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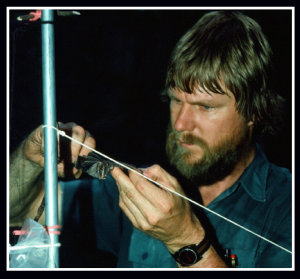
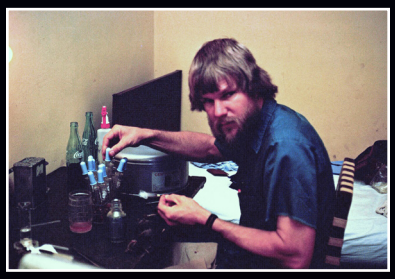
From Field to Laboratory: A Memorial Volume In Honor of Robert J. Baker





Edited by

Robert D. Bradley, Hugh H. Genoways, David J. Schmidly, and Lisa C. Bradley

Front cover: A selection of photos representing Robert J. Baker's career. The editors of this volume sincerely thank the many colleagues, friends, and students of Robert J. Baker that contributed 300+ photographs for the Baker retirement celebration in 2015 and for the memorial service in 2018, from which the images featured on this cover were selected.

Top row, left to right: Baker in his Biology lab, 2006, photo by Neal Hinkle, courtesy of Texas Tech University; preparing to karyotype a *Sundamys muelleri*, Sabah, Malaysian Borneo, 2006, photo by Peter A. Larsen; pinning bat specimens, Rattlesnake Canyon, Val Verde County, Texas, 2005, photo by Bill D. Mueller, courtesy of Museum of Texas Tech University.

Middle row, left to right: examining a *Macrotus californicus*, 2011, Picacho Peak State Park, Arizona, photo by Lizette Siles; setting a mist net, 2009, Placitas, New Mexico, photo by Lizette Siles; removing a *Uroderma bilobatum* from a mist net, 1977, Guatemala, photo by Ira F. Greenbaum.

Bottom row, left to right: admiring a *Hipposideros galeritus*, Sarawak, Malaysian Borneo, 2006, photo by Peter A. Larsen; karyotyping, Guatemala, 1977, photo by Ira F. Greenbaum; with Chernobyl specimens at NSRL, 1990, photo by Artie Limmer, courtesy of Texas Tech University.

SPECIAL PUBLICATIONS

Museum of Texas Tech University

Number 71

From Field to Laboratory: A Memorial Volume in Honor of Robert J. Baker

EDITED BY

ROBERT D. BRADLEY, HUGH H. GENOWAYS, DAVID J. SCHMIDLY, AND LISA C. BRADLEY

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From Field to Laboratory: A Memorial Volume in Honor of Robert J. Baker

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Table of Contents

Overture

Herein, we use the word "Overture" not in its first meaning, but rather in its second—"An introduction to something more substantial" ([Def. 2], *Oxford Dictionaries*, Oxford University Press 2018). In our current case, this is the overture to a memorial volume in honor of our friend and colleague Robert J. Baker. Here, his former students and colleagues have taken time and effort to write 43 essays and scientific articles and 54 personal encomia in demonstration of their respect for a mentor and friend. We hope that you will enjoy, reflect, remember, and be enlightened by the contents of this volume.

We chronicled Robert's life and accomplishments, both from a personal and academic standpoint, in our recent obituary for him (Genoways et al., *Journal of Mammalogy* 99:983–1012, 2018). As we worked on his obituary, and more recently as we worked on this memorial volume, we heard Robert described in many different terms, such as: advocate, bat-netter, brilliant, builder, companion, complimentary, conservationist, crotchety, defender, demanding, driven, driver (although he had a tendency to use the entire roadway and shoulders), editor, emotional, father, fatherly, fisherman, focused, friend, geneticist, grandfather, gregarious, hunter, husband, intellectual, leader, loyal (officially on faculty for 48 years although he could have left many times), mammalogist, mentor, mousetrapper, pain-in-the-ass, passionate, poetry-lover, prima donna, procrastinator, professor, promoter, raconteur, rancher, Red Raider, researcher, romantic (hundreds of pounds of chocolates distributed to the office ladies, graduate students, and friends every Valentine's Day), scheming, scholar, scientist, spontaneous, sportsman, storyteller, successful, supportive, teacher, 10 feet tall and bullet proof, Texan, tireless, uncompromising, unorganized, unpredictable, unrelenting, visionary, and writer. He was all of these things and much more, because his relationship with every person was different and had many facets.

The currency of Robert's academic work was a "publication." He believed deeply that publishing was the cornerstone of both teaching and research. To him it was the most significant accomplishment of any project. He implored his students to publish their work, and he was known to say that a project was never completed until a reprint of the publication of its results was in hand! He used this approach in his mentoring of both undergraduate and graduate students, emphasizing the importance of proper organization of a research project along with the discipline of organizing thoughts and coherent expression via written communication. Robert enjoyed publishing and he liked to publish with both students and colleagues. To him, it was a tool to get to know people, how they thought, and for developing a strong bond. As a result, he authored very few papers by himself. Most of his publications, and particularly his most cited papers, involved work with other people. He believed the more authors on a paper, the better the paper would be.

He also emphasized formal presentation of research work at scientific meetings. He felt that students benefited from the pressure of presenting and defending the results of their work in the presence of a group of peer scientists. He, as well as most of his students, presented either papers or posters at the annual meeting of the American Society of Mammalogists, the Southwestern Association of Naturalists, or the Texas Society of Mammalogists. Many of his students received awards for their presentations, and it was a point of pride to him that his students were competitive and successful in receiving recognition for their research work.

Robert's contributions to the publication record of science and mammalogy are legendary by any measure. His publication count (including several papers in this volume) stands at 449 with a few more papers still to come; his papers have been cited about 16,000 times, and his H index of 65 is very high for a person who worked in natural history and systematics. He is best known for his papers on karyotypic variation and evolution in mammals; his contributions to the systematics and classification of bats; his pioneering work on the impact of low-level radiation on mammals at the site of the Chernobyl nuclear accident; and his work on the genetic species concept in mammals. The two papers he co-authored on the latter subject have been cited more than 1,200 times. We would like to think he would be proud of the "good science" presented in this honorary volume.

Another point of pride for Robert was Texas Tech University, where he spent 48 years on the regular faculty and an additional three as an emeritus faculty member in the Department of Biological Sciences. When he joined the faculty there in 1967, it was still a

fledgling university without a Ph.D. program in biology. Research and publication were not stressed. Consequently, Robert had fewer resources at his disposal than faculty who worked at more prestigious universities, which make his achievements even more remarkable. A former President of Texas Tech, Grover Murray, told one of us (DJS) the story of a young faculty member (Robert Baker) who tapped on his office window one Saturday morning wanting to know if he could use the xerox machine in the President's office to copy a proposal to meet a NSF deadline because the machine in the library was not working! Robert stayed at Texas Tech, although he had numerous offers to leave, and pored his "heart and soul" into building the university. His contributions were not only in academics, but also in other leadership areas including fund-raising and athletics. He was very loyal to "his" institution and took great pride in seeing its growth and evolution into a major academic and research university.

Production of this memorial volume followed the format of the *Special Publications* series of the Museum of Texas Tech University. Each manuscript was peer-reviewed and critically edited for format and style following the standards that Robert helped establish for the *Special Publications* and *Occasional Papers* series. Because the volume is a part of a publication series, we attempted to corral the 43 varied manuscripts into a common format, and yet allow for the uniqueness of many of the manuscripts. In fact, throughout the Overture we refer to "essays and scientific articles" because the 43 manuscripts do not fit nicely under a single heading. There are some articles that examine Robert's career in research, teaching, and institutional service. Robert is on the author line of seven articles in this volume as his former students and colleagues have completed projects that had been initiated with Robert's involvement, but only now are they being published. Many are works that reflect the types of studies and the groups of mammals that occupied Robert's thinking for more than half a century. However, all of the articles in this volume serve as tributes to a colleague and friend who has left our midst. This volume, we hope, will be a strong and lasting memorial, which will help keep alive the memory of Robert J. Baker's contributions to mammalogy, science, and education.

Although it may not be readily apparent to readers, we have arranged the contributions in this volume into a loose order. The lead paper in the series is a scientometric examination of the professional career of Robert J. Baker. This article details many of the academic and professional contributions that Robert J. Baker made during his 48-year career at Texas Tech University. If you are not familiar with scientometric studies, you will find this analysis fascinating. By featuring this paper as the lead, our goal was to provide an introductory synthesis of Robert J. Baker as a multifaceted person who made significant contributions to a scientific community, university, professional society, and beyond as a researcher, educator, mentor, faculty athletic representative, university advocate, colleague, and friend.

The other two contributions in this initial group of articles also are hominal in nature; that is, they deal with humans, especially humans as a species. The next article in this group is an essay describing the past roles and future opportunities for mammalogists working in government teams combating biowarfare. The final paper of this initial group describes a low-cost, low-tech process by which species of origin may be determined for severely compromised skeletal specimens. This assay has become an invaluable tool for human identification efforts at Armed Forces DNA Identification Laboratory because it allows resources to be focused on samples that are human in origin.

The core of this memorial volume is the next 33 scientific contributions that are based on the study of mammals. We have chosen to arrange these articles in taxonomic order of their subject organisms. The article first up in this group is our single contribution on shrews of the order Soricomorpha. This is an in-depth set of analyses of the taxonomic relationships of *Sorex ornatus* and *S. vagrans* in the San Francisco Bay Area.

Bats of the Order Chiroptera are the topic of the next 13 contributions in this volume. The first six of these articles concern the critters that consumed so much of Robert's career, bats of the family Phyllostomidae. The lead article details the many new species described and the taxonomic changes that have occurred in members of the family in the 21st century. Next is a review of some of the most spectacular members of the family—species of the genus *Lonchorhina* with that amazingly pointed nose-leaf (we have pictures). Genetic variations in the pollenivorous genera

Leptonycteris and *Glossophaga* (respectively) are the subject of the two following articles. In another study, two closely related species of *Uroderma* were tested for adaptive divergence using transcriptomes. And the final article in this series of six presents data from a broad ecological study of *Platyrrhinus lineatus* in the Atlantic Forest of Paraguay.

Included in the volume are two papers dealing with insectivorous bats of the family Vespertilionidae. Both of these articles describe new species. One species is in the genus *Myotis* and is a patronym for Dr. Baker, whereas the other is in the genus *Rhogeessa* based on specimens from Nicaragua. The next two studies are surveys for bats conducted under difficult circumstances and in areas not known for rich chiropteran faunas. The first study was conducted on the High Plains of Texas in the vicinity of Lubbock, whereas the other was undertaken at the Tar Creek Superfund Site in northeastern Oklahoma. Despite the challenges, both studies report some interesting results. We also have two studies dealing with the zoogeography of bat faunas. The first study is a test of previously established zoogeographic units in Ecuador using distribution data from phyllostomid bats. The other investigation was to determine factors that influence species richness of the bat faunas on islands in the Caribbean Basin. The final contribution concerning bats is a genomic survey of the Order Chiroptera exploring evolutionary relationships based on LINE-1 transposable elements.

Our authors submitted three studies based on members of the Order Carnivora. Two of the studies deal with the biology of the coyote in the western United States. One of the studies is of the diet of the coyote in Joshua Tree National Monument and the interaction between this predator and its prey. The other considered the relationship of coyotes and gray wolves in northeastern Washington State where wolves are reoccupying the area and coyote populations are increasing. A morphometric analyses of craniodental characters and qualitative comparisons of pelage and other external features of bobcat/Canada lynx hybrids is the topic of the third carnivore study. The next article is the single contribution in the volume concerning whales of the Order Cetacea (yes, we still believe that whales are cetaceans and not even-toed ungulates). The study is based on four mtDNA genes comparing eastern and western stocks of the gray whales in the northern Pacific Ocean.

The next large group of articles is 11 based on studies of members of the Order Rodentia. The first of these articles is an investigation of the impact that anthropogenic and climate changes have had on hybridization between ground squirrels of the genus *Ictidomys*. The next three articles are taxonomic in nature including the descriptions of two new subspecies and two new species. The first of these articles discusses the Neotropical variegated squirrel in Nicaragua and describes a new subspecies from Isla de Ometepe, and the second article describes a new subspecies, which is a patronym for Dr. Baker, of Botta's pocket gopher from Texas. The third article describes two new species of deer mice of the *Peromyscus mexicanus* group—one is from Chiapas, Mexico, and the other is from Guatemala; the former species also is a patronym for Dr. Baker.

The next three papers continue the study of members of the genus *Peromyscus* using an array of molecular genetic techniques. In order, these cover topics dealing with the *P. maniculatus* group, *P. truei*, and relationships of members of genera *Isthmomys* and *Peromyscus*. The next two papers concern rodents of the subfamily Sigmodontinae. The karyotype of *Sigmodon hispidus* was examined using chromosome paints and fluorescent G-bands to see if it is primitive for the subfamily. In the subsequent molecular paper, the relationships within the Oryzomyini, one of the tribes within the subfamily, were studied using both mitochondrial and nuclear datasets.

The final two papers concerning rodents are based on morphological studies. In the first of these studies, geometric morphometrics were used to screen for fluctuating asymmetry in bank voles from the Chernobyl nuclear exclusion zone. In the other study, the size of m1 was analyzed as a proxy for variation in body size of muskrats in the transition period from the late Pleistocene to early Holocene on the southern High Plains of Texas.

The final four contributions based on mammals report the results of faunal surveys (based on field work and/or museum records). The first is a faunal report of

the mammals of the Chinati Mountains State Natural Area, Texas. The second is a comparison of pre- and post-hurricane Katrina faunal surveys conducted in the Barataria Preserve of Jean Lafitte National Historical Park, Louisiana, to gain an understanding of the impact of this storm on the biodiversity of the study site. The third study is based on the recently developed concept of data repatriation, which in this case was data on Mexican mammals housed in the Natural History Museum (London). The final faunal study describes the karyotypes of 17 small mammals as part of an ongoing survey of the mammals of the country of Botswana.

We did receive one contribution on a lower vertebrate group, and it is appropriate because Robert and one of his former Ph.D. students were among the authors. This is a detailed analysis of the evolution of rDNA of lizards of the genus *Aspidoscelis* to gain insight into the relationship of the unisexual and bisexual species in the genus.

The final six contributions of this volume are essays that pertain to teaching and institutional service, of which several are directly about Robert, and others were inspired by him. In the first essay one of Robert's former students attributes her training in evolutionary biology to her ability to effect change in a mid-sized educational institution. The second essay details Robert's institutional contributions as Texas Tech's Faculty Athletics Representative to the Big 12 Conference and the National Collegiate Athletic Association. Next, one of Robert's former undergraduate students who is now a member of the National Academy of Sciences muses on what and how to teach science to non-science majors at the university level, drawing on his inspiration from his association with Robert. The following essay explores Robert's impact as a mentor to graduate students and lays out lessons learned by one of his former Ph.D. students. The next essay explores what it means to be a naturalist and what is the future of naturalists; that essay concluded "Robert J. Baker was a naturalist" because "he exuded an enthusiasm for life, both in academia and in the natural world as a field biologist." The final essay in this memorial volume is an appreciation of Robert written by internationally known environmental writer Barry Lopez, who won the National Book Award for Nonfiction for *Arctic Dreams* (1986), and his *Of Wolves and Men* (1978) was a National Book Award finalist.

Although as editors we have committed considerable time to the organization and production of this volume, we would not have been successful without the time and support of many other people. First, and foremost, we appreciate the involvement of our authors who created the research that is presented here. They were not motivated to undertake this work for us, but rather because of their love, respect, and loyalty to Robert J. Baker. The funding necessary for the production of this volume has come from many people, including our authors, Robert's former students, former colleagues and administrators, and friends of Robert. In recognition of their financial contributions supporting this project, their names appear on our "Sponsors and Donors" page. As you will see there are 63 names on this list. We truly extend our gratitude to them. We especially thank Laura Baker for her sponsorship, in her husband's memory, of this volume.

Each article in this volume has undergone evaluation by at least two external reviewers. With 43 articles, this work has involved a large cadre of our fellow scientists, by our count 77 people (see "Reviewers" page). By our estimation, it took at least four to five hours to perform each of these reviews meaning that this a commitment of nearly 400 hours. These reviews were done anonymously, but here we wish to recognize all reviewers without reference to the particular article(s) that they handled. These reviews allowed us, as editors, to make decisions on accepting, revising, or returning the submitted manuscripts. These reviews also were extremely valuable to the authors, aiding them in revising and improving their contributions to this volume. We extend our gratitude to these reviewers for their time, their input, and their expertise.

In our obituary for Robert, we enumerated his contributions, but our lists ended with those things that occurred or appeared prior to 1 July 2018. Here we can update that information with what has happened in the last year so there is a more complete permanent record of his accomplishments. His obituary listed 438 publications, but since that time four additional papers have appeared and seven more appear in this volume, to bring his total publications to 449. There are an additional four manuscript that are in draft form that we believe will ultimately be published, so we believe that Robert's total publications will reach at least 453 (see

Schmidly et al. below for the lists). No description of a new taxon has appeared, but one new species will be recognized in one of the draft manuscripts. When that article appears, it will bring to 19 the number of new species and subspecies that he has described and named. Finally, as mentioned previously, three new patronyms have been described and named in Robert's honor herein, bringing his total to 10.

We now close our overture, hoping that it has set the mood for the 43 articles that follow. It is our hope that like a finely tuned orchestra these articles will flow from one group to another while making the whole larger than the sum of its parts. These are the best efforts of 121 authors working to honor and memorialize their friend, colleague, and mentor, Robert J. Baker. Following these articles, there are 54 encomia—small songs/speeches of praise or victory—which are very personal messages from those that knew him best.

As we worked on organizing and compiling this memorial volume, we tried to keep in mind Robert's favorite motto—often uttered as a challenge to his students and colleagues—that "anything worth doing is worth overdoing." We hope that Robert would be satisfied that this volume, at more than 900 pages, has fulfilled that challenge!

The Editors –

Robert D. Bradley, Lubbock, TX
Hugh H. Genoways, Lincoln, NE
David J. Schmidly, Placitas, NM
Lisa C. Bradley, Lubbock, TX

1 August 2019

Robert was a firm believer in the power of the DNA molecule; he even named his ranch "DNA Works" and used a modification of the DNA double helix as his cattle brand. Also note his devotion to hunting and fishing and his overall appreciation of wildlife and natural beauty, as illustrated by the gate art at his ranch entrance. (Photo by Robert D. Bradley)


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A Scientometric Appreciation of Robert J. Baker's Contributions to Science and Mammalogy

David J. Schmidly, Robert D. Bradley, Emma K. Roberts, Lisa C. Bradley, and Hugh H. Genoways

Abstract

This article describes Robert James Baker's academic pedigree and genealogy, his scientific productivity (number of publications), his citations, his students, his contributions to his university and scientific societies, his personality in relation to his scientific achievements, his legacy, and a personal note of appreciation by individuals who worked with him and knew him well. His accomplishments are compared with other dominant personalities in the field of mammalogy, both historical and contemporary. The paper builds on the 2018 obituary authored by Hugh Genoways and others that was published in the *Journal of Mammalogy,* but includes a much more quantitative and qualitative analysis of his scientific accomplishments and research productivity.

Key words: citation counts, contracts, grants, h-index, m-value, personality, publications, Robert James Baker, students

Introduction

This article explores the remarkable career of Robert James Baker (RJB), who died quietly at his home on 30 March 2018, thereby ending a career that spanned six decades at one institution, Texas Tech University (TTU). RJB's obituary was published shortly after his death, and it chronicles his remarkable career, including a listing of his publications, his numerous master's, doctoral, and post-doctoral students, as well as other highlights of his personal and professional life (Genoways et al. 2018). By any measure, his scientific achievements were substantial, and one could even say legendary—449 scientific publications, 98 graduate students produced, thousands of undergraduates taught and introduced to science, and numerous awards and honors bestowed during his career in recognition of his many achievements.

Using a scientometric approach to examine quantitatively and qualitatively his scientific achievements and research productivity, we delve much more deeply to interpret them in light of the recent literature regarding the careers of other highly productive and creative scientists. Each of the authors knew RJB for many years, in two cases (DJS and HHG) for more than 50 years. And because all of us worked and socialized with him and knew him well, we provide our perspective of his personality traits and strengths that contributed to his scientific creativity and impacts on the broad field of science and particularly mammalogy.

The notion of how to identify or readily measure scientific excellence has been elusive and argumentative (see Jackson and Rushton 1986), although several indicators of scientific excellence have been proposed in the past two decades to assess productivity and impact. These include: total number of publications in refereed journals; total number of citations; journal impact or index factors; frequency of appearance as first, middle, or senior author in collaborations; the number of different journals in which the research has been published; the number of grants awarded each year; and the number of papers presented at national scientific meetings (e.g., see Bartholomew 1982; Babu and Singh 1998; Panarctos and Malesios 2009; Kreiman and Maunsell 2011; Acuna et al. 2012; and Gibson et al. 2015).

Biologists have largely followed this model for professional credit, although those interested in systematics and evolutionary biology also contribute knowledge in nontraditional ways that are typically more difficult to quantify or assess in terms of scien-

tific merits, such as collecting biological specimens for natural history collections. Collecting and curating biological specimens builds and strengthens the basic infrastructure on which biodiversity knowledge is built, and this knowledge provides data critical for many disciplines beyond systematic biology (McDade et al. 2011).

We have considered all of these facets in examining the life and career of RJB. We draw attention to his publications and citation counts, his work with a legion of undergraduate and graduate students, his contributions to natural history collections, and his success in acquiring funding to support his research and that of his students. In addition, we have provided an overview of his academic pedigree and his personality traits as they contributed to his legacy. Finally, we have compared his research record with deceased highly published mammalogists as well as with some contemporary colleagues with highly regarded credentials and accomplishments.

Baker's Academic Pedigree, Genealogy, and Early Collaborators

Figure 1 presents the academic pedigree for RJB. It was generated utilizing various sources, including two articles (Jones 1991; Whitaker 1994) about the academic propinquity and genealogy of 20th century mammalogists, and by examining curriculum vitae, university and faculty webpages, pedigrees, obituaries, and biographies of many scientists included in the pedigree (e.g., RJB, Joseph Grinnell, J. Knox Jones, Jr.).

RJB's academic pedigree and genealogy (see Fig. 1) trace back to two prominent academic programs in mammalogy in the first half of the 20th Century—at the Museum of Vertebrate Zoology (MVZ), University of California Berkeley, and at the Museum of Natural History, University of Kansas (KU).

The MVZ program at Berkeley was led by Joseph Grinnell, considered by many to be the academic father of mammalogy (Jones 1991; Schmidly et al. 2017; Schmidly and Naples 2019). Grinnell began training doctoral students in mammalogy, and three of his best-known students became important figures in the genealogy of Baker. Walter P. Taylor was Grinnell's first Ph.D. student in mammalogy, and after leaving Berkeley he went on to establish the Cooperative Wildlife Units at Texas A&M University and then at Oklahoma State University. William B. Davis, another Ph.D. student of Grinnell, left Berkeley in 1938 to start the mammalogy program at Texas A&M University, and E. Raymond Hall, probably Grinnell's best-known student, left Berkeley in 1944 to establish a program at the Museum of Natural History at KU. Taylor, Davis, and Hall were the academic forefathers of RJB.

One of Davis' master's students at Texas A&M, Bryan Glass, assumed a position at Oklahoma A&M University, now Oklahoma State University (OSU), in 1946 and later completed his Ph.D. there in 1952 under the direction of Walter Taylor, who ran the Coop Unit at OSU. In 1963, after completing his bachelor's degree from Arkansas A&M College (now the University of Arkansas at Monticello), young Baker (then 21 years of age) entered the program at Oklahoma State and completed his Master's degree under Glass in 1965. The title of his thesis was "Systematics and Variation of *Myotis subulatus*." This was the beginning of Baker's long-standing "love affair" with the biology of bats.

Hall, following his move from Berkely to KU in 1944, established a dynasty in mammalogy that lasted three decades (see Schmidly and Naples 2019). One of his most successful Ph.D. students, E. Lendell Cockrum, took a position at the University of Arizona where he, too, established a graduate program in mammalogy. Following the completion of a master's degree, RJB entered that program and completed his Ph.D. work in two years in 1967. His Ph.D. dissertation involved nectar-feeding bats and was titled "Karyotypes of Phyllostomid Bats (Class, Mammalia; Family, Phyllostomidae) and Their Evolutionary Implications." At the time, this was considered to be pioneering research and it directly impacted future research on the systematics and evolution of mammals.

After receiving his doctoral degree, RJB accepted employment as an assistant professor in the Department of Biology at Texas Tech University. The university had an incipient program in mammalogy that was started in 1962 by Robert L. Packard, another doctoral student of Hall. Packard, who was directing master's students in mammalogy, was a prominent figure in the decision to hire Baker.

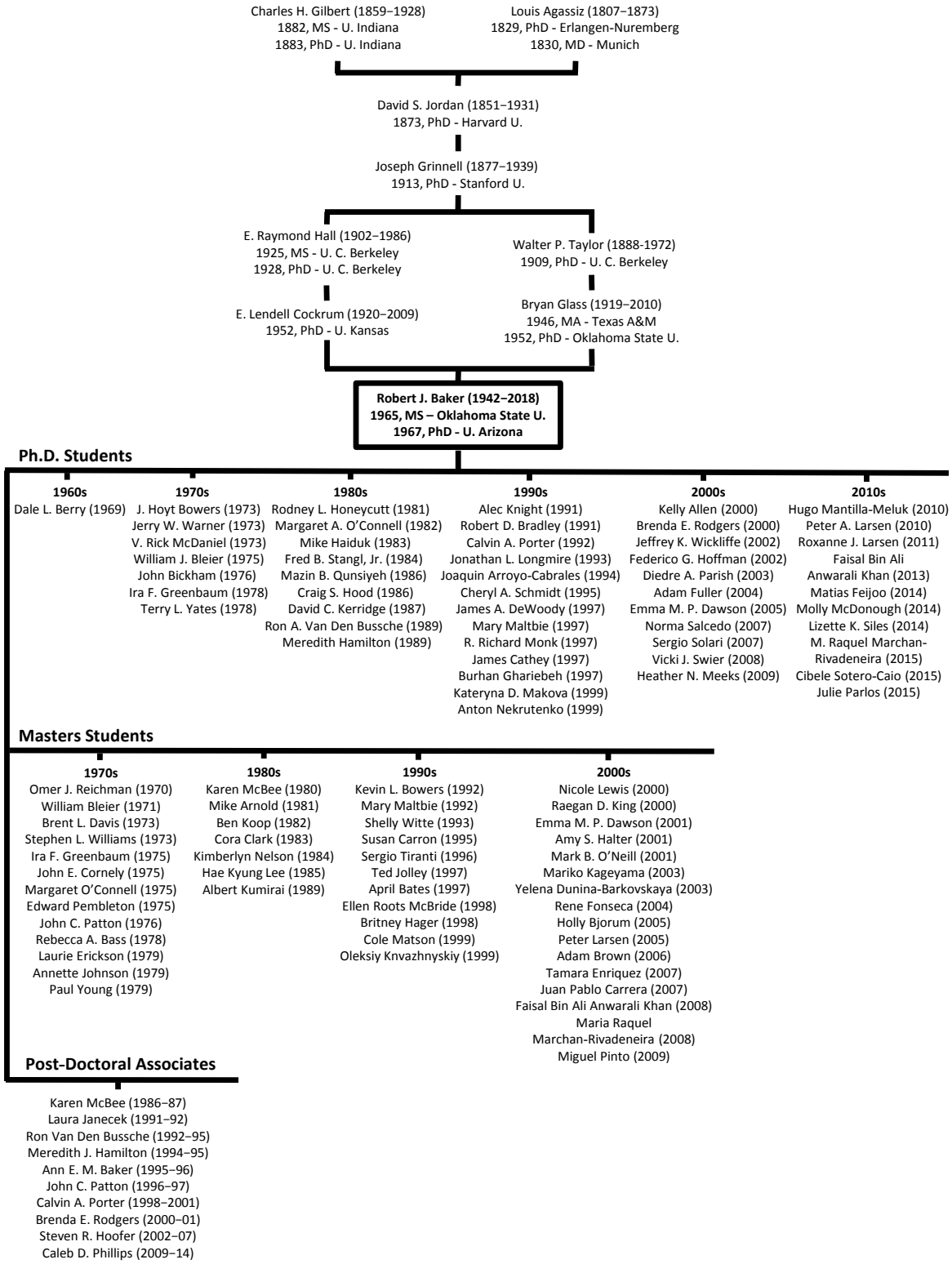

Figure 1. Robert J.Baker's academic pedigree, including his master's and doctoral students and post-doctoral associates.

Shortly after RJB joined Packard on the faculty, TTU made an institutional commitment to establish mammalogy as a major education and research focus of the university. Following the model used by E. Raymond Hall at KU, the institution made infrastructure investments to support the mammal collection and established three major publication outlets (*Occasional Papers*, *Special Publications*, and *Museology*). Other mammalogists soon followed Packard and Baker to TTU, most notably J. Knox Jones Jr., who was recruited as Dean of the Graduate School and Professor of Biological Sciences (and later became Vice President for Research) in 1971. The three mammalogists, together with a few other people, were instrumental in efforts to expand the Museum at the university and to establish the Natural Science Research Laboratory (NSRL) as a major research center and collection repository for mammal specimens. Over RJB's career at TTU, 13 other professional mammalogists joined the TTU faculty or staff. As explained below, RJB took great advantage of this institutional commitment by enhancing his publication horizons and recruiting outstanding students to participate in his graduate research program (see L. Bradley et al. 2005 for a history of mammalogy at TTU).

Another important association that RJB had during his graduate studies at University of Arizona and beyond was with James Patton, a fellow graduate student, and T. C. Hsu, Director of the Division of Cell Biology at M. D. Anderson Cancer Center in Houston. Hsu was instrumental in training a number of mammalogy graduate students in the new methods of mammalian cytogenetics. Besides Baker and Patton, they included Alfred Gardner, Dean Stock, and James Mascarello, all of whom made important contributions to the emerging field of mammalian cytosystematics (Hsu 1979). In the early 1960s, Hsu, with his research partner Sen Pathak, discovered how to isolate mitotic chromosomes of human tissue culture cells using a hypotonic solution, which led to the modern method for preparation of non-overlapping chromosomes in mammalian karyotypes. A significant breakthrough occurred in 1966, when Hsu, Baker, and Patton and a few others participated in a research trip to the Patagonia Mountains in Arizona where a major step was taken in adapting this technique to work under field conditions (Patton 2005). RJB continued his association with Hsu for many years, which included publishing three papers together in 1968 and 1970 that focused on the sex-chromosome systems of phyllostomid bats (see RJB bibliography in Genoways et al. 2018). In 2014, RJB and some of his students described and named a new genus (*Hsunycteris*) and tribe (Hsunycterini) of phyllostomid bats in honor of Hsu (Parlos et al. 2014).

METHODS

The two major quality indicators in our scientometric analysis of RJB's academic career are based on publication counts and citation counts, respectively. In addition, we have considered his students and their careers, his grant and funding sources, and his specimen and ancillary collection contributions to natural history museum collections. This information was obtained from several sources, including his published obituary (Genoways et al. 2018), his personnel file in the Department of Biology at TTU, his curriculum vitae, specimen catalogs and other documentation associated with the TTU mammal collection at the NSRL, and the personal knowledge of the five authors of this paper who knew RJB, collectively, for almost 150 years.

A yearly data matrix (1965–2018) of his publications was created based on the following information: total number of papers published; number of papers for which citation counts were available; number of database papers published in peer-reviewed outlets; total number of citations; and the average number of citations per paper. Each of his 445 papers was coded as follows: (1) journal or outlet of publication including the name of journal/outlet and year published; (2) sequence of authors for each paper—whether the paper was sole authored, co-authored (with RJB as either lead or second author), or multiple authored by more than two individuals (with RJB as the lead, secondary, or last author); (3) nature of the relationship of RJB to other publication authors—whether the paper was authored with a professional colleague (from Texas Tech or another institution), an undergraduate student, a graduate student, or a post-doctoral associate, or some combination of these groups; (4) subject organism of the paper,

whether it was a non-organism paper or about a specific group of organisms (plants, parasites, invertebrates, or vertebrates—fish, amphibians and reptiles, birds, or mammals); papers on mammals were further broken down into mammals in general, bats, rodents, or other (insectivores, primates, carnivores, edentates, or ungulates); and (5) *s*ubject area of the paper was assigned according to the following areas: an edited volume, book review, letter, encomia or obituary; taxonomy, systematics, evolution; natural history; genetic mechanism; ecotoxicology-radiation; collection management; wildlife-resource management; zoonoses-disease; or history of science. (Note: At the time of preparation of this article, the authors were aware of 445 total papers that were published or in press. Therefore, all data and calculations throughout this paper are based on that total of 445, and do not reflect the additional four papers, published in this volume and listed in the text of the Results, herein, that had not yet been submitted or accepted.)

From these data we made numerous tabulations, including number of publications per year, articles in 5-year aggregated intervals, and total publications each decade of his professional career (age 23–33; 34–44; 45–55; 56–66; and 67–76); the 20 journals that published at least five of his articles; and the number of papers published according to the sequence of authors, the group of organisms discussed, and the scientific subject of the paper. In addition, we made several calculations, including average number of papers published per year; percent and average number of data-based articles (i.e., excluding book reviews, obituaries, and other non-data publications) in peer-reviewed journals per year; and percent and average number of papers with citation counts per year. The results of these calculations and tabulations are presented in a series of tables or graphs (see Results).

Citation counts were determined for each of his papers using the Web of Science database (WOS). The total number of citations for each paper was determined for each year (1965 to 2017) and then arrayed into a citation increment range as follows—0–50; 51–100; 101–150, and so on thru 650. Citation counts were summed for each decade of his career (1960s, 1970s, 1980s, 1990s, 2000s, and 2010s), and the average annual rate of citations (calculated as the sum of citations divided by the number of years since first publication) was determined for each of those decades. The average article rate of citation (calculated by dividing the total number of citations for that year by the number of papers published that year) and the median of the average article rate of citation were determined. These data also are presented in either tables or graphs.

The Thompson Reuters Impact Factor (IF) was used to rank peer-reviewed journals. The IF is a metric of mean citations per article in a given journal and is calculated annually based on the number of citations in a given year of those citable articles that were published during the two preceding years (see McDade 2011). The IF was determined for each of the scientific journals that published his papers using information from the most current year.

Google Scholar, a web-based search engine that indexes scholarly literature, was used to calculate RJB's h-index. A scientists' h-index is defined as the highest number of his or her articles that have each received at least that number of citations (Hirsch 2005). For example, if you have an h-index of 20, that means that you have 20 papers with at least 20 citations. To make this calculation, the citation indices for each of RJB's articles were ranked in descending order. The largest number of articles that were cited at least that many times generated the h-index. The advantage of the h-index is that it combines productivity (number of papers produced) and impact (number of citations) into a single index number. Both high productivity and impact are required for a high h-index; neither a few highly cited papers nor a long list of papers with only a handful of (or no) citations will yield a high h-index. Thus, the h-index is the result of the balance between the number of publications and the number of citations per publication, and it has been promoted by many, including *Science* (Holden 2005) and *Nature* (Ball 2005), as a new measure of research performance that provides a robust evaluation of the scientific output of a researcher. Because h depends on scientific age, it has been determined that dividing the index number by scientific age, to calculate the m value, creates a more accurate picture of research performance (Hirsch 2005; Kelly and Jennions 2006).

For comparative purposes, a literature search was conducted to determine h- and m-values for other evolutionary biologists, and the h-index was calculated

for three other distinguished biologists, and contemporaries of RJB, who published important papers about mammals—John Avise at the University of Georgia, James Brown at the University of New Mexico, and James Patton at the University of California at Berkeley. Avise and Brown are members of the National Academy of Sciences, and Brown and Patton, along with RJB, served as President of the American Society of Mammalogists.

Information was obtained for 120 students who worked in RJB's lab, including 22 undergraduate, 48 master's, and 50 doctoral students, as well as 10 post-doctoral associates. The number of students who published with him was determined, and the career of each student was assigned to one of the following categories: academia, government agency, doctor or dentist, private sector, museum-zoo, public education, and NGO or foundation.

A complete list of RJB's grants and contracts, along with the sponsoring entity, was obtained from his curriculum vitae and personnel file, including specific awards from the National Science Foundation (NSF) and the National Institutes of Health (NIH).

The TTU specimen catalogs were used to determine the number of specimens he collected, including the number of tissue vials deposited in the Genetic Resources Collection (GRC) at the NSRL. The number of specimens prepped and deposited as vouchers, including the number of tissue vials preserved from voucher specimens, was determined directly from RJB's personal catalog. He also deposited specimens and tissues in other museums and collections, but those data were not readily available.

Finally, to assess RJB's publication legacy in mammalogy, we examined the published obituaries for 17 deceased, well-published naturalists/mammalogists, and determined for each the total number of papers published as well as the number and nature of papers published in the *Journal of Mammalogy* (feature article or note versus a book review, letter to the editor, or obituary).

RESULTS

The basic data about RJB's publication and citations counts are presented in Table 1. Tables 2–10 and Figures 2–5 present various tabulations, calculations, and graphed depictions of the data as described below.

RJB's Publications

Robert J. Baker's list of publications, as reprinted in his obituary (Genoways et al. 2018), included 438 titles over his career from 1965 to 2017. Since his death, four additional papers have appeared in print, bringing the total number to 442. The titles of these papers are as follows;

439. Montero, B. K., M. Sagot, C. D. Phillips, R. J. Baker, and E. H. Gillam. 2018. Geographic variation of contact calls suggest distinct modes of vocal transmission in a leaf-roosting bat. Behavioral Ecology and Sociobiology 72:125. https://doi.org/10,1007/s00265-018-2543-1.

440. Kwiecinski, G. G., S. C. Pedersen, H. H. Genoways, P. A. Larsen, R. J. Larsen, J. D. Hoffman, F. Springer, C. J. Phillips, and R. J. Baker. 2018. Bats of Saint Vincent, Lesser Antilles. Special Publications, Museum of Texas Tech University 68:1–68.

441. Pedersen, S. C., G. G. Kwiecinski, H. H. Genoways, R. J. Larsen, P. A. Larsen, C. J. Phillips, and R. J. Baker. 2018. Bats of Saint Lucia, Lesser Antilles. Special Publications, Museum of Texas Tech University 69:1–61.

442. Solari, S., C. G. Sotero-Caio, and R. J. Baker. 2019. Advances in systematics of bats: towards a consensus on species delimitation and classifications through integrative taxonomy. Journal of Mammalogy 100:838-851.

In addition, seven papers that include RJB on the author-line are included in this volume, thus bringing his total publication record to 449.

443. Hoffmann, F. G., R. N. Platt II, H. Mantilla-Meluk, R. A. Medellín, and R. J. Baker. Geographic and genetic variation in bats of the genus *Glossophaga*. This volume.

444. Parlos, J. A., M. A. Madden, L. Siles, F. A. Anwarali Khan, C. G. Sotero-Caio, K. L. Phelps, R. J. Baker, and R. D. Bradley. Temporal patterns of bat activity on the High Plains of Texas. This volume.

445. Wichman, H. A., L. Scott, E. K. Howell, A. R. Martinez, L. Yang, and R. J. Baker. Flying around in the genome: characterization of LINE-1 in Chiroptera. This volume.

446. Thompson, C. W., F. B. Stangl, Jr., R. J. Baker, and R. D. Bradley. Ecological niche modeling identifies environmental factors influencing hybridization in ground squirrels (Genus *Ictidomys*). This volume.

447. Swier, V. J., R. D. Bradley, F. F. B. Elder, and R. J. Baker. Primitive karyotype for Muroidea: evidence from chromosome paints and fluorescent G-bands. This volume.

448. Marchán-Rivadeneira, M. R., D. F. Alvarado-Serrano, B. Mueller, R. Strauss, and R. J. Baker. Patterns of fluctuating asymmetry and shape variation in *Myodes glareolus* from Chernobyl, Ukraine. This volume.

449. Porter, C. A., O. G. Ward, C. J. Cole, and R. J. Baker. Distribution and expression of ribosomal DNA in the composite genomes of unisexual lizards of hybrid origin (Genus *Aspidoscelis*). This volume.

Also, we are aware of another four papers that are under preparation and, if eventually published, that would increase the publication count to 453. Those potential papers include the following:

450. Siles, L., and R. J. Baker. Revision of the pale-bellied *Micronycteris* (Chiroptera, Phyllostomidae) with a description of a new species from Central America. In preparation.

451. Parlos, J. A., C. D. Phillips, J. C. Cokendolpher, S. J. Robertson, J. K. Krejca, and R. J. Baker. Genetic boundaries in endemic, troglobitic *Cicurina* spiders from Bexar County, Texas. In preparation.

452. Parlos, J. A., C. D. Phillips, S. Solari, and R. J. Baker. Phylogenetic reconstructions and multiple lines of evidence for species of *Dermanura.* In preparation.

453. Korstian, J., R. N. Platt II, B. Faircloth, T. C. Glenn, D. A. Ray, and R. J. Baker. Ultraconserved elements reveal the complexity of genus *Myotis* in the New World. In preparation.

RJB published at least one paper in every year of his career from 1965 to 2018 (Table 1 and Figure 2) with an average of 8.4 papers per year. Eighty-three percent of his papers were data-based and published in peer-reviewed journals (average of 6.9 per year). Ninety-one percent of his papers had citation counts available (average of 7.6 per year). The grand total of published pages in his papers was 6,483; subtracting out the pages of the 4 edited volumes lowers that number to just over five thousand (5,067), averaging just under 12 pages per article (11.7).

The fewest number of papers he published in a single year was two (1965, 1966, 1969, and 2015); the highest number was 17 in 2001 and 2003 (Table 1). In 19 different years (1978–1981, 1984–1985, 1988, 1991, 1996, 1998, 2000–2001, 2003, 2006–2007, 2009, and 2012–2014) he published 10 or more papers. Over a 45-year period from 1970 to 2015, he published 410 papers (92.8% of the total). His most productive periods were 1978–1982 and 2000–2004, with 59 and 61 publications, respectively, followed by 2006–2010 (52 papers, see Table 1). His period of peak publication productivity (almost 120 publications) occurred when he was between 56 and 66 years old (Fig. 3). A comparison of his research productivity in the first half of his career (1965–1991) with that of the second half (1992–2018/19) again speaks to his consistency with 203 papers (45.6% of the total) published in the former period compared to 239 (54.4%) in the latter.

RJB published in 127 different publication outlets, including 97 different peer-reviewed journals. During most of his tenure at Texas Tech, the university maintained a large number of mammalogists on its faculty and staff, and RJB took strategic advantage of this by publishing with many of these individuals, such as Hugh H. Genoways (48 publications), Robert D. Bradley (43; some as a graduate student, see below, and some as a faculty colleague), Ronald K. Chesser (37), Carleton J. Phillips (25), Clyde Jones (12), J. Knox Jones, Jr. (11), and David J. Schmidly (11). He also published with non-TTU faculty from other institutions, including 13 papers with Holly A. Wichman (University

Table 1. Publication and citation counts for Robert J. Baker's scientific articles, 1965−2018. Number of data-based papers indicates those containing original data. Total citations per paper were determined from the Web of Science online indexing service.

Year of publication	Number of papers published or in press	Number of papers with citation counts	Number of data-based papers	Total citations	Average citations per paper
1965	2	2	1	24	12.0
1966	2	2	2	47	23.5
1967	4	4	4	256	64.0
1968	6	5	5	207	41.4
1969	2	2	2	95	47.5
1970	8	8	6	398	49.8
1971	6	6	6	169	28.2
1972	11	11	11	353	32.1
1973	6	6	6	224	37.3
1974	6	6	6	220	36.7
1975	7	7	7	250	35.7
1976	9	9	9	337	37.4
1977	4	4	2	90	22.5
1978	12	11	10	472	42.9
1979	16	13	7	805	61.9
1980	11	11	10	443	40.3
1981	13	11	11	478	43.4
1982	7	7	7	521	74.4
1983	7	7	7	278	39.7
1984	11	10	9	298	29.8
1985	7	6	6	138	23.0
1986	4	4	4	397	99.2
1987	4	4	4	188	47.0
1988	12	10	12	321	32.1
1989	4	4	4	234	58.5
1990	6	6	6	738	123.0
1991	16	14	13	936	66.8
1992	9	6	6	253	42.2
1993	5	5	5	163	32.6
1994	5	5	4	161	32.2
1995	3	3	3	97	32.3
1996	14	10	12	319	31.9
1997	7	5	4	375	75.0
1998	14	12	9	304	25.3
1999	8	7	6	256	36.6
2000	11	11	9	576	52.4

Table 1. (cont.)

Year of publication	Number of papers published or in press	Number of papers with citation counts	Number of data-based papers	Total citations	Average citations per paper
2001	17	17	13	1,221	71.8
2002	8	8	8	481	60.1
2003	17	16	13	746	46.6
2004	8	6	6	144	24.0
2005	7	6	4	98	16.3
2006	12	10	10	821	82.1
2007	11	9	9	191	21.2
2008	8	8	7	207	25.9
2009	12	12	9	233	19.4
2010	9	9	9	214	23.8
2011	9	8	6	123	15.4
2012	10	10	10	225	22.5
2013	10	10	9	131	13.1
2014	10	10	6	131	21.8
2015	2	2	2	13	6.5
2016	5	4	3	39	9.8
2017	4	4	4	8	2.0
2018–2019*	7	4	6	NA	NA
Totals	445	403	367	16,447	NA

* Includes publications in press.

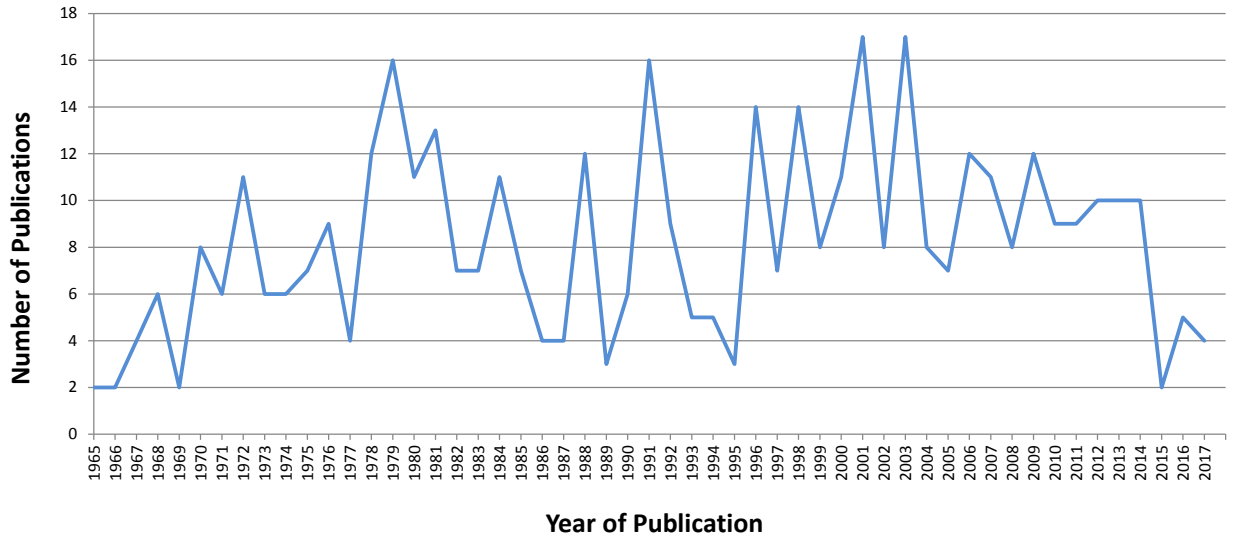

Figure 2. Robert J. Baker's publications by year.

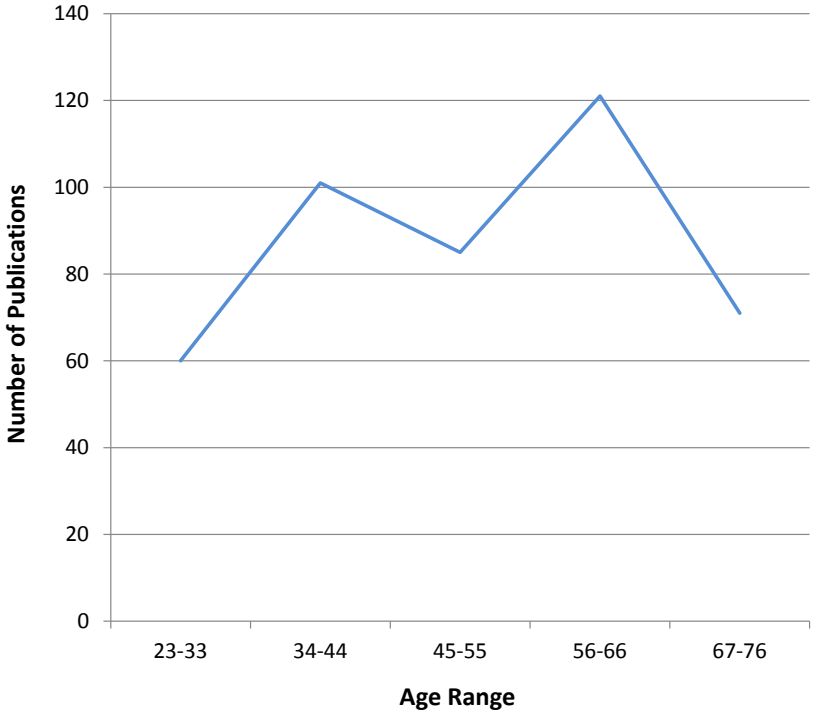

Figure 3. Robert J. Baker's publications by his age.

of Idaho), 12 with Sergey P. Gaschak (International Radiological Laboratory, Ukraine), nine with Michael H. Smith (University of Georgia), and five with Loren K. Ammerman (Angelo State University).

Table 2 lists the 20 journals that published the greatest number of his papers. He published approximately 15 percent of his papers (total of 66) in the *Journal of Mammalogy*, more than any other mammalogist of his generation. These papers have been cited 3,263 times for an average citation rate of 55.3 citations per article (Table 2). Ninety of his papers (20%) appeared in Texas Tech sponsored publications (e.g., *Occasional Papers* and *Special Publications*) and 354 (80%) appeared in other outlets. He had numerous papers in *Systematic Biology* (21 papers) and *Evolution* (13 papers), two high impact journals in his field; these papers have been cited 1,117 and 747 times, respectively. *The Southwestern Naturalist* and *Mammalian Species* each published 14 papers in which he was an author. He published 11 papers, collectively, in the journals *Science*, *Nature*, *BioScience*, and *Proceedings of the National Academy of Science*, all considered among the most prestigious journals in the biological sciences. These papers have been cited 1,383 times (Table 2). Toward the end of his career, as his research interests broadened, he published in other journals, including *Environmental Toxicology & Chemistry* (11 papers), *Molecular Ecology* (8 papers), and the *Journal of Heredity* (8 papers).

Mammals were by far the most common subjects of his publications, accounting for 360 (80.9%) of the total number of papers published (Table 3). Among his mammal papers, 194 (53.9%) were about bats, 110 (30.6%) were about rodents, 41 (11.4%) addressed mammals in general, and 15 (4.2%) were about other groups of mammals (insectivores, primates, carnivores, edentates, and ungulates). He published 20 papers (4.5% of the total) on reptiles, birds, fish, and vertebrates in general; two papers on plants; and five about invertebrates. Sixty of his papers (13.5%) did not involve a specific group of organisms.

Analysis of his papers by subject matter (Table 4) reveals that almost half of them (203 or 45.6%) were in the fields of taxonomy, systematics, and evolution. Another 35% covered general natural history (19%) and genetic mechanisms (16%). The remaining 19% covered a broad array of topics from ecotoxicology

Table 2. Journal and citation counts for journals with at least five scientific articles published by Robert J. Baker. Journal impact factors are provided in parentheses after the title, where available.

Journal	No. of papers and percent of total	Citation count	Citations/article
Journal of Mammalogy (2.139)	66 (14.8%)	3,263	55.3
Occasional Papers, Museum of TTU	63 (14.2%)	1,941	32.4
Systematic Zoology-Biology (8.523)	21 (4.7%)	1,117	58.8
Special Publications, Museum of TTU	17 (3.8%)	492	44.7
The Southwestern Naturalist (0.244)	14 (3.2%)	335	23.9
Mammalian Species	14 (3.2%)	745	57.3
Evolution (4.201)	13 (2.9%)	367	26.2
Environmental Toxicology and Chemistry (2.951)	12 (2.7%)	518	43.2
TTU, other publications	10 (2.2%)	43	7.2
Cytogenetics and Cell Genetics (1.455)	10 (2.2%)	519	51.9
Journal of Heredity (3.961)	8 (1.8%)	231	28.9
Molecular Ecology (6.086)	8 (1.8%)	409	51.1
Annals of the Carnegie Museum (0.750)	5 (1.1%)	137	27.4
Proceedings and Transactions, National Park Service	5 (1.1%)	131	26.2
Genetica (1.207)	5 (1.1%)	201	43.2
Molecular Phylogenetics and Evolution (4.419)	5 (1.1%)	152	30.4
Science (37.205), Nature (40.137), Bioscience (5.378), and PNAS (9.661)	11 (2.5%)	1,383	125.7
Totals	287 (64.6%)	11,984	36.5

and radiation (6%) to collection management (3%) and wildlife management (2.5%).

RJB was sole author of only 23 papers (5.2%) compared to 113 (25.4%) that were co-authored and 309 (69.4%) that were multiple authored (Table 5). Of the latter group, he was the last author on 162 (52.4%) of his papers. In total, he was sole or lead author for about a third of his papers (131 papers; 29.4% of the total), and he was a secondary or last author on 314 (70.6%). He was last author on 237 (53.3%) of his total publications.

For those that knew RJB, this statistic should not come as a surprise. Robert did not like authoring papers by himself. He wanted input from others—he believed in the adage of surrounding yourself with the best people possible and borrowing their brains! He felt bouncing ideas around and challenging others to think would help improve his papers. Further, he liked to share the credit. He wanted others to be involved so that they could improve their CVs, and he truly enjoyed writing with others.

Citation Counts of RJB's Publications

Citation counts from the Web of Science (WOS), an online scientific citation indexing service of Clarivate Analytics, were available for 403 of RJBs 445 papers (90.6%). Papers that could not be counted included

Table 3. Tabulations of Robert J. Baker's papers by topic and groups of organisms.

Category	No. of papers	% of total papers
Non-organism paper	60	13.5
Mammals	360	80.9
Bats	194 (53.9%)	
Rodents	110 (30.6%)	
Other (insectivore, primate, carnivore, ungulate)	15 (4.2%)	
Mammals in general (checklists, surveys)	41 (11.4%)	
Other vertebrates	20	4.5
Reptiles	9 (45.0%)	
Birds	7 (35.0%)	
Fish	1 (5.0%)	
Vertebrates in general	3 (15.0%)	
Invertebrates	3	0.7
Plants	2	0.4
Totals	445	100.0

Table 4. Tabulation of Robert J. Baker's papers according to subject areas.

Subject	No. of papers	% of papers
Taxonomy, systematics, evolution	203	45.6
Natural history	85	19.1
Genetic mechanisms	71	16.0
Ecotoxicology, radiation	27	6.1
Edited volumes, reviews, letters, obituaries	26	5.8
Collection management	14	3.1
Wildlife resource management	11	2.5
Zoonoses, diseases	4	0.9
History of science	4	0.9
Totals	445	100.00

Table 5. Tabulation of Robert J. Baker's papers according to the number of authors and his position on the author line.

Category	No. of papers	% of papers
Sole author	23	5.2
Co-author	113	25.4
Lead	(38)	(33.6)
Second	(75)	(66.4)
Multiple authored (more than 2)	309	69.4
Lead	(70)	(22.6)
Secondary	(77)	(25.0)
Last	(162)	(52.4)
Totals	445	100.00

some book reviews and letters to editors, chapters in edited volumes, species accounts in mammal books, contributions to newsletters, certain checklists of species, a few Texas Tech publications, some government proceedings and transactions, and papers in press or newly published.

A search of each of his publications in the WOS revealed a total citation count of 16,447 (Table 1). The average annual rate of citations for his papers was 310.3. A search in Google Scholar produced slightly fewer citations (15,853). These two databases use slightly different time frames and they index different journals, which accounts for the discrepancy.

The average and median annual rate of citation for his papers was 39.3 and 36.2, respectively. The distribution of the citations was significantly skewed, with 76% of the articles cited fewer than 50 times; 16% between 51 and 100 times; 4% between 101 and 150 times; 3% between 151 and 200 times; and 2% more than 200 times (Table 6). According to the WOS search results, eleven of his papers were never cited and an additional 11 were cited only one time.

The peak years for citations (Fig. 4) were: 2001 (1,221 citations; mean = 82.1 citations/article); 1991 (936; mean = 66.8); 2006 (821; mean = 82.1); 1979 (805; mean = 61.9); and 1990 (738; mean = 123). The average number of citations per article over RJB's career was generally consistent except for the last few years of his life (Table 7). The average annual rate of citations (calculated as the sum of citations divided by the number of years since the first publication) steadily increased from the 1960s until the end of the first decade of the 21st century, after which it also declined (Table 7).

RJB's 10 most cited papers are listed in Table 8. The two most highly cited papers were theoretical contributions about the genetic species concept in mammals that appeared in the *Journal of Mammalogy*. Four of the most highly cited papers appeared in the first decade of the 21st century, three in the 1990s, two in the 1980s, and one in the 1960s (Table 8).

The top journals, in terms of impact factor, in which RJB papers appeared were: *Nature*, *Science*, *Proceedings of the National Academy of Science*, *Systematic Biology*, *Molecular Ecology*, *Bioscience*, and *Molecular Phylogenetics and Evolution* (see Table 2). His most impactful papers (calculated by dividing the number of citations by the publishing journal's impact factor for that year, divided by the number of years since the article was published) were the two papers on the genetic species concept (co-authored with Robert D. Bradley) that appeared in the *Journal of Mammalogy* in 2001 and 2006.

Table 6. Analysis of citation counts for Robert J. Baker's 403 indexed papers. Citation counts were obtained from the Web of Science online indexing service.

Citation count range	No. of papers	% of papers
0-50	308	76.42
51-100	65	16.12
101-150	16	3.97
151-200	5	1.24
201-250	2	0.49
251-300	1	0.25
301-350	2	0.49
351-400	1	0.25
401-450	0	0.00
451-500	0	0.00
501-550	0	0.00
551-600	2	0.49
601-650	1	0.25
Total	403	

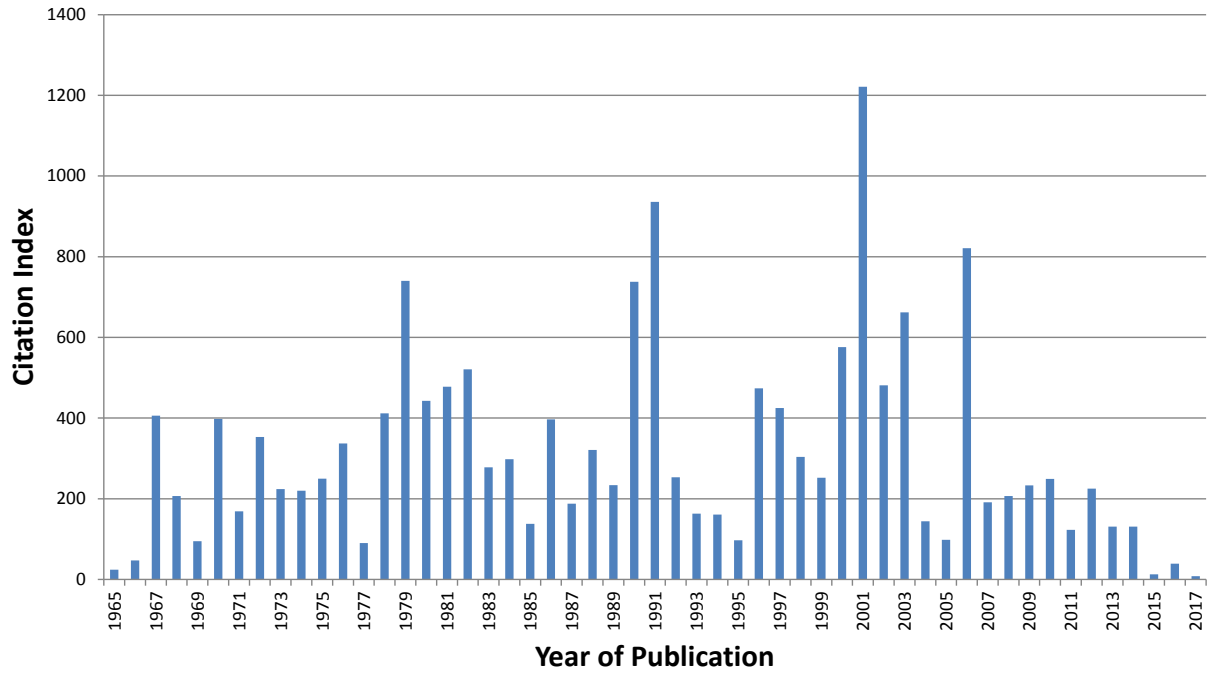

Figure 4. Annual citation counts for Robert J. Baker's publications.

Table 7. Publication and citation counts of Robert J. Baker's 403 indexed papers by decade. Citation counts were obtained from the Web of Science online indexing service.

Decade	No. of papers	Citation count	Average citation count per article	Average annual rate of citation*
1960s	15	629	42.0	125.8
1970s	81	3,318	41.0	331.8
1980s	74	3,296	44.5	329.6
1990s	73	3,602	49.3	360.2
2000s	103	4,718	45.8	471.8
2010s	57	884	15.5	110.5
Totals	403	16,447		

* Calculated as the sum of citations divided by the number of years since first publication.

Table 8. The 10 most cited articles published by Robert J. Baker.

Title	Journal	Year	Journal Impact Factor	No. of citations
A test of the genetic species concept: cytochrome-*b* sequences and mammals	Journal of Mammalogy	2001	1.630	642
Speciation in mammals and the genetic species concept	Journal of Mammalogy	2006	1.630	597
Distribution of non-telomeric sites of the $(TTAGGG)_n$ telomeric sequence in vertebrate chromosomes	Chromosoma	1990	4.021	586
Evidence for biased gene conversion in concerted evolution in ribosomal DNA	Science	1991	37.205	392
Use of "lysis buffer" in DNA isolation and its implications for museum collections	Occasional Papers, Museum of Texas Tech University	1997	NA	336
The ecology and evolutionary history of an emergent disease: hantavirus pulmonary syndrome	Bioscience	2002	5.378	310
Speciation by monobrachial centric fusions	Proceedings of the National Academy of Science	1986	9.661	287
Karyotypic evolution in bats: evidence of extensive and conservative chromosomal evolution in closely related taxa	Systematic Biology	1980	8.917	217
Diversification among New World leaf-nosed bats: an evolutionary hypotheses and classification inferred from digenomic congruence of DNA sequence	Occasional Papers, Museum of Texas Tech University	2003	NA	184
Karyotypes and karyotypic variation of North American vespertilionid bats	Journal of Mammalogy	1967	1.630	180
Total				3,731

H-index and M-value

The h-index for all of RJB's publications for which citations were available (15,853 in the Google Scholar database) was 65, meaning that 65 of his papers were cited at least 65 times. The m-value, derived by dividing the h-index score by his scientific age (53) was 1.23. By way of comparison, the h-indices and the m-values of Avise and Brown were higher (h = 102 and 106; m = 2.27 and 2.08, respectively). Patton's (h = 63; m =1.2) was nearly identical although slightly lower than that of RJB.

RJB's Influence in Teaching and Mentoring Students

RJB began working with graduate students soon after his arrival at Texas Tech. A list of his 48 Master's and 50 Ph.D. students was provided in his published obituary (Genoways et al. 2018), and they also are listed in Figure 1 of this publication. In his 48 years on the Texas Tech faculty, there were only seven years (1967, 1968, 1972, 1974, 1977, 1988, and 2012) in which he did not graduate a master's or a doctoral student.

In the early stages of his academic career, as might be expected, he worked more with master's than doctoral students, but this changed in the 1980s when he became more involved with doctoral students (Fig. 5). His production of Ph.D. students peaked in the 1990s and early part of the 21st century. Beginning with the 1990s and continuing throughout the remainder of his career, RJB also became involved with several post-doctoral associates who worked in his laboratory. These, too, were listed in his obituary (Genoways et al. 2018) and have been included in Figure 1.

He published papers with all but six of his Ph.D. students, and he had more than 10 publications with 14 of them, including 43 with Robert D. Bradley, 32 with Ronald A. Van Den Bussche, 21 with Jeffrey K. Wickliffe, 20 with Meredith Hamilton, and 17 with Calvin A. Porter. He published with 37 of his master's students; the largest number of papers was written with John C. Patton (9 papers), Stephen L. Williams (8), and Ben F. Koop (7). He published with all but one of his 10 post-doctoral associates, including 16 papers with Brenda Rodgers, 12 with Steven R. Hoofer, and 11 with Caleb D. Phillips.

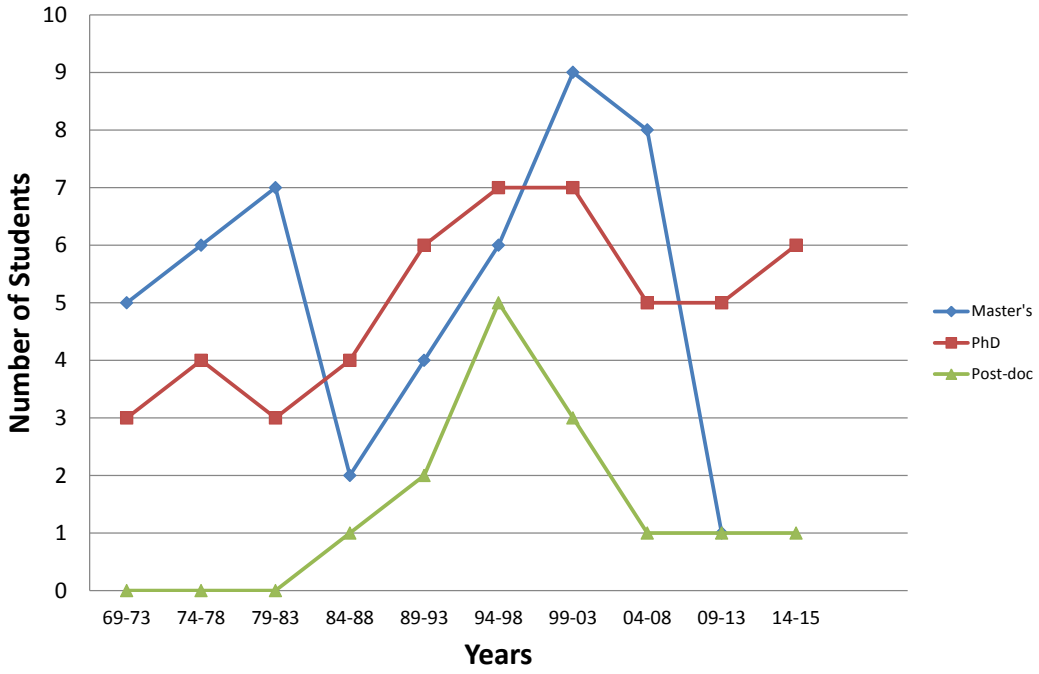

Figure 5. Robert J. Baker's master's and doctoral students and post-doctoral associates in 5-year periods throughout his career.

Table 9. Educational achievements and career fields of undergraduate and graduate students of Robert J. Baker.

Category	Undergraduate student*	Master's student	Doctoral student
Education			
Obtained Master's degree	2	48	NA
Obtained Ph.D. degree	10	24	50
Obtained medical/dental degree	7	1	0
Employment			
Academia	8	21	39
Federal/State agency	2	2	3
Private Sector	1	6	3
Medical Doctor or Dentist	8	1	0
Public Education	0	0	1
Museum/Zoo	0	5	2
Foundation/NGO	0	1	0
Unknown/deceased	3	12	2

* Undergraduate students who published with Robert J. Baker while an undergraduate.

An examination of the careers of RJB's graduate students (Table 9) reveals that of his 50 doctoral students, 39 (78%) have had careers in academia; nine others worked in federal agencies, the private sector, public education, or museums. Of his 48 master's students, 24 completed Ph.D. programs (7 under RJB at Texas Tech) and 21 ultimately became employed in academia; others went to work in museums or zoos, federal or state agencies, the private sector, NGO foundations, or in public education. All total, 60 of his graduate students (61.2%) received a Ph.D. at Texas Tech or some other institution and worked in academia.

The academic institutions where RJB's students worked include well-known public and private universities, several smaller state and regional universities, community colleges, and international institutions. The list of public and private colleges and universities in the U.S. where his students worked or currently work includes the following: University of California-Santa Barbara, North Dakota State University, Baylor University, Texas A&M University, Eastern Washington State University, Purdue University, Hebrew Theological College, Oklahoma State University, University of Georgia, Penn State University, University of Utah, Duke University, University of Minnesota, University of Michigan, the City University of New York, Wayland Baptist University, Northern Kentucky University, Arkansas State University, University of New Mexico, Pepperdine University, Harvard University, Lamar University, Midwestern State University, Loyola University, Sul Ross State University, Texas Tech University, Xavier University of Louisiana, Colorado State University, University of Pittsburgh, Tulane University, Mississippi State University, and the College of Charleston. Three of his former students are employed at community colleges (Lone Star College and Richland College in Texas and Tulsa Community College in Oklahoma). RJB also placed students at international universities in seven different countries: Universidad de Antigua (Medellin, Colombia), Universidad del Quindo (Colombia), Universidad de la Republica (Uruguay), Universidade Federal de Pernambuco (Recife, Brazil), Universidad Nacional de la Pampa (Argentina), University of Malaysia (Sarawak, Malaysia), Bethlehem and Birzeit universities (Pales-

tine), Malaspina College (British Columbia, Canada), and the University of Victoria (Canada).

RJB began teaching undergraduate students as soon as he arrived at Texas Tech, offering courses in histology, cytology, general zoology, the Biological Status of Man, but his favorite course was Freshman Biology for Non-majors, which he taught for more than 20 years (Genoways et al. 2018). It has been estimated that he taught several thousand students in this course (including, curiously, John Hinckley, Jr., who shot President Ronald Reagan on 30 March 1981).

He was also a huge supporter of undergraduate research, and many undergraduates worked in his laboratory. His curriculum vitae listed 22 undergraduate students that authored research papers based on work they did in his laboratory, including eight papers by Laura E. Wiggins, five by Amanda J. Wright, and four by Amy B. Baird. Of those 22 undergraduate research students, 19 pursued and obtained graduate degrees. Two obtained Master's degrees, and ten received Ph.D. degrees and work at the following academic institutions: University of Texas at Brownsville, University of Texas at Austin, U.S. Military Academy-West Point, Baylor University, Purdue University, University of Georgia, University of North Texas, University of Houston Downtown, and Texas Tech University. In addition, seven of the 22 undergraduate researchers went to medical or dental school and are now practicing in those professions.

RJB's Grants, Contracts, and Financial Support

Throughout his career, RJB was able to secure funding to support his research and graduate education programs. Through grants and contracts, he was awarded nearly $16 million (in 2018 dollars) from 31 different granting agencies (Table 10). He received 15 grants from the National Science Foundation (NSF), with almost 30 years of continuing funding from that agency totaling almost 3 million dollars. His NSF grants included the following:

1. Karyotypic studies of phyllostomid bats, 1968–1970;

2. Karyotypic studies of the Phyllostomidae, 1971–1972;

3. Extension of karyotypic studies of the Phyllostomidae, 1973;

4. Evolutionary studies of phyllostomatid bat faunas in Caribbean Islands (with Hugh H. Genoways), 1974–1975;

5. Chromosomal change in mammalian evolution (Chiroptera: Phyllostomatidae), 1976–1978;

6. Chromosomal studies of Phyllostomatidae, 1980–1982;

7. Chromosomal races of the white-footed mouse, *Peromyscus leucopus*, 1983–1984;

8. Updating and enhancement of the Recent mammal collections, Texas Tech University (with Robert Owen), 1986–1988;

9. Dynamics of a hybrid zone between chromosomal races of the white-footed mouse, *Peromyscus leucopus*, 1986–1989;

10. REU: Evolutionary genetics and dysgenesis in a naturally occurring hybrid zone in *Peromyscus leucopus*, 1990;

11. Repetitive DNA sequences in genome organization of phyllostomid bats: test of a molecular model for chromosomal divergence, 1992–1995;

12. Enhancement of collections and safety at the Museum of Texas Tech University (with Robert D. Bradley [P.I.], Clyde Jones, David J. Schmidly, and Richard Monk), 1998–1999;

13. Development of an integrated network for distributed databases of mammal specimens, 2001–2003;

14. Collection enhancement, enlargement, and compactorization at the Natural Sciences Research Laboratory (with Robert D. Bradley), 2006–2008; and

15. Natural history: Development of a liquid nitrogen system for the Genetic Resources Collection, Natural Sciences Research Laboratory, Museum of Texas Tech University (with Robert D. Bradley), 2015–2018.

RJB also received two funded grants from the National Institutes of Health:

Table 10. Categories of research funding for Robert J. Baker. All values have been converted to 2018 dollars.

Agency and Other Sources	Total funding
Federal Research Agencies	
National Science Foundation	$2,980,500
National Institutes of Health	$359,000
Smithsonian Foreign Currency Program	$578,000
U.S. Department of Agriculture	$354,000
U.S. Department of Commerce, Advanced Technology Program	$270,000
U.S. Fish and Wildlife Service	$36,000
Sandia National Laboratories	$175,000
National Park Service	$143,000
U.S. Department of Defense	
Fort Bliss	$873,000
Defense Threat Reduction Agency	$200,500
U.S. Department of Energy	
Pantex Treatment Facility	$125,500
Chernobyl	$1,308,500
Texas State Agencies	
Texas Parks and Wildlife Department	$315,750
Texas Tech University Office of Research Services	$123,500
Texas Department of Transportation	$72,000
Texas State Line Item (Biodiversity Database)	$3,680,000
Texas State Line Item (Genetic Identification of Cotton Cultivars)	$3,510,000
Texas Tech University faculty grants	$72,000
Texas Agricultural Experiment Station	$30,500
Texas Higher Education Coordinating Board	$78,500
Foreign Governments/Agencies	
New Brunswick Wildlife Trust Fund	$7,000
Health Protection Agencies, United Kingdom	$40,500
Private Sources	
Individuals - James Sowell	$230,000
Unidentified companies	$21,000
Foundations	
American Philosophical Society	$7,100
CH Foundation	$131,500

Table 10. (cont.)

Agency	Total funding
Conservation Organizations and Other	
Welder Wildlife Foundation	$40,000
National Fish and Wildlife Foundation	$58,000
National Geographic Society	$31,000
Texas Nature Conservancy	$23,000
State of Alaska (bear research)	$44,000
Total (approximately; in 2018 dollars)	$16 million

1. Ecology of emerging arena viruses in southwestern U. S., 1997–2000;

2. Mammalian genomes: stasis and change, 2001–2005.

Several other sources of funding for RJB also deserve mention because they provided support not only for his own research but also for institutional building at Texas Tech. He received funding from two line items provided by the Texas Legislature. Line item funding was the state equivalent to directed federal appropriations or "earmarks." Unless rescinded, this money was included annually in the TTU budget for the stated purpose of the work. One of the line item projects involved the development of a biological inventory and database of mammals on state-owned properties with the primary goal of providing an archival record of the mammalian biodiversity that was present in Texas at the turn of the 21st century and developing an electronic database of Texas mammals that could be accessible to state biologists and those in leadership roles in the development of wildlife management and conservation policies (see L. Bradley et al. 2005). This project supported the growth of the research collections at the Natural Science Research Laboratory (NSRL) at Texas Tech. The second line item project was for the genetic identification of species and cotton cultivars, and it was used to support the work of graduate students in his genetics lab in the Department of Biology who worked on the project.

One of us (DJS) introduced RJB to Jim Sowell (JS), a member of the Board of Regents at Texas Tech and a leading benefactor of the institution. When Professor Baker showed him the collections at the NSRL and explained the nature of his work and the numerous student publications that had resulted from that work, Sowell was so impressed that he offered to financially underwrite the cost of RJB's field trips to foreign countries to support his program. Overall, JS provided $230,000 in support for field studies, and in recognition of this support, RJB named a species of bat, *Carollia sowelli*, in his honor.

RJB received more than $1 million in funding to collaborate on a project at Chernobyl, the site of the world's largest nuclear accident. For this work Robert had to educate himself on methods and theory in ecotoxicology and radiation biology, recruit and train students from Ukrainian universities, and establish international collaborations. These collaborations continued for several years and resulted in more than 40 scientific publications focused on Chernobyl research.

RJB's Field Work and Contributions to Natural History Collections

Robert's fieldwork took him around the world, including five continents and 26 countries. He spent almost three years in the Neotropics, including the Caribbean Islands, collecting bats, as well as five total months, over a several year period, in the Chernobyl nuclear disaster zone, studying the impact of radiation on mammalian populations (for details of his field work, see Genoways et al. 2018). From these trips he accumulated a large amount of data and specimens that have been deposited in various natural history collections.

In his fieldwork, RJB emphasized special collections that included more than the traditional "skin and

skull" specimens for mammals. He pioneered the idea of cross-referencing museum specimens with information on karyotypes and various tissues. The frozen tissue collections he started are invaluable because many of the samples came from species and regions that are now heavily depleted. Without such a resource, studies of the evolution and systematics of mammals would be next to impossible to conduct, especially given the political and financial cost of expeditions. As a result of his vision, several other collections, including those at Texas A&M University, the Museum of Southwestern Biology (University of New Mexico), and Carnegie Museum of Natural History, now have special collections based on the model promoted by Robert. Other collections also have mimicked Robert's approach.

The NSRL contains specimens or specimen parts from 10,131 individuals that RJB was given at least partial credit for collecting. Materials archived from these specimens include standard museum vouchers, specimens preserved in ethanol, karyotypes, frozen tissues, lysis-preserved tissues, blood samples, parasites, fecal matter, and stomach contents. He also deposited an unknown number of specimens at other institutions in the United States and in foreign countries (e.g., Ukraine, Mexico, and Ecuador) where specimen sharing was required in order to obtain collecting permits. He spent a lot of time conducting field work in the Neotropics, including the Caribbean, Mexico, and Central America, where he conducted research on the evolution and systematics of New World bats.

Baker's personal catalog listed 4,711 specimens as the total number of voucher specimens that he prepared (standard museum specimens and those preserved in ethanol). Of those, 2,911 were deposited at the NSRL with the remainder, because of collaborative research arrangements, housed at the Carnegie Museum of Natural History in Pittsburgh, Pennsylvania, and the Texas Cooperative Wildlife Collection at Texas A&M University. For much of his career, RJB conducted karyotype work using both field and laboratory preparations of stained chromosomes. The NSRL houses an estimated 475 boxes of karyotype slides from this work with up to 100 slides per box. There are also thousands of negatives and printed photographs of karyotype preparations. Many frozen tissues in the GRC at the NSRL came from RJB's work. These include 16,453 tissue vials from specimens he collected and another 3,005 from specimens that he prepped. A large number of other tissues resulted indirectly from his work in the form of specimens and samples provided by graduate students and collaborators on funded research projects. All of these specimens and ancillary materials are available for other scientists to access and study.

RJB's Record in Mammalogy and Service to Scientific Societies

When his publication record is compared with that of other deceased, well-published naturalists-mammalogists, RJB clearly emerges among the individuals at the top of the list (Table 11). Of the 17 mammalogists listed, he ranks number 3 behind only Joseph Grinnell and C. Hart Merriam, two of the early giants in the field. (It should be noted that only 12% of Grinnell's papers were about mammals; most of his work was on birds.) So, by any measure, RJB was one of the most prolific mammalogists of his era. In many respects, Robert had an impact on mammalogy equivalent to that of Grinnell and Merriam. Grinnell made a lasting impact on the legacy of mammalogy by the students he taught and trained, whereas Merriam contributed more to biological surveys and the cataloging of diversity throughout the United States. Robert's career encompassed both of the contributions made individually by these two men. First and foremost, he was an educator and contributed to the next generation of mammalogists. At the same time, his studies of biodiversity and commitment to museum science overlapped with Merriam's main emphasis.

RJB was a major contributor and leader in the American Society of Mammalogists (ASM). As shown in Table 2, during his career he was the leading publisher of articles in the ASM's publication outlet, the *Journal of Mammalogy*. Also, between 1965 and 2016 he attended every annual meeting of the ASM and at most of them either he or one of his students presented scientific papers or posters. By examining the index of abstracts for the annual meetings, we determined that papers or posters were presented by RJB or his students every year except for 1973–74, 1980, 1994, 2000, 2007–2008, 2010, 2012, and 2014. Over a 6-year period from 2000 to 2006, the Baker group presented 37 papers or posters. He served in many leadership positions in the ASM, including elected and editorial positions as chronicled in his obituary. He served as President of ASM from 1994 to 1996, and he received the three major awards given by the society (Merriam,

Table 11. The publication records of deceased well-published naturalists/mammalogists.

Name	No. Papers	No. in JM	Feature/Note	Other*
Grinnell, J.**	554	12	11	1
Merriam, C. H.	490	9	9	1
Baker, R. J.	**445**	**66**	**57**	**9**
Miller, G. S., Jr.	399	49	33	16
Jones, J. K., Jr.	368	73	39	34
Hall, E. R.	349	61	48	13
Hoffmann, R. S.	247	29	13	16
Hamilton, W. J.	233	45	36	9
Layne, J. N.	229	23	21	2
Goldman, E. A.	206	47	43	4
Osgood, W. H.	205	29	22	7
Choate, J. R.	201	33	28	5
Jones, C.	200	36	21	15
Davis, W. B.	191	31	30	1
Hoffmeister, D. F.	137	31	28	3
Yates, T. L.	130	15	15	0
Findley, J. S.	100	49	46	3
Hooper, E. T.	90	34	29	5

* Includes book reviews, letters to the editor, and obituaries.

** Only 67 of Grinnell's 554 papers (12%) were about mammals.

Grinnell, and Jackson) and was elected Honorary Member—the only person in the history of the ASM to accomplish this.

He also was active in numerous other scientific societies, including the Southwestern Association of Naturalists and the Texas Society of Mammalogists, where he held important elected positions and received recognition for his contributions and leadership. His work in various scientific organizations is discussed in more detail in his obituary (Genoways et al. 2018).

DISCUSSION

What makes a good scientist and what constitutes evidence of scientific excellence? According to the Mertonian sociology of science, the primary criterion for a scientist's quality derives from the objective of science—extending certified knowledge (Sonnert 1995). The scientists who contribute the most to the growth of scientific knowledge are thought to perform their role as scientists the best. Because the standard way of communicating scientific research findings is through publication, this metric is widely adopted as the appropriate measure of a scientist's performance.

We also know that superior scientific performance is a disproportionately rare phenomenon, with a small minority accounting for a disproportionate impact (Jackson and Rushton 1986; Rushton 1988). Most significant publications are authored by a small proportion of researchers, and the majority of citations reference a relatively small pool of articles. This is why highly cited researchers wield a vastly disproportionate influence in their fields (Parker et al. 2010).

Two theories, based on research by social scientists, have emerged about how to best predict scientific productivity and creativity. D. K. Simonton (2004) has argued that highly prolific scientists are more successful in producing high-impact work compared with their less productive peers. He also concluded that scientists can increase their number of creative and high-impact works only by increasing their publication output; in other words, scientific creativity is a "probabilistic consequence" of research quantity. The second theory, developed by R. S. Burt (1992, 2004) and known as the theory of "structural holes," argues that individuals who live in the intersection of "social worlds" are more likely to select and synthesize cognitive alternatives into "good ideas." According to Burt's theory, individuals who bridge "structural holes" have access to multiple views, information, and perspectives, a fact that explains why they develop more novel and better ideas than their peers.

Heinze and Bauer (2007) have combined elements of both of these theories into a flowchart to illustrate the factors associated with highly creative scientists (see Figure 6). The premise behind this chart is that it is not only the sheer quantity of publications that causes scientists to produce pieces of work; in addition, their ability to effectively communicate with their colleagues and address a broader work spectrum creates important dimensions of the creative process.

Overlaying RJB's achievements on this chart (Fig. 6) demonstrates his research creativity. His number of publications (445) is prodigious for a naturalist-mammalogist. Publication is regarded as an indispensable part of science, and sustained and substantial publication favors creativity (Bartholomew 1982). The more research one completes, the more apt one is to make an original contribution. The simple number of peer-reviewed journal papers has been shown to be strongly and significantly associated with the number of collaborators and thus the size of the co-author network (Heinze and Bauer 2007). Furthermore, the number of publications and annual productivity rate of a scientist is known to widen the spectrum both of the journals that scientists publish in and the amount of citations their articles achieve (Sonnert 1995).

In many fields a scientist's annual productivity rate has been demonstrated to be a powerful predictor of quality, with a large number of publications being indicative of a larger number of higher-quality publications (Sonnert 1995). RJB averaged more than eight-papers per year over his 53-year publishing career, but he had several periods in his career where he sustained a much higher rate of publication. Creative individuals have been shown to go through "hot streaks" of peak productivity over a relatively short period when they produce their best work (Timmer 2018). The average hot streak for a scientist has been estimated to last 3.7 years (Timmer 2018), and RJB certainly had his "hot streaks" (see Table 1). From 2000 to 2004, for example, he authored a total of 61 papers, which equates to an average of one paper per month over a 5-year period. Similarly, from 1978 to 1982, he nearly matched this output, publishing 59 papers. Another era of extremely high productivity occurred from 2006 to 2010 when he appeared on the author-line of 52 papers. Three "hot streaks," over a span of four decades, is far above the average for most scientists. The period from 1978 to 1982 was the time that chromosome banding studies came to fruition in Robert's lab, and he and his students began publishing papers on the theoretical aspects of chromosome evolution and speciation, as well as many data-oriented chromosome papers. The periods 2000–2004 and 2006–2010 were when RJB was heavily involved in the Chernobyl work, with many papers being published about both genetics and ecotoxicology.

George Bartholomew, the eminent zoologist, has noted another and even more important reason for publishing. The more deeply, continuously, and productively one is immersed in research, including the final and compelling discipline of publishing, the greater the opportunity for favorable serendipity (Bartholomew 1982). We see this in many aspects of RJB's career. While collecting material on field trips in support of his numerous grants to study karyotypic and genetic evolution in mammalian populations, RJB and

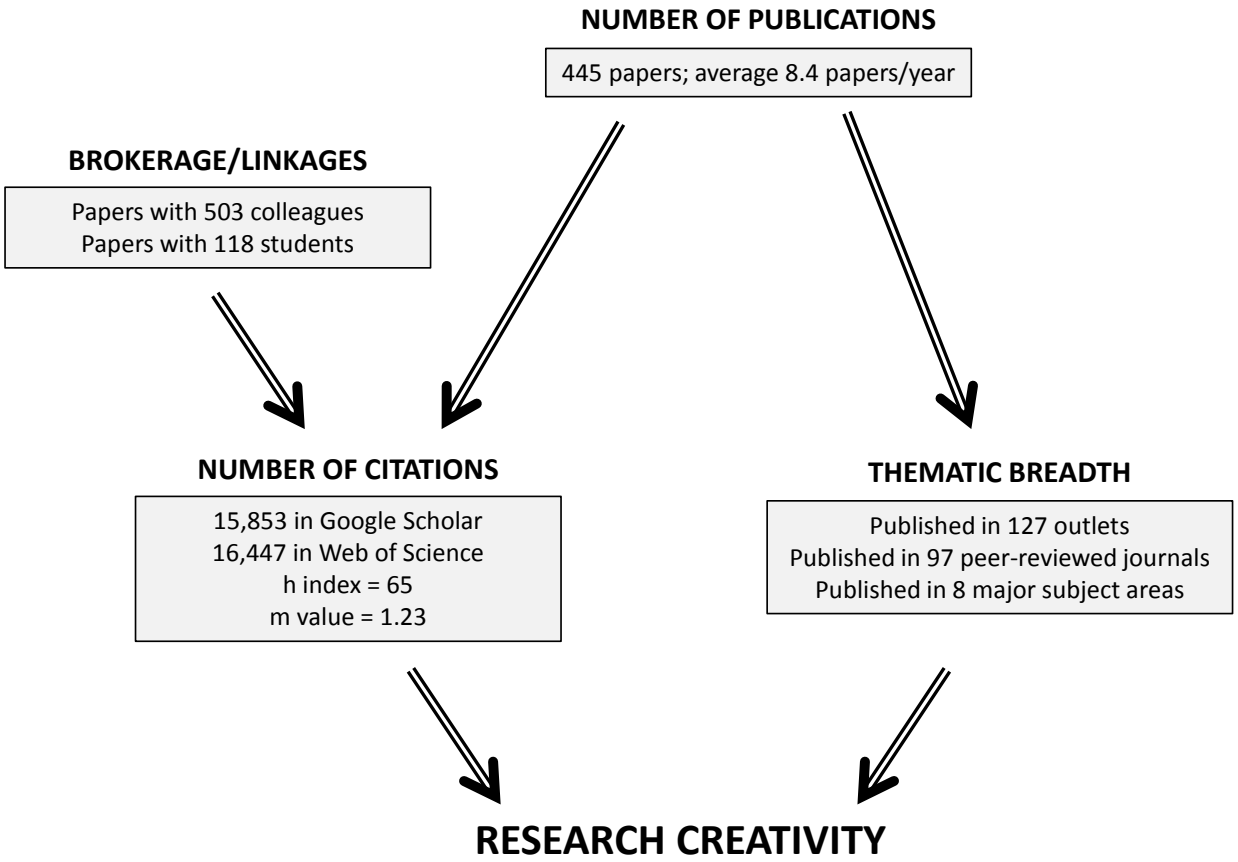

Figure 6. Flow chart of four key factors in determining research creativity. Information shaded in gray depicts key RJB data for each factor. Adapted from Heinze and Bauer (2007).

his collaborators made basic natural history observations for which specimens were collected to document findings about the distribution and natural history of species. He published these results in addition to his work on systematics and evolution, thereby expanding the publication horizon of his field research.

RJB's preferred method of publication was clearly collaborative; 416 of his 445 papers involved collaborators, including many of the 130 students who worked in his program. He published with all but 18 of his 130 students and post-docs (six doctoral students, 11 master's students, and one post-doc), and the author-line of his papers included an almost unbelievable number of 503 different individuals! On many papers he took the last authorship, especially toward the end of his scientific career. Last place on the author list is often reserved by senior biologists for all publications coming out of their labs or research programs (see Sonnert 1995).

A key aspect of the publication record of any scientist is the popularity and prestige of the journal(s) where the research is published (Olden 2007). Currently, the Thompson Reuters Impact Factor (IF), calculated as the average number of times that articles published in a specific journal in the past two years were cited in the current year, is recognized as the de facto measure of journal "quality," despite its known limitations (see Alberts 2013). However, the quality of an article is not necessarily correlated with the quality of the journal in which it is published (McDade et al. 2011), and in many fields the average journal prestige does not always correlate significantly with publication productivity and the average rate of citations per article (Sonnert 1995).

The IF was never intended to evaluate individual scientists, but rather as a measure of journal quality (Garfield 2006). Also, the IF (along with the Science Citation Index [SCI] and h-index) shares the shortcoming that not all serials are indexed for the system, thereby artificially reducing the estimated impact of biodiversity publications. Notably, a number of important journals in systematic biology, especially those that publish monographs, are not included. Books—whether edited volumes or individual contributions—are not included in the SCI. Also, impact factors have been shown not to work very well for taxonomic journals (Krell 2000), and there is some suggestion of a taxon bias with higher citation rates for biologists working on 'popular' organisms (Kelly and Jennions 2006).

RJB published in many prestigious journals including *Science*, *Nature*, *Proceedings of the National Academy of Science*, *Systematic Biology*, *Bioscience*, and *Evolution*. But some of his most important papers in mammalogy were published in the *Journal of Mammalogy*, which has a lower journal impact factor than the journals listed above. Also, 20 percent of his papers were published in Texas Tech publications (primarily *Occasional Papers* and *Special Publication* series at the Museum), which include many longer taxonomic revisions and biodiversity papers, and these publications are not indexed for impact factors.

From basic accounts about the distribution and natural history of bats and other mammals to insightful, paradigm-making papers, RJB's work covered many groups of taxa (mammalogy, ornithology, herpetology, ichthyology, parasitology, malacology, and botany) and several biological disciplines (genetics, systematics, taxonomy, evolution, biogeography, ecotoxicology, radiation biology). However, the majority of his publications were about mammalian systematics and evolution.

Few would argue that some publications contribute more than others to scientific knowledge and are thus of higher quality. For this reason, citation counts have been proposed as another good indicator of scholarly impact and excellence in research, even though the rate at which papers accumulate citations varies across disciplines (Kelly and Jennions 2006). Robert's number of citations (16,624 in Web of Science and 15,853 in Google Scholar) is quite high for any published naturalist. His 10 most cited papers (see Table 8) have been cited 3,731 times, which makes up almost a quarter of his total number of citations.

Despite the alleged limitations of the measure (see above), RJB's h-index of 65 is considered quite high. Inspection of a sample of 18 evolutionists and ecologists ranked by Thompson Scientific as "highly cited" yielded a mean h-index of 45.0 with an 11.45 standard deviation (Kelly and Jennions 2006). Likewise, his m-value of 1.23 is considered high for his scientific discipline. For example, William D. Hamilton, Edward O. Wilson, and Stephen J. Gould all have m-values of less than 1.0, and no one would argue about their ranking as highly influential evolutionary biologists (Kelly and Jennions 2006). However, when compared with John Avise, an evolutionary biologist at the University of Georgia, and James Brown, an ecologist at the University of New Mexico (both in the National Academy of Sciences), RJB's h/m values are quite a bit lower (65/1.23 for RJB compared to 102/2.27 and 106/2.08 for Avise and Brown, respectively), although Robert published more papers than either one of them. Both Avise and Brown wrote numerous papers that provided broad overviews of phylogeography and macroecology, respectively, and they also published books. RJB's more synthetic papers (e.g., genetics species concept and ideas about chromosome evolution across groups) received considerable attention, but Avise and Brown reached a broader audience, thus enhancing exposure of their writings. RJBs h-index of 65 is virtually the same as that of James Patton's (h index of 63 and m value of 1.21) among current systematic mammalogists, as these two contemporaries and colleagues generally published in the same subject area, with many papers appearing in the *Journal of Mammalogy*.

The advantages that h-index and m-value are thought to have over other citation-based indices of counting publications is to favor those authors who produce a series of influential papers rather than those authors who either produce many papers that are soon forgotten or produce a few that are uncharacteristically influential (Kelly and Jennions 2006). However, while they are easily computable, the validity of using h-index and m-value has been questioned for some scientific fields because the rate at which papers accumulate citations varies across disciplines (Kelly and Jennions 2006). For example, comparisons among highly cited

scientists have revealed that h-index values tend to be lower for evolutionary biologists and ecologists than for researchers in other fields (e.g., cell and biomedical scientists). Also, works in systematics often remain in use for decades, and longevity of impact may be a particularly valuable metric (McDade et al. 2011). For these reasons, in the fields of ecology and evolution the h index and associated values should be considered alongside other indices that rely on citation and publication count to assess research performance (Kelly and Jennions 2006).

RJB's thematic breadth is reflected in the 127 different publication outlets, including 97 different peer reviewed journals, and the broad subject matter coverage of his papers, ranging from contributions in systematics and taxonomy to ecotoxicology, radiation biology, and collection management. Publishing in many different journals and on many different subjects leads to fewer overlapping populations of scientists who cite the work, and hence higher growth potential for articles. Also, it has been demonstrated that the number of publications in leading journals can increase the visibility of a scientist's other papers, past and future (Acuna et al. 2012). Scientists who connect disciplinary communities or research fields also have a higher probability of exposure to alternative ways of thinking and behaving, and their linkages to otherwise disconnected researchers produces a broader disciplinary spectrum in their scientific work (Heinze and Bauer 2007). Evidence of all of these trends appears in RJB's scientific accomplishments.

According to Goodenough (1993), the goal of every scientist is the achievement of "eureka" moments, the ineffable experience of discovering some of the "truths" of nature, of finding the "unity of life." Because field work was a major component of his scientific work, and because of his intense interest in speciation, some of RJB's biggest "eureka" moments came in discovering taxa of mammals new to science. He described and named 18 new species and subspecies as well as 11 higher-level taxa. All of these are listed in his obituary (Genoways et al. 2018).

Examining hypothetical phylogenetic trees also produced "eureka" moments for him. One of his greatest joys was looking at the latest and greatest phylogenetic tree that was produced in his lab. In the early days, when phylogenies were deduced mentally and trees were drawn by hand, Robert could be a royal "pain in the ass." Sometimes it would take days to generate the synapomorphies and pathways for a phylogeny and another day or so to actually draw the tree. Once computer algorithms (i.e. PAUP) and graphic programs (i.e., MacDraw and later PowerPoint) became available, the student work load decreased somewhat—but Robert made up for it by redoubling his directives to "try this outgroup" or "add these to the ingroup"! The increase in data analyses unleashed the "Baker monster" in an entirely new dimension!

Robert's ability to distill or identify a publishable unit was uncanny. He could assess the importance of a dataset and calculate whether sufficient evidence was there to move the manuscript forward or if additional data were needed. Typically this calculation was made earlier in the experimental design state; therefore, most of his projects had a definitive termination point. Many of his graduate students (e.g., Robert Bradley, John Bickham, and Rodney Honeycutt, personal communication) think that this is one of the most important things that Robert taught his graduate students.

Many scientists reach their highest level of creativity when they face the need to improvise, when they lack adequate large infrastructure, and when they work with deficient funding (Medina 2006). We see this in RJB's career. In 1986, at the pinnacle of his publishing career, when his funding for chromosome research was winding down, he took a leave of absence from Texas Tech and spent a year with Rodney Honeycutt, one of his former Ph.D. students, at Harvard University learning some of the new techniques of molecular biology. He did this to prepare his students to be more "cutting edge," but also to open new vistas for his own research. This new learning opportunity opened the door for expanding his research horizons and led to a period of enormous publication activity in the 1990s and the first decade of the 20th century (see Table 1). He also learned to wear a sport coat and tie at Harvard!

Robert was often criticized, especially by some administrators during his annual evaluations, for publishing too many multiple authored papers and for publishing too many papers with his students. His response was always to note that he was in the business of education and that experience in completing

the publication process in research was critical to a student's ultimate success. He was known to say "the research is never completed until the published reprint of the paper is in your hand." To him, one of the greatest accomplishments was to see a student complete the hard work of publishing a paper. He was certainly successful in his endeavor, as he published more than 100 papers with his students, and he continued to publish papers with many of them after they had left his program and established their own careers. For example, he appears on the author-line of 112 papers with four of his graduate students (Robert Bradley, Ronald Van Den Bussche, Meredith Hamilton, and Calvin Porter) published while they were students and after they had completed their doctoral programs. Interestingly, these four students were contemporaries from 1986 to 1990. They represented a synergistic group in an exceptionally collaborative phase of RJB's program.

Several aspects of Robert's career go against the dogma in the literature about creativity in scientists. For example, several studies have pointed out that individuals who receive doctorates from and/or are appointed to high prestige universities are more likely to be productive and win recognition than scientists at universities lower in prestige (Rushton 1988; Babu and Singh 1998). Clearly, Robert J. Baker did not fit that profile. Neither Oklahoma State nor the University of Arizona, at the time that Baker attended, was considered a prestigious university. Similarly, Texas Tech University (then known as Texas Technological College) lacked a Ph.D. program in biology and most of the other sciences. He joined a university better known for undergraduate education programs, and he spent his entire academic career there helping to build the university into a significant academic and research university that is now recognized among the top 100 research institutions in the United States. Today, Texas Tech is recognized as one of the leading centers for mammalogy in the country, and RJB played a primary role in creating that reputation (L. Bradley et al. 2005).

The literature on scientific publication in many fields shows a relationship between aging and research productivity in academic scientists, with some suggestion that, on average, scientists become less productive as they age (Levin and Stephan 1991). Whether productivity peaks early or builds slowly, much of the data reveals a decline in productivity for many scientists from the ages of 25 to 65 (Horner et al. 1986). Clearly, that was not the pattern for RJB, who was remarkably consistent in authorship of papers. In fact, some of his most productive years were between the ages of 58 and 68. Scientists who are productive and publish many papers tend to remain productive throughout their careers although some decrease their publication rates after middle age because of competing commitments. Some scientists as they age spend less time in research and a larger proportion of time in administrative positions. This was not the case for RJB.

Social scientists have estimated that the age at which highly cited scientists produce their most cited papers is between 37 and 50 years (Garfield 1981). Again, we see an exception in RJB. His two most highly cited papers about the genetic species concept in mammals (discussed above) appeared in 2001 and 2006 when he reached the age of 59 and 64, respectively. He remained highly productive (both in number of publications and citation counts) until his retirement in 2015. This followed the tragic death of his son Bobby in 2012, which had a dramatic impact on both Robert and his wife Laura, and the onset of major health challenges following years of fighting diabetes and heart problems.

Highly creative scientists often seem to experience a midlife transition from a more empirical to a more theoretical focus in publications (Jackson and Rushton 1986). Most scientists prefer research driven by theoretical concerns rather than social benefits, as scientific reputations are typically founded on contributions to ongoing scientific debates (Kelly and Jennions 2006). We see evidence of this early in RJB's career. In 1978, he and Hugh Genoways published a paper in the *Special Publications of the Philadelphia Academy of Sciences* (cited 150 times) describing the island biogeography of bats in the Caribbean Basin. This was the first comprehensive account of the distribution of bats across a large oceanic archipelago, and it formed the basis for numerous comparative analyses in island biogeography that continue today (Schmidly et al. 2017).

Beginning in 1979, at the age of 37 (a dozen years after receiving his doctoral degree), RJB began to publish papers about theoretical issues in systematics and evolution. The first of these publications emphasized systematics and chromosomal evolution in mammals, including three seminal papers published with one

of his Ph.D. students, John Bickham, "Canalization model of chromosomal evolution" (published in 1979 in the *Bulletin of the Carnegie Museum of Natural History* and cited 153 times), "Karyotypic megaevolution model of chromosomal evolution" (1980 in *Systematic Zoology* with 217 citations), and "Monobrachial model of chromosomal speciation" (1986 in *Proceedings of the National Academy of Science* with 287 citations).

These early theoretical papers were followed by numerous contributions refuting the dogma of deme size models of chromosomal evolution. These papers included an article published in *Cytogenetics and Cell Genetics* with Michael Haiduk, Lynn Robbins, and Duane Schlitter (1981, "Chromosomal evolution in African megachiroptera: G- and C-band assessments of the magnitude of change in similar standard karyotypes") that was cited 32 times, a paper published in *Systematic Zoology* with Ben Koop and Michael Haiduk (1983, "Resolving systematic relationships with G-bands: a study of five genera of South American cricetine rodents") that was cited 78 times, and an article in *Evolution* with Ronald Chesser (1986, "On factors affecting the fixation of chromosomal arrangements and neutral genes") that was cited 44 times. At about the same time another series of papers followed that addressed computer modeling of chromosomal and genetic evolution. These included a paper with Ronald Chesser, Ben Koop, and R. A. Hoyt in the journal *Genetica* (1983, "Adaptive nature of chromosomal rearrangements: differential fitness in pocket gophers") that was cited 35 times, a paper in *Systematic Zoology* (1984, "Karyotypic megaevolution by any other name: a response to Marks") that was cited 12 times, and a paper published in *Current Mammalogy* (1987, "Role of chromosomal banding patterns in understanding mammalian evolution") that was cited 96 times. He also continued to publish papers proposing classifications for phyllostomid bats, including a paper published in *Systematic Zoology* in 1989 that has been cited 111 times. In that same year he published an article in the journal *Evolution* (cited 119 times) concerning hybrid zones between genetically distinct populations. At the time, it was considered the premier study of that subject.

In the 1990s, RJB began publishing papers about gene conversion and genome evolution and organization. The most highly cited of these papers ("Evidence for biased gene conversion concerted evolution in ribosomal RNA") was published in 1991 with David Hillis, Craig Moritz, and Calvin Porter in *Science* and was cited 392 times. He published several papers on genome evolution and organization, the most cited of which was a paper published in *Chromosoma* in 1990 and written with nine other authors, "Distribution of non-telomeric sites of the $(TTAGGG)_n$ telomeric sequence in vertebrate chromosomes," that received 586 citations.

In 1994, RJB initiated his collaborative work at Chernobyl, resulting in 40 publications about the impact of low-level radiation on mammals. Overall, this research showed that current radiation doses near Chernobyl were not sufficient to yield high mutation rates or prevent population maintenance, which was contrary to the scientific dogma at that time (Genoways et al. 2018). Initially, however, RJB's Chernobyl research resulted in a publication in *Nature* in 1996 about levels of genetic change in rodents that was featured on the cover of the magazine and received 87 citations. Unfortunately, that paper had to be retracted because of bad data (see Genoways et al. 2018 for a full discussion), and a 1997 paper in the same journal included the corrected data. The lack of any significant mutation rate, documented in the retracted paper, was met with opposition by several groups wanting to use the Chernobyl accident as an activist campaign against nuclear energy. Robert, with his colleague Ronald Chesser, eventually responded with an article in 2000 in the journal *Environmental Toxicology and Chemistry* (cited 67 times) suggesting that protection from human impact provided by the exclusion zone was actually beneficial to wildlife and an unintended consequence of the accident.

In 2001 and 2006, Robert, along with his former student and subsequent colleague Robert Bradley, proposed the genetic species concept for mammals in two seminal papers ("A test of the genetic species concept…" and "Speciation in mammals and the genetic species concept") that were published in the *Journal of Mammalogy* and have been cited 597 and 642 times, respectively. These are the two most highly cited papers for which RJB was on the author-line.

In 2014, toward the end of his career, RJB joined with several of his colleagues and students to produce two important papers in the area of collection manage-

ment. These papers (Baker at al. 2014 and R. Bradley et al. 2014) addressed the value of natural history collections, issues regarding their long-term growth and care, and the cost of curation and long-term care of mammal specimens in natural history collections. These papers were among the most comprehensive ever published about this subject.

RJB conducted his work in what has been termed the lab-field border of biology (see Kohler 2002 for a discussion), and he worked within the paradigm of evolution. While much of his fieldwork involved picking field sites that could provide "natural experiments" to test evolutionary theory, his work in the lab focused on the application of modern scientific technological advances to test hypotheses based upon his field data. His creativity appeared early and was evident in every decade of his career. He was an early pioneer in the adoption of karyotypes and the study of chromosomes for use as population markers to determine species distinction and interpret phylogenetic relationships in mammals, particularly bats. One of his earliest papers, "Karyotypes and karyotypic variation in North American vespertilionid bats," published in the *Journal of Mammalogy* in 1967, remains on the list of his most cited papers with 180 citations (see Table 8).

At critical junctions in his career, he adopted new pioneering techniques to keep his lab on the "cutting edge" of scientific work about important questions in systematics and evolution. In the decade of the 1970s, he advanced his chromosome research to include the use of in situ hybridization and G- and C-banding techniques. This resulted in several research papers in high-quality journals such as *Systematic Zoology* (e.g., 1979 with John Patton, "Chromosomal homology and evolution of phyllostomatoid bats" that received 117 citations) and in the journal *Evolution* (e.g., 1978 with Ira Greenbaum and Paul Ramsey, "Chromosomal evolution and the mode of speciation in three species of *Peromyscus"* that was cited 60 times). Keeping up with advances in technology, especially in such a dynamic field as genetics, is one of the most difficult challenges that anyone can have in their career, and Robert was obviously very good at it.

Also in the 1970s, he incorporated starch gel electrophoresis to produce several important papers that contributed to his growing reputation in science. These articles were published in *Evolution* (1975 with Robert Selander, Donald Kauffman, and Stephen Williams, "Genic and chromosomal differentiation in pocket gophers of the *Geomys bursarius* group" that received 84 citations), *Systematic Zoology* (1976 with Ira Greenbaum, "Evolutionary relationships in *Macrotus*…" that was cited 57 times), and *Comparative Biochemical Physiology* (1976 with Donald Straney, Michael Smith, and Ira Greenbaum, "Biochemical variation and genic similarity of *Myotis velifer* and *Macrotus californicus*" that received 12 citations).

In 1986, he took a one-year sabbatical from Texas Tech to work at Harvard with one of his former students, Rodney Honeycutt, to learn some of the techniques of modern molecular biology. This move helped to further broaden his scientific repertoire, which began to show up in his publication record in the 1990s; this was one of the most productive periods of his career. Significant papers from this era included topics such as in situ hybridization, restriction enzyme mapping, and eventually DNA sequences. Some of his most important papers were published in the journals *Evolution* (1989 with Scott Davis, Robert Bradley, Meredith Hamilton, and Ronald Van Den Bussche, "Ribosomal DNA, mitochrondrial DNA, chromosomal and allozymic studies on a contact zone in the pocket gopher, *Geomys*" that was cited 119 times), *Chromosoma* (1990 with Meredith Hamilton and Rodney Honeycutt, "Intragenomic movement, sequence amplification and concerted evolution in satellite DNA in harvest mice, *Reithrodontomys* …" that received 70 citations), and a special volume published by the American Museum of Natural History to honor the contributions of Karl F. Koopman (1991 with Rodney Honeycutt and Ronald Van Den Bussche, "Examination of monophyly of bats: restriction map of the ribosomal DNA cistron" that has been cited 32 times).

Systematic biologists increasingly contribute knowledge in nontraditional ways that were previously ignored in the broader scientific arena (see McDade et al. 2011). For example, they submit data to central repositories from which data can be retrieved and used by others (e.g. GenBank), and through their field and curatorial work in collections help to build basic infrastructure to study biodiversity. We see evidence of these contributions through RBJ's work. As described in his obituary (Genoways et al. 2018), he was a tireless

collector of scientific specimens and associated ancillary data (tissues, karyotypes, etc.). At the time that Baker joined the biology faculty at TTU, the mammal collection contained about 5,000 specimens; today, the collection numbers more than 140,000 specimens. While other mammalogists who worked at Texas Tech and their students contributed to the growth of the mammal collection, RJB certainly played a prominent role not only in contributing specimens but also by securing institutional and outside funding to provide critically needed infrastructure to support the collections (L. Bradley et al. 2005).

Similarly, he worked on interdisciplinary and transdisciplinary research projects, using bioinformatics and genomics, to link heretofore disparate fields of science to address broader societal problems associated with natural resource management issues. For example, he and his colleague, Nick Parker, joined with one of us (DJS) in the use of bioinformatics as a major tool for planning how the Texas Parks and Wildlife Department might address conservation and recreation issues in the State in the 21st century (see Schmidly et al. 2002). Unfortunately, the results of this work were completely ignored by Texas politicians and as a result the park system is dealing with many problems today. This really rankled Baker, who told one of us (DJS) that he never wanted to be involved again with a project in which good science was ignored in favor of bad politics!

During the last few years of his research career, Robert was obsessed with being able to use genomics and next generation sequencing methods to address research questions in the context of phyllostomid bat evolution and the genetic architecture of chromosomes. Although his untimely death precluded the fruition of his dream, he did see some projects published, including a paper published in the journal *Molecular Ecology* (2012 with 10 different authors, "Microbiome analysis among bats describes influences of host phylogeny, life history, physiology and geography" with 70 citations), a second paper in the journal *PLoS ONE* (2014 with nine authors, "Dietary and flight energetic adaptations in a salivary gland transcriptome of an insectivorous bat" with six citations), and a third paper in *Frontiers in Ecology and Evolution* (2015 with Caleb Phillips, "Secretory gene recruitments in vampire bat salivary adaptation and potential convergences with sanguivorous leeches" with seven citations).

Some scientists make huge contributions through their mentoring of students and generosity with ideas, skills, and time (Kelly and Jennions 2006). Although RJB made major scientific accomplishments through his research and publications, his greatest impact may well be through the students (undergraduate and graduate) that he trained. As John Steinbeck once said, "I have come to believe that a great teacher is a great artist, and that there are as few as there are other great artists. Teaching might even be the greatest of the arts since the medium is the human mind and spirit." (Steinbeck 2003).

The supervision of Ph.D. students, who have projects related to their supervisor's research, has been found to have an independent effect on scientific productivity. Graduate students are regarded as an important resource in research activities. They do much of the time-consuming data collection and data analysis work, and as supervisors, faculty may become co-authors of publications with graduate students. Recent studies have shown that more productive scientists are more than twice as likely to have large groups of graduate students than are less productive scientists. Similarly, a positive correlation has been demonstrated between the number of graduate students faculty supervise and their productivity (Kyrik and Smeby 1994).

Although it is difficult to obtain comparable numbers, it seems doubtful that any mammalogist has produced more undergraduate and graduate students and post-docs (130) who published on mammals than RJB. More than three-quarters of his Ph.D. students hold academic appointments at American and international universities and continue to publish work on mammals. The most effective graduate supervisors tend to be dedicated, productive researchers who have achieved eminence in their own fields, and they work closely with their students, often in the form of collaboration on published research (Morales et al. 2017). Through close personal interaction and collaboration, an eminent graduate supervisor models and transmits to the student an insider's tacit knowledge of how science is pursued and what it takes to be successful in scientific research (Schwartz no date). Clearly, RJB exhibited all of these attributes in his work with students.

Participation of women in the field-oriented vertebrate biological sciences was almost non-existent prior to 1960, and mammalogy certainly followed this

trend. The reasons for this are myriad—not many women in any of the sciences, family obligations, belief that women could not withstand the rigors of domestic and international fieldwork, lack of opportunities, and the difficulty of breaking through in a male-dominated area of study. However, beginning in the late 1960s and early 1970s, women were entering these fields, including mammalogy (Genoways and Freeman 2001). RJB did not start this trend, but as graduate advisor he certainly accepted and supported women graduate students. His first female graduate student was Margaret A. O'Connell, who entered his program in 1973, completing a MS in 1975 and a Ph.D. in 1982. Her graduate work included rigorous fieldwork in West Texas and New Mexico and in Venezuela. She is currently Professor in the Department of Biology at Eastern Washington University. Several other "pioneering" women received graduate degrees during the 1970's and 1980's, including MS students Rebecca A. Bass, Laurie Erickson, Anette Johnson, Karen McBee, Kim Nelson, and Hae Kuyng Lee, and Ph.D. student Meredith J. Hamilton. In total, 22 of RJB's MS graduates (46%), 18 of his Ph.D. graduate students (36%), and five (50%) of his post-doctoral associates were women. In later years, more women were probably attracted to mammalogy as the laboratory phases of the work came to dominate studies in the discipline. However, all of RJB's female students, and in fact all his students' incorporated strong field-oriented elements as well as the laboratory studies.

Robert's graduate students also were very successful in receiving awards for their research work. Between 1972 and 2015, the American Society of Mammalogists (ASM) granted 45 Shadle fellowships, recognizing accomplishments in mammalogy by a graduate student, and six of these went to RJB students (William Blier in 1972, Ira Greenbaum in 1977, Craig Hood in 1984, Ronald Van Den Bussche in 1988, Robert Bradley in 1990, and Sergio Solari in 2005). Two of his students, Sergio Solari (2006) and Peter Larsen (2010) also won ASM Fellowships, the highest student award given by the society (first awarded in 2001).

It was one of the disappointments of his career that Robert was not admitted to the National Academy of Sciences (NAS). For most of his years at TTU, the university did not have any faculty members in any of the national academies, and Robert wanted to be the first. Two of us (DJS and RDB) made attempts to promote his candidacy but we were not successful for reasons that were never divulged. Our opinion was that without anyone inside the academy to promote his cause that it would be difficult to achieve. Today, TTU has faculty members in the National Academy of Engineers and recently hired its first member of the NAS (Texas Monthly 2018). The institution still lacks a "home-grown" member of the NAS.

RJB's Personality

What personality traits accounted for RJB's prodigious productivity? If you knew him well, and understood his personality, it is not difficult to ascertain why he was so successful. And, from the literature (see below) it becomes evident that his profile is not unlike that of many other highly productive and creative scientists.

Using the Disc Model of Human Behavior (Rohm 2005), RJB would be characterized as having a "high D personality style" (dominant, direct, demanding, decisive, determined, doer). High Ds are a powerful group of people who are made to be world-changers with a vision (Rohm 2005). They are known to be intense, knowing two speeds in life—zero and full throttle… mostly full throttle. They communicate in a very direct manner, saying what they mean and meaning what they say. They decide quickly—almost effortlessly and with confidence, and they like control and choices. They would rather do something and take a risk versus doing nothing at all. They are results-oriented and are willing to overcome challenges as necessary to meet their goals. D's are passionate, and they can be tremendously loyal. While they can be seen as being all about "getting-it-done," they also have feelings and personal needs that may not be apparent. Those who work with a high D learn not to take everything that a D does or says personally—especially when a D is on-task. They are wired to achieve their goals, but it is amazing how much a D type person can relax after checking off the task at hand. Until then, they are focused and determined. Even with an orientation toward task, D types can be very caring. They often express their feelings by doing something for others—often behind the scenes.

Several studies have attempted to explore the personality disposition in the creativity of university scientists who produce superior scientific work (Rush-

ton et al. 1986; Parker et al. 2010). Many, like RJB, seem to exhibit classic type A behavior (aggressive, incessantly struggling, time oriented, hostile when frustrated). Other factors identified that influence research productivity and distinguish creative individuals from their peers are: a high level of initiative and radical imagination; energy, curiosity, and motivation; a strong personality and well-articulated self-concept; intelligence and learning capability; professional commitment and preparedness to take risks; persistence in situations of failure; cognitively complex with a particular thinking style; fortunate to enjoy a supportive institutional context; and distinctive goal orientations and concerns for advancement. RJB exhibited all of these traits, and with his type A and high D personality styles, he was driven to set high standards for himself and his students.

Variations, of course, can be expected but anyone who knew RJB well would recognize these traits both in how he perceived his work and his life. He was more than willing to admit to his "type A" personality and he seemed to try to live daily by his motto "anything worth doing is worth overdoing." To those who did not know him well and could not appreciate his strong personality and put his forthrightness into context, he could come across as intimidating when, in fact, he never intended to convey that impression. As a type 1 diabetic, he sensed that he had a limited amount of time to accomplish what he wanted in life (see his obituary for more detail about how this disease impacted his life; also see Baker 2005). He moved at top speed, especially when he was on a field trip. He had incredible drive and talent. Whenever he decided to act, he expected everyone to get on board. One of his favorite mantras came from General George Patton, "Lead, follow, or get the hell out of the way."

He also knew how to relax and have fun, which contributed to his creativity. He loved his ranch and being outdoors on his property, and he loved to train dogs and work with cattle. Hunting was a favorite pastime, and some of his best ideas came from discussing science with colleagues while on duck, pheasant, deer, or elk hunting excursions. He also loved his family, including his children April and Bobby, Laura, his wife of 39 years, and his grandchildren. The greatest tragedy of his life was the death of his son at the young age of 26. This affected both him and Laura in profound ways, both personally and professionally, from which they never fully recovered. More about RJB's personality and life can be found in his published obituary (Genoways et al. 2018).

A Personal Note of Appreciation

The purpose of this article was not to portray RJB as a genius or a saint, for he was not. Like most of us he had his demons and issues. He could be "quick tempered" and "go off" at a moment's notice, especially if he was in the midst of an intense productive period or under stress. There could be considerable lightning and thunder, but usually the mood quickly shifted to a gentle rain. But he had many good qualities—he enjoyed life, both professionally and personally—and he loved his friends, both professional and personal. We wish we had a nickel for every occasion that he bought flowers and sent them to someone he thought he may have offended or who took the time to help him out.

He especially enjoyed the outdoors and fieldwork. He loved the land and all of its products. In many ways, he was happiest while in the field, collecting bats, rodents, or other critters, but he also loved his work in the lab and he had a passion for collections and scientific databases. He adored his family, with all his heart, and his golden retrievers. He was equally at home on a farm or ranch, working cattle and raising crops, fishing, hunting for waterfowl, game birds, and large mammals. And, he enjoyed sharing these passions with his friends.

His record of achievement includes not only the sheer quantity of publication and citation counts, but also training and mentoring students to effectively communicate and work with other colleagues to address a broader work spectrum in biology. By any reasonable definition and criteria, he was a productive, creative scientist and one of the most successful mammalogists ever to live. He left a strong legacy in mammalogy with the many students that he mentored that continue to work in the field. In all of these regards, he will be remembered and missed.

No greater accolade can be bestowed on a professor than that from his students. One of RJB's doctoral students, Rodney Honeycutt (personal communication to DJS), provided these comments in a letter of appre-

ciation that was written to RJB on the occasion of his retirement from Texas Tech in 2015:

> "Robert, I thought this day would never come. I guess I always assumed you were invincible in terms of never actually standing down from your position at Texas Tech University. Perhaps it is just my way of being sad for the fact that all of us are getting older and beginning to realize that we are fast approaching the twilight of our careers.
>
> Throughout my 31 years as a university professor, I truly believe that one's greatest legacy is the contribution made to the next generation of scientists. Remembrance through publications and science citation indices are ephemeral, and as I am constantly reminded by my undergraduates, even great scientists are seldom recollected, unless their names will appear on impending exams. Although you have amassed an exceedingly impressive academic record, I feel that the best memories of you will be in the hearts and minds of all gathered to celebrate your retirement. Clearly, Robert, you are both loved and respected.
>
> Each of us [your students] came to Texas Tech as unfinished canvases, exposing promising outlines and many imperfections. In essence, we were like Michelangelo's unfinished sculptures struggling to become free from the marble. I remember talking with you for the first time about coming to Texas Tech and working in your program. You said, "If you are not already a good scientist, I cannot make you one. All I can do is knock off the rough edges." Well, I had a hell of a lot of rough edges, and you did not spare the hammer and chisel.
>
> When I was a postdoc with M. J. D. White in Australia, he lamented about his lack of ability to attract outstanding graduate students during his tenure as Professor of Genetics at Melbourne University. In contrast, Michael said that Spencer 'Spinny' Smith-White, a botanist at the Sydney University, was the major advisor for many of the prominent geneticists in Australia at that time. This was despite the fact that 'Spinny' was neither a Fellow of the Royal Society nor a foreign member of the National Academy of Science in the United States. Michael was both. After meeting 'Spinny,' it became clear to me why he was such a successful mentor. He created an academic atmosphere that encouraged his students to be independent, creative, argumentative, and enthusiastic. Many of his students worked on projects far from 'Spinny's' interest, but all were first class thinkers and scientists.
>
> Robert, I am unsure as to how much planning went into the establishment of your program at Texas Tech, but to me the program definitely mirrored 'Spinny's' program in Australia. You always demonstrated an uncanny ability to get the best from us without micromanaging. You allowed us to grow and to take a leadership role in the program. We learned how to work as a team, how to both present and defend our research, and how to become active members of our discipline. I can tell you that many of my junior professors would benefit from exposure to Robert Baker's program. It taught me how to be self-sufficient as a scientist, and I am personally grateful for your support, encouragement, and guidance.
>
> Finally, Robert, one of the greatest honors I received is when you took your sabbatical with me at Harvard. It was a role reversal, and I appreciate the humble way you approached learning new things. You even got to see me throw a Baker temper tantrum. The apple does not fall far from the tree.
>
> Thanks, Robert, for being my mentor and friend. You changed my life, and I will always have fond memories of my time in the Baker program. In fact, I have your picture with a bat net that stands behind my desk. When I look at that photo, I wait for that chisel to knock off another rough edge.
>
> I remember the lifelong friends and colleagues that I made at Texas Tech. We were and are a family, and you are definitely our academic father. Have a great retirement, Robert!
>
> Love, Rodney"

Another one of Robert's Ph.D. students, John Bickham, made these remarks in his encomium statement about Robert, which is germane to his remarkable talent:

> "A great thing about working in the field of science is that you get to meet many brilliant people. Some are humble, others are not. Some are fun to be with, and to work with, and others are not. Some you want to be friends with, and others you don't. Robert was definitely one that you wanted to be around! Like all successful scientists, Robert had a brilliant mind and was a deep thinker. But you might not detect it in casual conversation because he had a very down-to-earth way of talking to people. But the sharpness of his mind became apparent when you worked together on papers, or if you challenged him to any kind of serious discussion from politics to poetry. But that is not what made him great in my view. Rather it was his intelligence in combination with his tireless drive, outstanding leadership ability, and his personal charisma that set him apart from many of the greats of our field of science. In mammalogy, he will always be a legendary figure. With his passing, he takes his place among the legends, among the people on whose shoulders we stand."

Finally, there is this testimonial from Amy Bickham Baird, an undergraduate student who worked in Robert's lab:

> "When I decided to go to Texas Tech for my bachelor's degree, Robert became my mentor. Robert treated me like his graduate students, assigning me independent research projects and requiring me to present my results at local, national, and international meetings. At first, I was terrified of public speaking, but Robert knew that challenging me to do it would be valuable for my future. Of course, he was right, and I am so thankful that he pushed me out of my comfort zone. As a sophomore, he let me travel to Chernobyl to participate in a conference and see my research sites first-hand. I did not know how unique my undergraduate research experience was at the time, I just knew that I loved it. I ended up publishing 4 papers and giving about 10 talks at meetings in my 3 years at Tech. No other mentor could get that kind of productivity from an undergraduate!"

Amy went on to complete her Ph.D. at the University of Texas, and is currently a tenured faculty member at the University of Houston Downtown Campus.

CONCLUSION

Robert's publication record along with the citations of his work speaks for itself. By any definition he was prolific and creative. Although evaluating his mentoring of graduate students was more subjective, the sheer volume of students and their placement in academic institutions attest to arguably his most significant long-term influence on biological science and on mammalogy.

Robert clearly was one of the most influential mammalogists of the latter half of the 20th century and the early part of the 21st century. His cadre of students and extended program seeded through these students, who became established at other institutions, led the approach to evolution and systematics into the 21st century and were instrumental in incorporating the latest laboratory techniques in genetics, adding arrow after arrow of evidence to the systematist's quiver. Starting with karyotyping and chromosome banding, through the heyday of protein electrophoresis, restriction enzyme mapping, initial forays into DNA sequencing, to incorporation of a genomic approach, Robert was at the forefront throughout his career. The only other person with similar impact during this same time period would be James L. Patton of the Museum of Vertebrate Zoology at the University of California-Berkeley, who was a fellow Ph.D. student, colleague, and friend of Robert's. Both became giants in the field of mammalogy and systematic biology.

We close our tome to Robert James Baker with a poem about both life and death. Robert enjoyed poetry (his favorite poet was Nikki Giovanni, for whom he named a new species of bat, *Micronycteris giovanniae*)

and to us it represents a fitting tribute to a friend that we loved and respected both in life and death. The poem, shown below, was written in 1903 by Edmund Vance Cooke. One of us (DJS) showed this poem to Robert, and he agreed that it was pertinent. We believe that he would appreciate having it included in a volume honoring his work.

"How Did You Die?"

Did you tackle that trouble that came your way
With a resolute heart and cheerful?
Or hide your face from the light of day
With a craven soul and fearful?
Oh, a trouble's a ton, or a trouble's an ounce,
Or a trouble is what you make it.
And it isn't the fact that you're hurt that counts
But only how did you take it?

You are beaten to earth? Well, well, what's that?
Come up with a smiling face,
It's nothing against you to fall down flat,
But to lie there—that's disgrace.
The harder you're thrown, why the higher you bounce;
Be proud of your blackened eye!
It isn't the fact that you're licked that counts;
It's how did your fight and why?

And though you be done to death, what then?
If you battled the best you could;
If you played your part in the world of men,
Why, the critic will call it good,
Death comes with a crawl, or comes with a pounce,
And whether he's slow or spry
It isn't the fact that you're dead that counts
But only, how did you die?

This poem says volumes about RJB and the way he lived life. He lived with passion, courage, and intensity. He fought a terrible disease for most of his life, but refused to let it define him or bring him down. He committed his life to the good work of science and efforts to better understand the natural world. He died with dignity, and we believe knowing that he had done his best! To us he was not only a good friend but a valued colleague and inspiring mentor.

LITERATURE CITED

Acuna, D. E., S. Allesina, and K. P. Kording. 2012. Predicting scientific success. Nature Comments 489:201–202.

Alberts, B. 2013. Impact factor distortions. Science 340:787.

Babu, A. R., and Y. P. Singh. 1998. Determinants of research productivity. Scientometrics 43:309–329.

Baker, R. J. 2005. If you don't clean up your act, you'll end up at Texas Tech. Pp. 245–261 in Going afield (C. J. Phillips and C. Jones, eds.). Museum of Texas Tech University, Lubbock.

Baker, R. J., and J. W. Bickham. 1980. Karyotypic evolution in bats: evidence of extensive and conservative chromosomal evolution in closely related taxa. Systematic Zoology 29:239–253.

Baker, R. J., and J. W. Bickham. 1984. Karyotypic megaevolution by any other name: a response to Mark's. Systematic Zoology 33:339–341.

Baker, R. J., and J. W. Bickham. 1986. Speciation by monobrachial centric fusions. Proceedings of the National Academy of Science 83:8245–8248.

Baker, R. J., L. C. Bradley, H. J. Garner, and R. D. Bradley. 2014. "Draw to drawer" costs of curation, installation, documentation, databasing, and long-term care of mammal voucher specimens in natural history collections. Occasional Papers, Museum of Texas Tech University 323:i+1–15.

Baker, R. J., and R. D. Bradley. 2006. Speciation in mammals and the Genetic Species Concept. Journal of Mammalogy 87:643–662.

Baker, R. J., and R. K. Chesser. 2000. [Letter to the Editor] The Chornobyl nuclear disaster and subsequent creation of a wildlife preserve. Environmental Toxicology and Chemistry 19:1231–1232.

Baker, R. J., R. K. Chesser, B. F. Koop, and R. A. Hoyt. 1983. Adaptive nature of chromosomal rearrangement: differential fitness in pocket gophers. Genetica 61:161–164.

Baker, R. J., S. K. Davis, R. D. Bradley, M. J. Hamilton, and R. A. Van Den Bussche. 1989. Ribosomal DNA, mitochrondrial DNA, chromosomal and allozymic studies of a contact zone in the pocket gopher, *Geomys*. Evolution 43:63–75.

Baker, R. J., and H. H. Genoways. 1978. Zoogeography of Antillean bats. Pp. 53–97 in Zoogeography in the Caribbean. The 1975 Leidy Medal Symposium (F. B. Gill, ed.). Academy of Natural Science of Philadelphia, Special Publication 13:1–128.

Baker, R. J., R. L. Honeycutt, and R. A. Van Den Bussche. 1991. Examination of monophyly of bats: restriction map of the ribosomal DNA cistron. Pp. 42–53 in Contributions to mammalogy in honor of Karl F. Koopman (T. Griffiths and D. Klingener, eds.). Bulletin of the American Museum of Natural History 206:1–432.

Baker, R. J., C. S. Hood, and R. L. Honeycutt. 1989. Phylogenetic relationships and classification of the higher categories of the New World bat family Phyllostomidae. Systematic Zoology 38:228–238.

Baker, R. J., B. F. Koop, and M. W. Haiduk. 1983. Resolving systematic relationships with G-bands: a study of five genera of South American cricetine rodents. Systematic Zoology 32:403–426.

Baker, R. J., and J. L. Patton. 1967. Karyotypes and karyotypic variation of North American vespertilionid bats. Journal of Mammalogy 48:270–286.

Baker, R. J., M. B. Qumsiyeh, and C. S. Hood. 1987. Role of chromosomal banding patterns in understanding mammalian evolution. Pp. 67–96 in Current Mammalogy, Vol. 1 (H. H. Genoways, ed.). Plenum Publishing Corporation.

Baker, R. J., et al. 1996. High levels of genetic change in rodents of Chernobyl. Nature 380:707–708.

Baker, R. J., et al. 1997. [Retraction] High levels of genetic change in rodents of Chernobyl. Nature 390:100.

Ball, P. 2005. Index aims for fair ranking of scientists. Nature 436:900.

Bartholomew, G. A. 1982. Scientific innovation and creativity: a zoologist's point of view. American Zoologist 22:227–235.

Bickham, J. W., and R. J. Baker. 1979. Canalization model of chromosomal evolution. Pp. 70–84 in Models and methodologies in evolutionary theory (J. H. Schwartz and H. B. Rollins, eds.). Bulletin of the Carnegie Museum of Natural History 13:70–84.

Bradley, L. C., et al. 2005. Mammalogy at Texas Tech University: a historical review. Occasional Papers, Museum of Texas Tech University 243:1–30.

Bradley, R. D., and R. J. Baker. 2001. A test of the Genetic Species Concept: Cytochrome-*b* sequences and mammals. Journal of Mammalogy 82:960–973.

Bradley, R. D., L. C. Bradley, H. J. Garner, and R. J. Baker. 2014. Assessing the value of natural history collections and addressing issues regarding long-term growth and care. Bioscience 64:1150–1158.

Burt, R. S. 1992. Structural Holes. The social structure of competition. Harvard University Press, London.

Burt, R. S. 2004. Structural holes and good ideas. American Journal of Sociology 110:349–399.

Chesser, R. K., and R. J. Baker. 1986. On factors affecting the fixation of chromosomal rearrangements and neutral genes: computer simulations. Evolution 40:625–632.

Chesser, R. K., and R. J. Baker. 1996. Effective sizes and dynamics of uniparentally and diparentally inherited genes. Genetics 144:1225–1235.

Garfield, E. 1981. The 1000 contemporary scientists most cited 1965–1978. Part 1. The basic list and introduction. Current Contents 41:5–14.

Garfield, E. 2006. The history and meaning of the journal impact factor. JAMA 295:90–93.

Genoways, H. H., et al. 2018. [Obituary] Robert James Baker (1942–2018). Journal of Mammalogy 99:983–1012.

Genoways, H. H., and P. W. Freeman. 2001. Evolution of a scientific meeting: eighty annual meetings of the American Society of Mammalogists, 1919–2000. Journal of Mammalogy 82:582–603.

Gibson, D. S., C. B. Thompson, and U. S. Neill. 2015. Assessing research productivity. Scientist Magazine 1/1/2015:1–3.

Goodenough, U. W. 1993. Creativity in science. Zygon 28:399–414.

Greenbaum, I. F., and R. J. Baker. 1976. Evolutionary relationships in *Macrotus* (Mammalia: Chiroptera). Systematic Zoology 25:15–25.

Greenbaum, I. F., R. J. Baker, and P. R. Ramsey. 1978. Chromosomal evolution and the mode of speciation in three species of *Peromyscus*. Evolution 32:646–654.

Haiduk, M. W., R. J. Baker, L. W. Robbins, and D. A. Schlitter. 1981. Chromosomal evolution in African megachiroptera: G- and C-band assessment of the magnitude of change in similar standard karyotypes. Cytogenetics and Cell Genetics 29:221–232.

Hamilton, M. J., R. L. Honeycutt, and R. J. Baker. 1990. Intragenomic movement, sequence amplification and concerted evolution in satellite DNA in harvest mice, *Reithrodontomys*: evidence from in situ hybridization. Chromosoma 99:321–329.

Heinze, T., and G. Bauer. 2007. Characterizing creative scientists in nano-S&T: productivity, multidisciplinarity, and network brokerage in a longitudinal perspective. Scientometrics 70:811–830.

Hillis, D. M., C. Moritz, C. A. Porter, and R. J. Baker. 1991. Evidence for biased gene conversion in concerted evolution in ribosomal DNA. Science 251:308–310.

Hirsch, J. E. 2005. An index to quantify an individual's scientific research output. Proceedings of the National Academy of Sciences 102:16569–16572.

Holden, C. 2005. Random samples: data point—impact factor. Science 309:1181.

Horner, K. L., J. P. Rushton, and P. A. Vernon. 1986. Relation between aging and research productivity of academic psychologists. Psychology and Aging 1:319–324.

Hsu, T. C. 1979. Human and mammalian cytogenetics: An historical perspective. Springer-Verlag, New York.

Jackson, D. N., and J. R. Rushton (eds.). 1986. Scientific excellence origins and assessment. Sage Publications, Newbury Park, Beverly Hills, California.

Jones, J. K., Jr. 1991. Genealogy of twentieth-century systematic mammalogists in North America: the descendants of Joseph Grinnell. Pp. 48–55 in Latin American mammalogy: history, biodiversity, and conservation (M. A. Mares and D. J. Schmidly, eds.). University of Oklahoma Press, Norman.

Kelly, C. D., and M. D. Jennions. 2006. The h index and career assessment by numbers. Trends in Ecology and Evolution 21:167–170.

Kohler, R. E. 2002. Landscapes and labscapes: exploring the lab-field frontier border in biology. University of Chicago Press, Chicago, Illinois.

Kreiman, G., and J. H. R. Maunsell. 2011. Nine criteria for a measure of scientific input. Frontiers in Computational Science 5:1–6.

Krell, F-T. 2000. Impact factors aren't relevant to taxonomy. Nature 405:507–508.

Kyvik, S., and J-C Smeby. 1994. Teaching and research: the relationship between the supervision of graduate students and faculty research performance. Higher Education 28:227–239.

Levin, S. G., and P. E. Stephan. 1991. Research productivity over the life cycle: evidence for academic scientists. The American Economic Review 81:114–132.

McDade, L. A., et al. 2011. Biology needs a modern assessment system for professional productivity. Bioscience 61:619–625.

Medina, M. A. 2006. The pursuit of creativity in biology [editorial]. BioEssays 28:1151–1152.

Meyne, J., et al. 1990. Distribution of non-telomeric sites of the $(TTAGGG)_n$ telomeric sequence in vertebrate chromosomes. Chromosoma 99:3–10.

Morales, D. X., S. E. Grineski, and T. W. Collins. 2017. Increasing research productivity in undergraduate research experiences: exploring predictors of collaborative faculty-staff publications. CBE-Life Sciences Education 16:ar42, 1–9.

Olden, J. D. 2007. How do ecological journals stack up? Ranking of scientific quality according to the h index. Ecoscience 14:370–376.

Panarctos, J., and C. Malesios. 2009. Assessing scientific research performance and impact with single indices. Scientometrics 81:635–670.

Parker, J. N., C. Lortie, and S. Allesina. 2010. Characterizing a scientific elite: the social characteristics of the most highly cited scientists in science and ecology. Scientometrics 85:129–143.

Parlos, J. A., R. M. Timm, V. J. Swier, H. Zeballos, and R. J. Baker. 2014. Evaluation of paraphyletic assemblages within Lonchophyllinae, with description of a new tribe and genus. Occasional Papers, Museum of Texas Tech University 320:1–23.

Patton, J. L. 2005. Species and speciation: changes in a paradigm through the career of a rat trapper. Pp. 263–276, in Going afield (C. J. Phillips and C. Jones, eds.). Museum of Texas Tech University, Lubbock.

Patton, J. C., and R. J. Baker. 1979. Chromosomal homology and evolution of phyllostomatoid bats. Systematic Zoology 27:449–462.

Phillips, C. D., and R. J. Baker. 2015. Secretory gene recruitments in vampire bat salivary adaptation and potential convergences with sanguivorous leeches. Frontiers in Ecology and Evolution, vol. 3 article 122. doi:10,3389/fevo.2015.00122.

Phillips, C. D., et al. 2012. Microbiome analysis among bats describes influences of host phylogeny, life history, physiology and geography. Molecular Ecology 21:2617–2627.

Phillips, C. J., et al. 2014. Dietary and flight energetic adaptations in a salivary gland transcriptome of an insectivorous bat. PLoS ONE 9(1):e83512. Doi:10.1371/journal.pone.0083512.

Rohm, R. 2005. Positive Personality Profiles—D-I-S-C-over personality insights to understand your self and others. Voyages Press Inc., Atlanta, Georgia.

Rushton, J. P. 1988. Scientific creativity: an individual differences perspective. Journal of Social and Biological Structures 11:140–143.

Rushton, J. P., H. G. Murray, and S. V. Paunonen. 1986. Personality characteristics associated with high research productivity. Pp. 129–148 in Scientific excellence origins and assessment (D. N. Jackson and J. P. Rushton, eds.). Sage Publications, Newbury Park, Beverly Hills, California.

Schmidly, D. J., R. D. Bradley, L. C. Bradley, and R. D. Stevens. 2017. A timeline of significant events in the development of North American mammalogy. Special Publications, Museum of Texas Tech University 66:1–37.

Schmidly, D. J., and V. Naples. 2019. North American mammalogy: early history, dominant personalities, and significant milestones (1850–1960). Journal of Mammalogy 100:701-718.

Schmidly, D. J., N. C. Parker, and R. J. Baker. 2002. Texas Parks and Wildlife for the 21st century: an overview of the Texas Tech University studies in conservation and recreation for the coming decades. Texas Tech University, Lubbock.

Schwartz, M. no date. Best practices in graduate supervision. Ryerson University, Learning & Teaching Office.

Selander, R. K., D. W. Kauffman, R. J. Baker, and S. L. Williams. 1975. Genic and chromosomal differentiation in pocket gophers of the *Geomys bursarius* group. Evolution 28:557–564.

Simonton, D. K. 2004. Creativity in science: chance, logic, genius, and zeitgeist. Cambridge University Press, Cambridge.

Sonnert, G. 1995. What makes a good scientist?: determinants of peer evaluation among biologists. Social Studies of Science 25:35–55.

Steinbeck, J. 2003. America and Americans and selected nonfiction. Penguin Press, UK, p. 123.

Straney, D. O., M. H. Smith, R. J. Baker, and I. F. Greenbaum. 1976. Biochemical variation and genic similarity of *Myotis velifer* and *Macrotus californicus*. Comparative Biochemical Physiology 54B:243–248.

Texas Monthly Magazine. September 2018 issue, p. 55, Austin, Texas.

Timmer, J. 2018. Creative types all go through hot streaks of superior production. Arstechnica.com/science/2018/07. Last accessed 7 September 2018.

Whitaker, J. O., Jr. 1994. Academic propinquity. Pp. 121–138 in Seventy-five years of mammalogy (1919–1994) (E. C. Birney and J. R. Choate, eds.). Special Publication, American Society of Mammalogists 11:1–433.

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Bioterrorism and Biowarfare: A Continuing Defensive Role for Mammalogists

An Essay

Carleton J. Phillips

Abstract

The idea of using biological agents or their toxins as weapons (biological warfare—BW) is an ancient concept of warfare—a combination of terrorizing, killing, or demoralizing an enemy. In the modern era, biology provides opportunities for national governments and transnational terrorists to access weapons that have noteworthy political impact. In this essay, I share some of my own experience while serving with the rank of Senior Executive Service (SES) in the United States Government, and as a Foster Fellow recruited to the Office of Proliferation Threat Reduction at the Department of State and as a Special Advisor on Nonproliferation to the Coalition Provisional Authority in Iraq. In addition to discussing the political atmospherics in Washington, DC, I also include information about the search for BW in Iraq. The main theme of this essay is simple: the United States needs an organized BW response team that includes mammalogists with field skills and the capability of conducting research on genetics. This is true because mammal species' distributions are a critical part of understanding zoonotic (animal-borne) diseases that have potential value as biological weapons. Historically, mammalogists have had noteworthy roles in United States defense and preparedness against BW. In 1943, the United States created the Naval Medical Research Units (NAMRU-2 served in the South Pacific) that could detect and respond to Japanese bacteriological warfare. In the Korean conflict and again during the Cold War, mammalogists conducted research on such diseases as hemorrhagic fever, attempting to distinguish natural and intentional sources of disease on the battlefield. The United States organized and led two international coalitions; the first attacked Iraqui forces in Kuwait (1991, Operation Desert Storm), and the second invaded Iraq (2003, Operation Iraqui Freedom).

Key words: anthrax, Baghdad, Rollin H. Baker, Coalition Provisional Authority in Iraq, Cold War, glanders, E. Raymond Hall, Iraq, ISIS, Japan, J. Knox Jones, Jr., Korean conflict, NAMRU-2, Office of Proliferation Threat Reduction, plague, Russia, Robert Traub, World War II, zoonotic disease

Introduction

"On a bleak island in the Aral Sea, one hundred monkeys are tethered to posts set in parallel rows stretching out toward the horizon. A muffled thud breaks the stillness. Far in the distance, a small metal sphere lifts into the sky then hurtles downward, rotating, until it shatters in a second explosion. Some seventy-five feet above the ground, a cloud the color of dark mustard begins to unfurl, gently dissolving as it glides down toward the monkeys. They pull at their chains and begin to cry. Some bury their heads between their legs. A few cover their mouths or noses, but it is too late: they have already begun to die. At the other end of the island, a handful of men in biological protective suits observe the scene through binoculars, taking notes. In a few hours they will retrieve the still-breathing monkeys and return them to cages where the animals will be under continuous examination for the next several days until…[they all die, one by one]… of anthrax or tularemia, Q fever, brucellosis, glanders, or plague." (Alibek and Handelman 1999).

One morning in summer of 2003, I received an odd telephone call in my office at the United States Department of State. In a goofy voice caused by the scrambler electronics, a friend simply said: "let's have lunch today at such and such restaurant near the Pentagon." Agreeable, I took the train from Foggy Bottom and arrived in Crystal City Mall at the appointed time. My friend was sitting with another person, unknown to me. He did however have the look of being a scientist and maybe even a biologist—sort of rumpled, unshaven, and inelegant in contrast to the earnest Pentagon civilians in their business attire and the clean-cut, uniformed colonels cruising the mall by the dozens. This stranger was too nervous and edgy to eat his hamburger. But my guess was a good one; the fellow was a microbiologist and that alone explained his insistence on ordering his burger well done and still being loath to consume it. A fear of bacterial attack is the bane of the microbiologist. It can be awful to know too much.

All of the precautions seemed unnecessary, although the microbiologist was convinced that he was being followed and watched by persons unknown. It was clear that his concern was that he might be seen by 'friends' in the intelligence community. Fear of friends is always more acute than fear of the enemy—which in this case I suppose was mainly Iraqi spies or al Qaeda watch dogs.

Friends, no friends, friends in high places, dangerous friends (armed), and goofy characters were just some of the categories that were filled easily in Iraq during the search for the nonexistent Bioweapons Program. I spent almost ten years (2003–2011) living in a world where I witnessed embarrassing ignorance, willful misdirection, or blatant misinformation linked to Iraq. I witnessed all of these things promulgated by people both in and outside of government. But undoubtedly the most disturbing examples—which still make me cringe—were instances in which major players with legal obligations to the President simply refused to support his Iraq project. I never got used to the fact that certain Federal agencies and many of the United States National Laboratories with important expertise generally refused to participate on the ground in Iraq.

The first year and a half (until July 2004), I worked at the Department of State as a William Foster Fellow on leave from a professorship and senior administrative position (Assistant Vice President for Research) at Texas Tech University. Simultaneously, I served as the Special Advisor to the Coalition Provisional Authority (CPA) on Nonproliferation. I operated independently of David Kay's Iraq Survey Group so I avoided the misery of the public and private battle between David Kay and George Tenet, who served as DCI (Director of the CIA) under President Bush (Tenet 2007). Instead, I worked independently and very quietly on the development and implementation of a Redirection Program for Iraqi scientists. The present essay explores aspects of how my professional life in the discipline of mammalogy related to that position and how mammalogy historically has been a large part of the United States' defense against bioweapons. The largest and most comprehensive bioweapons program was run by the former Soviet Union (FSU), which maintained a vast network of facilities, including BSL-4's at the Russian State Research Center of Virology and Biotechnology (widely known as Vector) in Koltsovo, Siberia, and their super-secret testing facility, Aralsk-7, on Vozrozhdeniya Island (known as Voz Island) in the Aral Sea, and elsewhere. The Voz Island facility was the source of accidental release of weaponized smallpox in 1971.

The old Soviet Program was based on the public health system, which in turn was based on having mammalogists and microbiologists stationed around the FSU where they could collect specimens of mammals, especially rodents. For their part, the species and habits of mammals affected the characteristics of their associated pathogens. The staff scientists regularly conducted collecting tours to sample whatever was showing up in nature. These same people also responded and collected whenever human cases of plague were reported. This especially was the case if there was a report of anything unusual about an infection. For example, on the basis of these types of monitoring the Soviets gradually obtained genetically diversified plague bacteria. Likewise, they sought genetically diversified anthrax by collecting organisms near the entrances to uranium mines. The thinking apparently was that exposure to radioactivity would promote mutations and thus create over time a genetically diversified local anthrax population. An unknown and unexpected strain of anthrax could create problems even for a country that had an off-the-shelf vaccine. The Soviets thus had an extremely dangerous BW Program.

Not long before the Soviet government collapsed, Ken Alibeck, an Army medical officer from Kazakhstan, was called to a meeting in Moscow. This is how he described the outcome: "Lebedinsky quickly explained the reason for the special meeting. A decision had been made at the highest levels…[in the Soviet Government]… he said, to arm SS-18 missiles with disease agents…the giant SS-18 missiles, which could carry ten five-hundred-kiloton warheads apiece over a range of six thousand miles, had never been considered before as delivery vehicles for a biological attack…Mikhail Gorbachev and his team of self-described reformers were publically heralding a new era of rapprochement with the West. We joked that the mysteries of *perestroika* were beyond the scope of simple military men. I don't remember giving a moment's thought to the fact that we had just sketched out a plan to kill millions of people." (Alibeck and Handelman 1999).

Meanwhile, back at lunch in Washington, DC, my new acquaintance explained his rumpled look by telling me about arriving at Dulles on his way home from Baghdad, Iraq, that very morning. It was not an easy trip; it involved flying on a United States Air Force C-130 from Iraq to Kuwait followed by commercial flights through Europe. The first part of the trip was always tough and exhausting and secretive. Iraqi insurgents affiliated with al Qaeda carried Strella-7 shoulder-fired, heat-seeking, antiaircraft missiles so combat tactical takeoffs and landings were used in Iraq, and that added stress to a typical mission for both flight crew and passengers (Phillips 2004). Landings in particular were tough on civilians, especially those with weak stomachs and little experience. It was not unusual to land in Baghdad amid the sounds of retching, the prop blades reversing on the four turbo-prop engines, and the rattling of the wheels touching down on concrete. It did not help that whenever I shuffled aboard the aircraft in Kuwait, a fellow named Jones who worked for Kellogg, Brown, and Root (KBR), would come aboard and shout out something like, "remember folks, where you're going there are people who want to kill you, so take care." What strange, but honest, advice.

The Iraqi bioweapons program was uncovered in 1996 after the conclusion of the Second Gulf War (Desert Shield and Desert Storm in 1991). And the bioweapons program was what the microbiologist wanted to speak about. In particular he wanted to tell me about the "mobile biological laboratories" that Secretary of State Collin Powell had described in a retrospectively embarrassing speech at the United Nations in New York on 5 February 2003. Secretary Powell had argued at the UN that the mobile laboratories were a key and sinister feature of the current Iraqi bioweapons capability. My new acquaintance, whose well-done burger was now cold, had gone to Iraq to examine one of the mobile laboratories that had been captured by our Special Ops personnel. He explained in detail how he had meticulously gone over the laboratory. Then came his stunning conclusion—the mobile biology laboratory was not a *biology laboratory* at all. No trace of disease-causing spores or other chemical contamination was found on any of the surfaces. In particular he and his team could find no trace of *Anthrax* regardless of the markers that they tested for in their swipe samples.

I recalled the Secretary of State's testimony before the U.N. Security Council. Secretary Powell had shown some drawings of a mobile biology laboratory. A trailer carried a steel box with generic metal cabinetry and work-surfaces. One piece of equipment could have been a large fermenter for growing microbes, but aside from this device, nothing about the trailer seemed connected to biology. I was not the least bit surprised, which amazed and depressed the microbiologist. I had already concluded that Iraq had dropped its BW program after it was discovered by UN inspectors in 1996—seven years before the invasion called Iraqi Freedom and capture of a mobile laboratory. "I told you so," is an unpleasant thing to say, but a fairly large number of people who studied such things on behalf of the intelligence community were in quiet, unspoken, agreement on the subject.

The microbiologist glanced about furtively as he expressed concern if word got out that he had been sharing what then was closely held information. His concern centered on the fact that the Department of Defense claimed ownership of whatever he had discovered in Iraq—which meant that it was more like a kind of intellectual property dispute than anything else. President Bush had made the mistake of announcing the capture of the mobile lab and then doubled his mistake by implying that it was the equivalence of finding WMD on Iraqi soil. So, some of the sensitivity was clearly associated with the President being misled

by his own Administration. Knowledge of what the mobile lab really was used for possibly was being kept from the President. Moreover, it also clearly cast doubt on the veracity of the positions taken by some of the Neocons in the Bush Administration. Two examples will suffice: Undersecretary of State John Bolton and Undersecretary of Defense for Policy, Douglas Feith. Contrary to what Feith argued publically (Feith 2008) and used as a foundation for decision-making, Iraq did not have an active bioweapons (BW) program in 2002, or later. Bolton had his own issues, one of which resulted from less than truthful statements about an imaginary BW program in Cuba. His mistake was to try to draw in and blame an intelligence officer who worked at the State Department. John Bolton tried to force the analyst to support his unfounded claims and when he refused, Bolton then tried unsuccessfully to fire him. I was present when Secretary Powell expressed his full support for the analyst and his honesty—a statement that left John Bolton standing alone, but not in the least embarrassed (Dafina 2005; Rotella 2018).

And as the word spread that Iraq did not have active programs, Feith took the position that, oh well, getting rid of Saddam Hussein and his regime was good enough reason for the invasion (Feith 2008). And of course it *only* cost the United States the lives of more than 4,000 men and women (with another 32,000 WIA) and three trillion dollars (Stiglitz and Bilmes 2008).

Neither Bolton nor Feith knew enough about science and bioweapons and disease processes to make educated guesses about Iraq or Cuba or any other country for that matter. These two men qualify as examples of how seemingly intelligent, well-educated people can be completely blank when it comes to science. The blame falls with the universities where they were educated.

My goal in writing this essay is to share some of what I know from my own experience as a diplomat and scientist. Biological warfare is ugly and uncompromisingly dangerous, whether conducted by well-organized wealthy nation-states, or rouge nations lacking central government and control, or transnational terrorists such as ISIS, or by demented individuals or loud-mouthed, small political entities. In order to defend itself, the United States needs to employ a complicated strategy that includes treaties, planning, vaccine development, and diplomacy (especially science diplomacy) (Lederberg 2001). A role for mammalogists is unique and unheralded and that is what I will present here.

By chance alone in my career I worked with many key individuals involved in developing the United States response and preparation for defense against bioweapons. This unique experience is worth talking about because the threat of bioweapons is real and expanding. Beginning with WWII, the first response to bacteriological warfare, as it was then known, included field teams led by Colonel Robert Traub (US Army-ret.) and Lt. Rollin H. Baker (US Navy-ret.). I did my own first real fieldwork as an undergraduate (Michigan State University) in mammalogy with Rollin Baker in Mexico, and as a graduate student (University of Kansas) I worked in Pakistan near the Soviet border with Robert Traub. My Ph.D. graduate mentor, Lt. J. Knox Jones, Jr. (USA-ret.), was the key mammal expert in Korea and helped sort the important question of whether BW was used by the Chinese or Soviet Union in an attempt to break down the UN resolve. My M.S. mentor at the University of Kansas was E. Raymond Hall, who had the idea to get funding from the Navy to create the critically important publication, *Mammals of North America*. In 1965, I trained a group of Navy medical and biological specialists headed for Viet Nam as part of a NAMRU-2 unit. And finally, I had the good fortune to have conducted research and diplomacy on BW in Iraq and Kyrgyzstan. Collectively, I have been near the center of BW as a threat to United States National Security, and one objective of this essay is to share some of what I learned from that unique vantage point.

MAMMALOGISTS AND HISTORICAL LINKS TO BW

A movie titled *Green Zone* with Matt Damon in a lead role was released in 2010. A movie seems like an odd place to look for explanations of why mammalogy is a particularly valuable scientific discipline when it comes to defending ourselves against bioweapons. But please stay with me. The implication in the movie

script was that our government knew that there were no biological weapons or even an active WMD program, and were willing to kill to protect that secret. I gathered that the movie script was an unexpected consequence of my colleague Alex Dehgan's interview with a *Washington Post* journalist named Rajiv Chandrasekaran. In his book, *Imperial Life in the Emerald City*, Chandrasekaran wrote about the dangers to Alex and me in Iraq in early 2004 (Chandrasekaran 2006). I do not believe that Chandrasekaran fully understood our situation working in Baghdad, although he did report the threats that we both received. We even had some difficulties with our colleagues.

The scientists who were part of the team in the Iraq Survey Group (ISG) were highly stressed by their inability to uncover hidden biological (and chemical and nuclear) weapons, and the impact of that stress was felt all the way from Baghdad to Washington, DC, and back again (Tenet 2007). The scientists were excellent, but the ISG was so large that individuals had little influence, and their boss reported to George Tenet, the DCI. Reporting to the DCI or another head of an intelligence agency has the feeling of reporting to a black hole in the sense that such people tend to collect information, and then bury it within the organizational back rooms and darkened hallways.

The movie version of our lives was full of adventure and Alex and I argued—always in good nature—over which of us was played by Damon. We were both wrong. It turned out that Damon's character was not based on us even though we knew (or believed) that we belonged at the epicenter of the story. More important to my essay, Alex Dehgan is a mammalogist, one of Bruce Patterson's Ph.D. students. Alex studied lemurs in Madagascar and is the author of *The Snow Leopard Project and Other Adventures in Warzone Conservation* (Dehgan 2019). Alex also has a law degree, which makes for an interesting and rare combination with his Ph.D in mammalogy.

Chandrasekaran was baffled to hear about the field of mammalogy, and thought that it made no sense for me to have hired Alex to deal with bioweapons personnel still loose in Iraq. As a partial explanation of why Alex, a mammalogist lawyer, got his job, Chandrasekaran wrote that "…[Phillips] took a shine to Dehgan, recommending that he be sent to Baghdad to open a science center, a place where Iraqis who had worked on weapons programs could interact with one another and learn about new jobs." This is sort of how we describe "Redirection."

Incidentally, the notion is preposterous that I would hire a person whom I liked and then show that I liked them by sending him or her to a warzone. But Chandrasekaran probably did not mean to say what he said. Demanding a more complete explanation of why mammalogists were piling up at my office door, Chandrasekaran pressed Alex Dehgan for an answer and finally got him to say, "it…[the job in Baghdad] was all based in the fact that [both of us] study animals that give milk and have a certain number of ear bones." Well put, I thought. This should keep Ravi happy.

When I hired a third mammalogist, Peter Smallwood of Richmond University, in late spring of 2004, I failed to mention it to Ravi Chandrasekaran or anyone else at the *Washington Post*. As with Alex, Peter came to us at the Department of State as an AAAS (American Association for the Advancement of Science) Congressional Fellow. Peter had spent the year working on environmental legislation in Senator Joe Lieberman's office. Later (2004–2005), Peter served as Executive Director of the Department of State Redirection Program for Iraqi scientists (Smallwood and Liimatainen 2011).

Having a group of three mammalogists prompted a senior Iraqi molecular biologist to paraphrase J. B. S. Haldane's famous remark about the incredible number of beetle species—Professor Ali al-Zaak said that: "Obviously, Secretary of State Collin Powell has an inordinate fondness for mammalogists, having sent so many to us here in Baghdad." The Secretary of State appreciated the joke. Or, as mammalogist David J. Schmidly (whose career included serving as President of Texas Tech University, Oklahoma State University, and the University of New Mexico) is fond of saying, "if you want a job done right, hire a mammalogist" (Schmidly 2005).

A lack of knowledge of the historical role or potential future role of mammalogists in contributing to ways of dealing with bioweapons programs was typical of most of my CIA colleagues and DOD-Policy personnel. The same thing was true of academics interested in

the challenge of controlling or defending against BW. A surprising number of scholars "study" issues without actually being involved in the fieldwork or diplomacy needed. Collectively, all of these issues were a source of significant frustration on my part, particularly when I encountered scientific ignorance mixed in with politics. The historical role of mammalogy in its most basic form is one key to a successful strategy for preparation and response to a biological attack based on a zoonotic—animal-borne—disease.

Mammalogy as a scientific discipline provides many of the tools and experience needed to support a national strategy. More important, perhaps, our look at historical data reveals that mammalogy was a core discipline in one of the first U. S. Government (USG) responses to the realization that disease could cripple an attacking force and, thus, the potential of BW as a threat to our troops in the Pacific—namely the creation in 1944 of Navy Medical Research Units (known as NAMRU). And for starters, this was the beginning of mammalogists' association with an aspect of response to biological warfare. In the 1930's and 1940's, academic biology was not particularly diversified even though there were basic disciplines such as bacteriology, zoology, entomology, ornithology, and mammalogy—just to name a few of the "ologies." In American universities many of these subjects were represented by single courses rather than curricula. All of these factors are important because the historical perspective gives us a sense of the past and how it compares to the present. There was not much of a gap between disciplines, especially in comparison to what we find today. The combination of mammalogy with bacteriology made sense back in the early 1940's. Unfortunately, it makes less sense today because newly acquired data accumulate rapidly, and generally speaking, such data have tended to push disciplines apart. Academically, the organismic and molecular curricula have been restructured in such a way as to emphasize the distinctions between them. And it has proven to be impossible to bring all of biology back into an integrated whole.

In North America, the discipline of mammalogy rests intellectually upon E. Raymond Hall and Keith R. Kelson's massive writing project on North American mammals. It was unique in that it listed all named species of North American mammals—treated in taxonomic synonymies as dynamic versus static data, and illustrated the geographic distribution of each species (Hall and Kelson 1959). This work, which laid the foundation for studying mammals comparatively, at the most basic level, provided the raw materials for thousands of testable hypotheses for mammalogists, ecologists, biogeographers, and others. In terms of its science, "Hall and Kelson" [as the original 2-volume set of *Mammals of North America* came to be known] was unique in scope, purposes, and coverages. It is relevant to our discussion because the project was mostly funded by the Office of Naval Research (ONR). And the ultimate goal of ONR was to create a database in support of defensive strategies to counter the threat of zoonosis-based bioweapons developed and deployed by the former Soviet Union. The late James Findley, a mammalogist and professor for decades at the University of New Mexico, wrote in his autobiography in *Going Afield* a humorous description of the inevitable interactions among mammalogists and the ONR (Findley 2005). Findley honed in on the relationships among the famous American mammalogist E. Raymond Hall and his basic research on mammal species distributions on the one hand, and official government fears about BW and the Soviet Union on the other.

The thinking behind getting the ONR involved in funding the research that went into writing *Mammals of North America* was basic and fairly simple. The argument in the early 1950's was as follows. The Soviet Union was thought (or known, depending on the quality of your intelligence information) to have a BW Program that involved the collection and ultimate weaponization of zoonotic disease pathogens. These zoonotic agents primarily were bacterial pathogens at first, but later included deadly viruses such as smallpox, Marburg, and Ebola. At least some zoonotic agents were likely to be species-specific, which meant that their geographic distribution possibly matched that of their mammalian hosts. The weaponization and release into a novel environment of such an agent potentially could be devastating (Phillips et al. 2009). When one reads this paragraph it is essential to remember the timing. This discussion about "genetic" correlations among mammal species and associated zoonotic agents occurred at almost the same time as the discovery of the molecular structure and mode of replication of

DNA and at least 25 years before the development of processes to test a hypothesis about zoonotic agents and their genetic diversity. In 2003, the year when I had my secret lunch regarding an imaginary Iraqi mobile biology laboratory, 50 years already had passed since the ONR's funding of *Mammals of North America*.

As an academic activity, developing an understanding of the species distributions of mammals is extremely important. Such databases can reveal the potential sources or reservoirs of zoonotic diseases. It also is the case that a combination of fieldwork and laboratory time has been essential to being able to conduct research in mammalogy. Although it might not be obvious, the ability of field biologists to work under incredibly difficult field conditions and to create techniques for mating field and laboratory work is significant. In fact, the ability to conduct fieldwork is a necessary ingredient to success in many, but perhaps literally *not all*, sub-disciplines of mammal research, and is common enough that most of us do not even think about it (Phillips 2005). Alex Dehgan's book captures much of this perspective by illustrating what can be done in a warzone such as what he encountered in both Iraq and Afghanistan (Dehgan 2019). Again, there is a tradition among mammalogists to work in places where one practically wears a target.

Rollin H. Baker was persuaded that in future times it would make sense for civilian scientists and mammalogists in particular to conduct fieldwork alongside the military (Baker 1994). And I agreed strongly in an invited speech to the Strategic Studies Institute, where I was asked to speak about future joint work between civilian scientists and military units. In reality, only a very small number of people have had experience of working in a war zone with a mixed group of military and civilian personnel. Historically, such a group was not unusual in the American West, where George Bird Grinnell did much of his bird specimen collecting while afield as a graduate student. What gave Grinnell an edge and stimulated his intellect was the fact that sometimes he accompanied General George A. Custer and the 7th cavalry. One exception was Custer's infamous last expedition to the Little Big Horn in Montana. In this case, in a moment of prescience, young Grinnell turned down Custer's offer of going afield with him as the civilian graduate student "naturalist" traveling with the Army.

In my experience, in Iraq, coordination with the U.S. Army was complicated, but rewarding for both parties. The costs of such fieldwork are astronomical in comparison to the typical National Science Foundation-funded field project involving only academic (university) personnel, although much of the added costs can be covered by the military team. In the Iraq warzone a typical joint mission between Texas Tech University and the United States Army involved rotary wing aircraft and sometimes unmanned aerial vehicles (UAV's). On-the-ground military personnel, plus backups on stand-by, vehicles (either Humvees or MWRAPs, or both, and specialized equipment (e.g., portable air conditioning units) were used.

One possibility is that the military side of the equation eventually will create a special group or unit that can undertake joint projects with the civilian side. The civilian side will include personnel suitable for such work and knowledgeable about mammals, ecology, and related subjects. One down side, which plagued some of my own joint work in Iraq (2003–2011), is that military units tend to have definite, short, deployment periods of about one year. By way of comparison, civilian scientists tend to occupy essentially the same position for years. In the case of academic-based personnel, the time-frame is even longer—decades—on account of low mobility.

The word "dangerous" falls short of describing real-world working environments in which a mixed group of military and civilian personnel might be deployed. Possibly the closest analog in other fields would be the microbe hunters seeking species that exist under the most extreme situations—deep sea vents, cave soils, and the Atacama desert in Chile are just three examples of places where fieldwork is conducted. In principle, all of this type of research into extreme environments plays into astrobiology and the possibility of extraterrestrial life on Mars, or Jovian or Saturnine moons, or elsewhere in the solar system.

BIOLOGY AS A WEAPON OF WAR

To most of us, "biology" is not a weapon—far from it. Biology is a term that we use to describe a discipline of scientific study that includes all living things ranging from familiar mammal species to bacteria and even the strangest of viruses. If one reads the articles published in this memorial book or peruses some of the articles or simply looks at the titles, there is no hint of weapons or terrorism or anything sinister about the study of mammalian biology.

Historians are fascinated with the challenge of seeing how far back in time humans have used biologically-based weapons (Hilleman 2002). Usually the data support the idea that dead bodies were used to contaminate water sources at least several thousand years before the "germ theory" became common knowledge. Animals have served unwittingly as war-fighting partners of human beings and these partnerships were very common in antiquity (Hilleman 2002). Most of the animal species involved—in a general sense because in most cases we do not know exactly which species were used—were either directly or indirectly threatening. Animals such as venomous snakes, insects, and scorpions can frighten hardened soldiers even more than bullets, and species such as domestic dogs (war-dogs), and cattle, elephants, and goats all have been used as "biological weapons."

An Iraqi microbiologist, Nassir al-Hindawi, who helped weaponize anthrax for Saddam Hussein and who introduced himself to me as the "father" of the Iraq BW Program, insisted to me that snakes could be used as biological weapons. In particular he told me of a species of snake known only in Iraq that could transform itself into a spear. This imaginary snake supposedly could launch itself and penetrate the torso or limbs of a human being. The triangular-shaped head on this snake species was offered as proof that it was the tip of the spear. It was an interesting cultural phenomenon to find a supposedly well-trained scientist so able to dismiss his credulity and somehow live in a technical world side-by-side with a world of folklore. I must add, however, that it is equally improbable to meet this elderly gentleman and hear him talk about his American-born children and wonder how in the same breath he could be so proud of his designation—father of Iraq's BW Program. I brought him to Texas in early 2004 and asked him to speak to mammalogy graduate students at Texas Tech University. His assigned topic was "ethics in science." Afterwards, for good measure I took him to my house for dinner, which he earned by mucking out the horse stalls.

Dr. Hindawi also had the distinction of being the faculty mentor of Dr. Huda S. Ammash (aka, Mrs. Anthrax or Chemical Sally or the Five of Hearts), who was a Dean of Science at the University of Baghdad when Operation Iraqi Freedom was launched in 2003. Huda was famous more for her propaganda writing ability than for her science, which she partly learned at the University of Missouri, which presented her with a Ph.D. in microbiology. So, in a bizarre sense she was well-trained. Perhaps her most famous paper was entitled, *Toxic Pollution*, *the Gulf War, and Sanctions: The Impact on the Environment and Health in Iraq*. As one example of her writing we have the following absurd statement: "…the military bombardment altered the physical conditions of surface soil and incinerated many areas of plant cover. This inevitably affected the seed bank, which in turn reduced the density and composition of Iraq's plant life…new fields of sand dunes were created, with simultaneous increase of dust storms and dust falls…since all the components of the ecosystem were changed, Iraq has seen an *increase in rodents and scorpions* [my italics], which has caused considerable problems for health and agriculture" (Ammash 2002). I looked for an Iraqi mammalogist who could identify reliably, which species of rodents had increased in population density, but discovered that Iraq lacked anyone with mammal species identification skills.

The use of scorpions as a threat is my favorite example of ancient biological warfare. At the Roman siege of Hatra (near Mosul in modern-day northern Iraq) in Ninawa Province in 198 A.D., scorpions in clay pots were tossed like hand grenades. Ironically, when ISIS (Islamic State) occupied northern and northwestern Iraq, beginning in 2014, their leadership claimed that they would be using scorpion "bombs" just as they were used to defeat the Roman Legions of Septimius Severus at Hatra in 198 A.D.

Although scorpions and vipers might have been used in ancient warfare, rodents were the most written about sources of pestilence, even 2,000 years ago. Rodent outbreaks characterized by rapid and visible increases in population density were preludes to trouble. It thus is not surprising that stories, myths, and religious beliefs grew up in connection to rodents. It also is not surprising that one can discover evidence that humans looked for ways to use rodents to spread disease. Bubonic plague is an important example. Although the details are incomplete, there is evidence that plague showed up in the Mediterranean cities from Central Asia. But the working hypothesis is that plague used as a novel weapon came into the Mediterranean from Crimea, which was an important connection to the Silk Road. The message here is that zoonotic diseases can be defined in terms of their geographic distributions. The determining factors are mostly unknown, but as mammalogists interested in the host mammals, our hypothesis is that hosts have genetic relationships with the zoonotic agents and consequently they must share both history and distributional patterns.

The thing about zoonotic diseases is that they seem to make themselves available at times that favor their collection and weaponization. Plague is just one example. Population expansion and habitat modification are often thought of as two factors that have contributed to species "jumps" on the part of zoonotic agents. Most—essentially all—of these zoonotic agents were unknown or poorly known prior to infecting human beings. So, the danger exists and demands a response involving research on newly discovered agents, which in turn produces exactly the type of information needed to weaponize the agents. An awful reality is that thanks to evolution, nature produces new versions of old variants fairly frequently (Phillips et al. 2009). The birth of modern concepts of BW occurred during the First World War (WWI). The German Army deliberately and secretly infected horses with *Burkholderia mallei*, which is an intracellular bacterial causative agent of glanders. Glanders is contagious, usually attacks equids, and as a zoonotic disease can under unknown conditions infect human beings. And so it began.

ZOONOTIC (ANIMAL-BORNE) DISEASES

Zoonotic diseases also are referred to as animal-borne diseases. This means that the pathogen typically is associated with a particular host other than human beings (Phillips et al. 2009). Among such diseases, the pathogenic organisms associated with mammals rather than birds or other vertebrates usually are the most dangerous to us. It is in this area of knowledge that mammalogists can make obvious contributions. In an ideal situation, all species of mammals, their geographic distributions, and the genetics of their particular associated zoonotic agents already would be known. But of course we are not even close to knowing basic information for all mammal species. Moreover, in dealing with science there always is the challenge of contingency. Species recognition alone is an excellent example—systematics is an ever-changing playing field. The contingent nature of species does not bother the systematist, but can be a source of consternation or wonder on the part of a non-expert responsible for policy decision-making.

It would be our good fortune if it turned out that not all species were equally likely to carry zoonotic agents capable of jumping to humans. Until now at least such an hypothesis seems to be supported; for instance, bats and rodents are more likely to carry zoonotic diseases than are other mammals. But these two orders account for ~50% of all mammal species, so that leaves a large number of species that are important. The role of bat species as potential reservoirs for zoonotic viruses is especially notable, and the "why" questions need to be answered (Chua et al. 2003; Li et al. 2005; Phillips et al. 2009).

The former Soviet Union had the largest BW Program, as measured by the variety of bacterial, rickettsial, and viral agents that were studied and in many cases weaponized. Their program also was based on evolutionary principles, which gave it uniqueness in comparison to any other national BW programs (Miller et al. 2002; Phillips et al. 2009).

Using a zoonotic, or animal-borne, disease as a weapon minimally requires two basic kinds of knowledge or ability. These are: 1) knowledge of the disease epidemiology; and 2) the ability to isolate, maintain,

and manipulate the pathogen collected from tissues of infected animals (Phillips et al. 2009). The same things can be said about the basic knowledge required for prevention of virtually any zoonotic disease, without regard to its use or potential use as a biological weapon. Obviously, at some level of comparison there is no difference between what we need to know about a disease in order to control or eliminate it on the one hand and what BW personnel need to know in order to weaponize it on the other. Ironically, then, ordinary research on zoonotic diseases such as glanders in the late nineteenth and early twentieth centuries was conducted originally because it was dangerous to livestock and thus important economically. But this research had two purposes—one legitimate to veterinary medicine and one (actually the same research) for use in an illegitimate biological weapons program. Two purposes, or uses, of research—one legitimate and one illegitimate—can be referred to as *dual use*.

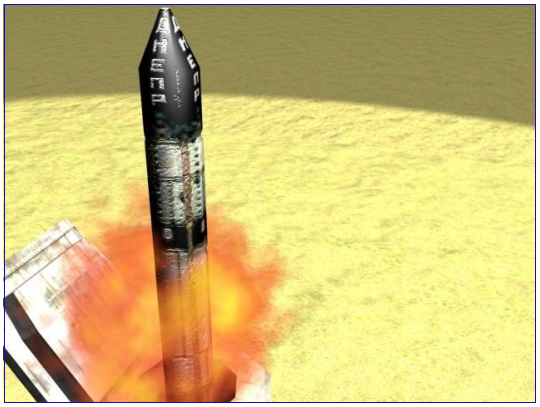
The term "dual use" arose from the realization that certain categories of research, and technology that supported such research, could make it easy to misuse data and technologies that had been obtained legitimately. Dual use has become a source of dread, especially among non-scientists with foreign policy responsibilities. As a concept it has affected foreign policy in that it quickly has become the basis for not allowing U.S. companies to sell many kinds of instruments abroad. The PCR (polymerase chain reaction) device is a simple, fairly recent example of laboratory equipment with potential for dual use. It was not only the sale or rental of such equipment that was a cause for concern. Instead, it was the small size and ease of use that made policy-people nervous. This especially was the case as the potential for transnational bioterrorism emerged. It occurred to everyone concerned that dual use equipment or research could make it practical for deranged individuals or political anarchists to obtain raw materials for creating their own bioweapons.

One lucky event arose from the failure of the Nazi's Germany to continue to put an emphasis on a bioweapons program. The main reason is thought to be the fact that Germany devoted heavy resources to the famous (or infamous, depending on your position regarding ex-Nazis) Wernher von Braun's rocket technology as a weapon that might save the Third Reich. Another reason might have been that livestock obviously had been deemphasized as a tactical component of Germany's military strategy. Some students of the subject have speculated that infecting horses with glanders was regarded as "fair game," whereas infecting humans was unacceptable. Such thinking of course is inconsistent with the typical criminal behavior of the German Nazi government.

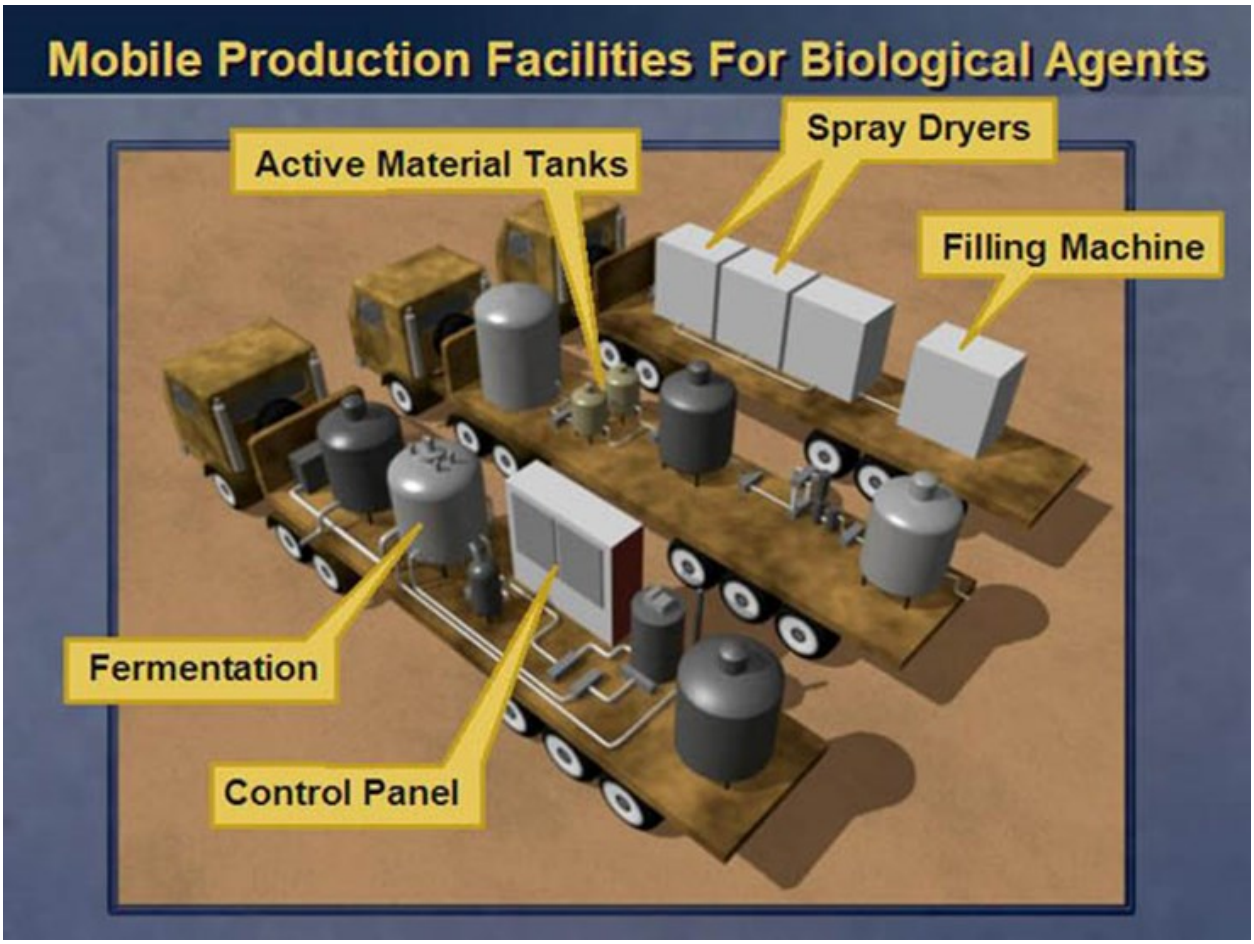
One of the basic issues regarding BW will have to be treated as an aside in this short essay. But aside or not, it is the question of United States policy toward enemy scientists following the defeat of their government. There is good historical evidence that in at least some cases, the intelligence gatherers managed to "protect" enemies regardless of their heinous crimes against humanity. In each instance the argument could be made that their knowledge and experience was extremely valuable to the United States and, therefore, their value as a resource to the United States exceeded their value as a defendant or a corpse.

IRAQ, NATIONAL SECURITY COUNCIL MEETINGS, AND WHITE HOUSE BATTLES

Late one summer morning in 2003, I set out for the White House, which is a reasonable (~12 city blocks) walk from the Department of State. Accompanying me was my colleague, Anne Harrington. Anne was Deputy Director of the State Department's Office of Proliferation Threat Reduction (PTR at that time, but now known as Cooperative Threat Reduction—CTR) and together we were writing a personnel redirection plan for Iraq. A few years later, after a stint at the United States National Academy of Sciences, President Obama appointed Anne to serve as Deputy Administrator for Nuclear Nonproliferation of the National Nuclear Security Administration (NNSA). In the course of her career, Anne published and spoke about United States policy relevant to BW. The third member of our party was a Foreign Service Officer, Richard Jarvis, who had flown more than a hundred combat missions in F-4 phantoms in Southeast Asia and who also was very experienced as a diplomat in the Middle East. Our shared goal was to modify the behavior of



Images of Soviet ICBMs, including an artist's rendering of the lift-off of an SS-18 Satan (lower left). According to Ken Alibek, the multiple warheads in a Satan ICBM were replaced by containers filled with *Anthrax* spores adequate for killing millions of Americans in the event of war with the United States.



The drawings that were part of the United States presentation to the United Nations Security Council (UNSC) by Secretary of State Colin Powell. Too good to be true, and they were that. Post-war, it turned out that a notorious character code-named "curve-ball" invented some of the evidence used to convince the United States Government that Iraq had weapons programs of concern.

The horror of Biological Warfare (BW) as well as Chemical Warfare (CW) are easily seen in these images. The top image reminds us that ISIS used chemicals in the residential zones in Mosul, Iraq. The bottom image is from the 1980s and shows Iranian soldiers suited up and awaiting a BW attack by Iraqui forces. It presently is unknown whether the Iraquis used both BW and CW weapons in the war between the two.

(Upper left) Ian Hay and Roger Hewson, two microbiologists, conducting research with mammalogists in Kyrgyzstan.

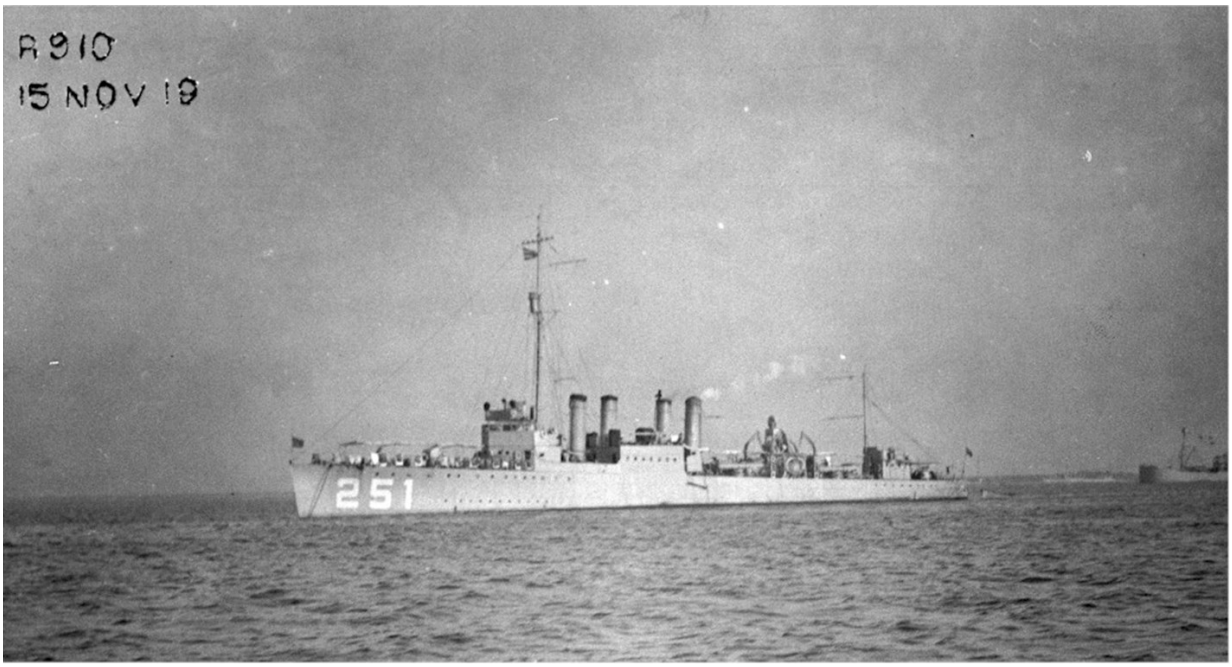
(Upper right) Rollin H. Baker with his collection of mammal specimens at Michigan State University.

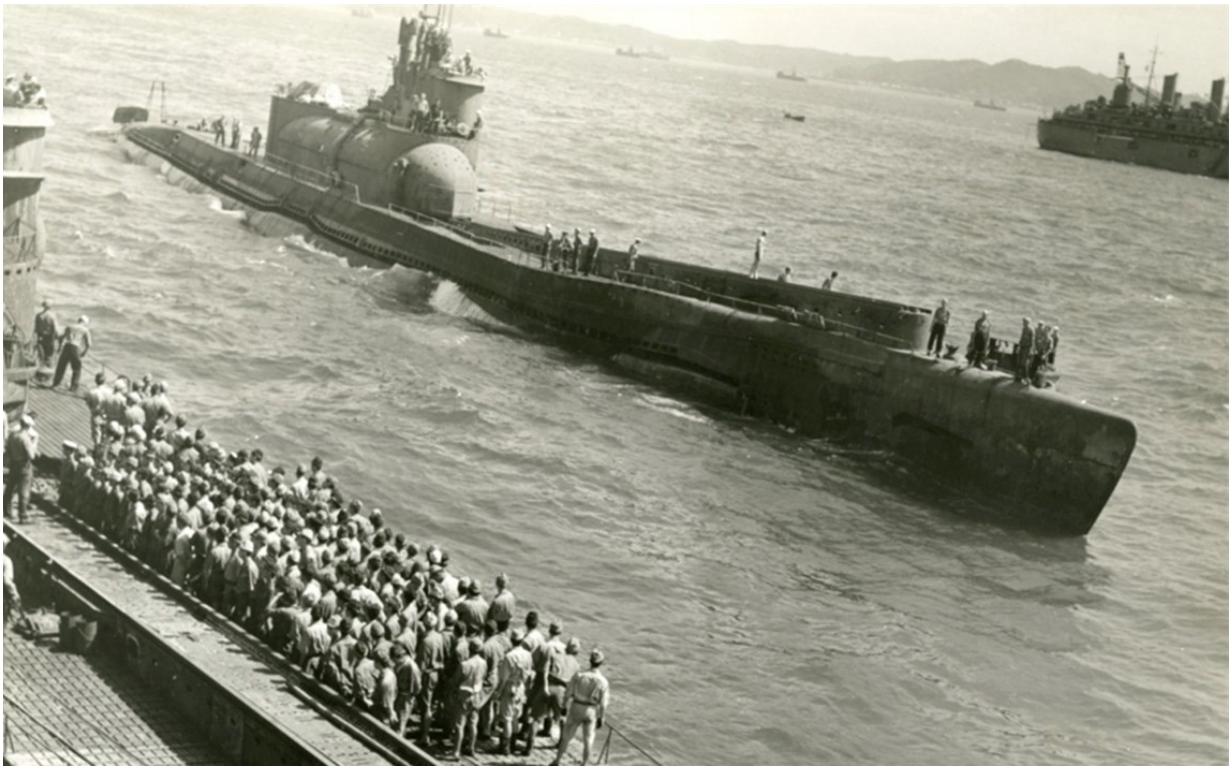
(Lower left) J. Knox Jones, Jr., preparing a mammal specimen in Nicaragua in 1966.

(Lower right) Robert Traub examining mammal specimens for fleas in 1966.

Lt. Rollin H. Baker on board the Belknap while hunting Nazi U-boats in the North Atlantic. This photograph was probably taken in 1943. In 1944, Baker was assigned to NAMRU-2 and deployed to Guam in the Pacific. Ironically, his ship was also assigned to the Pacific, where she was sunk by Japanese aircraft.

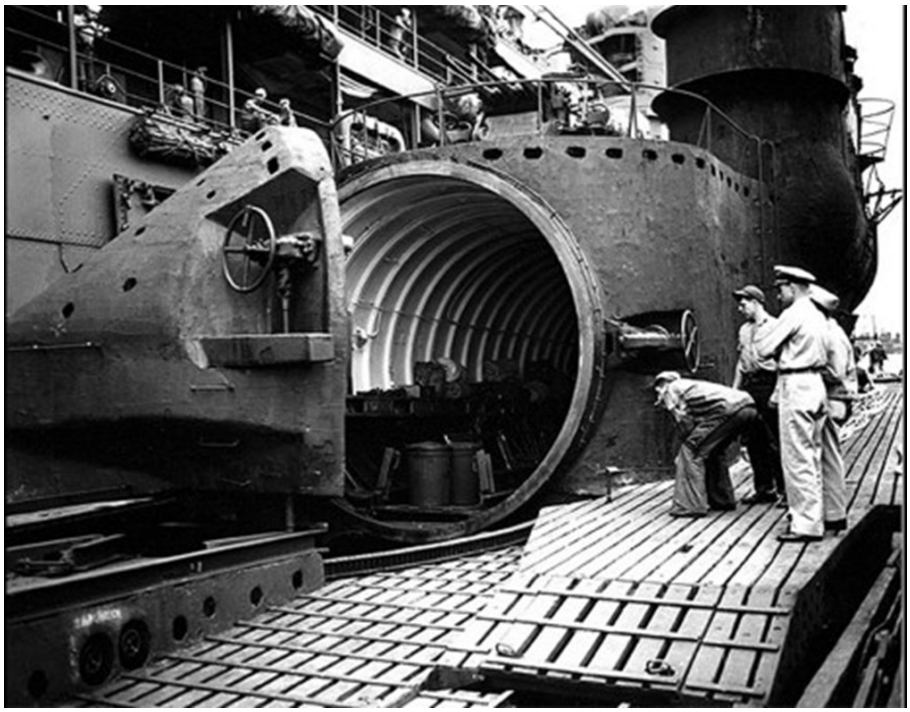
The author (Carleton Phillips) stands to the right of Colonel Henry Franke, in the second position in front row. Professor Ronald Chesser stands behind and to the left (from viewer perspective) of Phillips. Professor Peter Smallwood is kneeling in the foreground. The mixed military-civilian scientist team had just returned from fieldwork at the Al-Tuwaitha Nuclear Center 18 km south of Baghdad, Iraq. Photograph taken in 2005.





(Top) Photograph of the USS Belknap underway. This is the destroyer on which Rollin H. Baker served as a line officer prior to his service with NAMRU-2.

(Bottom) A captured Japanese Sentoku I-400. The hangar is readily seen on the deck. The crowd illustrates the interest on the part of U.S. Navy personnel, who are standing on the deck of a U.S. submarine.



(Top) U.S. Navy photograph of captured Sentoku submarine. This view shows the open hatch for the aft end of the seaplane hangar mounted on the top deck mid-line.

(Lower left) A painting showing the catapult rails and a Seiran aircraft ready for takeoff.

(Lower right) Model seaplane showing wing-folding mechanism.

A laboratory tent was provided to our Texas Tech team in the desert west of Mosul, Iraq. This tent, which was pitched each day as needed, is an excellent example of the support provided to a civilian team of scientists by the U.S. Army.

An an example of the types of equipment provided by the U.S. Army, this vehicle is called an MWRAP. Here it is shown near Mosul in 2011.

It is not apparent, but on this day in 2011 it was close to 100 degrees F. Thus, in addition to the lab tent, this portable air conditioning system had to be brought into the field.

Two HUMVEES await our team arrival via helicopter.

former weapons scientists by involving the Iraqis in a new, strictly civilian government. This process is called "redirection" and was invented when the Soviet Union collapsed in 1991. Anne Harrington and her colleague Andrew Weber (former Assistant Secretary of Defense under President Obama's Administration) played very prominent roles in the creation and application of redirection as a mechanism for dealing with the thousands of Soviet biological scientists affiliated with BW (Miller et al. 2002). Essentially all of these people were still employed, or behaved as though they were still employed, but without pay. The majority of these persons were patient—a consequence of living under a repressive Communist-style government for all of their lives. When I was in the Soviet Union, in 1984, as an Inter-academy Exchange Scientist (the USSR Academy of Sciences and the United States National Academy of Sciences), there was a mammalogist joke that was popular in the Moscow laboratory. The joke was about a Russian mammalogist whose book about the biology of wild Koryak snow sheep was banned in the Soviet Union (and he was sent to the Gulag) when political authorities discovered the title in English was *The Sheep of the Soviet Union*. The person telling the joke generally would end it by remarking on the subtlety of multiple meanings of the word "sheep" and by remarking, sheep-like, that I should not worry about the mammalogist because he had after all embarrassed the political authorities in the Soviet Academy of Sciences.

In Washington, where not everyone understood Russian scientists or their jokes, the main fear was that individuals with special skills would leave the FSU and sell their abilities and knowledge to potential or real adversaries of the United States. Moreover, there was a special fear that some renegade character would sell off samples of weaponized biologicals. This calculus led the United States to offer its assistance in creating secure facilities for storage of Russian samples or, better yet, the U.S. side offered to purchase the Russian samples. Although the Russian Federation, successor to the Soviet Union, was willing to allow the United States Department of Defense (DoD) to significantly improve sample security, they refused to sell samples to the DoD.

There were two additional, related topics that added to the concern, at least in some quarters of the U.S. Government. During the Cold War, the Soviet strategy involved spreading their influence. In particular, the Soviets wished to create a broad buffer zone around their country, which already either owned or controlled or at least influenced a huge amount of real estate. So, the question we might ask is: Did the Soviets share their expertise in BW? The answer was assumed to be no, especially in view of their paranoia about state's secrets. However, what they did do was provide training in such things as "industrial microbiology." And Iraq under Saddam was one of the recipients, at least according to one of the participants from Baghdad. Such relationships left a footprint, namely in this case a footprint that committed the Russian Federation to support Iraq versus the United States.

Anne Harrington and I reached the White House in a driving summer rainstorm, checked in at the gate, got our A passes, and went well-soaked to our meeting room. As was usual, today we met in the Eisenhower (Executive) Office Building across the driveway from the West Wing. It is a beautifully restored old building, with an office complex for the Vice President and the National Security Council (NSC). This particular meeting was hosted by William Tobey of the NSC, which at that time was headed by Condoleezza Rice. One of the roles of the NSC is to arbitrate security-related disagreements between Federal Agencies. Anne and I represented the Department of State and were in constant tugs-of-war with the Department of Defense, and, according to Condoleezza Rice, Will Tobey's job was to keep things moving along in a fair and balanced way (Rice 2011). Our project was not the only one that received such a treatment. Larry Diamond, an academic expert on democracy and Professor at Stanford University, dealt with the same impossible challenges in winter of 2003 (Diamond 2005). Diamond had the presumed advantage of being a professional colleague and personal friend of Condoleezza Rice, but that set of credentials did not help him. In fact, in his narrative he expressed surprise at the fact that his connection with Professor Rice had no positive influence at all (Diamond 2005). "Welcome to the club" is what we said.

On this particular day, Anne and Richard Jarvis and I sat across the table from a couple of our favorite people from OSD-Policy (Office of the Secretary of Defense for Policy). In chairs set along the walls we were joined by colleagues from the Joint Chiefs of Staff, the State Department, and the CIA. These wall

hangers rarely spoke. Although their choice of seats and body language and what they said to us in private was strongly supportive of our positions.

Our disagreement and our discussion was about Iraqis and whether or not the former weapons personnel would leave Iraq for greener pastures, the same concern that had driven the United States Department of State in dealing with former Russian BW scientists. The Israelis naturally claimed that they knew with certainty that key Iraqi weapons personnel had already fled to Syria or Libya or Iran. The Israelis were good at playing the Americans, especially those who were poorly informed about geography or history.

Undersecretary of Defense for Policy Douglas Feith agreed with the Israelis' assessment. But he and his representatives to our meeting also were opposed to nation building. Confronted with certain kinds of intelligence, Anne and I felt that it would be critically important to draft a statement for Paul Bremer (Bremer and McConnell 2006) that covered the question of whether the United States felt that any weapons person would be tried for crimes against humanity. Presumably, no one was likely to be charged with such a crime in apparent absence of an active BW program. And so with assistance of Feith's two representatives and the NSC attendees to our meeting, but with no input from the Iraq Survey Group, we drafted and "cabled" our statement for Bremer to read at a news conference at the CPA headquarters in Baghdad.

After collapse of the Soviet Union in 1991, there were persistent rumors about certain individual Russians who might have gone to Iran, or Egypt, or South Africa, or Israel to ply their BW expertise. And of course there were several prominent scientists who came to the United States. Funding for this remarkable challenge was needed to keep the Russians employed in civilian positions doing projects that were important to human health. Two members of Congress, Sam Nunn (D, North Carolina) and Richard Lugar (R, Indiana) created bipartisan legislation to fund the program that Anne Harrington and Andy Weber created (described by Miller et al. 2002; Hoffman 2009; Dehgan 2019). This experience was the cornerstone, or "mother," of what we tried to do in Iraq.

The basic idea with the former Soviet biological warfare specialists was to create a secure situation by focusing on the people and the fact that they could never unlearn whatever they had learned on the job. Another, less well known part of the program involved investments in securing the Russian's collections of pathogens and potential pathogens. As I mentioned previously, when their government collapsed, and the labs suddenly were without funding, the collections of microbes abruptly seemed to have value. The United States was willing to purchase or secure or help study these dangerous organisms. Although this latter program was an important part of Andy Weber's work at the Department of Defense (Defense Threat Reduction Agency—DTRA) it did not have much of an analog in Iraq. It did, however, set the stage for joint and international attempts at disease surveillance (see Phillips et al. 2009).

Fundamentally, biological weapons fall into two categories—those that involve live or active (in the case of viruses) pathogens such as plague, Ebola, or anthrax; and those that involve the use of biologically-derived toxic chemicals such as ricin or botulinum toxins. The strategic value in both cases is defensive, at least in my view. By defensive I am thinking strategically about these weapons being deployed and used by a nation or military unit attempting to defend itself from an attack with conventional weapons. In 1991, when the United States and Coalition forces attacked Iraq Army positions in Kuwait (operation Desert Storm) one could imagine the Iraqis resorting to biological weapons. At the same time, the Coalition forces moved so quickly that BW of any type most likely would have failed to be effective. And this is an important point—namely that BW is not an ideal battlefield weapon except under very specific conditions in which opposing forces are stalemated. Such situations, like trench warfare in WWI, would occur very rarely, if ever, when United States troops are involved.

Now we also need to consider another way that the word defensive can be applied to BW. The United States gave up its offensive weapons program under the direct orders of President Richard Nixon. On 25 November 1969, Nixon announced that, "the U.S. shall renounce the use of lethal biological agents and

weapons, and all other methods of biological warfare. The U.S. will confine its biological research to defensive measures (Miller et al. 2002)." In this example, the term defensive refers to the ways and means of protecting—defending—troops or the civilian population from BW attack or an attack from transnational bioterrorists. The way that it worked out, the United States determined that a defensive program primarily would involve rapid development of vaccines. The FDA, which regulates vaccines, has a reputation for being slow. So slow in fact that it would be a factor in responding to unknown zoonotic agents. Moreover, it was a matter of substantial concern if our national strategy was to be based primarily on vaccines.

The likelihood of conflict with Iraq or other nations with weaponized *Anthrax* triggered a response in the United States, which fairly quickly began producing vaccine. The Iraqis had biological weapons from the 1980's until at least 1996, and the man who took credit for developing them told me that he anticipated that *Anthrax* might be used against Iranians during the First Gulf War, which was fought between Iraq and Iran in the 1980's. But even so, he personally had been assured by Saddam Hussein that BW was only a last ditch defensive move against Iranians on Majnoon Island and the Al Faw Peninsula during Operation Kheibar in 1984. He insisted to me that his weaponized anthrax was not ready for use during Operation Kheibar, although the Iraqis did use chemical weapons to defend themselves from the Iranians. Finally, he also insisted that he never imagined that his biological weapons would be used against Americans. The truth might never be known, particularly the answer to our question about whether or not Iraq actually did use BW against the Iranians essentially trapped in place on Majnoon Island. In retrospect it seemed like the ideal physical situation for using anthrax, but according to my source, the anthrax spores coagulated due to charge and clogged the exit ports in the containers carried by fixed-wing aircraft or helicopters set up to disperse the disease-causing pathogenic bacteria. If that is true, then it is likely by luck alone the Iranians were not exposed to BW agents such as anthrax.

But there is an exception—a situation when the anticipated strategic use of a biological weapon changes dramatically. This occurs when either type of weapon—one with live pathogens or one consisting of a toxic bio-product—falls into the hands of transnational terrorists. All rules are off. A frightening hint of what that is like was provided by ISIS when they invaded northern Iraq and easily and quickly captured Mosul. Several years before ISIS declared a Caliphate, our team from Texas Tech University conducted fieldwork near Mosul. As a result we knew the entire story of radioactive materials buried or stored in various places near Mosul. As ISIS moved in, they conducted publicized searches for these materials with an oft-stated intention to create radioactive dispersal devices for use against their many enemies. Although the ISIS strategy included trying to actually create a governmental infrastructure and eventually a caliphate-like nation, they represented something more like transnational bioterrorists. As such, they provided insight into how dangerous such an organization can be. Fortunately, biological weapons seem to have remained out of their grasp.

THE JAPANESE BW PROGRAM

The World was fortunate that Germany did not seriously pursue BW during the Second World War (Cornwell 2003). However, the Japanese more than made up for the absence of a major German Program in Europe. The Japanese Program developed well before the attack on Pearl Harbor. Unlike other countries with similar programs, the Japanese eagerly deployed their biological weapons, especially against Chinese civilians (Harris 2002). The Japanese BW Program is a large and complex story that goes far beyond the present essay. The single most important point about the Japanese BW Program is that they deployed and actually used their weaponry.

The Japanese program was administered through Imperial Army Unit number 731, which was deployed principally to Manchuria from the 1930s on to the end of the war. Unit 731 operated in a deeply secretive space consistent with their mission, which included research and testing on human subjects and use of BW for military or quasi-military purposes. There is no way to know for certain how many thousands of people were

killed by Unit 731, but the number 200,000 is conservative. The awful legacy of Unit 731 was complex and many faceted. The Unit 731 staff created projects to study the effects and symptoms of diseases including water-borne bacterial diseases and zoonotic diseases such as plague. In addition to using Chinese civilians as human test subjects, the Unit also used prisoners of war (including, reportedly, American POW's). And as if to underscore the pure evil, the Unit also undertook vivisection "experiments" on human beings. It was not unusual for these dissections to be conducted on live, un-sedated individuals who died in agony as their internal organs were being cut out (Harris 2002). Today there is a museum built by the Chinese near the city of Harbin that commemorates the Chinese who suffered terribly at the hands of Japanese Army Unit 731.

A sociopathic individual named Ishii Shiro was the "father" of Japanese biological warfare in the sense that he pushed the government to create the program and when that occurred he was the man who led the program. His background was in Army medicine, and ironically he was an expert on clean water. But his expertise was misspent and he used his understanding of water purification for the exact opposite—he developed projects to contaminate Chinese water supply with typhus, paratyphus, and cholera-causing bacteria (Harris 2002).

Why were the Japanese so willing to develop a BW Program and in doing so commit unspeakable atrocities? This important question has been discussed, but still is an unsolved mystery. Some attribute the behavior of the Japanese to racism directed at Western peoples. There seems to be some reality in this explanation; in fact in Manchuria the Japanese referred to humans used in experiments as "logs," which indicates that they were regarded as alive but non-human (Harris 2002). According to the late Col. Robert Traub [in several conversations with me while we conducted field work in northern Pakistan], the Japanese also referred to Chinese as "Manchurian monkeys" when they wrote reports and scientific articles. Cultural, religious, and racial factors seem to have interacted in providing Japanese military officers with whatever they needed in order to explain and be comfortable with their own behaviors (Dower 1986; Hanson 2002). According to Harris (2002), the prevailing line of thinking, among the Japanese leadership, was as follows: "The superior Japanese race would benefit immeasurably from the sacrifices of people who were, in general, of little value to mankind. The world would be a better place to live…they reasoned, without so many sub-humans wasting the planet's limited resources."

One important consequence of the Japanese BW Program was that the United States very quickly established its own Program at Camp (later "Fort") Detrick in Maryland. Research was conducted there and at the home universities of funded bacteriologists. Officially, in 1943, Detrick was named the U.S. Army Biological Warfare Laboratories (USBWL). Angst prevailed among interested parties in the United States, with some individuals believing that the United States should quickly develop and then use BW weapons against the Japanese. This thinking was typical of most of the people who formally or informally visited with President Roosevelt. One notable exception was Admiral William Leahy, President Roosevelt's Military Chief of Staff. Admiral Leahy said that using BW against Japan "would violate every Christian ethic I have ever heard of and all the known laws of war" (Leahy 1950).

One thing about BW seems certain. People involved in bioweapons research and development, or in power positions about R&D policies, feel that it is important to explain themselves. The Japanese started with their supposed racial superiority and built toward an explanation that exonerated them as barbarians—in a sense they were doing the "right" thing and, furthermore, the sub-humans murdered through crude experimentation were given an opportunity for redemption (Dower 1986). The belief that non-Japanese were a "lower life form" and expendable to a higher cause thus gave comfort to any Japanese that had second thoughts about vivisection and other atrocities perpetuated on Chinese civilians, Russian soldiers, and American POW's. For its part, the United States also pursued creation of an awful, cartoonish characterization of Japanese that essentially painted them as ape-like or other non-human (Dower 1986).

Although secretive, the main goals of Unit 731 were known to American intelligence by the early 1940's. As data were collected from various sources, concern about the supposed successes of the Japanese use of plague bacterium to attack rural Manchurian villages had reached the White House and President

Franklin D. Roosevelt personally by sometime in early months of 1942, if not before. There was a growing concern about the Japanese BW Program within a very few highly selective offices of the United States Government. The White House was one such place, and that level of concern was reinforced when the President signaled his support for R&D that would both develop a United States offensive program and prepare the military and nation for possible Japanese attacks. Organization was one of several huge management and administrative challenges. What agency would handle strategic planning and who could do the biological and disease-associated research and laboratory work to create an American program? How would intelligence fit into the puzzle and how would information be filed and kept accessible? The complexity was real. To understand this basic fact, it might be necessary to remind ourselves that personal computers and desk-top data storage simply did not exist in the early 1940's. From a leadership point-of-view, the concern about a Japanese bacteriological attack and response to the situation was the second major unanticipated challenge within three years. The first such challenge was outlined in a letter written to President Roosevelt by Albert Einstein in 1939. It was this famous letter in which Einstein both warned and alerted the President about the potential of atomic weapons and recommended that the United States start stockpiling uranium. So, this instance did the same thing in regard to United States response to Japanese biological weapons.

One response on the part of the White House was to appoint in 1942 a War Reserve Service Committee (the WRS) Chaired by George W. Merck, whose family had created and operated Merck Pharmaceutical Company. In October 1942, at the request of Secretary of War Henry Stimson, the United States National Academy of Sciences created an ABC Committee of scientific consultants, mostly representing the academic field of bacteriology in a variety of American universities. At the time, the term "bacteriology" covered bacteria, which were too small to be investigated easily with optical microscopy. Viruses still were very poorly known, although this would change dramatically in the next decade.

The "ABC" designation was intentional; it held no actual meaning and presumably helped obscure the secret purpose of the organization, which was to rapidly develop the fledgling United States BW Program. Meanwhile, the United States Army medical command deployed a field team to the southern border of China. The thinking behind this strategic deployment is obvious as soon as one notices the presence of Colonel Robert Traub, an American expert on fleas and, ultimately, plague. After the war, Traub also was nominated for a Nobel Prize in Medicine for his wartime work on typhus as a member of a team led by Theodore Woodward (also a nominee). Although neither Traub nor Woodward (or other members of their team) was awarded a Nobel Prize in Medicine, Traub continued to conduct field research in Asia. In conducting research on zoonotic disease, he always included a mammalogist.

ROLLIN H. BAKER—MAMMALOGIST AND NAVAL OFFICER

The involvement of the United States Army in medical research relevant to BW had been underway for many years prior to WWII. If we were to select a starting point it probably would be the battle with mosquito-borne yellow fever virus, which was encountered during the construction of the Panama Canal, begun by France in 1881. But France withdrew from the complex engineering project on account of the loss of personnel. The main culprit was yellow fever, which could be deadly and was not yet understood other than in terms of its basic pathways. The United States took up the challenge in 1904 and opened the Canal to ship traffic about a decade later. Interest in Yellow Fever and other zoonotic diseases outlived the construction phase, and a large number of biologists of various stripes worked in the Canal Zone conducting research on zoonotic disease and host species. All of this work gave the U. S. Army the edge in experience relevant to creating a response to the Japanese BW Program when the Second World War began for the United States.

The Army medical experience with yellow fever in Panama was applied to the creation and deployment to the Pacific of a Naval Medical Research Unit (NAM-

RU-2). The naval personnel in this adventure included mammalogists Rollin H. Baker and David M. Johnson. Rollin Baker had enlisted in 1942 with a partially completed graduate experience at the University of Texas (Austin). He was assigned to an ancient destroyer (commissioned in 1919), the USS Belknap (DD-251), which operated on antisubmarine patrol in the North Atlantic. But unbeknownst to Baker, NAMRU-2 was being organized while he was chasing and destroying German U-boats. By luck alone, the Belknap stopped at Norfolk and Baker went ashore to visit Remington Kellogg at the Smithsonian. On this particular occasion (March or April 1944), Kellogg surprised Baker by requesting that he visit with Commander James Shapiro at the Navy Bureau of Medicine located at the Naval Observatory, which was about a forty minute walk from the U.S. Museum of Natural History.

"I walked into Jim Shapiro's office. He looks at me and grins and said, 'are you a mammalogist?' And I said, yes sir. That is what I am. He said, 'would you like to get out of sea duty and become a mammalogist for U.S. Naval Medical Research Unit number two?' And he explained what they're going to do out [in] the Pacific. Oh my Lord…the right place at the right time. So Shapiro said, 'okay,' and he got a lot of information from me. He said, 'go on back to your ship…go on back to your duty [station] and you'll get orders.' I got on back to the ship and I told them what happened. They didn't believe me, [and] said, 'you can't get ordered from sea duty. You've been trained to be a fighting officer type of person. How in the Sam Hill can you be transferred to a less priority position?' Which [authority] BuMed would have, you know what I mean? Everybody wanted line officers and I was qualified not only [for] deck duty, but qualified [also] for destroyers underway. I was in line to be exec [XO, the Executive Officer] you know, Captain or something if I had stayed around long enough. Anyway, in June I received these orders to report to the Rockefeller Institute for Medical Research…" (Herman 1994).

As the NAMRU-2 team assembled at Rockefeller, they had other surprises too. According to Baker, he was handed cash ($25,000) to spend on equipment and supplies for their Pacific Island venture. He always claimed to me (Phillips) that he never heard any mention of the Japanese BW Program, although in retrospect it was obvious that he had been selected for a special team and very specific job. Still, very few people knew what was coming. The Japanese Program had been more of a curiosity in the opening year of the war; now it was a tangible threat. The causality rate could be extreme if one assumed that the United States ultimately would have to lead the Allies in an invasion of the Japanese "Home Islands." Insofar as BW was concerned, an obvious question was: Under what circumstances would their government authorize the use of BW? Intelligence agents, including personnel in NAMRU-2, interviewed Japanese prisoners, especially those in the military medical units—typically the only ones to survive and surrender to American forces (Harris 2002). Overall, there was general agreement that Japan was preparing, or at least likely to prepare, for bacterial warfare against the U.S. troops that were expected to invade Okinawa. Rollin Baker and many of his NAMRU-2 colleagues were on Guam, studying the species inhabiting the island and, in the case of rodents, the species associated with scrub typhus, a mite-borne zoonotic disease that at that time seemed to have potential as a weapon (Herman 1994).

In 1945, Rollin Baker's thinking was that "the idea of the NAMRU-2 group going in there [as part of the invasion force landing on Okinawa] was to determine how feasible [it was] for a research team to go in during invasion times and carry on preventive medicine, research studies [on] some of the conditions that the troops were finding and their health problems and how to alleviate them….This was part of the reason that the Okinawa invasion was made. Of course the pre-spraying was done," he added, cryptically, in reference to a project to prepare the battlefield by heavy DDT spraying before D-day and the landing. What Rollin Baker left unsaid was the real reason why the pre-invasion spraying was done.

Captain Thomas Rivers, the first commander of NAMRU-2, worked out a battle plan for Okinawa. The first idea was based on the high expectation that the Japanese would likely use bacteriological warfare—specifically their ceramic shell bomblets filled with plague-infected rodent fleas. The NAMRU-2 intention was to alter the battlefield by spraying DDT on the landing beaches, fields, and other logical targets where the Japanese were likely to bomb with plague. Lt. John Maple was assigned this task—the aircraft flew low and slow and sprayed DDT. The spraying

was effective, but unfortunately Lt. Maple was killed in action. The second idea was that it would be critical to rapidly recognize that a BW attack had occurred. For this reason, Captain Thomas Rivers decided to send ashore a team that could directly study any unanticipated zoonotic diseases, and perform autopsies on troops who died from illness rather than from combat during the invasion. Thus, a team led by Dr. Richard Shope, a Naval Officer and famous virologist who was a member of the original NAMRU-2 team, went ashore on D-day + 6. Shope was particularly interested in Japanese encephalitis b virus, which was thought to be another zoonotic agent that could have been weaponized, but was not.

In this interview (conducted by the U.S. Navy), Rollin Baker was asked if he had gone into Okinawa with the landing force (Herman 1994). His answer is enlightening. "A young man named Merle Markley, who had a Master's degree in wildlife biology from Oregon State University, was a first-class Pharmacist's Mate that my lab picked out of the fleet [when NAMRU-2 was formed]. They wouldn't let me go. They had enough officers, I guess, coming, so [Markley] went and represented our lab." Markley reached Okinawa on D-day plus 6, and quickly started putting out mouse and rat traps to see which species of small mammals were there and what was their habitat association(s). Thanks to previous research on scrub typhus the NAMRU mammalogists had some ideas of what to look for in a new locality, be it island or mainland. As it turned out, Markley was briefly famous because Japanese troops watched him set his trap line and then launched mortar rounds where they had seen him doing something in the jungle. As Rollin Baker put it, "the great stunt was that [Markley] had a bunch of traps down in this little valley, and the Japs [mortared] it during the night, and [Markley] claims he lost all of his traps" (Baker 1994; Herman 1994).

After Okinawa, Rollin Baker expressed sarcastic, tongue-in-cheek disappointment that the war ended so abruptly in August 1945. He had hoped to finish some of his field projects and was pleased when he was asked to stay on and conduct research for almost a year post-conflict. He and his shipmate scientists were reassigned to Rockefeller.

The Japanese Plan for a BW Sneak Attack

As it turned out, separate from the defense of Okinawa, the Japanese Command also had planned a BW attack on California for 22 September 1945. It was a truly exotic mission that relied on a combination of secret technologies and combinations of equipment and techniques. The Japanese had created a huge Sentoku I-400 Class submarine in order to accomplish complex, combined missions. Aside from its size, speed, and diving, this submarine essentially was a submersible aircraft carrier. With a keel length of slightly more than 400 feet, the Sentoku submersibles were 60% larger than the largest contemporaneous United States submarine. In fact, the size of the boat was similar to modern nuclear-powered ballistic missile-carrying submarines. The typical WWII American submarine was diminutive alongside the I-400.

The I-400 had four diesel motors that generated 7,000 hp for surface running and electric motors that generated 2,400 hp for submerged running. With these power plants, the submarine could cruise at 18.7 knots with a range of 37,500 nautical miles (while averaging 14 knots).

The Sentoku carried three Aichi M6A1 Seiran seaplanes, along with parts for a fourth aircraft. These amphibian aircraft were carried with their wings rotated 90° (leading edge down, trailing edge up) and then folded back along the fuselage sides. When the giant submersible was underway, the three airplanes and maintenance gear were carried in a 115-foot cylindrical hangar. This capsule-like hangar was positioned slightly to starboard of the mid-line and attached to the deck of the submarine. A long, upswept catapult occupied the sub's foredeck.

The planned attack on the United States was called *Operation Cherry Blossoms at Night.* The idea was to terrorize American citizens living along the west coast. Any guess about what would have happened if the Japanese had brought their giant submarines (three were expected to participate) to within range for the

nine seaplanes and their cargos of plague-infected fleas to attack San Diego? The timing (just after the United States invasion of Okinawa) and intent (terrorize and kill thousands of civilians) might be interpreted as a fore-planned response to the expected United States invasion of Japanese property. The United States focus was on blunting a Japanese attack with BW on Okinawa, whereas the Japanese wished to hit the U.S. mainland with plague-infected fleas in retaliation for the allied invasion of Okinawa. In any case, as it turned out the timing was off and Japan surrendered after two horrific atomic explosions. With the surrender of Japan, the United States took possession of the giant submarines, and rather than allow the Russians access to the subs, they were secretly scuttled by the United States Navy.

MAMMALOGY NEEDED

The surrender of Japan marked the beginning of a new and dangerous era. What should have been an ending to the worst of human behavior conducted on a massive scale was instead an open door to even worse behavior. Although the United States was clearly a major winner of the war, within a few short years the country slipped into a new conflict. And this time it was on the Korean Peninsula. Surprisingly, although the government responded perfectly during the Second World War, it failed to develop and implement a comprehensive plan for reconstruction and redirection of talent into reconstruction after the war. This reactive approach led to some embarrassing actions on the part of the United States. For instance, on at least one occasion an attempt was made to secure secretly the slides and preserved tissues from human beings used in Japanese BW experiments. Moreover, ultimately the United States ignored its own legal traditions and failed to prosecute the Japanese involved in the atrocities of Unit 731. Even the sociopathic Ishii Shiro was protected from legal action, presumably in exchange for information that he had accumulated.

Conflict on the peninsula was significant by every measure. For our purposes it was an outbreak of an unknown disease in troops that became a pivotal moment. The United States Marines had barely survived the huge counterattack by Chinese troops who streamed into Korea from the north during the fall and bitterly cold winter of 1950 (Sides 2018). In June 1951 there was an outbreak of an unknown, but serious, disease. A total of 55 cases were reported at the same time in a single regiment of United States Army infantry engaged in combat with North Korean and Chinese troops north of Seoul. Two possibilities were considered: 1) an unknown but naturally occurring zoonotic disease had been encountered; 2) an unknown zoonotic disease had been introduced to the battlefield intentionally by the enemy forces. In some ways the practical, medical response had to be the same, so these two options were not mutually exclusive (Phillips et al. 2009). Politically, however, the response had to be distinct, and there was a great deal of excitement and concern about it. The main response was to form an Army team that included a mammalogist, Lt. J. K. Jones, Jr., and other types of field-oriented biologists all of whom worked on the technical (non-political) issues. Politically, the North Koreans and Chinese announced that the United States, backed by the Japanese BW personnel left over from the previous war, had introduced BW to the Korean conflict. Some Americans and Canadians accepted that notion and were happy to blame the United States (Endicott and Hagerman 1998; Phillips et al. 2009). Scientifically, it ultimately was easy to prove that the illness was natural and not due to an intentional release of a previously unknown strain of hemorrhagic fever virus (Lee et al. 1978; Johnson 2004). The mammalogy piece of the puzzle involved the discovery that the virus was associated with a particular species of wild mouse, genus *Apodemus*. The political piece of the story has not been resolved; presumably it contributes to the bellicose behavior of North Korean (DPRK) leader Kim Jong Un and might explain the source of his disproportionate fear of the United States.

The Soviet Union emerged from the Korean conflict with a growing BW Program. When the Soviet Union collapsed in 1991, the government and the economy of the huge, cobbled-together nation state were left in disarray. The significance of this tumultuous outcome was multifaceted—it meant one thing to political observers and quite another to scientists. But

most important of all, there was the danger to United States national security posed by thousands of suddenly unemployed weapons experts. This is where Anne Harrington and Andrew Weber took on significant roles in building contacts with former weapons experts and developing redirection of former weapons personnel into civilian pathways. Their original thinking included the creation of grant opportunities and through diplomacy the creation of two unique international organizations—the International Science and Technology Center (ISTC) and the Science and Technology Center in Ukraine (STCU). Although these twin organizations had similar charters and purposes, the existence of two—one headquartered in Moscow and one headquartered in Kiev—reflects quite well the political divide between these two countries after the dissolution of several of the old republics. The science centers functioned in both cases as a mechanism for funding former weapons personnel with the idea of keeping them associated with their original laboratories.

IN CONCLUSION

So, what have we learned since the 1930's? There is good news and bad news, which often is the case as we learn lessons. The good news is that mammalogists have played an important role from 1938 up until now. The contributions to field technologies have kept pace with the broader laboratory-based technologies. Moreover, there are plenty of examples to support the idea that mammalogists continue to learn the secrets of fieldwork, which is the key to their role(s) in fighting bioterrorism and biological warfare.

The bad news is that there is no reason to expect either bioterrorism or biological warfare to disappear on its own accord. In fact it seems as though the situation is becoming more and more dangerous. If we were to look for culprits we almost certainly would point to the Russian Federation and their current client-state, Syria. The willingness of the Russians to continue to support the Syrian government despite its use of chemical weapons is truly appalling. There is no reason to doubt that Syria would use biological weapons if they had them in functioning order. ISIS remains an existential threat as a transnational terror organization. There also seems to be no doubt that they used chemical weapons in the vicinity of Mosul, Iraq. Their behavior as terrorists means that they would be more likely than not to use whatever weapons they obtained.

There are several challenges that confront the United States and our allies. The most important of these is to create an appropriate, agile, decision-making tree. The United States is too large and the government is too slow-moving to respond quickly to an attack either from an established nation or transnational bioterrorists. It is ironic that a country like the United States, blessed as it is with scientific and technical talent, is also vulnerable to a biological attack. It would be highly appropriate for a new agency, or team of persons, to compose an interagency unit to look at the dilemma created by the size and a kind of paralysis that threatens to overwhelm our ability to respond rapidly to a crisis caused by bioweapons or bioterrorism.

It would be helpful if the United States had an agency that focused on BW and preparations for a national response to an attack. Such an agency might be located within the framework of the State Department, which could provide existing experience in science diplomacy and expertise in international science, including non-proliferation specialists.

There are several roles for mammalogists, especially people with training in fieldwork, both traditional and modern. Clearly, there are plenty of basic questions about the interplay of mammals as reservoirs and the factors involved with the zoonotic agents associated with them. Hopefully mammalogists will assert themselves into the process and show what can be done and how it relates to basic microbiology and virology, which are important areas of knowledge but not independent of mammalogy.

ACKNOWLEDGMENTS

I am pleased to dedicate this paper to two close friends, both of whom have passed: Robert J. Baker and Jim Mollen. Robert Baker of course is the individual in whose honor this particular volume of papers, essays, and purely scientific articles was published. I knew Robert for at least 53 years, and maybe a few more. We shared some funding and lots of interests, particularly in regard to mammalian evolution. We both were fascinated by the technology that might be deployed for fieldwork. While Robert was developing the techniques for field preparation of chromosomes, including G- and C-banding, I was developing the technology for transmission electron microscopy of field-fixed tissues. I think that more than anything else, our respective interests reflect our tendency to try to work at the cutting edge. Some of the most enjoyment that we had working together came from our joint research in Ecuador. I described some aspects of this important international activity in my autobiography in *Going Afield* (Phillips 2005). In 2008, I invited Robert to join my team conducting field mammalogy in Kyrgyzstan, which previously had been a republic in the Soviet Union and had been part of the old BW Program. In any case, Robert and I shared a lot, but my favorite time still is our October walk at Chernobyl, which I described in my autobiography (Phillips 2005).

Jim Mollen is not a name that will ring a bell with readers who are mammalogists. But it would be disgraceful for me to write about Iraq without honoring Jim. Jim served as a CPA Advisor on Higher Education in Iraq from shortly after the invasion (2003) until late summer of 2004 (Agresto 2007). Jim and me, and Alex Dehgan, were the only three civilians who had our own vehicles and regularly traveled on our own in and out of the green zone. It was the nature of our respective jobs that took us into so dangerous a way to spend time. It caught up with Jim. He was ambushed in Baghdad and his death left his many friends with a profound sense of loss. Jim was the real McCoy—a true American hero—a political (President George W. Bush) appointee at the Department of State who volunteered to serve in Iraq.

Numerous other talented people worked closely with me and the following persons in particular have earned my deep appreciation of their skills, sense of responsibility, their companionship and frequently their bravery. These people are: A. Dehgan, A. M. Harrington, A. Weber, R. Kadlec, D. Kenagy, B. Patton, B. Cobb, C. Mentz, I. Hay, E. Howell, P. Smallwood, D. Nagel, R. Jarvis, D. Elliott, B. Briggs, G. Atkinson, H. Franke, R. Hewson, B. Rodgers, and R. K. Chesser.

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LITERATURE CITED

Agresto, J. 2007. Mugged by reality: the liberation of Iraq and the failure of good intentions. Encounter Books, New York.

Alibek, K., and S. Handelman. 1999. Biohazard: the chilling true story of the largest covert biological weapons program in the world—told from the inside by the man who ran it. Random House, New York.

Ammash, H. S. 2002. Toxic pollution, the Gulf War, and sanctions. Pp. 205–214 in Iraq under siege: the deadly impact of sanctions and war (A. Arnove, ed.). South End Press, Cambridge, Massachusetts.

Baker, R. H. 1994. Recollections of NAMRU-2: 1944–1946. Navy Medicine 85:18–22.

Bremer, L. P., and M. McConnell. 2006. My year in Iraq: the struggle to build a future of hope. Simon & Schuster, New York.

Chandrasekaran, R. 2006. Imperial life in the Emerald City: inside Iraq's Green Zone. Alfred A. Knopf, New York.

Chua, K. B. 2003. A novel approach to collecting samples from fruit bats for isolation of infectious agents. Microbes and Infection 5:487–490.

Dehgan, A. 2019. The snow leopard project: and other adventures in warzone conservation. Public Affairs, New York.

Diamond, L. 2005. Squandered victory. The American occupation and the bungled effort to bring democracy to Iraq. Times Books, Henry Holt and Company, New York.

Dower, J. W. 1986. War without mercy: race and power in the Pacific war. Pantheon Books, New York.

Endicott, S. L., and E. Hagerman. 1998. he United States and biological warfare: secrets from the early Cold War and Korea. Indiana University Press, Bloomington.

Feith, D. J. 2008. War and decision: inside the Pentagon at the dawn of the war on terrorism. Harper Collins, New York.

Findley, J. S. 2005. Mammalogical reminiscences. Pp. 171–183 in Going afield (C. J. Phillips and C. Jones, eds.). Museum of Texas Tech University.

Hall, E. R., and K. R. Kelson. 1959. The mammals of North America. The Ronald Press Company, New York.

Hanson, V. D. 2002. Carnage and culture. Anchor Books, New York.

Harris, S. H. 2002. Factories of death: Japanese biological warfare, 1932–1945, and the American cover-up. Routledge, New York, New York.

Herman, J. 1994. This is a taped interview (July 18, 1994) with Rollin H. Baker. It is titled: Interview with ornithologist and zoologist Dr. Rollin H. Baker (November 11, 1916–November 12, 2007) who was aboard the USS Belknap, then worked with NAMRU-2 in the Pacific during World War II. It was available on 9 December 2018 at https://archive.org/stream/BAKERRollin/Final.

Hilleman, M. R. 2002. Overview: cause and prevention in biowarfare and bioterrorism. Vaccine 20:3055–3067.

Hoffman, D. E. 2009. The dead hand: the untold story of the Cold War arms race and its dangerous legacy. Doubleday, New York.

Johnson, K. M. 2004. The discovery of Hantaan Virus: comparative biology and serendipity in a world at war. Journal of Infectious Diseases 190:1708–1710.

Leahy, W. D. 1950. I was there: the personal story of the Chief of Staff to Presidents Roosevelt and Truman based on his notes and diaries made at the time. Whittlesey House, New York.

Lee, H. W., P. W. Lee, and K. M. Johnson. 1978. Isolation of the etiologic agent of Korean hemorrhagic fever. Journal of Infectious Diseases 137:298–308.

Li, W., Z. Shi, M. Yu, W. Ren, C. Smith, J. H. Epstein, H. Wang, G. Crameri, Z. Hu, H. Zhang, J. Zhang, J. McEachern, H. Field, P. Daszak, B. T. Eaton, S. Zhang, and L. Wang. 2005. Bats are natural reservoirs of SARS-like coronaviruses. Science 310:676–679.

Miller J., S. Engelberg, and W. J. Broad. 2002. Germs: biological weapons and America's secret war. Simon & Schuster, New York.

Phillips, C. J. 2004. Capturing Saddam: Iraq, December 2003. Virginia Quarterly Review 80(20):1–10.

Phillips, C. J. 2005. Ken Ward in the jungle: making scientific sense of field work. Pp. 85–128 in Going afield (C. J. Phillips and C. Jones, eds.). Museum of Texas Tech University, Lubbock.

Phillips, C. J., A. M. Harrington, T. L. Yates, G. L. Simpson, and R. J. Baker. 2009. Global disease surveillance, emergent disease preparedness, and national security. Museum of Texas Tech University, Lubbock. [out of print but available in PDF format at www.nsrl.ttu.edu].

Rice, C. 2011. No higher honor: a memoir of my years in Washington. Crown Publishers, New York.

Rotella, S. 2018. John Bolton skewed intelligence people who worked with him. ProPublica, 30 March, last accessed at https://www.propublica.org/article/john-bolton-national-security-adviser-intelligence on 20 May 2019.

Schmidly, D. J. 2005. Where have you gone Vernon Bailey: the laments of a mammalogist. Pp. 37–53 in Going afield (C. J. Phillips and C. Jones, eds.). Museum of Texas Tech University, Lubbock.

Sides, H. 2018. On desperate ground: the marines at the reservoir, the Korean War's greatest battle. Doubleday, New York, New York.

Smallwood, P. D., and W. T. Liimatainen. 2011. Securing WMD expertise: lessons learned from Iraq. Arms Control Association, presented on 11 July 2011 and available through the Arms Control Association (https://www.armscontrol.org) as of December 2018.

Stiglitz, J. E., and L. J. Bilmes. 2008. The three trillion dollar war: the true cost of the Iraq conflict. W. W. Norton, New York.

Tenet, G. 2007. At the center of the storm: my years at the CIA. Harper Collins, New York.

Zaak, A. 2018. Are you brown name in a black list? Mesopotamian memoir. Outskirts Press, Denver, Colorado.

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Use of Mitochondrial 12S rRNA Gene Sequencing in a Human Identification Laboratory for Species Determination of Compromised Skeletal Remains

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Abstract

In the field of human identification, remains may be skeletonized and highly fragmented. This damage to remains often precludes identification as to the species of origin. PCR-based amplification of a portion of the mitochondrial 12S rRNA gene is a quick, inexpensive method for determination of a species. This chapter describes the development of such an assay at the Armed Forces Medical Examiner System–Armed Forces DNA Identification Laboratory (AFMES–AFDIL) and the subsequent implementation of the protocol into regular casework. The species identified from 605 samples tested are described, along with the impact of this protocol on the streamlining of testing osseous materials in a human identification laboratory.

Key words: 12S rRNA, BLAST, DNA, human identification, protocol development, skeletal remains, species identification

Introduction

In modern mass fatality events, the remains presented for analysis typically are intact, and are, at the least, visually identifiable as human. Remains from past events, however, can be fully skeletonized and may be found in fragments or in a highly damaged state in which the species of origin is not readily apparent. Fully skeletonized remains may be subjected to fragmentation post-mortem from human impact (e.g., farming, industrial activities, road building) or simply age. Additionally, remains may undergo fragmentation at time of death, particularly in events that involve plane crashes or explosions. This can be particularly true in times of conflict, when high-energy events are more common.

The specimens submitted to the Armed Forces Medical Examiner System–Armed Forces DNA Identification Laboratory (AFMES–AFDIL, a.k.a. AFDIL) by the Defense POW/MIA Accounting Agency (DPAA) include remains excavated from decades-old events world-wide. Some locations, particularly those in Southeast Asia, experience an annual variability in soil temperature and moisture combined with high soil acidity that rapidly erodes any skeletal fragments. In many cases, the only conclusion that can be drawn from osteological analysis of these remains is that they are consistent with, but not exclusive to, human in origin.

In 2007, AFDIL implemented into casework a complete demineralization protocol for the extraction of DNA from osseous materials (Loreille et al. 2007). This protocol involves a complete dissolution of the skeletal materials, and a more efficient extraction of DNA than presented in Edson, et al. (2004). This protocol allowed for the reduction in size of samples submitted to AFDIL by the DPAA Laboratory. Prior to 2007, the requested size of the element sampled was 5.0 g or greater, as the required input for DNA extraction was 2.0–2.5 g of material (Edson et al. 2004). With complete demineralization, coupled with an organic purification, the input size was reduced to 0.2 g, allowing the DPAA to re-evaluate cases previously thought to be untestable due to the small size of fragments recovered. Excluding teeth, the average size of a sample submitted to AFDIL decreased from 7.66 g to 4.11 g with the implementation of complete demineralization. The trend has continued with implementation of an inorganic purification coupled with the

complete demineralization (Loreille et al. 2010; Edson and McMahon 2016), to an average sample size of 3.59 g. Therefore the size of the samples submitted to the AFDIL decreased by approximately 50% and, although DPAA anthropologists examine the remains prior to submission, accurate determination of species origin is often precluded by the small and severely compromised condition of the elements.

Since the implementation of the demineralization protocol, AFDIL successfully has reported mtDNA sequence data for 86% of the more than 9,000 samples tested. When reportable sequence data cannot be produced, one possible explanation is that the endogenous DNA is either too fragmented or too limited in quantity to be recovered with currently validated assays. Another possibility is that the fragments are not of human origin. When preliminary attempts to obtain mtDNA are unsuccessful, modifications are made to the amplification reaction to accommodate DNA fragmentation, inhibition, and low quantities of DNA. Additionally, standard practice at DPAA has been, when possible, to resample those skeletal elements that did not yield reportable sequence data so that AFDIL can attempt to successfully obtain a mtDNA profile. These processes are time-consuming and costly, and may also continue to be unsuccessful if the specimen is not of human origin. To prevent needless DNA testing, and to provide critical information to DPAA, it is important to determine if the failure to produce conclusive data is due purely to sample degradation, and thus low quantity/quality DNA, or is instead due to the non-human origin of the skeletal element. This is extremely vital in cases for which small fragments of uncertain origin are the only biological remains recovered for a particular incident.

Human identification efforts may not seem to have that much in common with wildlife biology; however, the DNA analysis tools that can be used are very similar. Although advanced methods exist for precise species identification of biological materials (e.g., melt curve analysis: Kitpipit et al. 2016; cytochrome-*b*: Tobe and Linacre 2010; Ciavaglia et al. 2015; Linacre and Lee 2016), AFDIL uses the amplification of the 12S ribosomal (rRNA) gene as a rapid screen to determine if smaller skeletal elements are human in origin. In 2005, primers that amplified the cytochrome-*b* gene on the mitochondrial genome were evaluated for use in casework (Freeman, internal validation). Although cytochrome-*b* has been found to be successful for determination in forensics settings (Branicki et al. 2003), the size of the amplicon (300 bp) is too large for usage with degraded skeletal remains, leading to the evaluation of the 12S rRNA gene. Polymerase chain reaction (PCR) primers developed by Balitzki-Korte et al. (2005) target this gene within mtDNA. These primers bind to a small, highly conserved region across a range of species and amplify a short (146 bp), yet variable portion of the mitochondrial 12S rRNA gene, allowing for the development of a species identification assay tailored for highly compromised remains. The size of this amplicon is comparable to the primer sets commonly used on the most degraded DNA that target small fragments (typically 150 bp or less) of the human mtDNA control region (Gabriel et al. 2001). Although small, this portion of the 12S rRNA gene has been shown to provide information sufficient to differentiate taxa at the species level (Balitzki-Korte et al. 2005; Melton and Holland 2007). The following text provides a description of the protocol development and a summary of the usage of the technique in casework.

PROTOCOL DEVELOPMENT

Morphological and histological determination of human versus non-human origin.—Prior to DNA testing, anthropologists assess human versus non-human origin of skeletal remains based on macro- and microscopic morphological characteristics. Larger elements generally can be characterized as either human or non-human based upon morphological features. However, when small bone fragments are encountered, size may preclude a human or non-human designation based upon bone morphology. In these cases, a thin section cut from the fragment may be examined microscopically, and qualitative analyses, which include determination of different types of micromorphology such as plexiform bone or osteon banding, are used to determine whether or not the bone is consistent with non-human origin (Mulhern and Ubelaker 2001; Benedix 2004; Hillier and Bell 2007). Although the presence of plexiform bone or osteon banding defini-

tively classifies a bone as non-human, the absence of this bone type does not automatically indicate human origin. According to DPAA procedures utilized during this study, histological analysis results in a judgment of either "match to non-human" or "inconclusive." If the osseous material cannot be identified conclusively as non-human based upon microscopic analyses or the sample is not large enough to examine its histology, a fragment is submitted to AFDIL for 12S mtDNA testing.

Extraction of DNA from the bone.—Upon arrival at AFDIL from DPAA, osseous fragments are cleaned using a Dremel® tool (Dremel, Racine, Wisconsin), washed with sterile deionized water (diH_2O) and 100% (v/v) ethanol (Pharmco-Aaper, Brookfield, Connecticut), and allowed to air dry. After cleaning, the osseous sample is sectioned for pulverization. Samples submitted to AFDIL are typically 2.0–5.0 g, but the desired input for the extraction protocol is 0.25–0.5 g. Pulverization is performed using a Waring blender with a professional base (MC2 cup; Waring, Stamfield, Connecticut).

Samples in this study used two different extraction protocols: complete demineralization coupled with an organic purification, and complete demineralization coupled with an inorganic purification (Edson and McMahon 2016; Edson 2019). For both protocols, the pulverized bone material is incubated overnight at 56°C using an extraction buffer (0.5 M EDTA, pH 8.0; 1% *N*-Lauroylsarcosine) and Proteinase K (200 mg/mL; Ambion™, Thermo Fisher, Gaithersburg, Maryland). Purification follows with either an organic purification using 25:24:1 phenol:chloroform:isoamyl alcohol (Sigma-Aldrich, St. Louis, MO) followed by a wash with *n*-Butanol (Sigma-Aldrich) or an inorganic purification with the QIAquick PCR purification kit (QIAGEN, Hilden, Germany). Samples were concentrated using Amicon Ultra-4/30K centrifugal filter units (Millipore, Billerica, Massachusetts) and the extracted DNA is brought to a final volume of 100–200 µL with TE (10 mM Tris, 0.1 M EDTA; pH 8.5).

12S amplification, sequencing, and data analysis.—PCR was conducted using primers that target a 146-bp region of the 12S rRNA gene described in Balitzki-Korte et al. (2005). Amplification of 1–3 µL DNA extract was performed in a 50 µL reaction containing 10 units AmpliTaq® Gold DNA polymerase (Life Technologies, Gaithersburg, Maryland); 1X GeneAmp® PCR Buffer I (Life Technologies); 200 µM dNTPs (Life Technologies); and 0.4 µM of each primer. Non-acetylated bovine serum albumin (BSA; 0.025 mg/mL, Sigma-Aldrich, St. Louis, MO) was eliminated from the amplification after it was found that there was cross-reactivity with the primers, giving false results of *Bos taurus* DNA. In accordance with in-house quality control standards, appropriate extraction and amplification controls were included. Thermal cycling for both amplification and sequencing reactions was carried out in a GeneAmp® 9700 (Life Technologies) using the 9600 emulation mode. The optimized cycling conditions for amplification were 96˚C for ten minutes followed by 38 cycles of 94˚C for 30 seconds, 50˚C for 30 seconds, and 72˚C for one minute with a final extension step of 72˚C for seven minutes. The PCR products were confirmed using a 2% agarose gel stained with Ethidium bromide (5 mg/mL). If a positive result was obtained, purification was performed using 1.5 µL Exo-SAP-IT® (Affymetrix, Santa Clara, California) and 17.5 µL dilution buffer (50 mM Tris; pH 8.0).

Cycle sequencing was conducted in 20 µL reactions with 3.6 µL BigDye® Terminator v1.1 Cycle Sequencing Kit (Life Technologies), 0.4 µL dGTP BigDye® Terminator v1.0 (Life Technologies), 4 µL dilution buffer (400 mM Tris, 10 mM $MgCl_2$; pH 9.0), and 0.5 µM of sequencing primer. Both amplification primers were utilized to generate sequence data from both strands for each sample. Input volume of purified product was either 1 µL or 7 µL depending on band intensity observed on the agarose gel. Sequencing products were purified with Performa® DTR V3 Short or Ultra 96-Well Plates (Edge Biosystems, Gaithersburg, Maryland) and dried down in an evaporator/concentrator centrifuge. Samples were resuspended with 10 µL Hi-Di™ Formamide (Life Technologies) prior to separation on an Applied Biosystems 3130*xl* and/or 3500 Genetic Analyzer (Life Technologies).

Sequences were aligned using Sequencher™ version 4.1 or higher (GeneCodes, Ann Arbor, Michigan) and a consensus sequence of approximately 109 bases, depending on species origin, was generated for each sample. Once the 12S consensus sequence was established, the Basic Local Alignment and Search

Tool (BLAST) available online (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al. 1990; Zhang et al. 2000) was used to search the National Center of Biotechnology Information (NCBI) database. The consensus sequence string, which excludes the primers, was entered into the nucleotide-nucleotide BLAST (BLASTN) program and searched against the "Nucleotide collection (nr/nt)" (NCBI Nucleotide) database using the default search parameters. The BLASTN search results were then reviewed to assess sample origin. For each sequence returned from the database search, BLAST generates statistics that reflect the similarly of the alignment (bit score, or "Max Score"), the statistical significance (Expect value, or "E-value") of the database hit, as well as the percentage of identical ("Max Ident") and covered ("Query Coverage") bases (Madden 2002). The sequence homology (reported as the "Max Ident" in BLASTN search results) was used to establish the thresholds described in Table 1. A 12S sequence of 75 or more bases can be reported as "human", "non-human", or "inconclusive." All mixed sequences are reported as "inconclusive" due to the inability to definitively determine human or non-human origin, and sequences less than 75 bases are reported as "insufficient data". Samples determined to be of human origin are reported as "consistent with" or "presumed to be" human depending on the sequence homology with the *Homo sapiens* sequence (100% and ≥90%, respectively). "Non-human" sequences are reported similarly, and are classified as the common taxon (e.g. genus, family) opposed to a specific species when more than one non-human species is homologous with the searched sequence. These BLASTN interpretation guidelines, though developed primarily to distinguish between human and non-human origin, also permit more specific classifications to be made by the analyst. Further, any identification as "human" using this assay is considered with caution as exogenous modern human DNA has the potential to contaminate lower quality specimens.

Table 1. Classification guidelines implemented at AFDIL for the 12S species identification assay. The sequence homology refers to the maximum identity ("Max Ident") reported for each alignment generated by the BLASTN query. Regardless of the sequence homology, a sample is classified as "Inconclusive" if *both* human and non-human species are present in the search results or if no human or animal species are homologous with the queried sequence.

Classification	Sequence Homology	BLASTN Search Results
Human	100%	*Homo sapiens* (and *Homo neanderthalensis*) ONLY
Presumed to be Human	≥ 90%	*Homo sapiens* (and *Homo neanderthalensis*) ONLY
Non-Human	100%	One or more taxa (other than *Homo sapiens*)
Presumed to be Non-Human	≥ 90%	One or more taxa (other than *Homo sapiens*)
Inconclusive	-	*Homo sapiens* and other animal taxa
	-	Neither animal nor *Homo sapiens* (e.g., bacteria)
	< 90%	One or more taxa
	N/A	"No significant similarity found"
Insufficient Data	Mixed sequence	
	Sequence less than 75 bases	

BLIND STUDY

Thirty-eight skeletal fragments were selected by DPAA anthropologists and submitted blindly to AFDIL for species identification using the 12S assay. The samples varied by species, age, preservation, and geographic origin. Of the 38 samples submitted for this study, 37 (97%) produced sequence data resulting in an

unambiguous match in the NCBI Nucleotide database (Table 2). In 76% (29) of cases, 12S testing produced sequence data consistent with the species determination made via anthropological analysis. Eight of the remaining samples (4, 8, 16, 23, 25, 26, 29, and 33) generated reproducible 12S sequence data and were successfully associated with a species in the NCBI Nucleotide database, but were inconsistent with the species assignment made by the DPAA anthropologists. DPAA anthropologists subsequently performed more extensive physical examinations and determined that six of the eight samples were consistent with the genera, if not the species, indicated by the 12S assay. The osteological reassessment of the other two samples (8 and 23) indicated that they were consistent with the 12S determinations. Regardless, all eight samples were non-human according to both the 12S and anthropological taxonomic classifications, which is the foremost purpose of the assay.

Of particular interest were the species identification results for Sample 9. Replicate amplifications produced the same 12S sequence for which the BLASTN search produced a best match inconsistent with the geographical location of the recovery site. *Acinonyx jubatus* (Cheetah) was the top hit but there were three mismatches (94% identity) between the queried and database sequences. A subsequent BLASTN query performed less than a year later resulted in a 100% match to *Viverricula indica* (Small Indian Civet), a more likely origin based on the sample metadata and consistent with the anthropological classification. Nevertheless, the sequence data clearly indicated a non-human source and initially would have been reported as "Non-Primate" based on the previously stated guidelines (Table 1).

Sample 19 was the only blind study bone specimen that remained unresolved at the conclusion of this study. Initial 12S testing classified the bone fragment as human. However, the skeletal element was a complete right radius from a medium-sized canid and had been unequivocally identified as non-human by DPAA anthropologists. Extraction of a re-sampled fragment revealed the presence of a mixture between two species, *Homo sapiens* and *Canis lupus familiaris* (Domestic Dog). The major contributing sequence of the 12S mixture was human and assumed to be a contaminant that dominated the endogenous canid DNA. In these situations, when a 12S "inconclusive" classification results from a human:non-human mixture, case-specific details would be examined in order to establish the best course of action for the sample and to determine which component of the mixture is the contaminating species. Possible strategies include 12S testing of a new cutting of the same bone or re-extraction of the original sample if additional material is available.

USE IN CASEWORK

Since the validation of this testing protocol in 2010 and through the spring of 2018, 605 samples have been tested using the 12S assay. Of these, 254 (42%) were reported as inconclusive and 95 (16%) were determined to be human. Those shown to be human continued though the regular casework processing of mtDNA Sanger sequencing. The remaining 256 (42%) were determined to be non-human. It is most common for non-specific elements (i.e., long bones or bone fragments) to be found to be non-human (Table 3). There appears to be little or no correlation between size of the fragment submitted and whether it is non-human in origin.

Samples recovered from Southeast Asia are most commonly tested using the 12S amplification strategy and also are more likely to be non-human (Table 4). Remains recovered from Southeast Asia often can be highly fragmented due to the circumstances surrounding the loss and/or the acidic nature of the soil, which can break down osseous material. It is more difficult to accurately ascribe smaller fragments as human or non-human. In addition, very small fragments may be all that is recovered and it becomes a choice as to whether to use the entire sample for microscopic or DNA analysis. In two different cases, the results were a mixture of human and pig (*Sus scrofa*). The low-quality mtDNA profiles generated from the samples were determined to be consistent with the profiles of members of the field recovery team (Edson and Christensen 2013). Other mixtures of animal and human, or animal and animal, are thought to have occurred via excessive handling or

Table 2. Species identification results from the blind study samples. Taxonomic classifications are shown for the 12S assay as the top BLASTN species and the classification determined by AFDIL guidelines. For the DPAA species identification, the human/non-human determination is provided as well as the presumed species based upon anthropological analysis. Animals noted with "[ ]" identify the revised determinations made by faunal experts in cases of discordance between the 12S and DPAA species. The geographic origin is the location in which the specimens were recovered.

	12S Taxonomic Classification		DPAA Taxonomic Classification		
Sample	*Top BLASTN Match*	*AFDIL Guidelines*	Species	Human/Non-Human	Geographic Origin
1	*Cervus elaphus*	Red Deer	Elk	Non-human	Contiguous United States
2	*Castor canadensis*	Beaver	Beaver	Non-human	Contiguous United States
3	*Odocoileus hemionus*	Mule Deer	Deer	Non-human	Contiguous United States
4	*Canis lupus familiaris*	Dog	Civet [Canid]	Non-human	Vietnam
5	*Sus scrofa*	Pig	Pig	Non-human	Vietnam
6	*Homo sapiens*	Human	-	Human	Vietnam
7	*Bos taurus*	Cow	Cow/Buffalo	Non-human	Papua New Guinea
8	*Muntiacus muntjak*	Indian Muntjac	Sheep/Goat	Non-human	Vietnam
9	*Viverricula indica*	Small Indian Civet	Civet	Non-human	Vietnam
10	*Bos taurus*	Cow	Cow/Buffalo	Non-human	Hawaii
11	*Bos taurus*	Cow	Cow/Buffalo	Non-human	Laos
12	*Bos taurus*	Cow	Cow/Buffalo	Non-human	Laos
13	*Homo sapiens*	Human	-	Human	Vietnam
14	*Sus scrofa*	Pig	Pig	Non-human	Luxemburg
15	*Bos taurus*	Cow	Cow	Non-human	Luxemburg
16	*Ovis aries* or *ammon*	Sheep	Pig [Sheep/Goat]	Non-human	Solomon Islands
17	*Bos taurus*	Cow	Cow/Buffalo	Non-human	China
18	*Homo sapiens*	Human	-	Human	Vietnam
19	Mixture	Inconclusive	Dog	Non-human	Hawaii
20	*Odocoileus hemionus*	Mule Deer	Deer	Non-human	Contiguous United States
21	*Equus grevyi*	Zebra	Zebra	Non-human	Kenya
22	*Cervus elaphus*	Red Deer	Elk	Non-human	Contiguous United States
23	*Sus scrofa*	Pig	Sheep/Goat	Non-human	Laos
24	*Bos taurus*	Cow	Cow	Non-human	Hungary

Table 2. (cont.)

Sample	12S Taxonomic Classification		DPAA Taxonomic Classification		Geographic Origin
	Top BLASTN Match	*AFDIL Guidelines*	Species	Human/Non-Human	
25	*Cervus elaphus*	Red Deer	Cow [Deer]	Non-human	Hungary
26	*Vulpes vulpes*	Fox	Dog [Fox]	Non-human	Hungary
27	*Capra hircus*	Goat	Sheep/Goat	Non-human	Hungary
28	*Felis catus*	Cat	Cat	Non-human	Hungary
29	*Capreolus capreolus*	Western Roe Deer	Dog [Deer]	Non-human	Hungary
30	*Capreolus capreolus*	Western Roe Deer	Deer	Non-human	Hungary
31	*Homo sapiens*	Human	-	Human	Thailand
32	*Lama pacos, glama* or *guanicoe**	Camelid	Llama	Non-human	Bolivia
33	*Felis catus*	Cat	Sheep/Goat [Cat]	Non-human	Kwajalein Island
34	*Homo sapiens*	Human	-	Human	Thailand
35	*Bos taurus*	Cow	Cow/Buffalo	Non-human	Kwajalein Island
36	*Bubalus bubalis*	Asian Water Buffalo	Cow/Buffalo	Non-human	Laos
37	*Homo sapiens*	Human	-	Human	Thailand
38	*Ovis aries* or *ammon*	Sheep	Sheep/Goat	Non-human	Contiguous United States

*As of 2001, the genus for *Lama pacos* (Alpaca) was changed to *Vicugna* based on genetic evidence that supported the hypothesis that the Alpaca is derived from the Vicuña not the Guanaco (Kadwell et al. 2001). However, the database hits that were 100% homologous with the Sample 32 sequence at the time of the search were noted as *Lama pacos* in the NCBI Nucleotide database even though they were added in 2006. Species of the *Vicugna* and *Lama* genera are closely related and the common non-human taxon, family Camelidae, would be reported.

Table 3. The 12S rRNA testing results for each type of skeletal element, and the average weight of each type of element. "Long Bones" and "Bone Fragments" are listed independently of each other as a type of element as the former implies there was enough of the osseous material present to determine element was a long bone, wheras the latter is a non-specific catch-all for small fragments.

	Human		Inconclusive		Non-Human	
	Number Tested	Avg. Weight (g)	Number Tested	Avg. Weight (g)	Number Tested	Avg. Weight (g)
Bone Fragment	47	0.94	78	1.23	107	1.30
Calcaneus	--	--	2	3.45	--	--
Clavicle	--	--	2	1.0	--	--
Cranium (general)	3	1.31	31	1.92	4	1.91
Cuneiform	--	--	1	1.9	--	--
Femur	2	4.39	13	3.94	2	2.7
Fibula	--	--	2	1.89	--	--
Frontal	--	--	4	1.67	--	--
Humerus	1	3.10	10	2.99	4	1.46
Long Bone	26	1.28	58	1.77	101	1.75
Mandible	2	2.25	4	2.05	3	2.19
Metacarpal	1	0.40	2	0.80	3	0.54
Metatarsal	1	0.99	2	1.65	1	0.60
Occipital	--	--	1	4.2	--	--
Os coxa	--	--	3	3.09	2	1.87
Parietal	--	--	3	2.46	--	--
Phalanx	--	--	2	0.44	--	--
Radius	1	2.50	7	2.36	1	3.00
Rib	5	0.83	6	1.07	23	1.08
Scapula	1	2.45	1	1.4	1	1.35
Talus	--	--	3	2.33	--	--
Temporal	--	--	4	4.04	2	0.86
Tibia	1	1.50	4	3.64	2	1.98
Tooth (Molar)	--	--	2	n/r	--	--
Ulna	--	--	6	2.22	--	--
Vertebra (Any)	3	1.86	1	4.5	--	--
Zygomatic	--	--	1	0.78	--	--

Table 4. Summary of animals detected in the 12S rRNA testing and the conflict of origin. The original species designations are indicated and were not adjusted with more recent searches. Oftentimes, the geographic location of a country from which the remains were recovered will provide clues as to the animal, even if the 12S results are more general. Some of the results seem unlikely (e.g., the Common House Gecko); however, the sequence was duplicated through either extraction or amplification and confirmed prior to searching in BLAST and being reported. Remains from Southeast Asia were typically recovered from Vietnam, Laos, or Cambodia; those recovered from the Korean War were from the Korean peninsula; and those from World War II were from world-wide locations (e.g., Tarawa Atoll, Germany, Papua New Guinea).

	WWII	Southeast Asia	Korean War
Human (*Homo sapiens*)	21	70	4
Arctic cod (*Arctogadus glacialis*)		1	
Order Artiodactyla (non-specific)		2	
Asian Black Bear (*Ursus thibetanus*)		1	
Family Bovidae		7	
Family Cercopithecidae		1	
Family Cervidae		5	
Chicken (*Gallus gallus*)		1	
Cow (*Bos taurus*)	30	90	
Deer (non-specific)	2	9	
Order Diprotodontia	1		
Dog (*Canis familiaris*)		2	
Dolphin (non-specific)		1	
Giant Grouper (*Epinephalus lanceolatus*)		1	
Goat (*Capra hircus*)		2	
Common house gecko (*Hemidactylus frenatus*)		1	
Horse (Genus *Equus*)	1		1
Edward's Giant Rat (*Leopoldamys edwardsi*)		1	
Macaque (Genus *Macaca*)		5	
Muntjac (Genus *Muntiacus*)		2	
Family Phasianidae	1		
Pig / Wild Boar (*Sus scrofa*)	23	35	1
Rat (Genus *Rattus*)	1		
Sea Turtle (Superfamily Chelonioidea)		1	
Softshell Turtle (*Palea steindachneri*)		1	
Sheep (*Ovis aries*)	4		
Water Buffalo (*Bubalus bubalis*)		18	
Non-human (non-specific)		2	
Inconclusive	82	169	3

gnawing. Although not a validated protocol, the observed mixtures can be separated visually by an analyst and searched in BLAST if so desired. This is a fairly simple process since the human sequence is known.

One of the difficulties with using BLAST is that the NCBI database it accesses is self-curated and continuously being updated. The assignment of 'non-human' will not change; however, the species assignment may be different. This is particularly notable for rare species that may not be commonly added to the database. For example, a recent search of the NCBI database for the purposes of this study revealed that thirteen of fifteen samples are now classified as a more specific species (e.g., original search result Family Cervidae and new search result (*Rusa unicolor*, Sambar Deer) and one sample did not change (Genus *Muntiacus*, muntjacs). However, one sample changed to a more general category. This sample was previously determined in 2014 to be a Wattle-necked Softshell Turtle (*Palea steindachneri*); re-running the search in 2019 resulted in a 100% match to not only *P. steindachneri*, but also the Asiatic Softshell Turtle (*Amyda cartilaginea*), an IUCN threatened species. By the calling criteria of AFDIL, the sample would now be reported to DPAA as being Family Trionychidae, rather than a specific species.

In addition, those who upload sequences are on their own to provide accurate information on the taxa to which the sequence belongs. The standards put in place as part of the AFDIL validation tend to eliminate incorrect 'matches'. However, there are cryptids that are part of the NCBI database that occasionally match to samples submitted by DPAA. The most common 'match' is to the Kting Voar (*Pseudonovibos spiralis*), also known as the Snake-eating Cow or the Spiral-horned Ox. Although the designation is subject to controversy (Olson and Hassanin 2003), testing has indicated that the specimens are most likely from Domestic Cows (Hassanin et al. 2001) or Water Buffalo (Kuznetsov et al. 2001) rather than a mythical beast. Nonetheless, the sequences are still present in the NCBI database (e.g., GenBank Accession No. AF231029). Matches to the Kting Voar include other Bovids and are usually attributed to the Family Bovidae.

DISCUSSION

The use of highly sensitive methods, such as those employed at AFDIL including the 12S assay, is a necessity in cases involving decades-old skeletal remains. Unfortunately, with this type of testing, modern contaminating DNA is always a concern (Malmstrom et al. 2005; Gilbert et al. 2006; Pilli et al. 2013) despite precautions taken to minimize contamination during remains recovery and laboratory processing (Edson et al. 2004; Kemp and Smith 2005; Barta et al. 2013; Edson and Christensen 2013; Edson and McMahon 2016). Consequently, an identification of human should be considered in the context of other case information and molecular data including any human mtDNA testing since exogenous modern human DNA may mask the authentic DNA from the non-human species, which is likely only present at low levels in poor quality specimens. The classification guidelines established at AFDIL for the interpretation of the 12S data aim to ensure the greatest level of confidence in the resulting species identification. However, all information must be considered if contamination from an exogenous source, human or non-human, is suspected.

The comparison between 12S and osteological taxonomic assignment of the blind study samples demonstrates how difficult it can be for anthropologists to accurately differentiate between various non-human species in situations involving small, severely compromised skeletal fragments. Although immunological and histological analyses have been shown to facilitate the determination of human or non-human origin (Cattaneo et al. 1999; Ubelaker et al. 2004; Lowenstein et al. 2006; Hillier and Bell 2007), reliable species identification based solely on these analyses may still be limited. Morphological determination of species is dependent on the experience and knowledge of the anthropologist in addition to the size of the fragment. The reproducibility of the 12S result is not reliant on the analyst but rather on the BLAST alignment algorithm and composition of the NCBI Nucleotide database. Therefore, the sequence data generated by the 12S assay enables an unbiased determination of taxonomic origin, and in particular whether a sample is or is not human.

Species identification using this 12S assay, though superior to osteological analyses, is limited by the composition of the NCBI Nucleotide database and, depending on the application, the inter-species variation of the targeted mtDNA region. As evidenced by the initial BLASTN search for Sample 9 of the blind study, a 100% homologous sequence may not be returned by the search if the exact taxon has not been captured in the database. In these situations, the most closely related species represented in the database will be returned as the most significant alignment. This was the case for Sample 9 in which the *V. indica* sequence was not present in the database at the time of the initial query (May 2010) and was added approximately seven months later (December 2010). Although the database continues to grow, no doubt facilitating sequence identifications at the species level over time, current designations using this 12S assay should be weighted heavily on sequence homology. This consideration is reflected in the classification guidelines employed at AFMES-AFDIL (Table 1) in which 100% homology is required in order to report a specific species. With that being said, identical queried and searched sequences may not definitively identify the exact taxon since this small region of the 12S rRNA gene could potentially be conserved among closely related species.

Because DPAA recovery missions take place across the globe, often in areas with indigenous primate populations, AFDIL may receive skeletal fragments from other primates commingled with human remains. In fact, the 12S sequence generated from several samples in a case from the Vietnamese province of Quang Binh was classified as genus *Macaca* (macaque), as it was 100% consistent with two macaque species. Macaques, though primates, are members of the Cercopithecidae family. Humans are much more similar to other apes within the Hominidae family. Minimal differences and large regions of homologous bases also are observed between the sequences of *Homo*, *Gorilla*, and *Pongo* genera. Based on the similarity of 12S sequences among hominids, AFDIL guidelines require at least 75 bases of sequence and 100% homology to conclusively classify a sample as having originated from a human.

Conclusions

Based on the results obtained from the application of this protocol to the blind study as well as routine case samples, the 12S assay described here is a robust and reliable method for the species identification of degraded bone fragments. This protocol easily could be implemented into any forensic laboratory already performing standard mtDNA sequence analysis. The 12S assay remains a low-cost, low-tech process by which species of origin may be determined. This species identification assay has become an invaluable tool for human identification efforts at AFDIL due to its ability to determine the species origin of severely compromised skeletal specimens and thereby allow laboratory resources to be focused on samples that are human in origin.

Disclaimer

The opinions or assertions presented are the private views of the authors and should not be construed as official or as reflecting the views of the Department of Defense; the Defense Health Agency; the Armed Forces Medical Examiner System; or the Defense POW/MIA Accounting Agency.

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LITERATURE CITED

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. Journal of Molecular Biology 215:403–410.

Balitzki-Korte, B., K. Anslinger, C. Bartsch, and B. Rolf. 2005. Species identification by means of pyrosequencing the mitochondrial 12S rRNA gene. International Journal of Legal Medicine 119:291–294.

Barta, J. L., C. Monroe, and B. M. Kemp. 2013. Further evaluation of the efficacy of contamination removal from bone surfaces. Forensic Science International 231:340–348.

Benedix, D. C. 2004. Differentiation of fragmented bone from Southeast Asia: The histological evidence, Ph.D. Dissertation, University of Tennessee, Knoxville, TN.

Branicki, W., T. Kupiec, and R. Pawlowski. 2003. Validation of cytochrome *b* sequence analysis as a method of species identification. Journal of Forensic Sciences 48:1–5.

Cattaneo, C., S. DiMartino, S. Scali, O.E. Craig, M. Grandi, and R.J. Sokol. 1999. Determining the human origin of fragments of burnt bone: a comparative study of histological, immunological and DNA techniques. Forensic Science International 102:181–191.

Ciavaglia, S. A., S. S. Tobe, S. C. Donnellan, J. M. Henry, and A. Linacre. 2015. Molecular identification of python species: development and validation of a novel assay for forensic investigations. Forensic Science International: Genetics 16:64–70.

Edson, S. M. 2019. Extraction of DNA from skeletonized postcranial remains: A discussion of protocols and testing modalities. Journal of Forensic Sciences. Published on-line 29 March 2019. https://doi.org/10.1111/1556-4029.14050.

Edson, S. M., and A. F. Christensen. 2013. Field contamination of skeletonized human remains with exogenous DNA. Journal of Forensic Sciences 58:206–209.

Edson, S. M., and T. P. McMahon. 2016. Extraction of DNA from skeletal remains. Pp. 69–87 in Forensic DNA typing protocols: Methods in molecular biology, Vol 1420 (W. Goodwin, ed.). Humana Press, New York, New York.

Edson, S. M., J. P. Ross, M. D. Coble, T. J. Parsons, and S. M. Barritt. 2004. Naming the Dead: Confronting the realities of rapid identification of degraded skeletal remains. Forensic Science Review 16:63–90.

Gabriel, M. N., E. F. Huffine, J. H. Ryan, M. M. Holland, and T. J. Parsons. 2001. Improved mtDNA sequence analysis of forensic remains using a "mini-primer set" amplification strategy. Journal of Forensic Sciences 46:247–253.

Gilbert, M. T. P., A. J. Hansen, E. Willerslev, G. Turner-Walker, and M. Collins. 2006. Insights into the processes behind the contamination of degraded human teeth and bone samples with exogenous sources of DNA. International Journal of Osteoarchaeology 16:156–164.

Hassanin, A., A. Seveau, H. Thomas, H. Bocherens, D. Billiou, and B. X. Nguyen. 2001. Evidence from DNA that the mysterious 'linh duong' (*Pseudonovibos spiralis*) is not a new bovid. Comptes Rendus de L'Academie des Sciences, Life Sciences 324:71–80.

Hillier, M. L., and L. S. Bell. 2007. Differentiating human bone from animal bone: a review of histological methods. Journal of Forensic Sciences 52:249–263.

Kadwell, M., et al. 2001. Genetic analysis reveals the wild ancestors of the llama and the alpaca. Proceedings of the Royal Society B: Biological Sciences 268:2575–2584.

Kemp, B. M., and D. G. Smith. 2005. Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. Forensic Science International 154:53–61.

Kitpipit, T., K. Penchart, K. Ouithavon, C. Satasook, A. Linacre, and P. Thanakiatkrai. 2016. A novel real time PCR assay using melt curve analysis for ivory identification. Forensic Science International 12:210–217.

Kuznetsov, G. V., et al. 2001. The "linh duong" *Pseudonovibos spiralis* (Mammalia, Artiodactyla) is a new buffalo. Naturwissenschaften 88: 123–125.

Linacre, A., and J. C. Lee. 2016. Species determination: The role and use of cytochrome b gene. Methods in Molecular Biology 1420: 287–296.

Loreille, O. M., T. M. Diegoli, J. A. Irwin, M. D. Coble, and T. J. Parsons. 2007. High efficiency DNA extraction from bone by total demineralization. Forensic Science International: Genetics 1:191–195.

Loreille, O. M., et al. 2010. Integrated DNA and fingerprint analyses in the identification of 60-year-old mummified human remains discovered on an Alaskan glacier. Journal of Forensic Sciences 55:813–818.

Lowenstein, J. M., J. D. Reuther, D. G. Hood, G. Scheuenstuhl, S. C. Gerlach, and D. H. Ubelaker. 2006. Identification of animal species by protein radioimmunoassay of bone fragments and bloodstained stone tools. Forensic Science International 159:182–188.

Madden, T. 2002. The BLAST sequence analysis tool. In The NCBI handbook [Internet] (J. McEntyre and J. Ostell, eds.). National Center for Biotechnology Information, Bethesda, Maryland.

Malmstrom, H., J. Stora, L. Dalen, G. Holmlund, and A. Gotherstrom. 2005. Extensive human DNA contamination in extracts from ancient dog bones and teeth. Molecular Biology and Evolution 22:2040–2047.

Melton, T., and C. Holland. 2007. Routine forensic use of mitochondrial 12S ribosomal RNA gene for species identification. Journal of Forensic Sciences 52:1305–1307.

Mulhern, D. M., and D. H. Ubelaker. 2001. Differences in osteon banding between human and nonhuman bone. Journal of Forensic Sciences 46:220–222.

Olson, L. E., and A. Hassanin. 2003. Contamination and chimerism are perpetuating the legend of the snake-eating cow with twisted horns (*Pseudonovibos spiralis*): A case study of the pitfalls of ancient DNA. Molecular Phylogenetics and Evolution 27:545–548.

Pilli, E., et al. 2013. Monitoring DNA contamination in handled vs. directly excavated ancient human skeletal remains. PLoS One 8:e52524.

Tobe, S. S., and A. Linacre. 2010. DNA typing in wildlife crime: recent developments in species identification. Forensic Science, Medicine, and Pathology 6:195–206.

Ubelaker, D. H., J. M. Lowenstein, and D. G. Hood. 2004. Use of solid-phase double-antibody radioimmunoassay to identify species from small skeletal fragments. Journal of Forensic Sciences 49:924–929.

Zhang, Z., S. Schwartz, L. Wagner, and W. Miller. 2000. A greedy algorithm for aligning DNA sequences. Journal of Computational Biology 7:203–214.

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Diversification within the Ornate (*Sorex ornatus*) and Vagrant Shrew (*Sorex vagrans*) Complex in the San Francisco Bay Area of California

James L. Patton

Abstract

Shrews of the *Sorex ornatus – Sorex vagrans* complex, while distinguished by morphological, molecular, and karyotypic traits over most of their respective ranges, present a conundrum of character discordances in the greater San Francisco Bay region of central California that historically has been difficult to untangle. The utility of cranio-mandibular characters to diagnose both species is documented, and additional mitochondrial sequences are added to expand the current understanding of phylogeographic units within the complex. Patterns of character variation in dorsal pelage color, upper incisor tine pigment pattern, and cranio-mandibular mensural variables are then examined for currently recognized taxa of both species from the San Francisco Bay Area, with specific comparisons of shrews from wetland and tidal marshes around the Bay using a combination of univariate and multivariate methods. These analyses highlight the discordance between phenotype and the limited genotypic assessments of multiple populations, patterns that likely result from a history of repeated hybridization leading to mitochondrial capture, a hypothesis that must await future studies of these remarkably variable shrews.

Key words: ornate shrew, San Francisco Bay Area, *Sorex ornatus*, *Sorex vagrans*, taxonomy, vagrant shrew

Introduction

Sorex ornatus (ornate shrew) and *Sorex vagrans* (vagrant shrew), two of the 11 species in the genus whose ranges encompass at least part of California, have among the widest distributions in the state. These two are morphologically very similar sister species (Junge and Hoffmann 1981; Willows-Munro and Matthee 2011), but differ by a mean molecular divergence (mtDNA cytochrome-*b* gene [*Cyt*b]) of 6.93% and an estimated divergence date ranging from 2.5 mya (Esteva et al. 2010) to 0.8 mya (Hope et al. 2014).

By current understanding, the two species have primarily complementary, non-overlapping distributions in California (Fig. 1). *Sorex ornatus* is broadly distributed through central California west of the foothills of the Sierra Nevada, in southern California from the Transverse Ranges south to San Diego County and beyond in Baja California, and extends into wetland pockets in the western Mojave Desert around the southern terminus of the Sierra Nevada. It occurs in tidal marshes that fringe the greater San Francisco Bay region and estuary marshes along parts of the central and southern coasts, on Santa Catalina Island, in isolated wetland pockets on the floor of the San Joaquin Valley, and in the Sierra de la Laguna at the southern end of Baja California Sur. *Sorex vagrans*, in contrast, is distributed in northern California, along the Pacific coast from Monterey Bay to the Oregon border and further north; across northern California into the southern Cascade Range and isolated Great Basin ranges to the east; and south along the crest and eastern slopes the Sierra Nevada to the vicinity of Lake Tahoe. It is also found in isolated wetland pockets east of the Sierra Nevada in Mono Basin, Adobe Valley, Long Valley, northern Owens Valley, and Deep Springs Valley. Range limits of this species outside of California are unclear, largely because of a general lack of detailed analyses of both morphological and molecular

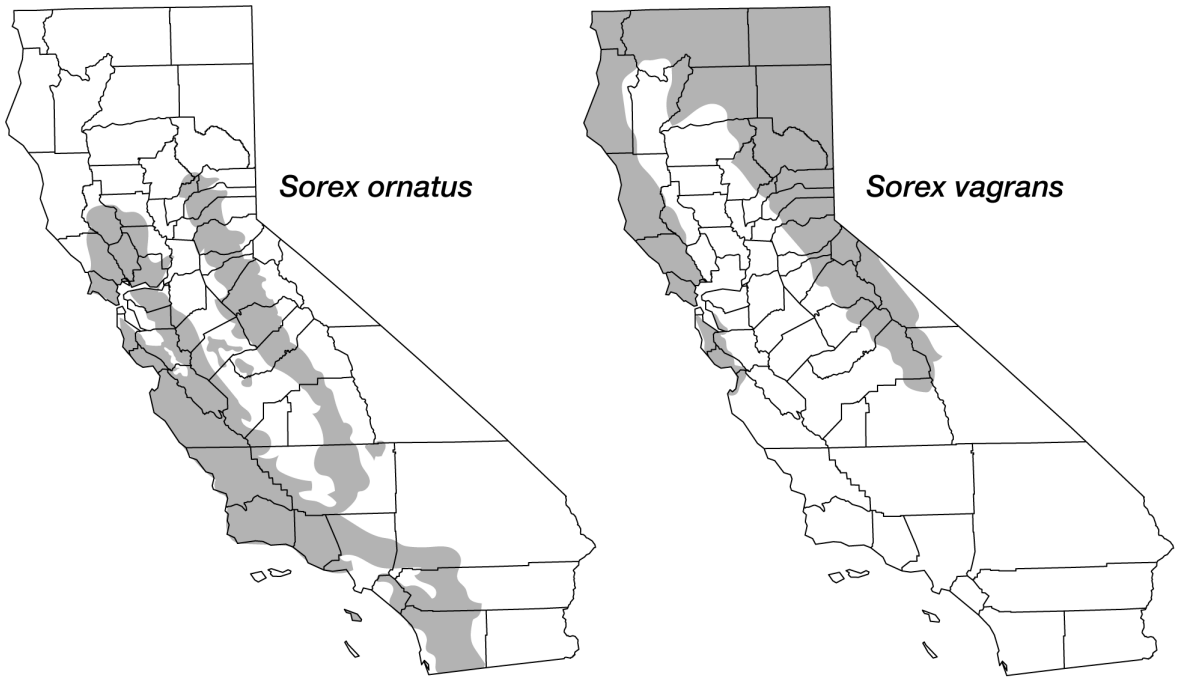

Figure 1. Approximate distributions of *Sorex ornatus* and *S. vagrans* in California, as currently understood (maps redrawn from the California Gap Analysis Project, University of California Santa Barbara; Davis et al. 1998).

characterization of member populations over the broad distribution mapped by Findley (1955) and Hall (1981). *Sorex ornatus* and *S. vagrans* contact one another, or overlap, north of the Golden Gate around the edge of San Pablo Bay, in marshes around San Francisco Bay in Contra Costa, Alameda, Santa Clara, and San Mateo counties, and in salt marshes that fringe Monterey Bay in Monterey County.

A third taxon in the *S. ornatus – S. vagrans* complex is *Sorex sinuosus*, described originally as a species (Grinnell 1913) limited to the salt marshes of Grizzly Island in Solano County, California. Rudd (1955) treated this taxon as a valid species, but one that hybridized with both *S. ornatus* to the east and south in Sacramento and Contra Costa counties and *S. vagrans* in Sonoma and Marin counties. Brown and Rudd (1981), Junge and Hoffmann (1981), and Owen and Hoffmann (1983) subsequently subsumed *sinuosus* Grinnell as a subspecies of *S. ornatus*, a decision maintained by Hutterer (2005) but not by Woodman (2018), who assigned *sinuosus* Grinnell as a subspecies of *S. vagrans*.

The analyses presented here focus on *S. ornatus* and *S. vagrans* of the greater San Francisco Bay region, an area of substantial diversity but with discordant patterns of relationship based on limited published morphological, karyological, and molecular data (e.g., Rudd 1955; Brown 1974; Brown and Rudd 1981; Junge and Hoffmann 1981; Owen and Hoffmann 1983; Maldonado et al. 2001, 2004). The data employed are largely limited to morphological, morphometric, and colorimetric variables obtained from the large collections housed in the mammal collection of the Museum of Vertebrate Zoology, critical samples of which were collected and used by Robert L. Rudd in his seminal published contributions. In addition, the phylogeographic clade structure delineated by Maldonado et al. (2001, 2004) was supplemented by adding new sequences from key areas on the north side of San Pablo Bay and throughout the range of *S. vagrans* from the southern Cascades, Warner Mts., and the Sierra Nevada and adjacent desert valleys.

An expanded phylogeographic assessment of clade structure and distribution of members of the *S.*

ornatus – *S. vagrans* complex in California is presented first and then followed by an assessment of the diagnostic utility of characters posited in the literature to distinguish *S. ornatus* from *S. vagrans*. These include univariate external and cranio-dental measurements, upper first incisor medial tine states, and dorsal pelage color attributes. Lastly, variability is reviewed for these same traits among samples from the greater San Francisco Bay region where discordance in the distribution of molecular haplotypes, karyotypes, and literature assessments of species boundaries occurs (e.g., contrast Junge and Hoffmann 1981 and Brown and Rudd 1985 with Maldonado et al. 2001, 2004), with special reference to the allocation of tidal marsh specimens to either *S. ornatus* or *S. vagrans*.

CURRENT TAXONOMY

Hutterer (2005; see also Owen and Hoffmann 1983) allocated nine taxa to *S. ornatus* and Woodman (2012) added a 10th. Eight of these have their type localities, and all or part of their distributions, within California. Two occur within the San Francisco Bay Area: (1) *californicus* Merriam, 1895:80; type locality "Walnut Creek, Contra Costa County, Calif[ornia]," with a current range that extends from the Santa Lucia and Gabilan ranges in the central coastal region northward to the San Francisco Bay, east through the Sacramento-San Joaquin Delta, and north into the Sacramento Valley (Grinnell 1933). And (2) *sinuosus* Grinnell, 1913:187; type locality "Grizzly Island, near Suisun, Solano County, California," with its range restricted to the islands and tidal salt marshes that fringe the northern edges of Suisun Bay in Solano County (Brown and Rudd 1981). Woodman (2018) assigned *sinuosus* Grinnell to *S. vagrans*, based on a shared mitochondrial DNA relationship, but the more traditional assignment of Grinnell's *sinuosus* to *S. ornatus* is retained here.

There has been no comprehensive review of *S. vagrans* since Findley (1955), with the exception of Hennings and Hoffmann (1977), who separated *S. monticolus* Merriam as a species, and Carraway (1990), who revised *vagrans* complex members along the north coast of California, Oregon, and Washington. Currently, there are three subspecies listed within California (Gillihan and Foresman 2004; see also Woodman 2018), all of which occur in the San Francisco Bay Area: (1) *vagrans* Baird, 1857:15; type locality "Shoalwater Bay, W.T. [=Willapa Bay, Pacific Co., Washington];" range across northern California from Marin County to Humboldt County (Carraway 1990), the southern Cascade Range and the northern Sierra Nevada. (2) *halicoetes* Grinnell, 1913:183; type locality "Palo Alto, Santa Clara County, California;" range restricted to salt marshes around San Francisco Bay, from Belmont (San Mateo County) to San Pablo Creek marsh, Contra Costa County. And, (3) *paludivagus* von Bloeker, 1939:93; type locality "salt-marsh at the mouth of Elkhorn Slough, Moss Landing, Monterey County, California;" range includes coastal salt marsh and wetland areas in west-central California, from San Gregorio, San Mateo County south to at least Seaside Lagoon, Monterey County. Samples from upland habitats along the San Francisco Peninsula are referred to this taxon (e.g., MVZ records, by Seth B. Benson).

Species Recognition

Morphological diagnoses.—*Sorex ornatus* and *S. vagrans* are stated to differ in overall size, tail length, general dorsal pelage coloration, and several cranial features (Table 1; Grinnell 1913; Jackson 1928; and Ingles 1965).

Junge and Hoffmann (1981) and Owen and Hoffmann (1983) stressed the utility of the pigmentation pattern on the medial tine of the 1st upper incisor as diagnostic for many pairs of similar shrews. Specifically, they characterized *S. ornatus* by a large tine whose pigment is completely confluent with that of the incisor, and *S. vagrans* by a smaller tine with the pigment area elevated above, and usually separated from, that of the incisor. The utility of this trait is examined below, but three character states are identified instead of two (see Methods and Materials).

Karyotypic differences.—The two species in the San Francisco Bay Area have karyotypes that differ in

Table 1. Morphological characters distinguishing *S. ornatus* and *S. vagrans* in California. Variable names following each attribute refer to those identified in the Materials and Methods.

Sorex ornatus	*Sorex vagrans*
Summer pelage color relatively pale, grayish brown	Summer pelage color relatively dark, brownish gray
Body size smaller (TOL)	Body size larger (TOL)
Tail absolutely shorter (TAL)	Tail absolutely longer (TAL)
Skull flat in lateral profile (CD; also CHo)	Skull taller in lateral profile (CD; also CHo)
Braincase narrower in dorsal view (CB)	Braincase broader in dorsal view (CB)
Foramen magnum positioned higher on occiput (FMoH, and ratio FMoH/CHo)	Foramen magnum positioned more ventrally on occiput (FMoH, and ratio FMoH/CHo)
Foramen magnum shallower in ventral view (FMvL)	Foramen magnum deeper in ventral view (FMvL)

autosomal arm number but not in diploid complement (Brown 1974; Brown and Rudd 1981): *S. ornatus* – $2n = 54$, FN = 76, with a haploid autosomal set of three metacentric (M), nine submetacentric (SM), and 14 acrocentric (A) chromosomes, specimens from Monterey, Solano, Sonoma, and Yolo counties; and *S. vagrans* – $2n = 54$, FN = 62, with a haploid autosomal set of 3M, 2SM, 21A; specimens from Marin [*S. v. vagrans*] and San Mateo [*S. v. halicoetes*] counties). Importantly, Grizzly Island specimens of Grinnell's *sinuosus* possessed a karyotype identical to that of *S. ornatus*, as did specimens from the tidal marshes along the North Bay at least as far west as Novato Creek in Marin County that Rudd (1955) regarded as hybrids.

mtDNA phylogeography.—Maldonado et al. (2001, 2004) defined three cytochrome-*b* (*Cyt*b) clades within the *S. ornatus* – *S. vagrans* complex. (1) A Southern clade distributed throughout southern California from Santa Barbara on the coast, Santa Catalina Island, and the Transverse Ranges south into northern Baja California, with an isolate in the Sierra de la Laguna in southern Baja California Sur; (2) a Central clade that unified samples from the western foothills of the Sierra Nevada, San Joaquin Valley, and Monterey Bay; and (3) a Northern clade that included topotypes and near-topotypes of *sinuosus* Grinnell, a sample from Tehama County in the northern Sacramento Valley Maldonado et al. (2001, 2004) attributed to *S. ornatus*, and samples of *S. vagrans* from localities as distant as the north coast of Sonoma County (Bodega Bay) and the Sweetwater Mts. in Mono County on the border with Nevada east of the Sierra Nevada. These samples included 29 unique sequences (25 from California), 343 to 392 bp in length, from 161 specimens and 21 localities.

METHODS AND MATERIALS

Bay Area geographic terminology.—For readers unfamiliar with the geography of the San Francisco Bay region, regional areas referred to herein include: (1) San Francisco Peninsula, bounded on the west by the Pacific Ocean and on the east by San Francisco Bay, and comprising San Francisco, San Mateo, and parts of Santa Clara counties; (2) East Bay, Alameda and Contra Costa counties bounded on the west by San Francisco Bay and part of San Pablo Bay, on the north by the Carquinez Strait and Suisun Bay, and on the east by the Diablo Range; and (3) North Bay, which groups Solano, Napa, Sonoma, and Marin counties on the north side of Suisun Bay, the Carquinez Strait, and San Pablo Bay. On the west, the North Bay is separated from the San Francisco Peninsula by the Golden Gate, the strait that connects the Pacific Ocean with San Francisco Bay.

Molecular analyses.—The data set developed by Maldonado et al. (2001, 2004) was expanded by obtaining between 801 and 1,140 bp sequence of *Cyt*b from 127 specimens from 61 localities in California (localities and voucher catalog numbers or GenBank

accession numbers for all available sequences are listed in Appendix A, S1). New, unique sequences were submitted to GenBank (accession numbers: MK691325–MK601381). DNA extraction, amplification, *Cyt*b primers, and sequencing protocols followed procedures provided in previous publications (e.g., Smith and Patton 2007).

Because the data originally collected by Maldonado et al. (2001) were limited in sequence length, new sequences were pared to 801 bp of *Cyt*b for analysis and missing data in the shorter Maldonado et al. sequences were replaced with a null. Since the focus here is on morphological similarity and disparity among shrews of the *S. ornatus* – *S. vagrans* complex, only a gross overview of statewide phylogeographic patterns is provided. To do this, a minimum evolution tree was generated, with bootstrap support calculated from 1,000 replicates, with replacement, using MEGA7 (Kumar et al. 2016). The complete dataset contained 152 individual sequences.

Morphological samples.—Approximately 1,100 specimens of California *S. ornatus*, from 73 separate localities, and *S. vagrans*, from 94 localities from the San Francisco Bay Area were examined. All material is housed in the mammal collection of the Museum of Vertebrate Zoology. These include the holotypes of *S. halicoetes* Grinnell (MVZ 3638) and *S. sinuosus* Grinnell (MVZ 16470). Specimens were grouped into local samples for each of the following analyses (sample sizes and locality data for these samples are given in Appendix A, S2–S3).

Morphometric variables.—External measurements (total length [TOL], tail length [TAL], hind foot length, with claw [HF], ear height, from notch [E], and body mass [MASS]) were obtained from specimen labels. Twenty-five cranio-mandibular variables were measured using a calibrated micrometer with a Dino-Lite© AD4113TL digital microscope (AnMo Electronics Corp, New Taipei City, Taiwan), with pixels converted to a metric scale at a precision of 0.001 mm. To minimize measurement error, each skull and mandible was repositioned, imaged, and measured five times, with the mean of those repeated measurements then used in all analyses. The position of the pair of digitized points that delimit each measurement is illustrated in Figure 2. Measurements chosen largely follow those defined by Woodman and Timm (1993): greatest skull length (GSL – anterior margin of upper incisors to posterior margin of cranium); cranial breadth (CB – greatest distance across the mid-points of the cranium); interorbital constriction (IOC – least distance across the frontal bones between the orbits); rostral breadth (RB – breadth across the premaxilla); condylobasal length (CBL – posterior margin of upper incisor, in ventral view, to posterior margin of occipital condyles); palatal length (PL – length of palate from posterior margin of upper incisor to anterior margin of mesopterygoid fossa); length of the upper unicuspid row (UniTRL – distance from anterior margin of U1 and posterior margin of U5); length of molariform teeth (P4-M3L – distance from anterior margin of P4 and posterior margin of M3); length of the upper tooth row (upperTRL – distance from anterior margin of U1 and posterior margin of M3); breadth across upper first unicuspids (U1B – breadth across the outer margins of the left and right U1s); breadth across 2nd upper molars (M2B – breadth across outer margins of left and right M2s); basioccipital width (basiW – least width across the basioccipital); length of the foramen magnum, in ventral view (FMvL – length of foramen magnum from posterior margins of occipital condyles to anterior margin of foramen); width of the foramen magnum, in ventral view (FMvW – width of foramen magnum from inner margins of occipital condyles in ventral view); cranial depth (CD –height of cranium, in lateral view); width of the zygomatic plate (ZPW – minimal distance across the zygomatic plate, in lateral view); mandibular length (manL – distance from anterior margin of 1st lower incisor [i1]alveolus to posterior margin of ramus); length of the mandibular tooth row (manTRL – distance from anterior margin of 1st lower incisor [i1] alveolus to posterior margin of m3); length of first lower molar (m1L – distance between anterior and posterior margins of ml); height of the coronoid process (HCP – least distance from ventral margin of ramus to distal margin of the coronoid process); height of coronoid valley (HCV – least distance from ventral margins of ramus and sigmoid notch between coronoid and articular condyles); height of articular condyle (HAC – least distance from ventral margin of ramus and distal margin of articular condyle); height of cranium, in occipital view (CHo – height of cranium from ventral margins of occipital condyles to top of cranium, in occipital view); height of the foramen magnum, in occipital view (FMoH – height of foramen magnum

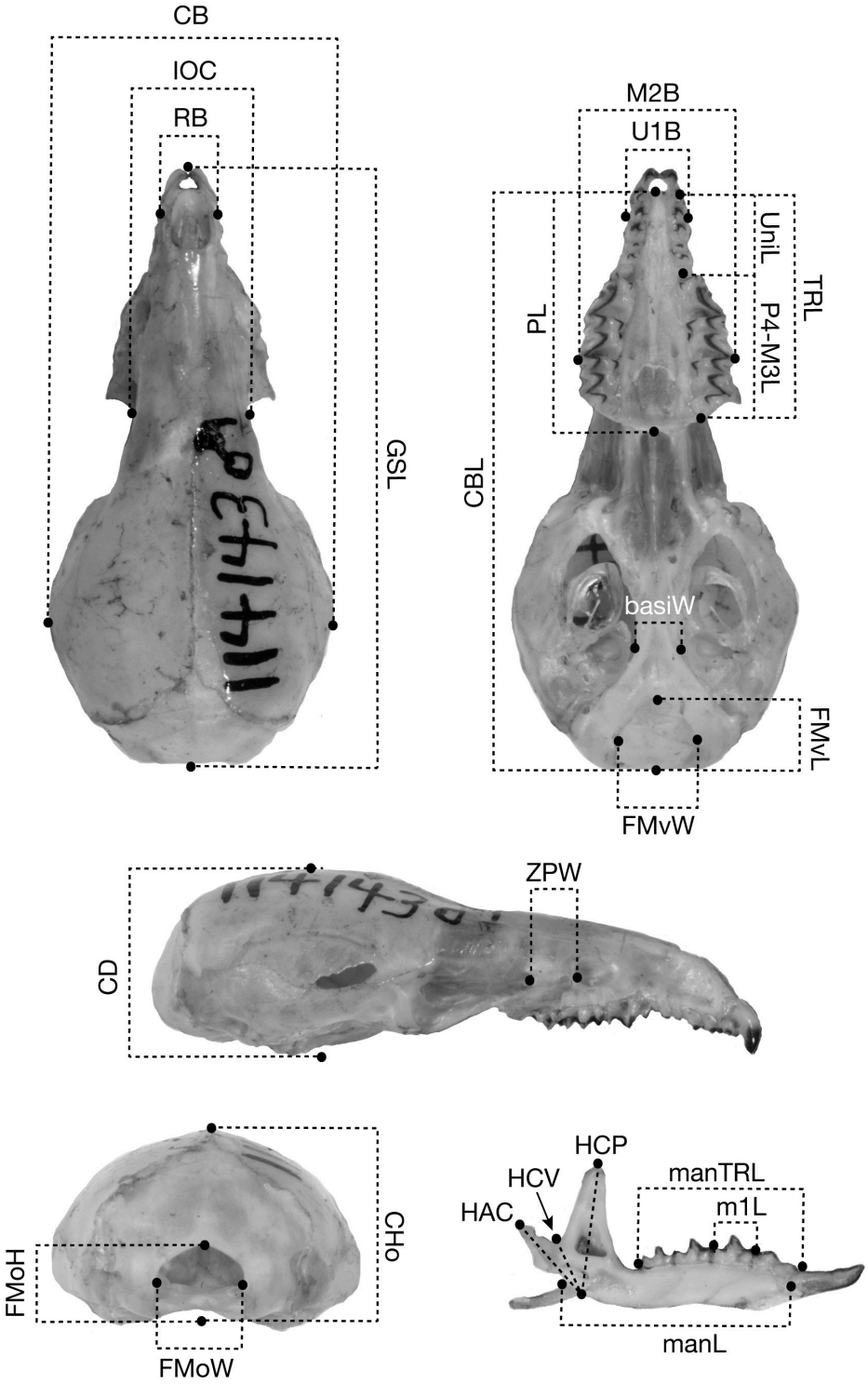

Figure 2. Twenty-five cranio-mandibular variables taken with a Dino-Lite® AD4113TL digital microscope; see text for variable abbreviations. Solid circles represent the approximate position of the pair of digitized points that bound each variable measurement.

from ventral margins of occipital condyles to anterior margin of foramen, in occipital view); foramen magnum width, in ventral view (FMoW – width of foramen magnum from inner margins of occipital condyles).

To augment the tail length comparison, the number of caudal vertebrae were counted in 278 skeletons collected from throughout the California ranges of both *S. ornatus* (specimens pooled from Alameda, Contra Costa, Kern, Mariposa, Orange, and San Diego counties) and *S. vagrans* (El Dorado, Inyo, Lassen, Marin, Plumas, Shasta, Siskiyou, and Tehama counties; locality and specimen data accessible from http://arctos.database.museum/SpecimenSearch.cfm). The sacrum of all specimens consisted of five elements, all fused in older individuals but with the fifth element often unfused in younger ones; in 91% of specimens, each sacral element had a neural spine, those that did not were invariably the fifth element. The first vertebra in the caudal series was regarded as the first free element posterior to the five sacral ones, and could be identified unambiguously by the lack of a neural spine, the lack of prezygapophyses resulting in contact between adjacent elements at the centrum alone, and the presence of a sesamoid chevron at the ventral junction of each centrum pair, beginning with that of the fifth sacral and first caudal elements (terminology from Thorington 1966).

Only specimens judged to be adult by tooth wear supplemented by available reproductive data on specimen labels were included in the analyses. Four age categories based on the degree of wear on the maxillary and mandibular teeth were identified, as follows: (1) all teeth in place but with sharp, largely unworn cusps; pigment present on all cusps of PM4-M3 and on all unicuspids (rarely including U5). (2) Teeth moderately worn; some pigment, visible in lateral view, remaining on upper incisor, most unicuspids, and taller cusps of PM4-M2. (3) Teeth worn and largely devoid of any pigmented cusps (minimal pigment still present on upper incisor and highest cusps of U1-U2 and PM4). (4) Teeth heavily worn; none retaining even a vestige of pigment. For the largest single locality sample (Martinez marsh *S. ornatus*, $n = 122$; Appendix A, S3), none of the 25 cranio-mandibular variables exhibited significant differences attributable to age, sex, or age*sex interaction (two-way ANOVA with Bonferroni corrected *P*-values at $\alpha < 0.002$). Hence, all individuals were pooled in the following analyses.

Medial tine of upper 1st incisor.—Junge and Hoffmann (1981) emphasized the relationship of the pigmented area of the accessory medial tine to that of the main pigmented area of the first upper incisor as an important character useful for distinguishing several species of soricine shrews. They noted a lack of ontogenetic change in tine size or pigmentation and that there appeared "to be little individual variation of the tine within species (p. 5)." This latter assertion has remained largely untested, except by Carraway (1990) who identified 11 states in this character for *S. vagrans* and related shrews in the Pacific Northwest.

Three classes of medial tine to shaft pigment patterns could be differentiated, with reasonable consistency: state 1 – upper pigment boundary of medial tine confluent with upper pigment boundary of main shaft of I1, even if the two pigment areas are separated by a pale gap (Fig. 3a,b); state 2 – upper pigment boundary of tine above upper pigment boundary of main shaft of I1, but tip of tine well within that pigmented area; the two pigment areas may or may not be separated by pale gap (Fig. 3c,d); and state 3 – both the upper pigment boundary of tine and its tip are at or above the upper pigment boundary of main shaft of I1; the two areas always separated by a pale gap (Fig. 3e,f). State 1 has been posited to characterize *S. ornatus*, and state 3 *S. vagrans* (Junge and Hoffmann 1981; Owen and Hoffmann 1983).

Colorimetric variables.—The three CIE color variables L* (lightness, measured on a scale from 0 [= black] to 100 [= diffuse white]), a* (the position on the color spectrum between red/magenta and green [negative values indicate green while positive values indicate magenta]), and b* (the position on the color spectrum between yellow and blue [negative values indicate blue and positive values indicate yellow]) were examined. To obtain these values, each shrew skin was photographed at a distance of 25 cm using a Nikon DX SWM micro 1:1 lens and under standard lighting conditions at 4600°K; the brightness of each photograph was then increased by a factor of 150 to yield a uniform white background with L* = 90, a* = 0, and b* = 1. Color values were then recorded at five points along the mid-dorsum on each photograph using the Lab color mode in Adobe PhotoShop CC™ (Adobe Systems Inc., San Jose, California), and averaged to provide a single value for each variable in subsequent

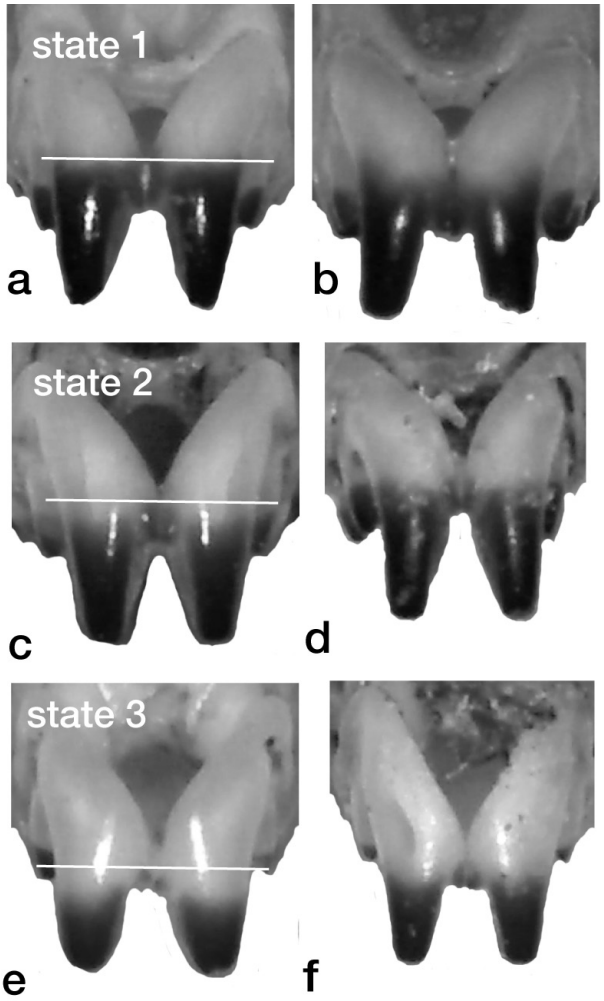

Figure 3. Two examples each of the three medial tine pigment states recognized: state 1 (a – MVZ 115195, *S. ornatus*, Tilden Park, Contra Costa Co., California; b – MVZ 119097, *S. ornatus*, salt marsh, 2 mi E Martinez, Contra Costa Co., California); state 2 (c – MVZ 107816, *S. ornatus*, mouth Salinas River, Monterey Co., California; d – MVZ 96141, *S. vagrans*, 3 mi W Inverness, Marin Co., California); and state 3 (e – MVZ 101800, *S. vagrans*, Pierce Ranch, Tomales Point, Marin Co., California); f – MVZ 3639, *S. vagrans*, Palo Alto, Santa Clara Co., California). The horizontal white lines are tangential to the upper boundary of the medial tine pigment; note that in state 1, this boundary is also confluent with the main upper boundary, but in state 3, the medial tine pigment is elevated above and separated from that of the main incisor shaft.

analyses. Values for a* and b* were converted to C* (chroma, or relative saturation) as the square root of $a^{*2} + b^{*2}$, and h^o (hue, or angle of the hue in the CIELab color wheel) was converted as $h^o = \arctan (b^*/a^*)$.

Statistical procedures.—Univariate and multivariate routines in JMP-Pro (version 14; SAS Institute Inc., Cary, North Carolina) were used for all morphological analyses. These included univariate summaries of cranio-mandibular and colorimetric variables and frequencies of caudal elements and upper incisor tine scores. Multivariate principal components (PCA) and canonical variates analyses (CVA) of $\log_{10}$ transformed cranio-mandibular variables generated reduced-axis perspectives of overall variable differences among pre-defined reference groups. In several CVA, some locality samples were treaded as unknown, with their posterior probabilities used to determine the relationship of each unknown specimen to the respective reference groups. Finally, X^2 contingency tests were used to compare categorical variable distributions among samples, and the null hypothesis was evaluated by likelihood ratio tests. All skulls were examined under a dissecting microscope for the presumptive diagnostic traits (Table 1) before assembling the sets of samples used throughout. To minimize the likelihood of Type 1 error, all multiple comparisons used Bonferroni corrected *P*-values for the rejection of the null hypothesis (Dunn 1961; Rice 1989).

RESULTS

mtDNA Phylogeography

Phylogeographic groups recovered from all available *Cyt*b sequence data and their geographic distributions are depicted in Figure 4 (localities and voucher specimens number of the new sequences are listed in Appendix A, S1). Note that the three (Southern, Central, and Northern) clades delineated by Maldonado et al. (2001) remain intact, each with substantial internal support (95 to 100% bootstrap support). The Northern clade, however, now expands geographically to include all specimens of *S. vagrans* from northern California, including those from the Sierra Nevada and adjacent wetlands to the east of that montane axis. The few new specimens from localities in Marin, Sonoma, and Solano counties also possess Northern clade haplotypes, as did those of *S. o. sinuosus* examined by Maldonado et al. (2001).

Trait Differences between *S. ornatus* and *S. vagrans*

Univariate character differences.—The utility of univariate characters posited by previous authors (Table 1; Grinnell 1913; Jackson 1928; Ingles 1965; Junge and Hoffmann 1981) as diagnostic in distinguishing these two species was evaluated from pooled data for 408 *S. ornatus* and *S. vagrans* from the San Francisco Bay Area. The *S. ornatus* specimens included those from Alameda and Contra Costa counties, both upland and Martinez marsh samples (combined n = 196); the *S. vagrans* sample included all specimens from coastal Marin County (combined n = 212; localities and included specimens listed in Appendix A, S3).

Early workers had a perceptive eye. Bonferroni corrected P-values at $\alpha = 0.005$ derived from a one-

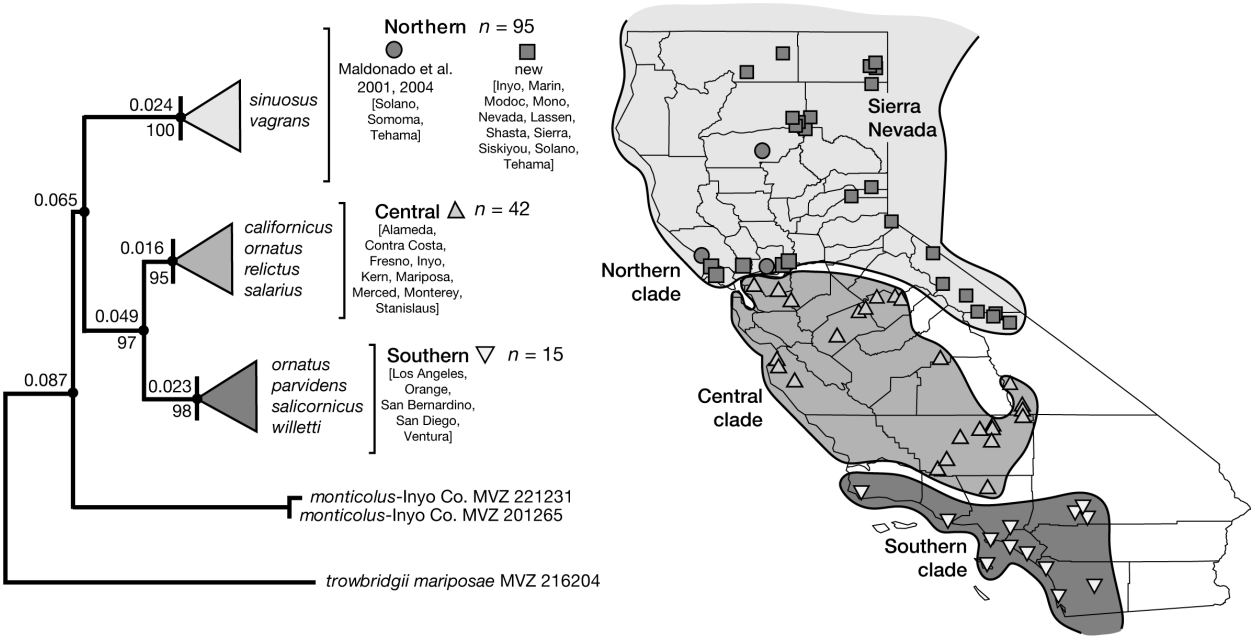

Figure 4. Left: Simplified minimum evolution phylogram of clade relationships among California samples of the *S. ornatus–S. vagrans* complex. Numbers above each node are mean p-distances for each included group of sequences; those below nodes are bootstrap values when > 95. Clades are identified as per Maldonado et al. (2001), with numbers of sequences included in each indicated. For the Northern clade, circles identify the original data from Maldonado et al. and squares are new sequences from North Bay and Sierra Nevada specimens of *S. vagrans*. Infraspecific taxa currently assigned to specimens in each clade are listed, as are the counties within California where specimens were collected. Note that the Northern clade remains paraphyletic, as it includes all *S. vagrans* sequences as well as those allocated to *S. o. sinuosus* (Solano County). The tree is rooted by sequences of *S. trowbridgii* and *S. monticolus*. Right: Distribution of sample localities of each of clade depicted in the phylogram.

way ANOVA reject the null hypothesis of statistical equivalence in the comparison of the two species for each variable listed in Table 1. *Sorex ornatus* is a smaller shrew with a slightly, but significantly shorter tail (as indicated by tail length obtained from specimen labels or counts of the number of caudal vertebrae in preserved skeletons; Table 2). It also has a flatter skull (as indexed by lesser cranial depth in lateral view [CD] and shorter cranial height in occipital view [CHo]), a narrower braincase (CB), a foramen magnum extending higher onto the occiput (FMoH and ratio FMoH/CHo), and, as a corollary, a shallower foramen magnum when viewed ventrally (FMvL). Furthermore, and consistent with statements by Junge and Hoffmann (1981) and Owen and Hoffmann (1983), these two species differ in the distribution of I1 medial tine pigmentation states, with state 1 characterizing the majority of *S. ornatus* and either state 2 or 3 characterizing *S. vagrans* (Table 2; raw counts of all samples given in Appendix B, Table SB1).

Color differences between San Francisco Bay Area S. ornatus *(including* sinuosus *Grinnell) and* S. vagrans.—Color differences in the dorsal pelage among populations of shrews in the San Francisco Bay region have formed much of the basis for their current taxonomic assignments. Rudd (1955) posited that shrews from tidal marshlands along the northern shore of San Pablo and Suisun bays that were intermediate in dorsal pelage darkness were hybrids between what he regarded as three species, the melanic *S. sinuosus* from Grizzly Island and both the paler *S. ornatus* from the East Bay Contra Costa County and *S. vagrans* from Marin County. Junge and Hoffmann (1981:31) subsequently stated that dark individuals of both *S. ornatus* and *S. vagrans* occupied all salt marshes fringing the San Francisco Bay Area. These authors also concluded "the salt marshes of the 'North Bay' from Martinez to Tolay Creek are occupied by *S. ornatus sinuosus*."

CIELab color attributes were examined for 125 shrews of *S. o. californicus*, S. *o. sinuosus*, *S. v. paludivagus*, *S. v. halicoetes*, and *S. v. vagrans* (localities and included specimens are listed in Appendix A, S2 and sample statistics in Appendix B, Table SB2). Seasonal differences were first tested for all color parameters by separating specimens into wet (= winter: October through March) and dry (= summer: April through September) seasonal cohorts, consistent with California's Mediterranean annual climate pattern. In separate analyses for each sample with 17 or more specimens, and with similar numbers of specimens from each season, seasonal color differences were found only in the sample of *S. o. sinuosus* from Grizzly Island, Solano County. For this taxon, winter specimens were darker (mean L* = 8.6) and less saturate (mean C* = 6.1) than summer specimens (mean L* = 10.4 and mean C* = 8.4), both significant by one-way ANOVA at $P = 0.007$ and 0.002, respectively. No sample of *S. vagrans* from Marin (*S. v. vagrans*), San Francisco (*S. v. paludivagus*), or Santa Clara (*S. v. halicoetes*) counties exhibited seasonal differences, nor did the two samples of *S. ornatus* from Contra Costa County (upland *S. o. californicus* and Martinez marsh). Among the three samples of *S. vagrans*, *P*-values obtained from one-way ANOVA for L*, C*, and h^o were 0.292, 0.088, and 0.201, respectively; corresponding *P*-values for the two *S. ornatus* samples were 0.186, 0.154, and 0.170. Despite the seasonal differences in the *S. o. sinuosus* sample, pooling these in the comparison among the five Bay Area shrew taxa did not affect the following results.

Based on one-way ANOVAs and pairwise Tukey post-hoc tests, with Bonferroni corrected *P*-values at $\alpha = 0.005$, Grizzly Island *S. o. sinuosus* is uniformly separated from the other four taxon samples for all three dorsal pelage color characters, significantly darker (mean L* = 9.12; $P < 0.0001$ in each pairwise comparison), less saturate (mean C* = 6.79; $P < 0.0001$), and with a more reddish hue (mean $h^o = 0.932$; *P* ranged from 0.0028 [*S. o. sinuosus* vs. *S. v. vagrans*] to < 0.0001 [all other comparisons]; Table 3). Differences in lightness and saturation are visible to the eye; hue differences are slight and were not apparent when study skins are compared. San Francisco Bay marsh *S. v. halicoetes* also is significantly darker and less saturate than either other samples of this species or *S. o. californicus* (mean L* = 14.72; *P* ranged from 0.0009 [*S. v. halicoetes* vs. *S. v. paludivagus*] to < 0.0001 [*S. v. halicoetes* to both *S. o. californicus* and *S. v. vagrans*]; mean C* = 14.03; $P < 0.0001$ in all three pairwise comparisons]), but does not differ from any of them in hue (*P* ranged from 0.294 [comparison to *S. v. vagrans*] to 0.687 [comparison to *S. o. californicus*]). *Sorex o. californicus* cannot be distinguished from *S. v. vagrans* by any dorsal color variable (Table 3).

Table 2. Mean, standard error, range, and sample sizes for each of the seven traits listed in Table 1 that have been posited as diagnostic for *S. ornatus* and *S. vagrans*. Samples are those from the San Francisco Bay Area: *S. ornatus* from the East Bay Alameda and Contra Costa counties, and *S. vagrans* from the San Francisco Peninsula, San Francisco Bay marshes, and Point Reyes in Marin County. Significance levels are derived from one-way ANOVA for mensural variables, at a Bonferroni corrected $\alpha = 0.005$. Data for both the number of caudal vertebrate and upper incisor 1 medial tine states are the median and range; significance is based on X^2 contingency tests.

Variable	*S. ornatus*	F-value / p-value	*S. vagrans*
total length (TOL)	97.8 ± 0.5 82 – 108 *n* = 101	$F_{1, 292} = 28.02$ $P < 0.0001$	101.2 ± 0.4 87 – 114 *n* = 292
tail length (TAL)	35.3 ± 0.3 27 – 41 *n* = 101	$F_{1, 292} = 220.55$ $P < 0.0001$	40.4 ± 0.2 32 – 49 *n* = 292
number of caudal vertebrae	14 11 – 15 *n* = 53	$X^2_5 = 62.28$ $P < 0.0001$	15 13 – 16 *n* = 225
lateral profile (CD)	4.66 ± 0.02 3.61 – 5.46 *n* = 196	$F_{1, 407} = 932.10$ $P < 0.0001$	5.55 ± 0.02 4.47 – 6.20 *n* = 212
occipital profile (CHo)	4.05 ± 0.02 3.48 – 4.78 *n* = 196	$F_{1, 407} = 892.95$ $P < 0.0001$	4.85 ± 0.02 3.97 – 5.41 *n* = 212
braincase breadth (CB)	7.75 ± 0.02 7.22 – 8.58 *n* = 196	$F_{1, 407} = 434.63$ $P < 0.0001$	8.18 ± 0.01 7.59 – 8.77 *n* = 212
height of foramen magnum, occipital view (FMoH)	2.47 ± 0.02 1.95 – 3.03 *n* = 196	$F_{1, 407} = 13.97$ $P = 0.0002$	2.38 ± 0.01 1.84 – 2.91 *n* = 212
ratio FMoM/CHo	0.609 ± 0.004 0.432 – 0.789 *n* = 196	$F_{1, 407} = 431.15$ $P < 0.0001$	0.493 ± 0.0049 0.391 – 0.626 *n* = 212
length of foramen magnum, ventral view (FMvL)	1.71 ± 0.01 1.29 – 2.22 *n* = 196	$F_{1, 407} = 503.92$ $P < 0.0001$	2.12 ± 0.01 1.56 – 2.65 *n* = 212
upper incisor 1 medial tine	1 1 – 2 *n* = 141	$X^2_2 = 271.1$ $P < 0.0001$	3 1 – 3 *n* = 252

Table 3. Minimally non-significant subsets for the five samples of Bay Area taxa (species or subspecies) of the *Sorex ornatus–Sorex vagrans* complex, based on one-way ANOVA and Tukey post-hoc tests, with significance among subsets at a Bonferroni corrected $\alpha < 0.001$, for each of the three dorsal color attributes (L* = paleness; C* = chroma; and h^o = hue) and canonical variates 1 scores (CV-1). Sample means of each color variable and mean CV-1 score for each *a posteriori* defined taxon are given.

	L* (paleness)			C* (saturation)			h^o (hue)			CV-1 scores		
	subset	mean		subset	mean		subset	mean		subset	mean
vagrans	A	21.95	*paludivagus*	A	21.51	*halicoetes*	A	1.143	*vagrans*	A	1.462
californicus	A	21.18	*vagrans*	A	21.13	*californicus*	A	1.105	*californicus*	A	1.218
paludivagus	B	18.09	*californicus*	A	20.39	*paludivagus*	A	1.099	*paludivagus*	B	0.253
halicoetes	C	14.72	*halicoetes*	B	14.03	*vagrans*	A	1.077	*halicoetes*	C	-0.878
sinuosus	D	9.12	*sinuosus*	C	6.79	*sinuosus*	B	0.932	*sinuosus*	D	-2.773

In a CVA (not shown) comparing these five taxon samples based on the dorsal pelage color parameters L*, C*, and h^o, the first axis explains 79.4% of the total pool of variation with L* contributing by far the most heavily (standardized L* coefficient = 0.959 compared to 0.049 and 0.027, respectively). This analysis strongly separates *S. o. sinuosus* from each of the other four samples along the first canonical axis, with CV-1 scores identifying the same minimally non-significant subsets as the single variable L* alone, again defined by Tukey post-hoc tests following a one-way ANOVA at a Bonferroni corrected α of 0.005 (Table 3). Thus, Grinnell's *sinuosus* differs equally strongly (at $P < 0.0001$) from *S. o. californicus* and *S. v. vagrans*, and less so from both *S. v. paludivagus* and especially *S. v. halicoetes*, the latter which Grinnell (1913:184) remarked "resembles *S. sinuosus* in notably blackish coloration; but *sinuosus* is most extreme in this respect."

S. ornatus and *S. vagrans* of the San Francisco Bay Area

Shrews of the *S. ornatus – S. vagrans* complex from the San Francisco Bay Area present a complex set of character discordances, several of which have been either alluded to (e.g., Junge and Hoffmann 1981; Owen and Hoffmann 1983) or detailed (e.g., Rudd 1955; Brown and Rudd 1981) in earlier publications. The following is a three-part analysis of the shrews from this region, each focused on a set of prior questions or hypotheses concerning shrews in smaller geographic areas within this large area. Sample localities are mapped in Figs. 5 and 6 (see Appendix A, S3 for list of localities and specimen catalog numbers). Appendix B provides state distributions for the upper incisor medial tine pigment patterns (Table SB1), sample means, standard error, range, and samples sizes for dorsal pelage color (Table SB2) and cranio-dental variables (Table SB3).

*Five reference groups (*S. v. vagrans*,* S. v. paludivagus, S. v. halicoetes, S. o. californicus, *and* S. o. sinuosus*).*— Principal Components Analysis (PCA) was used to reduce the 25 log-transformed craniomandibular variables to a few multivariate axes and tested the commonality of the five-taxon samples in *a posteriori* comparisons (Table 4 provides eigenvectors, eigenvalues, and percent contribution for the first four PC axes, which combine to explain 62.02% of the variation). This analysis included 483 specimens (taxon

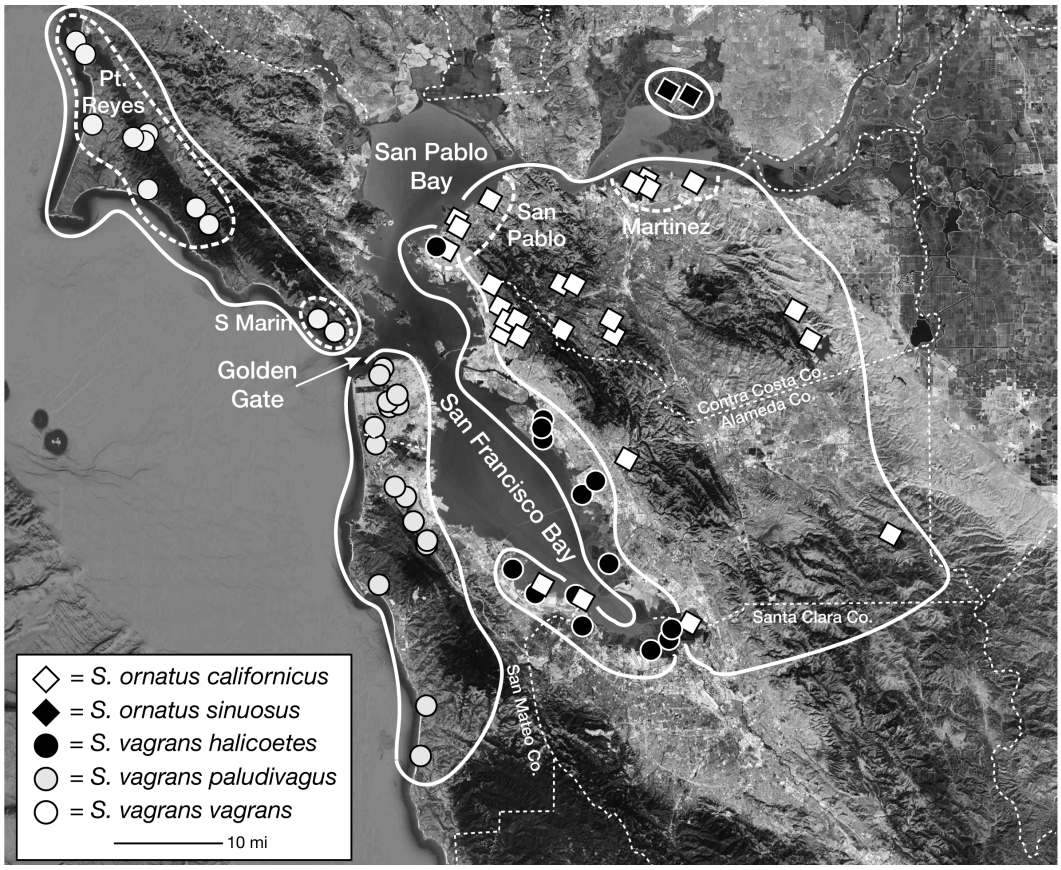

Figure 5. Sample localities of *S. vagrans* and *S. ornatus* in the greater San Francisco Bay Area, excluding those from the tidal marshes of the North Bay mapped in Figure 6. The two coastal Marin County samples of *S. v. vagrans* are bounded separately, as are the San Pablo and Martinez marsh samples of *S. o. californicus* (heavy dashed lines), which initially were treated as unknown in a CVA designed to allocate them to either *S. o. californicus* or *S. o. sinuosus* (see text; localities listed in Appendix A, S3).

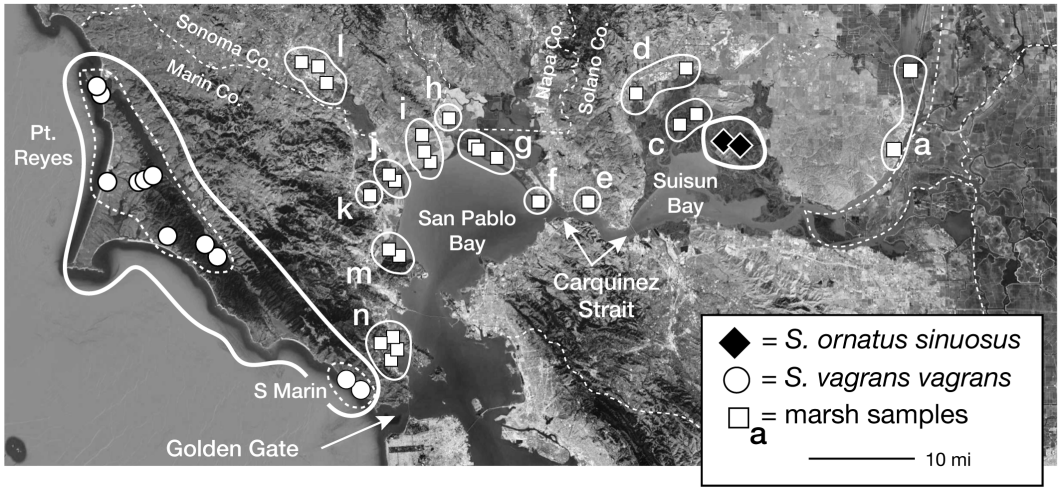

Figure 6. Map of the North Bay shrew localities. Reference samples of *S. o. sinuosus* and *S. v. vagrans* (S Marin and Pt. Reyes) are bounded by heavy white lines; letters identify tidal and other marsh samples treated as unknown in the CVA (see text; localities listed in Appendix A, S3).

Table 4. Standardized scoring coefficients of five taxa (*S. o. californicus*, *S. o. sinuosus*, *S. v. halicoetes*, *S. v. paludivagus*, and *S. v. vagrans*) of shrews from the greater San Francisco Bay Area for the first four principal components (PC) axes, with eigenvalues and percent explained variation given below. Variable scores highlighted in bold are those that contribute most to specimen dispersion on PC-1 and PC-2.

Variable	PC-1	PC-2	PC-3	PC-4
Log_{10} [GSL]	**0.297**	-0.025	-0.044	-0.037
Log_{10} [CB]	**0.259**	-0.097	0.192	-0.052
Log_{10} [IOC]	0.179	0.133	0.154	0.065
Log_{10} [RB]	0.042	**0.371**	0.280	-0.141
Log_{10} [CBL]	**0.297**	-0.022	-0.083	-0.036
Log_{10} [PL]	**0.252**	0.095	-0.242	-0.062
Log_{10} [UniTRL]	0.141	0.037	-0.318	0.001
Log_{10} [P4-M3L]	**0.245**	0.127	-0.180	-0.005
Log_{10} [upperTRL]	**0.259**	0.119	-0.308	0.020
Log_{10} [U1B]	0.013	**0.444**	0.245	-0.095
Log_{10} [M2B]	0.184	**0.332**	0.165	-0.013
Log_{10} [basiW]	0.141	0.050	0.232	0.080
Log_{10} [FMvL]	0.165	**-0.424**	0.107	-0.082
Log_{10} [FMvW]	0.174	-0.204	0.129	0.428
Log_{10} [CD]	**0.226**	**-0.296**	0.128	-0.041
Log_{10} [ZPW]	0.155	-0.103	-0.036	-0.209
Log_{10} [manL]	**0.270**	0.089	-0.176	-0.018
Log_{10} [manTRL]	**0.232**	0.127	-0.269	0.042
Log_{10} [m1L]	0.139	0.131	-0.200	0.107
Log_{10} [HCP]	0.211	0.047	0.265	-0.135
Log_{10} [HCV]	0.178	0.017	0.314	-0.104
Log_{10} [HAC]	0.205	0.019	0.182	-0.099
Log_{10} [CHo]	**0.232**	**-0.276**	0.104	0.026
Log_{10} [FMoH]	-0.016	0.208	-0.019	0.413
Log_{10} [FMoW]	0.088	0.022	0.149	0.702
eigenvalue	9.639	2.498	1.992	1.274
% contribution	38.56	9.99	7.97	5.50

samples and included localities are given in Appendix A, S3). Cranial and mandibular length variables, such as GSL, CBL, PL, upperTRL, manL, and manTRL, have the greatest influence on the dispersion of individual specimen scores on the first axis; rostral width variables (RB and U1B) and those of both cranial height (CD, CHo) and foramen magnum height, width, and length (FMoH, FMvW, FMvL) provide greatest influence on the second axis (Table 4). This set of variables includes those treated by earlier workers, and confirmed above, as diagnostic in distinguishing these two species of shrews (see Tables 1 and 2 and accompanying text).

The two samples of *S. v. vagrans* (Pt. Reyes and S Marin; Fig. 5) do not differ in a one-way ANOVA comparing their respective PC-1 and PC-2 scores ($P = 0.853$ and 0.301, respectively). These specimens were thus combined into a single *S. v. vagrans* sample for comparison to other Bay Area taxa in subsequent analyses.

Combined PC-1 and PC-2 scores do perform well in distinguishing between the five Bay Area taxa of the *S. ornatus – S. vagrans* complex when their respective sample scores were grouped in an *a posteriori* one-way ANOVA using pairwise Tukey post-hoc tests, with Bonferroni corrected *P*-values at $\alpha = 0.005$ (Table 5). PC-1 groups *S. v. halicoetes* and *S. v. paludivagus*, separates *S. o. californicus* from all other samples, and the *S. v. vagrans* sample provides a bridge between the other two *S. vagrans* samples and *S. o. sinuosus*. PC-2 groups the two samples of *S. ornatus* as distinct from the three *S. vagrans*. For PC-3, while the sample of *S. o. californicus* separates from *S. o. sinuosus* (at $P = 0.0005$), the three samples of *S. vagrans* provide an overlapping bridge between these two extremes.

A CVA using all 25 cranio-mandibular variables delineated two axes that combine to explain 91.1% of the total variance (Table 6, which also highlights those variables with the highest loadings on both axes). The bivariate plot depicting 50% ellipses of CV-1 and CV-2 specimen scores clearly separated *S. o. californicus* from the three samples *of S. vagrans*, with *S. o. sinuous* placed in a somewhat intermediate position (Fig. 7). Tukey post-hoc tests from one-way ANOVA on the distribution of CV-1 scores (with Bonferroni corrected *P*-values at $\alpha = 0.005$) indicate that of the three *S. vagrans* samples, shrews from the San Francisco Peninsula (both the upland *S. v. paludivagus* and marsh *S. v. halicoetes*) are statically homogeneous ($P = 0.487$) and that *S. v. paludivagus* is identical to *S. v. vagrans* ($P = 0.452$) but *S. v. halicoetes* and *S. v. vagrans* are divergent ($P < 0.0001$). Nevertheless, the three *S. vagrans* samples are strongly separated from both *S. o. sinuosus* and *S. o. californicus* ($P < 0.0001$ in each pairwise comparison), as are the latter two from each other (also $P < 0.0001$). CV-2 scores fail to separate *S. o. sinuosus* from *S. o. californicus* ($P = 0.167$) but the three *S. vagrans* samples are separable on this axis (pairwise $P < 0.0001$ in each comparison).

Both *S. o. californicus* and *S. o. sinuosus* lack character state 3 of the first upper incisor medial tine (Appendix B, Table SB1), although these two samples differ notably in the number and proportion of states

Table 5. Minimally non-significant subsets for the five samples of Bay Area taxa (species or subspecies) of the *Sorex ornatus – Sorex vagrans* complex, based on one-way ANOVA and Tukey post-hoc tests, with significance among subsets at a Bonferroni corrected $\alpha < 0.001$, for the first three Principal Components axes. Mean sample scores for each *a posteriori* defined taxon are given for each PC axis.

PC-1			PC-2			PC-3		
	subset	mean		subset	mean		subset	mean
halicoetes	A	2.557	*sinuosus*	A	1.208	*californicus*	A	0.984
paludivagus	A	2.525	*californicus*	A	1.177	*halicoetes*	AB	0.659
vagrans	AB	1.587	*paludivagus*	B	-0.749	*halicoetes*	BC	0.260
sinuosus	B	0.413	*halicoetes*	B	-0.924	*vagrans*	BC	-0.050
californicus	C	-2.127	*vagrans*	B	-1.028	*sinuosus*	C	-0.493

Table 6. Standardized scoring coefficients of five taxa (species or subspecies) of shrews from the greater San Francisco Bay Area for the first three conical variates (CV) axes, with eigenvalues and percent explained variation given below. Coefficients highlighted in bold are those that contribute most to the dispersion of the five taxa on CV-1 and CV-2 axes depicted in Figure 5.

Variable	CV-1	CV-2	CV-3	CV-4
Log_{10} [GSL]	0.125	**0.335**	-0.344	0.005
Log_{10} [CB]	**0.438**	**0.332**	-0.230	-0.002
Log_{10} [IOC]	0.193	-0.203	-0.046	0.227
Log_{10} [RB]	**-0.257**	-0.123	-0.132	-0.112
Log_{10} [CBL]	-0.166	-0.061	0.289	-0.350
Log_{10} [PL]	-0.195	0.181	-0.271	0.488
Log_{10} [UniTRL]	0.003	**-0.460**	0.184	0.422
Log_{10} [P4-M3L]	**0.325**	-0.010	0.009	-0.349
Log_{10} [upperTRL]	**-0.248**	0.190	-0.216	-0.165
Log_{10} [U1B]	**-0.388**	0.184	0.291	0.174
Log_{10} [M2B]	0.019	0.076	0.252	-0.222
Log_{10} [basiW]	0.009	**0.452**	0.169	0.077
Log_{10} [FMvL]	0.173	0.205	0.626	-0.061
Log_{10} [FMvW]	-0.077	0.123	-0.292	-0.021
Log_{10} [CD]	**0.410**	-0.076	0.372	0.246
Log_{10} [ZPW]	0.105	0.155	-0.069	-0.213
Log_{10} [manL]	**0.335**	-0.132	-0.369	0.615
Log_{10} [manTRL]	0.012	0.059	0.092	-0.531
Log_{10} [m1L]	-0.057	-0.260	-0.122	0.279
Log_{10} [HCP]	-0.095	**-0.692**	0.611	0.265
Log_{10} [HCV]	-0.043	**0.661**	-0.413	0.079
Log_{10} [HAC]	0.055	**-0.398**	0.170	-0.229
Log_{10} [CHo]	0.290	**-0.433**	-0.339	-0.030
Log_{10} [FMoH]	0.020	0.264	-0.111	0.417
Log_{10} [FMoW]	-0.067	0.250	0.601	0.207
eigenvalue	3.221	0.839	0.234	0.161
% contribution	72.29	18.84	5.27	3.61

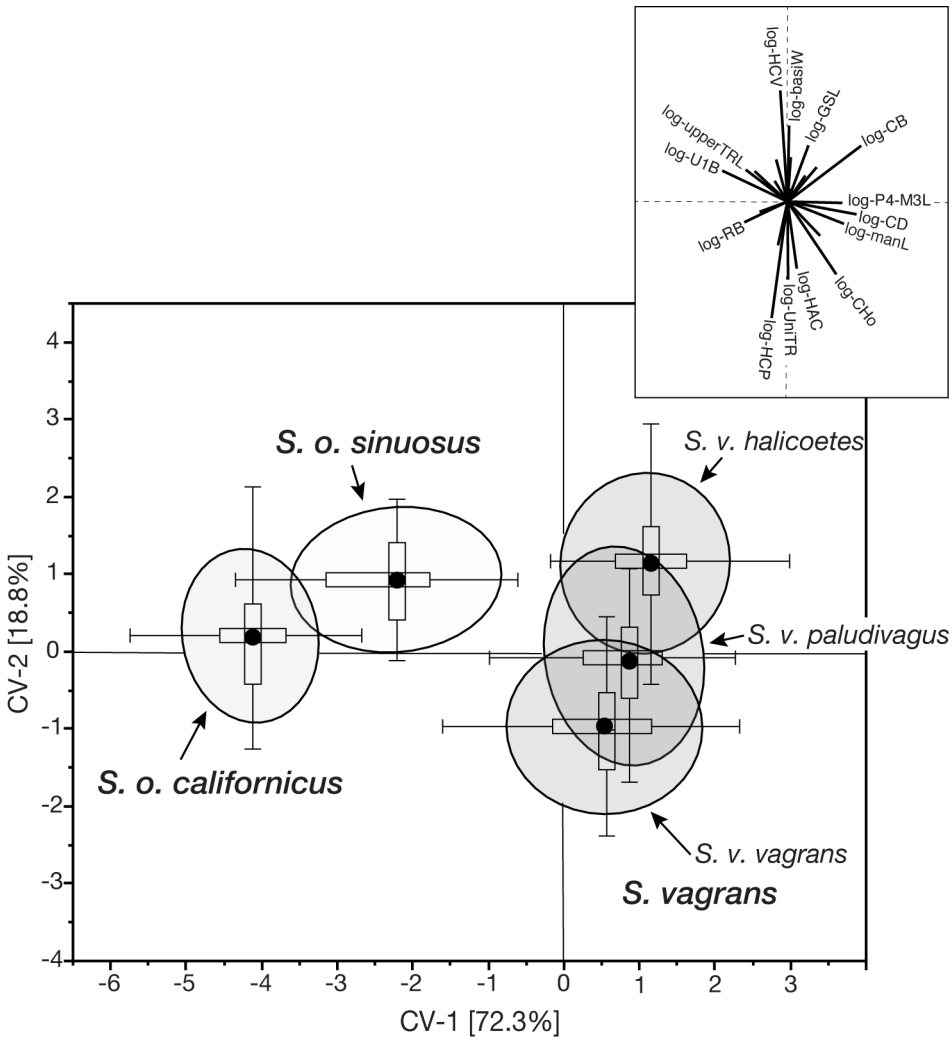

Figure 7. Bivariate plot of CV–1 and CV–2 scores for samples of *S. o. californicus* (East Bay upland, on the left), *S. o. sinuosus* (Grizzly Island, in the middle), and the three samples of *S. vagrans* on the right (*S. v. halicoetes* [San Francisco Bay marshes], *S. v. paludivagus* [San Francisco Peninsula upland], and *S. vagrans* [coastal Marin County; S Marin and Pt. Reyes combined]). Data are presented as ellipses that encompass 50% of sample specimen scores; mean sample scores (black circles); and overlapping box-plots that illustrate the skew in sample scores on both CV axes. These two axes combine to explain 91.1% of the total variation in the data. The inset in the upper right illustrates character vectors that determine the dispersion of specimen scores in this plot; only those expressing the highest loadings, as indicated by line length, are identified (see Table 6).

1 (83%, n = 35 vs. 40%, n = 15, respectively) and 2 (17% vs. 60%). All three samples of *S. vagrans* contain specimens with tine state 3, which has been considered diagnostic for this species, but a few individuals in each sample exhibit state 1 and a substantial number have the intermediate state 2. The distribution of the character states for the five Bay Area reference samples in three separate X^2 contingency analyses was compared using a likelihood ratio test. Overall, the five taxa exhibited highly significant differences in character state distributions ($X^2_8 = 155.28$, $P < 0.0001$). In separate analyses, tine state distributions were significantly different between *S. o. californicus* and *S. o. sinuosus* ($X^2_1 = 8.83$, $P = 0.003$) but not among the three samples of *S. vagrans* ($X^2_4 = 9.03$, $P = 0.060$). Each of the three *S. vagrans* samples differed in tine state distribution from both *S. o. californicus* (X^2_2 ranged from 55.78 to 107.69, $P < 0.0001$ in each pairwise comparison) *and* *S. o. sinuosus* (X^2_2 ranged from 22.79 to 29.04, $P < 0.0001$ in pairwise comparisons).

East Bay Martinez marsh S. ornatus *vis-à-vis upland* S. o. californicus *and* S. o. sinuosus.—Junge and Hoffmann (1981) and Owen and Hoffmann (1983), based primarily on stated similarity of dark dorsal pelage, assigned specimens from the salt marshes near Martinez on the south side of Suisun Bay to *S. o. sinuosus*. These authors, however, provided neither data nor analyses to support their assertion. Their hypothesis was evaluated by comparing the three CIE color attributes of the Martinez marsh *S. ornatus* (n = 31) to both upland *S. o. californicus* (n = 33) and the melanic Grizzly Island *S. o. sinuosus* (n = 17; sample localities and specimen numbers listed in Appendix A, S2, and color values in Appendix B, Table SB2).

Martinez marsh *S. ornatus* differ neither in pelage lightness (L*) nor chroma (C*) from *S. o. californicus* ($F_{1, 63} = 0.435$; $P = 0.512$ and $F_{1, 63} = 0.066$; $P = 0.799$, respectively) but is decidedly divergent from *S. o. sinuosus* in both attributes (L*: $F_{1, 47} = 227.60$, $P < 0.0001$; C*: $F_{1, 47} = 117.76$, $P < 0.0001$). Hue differs among all three samples (h^o: $F_{2, 80} = 10.25$, $P = 0.0001$). In a CVA based on these three attributes, and treating the Martinez specimens as unknown, these individuals are uniformly assigned to *S. o. californicus* (mean posterior probability = 0.976, 95% confidence limits 0.943–1.000) and not to *S. o. sinuosus* (mean posterior probability = 0.024, 95% confidence limits 0.000–0.057). Martinez marsh *S. ornatus* cannot be assigned to *S. o. sinuosus* by color attributes alone, as originally posited by Junge and Hoffmann (1981).

Other morphological characters also indicate that specimens from the Martinez marsh are best allocated to *S. o. californicus* than *S. o. sinuosus*. For example, the predominant tine state for both the Martinez marsh sample and *S. o. californicus* is state 1 (Appendix B, Table SB1), and the distribution of states in these two samples is not significantly different ($X^2_1 = 3.69$, $P = 0.055$). Alternatively, the majority of *S. o. sinuosus* specimens have tine state 2 and a state distribution different from the Martinez marsh sample ($X^2_1 = 20.27$, $P < 0.0001$). In a CVA of the 25 cranio-mandibular variables using the *S. o. californicus* and *S. o. sinuosus* samples as reference groups to which each specimen from the Martinez marsh sample was then compared (analysis not shown), the latter unambiguously align with *S. o. californicus* (mean posterior probability = 0.922; 95% confidence limits 0.884–0.960) and not to *S. o. sinuosus* (mean posterior probability = 0.078; 95% confidence limits 0.040–0.116).

A global view of S. ornatus *and* S. vagrans *of the San Francisco Bay Area*.—The unequivocal linkage of the Martinez marsh sample of *S. ornatus* to the adjacent upland *S. o. californicus* based on color characteristics of their dorsal pelage forced a reconsideration of the status of *sinuosus* Grinnell as a potentially valid species-level entity. To do this, the five-taxon CVA described above and depicted in Fig. 7 was rerun using the same samples of *S. o. californicus*, *S. o. sinuosus*, *S. v. halicoetes*, *S. v. paludivagus*, and *S. v. vagrans* as reference groups but treating as unknown all marshland samples that fringe San Pablo and Suisun bays and the Sacramento–San Joaquin river delta (localities and samples mapped in Figs. 5 and 6). This analysis included 975 specimens (localities and specimens listed in Appendix A, S3).

The first CV axis explained 72.3% of the total pool of variation (Table 6). Sample CV-1 scores were compared by one-way ANOVA and Tukey post-hoc tests to visualize the pattern of similarity and dissimilarity of cranio-mandibular relationships of all samples from west to east across the San Francisco Bay Area (Fig. 8). Significance tests were applied to each geographically adjacent pair of samples across

the region, with a Bonferroni corrected α of 0.0025. Four geographically aligned sets of samples are apparent (separated by arrows in Fig. 8), each internally homogeneous, or mostly so, while differing from other groups at $P < 0.0001$: (1) the three taxa of *S. vagrans* (*S. v. halicoetes*, S. *v. paludivagus*, and *S. v. vagrans*; pairwise P values = 0.991 and 0.986); (2) the East Bay *S. o. californicus*, including upland and the San Pablo and Suisun Bay marsh samples plus the small sample a-Rio Vista from the Sacramento River delta (pairwise P values = 0.992 to 0999); (3) *S. o. sinuosus* and those to the immediate west around the northern

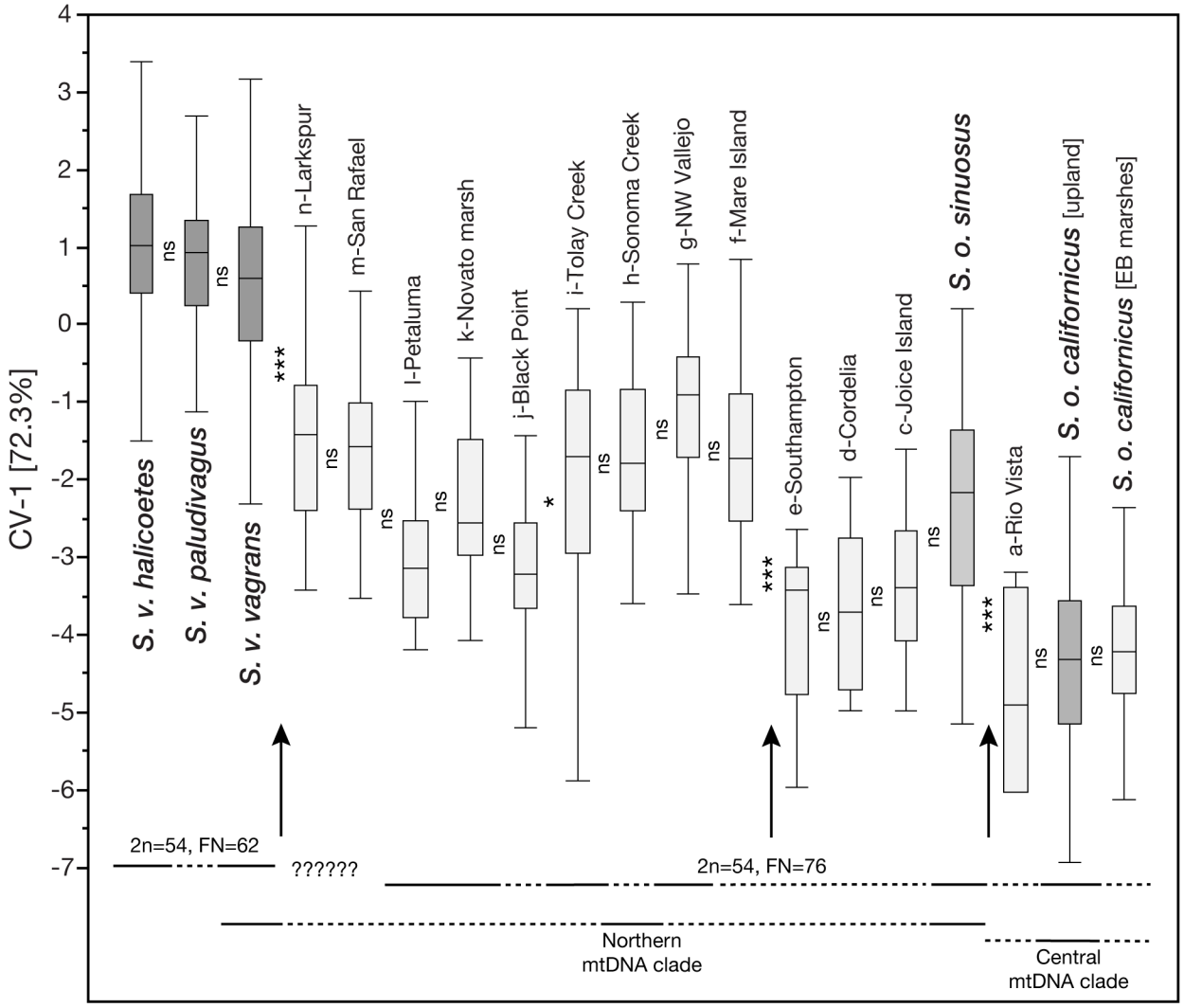

Figure 8. Box plots of CV-1 scores for all greater San Francisco Bay Area samples of the *S. ornatus–S. vagrans* complex. Samples are arranged generally from west to east (see Figures 5 and 6), except that the two East Bay *S. ornatus californicus* samples (upland Alameda and Contra Costa country and Martinez and San Pablo Bay marshes) are positioned on the far right, and the two San Francisco Bay *S. vagrans* taxa (marsh *S. v. halicoetes* and upland *S. v. paludivagus*) are positioned to the far left. Vertical arrows indicate major statistical break points, where geographically adjacent pairwise comparisons are *** = $P < 0.0001$. A minor break occurs between Tolay Creek and Black Point (pairwise * = $P = 0.002$). Across the bottom, solid lines encompass localities where the *S. vagrans* or *S. ornatus* karyotypes and/or mtDNA sequences have been described (karyotypes from Brown 1974 and Brown and Rudd 1981; mtDNA clades from Maldonado et al. 2001 and Figure 4).

side of Suisun Bay (c-Joice Island, d-Cordelia marsh, and e-Southampton Bay; pairwise P values = 0.032 to 0.177); and (4) samples from the tidal marshes that rim the north shore of San Pablo Bay (from f-Mare Island west to n-Larkspur; all but a single P-value ranged from 0.011 to 0.999). A minor difference (P = 0.0003) between i-Tolay Creek and j-Black Point disrupts the otherwise consistent similarities among this set of samples.

Figure 8 also illustrates the complexities of varying phenotypic intermediacy these samples represent and accentuate the discordance, based on available data, of both karyotype and mtDNA clade membership. The East Bay *S. o. californicus* differ from all others in belonging to the Central mtDNA clade (Fig. 4) yet share the same karyotype with *S. o. sinuosus*. All other North Bay samples, including *S. o. sinuosus*, for which karyotypes are known (Brown 1974; Brown and Rudd 1981) belong to the Northern mtDNA clade.

To further illustrate the degree of morphological intermediacy expressed by individual specimens from all population samples across the North Bay, ternary diagrams were constructed from posterior probabilities derived from a separate CVA using single reference samples of three Bay Area taxa (*S. o. californicus*, *S. o. sinuosus*, and *S. vagrans* [pooled samples of *S. v. halicoetes*, *S. v. paludivagus*, and *S. v. vagrans*]; sample statistics provided in Appendix B, Table SB4). Each reference sample, not surprisingly given that CVA minimizes within-group variance but maximizes that between groups, have high mean posterior probabilities to themselves (> 0.94 in each case), even though a few individuals do express some similarity to one or the other reference taxa (apparent in the distribution of individual specimen posterior probabilities visualized in ternary plots, Figs. 9 to 11). Importantly, though, individuals from each unknown sample exhibit different patterns of association with the reference taxa based on the geographic positions of their respective localities. Individuals of *S. ornatus* from San Pablo and Suisun Bay marshes in Contra Costa County align strongly with the geographically adjacent upland *S. o. californicus* (mean posterior probabilities of 0.959), a recapitulation of the results for the Martinez marsh samples on the south side of Suisun Bay described above. Note that each specimen posterior probability falls within, or very close to, the distribution of posterior probabilities of reference *S. o. californicus* (Fig. 9).

In contrast, North Bay tidal marsh samples comprise individuals with widely disparate posterior probabilities, but their separate distributions are more complex as their level of intermediacy may involve only two, or all three, reference samples. The eastern samples from Rio Vista (a) to Southampton Bay (e; see map, Fig. 6) group morphologically with either *S. o. californicus* or *S. o. californicus* and the *S. o. sinuosus* reference sample (Figs. 8 and 10). Average posterior probabilities of individual assignments of these four marsh samples to *S. o. californicus* and *S. o. sinuosus* are 0.466 and 0.517, respectively; values that are statistically indistinguishable (one-way ANOVA, Tukey post hoc tests, P = 0.720). These four samples exhibit virtually no influence from *S. v. vagrans* (average posterior probability, 0.017). In contrast, individuals from the marshes around the northern margins of San Pablo Bay express posterior probabilities that fall between the three reference samples (Fig. 11). Average individual posterior probabilities are statistically higher to *S. o. sinuosus* (mean posterior probability = 0.561; one-way ANOVA, Tukey post hoc tests, $P < 0.0001$) while assignments to *S. o. californicus* (mean posterior probability = 0.208) and *S. o. vagrans* (mean posterior probability = 0.231) are homogeneous (one-way ANOVA, Tukey post hoc test, P = 0.407).

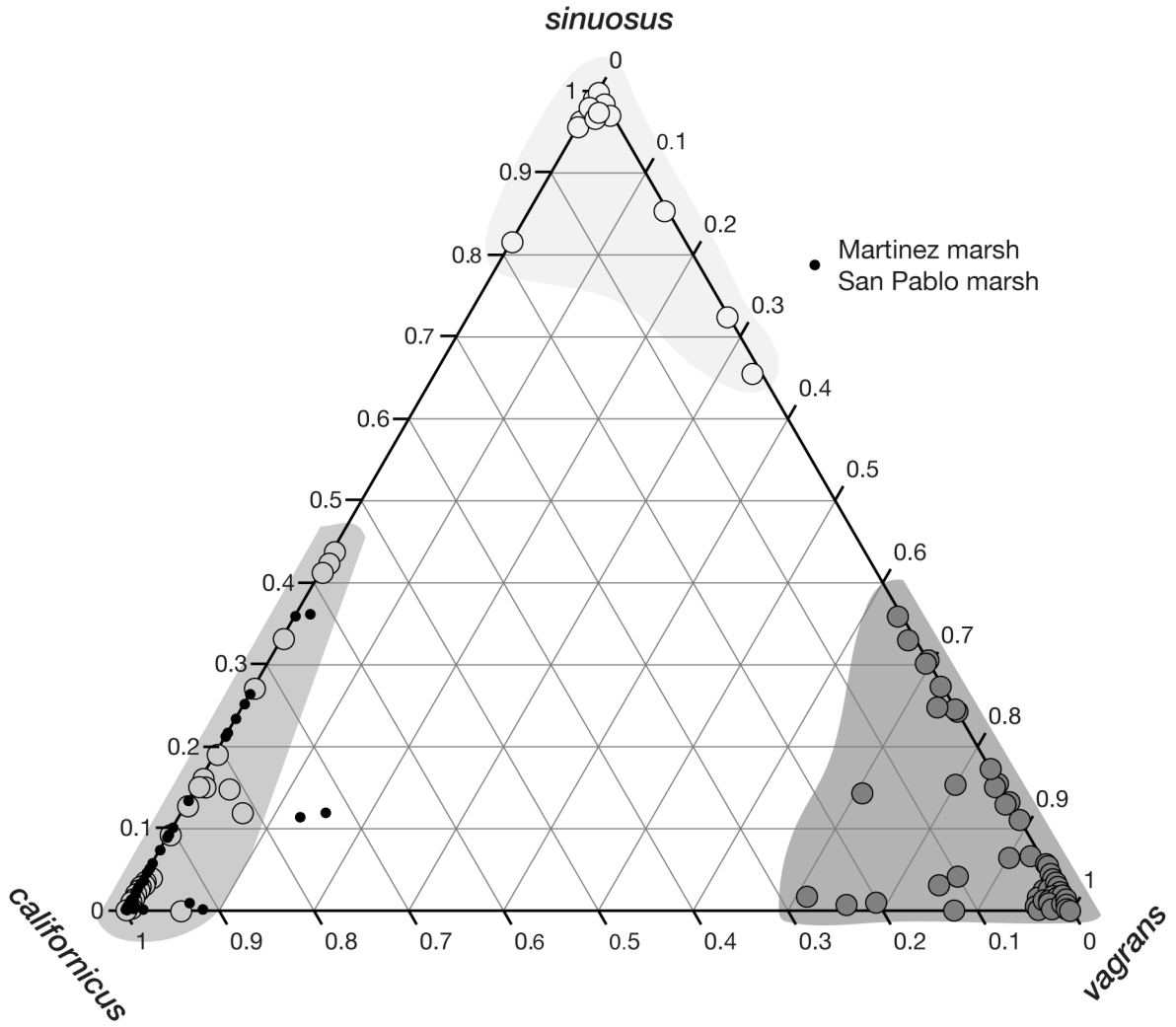

Figure 9. Ternary plot of the distribution of individual specimen posterior probabilities of the three reference samples (*S. o. californicus*, *S. o. sinuosus*, and the pooled *S. vagrans*) and both the Martinez and San Pablo Bay marsh samples treated as unknown. Large circles with unique shades of gray, and surrounded by similar shaded ellipses, identify each reference sample specimen; small black circles identify each individual from two marsh samples treated as unknowns in the CVA (see maps, Figures 5 and 6).

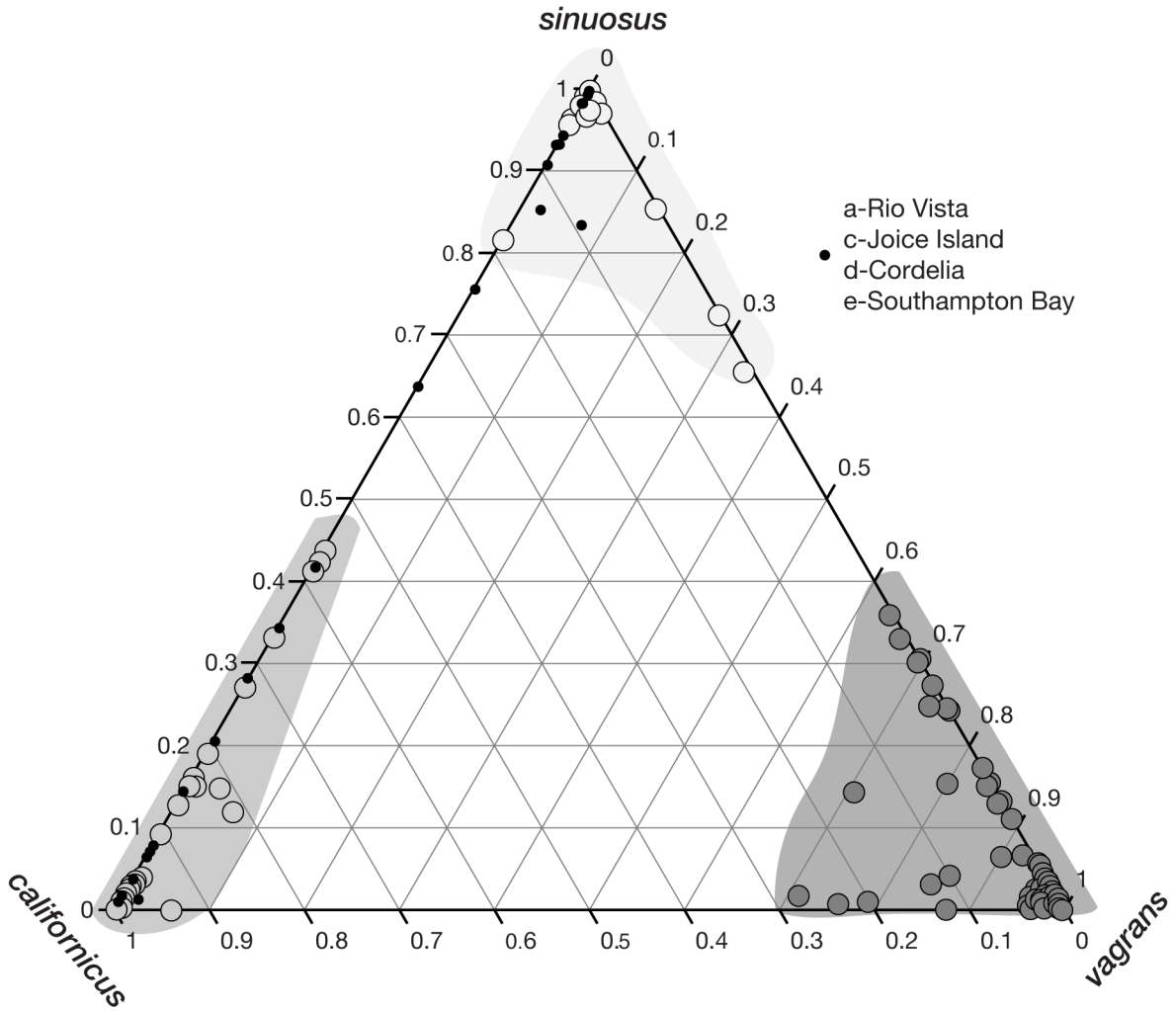

Figure 10. Ternary plot of the distribution of individual specimen posterior probabilities for marsh samples from the North Bay from Southampton Bay through Suisun Bay to the Sacramento River delta. As in Figure 9, large circles with unique shades of gray, and surrounded by similar shaded ellipses, identify each reference sample specimen; small black circles identify each individual from the four eastern North Bay marshes treated as unknowns in the CVA (see map, Figure 6).

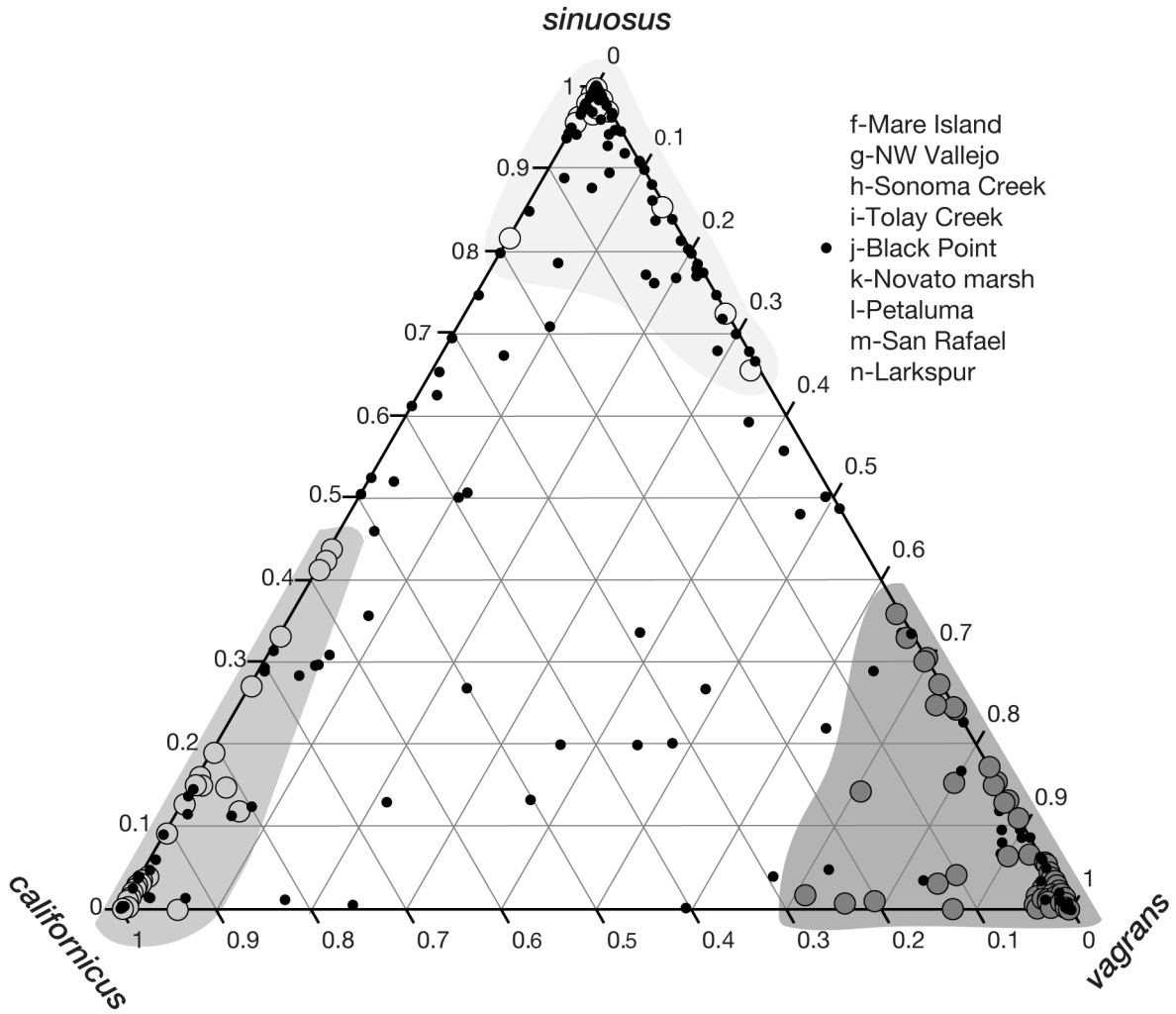

Figure 11. Ternary plot of the distribution of individual specimen posterior probabilities for tidal marsh that rim the northern margin of San Pablo Bay. As in Figure 9, large circles with unique shades of gray, and surrounded by similar shaded ellipses, identify each reference sample specimen; small black circles identify each individual of the nine San Pablo Bay samples, from Mare Island on the east to Larkspur on the west, treated as unknowns in the CVA (see map, Figure 6).

DISCUSSION

Two obvious conclusions derive from the results presented above; neither is novel, nor probably unexpected.

First, and excluding shrews from the tidal marshes around the northern rim of the greater San Francisco Bay region, the species *S. ornatus* and *S. vagrans* are delineated by trenchant morphological differences. The utility of those diagnostic traits enumerated in early publications and listed in Table 1, for example, is upheld — *S. ornatus* has a shorter, flatter skull, with a narrower braincase and a foramen magnum placed higher on the occiput so that it also is anteroposteriorly shorter in ventral view, and is characterized typically by pigmentation of the upper incisor medial tine contained wholly within that of the main incisor shaft (state 1) or, more rarely, with its proximal edge slightly elevated above (state 2; Fig. 3). In contrast, *S. vagrans* has a longer, more domed skull, wider braincase, and a foramen magnum placed lower on the occiput making it more expansive in ventral view. Its upper incisor medial tine is characteristically small with the pigmented portion typically separate from, and elevated above, that of the main incisor shaft (state 3). Additional, non-morphological characters, as best understood with available sampling, and as described in the literature or presented herein, are largely concordant. The two species possess distinct karyotypes (Brown 1974; Brown and Rudd 1981) and belong to separate, well-supported mtDNA clades (Maldonado et al. 2001; Fig. 4).

Second, character discordance is present among tidal marsh samples around the northern margin of San Pablo Bay east to Grizzly Island in Suisun Bay, at least as far as is now known. Here are found individuals that have the *S. ornatus* karyotype but, with the limited available mtDNA sequence data, are part of the well-supported Northern clade otherwise comprising all samples of *S. vagrans*. Furthermore, the population samples especially around the north rim of San Pablo Bay exhibit a complex pattern of intermediacy between *S. ornatus* and *S. vagrans* in all morphological characters, be these cranio-mandibular measurements, upper I1 medial tine scores, or dorsal pelage color attributes. There are too few specimens from localities farther east in the Sacramento-San Joaquin river delta or the Sacramento Valley and adjacent uplands for statistical comparisons (MVZ has only 11 from eight localities throughout this large area). While each of these share the qualitative cranio-mandibular attributes of *S. ornatus*, it remains to be determined if larger samples will exhibit the type of character intermediacy and discordance that typifies shrews from the North Bay.

The fact of substantial character discordance leaves several unanswered questions with regard to the *S. ornatus – S. vagrans* complex from the San Francisco Bay Area. The most obvious of these are: (1) what might explain the full range of phenotypes present in most of the North Bay samples (Figs. 9-11), the stair-stepped character change among them (Fig. 8), and the discordance among morphological phenotype, karyotype, and molecular clade assignments (also Fig. 8)? (2) What taxonomy would best capture the diversity of shrews in this area?

The hybridization hypothesis.—Rudd (1955) used several cranial and pelage characteristics to describe zones of intergradation between Grizzly Island *S. o. sinuosus* and upland populations of *S. o. californicus* from the East Bay Contra Costa County. He also grouped three populations (Southampton Bay [my sample e-Southampton Bay; Fig. 6], Van Sickle Island [south of Grizzly Island; not examined here], and Sears Point Road [sample g-NW Vallejo; Fig. 6]) with *S. o. sinuosus* based on these attributes, but emphasized that each contained some influence from *S. o. californicus*. And he concluded that western-most populations contacted and hybridized with *S. vagrans* in Marin County to form a hybrid swarm between these taxa at Tolay Creek (sample j-Tolay Creek; Fig. 6). This set of conclusions generally mirrors the patterns of character distribution, and sample intermediacy, previously described and depicted in Figs. 8 to 11, except that the Tolay Creek sample is no more a "hybrid swarm" than are most of the other San Pablo Bay tidal marsh samples along the transect.

The discordance between phenotype-karyotype and mtDNA clade assignments can be explained by mitochondrial capture following an earlier episode of hybridization, an increasingly common finding in detailed studies of closely related species of mammals and many other organisms. Discordance due to dif-

ferent evolutionary histories of the mitochondrial and nuclear genomes can be tested by current laboratory methods and remains a problem for future investigation. Another important issue for future resolution is an explanation for the full array of character phenotypes, encompassing those of both reference species and spanning nearly the entire intervening morphospace present in so many of the tidal marsh samples (e.g., Figs. 6 and 9-11). Assuming that phenotype is under substantial genetic control, there are two likely, but not mutually exclusive, explanations. First, both species were present and hybridizing, at least limitedly, at each locality before the time of sample collection. Second, individuals with intermediate phenotypes were not at a strong selective disadvantage, so that the range of observed phenotypes represents a long period of multiple generations of backcrossing, which would inevitably reconstitute parental-appearing phenotypes. With the limited information now available, the latter hypothesis is at least partially supported. Using the upper incisor medial tine pigmentation states as a species-diagnostic attribute (i.e., state 1 = *S. ornatus*, state 3 = *S. vagrans*; Table 2 and Junge and Hoffmann 1981), no specimens from Mare Island (sample f) west along northern San Pablo Bay to San Rafael (sample m) possess the *S. vagrans* state 3. Furthermore, the average cranio-mandibular CVA posterior probability of these samples to *S. o. sinuosus* is 0.561 but only 0.231 to *S. v. vagrans*. Both results suggest that the influence of hybridization among these serially adjacent and interacting populations has been asymmetric favoring *S. o. sinuosus* and that some stability in each had been achieved by the time of collection. Molecular technology today would permit one to examine the array of underlying genotypes in these historic samples and detail whether or not, for example, hybridization had affected the entire genome or had been selectively restricted to certain parts, and if there had been differential introgression from *S. o. sinuosus*, or even *S. o. californicus* across the Carquinez Strait, as sources. And a follow-up study, based on newly collected specimens, could address whether changing environmental conditions over the past half-century since these samples were collected have changed both the nature and consequences of hybridization, if that hypothesis were supported.

Another corollary question that remains is why there is no apparent evidence of hybridization between *S. o. californicus* and *S. v. halicoetes*, whose distributions overlap in the salt marshes at several points around San Francisco Bay. What is it about these taxa, or the ecological relationships where they co-occur, that has apparently generated a different evolutionary history than that between *S. o. sinuosus* and *S. v. vagrans* around San Pablo Bay? These shrews resident in the Bay Area offer a rich arena for evolutionary studies.

Taxonomic resolutions.—There is no question that *S. ornatus* and *S. vagrans* in the San Francisco Bay Area represent separate species. Excepting the discordances across the North Bay marshes, the two are distinct in cranial morphometrics, karyotypes, and mitochondrial DNA. They are also sympatric, and even syntopic, at a number of localities in the marshes rimming Monterey Bay (von Bloeker 1939; unpublished data) as well as both San Francisco and San Pablo bays (e.g., Aviso marsh in Santa Clara County and San Pablo marsh in Contra Costa County). In each of these areas of overlap, there is complete concordance between morphological and mtDNA clade assignments, and no evidence of morphological intermediacy.

Of the taxa of *S. ornatus* examined herein, Grinnell's *sinuosus*, despite its membership in the Northern, otherwise uniquely *S. vagrans*, mtDNA clade, possesses an *S. ornatus* karyotype and is phenotypically closer to *S. ornatus* in cranio-mandibular (Fig. 8) and upper incisor tine traits, even if unique in dorsal pelage color. Given that the discrepancy between phenotypic-karyotypic and molecular assignments can be explained by past mitochondrial capture, the assigmnent by previous authors of *sinuosus* Grinnell to *S. ornatus* and not to *S. vagrans* (*sensu* Woodman 2018) is supported. Furthermore, Grinnell's taxon is sufficiently distinct from other regional samples of *S. ornatus*, particularly the adjacent *S. o. californicus*, in morphological attributes (cranio-mandibular measurements, upper incisor tine pigmentation, and dorsal pelage coloration) to warrant subspecies status. This decision follows the conceptual argument and analytical framework advocated for trinomial use by Patton and Conroy (2017). The range of *S. o. sinuosus* should also be expanded from Grizzly Island west to at least Southampton Bay (see Fig. 8), a conclusion also reached by Brown and Rudd (1981).

The East Bay *S. o. californicus* also is sufficiently distinct, at least in the geographically limited analyses presented, to warrant recognition (contra Hutterer

2005). Junge and Hoffmann's (1981; restated in Owen and Hoffmann 1983) assignment of the Martinez marsh samples to *S. o. sinuosus* is not supported; these specimens are clearly part of *S. o. californicus*. Thus, *S. o. californicus* includes individuals living in both upland and marsh habitats, and extends to the northeast at least as far as the Rio Vista area in the marshlands that border the lower Sacramento River delta. Whether it continues further north into Yolo and Sacramento counties (as suggested by Brown and Rudd 1981), or beyond, must await further study.

The geographically confined samples of *S. vagrans* that were examined, especially given the very large range of this species (Gillihan and Foresman 2004), limit useful comments about subspecific validity. Hutterer (2005) recognized both *halicoetes* Grinnell and *paludivagus* von Bloeker apart from nominotypical *S. v. vagrans*. Grinnell's *halicoetes*, which nearly circumscribes San Francisco Bay in its tidal marshes and estuaries, is only minimally separable from upland *S. v. paludivagus* in cranio-mandibular multivariate space (they do not differ along any PCA axis [Table 5] and only slightly on CV-2, which explains only 18.8% of the total variation [Table 6]). The two do differ in dorsal pelage color (*S. v. halicoetes* is significantly darker [mean L* = 14.73 versus 18.07] and more saturate [mean C* = 14.03 versus 21.51]). If color alone is sufficient for taxonomic recognition, then *S. v. halicoetes* warrants that status.

For the most part, the shrews examined herein do fall into recognizable, and supportable, taxonomic units to which available names appropriately apply. The nagging question that remains, however, is to how to treat the tidal marsh samples around the northern rim of San Pablo Bay (those from Mare Island [sample f in Fig. 6] to Larkspur [sample n]). Brown and Rudd (1981:34) recognized the problem of "a less definable westerly identification" with these populations "neither *S. o. sinuosus* nor *S. o. californicus*." They also wrote (p. 34) that while "some populations are clearly separable... a new subspecific name would disguise their individuality...[and] since all populations are distributed allopatrically, they are better referred to as *S. o. californicus*."

Based on the analyses presented herein, the statement above by Brown and Rudd (1981) is supported, with the exception of that last phrase. Assigning samples with such "individuality" to a particular taxon (e.g., *S. o. californicus*) is incompatible with the range of phenotypes expressed in each. These samples share only the apparently global *S. ornatus* karyotype and possess a broad mixture of phenotypes with less *S. o. californicus* influence than that of the other adjacent taxa. Nor does assignment to any of the other named taxa in the San Francisco Bay Area. Until, and if, genomic tools might uncover the genetic basis for the intermediacy expressed in samples from Mare Island to Larkspur (e.g., Fig. 11), the results suggest treating each of these samples as *S. ornatus – S. vagrans* hybrid populations.

Future Directions

As has been suggested at several points in this paper, shrews of the *S. ornatus – S. vagrans* complex, both globally throughout their respective ranges or within limited geographic areas such detained herein, are ripe for a concerted field and museum program that would take advantage of the laboratory and analytical methods now available. A host of evolutionary, ecological, and systematic questions remain to be addressed; collectively these would underscore an exciting research program for a young scholar. Such studies could combine renewed collections at key geographic sites around the San Francisco Bay Area, and in other areas within California where the two species co-occur (e.g., marshes around Monterey Bay), and targeted sampling of historical specimens housed in museum collections. It is now possible to sample preserved specimens in ways that hardly impact the specimen (for example, Bi et al. 2013).

However, continuing work on these shrews in their natural habitats across the San Francisco Bay region, or elsewhere in California, is hampered by the extreme loss of wetland habitats to urban development and water distribution policies supporting agricultural expansion in recent decades. As a consequence, several taxa included in the group now are listed as endangered, threatened, and/or of special concern by regional, state, and/or federal agencies (lists of California threatened or endangered taxa and those of special concern available at https://www.wildlife.ca.gov/Conservation). Additionally, documented climate change that already has led to a sea level rise of eight inches along the California

coast and bays in recent decades is a detrimental force that will only increase into the future (see, for example, Ackerly et al. [2018], Pierce et al. [2018] and the regional reports of *California's Fourth Climate Change Assessment* available at http://www.climateassessment.ca.gov/regions/). The compounding impact of rising waters coupled with preventive measures to preserve adjacent urban, commercial, and agricultural lands will undoubtedly result in continued loss of shrew habitat in coastal marsh systems.

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I dedicate this paper to the memory of Robert J. Baker, a superbly gifted and prolific scholar, and a close friend of more than 50 years. We first met in graduate school at the University of Arizona in the summer of 1965 and remained friends and colleagues until the moment of his untimely passing. I miss him terribly, but know that he continues to dream of the beauty of the natural world he loved so much, and, of course, about bats. My special thanks go to L. Bradley and R. Bradley for inviting me to participate in a festschrift appropriately honoring my friend; and to H. Genoways and an anonymous reviewer for substantially improving the quality of this manuscript.

This paper could not have been contemplated without the heroic field efforts of my predecessors at the MVZ who collected the majority of the *S. ornatus* and *S. vagrans* housed in the collections, notably J. Grinnell, A. Alexander, L. Kellogg, J. Dixon, and especially S. Benson, O. Pearson, and R. Rudd. The continued augmentation of collections like this one will only occur if similarly motivated individuals exit the lab and go to the field, something sadly, to me, not done as often as it once was. Many individuals over the years have helped in the lab to assemble the cytochrome-*b* sequence data summarized herein, including H. Shohfi, E. Rubidge, A. Sellas, B. Sousa, J. Castillo, K. Rowe, M. Lim, M. Phuong, and B. Bach. I am indebted to each of them.

LITERATURE CITED

Ackerly, D., A. Jones, M. Stacey, and B. Riordan. 2018. San Francisco Bay Area, summary report. California's Fourth Climate Change Assessment, Publication No. CCCA4-SUM-2018-005.

Baird, S. F. 1857. Mammals. Reports of explorations and surveys to ascertain the most practicable and economical route for a railroad from the Mississippi River to the Pacific Ocean 8(1):i–xlviii, 1–757, The U.S. House of Representatives, Washington, DC.

Bi, K., T. Linderoth, D. Vanderpool, J. M. Good, R. Nielsen, and C. Moritz. 2013. Unlocking the vault: next-generation museum population genomics. Molecular Ecology 22:6018–6032.

Brown, R. J. 1974. A comparative study of the chromosomes of three species of shrews, *Sorex bendirii*, *Sorex trowbridgii*, and *Sorex vagrans*. Wasmann Journal of Biology 32:303–326.

Brown, R. J., and R. L. Rudd. 1981. Chromosomal comparisons within the *Sorex ornatus–S. vagrans* complex. Wasmann Journal of Biology 39:30–35.

Carraway, L. N. 1990. A morphological and morphometric analysis of the "*Sorex vagrans* species complex" in the Pacific Coast region. Special Publications, Museum of Texas Tech University 32:1–76.

Davis, F. W., et al. 1988. The California Gap Analysis Project – Final Report. University of California, Santa Barbara, California. [http:legacy.biogeog.ucsb.edu/projects/gap/gap_rep.html]

Dunn, O. J. 1961. Multiple comparisons among means. Journal of the American Statistical Association 56:52–64.

Esteva, M., F. A. Cervantes, S. V. Brant, and J. A. Cook. 2010. Molecular phylogeny of long-tailed shrews (genus *Sorex*) from México and Guatemala. Zootaxa 2615:47–65.

Findley, J. A. 1955. Speciation of the wandering shrew. University of Kansas Publications, Museum of Natural History 9:1–68.

Gillihan, S. W., and K. R. Foresman. 2004. *Sorex vagrans*. Mammalian Species 744:1–5.

Grinnell, J. 1913. The species of the mammalian genus *Sorex* in west-central California. University of California Publications in Zoology 10:179–195.

Grinnell, J. 1933. Review of the Recent mammal fauna of California. University of California Publications in Zoology 40:71–234.

Hall, E. R. 1981. The mammals of North America, vol.1. John Wiley & Sons, New York.

Hennings, D., and R. S. Hoffmann. 1977. A review of the taxonomy of the *Sorex vagrans* complex from western North America. Occasional Papers of the Museum of Natural History, University of Kansas 68:1–35.

Hope, A. G., N. Panter, J. A. Cook, S. L. Talbot, and D. W. Nagorsen. 2014. Multilocus phylogeography and systematic revision of North American water shrews (genus: *Sorex*). Journal of Mammalogy 95:722–738.

Hutterer, R. 2005. Order Soricomorpha. Pp. 220–311 in Mammal species of the world. A taxonomic and geographic reference (D.E. Wilson and D.M. Reeder, eds.), 3rd ed. Johns Hopkins University Press, Baltimore, Maryland.

Ingles, L. G. 1965. Mammals of the Pacific states. Stanford University Press, Stanford, California.

Jackson, H. H. T. 1928. A taxonomic review of the American long-tailed shrews (genera *Sorex* and *Microsorex*). North American Fauna 51:1–218.

Kumar, S., G. Strecher, and K. Tamura. 2016. MEGA 7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870–1874.

Junge, J. A., and R. S. Hoffmann. 1981. An annotated key to the long-tailed shrews (genus *Sorex*) of the United States and Canada, with notes on Middle American *Sorex*. Occasional Papers of the Museum of Natural History, University of Kansas 94:1–48.

Maldonado, J. E., F. Hertel, and C. Vilà. 2004. Discordant patterns of morphological variation in genetically divergent populations of ornate shrews (*Sorex ornatus*). Journal of Mammalogy 85:886–896.

Maldonado, J. E., C. Vilà, and R. K. Wayne. 2001. Tripartite genetic subdivisions in the ornate shrew (*Sorex ornatus*). Molecular Ecology 10:127–147.

Merriam, C. H. 1895. Synopsis of the American shrews of the genus *Sorex*. North American Fauna 10:57–98.

Owen, J. G., and R. S. Hoffmann. 1983. *Sorex ornatus*. Mammalian Species 212:1–5.

Patton, J. L., and C. J. Conroy. 2017. The conundrum of subspecies: morphological diversity among desert populations in the California vole (*Microtus californicus*, Cricetidae. Journal of Mammalogy 98:1010–1026.

Pierce, D. W., J. F. Kalansky, and D. R. Cayan. 2018. Climate, drought, and sea level rise scenarios for the Fourth California Climate Assessment. California's Fourth Climate Change Assessment, California Energy Commission. Publication No. CNRA-CEC-2018-006.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Rudd, R. L. 1955. Population variation and hybridization in some Californian shrews. Systematic Zoology 4:21–34.

Smith, M. F., and J. L. Patton. 2007. Molecular phylogenetics and diversification of South American grass mice, genus *Akodon*. Pp. 827-858 in The quintessential naturalist: honoring the life and legacy of Oliver P. Pearson (D. A. Kelt, E. P. Lessa, J. Salazar-Bravo, and J. L. Patton, eds.), University of California Publications in Zoology, 134:v–xii + 981 pp.

Thornington, R. W., Jr. 1966. The biology of rodent tails: a study of form and function. Arctic Aeromedial Laboratory, Air Force Systems Command, Fort Wainright, Alaska, AAL-TR-65-8.

von Bloeker, J. C. Jr. 1939. Two new shrews from west-central California. Proceedings of the Biological Society of Washington 52:93–96.

von Bloeker, J. C. Jr. 1941. A new shrew from Santa Catalina Island, California. Bulletin of the Southern California Academy of Science 40:163–164.

Willows-Munro, S., and C. A. Matthee. 2011. Exploring the diversity and molecular evolution of shrews (family Soricidae) using mtDNA cytochrome *b* data. African Zoology 46:246–262.

Woodman, N. 2012. Taxonomic status and relationships of *Sorex obscurus parvidens* Jackson, 1921, from California. Journal of Mammalogy 93:826–838.

Woodman, N. 2018. American Recent Eulipotyphla, nesophontids, solenodons, moles, and shrews in the New World. Smithsonian Contributions to Zoology, 650:iii–vi + 1–107.

Woodman, N., and R. M. Timm. 1993. Intraspecific and interspecific variation in the *Cryptotis nigrescens* species complex of small-eared shrews (Insectivora: Soricidae), with the description of a new species from Colombia. Fieldiana: Zoology, new series, 74:1–30.

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APPENDIX A

Sample groups and included localities for each separate analysis.

S1 – Specimens and included localities for which cytochrome-*b* sequence is available; AF numbers refer to GenBank sequences deposited by Maldonado et al. (2001). Localities are mapped in Figure 2.

Central clade, *S. ornatus* (*n* = 42): Alameda Co., Livermore (MVZ 219053). Contra Costa Co., Orinda (MVZ 208913, 216011–216012); Mallory Ridge (MVZ 219054). Fresno Co., Mill Creek, 1 mi S Dunlop (MVZ 223956). Inyo Co., Cartago, Owens Lake (MVZ 226259–226260; MVZ 230724–230728); Little Lake (MVZ 230729). Kern Co., Bodfish (MVZ 222857); Dock Williams Canyon (MVZ 219126, 219132); Fay Creek MVZ 223902–03); Kern Lake (AF300669); Landers Creek, Piute Mts. (MVZ 227879–227883); Mill Creek, 12 mi S Bodfish (MVZ 223904); Pozo Creek, 8 mi NE Bakersfield (MVZ 223905); S fork Kern River (MVZ 222858–222860). Mariposa Co., Coulterville (MVZ 207131); Domingo Flat (MVZ 225822); El Portal (AF300671–AF300672). Merced Co., Kelsey Ranch, 5.2 mi E Snelling (MVZ 207047); Merced River Ranch, Snelling (MVZ 207046). Monterey Co., Fort Ord (MVZ 216781–216782); Moss Landing (MVZ 199736); mouth Salinas River (AF300668). Stanislaus Co., 1 mi W La Grange (MVZ 207045). Tulare Co., Trout Creek, Smith Meadow (MVZ 222647).

Northern clade, *S. vagrans* + *S. ornatus* (*n* = 95): El Dorado Co., Trout Creek Meadow, South Lake Tahoe (MVZ 229760–229762, 229771–229772, 229783, 229791, 229816, 229826). Inyo Co., Buckhorn Springs, Deep Springs Valley (MVZ 221232, 230730, 230732–230738); Lower Rock Creek, Rovana (MVZ 224838); Silver Canyon, White Mts. (MVZ 217258, 225011). Lassen Co., Blue Lake (MVZ 227404); Pole Spring (MVZ 220156–220158). Marin Co., Abbott's Lagoon (MVZ 191627–191628); 7.2 km WSW Olema (MVZ 191541). Modoc Co., Pepperdine Camp, Warner Mts. (MVZ 206915); north fork Parker Creek, Warner Mts (MVZ 218821); Shields Creek, Warner Mts (MVZ 218832). Mono Co., Benton Crossing (MVZ 230739); Bohler Creek (MVZ 216216–216218); Sweetwater Mts (AF300656). Placer Co., Sixmile Valley (MVZ 224680–224681). Plumas Co., Drakesbad (MVZ 220649); Willow Lake (MVZ 220651–220663). Shasta Co., Emerald Lake, Lassen Volcanic National Park (MVZ 220427); Lake Helen, Lassen Volcanic National Park (MVZ 220429); Mt. Shasta (AF300654); Trapline B-1, Lassen Volcanic National Park (MVZ 199183); Trapline B–2, Lassen Volcanic National Park (MVZ 220521, 206294); Trapline F-2, Lassen Volcanic National Park (MVZ 196627); Trapline R-2, Lassen Volcanic National Park (MVZ 196632, 196636–116639, 206301, 206305); Trapline S-2, Lassen Volcanic National Park (MVZ 196644, 200643, 206306). Sierra Co., Independence Lake (MVZ 222748); Sierraville (MVZ 217510). Siskiyou Co., Wild Horse Mtn. (MVZ 223027). Solano Co., Collinsville (MVZ 230314); Grizzly Island (AF300665–AF300667); Montezuma Slough, near Collinsville (MVZ 218666); Montezuma wetlands, 1 km W Collinsville (MVZ 230315). Sonoma Co., Bodega Bay (AF300657–AF330660); W side Sonoma Creek bridge (MVZ 218665, 218667). Tehama Co., Dye Creek (AF300661–AF300664); Trapline R-1, Lassen Volcanic National Park (MVZ 196649, 196651, 196664, 196667, 200575, 206315–206316).

Southern clade, *S. ornatus* (*n* = 15): Los Angeles Co., Catalina Island (AF300673); Rancho Palos Verdes (AF300680). Orange Co., Bolsa Chica State Beach (AF300681); Puente Hills (MVZ 216851). San Bernardino Co., Bluff Lake, San Bernardino Mts. (AF300667, AF300670); Fawnskin, San Bernardino Mts. (MVZ 198733–198734); Metcalf Meadows, S of Big Bear (MVZ 198735–198736). San Diego Co., Camp Pendleton (AF300679), Santa Isabella (MVZ 222138–222139), Torrey Pines (AF300678). Ventura Co., Point Mugu (AF300672).

S2 – Samples and included localities examined for differences in dorsal color attributes between San Francisco Bay Area taxa of *S. ornatus* (including *sinuosus* Grinnell) and *S. vagrans*. Localities are mapped in Figures 5 and 6.

S. o. californicus (*n* = 33): Contra Costa Co., Tilden Park (MVZ 114126, 115099. 115111–115116, 115118–115121, 115571).

S. o. sinuosus (*n* = 17): Solano Co., Grizzly Island (MVZ 16467–16469, 16471–16472, 18700–18703, 35318–35319, 141183, 145804).

S. v. halicoetes (*n* = 32): Santa Clara Co., 1.75 mi NE Alviso; Palo Alto (MVZ 115005–115032); Palo Alto (MVZ 3635–3637, 3639).

S. v. paludivagus (*n* = 23): San Francisco Co., near Laguna Honda Home, Sutro Forest, San Francisco (MVZ 113458–113462, 113464); Lake Merced (MVZ 113464); Twin Peaks, San Francisco (MVZ 114340). San Mateo Co., Thornton, Skyline Blvd and Aleman Rd (MVZ 114346); Skyline Blvd, 4.5 mi S Lake Merced (MVZ 107732, 112875, 115065, 115578–115583); San Gregorio (MVZ 30361–30362); 2.25 mi E, 1.5 mi N Rockaway Beach (MVZ 107706–107707).

S. v. vagrans (*n* = 20): Marin Co., Fort Barry (MVZ 101439–101440, 101542–101548, 101550–101553, 102810–102812, 102814–102817).

Martinez marsh [n = 31]: Contra Costa Co., 1.0 mi N and 2.0 mi E Martinez, salt marsh (MVZ 123595–123597, 123639–123656, 123791, 123823, 124184–124186, 124344, 125220, 126075, 126515).

c-Joice Island [*n* = 25]: Solano Co., Cutoff Slough marsh, 0.1 mi N Joice Island (MVZ 125536–125546); 0.1 mi N Joice Island (MVZ 125548–125555, 125557–125562).

d-Suisun Marsh [*n* = 4]: Solano Co., Suisun City, salt marsh adjacent to Cordelia Street (MVZ 115592–115595).

e-Southampton Bay [*n* = 14]: Solano Co., Southampton Bay, near Solano Public Dump (MVZ 115163–115167, 115562–115570).

g-NW Vallejo [*n* = 26]: Solano Co., Sears Point Rd, 6 mi NW Vallejo (MVZ 113341–113353, 114141, 114143–114153); Sears Point Rd., W side Sonoma Creek Bridge (MVZ 183410).

h-Sonoma Creek [*n* = 12]: Sonoma Co., 50 m from W side Sonoma Creek bridge at Hwy 37 (MVZ 218667 218672–218675, 218677); Hwy 37 at W side Sonoma Creek (MVZ 218665); Sears Point Road, W side Sonoma Creek Bridge (MVZ 183410).

i-Tolay Creek [*n* = 31]: Sonoma Co., Tolay Creek, 1 mi N Sears Point (MVZ 115105–115106); Tolay Creek, 0.5 mi S Sears Point (MVZ 115468–115470, 115599–115604); Tolay Creek, 0.75 mi S Sears Point (MVZ

115598); Tolay Creek, 1.5 mi S Sears Point (MVZ 115108–115110, 115453–1154467); Tolay Creek, 2.5 mi S Sears Point (MVZ 115107).

j-Black Point [*n* = 38]: Sonoma Co., E side Bascule Bridge, 0.75 mi NE Black Point (MVZ 114212–114213); mouth Petaluma Creek, 0.5 mi NE Black Point (MVZ 115100–115104, 115471–115498, 115588–115590).

k-Novato marsh [*n* = 17]: Marin Co., 5 mi E Novato [salt marsh], mouth San Antonio Creek (MVZ 113354–113357, 114176–114188).

l-Petaluma [*n* = 10]: Marin Co., Petaluma (MVZ 3642, 10847, 10849); 1.4 mi S Petaluma, salt marsh (MVZ 94688); 1.5 mi S Petaluma (38812–38814); 150 yds E McNear Bridge, 1.7 mi SE Petaluma (MVZ 90481); McNear Bridge, 1.5 mi SE Petaluma, salt marsh (MVZ 15572–15577).

m-San Rafael marsh [*n* = 49]: Marin Co., 3.5 mi N San Rafael (in marsh) (MVZ 114191–114202, 114204–114207, 114208–114211, 115081–115087, 115092–115098, 115499–115511, 115596–115597, 116966).

n-Larkspur [*n* = 30]: Marin Co., Point San Quentin (marshes) (MVZ 115075–115080); San Quentin (112880–112882, 114190).

S3 – Samples and included localities examined for all samples of *S. ornatus* and *S. vagrans* from the greater San Francisco Bay Area. Localities are mapped in Figures 5 and 6.

Reference samples:

S. o. californicus (*n* = 70): Alameda Co., Arroyo Mocho, 11.5 mi SE Livermore (MVZ 116948); Berkeley (MVZ 23864, 29959–29960, 29969, 102076, 108937); Berkeley, 1 mi E Stadium, Strawberry Canyon (MVZ 181437–181438); 0.5 mi NE California School for the Blind, near Berkeley (MVZ 81121); Hayward (MVZ 16614); Lawrence Livermore National Laboratory (MVZ 219052); N slope Strawberry Canyon, near Berkeley (MVZ81120); N wall of Claremont Canyon, near Berkeley (MVZ 66416); University of California Botanical Garden, Strawberry Canyon (MVZ 101662). Contra Costa Co., Camp Padre, Tilden Park (MVZ 114121); near golf course, Tilden Park (MVZ 115111–115116, 115120, 115571); Lafayette Terrace, Lafayette (MVZ 47311, 84864); 1.25 mi S Lafayette (MBVZ 104561); Observatory Plot, Russell Tree Farm (MVZ 129192–139193); Orinda (MVZ 98940, 112876–112877, 122407, 135345)141184, 216012, 225266); Richmond (MVZ 78328); Russell Tree Farm, Layafette (MVZ 107970); Mallory Ridge, Contra Costa Water District (MVZ 219054–219055); Tilden Park (115099, 115190, 115192–115194); Tilden Park, Camp Padre (MVZ 114121); Tilden Park, 0.25 mi S Inspiration Point (MVZ 115121); Tilden Park, 0.25 mi SW Inspiration Point (MVZ 114126); Tilden Park, 0.75 mi S Inspiration Park (MVZ 115122, 115124–115141); Tilden Park, vicinity of gold course (MVZ 183408); Tilden Park, near gold course MVZ 115111–115116, 115120, 115571); Tilden Park, 1 mi NE gold course (MVZ 191738).

S. o. sinuosus (*n* = 14): Solano Co., E side, 3 mi NE Oakley, Grizzly Island (MVZ 138736); Grizzly Island (MVZ 16468–16471, 18701–18702, 113334–113337, 141183, 145804–145805).

S. v. halicoetes (*n* = 154): Alameda Co., 1 mi N Bay Farm Island, Melrose Marsh (MVZ 77513, 87900); 1 mi NE Newark, salt marsh (MVZ 114162); Elmhurst (MVZ 3628, 3630–3631); Hayward Landing, end of Russell City Rd., salt marsh (MVZ 115059–115061, 115063, 115196); Oakland Airport (MVZ 112145); S side Oakland Airport (MVZ 114163–114172, 114174–114175). Contra Costa Co., Giant, Atlas Power Company salt marsh (MVZ 114175); San Pablo Creek salt marsh, Richmond (MVZ 115064, 120331–120334, 123588–124592, 123704–123706, 124290); San Pablo marsh (MVZ 183420–183424). San Mateo Co., Belmont (MVZ 3627);

Redwood City (MVZ 10845); W approach Dumbarton Bridge (MVZ 115056–115058). Santa Clara Co., 1 mi SSW Alviso, salt marsh (MVZ 115053–115055); 1.5 mi NE Alviso (MVZ 139194–139195); 1.75 mi NE Alviso (MVZ 114981–114992, 114994–115033, 115189, 115512–115516, 183425); 1.75 mi NE Alviso, jct Garbage Dump Rd and railroad tracks (MVZ 115521–115523, 115525115539); Los Esteros Rd, 0.5 mi NE Alviso, salt marsh (MVZ 114979–114980, 115034–115047, 114049–115051, 115188); Los Esteros Rd, 1 mi NE Alviso (MVZ 115052); Palo Alto (3635–3636, 3638–3639).

S. v. paludivagus (*n* = 44): San Francisco Co., 1 mi S Fort Point, San Francisco (MVZ 189901–189907); Lake Merced (113463–113464); near Laguna Honda Home, Sutro Forest, San Francisco (MVZ 113458, 113460–113462); San Francisco (MVZ 89398); Sutro Forest, San Francisco (MVZ 113466–113467, 113481–113482); Twin Peaks, San Francisco (MVZ 114340); US Presidio, 0.75 mi N Marine Hospital, San Francisco (MVZ 57245); W side Twin Peaks, San Francisco (MVZ 113480). San Mateo Co., W San Bruno; mouth Pescadero Creek (MVZ 103572); Princeton (MVZ 113468–113473); 1.5 mi E and 2.75 mi N Rockaway Beach (MVZ 107706); 2.25 mi E and 1.5 mi N Rockaway Beach (MVZ 107707); 2.5 mi E Daly City [San Benito Mtn] (MVZ 132177, 132233–132234); 4.5 mi S Lake Merced, Skyline Blvd (MVZ 115578–115583); 4.5 mi S Lake Merced, Skyline Blvd, marshy edge (MVZ 115065); 2 mi S Sharp Peak, Skyline Blvd (MVZ 112875); Thornton, Skyline Blvd and Aleman (MVZ 114346); W San Bruno, 1 mi head San Andreas Lake (MVZ 181439).

S. v. vagrans – Pt. Reyes (*n* = 127): Marin Co., 0.2 mi NW Abbott's Lagoon parking area, 7.8 km NW Inverness (MVZ 191627–191667, 191669–191670); 1.5 mi NW Inverness (MVZ 114515–114516); 1.6 mi S Inverness (MVZ 101284–101285); 2.6 mi WNW Inverness (MVZ 191688); 2 mi WNW Inverness (MVZ 96143); 2.7 km WNW Inverness (MVZ 191689, 191691–191696, 191699–191722); 3 mi W Inverness (MVZ 19589–19592, 19594, 67187, 96141–96142); 4 mi W Inverness (MVZ 19582); 3.3 km SSW Olema (MVZ 191671); 7.2 km WSW Olema (MVZ 191541, 191672–191687); 5.2 km S Olema (MVZ 191723–191728); Pierce Ranch, Tomales Point (MVZ 101788–101799); White Gulch, Tomales Point (MVZ 101282–101283).

S. v. vagrans – S Marin (*n* = 74): Marin Co., Fort Barry (MVZ 101629); Mendell Ordance Depot (MVZ 99749); Rodeo Valley, Fort Barry Military Reserve (MVZ 101436–101438); W Portal area, Fort Barry Military Reserve; Fort Barry; Tennessee Valley [= Elk Valley] (MVZ 101185–101886); W end Tennessee Valley [= Elk Valley] (MVZ 101230–101234, 101287, 101289–101301, 101381–101382, 101555, 101786–101787, 122253–122258, 122260–122271, 122273–1222275, 101439–101440, 101542–1–1553, 102809–102812, 102814–102817).

Samples treated as unknown in CVA analyses:

Martinez marsh (*n* = 122): Contra Costa Co., Bull's Head Marsh, 1.5 mi NNE Martinez (MVZ 215533); 1.0 mi N and 2.0 mi E Martinez (MVZ 121286, 123595–123597, 123631–123632, 123634–123656, 123790–123791, 123823, 124184–124186, 124344, 125220, 126075, 126514); 2 mi E Martinez (MVZ 119041, 119470–119475, 119487–119498, 119500–119503, 119505–119523); 2 mi E Martinez [salt marsh] (MVZ 119087–119116, 119118, 119120–119125); 3 mi ESE Port Chicago (MVZ 114124–114125).

San Pablo marsh [*n* = 12]: Contra Costa Co., Pinole, salt marsh near S. P. Railroad tracks (MVZ 114122–114123); Point Isabel [E Shore Hwy] (MVZ 115517–115520, 115586); Point Pinole Regional Park (MVZ 216719); salt marsh, Giant (MVZ 122074); San Pablo Creek salt marsh, Richmond (MVZ 123702, 124289, 126076).

a-Rio Vista [*n* = 5]: Solano Co., 11 mi N Rio Vista (MVZ 97856); Rio Vista, tule marsh S end of Second Street (MVZ 115612–115615).

c-Joice Island [*n* = 25]: Solano Co., Cutoff Slough marsh, 0.1 mi N Joice Island (MVZ 125536–125546); 0.1 mi N Joice Island (MVZ 125548–125555, 125557–125562).

d-Suisun Marsh [*n* = 9]: Solano Co., 0.5 mi NE Cordelia, salt marsh (MVZ 115158–115161; Suisun City, salt marsh adjacent to Cordelia Street (MVZ 115587, 115592–115595).

e-Southampton Bay [*n* = 11]: Solano Co., Southampton Bay, near Solano Public Dump (MVZ 115162, 115164–115165, 115167, 115562–115566, 115568–115570).

f-Mare Island [*n* = 20]: Solano Co., Mare Island Naval Shipyard, Vallejo (MVZ 179828–179847).

g-NW Vallejo [*n* = 68]: Solano Co., 2.7 mi W jct Napa River and Hwy 37 (MVZ 149671); Sears Point Rd, 6 mi NW Vallejo (MVZ 113338–113349, 113351–113353, 114127–114128, 114131–114140, 114142–114154, 114189, 115168–115171, 115173–115185, 115584–115585); Sears Point Rd., 8 mi NW Vallejo (MVZ 114155–114161).

h-Sonoma Creek [*n* = 12]: Sonoma Co., 50 m from W side Sonoma Creek bridge at Hwy 37 (MVZ 218668–128669, 218872–218879); Hwy 37 at W side Sonoma Creek (MVZ 218665); Sears Point Road, W side Sonoma Creek Bridge (MVZ 183410).

i-Tolay Creek [*n* = 30]: Sonoma Co., Tolay Creek, 1 mi N Sears Point (MVZ 115105–115106); Tolay Creek, 0.5 mi S Sears Point (MVZ 115468–115470, 115599–115604); Tolay Creek, 0.75 mi S Sears Point (MVZ 115598); Tolay Creek, 1.5 mi S Sears Point (MVZ 115108, 115110, 115453–115467).

j-Black Point [*n* = 39]: Sonoma Co., E side Bascule Bridge, 0.75 mi NE Black Point (MVZ 114212–114213); mouth Petaluma Creek, 0.5 mi NE Black Point (115100–115104, 115471–115498, 115588–115591).

k-Novato marsh [*n* = 17]: Marin Co., 5 mi E Novato [salt marsh], mouth San Antonio Creek (MVZ 113354–113357, 114176–114188).

l-Petaluma [*n* = 10]: Marin Co., Petaluma (MVZ 10847); 1.4 mi S Petaluma, salt marsh (MVZ 94688); 1.5 mi S Petaluma (MVZ 38812); 150 yds E McNear Bridge, 1.7 mi SE Petaluma (MVZ 90481); McNear Bridge, 1.5 mi SE Petaluma, salt marsh (MVZ 115572–115577).

m-San Rafael [*n* = 53]: Marin Co., 3.5 mi N San Rafael [in marsh] (MVZ 114191–114202, 114204–114207, 114209–114211, 115081–115095, 115097–115511, 115596–115597, 116966); S bank mouth Galinas Creek (MVZ 125221).

n-Larkspur [*n* = 30]: Marin Co., 1 mi E Corte Madera (MVZ 101708); 2 mi E Corte Madera (MVZ 115066–115067); 2 mi E Mill Valley [salt marsh] (MVZ 115072–115073); Corte Madera Creek, W end Bon Air Tract (MVZ 101383); Larkspur [salt marsh, 0.75 mi W Bon Air Hill] (MVZ 125222–125223, 125225–125233); Larkspur, salt marsh (MVZ 101709–101710, 113405); Point San Quentin [marshes] (MVZ 115075–115080); San Quentin (MVZ 112880–112882, 114190).

APPENDIX B

Data (counts or mean, standard error, and range, plus sample sizes) for each geographic group of *S. ornatus* and *S. vagrans* examined. Separate tables are given for each analysis.

Table SB1. Distribution of the 1st upper incisor medial tine states for samples of *S. ornatus* and *S. vagrans*. Data given are sample size and numbers of each tine state (states are described in the Methods and Materials and illustrated in Fig. 3).

Sample	*n*	state 1	state 2	state 3
S. o. californicus	35	29	6	0
S. o. sinuosus	15	6	9	0
S. v. halicoetes	127	3	67	57
S. v. paludivagus	40	1	20	19
S. v. vagrans	164	10	61	93
S Marin	47	1	23	23
Pt. Reyes	116	9	38	70
Martinez marsh	109	99	9	1
San Pablo Bay	12	11	1	0
a-Rio Vista	5	5	0	0
c-Joice Island	22	14	8	0
d-Cordelia	5	5	0	0
e-Southampton	12	8	4	0
f-Mare Island	16	4	11	0
g-NW Vallejo	25	15	10	0
h-Sonoma Creek	11	8	3	0
i-Tolay Creek	30	24	6	0
j-Black Point	34	21	13	0
k-Novato marsh	14	9	5	0
l-Petaluma	11	4	7	0
m-San Rafael	46	29	17	0
n-Larkspur	6	0	3	3

Table SB2. Standardized sample statistics (mean ± standard error, range, and sample size) for dorsal CIE color variables for Bay Area samples of shrews. L* = paleness; C* = chroma; and h^o = hue; a* and b* are the respective positions in the color spectrum between red/magenta and green and between yellow and blue (see Methods and Materials).

Sample	L*	a*	b*	C*	h^o
S. o. californicus	21.18 ± 0.61	9.00 ± 0.40	18.20 ± 0.75	20.39 ± 0.78	1.10 ± 0.02
n = 33	14 – 28	4 – 14	10 – 29	12.8 – 31.4	0.90 – 1.35
S. o. sinuosus	9.12 ± 0.33	4.00 ± 0.32	5.35 ± 0.36	6.79 ± 0.38	0.93 ± 0.05
n = 17	7 – 13	1 – 6	4 – 8	4 – 12	0.67 – 1.33
S. v. halicoetes	14.73 ± 0.33	5.77 ± 0.30	12.72 ± 0.53	14.03 ± 0.56	1.14 ± 0.02
n = 32	11 – 20	38 – 125	9 – 22	10.0 – 24.3	0.94 – 1.32
S. v. paludivagus	18.09 ± 0.83	9.48 ± 0.68	19.13 ± 1.31	21.51 ± 1.37	1.10 ± 0.03
n = 23	11 – 2268	4 – 18	18 – 28	10.8 – 31.3	0.79 – 1.31
S. v. vagrans	21.95 ± 0.33	9.95 ± 0.32	18.60 ± 0.36	21.13 ± 0.38	1.08 ± 0.02
n = 20	17 – 28	6 – 15	8 – 28	10.0 – 31.8	0.93 – 1.18
S. v. halicoetes	14.73 ± 0.33	5.77 ± 0.30	12.72 ± 0.539	14.03 ± 0.56	1.14 ± 0.02
n = 32	11 – 20	38 – 125	9 – 22	10.0 – 24.3	0.94 – 1.32
Martinez marsh	20.55 ± 0.53	10.39 ± 0.41	17.77 ± 0.93	20.70 ± 0.92	1.03 ± 0.02
n = 31	16 – 27	5 – 15	10 – 26	11.2 – 30.0	0.84 – 1.25
c–Joice Island	14.16 ± 0.61	4.76 ± 0.56	11.76 ± 0.86	12.91 ± 0.90	1.18 ± 0.04
n = 25	10 – 22	0 – 10	4 – 21	4.12 – 22.14	0.84 – 1.46
d–Suisun Marsh	12.50 ± 0.87	3.50 ± 0.96	9.25 ± 0.89	10.01 ± 0.92	1.21 ± 0.09
n = 4	11 – 14	1 – 5	5 – 14	6.09 – 14.32	1.01 – 1.37
e–Southampton	17.64 ± 0.93	8.64 ± 0.75	17.57 ± 1.02	19.66 ± 1.17	1.12 ± 0.03
n = 14	12 – 25	4 – 14	12 – 26	13.60 – 29.53	0.98 – 1.31
g–NW Vallejo	11.65 ± 0.43	5.29 ± 0.31	8.92 ± 0.59	10.50 ± 0.57	1.02 ± 0.03
n = 26	7 – 17	3 – 8	4 – 17	5.83 – 17.89	0.52 – 1.30
h–Sonoma Creek	16.33 ± 2.16	7.00 ± 0.68	15.33 ± 2.59	16.94 ± 2.57	1.11 ± 0.06
n = 12	12 – 24	5 – 10	7 – 23	9.22 – 25.08	0.86 – 1.25
i–Tolay Creek	17.52 ± 0.677	9.16 ± 0.54	15.32 ± 0.74	17.97 ± 0.83	1.04 ± 0.02
n = 31	10 – 23	2 – 15	8 – 24	9.21 – 25.81	0.84 – 1.35
j–Black Point	20.61 ± 0.58	7.71 ± 0.51	18.05 ± 0.92	19.84 ± 0.94	1.15 ± 0.02
n = 38	10 – 26	2 – 15	6 – 26	7.21 – 27.73	0.85 – 1.45
k–Novato marsh	19.76 ± 0.65	9.29 ± 0.57	16.88 ± 0.88	19.39 ± 0.90	1.06 ± 0.03
n = 17	15 – 23	6 – 15	12 – 24	15.00 – 26.62	0.87 – 1.25
l–Petaluma	15.86 ± 0.53	8.71 ± 0.42	15.86 ± 0.83	18.15 ± 0.87	1.06 ± 0.02
n =10	15 – 23	6 – 15	12 – 24	15.00 – 26.62	0.87 – 1.25
m–San Rafael marsh	14.96 ± 0.37	8.02 ± 0.39	13.61 ± 0.66	18.00 ± 0.68	1.02 ± 0.02
n =49	11 – 22	2 – 14	7 – 25	9.22 – 26.0	0.73 – 1.4
n–Larkspur	16.90 ± 1.14	8.40 ± 0.91	13.20 ± 1.14	15.78 ± 1.27	1.00 ± 0.04
n =30	12 – 25	5 – 14	7 – 19	8.80 – 21.26	0.84 – 1.18

Table SB3, part 1. Cranio-mandibular variable sample means, standard errors, range, and sample size of shrews of the *Sorex ornatus–vagrans* complex from the greater San Francisco Bay Area, including *S. o. californicus*, *S. o. sinuosus*, *S. v. paludivagus*, *S. v. halicoetes*, and each marsh sample rimming the East Bay and North Bay.

Variable	*S. o. californicus* East Bay uplands $n = 74$	San Pablo Bay $n = 12$	Martinez marsh $n = 122$	*S. o. sinuosus* Grizzly Island $n = 14$	a-Rio Vista $n = 5$	c-Joice Island $n = 25$
GSL	16.15 ± 0.05 15.16 – 16.85	15.77 ± 0.00 15.30 – 16.26	15.89 ± 0.03 14.96 – 17.06	16.62 ± 0.06 16.29 – 17.05	15.78 ± 0.09 16.06 – 17.64	16.30 ± 0.08 15.53 – 16.96
CB	7.82 ± 0.02 7.37 – 8.36	7.76 ± 0.038 7.59 – 7.89	7.70 ± 0.029 7.22 – 8.13	8.03 ± 0.07 7.65 – 8.58	7.65 ± 0.10 7.36 – 7.95	7.87 ± 0.03 7.47 – 8.17
IOC	3.21 ± 0.10 2.98 – 3.44	3.14 ± 0.09 3.02 – 3.31	3.20 ± 0.01 2.90 – 3.49	3.27 ± 0.13 3.05 – 3.55	3.01 ± 0.06 2.84 – 3.15	3.12 ± 0.02 2.95 – 3.38
RB	1.61 ± 0.08 1.47 – 1.84	1.57 ± 0.01 1.53 – 1.65	1.56 ± 0.01 1.39 – 1.77	1.60 ± 0.02 1.47 – 1.72	1.58 ± 0.04 1.47 – 1.67	1.52 ± 0.01 1.43 – 1.70
CBL	16.25 ± 0.04 15.23 – 16.81	15.88 ± 0.09 15.40 – 16.65	15.97 ± 0.03 15.02 – 16.94	16.69 ± 0.08 16.00 – 17.08	15.84 ± 0.13 15.51 – 16.32	16.33 ± 0.09 15.63 – 17.19
PL	6.70 ± 0.03 6.17 – 7.28	6.61 ± 0.04 6.38 – 6.87	6.61 ± 0.02 6.09 – 7.00	6.94 ± 0.04 6.59 – 7.21	6.58 ± 0.10 6.42 – 6.95	6.85 ± 0.05 6.45 – 7.30
UniTRL	2.46 ± 0.01 1.82 – 2.46	2.02 ± 0.02 1.93 – 2.17	2.07 ± 0.01 1.80 – 2.31	2.08 ± 0.02 1.89 – 2.21	2.05 ± 0.06 1.87 – 2.18	2.13 ± 0.035 1.87 – 2.35
PM4-M3L	3.88 ± 0.01 3.54 – 4.08	3.85 ± 0.02 3.70 – 3.96	3.85 ± 0.01 3.54 – 4.08	3.99 ± 0.02 3.86 – 4.13	3.82 ± 0.02 3.76 – 3.88	3.96 ± 0.03 3.77 – 4.40
upperTRL	5.83 ± 0.02 5.39 – 6.13	5.76 ± 0.04 5.56 – 5.95	5.81 ± 0.01 5.31 – 6.23	5.99 ± 0.04 5.63 – 6.23	5.75 ± 0.07 5.53 – 5.92	5.94 ± 0.04 5.60 – 6.43
U1B	1.62 ± 0.01 1.41 – 1.78	1.54 ± 0.02 1.40 – 1.64	1.56 ± 0.017 1.40 – 1.79	1.54 ± 0.02 1.42 – 1.68	1.52 ± 0.029 1.47 – 1.56	1.59 ± 0.02 1.40 – 1.74
M2B	4.21 ± 0.02 3.77 – 4.46	4.16 ± 0.07 4.05 – 4.26	4.16 ± 0.01 3.89 – 4.50	4.19 ± 0.04 3.97 – 4.42	3.99 ± 0.05 3.82 – 4.12	4.21 ± 0.00 3.99 – 4.43
BasiW	1.27 ± 0.01 1.11 – 1.43	1.21 ± 0.02 1.10 – 1.34	1.21 ± 0.01 1.04 – 1.38	1.27 ± 0.03 1.14 – 1.43	1.18 ± 0.02 1.12 – 1.25	1.29 ± 0.038 1.02 – 1.67
FMvL	1.76 ± 0.02 1.38 – 2.22	1.68 ± 0.04 1.46 – 1.95	1.67 ± 0.02 1.29 – 21853	1.72 ± 0.08 1.15 – 2.09	1.61 ± 0.08 1.39 – 1.88	1.54 ± 0.05 1.05 – 1.85

Table SB3, part 1. (cont.)

Variable	*S. o. californicus* East Bay uplands $n = 74$	San Pablo Bay $n = 12$	Martinez marsh $n = 122$	*S. o. sinuosus* Grizzly Island $n = 14$	a-Rio Vista $n = 5$	c-Joice Island $n = 25$
FMvW	2.25 ± 0.01 1.96 – 2.63	2.25 ± 0.04 2.08 – 2.45	2.19 ± 0.01 1.80 – 2.50	2.28 ± 0.03 2.09 – 2.49	2.15 ± 0.04 2.03 – 2.29	2.18 ± 0.10 2.01 – 2.36
CD	4.72 ± 0.03 4.31 – 5.24	4.79 ± 0.07 4.25 – 5.06	4.61 ± 0.02 3.61 – 5.22	4.81 ± 0.09 4.14 – 5.30	4.50 ± 0.10 4.29 – 4.78	4.83 ± 0.04 4.42 – 5.27
ZPW	0.97 ± 0.01 0.60 – 1.27	0.91 ± 0.03 0.79 – 1.09	0.92 ± 0.01 0.69 – 1.19	1.05 ± 0.04 0.72 – 1.20	0.91 ± 0.04 0.82 – 1.04	1.04 ± 0.02 0.85 – 1.23
manL	6.67 ± 0.02 6.21 – 7.07	6.60 ± 0.045 6.38 – 6.77	6.60 ± 0.02 6.14 – 7.06	6.96 ± 0.04 6.62 – 7.21	6.63 ± 0.10 6.35 – 6.89	6.66 ± 0.05 6.07 – 7.17
manTRL	4.57 ± 0.02 4.19 – 4.90	4.56 ± 0.03 4.30 – 4.75	4.59 ± 0.01 4.29 – 4.89	4.73 ± 0.03 4.55 – 4.99	4.53 ± 0.04 4.46 – 4.65	4.55 ± 0.04 4.14 – 4.91
m1L	1.25 ± 0.01 1.11 – 1.43	1.23 ± 0.01 1.17 – 30	1.25 ± 0.01 1.12 – 1.42	1.29 ± 0.02 1.10 – 1.42	1.17 ± 0.03 1.09 – 1.21	1.24 ± 0.01 1.11 – 1.38
HCP	3.64 ± 0.02 3.27 – 3.98	3.50 ± 0.06 2.96 – 3.78	3.54 ± 0.02 3.04 – 3.88	3.61 ± 0.04 3.37 – 3.85	3.49 ± 0.04 3.34 – 3.58	3.57 ± 0.02 3.39 – 3.75
HCV	1.79 ± 0.01 1.51 – 2.04	1.74 ± 0.038 1.55 – 1.90	1.74 ± 0.01 1.55 – 2.03	1.85 ± 0.03 1.71 – 2.10	1.68 ± 0.05 1.51 – 1.77	1.79 ± 0.02 1.61 – 1.97
HAC	2.58 ± 0.02 2.18 – 2.95	2.58 ± 0.03 2.33 – 2.70	2.55 ± 0.01 2.30 – 2.86	2.61 ± 0.03 2.43 – 2.76	2.34 ± 0.06 2.18 – 2.50	2.62 ± 0.03 2.41 – 2.91
CHo	4.18 ± 0.03 3.61 – 4.77	4.06 ± 0.05 3.80 – 4.45	3.97 ± 0.02 3.48 – 4.78	4.35 ± 0.06 3.96 – 4.61	4.01 ± 0.06 3.56 – 4.40	4.32 ± 0.04 3.80 – 4.66
FMoH	2.47 ± 0.03 1.95 – 2.92	2.51 ± 0.06 2.13 – 2.77	2.46 ± 0.02 2.00 – 3.03	2.58 ± 0.04 2.29 – 2.95	2.36 ± 0.05 2.20 – 2.46	2.53 ± 0.05 2.05 – 2.98
FMoW	2.55 ± 0.02 2.26 – 2.94	2.53 ± 0.04 2.34 – 2.74	2.51 ± 0.01 2.13 – 2.94	2.51 ± 0.04 2.26 – 2.79	2.36 ± 0.05 2.20 – 2.46	2.46 ± 0.03 2.06 – 2.73
FMoH/CHo ratio	0.593 ± 0.006 0.482 – 0.749	0.618 ± 0.01 0.547 – 0.677	0.622 ± 0.01 0.432 – 0.789	0.594 ± 0.012 0.530 – 0.700	0.597 ± 0.0255 0.533 – 0.676	0.588 ± 0.012 0.463 – 0.677

Table SB3, part 2. Cranio-mandibular variable sample means, standard errors, range, and sample size of shrews of the *Sorex ornatus–vagrans* complex from the greater San Francisco Bay Area, including *S. o. californicus*, *S. o. sinuosus*, *S. v. paludivagus*, *S. v. halicoetes*, and each marsh sample rimming the East Bay and North Bay.

Variable	d-Cordelia Marsh *n* = 9	e-Southhampton *n* = 11	f-Mare Island *n* = 20	g-NW Vallejo *n* = 68	h-Sonoma Creek *n* = 12	i-Tolay Creek *n* = 30
GSL	16.09 ± 0.12 15.39 – 16.45	16.06 ± 0.11 15.58 – 16.61	16.47 ± 0.08 15.79 – 17.03	16.77 ± 0.04 15.75 – 17.50	16.49 ± 0.12 15.81 – 17.11	16.48 ± 0.06 15.44 – 17.01
CB	7.90 ± 0.07 7.55 – 8.19	7.76 ± 0.07 7.27 – 8.07	8.10 ± 0.05 7.74 – 8.67	8.15 ± 0.02 7.75 – 8.59	8.03 ± 0.06 7.66 – 8.32	7.94 ± 0.04 7.55 – 8.27
IOC	3.12 ± 0.04 2.94 – 3.32	3.09 ± 0.03 2.95 – 3.27	3.29 ± 0.03 3.08 – 3.47	3.34 ± 0.01 2.97 – 3.55	3.16 ± 0.02 3.06 – 3.34	3.19 ± 0.02 2.98 – 3.44
RB	1.58 ± 0.03 1.42 – 1.70	1.54 ± 0.02 1.45 – 1.67	1.61 ± 0.02 1.50 – 1.80	1.60 ± 0.01 1.44 – 1.78	1.56 ± 0.03 1.44 – 1.78	1.54 ± 0.01 1.36 – 1.66
CBL	16.14 ± 0.13 15.32 – 16.53	16.14 ± 0.11 15.59 – 16.74	16.61 ± 0.08 15.88 – 17.19	16.78 ± 0.04 15.85 – 17.48	16.53 ± 0.12 15.78 – 17.22	16.56 ± 0.07 15.41 – 17.09
PL	6.66 ± 0.07 6.25 – 6.88	6.68 ± 0.05 6.39 – 6.95	6.92 ± 0.04 6.61 – 7.16	6.98 ± 0.02 6.49 – 7.24	6.83 ± 0.05 6.53 – 7.15	6.86 ± 0.03 6.42 – 7.13
UniTRL	2.07 ± 0.04 1.82 – 2.25	2.11 ± 0.04 1.88 – 2.30	2.14 ± 0.02 1.92 – 2.33	2.15 ± 0.03 1.87 – 2.39	2.11 ± 0.02 2.01 – 2.25	2.11 ± 0.02 1.91 – 2.31
PM4-M3L	3.91 ± 0.03 3.74 – 4.02	3.95 ± 0.02 3.82 – 4.05	4.08 ± 0.03 3.82 – 4.31	4.17 ± 0.02 3.85 – 4.55	4.02 ± 0.03 3.81 – 4.17	3.98 ± 0.02 3.75 – 4.28
upperTRL	5.88 ± 0.07 5.43 – 6.16	5.90 ± 0.05 5.68 – 6.12	6.05 ± 0.04 5.75 – 6.33	6.19 ± 0.029 5.77 – 6.56	6.02 ± 0.049 5.74 – 6.20	5.99 ± 0.03 5.64 – 6.34
U1B	1.56 ± 0.02 1.43 – 1.65	1.57 ± 0.02 1.47 – 1.76	1.61 ± 0.02 1.51 – 1.73	1.61 ± 0.01 1.39 – 1.78	1.60 ± 0.03 1.49 – 1.73	1.47 ± 0.01 1.27 – 1.64
M2B	4.14 ± 0.04 3.97 – 4.37	4.19 ± 0.03 3.99 – 4.33	4.25 ± 0.02 4.11 – 4.41	4.35 ± 0.01 4.13 – 4.74	4.18 ± 0.04 3.96 – 4.41	4.14 ± 0.02 3.87 – 4.34
BasiW	1.20 ± 0.03 1.06 – 1.33	1.19 ± 0.02 1.08 – 1.31	1.31 ± 0.02 1.17 – 1.54	1.33 ± 0.01 1.19 – 1.52	1.30 ± 0.02 1.22 – 1.40	1.21 ± 0.01 1.03 – 1.38
FMvL	1.51 ± 0.06 1.21 – 1.75	1.42 ± 0.07 1.18 – 1.82	1.69 ± 0.04 1.36 – 2.03	1.70 ± 0.03 1.26 – 2.20	1.92 ± 0.07 1.63 – 2.28	1.84 ± 0.04 1.34 – 2.13

Table SB3, part 2. (cont.)

Variable	d-Cordelia Marsh *n* = 9	e-Southhampton *n* = 11	f-Mare Island *n* = 20	g-NW Vallejo *n* = 68	h-Sonoma Creek *n* = 12	i-Tolay Creek *n* = 30
FMvW	2.21 ± 0.04 2.05 – 2.36	2.18 ± 0.03 2.02 – 2.29	2.27 ± 0.02 2.13 – 2.52	2.33 ± 0.01 2.06 – 2.58	2.31 ± 0.04 2.16 – 2.70	2.24 ± 0.03 1.94 – 2.52
CD	4.65 ± 0.12 3.98 – 5.11	4.75 ± 0.08 4.93 – 5.02	5.16 ± 0.05 4.75 – 5.53	5.14 ± 0.03 4.47 – 5.91	5.23 ± 0.06 4.81 – 5.54	4.46 ± 0.08 3.67 – 5.32
ZPW	1.09 ± 0.04 0.94 – 1.34	1.09 ± 0.06 0.84 – 1.42	1.16 ± 0.02 0.99 – 1.31	1.11 ± 0.01 0.85 – 1.37	1.07 ± 0.04 0.80 – 1.21	1.07 ± 0.03 0.83 – 1.31
manL	6.65 ± 0.05 6.42 – 6.86	6.63 ± 0.06 6.35 – 7.04	6.88 ± 0.05 6.47 – 7.29	6.99 ± 0.02 6.56 – 7.31	6.84 ± 0.05 6.44 – 7.05	6.87 ± 0.03 6.48 – 7.23
manTRL	4.54 ± 0.05 4.25 – 4.73	4.52 ± 0.04 4.32 – 4.67	4.66 ± 0.03 4.43 – 4.91	4.79 ± 0.02 4.50 – 5.11	4.64 ± 0.045 4.38 – 4.83	4.71 ± 0.03 4.38 – 4.96
m1L	1.23 ± 0.02 1.11 – 1.28	1.23 ± 0.01 1.15 – 1.28	1.27 ± 0.01 1.19 – 1.35	1.30 ± 0.016 1.21 – 1.42	1.27 ± 0.02 1.20 – 1.36	1.27 ± 0.01 1.18 – 1.38
HCP	3.54 ± 0.06 3.18 – 3.81	3.58 ± 0.05 3.34 – 3.97	3.64 ± 0.05 3.03 – 4.08	3.64 ± 0.02 3.25 – 3.91	3.56 ± 0.03 3.36 – 3.72	3.51 ± 0.03 3.26 – 3.81
HCV	1.77 ± 0.03 1.64 – 1.90	1.76 ± 0.03 1.58 – 1.97	1.83 ± 0.03 1.62 – 2.04	1.79 ± 0.01 1.56 – 2.02	1.75 ± 0.02 1.68 – 1.88	1.75 ± 0.02 1.58 – 1.95
HAC	2.52 ± 0.04 2.35 – 2.74	2.57 ± 0.04 2.37 – 2.79	2.65 ± 0.03 2.35 – 2.94	2.72 ± 0.01 2.39 – 3.05	2.61 ± 0.03 2.32 – 2.74	2.57 ± 0.02 2.32 – 2.77
CHo	4.20 ± 0.108 3.86 – 4.67	4.23 ± 0.09 3.66 – 4.71	4.47 ± 0.06 3.75 – 4.90	4.58 ± 0.04 3.52 – 5.18	4.48 ± 0.10 3.88 – 4.86	4.40 ± 0.05 3.86 – 4.81
FMoH	2.63 ± 0.04 2.37 – 2.75	2.52 ± 0.06 2.18 – 2.76	2.47 ± 0.05 2.01 – 2.93	2.47 ± 0.03 1.94 – 2.89	2.40 ± 0.08 1.81 – 2.72	2.53 ± 0.03 2.21 – 2.90
FMoW	2.48 ± 0.04 2.32 – 2.73	2.47 ± 0.05 2.10 – 2.64	2.50 ± 0.04 1.91 – 2.87	2.54 ± 0.02 1.98 – 2.85	2.45 ± 0.04 2.17 – 2.59	2.42 ± 0.02 2.21 – 2.63
FMoH/Cho ratio	0.629 ± 0.012 0.585 – 0.672	0.598 ± 0.017 0.516 – 0.727	0.554 ± 0.012 0.471 – 0.705	0.541 ± 0.007 0.407 – 0.642	0.538 ± 0.018 0.465 – 0.671	0.578 ± 0.011 0.491 – 0.708

Table SB3, part 3. Cranio-mandibular variable sample means, standard errors, range, and sample size of shrews of the *Sorex ornatus–vagrans* complex from the greater San Francisco Bay Area, including *S. o. californicus*, *S. o. sinuosus*, *S. v. paludivagus*, *S. v. halicoetes*, and each marsh sample rimming the East Bay and North Bay.

Variable	j-Black Point *n* = 39	k-Novato marsh *n* = 17	l-Petaluma *n* = 10	m-San Rafael *n* = 53	n-Larkspur *n* = 30	*S. v. vagrans* S Marin *n* = 72
GSL	16.11 ± 0.05 15.22 – 16.78	16.34 ± 0.11 15.55 – 17.24	16.05 ± 0.11 15.63 – 16.70	16.34 ± 0.07 15.75 – 17.27	16.57 ± 0.07 15.90 – 17.24	17.64 ± 0.049 16.06 – 17.64
CB	7.87 ± 0.03 7.41 – 8.35	8.01 ± 0.06 7.37 – 8.52	7.85 ± 0.06 7.62 – 8.28	7.94 ± 0.03 7.48 – 8.40	8.05 ± 0.04 7.68 – 8.48	8.14 ± 0.02 7.74 – 8.36
IOC	3.19 ± 0.02 2.99 – 3.38	3.18 ± 0.03 3.07 – 3.36	3.18 ± 0.03 2.97 – 3.30	3.24 ± 0.02 2.86 – 3.41	3.41 ± 0.03 3.10 – 3.66	3.30 ± 0.01 3.03 – 3.57
RB	1.53 ± 0.01 1.37 – 1.69	1.59 ± 0.02 1.46 – 1.80	1.57 ± 0.02 1.45 – 1.72	1.53 ± 0.01 1.38 – 1.67	1.59 ± 0.02 1.42 – 1.85	1.55 ± 0.01 1.40 – 1.69
CBL	16.23 ± 0.05 15.34 – 17.02	16.48 ± 0.12 15.63 – 17.30	16.12 ± 0.10 15.71 – 16.70	16.38 ± 0.06 15.07 – 17.27	16.63 ± 0.07 16.02 – 17.14	16.76 ± 0.04 15.90 – 17.66
PL	6.76 ± 0.03 6.35 – 7.16	6.80 ± 0.05 6.50 – 7.22	6.59 ± 0.076 6.32 – 6.90	6.80 ± 0.02 6.22 – 7.19	6.89 ± 0.04 6.50 – 7.30	6.82 ± 0.02 6.46 – 7.22
UniTRL	2.10 ± 0.01 1.96 – 2.30	2.06 ± 0.03 1.88 – 2.31	2.08 ± 0.046 1.90 – 2.25	2.09 ± 0.01 1.79 – 2.19	2.11 ± 0.02 1.91 – 2.37	2.10 ± 0.01 1.90 – 2.29
PM4-M3L	3.92 ± 0.027 3.63 – 4.16	3.93 ± 0.045 3.69 – 4.16	3.88 ± 0.03 3.72 – 4.04	3.90 ± 0.02 3.58 – 4.21	3.92 ± 0.01 3.63 – 4.14	4.00 ± 0.01 3.66 – 4.23
upperTRL	5.91 ± 0.02 5.58 – 6.17	5.88 ± 0.05 5.44 – 6.34	5.80 ± 0.07 5.58 – 6.13	5.87 ± 0.02 5.25 – 6.23	5.95 ± 0.03 5.57 – 6.28	5.99 ± 0.12 5.75 – 6.28
U1B	1.49 ± 0.01 1.28 – 1.66	1.54 ± 0.02 1.40 – 1.66	1.56 ± 0.03 1.43 – 1.71	1.43 ± 0.01 1.28 – 1.62	1.55 ± 0.027 1.38 – 1.80	1.49 ± 0.01 1.28 – 1.67
M2B	4.14 ± 0.028 3.83 – 4.40	4.19 ± 0.03 3.92 – 4.41	4.19 ± 0.04 4.06 – 4.35	4.07 ± 0.02 3.71 – 4.33	4.22 ± 0.036 3.96 – 4.50	4.23 ± 0.01 4.01 – 4.45
BasiW	1.19 ± 0.01 1.03 – 1.35	1.19 ± 0.02 1.07 – 1.36	1.20 ± 0.03 0.99 – 1.34	1.25 ± 0.01 1.09 – 1.42	1.23 ± 0.01 1.11 – 1.41	1.25 ± 0.01 1.05 – 1.40
FMvL	1.66 ± 0.03 1.28 – 1.99	1.81 ± 0.04 1.46 – 2.16	1.76 ± 0.07 1.30 – 2.03	1.94 ± 0.03 1.55 – 2.38	1.87 ± 0.04 1.33 – 2.28	2.11 ± 0.02 1.56 – 2.56

Table SB3, part 3. (cont.)

Variable	j-Black Point *n* = 39	k-Novato marsh *n* = 17	l-Petaluma *n* = 10	m-San Rafael *n* = 53	n-Larkspur *n* = 30	*S. v. vagrans* S Marin *n* = 72
FMvW	2.21 ± 0.02 1.91 – 2.36	2.19 ± 0.02 1.93 – 2.34	2.22 ± 0.03 2.09 – 2.39	2.28 ± 0.02 2.07 – 2.59	2.25 ± 0.02 2.03 – 2.53	2.33 ± 0.01 2.11 – 2.52
CD	4.59 ± 0.03 3.82 – 4.90	4.97 ± 0.08 4.48 – 5.66	4.83 ± 0.073 4.51 – 5.17	5.01 ± 0.03 4.48 – 5.64	5.22 ± 0.05 4.64 – 5.72	5.44 ± 0.04 4.71 – 6.19
ZPW	1.03 ± 0.01 0.85 – 1.21	1.10 ± 0.02 0.96 – 1.28	1.06 ± 0.037 0.91 – 1.28	1.11 ± 0.01 0.87 – 1.33	1.08 ± 0.02 0.91 – 1.33	1.10 ± 0.01 0.79 – 1.18
manL	6.70 ± 0.03 6.31 – 7.02	6.74 ± 0.075 6.39 – 7.26	6.63 ± 0.070 6.32 – 6.94	6.67 ± 0.03 6.02 – 7.18	6.82 ± 0.03 6.89 – 7.14	6.95 ± 0.02 6.62 – 7.33
manTRL	4.60 ± 0.02 4.26 – 4.80	4.59 ± 0.04 4.32 – 4.91	4.58 ± 0.942 4.42 – 4.84	4.60 ± 0.02 4.25 – 4.87	4.63 ± 0.03 4.33 – 4.90	4.75 ± 0.01 4.42 – 4.96
m1L	1.26 ± 0.01 1.12 – 1.35	1.22 ± 0.02 1.08 – 1.33	1.23 ± 0.016 1.16 – 1.31	1.23 ± 0.01 1.11 – 1.31	1.22 ± 0.01 1.06 – 1.42	1.29 ± 0.01 1.19 – 1.43
HCP	3.46 ± 0.03 2.97 – 3.88	3.67 ± 0.04 3.3434 – 3.95	3.63 ± 0.05 3.44 – 3.77	3.60 ± 0.02 3.20 – 3.84	3.71 ± 0.03 3.45 – 4.06	3.72 ± 0.01 3.47 – 4.08
HCV	1.74 ± 0.02 1.55 – 2.06	1.79 ± 0.02 1.66 – 1.92	1.78 ± 0.03 1.61 – 1.97	1.75 ± 0.01 1.59 – 1.93	1.86 ± 0.02 1.63 – 2.12	1.83 ± 0.01 1.60 – 2.02
HAC	2.53 ± 0.02 2.22 – 2.85	2.58 ± 0.03 2.39 – 2.83	2.53 ± 0.05 2.35 – 2.84	2.56 ± 0.01 2.36 – 2.83	2.61 ± 0.02 2.36 – 2.86	2.66 ± 0.01 2.33 – 2.95
CHo	4.09 ± 0.04 3.31 – 4.95	4.33 ± 0.06 3.95 – 4.80	4.24 ± 0.08 3.85 – 4.74	4.32 ± 0.04 3.83 – 5.15	4.38 ± 0.05 3.90 – 4.86	4.76 ± 0.03 3.97 – 5.32
FMoH	2.50 ± 0.03 2.09 – 2.91	2.45 ± 0.06 1.90 – 2.87	2.57 ± 0.03 2.41 – 2.76	2.41 ± 0.03 1.90 – 2.82	2.54 ± 0.04 1.96 – 3.01	2.41 ± 0.02 1.86 – 2.79
FMoW	2.42 ± 0.03 2.07 – 2.70	2.41 ± 0.04 2.09 – 2.65	2.50 ± 0.04 2.30 – 2.70	2.49 ± 0.02 2.12 – 2.85	2.49 ± 0.03 2.04 – 2.80	2.51 ± 0.01 1.98 – 2.73
FMoH/CHo ratio	0.613 ± 0.008 0.518 – 0.714	0.567 ± 0.014 0.448 – 0.687	0.607 ± 0.016 0.558 – 0.689	0.560 ± 0.008 0.458 – 0.703	0.581 ± 0.010 0.449 – 0.659	0.506 ± 0.005 0.401 – 0.623

Table SB3, part 4. Cranio-mandibular variable sample means, standard errors, range, and sample size of shrews of the *Sorex ornatus–vagrans* complex from the greater San Francisco Bay Area, including *S. o. californicus*, *S. o. sinuosus*, *S. v. paludivagus*, *S. v. halicoetes*, and each marsh sample rimming the East Bay and North Bay.

Variable	*S. v. vagrans* Pt. Reyes *n* = 128	*S. v. halicoetes* SF Bay *n* = 154	*S. v. paludivagus* SF peninsula *n* = 45
GSL	16.63 ± 0.03 15.42 – 17.25	16.86 ± 0.03 16.08 – 17.70	16.83 ± 0.05 15.96 – 17.41
CB	8.21 ± 0.02 7.58 – 8.77	8.34 ± 0.02 7.92 – 8.90	8.27 ± 0.04 7.48 – 8.66
IOC	3.26 ± 0.01 3.01 – 3.51	3.29 ± 0.01 3.08 – 3.57	3.30 ± 0.09 3.17 – 3.54
RB	1.52 ± 0.01 1.31 – 1.72	1.54 ± 0.01 1.35 – 1.69	1.53 ± 0.01 1.42 – 1.73
CBL	16.25 ± 0.04 15.23 – 16.81	16.89 ± 0.03 16.08 – 17.74	16.86 ± 0.05 15.84 – 17.45
PL	6.70 ± 0.03 6.17 – 7.28	6.89 ± 0.02 6.26 – 7.42	6.93 ± 0.02 6.58 – 7.25
UniTRL	2.14 ± 0.019 1.93 – 2.40	2.08 ± 0.01 1.53 – 2.37	2.16 ± 0.01 1.96 – 2.35
PM4-M3L	4.03 ± 0.01 3.68 – 4.35	4.05 ± 0.01 3.76 – 4.30	4.01 ± 0.026 3.73 – 4.21
upperTRL	6.03 ± 0.01 5.59 – 6.38	6.02 ± 0.02 5.43 – 6.39	6.04 ± 0.02 5.58 – 6.35
U1B	1.48 ± 0.01 1.30 – 1.67	1.52 ± 0.01 1.29 – 1.71	1.51 ± 0.01 1.38 – 1.80
M2B	4.19 ± 0.01 3.81 – 4.44	4.25 ± 0.01 3.90 – 4.59	4.22 ± 0.027 3.87 – 4.50
BasiW	1.24 ± 0.01 1.05 – 1.41	1.32 ± 0.01 1.14 – 1.58	1.30 ± 0.02 1.05 – 1.57
FMvL	2.13 ± 0.02 1.73 – 2.65	2.20 ± 0.01 1.75 – 2.67	2.14 ± 0.03 1.70 – 2.53
FMvW	2.31 ± 0.01 2.04 – 2.71	2.39 ± 0.019 2.06 – 2.63	2.37 ± 0.02 1.97 – 2.69
CD	5.60 ± 0.03 4.47 – 6.20	5.64 ± 0.02 4.26 – 6.38	5.70 ± 0.05 4.55 – 6.21
ZPW	1.07 ± 0.019 0.82 – 1.31	1.12 ± 0.018 0.88 – 1.38	1.08 ± 0.01 0.83 – 1.28
manL	6.84 ± 0.02 6.43 – 7.27	6.93 ± 0.01 6.54 – 7.27	6.96 ± 0.03 6.60 – 7.53
manTRL	4.68 ± 0.01 4.21 – 5.07	4.72 ± 0.00 4.31 – 5.02	4.70 ± 0.02 4.37 – 4.96
m1L	1.27 ± 0.01 1.14 – 1.37	1.27 ± 0.01 1.14 – 1.43	1.28 ± 0.01 1.16 – 1.39
HCP	3.73 ± 0.01 3.44 – 3.95	3.70 ± 0.01 3.41 – 3.99	3.76 ± 0.02 3.41 – 4.12

Table SB3, part 4. (cont.)

Variable	*S. v. vagrans* Pt. Reyes *n* = 128	*S. v. halicoetes* SF Bay *n* = 154	*S. v. paludivagus* SF peninsula *n* = 45
HCV	1.83 ± 0.019 1.45 – 2.10	1.87 ± 0.01 1.64 – 2.20	1.86 ± 0.01 1.67 – 2.10
HAC	2.73 ± 0.01 2.37 – 3.07	2.69 ± 0.01 2.36 – 2.99	2.70 ± 0.02 2.04 – 2.94
CHo	4.90 ± 0.02 4.09 – 5.41	4.84 ± 0.02 3.60 – 5.70	4.88 ± 0.04 4.50 – 5.54
FMoH	2.37 ± 0.028 1.87 – 2.91	2.43 ± 0.02 1.88 – 3.05	2.54 ± 0.04 2.10 – 3.01
FMoW	2.48 ± 0.02 1.95 – 2.95	2.59 ± 0.01 2.06 – 2.96	2.61 ± 0.02 2.12 – 2.84
FMoH/CHo ratio	0.486 ± 0.004 0.391 – 0.626	0.504 ± 0.004 0.346 – 0.669	0.521 ± 0.008 0.398 – 0.612

Table SB4. Mean, standard error, and range of posterior probabilities (pprob) of all greater San Francisco Bay Area samples to each of three reference samples (*S. o. californicus*, *S. sinuosus*, and *S. vagrans* [combined samples of *S. v. halicoetes*, *S. v. paludivagus*, and *S. v. vagrans*]) derived from a CVA using 25 cranio-mandibular variables.

Sample	pprob *californicus*	pprob *sinuosus*	pprob *vagrans*
S. o. californicus	0.949 ± 0.002	0.047 ± 0.012	0.006 ± 0.004
n = 70	0.560 – 1.000	0.000 – 0.439	0.000 – 0.271
S. o. sinuosus	0.022 ± 0.014	0.944 ± 0.025	0.034 ± 0.023
n = 14	0.000 – 0.181	0.725 – 0.999	0.000 – 0.275
S. vagrans	0.004 ± 0.001	0.014 ± 0.003	0.982 ± 0.003
n = 325	0.000 – 0.271	0.000 – 0.664	0.310 – 1.000
S. ornatus – Martinez marsh	0.959 ± 0.012	0.036 ± 0.007	0.006 ± 0.010
n = 121	0.624 – 1.000	0.000 – 0.361	0.000 – 0.193
S. ornatus – San Pablo marsh	0.999 ± 0.0001	0.000 ± 0.0001	0.000 ± 0.0001
n = 12	0.999 – 1.000	0.000 – 0.001	0.000 – 0.001
a–Rio Vista	0.727 ± 0.171	0.273 ± 0.171	0.000 ± 0.0002
n = 5	0.074 – 0.994	0.006 – 0.927	0.000 – 0.001
c–Joice Island	0.457 ± 0.079	0.532 ± 0.078	0.011 ± 0.007
n = 25	0.000 – 0.996	0.004 – 0.999	0.000 – 0.144
d–Suisun Marsh	0.279 ± 0.004	0.710 ± 0.126	0.011 ± 0.008
n = 9	0.432 – 0.789	0.037 – 0.998	0.000 – 0.074
e–Southampton	0.501 ± 0.129	0.497 ± 0.130	0.002 ± 0.002
n = 11	0.000 – 0.998	0.002 – 0.999	0.000 – 0.017
f–Mare Island	0.208 ± 0.078	0.561 ± 0.099	0.231 ± 0.086
n = 20	0.000 – 0.963	0.002 – 0.999	0.000 – 0.998
g–NW Vallejo	0.045 ± 0.018	0.563 ± 0.052	0.392 ± 0.051
n = 68	0.000 – 0.999	0.000 – 0.999	0.000 – 0.999
h–Sonoma Creek	0.247 ± 0.110	0.377 ± 0.114	0.376 ± 0.127
n = 12	0.000 – 0.998	0.002 – 0.969	0.000 – 0.997
i–Tolay Creek	0.051 ± 0.026	0.810 ± 0.048	0.140 ± 0.044
n = 30	0.000 – 0.682	0.051 – 0.999	0.000 – 0.948
j–Black Point	0.197 ± 0.097	0.797 ± 0.046	0.006 ± 0.003
n = 39	0.001 – 0.997	0.000 – 0.995	0.000 – 0.130
k–Novato marsh	0.408 ± 0.004	0.358 ± 0.092	0.235 ± 0.0937
n = 17	0.432 – 0.789	0.000 – 0.995	0.000 – 0.999
l–Petaluma	0.722 ± 0.112	0.177 ± 0.087	0.101 ± 0.083
n = 10	0.069 – 0.999	0.000 – 0.751	0.000 – 0.921

Table SB4. (cont.)

Sample	pprob *californicus*	pprob *sinuosus*	pprob *vagrans*
m–San Rafael marsh *n* = 53	0.325 ± 0.048 0.000 – 0.996	0.369 ± 0.0450 0.000 – 0.999	0.306 ± 0.051 0.000 – 0.999
n–Larkspur *n* = 30	0.238 ± 0.057 0.000 – 0.999	0.353 ± 0.069 0.000 – 0.999	0.409 ± 0.079 0.000 – 0.994

Suggested citation format:

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Family Phyllostomidae Gray 1825 (Chiroptera): Summary 2000 to 2018

Lizette Siles and Rodrigo S. Rios

Abstract

The bat family Phyllostomidae has undergone one of the largest known adaptive radiations among mammals; currently it is the second most diverse family of bats after Vespertilionidae and the most diverse with respect to feeding habits. Consequently, the Phyllostomidae plays a vital role in ecosystem processes, which include forest regeneration, plant pollination, and insect predation. These characteristics make phyllostomids a fascinating group to study and current research is very dynamic. In this review, a summary of the state of knowledge regarding three main aspects of phyllostomids is provided—rapid diversification, systematics, and recent taxonomic changes. First, the rapid diversification in the family is explored, and then the morphological, ecological, historical, and genetic processes that allowed this diversification to occur are discussed. Systematics and taxonomy in Phyllostomidae have been influenced by the intense molecular work carried out in recent decades. Early results showed that molecular and morphological phylogenies of Phyllostomidae were not congruent and revealed that feeding guilds were not necessarily monophyletic. Since then, numerous efforts have been made to resolve the family comprehensively and a discussion of the most recently published phylogenies is included. Taxonomically, new species have been described every year since the last revision in 2005, which resulted in a 34% increase in number of species, accounting for approximately 60% of all bats described in the Neotropics. A detailed review of these changes is presented, which includes described species, elevated subspecies or synonyms, and synonymized species. Taxonomic revisions were focused on only 10 of the 60 phyllostomid genera, thus research opportunities in this area are extensive. Future work promises to be equally intense to obtain an accurate description of the family's diversity, define speciation patterns, provide an accurate taxonomy for ecological and behavioral studies, and delimit species distributions.

Key words: Chiroptera, Neotropics, Phyllostomidae, rapid diversification, systematics, taxonomy

Introduction

One of biology's most fundamental and recognizable patterns is that species diversity is highest in the tropical regions of the world, with a few exceptions (Willlig et al. 2003). Explanations for this pattern date to Dobzhansky (1950) and competitive arguments have been circular or contradictory (Lugo 1988). A review by Mittelbach et al. (2007) summarized three kinds of explanations for the pattern: ecological hypotheses that focus on species coexistence and diversity maintenance, evolutionary hypotheses that focus on rates of diversification, and historical hypotheses that focus on the duration and extent of tropical environments in Earth's history.

The latitudinal diversity gradient is particularly true for mammals in the New World tropics. Originally defined by Wallace (1876), the Neotropical region includes South America, tropical North America, and the Antilles, and is defined by its large proportion of lowlands, tropical forests, a large mountain range, favorable climate, and a high diversity of genera and species. Currently, more than 1,500 species of mammals have been described from this region, which is approximately 30% of all extant mammal species (Patterson and Costa 2012). In the case of Chiroptera, approximately 100 species of bats have been estimated to occur in sympatry (Voss and Emmons 1996), but the highest bat diversity sampled has yielded 78 species in a 3 km radius (Simmons and Voss 1998). A single area can only support this many species through an effective resource partitioning among competing species (Hutchinson 1959; Giller 1984).

There are several speciose endemic mammalian groups that occur in the Neotropics, e.g. platyrrhine monkeys, caviomorph rodents, and sigmodontine rodents. However, bats of the family Phyllostomidae are recognized as being the most ecologically diverse because they comprise species with all the dietary strategies used by Chiroptera (Baker et al. 2012). Bat families that are also endemic to this region, are very poor in diversity (Furipteridae, Mormoopidae, Natalidae, Noctilionidae, and Thyropteridae) (Table 1). Other bat families that have a cosmopolitan distribution are less diverse than phyllostomids in the Neotropics: Emballonuridae has 23 species, Molossidae 51, and Vespertilionidae 85 (Solari and Martínez-Arias 2014). Overall, the Phyllostomidae has the largest number of genera and is the second most speciose bat family after Vespertilionidae (Table 1). It is distributed mostly across the Neotropics, but can also be found in the extreme southwestern United States (Villalobos and Arita 2010).

The Phyllostomidae is one of the most highly studied bat families encompassing almost every aspect of its biology. Its high species diversity, adaptations, feeding guilds, and rapid diversification make it a fascinating group to study. Perhaps another contributing factor is that its members are the most easily captured species using traditional mist-netting techniques. Dr. Robert J. Baker (1942–2018) was captivated by phyllostomid bats from the beginning of his career (Genoways et al. 2018) and contributed enormously to our current knowledge of the family. This review focuses on three topics of Dr. Baker's work and legacy within the group. The first topic describes the morphological, historical, ecological, and genetic processes that have contributed to the rapid diversification in phyllosomid bats. The second topic summarizes the recent systematics of the family, and the third topic details recent taxonomic changes.

Table 1. Current taxa within bat families based on information from www.itis.gov, excluding Phyllostomidae, which is based on published records through November 2018.

Bat Families	Subfamilies	Genera	Species	Subspecies
Vespertilionidae	5	50	456	397
Phyllostomidae	11	60	218	126
Pteropodidae	2	44	195	205
Molossidae	2	16	119	100
Rhinolophidae		1	95	144
Hipposideridae		9	94	89
Emballonuridae		14	53	61
Miniopteridae	1	1	31	34
Nycteridae		1	16	18
Natalidae		3	12	
Mormoopidae		2	10	22
Rhinopomatidae		1	6	10
Megadermatidae		4	5	22
Thyropteridae		1	5	5
Furipteridae		2	2	
Mystacinidae		1	2	
Myzopodidae		1	2	
Noctilionidae		1	2	6
Cistugidae		1	2	
Craseonycteridae		1	1	

Rapid Diversification in Phyllostomid Bats

It is evident that the family Phyllostomidae has undergone one of the largest known adaptive radiations among mammals (Baker et al. 2003, 2012; Freeman 2000; Dumont et al. 2012). The origin of Phyllostomidae occurred approximately 35 million years ago (MYA) (Baker et al. 2012; Amador et al. 2018), but most species have diversified in a short time period. Of the 154 valid species analyzed by Amador et al. (2018), 58.4% evolved in the last 5 million years and 80.5% in the last 10 million years (58% of all phyllostomids), leaving a small window of evolutionary time for these major adaptive changes to have accumulated (Fig. 1). The possible scenario that allowed this diversification includes eco-morphological, historical and ecological, and genetic processes.

Eco-morphological processes.— The most striking and perceptible characteristic of the species in Phyllostomidae is their morphological adaptations and how these relate to the ecological role they perform in the environment, specifically regarding their diet. Based on phylogenetic data and the widespread occurrence of insectivory, it is more parsimonious to assume that this was the feeding strategy of the common ancestor of all members of this family (Freeman 2000; Baker et al. 2012). The primitive insectivore of the Phyllostomidae was not very differentiated in morphology from the modern insectivorous species that eat some plant material and are located at the base of the phylogenetic tree, i.e. *Macrotus* and *Micronycteris* (Freeman 2000; Baker et al. 2012). These genera have retained ancestral characteristics for teeth, reproductive histomorphology (Hood and Smith 1982), and post-cranial anatomy (Walton and Walton 1968). It is likely that a minor change in jaw mechanics occurred, which had major implications at the tooth-food interface (Freeman 2000), producing an unusual insectivorous bat that was able to eat at least some plant material (Freeman 2000; Baker et al. 2012). This change in ecology sets the bases for further variation in diet and great morphological diversification, allowing phyllostomids to escape the insectivore morphospace into different feeding guilds.

In the case of carnivores, changes in tooth morphology, such as larger teeth relative to the palate and an enlarged protoconid in the lower molars (Freeman 1998) allowed these bats to feed on small vertebrates. This change in diet allowed an increase in the body mass due to the larger prey, therefore escaping the insectivorous morphospace by significantly increasing its body size (Freeman 2000), e.g. *Vampyrum spectrum* (171–180 g) and *Chrotopterus auritus* (62–77 g). However, Santana et al. (2011) discovered that the much smaller *Micronycteris microtis* (7 g) could feed on anole lizards, which not only makes it the smallest carnivore reported to date, but also broadens significantly their ecological niche and exhibits their plasticity.

Bats that shifted their diet from insects to fruit are likely to share this derived character of Phyllostomidae, therefore frugivory may be the dietary pleisiomorphy of the family (Baker et al. 2012). Currently, frugivorous bats comprise the most speciose feeding guild within the family (3 subfamilies, 107 species). Some frugivores retained the ancestral cranial and tooth morphology (e.g., *Carollia,* Freeman 2000) and a portion of their diet includes insects, whereas others have evolved teeth specialized for processing only fruit (e.g., *Centurio*), which in turn modified greatly the overall skull pattern towards a short rostrum and round braincase.

For nectar and pollen-feeding bats, most of the morphological changes included the lengthening of the rostrum and a size reduction of the teeth (Fig. 2) (Freeman 2000). The specialization of the tongue (elongated and hirsute) to collect nectar is one of the most extreme among mammalian nectivores (Freeman 1998). Similarly to *Carollia* for the frugivores, some species like *Glossophaga soricina* have retained the most ancestral form, whereas other species have evolved extreme morphological modifications (Freeman 2000), e.g. *Anoura fistulata* with a tongue that is 150% its body length (Muchhala et al. 2005), and the elongated skulls of *Musonycteris harrisoni* and *Platalina genovensium* (Fig. 2). A third morphological pattern can be observed in the island-dwellers of the tribe Brachyphyllini (Fig. 2), which evolved away from specialized nectarivory to a more frugivorous, florivorous, and generalized diet (Freeman 2000).

Only two groups of vertebrates are obligate blood-feeders, catfish of the subfamily Vandellinae,

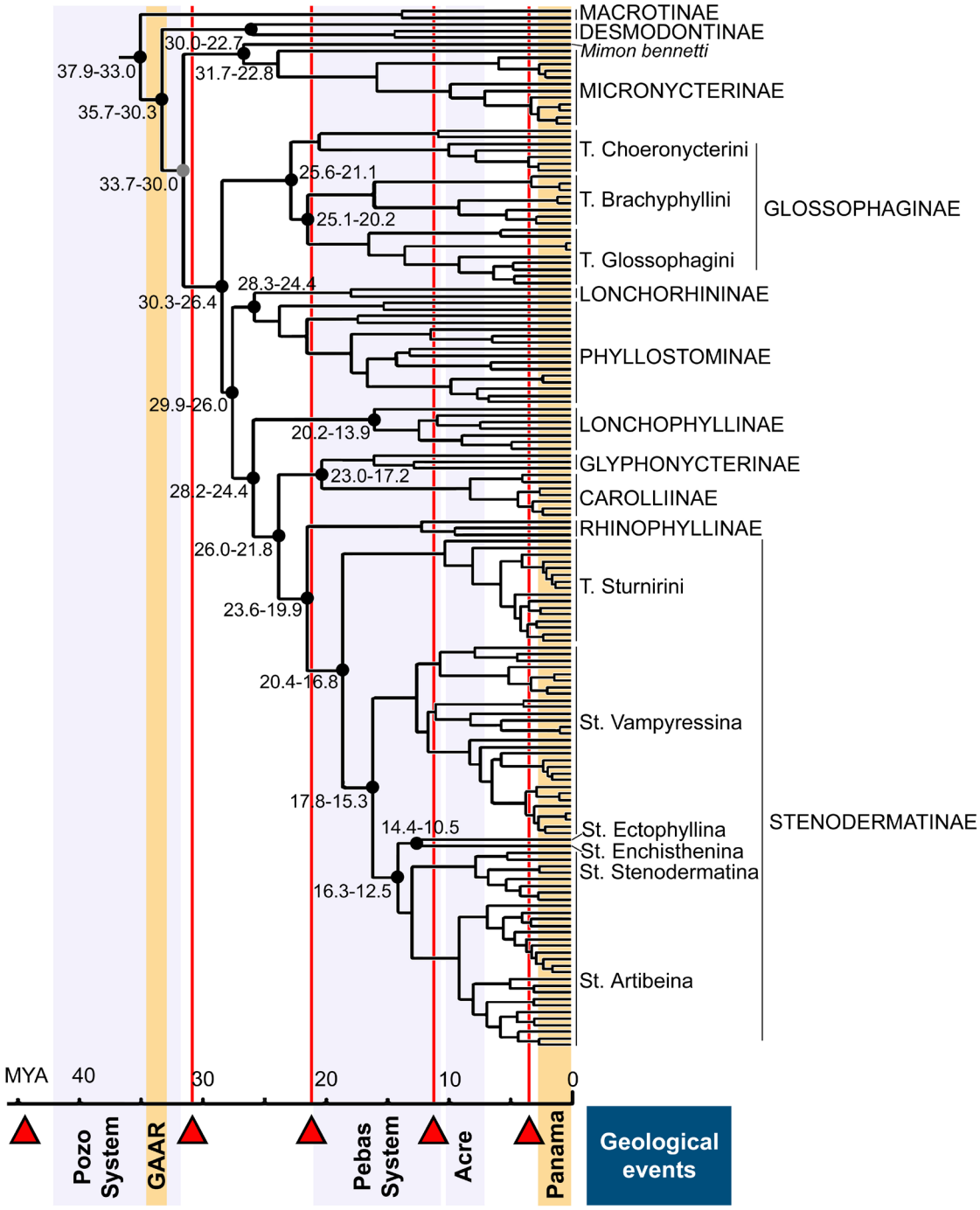

Figure 1. Dated phylogeny of the family Phyllostomidae (modified from Amador et al. 2018). Black filled circles: posterior probability ≥ 0.95, gray filled circles: posterior probability < 0.95, with high posterior density intervals for node ages. Taxonomic arrangement follows Baker et al. (2016). Geological events are modified from Hoorn et al. (2010). Red triangles and lines: periods of intensified Andean uplift; Panama: closing of Panama Isthmus and start of GABI; Acre: fluvial Western Amazonian wetland; Pebas system: large wetland of shallow lakes and swamps in Western Amazonia; GAAR: Greater Antilles-Aves Ridge; and Pozo system: large extension of Amazonia over most of northern South America.

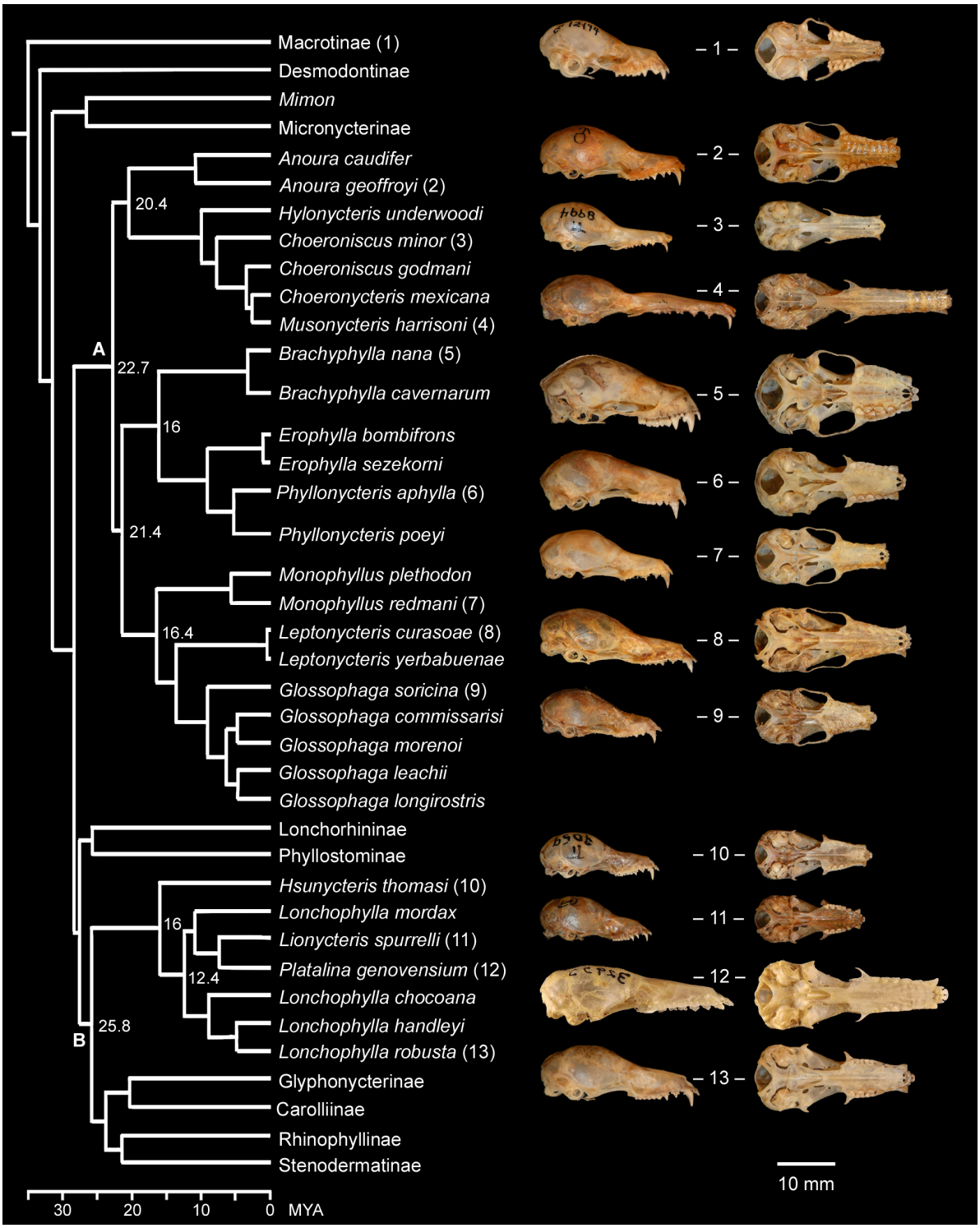

Figure 2. Skull morphology of representative members of nectar-feeding bats of the subfamilies Glossophaginae (clade A) and Lonchophyllinae (B). The dated phylogeny with node ages is modified from Amador et al. (2018). The skull of *Macrotus californicus* (1) is shown as a reference for the ancestral morphotype. Photos and specimen information are available in Morphobank: https://morphobank.org/permalink/?P3449.

family Trichomycteridae (Nelson 2006) and vampire bats of the subfamily Desmodontinae, represented by three highly specialized species of the genera *Desmodus*, *Diaemus*, and *Diphylla* (Emmons and Feer 1999). To allow for this specialized diet, morphological changes included an extreme reduction of the molars and enlargement of the incisors and canines to occupy 80% of the tooth area (Freeman 1998), as well as physiological changes to be able to digest blood and behavioral changes to obtain it (Baker et al. 2012). Baker et al. (2012) hypothesized that this group is likely to have undergone the largest magnitude of directional selection within a restricted time frame, from the insectivore common ancestor to a specialized sanguivore in approximately 4 million years. However, lack of fossils or extant intermediate forms that link these bats to the ancestral insectivore or to any other group within the family make the study of their origins more difficult (Baker et al. 1989; Freeman 2000).

Historical and ecological processes.—The environment may play a key role in a given speciation event, and in order to understand how the evolutionary processes took place, a geographic and ecological setting is needed. From a broad perspective, the mammals of the Neotropics evolved largely within the context of isolation, punctuated by rare episodes of waif dispersal and faunal interchange (Patterson and Costa 2012). The most significant transitions for mammals occurred: (a) near the end of the Age of Dinosaurs (~ 65 MYA) when most early mammals were replaced by marsupials and placentals (Meredith et al. 2011; Croft 2012), (b) when South America was isolated from Antarctica and Australia (~ 35 MYA), which was followed by a substantial drop in global temperatures (Croft 2012) and significant changes in terrestrial habitats (Flynn and Weiss 1998), and (c) during the Great American Biotic Interchange (GABI) between North and South America, when these land masses became connected (~ 3 MYA) (Croft 2012). Phyllostomids were most likely affected by the last two transitions, given that its origin was 25 to 38 MYA (Baker et al. 2012), which also coincides with the formation of the Andes in the Neogene. This event had a profound impact on the history of the South American continent, because it changed the course of the Amazon system (from northwestwards to eastwards), and affected the climate of the region by forming the only barrier to atmospheric circulation in the Southern Hemisphere (Antonelli et al. 2009; Hoorn et al. 2010). Geological reconstructions (Hoorn et al. 1995; Gregory-Wodzicki 2000) indicate that the uplift took place in discrete periods, progressing from south to north and from west to east (in northern South America), affecting different regions at different times (Antonelli et al. 2009) (Fig. 1). Furthermore, marine floods related to the sea level rise followed the periods of major uplift, having an important impact on drainage patterns of the region and possibly acted as barriers to dispersal, fragmenting the ranges of terrestrial animals and plants (Antonelli et al. 2009). An example of this is the fluvial system "paleo-Orinoco" that occurred at the end of the Oligocene (~24 MYA), which, coupled with the uplift of the Eastern Cordillera in the Central Andes (~23 MYA) caused western Amazonia to become submerged. These events created a huge system (more than 1 million km^2) of long-lived (until 17 or 11 MYA) lakes and wetlands known as "Lake Pebas" (Antonelli et al. 2009; Hoorn et al. 2010). The most intense periods of the Andean uplift took place in the middle Miocene (~ 12 MYA) and early Pliocene (~ 4.5 MYA), which were followed by the closing of the Panama Isthmus (~ 3.5 MYA) and the Quaternary ice ages (2.5 to 0.01) (Hoorn et al. 2010) (Fig. 1).

The oldest phyllostomid fossils date to the middle Miocene and include two extinct genera (*Notonycteris magdalenensis*, *N. sucharadeus*, *Palynephyllum antimaster*) and an undetermined species of *Tonatia* or *Lophostoma* (Czaplewski et al. 2003a). Additionally, there is a vast diversity of Quaternary phyllostomid records from the Caribbean (Velazco et al. 2013; Soto-Centeno et al. 2017), Central America (Czaplewski et al. 2003b), and South America (de Aguiar and de Oliveira 2005; Hadler et al. 2018).

Genetic processes.— The underlying genetic changes involved in the diversification of this family possibly started with a small change in the skull, leading to an adaptation to feed on plant material (an available niche space), which in turn awarded these bats with increased fitness and the ability to explore other feeding guilds (Freeman 2000; Baker et al. 2012). It has been long recognized that phyllostomids have undergone intense chromosomal rearrangements in most of the taxa (Baker 1967; Baker and Bickham 1980). Furthermore, a study by Sotero-Caio et al. (2013) of nectarivores found that bat Evolutionary Conserved Units (ECUs) were rearranged differently in each nectar-feeding

clade, which is compatible with the hypothesis that rearrangements might have played a key role in genome organization and eco-morphological evolution of the family. Modern methodologies in karyotypic studies, such as chromosome painting, will help reconstruct ancestral chromosomal associations between species and determine the role of chromosomal evolution in phyllostomid diversification (Volleth and Eik 2012; Sotero-Caio et al. 2013).

A series of phylogenetic studies on phyllostomid species have revealed species-level clades, which resulted in descriptions of new species or the elevation of recognized subspecies, e.g. *Artibeus* (Larsen et al. 2007); *Carollia* (Hoffmann and Baker 2003; Solari and Baker 2006); *Dermanura* (Solari et al. 2009); *Glossophaga* (Hoffmann and Baker 2001); and *Vampyressa* (Porter and Baker 2004). Most of these studies relied on the genetic species concept based on the Bateson-Dobzhansky-Muller (BDM) process (Baker and Bradley 2006) to explain diversification in these groups. Speciation via the BDM model occurs when populations become sufficiently isolated to allow different evolutionary and ecological forces to shape their genomes independently, and in time become reproductively incompatible (Baker and Bradley 2006). The evolutionary history of the Neotropics set the ecological and geographical setting for such a process to take place and the phyllostomids contributed a sufficiently diverse genome.

Phyllostomidae Systematics

Phylogenetic relationships within the family have been widely studied (reviewed by Wetterer et al. 2000), but controversial because early research based on morphology was incongruent with the first multi-locus molecular phylogenies by Baker et al. (2000, 2003), and therefore, several recognized subfamilies and genera were not recovered as monophyletic. For example, Phyllostominae, which contained all the insectivores and carnivores, was paraphyletic and has been split in five subfamilies (Macrotinae, Micronycterinae, Lonchorhininae, Glyphonycterinae, and Phyllostominae *sensu stricto*) that are spread across the family tree. The frugivore Carolliinae was also paraphyletic, with the genus *Carollia* sister to *Glyphonycteris*/*Trinycteris*, and *Rhinophylla* sister to the subfamily Stenodermatinae. One of the most prominent molecular results was that the nectar-feeding bats were not monophyletic, meaning that one of the most extreme specializations in mammals occurred (at least) twice in the Neotropics (Figs. 1 and 2) and are now represented by the subfamilies Glossophaginae *sensu stricto* and Lonchophyllinae. Since the first molecular phylogeny, numerous efforts have been made to resolve the family comprehensively (Datzmann et al. 2010; Baker et al. 2012; Dumont et al. 2012; Dávalos et al. 2014; Rojas et al. 2016), and also resolve the cases of specific genera independently (e.g., *Artibeus* – Van Den Bussche et al. 1998, Redondo et al. 2008; *Lonchophylla* – Parlos et al. 2014; *Micronycteris* – Porter et al. 2007; *Mimon* – Hurtado-Miranda and Pacheco-Torres 2014; *Platyrrhinus* – Velazco et al. 2018; *Sturnira* – Velazco and Patterson 2013). Baker et al. (2012) explored the diversification of feeding strategies from a phylogenetic perspective. Baker et al. (2016) and Cirranello et al. (2016) presented a much-needed revised classification that encompasses all of these efforts. Dávalos et al. (2014) evaluated the phylogenetic position of the Miocene fossils *Notonycteris* and *Palynephyllum*.

The most recent Chiroptera phylogeny (Amador et al. 2018) was based on unconstrained analyses of a comprehensive dataset regarding taxonomic (90% of extant genera, 64% extant species) and character sampling (four mitochondrial, five nuclear), with updated taxonomy and no chimeric terminals. The results showed a highly congruent phylogeny (Fig. 1) with the classification proposed by Baker et al. (2016), with a single exception in the position and membership of *Mimon bennettii*, which is classified as a phyllostomine (tribe Vampyrini), but in the tree it is sister to the subfamily Micronycterinae. Additionally, in contrast with the Baker et al. (2003, 2012) phylogenies, another incongruence is found in the positions of the subfamilies Desmodontinae and Micronycterinae. The authors compare in detail the phylogenetic relationships at all levels with the most recent study of Rojas et al. (2016) and Baker et al. (2003), as well as with research of specific genera (cited above). The Amador

et al. (2018) dated phylogeny is presented here (Fig. 1) relative to the geological events (by Hoorn et al. 2010) that occurred in the Neotropics, which have shaped the systematics of the family Phyllostomidae.

RECENT TAXONOMIC CHANGES IN PHYLLOSTOMIDAE

A 200-year taxonomic history of the family Phyllostomidae has been summarized by Wetterer et al. (2000). The last taxonomic revision of the order Chiroptera included 55 genera and 159 extant species in the family Phyllostomidae (Simmons 2005), comprising the highest number of genera and the third largest number of species among bat families (Baker et al. 2012). Currently, there are 60 genera and 218 species (last update November 2018), which represent an increase of 34% species that were described or elevated in a period of 14 years (2004 to 2018). Phyllostomidae is currently the second most speciose bat family after Vespertilionidae (~ 456 spp.), and remains with the largest number of genera (Table 1).

Taxonomic changes in chiropteran higher taxa at the subfamily, family, tribe, and subtribe levels were mostly due to the intense molecular work carried out in recent decades, a process that took time as rare representatives were gradually added to the analyses. In the case of Phyllostomidae, of the 11 current subfamilies, four were recognized (Lonchophyllinae, Lonchorhininae, Macrotinae, Micronycterinae) and two were described (Glyphonycterinae, Rhinophyllinae), most of which were previously considered phyllostomines. Of the current 12 tribes, six have been validated (Brachyphyllini, Choeronycterini, Desmodontini, Macrophyllini, Phyllostomini, Vampyrini) and two described (Diphyllini, Hsunycterini); and of the nine subtribes, four have been validated (Artibeina, Brachyphyllina, Choeronycterina, Phyllonycterina) and four described (Anourina, Ectophyllina, Enchisthenina, Vampyressina). Four new genera (*Dryadonycteris*, *Gardnerycteris*, *Hsunycteris*, and *Xeronycteris*) and two new subgenera (*Leuconycteris* and *Schizonycteris*) have been described, one subgenus was elevated to genus (*Vampyriscus*), and three subgenera have been validated (*Dermanura*, *Micronycteris*, and *Xenoctenes*). Invalidated higher taxa include: subfamily Brachyphyllinae (currently a tribe), subfamily Phyllonycterinae, genus *Dermanura* (currently a subgenus in *Artibeus*), and subgenus *Koopmania* (currently a junior synonym of *Artibeus*). These taxonomic changes, descriptions, and references are detailed in Baker et al. (2016) and Cirranello et al. (2016).

At the species level, there has been an increase of 60 species since Simmons (2005), which includes 41 new species described (Table 2), 19 subspecies/synonyms that were elevated (Table 3), and seven species that were synonymized (Table 4). From 2004 to 2018 at least one species has been described per year, which accounts for approximately 60% of all bats described in the Neotropics. The genera with most species described are *Platyrrhinus* (8), *Sturnira* (6), and *Lonchophylla* (6) (Fig. 3). The genera with most subspecies elevated or synonyms recognized are *Artibeus* (6) and *Sturnira* (4) (Fig. 3). Taxonomic revisions were focused on 10 genera (Fig. 3), which accounts for 17% of all phyllostomid genera. Throughout the years there were two species description peaks, one from 2004 to 2006 (17 described) and the second in 2014 (six described). There were two revision peaks in 2008 and 2013, which recognized seven and three species, respectively. The new species distributions at the time of the description were mostly located in Ecuador (17 species), Colombia (16), Peru (10), Bolivia (7), and Brazil (7). Country endemics were mostly described for Brazil (5 species), Colombia (5), and Ecuador (5).

Table 2. Phyllostomid species described from 2004 to November 2018 that are currently considered to be valid. Geographic distribution as in the species description, may be currently extended for some species. *Authoritative reference different than publication: [1]Fonseca et al. (2007) and [2]Siles et al. (2013).

Species	Authoritative Reference	Distribution
Anoura cadenai	Mantilla-Meluk and Baker 2006	Colombia
Anoura carishina	Mantilla-Meluk and Baker 2010	Colombia
Anoura fistulata	Mucchala, Mena, and Albuja 2005	Ecuador
Anoura javieri	Pacheco, Sánchez-Vendizú, and Solari 2018	Peru
Carollia benkeithi	Solari and Baker 2006	Bolivia, Brazil, Peru
Carollia manu	Pacheco, Solari, and Velazco 2004	Bolivia, Peru
Carollia monohernandezi	Muñoz, Cuartas-Calle, and González 2004	Colombia
Chiroderma vizottoi	Taddei and Lim 2010	Brazil (Piaui)
Dryadonycteris capixaba	Nogueira, Lima, Peracchi, and Simmons 2012	Brazil (Espirito Santo)
Hsunycteris cadenai	(Woodman and Timm 2006)	Colombia, Ecuador
Hsunycteris dashe	Velazco, Soto-Centeno, Fleck, Voss, and Simmons 2017	Peru
Hsunycteris pattoni	(Woodman and Timm 2006)	Bolivia, Colombia, Ecuador, Peru
Lonchophylla chocoana	Dávalos 2004	Colombia, Ecuador
Lonchophylla fornicata	Woodman 2007	Colombia, Ecuador
Lonchophylla inexpectata	Moratelli and Dias 2015	Brazil (Bahia, Pernambuco)
Lonchophylla orcesi	Albuja and Gardner 2005	Ecuador
Lonchophylla orienticollina	Dávalos and Corthals 2008	Colombia, Ecuador, Venezuela
Lonchophylla peracchii	Dias, Esbérard, and Moratelli 2013	Brazil (Rio de Janeiro)
Lonchorhina mankomara	Mantilla-Meluk and Montenegro 2016	Colombia
Lophostoma kalkoae	Velazco and Gardner 2012	Panama
Micronycteris buriri	Larsen, Siles, Pedersen, and Kwiecinski 2011	Saint Vincent and the Grenadines
Micronycteris giovanniae	Baker and Fonseca 2007* [1]	Ecuador
Micronycteris yatesi	Siles and Brooks 2013* [2]	Bolivia
Platyrrhinus albericoi	Velazco 2005	Ecuador, Peru, Bolivia
Platyrrhinus angustirostris	Velazco, Gardner, and Patterson 2010	Colombia, Ecuador, Peru, Venezuela,
Platyrrhinus fusciventris	Velazco, Gardner, and Patterson 2010	Brazil (Amapa, Para), Ecuador, French Guiana, Guyana, Suriname, Trinidad and Tobago, Venezuela
Platyrrhinus guianensis	Velazco and Lim 2014	Guyana, Suriname
Platyrrhinus ismaeli	Velazco 2005	Colombia, Ecuador, Peru
Platyrrhinus masu	Velazco 2005	Bolivia, Peru
Platyrrhinus matapalensis	Velazco 2005	Ecuador, Peru
Platyrrhinus nitelinea	Velazco and Gardner 2009	Colombia, Ecuador
Sturnira adrianae	Molinari, Bustos, Burneo, Camacho, Moreno, and Fermín 2017	Colombia, Venezuela

Table 2. (cont.)

Species	Authoritative Reference	Distribution
Sturnira bakeri	Velazco and Patterson 2014	Ecuador
Sturnira burtonlimi	Velazco and Patterson 2014	Costa Rica, Panama
Sturnira koopmanhilli	McCarthy, Albuja, and Alberico 2006	Colombia, Ecuador
Sturnira perla	Jarrín and Kunz 2011	Ecuador
Sturnira sorianoi	Sánchez-Hernández, Romero-Almaraz, and Schnell 2005	Bolivia, Venezuela
Uroderma bakeri	Mantilla-Meluk 2014	Colombia, Venezuela
Vampyressa elisabethae	Tavares, Gardner, Ramírez-Chaves, and Velazco 2014	Panama
Vampyressa sinchi	Tavares, Gardner, Ramírez-Chaves, and Velazco 2014	Colombia
Xeronycteris vieirai	Gregorin and Ditchfield 2005	Brazil

Table 3. Subspecies or synonyms within Phyllostomidae that were elevated or recognized in the past 14 years with the reference(s) that support this decision.

Species and Original Author	Reference
Anoura aequatoris (Lönnberg 1921)	Mantilla-Meluk and Baker 2006
Anoura peruana (Tschudi 1844)	Mantilla-Meluk and Baker 2010
Artibeus aequatorialis K. Andersen 1906	Larsen et al. 2010
Artibeus bogotensis (K. Andersen 1906)	Hoofer et al. 2008, Lim et al. 2008, Solari et al. 2009
Artibeus planirostris (Spix 1823)	Larsen et al. 2007
Artibeus rava Miller 1902	Hoofer et al. 2008, Solari et al. 2009
Artibeus rosenbergi Thomas 1897	Hoofer et al. 2008, Solari et al. 2009
Artibeus schwartzi Jones 1978	Larsen et al. 2007
Gardnerycteris keenani (Handley 1960)	Hurtado and D'Elía 2018
Lichonycteris degener Miller 1931	Griffiths and Gardner 2008
Lonchophylla concava Goldman 1914	Albuja and Gardner 2005
Lophostoma occidentalis (Davis and Carter 1978)	Velazco and Cadenillas 2011
Platyrrhinus aquilus (Handley and Ferris 1972)	Velazco and Gardner 2009
Platyrrhinus incarum (Thomas 1912)	Velazco and Patterson 2008
Sturnira angeli de la Torre 1961	Velazco and Patterson 2013
Sturnira hondurensis Goodwin 1940	Iudica 2000, Gardner 2008
Sturnira parvidens Goldman 1917	Iudica 2000, Velazco and Patterson 2013
Sturnira paulsoni de la Torre and Schwartz 1966	Velazco and Patterson 2013
Uroderma convexum Lyon 1902	Mantilla-Meluk 2014
Uroderma davisi Baker and McDaniel 1972	Mantilla-Meluk 2014
Vampyrodes major G.M. Allen 1908	Velazco and Simmons 2011

Table 4. Species within Phyllostomidae that were synonymized in the past 14 years, the reference mentions it as a valid species, the current valid species, and the reference that supports the decision. *Micronycteris homezorum* Pirlot, 1967 is erroneously referred to *M. homezi* in some publications. *Platyrrhinus nigellus* was included in Simmons (2005) as a subspecies.

Synonym	Valid species reference	Currently valid species	Revision
Artibeus incomitatus Kalko and Handley 1994	Simmons 2005	*A. watsoni*	Solari et al. 2009
Carollia colombiana Cuartas et al. 2001	Simmons 2005	*C. brevicauda*	McLellan and Koopman 2008
Lophostoma aequatorialis Baker et al. 2004	Williams and Genoways 2008	*L. occidentalis*	Velazco and Cadenillas 2011
Lophostoma yasuni Fonseca and Pinto 2004	Williams and Genoways 2008	*L. carrikeri*	Camacho et al. 2016
Micronycteris homezorum Pirlot 1967	Simmons 2005	*M. minuta*	Ochoa and Sánchez 2005
Platyrrhinus nigellus (Gardner and Carter 1972)	Velazco and Solari 2003	*P. umbratus*	Velazco et al. 2018
Sturnira thomasi de la Torre and Schwartz 1966	Simmons 2005	*S. angeli*	Velazco and Patterson 2013

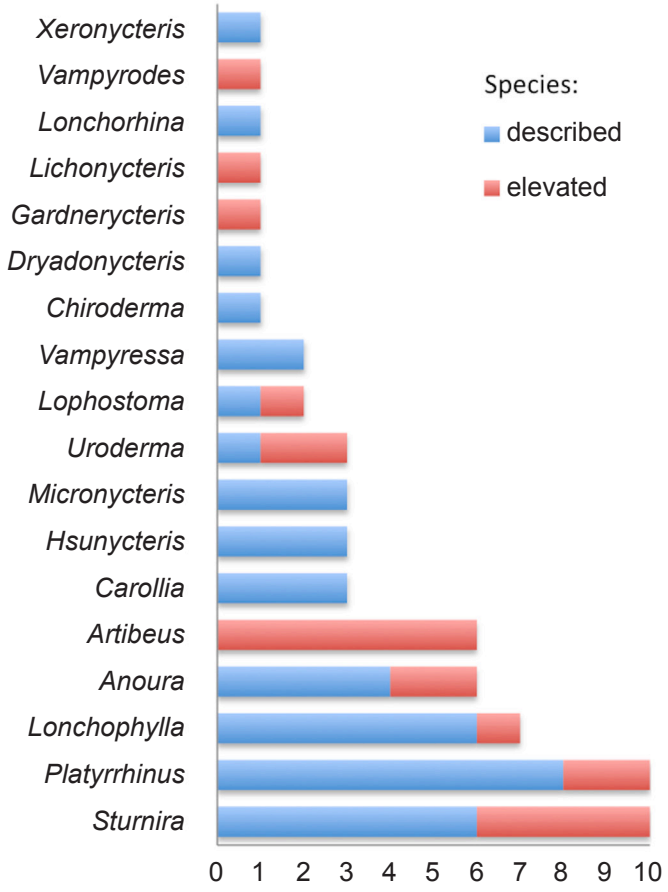

Figure 3. Number of species described and elevated in phyllostomid genera from 2004 to 2018.

Conclusion

The constant increase in the number of species, the highly debated and not-fully resolved systematics, and unanswered questions regarding rapid diversification emphasizes that a great deal remains to be learned about Phyllostomidae. Further research is needed to obtain an accurate description of the family's diversity, define speciation patterns, provide an accurate taxonomy for ecological and behavioral studies, and delimit species distributions. This information is needed to contribute to conservation and management efforts. In the Neotropical region, phyllostomid bats are the most diverse family of mammals with respect to feeding habits, and therefore play an incomparable role in ecosystem processes, which include forest regeneration, plant pollination, and predation on insects (Medellin et al. 2000; Muscarella and Fleming 2007; Jones et al. 2009). These services are highly valuable in the Neotropics, where many ecoregions and habitats are experiencing increasing levels of disturbance and destruction (Myers 1988; Myers et al. 2000).

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Literature Cited

Albuja, V. L., and A. L. Gardner. 2005. A new species of *Lonchophylla* Thomas (Chiroptera: Phyllostomidae) from Ecuador. Proceedings of the Biological Society of Washington 118:442–449.

Amador, L. I., R. L. M. Arévalo, F. C. Almeida, S. A. Catalano, and N. P. Giannini. 2018. Bat systematics in the light of unconstrained analyses of a comprehensive molecular supermatrix. Journal of Mammalian Evolution 25:37–70.

Antonelli, A., J. A. A. Nylander, C. Persson, and I. Sanmartin. 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. Proceedings of the National Academy of Sciences 106:9749–9754.

Baker, R. J. 1967. Karyotypes of bats of the family Phyllostomidae and their taxonomic implications. The Southwestern Naturalist 12:407–428.

Baker, R. J., and J. W. Bickham. 1980. Karyotypic evolution in bats: evidence of extensive and conservative chromosomal evolution in closely related taxa. Systematic Zoology 29:239–253.

Baker, R. J., O. R. P. Bininda-Emonds, H. Mantilla-Meluk, C. A. Porter, and R. A. Van Den Bussche. 2012. Molecular timescale of diversification of feeding strategy and morphology in New World leaf-nosed bats (Phyllostomidae): a phylogenetic perspective. Pp. 385–409 in Evolutionary history of bats: fossils, molecules and morphology (G. F. Gunnell and N. B. Simmons, eds.). Cambridge University Press, Cambridge, UK.

Baker, R. J., and R. D. Bradley. 2006. Speciation in mammals and the genetic species concept. Journal of Mammalogy 87:643–662.

Baker, R. J., C. S. Hood, and R. L. Honeycutt. 1989. Phylogenetic relationships and classification of the higher categories of the New World bat family Phyllostomidae. Systematic Zoology 38:228–238.

Baker, R. J., S. R. Hoofer, C. A. Porter, and R. A. Van Den Busche. 2003. Diversification among the New World leaf-nosed bats: an evolutionary hypothesis and classification inferred from digenomic congruence of DNA sequence. Occasional Papers, Museum of Texas Tech University 230:1–32.

Baker, R. J., C. A. Porter, J. C. Patton, and R. A. Van Den Bussche. 2000. Systematics of bats of the family Phyllostomidae based on *RAG2* DNA sequences. Occasional Papers, Museum of Texas Tech University 202: i+1–16.

Baker, R. J., S. Solari, A. Cirranello, and N. B. Simmons. 2016. Higher level classification of phyllostomid bats with a summary of DNA synapomorphies. Acta Chiropterologica 18:1–38.

Camacho, M. A., D. Chávez, and S. F. Burneo. 2016. A taxonomic revision of the Yasuni round-eared bat, *Lophostoma yasuni* (Chiroptera: Phyllostomidae). Zootaxa 4114:246–260.

Cirranello, A., N. B. Simmons, S. Solari, and R. J. Baker. 2016. Morphological diagnoses of higher-level phyllostomid taxa (Chiroptera: Phyllostomidae). Acta Chiropterologica 18:39–71.

Croft, D. A. 2012. Punctuated isolation: the making and mixing of South America's mammals. Pp. 9–19 in Bones, clones, and biomes: the history and geography of recent Neotropical mammals (B. D. Patterson and L. P. Costa, eds.). The University of Chicago Press, Chicago and London.

Czaplewski, N. J., J. Krejca, and T. E. Miller. 2003b. Late Quaternary bats from Cebada Cave, Chiquibul cave system, Belize. Caribbean Journal of Science 39:23–33.

Czaplewski, N. J., M. Takai, T. M. Naeher, N. Shigehara, and T. Setoguchi. 2003a. Additional bats from the middle Miocene La Venta fauna of Colombia. Revista de la Academia Colombiana de Ciencias 27:263–282.

Dávalos, L. M. 2004. A new Chocoan species of *Lonchophylla* (Chiroptera: Phyllostomidae). American Museum Novitates 3426:1–16.

Dávalos, L. M., and A. Corthals. 2008. A new species of *Lonchophylla* (Chiroptera: Phyllostomidae) from the eastern Andes of northwestern South America. American Museum Novitates 3635:1–16.

Dávalos, L. M., P. M. Velazco, O. M. Warsi, P. D. Smits, and N. B. Simmons. 2014. Integrating incomplete fossils by isolating conflicting signal in saturated and non-independent morphological characters. Systematic Biology 63:582–600.

Datzmann, T., O. von Helversen, and F. Mayer. 2010. Evolution of nectarivory in phyllostomid bats (Phyllostomidae Gray, 1825, Chiroptera: Mammalia). BMC Evolutionary Biology 10:165.

de Aguiar, M. P., and de Oliveira, L. 2005. Diversity of Quaternary bats from Serra da Mesa (State of Goiás, Brazil). Zootaxa, 817:1–19.

Dias, D., C. E. L. Esbérad, and R. Moratelli. 2013. A new species of *Lonchophylla* (Chiroptera, Phyllostomidae) from the Atlantic Forest of southeastern Brazil, with comments on *L. bokermanni*. Zootaxa 3722:347–360.

Dobzhansky, T. 1950. Evolution in the Tropics. American Scientist 38:209–221.

Dumont, E. R. 2004. Patterns of diversity in cranial shape among plant-visiting bats. Acta Chiropterologica 6:59–74.

Dumont, E. R., L. M. Dávalos, A. Goldberg, S. E. Santana, K. Rex, and C. C. Voigt. 2012. Morphological innovation, diversification and invasion of a new adaptive zone. Proceedings of the Royal Society B 279:1797–1805.

Emmons, L. H., and F. Feer. 1999. Mamíferos de los bosques húmedos de América tropical. Una guía de campo. Editorial F.A.N. Bolivia.

Flynn, J. J., and A. Wyss. 1998. Recent advances in South American mammalian palaeontology. TREE 13:449–454.

Fonseca, R. M., et al. 2007. Morphological and molecular variation within little big–eared bats of the genus *Micronycteris* (Phyllostomidae: Micronycterinae) from San Lorenzo, Ecuador. Pp. 721–746 in The quintessential naturalist: honoring the life and legacy of Oliver P. Pearson (D. A. Kelt, E. P. Lessa, J. Salazar–Bravo, and J. L. Patton, eds.). University of California Publications in Zoology 134:1–981.

Freeman, P. W. 1998. Form, function, and evolution in skulls and teeth of bats. Pp. 140–156 in Bat biology and conservation (T. H. Kunz and P. A. Racey, eds). Smithsonian Institution Press, Washington, DC.

Freeman, P. W. 2000. Macroevolution in Microchiroptera: recoupling morphology and ecology with phylogeny. Evolutionary Ecology Research 2:317–335.

Gardner, A. L. 2008. Tribe Sturnirini. Pp. 363–376 in Mammals of South America. Volume 1. Marsupials, xenarthrans, shrews, and bats (A. L. Gardner, ed.). University of Chicago Press, Chicago.

Genoways, H. H., et al. 2018. Obituary: Robert James Baker (1942–2018). Journal of Mammalogy 99:983–1012.

Gregorin, R., and A. D. Ditchfield. 2005. New genus and species of nectar-feeding bat in the tribe *Lonchophyllini* (Phyllostomidae: Glossophaginae) from northeastern Brazil. Journal of Mammalogy 86:403–14.

Griffiths, T. A., and A. L. Gardner. 2008. Subfamily Glossophaginae Bonaparte, 1845. Pp. 224–244 in Mammals of South America. Volume 1. Marsupials, xenarthrans, shrews, and bats (A. L. Gardner, ed.). The University of Chicago Press, Chicago.

Giller, P. S. 1984. Community structure and the niche. Chapman Hall, New York.

Gregory-Wodzicki, K. M. 2000. Uplift history of the Central and Northern Andes: a review. Geological Society of America Bulletin 112:1091–1105.

Hadler, P., E. L. Mayer, F. Motta, and A. M. Ribeiro. 2018. Fossil bats from the Quaternary of Serra da Capivara, northeast Brazil. Quaternary International 464:411–416.

Hoffman, F. G., and R. J. Baker. 2001. Systematics of bats of the genus *Glossophaga* (Chiroptera: Phyllostomidae) and phylogeography in *G. soricina* based on the cytochrome-*b* gene. Journal of Mammalogy 84:1092–1101.

Hoffmann, F. G., and R. J. Baker. 2003. Comparative phylogeography of short-tailed bats (*Carollia*: Phyllostomidae). Molecular Ecology 12:3403–3414.

Hood, C. S., and J. D. Smith. 1982. Cladistical analysis of female reproductive histomorphology in phyllostomatoid bats. Systematic Zoology 31:241–251.

Hoofer, S. R., S. Solari, P. A. Larsen, R. D. Bradley, and R. J. Baker. 2008. Phylogenetics of the fruit-eating bats (Phyllostomidae: Artibeina) inferred from mitochondrial DNA sequences. Occasional Papers, Museum of Texas Tech University 277:1–15.

Hoorn, C., J. Guerrero, G. A. Sarmiento, and M. A. Lorente. 1995. Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. Geology 23:237–240.

Hoorn, C., et al. 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. Science 330:927–931.

Hurtado, N., and G. D'Elía. 2018. Taxonomy of the genus *Gardnerycteris* (Chiroptera: Phyllostomidae). Acta Chiropterologica 20:99–115.

Hurtado-Miranda, N. E., and V. Pacheco-Torres. 2014. Análisis filogenético del género *Mimon* Gray, 1847 (Mammalia, Chiroptera, Phyllostomidae) con la descripción de un nuevo género. Therya 5:751–791.

Hutchinson G. E. 1959. Homage to Santa Rosalia or why are there so many kinds of animals? American Naturalist 93:145–159.

Iudica, C. A. 2000. Systematic revision of the Neotropical fruit bats of the genus *Sturnira*: a molecular and morphological approach. Unpublished PhD dissertation, University of Florida, Gainesville.

Jarrín, P., and T. H. Kunz. 2011. A new species of *Sturnira* (Chiroptera: Phyllostomidae) from the Choco forest of Ecuador. Zootaxa 2755:1–35.

Jones, G., D. S. Jacobs, T. H. Kunz, M. R. Willig, and P. A. Racey. 2009. Carpe noctem: the importance of bats as bioindicators. Endangered Species Research 8:93–115.

Larsen, P. A., et al. 2007. Phylogenetics and phylogeography of the *Artibeus jamaicensis* complex based on cytochrome-*b* DNA sequences. Journal of Mammalogy 88:712–727.

Larsen, P. A., M. R. Marchán-Rivadeneyra, and R. J. Baker. 2010. Taxonomic status of Andersen's fruit-eating bat (*Artibeus jamaicensis aequatorialis*) and revised classification of *Artibeus* (Chiroptera: Phyllostomidae). Zootaxa 2648:45–60.

Larsen, P.A., L. Siles, S. C. Pedersen, and G. G. Kwiecinski. 2011. A new species of *Micronycteris* (Chiroptera: Phyllostomidae) from Saint Vincent, Lesser Antilles. Mammalian Biology 76:687–700.

Lim, B. K., M. D. Engstrom, J. C. Patton, and J. W. Bickham. 2008. Systematic review of small fruit-eating bats (*Artibeus*) from the Guianas, and a re-evaluation of *A. glaucus bogotensis.* Acta Chiropterologica 10:243–256.

Lugo, A. E. 1988. Diversity of tropical species: questions that elude answers. Biology International, Special Issue 19:1–37.

Mantilla-Meluk, H. 2014. Defining species and species boundaries in *Uroderma* (Chiroptera: Phyllostomidae) with a description of a new species. Occasional Papers, Museum of Texas Tech University 325:1–25.

Mantilla-Meluk, H., and R. J. Baker. 2006. Systematics of small *Anoura* (Chiroptera: Phyllostomidae) from Colombia, with description of a new species. Occasional Papers, Museum of Texas Tech University 261:1–18.

Mantilla-Meluk, H., and R. J. Baker. 2010. New species of *Anoura* (Chiroptera: Phyllostomidae) from Colombia, with systematic remarks and notes on the distribution of the *A. geoffroyi* complex. Occasional Papers, Museum of Texas Tech University 292:1–19.

Mantilla-Meluk, H., and O. Montenegro. 2016. Nueva especie de *Lonchorhina* (Chiroptera: Phyllostomidae) de Chiribiquete, Guayana colombiana. Revista Biodiversidad Neotropical 6:171–187.

McCarthy, T. J., L. Albuja, and M. Alberico. 2006. A new species of Chocoan *Sturnira* (Chiroptera: Phyllostomidae: Stenodermatinae) from western Ecuador and Colombia. Annals of Carnegie Museum 75:97–110.

McLellan, L. J., and Koopman. 2008. Subfamily Carollinae. Pp. 208–218 in Mammals of South America. Volume 1. Marsupials, xenarthrans, shrews, and bats (A.L. Gardner, ed.). University of Chicago Press, Chicago.

Medellin, R. A., M. Equihua, and M. A. Amin. 2000. Bat diversity and abundance as indicators of disturbance in Neotropical rainforests. Conservation Biology 14:1666–1675.

Meredith, R. W., et al. 2011. Impacts of the Cretaceous terrestrial revolution and KPg extinction on mammal diversification. Science 334:521–524.

Mittelbach, G. G., et al. 2007. Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. Ecology Letters 10:315–331.

Molinari, J., X. E. Bustos, S. F. Burneo, M. A. Camacho, S. A. Moreno, and G. Fermin. 2017. A new polytypic species of yellow-shouldered bats, genus *Sturnira* (Mammalia: Chiroptera: Phyllostomidae), from the Andean and coastal mountain systems of Venezuela and Colombia. Zootaxa 4243:75–96.

Moratelli, R., and D. Dias. 2015. A new species of nectar-feeding bat, genus *Lonchophylla*, from the Caatinga of Brazil (Chiroptera, Phyllostomidae). ZooKeys 514: 73.

Muchhala, N., P. Mena, and L. Albuja. 2005. A new species of *Anoura* (Chiroptera: Phyllostomidae) from the Ecuadorian Andes. Journal of Mammalogy 86:457–461.

Muñoz, J., C. A. Cuartas-Calle, and M. González. 2004. Se describe una nueva especie de murciélago del género *Carollia* Gray, 1838 (Chiroptera: Phyllostomidae) de Colombia. Actualidades Biológicas 26:80–90.

Muscarella, R., and T. H. Fleming. 2007. The role of frugivorous bats in tropical forest succession. Biological Review 82:573–590.

Myers, N. 1988. Threatened biotas: "Hot Spots" in tropical forests. The Environmentalist 8:187–208.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature 403:853–858.

Nelson, J. S. 2006. Fishes of the World. John Wiley & Sons, Inc., New Jersey.

Nogueira, M. R., I. P. Lima, A. L. Peracchi, and N. B. Simmons. 2012. New genus and species of nectar-feeding bat from the Atlantic Forest of southeastern Brazil (Chiroptera: Phyllostomidae: Glossophaginae). American Museum Novitates 3747:1–30.

Ochoa, J. G., and J. Sánchez H. 2005. Taxonomic status of *Micronycteris homezi* (Chiroptera, Phyllostomidae). Mammalia 69:323–336.

Pacheco, V., P. Sánchez-Vendizú, and S. Solari. 2018. A new species of *Anoura* Gray, 1838 (Chiroptera: Phyllostomidae) from Peru, with taxonomic and biogeographic comments on species of the *Anoura caudifer* complex. Acta Chiropterologica, 20:31–50.

Pacheco, V., S. Solari, and P. M. Velazco. 2004. A new species of *Carollia* (Chiroptera: Phyllostomidae) from the Andes of Peru and Bolivia. Occasional Papers, Museum of Texas Tech University 236:1–15.

Parlos, J. A., R. M. Timm, V. J. Swier, H. Zeballos, and R. J. Baker. 2014. Evaluation of the paraphyletic assemblage within Lonchophyllinae, with description of a new tribe and genus. Occasional Papers, Museum of Texas Tech University 320:1–23.

Patterson, B. D., and L. P. Costa. 2012. Introduction to the history and geography of Neotropical mammals. Pp. 1–5 in Bones, clones, and biomes: the history and geography of recent Neotropical mammals (B. D. Patterson and L. P. Costa, eds.). The University of Chicago Press, Chicago and London.

Porter, C. A., and R. J. Baker. 2004. Systematics of *Vampyressa* and related genera of phyllostomid bats as determined by cytochrome-*b* sequences. Journal of Mammalogy 85:126–132.

Porter, C. A., S. R. Hoofer, C. A. Cline, F. G. Hoffmann, and R. J. Baker. 2007. Molecular phylogenetics of the phyllostomid bat genus *Micronycteris* with descriptions of two new subgenera. Journal of Mammalogy 88:1205–1215.

Redondo, R. A., L. P. Brina, R. F. Silva, A. D. Ditchfield, and F. R. Santos. 2008. Molecular systematics of the genus *Artibeus* (Chiroptera: Phyllostomidae). Molecular Phylogenetics and Evolution 49:44–58.

Rojas, D., O. M. Warsi, and L. M. Dávalos. 2016. Bats (Chiroptera: Noctilionoidea) challenge a recent origin of extant neotropical diversity. Systematic Biology 65:432–448.

Sánchez-Hernández, C., M. L. Romero-Almaraz, and G. D. Schnell. 2005. New species of *Sturnira* (Chiroptera: Phyllostomidae) from northern South America. Journal of Mammalogy 86:866–872.

Santana, S. E., I. Geipel, E. R. Dumont, M. B. Kalka, and E. K. V. Kalko. 2011. All you can eat: high performance capacity and plasticity in the common big-eared bat, *Micronycteris microtis* (Chiroptera: Phyllostomidae). PloS ONE 6:e28584.

Simmons, N. B. 2005. Order Chiroptera. Pp. 312–529 in Mammal species of the world, Vol. 1 (D. E. Wilson and D. M. Reeder, eds.). Johns Hopkins University Press, Baltimore, Maryland.

Simmons, N. B., and R. S. Voss. 1998. The mammals of Paracou, French Guiana, a Neotropical lowland rainforest fauna. Part 1, Bats. Bulletin of the American Museum of Natural History 237:1–219.

Siles, L., et al. 2013. A new species of *Micronycteris* (Chiroptera: Phyllostomidae) from Bolivia. Journal of Mammalogy 94:881–896.

Solari, S., and R. J. Baker. 2006. Mitochondrial DNA sequence, karyotypic, and morphological variation in the *Carollia castanea* species complex (Chiroptera: Phyllostomidae) with description of a new species. Occasional Papers, Museum of Texas Tech University 254:1–16.

Solari, S., et al. 2009. Operational criteria for genetically defined species: analysis of the diversification of the small fruit-eating bats, *Dermanura* (Phyllostomidae: Stenodermatinae). Acta Chiropterologica 11:279–288.

Solari, S., and V. Martínez-Arias. 2014. Cambios recientes en la sistemática y taxonomía de murciélagos Neotropicales (Mammalia: Chiroptera). Therya 5:14–180.

Sotero-Caio, C. G., et al. 2013. Chromosomal evolution among leaf-nosed nectarivorous bats – evidence from cross-species chromosome painting (Phyllostomidae, Chiroptera). BMC Evolutionary Biology 12:1–12.

Soto-Centeno, J. A., N. B. Simmons, and D. W. Steadman. 2017. The bat community of Haiti and evidence for its long-term persistence at high elevations. PloS ONE 12:e0178066.

Taddei, V. A., and B. K. Lim. 2010. A new species of *Chiroderma* (Chiroptera, Phyllostomidae) from northeastern Brazil. Brazilian Journal of Biology 70:381–386.

Tavares, V. D. C., A. L. Gardner, H. E. Ramírez-Chaves, and P. M. Velazco. 2014. Systematics of *Vampyressa melissa* Thomas, 1926 (Chiroptera: Phyllostomidae), with descriptions of two new species of *Vampyressa*. American Museum Novitates 3813:1–27.

Van Den Bussche, R. A., J. L. Hudgeons, and R. J. Baker. 1998. Phylogenetic accuracy, stability, and congruence: relationships within and among the New World bat genera *Artibeus*, *Dermanura*, and *Koopmania*. Pp. 59-71 in Bat biology and conservation (T. H. Kunz and P. A. Racey, eds). Smithsonian Institution Press, Washington, DC.

Velazco, P. M. 2005. Morphological phylogeny of the bat genus *Platyrrhinus* (Chiroptera: Phyllostomidae) with the description of four new species. Fieldiana Zoology 42(75):1–53.

Velazco, P. M., and R. Cadenillas. 2011. On the identity of *Lophostoma silvicolum occidentalis* (Davis and Carter, 1978) (Chiroptera: Phyllostomidae). Zootaxa 2962:1–20.

Velazco, P. M., and A. L. Gardner. 2009. A new species of *Platyrrhinus* (Chiroptera: Phyllostomidae) from western Colombia and Ecuador, with emended diagnoses of *P. aquilus*, *P. dorsalis*, and *P. umbratus*. Proceedings of the Biological Society of Washington 122:249–281.

Velazco, P. M., and A. L. Gardner. 2012. A new species of *Lophostoma* d'Orbigny, 1836 (Chiroptera: Phyllostomidae) from Panama. Journal of Mammalogy 93:605–614.

Velazco, P. M., A. L. Gardner, and B. D. Patterson. 2010. Systematics of the *Platyrrhinus helleri* species complex (Chiroptera: Phyllostomidae), with descriptions of two new species. Zoological Journal of the Linnean Society 159:785–812.

Velazco, P. M., L. Guevara, and J. Molinari. 2018. Systematics of the broad-nosed bats, *Platyrrhinus umbratus* (Lyon, 1902) and *P. nigellus* (Gardner and Carter, 1972) (Chiroptera: Phyllostomidae), based on genetic, morphometric, and ecological niche analyses. Neotropical Biodiversity 4:118–132.

Velazco, P. M., and B. K. Lim. 2014. A new species of broad-nosed bat *Platyrrhinus* Saussure, 1860 (Chiroptera: Phyllostomidae) from the Guianan Shield. Zootaxa 3796:175–193.

Velazco, P. M., et al. 2013. Quaternary bat diversity in the Dominican Republic. American Museum Novitates, 3779:1–20.

Velazco, P. M., and B. D. Patterson. 2008. Phylogenetics and biogeography of the broad-nosed bats, genus *Platyrrhinus* (Chiroptera: Phyllostomidae). Molecular Phylogenetics and Evolution 49:749–759.

Velazco, P. M., and B. D. Patterson. 2013. Diversification of the yellow-shouldered bats, genus *Sturnira* (Chiroptera, Phyllostomidae), in the New World tropics. Molecular Phylogenetics and Evolution 68:683–698.

Velazco, P. M., and B. D. Patterson. 2014. Two new species of yellow-shouldered bats, genus *Sturnira* Gray, 1842 (Chiroptera, Phyllostomidae) from Costa Rica, Panama and western Ecuador. ZooKeys 402:43–66.

Velazco, P. M., and N. B. Simmons. 2011. Systematics and taxonomy of great striped-faced bats of the genus *Vampyrodes* Thomas, 1900 (Chiroptera: Phyllostomidae). American Museum Novitates 3710:1–35.

Velazco, P. M., and S. Solari. 2003. Taxonomía de *Platyrrhinus dorsalis* y *Platyrrhinus lineatus* (Chiroptera: Phyllostomidae) en Perú. Mastozoología Neotropical 10:303–319.

Velazco, P. M., J. A. Soto-Centeno, D. W. Fleck, R. S. Voss, and N. B. Simmons. 2017. A new species of nectar-feeding bat of the genus *Hsunycteris* (Phyllostomidae: Lonchophyllinae) from northeastern Peru. American Museum Novitates 3881:1–26.

Villalobos, F., and H. T. Arita. 2010. The diversity field of New World leaf-nosed bats (Phyllostomidae). Global Ecology and Biogeography 19:200–211.

Volleth, M., and G. Eick. 2012. Chromosome evolution in bats as revealed by FISH: the ongoing search for the ancestral Chiropteran karyotype. Cytogenetics and Genome Research 137:165–173.

Voss, R. S., and L. H. Emmons. 1996. Mammalian diversity in Neotropical lowland rainforests: a preliminary assessment. Bulletin of the American Museum of Natural History 230:1–115.

Wallace, A. R. 1876. The geographical distribution of animals. With a study of the relations of living and extinct faunas as elucidating the past changes of the Earth's surface. Vol II. Harper and Brothers Publishers, New York.

Walton, D. W., and G. W. Walton. 1968. Comparative osteology of the pelvic and pectoral girdles of the Phyllostomatidae (Chiroptera: Mammalia). Journal of the Graduate Research Center, Southern Methodist University 37:1–35.

Wetterer, A. L., M. V. Rockman, and N. B. Simmons. 2000. Phylogeny of phyllostomid bats (Mammalia: Chiroptera): data from diverse morphological systems, sex chromosomes, and restriction sites. Bulletin of the American Museum of Natural History 248:1–200.

Williams, S. L., and H. H. Genoways. 2008. Subfamily Phyllostominae. Pp. 255–300 in Mammals of South America. Volume 1. Marsupials, xenarthrans, shrews, and bats (A. L. Gardner, ed.). The University of Chicago Press, Chicago.

Willig, M. R., D. M. Kaufman, and R. D. Stevens. 2003. Latitudinal gradients of biodiversity: pattern, process, scale, and synthesis. Annual Review of Ecology, Evolution, and Systematics 34:273–309.

Woodman, N. 2007. A new species of nectar-feeding bat, genus *Lonchophylla*, from western Colombia and western Ecuador (Mammalia: Chiroptera: Phyllostomidae).

Proceedings of the Biological Society of Washington 120:340–358.

Woodman, N., and R. M. Timm. 2006. Characters and phylogenetic relationships of nectar-feeding bats, with descriptions of new *Lonchophylla* from western South America (Mammalia: Chiroptera: Phyllostomidae: Lonchophyllini). Proceedings of the Biological Society of Washington 119:437–476.

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Morphometric Variation of the Genus *Lonchorhina* (Chiroptera, Lonchorhininae), with Noteworthy Comments on Taxonomy and Distributional Range Extensions

Hugo Mantilla-Meluk, Natalia Perez-Amaya, and Paúl M. Velazco

Abstract

Insectivorous sword-nose bats *Lonchorhina* (Phyllostomidae: Lonchorhininae) are characterized by the most hypertrophied telic structures (noseleaf and pinna) within the family. Internally, enlarged ears and noseleaf are supported by a skull morphology that differs from that in other closely related genera. Except for *L. aurita*, widely distributed across both Central and South America, species in the genus *Lonchorhina* have relatively restricted distributions and are represented in museum collections by a limited number of specimens. Herein, morphological and morphometric variation is assessed in 115 *Lonchorhina* specimens representing the six recognized species in the genus from a significant portion of its geographic range, from Belize to Brazil, including the recently described *L. mankomara* from the isolated mountain range of Serrania de Chiribiquete in the Colombian Guayana. The first Peruvian records of *L. inusitata*, collected 9 km N of Aucayacu, province of Leoncio Prado, department of Huánuco in the piedmonts of the Peruvian Amazon, are reported herein; these records represent a significant extension in the geographic and ecological distribution of this taxon. In addition, the distribution of *L. mankomara* is extended into the northern portion of Colombian Guianan-Amazon. Finally, the morphometric affinities of medium-size representatives of the genus *L. aurita*, *L. inusitata*, and *L. orinocensis* are discussed and comments are made on the external and cranial morphological differentiation between *L. mankomara* and *L. marinkellei*.

Key words: cranial morphology, distribution extension, geographic variation, *Lonchorhina inusitata*, *Lonchorhina mankomara*, sword-nosed bats

Resumen

Los murciélagos insectívoros de hoja de espada en el género *Lonchorhina* (Phyllostomidae: Lonchorhininae), se caracterizan por poseer las estructuras télicas (hoja nasal y pina) más hipertrofidas en la familia. Internamente, estas estructuras están soportadas por una morfología craneal que difiere significativamente de aquella en los géneros más cercanos. Con excepción de *L. aurita*, ampliamente distribuida a lo largo de Centro y Sur América, las especies de *Lonchorhina* presentan distribuciones restringidas y están pobremente representadas en colecciones científicas. En este estudio investigamos la variación geográfica en la morfología y morfometría craneal en *Lonchorhina* a través del análisis de 115 especímenes representando las seis especies descritas para el género, provenientes de una porción significativa de su rango de distribución desde Belice a Brasil, incluyendo material de la recientemente descrita *L. mankomara* del sistema montañoso aislado de la Serranía de Chiribiquete en la Guayana Colombiana. Presentamos los primeros registros peruanos de *L. inusitata* colectados 9 km N de Aucayacu, en la provincia de Leoncio Prado, departamento de Huánuco, en el piedemonte de la

Amazonía peruana, que constituyen una extensión geográfica y ecológica significativa en la distribución de este taxón; y extendemos la distribución de *L. mankomara* al norte de la Guayana-Amazónica de Colombia. Finalmente discutimos sobre las afinidades morfológicas de representantes de talla media en el género: *L. aurita*, *L. inusitata* y *L. orinocensis*; y se adición comentarios sobre la diferenciación craneal y en caracteres externos entre *L. mankomara* y *L. marinkellei*.

Palabras clave: extensión en distribución, *Lonchorhina inusitata*, *Lonchorhina mankomara*, morfología craneal, murciélagos de hoja de espada, variación geográfica

INTRODUCTION

The phyllostomid genus *Lonchorhina* (sword-nosed bats) is the sole member of the subfamily Lonchorhininae (Baker et al. 2012; Dávalos et al. 2012) and is characterized by the most hypertrophied telic structures (noseleaf, ear, and tragus) within the family (Fig. 1) and a unique skull morphology. The genus *Lonchorhina* currently includes six species—*L. aurita* Tomes, 1863; *L. orinocensis* Linares and Ojasti, 1971; *L. marinkellei* Hernández-Camacho and Cadena, 1978; *L. fernandezi* Ochoa and Ibañez, 1984; *L. inusitata* Handley and Ochoa, 1997; and *L. mankomara* Mantilla-Meluk and Montenegro, 2016—that exhibit considerable variation in size and distributional patterns (Williams and Genoways 2008; Mantilla-Meluk and Montenegro 2016; Mantilla-Meluk et al. 2017). Except for *L. aurita*, species in the genus are poorly represented in museum collections and little is known about their morphometric variation. To date, the only available revision of the genus (Hernandez-Camacho and Cadena 1978) included three of the six currently recognized species. Although that revision provides one of the most detailed morphological assessments of a phyllostomid genus, it was limited in scope in terms of the localities examined and lacked information on geographic variation in skull morphology and morphometrics (Hernandez-Camacho and Cadena 1978).

In the present work, the skull morphometric variation in all currently recognized species in the genus was analyzed, with attention given to variation displayed by *L. aurita*. In addition, while examining *Lonchorhina* specimens deposited at two natural history museums in the USA, two relatively larger male specimens (GLS > 22 mm) were found from Peru, where only *L. aurita* (GLS < 21.5 mm) is known to occur. These two specimens were within the morphometric ranges of measurements reported for *L. inusitata*. The discrete character variation of these two *Lonchorhina* specimens from Peru was analyzed in order to determine their taxonomic status. Similarly, new material of *L. mankomara* was analyzed that not only extended this species' distribution into the northern portion of the Serrania de Chiribiquete in the department of Guaviare, Colombia, but also allowed an opportunity to document the external morphology of the largest species in the genus (Fig. 1).

MATERIALS AND METHODS

Specimens examined.—A total of 115 adult specimens of *Lonchorhina* representing the six species of the genus were examined: 83 specimens of *L. aurita*, 6 of *L. inusitata*, 1 of *L. fernandezi*, 9 of *L. mankomara*, 2 of *L. marinkellei*, and 14 of *L. orinocensis* (see Appendix for complete specimen data). The specimens examined in this study are deposited in the American Museum of Natural History (AMNH, New York, USA); Carnegie Museum of Natural History (CM, Pittsburgh, USA); Collection of Mammals of the University of Quindío (CMUQ, Armenia, Colombia); Field Museum of Natural History (FMNH, Chicago, USA); Instituto de Ciencias Naturales, Universidad Nacional de Colombia (ICN, Bogotá, Colombia); Museum of Texas Tech University (TTU, Lubbock, USA); and National Museum of Natural History (formerly the United States National Museum), Smithsonian Institution (USNM, Washington, DC, USA).

Figure 1. Images of various species of *Lonchorhina*, representing: *L. aurita* from Costa Rica, Central America (top left; photo courtesy of M. Tschapka) and Brazil (top center; photo courtesy of Tekbio); *L. inusitata* from Suriname (top right; photo courtesy of B. Lim); *L. orinocensis* from Serranía de La Lindosa, Guaviare, Colombia (bottom left; photo courtesy of R. Agudelo); *L. mankomara* from Serranía de Chirbiquete, Guaviare, Colombia (bottom center; photo courtesy of H. Mantilla-Meluk); and *L. marinkellei* from Serranía de La Lindosa, Guaviare, Colombia (bottom right; photo courtesy of D. Martínez-Morales).

Morphology and morphometrics.—External and osteological characters examined were defined based on, but not restricted to, Ochoa and Ibañez (1984) and Handley and Ochoa (1997). Dávalos et al. (2014) was followed in assigning homology for the premolars: first upper premolar (P4), second upper premolar (P5), first lower premolar (p1), second lower premolar (p4), and third lower premolar (p5).

Digital calipers were used to take 10 craniodental measurements to the nearest 0.01 mm on each specimen. Only adult specimens were used in this study and

age was determined based on the presence of closed phalangeal epiphyses. Descriptive statistics (mean and observed range) were calculated for all samples (Table 1). The craniodental measurements used in this study include: greatest length of skull (GLS): measured from the most posterior edge of the skull at the occipital crest to the most anterior point of the maxillary bone; condylobasal length (CBL): measured from the most posterior edge of the condyles to the most anterior point of the maxillary bone; palatal length (PAL): distance from the most anterior point of the palatal, usually in between the incisor (in ventral view), to the inflexion point of the arc draw by the joint of the palatines in the so-called mesopterygoid fossa; postorbital constriction (PO): smallest distance across the maxilla at the interorbital region; rostrum width (RO): maximum distance on the maxilla, across the base of the canines; braincase length (BL): distance between the point of inflexion delimiting the braincase anteriorly and the posterior end of the occipital; distance across tympanic bullae (BL–BL): maximum distance across the most lateral points of the tympanic bullae in dorsal view; braincase height (BCH): distance from the base of the cranium at the basisphenoidal region to the highest point at the joint of the parietals; tooth-row length (LTR): distance from the posterior edge of the third upper molar to the most anterior edge of the canine in the maxillary toothrow; distance across third upper molars (M-M): maximum distance across the third upper molars; and distance across canines including cingula (C–C): distance across the cingula of the upper canines.

Eleven craniodental measurements (GLS, CBL, PAL, PO, RO, BL, BL–BL, BCH, LTR, M–M, and C–C) of 115 *Lonchorhina* specimens from eight countries, including all recognized species from most of the known geographic range of the genus from Belize to Brazil, were tested for normality and homogeneity of variances using a Shapiro-Wilks and a Bartlett's, tests respectively, in the software R versión 3.4.3 (R Core Team 2017), with the package stats (R Core Team 2017) and car (Fox and Weisberg 2011). Two principal components analyses (PCA) were performed on the data. The first PCA included all species of *Lonchorhina*. For the second PCA, *L. fernandezi* (smallest species of the genus) and *L. mankomara* and *L. marinkellei* (largest species) were excluded. The range of size variation in the first PCA analysis was such that *L. aurita*, *L. inusitata*, and *L. orinocensis* were partially obscured by overlap. Therefore, a second PCA analysis of these three species was performed in an effort to ascertain the degree to which these taxa differed within the morphospace. Size discrimination follows the criteria in Williams and Genoways (2008). Subsequently, Discriminant Function Analyses (DFA) were performed in order to statistically determine the morphometric independence among medium-sized species: *L. aurita*, *L. inusitata*, and *L. orinocensis*. In addition, because previous publications had misidentified medium-sized *L. inusitata* as the large-sized *L. marinkellei* (Brosset and Charles-Dominique 1991; Brosset et al. 1996), a second DFA was performed in order to test for morphometric independence among *L. inusitata* (N = 6), and *L. marinkellei* (N = 2), and *L. mankomara* (N = 9). Finally, a DFA was conducted to assess the variation of the widespread *L. aurita* across its geographic range. Seventy-three specimens assigned to *L. aurita* from eight countries were included in this analysis: Belize (1), Brazil (10), Colombia (32), Guatemala (13), Honduras (2), Panama (1), Peru (3), and Trinidad (11). Morphometric analyses were performed using the statistical packages PAST (Hammer et al. 2001) for the PCA analyses, and Statgraphics Centurion XV (StatPoint Technologies Inc.) was used for the DFA analyses. Because *L. aurita* was the species with the highest number of individuals, a U-Mann-Whitney test was conducted to account for sexual dimorphism in the software R (R Core Team 2017).

To confirm the identification of *L. inusitata* specimens from Huánuco, Peru (TTU 46137♂ and CM 98592♂), cranial morphological affinities were analyzed among the *L. inusitata* specimens and representatives of all recognized species in the genus, in particular with the morphometrically closely related *L. aurita*. These analyses included the following localities and specimens: comparisons with *L. aurita* material from Trinidad, collected near the type locality of the species; specimens of *L. aurita* from several localities across its distributional range (Belize, Honduras, Panama, Colombia, Venezuela, Peru, and Brazil); the holotype of *L. a. occidentalis* (Ecuador); specimens of *L. inusitata* from Brazil and Venezuela; a specimen of the rare *L. fernandezi* from the only known locality of this species in Venezuela; representatives of *L. orinocensis* from several localities in Colombia and Venezuela; and the holotypes of the rare *L. mankomara* and *L. marinkellei* in Colombia, including new putative collecting localities for the species.

RESULTS

Craniometric variation in the genus Lonchorhina.—For the PCA analysis of all species of *Lonchorhina* (Fig. 2), the first two principal components (PC1 = 92.8%, PC2 = 2.9%) explained most of the variation, with GLS, CBL, and PAL having the highest loadings on PC1, reflecting the skull length variation among all the species of the genus. On PC2, BL-BL showed the highest loading, thus illustrating differences in skull width of *L. fernandezi* with respect to the other species in the genus (Fig. 2). The three medium-sized species (*L. aurita*, *L. inusitata*, and *L. orinocensis*) grouped in the same area of the morphospace, with some degree of overlap between *L. aurita* and *L. inusitata*, and *L. orinocensis* being the smallest of the three. Large species *L. mankomara* and *L. marinkellei* show no overlap with each other or with the rest of species in the genus.

Craniometric variation among medium-sized species of Lonchorhina.—The PCA analysis of *L. aurita*, *L. inusitata*, and *L. orinocensis* revealed the highest variation for the first principal component (PC1 = 85%, PC2 = 8.5%), with CBL showing the highest loading (CBL= 0.333, M–M = 0.329, GLS = 0.327). Correspondingly, three clusters were observed along PC1, representing samples of: *L. orinocensis*, placed at the negative end of the axis (lower scores); *L. aurita*, placed in the middle of the axis with some individuals presenting negative and other positive scores; and *L. inusitata*, with all its individuals grouped at the positive end of the axis.

Craniometric comparison of medium-sized Lonchorhina *from Peru.*—Measurements of the two

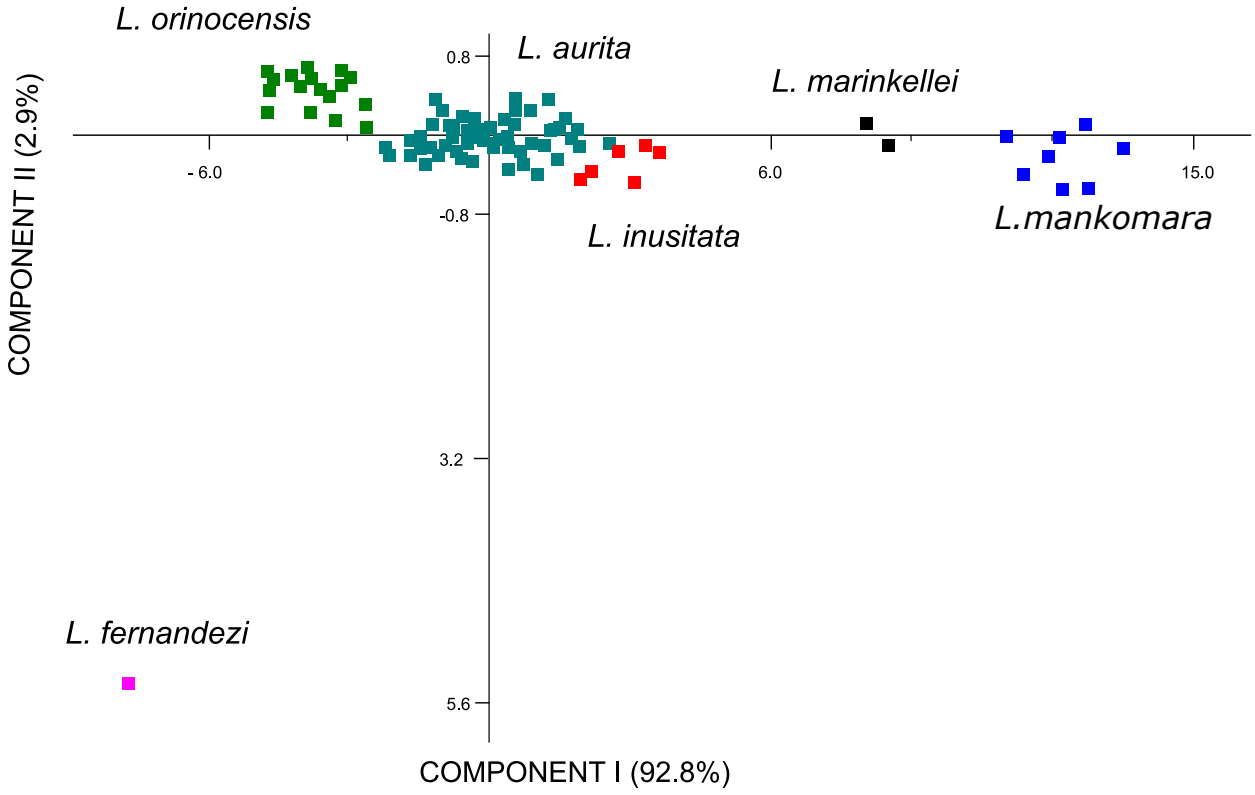

Figure 2. Principal Component Analysis of 10 cranial measurements of 115 individuals of the genus *Lonchorhina*, representing the six recognized species: *L. aurita* (aquamarine squares); *L. fernandezi* (pink square); *L. inusitata* (red squares); *L. mankomara* (blue squares); *L. marinkellei* (black squares); and *L. orinocensis* (green squares).

male specimens (TTU 46137 and CM 98592) from Peru fell within the range of *L. inusitata* proposed by Handley and Ochoa (1997) (see Table 1), and clustered together with other *L. inusitata* specimens from Brazil and Venezuela in the factorial plane of both PCA's (PCA of all species in the genus and PCA on medium-sized species). The DFA of the medium-sized species indicated that all the specimens *a priori* identified as *L. inusitata*, *L. aurita,* and *L. orinocensis* were correctly assigned (Wilks´s λ=0.66, 0.64; χ^2=201.71, 33.2; $P >$ 0.001, 0.001) and showed no overlap on the factorial plane (Fig. 3). Based on these analyses, *L. aurita* and *L. inusitata* were more morphologically aligned relative to *L. orinocensis*.

The discriminate function analysis that included *L. inusitata* and the larger species *L. mankomara* and *L. marinkellei* indicated that all the specimens identified *a priori* were correctly assigned to their taxonomic groups (Wilks´s λ=0.78, 0.73; χ^2=231.014, 47.2; $P >$ 0.001, 0.001) and showed no overlap on the factorial plane (Fig. 4).

Evaluation of the status of divergent Lonchorhina *specimens from Peru.*—Specimens TTU 46137 and CM 98592 from Peru were within the morphometric ranges established for *L. inusitata*, and were placed with other specimens of *L. inusitata* from Brazil and Venezuela in both PCA analysis (Figs. 2, 3), as well as confirmed as part of this taxon in our DFA analysis (Fig. 4).

Geographic craniometric variation of the widespread L. aurita.—The U-Mann-Whitney test conducted to account for secondary sexual dimorphism showed no statistical differences in this taxon. The DFA revealed high variability and some geographic structure in the skull morphology of *L. aurita* (Fig. 5). Based on the DFA, specimens from Trinidad, the type locality of *L. a. aurita*, were morphologically different from specimens from Central America and Brazil.

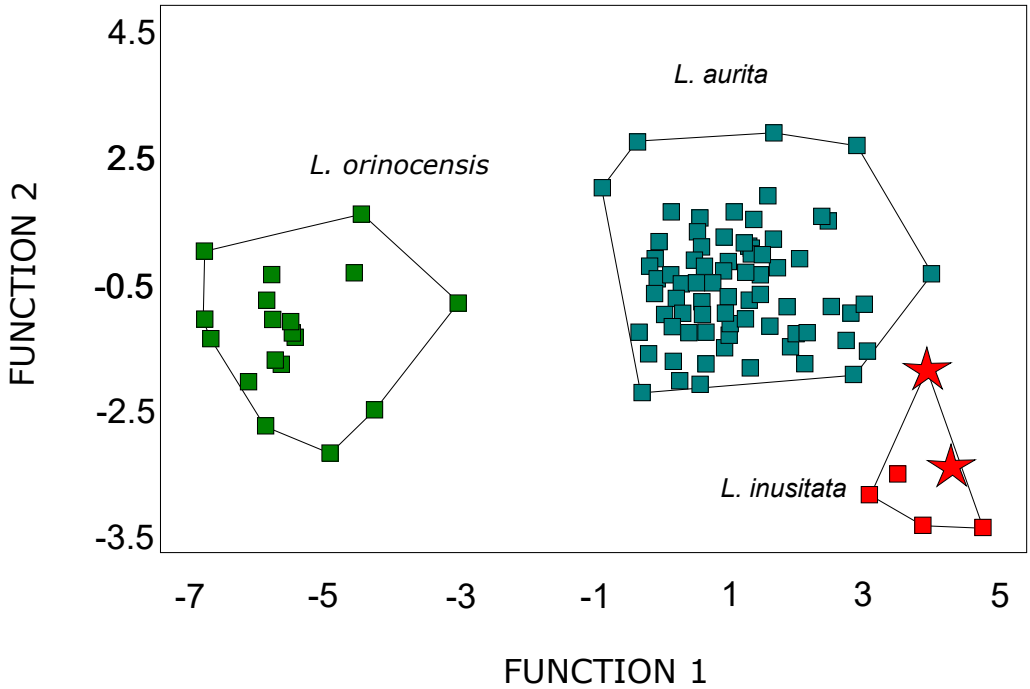

Figure 3. Discriminant Function Analysis performed on 10 cranial measurements of *Lonchorhina aurita* (blue squares), *L. orinocensis* (green squares), and *L. inusitata* (red squares). Red stars represent Peruvian specimens of *L. inusitata* (TTU 46137 ♂ and CM 98592 ♂).

Table 1. Cranial measurements analyzed among representatives of the five recognized species in the genus *Lonchorhina*. Abbreviations of measurements: Greatest length of skull (GLS); condylobasal length (CBL); palatal length (PAL); postorbital constriction (PO); rostrum (RO); braincase length (BL); distance across tympanic bullae (BL–BL); braincase length (BCH); tooth row length (LTR); distance across third upper molars (M–M); distance across canines, including cingula (C–C).

Statistics or catalog number	GLS	CBL	PAL	PO	RO	BL	BL–BL	BCH	LTR	M–M	C–C
Lonchorhina aurita (N = 83)											
Mean±SD	20.46±0.60	19.01±0.62	9.62±0.51	4.95±0.22	5.86±0.36	10.49±1.39	11.22±0.38	6.86±0.22	6.84±0.31	7.27±0.55	4.55±0.24
Range	19.3–21.55	17.56–20.09	8.40–10.87	4.38–5.50	5.13–6.84	8.51–12.30	10.47–11.96	6.28–8.32	6.18–7.54	6.50–7.89	4.12–5.12
Lonchorhina fernandezi (N = 1)											
ICN 12276♂	17.92	15.53	6.70	3.90	4.70	11.64	3.95	6.53	5.46	5.81	3.45
Lonchorhina inusitata (N = 6)											
Mean±SD	22.21±0.43	20.65±0.42	10.75±0.27	5.26±0.22	6.39±0.12	12.56±0.48	11.87±0.23	7.10±0.22	7.64±0.14	7.91±0.17	5.08±0.07
Range	21.04–22.69	19.96–21.09	10.28–11.20	5.02–5.59	6.04–6.59	12.11–13.36	11.38–12.15	6.89–7.43	7.08–7.78	7.45–8.20	4.81–5.17
Lonchorhina mankomara (N = 9)											
Mean±SD	27.78±0.08	25.87±0.46	13.11±0.34	6.89±0.08	8.69±0.14	14.61±0.45	14.35±0.15	8.04±0.03	9.19±0.29	9.44±0.31	6.02±0.11
Range	26.54–27.95	25.57–26.59	12.20–13.71	6.26–6.96	8.39–8.83	14.00–15.23	14.25–14.62	8.02–8.09	8.73–9.43	9.35–9.91	5.92–6.12
Lonchorhina marinkellei (N = 2)											
ICN 12587♂	25.08	24.00	11.81	6.07	8.26	14.40	13.79	7.35	8.08	8.82	5.70
ICN 5459♀	25.91	24.31	12.00	6.11	8.25	14.47	13.81	8.11	8.63	9.17	5.52
Lonchorhina orinocensis (N = 14)											
Mean±SD	18.67±0.24	16.81±0.29	8.07±1.01	4.04±0.08	4.64±0.11	11.10±0.28	10.51±0.26	6.32±0.22	6.04±0.09	6.03±0.14	3.53±0.10
Range	18.33–19.82	16.24–17.64	7.07–9.05	3.87–4.87	4.09–5.30	10.53–11.74	9.82–10.90	5.94–6.97	5.92–6.32	5.76–6.69	3.37–4.35

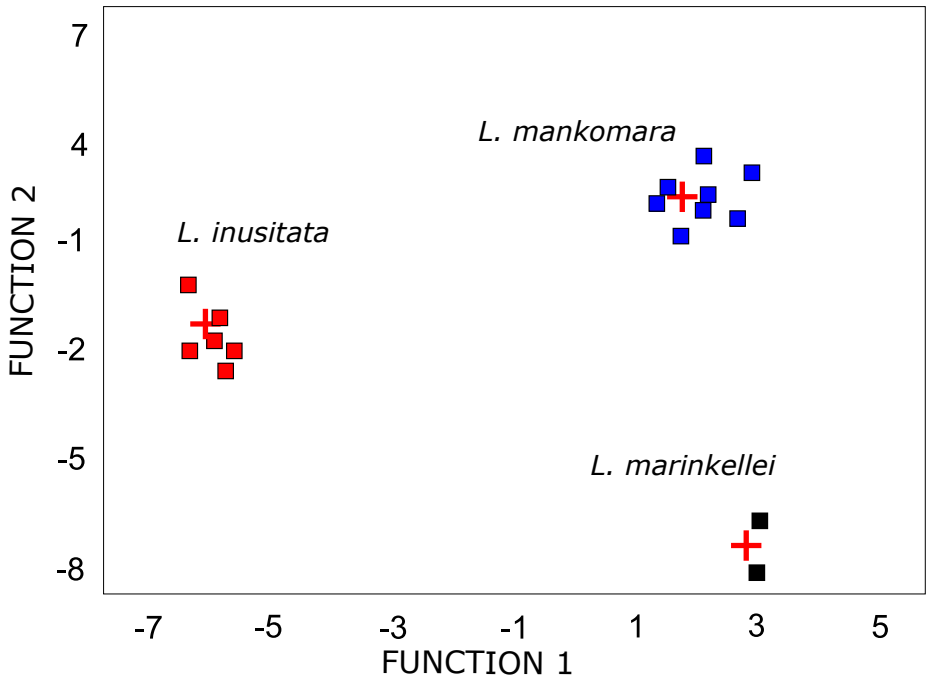

Figure 4. Discriminant Function Analysis of large species *L. mankomara* (blue squares) and *L. marinkellei* (black squares), as well as the medium sized *L. inusitata* (red squares). Centroids are designated by a plus symbol (+).

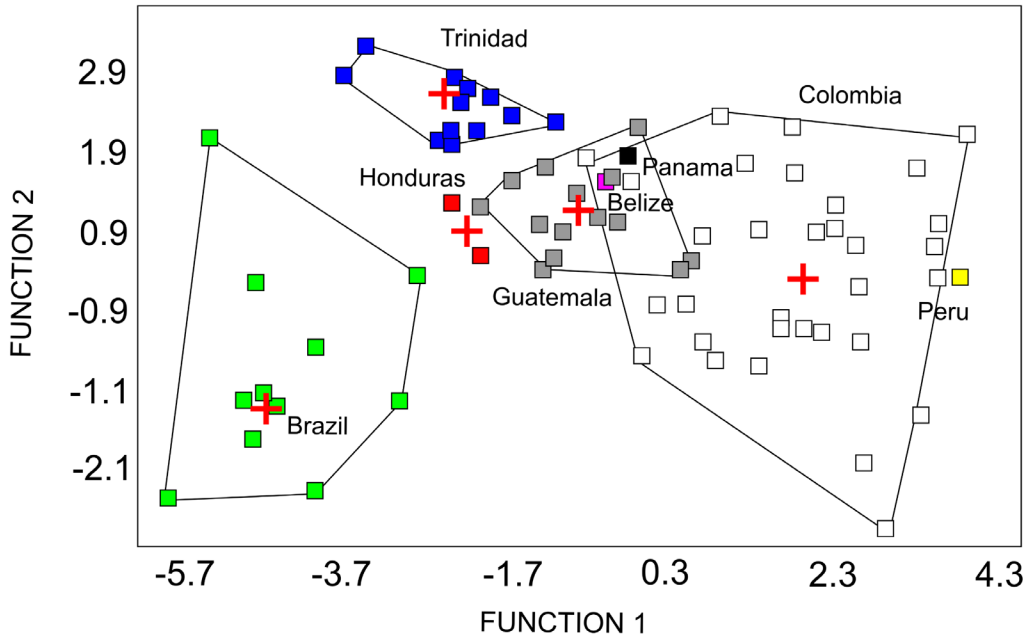

Figure 5. Discriminant Function Analysis (DFA) performed on 10 cranial measurements of analyzed specimens of *Lonchorhina aurita* across its distributional range: Belize (pink square); Brazil (green squares); Colombia (white squares); Guatemala (grey squares); Honduras (red squares); Panama (black square); Peru (yellow square); and Trinidad (blue squares). Centroids are designated by a plus (+) symbol.

DISCUSSION

Molecular data place the genus *Lonchorhina* in its own subfamily Lonchorhininae with a deep node that probably diverged from other phyllostomids between 24.7 and 21.6 mya (Baker et al. 2003, 2012). As mentioned, species in the genus *Lonchorhina* are characterized by extreme morphological modifications, including hypertrophied telic structures, with an enormous and extremely complex sword-shaped noseleaf, that in some species could reach one and a half times the dimensions of the skull (Hernández-Camacho and Cadena 1978; Mantilla-Meluk and Montenegro 2016) (Figs. 1, 7). Modifications of external features in *Lonchorhina* are accompanied by a unique skull morphology, found only in this genus. In *Lonchorhina*, the anterior portion of the skull, particularly the nasal and maxillae bones, are enlarged, providing support to the hypertrophied noseleaf, a structure that has been associated with the direction and intensity of the echolocation among bats in the family Phyllostomidae. The noseleaf among phyllostomids aids in echolocation and navigation inside a complex understory of Neotropical mature stratified forests. In addition, although poorly investigated, a larger and more complex noseleaf is a characteristic of some of the most specialized insectivore phyllostomid genera (i.e., *Gardnerycteris*, *Lophostoma*, *Macrophyllum*, and *Mimon*), suggesting that prey detection may play an important role in the evolution of more elaborate nose leaves and consequently can be associated with the accumulation of differences in skull morphologies in the genus *Lonchorhina*. Therefore, two non-excluding hypotheses can be suggested for the origin of the unique morphology in *Lonchorhina*: 1) a red queen effect, that includes a rapid channelization of the echolocation system (external and internal) in a predator-prey arms race (Mantilla-Meluk and Montenegro 2016); and 2) trophic niche displacement. Based on these arguments, prey size could be one of the drivers of the skull morphometric variation among recognized species in the genus.

Currently, the genus includes six species, five of which were subdivided by Williams and Genoways (2008) into three size groups: 1) large-sized (greatest length of the skull, GLS > 25 mm), including *L. marinkellei* and, in the study reported herein, the recently described *L. mankomara*, which is the largest species in the genus; 2) medium-sized (GLS 19–23 mm), which includes *L. aurita*, *L. inusitata*, and *L. orinocensis*; and 3) small-sized (GLS < 19 mm), *L. fernandezi*. The analyses reported herein support the proposed size classes among *Lonchorhina* species, and point to the extreme divergence between the smallest, *L. fernandezi*, and the largest, *L. mankomara* and *L. marinkellei*, with a greater morphometric overlap among medium-sized taxa. In addition, the results of this study suggest an association between size of the noseleaf and complexity of its ornamentations and species body size. This is partially explained by the need for major structural support of larger noseleaves, represented by an also larger and more elaborate sellas (for images and nomenclature of *Lonchorhina* noseleaf structures see Hernandez-Camacho and Cadena 1978; Mantilla-Meluk and Montenegro 2016). Based on the general morphology of the *sellas*, three groups can be differentiated: 1) the simplest sella expressed in *L. fernandezi*; 2) intermediate ornamentation of the sella, expressed in *L. orinocensis*, which also has a distinctly characteristic serrate border of the ear pinna; and hyperelaborated sellas in *L. aurita*, *L. inusitata*, *L. mankomara*, and *L. marinkellei*, species that exhibit size differences in noseleaf and ear pinna (Fig. 1).

Although analyses herein provide evidence of morphological separation of the six recognized species of *Lonchorhina*, except for *L. aurita*, most species (especially *L. fernandezi*, *L. inusitata*, *L. mankomara,* and *L. marinkellei*) are known from a few specimens and a limited number of localities. This makes a detailed morphological assessment of intraspecific variation and delineation of the distribution of species challenging (see Williams and Genoways 2008; Mantilla-Meluk and Montenegro 2016; Mantilla-Meluk et al. 2017).

Variation in Lonchorhina aurita *and taxonomic remarks.*—Morphometric analyses in this study indicated that specimens of *L. aurita* from near the type locality of the species in Trinidad were differentiated as an independent unit from two groups showing no overlap on the factorial plane—specimens from Brazil that were consistently smaller with respect to the hypodigm of *L. aurita* but clearly differentiated from *L. orinocensis*; and a group including representatives

in the remainder of the geographic range of the species (Central and northern South America). To better understand the morphological relationships among populations of *L. aurita*, a discriminant function analysis was performed on the *L. aurita* subset of specimens. *Lonchorhina aurita* from Brazil proved to be morphologically different from *L. aurita* from several localities across its distribution, including material from near the type locality of the species in Trinidad (Fig. 5). These morphometric differences suggest that *Lonchorhina* specimens from Brazil may represent a distinct species.

The only species in the genus that has a wide distribution is *Lonchorhina aurita*, originally described from Trinidad (Tomes, 1863), with a distribution extending from Oaxaca, Mexico, south to South America (Colombia, Ecuador, Peru, Bolivia, and Brazil), and a portion of the Caribbean (Trinidad; and the New Providence Islands [Bahamas Islands]) (Jones and Carter 1976; Lassieur and Wilson 1989; Nogueira et al. 2007; Williams and Genoways 2008; Reid 2009). Intraspecific variation in the genus has been suggested only for *L. aurita*. Anthony (1923) described the subspecies *L. a. occidentalis* based on three specimens collected in Guayas, Ecuador, claiming that this taxon could be differentiated from *L. a. aurita* (from Trinidad) by external characters (e.g., presence of white markings on the wings and length of the noseleaf). Soon after the description, several authors treated *occidentalis* as a subspecies of *L. aurita* (Goodwin and Greenhall 1961; Koopman 1978; Sanborn 1932; Tuttle 1970), with Linares and Naranjo (1973) and Hernández-Camacho and Cadena (1978) accepting *L. a. occidentalis* as a subspecies of *L. aurita* but restricting this consideration until additional material was available to test its specific status. However, since Cabrera (1958) overlooked this taxon in his revision of mammals from South America, other researchers considered it as a junior synonym of *L. aurita*, which is its currently accepted taxonomic status (Solmsen 1985; Williams and Genoways 2008). Based on the results of this study, some degree of morphometric differentiation is recognized between northern South American and Central American specimens of *L. aurita* and representatives of this taxon from Trinidad.

Also noteworthy is that Colombian specimens of *L. aurita*, particularly those from the departments of Caqueta and Meta, in the northeastern portion of the Colombian Amazon, proved to have larger skulls than typical *L. aurita* from Trinidad, Brazil, and Ecuador but were part of a group including specimens from Central and northern South America, on the factorial plane. Although larger than typical *L. aurita* from Trinidad, the overall skull morphology of *L. aurita* material from Caqueta differed from that of herein analyzed *L. inusitata* specimens from Brazil, Peru, and Venezuela. These results point again to the need to conduct more detailed analyses using data sets other than morphology, including DNA analyses, to better understand the phylogenetic and phylogeographic affinities within the genus.

Large-sized Lonchorhina.—*Lonchorhina* species with a larger skull size in this study (medium-sized *L. inusitata* and large-sized *L. mankomara* and *L. marinkellei*) have a height of the rostrum comparable to braincase height, a character associated with size increase and complexity of the noseleaf supporting structures (cartilage and muscles). The overall enlargement of the rostrum (rostrum width, depth, and degree of swollen) is one of the major and consistent modifications separating medium-sized *L. inusitata* from *L. aurita* and *L. orinocensis*, and also works as diagnostic characters distinguishing *L. mankomara* from *L. marinkellei* (Figs. 6, 7). In addition, Mantilla-Meluk and Montenegro (2016) mention the following as modifications of the anterior portion of the skull in *L. mankomara*—the presence of projections of palatine; a massive hamulus pterygoideus; and an angled occipital region (all characters observed from a ventral view; see images in Mantilla-Meluk and Montenegro 2016). *Lonchorhina mankomara* has an overall more massive dentition than *L. marinkellei*; central upper incisors longer and wider; massive canines with wide cingula; enlarged first upper premolar, double the size of that in *L. marinkellei* (Fig. 6); wide molars with elongated lingual bases (Fig. 6); and bilobed lower incisors (Figure 8b in Mantilla-Meluk and Montenegro 2016). From a craniometric standpoint, significant values in the DFA´s of medium and large size *Lonchorhina* support the currently accepted taxonomic differentiation and the recognition of *L. inusitata*, *L. mankomara*, and *L. marinkellei* as craniometrically distinct groups, also supported by discrete morphological characters.

In their assessment of representatives of the genus *Lonchorhina* from Colombia, Morales-Martínez and

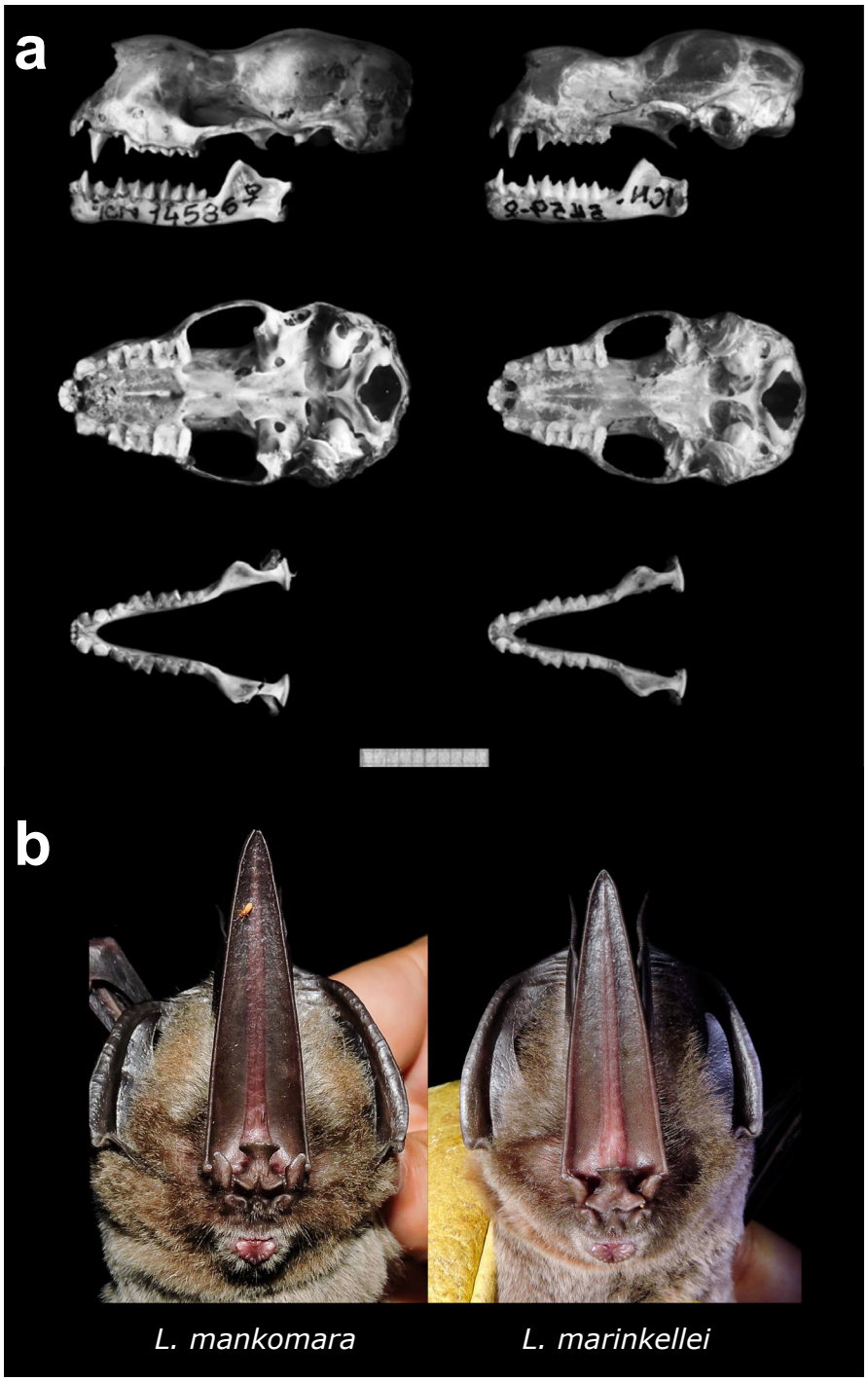

Figure 6. a) Comparison of lateral (top) and ventral (bottom) skull views of the holotypes of the recently described *Lonchorhina mankomara* (ICN 14586♀; Mantilla-Meluk and Montenegro 2016) (left) and *L. marinkellei* (ICN 5459; Hernandez-Camacho and Cadena 1978) (right). b) Comparison of external characters distinguishing *L. mankomara* and *L. marinkellei*, showing differences in size and shape of the nose-leaf, tragus, and pinna documented at the Serrania de la Lindosa where these two species occur in sympatry.

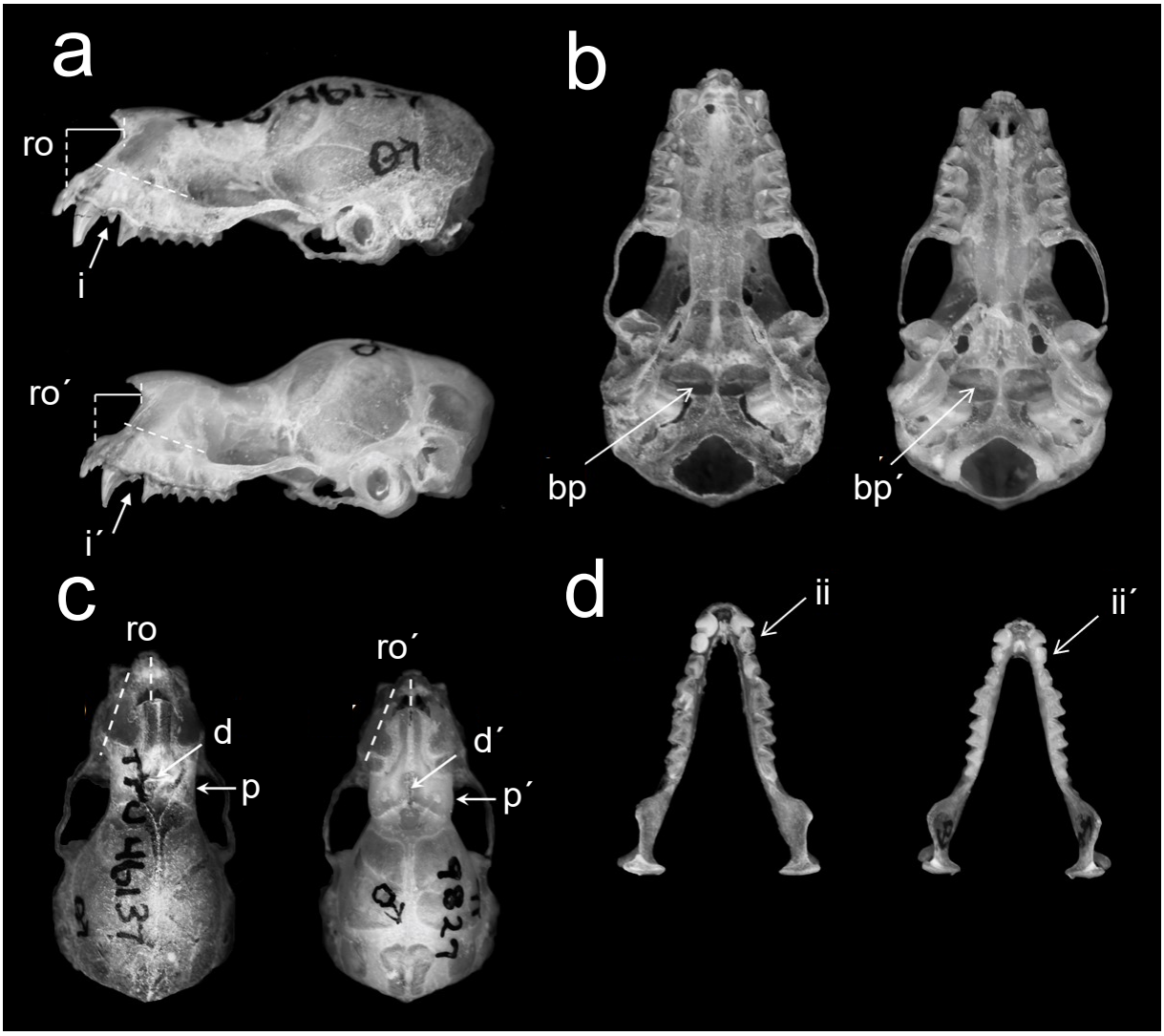

Figure 7. Comparison of discrete skull characters between the *Lonchorhina inusitata* (TTU 46137 ♂) from Peru and *L. aurita* (TTU 9827 ♂) from the island of Trinidad, type locality of the species: a) lateral view, showing the enlarged second premolar in *L. inusitata* (i) versus the small upper premolar in *L. aurita* (i´); b) ventral view, showing the wide and deep basisphenoid pits in *L. inusitata* (bp) versus the narrow and shallow basisphenoid pits in *L. aurita* (bp´); c) dorsal view showing the deep postorbital depression in *L. inusitata* (dp) versus a shallow postorbital depression in *L. aurita* (dp´), and a flat postorbital lateral profile in *L. inusitata* (p) versus a swollen postorbital region in *L. aurita* (p´); d) dorsal view of the mandible showing the enlarged first lower premolar in *L. inusitata* (ii) versus the small lower premolar in *L. aurita* (ii´). Dashed lines in 7a and 7c show deeper rostrum in *L. inusitata* (ro) versus a shallow rostrum in *L. aurita* (ro´).

López-Arévalo (2018) omitted records of *L. mankomara and L. marinkellei* that were reported in Agudelo et al. (2018) and Mantilla-Meluk et al. (2017), alluding that: "being these two species externally undistinguishable, we consider that records in Agudelo et al. (2018) and Mantilla-Meluk et al. (2017) do not count with enough certainty to be considered within our work." In the present assessment we verified not only the already reported differences in size of telic structures recorded for these two taxa (Mantilla-Meluk and Montenegro 2016) but also differences in shape and complexity of the sella, the cornus minus, and majus, as well as general shape of the excrescences at the base of the noseleaf López-Arévalo (2018). Herein, the lack of resolution in Morales-Martínez and López-Arévalo (2018) is interpreted as a potential consequence of the limited number (or absence) of *in vivo* specimens. However, although Morales-Martínez and López-Arévalo (2018) included a table with a detailed analysis of the external morphology of representatives of the genus, they failed to identify conspicuous external differences between *L. mankomara* and *L. marinkellei* (Fig. 6).

Finally, it is important to mention that the morphology of external characters is compromised in museum specimens preserved as dry skins, preventing an appropriate description of the actual complexity of soft anatomy structures.

First record of Lonchorhina inusitata *for Peru*.—To date, the only species of the genus *Lonchorhina* reported in official lists of mammals from Peru is *L. aurita* (Pacheco et al. 2009); however, specimens from Huánuco (TTU 46137 and CM 98592) proved to differ in all craniometric measurements but to have a distinct morphology when compared with typical *L. a. aurita* from Trinidad and the holotype of *L. a. occidentalis* (AMNH 62101♂). The craniometric ranges of specimens TTU 46137 and CM 98592 fell into those described for *L. inusitata*. As mentioned, *L. inusitata* is among the medium-sized species in the genus (Table 1) and it can be distinguished easily by forearm and skull size from the smaller *L. fernandezi* and *L. orinocensis* and the much larger *L. mankomara* and *L. marinkellei* (Williams and Genoways 2008; Mantilla Meluk and Montenegro 2016). No differences were found in overall craniometrics of specimen TTU 46137 in comparison to analyzed specimens of *L. inusitata* from Brazil and Venezuela (Figs. 2, 3).

Comparative analysis of discrete skull characters between L. inusitata *and* L. aurita.—The combination of discrete skull characters of *L. inusitata* are unique among recognized species of *Lonchorhina* and represent significant morphological evolution of characters historically assumed as conserved among mammals, such as modifications in size and placement of dental elements, as well as cusp development. Many specimens of *L. inusitata* have been either misidentified with the smaller and more common *L. aurita* (Genoways et al. 1981, material from Suriname) or with the much larger and less common *L. marinkellei* (Brosset and Charles-Dominique 1991; Brosset et al. 1996; material from French Guiana).

Besides differences in size between *L. aurita* and *L. inusitata* (Table 1), specimen TTU 46137 herein identified as *L. inusitata* is morphologically different from the typical *L. aurita* from Trinidad and Ecuador (countries from where the two subspecies of *L. aurita* have been described—*L. a. aurita* and *L. a. occidentalis*), and proved to be closely related in its morphology with typical *L. inusitata* from Brazil and Venezuela. However, in the Peruvian *L. inusitata* the anteriorly excavated portion of the basisphenoidal pits, also called inter-auditory pits in Handley and Ochoa (1997), were deeper than those of analyzed *L. inusitata* specimens from Brazil and Venezuela. Peruvian specimens of *L. inusitata* can be distinguished easily from the morphometrically closely related *L. aurita* by a suite of discrete characters. These include a more robust dentition, with particularly enlarged canines and premolars which are almost double the size of those in *L. aurita* (Figs. 7, 8), and bilobed inner incisors that contrast the entire cutting edges of the inner incisors of *L. aurita*. Although both *L. inusitata* and *L. aurita* have subequal lateral incisors (I2s), the inner lobes on I2 in *L. inusitata* are more acute than those in *L. aurita*, with the inner lobes almost twice the size of the lateral lobes. The canines of the Peruvian *L. inusitata* are larger than those in *L. aurita* and have a wider frontal surface. Some of the most contrasting characters between Peruvian *L. inusitata* and typical *L. aurita* from Trinidad are found on P4. Size, placement, shape, and disposition of the cusps on P4 differ substantially as follows—the paracone of P4 in Peruvian *L. inusitata* is larger in comparison with the paracone of *L. aurita*; and, the P4s in Peruvian *L. inusitata* are in contact with the canines and P5, whereas in *L. aurita* from Trinidad the P4 possesses diastemata between adjoining teeth (Figs. 7, 8).

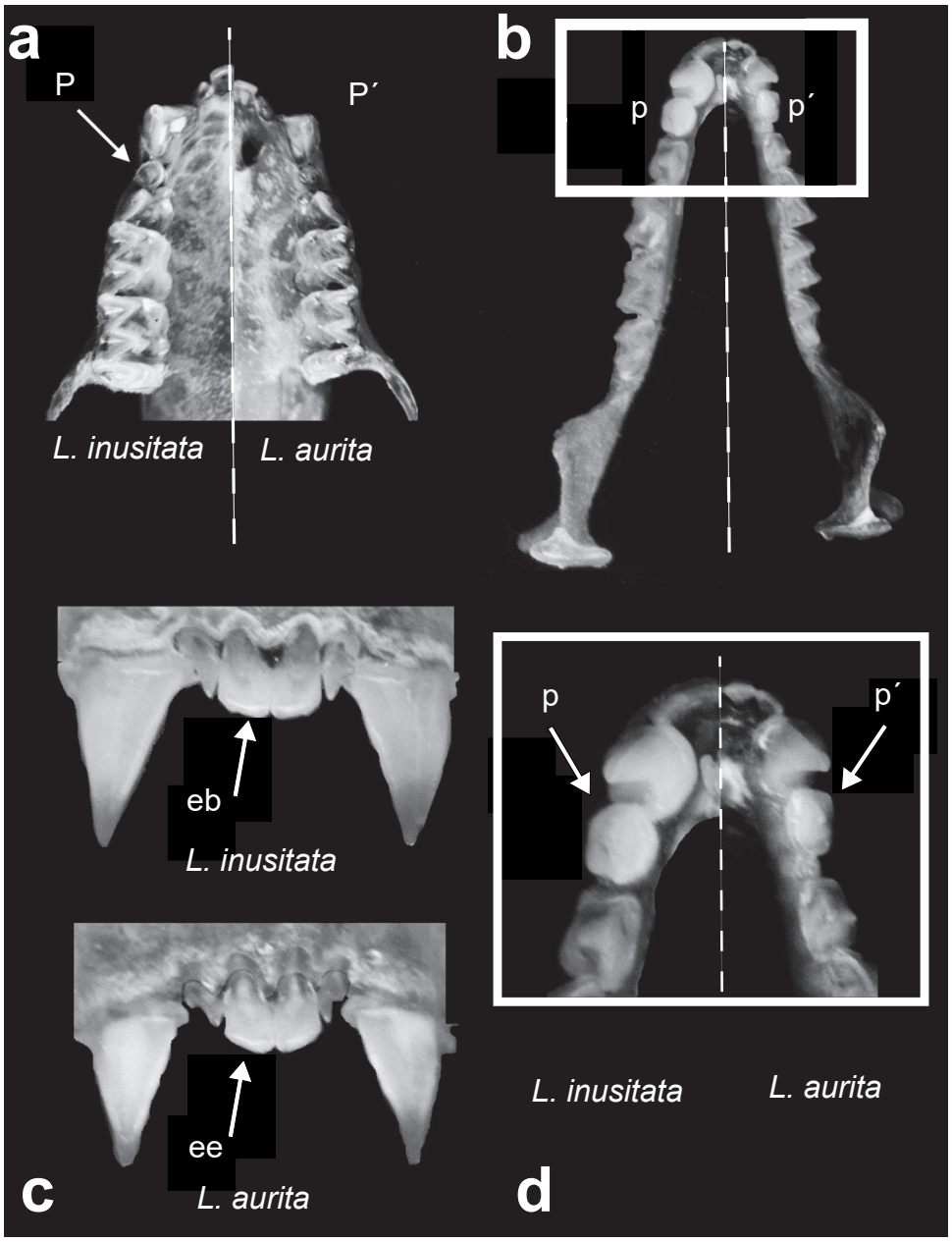

Figure 8. Comparison of discrete skull characters differentiating *L. aurita* from Trinidad and *L. inusitata* from Peru (TTU 46137). Skull images are composites, in which the left half of the axis (dashed line) corresponds to *L. inusitata*, while the right half corresponds to *L. aurita*: a) skull ventral view showing the enlarged first upper premolar in *L. inusitata* (P) versus the smaller first upper premolar in *L. aurita* (P´); b) dorsal view of the mandible showing the enlarged first lower premolar in *L. inusitata* (p) versus the smaller lower premolar of *L. aurita* (p´); c) frontal view of the bilobed edge inner incisors of *L. inusitata* (upper) (eb) versus the entire edge of inner incisors in *L. aurita* (bottom) (ee); and d) detail of the first lower premolars of *L. inusitata* (left of the axis) versus *L. aurita* (right of the axis) showing differences in size of the paracone of P4, larger *L. inusitata* (p) and in contact with the canines and P5, than that in *L. aurita* (p´), which also has a diastemata between adjoining teeth.

In addition to size differences in all measurements analyzed between *L. inusitata* and *L. marinkellei* (Table 1), Williams and Genoways (2008:264) mentioned that *L. inusitata* is internally similar to *L. marinkellei* in shape of rostrum, with the basisphenoid pits shallow anteriorly, in contrast to the deep anteriorly pits in *L. marinkellei*. In addition, the rostrum is higher than the braincase in *L. marinkellei*, but of about equal height or slightly lower than the braincase in *L. inusitata*. Externally, both species are similar in the size, shape, and degree of hairiness of ears, noseleaf, and facial excrescences; however, the underparts are dark in *L. inusitata*, while in *L. marinkellei* the venter is heavily washed with white. All the above-mentioned characters were identified in both *Lonchorhina* specimens from Huánuco (TTU 46137 and CM 98592).

Based on the evidence provided herein from the craniometric analyses, as well as morphological comparison of discrete characters, the Peruvian specimens TTU 46137 and CM 98592 from the department of Huánuco, previously identified as *L. aurita*, should be recognized as *L. inusitata*. Thus, they represent the first record of the species in the country, as well as a significant distribution extension in its range of more than 1,360 km (860 mi) southeast from records in Venezuela (Fig. 9). Pacheco et al. (2018) reported 181 bat species occurring in Peru. With the new records of *L. inusitata* for Peru, the bat diversity increases to 182 species.

The specimens of *L. inusitata* from Peru (TTU 46137 and CM 98592) were caught the same night in the same mist net, and are in good condition and preserved as study skins with clean skulls. The specimens were the product of a field trip organized by D. E. Wilson as Director of Biodiversity Programs at the National Museum of Natural History (NMNH). The trip was conducted under the auspices of the Smithsonian Institution/Man and the Biosphere Program, with Francisco Dallmeier as Director of that program. Don Wilson invited Robert Baker, curator of mammals at the and director of the Natural Science Research Laboratory of the Museum of Texas Tech University at the time, to go to a Shell Oil Company drilling platform in the lower Urubamba region of Peru, in the company of M. O´Connell of the Carnegie Museum of Natural History, which is the institution that houses one of the two collected *L. inusitata* specimens. Robert had wanted to go to the field with his good friend Don Wilson, and the trip to Urubamba seemed like a good opportunity. The site was very isolated, and accessible only by helicopter. The trip was very successful in terms of the number collected specimens (D. Wilson, comm. pers.). As a result of Dr. Baker's and Dr. Wilson's commitment to science, and their profound personal and academic friendship, herein we add another species to the list of Peruvian mammals. One author of this publication (H. Mantilla-Meluk), had been introduced to R. J. Baker (his former PhD advisor) by D. Wilson, who also supported the visit to the NMNH mammal collections that allowed the *Lonchorhina* craniometric data gathering years later. The authors chose to include this manuscript as part of this memorial volume, in honor of Dr. Robert J. Baker, because it depicts a crucial aspect of mammalogy as a science, as well as our academic family bonds. In his life, Robert J. Baker understood what academia is, a web of bridges that facilitate the construction of knowledge; connections that can only be generated with generosity, as he used to say: "as many brains as you need" (R. J. Baker 1942–2018).

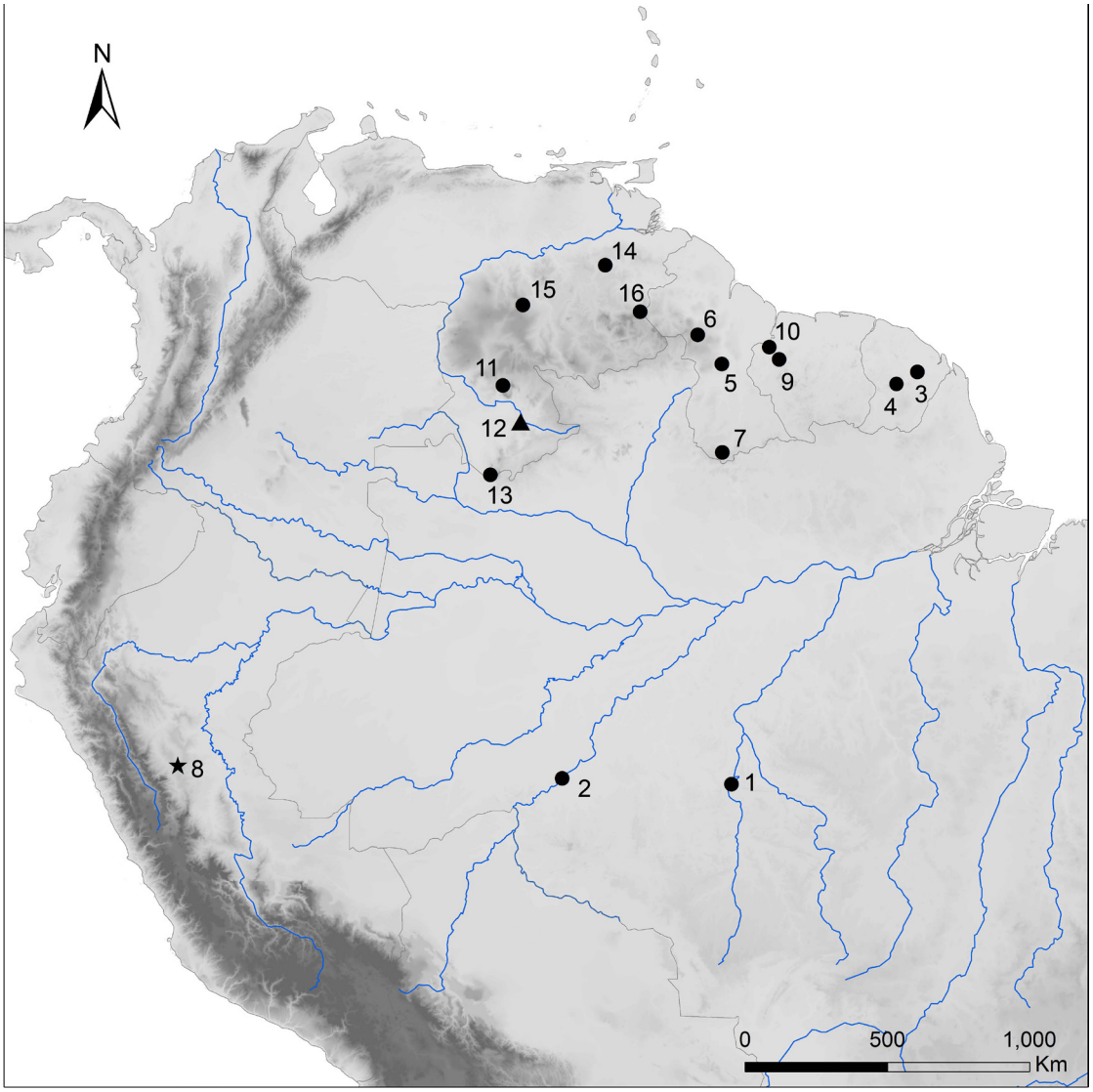

Figure 9. Map showing known localities of *Lonchorhina inusitata*, including the first record of this taxon for Peru (black star). 1) Brazil: Mato Grosso, Juruena National Park, São João River trail; 2) Brazil: Rondônia, Pôrto Velho ["19 km da Cidade"]; 3) French Guiana: Cayenne, 100 km SSW Cayenne, Aratoi, Estación les Nouragues; 3) French Guiana: Cayenne, Grotte du Bassin du Tapir, Les Nouragues; 4) French Guiana: St. Laurent Du Maroni, Saül; 5) Guyana: Potaro-Siparuni, 30 Km NE of Surama; 6) Guyana: Potaro-Siparuni, Kaieteur National Park, Kaieteur Falls; 7) Guyana: Upper Takutu-Upper Essequibo, Kamoa River, 50 Km SWW of Gunn's Strip; 8) Peru: Huánuco, Leoncio Prado, 9 km N Aucayacu; 9) Suriname: Nickerie, Sipaliwini, Bakhuis, Transect 9; 10) Suriname: Sipaliwini, Avanavero; 11) Venezuela: Amazonas, 56 km NNW La Esmeralda, Caño Culebra, Belén; 12) Venezuela: Amazonas, Boca Mavaca, 84 km SSE Esmeralda; 13) Venezuela: Amazonas, Río Mawarinuma, Parque Nacional Serranía de la Neblina; 14) Venezuela: Bolívar, 12 km S of El Manteco; 15) Venezuela: Bolivar, Alto Rio Tawadu, Monumento Natural Sierra de Maigualida; and 16) Venezuela: Bolivar, Km 85, about 65 km SSE El Dorado.

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LITERATURE CITED

Agudelo R., V. Giraldo, and V. J. Setina. 2018. Murciélagos de San José del Guaviare. Field Guides of Field Museum # 1006. [Reviewed on: 15 June 2018] https://fieldguides.fieldmuseum.org/sites/default/files/rapid-color-guides-pdfs/1006_colombia_bats_of_san_jose_del_guaviare.pdf.

Anthony, H. E. 1923. Preliminary report on Ecuadorean mammals, no. 3. American Museum Novitates 55:1–14.

Baker, R. J., O. R. P. Bininda-Emonds, H. Mantilla-Meluk, C. A. Porter, and R. A. Van den Bussche. 2012. Molecular timescale of diversification of feeding strategy and morphology in New World leaf-nosed bats (Phyllostomidae): a phylogenetic perspective. Pp. 385–409 in Evolutionary history of bats: fossils, molecules and morphology (G. F. Gunnell and N. B. Simmons, eds.). Cambridge University Press, Cambridge, United Kingdom.

Baker, R. J., S. R. Hoofer, C. A. Porter, and R. A. Van den Bussche. 2003. Diversification among New World leaf nosed bats: an evolutionary hypothesis and classification inferred from digenomic congruence of DNA sequence. Occasional Papers, Museum of Texas Tech University 230:1–32.

Brosset, A., and P. Charles-Dominique. 1991. The bats from French Guiana: a taxonomic, faunistic, and ecological approach. Mammalia 54:509–560.

Brosset, A., J. -F. Cosson, P. Gaucher, and D. Masson. 1996. Les chiropteres d'un marecagecotier de Guyane; composition du peuplement. Mammalia 59:527–537.

Cabrera, A. 1958. Catalogo de los mamíferos de America del Sur. I (Metatheria, Unguiculata, Carnivora). Revista del Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" 4:1–307.

Dávalos, L. M., A. L. Cirranello, J. H. Geisler, and N. B. Simmons. 2012. Understanding phylogenetic incongruence: lessons from phyllostomid bats. Biological Reviews 87:991–1024.

Dávalos, L. M., P. M. Velazco, O. M. Warsi, P. D. Smits, and N. B. Simmons. 2014. Integrating incomplete fossils by isolating conflictive signal in saturated and non-independent morphological characters. Systematic Biology 63:582–600.

Fox, J., and S. Weisberg. 2011. An {R} companion to applied regression, Second Edition. Thousand Oaks California: Sage. URL:http://socserv.socsci.mcmaster.ca/jfox/Books/Companion.

Genoways, H. H., S. L. Williams, and J. A. Groen. 1981. Results of the Alcoa Foundation–Suriname expeditions. V. Noteworthy records of Surinamese mammals. Annals of Carnegie Museum 50:319–332.

Goodwin, G. G., and A. M. Greenball. 1961. A review of the bats of Trinidad and Tobago. Bulletin of the American Museum of Natural History 122:187–302.

Hammer, Ø., D. A. T. Harper, and P. D. Ryan. 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4:1–9.

Handley, C. O., Jr., and J. Ochoa G. 1997. New species of mammals from northern South America: a sword-nosed bat, genus *Lonchorhina* Tomes (Chiroptera: Phyllostomidae). Memoria Sociedad de Ciencias Naturales La Salle 57:71–82.

Hernández-Camacho, J., and A. Cadena G. 1978. Notas para la revisión del género *Lonchorhina* (Chiroptera, Phyllostomidae). Caldasia 7:199–251.

Jones, J. K., Jr., and D. C. Carter. 1976. Annotated checklist, with keys to subfamilies and genera. Pp. 7–38 in Biology of bats of the New World family Phyllostomatidae. Part I (R. J. Baker, J. K. Jones, Jr., and D. C. Carter, eds.).

Special Publications, Museum of Texas Tech University 10:1–218.

Koopman, K. F. 1978. Zoogeography of Peruvian bats with special emphasis on the role of the Andes. American Museum Novitates 2651:1–33.

Lassieur, S., and D. E. Wilson. 1989. *Lonchorhina aurita*. Mammalian Species 347:1–4.

Linares, O. J., and C. J. Naranjo. 1973. Notas acerca de una colección de murciélagos del género *Lonchorhina* de la cueva de Archidona, Ecuador (Chiroptera). Boletín de la Sociedad Venezolana de Espeleología 4:175–180.

Linares, O. J., and J. Ojasti. 1971. Una nueva especie de murciélago del género *Lonchorhina* (Chiroptera: Phyllostomatidae) del sur de Venezuela. Novedades Científicas, serie zoología 36:1–8.

Mantilla-Meluk, H., and O. L. Montenegro. 2016. A new species of *Lonchorhina* (Chiroptera: Phyllostomidae) from Chiribiquete, Colombian Guayana. Biodiversidad Neotropical 6:171–187.

Mantilla-Meluk, H., F. Mosquera-Guerra, F. Trujillo, N. Pérez-Amaya, A. Velásquez-Valencia, and A. Vargas Pérez. 2017. Mamíferos del Sector Norte del Parque Nacional Natural Serranía de Chiribiquete. Colombia Amazónica 10:99–134.

Morales-Martínez, D. M., and H. F. López-Arévalo. 2018. Distribución y conservación de los murciélagos del género *Lonchorhina* (Chiroptera: Phyllostomidae) en Colombia. Caldasia 40:349–365.

Nogueira, M. R., A. L. Peracchi, and R. Moratelli. 2007. Subfamília Phyllostominae. Pp. 61–97 in Morcegos do Brasil (N. R. Reis, A. L. Peracchi, W. A. Pedro, I. P. Lima, eds.). Nélio Roberto dos Reis, Londrina, Brazil.

Ochoa G., J., and C. Ibañez. 1984. Nuevo murciélago del género *Lonchorhina* (Chiroptera: Phyllostomidae). Memoria de la Sociedad de Ciencias Naturales La Salle 118:145–159.

Pacheco, V., R. Cadenillas, E. Salas, C. Tello, and H. Zeballos. 2009. Diversidad y endemismo de los mamíferos del Perú. Revista Peruana de Biología 16:5–32.

Pacheco, V., P. Sánchez-Vendizú, and S. Solari. 2018. A new species of *Anoura* Gray, 1838 (Chiroptera: Phyllostomidae) from Peru, with taxonomic and biogeographic comments on species of the *Anoura caudifer* complex. Acta Chiropterologica 20:31–50.

R Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reid, F. A. 2009. A field guide to the mammals of Central America and Southeast Mexico. 2nd ed. Oxford University Press, New York.

Sanborn, C. C. 1932. Neotropical bats in the Carnegie Museum. Annals of the Carnegie Museum 21:171–183.

Solmsen, E.-H. 1985. *Lonchorhina aurita* Tomes, 1863 (Phyllostominae, Phyllostomidae, Chiroptera) im westlichen Ecuador. Zeitschrift für Säugetierkunde 50:329–337.

Tomes, R. F. 1863. On a new genus and species of leaf-nosed bats in the Museum at Fort Pitt. Proceedings of the Zoological Society of London 1863:81–84.

Tuttle, M. D. 1970. Distribution and zoogeography of Peruvian bats, with comments on natural history. The University of Kansas Science Bulletin 49:45–86.

Williams, S. L., and H. H. Genoways. 2008 (2007). Subfamily Phyllostominae Gray, 1825. Pp. 255–300 in Mammals of South America, Volume 1: Marsupials, xenarthrans, shrews, and bats (A. L. Gardner, ed.). The University of Chicago Press, Chicago, Illinois.

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APPENDIX

Specimens of *Lonchorhina* used in this study. See Materials and Methods for collection acronyms.

Lonchorhina aurita (83).—BELIZE: Toledo: Bladen Nature Reserve, Teakettel camp, on Bladen Branch (USNM 583007♂). BRAZIL: Maranhão: Alto Parnaiba (FMNH 26449♂). Para: Altamira 9 km SE (by road) Caverna do Valdeci (USNM 549340, USNM 549343–549345♀, USNM 549339♂, USNM 549341–549342♂); Altamira 85 km SW Eastern bank Rio Iriri (USNM 549346♀). Pernambuco: Toritama, Fazenda Matumbo, Pedrao Dos Pontais (USNM 536441♂). COLOMBIA: Antioquia: Zaragoza 26 km W Aljibes (USNM 799290–799292♂); 26 km S, 22 km W of Zaragoza (Aljibes) (ICN 12697♂, 12698♂); Zaragoza 25 km W La Tirana (USNM 549340♀). Caldas: Samana, Norcasia, Campamento Profesionales I, Proyecto La Miel I (ICN 14277♀); Samana, Vereda La Miel, near Campamento Tasajos (ICN 14306–14307♀); Samana, Corregimiento Norcasia, surroundings Campamento CHEC (ICN 15953♀); Samana, Corregimiento Norcasia, Vereda La Pradera, Campamento CHEC, Corporación Hidroeléctrica de Caldas, La Miel I (ICN 12488–12489♀, ICN 12490♂, ICN 12571♂). Caqueta: Municipio Montañitas; Vereda Santuario, Finca Ceilán (ICN 16896–16897♀); Rio Cuñare, Raudal El Tubo, E Serrania de Chiribiquete, Parque Nacional Natural (PNN) Chiribiqueté (ICN 14716♀, ICN 14584–14587♀); Rio Mesay, Puerto Abeja, SE Serranía de Chiribiquete (ICN 14583♂). Meta: San Juan de Arama, Northern portion Serranía La Macarena, Caño Guamalito (ICN 12041–12042♀); San Juan de Arama, northern portion Serranía La Macarena, Caño La Curia (ICN 10215♂, ICN 10217♂, ICN 10218♀, ICN 10219♂). Risaralda: Pueblo Rico, camino a la Bocatoma (ICN 11458–11459♂). Valle del Cauca: 29 km SE Buenaventura (USNM 483327♂). ECUADOR: Chimborazo: Chunchi, Puente de Chimbo (AMNH 62101♂ [holotype of Lonchorhina aurita occidentalis]). GUATEMALA: Izabal: Quebrados (FMNH 41891–41892 ♂, 41893 ♀, 41894–41897♂, 41898–41900♂, 41901–41904, 41906♀, 41911–41913 sex undetermined). HONDURAS: Colón: Trujillo, Parque Nacional Caprio y Calenturas (TTU 104265–104266♀). PANAMA: Colón: Gamboa, Coco Plantation, Mine Shaft (FMNH 92642♂); Fort Sherman (FMNH 92663♂). PERU: Cuzco: Paucartambo, Consuelo, 15.9 km SW Pilcopata (FMNH 174715♂, 174717–174718♀). Madre de Dios: Maskoitania, 13.4 km NNW Atalaya, left bank Rio Alto Madre de Dios (FMNH 174716♀). Pasco: Oxapampa, San Juan (USNM 364269–364270♀, 364268♂). TRINIDAD: Saint George: (TTU 5233♀, 5221♂, 5223♂, 5224♀, 8983♂, 8984♂, 9826–9829♂). VENEZUELA: (FMNH 20637 sex undetermined).

Lonchorhina inusitata (6).—BRAZIL: Rondônia: Porto Velho (NMNH 554575♀). PERU: Huánuco: Leoncio Prado, 9 km N Aucayacu (TTU 46137♂ [tissues available, TK 22878]; CM 98592♂). VENEZUELA: Amazonas: Belém, 56 Km NNW Esmeralda, Caño Culebra (USNM 388736♂); Cerro Neblina, Base Campamento (USNM 560553♀, 560774♀).

Lonchorhina fernandezi (1).—VENEZUELA: Bolívar: Puerto Cedeño (ICN 12276♂).

Lonchorhina mankomara (9).—COLOMBIA: Caqueta: Rio Mesay, Puerto Abeja, SE Serrania de Chiribiquete (ICN 14584–14587♀). Guaviare: Serrania de Chiribiquete, Sector Norte (UQ-HMM 1049♀, 1960♀, 1062♀, 1072♀, 1081♂).

Lonchorhina marinkellei (2).—COLOMBIA: Vaupés: Mitu, 10 km E Durania (ICN 5459♀); Mitu, Cueva Superior Primer Cerro, Finca Urania (ICN 12587♂).

Lonchorhina orinocensis (14).—COLOMBIA: Meta: Serranía de la Macarena, Caño Cristales (FMNH 58672♂, 8675♂, 58676♀). VENEZUELA: Apure: Hato Cariben, 32 km NE Puerto (USNM 373290♀, 373291♀, 373292♀, 373293♂, 373294♀, 373295♂, 373296–373298♀, 373299, 373302♀).

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Genetic Variation and Structure in the Endangered Mexican Long-nosed Bat (*Leptonycteris nivalis*): Mitochondrial and Nuclear Perspectives

Loren K. Ammerman, Carson M. Brown, Rodrigo A. Medellín, Arnulfo Moreno-Valdez, Russell S. Pfau, Randi Lesagonicz, and Amy L. Russell

Abstract

The endangered Mexican long-nosed bat (*Leptonycteris nivalis*) is a migratory nectar-feeder that occurs in high-elevation, semi-arid, pine-oak woodlands and Chihuahuan Desert of central and northern Mexico as well as two localities within the southwestern United States. Little is known about migratory movements and population structure of this species. The primary objectives of this study were to measure variation and patterns of subdivision in maternally-inherited mtDNA, particularly addressing the hypothesis of female philopatry, and to compare this with the bi-parentally inherited AFLP (Amplified Fragment Length Polymorphism) data. A second objective was to infer historical demographics based on patterns of sequence variation. Genetic analysis of mitochondrial DNA (control region) and nuclear DNA (AFLP) revealed an absence of genetic structuring within *L. nivalis*. Nucleotide (π = 0.013) and haplotype (h = 0.810) diversity values for genetic data were comparable to other species of migratory bats and were moderately high for a species believed to have undergone a recent drastic decline in population size. Some patterns of mtDNA sequence variation (Fu's F_S and a network analysis) along with a lack of structure in the analysis of AFLP loci suggest a historic population expansion, but other analyses (Tajima's D, Ramos-Onsins and Rozas' R_2, and a mismatch analysis) cannot reject stasis. It is concluded that individuals of *L. nivalis* form a panmictic population over a large geographic area. In addition, the geographic distribution of mtDNA control region haplotypes does not support the hypothesis of female philopatry.

Key words: AFLP, control region, genetic variation, *Leptoncyteris nivalis*, Mexican long-nosed bat, mitochondrial DNA

Resumen

El murciélago magueyero mexicano (*Leptonycteris nivalis*), que se encuentra en peligro de extinción, es una especie nectarívora migratoria que se encuentra en bosques abiertos semiáridos de pino-encino de gran altitud, y en el desierto Chihuahuense en el centro y norte de México, así como en dos localidades del suroeste de los Estados Unidos. Se sabe poco sobre los movimientos migratorios y la estructura poblacional de esta especie. Los principales objetivos de este estudio fueron medir la variación y los patrones de subdivisión en el ADN mitocondrial hereditario por línea materna, abordando particularmente la hipótesis de filopatría de las hembras, y comparar esto con los datos de AFLP (Polimorfismo de Longitud de Fragmentos Amplificados) heredados biparentalmente. Un segundo objetivo fue inferir datos demográficos históricos basados en patrones de variación de secuencia. El análisis genético del ADN mitocondrial (región control) y el ADN nuclear (AFLP) reveló una ausencia de estructuración genética en *L. nivalis*. Los valores de diversidad de nucleótidos (π = 0.013) y haplotipo (h = 0.810) para los datos genéticos fueron comparables a otras especies de murciélagos migrato-

rios y fueron moderadamente altos para una especie que se cree que sufrió un descenso drástico reciente. Algunos patrones de variación de la secuencia de mtDNA (el F_s de Fu y un análisis de red) junto con una falta de estructura en el análisis de los loci de AFLP sugieren una expansión histórica de la población, pero otros análisis (*D* de Tajima, R_2 de Ramos-Onsins y Rozas, y un análisis de inconsistencia) no pudieron rechazar la estasis. Se concluye que los individuos de *L. nivalis* forman una población panmíctica en una gran área geográfica. Además, la distribución geográfica de los haplotipos de la región control de ADNmt no apoya la hipótesis de la filopatría de las hembras.

Palabras clave: AFLP, Polimorfismo de Longitud de Fragmentos Amplificados, región control, variación genético, *Leptoncyteris nivalis*, murciélago magueyero mexicano, ADN mitocondrial

INTRODUCTION

The Mexican long-nosed bat (*Leptonycteris nivalis*) is the largest nectar-feeding bat of the New World and a member of the family Phyllostomidae (subfamily Glossophaginae). Typical of the glossophagines, *L. nivalis* is nectarivorous, and, like its congener the lesser long-nosed bat (*L. yerbabuenae*), *L. nivalis* migrates seasonally to feed on the blooms of chiropterophilous plants (Easterla 1972; Sanchez and Medellín 2007). The southern half of the range of *L. nivalis*, which spans central Mexico, is occupied year-round, whereas the northern half, which includes northern Mexico and two localities in the southwestern United States, is occupied only in the summer (Easterla 1972; Moreno-Valdez et al. 2004). The summer colonies in the northern half of their range consist almost entirely of adult females and their young of the year (Borrell and Bryant 1942; Easterla 1972; Moreno-Valdez et al. 2004; Adams 2015), suggesting that males do not migrate.

It has long been recognized that *L. nivalis* is rare throughout its range (Allen 1939; Barbour and Davis 1969; Easterla 1972). Wilson (1985) conducted a survey of *L. nivalis* throughout its range and observed that several sites with historically high numbers of *L. nivalis* were then unoccupied, concluding that this species had experienced declines and suggesting that *L. nivalis* be listed as threatened. In 1988, *L. nivalis* was added, along with *L. yerbabuenae*, to the U.S. Endangered Species List (Shull 1988). Cockrum and Petryszyn (1991) questioned the listing of both *Leptonycteris* species, and although their criticism focused on the listing of *L. yerbabuenae*, they argued that the listing of *L. nivalis* was unfounded as well.

Although *L. nivalis* remains listed as an endangered species, Cockrum and Petryszyn (1991) successfully opened up to debate the idea that *L. nivalis* had experienced recent population declines. In the *L. nivalis* Species Recovery Plan (U. S. Fish and Wildlife Service 1994), it was acknowledged that seasonal movements create difficulties in obtaining accurate census data and highlighted the need to identify what constitutes a population in *L. nivalis*, as a prerequisite to estimating population sizes. Unfortunately, a current lack of understanding concerning the migratory habits of *L. nivalis* has made it impossible to determine population boundaries and sizes.

Traditionally, placing metal or plastic bands on the forearm has been used to determine migratory movements and estimate population size in bats (e.g., Kurta and Murray 2002; Rivers et al. 2006). Easterla (1972) banded 568 *L. nivalis* at Emory Cave, Big Bend National Park, Texas, but none of the banded *L. nivalis* have been recaptured or recovered. The majority of these (512/568; 90%), however, were not banded until the last year of the 5-year study (Easterla 1972) and little work on this species has been conducted since. Nearly 30 years later, Arnulfo Moreno-Valdez searched the floor of El Infierno Cave, Nuevo Leon, Mexico (a maternity roost for *L. nivalis*) with a metal detector and recovered no bands (Moreno-Valdez, pers. obs.). More recently, passive integrated transponders (PIT tags) have been used to mark individuals at Emory Cave and monitor their movements (Adams 2015). Monitoring efforts in Mexican roost sites have not detected any PIT-tagged bats from Emory Cave and vice versa

(Adams 2015). Therefore, determining the extent of migratory corridors or substructure across the range of the species has not been possible.

Molecular markers have gained considerable traction in the study of population subdivision, sex-biased dispersal, and migratory movements in elusive animals such as bats (reviewed in Burland and Worthington Wilmer 2001; Ruedi and McCracken 2009). Additionally, the combination of maternally-inherited and bi-parentally inherited markers can be used to evaluate population structure and potentially to reveal philopatry. Recent work with the pallid bat (*Antrozous pallidus*) using maternally and bi-parentally inherited markers, has revealed strong female natal philopatry (Arnold and Wilkinson 2015) and male dispersal. If female *L. nivalis* exhibit philopatry for maternity sites, divergent matrilines are expected to correspond to roost sites. No genetic studies have been published for *L. nivalis*, but some previous work exists for a close relative, *L. yerbabuenae*. For example, Wilkinson and Fleming (1996) used maternally-inherited mitochondrial DNA (mtDNA) to identify population subdivision and infer migratory movements in *L. curasoae* [=*yerbabuenae*]. Morales-Garza et al. (2007) identified migratory and non-migratory populations within *L. yerbabuenae* using a biparentally inherited marker, random amplified polymorphic DNA (RAPD). Since that time, however, Ramirez (2011) and Arteaga et al. (2018) found low levels of genetic structure and no evidence for separate migratory populations in *L. yerbabuenae* based on microsatellite loci and mtDNA. Other studies on the endangered Ozark big-eared bat (*Corynorhinus townsendii ingens*; Weyandt et al. 2005) also have combined maternally and biparentally inherited markers to better understand movement and gene flow. Given the widespread application of molecular markers to the study of movement in natural populations, it follows that our understanding of *L. nivalis* population boundaries and migration would benefit from a molecular-based approach.

One such beneficial molecular-based approach is through the use of amplified fragment length polymorphism (AFLP) DNA fingerprinting, which is a versatile genome-wide multi-locus molecular method (Meudt and Clarke 2007) that has been used in the study of various animals (Bensch and Åkesson 2005), including bats (Ammerman et al. 2016), rabbits (Lee et al. 2010), and snakes (Strickland et al. 2014). The AFLP method involves a restriction digest of whole genomic DNA, followed by the ligation of known sequence adapters. A two-step series of PCR amplifications follows that takes advantage of the known adapter sequences and incorporates a "selective" component that effectively reduces the number of restriction fragments to a manageable number. The fragments are then visualized via gel-electrophoresis; this produces a unique fingerprint of dominant loci (Vos et al. 1995). In principle, this method is similar to the RAPD technique; however, the use of known sequence adapters in the AFLP method results in a greater reproducibility than the RAPD method (Allendorf and Luikart 2007). The AFLP method has a number of applications and is well suited for this study, as no prior genetic sequence data are required and the level of resolution is appropriate for intra-specific studies, especially when genetic variability is expected to be low, such as with endangered species (Meudt and Clarke 2007).

In addition, mitochondrial DNA sequencing is a well-established molecular tool for examining animal phylogenetics and phylogeography. The high mutation rates (relative to nuclear markers) and maternal transmission make it particularly well suited to study shallow phylogenetics and population genetics (Avise 2000). The mtDNA control region is an exceptionally variable portion of the mitochondrial genome and is often used to study population subdivision within a species (Hartl and Clark 2007). Prior to this study, the genetic data for *L. nivalis* were limited and the only published mtDNA control region sequence on GenBank (accession number U95324) is from Wilkinson et al. (1997). Interestingly, the International Union for the Conservation of Nature conservation action plan for *L. nivalis* (Hutson et al. 2001) reports, "Examination of mitochondrial DNA from animals trapped at migratory stations has demonstrated the presence of discrete populations." However, this statement actually refers to Wilkinson and Fleming's (1996) work on *Leptonycteris curasoae* [=*yerbabuenae*] (Paul Racey, pers. comm.).

The objectives of this study were to measure variation and patterns of subdivision in maternally-inherited mtDNA, particularly addressing the hypothesis of female philopatry, and to compare this with the

bi-parentally inherited AFLP data, so that male and female mediated genetic structure can be contrasted and to provide insights into the migratory patterns of this species. A secondary objective was to infer historical demographics based on patterns of sequence variation.

METHODS

Sample collection and DNA extraction.—*Leptonycteris nivalis* were captured between 5 June and 28 August 2006 using mist-nets placed near the cave entrance or a single harp trap placed at the cave entrance at eight localities spanning the range of *L. nivalis* (Fig. 1). These localities, along with their corresponding code used in this study, are as follows: Cueva del Diablo, Tepoztlán, Morelos (DIA); Emory Cave, Brewster County, Texas (EMO); El Infierno Cave, Laguna de Sanchez, Nuevo Leon (INF); OK Bar Hill, Hidalgo County, New Mexico (NM); Cueva de San Lorenzo, Tehuacan, Puebla (TEH); Grutas de Tziranda, Ciudad Hidalgo, Michoacan (TZR); Grutas de Xoxafi, Xoxafi, Hidalgo (XOX); and Cueva del Durazno, Concepción del Oro, Zacatecas (ZAC). Because *L. nivalis* is an endangered species and the roost sites are sensitive sites, specific geographical coordinates are not provided. Once bats were captured, sex was recorded and a biopsy was taken from the plagiopatagium using a sterile 3 mm biopsy punch (Miltex, Inc., York, Pennsylvania) following the procedure described by Worthington Wilmer and Barratt (1996). Biopsied tissues were stored in either lysis buffer (Longmire et al. 1997) or 95% ethanol; no voucher specimens were collected. After processing, individuals were fed sugar-water solution (10-15% sucrose) and released. These procedures adhered to the guidelines of the American Society of Mammalogists (Gannon et al. 2007). Whole genomic DNA was isolated from wing biopsy tissues using a DNeasy Blood & Tissue Kit (QIAGEN, Inc., Valencia, California) per the manufacturer's protocol, including a single elution in 50 µL of the provided AE buffer.

Mitochondrial control region.—A fragment of approximately 300 base pairs (bp) of the mitochondrial control region (CR) was amplified using two primers known to work in bats, CRP-L, 5'–TCCTACCATCAGCACCCAAAGC–3' and CRF-H, 5'–GTTGCTGG TTTCACGGAGGTAG–3' (Wilkinson and Chapman 1991). Preliminary sequence revealed the presence of multiple nuclear integrations of mitochondrial DNA (numts; data not presented). To avoid numt contamination, a two-step amplification procedure was devised to exploit the understanding that numts occur less frequently at larger sizes (Triant and DeWoody 2007). To reduce the possibility of amplifying nuclear copies of the target CR, a much larger amplicon (~5 kilobases) was amplified, which included the CR, from whole genomic DNA. Primers were designed based on the GenBank sequences of cytochrome-*b* and 12s rRNA genes for *L. yerbabuenae* (accession number AY395814): FRWD (5'–TTTGCCTTCCACTTCCTACTC–3') and REV (5'–CCCTGTCTCTCTTGTCCTTTC–3'). Additionally, Phusion Polymerase (Finnzymes from New England BioLabs, Inc., Ipswich, Massachusetts) was used, which has proofreading capabilities, in place of *Taq* polymerase to reduce the chance of producing erroneous haplotypes (Cline et al. 1996; Kobayashi et al. 1999).

The large fragment amplification was performed in 20 µL reaction volumes with ~20 ng template DNA, 1X HF Buffer (Finnzymes Oy, Finland), 200 µM each dNTP (Fermentas Corp., Glen Burnie, Maryland), 0.5 µM of each of the above long-read primers (Alpha DNA Montreal, Quebec), a final $MgCl_2$ concentration of 2.0 mM, and 1 U Phusion Hot Start DNA Polymerase (Finnzymes) using the following cycling conditions: initial denaturation at 99°C for 3 min, followed by 35 cycles of 99°C for 15 s, 60°C for 30 s, 72°C for 4 min.

From this large (~5 kb) amplicon PCR product, the original 300 bp CR was amplified using the above Wilkinson and Chapman (1991) primers and the following cycling conditions: initial denaturation at 99°C for 3 min, then 33 cycles of 99°C for 20 s, 64.5°C for 15 s, 72°C for 25 s. The PCR conditions for the CR amplicon were the same as for the 5 kb amplicon, except the template for this reaction was 0.5 µL of the 5kb PCR product and a slightly higher final concentration of $MgCl_2$ (2.25 mM) was used. All amplification reactions were carried out in a Bio-Rad MyCycler Thermal Cycler (Bio-Rad Laboratories, Inc. Hercules, California).

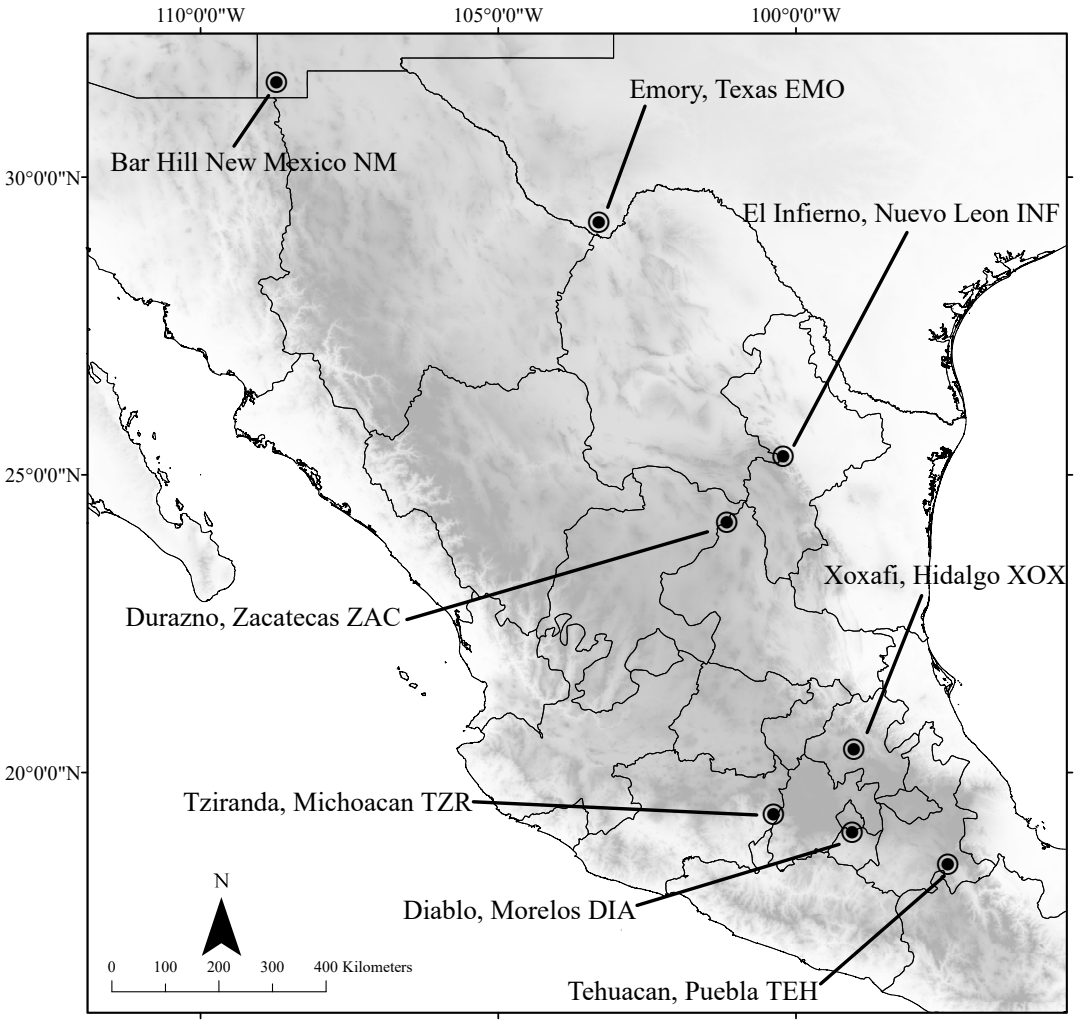

Figure 1. Location of Mexican long-nosed bat (*Leptonycteris nivalis*) sampling sites in the U.S. and Mexico used in genetic analyses. Darker shading indicates higher elevation compared to light shading. Site abbreviations following each line are used in Table 1 and 2.

The PCR products were gel purified using a low-melt agarose (1.0% w/v) containing 0.05% (10µg/mL) ethidium bromide and a Gel-prep PCR Wizard kit (Promega Corp., Madison, Wisconsin). In preparation for cloning with the TOPO TA PCR 2.1 Vector Cloning Kit (Invitrogen Corp., Carlsbad, California), an adenine overhang was added to the blunt-end PCR amplicon in a 5 µL reaction containing 50-100 ng of purified PCR product, 4 mM dATP (Fermentas Corp.), 1X Standard *Taq* Reaction Buffer (New England BioLabs, Inc.), and 0.5 U *Taq* DNA polymerase (New England BioLabs, Inc.). Purified PCR products were cloned using the TOPO TA Cloning Kit per the manufacturer's protocol (the salt solution, which is provided as an optional reagent, was used in the ligation reactions along with an extended ligation time of 30 min.) (Invitrogen Corp.). Plasmids containing PCR product inserts were extracted and purified following the protocol of the QIAprep Spin Miniprep Kit (QIAGEN, Inc., Valencia, California). The target CR insert was amplified and sequenced using the Thermo Sequenase Cycle Sequencing Kit (USB, Corp., Cleveland, Ohio) and a NEN Global IR2 sequencing system (LI-COR Corp., Lincoln, Nebraska). Sequences for the control region fragment that was analyzed were submitted to GenBank (accession numbers MK572876–MK572947).

Amplified fragment length polymorphism.—The original AFLP procedure as described by Vos et al. (1995) was followed. However, based on the suggestion of Vos and Kuiper (1997) the restriction enzyme *Taq*I was used instead of *Mse*I. Whole genomic DNA (> 50 ng/µL) samples were first digested with restriction enzymes in a two-step digestion procedure (all restriction digest reagents from New England BioLabs, Inc.). In 20 µL reactions, 200 ng of whole genomic DNA was combined with 20 U *Eco*RI enzyme, 1X NEBuffer 3, and 1X Bovine Serum Albumin and incubated at 37ºC for 3 hrs. Then, 20 U *Taq*I were added to the reaction and digestion proceeded for 3 hrs at 65ºC.

After restriction digest, the double stranded oligonucleotide adapters (Alpha DNA, Montreal, Quebec) were ligated to their complementary restriction sites as described in Brown (2008). Ligation reactions were carried out by adding Ligase Buffer (1X final concentration) (Promega Corp., Madison, Wisconsin), 75 pmol of the *Eco*RI adapter, 175 pmol of the *Taq*I adapter, and 3 U T4 DNA Ligase (Promega Corp.) directly to the restriction digestion reaction product. The ligation reaction was incubated for 13 hrs at 16ºC.

The ligation reactions were then diluted 1/8 in 10 mM Tris-HCl in preparation for the first of two rounds of PCR amplification. The first amplification, termed preselective amplification, was accomplished using the following preselective primers: *Eco*RI-C, 5'–CTGCGTACCAATTCC–3' and *Taq*I-T, 5'–ATGAGTCCTGACCGAT–3' (Alpha DNA). Preselective amplification was carried out in 50 µL reaction volumes, including 10 µL of diluted ligation reaction product as template, 1X Standard *Taq* Reaction Buffer (New England BioLabs, Inc.), 200 µM each dNTP (Fermentas Corp.), 0.15 µM of the *Eco*RI-C primer and 0.2 µM of the *Taq*I-C primer, a final $MgCl_2$ concentration of 1.5 mM, and 2.5 U *Taq* DNA Polymerase (New England BioLabs, Inc.) using the following cycling conditions: initial denaturation at 72°C for 1 min, followed by 20 cycles of 94°C for 50 s, 56°C for 60 s, 72°C for 2 min.

Preselective amplification products were diluted 1/10 in 10 mM Tris-HCl in preparation for the second amplification step, selective amplification. Selective amplification was accomplished with a fluorescently labeled *Eco*RI-CNN selective primer (either *Eco*RI-CAC or *Eco*RI-CAT) (Sigma-Proligo Corp., The Woodlands, Texas) used in combination with one of five *Taq*I-TNN selective primers (Alpha DNA) (Brown 2008). PCR conditions for the selective amplification were identical to the preselective amplification, except the template for the selective amplification was 5 µL of diluted preselective PCR product and primer concentrations were 0.12 µM for both *Eco*RI-CNN and *Taq*I-TNN selective primers. The following touchdown cycling protocol was used: 35 cycles of 94°C for 50 s, 65-56.6°C (0.7°C reduction for 2nd through 13th cycle) for 60 s, 72°C for 120 s.

The incorporation of the fluorescently labeled *Eco*RI selective primers in the selective amplification reactions allowed the detection of fragments using a Beckman-Coulter CEQ8000 Automated DNA Analysis System (Beckman-Coulter, Inc., Fullerton, California). Beckman-Coulter software was used to size fragments using an internal size standard. Fragment profiles were scored manually for each individual. Only fragments that could be scored unambiguously as present or absent were included in the data set, which consisted of a binary data matrix; fragments were either present (1) or absent (0) for each individual for each fragment size. Replicates were amplified and analyzed for ~1/4 of the samples and confirmed the repeatability of this protocol.

Statistical analyses.—Complementary mitochondrial CR sequences were aligned and trimmed using Sequencher 4.7 (Gene Codes Corp.) to form a consensus sequence for each individual. These sequences were aligned using ClustalW (Thompson et al. 1994). PAUP* version 4.0b10 (Swofford 2002) was used to convert the alignment into various formats for analyses. The best-fitting among 88 models of evolution was determined using jModelTest version 2.1.7 (Darriba et al. 2012). DnaSP version 5.10.01 (Librado and Rozas 2009) was used to analyze the mtDNA data by calculating standard measures of sequence diversity (number of segregating sites, haplotype diversity, nucleotide diversity (π), and Watterson's θ_W). Neutrality tests, including Tajima's (1989) *D*, Fu's (1997) F_S, and Ramos-Onsins and Rozas' (2002) R_2 were used as well as a mismatch distribution analysis (Rogers and Harpending 1992; Rogers et al. 1996) to infer historical population demographic trends. An analysis of molecular variance (AMOVA; Excoffier et al. 1992) was used in the GenAlEx software package to test hypothesized patterns

of population structure that were proposed in the *L. nivalis* recovery plan (U.S. Fish and Wildlife Service 1994); specifically, considering our sampling sites and sizes, the samples were partitioned into three regions consisting of (1) NM and EMO, (2) ZAC and INF, and (3) TZR, XOX, DIA, and TEH. Based on the results of the jModelTest analysis, a Tamura model of evolution with $\alpha = 0.711$ was specified. Statistical significance for the AMOVA was determined using 16,000 iterations of a permutation test. A median-joining haplotype network was generated using POPART (Leigh and Bryant 2015) to determine if haplogroups corresponded to geographic sampling.

The AFLP data were tested for patterns of genetic structure in several ways. Levels of structure and patterns of differentiation among sampling localities were visualized using principal coordinates analysis (PCoA) in GenAlEx, version 6 (Peakall and Smouse 2006, 2012). For this dataset, an analysis of molecular variance (AMOVA; Excoffier et al. 1992) was conducted in the GenAlEx software package to test hypothesized patterns of population structure with regions defined as specified for the mtDNA data. Differences within and among regions were quantified using a simple pairwise difference measure. Statistical significance for the AMOVA was determined using 16,000 iterations of a permutation test. Population structure was further assessed using the Bayesian assignment test in STRUCTURE version 2.3.4 (Pritchard et al. 2000, Falush et al. 2007). The admixture model with correlated allele frequencies was used for this analysis; AFLP band presence was specified as the dominant allele for all loci. A burn-in of 100,000 steps was used followed by a Markov chain Monte Carlo (MCMC) search of 900,000 steps. All 159 samples were included in our main implementation of STRUCTURE, which considered K values from 1 to 8 and included 10 replicate analyses per K value. Because the STRUCTURE algorithm has been shown to be sensitive to uneven sample sizes (Puechmaille 2016), a second analysis was performed in which sites with larger sample sizes (DIA) were randomly subsampled to a sample size of 10 and sites with small sample sizes (NM and XOX) were excluded from the analysis. For this second implementation of STRUCTURE, K values from 1 to 6 were evaluated; other settings were as described for the full analysis. In all cases, the best-supported value of K was evaluated using Puechmaille's (2016) MaxMeanK with a threshold assignment value of 0.5.

RESULTS

Mitochondrial control region.—A 300 bp region of the mitochondrial CR was sequenced for 72 individuals spanning the geographic range of *L. nivalis*. Sites were not represented equally, as sample sizes ranged from 2 to 22 (NM = 3; EMO = 21; INF = 21; XOX = 4; TEH = 6; DIA = 8; TZR = 7; and ZAC = 2). These sequences were trimmed to 288 bp for analysis to remove ambiguous base calls at the ends of the sequences. Alignment of sequences revealed 27 variable sites (*S*) conforming to the infinite sites mutation model, with no insertions or deletions (Table 1). An invariable 73 bp region was observed from position 7 to 80. Nucleotide diversity (π) was 0.013 ($\pm$ 0.001 *SD*) and theta (θ_W) per site was 0.019. Average uncorrected pairwise sequence divergence within *L. nivalis* was 1.28% ($\pm$ 0.95% *SD*) with a maximum of 3.47%.

Twenty-nine haplotypes were recorded; 19 of these were found only in a single specimen. Haplotype diversity was relatively high ($H = 0.81 \pm 0.05$ *SD*). One haplotype (H1) was abundant and widely encountered with 43% of individuals sharing this haplotype at 6 of the 8 sampling sites. The two sites where this haplotype was not recorded (ZAC and NM) had low sample sizes ($n = 2$ and 3, respectively; Table 1, Fig. 2). Our sequence designated H1 was identical to an unpublished sequence in GenBank for *L. nivalis* (accession GU473259). The next most common haplotype (H4) was shared among five individuals at three sites.

No geographic patterns or patterns of philopatry were revealed in the median-joining haplotype network (Fig. 2). The haplotype network displayed two weakly formed haplogroups. At the center of each of these groups was a central haplotype (H1 and H21) from which other haplotypes are connected via one or two mutational steps (Fig. 2). The second (H4) and third (H25) most common haplotypes were both

Table 1. Variable nucleotide positions for all unique *Leptonycteris nivalis* haplotypes sampled at each site (abbreviations in Fig. 1). Dots indicate identical nucleotide to that found in H1. Numbers in parentheses indicate number of individuals with identical haplotypes. Complete sequences are deposited in GenBank (accession numbers MK572876–MK572947).

Haplotype	Site	Sequence* at variable sites†
H1	INF (10), EMO (10), DIA (3), TZR(2), TEH (4), XOX (2)	TTTTAAAGAATCTTTACGGGGTCTCCA
H2	NM	.....G.....
H3	INF	.....T..
H4	EMO (3), INF, ZAC	CC.C.....C.....A.T..T.
H5	DIA, TEH	.....G.....
H6	TZR	C.....C.....
H7	EMO	.....A.....C.....
H8	EMO	...C.....C.....T....
H9	INF, DIA	.....T....
H10	INF	...C.....T.TT.
H11	INF, EMO	C.....C.....CT..TG
H12	NM (2)	C..C.....C.....T..TG
H13	INF	...C.....C.....CT..TG
H14	TZR	C..C.....C.....T.TTG
H15	INF, TZR	C.CC.....C.....T..TG
H16	INF	C.....G.....C.....T..TG
H17	TZR	C.....C.....TC.TG
H18	TEH	C.....C...A....T..TG
H19	XOX	...C.....T.....
H20	INF, DIA	.....C.....
H21	DIA	C.....C.....T..TG
H22	XOX	C.....C....A...T..TG
H23	TZR	C.....C.....A..T.TTG
H24	EMO	.....T.TT.
H25	EMO (2), ZAC	C..C....G..T.....
H26	INF	...C.....T....
H27	EMO	....G.....T.
H28	INF	.....G.....CT..TG
H29	EMO, DIA	...C.....

*H1 complete sequence: CTGCTTATGTTATTCGTGCATAAATCTTATTACCCCATATTAATGAATGACATATATGTATA-ATAGTACATTATATTATGTACCTCATGAATATTAAGCAAGTAATTTAATTCAATGTATGAATGATATATTATTATATAT-TAACAGTCAAAATTATATCAATATGGATAATTCATTAATGATTATATATGATTAATCAGCTGTAGGACATAATATTTAT-TAATCGTGCATAGACCATTCCATTATATTTAATTCTTATCAATACGCCTATCCCCCACCAAAGGGTGT

†Variable sites: 5, 6, 81, 85, 90, 104, 119, 121, 122, 123, 142, 149, 165, 176, 177, 185, 198, 200, 203, 206, 233, 239, 241, 254, 267, 276, and 277.

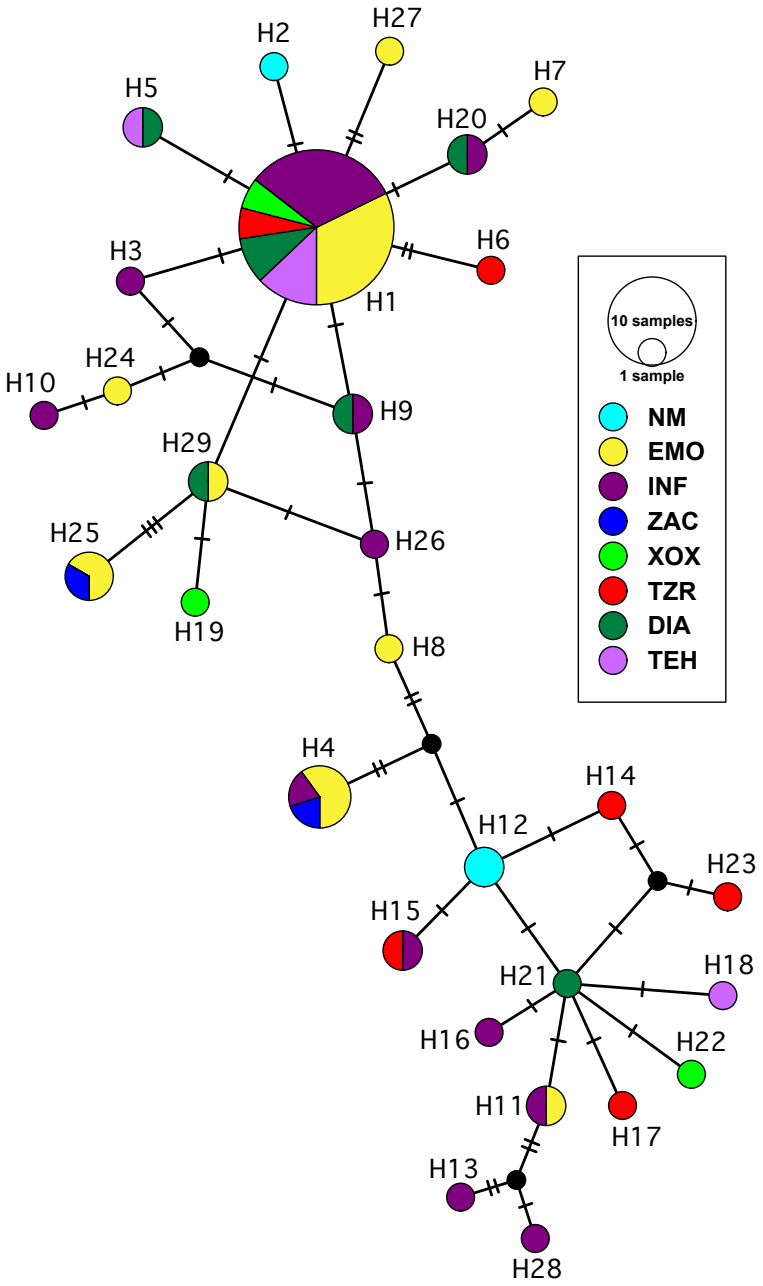

Figure 2. Median-joining network of *Leptonycteris nivalis* mtDNA control region sequence data. Haplotypes are represented by circles. The area of each circle is proportional to the haplotype frequencies, and the proportion of each site recorded is represented by the shaded areas. Site abbreviations: DIA = Diablo, Morelos; EMO = Emory, Texas; INF = El Infierno, Nuevo Leon; NM = Bar Hill, New Mexico; TEH = Tehuacan, Puebla; TZR = Tziranda, Michoacan; XOX = Xoxafi, Hidalgo; and ZAC = Durazno, Zacatecas. Tick marks on branches represent number of mutations required to connect haplotypes. The small nodes in black represent hypothetical haplotypes.

disconnected from these central haplotypes by at least four mutations (Fig. 2). No haplotypes differed from their nearest neighbor by more than three mutational steps. The AMOVA revealed no significant structure either among regions (Φ_{CT} = -0.078; P = 0.965), among sampling sites within regions (Φ_{SC} = 0.099; P = 0.063), or within sites (Φ_{ST} = 0.029; P = 0.124).

Patterns of mtDNA control region sequence variation were conflicted as to signals of a historic expansion. Whereas neither Tajima's (1989) D (D = -1.017; P = 0.141) nor Ramos-Onsins and Rozas' (2002) R_2 (R_2 = 0.065; P = 0.156) were significant, Fu's (1997) F_S (F_S = -17.344, P < 0.00001) was significantly negative, as is expected with population growth. The mismatch distribution pattern of pairwise differences (Fig. 3) resembled that expected for a population having experienced expansion (Rogers and Harpending 1992; Rogers et al. 1996), but this distribution was not significantly different (raggedness index r = 0.031, P = 0.140; SSD = 0.020, P = 0.410) from the null hypothesis of a constant population size, and confidence intervals for pre-expansion population size (θ_0 = 0.002, 95% CI = 0.000-5.015) and post-expansion population size (θ_1 = 6.057, 95% CI = 3.294-321.684) overlapped considerably.

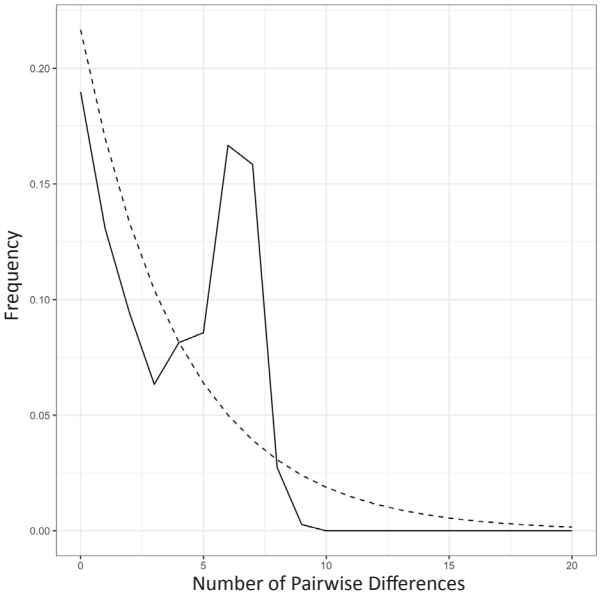

Figure 3. Mismatch distribution of pairwise differences among *Leptonycteris nivalis* mtDNA sequences. The dashed line indicates the expected distribution of pairwise differences for a constant size population. The solid line represents the distribution of pairwise differences observed for *Leptonycteris nivalis*.

Amplified fragment length polymorphism.— AFLP data were produced for 74 individuals (Table 2). Five primer pairs (*Eco*RI-CAC & *Taq*I-TGC; *Eco*RI-CAC & *Taq*I-TCG; *Eco*RI- CAC & *Taq*I-TCA; *Eco*RI-CAC & *Taq*I-TAC; and *Eco*RI-CAT & *Taq*I-TAC) generated data on 159 loci, of which 47.2% were polymorphic overall. Polymorphism ranged from 13.8 to 31.5% across sampling sites (Table 2). A PCoA (n = 74) revealed extensive overlap of each geographic group and a low percentage of the variation was explained (Fig. 4). An AMOVA revealed no significant partitioning of genetic differentiation among regions (Φ_{CT} = 0.015, 2% of total variance; P = 0.071) or among sites within regions (Φ_{SC} = 0.010, 1% of total variance; P = 0.223). Results from STRUCTURE analyses of the subsampled dataset did not differ from those of the full dataset, thus, results from the full dataset are presented here. Evaluations of K in almost all cases showed very high assignment probabilities of all individuals to one cluster (Fig. 5), and MeanMaxK universally supported a panmictic model with K = 1.

Table 2. Percentage of polymorphism for *Leptonycteris nivalis* individuals at each site based on 159 AFLP loci. Site abbreviations: DIA = Diablo, Morelos; EMO = Emory, Texas; INF = El Infierno, Nuevo Leon; NM = Bar Hill, New Mexico; TEH = Tehuacan, Puebla; TZR = Tziranda, Michoacan; XOX = Xoxafi, Hidalgo; and ZAC = Durazno, Zacatecas.

Site	% Polymorphism	Sample size
DIA	31.5	19
EMO	30.8	11
INF	28.9	11
NM	26.4	3
TEH	23.9	8
TZR	26.4	9
XOX	13.8	3
ZAC	24.5	10
	Mean: 25.7	Total: 74

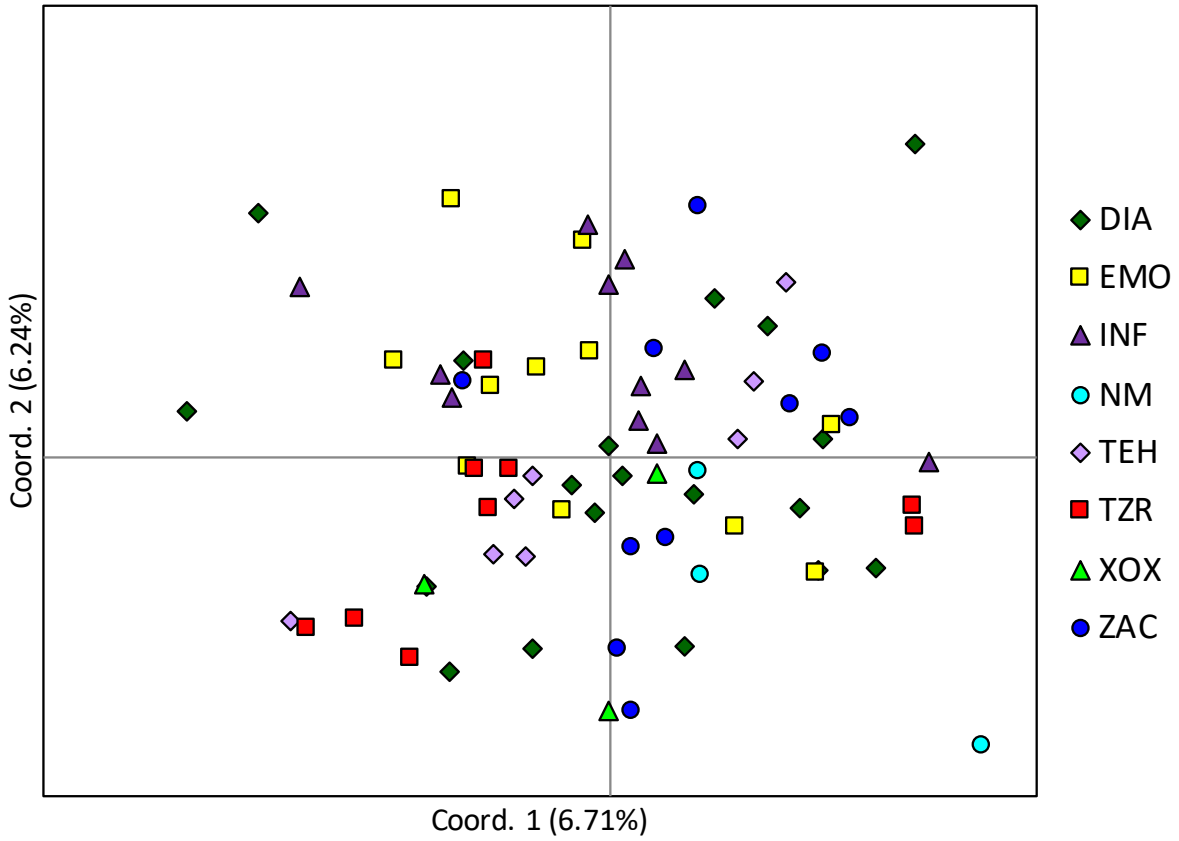

Figure 4. Principal coordinates analysis of amplified fragment length polymorphism data for Leptonycteris nivalis individuals from eight sites. Site abbreviations: DIA = Diablo, Morelos; EMO = Emory, Texas; INF = El Infierno, Nuevo Leon; NM = Bar Hill, New Mexico; TEH = Tehuacan, Puebla; TZR = Tziranda, Michoacan; XOX = Xoxafi, Hidalgo; and ZAC = Durazno, Zacatecas.

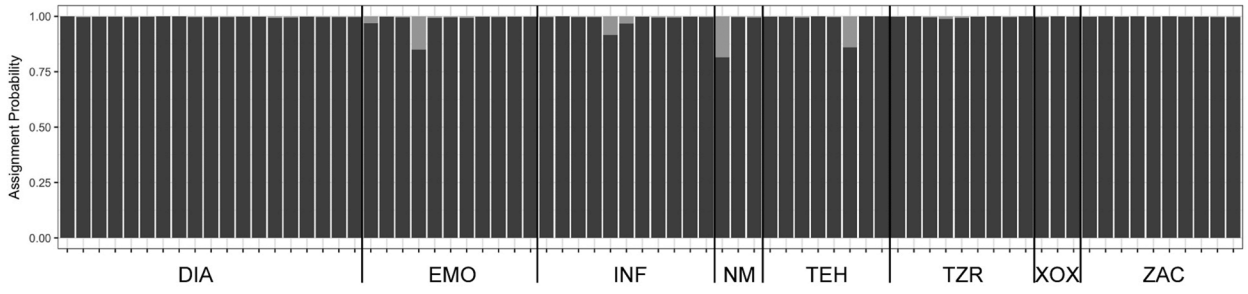

Figure 5. Representative STRUCTURE plot from an analysis testing $K = 2$. Each individual is represented by a column, grouped by sample site, with the probability of assignment to cluster 1 (dark grey) and cluster 2 (light grey). All individuals are assigned to the same cluster with a probability ≥ 0.85, indicating support for only a single genetic cluster. Site abbreviations: DIA = Diablo, Morelos; EMO = Emory, Texas; INF = El Infierno, Nuevo Leon; NM = Bar Hill, New Mexico; TEH = Tehuacan, Puebla; TZR = Tziranda, Michoacan; XOX = Xoxafi, Hidalgo; and ZAC = Durazno, Zacatecas.

DISCUSSION

Genetic diversity.—*Leptonycteris nivalis* has relatively low to moderate levels of genetic diversity for a migratory bat, but not alarmingly low as might be expected for a species thought to have undergone a recent and severe population decline. The estimated nucleotide diversity (π) of 1.3% in our mtDNA data was comparable to that recovered by Wilkinson and Fleming (1996) in *L. yerbabuenae* (1.6%), a species that is more common than *L. nivalis* (Arita and Humphrey 1988) and recently has been downlisted from endangered (U.S. Fish and Wildlife Service 2018). A conserved region of 73 bases was unique to *L. nivalis*, as *L. yerbabuenae* (Wilkinson and Fleming 1996) and *L. curasoae* (Newton et al. 2003) samples contained variable sites within the control region. The noctule bat (*Nyctalus noctula*; Petit and Mayer 2000), a widespread migratory species of Britain, had π values of 0.9-1.3%. Our estimates of diversity values for *L. nivalis* (π = 1.3%; h = 0.81) were higher than those of the endangered Ozark big-eared bat (*Corynorhinus townsendii ingens*; Weyandt et al. 2005) with π = 0.0-0.8% and h = 0.0-0.47, and were much lower than those of very abundant species such as the Mexican free-tailed bat (*Tadarida brasiliensis mexicana*; Russell et al. 2005) with π = 4.5% and h = 0.998 and the threatened greater mouse-eared bat (*Myotis myotis*; Castella et al. 2001) with π = 7.1% and h = 0.45. Genetic diversity values, however, are influenced by species-specific historical demography and evolutionary processes, as well as ecological factors, current population sizes, and even sampling methodologies (Allendorf and Luikart 2007), making it difficult to draw strong conclusions from inter-study comparisons of diversity values.

Migratory movements.—That female *L. nivalis* exhibit philopatry, returning year after year to their natal roost, either Emory Cave or El Infierno Cave, was thought to be a likely scenario based on observations suggesting that parturition is occurring at both Emory Cave (Ammerman, pers. obs.) and El Infierno Cave (Moreno-Valdez et al. 2004), as well as the lack of observed movement of banded or tagged individuals between these caves. However, the lack of geographically-based partitioning in the network analysis of mtDNA haplotypes and low estimates of Φ-statistics from these data suggest that *L. nivalis* does not exhibit female philopatry (Fig. 2). An alternative scenario that is consistent with the data is that a single colony of reproductive female *L. nivalis* migrate north each summer, first arriving at El Infierno Cave where parturition occurs for the majority of individuals. A minority of early-term females might continue northward to Emory Cave to give birth. Possibly, these females are joined later in the season (June-July) by the adult females and their newly volant young from El Infierno. If true, individuals at Emory Cave might then move to the roost in New Mexico and continue southward after the peak of agave blooms in New Mexico. Currently, data on arrival times at caves is scarce, variable from year to year, and rarely available for more than one cave in a single year. Such a circuitous migratory route should be tested through a large-scale bi-national survey conducted at the major maternity roosts, i.e., El Infierno, Emory Cave, and New Mexico. This would need to be conducted in the same year and span the earliest documented arrivals and latest documented departures at each cave.

Genetic structure.—In addition to a lack of population subdivision in mtDNA at the northern maternity colonies, a complete lack of AFLP subdivision (AFLP AMOVA and STRUCTURE analyses) was observed throughout their range. The lack of genetic structure in the AFLP data among regions (Φ_{CT} = 0.015) and among sites within regions (Φ_{SC} = 0.010) in *L. nivalis* is in striking contrast to the overall genetic structure (Φ_{ST} = 0.608) observed in *L. yerbabuenae* (Morales-Garza et al. 2007) based on RAPDs. The evolutionary ecological question of why these two congeners with similar distributions have such dissimilar patterns of population structure is interesting. The obvious ecological difference between these two species of *Leptonycteris* is the typical elevation they inhabit. *L. nivalis* is a denizen of the mountains whereas *L. yerbabuenae* inhabits the lowlands (Baker and Cockrum 1966; Arita 1991). This difference in elevation might be related to differences in resource availability. Furthermore, *L. nivalis* is able to facultatively compensate their energy intake using nectar with a sugar concentration as low as 5%, whereas *L. yerbabuenae* enters a physiological deficit when ingesting nectar concentrations below 15% (Ayala-Berdon et al. 2013). This suggests that *L. nivalis* is adapted to

colder environments where plants tend to produce less concentrated nectar (Ayala-Berdon et al. 2013). The among-site genetic differentiation that Morales-Garza et al. (2007) observed in *L. yerbabuenae* was caused by the presence of a migratory population in the north and a non-migratory population in the south. Morales-Garza et al. (2007) suggested that the population boundary observed in *L. yerbabuenae* was, in effect, the result of abundant food resources in the western corridor; that is, northern migrants do not return to the southern extent of the range because year-round food resources are available further north. Because *L. nivalis* occurs at higher elevations, it could be that these same food resource patterns do not occur, and all *L. nivalis* return to central Mexico to winter and breed. Currently, Cueva del Diablo is the only cave where breeding has been observed in *L. nivalis* (Medellín, pers. obs.). The lack of subdivision observed in our data suggests that Cueva del Diablo might actually be the only known breeding site for this species (but see Torres-Knoop 2014 for further discussion on potential additional mating roosts in the southern portion of the range).

The lack of subdivision in our AFLP data is in keeping with the pattern observed in many migratory bat species (Moussy et al. 2013) and, in the case of *L. nivalis*, is likely due to concurrent mating in a small number of winter roosts followed by some unknown level of migratory coherence among females during their movements to maternity colonies. In this way, *L. nivalis* is similar to species such as *Tadarida brasiliensis* (Russell et al. 2005) and *Lasiurus borealis* (Korstian et al. 2015; Vonhof and Russell 2015) that are migratory and lack detectable population structure. Although Morales-Garza et al. (2007) found significant substructuring in *Leptonycteris yerbabuenae* using RAPD markers, later analyses of this same species with microsatellite data found only weak genetic differentiation (Ramirez 2011; Arteaga et al. 2018) similar to that recovered in this study for *L. nivalis*. In Venezuela, *Leptonycteris curasoae*, a close relative to *L. nivalis*, was found to have low F_{ST} values and low genetic differentiation compared to *Glossophaga longirostris* (Newton et al. 2003), a difference that was attributed to the higher dispersal capabilities of *Leptonycteris*. Overall, the high level of genetic connectivity inferred across the range of *L. nivalis* is attributed to the gene flow occurring at mating roosts in the southern part of their range, a pattern that also has been seen in *Myotis lucifugus* (Burns et al. 2014), a temperate species that forms mixed-sex swarms and engages in mating behavior at a small number of hibernation sites.

Historic demographics.—The high mtDNA haplotype diversity and high proportion of singleton haplotypes (66%), combined with a lack of nuclear and mitochondrial genetic structure, suggests that *L. nivalis* has experienced a historical population expansion. The hypothesis of historical expansion in *L. nivalis* also is indicated in the mtDNA data by the star-like shape of subsets of the median joining network (Fig. 2), and the significantly negative value of Fu's F_S. However, this hypothesis of historical growth is thrown into question by results from other neutrality tests (Tajima's D and R_2) and the mismatch distribution. Although the mismatch distribution was not significantly ragged (Fig. 3) and estimates of pre- and post-expansion population sizes were not significantly different, the shape of the distribution of observed differences suggests the single peak expected of a population having experienced expansion. Because mutation rates are known to vary widely in bats (Nabholz et al. 2007), because no mutation rates have been determined for *L. nivalis* or closely related species, and because generation times are not known for *L. nivalis*, neither effective population sizes nor dates of expansion were estimated from our data. However, the large number of singleton haplotypes suggests that a population expansion, if it occurred, was not recent and its signal may be fading from these genetic data. To strengthen these conclusions, it is recommended that codominant data, such as microsatellites or SNPs, be used to test more complex demographic hypotheses that include a potential historical expansion as well as a recent population bottleneck, as has been suggested by census records.

In summary, the findings of this study suggest that *L. nivalis* constitutes a single panmictic population with a moderate level of genetic diversity. The absence of female- or male-mediated population genetic subdivision in *L. nivalis* provides evidence against the hypothesis of female philopatry. Several lines of evidence support the idea that *L. nivalis* has undergone a historic population expansion. These analyses may appear to contradict the idea that *L. nivalis* has experienced a recent decline; however, mutation is a slow process and even relatively quickly-evolving markers such as microsatellites can be slow to respond to recent reductions

in population size (Dinerstein and McCracken 1990; Munster 2015). Similar patterns have been observed in the little brown bat (*Myotis lucifugus*), a species that has a genetic signature of recent population expansion (following Pleistocene glaciation) but has experienced drastic declines in the past decade due to white-nose syndrome (Burns et al. 2014). Although a confirmation or rejection of the hypothesized recent population decline could not be made based on these data, it is evident that current levels of genetic diversity are not alarmingly low. Nevertheless, this species seems to face greater extinction risk than *L. yerbabuenae*, and further studies should proceed with care so as not to negatively affect this sensitive species.

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Literature Cited

Adams, E. R. 2015. Seasonal and nightly activity of Mexican long-nosed bats (*Leptonycteris nivalis*) in Big Bend National Park, Texas. M.S. Thesis, Angelo State University, San Angelo, Texas.

Allen, G. M. 1939. Bats. Dover Publications, New York.

Allendorf, F. W., and G. Luikart. 2007. Conservation and the genetics of populations. Blackwell Publishing, Malden, Massachusetts.

Ammerman, L. K., D. N. Lee, and R. S. Pfau. 2016. Patterns of genetic divergence among *Myotis californicus*, *M. ciliolabrum*, and *M. leibii* based on amplified fragment length polymorphism. Acta Chiropterologica 18:337–347.

Arita, H. T. 1991. Spatial segregation in long-nosed bats, *Leptonycteris nivalis* and *Leptonycteris curasoae*, in Mexico. Journal of Mammalogy 72:706–714.

Arita, H. T. and S. R. Humphrey. 1988. Revisión taxonómica de los murciélagos magueyeros del género *Leptonycteris* (Chiroptera: Phyllostomidae). Acta Zoológica Mexicana 29:1–60.

Arnold, B. D. and G. S. Wilkinson. 2015. Female natal philopatry and gene flow between divergent clades of pallid bats (*Antrozous pallidus*). Journal of Mammalogy 96:531–540.

Arteaga, M. C., R. A. Medellín, P. A. Luna-Ortíz, P. A. Heady III, and W. F. Frick. 2018. Genetic diversity distribution among seasonal colonies of a nectar-feeding bat (*Leptonycteris yerbabuenae*) in the Baja California Peninsula. Mammalian Biology 92:78–85.

Avise, J. C. 2000. Phylogeography: the history and formation of species. Harvard University Press, Cambridge, Massachusetts.

Ayala-Berdon, J., R. Galicia, C. Flores-Ortíz, R. A. Medellín, and J. E. Schondube. 2013. Digestive capacities allow the Mexican long-nosed bat (*Leptonycteris nivalis*) to live in cold environments. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 164:622–628. doi:10.1016/j.cbpa.2013.01.015

Baker, R. J., and E. L. Cockrum. 1966. Geographic and ecological range of the long-nosed bats, *Leptonycteris*. Journal of Mammalogy 47:329–331.

Barbour, R. W., and W. H. Davis. 1969. Bats of America. University Press of Kentucky, Lexington.

Bensch, S., and M. Åkesson. 2005. Ten years of AFLP in ecology and evolution: why so few animals? Molecular Ecology 14:2899–2914.

Borrell, A. E., and M. D. Bryant. 1942. Mammals of the Big Bend area of Texas. University of California Publications in Zoology 48:1–62.

Brown, C. M. 2008. Natural history and population genetics of the endangered Mexican long-nosed bat, *Leptonycteris nivalis* (Chiroptera:Phyllostomidae). M.S. thesis, Angelo State University, San Angelo, Texas.

Burland, T. M., and J. Worthington Wilmer. 2001. Seeing in the dark: molecular approaches to the study of bat populations. Biological Reviews 76:389–409.

Burns, L. E., T. R. Frasier, and H. G. Broders. 2014. Genetic connectivity among swarming sites in the wide ranging and recently declining little brown bat (*Myotis lucifugus*). Ecology and Evolution 4:4130–4149.

Castella, V., M. Ruedi, and L. Excoffier. 2001. Contrasted patterns of mitochondrial and nuclear structure among nursery colonies of the bat *Myotis myotis*. Journal of Evolutionary Biology 14:708–720.

Cline, J., J. C. Braman, and H. H. Hogrefe. 1996. PCR fidelity of *Pfu* DNA polymerase and other thermostable DNA polymerases. Nucleic Acids Research 24:3546–3551.

Cockrum, E. L., and Y. Petryszyn. 1991. The long-nosed bat, *Leptonycteris*: an endangered species in the Southwest? Occasional Papers, The Museum, Texas Tech University 142:1–32.

Darriba, D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9:772.

Dinerstein, E., and G. F. McCracken. 1990. Endangered greater one-horned rhinoceros carry high levels of genetic variation. Conservation Biology 4:417–422.

Easterla, D. A. 1972. Status of *Leptonycteris nivalis* (Phyllostomidae) in Big Bend National Park, Texas. The Southwestern Naturalist 17:287–292.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Falush, D., M. Stephens, and J. K. Pritchard. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology Notes 7:574–578.

Fu, Y.-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking, and background selection. Genetics 147:915–925.

Gannon, W. L., R. S. Sikes, and the Animal Care and Use Committee of the American Society of Mammalogists. 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 88:809–823.

Hartl, L. D., and A. G. Clark. 2007. Principles of population genetics. Sinauer Associates, Inc., Sunderland, Massachusetts.

Hutson, A. M., S. P. Mickleburgh, and P. A. Racey (Compil.). 2001. Microchiropteran bats: global status survey and conservation action plan. IUCN/SSC Chiroptera Specialist Group. IUCN, Cambridge, United Kingdom.

Kobayashi, N., K. Tamura, and T. Aotsuka. 1999. PCR error and molecular population genetics. Biochemical Genetics 37:317–321.

Korstian, J. M., A. M. Hale, and D. A. Williams. 2015. Genetic diversity, historic population size, and population structure in 2 North American tree bats. Journal of Mammalogy 96:972–980.

Kurta, A., and S. W. Murray. 2002. Philopatry and migration of banded Indiana bats (*Myotis sodalis*) and effects of radio transmitters. Journal of Mammalogy 83:585–589.

Lee, D. N., R. S. Pfau, and L. K. Ammerman. 2010. Taxonomic status of the Davis Mountains cottontail, *Sylvilagus robustus*, revealed by amplified fragment length polymorphism. Journal of Mammalogy 91:1472–1483.

Leigh, J. W., and D. Bryant. 2015. POPART: full-feature software for haplotype network construction. Methods in Ecology and Evolution 6:1110–1116.

Librado, P., and J. Rozas. 2009. DnaSp v.5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452.

Longmire, J. L., M. Maltbie, and R. J. Baker. 1997. Use of "lysis buffer" in DNA isolation and its implications for museum collections. Occasional Papers, Museum of Texas Tech University 163:1–3.

Meudt, H. M., and A. C. Clarke. 2007. Almost forgotten or latest practice? AFLP applications, analyses and advances. Trends in Plant Sciences 12:1360–1385.

Morales-Garza, M. R., M. del C. Arizmendi, J. E. Campos, M. Martinez-Garcia, and A. Valiente-Banuet. 2007. Evidences on the migratory movements of the nectar-feeding bat *Leptonycteris curasoae* in Mexico using random amplified polymorphic DNA (RAPD). Journal of Arid Environments 68:248–259.

Moreno-Valdez, A., R. L. Honeycutt, and W. E. Grant. 2004. Colony dynamics of *Leptonycteris nivalis* (Mexican long-nosed bat) related to flowering *Agave* in northern Mexico. Journal of Mammalogy 85:453–459.

Moussy, C., D. J. Hosken, F. Mathews, G. C. Smith, J. N. Aegerter, and S. Bearhop. 2013. Migration and dispersal patterns of bats and their influence on genetic structure. Mammal Review 43:183–195.

Munster, S. K. 2015. Evaluating accuracy and bias in genetic monitoring under scenarios of population decline. M.S.

thesis, Grand Valley State University, Allendale, Michigan.

Nabholz, B., S. Glemin, and N. Galtier. 2007. Strong variations of mitochondrial mutation rates across mammals—the longevity hypothesis. Molecular Biology and Evolution 25:120–130.

Newton, L. R., J. M. Nassar, and T. H. Fleming. 2003. Genetic population structure and mobility of two nectar-feeding bats from Venezuelan deserts: inferences from mitochondrial DNA. Molecular Ecology 12:3191–3198.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295.

Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537–2539.

Petit, E., and F. Mayer. 2000. A population genetic analysis of migration: the case of the noctule bat (*Nyctalus noctula*). Molecular Ecology 9:683–690.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Puechmaille, S. J. 2016. The program STRUCTURE does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. Molecular Ecology Resources 16:608–627.

Ramirez, J. 2011. Population genetic structure of the lesser long-nosed bat (*Leptonycteris yerbabuenae*) in Arizona and Mexico. M.S. thesis, University of Arizona, Tucson.

Ramos-Onsins, S. E., and J. Rozas. 2002. Statistical properties of new neutrality tests against population growth. Molecular Biology and Evolution 19:2092–2100.

Rivers, N. M., R. K. Butlin, and J. D. Altringham. 2006. Autumn swarming behaviour of Natterer's bats in the UK: Population size, catchment area and dispersal. Biological Conservation 127:215–226.

Rogers, A. R., A. E. Fraley, M. J. Bamshad, W. S. Watkins, and L. B. Jorde. 1996. Mitochondrial mismatch analysis is insensitive to the mutational process. Molecular Biology and Evolution 13:895–902.

Rogers, A. R., and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9:552–569.

Ruedi, M., and G. F. McCracken. 2009. Phylogeographic analysis of bats. Pp. 739–756 in Ecological and behavioral methods for the study of bats. (T. H. Kunz and S. Parsons, eds.). Johns Hopkins University Press, Baltimore.

Russell, A. L., R. A. Medellín, and G. F. McCracken. 2005. Genetic variation and migration in the Mexican free-tailed bat (*Tadarida brasiliensis mexicana*). Molecular Ecology 14:2207–2222.

Sánchez, L. R., and R. Medellín. 2007. Food habits of threatened bat *Leptonycteris nivalis* (Chiroptera: Phyllostomidae) in a mating roost in Mexico. Journal of Natural History 41:1753–1764.

Shull, A. M. 1988. Endangered and threatened wildlife and plants; determination of endangered status for two long-nosed bats. Federal Register 53:38456–38460.

Strickland, J. L., C. L. Parkinson, J. K. McCoy, and L. K. Ammerman. 2014. Phylogeography of *Agkistrodon piscivorus* with emphasis on the western limit of its range. Copeia 2014:639–649.

Swofford, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. ClustalW: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673–4680.

Torres-Knoop, L. 2014. Refugio de apareamiento de *Leptonycteris nivalis*: modelación y búsqueda de un recurso limitante. Unpublished Undergraduate thesis, Facultad de Ciencias, UNAM, 18 August 2014.

Triant, D. A., and J. A. DeWoody. 2007. The occurrence, detection, and avoidance of mitochondrial DNA translocations in mammalian systematics and phylogeography. Journal of Mammalogy 88:908–920.

U. S. Fish and Wildlife Service. 1994. Mexican long-nosed bat (*Leptonycteris nivalis*) recovery plan. United States Fish and Wildlife Service, Albuquerque, New Mexico.

U. S. Fish and Wildlife Service. 2018. Endangered and Threatened Wildlife and Plants: Removal of the Lesser Long-nosed bat from the Federal List of Endangered and Threatened Wildlife. 18 April 2018. Federal Register 50 CFR 17: 17093–17110.

Vonhof, M. J., and A. L. Russell. 2015. Genetic approaches to the conservation of migratory bats: a study of the eastern red bat (*Lasiurus borealis*). PeerJ 3:e983.

Vos, P., et al. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23:4407–4414.

Vos, P., and M. Kuiper. 1997. AFLP Analysis. Pp. 115–131 in DNA markers: protocols, applications, and overviews. (G. Caetano-Anollés and P. M. Gresshoff, eds.). Wiley and Sons, Inc., New York.

Weyandt, S. E., R. A. Van Den Bussche, M. J. Hamilton, and D. M. Leslie, Jr. 2005. Unraveling the effects of sex and dispersal: Ozark big-eared bat (*Corynorhinus townsendii ingens*) conservation genetics. Journal of Mammalogy 86:1136–1143.

Wilkinson, G. S., and A. M. Chapman. 1991. Length and sequence variation in evening bat D-loop mtDNA. Genetics 128:607–617.

Wilkinson, G. S., and T. H. Fleming. 1996. Migration and evolution of lesser long-nosed bats *Leptonycteris curasoae*, inferred from mitochondrial DNA. Molecular Ecology 5:329–339.

Wilkinson, G. S., F. Mayer, G. Kerth, and B. Petri. 1997. Evolution of repeated sequence arrays in the D-loop region of bat mitochondrial DNA. Genetics 146:1035–1048.

Wilson, D. E. 1985. Status report: *Leptonycteris nivalis* (Saussure). Mexican long-nosed bat. Final Report submitted to Office of Endangered Species, United States Fish and Wildlife Service.

Worthington Wilmer, J., and E. Barratt. 1996. A non-lethal method of tissue sampling for genetic studies of chiropterans. Bat Research News 37:1–3

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Geographic and Genetic Variation in Bats of the Genus *Glossophaga*

Federico G. Hoffmann, Roy N. Platt II, Hugo Mantilla-Meluk, Rodrigo A. Medellín, and Robert J. Baker

Abstract

Nectar-feeding bats of the genus *Glossophaga*, with five recognized species, are distributed throughout the Neotropics, from northern Mexico to northern Argentina, with populations found on islands along the Pacific coast of Mexico and the Caribbean. Studies based on morphology, morphometrics, isozymes, and mitochondrial DNA uncovered high levels of genetic variation in *Glossophaga soricina*, the species with the widest geographical distribution in the group. Genetic and geographic assessment of intra- and inter-specific variation were combined to better understand the systematics of this genus, with emphasis on *G. soricina.* Initially, sequence data from the mitochondrial DNA cytochrome-*b* (*Cyt*b) gene was used to assess intraspecific variation within *Glossophaga* focusing on assessing how sequence variation partitions relative to the currently recognized subspecies of *G. soricina*. Data from the nuclear 7th intron of the β-fibrinogen gene were then incorporated to compare the patterns of variation at the mitochondrial and nuclear DNA level. Next, geographic information systems based computer modeling was applied to evaluate the biogeographic context of genetic differentiation within and between the different species in the genus. Lastly, the information from geographic structure and variation in the *Cyt*b gene was used to examine taxonomic boundaries in *G. soricina*. Based on patterns of nuclear and mitochondrial variation, the results reported herein suggest that what currently is recognized as *G. soricina* should be split into two separate species and that the nature of the subspecies of *G. soricina* should be reassessed. From a historical standpoint, these analyses indicate that geological changes during the Miocene-Pliocene transition played a major role in shaping diversity in the genus, and from a biogeographic standpoint the analyses identify two areas of high endemicity in Central America. The lack of congruence between molecular data and subspecies boundaries in Central America highlights the need for further studies on the area.

Key words: beta-fibrinogen, biogeography, cytochrome-*b*, *Glossophaga*, *G. soricina*, New World tropics, systematics, taxonomy

Introduction

Nectar-feeding bats of the genus *Glossophaga* are widely distributed in the Neotropics, from northern Mexico to northern Argentina, with populations on islands along the Pacific coast of Mexico and the Caribbean (see Fig. 1 for geographic distributions). These bats feed primarily on nectar, but also include pollen, fruit, and insects in their diet (Eisenberg and Redford 2000). There are five currently recognized species within the genus: *G. commissarisi* Gardner, 1962; *G. leachii* Gray, 1844; *G. longirostris* Miller, 1898; *G. morenoi* Martinez and Villa, 1938; and *G. soricina* Pallas, 1766 (Webster 1993). The last is the type species of the genus and is the most widespread, ranging from Mexico to northern Argentina, and includes populations from Jamaica, Trinidad, and the Tres Marías Islands (Alvarez et al. 1991). *Glossophaga commissarisi* also is widespread, with a disjunct distribution that has Central and South American components (Webster and Jones 1980, 1982, 1987). By contrast, the other three species in the genus, *G. leachii*, *G. longirostris*, and

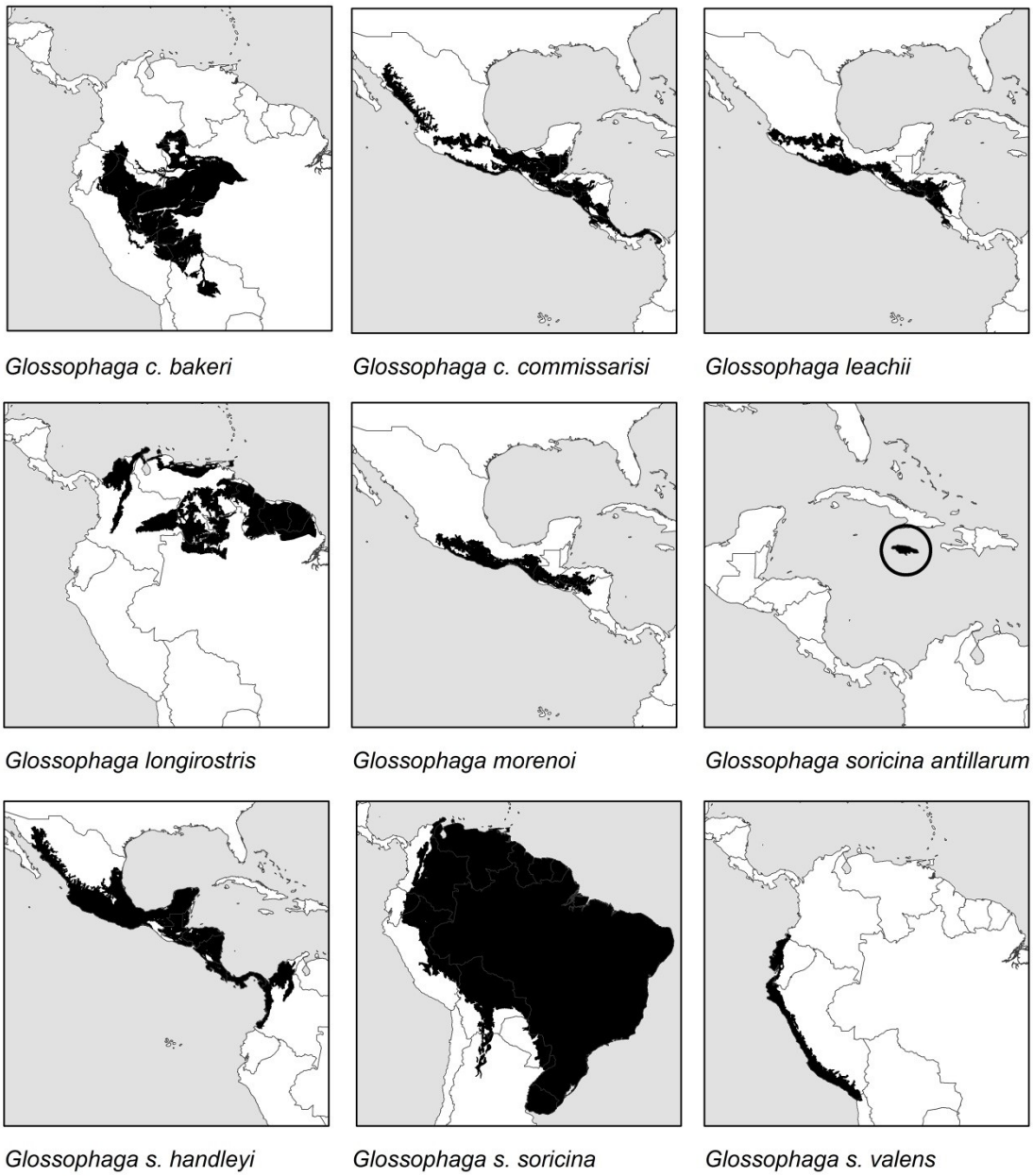

Figure 1. Models of potential distribution generated for *Glossophaga* species and subspecies included in the present work, following the subspecies arrangement from Webster (1993). Because samples were not available to determine the phylogenetic affinities among *G. longirostris* proposed variants, the six subspecies in *G. longirostris* recognized by Webster (1993) were included within a single model. *Glossophaga longirostris* variants present adjacent distributions in northern South America as follows: *G. l. longirostris* Colombian Guajira and adjacent islands; *G. l. campestris* Guyana and Venezuela; *G. l. major* Venezuela; and *G. l. reclusa* Magdalena Valley. Models were built based on modified marginal localities in Gardner (2007) and Webster (1980).

G. morenoi, have more restricted distributions. *Glossophaga leachii* occurs from western Mexico to Costa Rica (Webster and Jones 1984a), *G. longirostris* occurs in northern South America and some Caribbean islands (Webster and Handley 1986), and *G. morenoi* occurs from southern Mexico to Central America (Webster and Jones 1984b, 1985; Gardner 1986).

Webster (1993) assessed the taxonomy and systematics of the species included in the genus *Glossophaga* based on morphology, morphometrics, and isozyme variation. He found strong support for the identity of the five currently recognized species and uncovered a relatively high level of intraspecific variation within *G. soricina*. Later, Webster's findings were substantiated by analyses of sequence variation in the mitochondrial cytochrome-*b* (*Cyt*b) gene (Hoffmann and Baker 2001). Within *G. soricina*, Hoffmann and Baker (2001) encountered a deep phylogeographic subdivision that separated samples of *G. soricina* coming from Central America, Mexico, and the western slope of the Andes in South America from samples coming from the Atlantic side of South America, probably derived from a vicariant event generated by the uplifting of the Andes mountains. This subdivision was congruent with Webster's (1993) results, and groups the subspecies *G. s. antillarum* (Jamaica), *G. s. handleyi* (Central America and Mexico), and *G. s. valens* (western South America) in one clade, and *G. s. soricina* (Atlantic slope of South America) in the other clade. Due to the absence of samples, Hoffmann and Baker (2001) were not able to resolve the position of *G. s. mutica*, which is restricted to the Tres Marías Islands off the Pacific coast of Mexico.

The previous work left unanswered questions regarding variation within *G. soricina* (Webster 1993; Hoffmann and Baker 2001). First, the level of genetic divergence (*Cyt*b sequences) reported between populations of *G. soricina* from opposite sides of the Andes was high, ~6%, similar in magnitude to distances observed between sister species in other phyllostomid bat genera (reviewed in Baker and Bradley [2006] and Bradley and Baker [2001]). Further, specimens of *G. s. mutica* were absent in the phylogenetic trees from Hoffmann and Baker (2001), and thus, their position in the tree remained unknown. In addition, field work in 2004 revealed previously unseen variation in pelage color in specimens of *Glossophaga* collected in the dry coastal region of Ecuador that ranged from light red to dark brown in adult individuals. Because *G. soricina* is the only species known to occur on the western side of Andes, the observed color variation raised the possibility of the presence of a second species of *Glossophaga* in that area.

The current study focused on examining the evolutionary history of *Glossophaga* using a combination of genetic and geographic assessments of intra- and inter-specific variation. Specifically, *Cyt*b sequences were used to study intraspecific variation within *Glossophaga* focusing on: 1) assessing the phylogenetic affinities of the samples of *G. s. mutica*; 2) elucidating the taxonomic status of the samples of *Glossophaga* from western Ecuador; and 3) assessing how sequence variation partitions relative to the currently recognized subspecies of *G. soricina*. Because mitochondrial *Cyt*b only reflects matrilineal history, data from the autosomal 7th intron of the nuclear β-fibrinogen gene (*Fgb*) was collected so as to compare patterns of phylogenetic variation at the mitochondrial and nuclear DNA level, and to determine if the nuclear fragment supports the *Cyt*b tree proposed by Hoffmann and Baker (2001) or the phylogeny proposed by Webster (1993) based on morphology and allozymes. Then, the biogeographic context of genetic differentiation within and between the different species in the genus was investigated, applying geographic information systems (GIS) to do a Parsimony Analysis of Endemicity (PAE), which is an explicit biogeographic analysis integrating phylogenetic and distributional information. The resulting analyses of geographic structure and the *Cyt*b gene were used to examine subspecies boundaries in *G. soricina*.

METHODS

Data Collection

DNA sequence data.—For all new sequence data, DNA was extracted using a phenol/PCI protocol (Longmire et al. 1997), SDS/ proteinase K/NaCl extraction with alcohol precipitation protocol (Miller et al. 1988; Maniatis et al. 1992), or the DNAeasy DNA Purification Kit (Qiagen Inc., Chatsworth, California).

The mitochondrial *Cyt*b gene was sequenced for 12 individuals from western Ecuador, covering the range of color and morphological variation observed, plus two specimens of *G. s mutica* from Tres Marías Islands using primers and conditions described by Hoffmann and Baker (2001). Additionally, the 7th intron of the fibrinogen β-polypeptide (*Fgb*)was sequenced for a subset of the specimens of *Glossophaga*, *Monophyllus plethodon*, and *Leptonycteris* included in Hoffmann and Baker (2001): seven specimens of *G. soricina*, two of *G. commissarisi*, one of *G. leachii*, two of *G. longirostris*, and one of *G. morenoi*, plus a sample of *Macrotus waterhousii* as the outgroup. *Fgb* amplicons were obtained using primers FIB-βI7L-Rod and FIB-βI7U from Wickliffe et al. (2003) and sequenced with the PCR primers. PCR products were checked in a 1% agarose gel, purified using the QUIAQuick purification kit (Qiagen Inc., Chatsworth, California) following manufacturer's instructions, and sequenced with the ABI Big Dye chemistry (Applied Biosystems, Inc., Foster City, California). We were unable to obtain *Fgb* amplicons from several of the specimens, including the two specimens from the Tres Marías Islands. Sequences were deposited in GenBank with accession numbers FJ392505–FJ392532. A list of the specimens sequenced is provided in the Appendix. Because no data were available from the South American populations of *G. commissarisi*, assigned to the *G. c. bakeri* subspecies, this subspecies was not considered in our analyses.

Phylogenetic Analysis

Mitochondrial DNA sequence analyses.—Systematic relationships among subspecies of *G. soricina* and the taxonomic position of the specimens of *Glossophaga* from western Ecuador and the Tres Marías Islands were evaluated via distance comparisons and phylogenetic analyses. The 14 newly sequenced specimens of *G. soricina* from western Ecuador were added to the 57 specimens of *Glossophaga* included in Hoffmann and Baker (2001), for a total of 71 *Glossophaga* representatives and four outgroup sequences, represented by *Leptonycteris curasoae*, *Monophyllus redmani*, *M. plethodon*, and *Macrotus waterhousii*. The alignment of these 75 sequences was straightforward, as there were no insertions or deletions, and is referred to as the complete data set. Pairwise distance comparisons were calculated as the proportion of nucleotide sites at which two sequences differ (*p*-distance). Phylogenetic relationships were estimated with maximum likelihood and Bayesian analyses. Bayesian estimation of phylogenies was conducted in MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003) under a GTR+Γ model of nucleotide substitution, running four simultaneous chains for 5 x 10^6 generations, sampling trees every 1,000 generations, and using default priors. Chains were considered to have converged once the average split frequency was lower than 0.01. Results were summarized by a majority-rule consensus of the 2,500 trees collected after convergence was reached. Maximum likelihood searches were conducted using IQ-Tree ver 1.6.8 (Nguyen et al. 2015) in the implementation of IQ-Tree available from the IQ-Tree web server (Trifinopoulos et al. 2016) last accessed in November 2018, and support for the nodes was evaluated with 1,000 pseudoreplicates of the ultrafast bootstrap procedure (Minh et al. 2013). The best-fitting model of substitution was selected using the ModelFinder subroutine from IQ-Tree (Kalyaanamoorthy et al. 2017).

Nuclear + mtDNA sequence analysis.—This analyses were based on a subset of 17 of the specimens included in the previous section for which it was possible to collect sequence data from both the nuclear *Fgb* gene and the mitochondrial *Cyt*b gene (reduced data set). These analyses were conducted to evaluate whether phylogeographic variation within *G. soricina* at the mtDNA and nuclear level are similar and to resolve relationships among species of *Glossophaga*. Phylogenetic relationships were estimated in Bayesian and maximum likelihood analyses for the two genes independently, following the analytical procedures described above. Because there were no topological incompatibilities as defined by Wiens (1998), the two alignments were concatenated for phylogenetic reconstruction as well. In this study, trees were considered compatible if there were no strongly supported conflicting nodes, ≥ 95% Bayesian posterior probabilities, or ≥ 80% Maximum Likelihood bootstrap support. The *Fgb* sequences were aligned with MUSCLE ver. 3.8.31 (Edgar 2004), and Maximum likelihood and Bayesian analyses were conducted using the approach described in the previous section.

Geographic Analysis

Geographic distribution analysis.—Models of potential distribution generated for *Glossophaga* species and subspecies included in the present work were based on peripheral localities reported in Gardner (2007) and Webster (1993). Given that samples to determine the phylogenetic affinities among the six different subspecies of *G. longirostris* recognized by Webster (1993) were not available, these subspecies were included within a single model. Monophyletic groups recovered from our molecular analyses were used to discriminate among localities of species and subspecies presented by Webster (1993). Discriminated localities were used as input to generate models of potential distribution following protocols in Mantilla-Meluk et al. (2009). In the present analysis, phylogenetic methods were applied to determine monophyletic groups, later used as biotic units. In the case of *G. soricina*, because of issues with the current classification as described below, the subspecies *G. s. antillarum*, *G. s. handleyi*, *G. s. mutica*, and *G. s. valens* were considered as one single unit, and *G. s. soricina* as a second unit. Localities associated with museum voucher specimens listed by Webster (1993) were provided as input for the construction of distribution models.

Parsimony analysis of endemicity (PAE).—The PAE relates areas historically, based on the composition of their biota or part of it (Rosen 1988). Harold and Mooi (1994) define an area of endemism as a geographic region comprising the distributions of two or more monophyletic taxa that exhibit a phylogenetic and distributional congruence and having their respective relatives occurring in other such-defined regions or operational geographic units (OGU´s).

For the purpose of this analysis, the biogeographic districts defined in Morrone (1994) were used as Operational Geographic Units (OGUs). To construct a matrix of presence/absence for all taxa considered, the models of predicted distribution were superimposed on a map describing the extent of Neotropical biogeographic districts proposed by Morrone (1994) in ArcGIS 9.3 (Redlands, California: Environmental Systems Research Institute). To determine the taxonomic affinities among analyzed biogeographic districts, we then performed a PAE following protocols in Goldani et al. (2006). A majority rule consensus tree was calculated among equally parsimonious trees obtained in our analysis. Finally, clades recovered in the tree were represented in a species-area map. Although in this analysis monophyletic taxa are assumed to be the product of vicariant events, areas of endemism can be recognized despite dispersal or expansion events of some taxa.

RESULTS

Data Description

The analyses of molecular variation in this study were focused on two overlapping data sets. The first was a *Cyt*b sequence data set for all 75 specimens in the study, comprising 71 specimens of *Glossophaga* plus four outgroup sequences. The second data set was restricted to specimens for which both *Cyt*b and *Fgb* sequences were collected; this second data set included 15 representatives of the genus *Glossophaga*. Thus, the analysis of mitochondrial sequences included all specimens, but the analyses that combined and contrasted phylogenetic trees from nuclear and mitochondrial sequence data were restricted to those specimens for which such data were available for the two markers.

Cytochrome-b.—Alignment of the protein-coding mitochondrial *Cyt*b sequences was straightforward as all copies of the gene were 1,140 base pairs long. There were 438 variable sites out of the 1,140 base pairs sequenced for the full data set, whereas in the small data set for which we also obtained *Fgb* sequence there were 403 variable sites. The two sets of newly sequenced specimens were highly similar. The average pairwise distance among specimens from western Ecuador was 0.7% and the distance between the two specimens from Tres Marías Islands was ~ 0.1%. The specimens from western Ecuador were most similar in *Cyt*b sequence distance comparisons to three *G. soricina* specimens from western Peru, with an average genetic distance between specimens from western Ecuador and samples

from western Peru of 1.1%, consistent with the *a priori* expectations. In turn, samples of *G. s. mutica*, from Tres Marías Islands, were most similar to a sample from San Luis Potosí, in Mexico, with an average distance of 0.8%. In all cases, distances within the different subspecies of *G. soricina* were lower than 3%, whereas average distances among them ranged from 1 to 6.4% (Table 1). Mean intraspecific distances were lowest in *G. longirostris* where they averaged 0.6%, and highest in *G. soricina* where they averaged 4.3% (Table 2). All intraspecific pairwise comparisons were lower than 3.2%, except for comparisons within *G. soricina* that ranged from 0 to 7.9%. The latter value is registered in comparisons between samples of *G. soricina* from Venezuela and Western Peru or Jamaica, and it is similar to interspecific distances within *Glossophaga*, which ranged from 8.1% (*G. longirostris* vs *G. leachii*) to 14% (*G. morenoi* vs *G. soricina*).

Fgb.—Alignment of the 7th intron of the fibrinogen polypeptide was unambiguous, and resulted in a 580 base-pair alignment, of which 100 were variable. Mean intraspecific distances ranged from 0.2% in *G. commissarisi* to 0.5% in *G. soricina* (Table 2). Pairwise comparisons within *G. soricina* ranged from 0 to 0.9 %, and as for the *Cyt*b, the highest intraspecific comparisons within *G. soricina* were similar to other interspecific comparisons within *Glossophaga*, which ranged from 0.9% (*G. commissarisi* vs *G. leachii*) to 2.1% (*G. morenoi* vs *G. soricina*), whereas distance comparisons between *Glossophaga* and the outgroups ranged from 3.0% in comparisons between *Glossophaga* and *Leptonycteris* to 10.7% in comparisons between *Glossophaga* and *Macrotus*.

Phylogenetic Analysis

Intraspecific variation.—Maximum likelihood, Bayesian analyses, and distance comparisons place all specimens from western Ecuador within the species *G. soricina* with strong bootstrap support (bs), and high posterior probabilities (pp). Bayesian and ML analyses of the complete dataset support the monophyly of each of the currently recognized species of *Glossophaga* with strong support (Fig. 2). In the complete dataset, comprising 71 specimens of *Glossophaga*, specimens within *G. soricina* were grouped as follows: three clades representing the subspecies *G. s. antillarum*, *G. s. mutica*, and *G. s. valens*, each in a monophyletic group with strong support (bs = 100%, pp = 1.00); one specimen identified as *G. s. valens* from western Peru grouped with specimens from *G. s. soricina* with moderate bootstrap support (bs = 63%); and finally, specimens that would be assigned to *G. s. handleyi* are split into two separate lineages. The first lineage within *G. s. handleyi* includes three specimens from Panama in a strongly supported clade. The second lineage of *G. s. handleyi* is paraphyletic relative to the two specimens of *G. s. mutica*. Most nodes resolving relationships among the different subspecies of *G. soricina* are poorly resolved, with the exception of the one splitting *G. s. soricina* from the four other subspecies. Overall, results from the *Fgb* (Fig. 2b) are congruent with the results obtained for the complete data set based on the *Cyt*b. Within *G. soricina*, the two samples of *G. s. antillarum* are in a clade with maximal support (bs = 100%, pp 1.00), and the samples from *G. s. soricina* are in another clade with strong support (bs = 90%, pp 1.00).

Table 1. Mean pairwise p-distances among and within the different subspecies of *G. soricina* expressed as a percentage. Note that we included all specimens of *G. s. handleyi*.

	G. s. mutica	*G. s. handleyi*	*G. s. valens*	*G. s. antillarum*	*G. s. soricina*
G. s. mutica	0.1				
G. s. handleyi	1.8	2.1			
G. s. valens	3.3	3.3	0.8		
G. s. antillarum	3.8	3.6	3.7	1.1	
G. s. soricina	6.1	6.1	6.6	6.4	2.7

Table 2. Pairwise p-distance distance comparisons in *Glossophaga* based on *Fgb* (top) and cytochrome-*b* (bottom). Distances are expressed as percentages.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Intron 7 *Fgb*															
1- *G. s. soricina* Venezuela																
2- *G. s. soricina* Bolivia	0.18															
3- *G. s. soricina* Trinidad	0.18	0.17														
4- *G. s. valens* W Peru 1	0.88	0.87	0.70													
5- *G. s. valens* W Peru 2	0.53	0.52	0.35	0.17												
6- *G. s. antillarum* Jamaica 1	0.88	0.86	0.69	0.35	0.17											
7- *G. s. antillarum* Jamaica 2	0.70	0.69	0.52	0.35	0.18	0.00										
8- *G. s. mutica* Mexico	0.88	0.87	0.69	0.35	0.00	0.35	0.35									
9- *G. s. soricina* Grenada	1.76	1.73	1.56	1.56	1.22	1.56	1.56	1.21								
10- *G. s. soricina* Trinidad	2.11	2.08	1.90	1.90	1.57	1.90	1.90	1.56	0.35							
11- *G. leachii* Mexico	1.59	1.56	1.39	1.39	1.05	1.39	1.39	1.04	1.04	1.39						
12- *G. morenoi* Mexico	2.04	2.21	2.03	2.40	1.86	2.39	2.21	2.03	1.29	1.66	1.85					
13- *G. commissarisi* Mexico	1.42	1.39	1.22	1.22	0.87	1.22	1.22	0.87	1.05	1.40	0.88	1.87				
14- *G. commissarisi* Panama	1.59	1.56	1.40	1.39	1.04	1.38	1.39	1.04	1.21	1.56	0.87	2.04	0.17			
15- *Monophyllus*	5.47	5.57	5.40	5.22	4.89	5.22	5.04	5.23	5.56	5.72	5.41	5.74	5.25	5.56		
16- *Leptonycteris*	3.00	3.12	2.95	3.12	2.79	3.12	2.95	3.13	2.76	3.11	3.30	3.13	2.78	3.12	5.56	
17- *Macrotus*	10.04	10.40	10.59	10.57	10.45	10.75	10.58	10.77	10.89	11.24	11.29	11.30	10.80	10.75	11.30	10.73

Table 2. (cont.)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Cytochrome-*b*															
1- *G. s. soricina* Venezuela																
2- *G. s. soricina* Bolivia	4.21															
3- *G. s. soricina* Trinidad	3.25	3.25														
4- *G. s. valens* W Peru 1	4.39	3.51	3.25													
5- *G. s. valens* W Peru 2	7.89	6.75	6.67	6.93												
6- *G. s. antillarum* Jamaica 1	7.54	5.61	6.4	6.32	4.47											
7- *G. s. antillarum* Jamaica 2	7.89	6.14	6.75	6.67	4.3	1.05										
8- *G. s. mutica* Mexico	6.84	5.26	6.05	5.61	3.42	3.16	3.33									
9- *G. s. soricina* Grenada	13.07	12.02	12.72	12.72	13.07	11.84	12.19	12.02								
10- *G. s. soricina* Trinidad	12.98	11.84	12.54	12.37	12.89	11.84	12.19	11.84	0.7							
11- *G. leachii* Mexico	13.42	11.75	12.72	12.54	12.98	12.81	12.46	12.89	8.16	7.81						
12- *G. morenoi* Mexico	14.12	13.86	13.86	14.3	14.82	13.51	13.68	13.77	10.7	10	10.53					
13- *G. commissarisi* Mexico	13.6	12.19	12.72	12.98	13.07	12.19	12.37	12.46	10	9.47	10.18	10.7				
14- *G. commissarisi* Panama	14.04	12.72	12.72	13.33	13.68	12.72	12.89	12.98	10.18	9.65	10	11.05	3.16			
15- *Monophyllus*	16.23	16.32	16.49	15.61	16.84	16.75	17.11	16.4	15.53	15.35	15.18	16.93	15.18	15.44		
16- *Leptonycteris*	17.19	17.02	17.19	16.49	17.02	17.46	17.63	16.93	16.14	15.61	15.35	16.14	15.96	15.61	15.79	
17- *Macrotus*	19.39	18.95	19.12	18.95	19.21	19.21	19.21	18.77	17.54	17.46	18.07	18.86	18.42	18.16	18.6	18.16

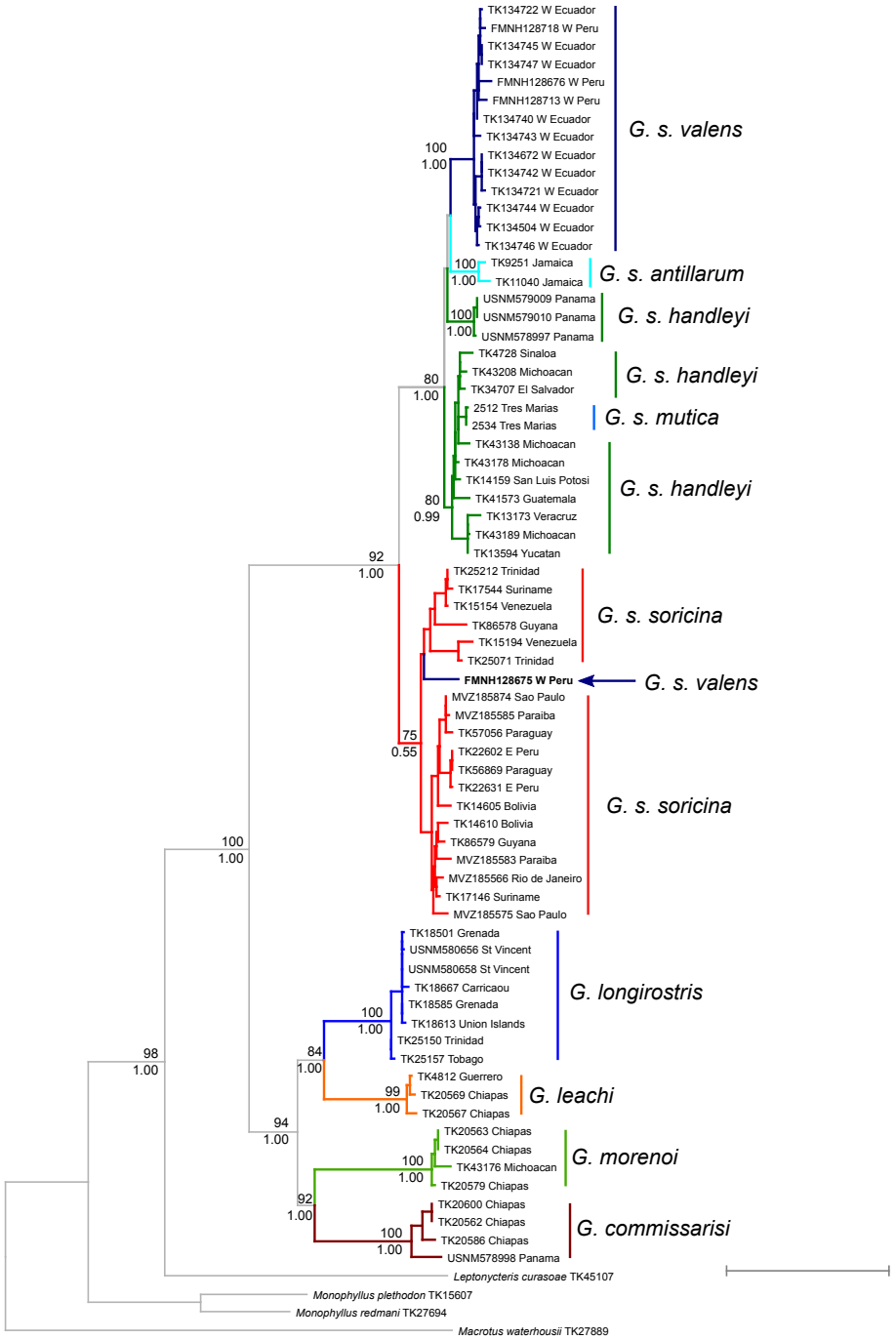

Figure 2. Maximum likelihood phylogram describing relationships among species of nectar-feeding bats in the genus *Glossophaga*, with samples of *Macrotus*, *Leptonycteris*, and *Monophyllus* included as the outgroup. Support for the relevant nodes is presented as bootstrap values (above the nodes) and as Bayesian posterior probabilities (below the nodes). The vertical bars identify the different lineages recognized in this study. Specimens that were previously assigned to *G. s. handleyi* are shown in three separate boxes, and the specimen that shows evidence of introgression between *G. s. valens* and *G. s. soricina* is in bold.

Interspecific relationships.—Maximum likelihood and Bayesian phylogenies derived from analysis of the small dataset restricted to specimens for which we obtained both *Cyt*b and *Fgb* sequence data differed in topology, but no statistically supported conflicting nodes were found among them. In the *Cyt*b based phylogeny (Fig. 3a), *Leptonycteris* was placed as sister to *Glossophaga* with weak support (pp = 0.73), and there was strong support for grouping all samples of *G. soricina* in one clade, and the other four species in the other clade. By contrast, in the *Fgb* phylogeny (Fig. 3b) there was strong support for placing *Leptonycteris* as sister to *Glossophaga*, but not to resolve relationships among species within *Glossophaga*. Finally, in the phylogenies based on the concatenated sequences, *Leptonycteris* is sister to *Glossophaga* with strong support in both Bayesian and ML analyses (Fig. 3c), and the grouping of the species of *Glossophaga* into two clades is strongly supported as well. Relationships within the clade that included *G. commissarisi*, *G. leachii*, *G. longirostris*, and *G. morenoi* were weakly supported.

Geographic Analysis

Distribution analysis.—Because of the large extent of their geographic distributions and the level of genetic differences among them, the distribution analysis grouped the subspecies within *G. soricina* into two independent lineages: one including *G. s. soricina*, and the other one including all four other subspecies. Central America is the region with the highest diversity of *Glossophaga*, as four evolutionary independent lineages are present there: the subspecies *G. s. handleyi* and the species *G. commissarisi*, *G. leachii*, and *G. morenoi* (Fig. 1). Note that revisions of *G. s. handleyi* would increase diversity, so the results from these analyses are conservative. Two subspecies of *G. soricina* are restricted to insular populations, *G. s. antillarum* from Jamaica and *G. s. mutica* from

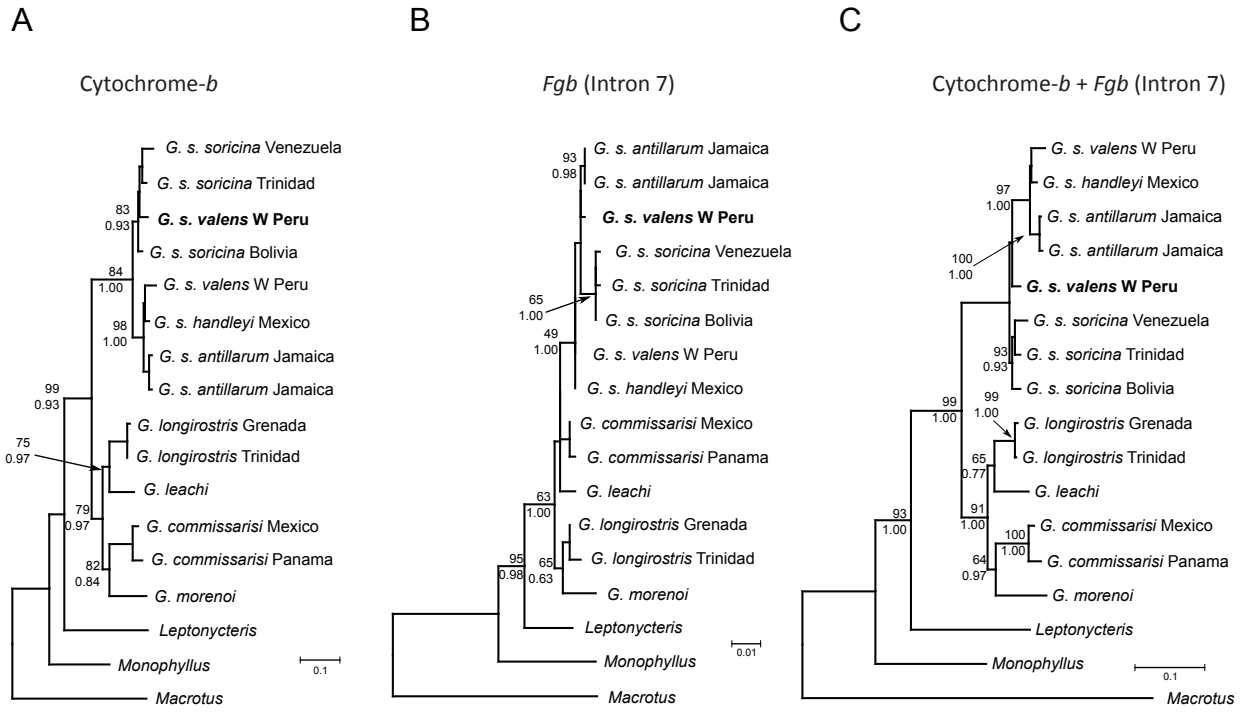

Figure 3. Maximum likelihood phylograms describing relationships among species of nectar-feeding bats in the genus *Glossophaga*, with samples of the genera *Leptonycteris* and *Monophyllus*. Sequence data from the genus *Macrotus* was included as the outgroup. Analyses were based on the cytochrome-*b* (A), the 7th intron of *Fgb* (B), and the concatenated data (C). Support for the relevant nodes is presented as bootstrap values (above the nodes) and as Bayesian posterior probabilities (below the nodes). The specimen that shows evidence of introgression is identified by an arrow.

Tres Marías Islands, that lack sympatric lineages. Two lineages of *Glossophaga* are restricted to South America: *G. longirostris*, which is distributed across the Colombian-Venezuelan Caribbean coasts, and *G. s. soricina which is* widespread on the eastern side of the Andes (Fig. 1). Samples from *G. c. bakeri*, the disjunct subspecies of *G. commissarisi* from South America, were not available to us and would be required to further refine this picture.

Parsimony Analysis of Endemicity (PAE).—The PAE grouped populations of bats in the genus *Glossophaga* into four major areas but was unable to resolve the polarity of putative vicariant events among them. The four areas defined are shown in Figure 4 and correspond to: 1) the Caribbean, where only *G. s. antillarum* is found; 2) the dry environments on the western side of the Andes in the biogeographic dis-

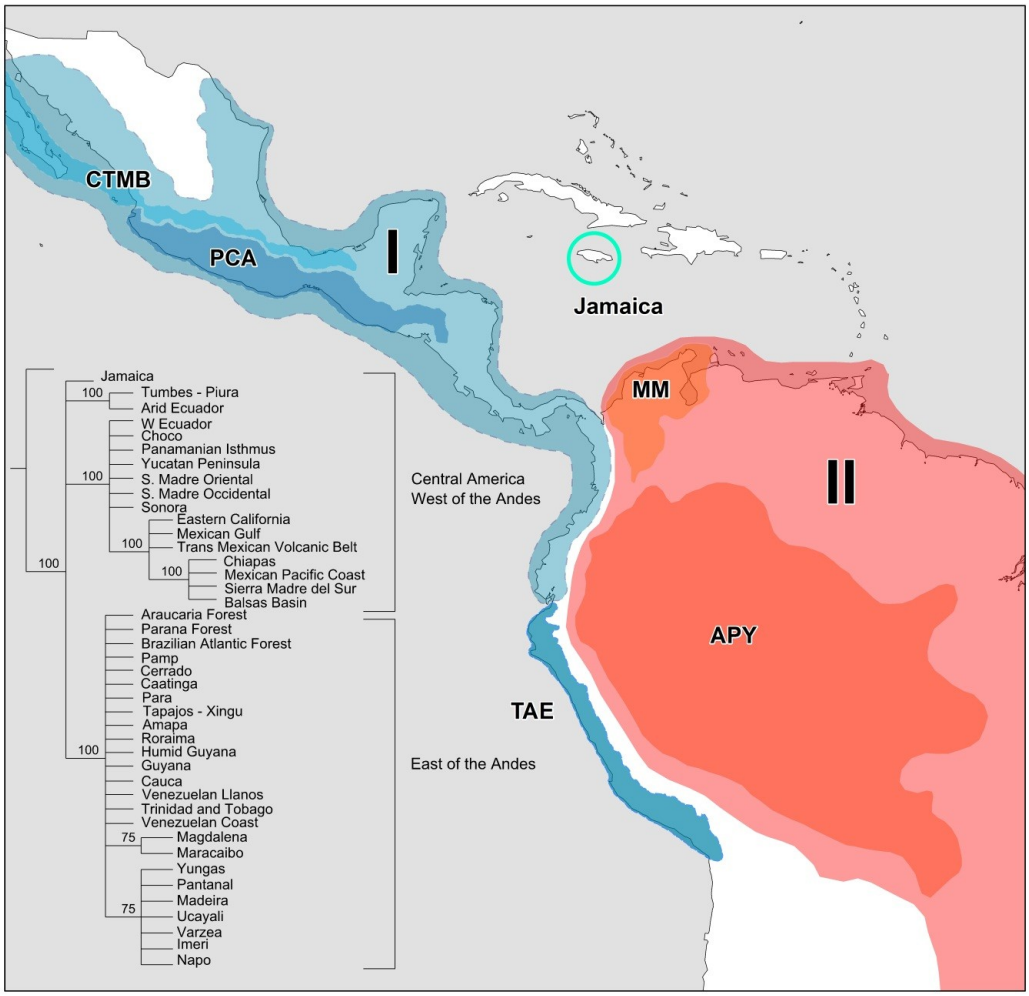

Figure 4. Results of the Parsimony Analysis of Endemicity for the genus *Glossophaga*, showing zonation obtained from the analysis of models of the distribution of monophyletic groups recognized in our phylogenetic analyses. Abbreviations refer to biogeographic areas that cluster in the analysis as follows: I. Central American-Western South American-Caribbean Domain; and II. Eastern South American Domain; California-Trans-Mexican Volcanic Belt (CTMB); Pacific Central America (PCA); Tumbes-Piura-Arid Ecuador (TAE); Magdalena-Maracaibo (MM); and Amazon-Pantanal-Yungas (APY). The majority rule consensus tree, constructed out of the four most parsimonious trees obtained in the analysis, is shown in the inset. Spatial boundaries of the zones defined were based upon the biogeographic zonation proposed by Morrone (2004).

tricts of Tumbes, Piura, and Arid Ecuador, where only *G. s. valens* is found; 3) mainland Central America, where *G. commissarisi*, *G. leachii*, *G. morenoi*, and *G. s. handleyi* are found; and 4) the eastern side of the Andes, where *G. longirostris* and *G. s. soricina* are found (Fig. 4). These four areas were arranged into two separate domains, the first one including samples from Central and North America, Jamaica, and the western side of South America, whereas the second one was restricted to South America east of the Andes (Fig. 4). In Central America, two subareas were identified with maximal support, the California-TransMexican Volcanic Belt (CTMB) and Pacific Central America (PCA); whereas two subareas were identified in South America, Magdalena-Maracaibo subarea in northern South America, and the Amazon-Pantanal-Yungas subarea on the eastern side of the Andean System (Fig. 4).

DISCUSSION

The current study is an assessment of variation in mitochondrial and nuclear markers across a broad taxonomic sample of the genus *Glossophaga*. This approach was implemented to reveal mechanisms and history of diversification, assess species definitions and isolating mechanisms, and explore whether partitioning of genetic variation corresponds to species and subspecies boundaries. Further, by incorporating climate models into the resulting phylogeny it was possible to infer areas of original diversification, link divergences to particular geological times, and explore patterns of endemism.

Intraspecific variation.—Based on the observed patterns of nuclear and mitochondrial genetic variation analyzed in this study, in addition to morphologic and morphometric variation from previous research (see Webster 1993), it seems clear that what currently is recognized as *G. soricina* should be split into two separate species. The evidence in support of this arrangement is summarized in Table 3. Morphological and molecular evidence indicates that levels of intraspecific variation are substantially higher in *G. soricina* than in any of the other currently recognized species in the genus. This especially is true of *Cyt*b sequence divergences between the subspecies *G. s. soricina*, from the Atlantic side of the Andes, and populations currently assigned to *G. soricina* from the western side of the Andes and Central America, which averaged 6.4%. This value is higher than the mean distance observed between sister species in most phyllostomid bats (Bradley and Baker 2001; Baker and Bradley 2006).

The phylogenies in this study partition what currently is recognized as *G. soricina* into two separate monophyletic clades, with *G. s. antillarum*, *G. s. handleyi*, *G. s. mutica*, and *G. s. valens* in a strongly supported monophyletic clade, and *G. s. soricina* in a moderately supported one. In addition, *G. s. antillarum* appears to be distinct in both *Cyt*b and *Fgb* analyses, and might also represent a different species. Thus, the name *G. soricina* should probably be restricted to populations from South America on the eastern side of the Andes, and further studies should be undertaken to resolve the systematics and taxonomy of what is now placed in *G. s. antillarum*, *G. s. handleyi*, *G. s. mutica*, and *G. s. valens*. It is clear that current subspecies definitions do not match patterns of genetic variation, as *G. s. handleyi* involves several monophyletic groups that may be genetically isolated from each other (Fig. 2), and that do not correspond well with the currently recognized subspecies. For example, *G. s. mutica* appears to be a rather recent migrant to the Tres Marías Islands that might not deserve taxonomic recognition as a subspecies, as it is embedded within the clade that includes samples from Central America and Mexico. On the other hand, what currently is recognized as *G. s. handleyi* appears to be composed of at least two separate lineages.

A full review of subspecific taxonomy in *G. soricina* will require careful re-examination of specimens to explore patterns of morphological variation and identify potential diagnostic morphological characters that may better resolve the use of trinomial names within this species. Of the names currently available for the *G. soricina* subspecies from South America west of the Andes, Central America, and the antilles, *G. s. mutica* would be the oldest applicable name to the group; but because *G. s. mutica* does not seem to be a valid subspecies and the status of *G. s. handleyi* requires further study, it seems that more data would

Table 3. Summary of the available evidence distinguishing the six distinct evolutionary lineages within *Glossophaga*. Note that for the purpose of these pairwise comparisons the subspecies *G. s. antillarum*, *G. s. handleyi*, *G. s. mutica*, *G. s. valens* were considered as a single unit labeled as *G. s. soricina* II. The specimen FMNH128675, which shows evidence of introgression, was excluded from these comparisons.

Pairwise comparison		p-distance cytochome-*b*	Reciprocal monophyly cytochrome-*b*	Sister species? cytochrome-*b*	Reciprocal monophyly *Fgb*	Morphologically distinct	Ecologically distinct
G. s. soricina II	*G. commissarisi*	12.4	Y	N	Y	Y	Y
G. s. soricina II	*G. leachi*	12.7	Y	N	?	Y	Y
G. s. soricina II	*G. longirostris*	12.0	Y	N	Y	Y	Y
G. s. soricina II	*G. morenoi*	13.1	Y	N	?	Y	Y
G. s. soricina II	*G. s. soricina*	6.4	Y	Y	*G. s. soricina*	Y	Y
G. commissarisi	*G. leachi*	9.8	Y	N	?	Y	Y
G. commissarisi	*G. longirostris*	9.6	Y	N	Y	Y	Y
G. commissarisi	*G. morenoi*	10.3	Y	Y	?	Y	Y
G. commissarisi	*G. s. soricina*	12.3	Y	N	Y	Y	Y
G. leachii	*G. longirostris*	8.1	Y	Y	?	Y	Y
G. leachii	*G. morenoi*	9.9	Y	N	?	Y	Y
G. leachii	*G. s. soricina*	12.1	Y	N	?	Y	Y
G. longirostris	*G. morenoi*	9.9	Y	N	?	Y	Y
G. longirostris	*G. s. soricina*	12.3	Y	N	Y	Y	Y
G. morenoi	*G. s. soricina*	13.1	Y	N	?	Y	Y

be necessary to align the systematics and taxonomy of what is currently recognized as *G. soricina*. Because of the observed variation, it would seem that sampling populations from Panama, Costa Rica, and Colombia would provide critical information for this purpose.

At a narrower geographic scale, specimens from a particular location tend to cluster together on a phylogenetic tree for the most part. The only exception here is the presence of a *G. s. valens* specimen, FMNH 128675 from western South America, that is placed within the *G. s. soricina* clade. The likely explanation is that specimen FMNH 128675 is probably derived from historical introgression between *G. s. valens* and *G. s. soricina.* Results from analyses of the nuclear *Fgb* (Fig. 2b) provide further evidence for this hypothesis, as the specimen FMNH 128675 groups with the samples of *G. s. antillarum* from Jamaica, whereas in the *Cyt*b based analyses (Figs. 1 and 2a) it groups with samples from South America east of the Andes. Thus, it seems clear that this complex needs additional research that should include additional geographic samples for genetic studies, which are currently unavailable.

Interspecific relationships.—Two previous studies produced alternative hypotheses regarding interspecific phylogenetic relationships within *Glossophaga*. In the first hypothesis, analyses of morphological, morphometric, and isozyme variation by Webster (1993) identified three different clades: 1) *G. soricina*; 2) *G. commissarisi* and *G. leachii*; and 3) *G. longirostris* and *G. morenoi* (Fig. 3A). This phylogenetic arrangement, however, was not supported by analyses of *Cyt*b sequence variation (Hoffmann and Baker 2001), where *G. soricina* was sister to the remaining species in the genus, and grouped *G. commissarisi* with *G. morenoi* and *G. leachii* with *G. longirostris*. As expected, analyses based on the mitochondrial *Cyt*b, in Figure 2A, resulted in species-level relationships similar to the results of Hoffmann and Baker (2001). By contrast, analyses based on the nuclear *Fgb*, in Figure 2B, yielded a tree where species-level relationships matched those retrieved by Webster (1993), although resolution was not significant. The combined gene sequence data analyses produced results that are similar to those obtained with the *Cyt*b, with stronger support for the monophyly of the clade that included *G. commissarisi*, *G. morenoi*, *G. leachii*, and *G. longirostris*. Support for this arrangement is not statistically significant and is dependent heavily on the *Cyt*b sequence data, as information derived from the nuclear genome (either sequences from the *Fgb* fragment, or the isozyme electrophoresis data) is swamped by the magnitude of informative sites in the *Cyt*b gene data. Thus, the resolution of relationships among species of *Glossophaga* will require the addition of mitochondrial and nuclear sequence data. The available evidence indicates that the species *G. commissarisi*, *G. leachii*, *G. longirostris*, and *G. morenoi* separated from each other at a similar geologic time, and this makes it difficult to discern among the alternative possible relationships.

Geographic analyses.—Central America is the geographic area with the highest diversity of *Glossophaga* lineages, with *G. commissarisi*, *G. leachii*, *G. soricina*, *G. morenoi*, and *G. leachii* all occurring there. *Glossophaga leachii* and *G. morenoi* have a distribution restricted to this area, which is their most likely place of origin; *G. longirostris* is found over the northeast of South America and the Antilles, and the analysis in this study cannot shed light on the geographic origin of this species. In turn, *G. commissarisi* has a more widespread distribution, and today it is represented by apparently disjunct populations (Webster 1993; Gardner 2007). In order to understand whether the two disjunct populations represent a single species, as suggested by Gardner (2008), additional specimens of *G. commissarisi* from South America would be required, but are currently unavailable to the best of the authors' knowledge.

Models of predicted distribution in conjunction with molecular evidence suggest that the observed distributional patterns among species of *Glossophaga* were influenced strongly by the Miocene-Pliocene transition associated with the closure of the Isthmus of Panama and the uplift of the Andes (O´Dea et al. 2016). Most of the diversity in the genus concentrates in Central America, where four of the five recognized species are found. *Glossophaga leachii* and *G. morenoi* are restricted to northern Central America; interestingly, the southern end of the distribution of *G. leachii* and *G. morenoi* coincides with the extent of the Cohortis Block formation, which was the southernmost landmass of Central America prior to the completion of the Panamanian Isthmus (Dengo 1969, 1985; Donnelly et al. 1990; Francis 2005; Montes et al. 2012 a,b; Montes et al. 2015). The entrance of *Glossophaga* into South

America probably occurred after the formation of the Chocoan and Chorotega Blocks that emerged as a product of the subduction of the Cocos Ridge and the collision of the arc with South America. The completion of the isthmus provided a landmass connection between Central and South America, but the establishment of suitable environments for nectar-feeding bats in the genus *Glossophaga* may have been more recent. Two of the groups analyzed, one comprised of the subspecies *G. s. antillarum*, *G. s. handleyi*, *G. s. mutica*, and *G. s. valens*, plus the species *G. commissarisi*, are distributed in both Central and South America. The first group has the widest latitudinal range, as it occurs from Mexico to southern Peru along the Pacific side of the Andes, and it also is present in the island of Jamaica (Fig. 1), and the analyses herein indicate that the colonization of Jamaica by *G. s. antillarum* occurred via the Yucatán peninsula, as has been documented for other vertebrates including bats (Gannon et al. 2005). As currently recognized, *G. commissarisi* has a disjunct distribution with the southernmost limit of Central American populations in Panama, and the northernmost limit of South American populations in Venezuela. Although further analyses are necessary to identify the actual limits of this taxon in Colombia, where the species is apparently rare, it is likely that the northern Andes serve as a barrier separating populations of *G. commissarisi* from both sides of the system.

Two species of *Glossophaga*, *G. longirostris* and *G. soricina*, are restricted to South America. *Glossophaga longirostris* is distributed along the Colombian Arid Peri-Caribbean belt, a zone of low precipitation which extends eastward to the Guianas and the Lesser Antilles (Hernández-Camacho et al. 1992). This region is characterized by the Cardonales vegetation formation dominated by columnar cacti (Rangel and Garzón 1994). Caribbean columnar cacti are typically pollinated by *G. longirostris* (Petit 1995; Nassar et al. 1997). *Glossophaga soricina* appears to be restricted to the eastern side of Andes across the Guiana shield, the Amazon River basin southward to Brazil, and northern Argentina.

Although representatives in the genus *Glossophaga* have wide distributions, geographic distributions of the different groups do not show overlaps larger than 50%, suggesting strong geographic partitioning among the lineages recognized in this study. Webster (1993) hypothesized that cycles of expansion and contraction of wet and dry habitats during the Quaternary could explain relationships within *Glossophaga* and the present distribution of members of the genus. By contrast, the analyses herein suggest an alternative scenario where geological changes during the Miocene-Pliocene transition played a major role in shaping present-day patterns of genetic diversity in the genus, and could account for the difficulties in resolving interspecific relationships.

Parsimony Analysis of Endemicity (PAE).—The CTMB and PCA subareas recovered within Central America coincide in their general boundaries with those identified in previous analyses of Mexican biotic provinces for reptiles (Smith 1940), birds (Moor 1945) and mammals (Goldman and Moore 1946). All these analyses agree that the Sierra de Anahuac (Smith 1940) or the Transverse Volcanic Biotic Province (Moore 1945; Goldman and Moore 1946) is an area of significant species richness and exceptional endemism. The area is associated with the Clarion and San Andrés fractures, and its topographic complexity is associated with intricate distribution patterns of species (Fa and Morales 1991) and has promoted the isolation of several mammals such as gophers in the genus *Thomomys* and *Pappogeomys* (Merriam 1895; Russell 1968a, 1968b). The Trans-Mexican Volcanic Belt constitutes a barrier promoting vicariant events among populations north and south of the system (Fa and Morales 1991). As its name implies, the area comprises a belt of active volcanoes and its origin has been dated to the Upper Miocene-Pliocene (Lorenzo 1964).

The second area in Central America highlighted in the analysis in this study, the Pacific Coast of Central America, has its southernmost limit at the Gulf of Fonseca at the meeting point of the borders of El Salvador, Honduras, and Nicaragua. This area also coincides with the boundary between the Chortis block and the southern portion of the North American Plate (McBirney and Williams 1965; Mann and Burke 1984; Mann et al. 1990). This area remained isolated from South America until the Paleocene-Eocene when the island arc of the Chorotega block was built on a primitive basaltic crust evolving into the modern arc that completed the connection between Central and South America (Coates et al. 1992; Collins et al. 1995; Coates and Obando 1996). The area of the Gulf of Fonseca is also recognized as a transitional zone separating two subspecies of the phyl-

lostomid frugivorous bat *Uroderma bilobatum*: *U. b. davisi* distributed north of the Gulf on the Pacific Coast up to Mexico (Baker and McDaniel 1972; Cuadrado-Ríos and Mantilla-Meluk 2016) and *U. b. convexum* occurring south of the Gulf and extending its distribution into South America (Hoffmann et al. 2003). These two subspecies hybridize on a contact zone at the Gulf of Fonseca, the origin of which has been explained as secondary contact after the completion of the physical connection between South and Central America and the gradual establishment of suitable environments for bat populations in what was suggested as a relatively recent event (Hoffmann et al. 2003).

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LITERATURE CITED

Alvarez, J., M. R. Willig, J. K Jones, Jr., and W. D. Webster. 1991. *Glossophaga soricina*. Mammalian Species 379:1–7

Baker, R. J. 1981. Chromosome flow between chromosomally characterized taxa of a volant mammal, *Uroderma bilobatum* (Chiroptera: Phyllostomatidae). Evolution 35:296–305.

Baker, R. J., and R. D. Bradley. 2006. Speciation in mammals and the Genetic Species Concept. Journal of Mammalogy 87:643–662

Baker, R. J., and R. V. Mc Daniel. 1972. A new subspecies of *Uroderma bilobatum* (Chiroptera, Phyllostomatidae) from Middle America. Occasional Papers, Museum of Texas Tech University 7:1–4.

Bradley, R. D., and R. J. Baker. 2001. A test of the Genetic Species Concept: cytochrome-*b* sequences and mammals. Journal of Mammalogy 82:960–973.

Coates, A. G., J. B. C.Jackson, and L. S. Collins. 1992. Closure of the Isthmus of Panama: the near-shore marine record of Costa Rica and Western Panama. Geological Society of America Bulletin 104:814–828.

Coates, A. G., and J. A. Obando. 1996. The geologic evolution of the Central American Isthmus. Pp. 21–56 in Evolution and environment in tropical America (J. B. C. Jackson, A. F. Budd, and A. G. Coates, eds.). The University of Chicago Press.

Collins, L. S., A. G. Coates, J. B. C. Jackson, and J. A. Obando. 1995. Timing and rates of emergence of the Limón and Bocas del Toro basins: Caribbean effects of Cocos Ridge subduction? Pp. 263–290 in Geologic and tectonic development of the Caribbean Plate Boundary in southern Central America (P. Mann, ed.). Geological Society of America Special Papers Vol. 295.

Cuadrado-Ríos, S., and H. Mantilla-Meluk. 2016. Timing the evolutionary history of tent-making bats, genus *Uroderma* (Phyllostomidae): a biogeographic context. Mammalian Biology 81:579–586.

Dengo, G. 1969. Problems of tectonic relations between Central America and the Caribbean: transactions of the Gulf Coast Association of Geological Societies 19:311–320.

Dengo, G. 1985. Mid America; tectonic setting from the Pacific margin from southern Mexico to northwestern Colombia. Pp. 123–180 in The ocean basins and margins, Vol. 7 (A. E. M. Nairn, and F. G. Stehli, eds.). Plenum Press, New York.

Donnelly, T. W., G. S. Horne, R. C. Finch, and E. López-Ramos. 1990. Northern Central America: the Maya and Chortís blocks. Pp. 37–76 in The geology of North America, Vol. H, The Caribbean region (G. Dengo and J. E. Case, eds.). Geological Society of America, Boulder, Colorado.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797.

Eisenberg, J. F., and K. H. Redford. 2000. Mammals of the Neotropics, Vol. 3, Ecuador, Bolivia, Brazil. The University of Chicago Press, Chicago, Illinois.

Fa, L. E., and L. M. Morales. 1991. Mammals and protected areas in the Trans-Mexican Neovolcanic Belt. Pp. 199–226 in Latin American mammalogy, history, biodiversity and conservation (M. A. Mares and D. J. Schmidly, eds.). University of Oklahoma Press, Norman.

Francis, A. H. 2005. Deformation history of the Maya and Chortís blocks: insight to the evolution of the Motagua fault zone, Guatemala. Master's thesis, Rice University, Houston, Texas.

Gannon, M. R., A. Kurta, A. Rodríguez-Durán, and M. R. Willig. 2005. Bats of Puerto Rico: an island focus and a Caribbean perspective. Texas Tech Press, Lubbock.

Gardner, A. L. 1986. The taxonomic status of *Glossophaga morenoi* Martinez and Villa, 1938 (Mammalia: Chiroptera: Phyllostomidae). Proceedings of the Biological Society of Washington 99:489–492.

Gardner, A. L. 2007. Family Phyllostomidae. Pp. 207–218 in Mammals of South America,Vol. 1, Marsupials, Xenarthrans, Shrews, and Bats (A. L. Gardner, ed.). University of Chicago Press, Chicago, Illinois.

Goldani, A., G. S. Carvalho, and J. C. Bicca-Marques. 2006. Distribution of Neotropical primates (Platyrrhini) based on parsimony analysis of endemicity. Brazilian Journal of Biology 66:61–74.

Goldman, E. A., and R. T. Moore. 1946. Biotic provinces of Mexico. Journal of Mammalogy 26:347–360.

Harold, A. S., and R. D. Mooi. 1994. Areas of endemism: definition and recognition criteria. Systematic Biology 43:439–441.

Hernández-Camacho, J., T. Walschburger, R. Ortiz Quijano, and A. Hurtado Guerra. 1992. Origen y distribución de la biota suramericana y colombiana. Pp. 55–104 in La Diversidad Biológica Iberoamericana (I. G. Halffter, ed.). Acta Zoológica Mexicana, Volumen Especial.

Hoffmann, F. G., and R. J. Baker. 2001. Systematics of the bats of the genus *Glossophaga* (Chiroptera: Phyllostomidae) based on cytochrome-*b*. Journal of Mammalogy 82:1092–1101.

Hoffmann, F. G., J. Owen, and R. J. Baker. 2003. mtDNA perspective of chromosomal diversification and hybridization in Peter's tent-making bat (*Uroderma bilobatum*: Phyllostomidae). Molecular Ecology 12:2981–2993.

Kalyaanamoorthy, S., B. Q. Minh, T. K. F. Wong, A. Von Haeseler, and L. S. Jermiin. 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14:587–589.

Longmire, J. L., M. Maltbie, and R. J. Baker. 1997. Use of "lysis buffer" in DNA isolation and its implication for museum collections. Occasional Papers, Museum of Texas Tech University 163:1–3.

Lorenzo, J. 1964. Los Glaciares de México. Monografías del Instituto de Geología, No. 1. Universidad Nacional Autónoma de México.

Mann, P., and K. Burke. 1984. Neotectonics of the Caribbean. Review of Geophysics 22:309–62.

Mann, P., C. Schubert, and K. Burke. 1990. Review of Caribbean neotectonics. Pp. 307–338 in The geology of North America, Vol. H, The Caribbean Region (G. Dengo and J. E. Case, eds.). Geological Society of America, Boulder, Colorado.

Maniatis, T., E. F. Fritsch, and J. Sambrock. 1992. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory, New York.

Mantilla-Meluk, H., and A. M. Jiménez-Ortega. 2006. Estado de conservación y algunas consideraciones biogeográficas sobre la quiropterofauna del Chocó Biogeográfico. Revista Institucional Universidad Tecnológica del Chocó Diego Luis Córdoba 25:10–17.

Mantilla-Meluk, H., A. M. Jiménez-Ortega, and R. J. Baker, 2009. Phyllostomid bats of Colombia: annotated checklist, distribution, and biogeography. Special Publications, Museum of Texas Tech University 56:1–37.

McBirney, A. R., and H. Williams. 1965. Volcanic history of Nicaragua. University of California Publications in Geological Sciences 55:1–65.

Merriam, C. H. 1985. Monographic revision of pocket gophers Geomyidae (exclusive of the species *Geomys*). North American Fauna 8:1–258.

Miller, S. A., D. D. Dikes, and H. F. Polesky. 1988. A simple 'salting out' procedure for extracting DNA for human nucleated cells. Nucleic Acids Research 16:215.

Minh, B. Q., M. A. T. Nguyen, and A. Von Haeseler. 2013. Ultrafast approximation for phylogenetic bootstrap. Molecular Biology and Evolution 30:1188–1195.

Montes, C., et al. 2012a. Evidence for middle Eocene and younger land emergence in central Panama: implications for Isthmus closure. Geological Society of America Bulletin 124:780–799.

Montes, C., et al. 2012b. Arc-continent collision and orocline formation: closing of the Central American seaway. Journal of Geophysical Research: Solid Earth 117(B4).

Montes, C.., et al. 2015. Middle Miocene closure of the Central American seaway. Science 348:226–229.

Moore, P. T. 1945. The Transverse Volcanic Biotic Province of Central Mexico and its relationship to adjacent provinces. Transactions of the San Diego Society of Natural History 10:217–236.

Morrone, J. J. 1994. On the identification of areas of endemism. Systematic Biology 43:438–441.

Nassar, J. M., N. Ramírez, and O. Linares. 1997. Comparative pollination biology of Venezuelan columnar cacti and the role of nectar-feeding bats in their sexual reproduction. American Journal of Botany 84:918–927.

Nguyen, L. T., H. A. Schmidt, A. von Haeseler, and B. Q. Minh. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32:268–274

O'Dea, A., et al. 2016. Formation of the Isthmus of Panama. Science Advances 2, e1600883, 1–11.

Petit, S. 1995. The pollinators of two species of columnar cacti on Curacao, Netherlands Antilles. Biotropica 27:538–541.

Rangel, J.O., and C. A. Garzón. 1994. Aspectos de la estructura, de la diversidad y de la dinámica de la vegetación del Parque Regional Natural Ucumarí. Pp. 85–108 in Ucumarí: Un caso típico de la diversidad biótica andina (J. O. Rangel, ed.). Publicaciones de la Carder, Pereira, Colombia.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Rosen, B. R. 1988. From fossils to earth history: Applied historical biogeography. Pp. 437–481 in Analytical biogeography: an integrated approach to the study of animal and plant distributions (A. A. Myers and P. Giller, eds.). Chapman and Hall, London.

Russell, R. J. 1968a. Evolution and classification of the pocket gophers of the subfamily Geomyinae. University of Kansas Publications of the Museum of Natural History 6:473–579.

Russell, R. J. 1968b. Revision of pocket gophers of the genus *Pappogeomys*. University of Kansas Publications of the Museum of Natural History 16:581–776.

Smith, H. M. 1940. An analysis of the biotic provinces of Mexico, as indicated by the distribution of lizards of the genus *Scleropus*. Anales de la Escuela Nacional de Ciencias Biológicas de México 2:95–110.

Trifinopoulos, J., L.-T. Nguyen, A. von Haeseler, and B. Q. Minh. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research 44:1–4.

Webster, W. D. 1993. Systematics and evolution of bats of the genus *Glossophaga*. Special Publications, Museum of Texas Tech University 36:1–184.

Webster, W. D., and C. O. Handley, Jr. 1986. Systematics of Miller's long-tongued bats, *Glossophaga longirostris*, with the description of two new subspecies. Occasional Papers, Museum of Texas Tech University 100:1–22.

Webster, W. D., and J. K. Jones, Jr. 1980. Taxonomic and nomenclatorial notes on bats of the genus *Glossophaga* in North America, with the description of a new species. Occasional Papers, Museum of Texas Tech University 71:1–12.

Webster, W. D., and J. K. Jones, Jr. 1982. A new subspecies of *Glossophaga commissarisi* (Chiroptera: Phyllostomidae) from western México. Occasional Papers, Museum of Texas Tech University 76:1–6.

Webster, W. D., and J. K. Jones, Jr. 1984a. *Glossophaga leachii*. Mammalian Species 226:1–3.

Webster, W. D., and J. K. Jones, Jr. 1984b. A new subspecies of *Glossophaga mexicana* (Chiroptera: Phyllostomidae) from southern México. Occasional Papers, Museum of Texas Tech University 91:1–5.

Webster, W. D., and J. K. Jones, Jr. 1985. *Glossophaga mexicana*. Mammalian Species 245:1–2.

Webster, W. D., and J. K. Jones, Jr. 1987. A new subspecies of *Glossophaga commissarisi* (Chiroptera: Phyllostomidae) from South America. Occasional Papers, Museum of Texas Tech University 109:1—-6.

Wickliffe, J. K., F. G. Hoffmann, D. S. Carroll, Y. V. Dunina-Barkovskaya, R. D. Bradley, and R. J. Baker. 2003. Intron 7 (*Fgb*-I7) of fibrinogen, B Beta polypeptide (*Fgb*): a nuclear DNA phylogenetic marker for mammals. Occasional Papers, Museum of Texas Tech University 219:1–6.

Wiens, J. J. 1998. Combining data sets with different phylogenetic histories. Systematic Biology 47:568–581.

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APPENDIX

Specimens examined for the combined analyses of nuclear and mitochondrial data and their geographic localities are given below. TK numbers correspond to samples from the Genetic Resources Collection at the Natural Science Research Laboratory, Museum of Texas Tech University, Lubbock, Texas; MVZ numbers correspond to samples from the Museum of Vertebrate Zoology, Berkeley, California; FMNH numbers correspond to samples from the Field Museum of Natural History, Chicago, Illinois; and USNM numbers correspond to samples from the National Museum of Natural History, Washington, D.C.

Glossophaga commissarisi.—TK 20586, MEXICO, Chiapas, Huixtla; USNM 578998, PANAMA, Bocas del Toro, Isla Popa.

Glossophaga leachii.—TK 4812, Mexico, Guerrero, El Carnizal.

Glossophaga longirostris.—TK 18585, GRENADA, St. George, St. Paul's; TK 25150, TRINIDAD AND TOBAGO, Tobago, St Patrick, Grange

Glossophaga morenoi.—TK 43176, Mexico, Michoacan, Aquila, Maruata.

Glossophaga soricina antillarum.—TK 9251, JAMAICA, St. Catherine Parish, Watermount; TK 11040, JAMAICA, St. Ann Parish, Discovery Bay, Green Grotto; TK 14159, MEXICO, San Luis Potosi, Ciudad Valles; FMNH 128675, FMNH 128676, PERU, Amazonas, Chachapoyas.

Glossophaga soricina soricina.—TK 25212, TRINIDAD AND TOBAGO, Trinidad, Mayaro, Guayaguayare; TK 15194, VENEZUELA, Guarico, Calabozo.

Leptonycteris curasoae.—TK 45107.

Monophyllus plethodon.—TK 15607.

Macrotus waterhousii.—TK 27889.

Additional specimens of *Glossophaga* included in this study relative to the previous one by Hoffmann and Baker (2001): *Glossophaga soricina valens.*—TK134501, TK134504, TK134721, TK134722, TK134740, TK134742, TK134743, TK134744, TK134745, TK134746, TK134747, TK134672, ECUADOR, Guayas, Manglares Churute.

Sequences 2512 and 2534 correspond to skin clips of bats sampled in the Tres Marias Islands, MEXICO, and have no associated vouchers.

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The Transcriptomes of Tent-making Bats (*Uroderma*): Testing for Adaptive Divergence in Recently Diverged Species

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Abstract

Molecular evidence of adaptation is of interest to evolutionary biologists trying to understand the role of adaptive divergence in the speciation process. In bats (Order Chiroptera), evidence of directional selection has been associated with drastic changes in lifestyle, especially those related to the evolution of flight and echolocation. Here, the transcriptomes of submandibular glands of two closely related species of tent-making bats (*Uroderma davisi* and *U. convexum*) were reported, annotated, and described. Also, episodic directional selection was tested in 21 genes reported to be involved in lipid lysis, transport, and usage for energetics in a phylogenetic framework centered on bats (Chiroptera), using novel genomic and transcriptomic data. Transcriptomes of the two *Uroderma* species were highly similar. Ectopic expression (in the submandibular salivary glands) of genes of interest (*C3*, *Lcn2*, *Psap*, and *Clu*) in tent-making bats was found, expanding an observation made earlier in *Myotis*. Eight out of the twenty-one genes screened had at least one codon under positive selection as detected by aBSREL. Four (*Apoe*, *Atgl*, *Hadh*, and *Lcn2*) were exclusive to some branches of the tree and four (*C3*, *Lsr, Plin1*, and *Psap*) appeared in more than one branch. These results indicate that these selective processes affecting the target loci are scattered along several branches, including those leading to species of *Uroderma*, rather than being restricted to the early divergence of bats from other mammals, where flight evolved. Results also indicate that the strength of selection over the energy generation systems in diverse lineages of bats involve both recruitment of new genes for ectopic expression and adaptive divergence of coding sequences.

Key words: Chiroptera, ectopic expression, episodic positive selection, free fatty acids, lipolysis, Phyllostomidae, submandibular salivary glands

Supplementary material related to this manuscript is available online at https://drive.google.com/open?id=1ZKXiSYfhbgIgPMsSkcAak3YNcDVjlEGx.

Introduction

With at least 59 genera and more than 200 species (Koopman 1993; Simmons 1998; Wetterer et al. 2000; Baker et al. 2003; Baker et al. 2016; Cirranello et al. 2016), the New World leaf-nosed bat family Phyllostomidae is the second largest of the extant bat families. It also exhibits more variation in morphological features than any other family-level group of mammals, including modifications associated with feeding habits that are also unusually diverse (Wetterer et al. 2000; Baker et al. 2003). Dietary specializations include sanguivory (blood-feeding), insectivory, carnivory, omnivory, nectarivory, palynivory (pollen-feeding), and frugivory. These dietary specializations occur in addition to those required by flight (an energetically demanding lifestyle [Speakman and Thomas 2003]).

Evolutionary adaptations can be manifested through changes in gene sequence and/or gene expression that contribute to locally adaptive phenotypes (Feder and Mitchell-Olds 2003; Coyne and Orr 2004). Transition from local divergence within species to incipient species is the first step of ecological specia-

tion, but early divergence is often subtle and difficult to characterize (Andrew and Riesberg 2013). In recent years, the advent of massively paralleled sequencing technologies (see, Metzker 2010 for a review) has permitted the generation of data providing new insights into chiropteran adaptations, using both genomic (Parker et al. 2013; Zhang et al. 2013) and transcriptomic (Shaw et al. 2012; Phillips et al. 2014) data analyses. Zhang et al. (2013) found a concentration of positively selected genes among those possibly associated with the evolution of flight. Parker et al. (2013) highlighted the convergence of directional selection signals throughout mammalian evolution in genes related to echolocation and vision. Further, Shaw et al. (2012) detected a smaller set of genes associated with structural development and highlighted a list of interesting genes for the study of defense against viral infections. Finally, Phillips et al. (2014) detected the ectopic expression of seven genes related to lipid metabolism and insulin resistance in the submandibular salivary gland in the insectivorous *M. lucifugus* and pointed to a relationship between diet and flight in gene expression. The results of Phillips et al. (2014) were consistent with those of Voigt et al. (2010), who reported that rapid combustion of recently ingested lipids was used to cover flight energetic demands by the insectivorous bat *Noctilio albiventris*. Undoubtedly, lipid energetics has been of central importance in the dietary specialization and adaptation to flight in bats. Examination of recently diverged bats that are not strictly insectivorous might help understand whether natural selection on genes related to lipid metabolism is continuing to operate at this level.

The *Uroderma bilobatum* (Peters' tent-making bat) species complex (Chiroptera: Phyllostomidae: Stenodermatini) offers an opportunity to identify genomic targets of selection among recently diverged taxa. This complex is distributed widely in the New World tropics. Five species of Peters' tent-making bat currently are recognized (*U. bilobatum*, *U. convexum*, *U. davisi*, *U. magnirostrum*, and *U. bakeri*; Mantilla-Meluk 2014 and references therein), supported by chromosomal differences (Baker et al. 1972; 1975 and Baker 1979; 1987), mitochondrial DNA sequences (Cuadrado-Ríos and Mantilla-Meluk 2016), and morphology (Mantilla-Meluk 2014). Among these, *U. davisi* (2n = 44) is found along the Pacific versant of El Salvador, Guatemala, Honduras, and Mexico, and, *U. convexum* (2n = 38) can be found in the remainder of Central America and along the Pacific versant of Colombia and northern Ecuador. These two species come into contact and hybridize at only one locality (Honduras, Departamento Valle, 17 km SSW of Nacaome) in Central America. These are not sister species, as *U. convexum* is sister to (*U. davisi* + *U. bakeri*) (Cuadrado-Ríos and Mantilla-Meluk 2016). However, average divergence between *U. davisi* and *U. convexum* is only 2.5% in the mitochondrial cytochrome-*b* gene (Hoffmann et al. 2003), and the most recent common ancestor was estimated at 3.8 MYA (Cuadrado-Ríos and Mantilla-Meluk 2016). Studies of genetic markers have shown limited hybridization and suggested diversifying selection might be at work in limiting introgression (proposed by Baker 1981; Greenbaum 1981; Barton 1982; see also Lessa 1990; and Hoffmann et al. 2003).

The recent divergence among species and the plausible role of diversifying selection in this process offers a suitable scenario to identify genetic targets of selection acting in early stages of speciation and to compare them with those found in other cases such as the chiropteran basal lineage or the major chiropteran clades, namely Yangochiroptera, and Yinpterochiroptera (Springer et al. 2001; Teeling et al. 2005). The study herein focused on two sets of candidate genes related to lipid catalysis and fatty acid energetics: a) a group of twenty-two loci posited to be important in fatty acid energetics in mammals (*e.g.* Schoiswohl et al. 2014); and b) a group of seven genes related to the regulation and processing of lipids that were found to express ectopically in salivary glands of the little brown bat (*M. lucifugus*, Phillips et al. 2014) (supp Table S1). The aims of this study were twofold. First, the genetic divergence between the hybridizing species of *Uroderma* using transcriptomes of submandibular salivary glands was characterized. Second, the role of directional selection on the aforementioned genes in the divergence of bats in a broader phylogenetic framework was examined to gain insights into the phylogenetic localization and functional significance of adaptive change at the molecular level.

MATERIALS AND METHODS

Sample collection, RNA extraction, and sequencing.—The submandibular gland (SMG) of one specimen each of *U. davisi* (TK169381) and *U. convexum* (TK165187) were collected and preserved in liquid nitrogen for posterior total RNA isolation with Trizol Reagent (Invitrogen, Carisbad, California, US). TK numbers correspond to samples archived in the Genetic Resources Collection of the Natural Science Research Laboratory, Museum of Texas Tech University, Lubbock, Texas. RNA and library preparation quality, purity and integrity were evaluated using a NanoDrop Spectrophotometer (Nanodrop Technologies, Wilmington, Delaware, USA) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, California, United States) (see supp. Appendix S1 for detailed estimates of quality, purity and integrity of samples, RIN, rRNA ratio, and 260/280 rate minimum values required). Poly-A based mRNA enrichment and paired-ends library preparation were conducted using Illumina TruSeqTM RNA sample preparation kits. Sequencing was performed on a Hiseq2000 and a Genome Analyzer IIx (*U. convexum* and *U. davisi*, respectively), using a complete lane for each sample.

Assembly and annotation.—Sequence quality descriptors were generated using FastQC (Andrews 2010), version 0.10.1, http://www.bioinformatics.babraham.ac.uk/projects/fastqc) which were used to guide read trimming with Fastx-toolkit (Gordon and Hannon 2010), version 0.0.13, http://hannonlab.cshl.edu/fastx_toolkit). Post-processed reads were assembled *de novo* using Trinity (Grabherr et al. 2011), separately for each sample. This is a method for efficient and robust *de novo* reconstruction of transcriptomes from RNA-seq data (Grabherr et al. 2011; Henschel et al. 2012); the algorithm partitions the sequence data into many individual de Bruijn graphs (de Bruijn 1946; Good 1946), each representing the transcriptional complexity at a given gene or locus, and then processes each graph independently to extract full-length splicing isoforms and to identify transcripts derived from paralogous genes.

Contigs from each assembly were annotated using a BLASTx search (*e-value* $\leq 1.00\times10^{-10}$) against *M. lucifugus* CDSs extracted from the OMA Browser (Altenhoff et al. 2018). For annotated contigs, the gene ontology distribution was assigned using the Blast2GO software function (Conesa et al. 2005; Götz et al. 2008) against the Swissprot database with stringency conditions similar to those used for blastx search. Through Blast2GO, contigs were classified into the three main GO (Gene Ontology) categories: molecular functions, biological processes, and cellular components. Each category also contains detailed inner GO terms assignments, allowing examination of function, processes and components.

Positively selected genes.—An initial set of 29 candidate genes (potential targets of positive selection) related to lipid catalysis and fatty acid energetics was defined from two sources: a) twenty-two loci previously identified as functionally important by Schoiswohl et al. (2014) in lipid catalysis or linked to the same GO terms (functions); and b) a set of seven genes that were found to express ectopically in the submandibular salivary glands of the little brown bat (*M. lucifugus)* by Phillips et al. (2014) (supp. Table S1). To identify orthologs for subsequent analysis of molecular adaptation, coding sequences from species of Chiroptera available on public databases were surveyed. Some were available as CDSs already annotated, whereas others were obtained from raw reads in transcriptome sequencing data (in these cases, filtering, assembly, and annotation was performed as previously described for the two species of *Uroderma*). These data were combined with those for major Laurasiatheria representatives and the assemblages of *U. davisi* and *U. convexum* (see Table 1). Only 1 to 1 orthology class, as classified by OMA-Browser v.24 (Altenhoff et al. 2018) was used for subsequent analyses. Nucleotide sequence alignments were combined with the corresponding aligned protein sequences using Pal2nal (Suyama et al 2006) and indels were retained. After excluding loci that were poorly represented in the targeted taxa, analyses were narrowed to 21 loci (see Table 2). Alignments for these genes were inspected visually and adjusted manually.

Phylogenetic relationships within Chiroptera were specified as in Agnarsson et al. (2011) and Feijoo and Parada (2017); the former defines all major clades, and the latter adds all additional taxa used in the study reported herein (Fig. 1). aBSREL (adaptive Branch-Site Random Effects Likelihood) (Smith et al. 2015)

was used to detect individual branches and sites subject to positive selection within Chiroptera, which was set as the foreground group. Computations were performed using the Datamonkey online suite (Weaver et al. 2018) using default validation parameters.

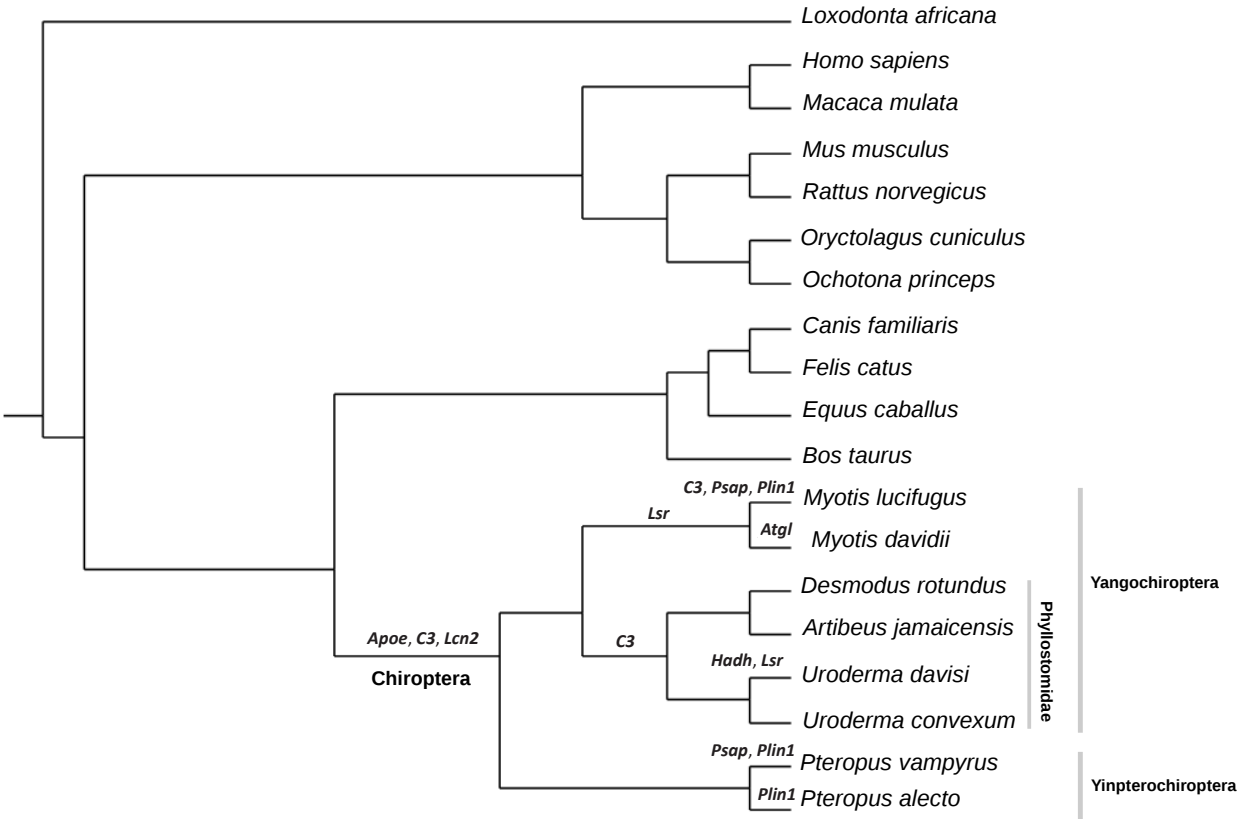

Figure 1. Phylogenetic relationships of taxa used in this study based on Bayesian analysis of multilocus data (following Agnarsson et al. 2011, Song et al. 2012, and Feijoo and Parada 2017). Some branches (Chiroptera, Yangochiroptera, and Yinpterochiroptera) are named as the corresponding crown groups. Name of loci that were recovered to be under positive selection are located along specific branches of the tree (see Table 2 for details on these loci).

RESULTS

Data generation and assembly.—One transcriptome was sequenced from the submandibular salivary gland of each of the hybridizing species, *U. convexum* and *U. davisi* with Illumina-SOLEXA (Bentley et al. 2008) technology, using adult specimens. Raw sequences generated herein also were used for a phylogenomic study (Feijoo and Parada 2017) and deposited in NCBI-SRA database (Table 1). After removal and trimming of reads, sequencing provided a total of 138.5 million high-quality (> 30 phred, each base) paired-end reads with average read-length of 74 bp. *De novo* assembly using Trinity (Grabherr et al. 2011) resulted in an average number of contigs assembled and average contig lengths of ~121,000 and 1,093 bp, respectively. By way of size comparison to a reference transcriptome of a related species (*M. lucifugus* 10.4 million bp), and assuming a similar transcriptome size for *U. bilobatum*, an average depth of 175x of bases sequenced was obtained for *U. davisi* and *U. convexum*. Completness of assembly assessed using BUSCO (Simao et al. 2015) with default parameters in gVolante (Nishimura and Kuraku 2017) with mammal references was similar, and sufficient for *de novo transcriptome* in both samples (63% and 75%, *U*

Table 1. Source of data for the 19 taxa used for analyses. For each species, the database for annotated sequences or accession number for raw sequences and the referenced article are provided.

Taxa	Database	Observations	Reference
Ingroup			
Major Lausrasiatheria representatives			
Bos taurus	OMA Browser 1:1 orthology	OMA Release v24	Altenhoff et al. 2018
Canis familiaris	OMA Browser 1:1 orthology	OMA Release v24	Altenhoff et al. 2018
Eqqus caballus	OMA Browser 1:1 orthology	OMA Release v24	Altenhoff et al. 2018
Felis catus	OMA Browser 1:1 orthology	OMA Release v24	Altenhoff et al. 2018
Homo sapiens	OMA Browser 1:1 orthology	OMA Release v24	Altenhoff et al. 2018
Macaca mulata	OMA Browser 1:1 orthology	OMA Release v24	Altenhoff et al. 2018
Mus musculus	OMA Browser 1:1 orthology	OMA Release v24	Altenhoff et al. 2018
Ochotona princeps	OMA Browser 1:1 orthology	OMA Release v24	Altenhoff et al. 2018
Oryctolagus cuniculus	OMA Browser 1:1 orthology	OMA Release v24	Altenhoff et al. 2018
Rattus norvegicus	OMA Browser 1:1 orthology	OMA Release v24	Altenhoff et al. 2018
Chiroptera			
Artibeus jamaicensis	SRA-NCBI SRR539297	de novo assembly-anotation for this article	Shaw et al. 2012
Desmodus rotundus	SRA-NCBI SRR606911	de novo assembly-anotation for this article	Francischetti et al. 2013
Myotis davidii	SRA-NCBI SRR628072	de novo assembly-anotation for this article	Zhang et al. 2013
Myotis lucifugus	OMA Browser 1:1 orthology	OMA Release v24	Altenhoff et al. 2018
Pteropus alecto	SRA-NCBI SRR628071	de novo assembly-anotation for this article	Zhang et al. 2013
Pteropus vampyrus	OMA Browser 1:1 orthology	OMA Release v24	Altenhoff et al. 2018
Uroderma convexum (2n = 38 cytotype)	SRX768594	de novo assembly-anotation for this article	Feijoo and Parada 2017; This article
Uroderma davisi (2n = 44 cytotype)	SRX768593	de novo assembly-anotation for this article	Feijoo and Parada 2017; This article
Outgroup			
Afrotheria			
Loxodonta africana	OMA Browser 1:1 orthology	OMA Release v24	Altenhoff et al. 2018

davisi and *U convexum,* respectively). Also, mapping reads to assembled transcripts indicated high-quality assemblies for which mapping rates were 73% and 75% for *U. davisi,* and *U. convexum,* respectively.

For phylogenetic analysis of positive selection at the 21 genes set along major lineages, a sequence data set of 19 taxa (Table 1) was constructed. This data set included the two *Uroderma* species, the *de novo* assembly and annotation of transcriptome raw data available on NCBI-SRA database of four additional bat species, and the OMA-Browser (Altenhoff et al. 2018) 1:1 orthologous genes of 13 species (*Myotis lucifugus, Pteropus vampyrus,* and ten additional species selected to represent major clades of Laurasiatheria, and one Probosciidea as outgroup).

Functional annotation of Uroderma *transcriptomes.*—Annotation of contigs against *M. lucifugus* CDSs from OMA database through Blastn (Altschul et al. 1990) analysis, resulted in 9,691 unique IDs for *U. davisi* and 11,783 for *U. convexum*. From these, 9,379 CDSs were shared between species, whereas 312 and 2,404 were restricted to *U. davisi* and *U. convexum*, respectively. For functional annotation, the international standardized gene functional classification system (Gene Ontology–GO–, Ashburner et al. 2000), using Blast2GO (Conesa et al. 2005) against the SwissProt (Uniprot Consortium 2018) database. This system offers a dynamic-updated controlled vocabulary and a strictly defined concept to comprehensively describe the properties of genes and their products in any organism using three main categories: Biological process, Molecular function and Cellular component. Then, the graphical data for comparisons of functional categories between species was done. In total, for both species, out of the 88% and 86% of the SwissProt/GO sequences with positive hits for *U. davisi* and *U. convexum*, respectively, 32% were classified for biological process, 32% for molecular function, and 36% for cellular component categories.

Positive selection.—From the initial list of 29 candidate genes, 21 were recovered for analyses (Table 2). Of these, 8 genes (38%) were found to be significantly selected for at least one branch. These included: a) four genes positive selected at individual lineages and b) four genes positive selected in multiple lineages. Three genes were positively selected along the branch leading to Chiroptera (*Apoe*, *C3*, and *Lcn2*), three genes were exclusively selected within Yangochiroptera (*Atgl*, *Lsr,* and *Hadh*) and two were positively selected in both Yango- and Yinpterochiroptera (*Plin1* and *Psap*). No genes were positive selected solely within Yinpterochiroptera. The genes *Lsr* and *Hadh* were positively selected in *U. davisi*, and *C3* was positively selected in *U. convexum.*

DISCUSSION

Comparison of the two Uroderma *transcriptomes.*—With 19,862 sequences of protein-coding DNA available for *M. lucifugus* in the OMA-Browser database, and assuming similar gene contents for the two *Uroderma* species, the submandibular gland transcriptome included ~50% (*U. davisi*) and ~60% (*U. convexum*) of genes currently identified in *M. lucifugus*. Their functional annotation showed minimal differences in biological functional categories assignments against the GO database (Fig. 2), as expected for two closely related species that have experienced recent divergence (Hoffmann et al. 2003). It was found, as expected, that the majority of sequences expressed in the submandibular gland were associated with housekeeping and that expressed loci tied to specific tissue functions were mostly shared between species (supp. Appendix S2). Nucleotide divergence between species calculated for genes of interest was, as expected, very low, 1 every 3650 bp (0,027%). Some genes were uncovered uniquely in one of these species, but transcriptomes of additional individuals are needed to understand whether such apparent differences in expression are characteristic of species or represent individual or temporal differences.

For *M. lucifugus*, a strictly insectivorous bat, Phillips et al. (2014) identified a set of proteins recruited for ectopic expression in the submandibular glands associated with lipids hydrolysis (CEL), insulin resistance to avoid lipid storage (C3, LCN2, and RETNLB) and lipid transport and receptor-mediated endocytosis (PSAP, APOE, and CLU). The analyses also uncov-

Table 2. Genes examined for positive selection in 19 taxa. The genes are identified as in the Ensembl database for *M. lucifugus* (* indicates *P. vampyrus* id where *M. lucifugus* ortholog was not found) and named following Uniprot. Lineages under positive selection are those for which analyses using aBSREL (Smith et al. 2015) uncovered at least some codons under positive selection. The first five loci are those selected from Phillips et al. (2014) and last 16 are taken from Schoiswohl et al. (2014) and from GO related term search.

Ensmbl gene ID (*M. lucifugus*)	Gene name	Lineages under positive selection (aBSREL)
ENSMLUG00000006546	*Apoe*	Chiroptera basal branch
ENSMLUG00000006721	*Clu*	None
ENSMLUG00000011254	*C3*	Chiroptera and Phyllostomidae basal branches, and *M. lucifugus*
ENSMLUG00000015746	*Psap*	*P. vampyrus* and *M. lucifugus*
ENSMLUG00000016210	*Lcn2*	Chiroptera basal branch
ENSMLUG00000006040	*Acsa*	none
ENSMLUG00000007408	*Appl2*	none
ENSPVAG00000014851*	*Atgl*	*M. davidii*
ENSMLUG00000024511	*Bscl2*	none
ENSMLUG00000001134	*Elmo3*	none
ENSMLUG00000008844	*Hadh*	*U. davisi*
ENSMLUG00000016739	*Hsl*	none
ENSMLUG00000000120	*Kdsr*	none
ENSMLUG00000029908	*Lsr*	*Myotis* basal branch and *U. davis*i
ENSMLUG00000017152	*Mgll*	none
ENSMLUG00000015655	*Pip4k2b*	none
ENSMLUG00000012035	*Plin1*	*P. alecto, P. vampyrus* and *M. lucifugus*
ENSMLUG00000010899	*Plin2*	none
ENSMLUG00000011457	*Plin3*	none
ENSMLUG00000008255	*Prkaca*	none
ENSMLUG00000012359	*Unv119*	none

ered, through reads mapping counts using RSEM (Li and Dewey 2011), consistent expression of *C3*, *Lcn2*, *Psap*, and *Clu* in the transcriptome of submandibular glands of both *Uroderma* specimens. *Uroderma* is known to be primarily frugivorous, although its diet includes insects as well (Fleming et al. 1972). It thus seems that ectopic expression of at least some of the genes discussed by Phillips et al. (2014) is not limited to insectivorous bats, such as the little brown bat (*Myotis*). The phylogenetic distribution of ectopic expression of these genes in the submandibular glands of bats warrants additional studies.

Phyllostomidae is the most diverse extant chiropteran family in terms of ecological and morphological features (Wetterer et al. 2000; Baker et al. 2003). Among Phyllostomidae, the *Uroderma bilobatum* complex is a particularly interesting group to study ongoing speciation and divergent selection processes. Two chromosomally distinct species (*U. davisi* and *U. convexum*) of this complex are known to hybridize in a contact zone, reflecting their phylogenetic affinity and some level of genetic compatibility. *U. davisi* occupies a somewhat more arid habitat than *U. convexum* (Baker et al. 1975). However, if the entire range of the

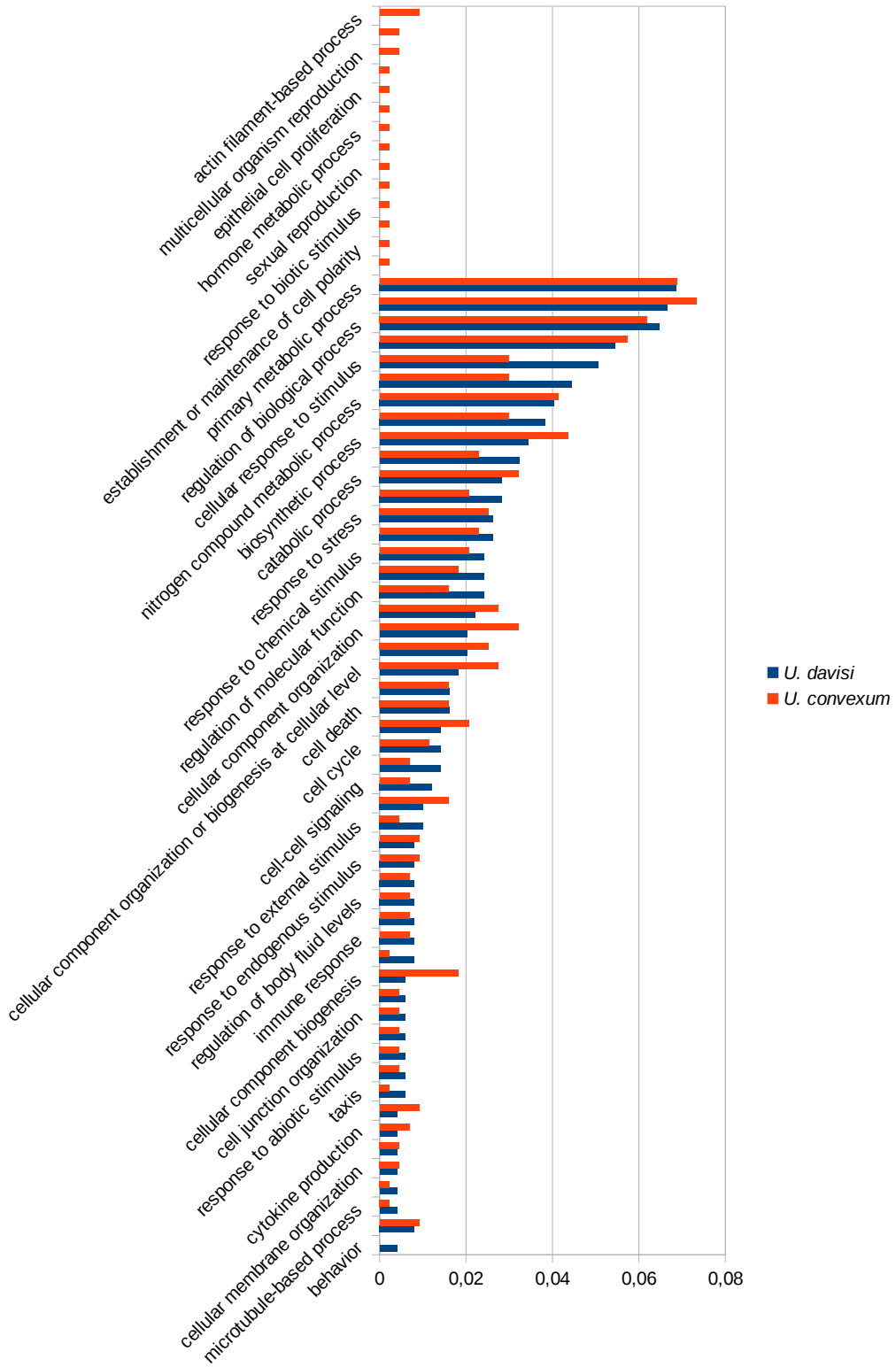

Figure 2. Comparison of the proportion of genes assigned to each biological category with Blast2GO (level 3, biological processes) in the transcriptomes of *U. davisi* and *U . convexum*. The first 14 and last 1 terms are uniquely represented in *U . convexum* and *U. davisi*, respectively.

two respective species is considered, there are no clear ecological factors to readily distinguish the habitat of the two chromosomal races (Baker et al. 1975). No differences have been reported, for example, in diet, roost characteristics or reproduction and life history. These general similarities do not rule out yet to be discovered ecological differences, but nonetheless suggest ecological divergence is limited. The hybrid zone formed by these two species has been extensively studied, and several studies suggest some level of selection against introgression (Baker 1981; Greenbaum 1981; Barton 1982; Lessa 1990; Owen and Baker 2001; Hoffmann et al. 2003). However, the role of diversifying selective forces in the generation and maintenance of divergence has been difficult to assess.

This study of a small set of 21 loci related to lipid processing and metabolism uncovered three loci under positive selection for phyllostomids. Of these, LSR (*lipolysis stimulated lipoprotein receptor*) was positively selected for *U. davisi* and also was uncovered as positively selected in the branch leading to *Myotis*. *C3* (*Complement component 3*) was positively selected in several bat lineages (including the branch leading to Phyllostomidae), and *Hadh* was positively selected in *U. davisi*. *Hadh* (Hydroxyacyl-CoA Dehydrogenase) is a member of the 3-hydroxyacyl-CoA dehydrogenase gene family. The encoded protein functions in the mitochondrial matrix to catalyze the oxidation of straight-chain 3-hydroxyacyl-CoA as part of the beta-oxidation pathway, where fatty acid molecules are broken down to generate acetyl-CoA. Then, acetyl-CoA enters the citric acid cycle and NADH and FADH2 co-enzymes, which are used in the electron transport chain (Yang et al. 2005; Houten et al. 2010). The lipolysis-stimulated lipoprotein receptor, LSR, is a multimeric protein complex in the liver that undergoes conformational changes upon binding of free fatty acids, thereby revealing a binding site (s) that recognizes both apoB and apoE. Its central role, described for rat and human, is the clearance of triglyceride-rich lipoprotein from blood (Yen et al. 2008). With regard to expression of a *C3* gene, for *M. lucifugus* it was reported that salivary gland uses the anaphylatoxin processing pathway (C3a–C3b) typically observed in hepatocytes. This is the enzymatic cleavage within the N-terminal of the nascent C3 protein that produces a 77 amino acid anaphylatoxin peptide (C3a) with immunological functions (Caporale et al. 1980; De Brujin and Frey 1985). The C3 protein that remains after cleavage is termed C3b. Consequently, hepatocytes secrete two proteins -the anaphylatoxin peptide (C3a) and a large C3b protein- both of which are processed from a single precursor. Besides the inmunological function of C3a, the C3b protein is associated with insulin resistance and free fatty acids trapping. Over-production of the C3b protein has been linked to hyperlipidemia in humans (Verseyden et al. 2003). In this sense, the hyperlipidemia resulting from the abundant secretion of the C3b protein would be advantageous in processing and using insect lipids while foraging, being used not only by insectivorous bats but also by omnivorous taxa.

Three of the 21 loci (14%) analyzed were determined to be under positive selection in *Uroderma*, and a total of eight of 21 loci (38%) were positively selected somewhere in the bat radiation. However, focus was set on a group of candidate genes associated with lipid processing and metabolism, which are known to be of particular physiological importance in bats. Most likely, therefore, the fraction of genes under positive selection across the bat genome in general is much smaller. On the other hand, phylogenetic methods for detecting selection aim at reducing type I error and will fail to detect selection in many instances (Anisimova and Yang 2007; Kosiol et al. 2008). Therefore, it seems that selective pressures on genes related to lipid metabolism have been very significant in the evolutionary diversification of bats. Additionally, the results show that these selective processes are scattered along several branches, including those leading to species of *Uroderma*, rather than being restricted to the early divergence of bats from other mammals, where flight evolved.

Positively selected genes.—Although identifying signatures of positive selection is only the first step in defining the genetic basis of species differentiation, such analyses are able to identify potential candidate genes of ecological, behavioral, morphological, physiological, or other functional significance. The aBSREL method allows w (=*dn*/*ds*) to change among lineages, as well as among codons. This is a more realistic assumption on how selective pressure acts and results in greater power for identifying selected sites when compared with other molecular selection algorithms (Murrell et al. 2012).

In addition to the genes positively selected in Phyllostomidae discussed above, five genes were positively selected somewhere in the bat radiation: *Atgl* (in *M. davidii*), *Plin1* (in *Pteropus vampyrus*, *Pteropus alecto*, and *M. lucifugus*), *Apoe* and *Lcn2* (in the Chiropteran basal lineage), and *Psap* (in *P. vampyrus* and *M. lucifugus*). The sequential hydrolysis of triacylglycerols in adipocytes producing free fatty acids is catalyzed by a cascade of lipolytic enzymes, with different substrate preferences (Watt 2008). The committed enzyme catalyzing the first step of triacylglycerol hydrolysis is ATGL (*Adipose triglyceride lipase*). ATGL is the major triacylglycerol lipase in adipose tissue and expression in other tissues is rather low (Morak et al. 2012). PLIN1 (*Perilipin 1*) is the major lipid droplet coat protein in mature adipocytes and plays a critical role in the regulation of lipolysis, the process via which fatty acids and glycerol are liberated from triglyceride in the lipid droplet (Girousse and Langin 2012; Zechner et al. 2012). The majority of perilipin is associated with the lipid droplet, although a small but significant proportion has been reported to be bound to the endoplasmic reticulum membrane, where it may be involved in the function of lipids droplets that bud from this organelle (Rochford 2014). PLIN1 controls the access to the adipocyte triglyceride stores and thus plays an important role in energy homeostasis. Depending on the energy state of the organism, PLIN1 either limits lipase access to stored triglyceride (in the fed state) or facilitates hormonally stimulated lipolysis (in the fasted state) (Ordovas 2017).

The secreted form of APOE (Apolipoprotein E) consists of two domains—the NH2-terminal domain that binds to the low-density lipoprotein (LDL) cell surface receptor, and the COOH-terminal that binds to LDLs (Mahley 1998). In humans, three *Apoe* alleles encode proteins that are associated with differing lipoprotein plasma concentrations (Elosua et al. 2003). Consequently, *Apoe* manly participates in the distribution or redstribution of lipids among various tissues and cells of the body (Huang and Mahley 2014). *Lcn2* (Lipocalin 2) is a cytokine with a role in regulating lipid metabolism and increasing insulin resistance (Guo et al. 2010; Jin et al. 2012). The protein has the b-barrel motif similar to other lipocalins, including an array of secretory and intracellular lipid-binding proteins (Flower 1996; Flower et al. 2000). In the extracellular milieu, the LCN2 protein can bind to fatty acids or iron and has been investigated from in connection with obesity, insulin resistance, and inflammation (Wang et al. 2007; Zhang et al. 2008). The LCN2 protein also modulates the peroxisome proliferator-activated receptor-c, which in turn regulates energy expenditure and lipid homeostasis in adipocytes (Spiegelman 1998). The PSAP (Prosaposin) protein is thought to be secreted before its final processing and the secreted form serves as a lipid transporter that delivers bound sphingolipids to cell plasma membranes and into an endocytotic pathway (Hiraiwa et al 1992). The intracellular prosaposin peptides—referred to as saposins (termed A, B, C, and D)—enhance lysosomal hydrolytic activity (Yuan et al. 2007).

Bat specializations related to lipid metabolism could be explained as related to hibernation and/or daily torpor mechanisms presented in major chiropteran lineages (Yuan et al. 2011), where efficient usage of adipose tissue for heat production and control is required. Another interesting mechanism is energetic performance during active flight. During flight, an elevated metabolic rate must be balanced by a great nutrient combustion for energy (Speakman and Thomas 2003). In this context, Voigt et al. (2010) proposed, in a study of metabolism in *Noctilio albiventris*, that bats might quickly mobilize and combust just-ingested nutrients instead of utilizing endogenous lipids. This may have enabled bats to conquer the nocturnal niche of aerial insectivory. Given the diversity of feeding habits that exist, these authors suggest that all bats might be using predominantly newly ingested nutrients to meet the high energy requirements of flapping flight. Therefore, genes associated with these processes might be expected to be under positive selection. In contrast to *M. lucifugus,* the diet of *U. bilobatum* consists of fruits and a small fraction of insects (e.g. Fleming et al. 1972). These findings are in line with the views of Voigt et al. (2010) in that selection acting on energy generation systems may be widespread in diverse lineages of bats.

De novo assembly of sequences of non-model species is a rapidly growing area of research and of methodological development (reviewed in Martin and Wang 2011) and evaluation (Misner et al. 2013; O'Neil and Emrich 2013). With increased taxonomic density and better annotation, the number of orthologous genes that can be reliably identified is expected to

increase rapidly. The present work limited analyses to candidate genes with previously published evidence of functional importance. The representation of lineages of bats is significantly increased relative to that of Shen et al. (2010); in relation to Parker et al. (2013), bat taxa sampled were increased and a more recent and sensitive method for testing positive selection was used. As illustrated by this work, taxonomic density may be greatly increased by generating orthologous sets of genes via transcriptomics and combining them with genomic data (see also Feijoo and Parada 2017).

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LITERATURE CITED

Agnarsson, I., C. M. Zambrana-Torrelio, N. P. Flores-Saldana, and L. J. May-Collado. 2011. A time-calibrated species-level phylogeny of bats (Chiroptera, Mammalia). PLoS Currents 10:RRN1212.

Altenhoff, A. M., et al. 2018. The OMA orthology database in 2018: retrieving evolutionary relationships among all domains of life through richer web and programmatic interfaces. Nucleic Acids Research 46:D477–D485.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. Journal of Molecular Biology 215:403–410.

Andrew, R. L., and L. H. Riesberg. 2013. Divergence is focused on few genomic regions early in speciation: incipient speciation of sunflower ecotypes. Evolution 67:2468–2482.

Andrews, S. 2010. FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Anisimova, M., and Z. Yang. 2007. Multiple hypothesis testing to detect lineages under positive selection that affects only a few sites. Molecular biology and evolution 24:1219–1228.

Ashburner, M., et al. 2000. Gene ontology: tool for the unification of biology. Nature genetics 25:25.

Baker, R. J. 1979. Karyology. Pp. 107-155 in Biology of the Bats of the New World Family Phyllostomatidae, Part III (R. J. Baker, J. K. Jones, Jr. and D. C. Carter, eds.). Special Publications 16, Museum of Texas Tech University, Lubbock.

Baker, R. J. 1981. Chromosome flow between chromosomally characterized taxa of a volant mammal, *Uroderma bilobatum* (Chiroptera: Phyllostomidae). Evolution 35:296–305.

Baker, R. J., W. R. Atchley, and V. R. McDaniel. 1972. Karyology and morphometrics of Peters tent-making bat *Uroderma bilobatum* (Chiroptera: Phyllostomidae). Systematic Zoology 21:414–429.

Baker, R. J., W. J. Bleier, and W. R. Atchley. 1975. A contact zone between characterized taxa of *Uroderma bilobatum* (Chiroptera: Phyllostomidae). Systematic Zoology 24:133–142.

Baker, R. J., and C. L. Clark. 1987. *Uroderma bilobatum*. Mammalian Species 279:1–4.

Baker, R. J., S. R. Hoofer, C. A. Porter, and R. A. Van Den Bussche. 2003. Diversification among New World Leaf-Nosed Bats: an evolutionary hypothesis and classification inferred from digenomic congruence of DNA sequence. Occasional Papers, Museum of Texas Tech University 230:1–32.

Baker, R. J., S. Solari, A. Cirranello, and N. B. Simmons. 2016. Higher level classification of phyllostomid bats with a summary of DNA synapomorphies. Acta Chiropterologica 18:1–38.

Barton, N. H. 1982. The structure of the hybrid zone in *Uroderma bilobatum* (Chiroptera: Phyllostomidae). Evolution 36:863–866.

Bentley, D. R., S. Balasubramanian, H. P. Swerdlow, G. P. Smith, J. Milton, C. G. Brown, ... and J. M. Boutell. 2008. Accurate whole human genome sequencing using reversible terminator chemistry. Nature 456:53.

Caporale, L. H., P. S. Tippett, B. W. Erickson, and T. E. Hugli. 1980. The active site of C3a anaphylatoxin. Journal of Biological Chemistry 255:10758–10763.

Cirranello, A., N. B. Simmons, S. Solari, and R. J. Baker. 2016. Morphological diagnoses of higher-level phyllostomid taxa (Chiroptera: Phyllostomidae). Acta Chiropterologica 18:39–71.

Conesa, A., S. Götz, J. M. Garcia-Gomez, J. Terol, M. Talon, and M. Robles. 2005. "Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research". Bioinformatics 21:3674–3676.

Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer Associates, Sunderland, Massachusetts.

Davis, W. D. 1968. Review of the genus *Uroderma* (Chiroptera). Journal of Mammalogy 49:676–698.

De Bruijn, M. H., and G. H. Fey. 1985. Human complement component C3: cDNA coding sequence and derived primary structure. Proceedings of the National Academy of Sciences 82:708–712.

De Bruijn, N. G. 1946. A combinatorial problem. Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen 49:758–764.

Elosua, R., et al. 2003. Obesity modulates the association among APOE genotype, insulin, and glucose in men. Obesity Research 11:1502–1508.

Feder, M. E., and T. Mitchell-Olds. 2003. Evolutionary and ecological functional genomics. Nature Reviews Genetics 4:651–657.

Feijoo, M., and A. Parada. 2017. Macrosystematics of eutherian mammals combining HTS data to expand taxon coverage. Molecular Phylogenetics and Evolution 113:76–83.

Fleming, T. H., E. T. Hooper, and D. E. Wilson. 1972. Three Central American bat communities: structure, reproductive cycles and movement patterns. Ecology 53:555–569.

Flower, D. R. 1996. The lipocalin protein family: structure and function. Biochemical Journal 318:1–14.

Flower, D. R., A. C. North, and C. E. Sansom. 2000. The lipocalin protein family: structural and sequence overview. Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology 1482:9–24.

Francischetti, I., et al. 2013. The "Vampirome": Transcriptome and proteome analysis of the principal and accessory submaxillary glands of the vampire bat *Desmodus rotundus*, a vector of human rabies. Journal of Proteomics 82:288–319.

Girousse, A., and D. Langin. 2012. Adipocyte lipases and lipid droplet-associated proteins: insight from transgenic mouse models. International Journal of Obesity 36:581–594.

Grabherr, M. G., et al. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nature Biotechnology 29:644–652.

Good, I. J. 1946. Normal recurring decimals. Journal of the London Mathematical Society 21:167–169.

Gordon, A., and G. J. Hannon. 2010. Fastx-toolkit. http://hannonlab.cshl.edu/fastx_toolkit.

Götz, S., et al. 2008. High-throughput functional annotation and data mining with the Blast2GO suite. Nucleid Acids Research 36:3420–3435.

Greenbaum, I. F. 1981. Genetic interactions between hybridizing cytotypes of the tent-making bat (*Uroderma bilobatum*). Evolution 35:305–320.

Guo, H., et al. 2010. Lipocalin 2 deficiency impairs thermogenesis and potentiates diet-induced insulin resistance in mice. Diabetes 59:1376–1385.

Henschel, R., P. M. Nista, M. Lieber, B. J. Haas, L. S. Wu, and R. D. LeDuc. 2012.Trinity RNA-Seq assembler performance optimization. P. 45 in Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment: Bridging from the eXtreme to the campus and beyond. ACM.

Hiraiwa, M., S. Soeda, Y. Kishimoto, and J. S. O'Brien. 1992. Binding and transport of gangliosides by prosaposin. Proceedings of the National Academy of Sciences 89:11254–11258.

Hoffmann, F. G., J. G. Owen, and R. J. Baker. 2003. mtDNA perspective of chromosomal diversification and hybridization in Peters' tent-making bat (*Uroderma bilobatum*: Phyllostomidae). Molecular Ecology 12:2981–2993.

Houten, S. M., and R. J. Wanders. 2010. A general introduction to the biochemistry of mitochondrial fatty acid β-oxidation. Journal of Inherited Metabolic Disease 33:469–477.

Huang, Y., and R. W. Mahley. 2014. Apolipoprotein E: structure and function in lipid metabolism, neurobiology, and Alzheimer's diseases. Neurobiology of Disease 72:3–12.

Jin, D., et al. 2011. Lipocalin 2 is a selective modulator of peroxisome proliferator-activated receptor-γ activation and function in lipid homeostasis and energy expenditure. The FASEB Journal 25:754–764.

Kosiol, C., et al. 2008. Patterns of positive selection in six mammalian genomes. PLoS Genetics 4:e1000144.

Koopman, K. F. 1993. Order Chiroptera. Pp. 137–241 in Mammal Species of the World, a Taxonomic and Geographic Reference (D Wilson and DM Reeder, eds.). Second Edition, Smithsonian Institution Press, Washington.

Lessa, E. P. 1990. Multidimensional analysis of geographic genetic structure. Systematic Biology 39:242–252.

Li, B., and C. N. Dewey. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12:323.

Mahley, R. W. 1988. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 240:622–630.

Martin, J. A., and Z. Wang. 2011. Next-generation transcriptome assembly. Nature Reviews Genetics 12:671–682.

Metzker, M. L. 2010.Sequencing technologies-the next generation. Nature Reviews Genetics 11:31–46.

Misner, I., C. Bicep, P. Lopez, S. Halary, E. Bapteste, and C. E. Lane. 2013. Sequence Comparative Analysis using Networks (SCAN): software for evaluating de novo transcript assembly from next generation sequencing. Molecular Biology and Evolution 30:1975–86.

Morak, M., et al. 2012. Adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) deficiencies affect expression of lipolytic activities in mouse adipose tissues. Molecular and Cellular Proteomics 11:1777–1789.

Murrell, B., J. O. Wertheim, S. Moola, T. Weighill, K. Scheffler, and S. L. Kosakovsky Pond. 2012. Detecting individual sites subject to episodic diversifying selection. PLoS Genetics 8:e1002764.

Nishimura, O., Y. Hara, and S. Kuraku. 2017. gVolante for standardizing completeness assessment of genome and transcriptome assemblies. Bioinformatics 33:3635–3637.

O'Neil, T. S., and S. J. Emrich. 2013. Assessing De Novo transcriptome assembly metrics for consistency and utility. BMC Genomics 14:465.

Ordovas, J. M. 2017. Nutritional Genomics and Biological Sex. Pp. 557–568 in Principles of Gender-Specific Medicine (third edition), Academic Press, London.

Owen, J. G., and R. J. Baker. 2001. The *Uroderma bilobatum* (Chiroptera: Phyllostomidae) cline revisited. Journal of Mammalogy 82:1102–1113.

Parker, J., et al. 2013. Genome-wide signatures of convergent evolution in echolocating mammals. Nature 502:228–231.

Phillips, C. J., et al. 2014. Dietary and Flight Energetic Adaptations in a Salivary Gland Transcriptome of an Insectivorous Bat. PloS ONE 9:e83512.

Rochford, J. J. 2014. Mouse models of lipodystrophy and their significance in understanding fat regulation. Pp. 53–96 in Current topics in developmental biology, Vol. 109. Academic Press.

Schoiswohl, G., J. Aljammal, and E. E. Kershaw. 2014. Fat tissue. Pp. 191-198 in Metabolism of Human Diseases: Organ Physiology and Pathophysiology (E. Lammert and M. Zeeb, eds). Springer, New York.

Shaw, T. I., et al. 2012. Transcriptome sequencing and annotation for the Jamaican Fruit Bat (*Artibeus jamaicensis*). PLoS ONE 7:e48472.

Shen, Y. Y., L. Liang, Z. H. Zhu, W. P. Zhou, D. M. Irwin, and Y. P. Zhang. 2010. Adaptive evolution of energy metabolism genes and the origin of flight in bats. Proceedings of the Natural Academy of Science USA 107:8666–8671.

Simão, F. A., R. M. Waterhouse, P. Ioannidis, E. V. Kriventseva, and E. M. Zdobnov. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31:3210–3212.

Simmons, N. B. 1998. A reappraisal of interfamilial relationships of bats. Pp. 3–26 in Bat Biology and Conservation (T. H. Kunz and P. A. Racey, eds.). Smithsonian Institution Press, Washington, DC.

Smith, M. D., J. O. Wertheim, S. Weaver, B. Murrell, K. Scheffler, and S. L. Kosakovsky Pond. 2015. Less is more: an adaptive branch-site random effects model for efficient detection of episodic diversifying selection. Molecular Biology and Evolution 32:1342–1353.

Song, S., L. Liu, S. V. Edwards, and S. Wu. 2012. Resolving conflict in eutherian mammal phylogeny using phylogenomics and the multispecies coalescent model. Proceedings of the Natural Academy of Science USA 109:14942–14947.

Speakman, J. R., and D. W. Thomas. 2003. Physiological ecology and energetics of bats. Pp. 430–492 in Bat Ecology (T. H. Kunz and M. B. Fenton, eds.). University of Chicago Press, Chicago.

Spiegelman, B. M. 1998. Perspectives in diabetes PPAR-c: Adipogenic regulator and thiazolidinedione receptor. Diabetes 47:507–514.

Springer, M. S., E. C. Teeling, O. Madsen, M. J. Stanhope, and W. W. de Jong. 2001. Integrated fossil and molecular data reconstruct bat echolocation. Proceedings of the National Academy of Sciences 98:6241–6246.

Suyama, M., D. Torrents, and P. Bork. 2006. PAL2NAL: robust conversion of protein sequence alignments into the corresponding codon alignments. Nucleid Acids Research 34:W609–W612.

Teeling, E. C., M. S. Springer, O. Madsen, P. Bates, S. J. O'brien, and W. J. Murphy. 2005. A molecular phylogeny for bats illuminates biogeography and the fossil record. Science 307:580–584.

UniProt Consortium. 2018. UniProt: a worldwide hub of protein knowledge. Nucleic acids research, 47:D506–D515.

Verseyden, C., S. Meijssen, H. van Dijk, H. Jansen, and M. C. Cabezas. 2003. Effects of atorvastatin on fasting and postprandial complement component 3 response in familial combined hyperlipidemia. Journal of Lipid Research 44:2100–2108.

Voigt, C. C., K. SÖrgel, and D. K. N. Dechmann. 2010. Refueling while flying: foraging bats combust food rapidly and directly to power flight. Ecology 91:2908–2917.

Wang, Y., et al. 2007. Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. Clinical Chemistry 53:34–41.

Watt, M. J., and G. R. Steinberg. 2008. Regulation and function of triacylglycerol lipases in cellular metabolism. Biochemical Journal 414:313–325.

Weaver, S., S. D. Shank, S. J. Spielman, M. Li, S. V. Muse, and S. L. Kosakovsky Pond. 2018. Datamonkey 2.0: a modern web application for characterizing selective and other evolutionary processes. Molecular Biology and Evolution 35:773–777.

Wetterer, A. L., M. V. Rockman, and N. B. Simmons. 2000. Phylogeny of Phyllostomid Bats (Mammalia: Chiroptera): Data from diverse morphological system, sex chromosomes, and restriction sites. Bulletin of the American Museum of Natural History 248:1–200.

Yang, S. Y., X. Y. He, and H. Schulz. 2005. 3-Hydroxyacyl-CoA dehydrogenase and short chain 3-hydroxyacyl-CoA dehydrogenase in human health and disease. The FEBS Journal 272:4874–4883.

Yen, F. T., et al. 2008. Lipolysis stimulated lipoprotein receptor: a novel molecular link between hyperlipidemia, weight gain and atherosclerosis in mice. Journal of Biological Chemistry 283:25650–25659.

Yuan, L., et al. 2011. Adaptive Evolution of Leptin in Heterothermic Bats. PLoS ONE 6:e27189.

Yuan, W., et al. 2007. Saposin B is the dominant saposin that facilitates lipid binding to human CD1d molecules. Proceedings of the National Academy of Sciences 104:5551–5556.

Zechner, R., et al. 2012. FAT SIGNALS-lipases and lipolysis in lipid metabolism and signaling. Cell Metabolism 15:279–291.

Zhang, G., et al. 2013. Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. Science 339:456–460.

Zhang, J., Y. Wu, Y. Zhang, D. LeRoith, D. A. Bernlohr, and X. Chen. 2008. The role of lipocalin 2 in the regulation of inflammation in adipocytes and macrophages. Molecular Endocrinology 22:1416–1426.

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Ecology of *Platyrrhinus lineatus* in the Atlantic Forest of Paraguay: Reproductive Patterns, Activity, Seasonality, and Morphometric Variability

John D. Stuhler, Ashlyn N. Kildow, and Richard D. Stevens

Abstract

Frugivorous bats are some of the most abundant and species-rich chiropterans in the Neotropics and are critical to maintenance and function of ecological processes in many tropical and subtropical forests. Nonetheless, basic information on ecology of most species is lacking. Sexual and geographic morphometric variation, nightly and monthly activity, and reproductive patterns of *Platyrrhinus lineatus* were characterized from two sites in the Atlantic Forest of eastern Paraguay. No differences were found in cranial or external characters between sites, nor differences in cranial characters between sexes. However, significant differences were found in external characters between sexes, with females being larger than males for six of the 14 measurements. *Platyrrhinus lineatus* exhibited considerable seasonal variation in activity, with the greatest number of bats active during the summer months. Moreover, activity peaked in the middle of the night. The proportion of reproductive males peaked in April and May. Although in other months the proportion of reproductively active males was variable, none exhibited descended testes in August. Pregnancy increased from the end of winter into summer, with all captured females being pregnant during the three months from August to October. Lactation exhibited a similar but lagging pattern. The observation of females simultaneously pregnant and lactating demonstrated a seasonally polyestrous breeding strategy. Together, these results help characterize understudied characteristics of a common frugivorous bat from the Atlantic Forest.

Key words: activity patterns, Atlantic Forest, Paraguay, *Platyrrhinus lineatus,* Reserva Natural del Bosque Mbaracayú, secondary sexual dimorphism, white-lined broad-nosed bat, Yaguareté Forests

Introduction

Environmental heterogeneity measured at scales ranging from individual habitat patches to the biosphere is an important determinant of diversity of organisms (Huston 1994). One growing contemporary form of environmental heterogeneity is anthropogenic habitat modification. Natural systems are being transformed for human benefit at the fastest rate in recorded time (Fargione et al. 2008). Moreover, the biota in general and bats in particular respond rapidly and robustly to human-modified changes (García-Morales et al. 2013; Jung and Threlfall 2018; Ramos Pereira 2018). Another important and more natural form of environmental heterogeneity important to the structure of ecological communities is climatic seasonality (Mello 2009; Stevens and Amarilla-Stevens 2012). At higher latitudes, distinct summer and winter seasons impose very different environmental regimes on the biota that require various adaptations for species to persist, in particular those that allow effects of freezing temperatures and resource reductions to be overcome (Wiens et al. 2010). Bats of the family Phyllostomidae exhibit strong responses to environmental heterogeneity. Abundance of individual species and overall diversity of communities changes when landscapes become more fragmented (Gorresen and Willig 2004; Meyer et al. 2016) and when seasons change from summer

to winter (Stevens and Amarilla-Stevens 2012; Sicotti Maas et al. 2018). In the Neotropics, variation in bat communities stems from responses of frugivorous bats to environmental heterogeneity. Indeed, frugivorous bats are numerous and critical to the maintenance and function of ecological processes in many tropical and subtropical forests (e.g., Fleming and Heithaus 1981; Muscarella and Fleming 2007).

Despite their importance, basic information on the ecology of most frugivorous bat species is lacking. *Platyrrhinus lineatus* (Geoffroy St.-Hilaire 1810) is a medium-sized (25 g; Willig 1983) frugivorous bat that is distributed from northeastern Brazil to Bolivia, Paraguay, and northern Argentina and into northern Uruguay (Gardner 2008). *Platyrrhinus lineatus* is most abundant in moderately fragmented forest landscapes, particularly those with many small forest patches and abundant secondary vegetation (Gorresen and Willig 2004). This is possibly due to greater availability of diverse food items (e.g., *Ficus* and *Vismia* spp.; Willig and Hollander 1987; Willig et al. 1993). Importantly, although much is known about diet of *P. lineatus* (e.g., Sazima 1976; Gardner 1977; Aguiar 2005; Silvestre et al. 2016), less is known about other characteristics of its ecology (but see Aguiar and Marinho-Filho 2004). Morphometric variation (i.e., cranial and external characters), nightly and monthly activity, and reproductive patterns of *P. lineatus* were characterized from two sites in the Atlantic Forest of eastern Paraguay.

METHODS

Study sites.—Fieldwork was conducted at two sites in interior Atlantic Forest of Paraguay—Reserva Natural del Bosque Mbaracayú (RNBM) and Yaguareté Forests (Fig. 1). The RNBM is a 66,000 hectare reserve located approximately 30 km east of the village of Ygatimí in the Department of Canindeyú (24°07.69'S, 55°30.34'W) at an elevation of 250 m (Willig et al. 2000). The RNBM was established through a federal mandate in 1992 to conserve in perpetuity one of the largest contiguous parcels of interior Atlantic Forest that remains in South America. Although the reserve includes mesophytic broadleaf tall, medium, and short forests, tall forest is most common. The five most common plant species at this site were *Sorocea bonblandii*, *Campomanasia xanthocarpa*, *Chrysophyllum. gonocarpum*, *Myrciaria baporeti*, and *Balfourodendron reidelianum* (Keel et al. 1993), all of which represent plant families that are included in diets of frugivorous bats (Gardner 1977).

Yaguareté Forests was located approximately 40 km east of Santa Rosa de Lima in the Department of San Pedro (23°48.50'S, 56°07.68'W) at an elevation of approximately 250 m (Willig et al. 2000). At the time of data collection, this private reserve was established to operate an economically viable but environmentally sound sustainable timber management and wildlife conservation program. Ostensibly, harvest of trees was light; only a portion of the property was logged selectively on a 40-year rotation scheme (Yaguareté Forests LDC 1996). Yaguareté Forests occupied approximately 16,000 ha and was bordered to the south and east by the Aguaray-Guazú River and on the west by the Verde River; both rivers are tributaries of the Jejui-Guazú River. This site was embedded in a transition zone between the Amambay and Central Paraguay ecoregions defined by Keel et al. (1993), and the property was characterized by mesic tree species of low stature, although some tall-dry forest habitats dominated by peroba (*Aspidosperma polyneuron*) occurred as well (Yaguareté Forests LDC 1996). Grassland-savanna habitats (Campos Cerrados) interdigitated with these forested habitats, enhancing spatial heterogeneity. Accordingly, 66% of the site was forested and 34% was mixed natural grassland and riparian habitats. The five most common plant species in rank abundance in the overstory were *M. baporeti*, *Ch. gonocarpum*, *Coutarea hexandra*, *Sweetia elegans*, and *Dendropanex cuneatus*, whereas the five most common species in the understory were *Coussarea platyphylla*, *So. bondplandii*, *Fagara naranjillo*, *Co. hexandra*, and *Sw. elegans* (Jin and Oren 1997).

In general, 10 standard mist nets (3 by 12 m, 1.5-inch mesh) were erected on roads and trails and in open areas along edges of emergent vegetation each night. Netting was conducted only on nights that had a half-moon or less to minimize effects of lunar phobia. Nets were checked hourly; bats were sacrificed and prepared as standard museum specimens or released

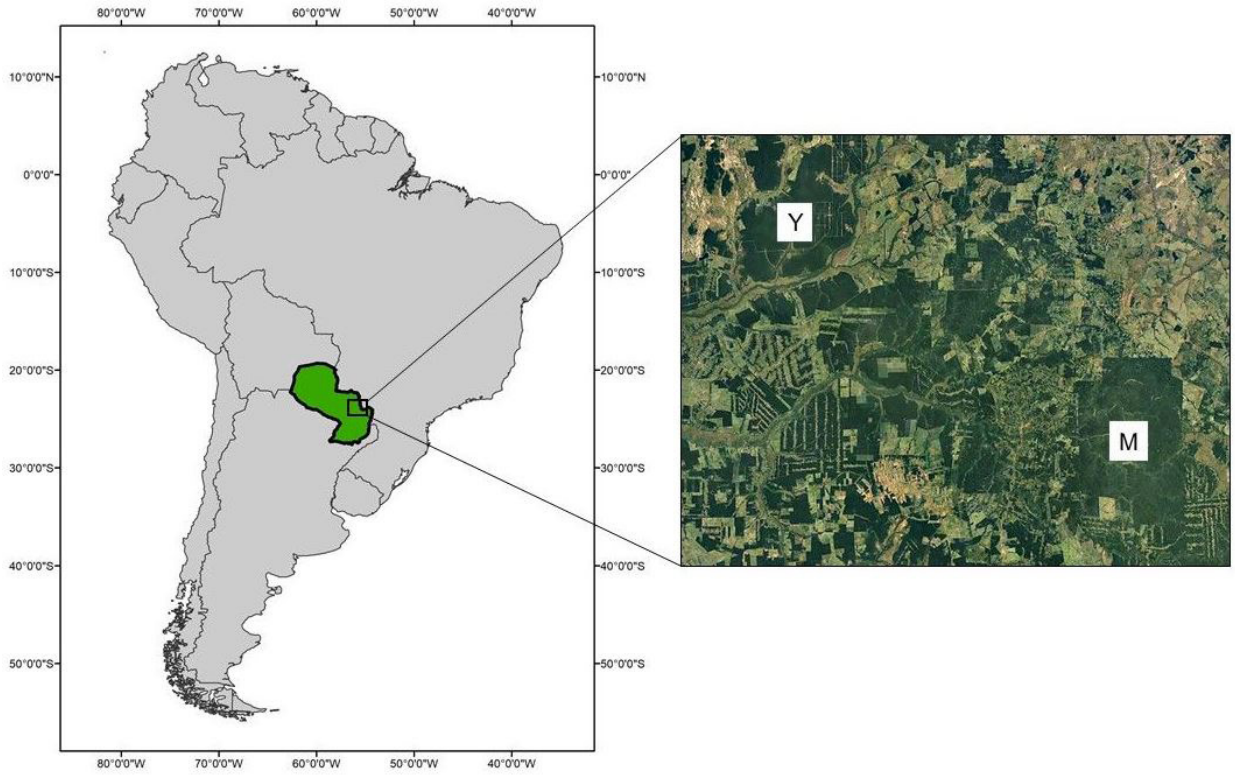

Figure 1. Locations of the Reserva Natural del Bosque Mbaracayú (M) and Yaguareté Forests (Y) in eastern Paraguay. Inset illustrates the pattern of human land-use at the time of sampling (1996–1998). Imagery was obtained from the Time Timelapse website http://world.time.com/timelapse/, accessed 4 February 2019.

after identification. To understand monthly activity patterns, nets were typically opened at dusk and monitored until midnight. Only data from mist nets that were monitored from dusk until dawn (RNBM: 8 nights; Yaguareté Forests: 16 nights) were used to quantify nightly activity patterns. In total, 4,516 bats were captured at the RNBM and 2,740 bats were captured at Yaguareté Forests. One-half of the collection of specimens was deposited at the Museum of Texas Tech University, and the other half at the Museo Nacional de Historia Natural del Paraguay. Characteristics of the entire bat community at each site are described in detail in Stevens et al. (2004).

Upon capture, age (juvenile, subadult, or adult, based on Brunet-Rossinni and Wilkinson 2009), sex, and reproductive condition (males: testes descended or not; females: pregnant or not and lactating or not) was determined for each individual. For morphometric and reproductive analyses, only adults were used. Fourteen external morphological characters of each individual were measured with a ruler to the nearest 1 mm, as follows: total body length, forearm length, foot length, calcar length, tibia length, ear length, tragus length, lengths of digits 1–5, nose-leaf length, and nose-leaf width.

Morphometrics.—A total of 94 adult specimens (44 females, 50 males) of *P. lineatus* were examined from the RNBM and Yaguareté Forests. A series of 13 cranial and three mandibular characters (breadth across upper canines, breadth across upper molars, breadth of braincase, condylobasal length, greatest length of skull, greatest length of mandible, height of braincase, length of coronoid process, length of mandibular toothrow, length of maxillary toothrow, length of molariform toothrow, mastoid breadth, postorbital constriction, rostral breadth, width of widest molar, and zygomatic breadth; Willig 1983) were measured on each specimen using digital calipers, recorded to the nearest 0.01 mm. Each specimen was measured three times. Order was randomized to minimize serial non-independence.

Means for the three measurements of each character for each specimen were used in analyses after a log transformation (LaBarbera 1989).

Two different multivariate analyses of variance (MANOVA) were conducted. In the first, the 14 external measurements were dependent variables and sex (female versus male) and site (RNBM versus Yaguareté Forests) were independent variables. In the second, the same independent variables were used but the 13 cranial and three mandibular characters were dependent variables. Upon significance of the two-way MANOVA (i.e., $P < 0.05$), univariate two-way analyses of variance (ANOVA) were used for each character separately to estimate which of the individual characters exhibited significant differences and were likely contributing to the multivariate difference.

Nightly activity, monthly activity, and reproductive patterns.—To characterize nightly activity patterns, the number of individuals caught across each hour for each site was counted, as well as a check 30 minutes after the initial opening of the nets. Sample sizes for each site were relatively small, and because of close spatial proximity (~ 65 km) there was no reason to believe that nightly patterns would be different between the two sites. To this end, information from both sites were combined to characterize nightly activity. Then the number of individuals captured per check (i.e., hours after sunset; Aguiar and Marinho-Filho 2004) was summed, using differences in activity between months to characterize seasonality. Monthly activity was determined by counting the number of individuals caught and dividing this by the number of mist-net-meter hours of effort for that month.

Reproductive patterns for adult males and females were characterized by using proportional values (i.e., for each sex separately, the proportion of individuals each month that exhibited a particular reproductive state out of the total number of individuals of that sex). Reproductive states were pregnant or not and lactating or not for females and testes descended or not for males.

RESULTS

Netting was conducted for a total of 119 nights at the RNBM and 99 nights at Yaguareté Forests from June to August 1996 and January 1997 to February 1998. In total, 40 *P. lineatus* were captured at the RNBM and 89 were captured at Yaguareté Forests.

Morphometrics.—MANOVA indicated highly significant differences between sexes in terms of external characters (Table 1). Differences among sites and the site by sex interaction were nonsignificant. Two-way ANOVAs indicated that significant differences between sexes involved six of the 14 external characters (Table 2). In all cases, females were larger than males (Table 2). Differences between sites, sexes, and the site by sex interaction were nonsignificant based on cranial and mandibular characters (Table 1).

Nightly and monthly activity.—Nightly activity of *P. lineatus* was unimodal and peaked five hours after sunset (Fig. 2). Across months, *P. lineatus* exhibited considerable variation in seasonal activity. The greatest number of bats were captured during the austral summer (i.e., December; Fig. 3). Moreover, between the two peaks of highest activity, in April and December 1997, there was a fairly consistent decrease through winter (e.g., July and August 1997) and then an increase into the summer (Fig. 3).

Table 1. Results from MANOVA determining significant differences between sexes and sites and their interaction for both cranial and external characteristics separately.

	Cranial			External		
Factor	Pillai's Trace	F	*P*	Pillai's Trace	F	*P*
Sex	0.22	1.35	0.20	0.45	3.85	< 0.001
Site	0.21	1.25	0.26	0.16	0.89	0.57
Sex x Site	0.15	0.84	0.64	0.13	0.75	0.71

Table 2. Summary statistics for cranial (Mbaracayú: 24 males, 15 females; Yaguareté: 26 males, 29 females) and external (Mbaracayú: 22 males, 14 females; Yaguareté: 24 males, 26 females) characters of adult *Platyrrhinus lineatus* males and females. A two-way analysis of variance (based on log-transformed data) is presented for each character.

	Mbaracayú		Yaguareté		Analysis of variance				
	♂♂	♀♀	♂♂	♀♀	Factor	df	MS	F	Significance
					Breadth across upper canines				
Mean	7.57	8.29	7.20	7.60	Sex	1	0.02	1.41	0.24
SD	2.08	2.32	2.06	2.19	Site	1	0.02	1.42	0.24
n	24	15	26	29	Sex × Site	1	0.00	0.10	0.75
					Error	90	0.01		
					Breadth across upper molars				
Mean	10.47	10.6	10.27	10.45	Sex	1	0.001	4.36	0.04
SD	0.38	0.36	0.34	0.34	Site	1	0.001	5.37	0.02
n	24	15	26	29	Sex × Site	1	< 0.001	0.11	0.74
					Error	90	< 0.001		
					Breadth of braincase				
Mean	9.05	8.30	9.42	9.10	Sex	1	0.016	0.96	0.33
SD	2.49	2.44	2.37	2.47	Site	1	0.02	1.20	0.28
n	24	15	26	29	Sex × Site	1	0.002	0.14	0.71
					Error	90	0.017		
					Condylobasal length				
Mean	22.08	22.23	22.10	22.32	Sex	1	< 0.001	3.69	0.06
SD	0.40	0.54	0.51	0.38	Site	1	< 0.001	0.33	0.57
n	24	15	26	29	Sex × Site	1	< 0.001	0.16	0.69
					Error	90	< 0.001		
					Greatest length of skull				
Mean	24.71	24.74	24.65	24.79	Sex	1	< 0.001	0.83	0.36
SD	0.35	0.42	0.55	0.39	Site	1	< 0.001	0.01	0.94
n	24	15	26	29	Sex × Site	1	< 0.001	0.40	0.54
					Error	90	< 0.001		
					Greatest length of mandible				
Mean	15.67	15.86	15.64	15.9	Sex	1	< 0.001	9.75	< 0.01
SD	0.31	0.38	0.32	0.34	Site	1	< 0.001	0.003	0.96
n	24	15	26	29	Sex × Site	1	< 0.001	0.35	0.56
					Error	90	< 0.001		

Table 2. (cont.)

	Mbaracayú		Yaguareté		Analysis of variance				
	♂♂	♀♀	♂♂	♀♀	Factor	df	MS	F	Significance
					Height of braincase				
Mean	11.01	10.88	10.99	11.09	Sex	1	< 0.001	0.08	0.78
SD	0.23	0.23	0.27	0.31	Site	1	< 0.001	2.71	0.10
n	24	15	26	29	Sex × Site	1	< 0.001	3.73	0.06
					Error	90	< 0.001		
					Length of coronoid process				
Mean	5.82	5.86	5.78	5.94	Sex	1	< 0.001	3.93	0.05
SD	0.22	0.18	0.35	0.17	Site	1	< 0.001	0.09	0.77
n	24	15	26	29	Sex × Site	1	< 0.001	1.14	0.29
					Error	90	< 0.001		
					Length of mandibular toothrow				
Mean	9.70	9.83	9.72	9.80	Sex	1	< 0.001	4.66	0.03
SD	0.25	0.18	0.20	0.26	Site	1	< 0.001	0.02	0.90
n	24	15	26	29	Sex × Site	1	< 0.001	0.16	0.69
					Error	90	< 0.001		
					Length of maxillary toothrow				
Mean	8.88	8.92	8.88	8.95	Sex	1	< 0.001	1.32	0.25
SD	0.23	0.23	0.22	0.22	Site	1	< 0.001	0.06	0.81
n	24	15	26	29	Sex × Site	1	< 0.001	0.14	0.71
					Error	90	< 0.001		
					Length of upper molariform toothrow				
Mean	6.12	6.10	6.08	6.15	Sex	1	< 0.001	0.29	0.59
SD	0.19	0.20	0.17	0.21	Site	1	< 0.001	0.03	0.87
n	24	15	26	29	Sex × Site	1	< 0.001	1.25	0.27
					Error	90	< 0.001		
					Mastoid breadth				
Mean	12.21	12.28	12.17	12.26	Sex	1	< 0.001	2.24	0.14
SD	0.25	0.18	0.27	0.29	Site	1	< 0.001	0.26	0.61
n	24	15	26	29	Sex × Site	1	< 0.001	<0.01	0.95
					Error	90	< 0.001		

Table 2. (cont.)

	Mbaracayú		Yaguareté		Analysis of variance				
	♂♂	♀♀	♂♂	♀♀	Factor	df	MS	F	Significance
					Postorbital constriction				
Mean	6.35	6.19	6.29	6.35	Sex	1	< 0.001	1.50	0.22
SD	0.19	0.19	0.17	0.21	Site	1	< 0.001	1.67	0.20
n	24	15	26	29	Sex × Site	1	0.001	7.27	0.01
					Error	90	< 0.001		
					Rostral breadth				
Mean	6.72	6.74	6.75	6.82	Sex	1	< 0.001	0.95	0.33
SD	0.25	0.24	0.23	0.22	Site	1	< 0.001	1.15	0.29
n	24	15	26	29	Sex × Site	1	< 0.001	0.22	0.64
					Error	90	< 0.001		
					Width of widest molar				
Mean	2.17	2.16	2.14	2.17	Sex	1	< 0.001	0.18	0.67
SD	0.08	0.12	0.08	0.11	Site	1	< 0.001	0.14	0.71
n	24	15	26	29	Sex × Site	1	< 0.001	1.23	0.27
					Error	90	< 0.001		
					Zygomatic breadth				
Mean	14.35	14.45	14.26	14.38	Sex	1	< 0.001	2.57	0.11
SD	0.26	0.37	0.34	0.33	Site	1	< 0.001	1.34	0.25
n	24	15	26	29	Sex × Site	1	< 0.001	0.01	0.92
					Error	90	< 0.001		
					Total length				
Mean	66.95	68.86	66.42	68.27	Sex	1	< 0.001	14.13	< 0.01
SD	1.40	3.01	2.04	2.46	Site	1	< 0.001	1.31	0.26
n	22	14	24	26	Sex × Site	1	< 0.001	0.00	0.99
					Error	82	< 0.001		
					Forearm length				
Mean	47.27	48.14	47.04	47.92	Sex	1	< 0.001	11.02	0.001
SD	1.12	1.10	1.27	1.20	Site	1	< 0.001	0.75	0.39
n	22	14	24	26	Sex × Site	1	< 0.001	0.00	0.98
					Error	82	< 0.001		

Table 2. (cont.)

	Mbaracayú		Yaguareté		Analysis of variance				
	♂♂	♀♀	♂♂	♀♀	Factor	df	MS	F	Significance
					Hindfoot length				
Mean	15.05	15.29	14.79	15.23	Sex	1	< 0.001	8.54	0.004
SD	0.49	0.47	0.51	0.59	Site	1	< 0.001	1.86	0.18
n	22	14	24	26	Sex × Site	1	< 0.001	0.73	0.39
					Error	82	< 0.001		
					Calcar length				
Mean	5.77	5.50	5.38	5.54	Sex	1	< 0.001	0.02	0.89
SD	1.27	0.65	0.58	0.58	Site	1	0.003	0.86	0.36
n	22	14	24	26	Sex × Site	1	0.004	1.46	0.23
					Error	82	0.003		
					Tibia length				
Mean	18.14	18.36	17.67	18.35	Sex	1	0.003	2.84	0.10
SD	0.77	0.63	1.76	0.75	Site	1	0.001	0.97	0.33
n	22	14	24	26	Sex × Site	1	0.001	0.88	0.35
					Error	82	0.001		
					Ear length				
Mean	19.59	19.86	19.46	19.92	Sex	1	< 0.001	6.42	0.01
SD	0.67	0.66	0.72	0.56	Site	1	< 0.001	0.05	0.82
n	22	14	24	26	Sex × Site	1	< 0.001	0.49	0.49
					Error	82	< 0.001		
					Tragus length				
Mean	7.59	7.71	7.42	7.69	Sex	1	0.003	2.57	0.11
SD	0.50	0.61	0.50	0.55	Site	1	0.001	0.63	0.43
n	22	14	24	26	Sex × Site	1	< 0.001	0.44	0.51
					Error	82	< 0.001		
					Length of digit one				
Mean	11.95	12.07	11.92	12.00	Sex	1	< 0.001	0.40	0.53
SD	0.65	0.83	0.72	0.63	Site	1	< 0.001	0.11	0.74
n	22	14	24	26	Sex × Site	1	< 0.001	0.01	0.95
					Error	82	< 0.001		

Table 2. (cont.)

	Mbaracayú		Yaguareté		Analysis of variance				
	♂♂	♀♀	♂♂	♀♀	Factor	df	MS	F	Significance
					Length of digit two				
Mean	40.09	40.38	39.58	41.19	Sex	1	0.002	3.50	0.07
SD	4.66	1.66	1.74	1.63	Site	1	< 0.001	0.17	0.68
n	22	14	24	26	Sex × Site	1	0.001	1.06	0.31
					Error	82	0.001		
					Length of digit three				
Mean	95.41	97.21	94.50	96.88	Sex	1	0.002	11.09	0.001
SD	2.24	1.85	4.06	2.16	Site	1	< 0.001	1.06	0.31
n	22	14	24	26	Sex × Site	1	< 0.001	0.26	0.61
					Error	82	< 0.001		
					Length of digit four				
Mean	71.68	69.14	71.38	73.62	Sex	1	0.01	0.71	0.40
SD	1.73	16.51	2.08	1.88	Site	1	0.02	2.13	0.15
n	22	14	24	26	Sex × Site	1	0.02	2.44	0.12
					Error	82	0.01		
					Length of digit five				
Mean	67.82	69.64	67.75	69.73	Sex	1	0.003	22.49	< 0.001
SD	1.74	1.78	1.89	1.80	Site	1	< 0.001	< 0.001	0.99
n	22	14	24	26	Sex × Site	1	< 0.001	0.04	0.84
					Error	82	< 0.001		
					Nose length				
Mean	12.27	12.43	12.54	12.5	Sex	1	< 0.001	0.04	0.84
SD	0.70	0.65	0.78	1.10	Site	1	0.001	0.51	0.48
n	22	14	24	26	Sex × Site	1	< 0.001	0.32	0.57
					Error	82	0.001		
					Nose width				
Mean	9.18	9.00	9.13	9.23	Sex	1	< 0.001	0.17	0.68
SD	0.50	0.39	0.34	0.43	Site	1	< 0.001	0.97	0.33
n	22	14	24	26	Sex × Site	1	0.001	2.34	0.13
					Error	82	< 0.001		

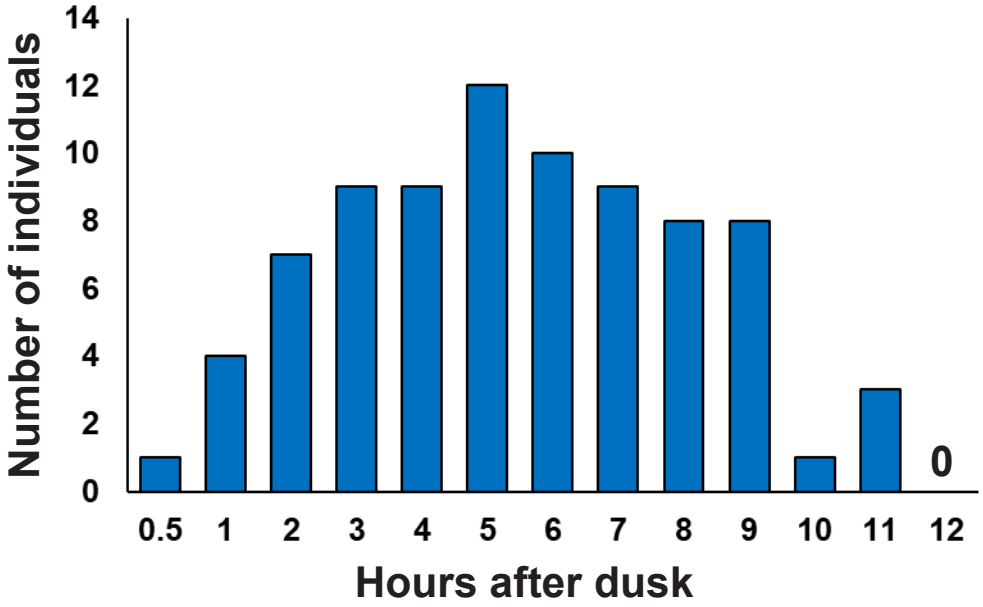

Figure 2. Nightly activity patterns of *Platyrrhinus lineatus* from Reserva Natural del Bosque Mbaracayú and Yaguareté Forests. Bars represent the number of individuals captured per hour, following dusk.

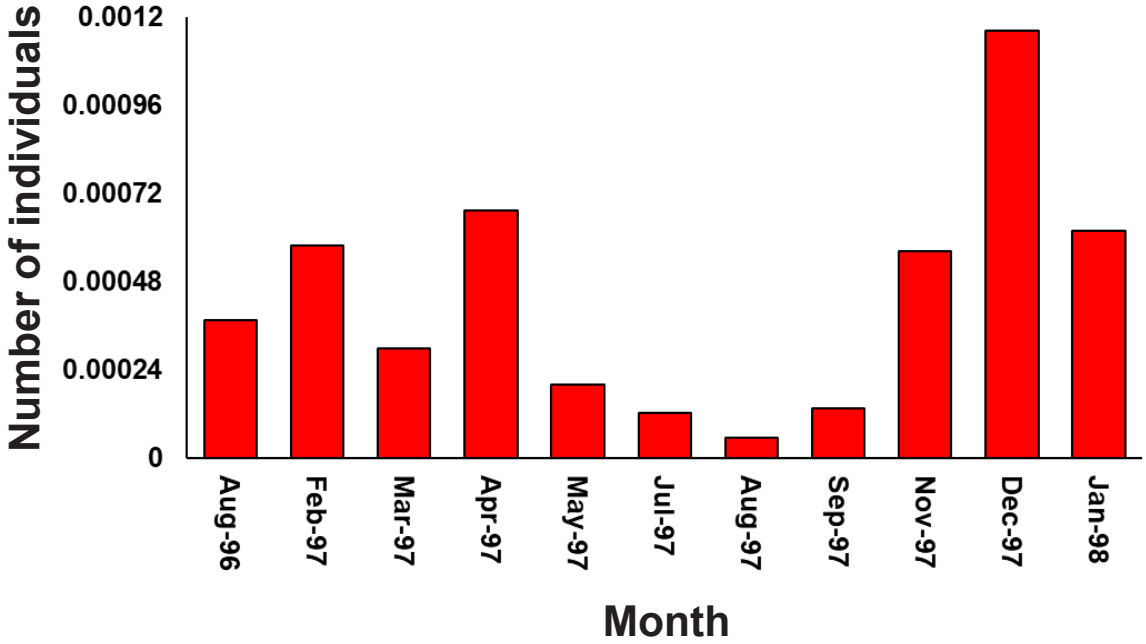

Figure 3. Monthly activity patterns of *Platyrrhinus lineatus* from Reserva Natural del Bosque Mbaracayú and Yaguareté Forests. Bars represent the total number of individuals captured per month, standardized by sampling effort (i.e., mist-net-meter hours).

Reproductive patterns.—Females exhibited a peak in pregnancy from August to October and a secondary peak in December (Fig. 4a). This was accompanied by complimentary peaks in lactation in April and November with the latter peak higher than the former (Fig. 4a). Three females captured at Yaguareté Forests were simultaneously pregnant and lactating. These individuals were caught during the secondary peak of reproduction, one on 4 November and two on 29 December. These observations taken together indicate that *P. lineatus* is seasonally polyestrous, at least at Yaguareté Forests. Males showed a similar reproductive pattern—there was a large peak in the proportion of adult males with testes descended between April and May and a smaller peak in December (Fig. 4b).

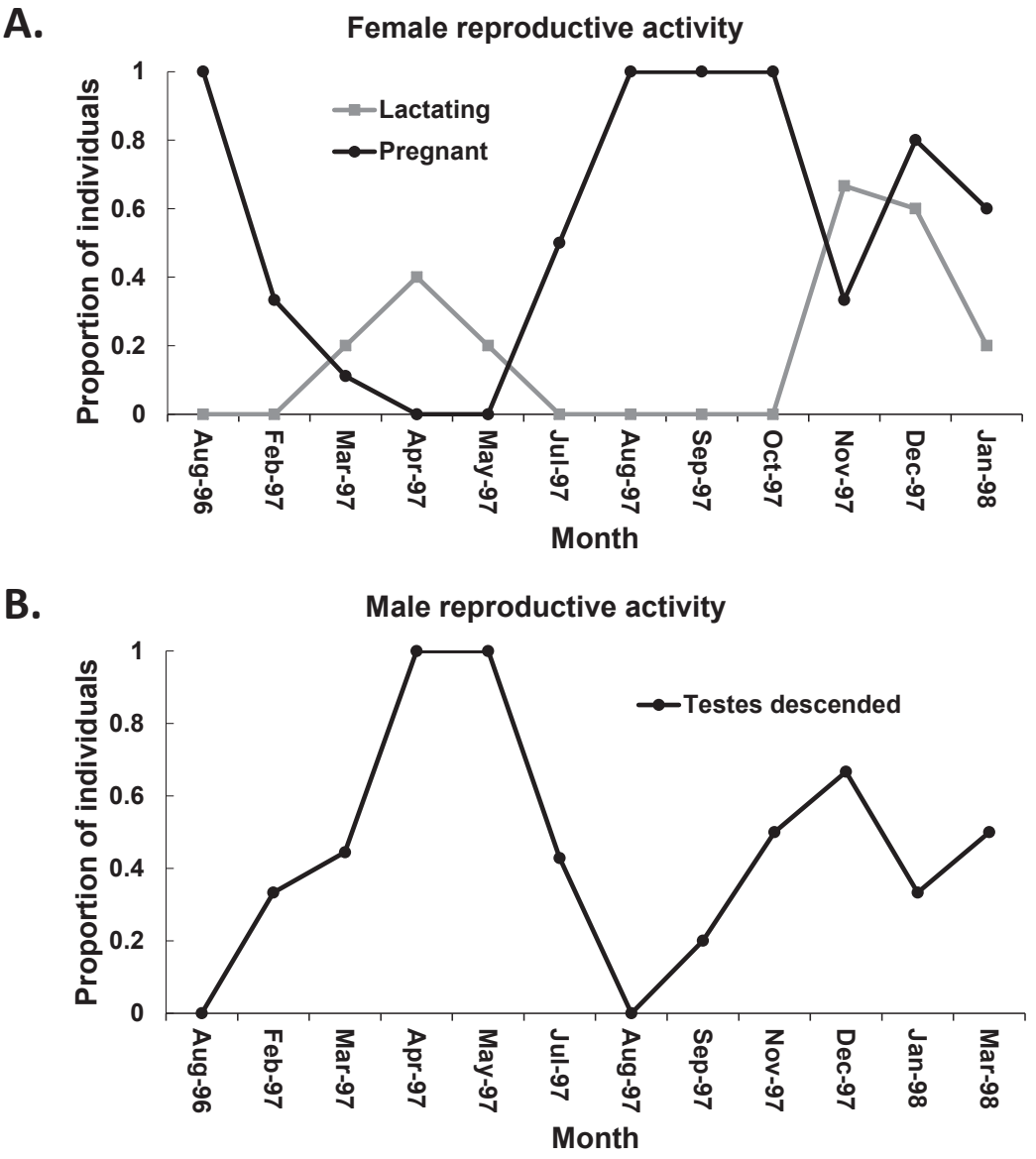

Figure 4. Proportion of adult A) female and B) male *Platyrrhinus lineatus* per month from Reserva Natural del Bosque Mbaracayú and Yaguareté Forests that were reproductively active (i.e., females: pregnant or lactating; males: testes descended).

DISCUSSION

The ecology of many Neotropical bats is poorly understood. As habitat in general and those in the Atlantic Forest in particular continue to become more degraded and fragmented, it is becoming increasingly necessary to understand ecological characteristics important to distribution and abundance. In particular, it is vital to monitor frugivorous bats as they are often the most abundant mammals in tropical and subtropical forests, facilitate colonization of plants and succession of forests in areas disturbed by anthropogenic activities (Galindo-González et al. 2000; Medellin and Gaona 2006), and are critical to the maintenance and function of ecological processes in many of these systems (e.g., Fleming and Heithaus 1981; Muscarella and Fleming 2007). Herein, a number of ecological characteristics are reported for an important frugivorous bat (*Platyrrhinus lineatus*) in the Atlantic Forest of Paraguay.

Morphometrics.—*Platyrrhinus lineatus* in eastern Paraguay was not geographically variable regarding any aspect of its morphology. This is in contrast to findings in northeastern Brazil where *P. lineatus* exhibited significant multivariate geographic morphometric variation and significant univariate differences regarding seven characters: hindfoot length, forearm length, length of digit 4, length of digit 5, rostral breadth, breadth across the upper molars, width of the widest molar, and greatest length of mandible (Willig 1983; Willig et al. 1986). The geographic distance between the RNBM and Yaguareté Forests is approximately 65 km, whereas the distance between the Caatingas and Cerrado sites described in Willig (1983) is approximately 45–50 km. Thus, this difference in degree of geographic variation in morphology likely is not due to differences in the geographic scales of these two studies. Willig (1983) did have smaller samples sizes (20 males and 20 females at each site) relative to those of this study for both cranial (RNBM: 24 males and 15 Females; Yaguareté Forests: 26 males and 29 females) and external (RNBM: 22 males and 14 Females; Yaguareté Forests: 24 males and 26 females) measurements, so differences in statistical power cannot be the reason for the difference between this study and his. A number of phyllostomid species exhibit significant geographic variation in one portion of their distribution and not the other (Swanepoel and Genoways 1979). Moreover, more tropical populations may be smaller and experience a greater number or more intense biotic interactions and therefore manifest more microgeographic variation than those in more temperate areas (MacArthur 1972). In a similar vein, climatic variability and the responses of bats to such variability may prevent microgeographic differentiation among populations. For example, in eastern Paraguay there are considerable fluctuations in temperature and precipitation that ultimately translate into seasonality of fruit production. It is possible that frugivourous bats such as *P. lineatus* migrate seasonally. Such regional movements may result in relatively more gene flow that homogenizes differences among populations.

Significant secondary sexual dimorphism was detected with respect to external characteristics but not cranial or mandibular characteristics. Characters that were significant were: total length, forearm length, hindfoot length, ear length, length of digit 3, and length of digit 5. Females were larger than males in all measures that displayed a significant difference, and half of the characters that were significant were associated with wing morphology. In bats, it is common for females to be larger than males (Ralls 1976; Stevens and Platt 2015). A number of hypotheses have been posed including more intense competition among females, differential resource utilization by males and females, or that larger females are more capable of rearing an offspring (big mother hypothesis, Ralls [1976]). The "big mother" effect can manifest as dimorphism in wing morphology in bats because of the aerodynamic constraints of carrying a large fetus and subsequently a large neonate during flight (Stevens et al. 2013).

Nightly activity.—In eastern Paraguay, *P. lineatus* was most active during the middle of the night. The peak in the middle of the night likely reflects a peak in the number of individuals on the landscape at that time. The temporal duration of the night, bounded by dusk and dawn, may create a temporal constraint on foraging duration similar to a geometric constraint on position of geographic ranges (Colwell and Lees 2000). Thus, if individuals are variable in how long they spend away from day roosts foraging throughout the night and there is no systematic variation as to when they begin to

forage or when they end, then a peak in activity could result in the middle of the night. A number of spatial and nonspatial biological characteristics of species are characterized by midpoint attractors (Colwell et al. 2016). These include latitudinal and elevational gradients in species richness, responses of abundance to edge effects (Prevedello 2013), and movements of individuals within populations (Tiwari et al. 2005). Of course, this remains a hypothesis to be tested using data on overlapping nightly distributions of individuals, which were not available in this study.

In contrast to insectivores and sanguinivores, frugivores as a group have been demonstrated to peak in abundance in the middle of the night (Arriaga-Flores et. al. 2007; Mancina and Castro-Arellano 2013) and in Paraguay the same appears to be true for *P. lineatus*. Nonetheless, in other parts of the Atlantic Forest *P. lineatus* has been documented to peak in activity early in the night, before midnight (Pedro 1992; Aguiar 1994; Mikalauskas 2007; Duarte 2008) or to exhibit no discernable pattern (Gazarini 2008; Ortêncio-Filho et al. 2010), although Aguiar and Marinho-Filho (2004) similarly saw a peak in activity in the middle of the night for *P. lineatus* in southeastern Brazil. Across much of the Atlantic Forest, *P. lineatus* is one of the less numerous but common bats that requires considerable effort to obtain large sample sizes. The main limitation to these other studies is that they either sampled only halfway through the night or they are based on so few individuals that activity through time relationships are so noisy that there does not appear to be a pattern. Activity patterns of *P. lineatus* may be different across its geographic distribution. Many environmental characteristics can influence activity patterns in bats such as amount of lunar illumination (Mello et al. 2013; Saldana-Vazquez and Munguia-Rosas 2013; Peche-Canche et al. 2018) or anthropogenic habitat modification (Castro Arellano et al. 2009). Indeed, better understanding of nightly activity patterns will come from more intensive sampling across a number of different environmental regimes.

Monthly activity.—*Platyrrhinus lineatus* was most active during spring and summer, with markedly reduced numbers throughout winter. This appears to be a general pattern throughout the Atlantic Forest. Along the upper Parana River in Brazil, numbers of captures of *P. lineatus* decreased fairly linearly from summer through spring (Ortêncio-Filho et al. 2010). No *P. lineatus* were captured during winter months (i.e., July through September) in Maringá (Gazarini 2008) or Rancho Alegre (Gallo 2008), Paraná state. A similar pattern was true at Caratinga (Aguiar 1994) and essentially the same (on capture) in Panga (Pedro 1992), both in Minas Gerais in Brazil. Much of the Atlantic Forest is at sufficiently high latitudes that climatic seasonality is a conspicuous form of environmental heterogeneity. Indeed, in eastern Paraguay a typical year experiences at least a few nights of freezing temperatures (Sanchez 1973). Such seasonality of temperature causes seasonality in bat community structure (Mello 2009; Stevens and Amarilla-Stevens 2012). In fact, *P. lineatus* is nearly absent from esatern Parguay during the winter months. What *P. lineatus* in particular, and many other bats in general, do during the winter months remains a mystery. Future studies should investigate potential short-term torpor or regional scale seasonal movements as means whereby bats mitigate temperature seasonality in this system.

Reproductive patterns.—Reproductive patterns of *P. lineatus* are not well documented. In eastern Paraguay this species exhibits seasonal polyestry with both a primary and a secondary breeding season. The primary season is during summer–fall from March to July and the smaller one in spring during November and December. In the state of São Paulo, Taddei (1973, 1976) found that females were pregnant in all months but April, and Peracchi and Albuquerque (1971) found pregnant females in the State of Rio de Janeiro in December, January, and March. All of these observations are consistent with patterns from eastern Paraguay. The reproductive patterns of *P. lineatus* in the Cerrado of eastern Brazil (Willig 1985) also are similar to those described herein for eastern Paraguay.Comparisons with Willig (1983) suggest the ability to exhibit polyestry may be similar across the entire range of *P. lineatus*.

It is important to improve our understanding of the effects of environmental heterogeneity on the ecology of frugivorous bats, especially in the subtropics where heterogeneity has the added dimension of climatic seasonality. This is especially true because bats are important indicators of environmental health (Jones et al. 2009) and they perform ecosystem services that other species do not, such as nocturnal seed dissemination and flower pollination (Kunz et al.

2011). *Platyrrhinus lineatus* is common throughout the Atlantic Forest of South America and is a species that responds strongly to environmental heterogeneity. Continued investigation at other locations within the Atlantic Forest will help illuminate effects of environmental heterogeneity on distribution and abundance of bats in general.

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LITERATURE CITED

Aguiar, L. M. S. 1994. Comunidades de Chiroptera em três áreas de Mata Atlântica em diferentes estádios de sucessão—Estação Biológica de Caratinga, Minas Gerais. Unpublished Dissertation, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

Aguiar, L. M. S. 2005. First record of the use of leaves of *Solanum lycocarpum* (Solanacea) and fruits of *Emmotum nitens* (Icacinacea) by *Platyrrhinus lineatus* (E. Geoffroy) (Chiroptera, Phyllostomidae) in the Brazilian Cerrado. Revista Brasileira de Zoologia 22:509–510.

Aguiar, L. M. S., and J. Marinho-Filho. 2004. Activity patterns of nine phyllostomid bat species in a fragment of the Atlantic Forest in southeastern Brazil. Revista Brasileira de Zoologia 21:385–390.

Arriaga-Flores, J. C., I. Castro Arellano, A. Moreno-Valdez, and A. Correa-Sandoval. 2007. Temporal niche overlap of a riparian forest bat assemblage in subtropical Mexico. Revista Mexicana de Mastozoologia 2:3–17.

Brunet-Rossinni, A. K., and G. D. Wilkinson. 2009. Methods of ages estimation and the study of senescence in bats. Pp. 315–328 in Ecological and behavioral methods for the study of bats, Second edition (T. H. Kunz and S. Parsons, eds.). The Johns Hopkins University Press, Baltimore, Maryland.

Castro-Arellano, I., S. J. Presley, M. R. Willig, J. M. Wunderle, and L. N. Saldanha. 2009. Reduced-impact logging and temporal activity of understorey bats in lowland Amazonia. Biological Conservation 142:2131–2139.

Colwell, R. K., et al. 2016. Midpoint attractors and species richness: modelling the interaction between environmental drivers and geometric constraints. Ecology Letters 19:1009–1022.

Colwell, R. K., and D. C. Lees. 2000. The mid-domain effect: geometric constraints on the geography of species richness. Trends in Ecology and Evolution 15:70–76.

Duarte, A. C. 2008. Comunidade de Quirópteros (Mammalia, Chiroptera) do Parque Natural Municipal da Prainha, Rio de Janeiro, RJ, Brasil. Unpublished Dissertation, Univerdidade Federal Rural do Rio de Janeiro, Rio de Janeiro, Brazil.

Fargione, J., J. Hill, D. Tilman, S. Polasky, and P. Hawthorne. 2008. Land clearing and the biofuel carbon debt. Science 319:1235–1238.

Fleming, T. H., and E. R. Heithaus. 1981. Frugivorous bats, seed shadows and the structure of tropical forests. Biotropica 13:45–53.

Galindo-González, J., S. Guevara, and V. J. Sosa. 2000. Bat- and bird-generated seed rains at isolated trees in pastures in a tropical rainforest. Conservation Biology 14:1693–1703.

Gallo, P. H. 2008. Morcegos (Mammalia: Chiroptera) em um fragmento de Mata Atlântica e reflorestamento no mu-

nicípio de Rancho Alegre, Paraná, Brasil. Unpublished Dissertation, Universidade Estadual de Londrina, Londrina, Paraná, Brazil.

García-Morales, R., E. L. Badano, and C. E. Moreno. 2013. Response of Neotropical bat assemblages to human land use. Conservation Biology 27:1096–1106.

Gardner, A. L. 1977. Feeding habitats. Pp. 293–350 in Biology of bats of the New World family Phyllostomatidae, Part II (R. J. Baker, J. K. Jones, Jr., and D. C. Carter, eds.). Special Publications, Museum of Texas Tech University 13:1–364.

Gardner, A. L. (ed.). 2008. Mammals of South America. University of Chicago Press, Chicago, Illinois.

Gazarini, J. 2008. Estrutura de comunidade de morcegos (Mammalia, Chiroptera) em fragmentos urbanos de Maringá, Paraná, Brasil. Unpublished Dissertation, Universidade Estadual de Londrina, Londrina, Paraná, Brazil.

Geoffroy St.-Hilaire, E. 1810. Sur les phyllostomes et les megadermes, deux genres de la famille des chauve-souris. Annals del Museo Natural Historia, Paris 15:157–198.

Gorresen, P. M., and M. R. Willig. 2004. Landscape responses of bats to habitat fragmentation in Atlantic Forest of Paraguay. Journal of Mammalogy 85:688–697.

Huston, M. A. 1994. Biological diversity: the coexistence of species on changing landscapes. Cambridge University Press, Cambridge, UK.

Jin, V. L., and R. Oren. 1997. Analysis of spatial variability in forest structure and composition in Yaguarete Forest, San Pedro department, Paraguay. Unpublished B. S. Thesis, Duke University, Durham, NC.

Jones, G., D. S. Jacobs, T. H. Kunz, M. R. Willig, and P. A. Racey. 2009. Carpe noctem: the importance of bats as bioindicators. Endangered Species Research 8:93–-115.

Jung, K., and C. G. Threlfall. 2018. Trait-dependent tolerance of bats to urbanization: a global meta-analysis. Proceedings of the Royal Society B 285:20181222.

Keel, S., A. H. Gentry, and L. Spinzi. 1993. Using vegetation analysis to facilitate the selection of conservation sites in eastern Paraguay. Conservation Biology 7:66–75.

Kunz, T. H., E. B. de Torrez, D. Bauer, T. Lobova, and T. H. Fleming. 2011. Ecosystem services provided by bats. Year in Ecology and Conservation Biology 1223:1–38.

LaBarbera, M. 1989. Analyzing body size as a factor in ecology and evolution. Annual Review of Ecology, Evolution, and Systematics 20:97–117.

MacArthur, R. H. 1972. Geographical ecology: patterns in the distribution of species. Harper and Row, Publishers, Inc., Princeton, New Jersey.

Mancina, C. A., and I. Castro-Arellano. 2013. Unusual temporal niche overlap in a phytophagous bat ensemble of western Cuba. Journal of Tropical Ecology 29:511–521.

Medellin, R. A., and O. Gaona. 2006. Seed dispersal by bats and birds in forest and disturbed habitats of Chiapas, Mexico. Biotropica 31:478–485.

Mello, M. A. R. 2009. Temporal variation in the organization of a Neotropical assemblage of leaf-nosed bats (Chiroptera: Phyllostomidae). Acta Oecologica 35:280–286.

Mello, M. A. R., E. K. V. Kalko, and W. R. Silva. 2013. Effects of moonlight on the capturability of frugivorous bats (Chiroptera: Phyllostomidae) at different time scales. Zoologia 30:397–402.

Meyer, C. F. J., M. L. Struebig, and M. R. Willig. 2016. Responses of tropical bats to habitat fragmentation, logging, and deforestation. Pp. 63–103 in Bats in the Anthropocene: conservation of bats in a changing world (C. C. Voigt and T. Kingston, eds.). California Academy of Sciences, San Fransisco.

Mikalauskas, J. S. 2007. Diversidade, dieta, e reprodução de morcegos (Mamalia, Chiroptera) de Serra de Itabaiana, Sergipe. Unpublished Dissertation, Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, Brazil.

Muscarella, R., and T. H. Fleming. 2007. The role of frugivorous bats in tropical forest succession. Biological Reviews 82:573–590.

Ortêncio-Filho, H., N. R. Reis, and C. V. Minte-Vera. 2010. Time and seasonal patterns of activity of phyllostomid bats in fragments of a stational semidecidual forest from the Upper Paraná River, Southern Brazil. Brazilian Journal of Biology 70:937–945.

Peche-Canche, J. M., P. D. C. Villegas, I. A. Chamorro-Florescano, J. L. A. Mendez, and M. A. Lozano Rodriguez. 2018. Lunar phobia in phyllostomid bats at La Ceiba, Tuxpan, Vera Cruz. Ecosystemas y Tecursos Agropecuarios 5:165–170.

Pedro, W. A. 1992. Estrutura de uma taxocenose de morcegos da Reserve do Panga (Uberlândia, M. G.), com ênfase nas relações tróficas em Phyllostomidae (Mammalia: Chiroptera). Unpublished Dissertation, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

Peracchi, A. L., and S. T. Albuquerque. 1971. Lista provisória dos quirópteros dos estados do Rio de Janeiro e Guanabara, Brasil (Mammalia, Chiroptera). Revista Brasileira de Biologia 31:405–413.

Prevedello, J. A., M. S. L. Figueiredo, C. E. V. Grellle, and M. V. Viera. 2013. Rethinking edge effects: the unaccounted role of geometric constraints. Ecography 36:287–299.

Ralls, K. 1976. Mammals in which females are larger than males. Quarterly Review of Biology 51:245–276.

Ramos Pereira, M. J., C. Fonseca, and L. M. S. Aguiar. 2018. Loss of multiple dimensions of biodiversity under land-use intensification in the Brazilian Cerrado. Hystrix 29: 25–32.

Saldana-Vazquez, R., and M. A. Munguia-Rosas. 2013. Lunar phobia in bats and its ecological correlates: a metanalysis. Mammalian Biology 78:216–219.

Sazima, I. 1976. Observations on the feeding habits of phyllostomatid bats (*Carollia*, *Anoura* and *Vampyrops*) in southeastern Brazil. Journal of Mammalogy 57:381–382.

Sanchez, T. F. 1973. The climate of Paraguay. Pp. 33–38 in Paraguay: ecological essays (J. R. Gorham, ed.). Academy of the Arts and Sciences of America, Miami.

Sicotti Mass, A. C., et al. 2018. Bats in a Cerrado landscape of northern Brazil: species occurrence, influence of environmental heterogeneity and seasonality, and eight new records for the state of Tocantins. Mammalia 82: DOI: https://doi.org/10.1515/mammalia-2017-0023.

Silvestre, S. M., P. A. da Rocha, M. A. da Cunha, J. P. Santana, and S. F. Ferrari. 2016. Diet and seed dispersal potential of the white-lined bat *Platyrrhinus lineatus* (E. Geoffroy, 1810), at a site in northeast Brazil. Studies in Neotropical Fauna and Environments 51:37–44.

Stevens, R. D., and H. N. Amarilla-Stevens. 2012. Seasonal environment, episodic density compensation and dynamics of structure of chiropteran frugivore guilds in Paraguayan Atlantic Forest. Biodiversity and Conservation 21:267–279.

Stevens, R. D., M. E. Johnson, and E. S. McCulloch. 2013. Absolute and relative secondary-sexual dimorphism in wing morphology: a multivariate test of the "Big Mother" hypothesis. Acta Chiropterologica 15:163-170.

Stevens, R. D., and R. N. Platt. 2015. Patterns of secondary sexual dimorphism in New World Myotis and a test of Rensch's Rule. Journal of Mammalogy 96:1128–1134.

Stevens, R. D., M. R. Willig, and I. G. de Fox. 2004. Comparative community ecology of bats from eastern Paraguay: taxonomic, ecological, and biogeographic perspectives. Journal of Mammalogy 85:698–707.

Swanepoel, P., and H. H. Genoways. 1979. Morphometrics. Pp. 13–106 in Biology of bats of the New World Phyllostomatidae, Part III (R. J. Baker, J. K. Jones, Jr., and D. C. Carter, eds). Special Publications, Museum of Texas Tech University 16:1–441.

Taddei, V. A. 1973. Phyllostomidae de região norte-occidental do estado de São Paulo. Unpublished Dissertation, Universidade de São Paulo, São Paulo, Brazil.

Taddei, V. A. 1976. The reproduction of some Phyllostomidae (Chiroptera) from the northwestern region of the state of São Paulo. Boletim de Zoologia 1:313–330.

Tiwari, M., K. A. Bjorndal, A. B. Bolten, and B. M. Bolker. 2005. Intraspecific application of the mid-domain effect model: spatial and temporal nest distributions of green turtles. Ecology Letters 8:918–924.

Wiens, J. J., et al. 2010. Niche conservatism as an emerging principle in ecology and conservation biology. Ecology Letters 13:1310–1324.

Willig, M. R. 1983. Composition, microgeographic variation, and sexual dimorphism in Caatingas and Cerrado bat communities from northeast Brazil. Bulletin of Carnegie Museum of Natural History 23:1–131.

Willig, M. R. 1985. Ecology, reproductive biology, and systematics of Neoplatymops mattogrossensis (Chiroptera: Molossidae). Journal of Mammalogy 66:618–628.

Willig, M. R., G. R. Camilo, and S. J. Noble. 1993. Dietary overlap in frugivorous and insectivorous bats from edaphic cerrado habitats of Brazil. Journal of Mammalogy 74:117–128.

Willig, M. R., and R. R. Hollander. 1987. *Vampyrops lineatus*. Mammalian Species 275:1–4.

Willig, M. R., R. D. Owen, and R. L. Colbert. 1986. Assessment of morphometric variation in natural populations: the inadequacy of the univariate approach. Systematic Zoology 35:195–203.

Willig, M. R., S. J. Presley, R. D. Owen, and C. Lopez-Gonzalez. 2000. Composition and structure of bat assemblages in Paraguay: a subtropical-temperate interface. Journal of Mammalogy 81:386–401.

Yaguareté Forests LDC. 1996. Management plan. Yaguareté Forests, Asunción, Paraguay.

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A New Species of *Myotis* (Chiroptera, Vespertilionidae) from Peru

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Abstract

Eight species of *Myotis* are known to occur west of the Andes, of which four are from western Peru. Based on four vouchers from the arid coast of northern and central Peru, a new species is added to this list. *Myotis bakeri* species novum externally resembles *M. atacamensis* but it can be distinguished from this species and all other South American congeners by a unique set of external and craniodental qualitative and quantitative characters. Herein, this new species that inhabits the lowland arid regions on the northern and central coasts of western Peru is named and described. As a result, 22 species of *Myotis* are now known from South America, nine of which occur in Peru.

Key words: desert, Myotinae, *Myotis bakeri* species novum, neotropics, South America

Introduction

The genus *Myotis* Kaup, 1829 in South America currently comprises 22 species (Moratelli and Wilson 2014; Moratelli et al. 2017). The Andean Cordillera plays an important role in the distribution of most of this assemblage, acting as a corridor for a few species and a partial or complete barrier for others (LaVal 1973; Moratelli et al. 2013). Among the South American assemblage, *Myotis albescens* (É. Geoffroy, 1806), *Myotis nigricans* (Schinz, 1821), and *Myotis riparius* Handley, 1960, are widespread on the continent, occurring on both sides of the Cordillera. Southernmost records on the west side of the Andes are in southwestern Ecuador for *Myotis riparius*, and northwestern Peru for *Myotis albescens* and *Myotis nigricans*. *Myotis caucensis* J. A. Allen, 1914, *Myotis keaysi* J. A. Allen, 1914, *Myotis oxyotus* (Peters, 1866), and *Myotis pilosatibialis* LaVal, 1973, occur throughout the Cordillera. *Myotis keaysi* and *M. pilosatibialis* are restricted to mid-elevations (≈ 900–2000 m), whereas *M. oxyotus* reaches the lowlands on the west side of the Cordillera, and *M. caucensis* occurs in intermontane valleys and adjacent Amazon lowlands on the east side. *Myotis levis* (I. Geoffroy, 1824), *Myotis ruber* (É. Geoffroy, 1806), and *Myotis izecksohni* Moratelli et al., 2011, are restricted to eastern South America. *Myotis izecksohni* occurs in the mountains of eastern Brazil and Argentina, whereas *M. levis* and *M. ruber* occur from Paraguay eastward to the lowlands of southern Brazil and Argentina, and northward throughout the mountains of eastern Brazil. Apparently, *Myotis simus* Thomas, 1901, is restricted to the lowlands of the Amazon River. One of the authors (RM) has reanalyzed all vouchered specimens of *Myotis simus* Thomas, 1901, supporting the occurrence of the species for the west side of the Andes, and found that they correspond to misidentifications of other species in the *ruber* group (R. Moratelli, unpublished data). *Myotis midastactus* Moratelli and Wilson, 2014, is known from only the Cerrado in Bolivia and Alto Chaco in Paraguay. *Myotis chiloensis* (Waterhouse, 1840) is restricted to localities in southwestern Chile and Argentina. *Myotis dinellii* Thomas, 1902, occurs in Argentina and southern Bolivia. *Myotis atacamensis* (Lataste, 1892) occupies arid and semiarid habitats in western Peru and Chile. *Myotis diminutus* Moratelli and Wilson, 2011, is in western Colombia and Ecuador, where the species appears to be restricted to the Chocó Ecoregion. *Myotis handleyi* Moratelli et al., 2013, is known from two cordilleras in northern Venezuela, and *M. nesopolus* Miller, 1900, occurs in a restricted arid zone in northwestern Venezuela (*M. nesopolus larensis* LaVal, 1973) and on Curaçao (*M. nesopolus nesopolus*). *Myotis attenboroughi* Moratelli et al., 2017, is endemic to the Island of Tobago, Trinidad and Tobago, and *Myotis clydejonesi* Moratelli et al., 2016, is known from a single individual collected in tropical lowland forest in Sipaliwini, Suriname. Finally, *Myotis lavali* Moratelli et al., 2011, occurs throughout the South American dry corridor (LaVal 1973; Wilson 2008; Stevens et al. 2010; Muñoz-Garay and Mantilla-Meluk 2012; Mantilla-Meluk and Muñoz-Garay 2014; Moratelli and Wilson 2014, 2015; Moratelli et al. 2013, 2015a,b, 2016, 2017; Barquez et al. 2017).

Moratelli and Wilson (2014) and Moratelli et al. (2013) arranged this assemblage into the *albescens* and *ruber* groups based on the oldest available names. Species in each of these groups share external and cranial features, and have been partially retrieved as closely related in the molecular phylogenies of Stadelmann et al. (2007) and Larsen et al. (2012a), but several species are still pending positioning in phylogenies. Species in the *albescens* group generally have silky fur, rounded occipital region, and either lack a sagittal crest or the crest is low and weakly developed. This species group includes *M. albescens*, *M. atacamensis*, *M. caucensis*, *M. chiloensis*, *M. diminutus*, *M. dinellii*, *M. izecksohni*, *M. lavali*, *M. levis*, *M. nesopolus*, *M. nigricans*, and *M. oxyotus*. Species in the *ruber* group are characterized by woolly fur, generally flattened occipital region, and the presence of a sagittal crest, which usually varies from moderately developed to high. The *ruber* group includes *M. keaysi*, *M. midastactus*, *M. pilosatibialis*, *M. riparius*, *M. ruber*, and *M. simus*.

Seven species are found west of the Andes—*albescens*, *atacamensis*, *chiloensis*, *diminutus*, *nigricans*, *oxyotus*, and *riparius*—with all but *M. riparius* in the *albescens* group (LaVal 1973; Wilson 2008; Moratelli et al. 2013). Based on four vouchers from desert localities in western Peru (Lambayeque and Lima), a new species of *Myotis,* named and described herein, is added to this list. The new species is morphologically a member of the *albescens* group, and particularly allied with *M. atacamensis*—the name under which the vouchers were misidentified. Although similar in external appearance, this new species shows a particular set of external and cranial traits that distinguish it from *M. atacamensis* and all other South American congeners.

METHODS

This research is part of a critical review of collections of Neotropical *Myotis*, and more than 3,800 specimens from different localities in South America, covering all species currently recognized, have been examined. The new species was compared with 286 vouchers representing all species currently recognized from Peru. These specimens are preserved in the collections of the American Museum of Natural History (AMNH, New York), Carnegie Museum of Natural History (CM, Pittsburgh, Pennsylvania), The Field Museum (FMNH, Chicago, Illinois), Muséum d'histoire naturelle (MHNG, Geneva, Switzerland), Museum of Natural Science, Louisiana State University (LSUMZ, Baton Rouge), Museum of Texas Tech University (TTU, Lubbock), Museum of Vertebrate Zoology (MVZ, Berkeley, California), Natural History Museum, University of Kansas (KU, Lawrence), and National Museum of Natural History, Smithsonian Institution (USNM, Washington, DC).

Four vouchers, three adult males and one adult female, constitute the source material for the description of this new species. They were collected in the departments of Lambayeque (LSUMZ 21306 ♂, 21307 ♀) and Lima (MVZ 137906 ♂, 137907 ♂), western Peru, between 1969 and 1981. The complete list of specimens from Peru analyzed here is presented in Appendix I, including those that could not be identified. Measurements are in millimeters (mm) and the body mass in grams (g), and are from adults and one subadult (the holotype of *M. diminutus*). The total length (TL), tail, hind foot, ear, and body mass were recorded from skin labels, and reported to the nearest millimeter or nearest gram. Other measurements were taken using digital callipers accurate to 0.02 mm. Craniodental measurements were taken under a binocular microscope at low magnification (usually 6x). These dimensions were recorded and analyzed to the nearest 0.01 mm, but values were rounded off to 0.1 mm throughout the text because this is the smallest unit that allows accurate repeatability with calipers (Voss et al. 2013). Measurements include forearm length (FA), third metacarpal length (3MC), length of dorsal hair (LDH), length of ventral hair (LVH), greatest length of skull (GLS), condylocanine length (CCL), condylobasal length (CBL), condylo-incisive length (CIL), basal length (BAL), zygomatic breadth (ZB), mastoid breadth (MAB), braincase breadth (BCB), interorbital breadth (IOB), postorbital breadth (POB), breadth across canines (BAC), breadth across molars (BAM), maxillary toothrow length (MTL), length of the upper molars (M1–3), mandibular length (MAL), and mandibular toothrow length (MAN). These measurements are defined in Moratelli et al. (2013). The cranial index—CRI = ((IOB + BCB) x GLS)/2—used by Moratelli and Wilson (2011, 2014), was applied

to compare skull shape. Descriptive statistics (mean and range) were calculated for all dimensions. The statistical significance of craniometric differences among samples was assessed by single analyses of variance (one-way ANOVA). Based on a subset of the craniodental dimensions (GLS, CCL, BCB, POB, BAM, MTL, M1–3, MAL, MAN, BAC), a discriminant function analysis (DFA) was performed to compare the new species with *M. atacamensis*, *M. diminutus*, and *M. nigricans*. *Myotis atacamensis* and *M. nigricans* were included because they are the most similar species in external and craniodental features, respectively. *Myotis diminutus* was included due to its geographic proximity, although it is less similar as compared to the other taxa. DFA was performed using MASS and Lattice packages in R software (R Development Core Team 2008). Multivariate procedures require complete datasets, thus missing data (approximately 5% of total dataset) were estimated from the existing raw data using the Amelia II packpage (Honaker et al. 2011) implemented in the R software. The complete list of specimens used in the statistical analyses is in Appendix II. Capitalized color nomenclature is from Ridgway (1912).

RESULTS

Myotis bakeri, species novum

Baker's Myotis, *Myotis de Baker*
Figs. 1, 2, and 4; Tables 1 and 2

Myotis atacamensis Wilson 2008:472; part, not *M. atacamensis* (Lataste, 1892).

Myotis atacamensis Rodríguez-San Pedro et al. 2014; part, not *M. atacamensis* (Lataste, 1892).

Holotype and type locality.—The holotype (MVZ 137907) consists of the skin and skull of an adult male (Figs. 1 and 2) collected by M. L. Hawes (field number 232) on 30 July 1969 at about 7 km SE of Chilca (≈ 12°33′S, 76°41′W; ca. 200–250 m altitude, obtained from Google Earth), department of Lima, Peru.

Paratypes.—One adult female (LSUMZ 21307) and two adult males (LSUMZ 21306, MVZ 137906). MVZ 137906 is from the type locality. LSUMZ 21306 and 21307 are from the department of Lambayeque, Peru, about 12 km N of Olmos. See Table 1 for external and craniodental measurements and body mass for the type series. These vouchers include skin and skull, and those from Lambayeque (LSUMZ 21306, 21307) include parts of the post-cranial skeleton.

Distribution and habitat.—*Myotis bakeri* is known from single localities in the departments of Lima and Lambayeque, Peru (Fig. 3). Specimens from Lima are from ca. 7 km SE of Chilca (≈ 12°33′S, 76°41′W; Google Earth), and those from Lambayeque are from ca. 12 km N of Olmos (05°55′S, 79°47′W; Gardner's Gazetteer of marginal localities [Gardner 2008]). Information about elevation is available only for specimens from Lambayeque, 12 km N of Olmos (ca. 335 m [1100 ft] above sea level). The elevation obtained from Google Earth for Lima, 7 km SE of Chilca, is ca. 200–250 m above sea level.

Based on these records, *M. bakeri* is restricted to western Peru, where it inhabits the lowland arid formations on the northern and central Pacific coast. Localities are in the extreme north of the South American Transition Zone, Desert province (see Morrone 2014:24, fig. 12). Morrone's (2014) Desert province corresponds to Cabrera and Willink's Desert province (Cabrera and Willink 1973). In this region, vegetation is scarce and usually found along margins of rivers and near the ocean (Morrone 2001).

Diagnosis.—Similar to most species of *Myotis*, a single character was not found that could distinguish *M. bakeri* from all other American congeners. However, *M. bakeri* can be distinguished from other Neotropical species in the genus by the unique combination of the following set of traits: silky fur; dorsal pelage strongly bicolored, with Mummy Brown bases (2/3), and Buckthorn Brown tips (1/3 of the total fur length); absence of a fringe of hairs along the trailing edge of uropatagium (although a few sparse hairs may be present); plagiopatagium broadly attached to the side of the foot at the level of the toes; rounded occipital region; low, weakly developed sagittal crest; inflated, but not rounded braincase.

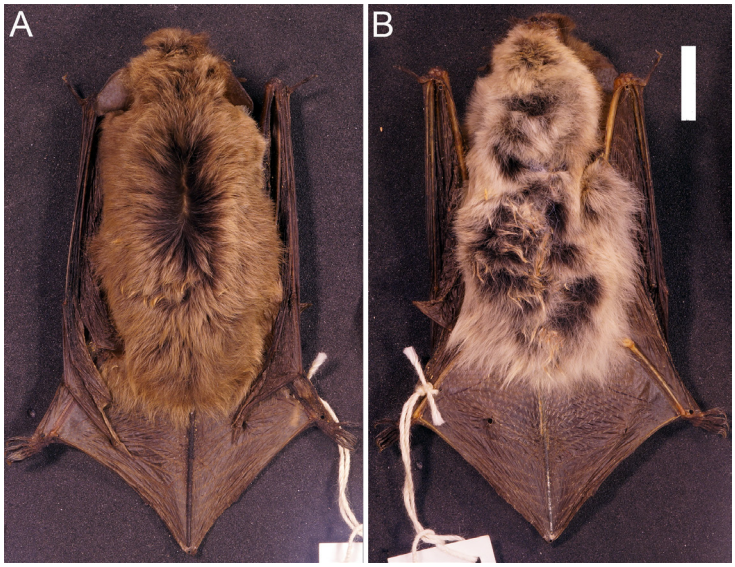

Figure 1. Dorsal (A) and ventral (B) views of the skin of the holotype of *Myotis bakeri* (MVZ 137907). Scale bar = 10 mm. See Table 1 for measurements.

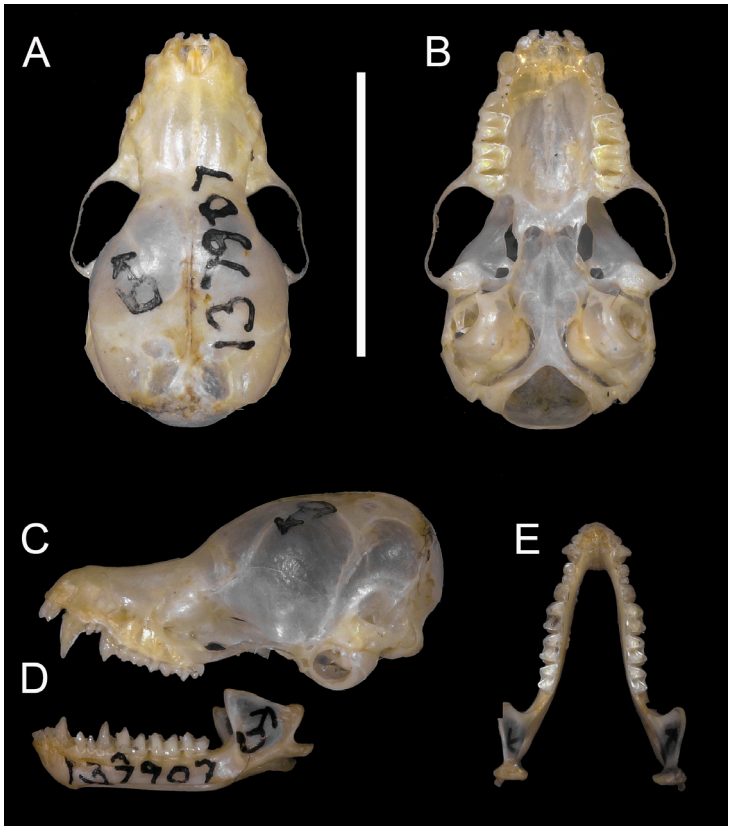

Figure 2. Dorsal (A), ventral (B), and lateral (C) views of the skull, and lateral (D) and dorsal (E) views of the mandible of the holotype of *Myotis bakeri* (MVZ 137907). Scale bar = 10 mm. See Table 1 for measurements.

Table 1. Selected measurements (mm) and body mass (g) of the holotype (MVZ 137907) and paratypes of *Myotis bakeri* (LSU 21306, 21307, MVZ 137906). See Methods for variable abbreviations and Appendix I for localities of specimens.

Characters	MVZ 137907 ♂	MVZ 137906 ♂	LSU 21306 ♂	LSU 21307 ♀
TL	79	74	73	72
Tail	30	26	30	31
Hind foot	7	6	7	7
Ear	14	12	12	12
Body mass	4.6	5.0	3.3	3.3
FA	30.8	31.6	29.0	30.5
3ML	30.6	29.9	28.4	29.9
LDH	7.1	6.7	8.4	8.9
LVH	6.9	–	6.4	6.1
GLS	13.8	13.5	13.4	13.1
CCL	12.2	11.8	11.7	11.7
CBL	12.7	12.4	12.3	12.3
CIL	12.9	12.6	12.5	12.5
BAL	11.4	11.3	11.2	11.1
ZB	–	–	–	–
MAB	7.2	7.0	6.8	6.8
BCB	6.8	6.5	6.4	6.3
IOB	4.4	4.1	4.2	4.0
POB	3.7	3.7	3.7	3.5
BAC	3.5	3.5	3.3	3.3
BAM	5.3	5.3	5.1	5.1
MTL	5.0	4.8	4.9	5.1
M1–3	2.8	2.8	2.8	2.9
MAL	9.5	9.2	9.3	9.2
MAN	5.2	5.2	5.3	5.4
CRI	77.8	72.0	71.4	67.3

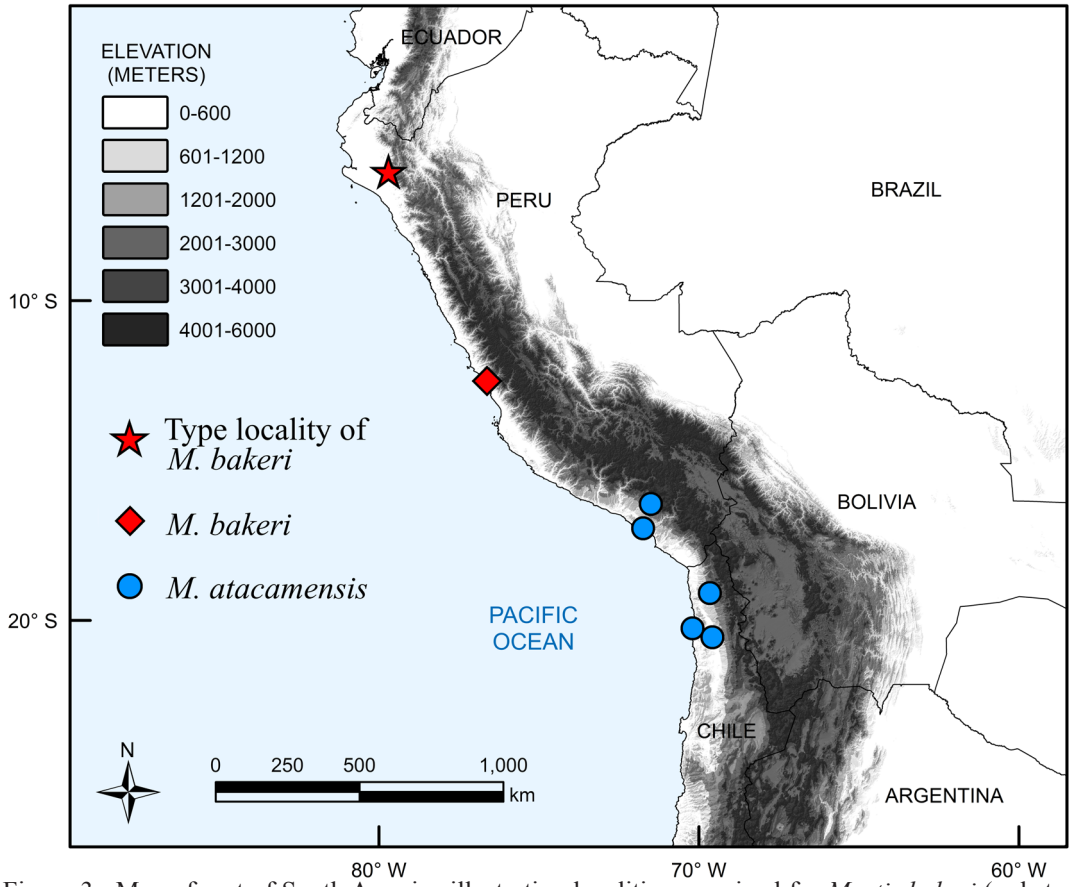

Figure 3. Map of part of South America illustrating localities examined for *Myotis bakeri* (red star and diamond) and *M. atacamensis* (blue circles). See Appendix I for localities.

Morphological description and comparisons.—*Myotis bakeri* is a small species of *Myotis* (FA 29.0–31.6 mm, other measurements in Table 1), with external size overlapping with *M. atacamensis*, *M. diminutus*, and smaller individuals of other species such as *M. nigricans*, *M. albescens*, and *M. riparius*. Like most other New World species, the dorsal surfaces of wing membranes and uropatagium appear to be naked, but a few hairs can be seen under magnification. These membranes are medium brown or slightly paler. Ears are medium brown as well and comparatively small to medium-sized (EL 12–14 mm). Dorsal and ventral fur is silky and long (LDH 7–9 mm, LVH 6–7 mm). The dorsal pelage is strongly bicolored with dark-brown bases (near Mummy Brown) and yellowish tips (near Buckthorn Brown). The darker base comprises 2/3 of the total fur length and changes gradually from darker to paler, with the paler tip comprising about 1/3 of fur length. Thus, there are only two color bands in the dorsal fur. The ventral pelage is strongly bicolor as well, with the same proportion of blackish base to whitish tip. The whitish venter strongly contrasts with the yellowish dorsum. The plagiopatagium is attached to the foot at the level of the toes by a broad band of membrane (see López-González et al. 2001:141, fig. 1a). The dorsal surfaces of elbow and tibia are naked or nearly naked. The uropatagium lacks fringing hairs along the trailing edge, although sparsely distributed hairs are present in half of the sample (LSUMZ 21306, 21307). The tragus is long and slender. Bases of the nails are pale brown. Skull and mandible are medium-sized in length and resemble those of most Neotropical *Myotis* (GLS 13.1–13.8 mm, MAL 9.2–9.5 mm). The dental formula is typical of most species of *Myotis*: 2/3, 1/1, 3/3, 3/3 = 38. The 2nd upper premolar (P3) is aligned in the toothrow, not displaced lingually. The 1st lower

molar (m1) is myotodont, with the postcristid connecting hypoconid and entoconid instead of connecting hypoconid and hypoconulid (this latter condition is known as nyctalodont [see Ruedi et al. 2013:fig. S3] and apparently does not occur in Neotropical species of *Myotis*). Sagittal and lambdoidal crests are low and weakly developed. The occipital region is rounded, and projects behind the level of the condyles. Frontals are steeply sloping.

Considering either the assemblage of *Myotis* that occurs west of the Andes (*albescens*, *atacamensis*, *chiloensis*, *diminutus*, *nigricans*, *oxyotus*, and *riparius*) or the taxa that occur in Peru (*albescens*, *atacamensis*, *caucensis*, *keaysi*, *nigricans*, *oxyotus*, *riparius*, and *simus*), *M. bakeri* can be distinguished from all by the set of diagnostic traits reported above. Among them, *M. bakeri* is externally closer to *M. atacamensis*, from which it can be distinguished by slightly darker dorsal fur (near Mummy Brown basally and Buckthorn Brown on the tips in *bakeri*; black on the bases and Light Ochraceous Buff on the tips in *atacamensis* [Fig. 4]); absence of a fringe of hairs along the trailing edge of uropatagium (present in *atacamensis*); and more robust skull (Fig. 5), with most dimensions representing length (CCL, CBL, CIL,MTL, M1–3, MAL, and MAN) and width (MAB, BCB, IOB, POB, BAC, and BAM) of skull differing significantly from those of *atacamensis* (Table 2).

Myotis bakeri can be distinguished from *M. albescens* by the absence of a fringe of hairs along the trailing edge of uropatagium, and paler dorsal fur color (dorsal fur medium to dark brown on the bases [≈ 4/5 of the total fur length] and yellowish on the tips [≈ 1/5] in *albescens*, conveying a brownish general appearance to the dorsum, in contrast with the yellowish appearance in *bakeri*). Additionally, most individuals of *M. albescens* have a globular braincase and short rostrum, and the throat is yellowish, grading to whitish towards the abdomen and sides of the body (Moratelli and Oliveira 2011), whereas in *M. bakeri* the skull is robust, the braincase is not globular, the rostrum is comparatively longer, and the entire venter, from the throat to the abdomen, is whitish.

Myotis bakeri can be distinguished from *M. caucensis*, *M. diminutus*, *M. nigricans*, and *M. oxyotus* by the dorsal fur paler and strongly bicolored, and the ventral fur whitish (dorsal fur weakly bicolored and darker [medium-brown], and ventral fur yellowish in *caucensis*, *diminutus*, *nigricans,* and *oxyotus*). *M. bakeri* averages smaller than *M. caucensis*, *M. nigricans*, and *M. oxyotus*, and it is smaller than *M. diminutus* in forearm length (FA: 29.0–31.6 mm in *bakeri*; 33.3, 33.4 mm in *diminutus*), but averages larger in all craniodental dimensions. *M. bakeri* also can be distinguished from *M. diminutus* by the larger cranial index (CRI: *bakeri* = 67.3–77.8; *diminutus* = 59.2, 63.8), a ratio that reflects the wider cranial conformation of the former (which overlaps with *M. nigricans*). Moratelli et al. (2013) and Moratelli and Wilson (2015) provide external and craniodental measurements and illustrations of the dorsal and ventral fur of *M. caucensis*, *M. diminutus*, and *M. nigricans*. No evidence was found for the occurrence of *M. nigricans* in arid or semiarid zones in Peru. The southernmost records for the species on the west side of the Andes are in the Western Ecuador and Ecuadorian provinces in the Pacific dominion (see Morrone 2014:24, fig. 12). In addition to the distinctive dorsal and ventral fur coloration (Fig. 4), *M. bakeri* can be distinguished from *M. nigricans* from western localities in Ecuador ($N = 7$) by the moderately steeply sloping frontals (frontals not steeply sloping in *nigricans* [Fig. 5]), wider post-orbital breadth (POB: 3.5–3.7 mm in *bakeri*; 3.2–3.5 mm in *nigricans* [$P = 0.003$]), and shorter mandibular length (MAL: 9.2–9.5 mm in *bakeri*; 9.4–9.6 mm in *nigricans* [$P = 0.036$]). In contrast to *M. oxyotus*, *M. bakeri* has frontals less inclined in profile.

Myotis bakeri can be distinguished from *M. keaysi*, *M. riparius*, and *M. simus* by its paler, silky fur (woolly, and either brownish or reddish-brown in *keaysi*, *riparius*, and *simus*). In addition, it can be distinguished from *M. simus* by the plagiopatagium broadly attached to the side of the foot at the level of the toes (usually attached to the ankle or to the side of the foot by a narrow band of membrane [< 1.5 mm] in *simus*); and it can be distinguished from *M. keaysi* by the tibia not furred (fur on uropatagium extending on to the tibia in *keaysi*).

From *M. aelleni* and *M. chiloensis*, *M. bakeri* can be distinguished by its silky, brighter, and longer fur (woolly and short in *aelleni* and *chiloensis*). *M. aelleni* and *M. chiloensis* vary in color from medium brown to pale yellowish, but the pelage is consistently brighter in *M. bakeri*.

Table 2. External, dental, and cranial measurements (mm), body mass (g), and cranial index of males and females of *Myotis bakeri* from Peru, and *M. atacamensis* from Chile and Peru, and *F* values for skull dimensions (one-way ANOVA: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

	Myotis bakeri	*Myotis atacamensis*	
Characters	Mean (Min.–Max.) *N*	Mean (Min.–Max.) *N*	*F*
HB	75 (72–79) 4	79 (74–85) 3	–
Tail	29 (26–31) 4	37 (34–42) 3	–
Hind foot	7 (6–31) 4	(6–7) 2	–
Ear	13 (12–14) 4	14 (13–14) 3	–
Body mass	4.1 (3.3–5.0) 4	3.9 (3.8, 4.0) 2	–
FA	30.5 (29.0–31.6) 4	32.2 (30.8–34.1) 3	–
3ML	29.7 (28.4–30.6) 4	30.4 (29.1–32.9) 3	–
LDH	7.8 (6.7–8.9) 4	7.7 (7.0–8.7) 3	–
LVH	6.5 (6.1–6.9) 3	6.3 (5.9–6.7) 3	–
GLS	13.5 (13.1–13.8) 4	12.9 (12.6–13.6) 8	4.108
CCL	11.8 (11.7–12.2) 4	11.2 (10.9–11.8) 8	7.896*
CBL	12.4 (12.3–12.7) 4	11.9 (11.5–12.5) 8	5.390*
CIL	12.6 (12.5–12.9) 4	12.0 (11.7–12.6) 8	7.238*
BAL	11.2 (11.1–11.4) 4	10.8 (10.5–11.4) 8	4.061
ZB	–	6.7	–
MAB	6.9 (6.8–7.2) 4	6.5 (6.3–6.7) 6	6.839*
BCB	6.5 (6.3–6.8) 4	6.2 (6.1–6.5) 7	5.247*
IOB	4.2 (4.0–4.4) 4	3.7 (3.6–3.8) 8	12.216**
POB	3.6 (3.5–3.7) 4	3.1 (3.0–3.2) 8	30.264***
BAC	3.4 (3.3–3.5) 4	2.9 (2.8–3.0) 7	13.754**
BAM	5.2 (5.1–5.3) 4	4.7 (4.6–4.9) 7	21.114**
MTL	4.9 (4.8–5.1) 4	4.7 (4.4–5.1) 7	5.057*
M1–3	2.8 (2.8–2.9) 4	2.7 (2.6–2.9) 7	5.992*
MAL	9.3 (9.2–9.5) 4	8.8 (8.6–9.3) 5	6.297*
MAN	5.3 (5.2–5.4) 4	4.9 (4.8–5.3) 7	5.879*
CRI	72.1 (67.3–77.8) 4	64.2 (61.9–69.4) 7	15.028**

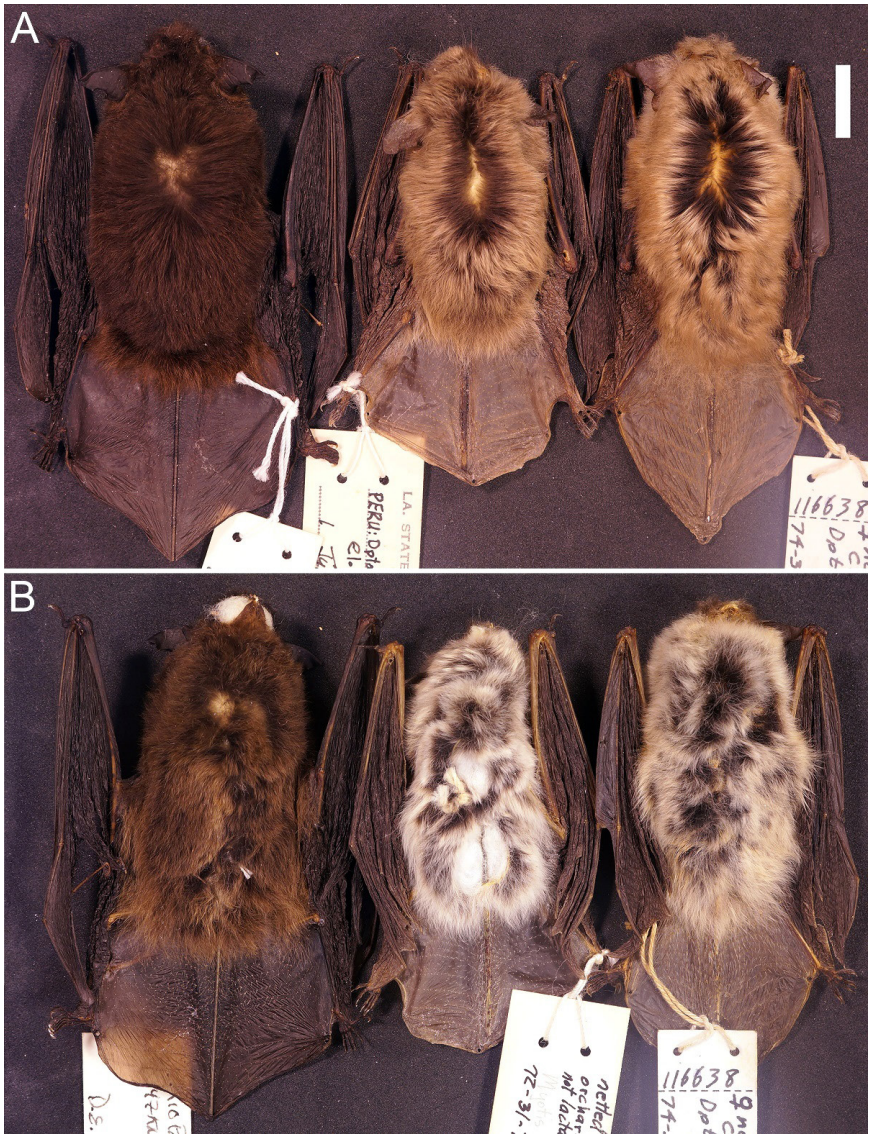

Figure 4. Dorsal (A) and ventral (B) views of the skins of *Myotis nigricans* (USNM 525866 [left]), *M. bakeri* (LSUMZ 21307 [center]), and *M. atacamensis* (MVZ 116638 [right]). Scale bar = 10 mm. See Table 1 for measurements.

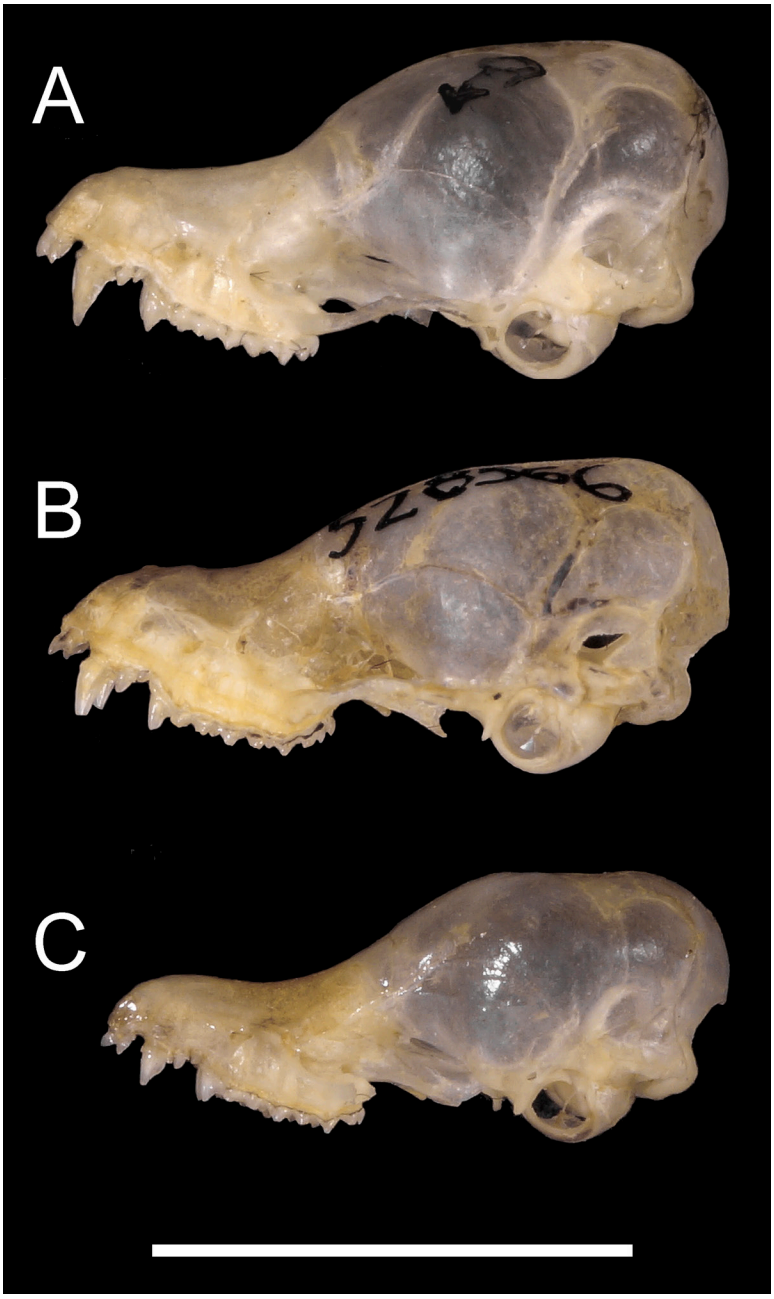

Figure 5. Lateral views of the skulls of *Myotis bakeri* (A, MVZ 137907), *M. nigricans* (B, USNM 525866), and *M. atacamensis* (C, MVZ 116638). Scale bar = 10 mm. See Table 1 for measurements.

Myotis nigricans nicholsoni Sanborn, 1941 was described based on three specimens from Arequipa, southern Peru. Based on the original description, this name could be a possible available name for the taxon described here. Sanborn's description of *M. n. nicholsoni* emphasizes the pale fur, with the almost black bases of the dorsal and the ventral hairs contrasting strongly with the "slightly lighter than Buffy Brown" tips on the dorsum and the grayish white tips on the venter (Sanborn 1941). In addition, most of the craniodental measurements he provides for the holotype are below (IOC) or in the range (CBL, MAB, MTL, BAC, and MAN) of the measurements retrieved in this study for *M. atacamensis*. The primary types of *M. n. nicholsoni* (FMNH 50783) and *M. atacamensis* (FMNH 49790) were compared and found to be indistinguishable from one another, and distinguishable from *M. bakeri* on the basis of the same characters reported above as differences from *M. atacamensis*.

Multivariate analysis.—In a discriminant function analysis including *M. bakeri* ($N = 4$), *M. atacamensis* ($N = 8$), *M. diminutus* ($N = 2$), and *M. nigricans* ($N = 7$), the first and second discriminant functions (DF1, DF2) accounted for 70.4% and 19.7% of the total variation, respectively (Fig. 6, Table 3). Along these axes, *M. bakeri* is fully distinct from all analyzed species (Fig. 6). Measurements of the length (MTL and MAL) and width (POB, BAM, BAC, M1-3, and MAN) of skull were the best to discriminate samples.

Etymology.—*Myotis bakeri* honors Robert J. Baker, in recognition of his outstanding contributions to mammalogy.

Remarks.—The identity of three vouchers (LSUMZ 21308, LSUMZ 25009, and AMNH 216119) could not be confirmed. LSUMZ 25009 (adult male) is from Cerro La Vieja, 7 km S of Botupe, Lambayeque, Peru. Although its external and craniodental measurements (FA 31.0, 3ML 29.4, LDH 8, LVH 6, GLS 13.8, CCL 11.9, CBL 12.6, CIL 12.7, BAL 11.4, MAB 7.0, BCB 6.4, IOB 4.3, POB 3.5, BAC 3.5, BAM 5.2, MTL 5.0, M1–3 2.8, MAL 9.2, MAN 5.3, and CRI 74.0 [all but CRI in mm]) fit with those from the type series of *M. bakeri* and the fringe of hairs along the trailing edge of uropatagium seems to be absent (only a few hairs sparsely distributed), this voucher was examined before the neotype of *M. atacamensis* was available for comparison—that comparison was key to determine that specimens from northernmost localities in Peru should be assigned to the new species described here.

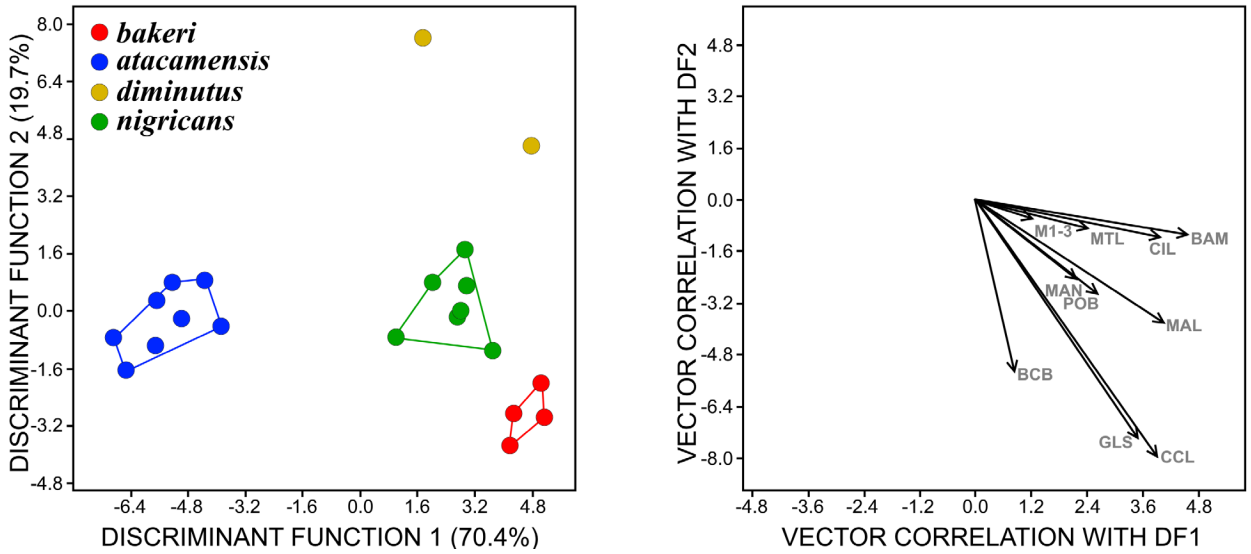

Figure 6. Plots of multivariate individual scores in the first and second discriminant functions (DF1, DF2; left) and corresponding vector correlations of craniometric characters with the first 2 eigenvectors (right). Samples: *Myotis bakeri* (red circles, $N = 4$), *M. atacamensis* (blue circles, $N = 8$), *M. diminutus* (dark golden circles, $N = 2$), and *M. nigricans* (green circles, $N = 7$).

Table 3. Vector correlation coefficients between original variables and discriminant functions (DF1 and DF2) for samples of *Myotis bakeri*, *M. atacamensis*, *M. diminutus*, and *M. nigricans*.

Measurements	DF1	DF2
MAL	-2.071	9.848
GLS	3.620	-4.674
CCL	1.457	-4.902
BCB	3.588	-2.063
POB	-9.668	-0.592
BAM	-4.445	13.813
MTL	-5.003	19.190
M1-3	2.403	-25.527
MAN	-2.378	-9.392
BAC	-5.889	-14.288

Voucher AMNH 216119 (male, adult?) is from Lima, Peru. Similarly, this voucher was examined before the neotype of *M. atacamensis* was available for comparison. Its skull measurements (GLS 12.9, CCL 11.3, CBL 11.9, CIL 12.0, BAL 10.8, MAB 6.3, BCB 6.2, IOB 3.8, POB 3.2, BAC 3.0, BAM 4.6, MTL 4.5, M1–3 2.6, MAL 8.8, MAN 4.9, and CRI 64.7 [all but CRI in mm]) fit with those from *M. atacamensis*, but the skin (preserved in alcohol) was not examined. Because fur color and skull shape are decisive for confirming identifications, identifications of these specimens are postponed until skins and skulls can be analyzed again. Voucher LSUMZ 21308 (adult male) also could not be identified. This voucher is from 12 km N of Olmos, Lambayeque, Peru (the same locality of the paratypes LSUMZ 21306, 21307). It is a very small individual with external and craniodental measurements (FA 27.5, 3ML 26.4, LDH 7, LVH 6, GLS 12.5, CCL 10.9, CBL 11.5, CIL 11.6, BAL 10.3, MAB 6.1, BCB 6.0, IOB 3.8, POB 3.3, BAC 3.0, BAM 4.6, MTL 4.6, M1–3 2.6, MAN 5.0, and CRI 61.0 [all but CRI in mm]) close to *M. diminutus* and smaller specimens of *M. atacamensis*. However, the absence of a fringe of hairs on the trailing edge of uropatagium (with only a few hairs sparsely distributed), and the fur color distinguish it from *M. atacamensis* and *M. diminutus*, and suggest *M. bakeri*.

DISCUSSION

Sanborn (1941) described *M. n. nicholsoni* as a pale race of *M. nigricans*, without commenting on its distinction from *M. atacamensis*—which at that time was considered to be a synonym of *M. chiloensis* (see Miller and Allen 1928). LaVal (1973) raised *M. atacamensis* to the species level, and LaVal (1973) and Wilson (2008) placed *M. n. nicholsoni* in the synonymy of *Myotis atacamensis*. Based on the results of this study, *M. n. nicholsoni* is not applicable for the populations described here as a new species, and should be kept as a junior synonym of *M. atacamensis*. In regard to *M. n. nicholsoni*, Lataste's description of *M. atacamensis* was published in Tome 1 of the *Actes de la Société Scientifique du Chili* (Lataste 1892). LaVal (1973) reported 1891 as the year of publication, but Cabrera (1958) and Wilson (2008) reported 1892. The original volume was examined, and the year of publication shown on page iii of the journal is 1892. According to Friedländer and Sohn, pages 58 to 127 along with 18 plates were published in 1892.

With the description of *M. bakeri*, 22 species of *Myotis* currently are known from South America, nine of which occur in Peru. Within the Peruvian assemblage, *M. albescens*, *M. nigricans*, *M. riparius*, and *M. simus* are on the eastern side of the Andes; *M. caucensis*, *M. keaysi*, and *M. oxyotus* occur along the Cordillera, with *M. caucensis* reaching the eastern lowlands, and *M. oxyotus* reaching mid-elevations on the western side; and *M. bakeri* and *M. atacamensis* inhabit arid and semiarid habitats in the lowlands along the Pacific coast.

Myotis bakeri occurs from northern to central Peru, and *M. atacamensis* occurs from southern Peru to northern Chile. These species occur in the Desert Province (*bakeri*), and in the Desert and the Atacama provinces (*atacamensis*) of the South American transition zone (see Morrone 2014:fig. 2). Based on the material analyzed in this study, northernmost records of *M. atacamensis* are ca. 700 km south of the south-

ernmost known locality for *M. bakeri* (Fig. 3). On the other hand, the western distribution of *M. nigricans* apparently extends southward into the Western Ecuador and Ecuadorian provinces of the Pacific dominion (see Wilson's map of marginal localities for *M. nigricans* [Wilson 2008:476, map 277]). These three species can be distinguished easily on the bases of external and cranial traits. Fieldwork in Peru and western Ecuador to acquire additional specimens and a critical review of material previously assigned to *M. atacamensis* and *M. nigricans* from western Peru might be decisive in determining the extent of their geographic distributions.

Specimens labeled AMNH 216119 and LSUMZ 25009 appear to be representative of *M. atacamensis* and *M. bakeri*, respectively. On the other hand, the identity of LSUMZ 21308 is uncertain, and the possibility that it represents another undescribed species from western Peru cannot be discarded. Likewise, through the critical review of specimens in collections, many other individuals of *Myotis* from several Neotropical localities have been found that cannot be assigned with confidence to any of the currently known species or, as yet, recognized as undescribed taxa. Larsen et al. (2012a) used molecular data to show that the taxonomic diversity currently recognized for the genus in South America is only a fraction of the real diversity. Moreover, molecular and morphological analyses previously conducted by the authors have revealed that some widespread Neotropical species of *Myotis* constitute complexes of cryptic species (Moratelli et al. 2016, 2017). Based on the combined evidence, it is speculated that the 22 recognized South American species of *Myotis* constitute about half or two-thirds of the real diversity of South American *Myotis*. Field and museum-based research are still necessary to more accurately determine taxonomic and geographic limits of species, and molecular and morphological approaches combined will certainly reveal many new cryptic species.

Étienne Geoffroy St.-Hilaire described the first two South American species of *Myotis* in 1806 based on Azara's descriptions of Paraguayan bats (Geoffroy 1806). Based on museum research, Miller and Allen (1928) recognized five South American species, with most of the currently valid names under synonymy or as subspecific categories. LaVal (1973) also studied collections extensively to improve resolution and recognized 14 South American species, a few of which he divided into subspecies. Subsequently, other species also have been revealed during museum-based research (Larsen et al. 2012b; Moratelli and Wilson 2015; Moratelli et al. 2011; 2013, 2016, 2017), and emerging evidence suggests that many other undescribed species are awaiting discovery and description. After more than two centuries of study, we still know only a fraction of the real diversity of this genus in South America. The scenario for *Myotis* seems not to be an exception, and the systematics and geography of only a few taxa of Neotropical mammals can be considered well resolved. Throughout the previous centuries, we have relied on the service of competent field biologists—such as Robert J. Baker—who have continually performed fieldwork and deposited well-documented samples in collections. These samples are unique representations of local faunas at a given time, and function as the primary research resource for understanding and preserving life on our planet (Moratelli 2014; Rocha et al. 2014).

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LITERATURE CITED

Barquez, R. M., M. D. Miotti, F. M. Idoeta, and M. M. Díaz. 2017. Two new species of *Myotis* (Chiroptera: Vespertilionidae) from Argentina. Papeis Avulsos de Zoologia 57(22):287–294.

Cabrera, A. 1958 [1957]. Catálogo de los mamíferos de América del Sur. Revista del Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Ciencias Zoológicas 4(1):1–308. [Date of issue: March 27, 1958, see notice on p. 308.]

Cabrera, A. L., and A. Willink. 1973. Biogeografia de America Latina. Washington, DC: The General Seretariat of the Organization of American States. Serie de Biologia, Monografia 13.

Gardner, A. L. 2008 [2007]. Gazetteer of marginal localities. Pp. 581–628 in Mammals of South America. Vol. 1, Marsupials, xenarthrans, shrews, and bats (A. L. Gardner, ed.). University of Chicago Press, Chicago.

Geoffroy, É. 1806. Mémoire sur le genre et les espècies de Vespertilion, l'un des genres de la famille des chauve-souris. Annales du Muséum d'Histoire Naturelle 8:187–205.

Honaker, J., G. King, and M. Blackwell. 2011. Amelia II: a program for missing data. Journal of Statistics Software 45:1–47.

Larsen, R. J., et al. 2012a. Genetic diversity of Neotropical *Myotis* (Chiroptera: Vespertilionidae) with an emphasis on South American species. PLoS ONE 7:e46578.

Larsen, R. J., et al. 2012b. Evolutionary history of Caribbean species of *Myotis*, with evidence of a third Lesser Antillean endemic. Mammalian Biology 77:124–134.

Lataste, F. 1892. Etudes sur la faune Chilienne. II — Note sur les chauve-souris (Ordre des Chirotères). Actes de la Société Scientifique du Chili 1:70–91.

LaVal, R. K. 1973. A revision of the Neotropical bats of the genus *Myotis*. Natural History Museum of Los Angeles County, Science Bulletin 15:1–54.

López-González, C., S. J. Presley, R. D. Owen, and M. R. Willig. 2001. Taxonomic status of *Myotis* (Chiroptera: Vespertilionidae) in Paraguay. Journal of Mammalogy 82:138–160.

Mantilla-Meluk, H., and J. Muñoz-Garay. 2014. Biogeography and taxonomic status of *Myotis keaysi pilosatibialis* LaVal 1973 (Chiroptera: Vespertilionidae). Zootaxa 3793:60–70.

Miller, G. S., Jr., and G. M. Allen. 1928. The American bats of the genera *Myotis* and *Pizonyx*. Bulletin of the United States of Natural Museum 144:1–128.

Moratelli, R. 2014. Wildlife biologists are on the right track: a mammalogist's view of specimen collection. Zoologia 31:413–417.

Moratelli, R., M. Dewynter, M. Delaval, F. Catzeflis, and M. Ruedi. 2015a. First record of *Myotis albescens* (Chiroptera, Vespertilionidae) in French Guiana. Biodiversity Data Journal 3:e5314.

Moratelli, R., A. L. Gardner, J. A. Oliveira, and D. E. Wilson. 2013. Review of *Myotis* (Chiroptera, Vespertilionidae) from northern South America, including description of a new species. American Museum Novitates 3780:1–36.

Moratelli, R., L. Idárraga, and D. E. Wilson. 2015b. Range extension of *Myotis midastactus* (Chiroptera, Vespertilionidae) to Paraguay. Biodiversity Data Journal 3:e5708.

Moratelli, R., and J. A. Oliveira. 2011. Morphometric and morphological variation in South American populations of *Myotis albescens* (Chiroptera: Vespertilionidae). Zoologia 28:789–802.

Moratelli, R., A. L. Peracchi, and J. A. Oliveira. 2011. Morphometric and morphological variation in *Myotis simus* Thomas (Chiroptera, Vespertilionidae), with an appraisal of the identity of *Myotis guaycuru* Proença based on the analysis of the type material. Zootaxa 2985:41–54.

Moratelli, R., and D. E. Wilson. 2011. A new species of *Myotis* Kaup, 1829 (Chiroptera, Vespertilionidae) from Ecuador. Mammalian Biology 76:608–614.

Moratelli, R., and D. E. Wilson. 2014. A new species of *Myotis* (Chiroptera, Vespertilionidae) from Bolivia. Journal of Mammalogy 95:E17–E25.

Moratelli, R., and D. E. Wilson. 2015. A second record of *Myotis diminutus* (Chiroptera: Vespertilionidae): its bearing on the taxonomy of the species and discrimination from *M. nigricans*. Proceedings of the Biological Society of Washington 127:533–542.

Moratelli, R., D. E. Wilson, A. L. Gardner, R. D. Fisher, and E. E. Gutiérrez. 2016. A new species of *Myotis* from Suriname (Chiroptera, Vespertilionidae). Occasional Papers, Museum of Texas Tech University 65:49–63.

Moratelli, R., D. E. Wilson, R. L. M. Novaes, K. M. Helgen, and E. E. Gutiérrez. 2017. Caribbean *Myotis* (Chiroptera, Vespertilionidae), with description of a new species from Trinidad and Tobago. Journal of Mammalogy 98:994–1008.

Morrone, J. J. 2001. A formal definition of the Paramo-Punan biogeographic subregion and its provinces, based mainly on animal taxa. Revista del Museo Argentino de Ciencias Naturales n.s. 3:1–12.

Morrone, J. J. 2014. Biogeographical regionalization of the Neotropical region. Zootaxa 3782:1–110.

Muñoz-Garay, J., and H. Mantilla-Meluk. 2012. First record of *Myotis nesopolus* from Colombia. Occasional Papers, Museum of Texas Tech University 312:1–9.

R Development Core Team. 2008. R: a language and environment for statistical computing. Vienna: R Foundation for statistical computing. Available at http://www.rproject.org.

Ridgway, R. 1912. Color standards and color nomenclature. Washington, DC: privately published.

Rocha, L. A., A. Aleixo, G. Allen, et al. 2014. Specimen collection: an essential tool. Science 344:814–815.

Rodríguez-San Pedro, A., J. L. Allendes, M. L. C. Castillo, D. A. Peñaranda, and F. T. Peña-Gómez. 2014. Distribution extension and new record of *Myotis atacamensis* (Lataste, 1892) (Chiroptera: Vespertilionidae) in Chile. Check List 10:1164–1165.

Ruedi, M., et al. 2013. Molecular phylogenetic reconstructions identify East Asia as the cradle for the evolution of the cosmopolitan genus *Myotis* (Mammalia, Chiroptera). Molecular Phylogenetics and Evolution 69:437–449.

Sanborn, C. C. 1941. Descriptions and records of Neotropical bats. Field Museum of Natural History, Zoological Series 20: 371–387.

Stadelmann, B., T. H. Kunz, L. K. Lin, and M. Ruedi. 2007. Molecular phylogeny of New World *Myotis* (Chiroptera, Vespertilionidae) inferred from mitochondrial and nuclear DNA genes. Molecular Phylogenetics and Evolution 43:32–48.

Stevens, R. D., C. López-González, E. S. McCulloch, and F. Netto. 2010. *Myotis levis* (Geoffroy Saint-Hilaire) indeed occurs in Paraguay. Mastozoología Neotropical 17:195–200.

Voss, R. S., B. K. Lim, J. F. Díaz-Nieto, and S. A. Jansa. 2013. A new species of *Marmosops* (Marsupialia: Didelphidae) from the pakaraima highlands of Guyana, with remarks on the origin of the endemic Pantepui mammal fauna. American Museum Novitates 3778:1–27.

Wilson, D. E. 2008 [2007]. Genus *Myotis* Kaup 1829. Pp. 468–481 in Mammals of South America, Vol. 1, Marsupials, xenarthrans, shrews, and bats (A. L. Gardner, ed.). University of Chicago Press, Chicago.

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APPENDIX I

Listed below are localities with specimens examined from Peru, including those pending identification. Abbreviations are in "Material and Methods." Localities are arranged by species and alphabetically by major political unit (region/department/province), and may include precise locality.

Myotis albescens (*N* = 104; 67 females, 37 males).—Ayacucho, Río Apurimac, Hacienda Luisiana (LSUMZ 16621, 16622); Ayacucho, San José, Río Santa Rosa (LSUMZ 16623–16625); Cusco, Quispicanchi (FMNH 68471, 68473–68478); Huánuco, Leonicio Prado, 1 km S of Tingo Maria (CM 98854); Huánuco, Río Huallaga, ca. 4 km NE of Tingo Maria (LSUMZ 14265); Loreto, Río Curaray (AMNH 71643); Loreto, Maynas, Puerto Indiana, Amazon River (AMNH 73235, 73237, 73239, 73242); Loreto, Maynas, Orosa, Amazon River (AMNH 74018, 74019, 74021); Loreto, San Jacinto (KU 158160); Madre de Dios, Manú, Maskoitania, 13.4 km NW Atalaya, left bank of Rio Alto Madre de Dios (FMNH 174919, 174921); Madre de Dios, Manú, Quebrada Aguas Calientes, left bank, Rio Alto Madre de Dios, 2.75 km E of Shintuya (FMNH 170275); Madre de Dios, Mouth of Rio La Torre, south bank of Rio Tambopata (LSUMZ 24562); Madre de Dios, Pakitza (USNM 564391, 564392, 566560–566563); Pasco, Oxapampa (AMNH 230746–230748, 230750–230757); Pasco, San Juan (USNM 364442–364480); Pasco, unknown localities (AMNH 213428, 213430); Ucayali, Balta, Río Curanja (LSUMZ 12272, 12274–12279); Ucayali, Coronel Portillo, Yarinacocha (LSUMZ 12253, FMNH 62178–62188).

Myotis atacamensis (*N* = 6; 5 females, 1 male).—Arequipa, Chucarapi (MVZ 116638); Arequipa, Arequipa, Patasagua, 3 km W of Tiabaya (FMNH 49790–49792); Arequipa, Valle del Tambo (FMNH 50783, 51063).

Myotis bakeri (*N* = 4; 1 female, 3 males).—Lambayeque, 12 km N of Olmos (LSUMZ 21306, 21307); Lima, 7 km SE of Chilca (MVZ 137906, 137907).

Myotis caucensis (*N* = 35; 21 females, 14 males).—Ayacucho, Yuraccyacu (LSUMZ 16632, 16633); Huánuco, Santa Elena, 35 km NE of Tingo Maria, on Carretera Central (LSUMZ 12567, 12570, 12574, 12575, 12576); Huánuco, Rio Huallaga, ca. 4 km NE of Tingo Maria (LSUMZ 14269); Huánuco, Rio Chincao (FMNH 24875–24882); Huánuco, Vista Alegre (FMNH 24885–24892); Madre de Dios, Reserva Amazónica, 15 km E of Puerto Maldonado (KU 144288–144291); Ucayali, Coronel Portillo, Yarinacocha (LSUMZ 12252, 12254–12259).

Myotis keaysi (*N* = 32; 17 females, 15 males).—Amazonas, ca. 20 km of La Peca by trail (LSUMZ 21488); Ayacucho, Puncu, ca. 30 km NE of Tambo (15688); Cusco, Cordillera Vilcabamba (AMNH 214371); Cusco, Cordillera Vilcabamba, west side (AMNH 233850, 233851, 233853, 233854, 233857, 236134); Cusco, Hacienda Cadena (FMNH 78686); Huánuco, Bosque Cutirragra, S of Huaylaspampa (LSUMZ 17898); Huánuco, Trail to Hacienda Paty below Carpish Pass (LSUMZ 18434); Huánuco, 7 km NW of Carpish Pass by road (AMNH 216117); Junín, Chanchamayo (FMNH 65751); Lambayeque, 16 km N and 25 km E of Olmos (MVZ 135620, 135621); Pasco, Santa Cruz, ca. 9 km SE of Oxapampa (LSUMZ 25907); Piura, Batan, on Zapalache-Carmen trail (LSUMZ 26898); Piura, 15 km E of Canchaque by road (LSUMZ 19213); Puno, Inca Mines (AMNH 15814); Puno, Oconeque, 10 miles N of Limbani (MVZ 116050).

Myotis nigricans (*N* = 26; 13 females, 13 males).—Amazonas, 43 km NE of Chiriaco by road (LSUMZ 21549); Amazonas, Cordillera Colán, E of La Peca (LSUMZ 21489); Amazonas, Cordillera del Condor, Valle del Rio Comaina, Puesto Vigilancia Comaina (USNM 581966, 581967); Ayacucho, Río Apurimac, Hacienda Luisiana (LSUMZ 16628); Ayacucho, San José, Río Santa Rosa (LSUMZ 16629, 166230); Cusco, Camisea, San Martín-3 (USNM 582874); Huánuco, Santa Elena, 35 km NE of Tingo Maria on Carretera Central (LSUMZ 12566, 12571, 12573); Madre de Dios, Pampa del Heath, ca. 50 km S of Puerto Pardo by river (LSUMZ 22132, 22133); Madre

de Dios, Mouth of Rio La Torre, south bank of Rio Tambopata (LSUMZ 24560); Ucayali, Chicosa, upper Río Ucayali (LSUMZ 12260–12267); Ucayali, Yarinacocha (LSUMZ 12577, 14266–14268, 16626).

Myotis oxyotus (*N* = 18; 10 females, 6 males, 2 sex undetermined).—Ancash, 31 km E of Pariacoto by road (LSUMZ 22131); Cajamarca, Celendin, Hacienda Limón (FMNH 19969); Cusco, Iquente (USNM 195146); Cusco, Marcapata (FMNH 66375, 66376); Cusco, Santa Ana (USNM 194452, 194453, 195141, 195147, 195149); Cusco, 6 miles N of Paucartambo (MVZ 116008); Huancavelica, Rumicruz (AMNH 60598); Huánuco, Ambo (FMNH 24864–24866); Junín, Rio Palca, 15 Km W of San Ramon (USNM 507204); Lima, Bujama Baja, 95 km south of Lima by road (AMNH 216118); Lima, Huaros, Bosque de Zarate, San Bartolomé (FMNH 129208).

Myotis riparius (*N* = 30; 15 females, 15 males).—Ayacucho, San José, Río Santa Rosa (LSUMZ 16631); Cusco, Camisea, Armihuari (USNM 582875, 582876); Cusco, Camisea, San Martín-3 (USNM 582877, 582878); Cusco, Cordillera Vilcabamba (AMNH 233859, 233860); Cusco, Marcapata (FMNH 68479–68482); Cusco, Paucartambo, Consuelo (FMNH 174941); Cusco, Paucartambo, San Pedro (FMNH 172162); Cusco, Quispicanchi, Hacienda Cadena (FMNH 75150); Cusco, Ridge Camp (USNM 588040); Huánuco, Agua Caliente, Río Pachitea (FMNH 55400); Huánuco, Leoncio Prado (TTU 46348); Loreto, Río Curaray (AMNH 71645); Loreto, San Jacinto (KU 158162); Madre de Dios, Lago Sandoval, Río Madre de Dios (MVZ 157782); Madre de Dios, Manú, Maskoitania (FMNH 174933); Madre de Dios, Mouth of Rio La Torre, south bank of Rio Tambopata (LSUMZ 24561); Madre de Dios, Rio Tambopata, 30 km up from Mouth (USNM 530919); Pasco, Oxapampa, San Pablo (AMNH 230775–230777); Ucayali, Balta, Río Curanja (LSUMZ 12268–12271).

Myotis simus (*N* = 34; 19 females, 15 males).—Huánuco, Rio Huallaga, ca. 4 km NE of Tingo Maria (LSUMZ 14262, 14263); Loreto, Maynas (AMNH 74105, 74109, 74110, 74378–74381); Loreto, Ucayali (AMNH 76240–76249, 76252, 76253); Pasco, San Juan (USNM 364481, 364482); Loreto, Pacaya Samiria National Reserve, Pithecia Biological Station (MHNG 1694.41, 1694.42); Ucayali, Balta, Rio Curanja (LSUMZ 12249, 12250); Ucayali, Yarinacocha (LSUMZ 12251, 14264).

Myotis sp. (*N* = 3; 3 males).—Lambayeque, 12 km N of Olmos (LSUMZ 21308); Lambayeque, Cerro La Vieja, 7 km S of Motupe (LSUMZ 25009); Lima, Bujama Baja, 95 km S of Lima by road (AMNH 216119).

APPENDIX II

Listed below are localities with specimens used in the statistical analyses. Abbreviations are in "Methods." Localities are arranged by species and alphabetically by country, major political unit (region/department/province), and may include precise locality.

Myotis atacamensis (N = 8; 6 females, 1 male, 1 sex undetermined)).—CHILE: Tarapacá, Canchones (FMNH 23618); Tarapacá, Miñimiñi (USNM 391786). PERU: Arequipa, Chucarapi (MVZ 116638); Arequipa, Arequipa, Patasagua, 3 km W of Tiabaya (FMNH 49790–49792); Arequipa, Valle del Tambo (FMNH 50783, 51063).

Myotis bakeri (N = 4; 1 female, 3 males).—PERU: Lambayeque, 12 km N of Olmos (LSUMZ 21306, 21307); Lima, 7 km SE of Chilca (MVZ 137906, 137907).

Myotis diminutus (N = 2; 1 female, 1 male [sub-adult]).—COLOMBIA: Nariño, La Gayacana (LACM 18761). ECUADOR: Pichincha, Santo Domingo, 47 km S of Rio Palenque Science Center by road (USNM 528569).

Myotis nigricans (N = 7; 5 females, 2 males)).—ECUADOR: Esmeraldas, Cachabí (LACM 15729); Esmeraldas, Cristal, over stream in pasture (CM 112854); Esmeraldas, Esmeraldas (AMNH 33239); Esmeraldas, Malimpia, Pambilar (USNM 113345); Guayas, Isla de Silva (LACM 8448); Imbiura, Paramba (USNM 113349); Pichincha, Santo Domingo, 47 km S of Rio Palenque Science Center by road (USNM 528566).

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A New Species of *Rhogeessa* from Nicaragua Based on Morphological, Karyotypic, and Molecular Data

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Abstract

New molecular and morphological data along with previously described karyotypes of *Rhogeessa* from eastern Nicaragua were examined. Currently, this population is classified as *R. tumida*. Based on genetic divergence, karyotypic distinction, and morphology, it is herein designated as a new species. The recognition of this new taxon brings the number of species in the *Rhogeessa tumida* complex to nine. The other species in the complex include *R. aeneus*, *R. bickhami*, *R. genowaysi*, *R. hussoni*, *R. io*, *R. menchuae*, *R. tumida*, and *R. velilla.* Now, only a single karyotype is present in each species in the *R. tumida* complex (although the karyotype is not necessarily unique to each species), and species delimitations are all consistent with expectations of genetic isolation as described by the Genetic Species Concept. Multivariate analysis of mensural characters is shown to separate nearly all of the taxa within the species complex.

Key words: cryptic species, cytochrome-*b*, genetic species, karyotype, Nicaragua, *Rhogeessa*, *Rhogeessa tumida* complex

Introduction

The rate of new species discoveries in mammalian taxa, including bats, is higher than expected given the relatively low biodiversity in mammals compared to other organismal groups (Ceballos and Ehrlich 2009; Reeder et al. 2007). A major factor in our ability to more accurately detect species differences is improvement in technology. Molecular studies, for instance, have allowed scientists to uncover previously unknown cryptic species (those that are morphologically indistinguishable), and these discoveries have had a profound impact on conservation (Bickford et al. 2007). An estimated 60% of new mammalian taxonomic discoveries between 1993 and 2008 involved cryptic species (Ceballos and Ehrlich 2009) and it has been suggested that species richness may be vastly underestimated in mammals (Francis et al. 2010; Honeycutt et al. 2010).

Among recently described cryptic species of bats, distinct echolocation calls and molecular divergence are likely the top means of differentiating cryptic taxa. Mammalogists have used the Genetic Species Concept (GSC; Baker and Bradley 2006) widely as a criterion for species definitions. Under this concept, species can be recognized as distinct when genetic divergence between lineages reaches a level expected to result in genetic incompatibility. Among bats, there are many examples of new species descriptions based at least in part on the GSC, including species of *Scotophilus* (Brooks and Bickham 2014), *Eumops* (Gregorin et al. 2016), *Pipistrellus* (Benda et al. 2004), and many others. Perhaps the most relevant example to the present study comes from the description of *Eumops wilsoni* (Baker et al. 2009). *Eumops wilsoni* is genetically distinct from its nearest relatives at both mitochondrial DNA sequences and Amplified Fragment Length Polymorphisms, and also possesses a unique karyotype. It is morphologically distinct from some, but not all, closely related species. The patterns of distinction in *Eumops wilsoni* closely mirror the findings presented herein to justify a new species of *Rhogeessa*.

Multiple members of the vespertilionid genus *Rhogeessa* have been described based on the GSC (Baird et al. 2012). The *Rhogeessa tumida* complex contains eight species: *R. aeneus*, *R. bickhami*, *R. genowaysi*, *R. hussoni*, *R. io*, *R. menchuae*, *R. tumida*, and *R. velilla* (Baker 1984; Audet et al. 1993; Genoways and Baker 1996; Baird et al. 2008; Baird et al. 2012).

All of these taxa represent cryptic species and at one time were considered as populations of *R. tumida* (LaVal 1973b). They generally are morphologically indistinguishable but karyotypic differences and/or molecular divergence distinguish them. The unique pattern of karyotypic rearrangements in *Rhogeessa*, all involving centric fusions, inspired the model of speciation by monobrachial centric fusions to explain how the chromosomal rearrangements themselves may have led to much of the biodiversity within the complex (Bickham and Baker 1977; Baker et al. 1985; Baker and Bickham 1986). Recognition of two of the species within the *R. tumida* complex (*R. bickhami* and *R. menchuae*) that are karyotypically identical to *R. tumida*, however, demonstrate that karyotypic rearrangements are not required for speciation in the group, as genetic divergence has taken place in their absence for these taxa (Baird et al. 2009; Baird et al. 2012).

Bickham and Baker (1977) and Baker et al. (1985) described a unique karyotype of 2n=32 from eastern Nicaraguan *R. tumida* that differed from the 2n=32 form in the Yucatan peninsula by eight chromosomal rearrangements. The Nicaraguan karyotype was referred to in Baker et al. (1985) as "2n=32N" to distinguish it from "2n=32B" in Belize, now recognized as *R. aeneus* (Audet et al. 1993). The status of the eastern Nicaraguan population has not been explored with additional data. The goal of the present study is to evaluate the taxonomic status of the Nicaraguan 2n=32N population using new molecular and morphological data.

MATERIALS AND METHODS

Molecular analysis.—Sampling for the molecular analysis was limited due to a lack of fresh or frozen tissue samples of Nicaraguan *Rhogeessa* (from the locality known to have 2n=32N karyotypes). Wing clips were taken from several dried specimens at the Biodiversity Research and Teaching Collections (BRTC; specimens are labeled with a TCWC acronym for the previous name of the collection) at Texas A&M University and the Natural Science Research Laboratory (NSRL) at Texas Tech University. A list of specimens examined is contained in Appendix I.

DNA from the clips was extracted using a Qiagen DNeasy blood and tissue kit, following the manufacturer's protocol for tissue extractions. Only specimen number TCWC 19756 yielded usable DNA. A portion of the mitochondrial DNA (mtDNA) cytochrome-*b* (*Cyt*b) gene was amplified in small, overlapping fragments using Polymerase Chain Reaction (PCR) primer combinations listed in Table 1 and sequenced with Sanger sequencing methods using an ABI 3730xl DNA analyzer.

The *Cyt*b fragment was aligned with previously published *Rhogeessa Cyt*b sequences (Baird et al. 2008; Appendix I) using Geneious 9.0 (http://geneious.com; Kearse et al. 2012). Only members of the *R. tumida* complex were included in the alignment to better understand the relationship of the Nicaraguan specimen to this group. *Bauerus dubiaquercus* was used as an outgroup (all specimens used in the molecular analysis are listed in Appendix I). Only the portion of the *Cyt*b gene that was sequenced across all specimens (844 base-pairs) was used in the analysis. The program jModelTest 2.1.10 (Darriba et al. 2012; Guindon and Gascuel 2003) was used to find the most appropriate model of evolution under the Akaike Information Criteria (AIC) method. MrBayes version 3.2.6 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) was used to conduct a Bayesian phylogenetic analysis of the data. A total of 6 million generations were run in the Bayesian analysis with a burnin rate of 25%. The phylogenetic results were visualized using FigTree version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Mophological analysis.—Fifteen cranial and mandibular characters were measured using hand-held calipers to the nearest 0.01 or 0.05 mm from 49 specimens (35 females and 14 males; Appendix I and II), representing nine *Rhogeessa* species (and all representatives of the *R. tumida* complex except *R. hussoni*): *R. aeneus* (n = 5), *R. bickhami* (n = 22), *R. genowaysi* (n = 5), *R. io* (n = 1), *R. menchuae* (n =2), *R. parvula* (n = 2), *R. tumida* (n = 1), *R. velilla* (n = 3), and Nicaraguan *Rhogeessa* (n = 8). Thirty of these specimens also were included in the molecular analysis (Appendix I). Measurements followed LaVal (1973a;

Table 1. PCR primers used to amplify small fragments of the *Cyt*b gene from museum vouchers of *Rhogeessa*. Primers paired together for PCR are listed together, with the forward primer (F) first and the reverse primer (R) second. The LGL 765 primer was published previously (Bickham et al. 1995, 2004).

Primer Name	Sequence (5'-3')
RhogCyt*b*256F	CGGAGCCTCCATATTTTTCA
RhogCyt*b*417R	GATATTTGGCCTCATGGGAGT
RhogCyt*b*609F	CAGGATCMAACAACCCAACR
RhogCyt*b*783R	GGGTTGGCTGGYGTRTARTT
RhogCyt*b*853F	RCCCAATAAACTRGGMGGAG
RhogCyt*b*1039R	TGGGTGTCCRGGTTGTC
LGL765 (F)	GAAAAACCAYCGTTGTWATTCAACT
Rho Cyt*b*270 R	ACCTCGTCCTACGTGTAGATA
Rho Cyt*b*400 F	CGTTATAGCAACAGCCTTTAT
Rho Cyt*b*620 R	GTGGAAAGGGATTATATCTATGT

1973b) and Baird et al. (2012) and included: greatest length of skull including incisors (GLS), condylobasal length (CBL), mastoid width (MW), depth of braincase (DB), zygomatic width (ZW), postorbital width (POW), width across upper canines (C1-C1), width across first upper incisors (I1-I1), width across second upper molars (M2-M2), palatal length (PL), maxillary toothrow (MAXT), mandible length including incisors (ML), coronoid height (CH), mandibular toothrow (MAND), and width across lower canines (c1-c1). Four external measurements taken from specimen tags also were recorded: overall total length (TL), length of tail (LT), length of hind foot (LHF), and length of ear (LE).

Univariate and multivariate statistical analyses were performed using SYSTAT 13.2 (Systat Software, Inc., San Jose, California, USA). All characters were examined using univariate analyses, whereas only cranial and mandibular characters were examined using multivariate analyses (to avoid substantial missing data and measurement error associated with external characters; Blackwell et al. 2006). To decrease the effect of individual size variation, all cranial and mandibular characters were transformed logarithmically (e.g., Gould 1966; Corrucini 1975; dos Reis et al. 1990; Burbrink 2001). Non-parametric tests were used to explore secondary sexual size dimorphism within each *a priori* defined species. Principal component analysis (PCA) was performed on the covariance matrix of the log-transformed characters to explore variation in the samples examined, which was summarized by the first two axes (PC) of the PCA. Lastly, discriminant function analyses (DFAs) were performed on the size-adjusted characters to determine if *a priori* group membership (i.e., "species") could be predicted and if individuals could be correctly assigned to their groups.

RESULTS

Molecular results.—When all fragments from specimen TCWC 19756 were aligned, they resulted in an 844 base-pair segment spanning positions 121-964 of the *Cyt*b gene. Because the DNA was derived from dried museum skins (rather than the traditional method of frozen tissue), DNA damage and/or contamination was a concern. However, no stop codons were observed within the sequence and the raw data were relatively

clean, alleviating most of the worry that the resulting sequence analysis may be misleading. The new sequence was submitted to GenBank under accession number MK410433.

The results of the Bayesian phylogenetic analysis are shown in Figure 1. Several clades are highly supported in this analysis and were also highly supported in Baird et al. (2008), which was based on the complete *Cyt*b gene (1,140 base pairs). These include the clade containing *R. aeneus*, *R. tumida*, and *R. menchuae* (the relationships among these taxa remain unresolved); *R. velilla* supported as being sister to the previously mentioned clade; and a clade containing *R. io* from South America, *R. io* from Panama, and the Nicaraguan sample (the relationships among these lineages are also unresolved).

The Nicaraguan specimen is clearly genetically distinct from all other described species in the *R. tumida* complex. It differs from its most closely related lineages, *R. io* from South America and *R. io* from Panama, by 5–6%. By comparison, *R. aeneus*, *R. tumida*, and *R. menchuae* differ from each other by 2.5% to less than 4% across the entire *Cyt*b gene (Baird et al. 2008).

When the *Cyt*b sequence from the Nicaraguan specimen is compared to the geographically and genetically close species *R. io* from Panama, there are 48 out of 844 bp that are different. These differences are mainly synonymous changes in Cyt*b*. However, there are two nonsynonymous changes resulting in amino acid differences between these two lineages. The first occurs at *Cyt*b position 475–477 (numbers reflect the position relative to the complete gene sequence), where *R. io* (Panama) contains an asparagine and the Nicaraguan sample contains an aspartic acid. The second occurs at position 706–708 where *R. io* (Panama) contains a valine and the Nicaraguan specimen contains an isoleucine.

Morphological results.—Non-parametric tests exploring secondary sexual size dimorphism within each *a priori* defined species resulted in no significant difference between sexes for all characters and species, except for *R. bickhami* where analyses resulted in inconsistent patterns among characters. Therefore,

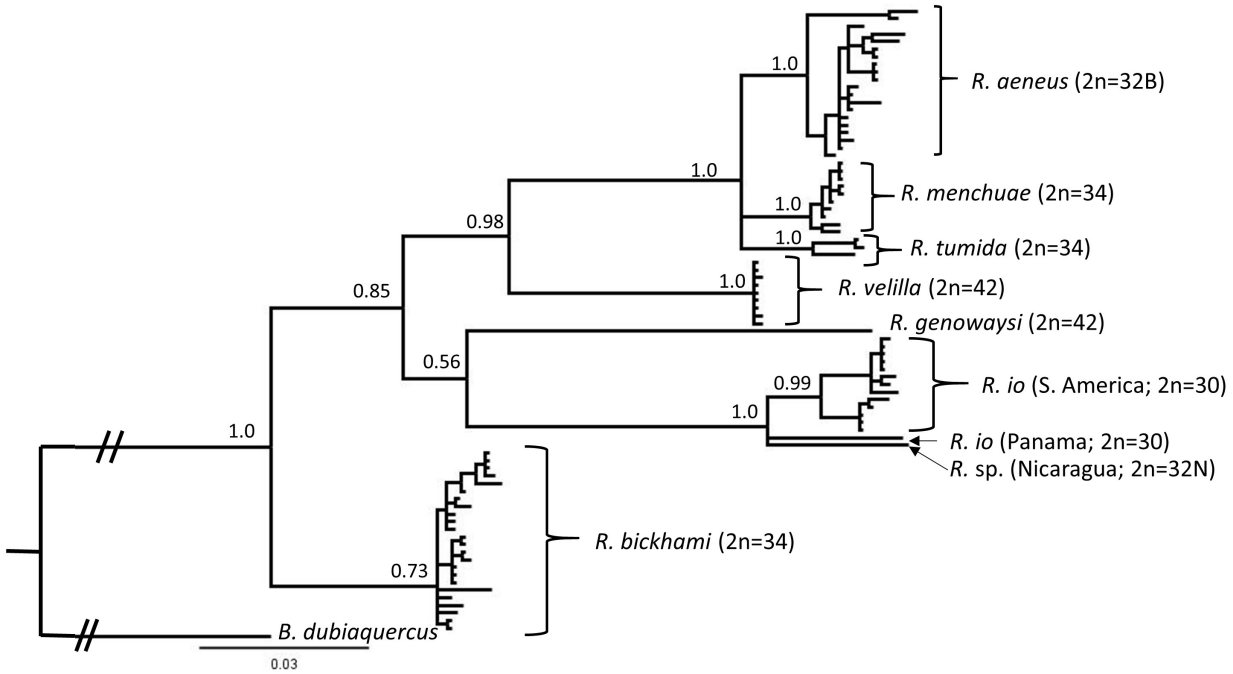

Figure 1. Bayesian phylogeny of *Rhogeessa* species based on partial *Cyt*b sequences (844 base-pairs). Numbers above nodes represent Bayesian posterior probabilities. Diploid numbers are noted next to species names.

males and females were combined for all subsequent analyses. Although 49 specimens were measured for inclusion in the analysis, specimens missing data were omitted from multivariate analyses resulting in a sample size of 32 specimens (*R. aeneus*, *n* = 4; *R. bickhami, n* = 13; *R. genowaysi*, *n* = 3; *R. io*, *n* = 1; *R. menchuae*, *n* =1; *R. parvula*, *n* = 2; *R. velilla, n* = 3; and Nicaraguan *Rhogeessa, n* = 5). Raw measurements are provided in Appendix II and a summary of the results is shown in Table 2. PCA of the transformed data resulted in the first two principal components accounting for 69.3% of the total variation in the sample (57.9% and 11.4% for PC1 and PC2, respectively). No eigenvalues were ≥ 1 and the plot of the first two principal component factors indicated some level of overlap among the species (results not shown). DFAs did result in differentiation among the *a priori* defined species (Fig. 2). *A posteriori* rates of correct classification by species were 100% for all species (jackknifed classification was lower, ranging from 0% to 100%). The first five eigenvalues of the DFA were all > 1, with the first discriminant axis explaining 51.7% of the variation (first and second discriminant axes explained a total of 80.2% of the variation). GLS, I1-I1, and c1-c1 all had high and positive loading on the first discriminant axis (with C1-C1, M2M2, and MAND loading high and negatively on this axis). MAXT, POW, GLS, and c1-c1 loaded high and positively on the second discriminant axis (ML, M2M2, and MAND loaded high and negatively on the second axis).

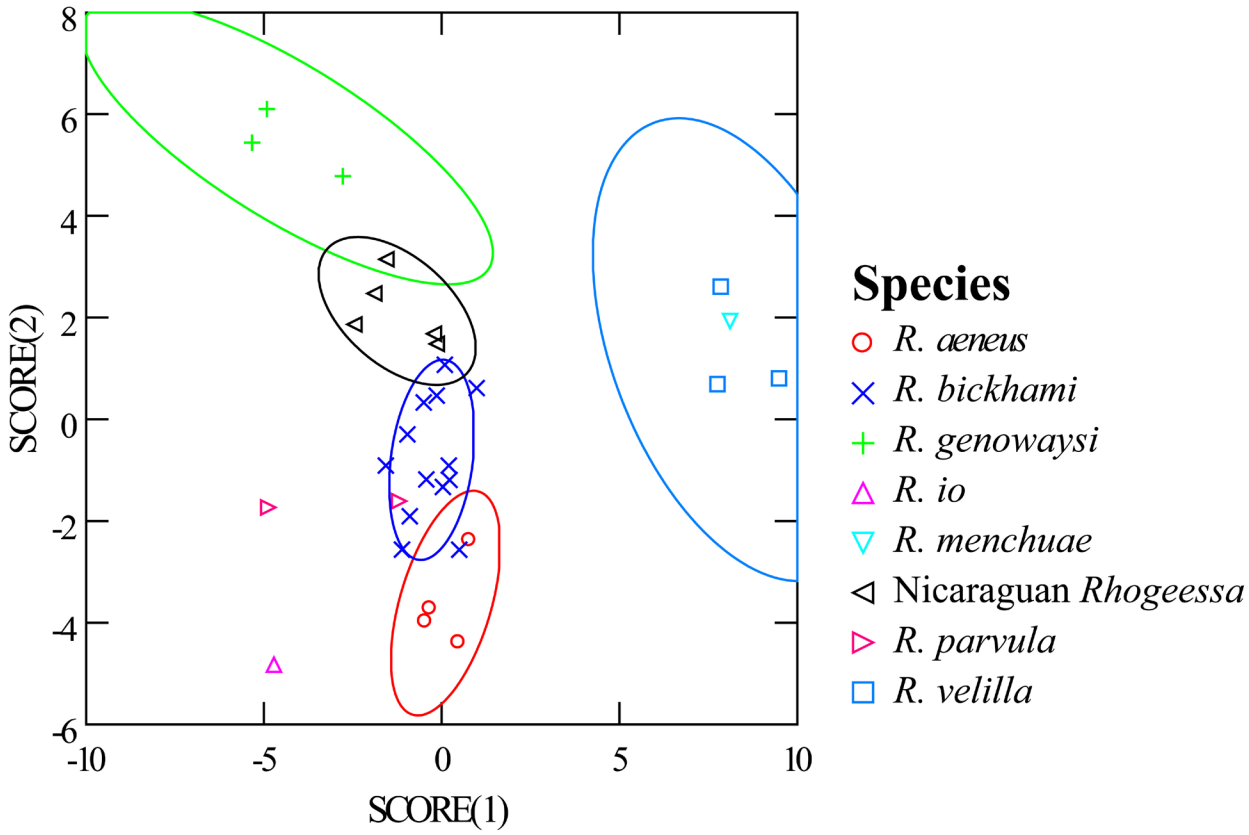

Figure 2. Discriminant function plot of standardized cranial measurements for 32 specimens of *Rhogeessa* with *a priori* groups defined as species, including the new species recognized as part of this study. Note that there is no overlap between the Nicaraguan *Rhogeessa* and its closest relative, *R. io* from Panama.

Table 2. Descriptive statistics for four external and 15 cranial and mandibular measurements for nine species of *Rhogeessa*. Acronyms for the characters are given in the Materials and Methods. For each character, the first row of measurements corresponds to the mean and standard deviation and the second row corresponds to the range. Sample size is indicated under the species name and all measurements are in millimeters.

Variable	*R. aeneus* (*n* = 5)	*R. bickhami* (*n* = 22)	*R. genowaysi* (*n* = 5)	*R. io* (*n* = 1)	*R. menchuae* (*n* = 2)	Nicaraguan *Rhogeessa* (*n* = 8)	*R. parvula* (*n* = 2)	*R. tumida* (*n* = 1)	*R. velilla* (*n* = 3)
TL	74.25 ± 2.22 (72.00–77.00)	72.50 ± 3.61 (66.00–78.00)	78.50 ± 4.95 (75.00–82.00)	72.00	71.00 ± 4.24 (68.00–74.00)	74.75 ± 4.35 (71.00–79.00)	70.00	--	67.00 ± 1.73 (65.00–68.00)
LT	30.75 ± 6.55 (21.00–35.00)	30.07 ± 4.03 (22.00–38.00)	31.00 ± 1.41 (30.00–32.00)	30.00	30.50 ± 2.12 (29.00–32.00)	31.75 ± 0.96 (31.00–33.00)	30.00	--	26.00 ± 3.46 (22.00–28.00)
LHR	6.00 ± 0.00	5.93 ± 0.73 (5.00–7.00)	6.50 ± 0.71 (6.00–7.00)	5.00	5.00 ± 0.00	6.25 ± 0.96 (5.00–7.00)	4.00	--	7.67 ±2.08 (6.00–10.00)
LE	12.75 ± 0.50 (12.00–13.00)	11.86 ± 1.75 (8.00–14.00)	11.00 ± 0.00	11.00	10.00 ± 0.00	12.75 ± 1.26 (11.00–14.00)	12.00	--	13.00 ± 2.65 (11.00–16.00)
GLS	12.41 ± 0.27 (12.18–12.75)	12.43 ± 0.48 (11.61–13.32)	13.13 ± 0.36 (12.70–13.60)	13.07	12.35 ± 0.33 (12.11–12.58)	12.99 ± 0.39 (12.30–13.42)	12.63 ± 0.45 (12.31–12.95)	11.76	12.41 ± 0.19 (12.20–12.58)
CBL	11.93 ± 0.16 (11.71–12.13)	11.98 ± 0.46 (11.08–12.89)	12.45 ± 0.46 (12.02–13.10)	12.56	12.07	12.33 ± 0.35 (11.89–12.99)	11.99 ± 0.58 (11.58–12.40)	11.10	11.98 ± 0.26 (11.80–12.27)
MW	6.56 ± 0.09 (6.43–6.65)	6.56 ± 0.27 (6.09–7.01)	6.94 ± 0.29 (6.49–7.17)	7.07	6.65 ± 0.23 (6.48–6.81)	6.57 ± 0.50 (5.71–7.20)	6.72 ± 0.09 (6.66–6.78)	6.21	6.72 ± 0.06 (6.66–6.77)
DB	4.23 ± 0.17 (4.03–4.49)	4.25 ± 0.23 (3.70–4.60)	4.14 ± 0.31 3.68–4.43	4.46	4.70	4.22 ± 0.29 (3.63–4.58)	4.10 ± 0.06 (4.06–4.14)	3.66	4.21 ± 0.03 (4.18–4.24)
ZW	7.62 ± 0.25 (7.35–8.02)	7.67 ± 0.48 (6.96–8.57)	8.62 ± 0.33 (8.37–9.00)	8.50	7.25	8.01 ± 0.61 (7.00–8.67)	7.87 ± 0.33 (7.63–8.10)	7.93	7.22 ± 0.22 (7.09–7.47)
POW	3.05 ± 0.09 (2.90–3.13)	3.21 ± 0.16 (2.95–3.59)	3.34 ± 0.10 (3.16–3.42)	3.18	3.39 ± 0.13 (3.30–3.48)	3.33 ± 0.15 (3.11–3.58)	3.11 ± 0.15 (3.00–3.21)	3.29	3.46 ± 0.04 (3.43–3.51)
C1–C1	3.54 ± 0.08 (3.43–3.65)	3.53 ± 0.17 (3.18–3.86)	3.75 ± 0.27 (3.34–3.96)	3.68	3.53 ± 0.28 (3.33–3.72)	3.62 ± 0.26 (3.33–3.97)	3.54 ± 0.07 (3.49–3.59)	3.49	3.40 ± 0.07 (3.32–3.46)
I1–I1	2.11 ± 0.12 (2.00–2.22)	2.19 ± 0.25 (1.88–2.75)	2.12 ± 0.16 (1.88–2.24)	2.18	2.24 ± 0.16 (2.13–2.35)	2.19 ± 0.19 (1.87–2.48)	2.13 ± 0.01 (2.12–2.14)	1.91	2.27 ± 0.07 (2.21–2.34)
M2M2	4.97 ± 0.09 (4.90–5.12)	5.09 ± 0.20 (4.82–5.52)	5.41 ± 0.23 (5.14–5.66)	5.45	5.10 ± 0.21 (4.95–5.25)	5.24 ± 0.18 (5.02–5.53)	5.09 ± 0.09 (5.02–5.15)	5.22	5.01 ± 0.06 (4.96–5.07)
PL	4.88 ± 0.14 (4.64–5.00)	4.76 ± 0.28 (4.28–5.38)	4.97 ± 0.32 (4.50–5.25)	5.01	4.89 ± 0.13 (4.80–4.98)	4.81 ± 0.26 (4.44–5.10)	5.30 ± 0.16 (5.19–5.41)	4.59	4.52 ± 0.10 (4.46–4.63)

Table 2. (cont.)

Variable	*R. aeneus* (*n* = 5)	*R. bickhami* (*n* = 22)	*R. genowaysi* (*n* = 5)	*R. io* (*n* = 1)	*R. menchuae* (*n* = 2)	Nicaraguan *Rhogeessa* (*n* = 8)	*R. parvula* (*n* = 2)	*R. tumida* (*n* = 1)	*R. velilla* (*n* = 3)
MAXT	4.30 ± 0.13 (4.15–4.45)	4.40 ± 0.18 (4.14–4.90)	4.72 ± 0.19 (4.47–4.97)	4.47	4.34±0.12 (4.25–4.42)	4.58 ± 0.16 (4.29–4.81)	4.43 ± 0.14 (4.33–4.53)	4.45	4.23 ± 0.04 (4.19–4.26)
ML	9.06 ± 0.29 (8.64–9.40)	9.07 ± 0.46 (8.30–10.07)	9.25 ± 0.35 (8.80–9.60)	9.58	8.93 ± 0.13 (8.84–9.02)	9.46 ± 0.25 (9.16–9.92)	9.20 ± 0.12 (9.11–9.28)	8.33	8.91 ± 0.09 (8.83–9.00)
CH	3.26 ± 0.08 (3.19–3.36)	3.23 ± 0.22 (2.81–3.58)	3.56 ± 0.15 (3.34–3.67)	3.28	3.34±0.06 (3.30–3.38)	3.27 ± 0.27 (2.86–3.55)	3.20 ± 0.14 (3.10–3.30)	--	2.99 ± 0.07 (2.91–3.04)
MAND	4.96 ± 0.12 (4.85–5.16)	5.08 ± 0.19 (4.80–5.08)	5.18 ± 0.23 (4.90–5.38)	5.31	5.11 ± 0.04 (5.08–5.14)	5.37 ± 0.16 (5.06–5.60)	5.01 ± 0.33 (4.78–5.01)	4.88	4.79 ± 0.10 (4.68–4.87)
c1–c1	2.42 ± 0.04 (2.36–2.45)	2.39 ± 0.18 (2.22–2.66)	2.52 ± 0.20 (2.29–2.72)	2.52	2.56 ± 0.12 (2.47–2.64)	2.50 ± 0.16 (2.26–2.70)	2.40 ± 0.04 (2.37–2.43)	2.33	2.35 ± 0.05 (2.31–2.40)

ORDER CHIROPTERA
Family Vespertilionidae Gray 1821
Genus *Rhogeessa* H. Allen 1866
***Rhogeessa permutandis*, new species**
Nicaraguan Little Yellow Bat

Holotype.—Adult female, TCWC 19756, deposited at the Biodiversity Research and Teaching Collections (BRTC) at Texas A&M University (Fig. 3). The holotype is preserved as skin, with skull and skeleton extracted in good condition. Specimen collected by D. C. Carter (collector's number 7602) on 28 April 1967. The following measurements (in mm) taken by the collector are recorded on the skin tag: overall total length, 79; length of tail, 32; length of hind foot, 7; and length of ear, 13. Additional cranial and mandible measurements were taken by JEL and are as follows: greatest length of the skull, 13.25; condylobasal length, 12.47; mastoid width, 7.10; depth of braincase 4.23; zygomatic width, 8.59; postorbital width, 3.33; width across first upper canines, 3.89; width across first upper incisors, 2.48; width across second upper molars, 5.44; palatal length, 5.10; maxillary toothrow, 4.69; mandible length including incisors, 9.36; coronoid height, 3.55; mandibular toothrow, 5.60; and width across first lower canines, 2.63 (Appendix II). Nucleotide sequence of the mitochondrial *Cyt*b gene (partial sequence) is deposited in GenBank under accession number MK410433.

Type Locality.—10 km W Rama, Zelaya [now the South Atlantic Autonomous Region], Nicaragua, 40 m elevation. GPS coordinates were not available at the time the holotype was collected. For a map indicating the location of samples from Rama, see Baker et al. (1985; their Fig. 1).

Type Series (7).—Seven individuals are included in the type series (2 males and 5 females): TTU 13313 (female, skin and skeleton), specimen collected by R. J. Baker et al. and prepared by V. R. MacDaniel (preparator's number 1632) on 27 July 1971 at 4.5 km NW Rama, Zelaya, Nicaragua; TTU 13317 (male, skin and skeleton), specimen collected by R. J. Baker et al. and prepared by W. J. Bleier (preparator's number 567) on 29 July 1971 at 3 km NW Rama, Zelaya, Nicaragua; TTU 13318 (female, skin and skeleton), specimen collected by R. J. Baker et al. and prepared by C. S. Rouk (preparator's number 362) on 28 July 1971 at 3

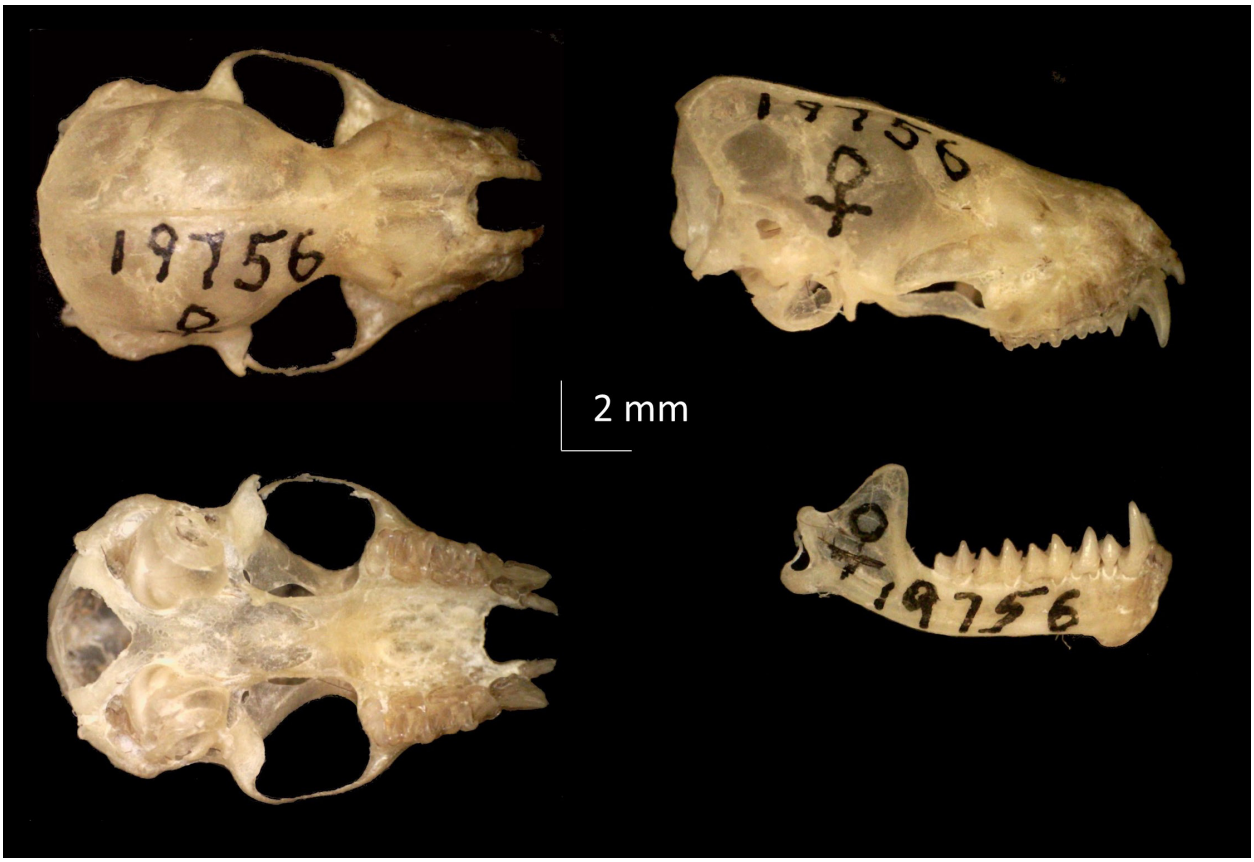

Figure 3. Dorsal, ventral, and lateral views of the skull and lower jaw of the holotype of *Rhogeessa permutandis* (TCWC 19756).

km NW Rama, Zelaya, Nicaragua; TTU 13316 (male, alcohol, skull removed), specimen collected by R. J. Baker et al. and prepared by R. J. Baker (preparator's number 1734) on 31 July 1971 at 4.5 km NW Rama, Zelaya, Nicaragua; TTU 29304 (female, alcohol, skull removed), specimen collected by P. G. Dolan (preparator's number 806) on 29 July 1971 at 4 km W Rama, Zelaya, Nicaragua; TTU 29305 (female, alcohol, skull removed), specimen collected by P. G. Dolan (preparator's number 805) on 29 July 1971 at Rama, Zelaya, Nicaragua; and TCWC 35148 (female, skin and skull), specimen collected by J. W. Bickham, I. F. Greenbaum et al. and prepared by W. B. Davis (preparator's number W. B. Davis 10047) on 25 May 1978 at 4.5 km NW Rama, Zelaya, Nicaragua, 100 m.

Distribution.—Rhogeessa permutandis has been collected only from Nicaragua near the town of Rama in the South Atlantic Autonomous Region. The extent of the distribution, therefore, is unknown. It does not occur in the southwestern part of Nicaragua, as those populations are known to have a 2n=34 karyotype (Bickham and Baker 1977) and are likely *R. bickhami*. Based on distribution patterns of other *Rhogeessa*, which are known to be limited to low elevations, it is unlikely to be present west of the central highlands of Nicaragua. Its range may spread along the Caribbean coast, with a northern limit somewhere in Honduras (where *R. menchuae* occurs). The Caribbean coast of Central America has not been well sampled for *Rhogeessa* south of Rama, so it is difficult to hypothesize how far its range may extend in that direction.

Etymology.—The specific epithet "permutandis" translates from Latin meaning "rearrangement" or "exchange." The rearrangements referenced by this name are the unique chromosomal differences among most of the species in the *R. tumida* complex, includ-

ing *R. permutandis*. These chromosomal changes are hypothesized to be the mechanism of speciation among many members of the *R. tumida* complex (Baker and Bickham 1986). "Nicaraguan Little Yellow Bat" is recommended as the English common name in reference to the type locality of this species.

Diagnosis.—Rhogeessa permutandis has a karyotype of 2n=32, although with different chromosomal fusions than the 2n=32 *R. aeneus* in the Yucatan peninsula (Bickham and Baker 1977; Baker et al. 1985). A detailed description of the difference between the karyotypes of *R. permutandis* and *R. aeneus* can be found in Baker et al. (1985). *Rhogeessa permutandis* is a large species of *Rhogeessa*. It is larger on average than all other members of the *R. tumida* complex except *R. genowaysi* (Table 2). The holotype was examined as part of LaVal's (1973b) study on morphology of *Rhogeessa*. He categorized the coloration of this specimen as having a fuscous-black dorsum and buffy brown venter following Ridgway (1912). The dental formula, as is the condition in all *Rhogeessa* species, is i 1/3, c 1/1, p 1/2, m 3/3, total 30.

Habitat and ecology. — The Caribbean lowlands of Nicaragua are hot and humid and characterized by tropical forests. The Rio Escondido runs through Rama, Nicaragua, and to the west branches into the Rio Mico and Rio Siquia. Based on recent satellite imagery (Google Earth), logging and deforestation appear to have been extensive to the north of Rama, but to the west (the direction of the type locality) natural habitat appears to be still present.

DISCUSSION

New data on morphology and *Cyt*b sequence from *Rhogeessa* specimens originating near Rama, Nicaragua, were presented herein. The *Cyt*b sequence is distinct from all other described forms of *Rhogeessa*. Karyotypes of samples near Rama, Nicaragua, have been reported in previous studies (Bickham and Baker 1977; Baker et al. 1985). There are a few errors in reporting the karyotypes of the Rama specimens in Bickham and Baker (1977) that were later corrected, though not explicitly discussed, in Baker et al. (1985). For clarity, we want to emphasize here that all specimens from eastern (the Caribbean versant) Nicaragua have the 2n=32N karyotype and all specimens from western (the Pacific versant) Nicaragua have the 2n=34 karyotype. Verified karyotypes for 2n=32N (*R. permutandis*) were derived from localities 4.5 km NE Rama (one individual) and 3 km NW Rama (two individuals; Bickham and Baker 1977). All available vouchers from eastern Nicaragua, including those with verified karyotypes, were included in the morphological analysis in this paper.

Although cranial and mandibular characters were not measured from a large number of specimens, the morphological results of this study support the genetic and karyotypic findings. This finding is in sharp contrast to previous studies on morphology of *Rhogeessa*, which have noted considerable morphological variation within the group but were not successful at delimiting most of the species within the complex (LaVal 1973b; Baker 1984; Baird et al. 2012). The DFA analysis here, which used *a priori* species definitions to test whether individuals could be assigned to those groups, was able to differentiate most of the species. The species in the *R. tumida* complex are still considered morphologically cryptic because they cannot be distinguished from one another by measuring individual specimens. Rather, samples from multiple individuals of each species must be analyzed using sophisticated statistical methods. It is still necessary to sequence genetic loci and/or karyotype *Rhogeessa* specimens to ensure their correct classification. This illustrates the utility of multiple datasets for taxonomic studies and the strength of combined morphological and genetic analyses.

Rhogeessa permutandis is distinct from all other members of the genus in molecular sequence and karyotypic rearrangements, and these differences are supported by morphology. Its elevation to species status is consistent with the treatment of other karyotypically and genetically distinct members of the *R. tumida* complex. Despite the low sample size of *R. permutandis* in the molecular aspect of this study, multiple specimens were previously karyotyped near the type locality and all were found to possess a 2n=32N karyotype (Bickham and Baker 1977). The descrip-

tion of *R. permutandis* based on karyotype and DNA sequence divergence is also consistent with the expectations of what constitutes a species under the Genetic Species Concept (GSC; Baker and Bradley 2006). As Baker et al. (1985) explained, the 2n=32B (*R. aeneus*) and 2n=32N (*R. permutandis*) karyotypes differ by a sufficient number of chromosomal rearrangements to lead to hybrid sterility if they were to interbreed. Thus, genetic isolation is virtually guaranteed among these two taxa, and to most other karyotypic forms present in the *R. tumida* complex, due to problems during meiosis of F1 hybrids. However, *R. permutandis* differs from *R. io* by a single centric fusion which likely would not lead to reproductive isolation (Baker et al., 1985). Nonetheless, mtDNA divergence levels of 5–6% between *R. permutandis* and its closest relatives, the two lineages of *R. io*, is on the order of divergence commonly observed in Cyt*b* between sister taxa in bats (Bradley and Baker 2001).

The combination of morphological, karyotypic, and molecular distinction of *R. permutandis* from its closest relative, *R. io* (and all other *Rhogeessa*), is compelling evidence for recognizing *R. permutandis*. No source of data we examined is ambiguous as to whether the eastern Nicaraguan specimens should remain classified as *R. tumida* (as they were prior to this study), nor should they be reclassified as synonymous with *R. io* (their sister taxon in the molecular phylogeny). Taken together, the morphological, karyotypic, and molecular datasets indicate that *R. permutandis* is an independently evolving lineage worthy of specific status.

With the description of this new taxon, each karyotypically distinct population in the *R. tumida* complex is now recognized and formally described as a distinct species. The chromosomal rearrangements that have been described in the complex have indeed resulted in speciation as predicted by the model of speciation by monobrachial centric fusions (Baker and Bickham 1986), at least among some species (Baird et al. 2009; Baird et al. 2012). *Rhogeessa permutandis* and its closest relative, *R. io*, do not differ by any monobrachial centric fusions (Baker et al. 1985), so it is unknown what the initial isolating mechanism and cause of speciation was among these two lineages.

The relationships shown in Figure 1 based on *Cyt*b sequence are identical to those predicted among karyotypic forms in Baker et al. (1985; their Fig. 2). There, the authors expected the 2n=32N form to be the sister taxon to the 2n=30 form (now *R. io*). They predicted that the 2n=32B form (now *R. aeneus*) would be the sister taxon to the 2n=34 form (*R. tumida* and now also *R. bickhami* and *R. menchuae*). Although sequence data has uncovered additional divergence within karyotypic forms, the expected patterns of Baker et al. (1985) generally hold true. *Rhogeessa permutandis* (2n=32N) is the sister taxon to *R. io* (2n=30), and *R. aeneus* (2n=32B) occurs in a clade with both *R. tumida* (2n=34) and *R. menchuae* (2n=34).

A literature search for synonyms of *Rhogeessa tumida* that could potentially apply to the newly described taxon did not reveal any candidate available names. *Rhogeessa tumida major* is currently applied as a synonym of *R. parvula* based on morphology (LaVal 1973b; Wilson and Reeder 2005). *Rhogeessa tumida riparia* and *R. bombyx* are considered synonyms of *R. io* (Genoways and Baker 1996; Wilson and Reeder 2005), and both were described from South America. Therefore, it was concluded that there was not a name available to be applied to the newly described taxon from Nicaragua.

The abundance and range extent of *R. permutandis* is unknown. The species is known only from a handful of individuals (see "type series" above). Additional study along the Caribbean coast of Central America is recommended to determine species ranges and abundance for all members of the *R. tumida* complex that occur there.

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especially helpful across many aspects of this study. L. Lindsay kindly measured holotypes of *Rhogeessa* housed at the NSRL for use in this study. J. Jacisin and L. Siciliano-Martina took images of the type specimen and D. Hafner provided helpful information on the morphology analyses. We thank A. Gardner and D. Wilson for comments on the name of this new species. This is publication 1599 of the Biodiversity Research and Teaching Collections at Texas A&M University.

This work would not have been possible without the groundwork laid by Robert J. Baker. His contributions to the fields of mammalian systematics and karyotypic evolution are exemplified in his early studies on *Rhogeessa*. We can think of no more appropriate paper to contribute to this volume in his memory than describing a new member of the *R. tumida* complex, which reflects his interests in chromosomal speciation and the Genetic Species Concept so uniquely.

LITERATURE CITED

Allen, H. 1866. Notes on the Vespertilionidae of tropical South America. Proceedings of the Academy of Natural Sciences of Philadelphia 18:279–288.

Audet, D., M. D. Engstrom, and M. B. Fenton. 1993. Morphology, karyology, and echolocation calls of *Rhogeessa* (Chiroptera: Vespertilionidae) from the Yucatan Peninsula. Journal of Mammalogy 74:498–502.

Baird, A. B., D. M. Hillis, J. C. Patton, and J. W. Bickham. 2008. Evolutionary history of the genus *Rhogeessa* (Chiroptera: Vespertilionidae) as revealed by mitochondrial DNA sequences. Journal of Mammalogy 89:744–754.

Baird, A. B., D. M. Hillis, J. C. Patton, and J. W. Bickham. 2009. Speciation by monobrachial centric fusions: a test of the model using nuclear DNA sequences from the bat genus *Rhogeessa*. Molecular Phylogenetics and Evolution 50:256–267.

Baird, A. B., M. R. Marchán-Rivadeneira, S. G. Pérez, and R. J. Baker. 2012. Morphological analysis and description of two new species of *Rhogeessa* (Chiroptera: Vespertilionidae) from the neotropics. Occasional Papers, Museum of Texas Tech University 307:1–25.

Baker, R. J. 1984. Mammalian sympatric, cryptic species: a new species of *Rhogeessa* (Chiroptera: Vespertilionidae). Systematic Zoology 33:178–183.

Baker, R. J., and J. W. Bickham. 1986. Speciation by monobrachial centric fusions. Proceedings of the National Academy of Sciences 83:8245–8248.

Baker, R. J., J. W. Bickham, and M. L. Arnold. 1985. Chromosomal evolution in *Rhogeessa* (Chiroptera: Vespertilionidae): possible speciation by centric fusions. Evolution 39:233–243.

Baker, R. J., and R. D. Bradley. 2006. Speciation in mammals and the genetic species concept. Journal of Mammalogy 87:643–662.

Baker, R. J., M. M. McDonough, V. J. Swier, P. A. Larsen, J. P. Carrera, and L. K. Ammerman. 2009. New species of bonneted bat, genus *Eumops* (Chiroptera: Molossidae) from the lowlands of western Ecuador and Peru. Acta Chiropterologica 11:1–13.

Benda, P., P. Hulva, and J. Gaisler. 2004. Systematic status of African populations of *Pipistrellus pipistrellus* complex (Chiroptera: Vespertilionidae), with a description of a new species from Cyrenaica, Libya. Acta Chiropterologica 6:193–217.

Bickford, D., et al. 2007. Cryptic species as a window on diversity and conservation. Trends in Ecology and Evolution 22:148–155.

Bickham, J. W., and R. J. Baker. 1977. Implications of chromosomal rearrangements in *Rhogeessa* (Chiroptera: Vespertilionidae). Journal of Mammalogy 58:448–453.

Bickham, J. W., J. C. Patton, D. A. Schlitter, I. L. Rautenbach, and R. L. Honeycutt. 2004. Molecular phylogenetics, karyotypic diversity and partition of the genus *Myotis* (Chiroptera: Vespertilionidae). Molecular Phylogenetics and Evolution 33:333–338.

Bickham, J. W., C. C. Wood, and J. C. Patton. 1995. Biogeographic implications of cytochrome-*b* sequences and allozymes in sockeye (*Oncorhynchus nerka*). Journal of Heredity 86:140–144.

Blackwell, G. L., S. M. Basset, and C. R. Dickman. 2006. Measurement error associated with external measurements commonly used in small-mammal studies. Journal of Mammalogy 87:216–223.

Bradley, R. D., and R. J. Baker. 2001. A test of the genetic species concept: cytochrome-*b* sequences and mammals. Journal of Mammalogy 82:960–973.

Brooks, D. M., and J. W. Bickham. 2014. New species of *Scotophilus* (Chiroptera: Vespertilionidae) from sub-Saharan Africa. Occasional Papers, Museum of Texas Tech University 326:1–21.

Burbrink, F. T. 2001. Systematics of the eastern ratsnake complex (*Elaphe obsolete*). Herpetological Monographs 15:1–53.

Ceballos, G., and P. R. Ehrlich. 2009. Discoveries of new mammal species and their implications for conservation and

ecosystem services. Proceedings of the National Academy of Sciences 106:3841–3846.

Corruccini, R. S. 1975. Multivariate analysis in biological anthropology: some considerations. Journal of Human Evolution 4:1–19.

Darriba, D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9:772.

dos Reis, S. F., L. M. Pessoa, and R. E. Strauss. 1990. Application of size-free canonical discriminant analysis of geographic differentiation. Brazilian Journal of Genetics 13:509–520.

Francies, C. M., et al. 2010. The role of DNA barcodes in understanding and conservation of mammal diversity in Southeast Asia. PLOS One 5:e12575.

Genoways, H. H., and R. J. Baker. 1996. A new species of the genus *Rhogeessa*, with comments on geographic distribution and speciation in the genus. Pp. 83–87 in Contributions to mammalogy: a memorial volume honoring Dr. J. Knox Jones, Jr. (H. H. Genoways and R. J. Baker, eds.). Museum of Texas Tech University, Lubbock.

Gould, S. J. 1966. Allometry and size in ontogeny and phylogeny. Biological Review 41:587–640.

Gray, J. E. 1821. On the natural arrangement of vertebrose animals. London Medical Repository 15:296–310.

Gregorin, R., et al. 2016. A new species of *Eumops* (Chiroptera: Molossidae) from southeastern Brazil and Bolivia. Mammalian Biology 81:235–246.

Guindon, S., and O. Gascuel. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Systematic Biology 52:696–704.

Honeycutt, R. L., D. M. Hillis, and J. W. Bickham. 2010. Biodiversity discovery and its importance to conservation. Pp. 1–34 in Molecular approaches in natural resource conservation and management (J. A. DeWoody, J. W. Bickham, C. H. Michler, K. M. Nichols, O. E. Rhodes, and K. E. Woeste, eds.). Cambridge University Press, New York.

Huelsenbeck, J. P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

Kearse, M., et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649.

LaVal, R. K. 1973a. A revision of the Neotropical bats of the genus *Myotis*. Bulletin of the Natural History Museum, Los Angeles County 15:1–54.

LaVal, R. K. 1973b. Systematics of the genus *Rhogeessa* (Chiroptera: Vespertilionidae). Occasional Papers of the Museum of Natural History, University of Kansas 19:1–47.

Reeder, D. M., K. M. Helgen, and D. E. Wilson. 2007. Global trends and biases in new mammal species discoveries. Occasional Papers, Museum of Texas Tech University 269:1–35.

Ridgway, R. 1912. Color standards and color nomenclature. Privately published, Washington, DC.

Ronquist, F., and J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Wilson, D. E., and D. M. Reeder (eds). 2005. Mammal species of the world: a taxonomic and geographic reference (3rd edition). Johns Hopkins University Press, Baltimore, Maryland.

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APPENDIX I

Specimens examined.—Species, localities, and catalog numbers of specimens examined in this study. All specimens examined for morphology are housed at the Biodiversity Research and Teaching Collections at Texas A&M University (BRTC; specimens have a TCWC acronym) or the Natural Science Research Laboratory at the Museum of Texas Tech University (TTU). Tissue numbers (AK for the BRTC, TK for TTU, ASNHC for Angelo State Natural History Collection, FN for Royal Ontario Museum, and SP for Carnegie Museum) and collector numbers (SGP for Sergio G. Perez) also are noted when available for specimens used in molecular analyses. For specimens used in molecular analyses, individual specimen numbers are as follows: museum catalog number/ tissue number/*Cyt*b GenBank number. Asterisks (*) correspond to type specimens; daggers (†) indicate specimens used in both molecular and morphological analyses.

Molecular Analyses

Rhogeessa aeneus (*n* = 18).—BELIZE: Belize District (TTU 40003/TK 20704/EF222329†, TTU 40010/ TK 20706/EF222361, TTU 40004/TK 20707/EF222363, TTU 40009/TK 20710/EF222395†, TTU 40012/TK 20712/EF222364†); Orange Walk (AK7771/EF222325). GUATEMALA: Peten (SGP1030/EF222418, SGP1140/ EF222419). MEXICO: Campeche (FN30223/EF222334, FN30225/EF222327, FN30226/EF222408, FN30677/ EF222333, FN30678/EF222337, ASNHC1414/EF222359); Yucatan (FN30462/EF22405, FN30463/EF222406, FN30464/EF222331).

Rhogeessa bickhami (*n* = 24).—COSTA RICA: Guanacaste (TCWC 47833/AK 7022/EF222335†). EL SALVADOR: San Salvador (TTU 60986/TK 34866/EF222380†, TTU 60987/TK 34867/EF222353†); La Paz (TTU 60985/TK 34902/EF222385†, TK 34980/EF222390). GUATEMALA: Zacapa (SP12771/EF222402, SP12772/ EF22403); El Progreso (AK 25022/EF222416, AK 25023/EF222413, AK25024/EF222414). HONDURAS: Valle (TTU 61231/TK 40186/EF222350†, TTU 83681/TK 101020/EF22235†1, TTU 83682/TK 101021/EF222352†, TTU 83705/TK 101044/EF222367†, TTU 83713/TK101052/EF222368†, TTU83927/TK 101266/EF222409†, TCWC 47833/AK 9585/EF222326, TCWC 49793/AK 9587/EF222372†, TCWC 49797/AK 9615/EF222373†, TCWC 49799/AK 9617/EF222374†); Comayagua (TTU 84027/ TK 101367/EF222383†, TTU 84030/TK 101370/ EF222411†). MEXICO: Chiapas (TTU 36161/ TK 20594/EF222338*†, TTU 36164/TK 20596/EF222365†).

Rhogeessa genowaysi (*n* = 1).—MEXICO: Chiapas: 23.6 mi N Huixtla (TTU 36171/TK 20597/EF222326†).

Rhogeessa io (*n*= 14).—PANAMA: Darién: Cana (TTU 39147/TK 22536/EF222369†). TRINIDAD: Trinidad Nariva (TK 25079/EF222379). VENEZUELA: Guarico (TTU 33400/TK 15163/EF222410, TK 15164/ EF222384, TK 15179/EF222392, TK 15209/EF222392); Guatopo (TTU 33402/TK 15286/EF222358); Bolivar (TK 19004/EF222393, TK 19005/EF222394, TK 19043/EF222347); Barinas (TK 19450/EF222404, TK 19458/ EF222348, TK 19459/EF222330, TK 19519/EF222407).

Rhogeessa menchuae (*n* = 12).—GUATEMALA: Izabal (AK 25065/EF222417, AK 25093/EF222415, SP12543/EF222396, SP12544/EF222397, SP12606/EF222398, SP12615/EF222399, SP12617/EF222400, SP12650/EF222401). HONDURAS: Atlantida (TCWC 49808/AK 7136/EF222370, AK 7137/EF222371, TTU 61229/ TK40345/EF222377†, TTU 61230/ TK40360/EF222378*†).

Rhogeessa tumida (*n* = 3).—MEXICO: Oaxaca (TTU 36168/TK 20515/EF222349); Tamaulipas (TTU 44867/TK 27068/EF222345†, AK 1638/EF222360).

Rhogeessa velilla (*n* = 9).—ECUADOR: Guayas (TTU 103525/TK 134692/EF222341†, TTU 103525/TK 134693/EF222342, TTU 103254/TK 13792/EF222339†, TTU 103292/TK 134868/EF222366†, TTU 103341/TK

134869/EF222365, TTU 103293/TK 134870/EF222386, TTU 103294/TK134871/EF222387, TTU 103295/TK 134872/EF222388, TTU 102429/TK 135175/EF222389).

Rhogeessa permutandis (*n* = 1).—NICARAGUA: Zelaya: (TCWC 19756/MK410433*†, TTU 13313, TTU 13318, TTU 13317). Note: in this species, only specimen TCWC 19756 resulted in DNA and was used in the molecular analysis.

Morphometric Analyses

Rhogeesa aeneus (*n* = 5).—BELIZE: Belize District: Belize City (TTU 40003†, TTU 40009†, TTU 40010†); Burrell Boom Village (TTU 40005, TTU 40012†).

Rhogeessa bickhami (*n* = 26).—COSTA RICA: Guanacaste: Finca La Pacifica (TCWC 47833†). EL SALVADOR: La Paz: Hacienda Escuintla, Zacatecaluca (TTU 60985†); San Salvador: near El Guaje (TTU 60986†, TTU 60987†). HONDURAS: Comayagua: Comayagua (Senasa) (TTU 84027†; TTU 84030†); 7.4 mi SSW San Lorenzo (TCWC 49805, TCWC 49806, TCWC 49807); 8.5 mi SSW San Lorenzo (TTU 61231†); Valle: 10.8 mi S, 2.6 mi W Jicaro Galan (TCWC 49791, TCWC 49792, TCWC 49793†, TCWC 49797†, TCWC 49799†); Valle: 13 km W, 3 km S Nacome (TTU 83927†); Valle: 3 km N, 12.5 km SW San Lorenzo (TTU 83681†, TTU 83682†, TTU 83705†, TTU 83713†). MEXICO: Chiapas: 23.6 mi N Huixtla (TTU 36161*†, TTU 36164†).

Rhogeessa genowaysi (*n* = 5).—MEXICO: Chiapas: 23.6 mi N Huixtla (TTU 36171†); Chiapas: 23.6 mi NW Huixtla (TTU 29103, TTU 29104, TTU 29106, TTU 29108).

Rhogeessa io (*n* = 1).—PANAMA: Darién: Cana (TTU 39147†).

Rhogeessa menchuae (*n* = 2).—HONDURAS: Atlántida: Lancitilla (TTU 61229†, TTU 61230*†).

Rhogeessa parvula (*n* = 2).—MEXICO: Guerrero: 24.1 mi NW Rio La Union Hwy 200 (TTU 46788); Jalisco: 2 km W Tomatlan (TTU 37726).

Rhogeessa tumida (*n* =1).—MEXICO: Tamaulipas: 2 mi W Calabazas Rio Sabinas (TTU 44867†).

Rhogeessa velilla (*n* = 3).—ECUADOR: Guayas: Bosque Protector Cerro Blanco, Centro de Visitantes (TTU 103254†, TTU 103292†); Manglares Churute, Cerro Cimalon (TTU 103525†).

Rhogeessa permutandis (*n* = 8).—NICARAGUA: Zelaya: Rama (TTU 29305); 3 km NW Rama (TTU 13317, TTU 13318); 4 km W Rama (TTU 29304); 4.5 km NW Rama (TCWC 35148, TTU 13313, TTU 13316); 10 km W Rama (TCWC 19756*†)

APPENDIX II

Morphological measurements for the *Rhogeessa* specimens used in this study. Abbreviations of measurements are found in the Materials and Methods section. Locality information is provided in Appendix I. All measurements are in millimeters. ND = no data available. Holotypes are indicated with an asterisk (*) after the museum number.

Species	Museum No.	Sex	TL	LT	LHF	LE	GLS	CBL	MW	DB	ZW	POW	C1-C1	I1-I1	M2-M2	PL	MAXT	ML	CH	MAND	c1-c1
aeneus	TTU 40003	F	73	35	6	13	12.75	12.13	6.64	4.49	7.6	2.9	3.49	2	4.93	5	4.45	9.21	3.2	5.16	2.44
aeneus	TTU 40005	F	75	34	6	13	12.66	12.03	6.65	4.27	7.49	3.12	3.58	2.19	4.98	4.9	4.23	9.4	3.36	4.91	2.36
aeneus	TTU 40009	M	72	21	6	12	12.27	11.84	6.43	4.18	7.35	3.13	3.65	2.22	5.12	4.92	4.24	9.15	3.21	4.85	2.44
aeneus	TTU 40010	F	77	33	6	13	12.21	11.94	6.52	4.03	7.62	3.08	3.43	2.01	4.92	4.92	4.15	8.92	3.33	4.9	2.43
aeneus	TTU 40012	F	ND	ND	ND	ND	12.18	11.71	6.54	4.16	8.02	3.03	3.54	ND	4.9	4.64	4.42	8.64	3.19	5	4.45
bickhami	TCWC 47833	F	ND	ND	ND	ND	12.55	12.18	6.81	4.4	ND	3.27	3.51	2.13	5.21	4.75	4.47	9.43	3.3	5.2	2.44
bickhami	TCWC 49791	M	ND	ND	ND	ND	11.68	11.08	6.17	4.48	7.13	3.13	3.35	ND	4.95	4.69	4.14	8.47	2.81	4.94	2.34
bickhami	TCWC 49793	M	ND	ND	ND	ND	12.05	11.9	6.38	4.54	7.33	3.33	3.35	1.95	4.87	5.04	4.21	8.73	3.06	4.8	2.28
bickhami	TCWC 49797	F	ND	ND	ND	ND	12.34	12.03	6.39	4.34	7.41	3.14	3.34	1.88	4.94	5.03	4.34	9.01	3.46	4.91	2.38
bickhami	TCWC 49799	F	ND	ND	ND	ND	11.61	11.18	6.13	4.3	7.24	3.15	3.36	2	4.84	4.5	4.29	8.54	3.3	4.99	2.27
bickhami	TCWC 49792	F	ND	ND	ND	ND	12.3	11.71	6.34	4.08	ND	3.16	3.42	2.33	5.11	4.43	4.42	8.67	2.94	5.05	2.23
bickhami	TTU 36161*	F	72	30	5	13	12.31	11.84	6.48	4.6	7.77	3.59	3.18	2.03	5.13	5.38	4.54	9.2	3.42	5.05	2.32
bickhami	TTU 36164	F	70	30	7	11	12.82	12.07	6.45	4.11	ND	3.02	3.53	2.1	5.08	4.64	4.58	8.9	3.06	5.09	2.34
bickhami	TTU 60985	F	66	25	6	12	12.54	11.87	6.62	4.2	ND	3.34	3.63	2.09	4.99	4.75	4.32	9.38	3.21	5.1	2.45

Species	Museum No.	Sex	TL	LT	LHF	LE	GLS	CBL	MW	DB	ZW	POW	C1-C1	I1-I1	M2-M2	PL	MAXT	ML	CH	MAND	c1-c1
bickhami	TTU 60986	F	73	29	6	13	12.97	12.12	6.55	4.01	ND	3.09	3.58	2.17	5.11	4.97	4.56	9.68	3.49	5.29	2.43
bickhami	TTU 60987	F	77	31	7	13	13.32	12.76	7	4.51	8.47	3.5	3.8	2.44	5.47	4.87	4.59	9.68	3.55	5.14	2.47
bickhami	TTU 83681	F	72	28	6	13	11.91	11.74	6.45	4.12	ND	3.26	3.51	2.05	5.31	4.36	4.27	9.12	3.14	5.06	2.22
bickhami	TTU 83682	M	71	32	7	12	12.27	12.12	6.6	4.24	ND	3.24	3.45	ND	4.87	4.95	4.22	8.85	3.1	4.96	2.37
bickhami	TTU 83705	F	74	33	6	13	12.12	11.76	6.58	4.11	ND	3.12	3.42	2.07	5	4.51	4.25	9	3.33	4.91	2.27
bickhami	TTU 83713	F	ND	ND	ND	ND	12.53	12.14	6.71	4.25	8.09	3.14	3.59	2.05	5.12	4.76	4.34	8.99	3.31	5.02	2.45
bickhami	TTU 84027	M	71	22	5	12	12.55	11.88	6.65	ND	7.94	3.09	3.72	2.7	5.04	4.75	4.44	9.27	3.08	5.14	2.37
bickhami	TTU 84030	F	78	32	6	14	13.1	12.64	7.01	4.44	ND	3.38	3.86	2.61	5.52	4.89	4.57	9.73	3.4	5.13	2.63
bickhami	TCWC 49805	F	78	38	6	9	12.78	12.57	6.75	4.45	7.97	3.36	3.67	2.19	5.18	4.75	4.58	9.18	3.45	5.42	2.51
bickhami	TCWC 49806	M	70	35	5	8	11.8	11.33	6.09	3.7	ND	2.95	3.6	2.1	4.82	4.49	4.26	8.3	2.92	4.98	2.34
bickhami	TCWC 49807	M	68	27	5	10	12.02	11.74	6.3	3.9	7.62	3.02	3.48	2	4.98	4.28	4.24	8.72	3.02	4.93	2.41
bickhami	TTU 61231	F	ND	ND	ND	ND	12.64	12.07	6.84	4.22	ND	3.12	3.62	2.1	5.07	4.64	4.3	8.6	3.02	5	ND
genowaysi	TTU 29103	F	75	32	6	11	13.6	13.1	7.17	4.43	9	3.36	3.96	2.17	5.66	5.23	4.97	9.6	3.67	5.38	2.72
genowaysi	TTU 29104	F	ND	ND	ND	ND	12.85	12.02	6.49	3.68	ND	3.38	3.34	1.88	5.21	4.78	4.64	8.96	3.63	5.33	2.29
genowaysi	TTU 29106	M	ND	ND	ND	ND	12.7	12.03	6.83	4.2	ND	3.16	3.61	ND	5.14	4.5	4.47	8.8	ND	4.98	2.37
genowaysi	TTU 29108	F	ND	ND	ND	ND	13.2	12.66	7.09	4.4	8.37	3.39	3.94	2.2	5.56	5.07	4.78	9.37	3.58	5.33	2.71
genowaysi	TTU 36171	F	82	30	7	11	13.3	12.45	7.14	4	8.5	3.42	3.89	2.24	5.48	5.25	4.76	9.51	3.34	4.9	2.5

Species	Museum No.	Sex	TL	LT	LHF	LE	GLS	CBL	MW	DB	ZW	POW	C1-C1	I1-I1	M2-M2	PL	MAXT	ML	CH	MAND	c1-c1
io	TTU 39147	F	72	30	5	11	13.07	12.56	7.07	4.46	8.5	3.18	3.68	2.18	5.45	5.01	4.47	9.58	3.28	5.31	2.52
menchuae	TTU 61229	F	74	32	5	10	12.11	ND	6.81	ND	ND	3.3	3.72	2.35	5.25	4.8	4.25	8.84	3.38	5.08	2.64
menchuae	TTU 61230*	M	68	29	5	10	12.58	12.07	6.48	4.7	ND	3.48	3.33	2.13	4.95	4.98	4.42	9.02	3.3	5.14	2.47
parvula	TTU 46788	F	ND	ND	ND	ND	12.95	12.4	6.78	4.06	8.1	3	3.59	2.12	5.15	5.41	4.53	9.11	3.3	5.24	2.43
parvula	TTU 37726	F	70	30	4	12	12.31	11.58	6.66	4.14	ND	3.21	3.49	2.14	5.02	5.19	4.33	9.28	3.1	4.78	2.37
tumida	TTU 44687	M	ND	ND	ND	ND	11.76	11.1	6.21	3.66	ND	3.29	3.49	1.91	5.22	4.59	4.45	8.33	ND	4.88	2.33
velilla	TTU 103254	M	68	28	7	12	12.45	11.8	6.66	4.18	7.09	3.51	3.46	2.21	5	4.46	4.26	9	2.91	4.87	2.4
velilla	TTU 103292	F	68	28	6	11	12.2	11.86	6.77	4.22	7.1	3.45	3.32	2.25	5.07	4.63	4.23	8.83	3.03	4.68	2.31
velilla	TTU 103525	M	65	22	10	16	12.58	12.27	6.74	4.24	7.47	3.43	3.42	2.34	4.96	4.47	4.19	8.91	3.04	4.81	2.34
permutandis	TTU 13313	F	78	33	6	13	13.11	12.24	6.36	4.03	7.71	3.52	3.48	2.16	5.13	4.56	4.47	9.33	3.14	5.33	2.31
permutandis	TTU 13317	M	71	31	7	11	12.89	12.24	6.4	4.37	7	3.27	3.44	1.87	5.1	4.91	4.5	9.66	ND	5.06	2.26
permutandis	TTU 13318	F	71	31	5	14	12.3	11.89	5.71	3.63	ND	3.35	3.33	2.08	5.24	4.44	4.29	9.54	3.09	5.41	2.53
permutandis	TTU 13316	M	ND	ND	ND	ND	12.65	12.08	6.63	4.2	ND	3.11	3.33	ND	5.02	4.54	4.66	9.16	2.86	5.38	2.45
permutandis	TTU 29304	F	ND	ND	ND	ND	12.88	12.11	6.22	4.23	8.01	3.28	3.66	2.14	5.1	4.84	4.61	9.23	3.2	5.28	2.45
permutandis	TTU 29305	F	ND	ND	ND	ND	13.4	12.99	6.97	4.58	ND	3.58	3.97	2.25	5.53	5.06	4.81	9.92	3.55	5.35	2.7
permutandis	TCWC 19756*	F	79	32	7	13	13.25	12.47	7.1	4.23	8.59	3.33	3.89	2.48	5.44	5.1	4.69	9.36	3.55	5.6	2.63
permutandis	TCWC 35148	F	ND	ND	ND	ND	13.42	12.65	7.2	4.45	8.67	3.23	3.87	2.32	5.33	5.04	4.62	9.46	3.53	5.53	2.69

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Temporal Patterns of Bat Activity on the High Plains of Texas

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Abstract

Texas is home to more wind turbines and more bat species than any other state in the United States. Insectivorous bats provide an important economical ecosystem service in this region through agricultural pest regulation. Unfortunately, bats can be impacted negatively by wind turbines, and migratory bat species particularly so. To understand how bat activity changes throughout the year in western Texas, activity was monitored through echolocation calls and opportunistic mist-netting efforts over a period of four years (2012–2015). Peaks in activity were observed from March through April, and again in September, which coincides with previously documented migratory periods for many species native to the High Plains of Texas. Findings presented herein suggest that urban habitats are preferred stopover sites for migratory bat species while traversing arid regions such as those occurring in western Texas. In addition to human-made structures, urban habitats harbor non-native trees that provide suitable roost sites, aggregations of insect prey swarming outdoor light sources, and artificial water sources. It is important to understand bat activity in western Texas, not only for the benefit of agricultural pest suppression, but also to predict how the expansion of wind energy may affect bat populations in this region.

Key words: active monitoring, agriculture, driving transect, echolocation calls, passive monitoring, West Texas, wind energy

Introduction

The High Plains ecoregion of western Texas is characterized by a relatively flat topography, an arid climate with scarce water sources, and a shortgrass prairie habitat that is largely depauperate of trees (Price et al. 1997; Griffith et al. 2007). Many bat species native to Texas are dependent on trees as roosting sites (Schmidly and Bradley 2016), thus the nearly treeless landscape of the High Plains may deter bats from using Lubbock and surrounding counties as stopover sites during migration; some bat species appear to migrate through the High Plains instead of inhabiting the region year-round. Moreover, as much as 80% of the High Plains ecoregion has been converted to agricultural lands, which contributes to the loss of native prairie habitats and reduction in water resources available for wildlife due to irrigation demands (Schmidly 2002).

Despite these inhospitable conditions, over a third of all bat species native to Texas (i.e., 12 of 33 species) have been recorded in Lubbock and adjacent counties (Ammerman et al. 2012). Museum records of three migratory bats that occur in western Texas (*Aeorestes cinereus*, *Lasiurus borealis*, and *Lasionycteris noctivagans*) suggest a seasonal peak in the presence of these species likely occurs across the High Plains (Cryan 2003). Although another bat species, *Tadarida brasiliensis*, also is migratory (Glass 1982), *A. cinereus*, *Lasiurus borealis*, and *Lasionycteris noctivagans* are the more frequent species found to collide with turbines (Arnett et al. 2008). Though museum records do not specify whether bats were caught in urban areas or not, one potential explanation for peaks in seasonal activity of migratory bats could be the availability of atypical roost sites in the region, specifically planted trees in urban green spaces (e.g., parks, golf courses, residential yards). Many bat species, including migratory species, also have been documented to roost in occupied and abandoned human-made structures, such as buildings

and homes (McGuire et al. 2012; Schmidly and Bradley 2016). Coleman and Barclay (2012) reported higher bat activity in the urban environments of Alberta, Canada, versus the surrounding non-urban landscapes; Alberta, Canada, is located in the northern Great Plains ecoregion and is an environment similar to western Texas. Furthermore, playas and dammed reservoir lakes in the Great Plains provide water sources known to be used by migratory birds as stopover sites (Davis and Smith 2001) and could feasibly be used as migratory stopovers by insectivorous bats, as well.

Whereas urban environments provide suitable roost sites and water sources, expanding agricultural production in the region provides native bats with additional food resources. Insectivorous bats consume agricultural pests (Cleveland et al. 2006; Kunz et al. 2011), including cotton bollworms that contribute to economic losses for local farmers exceeding millions of dollars in recent years (USDA 2019). However, along with the agricultural expansion, there has been a marked increase in the installation of wind turbine farms in western Texas. The High Plains ecoregion is ideal for wind turbines due to its consistently high wind speeds (AWS Truepower and National Renewable Energy Laboratory 2010), yet there is mounting evidence that wind turbines have negative effects on wildlife, including mortality in bats and birds (Arnett et al. 2007, 2008; Baerwald et al. 2008; Arnett and Baerwald 2013). Declining bat populations in the High Plains could result in an increase in agricultural pests, making it more costly for farmers to achieve profitable yields (Cleveland et al. 2006).

Although bats play a critical role in suppressing agricultural pests in western Texas, the rapid expansion of wind energy installations potentially could threaten the long-term viability of resident and migratory bat populations. To date, however, bats largely have been understudied in the region compared to central and eastern Texas (Cleveland et al. 2006; Boyles et al. 2011). Challenges associated with capturing free-flying, highly maneuverable bats in open space habitats characteristic of the High Plains ecoregion likely contributes to the limited research effort on bats in western Texas. Much of our current knowledge about bat species in the Texas Panhandle is based on dead and live bats recovered by local citizens and submitted to the Texas Department of Health for rabies testing and/or bats deposited in natural history museums, including the Natural Science Research Laboratory at the Museum of Texas Tech University.

To improve our understanding of the bat species native to the High Plains of western Texas, an ecoregion that has undergone historic and ongoing conversion of native prairie to agricultural croplands and wind energy installations, this study employed diverse methods to inventory species present in Lubbock and surrounding counties. Moreover, the study sought to understand if bat activity varied throughout the year using passive and active acoustic monitoring, which allows identification of seasonal peaks in activity corresponding with known migratory periods of native bat species. Bat activity is hypothesized to vary seasonally within Lubbock County; specifically, a greater number of bat calls were expected to be detected during early spring (i.e., March to May) and fall (i.e., August to November), corresponding with previous records of seasonal migratory patterns of native species (Cryan 2003). Taken together, findings from this study will guide development of comprehensive recommendations to mitigate bat mortalities attributed to wind energy installations during migratory periods when bat activity is greatest, particularly with respect to nearly treeless ecoregions expected to be inhospitable to bats.

METHODS

Study areas.—Bat monitoring activities were conducted at two sites located within the High Plains and Rolling Plains ecoregions of Texas (Fig. 1). The first site, Reese Technology Center (RTC), is located approximately 5 km west of Lubbock, Texas. The RTC, constructed in 1941, served as a United States Air Base (Reese Air Force Base) until 1997 when it was targeted for closure and the property was transferred to the Lubbock community. The RTC contains numerous buildings (occupied and abandoned), hangers, runways, a water tower, a weather tower, parks and green areas with mature trees, and an adjacent

golf course with a permanent water source. One wind turbine was installed prior to the initiation of the survey and functioned intermittently. Three additional wind turbines were installed but were not yet functional during the study period; therefore, bat deaths due to wind turbines was not the focus of the study reported herein. Given that the primary objective of the study was to identify bat activity patterns, the RTC formed the basis for most of the activities conducted. Monitoring was concentrated around wind turbine locations, buildings, permanent water sources, and other sites likely to harbor bats.

A second study site was comprised of a driving transect survey that extended from the northeastern portion of the city of Lubbock, through the Canyon Lakes (CL) system, and terminated at Buffalo Springs and Ransom Canyon (approximately 20 km east of Lubbock, Texas). The CL system was established in 1971 and consists of a series of impoundments along the Yellowhouse Canyon system located in the northeastern portions of Lubbock. These man-made structures produce a series of eight lakes that not only serve as a valuable water source but also support a substantial number of large trees, which are rare in the High Plains ecoregion. This site was selected to be a pseudo-replicate of activities being conducted at RTC. Both sites were located in or on the fringe of a large urban area that was surrounded by agricultural activities (primarily cotton production).

Mist-netting for presence of bats.—Mist-net surveys were used to determine the current bat species present at study sites. Attempts were made to mist-net bats at RTC from March 2012 through June 2014. Six mist-nets were set up around the Reese Golf Center (golf course) next to vegetation corridors and ponds. Multiple mist-nets (lengths 9 m to 18 m) and mist-netting methods were utilized in an attempt to improve capture success, including arranging mist-nets in an

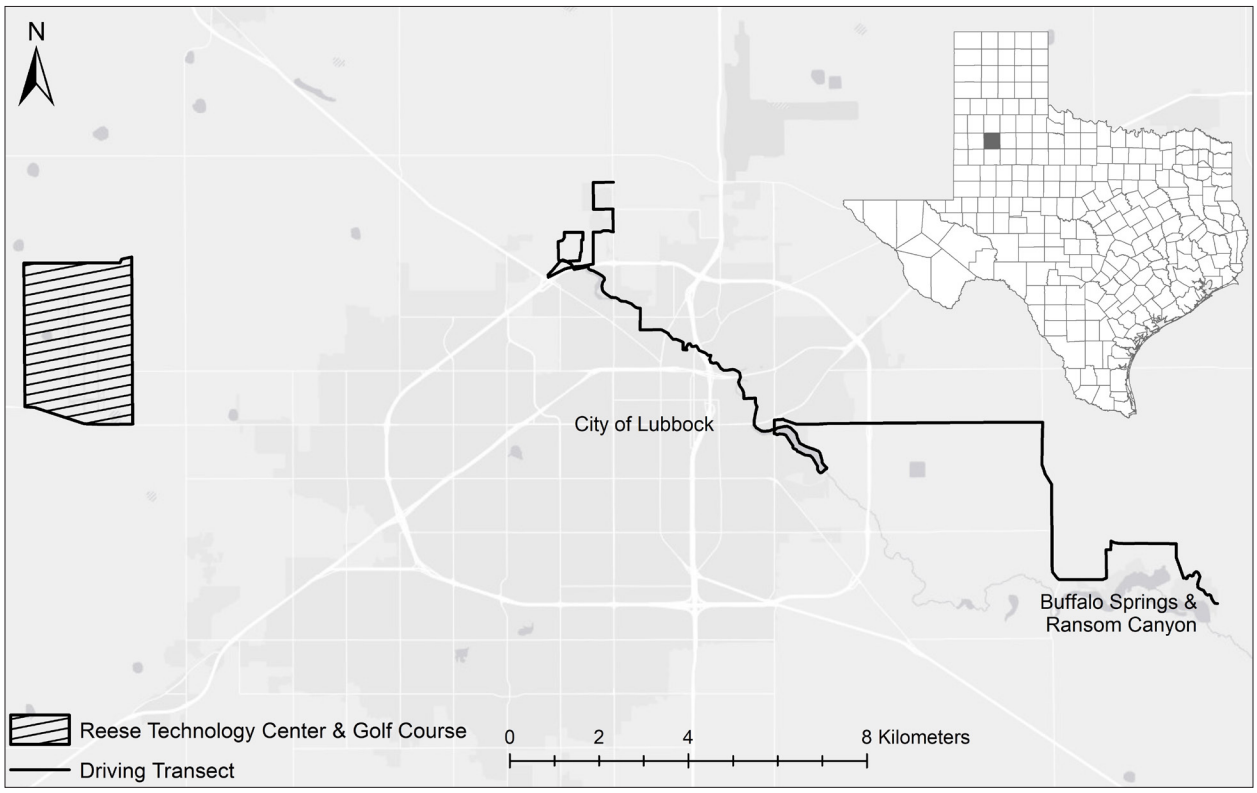

Figure 1. Study sites in Lubbock County. The area with diagonal lines indicates Reese Technology Center (RTC) and the black line indicates roads driven during the driving transect (48 km in length).

"L-shaped" formation and employing a triple high net system (BatNets.com) extending almost 7.5 m above the ground. Mist-netting was conducted twice per month from April to October, but were deployed only during periods when temperatures were above freezing during the winter months of November through March. Mist-nets were opened within 30 mins of sunset for a period of four to six hours and performed when weather conditions permitted, excluding evenings when temperatures decreased below freezing, rain occurred, or wind speeds exceeded 80.7 kmh. Additional mist-netting efforts were conducted elsewhere in Lubbock County and other locations across the High Plains to obtain reference echolocation calls not included in Adams (2003).

Acoustic monitoring of bat activity.—Anabat SD2 detectors (Titley Scientific, Columbia, Missouri) were used to establish four stationary (passive) acoustic monitoring stations at RTC from January 2012 through May 2015. Three Anabat detectors were set up at structures around RTC at the following locations: weather tower (approximately 50 m above the ground); water tower (approximately 40 m above the ground); and one building (approximately 20 m above the ground). The fourth Anabat detector was near a man-made pond at the golf course (approximately 2 m above the ground) which contained water year-round. The detector located at the building was connected directly to an outlet; others were powered by rechargeable 12-volt batteries attached to solar panel charging units. All detectors and batteries were protected against weather and solar influence by being placed inside an ammunition box. Anabat extension cables were used to set up microphones, which were placed parallel to the ground and protected with a plastic cover. Because bat species stratify by altitude, microphones were located at different heights to register as many species as possible in the study area. The detectors were configured to record bat calls from 1800 h to 0600 h.

The four Anabat units were checked at least once a month (more frequently during severe weather periods such as high winds), and files were downloaded and analyzed using AnalookW call analysis and data management software. Echolocation calls at both sites were evaluated for species identification following Adams (2003). In AnalookW, an "allbats.abf" filter was used to exclude all the noise from the files (i.e., wind, insects, machines). Bats were identified to species manually using search-phase calls and the following parameters: shape, duration, and frequencies (maximum, minimum, and characteristic). These parameters were compared to published records by Adams (2003), reference calls obtained from captured bats, and online libraries that were available at the time of this study (e.g., bat call library at Museum of Southwestern Biology and Bat Sound Services).

In May 2015, record-high monthly rainfall (30.8 cm) caused water damage to, and loss of, the Anabat detector at the golf course. The rainfall caused technical problems with the remaining three microphones, resulting in loss of data and cessation of data collection at RTC. Attempts were made to resolve the technical issues, but the damaged equipment was not replaced as this event occurred near the conclusion of the survey.

Acoustic monitoring with an Anabat SD2 with a PDA kit (Titley Scientific, Columbia, Missouri) also was conducted during all mist-netting activities at RTC, in an effort to determine if bats were in the area but were not netted.

In addition to the acoustic monitoring at RTC, a driving transect was conducted to examine bat activity on the eastern side of Lubbock, which followed the CL system and continued to Buffalo Springs Lake (Fig. 1). The 48-km transect route was selected to maximize the detection of bats by choosing wooded areas or established water sources (i.e., CL and Buffalo Springs Lake). Driving transect surveys occurred during two periods: June 2012 through June 2013, excluding July 2012 and February 2013; and January 2014 through September 2015. The first 12-month period was used to gather preliminary data before deciding to continue with a second study period. The driving transect was conducted using the Anabat SD2 with PDA kit and car mount following guidelines provided by Georgia Department of Natural Resources (Britzke and Herzog n.d.). Driving transects were conducted while traveling at a maximum speed of 32 kmh once per month when weather conditions were suitable. Evenings when the temperature decreased below freezing, rain occurred, or wind speeds exceeded 80.5 kmh were deemed poor weather conditions not conducive to active monitoring.

Literature records.—To examine historical records of bat species within Lubbock County, museum records were taken from the Natural Science Research Laboratory (NSRL) at the Museum of Texas Tech University. Records dated back to the 1930s and were current through 11 March 2016 (accessed April 2019). All bat species proposed to occur in Lubbock County according to Ammerman et al. (2012) and Schmidly and Bradley (2016) were included. Historical records were used only as a proportional comparison to the echolocation data, as is reported in Appendix I.

Statistical analysis.—Bat activity was quantified as the number of individual calls recorded monthly at the passive (i.e., four stationary detectors at RTC, combined) and active (i.e., driving transect) monitoring sites. Data were not normally distributed according to a Shapiro-Wilk normality test ($W = 0.25$, $P < 0.01$) and zero inflated, likely due to different survey methods and durations, therefore data were log-transformed to improve normality. To evaluate temporal variability in bat activity by month and year for RTC and driving transect sites, separate Kruskall-Wallis rank sum tests were conducted using the function kruskal.test in package stats (R Core Team 2014). Post hoc comparison analyses were conducted using the function posthoc.kruskal.dunn.test in package PMCMR (Pohlert 2014). All analyses were conducted in R version 3.1.2 (R Core Team 2014).

RESULTS

Species inventory of Lubbock County.—No bats were captured in the mist nets at the golf course, although bats were recorded with the active Anabat and PDA kit on multiple occasions at both the golf course and RTC (i.e., six days from June until October 2012 and 2013). Other than two unidentified echolocation calls, all calls were identified as *T. brasiliensis* ($n = 62$ calls). Echolocation calls recorded from passive monitoring were identified as *Lasionycteris noctivagans*, *A. cinereus*, *Lasiurus borealis*, *Myotis* sp., *Nycticeius humeralis*, *Nyctinomops macrotis*, *Perimyotis subflavus*, and *T. brasiliensis*. Not all calls could be identified to species, but of those identified the number of calls per species varied greatly. For example, 78% (1,011/1,291) of the calls recorded in 2012 were identified as *T. brasiliensis*, but only 13% (119/913) of the calls identified in 2013 were categorized as *T. brasiliensis*. Contrarily, 11% (146/1,291) of the calls recorded were identified as *L. borealis* in 2012 with a notable increase (61%; 557/913) of calls categorized as *Lasiurus borealis* in 2013 (data not shown).

In Lubbock County, 37 nights of opportunistic sampling using mist-nets captured two species of bats, *Eptesicus fuscus* ($n = 4$) and *A. cinereus* ($n = 1$). These species were captured near the dam at Dunbar Historical Lake in August 2013. In January 2014, four caves were visited in Hardeman and Wheeler Counties and 12 individuals of four species (*Corynorhinus townsendii*, *E. fuscus*, *M. velifer*, and *P. subflavus*) were captured. These captures assisted in confirming previously published echolocation calls of *C. townsendii* and *E. fuscus* (Adams 2003), as well as obtaining reference calls for *M. velifer* and *P. subflavus*.

While mist-net captures do not reflect the abundance of bat species in Lubbock, historical records do appear to reflect the variety of species (Appendix I). Although some species (e.g., *Nycticeius humeralis* and *Lasionycteris noctivagans*) were not found in the museum records, it is likely because the NSRL records are current only through March 2016; the authors know of *L. noctivagans* specimens that were collected recently (2019) in Lubbock County.

Seasonal patterns in bat activity.—Thousands of acoustic files were recorded with the Anabat detectors each month, but 99.7% of the recordings (850,649 of the total 853,199 files) were attributed to environmental noise (i.e., wind). Of those recordings that included bat calls, 1,369 echolocation calls were recorded in 2012, 962 calls in 2013, and 219 calls in 2014 (Appendix II).

Bat activity at RTC significantly differed by year ($H = 22.85$, $df = 3$, $P < 0.001$; Table 1; Fig. 2A) and by month ($H = 24.90$, $df = 11$, $P < 0.01$; Table 2; Fig. 3A). A Dunn's multiple comparison post hoc test indicates that 2012 was significantly different than any other

year (Table 3). The same post hoc test on bat calls per month reveals that April, May, August, September, and October all differ significantly from January, February, and December. Bat activity did not vary significantly by year ($H = 2.26$, $df = 3$, $P = 0.52$; Table 1; Fig. 2B) or month ($H = 14.23$, $df = 11$, $P = 0.22$; Table 2; Fig. 3B) based on the number of echolocation calls recorded along the active monitoring driving transect.

Table 1. Sample size in the total number of months (n), mean ($\bar{x}$) ± standard error (SE), and range in number of raw bat calls by sampling year for passive monitoring at RTC and active monitoring from the driving transects, across all years (2012–2015). Note that sampling effort differed between passive and active monitoring. Passive monitoring data from all four stationary detectors were combined by month within a year.

	Passive monitoring			Active monitoring		
Year	n	$\bar{x}$ ± SE	Range	n	$\bar{x}$ ± SE	Range
2012	12	114.1 ± 56.4	0–560	6	5.8 ± 2.4	0–14
2013	12	80.2 ± 47.4	0–578	5	5.0 ± 0.8	0–4
2014	12	18.3 ± 9.9	0–99	12	2.3 ± 0.9	0–11
2015	10	0 ± 0	0–0	9	1.8 ± 0.6	0–5

Table 2. Sample size in the total number of months (n), mean ($\bar{x}$) ± standard error (SE), and range in number of raw bat calls by sampling month for passive monitoring at RTC and active monitoring from the driving transects, across all years (2012–2015). Note that sampling effort differed between passive and active monitoring. Passive monitoring data from all four stationary detectors were combined by month within a year.

	Passive monitoring			Active monitoring		
Month	n	$\bar{x}$ ± SE	Range	n	$\bar{x}$ ± SE	Range
January	4	0 ± 0	0	3	3.7 ± 0.3	0–3
February	4	0 ± 0	0	3	2.7 ± 1.3	0–4
March	4	6.5 ± 3.3	0–14	2	0.5 ± 0.5	0–1
April	4	71.3 ± 29.8	0–137	2	0 ± 0	0
May	4	45.5 ± 16.9	0–79	3	0.33 ± 0.33	0–1
June	4	13.0 ± 7.5	0–27	2	1.0 ± 1.0	0–1
July	4	16.0 ± 14.7	0–60	4	3.3 ± 0.8	2–5
August	4	169.8 ± 133.1	0–560	3	5.3 ± 3.2	0–11
September	4	267.0 ± 154.2	4–578	3	1.3 ± 0.9	0–3
October	4	45.8 ± 31.6	0–134	2	0.5 ± 0.5	0–1
November	3	3.7 ± 3.7	0–11	2	6.0 ± 6.0	0–12
December	3	0 ± 0	0	3	6.3 ± 3.8	2–14

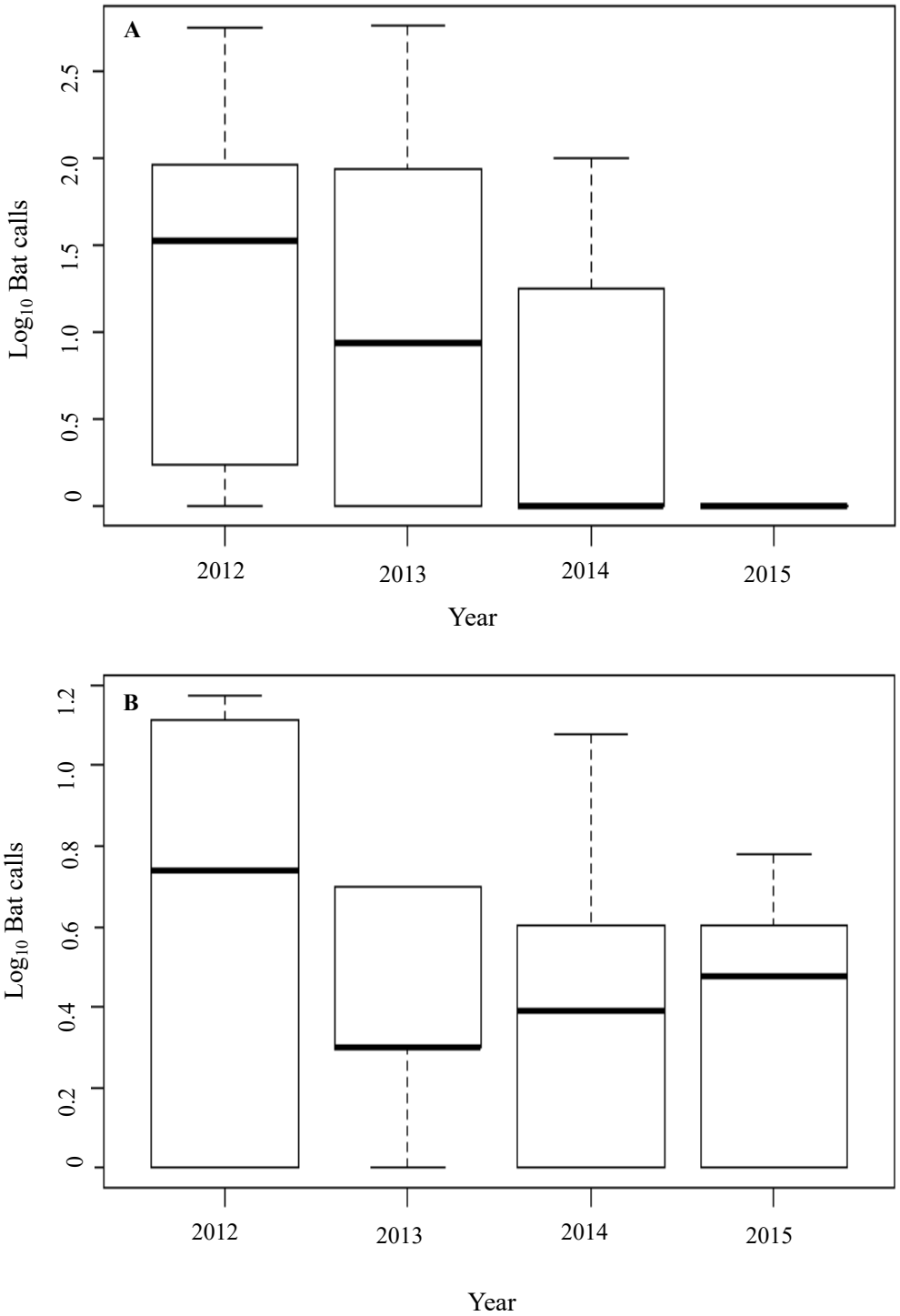

Figure 2. Comparison of bat activity among sampled years based on the number of log-transformed bat calls recorded by each monitoring protocol: A) passive monitoring at RTC; and B) active monitoring using driving transects. At RTC (A), the bat detectors were compromised by rain in May 2015.

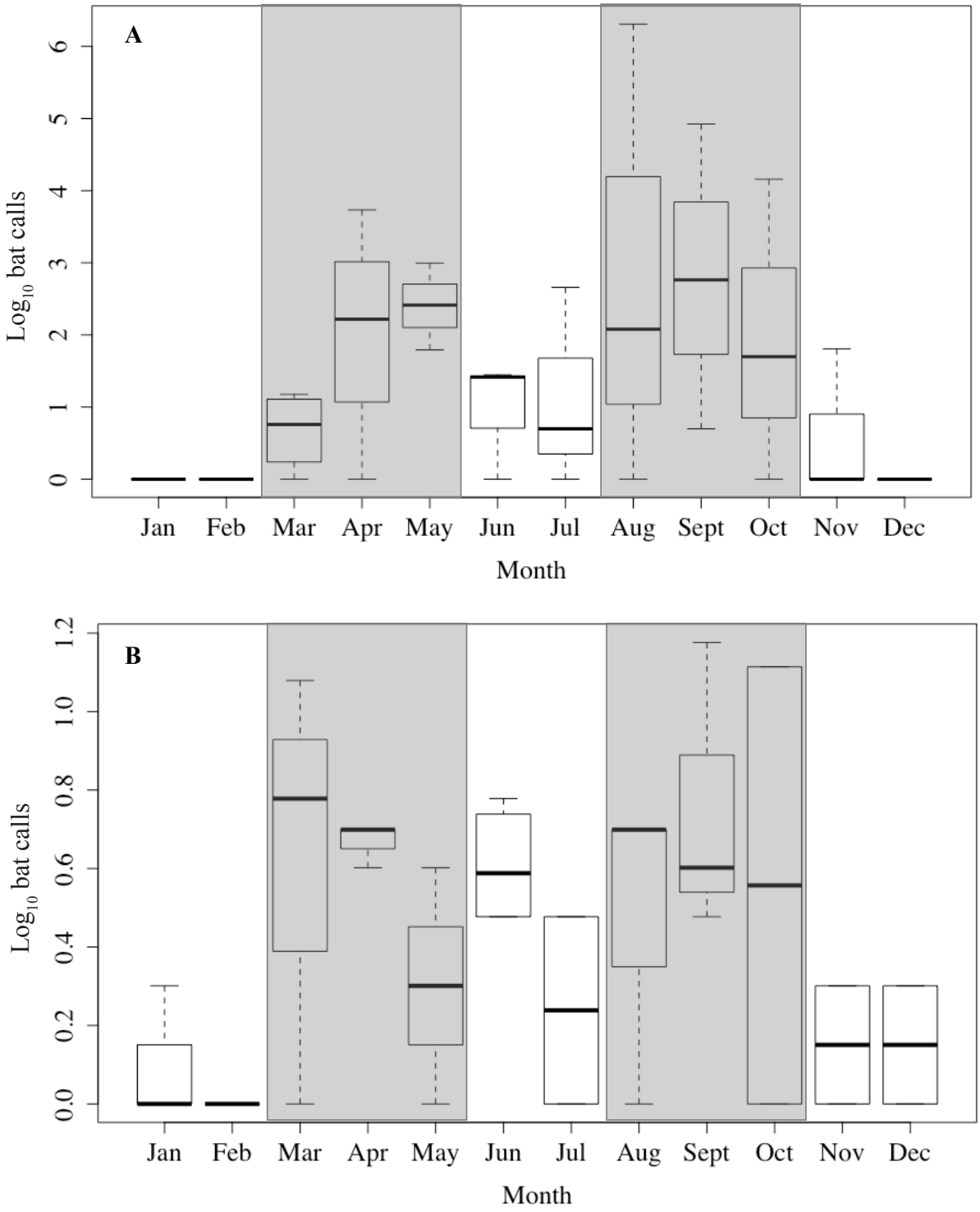

Figure 3. Comparison of bat activity across months as the number of log-transformed bat calls from years 2012–2015, combined, resulting from A) passive monitoring at RTC and B) active monitoring using driving transects. Shaded gray areas (March through May and August through October) indicate the migratory period for tree-roosting migratory species (Cryan 2003).

Table 3. Dunn's Multiple Comparisons post hoc test for the number of bat calls by year from RTC. The top value in each box of the matrix is the reported z-score and the bottom number is the *P*-value. Any *P*-value less than 0.025 ($\alpha = 0.05/2$) is considered significant; significant comparisons are highlighted in gray.

	2012	2013	2014
2013	3.55 0.000		
2014	3.72 0.000	0.17 0.431	
2015	3.79 0.000	1.29 0.099	1.16 0.123

DISCUSSION

Overall, differences in bat activity occurred by year and month at RTC, whereas no statistical differences were detected across months and years from the driving transects. Patterns of year at RTC likely were influenced by two factors. First, a possible contribution to decreased bat activity was that 2014 concluded the driest three-year period in the history of Lubbock County (NWS n.d.). Although other regions of the country may not have been impacted by this drought, wildlife, or insect, populations in this region likely declined. Second, equipment deterioration at RTC and eventual failure of the Anabat detectors in May 2015 is evident in the average decrease of log-transformed calls yearly. However, the post hoc comparison reveals that 2012 was significantly different from all other years (i.e., 2013, 2014, and 2015), suggesting that equipment failure was not the sole reason for decline.

Post hoc tests of the bat calls per month (Table 4) suggests two peaks in activity where the number of calls were significantly different from the winter months (December through February): April through May and August through October. These activity peaks are observable in Fig. 3A where activity was greatest in April and September at RTC. The number of calls per month for the driving transect, though not significantly different, does suggest peaks and in March and August (Fig. 3B). The lack of significance may be due to the low sample size of calls collected throughout the study period. The peaks in activity observed in April and September at RTC are shown in Appendix II. Due to the loss of Anabat detector(s) in May 2015, there was no spike in activity observed for September 2015.

The peaks in bat activity within Lubbock County coincide with seasonal migrations based on records of banded bats found in Lubbock as well as museum records. For example, Glass (1982) reported *T. brasiliensis* collected in West Texas from August through November. Furthermore, museum records of migratory tree-roosting species (*A. cinereus*, *Lasiurus borealis*, and *Lasionycteris noctivagans*) reveal that bats are present in West Texas during two "seasons": March through May and August through October (Cryan 2003). These are bats known to migrate over large distances (> 1,000 km), whereas other species (e.g., *P. subflavus*) are known to seasonally commute over small distances (Fleming and Eby 2003). Peaks in bat activity recorded during months of April and September from RTC (Fig. 3A), as well as August and March from the driving transect (Fig. 3B), match periods when long-distance migratory bats could potentially pass through the area. Our results from the bat calls per month at RTC confirm these periods of activity. These activity peaks suggest Lubbock County may be utilized as a stopover site, perhaps due to an increase in resource availability (i.e., roosting opportunities, food, water), particularly in the city of Lubbock. However, land use in Lubbock County and the surrounding region is primarily agricultural; thus, large trees for roosting opportunities are not prevalent.

Cotton production is an economically important industry in Texas, and the top three cotton-producing counties are in western Texas: Gaines, Lubbock, and Lynn (USDA 2014). In Lubbock County, 41% of the total acreage invested in farms is used for cotton pro-

Table 4. Dunn's Multiple Comparisons post hoc test for the number of bat calls by month from RTC. The top value in each box of the matrix is the reported z-score and the bottom number is the *P*-value. Any *P*-value less than 0.025 ($\alpha = 0.05/2$) is considered significant; significant comparisons are highlighted in gray.

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
Feb	0.00 0.500										
Mar	-1.14 0.128	-1.14 0.128									
Apr	-2.82 0.002	-2.82 0.002	-1.68 0.046								
May	-2.65 0.004	-2.65 0.004	-1.60 0.055	-0.04 0.483							
Jun	-1.85 0.149	-1.04 0.149	0.01 0.495	1.57 0.058	1.51 0.066						
Jul	-1.85 0.032	-1.85 0.032	-0.80 0.213	0.76 0.224	0.75 0.226	-0.76 0.224					
Aug	-2.35 0.010	-2.35 0.010	-1.29 0.098	0.26 0.397	0.29 0.387	-1.22 0.111	-0.47 0.321				
Sept	-2.67 0.004	-2.67 0.004	-1.62 0.052	-0.07 0.474	-0.02 0.492	-1.53 0.063	-0.77 0.220	-0.31 0.379			
Oct	-2.11 0.017	-2.11 0.017	-1.06 0.144	0.49 0.310	0.50 0.307	-1.00 0.158	-0.25 0.402	0.22 0.414	0.52 0.300		
Nov	-1.32 0.094	-1.32 0.094	-0.26 0.396	1.29 0.098	1.25 0.106	-0.26 0.398	0.50 0.309	0.96 0.168	1.27 0.102	0.75 0.228	
Dec	0.00 0.500	0.00 0.500	1.05 0.15	2.61 0.005	2.48 0.007	0.97 0.166	1.73 0.042	2.19 0.014	2.50 0.006	1.98 0.024	1.23 0.109

duction (USDA 2014). Furthermore, Lubbock County produced $78 million (all dollar amounts from hereafter are USD) in cotton during 2012, surpassed only by Gaines County (USDA 2014). The cotton bollworm (*Helicoverpa zea*) has been identified as one of the most destructive agricultural pests to cotton (Constable and Bange 2015) and is present in Lubbock County (Parajulee et al. 2009). Fortunately, insectivorous bats are known consumers of this pest species (Lee and McCracken 2005) and other agricultural pests (Kunz et al. 2011; Taylor et al. 2013; Brown et al. 2015). In Lubbock County alone, bats are purported to provide $20–$24 million worth of crop pest control each year (Boyles et al. 2011); this value was extrapolated from the original estimate of insect pest suppression costs provided by bats in south-central Texas (Cleveland et al. 2006). The value of this ecosystem service correlates with the economic cost that farmers would have to spend to control agricultural pests, including the cotton bollworm, if there were no bats performing crop pest consumption. Spending $20–$24 million on pest control in 2012 within Lubbock County could have resulted in a 26–31% decrease in cotton revenue (based on revenue reported in USDA 2014).

Whereas insectivorous bats are valuable to cotton farmers, other growing industries (e.g., wind energy) could be a major threat to bat populations of West Texas. Wind energy construction continues to grow exponentially in Texas and promises major importance

as an alternative energy, but with expanding research we understand more about the negative impacts on bat populations. Texas has the highest number of installed wind turbines and the highest number of turbines under construction (American Wind Energy Association 2017). Furthermore, total installed capacity in Texas (24,899 MW) exceeds capacity in any other U.S. state or territory and constitutes 25.8% of total capacity of the U.S. (American Wind Energy Association 2019). Wind energy, and the jobs it provides, is of economic importance (Reategui and Hendrickson 2011) to the High Plains and Rolling Plains ecoregions. A majority of wind turbines built in Texas occur within West Texas (Lund 2017). Alternative forms of energy are of growing interest for reasons including reducing fossil fuel consumption and decreasing contributors to climate change.

Research continues to show negative effects of wind turbines on wildlife, particularly bats and birds (Smallwood 2013). Over an 11-year period across the U.S. and Canada, between 650,000 and 1.3 million bats were estimated to have died due to impacts with wind turbines (Arnett and Baerwald 2013). Bat population estimates are difficult to obtain, but when these fatalities are combined with other pressures, such as White-nose Syndrome documented in the U.S. and Canada, endangerment to species increases. A majority (78%) of bat fatalities at wind turbines are migratory, tree-roosting species (*A. cinereus*, *Lasiurus borealis*, and *Lasionycteris noctivagans*) (Arnett and Baerwald 2013), all of which are documented in West Texas.

The results of this study suggest that: 1) there is a lack of tree bats in the Texas High Plains for a majority of the year; 2) reducing or curtailing wind turbine activity during the height of the migratory seasons could limit potential migratory bat deaths; and 3) if such a compromise is made, the High Plains could constitute an ideal site for the coexistence of green energy and bat species. Further research could focus on specific times that migratory species are active in Lubbock and surrounding counties to determine a time period to halt wind turbine activity.

The tradeoff between wind energy and bat populations also is important to cotton farmers. As previously stated, insectivorous bats provide an ecosystem service by consuming agricultural pests which, if left to farmers to combat, can have great cost to agricultural income and the environment as pesticide usage could increase. It is important to understand how we can encourage the ever-growing wind turbine industry without decreasing the benefits to the cotton industry that are provided by predators of agricultural pests (i.e., bats).

This study evaluated seasonal bat activity in Lubbock and also recorded bat species currently present in and surrounding Lubbock County. Future studies should investigate the route long-distance migrators use through Lubbock and determine: preferences in roosting structures (i.e., tree species and characteristics); distance to agricultural lands; possible interactions with wind turbines; and bat diets. Further, it would be of interest to direct efforts to characterize the migratory routes and understand patterns of occurrence of regional migrators, such as *P. subflavus*, that also are known to occur within and surrounding Lubbock County.

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LITERATURE CITED

Adams, R. A. 2003. Bats of the Rocky Mountain west: natural history, ecology, and conservation. University Press of Colorado, Boulder, Colorado.

American Wind Energy Association. 2017. Wind energy in Texas. http://awea.files.cms-plus.com/FileDownloads/pdfs/Texas.pdf.

American Wind Energy Association. 2019. U.S. wind industry fourth quarter report; 2018 market report. https://www.awea.org/resources/publications-and-reports/market-reports/2018-u-s-wind-industry-market-reports.

Ammerman, L. K., C. L. Hice, and D. J. Schmidly. 2012. Bats of Texas. Texas A&M University Press, College Station, Texas.

Arnett, E. B., and E. F. Baerwald. 2013. Impacts of wind energy development on bats: implications for conservation. Pp. 435–456 in Bat evolution, ecology, and conservation (R. A. Adams and S. C. Pederson, eds). Springer Science and Business Media, New York, New York.

Arnett, E. B., et al. 2007. Impacts of wind energy facilities on wildlife and wildlife habitat. Wildlife Society Technical Review, Bethesda, Maryland.

Arnett, E. B., et al. 2008. Patterns of bat fatalities at wind energy facilities in North America. Journal of Wildlife Management 72:61–78.

Baerwald, E. F., G. H. D'Amours, B. J. Klug, and R. M. R. Barclay. 2008. Barotrauma is a significant cause of bat fatalities at wind turbines. Current Biology 18:695–696.

Boyles, J. G., P. M. Cryan, G. F. McCracken, and T. H. Kunz. 2011. Economic importance of bats in agriculture. Science 332:41–42.

Britzke, E. R., and C. Herzog. [n.d.]. Using acoustic surveys to monitor population trends in bats, Atlanta, Georgia. https://georgiawildlife.com/sites/default/files/wrd/pdf/bats/Acoustic Transect Guidance.pdf.

Brown, V. A., E. Braun de Torrez, and G. F. McCracken. 2015. Crop pests eaten by bats in organic pecan orchards. Crop Protection 67:66–71.

Cleveland, C. J., et al. 2006. Economic value of the pest control service provided by Brazilian free-tailed bats in south-central Texas. Frontiers in Ecology and the Environment 4:238–243.

Coleman, J. L., and R. M. R. Barclay. 2012. Urbanization and the abundance and diversity of Prairie bats. Urban Ecosystems 15:87–102.

Constable, G. A., and M. P. Bange. 2015. The yield potential of cotton (*Gossypium hirsutum* L.). Field Crops Research 182:98–106.

Cryan, P. M. 2003. Seasonal distribution of migratory tree bats (*Lasiurus* and *Lasionycteris*) in North America. Journal of Mammalogy 84:579–593.

Davis, C. A., and L. M. Smith. 2001. Foraging strategies and niche dynamics of coexisting shorebirds at stopover sites in the southern Great Plains. The Auk 118:484–495.

Fleming, T. H., and P. Eby. 2003. Ecology of bat migration. Pp. 156–208 in Bat ecology (T. H. Kunz and M. B. Fenton, eds.). University of Chicago Press, Chicago.

Glass, B. P. 1982. Seasonal movements of Mexican freetail bats *Tadarida brasiliensis mexicana* banded in the Great Plains. The Southwestern Naturalist 27:127–133.

Griffith, G., S. Bryce, J. Omernik, and A. Rogers. 2007. Ecoregions of Texas. Texas Commission on Environmental Quality, Austin.

Kunz, T. H., E. B. de Torrez, D. Bauer, T. Lobova, and T. H. Fleming. 2011. Ecosystem services provided by bats. Annals of the New York Academy of Sciences 1223:1–38.

Lee, Y. F., and G. F. McCracken. 2005. Dietary variation of Brazilian free-tailed bats links to migratory populations of pest insects. Journal of Mammalogy 86:67–76.

Lund, A. 2017. Map of the month: wind energy in Texas. Retrieved from: https://nri.tamu.edu/blog/2017/december/map-of-the-month-wind-energy-in-texas/.

McGuire, L. P., C. G. Guglielmo, S. A. Mackenzie, and P. D. Taylor. 2012. Migratory stopover in the long-distance migrant silver-haired bat, *Lasionycteris noctivagans*. Journal of Animal Ecology 81:377–385.

National Weather Service. [n.d.] NWS Lubbock, Texas 2014 year-end summary. Retrieved from: https://www.weather.gov/lub/events-2014-20141231-summary.

Parajulee, M. N., D. R. Rummel, M. D. Arnold, and S. C. Carroll. 2009. Long-term seasonal abundance patterns of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) in the Texas High Plains. Journal of Economic Entomology 97:668–677.

Pohlert, T. 2014. The pairwise multiple comparison of mean ranks package (PMCMR), R Package. Retrieved from: http://cran.r-project.org/package=PMCMR.

Price, K. P., S. L. Egbert, M. D. Nellis, R. Y. Lee, and R. Boyce. 1997. Mapping land cover in a High Plains agroecosystem using a Multidate Landsat Thematic Mapper Modeling Approach. Transactions of the Kansas Academy of Science 100:21–33.

R Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, retrieved from http://www.r-project.org/.

Reategui, S., and S. Hendrickson. 2011. Economic development impact of 1,000 MW of wind energy in Texas. National Renewable Energy Laboratory, Technical Report NREL/TP-6A20-50400.

Schmidly, D. J. 2002. Texas natural history: a century of change. Texas Tech University Press, Lubbock, Texas.

Schmidly, D. J., and R. D. Bradley. 2016. The mammals of Texas, 7th ed. The University of Texas Press, Austin, Texas.

Smallwood, K. S. 2013. Comparing bird and bat fatality-rate estimates among North American wind-energy projects. Wildlife Society Bulletin 37:19–33.

Taylor, P. J., J. N. Steyn, and C. Schoeman. 2013. Bats eat pest green vegetable stink bugs (*Nezara viridula*): diet analyses of seven insectivorous species of bats roosting and foraging in macadamia orchards. Southern African Macadamia Growers' Association Yearbook 21:45–52.

USDA and N.A.S.S. 2014. 2012 Census of Agriculture, Vol. 1. https://www.nass.usda.gov/Publications/AgCensus/2012/

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APPENDIX I

Number of identified echolocation calls to species, bats captured, and museum records for Lubbock County, Texas. Echolocation calls were identified to species level only for RTC data. Bats were captured by mist-net at Dunbar Historical Lake. Museum records were retrieved from the Natural Science Research Laboratory (NSRL) at the Museum of Texas Tech University. Museum records dated back to the 1930s and were current through 11 March 2016 (accessed in April 2019).

Bat Species	Echolocation calls	Number of mist-net captures	Museum records from NSRL
Aeorestes cinereus	268 (11%)	1 (20%)	19 (33%)
Corynorhinus townsendii	0	0	0
Eptesicus fuscus	0	4 (80%)	1 (2%)
Lasionycteris noctivagans	59 (2%)	0	0
Lasiurus borealis	705 (29%)	0	6 (11%)
Myotis sp.	1 (0.04%)	0	1 (2%)
Nycticeius humeralis	4 (0.2%)	0	0
Nyctinomops macrotis	11 (0.5%)	0	2 (4%)
Perimyotis subflavus	118 (5%)	0	4 (7%)
Tadarida brasiliensis	1,163 (48%)	0	24 (42%)
Unidentified	98 (4%)	0	0

APPENDIX II

Bat calls, by year and station, recorded by monitoring systems (Anabat detectors) at Reese Technology Center and Golf Course, Lubbock, Texas, from 2012 through 2014. No bat activity was recorded during 2015 due to microphones being destroyed by rain in May of that year.

Month	Building	Weather tower	Water tower	Golf course	Total bat calls recorded
			2012		
January	0	0	0	0	0
February	0	0	0	0	0
March	2	0	0	0	2
April	5	1	14	29	49
May	0	0	6	36	42
June	0	0	0	25	25
July	56	1	0	3	60
August	336	30	0	194	560
September	436	3	0	47	486
October	21	5	0	108	134
November	7	1	0	3	11
December	0	0	0	0	0
			2013		
January	0	0	0	0	0
February	0	0	0	0	0
March	0	0	0	14	14
April	0	0	0	137	137
May	0	0	0	61	61
June	0	0	0	0	0
July	0	0	0	4	4
August	0	0	0	119	119
September	0	0	0	578	578
October	0	0	0	49	49
November	0	0	0	0	0
December	0	0	0	0	0

Appendix II. (cont.)

Month	Building	Weather tower	Water tower	Golf course	Total bat calls recorded
			2014		
January	0	0	0	0	0
February	0	0	0	0	0
March	0	0	0	10	10
April	0	1	0	98	99
May	0	14	0	65	79
June	0	0	0	27	27
July	0	0	0	0	0
August	0	0	0	0	0
September	0	0	0	4	4
October	0	0	0	0	0
November	0	0	0	0	0
December	0	0	0	0	0

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Community Structure of Bats (Chiroptera) at Tar Creek Superfund Site and the Behavioral Impact of Metal Concentrations in Tissues

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Abstract

The goals of this study were to determine community structure and population demographics of Chiroptera within Tar Creek Superfund Site, an area contaminated with lead (Pb), zinc (Zn), and cadmium (Cd); analyze liver, kidney, and hair for concentrations of these metals; and develop a behavioral assay to identify differences in flight ability in bats from the contaminated area and a reference area. It was hypothesized that the bat community from the contaminated area would have lower diversity and evenness; bats collected from the contaminated area would have higher levels of Pb, Zn, and Cd in tissues; and bats from contaminated areas would show lower maneuverability and willingness to fly compared to bats from reference sites. Mist netting occurred June–September 2012 and May–September 2013 at two sites within Tar Creek Superfund Site (TC, BC) and two reference sites within Oologah Wildlife Management Area (PLM, PAN). Both contaminated and reference communities were dominated by eastern red bats (*Lasiurus borealis*). Contaminated sites had lower, but not significantly different, species diversity (Simpson's *D*) compared to reference sites. Kidney Zn concentrations were significantly different for males from TC compared to BC males ($P = 0.02$), but were not significantly different from reference sites. Hair Zn concentrations in PAN males was significantly different compared to PLM males ($P = 0.04$), but not compared to contaminated sites. Hair Pb concentration in females from BC was significantly higher than females from TC ($P = 0.005$), and hair Pb concentrations in PAN males were significantly different compared to PLM males ($P = 0.007$). Bats from PLM showed predictably strong relationships between flight time and obstacles dropped ($r^2 = 0.695$), and between flight time and movement between sections of the cage ($r^2 = 0.976$), whereas bats from the contaminated area showed weaker relationships between flight time and obstacles dropped ($r^2 = 0.0004$) and flight time and movement between sections of the flight cage ($r^2 = 0.2422$). The study reported herein demonstrates that a noninvasive behavioral assay can distinguish differences in flight ability in bats from a contaminated site compared to a reference site.

Key words: cadmium, flight cage, *Lasiurus borealis*, lead, maneuverability, Oklahoma, Oologah Wildlife Management Area, zinc

Introduction

Between the late 1800s and the 1970s, an area of 500 sq mi known as the Tri-State Mining Region, which includes portions of southeastern Kansas, southwestern Missouri, and northeastern Oklahoma, was heavily mined for lead (Pb) and zinc (Zn; USFWS 2000). In Oklahoma, Pb and Zn ore were extracted from within the Boone Formation (also known as the Boone Aquifer). This aquifer sits approximately 500 ft above the Roubidoux Aquifer, which is the major source of drinking water in this area (USEPA 2005). Ore was removed via room-and-pillar mining techniques, meaning large underground cavities often 100 ft high, were cleared of ore leaving pillars to support the ceilings (USEPA 1994). At the peak of mining activity, annual extrac-

tion of Pb and Zn reached 130,410 tons and 749,254 tons, respectively. Because of the location of the mines within the aquifer, ground water continually had to be pumped out (USEPA 2005).

Under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) passed by Congress in 1980, the Environmental Protection Agency (EPA) has the authority to identify parties responsible for and enforce cleanup of abandoned hazardous waste sites by placing sites on the National Priorities List (NPL). The addition of Tar Creek Superfund Site (TCSFS) to the NPL was initially proposed in 1981, and formally occurred in 1983 (USFWS 2000). This 40 sq mi area, within the Tri-State Mining District, is located in northeastern Oklahoma and impacts communities including Picher, Quapaw, Cardin, Commerce, and Miami (USEPA 2000, 2010; ATSDR 2004). In the 1970's, when mining operations ceased, an estimated 100,000 acre-feet of underground cavities had been created. Exposed sulfide minerals oxidized while pumping occurred; however, with the termination of mine operations came cessation of water pumping. Ground water refilled the aquifer, thus creating acid mine water, which continues to flow from the mines today (USEPA 2000, 2005). Through the process of ore extraction, approximately 74 million tons of mine tailings, or chat, were created, with some piles reaching 200 feet in height (OWRB 1983). These chat piles are still a constant source of contamination.

In 1994, blood Pb levels were above 10 µg/dL in 34% of children tested within this area. Based on these findings, the EPA began remediating residential sites with soil contamination levels exceeding 500 ug/g Pb and 100 ug/g Cd within 0–12 inches in depth, and within 1,000 ug/g Pb and 100 ug/g Cd within 12–18 inches in depth (USEPA 2010). The Tar Creek Superfund Site area is not homogeneously contaminated, resulting in the EPA testing multiple sites and only remediating areas exceeding these levels. Of the locations tested, 61% of public sites and 65% of residential sites exceeded these levels and contaminated soil was excavated and clean topsoil was filled in (USEPA 2010). At the time of our study, remediation was still in progress through the EPA with management through Quapaw Tribe of Oklahoma (USEPA 2010; Quapaw Tribe of Oklahoma 2013).

The EPA identified major sources of exposure for humans as soil, mine tailings, ingestion of homegrown produce and tap water, airborne dust, and use of biota by Tar Creek area tribal populations (ATSDR 2004). Several of these pathways also have the potential to harm animal species living in this area. Past research at TCSFS included studies of invertebrates, fish, birds, reptiles, rodents, and deer (Conder and Lanno 1999; Beyer et al. 2004; Schmitt et al. 2005, 2006; Hays and McBee 2007; Phelps and McBee 2009, 2010); however, no research has yet investigated exposure or effects of metals on bat populations at TCSFS.

Bats have long been known to accumulate toxicants in contaminated environments (Geluso et al. 1976; Clark 1981) and may consume insects that are aquatic or that spend larval periods in the water or sediments; both types of arthropods are known to be sources of contamination for species that prey on them (Price et al. 1974; McBee and Bickham 1990; Currie et al. 1997). Zukal et al. (2015) reviewed metal contamination in bats and found that there have been few studies, with only four species having been analyzed more than five times. Although there are fewer reported cases, Pb appears to have similar effects on bats as on birds, including landing accidents associated with difficulty flying and walking (De Francisco et al. 2003). Lead accumulation in bats has resulted from pesticide poisoning, vehicle pollution, and Pb paint in zoo enclosures (Zook et al. 1970, 1972; Clark 1979; Thies and Gregory 1994). Sutton (1987) observed uncoordinated bats that were unable to fly and experienced muscle tremors resulting from lead poisoning most likely due to air pollution. Lead levels in liver and kidney from this group ranged from 12.1 to 47.1 ppm and 21.7 to 30.8 ppm, respectively (Sutton 1987). Thies and Gregory (1994) sampled Mexican free-tailed bats (*Tadarida brasiliensis*) at Carlsbad Caverns in New Mexico and Vickery Cave in Oklahoma for traces of Pb and Cd in liver tissues. They found measurable levels of Pb (0.74–49.44 µg/g WW) in all individuals examined. Liver Cd levels ranged from below detection limits in males from Oklahoma to 1.98 µg/g WW in females from Oklahoma. Clark (1979) compared Pb levels in bats and small terrestrial mammals collected near a major highway in Baltimore, Maryland. He found Pb levels in wild caught big brown bats (*Eptesicus fuscus*) and little brown bats (*Myotis lucifugus*) to be greater

than most terrestrial small mammals, other than shrews. Levels of Pb (µg/g WW) from frozen carcasses of *E. fuscus* were greater in males than females, and both sexes of *E. fuscus* had greater levels of Pb than the pooled sexes for *M. lucifugus*.

Clark et al. (1986) studied effects of metal contamination from a battery salvage plant in Jackson County, Florida. Livers and kidneys were collected for analysis of Pb, Zn, Cd, and chromium (Cr) from southeastern bats (*Myotis austroriparius*) roosting in Judges Cave, an important maternity colony of the endangered gray bat (*Myotis grisescens)*, and *M. austroriparius* roosting under a highway bridge in Gainesville, Florida, for use as reference bats. Liver levels of Zn and Cd from bats in Judges Cave were 1.1 and 2.4 times higher, respectively, than levels in bats from Gainesville. Kidney Cd levels were 3.3 times higher in bats from Judges Cave compared to bats from Gainesville. Lead was found in only five Gainesville bats and four Judges Cave bats. Gainesville bats had a higher mean Pb level (0.318 ppm WW) compared to Judges Cave bats (0.195 ppm WW), which may be due to heavy traffic on the bridge. Hickey et al. (2001) detected mercury (Hg), Zn, selenium (Se), Pb, aluminum (Al), and iron (Fe) in the hair of Canadian bats. They detected Hg and Zn most frequently, with concentrations varying between species. Levels of Zn ranged from below detection limits to 900 mg/kg among several species including *M. lucifugus*, northern long-eared bat (*Myotis septentrionalis*), *E. fuscus*, and eastern small-footed myotis (*Myotis leibii*). Mercury and Zn were detected in 98 and 96 % of samples, respectively, ranging from 2.0 to 7.6 mg/kg and 130–200 mg/kg, respectively, whereas Se and Pb were detected at a much lower frequency, in 12 and 8% of samples, ranging from 9.5 to 69 mg/kg and 1.6–8.8 mg/kg, respectively.

Behavioral assays have been used to determine effects of metals on wild species of vertebrates (Burger and Gochfield 1995; Marentette et al. 2012). Often, maneuverability is a measure used in these behavioral assays. Maneuverability in volant animals has been tested by use of an obstacle course within a flight cage (Brilot et al. 2009); however, no studies have addressed the impacts of metals on maneuverability in bats. Reports of bats suffering from Pb toxicosis describe them as lethargic, and unwilling to fly (Zook et al. 1972; Sutton and Wilson 1983). Many species of insectivorous bats require a high level of maneuverability to locate and reach prey, water sources, and roosts, as well as to avoid predators. Due to the high-energy requirements associated with flight, bats have a high metabolism, requiring them to consume close to their body weight in insects each evening (Kunz et al. 1995). Decreased maneuverability, for any reason, impacts foraging ability, which in turn can have a real effect on survival and fitness. Even low levels of exposure to a contaminant with neurological effects can potentially affect a bat's maneuverability. Bats within the TCSFS have great potential for exposure to Pb, a neurotoxin, which may impact their maneuverability and willingness to fly (Sutton 1987; De Francisco et al. 2003).

The goal of this study was to gain a better understanding of how the bat community is impacted by contaminants within TCSFS. First, mist netting was employed to test hypotheses that community structure and population demographics of bats inhabiting sites within TCSFS would be significantly different compared to uncontaminated reference sites. Second, an obstacle course within a flight cage was used to investigate maneuverability and willingness to fly. Flight trials were conducted to test the hypothesis that bats inhabiting sites within TCSFS will have significantly lower maneuverability/willingness to fly compared to bats inhabiting uncontaminated reference sites. Third, tissue samples, including hair, liver, and kidneys, were collected to test the hypothesis that metal levels in bats inhabiting sites within TCSFS would be significantly higher compared to bats inhabiting uncontaminated reference sites. Hair, liver, and kidney metal levels also were used for comparison with results from flight cage assays.

METHODS

Study sites.—Bats were collected from two sites within the contaminated TCSFS locality, along Tar Creek (TC; 36°57.495'N, 094°50.731'W) and along Beaver Creek (BC; 36°56.2026'N, 094°45.3846'W), and two sites within the reference locality, Oologah Wildlife Management Area (OWMA), along Plum

Creek (PLM; 36°35.5063'N, 095°32.4197'W) and along Panther Creek (PAN; 36°37.747'N, 095°31.372'W). The site along TC was located between private land and land bought out by the EPA, while the site along BC was located on land owned by the Quapaw Tribe of Oklahoma. Tar Creek Superfund Site and OWMA are approximately 67 km apart from one another and on different drainage systems, ensuring that OWMA is not contaminated via wind blowing metal contaminated chat dust or water-borne sediments, and that bat populations are unlikely to be shared between these two localities. Distance between the two TCSFS sites is approximately 8 km, as is the distance between the two OWMA sites. This close proximity allowed for netting to occur simultaneously at the two TCSFS sites, and simultaneously at the two OWMA sites.

Field methods.—Bats were collected via mist nets placed over riparian areas for three consecutive nights each month during June–September 2012 and May–September 2013. Three to four nets were opened at sunset and left in place for five hours or until 0100 h, and checked every 15 minutes. Total sampling effort was 278 net-nights, with 128 net-nights at reference sites and 150 net-nights at contaminated sites. Sampling was not possible during June 2013 due to flooding.

Captured bats were removed from the net, identified in the hand, and reproductive condition (scrotal/nonscrotal; pregnant/not pregnant; lactating/not lactating), and approximate age (as determined by shining a flash light through the extended fingers of the wing to determine degree of epiphyseal ossification) were recorded. All following analyses were conducted only on *L. borealis* because it was the most commonly captured species. Bats were placed in individual cloth bags until netting concluded for that evening. Weight, sex, and standard external measurements (total length, tail length, forearm length, ear length, tragus length, hindfoot length) were measured with digital calipers while animals were restrained by hand or while individually held in cloth bags.

As approved by the Animal Care and Use Committee of Oklahoma State University under Animal Care and Use Protocol #AS129, an annual maximum of five male and five females for *L. borealis* from each locality were euthanized for tissue collection. These bats were first anesthesized with isoflurane, then euthanized via cervical dislocation. Liver and kidney were removed and stored in sterile tubes and placed in liquid nitrogen for return to the laboratory where they were analyzed for Pb, Zn, and Cd. A 1 sq cm patch of hair also was clipped from the venter (Wimsatt et al. 2005) and stored in sterile tubes for analysis of metal content. Animals that were euthanized were prepared as voucher specimens and catalogued in the Oklahoma State University Collection of Vertebrates, adding the first collections data of Chiropteran species in Ottawa County (Eguren and McBee 2013). Bats exceeding the collection maximum were released. All field procedures were conducted following standards set forth by the American Society of Mammalogists (Sikes et al. 2011).

To account for potential impact of temperature and precipitation on capture frequencies, weather data were collected from the nearest weather stations, Nowata and Miami, for reference sites and TCSFS sites, respectively, through Oklahoma Mesonet environmental monitoring stations (Oklahoma Mesonet 2013). Specific data collected included daily maximum and minimum temperature for each sampling night, 10-year average maximum and minimum temperature by month, and precipitation by month, because temperature and rainfall are known to effect netting success (Kunz 1974).

Insect and water samples were collected in 2013. A light trap (Paulson 2005) was used to collect insect samples for one sampling night for approximately five hours, at each of the four sites. The trap was located 15–20 m downstream from each netting location and consisted of a white cloth (218 x 218 cm) illuminated by a UV light draped over a rope approximately 1.5 m above the ground. Twice per hour, insects present on the sheet were collected by using an aspirator and transferred to a collection jar. Insects were returned to the lab, transferred to 70% ethanol, identified to order, and quantified. A homogenized subsample was processed for metal analysis. Water samples were collected in triplicate (130 ml each) from each of the four sites. Sample jars were acid washed prior to collection and water was analyzed via ICP-MS for Pb, Zn, and Cd.

Metal analysis.—Materials used for tissue and insect digestion were acid washed. Tissue digestion

methods are similar to those of Sanchez-Chardi et al. (2007) except that only nitric acid was used for tissue digestion. Livers and kidneys from each euthanized animal were dried separately until a constant weight was maintained (60 °C). The entire liver (mean DW 0.063 g), both kidneys (mean DW 0.033 g), and hair (mean DW 0.006 g) were each digested with 1 ml HNO_3 (Fisher Scientific; Trace Metal Grade) using an Ethos EZ closed vessel microwave digester (Milestone; Hair and Animal Tissue Protocols). Insect samples, separated by site (mean mass (g) PLM – 0.46, PAN – 0.06, BC – 0.34, TC – 0.08), were digested with 10 ml HNO_3 (Hair Protocol). A 100 µl sample of each disgestate was diluted to a total volume of 5 ml with Ultrapure H_2O (Millipore) plus a 10 µl aliquot of internal standard (PerkinElmer Multi-element Calibration Standard 2% HNO_3). Analysis of water was conducted on a 5 ml sample of each triplicate sample plus 10 µl of internal standard made with Ultrapure H_2O.

Concentrations of Pb, Zn, and Cd were measured by an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) Perkin-Elmer ELAN-6000. Quality control practices included use of duplicates for each sample, as well as blanks, and internal standards and followed standard operating procedures for the OSU Metabolic and Nutrition Phenotyping Core Facility. Metal concentrations were determined based on a range of five concentrations of calibration standards (0.025 ppb, 1 ppb, 1 ppm, 10 ppm, and 20 ppm). Tissue concentrations were determined by averaging measured values in duplicate samples for each tissue. Concentrations that were below ½ of the lowest calibration standard were considered as below detection limits and were assigned values of **½** the lowest calibration standard (Sanchez-Chardi et al. 2007). Tissue and insect metal concentrations were calculated as µg/g on a dry weight basis, whereas metal concentrations in water samples were calculated as mg/L for Zn and µg/L for Cd and Pb.

Only *L. borealis* was used in maneuverability assays, which were conducted only during summer 2013. Bats were released, one at a time, into a portable flight cage made from a 10 ft x 15 ft pop-up canopy tent with an attachable mesh enclosed cage containing an obstacle course to test flight performance. The cage was divided into thirds, with obstacles present only in the middle third (Fig. 1). Three wooden poles (10 ft x 2 in x 1 in) were extended across the top of the mesh cage in the middle 5-foot section. Obstacles, consisting of lines made from size 9 nylon seine twine and weighted with small washers, were attached to the underside of the poles via magnets attached to each line. The two outer poles had six obstacles and the inner pole had five obstacles, which were off set to prevent open flyways through the course. Width between obstacles on a pole was 450 mm, slightly longer than the average wingspan for *L. borealis* (293 ± 0.001 mm; Salcedo et al. 1995). The magnets connecting obstacles to the poles were weak enough that when touched by a bat, lines dropped to the floor of the cage. Flight trials lasted 10 min and were video recorded with a thermographic camera (Flir Scout TS-32 Pro). Thermal markers were attached to the inside the cage with Velcro® to delineate sections of the cage on thermal video recordings. After recording for 10 min., number of obstacles dropped was counted by at least two people. After each trial, bats were recaptured with a hand net and returned to cloth bags until they could be processed for metal analysis.

Movements between sections of the cage and flight times were scored from the video files recorded by the thermographic camera. Each time a bat moved from one third of the cage into another (e.g., from a section with no obstacles into the section with obstacles; Fig. 1), a score of 1 was recorded. The amount of time a bat spent flying, rather than roosting on or crawling on the mesh, during the 10 min trial was recorded in sec. One person scored all videos for movement and flight time. Twenty percent of the videos were scored three times each to verify that scores and flight times were assessed accurately.

Statistical analyses.—Statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC). Species diversity of bat communities at each site was determined using Simpson's diversity index (*D*), which measures the probability that two individuals selected at random from a community will belong to the same species (Hair 1980). This index also takes into account the number of each species and is therefore indicative of evenness as well. Precipitation data were compared between years using a Student's *t*-test. Metal concentrations were non-normally distributed and therefore were analyzed via non-parametric tests (PROC NPAR1WAY, PROC GLM). Correlations between tissue metal levels were examined using Pearson correlation coefficients (PROC CORR) (Kalinska et al. 2014). Statistical anal-

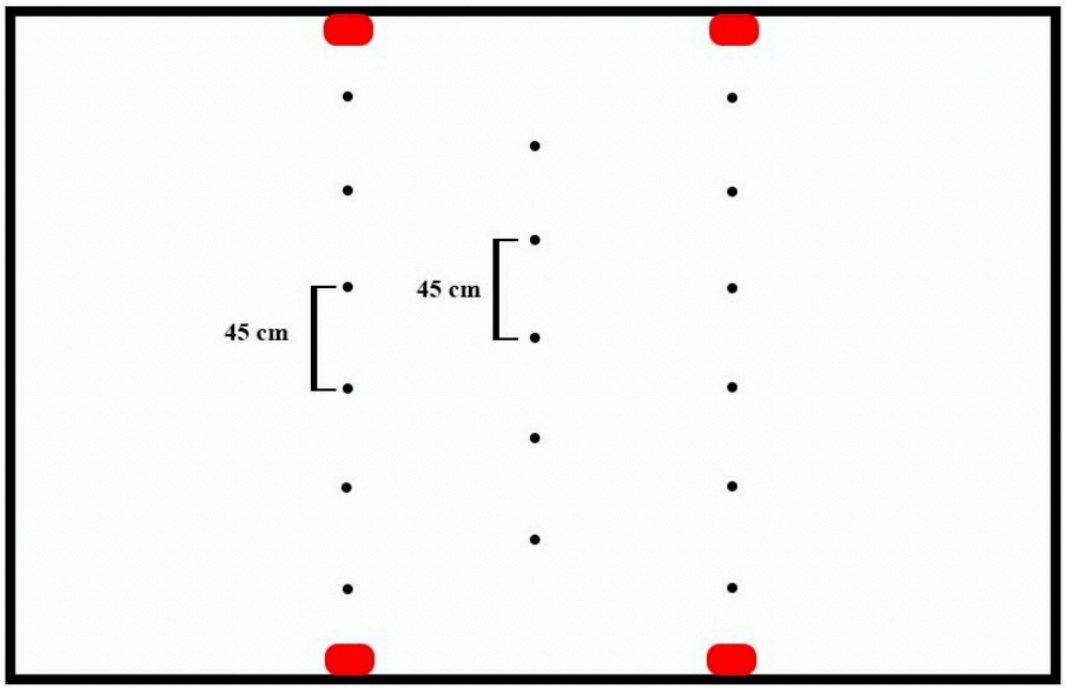

Figure 1. Flight cage diagram as viewed from above. Black dots represent hanging obstacles, spaced 45 cm apart, and red dots represent thermal markers that delineate cage into thirds when viewed through thermal camera. Movement is measured as passing from one section into another. Flight cage dimensions: 15 ft long x 10 ft wide x 6 ft high.

yses for maneuverability assays also were performed using SAS 9.3. Data were not normally distributed; therefore, nonparametric analyses were used. Due to low sample sizes, flight cage variables were compared between PLM and pooled TCSFS sites (PROC NPAR1WAY; PROC CORR). Metal concentrations in each tissue were compared between bats from reference and contaminated sites and correlations between metal concentrations and the three flight variables were determined using PROC NPAR1WAY and PROC CORR.

RESULTS

Community composition.—Due to heavy rains in late May/early June 2013, both PLM and PAN were flooded and it was not possible to reach reference sampling sites in June; therefore, all June 2013 sampling was eliminated. Forty-eight bats were collected, including five species (*Lasiurus borealis*—eastern red bat, *Perimyotis subflavus*—tricolored bat, *L. cinereus*—hoary bat, *Myotis lucifugus*—little brown bat, and *Nycticeus humeralis*—evening bat; Table 1). No pit-tagged bats were recaptured. The annual permitted numbers of bats for tissue metal analyses were exceeded at only two sites: seven female *L. borealis* were captured at TC in 2012, and six male *L. borealis* were captured at PLM in 2013. A single female captured at TC escaped before samples could be collected. A female *Nycticeus humeralis* was collected at PAN and released without data collection. *Lasiurus borealis* comprised 89.6% of all bats collected. All other species were represented by only one or two individuals (Table 1).

Table 1. Number of bats captured at each of four sites for 2012 and 2013 combined. Superscript r indicates a reference site and superscript c indicates a contaminated site. F indicates female and M indicates male. Simpson's diversity values (*D*) were calculated for each site. A net night equals one mist net set for one 5-hour period.

	Lasiurus borealis		*Lasiurus cinereus*		*Perimyotis subflavus*		*Myotis lucifugus*		*Nycticeus humeralis*				
Sites	F	M	F	M	F	M	F	M	F	M	Total	*D*	Bats per net night
Plum Creek[r]	6	10	0	0	0	0	0	0	1	0	17	0.12	0.265
Panther Creek[r]	2	2	0	0	1	0	0	1	0	0	6	0.55	0.09
Beaver Creek[c]	2	4	1	0	1	0	0	0	0	0	8	0.47	0.09
Tar Creek[c]	12	5	0	0	0	0	0	0	0	0	17	0.00	0.23
Total	22	21	1	0	2	0	0	1	1	0			
Species total	43		1		2		1		1				

Twenty-five bats, comprising three species, were collected from TCSFS sites, and 23 bats, comprising four species, were collected from OWMA sites. Simpson Diversity values were calculated for TCSFS sites (D = 0.16) and OWMA sites (D = 0.24). Diversity values for each site are presented in Table 1. Total bats per net-night from all sites combined was 0.17. Bats per net-night from individual sites are presented in Table 1. Field seasons 2012 and 2013 resulted in different capture numbers between contaminated and reference sites. In 2012, twice as many bats were collected from contaminated sites compared to reference sites (TCSFS n = 16, OWMA n = 8), whereas 2013 showed the opposite result (TCSFS n = 9, OWMA n = 15). Bats per net night separated by year are: 2012, TCSFS – 0.24, OWMA – 0.14; and 2013, TCSFS – 0.11, OWMA – 0.21.

Lasiurus borealis *age structure and reproduction.*—Both the TCSFS and OWMA communities were dominated by *L. borealis*; therefore, further analyses were conducted on only this species. Sex ratios (M:F) were equal at PAN, male biased at PLM and BC, and female biased at TC, where the number of females captured was twice that of males (Table 2). Seventeen percent of bats were identified as subadults based on epiphyseal closure (Brunet-Rossinni and Wilkinson 2009). Seventy-five percent of the subadults were collected from PLM, in June or early July; the other 25% were collected at TC in mid-July. No pregnant or lactating females were captured in 2012 at any site. In 2013, 15% of females captured were pregnant; all were collected in May and only from reference sites. Both pregnant bats carried four embryos with crown rump lengths of 4 and 6 mm, respectively. Twenty-three percent of females captured in 2013 were lactating; all were collected in July and only from reference sites. All adult males captured were scrotal (27 June–13 September). A single subadult male captured on 9 July 2013 from PLM was scrotal, with testis = 3 x 1 mm, of the same size as an adult male captured from the same location just one day prior.

Table 2. Sex ratios and average body mass (mean ± SE) separated by site and sex for *Lasiurus borealis* collected in 2012–2013 combined. Size of sample is included in parentheses. Superscript r indicates a reference site and superscript c indicates a contaminated site.

	Sex ratio	Average body mass (g)	
Sites	(male:female)	Males	Females
Plum Creek[r]	1.7:1	8.3 ± 0.4 (9)	11.5 ± 1.0 (6)
Panther Creek[r]	1:1	9.0 ± 0.0 (2)	12.3 ± 0.3 (2)
Beaver Creek[c]	2:1	9.6 ± 0.5 (4)	12.5 ± 2.0 (2)
Tar Creek[c]	0.42:1	7.7 ± 0.6 (5)	10.6 ± 0.4 (12)

Precipitation at all sites for 2012 was significantly lower than that recorded for 2013 ($P < 0.01$; Oklahoma Mesonet). Average rainfall level recorded in Nowata, Oklahoma, for June through September 2012 was 35.5% lower compared to the 30-year average, whereas average rainfall level recorded at the same station for May through September 2013 was 44.2% above the 30-year average. Although not significantly different, mean daily maximum and minimum temperatures recorded for 2012 were consistently higher compared to 2013 at all locations (Fig. 2).

Metals analysis.—Liver and kidney samples were analyzed from 38 *Lasiurus borealis*. Metal concentrations ($X \pm$ SE) in liver, kidney, and hair for *L. borealis* are given in Table 3. Male bats from PAN had significantly higher hair Zn concentrations compared to males from PLM ($P = 0.04$). Males from TC had significantly higher kidney Zn concentrations compared to BC males ($P = 0.03$). Significantly higher concentrations of hair Pb were found in BC females compared to TC females ($P = 0.02$). Also, PAN females had significantly higher hair Pb concentrations compared to PLM females ($P = 0.04$). Combined sexes from BC had significantly higher concentrations of Cd in liver ($P = 0.05$) and Pb in hair ($P = 0.05$) compared to combined sexes from TC. The only significant ($P < 0.05$) positive correlation between hair and internal tissues for the same metal was between hair Pb and liver Pb. Additional significant positive correlations for metal by tissue combinations included Liver Zn by Kidney Zn, Liver Cd by Kidney Cd, Kidney Cd by Hair Zn, Hair Zn by Hair Pb, and Hair Cd by Hair Pb (Table 4).

Although not significantly different, mean concentrations (μg/g) for all three metals in liver and Zn and Pb in kidney were higher in subadults ($n = 8$) compared to adults ($n = 30$; subadults liver Zn – 3.17, Cd – 0.03, Pb – 0.03, kidney Zn – 2.96, Pb – 0.011; adults liver Zn – 2.98, Cd – 0.02, Pb – 0.01, kidney Zn – 2.54, Pb – 0.003). In contrast, subadults showed lower mean concentrations (μg/g) for all three metals in hair and for kidney Cd (hair Zn – 2.98, Cd – 0.001, Pb – 0.001, kidney Cd – 0.01) compared to adults (hair Zn – 3.573, Cd – 0.005, Pb – 0.033, kidney Cd – 0.174).

Three to 18 times more insects were collected at reference sites (PLM – 268, PAN – 1,388) compared to contaminated sites (BC – 77, TC – 76). Insect orders listed by levels of abundance for each site are: PLM – Coleoptera>Diptera>Lepidoptera>Orthoptera; PAN – Diptera> Coleoptera>Trichoptera>Hemiptera; BC – Coleoptera>Lepidoptera>Diptera >Trichoptera; and TC – Trichoptera>Lepidoptera>Diptera>Coleop -tera. Insect metal concentrations by site are as follows: PLM (Zn – 3.00 μg/g, Cd – 0.01 μg/g, Pb – 0.01 μg/g); PAN (Zn – 4.92 μg/g, Cd – 0.03 μg/g, Pb – 0.00 μg/g); BC (Zn – 4.54 μg/g, Cd – 0.01 μg/g, Pb – 0.02 μg/g); and TC (Zn – 9.17 μg/g, Cd – 0.05 μg/g, Pb – 0.00 μg/g). Zn and Cd concentrations were highest at TC, but Pb concentrations were low at all sites.

Water metal concentrations for Zn (mg/L) and Cd and Pb (μg/L) from each of the four sites were: PLM (Zn – 0.01, Cd – 0.04, Pb – 0.22); PAN (Zn – 0.02, Cd – 0.35, Pb – 1.58); BC (Zn – 0.14, Cd – 0.12, Pb – BDL); and TC (Zn – 5.97, Cd – 6.55, Pb – 0.93). TC had the highest levels of Zn (5.97 mg/L) and Cd (6.55 μg/L) and the second highest level of Pb (0.93 μg/L).

Six *L. borealis* (4 M:2 F) from PLM, and eight *L. borealis* (4 M:4 F) from combined contaminated sites, were recorded in the flight cage. Females and males did not differ ($\alpha = 0.05$) for any of the flight variables; therefore, sexes were pooled for remaining analyses. Bats from TCSFS hit more obstacles (mean = 4.1 ± 0.72) than did bats from PLM (mean = 3.5 ±1.31). They were less likely to move through the obstacle course into the other sections of the cage (mean number of movements = 52) compared to bats from PLM (mean = 61 ± 37.6), but they spent on average nearly a minute more time in flight (51 s ± 14.5); however, none of the three variables was significantly different between the two groups. Bats from PLM showed strong relationships between amount of time spent flying and number of obstacles dropped ($r^2 = 0.6945$) and between amount of time spent flying and movement between sections of the cage ($r^2 = 0.9758$; Fig. 3a). Bats from TCSFS did not show strong relationships between flight variables (obstacles dropped and flight time—$r^2 = 0.0004$; movement and flight time—$r^2 = 0.2422$; Fig. 3b).

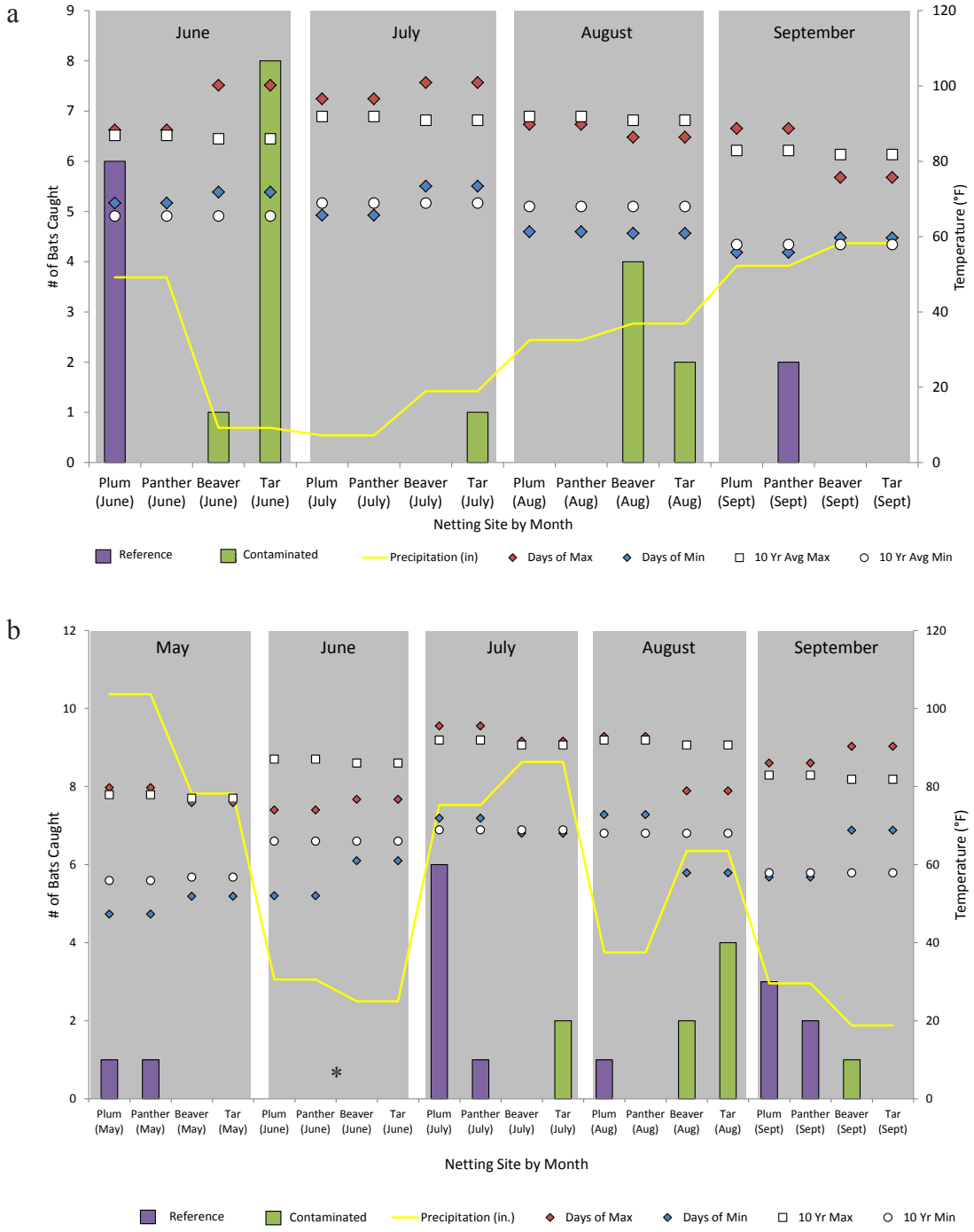

Figure 2. Number and location of captures for 2012 (a) and 2013 (b) by month in relation to weather variables recorded from closest Mesonet station. Number of bats (left y-axis) is represented by purple (reference sites) and green (contaminated sites) bars. Temperature (°F) is on the right y-axis. Diamonds represent three day maximum (red) and minimum (blue) temperatures for nights netted, whereas the 10-year average maximum (squares) and minimum (circles) temperatures are in white. Yellow lines represent inches of precipitation recorded for the month from the closest Mesonet station. *Nets were not set in June 2013 due to creek flooding during the scheduled netting dates.

Table 3. Mean (± SE) metal levels for three tissues in *Lasiurus borealis* (µg/g DW; *n* = 43), insects (µg/g DW; *n* = one sample night per site, means are for two independent analyses), and water (Zn—mg/L, Pb and Cd—µg/L, *n* = 3 per site). Sample sizes for bats are in parentheses followed by ranges. Superscript r indicates a reference site and superscript c indicates a contaminated site.

Metal	Plum[r]	Panther[r]	Beaver[c]	Tar[c]
		Liver		
Zn	3.05 ± 0.31 (14) 0.296–4.53	3.22 ± 0.61 (3) 2.11–4.22	2.82 ± 0.43 (6) 1.98–6.31	2.98 ± 0.29 (15) 1.98–6.31
Cd	0.02 ± 0.01 (13) 0.001–0.131	0.02 ± 0.01 (3) 0.012–0.030	0.02 ± 0.01 (6) 0.010–0.050	0.01 ± 0.00 (15) 0.028–0.002
PB	0.004 ± 0.000 (13) 0.000–0.014	0.006 ± 0.000 (3) 0.003–0.008	0.008 ± 0.000 (6) 0.002–0.016	0.008 ± 0.000(15) 0.002–0.026
		Kidney		
Zn	2.48 ± 0.137 (13) 1.682–3.314	2.364 ± 0.088 (2) 2.207–2.507	2.339 ± 0.176 (6) 1.874–3.022	2.884 ± 0.157 (15) 2.323–4.450
Cd	0.016 ± 0.003 (12) 0.002–0.038	0.049 ± 0.029 (4) 0.013–0.136	0.021 ± 0.008 (6) 0.009–0.061	0.015 ± 0.002 (15) 0.001–0.029
Pb	0.005 ± 0.002 (13) 0.000–0.033	0.002 ± 0.001 (3) 0.000–0.006	0.005 ± 0.001 (6) 0.000–0.011	0.003 ± 0.001 (15) 0.000–0.014
		Hair		
Zn	3.125 ± 0.227 (16) 0.942–4.662	4.438 ± 0.740 (4) 2.692–5.694	4.531 ± 0.823 (6) 1.440–7.552	3.146 ± 0.259 (16) 1.835–4.961
Cd	0.009 ± 0.009 (16) 0.000–0.143	0.003 ± 0.002 (4) 0.000–0.007	0.004 ± 0.003 (6) 0.000–0.017	0.002 ± 0.001 (16) 0.000–0.003
Pb	0.012 ± 0.011 (16) 0.000–0.171	0.030 ± 0.019 (4) 0.000–0.085	0.120 ± 0.085 (6) 0.000–0.526	0.007 ± 0.006 (16) 0.000–0.099
		Insects		
Zn	3.002 ± 0.002	4.922 ± 0.005	4.543 ± 0.053	9.173 ± 0.003
Cd	0.009 ± 0.014	0.028 ± 0.065	0.009 ± 0.374	0.049 ± 0.196
Pb	0.012 ± 0.149	0.001 ± 0.362	0.024 ± 0.666	0.001 ± 0211
		Water		
Zn	0.007 ± 0.001	0.020 ± 0.013	0.139 ± 0.005	5.969 ± 0.611
Cd	0.038 ±0.004	0.353 ± 0.148	0.115 ± 0.011	6.546 ± 0.952
Pb	0.219 ± 0.153	1.579 ± 1.356	BDL	0.934 ± 0.658

Table 4. Significant ($\alpha \leq 0.05$) Pearson Correlation Coefficients for relationships between tissue-metal combinations.

Tissues-metal combinations	Pearson Correlation Coefficient (*P*-value)
Liver Zn/Kidney Zn	0.5126 (0.001)
Liver Cd/Kidney CD	0.7883 (< 0.0001)
Liver Pb/Hair Pb	0.3165 (0.05)
Hair Zn/Hair PB	0.5902 (< 0.0001)
Hair Cd/Hair PB	0.3020 (0.01)

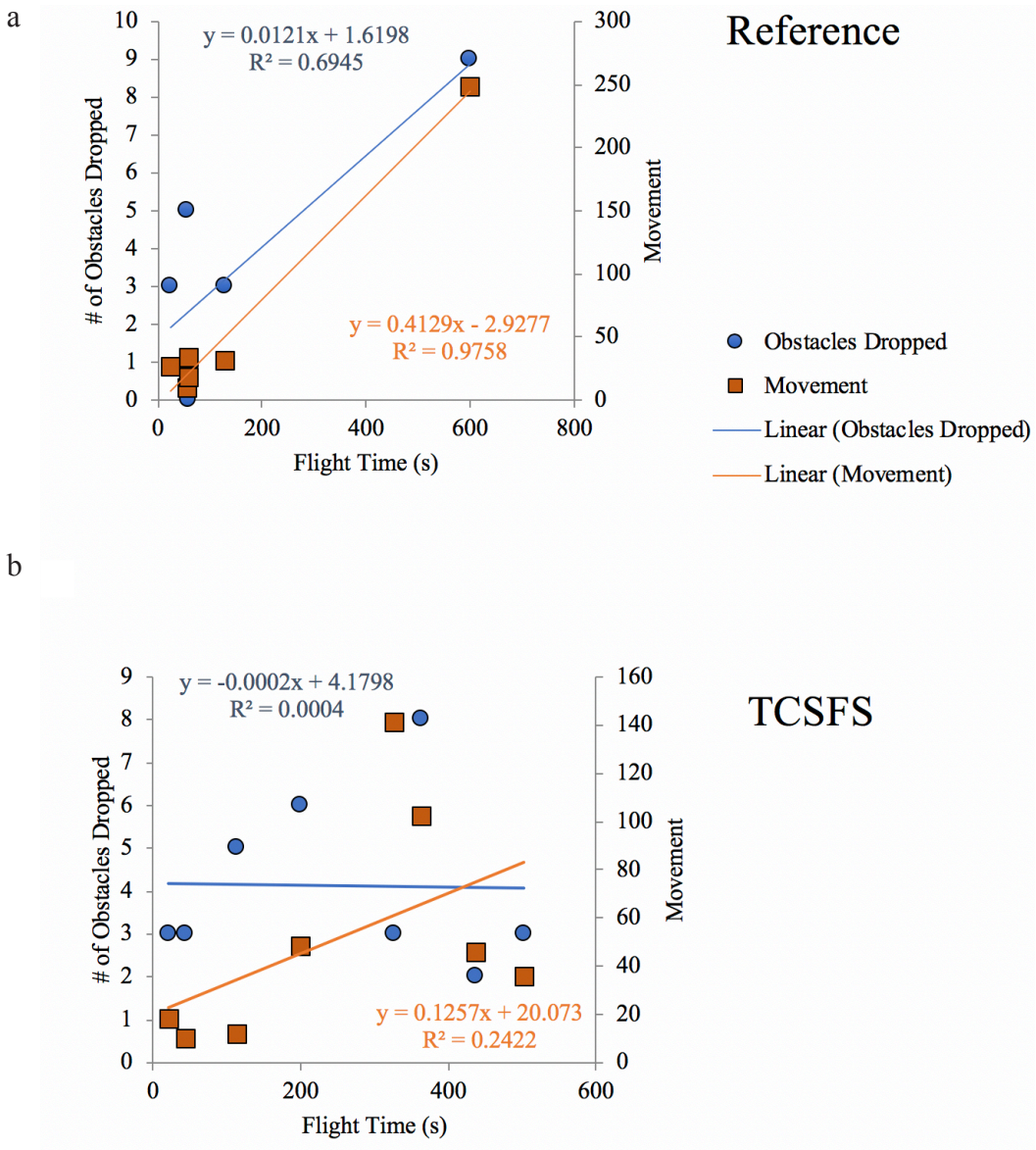

Figure 3. Flight cage scores for bats from a) PLM ($n = 6$) and b) TCSFS ($n = 8$). Blue dots represent number of obstacles dropped by flight time. Red squares represent movement scores by flight time (total $n = 14$).

DISCUSSION

Total number of bats collected at combined contaminated sites and combined reference sites over both years were nearly identical, although during 2012 twice as many individuals were collected at contaminated sites as at reference sites, and during 2013 nearly twice as many individuals were collected at reference sites. Had data only been collected during 2012, the results would have suggested that TCSFS had a bat community twice as large as OWMA. Creek levels within OWMA rise and fall with water levels in Lake Oologah, whereas Tar Creek and Beaver Creek largely are fed through overflow from flooded mines. During 2012, when precipitation levels were 35.5% below the 30-year average, OWMA creeks were well below normal levels by July; however, TCSFS water levels maintained normal levels through September. Bats are drawn to clear, running water for drinking, foraging, and as flyways. Therefore, low water levels at PLM and PAN most likely explains low capture success from reference sites during 2012. This study illustrates the importance of gathering data over multiple time periods and considering interactions between the focal species and abiotic factors, other than the presence of contaminants, that may influence population and community variables.

The hypotheses that the bat community within TCSFS would have lower diversity and evenness compared to the OWMA community was not supported. Although TCSFS sites had a lower combined diversity index ($D = 0.15$) compared to OWMA combined sites ($D = 0.24$), the diversity indices were not significantly different. Two species, represented by single individuals, were captured from reference sites but not at contaminated sites, and one species, also represented by a single individual, was collected only at a contaminated site. TC had a slightly higher number of captures, but lower species diversity, compared to BC, which had fewer captures, but higher diversity. Similarly, PLM had higher captures, but lower diversity, and PAN had a lower number of captures with greater diversity. Presence of contaminants has been related to lower levels of species diversity (Kendall et al. 2001). Lowest values for *D* were at TC, which had the highest Zn and Cd concentrations for both insect and water samples, and PLM, which had the second highest concentration of Pb in insect samples. *Lasiurus borealis* was the most common species at all four sites, which is not surprising. This species occurs in the eastern United States and southern Canada and is often the most frequently captured species within the Ouachita Mountains of eastern Oklahoma and western Arkansas (Saugey et al. 1989; Perry et al. 2010). Based on capture results from this study, this trend is consistent for northeastern Oklahoma as well, although number of individuals per net-night for this study were smaller than those reported in the Ouachita Mountains located further south (Saugey et al. 1989; Perry et al. 2010).

Male biased sex ratios are common for *L. borealis* (Saugey et al. 1989; Perry et al. 2010) in this part of the U.S. Although sample sizes in this study were small, TC had more than twice as many females (Table 2). Dumitrescu et al. (2008) found that sex ratio was female biased in the F_1 generation when mother Wistar rats *(Rattus norvegicus)* were exposed to lead acetate (100 and 150 ppb) before mating and during pregnancy. Bouland et al. (2012) observed a shift in sex ratios towards females in three bird species (*Megaceryle alcyon*, *Sialia sialis*, and *Tachycineta bicolor*) nesting along a mercury-contaminated river. These studies suggest that the female biased sex ratio observed at TC possibly is a subtle result of chronic exposure to low concentrations of metals.

Pregnant females were collected only in May and only from reference sites. All lactating females were collected in early July from reference sites, except for one *Perimyotis subflavus*, which was captured at BC on 27 May 2012. This suggests that she had already given birth, which, for this species, is consistent with findings from Florida (Fujita and Kunz 1984). Although the sample size is small, no reproductive females were collected from TC, which had water Cd concentrations six times higher than the other three sites. Acute Cd exposure interferes with steroidogenesis, thereby decreasing estrogen levels in Sprague-Dawley rats (*R. norvegicus)* when dosed during diestrus (Piasek and Laskey 1994); however, Cd doses used in that study were much higher than concentrations found in bats from either TCSFS or OWMA.

Lasiurus borealis subadults were captured as early as mid-June and into July. *Lasiurus borealis* is estimated to become volant at three to six weeks of

age, and may be weaned at four to six weeks of age (Barbour and Davis 1969) suggesting that the birth of the young captured on 16 June 2012 occurred in late May rather than mid-June, as reported from Indiana, Iowa, and lower Michigan (Shump and Shump 1982). This likely is due to Oklahoma's more southern location and warmer temperatures earlier in the year. Also, 75% of subadults were collected from PLM, with the other 25% collected from TC, which is consistent with the higher levels of female reproduction found from reference sites.

Tissue metal concentrations were converted to ppm WW for comparison to metal concentrations from *Myotis austroriparius* (southeastern myotis) collected by Clark et al. (1986) and *Tadarida brasiliensis* collected by Thies and Gregory (1994). Concentrations of Zn and Cd in liver and kidney found by Clark et al. (1986) were approximately 20–30 times higher for Zn and over 100 times higher for Cd compared to those found in this study. Liver Cd and Pb concentrations collected by Thies and Gregory (1994) were 50–100 times lower for Cd and about 1,000 times lower for Pb. These authors also found no reproductive effects in bats with Pb liver concentrations ranging from 0.74 to 49.44 μg/g WW. Walker et al. (2007) measured renal metal concentrations of bats in Britain and found that concentrations from *Plecotus auritus* (brown long-eared bats) were over 1,000 times higher for Pb and 55 times higher for Cd compared to concentrations from this study. These studies were from geographically distant areas, used different analytical methods, examined different tissue matrices, and focused on species with different diets. All of these factors may have influenced detectable concentrations, which emphasizes the need for consistent, standardized analytical protocols in development of monitoring programs for bats (Zukal et al. 2015).

Subadult bats from TCSFS and OWMA combined had higher concentrations of Zn and Pb in liver compared to adults from TCSFS and OWMA combined. In contrast to these results, Clark (1979) found no relation between age, based on tooth wear, and Pb concentrations in whole body analyses from *M. lucifugus* and *E. fuscus*. Thies and Gregory (1994) also found no correlation between age and Pb liver concentrations of *T. brasiliensis*. Tissue concentrations for kidney Zn and kidney Pb also were higher in subadults compared to adults; however, lower Cd concentrations in kidneys of subadults compared to adults is consistent with a study by Fritsch et al. (2010), in which Cd tended to increase in concentration with age in rodents and shrews. Possibly, subadults are foraging in less optimal habitat, due to best territories already being claimed by adults. Metals also may have been transferred from mothers to young, or young of the year may accumulate more metals because of higher growth rates.

It was hypothesized that bats collected within TCSFS would have higher tissue metal concentrations of Pb, Zn, and Cd in liver, kidney, and hair compared to bats collected from sites within OWMA; however, this hypothesis also was not supported. Although some tissue metal concentrations were higher from bats collected within TCSFS, other tissue metal concentrations detected in bats collected from within OWMA were higher than TCSFS levels (Table 3). Metal levels found in this study were low compared to other studies in bats from other locations, and compared to metal levels in other species from within TCSFS (Zook et al. 1972; Sutton and Wilson 1983; Thies and Gregory 1994; Beyer et al. 2004; Hays and McBee 2007). Beyer et al. (2004) reported that among multiple species of birds with elevated tissue metals concentrations from the Tri-State Mining District that encompasses TCSFS, doves did not have elevated concentrations and suggested that they may not be feeding in heavily contaminated areas. TCSFS is not a homogenously contaminated area, rather it is patchy with some highly contaminated areas, and others with typical background levels of metals (USEPA 1994, 2005), which may partially explain why similar tissue metal concentrations in bats were not detected in this study. Additionally, lower tissue metal concentrations in this volant species at TCSFS may be because species with elevated levels are ones that live in direct contact with chats, sediments, and water (e.g., Hays and McBee 2007).

Kidney Zn concentrations were higher from TC males compared to BC males ($P = 0.03$), which is consistent with insect and surface water concentrations. TC had the highest concentration of Cd of any site for both water and insects. Similarly PAN hair Zn concentrations in males were significantly higher compared to PLM concentrations in males, and PAN had the second highest concentration of Cd in water and insect samples. Lead concentrations from insects

were highest at BC, and BC females had significantly higher hair Pb concentrations compared to females from TC ($P = 0.005$). Females from both reference and contaminated sites had higher concentrations of Pb in all three tissues compared to males, with the exception of PAN liver Pb, PAN hair Pb, and TC hair Pb.

The EPA collected surface water samples from multiple monitoring points throughout TCSFS. Samples were analyzed for concentrations of Pb, Zn, and Cd in March 2009. Metal concentrations (µg/L) from monitoring sites located at TC and BC, respectively, reported Zn – 5360, 549, Cd – 5.5, < 5, and Pb – < 10, < 10. Concentrations of Zn exceed both the acute (379 µg/L) and chronic (343 µg/L) levels for the Oklahoma Water Quality Standards. Concentrations of Pb and Cd reported by the EPA are similar to the concentrations found in this study from BC and TC, and Zn levels are much lower than those reported by the EPA (2010).

Pearson correlation coefficients calculated for the three flight variables and each of seven metal/tissues combinations for all bats (Table 5) showed that liver, kidney, and hair Zn were all negatively correlated with amount of time spent in flight. Kidney and hair Zn were negatively correlated to movement scores. Liver Pb and Cd were positively correlated with number of obstacles dropped, whereas kidney Pb was negatively correlated with number of obstacles dropped. Although correlation coefficients indicated relationships between liver Pb and number of obstacles dropped and between liver Zn and amount of time spent in flight, none of the *P* values were below 0.05 level of significance.

Lasiurus borealis is considered an aerial hawker, meaning it hunts and catches insects in flight (Norberg and Rayner 1987). This type of foraging behavior requires a high level of maneuverability. For bats that are actively flying, one would expect to see a strong relationship between amount of time spent in flight, number of obstacles dropped, and number of movements between sections of the cage as was seen in bats from the reference site (Fig. 3a). Such relationships were not seen in bats from TCSFS (Fig. 3b). Interestingly, bats from TCSFS showed no obvious relationships between amount of time spent flying and number of obstacles dropped or number of movements between sections of the cage. The hypothesis that bats collected within TCSFS would have lower willingness to fly compared to bats from an uncontaminated reference site was not supported; however, they did drop more obstacles and were less willing to maneuver through the portion of the cage that contained the obstacles (i.e., number of movements between sections of the cage). Pb is a neurotoxin that can cause uncoordinated movement, muscle tremors, and a lack of appetite in bats (Sutton 1987). Shrews (*Blarina brevicauda)* exposed to 25 mg/kg lead acetate daily in drinking water were more hyperactive than animals that received sodium acetate in water (Punzo and Farmer 2003). A similar hyperactive response may explain why bats from TCSFS flew nearly a minute longer on average compared to bats from PLM. Although metal levels were not significantly different, negative correlation between liver, kidney, and hair Zn and time spent in flight suggests that bats spent less time flying with increased Zn levels. Concentrations of Pb and Cd in liver were positively correlated with number of obstacles dropped, suggesting that animals with higher Pb and Cd concentrations in liver hit more obstacles; however, kidney Pb was negatively correlated with number of obstacles.

Bats with higher Zn concentrations made fewer movements between sections of the cage and spent less time flying. Despite a tendency to make fewer movements between sections of the cage, bats with

Table 5. Pearson Correlation Coefficients for three flight variables by seven metal/tissue combinations. Metal concentrations for all hair Cd and Pb samples were below detection limit (BDL) and therefore not included in the analysis.

Flight variables	Liver Zn	Liver Cd	Liver Pb	Kidney Zn	Kidney Cd	Kidney Pb	Hair Zn	Hair Cd	Hair Pb
Lines	-0.107	0.396	0.456	-0124	0.239	-0.329	-0.049	BDL	BDL
Movements	-0.251	-0.046	0.126	-0.334	-0.049	-0.107	-0.323	BDL	BDL
Flight time	-0.451	0.003	0.294	-0.318	0.065	-0.145	-0.336	BDL	BDL

higher Cd levels in liver and kidney tended to drop more obstacles. Finally, higher concentrations of Pb in liver suggest a trend towards hyperactivity and poor maneuverability; with more movements, time in flight and obstacles dropped. Although tissue metal levels in *L. borealis* were not significantly different between sites, bats from TCSFS and bats from PLM showed differences in flight behavior. Despite lower tissue metal concentrations than those associated with Pb toxicity and loss of ability to fly in other bats (Zook et al. 1972; Sutton and Wilson 1983), bats from TCSFS did show decreased ability to maneuver through an obstacle course.

Although the hypotheses of this study were not supported, the results demonstrated that bats from within TCSFS had higher liver Pb levels and flew more erratically and for a longer amount of time than did bats from a reference site. The results herein illustrate that relationships among levels of environmental contamination, tissue metal concentrations, and ability and willingness to maneuver through an obstacle course are not straightforward. This study provided the first behavioral flight assay to assess impacts of environmental contaminants on bats by means of a lightweight, portable, field flight cage.

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LITERATURE CITED

ATSDR (Agency for Toxic Substances and Disease Registry). 2004. Report to Congress, Tar Creek Superfund Site, Ottawa County, Oklahoma. Centers for Disease Control and Prevention, Atlanta, Georgia.

Barbour, R. W., and W. H. Davis. 1969. Bats of American. University of Kentucky Press, Lexington, Kentucky.

Beyer, W. N., et al. 2004. Zinc and lead poisoning in wild birds in the Tri-State Mining District (Oklahoma, Kansas, and Missouri). Archives of Environmental Contamination and Toxicology 48:108–117.

Bouland, A. J., A. E. White, K. P. Lonabaugh, C. W. Varian-Ramos, and D. A. Cristol. 2012. Female-biased offspring sex ratios in birds at a mercury-contaminated river. Journal of Avian Biology 43:244–251.

Brilot, B. O., L. Asher, and M. Bateson. 2009. Water bathing alters the speed-accuracy trade-off of escape flights in European starlings. Animal Behaviour 78:801–807.

Brunet-Rossinni, A. K., and G. S. Wilkinson. 2009. Methods for age estimation and the study of senescence in bats. Pp. 315–325 in Ecological and behavioral methods for the study of bats, 2nd edition (T. H. Kunz and S. Parsons, eds.). Johns Hopkins University Press, Baltimore, Maryland.

Burger, J., and M. Gochfeld. 1995. Effects of varying temporal exposure to lead on behavioral development in herring gull (*Larus argentatus*) chicks. Pharmacology Biochemistry and Behavior 52:601–608.

Clark, D. R., Jr. 1979. Lead concentrations; bats vs. terrestrial small mammals collected near a major highway. Environmental Science and Technology 13:338–341.

Clark, D. R., Jr. 1981. Bats and environmental contaminants: a review. United States Department of the Interior Fish and Wildlife Service, Wildlife No. 235.

Clark, D. R., Jr., A. S. Wenner, and J. F. Moore. 1986. Metal residues in bat colonies, Jackson County, Florida, 1981–1983. Florida Field Naturalist 14:38–51.

Conder, J. M., and R. P. Lanno. 1999. Heavy metal concentrations in mandibles of white-tailed deer living in the Picher Mining District. Environmental Contamination and Toxicology 63:80–86.

Currie, R. S., W. L. Fairchild, and D. C. G. Muir. 1997. Remobilization and export of cadmium from lake sediments by emerging insects. Environmental Toxicology and Chemistry 16:2333–2338.

De Francisco, N., J. D. Ruiz Troya, and E. I. Agüera. 2003. Lead and lead toxicity in domestic and free living birds. Avian Pathology 32:2–13.

Dumitrescu, E., T. Alexandra, and P. Snejana. 2008. Lead acetate impact of some markers of female reproductive system performances (litter size, sex ratio) and physical development (vaginal opening) in rats. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Veterinary Medicine 65:283–287.

Eguren, R. E., and K. McBee. 2013. New records of three species of bats from Nowata, Rogers, and Ottawa counties, Oklahoma. Proceedings of the Oklahoma Academy of Science 93:13–18.

Fritsch, C., et al. 2010. Responses of wild small mammals to a pollution gradient: host factor influence metal and metallothionein levels. Environmental Pollution 158:827–840.

Fujita, M. S., and T. H. Kunz. 1984. *Pipstrellus subflavus*. Mammalian Species 228:1–6.

Geluso, K. N., J. S. Altenbach, and D. E. Wilson. 1976. Bat mortality: pesticide poisoning and migratory stress. Science 194:184–186.

Hair, J. D. 1980. Measurement of ecological diversity. Pp. 269–275 in Wildlife management techniques manual (S. D. Schemnitz, ed.). The Wildlife Society, Washington, DC.

Hays, K. A., and K. McBee. 2007. Flow cytometric analysis of red-eared slider turtles (*Trachemys scripta*) from Tar Creek Superfund Site. Ecotoxicology 16:353–361.

Hickey, M. B. C., M. B. Fenton, K. C. MacDonald, and C. Souliere. 2001. Trace elements in the fur of bats (Chiroptera; Vespertilionidae) from Ontario and Quebec, Canada. Bulletin of Environmental Contamination and Toxicology 66:699–706.

Kalisinska, E., et al. 2014. Hepatic and nephric mercury and selenium concentrations in common mergansers, *Mergus merganser*, from Baltic region, Europe. Environmental Toxicology and Chemistry 33:421–430.

Kendall, R. J., et al. 2001. Ecotoxicology. Pp. 1013–1045 in Casarett and Doull's toxicology: basic science of poisons, Sixth edition (C. D. Klaasen, ed.). McGraw-Hill Publishing, New York.

Kunz, T. H. 1974. Feeding ecology of a temperate insectivorous bat (*Myotis velifer*). Ecology 55:693–711.

Kunz, T. H., J. O. Whitaker, Jr., and M. D. Wadanoli. 1995. Dietary energetics of the insectivorous Mexican free-tailed bat (*Tadarida brasiliensis*) during pregnancy and lactation. Oecologia 101:407–415.

Marentette, J. R., et al. 2012. Behavior as biomarker? Laboratory versus field movement in round goby (*Neogobius melanostomus*) from highly contaminated habitats. Ecotoxicology 21:1003–1012.

McBee, K., and J. W. Bickham. 1990. Mammals as bioindicators of environmental toxicity. Current Mammalogy 2:37–88.

Nam, D., D. Yates, P. Ardapple, D. C. Evers, J. Schmerfeld, and N. Basu. 2012. Elevated mercury exposure and neurochemical alterations in little brown bats (*Myotis lucifugus*) from a site with historical mercury contamination. Ecotoxicology 21:1094–1101.

Norberg, U. M., and J. M. V. Rayner. 1987. Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 316:335–427.

Oklahoma Mesonet. 2013. [on-line]. Available from: http://www.mesonet.org/index.php/weather/category/past_data_files, accessed March 15, 2013.

OWRB (Oklahoma Water Resources Board). 1983. Groundwater investigation in the Picher Field. OWRB, Oklahoma City, Oklahoma.

Paulson, G. S. 2005. Handbook to the construction and use of insect collection and rearing devices: a guide for teachers with suggested classroom applications. Springer, New York.

Perry R. W., S. A. Carter, and R. E. Thill. 2010. Temporal patterns in capture rate and sex ratio of forest bats in Arkansas. American Midland Naturalist 164:270282.

Phelps, K. L., and K. McBee. 2009. Ecological characteristics of small mammal communities at a Superfund site. American Midland Naturalist l61:5768.

Phelps, K. L., and K. McBee. 2010. Populations parameters of *Peromyscus leucopus* (White-footed Deermice) inhabit-

ing a heavy metal contaminated superfund site. The Southwestern Naturalist 55:363373.

Piasek, M., and J. W. Laskey. 1994. Acute cadmium exposure and ovarian steroidogenesis in cycling and pregnant rats. Reproductive Toxicology 8:495507.

Price, P. W., B. J. Rathcke, and D. A. Gentry. 1974. Lead in terrestrial arthropods: evidence for biological concentration. Environmental Entomology 3:370372.

Punzo, F., and C. Farmer. 2003. Effects of lead on exploratory behavior and running speed in the shrew, *Blarina brevicauda* (Insectivora). Journal of Environmental Biology 24:411414.

Salcedo, H. D., M. B. Fenton, M. B. C. Hickey, and R. W. Blake. 1995. Energetic consequences of flight speeds of foraging red and hoary bats (*Lasiurus borealis* and *Lasiurus cinereus*; Chiroptera: Vespertilionidae). The Journal of Experimental Biology 198:22452251.

Sanchez-Chardi, A., M. J. López-Fuster, and J. Nadal. 2007. Bioaccumulation of lead, mercury, and cadmium in the greater white-toothed shrew, *Crocidura russula,* from the Ebro Delta (NE Spain); sex- and age- dependent variation. Environmental Pollution 145:714.

Saugey D. A., D. R. Heath, and G. A. Heidt. 1989. The bats of the Ouachita Mountains. Proceedings of the Arkansas Academy of Science 43:7177.

Schmitt, C. J., W. G. Brumbaugh, G. L. Linder, and J. E. Hinck. 2006. A screening-level assessment of lead, cadmium, and zinc in fish and crayfish from northeastern Oklahoma, USA. Environmental Geochemistry and Health 28:445471.

Schmitt, C. J., J. J. Shyte, W. G. Brumbaugh, and D. W. Tillitt. 2005. Chemical effects of lead, zinc, and cadmium from mining on fish in the tri-states district of northeastern Oklahoma, USA. Environmental Toxicology and Chemistry 24:14831495.

Shump, K. A., Jr., and A. U. Shump. 1982. *Lasiurus borealis*. Mammalian Species 183:16.

Sites, R. S., W. L. Gannon, and the Animal Care and Use Committee of the American Society of Mammalogists. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 92:235253.

Sutton, R. 1987. Lead poisoning in flying-foxes (Chiroptera: Pteropodidae). Australian Mammalogy 10:125126.

Sutton, R. H., and R. D. Wilson. 1983. Lead poisoning in Grey-headed fruit bats (*Pteropus poliocephalus*). Journal of Wildlife Diseases 19:294296.

Thies, M., and D. Gregory. 1994. Residues of lead, cadmium and arsenic in livers of Mexican free-tailed bats. Bulletin of Environmental Contamination and Toxicology 52:641648.

USEPA (United States Environmental Protection Agency), Region 6. 1994. First five-year review, Tar Creek Superfund Site, Ottawa County, Oklahoma. United States Environmental Protection Agency, Washington, DC.

USEPA (United States Environmental Protection Agency), Region 6. 2005. Third five-year review, Tar Creek Superfund Site, Ottawa County, Oklahoma. United States Environmental Protection Agency, Washington, DC.

USEPA (United States Environmental Protection Agency), Region 6. 2010. Five-year review, Tar Creek Superfund Site, Ottawa County, Oklahoma. United States Environmental Protection Agency, Washington, DC.

USFWS (United States Fish and Wildlife Service). 2000. Partial restoration plan and environmental assessment, addressing injuries to migratory birds and threatened and endangered species at the Tar Creek Superfund Site, Ottawa County, Oklahoma. Oklahoma Ecological Services Field Office, Tulsa, Oklahoma.

Walker, L. A., V. R. Simpson, L. Rockett, C. L. Weinburg, and R. F. Shore. 2007. Heavy metal contamination in bats in Britain. Environmental Pollution 148:483490.

Williams, D. F., and J. S. Findley. 1979. Sexual size dimorphism in vespertilionid bats. American Midland Naturalist 102:113126.

Wimsatt, J., T. J. O'Shea, L. E. Ellison, R. D. Pearce, and V. R. Price. 2005. Anesthesia and blood sampling of wild big brown bats (*Eptesicus fuscus*) with an assessment of impacts on survival. Journal of Wildlife Diseases 41:8795.

Zook, B. C., R. M. Sauer, and F. M. Garner. 1970. Lead poisoning in Australian fruit bats (*Pteropus poliocephalus*). Journal of the American Veterinary Medical Association 157:691–694.

Zook, B. C., R. M. Sauer, and F. M. Garner. 1972. Lead poisoning in captive wild animals. Journal of Wildlife Diseases 8:264–272.

Zukal, J., J. Pikula, and H. Bandouchova. 2015. Bats as indicators of heavy metal pollution: history and prospect. Mammalian Biology 80:220227.

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APPENDIX

Specimens examined.—Specimens are housed in the Oklahoma State University Collection of Vertebrates.

Lasiurus borealis (40).—UNITED STATES: Oklahoma; Nowata County, Panther Creek (36°37.747'N, 95°31.372'W), OSU 13297, 13319–13321; Ottawa County, Tar Creek (36°57.495'N, 94°50.731'W), OSU 13298–13306, 13322–13327; Beaver Creek (36°56.2026'N, 94°45.3846'W), OSU 13307–13309, 13328–13330; Rogers County, Plum Creek (36°35.5063'N, 95°31.4197'W), OSU 13310–13315, 13331–13339.

Lasiurus cinereus (1).—UNITED STATES: Oklahoma; Ottawa County, Beaver Creek (36°56.2026'N, 94°45.3846'W), OSU 13316.

Myotis lucifugus (1).—UNITED STATES: Oklahoma; Nowata County, Panther Creek (36°37.747'N, 95°31.372'W), OSU 13317.

Perimyotis subflavus (2).—UNITED STATES: Oklahoma; Nowata County, Panther Creek (36°37.747'N, 95°31.372'W), OSU 13340; Ottawa County, Beaver Creek (36°56.2026'N, 94°45.3846'W), OSU 13341.

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Phyllostomid Bats as a Model to Test Zoogeographic Units in Ecuador

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Abstract

Nine Zoogeographic Units (ZU) have been hypothesized for Ecuador, with seven of these units located within the continental portion of the country. Each ZU was defined by climate, topography, elevation, and vegetation type. In spite of their historical application, the validity of ZUs has not been tested. The goal of the present study was to treat the ZUs as hypotheses and test their validity. For this purpose, species of the bat family Phyllostomidae were used as a model. A total of 13,262 validated bat records, representing 109 species and six feeding guilds (with confirmed taxonomic identifications and geographic information), were analyzed using multivariate statistical analyses and geographic modeling. Results obtained from the Multidimensional Scaling Analysis, Detrended Correspondence Analysis, and Pairwise comparisons provided evidence that the ZUs as proposed were valid, based on information from phyllostomid bats, although no species was restricted to the Temperate Forests and High Andes. In addition, geographic models showed that Tropical Eastern Forests and Subtropical Eastern Forests were the most likely areas to be inhabited by phyllostomids. Bat species in the High Flying Frugivore and Insecti-Carnivore trophic guilds displayed a clear spatial pattern highly related to ZUs. The findings of this study provide important information regarding the validity of these ZUs for establishing priorities concerning research, conservation, and management in this group of mammals.

Key words: bats, Chiroptera, Ecuador, geographic distribution, Phyllostomidae, zoogeography

Resumen

En Ecuador se han propuesto nueve pisos zoogeográficos con siete de estas unidades localizadas en la porción continental del país. Cada piso zoogeográfico fue definido por su clima, topografía, elevación, y tipo de vegetación. A pesar de su aplicación histórica, la validez de estos pisos no ha sido puesta a prueba. El objetivo de este estudio fue tratar a los pisos zoogeográficos como hipótesis y evaluar su validez. Para este propósito, especies de la familia de murciélagos Phyllostomidae fueron utilizadas como modelo. Un total de 13,262 registros de murciélagos validados, representando a 109 especies y seis gremios tróficos (con identificación taxonómica e información geográfica confirmada) fueron analizados utilizando análisis estadísticos multivariados, y modelamiento geográfico. Los resultados obtenidos del Análisis de Escalamiento Multidimensional, Análisis de Correspondencia Sin Tendencia, y de Comparaciones por Pares, proporcionaron evidencias que soportan que los pisos zoogeográficos como están propuestos son válidos utilizando filostómidos. Aunque el Piso Templado y el Piso Altoandino no resgistran ninguna especie. Adicionalmente, los modelos geográficos mostraron que los Pisos Tropical Oriental y Subtropical Oriental fueron las áreas con más probabilidad para ser habitadas por los murciélagos filostómidos. Las especies de murciélagos de los gremios tróficos frugívoros de dosel y los insecto-carnívoros

mostraron un patrón espacial altamente relacionado con los pisos zoogeográficos. Los resultados de este estudio proveen información importante relacionada a la validez de los pisos zoogeográficos para el establecimiento de prioridades relacionadas con la investigación, conservación, y el manejo en este grupo de mamíferos.

Palabras clave: distribución geográfica, Ecuador, murciélagos, Phyllostomidae, Quiróptera, zoogeografía

INTRODUCTION

More than 1,500 species of mammals inhabit the Neotropics (Patterson and Costa 2012). This remarkable amount of speciation and diversity represents approximately 30% of extant mammals (Wilson and Reeder 2005) and includes newly described species (Reeder et al. 2007; Gardner 2008a; Patterson and Costa 2012; Patton et al. 2015; Moras et al. 2018). The Tropical Andes region of northern South America, which includes Ecuador, constitutes an important area in terms of biodiversity, endemism, scientific research, and conservation efforts (Dodson and Gentry 1991; Mittermeier et al. 1998; Myers et al. 2000; Rodríguez-Mahecha et al. 2004a, 2004b; Marchese 2015).

Patterns of biodiversity in this geographic area seem to have been influenced directly by a complex association of orographic, climatic, and geological factors (Montgomery et al. 2001; Richter et al. 2008; Anderson et al. 2011). Historically, this complexity has made it difficult to characterize, classify, and define life zones, ecoregions, and biogeographic units within this biologically diverse region (Albuja-V et al. 2012). The definition and establishment of such categories is crucial in setting priorities and planning research activities and conservation strategies at a global or regional scale, in planning effective natural resource management policies, or simply visualizing the geographic distribution of certain species or species assemblages in a defined region (Olson et al. 2001; Corace et al. 2012; Solari et al. 2012). However, abiotic features, such as elevation, have a different effect on the dispersion of birds, bats, and rodents (Patterson et al. 1996, 1998; Kattan et al. 2004). Furthermore, some of the standard biological datasets, including those deposited in natural history museums, are incomplete, which in turn makes it difficult to avoid bias in quantifying biodiversity. This issue also is confounded by the differing opinion of experts regarding the number of species recognized from the region. Systematic revisions published over the last decade have revealed the presence of cryptic species and undescribed species (i.e., Baker et al. 2009; Larsen et al. 2012; Velazco and Patterson 2013, 2014, 2019), most of which have not been included in recent studies. Further, Baker and Bradley (2006) and Reeder et al. (2007) indicated that the number of mammalian species is underestimated by about 40%. Thus, it has been difficult to estimate biodiversity.

In 1892, Theodore Wolf published *Geography and Geology of Ecuador*, which has been recognized as the first treatment on topography, natural formations, geology, climatology, and political geography of the country. Based on categories of vegetation, Wolf divided Ecuador into five regions: dry coastal forests, wet coastal forests, humid Andean forests, inter-Andean forests (valleys), and the high Andes (including paramo). Later, Chapman (1926) identified four life zones for birds, based on vegetation type and elevation: tropical, subtropical, temperate, and páramo. In Chapman's classification, the tropical and subtropical life zones were subdivided into Pacific and Amazonian divisions, and the Pacific Tropical division was further divided into humid and dry regions. Chapman's classification included previous observations of the bird fauna from Colombia (Chapman 1917) and established similarities based on previous studies by Wolf (1892).

Cabrera and Yépez (1940) in their book *Mamíferos Sudamericanos* partitioned South America into eleven zoogeographic provinces. The area corresponding to Ecuador included seven zoogeographic provinces: Galápagos, Pacific, Amazon, Arid, Yungas, High Andes, and Páramo. Subsequently, Hershkovitz (1958, 1969), based on geographic distribution of mammals across the Neotropical region, established four sub-regions: Brazilian subregion (including Middle

American and South American portions), Patagonian, West Indies, and Holarctic. He concluded that Ecuador is a confluence point between the Brazilian subregion and the Patagonian subregion.

Albuja et al. (1980) used elevational ranges to redefine the life zones of Chapman (1926). The elevational increments proposed by Albuja et al. (1980) were developed based on distributions of species of fish, reptiles, amphibians, birds, and mammals known to occur in Ecuador. The inclusion of elevation resulted in the formal recognition of eight distinct ecoregions for the country (Table 1): Tropical Northwestern Forests, Tropical Southwestern Forests, Subtropical Western forests, Temperate Forests, High Andes, Subtropical Eastern Forests, Tropical Eastern Forests, and Galápagos Islands. The classification proposed by Albuja et al. (1980) is considered as a geographic hypothesis for the distribution of Ecuadorian mammals (i.e., Albuja 1999; Tirira 1999; Anderson and Jarrín-V 2002; Anderson and Martínez-Meyer 2004; Albuja and Gardner 2005; Griffiths and Gardner 2008; Tirira et al. 2011; Palacios et al. 2018).

More recently, Solari et al. (2012) revisited previous classifications, including Hershkovitz' (1958, 1969) studies of geographic distributions, that incorporated > 900 species of endemic mammals. Those distributions were allocated by Solari et al. (2012) into 11 subregions following the system proposed by Cabrera and Willink (1980) for the Neotropics. The subregions including Ecuador are the Choco, Andes, and Amazonian lowlands.

Despite these previous studies, a formal assessment to examine the validity of this zoogeographic classification, for specific taxonomic groups, has not been completed. The order Chiroptera constitutes the most diverse, collected, and studied group of mammals in Ecuador (see Albuja 1999; Tirira 2007; Burneo and Tirira 2014; Tirira 2017) and provides an ideal taxonomic group for testing the usefulness of zoogeographic classifications. Several new species have been described in the last two decades (i.e., *Anoura fistulata* Muchhala et al. 2005; *Micronycteris giovanniae* Fonseca et al. 2007; *Lonchophylla fornicata* Woodman 2007; *Eumops wilsoni* Baker et al. 2009; *Myotis diminutus* Moratelli and Wilson 2011; *Sturnira perla* Jarrín-V. and Kunz 2011; *Sturnira bakeri* Velazco and Patterson 2014; *Cynomops tonkigui* Moras et al. 2018; and *Molossus fentoni* Loureiro et al. 2018). These new discoveries suggest that the study and understanding of bat biodiversity in the Neotropics, specifically in the Northern Andes, remains incomplete for several bat faunas and reinforces the need to continue studying, surveying, and protecting these ecosystems. In recent decades, the study of bats in Ecuador has been the subject of increasing attention by researchers interested in their ecology, diversity, evolution, and conservation (i.e., Albuja 1982, 1999; Reid et al. 2000; Muchhala and Jarrín-V 2002; Larsen et al. 2010; Burneo and Tirira 2014).

Table 1. Zoogeographic units (ZUs) proposed for Ecuador by Albuja et al. (1980).

Zoogeographic Unit	Acronym	Area	Elevation Range
Galápagos	GAL	7,850 km²	0–1,607 m
High Andes	HA	29,092 km²	> 3,000 m
Subtropical Eastern Forests	SEF	19,928 km²	1,000–2,000 m
Subtropical Western Forests	SWF	15,579 km²	1,000–2,000 m
Temperate Forests	TF	28,468 km²	1,800–3,000 m
Tropical Eastern Forests	TEF	80,884 km²	200–1,000 m
Tropical Northwestern Forests	TNWF	36,919 km²	0–1,000 m
Tropical Southwestern Forests	TSWF	36,449 km²	0–600 m

Further, the family Phyllostomidae constitutes the most diverse family of bats inhabiting the Neotropics and represents the most extensive radiation in feeding behaviors among the extant mammalian families (Baker et al. 2016; Cirranello et al. 2016). This extraordinary radiation allowed the emergence of different feeding behaviors and diets ranging from fruits, flowers, insects, crustaceans, blood, pollen, nectar, to small vertebrates (including some species of bats) (Gardner 1977) and plays a key role in the dynamics of Neotropical ecosystems. Six trophic categories (*sensu* Patterson et al. 1996; Patterson et al. 2003) are included in the Family Phyllostomidae: High-Flying Frugivore (HFF), Insecti-Carnivore (ICG), Low-Flying Frugivore (LFF), Omnivorous Nectarivore (OMG), Omnivorous Predator (OMP), and Sanguinivore (SAN). Furthermore, the presence and/or absence of certain species in a determined area provides a good estimate regarding the stage of conservation of that ecosystem (Fenton et al. 1992; Medellín et al. 2000; Jones et al. 2009).

Ecuadorian phyllostomid bats are well represented in natural history collections in Ecuador, in other countries worldwide, and in the scientific literature since the 19th century. To date, 111 species in the Phyllostomidae are recognized from Ecuador (modified from Carrera-Estupiñán 2016). In spite of this species richness, geographic distribution patterns for this family in the Northern Andes, which includes Ecuador, remain poorly known. The goal of the present study was to use robust data on geographic and spatial ecological patterns to determine distributional patterns for phyllostomid bats in Ecuador and to test the hypothesis by Albuja et al. (1980) that zoogeographic units define the distribution patterns ascribed to bats. Finally, our intention was to contribute new knowledge on biogeography, conservation, and management.

MATERIALS AND METHODS

Study area.—Ecuador is located in the northwestern region of South America and comprises a continental portion and the Galápagos Archipelago. It has an area of 256,370 km^2 and borders on Colombia to the north and Peru to the south. Continental Ecuador is located between 1º27' N to 5º01' S and 75º15' W to 81º00' W with an elevation gradient ranging from sea level to 6,310 m (Albuja-V 2002).

Ecuador constitutes a transition zone between the Northern (> 2º North) and Central Andes. This transition is marked by the tectonic rupture of Girón-Cuenca, which has been proposed as the northern limit of the Huancabamba depression and the main division between the Northern and Central Andes (Richter et al. 2008). The Ecuadorian Andes contain the narrowest portion of the Andes (~ 150–180 km wide) and dissect the country into two distinct units (Western and Eastern) each with their own biotic and abiotic characteristics (Coltori 2000). The uplift of the Andes Mountains influenced the topography, weather, soil types, watersheds, rivers, and vegetation types found in the country (Cañadas-Cruz 1983; Lenders and Cook 1995; Sierra 1999; Patterson et al. 2012). In terms of geology, Ecuador contains three geomorphological regions: Coast, Amazon, and Andes (Beck et al. 2008) with distinct alluvial and volcanic soil types in all three regions (Dodson and Gentry 1991).

Another relevant feature of Ecuador involves the movement of sea currents along the coast. The cold Humboldt Current coming from the southern hemisphere collides with the southern movement of warm currents coming from the north, causing the climatic effects known as the "El Niño" and "La Niña" (Bendix et al. 2011). The influence of these marine currents is evident in the annual rainfall cycles and the vegetation found in Ecuador. Numerous rivers, lakes, and watersheds also exist in Ecuador. The Río Napo, in eastern Ecuador, has been hypothesized to be the main natural barrier for animal populations occurring on each side of the river (Albuja 1999). All these features have had a strong influence on biodiversity of the bat fauna in Ecuador, resulting in high levels of species richness and endemism in this area.

Zoogeographic units.—Initially, Albuja et al. (1980) proposed eight zoogeographic units (ZU) for Ecuador (Table 1); however, Albuja-V et al. (2012) added the Pacific unit as a 9th ZU. Each ZU was de-

fined by climate, topography, elevation, and vegetation type. However, detailed information about the validity of these ZUs for different groups of vertebrates is not available and has not been examined thoroughly (Albuja-V et al. 2012). Herein, the Galápagos and the Pacific ZUs (Albuja-V et al. 2012) were not included, due to the lack of distributional records for phyllostomid bats from these areas.

Data sources: *Fieldwork*.—Bats were collected during two scientific expeditions to Ecuador. The first (Sowell Expedition 2001) occurred between July and August 2001, whereas the second (Sowell Expedition 2004) took place from June to August 2004 (see Carrera-E 2003; Fonseca et al. 2003; Carrera et al. 2010). Methods associated with the capture and preparation of scientific voucher specimens followed the guidelines of the American Society of Mammalogists (Sikes et al. 2016) and the Texas Tech Animal Care and Use Committee (Permit # 02217-02).

Data sources: Museum records.—Voucher specimens (skins and fluid preserved specimens with their associated skulls) were examined from the following repositories: United States National Museum (USNM); Museo de Zoología at the Pontificia Universidad Católica del Ecuador (QCAZ); Instituto Nacional de Biodiversidad (INABIO, formerly Museo Ecuatoriano de Ciencias Naturales MECN); and Colección Científica del Departamento de Biología, at the Escuela Politécnica Nacional del Ecuador (MEPN).

Data sources: *Scientific literatur*e.—Records of bats collected in Ecuador since the 19th century were obtained from several scientific journals and technical reports including: Thomas (1897); Allen (1916); Anthony (1921, 1923, 1924); Sanborn (1941); Brosset (1965); Linares and Naranjo (1973); Baker (1974); Carter and Dolan (1978); Hill (1980); Albuja (1982, 1999); Webster and Jones (1984); Pacheco and Patterson (1992); Rageot and Albuja (1994); Solmsen (1998); Tirira (1999, 2008, 2009, 2012a, 2012b); Reid et al. (2000); Iudica (2000); Jarrín-V (2001); Fonseca-N and Jarrín-V (2001); Bravo-Cabezas et al. (2003); Hoffmann et al. (2003); Baker et al. (2004); Dávalos (2004); Fonseca and Pinto (2004); Muchhala et al. (2005); Velazco (2005); Hoofer and Baker (2006); Lee et al. (2006a, 2006b, 2008, 2010); McCarthy et al. (2006); Solari and Baker (2006); Pinto et al. (2007); Fonseca et al. (2007); Woodman (2007); Dávalos and Corthals (2008); Gardner (2008); Hoofer et al. (2008); Velazco and Patterson (2008, 2013, 2014, 2019); Solari et al. (2009); Velazco and Gardner (2009); Carrera et al. (2010); Jarrín-V et al. (2010); Larsen et al. (2010); Clare et al. (2011); Jarrín-V and Kunz (2011); Jarrín-V and Menéndez-Guerrero (2011); McDonough et al. (2011); Guerra-M and Albuja-V. (2012); Jarrín-V and Coello (2012); Regalado and Albuja (2012); Tirira and Burneo 2012; Jarrín-V and Clare (2013); Hurtado and Pacheco (2014); Parlos et al. (2014); Tavarez et al. (2014); Bolzan et al. (2015); Calderón-Acevedo and Muchhala (2018); and Velazco et al. (2018). Records of *Sturnira aratathomasi* were not considered in the analyses due to the lack of detailed geographic information from Ecuador (see Peterson and Tamsitt 1968), nor records of *Micronycteris schmidtorum* (Morales-Martínez et al. 2018) because they were published after the statistic and geographic analyses for this study were performed. Further, phyllostomid bats records from Yasuní and Podocarpus National Parks (Rex et al. 2008) were not considered due to the lack of voucher specimens deposited in natural history museums.

Organization of the data.—A total of 13,262 records for phyllostomid bats, representing 109 species with confirmed taxonomic identifications and verified geographic information, were examined (see Appendix I). Detailed information regarding voucher specimens is described in Carrera-Estupiñán (2016). Geographic information for each bat record included was validated using ArcGIS 10.6 (ESRI 2017), Google Earth, and the Convert Geographic Units Website maintained by Montana State University (accessed on 23 April 2019) <http://www.rcn.montana.edu/resources/converter.aspx>. The matrix included in these analyses was based only on bat records with complete taxonomic identification, museum accession number, and verified geographic information (geographic coordinates including degrees, minutes, and seconds; standard UTM; decimal degrees; and elevation in meters).

Taxonomy in this study followed Gardner (2008) unless more recent revisions were available. Exceptions included: Velazco and Patterson (2008) for the recognition of species in the genus *Platyrrhinus*; Larsen et al. (2010) for the recognition of *Artibeus aequatorialis*; Velazco and Simmons (2011) for recognized species in the genus *Vampyrodes*; Jarrín-V and Clare

(2013) and Velazco and Patterson (2013) for taxonomic considerations in the genus *Sturnira*; Hurtado and Pacheco (2014) for the recognition of the new genus *Gardnerycteris*; Parlos et al. (2014) for new arrangements in the subfamily Lonchophyllinae; and Velazco et al. (2018) for the recognition of *Platyrrhinus umbratus*. The use of *Anoura aequatoris* (Mantilla-Meluk and Baker 2006) and *Anoura peruana* (Mantilla-Meluk and Baker 2010) was retained in this study.

Data analysis.—To examine the limits of the zoogeographic units (ZUs) proposed by Albuja et al. (1980) for Ecuador, all phyllostomid records were geo-referenced and assigned into each ZU. Species composition was generated for each ZU and unique species distributed within each ZU were considered to be representative of that ZU. Additionally, to examine the biogeographic affinities of each ZU, a non-metric Multidimensional Scaling analysis (MDS, de Leeuw and Heiser 1982; Stevens et al. 2004) was estimated using a binary Jaccard distance matrix based on species presence/absence. To confirm the strength of these analyses, a Detrended Correspondence Analysis (DCA, Hill and Gauch 1980; Ter Braack 1986) based on relative abundance was performed. This analysis has been used in ecological studies to measure the relationship between taxa and environmental variables (e.g., Owen 1990; Chase et al. 2000; Nakagawa et al. 2006). ZUs were used as the ecological variables and all analyses were performed using PAST 3.06 Software (Hammer et al. 2001).

Species modeling.—Spatial data (geolocations and localities) were filtered based on accuracy (i.e., locality described matched with coordinate location) and standardized to the same datum (WGS84) using ArcGIS 10.6. Species distribution models (SDM) were performed based upon a likelihood analysis for species habitat modeling (Phillips et al. 2006) using maximum entropy niche analysis software (MaxEnt-3.3.3).

Graduated spatial rarefying analyses of the occurrence data via principal component and climate heterogeneity analyses were conducted to eliminate spatial clusters and environmental biases (Boria et al. 2014; Brown 2014; Balaguera-Reina et al. 2019). A total of 24 environmental variables were selected: 19 bioclimatic (bio 1–19, Hijmans et al. 2005), two ecophysiological (mean annual potential evapotranspiration, and mean annual aridity index [Trabucco and Zomer 2009]), a digital elevation model (dem Lehner et al. 2008), and two variables regarding landscape attributes (lctype-landcover type [Broxton et al. 2014a] and mgvf-maximum green variation factor [Broxton et al. 2014b]) at ~1 km^2 resolution (Appendix II). These variables were examined for spatial autocorrelation using the band collection raster tool from ArcGIS 10.6 (Pearson comparison analysis; ESRI 2017), selecting a total of seven variables with $|r| \leq 0.5$ (bio 4, 7, 12, 15, dem, lctype, and mgvf).

Background selection of pseudoabsences was conducted via buffered local adaptive convex-hull analysis (Brown 2014) based upon a 10-km buffer and an alpha value of 3. This combination was selected after testing different values to define the bias file. It is also the most reliable one based on general bat ecology (Fleming and Eby 2003; Cryan and Diehl 2009). These background points also were compared with the rarefy occurrence data to ascertain environmental conditions in which bats can potentially occur, as well as to avoid commission errors and over-fitting the model (Anderson and Raza 2010; Brown 2014).

A geographically structured k-fold cross-validation analysis was performed, dividing the landscape into three regions based on spatial clustering of occurrence points. Five model feature class types (Linear, Quadratic, Hinge, Product, and Threshold) were examined, using 1 as a regularization multiplier to optimize the MaxEnt model performance. From these analyses, the best model was defined based on the omission rates (the lowest value), the area under the curve (AUC, the highest value; Boria et al. 2014), and model feature class complexity (the simplest one; Brown 2014). Finally, a jackknife test of variable importance was performed to define which variables contained the most useful information for the model, as well as which ones contained information not present in the other variables (Brown 2014).

A box-and-whiskers plot was used to define the probability of occurrence threshold based upon the distribution of the dataset via R (R Development Core Team 2012). This only included values above quartile group 1 (minimum) excluding outliers (Balaguera-Reina et al. 2019). Models were added to estimate hotspots (areas with high probability of presence for

phyllostomids) and richness across Ecuador throughout zoogeographic units. Shapiro-Wilk tests were performed to determine the normality of the data, and Kruskal-Wallis tests were run to analyze its variability across zoogeographic units. Dunn's-test for independent samples with a Bonferroni adjustment of *P*-values was used to determine pairwise differences of mean ranks when Kruskal-Wallis tests were significant ($P < 0.05$).

RESULTS

Bat diversity among zoogeographic units.—All phyllostomid species listed are included in Appendix I along with their appropriate zoogeographic units (ZU). The Tropical Northwestern Forest included 2,462 records representing 65 species of bats. The most abundant species in this ZU were: *Carollia perspicillata*, *Carollia castanea*, *Artibeus ravus*, *Artibeus rosenbergi, A. aequatorialis*, and *Glossophaga soricina*. *Choeroniscus periosus*, *Diaemus youngi*, *Hsunycteris cadenai*, *L. fornicata*, *M. giovanniae*, *Platyrrhinus vittatus*, and *Vampyriscus nymphaea* are known to occur only in this ZU (McCarthy et al. 2000; Dávalos 2004; Albuja-V and Gardner 2005; Fonseca et al. 2007; Woodman 2007; Lee et al. 2010; Jarrín-V and Kunz 2011; McDonough et al. 2011).

The Tropical Southwestern Forest included 1,182 records for 43 species with *Artibeus fraterculu*s, *A. aequatorialis*, and *G. soricina* being the most abundant species. In Ecuador, *S. bakeri* is endemic to this ZU (Velazco and Patterson 2013).

The Subtropical Western Forests included a total of 1,530 records from 54 species. The most abundant species were *A. fraterculus*, *C. perspicillata*, and *Sturnira ludovici*. *Lonchophylla hesperia* and *Lonchophylla orcesi* are restricted to this ZU (Albuja-V 1991; Albuja-V and Gardner 2005).

The Temperate Forests included 595 records from 21 species with *Anoura geoffroyi* and *Sturnira erythromos* being the most represented species. There were no species restricted to this ZU.

In the High Andes, a total of 193 records representing nine species were retrieved. *Anoura geoffroyi* and *S. erythromos* were the most abundant species. The species *Sturnira bogotensis* and *Sturnira bidens* were found at sampling localities over 3,000 m in the Ecuadorian Andes (Albuja 1982; Pacheco and Patterson 1992; Moreno-Cárdenas 2009; Jarrín-V and Clare 2013). In spite of no phyllostomid species restricted to this unit, it is important to mention that *Mormoops megalophylla* (Mormoopidae) has been recorded only in upper localities from the High Andes of Ecuador (Boada et al. 2003; Camacho et al. 2017).

Eastern Subtropical Forests were represented by a total of 2,179 records from 59 species, and *Carollia brevicauda* was identified as the most abundant species. The species *Vampyressa melissa* and *Glyphonycteris sylvestris* were restricted to this ZU and are known only from three sampling localities in Ecuador (Rageot and Albuja 1994; Tavares et al. 2014; Tirira et al. 2016).

The Tropical Eastern Forests is the most studied, collected, and diverse ZU in Ecuador, with 5,124 bat records and 71 species being recorded. The species *Artibeus lituratus, Artibeus obscurus*, *C. brevicauda*, *C. castanea*, *C. perspicillata*, *Desmodus rotundus*, *Lophostoma silvicolum*, *Phyllostomus elongatus*, *Platyrrhinus infuscus*, *Rhinophylla pumilio*, *Sturnira giannae* (historically *Sturnira lilium,* and *Sturnira* new sp. 3 *sensu* Velazo and Patterson 2013), *Sturnira magna*, *Uroderma bilobatum*, and *Vampyressa thyone* were represented by ≥ 100 records. There were 11 species endemic to the Tropical Eastern Forests: *Glossophaga commissarisi*, *Hsunycteris pattoni*, *Lampronycteris brachyotis*, *Lichonycteris degener*, *Lionycteris spurrelli*, *Lonchophylla orienticollina*, *Lophostoma carrikeri*, *Platyrrhinus angustirostris*, *Platyrhinus incarum*, *Sphaeronycteris toxophyllum*, and *Uroderma magnirostrum* (Baker 1974; Albuja 1982, 1999; Webster and Jones 1984; Albuja-V and Mena-V 1991; Solmsen 1998; Reid et al. 2000; Dávalos and Corthals 2008; Gardner 2008b; Velazco et al. 2010; Lim et al. 2010; McDonough et al. 2010; Tirira et al. 2010; Clare et al. 2011; Tirira 2012; Camacho et al. 2014, 2016).

Although no bats have been recorded from the Galapagos Islands, the geographic modeling depicted this ZU as an area suitable for phyllostomids. However, this model would require migratory or unusual dispersal events for bats to potentially reach/inhabit this region.

Geographic distribution of Ecuadorian phyllostomids across zoogeographic units includes species that are distributed in all the ZUs, and species that have been recorded in a single ZU. There are three species distributed broadly across all the ZUs (*C. perspicillata*, *Enchisthenes hartii*, *Micronycteris megalotis*) and 25 species have been recorded in only one ZU (Appendix I). The numbers of bat species restricted to each ZU are as follows: Tropical Northwestern Forests ($n = 7$), Tropical Eastern Forests ($n = 11$), Subtropical Western Forests ($n = 2$), Subtropical Eastern Forests ($n = 1$), and Tropical Southwestern Forest ($n = 1$). There were no species restricted to the Temperate Forests and High Andes ZUs.

Most recorded species exhibit broad distributions across the Neotropics (Gardner 2008; Reid 2009). However, several species are endemic to large neotropical ecoregions such as the Chocó, Amazonia, or the Andes. For example, *C. periosus* and *Rhinophylla alethina* are restricted to the Tropical Northwestern Forests, but also occur in the Chocó (*sensu* Solari et al. 2012). *Artibeus gnomus*, *Rhinophylla fischerae*, and *U. magnirostrum* are recorded from the Tropical Eastern Forests but also are representative of the broader Amazonian bat fauna. Several bat species, such as *L. orcesi*, *L. fornicata*, *M. giovanniae*, and *S. perla*, are known only from the collecting locality, or have a narrow geographic distribution. Therefore, their potential geographic ranges may be underestimated.

Spatial variation among Zoogeographic Units.—Multidimensional scaling (Fig. 1) and DCA (Fig. 2), depict more similarities in species composition between Pacific (Tropical Northwestern Forest, Tropical Southwestern Forest), Andean (High Andean and Temperate Forests), and Amazonian (Subtropical Eastern Forests and Tropical Eastern Forests) ZUs. Likewise, pairwise comparisons based on the Jaccard Index obtained from a comparison of species composition between ZUs revealed closer similarities between the Pacific, Amazonian, and Andean ZUs (Table 2).

Species modeling.—From the 109 species with geolocations recorded in Ecuador, 51 accurate models for species were generated (average AUC minus standard deviation > 0.5), mainly due to lack of accurate spatial information for the remainder. A total of 5,630 occurrence data points (average number of occurrences per species = 110 ± 116) were collected from reliable sources for all 51 species modeled. Of these, 1,657 geolocations (training samples) were selected after the graduated spatial rarefying analysis was performed (average number of occurrences per species = 32 ± 25 geolocations). An average of 32 ± 24 folds per species model were performed depending on the number of geolocations present. The maximum average area under the curve (AUC) value obtained was 0.975 and the minimum was 0.764. The environmental variable that contributed the most to all models was bio15 [precipitation seasonality; 17 species; percentage of contribution (PC) = 58 ± 13%; permutation importance (PI) = 54 ± 21] followed by the digital elevation model (DEM) (13 species; PC = 67 ± 19%; PI = 68 ± 20), bio12 (annual precipitation; 9 species; PC = 63 ± 16%; PI = 30 ± 28), and bio7 (temperature annual range; 8 species; PC = 62 ± 15%; PI = 51 ± 29).

Based on the results of the jackknife test of variable importance, bio15 (precipitation seasonality) had the highest gain when used in isolation for the majority of species (21) and appears to contain the most useful information, followed by DEM (10 species) and bio 12 and 7 (9 species, respectively). These same environmental variables decreased the gain the most when omitted and thus appear to contain information that is not present in the other variables.

Probability of presence (the chance a species can be found in a defined pixel based on the total pixels analyzed) based upon the rarefied occurrence data indicated that most of the species occurred with a 0.4 probability (40 % chance to be found in a defined pixel) and oscillated around 0.41 ± 0.10 and 0.69 ± 0.08 (upper and lower quartile ± standard deviation; Fig. 3). The minimum lower quartile reported for all species was 0.16 and the maximum upper quartile was 0.86.

Based on the zoogeographic units postulated by Albuja et al. (1980), the Tropical Eastern Forest had the highest probability of presence (13.64 ± 2.52) for the

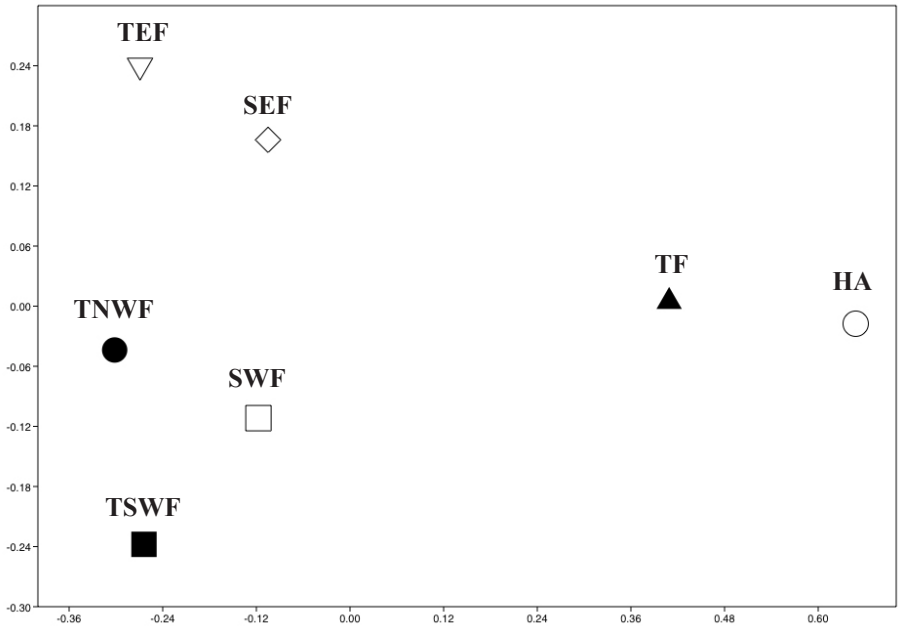

Figure 1. Proximity of seven Zoogeographic Units (ZUs) based on presence/absence of phyllostomid bat species. The graphic depicts first two axes of Non-metric Multidimensional Scaling based on Jaccard Index. ZU acronyms as follows: Tropical Northwestern Forest (TNWF); Tropical Southwestern Forest (TSWF); Subtropical Western Forest (SWF); Temperate Forest (TF); High Andean Forest (HA); Subtropical Eastern Forest (SEF); and Tropical Eastern Forest (TEF).

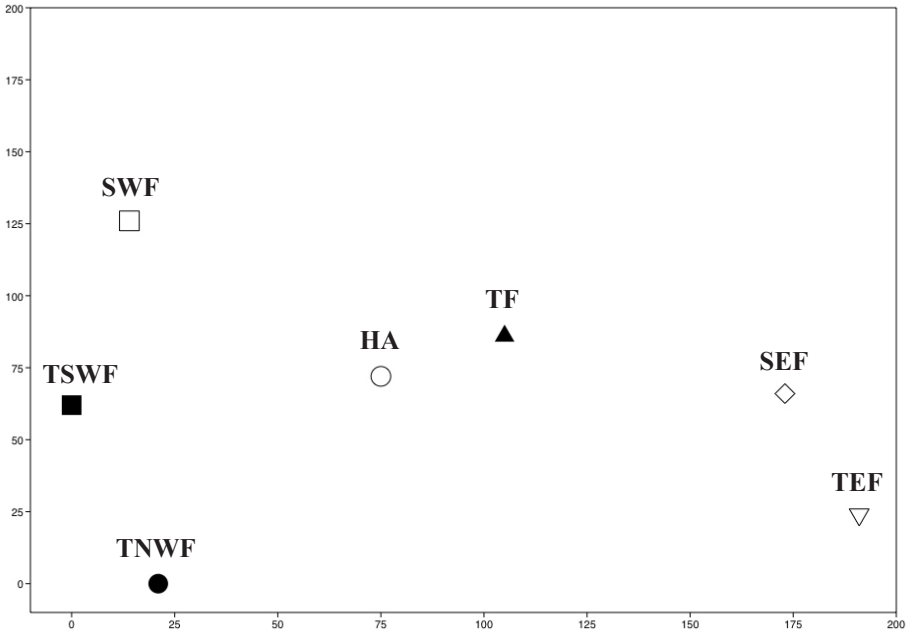

Figure 2. Detrended Canonical Correspondence Analysis (DCA) depicting changes in species composition among Zoogeographic Units (ZUs) defined by Albuja et al. (1980). Graphic is depicting first two DCA axes based on relative abundance of species of Ecuadorian bats. Acronyms of ZUs defined in Figure 1.

Table 2. Pairwise comparisons using the Jaccard index (Real and Vargas 1996) for the seven continental Zoogeographic Units (ZUs) proposed by Albuja et al. (1980). This index, ranging from 0 to 1, compares similarity based on species composition for each ZU. The higher the value, the more similar are the zoogeographic units. ZU acronyms as follows: Tropical Northwestern Forests (TNWF); Tropical Southwestern Forests (TSWF); Subtropical Western Forests (SWF); Temperate Forests (TF); High Andes (HA); Subtropical Eastern Forests (SEF); and Tropical Eastern Forests (TEF).

	TNWF	TSWF	SWF	TF	HA	SEF	TEF
TNWF	1	0.561	0.61	0.225	0.101	0.395	0.432
TSWF	0.561	1	0.65	0.196	0.12	0.371	0.352
SWF	0.61	0.65	1	0.305	0.137	0.444	0.372
TF	0.225	0.196	0.305	1	0.45	0.311	0.21
HA	0.101	0.12	0.137	0.45	1	0.131	0.094
SEF	0.395	0.371	0.444	0.311	0.131	1	0.67
TEF	0.432	0.352	0.372	0.21	0.0945	0.67	1

51 phyllostomid bats modeled, followed by Subtropical Eastern Forest (7.79 ± 2.49), Tropical Northwestern Forest (7.67 ± 2.78), Tropical Southwestern Forest (6.69 ± 2.30), Subtropical Western Forest (6.16 ± 2.37), Temperate Forest (4.62 ± 2.14), and High Andes (2.97 ± 1.55; Fig. 4 left, Fig. 5 left). Concomitantly, the richest zone based on our models is the Tropical Eastern Forest with 19.40 ± 5.47 species, followed by Tropical Northwestern Forest (10.62 ± 4.43 species), Tropical Southwestern Forest (8.74 ± 3.32 species), Subtropical Eastern Forest (8.72 ± 3.36 species), Tropical Southwestern Forest (6.59 ± 2.82 species), Temperate Forest (4.69 ± 2.65 species), and High Andes (3.03 ± 1.77 species; Fig. 4 right, Fig. 5 right).

Probability of presence and richness based on ZUs were significantly different (KW chi-squared = 22911, df = 7, *P*-value < 2.2e-16, and KW chi-squared = 50972, df = 7, *P*-value < 2.2e-16, respectively). The pairwise comparison using Dunn's-test shows that on average the probability of presence and richness across ZUs were significantly different with the exception of TF and TNWF and SEF (Dunn's Test *P* = 1.000).

Six trophic guilds were identified across the 51 species modeled: Sanguinivore (SAN); omnivorous predator (OMP); Omnivorous Nectarivore (OMG); Low-Flying Frugivore (LFF); Insecti-Carnivore (ICG); and High-Flying Frugivore (HFF). However, only four were included (HFF, LFF, ICG, and OMG) due to the limited number of species (≤ 5) in the SAN and OMP guilds (Fig. 6).

HFF and ICG were highly diverse in the Tropical Eastern Forest ZU as well as in the northern part of the Tropical Northwestern Forest and Tropical Southwestern Forest ZUs. ICG and OMG were not present in the High Andes and Temperate Forest but were present in lowland areas as well as LFF. There was a clear spatial pattern across HFF highly correlated with ZUs. Nevertheless, ICG were less correlated with ZUs. In contrast, LFF and OMG were diverse in lowlands without an identifiable pattern across ZUs. However, it is important to highlight that this lack of pattern could be influenced by the limited number of records and species in these two trophic guilds.

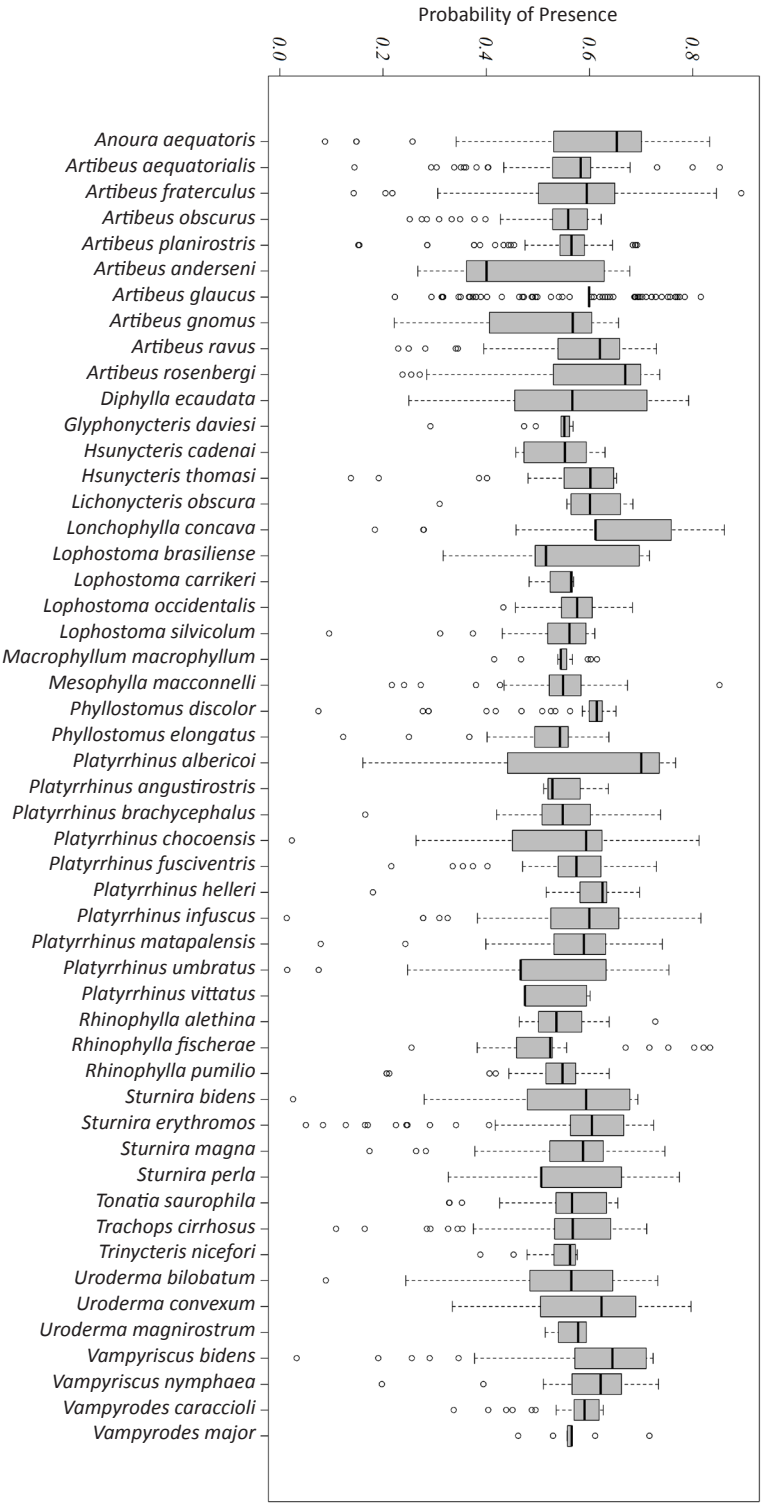

Figure 3. Box and whiskers analysis illustrating the probability of presence for the 51 species of phyllostomids modeled from the relative occurrence rate (ROR). These were based upon the rarefy occurrence data expressed as median and quartiles with whiskers at minimum and maximum values. Outliers are represented as open circles.

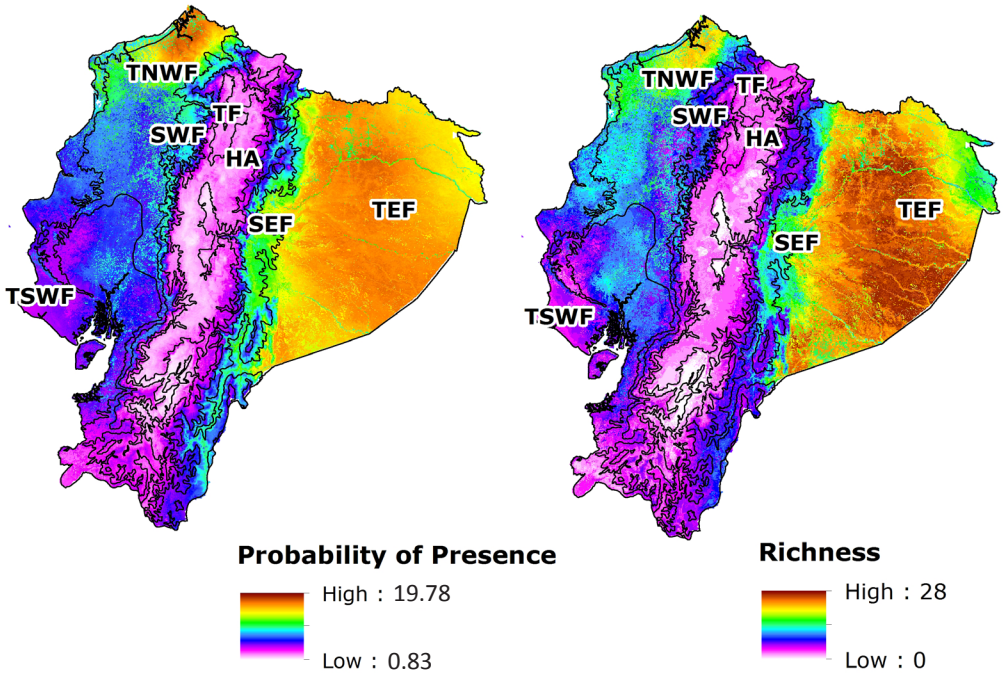

Figure 4. Probability of presence (left) and richness (right) maps based on the 51 phyllostomid bats modeled overlapping the ZUs (black lines and acronyms) defined by Albuja et al. (1980). Warm colors depict areas with high probability of presence, and richness cold colors low presence. Zoogeographic Units (ZUs) acronyms as follows: Tropical Northwestern Forest (TNWF); Tropical Southwestern Forest (TSWF); Subtropical Western Forest (SWF); Temperate Forest (TF); High Andean Forest (HA); Subtropical Eastern Forest (SEF); and Tropical Eastern Forest (TEF).

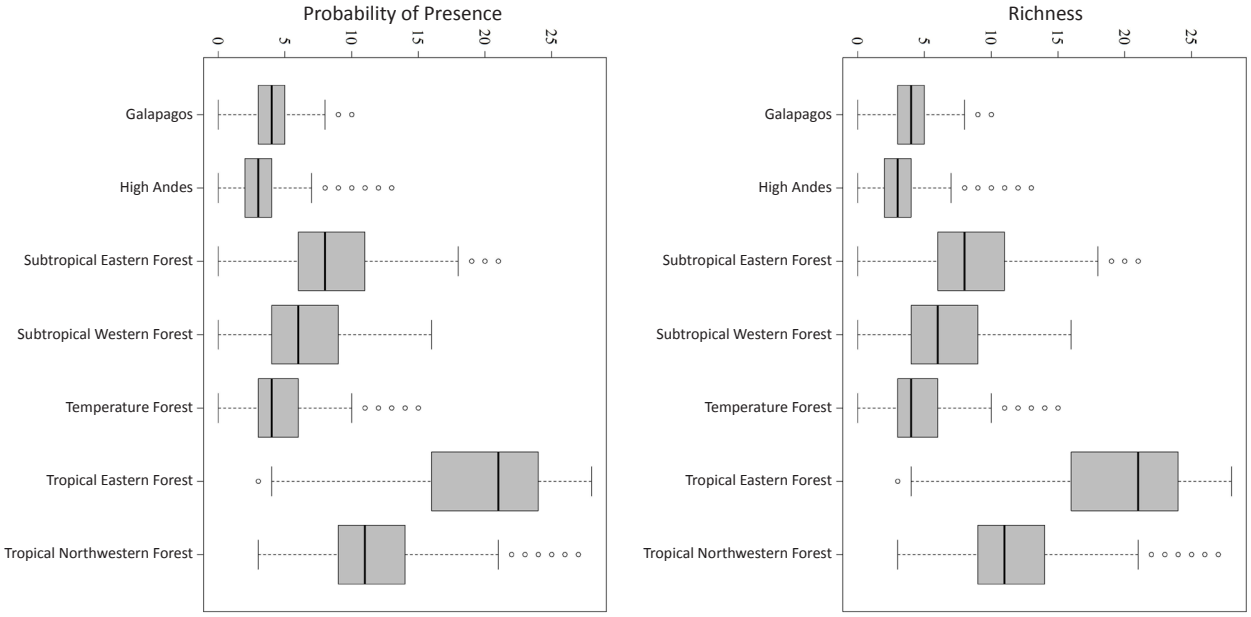

Figure 5. Box and whiskers analysis illustrating the probability of presence (left) and richness (right) for the 51 species of phyllostomids modeled by ZUs described by Albuja et al. (1980). These are expressed as median and quartiles with whiskers at minimum and maximum values. Outliers are represented as open circles.

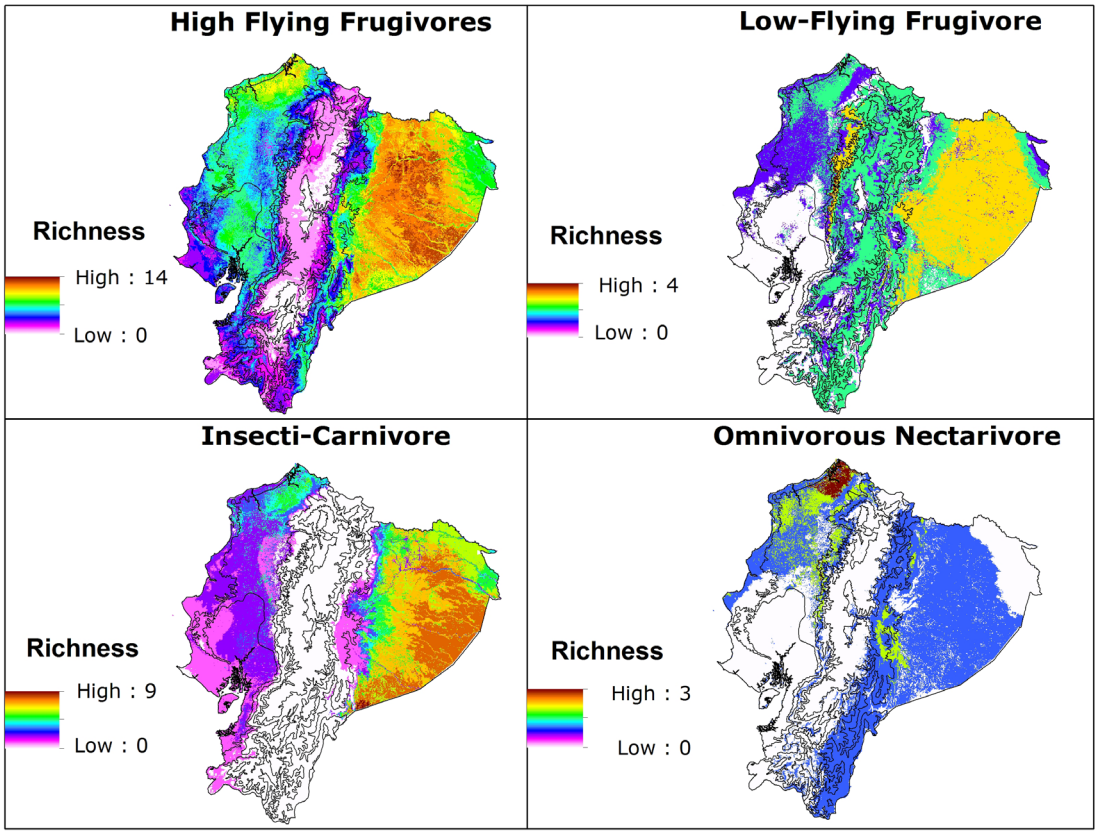

Figure 6. Richness maps based on the 51 phyllostomid bats modeled and classified by trophic guilds overlapping the Zoogeographic Units (ZUs) in black lines defined by Albuja et al. (1980).

DISCUSSION

The diversity of Phyllostomidae bats in Ecuador is a complex mosaic resulting from endemic, rare, common, and broadly distributed species across the Neotropics. Nonetheless, geographic distributions and statistical analyses of phyllostomid bats partially supported the validity of most ZUs proposed within the country. Further, it is possible to recognize differences based on species composition among units.

There are species whose distributions were found to be fully or partially restricted and/or endemic to these ZUs (Appendix I). There are "endemic" species in most of the ZUs with the exception of the Temperate and High Andean forests. However, some species that currently are restricted to a single ZU display broad distribution across the Neotropics. For example: *Vampyriscus nymphaea* recorded in TNWF is distributed broadly from the Pacific side of Ecuador and Colombia to Nicaragua (Arroyo-Cabrales 2008); *D. youngi* currently is known from one sampling locality in TNWF but is broadly distributed in South America, Trinidad and Tobago, and Central America (Kwon and Gardner 2008); *G. commissarisi* is recognized from one locality in Tropical Eastern Forest but recorded in sampling localities from the Amazon basin forest, Central America, and Mexico (Griffiths and Gardner 2008); *S. toxophyllum* is known from two sampling localities in Tropical Eastern Forest (Albuja-V and Mena-V 1991; this study) and currently recorded from Venezuela, Colombia, Peru, Brazil, and Bolivia (Gardner 2008b); *G. sylvestris*, recently reported in the Ecuadorian bat fauna from one sampling locality in Subtropical Eastern Forest (Tirira et al. 2016), is widely distributed from Mexico to Brazil (Williams and Genoways 2008); and *M. schmidtorum*, distributed from Brazil to central Mexico (Williams and Genoways 2008), recently was

reported from Ecuador (Morales-Martínez et al. 2018). There were two records of *S. aratathomasi* collected in Ecuador and deposited at the Royal Ontario Museum that unfortunately do not include geographic information (Peterson and Tamsitt 1968). This is a rare species, and records for this taxon are from 1,600 to 1,800 m on the eastern side of the Andes (Tamsitt et al. 1986). The confirmation of a sampling locality for this species in Ecuador is still pending.

In the last decade, several new species in the bat family Phyllostomidae were described based on morphological and/or genetic differences (e.g., *Lonchophylla chocoana*, *L. cadenai*, *L. orcesi*, *L. fornicata*, *L. orienticollina*, *M. giovanniae*, *S. perla*, and *S. bakeri*). In the majority of these descriptions, the new species were reported from a single collecting locality and were based only on a few specimens. This increasing diversity suggests it is necessary to continue surveying these sampling localities and to enforce conservation and management efforts in these ZUs. The rapid increase in recognized species suggests that biodiversity in the tropics is still underestimated for cryptic, poorly known taxa such as bats. Moreover, species limits in some Neotropical genera remain unclear (i.e., *Sturnira* and *Anoura*), suggesting the number of recognized species may increase in the near future. These uncertainties of biodiversity information will have an impact in establishing priorities for conservation and management in Ecuador.

In contrast to ZU endemics as indicators, three species have been recorded in all ZUs in Ecuador: *C. perspicillata*, *E. hartii*, and *M. megalotis.* These species are distributed broadly across the Neotropics (Gardner 2008a). The presence of the common vampire bat (*D. rotundus*) across all ZUs is likely an effect of the availability of food due to deforestation and increase of cattle (Albuja 1999).

The results obtained from the MDS, DCA, and Pairwise comparisons based on Jaccard's Index provide evidence that ZUs proposed by Albuja et al. (1980) are valid for phyllostomid bats, although there are no restricted species in Temperate Forests and the High Andes. ZUs also were supported by the species distribution model and statistical analyses performed based on the probability of presence obtained. Also, it is possible to distinguish closer affinities among bat assemblages occurring in the Pacific ZUs (Tropical Northwestern Forest, Tropical Southwestern Forest, and Southwestern Forest) versus Amazonian ZUs (Tropical Eastern Forest and Subtropical Eastern Forest) and Andean ZUs (High Andes and Temperate Forest). These differences in bat composition can be associated with the particular type of vegetation, climate, and soils present in each ZU. Based on the species distribution model, precipitation and elevation were the two most important variables for the majority of species (31 of 51 species) assessed in the present study.

Although it was not possible to recover models for all 109 species of phyllostomids present in Ecuador, a representative sample of this family (n =51) was generated to test the validity of ZUs defined by Albuja et al. (1980). Probability of presence values retrieved from all models showed Ecuador as a highly variable landscape with at least seven homogeneous areas (based on the variables used to model) that were significantly related with the ZUs defined by Albuja et al (1980). The Tropical and Subtropical Eastern Forests were the regions with the most suitable areas for phyllostomid bats (Amazon area) followed by Tropical Northwestern and Southwestern Forests. However, richness analyses showed only the northern part of the Amazon and Choco regions (Tropical Eastern Forest and Tropical Northwestern Forest) as the richest areas. This means that even though southern areas of these regions may be suitable for many of the species present in other ZUs, the current richness values present are lower than expected. Additional research should be done to define whether this lower richness and high suitability is due to a lower sampling effort or to an actual absence of species.

High Flying Frugivore and Insecti-Carnivore were the most common trophic guilds found across phyllostomid bats modeled, with a clear spatial pattern across the former and to a lesser extent with the latter. This pattern is relevant because ZUs may be related not only to species but also associated with the ecological role species play in ecosystems. However, the lack of modeled species in the other four trophic guilds identified for phyllostomids bats in Ecuador limited the analyses and conclusions regarding patterns and ZUs. Thus, more studies should be conducted to test how other trophic guilds are related to ZUs.

The significance in recognizing the validity of these ZUs, based on phyllostomid bat distribution, is important to Ecuador. A formal recognition of these categories might be required to establish research priorities and conservation efforts for chiropterans inside the country. Ecuador currently possesses the highest rate of deforestation in South America (Mosandl et al. 2008). This situation allows the expansion of agricultural lands, the advance of open mining, and the increasing use of natural resources to satisfy human needs. In the case of Temperate Forest and High Andes ZUs, it is imperative to continue research to provide a better estimate of bat diversity and the role of the Andes Mountains as a geographic barrier for dispersal and evolution of this group of mammals. Additionally, determining whether these ZUs are valid for other mammalian orders (such as Rodentia, Soricomorpha, and Didelphimorphia) and other taxonomic groups (such as amphibians, reptiles, and birds) is still pending. Future endeavors studying geographic distributions inside ZUs will contribute as well to the basic knowledge and determination of species as keystones for conservation priorities.

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LITERATURE CITED

Albuja-V., L. 1982. Murciélagos del Ecuador. Escuela Politécnica Nacional, Quito, Ecuador.

Albuja-V., L. 1991. Lista de vertebrados del Ecuador: mamíferos. Revista Politécnica (Biología 3) 16:163–203.

Albuja, L. 1999. Murciélagos del Ecuador. 2da edición. Departamento de Ciencias Biológicas, Escuela Politécnica Nacional, Quito, Ecuador.

Albuja-V., L. 2002. Mamíferos del Ecuador. Pp. 271–327 in Diversidad y conservación de los mamíferos neotropicales (G. Ceballos and J. A. Simonetti, eds.). CONABIO and Universidad Nacional Autónoma de México, México, DF.

Albuja-V., L., and A. L. Gardner. 2005. A new species of *Lonchophylla* Thomas (Chiroptera: Phyllostomidae) from Ecuador. Proceedings of the Biological Society of Washington 118:442–449.

Albuja-V., L., and P. Mena-V. 1991. Adición de dos especies de quirópteros a la fauna del Ecuador. Politécnica 16:93–98.

Albuja, L., M. Ibarra, J. Urgilés, and R. Barriga. 1980. Estudio preliminar de los vertebrados ecuatorianos. Escuela Politécnica Nacional, Quito, Ecuador.

Albuja-V, L., A. Almendariz-C., R. Barriga-S., L. D. Montalvo-E., F. Cáceres-F., and J. L. Román. 2012. Fauna de vertebrados del Ecuador. Instituto de Ciencias Biológicas, Escuela Politécnica Nacional, Quito, Ecuador.

Allen, J. A. 1916. List of mammals collected for the American Museum in Ecuador by William B. Richardson, 1912–1913. Bulletin of the American Museum of Natural History 35:113–125.

Anderson, E. P., et al. 2011. Consequences of climate change for ecosystems and ecosystem services in the Tropical

Andes. Pp. 1–18 in Climate change and biodiversity in the tropical Andes (S. K. Herzog, R. Martínez, P. M. Jørgensen, and H. Tiessen, eds.). Inter-American Institute for Global Change Research (IAI) (Brazil) and Scientific Committee on Problems of the Environment (SCOPE), São José dos Campos, SP, Brazil.

Anderson, R. P., and P. Jarrín-V. 2002. A new species of spiny pocket mouse (Heteromyidae: *Heteromys*) endemic to western Ecuador. American Museum Novitates 3382:1–26.

Anderson, R. P., and E. Martínez-Meyer. 2004. Modeling species' geographic distributions for preliminary conservation assessments: an implementation with the spiny pocket mice (*Heteromys*) of Ecuador. Biological Conservation 116:167–179.

Anderson, R. P., and A. Raza. 2010. The effect of the extent of the study region on GIS models of species geographic distributions and estimates of niche evolution: preliminary tests with montane rodents (genus *Nephelomys*) in Venezuela. Journal of Biogeography 37:1378–1393.

Anthony, H. E. 1921. Preliminary report on Ecuadorean mammals. No. 1. American Museum Novitates 20:1–6.

Anthony, H. E. 1923. Preliminary report on Ecuadorean mammals. No. 3. American Museum Novitates 55:1–14.

Anthony, H. E. 1924. Preliminary report on Ecuadorean mammals. No. 4. American Museum Novitates 114:1–6.

Arroyo-Cabrales, J. 2008. Genus *Vampyriscus* O. Thomas, 1900. Pp. 350–355 in Mammals of South America. Volume 1. Marsupials, xenarthrans, shrews, and bats (A. L. Gardner, ed.). University of Chicago Press, Chicago, Illinois.

Baker, R. H. 1974. Record of mammals from Ecuador. Publications of the Museum of Michigan State University, Biological Series 5:129–146.

Baker, R. J., and R. D. Bradley. 2006. Speciation in mammals and the genetic species concept. Journal of Mammalogy 87:643–662.

Baker, R. J., S. Solari, A. Cirranello, and N. B. Simmons. 2016. Higher level classification of phyllostomid bats with a summary of DNA synapomorphies. Acta Chiropterologica 18: 1–38.

Baker, R. J., R. M. Fonseca, D. A. Parish, C. J. Phillips, and F. G. Hoffmann. 2004. New bat of the genus *Lophostoma* (Phyllostomidae: Phyllostominae) from northwestern Ecuador. Occasional Papers, Museum of Texas Tech University 232:1–16.

Baker, R. J., M. M. McDonough, V. J. Swier, P. A. Larsen, J. P. Carrera, and L. K. Ammerman. 2009. New species of *Eumops* (Chiroptera: Molossidae) from the Andes. Acta Chiropterologica 11:1–13.

Balaguera-Reina, S. A., S. Bustillo, D. Zarrate-Charry, F. Charry, A. Cepeda-Mercado, J. F. González-Maya. 2019. Conservation status and distribution based on a species distribution model of the endemic yellow-striped poison frog, *Dendrobates truncatus* (Cope, 1861), in Colombia. Herpetological Review 50:25–57.

Beck, E., F. Makeschin, F. Haubrich, M. Richter, J. Bendix, and C. Valerezo. 2008. The ecosystem (Reserva Biológica San Francisco). Pp. 1–13 in Gradients in a tropical mountain ecosystem of Ecuador (E. Beck, J. Bendix, I. Kottke, F. Makeschin, and R. Mosandl, eds.). Springer, Berlin and Heidelberg, Germany.

Bendix, J., K. Trachte, E. Palacios, R. Rollenbeck, D. Gottlicher, T. Nauss, and A. Bendix. 2011. El Niño meets La Niña—anomalous rainfall patterns in the "traditional" El Niño region of southern Ecuador. Erdkunde 65:151–167.

Boada, C., S. Burneo, T. de Vries, and D. Tirira S. 2003. Notas ecológicas y reproductivas del murciélago rostro de fantasma *Mormoops megalophylla* (Chiroptera: Mormoopidae) en San Antonio de Pichincha, Pichincha, Ecuador. Mastozoología Neotropical 10:21–26.

Bolzan, D. P., L. M. Pessôa, A. L. Peracchi, and R. E. Strauss. 2015. Allometric patterns and evolution in Neotropical nectar-feeding bats (Chiroptera, Phyllostomidae). Acta Chiropterologica 17:59–73.

Boria, R. A., L. E. Olson, S. M Goodman, and R. P. Anderson. 2014. Spatial filtering to reduce sampling bias can improve the performance of ecological niche models. Ecological Modelling 275:73–77.

Bravo-Cabezas, J. J., G. A. Carrillo-Bilbao, R. M. Fonseca-Naranjo, and P. S. Jarrín-Valladares. 2003. Diversidad de mamíferos en la Reserva Ecológica Río Guajalito. Lyonia 3:37–44.

Brosset, A. 1965. Contribution a l'etude des chiropteres de l'ouest de l'Ecuador. Mammalia 29:211–227.

Broxton, P. D., X. Zeng, and W. Scheftic. 2014b. A MODIS-based global 1-km maximum green vegetation fraction dataset. Journal of Applied Meteorology and Climatology 53:1996–2004.

Broxton, P. D., X. Zeng, D. Sulla-Menashe, and P. A Troch. 2014a. A global land cover climatology using MODIS data. Journal of Applied Meteorology and Climatology 53:1593–1605.

Burneo, S. F., and D. G. Tirira. 2014. Murciélagos del Ecuador: un análisis de sus patrones de riqueza, distribución y aspectos de conservación. Therya 5:197–228.

Cabrera, A. L., and A. Willink. 1980. Biogeografia de America Latina. 2nd ed. OEA, Washington, DC.

Cabrera, A., and J. Yépez. 1940. Mamíferos Sudamericanos (vida, costumbres, y descripción). Historia Natural Ediar, Compañía Argentina de Editores, Buenos Aires.

Calderón-Acevedo, C. A., and N. C. Muchhala. 2018. Identification and diagnosis of *Anoura fistulata* with remarks

on its presumed presence in Bolivia. Journal of Mammalogy 99:131–137.

Camacho, M. A., D. Chávez, and S. F. Burneo. 2016. A taxonomic revision of the Yasuni Round-eared bat, *Lophostoma yasuni* (Chiroptera: Phyllostomidae). Zootaxa 4114:246–260.

Camacho, M. A., V. Leiva-D., R. López-Wilchis, and S. F. Burneo. 2017. Genetic diversity of the ghost-faced bat *Mormoops megalophylla* Peters, 1864 (Chiroptera: Mormoopidae) in Ecuador; implications for its conservation. Therya 8:223–232.

Camacho, M. A., D. G. Tirira, C. W. Dick, and S. F. Burneo. 2014. *Lophostoma carrikeri* (Allen, 1910) (Chiroptera: Phyllostomidae): first confirmed records in Ecuador. Check List 10:217–220.

Cañadas-Cruz, L. 1983. El mapa bioclimático y ecológico del Ecuador. Programa Nacional de Regionalización Agraria, MAG / Banco Central del Ecuador, Quito.

Carrera, J. P., S. Solari, P. A. Larsen, D. F. Alvarado, A. D. Brown, C. Carrión B., J. S. Tello, and R. J. Baker. 2010. Bats of the tropical lowlands of western Ecuador. Special Publications, Museum of Texas Tech University 57:1–37.

Carrera-E, J. P. 2003. Distribución de murciélagos (Chiroptera) a través de un gradiente altitudinal en las estribaciones orientales de los Andes ecuatorianos. Unpublished undergraduate thesis, Pontificia Universidad Católica del Ecuador, Escuela de Ciencias Biológicas, Quito.

Carrera-Estupiñán, J. P. 2016. Diversity, zoogeography, and community ecology of bats in the Ecuadorian Andes. Unpublished PhD dissertation, Texas Tech University, Lubbock.

Carter, D. C., and P. G. Dolan. 1978. Catalogue of type specimens of Neotropical bats in selected European museums. Special Publications, Museum of Texas Tech University 15:1–136.

Chapman, F. M. 1917. The distribution of bird-life in Colombia a contribution to a biological survey of South America. Bulletin American Museum of Natural History 36:1–729.

Chapman, F. M. 1926. The distribution of bird-life in Ecuador. Bulletin American Museum of Natural History 55:1–784.

Chase, M. K., W. B. Kristan III, A. J. Lynam, M. V. Price, and J. T. Rotenberry. 2000. Single species as indicators of species richness and composition in California coastal sage scrub birds and small mammals. Conservation Biology 14:474–487.

Cirranello. A., N. B. Simmons, S. Solari, and R. J. Baker. 2016. Morphological diagnoses of higher-level phyllostomid taxa (Chiroptera: Phyllostomidae). Acta Chiropterologica 18:39–71.

Clare, E. L., B. K. Lim, M. B. Fenton, and P. D. N. Hebert. 2011. Neotropical bats: estimating species diversity with DNA barcodes. PLoS ONE 6:e22648.

Coltorti, M. O., and C. D. Ollier. 2000. Geomorphic and tectonic evolution of the Ecuadorian Andes. Geomorphology 32:1–19.

Corace, R. G., III, L. M. Shartell, L. A. Schulte, W. L. Brininger, M. K. D. McDowell, and D. M. Kashian. 2012. An ecoregional context for forest management on National Wildlife Refuges of the Upper Midwest, USA. Environmental Management 49:359–371.

Cryan, P. M., and R. H. Diehl. 2009. Analyzing bat migration. Pp. 476–488 in Ecological and behavioral methods for the study of bats (T. H. Kunz and S. Parsons, eds). 2nd edition. Johns Hopkins University Press, Baltimore, Maryland.

Dávalos, L. M. 2004. A new Chocoan species of *Lonchophylla* (Chiroptera: Phyllostomidae). American Museum Novitates 3426:1–14.

Dávalos, L. M., and A. Corthals. 2008. A new species of *Lonchophylla* (Chiroptera: Phyllostomidae) from the eastern Andes of northwestern South America. American Museum Novitates 3635:1–16.

de Leeuw, J., and W. Heiser. 1982. Theory of multidimensional scaling. Pp. 285-316 in Handbook of statistics (P. R. Krishnaiah and L. N. Kanal, eds.) Volume 2. North-Holland, Amsterdam.

Dodson, C. H., and A. H. Gentry. 1991. Biological extinction in western Ecuador. Annals of the Missouri Botanical Garden 78:273–295.

ESRI (Environmental Systems Research Institute). 2017. Arc GIS Version 10.6. ESRI, Redlands, California. https://www.esri.com.

Fenton, M. B., L. Acharya, D. Audet, M. B. C. Hickey, C. Merriman, M. K. Obrist, D. M. Syme, and B. Adkins. 1992. Phyllostomid bats (Chiroptera: Phyllostomidae) as indicators of habitat disruption in the Neotropics. Biotropica 24:440–446.

Fleming, T. H., and P. Eby. 2003. Ecology of bat migration. Pp. 156–208 in Bat ccology (T. H. Kunz and M. B. Fenton, eds.). University of Chicago Press, Chicago, Illinois.

Fonseca-N., R., and P. Jarrín-V. 2001. Patrones reproductivos de las comunidades de murciélagos en dos bosques nublados de las estribaciones noroccidentales de los Andes ecuatorianos. Pp. 365–374 in Epiphytes and canopy fauna of the Otonga Rain Forest (Ecuador). Results of the Bonn-Quito Epiphyte Project, Vol. 2 (J. Nieder and W. Barthlott, eds.). Botanisches Institut der Universitat Bonn, Germany.

Fonseca, R. M. and C. M. Pinto. 2004. A new *Lophostoma* (Chiroptera: Phyllostomidae: Phyllostominae) from the Amazonia of Ecuador. Occasional Papers, Museum of Texas Tech University 242:1–9.

Fonseca, R. M., et al. 2007. Morphological and molecular variation within little big-eared bats of the genus *Micronycteris*

(Phyllostomidae: Micronycterinae) from San Lorenzo, Ecuador. Pp. 721–746 in The quintessential naturalist: honoring the life and legacy of Oliver P. Pearson (D. Kelt, E. P. Lessa, J. Salazar-Bravo and J. L. Patton, eds.). University of California Publications in Zoology 134:1–996.

Fonseca, R. M., et al. 2003. Identificación preliminar de un corredor ecológico para mamíferos entre los parques nacionales Llanganates y Sangay. Revista de la Pontificia Universidad Católica del Ecuador 71:201-216.

Gardner, A. L. 1977. Feeding habits. Pp. 293-350 in Biology of the bats of the New World family Phyllostomatidae. Part II (R. J. Baker, J. K. Jones, Jr., and D. C. Carter, eds.). Special Publications, Museum of Texas Tech University 13:1–364.

Gardner, A. L. (ed.) 2008a. Mammals of South America. Volume 1. Marsupials, xenarthrans, shrews, and bats. University of Chicago Press, Chicago, Illinois.

Gardner, A. L. 2008b. Genus *Sphaeronycteris* W. Peters, 1882. Pp. 361–363 in Mammals of South America. Volume 1. Marsupials, xenarthrans, shrews, and bats (A. L. Gardner, ed.). University of Chicago Press, Chicago, Illinois.

Griffiths, T. A., and A. L. Gardner. 2008. Subfamily Glossophaginae. Pp. 224–244 in Mammals of South America. Volume 1. Marsupials, xenarthrans, shrews, and bats (A. L. Gardner, ed.). University of Chicago Press, Chicago, Illinois.

Guerra-M, J., and L. Albuja-V. 2012. Registros adicionales de tres especies de murciélagos en el Yasuní, Amazonía ecuatoriana. Revista Politécnica 30:166–171.

Hammer, Ø., D.A.T. Harper, and P.D. Ryan. 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4(1):1–9.

Hershkovitz, P. 1958. A geographical classification of Neotropical mammals. Fieldiana Zoology 36:581–646.

Hershkovitz, P. 1969. The recent mammals of the Neotropical region: a zoogeographic and ecological review. Quarterly Review of Biology 44:1–70.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25:1965–1978.

Hill, J. E. 1980. A note on *Lonchophylla* (Chiroptera: Phyllostomatidae) from Ecuador and Peru, with the description of a new species. Bulletin of the British Museum of Natural History: Zoology 38:233–236.

Hill, M. O., and H. G. Gauch, Jr. 1980. Detrended correspondence analysis: an improved ordination technique. Vegetatio 42: 47–58.

Hoofer, S. R., and R. J. Baker. 2006. Molecular systematics of vampyressine bats (Phyllostomidae: Stenodermatinae) with comparison of direct and indirect surveys of mitochondrial DNA variation. Molecular Phylogenetics and Evolution 39: 424–438.

Hoofer, S. R., S. Solari, P. A. Larsen, R. D. Bradley, and R. J. Baker. 2008. Phylogenetics of the fruit-eating bats (Phyllostomidae: Artibeina) inferred from mitochondrial DNA sequences. Occasional Papers, Museum of Texas Tech University 277:1–15.

Hoffmann, F. G., J. G. Owen, and R. J. Baker. 2003. mtDNA perspective of chromosomal diversification and hybridization in Peters' tent-making bat (*Uroderma bilobatum*: Phyllostomidae). Molecular Ecology 12:2981–2993.

Hurtado, N., and V. Pacheco. 2014. Análisis filogenético del género *Mimon* Gray, 1847 (Mammalia, Chiroptera, Phyllostomidae) con la descripción de un nuevo género. Therya 5:751–791.

Iudica, C. A. 2000. Systematic revision of the Neotropical fruit bats of the genus *Sturnira*: a molecular and morphological approach. Unpublished PhD dissertation, Department of Zoology, University of Florida, Gainesville, Florida. 284pp.

Jarrín-V. P. 2001. Mamíferos en la niebla. Otonga, un bosque nublado del Ecuador. Publicación especial 5, Museo de Zoología, Centro de Biodiversidad y Ambiente, Pontificia Universidad Católica del Ecuador, Quito.

Jarrín-V., P., and E. L. Clare. 2013. Systematics of *Sturnira* (Chiroptera: Phyllostomidae) in Ecuador, with comments on species boundaries. Zootaxa 3630:165–183.

Jarrín-V., P., and D. Coello. 2012. Quantification of morphological variation within species of *Anoura* from Ecuador, with an emphasis on *A. fistulata* (Chiroptera: Phyllostomidae). Acta Chiropterologica 14:317-333.

Jarrín-V., P., and T. H. Kunz. 2011. A new species of *Sturnira* (Chiroptera: Phyllostomidae) from the Choco forest of Ecuador. Zootaxa 2755:1–35.

Jarrín-V., P., and P. A. Menéndez-Guerrero. 2011. Environmental components and boundaries of morphological variation in the short-tailed fruit bat (*Carollia* spp.) in Ecuador. Acta Chiropterologica 13:319–340.

Jarrín-V., P., C. Flores-C., and J. Salcedo-Q. 2010. Morphological variation in the short-tailed fruit bat (*Carollia*) in Ecuador, with comments on the practical and philosophical aspects of boundaries among species. Integrative Zoology 5:226–240.

Jones, G., D. S. Jacobs, T. H. Kunz, M. R. Willig, and P. A. Racey. 2009. Carpe noctem: the importance of bats as bioindicators. Endangered Species Research 8:93–115.

Kattan, G. H., P. Franco, V. Rojas, and G. Morales. 2004. Biological diversification in a complex region: a special analysis of faunistic diversity and biogeography of the Andes of Colombia. Journal of Biogeography 31:1829–1839.

Kwon, M., and A. L. Gardner. 2008. Subfamily Desmodontinae J. A. Wagner, 1840. Pp. 218-224 in Mammals of South America. Volume 1. Marsupials, xenarthrans, shrews, and bats (A. L. Gardner, ed.). University of Chicago Press, Chicago, Illinois.

Larsen, P. A., M. R. Marchán-Rivadeneira, and R. J. Baker. 2010. Taxonomic status of Andersen's fruit-eating bat (*Artibeus jamaicensis aequatorialis*) and revised classification of *Artibeus* (Chiroptera: Phyllostomidae). Zootaxa 2648:45–60.

Larsen, R. J., M. C. Knapp, H. H. Genoways, F. A. Anwarali Khan, P. A. Larsen, D. E. Wilson, and R J. Baker. 2012. Genetic diversity of Neotropical *Myotis* (Chiroptera: Vespertilionidae) with an emphasis on South American species. Plos ONE 7:e46578.

Lee, T. E., Jr., J. B. Packer, and D. Alvarado-Serrano. 2006a. Results of a mammal survey of the Tandayapa Valley, Ecuador. Occasional Papers, Museum of Texas Tech University 250:1–7.

Lee, T. E., Jr., D. Alvarado-Serrano, R. N. Platt, and G. G. Goodwiler. 2006b. Report on a mammal survey of the Cosanga River Drainage, Ecuador. Occasional Papers, Museum of Texas Tech University 260:1–10.

Lee, T. E., Jr., S. F. Burneo, T. J. Cochran, and D. Chávez. 2010. Small Mammals of Santa Rosa, southwestern Imbabura Province, Ecuador. Occasional Papers, Museum of Texas Tech University 290:1–16.

Lee, T. E., Jr., S. F. Burneo, M. R. Marchán, S. A. Roussos, and R. S. Vizcarra-Vácomez. 2008. The mammals of the temperate forests of Volcán Sumaco, Ecuador. Occasional Papers, Museum of Texas Tech University 276:1–10.

Lehner, B., K. Verdin, and A. Jarvis. 2008. New global hydrography derived from spaceborne elevation data. Eos 89:93–94.

Lenders, J. D., and K. H. Cook. 1995. Comments on "On the influence of the Andes on the general circulation of the Southern Hemisphere." Journal of Climate 8:2113-2115.

Linares, O. J., and C. J. Naranjo. 1973. Notas acerca de una colección de murciélagos del género *Lonchorhina* de la cueva de Archidona, Ecuador. Boletín de la Sociedad Venezolana de Espeleología 4:175–180.

Loureiro, L. O., B. K. Lim, and M. D. Engstrom. 2018. A new species of mastiff bat (Chiroptera, Molossidae, *Molossus)* from Guyana and Ecuador. Mammalian Biology 90:10–21.

Mantilla-Meluk, H., and R. J. Baker. 2006. Systematics of small *Anoura* (Chiroptera: Phyllostomidae) from Colombia, with description of a new species. Occasional Papers, Museum of Texas Tech University 261:1–17.

Mantilla-Meluk, H., and R. J. Baker. 2010. New species of *Anoura* (Chiroptera: Phyllostomidae) from Colombia, with systematic remarks and notes on the distribution of the A. *geoffroyi* complex. Occasional Papers, Museum of Texas Tech University 292:1–19.

Marchese, C. 2015. Biodiversity hotspots: a shortcut for a more complicated concept. Global Ecology and Conservation 3:297–309.

McCarthy, T. J., L. Albuja, and M. Alberico. 2006. A new species of Chocoan *Sturnira* (Chiroptera: Phyllostomidae: Stenodermatinae) from western Ecuador and Colombia. Annals of Carnegie Museum 75:97–110.

McDonough, M. M., A. W. Ferguson, L. K. Ammerman, L., C. Granja-Vizcaíno, S. F. Burneo, and R. J. Baker. 2011. Molecular verification of bat species collected in Ecuador: results of a country-wide survey. Occasional Papers, Museum of Texas Tech University 301:1–28.

Medellín, R. A., M. Equihua, and M. A. Amin. 2000. Bat diversity and abundance as indicators of disturbance in Neotropical rainforests. Conservation Biology 14:1666–1675.

Mittermeier, R. A., N. Myers, J. B. Thomsen, G. A. B. da Fonseca, and S. Olivieri. 1998. Biodiversity hotspots and major tropical wilderness areas: approaches to setting conservation priorities. Conservation Biology 12:516–520.

Montgomery, D. R., G. Balco, and S. D. Willett. 2001. Climate, tectonics, and the morphology of the Andes. Geology 29:579–582.

Morales-Martínez, D. M., M. A. Camacho, and S. F. Burneo. 2018. Distribution and morphometric variation of *Micronycteris schmidtorum* (Sanborn, 1935) (Chiroptera: Phyllostomidae) in north South America with the first record from Ecuador. Mastozoología Neotropical 25:391–398.

Moras, L. M., R. Gregorin, T. Sattler, and V. da C. Tavares. 2018. Uncovering the diversity of dog-faced bats of the genus *Cynomops* (Chiroptera: Molossidae), with the redescription of C. *milleri* and the description of two new species. Mammalian Biology 89:37–51.

Moratelli, R., and D. E. Wilson. 2011. A new species of *Myotis* Kaup, 1829 (Chiroptera, Vespertilionidae) from Ecuador. Mammalian Biology 76:608–614.

Moreno-Cárdenas, P. A. 2009. Micromamíferos voladores y no voladores del Distrito Metropolitano de Quito (DMQ). Pp. 53–76 in Guía de Campo de los Pequeños Vertebrados del Distrito Metropolitano de Quito (DMQ) (M. H. Yánez-Muñoz, P. A. Moreno-Cárdenas, and P. Mena-Valenzuela, eds.). Publicación Miscelánea No. 5. Serie de Publicaciones del Museo Ecuatoriano de Ciencias Naturales (MECN)-Fondo Ambiental del MDMQ, Imprenta Nuevo Arte, Quito, Ecuador.

Mossandl, R., S. Günter, B. Stimm, and M. Weber. 2008. Ecuador suffers the highest deforestation rate in South America. Pp. 37–40 in Gradients in a tropical mountain

ecosystem of Ecuador (E. Beck, J. Bendix, I. Kottke, F. Makeschin, and R. Mosandl, eds.). Ecological Studies 198, Springer-Verlag, Berlin, Heidelberg.

Muchhala, N., and P. Jarrín-V. 2002. Flower visitation by bats in cloud forests of western Ecuador. Biotropica 34:387–395.

Muchhala, N., P. Mena-V., and L. Albuja V. 2005. A new species of *Anoura* (Chiroptera: Phyllostomidae) from the Ecuadorian Andes. Journal of Mammalogy 86:457–461.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature 403:853–858.

Nakagawa, M., H. Miguchi, and T. Nakashizuka. 2006. The effects of various forest uses on small mammal communities in Sarawak, Malaysia. Forest Ecology and Management 231:55–62.

Olson, D. M., et al. 2001. Terrestrial ecoregions of the world: a new map of life on earth. Bioscience 51:933–938.

Owen, J. G. 1990. An analysis of the spatial structure of mammalian distribution patterns in Texas. Ecology 71:1823–1832.

Pacheco, V., and B. D. Patterson. 1992. Systematics and biogeographic analyses of four species of *Sturnira* (Chiroptera: Phyllostomidae) with emphasis on Peruvian forms. Memorias del Museo de Historia Natural 21:5–81.

Palacios, J., A. Naveda-Rodríguez, and G. Zapata-Ríos. 2018. Large mammal richness in Llanganates National Park, Ecuador. Mammalia 82:309–314.

Parlos, J. A., R. M. Timm, V. J. Swier, H. Zeballos, and R. J. Baker. 2014. Evaluation of paraphyletic assemblages within Lonchophyllinae, with description of a new tribe and genus. Occasional Papers, Museum of Texas Tech University 320:1–23.

Patterson, B. D., and L. P. Costa. 2012. Introduction to the history and geography of Neotropical mammals. Pp. 1-5 in Bones, clones, and biomes: the history and geography of Recent Neotropical mammals (B.D. Patterson and L.P. Costa, eds.). University of Chicago Press, Chicago, Illinois.

Patterson, B. D., V. Pacheco, S. Solari. 1996. Distributions of bats along an elevational gradient in the Andes of southeastern Peru. Journal of Zoology 240:637–658.

Patterson, B. D., S. Solari, and P. M. Velazco. 2012. The role of the Andes in the diversification and biogeography of Neotropical mammals. Pp. 351–378 in Bones, clones, and biomes: the history and geography of Recent Neotropical mammals (B. D. Patterson and L.P. Costa, eds.). University of Chicago Press, Chicago, Illinois.

Patterson, B. D., M. R. Willig, and R. D. Stevens. 2003. Trophic strategies, niche partitioning, and patterns of ecological organization. Pp. 536–579 in Bat ecology (T. H. Kunz and M. B. Fenton, eds.). University of Chicago Press, Chicago, Illinois.

Patterson, B. D., D. F. Stotz, S. Solari, J. W. Fitzpatrick, and V. Pacheco. 1998. Contrasting patterns of elevational zonation for birds and mammals in the Andes of southeastern Peru. Journal of Biogeography 25:593–607.

Patton, J. L., U. F. J. Pardiñas, and G. D'Elía (eds). 2015. Mammals of South America. Vol. 2. Rodents. University of Chicago Press, Chicago, Illinois.

Peterson, R. L., and J. R. Tamsitt. 1968. A new species of bat of the genus *Sturnira* from northwestern South America. Life Sciences Occasional Papers, Royal Ontario Museum 12:1–8.

Phillips, S. J., R. P. Anderson, R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190: 231–259.

Pinto, C. M., J. P. Carrera, H. Mantilla-Meluk, and R. J. Baker. 2007. Mammalia, Chiroptera, Phyllostomidae, *Diaemus youngi*: first confirmed record for Ecuador and observations on its presence in museum collections. Check List 3:244–247.

R Development Core Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rageot, R., and L. Albuja. 1994. Mamíferos de un sector de la alta Amazonía ecuatoriana: Mera, Provincia de Pastaza. Revista Politécnica, Serie Biología 19:165-208.

Real, R., and J. M. Vargas. 1996. The probabilistic basis of Jaccard's Index of Similarity. Systematic Biology 45:380-385.

Reeder, D. M., K. M. Helgen, and D. E. Wilson. 2007. Global trends and biases in new mammal species discoveries. Occasional Papers, Museum of Texas Tech University 269:1–35.

Reid, F. A. 2009. A field guide to the mammals of Central America and southeast Mexico. Oxford University Press, New York.

Reid, F. A., M. D. Engstrom, and B. K. Lim. 2000. Noteworthy records of bats from Ecuador. Acta Chiropterologica 2:37–51.

Regalado, J., and L. Albuja. 2012. Ampliación de la distibución de *Sturnira nana* y *Lophostoma brasiliense* en el Ecuador. Revista Politécnica 30:160–165.

Rex, K., D. H. Kelm, K. Wiesner, T. H. Kunz, and C. C. Voigt. 2008. Species richness and structure of three Neotropical bat assemblages. Biological Journal of the Linnean Society 94:0617–629.

Richter, M., K. H. Diertl, T. Peters, and R. W. Bussmann. 2008. Vegetation structures and ecological features of the upper timberline ecotone. Pp. 123–135 in Gradients in a tropical mountain ecosystem of Ecuador (E. Beck, J. Bendix, I. Kottke, F. Makeschin, and R. Mosandl, eds.). Ecological Studies 198, Springer-Verlag, Berlin, Heidelberg.

Rodríguez-Mahecha, J. V., et al. 2004a. Tropical Andes. Pp. 73–79 in Hotspots revisited: earth's biologically richest and most endangered ecoregions (R. A. Mittermeier, P. Robles-Gil, M. Hoffmann, J. Pilgrim, T. Brooks, C. Goettsch Mittermeier, J. Lamoreux, and G. A. B. Da Fonseca, eds). CEMEX, Mexico City.

Rodríguez-Mahecha, J. V., et al. Pp. 80–84 in Hotspots revisited: earth's biologically richest and most endangered ecoregions (R. A. Mittermeier, P. Robles-Gil, M. Hoffmann, J. Pilgrim, T. Brooks, C. Goettsch-Mittermeier, J. Lamoreux, and G. A. B. Da Fonseca, eds.). CEMEX, Mexico City.

Sanborn, C. C. 1941. Descriptions and records of Neotropical bats. Field Museum of Natural History, Zoological Series 27:371–387.

Sierra, R. (ed.). 1999. Propuesta preliminar de un sistema de clasificación de vegetación para el Ecuador continental. Proyecto INEFAN/GEF-BIRF and Ecociencia, Quito, Ecuador.

Sikes, R. S., and the Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy 97:663–688

Solari, S., and R. J. Baker. 2006. Mitochondrial DNA sequence, karyotypic, and morphological variation in the *Carollia castanea* species complex (Chiroptera: Phyllostomidae) with description of a new species. Occasional Papers, Museum of Texas Tech University 254:1–15.

Solari, S., P. M. Velazco, and B. D. Patterson. 2012. Hierarchical organization of Neotropical mammal diversity and its historical basis. Pp. 145–156 in Bones, clones, and biomes: the history and geography of Recent Neotropical mammals (B.D. Patterson and L.P. Costa, eds.). University of Chicago Press, Chicago, Illinois.

Solari, S., et al. 2009. Operational criteria for genetically defined species: analysis of the diversification of the small fruit-eating bats, *Dermanura* (Phyllostomidae: Stenodermatinae). Acta Chiropterologica 11:279–288.

Solmsen, E.-H. 1998. New World nectar-feeding bats: biology, morphology and craniometric approach to systematics. Bonner Zoologische Monographien 44:1–118.

Stevens, R. D., M. R. Willig, and I. G. de Fox. 2004. Comparative community ecology of bats from eastern Paraguay: taxonomic, ecological, and biogeographic perspectives. Journal of Mammalogy 85:698–707.

Tamsitt, J. R., A. Cadena, and E. Villaraga. 1986. Records of bats (*Sturnira magna* and *Sturnira aratathomasi*) from Colombia. Journal of Mammalogy 67:754–757.

Tavares, V. C., A. L. Gardner, H. E. Ramírez-Chaves, and P. M. Velazco. 2014. Systematics of *Vampyressa melissa* Thomas, 1926 (Chiroptera: Phyllostomidae), with description of two new species of *Vampyressa*. American Museum Novitates 3813:1–27.

Ter Braak, C. J. F. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology 67:1167–1179.

Thomas, O. 1897. Description of new bats and rodents from America. Annals and Magazine of Natural History, Series 6:544–553.

Tirira, D. 1999. Mamíferos del Ecuador. Publicación Especial 2. Museo de Zoología, Centro de Biodiversidad y Ambiente, Pontificia Universidad Católica del Ecuador, Quito, Ecuador.

Tirira, D. 2007. Guía de campo de los mamíferos del Ecuador. Ediciones Murciélago Blanco. Publicación Especial 6. Quito, Ecuador.

Tirira, D. G. 2008. Mamíferos de los bosques húmedos del noroccidente de Ecuador. Ediciones Murciélago Blanco. Publicación especial sobre mamíferos del Ecuador, 6. Quito, Ecuador.

Tirira, D. G. 2009. Mamíferos ecuatorianos en museos de historia natural y colecciones científicas. 1. El Museo de Historia Natural de Ginebra (Suiza). Boletín Técnico 8, Serie Zoológica Laboratorios IASA 4-5:74–100.

Tirira, D. G. 2012a. Presencia confirmada *de Lonchophylla cadenai* Woodman y Timm, 2006 (Chiroptera, Phyllostomidae) para el noroccidente de Ecuador. Pp. 185–194 in Investigación y conservación sobre murciélagos en el Ecuador (D. G. Tirira and S. F. Burneo, eds.). Publicación especial sobre los mamíferos del Ecuador 9. Pontificia Universidad Católica del Ecuador, Fundación Mamíferos y Conservación and Asociación Ecuatoriana de Mastozoología, Quito, Ecuador.

Tirira, D. G. 2012b. Murciélagos del Ecuador: una referencia geográfica, taxonómica y bibliográfica. Pp. 235–326 in Investigación y conservación sobre murciélagos en el Ecuador (D.G. Tirira and S. F. Burneo, eds.). Publicación especial sobre los mamíferos del Ecuador 9. Pontificia Universidad Católica del Ecuador, Fundación Mamíferos y Conservación and Asociación Ecuatoriana de Mastozoología, Quito, Ecuador.

Tirira, D. G. 2017. A field guide to the mammals of Ecuador: including the Galapagos Islands and the Ecuadorian Antarctic Zone. Special Publication 10. Asociación Ecuatoriana de Mastozoología y Editorial Murciélago Blanco, Quito, Ecaudor.

Tirira, D. G., and S. F. Burneo (eds.). 2012. Investigación y conservación sobre murciélagos en el Ecuador. Pontificia Universidad Católica del Ecuador, Fundación Mamíferos y Conservación, y Asociación Ecuatoriana de Mastozoología, Publicación Especial de los Mamíferos del Ecuador 9, Quito Ecaudor.

Tirira, D. G., C. E. Boada, and S. F. Burneo. 2010. Notes on geographic distribution. Mammalia, Chiroptera, Phyllostomidae, *Lampronycteris brachyotis* (Dobson 1879): first confirmed record to Ecuador. Check List 6:237–238.

Tirira, D. G., S. F. Burneo, C. E. Boada, and S. E. Lobos. 2011. Mammalia, Chiroptera, Phyllostomidae, *Lonchophylla hesperia* G. M. Allen, 1908: second record of the western nectar bat in Ecuador after 70 years. Check List 7:315–318.

Tirira, D. G., M. A. Camacho, N. Tinoco, M. F. Solórzano, and S. F. Burneo. 2016. Genus *Glyphonycteris* Thomas, 1896 (Mammalia: Chiroptera) in Ecuador: first confirmed record of G. *sylvestris* Thomas, 1896 and a geographical review *to G. daviesi* (Hill, 1965). Check List 12:1965.

Trabucco, A., and R. J. Zomer. 2009. Global aridity index (global-aridity) and global potential evapo-transpiration (global-PET) geospatial database. CGIAR Consortium for Spatial Information.

Velazco, P. M. 2005. Morphological phylogeny of the bat genus *Platyrrhinus* Saussure, 1860 (Chiroptera: Phyllostomidae) with the description of four new species. Fieldiana: Zoology, new series 105:1–53.

Velazco, P. M., and A. L. Gardner. 2009. A new species of *Platyrrhinus* (Chiroptera: Phyllostomidae) from western Colombia and Ecuador, with emended diagnoses of *P. aquilus*, *P. dorsalis*, and *P. umbratus*. Proceedings of the Biological Society of Washington 122:249–281.

Velazco, P. M., and B. D. Patterson. 2008. Phylogenetics and biogeography of the broad-nosed bats, genus *Platyrrhinus* (Chiroptera: Phyllostomidae). Molecular Phylogenetics and Evolution 49:749–759.

Velazco, P. M., and B. D. Patterson. 2013. Diversification of the yellow-shouldered bats, genus *Sturnira* (Chiroptera, Phyllostomidae), in the New World tropics. Molecular Phylogenetics and Evolution 68:683–698.

Velazco, P. M., and B. D. Patterson. 2014. Two new species of yellow-shouldered bats, genus *Sturnira* Gray, 1842 (Chiroptera, Phyllostomidae) from Costa Rica, Panama and western Ecuador. ZooKeys 402:43–66.

Velazco, P. M., and B. D. Patterson. 2019. Small mammals of the Mayo River basin in northern Peru, with the description of a new species of *Sturnira* (Chiroptera: Phyllostomidae). Bulletin American Museum of Natural History 429:1–67.

Velazco, P. M., and N. B. Simmons. 2011. Systematics and taxonomy of great striped faced bats of the genus *Vampyrodes* Thomas, 1900 (Chiroptera: Phyllostomidae). American Museum Novitates 3710:1–35.

Velazco, P. M., A. L. Gardner, and B. D. Patterson. 2010. Systematics of the *Platyrrhinus helleri* species complex (Chiroptera: Phyllostomidae), with descriptions of two new species. Zoological Journal of the Linnean Society 159: 785–812.

Velazco, P. M., L. Guevara, and J. Molinari. 2018. Systematics of the broad-nosed bats, *Platyrrhinus umbratus* (Lyon, 1902) and *P. nigellus* (Gardner and Carter, 1972) (Chiroptera: Phyllostomidae), based on genetic, morphometric, and ecological niche analyses. Neotropical Biodiversity 4:119–133.

Webster, W. D., and J. K. Jones, Jr. 1984. Notes on a collection of bats from Amazonian Ecuador. Mammalia 48:247–252.

Williams, S. L., and H. H. Genoways. 2008. Subfamily Phyllostominae Gray, 1825. Pp. 255-300 in Mammals of South America. Volume 1. Marsupials, xenarthrans, shrews, and bats (A. L. Gardner, ed.). University of Chicago Press, Chicago, Illinois.

Wilson, D. E., and D. M. Reeder (eds.). 2005. Mammal species of the world, a taxonomic and geographic reference. Third edition. The Johns Hopkins University Press, Baltimore, Maryland.

Woodman, N. 2007. A new species of nectar-feeding bat, genus *Lonchophylla*, from western Colombia and western Ecuador (Mammalia: Chiroptera: Phyllostomidae). Proceedings of the Biological Society of Washington 120:340–358.

Wolf, T. 1892. Geografía y geología del Ecuador. Brockhaus, Leipzig.

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Appendix I

Phyllostomid bat species distributed in Ecuador and their distribution based on zoogeographic units proposed by Albuja et al. (1980). Zoogeographic Units (ZUs) stand for: Tropical North Western Forests (TNWF); Tropical South Western Forests (TSWF); Subtropical Western Forests (SWF); Temperate Forests (TF); High Andes (HA); Subtropical Eastern Forests (SEF); and Tropical Eastern Forests (TEF). Feeding Guilds (based on Patterson et al. 1996 and Patterson et al. 2003): High-Flying Frugivore (HFF); Insecti-Carnivore (ICG); Low-Flying Frugivore (LFF); omnivorous nectarivore (OMG); omnivorous predator (OMP); and sanguinivore (SAN). * Previously known as *Sturnira lilium* and *S*. new species.

Species	TNWF 0–800m	TSWF 0–600m	SWF < 2 ,000m	TF < 3,000m	HA > 2,800m	SEF < 2,000m	TEF 0–1,000m	Feeding guild
Anoura aequatoris	X		X	X		X	X	OMG
Anoura caudifer	X		X	X		X	X	OMG
Anoura cultrata	X	X	X			X	X	OMG
Anoura fistulata	X		X	X		X	X	OMG
Anoura geoffroyi	X	X	X					OMG
Anoura peruana				X	X	X	X	OMG
Artibeus aequatorialis	X	X	X					HFF
Artibeus anderseni						X	X	HFF
Artibeus concolor						X	X	HFF
Artibeus fraterculus		X	X					HFF
Artibeus glaucus				X		X	X	HFF
Artibeus gnomus						X	X	HFF
Artibeus lituratus	X	X	X			X	X	HFF
Artibeus obscurus						X	X	HFF
Artibeus planirostris						X	X	HFF
Artibeus ravus	X	X	X					HFF
Artibeus rosenbergi	X	X	X					HFF
Carollia brevicauda	X	X	X	X		X	X	LFF
Carollia castanea	X	X	X	X		X	X	LFF
Carollia perspicillata	X	X	X	X	X	X	X	LFF
Chiroderma salvini	X	X	X			X	X	LFF
Chiroderma trinitatum	X	X					X	LFF
Chiroderma villosum	X	X	X			X	X	LFF
Choeroniscus minor	X	X	X				X	OMG

Appendix I (cont.)

Species	TNWF 0–800m	TSWF 0–600m	SWF < 2,000m	TF < 3,000m	HA > 2,800m	SEF < 2,000m	TEF 0–1,000m	Feeding guild
Choeroniscus periosus	X							OMG
Chrotopterus auritus		X	X			X	X	ICG
Desmodus rotundus	X	X	X	X		X	X	SAN
Diaemus youngi	X							SAN
Diphylla ecaudata						X	X	SAN
Enchisthenes hartii	X	X	X	X	X	X	X	HFF
Gardnerycterys crenulatum	X	X	X			X	X	ICG
Glossophaga commissarisi							X	OMG
Glossophaga soricina	X	X	X				X	OMG
Glyphonycteris daviesi	X		X				X	ICG
Glyphonycteris sylvestris						X		ICG
Hsunycteris cadenai	X							OMG
Hsunycteris pattoni							X	OMG
Hsunycteris thomasi						X	X	OMG
Lampronycteris brachyotis							X	OMG
Lichonycteris degener							X	OMG
Lichonycteris obscura	X	X	X					OMG
Lionycteris spurrelli							X	OMG
Lonchophylla chocoana	X		X					OMG
Lonchophylla concava	X	X	X					OMG
Lonchophylla fornicata	X							OMG
Lonchophylla handleyi						X	X	OMG
Lonchophylla hesperia			X					OMG
Lonchophylla orcesi			X					OMG
Lonchophylla orienticollina							X	OMG
Lonchophylla robusta	X	X	X			X	X	OMG
Lonchorhina aurita	X		X			X	X	OMP
Lophostoma brasiliense	X						X	ICG
Lophostoma carrikeri							X	ICG

Appendix I (cont.)

Species	TNWF 0–800m	TSWF 0–600m	SWF < 2,000m	TF < 3,000m	HA > 2,800m	SEF < 2,000m	TEF 0–1,000m	Feeding guild
Lophostoma occidentalis	X	X	X					ICG
Lophostoma silvicolum						X	X	ICG
Macrophyllum macrophyllum	X						X	ICG
Mesophylla macconnelli	X					X	X	HFF
Micronycteris giovanniae	X							ICG
Micronycteris hirsuta	X	X	X			X	X	ICG
Micronycteris megalotis	X	X	X	X	X	X	X	ICG
Micronycteris minuta	X	X				X	X	ICG
Phylloderma stenops	X	X	X			X	X	ICG
Phyllostomus discolor	X	X	X				X	OMP
Phyllostomus elongatus	X						X	OMP
Phyllostomus hastatus	X	X	X			X	X	OMP
Platyrrhinus albericoi	X	X	X			X		HFF
Platyrrhinus angustirostris							X	HFF
Platyrrhinus brachycephalus						X	X	HFF
Platyrrhinus chocoensis	X		X					HFF
Platyrrhinus dorsalis	X		X	X		X		HFF
Platyrrhinus fusciventris						X	X	HFF
Platyrrhinus helleri	X	X						HFF
Platyrrhinus incarum							X	HFF
Platyrrhinus infuscus						X	X	HFF
Platyrrhinus ismaeli			X	X		X		HFF
Platyrrhinus matapalensis	X	X	X					HFF
Platyrrhinus nitelinea		X	X					HFF
Platyrrhinus umbratus	X		X	X		X		HFF
Platyrrhinus vittatus	X							HFF
Rhinophylla alethina	X		X					LFF
Rhinophylla fischerae						X	X	LFF
Rhinophylla pumilio						X	X	LFF

Appendix I (cont.)

Species	TNWF 0–800m	TSWF 0–600m	SWF < 2,000m	TF < 3,000m	HA > 2,800m	SEF < 2,000m	TEF 0–1,000m	Feeding guild
Sphaeronycteris toxophyllum							X	HFF
Sturnira bakeri		X						LFF
Sturnira bidens			X	X	X	X		LFF
Sturnira bogotensis	X			X	X			LFF
Sturnira erythromos			X	X	X	X	X	LFF
Sturnira giannae *					X	X	X	LFF
Sturnira koopmanhilli	X		X					LFF
Sturnira ludovici	X	X	X	X	X			LFF
Sturnira luisi	X	X	X	X		X	X	LFF
Sturnira magna						X	X	LFF
Sturnira nana						X		LFF
Sturnira oporaphilum	X	X	X	X		X	X	LFF
Sturnira perla	X							LFF
Sturnira tildae	X	X	X			X	X	LFF
Tonatia saurophila	X	X				X	X	ICG
Trachops cirrhosus	X	X	X			X	X	ICG
Trinycteris nicefori	X						X	HFF
Uroderma bilobatum						X	X	HFF
Uroderma convexum	X	X						HFF
Uroderma magnirostrum							X	HFF
Vampyressa melissa						X		HFF
Vampyressa thyone	X	X	X	X		X	X	HFF
Vampyriscus bidens						X	X	HFF
Vampyriscus nymphaea	X							HFF
Vampyrodes caraccioli						X	X	HFF
Vampyrodes major	X		X					HFF
Vampyrum spectrum	X	X	X			X	X	ICG

APPENDIX II

Environmental variables used for modeling phyllostomids bats in Ecuador. These variables were tested for spatial autocorrelation; after this analysis a group of seven variables (*) were selected to run the final species distribution models.

Variable ID	Variable Description	Source
BIO1	Annual Mean Temperature	Hijmans et al. 2005
BIO2	Mean Diurnal Range [Mean of monthly (max temp - min temp)]	Hijmans et al. 2005
BIO3	Isothermality (BIO2/BIO7) (* 100)	Hijmans et al. 2005
BIO4 *	Temperature Seasonality (standard deviation *100)	Hijmans et al. 2005
BIO5	Max Temperature of Warmest Month	Hijmans et al. 2005
BIO6	Min Temperature of Coldest Month	Hijmans et al. 2005
BIO7 *	Temperature Annual Range (BIO5-BIO6)	Hijmans et al. 2005
BIO8	Mean Temperature of Wettest Quarter	Hijmans et al. 2005
BIO9	Mean Temperature of Driest Quarter	Hijmans et al. 2005
BIO10	Mean Temperature of Warmest Quarter	Hijmans et al. 2005
BIO11	Mean Temperature of Coldest Quarter	Hijmans et al. 2005
BIO12 *	Annual Precipitation	Hijmans et al. 2005
BIO13	Precipitation of Wettest Month	Hijmans et al. 2005
BIO14	Precipitation of Driest Month	Hijmans et al. 2005
BIO15 *	Precipitation Seasonality (Coefficient of Variation)	Hijmans et al. 2005
BIO16	Precipitation of Wettest Quarter	Hijmans et al. 2005
BIO17	Precipitation of Driest Quarter	Hijmans et al. 2005
BIO18	Precipitation of Warmest Quarter	Hijmans et al. 2005
BIO19	Precipitation of Coldest Quarter	Hijmans et al. 2005
PET	Mean annual potential evapotranspiration	Trabucco and Zomer 2009
Aridity	Mean annual aridity index	Trabucco and Zomer 2009
dem *	digital elevation model	Lehner et al. 2008
lctype *	landcover type	Broxton et al. 2014a
mgvf *	maximum green variation factor	Broxton et al. 2014b

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Predictors of Bat Species Richness within the Islands of the Caribbean Basin

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Abstract

Several mechanisms have been shown to influence species richness among island ecosystems, yet most studies limit their focus to a few predictor variables. The objective of this study is to investigate variation in Chiropteran richness across islands in the Caribbean Basin with an extensive set of predictor variables. Using recent faunal surveys, the most contemporary list of bat species per island was complied. Data were collected on 17 predictor variables, which summarized five general island characteristics including island area, isolation, habitat diversity, human impact, and climate. An information-theoretic approach was used by fitting alternative candidate models to determine which variable(s) best predicted bat species richness. Island area and timing of human colonization were most important when islands located on the continental shelf were included in the analysis. When these islands were removed, measures of habitat diversity and climate became the most important predictors for all island groups except the Bahamas, where no variables predicted species richness better than chance. The results of this analysis highlight the importance of island area, habitat heterogeneity, and climate in determining the bat species richness on Caribbean islands.

Key words: area, Caribbean Basin, Chiroptera, climate, habitat diversity, island biogeography, isolation, Mammalia, species richness

Introduction

Island ecosystems have provided valuable insight to the studies of ecology, biogeography, and evolution. Due to their variation in size, location, geologic history, and environmental conditions, islands provide ideal settings for natural experiments and were the major focus of seminal publications by Darwin (1859) and Wallace (1860, 1880). One of the most influential works on island dynamics in the 20th century is MacArthur and Wilson's equilibrium theory of island biogeography (MacArthur and Wilson 1963, 1967). This theory postulates that species richness on an island is the determinant of two dynamic processes—extinction and immigration. The authors hypothesized that rates of extinction would be lower on larger islands compared to small ones and that immigration rates would be higher to islands located closer to the mainland. Two prominent hypotheses that often have been proposed for explaining patterns of species richness on islands are "area *per se*" and "habitat heterogeneity" (MacArthur and Wilson 1963, 1967). The area *per se* hypothesis proposes that species richness is the result of the dynamic relationship between extinction and immigration. Specifically, it states that population sizes increase on large islands, which will subsequently decrease extinction rates. Additionally, larger islands should have higher immigration rates. Combined, these two dynamic processes should lead to higher species richness. The habitat heterogeneity hypothesis predicts that more complex habitats possess more niches that can be exploited by a higher variety of species. It is assumed that as island area increases, new habitats are added, thus resulting in more species. There has been much debate over which hypothesis better predicts species richness, with some recent studies suggesting that the two hypotheses are not mutually exclusive but rather complimentary (Kallimanis et al. 2008).

Quantifying habitat diversity, especially over larger spatial scales, can be problematic (Ricklefs and Lovette 1999). In lieu of reliable habitat data, a frequently used surrogate for habitat diversity is maximum elevation (Pedersen et al. 2018b). Higher elevations are often equated to more topographic diversity and are often strongly correlated with diversity in plant species (Tews et al. 2004). More advanced measures of habitat heterogeneity such as surface ruggedness (Grohmann et al. 2011) and the 3D volume of a habitat (Flaspohler et al. 2010; Davies and Asner 2014) have proved successful in predicting suitable habitat, but have yet to be used in studies of island biogeography.

Another aspect of island area that can effect species richness is the amount of area lost since the Last Glacial Maximum (LGM). The deglaciation events that occurred ~22,000 to ~13,000 years before present caused significant changes in climate and landscape features of the Northern Hemisphere including dramatic rises (~125 m) in sea level (Hag et al. 1993; Hearty 1998; Yokoyama et al. 2000; Curtis et al. 2001; Clark et al. 2002, 2009; Gehrels 2010). This resulted in significant land loss and has been linked to the extinction of certain species and subsequent loss of diversity on islands (MacFarlane et al. 1998; Morgan 2001; Dávalos and Russell 2012; Rijsdijk et al. 2014). However, there has been some debate on whether the LGM is the cause of these extinction events (Soto-Centeno and Steadman 2015; Stoetzel et al. 2016).

Island isolation and its effect on species richness is one of the two general patterns addressed by MacArthur and Wilson's (1963, 1967) equilibrium theory. As an island becomes more isolated, the immigration rates of species decrease, along with species richness. Island isolation is most often measured as distance to the nearest continent. However, isolation also can be a function of the islands immediately adjacent to the target island. Kalmar and Currie (2006) found that distance to nearest continent did have a negative relationship with insular avian richness but that these effects can be mitigated by the presence of neighboring islands. Similarly, Carvajal and Adler (2005) found that archipelagos in the South Pacific, which contained larger islands, possess higher mammal species richness than archipelagos made up of small islands.

Human impacts on island biodiversity have been well documented. These include historical colonization events (Cooke et al. 2017) and contemporary anthropogenic activities (Chown et al. 1998). Both situations commonly result in a loss of species diversity. Current human activities that threaten island biodiversity include hunting, land conversion, and the introduction of exotic species (Wiles and Brooke 2010; Valente et al. 2017; Turvey et al. 2017). Capture of animals for local consumption and trade often is uncontrolled on islands and can result in local extinctions (Riley 2002). The introductions of exotic species to islands have caused the loss of native island biota through direct predation, competition for resources, or spread of exotic diseases (Altizer et al. 2001; Wikelski et al. 2004). Although many native species have been impacted negatively by habitat loss and exotic introductions, some have benefited from these activities. It is likely that native frugivores in the Caribbean, including bats (i.e., *Artibeus* spp. and *Brachyphyla cavernarum*), benefited from the expansion of many fruit trees, such as mango, papaya, and banana, throughout the Caribbean Islands (García-Morales et al. 2013; Ávila-Gómez et al. 2015; Jung and Threlfell 2016).

Current climate conditions have a profound effect on species distributions and influence regional species diversity (Andrewartha and Birch 1954), however these variables (i.e., temperature and precipitation) are seldom used in studies of island biogeography. This is surprising because the physiological limits of species are determined by variables such as temperature and precipitation, which also can influence primary productivity on islands. In cases where these variables are included, a significant correlation often has been noted (Abbott 1974; Chown et al. 1998). Perhaps the most relevant findings to this study are those from Kalmar and Currie (2006) who analyzed global patterns of avian richness on islands. Being the only two groups of extant volant vertebrates, birds and bats likely have similar patterns of colonization on islands. They found that average annual precipitation and temperature on islands, along with area and distance from nearest continent, were significant predictor variables.

The chiropteran fauna of Caribbean islands has been the focus of several island biogeographical investigations. In an early study, Rickles and Lovette (1999) found that island area was correlated significantly with species richness of bats in the Lesser Antilles, whereas elevation and habitat diversity showed no significant relationship. Dávalos and Russell (2012) examined

the impact of modern island area and island area at the LGM on the richness of bat faunas. They concluded that bat species lost over the Holocene were the result of the loss of island area with rising sea levels. Willig and colleagues conducted two studies of island bat richness (Presley and Willig 2008; Willig et al. 2010) in the Bahamas, Greater Antilles, and Lesser Antilles. They found that inter-island distance was significant in predicting species richness in all three island groups and area was a significant predictor for the Greater and Lesser Antilles. They also noted that elevation was significant for the Greater and Lesser Antilles, but less so than area. Other investigations with a narrower focus have demonstrated the importance of island area and island elevation to the species richness of bat faunas in the Lesser Antilles (Genoways et al. 2001; Pedersen et al. 2018a). The studies discussed above share particular characteristics, including: no more than four variables were considered in any one study; each variable was considered individually for its impact on the bat species richness; and none considered the Outer Islands located on the continental shelf of North, Central, and South America. Further, since these findings were published several biological surveys have been conducted throughout the region providing updated species lists for several of the islands (e.g., Genoways et al. 2010, 2012; Kwiecinski et al. 2010, 2018; Larsen et al. 2012; Pedersen et al. 2013, 2018a, 2018b; Beck et al. 2016).

The study of insular species richness has progressed well beyond the traditional and simplistic variables of island area and isolation, yet few studies of island biogeography have attempted to include a large suite of predictor variables in their analysis. Chiropteran richness on Caribbean islands has been the focus of several investigations but have been limited in the number of predictor variables utilized. Further, recent surveys that resulted in updated species lists for many islands have not been included in any of the previous publications. The objective of this study is to determine which variable(s) best predict bat species richness for islands in the Caribbean Basin. This will be achieved by leveraging advanced GIS approaches and modern datasets to include a wide breadth of predictor variables and variable interactions, alongside the most contemporary list of bat species per island. These analyses provide unprecedented resolution to the biogeographic variables influencing bat faunas across all islands in the region.

METHODS

Study area.—The study area consists of islands in the Caribbean Basin, which has a complex geographical and geological history (Iturralde-Vinent and MacPhee 1999; Bachmann 2001). The basin contains the Caribbean Sea, which is differentiated from the North Atlantic Ocean on the northeast by the Bahamas Platform and along the east by the arc of the Lesser Antilles. Other boundaries of the basin are defined by the South American continent to the south and the mainland of Central America along the west. The Gulf of Mexico forms the northwestern boundary of the basin, being separated from the Caribbean Sea by a line running between the Yucatan and the Florida Keys (Nkemdirim 1997).

Much of the Caribbean Basin is underlain by the Caribbean Tectonic Plate, which is a small plate wedged between the North and South American plates. This plate had its origin during the Jurassic and Early Cretaceous and generally has moved in an eastward direction (Bachmann 2001; Giunta et al. 2006). The northern boundary of the Caribbean Plate is approximately at the northern edge of the Greater Antilles marked by a broad east-west strike-slip fault zone. To the south is a similar fault zone that approximates the north coast of South America. The western edge of the plate lies in the Pacific Ocean west of Central America and the eastern boundary of the plate lies in the Atlantic Ocean to the east of the Lesser Antillean arc. These last two boundaries are marked by: "Subduction and arc activity along the Lesser Antilles and Central America reflecting convergent interaction between the Caribbean Plate and the Atlantic and Pacific areas" (James 2005).

This study focused on multiple subsets of islands located in the region (Fig. 1). These consisted of the Lesser Antilles, Bahamas, Greater Antilles, and Outer Islands. The Lesser Antillean archipelagos are a volcanic Cenozoic arc, having formed west of where the Atlantic seafloor of the South American plate subducts

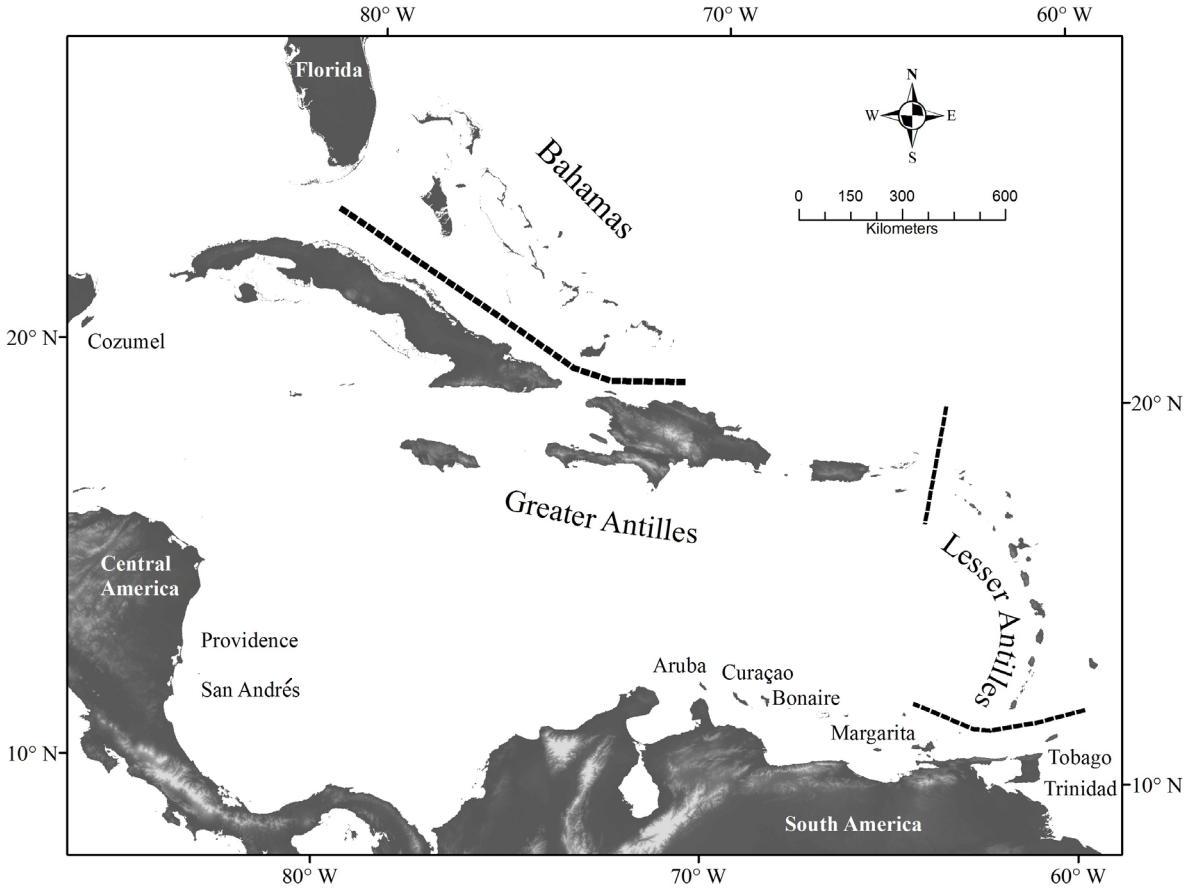

Figure 1. Map of islands in the Caribbean Basin. Dashed lines separate major island groups. Outer islands used in the analysis are individually labeled. Lighter shading indicates higher elevations.

under the Caribbean plate (Macdonald et al. 2000). These islands form an arc that extends from Anguilla in the north southward to Grenada, with most of these islands probably emerging in the Oligocene to early Miocene (Speed et al. 1993; Graham 2003a). The Lesser Antilles were never connected to a mainland, but some were connected to each other during the LGM. One island that often is grouped with the Lesser Antillean chain, but that has a separate geological origin, is Barbados. Barbados sits on an accretionary wedge of the eastern Caribbean plate boundary zone. As the South American plate moves under the Caribbean plate, the softer sedimentary materials of the Atlantic Ocean floor are scraped into the wedged-shaped Barbados Ridge, which surfaced about a million years ago (Speed and Keller 1993; Speed 1994). Genoways et al. (2011) contended that the chiropteran fauna of Barbados should be grouped with the Lesser Antillean fauna as the "Barbados subfauna."

The Bahamas (including the Turks and Caicos), which lie to the east of southern Florida and north of Cuba and Hispaniola, consist of 29 carbonate islands and 661 cays and shallow banks (Carew and Mylroie 1997). These islands are underlain by oceanic crust on which thick carbonate banks developed beginning in the Late Jurassic, including several platforms, such as the Bahama Platform, and other geological features. The Bahamas were never connected to North America during the Last Glacial Maximum and have since lost considerable landmass due to rising sea levels.

The Greater Antilles extends from Cuba in the west to the Virgin Islands in the east. These islands are associated with the strike-slip fault zone between the Caribbean Plate and the North American Plate, with the former moving to the east and the latter to the west. These islands have complex geological histories involving tectonic activity. Puerto Rico, Hispaniola,

and Jamaica are situated fully on the Caribbean Plate, whereas Cuba sits on both the Caribbean and North American plates. These islands have not been connected directly to the mainland at any time during their history (Potter 2004). The Virgin Islands all lie on the Puerto Rican bank except St. Croix and were connected to each other at the Last Glacial Maximum. Southeast of the northern Virgin Island Platform, St. Croix is a pinnacle of an east-trending submarine ridge, the St. Croix Platform (Kwiecinski and Coles 2007).

The last grouping of islands in our study was designated the "Outer Islands". All but one of these islands (Cozumel) are located on the continental shelf of South and Central America to which they were connected at the Last Glacial Maximum. They are located along the northern coast of South America from Trinidad and Tobago in the east to Aruba in the west and Cozumel lying just off the east coast of the Yucatan Peninsula of Mexico (Fig. 1). The other two islands in this group were San Andrés and Providencia, part of the country of Colombia, but located off the northeastern coast of Nicaragua in the western Caribbean. These two islands lie on the Nicaraguan Rise and are of volcanic origin (Pagnacco and Radelli 1962; Álvarez-Gutiérrez et al. 2014). During the height of Pleistocene glaciation, the lowered sea levels (~125 m) would have greatly reduced the distance between Central America and Jamaica by the emergence of the Nicaraguan Rise. At this point, it is unclear whether or not these two islands were ever directly attached to the mainland of Central America, but they would certainly have been much closer than at present (Hedges 1996; Graham 2003b). Previous studies of island biogeography in Caribbean bats (Presley and Willig 2008; Dávalos and Russell 2012) have excluded these outer islands from their analyses. The authors reasoned that the different bat biotas found on these islands, their proximity to the mainland, and differences in island origin may bias the results. It is true that certain characteristics of the outer islands are noticeably different than other islands in the Caribbean. However they are still islands, in the traditional sense, located within the Caribbean Basin. By including them in the analysis of all Caribbean islands and then analyzing them as a separate group, valuable information can be gathered on which variables are important in predicting bat richness throughout the region.

Data collection.—Bat species richness was determined through an extensive search of published literature and museum collections. Recently published species-occurrence matrices for the Caribbean Islands (Willig et al. 2010; Dávalos and Turvey 2012) were updated by including data from recent surveys and museum searches via VertNet (www.vertnet.org). Nomenclature largely followed Wilson and Reeder (2005) with the incorporation of recent taxonomic revisions (Kwiecinski et al. 2018; Pedersen et al. 2018a, 2018b; see also references in Appendix I).

For the purposes of this study, it was hypothesized that the number of bat species per island is dependent upon five general island characteristics—island area, island isolation, habitat diversity, human impact, and climate. For each category, data were collected on multiple predictor variables (Appendix II). Values published by Willig et al. (2010) were used for island area. In the cases of islands included in this study, but not in Willig et al. (2010), an island's area was obtained from various gazetteers and published sources. The change in island area since last glacial maximum (LGM) was determined by first downloading gridded bathymetric data of ocean depths (www.gebco.net) for the focus area. Next the gridded data was converted to contours at 5 m intervals. Assuming a 125 m drop in sea level during the LGM, the 125 m contour was isolated around each island and a polygon was created by tracing its border. Polygon area was calculated using the Zonal Statistics function in ArcGIS 9.3, divided into the current island's area, and subtracted from one. This provided an index of percent land lost since LGM.

Island isolation was measured in three ways. First, distance to mainland was recorded as the shortest distance (km) from an island to the nearest continent. Next, latitude and longitude were recorded from the middle point of each island. Finally, a nearest neighbor index was calculated to investigate the potential of neighboring islands as a source of colonization. This variable was obtained using equation 2 from Kalmar and Currie (2006) who hypothesized that the importance of neighboring islands as a source of colonization is proportional to its area (A) and inversely proportional to the distance (D) from the target island:

$$N = \sum \frac{A}{(D+1)^2}$$

The straight-line distance (km) from each island then was measured to all its adjacent islands. An island was determined adjacent if there were no other islands between it and the target island and if its distance was less than the nearest continent. If a target island had no island closer than a continent, a hypothetical neighbor was assigned at half the size of the smallest island in the data set and half the distance between the target island and the continent (Kalmar and Currie 2006).

Habitat diversity was characterized by collecting data on the highest elevation for an island, island volume, and island ruggedness. Elevation values reported in Willig et al. (2010) were used for most islands. For the islands included in this study, but not in Willig et al. (2010), highest elevation was determined using Google Earth (https://www.google.com/earth). Island volume and ruggedness allowed a higher level of detail in habitat diversity to be investigated on an island. The justification for including these variables is that island topography is extremely diverse across the Caribbean Islands. For instance, some islands are relatively flat (e.g., Bahamas), some possess a single peak often represented by a volcano, and others have multiple peaks and ridges. In general, the more changes there are in an island's topography the more variable the habitat should be. The volume of an island was determined by first importing a raster file of elevation from the WorldClim dataset (www.worldclim.org) into ArcGIS 9.3. The 3D Analyst tool was used to generate a triangular irregular network (TIN) file, which represents a 3D surface morphology of an island, and calculate the surface volume of each TIN above sea level. Island ruggedness was determined by finding the standard deviation of the slope of an islands elevation. The Spatial Analyst tool was used to calculate the slope of the elevation raster and then calculate its standard deviation. This measure of surface roughness has demonstrated favorable performance against other methods at multiple spatial scales (Grohmann et al. 2011).

The potential impact of human activities on bat species richness was assessed by determining the timing at which humans first colonized each island and the modern human population density on each island. There is gathering evidence that human occupation of islands in the Caribbean during the Holocene resulted in most recent extinction events for bats (Pregill et al. 1988; Soto-Centeno and Steadman 2015; Steadman et al. 2015). The earliest human occupation of each island was determined by examining the most recent archeological research for each island (Wilson 1989; Berman and Gnivecki 1995; Stokes and Keegan1995; Moure and Rivero de la Calle 1996; Drewett 2000; Davis and Oldfield 2003; Saunders 2005; Callaghan 2007; Steadman et al. 2007; Keegan et al. 2008, 2013; Davis 2011; Fitzpatrick 2011; Reid and Gilmore 2014; Cooke et al. 2016). There are notable shortcomings in these data—variation in intensity of archeological research, whether or not the earliest sites have been found on each island, variation in radiocarbon dating methods, and whether or not corrections have been made to the radiocarbon dates. However, it would be our hypothesis that the longer humans have occupied an island the more impact should be observed in the bat fauna. These impacts could be both negative (loss of habitat) and positive (introduction of fruit trees). Human population size on each island was taken from the official national websites. For many of the islands, this was sufficient, but for some nations that are composed of multiple islands (e.g., Bahamas), additional searching was required. These were found by searching the websites of the individual islands or island subgroups. Population numbers were taken from 2017 or 2018 when available, but for some of the smaller island nations the most recent population numbers were those from the last census in 2010. The raw population numbers for each island were divided by the island area to determine its population density.

Climate data for each island was downloaded from WorldClim Version2 (www.worldclim.org), which is a set of gridded global climate layers with a spatial resolution of 1 km^2 and uses temperature and rainfall averages from 1970 to 2000 (Fick and Hijmans 2017). The climate of each island was summarized by collecting data on average annual temperature and precipitation, along with the average temperature of the warmest and coldest month and the average precipitation of the wettest and driest month. These maps provide a gridded climate surface for each island. In order to capture the variation in climate of each island, five random points were generated within an island. Data for all six climate variables were collected at each point and averaged.

Data analysis.—A multi-model selection approach was used to investigate fluctuations in bat species richness across the islands of the Caribbean Basin

(Burnham and Anderson 2002). Model selection was applied to six combinations of islands—all islands, all islands minus the Outer Islands, Lesser Antilles, Greater Antilles, Bahamas, and Outer Islands. Several candidate models were constructed *a priori* with the number of bat species per island as the response variable. A Pearson correlation matrix was performed on all explanatory variables to test for multicollinearity before constructing the candidate models. Any variables with $r^2 > 0.7$ were considered highly correlated (Leathwick et al. 2005) and not included in the same model. The suite of candidate models included all single variable models and various combinations of predictor variables (Appendix III). There was some variation in the candidate models among the different island groups due to different variable correlations that arose when the data set was separated. A null model ("response = [1]") also was included, which predicts that the number of bat species per island are random with respect to all variables.

A generalized linear model (GLM) with a quasi-Poisson distribution and a "log" link function was used to fit the models to the data (Burnham and Anderson 2002). This method was chosen because it is an appropriate framework with which to fit alternative models with different combinations of covariates and compare their fit (Russell et al. 2004; Williams et al. 2009). To account for overdispersion, a common issue among count data, the quasi-Poisson link was used. The "log" link specifies how the log transformed mean of the response variable (species richness) relates to the linear predictors of the explanatory variable. A GLM assumes a linear relationship between the transformed response variable (via the link function) and explanatory variable. To determine if this assumption was violated, component-residual plots were generated for all variables in each island group. In two island groups (Caribbean Basin and Caribbean Basin minus outer islands), nonlinear relationships were found between species richness and the independent variables Area, Vol, and Rugg. Each of these variables were log transformed to achieve linearity. A quasi-Akaike Information Criteria with a correction for small sample sizes (QAICc) was used because of small sample sizes in island groups and because of the quasi-Poisson distribution. The model with the lowest QAICc value was considered best-approximating, and models with $\Delta QAIC < 2$ were significant and equally supported (Burnham and Anderson 2002). Akaike model weights (w) were included to represent the probability of best fit among all candidate models (Burnham and Anderson 2002). If more than one candidate model was equally supported, a model averaging procedure was performed to allow all significant models to be used for inference. Also reported are the average coefficient estimate, standard error, 95% confidence intervals, and relative importance (RI) of the parameters. Relative importance was determined for each variable by summing the model weights (w) for each model the variable was present. Values of RI range from 0 to 1 with higher values indicating higher importance in predicting species richness. All statistical analyses were performed using R (3.5.1) statistical software (R Development Core Team 2018). The R package "AICcmodavg" was used to implement the model selection and inference, whereas the package "car" was used to generate component residual plots.

RESULTS

Data were collected from 85 islands (28, Greater Antilles; 24, Lesser Antilles; 23, Bahamas; 9, Outer Islands). Species richness from all islands was 131 species that comprised nine families (Appendix I). The Outer Island Trinidad had the highest species richness (66) followed by Cuba (27) from the Greater Antilles. The highest species richness in the Lesser Antilles was equal on four islands (Dominica, St. Vincent, Grenada, and Guadeloupe), each with 12 species. Finally, species richness in the Bahamas was greatest on the islands of Great Exuma and Long Island (six species each).

The results showed variation among the best predictors of bat species richness on Caribbean islands. When all islands in the Caribbean Basin were included, one model best predicted ($\Delta QAICc < 2$) species richness (Table 1). Significant variables included island area (log(Area)) and timing of human colonization (YBP) (Table 2). Both variables had a significant and positive relationship with species richness suggesting that larger islands with early colonization by humans had more species of bats.

Table 1. Quasi-Akaike Information Criteria (QAICc) scores, differences in QAICc score between the i^{th} and top-ranked model (Δ QAICc), quasi-Akaike weights (w), and number of variables (k) for models predicting the number of bat species on various groups of Caribbean islands. Only models with Δ QAICc < 2.0 are reported.

Model variables	QAICc	Δ QAICc	*w*	k
Caribbean basin				
Area+YBP	227.93	0.00	86.00	4
Caribbean Basin (minus Outer Islands)				
Rugg	157.664	0.000	0.33	3
Vol	158.702	1.038	0.19	3
Rugg+Dist	158.725	1.061	0.19	4
Bahamas				
"1"	48.85	0.00	0.14	2
Rugg	50.00	1.15	0.08	3
Greater Antilles				
Area+Mint	69.41	0.00	0.48	4
Mint	69.54	0.13	0.45	3
Lesser Antilles				
Elev	60.83	0.00	0.22	3
Elev+YBP	62.46	1.63	0.10	4
Rugg+Maxt	62.61	1.78	0.09	4
Maxt	62.68	1.85	0.09	3
Annt	62.80	1.97	0.08	3
Outer Islands				
Rugg	40.14	0.00	0.40	3
Vol	41.26	1.12	0.23	3
Area	41.35	1.21	0.22	3

Table 2. Model average estimates of intercept and coefficients along with *P* values for the best fit models (Δ QAIC < 2) that predicts bat species richness for island groups in the Caribbean Basin.

	Estimate	SE	RI	*P*
Caribbean Basin				
(Intercept)	0.544	0.100		< 0.0001
Area	3.5×10^{-1}	4.1×10^{-4}	0.99	< 0.0001
YBP	3.5×10^{-4}	2.1×10^{-5}	0.99	< 0.0001
Caribbean Basin (minus Outer Islands)				
(Intercept)	0.081	0.660		< 0.0001
Rugg	4.9×10^{-1}	5.1×10^{-2}	0.52	< 0.0001
Vol	3.9×10^{-1}	2.8×10^{-2}	0.19	< 0.0001
Dist	-2.5×10^{-4}	1.9×10^{-4}	0.19	> 0.05
Bahamas				
(Intercept)	0.760	0.400	-	> 0.05
"1"	-	-	-	-
Rugg	4.9×10^{-4}	2.1×10^{-4}	-	0.061
Greater Antilles				
(Intercept)	8.670	1.240	-	< 0.0001
Mint	-0.040	0.010	0.93	< 0.0001
Area	5.3×10^{-6}	2.1×10^{-6}	0.48	0.014
Lesser Antilles				
(Intercept)	3.560	3.205	-	< 0.0001
Elev	6.7×10^{-4}	2.1×10^{-5}	0.32	< 0.0001
Maxt	-8.3×10^{-3}	8.3×10^{-3}	0.18	< 0.0001
YBP	8.98×10^{-5}	1.7×10^{-3}	0.10	0.117
Annt	-2.1×10^{-2}	1.6×10^{-4}	0.08	< 0.0001
Rugg	5.12×10^{-5}	1.2×10^{-3}	0.09	0.012
Outer Islands				
(Intercept)	0.600	0.910	-	< 0.0001
Rugg	-0.070	0.080	0.40	< 0.0001
Vol	-0.080	0.020	0.23	< 0.0001
Area	0.180	0.060	0.22	< 0.0001

When the Outer Islands were removed from the analysis, variable importance shifted considerably. Three models possessed ΔQAICc values of less than 2 (Table 1). The variables island ruggedness (log(Rugg)) and island volume (log(Vol)) had significant and positive relationships with species richness. The variable with the highest relative importance value (0.52) was log(Rugg) (Table 2). The other variable, distance from mainland (Dist), had a negative relationship with species richness; however, this relationship was not significant.

When island groups were analyzed separately, each group had different variables that best predicted bat species richness (Tables 1, 2). The Bahamas had two models that were equally supported (ΔQAICc < 2). Most notably among them was the Null ("1") model. This indicates that all of the candidate models used to predict bat species richness in the Bahama Islands did no better than chance. In the Greater Antilles, two models showed equal support. These two models consisted of two variables, island area (Area) and minimum temperature of the coldest month (Mint). Minimum temperature had the higher importance value (0.93) and a significant negative relationship with species richness. This suggests that Greater Antillean islands with cooler temperatures have higher species richness. Island area was also significant with a positive relationship to the number of species but a lower importance value (0.48) than minimum temperature. The Lesser Antilles had five equally supported models. Elevation and maximum temperature each occurred twice in the top models and had significant relationships. Elevation had the higher importance value (0.32) than maximum temperature (0.18). As in previous results, elevation was positively correlated with species richness and maximum temperature had a negative correlation. Other significant variables included annual temperature (Annt) and island ruggedness (Rugg). Annual temperature was negatively correlated and ruggedness was positively correlated with species richness. Both had low importance variables (0.08 and 0.09, respectively). Finally, the Outer Islands had three, single variable models which were equally supported. All three variables, island ruggedness (Rugg), volume (Vol), and area (Area), were significant and possessed positive relationships with species richness.

DISCUSSION

The Caribbean Basin possesses a wide range of islands with variable features (Appendix II). For instance, several orders of magnitude difference exist in island area (Cuba = 114,524 km^2; Grass Cay = 0.24 km^2). Islands of the Greater and Lesser Antilles range considerably in their elevation and climate conditions, whereas the Bahamas are mostly flat and arid. Finally, there is noticeable disparity in distance to the mainland, with many of the Bahamas and Greater Antilles located more than 800 km from the nearest continent. Comparatively, among the Outer Islands, none were found more than 125 km from the mainland.

Previous biogeographical studies of bats in the Caribbean have excluded these outer islands due to the potential confounding effects of continental proximity (Presley and Willig 2008; Willig et al. 2010; Dávalos and Russell 2012). One of the objectives of this study was to investigate this relationship by both including and excluding the Outer Islands from the analysis. The results herein confirm that the Outer Islands do influence the predictors of bat species richness. Island area (log(Area)) and timing of human colonization (YBP) were both significant and most important when the Outer Islands were included; however, once removed, island ruggedness log(Rugg) and island volume log(Vol) became most important. The importance of both log(Area) and YBP highlights an interaction related to the fundamental biogeographical processes that predicts that more species will be found on islands that are larger and/or located closer to the mainland (MacArthur and Wilson 1963, 1967). The inclusion of YBP as predictor variable was an attempt to capture some of the impact humans have had on the island ecosystems. However, it is logical to assume that patterns of colonization are similar for both bats and humans. Islands with the earliest colonization by humans either were close to the mainland or possessed large surface areas. These islands also possessed high numbers of bat species. For example, Trinidad was the first island to be colonized (8000 ybp) by humans, is only 24 km from the mainland, and has the highest

bat species richness (66). Whereas, Hispaniola is the third most remote island (933 km from mainland) but was colonized 6028 ybp by humans (4th earliest) and has the fifth most bat species (20).

When the Outer Islands were analyzed separately, the effect of human colonization was absent. These islands are located close to the mainland and most were colonized more than 2,100 years ago. When these effects were removed, the best predictors of bat species richness were island ruggedness (Rugg), volume (Vol), and area (Area). Similar results are seen in the remaining Caribbean Islands (minus the Outer Islands) where log(Rugg) and log(Vol) were most important. All these variables are highly correlated (Table 3) and can be considered indirect measures of habitat heterogeneity.

Among the Bahamas, the "Null" was the best predicting model, indicating that no variable(s) predicted bat species richness better than chance. Willig et al. (2010) found a similar result that species richness in the Bahamas was not significantly correlated to area, elevation, latitude, or hurricane disturbance. The Bahamas are a group of carbonate islands located on the Bahama platform that mostly consist of old coral cores around which sand has accumulated (Carew and Mylroie 1997). This has resulted in a group of relatively flat, dry islands (62.5 m maximum elevation; 93.7 cm average annual precipitation). The fossil record of bats from the Bahamas suggest that multiple dispersal events have been attempted and ultimately failed (Koopman et al. 1957; Buden 1986; Morgan 2001; Speer et al. 2015). These factors have produced a group of mostly depauperate islands, with the highest diversity (six species) on Great Exuma and Long Island. Presley and Willig (2008) noted that patterns of bat distribution in the Bahamas were idiosyncratic in regard to establishment of North American endemics and that the ranges of many species covered the entire region. Given the lack of habitat and species diversity, along with unpredictable establishment events in the Bahamas, the "Null" model is a logical result. Further,

Table 3. Pearson correlation values between island area and all other model variables among the different island groups. Asterisk (*) indicates all islands of the Caribbean Basin minus the Outer Islands.

Variable	Caribbean Basin	Caribbean Basin*	Bahamas	Greater Antilles	Lesser Antilles	Outer Islands
LGM	0.01	0.11	0.17	0.20	-0.41	0.29
Elev	0.60	0.61	0.12	0.76	0.71	0.66
Dist	-0.06	-0.10	-0.49	-0.46	-0.05	-0.45
Vol	0.80	0.80	0.95	0.79	0.93	0.99
NNI	0.11	0.11	0.08	0.06	-0.13	-0.13
Pop	-0.01	0.04	-0.12	0.09	0.15	-0.21
Rugg	0.84	0.83	0.86	0.83	0.90	0.97
YBP	0.37	0.45	-0.09	0.65	0.31	0.69
Lat	0.10	0.09	0.36	0.51	-0.03	-0.27
Long	-0.19	-0.22	-0.51	-0.34	0.33	0.45
Annt	-0.16	-0.15	-0.47	-0.31	-0.41	-0.30
Maxt	0.18	0.19	0.04	0.32	-0.38	-0.08
Mint	-0.35	-0.37	-0.51	-0.61	-0.45	-0.37
Pann	0.04	0.04	0.47	0.25	0.53	0.18
Pmax	0.15	0.19	0.33	0.46	0.48	0.05
Pmin	-0.04	-0.06	0.25	-0.14	0.51	0.48

the Bahamas are the most active hurricane region in the western Atlantic. The Caribbean Hurricane Network (2018) identified Abaco as the "Hurricane Capital of the Caribbean" because it has had more severe hurricanes (categories 3–5) in the period 1851 to 2010 (18 total) than any other island in the region. There are also five more of the islands in the top 25 of this list—Grand Bahama, 2nd ; New Providence, 7th ; San Salvador, 13th; South Caicos, 21st; and Grand Turk, 24th. Clearly, this is an unstable environment that has made it difficult for bats to establish reproductive populations.

In the Greater Antilles, the interaction of two variables, minimum temperature of the coldest month (Mint) and island area (Area), proved most important in predicting bat species richness. Minimum temperature had a negative relationship and Area a positive relationship with species richness. We suspect that the significance of these two variables is the result of the correlative interactions of area, temperature, and elevation. In the Greater Antilles, area and elevation are positively and significantly correlated (Table 3), whereas elevation and temperature possess a significantly negative relationship (Table 4). Overall temperature will decrease at higher elevations that are found on the larger islands in the Greater Antilles. Our data show that on the larger Greater Antillean islands (> 500 km^2), the average Mint is lower (16.7° C) than on islands <500 km^2 (20.7° C). Willig et al. (2008) noted that area, elevation, and latitude had significant effects on variation in bat species richness among the Greater Antilles. They found that area alone explained most of the variation followed by elevation and then latitude. Area was one of the important variables herein but less so than Mint. This could be due to the fact that Willig et al. (2008) used the log(Area) whereas the study reported here did not transform area for this island group (see Methods). Species richness was found to have a significant relationship with elevation ($\beta = 6.6 \times 10^{-4}$, $P < 0.0001$) and latitude ($\beta = 0.33$, $P < 0.0001$), although

Table 4. Pearson correlation values between island maximum elevation and all other model variables among the different island groups. Asterisk (*) indicates all islands of the Caribbean Basin minus the Outer Islands.

Variable	Caribbean Basin	Caribbean Basin*	Bahamas	Greater Antilles	Lesser Antilles	Outer Islands
Area	0.60	0.61	0.12	0.76	0.71	0.66
LGM	0.04	-0.14	-0.09	0.05	-0.69	0.63
Dist	-0.06	-0.07	-0.30	-0.20	0.04	-0.26
Vol	0.67	0.68	-0.12	0.85	0.80	0.72
NNI	0.08	0.08	-0.07	0.02	-0.09	0.25
Pop	0.13	0.38	-0.08	0.37	0.08	-0.30
Rugg	0.70	0.71	-0.12	0.88	0.84	0.80
YBP	0.55	0.59	-0.11	0.63	0.23	0.68
Lat	-0.28	-0.30	0.43	0.17	0.08	-0.63
Long	0.20	0.16	-0.27	-0.26	-0.03	0.75
Annt	-0.59	-0.64	-0.43	-0.75	-0.77	-0.21
Maxt	-0.48	-0.49	0.06	-0.18	-0.76	-0.21
Mint	-0.47	-0.52	-0.49	-0.81	-0.73	-0.21
Pann	0.45	0.52	0.44	0.50	0.58	0.14
Pmax	0.45	0.56	0.26	0.66	0.52	0.01
Pmin	0.50	0.53	0.68	0.35	0.72	0.41

neither predicted richness as well as the interaction of Area and Mint.

The Lesser Antilles arguably have seen the most attention with regards to bat research in the Caribbean. This includes large scale studies of island biogeography (Ricklefs and Lovette 1999; Morand 2000; Presley and Willig 2008; Willig et al. 2010; Dávalos and Russell 2012) as well as individual island surveys (e.g., Pedersen et al. 1996, 2003, 2005, 2006, 2007, 2013, 2018a, 2018b; Genoways et al. 2010, 2012; Kwiecinski et al. 2010, 2018; Lindsay et al. 2010; Larsen et al. 2012). Five models provided equal support for predicting bat species richness in the Lesser Antilles (Table 3), with four variables having a significant relationship (Table 4). Elevation (Elev) had the highest importance value of all significant variables, followed by temperature (Maxt and Annt) and island ruggedness (Rugg). Similar to the Greater Antilles, elevation has a relationship with temperature that is significant and negative (Table 4). Island ruggedness (Rugg), which was one of the measures of habitat diversity, also was highly correlated to both elevation ($r = 0.84$) and Area ($r = 0.90$).

Previous biogeographic studies of Lesser Antillean bats have produced varied results. Ricklefs and Lovette (1999) found significant correlations between bat richness and island area, but not for elevation and habitat diversity. Morand (2000) provided a complimentary study to Ricklefs and Lovette (1999) by conducting the same analysis with an additional variable of inter-island distances. He concluded that geographic distances between islands, along with area, was significant in explaining patterns of bat species richness. In contrast, Willig et al. (2010) determined that variation in bat species richness was significantly explained by island area and elevation, but that inter-island distance had no effect. This disparity was attributed to the improved distributional data set that was available to them but not Morand (2000). More recently, Pedersen et al. (2018b) produced species-area and species-elevation curves for the Lesser Antilles using the most current data on bat distributions. They found that both area and elevation were significant, but that area explained the most variation for the full set of Lesser Antillean islands. When they removed islands that had elevations <250 m ($n = 4$) and reran the analysis, island elevation explained more variation than area. The most notable difference between the results reported herein and others is the absence of island area as an important predictor variable. As mentioned above, this discrepancy is most likely the result of previous authors using log(Area) for the Lesser Antilles whereas this study used raw values of island area. Aside from that, the findings herein generally support those of others in that elevation and other proxies of habitat diversity (climate and ruggedness) are effective predictors of bat species richness.

In addition to current island area, the area of an island during LGM has been noted as a significant predictor of bat species richness (Dávalos and Russell 2012). That study used estimates of island size and richness during LGM and compared those to current conditions. They found that the change in island size explained levels of species loss in the Bahamas and Greater Antilles but not for the Lesser Antilles. Pedersen et al. (2018b) noted that the exclusion of data from recent surveys (see paragraph above) and paleontological studies (Stoetzel et al. 2016; Royer et al. 2017) in the Lesser Antilles could have affected their results.

The current study used percent of land lost since LGM to predict current species richness. Although LGM was not among the best predictors for any of the island groups, a significant relationship was detected between LGM and species richness, with the exception of the Bahamas (Caribbean Basin $\beta = -1.21$, $P < 0.0001$; Caribbean Basin (minus Outer Islands) $\beta = -1.81$, $P < 0.0001$; Bahamas $\beta = -0.418$, $P = 0.529$; Greater Antilles $\beta = -2.31$, $P < 0.0001$; Lesser Antilles $\beta = -1.041$, $P < 0.0001$; Outer Islands $\beta = 1.15$, $P = 0.0002$). Most islands have a negative relationship between LGM and species richness, indicating that fewer species were found on islands with a greater percentage of land lost. For example, in the Lesser Antilles many of the smaller islands were connected during the LGM (Fig. 2), whereas the larger islands with more species (i.e., Dominica, St. Lucia, St. Vincent) were not. Thus, the smaller islands experienced a much greater degree of area loss than larger islands. An exception to this trend was the Outer Islands, which possessed a positive relationship with LGM. This is due to high diversity islands (Trinidad, Tobago, Margarita) being connected to South America during LGM and less species rich islands (Aruba, Bonaire, Curaçao) remained isolated.

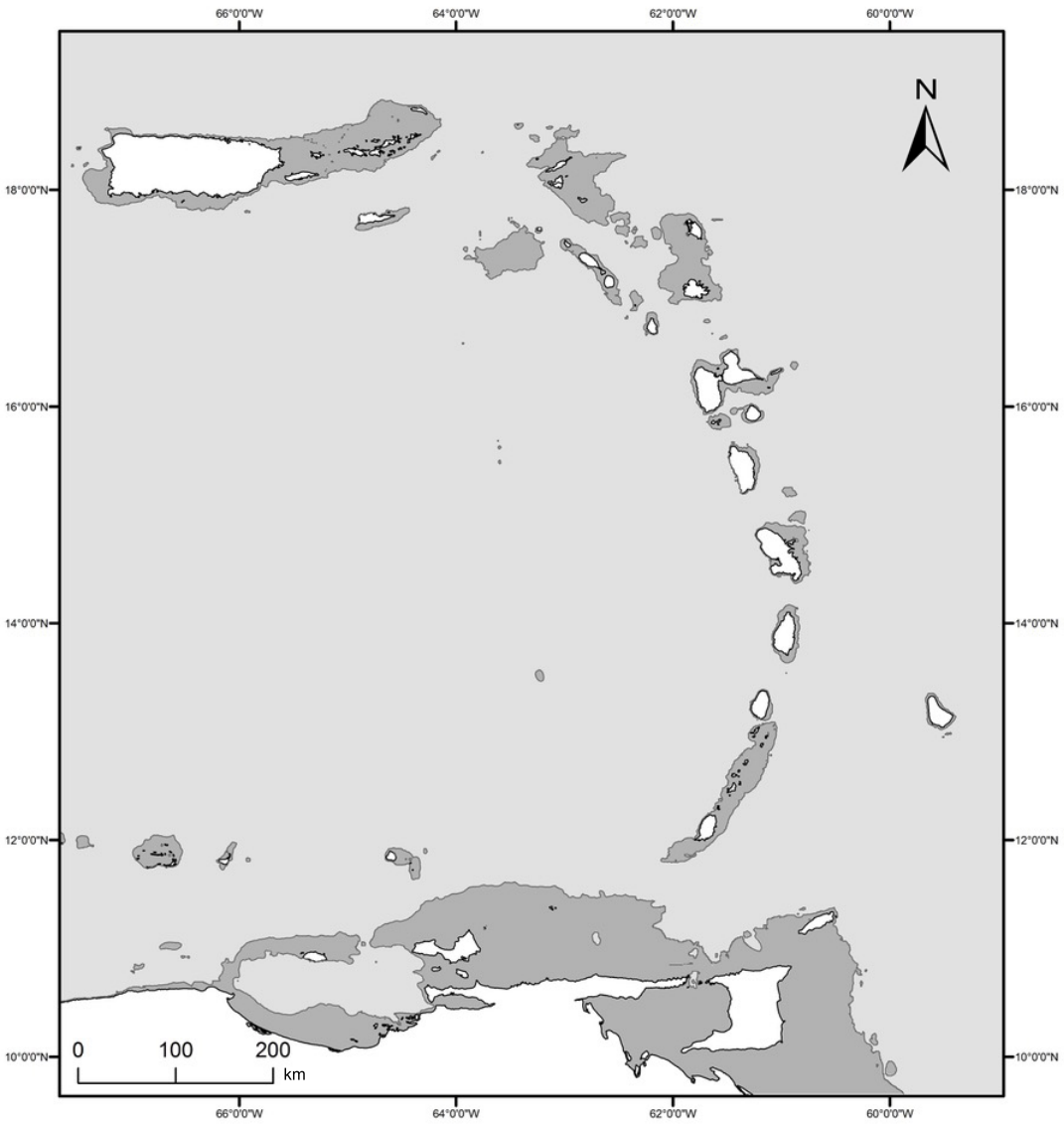

Figure 2. Bathymetric map of the Lesser Antilles showing the extent of exposed land during the last glacial maximum. Derived from Larsen et al. (2017).

The objective of this study was to determine which variable(s) best predicted bat species richness for islands in the Caribbean Basin. Although several variables had significant relationships with richness, those which corresponded to measures of island area, habitat diversity, and climate provided the best prediction of bat species richness. Habitat diversity on islands is often the results of island area and variation in elevation with its corresponding gradients of temperature and precipitation (Willig et al. 2010; Ricklefs and Lovette 1999). Along with elevation and climate, other estimates of habitat diversity (island ruggedness and volume) were shown to have high relative importance in predicting species richness. Recent studies have noted the importance of these variables, with many arguing they should be combined into a single framework when predicting species richness (Davidar et al. 2001; Carvajal and Adler 2005; Triantis et al. 2005; Panitsa et al. 2006; Báldi 2008; Kallimanis et al. 2008; Frick et al. 2008). The results provided in this

study support those arguments and suggest that future studies should be more inclusive of predictor variables for species richness, especially those that pertain to climate and habitat heterogeneity.

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LITERATURE CITED

Abbott, I. 1974. Numbers of plant, insect, and land bird species on nineteen remote islands in the Southern hemisphere. Biological Journal of the Linnean Society 6:143–152.

Alexander, I. R., and K. Geluso. 2013. Bats of Vieques National Wildlife Refuge, Puerto Rico, Greater Antilles. Check List 9:294–297.

Altizer, S., J. Foufopoulos, and A. Gager. 2001. Conservation and diseases. Pages 109–126 in S. Levin, editor. Encyclopedia of biodiversity. Academic Press, San Diego, California, USA.

Álvarez-Gutiérrez, Y., et al. 2014. Geological description and interpretation of Providencia and Santa Catalina islands. Boletín de Ciencias de la Tierra 35:67–81.

Andrewartha, H. G., and L. C. Birch. 1954. The distribution and abundance of animals. The Univeristy of Chicago Press, Chicago, Illinois.

Avila-Flores, R., J. J. Flores-Martínez, and J. Ortega. 2002. *Nyctinomops laticaudatus*. Mammalian Species 697:1–6.

Bachmann, R. 2001. The Caribbean Plate and the question of its formation. Institute of Geology, Department of Tectonophysics, Freiberg University of Mining and Technology, Freiberg, Germany.

Baker, R. J., and H. H. Genoways. 1978. Zoogeography of Antillean bats. Pp. 53–97 in Zoogeography in the Caribbean: The 1975 Leidy Medal Symposium (F. B. Gill, ed.). Special Publication of the Academy of Natural Sciences of Philadelphia 13:iii + 1–128.

Baker, R. J., H. H. Genoways, and J. C. Patton. 1978. Bats of Guadeloupe. Occasional Papers, Museum of Texas Tech University 50:1–16.

Báldi, A. 2008. Habitat heterogeneity overrides the species–area relationship. Journal of Biogeography 35:675–681.

Beck, J. D., A. D. Loftis, J. L. Daly, W. K. Reeves, and M. V. Orlova. 2016. First record of *Chiroderma improvisum* Baker & Genoways, 1976 (Chiroptera: Phyllostomidae) from Saint Kitts, Lesser Antilles. Check List, the Journal of Biodiversity Data, Volume 12, No. 2.

Bekker, J. P. 1996. The mammals of Aruba. Nijmegen: Vereniging voor Zoogdierkunde en Zoogdierbescherming.

Berman, M. J., and P. L. Gnivecki. 1995. The colonization of the Bahama Archipelago: a reappraisal. World Archaeology 26:421–441.

Borroto-Páez, R., and C. A. Mancina. 2011. Mamíferos en Cuba. Spartacus Foundation and la Sociedad Cubana de Zoología, Vasa, Finland.

Borroto-Páez, R., and C. A. Mancina. 2017. Biodiversity and conservation of Cuban mammals: past, present, and invasive species. Journal of Mammalogy 98:964–985.

Breuil, M., and D. Masson. 1991. Quelques remarques sur la biogéograhie des chauves-souris des Petites Antilles. Compte Rendu de la Société de Biogéographie 67:25–39.

Brown, J. H. 1971. Mammals on mountaintops: nonequilibrium insular biogeography. The American Naturalist 105:467–478.

Brown, R. J., et al. 1973. Animal disease survey of Navassa Island, West Indies. Naval Aerospace Medical Research Laboratory, Pensacola, Florida, Special Report 73-3:v + 1–28.

Buden, D. W. 1974. Prey remains of Barn Owls in the southern Bahama Islands. Wilson Bulletin 86:336–343.

Buden, D. W. 1975. *Monophyllus redmani* Leach (Chiroptera) from the Bahamas, with notes on variation in the species. Journal of Mammalogy 56:369–377.

Buden, D. W. 1977. First records of bats of the genus *Brachyphylla* from the Caicos Islands, with notes on geographic variation. Journal of Mammalogy 58:221–225.

Buden, D. W. 1986. Distribution of mammals of the Bahamas. Florida Field Naturalist 14:53–84.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference. 2nd ed. Springer-Verlag Inc., New York.

Callaghan, R. T. 2007. Prehistoric settlement patterns on St, Vincent, West Indies. Caribbean Journal of Science 43:11–22.

Carew, J. L., and J. E. Mylroie. 1995. Quaternary tectonic stability of the Bahamian archipelago: evidence from fossil coral reefs and ank margin caves. Quaternary Science Reviews 14:145–153.

Carew, J. L., and J. E. Mylroie. 1997. Geology of the Bahamas. Pp. 91–139 in Geology and hydrogeology of carbonate islands (H. L. Vacher and T. Quinn, eds.). Elsevier Science, New York.

Caribbean Hurricane Network. 2018. Hits & misses. (https://stormcarib.com/climatology/freq.htm) Accessed 14 December.

Carter, C. H., H. H. Genoways, R. S. Loregnard, and R. J. Baker. 1981. Observations on bats from Trinidad, with a checklist of species occurring on the island. Occasional Papers, Museum of Texas Tech University 72:1–27.

Carvajal, A., and G. H. Adler. 2005. Biogeography of mammals on tropical Pacific islands. Journal of Biogeography 32:1561–1569.

Chown, S. L., N. J. M. Gremmen, and K. J. Gaston. 1998. Ecological biogeography of southern ocean islands: species-area relationships, human impacts, and conservation. The American Naturalist 152:562–575.

Clark, P. U., et al. 2009. The Last Glacial Maximum. Science 325:710–714.

Clark, P. U., and A. C. Mix. 2002. Ice sheets and sea level of the Last Glacial Maximum. Quaternary Science Reviews 21:1–7.

Clarke, F. M., and P. A. Racey. 2003. Discovery of the Bartica bat *Glyphonycteris daviesi* (Chiroptera: Phyllostomidae) in Trinidad, West Indies. Acta Chiropterologica 5:151–160.

Cooke, S. B., L. M. Dávalos, A. M. Mychajliw, S. T. Turvey, and N. S. Upham. 2017. Anthropogenic extinction dominates Holocene declines of West Indian mammals**.** Annual Review of Ecology, Evolution, and Systematics 48:301–327.

Curtis, J. H., M. Brenner, and D. A. Hodell. 2001. Climate change in the circum-Caribbean (late Pleistocene to present) and implications for regional biogeography. Pp. 35–54 in Biogeography of the West Indies: patterns and perspectives (C. A. Woods and F. E. Sergile, eds.). CRC Press, Boca Raton, Florida.

Darwin, C. R. 1859. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. John Murray, London.

Dávalos, L. M. 2006. Short-faced bats (Phyllostomidae: Stenodermatina): a Caribbean radiation of strict frugivores. Journal of Biogeography 32:364–375.

Dávalos, L. M., and A. L. Russell. 2012. Deglaciation explains bat extinction in the Caribbean. Ecology and Evolution 12:3045–3051.

Dávalos, L. M., and S. Turvey. 2012. West Indian mammals: the old, the new, and the recently extinct. Pp. 157–202 in Bones, clones, and biomes: an extended history of recent neotropical mammals (B. D. Patterson and L. P. Acosta, eds.). University of Chicago Press, Chicago, Illinois.

Davidar, P., K. Yoganand, and T. Ganesh. 2001. Distribution of forest birds in the Andaman Islands: importance of key habitats. Journal of Biogeography 28:663–672.

Davies, A. B., and G. P. Asner. 2014. Advances in animal ecology from 3D-LiDAR ecosystem mapping. Trends in Ecology and Evolution 29:681–691.

Davis, D. D. 2011. Out of the shadows: a review of the archaeological evidence for the isolation, interaction, and abandonment of the British Virgin Islands in the pre-Columbian Caribbean. Unpublished Master of Arts thesis, Archaeology and Heritage, School of Archaeology and Ancient History, University of Leicester, England, UK.

Davis, D., and K. Oldfield. 2003. Archaeological reconnaissance of Anegada, British Virgin Islands. Journal of Caribbean Archaeology 4:1–11.

Drewett, P. L. 2000. Prehistoric settlements in the Caribbean: fieldwork in Barbados, Tortola, and the Cayman Islands. Barbados Museum and Historical Society, St. Ann's Garrison, Barbados, x + 174.

Engstrom, M. D., C. A. Schmidt, J. C. Morales, and R. C. Dowler. 1989. Records of mammals from Isla Cozumel, Quintana Roo, Mexico. The Southwestern Naturalist 34:413–415.

Eschelman, R. E., and G. S. Morgan. 1985. Tobagan Recent mammals, fossil vertebrates, and their zoogeographical implications. National Geographic Society Research Reports 21:137–143.

Fick, S. E., and R. J. Hijmans. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37:4302–4315.

Fitzpatrick, S. M. 2011. New radiocarbon dates for the Grenadine islands (West Indies). Radiocarbon 53:451–460.

Flaspohler, D. J., et al. 2010. Long-term effects of fragmentation and fragment properties on bird species richness in Hawaiian forests. Biological Conservation 143:280–288.

Frick, W. F., J. P. Hayes, and P. A. Heady III. 2008. Island biogeography of bats in Baja California, Mexico: patterns of bat species richness in a near-shore archipelago. Journal of Biogeography 35:353–364.

Gannon, M. R., A. Kurta, A. Rodríguez-Durán, and M. R. Willig. 2005. Bats of Puerto Rico. Texas Tech University Press, Lubbock.

García-Morales, R., W. I. Badano, and C. E. Moreno. 2013. Responses of neotropical bats assemblages to human land use. Conservation Biology 27:1096–1106.

Gardner, A. L. (ed.). 2007. Order Chiroptera Blumenbach, 1779. Pp. 187–484 in Mammals of South America, volume 1: marsupials, xenarthrans, shrews, and bats. University of Chicago Press, Chicago, Illinois.

Gehrels, R. 2010. Sea-level changes since the Last Glacial Maximum: an appraisal of the IPCC Fourth Assessment Report. Journal of Quaternary Science 25:26–38.

Geluso, K., M. J. Harner, C. A. Lemen, and P. W. Freeman. 2009. A survey of bats in northern Trinidad late in the rainy season. Occasional Papers, Museum of Texas Tech University 285:1–13.

Genoways, H. H., R. J. Baker, J. W. Bickham, and C. J. Phillips. 2005. Bats of Jamaica. Special Publications, Museum of Texas Tech University 48:1–154.

Genoways, H. H., et al. 2010. Bats of the Grenadine Islands, West Indies, and placement of Koopman's Line. Chiroptera Neotropical 16:501–521.

Genoways, H. H., P. A. Larsen, S. C. Pedersen, and J. J. Huebschman. 2007a. Bats of Saba, Netherlands Antilles. Acta Chiropterologica 9:91–114.

Genoways, H. H., R. J. Larsen, S. C. Pedersen, G. G. Kwiecinski, and P. A. Larsen. 2011. Bats of Barbados. Chiroptera Neotropical 17:1029–1054.

Genoways, H. H., S. C. Pedersen, P. A. Larsen, G. G. Kwiecinski, and J. J. Huebschman. 2007b. Bats of Saint Martin, French West Indies/Sint Maarten, Netherlands Antilles. Mastozoologia Neotropical 14:169–188.

Genoways H. H., S. C. Pedersen, C. J. Phillips, and L. K. Gordon. 2007c. Bats of Anguilla, northern Lesser Antilles. Occasional Papers, Museum of Texas Tech University 270:1–12.

Genoways, H. H., C. J. Phillips, and R. J. Baker. 1998. Bats of the Antillean island of Grenada: a new zoogeographic perspective. Occasional Papers, Museum of Texas Tech University 177:1–32.

Genoways, H. H., R. M. Timm, R. J. Baker, C. J. Phillips, and D. A. Schlitter. 2001. Bats of the West Indian island of Dominica: natural history, areography, and trophic structure. Special Publication, Museum of Texas Tech University 43:1–44.

Genoways, H. H., and S. L. Williams. 1979. Notes on bats (Mammalia: Chiroptera) from Bonaire and Curaçao, Dutch West Indies. Annals of the Carnegie Museum 48:311–321.

Giunta, G., L. Beccalua, and F. Siena. 2006. Caribbean Plate margin evolution: constraints and current problems. Geologica Acta 4:265–277.

Goodwin, G. G., and A. M. Greenhall. 1961. A review of the bats of Trinidad and Tobago. Bulletin of the American Museum of Natural History 122:187–302.

Graham, A. 2003a. Geohistory models and Cenozoic paleoenvironments of the Caribbean region. Systematic Botany 28:378–386.

Graham, A. 2003b. Historical phytogeography of the Greater Antilles. Brittonia 55:357–383.

Gregorin, R. 2009. Phylogeny of *Eumops* (Chiroptera: Molossidae) using morphological data. Acta Chiropterologica 11:247–258.

Griffiths, T. A., and D. Klingener. 1988. On the distribution of Greater Antillean bats. Biotropica 20:240–251.

Grohmann, C. H., M. J. Smith, and C. Riccomini. 2011. Multiscale analysis of topographic surface roughness in the Midland Valley, Scotland. IEEE Transactions on Geoscience and Remote Sensing 49:1200–1213.

Haq, B. U., J. Hardenbol, and P. R. Vail. 1993. Chronology of fluctuating sea levels since the Triassic. Science 235:1156–1167.

Hearty, P. J. 1998. The geology of Eleuthera Island, Bahamas: a Rosetta Stone of Quaternary stratigraphy and sea-level history. Quaternary Science Reviews 17:333–355.

Hedges, S. B. 1996. Historical biogeography of West Indian vertebrates. Annual Review of Ecology and Systematics 27:163–196.

Hummelinck, P. W. 1943. Zoögeografische opmerkingen over de Nederlandsche Benedenwindsche Eilanden. Pp. 168–180 in De West-Indische Gids, Royal Netherlands Institute of Southeast Asian and Caribbean Studies, Brill, Leiden, Netherlands.

Husson, A. M. 1960. Mammals of the Netherlands Antilles. Natuurwetenschappelijke Wekgroep Nederlandse Antillen, Curaçao 12: viii + 1–170.

Iturralde-Vinent, M. A., and R. D. E. MacPhee. 1999. Paleogeography of the Caribbean region: implications for Cenozoic biogeography. Bulletin of the American Museum of Natural History 238:1–95.

James, K. H. 2005. A simple synthesis of Caribbean geology. Caribbean Journal of Earth Science 39:69–82.

Jones, J. K., Jr. 1989. Distribution and systematics of bats in the Lesser Antilles. Pp. 645–660 in Biogeography of the West Indies: past, present, and future (C. A. Woods, ed.). Sandhill Crane Press, Inc., Gainesville, Florida.

Jones, J. K., Jr., J. D. Smith, and H. H. Genoways. 1973. Annotated checklist of mammals of the Yucatan Peninsula, Mexico. I. Chiroptera. Occasional Papers, Museum of Texas Tech University 13:1–31.

Jones, J. K., Jr., and C. J. Phillips. 1970. Comments on the systematics and zoogeography of the bats in the Lesser Antilles. Studies on the Fauna of Curaçao and Other Caribbean Islands 32:131–145.

Jung, K., and C. G. Threlfell. 2016. Urbanisation and its effects on bats—a global meta-analysis. Pp. 13–33 in Bats in the Anthropocene: conservation of bats in a changing world (C. C. Voigt and T. Kingston, eds.). Springer Open, Springer International Publishing, Switzerland.

Kallimanis, A. S., A. D. Mazaris, J. Tzanopoulos, J. M. Halley, J. D. Pantis, and S. P. Sgardelis. 2008. How does habitat diversity affect the species–area relationship? Global Ecology and Biogeography 17:532–538.

Kalmar, A., and D. J. Currie. 2006. A global model of island biogeography. Global Ecology and Biogeography 15:72–81.

Keegan, W. F., S. M. Fitzpatrick, K. S. Sealey, M. J. LeFebvre, and P. T. Sinelli. 2008. The role of small islands in marine subsistence strategies: case studies from the Caribbean. Human Ecology 36:635–654.

Keegan, W. F., C. L. Hofman, and R. Rodríguez Ramos. 2013. The Oxford handbook of Caribbean archaeology. New York: Oxford University Press.

Klingener, D., H. H. Genoways, and R. J. Baker. 1978. Bats of southern Haiti. Annals of Carnegie Museum 47:81–99.

Koopman, K. F. 1955. A new subspecies of *Chilonycteris* from the West Indies and a discussion of mammals of La Gonâve. Journal of Mammalogy 36:109–113.

Koopman, K. F. 1958. Land bridges and ecology in bat distribution on islands off the northern coast of South America. Evolution 12:429–439.

Koopman, K. F. 1959. The zoogeographical limits of the West Indies. Journal of Mammalogy 40:236–240.

Koopman, K. F. 1968. Taxonomic and distributional notes on Lesser Antillean bats. American Museum Novitiates 2333:1–13.

Koopman, K. F. 1975. Bats of the Virgin Islands in relation to those of the Greater and Lesser Antilles. American Museum Novitiates 2581:1–7.

Koopman, K. F., M. K. Hecht, and E. Ledecky-Janecek. 1957. Notes on mammals of the Bahamas with special reference to the bats. Journal of Mammalogy 38:164–174.

Kwiecinski, G. G., J.-P. Bacle, K. C. Lindsay, and H. H. Genoways. 2010. New records of bats from the British Virgin Islands. Caribbean Journal of Science 46:64–70.

Kwiecinski, G. G., and W. C. Coles. 2007. Presence of *Stenoderma rufum* beyond the Puerto Rican Bank. Occasional Papers, Museum of Texas Tech University 266:1–9.

Kwiecinski, G. G., et al. 2018. Bats of Saint Vincent, Lesser Antilles. Special Publications, Museum of Texas Tech University 68:1–68.

Larsen, R. J., et al. 2012. Evolutionary history of Caribbean species of *Myotis*, with evidence of a third Lesser Antillean endemic. Mammalian Biology 77:124–134.

Larsen, R. J., et al. 2017. Patterns of morphological and molecular evolution in the Antillean tree bat, *Ardops nichollsi* (Chiroptera: Phyllostomidae). Occasional Papers, Museum of Texas Tech University 345:1–28.

Larsen, P. A., H. H. Genoways, and S. C. Pedersen. 2006. New records of bats from Saint Barthélemy, French West Indies. Mammalia 70:321–325.

Leathwick, J. R., D. Rowe, J. Richardson, J. Elith, and T. Hastie. 2005. Using multivariate adaptive regression splines to predict the distributions of New Zealand's freshwater diadromous fish. Freshwater Biology 50:2034–2052.

Lindsay, K. C., G. G. Kwiecinski, S. C. Pedersen, J.-P. Bacle, and H. H. Genoways. 2010. First record of *Ardops nichollsi* from Antigua, Lesser Antilles. Mammalia 74:93–95

MacArthur, R. H., and E. O. Wilson. 1963. An equilibrium theory of insular zoogeography. Evolution 17:373–387.

MacArthur, R. H., and E. O. Wilson. 1967. The theory of island biogeography. Princeton University Press, Princeton, New Jersey.

Macdonald, R., C. J. Hawkesworth, and E. Heath. 2000. The Lesser Antilles volcanic chain: a study in arc magmatism. Earth-Science Reviews 49:1–76.

Mancina, C. A. 2009. *Phyllonycteris poeyi* (Chiroptera: Phyllostomidae). Mammalian Species 42:41–48.

Mantilla-Meluk, H., and J. Muñoz-Garay. 2014. Biogeography and taxonomic status of *Myotis keaysi pilosatibialis* LaVal 1973 (Chiroptera: Vespertilionidae). Zootaxa 3793:60–70.

McFarlane, D. A., R. D. MacPhee, and D. C. Ford. 1998. Body size variability and a Sangamonian extinction model for Amblyrhiza, a West Indian megafaunal rodent. Quaternary Research 50:80–89.

Monatelli, R., D. E. Wilson, R. L. Novaes, K. M. Helgren, and E. E. Gutiérrez. 2017. Caribbean *Myotis* (Chiroptera, Vespertilionidae), with description of a new species from Trinidad and Tobago. Journal of Mammalogy 98:994–1008.

Morand, S. 2000. Geographic distance and the role of island area and habitat diversity in the species–area relationships of four Lesser Antillean faunal groups: a complementary note to Ricklefs & Lovette. Journal of Animal Ecology 69:1117–1119.

Morgan, G. S. 1994. Mammals of the Cayman islands. Pp. 435–464 in The Cayman Islands: natural history and biogeography (M. A. Brunt and J. E. Davies, eds.). Kluwer Academic, Boston.

Morgan, G. S. 2001. Patterns of extinction in West Indian bats. Pp. 369–408 in Biogeography of the West Indies: patterns and perspectives (C. A. Woods and F. E. Sergile, eds.). CRC Press, Boca Raton, Florida.

Moure, R. D., and M. Rivero de la Calle. 1996. Art and archaeology of pre-Columbian Cuba. University of Pittsburgh Press, Pittsburgh [translated by D. H. Sandweiss and D. R. Watters].

Murray, K. L., E. Fraser, C. Davy, T. H. Fleming, and M. B. Fenton. 2009. Characterization of the echolocation calls of bats from Exuma, Bahamas. Acta Chiropterologica 11:415–424.

Nkemdirim, L. C. 1997. Climate and life in the Caribbean Basin. Pp. 177–201 in Climates and societies—a climatological perspective (M. Yoshino, ed.). Kluwer Academic Publishers, Boston, Massachusetts.

Pagnacco, P. F., and L. Radelli. 1962. Note on the geology of the isles of Providencia and Santa Catalina (Caribbean Sea, Colombia). Geologia Colombiana 3:125–132.

Panitsa, M., D. Tzanoudakis, K. A. Triantis, and S. Sfenthourakis. 2006. Patterns of species richness on very small islands: the plants of the Aegean archipelago. Journal of Biogeography 33:1223–1234.

Pavan, A. C., and G. Marroig. 2016. Integrating multiple evidences in taxonomy: species diversity and phylogeny of mustached bats (Mormoopidae: Pteronotus). Molecular Phylogenetics and Evolution 103:184–198.

Pedersen S. C., et al. 2006. Bats of Antigua, northern Lesser Antilles. Occasional Papers, Museum of Texas Tech University 249:1–18.

Pedersen, S. C., et al. 2009. Bats of Montserrat: population fluctuations in response to hurricanes and volcanoes, 1979–2004. Pp. 302–340 in Island bats: evolution, ecology, and conservation (T. H. Fleming and P. A. Racey, eds.). University of Chicago Press, Chicago.

Pedersen, S. C., et al. 2018a. Bats of St. Lucia, Lesser Antilles. Special Publication, Museum of Texas Tech University 69:1–61.

Pedersen, S. C., et al. 2018b. Bats of Sint Eustatius, Caribbean Netherlands. Occasional Papers, Museum of Texas Tech University 353:1–24.

Pedersen, S. C., H. H. Genoways, and P. W. Freeman. 1996. Notes on the bats from Montserrat (Lesser Antilles), with comments concerning the effects of Hurricane Hugo. Caribbean Journal of Science 32:206–213.

Pedersen, S. C., H. H. Genoways, G. G. Kwiecinski, P. A. Larsen, and R. J. Larsen. 2013. Biodiversity, biogeography, and conservation of bats in the Lesser Antilles. Pp. 62–73, 330 in Biodiversité insulaire: la flore, la faune et l'homme dans les Petites Antilles, eds. J.-L. Vernier and M. Burac. France: Schoelchers, Martinique, Direction de l'Environnement, de l'Aménagement et du Logement de Martinique et Université des Antilles et de la Guyane.

Pedersen, S. C., H. H. Genoways, M. N. Morton, J. W. Johnson, and S. E. Courts. 2003. Bats of Nevis, northern Lesser Antilles. Acta Chiropterologica 5:251–267.

Pedersen, S. C., H. H. Genoways, M. N. Morton, G. G. Kwiecinski, and S. E. Courts. 2005. Bats of St. Kitts (St. Christopher), northern Lesser Antilles, with comments regarding capture rates of Neotropical bats. Caribbean Journal of Science 41:744–760.

Pedersen, S. C., P. A. Larsen, H. H. Genoways, M. N. Morton, K. C. Lindsay, and J. Cindric. 2007. Bats of Barbuda, northern Lesser Antilles. Occasional Papers, Museum, Texas Tech University 271:1–19.

Petit, S. A. 1996. The status of bats on Curaçao. Biological Conservation 77:27–31.

Petit, S., A. Rojer, and L. Pors. 2006. Surveying bats for conservation: the status of cave-dwelling bats on Curaçao from 1993–2003. Animal Conservation 9:207–217.

Potter, R. B. 2004. The contemporary Caribbean. Pearson Prentice Hall, New York.

Pregill, G. K., D. W. Steadman, S. L. Olson, and F. V. Grady. 1988. Late Holocene fossil vertebrates from Burma Quarry, Antigua, Lesser Antilles. Smithsonian Contributions to Zoology 463:1–27.

Presley, S. J., and M. R. Willig. 2008. Composition and structure of Caribbean bat (Chiroptera) assemblages: effects of inter-island distance, area, elevation and hurricane-induced disturbances. Global Ecology and Biogeography 17:747–757.

R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reid, B. A., and R. G. Gilmore, III. 2014. Encyclopedia of Caribbean archaeology. University of Florida Press, Gainesville, Florida.

Ricklefs, R. E., and I. J. Lovette. 1999. The roles of island area per se and habitat diversity in the species–area relationships of four Lesser Antillean faunal groups. Journal of Animal Ecology 68:1142–1160.

Riley, J. 2002. Mammals on the Sangihe and Talaud Islands, Indonesia, and the impact of hunting and habitat loss. Oryx 36:288–296.

Rijsdijk, K. F., et al. 2014. Quantifying surface-area changes of volcanic islands driven by Pleistocene sea-level cycles: biogeographical implications for the Macaronesian archipelagos. Journal of Biogeography 41:1242–1254.

Rocha Dias, C. A., J. E. Santos Júnior, F. A. Perini, and F. R. Santos. 2017. Biogeographic scenarios for the diversification of a widespread Neotropical species, *Glossophaga soricina* (Chiroptera: Phyllostomidae). Systematics and Biodiversity 15:440–450.

Rodríguez-Durán, A., and E. Padilla-Rodríguez. 2010. New records for the bat fauna of Mona Island, Puerto Rico, with notes on their natural history. Caribbean Journal of Science 46:102–105.

Royer, A., et al. 2017. A high-resolution temporal record of environmental changes in the eastern Caribbean (Guade-

loupe) from 40 to 10 ka BP. Quaternary Science Reviews 155:198–212.

Russell, J. C., M. N. Clout, and B. H. McArdle. 2004. Island biogeography and the species richness of introduced mammals on New Zealand offshore islands. Journal of Biogeography 31:653–664.

Saunders, N. J. 2005. The peoples of the Caribbean. ABC-CLIO, Inc., Santa Barbara, California.

Silva Taboada, G. 1979. Los murciélagos de Cuba. Editorial Academica, La Habana, Cuba.

Simmons, N. B. 2005. Order Chiroptera. Pp. 312–529 in Mammals species of the world: a taxonomic and geographic reference, Third Edition (D. E. Wilson and D. M. Reeder, eds.). The John Hopkins University Press, Baltimore, Maryland.

Smith, S. R., N. Davaasuren, A. O. Debrot, F. Simal, and J. A. De Freitas. 2012. Preliminary inventory of key terrestrial nature values of Bonaire. IMARES Report number C003/12.

Smith, J. D., and H. H. Genoways. 1974. Bats of Margarita Island, with zoogeographic comments. Bulletin of the Southern California Academy of Sciences 73:64–79.

Soto-Centeno, J. A., N. B. Simmons, and D. W. Steadman. 2017. The bat community of Haiti and evidence for long-term persistence at high elevations. PLoS ONE 12(6):e0178066.

Soto-Centeno, J. A., and D. W. Steadman. 2015. Fossils reject climate change as the cause of extinction of Caribbean bats. Science Reports 5:7971.

Speed, R. C. 1994. Barbados and the Lesser Antilles forearc. Pp. 179–192 in Caribbean geology: an introduction (S. K. Donovan and T. A. Jackson, eds.). University of the West Indies Publishers' Association, Kingston, Jamaica.

Speed, R. C., and C. A. Keller. 1993. Synopsis of the geological evolution of Barbados. Journal of the Barbados Museum and Historical Society 41:113–139.

Speed, R. C., P. L. Smith-Horowitz, Kv. S. Perch-Nielsen, J. B. Saunders, and A. B. Sanfilippo. 1993. Southern Lesser Antilles arc platform: pre-late Miocene stratigraphy, structure, and tectonic evolution. Geological Society of America, Special Papers 277:1–98.

Speer, K. A., et al. 2017. Population structure of a widespread bat (*Tadarida brasiliensis*) in an island system. Ecology and Evolution 7:7585–7598.

Speer, K. A., J. A. Soto-Centeno, N. A. Albury, Z. Quicksall, M. G. Marte, and D. L. Reed. 2015. Bats of the Bahamas: natural history and conservation. Bulletin of the Florida Museum of Natural History 53:45–95.

Steadman, D. W., et al. 2007. Exceptionally well preserved Late Quaternary plant and vertebrate fossils from a Blue Hole on Abaco, the Bahamas. Proceedings of the National Academy of Sciences of the United States of America 104:19897–19902.

Steadman, D. W., et al. 2015. Vertebrate community on an ice-age Caribbean island. Proceedings of the National Academy of Sciences of the USA 112:44 E5963–E5971.

STINAPA. 2018. Bats (https://stinapabonaire.org/bat-species-on-bonaire). Accessed 28 July.

Stoetzel, E., A. Royer, D. Cochard, and A. Lenoble. 2016. Late Quaternary changes in bat palaeobiodiversity and palaeobiogeography under climatic and anthropogenic pressure: new insights from Marie-Galante, Lesser Antilles. Quaternary Science Review 143:150–174.

Stokes, A. V., and W. F. Keegan. 1995. A settlement survey for prehistoric archaeological sites on Grand Cayman. Miscellaneous Project Report, Florida Museum of Natural History 52:1–14.

Tejedor, A. 2011. Systematics of funnel-eared bats (Chiroptera: Natalidae). Bulletin of the American Museum of Natural History 353:1–140.

Tews, J., et al. 2004. Animal species diversity driven by habitat heterogeneity/diversity: the importance of keystone structures. Journal of Biogeography 31:79–92.

Thomas, R. 1966. A reassessment of the herpetofauna of Navassa Island. Journal of the Ohio Herpetological Society 5:73–89.

Timm, R. M., and H. H. Genoways. 2003. West Indian mammals from the Albert Schwartz Collection: biological and historical information. Scientific Papers of the Natural History Museum, University of Kansas 29:1–47.

Triantis, K. A., M. Mylonas, M. D. Weiser, K. Lika, and K. Vardinoyannis. 2005. Species richness, environmental heterogeneity and area: a case study based on land snails in Skyros archipelago (Aegean Sea, Greece). Journal of Biogeography 32:1727–1735.

Turvey, S. T., R. J. Kennerley, J. M. Nuñez-Miño, and R. P. Young. 2017. The last survivors: current status and conservation of the non-volant land mammals of the insular Caribbean. Journal of Mammalogy 98:918–936.

Valente L., R. S. Etienne, and L. M. Dávalos. 2017. Recent extinctions disturb path to equilibrium diversity in Caribbean bats. Nature ecology and evolution 1:0026

Varona, L. S. 1974. Catálogo de los mamiferos vivientes y extinguidos de las Antillas. Academia de Ciencias de Cuba, Habana.

Wallace, A. R. 1860. On the zoological geography of the Malay Archipelago. Journal of the Linnaean Society of the London 4:172–184.

Wallace, A. R. 1880. Island life, or the phenomena and causes of insular faunas and floras. Macmillan, London, UK.

Wikelski, M., J. Foufopoulos, H. Vargas, and H. Snell. 2004. Galápagos birds and diseases: invasive pathogens as threats for island species. Ecology and Society 9:5.

Wiles, G. J., and A. P. Brooke. 2010. Conservation threats to bats in the tropical Pacific islands and insular Southeast Asia. Pp. 405–459 in Island bats: evolution, ecology, and conservation (T.H. Fleming and P.A. Racey, eds.). University of Chicago Press, Chicago, Illinois.

Williams, M. R., B. B. Lamont, and J. D. Henstridge. 2009. Species-area functions revisited. Journal of Biogeography 36:1994–2004.

Willig, M. R., S. J. Presley, C. P. Bloch, and H. H. Genoways. 2010. Macroecology of Caribbean bats: effects of area, elevation, latitude, and hurricane-induced disturbance. Pp. 216–264 in Island bats: evolution, ecology, and conservation (T. H. Fleming and P. A. Racey, eds.). University of Chicago Press, Chicago, Illinois.

Wilson, D. E., and D. M. Reeder. 2005. Mammal species of the world: a taxonomic and geographic reference. John Hopkins University Press, Baltimore, Maryland.

Wilson, S. M. 1989. Prehistoric settlement pattern of Nevis, West Indies. Journal of Field Archaeology 16:427–450.

Yokoyama, Y., K. Lambeck, P. De Deckker, P. Johnston, and L. K. Fifield. 2000. Timing of the last glacial maximum from observed sea-level minima. Nature 406:713–716.

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APPENDIX I

Lists of bats occurring on four geographically defined groups of Caribbean islands—Bahamas, Greater Antilles and Virgin Islands, Lesser Antilles, and fringing islands.—These bat distribution lists were used as the basis of the analyses in this study. These lists were developed by consulting the following literature: Hummelinck (1943), Koopman (1955, 1958, 1959, 1968, 1975), Koopman et al. (1957), Husson (1960), Goodwin and Greenhall (1961), Thomas (1966), Jones and Phillips (1970), Brown et al. (1973), Jones et al. (1973), Buden (1974, 1975, 1977, 1986), Smith and Genoways (1974), Varona (1974), Baker and Genoways (1978), Baker et al. (1978), Klingener et al. (1978), Genoways and Williams (1979), Silva Taboada (1979), Carter et al. (1981), Eschelman and Morgan (1985), Griffiths and Klingener (1988), Engstrom et al. (1989), Jones (1989), Breuil and Masson (1991), Morgan (1994), Bekker (1996), Petit (1996), Genoways et al. (1998), Genoways et al. (2001), Avila-Flores (2002), Clarke and Racey (2003), Pedersen et al. (2003), Timm and Genoways (2003), Gannon et al. (2005), Genoways et al. (2005), Pedersen et al. (2005), Simmons (2005), Dávalos (2006), P. Larsen et al. (2006), Pedersen et al. (2006), Petit et al. (2006), Gardner (2007), Genoways et al. (2007a, 2007b, 2007c), Kwiecinski and Coles (2007), Pedersen et al. (2007), Presley et al. (2008), Geluso et al. (2009), Gregorin (2009), Mancina (2009), Murray et al. (2009), Pedersen et al. (2009), Willig et al. (2009), Genoways et al. (2010), Kwiecinski et al. (2010), Rodríguez-Durán and Padilla-Rodríguez (2010), Borroto-Páez and Mancina (2011, 2017), Genoways et al. (2011), Tejedor (2011), Dávalos and Turvey (2012), R. Larsen et al. (2012), Smith et al. (2012), Alexander and Geluso (2013), Mantilla-Meluk and Muñoz-Garay (2014), Speer et al. (2015), Pavan and Marroig (2016), R. Larsen et al. (2017), Monatelli et al. (2017), Rocha Dias et al. (2017), Soto-Centeno et al. (2017), Speer et al. (2017), Kwiecinski et al. (2018), Pedersen et al. (2018a, 2018b), STINAPA (2018), and VertNet (2018).

Lists are presented in multiple parts for layout purposes, as follows: A—Bahamas; B-1 and B-2—Greater Antilles and Virgin Islands; C—Lesser Antilles; and D—outer limits of the Caribbean.

Appendix I, Part A. Bat species occurring on the islands of the Bahamas.

Families and species of bats	Grand Bahama	Little Abaco	Great Abaco	Eleuthera	Andros	New Providence	Cat Island	San Salvador	Darby Island	Great Exuma	Little Exuma	Long Island	Crooked Island	Acklins	Long Cay	East Plana Cay	Mayaguana	North Caicos	Providenciales	Middle Caicos	East Caicos	Little Inagua	Great Inagua
Noctilionidae																							
Noctilio leporinus																							1
Phyllostomidae																							
Artibeus jamaicensis																	1		1			1	1
Brachyphylla nana																				1			
Erophylla sezekorni	1	1	1	1	1	1	1	1		1	1	1	1	1			1	1	1	1	1		1
Macrotus waterhousii				1	1	1	1	1	1	1		1	1	1		1		1	1	1	1		1
Monophyllus redmani													1	1				1	1	1			
Natalidae																							
Chilonatalus tumidifrons			1		1			1															
Nyctiellus lepidus				1			1			1	1	1											
Vespertilionidae																							
Eptesicus fuscus			1		1	1		1		1	1	1	1	1									
Lasiurus minor			1		1	1	1			1		1					1		1				1
Molossidae																							
Tadarida brasiliensis	1	1	1	1						1	1	1	1	1	1								
Totals	2	2	5	4	5	4	4	4	1	6	4	6	5	5	1	1	3	3	5	4	2	1	5

Appendix I, Part B-1. Bat species occurring on the islands of the Greater Antilles.

Families and species of bats	Cuba	Isla de la Juventud	Cayman Brac	Little Cayman	Grand Cayman	Jamaica	Navassa	Hispaniola	Gonâve	Isla de Mona	Puerto Rico
Noctilionidae											
Noctilio leporinus	1	1				1		1		1	1
Mormoopidae											
Mormoops blainvilli	1					1		1	1	1	1
Pteronotus macleayi	1	1				1		1			
Pteronotus parnellii	1					1					
Pteronotus portoricensis										1	1
Pteronotus pusillus								1	1		
Pteronotus quadridens	1					1		1			1
Phyllostomidae											
Ariteus flavescens						1					
Artibeus jamaicensis	1	1	1	1	1	1	1	1	1	1	1
Brachyphylla cavernarum											1
Brachyphylla nana	1	1			1			1			
Erophylla bombifrons								1			1
Erophylla sezekorni	1	1	1		1	1					
Glossophaga soricina						1					
Macrotus waterhousii	1	1	1	1	1	1	1	1	1		
Monophyllus redmani	1	1				1		1	1	1	1
Phyllonycteris aphylla						1					
Phyllonycteris poeyi	1	1						1			
Phyllops falcatus	1	1	1		1						
Phyllops haitiensis								1			
Stenoderma rufum											1
Natalidae											
Chilonatalus micropus	1	1				1		1			
Natalus jamaicensis						1					

Appendix I, Part B-1. (cont.)

Families and species of bats	Cuba	Isla de la Juventud	Cayman Brac	Little Cayman	Grand Cayman	Jamaica	Navassa	Hispaniola	Gonâve	Isla de Mona	Puerto Rico
Natalus major								1			
Natalus primus	1										
Nyctiellus lepidus	1	1									
Vespertilionidae											
Antrozous koopmani	1										
Eptesicus fuscus	1	1	1		1			1			1
Eptesicus lynni						1					
Lasiurus cinereus								1			
Lasiurus degelidus						1					
Lasiurus intermedius	1	1									
Lasiurus minor								1			1
Lasiurus pfeifferi	1										
Nycticeius cubanus	1										
Molossidae											
Eumops auripendulus						1					
Eumops glaucinus	1					1					
Eumops perotis	1										
Molossus molossus	1	1	1		1	1		1	1	1	1
Mormopterus minutus	1										
Nyctinomops laticaudatus	1										
Nyctinomops macrotis	1					1		1			
Tadarida brasiliensis	1	1	1		1	1		1			1
Totals	27	15	7	2	8	21	2	20	6	6	13

Appendix I, Part B-2. Bat species occurring on the islands of the Virgin Islands.

Families and species of bats	Vieques	Isla de Culebra	Anegada	Mosquito	Virgin Gorda	Great Camanoe	Guana	Tortola	Beef Island	Jost Van Dyke	Norman	Saint Thomas	Thatch Cay	Lovango Cay	Grass Cay	Saint John	Saint Croix
Noctilionidae																	
Noctilio leporinus	1	1				1	1	1	1		1	1				1	1
Mormoopidae																	
Mormoops blainvilli																	
Pteronotus macleayi																	
Pteronotus parnellii																	
Pteronotus portoricensis																	
Pteronotus pusillus																	
Pteronotus quadridens																	
Phyllostomidae																	
Ariteus flavescens																	
Artibeus jamaicensis	1	1	1		1		1	1		1		1		1		1	1
Brachyphylla cavernarum					1	1	1	1		1	1	1	1		1	1	1
Brachyphylla nana																	
Erophylla bombifrons																	
Erophylla sezekorni																	
Glossophaga soricina																	
Macrotus waterhousii																	
Monophyllus redmani																	
Phyllonycteris aphylla																	
Phyllonycteris poeyi																	
Phyllops falcatus																	
Phyllops haitiensis																	
Stenoderma rufum	1							1				1				1	1
Natalidae																	
Chilonatalus micropus																	
Natalus jamaicensis																	
Natalus major																	

Appendix I, Part B-2. (cont.)

Families and species of bats	Vieques	Isla de Culebra	Anegada	Mosquito	Virgin Gorda	Great Camanoe	Guana	Tortola	Beef Island	Jost Van Dyke	Norman	Saint Thomas	Thatch Cay	Lovango Cay	Grass Cay	Saint John	Saint Croix
Natalus primus																	
Nyctiellus lepidus																	
Vespertilionidae																	
Antrozous koopmani																	
Eptesicus fuscus																	
Eptesicus lynni																	
Lasiurus cinereus																	
Lasiurus degelidus																	
Lasiurus intermedius																	
Lasiurus minor																	
Lasiurus pfeifferi																	
Nycticeius cubanus																	
Molossidae																	
Eumops auripendulus																	
Eumops glaucinus																	
Eumops perotis																	
Molossus molossus	1	1	1	1	1	1	1	1	1	1		1				1	1
Mormopterus minutus																	
Nyctinomops laticaudatus																	
Nyctinomops macrotis																	
Tadarida brasiliensis							1		1							1	
Totals	4	3	2	1	3	3	5	5	3	3	2	5	1	1	1	6	5

Appendix I, Part C. Bats species occurring on the islands of the Lesser Antilles.

Families and species of bats	Anguilla	Saint Martin/Sint Maarten	Saint Barthélemy	Saba	Saint Eustatius	Saint Kitts	Nevis	Barbuda	Antigua	Montserrat	Guadeloupe	La Désirade	Marie-Galante	Dominica	Martinique	Saint Lucia	Saint Vincent	Barbados	Bequia	Mustique	Canouan	Mayreau	Union Island	Carriacou	Grenada
Emballonuridae																									
Peropteryx trinitatus																									1
Noctilionidae																									
Noctilio leporinus		1				1	1	1	1	1	1		1	1	1	1	1	1						1	1
Mormoopidae																									
Pteronotus davyi													1	1	1	1									1
Pteronotus fuscus																	1								
Phyllostomidae																									
Anoura geoffroyi																			1						1
Ardops nichollsi		1		1	1	1	1		1	1	1		1	1	1	1	1								
Artibeus jamaicensis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1							
Artibeus lituratus																	1		1				1	1	1
Artibeus planirostris																									1
Artibeus schwartzi																1	1		1	1	1		1	1	
Brachyphylla cavernarum	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1					
Chiroderma improvisum						1	1			1	1														
Dermanura sp.																									1
Glossophaga longirostris																	1		1	1	1	1	1	1	1

Appendix I, Part C. (cont.)

Families and species of bats	Anguilla	Saint Martin/Sint Maarten	Saint Barthélemy	Saba	Saint Eustatius	Saint Kitts	Nevis	Barbuda	Antigua	Montserrat	Guadeloupe	La Désirade	Marie-Galante	Dominica	Martinique	Saint Lucia	Saint Vincent	Barbados	Bequia	Mustique	Canouan	Mayreau	Union Island	Carriacou	Grenada
Micronycteris buriri																	1								
Micronycteris megalotis																									1
Monophyllus plethodon	1	1	1	1	1	1	1	1	1	1	1		1	1	1	1	1	1							
Sturnira angeli										1	1			1	1										
Sturnira paulsoni																1	1								
Natalidae																									
Natalus stramineus	1	1		1			1	1	1	1			1	1	1										
Vespertilionidae																									
Eptesicus guadeloupensis											1														
Eptesicus sp.														1											
Myotis dominicensis											1			1											
Myotis martiniquensis															1										
Myotis nyctor																		1							1
Molossidae																									
Molossus molossus	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Tadarida brasiliensis	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1								
Totals	6	8	5	7	5	8	9	7	8	10	12	4	9	12	11	10	12	6	4	3	3	2	4	5	12

Appendix I, Part D. Bats species occurring on the islands along the outer limits of the Caribbean. Many, but not all, of these islands are situated on the continental shelf.

Families and species of bats	Cozumel	Providencia	San Andres	Aruba	Bonaire	Curaçao	Isla de Margarita	Tobago	Trinidad
Emballonuridae									
Diclidurus albus									1
Peropteryx macrotis				1			1	1	1
Peropteryx trinitatis									1
Rhynochonycteris naso									1
Saccopteryx bilineata									1
Saccopteryx leptura							1	1	1
Noctilionidae									
Noctilio leporinus					1	1	1	1	1
Mormoopidae									
Mormoops megalophylla				1	1	1	1		1
Pteronotus davyi				1	1	1			1
Pteronotus fuscus							1	1	1
Pteronotus mesoamericanus	1								
Pteronotus personatus									1
Phyllostomidae									
Ametrida centurio					1				1
Anoura geoffroyi									1
Artibeus jamaicensis	1	1	1						
Artibeus lituratus	1						1	1	1
Artibeus planirostris						1	1	1	1
Carollia perspicillata							1	1	1
Centurio senex	1							1	1
Chiroderma trinitatum									1
Chiroderma villosum								1	1
Choeroniscus minor									1
Dermanura phaeotis	1								
Dermanura sp.								1	1
Desmodus rotundus							1		1
Diaemus youngii							1		1
Enchisthenes hartii									1
Glossophaga longirostris				1	1	1	1	1	1
Glossophaga soricina	1								1

Appendix I, Part D. (cont.)

Families and species of bats	Cozumel	Providencia	San Andres	Aruba	Bonaire	Curaçao	Isla de Margarita	Tobago	Trinidad
Glyphonycteris daviesi									1
Glyphonycteris sylvestris									1
Lampronycteris brachyotis									1
Leptonycteris curasoae				1	1	1	1		
Lonchorhina aurita									1
Lophostoma brasiliense									1
Mesophylla macconelli									1
Micronycteris hirsuta									1
Micronycteris megalotis	1						1	1	1
Micronycteris minuta									1
Micronycteris schmidtorum	1								
Mimon cozumelae	1								
Mimon crenulatum									1
Phylloderma stenops									1
Phyllostomus discolor							1		1
Phyllostomus hastatus								1	1
Platyrrhinus helleri									1
Sturnira sp. nov.								1	1
Sturnira tildae									1
Tonatia saurophila									1
Trachops cirrhosis									1
Trinycteris nicefori									1
Uroderma bilobatum									1
Vampyrodes caraccioloi								1	1
Vampyrum spectrum									1
Furipteridae									
Furipterus horrens									1
Thyropteridae									
Thyroptera tricolor									1
Natalidae									
Natalus mexicanus	1	1	1						
Natalus micropus		1	1						
Natalus tumidirostris				1	1	1		1	1

Appendix I, Part D. (cont.)

Families and species of bats	Cozumel	Providencia	San Andres	Aruba	Bonaire	Curaçao	Isla de Margarita	Tobago	Trinidad
Vespertilionidae									
Eptesicus brasiliensis								1	1
Lasiurus blossevillii	1							1	1
Lasiurus ega									1
Myotis attenboroughi								1	
Myotis keaysi	1								
Myotis nesopolus				1	1	1			
Myotis cf. *nigricans*									1
Myotis pilosatibialis									1
Myotis riparius									1
Rhogeessa io									1
Rhogeessa minutilla							1		
Rhogeessa parvula	1								
Molossidae									
Cynomops greenhalli									1
Eumops auripendulus									1
Eumops nanus	1								
Molossus bondae	1								
Molossus molossus				1	1	1	1	1	1
Molossus rufus	1								1
Molossus sinaloae									1
Nyctinomops laticaudatus	1								1
Promops centralis									1
Promops nasutus									1
Tadarida brasiliensis								1	
Totals	17	3	3	8	9	9	16	21	66

APPENDIX II

General island characteristics and predictor variables.—The number of bat species per island is hypothesized to be dependent upon five general island characteristics, and for each category data were collected on multiple predictor variables. The island characteristics and their predictor variables (abbreviations are used in charts below) used in the study were the following: Area—Island area (Area) and Change in island area since Last Glacial Maximum (LGM); Isolation—Minimum distance from mainland (Dist), Nearest island neighbor index (NNI), Latitude (Lat), and Longitude (Long); Habitat Diversity—Maximum elevation for each island (Elev), Island volume per surface area (Vol), and Island ruggedness determined by taking the standard deviation of slope per surface area (Rugg); Human impact—Human population density (Pop) and Number of years before present of human colonization (YBP); Climate—Annual temperature (annt), Maximum temperature of the warmest month (maxt), Minimum temperature of the coldest month (mint), Annual precipitation (annp), Maximum precipitation of the wettest month (maxp), and Minimum precipitation of the driest month (minp).

Islands and Richness			Area		Isolation				Habitat Diversity			Human Impact	
Islands	Island Group	Richness	Area	LGM	Dist	NNI	Lat	Long	Elev	Vol	Rugg	Pop	YBP
Grand Bahama	BA	2	1373	0.92	128	1.36	26.65	-78.37	5	5005962.10	222.93	37.70	800
Little Abaco	BA	2	1681	0.91	206	1.90	26.90	-77.71	7	219325.20	32.88	0.45	960
Great Abaco	BA	5	2010	0.89	286	0.70	26.52	-77.10	41	14972492.91	493.11	8.13	960
Eleuthera	BA	4	425	1.00	420	1.13	25.22	-76.27	51	3681842.54	631.00	25.88	900
Andros	BA	5	5957	0.95	245	2.31	24.35	-77.87	15	66101787.61	1579.00	1.29	1100
New Providence	BA	4	207	1.00	274	2.54	25.03	-77.40	37.5	1533533.82	101.03	61.63	1150
Cat Island	BA	4	388	1.00	468	0.32	24.41	-73.52	62.5	3744525.82	543.47	4.24	1150
San Salvador	BA	4	163	0.57	601	0.17	24.03	-74.49	43	1133756.90	103.82	5.71	1400
Darby island	BA	1	6.11	1.00	448	4.52	23.89	-76.26	19	7091.35	2.36	1.64	1150
Great Exuma	BA	6	186	1.00	474	2.20	23.57	-75.88	32	1800804.09	156.79	2.02	1150
Little Exuma	BA	4	25	1.00	522	2.34	23.43	-75.60	22	116505.84	26.71	15.00	1150
Long Island	BA	6	596	0.99	585	2.79	23.32	-75.11	54	4094302.53	497.56	5.19	1000
Crooked Island	BA	5	196	0.94	718	3.35	22.72	-74.19	47	1186755.38	110.48	1.79	800
Acklins	BA	5	389	0.88	711	2.58	23.42	-73.97	43	2195826.47	250.00	1.10	800
Long Cay	BA	1	21	0.99	680	4.17	22.59	-74.34	20	82564.58	13.10	1.38	1600
East Plana Cay	BA	1	5	0.97	759	0.60	22.61	-73.51	19	7386.82	3.01	2.00	1000

Appendix II, Part 1. (cont.)

Islands and Richness			Area		Isolation				Habitat Diversity			Human Impact	
Islands	Island Group	Richness	Area	LGM	Dist	NNI	Lat	Long	Elev	Vol	Rugg	Pop	YBP
Mayaguana	BA	3	281	0.55	796	1.79	22.38	-72.94	40	1062989.39	123.55	0.99	900
North Caicos	BA	3	106	0.99	919	0.88	21.90	-71.95	27	728061.97	89.55	19.49	1000
Providenciales	BA	5	97	0.99	906	0.38	21.79	-72.11	34	767115.88	113.74	245.04	1000
Middle Caicos	BA	3	124.3	0.98	948	2.10	21.80	-71.74	17	1637328.20	87.04	4.20	1000
East Caicos	BA	2	46.6	0.99	940	2.94	21.67	-71.54	50	902443.12	74.00	0.21	1000
Little Inagua	BA	1	127	0.95	849	4.84	21.50	-73.00	33.5	502810.58	44.31	0.08	960
Great Inagua	BA	5	1544	0.44	822	12.96	21.06	-73.29	33	7947220.57	338.29	0.59	960
Cuba	GA	27	114524	0.36	204	10.76	22.08	-79.13	1974	12836155218.16	295440.00	99.49	6460
Isla de la Juventud	GA	15	3059	0.98	381	16.23	22.61	-82.82	310	44469688.01	1785.64	28.30	1100
Cayman Brac	GA	7	36	0.87	623	2.93	19.72	-79.80	40	636785.98	152.34	70.69	500
Little Cayman	GA	2	28.5	0.90	594	2.56	19.69	-80.03	12	97484.90	21.76	5.96	500
Grand Cayman	GA	8	197	0.56	493	1.41	19.32	-81.24	43	1053507.48	97.38	298.56	500
Jamaica	GA	21	10991	0.29	629	7.43	18.13	-77.27	2256	3875225647.89	104829.00	263.73	1400
Navassa	GA	2	5	0.69	737	28.89	18.40	-75.01	77	24102.13	35.00	2.00	200
Hispaniola	GA	20	76070	0.14	933	16.14	18.87	-72.17	3175	34474686700.44	674425.00	280.12	6028
Gonâve	GA	6	743	0.99	719	69.08	18.84	-73.04	778	153767709.20	6783.23	117.20	1500
Isla de Mona	GA	6	49.2	0.56	699	18.49	18.09	-67.89	60	2754797.96	236.12	2.03	5000
Puerto Rico	GA	13	8897	0.53	715	6.25	18.23	-66.46	1338	2120149359.32	65440.00	373.82	5960
Vieques	GA	4	132	0.99	815	39.70	18.13	-65.43	301	4715787.87	799.83	70.83	3731
Isla de Culebra	GA	3	28.5	1.00	833	7.32	18.32	-65.29	198	527339.31	260.29	63.79	1330
Anegada	GA	2	34	1.00	882	0.95	18.73	-64.34	10	95589.66	14.81	8.38	750
Mosquito	GA	1	0.4	1.00	876	12.13	18.51	-64.39	75	4318.19	0.01	50.00	750
Virgin Gorda	GA	3	21	1.00	867	0.45	18.48	-64.40	417	1439648.76	468.63	187.14	1500
Great Camanoe	GA	3	3.37	1.00	871	6.78	18.48	-64.53	158	15069.11	11.17	14.84	750

Appendix II, Part 1. (cont.)

Islands and Richness			Area		Isolation				Habitat Diversity			Human Impact	
Islands	Island Group	Richness	Area	LGM	Dist	NNI	Lat	Long	Elev	Vol	Rugg	Pop	YBP
Guana	GA	5	2.97	1.00	870	10.29	18.48	-64.57	216	25157.39	29.26	33.67	2000
Tortola	GA	5	62	1.00	871	2.40	18.43	-64.63	542.5	6317673.12	1385.13	377.73	2000
Beef Island	GA	3	2.67	1.00	849	44.26	18.44	-64.53	174	19585.62	65.80	93.63	2000
Jost Van Dyke	GA	3	8	1.00	865	2.07	18.45	-64.38	294	228738.12	185.28	37.25	1300
Norman	GA	2	2.55	1.00	861	1.96	18.32	-64.61	100	633.16	0.74	39.22	750
Saint Thomas	GA	5	70	1.00	867	3.69	18.35	-64.93	472	6368426.70	1371.39	737.63	3940
Thatch Cay	GA	1	0.69	1.00	878	18.52	18.36	-64.86	147	6019.36	0.01	14.49	2500
Lovango Cay	GA	1	0.45	1.00	872	6.69	18.36	-64.81	75	4512.57	0.01	111.11	2000
Grass Cay	GA	1	0.24	1.00	870	4.86	18.36	-64.83	57	2415.56	0.01	41.67	2500
Saint John	GA	6	50	1.00	857	1.40	18.34	-64.74	388	5100634.20	1171.14	83.40	2456
Saint Croix	GA	5	212	0.64	800	0.77	17.73	-64.76	355	12508072.54	1721.24	238.68	2000
Anguilla	LA	6	91	0.98	807	0.83	18.22	-63.05	59	1019642.89	227.93	162.24	3600
Saint Martin	LA	8	96	0.98	810	0.60	18.04	-63.05	424	4206810.14	969.82	809.80	5129
Saint Barthélemy	LA	5	18	1.00	781	0.29	17.89	-62.83	281	583387.42	231.68	534.72	4000
Saba	LA	7	12	0.56	753	0.09	17.63	-63.24	869	2691208.00	767.00	165.92	3883
Sint Eustatius	LA	6	18	0.98	747	0.66	17.49	-62.98	603	1281001.00	551.21	177.39	2462
Saint Kitts	LA	8	173.5	0.82	714	3.89	17.33	-62.74	1156	33536104.19	2854.18	408.92	4152
Nevis	LA	9	93	0.91	706	1.54	17.32	-62.59	985	14238132.26	1283.17	154.81	2700
Barbuda	LA	7	162	0.96	763	0.09	17.64	-61.79	62.5	1319283.54	145.95	10.11	3772
Antigua	LA	8	280	0.92	700	0.43	17.07	-61.79	402	12201476.01	1479.25	362.19	5106
Montserrat	LA	10	104	0.54	658	0.56	16.74	-62.19	915	20663115.74	2195.35	50.02	2760
Guadeloupe	LA	12	1510	0.39	582	0.92	16.26	-61.57	1484	260376997.58	9401.00	288.82	3641
La Désirade	LA	4	20.64	0.99	638	12.93	16.32	-61.05	230	1348410.99	516.93	74.22	988

Appendix II, Part 1. (cont.)

Islands and Richness			Area		Isolation				Habitat Diversity			Human Impact	
Islands	Island Group	Richness	Area	LGM	Dist	NNI	Lat	Long	Elev	Vol	Rugg	Pop	YBP
Marie-Galante	LA	9	155	0.49	577	1.77	15.93	-61.27	204	13263364.88	1157.39	74.36	4899
Dominica	LA	12	750	0.28	499	0.64	15.42	-61.34	1447	269174299.12	12179.00	98.06	5000
Martinique	LA	11	1101	0.51	422	0.33	14.63	-60.97	1397	207880233.82	10828.70	351.03	3710
Saint Lucia	LA	10	609	0.46	343	0.92	13.90	-60.97	950	97774859.37	6557.16	292.31	1850
Saint Vincent	LA	12	344.5	0.31	273	0.22	13.25	-61.20	1234	108686391.89	5395.88	289.90	1800
Barbados	LA	6	422	0.35	368	0.08	13.17	-59.56	340	49206003.57	2738.00	678.64	4500
Bequia	LA	4	18	1.00	262	1.56	13.01	-61.23	268	627570.89	318.44	238.89	1690
Mustique	LA	3	5	1.00	245	0.56	12.88	-61.18	145	103964.20	56.16	100.00	965
Canouan	LA	3	7.3	1.00	224	0.06	12.71	-61.32	267	184712.68	111.71	232.88	1525
Mayreau	LA	2	3.76	1.00	214	0.33	12.64	-61.39	92	6627.03	1.44	72.07	1000
Union Island	LA	4	11	1.00	209	0.49	12.60	-61.44	305	140870.66	126.40	272.73	1290
Carriacou	LA	5	34	0.99	194	0.02	12.48	-61.45	291	1543004.89	466.80	178.85	1620
Grenada	LA	12	344	0.91	149	0.04	12.09	-61.68	838	63808923.68	3435.50	297.23	2040
Cozumel	OC	17	647	0.50	20	0.02	20.44	-86.91	14	6339621.53	228.63	154.56	1500
Providencia	OC	3	18	1.00	231	0.00	13.35	-81.37	360	2785.89	1.80	278.39	500
San Andrés	OC	3	26	1.00	199	0.00	12.54	-81.72	84	57195.08	24.56	3076.92	500
Aruba	OC	8	179	0.53	27	0.03	12.51	-69.98	173	6904399.51	505.23	590.28	2173
Bonaire	OC	9	294	0.35	87	0.21	12.19	-68.26	155	6693630.01	702.40	64.30	3500
Curaçao	OC	9	444	0.22	65	0.14	12.18	-68.99	320	14167712.92	1535.72	360.36	6500
Isla de Margarita	OC	16	1020	1.00	24	0.04	10.94	-64.06	920	79093477.25	7313.34	202.91	4225
Tobago	OC	21	300	1.00	125	3.12	11.24	-60.68	640	52655729.14	4387.63	480.31	5000
Trinidad	OC	66	4748	0.99	24	0.07	10.44	-61.26	940	413820809.17	22625.10	273.63	8000

Appendix II, Part 2.

Islands	Climate					
Islands	annt	maxt	mint	annp	maxp	minp
Grand Bahama	242	317	159	1487	238	50
Little Abaco	242	320	158	1452	207	62
Great Abaco	245	319	170	1135	188	40
Eleuthera	250	318	183	1042	190	28
Andros	250	320	179	1206	190	28
New Providence	247	317	178	1230	213	30
Cat Island	256	320	193	945	183	24
San Salvador	255	319	192	1072	218	30
Darby island	255	318	191	903	155	21
Great Exuma	257	319	194	936	159	21
Little Exuma	258	320	198	745	106	25
Long Island	259	321	196	895	172	26
Crooked Island	262	324	202	848	149	22
Acklins	262	323	202	831	141	25
Long Cay	264	325	203	843	145	20
East Plana Cay	260	322	201	822	140	28
Mayaguana	259	319	200	799	130	33
North Caicos	259	317	202	688	110	31
Providenciales	258	317	200	726	115	30
Middle Caicos	260	317	204	667	110	30
East Caicos	260	318	205	644	107	29
Little Inagua	258	319	196	943	166	22
Great Inagua	258	322	195	700	112	17
Cuba	252	330	167	1487	254	26
Isla de la Juventud	253	319	175	1539	242	28
Cayman Brac	261	323	195	1225	180	30
Little Cayman	263	324	197	1239	179	29
Grand Cayman	264	319	204	1384	220	25
Jamaica	215	268	161	1871	323	73
Navassa	265	319	207	1661	227	63
Hispaniola	242	324	160	1238	219	54
Gonâve	250	316	173	1037	153	23
Isla de Mona	255	313	190	1018	124	36
Puerto Rico	241	307	167	1786	242	68
Vieques	258	313	199	1348	174	51
Isla de Culebra	260	311	204	1155	152	44

Appendix II, Part 2. (cont.)

Islands	Climate					
Islands	annt	maxt	mint	annp	maxp	minp
Anegada	265	309	218	1039	137	49
Mosquito	266	311	219	1070	144	47
Virgin Gorda	243	291	194	1250	163	53
Great Camanoe	265	310	218	1123	150	48
Guana	265	311	217	1139	152	49
Tortola	245	294	195	1216	161	47
Beef Island	266	311	217	1129	151	47
Jost Van Dyke	265	314	215	1157	149	48
Norman	265	313	216	1127	152	44
Saint Thomas	258	308	206	1110	142	45
Thatch Cay	266	316	214	1096	142	44
Lovango Cay	266	317	214	1107	143	44
Grass Cay	266	317	214	1107	143	44
Saint John	259	309	208	1190	155	47
Saint Croix	263	312	211	1098	143	43
Anguilla	268	311	222	992	123	45
Saint Martin	248	292	201	1196	148	53
Saint Barthélemy	270	313	223	1015	125	45
Saba	230	275	181	1415	189	58
Sint Eustatius	262	305	215	1167	166	51
Saint Kitts	242	286	194	1577	193	68
Nevis	241	286	191	1683	200	76
Barbuda	266	308	218	924	107	38
Antigua	261	303	211	1133	148	41
Montserrat	245	295	193	1871	222	87
Guadeloupe	242	292	185	2807	324	105
La Désirade	250	300	195	1344	168	52
Marie-Galante	253	301	198	1444	195	57
Dominica	239	290	184	2551	293	115
Martinique	249	292	201	2169	281	74
Saint Lucia	236	284	183	2290	290	87
Saint Vincent	215	263	161	2834	340	117
Barbados	248	299	187	1446	194	45
Bequia	267	315	215	2144	282	77
Mustique	267	314	214	2095	275	74
Canouan	268	315	216	2053	263	70

Appendix II, Part 2. (cont.)

Islands	Climate					
Islands	annt	maxt	mint	annp	maxp	minp
Mayreau	268	315	218	2025	257	68
Union Island	262	308	211	2106	259	72
Carriacou	268	314	217	1991	247	65
Grenada	246	293	194	2215	268	76
Cozumel	260	326	189	1392	224	33
Providencia	263	308	218	2439	366	35
San Andrés	264	309	220	2273	345	30
Aruba	273	320	228	432	80	10
Bonaire	275	311	239	449	96	13
Curaçao	274	319	233	541	106	15
Isla de Margarita	276	325	223	590	110	11
Tobago	248	296	196	2258	291	67
Trinidad	256	308	200	2046	257	61

APPENDIX III

List of candidate models used to predict the number of bat species (Spp) on islands in the Caribbean Basin and the island group analyses they were used in: All = all island groups; BA = Bahamas; CB = all islands in the Caribbean Basin; GA = Greater Antilles; LA = Lesser Antilles; OC = islands located on or near the continental shelf.

Model	Island group
Spp = Area	All
Spp = Vol	All
Spp = Elev	All
Spp = Rugg	All
Spp = LGM	All
Spp = Dist	All
Spp = NNI	All
Spp = Pop	All
Spp = Lat	All
Spp = Long	All
Spp = Annt	All
Spp = Maxt	All
Spp = Mint	All
Spp = Pann	All
Spp = Pmax	All
Spp = Pmin	All
Spp = [1]	All
Spp = Rugg+Dist	All
Spp = Area+Dist	All
Spp = Pop+Rugg	All
Spp = Area+NNI+Annp	All
Spp = LGM+Pop+YBP	All
Spp = Rugg+Pop+YBP	All
Spp = NNI+vol+Pop+Mint	All
Spp = Dist+NNI+Rugg+Pop+YBP	All
Spp = Area+NNI+Annt	BA
Spp = Dist+NNI+Pmin	BA
Spp = Maxt+Mint+Pann	BA

Appendix III. (cont.)

Model	Island group
Spp = Dist+LGM	BA
Spp = Rugg+Pmin+Maxt+Dist+NNI	BA
Spp = Rugg+Pmin	BA
Spp = Long+Rugg+Pmin	BA
Spp = Annt+Mint+Pmax+Pmin	CB
Spp = Area+Maxt+Pmax	CB
Spp = Rugg+Annp+Maxt+Dist+NNI	CB
Spp = Vol+Pmax+Pmin	CB
Spp = Elev+Mint+Pmax	CB
Spp = Area+YBP	CB
Spp = Area+Elev	CB
Spp = Annt+Pmax	LA
Spp = Area+Dist+NNI	BA, GA
Spp = Area+YBP+Annt	BA, LA
Spp = Dist+NNI+Lat+Long	CB, LA
Spp = Annt+Mint+Pmax+Pmin	CB, LA
Spp = Dist+NNI+Annt+Pann	GA, OC
Spp = Maxt+Pmax	GA, OC
Spp = Annt+Vol+Pop+LGM	GA, OC
Spp = Area+Mint	GA, OC
Spp = Area+Elev+Dist+NNI	CB, LA, OC
Spp = Rugg+Pann+Maxt+Dist+NNI	GA, LA, OC
Spp = Rugg+Maxt	GA, LA, OC
Spp = Area+Annt+LGM+Dist	BA, GA, LA, OC
Spp = Elev+YBP	BA, GA, LA, OC
Spp = Area+Maxt+Pop	BA, GA, LA, OC
Spp = LGM+Dist+Elev+Annt+Pann	CB, GA, LA, OC

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Flying Around in the Genome: Characterization of LINE-1 in Chiroptera

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Abstract

L1s are transposable elements that move by a copy-and-paste mechanism that continuously increases their copy number in the genome, such that each genome has a record of the L1 history in that host lineage. They make up about 20% of the genomes of eutherian mammals and have played a major role in shaping genome evolution. Chiroptera has the lowest average genome size among mammalian orders and the only documented case of L1 extinction affecting an entire mammalian family. Herein, L1 activity and extinction are characterized in all families of the order Chiroptera using a method that enriches for the youngest lineages of L1s in the genome. In addition to the previously reported L1 extinction in Pteropodidae, L1 extinction was documented to occur in *Mormoops blainvilli*, but this event did not affect all species of Mormoopidae. Further, there was no evidence of concordance between the evolution of L1s and their chiropteran host. There were two L1 lineages present before the divergence of all extant bats. Both lineages are extinct in the Pteropodidae. One or the other L1 lineage is extinct in almost all bat families, but *Taphozous melanopogon* maintains active members of both. Most intriguingly, some families within the Rhinolophoidea retain one active L1 lineage whereas other families retain the other, creating a deep discontinuity between L1 phylogeny and chiropteran phylogeny. These results indicate that there have been numerous losses of active L1 lineages over the history of chiropteran evolution, but that all chiropteran families except Pteropodidae have retained L1 activity.

Key words: bat, Chiroptera, evolution, L1, LINE-1, phylogeny, retrotransposons, transposable elements

Introduction

L1 retrotransposons (LINE-1; Long INterspersed Element-1) have played a major role in shaping mammalian genomes (de Koning et al. 2011; Platt et al. 2018). In addition to retrotransposing their own sequence to new sites in the genome, L1s can provide the molecular machinery to move SINEs (Short INterspersed Elements) and processed pseudogenes (Dewannieux et al. 2003; Dewannieux and Heidmann 2005). Any of these sequences can cause mutations by inserting into genes, and retrotransposition can also move flanking sequences (Kazazian et al. 1988; Goodier et al. 2000; Ostertag and Kazazian 2001).

In mammals, full-length L1 elements are 6.5 to 7 kb and are made up of four major segments (Fig. 1): 5′ UTR, ORF1, ORF2, and 3′ UTR (Furano 2000). The 5′ UTR (untranslated region) includes the promoter; this region has been swapped out by recombination many times during mammalian evolution, so it is often non-orthologous between species and even for different subfamilies within a species (Boissinot and Sookdeo 2016). The ORF1 (open reading frame 1) segment encodes a nucleic acid binding protein that is associated with the L1 transcript as part of the retrotransposition complex. It has a hypervariable region (V) near the 5′ end that is either very rapidly evolving or also has been swapped out over the evolutionary history of the element. The ORF2 segment has four conserved domains: endonuclease (E), an octapeptide-containing sequence (Z), reverse transcriptase (RT), and a RNase-H-like zinc finger (C). The 3' UTR segment contains a G-rich polypurine tract and terminates with a poly-A tail. The proteins encoded by ORF1 and ORF2, along with host proteins, are responsible for retrotransposi-

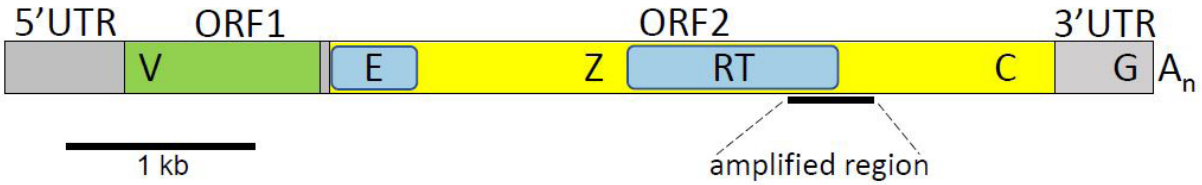

Figure 1. Structure of a typical mammalian L1. Full-length elements are ~7 kb in length and have four major segments: 5′ and 3′ untranslated regions (UTRs) and two open reading frames (ORFs). ORF1 has a 5′ hypervariable region (V) and ORF2 contains four conserved domains: endonuclease (E), an octapeptide-containing sequence (Z), reverse transcriptase (RT) and a RNase-H-like zinc finger (C). A G-rich polypurine tract (G) resides in the 3′ UTR and elements terminate with a poly-A tail. The region cloned for this study straddles the RT domain in ORF2 and was isolated by PCR with degenerate primers.

tion. Sequences generally are inserted into the genome starting at the 3′ end and most insertions are truncated, so there are relatively few full length L1s in the genome (Furano 2000).

Whole-genome sequencing has greatly expanded what is known about the evolution of mammalian L1s. These studies provide a broad overview of L1 evolution. L1s have persisted in the mammalian genome since before the divergence of placental mammals from marsupials, but are not found in monotremes (Ivancevic et al. 2016). Given the presence of multiple active elements retrotransposing in the genome at any given time, one would expect that over the course of evolutionary history the active elements would have diverged such that they form a bush-like phylogeny within each host species (Clough et al. 1996). Although this is true of other vertebrates that have retrotransposons related to LINE-1—fish, reptiles, and amphibians (Platt et al. 2018)—mammalian L1s from a given species generally form a pectinate tree with a single trunk, indicating that the active elements found in the genome (at any point in their history) within the host lineage are very closely related. The mechanism behind this unique mode of evolution within a genome is not well understood, but it is thought to indicate an ongoing arms race where the genome evolves to suppress retrotransposition and the L1 elements evolve to escape this control (Platt et al. 2018). Occasionally, multiple well-diverged L1 lineages persist over evolutionary time. For example, the deer mouse *Peromyscus* has two active lineages (Casavant et al. 1996), but these lineages arose subsequent to the origin of *Peromyscus* (Casavant et al. 1998) and are not found in all species of the genus.

Previously, a PCR-based approach was developed to enrich for relatively young L1 pseudogenes if they are present in the genome (Cantrell et al. 2000). If young elements are not present, older L1 pseudogenes are amplified. Using this technique, a comprehensive screen for L1 activity across all families of Chiroptera was conducted. In all species examined with active L1s, they evolve as one or two persistent lineages. In addition to the extinction event previously documented for the family Pteropodidae (Cantrell et al. 2008), an L1 extinction event was identified in *Mormoops blainvilli*, however, in this case it did not affect the entire family Mormoopidae.

METHODS

Specimens examined.—Genomic DNA from a total of 57 species of bats was examined by a PCR-based method that enriches for a conserved region of recently active L1s (Cantrell et al. 2000). Specimens examined and sources of material are provided in Table 1.

Degenerate PCR, L1 cloning, and colony screening.—A 575 bp region of L1 (Fig. 1) ORF2 homologous to bases 4989–5563 of a full-length *Mus* L1 (GenBank accession number M13002) was amplified and cloned from each species as described previously (Cantrell et al. 2000). This technique uses degenerate primers to regions that are highly conserved based on a previous alignment of reverse transcriptases from viruses and transposable elements plus alignments of L1s from a broad range of mammalian species. The

Table 1. Specimens examined in this study. Tissues with TK numbers were acquired from the Museum of Texas Tech University, gE numbers from the New Zealand Department of Conservation, and CT18 from University College Dublin, Belfield. L1 Activity indicates which ancestral L1 lineage is active within each species examined. An asterisk (*) indicates species that are included in the trees in Figure 3.

Family	Genus, Species	Tissue ID	L1 Activity
Pteropodidae	**Cynopterus sphinx*	TK21250	none
Rhinolophidae	**Rhinolophus eloquens*	TK33101	Lineage 2
Hipposideridae	**Hipposideros armiger*	TK21147	Lineage 2
Megadermatidae	**Megaderma lyra*	TK21292	Lineage 1
Craseonycteridae	**Craseonycteris thonglongyai*	CT18	Lineage 1
Rhinopomatidae	**Rhinopoma hardwickei*	TK40884	Lineage 1
Nycteridae	**Nycteris thebaica*	TK33153	Lineage 2
Emballonuridae	**Rhynchonycteris naso*	TK15108	Lineage 2
Emballonuridae	**Taphozous melanopogon*	TK21446	Lineages 1, 2
Phyllostomidae	**Artibeus jamaicensis*	TK27682	Lineage 2
Phyllostomidae	**Tonatia saurophila bakeri*	TK104519	Lineage 2
Mormoopidae	**Mormoops blainvilli*	TK32173	none
Mormoopidae	**Pteronotus quadridens*	TK9497	Lineage 2
Noctilionidae	**Noctilio albiventris*	TK17633	Lineage 2
Furipteridae	**Furipterus horrens*	TK17149	Lineage 2
Thyropteridae	**Thyroptera discifera*	TK104577	Lineage 2
Mystacinidae	**Mystacina tuberculata*	gE266	Lineage 2
Myzopodidae	**Myzopoda aurita*	gE172	Lineage 2
Vespertilionidae	**Antrozous pallidus*	TK44027	Lineage 2
Vespertilionidae	**Myotis velifer*	TK44032	Lineage 2
Molossidae	**Tadarida brasiliensis*	TK44001	Lineage 2
Natalidae	**Natalus stramineus*	TK15661	Lineage 2
Pteropodidae	*Dobsonia moluccensis*	TK20261	none
Pteropodidae	*Hypsignathus monstrosus*	TK21542	none
Pteropodidae	*Macroglossus* sp.	TK 20305	none
Pteropodidae	*Megaerops niphanae*	TK21085	none
Pteropodidae	*Megaloglossus woermanni*	TK21565	none
Pteropodidae	*Melonycteris melanops*	TK20071	none
Pteropodidae	*Nyctimene albiventer*	TK20056	none
Pteropodidae	*Pteropus hypomelanus*	TK20059	none
Pteropodidae	*Pteropus macrotis*	TK20310	none
Pteropodidae	*Rousettus amplexicaudatus*	TK20031	none
Phyllostomidae	*Ametrida centurio*	TK17743	Lineage 2
Phyllostomidae	*Anoura geoffroyi*	TK19385	Lineage 2

Table 1. (cont.)

Family	Genus, Species	Tissue ID	L1 Activity
Phyllostomidae	*Ardops nichollsi*	TK15576	Lineage 2
Phyllostomidae	*Artibeus cinereus*	TK19226	Lineage 2
Phyllostomidae	*Artibeus lituratus*	TK104427	Lineage 2
Phyllostomidae	*Artibeus planirostris*	TK15011	Lineage 2
Phyllostomidae	*Artibeus schwartzi*	TK82838	Lineage 2
Phyllostomidae	*Carollia perspicillata*	TK104347	Lineage 2
Phyllostomidae	*Choeroniscus godmani*	TK40021	Lineage 2
Phyllostomidae	*Choeronycteris mexicana*	TK27013	Lineage 2
Phyllostomidae	*Desmodus rotundus*	TK40368	Lineage 2
Phyllostomidae	*Diphylla ecaudata*	TK13508	Lineage 2
Phyllostomidae	*Glossophaga soricina*	TK9251	Lineage 2
Phyllostomidae	*Glyphonycteris sylvestris*	TK10454	Lineage 2
Phyllostomidae	*Hylonicteris underwoodi*	TK20540	Lineage 2
Phyllostomidae	*Lionycteris spurrelli*	TK22524	Lineage 2
Phyllostomidae	*Lonchophylla thomasi*	TK17580	Lineage 2
Phyllostomidae	*Lonchorhina aurita*	TK20560	Lineage 2
Phyllostomidae	*Macrotus waterhousii*	TK27889	Lineage 2
Phyllostomidae	*Micronycteris minuta*	TK15174	Lineage 2
Phyllostomidae	*Micronycteris nicefori*	TK25119	Lineage 2
Phyllostomidae	*Platyrrhinus helleri*	TK14577	Lineage 2
Phyllostomidae	*Rhinophylla pumilio*	TK10130	Lineage 2
Phyllostomidae	*Sturnira lucovici*	TK34856	Lineage 2
Phyllostomidae	*Trachops cirrhosus*	TK19132	Lineage 2

primers also contain 5′ clamps to increase specificity and introduce two restriction sites at each end of the amplified elements. Restriction digestion after amplification is followed by ligation into a modified *lac*Z reporter vector, pKSW, that was engineered such that the PCR product is cloned in-frame and in the sense orientation. Insertion of an L1 fragment from an element that has transposed so recently that it still contains an ORF results in production of an L1/β-galactosidase fusion protein. Insertion of an L1 region that has suffered stop mutations in the normal reading frame blocks production of the fusion protein. Thus, blue colonies are enriched for recently inserted L1 sequences that maintain ORFs, whereas white colonies generally have indels and stop codons.

For initial characterization of each species, clones were sequenced from both blue and white colonies. If identical clones were found, only one was included in the final dataset. Potential recombinants were detected as described previously (Cantrell et al. 2008) and were removed from the dataset. If primarily truncated ORFs were found due to internal restriction sites, PCR products were cloned with alternate enzymes. For each species, a minimum of 20 sequences was included in the final data set, generally from the first 10 blue and first 10 white colonies isolated except where unavailable. All L1 sequences isolated from species analyzed for Figures 2 and 3 of this study were deposited in GenBank (accession numbers EF437602–EF437898 and MK991326–MK991766).

Species were designated as having recently active L1s if at least two sequences were found with intact reading frames and in the correct reading frame across the entire length of the amplified region. In

cases where this criterion was not met, additional clones were sequenced in an attempt to detect elements containing ORFs.

Phylogenetic analysis.—For each species, 20 L1 sequences (usually 10 from blue colonies and 10 from white colonies) were aligned by the ClustalW algorithm (Thompson et al. 1994). Two young L1s from the most closely related sister taxon were included as outgroup. Alignments were adjusted manually. Phylogenetic analysis was carried out under maximum-likelihood criteria in PAUP* version 4.0b10 (Swofford 2003). To select the most appropriate model of evolution, the alignments were subjected to an iterative search strategy that estimated the parameters of 16 alternative maximum-likelihood models from an initial neighbor joining tree. The relative fit of the models was assessed using the χ^2-approximation to the null distribution as a likelihood-ratio test (Yang 1994). Heuristic searches with 100 replicate random addition sequences and tree bisection-reconnection branch swapping were then conducted under likelihood criteria with the fully defined, best-fit model, which was either HKY+G or GTR+G for all species. The trees were subsequently rooted with the outgroup and the taxa names and outgroup branches were removed for ease of viewing. Examples of species-specific L1 trees are shown in Figure 2 (see Results). Tree size was adjusted so that the height and scale bars were uniform. Black dots were added to indicate L1s with ORFs. To be considered an element with an ORF, the sequence was required to be full length, with intact reading frames maintaining the correct reading frame across the entire length of the amplified region. The same methods were used to build an L1 phylogeny representing all families of Chiroptera except that fewer sequences were used for each species, as described under Results.

RESULTS

A 575 bp region of L1 ORF2 (Fig. 1) was amplified, cloned, sequenced, and analyzed from 57 species of Chiroptera (Table 1). All families of bats were sampled and, when possible, the same genera used by Teeling (Teeling et al. 2005) to construct a phylogeny of all chiropteran families were included. Phylogenetic analysis was carried out on elements from each species separately and as well as collectively on species representing all families of Chiroptera. L1s for each of the 57 individual species were analyzed to determine if there was evidence of recent L1 activity and to assess the number of active L1 lineages. For the combined analysis of L1 from the order Chiroptera, one or two species were included for each family. Pteropodidae and Phyllostomidae were sampled more extensively (Table 1).

The targeted region was cloned in frame with *lacZ* such that a fusion protein was produced in clones where the reading frame of the 575 bp region was maintained, giving rise to blue colonies when clones were plated on β-galactosidase. This technique is extremely effective at enriching for young elements even in the presence of a vast excess of old L1 pseudogenes in the genome. To assess the sensitivity of the technique, DNA from *Rousettus amplexicaudatus*, a species of Pteropodidae with long extinct L1s, was seeded with quantities of a cloned mouse L1 element equivalent to 1, 3, 10, 100, or 1,000 young L1 copies per haploid genome. Using this PCR-based enrichment technique, no mouse L1 clones were found among 16 sequenced from the sample spiked with mouse L1 equivalent to 1 copy per haploid genome, but samples spiked with 3, 10, 100, or 1,000 copies per haploid genome yielded 25, 38, 94, and 100% mouse L1 clones, respectively (Cantrell et al. 2008). This reconstruction experiment suggested two points of interest: 1) young L1 copies were enriched even at far lower numbers than would be expected in a typical genome; and 2) the resulting phylogenies of L1 elements identified by this technique were more reflective of recent retrotransposition than of the complete history of L1 in that host species. The PCR relies on primers to conserved regions of L1 ORF2 and, thus, PCR amplified relatively young elements more readily than old degenerate elements. The colorimetric assay provides further enrichment for young elements by identifying elements with intact reading frames in the amplified region. The recent activity of L1s can be deduced from the structure of their phylogenetic trees. For example, if L1s have had recent bursts of retrotransposition in a species, this is reflected by the short terminal branch lengths

and abundance of open reading frames (ORFs) on the tree. Alternatively, if L1 activity is scant or absent, the past activity is revealed, and branch lengths tend to be longer and ORFs few or absent.

L1 activity within species.—As expected, species L1 trees tended to have a pectinate appearance with one or sometimes two lineages evident. Alternative L1 topologies in bats are shown in Figure 2. Single lineages are evident (Fig. 2A, B, and E), but a range of L1 activity can be implied in these species, from very active in *Tonatia saurophila bakeri* to low levels of recent activity in *Myzopoda aurita*. Extinction of L1 in megabats was reported previously (Cantrell et al. 2008) and is evident in these L1 phylogenies by the long terminal branch lengths and lack of ORFs in the two Pteropodidae (Fig. 2C and D). An independent L1 extinction event was evident in *Mormoops blainvilli* (Fig. 2F). Multiple lineages are evident in both L1 extinction events. Multiple lineages also are evident in species with active L1s. For example, *Rhinolophus eloquens* (Fig. 2G) had one active lineage and one extinct lineage, while *T. melanopogan* (Fig. 2H) had two very divergent active lineages. No L1 extinction events were found among the 27 species of Phyllostomidae examined, although some families possessed low levels of activity. As previously shown, L1 is extinct in all species of Pteropodidae (Cantrell et al. 2008).

L1 activity in Chiroptera.—To compare the evolution of L1s in Chiroptera to the phylogeny of their hosts, young L1s from genera examined by Teeling (Teeling et al. 2005) were analyzed. Five L1s with intact open reading frames from each species were included in the analysis; where multiple lineages were present, representatives from each L1 lineage were included. Five elements that lack intact reading frames from *Cynopterus sphinx* were included to represent the Pteropodidae. The reconstructed ancestors from both extinct Pteropodidae lineages (Pteropus 1, Pteropus 2) and from both extinct *Mormoops* lineages (Mormoops 1 and Mormoops 2) also were included.

Although there was an overall similarity between the L1 phylogeny and the bat phylogeny proposed by Teeling at al. (2005), there were many differences (Fig. 3). None of the superfamilies were conserved on the L1 phylogeny. Rhinolophidae and Hipposideridae clustered with the Yangochiroptera rather than the Yinpterochiroptera. Among the Yangochiroptera, L1s from Myzopodidae were sister to those from Vespertilionidae. The relationships among the Noctilionidae, Furipteridae, and Thyropteridae differed, and Nycteridae was not sister to Emballonuridae. *Taphozous* also was exceptional because of its two extremely divergent L1 active lineages (see below for further discussion of these lineages). One lineage clustered where expected with L1s from the other emballonurid, *Rhynchonycteris*. The other active L1 lineage in *Taphozous* clustered with L1s from the Yinpterochiroptera, and that lineage was the more active one in *Taphozous*. Although there were no active lineages in *M. blainvilli*, one of the two extinct lineages clustered with L1s from *Pteronotus quadridens*, consistent with its expected placement among the Mormoopidae.

There were two active L1 lineages present before the divergence of the families of bats. However, there must have been multiple extinctions within both ancestral L1 lineages over the course of chiropteran evolution, irrespective of which recently proposed chiropteran phylogeny is used for comparison. For example, one proposed phylogeny that supports the Yinptero- and Yangochiroptera groupings (Teeling et al. 2005) would require seven independent extinctions of L1 lineage 1 or lineage 2 to account for the active lineages observed in this study, whereas an alternative phylogeny (Van den Bussche and Hoofer 2004) would require eight L1 independent extinction events. The evolution of L1 in Chiroptera also was compared to phylogenies that support the monophyly of all microbats; this relationship required either seven (Jones et al. 2002) or nine (Agnarsson et al. 2011) independent extinction events. An example of mapping extinctions of L1 lineages onto the Teeling bat phylogeny is shown in Figure 4. Minimizing the number of lineage extinction events would require splitting the superfamily Rhinolophoidea so that 1) Megadermatidae, Craseonycteridae, and Rhinopomatidae were members of a clade with Pteropidae, and 2) Rhinolophidae and Hipposideridae were members of a clade with the Emballonuroidea, Noctillonoidea, and Verpertillonoidea (see Fig. 3B). This arrangement does not appear to be consistent with any proposed chiropteran phylogeny.

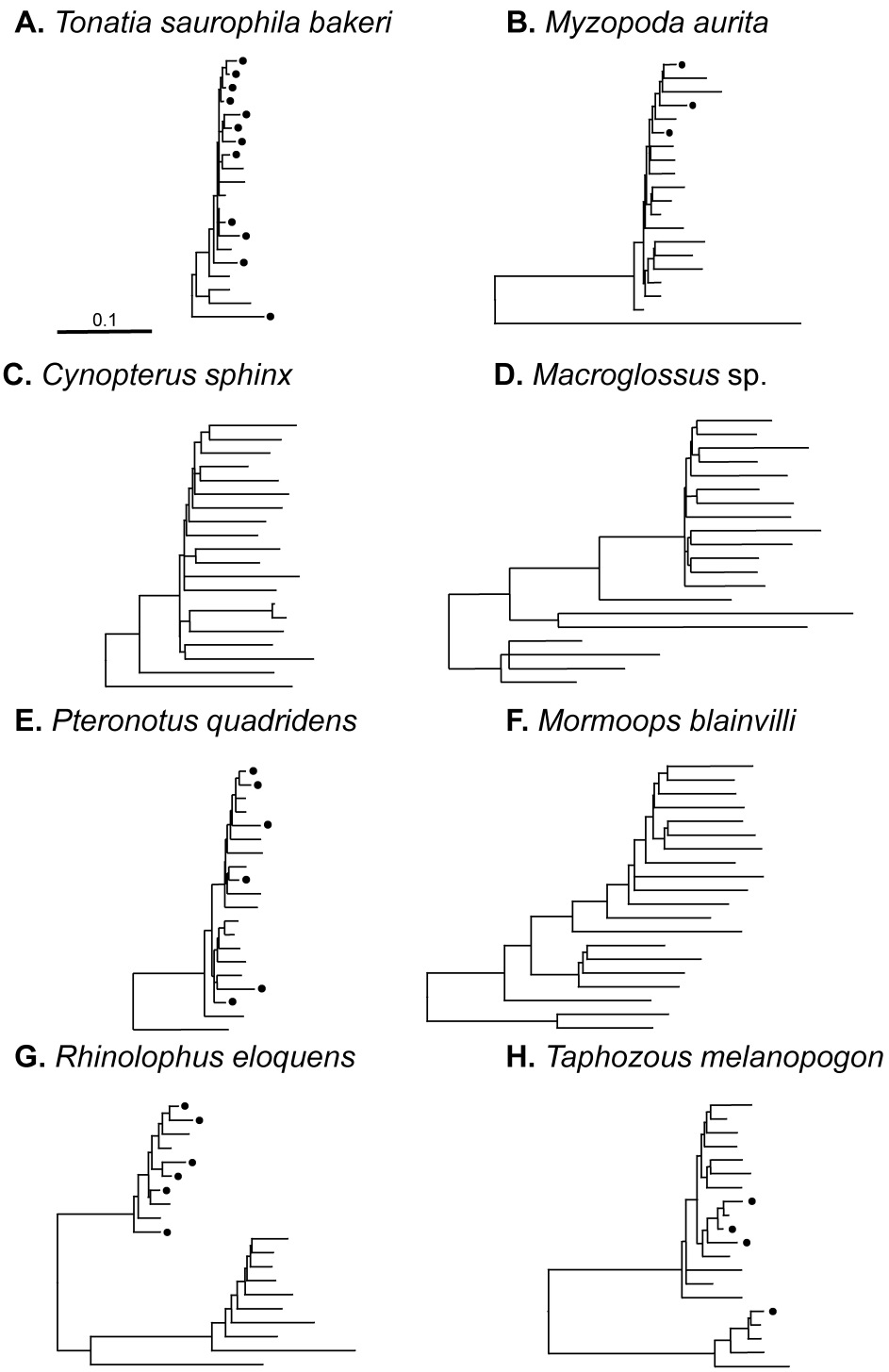

Figure 2. Example L1 phylogenies of 20 elements from eight bat species. Taxa names have been removed; a black dot represents an L1 with an open reading frame across the region of analysis, indicating recent L1 activity. Terminal branch lengths reflect relative time since insertion. The trees demonstrate the variation in bat L1 evolutionary dynamics: single and multiple lineages as well as cessation of activity.

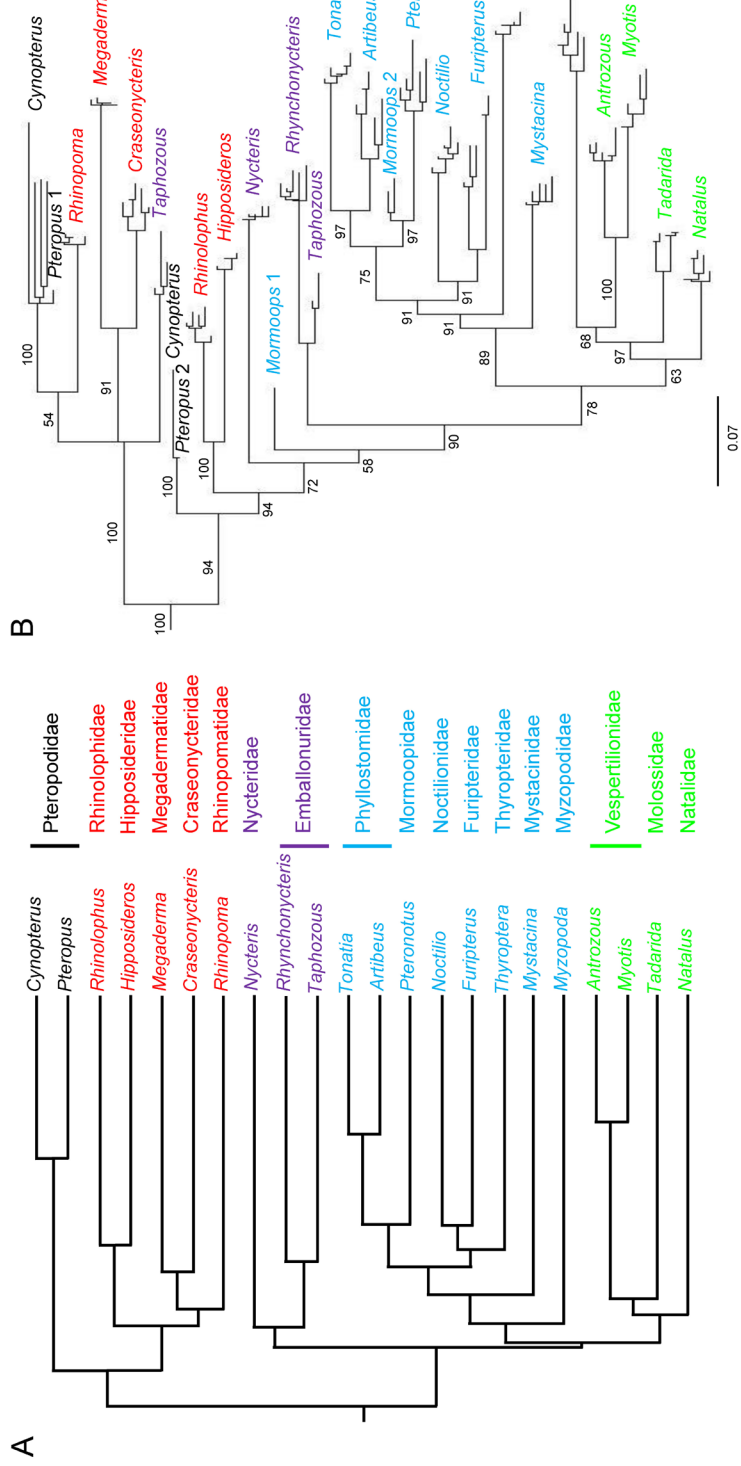

Figure 3. Comparison of phylogenies of bat families and bat L1 lineages. Taxa from all 18 bat families are included. Colors indicate families and genera within superfamilial groups: Rhinolophoidea, red; Emballonuroidea, purple; Noctillonoidea, blue; and, Verpertillonoidea, green. A. Family tree of Chiroptera derived from Teeling et al. Figure 2 (2005), from a maximum-likelihood analysis of a 13.7 kb concatenated data set. B. Maximum likelihood tree of L1s from the same genera as in tree A. Five L1s with open reading frames from each species plus reconstructed ancestral L1s from extinct lineages in *Pteropus* (Pteropus 1, Pteropus 2) and *Mormoops* (Mormoops 1 and Mormoops 2) are included in the analysis. Numbers at the nodes indicate majority rule bootstrap support values.

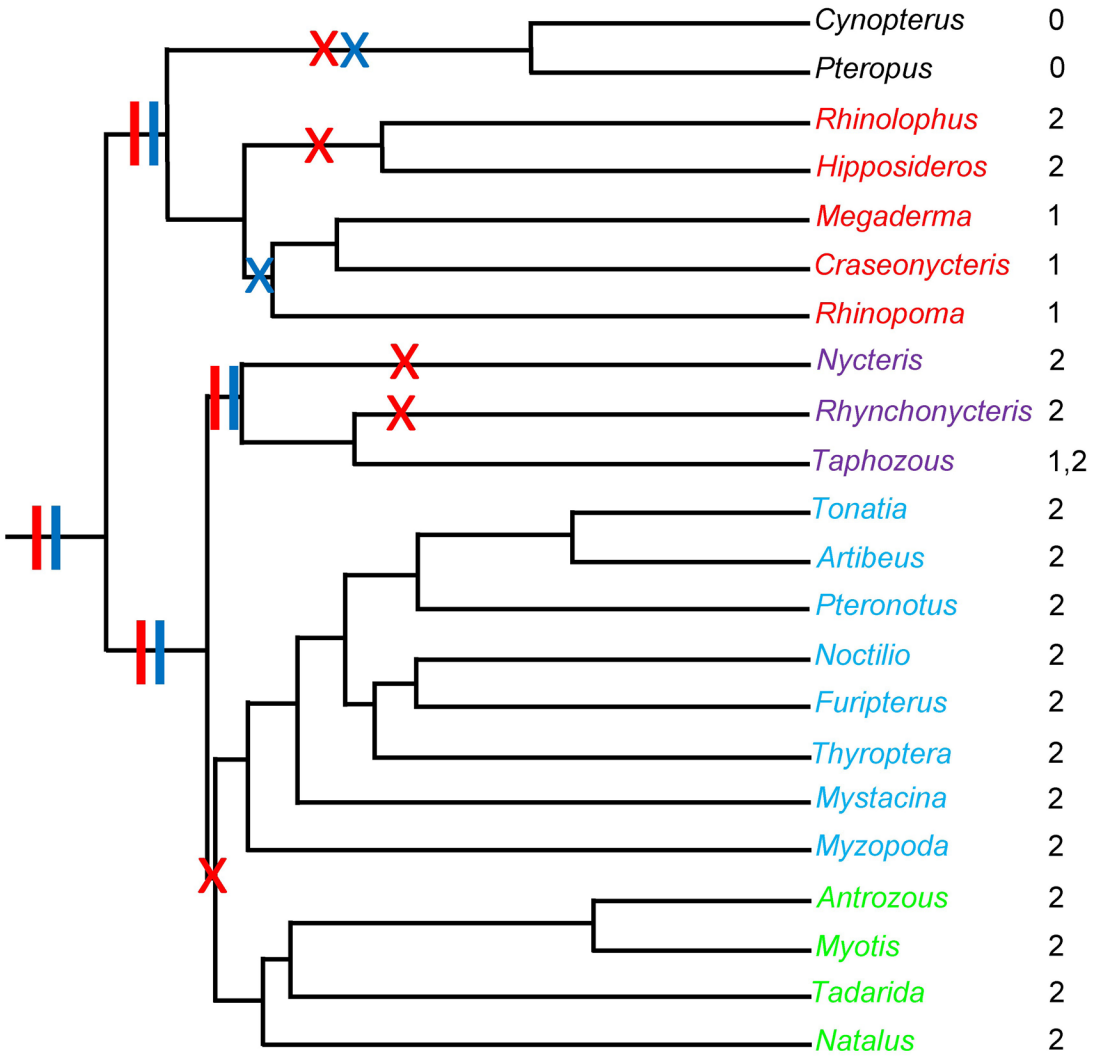

Figure 4. An example of activity and extinction of two ancient L1 lineages mapped onto the phylogeny of Chiroptera. Colors of taxa names indicate families and genera within superfamilial groups: Rhinolophoidea, red; Emballonuroidea, purple; Noctillonoidea, blue; and, Verpertillonoidea, green. Vertical bars represent activity of L1 lineages in common ancestors and Xs indicate extinction events: lineage 1, red; lineage 2, blue. Numbers to the right of the taxa are the lineage(s) active in the corresponding genus.

DISCUSSION

Persistence and extinction of L1s.—Persistence of L1 requires ongoing retrotransposition so that new active copies are inserted before debilitating mutations inactivate the minute fraction of L1s capable of replication; L1 lineages that do not replicate eventually will become extinct. Finding evidence of recent activity has not always been straightforward. Ancient L1s persist in the genome as molecular fossils that obscure the small subset of elements that are products of recent retrotransposition (Deininger et al. 1992; Deininger and Batzer 1993; Furano 2000). The method employed for this study is very sensitive for finding recently transposed L1s (Cantrell et al. 2000; Cantrell et al. 2008), but it does not uncover the complete history of L1s within a species because old elements generally are amplified only in the absence of younger elements. Although

this can be partially mitigated using the blue-white screening technique to enrich for clones both with and without intact reading frames over the region of interest, the phylogenies produced by this method should be considered a history of the most recent L1 activity rather than a complete history.

Occasionally, the active L1 lineages go extinct within a mammalian clade so that all subsequently derived species lack active L1s (Casavant et al. 2000; Cantrell et al. 2008; Sookdeo et al. 2018). Such extinctions may be underestimated because recognizing them requires that L1 copies remaining in the genome have acquired enough mutation to be clearly identifiable as inactive. Deeper extinctions are readily identifiable both because the fossil copies have accumulated more mutations and because cladogenesis after an L1 extinction event gives rise to more taxa that also lack active L1s. Why, then, have so few mammalian clades been discovered that lack active L1s? Certainly, sufficient mammalian clades to identify all L1 extinctions have not yet been examined, but among those mammals examined in this study, most were found to have active L1s. It is possible that this is just a historical accident—that L1 extinctions have occurred throughout mammalian evolution, but by chance few of those lineages gave rise to major mammalian radiations. This would make those extinction events harder to find because it would be necessary to locate one of a few species instead of one of many. For example, one could find the L1 extinction in Pteropodidae by looking at any one of the ~65 species in the family, but Mormoopidae contains only eight species and it is known that some of those still have active L1s. This study was very "lucky" to find the L1 extinction event in *M. blainvilli*.

Although only two complete extinctions of L1 activity were detected in Chiroptera, one in all Pteropodidae and one in *M. blainvilli*, a surprising number of L1 lineage extinctions in the group were identified. Additional sampling will be required to completely document the number of L1 lineage extinctions, but it seems likely that there have been at least seven independent deep extinctions (Fig. 4), as well as a number of more recent L1 lineage extinctions. For example, two lineage extinctions occurred in *M. blainvilli* to give rise to complete loss of L1 activity. Lineage extinction without loss of L1 activity likely occurred in several species where there was evidence of one active linage and one inactive one, such as *Hipposideros armiger* and *R. eloquens*. For reasons mentioned above, the methods used in this study likely underestimate the number of these extinctions. However, these lineage extinctions highlight what could be a major problem with using L1 phylogeny to reconstruct host phylogeny.

L1 activity and genome size in bats.—Among mammals, the genomes of Chiroptera are particularly interesting because average genome size is the lowest among mammalian orders—2.35 picograms in Chiroptera versus 3.5 picograms among all mammals (Smith et al. 2013). Although their small genome size seems exceptional, this has not hindered their evolutionary diversification. The order Chiroptera includes 20% of all extant species of placental mammals, second only to rodents (Wilson and Reeder 2005). Small genome size in both bats and birds has been proposed to be adaptive for flight (Hughes and Hughes 1995). Previous work has concluded that the reduced size of the chiropteran genome is due to extensive DNA loss due to deletions, rather than reduced gains due to retrotransposition (Kapusta et al. 2017). However, Pteropodidae have even smaller genomes than other bats—2.2 picograms—so lack of retrotransposition in these bats likely plays some role in restraining genome size (Smith et al. 2013).

Do L1s provide a function for the host?—Transposable elements are viewed widely as selfish parasites, but the long-term and widespread persistence of L1s has fueled speculation that they may provide a function for their mammalian hosts. Specific proposed functions include a role in chromosomal repair (Hutchison III et al. 1989; Morrish et al. 2002), X chromosome inactivation (Lyon 1998), modulating gene expression (Han et al. 2004; Elbarbary et al. 2016), and neuronal differentiation (Singer et al. 2010). However, if L1 elements play an essential function in their mammalian host, one must account for how that function would be maintained after the extinction of L1s, and that has not yet been documented for any of these proposed functions.

Whether L1s provide an essential function for the host is not known, but it may be that losing L1s could be deleterious in the long run. L1s account not only for their own retrotransposition but also for the movement of SINEs and processed pseudogenes, so losing the major source of retrotransposition in the genome

may be akin to drastically lowering the point mutation rate. In the short run, there may be no deleterious effect of losing L1 activity, and, in fact, the loss could be beneficial. But in the long run, the ability of species to evolve could be constrained by the reduction in the amount and type of genetic variation available. The central role of L1 in generating specific types of variation could be replaced by another retrotransposon. For example, sigmodontine rodents that lack active L1s have mysTR, a very active family of endogenous retroviruses (Erickson et al. 2011), but no such driver of retrotransposition has been found in the megabats.

L1s and their parasitic SINEs as phylogenetic markers.—"The only homoplasy-free phylogenetic marker is the new one" (Robert J. Baker)—meaning that each newly discovered phylogenetic marker is assumed to be homoplasy free, until sufficient data are generated that show otherwise. Given their vast representation in the genome, L1s and SINEs would seem to be ideal markers for reconstructing the history of their hosts. There are at least two ways by which retrotransposons might be used as phylogenetic markers for their mammalian hosts. First, the history of the L1s or SINEs can be reconstructed. At speciation events the active lineage will diverge and accumulate changes independently in the derived species (Sookdeo et al. 2018). Changes that accumulate in the active L1s can be used as markers to reconstruct the history of their hosts (Verneau et al. 1997; Casavant et al. 1998; Verneau et al. 1998). Second, individual insertions of L1s, SINEs, or other retrotransposons can be used as presence-or-absence characters that can be detected by PCR with flanking single copy primers (Shedlock and Okada 2000). Because there are so many L1 and SINE inserts in the genome, there is an almost unlimited supply of potential markers across a wide range of ages.

Neither of these approaches is completely homoplasy free. First, both may be subject to lineage sorting, as are all phylogenetic markers. As seen here, this may be more serious when reconstructing L1 (or SINE) history because multiple active lineages can coexist, and active lineages can go extinct in patterns that do not recapitulate species histories. It might be assumed that this would not be a problem when using individual insertions as presence-or-absence characters, but same-site insertions do occur. For example, a study of insertions sites of *mys* retrotransposons in the *Peromyscus* genome revealed both lineage sorting (Lee et al. 1996) and same-site insertions (Cantrell et al. 2001). One ancient *mys* insertion had accumulated 12 independent insertions of other retroelements among 13 alleles examined. At two sites, the insertions used identical initial nick sites to insert, but were clearly different events; in one case, two SINEs from different families inserted into the same site, and in another case, the insertions were resolved differently at the 5′ insertion site (Cantrell et al. 2001). Although allele size differences would have been detectable between some alleles in a presence-or-absence PCR assay, some alleles containing different insertions would have appeared to be the same size. It is unknown how common such insertional 'hot spots' are in mammalian genomes, but these findings caution against using a small number of insertion sites for phylogenetic reconstruction of the host. However, studies of millions of *Alu* SINE insertions in primates found that 0.01% or less exhibited homoplasy (Doronina et al. 2018). Phylogenies based on a large number of retrotransposon insertions sites distributed across the genome should be more phylogenetically robust than either studies based on single nucleotide polymorphisms (SNPs) or comparison of retrotransposons phylogenies to host phylogenies.

It was not the intent of this study to use L1s to reconstruct chiropteran phylogeny. Instead, the chiropteran phylogeny was used to better understand the biology of L1 elements. The findings of the study suggest that there may have been extensive lineage sorting of L1 elements in bats, along with a number of cases of multiple, highly diverged active lineages. It appears that the order began its history with two active lineages that were already ~27% divergent at the time of their extinction in the Pteropodidae. These two lineages gave rise to the active lineages in all Chiroptera, but through a lineage sorting process that did not result in L1 phylogeny recapitulating chiropteran phylogeny. Both lineages survived in at least one species, *Taphozous melanopogon*, where the two clades now differ by ~33%. The two complete extinctions of L1 activity in the order, along with the numerous extinctions of L1 lineages over time, may reflect the intensity of the ongoing arms race between L1 for its survival and strong selection on genome size in Chiroptera.

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LITERATURE CITED

Agnarsson, I., C. M. Zambrana-Torrelio, N. P. Flores-Saldana, and L. J. May-Collado. 2011. A time-calibrated species-level phylogeny of bats (Chiroptera, Mammalia). PLoS Currents 3:RRN1212.

Boissinot, S., and A. Sookdeo. 2016. The evolution of LINE-1 in vertebrates. Genome Biology and Evolution 8:3485–3507.

Cantrell, M. A., et al. 2001. An ancient retrovirus-like element contains hot spots for SINE insertion. Genetics 158:769–777.

Cantrell, M. A., R. A. Grahn, L. Scott, and H. A. Wichman. 2000. Isolation of markers from recently transposed LINE-1 retrotransposons. Biotechniques 29:1310–1316.

Cantrell, M. A., L. Scott, C. J. Brown, A. R. Martinez, and H. A. Wichman. 2008. Loss of LINE-1 activity in the megabats. Genetics 178:393–404.

Casavant, N. C., R. N. Lee, A. N. Sherman, and H. A. Wichman. 1998. Molecular evolution of two lineages of L1 (LINE-1) retrotransposons in the california mouse, *Peromyscus californicus*. Genetics 150:345–357.

Casavant, N. C., L. Scott, M. A. Cantrell, L. E. Wiggins, R. J. Baker, and H. A. Wichman. 2000. The end of the LINE?: Lack of recent L1 activity in a group of South American rodents. Genetics 154:1809–1817.

Casavant, N. C., A. N. Sherman, and H. A. Wichman. 1996. Two persistent LINE-1 lineages in *Peromyscus* have unequal rates of evolution. Genetics 142:1289–1298.

Clough, J. E., J. A. Foster, M. Barnett, and H. A. Wichman. 1996. Computer simulation of transposable element evolution: Random template and strict master models. Journal of Molecular Evolution 42:52–58.

de Koning, A. P., W. Gu, T. A. Castoe, M. A. Batzer, and D. D. Pollock. 2011. Repetitive elements may comprise over two-thirds of the human genome. PLoS Genetics 7:e1002384.

Deininger, P. L., and M. A. Batzer. 1993. Evolution of retroposons. Evolutionary Biology 27:157–196.

Deininger, P. L., M. A. Batzer, C. A. Hutchison, 3rd, and M. H. Edgell. 1992. Master genes in mammalian repetitive DNA amplification. Trends in Genetics 8:307–311.

Dewannieux, M., C. Esnault, and T. Heidmann. 2003. LINE-mediated retrotransposition of marked *Alu* sequences. Nature Genetics 35:41–48.

Dewannieux, M., and T. Heidmann. 2005. L1-mediated retrotransposition of murine B1 and B2 SINEs recapitulated in cultured cells. Journal of Molecular Biology 349:241–247.

Doronina, L., O. Reising, H. Clawson, D. A. Ray, and J. Schmitz. 2018. True homoplasy of retrotransposon insertions in primates. Systematic Biology 68:482–493.

Elbarbary, R. A., B. A. Lucas, and L. E. Maquat. 2016. Retrotransposons as regulators of gene expression. Science 351:aac7247.

Erickson, I. K., M. A. Cantrell, L. Scott, and H. A. Wichman. 2011. Retrofitting the genome: L1 extinction follows endogenous retroviral expansion in a group of muroid rodents. Journal of Virology 85:12315–12323.

Furano, A. V. 2000. The biological properties and evolutionary dynamics of mammalian LINE-1 retrotransposons. Progress in Nucleic Acid Research and Molecular Biology 64:255–294.

Goodier, J. L., E. M. Ostertag, and H. H. Kazazian, Jr. 2000. Transduction of 3'-flanking sequences is common in L1 retrotransposition. Human Molecular Genetics 9:653–657.

Han, J. S., S. T. Szak, and J. D. Boeke. 2004. Transcriptional disruption by the L1 retrotransposon and implications for mammalian transcriptomes. Nature 429:268–274.

Hughes, A. L., and M. K. Hughes. 1995. Small genomes for better flyers. Nature 377:391.

Hutchison III, C. A., S. C. Hardies, D. D. Loeb, W. R. Shehee, and M. H. Edgell. 1989. LINEs and related retroposons: long interspersed repeated sequences in the eucaryotic genome. Pp. 593–617 in Mobile DNA (D. E. Berg and M. M. Howe, eds.). American Society for Microbiology, Washington, DC.

Ivancevic, A. M., R. D. Kortschak, T. Bertozzi, and D. L. Adelson. 2016. LINEs between species: Evolutionary dynamics of LINE-1 retrotransposons across the eukaryotic tree of life. Genome Biology and Evolution 8:3301–3322.

Jones, K. E., A. Purvis, A. MacLarnon, O. R. P. Bininda-Edmonds, and N. B. Simmons. 2002. A phylogenetic supertree of bats (Mammalia: Chiroptera). Biological Reviews 77:223–259.

Kapusta, A., A. Suh, and C. Feschotte. 2017. Dynamics of genome size evolution in birds and mammals. Proceedings of the National Academy of Science USA 114:E1460–E1469.

Kazazian, H. H., Jr., C. Wong, H. Youssoufian, A. F. Scott, D. G. Phillips, and S. E. Antonarakis. 1988. Haemophilia A resulting from de novo insertion of L1 sequences represents a novel mechanism for mutation in man. Nature 332:164–166.

Lee, R. N., J. C. Jaskula, R. A. van den Bussche, R. J. Baker, and H. A. Wichman. 1996. Retrotransposon Mys was active during evolution of the *Peromyscus leucopus-maniculatus* complex. Journal of Molecular Evolution 42:44–51.

Lyon, M. F. 1998. X-chromosome inactivation: a repeat hypothesis. Cytogenetics and Cell Genetics 80:133–137.

Morrish, T. A., et al. 2002. DNA repair mediated by endonuclease-independent LINE-1 retrotransposition. Nature Genetics 31:159–165.

Ostertag, E. M., and H. H. Kazazian, Jr. 2001. Biology of mammalian L1 retrotransposons. Annual Review of Genetics 35:501–538.

Platt, R. N., II, M. W. Vandewege, and D. A. Ray. 2018. Mammalian transposable elements and their impacts on genome evolution. Chromosome Research 26:25–43.

Shedlock, A. M., and N. Okada. 2000. SINE insertions: powerful tools for molecular systematics. Bioessays 22:148–160.

Singer, T., M. J. McConnell, M. C. Marchetto, N. G. Coufal, and F. H. Gage. 2010. LINE-1 retrotransposons: mediators of somatic variation in neuronal genomes? Trends in Neuroscience 33:345–354.

Smith, J. D., J. W. Bickham, and T. R. Gregory. 2013. Patterns of genome size diversity in bats (order Chiroptera). Genome 56:457–472.

Sookdeo, A., C. M. Hepp, and S. Boissinot. 2018. Contrasted patterns of evolution of the LINE-1 retrotransposon in perissodactyls: the history of a LINE-1 extinction. Mobile DNA 9:12.

Swofford, D. L. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Sinauer Associates, Sunderland, Massachusetts.

Teeling, E. C., M. S. Springer, O. Madsen, P. Bates, S. J. O'Brien, and W. J. Murphy. 2005. A molecular phylogeny for bats illuminates biogeography and the fossil record. Science 307:580–584.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673–4680.

Van den Bussche, R. A., and S. R. Hoofer. 2004. Phylogenetic relationships among recent Chiropteran families and the importance of choosing appropriate out-group taxa. Jounal of Mammalogy 85:321–330.

Verneau, O., F. Catzeflis, and A. V. Furano. 1997. Determination of the evolutionary relationships in (Rodentia : Muridae) using L1 (LINE-1) amplification events. Journal of Molecular Evolution 45:424–436.

Verneau, O., F. Catzeflis, and A. V. Furano. 1998. Determining and dating recent rodent speciation events by using L1 (LINE-1) retrotransposons. Proceedings of the National Academy of Science USA 95:11284–11289.

Wilson, D. E., and D. M. Reeder. 2005. Mammal Species of the World. Johns Hopkins University Press, Baltimore.

Yang, Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. Journal of Molecular Evolution 39:306–314.

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Coyote Diet, Prey Densities, and Prey Biomass in Joshua Tree National Monument, California

John E. Cornely

Abstract

From May 1976 through January 1978, the diet of coyotes (*Canis latrans*) and the relationship of diet to prey density and biomass were examined in Joshua Tree National Monument, California. Coyote diets were estimated by identifying the remains of individual prey species in 215 scats from ten sampling periods from the study area. Leporids were censused seasonally using a spotlight area estimate method. Rodent populations were estimated by a mark and recapture assessment line technique. Prey biomass was estimated by multiplying the mean wet weight of each prey species from the study area times their density. Black-tailed jackrabbit (*Lepus californicus*) densities changed significantly from season to season. Eight species of rodents were captured, with the desert woodrat (*Neotoma lepida*) having the highest mean densities and Merriam's kangaroo rat (*Dipodomys merriami*) second. Although densities of *N. lepida* and *D. merriami* were nearly identical, the average biomass of *N. lepida* was more than twice as high. Total rodent density and biomass did not differ among seasons. Coyotes consumed *N. lepida* and *Chaetodipus/Perognathus* spp. roughly in proportion to their densities. *Dipodomys merriami, Peromyscus* spp., and southern grasshopper mice (*Onychomys torridus*) were taken less often than expected with respect to their densities, while *L. californicus* was taken more often than expected. *Lepus californicus* had the highest mass per scat. There were more *N. lepida* per ha than any other prey species, but they ranked third in mass per scat behind *L. californicus* and desert cottontail (*Sylvilagus audubonii*). Mass of prey per scat and biomass of prey per ha were significantly correlated whereas numbers of prey per scat and densities of prey were not. As prey biomass increased, the number of different species per scat decreased. Prey size and biomass are important parameters when interpreting coyote diet patterns.

Key words: *Canis latrans*, coyote diet, coyote foraging strategy, Joshua Tree National Monument, *Lepus californicus*, *Neotoma lepida*, prey densities and biomasses, seasonal changes, *Sylvilagus audubonii*

Introduction

The coyote (*Canis latrans)* was the most abundant and most widely distributed mesocarnivore in Joshua Tree National Monument (JTNM). For many decades the coyote has been the subject of numerous studies and the center of a great deal of controversy. Many of the research projects on coyotes have investigated their depredations on domestic livestock, game mammals and game birds. These studies have usually been conducted in areas where coyotes have been subjected to some measure of "population control." Because of widespread predator control in western states, little information is available concerning unexploited coyote populations. National Parks, therefore, offer unique opportunities for studies of unexploited populations and for comparisons with populations subject to lethal removal. Of particular value are large, remote tracts where the impact of human activities is relatively minimal.

Numerous coyote food habits or diet studies have been completed, but only since the 1970s have some researchers compared coyote diets and demography

with prey densities. The purpose of this study was to investigate the diet of coyotes in an area of JTNM and to compare the diet with prey densities and biomasses in the study area. These results provide insight into some aspects of the ecology of an unexploited coyote population and may be useful in comparison with populations subjected to lethal control or those whose behavior has been significantly modified by human activities.

JOSHUA TREE NATIONAL MONUMENT AND STUDY AREA

Joshua Tree National Monument, established in 1936, included about 233,950 ha of rugged desert terrain in San Bernardino and Riverside counties in southern California. The monument included parts of two large desert ecosystems delineated primarily on the basis of elevation: the Mojave or high desert and the Colorado or low desert (see Figure 1 for the boundaries and location of the monument in southern California during the study). In 1994, the monument was upgraded to Joshua Tree National Park and currently includes about 319,959 ha. The additions to what was JTNM were on the south and east sides.

Elevations within JTNM range from about 300 m to 1,772 m. The topography of JTNM consists of a series of rugged desert mountain ranges separated by relatively flat valleys such as Covington Flat, Lost Horse Valley, Queen Valley, Pleasant Valley, and the Pinto Basin.

Weather.—Table 1 summarizes weather data collected at Lost Horse Ranger Station in 1976 and 1977, the period of this study. Lost Horse weather station is on the edge of the study area, and these data are representative of the weather conditions during the study. The elevation at Lost Horse Ranger Station (1,252 m) is considerably higher than that the JTNM headquarters at Twentynine Palms (600 m). The higher elevations of the monument have milder summer temperatures, colder winter temperatures, and receive more precipitation. Precipitation during 1976 was well above average. Snowfall was recorded at Lost Horse in March 1976 and again in March 1977. In general, the climate of JTNM is characterized as a harsh, arid desert climate.

Flora and fauna.—Miller and Stebbins (1964) recognized three major plant belts corresponding with elevation. They are the creosote bush belt (300 m to 914 m), the yucca belt (914 m to 1,280 m) and the piñon

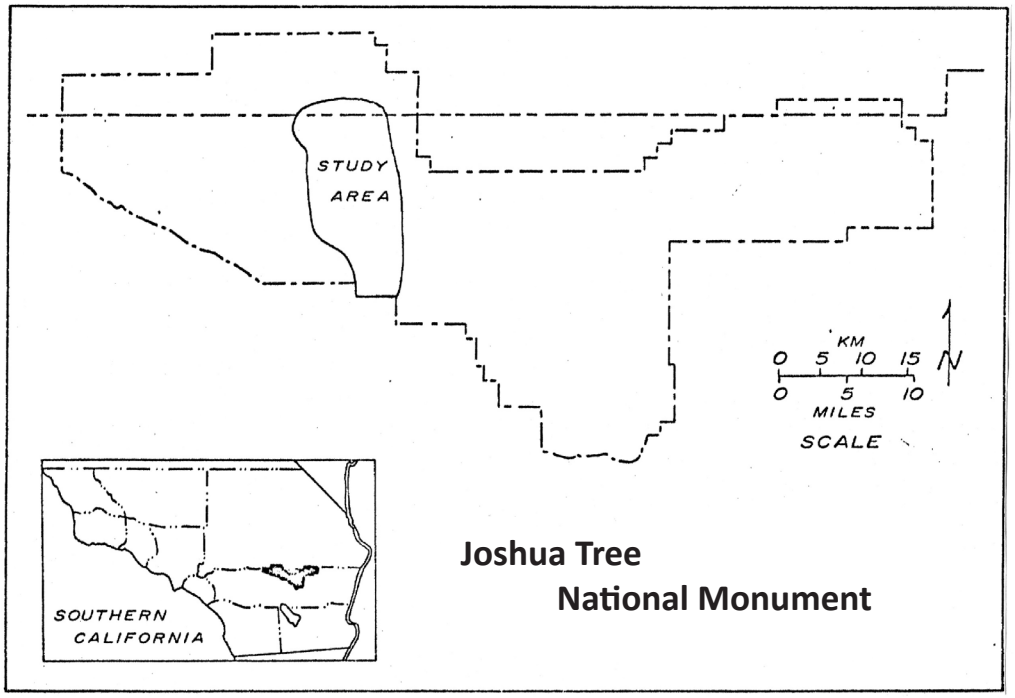

Figure 1. Location of the study area in southwestern Joshua Tree National Monument, California.

Table 1. Monthly precipitation and mean daily minimum and maximum temperatures at Lost Horse Ranger Station, elevation 1,252 m, Joshua Tree National Monument, 1976 and 1977.

	1976			1977		
Month	Precipitation (cm)	Mean Temperature (°C) Min	Max	Precipitation (cm)	Mean Temperature (°C) Min	Max
January	0.08	-3.19	15.04	3.05	-2.59	11.20
February	7.29	0.67	14.96	0.00	-2.74	17.76
March	0.89	0.67	15.82	0.76	-3.07	14.24
April	0.99	2.54	18.87	0.30	0.93	22.81
May	0.23	7.29	26.68	1.14	3.57	21.00
June	0.05	--	--	0.15	14.09	33.09
July	4.09	17.22	30.81	0.33	16.13	34.44
August	0.00	12.51	30.98	6.10	17.08	32.62
September	10.36	12.37	27.24	0.94	--	--
October	2.21	5.42	23.98	0.50	6.47	25.43
November	0.56	-0.56	17.64	0.00	0.30	20.52
December	0.66	-3.89	15.27	1.47	--	--
Total	27.36			12.33		

belt (1,280 m to 1,772 m). In addition, they recognized the following general habitat types: sand dune, desert wash, creosote bush, cholla cactus, oasis, yucca, desert grassland, rocky canyon, piñon, and chaparral. Leary (1977) investigated the plant communities on the monument and recognized four plant communities and 15 plant associations.

One of the most impressive plants is the Joshua tree (*Yucca brevifolia*) for which the monument was named. Also within the monument are six oases dominated by native desert fan palms (*Washingtonia filifera)*. According to Leary (1977), 634 taxa of vascular plants occur within the monument.

The climatic, topographical, and plant diversity has resulted in an interesting and diverse fauna. Miller and Stebbins (1964) reported the occurrence of five species of amphibians, 36 species of reptiles, 167 species of birds, and 42 species of mammals. A more recent JTNM animal list includes 44 species of reptiles, more than 260 species of birds, and 54 species of mammals (National Park Service 2017; Cornely 1977). Six species of rattlesnake (*Crotalus* spp.) have been reported. One of the mammals that generates the most public interest is the desert bighorn sheep (*Ovis canadensis nelsoni*).

Description of the study area.—The study area was located in the western half of JTNM and encompassed about 168 sq km (Fig. 1). To minimize interactions with park visitors and the influence they may have on coyote behavior and diet, much of the study area was located in a relatively remote area.

The entire study area was within the Yucca Belt described by Miller and Stebbins (1964). The following vegetative associations occurred within the area (Leary 1977): the *Yucca brevifolia* (Joshua tree)-*Hilaria rigida* (galleta grass) association; the *Coleogyne ramosissima* (blackbrush)-*Yucca schidigera* (Mojave yucca) association; the *Coleogyne ramosissima* (blackbrush)-*Yucca schidigera* (Mojave yucca)-*Juniperus californica* (California juniper) association; the *Yucca schidigera* (Mojave yucca)-*Yucca brevifolia* (Joshua tree)-*Coleogyne ramosissima* (blackbrush) association; the *Yucca schidigera* (Mojave yucca)-*Yucca brevifolia* (Joshua tree)-mixed shrub association; the

desert woodland association; the granitic outcrop association; the *Yucca schidigera* (Mojave yucca)-*Yucca brevifolia* (Joshua tree)-*Juniperus californica* (California juniper)-*Coleogyne ramosissima* (blackbrush) association; the *Hilaria rigida* (galleta grass)-*Oryzopsis hymenoides* (Indian ricegrass) association; and the *Larrea tridentata* (creosote bush)-*Ambrosia dumosa* (burr-sage) association. For a more detailed description of the vegetation, see Leary (1977).

METHODS AND MATERIALS

Coyote diet analysis.—The diet of coyotes was studied by the examination of prey remains found in scats on or near 32 km of unpaved roads in the center of the study area. Initially, all coyote scats were removed from roads so that scats collected subsequently could be dated. There were ten sampling periods between August 1976 and January 1978. Samples were collected at approximately bi-monthly intervals and stratified by season. Sampling seasons were Fall 1976, Winter 1976, Summer 1977, and Winter 1977–1978. All scats were sterilized in an autoclave to kill parasites and other disease organisms prior to laboratory analysis.

Sterilized scats were air-dried, broken apart by hand, and examined under a dissecting microscope. Samples of hair, feathers, and scales were removed for further analysis. The remaining material was soaked in a 2:1 mixture of water and liquid drain cleaner to dissolve the remaining hair and facilitate the examination and identification of bones and teeth. Osteological remains were identified by comparison with museum specimens. The number of individuals of each prey species in each scat was estimated by counting teeth and other diagnostic skeletal remains. For example, if three lower jaws of a species were identified in a single scat, then it was estimated that the sample included two individuals of that species. The presence of insect remains or plant material was noted, but because they occurred at such a low frequency they were not subject to detailed analysis.

Reptilian scales were compared to scales of museum specimens. Hairs were identified using keys developed by Mayer (1952), Adorjan and Kolenosky (1969), and Moore et al. (1974). Hair cross sections were prepared using the technique of Coman and Brunner (1971) and were compared to cross sections of hairs from museum specimens.

Percent occurrence for all food items was determined. Numbers of individuals of each prey species per scat and prey mass per scat were estimated. Estimates of prey mass per scat were calculated by multiplying the estimated number of each prey species per scat by the mean live weight of that species. Rodent weights were derived from animals captured during seasonal trapping. Because it was not possible to reliably distinguish between remains of San Diego pocket mouse (*Chaetodipus fallax*) and little pocket mouse (*Perognathus longimembris*) in scats, the mean weights of the two species from trapping results in the study area were added together and then divided by two. That number (12.43 g) was multiplied by the frequency per scat to calculate the mass. Leporid weights were mean live weights of specimens collected in the study area. Unidentified leporids were assigned a mass of 1,296 g. This was derived in the following manner: desert cottontails (*Sylvilagus audubonii)* comprised 67% of the identified leporids in scats while black-tailed jackrabbits (*Lepus californicus*) made up 33%. It was assumed that these two species made up those same percentages in the unidentified leporids. The 1,296 g figure is the weighted average mass of these two species derived from specimens collected in the study area. Analysis of variance (ANOVA) and Student-Newman-Keuls (SNK) tests were used to analyze seasonal changes in the number of individual prey per scat and differences in the number of individuals per scat between species (Sokal and Rolf 1969). These methods were also employed to detect differences in seasonal masses of prey per scat and differences in masses per scat between species. Wet mass estimates for *L. californicus* were derived by multiplying the mean wet weight of *L. californicus* collected seasonally in the study area by seasonal density estimates.

Prey population analysis.—Seasonal leporid and rodent sampling was used to estimate the population density and biomass of potential mammalian prey species. Leporid abundances were estimated using the area-estimate method of Flinders and Hansen (1973). Individuals were counted along five 1.61 km transects

adjacent to unpaved roads in the center of the study area. Each transect was traveled on three consecutive nights in October 1976 and May, August, and October 1977, beginning one hour after sunset during the dark phase of the moon. The beam from a hand-held spotlight was directed perpendicular to the line of travel by an observer in the bed of a pick-up truck. All leporids observed within 100 m of the observer were counted while the truck traveled at 8–16 kph. Seasonal *L. californicus* densities were subjected to ANOVA and SNK tests.

Rodent populations were estimated using a mark-recapture method involving two parallel transects of Sherman live traps with assessment lines (O'Farrell et al. 1977; O'Farrell and Austin 1978). Each transect line had 40 trap stations with 15 m between stations. They were 53 m apart and ran north and south. Six additional assessment trap lines were positioned at 45 degree angles on the outside of each of the transect lines. Each of these assessment lines consisted of 18 trap stations, 15 m apart. The assessment lines were used to determine how far outside the main transects individual rodents were traveling in order to determine the mean home range of each species. The trapping configuration was located near the south end of the study area and was characterized by the *Y. schidigera-Y. brevifolia-J. californica* association. The vegetation was sparse and the area had extensive open sandy soil areas.

One aluminum Sherman live trap (23x8x9 cm) was set at each trap station. Trapping was conducted during the dark phase of the moon to coincide with maximum nocturnal rodent activity. Traps were set in late afternoon and baited with rolled oats. While trapping on the main lines, traps were checked between 2100 and 2300 h and subsequently between 0600 and 0800 h the next morning. Traps remained closed during most of the day to avoid overheating captured animals. The main lines were trapped until fewer than 10% of the rodents caught during a session were unmarked. Traps were then moved to the assessment lines, opened for three nights, but checked only in the mornings in accordance with O'Farrell et al. (1977).

Captured animals were individually marked by toe clipping to identify individuals, then sexed, aged, weighed, and the reproductive condition was determined. Two age classes (juvenile and adult) were determined using pelage characteristics and weight. Rodents were weighed to the nearest 0.1 g with a Pesola spring scale. The data were processed using a computer program based on the calculations reported by O'Farrell et al. (1977). This program calculated density, mass, and mean weight for each species by sex and age class. Analysis of variance was used to analyze seasonal rodent densities and biomasses. Correlation coefficients were calculated comparing mean number of prey per scat and mean density of prey species, as well as mean mass of prey per scat and mean biomass of prey per ha. The relationship of seasonal changes of total prey biomass to the number of species detected per scat was analyzed using a Chi-square test of independence.

RESULTS

Coyote diet analysis.—Diet analysis was based on 215 scats from 10 bi-monthly sampling periods from August 1976 through January 1978. Sample size per period ranged from 13 to 29 scats. Remains of 419 individuals were identified, including two species of lagomorphs and at least eight species of rodents. Two species of *Peromyscus* were trapped on transects, but identification of *Peromyscus* in scats was not reliable to the species level.

Mammals were the vast majority of items identified, occurring in nearly 99% of scats. The percent occurrence of non-mammalian items was less than 10%. Overall, remains of insects, birds, lizards, and plants were encountered at such low frequencies that they did not constitute important components of coyote diets in the study area during the period of study.

The percent occurrence of desert woodrats (*Neotoma lepida)* was 47% compared to 25.1% for *Chaetodipus*/*Perognathus* spp. and 24.2% for *S. audubonii*. More than one species of pocket mouse probably occurred in the scats, but identification of these remains was not reliable below the genus level, therefore

pocket mouse remains were considered to be those of either *C. fallax* or *P. longimembris* and the results were combined. Next in order of percent occurrence was Merriam's kangaroo rat (*Dipodomys merriami*) (17.2%) followed by *L. californicus* (15.4%), white-tailed antelope squirrel (*Ammospermophilus leucurus*) (11.2%), unidentified leporids (8.4%), Botta's pocket gopher (*Thomomys bottae)* (3.3%), and *Peromyscus* spp. (2.8%). Combining the leporids results in a percent occurrence of 44.7%, just below the value for *N. lepida*.

Percent occurrence does not account for the presence of more the one individual of a species in the same scat. Individuals of *N. lepida* represented 34.8% of the total individuals identified in the scats. *Chaetodipus/ Perognathus* spp. followed with 19.3%. Next in order were *S. audubonii* (12.9%), *D. merriami* (9.6%), *A. leucurus* (7.6%), *L. californicus* (6.4%), unidentified leporids (4.8%), *T. bottae* (2.2%), and *Peromyscus* spp. (1.7%). Combined leporids accounted for 24.1% of all individuals identified. Although the percentages were different, the ranking by species was very similar when comparing percent occurrence to percent of individuals identified.

A more biologically meaningful way to analyze coyote diet is to estimate the mass ingested of each prey species detected per scat. This is the most appropriate method when investigating the ecological energetics of coyotes. Because of large differences in the average weights of prey species, percentages of the mass ingested per scat corresponding to each prey species differ considerably from the percent occurrences and the percent of individuals for these same species. Individuals of *L. californicus* made up 44.9% of the mass ingested per scat and *S. audubonii* made up 25.3%. Unidentified leporids were 16.8% of the total. *Neotoma lepida* made up 10.6%, *A. leucurus* 1.8%, *D. merriami* 1%, *Chaetodipus/Perognathus* spp. 0.6%, *T. bottae* 0.3%, and *Peromyscus* spp. 0.1%. Combined leporids comprised 87% of the mass ingested per scat. Leporids plus *N. lepida* made up 97.6% of the mass ingested. Table 2 gives the mean number of mammalian prey per scat and mean mass per scat. There were important temporal changes in coyote diet in the study area. Figure 2 presents the numbers of prey per scat by season. Through the first four sampling periods, *Chaetodipus/Perognathus* spp. were the most common prey item. Through the rest of the study, *N. lepida* remains are dominant numerically. The numerical contribution of *S. audubonii* to the diet increased steadily until the December 1976 sample, when it was detected in equal frequency with *Chaetodipus/Perognathus* spp. Through the remainder of the study, *S. audubonii* remains were second only to *N. lepida* in frequency.

The results of a two-way ANOVA on numbers of prey per scat resulted in a non-significant F-ratio (1.03, $P \leq 0.001$) between seasons indicating that the number of individuals per scat did not change significantly from season to season. A highly significant F-ratio (24.75, $P \leq 0.001$) between prey species points out that the number of individuals per scat differs between prey species, and a highly significant interaction (Prey Species x Seasons) F-ratio (3.65, $P \leq 0.001$) indicates that numerical contributions of the various prey species in the diet changed from season to season.

An SNK test ($P \leq 0.01$) showed that significantly more individuals of *N. lepida* were detected than any other prey. The number of leporids per scat was not significantly different from the number of *Chaetodipus/ Perognathus,* but was significantly higher than *Peromyscus* spp., *T. bottae*, *A. leucurus*, and *D. merriami*, but lower than *N. lepida*. The number of *Chaetodipus/ Perognathus* spp. was significantly higher than *Permyscus* spp., *T. bottae*, and *A. leucurus*, not different from *D. merriami*, and lower than *N. lepida*. Numbers of *D. merriami, A. leucurus, T. bottae*, and *Peromyscus* spp. were not significantly different.

Although the numbers of individual prey detected in coyote scats provides an interesting description of the coyote diet, an estimate of the mass of each prey group ingested is more important in examining the relative importance of the various prey to the diet. Figure 3 presents the changes in the total mass of prey ingested per scat by sampling period. The *Chaetodipus/Perognathus* mean combines the two species. The mean weights of the two species from the study area were added together and then divided by two. That number (12.43 g) was multiplied by the frequency per scat to calculate the mass.

Even though individuals of *L. californicus* were almost always found in the diet at lower frequencies than *N. lepida, Chaetodipus/Perognathus* spp., and *S. audubonii,* during four of the sampling periods the mass

Table 2. Mean number of prey individuals identified per scat and mass of prey per scat from *Canis latrans* scats collected in southwestern Joshua Tree National Monument, from Fall 1976 through Winter 1977–1978.

Species	Mean Number Per Scat	Mean Biomass Per Scat (g)
Neotoma lepida	0.68	75.4
Chaetodipus/Perognathus spp.	0.38	4.4
Sylvilagus audubonii	0.25	183.0
Dipodomys merriami	0.19	7.5
Lepus californicus	0.13	317.2
Ammospermophilus leucurus	0.15	14.5
Unidentified leporids	0.09	116.6
Thomomys bottae	0.04	2.0
Peromyscus spp.	0.03	0.5

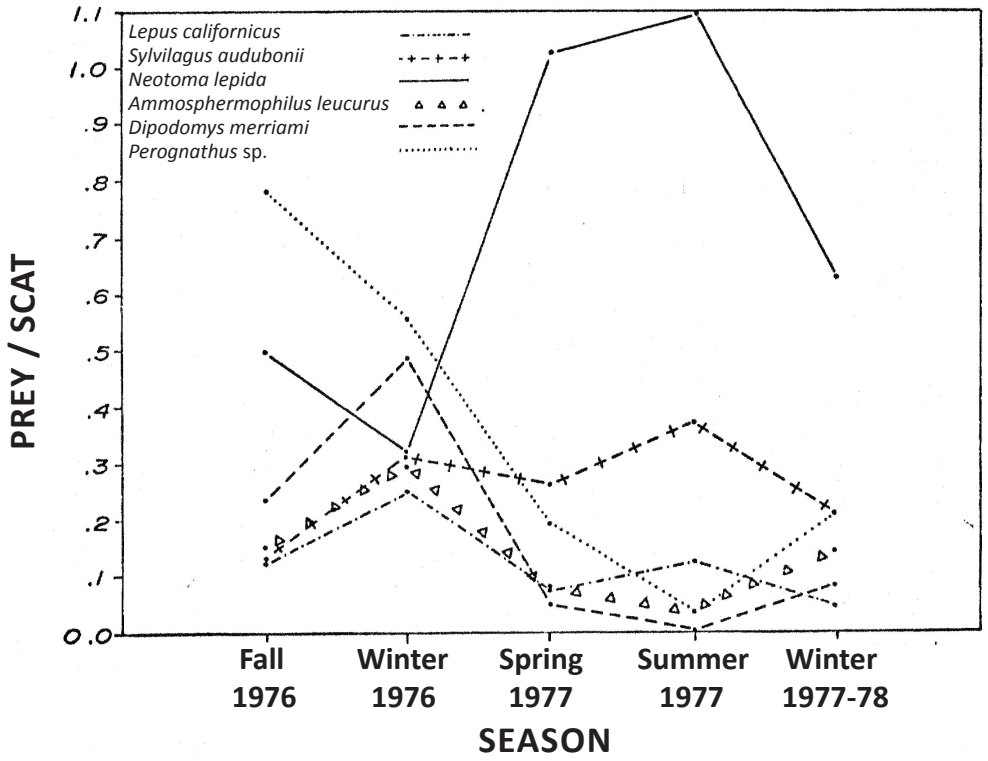

Figure 2. Individual prey per coyote (*Canis latrans*) scat from southwestern Joshua Tree National Monument, Fall 1976 to Winter 1977–78.

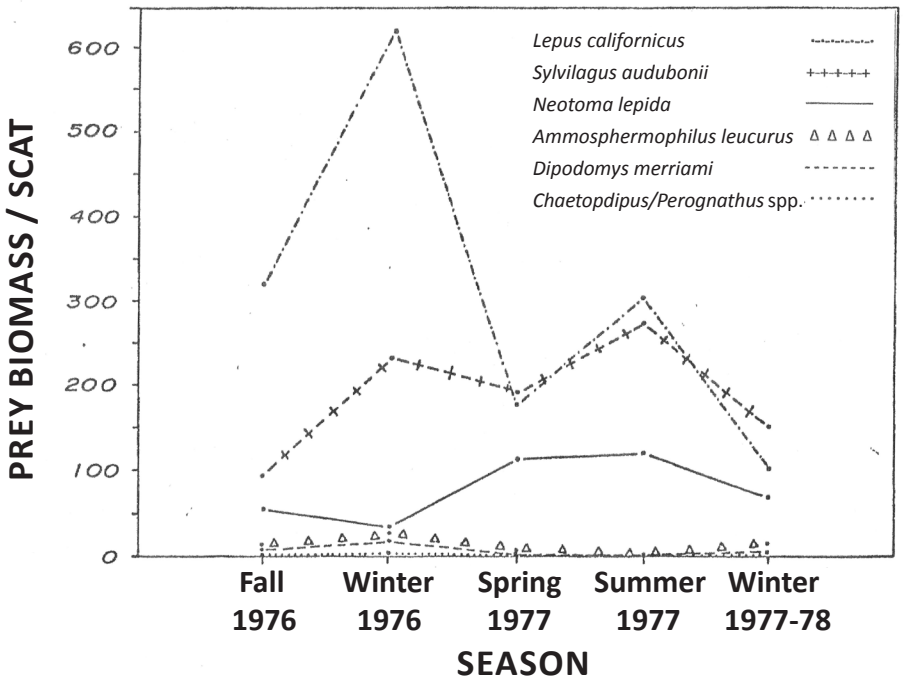

Figure 3. Prey biomass (g) per coyote (*Canis latrans*) scat from southwestern Joshua Tree National Monument, Fall 1976 to Winter 1977-78.

of *L. californicus* ingested was equal to or greater than the mass of all other species combined. On average, individuals of *S. audubonii* were detected with less frequency than those of *N. lepida* and *Chaetodipus/Perognathus* spp., but the mass ingested of *S. audubonii* exceeded that of *N. lepida* by a large margin and that of *Chaetodipus/Perognathus* by an even greater margin. *Ammospermophilus leucurus, D. merriami, Chaetodipus/Perognathus* spp., *T. bottae*, and *Peromyscus* spp. combined made up only 3.7% of the mass ingested throughout the study.

Lepus californicus, S. audubonii, and *N. lepida* comprised almost 98% of the estimated prey mass consumed by coyotes during the study. Leporid data for the May 1977 sampling period were excluded because of the difficulty in distinguishing between remains of young jackrabbits from adult cottontails in the sample.

The results of two-way ANOVA of prey mass per scat resulted in significant differences of prey mass per scat between seasons ($F = 3.151$, $P \leq 0.05$), and between species ($F = 104.163$, $P \leq 0.001$) and a significant interaction ($F = 2.965$, $P \leq 0.01$) between seasons and species. The interaction term suggested that the contribution of prey items varied seasonally. An SNK test on seasons indicated that mass per scat in the winter of 1976 and the spring of 1977 were significantly higher than in the winter of 1977–78 ($P \leq 0.05$). All other paired comparisons were not statistically significant. An SNK test on prey showed that the mass of leporids per scat was significantly greater ($P \leq 0.05$) than the mass of each of the other prey. All other species combined were only 17% of the mass per scat of that of leporids, and 12.4% of that 17% was *N. lepida.* All other paired comparisons were not statistically significant.

Prey abundance.—Leporids were counted along the transect routes in October 1976; May 1977; August 1977; and October 1977. *Sylvilagus audubonii* were uncommon along the transect routes and were not amenable to sampling by the area estimate method because the areas within 100 m of the roads required to use the method did not include sufficient cottontail habitat.

The transect routes were located in an area where *L. californicus* were relatively abundant and widely

distributed. *Lepus californicus* density was 0.09/ha in October 1976, 0.33/ha in May, 0.52/ha in August, and 0.32/ha in October of 1977. An analysis of variance test revealed that jackrabbit density varied significantly by seasons ($F = 42.61$, $P \leq 0.001$). A SNK test ($P \leq 0.01$) indicated that *L. californicus* densities in May 1977, and October of 1977 were not significantly different. October 1976 density was significantly lower than the other three periods ($P \leq 0.01$). The density in August 1977 was significantly higher than the other three periods ($P \leq 0.01$). Biomass per hectare was 223.7 g/ha in October 1976, 813.7 g/ha in May 1977, 1,276 g/ha in August 1977, and 790.5 g/ha in October 1977.

Rodent populations were sampled in May 1976; October 1976; March 1977; and August 1977. Eight species were sampled as follows: *A. leucurus*, *P. longimembris*, *C. fallax*, *D. merriami*, cactus mouse (*Peromyscus eremicus*), North American deermouse (*P. maniculatus*), southern grasshopper mouse (*O. torridus*), and *N. lepida*. *Thomomys bottae* was in the area, but was not sampled, because the traps were closed during most daylight hours. Observations of *A. leucurus* during periods when traps were closed strongly suggested that trapping data for the diurnal antelope ground squirrel were not representative of their numbers and were, therefore, excluded from the analysis.

The results of the seasonal rodent trapping are presented in Tables 3–5 and in Figures 4 and 5. Juvenile kangaroo rats and woodrats were captured in May 1976,

Table 3. Seasonal rodent densities per hectare in southwestern Joshua Tree National Monument in May and October 1976 and March and August 1977.

Species	May 1976	October 1976	March 1977	August 1977	Means
Neotoma lepida	9.97	1.82	11.76	7.02	7.643
Dipodomys merriami	8.38	8.82	5.80	7.56	7.64
Perognathus longimembris	5.48	2.19	0.55	2.74	2.74
Chaetodipus fallax	0.55	1.10	2.19	0.00	0.96
Onychomys torridus	0.55	1.10	0.41	0.98	0.76
Peromyscus maniculatus	0.55	0.36	1.37	0.44	0.68
Peromyscus eremicus	0.00	0.00	0.00	2.19	0.55

Table 4. Seasonal rodent biomass (grams per hectare) in southwestern Joshua Tree National Monument in May and October 1976 and March and August 1977.

Species	May 1976	October 1976	March 1977	August 1977	Means
*Neotoma lepid*a	921.10	216.57	1185.10	804.88	781.91
Dipodomys merriami	354.59	325.44	226.58	295.29	300.45
Perognathus longimembris	56.61	15.90	4.38	19.06	23.96
Chaetodipus fallax	12.10	20.28	33.96	0.00	16.59
Onychomys torridus	11.51	17.67	9.02	14.01	13.05
Peromyscus maniculatus	10.69	5.67	29.22	6.30	12.97
Peromyscus eremicus	0.00	0.00	0.00	39.99	10.00

Table 5. Seasonal mean weights of adult rodents by sex class in southwestern Joshua Tree National Monument in May and October 1976 and in March and August 1977.

Species	May 1976				October 1976				March 1977				August 1977				Mean
	Female		Male		Female		Male		Female		Male		Female		Male		
	n	wt	n	wt	n	wt	n	wt	n	wt	n	wt	n	wt	n	wt	
Neotoma lepida	6	103.7	10	123.3	4	87.2	2	140.2	21	114.4	10	115.0	15	103.7	53	121.7	116.05
Dipodomys merriami	30	42.4	64	48.8	59	36.3	106	37.6	60	38.0	88	40.21	45	38.7	33	40.0	39.61
Perognathus longimembris	5	11.5	5	9.2	1	6.0	3	7.7	--	--	1	8.0	3	8.0	2	9.0	9.11
Chaetodipus fallax	1	22.0	--	--	1	16.0	1	21.0	2	14.4	1	21.0	--	--	--	--	15.75
Onychomys torridus	1	21.0	--	--	1	16.0	1	16.3	6	23.2	5	20.4	6	14.8	2	14.0	18.70
Peromyscus maniculatus	--	--	1	19.5	--	--	1	15.7	2	20.5	2	18.3	2	15.0	2	14.0	16.95
Peromyscus eremicus	--	--	--	--	--	--	--	--	--	--	--	--	--	--	4	18.3	18.3

and juvenile deer mice and woodrats were trapped in August 1977. Because trapping was conducted only seasonally, a detailed analysis of reproductive patterns was not feasible. *Neotoma lepida* populations had the highest average densities during the study with *D. merriami* a very close second. *Neotoma lepida* populations were higher than those of *D. merriami* in May 1976 and March 1977, while *D. merriami* had higher densities in October 1976 and August 1977. Seasonal densities of *N. lepida* fluctuated much more than did those of *D. merriami.* These two species comprised almost 73% of the rodents captured during the study. *Perognathus longimembris* was the third most common rodent followed in order by *C. fallax, O. torridus, P. maniculatus,* and *P. eremicus. Peomyscus eremicus* was not captured during the first three sampling periods, but was caught in moderate numbers in August 1977. Densities of *L. californicus* averaged lower than those of any of the rodent species. The average density of jackrabbits between October 1976 and October 1977 was 0.32/ha compared to an average density of all nocturnal rodent species combined of 21/ha in May 1976 and August 1977. The average biomass of *L. californicus* was 776.0 g/ha, while the average biomass of nocturnal rodents was 1,145.9 g/ha.

Female *D. merriami* were captured at 1.6 times the number of males, female *N. lepida* outnumbered males by 1.5 times, while female *C. fallax* were twice as numerous as males. In contrast, there were 1.2 times as many male *P. longimembris* as females.

Although the biomass curves (Fig. 5) retain the same basic shapes as the density curves (Fig. 4), the positions of the curves change with respect to one another because of the differences in mean weight among species. *Neotoma lepida* had the highest average density and biomass during the study. However, *L. californicus*, which had lower densities than any of the rodent species, was second only to *N. lepida* biomass. *Dipodomys merriami* had an average density almost identical to that of *N. lepida*, but had an average biomass less than half of that of *N. lepida* or of *L. californicus*. Similarly, *P. longimembris* and *C. fallax* ranked higher in density than in biomass. An ANOVA test of seasonal rodent densities and mass indicated that total rodent densities ($F = 0.691$) and biomasses ($F = 0.882$) did not differ significantly among seasons. There were significant differences in both densities

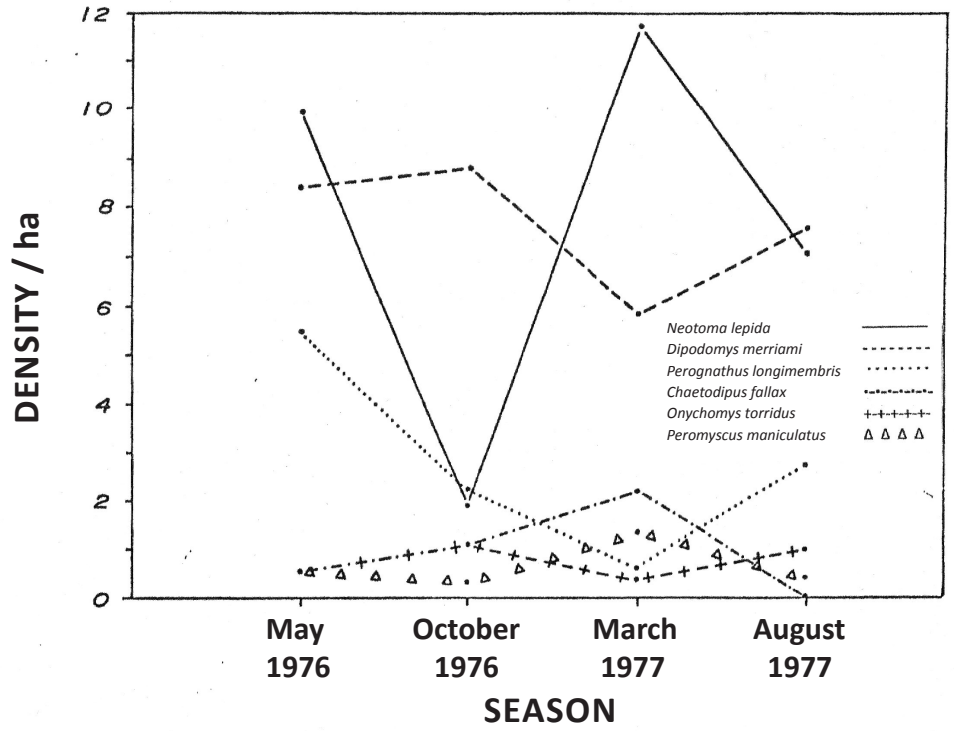

Figure 4. Rodent densities in southwestern Joshua Tree National Monument in May and October 1976 and in March and August 1977.

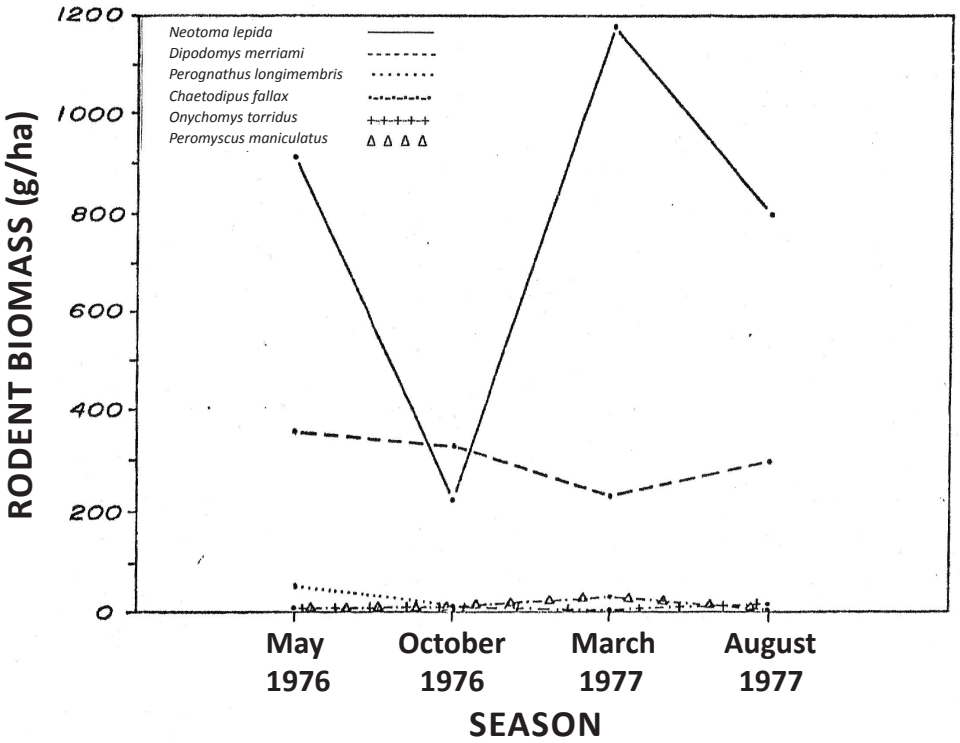

Figure 5. Rodent biomass in southwestern Joshua Tree National Monument in May and October 1976 and in March and August 1977.

($F = 9.9$, $P \leq 0.001$) and biomasses ($F = 13.274$, $P \leq 0.001$) among species, but it was not feasible to test for seasonal differences within species because of a lack of replications.

Comparisons of coyote diet to prey populations.—Comparisons of diet to prey populations were confounded by the fact that densities of three of the mammal species detected in scats could not be estimated accurately. *Sylvilagus audubonii* prefers brushy habitats that could not be accurately counted by the area estimate method. Because of the diurnal activity pattern of *A. leucurus,* this species was not estimated accurately because traps remained closed during most daylight hours to prevent mortality due to overheating. Pocket gopher mounds were uncommon and widely scattered, therefore counts of *T. bottae* was not feasible. Tables 6 through 9 compare the composition of coy-

Table 6. Mean number of prey individuals per scat compared to mean densities of prey species per hectare in southwestern Joshua Tree National Monument from May 1976 through January 1978.

Species	Mean Number Per Scat	Mean Density of Prey Per Hectare
Neotoma lepida	0.68	7.643
Chaetodipus/Perognathus spp.	0.38	3.70
Sylvilagus audubonii	0.25	--
Dipodomys merriami	0.19	7.640
Ammospermophilus leucurus	0.15	--
Lepus californicus	0.13	0.32
Thomomys bottae	0.04	--
Peromyscus spp.	0.03	1.23
Onychomys torridus	0.00	0.76

Table 7. Mean percent occurrence and mean percentage of prey identified per scat compared to mean percentages of prey population in southwestern Joshua Tree National Monument from May 1976 through January 1978.

Species	% Occurrence	% of Individuals Counted Per Scat	% of Prey Population
*Neotoma lepid*a	47.0	34.8	35.9
Chaetodipus/Perognathus spp.	25.1	19.3	17.4
Sylvilagus audubonii	24.2	12.9	--
Dipodomys merriami	17.2	9.6	35.9
Lepus californicus	15.4	6.4	1.5
Ammospermophilus leucurus	11.2	7.6	--
Thomomys bottae	3.3	2.2	--
Peromyscus spp.	2.8	1.7	5.8
Onychomys torridus	0.00	0.00	3.6

Table 8. Mean prey mass ingested per scat compared to mean prey biomass per hectare for *Canis latrans* in southwestern Joshua Tree National Monument from May 1976 through January 1978.

Species	Mean Prey Biomass Ingested Per Scat (g)	Mean Prey Biomass (g/ha)
Lepus californicus	317.2	776.0
Sylvilagus audubonii	183.0	---
Neotoma lepida	75.4	781.9
Ammospermophilus leucurus	14.5	---
Dipodomys merriami	7.5	300.5
Perognathus spp.	4.4	40.6
Thomomys bottae	2.0	---
Peromyscus spp.	0.5	23.0
Onychomys torridus	0.0	13.1

Table 9. Mean percent of mass of prey ingested per scat by *Canis latrans* compared to mean percent of prey biomass per hectare in southwestern Joshua Tree National Monument from May 1976 through August 1977.

Species	% of Biomass Ingested Per Scat	% of Prey Biomass Per Hectare
Lepus californicus	44.88	40.1
Sylvilagus audubonii	25.28	--
Neotoma lepida	10.60	40.4
Ammospermophilus leucurus	1.83	--
Dipodomys merriami	0.95	15.5
Chaetodipus/Perognathus spp.	0.57	2.1
Thomomys bottae	0.29	--
Peromyscus spp.	0.08	1.2
Onychomys torridus	0.00	0.7

ote diet to prey densities and biomasses for the entire study. Tables 6 and 7 focus on numbers of individual prey per scat and densities of prey species in the area. More individuals of *N. lepida* were encountered per scat than any of the other species, and *N. lepida* also had the highest mean density. About half as many *Chaetopidus/ Perognathus* were found per scat than *N. lepida* and the mean density was about half of that of *N. lepida* as well. Although the mean density of *D. merriami* was virtually equal to that of *N. lepida,* the mean number of individuals of *D. merriami* per scat was less than 25% of that of *N. lepida.* In contrast, the mean density of *L. californicus* was about 0.4% of that of *N. lepida,* but the mean number per scat was 19% of that of *N. lepida.* As noted above, the percentages of prey in the third column of Table 7 must be considered with caution because three known prey species were not included. In general, however, the picture presented is similar to that of Table 6. The proportion of the percent of *N. lepida* individuals per scat to *Chaetodipus/Perognathus* per scat was roughly the same as the proportion of their percentages of the prey population. The data in these tables suggest that coyotes consumed *N. lepida* and *Chaetodipus/Perognathus* spp. roughly in proportion to their densities. *Dipodomys merriami* was taken much less often than expected with respect to its density and *L. californicus* was taken more often than expected. *Peromyscus* spp. and *O. torridus* were consumed less often than expected. There is some evidence that small prey are underestimated in scat analyses and that larger prey are overestimated (Murie 1945; Floyd et al. 1978; Weaver 1977; Weaver and Hoffman 1979; Meriwether and Johnson 1980).

Tables 8 and 9 compare prey mass consumed to prey biomass per hectare in the area. These data present a different picture than presented above. *Lepus californicus* had the highest mean mass per scat and the second highest mean biomass per hectare. Although there were more *N. lepida* per hectare than any other prey species censused, *N. Lepida* ranked third in mass per scat behind *L. californicus* and *S. audubonii.* However, leporids are large enough that each one consumed may appear in more than one scat. *Dipodomys merriami* ranked third in biomass per hectare and fourth in mass per scat. Although *D. merriami* biomass per hectare was 38.4% of that of *N. lepida* and 39% of that of *L. californicus,* mass per scat was only 10% of *N. lepida* and 2.3% of *L. californicus.* This suggests that *N. lepida* and *D. merriami* were eaten at lower rates than expected from their biomass. However, with the exception of *N. lepida,* the ranking of prey in order of mean mass per scat seems to correspond with their ranking in order of mean biomass per hectare. A correlation analysis of mean number of prey per scat and mean densities of prey species resulted in a correlation coefficient of r=0.63 ($P \geq 0.1$) and correlation of mean biomass of prey per scat and mean biomass of prey per hectare resulted in a correlation coefficient of r=0.76 ($P \leq 0.1$). Coefficients ≥ 0.7 indicate a strong relationship.

It appears that analyses of prey mass consumed and prey biomass available is more biologically important than analyses of numbers of prey consumed and density of available prey. Logically, the mass of prey consumed should be more important energetically than actual numbers of individuals consumed.

Mean densities of *N. lepida* and *D. merriami* were almost the same during the study, but densities of *N. lepida* exhibited much greater variation (see Table 4). Although individuals of *D. merriami* comprised only 10% of the individuals per scat and 1% of the biomass per scat during the entire study, they comprised 21.74% of the prey per scat and 2.08% of the biomass in scat samples from October and December of 1976. Thus, *D. merriami* contributed more to the coyote diet when densities of other prey were relatively low. Even then, however, prey with densities and biomass lower than those of *D. merriami* made greater contributions to the diet. Coyotes seemed to prefer other prey species over *D. merriami*.

Total biomass of prey species increased from the winter of 1976 to the spring of 1977 and summer of 1977. The total rodent biomass was not significantly different between these seasons, but there were significant differences in seasonal jackrabbit biomass. A significant Chi-square value of 21.255 ($P \leq 0.05$) indicated that as available prey biomass increased, the number of different species detected per scat decreased.

Of the estimated prey mass ingested per scat, 97.9% was composed of leporids and *N. lepida.* In the winter of 1976, when *Lepus* and *N. lepida* biomass were the lowest, leporids and woodrats still made up 94.2% of the mass ingested per scat. The combined biomass of *L. californicus* and *N. lepida* per hectare increased by

453% between the fall of 1976 and the spring of 1977. During the same period, the combined percentage of leporid and *N. lepida* mass ingested per scat increased from 94.2% to 98.6%. The combined biomass of *L. californicus* and *N. lepida* increased again slightly from the spring of 1977 to the summer of 1977. This was again accompanied by an increase in the percentage of leporid and *N. lepida* mass in the diet to 99%.

Lepus californicus was the largest of the coyote prey species in the area followed, in order, by *S. audubonii*, *N. lepida*, *A. leucurus*, *D. merriami*, *O. torridus*, *P. eremicus*, *P. maniculatus*, *P. fallax*, and *C. longimembris*. The rankings of the five largest species according to estimated mass ingested per scat resulted in exactly the same order (see Table 8).

DISCUSSION

Coyote diet analysis.—Scat contents can be quantified in a variety of ways including percent occurrence, percent by weight, percent by volume, number of individual prey detected, presence or absence of a prey species, or an estimate of prey mass consumed. In scat analysis, it is difficult to interpret how percent occurrence, percent by weight, and percent by volume relate to what a coyote actually ingested. These have limited use for investigating foraging ecology, predator-prey relationships, or coyote energetics.

Johnson (1978) estimated the dry weight of each taxon contained in each scat. Each scat was separated into three fractions: hair and feathers, macrofragments, and microfragments. Each fraction was weighed and the ratios of the taxa in each fraction estimated. Ratios of taxa in the macrofragment were estimated by point analysis (Johnson and Hansen 1977a). In some of the earliest studies, diagnostic parts were counted to estimate the number of individual prey present in each scat (O. Murie 1935, 1945; Bond 1939; A. Murie 1940, 1951; Grater 1943). This technique was apparently ignored for many years. Weaver (1977) used this technique in analyzing coyote diets in Jackson Hole, Wyoming, and this was the technique that was used in this study. Estimates of the numbers and mass of prey species ingested were needed for comparison with densities and biomass of prey estimated in the study area. Recent development of molecular diet analysis techniques for predator scats (Mumma et al. 2016) rely on the detection of plant and animal DNA. Although molecular techniques tend to detect prey species at a greater percentage than morphological techniques, they do not provide reliable estimates of the number of individual prey per scat.

Scat analyses may be biased in number of ways. Non-digestible material from one meal may be eliminated in more than one scat (Gier 1968) and larger species may be detected more often than smaller ones (Weaver 1977; Weaver and Hoffman 1979). Thus, the actual number of individuals consumed may be overestimated for larger prey and underestimated for smaller prey. In a study of mammalian prey digestibility by coyotes, Meriwether and Johnson (1980) concluded that scat analysis may fail to detect small prey. Weaver (1977) used the results of feeding trials with captive coyotes to compute correction factors for prey of differing sizes. Feeding trials conducted in conjunction with field studies should result in correction factors that result in more accurate analysis of ecological energetics of predator-prey relationships (Weaver 1977; Floyd et al. 1978), but that was not feasible for this study. Sanchez et al. (2004) and Livingston et al. (2005) reported that scat removal by other animals is a potential source of bias. Degradation of scats over time also may be a factor (Sanchez et al. 2004). Frequent collection of scats can reduce the impact of degradation.

In this study, the ranking of prey species by percent occurrence and by percent of individual prey consumed resulted in nearly identical orders. However, when prey were ranked by percent of mass consumed, the results were strikingly different. Estimates of prey mass consumed are more meaningful in evaluating the feeding ecology of predators. Whenever possible, studies of coyote diet, coyote predator-prey relationships, and ecological energetics of coyotes should include estimates of prey mass consumed. The absence of these data may lead to serious errors in reporting the relative importance of prey species in the diet.

Importance of leporids in coyote diets has been reported from numerous studies (Bond 1939; Sperry 1941; Ferrel et al. 1953; Fichter et al. 1955; Gier 1968; Clark 1972; Hamilton 1974; Johnson and Hansen 1977b; Andrews and Boggess 1978; Johnson 1978; Windberg and Mitchell 1980; Toweill and Anthony 1988; Brillhart and Kaufman 1995; Prugh 2005). Some authors who concluded that leporids were unimportant to coyotes also suggested that leporids were uncommon in their study areas or comprised a relatively small portion of the available prey (Murie 1940, 1951; Meinzer et al. 1975). In a species with such a widespread distribution as the coyote, considerable geographic variation in diet is to be expected.

Prey populations.—In 1976 and 1977, *L. californicus* densities ranged from 0.09 to 0.52/ha in the study area. Jackrabbit abundance in this study peaked in the summer of 1977 and was significantly lower in October. However, the October 1977 density was 3.5 times than the previous October. This may have been a response to unusually high precipitation in 1976 and the resulting impressive increase in forage in 1977 that, in turn, influenced prey populations and prey selection.

The most abundant rodent in the study area was *N. lepida*. Although densities were relatively high, they were not the highest that have been reported from the JTNM. Cameron (1965) found 22.9 *N. lepida* per hectare in Cholla Garden about 15 km northeast of my census lines, and Brown et al. (1972) estimated up to 38.3 woodrats per hecture in Cholla Garden.

Because woodrats are not physiologically adapted to conserve water (Schmidt-Nielsen and Schmidt-Nielsen, 1952) it is not surprising that Brown et al. (1972) found a high correlation between densities of *N. lepida* and densities of the succulent *Opuntia bigelovii*. These plants provide a source of water, food, house sites, and house construction materials for the woodrats.

During this study, the average density of *D. merriami* was almost as high as that of *N. lepida*. However, the *D. merriami* population showed less seasonal variation. The highest density was 8.82/ha in October 1976. The highest densities in JTNM were reported by Soholt (1973) in Indian Cove with an average of 16.2/ha and a range of 12.3 to 19.5. In an earlier study in Indian Cove, Chew and Butterworth (1964) found densities ranging from 0.45 to 3.72/ha.

Perognathus longimembris was the third most abundant nocturnal rodent in the study area, averaging almost three per hectare. Unlike *N. lepida* and *D. merriami*, *P. longimembris* is only active seasonally (Chew and Butterworth 1964; Kenagy 1973). In Indian Cove in JTNM, Chew and Butterworth (1964) did not capture *P. longimembris* in October through January. In this study, this species was captured in October 1976 and March 1977. No trapping was conducted in the interim.

Densities of *C. fallax* during this study averaged about one per hectare. The pattern of seasonal densities was similar to that reported by MacMillen (1964) for this species in southern California chaparral. He found that *C. fallax* averaged 1.8 per/ha, with the highest density in winter and early spring and lowest in June. In JTNM, densities were highest in March and October and none were captured in August.

Numbers of *P. maniculatus* captured were low throughout this study. *Peromyscus eremicus* were captured only in August 1977, but they had the fourth highest density of any nocturnal rodent that month. According to MacMillen (1964), *P. eremcus* is capable of estivating to avoid the rigors of summer heat. It remains a mystery why this species was not captured in May, October, and March, then appeared in August. Densities of the *O. torridus* were low, but relatively stable throughout this study.

Ammospermophilus leucurus was captured only incidentally during this study in early evening or early morning. Traps were closed during most of their activity period because of the desert heat.

Although the mean density of *N. lepida* was nearly equal to that of *D. merriami*, the mean biomass of *N. lepida* was over 2.6 times as great. The mean biomass of *D. merriami* was 4.7 times that of all the remaining nocturnal rodents combined. The nocturnal rodent fauna was dominated by *N. lepida* and *D. merriami* numerically and in biomass.

Because rodent trapping was conducted only periodically, a detailed analysis of reproductive patterns

was not possible. It appears, however, that in 1976 and 1977 most reproductive activity occurred from March through the first two weeks in August. Several studies concluded that reproductive success of desert heteromyids is correlated with availability of green vegetation (Reynolds 1954, 1958; Chew and Butterworth 1964; Beatley 1969; Bradley and Mauer 1971; Van DeGraaff and Balda 1973; Reichman and Van DeGraaff 1975).

Precipitation in JTNM during 1976 was 2.5 times the average and also was above average in 1977. One half of the 1976 precipitation fell between September and December. This resulted in an explosion of annual vegetation in the spring of 1977. This probably contributed to the relatively high and relatively stable rodent populations during 1976 and 1977, and to the increase in *L. californicus* density in 1977. Total density of nocturnal rodents was not significantly different from season to season, but species rank by density changed somewhat. The *L. californicus* population exhibited significant seasonal fluctuations. Counts suggested a very good prey base for predators of small mammals during this study.

Coyote diet and prey availability.—During only a few studies, including this one, have prey abundance been related to coyote diet. Holle (1972) compared coyote diet to general availability of prey, and Nellis and Keith (1976) compared coyote diet to the relative abundance of microtines. Brown (1977) compared coyote diet to abundance of lagomorphs, rodents, insects, and fruit, whereas Weaver (1977) contrasted coyote food habits with relative abundance of rodents, grasshoppers, and estimates of quantities of ungulate carrion. Keith et al. (1977) described the responses of coyotes to changing snowshoe hare (*Lepus americanus*) numbers throughout a 10-year cycle.

Niebauer and Rongstad (1977) reported that their data indicated no clear relationship between abundance of a prey class and occurrence in coyote scats. They stated that when coyotes have a broad food base, changes in numbers of one food species have little impact on the total prey biomass available. However, they did not present prey biomass data to support this assumption. Some results from this study tend to support their assumption. Although seasonal densities and biomass of some rodent prey species changed markedly, no significant differences in seasonal densities or biomass of all rodent prey were detected. However, small sample size may have obscured relationships that might have been biologically important.

McKinney and Smith (2007) concluded that coyotes and bobcats partitioned food resources independent of varying rainfall. The data from this study suggest that significant changes in precipitation can result in changes in prey abundances and biomass and that coyotes may adjust their feeding patterns as a result. Bowyer et al. (1983) concluded the coyotes were feeding opportunistically, but they did not quantify prey densities or biomasses. They stated that seasonal fluctuations of food items should coincide with availability. That was not the case in this study. Literature on coyote food habits is full of statements describing the feeding behavior of coyotes as opportunistic (Bond 1939; Murie 1951; Johnson and Hansen 1977b; Mulder 1979; MacCracken and Uresk 1984; and others). The results of this study suggest that these statements oversimplify coyote-prey relationships. Prugh (2004) suggests that in northern areas where their foraging behavior and population dynamics are driven primarily by snowshoe hare abundance, coyotes may be considered specialists on the hares. MacCracken and Hansen (1982) noted that 15% of all available foods contributed to 80% of food ingested in southeastern Idaho, suggesting that coyotes are selective and prefer relatively few mammalian species. There is no question that coyotes are capable of utilizing a wide variety of food resources and that their diets exhibit a wide variance both geographically and temporally. That does not mean that they are not selective.

Optimal diet models (Schoener 1971) predict that the lower the absolute abundance of food, the greater the range of items eaten. In times of food abundance, coyotes should concentrate on prey that will yield the greatest return per unit of effort. The data from this study seem to support that hypothesis. MacCracken and Hansen (1987) ranked the three primary prey of coyotes based on energy and protein content, digestibility, and body size. They concluded that coyote diets were dominated by the most profitable foods. Their assumption was that handling cost of prey and resultant prey rank were directly proportional to relative body size. MacCracken and Hansen's (1987) data suggest that coyotes were selecting prey as predicted by optimal diet models (MacArthur and Pianka 1966; Schoener

1979; Pyke 1984). In an earlier paper, MacCracken and Uresk (1984) questioned the role that opportunistic behavior played in coyote prey selection. Windberg and Mitchell (1989) also suggested that coyotes were selecting prey based on relative profitability. In agreement with MacCracken and Hansen (1987) and MacCracken (1989), it is clear that more research, including controlled experiments, is warranted. The results of this study suggest that there may be situations where opportunistic foraging behavior may be the optimal choice.

Additional research is needed to understand how coyotes decide what to eat. A number of processes are involved in acquiring food. Each of these components requires the expenditure of energy. The choice of prey species must involve decisions based on energy return from a food item versus energy expended to aquire it. Prey size, population density, population biomass, ease of capture, and risk of injury are all potential factors. Coyotes should favor larger, easier to capture prey that are present at high densities and, therefore, high biomass. The five largest prey species in the study area were the most dominant coyote foods. Their ranks by weight and by biomass ingested per scat were nearly identical.

Simple rankings of prey present an oversimplified picture of coyote diet. A comparison of *N. lepida* and *L. californicus* illustrate this fact. On the average, a *L. californicus* from the study area weighed as much as 20 *N. lepida*, but the average density of *N. lepida* was 24 times greater. Four times as many *N. lepida* were detected per scat as *L. californicus*, but five times as much *L. californicus* mass was ingested. It appears, however, that coyotes were not eating prey simply in proportion to their size, density, or biomass. Another presumably very important parameter that has not been studied with coyotes is the time it takes to search for and capture different prey. This could account for some of the differences in the proportions of prey in coyote diets. Many authors have lumped all leporids together as a food class and lumped all rodents together as another class. Unless each species is considered separately, errors in interpretation of the data may occur. Lumping of data can obscure important patterns and lead to misleading conclusions (Fichter et al. 1955). Based on the results of this study and a review of numerous other coyote diet studies, the collection of the most detailed diet information possible is warranted.

Coyotes are one of the most successful, if not the most successful predator, in North America in recent history. They undoubtedly benefited from the near extermination of the gray wolf (*Canis lupus*). In addition, their behavioral plasticity and adaptability have not only allowed them to thrive in the face of concerted control efforts, but to significantly expand their range. In places like JTNM with an abundance of small prey, coyotes operate successfully solitarily. In other situations they have demonstrated the apparent ability to cooperate in packs to hunt larger prey (Camenzind 1978; Bowen 1981; Gese et al. 1988). Although numerous authors have studied coyote food habits, there is still much to learn about their foraging strategies and how they adapt to changes in food abundance, distribution, and vulnerability; how they are influenced by other sympatric predators and how they adapt to different habitats.

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LITERATURE CITED

Adorjan, A. S., and G. B. Kolenosky. 1969. A manual for identification of hairs of selected Ontario mammals. Ontario Department of Lands, Forest Research Report 90:1–64.

Andrews, R. D., and E. K. Boggess. 1978. Ecology of coyotes in Iowa. Pp. 249–266 in Coyotes: biology, behavior, and management (M. Bekoff, ed.). Academic Press, New York.

Beatley, J. C. 1969. Dependence of desert rodents on winter annuals and precipitation. Ecology 50:721–724.

Bond, R. M. 1939. Coyote food habits on the Lava Beds National Monument. Journal of Wildlife Management 3:180–198.

Bowen, W. D. 1981. Variation in coyote social organization in relation to prey size. Canadian Journal of Zoology 59:639–652.

Bowyer, R. T., S. A. McKenna, and M. E. Shea. 1983. Seasonal changes in coyote food habits as determined by fecal analysis. American Midland Naturalist 100:266–273.

Bradley, W. G., and R. A. Mauer. 1971. Reproduction and food habits of Merriam's kangaroo rat, *Dipodomys merriami*. Journal of Mammalogy 52:497–507.

Brillhart, D. E., and D. W. Kauffman. 1995. Spatial and seasonal variation in prey use by coyotes in northcentral Kansas. The Southwestern Naturalist 40:160–166.

Brown, J. H., G. A. Lieberman, and W. F. Dengler. 1972. Woodrats and cholla: dependence of a small mammal population on the density of cacti. Ecology 53:310–313.

Brown, K. L. 1977. Coyote food habits in relation to a fluctuating prey base in south Texas. Unpublished M.S. thesis, Texas A&M University, College Station.

Camenzind, F. J. 1978. Behavioral ecology of coyotes on the National Elk Refuge, Jackson, Wyoming. Pages 267–294 in Coyotes: biology, behavior, and management (M. Bekoff, ed.). Academic Press, New York.

Cameron, G. N. 1965. Ecology of the desert wood rat, *Neotoma lepida* Thomas, in Joshua Tree National Monument, California. Unpublished M.S. thesis, California State College, Long Beach.

Chew, R. M., and B. B. Butterworth. 1964. Ecology of rodents in Indian Cove (Mojave Desert), Joshua Tree National Monument, California. Journal of Mammalogy 45:203–225.

Clark, F. W. 1972. Influence of jackrabbit density on coyote population change. Journal of Wildlife Management 36:343–356.

Coman, B. C., and H. Brunner. 1971. Food-habits analysis using a fiber cross-sectioning technique. Journal of Wildlife Management 35:576–579.

Cornely, J. E. 1977. Checklist of mammals. Joshua Tree National Monument, California. National Park Service, Unpublished leaflet.

Cornely, J. E. 1980. Home ranges and diets of coyotes in Joshua Tree National Monument in relation to prey densities. Unpublished Ph.D. dissertation, Northern Arizona University, Flagstaff.

Ferrell, C. M., H. R. Leach, and D. F. Tillotson. 1953. Food habits of the coyote in California. California Fish and Game 39:301–341.

Fichter, E., G. Schildman, and J. H. Sather. 1955. Some feeding patterns of coyotes in Nebraska. Ecological Monographs 25:1–37.

Flinders, J. T., and R. M. Hansen. 1973. Abundance and dispersion of leporids within a shortgrass ecosystem. Journal of Mammalogy 54:287–291

Floyd, T. J., L. D. Mech, and P. A. Jordan. 1978. Relating wolf scat content to prey consumed. Journal of Wildlife Management 42:528–532.

Gese, E. M., O. J. Rongstad, and W. R. Mytton. 1988. Relationship between coyote group size and diet in southeastern Colorado. Journal of Wildlife Management 52:647–653.

Gier, H. T. 1968. Coyotes in Kansas. Kansas State University, Agricultural Experiment Station Bulletin 393:1–118.

Grater, R. K. 1943. Coyote foods near Boulder Dam. Journal of Wildlife Management 7:422–423.

Hamilton, W. J., Jr. 1974. Food habits of the coyote in the Adirondacks. New York Fish and Game Journal 21:177–181.

Holle, D. G. 1977. Diet and general availability of prey of the coyote (*Canis latrans*) at the Wichita Mountains National Wildlife Refuge, Oklahoma. Unpublished M.S. thesis, Oklahoma State University, Stillwater.

Johnson, M. K. 1978. Food habits of coyotes in south central Idaho. Unpublished Ph.D. dissertation, Colorado State University, Fort Collins.

Johnson, M. K., and R. M. Hansen. 1977a. Comparison of point frame and hand separation of coyote scats. Journal of Wildlife Management 41:319–320.

Johnson, M. K., and R. M. Hansen. 1977b. Foods of coyotes in the lower Grand Canyon, Arizona. Journal of the Arizona Academy of Science 12:81–83.

Keith, L. B., A. W. Todd, C. J. Brand, R. S. Adamcik, and D. H. Rusch. 1977. An analysis of predation during a cyclic fluctuation of snowshoe hares. International Congress of Game Biology 13:151–175.

Kenagy, G. J. 1973. Daily and seasonal patterns of activity and energetics in a heteromyid rodent community. Ecology 54:1201–1219.

Leary, P. J. 1977. Investigations of the vegetational communities of Joshua Tree National Monument, California. Unpublished M.S. thesis, University of Nevada, Las Vegas.

Livingston, T. R., P. S. Gipson, W. B. Ballard, D. M. Sanchez, and P. R. Krausman. 2005. Scat removal: a source of bias in feces-related studies. Wildlife Society Bulletin 33:172–178.

MacArthur, R. H, and E. R. Pianka. 1966. On optimal use of a patchy environment. American Naturalist 100:603–609.

MacCracken, J. G. 1989. Coyote prey choice: a reply. Journal of Wildlife Management 53:666–667.

MacCracken, J. G., and R. M. Hansen. 1982. Seasonal foods of coyotes in southeastern Idaho: A multivariate analysis. Great Basin Naturalist 42:45–49.

MacCracken, J. G., and R. M. Hansen. 1987. Coyote feeding strategies in southeastern Idaho: Optimal foraging by an opportunistic predator? Journal of Wildlife Management 51:278–285.

MacCracken, J. G. and D. W. Uresk. 1984. Coyote foods in the Black Hills, South Dakota. Journal of Wildlife Management 48:1420–1423.

MacMillen, R. E. 1964. Population ecology, water relations, and social behavior of a southern California semidesert rodent fauna. University of California Publications in Zoology 71:1–66.

McKinney, T., and T. W. Smith. 2007. Diets of sympatric bobcats and coyotes during years of varying rainfall in central Arizona. Western North American Naturalist 67:8–15.

Major, J. T, and J. A. Sherburne. 1987. Interspecific relationships of coyotes, bobcats, and red foxes in western Maine. Journal of Wildlife Management 51:606–616.

Mayer, W. V. 1952. The hair of California mammals with keys to the dorsal guard hairs of California mammals. American Midland Naturalist 48:480–512.

Meinzer, W. P., D. N. Ueckert, and J. T. Flinders. 1975. Foodniche of coyotes in the Rolling Plains of Texas. Journal of Range Management 28:22–27.

Meriwether, D., and M. K. Johnson. 1980. Mammalian prey digestibility by coyotes. Journal of Mammalogy 61:774-775.

Miller, A. H., and R. C. Stebbins. 1964. The lives of desert animals in Joshua Tree National Monument. University of California Press, Berkeley.

Moore, T. D., L. E. Spence, and C. E. Dugnolle. 1974. Identification of the dorsal guard hairs of some mammals of Wyoming. Wyoming Game and Fish Department Bulletin 14:174–183.

Mulder, J. B. 1979. Food selection by wild-caught captive coyotes. Transactions of the Kansas Academy of Sciences 82:63–66.

Mumma, M. A., J. R. Adams, C. Zieminski, T. K. Fuller, S. P. Mahoney, and L. P. Waits. 2016. A comparison of morphological and molecular diet analyses of predator scats. Journal of Mammalogy 97:112–120.

Murie, A. 1940. Ecology of the coyote in the Yellowstone. U. S. Department of Interior, National Park Service, Fauna of the National Parks 4:1–206.

Murie, A. 1951. Coyote food habits on a southwestern cattle range. Journal of Mammalogy 32:291–295.

Murie, O. J. 1935. Food habits of the coyote in Jackson Hole, Wyoming. U. S. Department of Agriculture Circular 362:1–24.

Murie, O. J. 1945. Notes on coyote food habits in Montana and British Columbia. Journal of Mammalogy 26:33–40.

National Park Service. 2017. Mammals. https://nps/jotr/learn/nature/mammals.htm.

Nellis, C. H., and L. B. Keith. 1976. Population dynamics of coyotes in central Alberta, 1964–68. Journal of Wildlife Management 40:389–399.

Niebauer, C. H., and O. J. Rongstad. 1977. Coyote food habits in northwestern Wisconsin. Pp. 237–251 in Proceedings of the 1975 Predator Symposium (Phillips, R. L., and C. Jonkel, eds.). University of Montana Forest and Conservation Experiment Station, Missoula.

O'Farrell, M. J., and G. T. Austin. 1978. A comparison of different trapping configurations with the assessment line technique for density estimations. Journal of Mammalogy 59:866–868.

O'Farrell, M. J., D. W. Kaufman, and D. W. Lundahl. 1977. Use of live-trapping with the assessment line method for density estimation. Journal of Mammalogy 58:575–582.

Prugh, L. R. 2005. Coyote prey selection and community stability during a decline in food supply. Oikos 110:253–254.

Pyke, G. H. 1984. Optimal foraging theory: a critical review. Annual Review of Ecological Systems 15:523–575.

Reichman, O. J., and K. M. Van DeGraaff. 1975. Association between ingestion of green vegetation and desert rodent reproduction. Journal of Mammalogy 56:503–506.

Reynolds, H. G. 1954. Some inter-relations of Merriam's Kangaroo Rat to velvet mesquite. Journal of Range Management 7:176–180.

Reynolds, H. G. 1958. Ecology of the Merriam's Kangaroo Rat (*Dipodomys merriami*) on the grazing lands of southern Arizona. Ecological Monographs 28:111–127.

Sanchez, D. M., P. R. Krausman, T. R. Livingston, and P. S. Gipson. 2004. Persistence of carnivore scat in the Sonoran Desert. Wildlife Society Bulletin 32:366–372.

Schmidt-Nielson, K., and B. Schmidt-Nielson. 1952. Water metabolism of desert mammals. Physiological Review 32:135–166.

Schoener, T. W. 1971. Theory of feeding strategies. Annual Review of Ecological Systems 2:369–404.

Schoener, T. W. 1979. Generality of the size-distance relation in models of optimal foraging. American Naturalist 114:902–914.

Soholt, L. F. 1973. Consumption of primary production by a population of kangaroo rats (*Dipodomys merriami*) in the Mojave Desert. Ecological Monographs 43:357–376.

Sokal, R. R., and F. J. Rolf. 1969. Biometry. W. H. Freeman and Company, San Francisco.

Sperry, C. C. 1941. Food habits of the coyote. U. S. Department of Interior, Fish and Wildlife Service, Wildlife Research Bulletin 4:1–70.

Toweill, D. E., and R. G. Anthony. 1988. Coyote foods in a coniferous forest in Oregon. Journal of Wildlife Management 52:507–512.

Van Degraaff, K. M., and R. P. Balda. 1973. Importance of green vegetation for reproduction in the kangaroo rat *Dipodomys merriami merriami*. Journal of Mammalogy 54:509–512.

Weaver, J. L. 1977. Coyote-food base relationships in Jackson Hole, Wyoming. Unpublished M.S. thesis, Utah State University, Logan.

Weaver, J. L., and S. W. Hoffman. 1979. Differential detectability of rodents in coyote scats. Journal of Wildlife Management 43:783–786.

Windberg, L.A., and C. D. Mitchell. 1990. Winter diets of coyotes in relation to prey abundance in southern Texas. Journal of Mammalogy 71:439–447.

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Coyotes, Wolves, and People: Coyote (*Canis latrans*) Occupancy Patterns across an Urban-Wildland Gradient in Northeastern Washington

Candace D. Bennett and Margaret A. O'Connell

Abstract

Increases in coyote abundance during the past century might be affected by urbanization and, in the western United States, by recolonizing gray wolf populations. Vocalizations were used to examine coyote occupancy in five land-use categories from an urban center to distant protected sites with recolonizing gray wolves in northeastern Washington from May 2009 to April 2010. Analysis of 401 coyote vocalizations revealed occupancy was greater in unfarmed rural and close protected sites surrounding the city compared to the urban center or distant protected sites. Detectability was least April through August during pup rearing and greatest September through December when individuals were dispersing and establishing new territories. This study provides baseline information on the combined effects of increasing urbanization and initial recolonization of wolves on coyote habitat occupancy in northeastern Washington.

Key words: *Canis latrans*, *Canis lupus*, detection, occupancy, return vocalization, seasons, urban-wildland gradient

Introduction

During the 20th century, coyotes (*Canis latrans*) expanded their geographic range from a restricted distribution in the arid-central regions of the United States to inhabit most of North America (Young and Jackson 1951; Andrews and Boggess 1978; Hilton 1978; Gaines et al. 1995). Since the 1950's alone, the coyote's geographic range has expanded by 40% (Hody and Kays 2018). This is due in part to the decline of the gray wolf (*Canis lupus*; Mech 1970; Switalski 2003) and in part to coyotes' ability to adapt to human-altered landscapes (Randa and Yunger 2006). Coyote populations during the past 100 years have increased both in areas of their historical range where gray wolf populations have been reduced (e.g., Newsome and Ripple 2015) as well as in newly-invaded regions (e.g., Lovell et al. 1998).

With the extirpation of gray wolves throughout most of the continental United States, coyotes were released from predation by and competition with wolves (e.g., Newsome and Ripple 2015). Gray wolves have been documented to kill and consume coyotes (consumptive predation; Mech 1970; Merkle et al. 2009). For example, predation of gray wolves on coyotes in Yellowstone National Park has been documented during 7% to 16% (Berger and Gese 2007; Merkle et al. 2009) of gray wolf-coyote confrontations. The presence of gray wolves also can affect coyote behavior (non-consumptive predation; Berger et al. 2008). Arjo and Pletscher (2004) observed that coyotes were more vigilant when gray wolves were present within home ranges. Atwood and Gese (2008) and Merkle et al. (2009) observed increased coyote vigilance at gray wolf carrion sites. Gray wolves also are direct competitors with coyotes (Mech 1970). Although coyotes have more generalist diets including primarily small to medium vertebrates, carrion, and berries (Bowyer et al. 1983) and gray wolves are more specialized for ungulates (American bison [*Bos bison*], deer [*Odocoileus* spp.], elk [*Cervus canadensis*], American moose [*Alces alces*]; Mech 1970), there can be diet overlap when coyotes hunt deer and scavenge on wolf-killed ungulates (Arjo et al. 2002).

Coyotes adapt readily to and have expanded into human-altered landscapes. Increased farming and grazing since the 1850's opened landscapes and

created habitats more closely associated with historic coyote habitat (Hilton 1978; Gompper 2002). With their generalist food habits, coyotes have exploited human-altered landscapes (Ozoga and Harger 1966; Berg and Chesness 1978; Bowyer et al. 1983; Arjo et al. 2002). The expansion of available habitat coupled with the coyote's potentially high fecundity rates under favorable conditions (Bekoff 1982) has contributed to the increased coyote populations observed during the past century. Coyotes, especially in the west, now face two factors that might impact abundance and distribution: recovery efforts for the gray wolf, and increased urbanization of agricultural areas.

Reintroduction and recovery efforts for the gray wolf began after its listing under the Endangered Species Act of 1973. Following the reintroduction of 66 wolves in 1995–1996 to Yellowstone National Park and central Idaho, wolves began recolonizing historical regions including eastern Washington (Wiles et al. 2011; Maletzke et al. 2016). Studies examining interactions between coyotes and reestablishing gray wolves have reported mixed effects on coyotes (Merkle et al. 2009). For example, Paquet (1992) demonstrated that due to prey selection differences between wolves and coyotes and the potential scavenger role of coyotes, these two predators can be sympatric. During Paquet's four-year study in Riding Mountain National Park in Manitoba, coyotes benefited from gray wolf-killed ungulates and did not avoid gray wolf home ranges (Paquet 1992). In contrast, Arjo and Pletscher (2004) found that in Glacier National Park, coyotes avoided home ranges of gray wolves except in mild winter months. During their 12-year study within Yellowstone National Park, Merkle et al. (2009) observed that gray wolves dominated encounters with coyotes by displacing, injuring, or in extreme cases killing coyotes. Arjo and Pletscher (2004) and Merkle et al. (2009) suggest that gray wolves have a negative impact on coyotes and that coyote abundance will decrease especially when wolves begin reestablishing home ranges where coyotes are abundant.

Coupled with recovering populations of the gray wolf, expanding urbanization might affect coyote populations. Human population within the United States has increased from around 4 million in 1790 to an estimate of more than 310 million (U.S. Census 2010). In Washington State, where the current study was conducted, the human population rose from 2.4 million in 1950 to around 7 million in 2010 (U.S. Census 2010). Although urbanization and exurban development affect local biodiversity and present challenges to native wildlife (e.g., Wait et al. 2018), responses of individual species are variable. Coyotes, for example, have been observed to inhabit urban areas such as Los Angeles County, California (Baker and Timm 1998) and Detroit, Michigan (Dodge and Kashian 2013) but comparisons of coyote abundance (e.g., Randa and Yunger 2006) and habitat occupancy (e.g., Wait et al. 2018) between urban and rural areas have found greater abundance and habitat occupancy in rural habitats.

In northeastern Washington, recolonizing gray wolves combined with an expanding urban landscape provide an opportunity to examine how these two factors might affect coyote habitat occupancy on an urban-wildland gradient. Zooarchaeological records and preserved specimens since 1850 indicate the presence of coyotes in this region since 10,000 BP (Hody and Kays 2018). Gray wolves were extirpated completely in Washington by the early 20th century and only began recolonizing eastern Washington in 2002 (Wiles et al. 2011). During this time frame, the human population in Spokane, Washington alone increased 12% between 2000 and 2010 (U.S. Census 2010). This study addresses the question of how coyotes respond to the recolonization of the gray wolf and expanding urbanization. The goal of this study is to examine coyote occupancy in five land-use categories and coyote detection in three seasons along an urban-wildland gradient using vocalization sampling.

MATERIALS AND METHODS

This study was conducted in northeastern Washington (Fig. 1) from south of Cheney, Washington (N47º20.913', W117º54.516') north to the Canadian border (N48º57.257', W117º54.588'). Prior to the beginning of fieldwork, all necessary landowner permissions, agency permits, and Eastern Washington

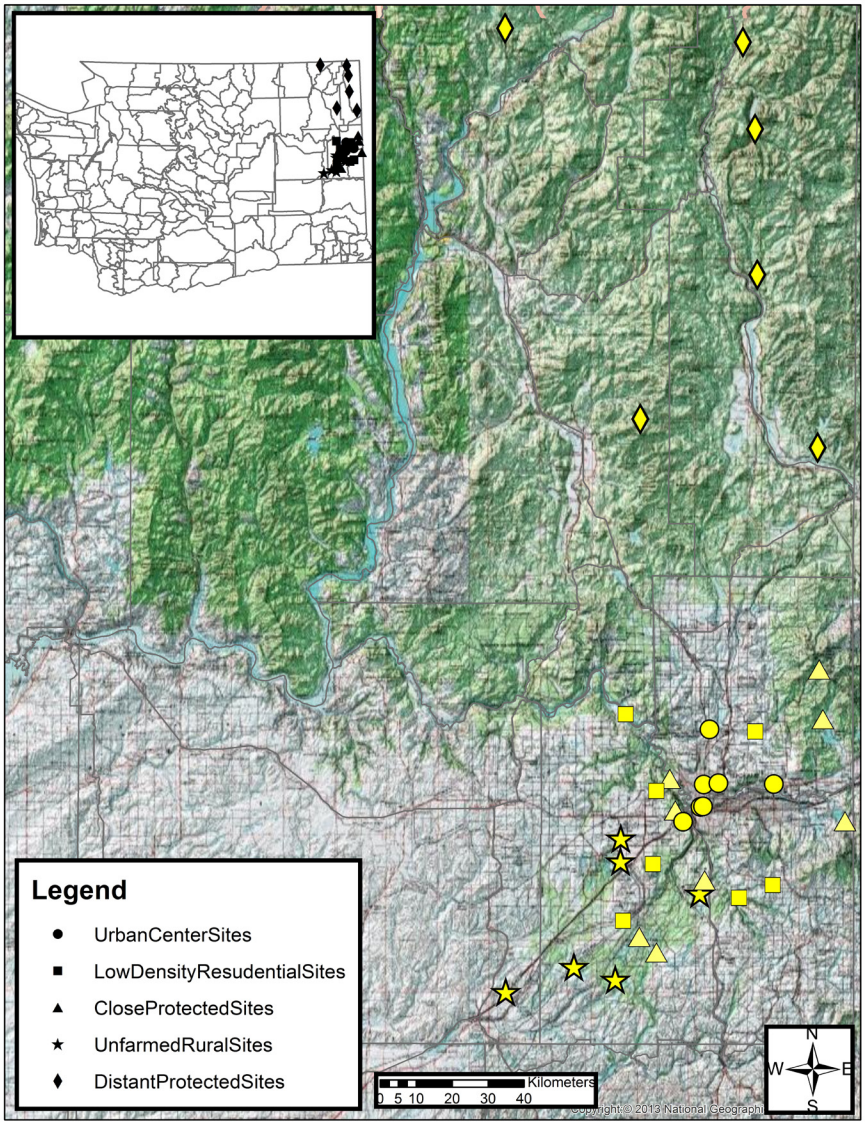

Figure 1. Location of study area in northeastern Washington showing distribution of sampling sites representing five land-use categories.

Institutional Animal Care and Use Committee (IACUC) authorizations were obtained.

Habitats surrounding the primary urban center of Spokane, Washington include dry ponderosa pine forest, mixed conifer forest in the mountains to the north and east, shrub-steppe to the west, and Palouse grasslands to the south. Coyotes are known to occur in all of these habitats (Young and Jackson 1951; Andrews and Boggess 1978; Hilton 1978; Gaines et al. 1995).

Five land-use categories were identified and mapped using ArcGIS (Llewellyn 2008 v9.2). Urban center sites were within the city limits of Spokane, Washington. Low-density residential sites were privately-owned properties from 2 to 8.1 ha within 80.5 km of city limits. Close protected sites were public lands >162 ha within 80.5 km of city limits. Unfarmed rural sites were privately-owned properties, >8.1 ha utilized for grazing or haying within 80.5 km of city limits. Distant protected land sites were public lands

>80.5 km from city limits and identified as locations with possible gray wolf presence.

Individual sampling sites were selected based on the five land-use categories and where landowner permission to access the sites was acquired. For each site, eight landscape characteristics were measured (Table 1). Three characteristics were measured at each site: elevation (km); distance to city limits (km); and distance to year-round water (km). Five landscape characteristics were measured within a 1.6 km radius of each site using ArcGIS (v9.2; U.S. Geological Survey 2010): maximum variation of elevation (total change in elevation in meters); percent tree cover; number of large commercial-use or multi-family dwellings; number of single-family homes; and length of two-lane paved roads (km). The radius for these five characteristics was determined based on pre-season sampling conducted to measure the minimum distance that a broadcast howl could be heard by an observer regardless of terrain (e.g., mountains, buildings). The mean values for these eight measurements were compared using one-way Analysis of Variance with a Tukey's means separation test. Discriminant Function Analysis (DFA; SAS v[9.2]) was conducted to determine if the measured landscape variables correctly differentiated the five land-use categories. A final variable, gray wolf presence or absence, was based on gray wolf return vocalizations recorded during this study.

Vocalization sampling was used to estimate the presence of coyotes and gray wolves. Vocalization sampling has used sirens as well as natural and simulated coyote and gray wolf vocalizations to elicit responses. Coyotes respond to sirens and to natural and simulated coyote and gray wolf vocalizations, whereas gray wolves respond only to natural and simulated gray wolf vocalizations (Harrington and Mech 1982; Okoniewski and Chambers 1984; Gaines et al. 1995). Because the goal was to document both coyote and gray wolf presence, simulated gray wolf broadcast vocalizations were used.

Similar to regional agencies, the protocol for call broadcasting was based on that developed by Harrington and Mech (1982). Each night's sampling session began with a 10-min period at each site to observe any canid activity before broadcasting began. Gray wolf vocalizations were broadcast with a Predator Master CD device. A vocalization was broadcast for 30 s and return vocalizations were recorded during the next 90 s using an Edirol R9 digital recording device. This procedure was repeated three times at three different volumes (low, medium, high) resulting in an 18 min sample session at each site per sample night. Decibel levels of the high volume broadcast howl was recorded at 96 dB in a lab with each lower volume decreasing incrementally. A minimum of six sample sites per land-use category were visited once monthly. With the exception of two urban center sites that were 4 km apart, all other sample sites were > 8 km apart. Monthly visits to each set of sites were selected by random. Sampling began 30 min after sunset, with the order of site visits relative to sunset reversed for each trip. Each site was sampled 12 times during May 2009–April 2010 for a minimum of 72 sample nights per land-use category. Sampling was conducted only on nights with no active rain or thunderstorms and with wind speeds <6 m/s (Harrington and Mech 1982).

After each night of sampling, vocalization data were downloaded into a bio-acoustic analysis software (Signal v4.0; Beeman 2006) to isolate howl frequency (kHz) and intensity (dB). The Signal waveform output for howl frequency was analyzed to identify species (e.g., domestic dog, coyote, gray wolf). The Signal spectrograph output for intensity (amplitude) was used to distinguish the number individual return vocalizations which indicated group size.

Single-season occupancy models in Presence 12.31 software (Hines 2016) were used to evaluate probability of site occupancy and detection models. A site was considered occupied if one recorded coyote vocalization was returned during a single sampling session. Covariates for site occupancy were the five land-use categories, and wolf presence (1 for a given category, 0 otherwise). Given that frequency of coyote howling has been observed to vary depending upon time of year relative to life history (Kenaga et al. 2013), we used three detection covariates based on seasons as defined by Okoniewski and Chambers (1984). Season 1 (May–August 2009; pup rearing) corresponded with the rearing of young when both parents and pups were closely associated with den areas. Season 2 (September–December 2009; dispersal) was identified as the dispersal-pairing when coyotes were leaving the natal area to establish new territories and find mates. Season

3 (January–April 2010; breeding) was characterized by mating and early pregnancy. Six a priori models incorporating different covariates were generated to assess the probability of site occupancy (ψ) and detection (p) for coyotes in relation to the five land-use categories, wolf presence, and three seasons.

RESULTS

The five land-use categories differed with respect to all landscape characteristics except distance to permanent water (Table 1). Urban center was relatively flat with highest distance of paved roads, number of houses, and commercial properties. Distant protected sites had the greatest elevation changes and lowest distance of paved roads. Tree cover was greater in both close and distance protected areas and lower in low-density residential and unfarmed rural sites. Based on landscape characteristics, Discriminant Function Analysis correctly classified each site as the land-use category assigned to it with a 91% accuracy (Wilk's lambda = 0.004, F = 7.5, df = 33, P < 0.0001)

A total of 408 sample sessions during the study year resulted in recordings of 401 coyote and nine gray wolf return vocalizations (Table 2). Coyote return vocalizations were recorded at 23 of the 34 sites and all land-use categories were represented. The percent of sampling nights that coyotes were detected varied between land-use categories from 2% (urban center) to almost 50% (close-protected). Gray wolf return vocalizations were recorded at only two distant protected land-use category sites. Coyote return vocalizations were recorded during all three seasons but the percent of sampling nights that coyotes were detected was much greater during Season 2 (dispersal). Gray wolf return vocalizations were only recorded during Seasons 1 and 2. Additionally, in four instances when coyotes saw observers before or during the broadcast sequence, there were no return vocalizations.

Of the five models representing different combinations of occupancy and detection covariates and one model with constant probability, two models had a combined model weight of 92%. (Table 3). The top model incorporated the five land-use categories and seasons and the second model added wolf presence. The naïve occupancy rate was 0.6765. The occupancy estimates (± SE) for the six occupancy covariates were urban center: -1.79 ± 1.080; low-density residential: 0.918 ± 0.838; distant protected: 1.10 ± 1.156; unfarmed rural: 1.612 ± 1.098; close protected: 1.950 ± 1.072; and wolf presence: 0.318 ± 1.519. Detection estimates (± SE) were -1.042 ± 0.237 for Season 1; 0.676 ± 0.221 for Season 2; and -0.352 ± 0.212 for Season 3.

Table 1. Number of sample sessions per land-use category and per season, number of coyote and gray wolf return vocalizations, and number of nights coyotes were detected per number of nights sampled in northeastern Washington, 2009–2010.

Land-use category	Sample sessions	Coyote return vocalizations	Nights detected/nights sampled (%)	Gray wolf return vocalizations
Urban center	84	4	2/84 (2.4%)	0
Low-density residential	84	64	27/84 (28.6%)	0
Close protected	96	139	43/96 (44.8%)	0
Unfarmed rural	72	116	35/72 (48.6%)	0
Distant protected	72	78	20/78 (25.6%)	9
Season				
1(May–Aug); Pup rearing	136	71	23/136 (16.9%)	7
2 (Sept–Dec); Dispersal	136	230	63/136 (46.3%)	2
3 (Jan–Apr); Breeding	136	100	41/136 (30.1%)	0

Table 2. ANOVA comparison of means (± SE) for eight landscape characteristics (defined in text) for five land-use categories in northeastern Washington. Means with different letters are significantly different.

Land-use category	Distance to urban center (km)	Elevation (m)	Δ Elevation (m)	% Tree cover	Distance to water (km)	Number of houses	Number of commercial	Road length per km
Urban center	6.53 ± 2.16 [a]	595.71± 22.23 [a]	65.31 ± 31.50[a]	21.29 ± 10.62 [a]	0.98 ± 0.43	1945.29 ± 501.79 [a]	41 ± 12.65[a]	85.99 ± 16.95[a]
Low-density residential	16.17 ± 2.45 [ab]	652.75 ± 42.97 [ab]	100.15 ± 33.07[ab]	14.43 ± 4.06 [ab]	0.84 ± 0.27	201.71 ± 55.53[b]	6.57 ± 5.16 [b]	14.48 ± 2.19 [b]
Close protected	21.35 ± 3.72 [abc]	698.26 ± 48.34 [ab]	209.55 ± 46.24[abc]	64.75 ± 6.91[c]	0.71 ± 0.28	61.25 ± 22.1[b]	0 [b]	8.77 ± 3.09 [b]
Unfarmed rural	29.13 ± 5.81 [abc]	711.76 ± 16.04 [ab]	86.36 ± 21.43[abc]	13.50 ± 6.49 [ab]	0.81 ± 0.21	33.5 ± 15.06[b]	0 [b]	5.70 ± 2.30 [b]
Distant protected	110.23 ± 13.84 [d]	836.42 ± 82.68 [b]	533.40 ± 55.51[d]	87.67 ± 3.32[c]	0.30 ± 0.11	3.83 ± 0.83 [c]	0 [b]	2.33 ± 0.77 [b]
ANOVA results	F = 42.91 df = 4 P<0.0001	F= 3.51 df 4 P=0.019	F = 23.66; df 4; P<0.0001	F = 23.05; df = 4; P<0.001	F= 0.74 df = 4 P= 0.57	F = 12.78 df = 4 p <0.0001	F = 7.9 df = 4 p < 0.0001	F= 16.65 df 4 p<0.0001

Table 3. Occupancy (ψ) and detection (p) models of coyotes based on vocalization sampling in northeastern Washington 2009–2010. Model descriptions with covariates, number of parameters (K), Aikaike's Information Criteria adjusted for small sample sizes (AICc), difference in AICc from most parsimonious model (ΔAICc), model weights (Wi) and -2log likelihood values are presented.

Model	K	AIC_c	ΔAIC_c	W_i	-2log likelihood values
ψLand-use categories; *p* Seasons	8	394.87	0.0	0.5792	378.87
ψLand-use categories, wolf presence; *p* Seasons	9	395.96	1.09	0.3359	377.96
p Seasons	4	398.71	3.84	0.0849	390.71
ψLand-use categories	6	422.27	27.40	0.0	410.27
ψLand-use categories; wolf presence	7	423.37	28.50	0.0	409.37
Constant ψ*p*	2	426.12	31.25	0.0	422.12

DISCUSSION

Coyote occupancy varied across the urban-wildland gradient in northeastern Washington and detection varied between seasons. Differences between landscape characteristics of the five land-use site categories provide insight into occupancy patterns and seasonal differences in life-history stages explain detection patterns.

Occupancy of coyotes was least within the city limits and low in the low-density residential sites. Length of paved roads and number of both single-family and commercial buildings were highest in the urban sites. The one urban center site that did receive return vocalizations had fewer paved roads. Avoidance of roads has been reported for coyotes in other regions (e.g., Kays et al. 2008; Greenspan et al. 2018). Furthermore, in Washington, coyotes might avoid roads because they are considered an unclassified wild animal (Washington Department of Wildlife 2019) and hunting was allowed even within city limits during the study period.

Although coyotes have colonized urban landscapes throughout the United States (Poessel et al. 2017), coyote use of urban areas is known to be variable. For example, Baker and Timm (1998) found that coyotes were common in urban and suburban areas of Los Angeles County, California. Within an 80-day period, 55 coyotes were removed from 0.8 km radius, indicating high coyote relative abundance within the urban area. Baker and Timm (1998) suggested that coyote relative abundance was high in Los Angeles County due to adequate shelter, food, and water. Greenspan et al. (2018) modeled the potential distributions of coyotes in metropolitan Chicago and suggested that the distribution of forested green spaces was sufficient to support coyotes. Behavior of individual coyotes might play a role in how likely they are to inhabit urban landscapes. Breck et al. (2019) observed that urban coyotes in Denver, Colorado, exhibited more "bold and exploratory" behaviors than coyotes in rural Utah.

Although coyotes certainly have adapted to urban landscapes, comparisons of coyote abundance between urban and surrounding rural areas suggest greater abundance in rural areas. For example, Randa and Yunger (2006) examined coyote relative abundance along an urban-wildland gradient of Chicago, Illinois, and found that coyotes were more abundant in the rural habitat surrounding the urban area than in the urban center. Using camera traps to predict habitat occupancy on an urban-rural gradient in the Flint Hills Region of Kansas, Wait et al. (2018), similar to the current study, concluded that coyotes were least likely to be found in urban landscapes. It could be argued that coyotes are less vocal in areas where they encounter humans frequently and therefore might bias estimates if using vocalizations for relative abundance. However, Randa and Yunger (2006) used scent stations and Wait et al. (2018) used camera traps to examine coyote abundance and not return vocalizations, but had similar results to this study.

Coyote occupancy increased in close-protected, and unfarmed rural sites surrounding the urban center. Within this region, length of paved roads and density of residential and commercial buildings were lower. Tree cover was variable; it was lowest in the unfarmed rural and higher in the close protected sites. How tree cover affects coyote habitat use varies and is probably related to the surrounding landscape matrix (Cherry et al. 2016). Quinn (1995) found that coyotes frequented areas where grasslands met moderately tree-covered areas. Using howl surveys and habitat modeling to examine coyote abundance in relation to land cover, Cherry et al. (2016) determined that although coyote abundance was greater in pastures and fallow fields, coyotes did not avoid forested areas. The land-use conditions that has been created in the unfarmed rural areas mimic historic habitat of coyotes (Gompper 2002; Cherry et al. 2016).

Coyote occupancy was low at the distant protected sties. These sites were characterized by greater elevation, change in elevation, and tree cover. Coyote abundance has been observed to be lower in forested mountain habitat (Ambrose 1996; Witczuk et al. 2015) and in the presence of larger carnivores (e.g., cougars [*Puma concolor*], black bears [*Ursus americanus*,], grizzly bears [*Ursus arctos*]]). Arjo (1998) found in Montana that 40% of radio-collared coyotes were consumed by cougars. With the recolonization of the gray wolf to northeastern Washington as early as February 2002 (Wiles et al. 2011), coyotes are encountering another direct predator and competitor. The presence of the three predators and the mountainous forested habitat of most of these distant protected lands might explain the lower occupancy of coyotes.

Gray wolves were recorded at two distant protected sites but coyote vocalizations varied between these two sites. At one site, coyotes were recorded during ten of the twelve sessions and at the other coyotes were never recorded. During July 2009, two gray wolves were radio-collared near the first site by area biologists (Wiles et al. 2011) and represented a newly established breeding pair with three offspring. The repeated recordings of multiple coyotes could be a direct consequence of the presence of a newly established gray wolf pack. Merkle et al. (2009) studied the response of coyotes to the reintroduced gray wolves in Yellowstone National Park. They observed that during initial interactions with gray wolves, coyotes formed larger, more aggressive groups to defend their territories. Also with the introduction of large-ungulate carrion from gray wolf kills, coyotes were able to sustain these larger group sizes. At the second distant protected site at which gray wolves were recorded, the wolf pack had been established longer and coyote presence was not detected. Further, Berger and Gese (2007) observed that after the establishment of gray wolves in Yellowstone National Park, coyote relative abundance decreased by 50% in areas of high gray wolf usage, suggesting that coyote occupancy at the first site might be temporary.

Results of this study also indicated that time of year affected detectability of coyotes. Many howl surveys (e.g., Lehnr 1982; Gaines et al. 1995; Bender et al. 1996) have been conducted during spring/summer and completed by October. However, in this study, detectability was greater during Season 2 (September–December) when coyotes are dispersing from natal areas and establishing new territories and lowest during Season 1 (May–August) when coyotes were rearing pups. Similar seasonal pattern in response vocalizations have been observed in metropolitan (Kenaga et al. 2103) and rural (Gese and Ruff 1998; Petroelje et al. 2013) areas. Given that resident coyotes tend to respond more to broadcast vocalizations than transient coyotes, low responses during pup rearing might reduce risk to pups at territory den sites (Petroelje et al. 2013). These observations indicate that it is important to use canid natural history events to define timing of howl surveys.

Results discussed herein suggest that with increasing gray wolf populations, coyotes will disperse away from newly recolonized gray wolf territories and demonstrate behavioral shifts to reduce encounters. With increasing urbanization and exurban sprawl in northeastern Washington, the unfarmed rural areas will shrink. Consequently, coyotes might become dependent upon a network of suitable habitats within an urban landscape matrix, as they have in other metropolitan areas. In addition, this study provides baseline data for managers monitoring changing canid population dynamics, and guidelines for best times of year for vocalization sampling.

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LITERATURE CITED

Ambrose, S. E. 1996. Undaunted courage: Meriwether Lewis, Thomas Jefferson, and the opening of the American West. Simon and Schuster, New York, New York.

Andrews, R. D., and E. K. Boggess. 1978. Ecology of coyotes in Iowa. Pp. 249–265 in Coyotes: biology, behavior, and management (M. Bekoff, ed.). Academic Press, New York, New York.

Arjo, W. M. 1998. The effects of recolonizing wolves on coyote populations, behaviors, and food habits. Ph.D. Thesis, University of Montana, Missoula.

Arjo, W. M., and D. H. Pletscher. 2004. Coyote and wolf habitat use in northwest Montana. Northwest Science 78:24–32.

Arjo, W. M., D. H. Pletscher, and R. R. Ream. 2002. Dietary overlap between wolves and coyotes in northwestern Montana. Journal of Mammalogy 83:754–766.

Atwood, T. C., and E. M. Gese. 2008. Coyotes and recolonizing wolves: social rank mediates risk-conditional behavior at ungulate carcasses. Animal Behaviour 75:753–762.

Baker, R. O., and R. M. Timm. 1998. Management of conflicts between urban coyotes and humans in southern California. Proceedings of the Vertebrate Pest Conference 18:299–312.

Beeman, K. 2006. Signal v4.0. Program distributed by Engineering Design. Berkeley, California

Bekoff, M. 1982. Coyotes. Pp. 447–459 in Wild mammals of North America: biology, management, economics (J. A. Chapman and G. A. Feldhamer, eds). John Hopkins University Press, Baltimore, Maryland.

Bender, D. J., E. M. Bayne, and R. M. Brigham. 1996. Lunar condition influences coyote (*Canis latrans*) howling. American Midland Naturalist 136:413–417.

Berg, W. E., and R. A. Chesness. 1978. Ecology of coyotes in northern Minnesota. Pp. 229–247 in Coyotes: biology, behavior, and management (M. Bekoff, ed.). Academic Press, New York.

Berger, K. M., and E. M. Gese. 2007. Does inference competition with wolves limit the distribution and abundance of coyotes? Journal of Animal Ecology 76:1075–1085.

Berger, K. M., E. M. Gese, and J. Berger. 2008. Indirect effects and tradition trophic cascades: A test involving wolves, coyotes, and pronghorn. Ecology 89: 818–828.

Bowyer, R. T., S. A. McKenna, and M. E. Shea. 1983. Seasonal changes in coyote food habits as determined by fecal analysis. American Midland Naturalist 109:266–273.

Breck, S. W., S. A. Poessel, P. Mahoney, and J. K. Young. 2019. The intrepid urban coyote: a comparison of bold and exploratory behavior in coyotes from urban and rural environments. Scientific Reports 9:14.

Cherry, M. J., P. E. Howell, C. D. Seagraves, R. J. Warren, and L. M. Conner. 2016. Effects of land cover on coyote abundance. Wildlife Research 43:662–670.

Dodge, W. B., and D. M. Kashian. 2013. Recent distribution of coyotes across an urban landscape in southeast Michigan. The Journal of Fish and Wildlife Management 4:377–385.

Gaines, W. L., G. K. Neale, and R. H. Naney. 1995. Response of coyotes and gray wolves to simulated howling in north-central Washington. Northwest Science 69:217–222.

Gese, E. M., and R. L. Ruff. 1998. Howling by coyotes (*Canis latrans*): variation among social classes, seasons, and pack sizes. Canadian Journal of Zoology 76:1037–1043.

Gompper, M. E. 2002. Top carnivores in the suburbs? Ecological and conservation issues raised by colonization

of north-eastern North America by coyotes. Bioscience 52:185–190.

Greenspan, E., C. K. Nielson, and K. W. Cassel. 2018. Potential distribution of coyotes (*Canis latrans*), Virginia opossum (*Didelphis virginiana*), striped skunks (*Mephitis mephitis*), and raccoons (*Procyon lotor*) in the Chicago metropolitan area. Urban Ecosystems 21:983–997.

Harrington, F. H., and L. D. Mech. 1982. An analysis of howling response parameters useful for wolf pack censusing. The Journal of Wildlife Management 46:686–693.

Hilton, H. 1978. Systematics and ecology of the eastern coyote. Pp. 209–228 in Coyotes: Biology, Behavior, and Management (M. Bekoff, ed.). Academic Press, New York.

Hines, J. E. 2016. Presence – software to estimate patch occupancy and related parameters. SGS-PWRC. https://www.usgs.gov/software/presence. Accessed 6 April 2019.

Hody, J. W., and R. Kays. 2018. Mapping the expansion of coyotes (*Canis latrans*) across North and Central America. Zookeys 759:81–97.

Kays, R. L., M. E. Gompper, and J. C. Ray. 2008. Ecology of eastern coyotes based on large-scale estimates of abundance. Ecological Applications 18:1014–1027.

Kenaga, B., R. A. Krebs, and W. B. Clapham, Jr. 2013. Coyote land use inside and outside urban parks. American Midland Naturalist 170:298–310.

Lehnr, P. N. 1982. Differential vocal response of coyotes to "group howl" and "group-yip howl" playbacks. Journal of Mammalogy 63:675–679.

Llewellyn, C. 2008. ArcGIS v9.3. Product distributed by ESRI. Redlands, California.

Lovell, C. D., B. D. Leopold, and C. C. Shropshire. 1998. Trends in Mississippi predator populations 1980–1995. Wildlife Society Bulletin 26:552–556.

Maletzke, B. T., R. B. Wielgus, D. J. Pierce, D. A. Martorello, and D. W. Stinson. 2016. A meta-population model to predict occurrence and recovery of wolves. The Journal of Wildlife Management 80:368–376.

McCarley, H. 1975. Long-distance vocalizations of coyotes (*Canis latrans*). Journal of Mammalogy 56:847–856.

Mech, L. D. 1970. The wolf: The ecology and behavior of an endangered species. University of Minnesota Press, Minneapolis, Minnesota. 384p.

Merkle, J. A., D. R. Stahler, and D. W. Smith. 2009. Interference competition between gray wolves and coyotes in Yellowstone National Park. Canadian Journal of Zoology 87:56–63.

Newsome, T. M., and W. J. Riddle. 2015. A continental scale trophic cascade from wolves through coyotes to foxes. Journal of Animal Ecology 84:49–59.

Okoniewski, J. C., and R. E. Chambers. 1984. Coyote vocal response to an electronic siren and human howling. The Journal of Wildlife Management 48:217–222.

Ozoga, J. J., and E. M. Harger. 1966. Winter activities and feeding habits of northern Michigan coyotes. The Journal of Wildlife Management 30:809–818.

Paquet, P. C. 1992. Prey use strategies of sympatric wolves and coyotes in Riding Mountain National Park, Manitoba. Journal of Mammalogy 73:337–343.

Petroelje, T. R., J. L. Belant, and D. E. Beyer. 2013. Factors affecting the elicitation of vocal responses from coyotes *Canis latrans*. Wildlife Biology 19:41–47.

Poessel, S. A., E. M. Gese, and J. K. Young. 2017. Environmental factors influencing the occurrence of coyotes and conflicts in urban area. Landscape and Urban Planning 157:259–269.

Quinn, T. 1995. Using public sighting information to investigate coyote use of urban habitat. The Journal of Wildlife Management 59:238–245.

Randa, L. A., and J. A. Yunger. 2006. Carnivore occurrence along an urban-rural gradient: A landscape-level analysis. Journal of Mammalogy 87:1154–1164.

SAS Institute Inc., 2008. SAS 9.3 Statistical analysis software. Cary, NC

Switalski, T. A. 2003. Coyote foraging ecology and vigilance in response to gray wolf reintroduction in Yellowstone National Park. Canadian Journal of Zoology 81:985.

U.S. Census. 2010. 2010 Census. http://www.census.gov/ Accessed September 2010. Revised August 2010.

U.S. Geological Survey. 2010. ArcGIS v9.2 map source. http://cumulus.cr.usgs.gov/services.php Accessed September 2010.

Washington Department of Fish and Wildlife 2019. Coyotes. https://wdfw.wa.gov/species-habitats/species/canis-latrans#conflict. Accessed 1 June 2019.

Wait, K. R., A. M. Ricketts, and A. A. Ahlers. 2018. Land-use change structures carnivore communities in remaining tallgrass prairie. The Journal of Wildlife Management 82:1491–2018.

Walsh, P. B., and J. M. Inglis. 1989. Seasonal and diel rate of spontaneous vocalization in coyotes in south Texas. Journal of Mammalogy 70:169–171.

Wiles, G. J., H. L. Allen, and G. E. Hayes. 2011. Wolf conservation and management plan for Washington. Washington Department of Fish and Wildlife, Olympia, Washington.

Witznuk, J., S. Pagacz, J. Gliwicz, and L. S. Mills. 2015. Niche overlap between sympatric coyotes and bobcats in highland zones of Olympic Mountains, Washington. Journal of Zoology 297:176–183.

Young, S. P., and H. H. T. Jackson. 1951. The clever coyote. The Stackpole Company, Harrisburg, Pennsylvania.

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Morphological and Craniodental Characterization of Bobcat × Canada Lynx (*Lynx rufus* × *L. canadensis*) F1 Hybrids from New Brunswick, Canada

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Abstract

Occurrences of hybridization between bobcats (*Lynx rufus*) and Canada lynx (*L. canadensis*) are rare but have been reported in Canada and the United States. Hybrids can prove problematic where conservation legislation is in place, as it is in some jurisdictions for Canada lynx, including New Brunswick. It is therefore important to determine how readily hybrids can be distinguished. In this study, morphometric analyses of craniodental characters and qualitative comparisons of pelage and other external features were reported for three genetically-confirmed bobcat × Canada lynx (*Lynx rufus* × *L. canadensis*) female F1 hybrids from New Brunswick. These bobcat × Canada lynx hybrids strongly resemble bobcats in pelage coloration. However, extent of ear tufts, size of hind feet, and tail coloration have characteristics of both parental species. Fifteen craniodental characters were measured for bobcats (n = 41), Canada lynx (n = 37), and the three genetically confirmed F1 hybrids. Multivariate analysis (principal components analysis) suggested that F1 hybrids are craniometrically intermediate between the parental species in size and key diagnostic craniodental characters but cluster more closely with bobcats in morphometric space. Altogether, although external and cranial characters can be strongly indicative of hybridization between bobcats and Canada lynx, genetic analyses should be used to confirm hybridization between these species.

Key words: bobcat, Canada lynx, craniodental, F1 hybrids, morphology, New Brunswick

Introduction

Bobcats (*Lynx rufus*) and Canada lynx (*Lynx canadensis*) are closely related felid species (Tumlinson 1987; Lariviere and Walton 1997; Johnson and O'Brien 1997) native to North America. Fossil records for bobcats (evolved from *L. issiodorensis*) and Canada lynx (evolved from *L. lynx*) date back 3.2–1.8 million (Pliocene) and 75,000–125,000 BP (Upper Pleistocene), respectively (Werdelin 1981). Generally, bobcats can be phenotypically distinguished from Canada lynx. Bobcats have significantly smaller and narrower feet than Canada lynx, a pelage that is reddish-brown with spots (especially on the flanks), a tail tipped with black and white, and ear tufts on the top of the pinnae that are shorter than those of Canada lynx (Tumlinson 1987; Novak 1999). In contrast, Canada lynx possess large, broad feet (a specialized adaptation for hunting Snowshoe Hare, *Lepus americanus*, a primary prey item throughout much of their range), grizzled white and gray pelage (camouflage during the winter), a black-tipped tail, and ear tufts that are longer than those of bobcats (Tumlinson 1987; Anderson and Lovallo 2003).

Both species have broad geographic ranges, exhibit considerable geographic variation with respect to phenotype (Hall 1981; Schmidly and Read 1986), and are largely allopatric with one another; the bobcat being the more austral generalist (southern Canada across the contiguous USA into southern Mexico) and the Canada lynx being a northern boreal specialist (northern USA to the treeline in Canada and Alaska). However, parts of their geographic ranges overlap across southern and Atlantic Canada, and the midwestern and northeastern USA (Banfield 1974; Anderson and Lovallo 2003) – i.e., the southern periphery of the geographic range for Canada lynx (Homyack et al. 2008). This range overlap is predicted to increase as the bobcat gradually expands its distribution northward into parts of southeastern and Atlantic Canada as a result of habitat modification and

global warming (Parker et al. 1983; Lavoie et al. 2010; Roberts and Crimmins 2010). As the range overlap between these two congeners increases, encounters between individuals and subsequent hybridization may become more frequent.

Hybridization may pose unique conservation problems for rare and/or threatened species (Rhymer and Simberloff 2003; Miller et al. 1989; but see Mallet 2007). Though hybridization occurs naturally among some species (and may be an important vector of speciation for some taxa; Dowling and Secor 1997; Larsen et al. 2010), it also can threaten the genetic integrity of populations when it arises from anthropogenic activity (Rhymer and Simberloff 2003). Though rare, hybridization between bobcats and Canada lynx has been detected in areas of sympatry; to date, a total of 15 cases of genetically confirmed bobcat × Canada lynx F1 hybrids (sequence of hybrid names used here reflects current knowledge of parentage; where known, all hybrids involve male bobcat crosses with female Canada lynx) have been reported in Canada (New Brunswick – Homyack et al., 2008; Saskatchewan, Manitoba – Koen et al. 2014) and the USA (Maine, Minnesota – Homyack et al. 2008; New York – Reding 2011 and Koen et al. 2014). However, because of the few reported cases of hybridization between these two congeners, relatively little is known about the dynamics of such reproductive interactions (Murray et al. 2008; Koen et al. 2014); questions regarding causal factors (e.g., ecological conditions that promote hybridization between bobcats and Canada lynx), fertility (though backcrosses have been detected; see Reding 2011 and Koen et al. 2014), and relative fitness of hybrids have yet to be addressed.

Homyack et al. (2008) provided an initial description of bobcat × Canada lynx F1 hybrid external morphology. However, no description was provided of associated skulls, an important component in mammalian studies that focus on anatomy and systematics (Elbroch 2006). In this study, external and craniodental morphology of bobcat × Canada lynx F1 hybrids are described and analysed. Through examinations of museum vouchers, initial qualitative descriptions of F1 hybrid pelts provided by Homyack et al. (2008) are expanded upon. In addition, the skulls of three genetically confirmed female F1 hybrids from New Brunswick (Homyack at al. 2008 had access to only two of these hybrid skulls from New Brunswick) were measured and morphometrically analysed; craniometrics were compared with individuals from New Brunswick that had previously been identified as either bobcat or Canada lynx based on cranial morphology and external characteristics. The matter of false-positive hybrids being reported is also considered, wherein trappers or wildlife managers identify carcasses as putative hybrids based on external morphology but further examination confirm these not to be of hybrid origin.

MATERIALS AND METHODS

The pelts of three genetically confirmed adult female bobcat × Canada lynx F1 hybrids from New Brunswick (deposited at the New Brunswick Museum) were examined and compared to pelts of both parental species from the same region. Lab work pertaining to genetic confirmation of hybridization (detection of F1 only, via microsatellite markers; see Schwartz et al. 2004) and determining matrilineage (via mitochondrial NADH-5 gene; see Johnson et al. 1998) were conducted at the Canadian Museum of Nature. In part following the original description provided by Homyack et al. (2008), detailed observations and notes were made on pelage coloration, the length of the ear tufts, comparative size (length) of the hind feet, and tail coloration.

For the craniodental analysis, 81 skulls (41 bobcats, 37 Canada lynx, and 3 genetically confirmed F1 hybrids) were examined and measured. Skulls examined were obtained from several museum collections: Smithsonian National Museum of Natural History (USNM); Royal Ontario Museum (ROM); Canadian Museum of Nature (CMN); and New Brunswick Museum (NBM). Both species have large, contiguous geographic ranges in North America (Fig. 1), but because the hybrids in this study originated from New Brunswick, the comparative aspects of this study were restricted to specimens collected only from New Brunswick and the immediate region (Nova Scotia and Maine). All specimens examined were confirmed

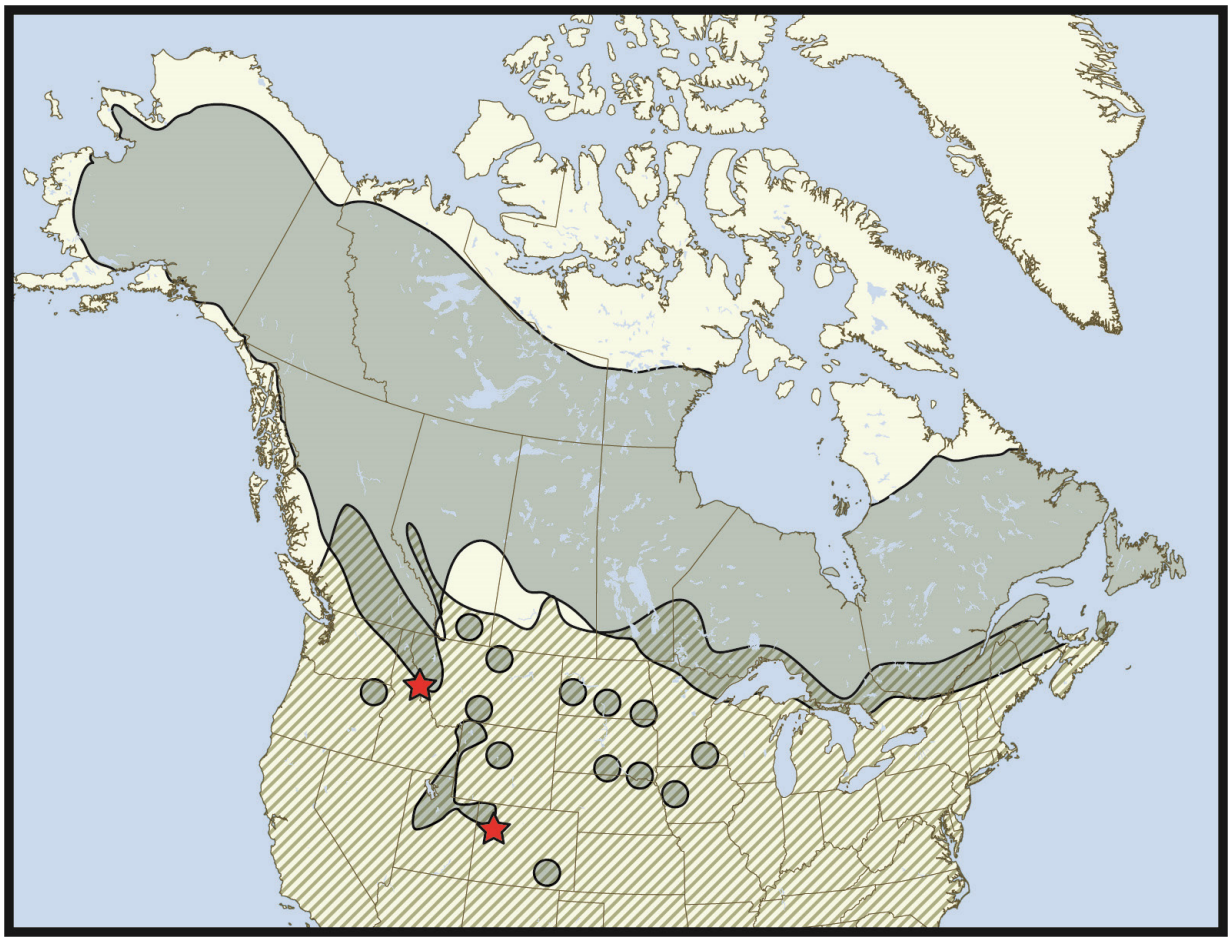

Figure 1. Geographic distribution of Canada lynx (shaded region) and bobcats (cross-hatched region). Circles indicate extra-limital records for Canada lynx and stars indicate areas where Canada lynx have been introduced.

as mature adults, either from museum records and/or qualitative examination of the skulls – i.e., overall size, toothwear, ossification patterns, development of the sagittal crest, and complete closure of the cranial sutures (Conley and Jenkins 1969; Morris 1972). Sexes of both parental species were relatively equal in the samples (bobcats: m = 24, f = 17; Canada lynx: m = 20, f = 17). All three bobcat × Canada lynx F1 hybrids were sexed as female and genetically verified either by others (Libby 2004; Homyack et al. 2008) or during this study.

Measurements for all skulls were recorded by digital calipers to the nearest 0.01 mm. Twelve cranial (skull and mandible) and three dental characters were measured, as follows: CBL = Condylobasal Length – length from the anterior edge of the premaxillae to the posterior-most projections of the occipital condyles; BCL = Braincase Length – length from the anterior edge of the basisphenoid to the anterior-most point on the lower edge of the foramen magnum; MB = Mastoid Breadth; BCB = Braincase Breadth – least width of the braincase; ZB = Zygomatic Breadth – maximum width across the skull at the posterior edge of the zygomatic arches; POC = Postorbital Constriction – least width at the top of the cranium posterior to the postorbital process; IOB = Interorbital breadth – least width of the bridge of bone between the orbits; NBW = Nasal Bone Width – greatest width of the nasal bone; ABW = Auditory Bullae Width – greatest width of the auditory bullae; PSW = Presphenoid Width – least width of the presphenoid bone; ML = Mandible Length – greatest length of the mandible/lower jaw; RH = Ramus Height – greatest height of the ramus (of the mandible); C–M1

= Canine–Molar 1 – distance from the upper canine to the first molar; MMRL = Mandibular Molariform Length – distance from the first premolar to the last molar on the mandible; and CCB = Canine–Canine Breadth – maximum breadth between the two upper canines. Measurements of cranial characters were taken at the edges of sutures. Measurements for dental characters were measured at the edge of the alveolus.

Principal components analysis (PCA) was performed on the covariance matrix (Sokal and Rohlf 1995) derived from the craniometric data. Because measurements for the presphenoid bone were highly variable and overlapped for bobcats and Canada lynx, it was excluded from the PCA; the remaining 14 craniodental characters were logarithmically transformed prior to the analysis. Because both bobcats (e.g., Larivière and Walton 1997; Anderson and Lovallo 2003) and Canada lynx (e.g., Quinn and Parker 1987; Khidas et al. 2013) are known to exhibit sexual dimorphism with respect to size, the PCA was conducted separately for males and females. Data for female hybrids were incorporated into the PCA for female bobcats and Canada lynx, whereas a series of four putative (i.e., initially identified as hybrids phenotypically, but not yet genetically confirmed) male hybrids, ultimately genetically confirmed as false-positives, were included in the PCA for male bobcat and Canada lynx. All multivariate analyses were conducted with SYSTAT©12 statistical program.

In addition to measuring the 15 quantitative variables, three qualitative traits for each hybrid skull were examined and recorded: (1) the shape of the presphenoid bone; (2) the presence of a septum that divides the auditory bullae in conjunction with the overall shape and conformation of the auditory bullae; and (3) the position of the hypoglossal canal relative to the jugular foramen. Typically, bobcats have a thin (medially constricted) presphenoid bone, no septum in large, greatly inflated auditory bullae, and a hypoglossal canal that is confluent with the jugular foramen (Elbroch 2006). In contrast, Canada lynx have medially wide presphenoid bones, a septum that divides relatively small auditory bullae, and a hypoglossal canal that is distinctly separate from the jugular foramen.

RESULTS

Detailed study of the three female bobcat × Canada lynx F1 hybrid pelts revealed one consistent pattern with respect to external morphology. F1 hybrids more closely resembled regional bobcats, manifesting a grizzled, rufous-brown and olive-grey dorsum and dark brown to light black spots along the slightly paler flanks. In contrast, typical Canada lynx found in New Brunswick exhibited a more grizzled, grayish-white dorsum interspersed with pale ochraceous hairs and much paler flanks that lack spots (Fig. 2).

Other external features blended the characters of both parental species. The hind feet of F1 hybrids were intermediate in length (average = 195 mm) between bobcats (average = 160 mm) and Canada lynx (average = 240 mm). Overall, hind feet from F1 hybrids were relatively small and slender, similar to but slightly larger than those of typical bobcats, but not as broad as typical Canada lynx (Fig. 3). The pinnae (external ear) of F1 hybrids possessed relatively long (> 25 mm) black ear tufts resembling those of Canada lynx, and the pronounced dull-white ear spot typical of bobcats was present (Fig. 4). We also noted that the tail of F1 hybrids was intermediate in appearance relative to both parental species – i.e., cream to ivory white-colored ventral tail hairs (typical of bobcats), suffusing into the posterior margin of the distal black tail tip (typical of Canada lynx; Fig. 5).

Most of the craniodental variation exhibited among individuals for both sexes was accounted for by the first two principal components – i.e., > 78% for males and > 84% for females (Table 1). The first principal component (PC1) accounted for most of the observed variation (~62% for males, Fig. 5; ~68% for females, Fig. 6). Loadings on PC1 revealed moderate variation for both sexes (Table 2). The highest loadings observed were ramus height (RH) for males and nasal breadth width (NBW) for females. Interorbital breadth (IOB) was the highest loading for PC2 for both sexes. Altogether, PCA plots showed distinct partitioning for bobcats and Canada lynx (Fig. 6), with genetically confirmed F1 hybrids morphometrically allied more closely with bobcats (Fig. 7).

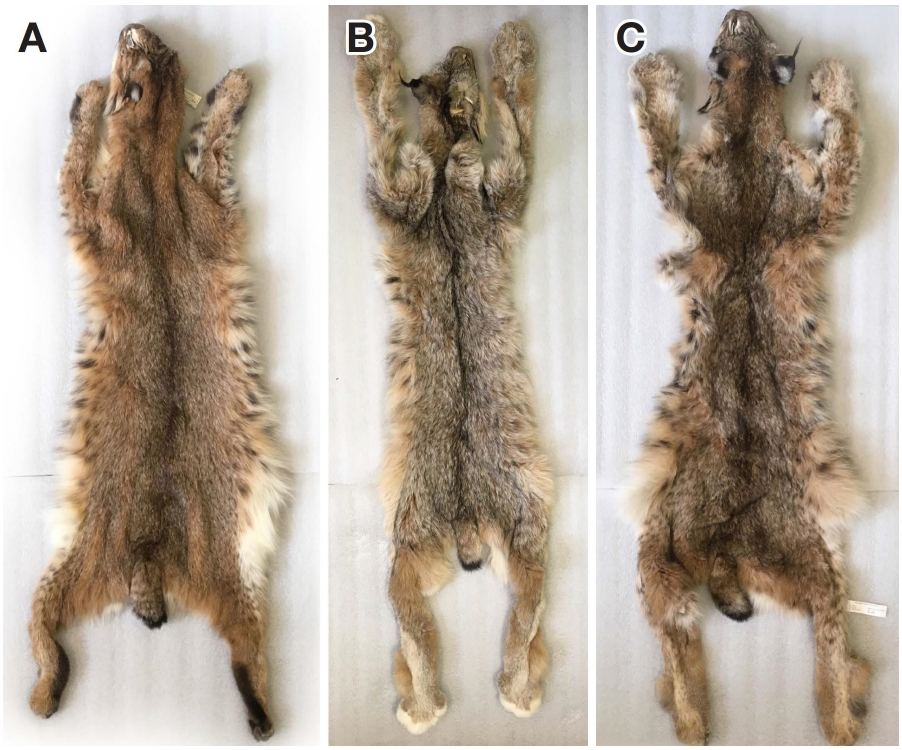

Figure 2. Pelts (deposited at the New Brunswick Museum) of a bobcat (A), a Canada lynx (B), and a bobcat × Canada lynx F1 hybrid (C). Note the F1 hybrid more closely resembles the bobcat in terms of general coloration and markings.

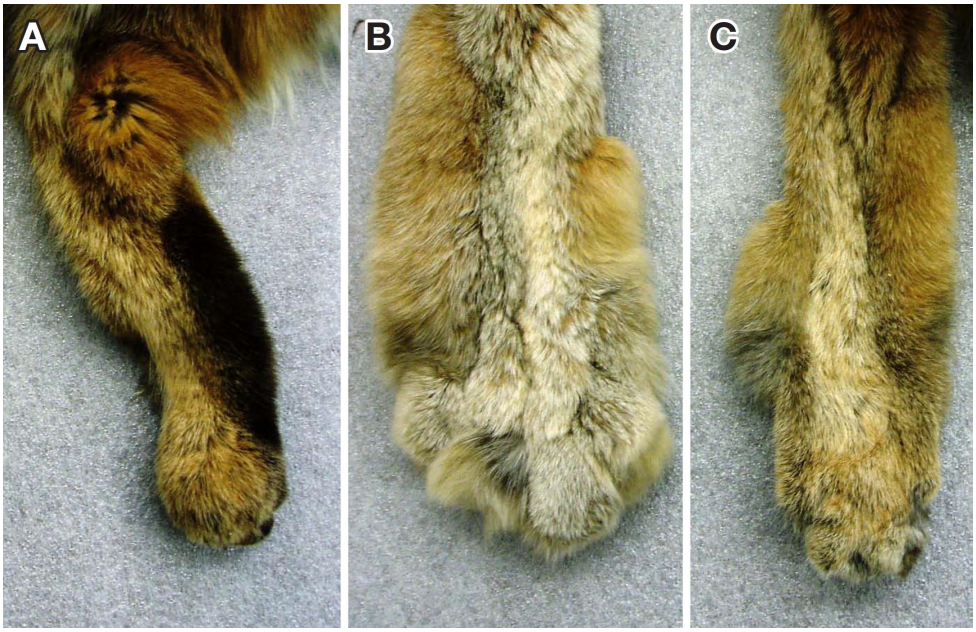

Figure 3. Hind feet of a bobcat (A), a Canada lynx (B), and a bobcat × Canada lynx F1 hybrid (C). Note that the hind foot of the F1 hybrid is slightly larger than that of the bobcat but is not as broad as the hind foot typical of Canada lynx.

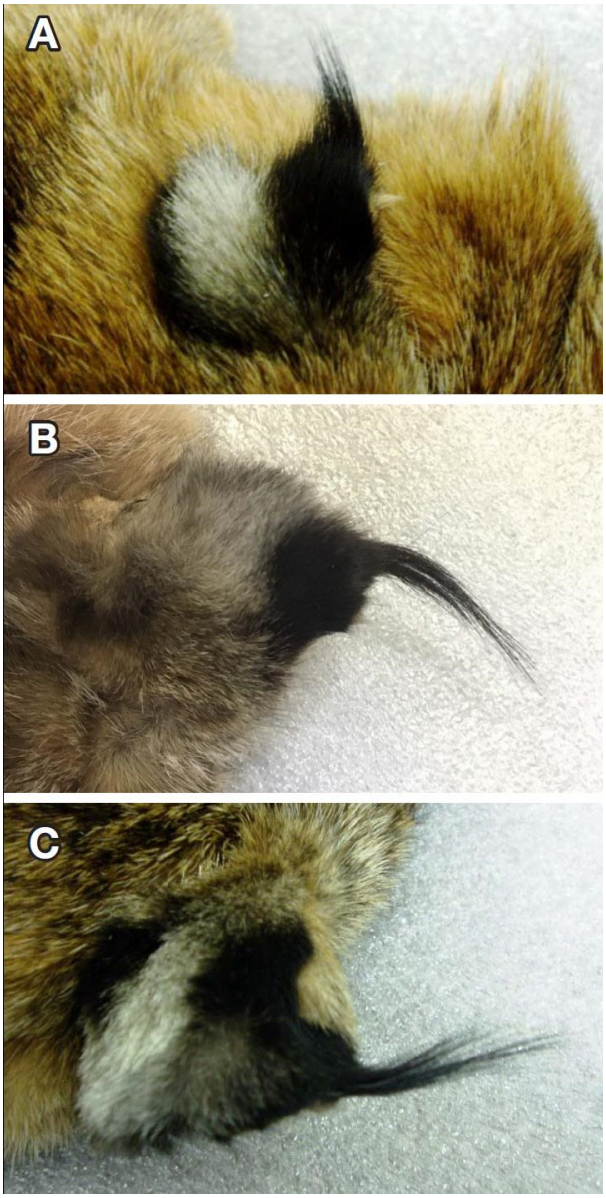

Figure 4. Ear tufts of a bobcat (A), a Canada lynx (B), and a bobcat × Canada lynx F1 hybrid (C). Note that the F1 hybrid possesses the relatively long (> 25 mm), black ear tuft of a typical Canada lynx and the pronounced white ear spot of a typical bobcat.

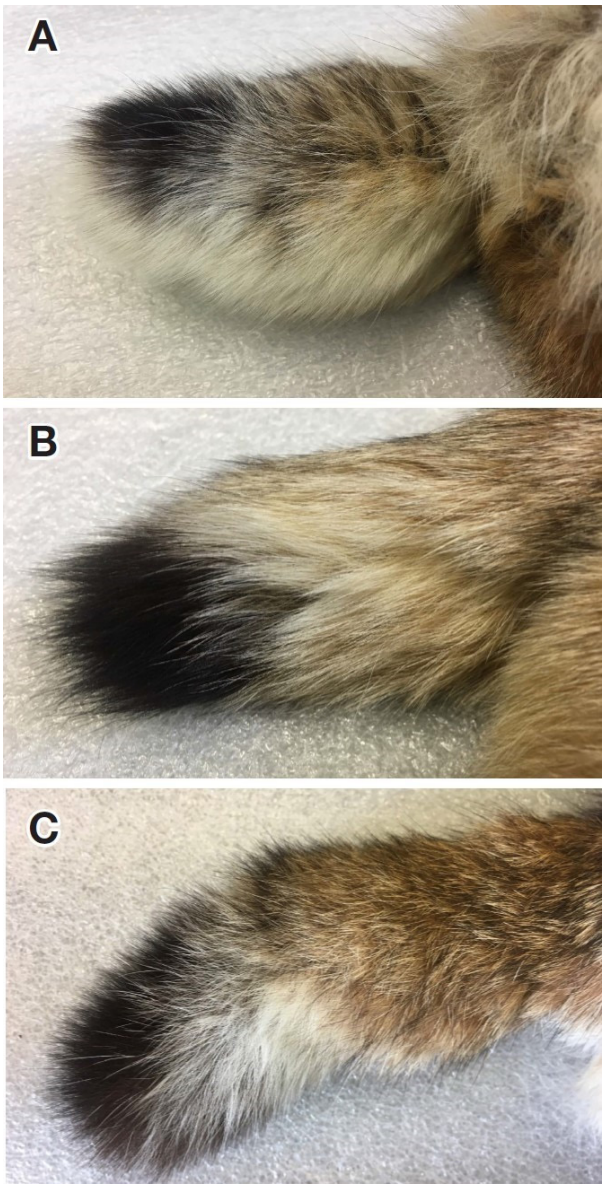

Figure 5. Tail morphology of a bobcat (A), a Canada lynx (B), and a bobcat × Canada lynx F1 hybrid (C). Note that the tail of the F1 hybrid is intermediate of both parental species, i.e., white-colored hair (typical of bobcat) suffusing into the black tip of the tail (typical of Canada lynx).

Table 1. Eigenvalues for the first three principal components (PC1–PC3) and percentage of variance explained for each PC. Data for 14 craniodental characters examined (Presphenoid Width excluded) in male and female bobcats, Canada lynx, and bobcat × Canada lynx F1 hybrids were logarithmically transformed before the PCA was conducted.

Males			Females		
PC	Eigenvalue	% of Variance explained	PC	Eigenvalue	% of Variance explained
1	0.006	61.86	1	0.007	67.92
2	0.002	16.26	2	0.002	16.85
3	0.001	7.42	3	0.000	3.23

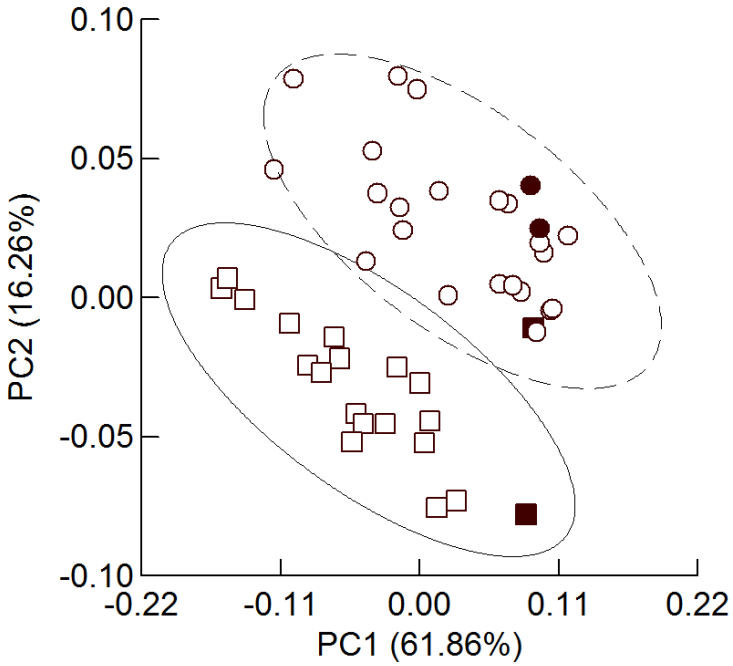

Figure 6. Principal Component plot (PC1 x PC2) of craniodental measurements (logarithmically transformed) of male bobcat, male Canada lynx, and male bobcat × Canada lynx false-positive hybrids. Empty circles = bobcats; empty squares = Canada lynx; solid circles and squares = false positive bobcat × Canada lynx hybrids – i.e., genetically confirmed pure bobcat (solid circles) and Canada lynx (solid squares) that were initially and putatively identified as hybrids based on external and craniodental morphology. A probability value of 0.9 is specified for each sample confidence ellipse.

Table 2. PCA factor loadings for 14 craniodental characters measured (Presphenoid Width excluded) for male and female bobcat, Canada lynx, and bobcat x Canada lynx F1 hybrid skulls. Data were logarithmically transformed before PCA was conducted. Bolded values denote variables with relatively high loadings (≥ 0.025). Carniodental characters abbreviated as defined in Methods.

	Males		Females	
Craniodental character	PC1	PC2	PC1	PC2
CBL	0.002	0.003	0.022	0.007
BCL	**0.025**	0.004	**0.028**	0.010
BCB	0.011	-0.001	0.018	-0.001
MB	0.017	0.000	0.020	0.003
ZB	0.024	0.002	0.020	0.000
POC	-0.008	-0.001	0.000	-0.003
IOB	0.012	**-0.031**	0.022	**-0.031**
NBW	**0.030**	-0.015	**0.033**	-0.012
ABW	0.012	0.004	0.016	0.005
ML	0.024	0.003	0.021	0.007
RH	**0.035**	0.011	**0.027**	0.011
C-M1	0.013	-0.003	0.022	0.005
MMRL	-0.002	-0.013	0.018	-0.012
CCB	**0.027**	0.004	0.022	0.006

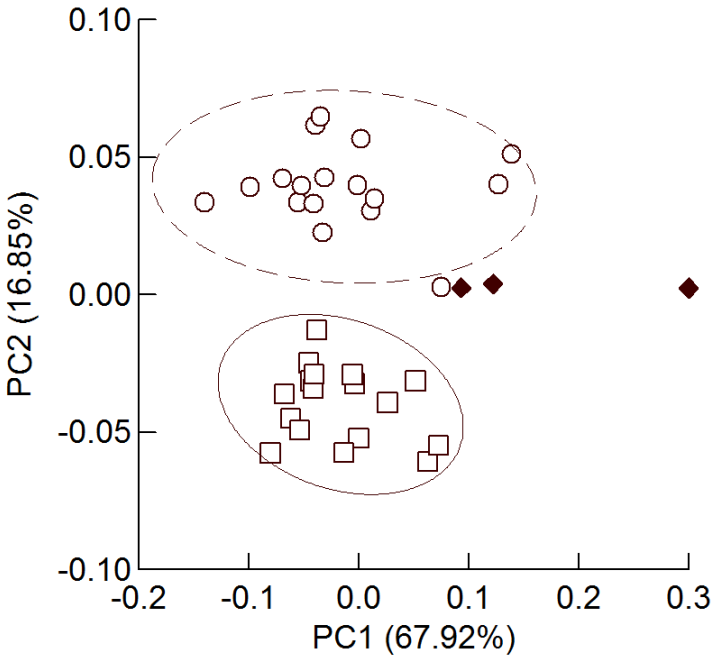

Figure 7. Principal Component plot (PC1 x PC2) of craniodental measurements (logarithmically transformed) of female bobcats, female Canada lynx, and female bobcat × Canada lynx F1 hybrids. Circles = bobcats; squares = Canada lynx; solid diamonds = bobcat × Canada lynx genetically confirmed F1 hybrids. A probability value of 0.9 is specified for each sample confidence ellipse.

Qualitatively, F1 hybrid skulls had several characters that were intermediate relative to both parental species. The presphenoid bone flared out medially – i.e., spade-like form – and was similar to but not as wide and pronounced as in Canada lynx. The bridge of bone that separates the hypoglossal canal from the jugular foramen was noticeably recessed, though not as far back as in bobcats. In contrast, the auditory bullae in F1 hybrids lacked a septum, were dorso-ventrally inflated, and much larger than those typically found in Canada lynx (i.e., strongly resembling those of bobcats; Fig. 8).

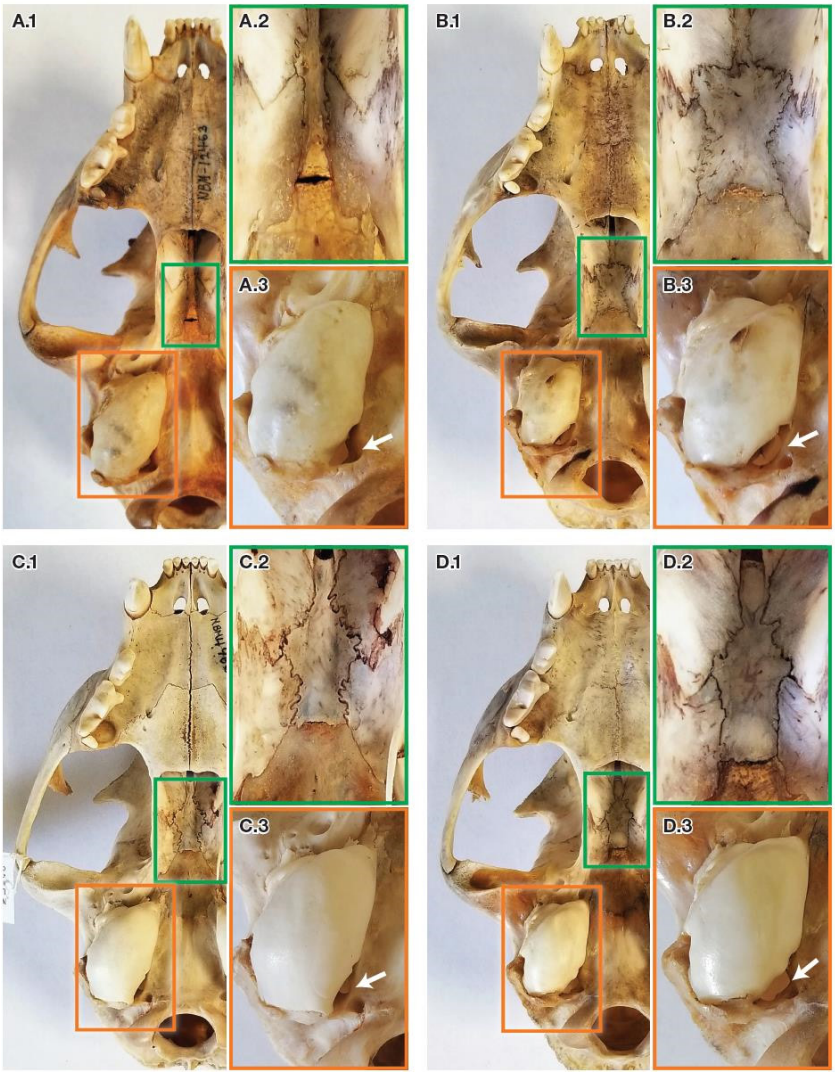

Figure 8. Ventral view of crania for a bobcat (A.1), a Canada lynx (B.1), and bobcat × Canada lynx F1 hybrids (C.1 & D.1). Note how the presphenoid bone is medially constricted in a typical bobcat (A.2) but flares out medially – i.e., spade-like form – in a Canada lynx (B.2). The presphenoid bone in F1 hybrids is intermediate in shape and form (C.2 & D.2). The bridge of bone that separates the hypoglossal canal from the jugular foramen is recessed in bobcats (A.3) but distinctive in Canada lynx (B.3); it is recessed in F1 hybrids (C.3 & D.3), but not as far back as in bobcats. The auditory bullae in bobcats lack a septum and are dorso-ventrally inflated (A.3), being much larger than those typically found in Canada lynx (B.3). The bullae in F1 hybrids (C.3 & D.3) strongly resemble those of bobcats.

DISCUSSION

Pelage coloration of bobcat × Canada lynx F1 hybrids strongly resembles that of a typical bobcat. Hence, in terms of general appearance, F1 hybrids superficially fit the profile for bobcats (Homyack et al. 2008). Thus hybrids may be overlooked and mistakenly identified as bobcats by trappers and wildlife managers if no concerted efforts are made to examine and contextualize other morphological features. The suite of external morphological characters examined in this study reveal that bobcat × Canada lynx F1 hybrids exhibit several traits that vary with respect to assignment to both or either parent species. The ears have markings similar to those of bobcats (not noted in Homyack et al. 2008), but the ear tufts strongly resemble those of Canada lynx with respect to length. Similarly, the tail tip manifests characters from both Canada lynx (black tip) and bobcats (ventral white hairs). Hind foot size in F1 hybrids is intermediate between bobcat and Canada lynx. Taken together, these morphological characters can identify individuals as possible hybrids, with the caveat that morphological characters can sometimes be misleading due to variation and subjective interpretation (e.g., see McKelvey et al. 2000). Naughton (2012) notes that the distinguishing features of the crania in bobcats and Canada lynx are variable and often no single character alone can ensure correct identification. There was inconsistency in the expression of characters associated with the auditory bullae of animals that were otherwise identifiable as bobcat or Canada lynx (i.e., Canada lynx with unconstricted bullae and one or more foramina that were confluent).

With respect to the PCA, most of the craniodental variation for both sexes was explained by the first two principal components, which largely reflect size (i.e., length, width, height). The PCAs for both sexes confirm that bobcats are craniometrically smaller than Canada lynx for most of the characters measured. For example, some length-wise axial characters, including braincase length (along with other allometric characters such condylobasal length; Radinsky 1984), are a surrogate measure of skull size, while other characters, such as interorbital breadth, are a reflection of skull proportions (e.g., wider interorbital breadth suggests more robust cranium; Sicuro and Oliveira 2011). These characters, which had relatively high loadings in the PCA, are all smaller in bobcat skulls when compared to Canada lynx skulls. Overall skull size, shape, and proportions in turn often are correlated with body size (Kurten and Rausch 1959; Radinsky 1984). Bobcats generally are smaller overall and of lower body weight than Canada lynx across most of their range (Buskirk et al. 2000b). Not surprisingly, F1 hybrid skulls examined in this study were intermediate between the two species in many of their craniodental characters. This includes width of the presphenoid bone and the position of the hypoglossal canal relative to the jugular foramen, features of the cranium that generally are reliable diagnostic characters when separating bobcats from Canada lynx (Elbroch 2006). Such blending of characters (morphological and molecular) is not unusual in the hybridization of animals, especially among closely related species (Barton 2001). Altogether, the three F1 hybrid skulls can be differentiated from bobcat or Canada lynx qualitatively (i.e., shape of bones, positioning of cranial foramina relative to other skull features), but because non-hybrid bobcats are only marginally smaller than non-hybrid lynx, differentiating hybrids on the basis of size alone, even with a multivariate approach, is problematical.

The matter of relying solely upon morphological traits to identify hybrids, even with rigorous statistical approaches, needs to be considered. The analysis presented in this study includes four males identified putatively as hybrids by trappers and wildlife managers based on external morphology. However, upon subsequent genetic testing, it was determined that these putative hybrids were either pure bobcat or Canada lynx. These false positives are significant and emphasize the difficulty, even for experienced wildlife biologists and trappers, in identifying bobcat × Canada lynx hybrids on the basis of pelage and/or craniodental characteristics. Although F1 hybrids appear to express pelage characters that strongly resemble those of bobcats, qualitative characters of the skulls of F1 hybrids appear to be more Canada lynx-like in the limited sample available in this study. Misidentifications are further evidence that though the morphological characters separating bobcat and Canada lynx are well recognized, there is some overlapping variation between the two congeners that potentially can confound positive iden-

tification of hybrids based on morphology alone (e.g., one individual, putatively identified as a hybrid, turned out to be a Canada lynx but exhibited character states that strongly resembled those of a bobcat, as noted in the PCA; Fig. 6). Such misidentifications can lead to over-estimation of hybridization rates in sympatric populations of bobcats and Canada lynx. Although a larger sample of hybrid crania from a wide geographic area may eventually allow more definitive statements on the extent of cranial variation in hybrids, genetic analysis should be used to confirm hybrid status between bobcat and Canada lynx (Homyack et al. 2008). It should be noted that although molecular genetic techniques (i.e., microsatellites; see Carmichael et al. 2000) are available to positively confirm the identity of F1 hybrids (see Schwartz et al. 2004), such methods may not always be feasible or readily accessible. Although multivariate statistical analyses of craniometric data can, as with external morphology, identify individuals as possibly hybrid, it cannot confirm hybridization between a bobcat and a Canada lynx. Hence, following Homyack et al. (2008), it is recommended that individuals that match the aforementioned morphological F1 hybrid profile be thoroughly examined and photographed, have measurements of features recorded (i.e., ear tuft length, hind foot length), and tissues sampled during necropsies that are then properly archived for subsequent molecular confirmation of identity.

Bobcats and Canada lynx exhibit substantial differences in ecology with respect to habitat selection, prey choice, and reproductive behavior (Parker et al. 1983; Lariviere and Walton 1997; Buskirk et al. 2000b; Anderson and Lovallo 2003). Ecomorphologically, some of the examined external characters strongly suggest that F1 hybrids have similar ecology to those of bobcats rather than Canada lynx. Most notably, the size of the hind feet of F1 hybrids suggest that they experience higher foot-loading on snow (i.e., the force exerted per unit area of the foot as the animal stands or moves; Buskirk et al. 2000a) than that of Canada lynx. This may infer reduced efficiency in traversing and exploiting areas that experience high snowfall (Murray and Boutin 1991). Hybridization among felids has been studied in detail (Dubost and Royere 1993; Pierpaoli et al. 2003; Trigo et al. 2008), and Reding (2011) and Koen et al. (2014) reported bobcat × Canada lynx hybrids back-crossing with the parental population, albeit at low levels. However, without direct observation of bobcat × Canada lynx hybrids living in the wild, positively identified a priori, it is difficult to ascertain the exact nature of the ecological interactions (e.g., competition for territory, food, and mates) among hybrids and the parental species.

Overall size alone is not always a reliable predictor of a species' biology and the resulting ecological interactions (McKelvey et al. 2008). Although Canada lynx are larger and heavier than bobcats, the latter are more aggressive with respect to acquisition and defense of territory (Kobalenko 1997). As such, bobcats competitively exclude Canada lynx in areas where both species are found (Peers et al. 2013). Thus far, all confirmed F1 hybrids are the result of bobcat males siring young with Canada lynx females (Schwartz et al. 2004; Homyack et al. 2008; unpublished data by authors) – i.e., unidirectional hybridization. It will be important to note if future recorded hybrids exhibit genetic profiles that reflect unidirectional hybridization and what this may actually portend with respect to species interaction and genetic introgression (hybrid-mediated gene flow) in areas of sympatry.

As bobcats expand their geographic range northward due to habitat modification (e.g., see Parker et al. 1983) and climate change (Peers et al. 2013), increased occurrences of hybridization between the two species may be inevitable. For example, the construction of the causeway from mainland Nova Scotia to Cape Breton Island in the 1950s permitted the dispersal and establishment of bobcats on Cape Breton Island. Canada lynx are now restricted to the Cape Breton Highlands due to competitive exclusion and displacement by bobcats (Parker et al. 1983). Increased frequency of encounters and interactions between the two species may lead to hybridization. If this is the case, then conservation and wildlife management plans may need to be revisited and revised. Canada lynx is a protected species in Maritime Canada, but the bobcat is not. Should hybridization between bobcats and Canada lynx become more prevalent in the future, the consequences for Canada lynx populations in Maritime Canada may be detrimental (e.g., genetic swamping). Thus to effectively monitor incidences and frequency of hybridization between bobcats and Canada lynx, proven identification methods and tools need to be utilized – i.e., in this case, initial morphological examination [pelts and skulls; Homyack et al. (2008) and

this study] confirmed with molecular genetic testing (especially with respect to detecting backcrossing and introgression; see Koen et al. 2014). Currently, no conservation agency in Canada has an official policy regarding the treatment of bobcat × Canada lynx hybrids. The situation is similar for the United States (Allendorf et al. 2001). If legal protection is afforded to hybrids, bobcat trapping in areas with Canada lynx could be problematic because both pure Canada lynx and hybrids can be incidentally removed from such populations (Schwartz et al. 2004). Such policy and actions could serve as an impediment to the protection and recovery of Canada lynx in certain areas, particularly at the southern periphery of their geographic range [see Lesica and Allendorf (1995) for a review of the conservation value of peripheral populations]. Indeed, any factors that may favor bobcats in Canada lynx habitat may lead to the production of hybrids and thus be potentially harmful to Canada lynx (e.g., integrity of the gene pool; Koen et al. 2014).

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LITERATURE CITED

Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenburg. 2001. The problems with hybrids: setting conservation guidelines. Trends in Ecology and Evolution 16:613–622.

Anderson, E. M., and M. J. Lovallo. 2003. Bobcat and lynx (*Lynx rufus* and *Lynx canadensis*). Pp. 758–787 in The wild mammals of North America (G.C. Feldhamer, B.C. Thompson, and J.A. Chapman, eds.). Johns Hopkins University Press, Baltimore, Maryland.

Barton, N. 2001. The role of hybridization in evolution. Molecular Ecology 10:551–568.

Banfield, A. W. F. 1974. The mammals of Canada. National Museums of Canada, University of Toronto Press, Toronto.

Buskirk, S. W., L. F. Ruggiero, K. B. Aubry, D. E. Pearson, J. R. Squires, and K. S McKelvey. 2000b. Comparative ecology of lynx in North America. Pp. 397–417 in Ecology and conservation of lynx in the United States (L.F. Ruggiero, K.B. Aubrey, S.W. Buskirk, G.M. Koehler, C.J. Krebs, K.S. McKelvey, and J.R. Squires, eds.). University Press of Colorado, Boulder.

Buskirk, S. W., L. F. Ruggiero, and C. J. Krebs. 2000a. Habitat fragmentation and interspecific competition: implications for lynx conservation. Pp. 83–100 in Ecology and conservation of lynx in the United States (L.F. Ruggiero, K.B. Aubrey, S.W. Buskirk, G.M. Koehler, C.J. Krebs, K.S. McKelvey, and J.R. Squires, eds.). University Press of Colorado, Boulder.

Carmichael, L. E., W. Clark, and C. Strobeck. 2000. Development and characterization of microsatellite loci from lynx (*Lynx canadensis*) and their use in other fields. Molecular Ecology 9:2197–2198.

Conley, R. M., and J. M. Jenkins. 1969. An evaluation of several techniques for determining the age of bobcats (*L. rufus*) in the south-east. Proceedings of the Southeastern Association of Game and Fisher Commissioners Conference 23:104–109.

Dowling, T. E., and C. L. Secor. 1997. The role of hybridization and introgression in the diversification of animals. Annual Review of Ecology and Systematics 28:593–619.

Dubost, G., and J.-Y. Royere. 1993. Hybridization between ocelot (*Felis pardalis*) and puma (*Felis concolor*). Zoo Biology 12:277–283.

Elbroch, M. 2006. Animal skulls: a guide to North American species. Stackpole Books, Mechanicsburg, Pennsylvania.

Hall, E. R. 1981. The mammals of North America, Vol. II. John Wiley & Sons, New York.

Homyack, J. A., et al. 2008. Canada lynx-bobcat (*Lynx canadensis* × *L. rufus*) hybrids at the southern periphery of lynx range in Maine, Minnesota, and New Brunswick. American Midland Naturalist 159:504–508.

Jackson, H. H. T. 1961. Mammals of Wisconsin. The University of Wisconsin Press, Madison.

Johnson, W. E., M. Culver, J. A. Iriarte, E. Eizirik, K. L. Seymour, and S.J. O'Brien. 1998. Tracking the evolution of the elusive Andean mountain cat (*Oreailurus jacobita*) from mitochondrial DNA. The American Genetic Association 89:227–232.

Johnson, W. E., and S. J. O'Brien. 1997. Phylogenetic reconstruction of the Felidae using 16S rRNA and NADH-5 mitochondrial genes. Journal of Molecular Evolution 44 (suppl. 1):S98–S116.

Khidas, K., J. Duhaime, and H. M. Huynh. 2013. Morphological divergence of continental and island populations of Canada lynx. Northeastern Naturalist 20(4):587–608.

Kobalenko, J. 1997. Forests cats of North America: cougars, bobcat, lynx. Firefly Books, Ontario, Canada.

Koen, E. L, J. Bowman, J. L. Lalor, and P. J. Wilson. 2014. Continental-scale assessment of the hybrid zone between bobcat and Canada lynx. Biological Conservation 178:107–115.

Kurten, B., and R. L. Rausch. 1959. Biometric comparisons between North American and European mammals: a comparison between the northern lynxes of Fennoscandia and Alaska. Acta Artica 11:21–44.

Lariviere, S., and L. R. Walton. 1997. *Lynx rufus*. Mammalian Species 563:1–8.

Larsen, P. A., M. R. Marchán-Rivadeneira, and R. J. Baker. 2010. Natural hybridization generates mammalian lineage with species characteristics. Proceedings of the National Academy of Sciences of the United States of America 107(25):11447–11452.

Lavoie, M., H. Jolicoeur, and S. Lariviere. 2010. Les hauts et les bas d'une espèce sudiste au Quebec: le lynx roux (*Lynx rufus*). Le Naturaliste canadien 134(2):54–64.

Lesia, P., and F. W. Allendorf. 1995. When are peripheral populations valuable for conservation? Conservation Biology 9(4):753–760.

Libby, C. 2004. Lynx-bobcat hybrid confirmed in New Brunswick. Biolink, Newsletter of the Atlantic Society of Fish and Wildlife Biologists, April 2004:1–2.

Mallet, J. 2007. Hybrid speciation. Nature 446:279–283. doi:10.1038/nature05706.

McKelvey, K. S., K. B. Aubry, and Y. K. Ortega. 2000. History and distribution of lynx in the contiguous United States. Pp. 207–264 in Ecology and conservation of lynx in the United States (L.F. Ruggiero, K.B. Aubrey, S.W. Buskirk, G.M. Koehler, C.J. Krebs, K.S. McKelvey, and J.R. Squires, eds.). University Press of Colorado, Boulder.

Miller, R. R., J. D. Williams, and J. E. Williams. 1989. Extinctions of North American fishes during the past century. Fisheries 14(6):22–38.

Morris, P. 1972. A review of mammalian age determination methods. Mammal Review 2:69–104.

Murray, D. L. and S. Boutin. 1991. The influence of snow on lynx and coyote movements: does morphology affect behavior? Oecologia 88(4):463–469.

Murray, D. L., T. D. Steury, and J. D. Roth. 2008. Assessment of Canada lynx research and conservation needs in the southern range: another kick at the cat. The Journal of Wildlife Management 72(7):1463–1472.

Naughton, D. 2012. The natural history of Canadian mammals. Canadian Museum of Nature and University of Toronto Press, Toronto.

Novak, R. M. 1999. Walker's mammals of the World, 6th ed. John Hopkins University Press, Baltimore, Maryland.

Ommundsen, P. D. 1991. Morphological differences between lynx and bobcat skulls. Northwest Science 65(5):248–250.

Parker, G. R. 1981. Winter habitat use and hunting activities of lynx (*Lynx canadensis*) on Cape Breton Island, Nova Scotia. Pp 221–248 in Proceedings of the worldwide furbearer conference (J. A. Chapman and D. Pursley, eds.). August 3–11, 1980. Frostburg, Maryland.

Parker, G. R., J. W. Maxwell, L. D. Morton, and G. E. J. Smith. 1983. The ecology of the lynx (*Lynx canadensis*) on Cape Breton Island. Canadian Journal of Zoology 61(4):770–786.

Peers, M. J. L., D. H. Thornton, and D. L. Murray. 2013. Evidence for large-scale effects of competition: niche displacement in Canada lynx and bobcat. Proceedings of the Royal Society B 280(1773):20132495. doi:10.1098/rspb.2013.2495.

Pierpaoli, M., et al. 2003. Genetic distinction of wildcat (*Felis silvestris*) populations in Europe, and hybridization with domestic cats in Hungary. Molecular Ecology 12:2585–2598.

Quinn, N. W. S., and G. R. Parker. 1987. Lynx. Pp. 683–694 in Wild furbearer management and conservation in North America. (M. Novak, J. A. Baker, M. E. Obbard, and B. Malloch, eds.). Ontario Trappers Association, North Bay.

Radinsky, L. B. 1984. Basicranial axis length v. skull length in analysis of carnivore skull shape. Biological Journal of the Linnean Society 22:31–41.

Reding, D. M. 2011. Patterns and processes of spatial genetic structure in a mobile and continuously distributed species, the bobcat (*Lynx rufus*). Dissertation, Iowa State University.

Rhymer, J. M., and D. Simberloff. 2003. Extinction by hybridization and introgression. Annual Review of Ecology and Systematics 27(1):83–109.

Roberts, N. M. and S. M. Crimmins. 2010. Bobcat population status and management in North America: evidence of large-scale population increase. Journal of Fish and Wildlife Management 1(2):169–174.

Schmidly, D. J., and J. A Read. 1986. Cranial variation in the bobcat (*Felis rufus*) from Texas and surrounding states. Occasional Papers, Museum of Texas Tech University 101:1–39.

Schwartz, M. K., et al. 2004. Hybridization between Canada lynx and bobcats: genetic results and management implications. Conservation Genetics 5:349–355.

Sicuro, F. L., and L. F. B. Oliveira.2011. Skull morphology and functionality of extant Felidae (Mammalia: Carnivora): A phylogenetic and evolutionary perspective. Zoological Journal of the Linnean Society 161:414–462.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: The Principles and Practice of Statistics in Biological Research, 3rd Edition. Freeman and Company, New York.

Trigo, T. C., et al. 2008. Inter-species hybridization among Neotropical cats of the genus *Leopardus*, and evidence for an introgressive hybrid zone between *L. geoffroyi* and *L. tigrinus* in southern Brazil. Molecular Ecology 17(19):4317–4333.

Tumlison, R. 1987. *Felis lynx*. Mammalian Species. 269:1–8.

Werdelin, L. 1981. The evolution of lynxes. *Annales Zoologici Fennici* 18:37–71.

Wirtz, P. 1999. Mother species–father species: unidirectional hybridization in animals with female choice. Animal Behavior 58(1):1–12.

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Population Structure of Sakhalin Gray Whales (*Eschrichtius robustus*) Revealed by DNA Sequences of Four mtDNA Genes

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Abstract

Two stocks of gray whales (*Eschrichtius robustus*), eastern and western, traditionally were considered to inhabit the North Pacific Ocean. The western gray whale migration was along the coast of Asia between summering grounds in the Sea of Okhotsk and wintering grounds presumed to be in the South China Sea. The eastern gray whale migration was along the coast of North America between summering grounds mainly in the Bering and Chukchi Seas and wintering grounds in the coastal lagoons of Baja, Mexico. The status of the current population of gray whales that summers in the Sea of Okhotsk, mainly near Sakhalin Island, Russia, is of uncertain affinity because the western stock previously was considered extinct, some members of the Sakhalin population are known to winter in Mexico, and an Asian wintering ground for this species has never been precisely located. A previously published analysis of 84 nuclear loci showed the Sakhalin population to be a mixed-stock aggregation comprised of animals with distinct "eastern" and "western" SNP genotypes. In the study reported here, a mitochondrial DNA (mtDNA) analysis of Sakhalin gray whales was conducted using control region and three protein coding gene sequences to test if animals with "western" genotypes might be descendants of the western stock. It was postulated that such a population would likely have distinct mtDNA haplotype lineages as observed in other marine mammals sundered in the North Pacific Ocean basin. From the mtDNA sequence data, haplotype networks were generated separately for the control region and the concatenated protein sequences. No clades of related haplotypes were found among the "western" genotype animals in either haplotype network. This is not consistent with long-term isolation during the Pleistocene as seen in many other marine mammals with similar distributions. Rather, the "western" and "eastern" genotypes likely have diverged recently, possibly since the end of commercial whaling in the early 20th century, but more likely post-Pleistocene.

Key words: gene flow, genetic structure, migration, western gray whales

Аннотация

Традиционно считалось, что в северной части Тихого океана обитают две популяции серых китов (*Eschrichtius robustus*): восточная и западная. Западная популяция серых китов мигрирует вдоль побережья Азии между летними участками нагула в Охотском море и предполагаемыми зимними участками нагула в Южно-Китайском море. Миграция восточной популяции серых китов происходит вдоль побережья Северной Америки между летними участками нагула, преимущественно в Беринговом и Чукотском морях, и зимними участками нагула в прибрежных лагунах мексиканского штата Баха. Принадлежность существующей популяции серых китов, находящихся в течение летнего сезона в Охотском море,

преимущественно у о. Сахалин, не установлена, так как ранее западное стадо считалось вымершим. При этом известно, что некоторые особи сахалинской популяции зимуют у побережья Мексики, а участки зимнего нагула данного вида в азиатском регионе никогда не были точно определены. Опубликованные ранее исследования по 84 ядерным локусам показали, что сахалинская популяция представляет собой смешанную популяционную группировку с отличающимися «восточными» и «западными» SNP-генотипами. Для проверки возможности происхождения животных с «западными» генотипами от животных западного стада нами проведен анализ митохондриальной ДНК (мтДНК) сахалинских серых китов с использованием контрольного региона и 3-х белок-кодирующих последовательностей. Предполагалось, что подобная популяция, по всей вероятности, имеет характерную гаплотипическую родословную мтДНК, как и в случае с другими видами морских млекопитающих, обитающих в водах северной части Тихого океана. По данным последовательностей мтДНК были построены гаплотипические сети отдельно для контрольного региона и отдельно на основе объединенных последовательностей белок-кодирующих генов. Ни в одной из гаплотипических сетей среди особей западного стада не было выявлено специфических «западных» гаплотипов , что противоречит гипотезе о долговременной изоляции в период плейстоцена, характерной для ряда других морских млекопитающих с аналогичными распределениями. Скорее всего, «западные» и «восточные» генотипы разошлись недавно; и это могло произойти после завершения китобойного промысла в начале XX века, однако более вероятным представляется разделение в пост-плейстоцен.

Ключевые слова: генетическая структура, генный поток, западная популяция серых китов, миграция

INTRODUCTION

In 2010, a satellite tag was placed on "Flex", a male gray whale (*Eschrichtius robustus*) summering in the Sea of Okhotsk near Sakhalin Island, Russia (Fig. 1) in order to track him to the unknown western North Pacific wintering grounds thought to be in the South China Sea. On 12 December 2010, Flex instead was tracked from Sakhalin to the coast of North America. In 2011, transmitters were placed on two females, "Agent" and "Varvara," that were feeding off of Sakhalin Island. Both were tracked travelling towards the east, and Varvara was tracked from Sakhalin to the eastern gray whale wintering grounds in the coastal lagoons of Baja California, Mexico, and then back to the Sea of Okhotsk (Mate et al. 2015). The study of Mate et al. (2015) was a landmark in gray whale science because it immediately upended the conventional wisdom of decades of North Pacific (NP) gray whale research. Specifically, it had been assumed two populations, or stocks, of gray whales are found in the North Pacific: western gray whales (WGWs) that migrate along the Asian coast between summering grounds in the Sea of Okhotsk and wintering grounds somewhere in Asia; and eastern gray whales (EGW) that migrate between summering grounds mainly in the Bering and Chukchi Seas and wintering grounds in Mexico. The result has been an exhaustive reappraisal of gray whale stock structure hypotheses, including a 5-year "Rangewide Review of the Population Structure and Status of North Pacific Gray Whales" (Rangewide Review) conducted by the Scientific Committee of the International Whaling Commission (IWC) (IWC 2014, 2018).

North Pacific gray whales were hunted extensively during the 1800's and early 1900's by commercial whalers. WGWs were hunted primarily by Korean and Japanese whalers in the first half of the 20th century (Rice and Wolman 1971; Brownell and Chun 1977). EGWs were hunted by United States and Canadian

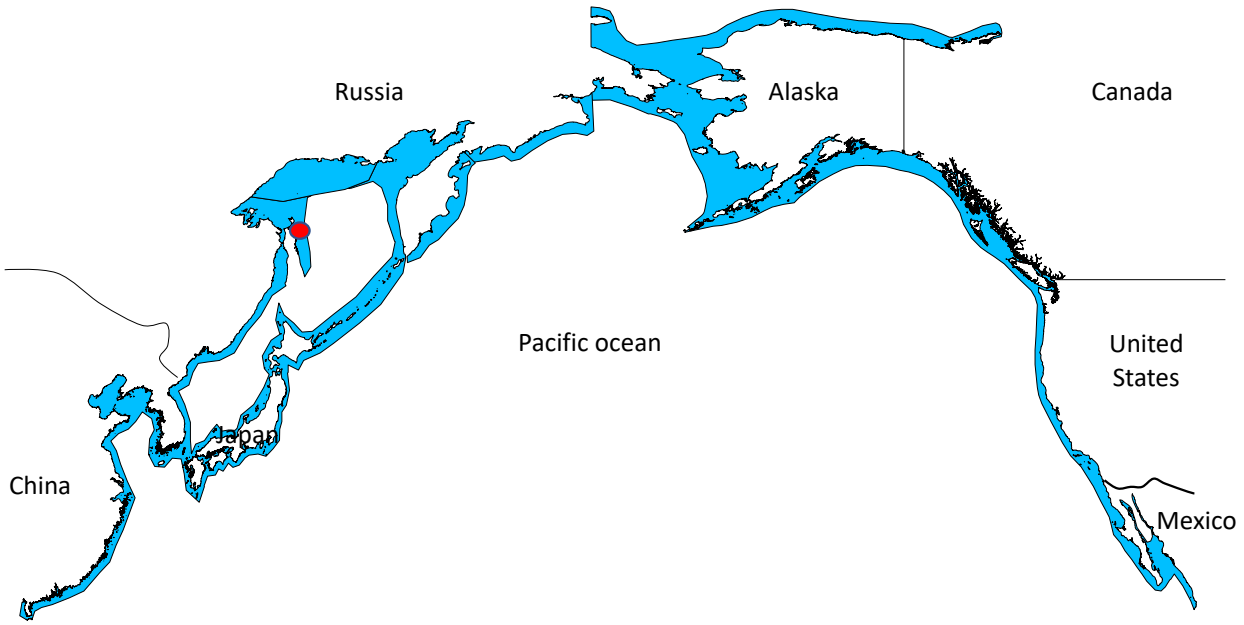

Figure 1. Distribution of North Pacific gray whales. Habitat range is shown with blue shade and sampling location for western gray whales off Sakhalin Island is shown with a red dot. This map is redrawn from the IUCN range map (https://www.iucnredlist.org/species/8097/50353881).

whalers, and the independence of the two stocks was established based on presumed geographic isolation across the NP Ocean basin, and because hunting pressure on one population did not impact the other. This is explained in the report of the first IWC Rangewide Review (IWC 2014, p. 11) which states, "Brownell reported that the large catches (> 1,750 gray whales) by Japanese modern whalers in the East Sea of Korea from 1890–1966, but mainly in the first third of the 20th century (Kato and Kasuya 2002), came at a time when the population of gray whales in the eastern Pacific was seriously depleted as a result of 19th century whaling. This mismatch in the timing of peak catches in the eastern and western North Pacific is consistent with the hypothesis of separate populations." This conclusion is corroborated by the historical catch data summarized in Appendix A of Bradford (2003). The Asian harvest was so great that by 1966 the WGW was considered by some to be extinct (Bowen 1974; Weller et al. 2002). However, this opinion was not shared by Brownell and Chun (1977) who documented sporadic sightings of gray whales in Asian waters during the early and mid-20th century. Nonetheless, the conclusion of Brownell and Chun (1977: p. 238) was as follows: "Therefore, we believe the western Pacific stock of gray whales will become extinct in the near future unless meaningful international protection is achieved." In the late 1960s and the 1970s, some gray whales were sighted in the Sea of Okhotsk, South China Sea, and the Sea of Japan (Omura 1974), and gray whales were observed in waters northeast of Sakhalin Island in the 1980s (Blokhin et al. 1985). It was assumed that the Sakhalin whales were surviving WGWs, and the population was estimated to be 100–200 individuals by Berzin and Yablokov (1978, cited in Ilyashenko 2011). While both WGW and EGW populations were hunted to near extinction, the EGW population has rebounded and now numbers approximately 27,000 (Durban et al. 2017), a number which exceeds most estimates of pre-whaling abundance (Henderson 1984). Based on photo-identification studies of the gray whales feeding off Sakhalin Island and the southern and eastern coasts of Kamchatka, the WGW population is estimated to consist of 320–410 individuals (Cooke et al. 2017) and is considered endangered by the IUCN (Cooke 2018).

The historical concept of separate stocks of gray whales on the eastern and western sides of the NP Ocean basin was supported by the catch data mentioned above, as well as genetics studies based on mtDNA and

nuclear microsatellites (LeDuc et al. 2002; Lang et al. 2011). A similar biogeographic track is shared with some other marine mammals also possessing genetically differentiated eastern and western populations in the NP, such as Steller sea lions (*Eumetopias jubatus*) (Bickham et al. 1996; Baker et al. 2005; Harlin-Cognato et al. 2006). However, recent genetic studies as well as the results of the IWC's 5-year Rangewide Review (IWC 2018) are indicative of alternative, plausible, stock-structure hypotheses. In a genetics study based on single nucleotide polymorphisms (SNPs, n=84 loci; Brüniche-Olsen et al. 2018a), Sakhalin gray whales were shown to comprise a mixed-stock aggregation containing individuals with either of two distinct genotypes as well as admixed individuals. Previous studies that have suggested dispersal between the eastern and western North Pacific within the last 10 ky based on mtDNA (Alter et al. 2015) are consistent with this. These distinct genotypes, as well as admixed genotypes, also were possessed by whales sampled at the EGW wintering grounds in Mexico but at substantially different frequencies. A "western" genotype was predominant in the Sakhalin population and an "eastern" genotype was predominant in Mexico. While it is tempting to assume that these two genotypes are representative of the historical EGW and WGW populations, the "western" genotype whales are of uncertain origin. Are these the descendants of the WGWs that migrated along the Asian coast that were previously believed to have been hunted to extinction? Alternatively, the "western" genotype whales might be a distinct subpopulation of EGW that has differentiated genetically because of a founder effect and/or genetic drift in a small population, a concept considered as plausible by the IWC's Rangewide Review (IWC 2018). Sakhalin whales with either of the "western" and "eastern" genotypes, as well as mixed, are known to migrate to Mexico. In this group are included reproductive females of both "eastern" and "western" genotypes (M. J. Scott, unpublished observations). Given the long migration between the Sea of Okhotsk and Mexico, and the fact that mating in gray whales takes place during the fall migration, within-group matings for the Sakhalin population might be a higher probability than outbreeding with EGWs who mainly begin the fall migration from the Bering and Chukchi Seas.

To determine if the Sakhalin whales with "western" genotypes are the descendants of the WGWs requires a different approach than simply estimating a statistically significant F_{ST} as was observed in the early studies that compared EGW and WGW samples (LeDuc et al. 2002; Lang et al. 2011). In those studies, it was established that the overall population of whales near Sakhalin have different microsatellite allele and mtDNA haplotype frequencies than EGWs. However, that is not informative about the historical identities of the whales that currently summer off the coast of Sakhalin. Moreover, the presence of the two distinct SNP genotypes in the Sakhalin population was not known at the time of those early studies, so all Sakhalin whales were grouped together for analyses.

In this paper the following question is posed: Are the gray whales that currently summer off the coast of Sakhalin Island descendants of WGWs that migrated along the Asian coast? This question is addressed by sequencing four mtDNA genes from Sakhalin gray whales characterized as having "eastern", "western", or admixed nuclear SNP genotypes. These data are used to test hypotheses of the historical origin of the Sakhalin whales. The null hypothesis is that the "western" and "eastern" genotypes detected with SNPs (Brüniche-Olsen et al. 2018a) differ as a result of long-term isolation of two populations on either side of the North Pacific Ocean during the Pleistocene, i.e., the traditionally recognized WGW and EGW stocks. A trans-NP distribution is shared with other marine mammals, fish, and even terrestrial organisms. This was illustrated by Harlin-Cognato et al. (2006) who studied the phylogeography of Steller sea lions in this region and stated that "Congruence in the distribution of genetic diversity for a wide variety of plants and animals suggests glacial vicariance shaped the history of these species in a similar fashion." If this is the case for NP gray whales, then one would expect to see clades of related haplotypes in the phylogeny of NP gray whales with some clades unique to the Sakhalin population, and more specifically to animals with "western" genotypes. Note that if the Sakhalin "western" genotype whales are the descendants of the WGWs, they represent WGWs that have dispersed into the EGW population (at least those that migrate to Mexico; it is unknown if all or only part of the Sakhalin whales migrate to Mexico; IWC 2018).

The alternative hypothesis is that the "western" and "eastern" genotypes have originated as a result

of recent dispersal of EGW into the Sea of Okhotsk, with subsequent divergence due to founder effect and/or drift. Although the isolation between these two genotypes might pre-date commercial whaling and be on the order of hundreds or a few thousands of years (i.e., post-Pleistocene), or possibly following the near-extirpation of the WGW stock in the 20th century, it would not show the degree of strong differentiation including clades of related haplotypes expected of populations isolated on opposite sides of an ocean basin through the Pleistocene. In the case of recent dispersal, haplotypes would be expected to be shared among the "eastern" and "western" genotype whales, but with different frequencies, and no clades of haplotypes unique to the "western" genome animals. Also, one would not expect to find many, if any, unique haplotypes with such recent divergence, but the small sample size of "eastern" genotype whales in this study prevents unique haplotypes from being a meaningful metric. It is implicit that the extinction of the WGW, or simply their absence from the samples because they are not found at Sakhalin, is what prevents the observation of clades of related haplotypes.

In order to test the two hypotheses of gray whale population structure, extended mtDNA sequences including the non-coding control region as well as three protein-coding genes (*CoI*, *Cyt*b, and *Nd2*) were produced from 65 Sakhalin gray whales. Previous studies of Sakhalin gray whale mtDNA have included only control region (LeDuc et al. 2002; Lang et al. 2011) or multiple mtDNA genes (Meschersky et al. 2015) and have shown no evidence of unique haplotype lineages in the Sakhalin whales. However, those studies predated Brüniche-Olsen et al. (2018a) and thus a mixed sample of "eastern" genotype and "western" genotype whales likely were included. MtDNA divergence, analyzed separately using the control region alone and using an extended sequence of three protein coding genes, was estimated for "eastern" and "western" genotype whales sampled near Sakhalin.

MATERIALS AND METHODS

A total of 75 skin samples of gray whales was obtained by the remote biopsy method in accordance with permission of the Russian Federal Supervisory Natural Resources Management Service (Rosprirodnadzor) along the coast of Sakhalin Island, Pil'tun Bay in August–September of 2012 (16 samples), 2013 (8 samples), 2014 (27 samples), 2015 (9 samples), and 2016 (15 samples). A total of 65 individual gray whales are represented among these samples as determined by unique SNP genotypes and photographic identification (Brüniche-Olsen et al. 2018a).

The tissues were stored in ethanol and shipped to the Laboratory of Genetics, National Scientific Center of Marine Biology, Far East Branch of the Russian Academy of Sciences (NSCMB FEB RAS, Vladivostok, Russia). Total genomic DNA was extracted using the standard phenol-chloroform method (Sambrook et al. 1989) or with a NucleoSpin® Tissue Kit (MACHEREY-NAGEL GmbH & Co.). Amplifications were performed using the DreamTaq DNA polymerase (Thermo Fisher Science, USA). The primers and conditions described by Alter et al. (2009) were used to amplify 621 base pairs of the mtDNA control region (CR) (including tRNA-Pro). The full 1,153 base pair sequence of the cytochrome-*b* (*Cyt*b) gene was obtained using overlapping pairs of primers developed by the Laboratory of Genetics, GW-CYTB F 5'- TACCATTAACCCCAGAAACGAACCAC -3' and GW-CYTB R 5'- GAGTCTTAGGGAGGTGTGGTTTGTCT -3'; and GW-CYTB F2 5'- ATGGGTCTGAGGCGGTTTTTCTGTAG -3' and GW-CYTB R2 5'- GAAGTGGAAGGCAAAGAAGCGTGTTA -3'. The following pair of primers was selected for the subunit 2 of the NADH dehydrogenase gene (*Nd2*): CET_ND2_F (5'-CATACCCCGAAAATGTTGGT-3') and CET_ND2_R (5'-TAGGGCTTTGAAGGCTCTTG-3') described in Meschersky et al. (2015), combined to produce a 1,058 base pair amplicon. Amplification conditions for *Cyt*b and *Nd2* were as follows: denaturation at 95°C - 3 minutes, followed by 37 cycles at 95°C - 30 sec, annealing of primers at 54°C - 60 se., chain extension at 72°C - 90 sec, and final extension at 72°C - 5 minutes. The cytochrome oxidase I (*CoI*) gene fragment with a length of 650 base pairs was amplified using the primers that were

also developed by the Laboratory of Genetics, GW-COI F 5'- ACCTACTCGGCCATCTTACCTA -3' and GW-COI R 5'- AAGCCTAAGAACCCGATGGATA -3'. Amplicons were subsequently purified using Exonuclease I (Exo I) and Shrimp Alkaline Phosphatase (rSAP) (New England Biolabs). The sequencing reactions were performed using BigDye Terminator v. 3.1 kit (Perkin-Elmer, Foster City, California, USA) in accordance with the manufacturer's recommendations. Capillary electrophoresis was performed using the automated ABI Prism GA3500 Genetic Analyzer using a 50 cm capillary assembly with POP-7 polymer. Sequences were assembled using the Geneious R11 software (v11.0.3, Biomatters Limited, Auckland, New Zealand). The similarity of the obtained sequences to those of other available sequences in GenBank was determined by a BLASTn search (Altschul et al. 1990). Sequence data were deposited in GenBank under accession numbers: MH046943-MH047185, MH064256–MH064334.

CLUSTALW (Thompson et al. 1994) was used for sequence alignment. We analyzed the control region and a concatenation of the three protein coding sequences separately. Summary statistics and demographic change parameters were calculated with DNASP v5.10.1 (Librado and Rozas 2009). Genetic diversity was quantified as the number of haplotypes (h), haplotype diversity (h_{div}), the number of segregating sites (S), average number of nucleotide differences (k), and nucleotide diversity (π). Demographic changes were quantified with Tajima's D (Tajima 1989) and Fu's F (Fu 1997).

POPART (Leigh 2015) was used to construct neighbor joining networks for each of the alignments. Genetic differentiation was measured using the fixation metric G_{ST} (Hedrick 2005) and differentiation metrics φ_{ST} (Excoffier et al. 1992; Meirmans 2006) and D (Jost 2008). These were estimated in R (Team 2017) using ADEGENET v2.1.1 (Jombart 2008) and MMOD v1.3.3 (Winter 2012). Estimates and 95% CI across all loci for $G_{ST,}$ φ_{ST} and D were based on 100 bootstraps replicates to identify variation across each point estimate. Fisher's exact test using 1,000 repetitions was used to test for allelic differentiation among subpopulations.

Of the 65 individuals, 46 were previously genotyped at 84 autosomal SNP loci (Brüniche-Olsen et al. 2018a). Genetic admixture coefficients (Q) results from Brüniche-Olsen et al. (2018a) were used to divide the dataset into three groups: "eastern", admixed or "western". The Q-values were estimated with LEA (Frichot and François 2015), which is similar to Bayesian clustering programs like STRUCTURE (Pritchard et al. 2000), where individual admixture coefficients are estimated from the genotypic matrix. The 46 individuals were grouped according to the following Q-values: eastern ($Q < 0.200$), admixed ($0.200 < Q < 0.800$), and western ($Q > 0.800$) for the summary statistics, demographic change and network analyses. Only individuals classified as "eastern" and "western" were included in the G_{ST}, φ_{ST} and D analyses. Our justification for using the LEA Q-values is based on the fact that independent LEA and STRUCTURE analyses produced highly similar results despite these two methods being based on different algorithms. In the LEA program, estimates of ancestry coefficients are calculated using least-squares estimates, whereas in STRUCTURE a likelihood model is used to calculate them. LEA and STRUCTURE were shown to produce similar results (Frichot et al. 2014), but LEA performed better under certain conditions. Specifically, the performance of LEA was better than that of the binomial model used by STRUCTURE where there are high levels of inbreeding, which appears to be the case in Sakhalin gray whales (Brüniche-Olsen et al. 2018b). Only a few individuals would have been assigned to different groups had the values based on the STRUCTURE analysis been used instead.

RESULTS

Of the 65 individuals, the control region (621 bp) was amplified for 64, and the protein coding regions (2,833 bp) were amplified for all individuals. There were 19 individuals with control region and protein coding regions sequenced for which SNP genotypes are not available. These are included in the haplotype networks (see Figs. 2 and 3) but not in Table 1. Variation in sample sizes from 5–32 individuals among the "western" genotype, "eastern" genotype, and admixed groups, respectively, are shown in Table 1. A total of

Table 1. Summary statistics for a) the mitochondrial control region sequence (621 bp) of 45 Sakhalin gray whales with known SNP genomes, and b) the mitochondrial protein coding sequences (2,833 bp) of 46 Sakhalin gray whales with known SNP genomes are given. Determination of groups was based on individual ancestry coefficients (see main text for details). For each group the number of individuals (*n*), number of haplotypes (*h*), haplotype diversity (h_{div}), number of segregating sites (*S*), average number of nucleotide differences (*k*), nucleotide diversity (π), Tajima's *D,* and Fu's *F* are given.

	Group	***n***	***h***	h_{div}	***S***	***k***	π	***D***	***F***
a)	Western	32	9	0.774	32	9.6	0.018	1.53	0.97
	Eastern	8	4	0.643	22	7.1	0.012	-0.83	-0.82
	Admixed	5	2	0.600	16	9.6	0.015	1.83	1.96*
	All	45	14	0.763	33	9.3	0.018	1.06	0.49
b)	Western	32	11	0.802	27	7.3	0.0036	0.31	-0.69
	Eastern	9	6	0.833	18	4.0	0.0014	-1.94*	-2.40*
	Admixed	5	3	0.800	13	7.6	0.0027	1.58	1.68
	All	46	18	0.808	29	6.6	0.0024	0.28	-0.28

* denotes $p < 0.05$

14 haplotypes were observed in the control region; of these, 9 were found in the "western" genotype group, 4 in the "eastern" genotype group, and 2 in the admixed group (Table 1a). Furthermore, the "western" genotype group was shown to have higher h_{div}, *S* and higher π than the eastern and admixed groups. Overall, no sign of demographic change was found for the entire dataset or in the "western" and "eastern" genotype groups; only in the admixed group was an indication of a population decline ($F > 0$) shown. A similar pattern was shown in the summary statistics for the protein coding regions with the "western" haplotype group having more haplotypes ("western" h = 11, "eastern" h = 3, and admixed h = 3) and higher S and higher π than the "eastern" haplotype and admixed groups (Table 1b), but a lower h_{div}. A population expansion ($F < 0$) was identified in the "eastern" group, but none of the other groups showed indication of demographic change.

Two high-frequency haplotypes were observed in both networks (Figs. 2 and 3). In the control region, the high frequency haplotypes were represented by 69% of the individuals (Fig. 2). In the protein coding network, they were represented by 60% of the individuals (Fig. 3). For the control region, there was no indication of individuals identified as "western", "eastern" or admixed to cluster together (Fig. 2). A slight indication was shown of "western" individuals clustering in part of the protein-coding network (Fig. 3).

Measures of G_{ST}, φ_{ST}, and D between whales with "western" and "eastern" genomes based on Q-values were higher for the control region $G_{ST} = 0.355$ (95% CI: 0.008–0.702), $\varphi_{ST} = 0.247$ (95% CI: -0.142–0.635), and D = 0.273 (95% CI: 0.001–0.545), than the protein coding sequences $G_{ST} = 0.129$ (95% CI: -0.204–0.462), $\varphi_{ST} = -0.044$ (95% CI: -0.410–0.322), and $D = 0.104$ (95% CI: -0.178–0.386). Fisher's exact test, $p = 0.034$ for the control region and $p = 0.013$ for the protein coding sequences, were suggestive of our predefined groupings being genetically differentiated according to maternally inherited DNA sequences.

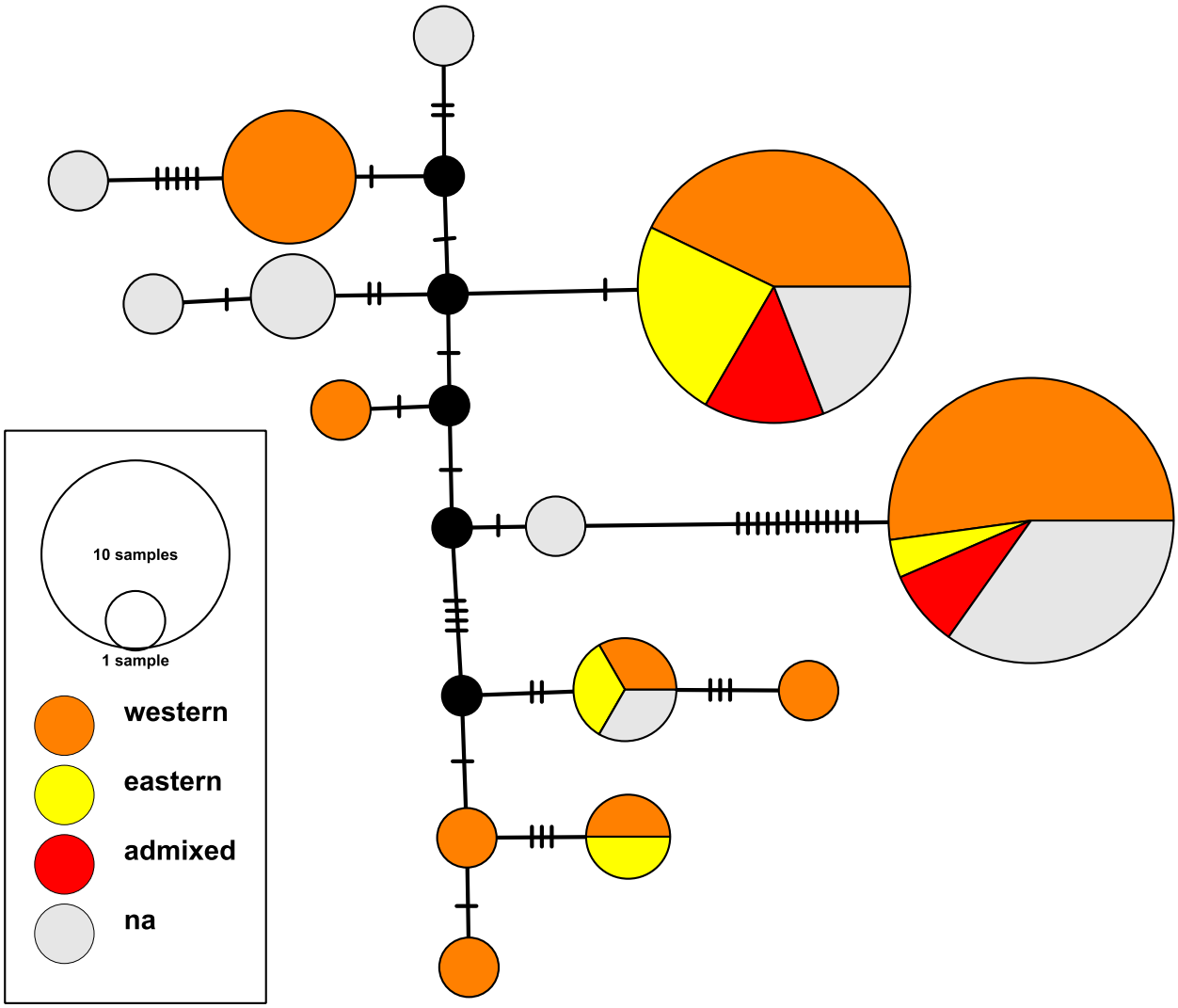

Figure 2. Haplotype network for the mitochondrial control region sequence. The haplotype frequency is indicated by the area of each circle and mutations are indicated on branches with hatch marks. The frequency of western (orange), eastern (yellow), and admixed (red) individuals based on autosomal SNPs (see main text for details) as well as individuals without autosomal SNP admixture coefficient information (gray) are indicated with color for each haplotype. Inferred haplotypes are represented by black dots.

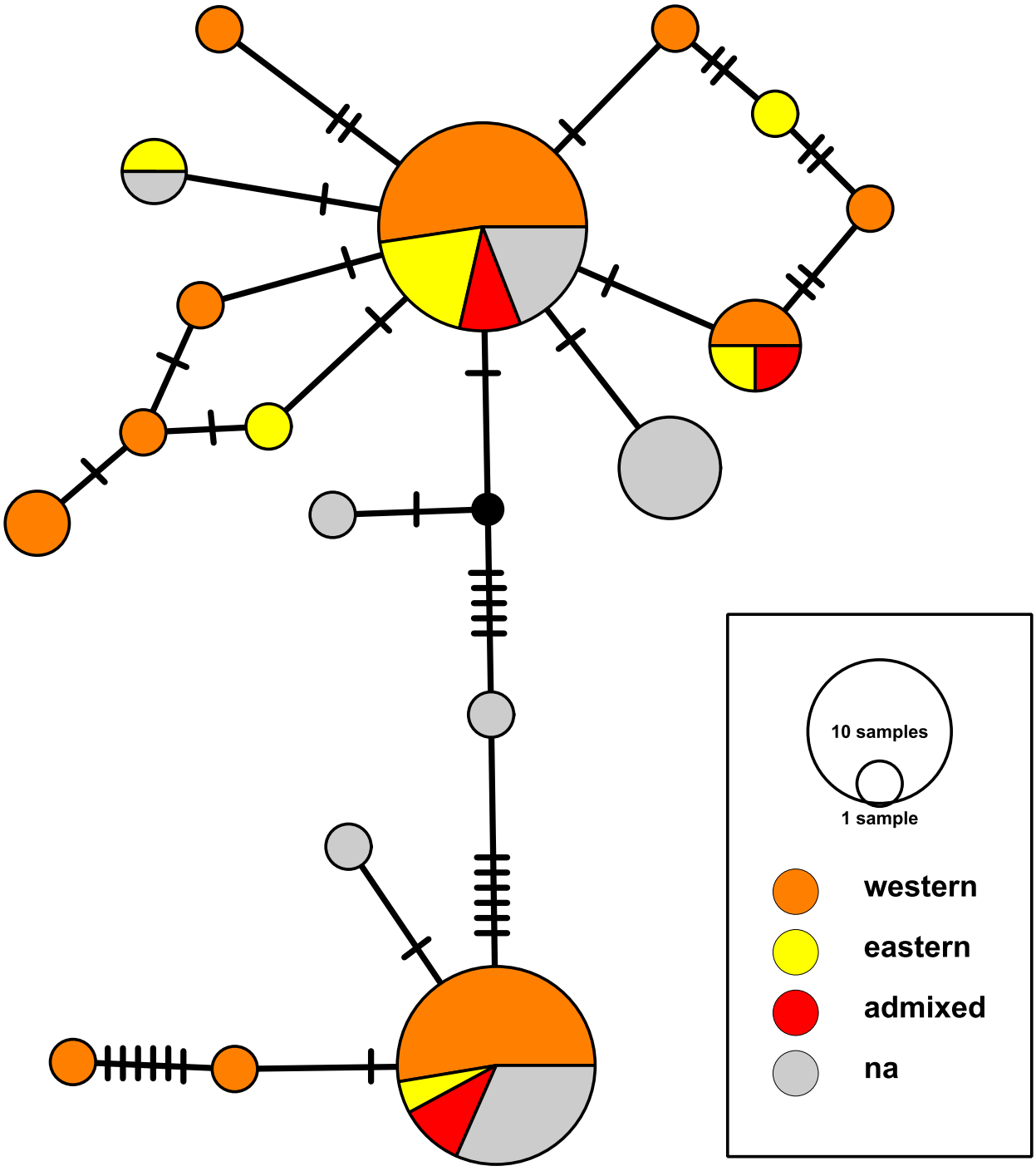

Figure 3. Haplotype network for the protein coding gene sequence. The haplotype frequency is indicated by the area of each circle and mutations are indicated on branches with hatch marks. The frequency of western (orange), eastern (yellow), and admixed (red) individuals based on autosomal SNPs (see main text for details) as well as individuals without autosomal SNP admixture coefficient information (gray) are indicated with color for each haplotype. Inferred haplotypes are represented by black dots.

DISCUSSION

Significant population structure in mtDNA sequences was revealed by Fisher's exact test for both the control region and protein coding genes. Thus, haplotype frequencies, whether identified by control region or protein coding sequences, were found to differ significantly between "eastern" and "western" genotype whales. This is indicative of the LEA scores measuring meaningful differences and that these two groups of whales likely have originated from different populations. The high degree to which there is ongoing or very recent gene flow (i.e., within a few generations) is indicated by the number of whales of admixed genotypes.

Measures of mtDNA diversity (*h, S, k, and* π) for both the control region and the protein coding genes are indicative of higher diversity in the "western" genotype whales compared to the "eastern" and admixed genotype whales (Table 1). However, limitations due to the small sample size of the "eastern" genome group that cause an underestimate of genetic variation in the very large EGW population, should be noted. Previous studies have shown higher mtDNA diversity for the EGW population than the small Sakhalin WGW population (LeDuc et al. 2002; Lang et al. 2011). More extensive sampling is needed to obtain better estimates of mtDNA diversity of the "eastern" and "western" genotype whales.

Haplotype networks for the control region and protein coding genes are shown in Figures 2 and 3, respectively. A comparison of the Figure 2 of this paper with Figure 3 in Lang et al. (2011), both of which are based on control region sequences, show that "western" genome whales from Sakhalin have haplotypes distributed throughout the network (Fig. 2), as does the larger sample of Sakhalin whales studied by Lang et al. (2011) but for which the SNP genotypes are not known. Haplotypes of "eastern" genotype animals also are found throughout the network in Figure 2, as are EGW haplotypes in Figure 3 of Lang et al. (2011).

The absence of any indication of a clade of similar haplotypes unique to the WGW in Lang et al. (2011) and in "western" genotype animals (Fig. 2) is consistent with the alternative hypothesis, that the "western" genotype whales are likely a subpopulation of the EGW population (i.e., not likely the descendants of the WGW population that migrated along the coast of Asia). It is also consistent with the mtDNA control region study of Alter et al. (2015), whose Figure 1 dates ostensible dispersal events between western and eastern Pacific gray whales to <10 kya. However, the hypervariable control region in mammals is known to be prone to extensive homoplasy in the form of recurrent substitutions at certain variable positions (Phillips et al. 2009). It was shown by Phillips et al. (2009) that accounting for the homoplastic substitutions is necessary to obtain a fully resolved haplotype network. To illustrate the significance of this, Phillips et al. (2011) were able to resolve ostensible long-range dispersal of Steller sea lions as being the result of homoplastic mutations, not dispersal. In this study, recurrent substitutions in the control region were not investigated, but it is likely that they are present, so the protein coding genes were analyzed separately. In the haplotype network shown in Figure 3, as in Figure 2, two common alleles that are distantly related are seen, and "western" genotype whales are found throughout the network. These patterns have been observed in all mtDNA studies of gray whales. An examination of Figure 1 in Meschersky et al. (2015), which is a haplotype network based on two protein coding genes and the control region, and the Figure 3 of this paper confirms this. Moreover, as shown in Figure 3, both "eastern" and "western" genotype whales are present in both of the distantly related common haplotypes. Therefore, there does not appear to be strong evidence of clades of related haplotypes that are specific to "western" genome whales, even with the extended three protein coding genes.

It is also useful to compare the phylogeographic patterns of gray whales to other baleen whales. While baleen whales often show distinct phylogeographic patterns including clades of related haplotypes in comparisons of populations between ocean basins (Archer et al. 2013; Jackson et al. 2014; Alter et al. 2015), comparisons within ocean basins, as in this study, might not show such a pattern due to the high vagility of and dispersal capability of these animals. For example, in neither the North Pacific right whale, *Eubalaena japonica*, nor the North Pacific humpback whale,

Megaptera novaeangliae, are clades of related haplotypes found in comparisons made between populations that are otherwise strongly differentiated by mtDNA haplotype frequencies (Baker et al. 2013; Pastene et al. 2013). Clearly, a long period of isolation is needed for the establishment of clades of related haplotypes, and in species with high dispersal capabilities inhabiting oceans without strong geographic boundaries it is possible that gene flow prevents this. Then why should we expect WGWs and EGWs to have clades of related haplotypes?

One unique aspect of gray whale distribution is that it is tightly correlated with coastal habitats. This is because gray whales are adapted to feed mainly on benthic organisms found in relatively shallow waters, and it is the only baleen whale species to do this. Thus, the Steller sea lion, which is also more closely tied to shallow waters and coastal habitats, seems to be a better comparison than pelagic species of baleen whales. And secondly, in the studies conducted on right whales and humpback whales only mtDNA control region sequences were used. Thus, the problem of recurrent mutations that mimic gene flow might obfuscate the finding of clades of related haplotypes if such have ever been established. The bowhead whale (*Balaena mysticetus*) might be an example of this. Two populations of bowhead whales are found in the North Pacific Ocean, one that inhabits the Sea of Okhotsk and the other in the Bering, Chukchi, and Beaufort Seas (BCB). As with the gray whales, the bowhead population in the Sea of Okhotsk is very small and endangered, and the BCB population is large. Studies that employed only control region sequences (Alter et al. 2012 and references therein) found four haplotypes among 24 Sea of Okhotsk bowhead whales, all of which were shared with BCB whales. Baird et al. (2018) examined control region and two protein coding gene sequences from seven Sea of Okhotsk whales and a large number of BCB whales and found five haplotypes of which three were unique to the Sea of Okhotsk whales. Of these five haplotypes, no clear examples of clades of related haplotypes were found, although two haplotypes were found that were one step different; one being a shared haplotype and the tip haplotype unique. Also, the five haplotypes found in the Sea of Okhotsk are restricted to one part of the very large 141-haplotype network. Thus, examination of the extended sequence allows for a higher degree of resolution and a greater probability of finding clades of related haplotypes.

It should be recognized that this study is based on a small sample size, especially of the "eastern" genotype whales (N = 8). Thus, the results and conclusions can be considered as preliminary, but they nonetheless are the best indicators available of the historical relationships of gray whales currently summering at Sakhalin Island. When other lines of evidence are considered, namely that a sizeable number of Sakhalin whales have been confirmed to migrate to North American waters (Mate et al. 2015) and the number of gray whales observed in Asian waters south of the Sea of Okhotsk is small (Weller et al. 2008), the weight of evidence seems to be mounting that there is continuity in the gray whale gene pool, in contrast to the established view of discontinuity.

In conclusion, the analysis of mtDNA control region and three protein coding genes of gray whales summering near Sakhalin Island in the Sea of Okhotsk, Russia, failed to reveal the presence of clades of related haplotypes specific to the "western" genotype whales as identified by nuclear SNP loci. Rather, both "western" and "eastern" genotype animals had haplotypes found throughout the network. This is inconsistent with the null hypothesis of historical divergence (e.g., due to Pleistocene isolation) but consistent with the alternative hypothesis that the "western" and "eastern" genotypes originated as a result of recent dispersal of EGW into the Sea of Okhotsk. In the absence of archaeological or historical samples from the range of the WGW in Asia, further testing of this hypothesis is needed by employing larger sample sizes of Sakhalin whales. In particular, more whales with the minority "eastern" genome need to be analyzed. Other approaches to explore the historical demography of the "western" and "eastern" genotypes that can be applied to whole genome sequences as well as mitogenomic analyses need to be extended beyond the three whole genomes analyzed in Brüniche-Olsen et al. (2018b). The significance of this study is that the current mtDNA and nuclear SNP data suggest that the Sakhalin whales with "western" genotypes may simply be a geographical isolate of the larger EGW gene pool.

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LITERATURE CITED

Alter, S. E., et al. 2012. Gene flow on ice: the role of sea ice and whaling in shaping Holarctic genetic diversity and population differentiation in bowhead whales (*Balaena mysticetus*). Ecology and Evolution 2:2895–2911.

Alter, S. E., et al. 2015. Climate impacts on transocean dispersal and habitat in gray whales from the Pleistocene to 2100. Molecular Ecology 24:1510–1522.

Alter, S. E., S. F. Ramires, S. Nigenda, J. U. Ramires, and S. R. Palumbi. 2009. Mitochondrial and nuclear genetic variation across calving lagoons in eastern North Pacific gray whales (*Eschrichtius robustus*). Journal of Heredity 100:34–46.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic logical alignment search tool. Journal of Molecular Biology 215:403–410.

Archer, F. I., et al. 2013. Mitogenome phylogenetics of fin whales (*Balaenoptera physalus*): Genetic evidence for revision of subspecies. PLOS One 8:e63396.

Baker, A. R., et al. 2005. Variation of Mitochondrial Control Region Sequences of Steller Sea Lions, *Eumetopias jubatus*: The Three-Stock Hypothesis. Journal of Mammalogy 86:1075–1084.

Baker, C. S., et al. 2013. Strong maternal fidelity and natal philopatry shape genetic structure in North Pacific humpback whales. Marine Ecology Progress Series 494:291–306.

Bickham, J. W., J. C. Patton, and T. R. Loughlin. 1996. High variability for control-region sequences in a marine mammal: implications for conservation and maternal phylogeny of Steller sea lions (*Eumetopias jubatus*). Journal of Mammalogy 77:95–108.

Blokhin, S.A., M.K. Maminov, and G.M. Kosygin. 1985. On the Korean-Okhotsk population of gray whales. Report of the International Whaling Commission 35:375–376.

Bowen, S.L. 1974. Probable extinction of the Korean stock of the gray whale (*Eschrichtius robustus*). Journal of Mammalogy 55:208–209.

Bradford, A.L. 2003. Population assessment of western North Pacific gray whales (*Eschrichtius robustus*). M.S. Thesis, University of Washington.

Brownell, R.L., Jr., and Chun C.I. 1977. Probable existence of the Korean stock of the gray whale (*Eschrichtius robustus*). Journal of Mammalogy 58:237–239.

Brüniche-Olsen A., et al. 2018b. The inference of gray whale (*Eschrichtius robustus*) population attributes from whole-genome sequences. BMC Evolutionary Biology 18:87. https://doi.org/10.1186/s12862-018-1204-3

Brüniche-Olsen, A., J. Urban R., V. V. Vertyankin, C. Godard-Codding, J. W. Bickham, and J. A. DeWoody. 2018a. Genetic data reveal mixed-stock aggregations of gray whales in the North Pacific Ocean. Biology Letters 14:20180399. http://dx.doi.org/10.1098/rsbl.2018.0399

Cooke, J. G. 2018. *Eschrichtius robustus*. The IUCN Red List of Threatened Species 2018: e.T8097A50353881. http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T8097A50353881.en

Cooke, J. G., et al. 2017. Population assessment update for Sakhalin, gray whales. with reference to stock identity. Paper SC/67a/NH11 presented to the IWC Scientific Committee (unpublished). [Available at http://www.iwcoffice.org]

Durban, J.W., D. W. Weller, and W. L. Perryman. 2017. Gray whale abundance estimates from shore-based counts off California in 2014/2015 and 2015/2016. Paper SC/A17/GW/06 presented to the IWC Scientific Committee (unpublished). [Available at http://www.iwcoffice.org]

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes – application to human mitochondrial-DNA restriction data. Genetics 131:479–491.

Frichot E. and O. François. 2015. LEA: an R package for landscape and ecological association studies. Methods in Ecology and Evolution 6:925–929.

Frichot E., F. Mathieu, T. Trouillon, G. Bouchard, and O. François. 2014. Fast and efficient estimation of individual ancestry coefficients. Genetics 196:973–983

Fu, Y-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925.

Harlin-Cognato, A., J. W. Bickham, T. R. Loughlin, and R. L. Honeycutt. 2006. Glacial refugia and the phylogeography of Steller's sea lion (*Eumatopias jubatus*) in the North Pacific. Journal of Evolutionary Biology 19:955–969.

Hedrick, P. W. 2005. A standardized genetic differentiation measure. Evolution 59:1633–1638.

Henderson, D. A. 1984. Nineteenth century gray whaling: grounds, catches and kills, practices and depletion of the whale population. Pp. 159–186 in The Gray Whale *Eschrichtius robustus* (M. L. Jones, S.L. Swartz, and S. Leatherwood, eds.). Academic Press, San Diego, California.

Ilyashenko V.Yu. 2011. Gray whale re-inhabits former species area. Paper SC/63/BRG24 presented to the IWC Scientific Committee (unpublished). [Available at http://www.iwcoffice.org]

International Whaling Commission. 2014. Report of the Workshop on the Rangewide Review of the Population Structure and Status of North Pacific Gray Whales. Paper SC/65b/Rep08 presented to the IWC Scientific Committee (Unpublished). [Available at http://www.iwcoffice.org/]

International Whaling Commission. 2018. Report of the 2018 IWC Scientific Committee. 24 April–6 May 2018, Bled, Slovenia. [Available at http://www.iwcoffice.org/]

Jackson, J. A., et al. 2014. Global diversity and oceanic divergence of humpback whales (*Megaptera novaeangliae*). Proceedings of the Royal Society B-Biological Sciences 281 (1786). pii: 20133222. doi: 10.1098/rspb.2013.3222.

Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405.

Jost, L. 2008. G(ST) and its relatives do not measure differentiation. Molecular Ecology 17:4015–4026

Kato, H. and T. Kasuya. 2002. Some analyses on the modern whaling catch history of the western North Pacific stock of gray whales (*Eschrichtius robustus*), with special reference to the Ulsan whaling ground. Journal of Cetacean Research and Management 4:277–82.

Lang, A. R., et al. 2011. Genetic analysis of stock structure and movements of gray whales in the eastern and western North Pacific. Paper SC/63/BRG10 presented to the IWC Scientific Committee (Unpublished). [Available at http://www.iwcoffice.org]

LeDuc, R. G., et al. 2002. Genetic differences between western and eastern gray whales (*Eschrichtius robustus*). Journal of Cetacean Research and Management 4:1–5.

Leigh, J. W., and D. Bryant. 2015. popart: full-feature software for haplotype network construction. Methods in Ecology and Evolution 6:1110–1116.

Librado, P, and J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452.

Mate, B., et al. 2015. Critically endangered western gray whales migrate to the eastern North Pacific. Biology Letters 11:20150071. http://dx.doi.org/10.1098/rsbl.2015.0071

Meirmans, P. G. 2006. Using the AMOVA framework to estimate a standardized genetic differentiation measure. *Evolution* 60:2399–2402.

Meschersky, I. G., et al. 2015. Occurrence and distribution of mitochondrial lineages of gray whales (*Eschrichtius robustus*) in Russian Far Eastern Seas. Biological Bulletin 42:34–42.

Omura, H. 1974. Possible migration route of the gray whale on the coast of Japan. Scientific Report of the Whales Research Institute, Tokyo 26:l–14.

Phillips, C. D., et al. 2009. Assessing substitution patterns, rates and homoplasy at HVRI of Steller sea lions, *Eumetopias jubatus*. Molecular Ecology 18:3379–3393.

Phillips, C. D., T. S. Gelatt, J. C. Patton, and J. W. Bickham. 2011. Phylogeography of Steller sea lions: relationships among climate change, effective population size, and genetic diversity. Journal of Mammalogy 92:1091–1104.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Rice, D. W., and A. A. Wolman. 1971. The Life History and Ecology of the Gray Whale (*Eschrichtius robustus*). American Society of Mammalogists, Special Publication No. 3, Stillwater, Oklahoma.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

Team, R. D. C. 2017 R: A language and environment for statistical computing, Vienna, Austria.

Thompson, J. D., D. G. Higgins and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673–4680.

Weller, D. W., A. M. Burdin, B. Wursig, B. L. Taylor and R. L. Brownell, Jr. 2002. The western gray whale: A review of past exploitation, current status and potential threats. Journal of Cetacean Research and Management 4:7–12.

Weller, D.W., A. L. Bradford, H. Kato, T. Bando, S. Ohtani, A. M. Burdin, and R. L. Brownell, Jr. 2008. Photographic match of a western gray whale between Sakhalin Island, Russia, and Honshu, Japan: First link between feeding ground and migratory corridor. Journal of Cetacean Research and Management 10:89–91.

Winter, D. J. 2012. MMOD: an R library for the calculation of population differentiation statistics. Molecular Ecology Resources 12:1158–1160.

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Ecological Niche Modeling Identifies Environmental Factors Influencing Hybridization in Ground Squirrels (Genus *Ictidomys*)

Cody W. Thompson, Frederick B. Stangl, Jr., Robert J. Baker, and Robert D. Bradley

Abstract

The Rio Grande ground squirrel (*Ictidomys parvidens*) and the thirteen-lined ground squirrel (*I. tridecemlineatus*) form a mosaic hybrid zone across portions of New Mexico and Texas. Recent studies have linked habitat modification to the formation of secondary contact; however, it has been suggested that ancient hybridization was a result of climate change. Therefore, ecological niche modeling (ENM) was used to assess the possible contributions of climate change and human-mediated habitat modification on present distributions of each species and contemporary hybridization, as well as to test the effect of climate on species' distributions during the last glacial maximum (LGM) and last inter-glacial (LIG). Specimen-occurrence records were obtained from natural history collections. Niche models were developed with Maxent for present, LGM, and LIG data and compared using niche-identity/equivalency tests and correlation analyses to assess differences between the two species and extent of overlap. Present models were similar to known species distributions. Models indicated that both climate and land use affected present distributions of each species. However, models that considered only land-use data overpredicted the distribution of each species, whereas models using only climatic variables were similar to known distributions. Models based on palaeoclimatic data further suggested the influence of climate on the distribution of both species. Present and LGM models were significantly different from each other, but niche overlap was greatest for present models. ENM revealed that present distributions of *I. parvidens* and *I. tridecemlineatus* were influenced primarily by climate change, although anthropogenic changes in natural habitat might have had a compounding effect on movement of either species leading to secondary contact and contemporary hybridization. In addition, secondary contact appears to be a recent phenomenon, as there was limited overlap of species' distributions during LGM and LIG. Therefore, gene flow has been restricted until recently.

Key words: climate change, habitat modification, *Ictidomys*, land-use, last glacial maximum, Maxent, mosaic hybrid zone, palaeoclimatic modeling, species distribution modeling

Introduction

Until recently, few studies have addressed the environmental component associated with hybrid zone formation and structure (Gaubert et al. 2006; Martínez-Freiría et al. 2008; Chatfield et al. 2010; Schukman et al. 2011; Hilbish et al. 2012; Taylor et al. 2015; Zhao et al. 2016). This is likely because few studies adequately assess the spatial structure of hybrid zones, leading to the misclassification of hybrid zones as clinal rather than mosaic (M'Gonigle and FitzJohn 2009). Ecological niche modeling (ENM) allows researchers the ability to address this problem (Kozak and Wiens 2006) through the prediction of a species' distribution (via inferred aspects of its ecological niche requirements) based on analyses of environmental variables and specimen-occurrence records. ENM attempts to model the fundamental niche of a species (Hutchinson 1959), defined as the theoretical range of possibilities where a species can exist (Grinnell 1917). However, ENM

cannot assess the realized niche of a species because of limitations in modeling the biotic aspects of species interactions, such as dispersal and competition, as well as aspects of species history, for example speciation and extinction (Peterson 2003). Therefore, models produced from ENM are predictions of the ecological niche of a species and are not meant to be a complete representation of the distribution of the species.

Applications using ENM have gained in popularity because of the increased accessibility of museum collection records and intuitive software packages (Feeley et al. 2011; McLean et al. 2015; Schindel and Cook 2018). In addition, advanced environmental datasets (e.g., Latifovic et al. 2002; Hjimans et al. 2005; Smith et al. 2016) have enabled researchers to construct more complicated models for addressing complex questions of biodiversity. Most ENM applications used in hybrid zone studies have focused primarily on discovering zones of contact, determining the extent of spatial overlap of hybridizing taxa, or identifying the environmental factors associated with hybridization (Kovak et al. 2008; Taylor et al. 2015). Although ENM studies have attempted to model the palaeoclimatic niche of many species (Carstens and Richards 2007; Fløjgaard et al. 2009; Vega et al. 2010; Waltari and Hickerson 2012; López-Alvarez et al. 2015), none have examined the environmental factors associated with hybrid zones over geological time. Ground squirrels of the genus *Ictidomys* (Helgen et al. 2009) offer an opportunity to test environmental and temporal effects on hybrid zones.

The Rio Grande ground squirrel (*I. parvidens*) and the thirteen-lined ground squirrel (*I. tridecemlineatus*) form a mosaic hybrid zone across portions of New Mexico and Texas. These two species hybridize in small, isolated populations in a mosaic pattern along a parapatric boundary in southeastern New Mexico and western Texas (Zimmerman and Cothran 1976; Stangl et al. 2012; Thompson et al. 2013, 2015). Although the extent of hybridization is limited between *I. parvidens* and *I. tridecemlineatus*, analyses of nuclear DNA data suggest intense admixture of the nuclear genome and limited genetic isolation to a few locations (Thompson et al. 2013).

Recent analyses of the mitochondrial cytochrome-*b* (*Cyt*b) and the Y-linked structural maintenance of chromosomes (*SmcY*) genes indicated that the two species experienced an ancient hybridization event that resulted in the mitochondrial capture of a *I. tridecemlineatus* mitochondrial genome within individuals of *I. parvidens* approximately 0.80 million years ago (mya—Thompson et al. 2015). This timeframe, as well as other divergence-date estimates for this genus (Harrison et al. 2003), are aligned closely with climate change as the result of the climatic oscillations of the Quaternary, creating conditions of sympatry allowing for opportunities of hybridization. Therefore, recent warming trends in global climate (Shurtliff 2011) may have contributed to contemporary hybridization between these two species by providing conditions favorable for secondary contact (Thompson et al. 2013).

An alternative hypothesis to climate change has implicated recent habitat modifications as being the primary cause of secondary contact between these two species (Cothran 1982; Stangl et al. 2012; Thompson et al. 2013, 2015). Much of the area in southeastern New Mexico and western Texas that supports hybridization between *I. parvidens* and *I. tridecemlineatus* has been affected by recent conversion of native habitat to row-crop agriculture (Choate 1997). As a result of the xeric nature of this region, much of this habitat conversion did not occur until the midpoint of the 20th century when the use of irrigation technology resulted in a drastic change of the native grasslands of the Great Plains to farmland (Mahmood et al. 2006), primarily cotton fields. The concomitant increase in roadways may have formed artificial dispersal routes by providing suitable roadside vegetation through this otherwise unsuitable habitat, allowing for the recent secondary contact to occur between *I. parvidens* and *I. tridecemlineatus* (Cothran 1982; Stangl et al. 2012; Thompson et al. 2013, 2015). This movement has been documented via voucher specimens in museum collections since the mid-1800s (Marcy 1856; Baird 1857). Although climate change influences land-use to a large degree (Dale 1997), the contribution of recent habitat modifications to the maintenance of contemporary hybridization between *I. parvidens* and *I. tridecemlineatus* is largely unknown.

In this study, ENM was used to assess the environmental factors that may have contributed to secondary contact and subsequent hybridization between *I. parvidens* and *I. tridecemlineatus* in recent time. The primary objective of this study was to develop ecologi-

cal niche models that incorporated variables of present climate and land-use data to estimate the influence of each variable on the ecological niche of *I. parvidens* and *I. tridecemlineatus*. If secondary contact were the result of climate change, climate variables used to construct ecological niche models would more drastically influence predictions of the current distribution of each species. However, if contemporary hybridization were the result of anthropogenic changes to habitat, then land-use variables describing human modifications to the environment would impact the distribution of either species more than either climate variables or natural land-use categories. In addition, palaeoclimatic models were developed by projecting the modern niche requirements of both species onto climatic data for the last glacial maximum (LGM; ~21,000 years before present [BP]) and the last inter-glacial (LIG; 120,000–140,000 years BP) to determine whether or not secondary contact was a recent phenomenon, thereby testing the ancient hybridization and mitochondrial capture hypotheses of Thompson et al. (2015). If secondary contact was recent, then paleoclimatic modeling for the LGM and LIG would indicate little distributional overlap. Finally, the extent of overlap of both species' predicted distributions and the equivalency of both species' niches were ascertained to determine the relative ability of both species to co-occur and potentially hybridize. High niche equivalency would suggest a high hybridization potential.

METHODS

Museum specimen records.—Specimen-occurrence records were obtained through a query of natural history collections via the Mammal Networked Information System (MaNIS, http://manisnet.org; Stein and Wieczorek 2004). Additional specimen-occurrence records were acquired from the Angelo State University Natural History Collections and from Midwestern State University. Results of the search were pruned for duplicate records, as well as those georeferenced outside of the known distribution of each species (Streubel and Fitzgerald 1978; Young and Jones 1982). Records with only textual locality data were georeferenced when high-precision estimates were possible. To avoid temporal influences of collecting efforts, this dataset was reduced to only include specimens collected post-1950. This date approximates the hypothesized timeframe of the recent dispersal and subsequent contact of *I. parvidens* and *I. tridecemlineatus* (Cothran 1982; Stangl et al. 2012). To avoid sampling bias and potential overfitting of the model (Reddy and Davalos 2003), a 50-km buffer was placed around each locality to ensure that they were distributed evenly over geographic space. When multiple records fell within the 50-km buffer, specimen records were systematically removed from the dataset with consideration given to preserving the most precise records. The Paleobiology Database (Behrensmeyer and Turner 2013) was queried for fossils of *I. parvidens* and *I. tridecemlineatus*, but they were determined to be inappropriate for this study because fossil ages were older than available paleoclimatic data (see below).

Climate and land-use data.—Bioclimatic (BIOCLIM) data were downloaded from WorldClim v1.4 (http://worldclim.org; Hjimans et al. 2005). BIOCLIM variables incorporate aspects of temperature and precipitation to represent different characteristics of the climate, which are thought to be more biologically meaningful than raw values. All 19 BIOCLIM variables for present climate conditions (1950–2000) were used to construct ENM models for each species. In addition, all 19 BIOCLIM variables for the LGM (~21,000 years BP) based on information from the Community Climate Model System (CCSM—Braconnot et al. 2007) and the LIG (120,000–140,000 years BP—Otto-Bliesner et al. 2006) were used for palaeoclimatic modeling. The present and LIG data had a 30 arc-second (~1 km) resolution, and the LGM data had a 2.5 arc-minute resolution (~20 km).

Land-use data were obtained from the Land Cover Database of North America (Latifovic et al. 2002). The land-use data for this database were generated in the year 2000 at ~1 km resolution. These data are divided into 29 land-use categories based on the Natural Vegetation Classification Standard and include both natural and modified classifications.

Ecological niche modeling.—Following Hope et al. (2012, 2016), Maxent version 3.3 (Phillips et al. 2004, 2006) was used to model both the present (1950–2000) and palaeoclimatic (i.e., LGM and LIG) distributions of *I. parvidens* and *I. tridecemlineatus*. Maxent uses specimen-occurrence data (i.e., presence-background) and environmental data to produce models based on the potential suitability of habitat. Therefore, Maxent offers a rigorous treatment and prediction of the species' potential ecological niche (Chatfield et al. 2010; Merow et al. 2013). However, Maxent does not incorporate biotic aspects of a species' niche (e.g., competition, predation, etc.), so there are limitations to the method (Hilbish et al. 2012).

To construct models, data layers (described above) first were extracted by using a combined polygon consisting of Canada, Mexico, and the United States of America as a mask. This area was considered to be the hypothetical 'M', defined as the area potentially accessible to the modeled taxa (Soberón and Peterson 2005). Although these two species are considered to be typical of the grasslands of central North America (Helgen et al. 2009), montane grassland populations do exist for each (*I. tridecemlineatus*, Strebel and Fitzgerald 1978; *I. parvidens*, Young and Jones 1982). Therefore, the potential areas for modeling were not limited to narrow grassland regions to avoid restraining the ability of the models to predict suitable areas that are currently unavailable to either species. In addition, because of the unknowns of each species' historical distribution for the purpose of palaeoclimate modeling, the hypothetical 'M' region was not overly limited.

For modeling the present distributions of each species, the 19 BIOCLIM variables from present climate conditions were combined with land-use data to assess the influence of both climate and land-use changes concomitantly. The model was trained using 75% of the occurrence records (randomly selected) and the remaining 25% of the occurrence records were used for testing the model. A jackknifing procedure was used to measure the importance of each variable. In addition, response curves were produced for each variable to estimate and visualize the effects of each variable independently. Maxent uses a regularization method to select variables, minimizing the need for pruning variables to avoid overfitting (Kalkvik et al. 2012). All other default options in Maxent (Phillips and Dudik 2008) were used in each run. Omission rates for both training and testing data were estimated using a binomial test to determine the significance of the prediction. Omission rates for all threshold parameters in Maxent were considered. The area under the receiver-operating curve (AUC) was calculated for both training and testing data to measure model performance. The criterion for model performance followed the suggestions of Swets (1988) and Elith (2002), where models were considered good if AUC values were > 0.75 and excellent if AUC values were > 0.90.

For palaeoclimatic modeling, it was assumed that the present climatic requirements of each species were similar to those in the past, and models were constructed using the 19 BIOCLIM variables for both the LGM and LIG data. Modeling procedures followed those described previously; however, palaeoclimatic models were constructed through projections based on the influence of the BIOCLIM variables on present distributions of each species. Present climatic models then were used to project models under the climate conditions of the LGM and LIG.

To measure the similarity between the predicted models of each species, the niche-identity and equivalency tests were used in ENMTools 1.3 (Warren et al. 2010). The niche-identity test (Warren et al. 2008) measures similarity of the predicted distributions for multiple species. The niche-equivalency test (Warren et al. 2008) is related to the niche-identity test. The former tests the hypothesis that two niche models are not significantly different (Graham et al. 2004), whereas the latter tests the hypothesis that two niche models are more similar than expected by chance (Peterson et al. 1999). However, the niche-equivalency test measures similarity by combining the specimen-occurrence datasets to determine if their niches are more different than expected due to chance by generating a random distribution for statistical comparisons. Each test uses three statistics: Schoener's *D* (Schoener 1968); the *I* statistic based on Hellinger distance (Warren et al. 2008); and relative rank (Warren and Seifert 2011). The *D* and *I* statistics measure the level of habitat suitability given the variables used to construct the model; whereas the relative rank (RR) statistic measures the relative quality of habitat found in overlapping cells for each species' models. All similarity statistics produced

range from zero to one, with zero indicating no overlap and one indicating identical distribution models. Random distributions of each statistic were approximated from 100 pseudoreplicates of the pooled localities of all samples. A z-score was used to estimate the level of significance. In addition, Pearson's correlation coefficient (r) was used to assess the similarity of models; correlation coefficients greater than ±0.5 were considered to be highly correlated. To visualize the extent of overlap, the fuzzy overlap function in ArcGIS v10 (ESRI, Redlands, California) was used. This function identifies overlap by finding cells that are occupied by two or more raster datasets.

RESULTS

Ecological niche models were developed for both species based on a dataset of 62 occurrence records for *I. parvidens* and 195 occurrence records for *I. tridecemlineatus* (Fig. 1 and Appendix). All models were trained with 46 and 143 occurrence records of *I. parvidens* and *I. tridecemlineatus*, respectively, and were tested with 16 and 52 occurrence records of *I. parvidens* and *I. tridecemlineatus*, respectively. Each model produced had significantly low omission rates ($p < 0.00001$) for all threshold parameters analyzed (Table 1). In addition, most models had AUC values greater than 0.9 for both training and testing data (0.814–0.992; Table 1), indicating that the models had more predictive power than random.

For models predicted from combined present (1950–2000) climate and land-use data, resulting predictions aligned closely with the known distributions of each species (Fig. 2). Some overprediction did occur, most notably for *I. parvidens* into areas north of its present distribution. This pattern likely is an indication that *I. tridecemlineatus* maybe prohibiting northerly expansion due to competition. The permutation tests indicated that the minimum temperature of the coldest month and the mean temperature of the driest quarter contributed most highly to the models of *I. parvidens* and *I. tridecemlineatus*, respectively. However, jackknifing tests indicated that models were best predicted by maximum temperature of the warmest month for *I. parvidens* and annual mean temperature for *I. tridecemlineatus*. Jackknifing tests also indicated that the land-use data had the most influence on the predicted distribution of both species; land-use categories that were the most influential varied among models. For *I. parvidens*, five land-use categories had a high probability of presence (> 0.50): herbaceous wetlands, urban and developed, disturbed areas, grasslands, and shrublands. For *I. tridecemlineatus*, three land-use categories had a high probability of presence: urban and developed, grasslands, and disturbed areas.

Independent models for both climate and land-use data also were developed for present data (not shown). Models for climate data only were similar to models constructed with both climate and land-use data; however, overprediction of climate data only models was more extensive than in the combined analyses. Permutation tests determined that temperature seasonality was the most important variable for *I. parvidens* and mean temperature of the driest quarter was the most important variable for *I. tridecemlineatus*. However, jackknifing tests indicated that maximum temperature of the warmest month was the best predictor of the present climate model for *I. parvidens*; whereas mean temperature of the wettest quarter was the best predictor of the model for *I. tridecemlineatus*. Jackknifing tests also determined that mean temperature of the driest quarter had the greatest influence on the model of each species. Models developed with only land-use data resulted in overprediction of the distributions of each species. Compared to other models, the land-use only models both had relatively low AUC values, indicating that the predictability of these models to be lower than random (see Table 1). Land-use categories with a high probability of presence were the same as above for both species.

For palaeoclimatic distribution projections, predicted models varied dramatically relative to the current known distribution of each species (Fig. 2). Palaeoclimatic models of *I. parvidens* predicted a reduction in distribution southward relative to that of the current distribution, but the largest portion of each prediction was within the current known distribution. In addition, palaeoclimatic models for *I. tridecemlineatus* indicated a reduction in distribution southward relative to the cur-

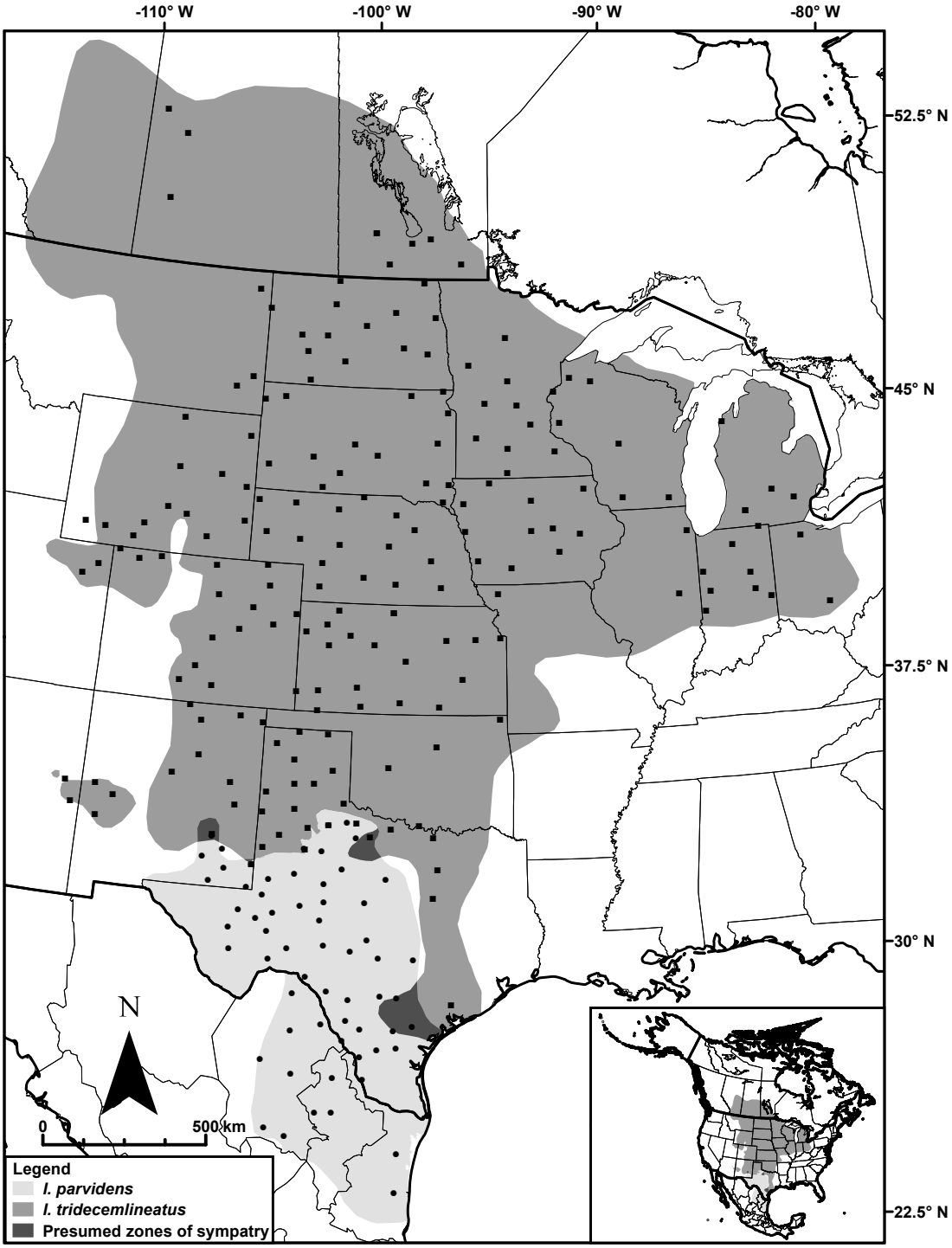

Figure 1. Maps illustrating present distributions of *I. parvidens* and *I. tridecemlineatus* and locations of specimen occurrences used for ecological niche modeling. Zones of sympatry are indicated by the darker shades. Circles represent locations of specimen occurrences for *I. parvidens*. Squares represent locations of specimen occurrences for *I. tridecemlineatus*. Catalog numbers and locality data are listed in the Appendix.

Table 1. Summary of evaluation statistics for the accuracy of predicted models. Omission rates with *p*-values < 0.05 are indicated with an asterisk (*). Models with area under the curve (AUC) estimates that were > 0.90 were indicated by a carat (^). Additional abbreviations are as follows: LGM = last glacial maximum; and LIG = last inter-glacial.

Species	Timeframe	Training Omission	Testing Omission	Training AUC	Testing AUC
I. parvidens	Present				
	all variables	0.000–0.085*	0.000–0.267*	0.991^	0.985^
	climate only	0.000–0.085*	0.000–0.267*	0.990^	0.986^
	land use only	0.000–0.106*	0.000–0.133*	0.923^	0.907^
	LGM	0.000–0.087*	0.000–0.267*	0.992^	0.987^
	LIG	0.000–0.085*	0.000–0.267*	0.990^	0.986^
I. tridecemlineatus	Present				
	all variables	0.000–0.097*	0.000–0.312*	0.957^	0.931^
	climate only	0.000–0.104*	0.000–0.312*	0.954^	0.922^
	land use only	0.000–0.125*	0.000–0.312*	0.880	0.814
	LGM	0.000–0.097*	0.000–0.271*	0.959^	0.926^
	LIG	0.000–0.104*	0.000–0.312*	0.954^	0.922^

rent known distribution of the species; however, models for both LGM and LIG were distributed broadly across geographic space. In addition, models for *I. tridecemlineatus* for LGM and LIG predicted the presence of this species into known areas of *I. parvidens*.

Measures of niche similarity were calculated only for models predicted with 2.5 arc-minute resolution, which required constructing present climate-only models at 2.5 arc-minute resolution. This limited comparisons to present and LGM models (see Table 2 and Fig. 3). Comparisons of models generated from present data were significantly different from each other for both Schoener's D ($D = 0.18979$, $p < 0.0001$) and the I statistic ($I = 0.42809$, $p < 0.0001$); however, models were not significantly different for the RR statistic (RR = 0.82078, $p = 0.0937$). Comparisons of the LGM models were significantly different for both Schoener's D ($D = 0.06545$, $p < 0.0001$) and the I statistic ($I = 0.21409$, $p < 0.0001$), and they were not significantly different for the RR statistic (RR = 0.81974, $p = 0.0715$). Pearson's correlation did not indicate a high level of correlation for either present ($r = 0.18069$) or LGM model comparisons ($r = 0.05331$). The extent of the areas of overlap from the predicted models was consistent with these tests (Fig. 4). The predicted overlap for present models occurred primarily in southeastern New Mexico and western Texas. Predicted areas of overlap for both the LGM and LIG models were restricted primarily to present-day southern Texas and northeastern Mexico.

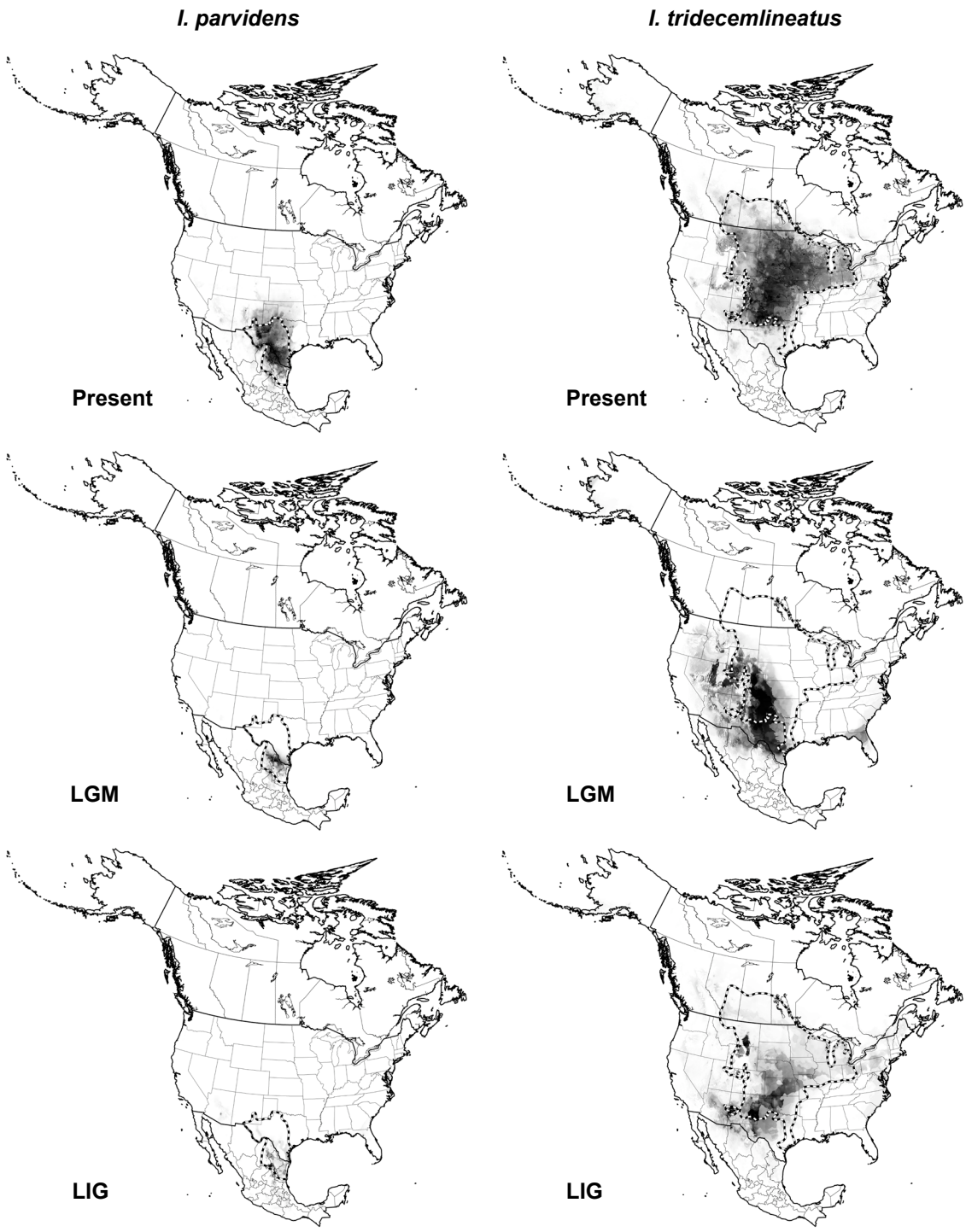

Figure 2. Present (1950–2000) and palaeoclimatic models for *I. parvidens* (left) and *I. tridecemlineatus* (right). Present models are based on 19 BIOCLIM variables and land-use data. Palaeoclimate models are from the last glacial maximum (LGM) and last inter-glacial (LIG). Shading indicates likelihood of occurrence, with the darkest shade being highest. Current known distributions of each species are outlined with a hashed line.

Table 2. Summary of statistics for measuring niche overlap. The statistics used include Schoener's *D* (Schoener 1968), the *I* statistic based on Hellinger distance (Warren et al. 2008), relative rank (RR—Warren and Seifert 2011), and Pearson's correlation (*r*).

	D	*I*	RR	*r*
Present	0.18979	0.42809	0.82078	0.18069
LGM	0.06545	0.21409	0.81974	0.05331

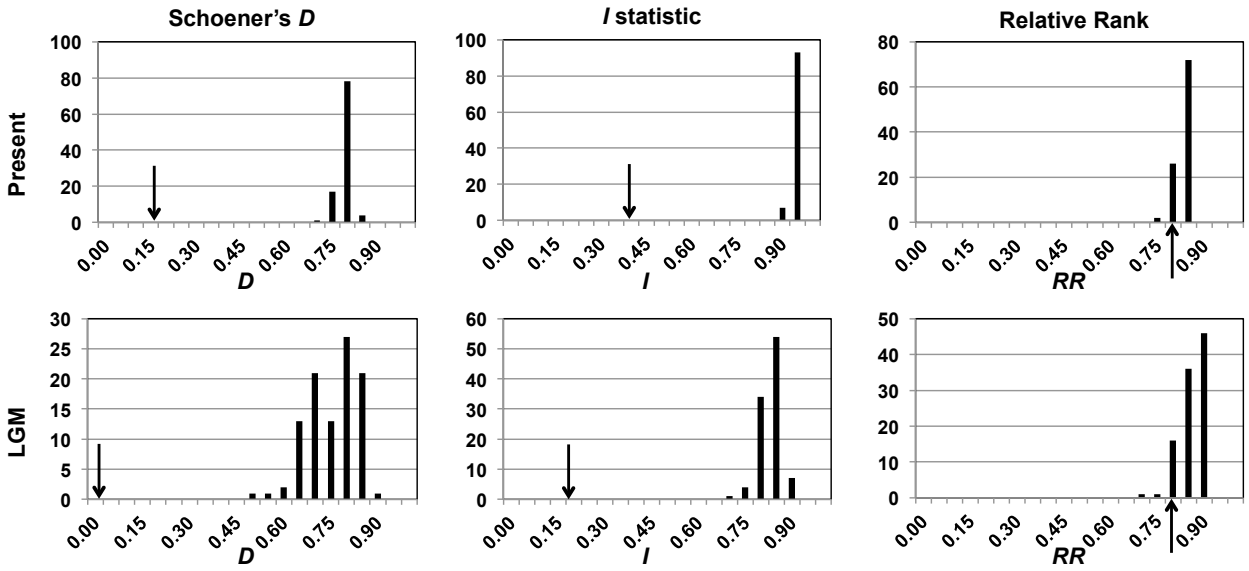

Figure 3. Results of niche-equivalency tests for *I. parvidens* and *I. tridecemlineatus*. Test statistics used are Schoener's *D* (Schoener 1968), the *I* statistic based on Hellinger distance (Warren et al. 2008), and relative rank (RR—Warren and Seifert 2011). Arrows indicate actual values calculated for each statistic using the niche-identity test in ENMTools 1.3 (Warren et al. 2010).

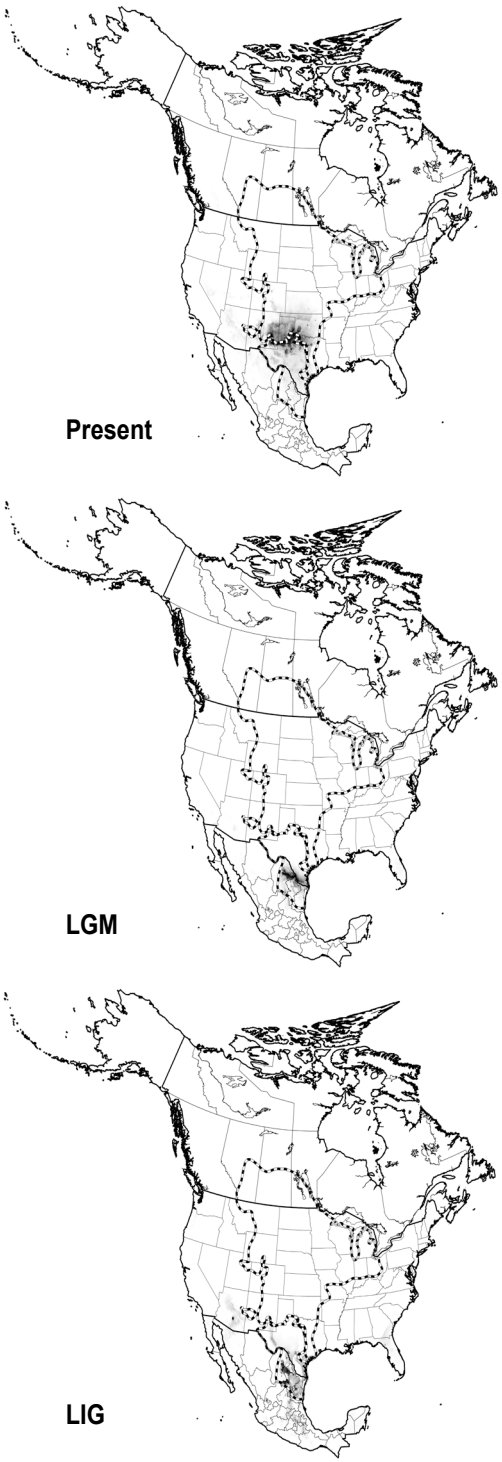

Figure 4. Predicted areas of overlap between *I. parvidens* and *I. tridecemlineatus* during the present (1950–2000), last glacial maximum (LGM), and last inter-glacial (LIG). Shading indicates the likelihood of occurrence, with the darkest shade being highest. Current known distributions of each species are outlined with a hashed line.

DISCUSSION

This study provides a comprehensive analysis of the influence of the environmental factors associated with the ecological niches of *I. parvidens* and *I. tridecemlineatus*. Results suggest that contemporary hybridization may be a product of the changes in modern climate and human-induced modifications to the natural habitat that led to the recent contact between these two species. However, the relative contributions of climate change or habitat modification is not obvious. In models developed with climate and land-use data, land use had the strongest influence on niche models produced for both *I. parvidens* and *I. tridecemlineatus*. In particular, land-use categories that had the most influence on each species were primarily those that were the result of anthropogenic changes to the environment (e.g., disturbed areas, urban and developed), supporting the hypothesis that contemporary hybridization is the result of human influence (Cothran 1982; Stangl et al. 2012).

This result is not without support, as both *I. parvidens* (Edwards 1946; Schmidly 1977) and *I. tridecemlineatus* (Jones 1964) have become habituated to modified landscapes. Additional "natural" categories were important components to the models of each species' modern distribution. For example, *I. parvidens* inhabits mesquite grasslands of Texas and northern Mexico (Young and Jones 1982), and models indicated such by specifying grasslands and shrublands as important variables. Models indicated that herbaceous wetlands were important to *I. parvidens* as well. Although this land-use category seems irrelevant to a semi-arid grassland species, *I. parvidens* thrives in well-irrigated areas (both natural and man-made). Similar results are shown for *I. tridecemlineatus*, as it is a well-known grassland inhabitant (Streubel and Fitzgerald 1978). Whether or not the predictions of these categories are a product of the localities selected in this study or collection bias is not known; however, models were produced in an unbiased manner and are a reflection of the known biology of each species (Streubel and Fitzgerald 1978; Young and Jones 1982; Schmidly and Bradley 2016).

Despite the apparent association of land use with the distribution of *I. parvidens* and *I. tridecemlineatus* and the implied effect of anthropogenic changes to these species' modern distributions, these analyses suggested that climate played an important role in determining the distribution of each species and potentially played a role in contemporary hybridization. Both present climate and palaeoclimatic models were able to demonstrate that this has been the case at least since the LIG. Models developed for the present climate performed well without the land-use dataset, whereas models for the land-use data did not perform well without climatic data and overpredicted the distribution of each species (see Table 1). In comparison, the AUC values for the climate only data indicated that the models had greater predicting power than random chance, whereas the land-use only models had AUC values indicating a predictive power lower than random chance. This result could be a product of the number of variables used to develop the climate-only model compared to the land-use only model. Climate data are described by continuous variables rather than categorical variables like those of the land-use data; therefore, the influence of the presence of a single specimen-occurrence record could have dramatic effects on the predicted ecological niche of a particular species. In addition, BIOCLIM variables are known to be highly correlated and occasionally are removed systematically (e.g., using correlation tests or principal component analyses) to minimize overfitting of the model (Rutishauser et al. 2012; Sede et al. 2012). Consequently, the correlations among the BIOCLIM variables might have reduced the ability of the jackknifing procedure to distinguish the importance of the climate variables relative to land use. However, test runs using different combinations of the least correlated BIOCLIM variables ($r < \pm 0.5$) did not alter the results of the jackknifing analysis, and land use systematically had the greatest influence on other variables. Although this suggests that land use is more important than climate in predicting current species' distributions, overprediction was still the highest for land-use only models in comparison to climate-only models no matter the number of BIOCLIM variables used.

Palaeoclimate models (Fig. 2) further support the importance of climate in shaping distributions of both species and suggest that their distributions have changed drastically over the course of recent time.

These models predict a reduction in the distribution of *I. parvidens* during the LIG, which expanded slightly during the LGM and even more so during the present time. Similar results were seen for *I. tridecemlineatus*; although, the LGM and LIG reductions in distribution for *I. tridecemlineatus* might have contributed to the displacement of *I. parvidens* from areas currently occupied by that species. These results are supported by divergence estimates from the mitochondrial *Cyt*b gene (Thompson et al. 2013). Increased within-species (or population-level) divergence for both species occurred approximately 0.15 to 0.25 mya, corresponding to the onset of the LIG. Recession of continental glaciers northward would have allowed both species to expand into territories previously unavailable during the Illinoian glacial period (Thompson et al. 2013), thereby increasing the opportunity for population expansion and subsequent divergence.

Although palaeoclimatic models support the contribution of climate change to distributions of both species over time (Thompson et al. 2013), they do not provide evidence as to the underlying cause of contemporary hybridization. In both LGM and LIG models, there was little overlap between the two species' distributions (Fig. 4); however, in present models, the extent of overlap is much broader. Despite the species' distributions being significantly different (Fig. 4), the difference between the models produced for each species was greater for LGM than for the present, implying that niche overlap and the corresponding distributional overlap has been greater during the present. This is reiterated in estimates of the RR statistic as comparisons of both present and LGM models indicated that suitable habitat for both species was not significantly different in areas of overlap. In areas of overlap, this would suggest that both species could co-occur in these predicted areas of overlap, increasing opportunities for hybridization and gene flow. Therefore, the lack of distributional overlap between both species during the LGM and LIG indirectly supports the hypothesis of an ancient hybridization event prior to 140,000 years BP (Fig. 4), enabling mitochondrial capture to occur and persist because of lack of gene flow between these species (Thompson et al. 2013). This would support the hypothesis that secondary contact is a recent phenomenon in these taxa (Cothran 1982; Stangl et al. 2012; Thompson et al. 2015).

Given these results, it is likely that modern distributional changes for both species are a result of climate, but that anthropogenic changes to the environment are a contributing factor (Vicente et al. 2010). Vicente et al. (2010) demonstrated that climate was the primary contributing factor for model prediction in invasive plant species; however, other variables such as land use were important in determining the structure of species' distributions. Therefore, in areas where climate predicted a strong presence of a particular species, it was exaggerated by favourable land-use categories (Vicente et al. 2010). Under this hypothesis, both *I. parvidens* and *I. tridecemlineatus* might have eventually come into contact independent of any anthropogenic influences (Thompson et al. 2015), but as a result of the drastic changes to native habitat over the course of the last half of the 20th century, that timeline was reduced dramatically, leading to redevelopment of areas of hybridization because of secondary contact (Cothran 1982; Stangl et al. 2012). Recent secondary contact has been driven primarily by the northward expansion of *I. parvidens*, which has enlarged its distribution locally since the LGM. In addition, the present model for *I. parvidens* overpredicted the distribution of the species primarily to the north, indicating its potential to inhabit more northern territories (Stangl et al. 2012).

In conclusion, this study is one of the first to address both the present and historic environmental factors associated with hybrid zone formation and structure. Some have attempted to identify environmental factors associated with modern hybridizing taxa (Gaubert et al. 2006; Martínez-Freiría et al. 2008; Chatfield et al. 2010; Schukman et al. 2011; Hilbish et al. 2012; Taylor et al. 2015; Zhao et al. 2016); however, few studies have given much consideration to the historical component to the formation and structure of hybrid zones (Hewitt 2011). Although museum occurrence records are available across multiple decades for many taxa, the majority of these records (and corresponding genetic data) are limited to the recent past. Therefore, misinterpretation of the formation, maintenance, and future of a hybrid zone can occur as a result of the limited ability to view the evolutionary history of species (Hewitt 2011). However, this does not appear to be the case for *I. parvidens* and *I. tridecemlineatus*, as detailed records have existed from the mid-1800s (Marcy 1856; Baird 1857) through the last century

(Bailey 1905; Blair 1954; Dalquest 1968), enabling researchers to track recent expansions of these two taxa (Cothran 1982; Stangl et al. 2012). Even though these records have added to the understanding of the environmental factors contributing to contemporary hybridization, the influence of climate change on each species' distribution would have been missed. With the future development of palaeoclimate datasets and the continued development of computational resources, it will be possible to more thoroughly address the environment's effect on species' evolution history.

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LITERATURE CITED

Bailey, V. 1905. Biological survey of Texas. North American Fauna 25:1–222.

Baird, S.F. 1857. General report upon the zoology of the Pacific Railroad routs, part I—mammals. Explorations and surveys for a railroad route from the Mississippi River to the Pacific Ocean. War Department, Washington, D. C.

Blair, W.F. 1954. Mammals of the mesquite plains biotic district in Texas and Oklahoma. Texas Journal of the Science 6: 235–264.

Bracannot, P., et al. 2007. Results of PMIP2 coupled simulations of the Mid-Holocene and Last Glacial Maximum – Part 1: experiments and large-scale features. Climate of the Past 3:261–277.

Carstens, B.C. and C. L. Richards. 2007. Integrating coalescent and ecological niche modeling in comparative phylogeography. Evolution 61:1439–1454.

Chatfield, M. W. H., K. H. Kozak, B. M. Fitzpatrick and P. K. Tucker. 2010. Patterns of differential introgression in a salamander hybrid zone: inferences from genetic data and ecological niche modelling. Molecular Ecology 19:4265–4282.

Choate, L. L. 1997. The mammals of the Llano Estacado. Special Publications of the Museum, Texas Tech University 40:1–240.

Cothran, E. G. 1982. Karyotypic, morphologic, and genic differentiation of hybridizing ground squirrels (*Spermophilus mexicanus* and *Spermophilus tridecemlineatus*). Ph.D. thesis, University of Oklahoma, Norman.

Dale, V. H. 1997. The relationship between land-use change and climate change. Ecological Applications 7:753–769.

Dalquest, W. W. 1968. Mammals of north-central Texas. The Southwestern Naturalist 13,:13–22.

Edwards, R. L. 1946. Some notes on the life hsitory of the Mexican ground squirrel in Texas. Journal of Mammalogy 27:105–121.

Elith, J. 2002. Quantitative methods for modeling species habitat: comparative performance and an application to Australian plants. Pp. 39–58 in Quantitative methods for conservation biology (S. Ferson and M. Burgman, eds.), Springer, New York.

Feeley, K. J., and M. R. Silman. 2011. Keep collecting: accurate species distribution modelling requires more collections than previously thought. Diversity and Distributions 17:1132–1140.

Fløjgaard, C., S. Normand, F. Skov and J.-C. Svenning. 2009. Ice age distributions of European small mammals: insights from species distribution modelling. Journal of Biogeography 36:1152–163.

Gaubert, P., M. Papeş, and A. T. Peterson. 2006. Natural History collections and the conservation of poorly known taxa: ecological niche modeling in central African rain-

forest genets (*Genetta* spp.). Biological Conservation 130:106–117.

Graham, C. H., S. R. Ron, J. C. Santos, C. J. Schneider, and C. Moritz. 2004. Integrating phylogenetics and environmental niche models to explore speciation mechanisms in dendrobatid frogs. Evolution 58:1781–1793.

Grinnell, J. 1917. Field tests of theories concerning distributional control. American Naturalist 51:115–128.

Helgen, K. M., F. R. Cole, L. E. Helgen, and D. E. Wilson. 2009. Generic revision in the Holarctic ground squirrel genus *Spermophilus*. Journal of Mammalogy 90:270–305.

Hewitt, G. M. 2011. Quaternary phylogeography: the roots of hybrid zones. Genetica 139:617–638.

Hilbish, T. J., F. P. Lima, P. M. Brannock, R. K. Fly, R. L. Rognstad, and D. S. Wethey. 2012. Change and stasis in marine hybrid zones in response to climate warming. Journal of Biogeography 39:676–687.

Hjimans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25:1965–1978.

Hope, A. G., et al. 2016. Revision of widespread red squirrels (genus: *Tamiasciurus*) highlights the complexity of speciation within North American forests. Molecular Phylogenetics and Evolution 100:170-182.

Hope, A. G., N. Takebayashi, K. E. Galbreath, S. L. Talbot, and J. A. Cook. 2013. Temporal, spatial, and ecological dynamics of speciation among amphi-Beringian small mammals. Journal of Biogeography 40:415-429.

Hutchinson, G. E. 1959. Homage to Santa Rosalia, or why are there so many kinds of animals? American Naturalist 93:145–159.

Jones, J. K., Jr. 1964. Distribution and taxonomy of mammals of Nebraska. University of Kansas Publications, Museum of Natural History 16:1–356.

Kalkvik, H. M., I. J. Stout, T. J. Doonan, and C. L. Parkinson. 2012. Investigating niche and lineage diversification in widely distributed taxa: phylogeography and ecological niche modeling of the *Peromyscus maniculatus* species group. Ecography 35:54–64.

Kozak, K. H., and J. J. Wiens. 2006. Does niche conservatism promote speciation? A case study in North American salamanders. Evolution 60:2604–2621.

Latifovic, R., Z.-L. Zhu, J. Cihlar, and C. Giri. 2002. Land cover of North America 2000. Natural Resources Canada, Canada Centre for Remote Sensing, U.S. Geological Survey EROS Data Centre.

López-Alvarez, D., et al. 2015. Environmental niche variation and evolutionary diversification of the *Brachypodium distachyon* grass complex species in their native circum-Mediterranean range. American Journal of Botany 102:1073–1088.

M'Gonigle, L. K., and R. G. FitzJohn. 2009. Assortative mating and spatial structure in hybrid zones. Evolution 64:444–455.

Mahmood, R., S. A. Foster, T. Keeling, K. G. Hubbard, C. Carlson, and R. Leeper. 2006. Impacts of irrigation on 20th century temperature in the northern Great Plains. Global and Planetary Change 54:1–18.

Marcy, R. B. 1856. Report of an expedition to the sources of the Brazos and Big Wichita rivers, during the summer of 1854. 34th Congress, Executive Document 60:1–48.

Martínez-Freiría, F., N. Sillero, M. Lizana, and J. C. Brito. 2008. GIS-based niche models identify environmental correlates sustaining a contact zone between three species of European vipers. Diversity and Distributions 14:452–461.

McLean, B. S., et al. 2015. Natural history collections-based research: progress, promise, and best practices. Journal of Mammalogy 97:287-297.

Merow, C., M. J. Smith, and J. A. Silander, Jr. 2013. A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. Ecography 36:1058-1069.

Otto-Bliesner, B. L., et al. 2006. Simulating Arctic climate warmth and icefield tretreat in the last interglaciation. Science 311:1751–1753.

Peterson, A. T. 2003. Predicting the geography of species' invasions via ecological niche modeling. Quarterly Review of Biology 78:419–433.

Peterson, A. T., J. Soberon, and V. Sanchez-Cordero. 1999. Conservatism of ecological niches in evolutionary time. Science 285:1265–1267.

Phillips, S. J., R. P. Anderson, and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190:231–259.

Phillips, S. J., and M. Dudík. 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography 31:161–175.

Phillips, S. J., M. Dudik, and R. E. Schapire. 2004. A maximum entropy approach to species distribution modeling. Pp. 655–662 in Proceedings of the 21st International Conference on Machine Learning, ACM Press, New York.

Reddy S., and L. M. Davalos. 2003. Geographical sampling bias and its implications for conservation priorities in Africa. Journal of Biogeography 30:1719–1727.

Rutishauser, M. D., F. Bontandina, V. Braunisch, S. Ashrafi, and R. Arlettaz. 2012. The challenge posed by newly discovered cryptic species: disentangling the environmental niches of long-eared bats. Diversity and Distributions 18:1107–1119.

Schindel, D. E., and J. A. Cook. 2018. The next generation of natural history collections. PLoS Biology 16:e2006125.

Schmidly, D. J. 1977. The mammals of the Trans-Pecos Texas. Texas A & M University Press, College Station.

Schmidly, D. J., and R. D. Bradley. 2016. The mammals of Texas. University of Texas Press, Austin.

Schoener, T. W. 1968. *Anolis* lizards of Bimini: resource partitioning in a complex fauna. Ecology 49:704–726.

Schukman, J. M., A. Lira-Noriega, and A. T. Peterson. 2011. Multiscalar ecological characterization of Say's and Eastern phoebes and their zone of contaxt in the Great Plains. Condor 113:372-384.

Sede, S. M., M. V. Nicola, R. Pozner, and L. A. Johnson. 2012. Phylogeography and palaeodistribution modelling in the Patagonian stepp: the case of *Mulinum spinosum* (Apiaceae). Journal of Biogeography 39:1041–1057.

Shurtliff, Q. R. 2011. Mammalian hybrid zones: a review. Mammal Review 43:1-21.

Smith, B. E., M. K. Johnston, and R. L. Lücking. 2016. From GenBank to GBIF: phylogeny-based predictive niche modeling test accuracy of taxonomic identifications in large occurrence data repositories. PLoS ONE 11:e0151232.

Soberón, J., and A. T. Peterson. 2005. Interpretation of models of fundamental ecological niches and species' distributional areas. Biodiversity Informatics 2:1–10.

Stangl, F. B., Jr., A. L. Snook, L. A. Harmon, and D. R. Mills. 2012. Historical zoogeography and current status of hybridizing ground squirrels (*Ictidomys parvidens* and *I. tridecemlineatus)* on the southern plains of Texas. Occasional Papers of the Museum, Texas Tech University 314:1–16.

Stein, B. R., and J. Wieczorek. 2004. Mammals of the world: MaNIS as an example of data integration in a distributed network environment. Biodiversity Informatics 1:14–22.

Streubel, D. P., and J. P. Fitzgerald. 1978. *Spermophilus tridecemlineatus*. Mammalian Species 103:1–5.

Swets, J. 1988. Measuring the accuracy of diagnostic systems. Science 240:1285–1293.

Taylor, S. A., E. L. Larson, R. G. Harrison. 2015. Hybrid zones: windows on climate change. Trends in Ecology and Evolution 30:398–406.

Thompson, C. W., F. A. Anwarali Khan, F. B. Stangl, Jr., R. J. Baker, and R. D. Bradley. 2013. Multi-locus analyses indicate a mosaic hybrid zone in ground squirrels (genus *Ictidomys*). Ecology and Evolution 3:4448–4460.

Thompson, C. W., F. B. Stangl, Jr., and R. D. Bradley. 2015. Ancient hybridization and subsequent mitochondrial capture in ground squirrels (genus *Ictidomys*). Occasional Papers of the Museum, Texas Tech University 331:1–24.

Vega, R., C. Fløjgaard, A. Lira-Noriega, Y. Nakazawa, J.-C. Svenning, and J. B. Searle. 2010. Northern glacial refugia for the pygmy shrew *Sorex minutus* in Europe revealed by phylogeographic analyses and species distribution modelling. Ecography 33:260–271.

Vicinte, J., P. Alves, C. Randin, A. Guisan, and J. Honrado. 2010. What drives invisibility? A multi-model inference test and spatial modelling of alien plant species richness patterns in northern Portugal. Ecography 33:1081–1092.

Waltari, E., and M. J. Hickerson. 2013. Late Pleistocene species distribution modelling of North Atlantic intertidal invertebrates. Journal of Biogeography 40:249–260.

Warren, D. L., R. E. Glor, and M. Turelli. 2008. Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. Evolution 62:2868–2883.

Warren, D. L., R. E. Glor, and M. Turelli. 2010. ENMTools: a toolbox for comparative studies of environmental niche models. Ecography 33:607–611.

Warren, D. L., and S. N. Seifert. 2011. Ecological niche modeling in Maxent: the importance of model complexity and the performance of model-selection criteria. Ecological Applications 21:335–342.

Young, C. J., and J. K. Jones, Jr. 1982. *Spermophilus mexicanus*. Mammalian Species 164:1–4.

Zhao, J.-L., P. F. Gugger, Y.-M. Xia, and Q.-J. Li. 2016. Ecological divergence of two closely related Roscoea species associated with late Quaternary climate change. Journal of Biogeography 43:1990–2001.

Zimmerman, E. G., and E. G. Cothran. 1976. Hybridization in the Mexican and 13-lined group squirrels, *Spermophilus mexicanus* and *Spermophilus tridecemlineatus*. Experientia 32:704–706.

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APPENDIX

Localities of catalogued voucher specimens used for ecological niche modeling analyses. Abbreviations are as follows: CAS = California Academy of Sciences; CRCM = Charles R. Conner Museum, Washington State University; CU = University of Colorado Museum of Natural History; DMNS = Denver Museum of Nature and Science; KU = University of Kansas Biodiversity Institute; LACM = Los Angeles County Museum of Natural History; LSUMZ = Louisiana State University Museum of Natural Science; MSB = Museum of Southwestern Biology; MVZ = Museum of Vertebrate Zoology at Berkeley; NMMNH = New Mexico Museum of Natural History; ROM = Royal Ontario Museum; PSM = James R. Slater Museum; TCWC = Texas Cooperative Wildlife Collection; TTU = Museum of Texas Tech University; UAM = University of Alaska Museum; UMMZ = University of Michigan Museum of Zoology; UMNH = Natural History Museum of Utah; UNSM = University of Nebraska State Museum; USNM = National Museum of Natural History, Smithsonian Institution; and UWBM = University of Washington Burke Museum.

Catalog #	Country	State	Latitude	Longitude
		Ictidomys parvidens		
KU35723	Mexico	Coahuila	29.22000	-101.56000
KU35724	Mexico	Coahuila	26.97000	-101.45000
KU48442	Mexico	Coahuila	28.39000	-100.60000
KU55466	Mexico	Coahuila	25.23000	-101.55000
KU56497	Mexico	Coahuila	28.19000	-101.55000
KU67568	Mexico	Coahuila	25.44000	-102.18000
NMMNH3679	Mexico	Coahuila	27.34500	-102.42000
KU48445	Mexico	Nuevo Leon	26.91000	-100.17000

Catalog #	Country	State	Latitude	Longitude
KU55482	Mexico	Nuevo Leon	25.95000	-100.16000
MSB14575	Mexico	Nuevo Leon	25.92609	-100.66798
KU55484	Mexico	Tamaulipas	23.76000	-98.20000
KU88451	Mexico	Tamaulipas	24.84000	-98.15000
KU131086	United States	New Mexico	33.40000	-104.52000
MSB146395	United States	New Mexico	32.55000	-104.03333
MSB191494	United States	New Mexico	33.04847	-104.13574
MSB191497	United States	New Mexico	32.06187	-103.25380
MSB65073	United States	New Mexico	32.18282	-104.51049
ROM87091	United States	New Mexico	32.82513	-104.78144
TTU35812	United States	New Mexico	32.70250	-103.13556
KU149776	United States	Texas	29.31000	-100.48000
KU51692	United States	Texas	27.98000	-99.93000
LSUMZ11183	United States	Texas	28.27717	-98.33986
MSB104003	United States	Texas	27.53545	-99.36095
MSB191509	United States	Texas	31.23237	-102.87943
MSB191511	United States	Texas	31.42844	-103.45885
MSB191514	United States	Texas	29.68438	-101.17344
MSB57449	United States	Texas	27.80500	-98.23800
MSB59364	United States	Texas	29.20470	-98.25560
PSM29035	United States	Texas	25.92400	-97.23800
TCWC26879	United States	Texas	30.94047	-103.74474
TCWC28574	United States	Texas	30.89248	-102.49966
TCWC29709	United States	Texas	27.73266	-98.83685
TCWC30007	United States	Texas	29.10722	-99.78618
TCWC36867	United States	Texas	30.14206	-102.39417
TCWC39500	United States	Texas	32.46932	-98.69254
TCWC43553	United States	Texas	26.90744	-99.25546
TCWC43607	United States	Texas	28.52166	-99.83558
TCWC43609	United States	Texas	28.29647	-99.38403
TCWC50404	United States	Texas	29.23294	-98.78012
TTU10237	United States	Texas	31.63600	-101.47000
TTU10279	United States	Texas	32.28300	-100.72400

Catalog #	Country	State	Latitude	Longitude
TTU2616	United States	Texas	30.34400	-103.66800
TTU35813	United States	Texas	30.26700	-97.74300
TTU3705	United States	Texas	31.77900	-100.69100
TTU374	United States	Texas	32.52200	-101.71300
TTU375	United States	Texas	31.79100	-99.36800
TTU40789	United States	Texas	33.58400	-99.73100
TTU44448	United States	Texas	31.40600	-102.33400
TTU44454	United States	Texas	30.57200	-100.64400
TTU47685	United States	Texas	30.44100	-101.81500
TTU48212	United States	Texas	33.98300	-100.04200
TTU54357	United States	Texas	33.90000	-100.66000
TTU56166	United States	Texas	31.88400	-102.71700
TTU56787	United States	Texas	32.33000	-102.55300
TTU56809	United States	Texas	33.22200	-101.43500
TTU57836	United States	Texas	30.28500	-98.87000
TTU57847	United States	Texas	30.77600	-99.25400
TTU59326	United States	Texas	32.69600	-100.15100
TTU63412	United States	Texas	31.27900	-100.80100
TTU6690	United States	Texas	33.16300	-100.84100
TTU76654	United States	Texas	30.45808	-99.78350
USNM138259	United States	Texas	28.40103	-97.74820
		Ictidomys tridecemlineatus		
MSB53284	Canada	Manitoba	49.43750	-96.27500
ROM102662	Canada	Manitoba	49.38549	-99.24378
ROM33147	Canada	Manitoba	50.00000	-98.33333
ROM86071	Canada	Manitoba	50.13333	-97.55000
UWBM32956	Canada	Manitoba	50.24583	-99.84167
UWBM32956	Canada	Manitoba	50.24583	-99.84167
ROM118448	Canada	Saskatchewan	52.64131	-108.30860
ROM94625	Canada	Saskatchewan	50.76448	-108.70092
TCWC27018	Canada	Saskatchewan	53.24735	-109.29514
MSB68643	United States	Arizona	34.47530	-109.60530
MSB78043	United States	Arizona	33.91667	-109.34167

Catalog #	Country	State	Latitude	Longitude
CU12095	United States	Colorado	39.99306	-105.03294
DMNS8312	United States	Colorado	39.73333	-103.75000
KU116729	United States	Colorado	39.29000	-102.99000
KU120990	United States	Colorado	39.12000	-104.20000
KU59767	United States	Colorado	37.50000	-105.00000
KU78924	United States	Colorado	40.79000	-105.21000
KU79097	United States	Colorado	40.92000	-103.34000
MSB103489	United States	Colorado	37.57911	-106.14819
MSB105764	United States	Colorado	40.74338	-108.06302
MSB107690	United States	Colorado	40.93334	-108.80654
MSB110618	United States	Colorado	40.87684	-107.24984
MSB112404	United States	Colorado	39.62527	-102.17436
MSB729	United States	Colorado	38.79643	-105.12225
TTU54369	United States	Colorado	38.00544	-105.63512
UMNH27105	United States	Colorado	40.36340	-103.20855
CAS27738	United States	Illinois	40.11661	-88.30132
KU131647	United States	Illinois	41.83000	-87.83000
KU112793	United States	Indiana	39.82000	-84.98000
TTU49333	United States	Indiana	39.57660	-87.39560
USNM313266	United States	Indiana	40.64681	-87.36283
USNM314656	United States	Indiana	40.51934	-85.63755
USNM314657	United States	Indiana	40.05704	-85.51832
USNM314660	United States	Indiana	41.31751	-86.20318
USNM317533	United States	Indiana	40.10793	-87.15975
UWBM73424	United States	Indiana	41.73780	-85.17240
KU104003	United States	Iowa	42.09000	-92.82000
KU107953	United States	Iowa	42.86000	-93.61000
KU107954	United States	Iowa	41.44000	-92.60000
KU107965	United States	Iowa	41.03000	-94.38000
KU116129	United States	Iowa	42.79000	-96.16000
KU116131	United States	Iowa	41.91000	-91.81000
TCWC11404	United States	Iowa	43.15733	-91.59904
TTU11697	United States	Iowa	43.36280	-95.17980

Catalog #	Country	State	Latitude	Longitude
TTU54398	United States	Iowa	42.04520	-93.62030
UMMZ162858	United States	Iowa	42.02741	-96.09630
UNSM15706	United States	Iowa	41.22955	-95.60860
KU100160	United States	Kansas	37.18000	-99.74000
KU102172	United States	Kansas	38.82000	-100.95000
KU113721	United States	Kansas	39.11000	-100.20000
KU135920	United States	Kansas	39.18000	-101.78000
KU160124	United States	Kansas	37.58000	-101.25000
KU160125	United States	Kansas	38.46000	-98.20000
KU160128	United States	Kansas	37.04000	-101.25000
KU160134	United States	Kansas	37.22000	-97.00000
KU160302	United States	Kansas	37.70000	-99.90000
KU38815	United States	Kansas	39.78000	-100.64000
KU38816	United States	Kansas	37.53000	-102.02000
KU45058	United States	Kansas	37.32000	-98.38000
KU73217	United States	Kansas	39.11000	-94.83000
KU98273	United States	Kansas	39.06000	-95.72000
TCWC50423	United States	Kansas	39.05326	-96.76407
TTU49343	United States	Kansas	37.97930	-96.18152
TTU54404	United States	Kansas	38.87330	-99.32980
TTU54425	United States	Kansas	39.39610	-101.04250
UWBM51165	United States	Kansas	39.77750	-98.67078
KU149846	United States	Michigan	42.43000	-83.72000
MSB73228	United States	Michigan	44.72500	-86.12500
TTU2485	United States	Michigan	42.71641	-84.51169
UWBM35241	United States	Michigan	42.20080	-85.58900
KU149850	United States	Minnesota	43.65000	-94.48000
LSUMZ7884	United States	Minnesota	46.61658	-95.96605
MSB90855	United States	Minnesota	46.18500	-94.43370
MVZ181285	United States	Minnesota	47.36540	-94.49500
TTU16700	United States	Minnesota	44.96770	-93.55790
TTU17871	United States	Minnesota	44.31250	-94.46030
TTU17872	United States	Minnesota	44.60890	-95.67390

Catalog #	Country	State	Latitude	Longitude
TTU17874	United States	Minnesota	45.56335	-95.33805
UAM50376	United States	Minnesota	44.20139	-92.64611
UMMZ105206	United States	Minnesota	45.49630	-94.07700
USNM554400	United States	Missouri	40.32451	-94.89506
KU123281	United States	Montana	46.05000	-104.49000
KU42663	United States	Montana	45.75000	-105.11000
PSM7972	United States	Montana	48.50130	-104.50390
KU134085	United States	Nebraska	42.45750	-98.66787
KU146945	United States	Nebraska	40.49000	-96.97000
KU49889	United States	Nebraska	41.60000	-98.92000
KU49892	United States	Nebraska	41.23000	-97.35000
KU73256	United States	Nebraska	42.56000	-100.84000
KU77944	United States	Nebraska	42.93000	-99.92000
MSB124335	United States	Nebraska	41.70267	-102.23403
TTU54426	United States	Nebraska	40.41870	-101.40870
TTU75920	United States	Nebraska	42.07050	-97.98390
UNSM12762	United States	Nebraska	42.70792	-103.84786
UNSM12852	United States	Nebraska	40.56855	-98.63674
UNSM16411	United States	Nebraska	41.60316	-100.75626
UNSM19636	United States	Nebraska	40.72220	-99.81679
UNSM21500	United States	Nebraska	42.68050	-102.46371
UNSM28024	United States	Nebraska	41.07200	-101.35637
UNSM28029	United States	Nebraska	41.84028	-103.49165
KU149835	United States	New Mexico	33.44000	-104.52000
MSB14139	United States	New Mexico	35.03360	-106.05860
MSB23630	United States	New Mexico	36.92360	-105.67030
MSB33018	United States	New Mexico	35.59389	-105.21667
MSB5357	United States	New Mexico	34.23520	-107.95160
MSB55707	United States	New Mexico	34.49960	-108.59000
MSB58788	United States	New Mexico	36.60743	-103.10117
MSB60556	United States	New Mexico	36.75438	-103.88779
MSB64444	United States	New Mexico	36.53333	-105.23333
MSB76233	United States	New Mexico	34.28822	-103.84578

Catalog #	Country	State	Latitude	Longitude
MSB89534	United States	New Mexico	33.64010	-108.46681
TTU58099	United States	New Mexico	34.90972	-104.05889
TTU58715	United States	New Mexico	32.70250	-103.13556
DMNS7783	United States	North Dakota	47.32892	-101.63149
KU108580	United States	North Dakota	47.07000	-98.56000
KU122584	United States	North Dakota	47.33000	-102.68000
MSB16700	United States	North Dakota	48.87850	-97.77790
MSB76277	United States	North Dakota	47.91670	-97.30000
MSB76278	United States	North Dakota	48.05690	-98.92060
MSB78338	United States	North Dakota	48.23000	-101.36000
MSB78339	United States	North Dakota	47.66670	-100.08330
MSB78550	United States	North Dakota	46.91760	-97.60010
MSB78745	United States	North Dakota	46.65060	-100.88360
MSB81881	United States	North Dakota	46.10000	-102.22000
TTU16701	United States	North Dakota	48.87010	-101.26500
TTU30799	United States	North Dakota	47.98830	-104.00010
UMMZ105208	United States	North Dakota	46.87141	-102.38997
KU154136	United States	Ohio	39.47000	-82.91000
LSUMZ15481	United States	Ohio	41.37830	-83.65390
KU41536	United States	Oklahoma	35.52000	-98.70000
LSUMZ13482	United States	Oklahoma	36.87400	-94.87700
UWBM42777	United States	Oklahoma	36.12136	-97.06805
KU100917	United States	South Dakota	45.46000	-103.93000
KU112971	United States	South Dakota	43.16000	-101.50000
KU115827	United States	South Dakota	42.84000	-96.93000
KU128338	United States	South Dakota	43.97000	-101.92000
KU147003	United States	South Dakota	44.46000	-97.15000
KU147010	United States	South Dakota	45.30000	-96.76000
KU153271	United States	South Dakota	45.77000	-98.22000
KU153278	United States	South Dakota	43.31416	-96.72263
KU153281	United States	South Dakota	45.89000	-96.97000
KU41674	United States	South Dakota	44.08000	-99.46000
KU86370	United States	South Dakota	45.58000	-103.14000

Catalog #	Country	State	Latitude	Longitude
TTU25708	United States	South Dakota	44.36430	-100.34120
UNSM26590	United States	South Dakota	43.35324	-97.57594
UNSM26631	United States	South Dakota	43.56818	-100.88454
USNM557714	United States	South Dakota	43.69315	-103.59898
KU139230	United States	Texas	34.98000	-101.88000
KU67108	United States	Texas	33.63000	-97.15000
TCWC25524	United States	Texas	32.74591	-96.99775
TCWC38207	United States	Texas	31.97007	-97.13721
TTU13516	United States	Texas	29.03100	-96.52300
TTU1778	United States	Texas	33.62600	-99.25900
TTU19843	United States	Texas	33.19300	-101.39900
TTU31681	United States	Texas	36.42700	-101.81600
TTU35052	United States	Texas	33.55500	-102.27900
TTU36992	United States	Texas	35.39207	-100.59940
TTU39699	United States	Texas	34.30100	-101.82800
TTU42040	United States	Texas	33.90000	-100.66000
TTU42733	United States	Texas	36.06400	-102.56500
TTU4588	United States	Texas	34.50500	-100.18000
TTU47158	United States	Texas	36.39100	-100.82600
TTU48225	United States	Texas	33.98500	-99.72400
TTU48227	United States	Texas	33.94900	-97.62400
TTU54440	United States	Texas	33.79600	-101.34300
TTU54516	United States	Texas	33.19300	-102.80900
TTU55918	United States	Texas	34.17400	-102.90300
TTU57978	United States	Texas	34.72500	-102.81500
TTU58341	United States	Texas	35.00800	-101.21700
TTU7052	United States	Texas	35.63800	-101.93100
UWBM42778	United States	Texas	33.84900	-98.56900
TTU54522	United States	Utah	40.45556	-109.52806
UMNH27948	United States	Utah	40.15210	-110.06092
CRCM59-410	United States	Wisconsin	44.97886	-92.43575
KU102239	United States	Wisconsin	42.77000	-88.40000
LACM28238	United States	Wisconsin	45.86250	-92.62360

Catalog #	Country	State	Latitude	Longitude
TTU49344	United States	Wisconsin	42.86000	-90.14032
UAM113814	United States	Wisconsin	46.20958	-91.97760
UAM113815	United States	Wisconsin	44.34155	-90.16430
UAM1703	United States	Wisconsin	46.10000	-91.14000
DMNS11030	United States	Wyoming	44.74410	-106.97270
DMNS8692	United States	Wyoming	41.52300	-109.46945
DMNS8693	United States	Wyoming	42.25640	-107.23920
DMNS8867	United States	Wyoming	41.59181	-110.22933
KU41679	United States	Wyoming	43.00000	-104.37000
KU87718	United States	Wyoming	41.73000	-108.05000
KU91094	United States	Wyoming	42.07000	-104.34000
MSB107633	United States	Wyoming	41.56168	-105.69536
MSB111547	United States	Wyoming	41.35102	-108.40308
MSB118318	United States	Wyoming	43.39308	-106.96174
MSB118319	United States	Wyoming	43.29490	-105.34481
MSB123264	United States	Wyoming	42.11734	-106.51740
TTU54528	United States	Wyoming	44.40239	-104.37528

Suggested citation format:

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The Neotropical Variegated Squirrel, *Sciurus variegatoides* (Rodentia: Sciuridae) in Nicaragua, with the Description of a New Subspecies

Hugh H. Genoways and Robert M. Timm

Abstract

The Neotropical variegated squirrel, *Sciurus variegatoides*, is represented in Nicaragua by five known subspecies—*adolphei*, *belti*, *boothiae*, *dorsalis*, and *underwoodi*. Analyses of morphometrics, color, and color patterns of 394 specimens from throughout the country and all available literature support the retention of these subspecies, but also reveal the presence of a sixth population of these squirrels, which is worthy of description and recognition as a new subspecies. This new subspecies is confined to Isla de Ometepe in Lago de Nicaragua. Variegated squirrels on Ometepe are on average the smallest variegated squirrels in the country in most cranial measures; however, in postorbital breadth, the island population averages larger than the samples from the surrounding mainland. This island population is the smallest and most distinctive of any population of variegated squirrels from throughout the species' geographic range. The baculum is distinct in size, shape, and angle of the disc. Ometepe variegated squirrels have a distinctive albeit a highly variable color pattern. Although there are some color differences between the populations found on the north island (Volcán Concepción) and the south island (Volcán Maderas), all specimens from Ometepe are regarded as belonging to a single subspecies because there are no discernable differences in cranial measures. Throughout Nicaragua's Pacific lowland dry tropical forest region, there is no evidence of integration between *S. variegatoides dorsalis* with *S. v. adolphei*, the subspecies occurring to the north; between *S. v. dorsalis* and *S. v. underwoodi*, the subspecies occurring to the east and northeast; or between *S. v. adolphei* and *S. v. underwoodi* in the northwest. The Central Depression region of Nicaragua appears to be a significant geographic barrier to gene flow between taxa. In the Central Highlands, there are regions of intergradation between *S. v. belti* and *S. v. underwoodi* and between *S. v. belti* and *S. v. boothiae*. The taxa of *S. variegatoides* found in the country are described and mapped by critically evaluating the historical collecting sites, all published literature, and data presented herein.

Key words: biodiversity, biogeography, Central America, color pattern variation, geographic variation, Mammalia, morphology, Neotropics, subspecies novum, taxonomy

Introduction

The Neotropical variegated squirrel *Sciurus variegatoides* Ogilby, 1839 (Rodentia: Sciuridae) is the largest and most widely distributed of the squirrels found in Central America. Variegated squirrels occur from southwestern Chiapas through central Panama and range in elevation from sea level to 2,600 m; however, they are most abundant at elevations below 1,500 m. *Sciurus variegatoides* occupies a wide array of habitats, including lowland dry and wet forests, as well as premontane and montane forests, but it favors second-growth, disturbed, and heterogeneous habitats where it tends to nest in the largest trees available.

This is often the most conspicuous squirrel in much of the Central American forests. Because of its size and at times abundance, variegated squirrels play important ecological roles in Neotropical ecosystems as they feeds on seeds, fruits, fungi, insects, and smaller

vertebrates. It can be the principal handler of seeds, especially as seed predators of palms, but can also increase seed dispersal and germination by caching. This species can be quite abundant locally, especially in agricultural situations. It can do considerable damage feeding on developing bananas (*Musa*, Musaceae), coconuts (*Cocos nucifera*, Arecaceae), coffee beans (*Coffea* sp., Rubiaceae), cacao pods (*Theobroma cacao*, Malvaceae), papaya (*Carica papaya*, Caricaceae), and other crops.

Sciurus variegatoides is one of the most variable species of all mammals with respect to color; hence the English common name, variegated squirrel, is especially appropriate. Fifteen subspecies, based primarily on color, currently are recognized (Harris 1937; McPherson 1971; Hall 1981), acknowledging the considerable geographic variation within the species, yet little systematic or ecological information is available for most populations (Best 1995). The species ranges in pelage color from nearly black to steely gray, or grizzled gray, to reds, and to having a broad, black dorsum with white, red, bright orange, or light orange sides and ventrum (Harris 1937; Reid 2009; Thorington et al. 2012).

As currently defined, this broadly distributed and highly variable squirrel has continued to be recognized as a single species, although there is considerable size and color variation among subspecies. Interestingly, there appears to be little, and in some cases no, introgression in color morphs between several of the subspecies; although between some, there is a narrow zone of introgression. There has been no revisionary work since Harris's classic review of the species (Harris 1937). A considerable number of specimens have become available since Harris's work and a re-assessment of distributions and status of several of the taxa are now possible. In the absence of genetic information for these subspecies, and given localities of intergradation between them as documented below, *S. variegatoides* is treated as a single, widespread, variable species herein.

In a recent review of the squirrels of Nicaragua, Medina-Fitoria et al. (2018) reported range extensions for southern flying squirrels (*Glaucomy volans*) and pygmy squirrels (*Microsciurus alfari*) and reviewed the then known distributions of these and *S. granatensis*, *S. richmondi*, and *S. variegatoides*. They provide descriptions and a useful key to the species of sciurids in the country, their conservation status, and color images of each. In their report on the variegated squirrels found on Isla de Ometepe, they provided two images of free-ranging animals demonstrating some of the color variation found in the island population and stated that these squirrels were of uncertain taxonomic status.

Between February 1964 and the spring of 1968, several field parties from University of Kansas led by J. Knox Jones, Jr. and James Dale Smith, collected mammals and their ectoparasites throughout Nicaragua. Herein, these collections are reported along with other specimens housed in museums to re-examine the systematic status of several populations of *S. variegatoides* focusing on the taxa found in Nicaragua. One goal is to build a better understanding of the geographic variation in this species and to re-evaluate the diversity that is currently recognized. The field parties from Kansas were the first to collect mammal specimens at several localities on Isla de Ometepe, the large volcanic island in Lago de Nicaragua. The tree squirrels on Ometepe represent a previously unrecognized taxon, which is described below as part of this species complex.

METHODS AND MATERIALS

A total of 394 specimens of *Sciurus variegatoides* from throughout Nicaragua comprise the basis of this report, with additional specimens examined from throughout the species' range and a review of the published literature to assess distributions, morphology, and color variation. All measurements in the accounts that follow are in millimeters and weights are given in grams (g) or kilograms (kg). Cranial measurements were taken by Genoways with digital calipers accurate to the nearest 0.1 mm as defined by Hall (1981) and include the following: greatest length of skull (GLS), condylobasal length (CBL), zygomatic breadth (ZB), interorbital breadth (IOB), postorbital breadth (POB), mastoid breadth (MB), length of nasals (LN), length of palatal bridge (PB), and length of maxillary toothrow (LTR). External measurements and reproductive data are those recorded on specimen labels by the collector.

Statistical analyses were performed using Minitab (Minitab, Inc., State College, Pennsylvania, USA). There is no secondary sexual dimorphism in size in these squirrels and given the small sample sizes, assessment of sexual dimorphism was not feasible; thus measurements of males and females were pooled in analyses of variation among groups. A number of principal component analyses were undertaken using either individual localities or small geographic areas as units beginning with 27 operational groupings. Individuals deemed intergrades as judged by external color characteristics were not included in morphometric analyses. Based on these results, six groups that represent taxonomic and geographic units were formed for the final principal components analysis. Discriminant function analysis was used to assess morphological differences and to maximize group discrimination (Strauss 2010). See Table 1 (in ANALYSES) for taxon-specific descriptive statistics (mean, standard deviation, and range). Differences among groups were analyzed for the six operational groupings and for pairwise comparisons.

Harris's (1937) color terminology was followed in describing the coloration of these squirrels to provide the user with a standard, uniform set of descriptors for the various colors and because Harris did an outstanding job of elucidating the patterns present.

Specimens reported in the systematics accounts below are housed in the following museums: American Museum of Natural History, New York (AMNH); British Museum (Natural History) London, UK [BM(NH)]; Muséum national d′Histoire naturelle, Paris, France (MNHN); Museum of Texas Tech University, Lubbock (TTU); University of Kansas Museum of Natural History, Lawrence (KU); and National Museum of Natural History, Washington, DC (NMNH). Timm's research on Central American mammals was undertaken with the approval of the University of Kansas Institutional Animal Care and Use Committee.

HISTORICAL REVIEW

The Irish naturalist William Ogilby first made the species *Sciurus variegatoides* known to science in 1839 based on a specimen sent to him by Captain Belcher from the coast of El Salvador (Ogilby 1839). Only three years later, Lesson (1842) reported the first specimen of this species from Nicaragua when he described *Macroxus Adolphei* from El Realejo near Chinandega on the Pacific coast of the country. This taxon, which is now known under the name *Sciurus variegatoides adolphei*, is believed to be confined to the tropical dry forest of extreme northwestern Nicaragua (Genoways and Timm 2005). The other subspecies of Neotropical variegated squirrel originally described from Nicaragua is *Sciurus boothiae belti* Nelson, 1899. Charles W. Richmond (1893:480–481), who collected the holotype on 22 November 1892, described the type locality as follows: "The International Planting Company's plantation, or 'I. P.,' as it is familiarly called, is 50 miles from Bluefields [along Escondido River]. A creek joins the river at this plantation, and affords an excellent means of reaching the heavy forest in the rear." This description places the type locality well within the lowland tropical wet forest that originally covered much of eastern Nicaragua.

John Edward Gray, long-serving botanist and zoologist at the British Museum, described several taxa of Mexican and Central American *Sciurus*, two of which are considered valid subspecies of *S. variegatoides* with geographic ranges extending into Nicaragua. The earliest was *Sciurus richardsoni* Gray, 1842 from Honduras; however, this name is preoccupied by *Sciurus richardsoni* Bachman, 1839, so in 1843 Gray renamed this taxon as *Sciurus boothiae*. He later described *Sciurus dorsalis* Gray, 1849, based on two specimens supposedly from Caracas, Venezuela, but he (Gray 1867) later corrected the type locality to Costa Rica, where (Nelson 1899:74) wrote "specimens from Liberia, Costa Rica, are typical." On this basis, Liberia, Costa Rica, is considered to be the restricted type locality of the strikingly and distinctly colored dry forest squirrel *Sciurus variegatoides dorsalis*.

The first systematic revision of *S. variegatoides* was undertaken by Edward W. Nelson in 1899. Within the squirrels now considered to represent *S. variegatoides*, Nelson recognized six species—*S. adolphei*, *S. boothiae*, *S. goldmani*, *S. managuensis*, *S. thomasi*, and *S. variegatoides*. Nelson (1899:79) treated *dorsalis*

as a subspecies of *S. adolphei* because specimens of *adolphei* appeared to "differ from *S. a. dorsalis* mainly in its darker color." Nelson (1899) described *Sciurus thomasi* as a distinct species because he concluded that specimens from La Carpintera, Costa Rica, represented both *thomasi* and *dorsalis*. On the other hand, Nelson (1899) described the taxon *belti* as a subspecies of *S. boothiae* because "*S. boothiae* grades into *S. b. belti* to the south." Also, Nelson (1899) placed two taxa, currently considered valid subspecies—*rigidus* and *melania*—as junior synonyms of *S. adolphei dorsalis*.

Subsequent to Nelson's revision of the group, Allen (1908) reported 12 specimens of these squirrels from five localities in western and northern Nicaragua. Two years later, Allen (1910) presented information on an additional 25 specimens from seven localities in Nicaragua and summarized the taxonomy of squirrels in these two collections. These specimens were all collected by William B. Richardson, who in many cases used local names for his work sites, which have challenged subsequent researchers to precisely locate (see below). Allen (1910) divided the specimens from Nicaragua into two species—*S. boothiae* and *S. variegatoides*—the former including the nominate subspecies and *belti* and the latter including the nominate subspecies and *adolphei*.

Between 1912 and 1933, five additional taxa, now associated with *S. variegatoides*, were described and named. Only one of these taxa—*underwoodi* Goldman, 1932—has ultimately been associated with squirrels in Nicaragua, with the type locality in Honduras. Two of the new taxa were from Costa Rica—*atrirufus* Harris, 1930 and *austini* Harris, 1933—one from Panama—*helveolus* Goldman, 1912—and the other from El Salvador—*bangsi* Dickey, 1928. Goldman (1912), Dickey (1928), and Harris (1933) used *variegatoides* as the specific epithet for the new taxa and Dickey (1928) placed earlier species names, such as *goldmani*, in the species *S. variegatoides*.

Harris (1937) revised this group of squirrels bringing them all into the species *S. variegatoides*, with 15 recognized subspecies. This is the same basic arrangement, with a few modifications, still used for the Neotropical variegated squirrel today (Hall 1981). Harris mapped the geographic ranges of five subspecies of *S. variegatoides* as including parts of Nicaragua—*adolphei*, *belti*, *boothiae*, *dorsalis*, and *underwoodi*. He considered *S. v. belti* to occupy much of eastern half of the country in the Caribbean Lowlands, an area of tropical wet forests. Two subspecies occurred on the Pacific Coast, with *S. v. dorsalis* west of Lago de Nicaragua along the southwest coast and *S. v. adolphei* along the northwest coast in the Department of Chinandega. These taxa occur in a much drier area than *belti* including much of the arid tropical scrub forests. The last two subspecies—*boothiae* and *underwoodi*—occur in the montane areas of central and northern Nicaragua.

Subsequent to Harris's revision (1937), only two taxonomic changes have been made regarding these squirrels. Hall and Kelson (1952) arranged *S. v. austini* as a junior synonym of *S. v. rigidus* in central Costa Rica. The distinctive populations from two valleys in southwestern Costa Rica separated by the Cordillera de Talamanca from more northern and eastern populations were described as *S. v. loweryi* McPherson, 1971, bringing the number of recognized subspecies back to 15.

HISTORICAL COLLECTING SITES

One of the early professional collectors of bird and mammal specimens in Nicaragua was William B. Richardson. In 1891, Richardson settled in Matagalpa, Nicaragua, to grow coffee, and over the years he collected a number of specimens for museums in England and the United States. The collections of mammals that he made in Nicaragua for the American Museum of Natural History from 1904 to 1908 contained a significant number of Neotropical variegated squirrels. Many of the Nicaraguan collecting localities visited by Richardson were small villages not found on most maps, then or now, or were given as nonspecific geographic features. "He never gave distance and direction from a locality and seldom included the Departamento. His handwriting was not always clear and he sometimes used cryptic abbreviations" (Howell 1993, 2010:3). Because the material collected by Richardson included a number of important scientific specimens, his localities have long been a challenge to researchers (Allen 1908, 1910; Buchanan and Howell 1965; Jones and

Genoways 1970, 1971; Jones and Yates 1983; Jones and Engstrom 1986; Howell 1993, 2010; McCarthy et al. 1999; Rossi et al. 2010).

Because Richardson's squirrel specimens are important to the study of *Sciurus variegatoides* in Nicaragua, the latest thinking on the placement of his relevant collecting locations are provided below.

Departmento de Boaco:

Chontales [probably near Tierra Azul, 30 km NNE Boaco, 12°41'N, 85°30'W; Jones and Genoways 1971; Jones and Engstrom 1986]

Departmento de Chinandega:

Volcán de Chinandega [Rossi et al. 2010 believed this to be the same as San Cristóbal volcanic complex, which is composed of five volcanoes including San Cristóbal (main cone), El Chonco, Moyotepe, Volcán Casita (site visited by a University of Kansas field party), and La Pelona (12°42'N, 87°01'W)]

Volcán Viejo [Volcán El Viejo is an alternative more local name for San Cristóbal]

Departmento de Jinotega:

Peña Blanca [in southern part of the Department of Jinotega at 13°15'N, 85°41'W; Buchanan and Howell 1965:549; Jones and Engstrom 1986]

San Rafael del Norte [13°13'N, 86°07'W on modern maps]

Río Coco [has been one of the most difficult of Richardson's localities to pinpoint, but Howell (1993, 2010) presented a persuasive case for the site to have been located in the vicinity of the village of Santa Cruz on modern maps, with its coordinates being 13°27'N and 85°55'W. The village is on the south side of the Río Coco and thus within Departmento de Jinotega. The birds and mammals collected by Richardson at "Santa Cruz" and "Rio Coco" are from a remarkably varied group of habitats in Nicaragua, including Caribbean slope, humid lowland forest, highland pine forest, humid montane (cloud) forest, and Pacific slope thorn scrub and deciduous forest edge. When Richardson collected some distance away from the village, with no other named place nearby, he appeared to have used only "Rio Coco" as his locality (Howell 1993, 2010). The squirrels appear to have a relationship with the Caribbean slope so probably were from near the river.]

Departmento de Madriz:

San Juan [San Juan de Telpaneca, 13°32'N, 86°17'W, on modern maps]

Departmento de Matagalpa:

Lavala [a misinterpretation of Richardson's spelling of Savala, located at 45 km ENE of Matagalpa; Buchanan and Howell 1965:549; Jones and Genoways 1970]

Matagalpa [12°56'N, 85°55'W on modern maps, but may cover more than one location according to Harris (1937)]

Río Grande [probably on the Río Grande de Matagalpa near the mouth of the Río Upá, 200 m, 13°15'N, 85°41'W; Jones and Genoways 1971; Jones and Engstrom 1986]

Río Tuma [probably near El Tuma on the Río Tuma, 13°08´N, 85°44'W; Rossi et al. 2010]

Sebaco [Sébaco, on modern maps at 12°51'N, 86°06'W in northwestern Departmento de Matagalpa]

Uluce [12°53'N, 85°37'W; Jones and Engstrom 1986]

Vijagua [= Bijagua, a small village near Guasaca, 13°07'N, 85°41'W, about 35 km NE Matagalpa; Buchanan and Howell 1965; Jones and Genoways 1971; Jones and Engstrom 1986]

Departmento de Nueva Segovia:

Jalapa [13°55'N, 86°07'W on modern maps]

Jicaro [13°43'N, 86°08'W on modern maps]

Departmento de Río San Juan:

> Los Sabalos [= Boca de Sabalos, at confluence of Río Sabalos and Río San Juan, 11°03'N, 84°28'W]

In addition to Richardson's localities, three other historic collecting sites in Nicaragua deserve comment. Dr. L. F. H. Birt of Greytown in the southeastern-most part of the country, who was associated with the Nicaragua Canal Company, collected a large number of mammals, reptiles, fishes, and birds in the late 1880s and donated them to the Smithsonian. His collections include a single *S. variegatoides* from Greytown. This specimen came to the National Museum of Natural History prior to 1888 from the Nicaragua Canal Company (True 1889). Greytown has changed names over the years from San Juan del Norte to San Juan de Nicaragua, but is now officially Greytown. Greytown appears on modern maps at 10°55'N, 83°41'W in the Departmento de Río San Juan.

A second site is Escondido River, "50 miles above Bluefields," where a series of Neotropical variegated squirrels was collected by Charles W. Richmond, Curator of Ornithology of the National Museum of Natural History, between August and November of 1892 (Richmond 1893). This site, which is the type locality for *S. v. belti*, was determined by Jones and Genoways (1971) to be the I. P. Plantation, 3 km S, 13 km E Rama currently located in the South Caribbean Coast Autonomous Region of the country.

Harris (1937) reported a specimen of *S. v. belti* from Edén, Departmento de Matagalpa. This specimen, which is deposited in the Carnegie Museum of Natural History, was not examined for this study and this locality is not shown on modern maps of Nicaragua. However, Ulmer (1995) gives a good description of this site and the history of specimens from there. Specimens from Edén were obtained in 1922 by an expedition from the Academy of Natural Sciences of Philadelphia lead by Wharton Huber and J. Fletcher Street. They described Edén as a gold mining town located at 14°00'N, 84°26'W (213 m), which places the site in the North Caribbean Coast Autonomous Region and not in Matagalpa.

PHYSIOGRAPHIC SETTING

Nicaragua is generally divided into three physiographic regions—Pacific, Central Highlands, and Caribbean Lowlands (Taylor 1963). Neotropical variegated squirrels occur in all three regions, and the climate and environment of the individual regions have influenced the variation in these squirrels. The Pacific region features a chain of 40 volcanoes extending from Volcán Cosigüina on the Golfo de Fonseca in the northwest to Volcán Maderas on Isla de Ometepe in the south (Arguello et al. 2018). Those volcanoes northwest of Lago de Managua are collectively known as the Cordillera de los Marrabios and the chain of volcanoes continues in Costa Rica as the Cordilleras de Guanacaste and Tilarán. This line of volcanoes lies just west of a large crustal rift formed by the subduction of the Cocos plate under the Caribbean plate (van Wyk de Vries 1993). This subduction zone forms the Central Depression of Nicaragua, which contains six freshwater lakes, with the largest being Lago de Managua and Lago de Nicaragua (Taylor 1963). Rainfall in this region is highly seasonal, with a marked rainy season from May to August, with the wettest period in July.

The Pacific region extends about 75 km inland from the coast and is characterized by relative flat, low-lying land except for the volcanic peaks. This region has high temperatures, moderate rainfall, and strong seasonal droughts. These low lands are characterized by dry tropical forest and grasslands (Sabogal 1992) or semi-evergreen rainforest (Taylor 1963), with common plants such as bull horn acacia (*Vachellia collinsii* = *Acacia collinsii*), Spanish elm (*Cordia alliodora*), white manjack (*Cordia dentata*), quickstick (*Gliricidia sepium*), and hog plum (*Spondias purpurea*) (Sabogal 1992). Since prehistoric times, the Pacific region has been heavily impacted by human activity, including significant harvests of large, valuable hardwood trees; agriculture—large ranches for raising cattle and mules, and crops primarily cotton, sugar cane, and rice in the lowlands and coffee at higher elevations; and building of communities and cities. The forests on the slopes of the volcanoes are taller because of the cooler temperatures and additional moisture as well as less timber harvest, but there are also treeless areas as a result of volcanic activity and landslides. Some of the larger

trees in these areas are kapok (*Ceiba pentandra*), hog plum, and chelate (*Ficus insipida*). Two subspecies of squirrels occur in this region—*S. v. adolphei* to the north and *S. v. dorsalis* to the south.

Lying in the Pacific region is Isla de Ometepe, which is situated in Lago de Nicaragua and comprised of two stratovolcanoes. Volcán Concepción (1,600 m), the larger of the two, is the northern island and connected to Volcán Maderas (1,400 m) by a narrow, low elevation isthmus (Istmo de Istián). Concepción is an active volcano that has erupted recently. Maderas, southernmost and smaller of the two, has not been active in historical times last erupting perhaps 3,000 years ago. Isla de Ometepe is home to a previously undescribed population of *S. variegatoides* described below.

The Central Highlands is a triangular-shaped area extending south from Honduras, that consists of three ancient major mountain ranges and several minor ones. These rugged mountains historically were covered in forest, but significant clearing has taken place. Dividing these mountains are deep valleys with rivers that generally flow to the east. The western slopes of these mountains are drier than the eastern slopes, with a flora that Taylor (1963) called Seasonal Evergreen Rainforest, but little of this mature forest remains. Regenerating forests have such trees as papelillo (*Miconia argentea*), pink shower tree (*Cassia grandis*), and aguacatillo (*Nectandra salicifolia*). The moister eastern slopes were covered in forests that Taylor (1963) classified as Lower Montane Rainforest, which grades toward the Caribbean Lowland forests. Some of the important trees in this zone are Mexican elm (*Ulmus mexicana*), oak (*Quercus lancifolia*), snowbell (*Styrax argenteus*), and mastic (*Mastichodendron capiri*). The subspecies *S. v. underwoodi* occurs in the lower elevations of the western slopes of the Central Highlands and *S. v. belti* seems to be found in the moister areas of the eastern slopes of the highlands probably following the valleys of the major river systems. Harris (1937) found that *belti* from this area show influences of *S. v. boothiae* and results presented below support this conclusion. Found in this region is highest point in Nicaragua, Pico Mogotón at 2,103 m, which is located on the Honduran border in the Departmento de Nueva Segovia (Arguello et al. 2018). The subspecies *S. v. boothiae* appears to be associated with these highest elevations in the Departmento de Nueva Segovia.

The Caribbean Lowlands occupy the eastern half of Nicaragua and are composed of low, level plains that at some points are 100 km wide (Arguello et al. 2018). South of Lago de Nicaragua Caribbean Lowland Tropical Moist Forest extends as far west–southwest as the Cordilleras de Guanacaste and Tilarán. This hot, humid region was covered in Lowland Evergreen Rainforest (Taylor 1963). Large areas of these forests are mature stands of trees, although species such as mahogany (*Swietenia*, Meliaceae) have been selectively logged and new areas have been opened recently in anticipation of the construction of a new canal. These forests are characterized by high biodiversity of plant species. Some of the major tree species in this area include cabbage bark (*Andira inermis*), crabwood (*Carapa nicaraguensis*), tamarindo montero (*Dialium guianense*), tonka bean tree (*Dipteryx panamensis*), Guácimo colorado (*Luehea seemannii*), and roble coral (*Terminalia amazonia*) (Taylor 1963). Occurring throughout these lowland areas are squirrels representing *S. v. belti*. This subspecies also penetrates the Central Highlands, probably following the large eastward flowing rivers.

ANALYSES

Morphometric variation.—To gain a broader understanding of the relationship among the populations of *Sciurus variegatoides* in Nicaragua, nine cranial measurements from specimens available for study were recorded. Three analyses on these measurements—derived standard univariate statistics, principal components analysis, and a discriminate function analysis were performed. Squirrels were grouped into taxonomic groups taking care to not include intergrades between them and a group from Isla de Ometepe where the squirrels have not been described previously. This created six groups for final analyses.

Table 1 presents univariate statistics for the six groups. The squirrels from Isla de Ometepe averaged smaller than the other five groups in seven of the

Table 1. Standard statistics for six subspecies of *Sciurus variegatoides* from Nicaragua. The samples are as follows: new subspecies, Isla de Ometepe; *S. v. adolphei*, departments of Chinandega and León; *S. v. belti*, North and South Caribbean Coast Autonomous Region; *S. v. boothiae*, Departmento de Nueva Segovia; *S. v. dorsalis*, departments of Carazo, Granada, Managua, Masaya, and Rivas; *S. v. underwoodi*, departments of Estelí, Jinotega, Madriz, and Matagalpa.

Statistics	GLS	CBL	ZB	IOB	POB	MB	LN	PB	LTR
				Isla de Ometepe					
Mean ± SE (N = 42)	55.3 ± 0.15	50.2 ± 0.16	32.3 ± 0.11	18.5 ± 0.10	18.8 ± 0.08	23.1 ± 0.08	17.1 ± 0.11	18.4 ± 0.10	10.6 ± 0.04
Range	52.6–57.1	48.4–52.2	30.0–33.7	17.2–20.1	17.8–19.8	22.3–24.7	15.3–19.1	16.7–19.7	9.8–11.2
				Sciurus variegatoides adolphei					
Mean ± SE (N = 33)	61.4 ± 0.22	56.5 ± 0.23	34.9 ± 0.14	20.5 ± 0.12	18.6 ± 0.13	25.2 ± 0.10	18.8 ± 0.17	20.5 ± 0.14	11.8 ± 0.07
Range	58.4–63.2	53.2–58.6	32.8–36.4	19.0–22.4	17.0–19.9	24.1–26.6	16.7–20.4	19.2–22.2	11.1–12.5
				Sciurus variegatoides belti					
Mean ± SE (N = 39)	57.1 ± 0.19	52.6 ± 0.20	33.4 ± 0.14	19.5 ± 0.11	18.5 ± 0.15	23.5 ± 0.21	16.9 ± 0.16	19.3 ± 0.11	11.6 ± 0.05
Range	54.9–59.6	49.7–54.5	31.8–34.9	18.2–21.6	17.4–19.3	22.8–25.1	14.8–18.8	17.4–20.8	11.0–12.2
				Sciurus variegatoides boothiae					
Mean ± SE (N = 19)	57.7 ± 0.36	52.9 ± 0.32	33.5 ± 0.23	19.7 ± 0.24	18.7 ± 0.19	24.1 ± 0.15	17.4 ± 0.20	19.5 ± 0.18	11.4 ± 0.11
Range	55.2–60.5	50.2–56.2	32.1–35.5	17.7–21.1	17.0–20.6	22.9–25.2	15.9–19.1	18.5–21.4	10.6–12.3
				Sciurus variegatoides dorsalis					
Mean ± SE (N = 52)	59.9 ± 0.16	54.6 ± 0.17	34.7 ± 0.14	20.5 ± 0.11	19.3 ± 0.12	24.7 ± 0.14	18.7 ± 0.12	20.4 ± 0.07	11.7 ± 0.06
Range	57.5–62.8	52.7–57.4	31.6–35.8	18.8–22.9	17.4–21.9	20.2–26.7	17.1–20.3	19.4–21.4	10.7–12.9
				Sciurus variegatoides underwoodi					
Mean ± SE (N = 28)	58.4 ± 0.21	53.7 ± 0.21	34.0 ± 0.16	19.7 ± 0.15	18.9 ± 0.13	24.1 ± 0.13	17.5 ± 0.14	19.6 ± 0.10	11.3 ± 0.08
Range	56.1–60.3	51.0–55.9	32.3–35.7	17.7–21.5	17.3–20.4	22.9–25.4	14.8–18.5	18.4–20.6	10.6–12.1

measurements, with the exceptions being postorbital breadth and length of nasals. In postorbital breadth, squirrels from Isla de Ometepe average larger than the samples of *adolphei*, *belti*, and *boothiae* and matched the average of the sample of *underwoodi*. Only specimens identified as *dorsalis* at 19.3 mm average larger than the island group at 18.8 mm. The sample of *belti* had on average the shortest nasal bones followed by the island population. It is worth noting that the range of measurements for greatest length of skull and condylobasal length for the sample from Isla de Ometepe does not overlap the range of these measurements from the other two groups of squirrels from western Nicaragua—*adolphei* and *dorsalis*.

The sample of *adolphei* from northwestern Nicaragua is on average the largest squirrels from Nicaragua in seven of the nine cranial measurements, the exceptions being postorbital breadth and interorbital breadth in which both *adolphei* and *dorsalis* average 20.5 mm. The samples of *dorsalis* from southwestern Nicaragua closely tracked the variation in *adolphei*, averaging the second largest in seven measurements, with the exceptions being postorbital breadth in which they averaged the largest and interorbital breadth where *dorsalis* averaged the same as *adolphei*. Examining the other three groups—*belti*, *boothiae*, and *underwoodi*—their mean values fall in the middle between the previous groups and so broadly overlap each other that there are no discernable morphometric differences.

A number of principal component analyses were undertaken either using individual localities or small geographic areas as units beginning with 27 operational groupings. Based on these results, six groups that represent taxonomic and geographic units for the final principal components analysis were formed. The results of this analysis are shown in Figure 1 and Table 2. Table 2 presents the component loadings for all characters. Loadings in PC 1 are all negative and fall between −0.294 and −0.398 except for postorbital constriction at −0.116. These results indicate that overall size is the dominant factor in this component and it accounts for just over 64% of the variation among these samples. The second component is dominated by a negative value of 0.903 for postorbital breadth. This component deals with shape of the cranium with all length measurements being negative and all breadth measurements being positive and accounts for just less than 12% of the variation among these samples. Each of the remaining components account for 7.9% or less of the variation. The units in Figure 1 do overlap, but form three groups across the first principal component. On the far left are representatives of *S. v. adolphei* and *S. v. dorsalis* with the highest negative values, indicating that they are the largest individuals for the species in Nicaragua as seen in the univariate analysis. These taxa are the two confined to the drier Pacific lowlands of western Nicaragua. These two taxa show some separation in the second component with *S. v. dorsalis* toward the top of the plot indicating heavier influence by breadth measurements and *S. v. adolphei* nearer the bottom of the plot indicating more influence from length measurements. Along PC 1, *S. v. adolphei* and *S. v. dorsalis* do not overlap the variation in the population from Isla de Ometepe. This is important because the Ometepe population is separated from the mainland Departmento de Rivas populations of *S. v. dorsalis* only by about a 6-kilometer water gap. In the middle of PC 1 are representatives of three taxa—*belti*, *boothiae*, and *underwoodi*—which are the taxa that occur in the Central Highlands and Caribbean Lowlands. These three taxa broadly overlap each other and fill the gap between the representatives of the other two groups. There is no separation morphometrically of these three taxa from each other. These three taxa also overlap with the larger taxa—*adolphei* and *dorsalis*—and larger individuals from Isla de Ometepe but the overlap is not extensive. No additional separation of the groups in PC 2 are discernable.

Discriminate function analyses were performed on the same groups used in the principal components analysis, resulting in a classification matrix presented in Table 3. The sample from Isla de Ometepe had the highest classification success with only one of the 42 squirrels being misclassified. At the opposite end of the scale was the sample of *S. v. boothiae* in which only five members of the sample of 19 squirrels were correctly identified, with misidentifications falling into all groups except *S. v. adolphei*. These results indicate that the taxon *S. v. boothiae* is not defined morphometrically. The remaining four samples have correct classification percentages falling between 64% and 80%, indicating that there is a certain level of morphometric definition to these groups. Half of the misidentified *adolphei* (4) were identified as *dorsalis* and half of the misidentified *dorsalis* (7) were identified as *adolphei* clearly indi-

Table 2. Principal component loadings for nine cranial measurements in 213 specimens of *Sciurus variegatoides* from Nicaragua.

Measurement	PC I	PC II
Greatest length of skull	− 0.398	− 0.118
Condylobasal length	− 0.395	− 0.129
Zygomatic breadth	− 0.373	0.092
Interorbital breadth	− 0.339	0.267
Postorbital breadth	− 0.111	0.903
Mastoid breadth	− 0.349	0.047
Length of nasals	− 0.293	− 0.187
Length of palatal bridge	− 0.354	− 0.135
Length of maxillary toothrow	− 0.294	− 0.139
Percent of variance explained	64.1%	11.9%

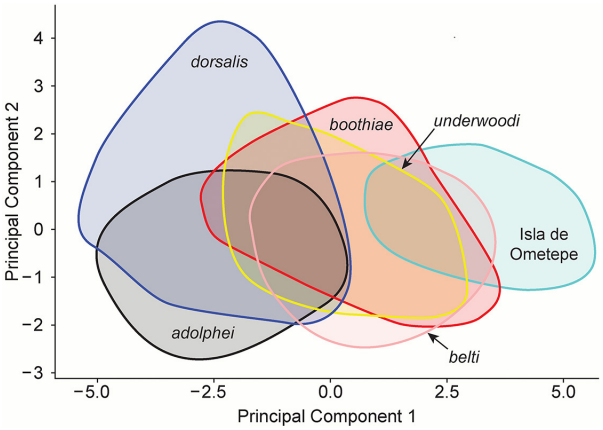

Figure 1. Specimen scores described as ellipses from principal component loadings circumscribing factor scores on CV 1 and CV 2 of nine cranial variables of the six subspecies of *Sciurus variegatoides* we recognize in Nicaragua. Principal component 1 accounts for 64.1% and principal component 2 accounts for 11.9% of the variance explained by specimen scores of 213 specimens for nine cranial measurements (GLS, CBL, ZB, IOB, POB, MB, LN, PB, and LTR) to show the relationships of these taxa. Axes are scaled relative to their eigenvalues (proportion of the variation explained). The colors used on the ellipses are as follows: black, *S. v. adolphei*; pink, *S. v. belti*; red, *S. v. boothiae*; blue, *S. v. dorsalis*; turquoise, *S. v. ometepensis*, the new subspecies described; and yellow, *S. v. underwoodi*.

cating that these two large-sized taxa of squirrels can be defined morphometrically but are actually closely related. The three medium-sized groups of squirrels occupying the Central Highlands and Caribbean Lowlands show in the discriminate analysis that they are close morphometrically, with *belti* being the most distinct of the group with nearly 80% correct identifications. Four of the misidentified *belti* classify as *boothiae*, which becomes understandable where there are several intergrades along the eastern edge of the Central Highlands (see subspecies accounts). Along the western edge of the Central Highlands, *boothiae* and *underwoodi* account for five misidentifications of each other.

Among the six groups studied morphometrically, it is the population from the Isla de Ometepe that represents a sixth taxonomic unit in Nicaragua. Its individuals are on average the smallest of the Neotropical variegated squirrels in the country, and indeed the smallest of any population of variegated squirrels from throughout the species' geographic range. This difference is particularly striking in comparison to the geographically adjacent populations of *S. v. dorsalis*.

Color variation.—Color in mammals is a combination of two forms of melanin. Eumelanin creates black, gray, and dark brown tones. Sulfur-containing

Table 3. Classification matrix resulting from a discriminate function analysis of nine cranial measurements from six subspecies of *Sciurus variegatoides* occurring in Nicaragua.

	Classification Group					
Input Group	*adolphei*	*belti*	*boothiae*	*dorsalis*	Isla de Ometepe	*underwoodi*
adolphei	25	0	0	7	0	1
belti	0	31	4	1	0	2
boothiae	2	4	5	2	1	5
dorsalis	4	1	3	39	0	2
Isla de Ometepe	0	1	2	0	41	0
underwoodi	2	2	5	3	0	18
Total N	33	39	19	52	42	28
N correct	25	31	5	39	41	18
Percentage	75.8%	79.5%	26.3%	75.0%	97.6%	64.3%

pheomelanin creates yellow, orange, and red tones. Hershkovitz (1968) attributed white coloration to bleaching or lack of deposition of melanin in the developing hair. Agouti colored hair is a pattern characterized by alternating blackish and reddish–tan bands. Production and deposition of one melanin can be switched off, and that of the other switched on, depending on hair type.

As Harris (1937) accurately described, the dorsal hairs are generally three banded (see exceptions below) and hairs on the ventrum generally are not banded. The terminal and middle band coloration of the dorsum hairs determines the overall coloration of the individual. Hairs along the sides are generally two banded, but this is highly variable between subspecies. The tail in all subspecies of *S. variegatoides* appears as a mix of long black and white hairs interspersed; however, all hairs are black basally and some have white tips.

Both males and females of most subspecies of *S. variegatoides* have conspicuous, distinctive, and similar tan, orange, or white ear patches. These are generally referred to as "postauricular patches," and as Harris (1937) notes, these are generally in sharp contrast to the dorsal coloration and interestingly often match the venter coloration. On all specimens of variegated squirrels examined from throughout the species' range, these distinctive hairs are on the posterior/medial surface of the pinna itself and generally proceed posteriorly appearing to be a full 10 mm or more post fleshy pinna, albeit in some, it is the length of the hairs on the ear that give the impression of a larger patch. Thus, they might best be termed "auricular patches." These hairs are generally silkier in texture than the more course surrounding guard hairs and are generally shorter in length. There is considerable variation in the size and color of the patch but in most taxa these are in sharp contrast to the body coloration. Thus, the auricular patch is a variable character both geographically as well as within populations, and, as a diagnostic character to identify geographic forms, is best used in combination with other characters. These distinctive auricular patches almost certainly have a social function (Ancillotto and Mori 2017).

The color and color pattern of the subspecific populations are discussed in the following individual systematic accounts. This information will emphasize the unique character of these colors and color patterns. The above analyses match closely those of Harris (1937) who was truly a keen observer and provided clear, succinct descriptions. The unique and highly variable population of squirrels on Isla de Ometepe is herein considered to be an undescribed subspecies and is described below.

SYSTEMATIC ACCOUNTS

Sciurus variegatoides ometepensis, new subspecies

Figs. 2–6

Holotype.—KU 115306, adult male skin and skull, obtained on 25 March 1968 and prepared by Jerry R. Choate (field number CJR 941). Skin and skull in excellent condition (Figs. 2–4). All teeth fully erupted and moderately worn, cranium with advanced fusion of basicranial synchondroses (Fig. 4). Skin with fleshy penis and baculum attached and extended (Fig. 3).

Measurements of the holotype.—KU 115306: total length, 500; length of tail, 223; length of hind foot, 62; length of ear, 30; greatest length of skull, 56.0; condylobasal length, 51.1; zygomatic breadth, 32.5; interorbital breadth, 19.3; postorbital breadth, 19.8; mastoid breadth, 23.4; length of nasals, 16.6; length of palatal bridge, 19.0; and length of maxillary toothrow, 10.3.

Type locality.—Nicaragua: Departmento de Rivas; Isla de Ometepe, 2 km N Mérida.

Geographic range.—This new subspecies is confined to Isla de Ometepe in Lago de Nicaragua, encompassing Volcán Concepción (1,610 m) to the northwest and Volcán Maderas (1,394 m) to the southeast and connected by a low, narrow isthmus (Istmo de Istián) into a single island (Fig. 5). The island has an area of some 276 square kilometers.

Paratypes (6).—Nicaragua: Departmento de Rivas; Isla de Ometepe, 2 km N Mérida (KU 115312, 115319); Mérida (KU 115337); Santa Ana (KU 115301); 3 km NE Moyogalpa (KU 110399); and 6 km E Moyogalpa (KU 110396).

Etymology.—The specific epithet is an adjective in the genitive case and formed by adding "ensis" to the stem of the island's name and is applied in reference to the subspecies distribution on Isla de Ometepe in Lago de Nicaragua. The island's name is derived from the Nahuatl words *ome* (two) and *tepetl* (mountain), meaning "two mountains." This gives the subspecies the common name of the Neotropical variegated squirrel from the place of two mountains.

Nomenclatural statement.—A life science identifier (LSID) number was obtained for the new subspecies (*Sciurus variegatoides ometepensis*): urn:lsid:zoobank.org:pub:F4DA74D8-5D54-46D8-95D0-FC3C73D60E34.

Diagnosis.—A large tree squirrel (order Rodentia, suborder Sciuromorpha, family Sciuridae, subfamily Sciurinae, tribe Sciurini, genus *Sciurus*, subgenus *Sciurus*) mass 400–500 g in mature adults; total length 500+ mm, variably colored with long tail (47–51% of total length). *Sciurus variegatoides* is the largest tree squirrel occurring in Central America. Cranial size smallest among populations of *S. variegatoides* in Nicaragua and throughout the geographic range of the species, with a proportionally broad postorbital region (Table 1). Incisors, 1/1; premolars, 2/1, anterior one minute; molars, 3/3. Jugal twisted posteriorly revealing medial surface when viewed from above. Mammary formula: 1 pectoral + 2 post-axillary + 1 inguinal. Dorsum variously colored ranging from brown–agouti to a lighter brown–tan, and in some individuals nearly blond appearing (Fig. 3). All dorsal hairs are black at base and either black or tan terminally, including on the blond-colored animals. Ventral fur bright orange–chestnut in all specimens examined except for very dark animals some of which have a black venter. Large, pronounced, and generally bright white auricular patches covering the basal 2/3s of pinnae extend as far posteriorly as 15+ mm. The tail is a mix of white tipped and black or pale brown hairs throughout giving an appearance typical of other Nicaraguan subspecies or considerably paler.

Description.—A small member of the *Sciurus variegatoides* complex confined to Isla de Ometepe in Lago de Nicaragua, with variable pelage color and color patterns, but centering around a pattern of a mixture of dark and tan producing a dark agouti-type or pale agouti-type appearance. Individuals range in color from blond to nearly black (see Fig. 3). Dorsally, all hairs have two or three bands, a black basal band and either tan, brown, or black (rarely) terminal band. Hairs of the ventrum are unicolor bright chestnut–orange. Hind feet are chestnut, orange, or dark agouti.

General anatomy of skull and dentition in *S. v. ometepensis* (Fig. 4) conforms in all major features with that in other species in the subgenus (e.g., see Harris 1937). Measurements of skull for nine cranial dimensions of 42 specimens of *S. v. ometepensis* are provided Table 1.

The baculum has a large, expanded base, circular in cross section; the shaft tapers to a narrow neck and terminates in a distal expanded, circular scoop-shaped disc at a 45° angle to the shaft (Fig. 6). The anterior and ventral margins of the disc are rounded with the edges slightly curled. The disc is concave on the right side and convex on the left. A bluntly pointed somewhat posteriorly curved dorsal spur is present dorsally. Measurements of the baculum of a paratype (KU 115312) are: length, 12.8 mm; height of base, 3.4 mm; width of base, 2.7 mm; and length of expanded tip, 2.2 mm. Total length and width of base of *S. v. ometepensis* were larger than the ranges for five individuals from other populations (length, 11.5–12.4, mean = 12.1 mm; height of base, 2.7–3.3, mean = 3.0 mm), whereas the length of expanded tip is smaller than the range (2.4–2.7, mean = 2.6 mm) provided by Burt (1960). On the other hand, width of base is within the range Burt provided (2.1–2.9, mean = 12 mm).

Comparisons.—Compared to all Nicaraguan Neotropical variegated squirrels most individuals of *ometepensis* are small, usually the smallest. In size, there is some overlap with the subspecies east and northeast of Lago de Nicaragua—*belti*, *boothiae*, and *underwoodi*. The only specific comparison that is really needed is to the geographically adjacent population of *S. v. dorsalis* occurring on the mainland of Departmento de Rivas about 6 km to the west of the island (*S. v. adolphei* farther to the northwest is even larger than *dorsalis* and *S. v. belti* to the east is also larger than *ometepensis*). The range of the measurements for greatest length of skull and condylobasal length for *ometepensis* do not overlap the ranges for the larger *dorsalis* or *adolphei*. The mass of mature *S. v. ometepensis* is in the range of 450–500 g for mature adults, whereas it is in the range of 500–600+ g for mature *S. v. dorsalis*.

In gross morphology, the baculum of *S. variegatoides ometepensis* differs from other northern members of *S. variegatoides* complex as provided by Burt (1960) in that the disc is at a 45° angle rather than a 90° angle, less curling of the margin of the disc, more sharply pointed spur, and lack of a tuberosity and notch posterior to the spur present in other *S. variegatoides* among other details. The baculum differs from that of the more southern taxon, *S. v. thomasi* (KU 26958), in the disc margin being less curled, and in having a more sharply pointed spur. It has a narrower attachment of the disc and is less rugose posterior to the disc. The disc on both is at a 45° angle to the shaft.

Sciurus v. ometepensis differs from *S. v. dorsalis* in that it lacks a broad dark dorsal stripe, which is present in all specimens of *dorsalis*, extending from the nape of the neck to the base of the tail. *Sciurus v. dorsalis* is paler in color (with exception of the dark dorsal stripe), often white laterally and ventrally.

Remarks.—*Sciurus variegatoides ometepensis* is the most variable in color and color patterns of the subspecies in the species complex. In addition to the overall description of color presented above, a single specimen (KU 115308, see Fig. 3) from 2 km N Mérida is black in overall appearance. The hairs on the dorsum are of two types—some primarily black throughout their length, and others are two banded with a long black basal band (8–10 mm) and a short tip of agouti to orange. These two color patterns of hair are not evenly distributed over the dorsum. Black hairs predominate on the head and shoulders and two-banded hairs predominate over the lower back and hips. The overall appearance is black, but on closer inspection, the brownish orange is clearly visible. The two-banded hairs continue along the dorsal tail, and the overall tail appearance is black. The ventrum has unicolored hairs as is typical of variegated squirrels and is black throughout. The auricular patches are confined to the back of the pinnae and the two-banded hairs are concentrated along the edges of the pinnae. Hind feet are black. The overall appearance of the tail is dark with a 40 mm tip of dirty white or tan color (see Fig. 3). The 50 mm prior to the blonde tip the agouti brown band becomes longer and imparts a brownish color to the tail. This individual or any of the other black appearing individuals described below are not considered as fully melanistic because all individuals evaluated have banded dorsal hairs with black predominating but with a tan or agouti band either centrally or terminally. Additional images of *S. v. ometepensis* are provided by Medina-Fitoria et al. (2018), who contributed significantly to knowledge

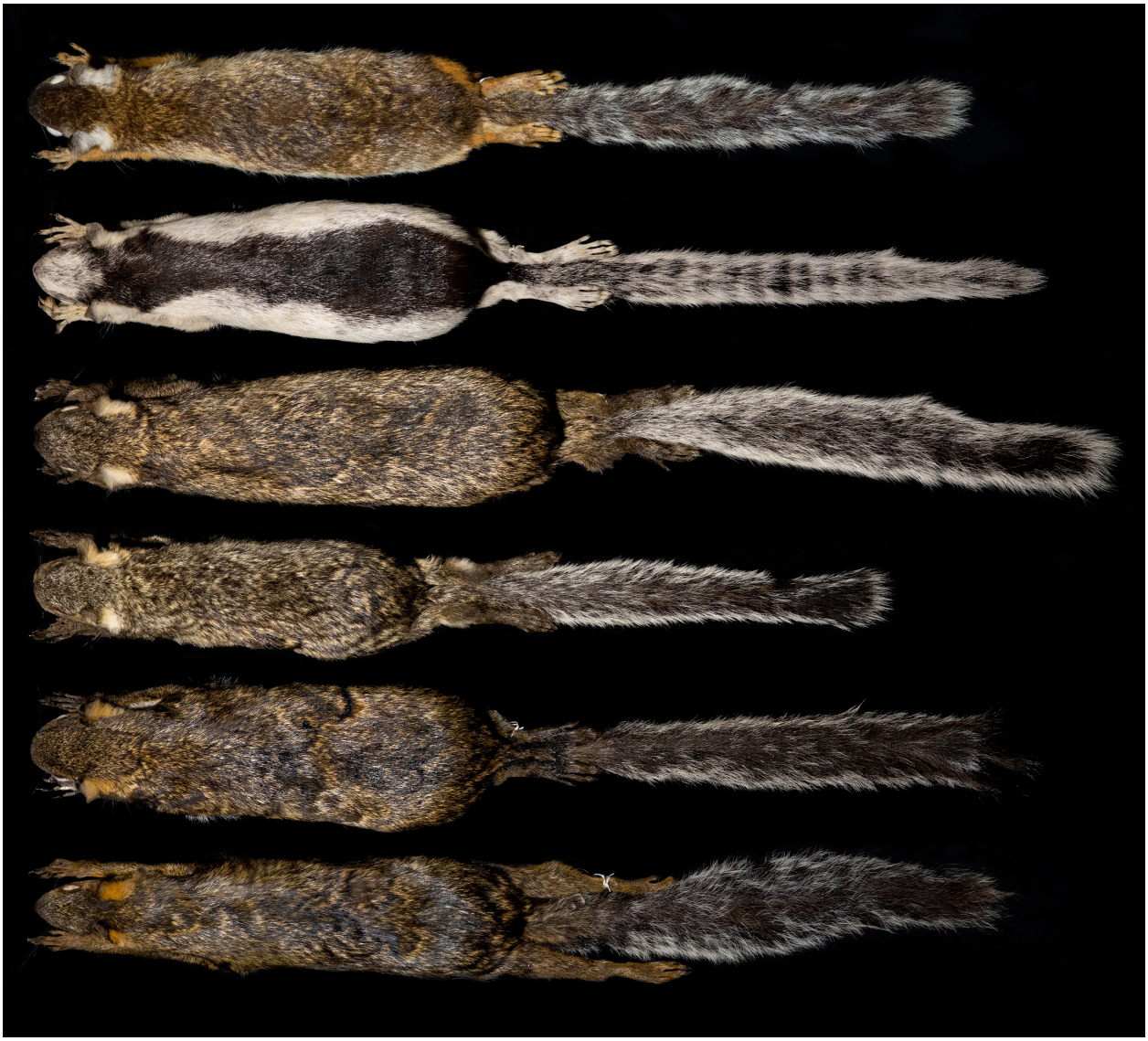

Figure 2. Variation in color and color patterns in the dorsum (left; this page) and ventrum (right; opposite page) of six subspecies of *Sciurus variegatoides* from Nicaragua. From top to bottom: *S. v. ometepensis*, KU 115306 (holotype), Departmento de Rivas, 2 km N Mérida, Isla de Ometepe; *S. v. dorsalis*, KU 110386, Departmento de Carazo, 3 km N, 4 km W Diriamba; *S. v. adolphei*, KU 106349, Departmento de Chinandega, Hacienda Bellavista, Volcán Casita; *S. v. underwoodi*, KU 97912, Departmento de Madriz, Darailí, 5 km N, 14 km E Condega; *S. v. boothiae*, KU 110361, Departmento de Nueva Segovia, 1.5 km N, 1 km E Jalapa; and *S. v. belti*, KU 99464, Departmento de Jinotega, Hacienda La Trampa, 5.5 km N, 16 km E Jinotega. Scale: total length of holotype = 500 mm.

of the color variation found in this subspecies. They report that these squirrels occur from the lowlands up to 1,000 m.

This new subspecies is confined to Isla de Ometepe in Lago de Nicaragua separated from the mainland of the Departmento de Rivas to the west by just over 6 km. The island consists of two volcanic cones, the associated low, flat volcanic aprons, and a narrow isthmus connecting the two islands into one. The aprons around the Ometepe volcanoes have been heavily impacted by human occupation and agricultural activities,

Figure 2. (cont.)

which has expanded extensively since the University of Kansas field research in the late 1960s. The large collection of squirrels from the southern island from 2 km N Mérida and the one individual from Mérida were taken from large trees that remained in the area fringing the dirt roads and footpaths that paralleled the coast. Among these trees were such species as West Indian cedar (*Cedrela odorota*), mango (*Manguifera indica*), sandbox tree (*Hura crepitans*), gumbo limbo (*Bursera simaraba*), and chelate (*Ficus trigonata*), along with an assortment of acacias and other shorter thorn-covered trees and bushes. The squirrels from 2 km N, 3 km E Mérida were obtained from trees on the slopes of Volcán Maderas. Some areas of the slopes were heavily vegetated, whereas other areas were more open as the result of past landslides. Among the important trees in this area were kapok (*Ceiba petranda*), molenillo (*Lueha candida*), balsa (*Ochroma pyramidale*), stinking toe tree (*Cassia grandis*), and spiny cedar (*Pachira quinata*). On the northern end of the island the situation was similar with squirrels from 3 km NE Moyogalpa coming from forest fragments on the apron of the volcano and those from 6 km E Moyogalpa coming from the western slope of Volcán Concepción and those from

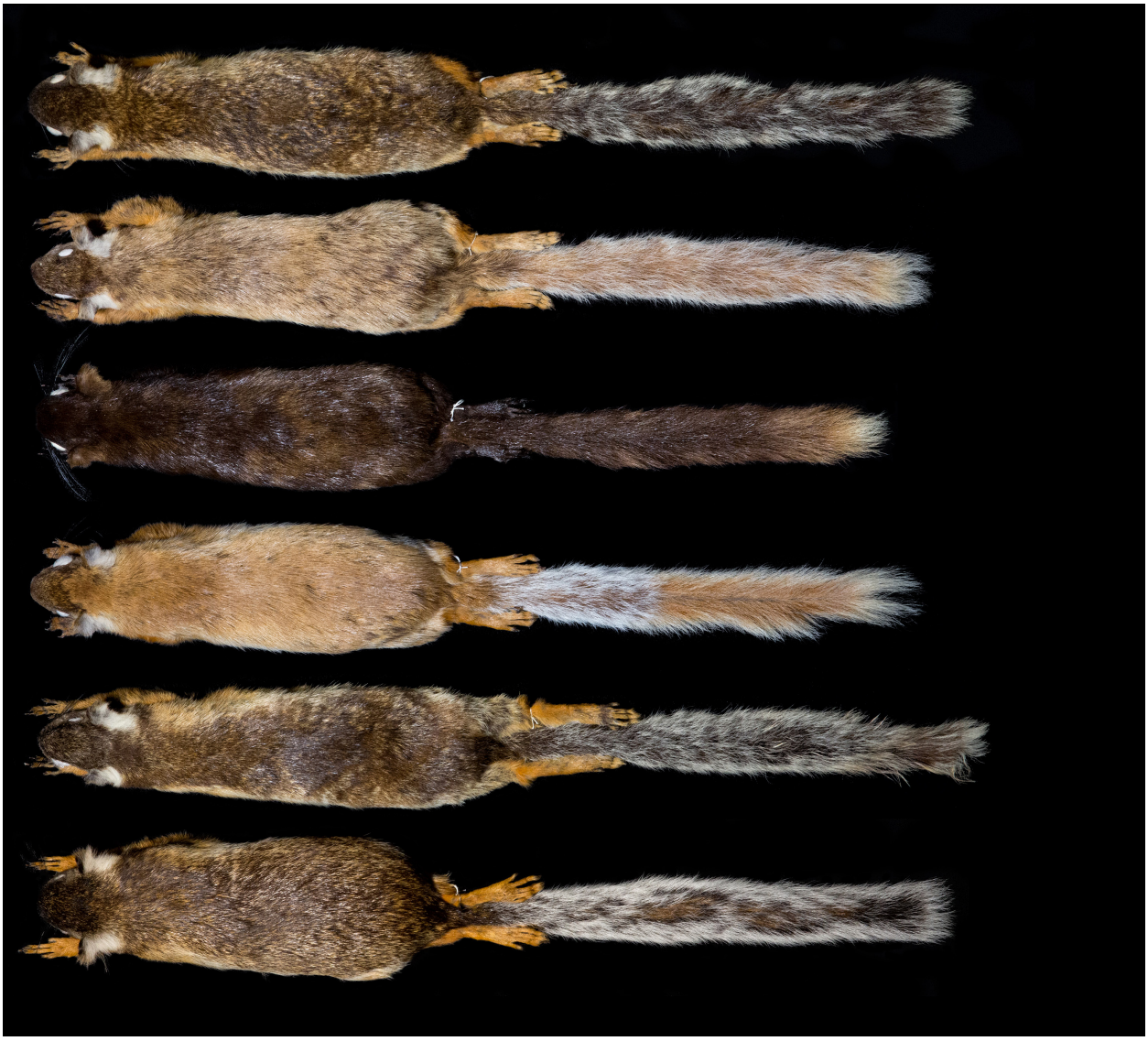

Figure 3. Variation in color and color patterns in the dorsum (left; this page) and ventrum (right; opposite page) of six individuals of *Sciurus variegatoides ometepensis* from Isla de Ometepe, Departmento de Rivas, Nicaragua. From top to bottom: South Island—KU 115306 (holotype), 2 km N Mérida; KU 115325, 2 km N Mérida; KU 115308, 2 km N Mérida; KU 115319 (paratype), 2 km N Mérida; Isthmus—KU 115301 (paratype), Santa Ana; and North Island—KU 110399 (paratype), 3 km NE Moyogalpa. Scale: total length of holotype = 500 mm.

1.5 km W Altagracia were from the northern slope. There was evidence of recent volcanic activity on this cone. The specimens from Santa Ana came from the area of the isthmus where soil conditions were moister and where stands of trees were in the wetter areas and agricultural fields occupied the intervening areas.

A specimen from northeast of Mérida (KU 115334) taken on 7 April 1968 is a juvenile with only M1 erupted and M2 starting to erupt. A dental variation was noted in KU 110396, which is missing the left upper P3. Based on the University of Kansas series of squirrels collected in Nicaragua, Emerson (1971)

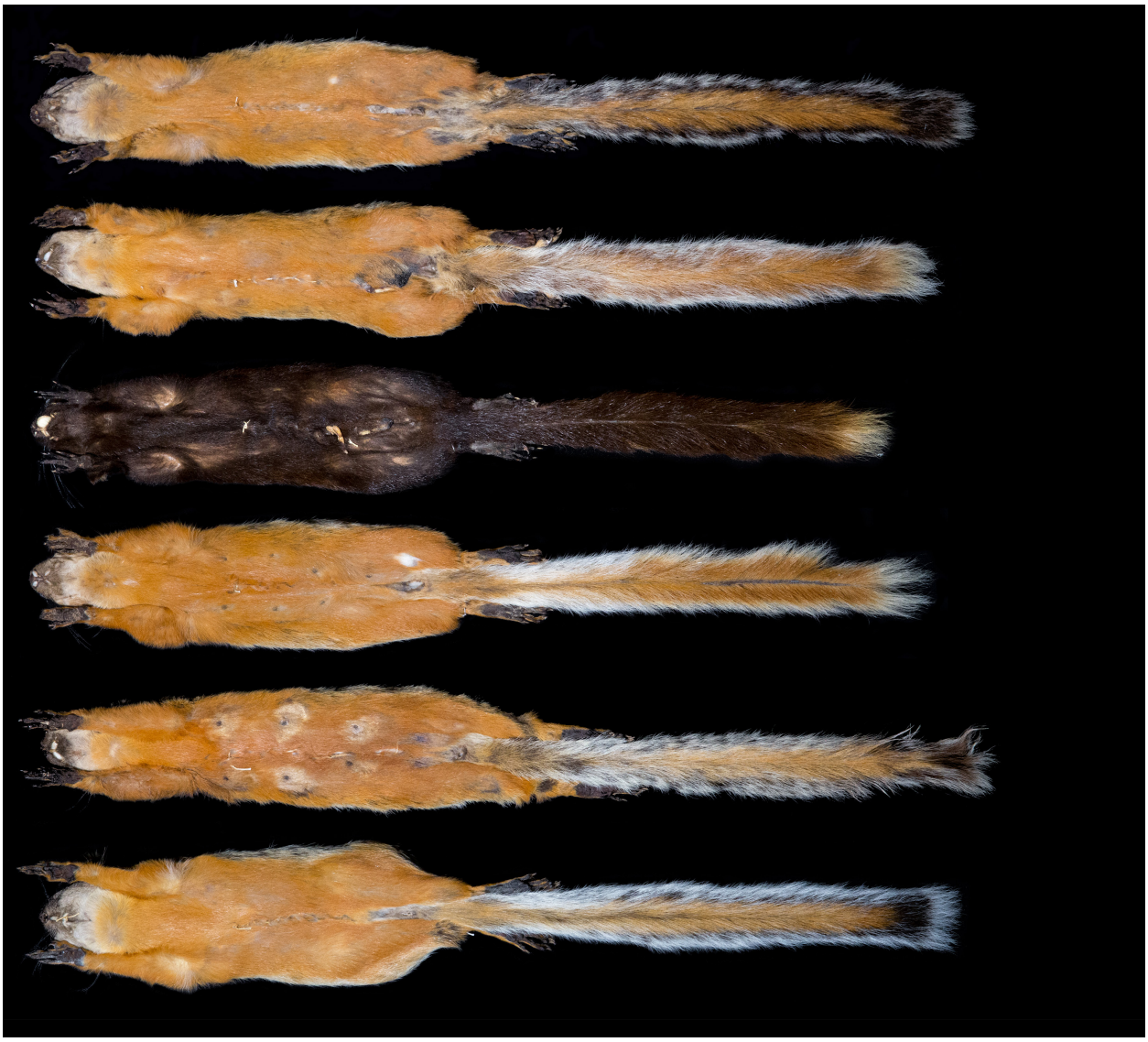

Figure 3. (cont.)

reported the squirrel sucking louse *Enderleinellus hondurensis* Werneck (Phthiraptera: Hoplopleuridae) from three subspecies in the *S. variegatoides* complex—*S. v. belti*, *S. v. underwoodi*, and a specimen of *S. v. ometepensis* from Mérida on Isla de Ometepe (KU 115326). The holotype of *E. hondurensis* is from a specimen of *S. v. underwoodi* from Honduras.

In addition to *Sciurus variegatoides*, species of mammals collected or observed on Isla de Ometepe include *Philander* sp., *Balantiopteryx plicata*, *Rhynchonycteris naso*, *Saccopteryx bilineata*, *Noctilio albiventris*, *Noctilio leporinus*, *Artibeus intermedius*, *Artibeus jamaicensis*, *Dermanura tolteca*, *Carollia perspicillata*, *Carollia subrufa*, *Chiroderma villosum*, *Desmodus rotundus*, *Glossophaga leachii*, *Glossophaga soricina*, *Phyllostomus discolor*, *Platyrrhinus helleri*, *Sturnira hondurensis*, *Sturnira parvidens*, *Uroderma convexum*, *Alouatta palliata*, *Cebus capucinus*, *Sylvilagus floridanus*, *Oligoryzomys fulvescens*, *Oryzomys couesi*, *Peromyscus nicaraguae*, *Peromyscus stirtoni, Sigmodon hirsutus*, *Liomys salvini*, *Odocoileus virginianus*, and the introduced black rat, *Rattus rattus*.

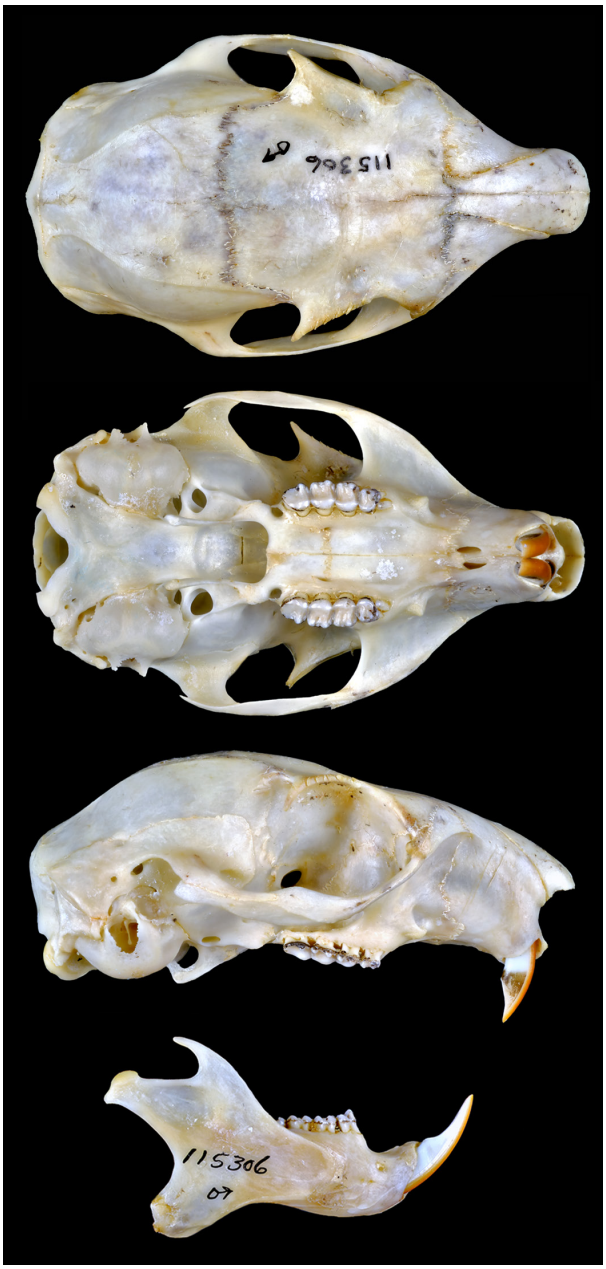

Figure 4. Dorsal, ventral, and lateral view of the cranium and lateral view of the right dentary of an adult male *Sciurus variegatoides ometepensis* from 2 km N Mérida, Isla de Ometepe, Departmento de Rivas, Nicaragua (holotype, KU 115306); greatest length of skull = 56.0 mm.

Specimens examined (50).—Departmento de Rivas: Isla de Ometepe [*North Island*], Volcán Concepción, 1.5 km W Altagracia [= Alta Gracia] (2, 1 ♂, 1 ♀, KU 115294–295); Volcán Concepción, 3 km NE Moyogalpa (4, 3 ♂♂, 1 ♀, KU 110397–400); Volcán Concepción, 6 km E Moyogalpa (2 ♂♂, KU 97914, 110396). Isla de Ometepe [*Istmo de Istián*], Santa Ana (7, 5 ♂♂, 2 ♀♀, KU 115296–302). Isla de Ometepe [*South Island*], 2 km N Mérida (31, 26 ♂♂, 5 ♀♀, KU 115303–333); 2 km N, 3 km E Mérida (3, 2 ♂♂, 1♀, KU 115334–336); Mérida (1 ♂, KU 115337).

Additional records.—Departmento de Rivas: Reserva Natural Volcán Maderas (Medina-Fitoria et al. 2018).

***Sciurus variegatoides adolphei* (Lesson, 1842)**

1842. *Macroxus Adolphei* Lesson, Nouveau Tableau du Règne Animal: Mammifères, Arthus Bertrand, Paris p. 112.

1920. *Sciurus variegatoides adolphei*, Goldman, Smithsonian Miscellaneous Collection 69(5):136.

Lectotype.—MNHN-ZM-MO-2000-611, adult female mounted skin with skull inside, collected by Adolphe Lesson, ship's surgeon of the French ship "La Pylade," prior to 1842.

Type locality.—El Realejo, Departmento de Chinandega, Nicaragua.

Remarks.—These are the largest of the variegated squirrels occurring in Nicaragua. They average the largest of all samples in seven of the nine cranial measurements analyzed (Table 1). This is a distinctive subspecies with a prominent white auricular patch that extends from near the tip of the ear to 10–15 mm posteriorly. Dorsally the overall appearance is a mixture of black or dark agouti and dark silver giving a unique overall dark appearance and that coloration extends down laterally (Fig. 2). The ventrum is strongly countershaded with white in most individuals although some have a mix of white and a light tan–orange color. The dorsal coloration of the hind feet is dark, most approach black although a few specimen are dark brown–agouti. These squirrels occur in extreme northwestern Nicaragua in the departments of Chinandega and León, and

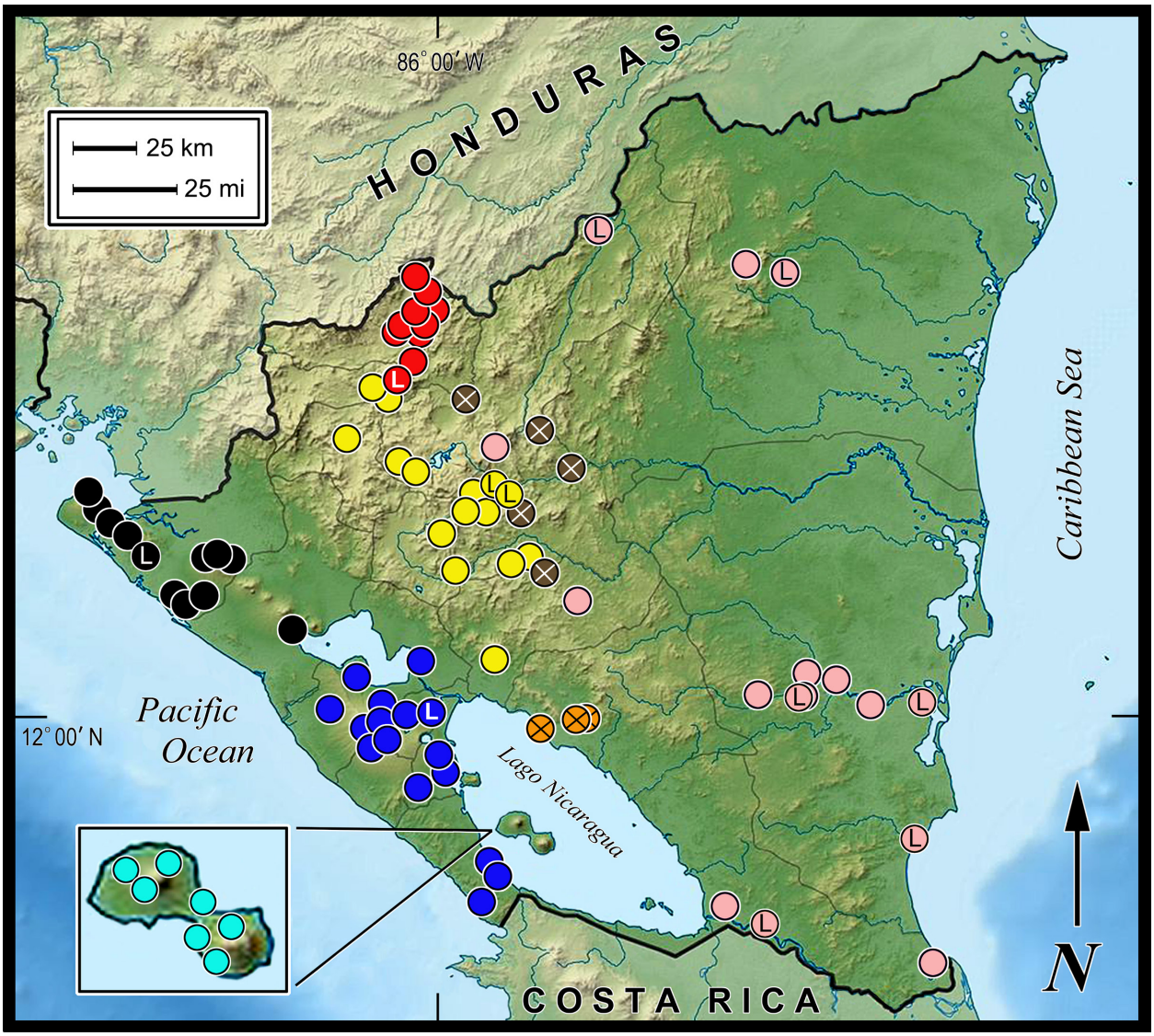

Figure 5. Map of Nicaragua showing the collecting sites and literature records for the six subspecies of *Sciurus variegatoides* in the country. The colors and symbols used on the map are as follows: black closed circles, *S. v. adolphei*; pink closed circles, *S. v. belti*; red closed circles, *S. v. boothiae*; blue closed circles, *S. v. dorsalis*; turquoise closed circles, *S. v. ometepensis*; yellow closed circles, *S. v. underwoodi*; brown symbols with "X", intergrades between *belti* and *boothiae*; orange symbols with "X", intergrades between *belti* and *underwoodi*; symbols marked with "L" indicate records from the literature. See text for details on localities, museum catalog numbers, and our identifications. (Base map courtesy of http://maplibrary.org, VMAP0).

are common on the volcanoes of the Cordillera de los Marrabios as far south as the northern end of Lago de Managua and the west in the dry Pacific Lowlands (Fig. 5).

Lesson (1842:112) described *Macroxus adolphei* based on "the male and female were killed by my brother Adolphe Lesson, in the forests around Realejo in Nicaragua province." Because neither specimen was selected as a holotype, the name was based on syntypes. Cecile Callou, curator in charge of the mammalian types, Muséum national d´histoire naturelle, Paris, France, reports that only the female specimen is present in the collections and they have no information on

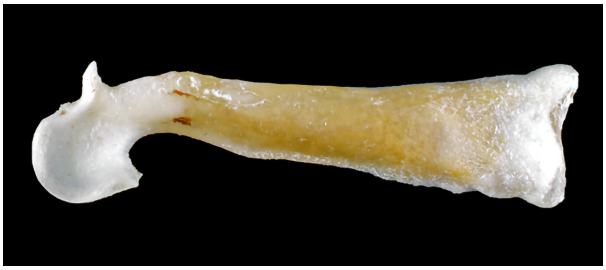

Figure 6. Baculum of *Sciurus variegatoides ometepensis* (KU 115312; paratype) from 2 km N Mérida, Isla de Ometepe, Departmento de Rivas, Nicaragua. Total length = 12.8 mm.

the male specimen. The female specimen, therefore, is designated as the lectotype for this taxon and the male will be the paralectotype if found.

Sciurus variegatoides adolphei was obtained at most collecting localities on the Cosigüina Peninsula and was common locally in the taller forests especially along streams on the peninsula. Field collectors noted that higher concentrations occurred in those areas that had tall evergreen trees growing along hot springs; however, in one case, an adult was shot while foraging in a mimosa tree (*Albizia*). The sample from Hacienda Las Colinas is the first record from the Departmento de León and the southernmost record for this subspecies. These squirrels match those from Departmento de Chinandega in overall large size and dorsal and ventral color patterns. There is no discernable evidence of integration of *S. v. adolphei* with *S. v. dorsalis* the subspecies occurring to the south (see account of that subspecies) or with *S. v. underwoodi*, the subspecies occurring directly to the east (see account of that subspecies).

As discussed under Historical Collecting Sites, the San Cristóbal volcanic complex in the Departmento de Chinandega, which is composed of five volcanoes, also has been known historically as Volcán Chinandega and Volcán El Viejo (Rossi et al. 2010). Harris (1937) reported two specimens as *S. v. adolphei* (AMNH 28439, 28443) from Volcán Chinandega and one specimen as *S. v. underwoodi* (AMNH 41231) from Volcán El Viejo. It seems an unlikely situation to have two subspecies occupying this volcanic complex. There is a series of eight squirrels from Hacienda Bellavista on Volcán Casita, which is one of five volcanoes that make up the San Cristóbal complex. These squirrels are somewhat variable in color, ranging from a dark, nearly black dorsum to dark browns and tans but in general matching those individuals from the coast and the Cosigüina Peninsula in both size and over all color pattern; therefore, all squirrels from San Cristóbal are assigned to *S. v. adolphei*, including AMNH 41231.

On 19 August 1967, two males were collected 7 km S, 1 km E of Cosigüina. The specimens weighed 472.2 and 610 g and had testes lengths of 8 and 27 mm. The smaller individual was judged to be a subadult based on its paler pelage and unfused cranial sutures. Between 1 and 6 March 1968, 12 additional specimens were obtained from the peninsula. Testes measurements and corresponding weights of four males are 20, 30, 31, and 34 mm and 576.4, 615.8, 642.2, and 673.3 g. Six adult females revealed no obvious reproductive activity, although they had enlarged teats, but were not lactating when taken on 2 and 3 March 1968, two on 6 March 1968, and on 14 and 16 July 1966. Lactating adults were taken on 4 and 6 March 1968; they weighed 740.5 and 761.7 g, respectively. Four other females had weights of 490.0, 656.0, 668.8, and 699.9 g. At Hacienda Las Colinas near Lago de Managua, three males and three females collected on 5 and 6 December 1962 had the following weights, respectively: 681, 681, 790; 795, 909, 909 g. Along the northwest coast near El Realejo, a male taken on 29 October 1967 weighed 526 g. A female from San Antonio, also along the coast, evinced no obvious reproductive activity on 9 March 1968.

Squirrels from the Cosigüina Peninsula showed multiple molt lines, which differed in distinctness and regularity, contributing to the variable appearance among individuals. Although molt does not always begin in the same area, it usually begins anteriorly and proceeds posteriorly. Single molt lines usually extend more posteriorly on the dorsum than on the lateral surfaces. No molt lines were observed on the venter (Genoways and Timm 2005). Molt from subadult pelage to adult pelage is evident in a specimen taken on 6 March.

Medina-Fitoria et al. (2018) reported that this subspecies is found from sea level to 800 m. Medina-Fitoria et al. (2018) assigned a photograph of a squirrel from Reserva Silvestre Privada Hato Nuevo,

Departmento de Chinandega to *S. v. underwoodi*. This record is from well within the geographic range of *S. v. adolphei*, to which it is now reassigned, and illustrates some of the problems of identifying mammals from a photograph. Only about half of the color and color pattern of the animal in this photograph is observable and while seemingly somewhat atypical for *S. v. adolphei*, without the specimen in hand, it is best assigned as such based on geographic location.

In a preliminary survey for leptospirosis in Nicaragua, Clark et al. (1966) obtained negative results after examining 18 specimens of *S. v. adolphei* from the vicinity of Puerto Momotombo.

Specimens examined (48).—Departmento de Chinandega: Corinto (1 ♂, AMNH 41229); 6 1/2 km N, 1 km E Cosigüina, 10 m (7, 4 ♂♂, 3 ♀♀, KU 115239–245); 4 1/2 km N Cosigüina, 15 m (2, 1 ♂, 1 ♀, KU 115246–247); 7 km S, 1 km E Cosigüina, 10 m (2 ♂♂, KU 110304–305); El Paraíso, 1 km N Cosigüina, 20 m (3 ♀♀, KU 115248–250); Hacienda Bellavista, Volcán Casita, 720 m (8, 4 ♂♂, 4 ♀♀, KU 106344–351); Hacienda San Isidro, El Realejo (3, 2 ♂♂, 1 ♀, KU 104590, NMNH 337570–571); San Antonio, 35 m (1 ♀, KU 115251); Volcán de Chinandega (2, 1 ♂, 1 ♀ AMNH 28439, 28443); Volcán Viejo (1 ♂, AMNH 41231). Departmento de León: Hacienda Las Colinas; 4 km WNW Puerto Momotombo (18, 11 ♂♂, 7 ♀♀, KU 104372–379, NMNH 334597–598, 337755–762).

Additional record.—Departmento de Chinandega: Reserva Silvestre Privada Hato Nuevo (Medina-Fitoria et al. 2018).

***Sciurus variegatoides belti* Nelson, 1899**

1899. *Sciurus boothiae belti* Nelson, Proceedings of the Washington Academy of Science 1:78.

1937. *Sciurus variegatoides belti*, Harris, Miscellaneous Publications of the Museum of Zoology, University of Michigan 38:13.

Holotype.—NMNH 36477/48847, female adult, skin and skull in good condition, collected by Charles W. Richmond, on 22 November 1892.

Type locality.—Escondido River, 50 miles from Bluefields [= I. P. Plantation, 3 km S, 13 km E Rama], South Caribbean Coast Autonomous Region, Nicaragua.

Remarks.—This subspecies is among the medium-sized variegated squirrels in Nicaragua, but on average, the nasals bones are the shortest in these squirrels (Table 1). These are the darkest of the Nicaraguan variegated squirrels. Dorsally, the three-banded hairs have a 6–7 mm black basal band, a 5–6 mm central silver or tan band, and a short, black terminal tip resulting in the appearance of a dark animal with silver to tan interspersed (Fig. 2). The dark dorsum coloration continues laterally over the sides to the ventrum. The ventrum on most individuals varies from dark orange–chestnut to deep chestnut; a few individuals have small patches of white. The auricular patch is a pale tan to chestnut and extends posteriorly 5–6 mm. The feet are dark agouti to black. A dark, approaching black, phase is present albeit rare in these squirrels.

This is the widest-ranging subspecies of the *S. variegatoides* complex in Nicaragua (Fig. 5), occurring throughout the Caribbean Lowlands east of the lakes between the Río Coco in the north and the Río San Juan in the south and into the eastern slopes and river valleys of the Central Highlands. Medina-Fitoria et al. (2018) reported that *S. v. belti* is found in the Caribbean Lowlands up to 1,500 m.

Specimens collected by Richardson in Nicaragua remain a challenge not only because of their locality data, but also because many come from zones of intergradation between combinations of the taxa *belti*, *boothiae*, and *underwoodi*. Nine specimens taken along the Río Coco in extreme northern Departmento de Jinotega at an altitude of 1,000 feet were assigned by Allen (1910:101) to *boothiae* but he remarked that they graded toward *belti*. However, Harris (1937) placed them with *belti* because the color of the back, ear patches, and the rufous tone the underparts were within the range of variation of that subspecies, and this arrangement is followed here. Both Allen (1908, 1910) and Harris (1937) assigned another group of thirteen specimens taken by Richardson in 1907–1909 at Matagalpa, Uluce, Peña Blanca, Savala, and Chontales to *belti*. These specimens are similar to specimens from Río Coco, but Harris (1937) believed they differ

from these in having more rusty rufous and less white on the underparts. However, as with other specimens from this region, they grade toward *boothiae*, and in agreement with Harris (1937) his assignment to *S. v. belti* is maintained herein. The assignment herein of the three specimens collected in the 1960s from Hacienda La Trampa, in this same region, to *belti* are in agreement with Harris that squirrels of this subspecies from the Caribbean Lowlands are making their way into the eastern slopes of the Central Highlands.

Nine specimens collected by Richardson at "Matagalpa," which was his home, were examined by Harris (1937) who assigned three of the specimens to *belti* and six to *underwoodi*, which seems an unlikely situation. As Howell (1993, 2010:3) stated: "Many of Richardson's Nicaraguan collecting localities were small villages not now found on most maps or were given as undelimited geographic features." Richardson never gave directions or distances from a locality, seldom listed the departments, and his elevations were estimates. Because Matagalpa was his home, he almost certainly collected in several directions from the town and probably listed the town for any sites within a day's travel from the town (see Historical Collecting Sites above). Harris (1937) surmised that specimens assignable to *belti* came from east of town and those assignable to *underwoodi* came from north or west of Matagalpa. The suggestion by Harris (1937) that the specimens assigned to *belti* were from east of Matagalpa seems less likely given the current sample of *underwoodi* from 3 mi SE San Ramon. The nearest specimen record considered *belti* to the east is from Uluce about 45 km east of Matagalpa and 34 km beyond San Ramon. The other potential direction for the source of these specimens would be to the northeast along the road to El Tuma. There are records of *S. v. belti* in this direction at Peña Blanca and La Trampa, which are at least 50 km to the northeast. These distances from Matagalpa all seem to be too far even for Richardson to have maintained the use of the "Matagalpa" locality designation, but the designation "Matagalpa" may have only been to the department name. Also one cannot discount the possibility that these specimens of *belti* designated as from "Matagalpa" were mislabeled either by Richardson or in the handling and shipping of the specimens to the American Museum of Natural History. Given this degree of uncertainty about the origins of these specimens, they are maintained herein in the list of specimens here, but not placed on the distribution map.

The single specimen from San Francisco, Departmento de Boaco, is the southern-most example of *S. v. underwoodi* (see account of that subspecies for further discussion). However, three specimens from Hato Grande approximately 42 km south of San Francisco appear to be intergrades between *belti* and *underwoodi*. Their dorsal coloration more closely matches that of *belti* as does the auricular patch; however, the ventrum is not typical of either one, being a dull chestnut and lacking white coloration. The series available from two localities in the vicinity of Villa Sandino, some 75 km southeast of San Francisco, also appear to be intergrades. Based on total evidence for squirrels from these three sites places them among the *S. v. belti* specimens examined. About 45 km northeast of San Francisco, a sample of seven squirrels from Santa Rosa in northeastern Departmento de Boaco is best assigned to *S. v. belti*, which is one of the western-most samples of this subspecies.

All specimens of variegated squirrels from Nicaragua's Caribbean Lowlands are treated herein as belonging to *S. v. belti*, although clearly additional specimens from throughout the lowlands are needed. Although *S. v. thomasi* has been reported from southeastern Nicaragua (Medina-Fitoria et al. 2018), the specimens of *S. v. thomasi* examined from Costa Rica, including the holotype and those from near the type locality, all differ from Nicaraguan *belti* in being considerably darker dorsally, possessing a bright orange ventrum, dark orange or black (or a combination of both) hind feet dorsally, and with a tan to dark orange auricular patch. The free-ranging individual photographed at Refugio Bartola along the Río San Juan (see Medina-Fitoria et al. 2018:fig. 24) clearly approaches this color pattern of *thomasi* and specimens from this area are needed to more fully access this population. Specimens reported from Departmento Río San Juan, as belonging to *S. v. thomasi* by Medina-Fitoria et al. (2018), are perhaps best considered assigned to *S. v. belti*. Harris (1937) assigned specimens from along the Río San Juan at Sebaco near the lake and from Greytown at the mouth of the river to *S. v. belti*. Because there are few specimens of squirrels (or other mammals) from the Nicaraguan–Costa Rican border region, a more complete understanding of this fauna will only

be possible when additional specimens are available. The few roads extending into Nicaragua's Caribbean Lowlands have clearly contributed to habitats being protected; however, few specimens of any species of mammals are available for study (Martínez-Fonseca et al. 2018).

A female taken at El Recreo (KU 115261) on 26 February 1968 carried three embryos with one in the right horn of the uterus and one in the left measuring 13 mm in crown–rump length and a second embryo in the left horn measuring 8 mm in crown–rump length. Four adult females were judged to be lactating on the following dates: 4 April 1968, 19 June 1967, 11 July 1967, and 4 August 1967. Females evincing no obvious reproductive activity were taken on the following dates: 16 June 1967, 2; 19 June 1967, 1; 22 June 1967, 1; 11 July 1967, 1; 13 July 1967, 2; 24 July 1967, 1; 6 August 1967, 2; and 7 August 1967, 1. Three individuals judged to be juveniles because the third upper molar had not erupted were taken on the following dates: two on 23 April 1963 and one on 28 April 1968. Testes lengths of adult male *S. v. belti* were as follows: 25–26 February 1968—7, 21, 21, 25 mm; 19–25 June 1967—6, 25, 30, 31 mm; 11 July 1967—24, 26 mm; 24 July 1966—23 mm; 4–7 August 1967—23, 24, 24, 26 mm. Adult females taken on 19 March 1963 and 23 April 1963 weighed, respectively, 454 and 577 g, whereas adult males taken on 16 and 23 April 1963 weighed 489.5 and 435 g, respectively.

A total of 394 specimens of *Sciurus variegatoides* from throughout Nicaragua were examined and only three are abnormally black—the specimen from Isla de Ometepe (KU 115308, described above), a young male from the Villa Sandino region (KU 110329), and an adult male from Greytown (NMNH 16412). The latter two localities are approximately 185 km apart. The specimen from Villa Sandino appears nearly black dorsally with a pale orange and white venter. Dorsally, most hairs have a short black basal band, a short agouti middle band, and a long black terminal band. Dorsal agouti-colored hairs with narrow terminal black bands give the overall coloration of a mostly black individual with some agouti interspersed. The adult male from Greytown has generally broad (> 50% length of hair) basal black bands, narrow deep orange middle bands, and broad black terminal bands. The venter and tail hairs are all black. The overall aspect is a black squirrel, showing orange highlights on close inspection. An additional black variegated squirrel from Refugio Bartola, Río San Juan, including a photograph of a free ranging animal, was reported by Medina-Fitoria et al. (2018). It too has some agouti colored hairs scattered throughout the dorsum. A single adult male from extreme northern Costa Rica (KU) is black dorsally with white and agouti colored hairs ventrally and agouti colored hairs laterally. All of the black-appearing squirrels from this region have some banded hairs and it is the terminal band that is longest and black in color. The *S. variegatoides* of the Caribbean Lowland Evergreen Rainforest are among the most variably colored individuals of the Neotropical variegated squirrel complex and exceeded only by *S. v. ometepensis*.

In a study of the use of dogs by indigenous hunters from Arang Dak in the Bosawas Biosphere Reserve in extreme northeastern Departmento de Jinotega, Koster (2008) presented a list of 20 species of mammals that were harvested among which were five individuals of *S. variegatoides*. Neotropical variegated squirrels were low on the target species list for the hunters, probably because of the low biomass return from the squirrels. Palmer (1945) reported that *butsong* was the Misquito name for the Neotropical variegated squirrel and *tete* was the Sumu name. Jones (1965:354) reported that the Miskito in the Caribbean Lowlands of Nicaragua had two indigenous names for *Sciurus variegatoides*, "butsung or tastas (the two names evidently are used to distinguish between different color phases of this species)." Clark et al. (1966) examined one Neotropical variegated squirrel from the vicinity of Villa Sandino and 35 from El Recreo for leptospirosis, but had only negative results from these squirrels.

Specimens examined (119).—Departmento de Boaco: Chontales (2 ♀♀, AMNH 28588, 28591); Santa Rosa, 17 km N, 15 km E Boaco (7, 2 ♂♂, 5 ♀♀, KU 110307–312). Departmento de Chontales: Hato Grande, 13 km S, 8 km W Juigalpa (3, 3 ♂♂, KU 115291–293); 1 km N, 2.5 km W Villa Sandino [= Villa Somoza] (10, 6 ♂♂, 4 ♀♀, KU 110327–336); Villa Sandino [= Villa Somoza] (1 ♂, KU 104474). Departmento de Jinotega: Hacienda La Trampa, 5.5 km N, 16 km E Jinotega (3, 2 ♂♂, 1 ♀, KU 99464, NMNH 338824–825); Río Coco (9, 8 ♂♂, 1 ♀, AMNH 29235–238, 29243–244, 29247–248, 29250). Departmento de Matagalpa: Matagalpa (3, 1 ♂, 2 ♀♀, AMNH 28319–321); Peña

Blanca (2, 1 ♂, 1 ♀, AMNH 29810–811); Savala (1 ♂, AMNH 28414); Uluce (5, 4 ♂♂, 1 ♀, AMNH 29805–809). Departmento de Río San Juan: Greytown (1 ♂, NMNH 16412/A23227); Los Sabalos on the Río San Juan (1 ♂, AMNH 41230). North Caribbean Coast Autonomous Region [= Zelaya]: Bonanza (5, 2 ♂♂, 3 ♀♀, KU 96366–367, 99463). South Caribbean Coast Autonomous Region [= Zelaya]: El Recreo (34, 15 ♂♂, 19 ♀♀, KU 104462–463, 104371, 106352–354, 110313–325, 115253–261, NMNH 337738–741, 337746, 337748, 337754); La Esperanza, Río Siguia (19, 13 ♂♂, 6 ♀♀, KU 104464–473); Cara de Mono (2 ♂♂, KU 110326, 115252); 4.5 km NW Rama (5, 1 ♂, 4 ♀♀, TTU 12593–597); Escondido River, 50 mi from Bluefields (6, 3 ♂♂, 3 ♀♀, NMNH 36477/A48847 [holotype], A48873, 51335–338).

Additional records.—Departmento de Jinotega: Arang Dak [14°30′56″N, 85°00′00″W] (Koster 2008). Departmento de Río San Juan: Refugio Bartola (Medina-Fitoria et al. 2018). North Caribbean Coast Autonomous Region: Edén (Harris 1937). South Caribbean Coast Autonomous Region: La Cruz de Río Grande (Medina-Fitoria et al. 2018); Río Sconfra (Medina-Fitoria 2016); Casa Vieja, Río Punta Gorda, Reserva Natural Punta Gorda (Medina-Fitoria et al. 2016).

Sciurus variegatoides boothiae Gray, 1843

1842. *Sciurus richardsoni* Gray, Annals and Magazine of Natural History, series 1, 10:264. Preoccupied by *Sciurus richardsoni* Bachman,1839, Proceedings of Zoological Society of London, for 1838, p. 100, now considered to be *Tamiasciurus hudsonius richardsoni*.

1843. *Sciurus boothiae* Gray, List of the Specimens of Mammalia in the Collection of the British Museum, Trustee of Museum, London p. 139.

1937. *Sciurus variegatoides boothiae*, Harris, Miscellaneous Publications of the Museum of Zoology, University of Michigan 38:12.

Holotype.—BM(NH) 1842.10.28.43, a juvenile of unknown sex, skin originally a taxidermy mount, but remade into a round museum specimen, with the skull remaining in the skin, received from Mr. Warwick's collection.

Type locality.—"From Honduras," restricted by Nelson (1899) to Honduras: Departmento de Cortés; San Pedro Sula.

Remarks.—These northern Nicaraguan squirrels are on average among the medium-sized variegated squirrels in the country (Table 1); however, as the classification matrix (Table 3) shows this taxon is poorly defined morphometrically. Records of this subspecies in Nicaragua are confined to the mountains of Departmento de Nueva Segovia and an adjacent part of the Departmento de Madriz (Fig. 5) in the extreme north-central part of the country. Harris's specimen from San Juan de Telpaneca, Departamento de Nueva Segovia, was not examined so Harris (1937) was followed in this designation.

This is a dark squirrel in overall in color pattern, with most specimens nearly as dark as *S. v. belti* (Fig. 2). Dorsally, the appearance is black with dark tan–agouti mix. All hairs on the dorsum are three banded having a black base (15–17 mm), an orange–tan middle band approximating 5 mm, and a black, 5–6 mm terminal band. The rusty–tan–cinnamon auricular patch contrasts with the dorsum with the paler color extending only 5–6 mm behind the basal center of the ear. The venter is white, and in many individuals, unicolored bright white. There is usually a sharp contrast between the dark dorsum and the white ventrum producing a strongly contrasting countershading. The hind feet are black with some agouti–tan hairs mixed in.

Harris (1937) described the relationship between *boothiae* and *belti* specimens collected by Richardson in the departments of Jinotega and Matagalpa. He assigned many to *belti*, including material from Río Coco, Matagalpa, Uluce, Peña Blanca, Savala, and Chontales. Although Harris assigned all of these specimens, except six from Matagalpa, to *belti*, he commented twice that these specimens "show intergradation with *boothiae*" and "all of which grade toward *boothiae*." Three specimens collected in the 1960s from Hacienda La Trampa in this region are assigned to *belti*. Therefore, in agreement with Harris, *belti* squirrels from the Caribbean Lowlands occur throughout the eastern side of the Central Highlands. These lowland squirrels perhaps migrated westward along the lowlands associated with major eastward-flowing rivers such as the Río Coco and

the Río Grande de Matagalpa and its major left tributary the Río Tuma. Along these rivers and their tributaries individuals of lowland *belti* encounter individuals of *boothiae* from the uplands thus forming a series of points of intergradation much as described by Howell (1993, 2010) in other groups from this region.

Reproductive data for a series of *S. v. boothiae* from the vicinity of Jalapa were collected between 21 and 27 July 1967. Two adult females were lactating, whereas three others evinced no obvious reproductive activity during this period. A juvenile squirrel with an unerupted upper M3 was taken on 24 July. Nine adult male from this period had a mean testes length of 23.9 (9–35 mm). Medina-Fitoria et al. (2018) reported that this subspecies is found from 500 to 2,000 m.

Specimens examined (35).—Departmento de Nueva Segovia: 7 km N, 4 km E Jalapa (1 ♂, KU 110339); 6.5 km N, 1 km E Jalapa (3, 2 ♂♂, 1 ♀, KU 110359–361); 5 km N, 2.5 km E Jalapa (4, 1 ♂, 3 ♀♀, KU 110340–343); 4.5 km N, 2 km E Jalapa (15, 9 ♂♂, 6 ♀♀, KU 110344–358); 1.5 km N, 1 km E Jalapa (3, 2 ♂♂, 1 ♀, KU 110259–261); Jalapa (4, 3 ♂♂, 1 ♀, AMNH 29241–242, 29273–274); 3.5 km S, 2 km W Jalapa (2, 1 ♂, 1 ♀, KU 110362–363); Jicaro (1 ♀, AMNH 29249); 2.5 km NE Totecacinthe (2 ♂♂, KU 110337–338).

Additional record.—Departmento de Madriz: San Juan de Telpaneca (AMNH 29239) (Harris 1937).

Sciurus variegatoides dorsalis Gray, 1849

1849. *Sciurus dorsalis* Gray, Proceedings of the Zoological Society of London [1848] Part 16:138.

1920. *Sciurus variegatoides dorsalis*, Goldman, Smithsonian Miscellaneous Collection 69(5):136.

Holotype.—BM(NH) 1848.10.26.4 (skin) and BM(NH) 1848.11.10.5 (skull), male, skin, originally a taxidermy mount, but remade into a round museum specimen, and skull damaged but some measurements can still be taken, received from M. Sallé via W. Cumming.

Type locality.—Originally given as Caracas, Venezuela (Gray 1848), but later restricted to Costa Rica by Gray (1867) and finally further restricted to Liberia, Guanacaste Province, Costa Rica by Nelson (1899).

Remarks.—Along with individuals of *S. v. adolphei* to the north, these are the largest variegated squirrels in Nicaragua. The extensive sample of *S. v. dorsalis* averaged the second largest to *adolphei* in seven of the nine cranial measurements and was the widest for postorbital breadth (Table 1). A strongly-marked, generally black, but in some individuals interspersed with brown, or rarely brown dorsal stripe is present in all specimens of this subspecies (Fig. 2; Medina-Fitoria et al. 2018:fig. 22). When the stripe is black, the dorsal hairs forming it are not banded but are black throughout. The overall white appearing sides and venter is formed by two-banded hairs, the basal half being gray and the terminal half white giving sharply contrasting white sides and venter to the black (or brown) dorsal stripe. The auricular patch is white and either consists of unicolored white hairs throughout or hairs with a gray base and white terminal band. There is not as sharp a contrast between the ear patches and body coloration in this subspecies as there is in all other subspecies because the auricular patch tends to be a continuation of the white sides. The interspersal of black and white hairs on the tail trends towards white hairs in this subspecies giving the overall appearance of a paler, whiter tail than in any other subspecies throughout the species' geographic range.

This distinctive subspecies occurs throughout much of the dry forest of western Nicaragua and south to Santa Cruz in Costa Rica's Guanacaste lowlands. In Nicaragua, *S. v. dorsalis* occurs in the area between Lago de Managua and Lago de Nicaragua, and Managua itself. Along the southeastern shore of Lago de Managua, the subspecies occurs as far north as Tipitapa and along the southwestern shore of the lake, as far north as Lago de Jiloa. It occurs from Lago de Managua southward along the western side of Lago de Nicaragua in the departments of Carazo, Granada, Managua, Masaya, and Rivas.

North of Lago de Jiloa along the Pacific coast, the next sample of *S. variegatoides* is from Hacienda Las Colinas near Puerto Momotombo at the northern edge of Lago de Managua in Departmento de León. These squirrels are typical of *S. v. adolphei* and show no characteristics of *S. v. dorsalis*. No samples between Lago

de Jiloa and Las Colinas are available to determine if there is a definable zone of intergradation. If such a zone occurred in the past, it may not be present today because this region has been heavily settled since the colonial period and is under intense agricultural use. From Las Colinas northward, *S. v. adolphei* occurs into the Departmento of Chinandega along the volcanoes of the Cordillera de los Marrabios (Genoways and Timm 2005). To the east of Managua and Tipitapa, the nearest record of variegated squirrels is a specimen from San Francisco, Departmento de Boaco, which are the southern-most *S. v. underwoodi* known. There are no specimens of squirrels in the intervening 45-km gap from Tipitapa to San Francisco and there is no indication of intergradation between these two taxa anywhere throughout the potential contact zones. The intervening area is part of the Central Depression and is under intensive agricultural use. *S. v. dorsalis* is a distinctive subspecies that seems to have a fairly well-defined distribution in the western dry forests of Costa Rica and Nicaragua. In Costa Rica, Harris (1937) noted a zone of integration with *S. v. atrirufus* on the Nicoya Peninsula. McPherson (1971, 1985) wrote that in the Pacific lowlands of Costa Rica "intergrades between *S. v. dorsalis* and *S. v. thomasi* are found … there is a complex zone of intergradation involving *S. v. rigidus*, *S. v. melania*, and *S. v. dorsalis*. The subspecies *S. v. austini*, described by Harris (1933), is an intergrade between *S. v. rigidus* and *S. v. melania*" (1985:162). However, in a series of 16 specimens obtained by Timm from the Upala area of northern Costa Rica just to the east of known specimens of *S. variegatoides dorsalis*, there is no evidence of integration of these squirrels with *S. v. thomasi*.

Reproductive data for *S. v. dorsalis* are not extensive, but the few data available are as follow. Lactating females were taken on 16 June 1966 and 11 August 1967. Adult females with enlarged teats but not obviously pregnant or lactating were taken on the following dates: 4 March 1956, 1; 31 March 1968, 2; 7 April 1956, 1; 13 June 1966, 2; 16 June 1956, 1; 16 June 1966 1; 26 June 1956, 1; 11 August 1967, 4; 13 August 1967, 1. Testes length for adult males are as follows: 31 March 1968—22, 22, 23, 24, 33 mm; 25 April 1968—23, 25 mm; 13 June 1966—7 mm; 21 June 1966—26 mm; 22 June 1966—27 mm; 11–13 August 1967—8, 9, 10, 24, 26 mm. Weights of two adult males were 681 (1 July 1964) and 596 g (9 July 1964), whereas the weights of two adult females were 900 (8 August 1963) and 455 g (9 October 1964). A juvenile nulliparous female weighing 213.7 g, molting, and with erupting permanent teeth was taken on 11 August 1967. A nulliparous female weighing 232.9 g, also molting, but with all permanent teeth in place, was taken on 31 March 1968.

The collecting site at 3 km N, 4 km W Diriamba was a large coffee finca with an elevation of about 550 m in the highlands of Departmento de Carazo. This was a typical coffee finca having the original large overstory trees remaining in place with the understory trees and bushes removed and replaced by the coffee trees. Neotropical variegated squirrels nested and carried on much of their activities in the tall overstory trees, but they were predatory on the developing coffee beans to the point that they were actively hunted by the managers of the finca. These squirrels also were a pest when the coffee beans were on the drying platforms. At other places in Nicaragua, these squirrels were considered to be pests in the cacao plantations. Medina-Fitoria et al. (2018) reported that *S. v. dorsalis* occurs up to 1,000 m and provided two images of free-ranging individuals of the characteristic color pattern.

Webb and Loomis (1970) described the chigger *Microtrombicula nicaraguae* from a specimen of *S. v. dorsalis* (KU 106357) collected at Finca Santa Cecilia, Departmento de Granada.

Medina-Fitoria et al. (2018:fig. 33) presented a photograph of a squirrel from El Abuelo, Departmento de Rivas, from the southern shore of Lago de Nicaragua. This is an area that would be just to the east of the peripheral geographic range of *S. v. dorsalis* as currently understood, but this individual does not appear to be a *dorsalis* and, in fact, it cannot be placed in any of the currently recognized groups of Nicaraguan squirrels. This individual is not mapped or the locality listed below and the identity of squirrels from this area of Nicaragua and adjacent Costa Rica will not be known until specimens are available for study.

Specimens examined (93).—Departmento de Carazo: 3 km N, 4 km W Diriamba (41, 24 ♂♂, 17 ♀♀, KU 110364–390, 115263–276); 3 mi NNW Diriamba (1 ♀, KU 71550). Departmento de Granada: Finca Santa Cecilia, 6.5 km SE Guanacaste (7, 1 ♂, 6 ♀♀

KU 106355–361); La Calera, 3 mi S, 5 mi W Nandaime (18, 11 ♂♂, 7 ♀♀, KU 108186–192); Mecatepe (1 ♀, NMNH 339949). Departmento de Managua: Hacienda Azacualpa (2, 1 ♂, 1 ♀ KU 108393; NMNH 361236); 1 mi SSE Las Conchitas (1 ♀, KU 71549); Lake Jiloa (2 ♂♂, AMNH 176694–695); 6 mi WSW Managua (2, 1 ♂, 1 ♀, KU 71545–546); 10 mi SW Managua (1 ♂, KU 71970); Tipitapa (1 ♂, AMNH 41232). Departmento de Masaya: 9 mi NW Masaya (2 ♂♂, KU 71547–548). Departmento de Rivas: Finca Amayo, 13 km S, 14 km E Rivas (11, 7 ♂♂, 4 ♀♀ KU 104701–704, 106362–364); 3 mi SE La Virgen (1 ♂, KU 71551); 8 km NE San Juan del Sur (2, 1 ♂, 1 ♀ KU 106365–366).

Additional record.—Departamento de Masaya: 21 km S Managua "common in the region" (J. Hruska, pers. comm.).

***Sciurus variegatoides underwoodi* Goldman, 1932**

1932. *Sciurus boothiae underwoodi* Goldman, Journal of the Washington Academy of Science 22(10):275.

1937. *Sciurus variegatoides underwoodi*, Harris, Miscellaneous Publications of the Museum of Zoology, University of Michigan 38:9.

Holotype.—NMNH 250219, adult male, skin and skull (left zygomatic arch broken), collected by Cecil F. Underwood on 8 December 1931.

Type locality.—Monte Redondo, about 30 miles NW Tegucigalpa, Departmento de Francisco Morazán, Honduras.

Remarks.—This is the third taxon of medium-sized variegated squirrels occurring in Nicaragua (Table 1). These squirrels average larger than the other medium-sized taxa, *belti* and *boothiae*, but only two-thirds of the specimens in the analyzed sample of *underwoodi* classify correctly in the discriminate function analyses. Five of the 10 misclassified squirrels are classified as *boothiae*, which was a taxon that Harris (1937) believed was influencing some of the specimens of *underwoodi* he examined.

Dorsally the overall appearance is a mixture of black and dark silver giving an overall dark, somewhat mottled appearance. Dorsal coloration extends over the sides to the ventrum, often extending further ventrally than the lateral coloration in other taxa (Fig. 2). Dorsal hairs are three banded with a black base (ca. 9 mm), dull silver middle band, and short black terminal band. The ventrum is countershaded, but highly variable with a moderate mix of chestnut and white patches or is uniformly dull white. The auricular patch is small, dull white and not contrasting as sharply as in other taxa, and extends back only some 5 mm. The dorsal hind feet are dark, with a mix of dark chestnut or dark silver hairs resulting mostly in an overall mixed agouti pattern.

Harris (1937:10) wrote: "When more material is available the relationships of *underwoodi* to other forms can be more clearly understood, and the limits of its range better defined." He had only material from San Rafael del Norte and Matagalpa for study, but the material available to us covers a much larger geographic area. In northern Nicaragua, specimens collected by the KU field parties provide new records from the departments of Madriz and Estelí. These sites place *underwoodi* to west and somewhat south of sites where *boothiae* occurs. These two subspecies probably approach each other most closely in eastern Departmento de Madriz at Venecia (*underwoodi*) and San Juan de Telpaneca (*boothiae*). To the south of these sites, there are two localities represented by KU material from near Yali and Richardson's specimens from San Rafael del Norte examined by Harris (1937).

Harris (1937) had the unusual situation of having Richardson's specimens labeled as "Matagalpa" that he assigned to *underwoodi* and others to *belti*. The six specimens from Matagalpa were the southernmost representatives of *S. v. underwoodi* that Harris (1937) had available for study and their relationship with *belti* was not readily apparent. With more material now available, the distributions of these taxa can now be refined. About 22 km north of Matagalpa near the border of Jinotega, there is a large sample from Santa Maria de Ostuma, which is a close match to the description and understanding of *S. v. underwoodi* in Nicaragua presented below. East of Matagalpa at 3 mi SE San Ramon, two specimens appear to be typical *underwoodi*. They are most similar to each other and similar to other specimens identified as *underwoodi*. Dorsal coloration and ear patches are typical of the subspecies, with feet grizzled agouti and ventrum

very light orange, almost tan. There appears to be no influence from the lowland *belti* in this color pattern. Southwest of Matagalpa there is a single specimen from Sebaco, but it is a juvenile and its color pattern is of no real value to this discussion. Between 50 and 60 km to the south of Matagalpa are three localities (11 mi SE Dario and two near Esquipulas) that are assigned to *S. v. underwoodi*.

A single specimen from San Francisco, Departmento de Boaco, at 135 km south of Matagalpa is treated as *S. v. underwoodi*. Dorsally, this specimen's coloration best matches that of *underwoodi* as does the auricular patch; however, the ventrum is a dull chestnut with no interspersed white, which is not typical of any taxon examined herein. This specimen from San Francisco is the southern-most record of *S. v. underwoodi*. As discussed in the *belti* account, the three specimens from Hato Grande approximately 42 km south of San Francisco and the series from the vicinity of Villa Sandino about 75 km southeast of San Francisco, combine color patterns of *belti* and *underwoodi*, but the predominance of evidence places them with *S. v. belti*, as assigned above. The latter two sites lie west and east, respectively, of the relatively low Cordillera Chontaleña, which forms the divide between rivers that flows directly into Lago de Nicaragua and those that flow eastward into the Caribbean. This small range of mountains represents the southern-most extension of the Central Highlands of Nicaragua and the southern-most topographic feature influencing the distribution of *S. v. underwoodi*.

After this review of the distribution of *S. v. underwoodi*, what can be said about the six specimens of *underwoodi* and the three specimens of *belti* labeled by Richardson from Matagalpa? In the available material, there are specimens of *underwoodi* in all directions from Matagalpa, which leads to the conclusion that within the environs of Matagalpa, *S. v. underwoodi* should be expected, including these six specimens. The difficulty of determining the source and relationships of the three *belti* specimens in the account for that subspecies are discussed above.

The distribution map of *S. variegatoides* in Hall and Kelson (1959) and Hall (1981) that shows the distribution of *S. v. underwoodi* extending along the dry forest west of Lago de Nicaragua into extreme northwestern Costa Rica is in error. This record was based on Goodwin (1946:360) who reported a single specimen from "Prov. Guanacaste: Liberia, Port Parker Bay." The online catalogue of the American Museum of Natural History lists this specimen, which is a skin only, as AMNH 140235, with the locality as "Port Parker Bay," Paquera, Puntarenas Province, collected by C. William Beebe on 23 January 1938. Paquera is located on the extreme southeastern coast of Costa Rica's Nicoya Peninsula, well within the geographic range of *S. v. atrirufus* (Timm et al. 2009). The American Museum's entry is in error when compared with the original handwritten catalogue where the locality for this specimen is given as "Costa Rica: Port Parker Bay?" with the remaining data in agreement with the online information. This brings into question the exact location of Port Parker Bay, Costa Rica. The marine survey in which Beebe was involved places this location at 10°56′N, 85°49′W (Fraser 1943), which is a long abandoned and now washed out port along the north shore of the Santa Elena Peninsula. Goodwin (1946) reported that William Beebe saw several squirrels at Port Parker, but the one he secured was shot in a gully about a mile back of the Port Parker beach. This specimen and the current populations of Santa Elena Peninsula should be reassigned to *S. variegatoides dorsalis* until additional material is available for study.

To the west of populations of *S. v. underwoodi* in Nicaragua are populations of *S. v. adolphei* in the departments of Chinandega and León. These populations are separated by the Central Depression of Nicaragua, which is an area where there are no specimens for study. Historically, there may have been variegated squirrels in this area, but if they occur there today, they are widely dispersed and in low numbers. This is an area of high human population and extensive agricultural crops and ranching operations. The specimens from Matagalpa and San Raphel del Norte, which J. A. Allen (1910) originally identified as the taxon *S. v. variegatoides*, were subsequently assigned to *S. v. underwoodi* by Harris (1937), although he did see some influence of *S. v. boothiae* in them.

Medina-Fitoria et al. (2018) determined that a photographed squirrel from Selva Negra was *S. v. boothiae* and one from Natural Reserve Cerro Arenal was an undetermined taxon. Variegated quirrels from northern Departmento de Matagalpa have been as-

signed here to *S. v. underwoodi.* Along with Harris (1937) who noted the influence of *S. v. boothiae* on the population in this part of Nicaragua, these squirrels are best placed with *underwoodi* based on color and over all color pattern.

A female *S. v. underwoodi* was pregnant when obtained on 14 March 1968 at La Danta. A single embryo was implanted in each uterine horn. Lactating females were recorded on 17 March 1968 and 26 June 1964. Females evincing no reproductive activity were taken on the following dates: 15–17 March 1968, 3; 11 April 1968, 1; 10–11 May 1956, 2; 25 June 1964, 1; 29–30 June 1966, 2; 4 July 1967, 1; 3 August 1966, 1. Juvenile individuals with unerupted third upper molars were taken at Sebaco on 26 January 1958 and at San Rafael del Norte on 1 February 1909. Testes length for adult males were as follows: 14–17 March 1968—19, 25, 26, 29, 30, 32 mm; 11 April 1968—5, 28 mm; 20–22 April 1968—8, 22, 25 mm; 29–30 June 1966—23, 25, 25 mm; 1–3 July—12, 26 mm; 4 July 1967—8, 26, 27 mm; 3 August 1966—25 mm. Medina-Fitoria et al. (2018) reported that *S. v. underwoodi* occurs from the lowlands up to 1,200 m.

Specimens examined (49).—Departmento de Boaco: San Francisco, 19 km S, 2 km E Boaco (1 ♀, KU 115262). Departmento de Estelí: 8 mi S Condega (1 ♀, KU 71553). Departmento de Jinotega: San Rafael del Norte (5, 2 ♂♂, 3 ♀♀, AMNH 28438, 29240, 29245–246, 41233); 2 km E Yali (2, 1 ♂, 1 ♀, KU 106335–336). Departmento de Madriz: Daraili, 5 km N, 14 km E Condega (3 ♀♀, KU 97911–913); Venecia, 7 km N, 16 km E Condega (1 ♂, KU 97910). Departmento de Matagalpa: 11 mi SE Dario (1 ♂, KU 71552); Santa Maria de Ostuma (15, 10 ♂♂, 5 ♀♀, KU 106337–343, 110391–395, 115277–279); 3 mi SE San Ramon (2 ♀♀, KU 71554–555); 1 km NE Esquipulas (3, 2 ♂♂, 1 ♀, KU 115288–290); La Danta, 1 km N, 5 km E Esquipulas (8, 4 ♂♂, 4 ♀♀, KU 115280–287); Sebaco (1 ♂, AMNH 176697); Matagalpa (6, 2 ♂♂, 4 ♀♀, AMNH 28411–412, 28444, 30753, 41388–389).

Additional records.—Departmento de Matagalpa: Reserva Natural Cerro Arenal [13°00′25"N, 85°54′16"W] (Medina-Fitoria et al. 2018); Selva Negra (Medina-Fitoria et al. 2018).

CONCLUSIONS AND INSIGHTS

Herein, the systematics, diversity, and distributions of the variegated squirrels of Nicaragua are assessed by critically evaluating historical collecting sites, all published literature, and new morphometric data. To provide insights into the diversity of Nicaragua's fauna, the taxa found in the country are described and mapped. The variegated squirrels found on Isla de Ometepe in Lago de Nicaragua are a here-to-for unrecognized distinctive subspecies that are described as *Sciurus variegatoides ometepensis*. There are now six subspecies of this species found in Nicaragua and 16 throughout the species' geographic range. How and when the small mammal fauna of the volcanic island of Ometepe was colonized from the mainland remains an open question.

Why is the study of subspecific variation valuable? In this modern era, studying subspecific variation, and the description of a population recognized as a subspecies has become controversial. Indeed, some authors have even questioned the value of describing species new to science. In a recent critique of the subspecies concept in mammalogy, Patton and Conroy (2017) using both morphological and genetic data, provide a valuable review of the history and use of the subspecies concept in mammals. Although morphology and genetic data do at times give somewhat different views of the evolutionary history of populations, both approaches can and do provide valuable insights into phylogenetic relationships.

In modern systematics of mammals, the use of color and color patterns has fallen into disfavor, with the emphasis on higher-level morphometrics, molecular genetics, and now genomics. One of the primary issues of using color as a character is that its genetic control is highly complex and it is not a simple one gene to one color situation (Caro 2005). There has been disagreement as to whether color patterns are adaptive or not (Hershkovitz 1968, 1970) when discussing these patterns' involvement in "social selection" (Lawlor 1969) or "intraspecific communication" (Caro 2005).

However, it is clear that for these diurnal squirrels in the *S. variegatoides* complex their color and color patterns are adaptive and function in social recognition as well as to convey protective value concealing the individual from predators (also see Ancillotto and Mori 2017).

In the variegated squirrels, there is almost certainly selection in coloration and probably intense selection. The color patterns of the Nicaraguan subspecies of *S. variegatoides* are stable over broad geographic areas and, when there is contact between some of the taxa, detectable intergrades are produced indicating a genetic control for the colors and patterns. The color of these squirrels tends to follow Golger's Rule, with the darker form *S. v. belti* occurring in the wetter more humid Caribbean lowlands, the paler colored sandy or reddish animals in some cases having a nearly white dorsum occurring in the western more arid lowlands, and animals with patches of white pelage being found in higher elevations. Neotropical variegated squirrels, as with other closely related squirrels in the genus *Sciurus*, are capable of color vision that is at least dichromatic, giving these squirrels the ability to discriminate among intraspecific colors and color patterns (Arden and Silver 1962; Michels and Shumacher 1968; Jacobs 1974; Yolton et al. 1974). There is no secondary sexual dimorphism in color and color patterns in variegated squirrels as occurs in other diurnal mammals, notable in primates (Caro 2005). As noted by Harris (1937) there is no distinctive seasonal change in pelage color.

Although the bright coloration of some of the subspecies would seemingly make individuals easily spotted by predators, these squirrels can be remarkably difficult to see in tropical forests. In closed canopy forests, often little light penetrates the canopy and it may be quite diffuse, making these dappled squirrels blend into the background (Figs. 2–3). Hayssen (2008) ascribed the distinctive tail coloration in some sciurids as having a role in "tail flagging" behavior, perhaps to induce a predator to strike there or as having a conspecific visual communication function. Throughout the subspecies of *S. variegatoides*, the tail color is remarkably similar despite the extreme variation seen in body color. The tails are long and have a sharply contrasting black and white or rarely black and agouti coloration; the contrasting coloration is dorsal and the ventral view is generally black (see Figs. 2–3).

The newly described *S. v. ometepensis* differs significantly in color from the other subspecies of variegated squirrels occurring in Nicaragua and throughout the geographic range of the species, as well as in cranial and bacular characters. One of the most interesting features in the color pattern of the Ometepe squirrels is the high variability in patterns and colors. Other subspecies do differ widely from each other, but variation within a population is quite limited. In addition, the new subspecies is the most distinct of all of the taxa in Nicaragua in morphometrics. These individuals are small and have unique cranial proportions compared to others subspecies to the point that 41 of 42 specimens examined were correctly identified in a discriminate analysis. The history of this distinctive population is obviously tied to its long isolation on Isla de Ometepe.

Borgia and van Wyk de Vries (2003; van Wyk de Vries 1993) place the origin of Volcán Concepión in the late Quaternary, which would place its origin at least 20,000 to 30,000 years ago. Volcán Concepción lies above a bed of Quaternary mudstone in Lago de Nicaragua, with no indication of earlier volcanic activity in the lake deposits. There is no evidence that the island has ever been in contact with the mainland; therefore, the method of dispersal of terrestrial mammals seems to have been over water. Currently, it is about 6 km west to the mainland of the Departmento de Rivas and 55 km to the east to the mainland of the Departmento de Río San Juan. The prevailing winds are from the west to east or southeast; therefore, the wave action on the lake would move in this same direction. Lago de Nicaragua slowly drains to the southeast to the Río San Juan and then on to Caribbean Sea (Fig. 5). A number of other small terrestrial mammals reported herein from the Isla de Ometepe, such as *Sylvilagus floridanus*, *Oligoryzomys fulvescens*, *Peromyscus nicaraguae*, *Peromyscus stirtoni*, *Sigmodon hirsutus*, and *Liomys salvini* have their affinities to the drier Pacific Coast of Nicaragua. If there truly was no contact between Volcán Concepción and the mainland, then Neotropical variegated squirrels and other small mammals must have reached Isla de Ometepe via over water dispersal from along the western shore of Lago de Nicaragua where currently *S. v. dorsalis* is the dominant form. Although there is no geological evidence for a land connection between the island and the mainland, the presence of arid-adapted rodents and the dry forest cottontail rabbit on the island

might suggest a terrestrial dispersal route. These species of mammals as well as four-eyed opossums and white-tailed deer, are common in disturbed habitats so mature forest would not be a necessary habitat connection. One of the species of *Peromyscus* on Ometepe is black in color, in contrast to the mainland form also attesting to the long isolation of this fauna. For mammals, or any other animals reaching Isla de Ometepe by swimming the water gap, there is always the added danger that Lago de Nicaragua hosts a robust freshwater population of bull sharks (*Carcharhinus leucas*).

One of the major zoogeographic barriers in Middle America is known as the Nicaraguan Gap, which refers to the lowland area in southern Nicaragua that creates a break in the mountainous spine of the region, separating the Central Highlands of Nicaragua and Costa Rica's Cordillera de Guanacaste–Cordillera de Tilarán ranges. In the Pliocene, this gap may have represented the southern terminus of North America, but more recently is seen as an area that terminates or interrupts the distribution of montane taxa both from the north and south. The impact of this "gap" has been studied in the montane sigmodontine rodent, *Scotinomys* (Buchanan and Howell 1967), montane birds (Howell 1969; Weir 2009), and scarabaeid beetles (Ratcliffe and Deloya 1992). In the most recent of these studies, Weir (2009:419) found that in montane birds the genetic differentiation across the Nicaraguan Gap "ranged l%–9% and had a similar distribution of divergence dates to the Isthmus of Tehuantepec" in Mexico.

The Neotropical variegated squirrels of Nicaragua illustrate some of the impacts of this lowland gap on their distribution. The two montane taxa—*S. v. boothiae* and *S. v. underwoodi*—are confined in their distribution to the Central Highlands of the north-central part of the country east of the Nicaraguan Depression (Fig. 5). The geographic range of *S. v. boothiae* terminates in the mountains of the Departmento de Nueva Segovia and adjacent parts of Madriz in the far north. Populations of *S. v. underwoodi* come further south along the western edge of the Central Highlands to some 50 km east of Managua at San Francisco in the Departmento de Boaco where the highlands are reduced to a few ridges. Finally, some influence of *underwoodi* just east of Lago de Nicaragua along the edges of the last remnant of highland, the Cordillera Chontaleña is represented in color. Here *underwoodi* is intergrading with *S. v. belti*, the taxon present throughout the eastern Caribbean Lowlands. This brings the eastern taxon to the eastern slopes of Lago de Nicaragua, ending the distribution of the montane forms. The remaining three taxa—*adolphei*, *dorsalis*, and *ometepensis*—are essentially isolated in the arid dry forest of the Pacific Lowlands of western Nicaragua. It is only *dorsalis* that enters Costa Rica and intergrades with other taxa there.

Although nearly 400 variegated squirrels from Nicaragua are now available for study, there is much to learn about the distributions and relationships of these squirrels. More intensive collecting needs to be done between Rama, near the type locality of *belti* and Talamanca in southeastern Costa Rica, which is the type locality of *thomasi*. The relationship of these two taxa occupying the Caribbean Lowlands is poorly understood, but if they represent distinct taxa there should be a zone of intergradation somewhere in this area and currently few specimens are known from Costa Rica. Precise and intensive collecting will be needed along the eastern slopes of Central Highlands and the valleys of the east-flowing rivers to gain a better understanding of the distribution of *belti*, *boothiae*, and *underwoodi* in this region. It would be of interest to learn if there is a zone of intergradation between *adolphei* and *dorsalis* west of Lago de Managua. Additionally, there may be undescribed taxa of variegated squirrels just south of Lago de Nicaragua in Nicaragua and northern Costa Rica.

The hypotheses presented throughout this work on systematic relationships of squirrels clearly need to be tested with genetic data, but herein a groundwork has been laid for additional productive research. The distinctive new variegated squirrel, *S. v. ometepensis*, a large, diurnal, and conspicuous tree squirrel, along with the recent discovery of the southern flying squirrel (*Glaucomys volans*), pygmy squirrel (*Microsciurus alfari*), and rufous tree rat (*Diplomys labilis*) in Nicaragua attests to how much remains to be learned about this interesting fauna (Martínez-Fonseca et al. 2018). Recent efforts by the Nicaraguan conservation community in establishing reserves to protect the country's rich fauna and associated habitats are to be applauded.

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LITERATURE CITED

Allen, J. A. 1908. Mammals from Nicaragua. Bulletin of the American Museum of Natural History 24:647–670.

Allen, J. A. 1910. Additional mammals from Nicaragua. Bulletin of the American Museum of Natural History 28:87–115.

Ancillotto, L., and E. Mori. 2017. Adaptive significance of coat colouration and patterns of Sciuromorpha (Rodentia). Ethology Ecology & Evolution 29:241–254.

Arden, G. B., and P. H. Silver. 1962. Visual thresholds and spectral sensitivities of the grey squirrel (*Sciurus carolinensis leucotis*). Journal of Physiology 163:540–557.

Arguello, R., F. D. Parker, M. S. Orozco, T. W. Walker, and B. Nietschmann. 2018. Nicaragua. Accessed at <www.britannica.com/place/Nicaragua/Introduction> on 20 November 2018.

Bachman, J. 1839. Monograph of the species of squirrel inhabiting North America. Proceedings of the Zoological Society of London [1838] Part 6:85–103.

Best, T. L. 1995. *Sciurus variegatoides*. Mammalian Species 500:1–6.

Borgia, A., and B. van Wyk de Vries. 2003. The volcano-tectonic evolution of Concepción, Nicaragua. Bulletin of Volcanology 65:248–266.

Buchanan, O. M., and T. R. Howell. 1965. Observations on the natural history of the thick-spined rat, *Hoplomys gymnurus*, in Nicaragua. Annals and Magazine of Natural History, series 13, 8:549–559.

Buchanan, O. M., and T. R. Howell. 1967. Zoogeography of *Scotinomys* in Middle America, with description of a new subspecies from Nicaragua. Journal of Mammalogy 48:414–419.

Burt, W. H. 1960. Bacula of North American mammals. Miscellaneous Publications Museum of Zoology, University of Michigan 113:1–76.

Caro, T. 2005. The adaptive significance of coloration in mammals. BioScience 55:125–136.

Clark, L. G., V. M. Varela-Diaz, C. R. Sulzer, R. R. Marshak, and C. J. Hollister. 1966. Leptospirosis in Nicaragua: preliminary report on the first year of study. American Journal of Tropical Medicine and Hygiene 15:735–742.

Dickey, D. R. 1928. Five new mammals of the rodent genera *Sciurus*, *Orthogeomys*, *Heteromys*, and *Rheomys*, from El Salvador. Proceedings of the Biological Society of Washington 41:7–14.

Emerson, K. C. 1971. Records of a collection of Mallophaga and Anoplura from Nicaraguan mammals. Journal of the Kansas Entomological Society 44:332–334.

Fraser, C. M. 1943. General account of the scientific work of the *Velero III* in the eastern Pacific, 1931–41. Part III. A ten-year list of *Velero III* collecting stations (Charts 1–115). Allan Hancock Pacific Expeditions 1(3):259–431.

Goodwin, G. G. 1946. Mammals of Costa Rica. Bulletin of the American Museum of Natural History 87:271–474.

Genoways, H. H., and R. M. Timm. 2005. Mammals of the Cosigüina Peninsula of Nicaragua. Mastozoología Neotropical 12:153–179.

Goldman, E. A. 1912. Descriptions of twelve new species and subspecies of mammals from Panama. Smithsonian Miscellaneous Collections 56(36):1–11.

Goldman, E. A. 1932. A new squirrel from Honduras. Journal of the Washington Academy of Sciences 22:274–275.

Gray, J. E. 1842. Descriptions of some new genera and fifty unrecorded species of Mammalia. Annals and Magazine of Natural History, series 1, 10:255–267.

Gray, J. E. 1843. List of the specimens of Mammalia in the collections of the British Museum. Trustees of the British Museum, London, xxviii + 1–216.

Gray, J. E. 1849. Descriptions of two species of Mammalia from Caracas. Proceedings of the Zoological Society of London [1848] Part 16:138.

Gray, J. E. 1867. Synopsis of the species of American squirrels in the collections of the British Museum. Annals and Magazine of Natural History, series 3, 20:415–434.

Hall, E. R. 1981. The mammals of North America. 2nd ed. John Wiley & Sons, New York, 1:1–600 + *90*.

Hall, E. R., and K. R. Kelson. 1952. Comments on the taxonomy and geographic distribution of some North American rodents. University of Kansas Publications, Museum of Natural History 5:343–371.

Hall, E. R., and K. R. Kelson. 1959. The mammals of North America. The Ronald Press Company, New York, 1: xxx + 1–546 + *79*.

Harris, W. P., Jr. 1930. A new squirrel from Costa Rica. Occasional Papers of the Museum of Zoology, University of Michigan 219:1–4.

Harris, W. P., Jr. 1933. A new tree squirrel and a new cottontail rabbit from Costa Rica. Occasional Papers of the Museum of Zoology, University of Michigan 266:1–4.

Harris, W. P., Jr. 1937. Revision of *Sciurus variegatoides*, a species of Central American squirrel. Miscellaneous Publications of the Museum of Zoology, University of Michigan 38:1–42.

Hayssen, V. 2008. Patterns of body and tail length and body mass in Sciuridae. Journal of Mammalogy 89:852–873.

Hershkovitz, P. 1968. Metachromism or the principle of evolutionary change in mammalian tegumentary colors. Evolution 22:556–575.

Hershkovitz, P. 1970. Metachromism like it is. Evolution 24:644–648.

Howell, T. R. 1969. Avian distribution in Central America. Auk 86:293–326.

Howell, T. R. 1993. W. B. Richardson's collecting localities "Santa Cruz" and "Rio Coco," Nicaragua. Accessed at http://www.bio-nica.info/Biblioteca/Howell1993.pdf on 29 October 2018.

Howell, T. R. 2010. Thomas R. Howell's check-list of the birds of Nicaragua as of 1993. Ornithological Monograph 68:1–108.

Jacobs, G. H. 1974. Scotopic and photopic visual capacities of an arboreal squirrel (*Sciurus niger*). Brain, Behavior and Evolution 10:307–321.

Jones, J. K., Jr. 1965. Some Miskito Indian names for mammals. Journal of Mammalogy 46:353–354.

Jones, J. K., Jr., and M. D. Engstrom. 1986. Synopsis of the rice rats (Genus *Oryzomys*) of Nicaragua. Occasional Papers, Museum of Texas Tech University 103:1–23.

Jones, J. K., Jr., and H. H. Genoways. 1970. Harvest mice (genus *Reithrodontomys*) of Nicaragua. Occasional Papers of the Western Foundation of Vertebrate Zoology 2:1–16.

Jones, J. K., Jr., and H. H. Genoways. 1971. Notes on the biology of the Central American squirrel, *Sciurus richmondi*. American Midland Naturalist 86:242–246.

Jones, J. K., Jr., and T. A. Yates. 1983. Review of the white-footed mice, genus *Peromyscus*, of Nicaragua. Occasional Papers, Museum of Texas Tech University 82:1–15.

Koster, J. 2008. The impact of hunting with dogs on wildlife harvests in the Bosawas Reserve, Nicaragua. Environmental Conservation 35:211–220.

Lawlor, T. E. 1969. The principle of metachromism: a critique. Evolution 23:509–512.

Lesson, R. P. 1842. Nouveau tableau du règne animal: Mammifères. Arthus Bertrand, Paris.

Martínez-Fonseca, J. G., F. A. Reid, J. Loza, L. Gutiérrez-López, and J. Sunyer. 2018. New records of *Diplomys labilis* (Bangs, 1901) (Mammalia, Rodentia, Echimyidae) from Nicaragua. Check List 14:555–558.

McCarthy, T. J., D. L. Anderson, and G. A. Cruz D. 1999. Tree sloths (Mammalia: Xenarthra) in Nicaragua and Honduras, Central America. The Southwestern Naturalist 44:410–414.

McPherson, A. B. 1971 [1972]. A new subspecies of squirrel *Sciurus variegatoides* Ogilby from Costa Rica. Revista de Biología Tropical 19(1–2):191–194.

McPherson, A. B. 1985. A biogeographical analysis of factors influencing the distribution of Costa Rican rodents. Brenesia 23:97–273.

Medina-Fitoria, A. 2016. Diversidad mastozoológica de la Cuenca del Río Sconfra en Bluefields, Nicaragua. Revista Nicaragüense de Biodiversidad 8:1–44.

Medina-Fitoria, A., et al. 2018. Las ardillas de Nicaragua (Rodentia, Sciuridae): una actualización basada en análisis fenotipíco. Revista Mastozoológica de México 8:48–80.

Medina-Fitoria, A., W. Silva, C. Molina, and J. Rodríguez. 2016. Diversidad de micromamíferos de la Cuenca del Río Punta Gorda, Bluefields-Nicaragua, RACCS. Revista Nicaragüense de Biodiversidad 10:1–58.

Michels, K. M., and A. W. Schumacher. 1968. Color vision in tree squirrels. Psychonomic Science 10:7–8.

Nelson, E. W. 1899. Revision of the squirrels of Mexico and Central America. Proceedings of the Washington Academy of Sciences 1:15–110.

Ogilby, W. 1839. On a new species of squirrel (*Sciurus variegatoides*) from the west coast of South America. Proceedings of the Zoological Society of London 1839:117.

Palmer, M. G. 1945. Through unknown Nicaragua: the adventures of a naturalist on a wild-goose chase. Jarrods Publishers, London.

Patton, J. L., and C. J. Conroy. 2017. The conundrum of subspecies: morphological diversity among desert populations of the California vole (*Microtus californicus*, Cricetidae). Journal of Mammalogy 98:1010–1026.

Ratcliffe, B. R., and A. C. Deloya. 1992. The biogeography and phylogeny of *Hologymnetis* (Coleoptera: Scarabaeidae: Cetoniinae) with a revision of the genus. The Coleopterists Bulletin 46:161–202.

Reid, F. A. 2009. A field guide to the mammals of Central America & southeast Mexico. Oxford University Press, New York.

Richmond, C. W. 1893. Notes on a collection of birds from eastern Nicaragua and the Rio Frio Costa Rica, with a description of a supposed new trogon. Proceedings of the National Museum 16:479–532.

Rossi, R. V., R. S. Voss, and D. P. Lunde. 2010. A revision of the didelphid marsupial genus *Marmosa*. Part 1. The species in Tate's '*mexicana*' and '*mitis*' sections and other closely related forms. Bulletin of the American Museum of Natural History 334:1–83.

Sabogal, C. 1992. Regeneration of tropical dry forests in Central America, with example from Nicaragua. Journal of Vegetation Science 3:407–416.

Strauss, R. E. 2010. Discriminating groups of organisms. Pp. 73–91 in Morphometrics for morphometricians (A. M. Elewa, ed.). Springer Press, Berlin, Germany.

Taylor, B. W. 1963. An outline of the vegetation of Nicaragua. Journal of Ecology 51:27–54.

Thorington, R. W., Jr., J. L. Koprowski, M. A. Steele, and J. F. Whatton. 2012. Squirrels of the world. Johns Hopkins University Press, Baltimore, Maryland.

Timm, R. M., D. Lieberman, M. Lieberman, and D. McClearn. 2009. Mammals of Cabo Blanco: history, diversity, and conservation after 45 years of regrowth of a Costa Rican dry forest. Forest Ecology and Management 258:997–1013.

True, F. W. 1889. On the occurrence of *Echinomys semispinosus*, Tomes, in Nicaragua. Proceedings of the United States National Museum [for 1888] 11:467–468.

Ulmer, F. A., Jr. 1995. Northward extension of the range of Richmond's squirrel, *Sciurus richmondi.* The Southwestern Naturalist 40:416–418.

van Wyk de Vries, B. 1993. Tectonics and magma evolution of Nicaraguan volcanic systems. Unpublished Ph.D. Thesis, Department of Earth Sciences, Open University, Milton Keynes, United Kingdom.

Webb, J. P., Jr., and R. B. Loomis. 1970. Four species of *Microtrombicula* (Acarina, Trombiculidae) Mexico and Nicaragua. Bulletin of the Southern California Academy of Sciences 69:133–144.

Weir, J. T. 2009. Implications of genetic differentiation in Neotropical montane forest birds. Annals of the Missouri Botanical Garden 96:410–433.

Yolton, R. L., D. P. Yolton, J. Renz, and G. H. Jacobs. 1974. Preretinal absorbance in sciurid eyes. Journal of Mammalogy 55:14–20.

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Systematic Review of Botta's Pocket Gopher (*Thomomys bottae*) from Texas and Southeastern New Mexico, with Description of a New Taxon

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Abstract

Morphometric variation was examined in 12 cranial and three mandibular characters from 625 specimens representing 14 subspecies of the *Thomomys bottae* complex in western Texas and southeastern New Mexico. Preliminary results revealed that age and secondary sexual differences existed, consequently only adult (age class 4 and 5 individuals) female specimens were included in analyses designed to examine geographic variation. Differences among subspecies were examined using multivariate (MANOVA, Discriminant Function Analysis, and Cluster Analysis) and univariate (ANOVA and Duncan's multiple range tests) analyses. Of the 14 currently recognized subspecies, eight were found to be statistically indistinct from adjacent populations. Data then were obtained from 24 additional specimens (from critical geographic localities) and combined with the original dataset to further test the validity of subspecific differentiation and to better access the distributional patterns among subspecies. Based on these results, taxa tentatively were reorganized into eight preexisting subspecies and a previously undescribed taxon from south-central Texas.

Key words: distribution of taxa, geographic variation, pocket gophers, subspecies, *Thomomys bottae*

Introduction

Members of the *Thomomys bottae* complex occupy much of the western United States and adjacent parts of northern Mexico. The most comprehensive attempt to summarize the taxonomic literature indicated that approximately 195 nominal taxa were recognized (Jones and Baxter 2004). This number represents a remarkable increase from the 18 subspecies recognized by the original taxonomic revision of the group (Bailey 1915). The proliferation of published taxonomic descriptions during the early 1900s has been attributed to extreme morphological variation among populations of *T. bottae*, adaptation to widely different geographic locations, a seeming lack of intermediate forms, and unclear intergradation of boundaries between populations (Bailey 1915; Goldman 1936, 1947; Durrant 1946; Anderson 1966; Hall 1981; Smith et al. 1983).

Subsequent studies by Smith and Patton (1980), Patton and Smith (1981, 1989, 1990), Smith (1998), and Wickliffe et al. (2005), using various genetic datasets, reveal that many of the subspecies initially described on morphological characters do not differ genetically. However, based on DNA sequences, Álvarez-Castañeda (2010) detected larger amounts of genetic variation and identified eight monophyletic groups from the southwestern U.S. and northern Mexico that he postulated should be recognized as phylogenetic species. Given these different interpretations, a comprehensive geographic review of the status of these pocket gophers is long overdue.

This group of pocket gophers has had a long and tortuous taxonomic history, particularly in the area of

study for this paper (see Table 1). When Bailey (1905) published his biological survey of Texas and his monograph of the genus *Thomomys* in 1915, four species (*T. baileyi*, *T. fulvus*, *T. lachuguilla*, and *T. perditus*) were recognized, an arrangement that persisted until Goldman (1936) made several changes, retaining *T. baileyi* as a distinct species, placing three other subspecies (*confinalus*, *lachuguilla*, and *limitaris)* under *T. lachuguilla*, arranging two subspecies (*T. b. ruidosae* and *T. b. texensis*) together with a newly described taxon (*T. b. guadalupensis*) under *T. bottae*, and adding *T. pectoralis* as a distinct taxon (see Table 1). These assignments represented the first use of *T. bottae* for the species designation of Texas populations. Two years later, Goldman (1938) assigned all of the subspecies of *lachuguilla* to *T. bottae*, although he continued to recognized *T. pectoralis* as a separate species. Of particular interest in all of these taxonomic reassignments and changes was the taxonomic status of *T. baileyi,* which had been described by Merriam in 1901 and retained as a distinct species until Anderson (1966) arranged it as a subspecies of *T. bottae*. At one time, three subspecies of *T. baileyi* (*T. b. baileyi* and *T. b. spatiosus* in Texas and *T. b. tularosae* in New Mexico) were recognized from the study area (Table 1).

Beginning with Hall and Kelson (1959) and continuing to the present, taxonomists have tended to arrange populations of *Thomomys* from this region under one of two species, *T. bottae* or *T. umbrinus* (Table 1). *T. bottae* was one of three species (with *umbrinus* and *townsendii)* combined with *T. umbrinus* by Hall and Kelson (1959) and Hall (1981), leading to the use of *T. umbrinus* for this taxon. Interestingly, mammalogists in Texas (Taylor and Davis 1947; Davis 1966, 1974) never recognized the assignment of *umbrinus* for Texas populations, instead referring to them as *bottae*. Beginning in the 1970s and continuing until the 1980s, taxonomists began to address the confusion regarding species boundaries in *Thomomys*. Several authors documented chromosome distinctions between *umbrinus* and *bottae* and argued that they should be treated as separate species (Patton and Dingman 1968; Patton 1973; Patton and Smith 1981, 1990, 1994), with all of the populations from our study area assigned to *T. bottae* (see Jones and Baxter 2004).

Currently, 14 nominal subspecies of *T. bottae* (*actuosus*, *baileyi*, *confinalis*, *guadalupensis*, *lachuguilla*, *limitarius*, *limpiae*, *pectoralis*, *pervarius*, *ruidosae*, *scotophilus*, *spatiosus*, *texensis*, and *tularosae*) are recognized from the rugged hills and mountains of southwestern Texas and the extension of that terrain along the Front Range into southeastern New Mexico (Findley et al. 1975; Manning and Jones 1988; Jones and Baxter 2004; Schmidly and Bradley 2016). Populations from this region have been reported to display marked morphological variability as a result of occupying a variety of habitats, ranging from lowland desert hillsides to montane meadows (Findley et al. 1975; Schmidly 1977, 2004; Stangl et al. 1994; Schmidly and Bradley 2016). Bailey (1905) described the distribution of *T. bottae* in Texas as confined mostly to the vicinity of type localities in the Trans-Pecos region of the Chihuahuan Desert, with a small, disjunct eastern population in south-central Texas. Hall and Kelson (1959) provided a geographic and taxonomic update on the known range and presumed boundaries of the species and the numerous subspecies. Subsequent decades of extensive collecting efforts in Texas have provided the requisite materials for more recent updates that have resulted in our current knowledge of the range of *T. bottae* across most of the Trans-Pecos and into the Edwards Plateau of south-central Texas (Hall 1981; Jones and Baxter 2004; Schmidly and Bradley 2016).

Only two comprehensive studies have been conducted in this geographic region since Bailey's (1915) revision of the systematic status of *Thomomys*. The first was a morphologic study (cranial and mandibular characters) of the 14 subspecies of *Thomomys bottae* recognized from southwestern Texas and southeastern New Mexico (Beauchamp 1998). In that study, Beauchamp (1998) recommended synonymizing six subspecies (*T. b. guadalupensis*, *T. b. limitaris*, *T. b. pectoralis*, *T. b. pervarius*, *T. b. ruidosae*, and *T. b. scotophilus*) and reallocating specimens identified as *T. b. confinalis* to a new subspecies. The second was the DNA sequence-based study of Wickliffe et al. (2005). Those authors assessed variation of the cytochrome-*b* gene for 25 specimens from portions of Texas and New Mexico. Their results indicated little evidence of genetic subdivisions that corresponded to historically recognized subspecific groups, although their conclusions were based on only seven of the 14 subspecies suggested to occupy this region (Hall 1981).

Table 1. A cursory synopsis of major changes in the taxonomic history of *Thomomys* from southwestern Texas and southeastern New Mexico. The names presented below were either described by or mentioned in the publications identified by the superscripted numbers. Abbreviations for taxa are as follows: *T. a.* = *Thomomys aureus*, *T. ba.* = *Thomomys baileyi*, *T. bo.* = *Thomomys bottae*, *T. f.* = *Thomomys fulvus*, *T. l.* = *Thomomys lachuguilla*, and *T. u.* = *Thomomys umbrinus*. Superscript numbers identify the authorities for the taxonomic histories.

Bailey 1905[1]	Bailey 1915[2]	Goldman 1936[3]	Goldman 1938[4]	Hall and Kelson 1959[5]	Anderson 1966[6]	Hall 1981[7]	Jones and Baxter 2004[8]
				T. u. actuosus		*T. u. actuosus*	*T. u. actuosus*
T. baileyi	*T. baileyi*		*T. ba. baileyi*	*T. ba. baileyi*	*T. bo. baileyi*	*T. u. baileyi*	*T. bo. baileyi*
		T. l. confinalis	*T. bo. confinalis*	*T. u. confinalis*		*T. u. confinalis*	*T. bo. confinalis*
		T. bo. guadalupensis		*T. u. guadalupensis*		*T. u. guadalupensis*	*T. bo. guadalupensis*
T. a. lachuguilla	*T. lachuguilla*	*T. l. lachuguilla*	*T. bo. lachuguilla*	*T. u. lachuguilla*		*T. u. lachuguilla*	*T. bo. lachuguilla*
		T. l. limitaris	*T. bo. limitaris*	*T. u. limitaris*		*T. u. limitaris*	*T. bo. limitaris*
				T. u. limpiae		*T. u. limpiae*	*T. bo. limpiae*
		T. pectoralis		*T. u. pectoralis*		*T. u. pectoralis*	*T. bo. pectoralis*
			T. bo. pervarius	*T. u. pervarius*		*T. u. pervarius*	*T. bo. pervarius*
		T. bo. ruidosae		*T. u. ruidosae*		*T. u. ruidosae*	*T. bo. ruidosae*
				T. u. scotophilus		*T. u. scotophilus*	*T. bo. scotophilus*
			T. ba. spatiosus	*T. ba. spatiosus*	*T. bo. spatiosus*	*T. u. spatiosus*	*T. bo. spatiosus*
T. f. texensis	*T. f. texensis*	*T. bo. texensis*	*T. bo. texensis*	*T. u. texensis*		*T. u. texensis*	*T. bo. texensis*
				T. ba. tularosae	*T. bo. tularosae*	*T. u. tularosae*	*T. bo. tularosae*
T. perditus	*T. perditus*						

[1]Bailey, V. 1905. Biological survey of Texas. North American Fauna 25:1–222.
[2]Bailey, V. 1915. Revision of pocket gophers of the genus *Thomomys*. North American Fauna 39:1–136.
[3]Goldman, E. A. 1936. New pocket gophers of the genus *Thomomys*. Journal of Washington Academy of Science 26:111–120.
[4]Goldman, E. A. 1938. Six new rodents from Coahuila and Texas and notes on the status of several described forms. Proceedings of the Biological Society of Washington 51:55–62.
[5]Hall, E. R., and K. R. Kelson. 1959. The mammals of North America. Ronald Press Co., New York.
[6]Anderson, S. 1966. Taxonomy of gophers, especially *Thomomys*, in Chihuahua, Mexico. Systematic Zoology 15:189–198.
[7]Hall, E. R. 1981. The mammals of North America. John Wiley & Sons, New York.
[8]Jones, C. A., and C. N. Baxter. 2004. *Thomomys bottae*. Mammalian Species 742:1–14.

Given that Wickliffe et al. (2005) was unable to examine all nominal taxa from this region and that most of the historical subspecific designations in gophers have been based on perceived differences in skull size, the goal of this study was to re-examine morphometric variation among specimens representing the 14 subspecies of *Thomomys* occurring in southwestern Texas and New Mexico. To accomplish this, we conducted a two-stage analysis. The first, based on the work of Beauchamp (1998), re-examines systematic status of populations of *T. bottae* based on multivariate and univariate morphometric analyses that support a reorganization of subspecies in Texas. The second analysis includes a partial dataset collected by one of us (DJS) but was never published. Given that the two independent datasets (Beauchamp's 1998 thesis and DJS's) had a subset of characters in common but also had several characters that were unique to each, the larger of the two datasets (Beauchamp's 1998) was used as the primary analysis and a combined dataset of both the Beauchamp (1998) and DJS data was used as a secondary confirmatory analysis. To have relied on the merged datasets as the primary analysis would have resulted in the eliminations of several characters, localities, and even taxa because of "missing data" due to the inclusion of different characters in each dataset.

METHODS AND MATERIALS

Characters examined.—Twelve cranial and three mandibular characters (Fig. 1) were measured with digital calipers to the nearest 0.01 mm. Measurements were obtained from 625 individuals (museum catalog numbers and specific collecting localities are provided in the Accounts of Subspecies) and are as follows: nasal length (a) – greatest length from tip of nasal to nasal suture; rostral length (b) – greatest distance from the tip of nasals to junction of lacrimal and maxillary bones; rostral breadth (c) – greatest width across rostrum; least interorbital constriction (d) – least distance between orbits; zygomatic breadth (e) – greatest distance across zygomatic arches parallel to skull; mastoidal breadth (f) – greatest distance from outermost extensions of mastoidal processes perpendicular to long axis of skull; upper incisor width (g) – greatest distance across incisor below alveolus; palatal length (h) – least distance from incisor alveolus to posteriormost extension of palate; condylobasal length (i) – measured from anterior-most point of premaxilla to posteriormost point of occipital condyles; occipital depth (j) – shortest distance between auditory bulla and temporal ridge; palatofrontal depth (k) – shortest distance from palate behind M3 to frontal bone; maxillary alveolar length (l) – greatest length between anterior P1 alveolus to posterior M3 alveolus; depth of ramus (m) – least distance from tip of the coronoid process to angle of mandible; mandibular alveolar length (n) – greatest length between anterior p1 alveolus to posterior m3 alveolus; and lower incisor width (o) – greatest distance across incisor above the alveolus.

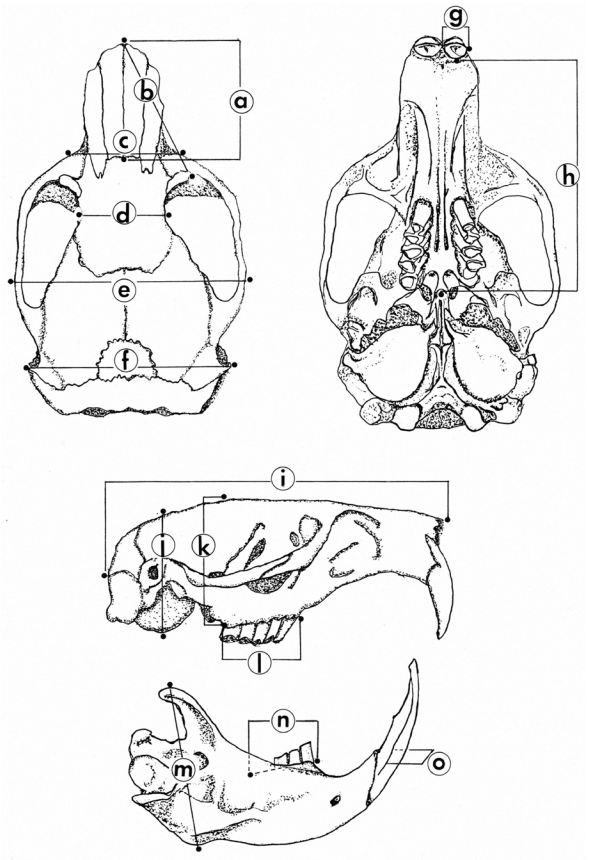

Figure 1. Dorsal, ventral, and lateral views of the skull and labial view of the mandible of an adult female *Thomomys bottae* (MWSU 20792) from 22 mi W Kent, Culberson Co., Texas. Letters illustrate the measurements described in the Methods and Materials.

Age and sexual dimorphism.—All specimens were assigned to one of four relative age categories, following the criteria of Hollander (1990) and Beauchamp (1998). Specifically, juveniles (age class 2) were characterized by small rounded skulls, lack of suture fusion, and presence of deciduous premolars; subadults (age class 3) possessed an increased angularity to the skull profile, partial fusion of cranial sutures, and newly erupted or erupting premolars; adults (age class 4) displayed an angular profile, development of occipital and sagittal crests, complete fusion of cranial sutures, and large incisors; and old adults (age class 5) exhibited a prominent cranial crest (especially for males) and complete obliteration of cranial sutures. A two-way MANOVA (sex, age) conducted by Beauchamp (1998) indicated that 10 of the 15 characters exhibited significant differences between the sexes ($P \leq 0.05$), prompting the removal of male specimens to prevent bias due to secondary sexual dimorphism. Similarly, Beauchamp (1998) indicated that age classes varied significantly (two-way MANOVA on sex and age; $P \leq 0.05$) and that a Duncan's multiple means test revealed that the highest similarity occurred between age classes 4 and 5. Based on these findings, only adult female specimens from age classes 4 and 5 ($n = 252$) were included in subsequent analyses.

Beauchamp (1998) analysis of systematic status and geographic variation.—To explore geographic variation among the 14 nominal subspecies of *T. bottae* occurring in southwestern Texas and adjacent areas of eastern New Mexico (Hall 1981), individual specimens were grouped into general sampling localities (Fig. 2) based on the taxonomic hypotheses interpolated from the range maps as depicted in Hall (1981) as follows: (a) *T. b. actuosus* (Lincoln and northern Otero counties, New Mexico); (b) *T. b. baileyi* (Sierra Blanca Mountains and Hudspeth Co., Texas); (c) *T. b. confinalis* (Concho, Edwards, Kimble, Mason, Menard, Schleicher, Sonora, Sutton, and Tom Green counties, Texas); (d) *T. b. guadalupensis* (Guadalupe Mountains, Beach Mountains, and Apache Mountains, Culberson Co., Texas); (e) *T. b. lachuguilla* (Franklin Mountains, El Paso Co., Texas and Dona Ana Co., New Mexico); (f) *T. b. limitaris* (eastern Presidio, southern Brewster, Terrell, Crockett, Irion, Reagan, Upton, Crane, and Val Verde counties, Texas); (g) *T. b. limpiae* (Limpia Canyon of Davis Mountains and eastern Jeff Davis Co., Texas); (h) *T. b. pectoralis* (Eddy Co., New Mexico); (i) *T. b. pervarius* (western Presidio Co., Texas); (j) *T. b. ruidosae* (Sacramento Mountains and Cloudcroft area of Otero Co., New Mexico); (k) *T. b. scotophilus* (Sierra Diablo Mountains, eastern Hudspeth and western Culberson counties, Texas); (l) *T. b. spatiosus* (Glass Mountains of northeastern Brewster Co., Texas); (m) *T. b. texensis* (Mt. Livermore and Sawtooth Mountain of Davis Mountains, western Jeff Davis Co., Texas); and (n) *T. b. tularosae* (Tularosa, Otero Co., New Mexico). A multivariate analysis of variance (MANOVA) was performed to determine differences among groups in the twelve cranial and three mandibular characters. These were followed by Duncans's multiple range tests (Sokal and Rohlf 1995) to examine which characters were different among the several taxa.

Confirmation of findings based on new data.—We obtained measurements from an additional 24 specimens from the following subspecies: *confinalis* (10), *lachuguilla* (11), *spatiosus* (1), and an apparently new taxon (2). From these specimens, the seven characters that were common between the Beauchamp and DJS datasets included: zygomatic breadth, mastoidal breadth, rostral breadth, rostral length, nasal length, least interorbital constriction, and maxillary alveolar length. These were combined with the same characters from the dataset of Beauchamp (1998) and this reduced dataset (i.e., in terms of number of characters) was used to test the generality of Beauchamp's findings.

Based on the unusual subdivision noted by Beauchamp (1998) among populations of *T. b. confinalis* located east of the Pecos River, all samples of *T. b. confinalis* and neighboring subspecies (*lachuguilla* and *spatiosus*) and a newly proposed subspecies (Beauchamp 1998) were further examined. To evaluate significant differences among these four taxa, including additional specimens from localities that were under-represented in the Beauchamp (1998) study, a multivariate analysis of variance (MANOVA) based solely on female specimens was employed. Initially, an omnibus test of all four taxa was used to infer whether at least one taxon was significantly different. Based on the significant results from the initial MANOVA, exploratory pairwise MANOVAs were conducted to assess what differences between taxa likely contributed to the significant initial MANOVA. These were followed by univariate ANOVA's to determine which individual characters were most different among taxa.

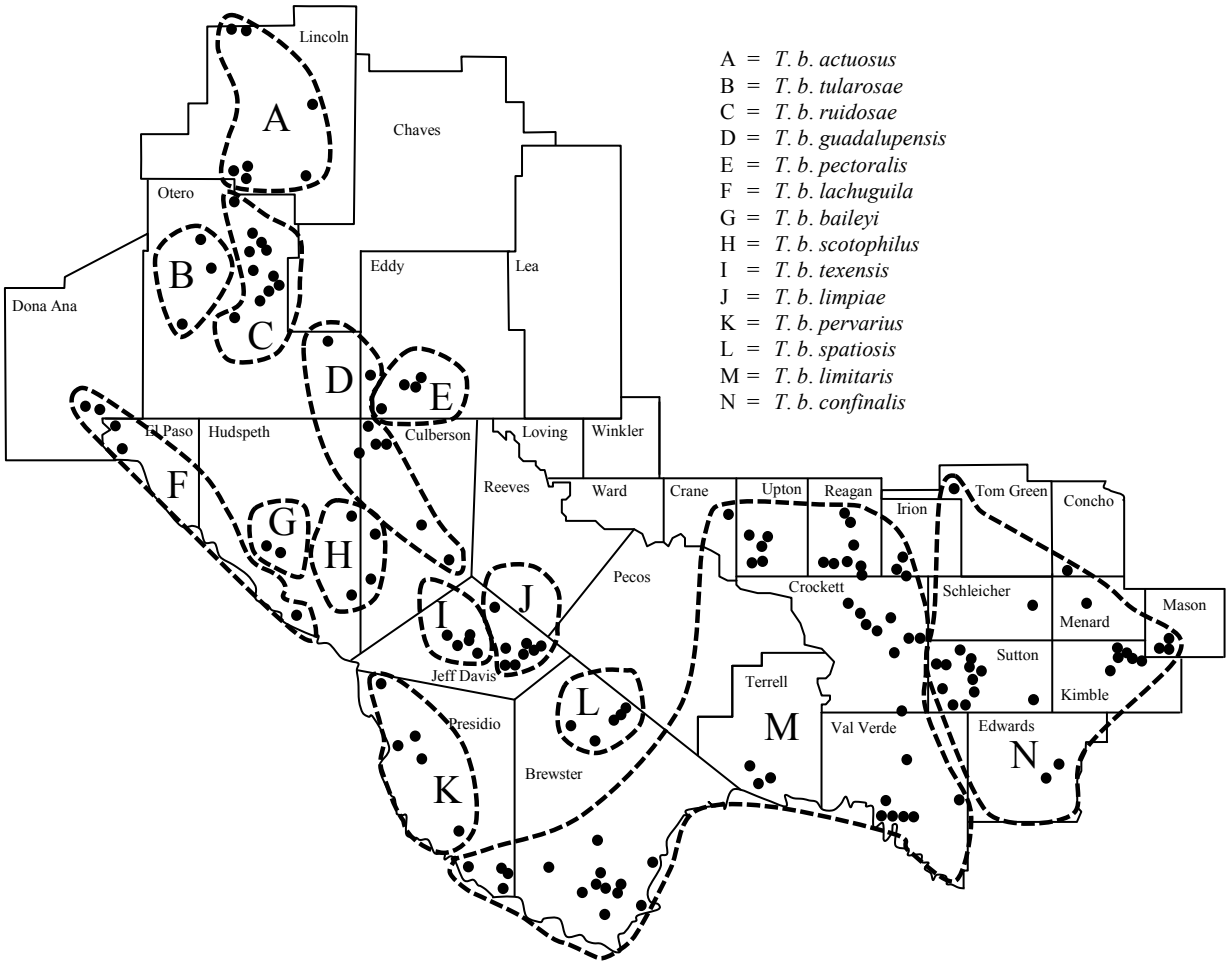

Figure 2. Map depicting the distribution of subspecies of *Thomomys bottae* from western Texas and south-central New Mexico. Subspecific boundaries are based on Hall (1981) and are delineated by dashed lines. Dots represent the approximate location of specimens examined in this study (see *Specimens Examined* for exact localities) and capital letters (A–N) correspond to subspecies as shown in the inset.

To confirm results from the MANOVA, a discriminant function analysis (DFA) was performed to illustrate differences between taxa and to identify individual characters most highly correlated with multivariate differences. Significant differences between groups separated by nodes of the resultant phenogram were determined by MANOVA. To display morphological relationships among taxa and estimate percent morphological similarity among newly interpreted subspecies (according to Beauchamp 1998), a cluster analysis based on log-transformed morphological characters based on an unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm was conducted. All analyses (e.g., MANOVA, ANOVA, UPGMA, and DFA) were conducted using SPSS version 24 (IBM Corp. 2016).

Assumptions and the conundrum of the subspecies concept.—Assignment of trinomens to species has been debated widely in the mammalian literature (Goldman 1936; Wilson and Brown 1953; Burt 1954; Durrant 1955; Lidicker 1962; Mayr 1982; Stangl and Baker 1984; Avise and Ball 1990; Cronin et al. 2015a, 2015b; Fredrickson et al. 2015; Weckworth et al. 2015; Patten and Remsen 2017). Although infraspecific variation underlying usage of trinomens is often seen as hierarchical reflecting descent of allopatric lineages, it is important to acknowledge that subspecific vari-

ants often reflect genealogical networks integrated by reduced gene flow (Braby et al. 2012; Patton and Conroy 2017). Most modern taxonomists would avoid the use of trinomens unless circumstances dictate that an official taxonomic designation would benefit the organism, population, ecological unit, etc., by placing attention upon the taxon. For example, whatever name is applied generally sends the message that this group of organisms is a recognizable unit that differs (morphologically, genetically, etc.) from other units within the species. Therefore, in some cases, the use of a trinomen may be extremely important when arguing for conservation or management of some genetically or morphologically discrete groups—as is the case for many of the isolated populations of *Thomomys* in southern New Mexico and western Texas. However, as discussed by Goldman (1936), assignment of trinomens is at best an individual judgment call without the benefit of coordinated field and laboratory studies, or multiple forms of evidence across diverse datasets such as genes, molecules, morphology, or coloration. Given that no non-arbitrary criterion (see Mayr and Ashlock 1991) are required for defining a subspecies, we hypothesized that populations possessing > 4 different morphological characters (determined to be significantly different in the morphometric analyses) were geographically isolated from other significantly different populations and that those differences were sufficient to be recognized as putative subspecies.

RESULTS

Beauchamp (1998) analysis of geographic variation and systematics.—Each nominal subspecies from the study area was treated as a geographically isolated group (Fig. 2) and female gophers were examined to ascertain if significant geographic variation was detectable. The resulting MANOVA revealed highly significant differences ($P \leq 0.001$) among morphological centroids of nominal taxa. Analysis of variance indicated that in 12 of the 15 characters examined, females exhibited significant univariate differences (excluding upper incisor width, rostral breadth, and occipital depth). Duncan's multiple range tests revealed distinct morphological differences between adjacent populations (see Fig. 3) of the nominal subspecies as follows: *T. b. actuosus* from *T. b. ruidosae* in condylobasal length, zygomatic breadth, mastoidal breadth, nasal length, least interorbital constriction, palatofrontal depth, upper incisor width, and lower incisor width; *T. b. tularosae* from *T. b. ruidosae* in condylobasal length, zygomatic breadth, mastoidal breadth, palatofrontal depth, upper incisor width, and depth of ramus, and from *T. b. lachuguilla* in condylobasal length, zygomatic breadth, and mastoidal breadth; *T. b. baileyi* from *T. b. lachuguilla* in condylobasal length, zygomatic breadth, mastoidal breadth, occipital depth, palatofrontal depth, palatal length, and depth of ramus, and from *T. b. scotophilus* in condylobasal length, zygomatic breadth, mastoidal breadth, nasal length, least interorbital constriction, palatofrontal depth, palatal length, mandibular alveolar length, depth of ramus, lower incisor width and upper incisor width; *T. b. limpiae* from *T. b. texensis* in condylobasal length, zygomatic breadth, mastoidal breadth, palatal length, and depth of ramus; *T. b. spatiosus* from *T. b. limpiae* in mandibular alveolar length, rostral length, least interorbital constriction, palatofrontal depth, upper incisor width, and depth of ramus, and from *T. b. limitaris* in mastoidal breadth, upper incisor width, depth of ramus, and lower incisor width; *T. b. limitaris* from *T. b. confinalis* in zygomatic breadth, rostral breadth, maxillary alveolar length, upper incisor width, depth of ramus, and lower incisor width; and *T. b. texensis* from *T. b. pervarius* in least interorbital constriction and nasal length.

Morphological characters (described above) that distinguished contiguous subspecific groups were plotted on the map illustrating the 14 nominal subspecies from western Texas and southeastern New Mexico (see Fig. 3). Populations that significantly differed by > 4 morphological characters were hypothesized to be geographically isolated and assumed to represent valid subspecies. Subspecies fitting this criterion included: *T. b. actuosus*, *T. b. baileyi*, *T. b. confinalis*, *T. b. limpiae*, *T. b. spatiosus*, and *T. b. tularosae*. The Duncan's test did not reveal any distinguishing characters among the contiguously distributed populations containing representatives of *T. b. ruidosae*, *T. b.*

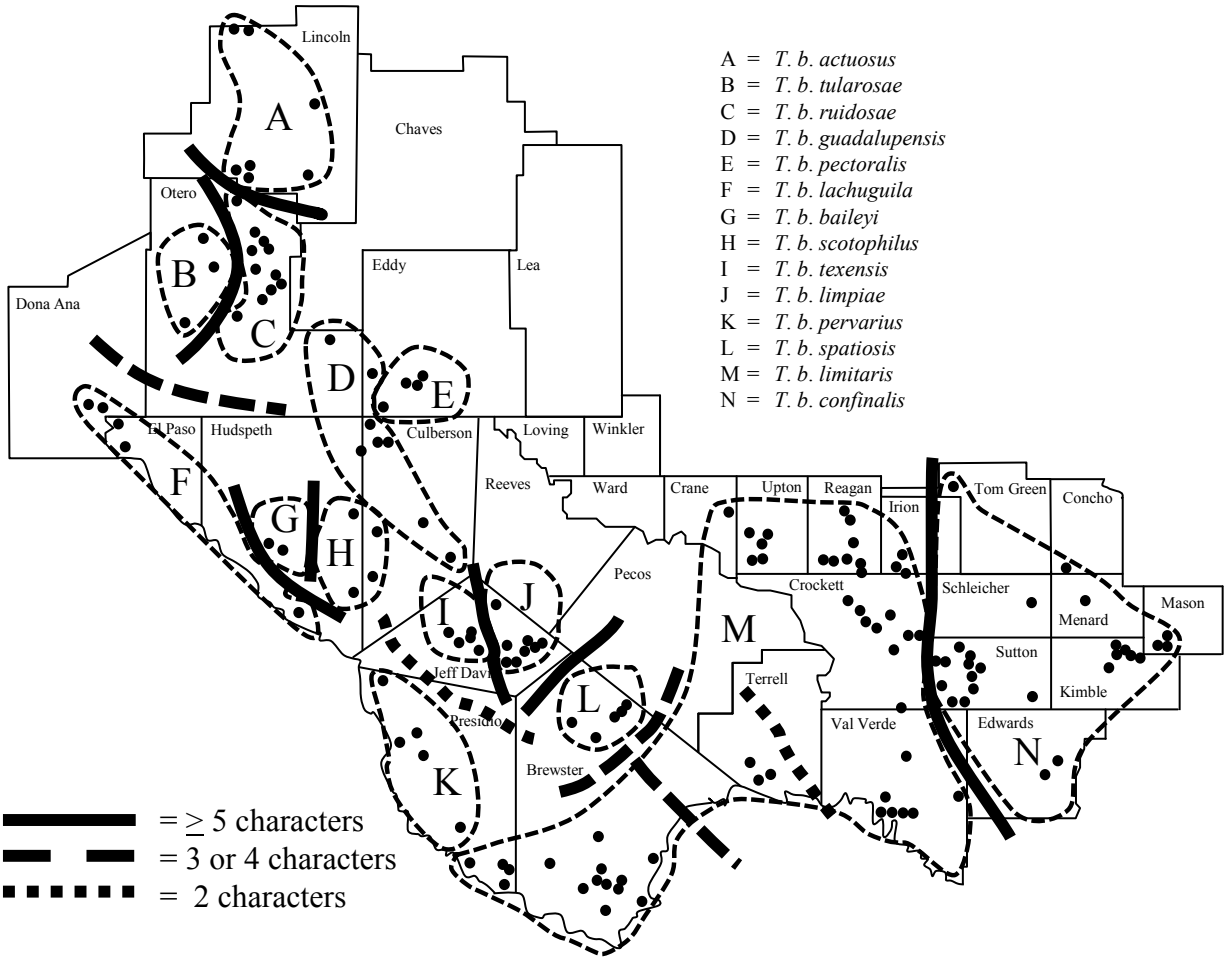

Figure 3. Morphological characters determined to be significantly different were overlaid onto the map depicting the distribution of subspecies of *Thomomys bottae* examined in this study. Thick, solid lines represent breaks between populations in which > 5 morphological characters were determined to differ significantly; thick, dashed lines represent breaks between populations in which three or four morphological characters were determined to differ significantly; and thick, dotted lines represent breaks between populations in which two morphological characters were determined to differ significantly. Dots represent the approximate location of specimens examined in this study (see Specimens Examined for exact localities) and capital letters (A–N) correspond to specific subspecies as shown in the inset.

guadalupensis, *T. b. pectoralis*, *T. b. scotophilus*, and *T. b. texensis*. There were no distinguishing characters identified between populations of *T. b. lachuguilla*, *T. b. pervarius*, and portions of *T. b. limitaris* from southeastern Presidio and southern Brewster counties. However, the MANOVA and Duncan's multiple range tests of 24 specimens of *T. b. limitaris* from Brewster, Terrell, and Val Verde counties indicated significant differences in mastoidal breadth, occipital depth, least interorbital constriction, mandibular alveolar length, and depth of ramus. Specimens from Terrell County differed significantly from specimens from Brewster County in occipital depth, palatofrontal depth, mandibular alveolar length, and depth of ramus; specimens from Terrell County were distinguished from Val Verde County specimens in least interorbital constriction and mandibular alveolar length.

Although it is possible that genetic drift or responses to environmental variables can influence morphological variation, the presence of significantly different measured characters between populations

of the same subspecies presumably indicate the presence of a barrier to gene flow. Further, the distribution of *T. b. limitaris* as presently defined (Hall 1981) does not clearly reflect the historical break between morphologically distinct specimens from Brewster County and Terrell County as indicated herein. The affiliation of *T. b. limitaris* from Brewster County to gophers from along the western portions of the Rio Grande (*T. b. lachuguilla* and *T. b. pervarius*), suggests that a new taxon should be recognized for specimens formerly referred as *T. b. limitaris* from Terrell County eastward to the range of *T. b. confinalis*. Therefore, samples unavailable to the Beauchamp (1998) study [University of Texas at El Paso, UTEP Biodiversity Collections, Mammal Division (UTEP), University of Illinois Museum of Natural History (UIMNH; currently at Museum of Southwestern Biology), National Museum of Natural History (USNM), Sul Ross State University Scudday Vertebrate Collection (SRSU), Texas A&M University, Biodiversity Research and Teaching Collection (TCWC), University of Michigan Museum of Zoology (UMMZ), and Collection of Recent Mammals, Museum of Texas Tech University (TTU)] were combined with all specimens occurring along the Rio Grande and a separate, more focused, assessment was conducted.

Confirmation based on new data.—Adult female gophers (categories 4 and 5) comprised the greatest subset of specimens examined. The MANOVA indicated highly significant differences among the four putative subspecies occurring in Terrell and Val Verde counties as well as along the Rio Grande River (including samples currently assigned to *T. b. confinalis*, *T. b. lachuguilla*, *T. b. pervarius*, and a possible new taxon) regarding morphological characters ($F_{45, 306.77} = 3.44$, $P < 0.001$). Of the six possible pairwise contrasts among the four subspecies, four were significant. *P*-values from significant pairwise MANOVA's ranged from 0.04 to < 0.001. Number of pairwise differences that were significant based on ANOVA ranged from two to seven characters. Discriminant function analysis identified three axes that significantly discriminated among groups. Canonical correlations describing the strength of the relationship between the linear combination of morphological variables defining the discriminant function and group affiliation were 0.716, 0.479, and 0.456 for DF1, DF2, and DF3, respectively. Discriminant function 1 was most highly correlated with upper incisor width ($r = 0.552$) and interorbital breadth ($r = 0.437$). Discriminant function 2 was most highly correlated with maxillary alveolar length ($r = -0.497$) and mastoidal breadth ($r = 0.472$). Discriminant function 3 was most highly correlated with zygomatic breadth ($r = -0.506$). All three bifurcations separating the four putative subspecies based on a UPGMA cluster analysis represented highly significant differences (Fig. 4).

Results from the combined but reduced data set for females were similar to those based on the original female dataset examined by Beauchamp (1998). The MANOVA indicated highly significant differences among the four putative subspecies regarding morphological characters ($F_{35, 561.91} = 2.73$, $P < 0.001$). Of the six possible pairwise contrasts among the four subspecies, five were significant, but only two were highly significant. *P*-values from significant pairwise MANOVA's ranged from 0.038 to < 0.001. Number of pairwise differences that were significant based on ANOVA ranged from one to four characters. Discriminant function analysis identified three axes that significantly discriminated among groups. Canonical correlations describing the strength of the relationship between the linear combination of morphological variables defining the discriminant function and group affiliation were 0.535, 0.349 and 0.299 for DF1, DF2, and DF3, respectively. Discriminant function 1 was most highly correlated with rostral breadth ($r = 0.713$) and interorbital breadth ($r = 0.586$). Discriminant function 2 was most highly correlated with mastoidal breadth ($r = 0.636$) and maxillary alveolar length ($r = -0.555$). Discriminant function 3 was most highly correlated with zygomatic breadth ($r = -0.410$). All three bifurcations separating the four subspecies based on a UPGMA cluster analysis represented highly significant differences based on morphology (Fig. 3). Although significantly different morphologically, the new taxon is most similar to the geographically adjacent *T. b. confinalis*, despite being separated by the Pecos River. *Thomomys bottae spatiosus* was the most morphologically distinct and *T. b. lachuguilla* was intermediate.

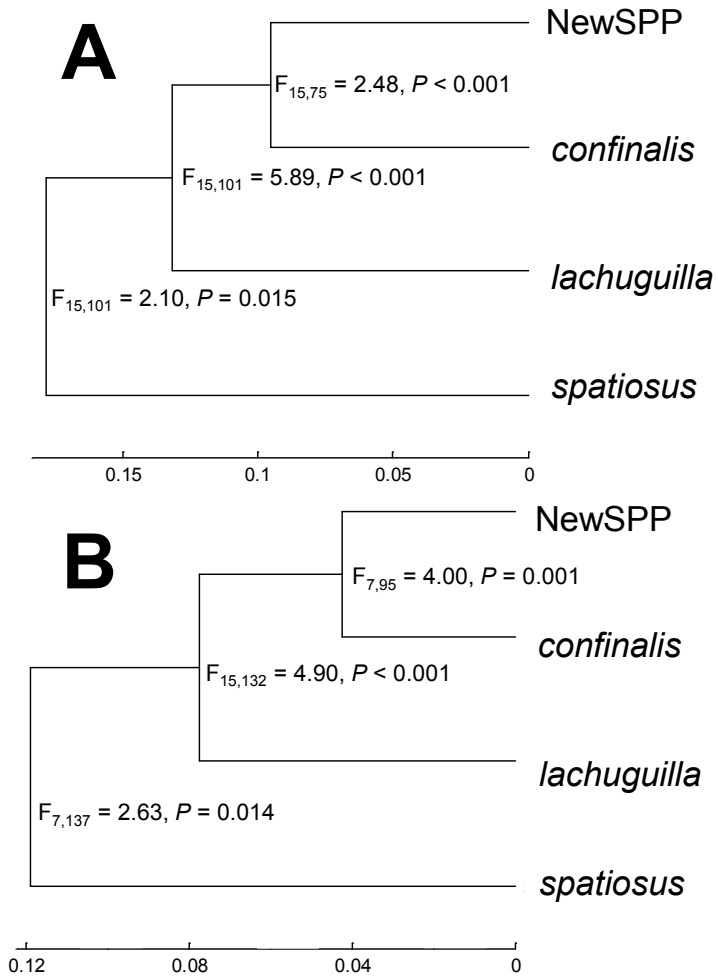

Figure 4. Results of a UPGMA cluster analysis classifying four subspecies of *Thomomys bottae* (*confinalis*, *lachuguilla*, *spatiosus*, and an undescribed taxon). Statistics at each node represent results from a MANOVA examining significant multivariate differences between the two groups separated by the node. A) Results for females from the original data set. B) Results from the dataset combining the Beauchamp study with 47 additional specimens measured based on a reduced number of cranial characteristics.

DISCUSSION

Of the 14 nominal subspecies examined in this study, only *T. b. limpiae* and *T. b. texensis* were originally validated based on statistical assessments (Davis and Buechner 1946). Moreover, inadequate sample size could have been an issue in historical examinations of subspecies of *T. bottae*. For example, 10 of the 14 subspecies examined in this study initially were described based on fewer than 11 specimens. Further, *T. b. pervarius, T. b. spatiosus, T. b. pectoralis, T. b. confinalis, T. b. scotophilus*, and *T. b. lachuguilla* were named on the basis of five or fewer specimens. Further complicating the issue is that subspecific recognition based on morphology may not necessarily reflect substantiated geographic distinctness based on genes or other aspects of the phenotype (see Patton and Conroy 2017 for a discussion).

Analyses of 252 adult female specimens revealed multiple contiguous subspecies were differentiated by ≥ four significant character differences (Fig. 5) and were determined to be worthy of subspecific consideration. Taxa meeting this criterion included: *T. b. actuosus, T. b. baileyi, T. b. confinalis, T. b. limpiae, T. b. spatiosus*, and *T. b. tularosae*. Lack of discriminating characters between *T. b. ruidosae, T. b. guadalupensis, T. b. pectoralis, T. b. scotophilus*, and *T. b. texensis* support the recognition of a single taxon referable to *T. b. texensis* (Bailey 1902). Further, specimens assigned to *T. b. lachuguilla*, *T. b. pervarius*, and *T. b. limitaris* (only *T. b. limitaris* samples from Brewster and southeastern Presidio counties) were morphologically indistinguishable and are best relegated to a single taxon, *T. b. lachuguilla* (Bailey 1902). In contrast, Terrell County specimens of *T. bottae* are distinguished from Val Verde County specimens in least interorbital constriction and mandibular alveolar length. The presence of significantly different measured characters between populations of the same subspecies indicates the presence of a barrier to free gene flow, possibly the Pecos River. Because there were few specimens examined from Terrell County, we tentatively retain all specimens from Terrell, Val Verde, Crockett, Irion, Reagan, Upton, and Crane counties and a portion of Pecos County in the same taxon.

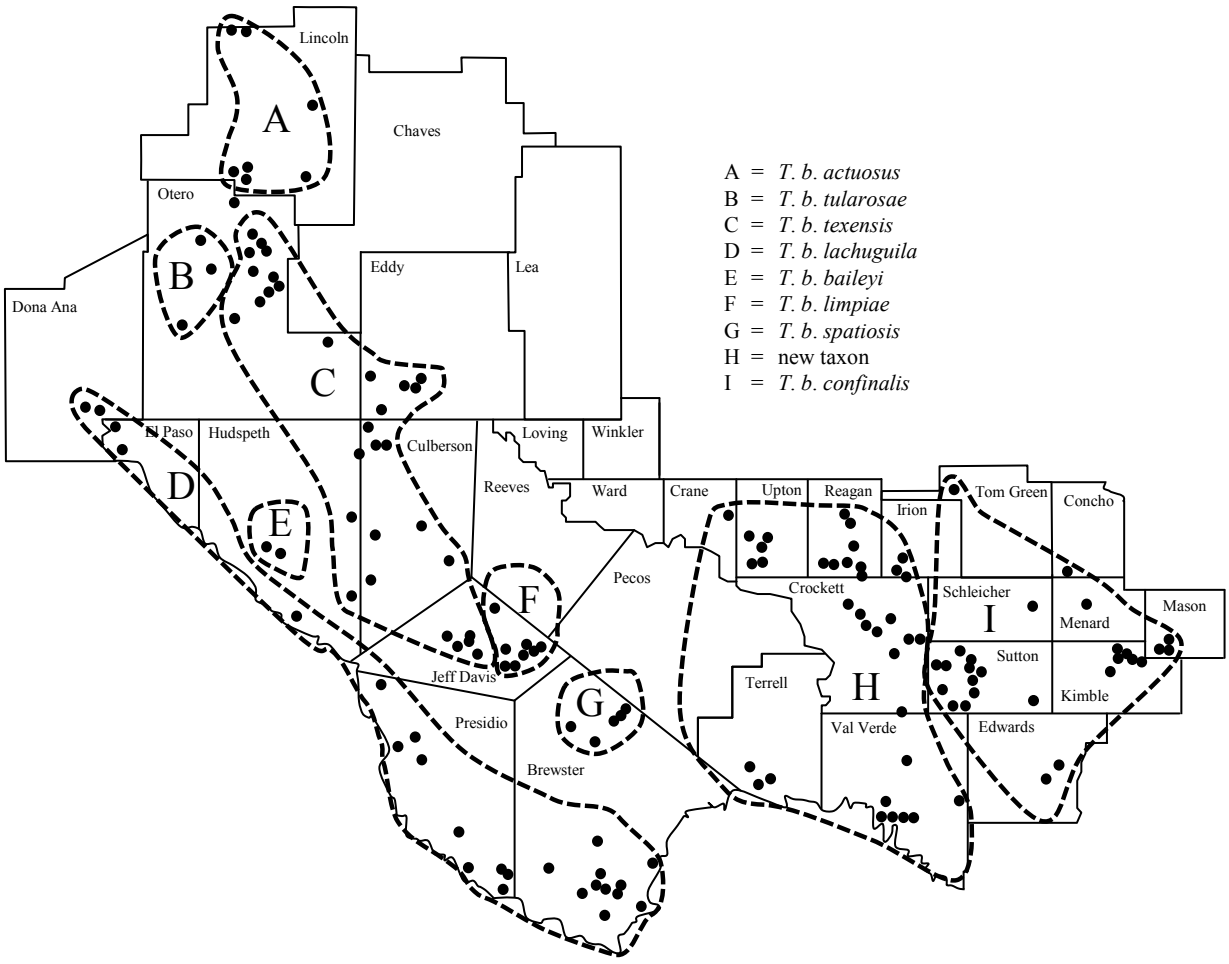

Figure 5. Map depicting the tentative distribution of subspecies (delineated by dashed lines) of *Thomomys bottae* as determined from analyses presented herein. Dots represent the approximate location of specimens examined in this study (see *Specimens Examined* for exact localities) and capital letters (A–I) correspond to subspecies as shown in the inset.

A complication to this solution involves the type locality for *T. b. limitaris*. The range of *T. b. lachuguilla,* as defined by the results presented herein, reaches its easternmost extent in Brewster County, and would include specimens from the type locality of *T. b. limitaris* (Boquillas, Texas). Therefore, the name *T. b. limitaris* becomes a junior synonym of *T. b. lachuguilla*, dictating that a new name be submitted for the specimens occupying the eastern portion of the former distribution of *T. b. limitaris* (following rules of zoological nomenclature ICZN 1999). Further, the former range of *T. b. limitaris* does not clearly reflect the natural break between morphologically distinct specimens from Brewster and Terrell counties. Therefore, a new taxon for all specimens from Terrell County eastward to the range of *T. b. confinalis* (those formerly assigned to *T. b. limitaris*) is proposed.

At this point, we conservatively suggest the following taxonomic considerations (presented below) and await confirmation from other datasets that could expand upon the data reported for the cytochrome-*b* gene (Wickliffe et al. 2005). Descriptive statistics (Beauchamp 1998) for the nine newly realigned subspecies were tabulated to lend validity to taxonomic conclusions presented herein (Appendix). MANOVA and Duncan's tests of the female samples revealed highly significant differences ($P \leq 0.001$) to exist for 13 of the 15 measured characters (exception occipital depth and nasal length). The basis for these decisions is discussed below. Descriptions, distributions, and zoogeographic analyses of the redefined subspecies (Fig. 5) and a previously undescribed taxon are provided. Within each Subspecies Account, the *Description* utilizes a combination of data generated herein as well as diagnostic information provided in the primary literature; accordingly, the descriptors for coloration often follow the system provided by Ridgeway (1912). Specimens examined (all from the United States) are listed under each taxonomic summary, with collections and museum designations following Dunnum et al. (2018): Angelo State Natural History Collection (ASNHC); Biodiversity Research and Teaching Collection, Mayborn Museum Complex, Baylor University (BU); University of Kansas, Natural History Museum and Biodiversity Research Center (KU); Midwestern State University (MWSU); Sul Ross State University Scudday Vertebrate Collection (SRSU); Texas A&M University (TCWC); Collection of Recent Mammals, Museum of Texas Tech University (TTU); University of Illinois Museum of Natural History (UIMNH) (these specimens now reside at the Museum of Southwestern Biology, University of New Mexico); University of Michigan Museum of Zoology (UMMZ); National Museum of Natural History (USNM); and University of Texas at El Paso, Biodiversity Collections, Mammal Division (UTEP).

ACCOUNTS OF SUBSPECIES

Thomomys bottae robertbakeri new subspecies

Holotype.—Adult female (no embryos), skin and skull specimen, The Museum, Texas Tech University, no. 43,737, from 2.5 mi. E McCamey, Upton County, Texas; obtained on 18 March 1986 by J. Knox Jones, Jr., original no. 6,135.

Measurements of holotype.—External measurements (mm) of the type are: total length, 209; length of tail vertebrae, 58; length of hind foot, 25; length of ear, 7; and weight, 115 grams. Selected cranial measurements (mm) are as follows: condylobasal length, 35.45; zygomatic breadth, 22.57; masoidal breadth, 17.85; occipital depth, 12.27; rostral breadth, 7.08; rostral length, 13.43; nasal length, 11.74; least interorbital constriction, 6.59; palatofrontal depth, 13.40; maxillary alveolar length, 7.41; palatal length, 19.45; upper incisor width, 2.03; mandibular alveolar length, 7.54; ramus depth, 14.55; and lower incisor width, 1.97.

Distribution.—Range mostly occupies the western Edwards Plateau, bordered by the Devil's River to the east and the Pecos River to the west, but provisionally includes Trans-Pecos populations from the proximal southeastern corner of the Stockton Plateau in southern Terrell County, Texas (Fig. 5).

Description.—The dorsal pelage of the holotype appears dull, light cinnamon in color and is distinguished by a darker cinnamon colored mid-dorsal line running from the head to the midback. The lateral

pelage uniformly lightens from dull cinnamon to buff. The venter is white to buff in color with visible gray underfur. Dark charcoal postauricular patches distinguish the ears. The mouth and nose are light gray, whereas the pouch margins are buff. The feet are white. The tail is not sharply bicolored; however, it appears light buff above and white below.

In general, characters analyzed in this study indicate that *T. b. robertbakeri* is a medium-sized subspecies of *Thomomys*, slightly smaller than the adjacent subspecies to the west (*T. b. lachuguilla*; see Appendix); whereas *T. b. robertbakeri* is generally larger than the adjacent eastern subspecies, *T. b. confinalis* (Appendix).

Comparisons.—Means for cranial measurements (Appendix) indicate that *T. b. robertbakeri* is statistically larger than *T. b. confinalis* in the following cranial measurements: zygomatic breadth, rostral breadth, maxillary alveolar length, upper incisor width, and ramus depth; but smaller than *T. b. lachuguilla* for rostral breadth, least interorbital constriction, maxillary alveolar length, upper incisor width, mandibular alveolar length, and upper incisor width.

Compared to *T. b. confinalis*, the dorsal pelage of *T. b. robertbakeri* is slightly lighter (medium cinnamon-ochre) with a slightly less marked mid-dorsal line. The nose and mouth of *T. b. confinalis* are of a darker gray than in *T. b. robertbakeri* and the pouch margins are cinnamon rather than buff. The complexity of pelage coloration in *T. b. lachuguilla* is discussed under that account, but in general, the dorsal pelage of *T. b. lachuguilla* is brighter in appearance and has a more tawny-yellow coloration with interspersed gray hairs compared to specimens of *T. b. robertbakeri*. The darker mid-dorsal line is not evident in *T. b. lachuguilla* as it is in *T. b. robertbakeri*. The lateral pelage of *T. b. lachuguilla* grades abruptly from tawny-yellow to buff, and the venter is white buff with very light gray visible underfur, whereas a darker brown pelage is found in specimens *T. b. robertbakeri*. Postauricular patches in *T. b. robertbakeri* are blackish rather than gray as seen in *T. b. lachuguilla*. In *T. b. robertbakeri*, the nose and mouth are dark gray in color and the pouch margins are orange in tone rather than buff; where these characters are lighter in appearance in *T. b. lachuguilla*.

Remarks.—*T. b. robertbakeri* encompasses populations of *T. bottae* from the eastern portion of the Trans-Pecos previously assigned to the subspecies *T. b. limitaris,* which in this study has been placed in synonomy under *T. b. lachuguilla* (see above).

Etymology.—The subspecies is named in honor of Horn Professor Robert J. Baker of Texas Tech University, in recognition of his many contributions to mammalogy, and specifically for his work in both the field and the laboratory with geomyid rodents.

Specimens examined (*n* = 96).—TEXAS; Crane Co., 6 mi S Crane (TTU 43110, 43111, 47187); Crockett Co., 20 mi S Big Lake (TTU 6781); 1 mi E Ozona (TTU 6699); 0.8 mi S Ozona (MWSU 9498); 4 mi N Ozona (TCWC 22793–22795); 4 mi W Ozona (TTU 6613); 5 mi N Ozona (TCWC 22797); 7 mi E Ozona (TTU 8322); 8 mi S Ozona (TTU 6700); 11 mi NW Ozona (TTU 6702); 14 mi N, 13 mi W Ozona (TTU 44474, 44475); 14 mi N, 16 mi W Ozona (TTU 44476); 15 mi N, 11 mi W Ozona (TTU 6701); 17 mi NW Ozona, 3 (TTU 6703, 6994, 12061); 25 mi NW Ozona (TTU 11999); 27 mi NW Ozona (TTU 6896); 33 mi N Ozona (listed as Val Verde Co. on locality tag) (SRSU 899); Irion Co., 0.5 mi W Barnhart (TTU 44477); 1.7 mi S Barnhart (MWSU 9499); 4 mi N Barnhart (TTU 6765, 6875). Reagan Co.: 1 mi W Best (TTU 44478); 1 mi S Big Lake (TTU 12001); 2 mi S Big Lake (TTU 44481) 3 mi W Big Lake (TTU 44479, 44480, 44482–44484); 4 mi S Big Lake (TTU 6780); 7 mi N Big Lake (TTU 6784, MWSU 6874); 8 mi N Big Lake (MWSU 8267); 12 mi N Big Lake (TTU 6614); 15 mi W Big Lake (TTU 6779); 3 mi SE Stiles (TTU 6238); 5 mi SE Stiles (TTU 6881); 6 mi SE Stiles (TTU 6885, 6894, 6895); Terrell Co., 15 mi S Dryden, 1,700 ft. (KU 52017, 52020, 52021); 15 mi S, 6 mi E Dryden, 1,700 ft (KU 52022–52025); 1 mi W Dryden, 2200 ft. (KU 52017, 52018); Upton Co., McCamey (TTU44649, 44650, 45540); McCamey Country Club (TTU 43740); 1 mi E McCamey (TTU 44651, 44652); 1.5 mi E McCamey (TTU 44653–44655); 2.5 mi E McCamey (TTU 43115, 43116, 43735–43739); 3 mi E McCamey (TTU 43112–43114); 4 mi N, 4 mi E McCamey (TTU 44656); 12 mi N, 5 mi E McCamey (TTU 43117–43119); and Val Verde Co., Comstock (USNM 31349, 31350, 108607); 1 mi E Comstock, 1,400 ft. (KU 52027); 5 mi E Com-

stock, 1,300 ft. (KU 52028); 8 mi E Comstock, 1,200 ft. (KU 52029–52032); 9 mi E Comstock, 1,100 ft (KU 52033, 52034); 8 mi N Comstock (MWSU 8266); 3 mi W Comstock, 1,600 ft. (KU 52026); 13 mi. below Juno (USNM 117572); 30 mi N Juno (SRSU 897); 5 mi N Hwy. 377 (SRSU 898, 900).

***Thomomys bottae actuosus* Kelson 1951**

1951. *Thomomys bottae actuosus* Kelson, University of Kansas Publication, Museum Natural History 5:67.

1959. *Thomomys umbrinus actuosus*, Hall and Kelson, Mammals of North America, Ronald Press, p. 48.

Holotype.—An adult male from Corona, Lincoln County, New Mexico.

Description.—Relatively large compared to other subspecies (Appendix), but averaging smaller than *tularosae* in all measurements except rostral length, nasal length, maxillary toothrow length, and mandibular toothrow length. Larger than *T. b. texensis* in all measurements except rostral breadth and interorbital width. The dorsal pelage is sayal brown to ochraceous-tawny, ventral pelage is clay or cinnamon, nose and ear patch blackish, tail bicolored (dark on top and lighter below) and white at tip, and feet similar to dorsal pelage.

Distribution.—Isolated between the Rio Grande and Pecos River of the San Andreas and Capital Mountains of New Mexico (Kelson 1951; see Fig. 5). These rivers may influence the distribution of *T. b. actuosus*, although they do not appear to contribute to the isolation of this subspecies.

Remarks.—A definitive geographic or ecological barrier is difficult to determine between *actuosus* and adjacent subspecies. It appears that *T. b. actuosus* is restricted to the rocky soils of the pinon-juniper woodlands below 2,300 m, whereas *T. b. texensis* occupies elevations above 2,300 m (Patton et al. 1979).

Specimens examined ($n = 18$).—NEW MEXICO; Lincoln Co., 3.3 mi. W Alto Village (UTEP 5345); 2.3 mi. W Alto Village (UTEP 5532); 4 mi. W Alto, Eagle Creek, 7,750 ft. (KU 95301-95305); 6 mi. SW Corona (MWSU 14841); 6 mi. SSW Corona (MWSU 14806); 7 mi. WSW Corona (MWSU 14814, 14840); 5 mi. S, 5 mi. W Glencoe, 6,000 ft. (KU 35156); 4 mi NW Lincoln (UM 79065, 79066); Oak Grove Camp, Sierra Blanca (TTU 16793); Ruidoso, 6,500 ft. (KU 35157-35159).

***Thomomys bottae baileyi* Merriam 1901**

1901. *Thomomys baileyi* Merriam, Proceedings Biological Society Washington 14:109.

1915. *Thomomys lachuguilla* (in part) Bailey, North American Fauna 39:89.

1932a. *Thomomys baileyi baileyi*, Hall, University of California Publication Zoology 38:411.

1966. *Thomomys bottae baileyi* Anderson, Systematic Zoology 15:195.

1981. *Thomomys umbrinus baileyi*, Hall, Mammals of North America, John Wiley & Sons, Inc., p. 477.

Holotype.—An adult female from Sierra Blanca, Hudspeth County, Texas.

Description.—A large subspecies, averaging largest of all subspecies in study area for most measurements (Appendix) and rivaled in overall cranial dimensions only by *T. b. tularosae*. Comparison of mean cranial measurements among *T. b. baileyi, T. b. texensis,* and *T. b. lachuguilla* reveal that *T. b. baileyi* is generally, and consistently, larger and has a more robust skull. In comparison to all other redefined subspecies, *T. b. baileyi* averages larger in all characters measured in the study except occipital depth and rostral length. The dorsal pelage is a dull ochraceous tawny or buffy fulvous, ventral pelage is a pale salmon or creamy white, nose and ear patch dusky, tail buffy to tip, and feet soiled whitish.

Distribution.—The only records of this gopher are from the area immediately surrounding the town of Sierra Blanca (Bailey 1905), suggesting that the population is a geographically isolated entity known only from that area (Fig. 5). The Sierra Blanca Mountains are an isolated range 1,500 to 2,100 m in elevation and are surrounded by relatively flat basin land. Previously, Goldman (1938) described populations of *Thomomys* from Alpine, Texas, as a separate subspecies of *baileyi*, *T. baileyi spatiosus*, which represented an eastern ex-

tension of the known range of the species. However, as discussed below (see Remarks), that taxonomic assignment is not supported by the analysis of this paper.

Remarks.—This taxon has had a long and convoluted taxonomic history. Originally described as a separate species (*T. baileyi*) by Merriam in 1901, it retained that status until Anderson (1966) presented evidence on the basis of his study of specimens from Chihuahua, Mexico, which led to his conclusion that *T. baileyi* likely was not a unique species. To quote Anderson (1966: 195), "Although I have not studied Texan and New Mexican material as intensively as the Chihuahuan specimens, I have looked at the types of *T. baileyi spatiosus*, *T. baileyi baileyi*, and *T. baileyi mearnsi* and would assign them to the same species as the surrounding gophers, which are *Thomomys bottae* of various subspecies."

Goldman (1938) described gophers from Alpine, Brewster County, Texas, as a separate subspecies of *baileyi*, *T. b. spatiosus*. Goldman also referred to *spatiosus* specimens from Paisano and two specimens from Presidio County (no specific locality) that were among four specimens collected by Bailey around 1890. In the 1938 paper, Goldman also described *T. bottae pervarius*, based on specimens collected from Lloyd Ranch, 35 miles south of Marfa, Presidio County.

An unpublished Ph.D. dissertation by Lane (1965), based on detailed cranial evidence with supporting statistical analysis of *Thomomys* populations from the borderlands of western Texas, southern New Mexico and Arizona, attempted to sort out some of the taxonomic problems relative to the subspecies of *T. bottae*. Lane suggested that the two specimens from Presidio County used in the description of *T. baileyi spatiosus* were in fact the other two specimens of *Thomomys* that had been collected by Bailey in 1890 (Bailey's 1915 monograph on *Thomomys* clearly indicated that he had collected four specimens of specimens from Lloyd's Ranch, 35 miles south of Marfa, Presidio County). Lane suggested that Goldman, in essence, named *T. bottae pervarius* entirely, and *T. baileyi spatiosus* in part, on specimens from the same locality. From his observations and analysis, Lane concluded that the two subspecies of *T. baileyi* (*baileyi* and *spatiosus*) and one subspecies of *bottae* (*T. b. pervarius*) should be combined under one subspecies, *T. bottae baileyi*, thus uniting under one subspecies the two former subspecies of *T. baileyi* and one of *T. bottae*. Thus, Lane (1965) and Anderson (1966) reached the same general conclusion that *T. baileyi* was a "composite taxon" that did not warrant separate species status. Apparently, Anderson was not aware of Lane's dissertation as he did not cite it, and unfortunately the dissertation was never published in a scientific journal. Only one of the four specimens discussed by Lane (1965) was included herein as it met the criteria age and sex requirement set forth in this study.

The analysis in this paper supports a slightly different taxonomic arrangement from that suggested by Lane, namely that gophers described by Goldman under the subspecies *T. b. pervarius* should be assigned to the subspecies *T. b. lachuguilla* (see below) and that *T. b. spatiosus* is, in fact, a valid subspecies of *T. bottae* separate from *T. b. baileyi*. Despite the low sample size for this subspecies, *T. b. baileyi* is recognized as a valid taxon due to the high number of significant cranial differences between it and adjacent populations.

Attempts over the past 75 years by mammalogists (including recent efforts by one of us, RDB) to find populations of *T. b. baileyi* have not produced any specimens, although another gopher, *Cratogeomys castanops*, has been obtained at the type locality (Sierra Blanca, Texas) of *T. b. baileyi*. At other places in the Trans-Pecos, *Cratogeomys* is known to have competitively replaced *Thomomys* (see Reichman and Baker 1972; Stangl et al. 1994; and discussion below), thus raising the possibility that *T. b. baileyi* is now extinct.

Specimens examined (n = 11).—TEXAS; Hudspeth Co., 1 mi. E Sierra Blanca (USNM 18072, 18255); 3 mi. N Sierra Blanca (TCWC 1624; recorded on locality tag as Culberson Co.); Sierra Blanca (USNM 18253, 247185, 247186, 24970, 24971, 25156–25158).

Thomomys bottae confinalis Goldman, 1938

1936. *Thomomys lachuguilla confinalis* Goldman, Journal Washington Academy Science 26:119.

1938. *Thomomys bottae confinalis* Goldman, Proceedings Biological Society Washington 51:55.

1959. *Thomomys umbrinus confinalis,* Hall and Kelson, Mammals of North America, Ronald Press, p. 423.

Holotype.—A subadult male from 35 mi. E Rock Springs, Edwards County, Texas.

Description.—As the easternmost representative of *T. bottae*, *T. b. confinalis* can be distinguished from its only adjacent taxon (described here as the new taxon, *T. b. robertbakeri*) by its smaller size. Comparison of means (Appendix) indicates that *T. b. confinalis* is statistically smaller than *T. b. robertbakeri* in the following cranial measurements: zygomatic breadth, rostral length, maxillary alveolar length, palatal length, upper incisor width, and ramus depth. The dorsal pelage of *confinalis* is slightly darker (dark cinnamon-ochre) than that of *T. b. robertbakeri* with a slightly darker mid-dorsal line. The nose and mouth of *T. b. confinalis* is dark gray and the pouch margins are cinnamon rather than buff.

Distribution.—Ranges over the eastern half of the Edwards Plateau seemingly bordered to the west by the Devil's River (Fig. 5).

Remarks.—A concise or natural geographic barrier that isolates *T. b. confinalis* from the new taxon *T. b. robertbakeri* is difficult to define. Dalquest and Kilpatrick (1973) suggested the area should be the line separating the Chihuahuan Desert from the Edwards Plateau, corresponding to the dividing line between the Chihuahuan and Balconian biotic provinces of Blair (1950). Several efforts to obtain specimens from the vicinity of the type locality (35 mi. E Rock Springs, Texas) have failed to produce specimens of *T. b. confinalis*; however, a small population resides near London in Kimble County, Texas.

Specimens examined (n = 137).—TEXAS: Concho Co., 6 mi S, 16 mi W Eden (TTU 25851); 18 mi SW Eden (TCWC 30682); Edwards Co., 2 mi S Rocksprings (MWSU 7578); 3 mi E Rocksprings (MWSU 7067, 7069, 7070, 7577, 7579); 30 mi N Rocksprings (MWSU 7576); Rocksprings (MSB 183420, 183421, 183423, 183414, 183415, 183417, 183418, 183424, 183425, 183426); Kimble Co., 1.5 mi S London (TTU 44472); 2 mi S London (TTU 44473); 2 mi W London (MWSU 7065); 2.5 mi SSW London (TTU 43732–43734); 3 mi S London (MWSU 13310); 3 mi W London (MWSU 5570–5573, 6866); 3 mi SE London (MWSU 8913–8915); 3 mi SSW London (MWSU 7570, TTU 45538); 4 mi SSW London (MWSU 6873, TTU 45539); 4 mi SSW London (MWSU 7571, 7572, 7574, 7575); 5 mi SW London (MWSU 8269); 6 mi SSW London (TTU 43731); 10 mi NE Junction (MWSU 5971, 6016–6019, 7573, 8376, 8277); 11 mi NE Junction (MWSU 14839); 0.9 mi S intersection RR 385 and Hwy 377 (BU 946); 1 mi S intersection RR 385 and Hwy 377 (BU 942); 3 mi W intersection RR 385 and Hwy 377 (BU 944); 2.7 mi S intersection RR 385 and Hwy 377 (BU 933); Mason Co., 0.25 mi S Llano River (BU 1250); 0.4 mi S Llano River (BU 1251); 0.6 mi Llano River (BU 1252); 1.5 mi S Llano River (BU 1253, 1254); 2.4 mi W Llano River (BU 1255); 2.5 mi Llano River (BU 1256); 2.7 mi W Llano River (BU 1257–1259); 2.9 mi W Llano River (BU 1260); 9 mi SSW Mason (BU 934); Menard Co., 22 mi W, 9 mi N Menard (TTU 7747); Schleicher Co., 5.1 mi W FM 2084, Napier Ranch (BU 935–940); Sutton Co., 0.9 mi W Jct 277 and FM 189 (TTU 8292, 8293); 5 mi W Jct 277 and FM 189 (TTU 8320); 7 mi W Jct 277 and FM 189 (TTU 12017, 12062); Sonora (MWSU 7580; TTU 6162, 6408, 6411, 6615, 6616, 6696, 7096, 7143, 8321, 8325, 8326); 1 mi SE Sonora (TTU 6415); 1.7 mi S Sonora (TTU 6409); 1.9 mi S Sonora (TTU 6401, 6414); 2 mi S Sonora (TTU 6402, 6430); 2 mi SE Sonora (TTU 6163); 2.4 mi S Sonora (TTU 6412); 2.9 mi S Sonora (TTU 6410); 2 mi N, 9 mi W Sonora (TTU 44485); 3 mi S Sonora (TTU 6405); 5 mi S Sonora (TTU 6997); 5.2 mi NE Sonora (TTU 7141); 6.2 mi S Sonora (TTU 6404); 6.4 mi S Sonora (TTU 6406); 6.5 mi S Sonora (TTU 7140); 7 mi E Sonora (TCWC 539, 540, 2321–2328); 13 mi W Sonora (TTU 44486); 15.5 mi S Sonora (TTU 8313, 8327, 8328); 20 mi SW Sonora (TCWC 46106); 20 mi SSW Sonora (TTU 8270–8274); 20 mi W Sonora (TTU 8314–8319); and Tom Green Co., 8 mi W Carlsbad (TCWC 30051, 30052).

Thomomys bottae lachuguilla Bailey, 1902

1902. *Thomomys aureus lachuguilla* V. Bailey, Proceedings Biological Society Washington 15:10.

1936. *Thomomys lachuguilla limitaris* Goldman, Journal Washington Academy Science 26:118 [type locality 4 mi. W Boquillas, Brewster Co., Texas].

1938. *Thomomys bottae lachuguilla* [sic], Goldman, Proceedings Biological Society Washington 51:55.

1938. *Thomomys bottae pervarius* Goldman, Proceedings Biological Society Washington 51:57 [type locality Lloyd Ranch, 35 mi. S Marfa, Presidio Co., Texas].

1959. *Thomomys umbrinus lachuguilla*, Hall and Kelson, Mammals of North America, Ronald Press, p. 42.

Holotype.—An adult male from "foothills near El Paso", El Paso County, Texas.

Description.—Compared to adjacent taxa, *T. b. lachuguilla* is a medium-sized gopher. On average, it is smaller than *T. b. baileyi* in all measured characters, but it is larger (Appendix) than both *spatiosus* and the new taxon *T. b. robertbakeri* in all characters except ramus depth, maxillary toothrow length, condylobasal length, zygomatic breadth, occipital depth, and palatal length. The dorsal pelage of *T. b. lachuguilla* is darker in the western part of the distribution and grades to a tawny-yellow coloration with interspersed gray in the eastern portions of the range. Similarly, the lateral pelage in *lachuguilla* grades abruptly from grayish brown in the western regions to tawny-yellow to buff or white in the eastern portion of the range. The venter is white buff with very light gray visible underfur in western specimens and tawny-yellow to creamy brown in the east. The nose and mouth are dark gray in color and the pouch margins are orange in tone rather than buff. The tail is not bicolored.

Distribution.—*T. b. lachuguilla* ranges along the rugged, mountainous terrain bordering the Rio Grande, from southeastern Dona Ana County, New Mexico, to the Big Bend Country of southern Brewster County (Fig. 5). The continuous, rugged topography and rocky, shallow soils of this region provide ideal habitat and do not present a barrier to distribution of gophers. This region is neither interrupted by large basins nor plains, which accounts for the more or less continuous distribution of the subspecies. There does not appear to be a distinct geographic barrier to the distribution of *T. b. lachuguilla* in eastern Brewster County, although the shear canyon walls along the Rio Grande at Boquillas Canyon could present a geographic isolating mechanism. However, the gradation of the terrain from the rugged Brewster County into the Stockton Plateau at the extent of its range may provide a more realistic geographic barrier for the subspecies.

Remarks.—*T. b. lachuguilla*, as defined in this study, includes populations previously referred to as *T. b. pervarius* from southern Presidio County and *T. b. limitaris* from the Big Bend region of southern Brewster County. There are no concordant character breaks among the cranial measurements examined that support taxonomic differentiation of gophers in southern Presidio County. With the inclusion of *T. b. limitaris* and *T. b. pervarius*, the differences in pelage coloration are no longer as diagnostic as discussed by Goldman (1936, 1938). In general, the dorsal pelage of specimens of *T. b. lachuguilla* are darker in the western part of the distribution (restricted to *T. b. lachuguilla* of previous studies) and grade to a tawny-yellow coloration with interspersed gray in the eastern portions of the range (inclusion of specimens formerly assigned to *T. b. limitaris* and *T. b. pervarius*).

Recent efforts (by RDB) to obtain samples of *T. b. pervarius* have proven difficult. A small population has been located near Shafter, Texas, and genetic assays are currently being conducted on this taxon to determine if specimens formerly assigned to *T. b. pervarius* are distinct from *T. b. lachuguilla*. For a further discussion of the status of *T. b. pervarius*, see the account of *T. b. baileyi*.

Specimens examined ($n = 90$).—NEW MEXICO; Dona Ana Co., N Anthony Cave (UTEP 4801); Ft. Bliss (UTEP 7706). TEXAS; Brewster Co., 3 mi S Bandera Mesa (MWSU 10583); Black Gap Wildlife Refuge Headquarters (MWSU 19814, 19843, 20284); 2.5 mi E Black Gap (TCWC 6075); Boquillas Canyon (USNM 110338); Chisos Mountains (TCWC 5957, 6067); Green Gulch of Chisos Mountains (UMMZ 103325); Grapevine, Big Bend National Park (TCWC 6079); Big Bend National Park, Harte Ranch, 0.1 mi S, 1.0 mi W Persimmon Gap Ranger Station (TTU 61276, 61277); Housetop Mountain (SRSU 1703); NE Laguna Meadow, Big Bend National Park (USNM 392123, 392131); 6.5 mi NE La Linda (MSWU 18799); 2.5 mi NW Lone Mountain, Big Bend National Park (TCWC 6077, 6078); Panther Junction, Big Bend National Park (TTU 3255); 10 mi N, 14 mi E Panther Junction (ASU 8679); 10 mi N, 15 mi E Panther Junction (ASU 8680, 8681); 11 mi N, 14 mi E Panther Junction (ASU 8676); 11 mi N, 14 mi E Panther Junction (ASU 8677, 8678); 0.2 mi N, 1.3 mi W Persimmon Gap Ranger Station

(TTU 61274, 61275); 1.1 mi N, 3.2 mi W Persimmon Gap (TTU 60103); Pine Canyon, Big Bend National Park (TCWC 46112); Terlingua (KU 52015, 52016); El Paso Co., 1 mi W Donaphuw; El Paso (USNM 25009, 25010, 25012, 35459, 35460, 37270, 37271, 64983, 64984, 110337); 2.5 mi. W Fort Bliss (MSB 183431, 183433, 183436, 183437); Franklin Mountains (USNM 120495–120498, 126112, 126298); foothills of Franklin Mountains (UM 104818); 1 mi. E 0.25 mi N Mt. Franklin (UTEP 2034); McCelligan Canyon Park (UINMH 183428, 183430); Near El Paso (USNM 37268, 18111, 20094); Hudspeth Co., 2.7 mi N Indian Hot Springs (SRSU 1839, 1840); Presidio Co., 5 mi E Bandera Mesa (MWSU 8275); Big Hill, Camino del Rio (SRSU 1295–1297); 8 mi NE Candelaria (TCWC 22880); 14 mi N, 3 mi E Candelaria (TTU 44487); Chinati Mountains (TCWC 2317, 2319, 2320); 10 mi NW Lajitas on mouth of Colorado Canyon (SRSU 2293–2295); 12 mi W Lajitas (ASU 521); 35 mi. S. Marfa (USNM 18202); 3 mi S, 6 mi E Presidio (KU 52013, 52014); 6 mi S, 1 mi W Shafter, 3600 ft. (KU 52247, 52248); 10 mis S. of Redford on river (SRSU 43, 45, 47, 51, 52, 55, 1612); Sierra Vieja Mountains (TTU 8462, 8583).

Thomomys bottae limpiae Blair 1939

1939. *Thomomys bottae limpiae* Blair, Occasional Papers of Museum of Zoology, University Michigan 403:2.

1959. *Thomomys umbrinus limpiae*, Hall and Kelson, Mammals of North America, Ronald Press, p. 427.

Holotype.—An adult male from Limpia Canyon, 1 mi. N Fort Davis, Jeff Davis County, Texas.

Description.—*T. b. limpiae* is distinguished from the other taxa by its comparatively moderate size (Appendix). Comparisons of mean cranial measurements reveals it is larger than adjacent taxa (*T. b. texensis* and *T. b. spatiosus*) and can be distinguished from both adjacent subspecies by its shorter nasal length, least interorbital constriction, and maxillary toothrow length. *T. b. limpiae* also is distinguished from the subspecies *T. b. texensis* by a shorter mandibular toothrow length. The dorsal pelage is a raw umber, sides and ventral pelage is a pinkish cinnamon, nose and ear patch blackish, tail white, and feet white.

Distribution.—*T. b. limpiae* is restricted to the isolated streambed association of Lower Limpia Canyon along Limpia Creek (see Fig. 5) situated at an elevation below 1,500 m (Blair 1940). Lower Limpia Canyon is characterized as a Chihuahuan Desert habitat, and is noted by its narrow, steep walls and intermittent water flow (Blair 1940; Williams and Baker 1976). The narrowly defined habitat of *T. b. limpiae* is indicative of its isolation from both *T. b. texensis* and *T. b. spatiosus*. Although *T. b. limpiae* occupies the streambed association of Lower Limpia Canyon, *T. b. texensis* occupies the same habitat in Upper Limpia Canyon above 1,500 m. A further factor restricting the habitat of *T. b. limpiae* is its range overlap and competitive interaction with *Cratogeomys castanops,* which also prevents contact with the adjacent subspecies, *T. b. texensis* (Reichman and Baker 1972; Stangl et al. 1994).

Remarks.—The ranges of *T. b. limpiae* and *T. b. texensis* appear to abut along Limpia Creek northeast of Ft. Davis where State Highway 118 runs between Davis Mountain State Park and Macdonald Observatory, with specimens at or below the state park being referable to *T. b. limpiae* and those at or above the observatory being assignable to *T. b. texensis*. Unfortunately, there are not enough specimens to conduct a detailed analysis of the zone of intergradation but that should be done in the future.

Specimens examined (n = 83).—TEXAS; Jeff Davis Co., Fort Davis (USBS 22512, 22515), 1 mi. N Fort Davis (TCWC 2290–2302); 1 mi. NW Fort Davis (UM 79103, 79104, 79112); 1 mi. W. Fort Davis (MVZ 91239); 4 mi. NE Fort Davis (TTU 14065); 7.5 mi. N, 6.5 mi. E Fort Davis (ASU 7743–7745); 9 mi. NE Fort Davis, Limpia Canyon (TTU 7744–7746, 8271, 14062, 14064); 9.5 mi. NE Fort Davis, Limpia Canyon (TTU 7789–7793); 10 mi. N Fort Davis, Limpia Canyon (TTU 8179, 8273, 8274, 8276–8280, 8283, 8287–8289, 8330–8332, 17261, 17741, 18283, 12018–12021); 11 mi. NE Fort Davis, Limpia Canyon (TTU 7782, 7787, 7898–7901, 8281, 8972, 10241, 12005, 12006, 12022, 14067, 17258); 11.2 mi. NE Fort Davis, Limpia Canyon (TTU 7783); 11.4 mi. NE Fort Davis (TTU 14066); 11.5 mi. NE Fort Davis (TTU 7794); 11.6 mi. NE Fort Davis (TTU 7795); 11.7 mi. NE Fort Davis (TTU 7796); 11.8 mi. N Fort Davis (TTU 7788); 12 mi. NE Fort Davis, Limpia Canyon (TTU 7784); 12.2 mi. NE Fort Davis

(TTU 7797); 14.1 mi. S Toyahvale, Reeves Co. in Jeff Davis County (MWSU 10577–10582); 0.5 mi. N Wildrose Pass, 13.5 mi. NE Fort Davis (TTU 7785); 1.7 mi. N Wildrose Pass, 14.6 mi. NE Fort Davis (TTU 7786).

Thomomys bottae spatiosus Goldman, 1938

1938. *Thomomys baileyi spatiosus* Goldman, Proceedings Biological Society Washington 51:58.

1966. *Thomomys bottae spatiosus,* Anderson, Systematic Zoology 15:195.

1981. *Thomomys umbrinus spatiosus*, Hall, Mammals of North America, John Wiley & Sons, Inc., p. 494.

Holotype.—An adult male from Alpine, Brewster County, Texas.

Description.—*T. b. spatiosus* averages smaller in most cranial measurements than the adjacent subspecies *T. b. limpiae* and *T. b. lachuguilla*. Measurements of *T. b. spatiosus* with means (Appendix) larger than those of *T. b. lachuguilla* and *T. b. limpiae* include maxillary toothrow length, ramus depth, upper incisor width, nasal length, interorbital width, and maxillary toothrow length. The dorsal pelage is dark with a mixture of cinnamon and black, sides are a cinnamon buff, ventral pelage is a pinkish buff, nose blackish, ears encircled by black, tail light brown above, grayish below and white at tip, and feet white.

Distribution.—*T. b. spatiosus* is restricted to the vicinity of Alpine, Brewster Co., Texas, a rugged area reaching elevations of approximately1,400 m at the type locatity (Fig. 5). *Thomomys b. spatiosus* is clearly isolated from the adjacent subspecies *T. b. limpiae* by factors indicated by its realignment. The geographic boundary is more difficult to define for specimens of *T. b. spatiosus* and *T. b. lachuguilla*, although the Marfa Plains southwest of Alpine may act as a barrier that isolates the two subspecies.

Remarks.—Originally described as a subspecies of *T. baileyi*, the analysis herein reveals that *spatiosus* is significantly smaller than specimens of *T. b. baileyi* in almost all of the cranial measurements. Futhermore, there are no obvious biogeographic factors (contiguous habitats or corridors) that link *T. b. baileyi* with *T. b. spatiosus*. Although at least four significantly different measured characters distinguish *T. b. spatiosus* from the adjacent subspecies *T. b. limpiae* and *T. b. lachuguilla*, the total number of specimens of *T. b. spatiosus* available for critical examination is insufficient to discount the possibility of sampling error. The subspecies is therefore only provisionally retained as a valid taxon. Several attempts by one of us (RDB) to obtain exemplars of *T. b. spatiosus* from the vicinity of the type locality (Alpine, Texas) have failed to produce specimens, although large numbers of *Cratogeomys castanops* were present at the type locality and surrounding areas. However, a small population of *T. bottae* was located south of Alpine at the Elephant Mt. Wildlife Management Area and genetic assays are currently being conducted on this taxon to determine if they are representative of *T. b. spatiosus*.

Specimens examined ($n = 20$).—TEXAS; Brewster Co., Marathon (USNM 108602); 11.5 mi N, 2 mi W Marathon (TTU 28845); 17.3 mi N, 0.6 mi E Marathon (TTU 22933); 17.9 mi N, 0.3 mi E Marathon (TTU 22931, 22932); 18.6 mi N, 1.2 mi E Marathon (TTU 22918–22923, 22925, 22926); 10 mi W, 4 mi N Marathon, 4,900 ft. (KU 52249–52255).

Thomomys bottae texensis Bailey, 1902

1902. *Thomomys fulvus texensis* V. Bailey, Proceedings Biological Society Washington 15:119.

1932b. *Thomomys bottae ruidosae* Hall, Proceedings Biological Society Washington 45:96. [type from Ruidoso, Lincoln Co., New Mexico].

1935. *Thomomys bottae texensis,* Goldman, Proceedings Biological Society Washington 48:157.

1936. *Thomomys bottae guadalupensis* Goldman, Journal Washington Academy Science 26:117 [type from McKittrick Canyon, 7800 ft., Guadalupe Mountains, Texas].

1936. *Thomomys pectoralis* Goldman, Journal Washington Academy Science 26:117 [type from vicinity Carlsbad Cave, Carlsbad Cave National Monument, Eddy Co., New Mexico].

1939. *Thomomys umbrinus texensis*, Blair, Occasional Papers of Museum of Zoology, University Michigan 403:2.

1940. *Thomomys bottae scotophilus* Davis, Journal of Mammalogy 21:204 [type from 1.5 mi. W Bat Cave, Sierra Diablo, Hudspeth Co., Texas].

Holotype.—An adult male from "head of Limpia Creek", Jeff Davis County, Texas.

Description.—*T. b. texensis* is a medium-sized gopher based on comparison to other subspecies examined in this study. On average, *T. b. texensis* is smaller in cranial measurements than adjacent taxa *T. b. actuosus*, *T. b. baileyi*, *T. b. limpiae*, and *T. b. tularosae* with the exception of nasal length (*T. b. limpiae* larger), interorbital constriction (*T. b. limpiae* and *T. b. actuosus* larger), maxillary toothrow length (*T. b. limpiae* and *T. b. tularosae* larger), mandibular toothrow length (*T. b. limpiae* and *T. b. tularosae* larger), rostral length (*T. b. baileyi* larger), and rostral breadth (*T. b. actuosus* larger).

Distribution.—Sacramento Mountains of Otero County, New Mexico, and south along the northern Front and Central Ranges of Texas, extending as far south and east as the western Davis Mountains of Jeff Davis County, Texas (Fig. 5). Intervening ranges include the Sierra Diablo, Beach, Wylie, Guadalupe, Delaware, and Apache mountains of Hudspeth and Culberson counties, Texas.

Remarks.—*T. b. texensis* is a broadly distributed subspecies that includes populations previously referred to the subspecies *T. b. ruidosae*, *T. b. guadalupensis*, *T. b. pectoralis*, and *T. b. scotophilus*. Throughout its range, *T. b. texensis* appears to reside in an upper montane habitat above 1,500 m in elevation. It is typically isolated to the stream-bed association of riparian-oak or Transitional to Canadian Zone conifer vegetation belts (Bailey 1905; Blair 1940; Davis and Robertson 1944; Patton et al. 1979). While the rugged topography of the range of *T. b. texensis* is interrupted by basins and flat land deserts (Davis and Robertson 1944), contact between populations of *T. b. texensis* is maintained more or less continuously between the mountain ranges. The Sacramento Mountain range of New Mexico is continuous with the Guadalupe Mountains of Texas. The continuous Front Range of Texas, including the Guadalupe, Apache, Delaware, and Davis Mountain ranges is bridged to the Central Range Sierra Diablo mountains by the Baylor, Beach, and Wylie mountain ranges (Stangl et al. 1994).

Pleistocene conditions allowed the extension of montane forms, such as *T. b. texensis*, to occupy desert regions in the lower Apache and Davis Mountain ranges (Dalquest and Stangl 1986). Pleistocene deposits from this study area indicate that *T. bottae* was common and continuously distributed across the range indicated for *T. b. texensis* (Davis 1940; Hafner et al. 1983; Stangl et al. 1994). Recent deposits indicate that *T. bottae* is being replaced by *Cratogeomys castanops* (Dalquest and Stangl 1986; Stangl et al. 1994). In response to climate changes in the post-Pleistocene and competition with *Cratogeomys*, *Thomomys* retreated to areas higher in elevation (Stangl et al. 1994), leaving *C. castanops* to occupy the xeric basin lands interrupting the mountain habitat of *Thomomys*. The depiction of *T. bottae* in this region by Hall (1981) is a misinterpretation of both the lack of suitable habitat for *Thomomys* and of the competitive interaction between *T. bottae* and *C. castanops*. Evidence suggested that *T. b. texensis* represents a continuous series of populations that were/are connected during mesic conditions rather than a series of isolated independently evolving populations.

Specimens examined (n = 160).—NEW MEXICO; Eddy Co., Carlsbad Caverns National Park, 4 mi W White's City, 3,700 ft. (KU 52010–52012, 52209, 52211); Carlsbad Caverns National Park, 6 mi W White's City (KU 52210); 30 mi SW Carlsbad, 1 mi up Rattlesnake Canyon (KU 8223); 5 mi E El Paso, Gap Fir Canyon (TTU 6774, 6775); 32'6"N;104'45"W, Dark Canyon, Guadalupe Mountains (TTU 8324, 12013–12015); Lincoln National Forest (TTU 8525, 8526); Otero Co., 0.7 mi E Cloudcroft (KU 149078); 1 mi E, 14 mi S Cloudcroft, Lightening Lake (TTU 6884, 6891–6893, 6897–6899); 2 mi E Cloudcroft (UTEP 1849); 9.5 mi E Cloudcroft (KU 149076, 149077); 2 mi NE Cloudcroft (TCWC 46114–46118); 2.4 mi W Cloudcroft (UTEP 2564); 3 mi E Cloudcroft (UTEP 1995); 10 mi N Cloudcroft (UM 999821, 99822); 10 mi N Cloudcroft (KU 149063–149075); 20 mi S Cloudcroft (TTU 5997, 5998, 6000, 6001); Lightening Lake, 12E township Section 18 (TTU 7417, 7419–7421, 7428–7432, 7443, 7454–7457); Lincoln

National Forest (UTEP 5533–5535); 0.25 mi E Mayhill (UTEP 1916); 15 mi E Mayhill (UTEP 1938, 1939); 24 mi N Orogrande (UTEP 1938); 3 mi S Jct. 241 and 130 in Sacramento Mountains (TTU10288); 6 mi N Timberon (UTEP 5969); Tularosa (TTU 8463, 8464); 2 mi NE Wofford Tower, Silver Spur Canyon (UTEP 4896). TEXAS; Culberson Co., Bear Canyon, Guadalupe Mountains National Park (TTU 20015, 20018, 20019); 2 mi SSE El Capitan (MWSU 13463, 14564, 14782); 2.5 mi SE El Capitan (MWSU 16928); 3 km. SSE El Capitan (MWSU 13462); Guadalupe Mountains (USNM 109222–109224); 6 mi N Kent (MWSU 12480; TTU 37796); 22 mi NNW Kent (MWSU 20792); Manzanita Spring, Guadalupe National Park (TTU 20016); Lower part of McKittrick Canyon, Guadalupe Mountains National Park (KU 84418–84423); McKittrick Canyon, Guadalupe National Park (TCWC 672–676); 1 mi N, 1 mi E Nickel, 5000 ft. (KU 52212); Nipple Hill, Guadalupe National Park (TTU 20017); North Pine Spring (TCWC 671); 5.3 mi N Van Horn (MWSU 18502–18507); 25 mi NE Van Horn (MWSU 12788); Hudspeth Co., Carrizo Mountains, 6 mi W Van Horn (KU 84415–84417); Diablo Mountains (TCWC 677–682); and Jeff Davis Co., 2 mi NW Fort Davis, near mouth of Limpia Canyon (KU 149046–149048); 10 mi. E. Ft. Davis (TTU 8220, 8333); 14 mi NW Fort Davis (TCWC 2306–2312); 14 mi W Fort Davis (TCWC 2313–2316); 3 mi N Mt. Livermore (TCWC 2303, 2304); Madera Canyon, 3 mi N. Mt. Livermore (TCWC 2305); 1 mi. N. Mt. Livermore (UMMZ 79017–79108); 5 mi. E. Mt. Livermore (UMMZ 79106); 5 mi E Mt. Livermore in Limpia Canyon (TCWC 2283–2289, 2279–2282); Sawtooth Mountains (TTU 8908, 8909, SRSU1023); Presidio Co., Paisano (USNM 31316).

Thomomys bottae tularosae Hall, 1932

1932a. *Thomomys baileyi tularosae* Hall, University California Publication Zoology 38:411.

1981. *Thomomys umbrinus tularosae,* Hall, Mammals of North American, John Wiley and Sons, Inc., p. 495.

Holotype.—An adult female from Cook Ranch, 0.5 mi. W Tularosa, Otero County, New Mexico.

Description.—*T. b. tularosae* is a subspecies distinguished from all other subspecies in the study region by its large average size (Appendix) and the robustness of its skull. It is, on average, larger than *T. b. actuosus* and *T. b. texensis* in all cranial measurements except for maxillary and mandibular toothrow length, rostral length, and nasal length, all of which are larger in *T. b. actuosus*.

Distribution.—Restricted to central Otero County of New Mexico, from eastern grasslands of the Tularosa Basin into the lower western foothills of the Sacramento Mountains (Fig. 5).

Remarks.—Because of its comparatively larger size, *T. b. tularosae* originally was described as a subspecies of *T. baileyi*. A clear natural distinction between *T. b. tularosae* and adjacent subspecies is difficult to define. In the study region, *T. b. tularosae* is a lower elevation montane subspecies associated with the Upper Sonoran life zone rather than the Canadian or Transitional Conifer zones of the adjacent subspecies *T. b. texensis*. Although significant differences were determined to exist between *T. b. tularosae* and its adjacent subspecies, total number of specimens examined is too low to discount sampling error. Therefore, it is provisionally maintained on this basis.

Specimens examined ($n = 10$).—NEW MEXICO; Otero Co., 5 mi W Agency (TCWC 90); Alamogordo (UM 58398, 58399); Alamagordo, 7,200 ft. SE of Post Office, 4,400 ft. (KU 87146–87149); 1.5 mi N Alamogordo, 4,400 ft. (KU 87145); 2 mi N Alamogordo, 4,400 ft. (KU 87143, 87144).

EPILOGUE AND FUTURE DIRECTIONS

Álvarez-Castañeda's (2010) study of 225 individuals of *Thomomys*, from 108 collecting sites across the southwestern U.S. and northwestern Mexico, revealed the presence of eight mtDNA phylogroups. Relative to our study, the samples from central and southeastern New Mexico and from western Texas

formed a genetic group that Álvarez-Castañeda (2010) referred to as the "Southwestern group". This group included individuals distributed from the southeastern portions of California and northeastern edge of Baja California across northern Mexico (Sonora to Nuevo León) southeastern New Mexico and southwestern Texas. Álvarez-Castañeda (2010) inferred two major distributions with samples from the Big Bend region of Texas, northern Coahuila, and western Nuevo León composing one group and samples from the Texas/New Mexico border southwestward to California and Baja California forming the second. However, the phylogenetic analyses, and ultimately the genetic data, did not support this discontinuous arrangement; presumably as a result of lack of adequate sampling, missing taxa (subspecies), and low genetic divergence between samples from this area. Similarly, Wickliffe et al. (2005) revealed that many of the subspecies distributed across the southwestern U.S. and northwestern Mexico, originally described based on morphological characters, did not differ substantially. Of interest is the fact that both of these genetic studies identified relatively small amounts of genetic variation among subspecies distributed in New Mexico and Texas; but genetic variation was sufficiently significant that Álvarez -Castañedas (2010) proposed eight potential phylogenetic species across southwestern U.S. and northern Mexico. Contrary to the findings of Álvarez -Castañeda (2010) and Wickliffe et al. (2005), other genetic studies (Sherwood and Patton 1982; Patton and Smith 1990) detected substantial variation among allozymic loci, karyotypes, and c-value levels in populations of *Thomomys* across the southern United States; therefore, it is paramount that the total genetic differences among "subspecies" be re-evaluated.

Further, there are a few instances where the molecular and morphological data were not concordant and additional data and consideration is needed in order to resolve the disagreements. For example, the morphological data generated, herein suggest that *T. b. ruidosae* be placed in synonymy with *T. b. texensis*. However, *T. b. ruidosae* is unique among all populations of *T. bottae* in eastern New Mexico and western Texas in that its karyotype is completely biarmed versus a substantially acrocentric autosomal complement in other subspecies of *Thomomys* from that region (Patton and Smith 1990). Additionally, divergent allozmic loci were identified in geographically neighboring populations of *T. b. actuosus*, *T. b. guadalupensis*, and *T. b. limpiae* (see fig. 3.3, Patton and Smith 1990) compared to *T. b. ruidosae*. Therefore, the question is: do *T. b. ruidosae* and *T. b. texensis* share a common ancestry or are their shared morphological attributes due to convergence? Further studies will be needed to resolve this issue.

Given the disparate results of Wickliffe et al. (2005), Álvarez -Castañeda (2010), and herein, it may not be surprising that morphometrically and genetically, *Thomomys* differs across the broader geographic context of the southwestern U.S. and northern Mexico but displays little differentiation across the smaller region that was the primary focus of this current study. Clearly, a need exists for a more detailed study across this region that involves a genetic assessment of pertinent type, or topotype, specimens to establish taxonomic boundaries.

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LITERATURE CITED

Álvarez-Castañeda, S. T. 2010. Phylogenetic structure of the *Thomomys bottae-umbrinus* complex in North America. Molecular Phylogenetics and Evolution 54:671–679.

Anderson, S. 1966. Taxonomy of gophers, especially *Thomomys*, in Chihuahua, Mexico. Systematic Zoology 15:189–198.

Avise, J. C., and R. M. Ball, Jr. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Oxford Survey of Evolutionary Biology 7:45–67.

Bailey, V. 1902. Seven new mammals from western Texas. Proceedings of the Biological Society of Washington 15:117–120.

Bailey, V. 1905. Biological Survey of Texas. North American Fauna 25:1–222.

Bailey, V. 1915. Revision of pocket gophers of the genus *Thomomys*. North American Fauna 39:1–136.

Beauchamp, S. L. 1998. Cranial and zoogeographic variation of Botta's pocket gopher (*Thomomys bottae*) from Texas and southeastern New Mexico. Unpublished master's thesis, Midwestern State University, Wichita Falls, Texas.

Blair, W. F. 1939. New mammals from Texas and Oklahoma with remarks on the status of *Thomomys texensis* Bailey. Occasional Papers of Museum of Zoology, University of Michigan 403:1–7.

Blair, W. F. 1940. A contribution to the ecological and faunal relationships of the Davis Mountain Region, Southwestern Texas. Miscellaneous Publications of Museum of Zoology, University of Michigan 46:1–45.

Blair, W. F. 1950. The biotic provinces of Texas. Texas Journal of Science 1:93–117.

Braby, M. F., R. G. Eastwood, and N. Murray. 2012. The subspecies concept in butterflies: has its application in taxonomy and conservation biology outlived its usefulness? Biological Journal of the Linnean Society of London 106:699–716.

Burt, W. H. 1954. The subspecies category in mammals. Systematic Zoology, 3:99–104.

Cronin, M. A., A. Cánovas, A. Islas-Trejo, D. L. Bannasch, A. M. Oberbauer, and J. F. Medrano. 2015a. Single nucleotide polymorphism (SNP) variation of wolves (*Canis lupus*) in southeast Alaska and comparison with wolves, dogs, and coyotes in North America. Journal of Heredity 106:26–36.

Cronin, M. A., A. Cánovas, A. Islas-Trejo, D. L. Bannasch, A. M. Oberbauer, and J. F. Medrano. 2015b. Wolf subspecies: reply to Weckworth et al. and Fredrickson et al. Journal of Heredity 106:417–419.

Dalquest, W. W., and C. W. Kilpatrick. 1973. Dynamics of pocket gopher distribution on the Edwards Plateau. The Southwestern Naturalist 18:1–9.

Dalquest, W. W., and F. B. Stangl, Jr. 1986. Post-Pleistocene mammals of the Apache Mountains, Culberson County, Texas, with comments on zoogeography of the Trans-Pecos Front Range. Occasional Papers, Museum of Texas Tech University 104:1–35.

Davis, W. B. 1940. A new *Thomomys* from Texas. Journal of Mammalogy 21:204–205.

Davis, W. B. 1966. The mammals of Texas. Bulletin of the Texas Game and Fish Commission 41:1–267.

Davis, W. B. 1974. The mammals of Texas. Bulletin of the Texas Parks and Wildlife Department 41:1–294.

Davis, W. B., and H. K. Buechner. 1946. Pocket gophers (*Thomomys*) of the Davis Mountains, Texas. Journal of Mammalogy 27:265–271.

Davis, W. B., and J. L. Robertson, Jr. 1944. The mammals of Culberson County, Texas. Journal of Mammalogy 25:254–273.

Dunnum, J. L., B. S. McLean, R. C. Dowler, and the Systematic Collections Committee of the American Society of Mammalogists. 2018. Mammal collections of the Western Hemisphere: a survey and directory of collections. Journal of Mammalogy 99:1307–1322.

Durrant, S. D. 1946. The pocket gophers (genus *Thomomys*) of Utah. University of Kansas Publications, Museum of Natural History 1:1–82.

Durrant, S. D. 1955. In defense of the subspecies. Systematic Zoology 4:186–190.

Findley, J. S., A. H. Harris, D. E. Wilson, and C. Jones. 1975. Mammals of New Mexico. University of New Mexico Press, Albuquerque.

Fredrickson, R., P. Hedrick, R. Wayne, B. vonHoldt, and M. Phillips. 2015. Mexican wolves are a valid subspecies and an appropriate conservation target. Journal of Heredity 106:415–416.

Goldman, E. A. 1935. Two new pocket gophers of the genus *Thomomys*. Proceedings of the Biological Society of Washington 48:149–152.

Goldman, E. A. 1936. New pocket gophers of the genus *Thomomys*. Journal of Washington Academy of Science 26:111–120.

Goldman, E. A. 1938. Six new rodents from Coahuila and Texas and notes on the status of several described forms. Proceedings of the Biological Society of Washington 51:55–62.

Goldman, E. A. 1947. The pocket gophers (Genus *Thomomys*) of Arizona. North American Fauna 59:1–39.

Hafner, J. C., D. J. Hafner, J. L. Patton, and M. F. Smith. 1983. Contact zones and the genetics of differentiation in the pocket gopher, *Thomomys bottae* (Rodentia: Geomyidae). Systematic Zoology 32:1–20.

Hall, E. R. 1932a. A new pocket gopher from New Mexico. University of California Publication Zoology 38:411–413.

Hall, E. R. 1932b. Three new species of pocket gophers from New Mexico and Arizona. Proceedings of the Biological Society of Washington 45:95–98.

Hall, E. R. 1981. The mammals of North America. John Wiley & Sons, New York.

Hall, E. R., and K. R. Kelson. 1959. The mammals of North America. Ronald Press Co., New York.

Hollander, R. R. 1990. Biosystematics of the yellow-faced pocket gopher, *Cratogeomys castanops* (Rodentia: Geomyidae) in the United States. Special Publications, Museum of Texas Tech University 33:1–62.

IBM Corp. 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, New York, IBM Corp.

ICZN. 1999. International code of zoological nomenclature. 4th ed. The International Trust for Zoological Nomenclature, London.

Jones, C. A., and C. N. Baxter. 2004. *Thomomys bottae*. Mammalian Species 742:1–14.

Kelson, K. R. 1951. Two new subspecies of *Thomomys bottae* from New Mexico and Colorado. University of Kansas Publications, Museum of Natural History 5:59–71.

Lane, J. D. 1965. Taxonomy of the pocket gopher, *Thomomys baileyi*. Unpublished PhD dissertation, University of Arizona 112 pages.

Lidicker, W. Z., Jr. 1962. The nature of subspecies boundaries in a desert rodent and its implications for subspecies taxonomy. Systematic Zoology 11:160–171.

Manning, R. W., and C. Jones. 1998. Annotated checklist of Recent land mammals of Texas, 1998. Occasional Papers, Museum of Texas Tech University 182:1–20.

Mayr, E. 1982. The growth of biological thought: diversity, evolution, and inheritance. The Belknap Press of Harvard University, Boston, Massachusetts.

Mayr, E., and P. D. Ashlock. 1991. Principles of systematic zoology. McGraw-Hill Inc., second edition.

Merriam, C. H. 1901. Descriptions of twenty-three new pocket gophers of the genus *Thomomys*. Proceedings of Biological Society of Washington 14:107–117.

Patten, M. A., and J. V. Remsen, Jr. 2017. Complementary roles of phenotype and genotype in subspecies delimitation. Journal of Heredity 108:462–464.

Patton, J. L. 1973. An analysis of natural hybridization between the pocket gophers *Thomomys bottae* and *Thomomys umbrinus* in Arizona. Journal of Mammalogy 54:561-585.

Patton, J. L., and C. J. Conroy. 2017. The conundrum of subspecies: morphological diversity among desert populations of the California vole (*Microtus californicus*, Cricetidae). Journal of Mammalogy 98:1010–1026.

Patton, J. L., and R. E. Dingman. 1968. Chromosome studies of pocket gophers, genus *Thomomys*. I. The specific status of *Thomomys umbrinus* (Richardson) in Arizona. Journal of Mammalogy 49: 1-13.

Patton, J. L., J. C. Hafner, M. S. Hafner, and M. F. Smith. 1979. Hybrid zones in *Thomomys bottae* pocket gophers: genetic, phenetic, and ecologic concordance patterns. Evolution 33:860–876.

Patton, J. L., and M. F. Smith. 1981. Molecular evolution in *Thomomys* pocket gophers: phyletic systematics, paraphyly, and rates of evolution. Journal of Mammalogy 62:493–500.

Patton, J. L., and M. F. Smith. 1989. Population structure and the genetic and morphologic divergence among pocket gopher species (genus *Thomomys*). Pp. 284-304 in Speciation and its consequences (D. Otte and J. A. Endler, eds.). Sinauer Associates Inc., Sunderland, Massachusetts.

Patton, J. L., and M. F. Smith. 1990. The evolutionary dynamics of the pocket gopher, *Thomomys bottae*, with emphasis on California populations. University of California Publications Zoology 123:vii + 1–161.

Patton, J. L., and M. F. Smith. 1994. Paraphyletic, polyphyly, and the nature of species boundaries in pocket gophers (genus *Thomomys*). Systematic Biology 43:11–26.

Reichman, O. J., and R. J. Baker. 1972. Distribution and movement of two species of pocket gophers (Geomyidae) in an area of sympatry in the Davis Mountains, Texas. Journal of Mammalogy 53:21–33.

Ridgway, R. 1912. Color standards and color nomenclature. Privately published, Washington, D.C.

Schmidly, D. J. 1977. The mammals of Trans-Pecos Texas. Texas A&M University Press, College Station.

Schmidly, D. J. 2004. The mammals of Texas. University of Texas Press, Austin.

Schmidly, D. J., and R. D. Bradley. 2016. Mammals of Texas. University of Texas Press, Austin.

Sherwood, S. W., and J. L. Patton. 1982. Genome evolution in pocket gophers (genus *Thomomys*). II. Variation in cellular DNA content. Chromosoma 85:163–179.

Smith, M. F. 1998. Phylogenetic relationships and geographic structure in pocket gophers in the genus *Thomomys*. Molecular Phylogenetics and Evolution 9:1–14.

Smith, M. F., and J. L. Patton. 1980. Relationships of pocket gopher (*Thomomys bottae*) populations of the lower Colorado River. Journal of Mammalogy 61:681–696.

Smith, M. F., J. L. Patton, J. C. Hafner, and D. J. Hafner. 1983. *Thomomys bottae* pocket gophers of the central Rio Grande Valley, New Mexico: local differentiation, gene flow, and historical biogeography. Occasional Papers, Museum of Southwestern Biology 2:1–16.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biology. 3rd Edition, W. H. Freeman and Co., New York.

Stangl, F. B., Jr., and R. J. Baker. 1984. A chromosomal subdivision in *Peromyscus leucopus*: implications for the subspecies concept as applied to mammals. Pp. 139–144 in Festchrift for Walter W. Dalquest in honor of his sixty-sixth birthday (N. V. Horner, ed.). Department of Biology, Midwestern State University.

Stangl, F. B., Jr., W. W. Dalquest, and R. R. Hollander. 1994. Evolution of a desert mammalian fauna: a 10,000-year history of mammals in Culberson and Jeff Davis counties, Trans-Pecos Texas. Midwestern State University Press, Wichita Falls, Texas.

Taylor, W. P., and W. B. Davis. 1947. The mammals of Texas. Bulletin of the Texas Game, Fish, and Oyster Commission 27:1–79.

Weckworth, B., N. Dawson, S. Talbot, and J. A. Cook. 2015. Genetic distinctiveness of Alexander Archipelago wolves (*Canis lupus ligoni*): reply to Cronin et al. (2015). Journal of Heredity 106:412–414.

Wickliffe, J. K., et al. 2005. Molecular systematics and phylogeographic history of *Thomomys bottae* in Texas. Pp. 507–522 in Contribuciones Mastozoologicas en homenaje a Bernardo Villa (V. Medellin and C. Sanchez-Cordero, eds.). UNAM Instituto de Ecologia, Mexico.

Williams, S. L., and R. J. Baker. 1976. Vagility and local movement of pocket gophers (Rodentia: Geomyidae). American Midland Naturalist 96:303–316.

Wilson, E. O., and W. L. Brown. 1953. The subspecies concept and its taxonomic applications. Systematic Zoology 2:97–122.

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APPENDIX

Summary of descriptive statistics for female, age class 4 and 5 specimens from eight realigned and one new subspecies of *Thomomys bottae*. Statistics include mean, standard deviation (STD), maximum value (MAX), and minimum value (MIN).

Measurements	Mean	STD	MAX	MIN
Thomomys bottae actuosus **(n = 6)**				
Condylobasal length	35.66	±1.78	38.67	33.96
Zygomatic breadth	20.93	±1.20	23.02	19.89
Mastoidal breadth	17.80	±0.67	18.65	16.91
Rostral breadth	6.77	±0.37	7.50	6.48
Rostral length	15.30	±1.03	16.81	14.37
Nasal length	12.16	±0.62	12.81	11.29
Least interorbital constriction	6.25	±0.31	6.61	5.81
Maxillary alveolar length	7.87	±0.43	8.49	7.34
Thomomys bottae baileyi **(n = 8)**				
Condylobasal length	36.07	±1.26	38.42	34.35
Zygomatic breadth	22.73	±1.09	23.78	20.54
Mastoidal breadth	18.89	±0.46	19.48	18.05
Rostral breadth	7.28	±0.23	7.60	6.93
Rostral length	13.88	±0.64	14.71	13.00
Nasal length	12.05	±0.35	12.55	11.50
Least interorbital constriction	6.79	±0.36	7.41	6.30
Maxillary alveolar length	7.45	±0.32	7.82	6.90
Thomomys bottae confinalis **(n = 65)**				
Condylobasal length	33.31	±1.69	37.10	28.93
Zygomatic breadth	19.91	±0.87	21.69	16.66
Mastoidal breadth	17.12	±0.73	19.49	15.08
Rostral breadth	6.71	±0.31	7.32	5.93
Rostral length	13.65	±0.79	15.33	11.14
Nasal length	11.57	±0.81	13.12	8.45
Least interorbital constriction	6.29	±0.35	7.12	5.45
Maxillary alveolar length	6.89	±0.40	7.92	5.84
Thomomys bottae lachuguilla **(n = 45)**				
Condylobasal length	32.91	±1.51	37.15	30.10
Zygomatic breadth	20.32	±1.11	22.75	18.30
Mastoidal breadth	17.53	±0.71	19.40	15.55
Rostral breadth	7.15	±0.34	8.03	6.52

Appendix (cont.)

Measurements	Mean	STD	MAX	MIN
Rostral length	13.72	±0.65	15.73	12.65
Nasal length	11.57	±0.71	13.40	10.30
Least interorbital constriction	6.58	±0.30	7.29	5.85
Maxillary alveolar length	7.15	±0.34	7.91	6.53
***Thomomys bottae limpae* (n = 43)**				
Condylobasal length	34.79	±1.40	37.29	31.20
Zygomatic breadth	20.91	±0.78	22.76	19.64
Mastoidal breadth	17.77	±0.63	19.10	16.78
Rostral breadth	7.04	±0.35	7.92	6.30
Rostral length	14.60	±1.01	18.89	12.90
Nasal length	11.90	±0.60	13.44	10.54
Least interorbital constriction	6.31	±0.30	7.09	5.60
Maxillary alveolar length	7.44	±0.46	8.47	6.66
***Thomomys bottae spatiosus* (n = 6)**				
Condylobasal length	32.85	±1.72	34.60	33.03
Zygomatic breadth	19.41	±1.38	21.61	17.82
Mastoidal breadth	16.41	±1.76	17.54	13.36
Rostral breadth	6.67	±0.22	7.03	6.54
Rostral length	13.26	±0.76	14.17	12.96
Nasal length	11.23	±0.64	12.05	10.88
Least interorbital constriction	6.37	±0.23	6.58	6.20
Maxillary alveolar length	7.63	±1.31	9.94	7.08
***Thomomys bottae robertbakeri* subspecies novum (n = 38)**				
Condylobasal length	33.99	±1.29	37.12	31.21
Zygomatic breadth	20.49	±1.01	22.78	18.78
Mastoidal breadth	17.44	±0.58	18.70	16.23
Rostral breadth	7.01	±0.60	9.82	6.29
Rostral length	13.61	±0.60	14.66	12.01
Nasal Length	11.33	±0.59	12.24	10.14
Least interorbital constriction	6.41	±0.28	6.98	5.76
Maxillary alveolar length	7.07	±0.50	7.96	5.97
***Thomomys bottae texensis* (n = 81)**				
Condylobasal length	34.17	±1.73	38.65	29.90
Zygomatic breadth	20.64	±1.26	24.75	17.80
Mastoidal breadth	17.60	±0.82	19.95	16.16
Rostral breadth	6.93	±0.42	8.10	6.18

Appendix (cont.)

Measurements	Mean	STD	MAX	MIN
Rostral length	14.38	±1.05	17.30	12.08
Nasal Length	11.83	±0.88	14.34	9.53
Least interorbital constriction	6.39	±0.33	7.17	5.66
Maxillary alveolar length	7.56	±0.46	8.54	6.55
***Thomomys bottae tularosa*e (n = 7)**				
Condylobasal length	38.00	±1.60	40.22	36.00
Zygomatic breadth	23.13	±1.14	25.22	21.75
Mastoidal breadth	19.31	±0.47	19.84	18.61
Rostral breadth	7.57	±0.30	7.96	7.08
Rostral length	15.22	±0.95	16.54	13.97
Nasal length	12.19	±0.81	13.48	10.95
Least interorbital constriction	6.62	±0.25	6.87	6.19
Maxillary alveolar length	7.49	±0.35	7.94	7.00

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Two New Species of *Peromyscus* from Chiapas, Mexico, and Guatemala

Sergio Ticul Álvarez-Castañeda, Consuelo Lorenzo, Cintya A. Segura-Trujillo, and Sergio G. Pérez-Consuegra

Abstract

Morphometric and molecular variation among specimens of *Peromyscus guatemalensis* were grouped into four pooled samples representing separate physiographic regions in Mexico and Guatemala. Mitochondrial sequence data identified three well-supported and reciprocally monophyletic clades. Specimens assigned to *P. guatemalensis* by current taxonomy also were divided into the same three well-supported clades distinguishable by univariate and multivariate analyses of craniodental morphometric variables. These three clades group as a strongly supported monophyletic lineage together with all other members of the *Peromyscus mexicanus* group of species (*gardneri, grandis*, *guatemalensis*, *gymnotis*, *mexicanus*, *nicaraguae*, *nudipes, salvadorensis*, *tropicalis*, and *zarhynchus*). Mitochondrial cytochrome-*b* gene (*Cytb*) p-distances among clades ranged from 3.46 to 3.57%. One of these clades is *P. guatemalensis* (herein restricted to the region encompassing its type locality in the Sierra de los Cuchumatanes, Guatemala). The other two clades are described as new species, one restricted to its type locality just east of the Rio Samala in the middle part of the Central American Volcanic Arc in Guatemala, and the other distributed from the Sierra Madre de Chiapas in Mexico eastward across the western part of the Central American Volcanic Arc in Guatemala to the Rio Samala.

Key words: Chiapas, Guatemala, Mexico, morphometric variation, *Peromyscus guatemalensis*, Rodentia

Resumen

La variación morfométrica y molecular de *Peromyscus guatemalensis* se agrupó en cuatro muestras representativas de diferentes regiones fisiográficas de México y Guatemala. Los datos de las secuencias mitocondriales identificaron tres clados monofiléticos recíprocos con alto soporte. Los especímenes asignados a *P. guatemalensis*, se dividieron en tres clados, los que se distinguen por análisis univariados y multivariados de las medidas cráneo-dentales. Los tres clados se agrupan como un linaje monofilético fuertemente sustentado, junto con todos los otros miembros del grupo de especies de *Peromyscus mexicanus* (*gardneri*, *grandis*, *guatemalensis*, *gymnotis*, *mexicanus*, *nicaraguae*, *nudipes*, *salvadorensis*, *tropicalis* y *zarhynchus*). Las distancias p del gen mitocondrial del citocromo-b (*Cytb*) tienen un porcentaje entre 3.46 y 3.57% entre clados. Un clado agrupa a *P. guatemalensis* (restringido a la Sierra de los Cuchumatanes, Guatemala, incluyendo la localidad tipo). Los otros dos clados se describen como nuevas especies; la primera restringida a su localidad tipo, justo al este del Río Samala en la parte media del Arco Volcánico Centroamericano en Guatemala. La segunda con distribuición desde la Sierra Madre de Chiapas, México hacia el este a través de la parte oeste del Arco Volcánico Centroamericano hasta el Rio Samala en Guatemala.

Key words: Chiapas, Guatemala, México, *Peromyscus guatemalensis*, Rodentia, variación morfologic

Supplemental material related to this manuscript is available online at www.ticulalvarez.com/Peromyscus.html.

INTRODUCTION

Peromyscus guatemalensis, the Guatemalan deer mouse, is a member of the *Peromyscus mexicanus* group (*sensu* Carleton 1989; Rogers and Engstrom 1992; Bradley et al. 2007), which also includes *P. gardneri, grandis*, *gymnotis*, *mexicanus*, *nicaraguae*, *nudipes, salvadorensis*, *tropicalis,* and *zarhynchus* (see Pérez-Consuegra and Vázquez-Domínguez 2015; Bradley et al. 2016; Lorenzo et al. 2016). Summarizing morphological and genetic differentiation among all species within the *mexicanus* group is difficult. Following the revision of Huckaby (1980), several subspecies are now considered as species and, in some cases, other new species have been described (see Bradley et al. 2016, Pérez-Consuegra and Vázquez-Domínguez 2017). Further, genetic differentiation has been correlated with geographic distance (Ordoñez-Garza et al. 2010; Lorenzo et al. 2016). Initially, Hall (1981) recognized two subspecies of *P. guatemalensis*, the nominate form (Merriam 1898) that ranged from southeastern Chiapas through southwestern Guatemala and *tropicalis*, known only from Chimoxan, Alta Verapaz, Guatemala (Goodwin 1932), despite Musser (1969) having referred Goodwin's *tropicalis* to *P. mexicanus*. Pérez-Consuegra and Vázquez-Domínguez (2015) elevated *tropicalis* to species status and recognized it as one of the basal lineages within the *mexicanus* group. Currently, *P. guatemalensis* is regarded as a monotypic species (e.g., Huckaby 1980; Musser and Carleton 2005) but it also is considered to consist of a small group of lineages that are paraphyletic in relation to *P. grandis* (Pérez-Consuegra and Vázquez-Domínguez 2015; Lorenzo et al. 2016).

Peromyscus guatemalensis is known only from montane areas at elevations from 1,300 to 3,000 m (Reid 2009), in relatively cool and humid cloud and pine-oak forest (Horváth 2014). Specimens collected and reported herein were from coffee plantations from 1,250 to 2,150 m, cloud forest from 1,500 to 2,950 m, and pine forest above 2,710 m. Populations currently allocated to *P. guatemalensis* (Fig. 1) are restricted to highland areas that have had had a rich, temporally varied, and diverse geological history. These highland areas are composed of two Paleozoic blocks (the Maya block on the North American plate, comprising the Sierra Norte de Chiapas, Montañas de Cuilco, and Sierra de los Cuchumatanes; and the Chortís block on the Caribbean plate, which includes the Sierra de Chaucús and Sierra de las Minas). Subduction of the Cocos plate along the Pacific coast gave rise to a third highland area, the chain of high (up to 4,220 m) volcanoes of Pleistocene age stretching from Chiapas (Volcán Tacaná, on the border with Guatemala) to El Salvador and beyond (the Central American Volcanic Arc). Intermountain valleys and canyons formed by activity along the Motagua-Polochic-Jocotán fault system (including the drier and warmer Motagua, Polochic, Cuilco, Selegua, Gijalva, Huixtla and Mazapa valley systems) divide the Maya block highlands from those of the Volcanic Arc in the Chortis block of the Caribbean plate (see Rogers et al. 2002; Marshall 2007). These low elevation, dry, and warm valleys serve as important biogeographic barriers for montane species. Intermittent bridges between these highlands likely formed and then disappeared during glacial-interglacial cycles, facilitating alternating opportunities for gene flow and divergence. The Sierra de los Cuchumatanes and Cerro Chirripó (in Costa Rica) are the only two areas in Central America known to have been glaciated during the Pleistocene (Orvis and Horn 2000).

Herein, craniodental morphology and mitochondrial DNA sequences of members of the *Peromyscus grandis – P. guatemalensis* complex recently collected in Guatemala and Chiapas, Mexico, were examined. The aims of this study were three-fold: first, to determine if significant morphological and molecular differentiation exists among sampled populations of *P. guatemalensis*; second, if so, are the differentiated population segments coincident with biogeographic barriers and thus the complex geographic history of the region; and third, do demonstrably differentiated populations deserve taxonomic recognition.

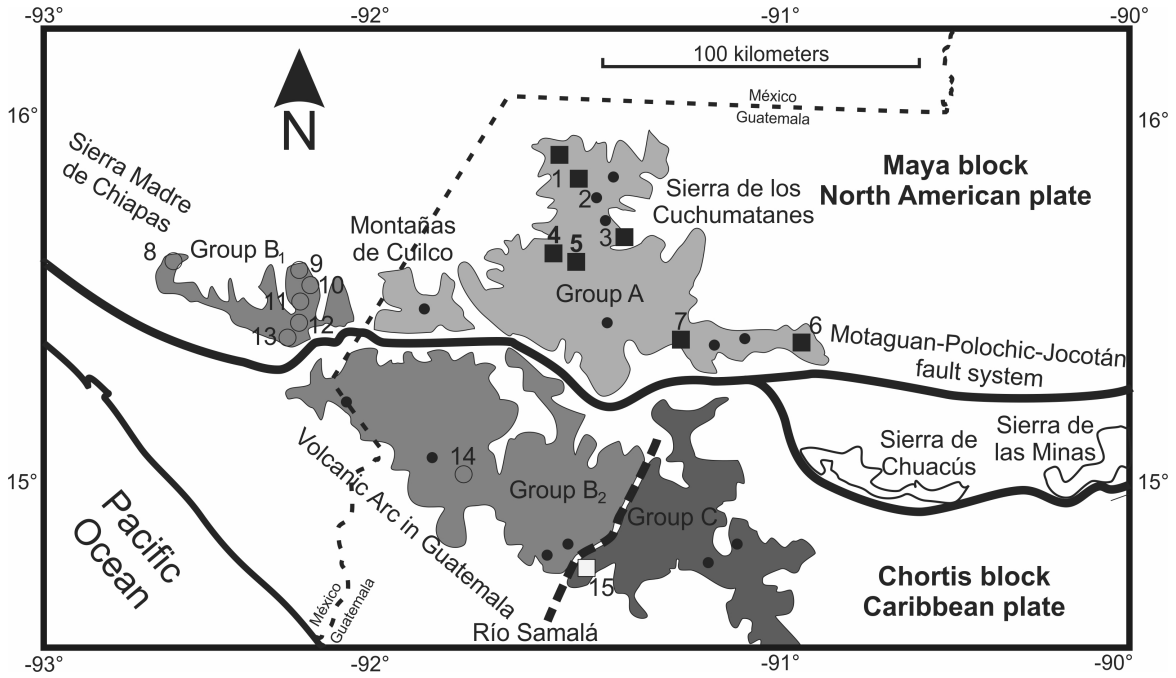

Figure 1. Distribution map of the three groups of *Peromyscus guatemalensis* in Chiapas, Mexico, and Guatemala. Group A (solid squares, light gray) includes topotypes of *P. guatemalensis* and *P. altilaneus*, localities 4 and 5 (numbers in bold), central Guatemala, Sierra de los Cuchumatanes north of the Motagua-Polochic-Jocotán fault system. Group B (open circles, medium gray) includes two subgroups with no habitat conection between them: Sierra Madre del Sur, Chiapas (B1), and Volcanic Arc in Guatemala, west of the canyon of the Río Samalá (B2). Group C (solid circle, dark gray) is Quetzaltenango, east of the Río Samalá. Localities are numbered as in the Specimens Examined (Supplement data SD1). The Motagua-Polochic-Jocotán fault system is indicated by a bold solid line, Río Samalá is indicated by a dashed line, and the contour line of 1,500 m is indicated by a thin line. Solid dots are other clusters of known localities for the species.

MATERIALS AND METHODS

Samples.—A total of 284 specimens (181 males and 103 females) of *P. guatemalensis* from seven municipalities in Chiapas, Mexico, and four departments in Guatemala were examined. All specimens were used in morphometric analyses of external and craniodental traits, whereas a subset of 45 individuals were sequenced for a molecular phylogenetic perspective. For all analyses, the localities were grouped into three geographic units: 1) Altos Cuchumatanes, located north of the Motagua-Polochic-Jocotán fault system (Group A, localities 1–7, Fig. 1), including topotypes of *P. guatemalensis* (locality 5, Supplemental Data SD1); 2) Sierra Madre de Chiapas (Group B_1, localities 8–13) and the part of the volcanic arc in Guatemala west of the Río Samalá (Group B_2, locality 14); and 3) near Zunil, Quetzaltenango, located east of the canyon of the Río Samalá (Group C, locality 15). Locality and specimen details are provided in Supplemental Data SD1. Voucher specimens are housed in the Centro de Investigaciones Biológicas del Noroeste S. C. (CIB), Colección Mastozoológica El Colegio de la Frontera Sur (ECOSUR), Colección de Mamíferos del Museo de Historia Natural, Universidad de San Carlos, Guatemala (USAC), and Museum of Vertebrate Zoology, University of California, Berkeley, California (MVZ). The Guatemalan and Mexican specimens are from the same localities as those examined in three previous molecular studies of Mesoamerican *Peromyscus* (Ordoñez-Garza et al. 2010; Pérez-Consuegra and Vázquez-Domínguez 2015, 2017). Capture and handling methods followed the animal care and use guidelines of the American Society of Mammalogists (Sikes et al. 2016).

Molecular analyses.—Mitochondrial sequences of the cytochrome-*b* gene (*Cyt*b) from 45 specimens collected from 15 localities distributed across three geographical regions were examined (Fig. 1; Supplemental Data SD1). Genomic DNA from muscle tissue preserved originally in either 95% ethanol or frozen (-80 °C) was extracted using the DNeasy Kit (QIAGEN, Inc., Valencia, California). A ~800 bp fragment of *Cyt*b using the primer pairs MVZ05 and MVZ16 (Smith and Patton 1993) was initially amplified. Double-stranded amplifications used the following methodologies and concentrations: 12.5 µl of template (10 ng), 4.4 µl ddH_2O, 2.5 µl of each primer pair (10 nM concentration), 0.474 µl (0.4 nM) dNTPs, 0.5 µl (3 mM) $MgCl_2$, 0.125 µl Taq polymerase (platinum, Invitrogen, Carlsbad, California), and 1× Taq buffer to a final volume of 25 µl. Amplification conditions included 3 min of initial denaturation at 94 °C followed by 37 cycles and 40, 1 minute annealing at 50 °C for *Cyt*b. Amplified products were purified using the QIAquick PCR Purification Kit (QIAGEN) and templates were cycle-sequenced with primer pairs MVZ05/MVZ16 for *Cyt*b, using Big Dye terminator chemistry (Applied Biosystems Inc., Foster City, California). All sequences were analyzed on an ABI 3730 sequencer (Applied Biosystems) at the Museum of Vertebrate Zoology. MVZ14 (Smith and Patton 1993) was used to sequence the reverse strand in all individuals. All haplotypes are deposited in GenBank (accession numbers provided in Supplemental Data SD1).

Nucleotide sequences were aligned in Sequencher ver. 3.1 (Gene Codes Corp., Ann Arbor, Michigan), checked by eye, and proofed for stop codons or gaps. Unique *Cyt*b haplotypes were identified in TCS ver. 1.18 (Clement et al. 2000). Arlequin ver. 2.001 (Schneider et al. 2000) was used to estimate haplotype and nucleotide diversity.

Phylogenetic analyses.—Bayesian and maximum likelihood (ML) approaches were used to estimate gene tree topologies. The best substitution model was determined using the Akaike information criterion (AIC) as implemented in MrAIC (Nylander 2004). All analyses were performed with the unique sequences (i.e., redundant haplotypes were eliminated). Bayesian analyses were implemented in MrBayes ver. 3.1.1 (Ronquist and Huelsenbeck 2003), with three separate runs with Markov chain Monte Carlo simulations starting from a random tree. Each run was conducted for 5,000,000 generations and sampled at intervals of 1,000 generations, with the first 1,000 samples of each run discarded as burn-in and all remaining sampled trees analyzed to find the posterior probability of resulting nodes. A consensus tree was generated with the 50% majority-rule algorithm in PAUP 4.0b10 (Swofford 2000), and the percentage of samples recovered in a particular clade was assumed to be the posterior probability of that clade. ML was performed in PAUP 4.0b10 using a heuristic search with 1,000 replicates and swapping with the TBR algorithm. Reliability of each codon position was assessed separately by applying equal weights. Nodal support was determined using nonparametric bootstrapping methods.

Cytochrome-*b* sequences from nine other species of the *Peromyscus mexicanus* group (*P. gardneri, grandis*, *gymnotis*, *mexicanus*, *nicaraguae*, *nudipes, salvadorensis*, *tropicalis,* and *zarhynchus*) obtained from GenBank (Supplemental Data SD1) were included. Sequence divergences among all pairs of geographic groups were summarized using uncorrected p-distances, which make no assumptions about substitution models, and Kimura 2-parameter (Kimura 1980) distances for comparison to other studies.

Morphological analyses.—Four external measurements were obtained from specimen labels: total length (ToL), tail length (TaL), hind foot length (LHF), and ear length (LE). Nineteen cranial measurements were recorded using digital calipers (0.01 mm resolution; measurements defined by Williams and Ramírez-Pulido [1984], Robinson and Dippenaar [1987], and Lorenzo et al. [2016]) as follows: greatest length of skull (GLS), skull height (SKH), condylobasal length (CBL), bullar length (BUL), shield-bullae depth (SBD), diastema length (DIL), rostral height (ROH), rostral breadth (BRR), palatine bridge length (PBL), postpalatal length (POL), basioccipital length (OCL), maxillary toothrow length (MTL), maxillary toothrow breadth (MTB), postdental breadth (PDB), zygomatic breadth (ZYB), braincase breadth (BAB), nasal length (NAL), interorbital breadth (IOB), and nasal breadth (NAB).

Specimens were assigned to age classes from 1 to 5 following tooth eruption and wear patterns following Hoffmeister (1951). Juveniles and subadults were assigned to age classes 1–3, and adults were assigned to age classes 4 and 5. Age and sex variation was examined for a pooled sample of 239 specimens

(Group B), with 76 individuals assigned to age classes 1–3 and 163 assigned to age classes 4 and 5. Based on this analysis, it was determined that juvenile individuals were significantly smaller, so they were excluded from all subsequent analyses. Examination of sexual variation was based on 163 adults (108 males, 55 females) using a least squares regression with both sex and age as covariates. All statistical analyses were conducted using SPSS (ver. 15.0.1 for Windows; SPSS Inc. 1989–2006), Paleontological Statistics PAST (ver. 3.11; Hammer et al. 2001), and STATISTICA (ver. 8.0; StatSoft, Inc. 2007).

A one-way ANOVA was conducted for each of the 19 cranial variables to determine if differences existed among the three geographic groups of *P. guatemalensis* (Fig. 1), specimens of each group belonging to the same *Cytb* clade, and *P. grandis*. External measurements from the multivariate analyses were excluded because an unknown proportion of their variance is due to differences in preparator measuring methods. The Tukey HSD with unequal N (Spjotvoll/Stoline) test was used to determine minimally non-significant geographic groups. Multivariate principal component analysis (PCA) and canonical variates analysis (CVA) were conducted on specimens from each physiographic region. Both multivariate analyses were implemented using log-transformations of the original variables.

RESULTS

Phylogenetic analysis.—The GTR +I + G (Tavaré 1986) model was selected by the Akaike Information Criterion (AIC) as the best-fit model of nucleotide substitution for each codon position separately as well as for the concatenation of all three positions (A = 33.65, C = 31.14, G = 10.95, and T = 24.25 for the latter), invariable sites = 0.5978, and gamma distribution = 2.2789). AIC = 9783.38, K = 10, -Lnl = 4881.69.

Maximum likelihood and Bayesian inference analyses (Fig. 2) identified reciprocally monophyletic clades within the *Peromyscus grandis – P. guatemalensis* complex: 1) *P. guatemalensis* group A from north of the Motagua-Polochic-Jocotán fault system in west-central Guatemala (includes topotypical specimens of *P. guatemalensis* and *P. altilaneus* [considered the latter as junior synonym of *P. guatemalensis* Huckaby 1980]), 2) *P. guatemalensis* group B from the Sierra Madre del Sur, Chiapas and the Volcano Arch west of the Rio Samala in Guatemala, 3) *P. guatemalensis* group C from the Volcanic Arc east of the Rio Samala in Guatemala, and 4) *P. grandis*. The two geographical subgroups of B are associated within the same lineage (Fig. 1).

The specimens from Quetzaltenango were 3.51% (p-distances) genetically divergent from those from the Altos Cuchumatanes, 3.13% divergent from those west of the Rio Samala in the Central American Volcano Arch, and 4.04% divergent from *P. grandis*. The specimens from west of the Rio Samala in the Central American Volcano Arch are 3.37% divergent from the specimens from the Altos Cuchumatanes and 4.68% divergent from *P. grandis* (Table 1).

Morphological comparisons.—Specimens from each of the three geographic areas (Fig. 1) share similar morphologies. Externally they agree with Merriam's (1898) description of the species. The dorsal coat is soft, varying from gray to dark grayish brown. The venter is pale whitish and the sides are sometimes tinted with light reddish brown. The legs are white with the proximal parts dark. The tail is long, usually longer than the body and head, slightly hairy, and bicolored. Eye rings are well marked (Hall 1981; Reid 2009).

None of the 19 craniodental variables of the large *P. guatemalensis* Group B sample were sexually dimorphic (*P* ranges from 0.086 [ANCC] to 0.995 [LOMC], one-way ANOVA; Supplemental Data SD2). Age differences, however, are substantial, with 18 or 19 variables significantly different between adults (class 3) and subadults (classes 4 and 5, 94.7%; Supplemental Data SD3).

Geographic variation.—Means and standard errors for each external and craniodental variable are given in Table 2, as are significance levels in comparisons among all three geographic samples of *P. guatemalensis* and *P. grandis* based on one-way ANOVAs (Supplemental Data SD4). The craniodental measure-

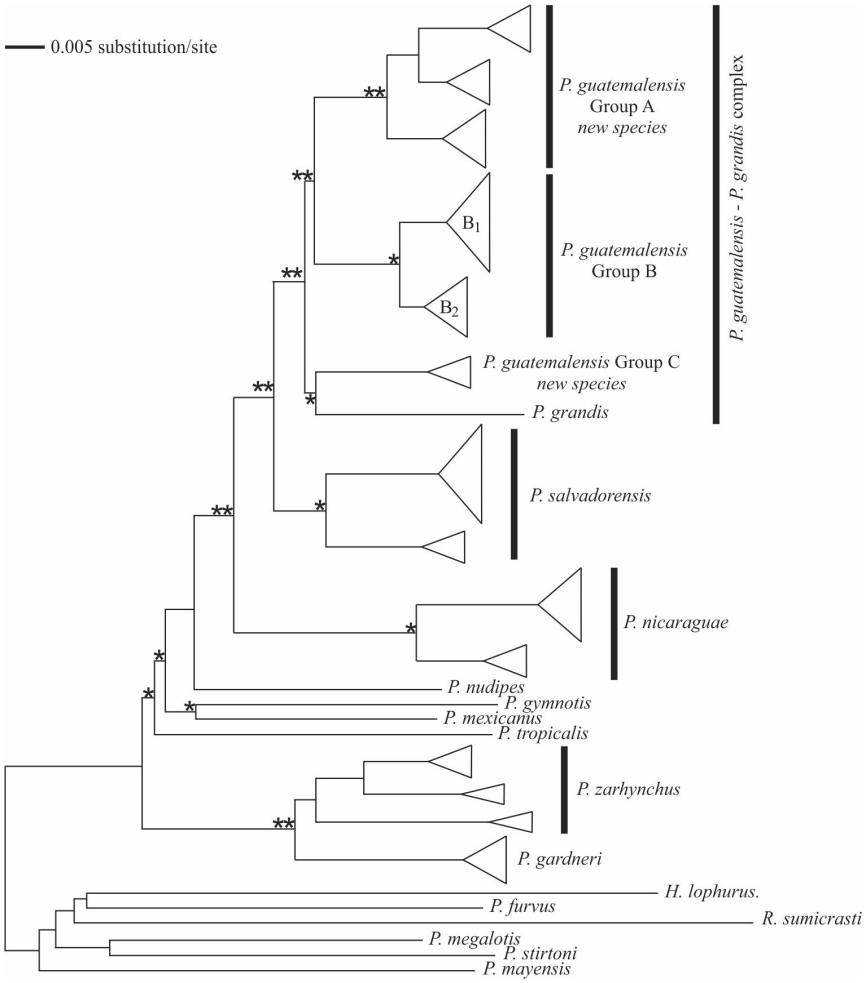

Figure 2. Bayesian inference tree of *Peromyscus mexicanus* group based on *Cyt*b haplotypes. The following were used as outgroup taxa: *Peromyscus furvus*, *P. mayensis*, *P. megalops*, *P. stirtoni*, *Habromys lophurus*, and *Reithrodontomys sumichrasti*. **Bayesian posterior probability values and bootstrap values = 100%;*Bayesian posterior probability values and bootstrap values ≥ 95%.

Table 1. Mean and range of uncorrected p-distance (above the diagonal) and Kimura 2-parameter distances (below the diagonal) expressed as percentages within and among the three geographic groups of *P. guatemalensis* (Fig. 1) and *P. grandis*. Numbers on the diagonal are paired p-values followed by Kimura 2-parameter values. Range is given in parentheses.

	P. guatemalensis Group A	*P. guatemalensis* Group B	*P. guatemalensis* Group C	*P. grandis*
P. guatemalensis Group A	1.13 / 1.15	3.37 (2.64–4.40)	3.51 (3.02–4.65)	4.28 (3.90–4.65)
P. guatemalensis Group B	3.48 (2.71–4.59)	0.75 / 0.76	3.13 (2.64–3.65)	4.49 (4.03–4.91)
P. guatemalensis Group C	3.62 (2.97–4.86)	3.22 (2.71–3.77)	0.25 / 0.25	3.90 (3.90–3.90)
P. grandis	4.45 (4.05–4.87)	4.68 (4.18–5.14)	4.04 (4.04–4.04)	0.00 / 0.00

ments of Group B (Southern Chiapas and Guatemala) are smaller ($P < 0.001$; Supplemental Data SD4) than those Group A (Central Guatemala) and Group C (from Southern Guatemala; Table 2).

The PCA on 19 craniodental variables indicated that loadings for PC1 were all positive and of similar magnitude, suggesting that this axis represents general size. The first three factors of the principal component analysis explained 59.1% of the total variation PC1 = 40.4%, PC2 = 10.5% and PC3 = 8.2% (Fig. 3; Supplemental Data SD 5). The PCA indicated that length of the skull (PC-1, condylobasal length) can be used to discriminate between the groups A, C, and *P. grandis*, but not with the group B. The rostrum, mainly the nasal bones (PC-2, nasal breadth), is the diagnostic characteristic that discriminated groups A and C from group B. Subclade B2 of Group B (locality 14 in Fig. 1; see Fig. 2) is completely enclosed within the ellipse enclosing all subgroup B1 specimens; these two mtDNA subclades share the same craniodental morphology and are treated together in subsequent analyses.

Table 2. Mean values for four external and 19 craniodental characters for adults of each of the four geographic groups of *Peromyscus guatemalensis* complex from Chiapas and Guatemala: (A) Central Guatemala, north of the Motagua-Polochic-Jocotán fault system ($n = 23$, includes type locality of *P. guatemalensis*); (B) Sierra Madre del Sur and the Volcanic Arch in Guatemala, north of the canyon of the Cuilco River ($n = 163$); (C) Zunil, Quetzaltenango, south of the Río Samalá ($n = 12$); and *P. grandis* ($n = 2$). *F*-values and significance levels for comparisons among samples by one-way ANOVA are given. Character abbreviations are given in the text; data are mean ± standard deviation.

	P. guatemalensis Group A	*P. guatemalensis* Group B	*P. guatemalensis* Group C	*P. grandis*	*F*	*P*
ToL	269.26±11.95	267.93±17.10	267.17±9.27	318.50±4.95	4.73	0.003
TaL	143.83±25.16	135.98±12.32	137.83±7.97	169.50±7.78	4.47	0.005
LHF	31.13±1.02	28.63±1.62	29.08±0.95	24.25±0.35	20.99	0
LE	23.78±1.38	22.06±2.89	25.23±1.36	33.5±0.71	15.70	0
GLS	35.06±0.96	34.09±0.87	33.59±0.71	40.21±0.22	35.91	0
SKH	12.63±0.41	14.37±1.57	11.34±0.33	12.75±0.18	27.06	0
CBL	28.92±0.79	27.84±0.82	27.86±0.60	32.93±0.61	33.73	0
BUL	5.17±0.16	4.69±0.26	4.91±0.21	5.32±0.39	28.20	0
SBD	7.80±0.27	7.32±0.27	7.47±0.19	8.36±0.00	27.99	0
DIL	9.58±0.37	8.97±0.34	9.11±0.28	11.34±0.11	41.22	0
ROH	6.13±0.27	5.90±0.34	5.96±0.18	7.29±0.38	15.03	0
BRR	5.79±0.29	4.57±0.40	5.34±0.29	6.22±0.15	84.69	0
PBL	5.21±0.21	5.05±0.27	5.08±0.26	5.74±0.39	4.39	0.005
POL	12.75±0.42	12.38±0.48	12.28±0.32	14.66±0.42	20.07	0
OCL	4.60±0.21	4.63±0.28	4.57±0.21	5.31±0.02	3.57	0.01
MTL	5.34±0.19	5.04±1.17	5.15±0.23	5.73±0.01	28.80	0
MTB	6.95±0.18	6.75±0.21	6.68±0.15	7.63±0.30	17.46	0
PDB	4.79±0.18	4.73±0.20	4.55±0.16	5.29±0.13	7.61	0
ZYB	17.03±0.36	16.36±0.50	16.12±0.38	18.88±0.16	34.84	0
BAB	15.02±0.38	14.58±0.34	14.58±0.33	15.89±0.13	21.20	0
NAL	14.10±0.68	13.56±0.62	13.54±0.62	17.16±1.36	22.56	0
IOB	5.53±0.19	5.34±0.18	5.25±0.15	5.83±0.22	13.41	0
NAB	2.50±0.23	3.50±0.34	2.4±0.10	2.93±0.03	105.00	0

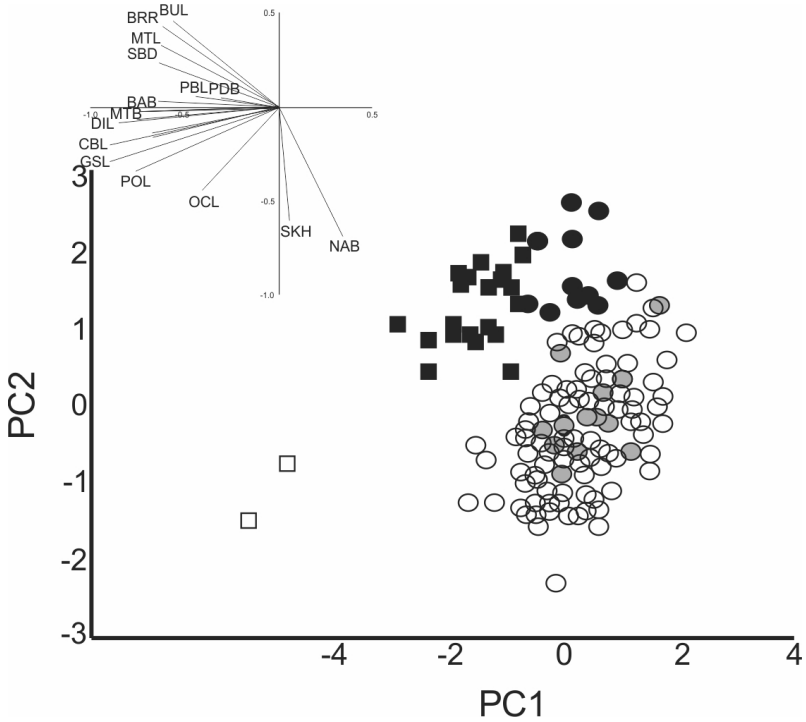

Figure 3. Bivariate plot of PC-I and PC-II scores extracted from a Principal Components Analysis of the 19 log-transformed craniodental variables with five a priori defined groups. Only outer bounds marks of each group are shown: Group A, Guatemalan samples north of the Motagua-Polochic-Jocotán fault system (Fig. 1, 1–7; solid squares); Group B, Chiapas samples (Fig. 1, 8–13; open circle) and the Guatemala sample (Fig. 1, 14; light gray circle); Group C, the sample east of the Río Samalá (Fig. 1, 15; solid circle); and *Peromyscus grandis* (open square).

The first three factors of the CVA explained 99.4% of the total variation CV1 = 86.2%, CV2 = 10.0% and CV3 = 3.2% (Fig. 4; Supplemental Data SD 6). CV-1 (nasal breadth contrasts with rostral breadth and, secondarily, diastema length) separated Group B from all others, whereas CV2 (greatest skull length contrasts with condylobasal length) separated *P. grandis* from Group A and C of *P. guatemalensis;* Group A and C overlap slightly. The two geographical areas in Group B overlapped completely (Fig. 4).

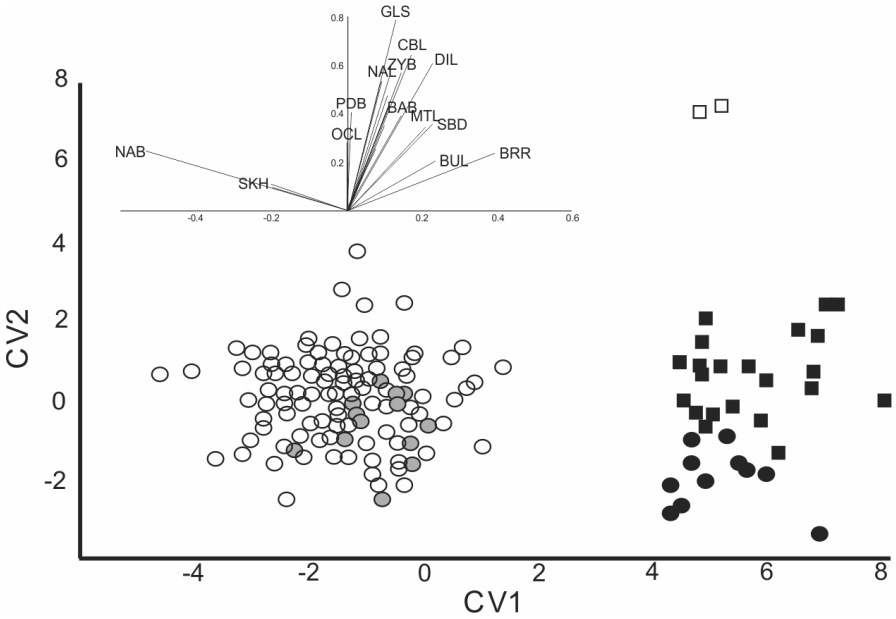

Figure 4. Bivariate plot of CV–I and CV–II scores from a Canonical Variates Analysis of 19 log-transformed craniodental variables, with five a priori defined groups. Only outer bounds marks of each group are shown: Group A, Guatemalan samples north of the Motagua-Polochic-Jocotán fault system (Fig. 1, 1–7; solid squares); Group B, Chiapas samples (Fig. 1, 8–13; open circle) and the Guatemala sample (Fig. 1, 14; light gray circle); Group C, the sample east of the Río Samalá (Fig. 1, 15; solid circle); and *P. grandis* (open square).

TAXONOMIC IMPLICATIONS

The genetic data of specimens from localities 4 and 5 of *P. guatemalensis* Group A are from the Todos Santos Cuchumatán area, which is the type locality of both *P. guatemalensis* (Merriam 1898) and *P. altilaneus* (Osgood 1904). Sequences from these specimens differ by < 1%. The holotype for *P. guatemalensis* nor other material referred to his taxon were not examined and therefore no decision regarding its association with either *P. guatemalensis* (*sensu* Huckaby 1980) or *P. mexicanus* (e.g., Musser and Carleton 2005) can be made at this time.

Peromyscus guatemalensis currently is known from the highlands of Chiapas in Mexico and Guatemala, occupying cloud and pine-oak forest above 1,300 m. This is not a continuous montane region, but one separated into three blocks by the Río Samalá valley and the Motagua- Polochic-Jocotán fault system (Fig. 1). In turn, these three blocks each delimit a molecularly and morphologically distinct subset of populations currently regarded as the single species *P. guatemalensis* (Fig. 2). One of these (Group C; Fig. 1) is known only from east of the Rio Samala in the Central American Volcanic Arc in Guatemala (Quetzaltenango). The second (Group B) occupies a larger distribution that includes the highlands of the Sierra Madre del Sur, Chiapas, and the Volcanic Arc in Guatemala west of the Río Samalá (Fig. 1). The last (Group A) occurs in west-central Guatemala north of the Motagua-Polochic-Jocotán fault system (the Altos Cuchumatanes which, as noted immediately above, contains the type locality of *P. guatemalensis*). The clade structure and high level of nodal support obtained from the analyses reported herein is consistent with the Phylogenetic Species Concept (Cracraft 1997), with each clade being morphologically distinct in multivariate ordination of craniodental variables (Figs. 3 and 4). Based on the combined morphological and molecular

distinctness of these groups, it appears that three species should be recognized in *P. guatemalensis* from Mexico and Guatemala: *P. guatemalensis* (restricted to the Sierra de los Cuchumatanes, the highland areas north of the Motagua-Polochic-Jocotán fault system; Group A in Fig. 1), and two new forms (Groups B and C), described herein.

***Peromyscus bakeri* sp. nov.**

Holotype.—USAC 4644/MVZ 224826, an adult male collected by Sergio G. Pérez (original number SGP 1588) on 25 April 2009 at Guatemala, Quezaltenango Fuentes Georginas, ca. 3.8 km S, 0.3 km E Municipio de Zunil, 2,433 m (14.7467, -91.4802); Group C, locality 15 in Figure 1. The specimen consists of a museum study skin with accompanying cranium, mandibles, and post-cranial skeleton (Fig. 5), and liver tissue preserved in 95% ethanol with frozen aliquots maintained at USAC and at MVZ; all parts are in good condition.

Paratypes.—USAC 4643–4645, 4656, 4685, 4702–4706, 4708, 4709, 4712 and MVZ 224823 (see Supplemental Data SD1 for localities).

Common name.—Baker´s Deermouse.

Diagnosis.—A member of the *Peromyscus mexicanus* group, *P. bakeri* is a medium sized mouse, smaller than *P. guatemalensis sensu stricto* from the Sierra de los Cuchumatanes, and larger than *P. gymnotis* from lower elevations in the same mountain range. It has uniformly medium grayish brown dorsal pelage, with a lighter brown color on the forehead; hairs on the back are basally gray; the eye ring is black; the lateral coat has a strip colored orange-brown that extends to the cheeks; and the venter is whitish gray with an indistinct buffy-brown patch on the chest. Both front and hind legs are whitish gray on the outside; inside of the hind legs darker brown with yellowish fingers and that of the front legs varies from whitish to yellowish. The tail is bicolored, darker grayish brown dorsally and white yellowish ventrally.

The skull is in overall appearance more robust in relation to other species of the *mexicanus* complex, less massive with the braincase more compressed. The posterior border of the incisive foramina does not reach the anterior border of the first upper molars and the axial and transverse axes of the foramen magnum are of similar dimensions.

Description and comparisons.—*Peromyscus bakeri* and *P. guatemalensis* are phylogenetically sister taxa (Fig. 2) and morphologically similar species. Besides being allopatric, these two species differ in the following: *P. bakeri* has a less distinct buffy-brown patch on the chest, which is much more prominent in *P. guatemalensis*, and much shorter mystacial vibrissae. Overall, *P. bakeri* has a smaller and less robust skull, with a narrower inter-orbital region, narrower zygomatic arches, and less inflated cranium (shorter cranial height); more prominent and longer incisive foramina; a more slender appearance to the rostrum; a smaller mesopterygoid fossa; slightly smaller maxillary teeth in both width and length; and a larger foramen magnum.

Peromyscus gynmotis, the only other species of the *Peromyscus mexicanus* group inhabiting the volcanic region near the type locality of *P. bakeri*, is smaller than *P. bakeri* and its skull possesses more pronounced supraorbital ridges (Huckaby 1980). This species inhabits the coastal plains and western foothills of the Volcanic Arc at elevations below that of the type locality of *P. bakeri*.

Compared with *P. guatemalensis* Group B, *P. bakeri* is smaller in body and tail length, larger in hind foot and ear lengths; is paler in colored, plumbeous gray in both the back and flanks; and lacks the pale orange pectoral patch of *P. guatemalensis* Group B. In *P. bakeri* the braincase is lower and the skull thicker, in general less massive; the posterior border of the incisive foramen is previous to the anterior border of the first upper molars, and the axial axis and transverse axis of the foramen magnum are similar.

Measurements.—Table 3 provides external and craniodental mensural values for the holotype and paratypic series.

Distribution.—*Peromyscus bakeri* currently is known only from the type locality at Fuentes Georginas, Zunil, Quetzaltenango (ca. 2,433 m), east of the Río Samalá on the Volcanic Arc in the southwestern highlands of Guatemala. This mouse is likely endemic to this small area, where it is associated with cloud

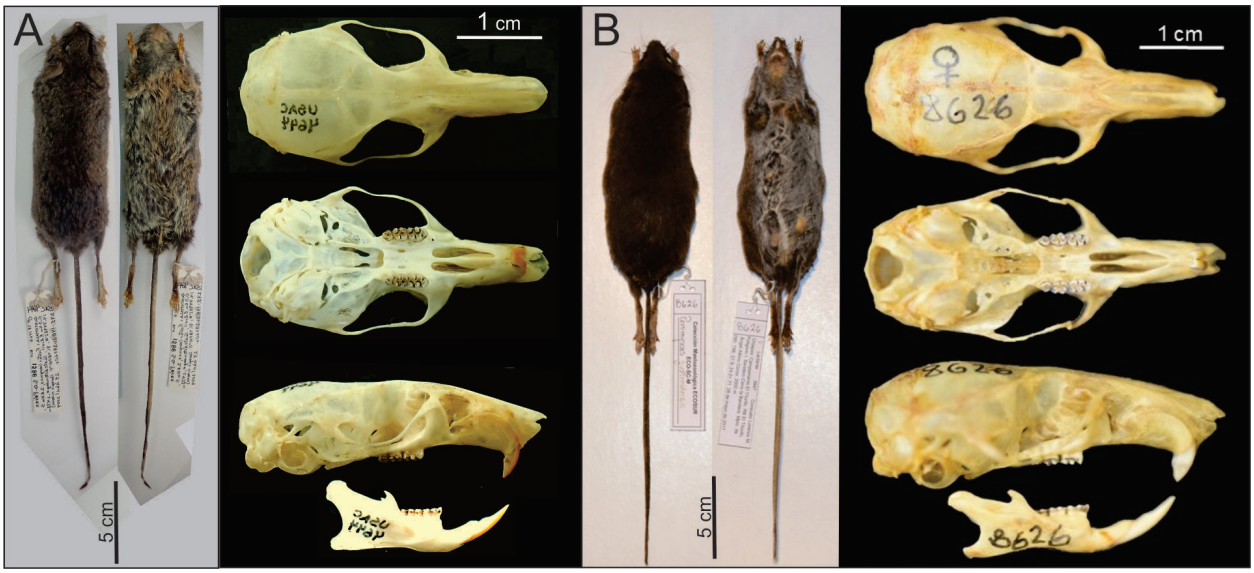

Figure 5. Dorsal, ventral, and lateral views of the skull and lateral view of the mandible of the holotypes of *P. bakeri* (A; USAC 4644) and *P. carolpattonae* (B; ECOSUR 8626).

forest dominated by oak, pine, and other typical cloud forest vegetation. It may be sympatric with, or at least occurs in close proximity to, *P. gymnotis* at or near the latter's upper elevational limit of 1,500 m, below which *P. gymnotis* first appears and begins to be abundant elsewhere in its range.

Ecology.—*Peromyscus bakeri* appears to be a locally abundant mouse. At the type locality, it was the most abundant species at the site, comprising >50% of all captures. Specimens were captured in the following habitats: on the floor of a pine-oak forest with old-growth trees laden with mosses, lichens, and bromeliads and an open understory but with many fallen trees; in open, disturbed forest with a larger mixture of microhabitats, including large banks of ferns, fallen logs covered with moss; on a steep south-facing slope covered with dense shrubs and short-stature trees, mostly small oaks; and in very wet meadows bordering a small stream in a narrow defile. Other species of terrestrial small mammals collected include *P. levipes*, *P. oaxacensis*, *Reithrodontomys tenuirostris*, and *Marmosa mexicana*.

Nomenclatural statement.— A life science identifier (LSID) number was obtained for new species described herein: urn:lsid:zoobank.org:act:564FB3F7-2CF3-4D6E-8BF3-2B4E1CD75589.

Etymology.—We honor Dr. Robert James Baker for his distinguished career in the mammalogy of different parts of the world and for his substantive training of scholars in the field, laboratory, and museum. His studies contributed substantially to our understanding of the systematics and taxonomy of mammals, especially Neotropical bats.

***Peromyscus carolpattonae* sp. nov.**

Holotype.—ECO-SC-M 8626, an adult female collected by C. Lorenzo (original number 3947) on Campamento El Triunfo, RB El Triunfo, Polígono I, Sendero Cerro la Bandera, at Mexico, Chiapas, El Triunfo (15.6568° N, -92.8093° W); Group B, locality 8 in Figure 1. The specimen consists of a stuffed museum study skin with accompanying cranium and mandibles (Fig. 5) and liver tissue preserved in 95% ethanol; all parts are in good condition.

Paratypes.—ECO-SC-M 901, 902, 905, 1183, 1185, 1283, 9023, 9024 (see Supplemental Data SD1 for localities).

Common name.—Carol Patton's Deermouse.

Diagnosis.—A member of the *Peromyscus mexicanus* group, *P. carolpattonae* is characterized exter-

Table 3. External and craniodental values for the holotype and paratypes of *Peromyscus bakeri* (USAC 4644) and *P. carolpattonae* (ECO-SC-M 8626); data for paratypes are mean and range.

	P. bakeri		*P. carolpattonae*	
Measurements	Holotype	Paratypes (n = 14)	Holotype	Paratypes (n = 8)
Total length	275.0	267.1 (246.0–281.0)	290.0	272.9 (257.5–287.0)
Tail length	148.0	137.8 (118.0–148.0)	146.0	143.2 (131.5–152.0)
Hind foot length	31.0	29.0 (28.0–31.0)	27.9	28.9 (27.1–31.0)
Ear length	27.0	25.2 (23.0–27.0)	24.2	20.9 (18.8–24.0)
Greatest skull length	34.52	33.5 (32.4–34.6)	34.69	34.3 (33.1–35.8)
Skull height	10.49	11.3 (10.4–11.7)	11.31	11.1 (10.4–11.4)
Condylobasal length	28.36	27.8 (26.8–28.7)	28.42	28.1 (27.2–29.9)
Bullar length	5.18	4.9 (4.6–5.2)	4.60	4.7 (4.3–4.9)
Shield–bullae depth	7.81	7.4 (7.2–7.8)	7.14	7.2 (6.6–7.6)
Diastema length	9.38	9.1 (8.6–9.4)	9.19	9.2 (8.3–9.6)
Rostral height	5.98	5.9 (5.5–6.2)	5.92	5.8 (5.5–6.3)
Rostral breadth	4.95	5.3 (4.9–5.7)	4.46	4.3 (4.0–4.6)
Palatine bridge length	5.5	5.0 (4.6–5.5)	4.99	4.9 (4.7–5.4)
Postpalatal length	12.41	12.2 (11.8–12.8)	12.91	12.6 (11.8–13.7)
Basioccipital length	4.41	4.5 (4–4.8)	4.97	4.7 (4.3–5.6)
Maxillary toothrow length	5.15	5.1 (4.8–5.7)	4.94	4.9 (4.8–5.1)
Maxillary toothrow breadth	6.77	6.6 (6.4–6.9)	6.78	6.9 (6.8–7.0)
Postdental breadth	4.67	4.5 (4.2–4.8)	4.81	4.7 (4.5–4.8)
Zygomatic breadth	16.36	16.1 (15.4–16.8)	17.44	16.5 (15.7–17.1)
Braincase breadth	14.56	14.5 (13.8–15)	14.78	14.6 (14.3–14.9)
Nasal length	13.95	13.5 (12.6–14.9)	14.17	14.2 (13.0–16.3)
Interorbital breadth	5.17	5.2 (4.9–5.4)	5.66	5.4 (5.2–5.7)
Nasal breadth	2.51	2.3 (2.2–2.5)	3.14	2.8 (2.7–2.9)

nally by a uniformly light grayish brown dorsal pelage, with the brown color paler on the forehead; hairs on the back are basally gray; the eye ring is black; the lateral coat has a strip of orange-brown color that extends to the cheeks; and the venter is whitish gray with a pale orange tuft on the chest. The outside color of the front and hind legs is whitish gray, the inside color of the hind legs is darker brown and that of the forelegs varies from white to yellowish; toes and fingers are yellowish. The tail is bicolor, brown dark dorsally and yellowish ventrally; the tip of tail is white in some specimens.

The skull is long and slender, more globose in specimens from San Marcos, with lacking well-developed supraorbital crests over the orbit, and nasals that extend beyond the premaxillae in El Triunfo but only to the premaxillae in those of San Marcos. The skull is robust in general appearance, more massive with taller braincase when viewed from the side. The posterior border of the incisive foramina reaches the anterior border of the first upper molars and the axial and transverse axes of the foramen magnum are similar in their dimensions.

Description and comparisons.—*Peromyscus carolpattonae* is compared only with those potentially sympatric species of the *P. mexicanus* group. *P. carolpattonae* differs externally from *P. guatemalensis* overall by smaller external measurements and all craniodental measurements, except the skull height and the basioccipital length.

Compared with *P. gymnotis*, *P. carolpattonae* differs externally by larger external measurements and larger dimensions for each craniodental measurement. The supraorbital crests of *P. carolpattonae* are not well developed, supraorbital region smooth, lacking both ledges and beading.

Compared with *P. mexicanus*, *P. carolpattonae* differs also externally by larger external and craniodental measurements to specimens from Volcán Agua, the closed *P. mexicanus* locality reported by Huckaby (1980). Externally *P. carolpattonae* differs from *P. mexicanus* from Chiapas and western Guatemala by lacking well-developed supraorbital crests over the orbit. Compared with *P. grandis*, *P. carolpattonae* has a shorter and more slender skull, with more prominent supraorbital crests, nasals that do not extend beyond the premaxillae, and proportionally larger bullae.

Compared with *P. bakeri*, *P. carolpattonae* is larger in body and has a longer tail, but has a shorter hind foot and ear; is more darkly colored, plumbeous gray on both the back and flanks; and lacks the pale orange pectoral patch of *P. bakeri.* In *P. carolpattonae*, the braincase is higher and the skull more robust; the posterior border of the incisive foramen is nearly at the level of the anterior border of the first upper molars; and the axial axis of the foramen magnum is longer than that of the transverse axis.

Compared with *P. gardneri*, the most resent species described in the *P. mexicanus* complex, *P. carolpattonae* has a shorter and more slender skull, supraorbital crests are better developed, and the nasals extend beyond the premaxilla.

Measurements.—Table 3 provides external and craniodental values for the holotype and paratypes series.

Distribution.—*Peromyscus carolpattonae* currently is known from the Sierra Madre de Chiapas and the western part of the Central American Volcano Arc in southwestern Guatemala at elevations from ca. 1,300 to 3,000 m. The Motagua-Polochic-Jocotán fault system forms the northern distributional limit and the canyon of the Río Samalá the eastern limit in Guatemala.

Ecology.–*Peromyscus carolpattonae* was abundant at Cerro Mozotal and Cerro Madron, municipality of El Porvenir, and near La Comunidad La Cascada, municipality of Siltepec. All specimens were captured in cloud and pine-oak forest in northern (1,500–2,950 m) and southeastern mountainous regions (Horváth 2014) pine forest in El Porvenir, pine-oak forest in San Marcos (2,710 m), and coffee plantations in Motozintla and Siltepec (1,140–1,530 m). At the type locality of Campamento El Triunfo, two lactating females were collected on 28 May 2017 and 1 March 2000, and a pregnant female (with a single embryo measuring 27.2 x 21.1 mm) on 19 March 2018. Two scrotal males were captured in February 1999. Other species of terrestrial small mammals collected included *Heteromys goldmani*, *P. aztecus*, *Habromys lophurus*, *Reithrodontomys sumichrasti* and *Handleyomys rostratus*.

Nomenclatural statement.—A life science identifier (LSID) number was obtained for new species described herein: urn:lsid:zoobank.org:act:C67D2CAB-6C09-4B02-93EA-6DBD82C57404.

Etymology.—We honor Carol Patton for distinguished contributions to mammalogy as the partner of James L. Patton over more than five decades of field research. Carol and Jim have been working side by side as a team and have made great contributions together.

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LITERATURE CITED

Bradley, R. D., and R. J. Baker. 2001. A test of genetic species concept: cytochrome-*b* sequence and mammals. Journal of Mammalogy 84:960–973.

Bradley, R. D., N. D. Durish, D. S. Rogers, J. R. Miller, M. D. Engstrom, and C. W. Kilpatrick. 2007. Toward a molecular phylogeny for *Peromyscus*: evidence from mitochondrial cytochrome-*b* sequences. Journal of Mammalogy 88:1146–1159.

Bradley R. D., M. Nuñez-Tabares, T. J. Soniat, S. Kerr, R. W. Raymond, and N. Ordóñez-Garza. 2016. Molecular systematics and phylogeny of *Peromyscus nudipes* (Cricetidae: Neotominae). Pp 201–214 in Contributions in natural history: A memorial volume in honor of Clyde Jones (R. W. Manning, J. R. Goetze, F. D. Yancey, II, eds.). Special Publications, Museum of Texas Tech University 65:1–273.

Carleton, M. D. 1989. Systematics and evolution. Pp. 7–141 in Advances in the study of *Peromyscus* (Rodentia) (G. L. Kirkland and J. N. Layne, eds.). Texas Tech University Press, Lubbock.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657–1660.

Cracraft, J. 1997. Species concepts in systematics and conservation biology -- an ornithological viewpoint. Pp. 325–340 in Species: the units of biodiversity (M. F. Claridge, H. A. Dawah, and M. R. Wilson, eds.). Chapman and Hall, London, U.K.

Goodwin, G. G. 1932.Three new *Reithrodontomys* and two new *Peromyscus* from Guatemala. American Museum Novitates 506:1–5.

Hall, E. R. 1981. Mammals of North America. 2nd ed. John Wiley & Sons, New York.

Hammer, Ø., D.A.T. Harper, and P. D. Ryan. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4:1–9. Available from: http://palaeo-electronica.org/2001_1/past/issue1_01.htm (accessed 15 June 2018).

Hoffmeister, D. F. 1951. A taxonomic and evolutionary study of the piñon mouse, *Peromyscus truei*. Illinois Biological Monographs 21:1–104.

Horváth, A. 2014. *Peromyscus gutemalensis*. In Ceballos, G. (ed.), Mammals of Mexico, John Hopkins University Press, Baltimore, Maryland.

Huckaby, D. G. 1980. Species limits in the *Peromyscus mexicanus* group (Mammalia: Rodentia: Muroidea). Contributions in Science, Natural History Museum of Los Angeles County 326:1–24.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111–120.

Lorenzo, C., S. T. Álvarez-Castañeda, J. L. Patton, and S. Pérez. 2016. Revision of the taxonomy of the Chiapan deer mouse, *Peromyscus zarhynchus*, with the description of a new species. Journal of Mammalogy 97:910–918

Marshall, J. S. 2007. The geomorphology and physiographic provinces of Central America. Pp 1–51 in Central America: geology, resources and hazards (J. Bundschuh and C. E. Alvarado, eds.). Taylor and Francis Group, London, UK.

Merriam, C.H. 1898. Descriptions of the twenty new species and a new subgenus of *Peromyscus* from Mexico and Guatemala. Proceedings of the Biological Society of Washington 12:115–125.

Musser, G. G. 1969. Notes on *Peromyscus* (Muridae) of Mexico and Central America. American Museum Notivates 2357:1–23.

Musser, G. G., and M. D. Carleton. 2005. Superfamily Muroidea. Pp. 894–1531 in Mammal species of the world: a taxonomic and geographic reference (Wilson, D. E., and D. M. Reeder, eds.). 3rd ed. Johns Hopkins University Press, Baltimore, Maryland.

Nylander, J. A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Center, Uppsala University, Sweden.

Ordoñez-Garza, N., J. O. Matson, R. E. Strauss, R. D. Bradley, and J. Salazar-Bravo. 2010. Patterns of phenotypic and genetic variation in three species of endemic Mesoamerican *Peromyscus* (Rodentia: Cricetidae). Journal of Mammalogy 91:848–859.

Orvis, K. H., and S. P. Horn. 2000. Quaternary glaciers and climate on Cerro Chirripó, Costa Rica. Quaternary Research 54:24–37.

Pérez-Consuegra, S. G., and E. Vázquez-Domínguez. 2015. Mitochondrial diversification of the *Peromyscus mexicanus* species group in nuclear Central America: biogeographic and taxonomic implications. Journal of Zoological Systematics and Evolutionary Research, published on-line 15 April 2015 (doi: 10.1111/jzs.12099).

Pérez-Consuegra, S. G., and E. Vázquez-Domínguez. 2017. Intricate evolutionary histories in montane species: a phylogenetic window into craniodental discrimination in the *Peromyscus mexicanus* species group (Mammalia: Rodentia: Cricetidae). Journal of Zoological Systematics and Evolutionary Research 55:57–72.

Reid, F. 2009. A field guide to the mammals of Central America and southeast Mexico. Oxford University Press, New York, USA.

Robinson, T. J., and N. J. Dippenaar. 1987. Morphometrics of the South African Leporidae. II: *Lepus* Linnaeus, 1758, and *Bunolagus* Thomas, 1929. Annals Transvaal Museum 34:379–404.

Rogers, D. S., and M. D. Engstrom. 1992. Evolutionary implications of allozymic variation in tropical *Peromyscus* of the *mexicanus* species group. Journal of Mammalogy 73:55–69.

Rogers, R., H. Karason, and R. van der Hilst. 2002. Epeirogenic uplift above a detached slab in northern Central America. Geology 30:1031–1034.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin ver. 2.000: a software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

Sikes, R. S., and the Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy 97:663–688.

Smith, M. F, and J. Patton. 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data of the akadontine tribe. Biological Journal of the Linnean Society 50:149–177.

SPSS Inc. 1989–2006. SPSS (ver. 15.0.1 for Windows), Chicago, Illinois.

StatSoft, Inc. 2007. STATISTICA (data analysis software system), version 8.0. www.statsoft.com.

Swofford, D. L. 2000. PAUP: Phylogenetic Analysis Using Parsimony, version 4.0b10. Sinauer, Sunderland, Massachusetts.

Tavaré, S. 1986. Some probabilistics and statistical problems in the analysis of DNA sequences. Pp. 57–86 in Some mathematical questions in biology – DNA sequence analysis (R. M. Miura, ed.). American Mathematics Society, Providence, Rhode Island.

Williams, S. L., and J. Ramírez-Pulido. 1984. Morphometric variation in the volcano mouse, *Peromyscus* (*Neotomodon*) *alstoni* (Mammalia: Cricetidae). Annals of Carnegie Museum 53:163–183.

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Taxonomy and Phylogenetics of the *Peromyscus maniculatus* Species Group

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Abstract

An overview is provided herein of advancements in the species composition and phylogenetics of the *Peromyscus maniculatus* species group since Carleton's 1989 review of the genus. These advancements primarily are the result of studies of chromosomal and nucleotide-sequence variation, with most sequence data derived from the mitochondrial genome. Evidence is summarized supporting the conclusion that variation in mitochondrial genes provides consistent and informative details relative to species-level identification and the phylogenetic relationships among major clades of deer mice. Based on studies of the mitochondrial cytochrome-*b* gene as well as previously published and new sequences of the mitochondrial ND3/ND4/ND4L genes, the number of species in the *P. maniculatus* group is increased to nine (*P. melanotis*, *P. polionotus*, *P. keeni*, *P. arcticus*, *P. gambelii*, *P. sejugis*, *P. sonoriensis*, *P. labecula*, and *P. maniculatus*). The newly identified species render *P. maniculatus* as being restricted to the northeastern United States and south-central Canada. The phylogenetic studies provide evidence of a sister-group relationship between the *P. leucopus* and *P. maniculatus* species groups and the placement of *P. melanotis* as basal to other members of the *P. maniculatus* group. The well-supported clade containing *P. keeni* and *P. gambelii*/*P. sejugis* appears to be best explained as having resulted from independent peripheral isolation. However, the available data fail to resolve the phylogenetic relationship of the clades corresponding to *P. maniculatus sensu stricto, P. sonoriensis*, *P. polionotus,* and *P. keeni*/*P. gambelii*/*P. sejugis*, and too little data exist to address the phylogenetic relationships of *P. arcticus* and *P. labecula* relative to the other species in the *P. maniculatus* species group. A more thorough resolution of the systematics of the species in the *P. maniculatus* group awaits broader and targeted geographic sampling and the inclusion of data from more rapidly evolving nucleotide sequences.

Key words: *Peromyscus maniculatus* species group, phylogenetics, systematics, taxonomy

Introduction

The genus *Peromyscus* represents the most ubiquitous genus of native North American rodents. One or more species of *Peromyscus* occur in nearly all habitats of North America from the Canadian taiga through central Mexico. In his revision of the genus, Osgood (1909) recognized the subgenus *Peromyscus* as a highly variable but distinct morphological group of species, and established species groups to reflect morphological discontinuities within the subgenus. Whereas some of Osgood's (1909) species groups and their constituent species have undergone substantial revision, his *Peromyscus maniculatus* group, containing *P. maniculatus*, *P. polionotus*, *P. melanotis*, and *P. sitkensis*, remained comparatively stable through Carleton's (1989) review of the genus. To Osgood's (1909) *P. maniculatus* group, Hooper (1968) added *P. sejugis* (Burt 1932) and tentatively *P. slevini* (Maillaird 1924), both from islands in the Gulf of California. Carleton (1989) retained the composition of Hooper's *P. maniculatus* group but followed Sheppe (1961), Gunn and Greenbaum (1986), and Allard et al. (1987) in recognizing and including *P. oreas* (Bangs 1897) as a distinct species in western Washington and southwestern British Columbia.

Traditional systematic studies of *Peromyscus* were based on evidence from morphology, pelage, geographic distribution, and ecology. Carleton (1989) noted that Hooper's (1968) bibliography contained no references to "nontraditional data as taxonomic evidence." As noted by Carleton (1989), the two decades between the review of Hooper (1968) and his review saw the inception and rapid growth of the application of chromosomal and biochemical techniques to studies of evolution, systematics, and taxonomy in general and to *Peromyscus* in particular. Carleton (1989) specified that of the more than 130 post-1968 references amassed for his review, nearly 70% reported results from karytoypic and/or biochemical studies. As such, Carleton (1989) provided a comprehensive review of both the traditional and nontraditional evidence relevant to the systematics of *Peromyscus* through 1987.

Subsequent to Carleton's (1989) review, most phylogenetic and systematic studies of the genus *Peromyscus* and the *P. maniculatus* group in particular have been based primarily on nucleotide sequence variation of mitochondrial DNA (mtDNA). The sole use of mtDNA for phylogenetic analyses and species delimitation has been criticized (for review see Galtier et al. 2009), and some authors (Yang and Kenagy 2009, 2011; Taylor and Hoffman 2012) have reported instances of mitonuclear discordance with respect to recent gene flow among populations of *P. maniculatus.* However, there is considerable evidence that mitochondrial sequence divergence of the cytochrome-*b* gene has proven useful for identifying species of *Peromyscus* and other mammals (Bradley and Baker 2001). Moreover, concordance between mitochondrial-sequence and nuclear-based evolution at the level of species differentiation within *Peromyscus* has been well documented. Hogan et al. (1993) found concordance among chromosomal, allozymic, and mtDNA variation in the recognition of *P. keeni* (formerly *P. oreas* and *P. sitkensis*). These characters also were concordant with the morphological cohesiveness of *P. keeni* relative to *P. maniculatus* (Allard et al. 1987; Allard and Greenbaum 1988; Sullivan et al. 1990). Analysis of microsatellite variation (Chirhart et al. 2005) recovered the same phylogenetic relationships among members of the *P. maniculatus* species group as obtained from analyses of mtDNA variation by Hogan et al. (1997). Combined analysis of mtDNA and nuclear sequences (Miller and Engstrom 2008; Platt et al. 2015) recovered the same phylogenetic relationships between and among species in the *P. leucopus* and *P. maniculatus* species groups as previously diagnosed from analyses of mtDNA sequence variation (Sullivan et al. 1995; Hogan et al. 1997; Engel et al. 1998; Durish et al. 2004; Bradley et al. 2007; Gering et al. 2009). Therefore, it is concluded that the available molecular data reflect reasonably accurate taxonomic inferences for the *P. maniculatus* species group.

The intent of this paper is three-fold. First, research since 1989 pertaining to the systematics of the *P. maniculatus* species group is reviewed and areas where new data have helped resolve systematic questions raised by Carleton (1989) are identified. Second, remaining taxonomic and phylogenetic issues that require further resolution are highlighted. Finally, new sequence data are introduced from the mitochondrial ND3/ND4/ND4L genes that are germane to defining the limits of *P. maniculatus sensu stricto*. Given the extraordinary popularity of deer mice as a research model for virtually all areas of organismal biology, discussion is limited to reports with direct relevance to the systematics and taxonomy of the *P. maniculatus* species group.

TAXONOMIC RESOLUTION SINCE 1989

Basal relation of the P. maniculatus *species group*.—Numerous molecular studies have included species of the *P. maniculatus* group as either outgroups or reference taxa in studies pertaining to either the systematics of the genus *Peromyscus* or as focal species for addressing questions other than the systematics of the *P. maniculatus* group (Table 1). However, studies that included species of both the *P. maniculatus* and *P. leucopus* species groups uniformly recovered these as highly supported and reciprocally monophyletic clades. Correspondingly, both Miller and Engstrom (2008) and Platt et al. (2015) entertained the notion that the clade containing the *maniculatus* + *leucopus* species groups will ultimately prove to constitute a distinct subgenus or genus.

Table 1. Studies since Carleton (1989) reporting molecular data for species of the *P. maniculatus* species group in which the data were used as outgroups or reference species and which were not designed as studies of the systematics of the *P. maniculatus* species group.

Reference	Data	Focus of study	Outgroup/reference species
Rogers and Engstrom 1992	allozymes	*P. mexicanus* group	*melanotis*
Sullivan et al. 1995	*Cyt*b, 12S rRNA	sigmodontine rodents	*melanotis*, *polionotus*, *keeni*
Engel et al. 1998	ND3/ND4/ND4L	sigmodontine rodents	*maniculatus*
Riddle et al. 2000	COIII	*P. eremicus* group	*maniculatus*
Tieman-Boege et al. 2000	*Cyt*b	*P. boylii* group	*melanotis*
Hafner et al. 2001	COIII	Sea of Cortez insular *Peromyscus*	*sejugis*, *maniculatus*
Bradley et al. 2004	*Cyt*b	neotomine-peromyscine rodents	*maniculatus*
Durish et al. 2004	*Cyt*b	*P. truei* group	*melanotis*, *maniculatus*
Dragoo et al. 2006	*Cyt*b	*P. maniculatus*	*melanotis*, *keeni*
Bradley et al. 2007	*Cyt*b	genus *Peromyscus*	*melanotis*, *keeni*, *polionotus, maniculatus*
Degener et al. 2007	*Cyt*b, microsatellites	*P. polionotus*	*melanotis*, *maniculatus*
Van Zant et al. 2007	*Cyt*b, D-loop	*P. polionotus*	*keeni*, *maniculatus*
Miller and Engstrom 2008	*Cyt*b, nuclear genes	genus *Peromyscus*	*melanotis*, *polionotus*, *maniculatus*
Gering et al. 2009	*Cyt*b	*Cyt*b evolution/adaptation	*melanotis*, *keeni*
Domingues et al. 2012	nuclear sequences	adaptation, *P. polionotus*	*maniculatus*
Kalkvik et al. 2012	*Cyt*b	phylogeography/niche modeling	*melanotis*, *polionotus, keeni*
Platt II et al. 2015	*Cyt*b, nuclear genes	genus *Peromyscus*	*melanotis*, *maniculatus*
Kingsley et al. 2017	SNPs, COIII–ND3	evolution of form/adaptation	*polionotus*, *keeni*
Cornejo-Latorre et al. 2017	*Cyt*b, COI, COIII	subgenus *Haplomylomys*	*sejugis*, *maniculatus*
Greenbaum et al. 2017	ND3/ND4/ND4L	*P. maniculatus*	*melanotis*, *keeni*
Kalkvik et al. 2018	*Cyt*b, microsatellites	*P. polionotus*	*melanotis*, *keeni*, *maniculatus*

Peromyscus slevini.—*P. slevini* is an island endemic restricted to Catalina Island in the Gulf of California (Álvarez-Castañeda and Cortés-Calva 2002). Although Carleton (1989) retained *P. slevini* in the *P. maniculatus* group, he noted that its "systematic position remains obscure." In their account of the species, Álvarez-Castañeda and Cortés-Calva (2002) suggested that *P. slevini* was derived from a *P. maniculatus* ancestral stock, but they failed to cite relevant molecular and chromosomal studies that clearly invalidate inclusion of *P. slevini* in the *P. maniculatus* group. Phylogenetic analyses of sequences of the mitochondrial ND3/ND4/ND4L genes (Hogan et al. 1997) grouped *P. slevini* outside a clade containing both the *P. leucopus* and *P. maniculatus* species groups. Smith et al. (2000) reported that *P. slevini* has an autosomally invariant chromosomal phenotype that lacks the chromosome 2 and chromosome 20 syapomorphies associated with members of the *P. maniculatus* group. The banded karyotype of *P. slevini* is unique among all banded karyotypes reported for *Peromyscus* but is similar to karyotypes reported for species in the *P. boylii* and *P. mexicanus* species groups. Based on analyses of craniofacial, exomorphological, and bacular variables, Carleton and Lawlor (2005) allocated *P. slevini* to the *P. melanophrys* species group.

Peromyscus melanotis.—Of the species in Carleton's (1989) *P. maniculatus* group (sans *P. slevini*), *P. melanotis*, distributed at higher elevations in south-

eastern Arizona (Bowers et al. 1973, Bowers 1974) and central Mexico (Fig. 1), has been the least affected by subsequent molecular phylogenetic studies. There is little morphological discontinuity among populations of *P. melanotis* (Martínez-Coronel et al. 1991), and chromosomal (Greenbaum et al. 1978; Robbins and Baker 1981; Stangl and Baker 1984) and most molecular data (Hogan et al. 1997; Chirhart et al. 2005; Walker et al. 2006; Gering et al. 2009; Kalkvik et al. 2012) identify it as the most basal member of the *maniculatus* group. That Dragoo et al. (2006) found *P. melanotis* to be weakly associated among clades of *P. maniculatus* is anomalous as three other studies (Gering et al. 2009; Kalkvik et al. 2012; Natarajan et al. 2015), using the same gene, recovered *P. melanotis* as the basal species of the group.

Peromyscus polionotus.—*Peromyscus polionotus* is restricted to sandy soils of the southeastern United States (Hall 1981) and is peripherally distributed relative to the other species in the *P. maniculatus* group (Fig. 1). Analysis of mtDNA variation (Van Zant et al. 2007; Kalkvik et al. 2018), microsatellites (Degener et al. 2007), and nuclear sequences (Domingues et al. 2012) supported *P. polionotus* as monophyletic with respect to other members of the *P. maniculatus* group, and cytosystematic analyses (Greenbaum et al. 1978; Robbins and Baker 1981; Stangl and Baker 1984) consistently identified *P. polionotus* as sister to *P. maniculatus*. Two studies of mtDNA sequence variation, however, recovered marginally supported associations of *P. polionotus* to clades within *P. maniculatus*. Kalkvik et al. (2012) obtained *P. polionotus* as sister to a clade of *P. maniculatus* from the central and western states, whereas Kingsley et al. (2017) obtained *P. polionotus* as sister to a clade (including *P. m. nubiterrae* and *P. m. gracilis*) from the eastern United States and Canada.

Peromyscus keeni (= *P. oreas* + *P. sitkensis*).—In support of the specific status of *P. oreas* and its inclusion in the *P. maniculatus* species group, Carleton (1989) cited its sympatry with (Sheppe 1961) and karytoypic (Gunn and Greenbaum 1986) and morphologic (Allard et al. 1987) distinction from *P. m. austerus*. *Peromyscus oreas* from Washington and coastal British Columbia are characterized by a largely biarmed karyotype (number of autosomal arms (FN) = 85–88), whereas *P. m. austerus* has karyotypes of FN = 74–76 (Gunn and Greenbaum 1986). This observation, however, left unanswered questions as to the range and island distribution of *P. oreas* and to its relationship with the chromosomally similar *P. sitkensis* (FN = 84–91; Thomas 1973; Pengilly et al. 1983). Gunn (1988) presented chromosomal homology data for a broad sampling of deer mice from Vancouver Island and islands in the Queen Charlotte Strait and Strait of Georgia and confirmed instances of sympatry without intermediates between the karytoypic groups representing *P. oreas* and *P. maniculatus,* respectively. Corresponding results were obtained for external, cranial, and mandibular (Allard and Greenbaum 1988) as well as genital (Sullivan et al. 1990) morphology. Hogan et al. (1993) incorporated results from previous studies, expanded the sampling to include additional localities from mainland British Columbia, the Queen Charlotte Islands, and southeastern Alaska, and analyzed chromosomal, allozymic, and mtDNA (ND3/ND4/ND4L) variation. Correspondingly, Hogan et al. (1993) subsumed *P. oreas*, *P. sitkensis*, and the *P. maniculatus* subspecies *algidus*, *hylaeus*, *macrorhinus*, and *prevostensis* under *P. keeni* (Fig. 1). With little data to clarify the taxonomic affinity of most of the insular subspecies of *P. maniculatus* in the Pacific Northwest (i.e., *beresfordi*, *crancrivorous*, *carli*, *doylei*, *maritimus*, *pluvialis*, *rubriventer*, *sartinensis*, and *triangularis*), Hogan et al. (1993) cited standard karytoypic data for *P. m. carli*, *P. m. doylei*, and *P. m. triangularis* (Thomas 1973) and morphological data presented by Cowan and Guiguet (1965) to suggest that all of these subspecies be referred to *P. keeni*. Chirhart et al. (2001) found that the mtDNA sequences of the deer mice from Triangle Island, British Columbia, confirmed that the subspecies *triangularis* is appropriately assigned to *P. keeni*. The classification by Musser and Carleton (2005) followed Hogan et al. (1993) in including *P. keeni* as a species in the *P. maniculatus* group.

Although inclusion of *P. keeni* in the *P. maniculatus* species group is uncontroversial, its phylogenetic relationship to *P. maniculatus* (*sensu* Musser and Carleton 2005) is incompletely resolved. Analyses including *P. keeni* reference sequences and widespread mtDNA variation in *P. maniculatus* generally have indicated a sister-group relationship between *P. keeni* and southwestern-most populations of *P. maniculatus* (Dragoo et al. 2006; Gering et al. 2009; Kalkvik et al. 2012; Natarajan 2015; Kingsley et al. 2017). More spe-

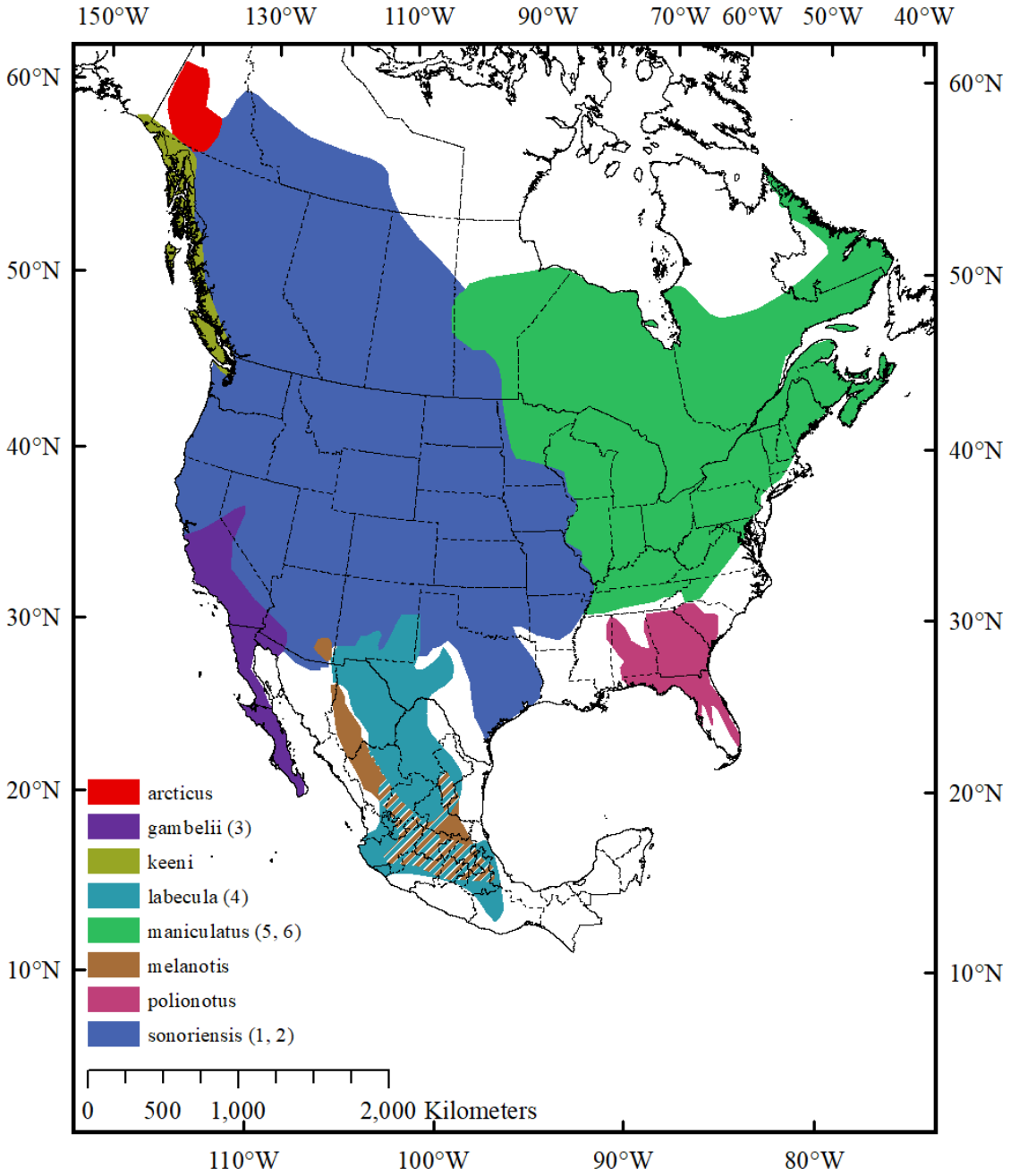

Figure 1. Map of the general distributions of species in the *P. maniculatus* group as discussed and recognized herein. *Peomyscus sejugis* is restricted to Isla Santa Cruz and Isla San Diego in the Gulf of California and is not figured. Numbers in parentheses refer to the DNA clades as designated by Dragoo et al. (2006), and cross hatching indicates distributional overlap of *P. melanotis* and *P. labecula*. The general distributions of species were determined by plotting the geographically marginal specimens reported in the following papers: Bowers et al. (1973), Allard and Greenbaum (1988), Hogan et al. (1993), Wike (1998), Zheng et al. (2003), Lucid and Cook (2004), Dragoo et al. (2006), Walker et al. (2006), Lucid and Cook (2007), Gering et al. (2009), Yang and Kenagy (2011), Domingues et al. (2012), Kalkvik et al. (2012), Natarajan et al. (2015), Greenbaum et al. (2017), Kingsley et al. (2017), Sawyer et al. (2017), and Kalkvik et al. (2018).

cifically, the combined results of Hogan et al. (1997), Chirhart et al. (2005) and Walker et al. (2006) indicated that *P. keeni* is sister to a clade including *P. sejugis* and *P. maniculatus* from Baja California (*P. m. coolidgei*) and southern California populations of *P. m. gambelii*; these *P. maniculatus* populations were elevated to species status as *P. gambelii* by Greenbaum et al. (2017). However, based on data from the mitochondrial ND3/ND4/ND4L genes, Greenbaum et al. (2017) found little difference in sequence divergence between *P. keeni* and *P. gambelii* (3.7%) and between *P. keeni* and *P. maniculatus* (3.8%) from eastern and northwestern California, Oregon, Washington, and Colorado (*P. m. austerus*, *artemisiae*, *rubidus*, *rufinus*, and *sonoriensis*). An analysis of single nucleotide polymorphisms (SNPs, Kingsley et al. 2017) provided no clarity, as it yielded a weakly supported association of *P. keeni* to a mixed variety of *P. maniculatus* subspecies from the western United States and Canada.

Peromyscus arcticus.—Phylogeographic analyses of deer mice from their northwestern-most range in the Yukon Territory, Canada (Wike 1998; Lucid and Cook 2007; Sawyer et al. 2017; Fig. 1) revealed the presence of a monophyletic clade distinct from *P. keeni* and *P. maniculatus*. Based on estimates of Kimura 2-parameter distances derived from cytochrome-*b* sequences, Lucid and Cook (2007) indicated that this clade was genetically equidistant from geographically proximate populations of *P. keeni* (4.32%) and *P. maniculatus* (4.56%). Wike (1998) and Lucid and Cook (2007) suggested that this third lineage represents an undescribed species for which the name *P. arcticus* (Wagner 1845) is available. Given the comparable sequence divergences among other species in this group, *P. arcticus* is recognized as a species in the *P. maniculatus* group.

Peromyscus sejugis.—*P. sejugis* is restricted to two small islands (Isla Santa Cruz and Isla San Diego) in the Gulf of California. Studies of mtDNA (Hogan et al. 1997; Hafner et al. 2001) and microsatellite (Chirhart et al. 2005) variation confirmed placement of *P. sejugis* in the *maniculatus* group but left the validity of this species open to question. Low mtDNA sequence divergence of *P. sejugis* relative to *P. maniculatus* from Baja California, as reported by Hogan et al. (1997), led Hafner et al. (2001) to speculate that additional sampling of *P. maniculatus* from Baja California would "demonstrate that *P. sejugis* should be included as a subspecies of *P. maniculatus*." Studies including *P. sejugis* and a geographic sampling of *P. maniculatus* from Baja California (Walker et al. 2006) and coastal western United States (Greenbaum et al. 2017) confirmed the close relationship between *P. sejugis* and deer mice from mainland Baja and southern California. Walker et al. (2006) considered a variety of factors in recommending retention of the specific status of *P. sejugis*. *Peromyscus sejugis* is larger than deer mice from mainland Baja California (Burt 1932), and both island populations of *P. sejugis* are fixed for a unique pericentric inversion of chromosome 13 (Smith et al. 2000). Despite a low level of mtDNA sequence divergence relative to deer mouse populations from mainland Baja California, *P. sejugis* populations have distinct mtDNA haplotypes (Walker et al. 2006; Greenbaum et al. 2017) and microsatellite alleles (Chirhart et al. 2005). Phylogenetic analyses of mtDNA and microsatellites consistently recover the two islands populations of *P. sejugis* as a single highly supported clade relative to deer-mouse populations from mainland Baja and southern California. Retention of the specific distinction of *P. sejugis* is further consistent with its restricted distribution and threatened status designated by the Government of Mexico (Alvarez-Castañeda 2001; Secretaría de Medio Amiente y Recursos Naturales SEMARNAT 2010).

Peromyscus gambelii.—Greenbaum et al. (2017) extended the work of Walker et al. (2006) in presenting a phylogeographic analysis of ND3/ND4/ND4L sequence variation for western deer mice from southern Baja California to Washington. These studies documented that deer mice from Baja California north to San Francisco Bay (corresponding to cytochrome-*b* clade 3, Fig. 1) comprise a phylogenetic lineage distinct from that including deer mice from eastern and northwestern California, Oregon, Washington, and Colorado. The mean nucleotide sequence divergence (based on p-distances) within these lineages was 0.8% and 0.9%, respectively, whereas that between these lineages was 3.7%. Considering that the San Francisco Bay and associated river drainages represent a physiographic boundary for numerous terrestrial genera and species and that these two lineages of deer mice occupy significantly different environmental spaces (Kalkvik et al. 2012), Greenbaum et al. (2017) referred the deer-mice from Baja and southern California to *P. gambelii*. As

such, *P. gambelii* includes all deer mouse populations previously recognized as *P. m. coolidgei* (Baja California) and those of *P. maniculatus gambelii* from south of the San Francisco Bay and west of the Sierra Nevada mountains (Fig. 1). The numerous insular subspecies of *P. maniculatus* (Hall 1981) along the Pacific coast of southern and Baja California are likely to prove to be representative of *P. gambelii.*

TAXONOMIC REVISIONS

The composite molecular data support the existence of paraphyly within *Peromyscus maniculatus* and call for a reevaluation of the specific integrity of the species. First noted by Lansman et al. (1983), patterns of variation of mtDNA restriction fragments identified five clonal assemblages representing the eastern states, northern Michigan, the central states, Texas-Mexico, and southern California. More recently, studies based on nucleotide sequences of the cytochrome-*b* gene (Dragoo et al. 2006; Gering et al. 2009; Kalkvik et al. 2012; Natarajan et al. 2015) identified six distinct clades distributed across the range of *P. maniculatus*. A study that included both cytochrome-*b* sequences and data from three nuclear genes recovered a corresponding pattern (Sawyer et al. 2017). These six clades (numbered according to Dragoo et al. 2006) and their general distributions (Fig. 1) are: 1) the Rocky Mountain states and including northern and central New Mexico, Washington, northern California and Michigan; 2) the Plains states; 3) the Pacific Coast including Southern and Baja California; 4) southern New Mexico and Mexico; 5) northeastern USA and eastern Canada; and 6) northeastern and north-central USA and south-central Canada.

Peromyscus labecula.—Several phylogeographic studies based on nucleotide sequences and single nucleotide polymorphisms or SNPs (Dragoo et al. 2006; Gering et al. 2009; Kalkvik et al. 2012; Natarajan et al. 2015; Kingsley et al. 2017) support the existence of an additional, geographically peripheral species within *P. maniculatus*. Each of these studies recovered a mtDNA lineage from southern New Mexico, southwestern Texas, and central Mexico to Oaxaca (clade 4, Fig. 1) as distinct and reciprocally monophyletic (posterior probabilities ranging between 92% and 100%) relative to the other mtDNA clades within *P. maniculatus* as well as to *P. keeni* and, when included in the phylogenetic analysis, to *P. melanotis*, *P. leucopus*, and *P. gossypinus*. The SNP analysis of Kingsley et al. (2017) recovered *P. m. blandus*, *P. m. fulvus*, and *P. m. labecula* as corresponding to mtDNA clade 4. Of these studies, only Gering et al. (2009) provided estimates of nucleotide divergence among the mtDNA clades within *P. maniculatus*. Nucleotide divergences (Kimura 2-parameter) estimated for cytochrome-*b* averaged 4.4% between the New Mexico/Mexico lineage (Clade 4) and the western/plains states lineages (clades 1 and 2). Clade 4 relative to the northeastern and north-central lineages (clades 5 and 6) averaged 3.5%. These values are consistent with estimates of ND3/ND4/ND4L gene divergence (Kimura 2-parameter) between *P. keeni* and western/central *P. maniculatus* (3.7%) and between *P. gambelii* and western/central *P. maniculatus* (3.8%, Greenbaum et al. 2017). Dragoo et al. (2006) suggested that the New Mexico/Mexico lineage (clade 4) might represent the formerly recognized species *P. blandus*. However, Kingsley et al. (2012) obtained a clade 4 cytochrome-*b* sequence for a specimen of *P. m. labecula* from Tepetitla, Tlaxcala, Mexico. The available data, both the phylogenetic and genetic species concepts (see review by Baker and Bradley 2006) and taxonomic priority, suggest that *P. labecula* (Elliot 1903, Fig. 1) represents a separate species that likely includes the subspecies *P. m. blandus*, *P. m. fulvus*, and *P. m. labecula*.

P. maniculatus and *P. sonoriensis*.—It is clear that the central issue for resolving the phylogeny and evolution of the *P. maniculatus* group requires addressing the residual paraphyly within *P. maniculatus*. Carleton (1989) recognized 67 subspecies of *P. maniculatus* with a composite distribution from the Atlantic to Pacific seaboards and from the Canadian taiga through south-central Mexico. Questions concerning the conspecificity of the various races of *P. maniculatus* date to Osgood (1909) and have historically centered on two distinctive morphological and ecological types, each of which comprises numerous subspecies (Blair 1950; Hooper 1968; Carleton 1989). Forest forms with long tails, large ears, and large hind feet range through the Appalachian Mountains, northeastern and boreal regions of Canada, and into the coastal forests of the western

United States. Grassland deer mice with short tails, small ears, and small feet generally occupy the prairies and grasslands of the continental interior and extend into the deserts of the western and southwestern United States. In regions where these forms come together, they generally maintain their morphological distinction and are assumed to not interbreed (Blair 1950; Hooper 1968). The genetic and morphological studies that resulted in the recognition of *P. keeni* (see Hogan et al. 1993 and references therein) confirmed the specific distinction of the northwestern-most forest form relative to *P. maniculatus,* and genome-wide SNP and mtDNA data partition the eastern and western forest forms of *P. maniculatus* into distinct and independently evolving clades (Kingsley et al. 2017). It does not appear, however, that the eastern forest forms are specifically distinct from eastern grassland forms. Nucleotide sequence divergence between the cytochrome-*b* lineages representing these forms was only 1.5% (Gering et al. 2009), and Kingsley et al. (2017) report successful reciprocal crossing (to the F2 generation) between corresponding short- and long-tailed forms of *P. maniculatus*. Carleton (1989) was apparently visionary when he wrote that "our tendency to pose the taxonomic dilemma of *maniculatus* as a species consisting of two contradistinctive sets of populations "the" long-tailed subspecies versus "the" short-tailed subspecies may mask the reticulate genealogical complexity of these organisms and hinder appreciation of the interrelationships and level of differentiation."

From their phylogeographic analysis, Dragoo et al. (2006) concluded that *P. maniculatus* (*sensu* Musser and Carleton 2005) is a complex of deeply divergent lineages and that the deepest genetic divergence is between the northeastern clades (5 and 6) and the central/western clades (1–4, Fig. 1). Each of the other sequence-based phylogeographic studies recovered the same strongly supported phylogenetic dichotomy between northeastern and central/western clades, and Gering et al. (2009) reported a mean nucleotide divergence of 3.9% between the two groups. Consistent with evolutionary independence of northeastern *P. maniculatus,* G- and C-banded karyotypes of deer mice representing five subspecies from the northeastern United States and eastern Canada (Myers Unice et al. 1998) indicated a unique (acrocentric or acrocentric with a heterochromatic short arm) condition of chromosome 10; all chromosomal homology analyses for central and western populations of *P. maniculatus* have reported the inverted and biarmed condition of chromosome 10 (Pathak et al. 1973; Murray and Kitchin 1976; Greenbaum et al. 1978a,b; Greenbaum and Reed 1984; Gunn and Greenbaum 1986; Gunn 1988; Hale and Greenbaum 1988a, b; Greenbaum et al. 1994; McAllister and Greenbaum 1997; Smith 1999).

Analysis of Northeastern versus Central/Western Clades of *P. maniculatus*

In an effort to contribute to the resolution of questions regarding the northeastern and central/western clades of *P. maniculatus,* mtDNA sequence variation (ND3/ND4/N4L) was analyzed from the populations reported by Myers Unice et al. (1998) and from a karyotypically characterized population from Kansas (McAllister and Greenbaum 1997). These sequences were compared to corresponding reference sequences from western/central populations of *P. maniculatus* and to reference sequences of *P. keeni*, *P. gambelii*, *P. sejugis*, *P. polionotus*, *P. melanotis*, and *P. leucopus.*

Materials and Methods

Specimens examined.—Specimens of northeastern *P. maniculatus* were live trapped from the following localities (Texas Cooperative Wildlife Collection (TCWC) accession and GenBank numbers in parentheses): CANADA: Ontario; 10 km N Moonbeam (*n*=3), 49.3432°N, 82.1541°W (56252–56254, MK122967); Quebec; 11.5 mi E of Havre-Saint-Pierre (*n*=3), 50.2418°N, 63.5986°W (59869, 59870, 59872, MK122965–MK122967). USA: Vermont;Washington Co., New Discovery Campground (*n*=10), 44.1987°N, 72.6973°W (56413,56415–56423, MK122965–MK122967, MK122971); Maine; Aroostook Co., Aroostook State Park (*n* =12), 46.6155°N, 68.0084°W (56385, 56398–56408, MK122966, MK122968–MK122970); Hancock Co., Mount Desert Island (*n* =1), 44.3924°N, 68.3021°W (56410, MK122970). Specimens representing central *P. maniculatus* (*n* =20) were live-trapped from 1 mi S, 2.3 mi W of Hayes, Ellis Co,

Kansas (56222, 56223, 56229–56232, 56234–56237, 56260, 56261, 56272–56279, 56328, and MK122972–MK122978). The capture and handling of animals followed the recommendations of Sikes et al. (2016).

Reference sequences were obtained from GenBank as follows: central/western *P. maniculatus*, Colorado, Gilpin Co. (U40250); California, Kern Co. (KC764393) and Humboldt Co. (KC764395); Oregon, Benton Co. (KC764399) and Harney Co. (KC764400); Washington, Gray's Harbor Co. (U40249) and Okanogan Co. (KC764408); *P. sejugis*, Isla San Diego, Baja California Sur (U40253); *P. gambelii*, Baja California, Mexico (DQ077697); *P. keeni*, Washington, Gray's Harbor Co. (U40062); *P. melanotis*, Durango, Mexico (U40247); and *P. polionotus*, South Carolina, Lexington Co. (U40254). A reference sequence for *P. leucopus*, Texas, Robertson Co. (U40252), was used as the outgroup in the phylogenetic analyses.

DNA isolation and sequencing.—The Sambrook et al. (1980) method was used to isolate total genomic DNA from liver and spleen tissues previously frozen at -80° C. A 1,439 base pair (bp) fragment containing the mitochondrial genes ND3/ND4L/ND4 as well as $tRNA^{Arg}$ and the 3' end of $tRNA^{Gly}$ were PCR (polymerase chain reaction) amplified following the techniques described in Arevalo et al. (1994). PCR primers included PI', Marg, ND4L, and Nap2, and amplifications were performed in a Perkin Elmer/Cetus DNA Thermal Cycler (Applied Biosystems, Foster City, California). Reaction conditions were as follows: 1 µL DNA (approximately 100 ng), 12.3 µL H_2O, 2.5 µL of 10X PCR Buffer II (PE Applied Biosystems), 2.5 µL of 25 mM $MgCl_2$, 0.5 µL BSA, 4 µL of 8 mM dNTPs (Amersham Pharmacia Biotech, Piscataway, New Jersey), 1.0 µL of forward and reverse primers, and 0.2 µL Takara *Taq* (TaKaRa, Japan). Conditions for PCR amplification were as follows: initial denaturation at 95° C for five min, followed by 35 cycles of 1 min each at 95° C (denaturation), 50° C (annealing), and 72° C (extension), and concluded with another extension cycle of 10 min at 72°C. Prior to sequencing, PCR amplification products were purified using Exonuclease I in combination with shrimp alkaline phosphatase (ExoSAP-IT, Affymetrix Inc., Santa Clara, California), and excess dye was removed using DyeEx spin columns (Qiagen, Germantown, Maryland).

Sequencing reactions were performed with a Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) in a Perkin Elmer/Cetus DNA Thermal Cycler, following the protocol recommended by the supplier. Prior to sequencing, DyeEx 2.0 spin columns (Qiagen Valencia, California) were used to remove excess dye. Sequencing was performed in an Applied Biosystems 377 automated sequencer. All PCR fragments were sequenced in both directions, and sequence contigs were produced using Sequencher 4.1.1 (Gene Codes, Ann Arbor, Michigan). Sequences were aligned by eye.

Phylogenetic analyses.—PAUP* version 4.0b10 (Swofford 2002) was used to compute p-distances for estimation of nucleotide divergence among the major groups identified by the phylogenetic analyses. Maximum parsimony (MP) was conducted in PAUP* and Bayesian inference (BI) was performed in Mr. Bayes version 3.2.6 (Ronquist et al. 2012). Using the Akaike Information Criterion (Akaike 1974), both the jModel Test 2.1.10 (Darriba et al. 2012) and the model test in PAUP* identified K81uf + I + G as being the most suitable substitution model for the BI analysis.

Maximum parsimony employed the branch and bound search option with 5,000 bootstrap replications. All characters were unordered with equal weights, and there were 143 parsimony-informative characters. Bayesian inference involved two separate runs, each consisting of four chains (1 cold and 3 hot), 10 million generations sampled every 1,000 generations with a 25% burn-in. The standard split frequency was 0.00, and convergence to a stationarity distribution was observed based on the analysis in TRACER version 1.7.1 (Rambaut et al. 2018). Posterior probabilities of branch support were obtained from a 50% majority rule tree.

Results

Estimates of sequence divergences (uncorrected p-distances) for the analysis of ND3/ND4/N4L variation are presented in Table 2. Sequence divergence among northeastern samples was 0.5% and among the individuals from the central (Kansas) population was 0.9%. Divergence between the central and western reference sequences was 1.7%. These values are consistent with those reported (Greenbaum et al. 2017) for

Table 2. Mean sequence divergences (uncorrected p-distances) between the ND3/ND4/ND4L mtDNA haplotypes of the *Peromyscus* reference sequences (*P. gambelii*, *P. keeni*, *P. polionotus*, *P. melanotis*, and *P. leucopus*) and those of the eastern, central, and western populations sampled. Northeast refers to deer mice from Canada (Quebec and Ontario), Maine, and Vermont. Central refers to deer mice from Kansas, and western refers to deer mice from Colorado, California, Oregon, and Washington. Specific localities, collections, and GenBank numbers are listed in the Materials and Methods.

	sejugis	*gambelii*	*keeni*	western	central	northeast	*polionotus*	*melanotis*
gambelii	0.017							
keeni	0.036	0.038						
western	0.041	0.040	0.043	0.010				
central	0.043	0.042	0.046	0.017	0.009			
northeast	0.044	0.044	0.048	0.037	0.036	0.005		
polionotus	0.046	0.046	0.054	0.040	0.041	0.043		
melanotis	0.070	0.069	0.079	0.075	0.076	0.075	0.069	
leucopus	0.134	0.136	0.138	0.140	0.135	0.130	0.127	0.134

intraspecific variation of ND3/ND4/ND4L for western populations currently recognized as *P. maniculatus* (0.9%) and *P. gambelii* (0.8%). Mean sequence divergence between the northeast and central/western populations was 3.7%; this is consistent with that between *P. keeni* and *P. gambelii* (3.8%) as well as between *P. keeni* and western populations currently recognized as *P. maniculatus* (4.3%). Mean divergence between the central/western populations and *P. keeni*, *P. gambelii*, *P. sejugis*, and *P. polionotus* was 4.2% and between the northeastern population and these species was 4.5%. The western/central and northeastern populations were essentially equidistant from *P. melanotis* (7.6% and 7.5%, respectively).

Maximum parsimony (MP) and Bayesian Inference (BI) analyses of the data (Fig. 2) recovered several well-supported monophyletic groups including: 1) a clade containing *P. sejugis*, *P. gambelii*, and *P. keeni* (consistent with Greenbaum et al. 2017); 2) a western clade containing haplotypes from Colorado, Oregon, northern California, and Washington; 3) a central clade representing haplotypes from Kansas; 4) monophyly of a clade containing both the western and central lineages; and 5) a northeastern clade representing haplotypes from eastern Canada and the northeastern United States. These groups are similar to those previously identified by other phylogenetic studies (Dragoo et al. 2006; Gering et al. 2009; Kalkvik et al. 2012; Natarajan et al. 2015; Sawyer et al. 2017). Although the MP bootstrap and posterior probability are somewhat lower, these groups appear monophyletic relative to *P. polionotus* and *P. melanotis*. With exception of monophyly of a western/central clade, the phylogenetic relationships among these lineages were not well-resolved, resulting in a trichotomy. The analyses did provide strong support for the basal position of *P. melanotis* as well as placement of *P. polionotus* as sister to the other members of the *P. maniculatus* group.

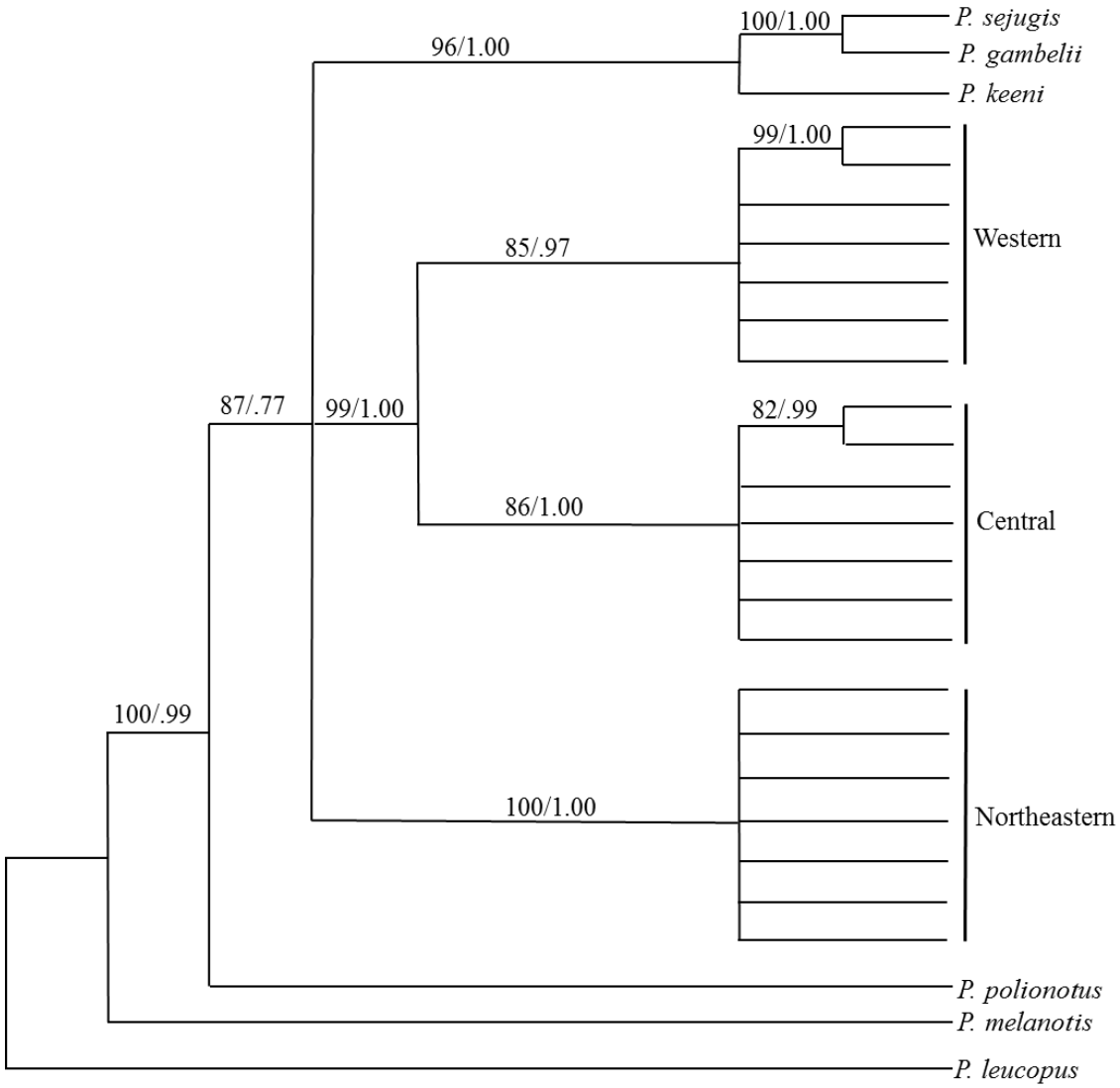

Figure 2. Maximum parsimony tree derived from sequence variation (ND3/ND4/ND4L) for the northeastern, central and western samples of *Peromyscus maniculatus* and reference sequences for *P. sejugis*, *P. gambelii*, *P. keeni*, *P. polionotus*, *P. melanotis*, and *P. leucopus*. Numbers associated with the branches are maximum parsimony bootstrap values and Bayesian posterior probabilities. Locality and GenBank references are given in the Materials and Methods.

DISCUSSION

Dragoo et al. (2006) noted that the type locality of *P. maniculatus* is from the Moravian Settlements in Labrador (Wagner 1845) and treated this taxon as restricted to eastern Canada and southward through the eastern United States. They correspondingly suggested that the central/western mtDNA clades of *P. maniculatus* might represent the previously described taxon *P. sonoriensis* (Le Conte 1853). Given the magnitude of sequence divergence (Table 2) and the consistent phylogenetic and apparent karytoypic dichotomy between the eastern and central/western mtDNA clades, the results of this study support the suggestions of Dragoo et al. (2006) and the recognition of *P. sonoriensis* as specifically distinct from *P. maniculatus* (Figs. 1 and 2). With

the other recommendations herein, the *P. maniculatus* species group is expanded to nine species (Table 3).

Table 3. Composition of the *P. maniculatus* species group as recognized by Carleton (1989) relative to changes in the number of species (recognized herein) resulting from the inclusion of chromosomal and molecular data. Carleton considered the conspecificity of all populations of *P. maniculatus* suspect and only tentatively included *P. slevini*. The indented species were formerly included as part of *P. maniculatus*.

Carleton 1989	This paper
P. slevini	
P. melanotis	*P. melanotis*
P. polionotus	*P. polionotus*
P. sejugis	*P. sejugis*
P. maniculatus	*P. maniculatus*
P. oreas	*P. keeni*
	P. articus
	P. gambelii
	P. labecula
	P. sonoriensis

Although the data supporting the specific recognition of *P. sonoriensis* and *P. maniculatus* include a broad sampling of deer mice from across the United States, large geographic expanses and many subspecies have not been correspondingly sampled. In addition to refining the distributional limits of *P. sonoriensis* and *P. maniculatus*, the specific affinity of many subspecies traditionally assigned to *P. maniculatus* (Hall 1981) will need to be investigated and evaluated. As with the mtDNA lineages within *P. maniculatus*, the genetic distances of the central and western lineages of *P. sonoriensis* (1.7% Gering et al. 2009; 1.2% Table 2) do not warrant taxonomic recognition. Based on the inferred geographic distributions (Fig. 1), it is suggested that the following subspecies formerly assigned to *P. maniculatus* (Hall 1981) be referred to *P. sonoriensis: alpinus*, *artemisiase*, *austerus*, *bairdii*, *borealis*, *gunnisoni*, *hollisteri*, *inclarus*, *luteus*, *nebrascensis*,*ozarkiarum, pallescens*, *rubidus*, *rufinus*, *saxamans*, *serratus*, and *sonoriensis.* Correspondingly, *P. maniculatus* would retain the subspecies: *abietorium*, *anticostiensis*, *argentatus*, *bairdii*, *eremus*, *gracilis*, *maniculatus*, *nubiterrae*, and *plumbeus.*

EVOLUTIONARY HISTORY

Previous studies concluded that the *P. maniculatus* species group evolved by peripheral isolation from a central stock (*maniculatus*-like ancestor) in response to effects of Pleistocene glaciation (Blair 1950; Bowers et al. 1973; Greenbaum et al. 1978; Carleton 1989). Given that the central and western range of deer mice corresponds to *P. sonoriensis*, it is most likely that *P. sonoriensis* is the modern-day remnant of the *P. maniculatus*-group central stock. Based on phylogenetic analyses of chromosomal banding data (Greenbaum et al. 1978; Robbins and Baker 1981; Stangl and Baker 1984), *P. melanotis* and *P. polionotus* are the most divergent lineages of the *P. maniculatus* species group, with the former being the most basal lineage. The basal position of *P. melanotis* has been widely supported by analyses of mtDNA sequences (Hogan et al. 1997; Walker et al. 2006; Gering et al. 2009; Kalkvik et al. 2012; Natarajan et al. 2015; Greenbaum et al. 2017; this paper; Fig. 2) and microsatellites (Chirhart et al. 2005). Overall the genetic data and the geographic distribution of *P. melanotis* (Fig. 1) support the hypothesis that this species was the earliest isolate off the *P. maniculatus*-group central stock.

The molecular data for *P. polionotus* are contradictory and fail to resolve its evolutionary derivation. Phylogenetic analysis of the ND3/ND4/ND4L sequences (Fig. 2) support the cytosystematic hypothesis (Greenbaum et al. 1978; Robbins and Baker 1981; Stangl and Baker 1984) in placing *P. polionotus* as the second most divergent lineage in the *P. maniculatus*-group. This hypothesis infers that *P. polionotus* diverged from the geographic central stock prior to the divergence of *P. sonoriensis* and *P. maniculatus*. Phylogeographic analysis of cytochrome-*b* sequences (Kalkvik et al. 2012; Natarajan et al. 2015) associated *P. polionotus* with samples referable to *P. sonoriensis*, and sequences of COIII-ND3 (Kingsley et al. 2017)

clustered *P. polionotus* to northeastern subspecies (*gracilis* and *nubiterrae*) of *P. maniculatus*. None of the above phylogenetic associations were strongly supported and each corresponds to a reasonable evolutionary hypothesis. It is noteworthy, however, that *P. polionotus*, *P. melanotis* (Greenbaum et al. 1978), and the northeastern populations of *P. maniculatus* (Myers-Unice et al. 1998) share the plesiomorphic condition of chromosome 10.

Although there can be little question that *P. sejugis* shares common ancestry with *P. gambelii*, the phylogeographic association of *P. keeni* and *P. gambelii* is less readily explained. Chirhart et al. (2005) postulated two alternative geographic scenarios relevant to the evolutionary history of *P. keeni* and *P. gambelii*. An "ancestral continuity" hypothesis proposes that these species diverged (north and south) from a common ancestor that occupied a Pacific coastal range after having been isolated from the geographic central stock. Alternatively, *P. gambelii* and *P. keeni* may have originated as independent peripheral isolates. In the latter case, the genetic similarities (Table 2) and apparent sister-group relationship between *P. gambelii* and *P. keeni* (Fig. 2) would be an artifact of coincidental founder effects and genetic drift. Despite the highly supported relationship between *P. keeni* and *P. gambelii*, the ancestral continuity hypothesis is unsupported by geography and the distributions of these species. Additionally, phylogeographic studies of *P. keeni* (Zheng et al. 2003; Lucid and Cook 2004; Sawyer et al. 2017) consistently support its isolation in Pleistocene refugia in coastal British Columbia and/or southeastern Alaska, and Greenbaum et al. (2017) cite physiographic and zoogeographic data that support the hypothesis that the San Francisco Bay and associated river drainages were the northern boundary of a southern California/Baja California refugium. As such, the results of this study support the conclusion of Greenbaum et al. (2017) that independent peripheral isolation is the more likely scenario for the evolution of *P. keeni* and *P. gambelii*. Although Sawyer et al. (2017) recovered sequences referable to *P. arcticus* as sister to those of *P. keeni*, too little else is known about the former to support its derivation as an isolate of the latter as opposed to its being an independent isolate of the geographic central stock.

All sequence analyses that included populations referable to *P. labecula* identified it as a distinct clade, but inferences of its phylogenetic association were inconsistent. Despite having included sequences from many of the same individuals, analyses of cytochrome-*b* variously but weakly linked *P. labecula* to a *keeni*, *sonoriensis*, *gambelii* clade (Dragoo et al. 2006); the northeast *maniculatus* clades (Gering et al. 2009); an unresolved trichotomy including *P. maniculatus*, *P. polionotus*, and *P. sonoriensis*/*gambelii* (Kalkvik et al. 2012); and as sister to *P. articus* (Natarajan et al. (2015). From sequences of COIII-ND3, Kinglsey et al. (2017) recovered *P. labecula* as basal to clades including *P. keeni*/*gambelii*, *P. polionotus*/*maniculatus*, and *P. sonoriensis*.

Most taxonomically significant, all sequence-based studies and the relevant chromosomal data support the specific and phylogenetic distinction of *P. sonoriensis* and *P. maniculatus* (Fig. 1). Absent inclusion of the complicating taxa *P. arcticus* and *P. labecula*, the ND3/ND4/ND4L analysis recovered *P. sonoriensis* and *P. maniculatus* as reciprocally monophyletic clades (Fig. 2). The level of sequence divergence (Gering et al. 2009, Table 2) and the distribution of *P. sonoriensis* and *P. maniculatus* support the hypothesis that during the Pleistocene glacial maximum the *P. maniculatus* group central stock was divided east and west by the Mississippi River. The modern distribution of these species would then have resulted from northward expansion following glacial recession. It is apparent that the inconsistency of inferences of the phylogeographic and phylogenetic history of the peripheral species *P. polionotus*, *P. arcticus*, and *P. labecula* reflects their recent and relatively contemporaneous divergence from the geographic central stock before or after divergence of *P. sonoriensis* and *P. maniculatus*. In particular, the phylogenetic inconsistency is apparently the result of the relative rate of the evolution of the mtDNA genes and the short internode branch lengths obtained. Detailed resolution of the evolutionary history of the *P. maniculatus* species group awaits greater sampling and analyses of characters with a more appropriate rate of evolution.

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LITERATURE CITED

Akaike, H. 1974. A new look at the statistical model identification. IEEE. Transactions on Automatic Control 19:716–723.

Allard, M. W., and I. F. Greenbaum. 1988. Morphological variation and taxonomy of chromosomally differentiated *Peromyscus* from the Pacific Northwest. Canadian Journal of Zoology 66:2734–2739.

Allard, M. W., S. J. Gunn, and I. F. Greenbaum. 1987. Mensural discrimination of chromosomally characterized *Peromyscus oreas* and *P. maniculatus*. Journal of Mammalogy 68:402–406.

Alvarez-Castañeda, S. T. 2001. Peromyscus sejugis. Mammalian Species 658:1–2.

Álvarez-Castañeda, S. T., and P. C. Cortés-Calva. 2002. Peromyscus slevini. Mammalian Species 705:1–2.

Arevalo, E., S. K. Davis, and J. W. Sites, Jr. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. Systematic Biology 43:387–418.

Baker, R. J., and R. D. Bradley. 2006. Speciation in mammals and the genetic species concept. Journal of Mammalogy 87:643–662.

Bangs, O. 1897. A new white-footed mouse from British Columbia. The American Naturalist 31:74–75.

Blair, W. F. 1950. Ecological factors in speciation of *Peromyscus*. Evolution 4:253–275.

Bowers, J. H. 1974. Genetic compatibility of *Peromyscus maniculatus* and *Peromyscus melanotis*, as indicated by breeding studies and morphometrics. Journal of Mammalogy 55:720–737.

Bowers, J. H., R. J. Baker, and M. H. Smith. 1973. Chromosomal, electrophoretic and breeding studies of selected populations of deer mice (*Peromyscus maniculatus*) and black-eared mice (*P. melanotis*). Evolution 27:378–386.

Bradley, R. D., and R. J. Baker. 2001. A test of the genetic species concept: cytochrome-*b* sequences and mammals. Journal of Mammalogy 82:960–973.

Bradley, R. D., N. D. Durish, D. S. Rogers, and J. R. Miller. 2007. Toward a molecular phylogeny for *Peromyscus*: evidence from mitochondria; cytochrome-*b* sequences. Journal of Mammalogy 88:1146–1159.

Bradley, R. D., C. W. Edwards, D. S. Carroll, and C. W. Kilpartick. 2004. Phylogenetic relationships of neotomine-peromyscine rodents: based on DNA sequences from the mitochondrial cytochrome-*b* gene. Journal of Mammalogy 85:389–395.

Burt, W. H. 1932. Description of heretofore unknown mammals from islands in the Gulf of California, Mexico. Transactions of the San Diego Society of Natural History 7:161–182.

Carleton, M. D. 1989. Systematics and evolution. Pp. 7–141 in Advances in the study of *Peromyscus* (Rodentia) (G. L. Kirkland Jr., and J. N. Layne, eds.). Texas Tech University Press, Lubbock.

Carleton, M. D., and T. E. Lawlor. 2005. *Peromyscus* from Santa Catalina Island, Sea of Cortez, Mexico: taxonomic identities and biogeographic implications. Journal of Mammalogy 86:814–825.

Chirhart, S. E., R. Arianpour, R. L. Honeycutt, and I. F. Greenbaum. 2001. Mitochondrial DNA sequence variation and the specific identification of deer mice (*Peromyscus*) from Triangle Island, British Columbia. Canadian Journal of Zoology 79:2257–2260.

Chirhart, S. E., R. L. Honeycutt, and I. F. Greenbaum. 2005. Microsatellite variation and evolution in the *Peromyscus maniculatus* species group. Molecular Phylogenetics and Evolution 34:408–415.

Cornejo-Latorre, C., P. Cortés-Calva, and S. T. Álvarez-Castañeda. 2017. The evolutionary history of the subgenus *Haplomylomys* (Cricetidae: *Peromyscus*). Journal of Mammalogy 98:1627–1640.

Cowan, I. M., and C. J. Guiget. 1965. The mammals of British Columbia. Handbook No. 11. British Columbia Provincial Museum, Victoria, British Columbia, Canada.

Darriba. D., G. L. Taboada. R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9:772

Degner, J. F., I. J. Stout, J. D. Roth, and C. L. Parkinson. 2007. Population genetics and conservation of the threatened southeastern beach mouse (*Peromyscus polionotus niveiventris*): subspecies and evolutionary units. Conservation Genetics 8:1441–1452.

Domingues, V. S., Y. Poh, B. K. Peterson, P. S. Pennings, J. D. Jensen, and H. E. Hoekstra. 2012. Evidence of adaptation

from ancestral variation in young populations of beach mice. Evolution 66:3209–3223.

Dragoo, J. W., J. A. Lackey, K. E. Moore, E. P. Lessa, J. A. Cook, and T. L. Yates. 2006. Phylogeography of the deer mouse (*Peromyscus maniculatus*) provides a predictive framework for research on hantaviruses. Journal of General Virology 87:1997–2003.

Durish, N. D., K. E. Halcomb, C. W. Kilpatrick, and R. D. Bradley. 2004. Molecular systematics of the *Peromyscus truei* species group. Journal of Mammalogy 85:1160–1169.

Elliot, D. G. 1903. A list of a collection of Mexican mammals with descriptions of some apparently new forms. Field Columbian Museum Publication 71, Zoology Series 3:141–149.

Engle, S. R., K. M. Hogan, J. F. Taylor, and S. K. Davis. 1998. Molecular systematics and paleobiogeography of South American sigmodontine rodents. Molecular Biology and Evolution 15:35–49.

Galtier, N., B. Nabholz, S. Glémin, and G. D. D. Hurst. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. Molecular Ecology 18:4541–4550.

Gering, E. J., J. C. Opazo, and J. F. Storz. 2009. Molecular evolution of cytochrome *b* in high- and low-altitude deer mice (genus *Peromyscus*). Heredity 102:226–235.

Greenbaum, I. F., R. J. Baker, and J. H. Bowers. 1978a. Chromosomal homology and divergence between sibling species of deer mice: *Peromyscus maniculatus* and *Peromyscus melanotis* (Rodentia, Cricetidae). Evolution. 32:334–341.

Greenbaum, I. F., R. J. Baker, and P. R. Ramsey. 1978b. Chromosomal evolution and the mode of speciation in three species of *Peromyscus*. Evolution 32:646–654.

Greenbaum, I. F., S. E. Chirhart, M. L. Walker, and R. L. Honeycutt. 2017. Molecular phylogenetics of western deer mice (*Peromyscus*): taxonomic and biogeographic implications. The Southwestern Naturalist 62:126–134.

Greenbaum, I. F., et al. 1994. Cytogenetic nomenclature of deer mice, *Peromyscus* (Rodentia): revision and review of the standardized karyotype. Cytogenetics and Cell Genetics 6 :181–195.

Greenbaum, I. F., and M. J. Reed. 1984. Evidence for heterosynaptic pairing of the inverted segment in pericentric inversion heterozygotes of the deer mouse (*Peromyscus maniculatus*). Cytogenetics and Cell Genetics 38:106–111.

Gunn, S. J. 1988. Chromosomal variation and differentiation among insular populations of *Peromyscus* from the Pacific Northwest. Canadian Journal of Zoology 66:2726–2733.

Gunn, S. J., and I. F. Greenbaum. 1986. Systematic implications of karytoypic and morphological variation in mainland *Peromyscus* from the Pacific Northwest. Journal of Mammalogy 67:294–304.

Hafner, D. J., B. R. Riddle, and S. T. Álvarez-Castañeda. 2001. Evolutionary relationships of white-footed mice (*Peromyscus*) on islands in the Sea of Cortéz, Mexico. Journal of Mammalogy 82:775–790.

Hale, D.W., and I. F. Greenbaum. 1988a. Chromosomal pairing in deer mice heterozygous for the presence of heterochromatic short arms. Genome 30:44–47.

Hale, D. W., and I. F. Greenbaum. 1988b. Synapsis of a chromosomal pair heterozygous for a pericentric inversion and the presence of a heterochromatic short arm. Cytogenetics and Cell Genetics 48:55–57.

Hall, E. R. 1981. The mammals of North America, second edition. John Wiley and Sons, New York, New York.

Hogan, K. M., S. K. Davis, and I. F. Greenbaum. 1997. Mitochondrial-DNA analysis of the systematic relationships within the *Peromyscus maniculatus* species group. Journal of Mammalogy 78:733–743.

Hogan, K. M., M. C. Hedin, H. S. Koh, S. K. Davis, and I. F. Greenbaum. 1993. Systematic and taxonomic implications of karytoypic, electrophoretic, and mitochondrial-DNA variation in *Peromyscus* from the Pacific Northwest. Journal of Mammalogy 74:819–831.

Hooper, E. T. 1968. Classification. Pp. 27-74 in Biology of *Peromyscus* (Rodentia) (J. A. King, ed.). Special Publication of the American Society of Mammologists 2:1–593.

Kalkvik, H. M., I. J. Stout, T. J. Doonan, and C. L. Parkinson. 2012. Investigating niche and lineage diversification in widely distributed taxa: phylogeography and ecology niche modeling of the *Peromyscus maniculatus* species group. Ecography 35:54–64.

Kalkvik, H. M., I. J. Stout, E. A. Hoffman, and C. L. Parkinson. 2018. Colonization and divergence: phylogeography genetics of the Atlantic coast beach mouse. Systematics and Biodiversity DOI: 10.1080/14772000.2018.1486339.

Kingsley, E. P., K. M. Kozak, S. P. Pfeifer, D. Yang, and H. E. Hoekstra. 2017. The ultimate and proximate mechanisms driving the evolution of long tails in forest deer mice. Evolution 71(2):261–273.

Lansman, R. A., J. C. Avise, C. F. Aquadro, J. F. Shapiro, and S. W. Daniel. 1983. Extensive genetic variation in mitochondrial DNA's among geographic populations of the deer mouse, *Peromyscucs maniculatus*. Evolution 37:1–16.

Le Conte, J., C. Girard, W. H. Harvey, and J. W. Bailey. 1853. Description of three new species of American Arcicolae, with remarks upon some other American rodents. Proceedings of the Academy of Natural Sciences of Philadelphia 6:404-432.

Lucid, M. K., and J. A. Cook. 2004. Phylogeography of Keen's mouse (*Peromyscus keeni*) in a naturally fragmented landscape. Journal of Mammalogy 85:1149–1159.

Lucid, M. K., and J. A. Cook. 2007. Cytochrome-*b* haplotypes suggest an undescribed *Peromyscus* species from the Yukon. Canadian Journal of Zoology 85:916–919.

Maillaird, J. 1924. A new mouse (*Peromyscus slevini*) from the Gulf of California. Proceedings of the California Academy of Sciences 12:1219–1222.

Martínez-Coronel, M., J. Ramírez-Pulido, and T. Álvarez. 1991. Variación intrapoblacional de *Peromyscus melanotis* (Rodentia: Muridae) en el Eje Volcánico Transverso, México. Acta Zoológica Mexicana, nueva serie 47:1–51.

McAllister B. F., and I. F. Greenbaum. 1997. How common are common fragile sites: variation of aphidicolin-induced chromosomal fragile sites in a population of the deer mouse (*Peromyscus maniculatus*). Human Genetics 100:182–188.

Miller J. R., and M. D. Engstrom. 2008. The relationships of major lineages within peromyscine rodents: a molecular phylogenetic hypothesis and systematic reappraisal. Journal of Mammalogy 89:1279–1295.

Murray, J. D., and R. M. Kitchin. 1976. Chromosomal variation and heterochromatin polymorphisms in *Peromyscus maniculatus*. Experientia 32:307–309.

Musser, G. G., and M. D. Carleton. 2005. Superfamily Muroidea. Pp. 894–1531 in Mammal species of the world: a taxonomic and geographic reference, 3rd ed. (D. E. Wilson and D. M. Reeder, eds.). Johns Hopkins University Press, Baltimore, Maryland.

Myers Unice S. M., D. W. Hale, and I. F. Greenbaum. 1998. Karyotypic variation in populations of deer mice (*Peromyscus maniculatus*) from eastern Canada and the northeastern United States. Canadian Journal of Zoology 76:584–588.

Natarajan, C., et al. 2015. Intraspecific polymorphism, interspecific divergence and the origins of function-altering mutations in deer mouse hemoglobin. Molecular Biology and Evolution 32:978–997.

Osgood, W. H. 1909. Revision of the mice of the American genus *Peromyscus*. North American Fauna No. 28:1–285.

Pathak, S., T. C. Hsu, and F. E. Arrighi. 1973. Chromosomes of *Peromyscus* (Rodentia, Cricetidae) IV. The role of heterochromatin in karyotypic evolution. Cytogenetics and Cell Genetics 12:315–326.

Pengilly, D., G. H. Jarrell, and S. D. MacDonald. 1983. Banded karyotypes of *Peromyscus sitkensis* from Baranof Island, Alaska. Journal of Mammalogy 64:682–685.

Platt II, R. N., B. R. Amman, M. S. Keith, C. W. Thompson, and R. D. Bradley. 2015. What is *Peromyscus*? Evidence from nuclear and mitochondrial DNA sequences suggests the need for a new classification. Journal of Mammalogy 96:708–719.

Rambaut A., A. J. Drummond, D. Xie, G. Baele, and M. A. Suchard. 2018. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67:901–904.

Riddle, B. R., D. J. Hafner, and L. F. Alexander. 2000. Phylogeography and systematics of the *Peromyscus eremicus* species group and the historical biogeography of North American warm regional deserts. Molecular Phylogenetics and Evolution 17:145-160.

Robbins, L. W., and R. J. Baker. 1981. An assessment of the nature of chromosomal rearrangements in 18 species of *Peromyscus* (Rodentia: Cricetidae). Cytogenetics and Cell Genetics 31:194–202.

Rogers, D. S., and M. D. Engstrom. 1992. Evolutionary implications of allozymic variation in tropical *Peromyscus* of the *mexicanus* group. Journal of Mammalogy 73:55–69.

Ronquist, F., et al. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61:539–542.

Sambrook, D. L., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual. Second edition. Cold Spring Harbor Press, Cold Spring Harbor, New York.

Sawyer, Y. E., M. J. Flamme, T. S. Jung, S. O. MacDonald, and J. A. Cook. 2017. Diversification of deermice (Rodentia: genus *Peromyscus*) at their north-western range limit: genetic consequences of refugial and island isolation. Journal of Biogeography 44:1572–1585.

Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT). 2010. Norma Oficial Mexicana NOM-059-ECOL-2010, Protección de especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. Diario Oficial de la Federación, México, December 30th:1–78. http://www.profepa.gob.mx/innovaportal/file/435/1/NOM_059_SEMARNAT_2010.pdf.

Sheppe, W., Jr. 1961. Systematics and ecological relations of *Peromyscus oreas* and *P. maniculatus*. Proceedings of the American Philosophical Society 105:421–446.

Sikes, R. S., and the Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy 97:663−688.

Smith, L. R. 1999. Phenotypic and genetic heterogeneity between sympatric deer mice from Washington State. Ph.D. dissertation, Texas A&M University, College Station.

Smith, L. R., D. W. Hale, and I. F. Greenbaum. 2000. Systematic implications of chromosomal data from two insular species of *Peromyscus* from the Gulf of California. Journal of Heredity 91:162–165.

Stangl, F. B., and R. J. Baker. 1984. Evolutionary relationships in *Peromyscus*: congruence in chromosomal, genic and classical data sets. Journal of Mammalogy 65:643–654.

Sullivan, J., K. E. Holsinger, and C. Simon. 1995. Among-site rate variation and phylogenetic analysis of 12S rRNA in

sigmodontine rodents. Molecular Biology and Evolution 12:988-1001.

Sullivan, R. M., S. W. Calhoun, and I. F. Greenbaum. 1990. Geographic variation in genital morphology among insular and mainland populations of *Peromyscus maniculatus* and *Peromyscus oreas*. Journal of Mammalogy 71:48–58.

Swofford, D. L. 2002. PAUP*: phylogenetic analysis using parsimony and other methods. Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Taylor, Z. S., and M. G. Hoffman. 2012. Microsatellite genetic structure and cytonuclear discordance in naturally fragmented populations of deer mice (*Peromyscus maniculatus*). Journal of Heredity 103:71–79.

Tiemann-Boege, I., C. W. Kilpatrick, D. J. Schmidly, and R. D. Bradley. 2000. Molecular phylogenetics of the *Peromyscus boylii* species group (Rodentia: Muridae) based on mitochondrial cytochrome *b* sequences. Molecular Phylogenetics and Evolution 16:366–378.

Thomas, B. 1973. Evolutionary implications of karytoypic variation in some insular *Peromyscus* from British Columbia, Canada. Cytologia 38:485–495.

Van Zant, J. L., and M. C. Wooten. 2007. Old mice, young islands and competing biogeographical hypotheses. Molecular Ecology 16:5070–5083.

Wagner, A. 1845. Diagnosen einiger neuen Arten von Nagren und Handflüglern. Archiv für Naturgeschichte 11:148.

Walker, M. L., S. E. Chirhart, A. F. Moore, R. L. Honeycutt, and I. F. Greenbaum. 2006. Genealogical concordance and the specific status of *Peromyscus sejugis.* Journal of Heredity 97:340–345.

Wike, M. J. 1998. Mitochondrial-DNA variation among populations of *Peromyscus* from Yukon, Canada and southeastern Alaska. M.S. thesis. Texas A&M University, College Station.

Yang, D.-S., and G. J. Kenagy. 2009. Nuclear and mitochondrial DNA reveal contrasting evolutionary processes in populations of deer mice (*Peromyscus maniculatus*). Molecular Ecology 18:5115–5125.

Yang, D.-S., and G. J. Kenagy. 2011. Population delimitation across contrasting evolutionary clines in deer mice (*Peromyscus maniculatus*). Ecology and Evolution 1:26–36.

Zheng, X., B. S. Arbogast, and G. J. Kenagy. 2003. Historical demography and genetic structure of sister species: deermice (*Peromyscus*) in the North American temperate rain forest. Molecular Ecology 12:711–724.

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Mitochondrial Cytochrome-*b* Variation Within *Peromyscus truei* Reveals Two Strongly Divergent Haplogroups

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Abstract

DNA sequences from the mitochondrial cytochrome-*b* gene representing 529 *Peromyscus truei* (pinyon mouse) from throughout the entire species range and including nine of the 11 recognized subspecies were evaluated. In addition, intron 7 of the fibrinogen beta chain (*Fgb*) gene was sequenced from 48 individuals. Phylogenetic analyses of mtDNA sequences document a strongly supported east–west split that is concordant with the distribution of *Pinus edulis* (common piñon) and *P. monophylla* (single-leaf piñon), with a relatively broad area of overlap in Utah in which both eastern and western mtDNA haplogroups are syntopic. Genetic divergence within these two genetic groups was minimal. However, within the western group, *P. truei* from the Baja California peninsula formed a well-support lineage relative to samples from Arizona, California, Nevada, Oregon, and Utah. Variation in *Fgb* essentially was non-existent; pinyon mice from California possessed *Fgb* sequences identical to those present in mice from the Texas Panhandle. Discriminant function analysis of 26 craniodental measurements between pinyon mice from California (western haplogroup) and mice from Arizona, Colorado, Oklahoma, and Texas (eastern haplogroup) clearly separate the two groups. However, *P. truei* from localities in central Utah and from which both genetic groups were syntopic were morphologically intermediate. It is hypothesized that central Utah represents an area of secondary contact and hybridization as evidenced by a relatively broad area of overlap between the eastern and western mtDNA haplogroups together with morphological intermediacy in craniodental characters. Aside from the populations from the Baja Peninsula, within-haplogroup genetic divergence is minor and therefore, the subspecific status of populations within the two haplogroups should be evaluated.

Key words: cytochrome-*b* gene, *Fgb* intron 7, intraspecific variation, morphology, *Peromyscus truei,* pinyon mouse, pinyon pine

Introduction

Peromyscus truei (pinyon mouse, Shufeldt, 1885) is a broadly distributed species, occurring in the western United States and Baja California, Mexico. The most recent, species-wide treatment of evolutionary and taxonomic relationships of the pinyon mouse was performed by Hoffmeister (1951), who examined morphological variation, described several new subspecies, and delimited a total of 12 subspecies. Several additional subspecies were described in the intervening years, and Hall (1981) recognized a total of 15, including several Mexican forms of uncertain taxonomic affinities. Based on their analysis of differentially stained chromosomes, Modi and Lee (1984) determined that populations from Mexico (including the subspecies *erasmus*, *gentilis*, *gratus*, and *zapotecae*) should be regarded as *P. gratus* and that the form *P. t. comanche* should be retained as a subspecies of *P. truei*. Restriction site data from mtDNA also supported a close relationship between *P. t. comanche* and populations of *P. t. truei* from Arizona and eastern Utah

(DeWalt et al. 1993), contra Janecek (1990). Thus, the pinyon mouse is now restricted to the western United States with the exception of populations in the Baja Peninsula of Mexico.

Durish et al. (2004) examined the systematics of the *P. truei* group using mtDNA sequence data from the cytochrome-*b* gene (*Cyt*b) and demonstrated that forms regarded as subspecies of *P. truei* (*gratus* and *zapotecae*), were divergent from all other populations of *P. truei* examined and were therefore regarded as specifically distinct; a general finding supported earlier by Janecek (1990). Within *P. truei* sensu stricto, Durish et al. (2004) found two genetic groups: two samples from California formed one group versus sequences of pinyon mice from Arizona, New Mexico, and Texas, which formed the second group.

This study extends our knowledge of the distribution of these two genetic groups by assessing mtDNA variation across the species range and including nine of the 11 recognized subspecies. Variation in nuclear DNA also was examined by sequencing intron 7 of the fibrinogen beta chain (*Fgb*) gene for a subset of individuals. In addition, the hypothesis that the two genetic groups differ morphologically was tested. Finally, the morphology of pinyon mice collected in Huntington Canyon, central Utah, a location from which both genetic groups are syntopic, was examined.

METHODS AND MATERIALS

DNA sequence data.—DNA was isolated from approximately 0.05 g of tissue (either frozen or preserved in ethanol) using the Qiagen DNeasy™ Tissue Kit (Qiagen Inc., Valencia, California). A single primer pair was used to amplify (polymerase chain reaction—PCR, Saiki et al. 1988) and sequence the first ~800 bp of the *Cyt*b gene: L14724 (Irwin et al. 1991) with CB3H (Palumbi 1996) or MVZ-16 (Smith and Patton 1993). Parameters for PCR reactions were as follows: one cycle of 94°C (3–5 min) was followed by 36 cycles of 94°C (1 min) denaturing, 46°C annealing (1 min), and 72°C (1 min) extension; the PCR was concluded by 1 cycle of 72°C (7 min). Intron 7 of the *Fgb* gene was amplified with primers B17 and Bfib (Wickliffe et al. 2003). Negative (no DNA) controls were run with amplifications to reveal instances of DNA contamination. PCR products were visualized on an agarose gel with ethidium bromide and the amplified products were purified using a Millipore Multiscreen™ PCR 96-Well Filtration System (Cat. No. MANU03050). These PCR products were then cycle sequenced using the primers described above, and sequenced products were purified using Millipore MultiscreenTM Filter Plates for High Throughput Separations (Cat. No. MAHVN4510). Light and heavy strand sequences were collected on an ABI 377 automated sequencer (Applied Biosystems), and the sequence data were then edited and compiled using Sequencher versions 3.1.1 and 4.1.2 (Gene Codes Corp.). Alignments for *Cyt*b and *Fgb* were unambiguous (no indels) and were performed by visual inspection or using the default parameters for the program MUSCLE ver. 3.8.31 as implemented at EMBI-EMB (http://www.ebi.ac.uk/Tools/msa/muscle/-Edgar 2004). The open reading frame for *Cyt*b was verified using the program MEGA ver. 5.2.2 (Tamura et al. 2011).

Wild-caught animals from which sequences were obtained were collected and processed according to the guidelines established by Sikes et al. (2016). A total of 484 new *Cyt*b sequences and 48 new *Fgb* sequences were submitted to GenBank (Appendix I). In addition, 57 GenBank *Cyt*b sequences were used from previous studies (Tiemann-Boege et al. 2000; Durish et al. 2004; Turner and Hoekstra 2008; Rodhouse et al. 2010; Hardy et al. 2013; Steppan and Schenk 2017). These were chosen to maximize the geographic (and phylogenetic) variation present among these sequences.

Of the 529 ingroup *Cyt*b sequences initially included in this study, 153 identical haplotypes were identified using RAxML and removed from the phylogenetic data set (Stamatakis 2014; Appendix I). Therefore, phylogenetic relationships were estimated based on a total of 376 ingroup and 16 outgroup *Cyt*b sequences using both maximum-likelihood (ML) and Bayesian inference (BI) optimality criteria. The ML analyses were performed in PhyML (Guindon et al. 2010) using GTR+I+G nucleotide substitution model, which was identified as the best model using Smart Model Selection (Lefort et al. 2017). Nodal support

was assessed by 1,000 bootstrap pseudoreplicates (Felsenstein 1985). Bayesian inference analysis coupled with Markov chain Monte Carlo (BMCMC) inference was performed in MrBayes v3.2.2 (Ronquist and Huelsenbeck 2003). Two independent BMCMC analyses, each consisting of four chains, were performed using the CIPRES Science Gateway portal (Miller et al. 2010). Each Markov chain was started from a random tree and run for 20 million generations using the default flat priors, sampling trees every 1,000 generations. Sequence evolution model parameters were treated as unknown variables with uniform default priors and were estimated as part of the analysis. Convergence was assessed based on examination of the standard deviation of split frequencies < 0.01 after 20 million generations and average effective sample size (ESS) values among runs was ≥ 1,000. The first 49% of the generations were conservatively deleted as burn-in. Results of *Cyt*b divergence were compared with those of previous using the Kimura 2-parameter substitution model (K2P—Kimura 1980) in PAUP* version 4.0b (Swofford 2003) by calculating average maximum and minimum divergence values for each of the main haplogroups in this study. The *Fgb* data were not evaluated phylogenetically due to lack of evolutionarily informative variation.

Morphometric data.—A total of 26 craniodental measurements were recorded to the nearest 0.01 mm of adult individuals (age classes 3–6 sensu Schmidly 1973b) using an Olympus SZX-16 dissecting microscope and processed using CellSens Standard 1.8 imaging platform (Olympus Corporation). These measurements were rounded to the nearest 0.05 mm for data analyses. The following dimensions between numbered landmarks were recorded: 1) nasal length (NL), measured as the greatest anteroposterior dimension of left nasal; 2) rostral length (RL), measured from the anteriormost point of the left nasal to the posteriormost point of the left premaxillae; 3) frontal length (FL), measured at the midline as the greatest anteroposterior dimension of frontal; 4) parietal length (PAL), measured at the midline as the greatest anteroposterior dimension of parietal; 5) interparietal length (IPAL), measured at the midline as the greatest anteroposterior dimension of interparietal; 6) greatest skull length (GSL), measured from the anteriormost post of the left nasal to the posteriormost point of the occipital; 7) frontal-occipital length (FOL), measured from the anteriormost point of the frontal to the posteriormost point of the occipital; 8) nasal breadth (NB), measured across the nasals at the point of appearance of the premaxillae as viewed dorsally; 9) posterior rostral breadth (RB), measured across the nasolacrimal capsules; 10) frontal breadth (FB), measured across the frontal from the left to the right zygomatic process of the squamosal; 11) zygomatic breadth (ZB), measured at the widest point across both zygomatic arches; 12) posterior frontal breadth (PFB), measured at the widest point of the posterior flanges of the frontal; 13) parietal breadth (PB), measured at the widest point of the frontal; 14) interparietal breadth (IPB), measured at the widest point of the interparietal; 15) naso-palatine length (NPL), measured from the anteriormost point of the left nasals to the posteriormost point of the left palatine foramen; 16) incisive foramen length (IFL), measured as the greatest anteroposterior dimension of left incisive foramen; 17) palatal length (PL), measured from the posteriormost point of the left incisive foramen to the anteriormost portion of the left postpalatine notch; 18) post-palatal length (PPL), measured from the anteriormost portion of the left postpalatine notch to the anteriormost potion of the ventral surface of the foramen magnum; 19) cranial breadth (CB), measured at the widest point across the ventral surface of the cranium (lateral protuberance of the auditory bullae); 20) occipital condyle breadth (OCB), measured at the widest point across the ventral surface of the occipital condyles; 21) bullar width (BW), measured across the maximum width; 22) bullar length (BL), measured from the protuberance of external auditory meatus to the end of the bulla immediately below the pterogoid process; 23) spheno-occipital length (SOL), measured from the posteriormost extent of the occipital to the suture demarking the presphenoid from the basispenoid; 24) maxillary toothrow length (MTL), measured from the anteriormost extent of M1 to the posteriormost extent of M3; 25) palatal width (PW), measured across palate at midpoint of M1; and 26) basiphenoid width (BW), measured across basiphenoid at the posteriormost extent of pterygoid processes.

SYSTAT 10 for Windows (SPSS Inc. 2000) was used in the multivariate analyses of log10-transformed cranial measurements. Quantitative phenetic variation and distinctiveness of geographic groups was assessed through discriminant function analysis (using backward stepwise estimation and jackknifed classification).

Discriminant functions with eigenvalues of less than 1 were considered as uninterpretable, and those with eigenvalues between 1 and 2 as marginal. A bivariate plot of discriminant functions was used to illustrate morphological differences among specimen groups.

Specimens were divided into four groups in the morphological analysis based on their haplogroup assignments (either genotyped or inferred; see results below). Skulls representing the western haplogroup were designated as WW, whereas skulls representing the eastern haplogroup were designated EE. In addition, skulls of pinyon mice from Huntington Canyon, Emery County, Utah, an area from which both haplogroups were syntopic, were genotyped and designated as either UE or UW (eastern or western haplogroup, respectively).

RESULTS

Molecular phylogenetics.—A total of 376 *Cyt*b sequences evaluated across the range of *Peromyscus truei* were recovered in two well supported haplogroups (Fig. 1). Pinyon mice from portions of northeastern and central Arizona, New Mexico, Oklahoma, Texas, and Utah formed one group (hereafter referred to as the eastern haplogroup), whereas individuals from Arizona north of the Colorado River, Baja California Peninsula, California, Nevada, Oregon, and roughly the western two-thirds of Utah formed the western haplogroup. The Colorado River separates these two hapolgroups in Arizona, whereas in Utah, the eastern haplogroup is distributed well west of the Colorado River but the reverse is not the case (Fig. 2). In addition, 15 localities were documented in Utah from which both the eastern and western haplogroups were syntopic: 71, 72, 73, 74, 76, 78, 81, 87, 90, 92, 99, 115, 120, 122, and 129 (Fig. 2). The majority of western haplogroup localities were divided into two strongly supported subgroups; all localities from the Baja California Peninsula versus localities spanning sites from northern California to western Utah and northern Arizona. In addition, seven samples representing one or more individuals from localities 12, 26, 28, and 30 in California were weakly associated with the Baja California haplogroup. Placement of five samples from two additional localities in California (16 and 33) was unresolved within the western haplogroup.

Intron 7 of the *Fgb* gene was sequenced for a total of 48 pinyon mice from Arizona, California, Nevada, Texas, and from several localities in Utah from which the eastern and western mtDNA groups were syntopic (Fig. 1 and Appendix I). This sampling design included populations of the eastern *Cyt*b haplogroup (Arizona and Texas), as well as representatives of the western haplogroup (California and Nevada). In addition, a series of 30 pinyon mice from near Huntington Canyon, Utah, also were sequenced; these individuals represented both haplogroups. Variation among sequences from California, Nevada, Utah, and eastward to Texas was minimal and consisted of the presence of autapomorphic heterozygote designations for 11 of the 44 individuals. There were no consistent differences attributable to the east–west genetic split as documented in *Cyt*b.

Kimura 2-Parameter genetic distances.—The majority of *Cyt*b genetic variation was partitioned between the eastern and western haplogroups. K2P distances between these two groups ranged from 3.97 to 6.53% (mean = 4.91 %). K2P distances within the eastern genetic group averaged 0.90% (range 0–1.94%) and within the western group K2P averaged 1.33% (range 0–3.05%).

Morphometric analysis.—A discriminant function analysis was conducted on 26 measurements from 89 adult specimens. Specimens were assigned to four *a priori* groups based on geography and genetic identity: EE – specimens from, northern Arizona, Colorado, western Oklahoma, northern Texas, and eastern Utah (n = 23, of which 15 were confirmed to represent to the eastern *Cyt*b haplogroup); WW – specimens from California (n = 29, including 26 confirmed to represent to the western haplogroup); UE – specimens from Huntington Canyon, Utah, confirmed to represent the eastern haplogroup (n = 13); and UW – specimens from Huntington Canyon confirmed to represent the western haplogroup (n = 24). Discriminant function analysis detected significant differences between the four specimen groups (Wilk's lambda = 0.092; ap-

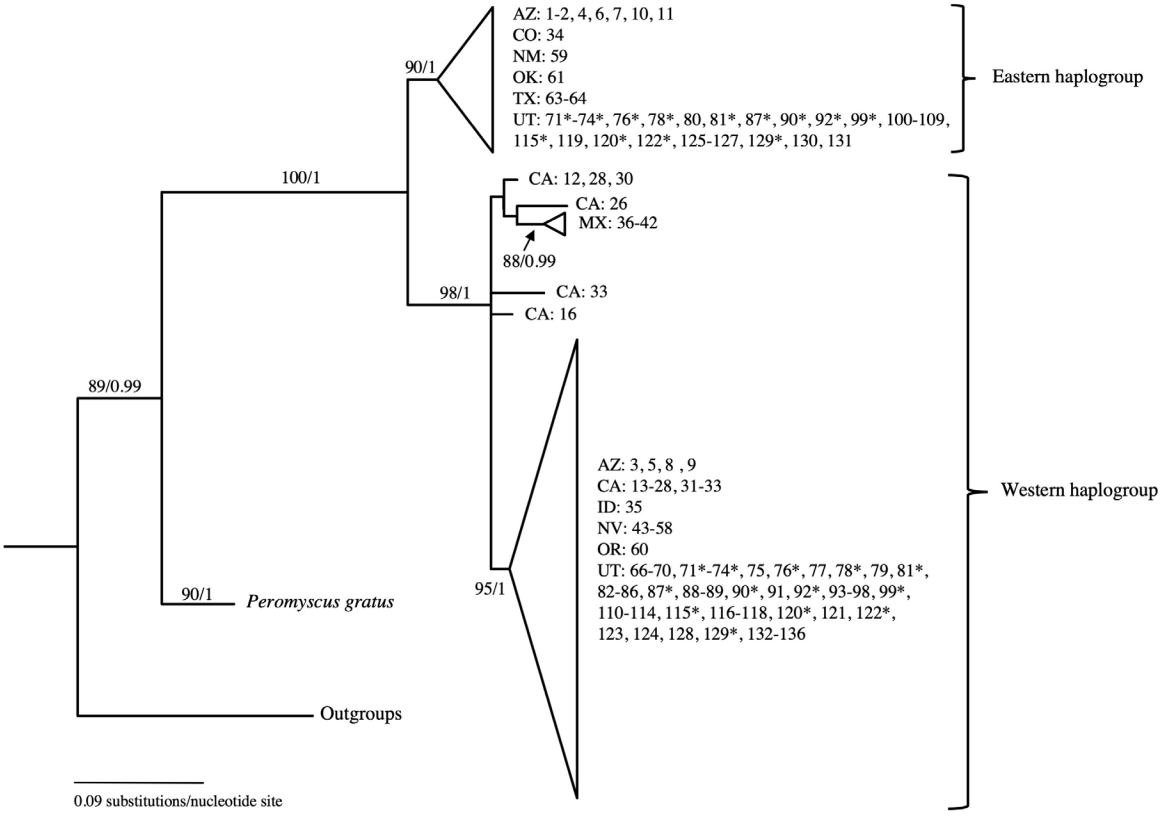

Figure 1. Maximum likelihood estimate of phylogenetic relationships among 376 *Peromyscus truei* collected from Arizona, Baja California Peninsula, California, Colorado, New Mexico, Oregon, Texas, and Utah based on a GTR+I+G model of evolution and evaluating 740 base pairs of the cytochrome-*b* gene. Branch lengths are shown proportional to the amount of evolutionary change. Values at nodes are bootstrap proportions followed by Bayesian posterior probabilities. Tree was midpoint rooted. Outgroup taxa included *P. boylii*, *P. crinitus*, *P. difficilis*, *P. gratus*, *Habromys lepturus*, and *Neotoma mexicana*. Major groups were condensed into triangles with sizes approximating the number of OTUs contained within each. Letters and numbers following terminal branches (or triangles) are abbreviations for states (MX = Baja California Peninsula) and localities as listed in Appendix I, respectively. Numbers with an asterisk indicate localities at which both the eastern and western haplogroups occurred in syntopy.

proximate $F = 2.818$, $d.f. = 78, 180$, $P < 0.001$). The first two discriminant functions (DFs) had eigenvalues of 2.656 and 1.222, canonical correlations of 0.852 and 0.742, and accounted for 63.1 and 29.0% of the explained variance, respectively (Table 1). DF1 was weighted most strongly by zygomatic breadth, distance from nasal tip to incisive foramen, posterior breadth of rostrum, anterior breadth of braincase, greatest length of skull, and length of palatal bridge. DF2 was only marginally interpretable, but was weighted by length of incisive foramen and length of nasal bones. Jackknifed classification correctly identified 57% of the specimens, including 76% of WW specimens and 61% of EE specimens. Classification success was poor for the Huntington Canyon specimens, with only 42% of the UW and 8% of the UE specimens correctly identified. In a plot of specimen scores on DF1 and DF2 (Fig. 3), groups EE and WW are non-overlapping, whereas groups UE and UW are indistinguishable and together overlap partially with EE and WW.

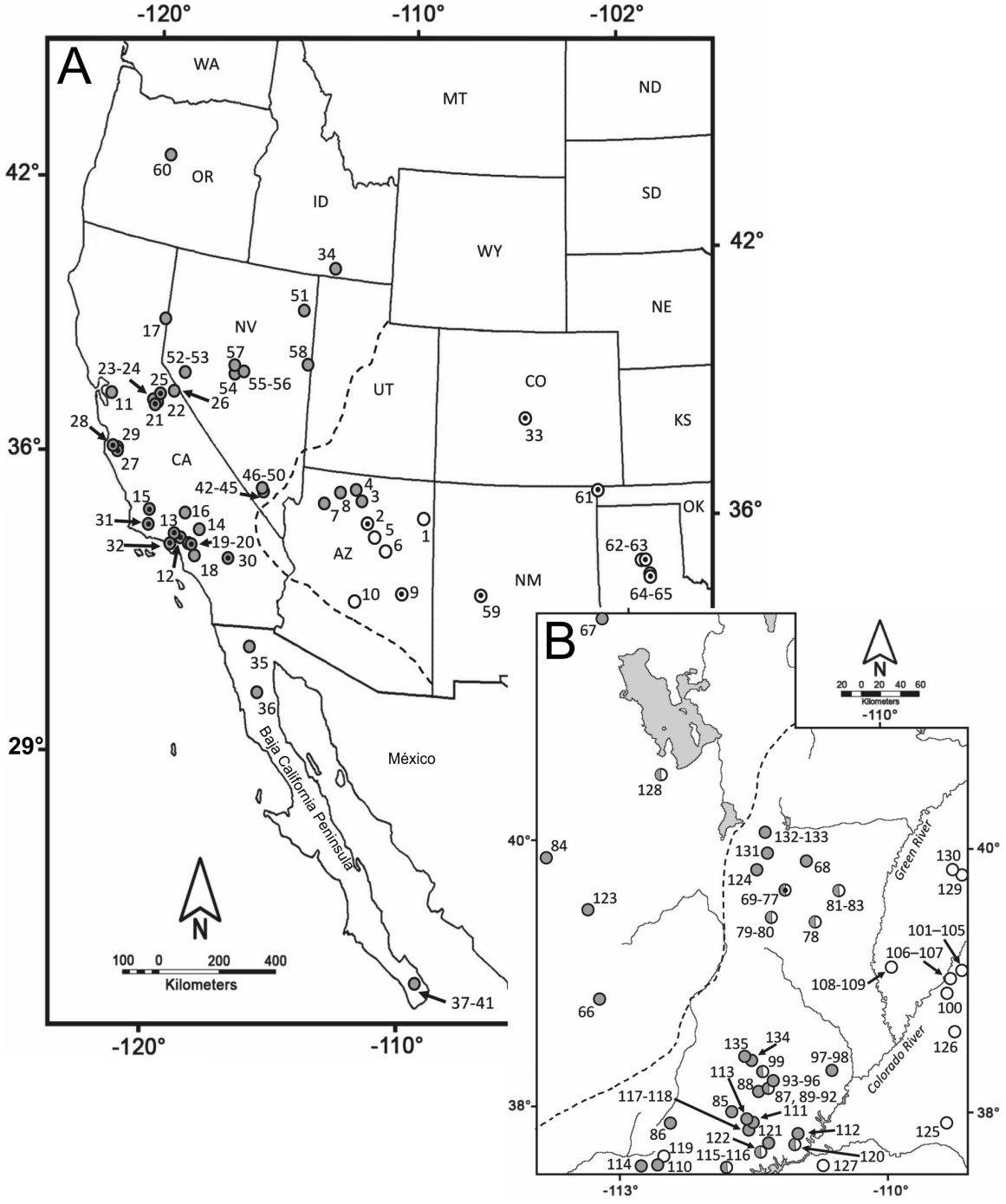

Figure 2. A) Map of the western United States depicting collecting localities for specimens examined in this study. Numbers indicate localities as referred to in Appendices I and II. Open circles denote eastern haplogroup localities, whereas gray circles show locations from which pinyon mice representing the western haplogroup were collected. Circles with centered black dots indicate localities that were used in the morphological analysis (see Appendix II). Dashed line indicates the contemporary demarcation between *Pinus monphylla* (west of the line) and *P. edulis* (east of the line—after Cole et al. 2015). B) Map of Utah depicting collecting localities as in Fig. 2A. Half gray-half open circles show locations at which both eastern and western haplogroup pinyon mice were collected. Dashed line delimits *P. monophylla* and *P. edulis* as in Fig. 2A.

Table 1. Loadings of the first three discriminant functions based on the analysis of 26 craniodental variables among four groups of *Peromyscus truei*. Variables, their abbreviations, and groups are defined in text.

	DF1	DF2	DF3
% variance explained	63.1	29.0	7.2
NL	0.047	0.071	0.129
RL	-0.315	-0.541	-0.329
FL	0.176	0.374	0.078
PAL	-0.035	0.381	0.310
IPAL	0.135	-0.139	-0.186
GLS	0.562	0.026	-1.146
FOL	0.466	0.321	0.300
RB	-0.628	-0.240	0.628
NB	-0.060	0.458	-0.039
FB	-0.314	-0.327	-0.048
ZB	1.014	-0.363	0.371
IPB	-0.268	-0.007	0.058
PB	0.203	-0.186	0.379
PFB	-0.609	0.257	-0.383
NPL	-0.654	-0.011	0.605
IFL	0.260	0.591	-0.381
PL	-0.539	0.249	0.076
PPL	-0.079	-0.402	0.788
CB	-0.123	0.134	-0.236
OCB	0.131	-0.733	-0.058
BW	0.174	-0.098	-0.208
BL	0.105	0.598	-0.443
SOL	-0.065	0.164	0.185
MTL	0.068	0.074	0.082
PW	-0.077	0.293	0.037
BW	0.109	0.357	0.262
Eigenvalue	2.656	1.222	0.335
Canonical Correlation	0.852	0.742	0.501

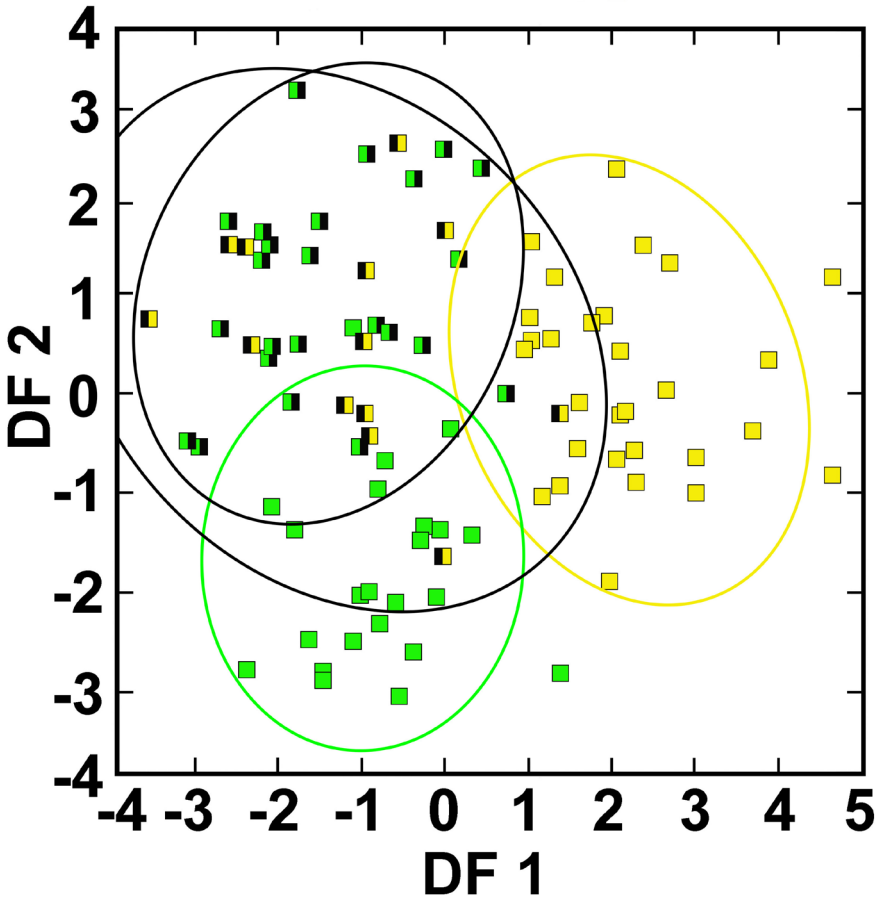

Figure 3. Discriminant function analysis of 26 craniodental variables for 89 *Peromyscus truei* skulls. Green squares indicate specimens belonging to the eastern haplogroup (EE in Appendix II), yellow squares correspond to the western haplogroup (WW in Appendix II), half green-half black squares are skulls from Huntington Canyon, Emery County, Utah, representing the eastern haplogroup (UE in Appendix II), and squares that are half yellow-half black represent skulls from Huntington Canyon representing the western haplogroup (UW in Appendix II).

DISCUSSION

Molecular phylogenetics and divergence.—Both ML and BI phylogenetic analyses of *Cyt*b recovered congruent tree topologies depicting two strongly supported haplogroups within *P. truei*. Within the western group, samples from Baja California peninsula-California formed a weakly-supported group relative to the majority of the samples. In addition, placement of some *Cyt*b sequences from California was unresolved (Fig. 1). The remaining western group sequences displayed little resolution, spanning a distance of more than 900 km from Alameda County in California to central Utah. Likewise, the lack of divergence within the eastern haplogroup is noteworthy. Sequences of *P. truei comanche* from the Texas Panhandle differed by only 0.38% from pinyon mice collected in southern Utah (locality 106). Moreover, identical *Cyt*b haplotypes shared between *P. truei* localities 106 and 122, representing sites on either side of the Colorado River in Utah, were recovered (Fig. 1).

The majority of genetic variation within *P. truei* was partitioned between the two haplogroups. Assuming a rate of 0.01 changes per base pair per million years for *Cyt*b as a lower bound (Arbogast et al. 2002) and 0.03 mutations per base pair per million years as an upper bound (Wu and Li 1985; Li et al. 1987, 1990), then the mean K2P distance of 4.91% between the eastern and western haplogroups translates to a divergence time between 1.64 and 4.91 million years ago. The magnitude of *Cyt*b genetic distance between these two haplogroups suggests a major vicariant event responsible for separating these lineages. K2P genetic distance between these two groups is on the high end for intraspecific variation (1.5–2.1% and 1.1–4.3%) among species in the *P. boylii* and *P. truei* species groups, respectively (Tiemann-Boege et al. 2000; Durish et al. 2004). According to Bradley and Baker (2001), the range of *Cyt*b variation for rodent sister taxa ranges from 2.70–19.23%. The mean K2P distance between the eastern and western haplogroups is greater than the distances between *P. carletoni*, *P. levipes*, and *P. schmidlyi* (range 3.25–3.50%), but less than the majority of distances separating sister species in the *P. boylii* and *truei* groups (Durish et al. 2004; Bradley et al. 2017). *Fgb* is one of the fastest evolving nuDNA markers and is one that typically provides useful phylogenetic signal (Prychitco and Moore 2000; Matocq et al. 2007; Platt et al. 2015; Almendra et al. 2018). Unfortunately, *Fgb* sequence data herein were uninformative because pinyon mice from California to Texas possessed essentially the same haplotype.

Systematic status of the eastern and western P. truei *haplogroups.*—Given the dynamism of the historical distributions of *P. edulis* and *P. monophylla* in the southwest (see discussion below), syntopy of the two *P. truei* haplogroups in central Utah may be the result of secondary contact (Coyne and Orr 2004). The possible evolutionary outcomes of such contact are three-fold: 1) the two haplogroups are reproductively isolated—no hybridization (Dobzhansky 1937; Mayr 1942); 2) reproductive barriers are incomplete and some interbreeding occurs, resulting in gene flow between the two haplogroups (Barton and Gale 1993); or 3) a relatively stable zone of hybridization forms due to partial reproductive isolation (Barton and Hewitt 1981). Unfortunately, the mtDNA sequence data reported herein cannot address the question of hybridization, nor can the *Fgb* sequence data due to lack of variation for that marker. However, both haplogroups of pinyon mice from Huntington Canyon in central Utah are morphologically indistinguishable from one another and, together, are intermediate in cranial size and shape compared to mice from further east in Utah, New Mexico, Oklahoma, and Texas or to pinyon mice further west in California (Fig. 3). Given that skull shape has a strong genetic component (Leamy et al. 1999), the morphological intermediacy in the zone of contact may represent evidence of hybridization between the eastern and western haplogroups.

The genetic data also provide some clues regarding the extent and nature of this apparent hybrid zone. Instances in which hybridization is relatively limited occur in either a rather narrow "tension zone", or over a selection gradient in which the hybrids occur in an intermediate habitat compared to the two parental environments (Barton and Hewitt 1985; Coyner et al. 2015). The data indicate that the potential hybrid zone between the eastern and western forms of *P. truei* is not narrow. Rather, both haplogroups are syntopic throughout central Utah (Fig. 2B), and the 15 locations from which both have been collected in the same trap line includes an area that spans more than 300 km north–south and approximately 200 km east–west. Likewise, there are no obvious edaphic or environmental gradient differences across this proposed hybrid zone. Therefore, there is no reason to argue that *P. truei* should be split into two species-level lineages. Moreover, the number of subspecies recognized (two within the eastern and nine within the western haplogroup) is not congruent with the amount of within-haplogroup genetic variation documented in this study.

Biogeography of Peromyscus truei *and members of the genus* Pinus.—According to Wells (1983), macrofossil evidence from *Neotoma* middens document a large latitudinal displacement of vegetation in the southern Great Basin during the Pleistocene and culminating in the last 40,000 years. For example, prior to 9,000 years ago, there was no evidence of pinyon-juniper habitat anywhere in Utah. Instead, areas north of ~37 degrees north latitude (Arizona–Utah border) were dominated by subalpine forests consisting of bristlecone pine (*Pinus longaeva*) and boreal juniper (*Juniperus communis*). Forty thousand years ago, *P. edulis* reached its northernmost distribution just north of present-day Lake Mead, where it apparently hybrid-

ized with *P. monophylla* (Cole et al. 2008). But in the last 9,000 years and as the climate continued to warm, pinyon-juniper vegetation migrated ~600 km northward (Wells 1979, 1983) and there is evidence of a continued northward expansion of both pinyons and pinyon mice. For example, *P. truei* recently expanded its range in Oregon (Carraway et al. 1993) and Nevada (Massey et al. 2017), and the two-leafed pinyon colonized Dutch John Mountain in Daggett County, Utah, about 800 years ago (Gray et al. 2006).

Pinyon mice occupy habitats other than pinyon-juniper woodlands. These include Douglass fir, madrone, coastal redwoods, and chaparral (McCabe and Blanchard 1950; Hoffmeister 1951, 1964, 1981), subalpine habitats (Yang et al. 2011) in California, juniper-sage in Oregon (Carraway et al. 1993), and cedar woodlands in Texas (Schmidly 1973a). But in Arizona, Colorado, Idaho, New Mexico, and Utah, especially areas with rocky substrates (Hall 1946; Armstrong 1972; Hoffmeister 1986), pinyon mice are more intimately associated with pinyon-juniper woodlands. Indeed, the distribution of the two *P. truei* haplogroups closely matches the distribution of *P. monophyla* and *P. edulis* (Fig. 2A and 2B; Cole et al. 2013) in these states, and the two pinyons hybridize readily in locations where they co-occur in central Utah (Lanner 1971; Lanner and Hutchison 1972; Cole et al. 2013). Moreover, all the localities from which syntopy between *P. truei* haplogroups was documented occurs to the east of the hybrid zone between the two pinyon pine species and within the range of *P. edulis*. The only exception is locality 129 from the Stansbury Range (Fig. 2B), which is located well within the range of *P. monophylla*. Based on these results, it appears likely that secondary contact and subsequent hybridization between the eastern and western haplogroups of *P. truei* in Utah is recent. This is because pinyon-juniper woodlands were not established in central Utah until several thousand years ago (Cole et al. 2013). Whether or not these two haplogroups hybridized in the past in areas further south is unanswerable at this time. At present, it appears that the Colorado River serves as a barrier between the two haplogroups in Arizona, but not further upstream in Utah where Pinyon mice representing the eastern haplogroup occur well to the west of the Colorado and Green Rivers.

A phylogeographic pattern best explained by a single southern refugium followed by a relatively rapid, post-glacial, northward expansion has been hypothesized for Clark's nutcracker (*Nucifraga columbiana*), another pinyon-juniper woodland specialist (Dohms and Burg 2013). Unfortunately, there are no other pinyon-juniper specialist species for which comparable phylogeographic data are available. However, the data reported herein suggest that *P. truei* was confined to two refugia at some point in the past, and that this separation must have been relatively lengthy given the amount of *Cyt*b divergence evidenced between the eastern and western haplogroups. An eastern refugium for *P. edulis* was hypothesized by Malusa (1992) as being located in southern New Mexico. Cole et al. (2013) proposed that extreme southern Nevada or northwest Arizona might have served as a Pleistocene refugium for pinyon-juniper woodland. Presumably this area would have been occupied by *P. monophylla*.

Summary

Two widely distributed and deeply divergent haplogroups within *P. truei* are indicative of early vicariant events followed by considerably more recent northward migrations that occurred during the Holocene. It is hypothesized that these two haplogroups tracked the northward movements of *P. edulis* and *P. monophylla* (Cole et al. 2013) during the most recent glacial maximum. Unfortunately, the available nuclear DNA sequence data are insufficiently variable to address the question of hybridization between the eastern and western haplogroups of *P. truei* directly. However, the morphological data support the hypothesis of hybridization following recent secondary contact. Testing this hypothesis would require the evaluation of relatively fast-evolving markers such as microsatellites.

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LITERATURE CITED

Almendra, A. L., F. X. González-Cózatl, M. D. Engstrom, and D. S. Rogers. 2018. Evolutionary relationships and climatic niche evolution in the genus *Handleyomys* (Sigmodontinae: Oryzomyini). Molecular Phylogenetics and Evolution 128:12–25.

Arbogast, B. S., S. V. Edwards, J. Wakeley, P. Beerli, and J. B. Slowinski. 2002. Estimating divergence times from molecular data on phylogenetic and population genetic timescales. Annual Review of Ecology and Systematics 33:707–740.

Armstrong, D. M. 1972. Distribution of mammals in Colorado. University of Kansas, Museum of Natural History, Lawrence, Kansas.

Barton, N. H., and K. S. Gale. 1993. Genetic analysis of hybrid zones. Pp. 13–45 in Hybrid zones and the evolutionary process (R. G. Harrison, ed.). Oxford University Press, New York, New York.

Barton, N. H., and G. M. Hewitt. 1981. Hybrid zones and speciation. Pp. 109–145 in Evolution and speciation: essays in honor of M. J. D. White (W. R. Atchley and D. S. Woodruff, eds.). Cambridge University Press, Cambridge, Massachusetts.

Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. Annual Review of Ecology and Systematics 16:113–148.

Bradley, R. D., and R. J. Baker. 2001. A test of the genetic species concept: cytochrome-*b* sequences and mammals. Journal of Mammalogy 82:960–973.

Bradley, R. D., N. Ordóñez-Garza, G. Ceballos, D. S. Rogers, and D. J. Schmidly. 2017. A new species in the *Peromyscus boylii* species group (Cricetidae: Neotominae) from Michoacán, Mexico. Journal of Mammalogy 98:1154–1165.

Carraway, L. N., E. Yensen, B. J. Verts, and L. F. Alexander. 1993. Range extension and habitat of *Peromyscus truei* in eastern Oregon. Northwestern Naturalist 74:81–84.

Cole, K. L., J. Fisher, S. T. Arundel, J. Cannella, and S. Swift. 2008. Geographical and climatic limits of needle types of one- and two-needled pinyon pines. Journal of Biogeography 35:257–269.

Cole, K. L., J. F. Fisher, K. Ironside, J. I. Mead, and P. Koehler. 2013. The biogeographic histories of *Pinus edulis* and *Pinus monophylla* over the last 50,000 years. Quaternary International 310:96–110.

Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer Associates, Inc., Sunderland, Massachusetts.

Coyner, B. S., P. J. Murphy, and M. D. Matocq. 2015. Hybridization and asymmetric introgression across a narrow zone of contact between *Neotoma fuscipes* and *N. macrotis* (Rodentia: Cricetidae). Biological Journal of the Linnean Society 115:162–172.

DeWalt, T. S., E. G. Zimmerman, and J. V. Planz. 1993. Mitochondrial-DNA phylogeny of species of the *boylii* and *truei* groups of the genus *Peromyscus*. Journal of Mammalogy 74:352–362.

Dobzhansky, T. 1937. Genetics and the Origin of Species. Columbia University Press, New York, New York.

Dohms, K. M., and T. M. Burg. 2013. Molecular markers reveal limited populations genetic structure in a North American corvid, Clark's nutcracker (*Nucifraga columbiana*). PLoS ONE 8: e79621. doi:10.1371/journal.pone.0079621

Dunnum, J. L., B. S. McLean, R. C. Dowler, and ASM Systematic Collections Committee. 2018. Mammal collections of the Western Hemisphere: a survey and directory of collections. Journal of Mammalogy 99:1307–1322.

Durish, N. D., K. E. Halcomb, C. W. Kilpatrick, and R. D. Bradley. 2004. Molecular systematics of the *Peromyscus truei* species group. Journal of Mammalogy 85:1160–1169.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

Gray, S. T., J. L. Betancourt, S. T. Jackson, and R. G. Eddy. 2006. Role of multidecadal climate variability in a range extension of pinyon pine. Ecology 87:1124–1130.

Guindon, S., J. F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, and O. Gascuel. 2010. New Algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59:307–321.

Hall, E. R. 1946. Mammals of Nevada. University of California Press, Berkeley, California.

Hall, E. R. 1981. The mammals of North America. 2nd ed. John Wiley & Sons, New York.

Hardy, D. K., F. X. González-Cózatl, E. Arellano, and D. S. Rogers. 2013. Molecular phylogenetics and phylogeographic structure of Sumichrast's harvest mouse (*Reithrodontomys sumichrasti*: Cricetidae) based on mitochondrial and nuclear DNA sequences. Molecular Phylogenetics and Evolution 68:282–292.

Hoffmeister, D. F. 1951. A taxonomic and evolutionary study of the piñon mouse, *Peromyscus truei.* Illinois Biological Monographs 21:1–104.

Hoffmeister, D. F. 1964. Mammals and life zones: time for re-evaluation. Plateau 37:46–55.

Hoffmeister, D. F. 1981. *Peromyscus truei*. Mammalian Species 161:1–5.

Hoffmeister, D. F. 1986. Mammals of Arizona. University of Arizona Press and Arizona Fish and Game Department, Tuscon, Arizona.

Irwin, D. M., T. D. Kocher, and A. C. Wilson. 1991. Evolution of the cytochrome *b* gene of mammals. Journal of Molecular Evolution 32:128–144.

Janecek, L. L. 1990. Genic variation in the *Peromyscus truei* group (Rodentia: Cricetidae). Journal of Mammalogy 71:301–308.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111–120.

Lanner, R. M. 1971. Conifer flora of the Bear Lake area and mountains south of the Great Salt Lake. Great Basin Naturalist 31:85–89.

Lanner, R. M., and E. R. Hutchison. 1972. Relict stands of pinyon hybrids in northern Utah. Great Basin Naturalist 32:171–175.

Leamy, L. J., E. J. Routman, and J. M. Cheverud. 1999. Quantitative trait loci for early− and late−developing skull characters in mice: a test of the genetic independence model of morphological integration. American Naturalist 153:201–214.

Lefort, V., J. E. Longueville, and O. Gascuel. 2017. SMS: Smart model selection in PhyML. Molecular Biology and Evolution 34:2422–2424. doi.org/10.1093/molbev/msx149.

Li, W.-H., M. Gouy, P.M. Sharp, C. O'Uhigin, and Y.-W. Yang. 1990. Molecular phylogeny of Rodentia, Lagomorpha, Primates, Artiodactyla, and Carnivora and molecular clocks. Proceedings of the National Academy of Sciences 87:6703–6707.

Li, W.-H., M. Tanimura, and P.M. Sharp. 1987. An evaluation of the molecular clock hypothesis using mammalian DNA sequences. Journal of Molecular Evolution 25:330–342.

Malusa, J. 1992. Phylogeny and biogeography of the pinyon pines (*Pinus* subsect. *Cembroides*). Systematic Botany 17:42–66.

Massey, A. L., E. A. Rickart, and R. J. Rowe. 2017. Habitat use of the piñon mouse (*Peromyscus truei*) in the Toiyabe Range, central Nevada. Western North American Naturalist 77:464–477.

Matocq, M. D., Q. R. Shurtliff, and C. R. Feldman. 2007. Phylogenetics of the woodrat genus *Neotoma* (Rodentia: Muridae): Implications for the evolution of phenotypic variation in male external genitalia. Molecular Phylogenetics and Evolution 42:637–652.

Mayr, E. 1942. Systematics and the origin of species. Columbia University Press, New York, New York.

McCabe, T. T., and B. D. Blanchard. 1950. Three Species of *Peromyscus*. Rood Associates, Santa Barbara, California.

Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. Pp. 1–8 in Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, Louisiana.

Modi, W. S., and M. R. Lee. 1984. Systematic implications of chromosomal banding analyses of populations of *Peromyscus truei* (Rodentia: Muridae). Proceedings of the Biological Society of Washington 97:716–723.

Palumbi, S. R. 1996. Nucleic acids I: The polymerase chain reaction. Pp. 205–247 in Molecular systematics, 2nd ed. (D. M. Hillis, C. Moritz, and B. K. Mable, eds.). Sinauer, Sunderland, Massachusetts.

Platt, R. N., II., R. B. Amman, M. S. Smith, C. W. Thompson, and R. D. Bradley. 2015. What is *Peromyscus*? Evidence from nuclear and mitochondrial DNA sequences suggest the need for a new classification. Journal of Mammalogy 96:708–719.

Prychitko, T. M., and W. S. Moore. 2000. Comparative evolution of the mitochondrial cytochrome *b* gene and nuclear *β*-fibrinogen intron 7 in woodpeckers. Molecular Biology and Evolution 17:1101–1111.

Rodhouse, T. J., R. P. Hirnyck, and R. G. Wright. 2010. Habitat selection of rodents along a piñon-juniper woodland-savannah gradient. Journal of Mammalogy 91:447–457.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Saiki, R. K., et al. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491.

Schmidly, D. J. 1973a. The systematic status of *Peromyscus comanche*. The Southwestern Naturalist 18:269–278.

Schmidly, D. J. 1973b. Geographic variation and taxonomy of *Peromyscus boylii* from Mexico and the southern United States. Journal of Mammalogy 54:111–130.

Shufeldt, R. W. 1885. Description of *Hesperomys truei*, a new species belonging to the subfamily Murinae. Proceedings of the United States National Museum 8:403–408.

Sikes, R. S., and the Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy 97:663–668.

Smith, M. F., and J. L. Patton. 1993. The diversification of South American muroid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. Biological Journal of the Linnaean Society 50:149–177.

SPSS, Inc. 2000. SYSTAT 10. SPSS Inc., Chicago, Illinois.

Stamatakis, A. 2014. RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. \Bioinformatics 30:1312–1313.

Steppan, S. J., and J. J. Schenk. 2017. Muroid rodent phylogenetics: 900-species tree reveals increasing diversification rates. PLoS ONE 12: e0183070.

Swofford, D. L. 2003. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.0b2.0a. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

Tamura, K., D. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28:2731–2739.

Tiemann-Boege, I., C. W. Kilpatrick, D. J. Schmidly, and R. D. Bradley. 2000. Molecular phylogenetics of the *Peromyscus boylii* species group (Rodentia: Muridae) based on mitochondrial Cytochrome *b* sequences.Molecular Phylogenetics and Evolution 16:366–378.

Turner, L. M., and H. E. Hoekstra. 2008. Reproductive protein evolution within and between species: maintenance of divergent ZP3 alleles in *Peromyscus*. Molecular Ecology 17:2616–2628.

Wells, P. V. 1979. An equable glaciopluvial in the west: Pleniglacial evidence of increase precipitation on a gradient from the Great Basin to the Sonoran and Chihuahuan deserts. Quaternary Research 12:311–325.

Wells, P. V. 1983. Paleobiogeography of montane islands in the Great Bain since the last glaciopluvial. Ecological Monographs 53:341–382.

Wickliffe, J. K., F. G. Hoffmann, D. S. Carroll, Y. V. Dunina-Barkovskaya, R. D. Bradley, and R. J. Baker. 2003. PCR and sequencing primers for intron 7 (*Fgb*-I7) of the fibrinogen, B beta polypeptide (*Fgb*) in mammals: a novel nuclear DNA marker. Occasional Papers of the Museum, Texas Tech University 219:1–8.

Wu, C.-I., and W.-H. Li. 1985. Evidence for higher rates of nucleotide substitution in rodents than in man. Proceedings of the National Academy of Sciences 82:1741–1745.

Yang, D-S., C. J. Conroy, and C. Moritz. 2011. Contrasting responses of *Peromyscus* mice of Yosemite National Park to recent climate change. Global Change Biology 17:2559–2566.

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APPENDIX I

Specimens examined in the genetic analyses.—Specimens from which a portion of the cytochrome-*b* and intron 7 of the fibrinogen beta chain genes were sequenced are listed below with map codes (corresponding numbers in Figs. 1 and 2), species and subspecies designations, collecting localities including latitude and longitude (either directly measured or estimated), specimen identification numbers (museum voucher or collector numbers), and GenBank Accession. Numbers with an asterisk designate specimens possessing identical cytochrome-*b* haplotypes and not included in the phylogenetic analyses to reduce computation times.

Museum abbreviations (following Dunnum et al. 2018) are as follows: BYU = Brigham Young University, Monte L. Bean Life Science Museum; CIB = Centro de Investigaciones Biológicas del Noroeste; CMC = Universidad Autónoma de Morelos; MVZ = University of California, Berkeley, Museum of Vertebrate Zoology; TCWC = Texas A&M University, Biodiversity Research and Teaching Collection (formerly Texas Cooperative Wildlife Collection); TTU = Collection of Recent Mammals, Museum of Texas Tech University; UMNH = University of Utah, Natural History Museum of Utah; UWBM = University of Washington, Thomas Burke Memorial Washington State Museum; and ZTNH = University of Vermont, Zadock Thompson Natural History Collections.

Collector or tissue number abbreviations are as follows: ALA = Ana L. Almendra (Brigham Young University); AWB = Andrew W. Bartlow (Natural History Museum of Utah); BMB = Brittany M. Bush (Brigham Young University); BWC = Bruce W. Christiansen (Brigham Young University); CWK = C. William Kilpatrick (University of Vermont); DSR = Duke S. Rogers (Brigham Young University); EA = Elizabeth Arellano (Universidad Autónoma del Estado de Morelos); EAR = Eric A. Rickart (Natural History Museum of Utah); ERM = Evelyn Rios-M (Centro de Investigaciones Biológicas del Noroeste); FC = Frozen Collection, University of California, Berkeley; FXG = Francisco X. González-Cózatl (Universidad Autónoma del Estado de Morelos); GK = Tissue series (Texas Cooperative Wildlife Collection); HAJ = Heather A. James (Brigham Young University); JLA = Jackie L. Alston (Brigham Young University); JLP = James L. Patton (University of California, Berkeley); JLS = Jared L. Stringer (Brigham Young University); JMH = Jason M. Harper (Brigham Young University); LDC = Loren D. Chase (Brigham Young University); LFA = Lois F. Alexander (Utah Museum of Natural History); LMT = Leslie M. Turner (Max Planck Institute for Evolutionary Biology); LWM = Lorie W. Milward (Natural History Museum of Utah); MEW = Merriam E. Ward (Brigham Young University), Mark Morris (Brigham Young University); NRM = Nathan R. Martinez (Brigham Young University); QRS = Quinn R. Shurtliff (Brigham Young University); RAK and RK = Romar A. Karl (Brigham Young University); STA-C = Sergio Ticul Álvarez-Castañeda (Centro de Investigaciones Biológicas de Noreste); TK = Museum of Texas Tech University; and ZLM = Zoe L. Mize (Brigham Young University).

Appendix I.

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
	Habromys lepturus	Oaxaca	Santa Maria Yacochi, 2400 m (17° 15" N 96° 00' 45" W)	DSR 5705	CMC 53	DQ861387	N/A	N/A
	Neotoma mexicana	Jalisco	3.4 km. W (by road) San Sebastián del Oeste, 1450 m (20° 46,146' N, 104° 52,263' W)	DSR 7381	CMC 1204	MK803421	N/A	N/A
	P. boylii	Utah: Garfield Co.	3.7 km N and 0.7 km W Tropic, 37° 39' 25.80" N, 112 05' 31.30" W, 2005 m	DSR 8776	BYU 33423	MK803423	N/A	N/A
	P. boylii	Utah, Kane Co.	Smokey Hollow Road, 37 09.5875' N, 111 32.0077' W, 1350 m	DSR 7312	BYU 23733	MK803422	N/A	N/A
	P. crinitus	Utah, Kane Co.	59 km E, 25 km N Kanab, 415045 E, 4121412 N, 1450 m	DSR 5644	BYU 18082	MK803424	N/A	N/A
	P. crinitus	Utah, Kane Co.	Smoky Hollow, 37 ° 09.41' N, 111° 32.10' W, 1270 m	DSR 7172	BYU 19453	MK803425	N/A	N/A
	P. crinitus	Utah, Washington Co.	9.2 km S, 0.1 km W Scarecrow Peak, 37.1386 N, 114.0271 W, 835 m	DSR 15133	BYU 40919	MK803426	N/A	N/A
	P. difficilis	Aguascalientes	6 miles W Rincon de Romas	GK 4129	TCWC no data	AY376418	N/A	N/A
	P. difficilis	Hidalgo	1.8 miles E Jonacapa	GK 3076	TCWC no data	AY376419	N/A	N/A
	P. difficilis	Puebla	8 miles SE Chignahuapan	CWK 2770	ZTNH no data	AY376414	N/A	N/A
	P. difficilis	Tlaxcala	Mt. Malinche	GK 3904	TCWC no data	AY376415	N/A	N/A
	P. difficilis	Tlaxcala	2 km NE Tepetitla	TK 93120	TTU 82690	AY376416	N/A	N/A
	P. gratus	Jalisco	2 km NW Mesoncito	TK 93079	TTU 82698	AY376420	N/A	N/A
	P. gratus	Michocán	5 km E Costzeo	TK 46354	TTU no data	AY376421	N/A	N/A
	P. gratus	Puebla	5 Km SE San Antonia	TK 93140 TK 93145	TTU 82701 no data	AY376422 AY376423	N/A N/A	N/A N/A
1	*P. truei truei*	Arizona: Apache Co.	no specific locality	TK 92372	TTU 104427	AY376433	E	N/A

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
2	*P. truei truei*	Arizona: Coconino Co.	Navajo Nation, 35.9300 N, 111.7229 W, 1925 m	DSR 15507 DSR 15508 DSR 15509	BYU 41011 BYU 41012 BYU 41013	MK871823 MK871824 MK871825	E E E	MK907209 MK907210 MK907211
3	*P. truei truei*	Arizona: Coconino Co.	east slope Kaibab Plateau, ca. 3 mi W House Rock Ranch [36.47997/ -112.00745]	JLP 18366 JLP 18367	MVZ 197293 MVZ 197294	KY754109 EU548404	W W	N/A N/A
4	*P. truei truei*	Arizona: Coconino Co.	2 mi SE Cameron, N side Little Colorado River [35.86377/ - 111.37848]	JLP 19036	MVZ 199481	EU568421	E	N/A
5	*P. truei truei*	Arizona: Coconino Co.	Ryan [36.69022/ -112.33742]	JLP 18349 JLP 18355	MVZ 197285 MVZ 197284	EU568401 EU568402	W W	N/A N/A
6	*P. truei truei*	Arizona: Coconino Co.	ca. 1 mi NE Tanner Tank [35.71277/ -111.53078]	JLP 18391 JLP 18392	MVZ 197295 MVZ 197296	EU548405 EU568406	E E	N/A N/A
7	*P. truei truei*	Arizona: Coconino Co.	E side Woodhouse Mesa [35.49001 / -111.35717]	JLP 19006 JLP 19007 JLP 19008 JLP 19009 JLP 19010 JLP 19011 JLP 19012 JLP 19013 JLP 19014 JLP 19015 JLP 19016 JLP 19017	MVZ 199469 MVZ 199470 MVZ 199471 MVZ 199472 MVZ 199473 MVZ199474 MVZ 199475 MVZ 199476 MVZ 199477 MVZ 199478 MVZ 199479 MVZ 199480	EU568409 EU568410 EU568411 EU568412 EU568413 EU568414 EU568415 EU568416 EU568417 EU568418 EU568419 EU568420	E E E E E E E E E E E E	N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A
8	*P. truei truei*	Arizona: Mohave Co.	Toroweap Valley [36.34078/ -113.05669]	JLP 18951	MVZ 199467	EU568407	W	N/A
9	*P. truei truei*	Arizona: Mohave Co.	Hack Canyon [36.60934 -112.84024]	JLP 18964	MVZ 199468	EU568408	W	N/A
10	*P. truei truei*	Arizona: Navajo Co.	3 mi S Woodruff [34.730120 -110.052399]	TK 77921	TTU 78507	AF155412	E	N/A
11	*P. truei truei*	Arizona: Yavapai Co.	Pine Flat [34.279279 - 112.350097]	TK 113804	no data	EU568383	E	N/A
12	*P. truei gilberti*	California: Alameda Co.	Strawberry Canyon, below Botanical Gardens [37.8727228 / -122.2429687]	FC 4313 FC 4316	MVZ 157330 MVZ 157332*	MK871830 MK871831	W W	N/A N/A

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
13	*P. truei montepinoris*	California: Kern Co.	Cuddy Canyon, 1 mi E Frazier Park [34.8213833333 / -118.9239166667]	JLP 17906	MVZ 196169	MK871834	W	N/A
14	*P. truei montepinoris*	California: Kern Co.	2 mi NNW Eagle Rest Peak, San Emigido Mts. [34.92801 / -119.1362]	JLP 18770	MVZ 198616	MK871846	W	N/A
15	*P. truei montepinoris*	California: Kern Co.	N side Hwy. 58, 6.8 mi NW [by road) Mojave [35.111565 / -118.2396183]	FC 3043 FC 3044	MVZ 158819 MVZ 158820*	MK871832 MK871833	W W	N/A N/A
16	*P. truei montepinoris*	California: Kern Co.	Temblor Range summit on Hwy. 58 [35.35564 / -119.82853]	JLP 18730 JLP 18731 JLP 18732 JLP 18733 JLP 18734	MVZ 198606 MVZ 198607 MVZ 198608 MVZ 198609* MVZ 198610	MK871841 MK871842 MK871843 MK871844 MK871845	W W W W W	N/A N/A N/A N/A N/A
17	*P. truei montepinoris*	California: Kern Co.	Rancheria Creek, east end Walker Basin [35.39925 / -118.45877]	JLP 18488 JLP 18489 JLP 18490 JLP 18491 JLP 18492	MVZ 197314 MVZ 197315 MVZ 197316 MVZ 197317 MVZ 197318*	MK871835 MK871836 MK871837 MK871838 MK871839	W W W W W	N/A N/A N/A N/A N/A
18	*P. truei truei*	California: Lassen Co.	Turtle Mt., N end Fort Sage Mts.[40.10348 / -120.08013]	JLP 18250	MVZ 197283	MK871847	W	N/A
19	*P. truei montepinoris*	California: Los Angeles Co.	Chatsworth Reservoir Park [34.258797 -118.627666	TK 92333	TTU 83290	AY376432	W	N/A
20	*P. truei montepinoris*	California: Los Angeles Co.	4.5 mi E (by road) Gorman [34.77825 / -118.7771]	JLP 18598	MVZ 198394	MK871840	W	N/A
21	*P. truei montepinoris*	California: Los Angeles Co.	0.4 mi W Gorman [34.79703 / -118.86111]	JLP 18589 JLP 18590	MVZ 198392 MVZ 198393	MK871848 MK871849	W W	N/A N/A
22	*P. truei gilberti*	California: Mariposa Co.	Hunter Valley Mountain [37.61872 / -120.18696]	JLP 20946 JLP 20947 JLP 20960 JLP 20970 JLP 20971	MVZ 208172 MVZ 208173 MVZ 208176 MVZ 208177 MVZ 208178*	MK871851 MK871852 MK871853 MK871854 MK871855	W W W W W	N/A N/A N/A N/A N/A
23	*P. truei gilberti*	California: Mariposa Co.	5.7 mi SE Coulterville [37.63654 / -120.15194]	JLP 20827	MVZ 208171	MK871850	W	N/A
24	*P. truei gilberti*	California: Mariposa Co.	Hunter Valley Mountain [37.64733 / -120.21127]	JLP 20979	MVZ 208180*	MK871856	W	N/A

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
25	*P. truei gilberti*	California: Mariposa Co.	Hunter Valley Mountain [37.63996 / -120.21697]	JLP 20982	MVZ 208181	MK871857	W	N/A
26	*P. truei gilberti*	California: Mariposa Co.	Blackstone Creek, 6.5 mi NE Coulterville [37.75496 / -120.09336]	JLP 20995 JLP 20996 JLP 20997 JLP 20998 JLP 20999 JLP 21000 JLP 21016 JLP 21017 JLP 21029	MVZ 208184 MVZ 208185 MVZ 208186* MVZ 208187 MVZ 208188 MVZ 208189 MVZ 208190* MVZ 208191* MVZ 208193*	MK871858 MK871859 MK871860 MK871861 MK871862 MK871863 MK871864 MK871865 MK871866	W W W W W W W W W	N/A N/A N/A N/A N/A N/A N/A N/A N/A
27	*P. truei truei*	California: Mono Co.	SE slope Williams Butte [37.90779 / -119.1158]	JLP 21566 JLP 21574 JLP 21601 JLP 21602 JLP 21603	MVZ 208477 MVZ 208478 MVZ 208479* MVZ 208480* MVZ 208481	EU568392 EU568393 EU568392 EU568394 EU568395	W W W W W	N/A N/A N/A N/A N/A
28	*P. truei gilberti*	California: Monterey Co.	Arroyo Seco, 7 mi SW Greenfield [36.271861 / -121.34763]	JLP 17552 JLP 17554 JLP 17566	MVZ 195335 MVZ 195337 MVZ 195338	MK871867 MK871868 MK871869	W W W	N/A N/A N/A
30	*P. truei gilberti*	California: Monterey Co.	Shirtail Canyon, 4.8 mi E Soledad [36.433446 / -121.227359]	JLP 17569	MVZ 195341	MK871870	W	N/A
31	*P. truei chlorus*	California:San Bernardino Co.	Cactus Flat, San Bernardino Mts. [34.31549 / -116.81038]	JLP 18839 JLP 18840 JLP 18841 JLP 18851 JLP 18853	MVZ 198703 MVZ 198704 MVZ 198705 MVZ 198706 MVZ 198708	MK871871 MK871872 MK871873 MK871874 MK871875	W W W W W	N/A N/A N/A N/A N/A
32	*P. truei montepinoris*	California: San Luis Obispo Co.	13.3 mi NW (by road) New Cuyama [35.04427 / -119.89468]	JLP 18142 JLP 18143	MVZ 196791* MVZ 196792	MK871876 MK871877	W W	N/A N/A
33	*P. truei montepinoris*	California: Ventura Co.	mouth Rose Valley [34.53403 / -119.23592]	JLP 18742 JLP 18743 JLP 18744 JLP 18745 JLP 18746	MVZ 198611* MVZ 198612 MVZ 198613 MVZ 198614* MVZ 198615*	MK871879 MK871880 MK871878 MK871881 MK871882	W W W W W	MK907219 MK907220 MK907221 MK907222 MK907223
34	*P. truei truei*	Colorado: Chaffee Co.	7.2 km E, 3.65 km N Poncha Mountain, 38° 30' 30.80" N, 105° 58' 52.65" W, 2190 m	DSR 11995 DSR 11996 DSR 11997 DSR 11998	BYU 37099 BYU 37100 BYU 37101 BYU 37102*	MK871883 MK871884 MK871885 MK871886	E E E E	N/A N/A N/A N/A

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
35	*P. truei nevadensis*	Idaho: Cassia Co.	City of Rocks National Reserve, 1922 m [42.0547 N -113.4109 W]	no data no data no data no data no data no data	UWBM 79645 UWBM 79646 UWBM 79659 UWBM 79661 UWBM 79674 UWBM 79677*	FJ800578 FJ800579 FJ800580 FJ800581 FJ800582 FJ800583	W W W W W W	N/A N/A N/A N/A N/A N/A
36	*P. truei martirensis*	Baja California	Laguna Juarez, 1652 m [32.0460 -115.9156]	STA-C 3476	CIB 3357	MK872273	W	N/A
37	*P. truei martirensis*	Baja California	10 mi E Rancho Melling [30.9667 -115.6000]	STA-C 3458	CIB 3367	MK872274	W	N/A
38	*P. truei lagunae*	Baja California Sur	Valle de la Laguna, Sierra de la Laguna, 1738 m [23.5450 -109.9756]	ERM 1151 ERM 1152 ERM 1155	CIB 10955* CIB 10956 CIB 10959	MK872275 MK872276 MK872277	W W W	N/A N/A N/A
39	*P. truei lagunae*	Baja California Sur	Agua de San Antonio, 9 km N, 26 km E Todos Santos, 1965 m [23.5336 -109.9531]	ERM 1159 ERM 1160 ERM 1161 ERM 1163 ERM 1164	CIB 10960 CIB 10961 CIB 10962 CIB 10964 CIB 10965	MK872278 MK872279 MK872280 MK872281 MK872282	W W W W W	N/A N/A N/A N/A N/A
40	*P. truei lagunae*	Baja California Sur	Los Pinitos, 17.5 km W Santiago, 1476 m [23.4906 -109.8847]	ERM 1167 ERM 1168	CIB 10966 CIB 10967*	MK872283 MK872284	W W	N/A N/A
41	*P. truei lagunae*	Baja California Sur	Palo extrano, Sierra de la Laguna, 1728 m [23.5167 -109.9350]	ERM 1082 ERM 1084 ERM 1092 ERM 1093 ERM 1094 ERM 1096 ERM 1097 ERM 1098 ERM 1117 ERM 1118	CIB 10979* CIB 10980* CIB 10968* CIB 10969 CIB 10970 CIB 10972 CIB 10973* CIB 10974 CIB 10979 CIB 10980*	MK872291 MK872292 MK872285 MK872286 MK872287 MK872288 MK872289 MK872290 MK872294 MK872295	W W W W W W W W W W	N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A
42	*P. truei lagunae*	Baja California Sur	4 km N, 22.5 km W Santiago, 1740 m [23.5167, -109.9358]	ERM 1081 ERM 1085 ERM 1086	CIB 10982 CIB 10986 CIB 10987	MK872293 MK872296 MC872297	W W W	N/A N/A N/A

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
43	*P. truei truei*	Nevada: Clark Co.	Spring Mountains, Kyle Canyon, 0.20 km N, 0.30 km E Little Falls, 36.25681 N, 115.65338 W, 2385 m	LFA 953 LFA 954 LFA 976 LFA 977 LFA 978	UMNH 40841 UMNH 40842 UNNH 40864 UMNH 40865* UMNH 40866	MK871903 MK871904 MK871905 MK871906 MK871907	W W W W W	N/A N/A N/A N/A N/A
44	*P. truei truei*	Nevada: Clark Co.	Spring Mountains, Kyle Canyon, 0.10 km N, 0.25 km E Little Falls, 36.25579 N, 115.65332 W, 2438 m	LFA 964	UMNH 40852*	MK871908	W	N/A
45	*P. truei truei*	Nevada: Clark Co.	Lower Big Falls Canyon, 0.40 km N, 0.17 km E Big Falls, 36.27266 N, 115.67632 W, 2562 m	LFA 894	UMNH 40782	MK871909	W	N/A
46	*P. truei truei*	Nevada: Clark Co.	Spring Mountains, Telephone Canyon, 0.4 km S, 4.2 km E summit Fletcher Peak, 36.28301 N, 115.57520 W, 2056 m	LFA 880 LFA 883 LFA 884	UMNH 40768 UMNH 40771 UMNH no data*	MK871910 MK871911 MK871912	W W W	N/A N/A N/A
47	*P. truei truei*	Nevada: Clark Co.	1.20 km N, 0.25 km W Willow Spring, 36.42722 N, 115.7686 W, 1750 m	DSR 13724 DSR 13725 DSR 13731 DSR 13732 DSR 13740 DSR 13741 DSR 13742 DSR 13853 DSR 13785	BYU 39546 BYU 39547* BYU 39548 BYU 39549 BYU 39550 BYU 39551 BYU 39552 BYU 39553* BYU 39554	MK871887 MK871888 MK871889 MK871890 MK871891 MK871892 MK871893 MK871894 MK871895	W W W W W W W W W	N/A N/A N/A N/A N/A N/A N/A N/A N/A
48	*P. truei truei*	Nevada: Clark Co.	Willow Creek, 1.24 km N, 010 km W Willow Spring, 36.42775 N, 115.76648 W, 1738 m	EAR 10045 EAR 10046	UMNH 40544 UMNH 40545	MK871899 MK871900	W W	N/A N/A
49	*P. truei truei*	Nevada: Clark Co.	1.10 km N, Willow Spring, 36.42644 N, 115.7656 W, 1750 m	DSR 13734 DSR 13747	BYU 39542 BYU 39544	MK871896 MK871897	W W	N/A N/A
50	*P. truei truei*	Nevada: Clark Co.	0.65 km E, 1.00 km Willow Spring, 36.42528 N, 115.7653 W, 1760 m	DSR 13749	BYU 39540	MK872298	W	N/A

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
51	*P. truei truei*	Nevada: Clark Co.	vicinity of Willow Creek, 1.25 km N, 0.10 km E Willow Spring, 36.42784 N, 115.76504W	EAR 10056 EAR 10065	UMNH 40546 UMNH 40547	MK871901 MK871902	W W	N/A N/A
52	*P. truei truei*	Nevada: Mineral Co.	Wassuk Range, Cottonwood Creek, 3 km W summit of Mount Grant, 38.57154 N, 118.82861 W, 2556 m	JLM 1302	UMNH 39141	MK871918	W	N/A
53	*P. truei truei*	Nevada: Mineral Co.	Wassuk Range, Cottonwood Creek, 2 mi. S, 4 mi. W Walker Lake, 38.62374 N, 118.82694 W, 2015 m	JLM 1160 JLM 1204 JLM 1205 JLM 1225 JLM 1240	UMNH 39127 UMNH 39129* UMNH 39130 UMNH 39132* UMNH 39133*	MK871913 MK871914 MK871915 MK871916 MK871917	W W W W W	MK907214 MK907215 MK907216 MK907217 MK907218
54	*P. truei truei*	Nevada: Nye Co.	Peavine Canyon, 0.92 km N, 0.27 km W mouth Horse Canyon, 38.61229 N, 117.30074 W, Toiyabe Range	DSR 9626 DSR 9673 DSR 9737 DSR 9792	BYU 33803 BYU 33804 BYU 33805* BYU 33806	MK871919 MK871920 MK871921 MK871922	W W W W	MK907178 N/A N/A N/A

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
55	*P. truei truei*	Nevada: Nye Co.	Pine Creek Canyon, 38.79616 N, 116.85276 W, 2358 m, Toquima Range	DSR 10110	BYU 34409*	MK871923	W	N/A
				DSR 10112	BYU 34411*	MK871924	W	N/A
				DSR 10113	BYU 34412	MK871925	W	N/A
				DSR 10114	BYU 34413	MK871926	W	N/A
				DSR 10115	BYU 34414	MK871927	W	N/A
				DSR 10116	BYU 34415	MK871928	W	N/A
				DSR 10117	BYU 34416	MK871929	W	N/A
				DSR 10118	BYU 34417	MK871930	W	N/A
				DSR 10119	BYU 34418	MK871931	W	N/A
				DSR 10188	BYU 34424	MK871932	W	N/A
				DSR 10196	BYU 34425	MK871933	W	N/A
				DSR 10197	BYU 34426	MK871934	W	N/A
				DSR 10198	BYU 34427*	MK871935	W	N/A
				DSR 10199	BYU 34428*	MK871936	W	N/A
				DSR 10201	BYU 34430	MK871937	W	N/A
				DSR 10202	BYU 34431*	MK871938	W	N/A
				DSR 10203	BYU 34432	MK871939	W	N/A
				DSR 10204	BYU 34433	MK871940	W	N/A
				DSR 10231	BYU 34434	MK871941	W	N/A
				DSR 10232	BYU 34435	MK871942	W	N/A
				DSR 10233	BYU 34436	MK871943	W	N/A
				DSR 10234	BYU 34437	MK871944	W	N/A
				DSR 10235	BYU 34438	MK871945	W	N/A
				DSR 10236	BYU 34439*	MK871946	W	N/A
				DSR 10237	BYU 34440	MK871947	W	N/A
				DSR 10238	BYU 34441*	MK871948	W	N/A
				DSR 10239	BYU 34442	MK871949	W	N/A
				DSR 10240	BYU 34443*	MK871950	W	N/A
56	*P. truei truei*	Nevada: Nye Co.	Monitor Valley near mouth of Pine Creek Canyon, 38.79655 N, 116.82616 W, 2179 m	DSR 10200	BYU 34344	MK871951	W	N/A
57	*P. truei truei*	Nevada: Nye Co.	5.55 km E, 3.56 km S of South Toiyabe Peak, 38.88816 N, 117.24146 W, 1910 m, Toiyabe Range	DSR 9286	BYU 33381	MK871961	W	N/A
				DSR 9362	BYU 33380*	MK871962	W	N/A

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
58	*P. truei nevadensis*	Nevada: White Pine Co.	5.3 km W Baker, 39° 0' 36.5" N, 114° 11' 05" W, 1885 m	DSR 13312 DSR 13427 DSR 13428 DSR 13429 DSR 13430 DSR 13431 DSR 13432 DSR 13433 DSR 13434	BYU 38265 BYU 38269* BYU 38270* BYU 38271 BYU 38272* BYU 38273 BYU 38274 BYU 38275 BYU 38276	MK871952 MK871953 MK871954 MK871955 MK871956 MK871957 MK871958 MK871959 MK871960	W W W W W W W W W	N/A N/A N/A N/A N/A N/A N/A N/A N/A
59	*P. truei truei*	New Mexico: Socorro Co.	32 mi S, 23.5 mi W Socorro	TK 13474	TTU36053	AY376434	E	N/A
60	*P. truei preblei*	Oregon: Deschutes Co.	5 km W Tumalo, 44° 02' N, 121° 34' W	RAK 14	BYU 21081	MK871963	W	N/A
61	*P. truei*	Oklahoma; Cimarron Co.	Black Mesa Nature Preserve/ State Park	TK 185648	TTU 136502	MN022904	E	N/A
63	*P. truei comanche*	Texas: Armstrong Co.	7 mi N, 6.25 mi E Wayside	TK 40209 TK 40211 TK 40215	TTU 61541 TTU 61543 TTU 61547	EU568384 AY376429 AY376428	E E E	N/A N/A N/A
64	*P. truei comanche*	Texas: Briscoe Co.	3 mi N Quitaque, Caprock Canyons State Park	TK 21841 TK 21856 TK 27737 TK 54856 TK 51107	TTU 47286* TTU 47296 TTU 47300 TTU 74991 TTU 69561	MK871967 MK871964 MK871965 AY376431 MK871966	E E E E E	N/A N/A MK907224 MK907225
66	*P. truei nevadensis*	Utah: Beaver Co.	San Francisco Mountains, 1.75 km N, 0.9 km E Frisco Peak summit, 38.53608 N, 113.27693 W, 2644 m	AWB 98 AWB 101 AWB 102 AWB 103 AWB 105	UMNH 37581 UMNH 37584* UMNH 37585 UMNH 37586* UMNH 37588*	MK871972 MK871973 MK871974 MK871975 MK871976	W W W W W	N/A N/A N/A N/A N/A
67	*P. truei nevadensis*	Utah: Box Elder Co.	3.6 km N, 8.8 km W Crystal Peak, 41.9508 N, 113.3341 W, 1995 m, Clear Creek Canyon	DSR 14963 DSR 14964 DSR 14965 DSR 14966 DSR 14904 DSR 14905 DSR 14937 DSR 14938	BYU 40191 BYU 40192 BYU 40193 BYU 40194 BYU 40195 BYU 40196 BYU 40197 BYU 40198	MK871979 MK871980 MK871981 MK871982 MK871983 MK871984 MK871985 MK871986	W W W W W W W W	N/A N/A N/A N/A N/A N/A N/A N/A
68	*P. truei nevadensis*	Utah: Carbon Co.	3.3 km E, 2.5 km N Castle Gate, 39° 45' 02.00" N, 110° 49' 48.50" W, 2005 m	DSR 13092 DSR 13093	BYU 38484 BYU 38485*	MK871970 MK871971	W W	N/A N/A

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
69	*P. truei nevadensis*	Utah: Emery Co.	7.35 km N, 11.75 km W Huntington, 39° 23' 29.95" N, 111° 06' 11.85" W, 2020 m	DSR 11822 DSR 11823 DSR 11877 DSR 12804 DSR 12807 DSR 12808 DSR 12809 DSR 12810 DSR 12811	BYU 36654 BYU 36655 BYU 36736* BYU 38022 BYU 38023* BYU 38024 BYU 38025* BYU 38026* BYU 38027*	MK872048 MK872049 MK872070 MK872050 MK872051 MK872052 MK872053 MK872054 MK872055	W W W W W W W W W	N/A N/A N/A N/A N/A N/A N/A N/A N/A
70	*P. truei nevadensis*	Utah: Emery Co.	Bear Creek Campground, 7.45 km N, 11.50 km W Huntington, 39° 23' 30.80" N, 111° 05' 58.75" W, 2000 m	DSR 11722	BYU 36466	MK871987	W	N/A
71	*P. truei nevadensis*	Utah: Emery Co.	7.85 km N, 11.85 km W Huntington, 39° 23' 42.40" N, 111° 06' 10.95" W, 2050 m	DSR 11754 DSR 11755 DSR 11756 DSR 11874 DSR 11875 DSR 11876	BYU 36665 BYU 36666* BYU 36667 BYU 36668 BYU 36669* BYU 36670*	MK872056 MK872057 MK872058 MK872059 MK872060 MK872061	W W W E W W	MK907179 MK907180 MK907181 MK907183 MK907184 MK907185
72	*P. truei nevadensis*	Utah: Emery Co.	7.95 km N, 12.30 km W Huntington, 39 23' 43.25" N, 111 06' 30.70" W, 2050 m	DSR 11825 DSR 11826 DSR 11834 DSR 11835 DSR 11836 DSR 11837 DSR 11838	BYU 36671* BYU 36672* BYU 36673 BYU 36674 BYU 36675 BYU 36676* BYU 36677	MK872062 MK872063 MK872064 MK872065 MK872066 MK872067 MK872068	E W E E E W E	MK907186 MK907187 MK907191 MK907192 MK907193 MK907194 MK907195
73	*P. truei nevadensis*	Utah: Emery Co.	8.00 km N, 12.35 km W Huntington, 39° 23' 44.35" N, 111° 06' 33.95" W, 2055 m	DSR 11830 DSR 11831 DSR 11832 DSR 11833 DSR 11839	BYU 36680* BYU 36681* BYU 36682 BYU 36683 BYU 36678	MK872071 MK872072 MK872073 MK872074 MK872069	E E E E W	MK907188 N/A MK907189 MK907190 MK907196
74	*P. truei nevadensis*	Utah: Emery Co.	8.05 km N, 12.25 km W Huntington, 39° 23' 46.45" N, 111° 06' 30.75" W, 2065 m	DSR 11869 DSR 11870 DSR 11871 DSR 11872 DSR 12791 DSR 12792 DSR 12793 DSR 12794	BYU 36684 BYU 36685 BYU 36686 BYU 36687* BYU 38037 BYU 38038 BYU 38039 BYU 38040	MK872045 MK872046 MK872047 MK872075 MK872076 MK872077 MK872078 MK872044	E E W W E W E W	MK907201 MK907202 MK907203 MK907204 MK907205 MK907206 MK907207 MK907208

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
75	*P. truei nevadensis*	Utah: Emery Co.	Rilda Canyon, 9.85 km N, 13.40 km W Huntington, 39° 24' 44.90" N, 111° 07' 22.60" W, 2150 m	DSR 11840 DSR 11841 DSR 11842 DSR 11843	BYU 36720 BYU 36721 BYU 36722 BYU 36723	MK872141 MK872142 MK872143 MK872144	W W W W	MK907197 MK907198 MK907199 MK907200
76	*P. truei nevadensis*	Utah: Emery Co.	Rilda Canyon, 9.80 km N, 13.25 km W Huntington, 39° 24' 50.75" N, 111° 07' 15.60" W, 2130 m	DSR 11775 DSR 11776 DSR 11777	BYU 36712* BYU 36713 BYU 36714*	MK872138 MK872139 MK872140	W E W	MK907182 N/A N/A
77	*P. truei nevadensis*	Utah: Emery Co.	Mill Fork Canyon, 11.00 km N, 14.15 km W Huntington, 39° 25' 24.00" N, 111° 08' 01.25" W, 2215 m	DSR 11749	BYU 36698*	MK872109	W	N/A
78	*P. truei nevadensis*	Utah: Emery Co.	8.8 km S, 0.5 km W Window Blind Peak, 38.9657 N, 110.6633 W, 1800 m	DSR 15240 DSR 15243 DSR 15226 DSR 15227 DSR 15228	BYU 40714 BYU 40706 BYU 40702 BYU 40703* BYU 40704	MK871826 MK871827 MK872135 MK872136 MK872137	W W W E E	N/A N/A N/A N/A N/A
79	*P. truei nevadensis*	Utah: Emery Co.	0.1 km N, 4.5 km W South Horn Mountain, 39° 08' 10.45" N, 111° 16' 05.25" W, 2000 m, Ferron Canyon	DSR 13229	BYU 38496	MK872164	W	N/A
80	*P. truei nevadensis*	Utah: Emery Co.	0.2 km N, 4.6 km W South Horn Mountain, 39° 08' 14.50" N, 111° 16' 10.50" W, 2000 m, Ferron Canyon	DSR 13228	BYU 38498	MK872165	E	N/A
81	*P. truei nevadensis*	Utah: Emery Co.	12.5 km N, 22.7 km W Last Chance Benches, 39° 26' 43.75" N, 110° 22' 01.80" W, 1880 m	DSR 13216 DSR 13217 DSR 13219 DSR 13220 DSR13221	BYU 38486 BYU 38487 BYU 38489 BYU 38490 BYU 38491	MK872102 MK872103 MK872104 MK872105 MK872106	W W E W W	N/A N/A N/A N/A N/A
82	*P. truei nevadensis*	Utah: Emery Co.	21.1 km N, 17.7 km W Last Chance Benches, 39° 27' 45.60" N, 110° 20' 24.10" W, 2060 m	DSR 13226	BYU 38492	MK907177	W	N/A
83	*P. truei nevadensis*	Utah: Emery Co.	21.4 km N, 17.9 km W Last Chance Benches, 39° 27' 52.05" N, 110° 20' 36.40" W, 1995 m	DSR 13171 DSR 13172 DSR 13173	BYU 38493 BYU 38494* BYU 38495	MK872094 MK872095 MK872101	W W W	N/A N/A N/A

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
84	*P. truei nevadensis*	Utah: Juab Co.	2.6 km E, 0.7 km S Indian Squaw Rock, 39° 52' 46.45" N, 113° 49' 11.50" W, 1910 m, Deep Creek Range	DSR 11946	BYU 37056	MK872079	W	N/A
85	*P. truei nevadensis*	Utah: Garfield Co.	Grosvenor Arch Day Use Area, 37° 27.1456' N, 111° 49.5453' W, 1870 m	DSR 7143 DSR 7144 DSR 7145 DSR 7147 DSR 7148 DSR 7168 DSR 7169 DSR 7170 DSR 7171	BYU 20176 BYU 20177 BYU 20178* BYU 20180 BYU 20181 BYU 20185 BYU 20186 BYU 20187* BYU 20188	MK872029 MK872030 MK872031 MK872032 MK872033 MK872034 MK872035 MK872036 MK872037	W W W W W W W W W	N/A N/A N/A N/A N/A N/A N/A N/A N/A
86	*P. truei nevadensis*	Utah: Garfield Co.	3.7 km N and 0.7 km W Tropic, 37° 39' 25.80" N, 112° 05' 31.30" W, 2005 m	DSR 8777 DSR 8778 DSR 8779 DSR 8780 DSR 8781	BYU 33424* BYU 33425 BYU 33426 BYU 33427* BYU 33428	MK872215 MK872216 MK872217 MK872218 MK872214	W W W W W	N/A N/A N/A N/A N/A
87	*P. truei truei*	Utah: Garfield Co.	Wolverine Petrified Forest, 37° 42.0902 N, 111° 12.3641 W, 1340 m	JLA 6 DSR 6051 DSR 6081 DSR 6082 DSR 6084 DSR 6087	BYU 20313 BYU 20316 BYU 20323 BYU 20324 BYU 20325 BYU 20328	MK872220 MK872223 MK872224 DQ861375 MK872221 MK872222	W W W E W W	N/A N/A N/A N/A N/A N/A
88	*P. truei truei*	Utah: Garfield Co.	Escalante River Trailhead, 37° 46.3305' N, 111° 25.1386 W, 1695 m	JLA 10 JLA 12 JLA 13	BYU 20112 BYU 20113 BYU 20114	MK872014 MK872016 MK872015	W W W	N/A N/A N/A
89	*P. truei truei*	Utah: Garfield Co.	1.85 km E, 3.75 km S Wolverine Bench, 37° 48' 11.05" N, 111°12' 27.35" W, 1650 m	DSR 11182 DSR 11183 DSR 11184 DSR 11185 DSR 11186 DSR 11187 DSR 11188	BYU 36063 BYU 36064 BYU 36065 BYU 36066* BYU 36067* BYU 36068* BYU 36069	MK872225 MK872226 MK872227 MK872228 MK872229 MK872230 MK872231	W W W W W W W	N/A N/A N/A N/A N/A N/A N/A

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
90	*P. truei truei*	Utah: Garfield Co.	2.45 km E, 3.20 km S Wolverine Bench, 37° 48' 32.50" N, 111° 12' 06.25" W, 1670 m	ALA 124	BYU 36072	MK872254	W	N/A
				ALA 125	BYU 36073	MK872255	W	N/A
				ALA 126	BYU 36074	MK872256	W	N/A
				ALA 127	BYU 36075	MK872257	W	N/A
				ALA 128	BYU 36076	MK872258	W	N/A
				ALA 129	BYU 36077*	MK872259	W	N/A
				ALA 130	BYU 36078*	MK872260	W	N/A
				DSR 11208	BYU 36095	MK872232	W	N/A
				DSR 11209	BYU 36096	MK872233	E	N/A
				DSR 11216	BYU 36101	MK872234	W	N/A
				DSR 11221	BYU 36105	MK872235	W	N/A
91	*P. truei truei*	Utah: Garfield Co.	3.10 km E, 2.60 km S Wolverine Bench, 37° 48' 53.55" N, 111° 11' 40.25" W, 1685 m	ALA 110	BYU 36139	MK872261	W	N/A
				ALA 111	BYU 36140	MK872262	W	N/A
				ALA 112	BYU 36141*	MK872263	W	N/A
				ALA 113	BYU 36142	MK872264	W	N/A
				ALA 114	BYU 36143	MK872265	W	N/A
				ALA 115	BYU 36144	MK872266	W	N/A
				ALA 116	BYU 36145	MK872267	W	N/A
				ALA 117	BYU 36146	MK872268	W	N/A
				ALA 118	BYU 36147*	MK872269	W	N/A
				ALA 119	BYU 36148*	MK872270	W	N/A
				ALA 120	BYU 36149	MK872271	W	N/A
				ALA 121	BYU 36150	MK872272	W	N/A
92	*P. truei truei*	Utah: Garfield Co.	0.50 km E, 1.30 km N Wolverine Bench, 37° 50' 56.55" N, 111° 13' 14.25" W, 1690 m	ALA 101	BYU 36038*	MK872236	W	N/A
				ALA 102	BYU 36039	MK872237	E	N/A
				ALA 103	BYU 36040	MK872238	E	N/A
				ALA 104	BYU 36041*	MK872239	W	N/A
				ALA 105	BYU 36042	MK872240	W	N/A
				ALA 106	BYU 36043	MK872241	W	N/A
				ALA 107	BYU 36044*	MK872242	W	N/A
				ALA 108	BYU 36045	MK872243	W	N/A
				ALA 131	BYU 36046*	MK872244	W	N/A
				ALA 132	BYU 36047	MK872245	W	N/A
				ALA 133	BYU 36048	MK872246	E	N/A
				ALA 134	BYU 36049*	MK872247	E	N/A
				ALA 137	BYU 36051	MK872248	W	N/A
				ALA 138	BYU 36052	MK872249	W	N/A
				ALA 139	BYU 36053	MK872250	W	N/A
				ALA 140	BYU 36054*	MK872251	W	N/A
				ALA 141	BYU 36055	MK872252	W	N/A
				ALA 142	BYU 36056	MK872253	W	N/A

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
93	*P. truei truei*	Utah: Garfield Co.	16.30 km E, 5.85 km S Steep Creek Bench, 37° 53' 13.90" N, 111° 06' 14.15" W, 2035 m	ALA 165	BYU 36016	MK872198	W	N/A
				ALA 166	BYU 36017	MK872199	W	N/A
				ALA 168	BYU 36019*	MK872200	W	N/A
				ALA 169	BYU 36020	MK872201	W	N/A
				ALA 170	BYU 36021	MK872202	W	N/A
				ALA 171	BYU 36022	MK872203	W	N/A
				ALA 172	BYU 36023	MK872204	W	N/A
94	*P. truei truei*	Utah: Garfield Co.	5.75 km E, 2.45 km S Steep Creek Bench, 37° 55' 12.60" N, 111° 13' 24.30" W, 1800 m	ALA 179	BYU 35936	MK872175	W	N/A
				ALA 180	BYU 35937	MK872176	W	N/A
				ALA 181	BYU 35938*	MK872177	W	N/A
				ALA 182	BYU 35939*	MK872178	W	N/A
				ALA 183	BYU 35940	MK872179	W	N/A
				ALA 184	BYU 35941	MK872180	W	N/A
				ALA 185	BYU 35942*	MK872181	W	N/A
				ALA 186	BYU 35943	MK872182	W	N/A
				ALA 187	BYU 35944	MK872183	W	N/A
				ALA 188	BYU 35945	MK872184	W	N/A
				ALA 189	BYU 35946	MK872185	W	N/A
				ALA 190	BYU 35947	MK872186	W	N/A
95	*P. truei truei*	Utah: Garfield Co.	5.70 km E, 1.65 km S Steep Creek Bench, 37° 55' 42.45" N, 111° 13' 21.80" W, 1820 m	DSR 11374	BYU 35921	MK872171	W	N/A
				DSR 11375	BYU 35922*	MK872172	W	N/A
				DSR 11376	BYU 35923	MK872173	W	N/A
				DSR 11377	BYU 35924*	MK872174	W	N/A
96	*P. truei truei*	Utah: Garfield Co.	11.00 km E, 0.90 km S Steep Creek Bench, 37° 56' 02.95" N, 111° 09' 47.70" W, 1965 m	ALA 143	BYU 35976*	MK872187	W	N/A
				ALA 144	BYU 35977	MK872188	W	N/A
				ALA 145	BYU 35978	MK872189	W	N/A
				ALA 146	BYU 35979	MK872190	W	N/A
				ALA 147	BYU 35980*	MK872191	W	N/A
				ALA 148	BYU 35981	MK872192	W	N/A
				ALA 149	BYU 35982	MK872193	W	N/A
				ALA 150	BYU 35983*	MK872194	W	N/A
				ALA 151	BYU 35984*	MK872195	W	N/A
				ALA 152	BYU 35985*	MK872299	W	N/A
				ALA 173	BYU 35986	MK872196	W	N/A
				ALA 174	BYU 35987	MK872197	W	N/A

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
97	*P. truei truei*	Utah: Garfield Co.	9.35 km E, 0.20 km N Mount Pennell, 37° 57' 49.25" N, 110° 41' 11.85" W, 1870 m	ALA 160 ALA 161 ALA 162 ALA 163 ALA 164 DSR 11476 DSR 11477	BYU 35900 BYU 35901* BYU 35902 BYU 35903 BYU 35904 BYU 35905 BYU 35906	MK872112 MK872113 MK872114 MK872115 MK872116 MK872117 MK872118	W W W W W W W	N/A N/A N/A N/A N/A N/A N/A
98	*P. truei truei*	Utah: Garfield Co.	7.55 km E, 0.50 km N Mount Pennell, 37° 57' 53.75" N, 110° 42' 23.40" W, 1950 m	ALA 195 DSR 11458	BYU 35857 BYU 35863*	MK872110 MK872111	W W	N/A N/A
99	*P. truei truei*	Utah: Garfield Co.	Lampstand (Circle Cliffs), 37° 59.515' N, 111° 10.448' W, 1950 m	DSR 7575 DSR 7576 DSR 7577 DSR 7578	BYU 23624 BYU 23625 BYU 23626 BYU 23627	MK872090 MK872091 MK872092 MK872093	E W E W	N/A N/A N/A N/A
100	*P. truei truei*	Utah Grand Co.	Onion Creek [38.7113 / -109.32825]	JLP 19040	MVZ 199482	EU568422	E	N/A
101	*P. truei truei*	Utah: Grand Co.	Rio Mesa field station, south side of Dolores River, 38.79653 N, 109.18188 W, 1291 m	EAR 9459 EAR 9460 EAR 9461	UMNH 38076* UMNH 38077 UMNH 38078*	MK872005 MK872006 MK872007	E E E	N/A N/A N/A
102	*P. truei truei*	Utah: Grand Co.	Utah Bottoms, Dolores River, 38.79771 N, 109.20597 W, 1275 m	EAR 8186	UMNH 34767	MK872002	E	N/A
103	*P. truei truei*	Utah: Grand Co.	Utah Bottoms, Dolores River, 38.79786 N, 109.1973 W, 1288 m	LWM 6	UMNH 35708	MK872010	E	N/A
104	*P. truei truei*	Utah: Grand Co.	Utah Bottoms, Dolores River, 38.79815 N, 109.20672 W, 1276 m	EAR 8193 EAR 8194	UMNH 34775 UMNH 34776	MK872003 MK872004	E E	N/A N/A
105	*P. truei truei*	Utah: Grand Co.	Utah Bottoms, Dolores River, 38.79862 N, 109.17841 W, 1277 m	LWM 1 LWM 2	UMNH 35706 UMNH 35707*	MK872008 MK872009	E E	N/A N/A
106	*P. truei truei*	Utah: Grand Co.	0.25 km S, 0.30 km E Dewey Bridge, south of USA: Colorado River, 38.80935 N, 109.2994 W, 1268 m	EAR 9445	UMNH 38144	MK872000	E	N/A

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
107	*P. truei truei*	Utah: Grand Co.	0.60 km N, 0.90 km E Dewey Bridge, north of USA: Colorado River, 38.8171 N, 109.29239 W, 1263 m	EAR 9629	UMNH 38127	MK872001	E	N/A
108	*P. truei truei*	Utah: Grand Co.	Rock Canyon Corral [38.8188 / -109.7728]	JLP 19057 JLP 19058 JLP 19059	MVZ 199483 MVZ 199484 MVZ 199485	EU568423 EU568424 EU568425	E E E	N/A N/A N/A
109	*P. truei truei*	Utah: Grand Co.	ca. 0.5 mi E Rock Canyon Corral [38.83132 / -109.76837]	JLP 19064 JLP 19065 JLP 19066	MVZ 199486 MVZ 199487* MVZ 199488	EU568426 EU568427 EU568428	E E E	N/A N/A N/A
110	*P. truei truei*	Utah: Kane Co.	Buckskin Mountain, 37° 02.4629' N, 112° 04.4549' W, 1920 m	QRS 180	BYU 23634	MK871969	W	N/A
111		Utah: Kane Co.	Camp Flat, 37° 29.4467' N, 111° 33.0377' W, 2000 m	DSR 7153 DSR 7318	BYU 23636 BYU 23637	MK871977 MK871978	W W	N/A N/A
112	*P. truei truei*	Utah: Kane Co.	Dance Hall Rock, 37° 21.0678' N, 111 06.042' W, 1440 m	NRM 92 NRM 152 NRM 156 NRM 158 NRM 159	BYU 20229* BYU 20232 BYU 20233* BYU 20234* BYU 20235*	MK871988 MK871989 MK871990 MK871991 MK871992	W W W W W	N/A N/A N/A N/A N/A
113		Utah: Kane Co.	Devil's Garden, 37° 35.0064' N, 111° 25.5047' W, 1475 m	QRS 5 JMH 4 JMH 5 JMH 16 JMH 17 JMH 19 JMH 20	BYU 23591 BYU 23573* BYU 23574 BYU 23576 BYU 23577 BYU 23579* BYU 23580*	MK871993 MK871994 MK871995 MK871996 MK871997 MK871998 MK871999	W W W W W W W	N/A N/A N/A N/A N/A N/A N/A
114	*P. truei truei*	Utah: Kane Co.	4.50 km E, 5.30 km S Elephant Butte, 37° 01' 30.80" N, 112 47' 0.10" W, 1755 m	DSR 11903 DSR 11904 DSR 11905	BYU 37450 BYU 37451 BYU 37452*	MK872011 MK872012 MK872013	W W W	N/A N/A N/A
115	*P. truei truei*	Utah: Kane Co.	6.50 km E, 9.20 km S Flag Point, 37° 0' 05.75" N, 112 10' 41.25" W, 1710 m	ZLM 145 ZLM 146 ZLM 147 ZLM 148 ZLM 149 ZLM 150	BYU 37475 BYU 37476 BYU 37477 BYU 37478 BYU 37479 BYU 37480	MK872018 MK872019 MK872020 MK872021 MK872022 MK872023	W E E W W W	N/A N/A N/A N/A N/A N/A

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
116	*P. truei truei*	Utah: Kane Co.	11.30 km E, 1.00 km S Flag Point, 37° 03' 07.20" N, 112 09' 10.95" W, 1730 m	ZLM 157 ZLM 158	BYU 37550 BYU 37551	MK872024 MK872025	W W	N/A N/A
117	*P. truei truei*	Utah: Kane Co.;	Fourmile Bench, 37° 23.1313' N, 111° 41.3450' W, 1765 m	MEW 6	BYU 23644	MK872027	W	N/A
118	*P. truei truei*	Utah: Kane Co.	Fourmile Bench, 37° 23.3933' N, 111 41.3311' W, 1770 m	LDC 87 QRS 183	BYU 23647* BYU 23648	MK872026 MK872028	W W	N/A N/A
119	*P. truei truei*	Utah: Kane Co.	59 km E, 25 km N Kanab, 415040 E, 4121500 N	DSR 5661	BYU 18075	MK872080	E	N/A
120	*P. truei truei*	Utah: Kane Co.	Kitchen Corral, 37° 13.3150' N 111° 07.0586' W, 2200 m	DSR 7300 DSR 7301 LDC 4 LDC 5 MEW 26 MEW 75 MEW 78 MEW 80 MEW 86	BYU 23747* BYU 23748* BYU 23649 BYU 23650 BYU 23656 BYU 23664 BYU 23665* BYU 23667 BYU 23671	MK872081 MK872082 MK872083 MK872084 MK872085 MK872086 MK872087 MK872088 MK872089	W W E W W W W W W	N/A N/A N/A N/A N/A N/A N/A N/A N/A
121	*P. truei truei*	Utah: Kane Co.	No Man's Mesa, 37° 15.4072' N, 111° 30.0367' W, 1680 m	LDC 7 LDC 51 LDC 52 LDC 53 LDC 55 LDC 58 LDC 61 LDC 62 MEW 33 MEW 37 MEW 43	BYU 23702 BYU 23688* BYU 23689* BYU 23690 BYU 23691* BYU 23693* BYU 23694* BYU 23695* BYU 23708* BYU 23710* BYU 23698	MK872119 MK872120 MK872121 MK872122 MK872123 MK872124 MK872125 MK872126 MK872127 MK872128 MK872129	W W W W W W W W W W W	N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
122	*P. truei truei*	Utah: Kane Co.	Smoky Hollow, 37° 09.41' N, 111° 32.10' W, 1270 m	DSR 6003	BYU 20288	HQ269735	W	N/A
				DSR 6037	BYU 20292	MK872145	W	N/A
				DSR 6040	BYU 20294*	MK872146	W	N/A
				DSR 7174	BYU 20307*	MK872147	W	N/A
				DSR 7175	BYU 20308	MK872148	W	N/A
				DSR 7176	BYU 20309	MK872149	E	N/A
				DSR 7311	BYU 23732*	MK872150	W	N/A
				DSR 7314	BYU 23734	MK872151	W	N/A
				HAJ 13	BYU 23713*	MK872152	W	N/A
				HAJ 14	BYU 23714*	MK872153	W	N/A
				HAJ 18	BYU 23718*	MK872154	W	N/A
				HAJ 20	BYU 23720	MK872155	W	N/A
				HAJ 23	BYU 23722	MK872156	W	N/A
				HAJ 24	BYU 23723*	MK872157	W	N/A
				HAJ 25	BYU 23724	MK872158	W	N/A
				MM 6	BYU 23730*	MK872159	W	N/A
				MM 7	BYU 23731	MK872160	W	N/A
				LDC 1	BYU 23712*	MK872161	W	N/A
				RK 6	BYU 23727*	MK872162	W	N/A
				RK 7	BYU 23728*	MK872163	W	N/A
123	*P. truei nevadensis*	Utah: Millard Co.	Ferguson Desert Snake Pass Rd., 3.7 km E Shotgun Knoll, 38° 54.732' N, 113° 37.139' W, 1747 m	BMB 71	BYU 24608	MK872107	W	N/A
				BMB 72	BYU 24609	MK872108	W	N/A
124	*P. truei nevadensis*	Utah: Sanpete Co.	4.3 km N, 1.1 km W, Fairview, 39° 40' 25" N, 111 27' 10" W, 1950 m	JLS 89	BYU 18440	MK872017	W	N/A
125	*P. truei truei*	Utah: San Juan Co.	4.5 km E, 7.6 km S South Peak, Abajo Mountains, 37° 44' 25.20" N, 109° 24' 50.25" W, 2185 m	DSR 13097	BYU 38499	MK871968	E	N/A
126	*P. truei truei*	Utah: San Juan Co.	La Sal Mtns, tributary to Brumley Creek, 38.4741° N, 109.34287° W, 2180 m	EAR 5841	UMNH 31487	MK872096	E	N/A
				EAR 5842	UMNH 31488*	MK872097	E	N/A
				EAR 5843	UMNH 31406	MK872098	E	N/A
				EAR 5844	UMNH 31407*	MK872099	E	N/A
				EAR 5849	UMNH 31408*	MK872100	E	N/A
127	*P. truei truei*	Utah: San Juan Co.	0.8 km E, 3.6 km N Navajo Mountain. 1830 m [37.0507 -110.7985]	EAR 10954	UMNH 43001	MK871828	E	MK907212
				EAR 10955	UMNH 43002	MK871829	E	MK907213

Appendix I. (cont.)

Map Code	Scientific Name	State/ County	Locality	Collector No. or Tissue No.	Museum Voucher No.	Cytochrome-*b* GenBank No.	Cytochrome-*b* haplogroup	*Fgb* GenBank No.
128	*P. truei nevadensis*	Utah: Tooele Co.	South Willow Canyon, 40° 30' 10.46" N, 112° 33' 33.13" W, 1875m, Stansbury Range	DSR 9907	BYU34137*	MK872166	W	N/A
129	*P. truei nevadensis*	Utah: Tooele Co.	South Willow Canyon, 40° 30' 6.85" N, 112° 33' 39.90" W, 1865m, Stansbury Range	DSR 12920 DSR 12921 DSR 12922 DSR 13054	BYU 38061* BYU 38062 BYU 38063* BYU 38065	MK872167 MK872168 MK872169 MK872170	E E E W	N/A N/A N/A N/A
130	*P. truei truei*	Utah: Uintah Co.	Rat Hole Canyon, 39° 35' 20" N, 109° 05' 30" W, 2160 m	EA 195	BYU 17166	MK872130	E	N/A
131	*P. truei truei*	Utah: Uintah Co.	Willow Creek Canyon, 39° 37' 15" N, 109° 33' 40" W, 1770 m	BWC 56	BYU 17799	MK872219	E	N/A
132	*P. truei nevadensis*	Utah: Utah Co.	17 km E, 2.8 km S, Thistle, 39° 48' 50" N, 111° 17' 40" W, 1975 m	JLS 31 JLS 35 JLS 36 JLS 45 JLS 49	BYU 18550 BYU 18551* BYU 18552 BYU 18553* BYU 18554*	MK872205 MK872206 MK872207 MK872208 MK872209	W W W W W	N/A N/A N/A N/A N/A
133	*P. truei nevadensis*	Utah: Utah Co.	5.7 km S, 1.3 km W Red Mountain, 40.0768 N, 111.3845 W, 1645 m	DSR 15209	BYU 40622	MK872131	W	N/A
134	*P. truei nevadensis*	Utah: Utah Co.	5.4 km S, 3.0 km W Red Mountain, 40.0802 N, 111.4028 W, 1630 m	DSR 15221 DSR 15223 DSR 15224	BYU 40634* BYU 40635* BYU 40636	MK872132 MK872133 MK872134	W W W	N/A N/A N/A
135	*P. truei truei*	Utah: Wayne Co.	Aquarius Plateau, Carcass Creek, 3.0 km S, 0.5 km W Grover	EAR 5341 EAR 5351 EAR 5352 EAR 5353 EAR 5354 EAR 5376	UMNH 30959* UMNH 30960 UMNH 31082 UMNH 30961 UMNH 31083* UMNH 30962	MK872038 MK872039 MK872040 MK872041 MK872042 MK872043	W W W W W W	N/A N/A N/A N/A N/A N/A
136	*P. truei truei*	Utah, Wayne Co.	Torrey [38.30081/-111.41954]	EAR 8576 EAR 8577 EAR 8595 EAR 8596	UMNH 36133 UMNH 36134* UMNH 36152* UMNH 36153*	MK872210 MK872211 MK872212 MK872213	W W W W	N/A N/A N/A N/A

APPENDIX II

Map codes (corresponding numbers in Fig. 1), collecting location, latitude and longitude (either directly measured or estimated), specimen identification numbers (museum voucher or collector numbers), and haplogroup designations for *Peromyscus truei* included in the morphological study. Haplogroup designations: EE – specimens from northern Arizona, Colorado, western Oklahoma, northern Texas, and eastern Utah; WW – specimens from California; UE and UW – specimens from Huntington Canyon, Emory County, Utah; "inferred" indicates that a particular specimen was not genotyped, but that its haplogroup designation was inferred based on the geographic proximity of specimens of known genotype.

Map Code	Scientific Name	Country: State/Province	Locality	Collector or Tissue No.	Museum Voucher No.	Haplogroup
2	*P. truei truei*	Arizona: Coconino Co., Navajo Nation	35.9300 N, 111.7229 W, 1925 m	DSR 15507 DSR 15508 DSR 15509	BYU 41011 BYU 41012 BYU 41013	EE EE EE
10	*P. truei truei*	Arizona: Navajo Co.	3 mi S Woodruff [34.730120 -110.052399]	TK 77921	TTU 78507	EE
13	*P. truei montepinoris*	California: Kern Co.	Cuddy Canyon, 1 mi E Frazier Park [34.8213833333 / -118.9239166667]	JLP 17906	MVZ 196169	WW
14	*P. truei montepinoris*	California: Kern Co.	2 mi NNW Eagle Rest Peak, San Emigido Mts. [34.92801 / -119.1362]	JLP 18770	MVZ 198616	WW
16	*P. truei montepinoris*	California: Kern Co.	Temblor Range summit on Hwy. 58 [35.35564 / -119.82853]	JLP 18730 JLP 18731 JLP 18734	MVZ 198606 MVZ 198607 MVZ 198610	WW WW WW
21	*P. truei montepinoris*	California: Los Angeles Co.	0.4 mi W Gorman [34.79703 / -118.86111]	JLP 18589 JLP 18590	MVZ 198392 MVZ 198393	WW WW
22	*P. truei gilberti*	California: Mariposa Co.	Hunter Valley Mountain [37.61872 / -120.18696]	JLP 20946 JLP 20947 JLP 20960 JLP 20970	MVZ 208172 MVZ 208173 MVZ 208176 MVZ 208177	WW WW WW WW
26	*P. truei gilberti*	California: Mariposa Co.	Blackstone Creek, 6.5 mi NE Coulterville [37.75496 / -120.09336]	JLP 20995 JLP 20997 JLP 20998 JLP 20999 JLP 21000	MVZ 208184 MVZ 208186 MVZ 208187 MVZ 208188 MVZ 208189	WW WW WW WW WW
28	*P. truei gilberti*	California: Monterey Co.	Arroyo Seco, 7 mi SW Greenfield [36.271861 / -121.34763]	JLP 17552	MVZ 195335	WW
29	*P. truei gilberti*	California: Monterey Co.	Hastings Natural History Reservation [36.379635 / - 122.565501]	no data no data no data	BYU 11186 BYU 11187 BYU 11188	WW (inferred) WW (inferred) WW (inferred)
31	*P. truei chlorus*	California, San Bernardino Co.	Cactus Flat, San Bernardino Mts. [34.31549 / -116.81038]	JLP 18851 JLP 18853	MVZ 198706 MVZ 198708	WW WW
32	*P. truei montipinoris*	California: San Luis Obispo	13.3 mi NW (by road) New Cuyama [35.04427/-119.89468]	JLP 18142 JLP 18143	MVZ 169791 MVZ 196792	WW WW
33	*P. truei montipinoris*	California: Ventura Co.	mouth Rose Valley [34.53403 / -119.23592]	JLP 18742 JLP 18743 JLP 18744 JLP 18745 JLP 18746	MVZ 198611 MVZ 198612 MVZ 198613 MVZ 198614 MVZ 198615	WW WW WW WW WW

Appendix II. (cont.)

Map Code	Scientific Name	Country: State/Province	Locality	Collector or Tissue No.	Museum Voucher No.	Haplogroup
34	*P. truei truei*	Colorado: Chaffee Co.	7.2 km E, 3.65 km N Poncha Mountain, 38° 30' 30.80" N, 105° 58' 52.65" W, 2190 m	DSR 11997 DSR 11998	BYU 37101 BYU 37102	EE EE
59	*P. truei truei*	New Mexico: Socorro Co.	32 mi S, 23.5 mi W Socorro	TK 13474	TTU 36053	EE
61	*P. truei*	Oklahoma; Cimarron Co.	Black Mesa Nature Preserve/State Park	TK 185647 TK 185648 TK 185650 TK 197089 TK 197293 TK 197294 TK 197331	TTU 136501 TTU 136502 TTU 136503 TTU 136505 TTU 136506 TTU 136507 TTU 136508	EE (inferred) EE EE (inferred) EE (inferred) EE (inferred) EE (inferred) EE (inferred)
62	*P. truei comanche*	Texas: Armstrong Co.	6 mi E Wayside [34.7849998 -101.4219971]	TK 27610	TTU 58444	EE (inferred)
63	*P. truei comanche*	Texas: Armstrong Co.	7 mi N, 6.25 mi E Wayside	TK 40209 TK 40211	TTU 61541 TTU 61543	EE (inferred) EE (inferred)
64	*P. truei comanche*	Texas: Briscoe Co.	3 mi N Quitaque, Caprock Canyons State Park	TK 21841 TK 54856 TK 21856	TTU 47286 TTU 74991 TTU 47296	EE EE EE (inferred)
65	*P. truei comanche*	Texas: Briscoe Co.	6 mi N, 4 mi W Silverton [34.5579987 -101.3779984]	TK 13487 TK 13490	TTU 36060 TTU 36056	EE (inferred) EE (inferred)
69	*P. truei nevadensis*	Utah: Emery Co.	7.35 km N, 11.75 km W Huntington, 39° 23' 29.95" N, 111° 06' 11.85" W, 2020 m	DSR 11877 DSR 12804 DSR 12807 DSR 12808 DSR 12809 DSR 12810 DSR 12811	BYU 36736 BYU 38022 BYU 38023 BYU 38024 BYU 38025 BYU 38026 BYU 38027	UW UW UW UW UW UW UW
71	*P. truei nevadensis*	Utah: Emery Co.	7.85 km N, 11.85 km W Huntington, 39° 23' 42.40" N, 111° 06' 10.95" W, 2050 m	DSR 11754 DSR 11755 DSR 11756 DSR 11874 DSR 11875 DSR 11876	BYU 36665 BYU 36666 BYU 36667 BYU 36668 BYU 36669 BYU 36670	UW UW UW UE UW UW
72	*P. truei nevadensis*	Utah: Emery Co.	7.95 km N, 12.30 km W Huntington, 39 23' 43.25" N, 111 06' 30.70" W, 2050 m	DSR 11825 DSR 11826 DSR 11834 DSR 11835 DSR 11836 DSR 11837 DSR 11838	BYU 36671 BYU 36672 BYU 36673 BYU 36674 BYU 36675 BYU 36676 BYU 36677	UE UW UE UE UE UW UE
73	*P. truei nevadensis*	Utah: Emery Co.	8.00 km N, 12.35 km W Huntington, 39° 23' 44.35" N, 111° 06' 33.95" W, 2055 m	DSR 11831 DSR 11832 DSR 11833	BYU 36681 BYU 36682 BYU 36683	UE UE UE
74	*P. truei nevadensis*	Utah: Emery Co.	8.05 km N, 12.25 km W Huntington, 39° 23' 46.45" N, 111° 06' 30.75" W, 2065 m	DSR 11869 DSR 11870 DSR 11871 DSR 12791 DSR 12792 DSR 12794	BYU 36684 BYU 36685 BYU 36686 BYU 38037 BYU 38038 BYU 38040	UE UE UW UW UW UW

Appendix II. (cont.)

Map Code	Scientific Name	Country: State/Province	Locality	Collector or Tissue No.	Museum Voucher No.	Haplogroup
75	*P. truei nevadensis*	Utah: Emery Co.	Rilda Canyon, 9.85 km N, 13.40 km W Huntington, 39° 24' 44.90" N, 111° 07' 22.60" W, 2150 m	DSR 11840 DSR 11841 DSR 11842 DSR 11843	BYU 36720 BYU 36721 BYU 36722 BYU 36723	UW UW UW UW
76	*P. truei nevadensis*	Utah: Emery Co.	Rilda Canyon, 9.80 km N, 13.25 km W Huntington, 39° 24' 50.75" N, 111° 07' 15.60" W, 2130 m	DSR 11775 DSR 11776 DSR 11777	BYU 36712 BYU 36713 BYU 36714	UW UE UW
80	*P. truei nevadensis*	Utah: Emery Co.	0.2 km N, 4.6 km W South Horn Mountain, 39° 08' 14.50" N, 111° 16' 10.50" W, 2000 m, Ferron Canyon	DSR 13228	BYU 38498	UE
89	*P. truei truei*	Utah: Garfield Co.	1.85 km E, 3.75 km S Wolverine Bench, 37° 48' 11.05" N, 111°12' 27.35" W, 1650 m	DSR 11186 DSR 11187 DSR 11188	BYU 36067 BYU 36068 BYU 36069	WW WW WW
131	*P. truei truei*	Utah: Uintah Co.	Willow Creek Canyon, 39° 37' 15" N, 109° 33' 40" W, 1770 m	BWC 56	BYU 17799	EE

Suggested citation format:

Rogers, D. S., N. Lewis-Rogers, S. Lewis-Rogers, S. T. Álvarez-Castañeda, and E. A. Rickart. 2019. Mitochondrial cytochrome-*b* variation within *Peromyscus truei* reveals two strongly divergent haplogroups. Pp. 577–612 in From field to laboratory: a memorial volume in honor of Robert J. Baker (R. D. Bradley, H. H. Genoways, D. J. Schmidly, and L. C. Bradley, eds.). Special Publications, Museum of Texas Tech University 71:xi+1–911.

Molecular Data Indicate That *Isthmomys* Is Not Aligned with *Peromyscus*

Megan S. Keith, Roy N. Platt II, and Robert D. Bradley

Abstract

The genus *Isthmomys* is composed of two species, *Isthmomys flavidus* and *Isthmomys pirrensis*. These rodents have been classified in either their own genus or as a subgenus of *Peromyscus* (*sensu lato*); however, recent molecular studies have alluded to a possible sister relationship with *Reithrodontomys*. In this study, maximum likelihood analyses were used to ascertain the phylogenetic relationship of *Isthmomys* to *Peromyscus* and *Reithrodontomys* using three approaches. First, a large-scale analysis of the mitochondrial cytochrome-*b* gene (*Cyt*b) was conducted for *Isthmomys pirrensis*, all species of *Reithrodontomys*, ≥ 53 species of peromyscine rodents (*Peromyscus* (*sensu lato*)), and other selected taxa. Second, topological constraints were applied to a reduced *Cyt*b dataset to reflect two competing hypotheses for the phylogenetic placement of *Isthmomys*: 1) *Isthmomys* is sister to a clade containing *Peromyscus* (*sensu lato*) and *Onychomys*; or 2) *Isthmomys* is sister to *Reithrodontomys*. Third, sequences of five nuclear gene segments (*Adh*1-I2, *Fgb*-I7, *Dmp*1, *Ghr*, and *Rbp*3) were analyzed individually and then combined with the *Cyt*b data. Analysis of the individual datasets resulted in three possible hypotheses explaining the relationships of *Isthmomys* to *Peromyscus* and *Reithrodontomys*; however, two of these relationships were not statistically supported and only the combined analysis (mitochondrial gene plus five nuclear genes) provided statistical support for a sister-taxa relationship between *Isthmomys* and *Reithrodontomys*.

Key words: combined dataset, *Isthmomys*, *Peromyscus*, *Reithrodontomys*

Introduction

The genus *Isthmomys* (family Cricetidae, subfamily Neotominae; Hooper and Musser 1964a) consists of two species, the Yellow Isthmus rat (*Isthmomys flavidus*; Bangs 1902) and the Mt. Pirri Isthmus rat (*Isthmomys pirrensis*; Goldman 1912). The phylogenetic relationships of these relict species have been difficult to resolve in relation to other genera of the subfamily Neotominae, in part, due to the rarity of voucher specimens and tissues in natural history collections. Previous studies have been unable to consistently place *Isthmomys* relative to *Peromyscus* and *Reithrodontomys*, with some datasets depicting a sister relationship with *Reithrodontomys* and others indicating that *Isthmomys* should be considered a subgenus of *Peromyscus* (Hooper and Musser 1964a). Whereas genetic data has been generated for *I. pirrensis*, no genetic data exists for *I. flavidus*, further complicating resolution of relationships for this genus. Questions concerning these taxa involve whether *Isthmomys* should be considered a distinct genus (*Peromyscus* (*sensu stricto*); Carleton 1980, 1989; Musser and Carleton 2005) or subgenus (Hooper and Musser 1964a; Hooper 1968) of *Peromyscus* (*sensu lato*, including *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys*) and how a taxonomic status change could affect the phylogeny and classification of *Peromyscus* (Platt et al. 2015).

Carleton's (1980) morphological study is the most inclusive analysis of morphology for this group of rodents. Carleton (1980) suggested that *Isthmomys* formed a sister-group relationship with *Megadontomys*, whereas other morphological studies placed it within the peromyscine rodents (Osgood 1909; Hooper and Musser 1964b; Hooper 1968). Further, *Isthmomys* lacks the grooved incisors characteristic of *Reithrodontomys* and it is considerably larger than even the

largest species of harvest mouse (Miller and Engstrom 2008; Reid 2009) indicating a plausible morphological affiliation with *Peromyscus* to the exclusion of *Reithrodontomys*. Alternatively, the karyotype of *I. pirrensis* consists of characters signifying its uniqueness relative to other species of *Peromyscus.* Stangl and Baker (1984) compared the karyotype of *I. pirrensis* to the inferred primitive karyotype for *Peromyscus* (*sensu lato*) and provided support for the hypothesis that *Isthmomys* may have been among the first lineages to have diverged from *Peromyscus.*

The allozyme study of Rogers et al. (2005) was the first molecular study to recover a sister relationship between *Isthmomys* and *Reithrodontomys*. In their study, these genera formed a clade sister to *Peromyscus* (*sensu lato*). More recently, Platt et al. (2015) and other authors (Bradley et al. 2007; Miller and Engstrom 2008) examined molecular variation in DNA sequences for the peromyscine rodents and proposed additional support for a sister relationship between *Isthmomys* and *Reithrodontomys.* In addition to recovering this phylogenetic relationship, Platt et al. (2015) reported that *Isthmomys* diverged from *Peromyscus* approximately 8 million years ago (MYA), whereas other peromyscine rodents originated 3.5 to 5.5 MYA.

Results of previous studies can be summarized as three alternative hypotheses characterizing the evolutionary relationship of *Isthmomys* to other members of the Neotominae (Musser and Carleton 2005): 1) *Isthmomys* is more closely related to *Peromyscus* (either monophyletic with *Peromyscus* (*sensu stricto*) - Hooper and Musser 1964a; Hooper 1968, or it represents one of the first lineages to diverge from *Peromyscus* - Stangl and Baker 1984); 2) *Isthmomys* forms a sister-group relationship with *Reithrodontomys* and is basal to *Peromyscus* and allied genera (Bradley et al. 2007; Miller and Engstrom 2008; Platt et al. 2015); or 3) *Isthmomys* is basal to a clade containing *Peromyscus* and allied genera and *Reithrodontomys.* This study utilizes extensive phylogenetic analysis of multiple genetic datasets to test the aforementioned hypotheses. To accomplish this, six genes that have been informative in other studies involving this group of rodents were analyzed to resolve the phylogenetic relationships of the genus *Isthmomys*: the mitochondrial cytochrome-*b* gene (*Cyt*b - Reeder et al. 2006; Bradley et al. 2007; Miller and Engstrom 2008; Platt et al. 2015); and five individual nuclear gene segments—intron 2 of the alcohol dehydrogenase gene (*Adh*1-I2 - Platt et al. 2015); intron 7 of the beta-fibrinogen gene (*Fgb*-I7- Reeder et al. 2006; Platt et al. 2015); exon 6 of the dentin matrix protein 1 gene (*Dmp*1- Reeder et al. 2006); exon 10 of the growth hormone receptor gene (*Ghr*- Miller and Engstrom 2008); and exon 1 of the interphotoreceptor retinoid-binding protein gene (*Rbp*3- Miller and Engstrom 2008; Platt et al. 2015)—were examined in a phylogenetic context separately and in combination.

MATERIALS AND METHODS

DNA sequences were generated in this study or were obtained from GenBank. For the majority of samples, genetic data were downloaded from GenBank's Nucleotide database using the following search terms—"*Peromyscus* OR *Reithrodontomys* OR *Isthmomys* OR *Onychomys* OR *Habromys* OR *Megadontomys* OR *Neotomodon* OR *Osgoodomys* OR *Podomys* OR *Baiomys* OR *Ochrotomys*" combined with the gene symbol for each gene (e.g., "AND *Cyt*b", "AND *Adh*1-I2", etc.). The purpose of these searches was to recover all available data for genera/species historically affiliated with *Peromyscus*, *Reithrodontomys*, and *Isthmomys* from each gene of interest as well as other selected genera within the Neotominae. Sequences that were utilized in both the individual and combined analyses are listed in the Appendix by GenBank accession numbers and museum catalog numbers.

Sequencing.—Genomic DNA was isolated from approximately 0.1g of frozen liver tissue using either the Puregene DNA isolation kit (Gentra, Minneapolis, Minnesota) or the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, California). DNA fragments were amplified using polymerase chain reaction (PCR, Saiki et al. 1988). Methods for PCR amplification and sequencing of *Cyt*b followed protocols developed by Bradley et al. (2007) and Tiemann-Boege et al. (2000) using primers MVZ05 (Smith and Patton 1993), CB40 (Hanson and Bradley 2008), and Pero3' (Tiemann-Boege et al. 2000) or LGL765 forward and LGL766

reverse (Bickham et al. 1995); *Adh*1-I2 followed the methods of Amman et al. (2006) using primers 2340-I, 2340-II, Exon II-F, and Exon III-R (Amman et al. 2006); *Fgb*-I7 followed Carroll and Bradley (2005) and Wickliffe et al. (2003) using primers *Fgb*-17U-*Rattus*, *Fgb*-17L-*Rattus* (Wickliffe et al. 2003) and B17-mammU and B17-mammL (Matocq et al. 2007); *Dmp*1 followed the methods of Reeder and Bradley (2004) using primers *Den-12* (F) and *Den-2* (R) (Toyosawa et al. 1999); *Ghr* followed Miller and Engstrom (2008) using primers GHREXON10 and GHREND (Adkins et al. 2001); and amplification of *Rbp*3 followed the protocols of Chambers et al. (2009) and Jansa and Voss (2000) using primers A and B (Stanhope et al. 1992).

PCR products were purified using the QIAquick PCR purification kit (Qiagen, Valencia, California) or ExoSAP-IT (USB Products, Cleveland, Ohio) and PCR amplicons were sequenced using ABI Prism Big Dye Terminator v3.1 ready reaction mix (Applied Biosystems, Foster City, California). Nucleotide sequences were determined on an ABI 3100 or 3130-*Avant* automated sequencer (Applied Biosystems, Foster City, California) with the following internal primers in addition to those used for PCR: *Cyt*b primers 400F (Edwards et al. 2001) and 870R (Peppers et al. 2002); *Adh*1-I2 primers Adh350F and Adh410R (Amman et al. 2006); *Fgb*-I7 primer Bfib300F (Carroll and Bradley 2005); *Dmp*1 primers 900F and 900R (Reeder and Bradley 2004); *Ghr* primers GHR7, GHR8, GHR9 and GHR10 (Adkins et al. 2001); and *Rbp*3 primers D (Stanhope et al. 1992), E2 (Weksler 2003), and 125F (DeBry and Sagel 2001). Sequences were edited using Sequencher 4.10 software (Gene Codes, Ann Arbor, Michigan) and aligned using MEGA v6.06 (Tamura et al. 2013). All sequences generated in this study are listed in the Appendix.

Data analyses.—The phylogenetic position of *Isthmomys* relative to *Peromyscus* and *Reithrodontomys* was tested using multiple phylogenetic approaches. For all datasets, sequences were aligned using MUSCLE (Edgar 2004), and jModelTest v2.1.6 (Darriba et al. 2012) was used to determine the appropriate nucleotide substitution model. Nucleotide substitution models were determined as follows: GTR+I+G for *Cyt*b, *Fgb*-I7, *Dmp*1, and *Rbp*3; and HKY+I+G for *Adh*1-I2 and *Ghr*. A maximum likelihood (ML) analysis and a total of 1,000 bootstrap replicates were used to analyze the sequence data in RaxML (Stamatakis et al. 2005). This approach was used due to the large amount of data for *Cytb* and to recover a phylogeny representing the best hypothesis for the relationship of *Isthmomys* to the other Neotominae rather than a consensus tree. All datasets were evaluated under the GTR+I+G model (option GTRGAMMAI) as the less parameter-rich HKY model was not available in the software package. *Sigmodon hispidus* was selected as the outgroup taxon based on previous studies (Bradley et al. 2004; Reeder and Bradley 2004; Bradley et al. 2007; Miller and Engstrom 2008).

For the *Cytb* dataset, only complete sequences (≥1,143 bp) were used when possible with the exception of *Isthmomys*, resulting in a dataset containing 1,063 sequences. This dataset was reduced to include a maximum of five sequences per species (284 total sequences) and was used to test the aforementioned competing hypotheses for the relationship of *Isthmomys* to *Reithrodontomys* and *Peromyscus*. An unconstrained analysis was performed (unconstrained = *Reithrodontomys*, *Isthmomys*, and *Peromyscus*) and subsequently the topology was constrained so that *Isthmomys* and *Peromyscus* formed a monophyletic group.

The approximately unbiased (AU) test (Shimodaira 2002) was conducted in CONSEL (Shimodaira and Hasegawa 2001) to assess the confidence of the tree selection by calculating *p*-values for the resulting phylogenies. The AU test is the primary result of CONSEL, which uses a multiscale bootstrap technique and provides less biased results (Shimodaira and Hasegawa 2001).

To further test the placement of *Isthmomys* relative to *Peromyscus* and *Reithrodontomys*, five nuclear gene segments were analyzed. The relationships of taxa were scored for each gene based on nodal support. Individual nuclear analyses included: 33 sequences for *Adh*1-I2 (1 *I. pirrensis*, 2 *Reithrodontomys*, 21 *Peromyscus*, 5 other peromyscine taxa [*Habromys*, *Neotomodon*, *Megadontomys*, *Osgoodomys*, and *Podomys*], 2 *Onychomys*, 1 *Ochrotomys*, and 1 outgroup); 35 sequences for *Fgb*-I7 (1 *I. pirrensis*, 4 *Reithrodontomys*, 21 *Peromyscus*, 5 other peromyscine taxa [*Habromys*, *Neotomodon*, *Megadontomys*, *Osgoodomys* and *Podomys*], 2 *Onychomys*, 1 *Ochrotomys*, and 1 outgroup); 32 sequences for *Dmp*1 (1 *I. pirrensis*, 3 *Reithrodontomys*,

20 *Peromyscus*, 5 other peromyscine taxa [*Habromys*, *Neotomodon*, *Megadontomys*, *Osgoodomys* and *Podomys*], 2 *Onychomys*, and 1 *Ochrotomys*); 35 sequences for *Ghr* (2 *I. pirrensis*, 3 *Reithrodontomys*, 21 *Peromyscus*, 5 other peromyscine taxa [*Habromys*, *Neotomodon*, *Megadontomys*, *Osgoodomys*, and *Podomys*], 2 *Onychomys*, 1 *Ochrotomys*, and 1 *outgroup*); and 36 sequences for *Rbp*3 (2 *I. pirrensis*, 4 *Reithrodontomys*, 21 *Peromyscus*, 5 other peromyscine taxa [*Habromys*, *Neotomodon*, *Megadontomys*, *Osgoodomys*, and *Podomys*], 2 *Onychomys*, 1 *Ochrotomys*, and 1 *outgroup*).

Individual datasets were reduced to include species (32) for which sequence data were available for all genes (mitochondrial and nuclear—see Appendix). These datasets were partitioned and evaluated in a combined analysis using the same parameters as above. Given that individual gene trees can differ from the underlying species tree due to incomplete lineage sorting and other processes (Maddison 1997; Kubatko and Degnan 2006), a combined dataset was used to overcome the effects of incomplete lineage sorting (Rokas et al. 2003) and to potentially increase resolution at multiple levels within a phylogeny. Under this scenario, the more quickly evolving *Cytb* should provide more resolution at terminal nodes, and nuclear markers should enhance resolution for the more basal nodes of the phylogeny (Bull et al. 1993; Adkins et al. 2001; Pereira et al. 2002; Platt et al. 2015). In this analysis, there was one representative for each species. An attempt was made to obtain mitochondrial and nuclear sequences from a single individual, but this was not possible for all nuclear gene segements. In some cases, sequences from conspecific individuals were used to complete the dataset. Concatenation of sequence data from conspecifics to represent a composite species rather than a single individual has been used successfully in other studies (Campbell and Lapointe 2009; Townsend et al. 2011; Haddrath and Baker 2012; Platt et al. 2015). The combined analysis totaled 5,345 base pairs and nodes were considered well-supported with a bootstrap value ≥ 80.

RESULTS

The *Cyt*b analysis (Fig. 1) generated a phylogeny in which *Isthmomys*, *Reithrodontomys*, and *Onychomys* formed a clade (Clade I) relative to *Peromyscus* and allied genera (Clade II). However, nodal support (boostrap value < 80) was not statistically significant for this relationship. The reduced mitochondrial dataset was constrained to test hypotheses concerning the phylogenetic placement of *Isthmomys*. The unconstrained analysis resulted in a phylogeny in which *Isthmomys* was sister to *Reithrodontomys* and the best tree score was -35559.90. A topological constraint was then applied to the dataset to force monophyly of *Isthmomys* and *Peromyscus* and the resulting phylogeny received a best tree score of -35576.12. The AU test (CONSEL-Shimodaira and Hasegawa 2001) indicated that the unconstrained topology (*Isthmomys* + *Reithrodontomys*) was not significantly better (*p*-value = 0.153) than the constrained topology (*Isthmomys* + *Peromyscus*).

Three different phylogenetic relationships were recovered when the five nuclear genes were examined independently (Fig. 2). Analyses of *Adh*1-I2 and *Fgb*-I7 sequences (Fig. 2A and 2B) each recovered a phylogeny in which *Isthmomys* was sister to a clade containing *Peromyscus* and allied genera and *Reithrodontomys*, analyses of *Ghr* and *Rbp*3 sequences (Fig. 2C and 2D) both recovered a sister relationship between *Isthmomys* and *Reithrodontomys*, and analysis of *Dmp*1 sequences (Fig. 2E) recovered a phylogeny in which *Isthmomys* was sister to *Peromyscus*. None of these analyses depicted statistical support for any relationship of *Isthmomys* to the other genera.

Finally, the combined analysis (Fig. 3) resulted in a phylogeny with a higher level of support than was recovered for the individual analyses. A well-supported (bootstrap value = 86) clade in which *Isthmomys* was sister to *Reithrodontomys* was recovered (Clade I). Clade I was sister to a monophyletic group containing *Peromyscus* (*sensu lato*) plus *Onychomys* (Clade II) with a bootstrap support value of 95.

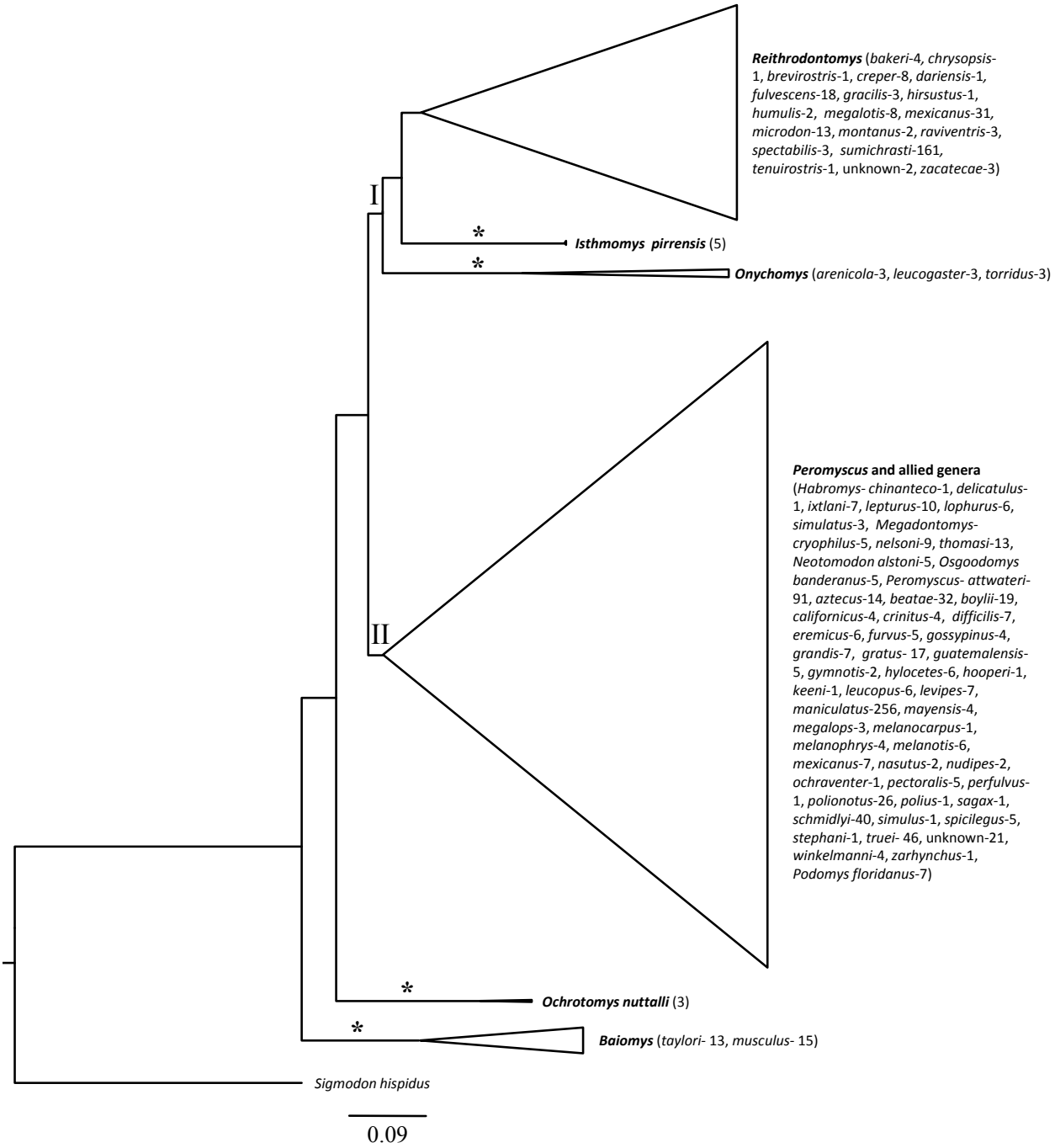

Figure 1. Phylogenetic tree obtained from the maximum likelihood analysis of the large scale sampling for the mitochondrial cytochrome-*b* gene (*Cyt*b). Support values were based on 1,000 bootstrap replicates of the maximum likelihood analysis. Values ≥ 80 are indicated by an asterisk. The number of individuals for each species included are provided to the right of the dash. Clade I reflects a sister relationship between *Reithrodontomys*, *Isthmomys*, and *Onychomys*, with *Peromyscus* as the sister group to these three genera in clade II; however, there was no statistical support for this relationship

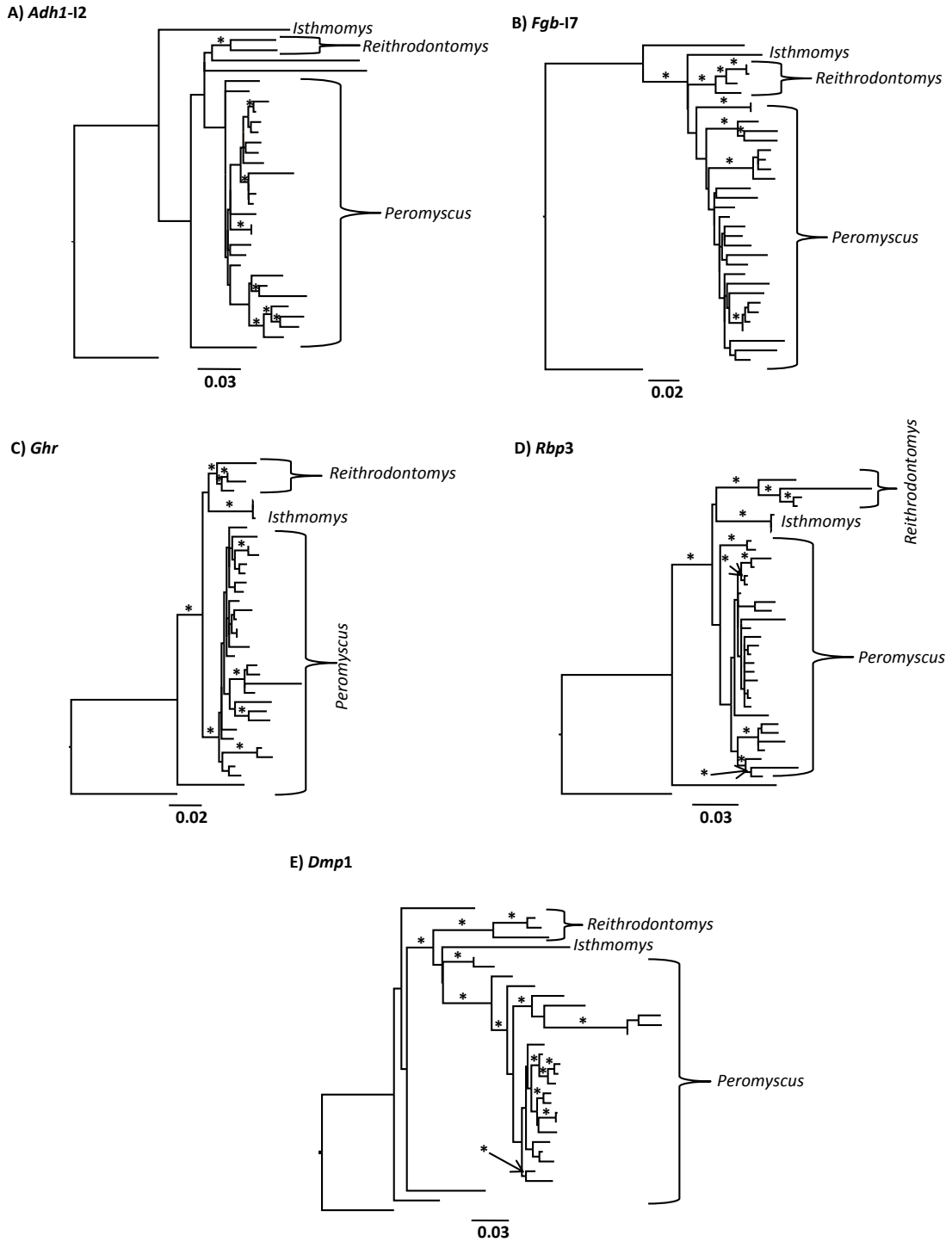

Figure 2. Phylogenetic trees based on likelihood analyses for individual gene segments. Support values were based on 1,000 bootstrap replicates. Values ≥ 80 are represented by an asterisk. Analyses of *Adh*1-I2 (A) and *Fgb*-I7 (B) sequences resulted in trees in which *Isthmomys* was sister to a clade containing *Peromyscus* and allied genera and *Reithrodontomys*; phylogenies for *Ghr* (C) and *Rbp3* (D) depict a clade uniting *Isthmomys* and *Reithrodontomys* with *Peromyscus* sister to this clade; and analysis of Dmp1 (E) resulted in an arrangement in which *Isthmomys* was sister to *Peromyscus*.

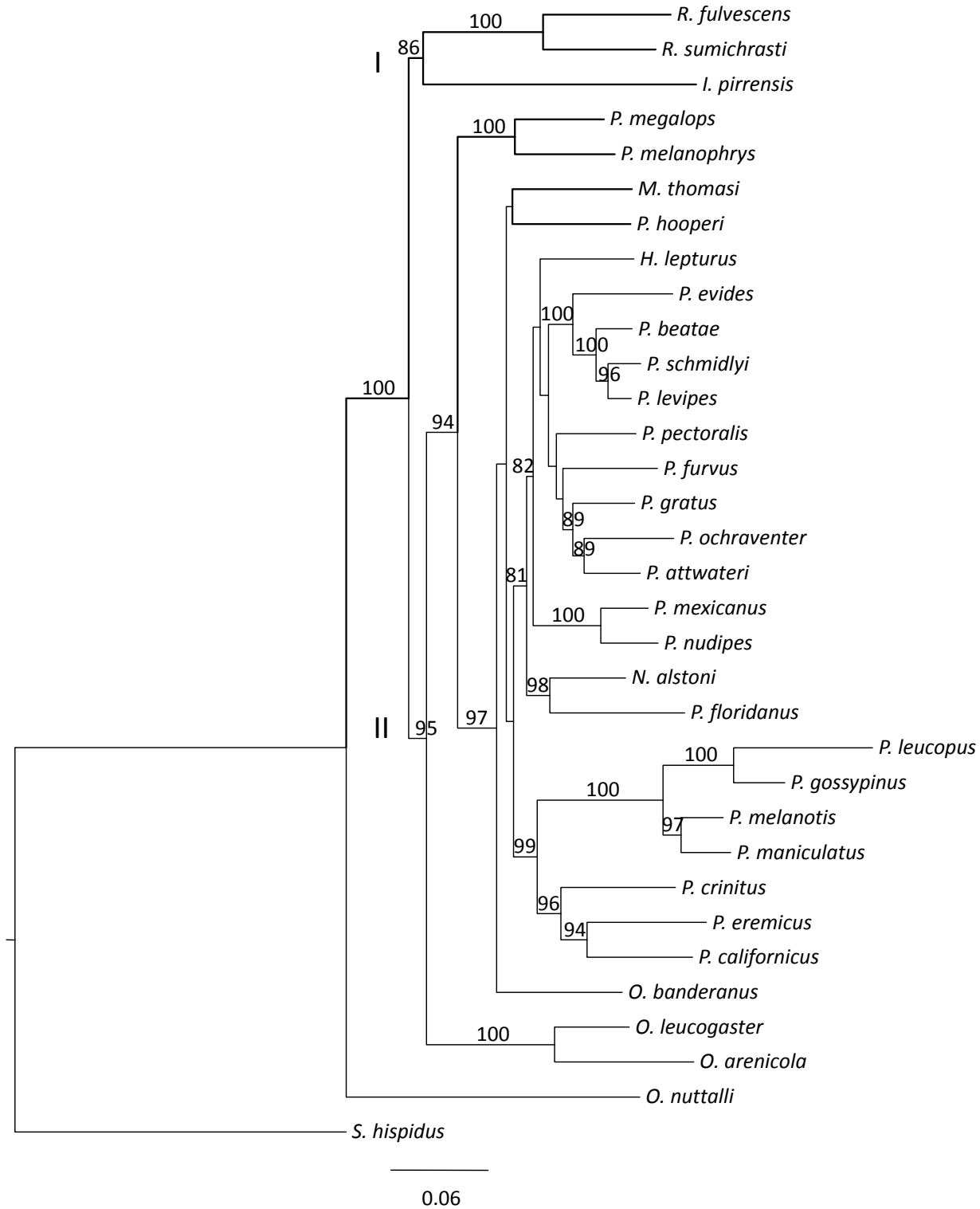

Figure 3. Phylogenetic tree obtained from maximum likelihood analysis of the combined dataset (*Cyt*b, *Adh*1-I2, *Dmp*1, *Fgb*-I7, *Ghr*, and *Rbp3*). Support values are based on 1,000 bootstrap replicates. Values ≥ 80 are shown above the supported node. Clade I reflects a well-supported sister relationship between *Reithrodontomys* and *Isthmomys*, with a monophyletic *Peromyscus* sister to *Onychomys* (Clade II).

DISCUSSION

The large-scale *Cyt*b analysis (1,063 sequences) resulted in a gene tree that was largely unsupported and indicated a sister relationship between *Isthmomys* and *Reithrodontomys*, which were sister to *Onychomys* (Clade I) and a clade containing *Peromyscus* and allied genera (Fig. 1). Therefore, greater taxon sampling for mitochondrial data beyond that of Bradley et al. (2007), Miller and Engstrom (2008), and Platt et al. (2015) did not increase resolution for estimating relationships between *Isthmomys* and other neotomine taxa.

Analysis of five individual nuclear genes resulted in three possible taxonomic arrangements of *Isthmomys* to *Peromyscus* and *Reithrodontomys* (Fig. 2): 1) *Isthmomys* was basal to both *Peromyscus* and allied genera and *Reithrodontomys* (*Adh1*-I2 and *Fgb*-I7; Fig. 2A and 2B); 2) *Isthmomys* formed a sister taxa relationship with *Reithrodontomys* (*Ghr* and *Rbp*3; Fig. 2C and 2D, Rogers et al. 2005; Bradley et al. 2007; Miller and Engstrom 2008; Platt et al. 2015); or 3) *Isthmomys* was sister to *Peromyscus* (*sensu lato* - including *Habromys*, *Megadontomys*, *Neotomodon*, and *Podomys* - *Dmp1*; Fig. 2E). Two of the recovered taxonomic arrangements (2 and 3) corresponded to existing hypotheses for the relationship of *Isthmomys* to other members of the Neotominae (Table 1), whereas analyses of *Adh1*-I2 and *Fgb*-I7 recovered a relationship that required a third, alternative hypothesis (i.e., *Isthmomys* is basal to a clade uniting *Peromyscus* and *Reithrodontomys*). However, none of the individual nuclear gene phylogenies indicated statistical support for any of the recovered phylogenetic relationships of *Isthmomys* with relation to *Reithrodontomys* and *Peromyscus* (Fig. 2). These results (both mitochondrial and nuclear) were a classic example of incongruencies between gene trees hindering the ability to estimate a phylogeny. Incongruencies between gene trees could be due to horizontal gene transfer, gene duplication, or incomplete lineage sorting (Avise and Ball 1990; Maddison 1997) that may have occurred during a rapid radiation event during the late Miocene that has increased the difficulty of placing some genera in the Neotominae (Bradley et al. 2004; Reeder and Bradley 2004; Reeder et al. 2006).

Incongruencies between individual gene trees and species trees often occur when divergence times are short relative to the effective population size of the ancestral population (Degnan and Rosenberg 2006; Kubatko and Degnan 2007; Belfiore et al. 2008). Increasing the number of independent loci sampled (six in this study) should increase resolution at different hierarchical levels (Bull et al. 1993; Adkins et al. 2001; Pereira et al. 2002), help overcome noise due to homoplasy (Barrett et al. 1991; de Queiroz 1993; Adkins et al. 2001), overcome stochastic lineage sorting (Rokas et al. 2003), and have an additive effect to reveal hidden relationships (Gatesy et al. 2004). Additionally, analyzing molecular markers with varying rates of evolution may also be beneficial. Sullivan (1996) concluded that when among-site rate variation is present, combining data may improve phylogenetic estimates and that the data is better understood if both individual and combined phylogenetic analyses are conducted. The combined ML analysis resulted in a strongly supported clade in which *Isthmomys* was sister to *Reithrodontomys* (Fig. 3, Clade I). A second clade formed a monophyletic *Peromyscus* (*sensu lato*; Fig. 3, Clade II). This analysis provided the greatest nodal support for a phylogenetic relationship for *Isthmomys*. These results were similar to those of Rogers et al. (2005), Reeder et al. (2006), Bradley et al. (2007), Miller and Engstrom (2008), and Platt et al. (2015).

Systematic conclusions. —Analysis of morphology, karyology, and genetic datasets generated different phylogenetic hypotheses concerning the relationship of *Isthmomys* to other peromyscine and reithrodontomyine genera. Morphological data indicated a sister relationship for *Isthmomys* to *Peromyscus* (Hooper and Musser 1964a; Hooper 1968; Carleton 1980), and karyotypic data (Stangl and Baker 1984) supported the hypothesis that *Isthmomys* may have been the first lineage to diverge from *Peromyscus*, indicating a possible sister relationship to *Peromyscus*. However, studies analyzing genetic data have recovered a sister relationship between *Isthmomys* and *Reithrodontomys* (Rogers et al. 2005; Bradley et al. 2007; Miller and Engstrom 2008; Platt et al. 2015).

Phylogenetic trees based on increased sampling and analysis of the mitochondrial cytochrome-*b* gene, as well as individual analyses of five nuclear markers, did not strongly support an association of *Isthmomys*

Table 1. The taxonomy of *Peromyscus* and its allied genera from previous studies, including the types of data used to infer taxonomy. Asterisks indicate genera not included in the analysis but included based on precedent. Table revised from Miller and Engstrom (2008).

Hooper and Musser (1964a, 1964b)—morphology	Carleton (1980)—morphology	Musser and Carleton (2005)	Reeder et al. (2006)—*Cyt*b, *Fgb*-I7, *Dmp*1	**Bradley et al. 2007—*Cyt*b	**Miller and Engstrom (2008)—*Cyt*b, *Ghr*, *Rbp*3	**Platt et al. (2015)—*Cyt*b, *Adh*1-I2, *Fgb*-I7, *Rbp*3
Peromyscini	Peromyscine	Reithrodontomyini	Peromyscini	Peromyscini	Reithrodontomyini	Reithrodontomyini
Baiomys	*Habromys*	*Habromys*	*Habromys**	*Habromys**	*Habromys*	*Habromys*
Nelsonia	*Isthmomys*	*Isthmomys*	*Isthmomys**	*Isthmomys**	*Isthmomys*	*Isthmomys*
Ochrotomys	*Megadontomys*	*Megadontomys*	*Megadontomys**	*Megadontomys**	*Megadontomys*	*Megadontomys*
Onychomys	*Neotomodon*	*Neotomodon*	*Neotomodon*	*Neotomodon*	*Neotomodon*	*Neotomodon*
Peromyscus^	*Ochrotomys*	*Onychomys*	*Onychomys*	*Onychomys*	*Onychomys*	*Onychomys*
Reithrodontomys	*Onychomys*	*Osgoodomys*	*Osgoodomys*	*Osgoodomys*	*Osgoodomys*	*Osgoodomys*
Scotinomys	*Osgoodomys*	*Peromyscus*	*Peromyscus*^	*Peromyscus*^	*Peromyscus*	*Peromyscus*+
	Peromyscus	*Podomys*	*Podomys**	*Podomys**	*Podomys**	*Podomys**
	Podomys	*Reithrodontomys*	*Reithrodontomys*	*Reithrodontomys*	*Reithrodontomys*	*Reithrodontomys*
	Reithrodontomys					

^ *Peromyscus* (*sensu lato*) including *Habromys*, *Isthmomys*, *Megadontomys*, *Osgoodomys*, and *Podomys* as subgenera.

+ *Peromyscus* (*sensu lato*) excluding *Isthmomys*. *Peromyscus* is paraphyletic and requires revision to the current taxonomy.

** Studies that recovered a sister taxa relationship between *Reithrodontomys* and *Isthmomys*. Bradley et al. 2007 indicated paraphyly for these two genera.

relative to *Peromyscus* and *Reithrodontomys*. Molecular studies examining the peromyscine rodents have recovered a sister group relationship between *Isthmomys* and *Reithrodontomys*; however, most of these studies did not indicate statistical support for this relationship (Rogers et al. 2005; Bradley et al. 2007, Platt et al. 2015) or statistical support was not consistent across all analyses performed in a single study (Miller and Engstrom 2008; this study). Analysis of a combined dataset appears to be the only situation where support for a sister relationship between *Isthmomys* and *Reithrodontomys* is realized (Miller and Engstrom 2008; this study). Phylogenetic signals from each dataset may have been additive to reveal an improved phylogenetic hypothesis for this group. However, these studies include a single representative of *Isthmomys* and it is unknown if including the second species in the genus (*I. flavidus*) would change the resulting phylogeny. A sister taxa relationship for *Isthmomys* and *Reithrodontomys* was recovered (Rogers et al. 2005; Bradley et al. 2007; Miller and Engstrom 2008; Platt et al. 2015) in a series of studies examining molecular data for rodents of the Neotominae. Therefore, this study further supports the recognition of *Isthmomys* as forming a sister relationship with *Reithrodontomys*, separate from *Peromyscus*.

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LITERATURE CITED

Adkins, R. M., E. L. Gelke, D. Rowe, and R. L. Honeycutt. 2001. Molecular phylogeny and divergence time estimates for major rodent groups: evidence from multiple genes. Molecular Biology and Evolution 18:777–791.

Amman, B. R., J. D. Hanson, L. K. Longhofer, S. R. Hoofer, and R. D. Bradley. 2006. Intron 2 of the alcohol dehydrogenase gene (ADH1-I2): a nuclear DNA marker for mammalian systematics. Occasional Papers, Museum of Texas Tech University 256:1–16.

Avise, J. C., and R. M. Ball. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Oxford Surveys in Evolutionary Biology 7:45–67.

Bangs, O. 1902. Chiriqui Mammalia. Bulletin of the Museum of Comparative Zoology at Harvard College, 39:17–51.

Barrett, M., M.J. Donoghue, and E. Sober. 1991. Against consensus. Systematic Zoology 40: 486-493.

Belfiore N.M., L. Liu and C. Moritz. 2008. Multilocus phylogenetics of a rapid radiation in the genus *Thomomys* (Rodentia : Geomyidae). Systematic Biology 57:29–310.

Bickham, J. W., C. C. Wood, and J. C. Patton. 1995. Biogeographic implications of cytochrome *b* sequences and allozymes in sockeye (*Oncorhynchus nerka*). Journal of Heredity 86:140–144.

Bradley, R. D., N. D. Durish, D. S. Rogers, J. R. Miller, M. D. Engstrom, and C. W. Kilpatrick. 2007. Toward a molecular phylogeny for *Peromyscus*: evidence from mitochondrial cytochrome-*b* sequences. Journal of Mammalogy 88:1146–1159.

Bradley, R. D., C. W. Edwards, D. S. Carroll, and C. W. Kilpatrick. 2004. Phylogenetic relationships of Neotomine-Peromyscine rodents: based on DNA sequences from the mitochondrial cytochrome-*b* gene. Journal of Mammalogy 85:389–395.

Bull, J. J., J. P. Huelsenbeck, C. W. Cunningham, D. L. Swofford, and P. J. Waddell. 1993. Partitioning and combining data in phylogenetic analysis. Systematic Biology 42: 384-397.

Campbell, V., and F.-J. Lapointe. 2009. The use and validity of composite taxa in phylogenetic analysis. Systematic Biology 58:560–572.

Carleton, M. D. 1980. Phylogenetic relationships in neotomine-peromyscine rodents (Muroidea) and a reappraisal of the dichotomy within New World Cricetinae. Miscellaneous Publications of the Museum of Zoology, University of Michigan 157:1–146.

Carleton, M. D. 1989. Systematics and evolution. Pp. 7–141 in Advances in the Study of *Peromyscus* (G. L. Kirkland, Jr., and J. N. Layne, eds.), Texas Tech University Press, Lubbock.

Carroll, D. S., and R. D. Bradley. 2005. Systematics of the genus *Sigmodon*: DNA sequences from beta-fibrinogen and cytochrome *b*. The Southwestern Naturalist 50:342–349.

Chambers, R. R., P. D. Sudman, and R. D. Bradley. 2009. A phylogenetic assessment of pocket gophers (*Geomys*): evidence from nuclear and mitochondrial genes. Journal of Mammalogy 90:537–547.

Darriba, D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9:772.

DeBry, R. W., and R. M. Sagel. 2001. Phylogeny of Rodentia (Mammalia) inferred from the nuclear-encoded gene *Irbp*. Molecular Phylogenetics and Evolution 19:290–301.

Degnan, J.H. and N.A. Rosenberg. 2006. Discordance of species trees with their most likely gene trees. PLos Genetics 2: e68. doi: 10.1371/journal.pgen.0020068.

de Queiroz, A. 1993. For consensus (sometimes). Systematic Biology 42: 368–372.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797.

Edwards, C. W., C. F. Fulhorst, and R. D. Bradley. 2001. Molecular phylogenetics of the *Neotoma albigula* species group: further evidence of a paraphyletic assemblage. Journal of Mammalogy 82:267–279.

Gatesy J., R.H. Baker and C. Hayashi. 2004. Inconsistencies in arguments for the supertree approach: supermatrices versus supertrees of Crocodylia. Systematic Biology 53:342–355.

Goldman, E. A. 1912. New small mammals from eastern Panama. Smithsonian Miscellaneous Collections 602:1–18.

Haddrath, O., and A. J. Baker. 2012. Multiple nuclear genes and retroposons support vicariance and dispersal of the palaeognaths, and an Early Cretaceous origin of modern birds. Proceedings of the Royal Society B: Biological Sciences 279:4617–4625.

Hanson, J. D., and R. D. Bradley. 2008. Molecular diversity within *Melanomys caliginosus* (Rodentia: Oryzomyini): evidence for multiple species. Occasional Papers, Museum of Texas Tech University 275:1–11.

Hooper, E. T. 1968. Classification. Pp. 27–74 in Biology of *Peromyscus* (Rodentia) (King, J. A. ed.). The American Society of Mammalogists.

Hooper, E. T., and G. G. Musser. 1964a. Notes on classification of the rodent genus *Peromyscus*. Occasional Papers of the Museum of Zoology, University of Michigan 635:1–13.

Hooper, E. T., and G. G. Musser. 1964b. The glans penis in Neotropical cricetines (Family Muridae) with comments on classification of muroid rodents. Miscellaneous Publications, Museum of Zoology, University of Michigan 123:1–57.

Jansa, S., and R. Voss. 2000. Phylogenetic studies on didelphid marsupials I. Introduction and preliminary results from nuclear IRBP gene sequences. Journal of Mammalian Evolution 7:43–77.

Kubatko, L. S. and J. H. Degnan. 2006. Inconsistency of phylogenetic estimates from concatenated data under coalescence. Systematic Biology 56:17–24.

Maddison, W. P. 1997. Gene trees in species trees. Systematic Biology 1997:523–536.

Matocq, M. D., Q. R. Shurtliff, and C. R. Feldman. 2007. Phylogenetics of the woodrat genus *Neotoma* (Rodentia: Muridae): implications for the evolution of phenotypic variation in male external genitalia. Molecular Phylogenetics and Evolution 42:637–652.

Miller, J. R., and M. D. Engstrom. 2008. The relationships of major lineages within Peromyscine rodents: A molecular phylogenetic hypothesis and systematic reappraisal. Journal of Mammalogy 89:1279–1295.

Musser, G. G. and M. D. Carleton. 2005. Superfamily Muroidea. Pp. 894–1531 in Mammal species of the world: a taxonomic and geographic reference (D. E. Wilson and D. M. Reeder, Eds.). 3rd Ed. Johns Hopkins University Press, Baltimore, Maryland.

Osgood, W. H. 1909. Revisions of the mice of the American genus *Peromyscus*. North American Fauna 28:1–285.

Peppers, L. L., D. S. Carroll, and R. D. Bradley. 2002. Molecular systematics of the genus *Sigmodon* (Rodentia: Muridae): evidence from the mitochondrial cytochrome-*b* gene. Journal of Mammalogy 83:396–407.

Pereira, S. L., A. J. Baker, and A. Wajntal. 2002. Combined nuclear and mitochondrial DNA sequences resolve genetic relationships within the Cracidae (Galliformes, Aves). Systematic Biology 51:946–958.

Platt II, R. N., B. R. Amman, M. S. Keith, C. W. Thompson, and R. D. Bradley. 2015. What is *Peromyscus*? Evidence from nuclear and mitochondrial DNA sequences suggests the need for a new classification. Journal of Mammalogy 96:708–719.

Reeder, S. A., and R. D. Bradley. 2004. Molecular systematics of Neotomine-Peromyscine rodents based on the Dentin Matrix Protein 1 gene. Journal of Mammalogy 85:1194–1200.

Reeder, S. A., D. S. Carroll, C. W. Edwards, C. W. Kilpatrick, and R. D. Bradley. 2006. Neotomine-peromyscine rodent systematics based on combined analyses of nuclear and mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 40:251–258.

Reid, F. A. 2009. A field guide to the mammals of Central America and southeast Mexico, second edition. Oxford University Press, New York.

Rogers, D. S., M. D. Engstrom, and E. Arellano. 2005. Phylogenetic relationships among Peromyscine rodents: allozyme evidence. Pp. 427–440 in Contribuciones mastozoológi-

cas en Homenaje a Bernardo Villa (V. Sanchez-Cordero and R. A. Medellin, eds.). Instituto de Biología e Instituto de Ecología, Universidad Nacional Autónoma de México, México.

Rokas, A., B. L. Williams, N. King, and S. B. Carroll. 2003. Genome-scale approaches to resolving incongruence in molecular phylogenies. Nature 425:798–804.

Saiki, R. K., et al. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491.

Shimodaira, H. 2002. An approximately unbiased test of phylogenetic tree selection. Systematic Biology 51:492–508.

Shimodaira, H. and M. Hasegawa. 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. Bioinformatics 17:1246–1247.

Smith, M. F., and J. L. Patton. 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the Akodontine tribe. Biological Journal of the Linnean Society 50:149–177.

Stamatakis, A., T. Ludwig, and H. Meier. 2005. RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. Bioinformatics 21:456–463.

Stangl, F. B., Jr., and R. J. Baker. 1984. Evolutionary relationships in *Peromyscus*: congruence in chromosomal, genic, and classical data sets. Journal of Mammalogy 65:643–654.

Stanhope, M. J., J. Czelusniak, J.-S. Si, J. Nickerson, and M. Goodman. 1992. A molecular perspective on mammalian evolution from the gene encoding interphotoreceptor retinoid binding protein, with convincing evidence for bat monophyly. Molecular Phylogenetics and Evolution 1:148–160.

Sullivan, J. 1996. Combining data with different distributions of among site rate variation. Systematic Biology 45: 375–380.

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30:2725–2729.

Tiemann-Boege, I., C. W. Kilpatrick, D. J. Schmidly, and R. D. Bradley. 2000. Molecular phylogenetics of the *Peromyscus boylii* species group (Rodentia: Muridae) based on mitochondrial cytochrome *b* sequences. Molecular Phylogenetics and Evolution 16:366–378.

Townsend, T. M., et al. 2011. Phylogeny of Iguanian lizards inferred from 29 nuclear loci, and a comparison of concatenated and species-tree approaches for an ancient, rapid radiation. Molecular Phylogenetics and Evolution 61:363–380.

Toyosawa, S., C. OhUigin, H. Tichy, and J. Klein. 1999. Characterization of dentin matrix protein 1 gene in crocodilia. Gene 234:307–314.

Weksler, M. 2003. Phylogeny of Neotropical Oryzomyine rodents (Muridae: Sigmodontinae) based on the nuclear IRBP exon. Molecular Phylogenetics and Evolution 29:331–349.

Wickliffe, J. K., F. G. Hoffmann, D. S. Carroll, Y. Duniniа-Barkovskaya, and R. D. Bradley. 2003. PCR and sequencing primers for intron 7 (*Fgb*-I7) of the fibrinogen, B beta polypeptide (*Fgb*) in mammals: a novel nuclear DNA phylogenetic marker. Occasional Papers, Museum of Texas Tech University 219:1–6.

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APPENDIX

Specimens examined in this study.—GenBank accession numbers are provided for each gene examined. Abbreviations are as follows: *Adh1*-I2 - intron 2 of alcohol dehydrogenase; *Cyt*b - cytochrome-*b*; *Fgb*-I7 - intron of the beta-fibrinogen; *Dmp*1 - dentin matrix protein 1; *Ghr*- growth hormone receptor; and *Rbp3* - interphotoreceptor retinoid binding protein. GenBank accession numbers (top) and museum catalog numbers (bottom) are given for each specimen. Museum acronyms are as follows: ASNHC (Angelo State Natural History Collection); BYU (Brigham Young University); CNMA (Colección Nacional de Mamíferos, Universidad Nacional Autónoma de México); CRD (Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Durango, Mexico); FSH (University of Texas Medical Branch at Galveston); PGSC (*Peromyscus* Genetic Stock Center); ROM (Royal Ontario Museum); TCWC (Texas Cooperative Wildlife Collection); and TTU (Museum of Texas Tech University). If museum catalog numbers were unavailable, specimens were referenced with the corresponding collector's numbers or TK (special number of the Museum of Texas Tech University). An asterisk indicates the individuals/sequences that were used in the combined analysis.

Taxon	*Adh*1-I2	*Cyt*b	*Fgb*-I7	*Dmp*1	*Ghr*	*Rbp*3
Sigmodon						
*S. hispidus**	KT318181 TTU80759	AF155420 FSH33	AY459371 TTU80759		KT964999 TTU80759	EU635710 TTU80759
Isthmomys						
*I. pirrensis**	FJ214668 TTU39162	FJ214681 TTU39162	FJ214692 TTU39162	MK862084 TTU39162	EF989746 ROM116308	EF989846 ROM116308
		EF989947 ROM116307				EF989848 ROM116307
		EF989945 ROM116308				
		EF989946 ROM116309			EF989747 ROM116309	
Peromyscus						
*P. attwateri**	AY994220 TTU55688	AF155384 TTU55688	AY274207 TTU55688	AY269978 TTU55688	KT950905 TTU55688	JX910128 TTU55688
*P. beatae**	AY994223 TK93279	AF131921 GK3954	FJ214696 TTU105037	MK862085 TK93279	KT950901 TTU105037	KT950924 TK93279
*P. californicus**	AY994211 TTU83292	AF155393 TTU81275	FJ214697 TTU83291	MK862086 TTU83291	EF989772 PGSCIS1590	EF989873 PGSCIS1590
*P. crinitus**	AY994213 DSR6171	AY376413 DSR6171	KT375572 TTU108167	MN057725 TTU108167	EF989773 BYU16629	EF989874 BYU16629
*P. eremicus**	AY994212 TTU81850	AY322503 TTU83249	FJ214699 TTU83249	MN057726 TTU83249	EF989775 BYU17952	EF989876 BYU17952

Appendix (cont.)

Taxon	*Adh*1-I2	*Cyt*b	*Fgb*-I7	*Dmp*1	*Ghr*	*Rbp*3
*P. evides**	FJ214670 TTU82696	FJ214685 TTU82696	FJ214700 TTU82696	MK970558 TTU82696	KT950904 TTU82696	JX910121 TTU82696
*P. furvus**	JX910116 FXG1168	KT965004 FXG1168	JX910113 FXG1168	MK970559 FXG1168	KT950907 FXG1167	JX910124 FXG1168
*P. gossypinus**	FJ214671 TTU80682	DQ973102 TTU80682	FJ214702 TTU80682	MN057727 TTU80682	KT950900 TTU80682	JX910126 TTU80682
*P. gratus**	AY994218 TK46354	AY376421 TK46354	FJ214703 TK46354	MK970560 TK46354	KT950906 TK46354	JX910129 TK46354
*P. hooperi**	FJ214672 TTU104425	DQ973103 TTU104425	FJ214704 TTU104425	MK970567 TTU104425	KT950909 TTU104425	JX910125 TTU104425
*P. leucopus**	AY994241 TTU115505	DQ000483 TTU101645	FJ214706 TTU101645	MK970571 TTU101645	EF989779 ROM101861	EF989880 ROM101861
*P. levipes**	AY994224 TK47819	AY322509 TTU82707	FJ214707 TTU105150	MK970561 TK47819	KT950902 TK47819	JX910123 TK47819
*P. maniculatus**	AY994242 TTU97830	AY322508 TTU83249	FJ214708 TTU97830	MK970562 TTU97830	EF989783 ROM98941	EF989884 ROM98941
*P. megalops**	AY994217 TTU82712	DQ000475 TTU82712	FJ214709 TTU82712	MN057728 TTU82712	KT950908 TTU82712	JX910127 TTU82712
*P. melanophrys**	AY994216 TTU75509	AY322510 TTU75509	FJ214710 TTU75509	MN057729 TTU75509	EF989789 PGSCXZ1073	EF989890 PGSCXZ1073
*P. melanotis**	FJ214673 CRD2025	AF155398 CRD2025	FJ214711 CRD2025	MK970563 CRD2025	EF989790 PGSC25	EF989891 PGSC25
*P. mexicanus**	AY994236 TTU97013	AY376425 TTU82759	AY274210 TTU82759	AY269981 TTU82759	EF989794 ROM113250	EF989895 ROM113250
*P. nudipes**	AY994238 TTU96972	FJ214687 TTU96972	FJ214713 TTU96972	MK970568 TTU96972	EF989792 ROM113216	EF989893 ROM113216
*P. ochraventer**	FJ214676 TTU104930	FJ214689 TTU104930	FJ214715 TTU104930	MN057730 TTU104930	KT950910 TTU104930	JX910130 TTU104930
*P. pectoralis**	AY994221 TK48645	DQ000476 TTU75575	FJ214716 TK48645	MK970564 TK48645	KT950911 TK48645	JX910131 TK48645
*P. schmidlyi**	KT318182 TTU81617	AY322524 TTU81703	FJ214718 TTU81617	MK970565 TTU81617	KT950903 TK72442	KT950925 TTU81703
Habromys						
*H. lepturus**	AY994239 TK93160	DQ973099 TTU82703	FJ214701 TTU82703	MN057731 TTU82703	EF989742 CMNA29970	EF989841 CNMA29970
Neotomodon						
*N. alstoni**	AY994210 TK45309	AY195796 TK45302	AY274202 TK45309	AY269973 TK45309	EF989751 TK45309	EF989851 ASNHC1595
Megadontomys						
*M. thomasi**	AY994208 TK93388	AY195795 TK93388	FJ214693 TK93388	MK970569 TK93388	EF989749 TK93388	EF989849 CNMA29186
Onychomys						
*O. arenicola**	JX910115 TTU67559	AY195793 TTU67559	AY274204 TTU67559	AY269975 TK46462	EF989755 ROM114904	EF989855 ROM114904
*O. leucogaster**	KT318183 TTU60605	AY195794 TTU60605	AY274205 TTU60605	AY26976 TTU60605	EF989758 ASNHC4348	EF989859 ASNHC4348

Appendix (cont.)

Taxon	*Adh*1-I2	*Cyt*b	*Fgb*-I7	*Dmp*1	*Ghr*	*Rbp*3
Osgoodomys						
*O. banderanus**	AY994209 TK45952	DQ000473 TK45952	AY274206 TK45401	AY269977 TK45401	EF989756 ASNHC2664	EF989857 ASNHC2664
Podomys						
*P. floridanus**	AY994214 TTU97867	KT965003 TTU97866	FJ214724 TTU97868	MN057732 TTU97868	KT950912 TTU97868	EF989878 TTU97866
Ochrotomys						
*O. nuttalli**	JX910114 TCWC31929	AY195798 TCWC31929	AY274203 TCWC31929	AY269974 TCWC31929	EF989761 ROM113008	EF989862 ROM113008
Reithrodontomys						
*R. fulvescens**	AY994207 TTU54898	AF176257 TTU54898	AY274211 TTU54898	AY269982 TTU54898	EF989800 ASNHC3465	EF989901 ASNHC3465
R. megalotis		AF176248 TTU40942	KT375573 TTU40942	MK970570 TTU40942	EF989808 ASNHC2133	EF989909 ASNHC2133
R. mexicanus						EF989911 ROM98468
*R. sumichrasti**	JX910117 TTU54952	AF176256 TTU54952	AY274212 TTU54952	MK970566 TTU54952	EF989823 ROM98383	EF989924 ROM98383

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Primitive Karyotype for Muroidea: Evidence from Chromosome Paints and Fluorescent G-bands

Vicki J. Swier, Robert D. Bradley, Frederick F. B. Elder, and Robert J. Baker

Abstract

The relationships among 22 chromosomal paints that were prepared by the Ferguson-Smith laboratory at Cambridge University from the karyotype of *Sigmodon hispidus* using DOP-PCR, flow-sorting are documented. In situ hybridization using these 22 paints was performed on the chromosomes of *S. hispidus* to define the nature of the paints and to establish estimates of homology among other selected representatives of the Sigmodontinae/Neotominae. Chromosomes that had been hybridized were pseudo-G banded using DAPI and identified to the published G-band karyotypes of *S. hispidus*. These baseline hybridization data were then used to establish homologies among the karyotypes of *S. hispidus*, *Melomys burtoni*, *Neotoma micropus*, *Peromyscus boylii*, and *Hylaeamys megacephalus* to determine the direction and nature of chromosomal rearrangements as well as to test the hypothesis that a *S. hispidus*-like karyotype is primitive for the Sigmodontinae.

Key words: chromosome rearrangements, in situ hybridization, *Sigmodon hispidus*, Sigmodontinae

Introduction

The karyotype of *Sigmodon hispidus* has been hypothesized to be similar to the ancestral karyotype for the genus (Zimmerman 1970; Elder 1980; Koop et al. 1984; Swier et al. 2009) and may be chromosomally identical to the species that invaded South America and gave rise to much of the South American sigmodontine fauna (Steppan 1995). The karyotype, proposed to be primitive, has 48 acrocentric and two metacentric autosomes, a subtelocentric X, and a metacentric Y resulting in a both a diploid and fundamental number of 52 (Hsu and Benirschke 1968). Generally, rodent karyotypes that have high diploid numbers and few biarmed chromosomes have been proposed to be ancestral to their respective rodent group (Nadler 1969).

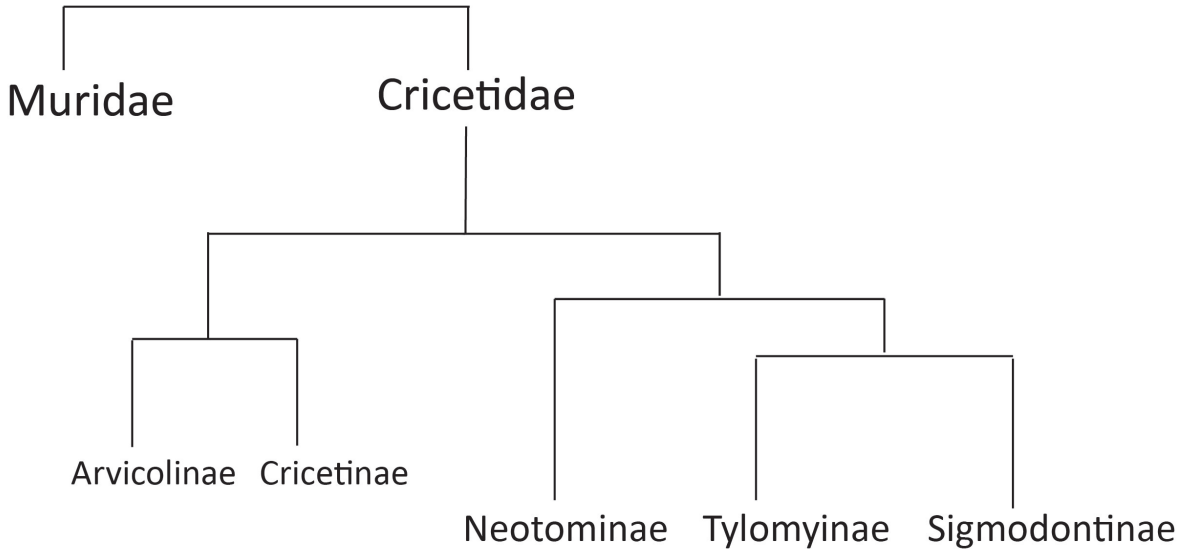
Molecular and morphological studies support this proposed ancestral hypothesis for sigmodontines because the genus *Sigmodon* appears to have originated basally to the sigmodontines (Engel et al. 1998; Weksler 2003); the 14 largest autosomes are considered acrocentric in the Cricetine ancestral karyotype (Baker and Mascarello 1969; Koop et al. 1984); and the diploid number of the ancestral karyotype (especially for the Akodontini) was proposed to be 52 or 54 (Bianchi and Merani 1984).

A hypothesis of the ancestral karyotype based on cladistic analysis of chromosomal banding patterns among various species of cricetids (New World rats, mice, voles, and hamsters) has been previously described (Baker et al. 1983; Koop et al. 1984). These two studies described the G-bands in two (Neotominae—North American rats and mice; Sigmodontinae—New World rats and mice) of the six subfamilies of Cricetidae and in one (Murinae—Old World rats and mice) of the five subfamilies of Muridae (Musser and Carleton 2005). Baker et al. (1983) compared G-bands among *Neotoma micropus*, *Holochilus brasiliensis*, *Nectomys squamipes*, *Neacomys guianae*, *Sigmodon hispidus*, and 11 species of *Oryzomys*; and Koop et al. (1984) compared the G-band patterns within *Rattus norvegicus*, *Apodemus sylvaticus*, *Melomys burtoni*, *Neotoma micropus*, *Oryzomys capito*, and *Peromyscus boylii*. Baker et al. (1983) found conservation of the

ancestral state in *N. micropus*, *S. hispidus*, *H. brasiliensis*, and many species of *Oryzomys* in chromosomes 5, 7, and 9, whereas Koop et al. (1984) described a shared ancestral state for chromosomes 2, 7, 9, 10, 12 and 14 for *N. micropus*, *P. boylii*, and *O. capito*. In both studies, the karyotype of *N. micropus* was hypothesized to be similar to an ancestral state like that of *S. hispidus*, with only four chromosomes modified from the ancestral state in *S. hispidus* (Baker et al. 1983). However, due to low banding resolution of smaller autosomes and inability to positively identify homologous chromosomes among species, the ancestral condition for a few chromosomes remained unknown. See Figure 1 for basic cladogram of Cricetidae and Muridae, and list of aforementioned species within Neotominae, Sigmodontinae, and Muridae.

The resolution of traditional G-banding has mixed results. Comparing the banded chromosomes of various species and compiling common characteristics may be difficult due to the variation of trypsin digestion for each chromosome preparation. Modern techniques such as chromosomal paints in conjunction with fluorescent G banding aid in the identification of chromosomes and the resolution of systematic relationships, with an ultimate goal of identifying homologous characters to the ancestral karyotype.

In this paper, chromosome paints isolated from *S. hispidus* were utilized to match the painted chromosomes of *S. hispidus* to their respective fluorescent G-banded chromosomes. The pairing of painted to banded chromosomes provides greater resolution to the



Muridae		Neotominae	Sigmodontinae
Rattus norvegicus		*Neotoma micropus*	*Holochilus brasiliensis*
Apodemus sylvaticus		*Peromyscus boylii*	*Nectomys squamipes*
Melomys burtoni			*Neacomys guianae*
			Sigmodon hispidus
			*Oryzomys capito**

Figure 1. A basic cladogram of the phylogenetic relationship of Muridae to Cricetidae. The subfamilies of Cricetidae are arranged as discussed in Steppan et al. (2004). **Oryzomys capito* is now recognized as *Hylaeamys megacephalus* in Weksler et al. (2006).

description of the G-banded karyotype of *S. hispidus*. Then, the traditional G-bands of *S. hispidus* (Elder 1980) and the fluorescent G-bands of *S. hispidus* were aligned with the G-bands of *M. burtoni* (Baverstock et al. 1983), *N. micropus*, *P. boylii*, and *O. capito* (now recognized as *Hylaeamys megacephalus*, Weksler et al 2006) from Koop et al. (1984) to better estimate characteristics and the numerical order of the primitive karyotype for the Muroidea.

METHODS AND MATERIALS

Animals.—Cell suspensions from the following *S. hispidus*, archived at Texas Tech University, were utilized: TK 93765 (Texas, USA); TK 93767 (Texas, USA); TTU 108169/TK 121529 (Texas, USA); and TTU 108155/TK 137315/GenBank# EU073177/EU652895/EU635708 (Tamaulipas, MX) (Henson and Bradley 2009). TK numbers represent the data set and tissues collected from the animal, and TTU numbers represent the skin and/or skull of the museum voucher. Museum vouchers do not exist for TK 93765 and TK 93767, but the cytochrome-*b* gene has been sequenced for these individuals and the GenBank numbers are FJ232944.1 and FJ232945.1, respectively.

Chromosome banding.—Karyotypes were prepared according to Baker et al. (2003). To establish a reference set of fluorescent G-banded chromosomes of *S. hispidus*, some chromosome preparations were only DAPI banded, whereas other chromosome preparations were DAPI stained following fluorescence in situ hybridization. Blazed-dried chromosome preparations were treated according to the fluorescent G-band methods developed by M. J. Hamilton as described in the "in situ hybridization and chromosome banding" section of Bowers et al. (1998), starting with the RNase treatment, through the pepsin digestion, and ending with the ethanol series. The chromosome denaturation step was omitted. After the ethanol series, slides were washed in three changes of 2XSSC and incubated in McIlvaine's buffer (0.2 M Na_2HPO_4, 0.1 M citric acid) with 0.01 M $MgCl_2$ for 15 minutes at room temperature. After draining the excess buffer from each slide, 100 µL of chromomycin A3 (100 µg/mL), a DNA binding guanine-specific antibiotic, was appplied to each slide for 15 minutes at room temperature. The slides were then washed in two changes of McIlvaine's buffer for 2 minutes per wash, rinsed with DI H_2O, and allowed to dry. Approximately 35 µL of Vectashield® (Vector Laboratories, Inc., Burlingame, California) mounting medium with DAPI was added to each slide and slides were covered with a coverslip.

Cell culture and fluorescence in situ hybridization.—Fibroblast cultures were established from ear cartilage biopsies from a male *S. hispidus* (TTU 108168/TK 121545) following standard tissue culture techniques. Briefly, biopsies were cleaned, finely minced, and introduced into T-25 culture flasks. Complete Eagle's Minimal Essential Medium (MEM, Invitrogen, Inc., Carlsbad, California) supplemented with 15% fetal bovine serum (Invitrogen, Inc.) was used. Cultures were maintained at 37° C in a tri-gas incubator maintaining a low oxygen (5% O_2), high carbon dioxide (5% CO_2) environment throughout the culturing process. Once the explants were actively proliferating fibroblasts, the cultures were spread, and then subcultured to additional flasks using a mild trypsin dissociation.

Chromosomes of a male *S. hispidus* (TTU 108168/TK 121545) were flow sorted, DOP-PCR amplified, and biotin labeled (Yang et al. 1995) into 22 separate probes at the Centre for Veterinary Science, University of Cambridge. Each probe was individually *in situ* hybridized back to metaphase spreads of *S. hispidus* following a modified version of Yang et al. (2003) as the probes were denatured at 70°C for 5 minutes and slides denatured in 50% formamide/2xSSC at 70°C for 2 minutes.

Analysis.—Each DAPI image and painted chromosome image of the same metaphase spread, as well as the reference DAPI images from different metaphase spreads, were viewed using an Olympus BX51 epifluorescence microscope. Images were photographed with an Applied Imaging® camera. These images were captured using the Genus™ System 3.7 from Applied Imaging Systems (San Jose, California). DAPI images

were inverted with Image Pro Plus 4.5.1 22 (Media Cybernetics Inc., Rockville, Maryland) so that areas that were stained brightly with DAPI became the dark bands of classical G-bands. The G-bands were then enhanced with the HiGauss filter of Image Pro Plus. Banded chromosomes were arranged into a karyogram using the Genus™ System 3.7 software and numerically classified according to previous literature (Elder 1980; Elder and Lee 1985). Separate chromosomes were selected from Elder (1980), Baker et al. (1983), Baverstock et al. (1983), Koop et al. (1984), and from the Texas Tech *S. hispidus* specimens, arranged numerically, and compared. At least 10 DAPI banded metaphase spreads and 10 non-differentially stained metaphase spreads from each of the 22 FISH experiments were photographed and analyzed.

RESULTS

Dr. Malcolm A. Ferguson-Smith's laboratory at the University of Cambridge Veterinary School, resource center for comparative genomics, amplified 22 whole chromosome paints from the karyotype of *S. hispidus*. These paints were labeled alphabetically (A–V) by the peaks generated during flow sorting. During the hybridization of these paints to the karyotype of *S. hispidus*, fifteen (A, C, E–K, M–Q, and T) hybridized to a single chromosome, whereas B, D, L, R, S, U, and V hybridized to multiple chromosomes. The *S. hispidus* karyotype is mainly acrocentric, causing multiple chromosomes to be included in the generation of paints and resulting in a single paint hybridizing to two different chromosomes. This would be expected since many of the smaller acrocentrics of *S. hispidus* are similar in size causing some chromosomes to be poorly distinguished from each other in the flow sorting process. Paint B hybridized to the X and Y, paint D to chromosome 7 and 11, L to chromosomes 8 and 9, R to chromosomes 13 and 22, S to chromosomes 12 and 14, U to chromosomes 16, 20, and 22, and V to chromosomes 19, 20, and 22. In some cases, whole chromosome paints shared chromosomes: chromosome 7 was shared among paints D and G, chromosome 20 among U and V, and chromosome 22 among R, U, and V. In Figure 2, ideograms of the painted chromosomes are matched to their respective DAPI banded chromosomes and arranged numerically according to Elder (1980). This shows the consistency of a specific paint to a chromosome or chromosomes and a corresponding G-banding pattern to the chromosome identified by the paint.

An individual G-banded chromosome from each paint was selected from Figure 2 to be compared to the G-bands of the chromosomes from *N. micropus*, *P. boylii*, *H. megacephalus* (Koop et al. 1984), and *M. burtoni* (Baverstock et al. 1983) (see Fig. 3 for G-band comparisons). Smaller autosomes (15–25) from *H. megacephalus* and *N. micropus* were taken from Baker et al. (1983). Koop et al. (1984) and Baker et al. (1983) produced a G-band numbering system for *Neotoma*, *Peromyscus*, *Oryzomys*, and *Sigmodon*; Baverstock et al. (1983) a system for *Melomys*; and Elder (1980) a system for *Sigmodon*. The numbering systems of Elder (1980) and Baverstock et al. (1983) are not identical to Koop et al. (1984) and Baker et al. (1983) for what is proposed as chromosome 1. For example, chromosome 1 in *S. hispidus* is homologous to chromosome 2 in *P. boylii* and *N. micropus* (Koop et al. 1984). Table 1 documents proposed homology of *S. hispidus* paints to the numbering system of Elder (1980), Baker et al. (1983), Baverstock et al. (1983), and Koop et al. (1984). The following describes the proposed homology in numerical chromosome order.

Chromosome 1—In Baker et al. (1983) and Koop et al. (1984), the primitive condition is retained in *N. micropus*, with varying amounts of G-band positive regions in *P. boylii* and *H. megacephalus*. Yet, all of these species have a large G-band negative block that matches the banding pattern of chromosome 2 in Elder (1980) and chromosome 5 of *M. burtoni* (Baverstock et al. 1983). Compared to the banding pattern of *N. micropus* (considered primitive for the Cricetidae), *S. hispidus* has a derived condition, namely the addition of euchromatin near the centromere, similar to *P. boylii*.

Chromosome 2—The proposed primitive condition is like that of *N. micropus*, *P. boylii*, and *H. megacephalus* (Koop et al. 1984) and closely resembles the pattern of *S. hispidus* chromosome 1 in Elder (1980) and chromosome 1 of *M. burtoni*. This pattern is composed of large blocks of G-positive bands distributed

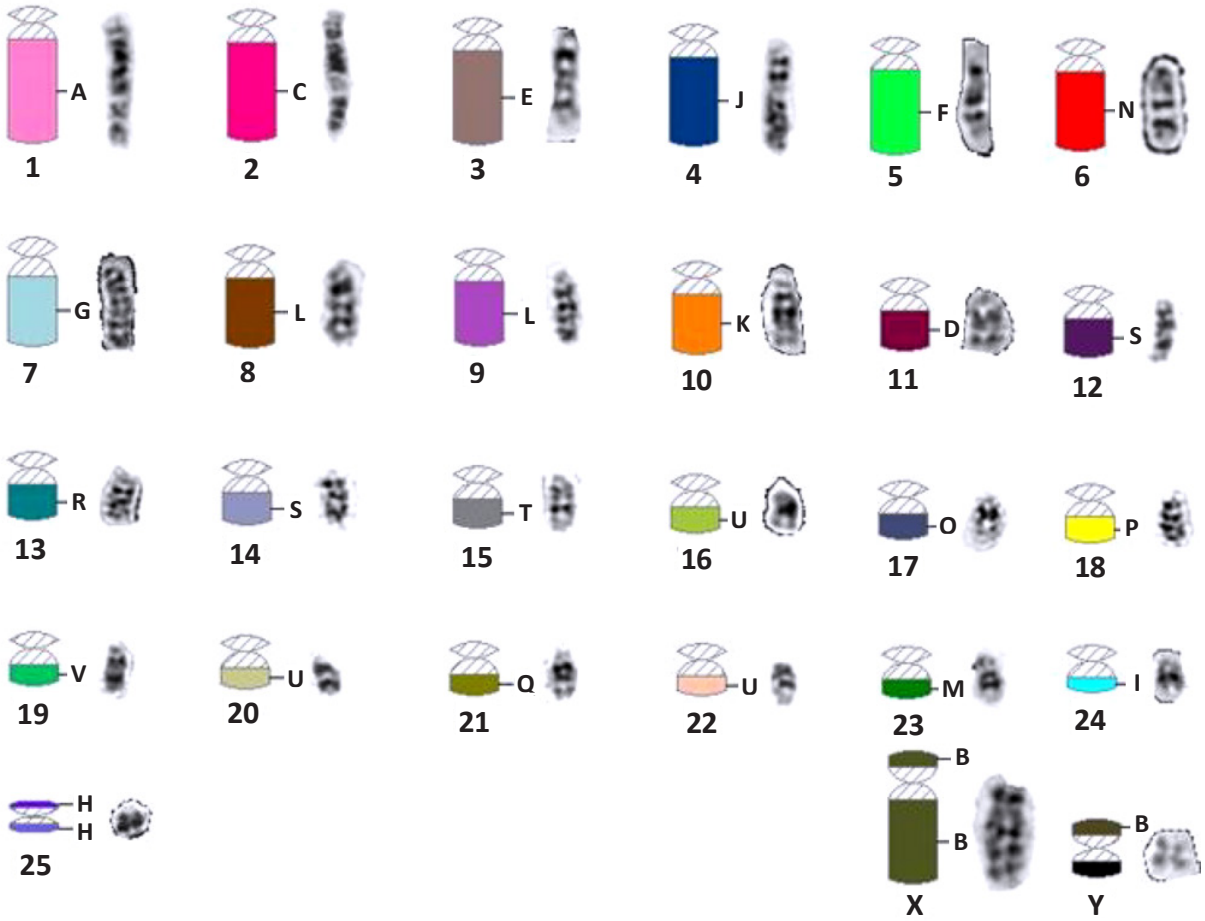


Figure 2. An ideogram of the painted chromosomes of *Sigmodon hispidus* following the numbering system of Elder (1980). Each painted chromosome is associated with its corresponding fluorescent G banded chromosome. The letters A–V represent the labels assigned to each whole chromosome paint.

along the length of the chromosome. Extra G-band positive blocks on the distal end of chromosome 1 of *S. hispidus* are not matched in the G-band patterns of *N. micropus*, *P. boylii*, and *H. megacephalus*.

Chromosome 3—All three species, *N. micropus*, *P. boylii*, and *H. megacephalus*, have a large G-band negative block near the distal end, similar to chromosome 1. The combination of the G-band negative block and an absence of G-positive bands around the centromere of chromosome 3 matches the banding pattern of the chromosome 5 of Elder (1980) and chromosome 2 of *M. burtoni* (Baverstock et al. 1983).

Chromosome 4—The proposed ancestral condition is like that of *N. micropus* and *P. boylii* (Koop et al. 1984). In *S. hispidus*, chromosome 3 (Elder 1980) appears to have a similar banding pattern as it shares a large block of interstitial G positive bands with the other three species. In *M. burtoni*, chromosome 3 (Baverstock et al. 1983) also shares the large block of interstitial bands with the other species.

Chromosome 5—Koop et al. (1984) was unsure about the primitive condition for this chromosome. It appears to be like that of *N. micropus,* as chromosome 4 in *S. hispidus* (Elder 1980) and chromosome 4 in *M. burtoni* (Baverstock et al. 1983) have a paracentromeric band and a darkly stained swath of G-positive bands at the distal end of their chromosomes. Chromosome 4 in *S. hispidus* resembles the ancestral condition as it lacks the derived rearrangement 5/6 as described in *H. megacephalus* (Koop et al. 1984).

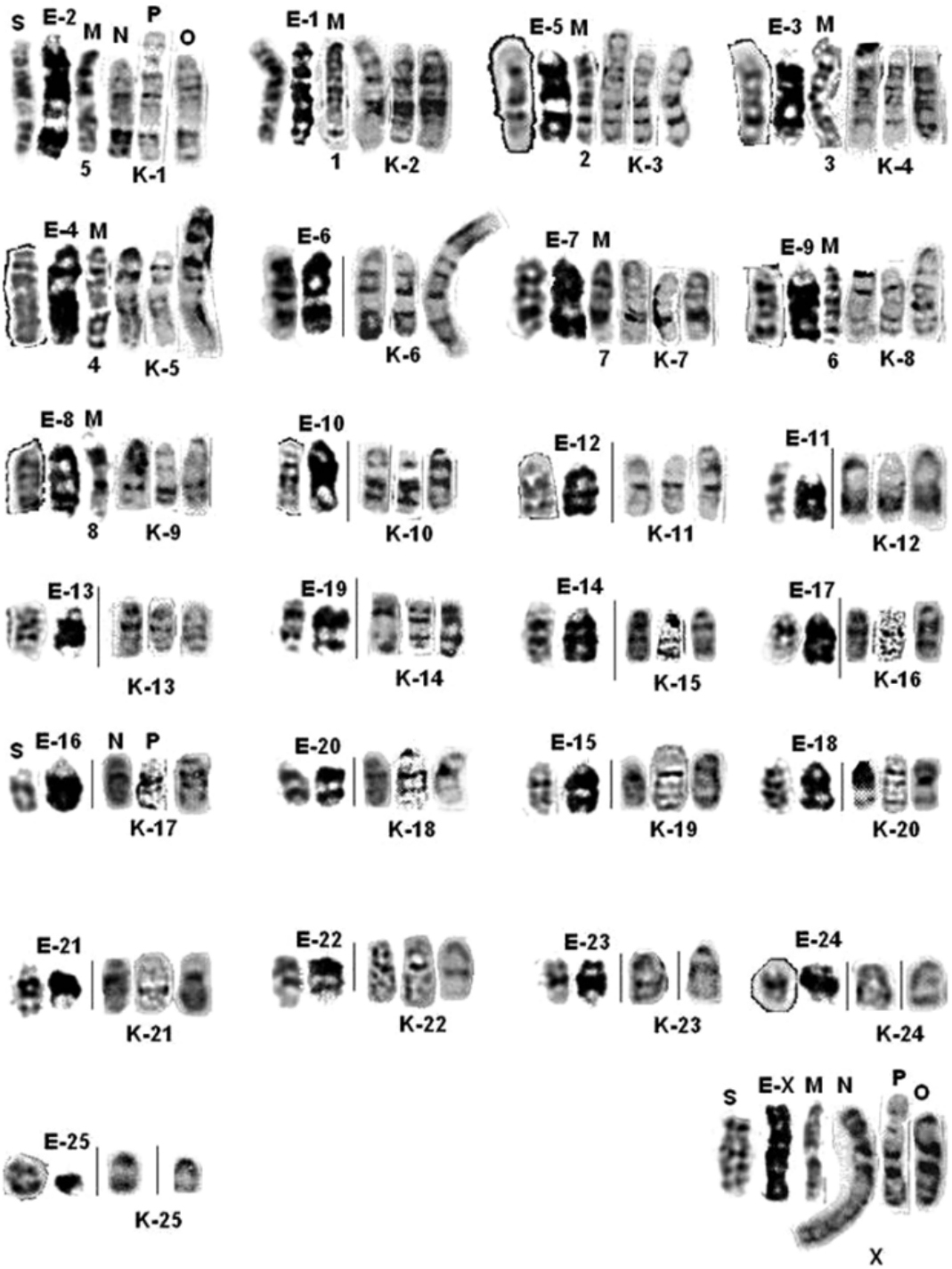


Figure 3. The fluorescent G-bands of *Sigmodon hispidus* and traditional G-bands of *S. hispidus* (Elder 1980), *Neotoma micropus*, *Peromyscus boylii*, *Hylaeamys megacephalus* (Koop et. al. 1984; Baker et al. 1983), and *Melomys burtoni* (Baverstock et al. 1983). S represents the chromosomes of *S. hispidus* that we banded in our lab, M represents the chromosomes of *M. burtoni* (Baverstock et al. 1983), E1–E25 represents the *S. hispidus* chromosomes from Elder (1980), N represents the chromosomes of *N. micropus*, P represents chromosomes of *P. boylii*, O represents chromosomes of *H. megacephalus* (Koop et al. 1984; Baker et al. 1983), and K1–25 represents Koop's numbering system.

Table 1. The paints isolated from *Sigmodon hispidus* labeled A–V and the chromosomes identified by the paints according to the numerical system of Elder (1980), Koop et al. (1984), Baker et al. (1983), and Baverstock et al. (1983). The question marks indicate at least three issues including the chromosomes that were not available in the publication, the resolution of the G-bands was too poor to make an accurate identification, or a chromosome with a similar banding pattern could not be found.

Paint	Elder 1980	Koop et al. 1984	Baker et al. 1983	Baverstock et al. 1983
A	#1	#2		#1
B	X and Y	X and Y		X and Y
C	#2	#1		#5
D	#7 and #11	#7 and #12		#7 and ?
E	#5	#3		#2
F	#3	#4		#3
G	#7	#7		#7
H	#25	?	?	?
I	#24	?	?	?
J	#4	#5		#4
K	#10	#10		?
L	#8 and #9	#9 and #8		? and #6
M	#23	?	?	?
N	#6	#6		?
O	#17	?	#16	?
P	#18	?	#20	?
Q	#21	?	?	?
R	#13 and #22	#13	?	?
S	#12 and #14	#11	#15	?
T	#15	?	#19	?
U	#16, #20, #22	?	#17, #18, ?	?
V	#19, #20, #22	#14	#18, ?	?

Chromosomes 6 and 7—The primitive condition was retained in *N. micropus* and *P. boylii* (Koop et al. 1984) and matches the banding pattern of chromosome 6 of Elder (1980). Chromosome 7 was identical in all three species *N. micropus*, *P. boylii*, and *H. megacephalus* and that pattern is the same as chromosome 7 of Elder (1980). In *M. burtoni*, a chromosome with a similar banding pattern to chromosome 6 of *S. hispidus*, *H. megacephalus*, *N. micropus*, and *P. boylii* could not be found, but chromosome 7 of *M. burtoni* matched the banding patterns of the chromosome 7 in the aforementioned species.

Chromosome 8—The G-band pattern of *N. micropus* and *P. boylii* were considered primitive for this chromosome (Koop et al. 1984). The chromosome designated as number 9 in *S. hispidus* (Elder 1980), and number 6 in *M. burtoni* (Baverstock et al. 1983) has at least four G-positive bands along its length. The location of at least three of these bands is shared to bands on chromosome 8 of *N. micropus*, *P. boylii*, and *H. megacephalus*. One of the median G-positive bands of *S. hispidus* 9 is not apparent on chromosome 8 of *N. micropus*.

Chromosome 9—The primitive condition was shared among *N. micropus*, *P. boylii*, and *H. megacephalus*. This pattern is an almost exact match to the banding pattern of chromosome 8 of *S. hispidus* (Elder 1980), any differences attributed to the resolution of traditional G-banding versus DAPI banding. Chromosome 8 of *M. burtoni* is aligned to these chromosomes in Figure 3, but the banding pattern is not entirely similar and hence this alignment is tentative. Only eight chromosomes of *M. burtoni* were aligned to the other species as these chromosomes were the only ones that had similar G-banding patterns to the chromosomes of *S. hispidus*, *H. megacephalus*, *N. micropus*, and *P. boylii*.

Chromosome 10—Chromosome 10 was identical in all three species, *N. micropus*, *P. boylii*, and *H. megacephalus,* and that pattern is the same as chromosome 10 of Elder (1980).

Chromosome 11—The banding pattern of *N. micropus* and *P. boylii* is identical and proposed to be primitive (Koop et al. 1984). This G-band pattern is similar to the bands of chromosome 12 in *S. hispidus* (Elder 1980). All three species have a G-positive region at the centromere and a dark interstitial G-positive band that stands out at the center of the chromosome. *Sigmodon hispidus* shares this interstitial band with *H. megacephalus* as well, but *H. megacephalus* has more chromatin near its centromere.

Chromosome 12—The G-bands of chromosome 12 were determined to be of the same pattern in all three species, *N. micropus*, *P. boylii*, and *H. megacephalus* (Koop et al. 1984), supporting the hypothesis that the primitive condition for this chromosome was retained. The chromosome designated as 11 in Elder (1980) seems homologous to chromosome 12 (Koop et al. 1984) as all species have a fairly thick, G-positive region on the distal end of the chromosome.

Chromosome 13—Koop et al. (1984) were unsure of the primitive condition of chromosome 13. It appears to be similar to that found in *N. micropus* and *P. boylii* as this G-banding pattern is similar to that found in chromosome 13 of *S. hispidus* (Elder 1980).

Chromosome 14—Chromosome 14 was identical in *N. micropus*, *P. boylii*, and *H. megacephalus* (Koop et al. 1984). Chromosome 19 in *S. hispidus* (Elder 1980) appears homologous to 14 as all the species have two G-positive paracentromeric bands, followed by a wide G-negative band, and another two G-positive bands. The smaller autosomes of *N. micropus* and *H. megacephalus* were not labeled numerically in Baker et al (1983) but arranged by centromeric position and size in their Figure 3. They were labeled left to right as 13–19 and 21–25.

Chromosome 15—Chromosome 15 appears to be identical in the species *N. micropus*, *P. boylii*, and *H. megacephalus* and homologous to chromosome 14 in *S. hispidus* (Elder 1980). All four species retain two interstitial G-positive bands on their respective chromosomes, though *S. hispidus* lacks the G-positive band at the centromere.

Chromosome 16—Chromosome 16 seems to be identical in all species and homologous to chromosome 17 (Elder 1980). The banding pattern consists of at least two interstitial G-positive bands are located near the centromere and a singular G-positive band near the distal end.

Chromosome 17— The G-banding pattern of *N. micropus*, *P. boylii*, and *H. megacephalus* is identical in 17 and homologous to chromosome 16 (Elder 1980). Only two interstitial G-positive bands located closer to the centromeric end seem to be the dominant pattern.

Chromosome 18—Chromosome 18 seems identical in all species and homologous to chromosome 20 (Elder 1980). Homologous G-bands are more difficult to distinguish in these smaller autosomes, but a pattern is still distinguishable of two G-positive bands separated by at least one G-negative band that is consistent among all the species.

Chromosome 19—The resolution in Elder (1980) for this chromosome is lacking in this particular association but after coupling it with the chromosome banded in this paper, a pattern of a darkly stained G-positive band near the centromere, followed by a G-negative band, and then two faintly stained G-positive bands was detected. This pattern in *S. hispidus* 15 matches the G-bands of chromosome 19 in *N. micropus*, *P. boylii*, and *H. megacephalus*.

Chromosome 20—This is the last autosome that was homologous among all the species. Chromosome 18 (Elder 1980) seems homologous to chromosome 20 (Baker et al. 1983). The G-banding pattern of *S. hispidus* and *P. boylii* consists of at least four G-positive bands along the length of the chromosome and at least three of these bands are retained in *N. micropus* and *H. megacephalus*.

Chromosome 21-25—Resolution is lacking and there was not confidence in assignment of chromosomal homology. Numerically similar chromosomes were aligned together but they may not be homologous to each other.

Chromosome X—G-banding pattern is well conserved throughout all the species. Most of the chromosomes have a similar G-band pattern in *N. micropus*, *P. boylii*, *M. burtoni*, and *S. hispidus*.

DISCUSSION

The karyotypes of *N. micropus*, *P. boylii*, *S. hispidus,* and *M. burtoni* have been considered ancestral for their respective genera (Mascarello and Hsu 1976; Elder 1980; Robbins and Baker 1981; Baverstock et al. 1983). After comparing the G-bands of these species from Elder (1980), Baker et al. (1983), Baverstock et al. (1983), Koop et al. (1984), and *S. hispidus* from this study, a similarity of banding patterns was documented to better estimate the ancestral Muroidea karyotype. The first 14 chromosomes and the X of *N. micropus*, *P. boylii*, *H. megacephalus*, *Apodemus sylvaticus*, and *Rattus norvegicus* were aligned together based on G-band homology (Koop et al. 1984) to determine an ancestral cricetid karyotype, and the G-bands of eight species of *Oryzomys* were compared to the outgroups of *Nectomys squamipes*, *Neacomys guianae*, *S. hispidus* from Elder (1980), and *H. brasiliensis* (Baker et al. 1983) to determine the ancestral karyotype for *Oryzomys*. Though the numbering system of *Sigmodon* from Elder (1980) does not match the chromosomal numbering system of Baker et al. (1983), Baverstock et al. (1983), and Koop et al. (1984), *Sigmodon* chromosomes were matched to homologous chromosomes of the other species. Resolution was lacking in some of the smaller autosomes and a complete study on chromosomal homology was not possible. Studies of chromosomal homologies are required to understand ancestral traits of a particular group of organisms and their systematic relationships. This study employs whole chromosome paints, traditional G-bands, and fluorescent G-bands to further resolve the hypothetical ancestral karyotype of the Muroidea. This study further supported the work of Baker et al. (1983) and Koop et al. (1984), recognizing that the G-band pattern of *N. micropus*, *P. boylii*, and *H. megacephalus* are so similar as to indicate common ancestry.

A common North American origin for all three genera (*Sigmodon*, *Neotoma*, and *Peromyscus*) is supported with fossil evidence (Lindsay 1972). The ancestry of neotomines, reithrodontines, and some genera of the subfamily Sigmodontinae may have evolved from a *Copemys*-like species, which existed in the mid-Miocene (Lindsay 1972; Jacobs and Lindsay 1984). Fossil data supports the derivation of *Peromyscus* from *Copemys* (Lindsay 1972; Baskin 1979); *Neotoma* (fossil *Repomys*) from *Peromyscus pliocenicus* (May 1981); and *Sigmodon* (fossil *Calomys*) from *Copemys* (Baskin 1978). Oldest fossils of Sigmodontini (species of *Sigmodon*), neotomines, and reithrodontines were collected in North America and dated from the Hemphillian stage, 4.75 to 9 mya (Marshall 1979; May 1981; Dalquest 1983).

Phylogenetic analyses also are compatible with a common ancestral position that gave rise to *Neotoma*, *Peromyscus*, and *Sigmodon*. In a phylogenetic analysis of muroid rodents, the clades of neotomine-peromycines, tylomyines, and sigmodontines are united by a common ancestor but the position of these clades in relation to each other is not well supported (Steppan et al. 2004). For the Neotomyinae (North American rodents), the basal position of *Neotoma* to clades of *Peromyscus* and *Reithrodontomys* is well supported by a 100% bootstrap value using sequencing data from nuclear genes (Steppan et al. 2004). Reeder et al. (2006) completed a maximum likelihood analysis of nuclear and mitochondrial sequences of neotomine-

peromyscine rodents to display a topology uniting the tribes Neotomini (*Neotoma*) and Peromyscini (now recognized as Reithrodontomyini) with a common ancestor (99% Bayesian support value). Smith and Patton (1999) determined that *S. hispidus* is basal in some of the phylogenetic analyses of South American sigmodontines, to the outgroup consisting of *Peromyscus* and *Neotoma*. Of note, the *S. hispidus* of Smith and Patton's study was from Costa Rica, a species now recognized as *S. hirsutus* (Peppers et al. 2002).

Other molecular work also supports *Sigmodon* as basal to all sigmodontines (Sarich 1985; Catzeflis et al. 1993; Engel et al. 1998; Steppan et al. 2004). The tribe Oryzomyini had been proposed to be the basal tribe in the subfamily Sigmodontinae (Gardner and Patton 1976; Reig 1980), but chromosomal data have difficulties in explaining such a relationship. At least four chromosomal events were proposed to have occurred from the primitive cricetid karyotype to the current karyotype of *H. megacephalus* (Koop et al. 1984), and at least 55 rearrangements were documented in *Oryzomys* compared to 33 in *Peromyscus* based on cladistic analysis of chromosome homology (Baker et al. 1983). This study supports previous studies as the karyotype of *H. megacephalus* was the most derived, rearrangements from the ancestral condition were documented in six chromosomes (1, 4, 5, 6, 8, and 11). *H. megacephalus* is considered to have a highly reorganized genome, with multiple rearrangements discovered when mapping whole chromosome paints isolated from *H. megacephalus* onto the karyotypes of other Sigmodontinae (Pereira et al. 2016).

In this study, the karyotypes of *N. micropus* and *P. boylii* only differed from the proposed ancestral condition by rearrangements in one chromosome (3 and 1, respectively). Rearrangements in two chromosomes (1 and 2) separate the karyotype of *S. hispidus* from the ancestral condition. These rearrangements also were described in Baker et al. (1983) and Koop et al. (1984). However, the karyotype of *S. hispidus* retains many G-band patterns of the Muroidea. The first seven chromosomes of *M. burtoni* were similar to the banding patterns of chromosomes 1–5, 7, and 9 of *S. hispidus*; and rearrangements in only two chromosomes of *S. hispidus* separate its karyotype from *P. boylii* and *N. micropus*. These data support the position that the *S. hispidus* karyotype has remained largely unchanged and similar to that proposed to be ancestral to the Muroidea.

The rearrangements found in chromosome 3 are not only specific to the species analyzed in this paper. The extra G-positive bands of chromosome 3 are retained in other species of *Neotoma*. In Mascarello et al. (1974), the G-bands of *N. micropus* and *N. phenax* were compared and the pattern of chromosome 3 was identical in both species (see their Figure 2). This particular rearrangement in chromosome 3 may be retained in all species of *Neotoma*, differentiating them from the proposed ancestral karyotype. In *Peromyscus*, the short arm addition to chromosome 1 is also documented in *P. californicus*, *P. attwateri*, *P. difficilis*, *P. pectoralis, P. ochraventer*, *P. truei*, and *P. leucopus* (Figure 1 of Robbins and Baker 1981). All the rearrangements detected in *Oryzomys* are not conserved throughout all species in this genus (Haiduk et al. 1979; Baker et al. 1983), but chromosomes 1, 2, and 11 are described as modified from the ancestral karyotype in the 11 species of Oryzomys analyzed in Baker et al. (1983).

Conservation of genetic material, especially in areas of the genome that are highly conserved, is important for the perpetuity of the species as chromosomal rearrangements in highly conserved area may be lethal to offspring. As this study documented that chromosomes 7, 9, 10, 12–20 were conserved in all cricetid species, Robbins and Baker (1980) explained a similar occurrence in *Reithrodontomys fulvescens* as chromosomes 6–14 were described as retaining the primitive condition. It is quite possible that chromosomes 7, 9, 10, and 12 have retained the ancestral condition in many cricetids. The G-band patterns of chromosome 7 were conserved among cricetids and murids (Koop et al. 1984; this paper) and among many species of cricetids (Baker et al. 1983); and chromosomes 10 and 12 have similar G-band patterns among cricetids and murids (Koop et al. 1984). Also, the first seven chromosomes of *M. burtoni* had similar banding patterns to *S. hispidus*, *H. megacephalus*, *N. micropus*, and *P. boylii*, to further support the primitive condition of the *S. hispidus* karyotype.

The conservation of genetic material on the smaller autosomes of many cricetids and murids of different geographic distributions and life histories documents an important characteristic of the alignment of genes upon a chromosome. The particular genetic

arrangement is possibly essential to the conservation of the genome and to the species. Some genetic mutations are deleterious, and drastic changes like chromosomal rearrangements produce reproductive isolating mechanisms that eventually either promote a new species or cause its extinction.

The lack of many chromosomal rearrangements in the grasshopper species *Warramaba virgo* led M. J. D. White to hypothesize that "natural selection against newly arisen rearrangements is extremely severe" as most rearrangements have deleterious effects (1975). This species is widely distributed across eastern and western Australia, has been in existence for many thousands of years, and its parthenogenetic lifestyle hypothetically should not restrict newly arisen rearrangements if they pass through mitosis. Yet, approximately only six major chromosomal rearrangements exist in natural populations. Selection for a particular gene order, usually a primitive gene order, may help to stabilize a genome. If the particular order was effective in the preservation of one species, it may also continue to preserve the existence of newly evolved species.

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LITERATURE CITED

Baker, R. J., M. Hamilton, and D. A. Parish. 2003. Preparations of mammalian karyotypes under field conditions. Occasional Papers, Museum of Texas Tech University 228:1–8.

Baker, R. J., B. F. Koop, and M. W. Haiduk. 1983. Resolving systematic relationships with G-bands: a study of five genera of South American Cricetine rodents. Systematic Zoology 32:403–416.

Baker, R. J., and J. T. Mascarello. 1969. Karyotypic analysis of the genus *Neotoma* (Cricetidae, Rodentia). Cytogenetics 8:187–198.

Baskin, J. A. 1978. *Bensonomys*, *Calomys*, and the origin of the phyllotine group of Neotropical cricetines (Rodentia: Cricetidae). Journal of Mammalogy 59:125–130.

Baskin, J. A. 1979. Small mammals of the Hemphillian age White Cone local fauna, northeastern Arizona. Journal of Paleontology 53:695–708.

Bianchi, N. O., and S. Merani. 1984. Cytogenetics of South American akodont rodents (Cricetidae). X. Karyological distances at generic and intergeneric levels. Journal of Mammalogy 65:206–219.

Bowers, K. L., M. J. Hamilton, S. M. White, and R. J. Baker. 1998. Origins of heterochromatic repatterning in white-footed mice, *Peromyscus leucopus*. Journal of Mammalogy 79:725–735.

Baverstock, P.R., C.H.S. Watts, M. Gelder, and A. Jahnke. 1983. G-banding homologies of some Australian rodents. Genetica 60:105–117.

Catzeflis, F. M., A. W. Dickerman, J. Michaux, and J. A. W. Kirsch. 1993. DNA hybridization and rodent phylogeny. Pp. 159–172 in Mammalian phylogeny: placentals (F.S. Szalay, M. J. Novacek, and M. C. McKenna, eds.). Springer-Verlag, New York.

Elder, F. F. B. 1980. Tandem fusion, centric fusion, and chromosomal evolution in the cotton rats, genus *Sigmodon*. Cytogenetics and Cell Genetics 26:199–210.

Elder, F. F. B., and M. R. Lee. 1985. The chromosomes of *Sigmodon ochrognathus* and *Sigmodon fulviventer* suggest a realignment of *Sigmodon* species groups. Journal of Mammalogy 66:511–518.

Dalquest, W. W. 1983. Mammals of the Coffee Ranch local fauna, Hemphillian of Texas. Pearce-Sellards Series, Texas Memorial Museum 38:1–41.

Engel, S. R., K. M. Hogan, J. F. Taylor, and S. K. Davis. 1998. Molecular systematics and paleobiogeography of the South American sigmodontine rodents. Molecular Biology and Evolution 15:35–49.

Gardner, A. L., and J. L. Patton. 1976. Karyotypic variation in oryzomine rodents (Cricetidae) with comments on chromosomal evolution in the Neotropical cricetine complex. Occasional Papers of the Museum of Zoology, Louisiana State University 49:1–48.

Haiduk, M. W., J. W. Bickham, and D. J. Schmidly. 1979. Karyotypes of six species of *Oryzomys* from Mexico and Central America. Journal of Mammalogy 60:610–615.

Henson, D. D., and R. D. Bradley. 2009. Molecular systematics of the genus *Sigmodon*: results from mitochondrial and nuclear gene sequences. Canadian Journal of Zoology 87:211–220.

Hsu, T. C., and K. Benirschke. 1968. An atlas of mammalian chromosomes, Volume 2. Springer-Verlag, Berlin, Heidelberg and New York.

Jacobs, L. L., and E. H. Lindsay. 1984. Holarctic radiation of Neogene muroid rodents and the origin of South American cricetids. Journal of Vertebrate Paleontology 4:265–272.

Koop, B. F., R. J. Baker, M. W. Haiduk, and M. D. Engstrom. 1984. Cladistical analysis of primitive G-band sequences for the karyotype of the ancestor of the Cricetidae complex of rodents. Genetica 64:199–208.

Lindsay, E. H. 1972. Small mammal fossils from the Barstow Formation, southern California. University of California, Publications in Geological Sciences 93:1–104.

Marshall, L. G. 1979. A model for paleobiogeography of South American cricetine rodents. Paleobiology 5:126–132.

Mascarello, J. T., and T. C. Hsu. 1976. Chromosome evolution in woodrats, genus *Neotoma* (Rodentia: Cricetidae). Evolution 30:152–169.

Mascarello, J. T., J. W. Warner, R. J. Baker. 1974. A chromosome banding analysis of the mechanisms involved in the karyological divergence of *Neotoma phenax* (Merriam) and *Neotoma micropus* (Baird). Journal of Mammalogy 55:831–834.

May, S. R. 1981. *Repomys* (Mammalia: Rodentia gen. nov.) from the late Neogene of California and Nevada. Journal of Vertebrate Paleontology 1:219–230.

Musser, G. G., and M. D. Carleton. 2005. Superfamily Muroidea. Pp. 894–1531 in Mammal species of the world (D. E. Wilson and D. M. Reeder, eds.). John Hopkins University Press, Baltimore, Maryland.

Nadler, C. F. 1969. Chromosomal evolution in rodents. Pp. 277–309 in Comparative mammalian cytogenetics (K. Benirschke, ed.). Springer-Verlag, New York.

Peppers, L. L., D. S. Carroll, and R. D. Bradley. 2002. Molecular systematics of the genus *Sigmodon* (Rodentia: Muridae): evidence from the mitochondrial cytochrome-*b* gene. Journal of Mammalogy 83:396–407.

Pereira, A. L., et al. 2016. Extensive chromosomal reorganization in the evolution of New World muroid rodents (Cricetidae, Sigmodontinae): searching for ancestral phylogenetic traits. PLoS One b11:e0146179.

Reeder, S. A., D. S. Carroll, C. W. Edwards, C. W. Kilpatrick, and R. D. Bradley. 2006. Neotomine-peromyscine rodent systematics based on combined analyses of nuclear and mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 40:251–258.

Reig, O. A. 1980. A new fossil genus of South American cricetid rodents allied to *Wiedomys*, with an assessment of the Sigmodontinae. Journal of Zoology (London) 192:257–281.

Robbins, L. W., and R. J. Baker. 1980. G- and C-band studies on the primitive karyotype for *Reithrodontomys*. Journal of Mammalogy 61:708–714.

Robbins, L. W., and R. J. Baker. 1981. An assessment of the nature of chromosomal rearrangements in 18 species *Peromyscus* (Rodentia: Cricetidae). Cytogenetics and Cell Genetics 31:194–202.

Sarich, V. M. 1985. Rodent macromolecular systematics. Pp. 423–452 in Evolutionary relationships among rodents, a multidisciplinary analysis (W. P. Luckett and J. L. Hartenberger, eds.). Plenum Press, New York.

Smith, M. F., and J. L. Patton. 1999. Phylogenetic relationships and the radiation of sigmodontine rodents in South America: evidence from cytochome *b*. Journal of Mammalian Evolution 6:89–117.

Steppan, S. J. 1995. Revision of the leaf-eared mice Phyllotini (Rodentia: Sigmodontinae) with a phylogenetic hypothesis for the Sigmodontinae. Fieldiana: Zoology 80:1–112.

Steppan, S. J., R. M. Adkins, and J. Anderson. 2004. Phylogeny and divergence-data estimates of rapid radiations in muroid rodents based on multiple nuclear genes. Systematic Biology 53:533–553.

Swier, V. J., R. D. Bradley, W. Rens, F. F. B. Elder, and R. J. Baker. 2009. Patterns of chromosomal evolution in *Sigmodon*, evidence from whole chromosome paints. Cytogenetics and Genome Research 125:54–66.

Weksler, M. 2003. Phylogeny of Neotropical oryzomyine rodents (Muridae: Sigmodontinae) based on the nuclear IRBP exon. Molecular Phylogenetics and Evolution 29:331–349.

Weksler, M., A. R. Percequillo, and R.S. Voss. 2006. Ten new genera of oryzomyine rodents (Cricetidae: Sigmodontinae). American Museum Novitates 3537:1–29.

White, M. J. D. 1975. Chromosomal repatterning-regularities and restrictions. Genetics 79:63–72.

Yang, F., E. Z. Alkalaeva, P. L. Perelman, A. T. Pardini, W. R. Harrison, P. C. M. O'Brien, B. Fu, A. S. Graphodatsky, M. A. Ferguson-Smith, and T. J. Robinson. 2003. Reciprocal chromosome painting among human, aardvark, and elephant (superorder Afrotheria) reveals the likely eutherian ancestral karyotype. Proceedings of the National Academy of Sciences 100:1062–1066.

Yang, F., N. P. Carter, L. Shi, and M. A. Ferguson-Smith. 1995. A comparative study of karyotypes of muntjacs by chromosome painting. Chromosoma 103:642–652.

Zimmerman, E. G. 1970. Karyology, systematics and chromosomal evolution in the rodent genus, *Sigmodon*. Publications of the Museum, Michigan State University 4:385–454.

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Molecular Phylogenetics of the Tribe Oryzomyini Using Multiple DNA Markers

J. Delton Hanson and Roy N. Platt II

Abstract

Taxonomic relationships of Oryzomyini rodents were examined to test phylogenetic hypotheses for the tribe and the placement of currently recognized genera. To accomplish this, taxonomic sampling and number of genes examined were increased relative to previous examinations. Phylogenetic relationships were estimated with maximum likelihood and Bayesian methods. A total of 84 individual samples representing all the genera of the tribe Oryzomyini as well as each of the recognized extant tribes of Sigmodontinae were examined for the first gene (interphotoreceptor retinoid binding protein). Although this was an increase of individuals from the most recent molecular work published on the whole tribe, the phylogenetic resolution provided did not increase accordingly. Phylogenetic resolution also was not increased with a second nuclear gene (alcohol dehydrogenase). When the tribe was examined using a mitochondrial gene (cytochrome-*b*), support was observed almost exclusively at recent nodes; however, when the mitochondrial and nuclear datasets were combined phylogenetic resolution was observed at most nodes. The multi-gene, combined analyses supported a monophyletic tribe Oryzomyini consistent with the most recent arrangement of the tribe. Additionally, all 10 genera previously elevated from *Oryzomys* (*Aegialomys*, *Cerradomys*, *Eremoryzomys*, *Euryoryzomys*, *Hylaeamys*, *Mindomys*, *Nephlomys*, *Oreoryzomys*, *Sooretamys*, and *Transandinomys*) were examined, as well as a previously unexamined taxon (*Microakodontomys*) and two recently named genera (*Drymoreomys* and *Tanyuromys*). All currently named genera in the tribe were supported except for reciprocal monophyly in *Sigmodontomys* and *Melanomys*. Further, additional recognition of generic groupings may be warranted based on genetic divergence observed herein.

Key words: alcohol dehydrogenase, Bayesian, cytochrome-*b*, interphotoreceptor retinoid binding protein, maximum likelihood, Oryzomyini, taxonomy

Supplementary material related to this manuscript is available online at https://github.com/jdelton/oryzomys-mutli-gene-phylo.

Introduction

The Oryzomyini is one of 11 tribes in the rodent family Cricetidae, subfamily Sigmodontinae, and contains 29 extant genera and more than 100 species (Musser and Carleton 2005; Weksler et al. 2006; Percequillo et al. 2011; Pine et al. 2012; Salazar-Bravo et al. 2016). It is the most widely distributed New World rodent tribe, occurring from the eastern coast of the United States to the southern tip of South America. Since the description of the Oryzomyini group by Hershkovitz (1944), this assemblage has received much attention relative to taxonomy and systematics. Historically, several different arrangements were proposed by different authors (Hershkovitz 1944, 1948; Reig 1986; Smith and Patton 1993, 1999; Bonvicino and Martins Moreira 2001; Weksler 2003; Musser and Carleton 2005; Weksler et al. 2006), and there was little agreement between them as to which taxa should be included in the Oryzomyini (Table 1). Although more recent

Table 1. Selected classifications for Oryzomyini. Subgenera are indented under generic name. Asterisk designates taxa treated by McKenna and Bell (1997) and Weksler (2003, 2006) but not Voss and Carleton (1993). Carat symbol designates taxa treated by Weksler (2003, 2006) but not McKenna and Bell (1997) or Voss and Carleton (1993). Plus sign designates taxa treated by McKenna and Bell (1997) but not Weksler (2003, 2006) or Voss and Carleton (1993).

Hershkovitz (1944, 1948)	Reig (1986)	Voss and Carleton (1993), McKenna and Bell (1997), and Weksler (2003, 2006)	Smith and Patton (1999)	Musser and Carleton (2005)
Oryzomyini	Oryzomini	Oryzomyini	Oryzomini	Oryzomini
Neacomys	*Oryzomys*	*Holochilus*	*Holochilus*	*Holochilus*
Nectomys	*Oligoryzomys*	*Melanomys*	*Melanomys*	*Melanomys*
Sigmodontomys	*Melanomys*	*Microryzomys*	*Microryzomys*	*Microakodontomys*
Oryzomys	*Microryzomys*	*Neacomys*	*Neacomys*	*Microryzomys*
Melanomys	*Sigmodontomys*	*Nesoryzomys*	*Nesoryzomys*	*Neacomys*
Microryzomys	*Thomasomys*	*Oecomys*	*Oecomys*	*Nesoryzomys*
Nesoryzomys	*Oecomys*	*Oligoryzomys*	*Oligoryzomys*	*Oecomys*
Oecomys	*Rhipidomys*	*Oryzomys*	*Oryzomys*	*Oligoryzomys*
Oligoryzomys	*Nectomys*	*Pseudoryzomys*	*Pseudoryzomys*	*Oryzomys*
Oryzomys	*Neacomys*	*Scolomys*	*Sigmodontomys*	*Pseudoryzomys*
Scolomys	*Delomys*	*Sigmodontomys*	*Zygodontomys*	*Sigmodontomys*
	Aepomys	*Zygodontomys*	Incertae sedis	*Zygodontomys*
	Nesoryzomys	*Amphinectomys**	*Microakodontomys*	*Incertae sedis*
	Phaenomys	*Microakodontomys*+	Independent Lineage	*Scolomys*
	Chilomys	*Handleyomys*^	*Scolomys*	
	Scolomys			
	Wilfredomys			
	Phyllotini			
	Pseudoryzomys			
	Sigmodontini			
	Holochilus			
	Incertae sedis			
	Zygodontomys			

molecular evidence has helped resolve which genera and species are included in the tribe, the inter-generic relationships are still uncertain and phylogenetically poorly resolved (Weksler 2006; Hanson and Bradley 2008; D'Elía et al. 2015).

The Oryzomyine group initially was classified by Hershkovitz (1944, 1948), who recognized four genera—*Neacomys*, *Nectomys*, *Oryzomys*, and *Scolomys*. Six subgenera were assigned to the genus *Oryzomys* (*Melanomys*, *Microryzomys*, *Nesoryzomys*, *Oecomys*, *Oligoryzomys*, and *Oryzomys*) and two subgenera (*Nectomys* and *Sigmodontomys*) were included in *Nectomys*. The other two genera (*Neacomys* and *Scolomys*) were considered to be monotypic (Hershkovitz 1944, 1948). Reig (1986) constructed an arrangement that differed from that of Hershkovitz (1944, 1948) with respect to the number of recognized genera and the taxonomic boundaries of the tribe. Reig (1986) placed *Oryzomys*, *Thomasomys*, *Oecomys*, *Rhipidomys*, *Nectomys*, *Neacomys*, *Delomys*, *Aepeomys*, *Nesoryzomys*, *Phaenomys*, *Chilomys*, *Scolomys*, and *Wilfredomys* in the tribe Oryzomyini, *Pseudoryzomys* in Phyllotini, *Holochilus* in Sigmodontini, and *Zygodontomys* as *incertae sedis*

within the subfamily Sigmodontinae. Reig (1986) considered *Oligoryzomys*, *Melanomys*, *Microryzomys*, and *Sigmodontomys* as subgenera within *Oryzomys* although he was "increasingly inclined to believe" that *Oligoryzomys* warranted generic status.

The subgenera of *Oryzomys* recognized by Reig (1986) were elevated to generic rank by Voss and Carleton (1993). In addition, they combined *Hesperomys molitor* and *Holochilus magnus* under a new genus *Lundomys* and placed *Lundomys* in the tribe Oryzomyini. Voss and Carleton's (1993) revision produced a classification in which *Holochilus*, *Melanomys*, *Microryzomys*, *Neacomys*, *Nesoryzomys*, *Oecomys*, *Oligoryzomys*, *Oryzomys*, *Pseudoryzomys*, *Scolomys*, *Sigmodontomys*, and *Zygodontomys* were included in the Oryzomyini. Although McKenna and Bell (1997) defined the tribe similarly to that of Voss and Carleton (1993), they differed by including two new genera: *Amphinectomys* (Malygin et al. 1994) and *Microakodontomys* (Hershkovitz 1993). Smith and Patton (1999) proposed similar associations as McKenna and Bell (1997), although *Scolomys* was considered a separate lineage of Sigmodontinae, and *Amphinectomys* was not included. Additionally, *Microakodontomys* was among genera Smith and Patton (1999) considered *incertae sedis* in the subfamily Sigmodontinae (with *Abrawayaomys*, *Phaenomys*, *Punomys*, and *Rhagomys)*.

Weksler (2003) examined all the genera placed in Oryzomyini by McKenna and Bell (1997) except for *Microakodontomys*. Additionally, he included *Handleyomys*, a new genus of Oryzomyini erected by Voss et al. (2002), which combined *Aepeomys fuscatus* and *Oryzomys intectus*. Weksler (2003) also included exemplar members of the tribes of the subfamily Sigmodontinae identified at the time, and four members of the subfamily (*Delomys*, *Irenomys*, *Juliomys*, and *Reithrodon*) considered either *incertae sedis* or as independent lineages by Smith and Patton (1999). Weksler's (2003) results supported an Oryzomyini tribe containing 16 genera; *Scolomys* and *Zygodontomys* were sister taxa and were sister to the remainder of the group; and none of the four Sigmodontine genera included as *incertae sedis* appeared to be affiliated with the Oryzomyini. Weksler's (2003) classification for Oryzomyini was similar to that of Musser and Carleton (2005) with the exception of *Scolomys*, placed *incertae sedis* within the subfamily Sigmodontinae by Musser and Carleton (2005), and *Microakodontomys*, which was not examined by Weksler (2003).

The tribe has been examined by many authors at different levels. A number of studies have used molecular data to examine phylogenetic relationships of the Oryzomyini in the broader context of relationships among Sigmodontinae (Smith and Patton 1993; Steppan 1995; Engel et al. 1998; Smith and Patton 1999; D'Elıa 2003; D'Elía et al. 2006; Richter et al. 2010; Parada et al. 2013; Salazar-Bravo et al. 2013; Vilela et al. 2014; Salazar-Bravo et al. 2016). Other studies focused only on relationships within or among genera of the tribe (Osgood 1933; Hershkovitz 1944,1955, 1962; Carleton 1989; Voss 1991; Carleton and Musser 1995; Dickerman and Yates 1995; Myers et al. 1995; Patton and Da Silva 1995; Bonvicino and Martins Moreira 2001; Gómez-Laverde et al. 2004; Hanson and Bradley 2008; Bonvicino et al. 2009; Rogers et al. 2009; Hanson et al. 2010; Hanson et al. 2011; Machado et al. 2014; Almendra et al. 2015; D'Elía et al. 2015). However, only three studies specifically investigated intertribal relationships of the entire tribe (Weksler 2003; Weksler 2006; Weksler et al. 2006).

In the examination performed by Weksler (2003), a single nuclear gene (interphotoreceptor retinoid binding protein, *Rbp*3) provided phylogenetic resolution for some groups, but poor resolution for others. Weksler (2006) later compared these nuclear data with morphological data and described similar results with both datasets. Although these two studies (Weksler 2003, 2006) provide a phylogenetic hypothesis that can be tested, many nodes on the phylogenetic tree supporting Weksler's classification were unresolved or possessed low nodal support. However, it is important to note that Weksler (2003, 2006) was the most through molecular examination of the tribe up to that point. Previous examinations were lacking in taxonomic sampling, which led to artificial discord between morphological and molecular taxonomies.

The most recent examination of phylogenetic relationships of the tribe (Weksler et al. 2006) resulted in the elevation of 10 new genera that had been suggested in the combined morphological and nuclear data analyses presented by Weksler (2006). The fact that 10 new genera were identified in a tribe is remarkable (D'Elıa and Pardinas 2007), but the fact that all 10 were

elevated from a single genus alludes to the extraordinary amount of diversity found not only within the tribe but also within genera in the tribe.

This high level of lineage diversity increases the importance of determining phylogenetic relationships within Oryzomyini. Without a strong understanding of the taxonomy and systematics of the tribe, it is difficult to adequately study the biology of this group. For example, *Oligoryzomys* and *Oryzomys* (*sensu* Weksler et al. 2006) are known to be reservoir hosts of several species of hantaviruses (Lopez et al. 1996; Torrez-Martinez et al. 1998; Powers et al. 1999; Vincent et al. 2000; Delfraro et al. 2003; Fulhorst et al. 2004), the cause of Hantavirus Pulmonary Syndrome in humans. Although it is beyond the scope of this paper, understanding the ecology and evolution of hantaviruses is dependent on understanding the phylogeny and ecology of their rodent hosts. For example, current understanding of host specificity (i.e., each unique virus should have a unique host species) and published host-virus relationships in Oryzomyini suggest that the current taxonomic nomenclature does not accurately represent the phylogenetic diversity found among hantavirus hosts (Torrez-Martinez et al. 1998; Vincent et al. 2000; Bohlman et al. 2002; Gonzalez Della Valle et al. 2002; Meissner et al. 2002; Fulhorst et al. 2004; Rogers et al. 2009; Hanson et al. 2011). In addition, recent examinations of *O. couesi* and *O. palustris* (Hanson et al. 2010) have shown that these two species are composite groups, as have examinations of many of the species of *Oligoryzomys* (Carleton and Musser 1995; Bonvicino and Weksler 1998; Weksler and Bonvicino 2005; Rogers et al. 2009; Palma et al. 2010; Richter et al. 2010; Hanson et al. 2011). Recently, it has been suggested that some of the *Oligoryzomys* species that are thought to be hosts for hantaviruses are either misidentified or are a part of a composite species (Andrades-Miranda et al. 2001; Rogers et al. 2009; Hanson et al. 2011).

Phylogenetic relationships within the Oryzomyini were evaluated here using three markers (two nuclear and one mitochondrial) and then combined. First, the *Rbp*3 gene (1,266 bp) has been shown to be effective at recovering phylogenies at many different levels of taxonomy (Stanhope et al. 1992; Stanhope et al. 1998; DeBry and Sagel 2001; D'Elıa 2003; DeBry 2003; Jansa and Weksler 2004), and in the tribe Oryzomyini *Rbp*3 provides support at primarily shallow nodes (Weksler 2003). Second, intron 2 of the alcohol dehydrogenase gene (*Adh*1-I2; 609 bp) has been used successfully to explore phylogenetic hypotheses within the genera *Neotoma* and *Peromyscus* (Amman et al. 2006; Platt II et al. 2015) and recently has been used in Sigmodontinae, *Holochilus*, *Oryzomys*, and Oryzomyini clade D (Machado et al. 2014; Vilela et al. 2014; D'Elía et al. 2015). Third, cytochrome-*b* (*Cyt*b; 1,143 bp) provided a maternally inherited gene and has been used in the past to explore relationships (Smith and Patton 1999). Finally, the *Rbp*3, *Adh*1-I2, and *Cyt*b sequence data were combined to develop a multi-gene phylogenetic hypothesis for the tribe Oryzomyini.

MATERIALS AND METHODS

Taxonomic sampling.—DNA sequences for *Rbp*3, *Adh*1-I2, and *Cyt*b were obtained from members of the Oryzomyini and from eight tribes in the subfamily Sigmodontinae (Table 2). *Neotoma* and *Tylomys*, members of Neotominae and Tylomyinae subfamilies, respectively, were used as outgroups in all analyses. The following genera were used to represent Sigmodontinae tribes (*sensu* D'Elıa 2007): *Abrothrix* (tribe Abrotrichini); *Akodon* (tribe Akodontini); *Calomys*, *Graomys*, and *Andalgaomys* (tribe Phyllotini); *Rhipidomys* and *Thomasomys* (tribe Thomasomyini); *Reithrodon* (tribe Reithrodontini); *Rheomys* and *Neusticomys* (tribe Ichthyomyini); *Wiedomys* (tribe Wiedomyini); and *Sigmodon* (tribe Sigmodontini). Samples from Weksler's (2003) study, in addition to others available on GenBank, were included to allow for consistency of taxa between studies.

DNA extraction, *PCR amplification*, *and DNA sequencing.*—DNA was extracted from preserved tissue by using the DNEasy kit (Qiagen, Inc., Valencia, California). The three respective genetic regions (*Rbp*3, *Adh*1-I2, and *Cyt*b) were amplified using standard Polymerase Chain Reaction amplification methods (PCR; Saiki et al. 1988) with the primers listed in Supplementary Data S1.

Table 2. Voucher and GenBank accession numbers of specimens examined for each of three genes analyzed.

	*Adh*1-I2		*Rbp*3		*Cyt*b	
Taxon[1]	Voucher no.[2]	Accession[3]	Voucher no.	Accession	Voucher no.	Accession
Abrothrix longipilis	MVZ155494	EU648965.1	MVZ155494	AY163577.1	MVZ155494	EU579470.1
Aegialomys galapogensis	ASK4105	EU648974.1	ASK4105	EU649039.1	ASK4105	EU579478.1
Aegialomys xanthaeolus	MVZ145533	EU648975.1	MVZ145533	AY163628.1	MVZ145533	EU579479.1
Aegialomys xanthaeolus	TK134912	EU648976.1	TK135790	EU273420.1	TK134912	EU340015.1
Akodon paranaensis	TK66311	EU648966.1	TK66311	EU649035.1	TK66311	EU579471.1
Amphinectomys savamis	MV97005	EU648977.1	MV97005	AY163579.1	MV97005	EU579480.1
Andalgalomys pearsoni	NS	NS	TK65697	EU649038.1	MSB55245	AF159285.1
Calomys lepidus	MVZ171562	EU648969.1	MVZ171562	AY163580.1	MVZ171562	EU579473.1
Cerradomys scotti	TK61881	EU648978.1	TK61881	EU649040.1	TK61881	EU579482.1
Cerradomys subflavus	MNRJ61885	EU648979.1	MNRJ61665	AY163626.1	MNRJ61885	EU579481.1
Ereoryzomys polius	FMNH129243	EU648980.1	FMNH129243	AY163624.1	FMNH129243	EU579483.1
Euryoryzomys macconnelli	NS	NS	AMNH272678	AY163620.1	AMNH272678	EU579484.1
Euryoryzomys nitidus	TK14571	EU648981.1	TK14571	EU649041.1	TK14571	EU579485.1
Euryoryzomys russatus	NS	NS	ORG67	AY163625.1	ORG67	EU579486.1
Graomys griseoflavus	TK65617	EU648968.1	TK65617	EU649037.1	TK65617	EU579472.1
Handleyomys alfaroi	NS	NS	TK93700	EU649044.1	TK93700	EU579489.1
Handleyomys alfaroi	TK135639	EU648983.1	TK135639	EU649043.1	TK135639	EU579488.1
Handleyomys intectus	CADV088	EU648984.1	ICN16093	AY163584.1	CADV088	EU579490.1
Handleyomys rostratus	ROM101843	EU648985.1	ROM101843	AY163622.1	ROM101843	EU579493.1
Handleyomys rostratus	TK113553/TTU104504	EU648986.1	TK113553	EU649045.1	TK113553	EU579491.1
Handleyomys rostratus	TK27527/TTU44930	EU648987.1	TK27527	EU649046.1	TK27527	EU579492.1
Handleyomys saturatior	ROM101537	EU648988.1	ROM101537	AY163615.1	ROM101537	EU579494.1
Handleyomys saturatior	TK113513/TTU101644	DQ207950.1	TK113513	EU649047.1	TK113513	DQ224410.1
Holochilus brasiliensis	GD071	EU648989.1	GD071	AY163586.1	GD071	EU579496.1
Holochilus brasiliensis	NS	NS	GD081	AY163585.1	GD081	EU579495.1
Holochilus brasiliensis	UACH7263	KJ614666.1	TK53509	EU273418.1	TK53509	EU074631.1
Holochilus chacarius	TK61941	DQ227456.1	TK61941	EU649048.1	TK61941	DQ227455.1
Holochilus sciureus	NK102248	EU648990.1	NK102248	EU649049.1	NK102248	EU579497.1

Table 2. (cont.)

	*Adh*1-I2		*Rbp*3		*Cyt*b	
Taxon[1]	Voucher no.[2]	Accession[3]	Voucher no.	Accession	Voucher no.	Accession
Hylaeamys laticeps	MVZ198262	EU648991.1	MVZ198262	EU649050.1	MVZ198262	EU579498.1
Hylaeamys megacephalus	MHNLS8061	EU648992.1	MHNLS8061	AY163621.1	MHNLS8061	EU579499.1
Hylaeamys perenensis	TK73011/TTU98606	DQ224408	TK73011/TTU98606	EU649051.1	TK73011/TTU98606	DQ207946
Hylaeamys yunganus	CM76926	EU648993.1	CMNH76926	AY163629.1	CM76926	EU579500.1
Lundomys molitor	MNHN4292	EU648994.1	MNHN4292	AY163589.1	MNHN4292	EU579501.1
Melanomys caliginosus	TK135894	EU648995.1	TK135894	EU649052.1	TK135894	EU340020.1
Melanomys chrysomelas	TK121417	EU648996.1	TK121417	EU649053.1	TK121417	EU340017.1
Melanomys columbianus	MHNLS7698	EU648997.1	MHNLS7698	AY163590.1	MHNLS7698	EU340022.1
Microakodontomys transitorius	NS	NS	MN25969	EU649054.1	NS	NS
Microryzomys minutus	QCAZ8353	EU648998.1	QCAZ8353	EU649055.1	QCAZ8353	EU579502.1
Microryzomys minutus	MVZ166666	EU648999.1	MVZ166666	AY163592.1	MVZ166666	EU258535.1
Mindomys sp.	ROM105820	MN061488	ROM105820	MN061490	ROM105820	MN061489
Neacomys minutus	AMNH272867	EU649000.1	AMNH272867	AY163595.1	AMNH272867	EU258536.1
Neacomys musseri	AMNH272676	EU649001.1	AMNH272676	AY163596.1	AMNH272676	EU579503.1
Neacomys spinosus	MVZ155014	EU649002.1	MVZ155014	AY163597.1	MVZ155014	EU579504.1
Nectomys apicalis	MVZ166700	EU649003.1	MVZ166700	EU649056.1	MVZ166700	EU340013.1
Nectomys squamipes	FMNH141632	EU649005.1	FMNH141632	AY163598.1	FMNH141632	EU340012.1
Nectomys squamipes	TK63841/TK108150	EU649004.1	TK63841/TK108150	EU273419.1	TK63841/TK108150	EU074634.1
Neotoma bryanti	NS	NS	MVZ195972	KC953408.1	NK77284	AF307835.1
Neotoma lepida	TK77284/TTU79131	AY817633.1	MVZ143946	AY163599.1	TK179750	KF250464.1
Nephlomys albigularis	AMNH268125	EU649006.1	AMNH268125	AY163614.1	AMNH268125	EU579505.1
Nephlomys morex	ACUNHC917	DQ207945.1	ACUNHC917	EU649057.1	ACUNHC917	DQ224407.1
Nesoryzomys fernandinae	ASNHC10580	EU649007.1	ASNCH10580	EU649058.1	ASNCH10580	EU579506.1
Nesoryzomys narboroughi	NS	NS	ASNHC8675	AY163600.1	ASNHC8675	GU126523.1
Nesoryzomys swarthi	ASNHC10003	EU649008.1	ASNHC10003	AY163601.1	ASNHC10003	EU340014.1
Neusticomys monticolus	NS	NS	TEL1531	EU649036.1	TEL1531	KF359516.1
Oecomys catherinae	MF29	EU649009.1	MF29	AY163605.1	MF29	EU579507.1
Oecomys concolor	NS	NS	MVZ155005	AY163606.1	MVZ155005	EU579508.1

Table 2. (cont.)

	*Adh*1-I2		*Rbp*3		*Cyt*b	
Taxon[1]	Voucher no.[2]	Accession[3]	Voucher no.	Accession	Voucher no.	Accession
Oecomys mamorae	NS	NS	JLP16961	AY163607.1	JLP16961	EU579509.1
Oligoryzomys chacoensis	TK63932	EU649010.1	TK63932	EU649059.1	TK62932	EU258543.1
Oligoryzomys delicatus	NS	NS	AMNH257262	AY163611.1	AMNH257262	GU126529.1
Oligoryzomys destructor	TEL1479	EU649012.1	TEL1479	EU649061.1	TEL1479	EU258544.1
Oligoryzomys eliurus	NS	NS	MVZ183088	EU649062.1	NK4226	EU192163.1
Oligoryzomys flavescens	CRB1405	EU649013.1	CRB1405	AY163609.1	CRB1405	EU258545.1
Oligoryzomys fulvescens	TK102042	EU649014.1	TK102042	EU649063.1	TK102042	EU258547.1
Oligoryzomys fulvescens	TK138080	EU649011.1	TK138080	EU649060.1	TK138080	DQ227457.1
Oligoryzomys longicaudatus	NS	NS	MVZ155463	EU649064.1	MVZ155463	FJ374766.1
Oligoryzomys longicaudatus	NS	NS	MSB55318	EU649065.1	NS	NS
Oligoryzomys microtis	NS	NS	MVZ193858	EU649066.1	MVZ193858	EU258549.1
Oligoryzomys nigripes	TK65938	EU649015.1	CRB1422	AY163612.1	TK65938	EU258550.1
Oligoryzomys stramineus	NS	NS	MNRJ46406	AY163613.1	MNRJ46873	GU126531.1
Oreoryzomys balneator	AMNH268144	EU649016.1	AMNH268144	AY163617.1	AMNH268144	EU579510.1
Oreoryzomys balneator	TEL1854	EU649017.1	TEL1854	EU649068.1	TEL1854	EU258534.1
Oryzomys couesi aquaticus	TK72660	EU649019.1	TK72661	EU273425.1	TK72661	EU074662.1
Oryzomys couesi azuerensis	NK101644	EU649020.1	NK101644	EU273429.1	NK101644	EU074668.1
Oryzomys couesi couesi	TK119183	EU649018.1	TK119183	EU273427.1	TK119183	EU074663.1
Oryzomys couesi goldmani	TK150231	EU649021.1	TK150231	EU649069.1	TK150231	EU074661.1
Oryzomys palustris palustris	EVGL05	EU649022.1	EVGL05	EU273432.1	EVGL06	EU074639.1
Oryzomys palustris texensis	TK91240	DQ207949.1	TK91240	EU273431.1	TK91240	DQ185382.1
Pseudoryzomys simplex	GD065	EU649023.1	GD065	AY163633.1	GD065	EU579517.1
Pseudoryzomys simplex	TK62425	EU649024.1	TK62425	EU649070.1	TK62425	EU579516.1
Reithrodon auritus	MVZ182704	EU648970.1	MVZ182704	AY163634.1	MVZ182704	EU579474.1
Rheomys raptor	NS	NS	KU159017	AY163635.1	KU159017	KF359512.1
Rhipidomys nitela	USNM448665	EU648971.1	MHNLS7820	AY163636.1	USNM448665	EU579475.1
Scolomys ucayalensis	AMNH272721	EU649025.1	AMNH272721	AY163638.1	AMNH272721	EU579518.1
Sigmodon hispidus	OK5840	EU665203.1	OK5840	EU635707.1	OK5840	AF425209.1

Table 2. (cont.)

	*Adh*1-I2		*Rbp*3		*Cyt*b	
Taxon[1]	Voucher no.[2]	Accession[3]	Voucher no.	Accession	Voucher no.	Accession
Drymoreomys albimaculatus	MVZ182088	EU648982.1	MVZ182088	EU649042.1	MVZ182088	EU579487.1
Sigmodontomys alfari	MUSNM449895	EU649027.1	MUSNM449895	AY163641.1	MUSNM449895	EU074635.1
Sigmodontomys alfari	TK135621	EU649026.1	TK135621	EU649071.1	TK135621	EU340016.1
Sooretamys angouya	MNRJ50234	EU649028.1	MNRJ50234	AY163616.1	MNRJ50234	EU579511.1
Sooretamys angouya	TK61763	EU649029.1	TK61763	EU649072.1	TK61763	EU579512.1
Tanyuromys aphrastus	NS	NS	KU161003	JF693878.1	KU161003	JF693878.1
Thomasomys erro	TEL1663	EU648972.1	NS	NS	TEL1663	EU579476.1
Transandinomys bolivaris	TK135687	EU649030.1	TK135687	EU649073.1	TK135687	EU579513.1
Transandinomys talamancae	TK135289	EU649031.1	TK135289	EU649074.1	TK135289	EU579514.1
Transandinomys talamancae	USNM449894	EU649032.1	USNM449894	AY163627.1	USNM449894	EU579515.1
Tylomys nudicaudus	TK41551	AY817625.1	ROM103590	AY163643.1	TK41551	DQ179812.1
Wiedomys pyrrhorhinos	MVZ197566	EU648973.1	CRB1839	AY163644.1	MVZ197566	EU579477.1
Zygodontomys brevicauda	AMNH257321	EU649033.1	AMNH257321	AY163645.1	AMNH257321	EU579521.1
Zygodontomys brevicauda	TTU76306	EU649034.1	TTU76306	EU649075.1	TTU76306	EU579519.1
Zygodontomys cherriei	NS	NS	USNM448665	AY163646.1	USNM448665	EU579520.1

[1] Generic taxonomy follows Weksler et al. (2006), and species taxonomy follows Musser and Carleton (2005).

[2] Museum and collectors acronyms as follows: ACUNHC, Abilene Christian University Natural History Collection; AMNH, American Museum of Natural History; ASNHC, Angelo State Natural History Collection; CM, Carnegie Museum of Natural History; CRB, Cibele R. Bonvincino, voucher at MNRJ; EVGL, Jane Indorf, no voucher; FMNH Field Museum of Natural History; GD, Guillermo D'Elia; ICN, Instituto de Ciencias Naturales, Bogota, Colombia; KU, University of Kansas Natural History Museum; MHNLS, Museo de Historia Natural La Salle, Caracas, Venezuela; MNHN, Museo Nacional de Historia Natural, Montevideo, Uruguay; MNRJ, Museu Nacional, Rio de Janeiro, Brazil; MSB, Museum of Southwestern Biology, University of New Mexico; MVZ, Museum of Vertebrate Zoology, University of California, Berkeley; OK, Collection of Tissues, Oklahoma State University; ROM, Royal Ontario Museum; QCAZ, Museo de Zoologia, Pontificia Universidad Catolica del Ecuador; TTU, Natural Science Research Laboratory Museum of Texas Tech University; TK, Tissue Collection, Museum of Texas Tech University no voucher; MUSNM, National Museum of Natural History.

[3] GenBank accession numbers listed as "NS" refer to samples which were not sequenced for the respective nucleotide region.

*Rbp*3 was amplified using primers A1 and B2 (Supplementary Data S1) and either GoTaq (Promega, Madison, Wisconsin) or AmpliTaq Gold (Applied Biosystems, Foster City, California). Thermal profiles, optimized for different taxa, were adapted from the following standard profile: one cycle of 95°C of either two min (GoTaq) or 10 min (AmpliTaq Gold), three stages of five cycles each with denaturation at 95°C for 20 sec, annealing at 58°C, 56°C, or 54°C for 15 sec, and extension at 72°C for 60 sec, one stage of 23 cycles of denaturation at 95°C for 20 sec, annealing at 52°C for 15 sec, and extension at 72°C for 60 sec, one cycle of 72°C for seven min. PCR products were cycle sequenced using primers listed in Supplementary Data S1.

*Adh*1-I2 was amplified using primers ExII-F and 2340-2 (Supplementary Data S1) and either GoTaq (Promega, Madison, Wisconsin) or AmpliTaq Gold (Applied Biosystems, Foster City, California). Thermal profiles, optimized for different taxa, were adapted from the following standard profile: one cycle of 95°C of either two min (GoTaq) or 10 min (AmpliTaq Gold), 35 cycles each with denaturation at 95°C for 45 sec, annealing at 52°C for 15 sec, and extension at 72°C for 60 sec, and one cycle of 72°C for eight min. PCR products were cycle sequenced using the primers listed in Supplementary Data S1.

The entire mitochondrial *Cyt*b gene (1,143 bp) was amplified using primers MVZ05 and CB40 (Supplementary Data S1) and GoTaq (Promega, Madison Wisconsin). Thermal profiles, optimized for different taxa, were adapted from the following standard profile: 35 cycles of 95°C for 45 sec, 52°C for 45 sec, and 72°C for one minute 30 sec and one cycle of 72°C for seven min. PCR products were cycle sequenced using the primers listed in Supplementary Data S1. For some samples that were more difficult to sequence, primer L14841 was used, 400F replaced F1, and 870R replaced 700H.

Cycle sequencing reactions were purified using isopropanol clean up protocols provided by the manufacturer (Applied Biosystems Inc., Beverly, Massachusetts). Sequences were generated with an ABI 3100-*Avant* with BigDye 3.1 terminator technologies then aligned and proofed using Sequencher 4.8 software (Gene Codes, Ann Arbor, Michigan) and deposited in GenBank (Table 2).

Data from some taxa that were already available from GenBank (Yates and Anderson 2000; Edwards and Bradley 2002; Weksler 2003; Amman et al. 2006; Carroll et al. 2005; Longhofer and Bradley 2006; Milazzo et al. 2006; Matocq et. al. 2007; Hanson and Bradley 2008; Henson and Bradley 2009; Hanson et al. 2010; Palma et al. 2010; Richter et al. 2010; Percequillo et al. 2011; Pine et al. 2012; Schenk et al 2013; Canon et al. 2014; Hanson et al. 2015; Milazzo et al. 2015) were included to increase sample size. Where possible all three gene fragments from the same individual were used to represent a species; however, some individual specimens may only be represented by a single gene. In these instances, markers from conspecifics were combined to create a composite taxon (Campbell and Lapointe 2009), which has been useful in other cricetid phylogenetic studies (Platt II et al. 2015).

Phylogenetic analysis.—The expected length of each gene marker was 1,266 bp, 609 bp, and 1,143 bp, respectively, for *Rbp*3, *Adh*1-I2, and *Cyt*b. Nucleotides were coded as A, C, T, G, - (gaps), ? (missing), or as heterozygous. Heterozygous sites in the nuclear data were coded using the International Union of Biochemistry (IUB) polymorphic code. Pseudogenes were tested for by checking for premature stop codons in each of the protein coding markers.

All genes were aligned using Muscle v3.4 (Edgar 2004). Each Muscle alignment was replicated 1,000 times. High confidence indels (those present in 90% or more alignments) were coded using 0 (absent) or 1 (present) using a local instance of reliINDEL v1.0 (Ashkenazy et al. 2014). Indels at the beginning of sequences, those due to incomplete representation of gene fragments, were excluded from analysis. Partition Finder v1.1.1 (Lanfear et al. 2012) was used to identify partitions within and among genes as well as appropriate models of substitution for each partition. Each of the protein coding sequences (*Rbp*3 and *Cyt*b) initially were partitioned by codon, and *Adh*1-I2 was a single partition. Twenty-four substitution models were tested for each initial partition with unlinked branch lengths.

DNA sequences from each gene were analyzed separately using Bayesian inference and maximum

likelihood, and the resultant topologies were compared for supported inconsistencies. Congruence between gene regions was evaluated by comparing Bayesian and maximum likelihood trees generated from individual gene region data. Nodes that differed between trees were not considered conflicting unless both nodes had a bootstrap support value greater than 75% (Helbig et al. 2005) or Bayesian posterior probabilities greater than 0.95. The partition homogeneity test in PAUP v4.0a164 (Swofford 2001), iterated over 1,000 replicates, was used to determine whether the single genes contained conflicting phylogenetic signal before they were combined into the dataset which included all three nucleotide regions (*Rbp*3, *Adh*1-I2, and *Cyt*b).

Analyses were conducted in MrBayes v3.2 (Ronquist et al. 2012) with the following options: two independent runs of four Markov chains (three hot, one cold), 10 million generations, sample frequency every 1,000th generation, and the 1st 25% of trees were discarded as "burnin". A consensus tree (50% majority rule) was constructed from the remaining trees. Nodal support was estimated as posterior probabilities in MrBayes 3.2 (Ronquist et al. 2012). Probabilities ≥95% were considered significant and used as evidence of supported clades. Maximum likelihood analyses were conducted in RAxML v8.2.2 (Aberer et al. 2014). Trees were generated for each gene as well as the combined data set. The GTR+I+Γ model of evolution was used for all nucleotide partitions and a binary model was used for the coded gaps. Nodal support for each tree was bootstrapped for 10,000 replicates using the fast bootstrapping option. Support values between 75 and 85 were considered moderate, and those between 86 and 100 were considered as evidence for strong nodal support.

RESULTS

Rbp3.—Nucleotide sequences from the *Rbp*3 gene were obtained for 98 individuals representing the 84 members of the tribe Oryzomyini, 12 individuals representing Sigmodontinae, and three outgroup individuals. A single, phylogenetically uninformative, 3bp indel at position 742–744 was identified in *Scolomys ucayalensis*. The best fit partitioning scheme from PartitionFinder was GTR+I+Γ in a single partition across the entire gene. Bayesian analysis was performed using the GTR+I+Γ model of evolution, and tree topologies (Supplementary Data S2) were similar to those obtained from ML analysis. Samples referable to Oryzomyini formed a monophyletic grouping within taxa referable to Sigmodontinae. This tribal topology contained two major clades (clade A and clade B nomenclature consistent with Weksler 2003). Clade A consisted of *Euryoryzomys*, *Handleyomys*, *Hylaeamys*, *Mindomys*, *Nephlomys*, *Oecomys*, and *Transandinomys*, whereas clade B comprised *Aegialomys*, *Amphinectomys*, *Cerradomys*, *Drymoreomys*, *Eremoryzomys*, *Holochilus*, *Lundomys*, *Melanomys*, *Microakodontomys*, *Microryzomys*, *Neacomys*, *Nectomys*, *Nesoryzomys*, *Oligoryzomys*, *Oreoryzomys*, *Oryzomys*, *Pseudoryzomys*, *Sigmodontomys*, *Sooretamys*, and *Tanyuromys*. *Scolomys* and *Zygodontomys* also were placed within the Oryzomyini, however they were not sister to each other in a supported clade. Within clade A, *Euryoryzomys* was the only genus represented by multiple members that was not supported as a monophyletic entity. The only intergeneric relationship that was phylogenetically supported was between *Transandinomys* and *Euryoryzomys*. Within clade B, all genera represented by multiple samples were depicted as monophyletic groups except *Aegialomys*, *Melanomys*, and *Sigmodontomys*. Additionally, *Holochilus* and *Pseudoryzomys* were supported as sister genera, as were *Amphinectomys* and *Nectomys*, and *Eremoryzomys* and *Drymoreomys*. *Sigmodontomys* was placed within *Melanomys* and this paraphyletic grouping was sister to *Nesoryzomys*. Together the *Nesoryzomys/Melanomys/Sigmodontomys* clade was sister to *Aegialomys*.

Adh*1-I2*.—Nucleotide sequences from *Adh*1-I2 were obtained for 79 individuals—68 members of the tribe Oryzomyini, 10 individuals of Sigmodontinae, and two outgroup members. One hundred and sixteen indels were identified by relINDEL; of these only 29 indels were present in ≥90% of alignment replicates generated by relINDEL. These 29 indels were coded as present or absent and included in subsequent analyses. A single partition with the GTR+Γ substitution model was the best partition scheme as indicated by PartitionFinder for *Adh*1-I2. As with the *Rbp*3 dataset, tree topologies produced in ML and Bayesian (GTR+Γ;

Supplementary Data S3) analyses were similar. The Oryzomyini was not reconstructed monophyletic to the rest of the Sigmodontinae; however, a clade containing all Oryzomyine genera except *Scolomys* and *Zygodontomys* was phylogenetically supported. Additionally, two clades (A and B) observed previously were inferred. Furthermore, *Adh*1-I2 nucleotide sequence data also provided support for a clade containing *Microryzomys* and *Oreoryzomys*, which was sister to *Neacomys*. The only conflict in comparisons of the phylogenetic topologies obtained from the *Rbp3* and *Adh*1-I2 datasets was support for a sister relationship between *Sigmodontomys* and *Nesoryzomys*.

Cyt*b*.—Nucleotide sequences from the mitochondrial *Cyt*b gene were obtained for 97 individuals of Oryzomyini, 12 individuals of Sigmodontinae, and three outgroup samples. PartitionFinder indicated that a single partition with the GTR+I+Γ was the best fit model for downstream phylogenetic analyses. The Bayesian (GTR+I+Γ; Supplementary Data S4) and maximum likelihood topologies were identical. Phylogenetically supported clades representing relationships above the generic level were rare; compared to the topologies for the nuclear datasets there were only two supported inconsistencies: first, the placement of *Oryzomys* sister to a *Tanyuromys/Melanomys/Sigmodontomys* group, and second, the placement of *Zygodontomys* sister to *Eremoryzomys*. Based on the limited conflict between the nuclear and mitochondrial genes, all three datasets were combined and analyzed as a single dataset.

Combined dataset.—A concatenated phylogenetic dataset was explored in an effort to increase the phylogenetic signal over that in any one single marker. A partition homogeneity test, reduced to common taxa in each dataset, and iterated over 1,000 replicates, showed no significant difference in gene tree lengths between *Adh*1-I2, *Rbp*3, and *Cyt*b ($p = 0.78$). The dataset composed of the two nuclear genes and the mitochondrial gene was analyzed for 84 individuals of the tribe Oryzomyini, 12 individuals of Sigmodontinae, and three outgroup individuals. The Bayesian (GTR+I+Γ; Fig. 1) and maximum likelihood topologies did not conflict at supported nodes. Combining all three datasets recovered clades A and B and provided statistical support (clade posterior probability < 0.95) at all but 11 nodes within the ingroup. Five of the 11 unsupported nodes represented interspecific relationships (within *Oligoryzomys*, *Oryzomys*, *Nesoryzomys*, and *Handleyomys*) that did not affect intergeneric relationships. The relationship between *Cerradomys*, *Sooretamys*, and a group comprising *Holochilus* and *Pseudoryzomys* was unresolved, as was the relationship between *Tanyuromys* and a paraphyletic group composed of *Melanomys* and *Sigmodontomys*.

DISCUSSION

Increasing sample size.—Much debate has been conducted on whether or not phylogenetic accuracy is improved by increasing either sample number or characters (Kim 1996; Graybeal 1998; Poe 1998; Rannala et al. 1998; Poe and Swofford 1999; Pollock and Bruno 2000; Rosenberg and Kumar 2001; Pollock et al. 2002; Zwickl and Hillis 2002; Hillis et al. 2003; Rosenberg and Kumar 2003; Hedtke et al. 2006). Hedtke et al (2006) suggested that increasing sampling is a more efficient way to improve phylogenetic accuracy. The sampling in the present project increased the number of ingroup taxa by 52% (from 44 to 84) compared to the number of ingroup members included in the study by Weksler (2003). Sampling of all genera represented by a single exemplar (except *Lundomys*, *Amphinectomys*, *Eremoryzomys*, and *Scolomys*) in Weksler (2003) was expanded to include at least two members (*Pseudoryzomys*, *Sigmodontomys*, *Sooretamys*, and *Oreoryzomys*), or if possible multiple species (*Melanomys*, *Nectomys*, *Cerradomys*, *Microryzomys*, and *Nephlomys*. Additionally, four genera not included in Weksler (2003) (*Microakodontomys*, *Mindomys*, *Drymoreomys*, and *Tanyuromys*) were examined herein.

Comparisons of the data from this study with the data from Weksler's (2003) examination of the Oryzomyini suggest that although increased taxonomic sampling did help resolve some generic relationships as monophyletic groups (e.g., *Cerradomys* and *Sooretamys*), most unresolved relationships remained unresolved and some were ambiguous (e.g., *Melanomys/Sigmodontomys*). Furthermore, resolution of clades

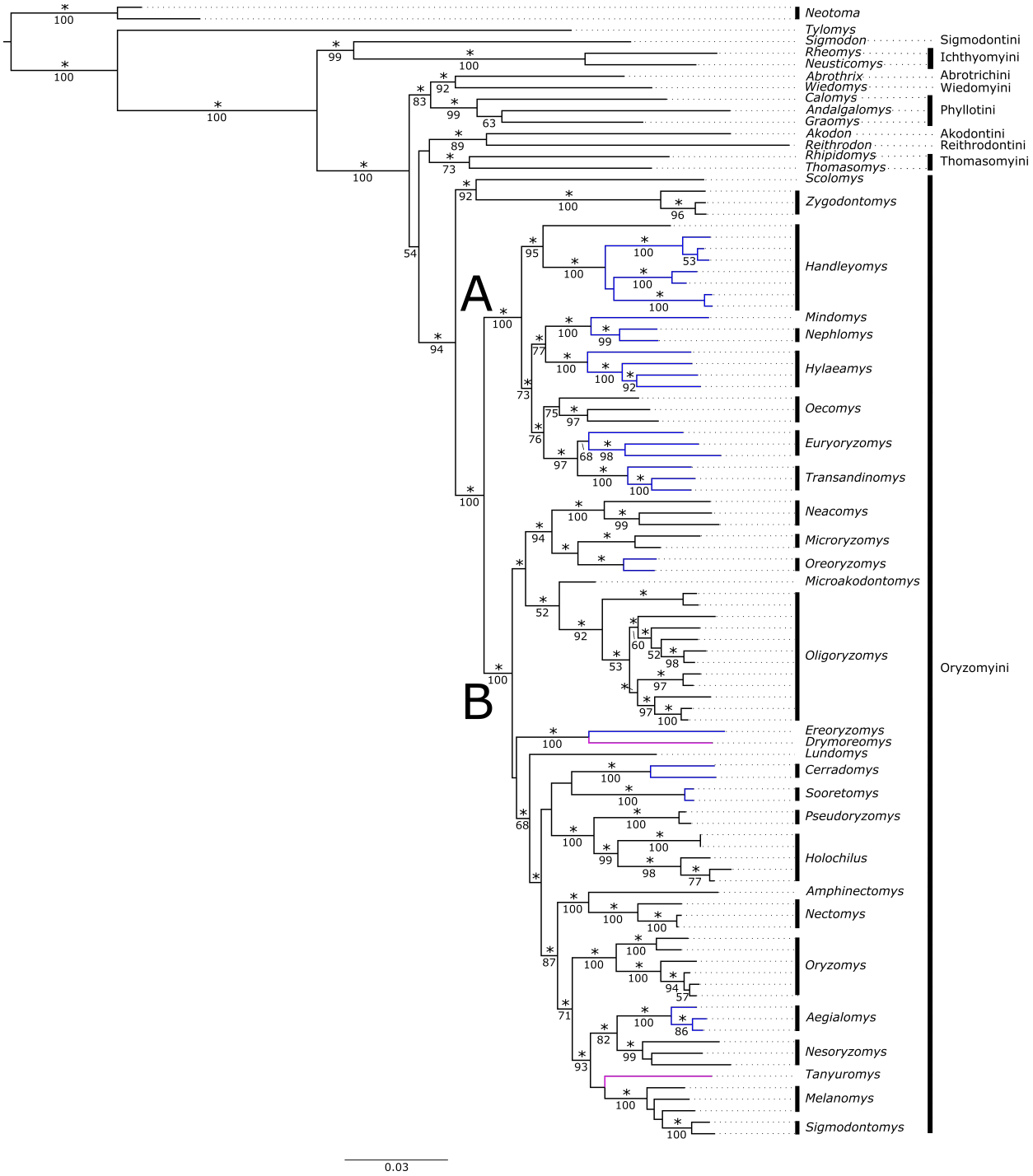

Figure 1. Phylogenetic tree obtained from the Bayesian analysis of DNA sequences for combined interphotoreceptor retinoid binding protein, alcohol dehydrogenase intron 2 (gaps informative), and cytochrome-*b* datasets. Asterisks above branches represent clade probabilities values > 95. Bootstrap support values > 50 (obtained from a parsimony analysis of the same dataset) are shown below branches. Taxa removed from *Oryzomys* by Weksler et al. 2006 are indicated by blue branches. Taxa elevated to generic status since 2006 are indicated with pink branches.

was not increased at higher taxonomic levels. Therefore, another method, such as including additional genes or different markers, must be utilized to provide an increase in resolution of relationships at multiple clades.

Increasing characters.—Not surprisingly, topologies based on multiple genes had support for more nodes than those based on single genes. The topology of the ingroup based on all three nucleotide regions (*Rbp*3, *Adh*1-I2, and *Cyt*b) had phylogenetic support at all but 11 nodes, compared to the topology based on the individual datasets which had 19 (*Cyt*b), 17 (*Adh*1-I2), and 26 (*Rbp*3) unresolved nodes within the ingroup. These results support the assertions of Rokas et al. (2003) that multiple characters are more important than multiple taxa. However, this assertion has a caveat; increasing taxon sampling will improve phylogenetic resolution if initial sampling does not represent all taxa. Previous studies involving this group that had incomplete taxon sampling (Smith and Patton 1999; Bonvicino and Martins Moreira 2001) failed to reconstruct a phylogenetic hypothesis congruent with morphological arrangements.

Although initial interpretations suggest that increasing characters allowed for an increase in nodal support, it is more likely that increased phylogenetic resolution was a result of appropriate taxon sampling combined with efficient character sampling. Rokas et al. (2003) sampled 106 genes to develop a phylogenetic topology of yeast, however 65 of those genes were highly invariable (Hedtke et al. 2006), thus requiring additional data to recover more phylogenetic signal. The three nucleotide regions used to develop the topologies presented herein, although less than the 20 suggested as necessary by Rokas et al. (2003), were still variable and were useful in estimating a supported phylogeny. The topology predicted from the combined dataset is the most robust, molecular-based topology for this group to date. Three explanations account for the increased phylogenetic resolution: 1) all extant named Oryzomyini genera were represented in the combined dataset; 2) the sampling of monotypic taxa was expanded when possible; and 3) multiple gene markers with moderate phylogenetic signal were sampled.

Mitochondrial vs nuclear datasets.—When nuclear sequences (*Rbp*3 and *Adh*1-I2) were examined independently, bootstrap values and clade probabilities (<0.95) provided support at various nodes in the topology. The highest level of phylogenetic support was observed at mid-level nodes (generic and intergeneric nodes) although support also was observed at terminal and basal nodes. The congruence between *Rbp*3 and *Adh*1-I2 suggests that *Adh*1-I2 is a phylogenetically informative marker at multiple evolutionary levels within Oryzomyini. In contrast, nodal support in the topology generated using *Cyt*b data was almost exclusively located at terminal branches. Additionally, relationships among Sigmodontinae tribes were not phylogenetically supported and only two basal nodes within the Oryzomyini had nodal support in the analyses of *Cyt*b. Lack of support for basal nodes in the *Cyt*b gene is congruent with previous studies in which *Cyt*b shows a decline in strength of utility as evolutionary depth increases (Yoder et al. 1996; Martin et al. 2000). The increase in support with each added dataset follows previously reported patterns for other mammalian groups (Gatesy et al. 1992; Ledje and Arnason 1996; Delpero et al. 2001; Kuznetsova et al. 2002; Olson et al. 2005; Ruedas and Morales 2005; Yu and Zhang 2005; Hafner et al. 2006; Hafner et al. 2007; Platt II et al. 2015) where strong nodal support values are observed at both deep and shallow levels only when phylogenetic regions are combined. Although *Cyt*b is useful for elucidating taxonomic relationships between terminal groups (genera and species), its utility for establishing deep phylogenetic relationships was poor.

Taxonomic implications.—The tribe Oryzomyini, as described by Weksler (2003) and Musser and Carleton (2005), was inferred as a monophyletic entity within the Sigmodontinae by three of the four analyses (*Rbp*3, *Cyt*b, and Combined data). Clades A and B as defined by Weksler (2003) were recovered in the combined dataset.

In addition to the estimation of groups inferred in previous examinations of the tribe (Weksler 2003, 2006), the combined data support taxonomic arrangements suggested by Weksler et al. (2006) in which 10 new genera were described. All the genera named by those authors were inferred in the combined analyses as monophyletic groups within the tribe Oryzomyini. In addition to the genera elevated by Weksler et al. (2006), the genus *Microakodontomys*, which had been considered an anomalous specimen of *Oligoryzomys*

by Weksler et al. (2006), was recovered as were *Tanyuromys*, *Mindomys*, and *Drymoreomys*. There may be other unnamed, generic-level groups still within Oryzomyini. Further investigation of the relationships within *Euryoryzomys*, *Handleyomys*, *Holochilus*, *Hylaeamys*, *Neacomys*, *Nectomys*, *Oecomys*, *Oryzomys*, and *Oligoryzomys* are warranted because of deep branches present within each group relative to other Oryzomyini. For example, *Holochilus* contains two clades that are as genetically distinct from each other as they each are from the next most closely related genus, *Pseudoryzomys*, and as genetically distinct as *Aegialomys* and *Nesoryzomys*, suggesting that an unrecognized genus currently may be present in *Holochilus*. The data presented herein also support the argument of Weksler et al. (2006) that *Handleyomys* (*sensu* Voss et al. 2002) and the *"alfaroi"* group (*sensu* Weksler 2003) represent two unique genera.

In contrast to the potential unrecognized genera suggested above, the validity of a number of genera recognized by Musser and Carleton (2005) and Weksler et al. (2006) need to be re-evaluated. One of the clades that were unsupported in the combined analysis involved the relationship between *Melanomys* and *Sigmodontomys*. The lack of phylogenetic resolution between these two genera also was predicted in a study examining relationships of taxa assigned to *Melanomys caliginosus* (Hanson and Bradley 2008). Although there is strong external morphological distinction (e.g., pelage color and tail length; Weksler and Percequillo 2011) between the two genera, skull characteristics are similar, and the molecular differentiation is lower than expected. These genera have a large geographic distribution; however, genetic data for either genus are sparse and no genetic data are available for two of the species of *Melanomys*. Additional specimens are necessary to better understand relationships within and between these genera.

Furthermore, if some of the genera named above (e.g., *Hylaeamys*, *Oecomys*, and *Neacomys*) are maintained as distinct units, the validity of others (*Mindomys/Nephlomys*, *Euryoryzomys/Transandinomys*, *Oreoryzomys/Microryzomys*) may need to be reexamined. For example, in the combined analyses, *Transandinomys* and *Euryoryzomys* were recovered in a well-supported clade. Weksler et al. (2006) discussed the external similarities between these groups and differentiated them based on dental structures. Similarly, *Oreoryzomys* and *Microryzomys* were inferred in a phylogenetically, well-supported sister relationship in the analyses of the combined and nuclear datasets. The amount of divergence observed between these two genera is less than that found within other generic level groups (*Hylaeamys* and *Oecomys*), and this clade was estimated in the *Cyt*b dataset, which was not informative at deeper nodes, suggesting a close relationship between these two taxa. Weksler et al. (2006) differentiated *Oreoryzomys* and *Microryzomys* based on pelage characteristics, toe length, and four skull characteristics, indicating that sufficient morphological variation may exist to recognize valid genera.

Finally, the combined dataset provided resolution for *Zygodontomys* and *Scolomys* within Oryzomyini. In the *Cyt*b dataset, *Zygodontomys* and *Scolomys* were resolved in the center of the Oryzomyini, which is different from other arrangements. The difficulty in resolving these two genera within any individual gene may be related to the depth of divergence times. This should be examined more fully with regards to the relation between the clade comprising these two genera and the rest of the tribe.

Previous arrangements of the Oryzomyini (Supplementary Data S1) did not identify the extent of genetic differentiation present in this tribe. As shown by Weksler et al. (2006), at least 10 genera had been unrecognized by previous authors and historical accounts of the tribe had failed to consistently recognize others (*Melanomys*, *Microryzomys*, *Nesoryzomys*, *Oecomys*, *Oligoryzomys*, and *Sigmodontomys*). This arrangement of the tribe served to minimize taxonomic characters separating species, which in turn relegated species to subspecific rank. The diversity contained within this tribe will be accentuated if many of these taxa are elevated. Finally, additional collecting efforts in the Neotropics are likely to recover species that are not present in museum collections. These serve to highlight the importance of broad-scale collection efforts (for examples see Lee et al. 2006, 2011; Brito and Ojala-Barbour 2016; Rossi et al. 2016), but may also require additional revisions to account for potentially inconsistent arrangements.

The data presented herein provide a clearer taxonomic understanding of the relationships within

one of the most diverse and enigmatic tribes of the subfamily Sigmodontinae. The use of two moderately evolving nuclear genes and a rapidly evolving mitochondrial gene essentially provided a robust topology for this group. Finally, the taxonomic arrangement and elevation of 10 new genera by Weksler et al. (2006) was supported phylogenetically. However, further examination of evolutionary relationships is needed within the genera *Oligoryzomys*, *Oecomys*, *Neacomys*, *Oryzomys*, and *Handleyomys*, as well as between the genera *Holochilus* and *Pseudoryzomys; Sigmodontomys* and *Melanomys; Microryzomys* and *Oreoryzomys*; and *Euryoryzomys* and *Transandinomys*.

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LITERATURE CITED

Aberer, A. J., K. Kobert, and A. Stamatakis. 2014. ExaBayes: Massively parallel Bayesian tree inference for the whole-genome era. Molecular Biology and Evolution 31:2553–2556.

Almendra, A. L., D. S. Rogers, and F. X. González-Cózatl. 2014. Molecular phylogenetics of the *Handleyomys chapmani* complex in Mesoamerica. Journal of Mammalogy 95:26–40.

Amman, B. R., J. D. Hanson, L. K. Longhofer, and R. D. Bradley. 2006. Intron 2 (*Adh1*-I2) of the alcohol dehydrogenase gene: a potential nuclear DNA phylogenetic marker for mammals. Occasional papers, Museum of Texas Tech University 256:1–16.

Andrades-Miranda, J. L., F. B. Oliveira, C. A. V. Lima-Rosa, A. P. Nunes, N. I. T. Zanchin, and M. S. Mattevi. 2001. Chromosome studies of seven species of *Oligoryzomys* (Rodentia: Sigmodontinae) from Brazil. Journal of Mammalogy 82:1080–1091.

Ashkenazy, H., O. Cohen, T. Pupko, and D. Huchon. 2014. Indel reliability in indel-based phylogenetic inference. Genome Biology and Evolution 6:3199–3209.

Bohlman, M. C., et al. 2002. Analysis of hantavirus genetic diversity in Argentina: S segment-derived phylogeny. Journal of Virology 76:3765–3773.

Bonvicino, C. R., P. R. Gonçalves, J. A. de Oliveira, L. F. B. de Oliveira, and M. S. Mattevi. 2009. Divergence in *Zygodontomys* (Rodentia: Sigmodontinae) and distribution of Amazonian savannas. Journal of Heredity 100:322–328.

Bonvicino, C. R. and M. A. Martins Moreira. 2001. Molecular phylogeny of the genus *Oryzomys* (Rodentia: Sigmodontinae) based on cytochrome b DNA sequences. Molecular Phylogenetics and Evolution 18:282–292.

Bonvicino, C. R. and M. Weksler. 1998. A new species of *Oligoryzomys* (Rodentia, Sigmodontinae), from Northeastern Brazil. Zeitschrift für Säugetierkunde 36:90–103.

Brito, J. and R. Ojala-Barbour. 2016. Mamíferos no voladores del Parque Nacional Sangay, Ecuador. Papéis Avulsos de Zoologia (São Paulo) 56.

Campbell, V. and F.-J. Lapointe. 2009. The use and validity of composite taxa in phylogenetic analysis. Systematic Biology 58:560–572.

Canon, C., D. Mir, U. F. J. Pardinas, E. P. Lessa, and G. D'Elia. 2014. A multilocus perspective on the phylogenetic relationships and diversification of rodents of the tribe Abrotrichini (Cricetidae: Sigmodontinae). Zoologica Scripta 43:443–454.

Carleton, M. D. 1989. Systematics and evolution. Pp. 7–141 in Advances in the Study of *Peromyscus* (Rodentia) (G. L. Kirkland and J. N. Layne, eds.). Texas Tech University Press, Lubbock.

Carleton, M. D., and G. G. Musser. 1995. Systematic studies of oryzomyine rodents (Muridae: Sigmodontinae): definition and distribution of *Oligoryzomys vegetus* (Bangs, 1902). Proceedings of the Biological Society of Washington 108:338–369.

Carroll, D. S., L. L. Peppers, and R. D. Bradley. 2005. Molecular systematics and phylogeography of the *Sigmodon hispidus* species group. Pp. 87–100 in Contribuciones mastozoológicas en homenaje a Bernardo Villa.

D'Elıa, G. 2003. Phylogenetics of Sigmodontinae (Rodentia, Muroidea, Cricetidae), with special reference to the akodont group, and with additional comments on historical biogeography. Cladistics 19:307–323.

D'Elıa, G. 2007. Definition and diagnosis of a new tribe of Sigmodontine rodents (Cricetidae: Sigmodontinae), and a revised classification of the subfamily. Gayana 71:187–194.

D'Elía, G., J. D. Hanson, M. R. Mauldin, P. Teta, and U. F. Pardiñas. 2015. Molecular systematics of South American marsh rats of the genus *Holochilus* (Muroidea, Cricetidae, Sigmodontinae). Journal of Mammalogy 96:1081–1094.

D'Elía, G., L. Luna, E. M. González, and B. D. Patterson. 2006. On the Sigmodontinae radiation (Rodentia, Cricetidae): An appraisal of the phylogenetic position of *Rhagomys*. Molecular Phylogenetics and Evolution 38:558–564.

D'Elıa, G., and U. F. J. Pardinas. 2007. Putting names to the phylogenetic diversity of Neotropical sigmodontine rodents: new genera for known species. Mammalia 71:143–145.

DeBry, R. W. 2003. Identifying conflicting signal in a multigene analysis reveals a highly resolved tree: The phylogeny of Rodentia (Mammalia). Systematic Biology 52:604–617.

DeBry, R. W. and R. M. Sagel. 2001. Phylogeny of rodentia (Mammalia) inferred from the nuclear-encoded gene IRBP. Molecular Phylogenetics and Evolution 19:290–301.

Delfraro, A., et al. 2003. Yellow pygmy rice rat (*Oligoryzomys flavescens*) and Hantavirus Pulmonary Syndrome in Uruguay. Emerging Infectious Diseases 9:846–852.

Delpero, M., J. C. Masters, P. Cervella, S. Crovella, G. Ardito, and Y. Rumpler. 2001. Phylogenetic relationships among the Malagasy lemuriforms (Primates: Strepsirrhini) as indicated by mitochondrial sequence data from the 12S rRNA gene. Zoological Journal of the Linnean Society 133:83–103.

Dickerman, A. W., and T. L. Yates. 1995. Systematics of *Oligoryzomys*: Protein-Electrophoretic Analyses. Journal of Mammalogy 76:172–188.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797.

Edwards, C. W., and R. D. Bradley. 2002. Molecular systematics of the genus *Neotoma*. Molecular Phylogenetics and Evolution. 25:489–500.

Engel, S. R., K. M. Hogan, J. F. Taylor, and S. K. Davis. 1998. Molecular systematics and paleobiogeography of the South American sigmodontine rodents. Molecular Biology and Evolution 15:35–49.

Fulhorst, C. F., M. N. B. Cajimat, A. Utrera, M. L. Milazzo, and G. M. Duno. 2004. Maporal virus, a hantavirus associated with the fulvous pygmy rice rat (*Oligoryzomys fulvescens*) in western Venezuela. Virus Research 104:139–144.

Gatesy, J., D. Yelon, R. DeSalle, and E. S. Vrba. 1992. Phylogeny of the Bovidae (Artiodactyla, Mammalia), based on mitochondrial ribosomal DNA sequences. Molecular Biology and Evolution 9:433–446.

Gómez-Laverde, M., R. P. Anderson, and L. F. García. 2004. Integrated systematic reevaluation of the Amazonian genus *Scolomys* (Rodentia: Sigmodontinae). Mammalian Biology - Zeitschrift für Säugetierkunde 69:119–140.

Gonzalez Della Valle, M., et al. 2002. Andes virus associated with hantavirus pulmonary syndrome in northern Argentina and determination of the precise site of infection. The American Journal of Tropical Medicine and Hygiene 66:713–720.

Graybeal, A. 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? Systematic Biology 47:9–17.

Hafner, J. C., et al. 2007. Basal clades and molecular systematics of Heteromyid rodents. Journal of Mammalogy 99:1129–1145.

Hafner, J. C., E. Reddington, and M. T. Craig. 2006. Kangaroo mice (*Microdipodops megacephalus*) of the Mono Basin: phylogeography of a peripheral isolate. Journal of Mammalogy 87:1204–1217.

Hanson, J. D., and R. D. Bradley. 2008. Molecular diversity Within *Melanomys caliginosus* (Rodentia: Oryzomyini): Evidence for multiple species. Occasional Papers (Texas Tech University Museum) 275:1–11.

Hanson, J. D., J. L. Indorf, V. J. Swier, and R. D. Bradley. 2010. Molecular divergence within the *Oryzomys palustris* complex: evidence for multiple species. Journal of Mammalogy 91:336–347.

Hanson, J. D., A. Utrera, and C. F. Fulhorst. 2011. The delicate pygmy rice rat (*Oligoryzomys delicatus*) is the principal host of Maporal virus (family Bunyaviridae, genus Hantavirus). Vector-Borne and Zoonotic Diseases 11:691–696.

Hanson, J.D., G. D'Elía, S. B. Ayers, S. B. Cox, S. F. Burneo, and T. E. Lee, 2015. A new species of fish-eating rat, genus *Neusticomys* (Sigmodontinae), from Ecuador. Zoological Studies 54:49.

Hedtke, S. M., T. M. Townsend, and D. M. Hillis. 2006. Resolution of phylogenetic conflict in large data sets by increased taxon sampling. Systematic Biology 55:522–529.

Helbig, A. J., A. Kocum, I. Seibold, and M. J. Braun. 2005. A multi-gene phylogeny of aquiline eagles (Aves: Accipitriformes) reveals extensive paraphyly at the genus level. Molecular Phylogenetics and Evolution 35:147–164.

Henson, D. D., and R. D. Bradley. 2009. Molecular sysematics of the genus *Sigmodon*: results from mitochondrial and nuclear gene sequences. Canadian Journal of Zoology 87:211–220.

Hershkovitz, P. 1944. Systematic review of the Neotropical water rats of the genus *Nectomys* (Cricetinae). Miscellaneous Publications of the Museum of Zoology University of Michigan 58:1–101.

Hershkovitz, P. 1948. Mammals of northern Colombia. Preliminary report no. 3. Water rats (genus *Nectomys*), with supplemental notes on related forms. Proceedings of the United States National Museum 98:49–59.

Hershkovitz, P. 1955. South American marsh rats, genus *Holochilus*, with a summary of sigmodont rodents. Fieldiana Zoology 37:639–687.

Hershkovitz, P. 1962. Evolution of Neotropical cricetine rodents (Muridae) with special reference to the phyllotine group. Fieldiana Zoology 46:1–524.

Hershkovitz, P. 1993. A new central Brazilian genus and species of Sigmodontine rodent (Sigmodontinae) transitional between Akodonts and Oryzomyines, with a discussion of muroid molar morphology and evolution. Fieldiana Zoology 75:1–18.

Hillis, D. M., D. D. Pollock, J. A. McGuire, and D. J. Zwickl. 2003. Is sparse taxon sampling a problem for phylogenetic inference? Systematic biology 52:124–126.

IUCN. 2016. The IUCN Red List of Threatened Species. http://www.iucnredlist.org. Accessed 5 April 2017.

Jansa, S. A., and M. Weksler. 2004. Phylogeny of muroid rodents: relationships within and among major lineages as determined by IRBP gene sequences. Molecular Phylogenetics and Evolution 31:256–276.

Kim, J. 1996. General inconsistency conditions for maximum parsimony: Effects of branch lengths and increasing numbers of taxa. Systematic Biology 45:363–374.

Kuznetsova, M. V., M. V. Kholodova, and A. A. Luschekina. 2002. Phylogenetic analysis of sequences of the 12S and 16S rRNA mitochondrial genes in the family Bovidae: New evidence. Russian Journal of Genetics 38:942–950.

Lanfear, R., B. Calcott, S. Y. W. Ho, and S. Guindon. 2012. PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29:1695–1701.

Ledje, C., and U. Arnason. 1996. Phylogenetic relationships within caniform carnivores based on analyses of the mitochondrial 12S rRNA gene. Journal of Molecular Evolution 43:641–649.

Lee, T. E., D. Alvarado-Serrano, R. N. Platt II, and G. Goodwiler. 2006. Report on a mammal survey of the Cosanga River drainage, Ecuador. Occasional Papers Museum of Texas Tech University. 260:1–10.

Lee, T. E., C. Boada-Teran, A. M. Scott, S. F. Burneo Núñez, and J. D. Hanson. 2011. Small Mammals of Sangay National Park, Chimborazo Province and Morona Santiago Province, Ecuador. Occasional Papers Museum of Texas Tech University. 305:1–14.

Longhofer, L. K. and R. D. Bradley. 2006. Molecular systematics of the genus *Neotoma* based on DNA sequences from Intron 2 of the Alcohol Dehydrogenase gene. Journal of Mammalogy 87:961–970.

Lopez, N., P. Padula, C. Rossi, M. E. LÁZaro, and M. T. FranzeFernÁNdez. 1996. Genetic identification of a new Hantavirus causing severe pulmonary syndrome in Argentina. Virology 220:223–226.

Machado, L. F., Y. L. Leite, A. U. Christoff, and L. G. Giugliano. 2014. Phylogeny and biogeography of tetralophodont rodents of the tribe Oryzomyini (Cricetidae: Sigmodontinae). Zoologica Scripta 43:119–130.

Malygin, V. M., V. M. Aniskin, I. S. Isaev, and A. Milishnikov. 1994. *Amphinectomys savamis* Malygin gen. et. sp.n., a new genus and a new species of water rat (Cricetidae, Rodentia) from Peruvian Amazonia. Zoologicheskii Zhurnal 73:195–208.

Martin, Y., G. Gerlach, C. Schlötterer, and A. Meyer. 2000. Molecular phylogeny of European Muroid rodents based on complete cytochrome b sequences. Molecular Phylogenetics and Evolution 16:37–47.

Matocq, M. D., Q. R. Shurtliff, and C. R. Feldman. Phylogenetics of the woodrat genus *Neotoma* (Rodentia: Muridae): implications for the evolution of phenotypic variation in male external genitalia. Molecular Phylogenetics and Evolution 42:637–652

McKenna, M. C., and S. K. Bell. 1997. Classification of mammals above the species level. Columbia University Press, New York.

Meissner, J. D., J. E. Rowe, M. K. Borucki, and S. C. St. Jeor. 2002. Complete nucleotide sequence of a Chilean hantavirus. Virus Research 89:131–143.

Milazzo, M. L., M. N. Cajimat, J. D. Hanson, R. D. Bradley, M. Quintana, C. Sherman, R. T. Velasquez, and C. F. Fulhorst.

2006. Catacamas virus, a hantaviral species naturally associated with *Oryzomys couesi* (Coues' Oryzomys) in Honduras. The American Journal of Tropical Medicine and Hygiene 75:1003–1010.

Milazzo, M. L., M. N. Cajimat, M. R. Mauldin, S. G. Bennett, B. D. Hess, M. P. Rood, C. A. Conlan, K. Nguyen, J. W Wekesa, R. D. Ramos, R. D. Bradley, C. F. Fulhorst. 2015. Epizootiology of Tacaribe serocomplex virus (Arenaviridae) associated with neotomine rodents (Cricetidae, Neotominae) in southern California. Vector Borne Zoonotic Diseases 15:156–166

Musser, G. G., and M. D. Carleton. 2005. Family Cricetidae. Pp. 894–1522 in Mammal Species of the World: A Taxonomic and Geographic Reference (D. E. Wilson and D. M. Reeder, eds.). Johns Hopkins University Press, Baltimore, Maryland.

Myers, P., B. Lundrigan, and P. K. Tucker. 1995. Molecular phylogenetics of Oryzomyine rodents: The genus *Oligoryzomys*. Molecular Phylogenetics and Evolution 4:372–382.

Olson, L. E., E. J. Sargis, and R. D. Martin. 2005. Intraordinal phylogenetics of treeshrews (Mammalia: Scandentia) based on evidence from the mitochondrial 12S rRNA gene. Molecular Phylogenetics and Evolution 35:656–673.

Osgood, W. H. 1933. The supposed genera *Aepomys* and *Inomys*. Journal of Mammalogy 14:161.

Palma, R. E., et al. 2010. Phylogenetic relationships of the pygmy rice rats of the genus *Oligoryzomys* Bangs, 1900 (Rodentia: Sigmodontinae). Zoological Journal of the Linnean Society 160:551–566.

Palma, R. E., E. Rodriguez-Serrano, E. Rivera-Milla, C. E. Hernandez, J. Salazar-Bravo, M. I. Carma, S. Belmar-Lucero, P. Gutierrez-Tapia, H. Zeballos, and T. L. Yates. 2010. Phylogenetic relationships of the pygmy rice rats of the genus *Oligoryzomys* Bangs, 1900 (Rodentia: Sigmodontinae). Zoological Journal of the Linnean Society 160:551–566.

Parada, A., U. F. Pardiñas, J. Salazar-Bravo, G. D'Elía, and R. E. Palma. 2013. Dating an impressive Neotropical radiation: molecular time estimates for the Sigmodontinae (Rodentia) provide insights into its historical biogeography. Molecular Phylogenetics and Evolution 66:960–968.

Patton, J. L., and M. N. F. Da Silva. 1995. A review of the spiny mouse genus *Scolomys* (Rodentia: Muridae: Sigmodontinae) with the description of a new species from the western Amazon of Brazil. Proceedings of the Biological Society of Washington 108:319–337.

Percequillo, A. R., M. Weksler, and L. P. Costa. 2011. A new genus and species of rodent from the Brazilian Atlantic Forest (Rodentia: Cricetidae: Sigmodontinae: Oryzomyini), with comments on oryzomyine biogeography. Zoological Journal of the Linnean Society 161:357–390.

Pine, R. H., R. M. Timm, and M. Weksler. 2012. A newly recognized clade of trans-Andean Oryzomyini (Rodentia: Cricetidae), with description of a new genus. Journal of Mammalogy 93:851–870.

Platt II, R. N., B. R. Amman, M. S. Keith, C. W. Thompson, and R. D. Bradley. 2015. What is *Peromyscus*? Evidence from nuclear and mitochondrial DNA sequences suggests the need for a new classification. Journal of Mammalogy. 96:708–719.

Poe, S. 1998. Sensitivity of phylogeny estimation to taxonomic sampling. Systematic Biology 47:18–31.

Poe, S., and D. L. Swofford. 1999. Taxon sampling revisited. Nature 398:299–300.

Pollock, D. D. and W. J. Bruno. 2000. Assessing an unknown evolutionary process: Effect of increasing site-specific knowledge through taxon addition. Molecular Biology and Evolution 17:1854–1858.

Pollock, D. D., D. J. Zwickl, J. A. McGuire, and D. M. Hillis. 2002. Increased taxon sampling is advantageous for phylogenetic inference. Systematic biology 51:664–671.

Powers, A. M., et al. 1999. Isolation and genetic characterization of a hantavirus (Bunyaviridae: Hantavirus) from a rodent, *Oligoryzomys microtis* (Muridae), collected in northeastern Peru. The American Journal of Tropical Medicine and Hygiene 61:92–98.

Rannala, B., J. P. Huelsenbeck, Z. Yang, and R. Nielsen. 1998. Taxon sampling and the accuracy of large phylogenies. Systematic Biology 47:702–710.

Reig, O. A. 1986. Diversity patterns and differentiation of high Andean rodents. Pp. 404–440 in High Altitude Tropical Biogeography (F. Vuilleumier and M. Monasterio, eds.). Oxford University Press, New York.

Richter, M. H., J. D. Hanson, M. N. Cajimat, M. L. Milazzo, and C. F. Fulhorst. 2010. Geographical range of Rio Mamore virus (family Bunyaviridae, genus Hantavirus) in association with the small-eared pygmy rice rat (*Oligoryzomys microtis*). Vector-Borne and Zoonotic Diseases 10:613–620.

Rogers, D. S., E. A. Arenas, F. X. Conzalez-Cozatl, D. K. Hardy, J. D. Hanson, and N. Lewis-Rogers. 2009. Molecular phylogenetics of *Oligoryzomys fulvescens* based on cytochrome b gene sequences, with comments on the evolution of the genus *Oligoryzomys*. Pp. 179–192 in 60 Anos de la Colección Nacional de Mamíferos del Instituto de Biología, UNAM Aportaciones al Conocimiento y Conservacíon de los Mamíferos Mexicanos (F. Cervantes, ed.). Universidad Autónoma de México, México, D.F.

Rokas, A., B. L. Williams, N. King, and S. B. Carroll. 2003. Genome-scale approaches to resolving incongruence in molecular phylogenies. Nature 425:798–804.

Ronquist, F., et al. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61:539–542.

Rosenberg, M. S., and S. Kumar. 2001. Incomplete taxon sampling is not a problem for phylogenetic inference. Proceedings of the National Academy of Sciences 98:10751–10756.

Rosenberg, M. S., and S. Kumar. 2003. Taxon sampling, bioinformatics, and phylogenomics. Systematic biology 52:119–124.

Rossi, R. V., C. L. Miranda, and T. B. F. Semedo. 2016. Rapid assessment of nonvolant mammals in seven sites in the northern State of Pará, Brazil: a forgotten part of the Guiana Region. Mammalia 81:465–487.

Ruedas, L. A. and J. C. Morales. 2005. Evolutionary relationships among genera of Phalangeridae (Metatheria: Diprotodontia) inferred from mitochondrial DNA. Journal of Mammalogy 86:353–365.

Swofford, D. L. 2001. PAUP*: Phylogenetic analysis using parsimony (and other methods) 4.0. B5.

Saiki, R., et al. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491.

Salazar□Bravo, J., U. F. Pardiñas, and G. D'Elía. 2013. A phylogenetic appraisal of Sigmodontinae (Rodentia, Cricetidae) with emphasis on phyllotine genera: systematics and biogeography. Zoologica Scripta 42:250–261.

Salazar-Bravo, J., U.F. Pardiñas, H. Zeballos, and P. Teta. 2016. Description of a new tribe of Sigmodontine rodents (Cricetidae: Sigmodontinae) with an updated summary of valid tribes and their generic contents. Occasional papers, Museum of Texas Tech University 338:1–23.

Schenk, J. J., K. C. Rowe, and S. J. Steppan. 2013. Ecological opportunity and incumbency in the diversification of repeated contenental colonizations by muroid rodents. Systematic Biology 62:837–864.

Smith, M. F., and J. L. Patton. 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. Biological Journal of the Linnean Society 50:149–177.

Smith, M. F., and J. L. Patton. 1999. Phylogenetic Relationships and the Radiation of Sigmodontine Rodents in South America: Evidence from Cytochrome b. Journal of Mammalian Evolution 6:89–128.

Stanhope, M. J., J. Czelusniak, J.-S. Si, J. Nickerson, and M. Goodman. 1992. A molecular perspective on mammalian evolution from the gene encoding interphotoreceptor retinoid binding protein, with convincing evidence for bat monophyly. Molecular Phylogenetics and Evolution 1:148–160.

Stanhope, M. J., et al. 1998. Highly congruent molecular support for a diverse superordinal clade of endemic African mammals. Molecular Phylogenetics and Evolution 9:501–508.

Steppan, S. J. 1995. Revision of the tribe Phyllotini (Rodentia:Sigmodontinae), with a phylogenetic hypothesis for the Sigmodontinae. Fieldiana Zoology 80:1–112.

Torrez-Martinez, N., et al. 1998. Bayou virus-associated hantavirus pulmonary syndrome in Eastern Texas: identification of the rice rat, *Oryzomys palustris*, as reservoir host. Emerging Infectious Diseases 4:105–111.

Vilela, J. F., B. Mello, C. M. Voloch, and C. G. Schrago. 2014. Sigmodontine rodents diversified in South America prior to the complete rise of the Panamanian Isthmus. Journal of Zoological Systematics and Evolutionary Research 52:249–256.

Vincent, M. J., et al. 2000. Hantavirus pulmonary syndrome in Panama: Identification of novel Hantaviruses and their likely reservoirs. Virology 277:14–19.

Voss, R. S. 1991. An introduction to the Neotropical muroid rodent genus *Zygodontomys*. Bulletin of the American Museum of Natural History 210:1–113.

Voss, R. S., and M. D. Carleton. 1993. A new genus for *Hesperomys molitor* Winge and *Holochilus magnus* Hershkovitz (Mammalia, Muridae) with an analysis of its phylogenetic relationships. American Museum Novitates 3085:1–39.

Voss, R. S., M. Gomez-Laverde, and V. Pacheco. 2002. A new genus for *Aepeomys fuscatus* Allen, 1912, and *Oryzomys intectus* Thomas, 1921: enigmatic murid rodents from Andean cloud forests. American Museum Novitates 3373:1–42.

Weksler, M. 2003. Phylogeny of Neotropical oryzomyine rodents (Muridae: Sigmodontinae) based on the nuclear IRBP exon. Molecular Phylogenetics and Evolution 29:331–349.

Weksler, M. 2006. Phylogenetic relationships of Oryzomine rodents (Muroidea: Sigmodontinae): Separate and combined analyses of morphological and molecular data. Bulletin of the American Museum of Natural History:1–149.

Weksler, M. and C. R. Bonvicino. 2005. Taxonomy of pigmy rice rats (genus *Oligoryzomys*, Rodentia: Sigmodontinae) of the Brazilian Cerrado, with the description of 2 new species. Arquivos do Museu Nacional 63:113–130.

Weksler, M., and A. R. Percequillo. 2011. Key to the genera of the tribe Oryzomyini (Rodentia: Cricetidae: Sigmodontinae). Mastozoología Neotropical 18:281–292.

Weksler, M., A. R. Percequillo, and R. S. Voss. 2006. Ten new genera of Oryzomyine rodents (Cricetidae: Sigmodontinae). American Museum Novitates:1–29.

Yates, T. L., and S. Anderson. 2000. A new genus and species of phylottine rodent from Bolivia. Journal of Mammalogy 81:18–36.

Yoder, A. D., M. Cartmill, M. Ruvolo, K. Smith, and R. Vilgalys. 1996. Ancient single origin for Malagasy primates. Proceedings of the National Academy of Sciences 93:5122–5126.

Yu, L., and Y.-p. Zhang. 2005. Phylogenetic studies of pantherine cats (Felidae) based on multiple genes, with novel application of nuclear β-fibrinogen intron 7 to carnivores. Molecular Phylogenetics and Evolution 35:483–495.

Zwickl, D. J., and D. M. Hillis. 2002. Increased taxon sampling greatly reduces phylogenetic error. Systematic Biology 51:588–598.

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Patterns of Fluctuating Asymmetry and Shape Variation in *Myodes glareolus* from Chernobyl, Ukraine

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Abstract

The effect of high doses of radiation on the morphological traits of wild populations of organisms is not well understood. The present study focuses on levels of fluctuating asymmetry and shape variation in the skull of the bank vole, *Myodes glareolus*, from Chernobyl, Ukraine. Specifically, geometric morphometric analyses were used to test for the expected increase of fluctuating asymmetry in relation to geographic proximity to the Chernobyl Nuclear Power Plant Reactor 4—the reactor that suffered the infamous 1986 meltdown. Morphological variation was decomposed into symmetric and asymmetric components to evaluate patterns of spatial distribution and describe the associated morphological changes in skull shape. The results from the Procrustes analyses of variance indicate a slight but weak decrease of fluctuating asymmetry levels related to Reactor 4 proximity. Morphological variation among individuals shows wide overlap across different sites located around the Nuclear Power Plant indicating that within population variation is equivalent to that seen across sites. Together these analyses show no significant effects on skull morphological patterns in *M. glareolus* associated with geographic proximity to the Reactor 4 in Chernobyl.

Key words: Chernobyl Nuclear Power Plant Reactor 4, fluctuating asymmetry, geometric morphometrics, radiation exposure, Rodentia, skull morphology, Ukraine

Resumen

El efecto de la radiación sobre los rasgos morfológicos en poblaciones silvestres de organismos no es bien entendido. El presente estudio se centra en los niveles de asimetría fluctuante y la variación de la forma en el cráneo del ratón de banco, *Myodes glareolus*, de Chernóbil, Ucrania. Específicamente, utilizamos análisis de morfometría geométrica para evaluar el aumento de asimetría con la proximidad geográfica al Reactor 4 de la Planta de Energía Nuclear de Chernóbil —el reactor que sufrió el infame colapso de 1986. Descomponemos la variación morfológica en sus componentes simétricos y asimétricos para evaluar sus patrones de distribución espacial y describir los cambios morfológicos asociados en la forma del cráneo. Los análisis de la varianza de Procrustes indican una asociación débil con una ligera disminución de los niveles de asimetría fluctuante con la proximidad al Reactor 4. La variación morfológica entre los individuos muestra una amplia superposición entre los diferentes sitios ubicados alrededor de la Central Nuclear de Chernóbil, lo que indica que la variación dentro de la población es equivalente a que se ve a través de los sitios. Estos análisis muestran que no se detectaron efectos significativos en los patrones morfológicos del cráneo en *M. glareolus* asociados con la proximidad geográfica al Reactor 4 en Chernóbil.

Palabras clave: asimetría fluctuante, exposicion a la radiación, morfología del cráneo, morfometría geométrica, Reactor 4 de la Planta de Energía Nuclear de Chernóbil, Rodentia, Ucrania

INTRODUCTION

Environmental effects on morphological traits can be assessed by evaluating the degree of asymmetry in bilateral characters (Palmer and Strobeck 1986; Møller 1993; Lens et al. 2002). Three asymmetry types characterized by differing combinations of differences between the right (R) and the left (L) sides of a trait have been recognized—directional asymmetry, antisymmetry, and fluctuating asymmetry (Van Valen 1962; Parsons 1990). Although both directional asymmetry (the same side is consistently larger) and antisymmetry (one of the sides is consistently larger) result in a bimodal distribution of the right minus left (R-L) differences with an approximate mean of zero, fluctuating asymmetry is characterized by a normal distribution of differences and refers to subtle random deviations from perfect symmetry in bilateral traits resulting from developmental perturbations (Parsons 1990). Thus, levels of fluctuating asymmetry in bilateral traits have traditionally been associated with the ability of an organism to adjust its development to an ideal symmetric pattern, which can be influenced by environmental perturbations (developmental instability). The underlying reasoning for the use of fluctuating asymmetry as a measure of developmental instability is that two sides of a bilateral feature represent independent replicates of the same developmental events. Therefore, differences between sides must reflect minor developmental "errors" or perturbations affecting one side over the other (Klingenberg 2003; Van Dongen 2006).

Previous studies have explored the association between fluctuating asymmetry and stress, fitness, and health (Clarke and McKenzie 1992a, 1992b; Ho et al. 2009; de Coster et al. 2013). Although fluctuating asymmetry has been related to these processes across a variety of taxa, a debate continues about the validity of how these conclusions have been reached (Lens et al. 2002; Beasley et al. 2013). For instance, there might be an overestimation of effects due to sample size, which affects the power of detecting slight differences between sides (Van Dongen and Gangestad 2011). In addition, the fact that there is a literature bias towards reporting positive associations also might influence how hypotheses have been formulated in the first place. As a result, new studies are needed that carefully investigate the effect of environmental disturbances on levels of fluctuating asymmetry.

A large number of studies across a variety of taxa have explored genetic and phenotypic consequences of radiation exposure from the nuclear event in Reactor 4 at Chernobyl's Nuclear Power Plant on 26 April 1986. For example, studies of birds (Møller 1993) and mammals (Gileva and Nokhrin 2001; Oleksyk et al. 2004) that evaluated the effect of radiation exposure on morphological traits (e.g., skull, feathers, and tail) in and around Chernobyl have shown a positive association between higher levels of radiation and an increase in fluctuating asymmetry in these traits. Yet the tendency has been to use linear measurements to assess the morphological changes, which could be strongly influenced by allometric differences among sample individuals. In contrast, overall shape variation can be analyzed through the use of geometric morphometrics, which can separate the effects of size and shape, and thus offer a methodological advance over traditional approaches (Coda et al. 2017).

Populations of the bank vole (*Myodes glareolus*) around Chernobyl are a good model system for evaluating the effects of environmental disturbance on morphological traits due to their over 30 years of exposure to high doses of radiation and radioactive contamination of the environment. *Myodes* (prev. *Clethrionomys*) *glareolus* is a small-bodied, arvicoline rodent with a Palearctic distribution extending south from Scandinavia into the temperate and boreal forests of Europe and western Russia (Meeks et al. 2009). This species has been the focus of numerous research efforts for many reasons: (1) its presence is a general indicator of ecosystem health (Appleton et al. 2000; Flowerdew

et al. 2004; Sundell et al. 2004); (2) it has the most radioactive body-burden of cesium and strontium in muscle and bone tissues (radioelements prevalent on food sources from this species) when compared to other species of mammals and birds that inhabit the Chernobyl region (Chesser et al. 2000); (3) it is likely that there have being more than 30 bank vole generations in the most radioactive regions of Chernobyl (Meeks et al. 2007; Baker et al. 2017); (4) the mitochondrial genetic diversity of Chernobyl's populations of this vole have been thoroughly studied (Meeks et al. 2007, 2009; Baker et al. 2017); and (5) voucher specimens collected in the region after the nuclear event are well represented in museum collections.

Meeks et al. (2009) assessed the genetic structure of populations of *M. glareolus* in northern, central, and southern Ukraine to determine the extent to which population dynamics contribute to the observed differences in contaminated regions resulting from chronic environmental radiation exposure. These authors found that genetic diversity in contaminated areas apparently is not significantly different from uncontaminated sites (but see Baker et al. 2017). Additional studies have suggested that resident populations from Chernobyl and the surrounding areas are experiencing an elevated mutational load as a result of exposure to ionizing radiation (Shevchenko et al. 1992; Pomerantseva et al. 1997; Ryabokon et al. 2005; Ryabokon and Goncharova 2006).

In this study, the effects of environmental stress related to radiation exposure on the skull morphology of *Myodes glareolus* around the Chernobyl Nuclear Power Plant were assessed. The specific hypothesis tested was: if stress resulting from excessive radiation increases fluctuating asymmetry, then individuals from populations within higher radiation areas will have more phenotypic asymmetry than populations with little to no known radiation exposure. Also tested was the subsequent prediction that if radiation stress increased developmental perturbations, greater random unstructured shape variation will be observed in populations with higher radiation levels. Thus, asymmetry components were evaluated in the skull of *M. glareolus* when viewed dorsally and ventrally and that of the mandible when viewed laterally.

MATERIALS AND METHODS

Experimental design and study specimens.—The analyses included only adult specimens. Sex and locality information was recorded for all specimens from skin tags and collectors' field notes. All specimens were genetically characterized from previous studies that monitored populations of *M. glareolus* from Ukraine and corresponded to specimens collected from 1995 to 2004 (Baker et al. 2001; Meeks et al. 2007, 2009). Voucher specimens (Appendix I) were deposited at the Natural Science Research Laboratory of Texas Tech University. Study specimens were collected from sites located within the most-contaminated zone (10 km radius) and outside the exclusion zone (> 30 km radius) from the Chernobyl Nuclear Power Plant Reactor 4 (Fig. 1). Previous studies considered localities within a 10 km radius from the contaminated Plant, but no study on *M. glareolus* has analyzed localities outside this 30-km exclusion zone, which have been regarded as uncontaminated or control plots (Rodgers et al. 2001; Meeks et al. 2009; Baker et al. 2017).

Data collection.—Three skull views (Fig. 2) were selected to document skull shape. Two replicate (non-consecutive) photograph series were taken of each skull to assess for imaging errors. Skull images were captured using a Besler CS copy stand with two 250 watt 3200 K photofloods, a Canon Rebel T1i digital camera, a Canon 100 mm macro lens, a Canon cable release, and a tray in which to position specimens (measuring 65 mm x 34 mm x 14 mm; 1.8 mm thick bottom). The camera was affixed to the copy stand (26 cm above the base). A small rectangular section of graph paper with centimeter and millimeter grids and a window cut into it for the subject was used for a scale. The tray was filled with 7 mm of sand to elevate the skulls, so the approximate middle of the skull was level with the graph paper.

Each skull was arranged in a standard position for each photograph. For each dorsal view, skulls were positioned such that the tooth row was oriented

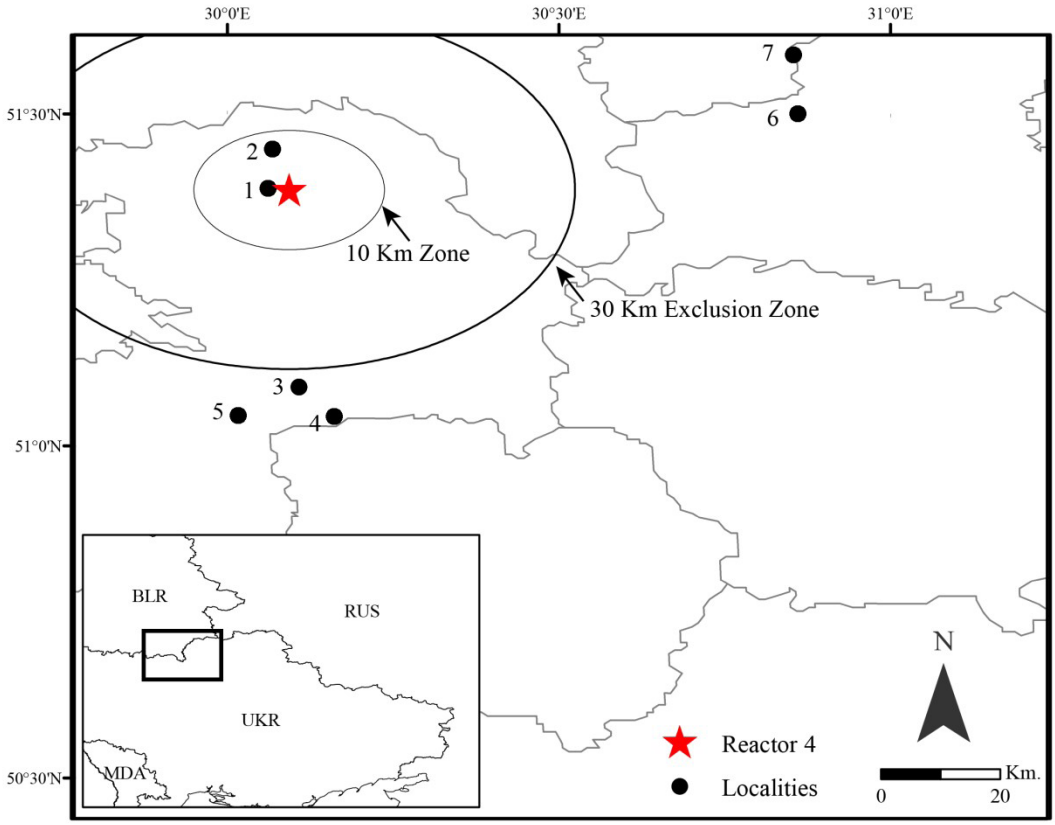

Figure 1. Map of geographic locations sampled in Ukraine. Collection sites for *Myodes glareolus* specimens included in this study are indicated by the numbers 1 to 7. Numbers 1 and 2 are located within the 10 km radius of Chernobyl Nuclear Power Plant Reactor 4. The remaining sites (3–7) are outside of the 30 km radius. 1 = Chernobyl, Red Forest; 2 = Chernobyl, Glyboke Lake; 3 = Ivanov, The Shop; 4 = Ivanov, Oranoe; 5 = Kiev, Pine Wood; 6 = Kiev, Nedanchichy; and 7 = Kiev, Chista.

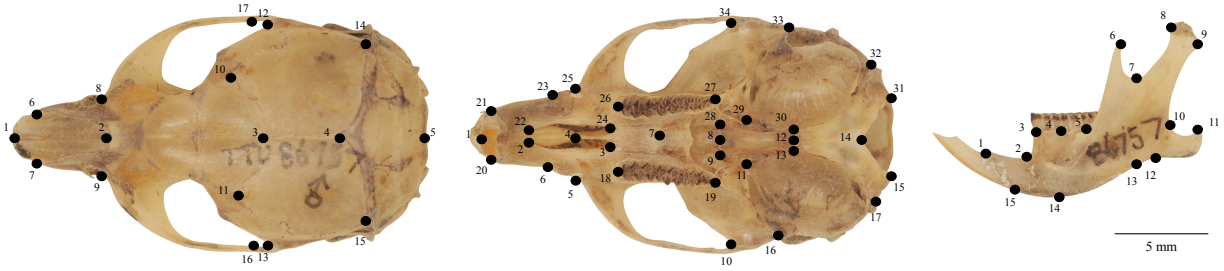

Figure 2. Three skull views of *Myodes glareolus*, illustrating landmark configurations used to characterize dorsal view of the skull, ventral view of the skull, and lateral view of the mandible. Landmark descriptions are provided in Appendix II.

to a horizontal position. The focal plane of the photographic frame was established below the center point and brought up to where the center point was in focus. For the photographs of the ventral surface, the skulls were placed so that the toothrow was oriented to the horizontal position. These images focused on the posterior margin of the palate. Lateral photographs of the mandible (right and left side) also were oriented using the tooth rows aligned to the horizontal position. The proximal and distal tooth rows (from the camera) were oriented as squarely as possible. The anterior margins and the occlusal surfaces were aligned. The center of the image was focused on the posterior visible portion of the tooth row.

Landmark data.—The relative positions of landmarks located in the three views of the skulls (Fig. 2) were digitized for each specimen with the TPS program series (software modules developed by J. Rohlf, http://life.bio.sunysb.edu/morph/). The dimensionality of each matrix depended on the number of specimens and landmarks included per view. The resulting matrices of landmark coordinates were used in all subsequent analyses.

Statistical analysis.—The landmark coordinate matrices were used to extract shape information. Variation in size, position, and orientation were removed by rescaling the matrix configurations to a standard size, position, and orientation and by conducting a Procrustes superimposition (Klingenberg et al. 2002). After superimposition, outliers were searched for in the dataset (i.e., individuals with landmarks that strongly deviated from the mean shape); outlier individuals were redigitized to assure they were not the product of a digitizing error. Then, to disentangle fluctuating asymmetry from directional asymmetry, a Procrustes ANOVA was performed following Palmer et al. (2010), where individuals were considered a random factor, and the left-right side of the skull was a fixed factor. The individual × side interaction in this model represents the morphological differences between the right and left halves of the skull, which is a measure of fluctuating asymmetry (Klingenberg 2015). Imaging error was included in the model thanks to the replicate photographs.

To investigate shape variation, a separate principal component analysis (PCA) was performed for each view of the skull. Principal component analysis simplifies the description of variation among individuals by reducing the variation encountered in the dataset into a smaller number of orthogonal dimensions (Zelditch et al. 2004). These analyses were run using an independent covariance matrix for each view employing the mean individual values (across replicate images) for the symmetric and asymmetric components. These components account for the repetition of parts in different positions of morphological structures (symmetry) and its deviation from symmetry (asymmetry; Klingenberg 2015). All analyses were carried out with the MorphoJ software package (http:/www.flywings.org.uk/MorphoJ_page.htm).

RESULTS

The Procrustes ANOVA indicates that in all skull views, most variation was accounted by individual (60% for dorsal view, 58% for ventral view, and 59% for lateral view of the mandible) and not by locality. This outcome was expected if the distance from the Reactor 4 was a main contributor to shape variation in these populations. In fact, locality was a source of variation with statistically significant differences across only the skull views ($P < 0.001$) but not the mandible view ($P < 0.06$). Imaging error was relatively high and accounted for 19%, 14% and 12% of the total variation of dorsal and ventral views of the skull and lateral view of the mandible, respectively (Table 1). No significant differences between sexes were found in any of the ANOVA models ($P > 0.05$; this finding was supported by a lack of statistical differences between sexes in centroid size). Sexes were thus pooled together for further analyses. Additionally, the interaction between individual × side, defined as the variation of asymmetric pattern from mean asymmetry, which is the measurement of fluctuating asymmetry (FA) used here, captures 8% of the variance ($F = 0.86$; $P = 1.00$) for the dorsal view, 10% ($F = 1.53$; $P < 0.0001$) for the ventral view, and 12% ($F = 2.16$; $P < 0.0001$) for the lateral view of the mandible.

Table 1. Procrustes ANOVA results per skull view. The interaction between individual × side, defined as the variation of asymmetric pattern from mean asymmetry, is the measurement of fluctuating asymmetry (FA) used here.

Effect	% Variation	SS	MS	df	F	P-value
Dorsal view of skull						
Locality	8.55	4.07E-02	4.52E-04	90	4.43	<0.0001
Sex	0.30	1.45E-03	9.67E-05	15	0.95	0.51
Individual	60.84	2.90E-01	1.02E-04	2835	7.78	<0.0001
Side	2.54	1.21E-02	8.05E-04	15	61.32	<0.0001
Individual × Side	8.11	3.86E-02	1.31E-05	2940	0.83	1.00
Error	19.66	9.36E-02	1.58E-05	5910		
Total	100.00	4.76E-01				
Ventral view of skull						
Locality	8.95	1.26E-02	7.87E-05	160	2.52	<0.0001
Sex	0.66	9.30E-04	2.91E-05	32	0.93	0.58
Individual	58.95	8.29E-02	3.12E-05	2656	6.08	<0.0001
Side	6.20	8.72E-03	2.73E-04	32	53.12	<0.0001
Individual × Side	10.39	1.46E-02	5.13E-06	2848	1.42	<0.0001
Error	14.85	2.09E-02	3.63E-06	5760		
Total	100.00	1.41E-01				
Lateral view of mandible						
Locality	9.82	4.65E-02	2.98E-04	156	1.19	0.06
Sex	2.62	1.24E-02	2.38E-04	52	0.95	0.57
Individual	59.02	2.79E-01	2.50E-04	1118	5.44	<0.0001
Side	3.49	1.65E-02	6.34E-04	26	13.81	<0.0001
Individual × Side	12.88	6.09E-02	4.59E-05	1326	2.16	<0.0001
Error	12.18	5.76E-02	2.13E-05	2704		
Total	100.00	4.73E-01				

Fluctuating asymmetry variation across localities was significant despite high intra-locality variation only in the dorsal and ventral views. Specifically, FA increases slightly as the distance to Chernobyl's Reactor 4 increases—dorsal ($R^2 = 0.03$; $P = 0.01$; Fig. 3a) and ventral ($R^2 = 0.04$; $P = 0.04$; Fig. 3b). No FA trend was evident in the lateral view of the mandible ($R^2 = 0.002$; $P = 0.75$; Fig. 3c). It is noteworthy that in all these cases, the strength of the relationship between FA index and geographic distance was very weak ($R^2 <= 0.04$).

In the PCA, the symmetric component of all skull views, the variance of PC1 and PC2 together account for between 35% and 50% (Figs. 4a, 5a, 6a). For the dorsal view (Fig. 4c), PC1 primarily account for length and width of the rostrum and zygomatic arch, whereas

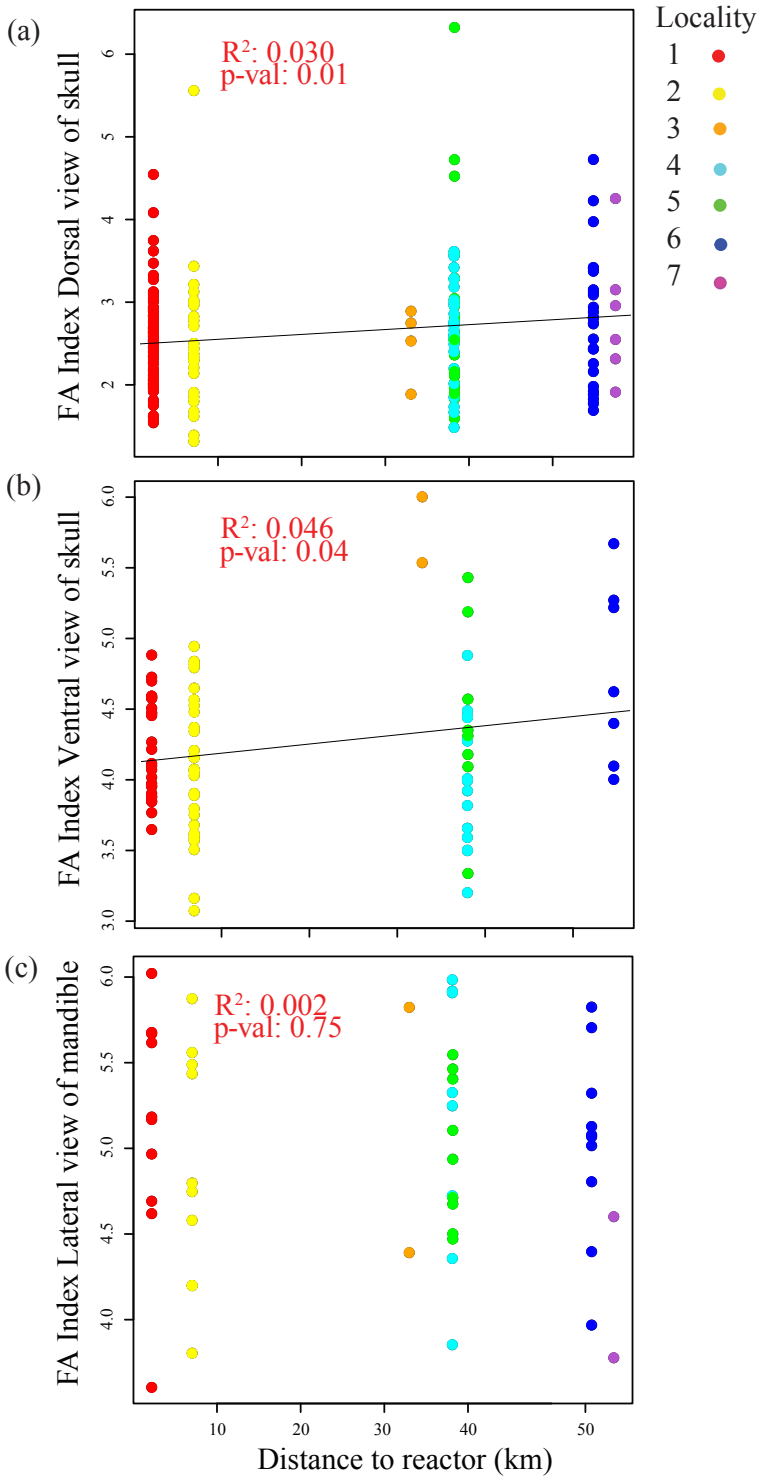

Figure 3. Variation in fluctuating asymmetry index values at increasing distances from Chernobyl Nuclear Power Plant Reactor 4 (see Fig. 1). From top to bottom: variation in dorsal view of the skull (a), ventral view of the skull (b), and lateral view of the mandible (c). Coefficients of determination and associated significance values for each regression are provided in red.

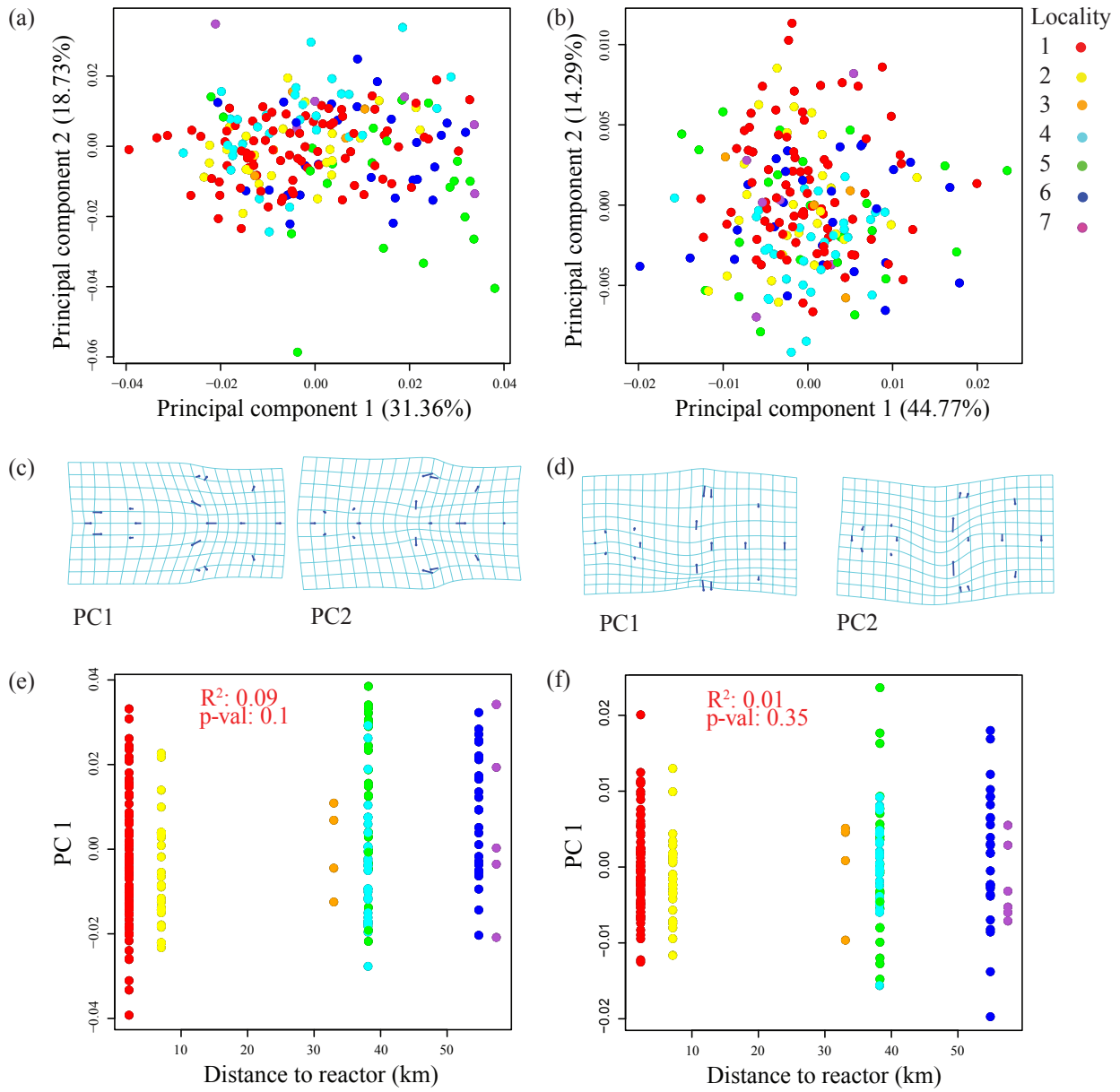

Figure 4. Individual variation in symmetric (left column) and asymmetric (right column) components in the dorsal view of the skull, summarized by principal component analyses (PCAs). From top to bottom: scatterplot of first two PCA components (a, b); associated shape changes along PC1 and PC2 (c, d); and regression plot of PC1 against distances from Chernobyl Nuclear Power Plant Reactor 4 (e, f).

PC2 primarily accounts for width of zygomatic arch and frontal region. For the ventral view (Fig. 5c), PC1 of the symmetric component corresponds mainly to a contrast between relative width of the skull and length and width of the pterygoid and basisphenoid. In contrast, PC2 in this analysis mainly represents variation in size and shape of the premaxilla, maxilla and palatal, and width of the zygomatic arch. Finally, in the PCA of the symmetric component variation in the lateral view of the mandible (Fig. 6c), PC1 is associated with the length of the mandible body and height of the coronoid, whereas PC2 in this latter analysis mainly accounts for variation in depth of the mandible.

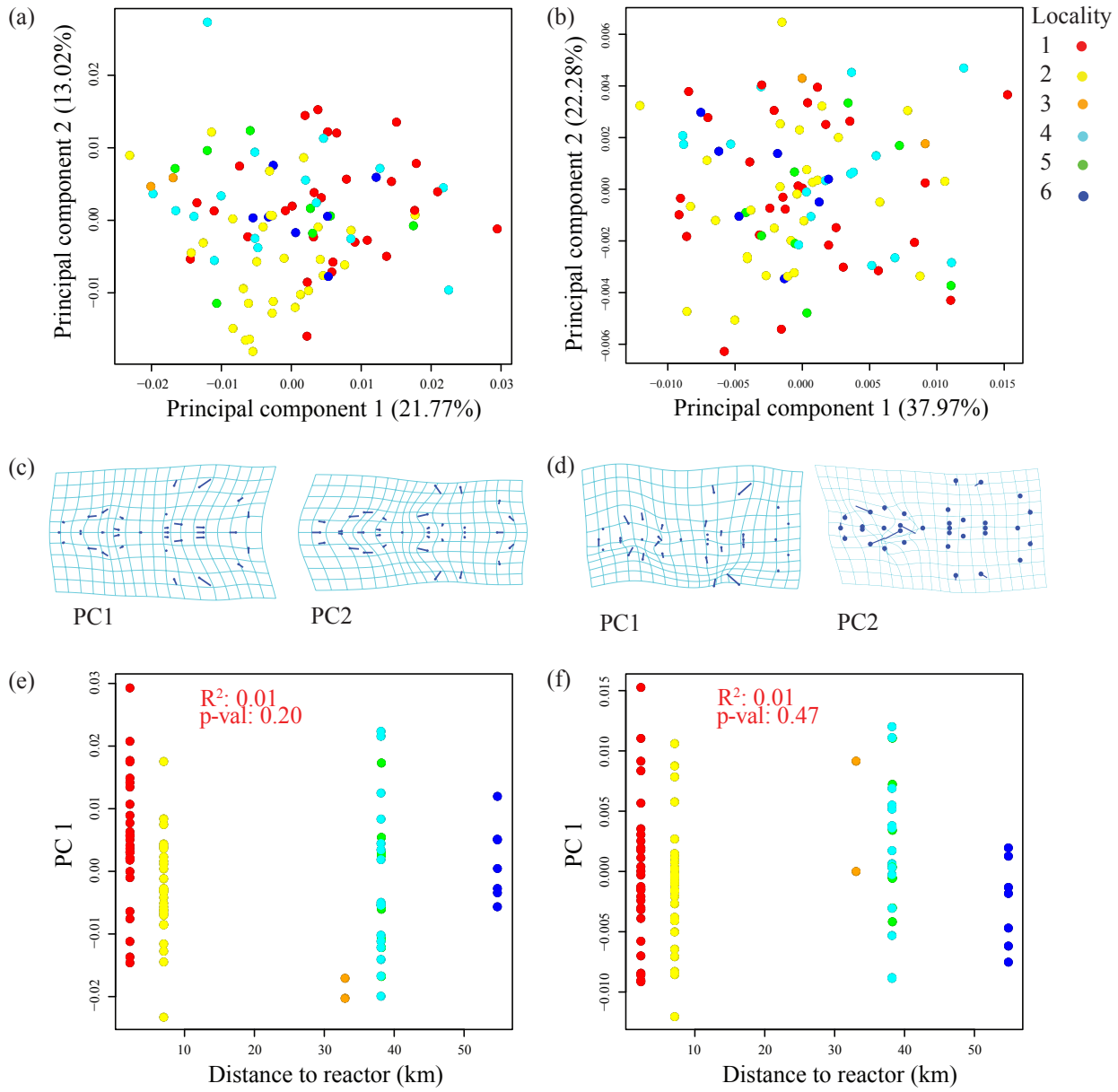

Figure 5. Individual variation in symmetric (left column) and asymmetric (right column) components in the ventral view of the skull, summarized by principal component analyses (PCAs). From top to bottom: scatterplot of first two PCA components (a, b); associated shape changes along PC1 and PC2 (c, d); and regression plot of PC1 against distances from Chernobyl Nuclear Power Plant Reactor 4 (e, f).

In all three views (Figs. 4a, 5a, 6a), variation among individuals in the PC1-PC2 scatter plots show wide overlap among individuals from different sites located around the Nuclear Power Plant and ample variation across individuals from each site. Remarkably, in all three PCA analyses, populations close to Reactor 4 (Pops. 1 and 2) fall well within the variation observed in more distant populations. Variation along PC1 in the dorsal view of the skull, ventral view of the skull and lateral view of the mandible showed no significant trend with distance to Reactor 4 (Figs. 4e, 5e, and 6e).

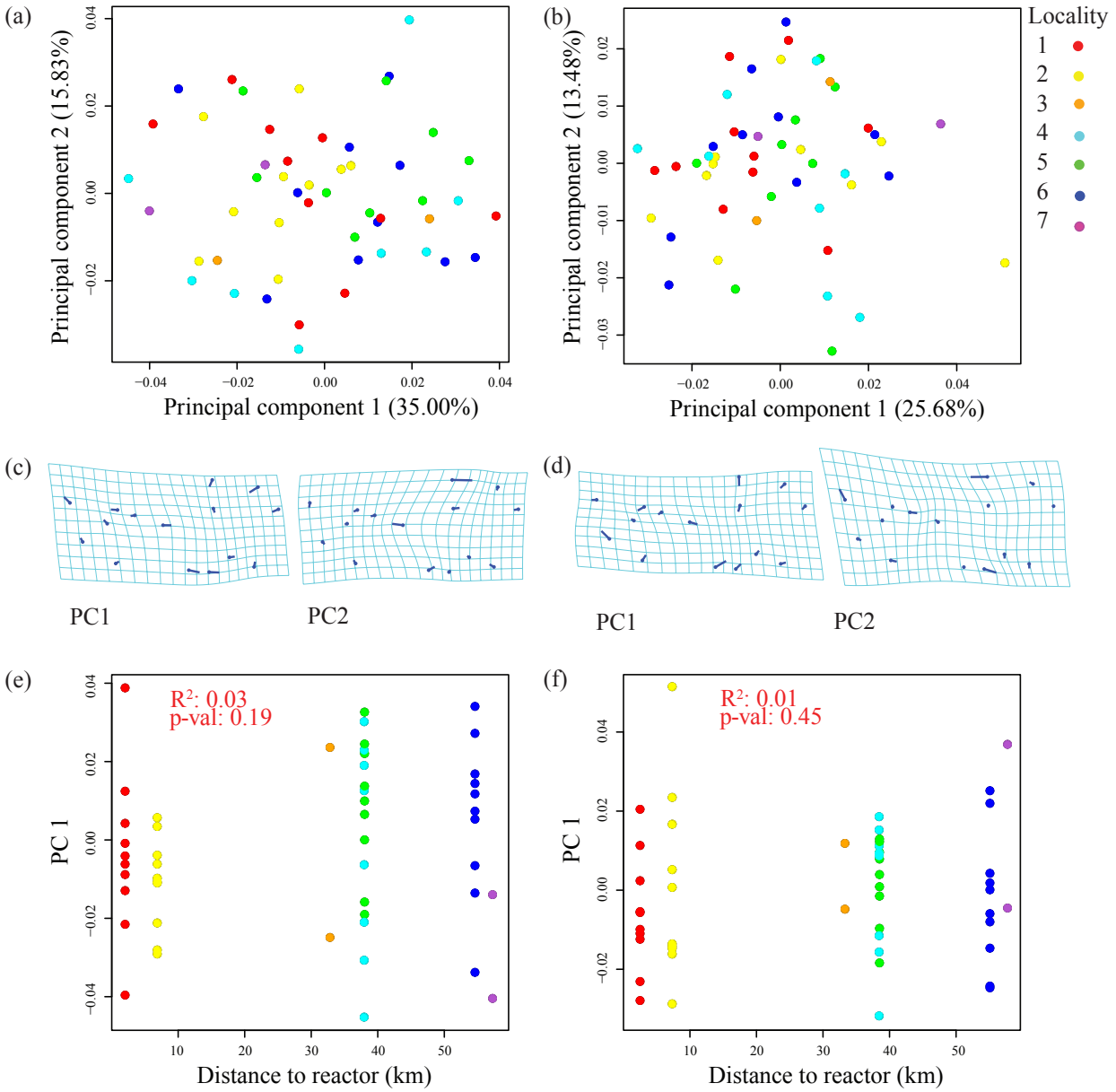

Figure 6. Individual variation in symmetric (left column) and asymmetric (right column) components in the lateral view of the mandible, summarized by principal component analyses (PCAs). From top to bottom: scatterplot of first two PCA components (a, b); associated shape changes along PC1 and PC2 (c, d); and regression plot of PC1 against distances from Chernobyl Nuclear Power Plant Reactor 4 (e, f).

On the other hand, the PCAs for the asymmetric component, which primarily summarize patterns of asymmetry, show that less than half of the variation is accounted by PC1 in all three views (Figs. 4b, 5b, 6b). The PC1 for the dorsal view mainly represents asymmetry in size and orientation of rostrum and zygomatic arch, whereas PC2 primarily features differences in the pre-orbital constriction (Fig. 4d). In contrast, the PC1 of the ventral view shows differences in relative size of the rostrum and braincase on either side, whereas PC2 mostly features asymmetry in orientation (Fig. 5d). Finally, patterns of asymmetric variation in the

mandible (Fig. 6d) accounted by PC1 primarily reflect differences in relative orientation and size of the coronoid and mandible body between sides, whereas PC2 primarily comprises differences in breadth of the mandible between sides. Remarkably, the association between individual scores on these asymmetry PC1s and distance to Chernobyl's Reactor 4 show similar patterns among views. In all three cases, there is no evidence of levels of asymmetry changing with proximity to the reactor: dorsal ($R^2 = 0.01$; $P = 0.35$; Fig. 4f), ventral ($R^2 = 0.01$; $P = 0.47$; Fig. 5f), and lateral view of the mandible ($R^2 = 0.01$; $P = 0.45$; Fig. 6f).

DISCUSSION

This study investigated variation in skull configuration of *M. glareolus* across specimens collected around Chernobyl's Nuclear Power Plant Reactor 4. The results indicate that, proportionally, most of the variation is accounted by variation across individuals. In addition, there is a restricted contribution of distance to the melted reactor. Taken together, these results present limited evidence of the effects of the Chernobyl disaster's radiation on skull and mandible morphological variation in *M. glareolus*.

Patterns of both fluctuating asymmetry and the symmetric and asymmetric components of shape variation among views do not support the hypothesis that populations within areas with higher radiation levels would have more phenotypic asymmetry and greater random unstructured variation than populations with little to no known radiation exposure (Table 1; Fig. 3). On one hand, the results from the Procustes ANOVA found statistically significant differences in fluctuating asymmetry (interaction between individual × side) in the ventral view of the skull and lateral view of the mandible, but these differences are not strongly associated with geographic distance. In fact, contrary to the study's first prediction, a slight decrease of fluctuating asymmetry levels exist as the geographic proximity to Reactor 4 increases based on both dorsal and ventral views—the lateral view of the mandible shows no significant association. However, the strength of these associations is very weak, and significant results can be driven by sample size effect. Further, in contrast to the study's second prediction, wide intra-location variation in fluctuating asymmetry scores exists across all localities (Fig. 3), which is paralleled in the patterns of overlap among individuals from different localities (including those close to Reactor 4) found in PCA analyses of both symmetric and asymmetric shape components (Fig. 4-6). Thus, patterns of variation do not support significant effects of radiation on skull morphological patterns in *M. glareolus* populations around the Nuclear Power Plant area in relation to the distance to Reactor 4.

The findings reported here fail to support a previously reported positive association between fluctuating asymmetry and the proximity to Reactor 4 in mice populations from Chernobyl (Oleksyk et al. 2004), as well as previous studies in bank voles that evaluated the effect of environmental factors, such as habitat fragmentation (Marchand et al. 2003). In contrast with Oleksyk et al. (2004), who identified a significant increase in fluctuating asymmetry in shape as the distance to Reactor 4 increases in a muroid rodent species from a different family (*Apodemus flavicollis*), we do not collapse individual estimates into central estimates. Hence, the present study does account for the individual variation component, which, as evidenced by the Procrustes ANOVA, encompasses variation in the sample. Therefore, further analyses are necessary to assess the extent to which the differences observed are driven by species-specific responses to radiation. For instance, it has been proposed that *M. glareolus* possesses an inherent quality for radio-resistance (Krapivko and Il'enko 1988; Il'enko and Krapivko 1994; Rodgers et al. 2001). This assertion is supported by the research of Jernfors et al. (2018), which discovered candidate genes that regulate genomic stability in bank voles exposed to environmental radionuclides and gave this mammal its value as a sentinel species in environmental studies of radiation contamination (Rodgers et al. 2001).

It is important to consider that geographic distance may not be an accurate proxy for increasing radiation exposure or that other factors might contribute to our inability to recover the effects of radiation in our analyses. Thus, further assessment is required concerning whether the asymmetric patterns observed in this study's data are an accurate indicator of limited

effects of radiation exposure on morphological traits in vole populations around Chernobyl. Alternatively, the limited radiation effects observed may be the result of a complex history of recolonization in the disaster's zone zero (Baker et al. 2017), which could obscure the disaster's impacts due to the influx of immigrants with limited radiation exposure. Yet, considering that the observed genetic variability of populations closest to Reactor 4 does not suggest source-sink dynamics at play (Baker et al. 2017), it seems likely that these populations have been able to maintain morphological consistency of the skull despite high radiation levels, presumably due to strong canalization. Such compensation may result from the high selective costs of deviant skull morphologies. Indeed, it is possible that the limited radiation effects we identify originate from the fact that the proposed association between fluctuating asymmetry as an indirect measurement of individual environmental quality (Palmer 1996; Møller 1998) might be expressed differently across morphological traits due to different levels of stabilizing selection (Ditchkoff and DeFreese 2010). In this scenario, some morphological traits (e.g., those related to survival) are expected to be highly canalized and, therefore, less susceptible to developmental disturbance (Polak 1993). Since skull configuration is a key adaptation of functional and evolutionary mechanisms, deviations on the right and left side in bilateral traits might be under high selective pressures. Yet, while the study's findings of lower FA levels closest to Reactor 4 are in line with this latter canalization possibility, compensating the effects of radiation, further studies are needed to confirm this hypothesis.

Limitations

Imaging error for all skull views was relatively large, accounting for more than 10% of the total variance across views. However, this error does not seem to compromise any of the analyses, as indicated by a robustness test. Specifically, the percentage of outliers for each landmark was used to evaluate the robustness of each landmark respect to the two replicates (image 1 and 2). Among skull views, the range of outliers per landmark ranged between 0 to 13%. Taking this finding into consideration, the analyses were repeated with all landmarks, removing a proportion of outliers above 5%. It is important to note that the same general patterns of variation with respect to the original data set used and the same limited effect of distance to Reactor 4 were recovered. Furthermore, a strong correlation in fluctuating asymmetry estimates between both sets (with and without outliers) was found ($R^2 > 0.7$; $P = 0.01$). Still, increasing the number of individuals and images in future analyses should increase the power to control imaging error and would allow for a more accurate test of significance in asymmetry patterns among skull views. More importantly, other proxies of individual radiation exposure should be included (e.g., the absorbed dose to bone) to better account and control for effects of radiation exposure in morphological configurations. Yet, despite these possible caveats, the robust lack of support across skull views for our predictions provides strong evidence of the limited effect of radiation on *M. glareolus* skull morphology.

Conclusion

In conclusion, this study suggests the limited impact of radiation on both fluctuating asymmetry and the symmetric and asymmetric components of skull shape on populations inhabiting the areas surrounding the Nuclear Power Plant's Reactor 4. Further, the effects of high levels of radiation on the bank vole, *M. glareolus*, in Chernobyl show fluctuating asymmetry patterns among skull projections that are inconsistent with the expected increase in asymmetry with decreasing distance to the Chernobyl disaster site. These results suggest that disentangling the effects of major environmental changes on wild populations is more complex than previously thought. Buffering mechanisms that control the phenotypic variability and mitigate the effect of environmental stressors (e.g., canalization and developmental stability; Vishalakshi and Singh 2008) presumably play a fundamental role in maintaining selectively important morphological structures. Nevertheless, characterizing shape variation patterns in skull symmetry should allow for estimates of the degree of change over time and can provide information about key ecomorphological adaptations to drastic environmental changes.

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LITERATURE CITED

Appleton, J., K. M. Lee, K. S. Kapusta, M. Damek, and M. Cooke. 2000. The heavy metal content of the teeth of the bank vole (*Clethrionomys glareolus*) as an exposure marker of environmental pollution in Poland. Environmental Pollution 110:441–449.

Baker, R. J., et al. 2001. Consequences of polluted environments on population structure: The bank vole (*Clethrionomys glareolus*) at Chornobyl. Ecotoxicology 10:211–216.

Baker, R. J., et al. 2017. Elevated mitochondrial genome variation after 50 generations of radiation exposure in a wild rodent. Evolutionary Applications 10:784–791.

Beasley, D. A. E., A. Bonisoli-Alquati, and T. A. Mousseau. 2013. The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: a meta-analysis. Ecological Indicators 30:218–226.

Chesser, R. K., et al. 2000. Concentrations and dose rate estimates of (134,137)cesium and (90)strontium in small mammals at Chornobyl, Ukraine. Environmental Toxicology and Chemistry 19:305–312.

Clarke, G., and J. McKenzie. 1992a. Coadaptation, developmental stability, and fitness of insecticide resistance genotypes in the Australian sheep blowfly, *Lucilia cuprina*: a review. Acta Zoologica Fennica 191:107–110.

Clarke, G., and L. McKenzie. 1992b. Fluctuating asymmetry as a quality control indicator for insect mass rearing processes. Journal of Economic Entomology 85:2045–2050.

Coda, J. A., J. J. Martínez, A. R. Steinmann, J. Priotto, and M. D. Gomez. 2017. Fluctuating asymmetry as an indicator of environmental stress in small mammals. Mastozoología Neotropical 24:313–321.

de Coster, G., S. Van Dongen, P. Malaki, M. Muchane, A. Alcántara-Exposito, H. Matheve, and L. Lens. 2013. Fluctuating asymmetry and environmental stress: understanding the role of trait history. PLoS ONE 8:1–8.

Ditchkoff, S. S., and R. L. DeFreese. 2010. Assessing fluctuating asymmetry of white-tailed deer antlers in a three-dimensional context. Journal of Mammalogy 91:27–37.

Flowerdew, J. R., R. F. Shore, S. M. C. Poulton, and T. H. Sparks. 2004. Live trapping to monitor small mammals in Britain. Mammal Review 34:31–50.

Gileva, E. A., and D. Y. Nokhrin. 2001. Fluctuating asymmetry in cranial measurements of east European voles (*Microtus rossiaemeridionalis* Ognev, 1924) from the zone of radioactive contamination. Russian Journal of Ecology 32:39–44.

Ho, G. W. C., K. M. Y. Leung, D. L. Lajus, J. S. S. Ng, and B. K. K. Chan. 2009. Fluctuating asymmetry of *Amphibalanus* (*Balanus*) *amphitrite* (Cirripedia: Thoracica) in association with shore height and metal pollution. Hydrobiologia 621:21–32.

Il'enko, A. I., and T. P. Krapivko. 1994. Radioresistance of populations of bank voles *Clethrionomys glareolus* in radionuclide-contaminated areas. Doklady Biological Sciences 26:262–266.

Jernfors, T., et al. 2018. Transcriptional upregulation of DNA damage response genes in bank voles (*Myodes glareolus*) inhabiting the Chernobyl exclusion zone. Frontiers in Environmental Science 5:95.

Klingenberg, C. P. 2003. A developmental perspective on developmental instability: theory, models and mechanisms. Pp. 14–34 in Developmental instability: causes and consequences (M. Polak, ed.). 1st ed. Oxford University Press.

Klingenberg, C. P. 2015. Analyzing fluctuating asymmetry with geometric morphometrics: concepts, methods, and applications. Symmetry 7:843–934.

Klingenberg, C. P., M. Barluenga, and A. Meyer. 2002. Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. Evolution 56:1909–1920.

Krapivko, T. P., and A. I. Il'enko. 1988. [First signs of radioadaptation in the population of bank voles (*Clethrionomys glareolus*) in the radiation biogeocenosis]. Doklady Akademii Nauk SSSR 302:1272–1274.

Lens, L., S. Dongen, S. Kark, and E. Matthysen. 2002. Fluctuating asymmetry as an indicator of fitness: can we bridge the gap between studies? Biological Reviews 77:27–38.

Marchand, H., G. Paillat, S. Montuire, and A. Butet. 2003. Fluctuating asymmetry in bank vole populations (Rodentia, Arvicolinae) reflects stress caused by landscape fragmentation in the Mont-Saint-Michel Bay. Biological Journal of the Linnean Society 80:37–44.

Meeks, H. N., R. K. Chesser, B. E. Rodgers, S. Gaschak, and R. J. Baker. 2009. Understanding the genetic consequences of environmental toxicant exposure: Chernobyl as a model system. Environmental Toxicology and Chemistry 28:1982–1994.

Meeks, H. N., J. K. Wickliffe, S. R. Hoofer, R. K. Chesser, B. E. Rodgers, and R. J. Baker. 2007. Mitochondrial control region variation in bank voles (*Clethrionomys glareolus*) is not related to Chernobyl radiation exposure. Environmental Toxicology and Chemistry 26:361–369.

Møller, A. P. 1993. Morphology and sexual selection in the barn swallow *Hirundo rustica* in Chernobyl, Ukraine. Proceedings: Biological Sciences 252:51–57.

Møller, A. P. 1998. Developmental instability of plants and radiation from Chernobyl. Oikos 81:444–448.

Oleksyk, T. K., J. M. Novak, J. R. Purdue, S. P. Gashchak, and M. J. Smith. 2004. High levels of fluctuating asymmetry in populations of *Apodemus flavicollis* from the most contaminated areas in Chornobyl. Journal of Environmental Radioactivity 73:1–20.

Palmer, A. R. 1996. Waltzing with asymmetry. Bioscience 46:518–532.

Palmer, A. R., and C. Strobeck. 1986. Fluctuating asymmetry: measurement, analysis, patterns. Annual Review of Ecology and Systematics 17:391–421.

Palmer, M., M. Linde, and B. Morales-Nin. 2010. Disentangling fluctuating asymmetry from otolith shape. Marine Ecology Progress Series 399:261–272.

Parsons, P. A. 1990. Fluctuating asymmetry: an epigenetic measure of stress. Biological Reviews 65:131–145.

Polak, M. 1993. Parasites increase fluctuating asymmetry of male *Drosophila nigrospiracula*: implications for sexual selection. Genetica 89:255–265.

Pomerantseva, M. D., L. K. Ramaiya, and A. V Chekhovich. 1997. Genetic disorders in house mouse germ cells after the Chernobyl catastrophe. Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis 381:97–103.

Rodgers, B. E., J. K. Wickliffe, C. J. Phillips, R. K. Chesser, and R. J. Baker. 2001. Experimental exposure of naive bank voles (*Clethrionomys glareolus*) to the Chornobyl, Ukraine, environment: a test of radioresistance. Environmental Toxicology and Chemistry 20:1936–1941.

Ryabokon, N. I., and R. I. Goncharova. 2006. Transgenerational accumulation of radiation damage in small mammals chronically exposed to Chernobyl fallout. Radiation and Environmental Biophysics 45:167–177.

Ryabokon, N. I., I. I. Smolich, V. P. Kudryashov, and R. I. Goncharova. 2005. Long-term development of the radionuclide exposure of murine rodent populations in Belarus after the Chernobyl accident. Radiation and Environmental Biophysics 44:169–181.

Shevchenko, V. A., M. D. Pomerantseva, L. K. Ramaiya, A. V Chekhovich, and B. V Testov. 1992. Genetic-disorders in mice exposed to radiation in the vicinity of the Chernobyl nuclear-power station. Science of the Total Environment 112:45–56.

Sundell, J., et al. 2004. Large-scale spatial dynamics of vole populations in Finland revealed by the breeding success of vole-eating avian predators. Journal of Animal Ecology 73:167–178.

van Dongen, S. V. 2006. Fluctuating asymmetry and developmental instability in evolutionary biology: past, present and future. Journal of Evolutionary Biology 19:1727–43.

van Dongen, S., and S. W. Gangestad. 2011. Human fluctuating asymmetry in relation to health and quality: a meta-analysis. Evolution and Human Behavior 32:380–398.

Van Valen, L. 1962. A study of fluctuating asymmetry. Evolution 16:125–142.

Vishalakshi, C., and B. N. Singh. 2008. Effect of mutations on developmental stability and canalization in morphological traits in *Drosophila ananassae*. The Journal of Heredity 99:539–45.

Zelditch, M. L., D. L. Swiderski, H. D. Sheets, and W. L. Fink. 2004. Geometric morphometrics for biologists: a primer. Elvesier, San Diego, California.

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APPENDIX I

List of specimens examined of *Myodes glareolus* from Ukraine. Vouchers are deposited at the Natural Science Research Laboratory, Museum of Texas Tech University. Specimens are organized by skull view and locality.

Dorsal view of the skull (females: 107; males: 90).—Chernobyl, Glyboke Lake (TTU86590, TTU86629, TTU86724, TTU86725, TTU86732, TTU86733, TTU86734, TTU86736, TTU86737, TTU86739, TTU86741, TTU86743, TTU86745, TTU86747, TTU86748, TTU86750, TTU86753, TTU86754, TTU86755, TTU86757, TTU86760, TTU86762, TTU86770, TTU86771, TTU86772, TTU87708). Chernobyl, Red Forest (TTU86685, TTU86687, TTU86688, TTU86699, TTU86700, TTU86701, TTU86702, TTU86706, TTU86708, TTU86714, TTU86716, TTU86932, TTU86966, TTU86967, TTU86968, TTU86970, TTU86973, TTU86977, TTU86979, TTU86980, TTU86981, TTU86983, TTU86988, TTU86989, TTU86990, TTU86991, TTU86993, TTU86998, TTU87000, TTU87001, TTU87013, TTU87018, TTU87019, TTU87024, TTU87025, TTU87207, TTU87211, TTU87212, TTU87213, TTU87215, TTU87218, TTU87227, TTU87232, TTU87245, TTU87247, TTU87248, TTU87255, TTU87257, TTU87259, TTU87263, TTU87264, TTU87288, TTU87292, TTU87314, TTU87321, TTU87322, TTU87329, TTU87330, TTU87331, TTU87339, TTU87367, TTU87425, TTU87437, TTU87662, TTU87666, TTU87676, TTU87677, TTU87680, TTU87899, TTU87908, TTU87917, TTU87922, TTU87929, TTU87993, TTU87994, TTU87995, TTU87996, TTU88000, TTU88002, TTU88003, TTU88009). Ivanov, Oranoe (TTU106089, TTU106091, TTU106094, TTU106098, TTU106101, TTU106102, TTU106108, TTU106109, TTU106111, TTU106113, TTU106115, TTU106117, TTU106118, TTU106119, TTU106120, TTU106121, TTU106128, TTU106130, TTU106132, TTU87187, TTU87491, TTU87505, TTU88008). Ivanov, The Shop (TTU106099, TTU86521, TTU86523, TTU87455). Kiev, Chista (TTU106144, TTU106146, TTU106158, TTU87959, TTU87960, TTU87968). Kiev, Nedanchichy (TTU106188, TTU106191, TTU106194, TTU106199, TTU106200, TTU106203, TTU106204, TTU106206, TTU106208, TTU106217, TTU106218, TTU106220, TTU106221, TTU106223, TTU106225, TTU106226, TTU106227, TTU106228, TTU106229, TTU106230, TTU106231, TTU87048, TTU87952, TTU87953, TTU87957, TTU87958, TTU87984). Kiev, Pine Wood (TTU87060, TTU87062, TTU87064, TTU87089, TTU87094, TTU87099, TTU87111, TTU87175, TTU87176, TTU87178, TTU87179, TTU87196, TTU87197, TTU87200, TTU87202, TTU87203, TTU87466, TTU87468, TTU87469, TTU87470, TTU87471, TTU87472, TTU87473, TTU87474, TTU87486, TTU87488, TTU87489, TTU87490, TTU87524, TTU87525).

Ventral view of the skull (females: 47; males: 43).—Chernobyl, Glyboke Lake (TTU86590, TTU86592, TTU86593, TTU86629, TTU86724, TTU86725, TTU86727, TTU86733, TTU86734, TTU86736, TTU86737, TTU86739, TTU86741, TTU86746, TTU86747, TTU86748, TTU86750, TTU86753, TTU86754, TTU86757, TTU86761, TTU86762, TTU86765, TTU86770, TTU86771, TTU86772, TTU86774, TTU86775, TTU86776). Chernobyl, Red Forest (TTU86685, TTU86687, TTU86688, TTU86696, TTU86698, TTU86699, TTU86700, TTU86701, TTU86702, TTU86707, TTU86708, TTU86714, TTU86716, TTU86721, TTU86932, TTU86966, TTU86967, TTU86968, TTU86970, TTU86973, TTU86977, TTU86979, TTU86981, TTU86988, TTU87013, TTU87018, TTU87024, TTU87025). Ivanov, Oranoe (TTU106094, TTU106113, TTU106116, TTU106118, TTU106119, TTU106120, TTU106130, TTU87187). Ivanov, The Shop (TTU86521, TTU86523). Kiev, Nedanchichy (TTU106204, TTU106206, TTU106208, TTU106217, TTU106218, TTU87047, TTU87048). Kiev, Pine Wood (TTU87060, TTU87062, TTU87064, TTU87083, TTU87094, TTU87099, TTU87111, TTU87116, TTU87175, TTU87176, TTU87178, TTU87179, TTU87180, TTU87182, TTU87196, TTU87197).

Lateral view of the mandible (females: 31; males: 20; undetermined: 1).—Chernobyl, Glyboke Lake (TTU86593, TTU86629, TTU86724, TTU86727, TTU86737, TTU86747, TTU86748, TTU86750, TTU86753, TTU86757). Chernobyl, Red Forest (TTU86685, TTU86687, TTU86688, TTU86700, TTU86708, TTU86714, TTU86716, TTU86932, TTU86948, TTU86966). Ivanov, Oranoe (TTU106088, TTU106091, TTU106113,

TTU106116, TTU106117, TTU106118, TTU87187, TTU87491, TTU87505). Ivanov, The Shop (TTU86521, TTU87455). Kiev, Chista (TTU87959, TTU87968). Kiev, Nedanchichy (TTU106190, TTU106191, TTU106197, TTU106203, TTU106204, TTU106206, TTU106212, TTU106214, TTU106222, TTU87048). Kiev, Pine Wood (TTU87060, TTU87062, TTU87064, TTU87086, TTU87094, TTU87099, TTU87178, TTU87180, TTU87182).

APPENDIX II

Description of landmarks digitized on the dorsal view of skull, ventral view of the skull, and lateral view of the mandible.

Dorsal view of skull:

1. Rostral tip of internasal suture
2. Intersection of nasal-frontal suture with the internasal suture
3. Intersection of frontal-parietal suture and the interparietal suture
4. Intersection of suture between left and right parietals, and parietal-interparietal suture
5. Midline point of caudal margin of the occipital

6, 7. Most rostral point of suture between nasal and premaxilla

8, 9. Rostral end of zygomatic plate

10, 11. Suture between frontal, parietal, and squamosal

12, 13. Tip of concavity of squamosal root of zygomatic arch

14, 15. Distal tip of lateral process of supraoccipital

16, 17. Suture between zygomatic process of maxillary and zygomatic process of squamosal

Ventral view of skull:

1. Anterior -most border of gnathic process

2, 22. Anterior-most point of incisive foramen

3, 24. Posterior-most point of incisive foramen

4. Point of convergence of ventral nasal concha

5, 25. Anterior extremity of the zygomatic plate

6, 23. Outer-most suture between the premaxilla and maxilla

7. Suture between maxilla and palatine along the midsagittal plane

8. Medial suture between the presphenoid and basisphenoid

9, 28. Outer-most suture between presphenoid and basisphenoid

10, 34. Anterior-most curvature of squamosal projection that creates the orbit

11, 29. Posterior extremity of foramen ovale

12. Midpoint of basisphenoid-basioccipital suture

13, 30. Point where the suture between the basisphenoid and basioccipital contacts the tympanic bulla

14. Anterior-most medial point of the foramen magnum

15, 31. Lateral tip of the occipital condyle

16, 33. Most-anterior external border of ectotympanic

17, 32. Intersection of basioccipital and bullae

18, 26. Intersection between the anterior end of the premolar and maxillary

19, 27. Intersection between the posterior end of the third molar and maxillary

20, 21. Lateral margin of incisive alveolus where it intersects

Lateral view of mandible:

1. Antero-dorsal border of the incisive alveolus
2. Extreme of the diastema invagination
3. Anterior edge of the molar tooth-row
4. Intersection of lower first molar and lower second molar
5. Posterior intersection of the molar tooth-row with the coronoid surface
6. Tip of the coronoid process
7. Maximum of curvature between coronoid and condylar processes
8. Anterior tip of the condyle
9. Posterior tip of the condyle
10. Maximum of curvature on the curve between the condylar and angular processes
11. Tip of the angular process
12. Ventral-most point on the ventral border of angular process
13. Dorsal-most point on the ventral border of the mandible
14. Intersection of posterior alveolar and angular regions
15. Antero-ventral border of the incisive alveolus

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Molar Microevolution in Late Quaternary *Ondatra zibethicus* (Arvicolinae, Rodentia)

Eileen Johnson, Patrick J. Lewis, and John A. Moretti

Abstract

The North American muskrat (*Ondatra zibethicus*) exhibits a chronocline of body size change from the late Pliocene through the early Holocene. On the Southern High Plains of Texas, dramatic changes in muskrat habitat from the latest Pleistocene through early Holocene (~11,100 ^{14}C yrs BP to ~8,500 ^{14}C yrs BP) are concurrent with changes in molar morphology. The present study expands upon earlier work with analysis of additional samples of lower first molars from Lubbock Lake and new samples from two other regional localities, that of Paul and Macy Locality 100. The total new specimens (n=55) represents an 85.5% increase from the sample used in that earlier work. Geographic and temporal variation in muskrat m1 morphology is examined further in these additional samples using ANOVA and Mann-Whitney U pairwise tests. Variation in body size (i.e., mass**)** also is examined using m1 lengths as a proxy. Results of this analysis support previous findings of decreasing m1 length to width ratios through time, indicating a change in m1 morphology. In contrast to other studies, no significant change in body size is observed. Southern High Plains muskrat samples are large, come from radiometrically well-constrained strata, and are correlated with extensive paleoenvironmental records. These attributes facilitate examination of the relationship between observed morphological changes and known paleoenvironmental changes during the late Quaternary. Change in m1 morphology is interpreted as a response to multiple, concurrent ecological pressures including declines in precipitation, changes in flora, and reduced habitat quality. These pressures culminate in the extirpation of muskrat from the Southern High Plains at ~8,500 ^{14}C yrs BP.

Key words: early Holocene, environmental change, late Pleistocene, morphological change, muskrats, *Ondatra zibethicus*, Southern High Plains

Introduction

The diagnosis of genera, species (Hollister 1911; Semken 1966; L. Martin 1979), and subspecies (Wilson 1933; Lawrence 1942; Lewis 1998) of fossil muskrats (*Ondatra zibethicus*) is based primarily on morphological variation in the lower first molar (m1). The m1 also has been used to study patterns of evolution (L. Martin 1979; R. Martin 1993, 1996, 2017), biogeographic variation (Semken 1966; Nelson and Semken 1970), and variation due to habitat (L. Martin 1979; Lewis 1998; Lewis et al. 2000; Mihlbachler et al. 2002; R. Martin et al. 2009; Mihlbachler 2012). The primary finding of these various investigations of muskrat from across North America is a relationship between changes in body size (i.e., mass as denoted by weight) and in m1 morphology and paleoenvironmental fluctuations (Nelson and Semken 1970; R. Martin 1996, 2017). Population replacements (Lewis et al. 2000; Lewis and Johnson 2002) and regional extirpations (Lewis et al. 2000; Mihlbachler et al. 2002) occurring in muskrat across the Pleistocene-Holocene boundary also are interpreted as being driven by contemporaneous climatic and environmental transitions.

Muskrat remains are widespread, relatively large for rodents, and readily identifiable. These variables, in combination with the demonstrated environmentally

associated size change and obligate aquatic ecology, make muskrats a valuable proxy for studying late Quaternary changes and microevolutionary patterns (Nelson and Semken 1970; L. Martin 1979; Lewis 1998; R. Martin 2017).

In general, muskrats experience increasing body mass through most of the Plio-Pleistocene, with significant episodic body mass increases occurring in middle to late Pleistocene (R. Martin 1996, 2017). The trend of body size increase culminates in particularly large (1.68 kg) late Pleistocene forms that are replaced rapidly by smaller forms in the latest Pleistocene into the early Holocene (R. Martin 1996, 2017; Lewis and Johnson 2002). This series of size changes lead Pleistocene muskrats toward the body mass of extant forms (Nelson and Semken 1970).

The physical and biogeographic changes observed in muskrats are emblematic of the influence of late Quaternary abiotic change on extant North American biota (Lundelius et al. 1983; Blois et al. 2010; Johnson 2017). Detailed examination of these changes offer insight into the relationship between pattern, process, and time. They also provide historical context for understanding how the physical characteristics and geographic distribution of the living biota arose.

Southern High Plains Muskrat Research

Muskrat remains are prevalent at Lubbock Lake and other Southern Plains localities (Fig. 1) in the late Pleistocene and early Holocene (Green 1961; Slaughter 1975; Johnson 1986, 1987c, 2009, 2010, 2017; Lewis and Johnson 2002; Moretti 2018). Remains are unknown from the Southern High Plains after ~8,500 ^{14}C yrs BP, and muskrat do not inhabit the region today (Johnson 1987b; Schmidly and Bradley 2016).

Based on a sample from Lubbock Lake, Lewis (1998) and Lewis and Johnson (2002) demonstrated a correlation between Southern High Plains muskrat m1 morphology and major paleoenvironmental shifts, specifically, increasing temperatures, reduction in regional effective precipitation, and the deterioration of the regional fluvial system. A concomitant dietary shift occurred based on a microwear study (Gutierrez et al. 1998; Lewis et al. 2000) of muskrat m1s from the Lubbock Lake sample.

The present study seeks to re-evaluate and expand upon the findings of Lewis and Johnson (2002) using an increased sample of muskrat m1s collected in the past 20 years from three Southern High Plains localities (Fig.1). To understand better the pattern of molar evolution and Southern High Plains muskrat populations during the transition from the late Pleistocene into the early Holocene, the molar morphology of five subfossil Lubbock Lake muskrat populations now have been examined. The samples represent discrete populations of muskrat because each is from a different, well-dated stratigraphic subunit from Lubbock Lake. These subfossil populations span from ~11,100 ^{14}C yrs BP to ~8,500 ^{14}C yrs BP (Table 1), and are associated with dramatic changes in the environment and climate (Johnson 1986, 1987a, 2017). In addition, samples from two other regional localities expand the database and regional perspective. The present study, therefore, tests whether new samples of muskrat exhibit morphological patterns that are consistent with, or diverge from, the existing model of correlated changes in m1 morphology and environments from Lubbock Lake (Lewis and Johnson 2002).

Setting and Stratigraphy

The Southern High Plains (or Llano Estacado) is a distinctive geographic region that spans northwestern Texas and eastern New Mexico (Fig. 1). Situated within the Great Plains province of North America (Fenneman 1931; Hunt 1967; Holliday et al. 2002), the region is a flat, expansive plateau that covers ~130,000 km^2. The current, now-dry river valleys, locally known as draws, are the headwaters for the Red, Brazos, and Colorado rivers that flow through Texas (Fig. 1). These draws were downcut after ~20,000 ^{14}C yrs BP (all radiocarbon ages are uncorrected and isotopic fractionation-corrected radiocarbon ages that are not calibrated; expressed as ^{14}C ages) at the end of the Last Glacial Maximum (Holliday 1995) and form the modern regional drainage system. Aggrading and infilling of the valleys started ~12,000 ^{14}C yrs BP and continues today (Holliday 1995). Springs fed the regional drainage system and provided free-flowing and ponded waters through time (Brune 1981; Holliday 1995), some of which still are active today.

Yellowhouse Draw is the southernmost of three major draws that form the central drainage basin for

Table 1. Locality, age, and associated data for Southern High Plains muskrat (*Ondatra zibethicus*) samples.

Locality Name	Substratum	# of Specimens	Period	Age (^{14}C yrs BP)	Paleoenvironment	References
Lubbock Lake	upper 2B	8	early Holocene	~9,500 - 8,500	aggrading marsh	Holliday 1995; Johnson 1987a
	2B cienega	18	early Holocene	~10,000 - 9,500	ponds	Holliday 1995; Holliday et al. 1983, 1985; Johnson 1987a
	2s	38	early Holocene	~10,250 - 9,780	nearshore interface of 2A & lower 2B	Holliday et al. 1985; Johnson 1987a; Knudson et al. 1998
	2A	21	early Holocene	~10,880 - 10,000	ponds	Holliday 1995; Holliday et al. 1983; Johnson 1987a
	1B	8	latest Pleistocene	~11,100	meandering stream	Holliday 1995; Johnson 1987a
Paul	lower 2B	2	early Holocene	~9,960 – 9,025	aggrading marsh	Johnson 2010
	2A	3	early Holocene	~10,700 - 9,900	ponds	Johnson 2010
Macy Locality 100	fluvio-lacustrine 1	2	latest Pleistocene	~11,300 - 11,000	pond, flowing marsh	Moretti 2018
	basal gravel	2	latest Pleistocene	~11,550 - 11,300	stream	Moretti 2018

the Southern High Plains and the upper Brazos River basin (Fig. 1). The now ephemeral Yellowhouse Creek flows through the draw. Downstream, Yellowhouse Canyon begins at the confluence of Yellowhouse Draw and Blackwater Draw. At that point, the stream becomes the North Fork of the Double Mountain Fork of the Brazos River and flows out onto the westernmost Rolling Plains (i.e., Central Lowlands; Holliday et al. 2002). Lubbock Lake is located within Yellowhouse Draw (above the confluence) along the eastern edge of the Southern High Plains (Fig. 1).

Lubbock Lake has an extensive late Quaternary cultural and natural history record covering the last ~12,000 radiocarbon years (Johnson 1987a). The paleoenvironment for this time period is well-documented and the muskrat-bearing substrata are dated with multiple radiocarbon ages (Table 1; Holliday et al. 1983, 1985; Johnson 1987a; Knudson et al. 1998).

Extensive geologic, stratigraphic, and radiometric dating have been undertaken at Lubbock Lake, Paul, and Macy Locality 100 that place the muskrat remains within a geochronologic framework. While direct bone dates on muskrat remains are not available, charcoal and organic sediment dates provide a robust, constrained age framework in which the remains are found (Table 1). More than 100 radiocarbon ages date the muskrat-bearing strata at these localities and regionally and provide the age ranges used in Table 1. These dates have been published (Table 1) and details are not repeated here. The focus is the geochronologic framework rather than an evaluation of specific dates. Another significant aspect of the framework is that it is regional and not confined to a specific locality (Holliday 1995). Depositional units and designations used occur across the region so that, for example, 2A at Lubbock Lake and at Paul are the same unit.

Muskrats inhabited Lubbock Lake during deposition of strata 1 and 2. These lower strata record extensive sedimentological, faunal, floral, environmental, and climatic changes (Holliday et al. 1983, 1985; Holliday 1985; Holliday and Allen 1987; Johnson 1987a, 2007). These changes also occur on a regional scale (Johnson 1986, 2017; Holliday 1995; Johnson and Holliday 2004; Moretti et al. 2013; Moretti and Johnson 2015). The current Lubbock Lake muskrat sample is composed of 93 m1s from five substrata (Table 1).

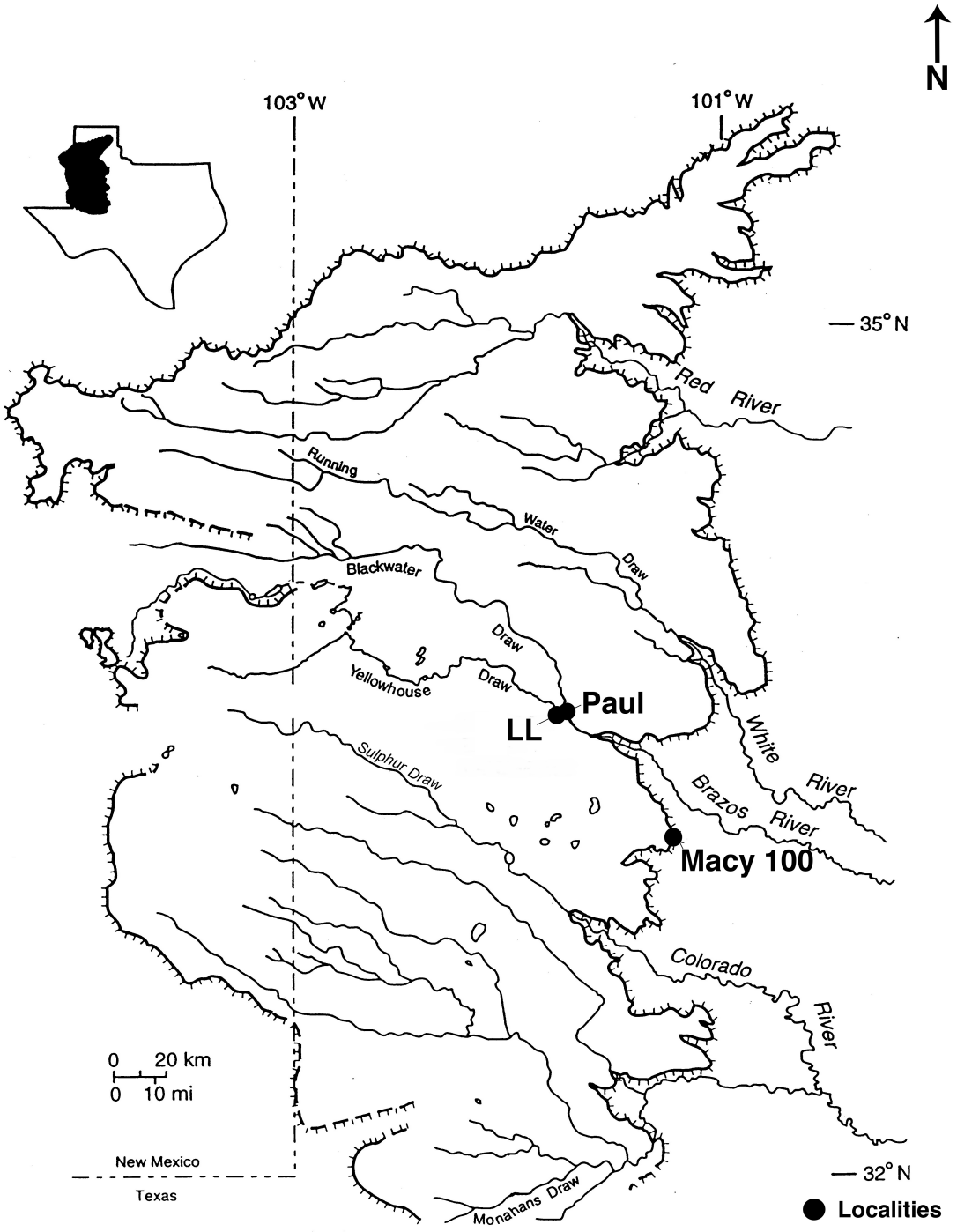

Figure 1. Southern High Plains with the location of the localities involved in the subfossil muskrat (*Ondatra zibethicus*) m1 analyses (LL = Lubbock Lake).

Stratum 1 (i.e., latest Pleistocene), composed of bedded sand and gravel, was deposited in most draws, indicative of competent streams flowing in the drainages (Holliday 1995). At Lubbock Lake, this stratum represented a meandering stream deposit with point bar sediments (1A), cross-bedded sands (1B), and clay drape/overbank deposits (1C) (Holliday and Allen 1987). Based on proxy data, an equable, humid, maritime paleoclimate existed across the Southern High Plains with a lower mean annual temperature than today, and cooler summers and warmer winters that lacked extended freezing conditions (Johnson 1987b, 1991, 2007). A parkland (grassland interrupted by small stands of trees) existed along the draw at Lubbock Lake. A low gradient stream, with emergent vegetation and sedge beds along the banks and margins, meandered through the valley (Johnson 1986, 1987b, 2007). Hackberry, willow, and American elm grew in the riparian draw (Johnson 1987b, 2007, unpublished data). Geologic evidence indicates an abrupt hydrologic change from flowing to standing water in the draws around 11,000 ^{14}C yrs BP, altering the course of the stream to a ponded environment and marking a major environmental shift.

Lacustrine deposition of stratum 2 (early Holocene), in the form of diatomite and sapropelic mud, began conformably about 11,000 ^{14}C yrs BP in some reaches in a number of draws. At Lubbock Lake, substratum 2A consisted of beds of pure diatomite and interbedded peaty muds (local beds). Regionally, substratum 2A deposition was associated with springs (Holliday 1995). Water level in these ponds fluctuated in response to short-term droughts, from centimeters to meters deep and periodically at or below the surface, exposing the floor of the draw (Holliday 2000). Wet meadow grasses and sedge beds around the ponds graded into a better-drained mixed-grassland along the valley floor. Occasional hackberry trees grew in the draw (Johnson 1987b, 2007).

Deposition of substratum 2B, a homogeneous sapropelic mud, began around 10,000 ^{14}C yrs BP and continued until about 8,500 ^{14}C yrs BP after which a soil developed in upper 2B. That soil marked a stable land surface with little deposition or erosion (Holliday and Allen 1987). Substratum 2B represented a slowly aggrading boggy waterway within the draw. A mixed-grassland dominated the draw, with occasional hackberry, sumac, and American elm interspersed (Johnson 2007; unpublished data).

Substratum 2s is a sandy, near-shore facies of stratum 2 that was deposited along the valley margin for most of the timespan of stratum 2 (Table 1). At Lubbock Lake, 2s consists of various lenses. Those of carbonate and quartz sand-size particles have washed off the valley wall (slope wash). The lenses of clayey, organic rich lacustrine sediments represent transgressions of the stratum 2 ponds and marshes across the valley floor. Substratum 2s is subdivided into three local beds (LB). Lowermost 2sLBa and uppermost 2sLBc consists of marsh sediments. In between, 2sLBb has a much greater slope wash content but has thin, organic-rich lenses within it (Holliday and Allen 1987; Knudson et al. 1998). Substratum 2s, then, represents the horizontal fluctuation of the ponds and marshes of stratum 2.

The ponds of 2A and basal 2B (i.e., 2B cienega) changed into extensive, shallow, freshwater, wet meadows-marshlands with emergent vegetation and sedge beds. This slowly aggrading freshwater bog had little to no standing water (Holliday 1985, 1995; Johnson 1986; Johnson and Holliday 2004). Continued periodic droughts and disappearing surface-water resources denoted the trend toward more temperate climatic conditions (Johnson 2017). Sand sheets formed, mainly on the western half of the Southern High Plains, indicating a regional reduction in vegetative cover (Holliday 1997; Johnson and Holliday 2004). Effective precipitation decreased and maximum summer temperatures rose.

After 8,500 ^{14}C yrs BP, what remained of the freshwater marshland turned brackish and, regionally, alkaline marshes began to dominate the floors of the draws. These hydrologic changes resulted both from warming of water and from reduction in effective precipitation that decreased the discharge of springs and seeps (Holliday 1995). The muskrat that had inhabited the freshwater ponds and marshes disappeared.

The Paul locality, located ~5.2km downstream from Lubbock Lake in Yellowhouse Draw (Fig. 1), contains a stratified record of late Quaternary valley fill (Johnson 2010). The record of sediments, fauna, and flora reflects the patterns observed upstream at Lubbock Lake, as well as elsewhere in the region

(Johnson 2010, 2017). The Paul muskrat samples come from early Holocene substrata 2A and lower 2B (Table 1; Johnson 2010). The Paul stratum 2 samples come from environmental settings similar to Lubbock Lake stratum 2.

Macy Locality 100 is located on the eastern edge of the Southern High Plains within the Macy Fork of Spring Creek, an ephemeral tributary to the South Fork of the Double Mountain Fork of the Brazos River (Fig. 1). While in the same upper Brazos River basin, the North Fork and the South Fork of the Double Mountain Fork join 101.5 km downstream off the Southern High Plains in the westernmost Rolling Plains. Muskrat remains come from two units of latest Pleistocene alluvium: the basal gravel and fluvio-lacustrine 1 (Table 1; Moretti 2018). These two units are temporally and geologically equivalent to stratum 1 at Lubbock Lake and regionally. The basal gravel unit is composed of gravel zones with discontinuous layers of sands and muds. The unit represents a strongly flowing stream with a more incised, higher gradient channel than the stratum 1 stream in Yellowhouse Draw. Fluvio-lacustrine 1 is composed of light to dark gray silty and sandy muds. These muds reflect a decrease in water flow, with pond and flowing marsh settings (Johnson et al. 2018). Sedge beds grow along the banks with the occasional hackberry and willow trees (Johnson unpublished data). The depositional environments represent a variation of the regional pattern, with the shift to slow aggrading marsh habitats occurring within the latest Pleistocene, earlier than elsewhere in the region (Johnson et al. 2018).

MATERIALS AND METHODS

Excavations at Lubbock Lake and Macy Locality 100 took place within a meter grid system, hand-troweling in 2.5 cm levels within the least defined substratigraphic unit. Sediments were not mixed between these substratigraphic units, boundaries are clearly defined, and burrowing that may cause mixing of sediments is not an issue. Geologic trenching in stratum 2 at the Paul locality was undertaken within 10 cm increments within the defined subunits. All excavated sediments from these localities had their provenience information recorded and were water-processed through nested fine-mesh screen to recover any microfaunal remains not found in-situ.

The Lubbock Lake muskrat collection contains over 1,000 cataloged elements including 172 m1s. This subfossil muskrat collection is the largest on the Southern Plains (Dalquest and Schultz 1992; Lewis 1998) and may be the largest fossil or subfossil muskrat collection excavated from a single locality (R. Martin 2017). All Lubbock Lake late Pleistocene m1s used in this study come from substratum 1B (Table 1). The Lubbock Lake sample combined with nine m1s from Paul and Macy Locality 100 produces a total sample size of 181 m1s.

For this analysis, m1s have been selected based on parameters of completeness and wear category. Samples having broken triangles or loops, or other significant damage, are omitted from all analyses. All complete molars have been placed in appropriate wear categories (initial, intermediate, and advanced). Only those in the intermediate wear stage have been used in the analysis to minimize the effects of wear on patterns of variation. Juvenile teeth then are excluded from analysis, as are heavily worn m1s. The results of this culling provide the present study sample that consists of 102 specimens drawn from 93 Lubbock Lake specimens, four Macy Locality 100 specimens, and five Paul specimens (Table 1; Fig. 1). Of the 93 Lubbock Lake specimens, 46 are new (not represented in previous study of Lewis 1998, Lewis and Johnson 2002), doubling the Lubbock Lake sample. Those from Macy Locality 100 (n=4) and Paul (n=5) also are new. The total new specimens (n=55) represents an 85.5% increase from the sample used in the previous study.

The modern muskrat (*Ondatra zibethicus*) m1 is comprised of an anterior loop, a posterior loop, and seven triangles distributed along the lingual and buccal surfaces of the molar (Fig. 2). Re-entrant angles separate the triangles and accumulate cement following eruption. Dentine tracks are present on the buccal and lingual sides of the molar (Galbreath 1954). Modern molars are hypsodont and show no sexual dimorphism (Lewis et al. 2002). Small m1 length-to-width ratios

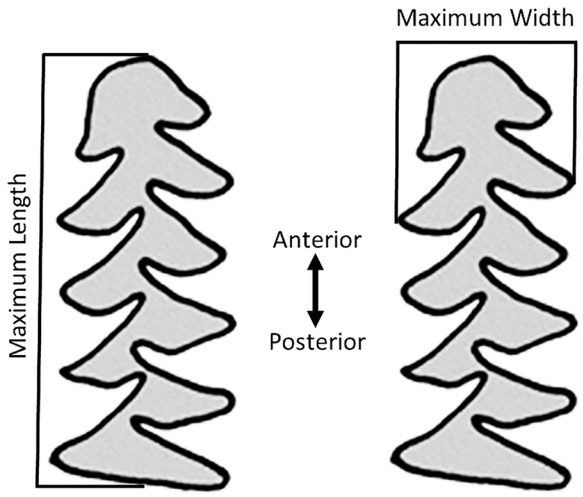

Figure 2. Idealized occlusal view of muskrat (*Ondatra zibethicus*) m1 with illustration of length and width measurements.

are associated with muskrats from warm climates while large ratios are associated with colder climates (Semken 1966; Nelson and Semken 1970).

Molars were selected randomly (i.e., not knowing locality or side) for measuring purposes. The measurements of length and width on the various populations were taken using digital calipers (Lewis 1998) in accordance with methods established by Semken (1966; Nelson and Semken 1970). Measurements were taken at the girth of the molar, with the most extreme points used for both the length and width measurements (Fig. 2).

Univariate and bivariate methods tested for differences between the Lubbock Lake populations and allowed for comparisons with the Paul and Macy Locality 100 samples. In order to test for variation between localities and substrata, m1 length/width (L/W) ratios from individual samples were tested with a one-way ANOVA and a Mann-Whitney U pairwise test. A one-way ANOVA also was used to examine variation in molar length as a proxy for body size (R. Martin 1996, 2017). Commonly used as a proxy for body size (Alroy 1998, 1999, 2000), variation in the length of m1 in muskrats was considered to be indicative of differences in their overall body size. Scattergrams and a bivariate plot were employed to detect trends across the various samples. All statistics were performed with the software PAST v3.21 (Hammer et al. 2001).

RESULTS

During analysis, a m1 in a mandible from substratum 2A at the Paul locality consistently emerges as an outlier, exhibiting a very large length/width ratio of 2.79 mm. This specimen (TTU-A1-123978), however, is a relatively small adult individual with lightly worn molars. The m1 is relatively long and wide in relation to the other Paul specimens and most of the specimens in the analysis. It appears that width may be why the ratio appears anomalous. This outlying specimen masks otherwise evident and significant trends and, as a result, was removed from the current study sample.

Plotting length/width ratios for all specimens arranged by locality/deposit reveals a general trend of decreasing ratio through time, from the latest Pleistocene to early Holocene (Fig. 3). The late Pleistocene mean ratios for Lubbock Lake substratum 1B are 2.45 and 2.43 for Macy Locality 100. The early Holocene mean ratios for Lubbock Lake substrata are: 2A=2.37;

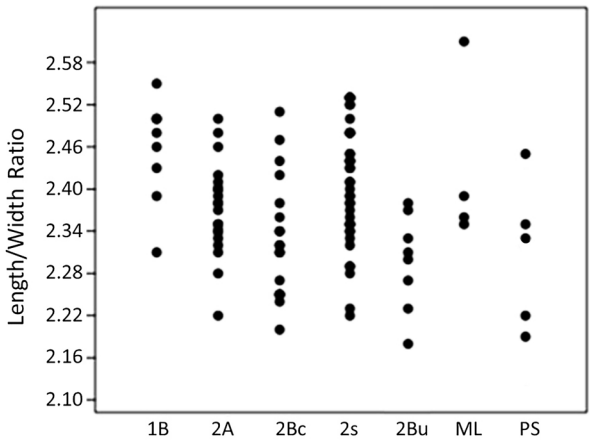

Figure 3. Scattergram of muskrat (*Ondatra zibethicus*) m1 length/width ratios. Abbreviations refer to localities and substratum: 1B = Lubbock Lake 1B; 2A= Lubbock Lake 2A; 2Bc = Lubbock Lake 2B cienega; 2Bu = Lubbock Lake upper 2B; ML = Macy Locality 100; and PS = Paul.

2s=2.40; 2B cienega (2Bc)=2.33; upper 2B (2Bu)=2.30. The early Holocene mean ratios for Paul are 2.33. A one-way ANOVA indicates significant differences between group means (Table 2). Latest Pleistocene specimens from Lubbock Lake (substratum 1B) exhibit the largest L/W ratio. Macy Locality 100 specimens, also latest Pleistocene in age, have ratios similar to substratum 1B specimens as would be expected based on the comparable time period. The sample size of Macy Locality 100, however, is small (*n*=4) and may not be an accurate reflection of that population. The length and width ratios of the subfossil upper 2B molars were among the smallest molars studied (Fig. 3).

Table 2. ANOVA results for muskrat (*Ondatra zibethicus*) m1 length/width ratios.

	Sum of sqrs	df	Mean square	F	p (same)
Between groups	0.179	6	0.030	4.515	> 0.001
Within groups	0.620	94	0.007		
Total	0.798	100			

In a Mann-Whitney U pairwise test, several significant results are found (Table 3). The substratum 1B sample is different than 2B cienega (2Bc) and upper 2B (2Bu). The latest Pleistocene substratum 1B specimens again are distinct, with a larger mean ratio (2.45) than any other population. This situation is seen in the significant results involving 1B and the populations with the smallest mean ratios (i.e., 2A, 2B cienega, and 2B upper). Lubbock Lake populations 2B cienega and upper 2B both have significantly different ratios compared to the 1B and 2s populations. The Lubbock Lake 2A population ratio is intermediate (2.37) and significantly different from the 1B population ratio (2.45) and the upper 2B population (2.30). Although the Paul specimens are contemporaneous with the early Holocene Lubbock Lake populations, the Paul ratio (2.33) is similar to Lubbock Lake populations 2B cienega (2.33) and upper 2B (2.30). Paul specimens are not significantly different from any other early Holocene population, likely due to the small sample size (*n*=4). While Macy Locality 100 (2.43) is similar to Lubbock Lake 1B (2.45) and 2s (2.40), it is not significantly different from any other group, most likely because of the small sample size (*n*=4).

The 2s local beds are sequential (2sLBa being the oldest) and differ in age within the overall ~1,100 radiocarbon years age range provided in Table 1. The large 2s sample (*n*=38) dominates the new specimens from Lubbock Lake that were added into this current analysis. The 2s specimens have been examined by local beds (Fig. 4) to see if significant variation may have occurred in m1 length/width ratios over the 2s time span. Specimens from local bed b (2sLBb; *n*=12; ~9950 ^{14}C yrs BP) skew more toward the 1B population

Table 3. Mann-Whitney U pairwise test of muskrat (*Ondatra zibethicus*) m1 length/width ratios with significant results italicized. Sample abbreviations: 1B = Lubbock Lake 1B; 2A = Lubbock Lake 2A; 2Bc = Lubbock Lake 2B cienega; 2s = Lubbock Lake 2s; 2Bu = Lubbock Lake upper 2B; ML = Macy Locality 100; and PS = Paul.

	1B	2A	2Bc	2s	2Bu	ML	PS
1B		*0.018*	*0.007*	0.147	*0.003*	0.495	0.074
2A	*0.018*		0.071	0.130	*0.011*	0.552	0.435
2Bc	*0.007*	0.071		*0.005*	0.388	0.088	0.831
2Bu	*0.003*	*0.011*	0.388	*0.003*		0.051	0.496
2s	0.147	0.130	*0.005*		*0.003*	0.949	0.183
ML	0.495	0.552	0.088	0.949	0.051		0.245
PS	0.074	0.435	0.831	0.183	0.496	0.245	

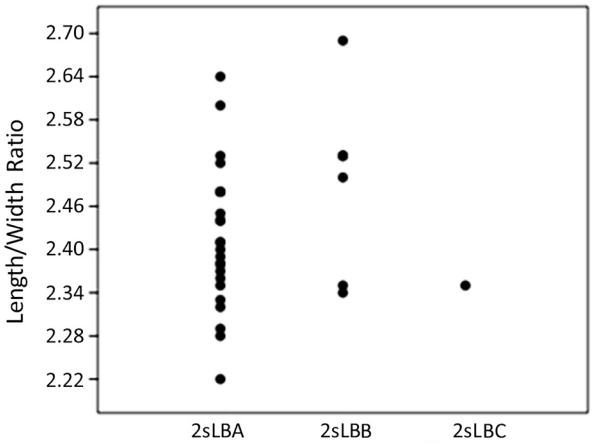

Figure 4. Scattergram of muskrat (*Ondatra zibethicus*) m1 length/width ratios for Lubbock Lake substratum 2s specimens. Abbreviations refer to relevant 2s substrata: 2sLBA = 2s local bed a; 2sLBB = 2s local bed b; and 2s local bed c = 2sLBC.

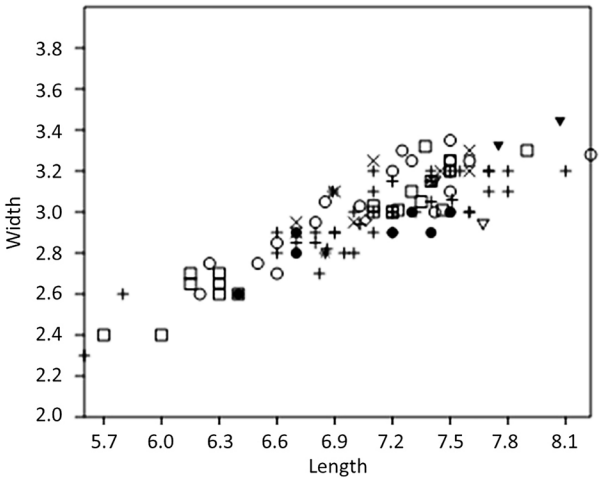

Figure 5. Bivariate plot of muskrat (*Ondatra zibethicus*) m1 length and width (measurements in mm). Symbols as follows: Lubbock Lake 1B = dot; 2A = square; 2Bc = circle; 2s = +; 2Bu = x; Macy Locality 100 = diamond; and Paul = *.

than those from local bed a (2sLBa; n=25; ~10,250 to 9950 ^{14}C yrs BP). Because it is not a significant difference (p = 0.08), the 2s specimens have been treated as a single population.

The larger samples sizes for the Lubbock Lake populations have reinforced earlier interpretations (Lewis 1998; Lewis and Johnson 2002), with 1B still significantly different in mean L/W ratio than the younger early Holocene populations of 2A, 2B cienega, and upper 2B. The large sample from Lubbock Lake substratum 2s (n=38; 2.40) appears heavily influenced by the 2sLBb specimens and is not similar to its 2A (2.37) and 2B cienega (2.33) facies populations.

In a bivariate analysis, the relationship between length and width (Fig. 5) is consistent between all groups when viewed geographically, through time, and habitat. The R^2 is 0.74, indicating a strong correlation between length and width through the combined sample of molars.

The m1 lengths have been examined by locality and stratigraphy (Fig. 6). The single longest m1 belongs to the Lubbock Lake 2B cienega population while the shortest m1 comes from the Lubbock Lake 2s population. Although considerable overlap exists in the data sets, in examining the mean, Macy Locality 100 and Paul specimens both have the longest average

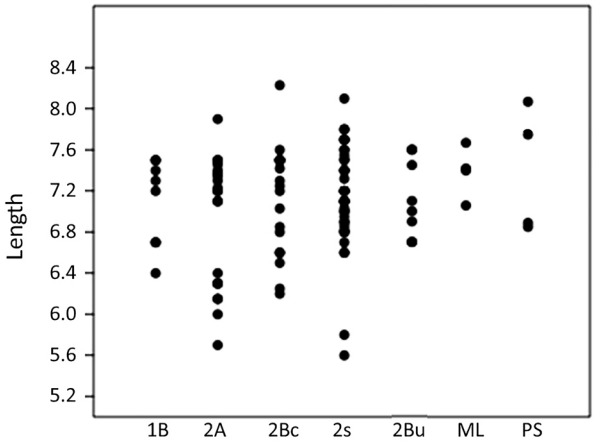

Figure 6. Scattergram showing muskrat (*Ondatra zibethicus*) m1 length (measurements in mm). Abbreviations refer to localities and substratum: 1B = Lubbock Lake 1B; 2A= Lubbock Lake 2A; 2Bc = Lubbock Lake 2B cienega; 2Bu = Lubbock Lake upper 2B; 2Bu = 2B upper; ML = Macy Locality 100; and PS = Paul.

m1s at 7.39 mm. Using m1 length as a measure of overall body size, Macy Locality 100 and Paul muskrat are the largest, although the sample sizes are too small (n=4 for each locality) to be confident that this result truly represents the populations. The Lubbock Lake populations exhibit a general trend that nonetheless is not unilineal. Substratum 1B samples have a mean m1

length of 7.10 mm. Substratum 2A samples have the smallest mean m1 length at 6.90 mm. Samples from 2B cienega have a mean m1 length of 7.10 mm while the mean m1 length for those from 2s is 7.15 mm and 7.13 mm for upper 2B. An ANOVA performed on m1 length finds no significant variation (p=0.48). Likewise, a Mann-Whitney U test has found no population significantly different from any other. Although fluctuations occur with the average m1 lengths by substratigraphic unit, it appears that body size remains fairly constant rather than a trend towards decreasing size.

DISCUSSION

The microstratigraphic record of lower stratum 2 coupled with radiocarbon dating allow for a view of muskrat paleodemographics on the Southern High Plains. A gradual and directional change in m1 morphology is indicated by the univariate and bivariate analyses (Tables 2, 3; Figs. 3–6). Overall, the m1 length/width ratio is decreasing from the larger ratios of late Pleistocene samples to the increasingly smaller ratios of early Holocene populations.

Sample size is low from Paul and Macy Locality 100 and may be biasing the results. Nevertheless, the Lubbock Lake sample size is large and provides a strong anchor point for comparisons. Contemporary latest Pleistocene populations from Lubbock Lake 1B and Macy Locality 100 are similar in m1 ratio.

The length/width ratio of the Lubbock Lake 2s population as a whole (2.40) is not similar to its 2A (2.37) and 2B cienega (2.33) facies populations. The 2s local bed b subpopulation (dated ~9,950 ^{14}C yrs BP; Knudson et al. 1998) is more like the latest Pleistocene 1B population (dated ~11,100 ^{14}C yrs BP). The 2s local bed b subpopulation, then, tends to be larger than its Lubbock Lake cohorts. On the other hand (and keeping in mind the low sample size), 2A and lower 2B populations at the Paul locality (Table 1), ~5.2km downstream from Lubbock Lake, are smaller (2.33) than the contemporaneous 2A population (2.37) at Lubbock Lake.

Although Boyce (1978:4) observed that muskrats exhibited slight sexual dimorphism, sexual dimorphism statistically is absent in modern muskrats (Lewis et al. 2002). Sexual dimorphism, then, is not considered to be a factor in the length/width ratio differences among contemporaneous early Holocene populations. The trend towards smaller ratios indicates a gradual change in m1 morphology in a regional resident population.

Muskrats are phenotypically plastic and exhibit a chronocline through the Plio-Pleistocene, with the rapid decrease in size from late Pleistocene to the early Holocene as one aspect (R. Martin 1996; Mihlbachler et al. 2002). Size reduction and chronoclines are seen in other late Quaternary larger vertebrates, such as bison (Lewis et al. 2010), responding to vegetational changes brought about by climatic changes. While some variation in body size (as indicated by the proxy of m1 length; Alroy 1998, 1999, 2000) is evident within the current samples, no significant change in body size is detected. Rather than a total population replacement, the detailed Southern High Plains record illuminates a transformation in m1 morphology from one chronomorph to another within the chronocline of the evolving resident regional population.

A gap in muskrat remains occurs between the disappearance of the 1B stream and the initial 2A ponding event (2A local bed 1/2ALB1). To date, this local bed has produced no muskrat skeletal material although remains from fish and water birds were recovered (Johnson 1987c). This gap is the only period with no remains in an otherwise continuous sequence of muskrat fossils (Johnson 1987b). This situation suggests a potential regional absence of muskrat (Lewis and Johnson 2002) for a short period (~300 radiocarbon years) during the Pleistocene-Holocene transition (i.e., post-11,100 ^{14}C yrs BP and pre-10,800 ^{14}C yrs BP).

Although muskrats may have retreated downstream, sample bias more likely is a factor due to limited excavation (40 m^2) within 2ALB1 deposits. Persistence of other aquatic forms through this time period indicates that suitable habitat remained. The 2ALB1 deposit represents deep ponds (Holliday 2000) and should have been a preferred habitat for muskrats during this period of environmental change. Despite the deterioration of aquatic settings during the early

Holocene, appropriate muskrat habitats persist regionally for another two millennia (Johnson 1987b). Only the complete loss of appropriate aquatic habitats in the regional draws ~8,500 ^{14}C yrs BP causes extirpation of muskrat from the Southern High Plains. Muskrats do not occur in the Brazos River drainage system today (Schmidly and Bradley 2016). They occur further north of the Southern High Plains (Hall and Kelson 1959) and historically occurred in the Canadian River drainage of the Texas Panhandle (Schmidly and Bradley 2016). Such a geographic pattern of extirpation would appear to eliminate the potential for dispersal back into the drainages of the Southern High Plains except through overland routes.

In addition to molar morphology, habitat served to separate the 1B and stratum 2 populations. A dramatic habitat shift occurred between the deposition of substrata 1B and 2A, with the stream of 1B replaced by a series of ponds (Holliday 1985, 1997; Holliday and Allen 1987). A change in flora and fauna accompanied this shift from stream to pond, with many species disappearing (Johnson 1987a, 2007, 2017). This alteration in flora affected muskrat diet, as documented by differential dental microwear patterns. The 1B molars exhibited fine-pitting, while the upper 2B molars have enamel patterns dominated by striations (Gutierrez et al. 1998; Lewis et al. 2000). This change in pattern was indicative of a switch from harder food items (i.e., bark, twigs, or vertebrates) to softer items (emergent and submergent plants) (Teaford 1991).

A recent *Microtus* m1 microwear study under laboratory conditions (Zykov et al. 2018) corroborates this interpretation. While the vole species are different, *Microtus* is abundant in the 1B and stratum 2 deposits. A similar microwear analysis with *Microtus* would help determine if these voles responded in the same manner as muskrat.

Climatic and environmental changes have been posited as the driving forces in the overall trend to smaller body size and decreasing m1 length/width ratios, including that of the Southern High Plains muskrat (Nelson and Semken 1970; R. Martin 1996, 2017; Lewis et al. 2000; Lewis and Johnson 2002; Mihlbachler et al. 2002). The detailed record at Lubbock Lake provides an opportunity to outline what some of those climatic and environmental factors may have been for the Southern High Plains populations. Body size can vary with temperature and seasonality (climatic factors) as well as drought, water-level fluctuations, intraspecific strife, and quality and quantity of food (environmental factors) (Friend et al. 1964; Boyce 1978; Willner et al. 1980; Proulx and Buckland 1986; Virgil and Messier 1996; Mihlbachler 2012). The temperature regime appears the key factor in changes in m1 length/width ratios for both fossil (Nelson and Semken 1970) and modern muskrats (Lewis 1998). The muskrat's phenotypic flexibility allows them to respond to short-term localized environmental events, such as those observed in the early Holocene record from the Southern High Plains. Muskrat populations appear to rebound under good years.

In modern muskrats, body size is correlated with rainfall patterns. The largest forms occur in areas of high annual precipitation and low seasonality coupled with rainfall predictability (Boyce 1978:9). In general, these are the climatic conditions reconstructed for the Southern High Plains during the late Pleistocene (Johnson 1987b, 1991). Decreasing body size also is a response in muskrat to other environmental stressors such as low food availability, seasonality, and drought (Errington 1963; Boyce 1978). During the early Holocene on the Southern High Plains, climatic conditions shift towards greater seasonality with higher summer temperatures and lower winter temperatures. Rainfall patterns shift with decreasing annual precipitation. The early Holocene warming trend brings increasing aridity, decreasing rainfall predictability, and droughts (Johnson 1987b, 2017; Holliday 2000). Although not statistically significant, the decrease in m1 length for the Lubbock Lake 2A population may reflect the drought, decreased rainfall, and increased temperature conditions at that time.

Modern muskrats appear highly sensitive to droughts and fluctuating water levels, with one effect being a reduction in body size (Errington 1939; Bellrose and Brown 1941; Bellrose and Low 1943). Droughts affect the water levels in the regional 2A ponds and 2s recorded the horizontal fluctuations of ponds and marshes across the valley floor (Holliday 1995, 2000). Droughts affect muskrat resources (both for food and construction materials) (Errington 1939; Bellrose and Brown 1941; Bellrose and Low 1943; Boyce 1978). Modern muskrats show a preference for a stable water

depth and certain emergent vegetation but are more affected by fluctuating water levels than the types of marsh vegetation present (Bellrose and Brown 1941).

Modern muskrats are reluctant to leave during drought and will switch to less preferred food once higher priority foods have been depleted. Die-off of cattail in particular occurs as the water level drops below 15 cm (Bellrose and Brown 1941). Small body size during extended drought periods enhances survival under low food resource availability by allowing them to attain required nutritional resources (Boyce 1978). Reducing body size is a muskrat response to diminishing quality and quantity of emergent vegetation that are the muskrat's food resources. It also is a response in minimizing intraspecific competition in food stressed areas (Boyce 1978).

Muskrats build feeding houses and larger residential lodges for protection (Errington 1939, 1963; Bellrose and Brown 1941; Bellrose and Low 1943; Bellrose 1950). When water levels fall to the extent that the bases of their houses are exposed or houses are in shallow water, muskrats are more exposed when foraging for food. Exposed muskrats are the most vulnerable and suffer higher mortality rates than animals less exposed. They become easy prey and subject to predation by a number of mammals and birds (Errington 1939; Bellrose and Low 1943), including the marsh hawk (*Circus cyaneus*) known from Lubbock Lake 2A deposits (Johnson 1987c). Mortality rates increase for exposed individuals during droughts and are exacerbated during winter months when forced to leave their lodges to forage. Further, fewer young are born in the fall during summer low water/drought periods, affecting population size and density (Bellrose and Brown 1943; Errington 1963). As conditions worsen, intraspecific strife increases and the highest level is among muskrats most exposed. Intraspecific strife affects body size and such strife increases the mortality rate (Errington 1939; Bellrose and Low 1943).

All of these environmental factors may have combined during Lubbock Lake 2A times to affect the slight decrease seen in muskrat body size. But, muskrat body size rebounds post-2A that would appear to indicate improved environmental conditions despite the increasing temperature. The average m1 lengths through the strata 1 and 2 record are not statistically different and the fairly constant body size does not fit the general trend seen elsewhere in North America (R. Martin 1996, 2017; Milbachler et al. 2002; R. Martin et al. 2009; Milbachler 2012). Why the Southern High Plains muskrat does not reflect the change in body size pattern is not yet understood and is a matter for further research. Southern High Plains muskrat length/width ratios, however, show a decreasing trend through the strata 1 and 2 record that fits the Nelson and Semken (1970) model. Muskrat m1 morphology changes through time that according to the model reflect changes in temperature regimes. The Southern High Plains trend is in concert with rising regional temperatures from the late Pleistocene through the early Holocene (Johnson 1987b; Holliday 1995, 2000).

CONCLUDING REMARKS

Lubbock Lake excavations have produced one of the largest collections of subfossil muskrat molars known and that assemblage has permitted statistical analyses. The detailed paleoenvironmental reconstruction and well-dated stratigraphy further allows evolutionary changes to be viewed in chronological and environmental contexts at Lubbock Lake as well as within a regional perspective. Southern High Plains muskrat populations persist through a sequence of climatic, environmental, and floral changes during the latest Pleistocene to early Holocene. This persistence likely is facilitated by the great phenotypic plasticity of the species. A decreasing m1 length/width ratio reflecting changes in m1 morphology represents one aspect of the regional adaptive response of the muskrat to changes during the Pleistocene-Holocene transition. These changes include water level fluctuations, increasing aridity, decreasing rainfall, and related floral community alterations. These disruptions are known to drive morphological and behavioral changes in extant muskrat populations. The paleoenvironmental and geochronological context of the Southern High Plains record illustrates a link between ecological variables and m1 morphological changes of muskrat populations during the early Holocene.

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LITERATURE CITED

Alroy, J. 1998. Cope's rule and the dynamics of body mass evolution in North American fossil mammals. Science 280:731–734.

Alroy, J. 1999. The fossil record of North American mammals: evidence for a Paleocene evolutionary radiation. Systematic Biology 48:107–118.

Alroy, J. 2000. New methods for quantifying macroevolutionary patterns and processes. Paleobiology 26:707–733.

Bellrose, F. C. 1950. The relationship of muskrat populations to various marsh and aquatic plants. Journal of Wildlife Management 14:299–315.

Bellrose, F. C., and L. G. Brown. 1941. The effect of fluctuating water levels on the muskrat population of the Illinois River Valley. Journal of Wildlife Management 5:206–212.

Bellrose, F. C., and J. B. Low. 1943. The influence of flood and low water levels on the survival of muskrats. Journal of Mammalogy 24:173–188.

Blois, J. L., J. L. McGuire, and E. A. Hadly. 2010. Small mammal diversity loss in response to late-Pleistocene climatic change. Nature 465:771–775.

Boyce, M. S. 1978. Climatic variability and body size variation in the muskrats (*Ondatra zibethicus*) of North America. Oecolgia 36:1–19.

Brune, G. 1981. Springs of Texas. Branch-Smith, Fort Worth, Texas.

Dalquest, W. W., and G. E. Schultz. 1992. Ice Age Mammals of Northwestern Texas. Midwestern State University Press, Wichita Falls, Texas.

Errington, P. L. 1939. Reaction of muskrat populations to drought. Ecology 20:168–186.

Errington, P. L. 1963. Muskrat Populations. Iowa State University Press, Ames, Iowa.

Fenneman, N. M. 1931. Physiography of Western United States. McGraw-Hill, New York.

Friend, M., G. F. Cummings, and J. S. Morse. 1964. Effect of changes in winter water levels on muskrat weights and harvest at the Montezuma National Wildlife Refuge. New York Fish and Game Journal 11:125–131.

Galbreath, E. C. 1954. Growth and development of teeth in the muskrat. Transactions of the Kansas Academy of Science 57:238–241.

Green, F. E. 1961. The Monahans Dunes Area. Pp. 22–47 in Paleoecology of the Llano Estacado (F. Wendorf, ed.). Museum of New Mexico Press, Santa Fe.

Gutierrez, M., P. Lewis, and E. Johnson. 1998. Evidence of paleoenvironmental change from muskrat dental microwear patterns. Current Research in the Pleistocene 15:107–109.

Hall, E. R., and K. R. Kelson. 1959. Mammals of North America. The Ronald Press Company, New York.

Hammer, O., D. A. T. Harper, and P. D. Ryan. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologica Electronica 4:1–9.

Holliday, V. T. 1985. Archaeological geology of the Lubbock Lake site, Southern High Plains of Texas. Geological Society of America Bulletin 96:1483–1492.

Holliday, V. T. 1995. Stratigraphy and Paleoenvironments of late Quaternary valley fills on the Southern High Plains. Geological Society of America Memoir 186:1–131.

Holliday, V. T. 1997. Paleoindian geoarchaeology of the Southern High Plains. University of Texas Press, Austin, Texas.

Holliday, V. T. 2000. Folsom drought and episodic drying on the Southern High Plains from 10,900–10,200 ^{14}C yr B.P. Quaternary Research 53:1–12.

Holliday, V. T., and B. L. Allen. 1987. Geology and soils. Pp. 14–21 in Lubbock Lake. Late Quaternary studies on the Southern High Plains (E. Johnson, ed.). Texas A&M University Press, College Station, Texas.

Holliday, V. T., E. Johnson, H. Haas, and R. Stuckenrath. 1983. Radiocarbon ages from the Lubbock Lake site,

1950–1980: Framework for cultural and ecological change on the Southern High Plains. Plains Anthropologist 28:165–182.

Holliday, V. T., E. Johnson, H. Haas, and R. Stuckenrath. 1985. Radiocarbon ages from the Lubbock Lake site, 1981–1984. Plains Anthropologist 30:277–291.

Holliday, V. T., J. C. Knox, G. L. Running IV, R. D. Mandel, and C. R. Ferring. 2002. The Central Lowlands and Great Plains. Pp. 335–362 in The Physical Geography of North America (A. Orme, ed.). Oxford University Press, New York.

Hollister, N. 1911. A systematic synopsis of the muskrats. North American Fauna 32:1–47.

Hunt, C. B. 1967. Physiography of the United States. W. H. Freeman, San Francisco, California.

Johnson, E. 1986. Late Pleistocene and early Holocene paleoenvironments and vertebrates on the Southern High Plains (USA). Geographic Physique et Quaternaire 40:249–261.

Johnson, E. 1987a. Lubbock Lake: Late Quaternary studies on the Southern High Plains. Texas A&M University Press, College Station.

Johnson, E. 1987b. Paleoenvironmental overview. Pp. 90–99 in Lubbock Lake. Late Quaternary studies on the Southern High Plains (E. Johnson, ed.). Texas A&M University Press, College Station, Texas.

Johnson, E. 1987c. Vertebrate remains. Pp. 49–89 in Lubbock Lake. Late Quaternary studies on the Southern High Plains (E. Johnson, ed.). Texas A&M University Press, College Station, Texas.

Johnson, E. 1991. Late Pleistocene cultural occupation of the Southern Plains. Pp. 215–236 in Clovis origins and adaptations (R. Bonnichsen and K. L. Turnmire, eds.). Center for the Study of the First Americans, Oregon State University, Corvallis, Oregon.

Johnson, E. 2007. Grassland ecosystems of the Llano Estacado. Pp. 11–23 in Proceedings: Shrubland dynamics – Fire and Water (R. E. Sosebee, D. B. Wester, C. M. Britton, E. D. McArthur, and S. G. Kitchen, eds.). U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station RMRS-P-47:1–173.

Johnson, E. 2009. Return to the Nash Site (41LU35), in the Yellowhouse System on the Southern High Plains. Lubbock Lake Landmark Quaternary Research Center Series 16:1–472.

Johnson, E. 2010. The Paul Site (41LU136), late Quaternary hunter-gatherer occupations in the Yellowhouse System on the Southern High Plains. Lubbock Lake Landmark Quaternary Research Center Series 18:1–397.

Johnson, E. 2017. The Younger Dryas and Plainview paleoecology on the Llano Estacado. Pp. 103–121 in Plainview. The Enigmatic Paleoindian Artifact Style of the Great Plains (V. T. Holliday, E. Johnson, R. Knudson, eds.). University of Utah Press, Salt Lake City, Utah.

Johnson, E., and V. T. Holliday. 2004. Archaeology and late Quaternary environments of the Southern High Plains. Pp. 283–295 in Prehistoric Archaeology of Texas (T. Perttula, ed.). Texas A&M University Press, College Station, Texas.

Johnson, E., S. Hurst, and J. A. Moretti. 2018. Spring Creek drainage—geoarchaeological explorations along the Southern High Plains eastern escarpment, northwest Texas. Symposium paper presented at the 83rd annual meeting of the Society for American Archaeology, Washington, D.C.

Knudson, R., E. Johnson, V. T. Holliday. 1998. A 10,000 year old Lubbock artifact assemblage. Plains Anthropologist 43:239–256.

Lawrence, B. 1942. The muskrat in Florida. Proceedings of the New England Zoological Club 19:17–20.

Lewis, P. J. 1998. Paleoenvironmental change and microevolution of muskrat from Lubbock Lake. M.S. Thesis, Texas Tech University, Lubbock.

Lewis, P. J., M. Gutierrez, and E. Johnson. 2000. *Ondatra zibethicus* (Arvicolinae, Rodentia) dental microwear patterns as a potential tool for palaeoenvironmental reconstruction. Journal of Archaeologial Science 27:789–798.

Lewis, P. J., and E. Johnson. 2002. Biogeography and paleoenvironments of late-Pleistocene muskrats on the Southern High Plains. The Southwestern Naturalist 47:121–125.

Lewis, P. J., E. Johnson, B. Buchanan, and S. E. Churchill. 2010. The impact of changing grasslands on late Quaternary bison of the Southern Plains. Quaternary International 217:117–130.

Lewis, P. J., R. Strauss, E. Johnson, and W. C. Conway. 2002. Absence of sexual dimorphism in molar morphology of muskrats. Journal of Wildlife Management 66:1189–1196.

Lundelius, E. L., et al. 1983. Terrestrial Vertebrate Faunas. Pp. 311–353 in Late Quaternary environments of the United States, Volume 1, the late Pleistocene (H. E. Wright, Jr. and S. C. Porter, eds.). University of Minnesota Press, Minneapolis, Minnesota.

Martin, L. D. 1979. The biostratigraphy of arvicoline rodents in North America. Transactions of the Nebraska Academy of Sciences 7:91–100.

Martin, R. A. 1993. Patterns of variation and speciation in Quaternary rodents. Pp. 226–280 in Morphological change in Quaternary mammals in North America (R. A. Martin and A. D. Barnosky, eds.). Cambridge University Press, Cambridge.

Martin, R. A. 1996. Dental evolution and size change in the North American muskrat: Classification and tempo of a presumed phyletic sequence. Pp. 431–457 in Palaeoecol-

ogy and palaeoenvironments of late Cenozoic mammals: Tributes to the career of C. S. (Rufus) Churcher (K. M. Stewart and K. L. Seymour, eds.). University of Toronto Press, Toronto.

Martin, R. A. 2017. Body mass and correlated ecological variables in the North American muskrat lineage: evolutionary rates and the tradeoff of large size and speciation potential. Historical Biology DOI: 10.1080/08912963.2017.1384474.

Martin, R. A., F. Marcolini, and F. Grady. 2009. The early Pleistocene Hamilton Cave muskrats and a review of muskrat size change through the late Neogene. Paludicola 7:61–66.

Mihlbachler, M. C. 2012. Palaeodemographics of small-bodied mammals in Pleistocene environments: A case study of muskrats (*Ondatra zibethicus*: Rodentia: Muridae) from north Florida. Biological Journal of the Linnean Society 106:41–56.

Mihlbachler, M. C., C. A. Hemmings, and S. D. Webb. 2002. Morphological chronoclines among late Pleistocene muskrats (*Ondatra zibethicus*: Muridae, Rodentia) from northern Florida. Quaternary Reserch 58:289–295.

Moretti, J. A. 2018. The vertebrate fauna of Macy Locality 100: Exploring late Pleistocene community composition in non-analog North America. M. S. Thesis, Texas Tech University, Lubbock.

Moretti, J. A., and E. Johnson. 2015. The first record of the jumping mouse *Zapus* from the Southern High Plains. PaleoAmerica 1:121–123.

Moretti, J., E. Johnson, and S. Hurst. 2013. Macy Locality 100 – A record of environmental change from the latest Pleistocene to early Holocene along the Southern High Plains of Texas. Poster presented at the 71st Plains Anthropology conference, Ft. Collins, Colorado.

Nelson, R. S., and H. A. Semken. 1970. Paleoecological and stratigraphic significance of the muskrat in Pleistocene deposits. Geological Society of America Bulletin 81:3733–3738.

Proulx, G., and B. M. L. Buckland. 1986. Productivity and mortality rates of southern Ontario pond- and stream-dwelling muskrat, *Ondatra zibethicus*, populations. The Canadian Field-Naturalist 100:378–380.

Schmidly, D. J., and R. D. Bradley. 2016. The Mammals of Texas. University of Texas Press, Austin.

Semken, H. A. 1966. Stratigraphy and paleontology of the McPherson *Equus* Beds (Sandahl local fauna), McPherson county, Kansas. The University of Michigan, Contributions from the Museum of Paleontology 20:121–178.

Slaughter, B. H. 1975. Ecological Interpretation of the Brown Sand Wedge Local Fauna. Pp. 179–192 in Late Pleistocene Environments of the Southern High Plains (F. Wendorf and J. J. Hester, eds.). Fort Burgwin Research Center, Southern Methodist University.

Teaford, M. F. 1991. Dental microwear: What can it tell us about diet and dental function? Pp. 341–356 in Advances in Dental Anthropology (M. A. Kelly and C. S. Larsen, eds.). Wiley-Liss, New York.

Virgil, J. A., and F. Messier. 1996. Population structure, distribution, and demography of muskrats during the ice-free period under contrasting water fluctuations. Ecoscience 3:54–62.

Willner, G. R., G. A. Feldhamer, E. E. Zucker, and J. A. Chapman. 1980. Ondatra zibethicus. Mammalian Species 141:1–8.

Wilson, R. W. 1933. A rodent fauna from later Cenozoic beds of southwestern Idaho. Carnegie Institution of Washington Publication 440:119–135.

Zykow, S. V., Y. E. Kropacheva, N. G. Smirnov, and Y. V. Dimitrova. 2018. Molar microwear of narrow-headed vole (*Microtus gregalis* Pall., 1779) depending on the feed abrasiveness. Doklady Biological Sciences 478:16–18.

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Update on the Mammals of Chinati Mountains State Natural Area, Texas

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Abstract

In order to better document occurrence of mammalian species within Chinati Mountains State Natural Area, located in Presidio County, Texas, remotely-activated game cameras were deployed at four locations of the natural area over a period of 19 months. Sites for camera traps were selected primarily based upon availability of water sources and presence of mammal sign, and cameras were arranged at appropriate heights and locations for targeting mid- to large-sized mammals. Camera trap stations remained in operation from January 2016 until July 2017. During this period, cameras captured images of 18 species of mammals, 11 of which previously were not documented by verifiable means to occur at the natural area. In addition, specimens of two species of mammals previously unreported from the area were salvaged and retained as voucher specimens. In total, 13 previously unverified species of mammals were documented. As a result, the checklist of mammals known from CMSNA was updated.

Key words: camera traps, Chinati Mountains, mammals, Texas

Introduction

Jones et al. (2011) conducted research on mammals at Chinati Mountains State Natural Area (hereafter, CMSNA), Texas, in order to generate baseline inventories of the mammalian fauna. Although these researchers developed a general assessment of mammalian diversity and natural history of mammals at CMSNA, the authors suggested the need for further research to document possible additions to the mammalian fauna, as well as changes in abundance and distribution of mammalian taxa.

Jones et al. (2011) reported on 44 native species of mammals at CMSNA. Of these reported species, 38 were documented by verifiable vouchers. However, the remaining six species were considered present based only on sight records, presence of signs (e.g., tracks, scats, and trail markings), or auditory detection by the researchers. In addition, one introduced species was reported based on visual observations.

Traditional methods of sampling were the primary means utilized to verify presence of mammals within CMSNA during the initial investigation by Jones et al. (2011). These methods included rodent trapping using Sherman live traps, collecting sciurids and lagomorphs with firearms, and capturing bats with mist nets. Due to the limitations of these sampling methods, carnivores and other medium to large-sized mammals were not specifically targeted, and, hence, were largely undetected and underreported. For example, Jones et al. (2011) reported only five species of carnivores from CMSNA, all based on visual sightings, vocalizations, or detection of sign.

Technological advances have resulted in affordable automated game cameras (i.e., camera traps) that are designed to capture digital images of an animal whenever triggered remotely by animal movements and temperature. The primary goal for this study was to deploy camera traps at strategic locations in CMSNA in an effort to add to the verified occurrence of mammal species (especially carnivores and other medium- to large-sized mammals) within the natural area.

STUDY SITE

Chinati Mountains State Natural Area is located on the western slope of the Chinati Mountains, which are a subunit of the larger Central Range of Trans-Pecos Texas (Schmidly 1977). The site is administered by Texas Parks and Wildlife Department (TPWD), and is situated in Presidio County north of the Rio Grande (Fig. 1.) Climate there is similar to that of nearby Big Bend Ranch State Park (see Yancey 1996; 1997), but with slightly cooler and wetter summer conditions and colder winter temperatures. The physiography of the natural area includes high, steep mountains with intermittent valleys and arroyos. Permanent natural water sources are present but uncommon. General habitat types at CMSNA include Chihuahuan Desert scrub, disturbed desert grassland, riparian, and juniper and oak woodland. Vegetation is typical for the northern Chihuahuan Desert. For a complete overview of the habitats and associated vegetation at CMSNA, see Jones et al. (2011).

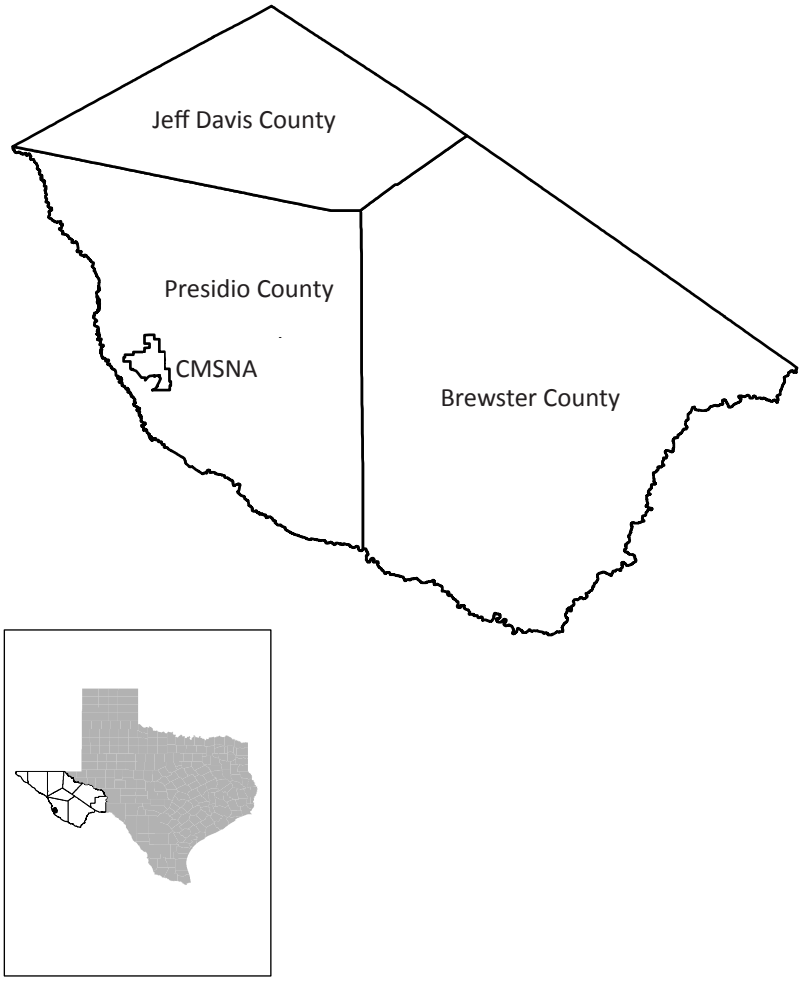

Figure 1. Location of CMSNA, situated in west-central Presidio County, Texas.

MATERIALS AND METHODS

Camera traps (Reconyx ® PC800 and Hyperfire 600) were set at four localities on CMSNA. Sites were chosen based upon accessibility, the presence of a water source that might attract mammals, and signs that indicated mammal activity in the area. Batteries and SD cards in cameras were replaced as necessary during each visit to the study site. Following each trip, all images captured were downloaded to an external hard drive, examined, and sorted by locality and species.

Mammals incidentally found dead during fieldwork were collected, prepared, and retained as voucher specimens. The type of voucher varied due to the nature of the specimen. Voucher specimens were deposited in the Collection of Recent Mammals, Natural Science Research Laboratory of the Museum, Texas Tech University (hereafter, NSRL).

Localities of camera sites and voucher specimens were recorded as UTM coordinates using a handheld GPS unit (Garmin Vista®). Fieldwork and collection of specimens were conducted in accordance with the specifications of scientific collecting permits (2015-07 and 2017-R1-15) issued by TPWD. Sequence of taxa and names (scientific and vernacular) of mammals presented herein follow those of Schmidly and Bradley (2016).

RESULTS AND DISCUSSION

Between January 2015 and July 2017, eight fieldtrips were conducted to install and monitor camera traps at CMSNA. Cameras were set at four sites in CMSNA (Fig. 2). Vegetation surrounding the region of the four camera sites generally was open mixed desert scrub in mid-elevation grassland dominated by tanglehead (*Heteropogon contortus*), sideoats grama (*Bouteloua curtipendula*), blue grama (*B. gracilis*), sotol (*Dasylirion leiophyllum*), bear grass (*Nolin*a sp.), yucca (*Yucca* sp.), and skeleton-leaf goldeneye (*Viguiera stenoloba*). Specifically, Camera Site 1 was situated in an area of CMSNA known as La Ciénega. Habitat at Site 1 was salt grass ciénega surrounded by open mixed desert scrub. The ciénega is dominated by salt grass (*Distichlis spicata*), bulrush (*Scirpus* sp.), and cattails (*Typha* sp.). Camera Site 2 was located in San Antonio Canyon. Habitat at Site 2 was shallow arroyo dominated by dense thorn-scrub vegetation, which included white-thorn acacia (*Acacia neovernicosa*), honey mesquite (*Prosopis glandulosa*), catclaw acacia (*Senegalia greggii*), and granjeno (*Celtis pallida*). Camera Site 3 was located in Cinco-de-Mayo Canyon. Habitat at Site 3 also was shallow arroyo with a similar plant community to that of Camera Site 2, but with an intermittent spring that supported riparian species such as western cottonwood (*Populus deltoids*). Camera Site 4 was positioned at Pelillos Arroyo Waterfall. This site was at the base of an intermittent waterfall in a deep and wide arroyo that supported some desert scrub as well as sparse riparian shrubs, including buttonbush (*Cephalanthus occidentalis*), evergreen sumac (*Rhus virens*), and seepwillow (*Baccharis salicifolia*).

A total of 41,006 images was captured over 1,412 camera-days. The general areas, UTM coordinates, elevations, time periods that cameras were active, number of trap-days, and number of images captured for each camera site are presented in Table 1. Kinds of images captured include wild mammals (as intended), as well as unintended targets such as humans, birds, and vegetation swaying in the wind. Eighteen species of wild mammals were photo-captured during the study. Species of mammals detected at specific camera-trap sites in CMSNA are presented in Table 2. Camera-trap efforts resulted in 11 new verifiable species of mammals for CMSNA.

While conducting routine camera-trap monitoring in CMSNA, specimens of two previously unreported species from CMSNA, *Notiosorex crawfordi* and *Spilogale gracilis*, were found dead. These specimens were collected and prepared as voucher specimens. They represent the first records for both species in CMSNA.

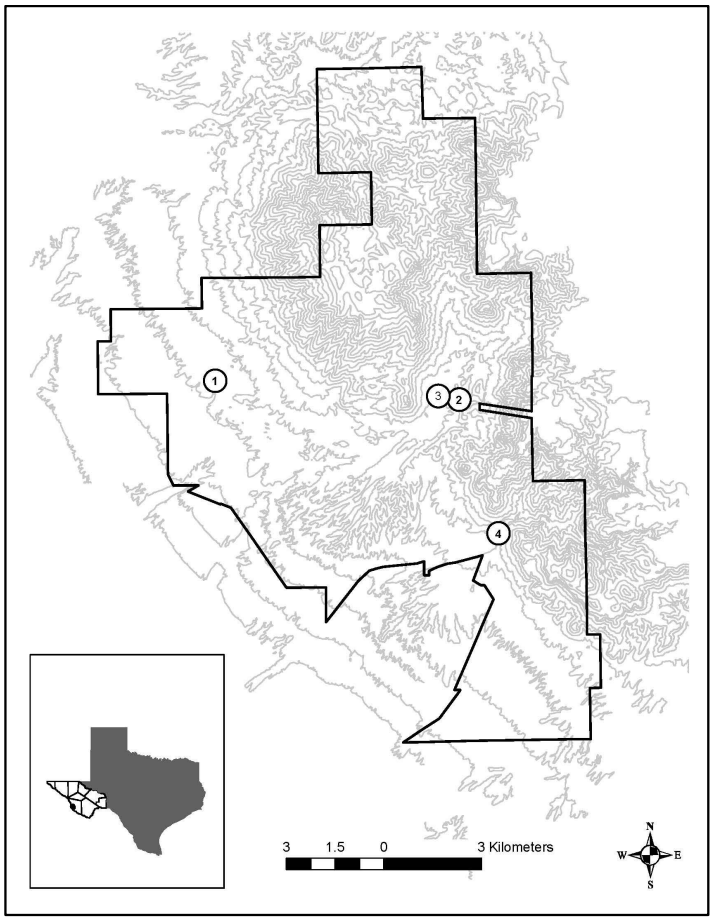

Figure 2. Location of CMSNA Camera Sites 1–4.

Table 1. Locations and summary of results for camera traps set at Chinati Mountains State Natural Area, Texas, during 2016–2017.

Camera Number	Place Name Location	UTM Coordinates and Elevation	Time Period Active	Number of Trap-Days	Number of Images
1	La Ciénega	13 0541855E 3308161N 1,077 m	01/02/2016 to 06/23/2016	173	6,932
2	San Antonio Canyon	13 0549353E 3307590N 1,237 m	01/02/2016 to 07/25/2017	570	16,890
3	Cinco-de-Mayo Canyon	13 0548714E 3307696N 1,209 m	01/02/2016 to 07/25/2017	570	13,615
4	Pelillos Arroyo Waterfall	13 0550564E 3303466N 1,149 m	04/17/2017 to 05/13/2017	99	3,569
Totals				1,412	41,006

Table 2. Species of mammals photographed by camera traps at CMSNA during 2016–2017. See Table 1 for camera site specifics. See checklist below for common names of species listed.

	Camera Site Number			
Species	1	2	3	4
Lepus californicus		X		
Sylvilagus audubonii		X	X	
Canis latrans		X	X	
Urocyon cinereoargenteus		X	X	X
Lynx rufus	X	X	X	
Puma concolor		X	X	X
Conepatus leuconotus		X		
Mephitis mephitis		X	X	
Bassariscus astutus			X	
Procyon lotor		X	X	X
Ursus americanus		X		
Ammotragus lervia		X	X	X
Taurotragus oryx		X		
Odocoileus hemionus	X	X	X	X
Odocoileus virginianus		X	X	
Pecari tajacu		X	X	X
Otospermophilus variegatus		X	X	X
Ammospermophilus interpres		X		

ADDITIONS TO THE VERIFIED MAMMALIAN FAUNA OF CMSNA, WITH ANNOTATIONS

The following Species Accounts are of mammals verified by this study to occur within the boundaries of CMSNA. Some of the species listed below have not been reported to occur at the site. Other species have been reported previously at CMSNA based on non-verifiable means such as tracks, vocalizations, sightings, or odors. New additions are considered to be species that, prior to our study, had not been documented by verifiable means that can be referenced and examined. All new additions presented are verifiable by a museum-cataloged voucher specimen or a confirmable date/time-stamped photograph. Accounts of 13 new additions for CMSNA are presented.

Notiosorex crawfordi
Crawford's Desert Shrew

Jones et al. (2011) did not encounter Crawford's desert shrew and did not list it as a component of the mammalian fauna at CMSNA. On 26 July 2017 one individual was found dead at San Antonio Cabin: CMSNA, UTM: 13 0548278E 3306080N. The skeleton was salvaged and cataloged into the NSRL (TTU-M 135936). This is the first record of this shrew from CMSNA. Although this shrew may be more abundant in the Trans-Pecos than indicated by captures (Schmidly and Bradley 2016), it should be considered uncommon

at CMSNA. Carraway (2014) indicated that *N. crawfordi* was widespread within adjacent northern Mexico, but it is considered a threatened species there because of its uncommon occurrence.

Canis latrans
Coyote

Jones et al. (2011) included the coyote as a member of the CMSNA mammal fauna based on vocalizations presumed to be from a single individual. During this study, *Canis latrans* was photo-verified at CMSNA Camera Site 2 in San Antonio Canyon on 9 January 2016, 4 February 2016, 2 June 2016, 11 December 2016, 16 December 2016, 17 December 2016, 20 December 2016, 26 December 2016, 27 December 2016, 31 December 2016, 3 January 2017, 4 January 2017, 7 January 2017, 9 February 2017, 10 February 2017 (Fig. 3), 19 February 2017, 20 February 2017, 21 February 2017, 8 April 2017, 19 May 2017, and 22 May 2017; Camera Site 3 in Cinco-de-Mayo Canyon on 8 December 2016, 11 December 2016, 19 December 2016, 28 December 2016, 29 December 2016, 6 January 2017, 11 January 2017, 5 February 2017, 10 February 2017, and 5 March 2017. Based on these data, *Canis latrans* should be considered common in CMSNA. This species also is common throughout much of northern Mexico and is listed by the International Union for Conservation of Nature (IUCN) as a species of least concern (Servin et. al. 2014).

Urocyon cinereoargenteus
Common Gray Fox

Jones et al. (2011) reported the common gray fox from CMSNA based upon two visual observations. In addition, a photograph was taken of a young individual, but the image was not archived and is not available for reference. During this study, the common gray fox was photo-verified at CMSNA Camera Site 2 in San Antonio Canyon on 3 January 2016, 4 January 2016, 23 March 2016, 24 March 2016, 26 March 2016, 28 March 2016, 29 March 2016, 31 March 2016, 7 May 2016, 10 May 2016, 27 May 2016, 31 May 2016, 2 June 2016, 6 June 2016, 4 August 2016, 8 August 2016, 25 August 2016, 17 September 2016, 18 September 2016, 19 September 2016, 21 September 2016, 23 September 2016, 25 September 2016, 21 October 2016, 28 October 2016, 13 November 2016, 15 November 2016, 19 November 2016, 25 December 2016, 26 December 2016, 29 January 2017, 31 January 2017, 2 February 2017, 3 February 2017, 4 February 2017, 5 February 2017, 6 February 2017 (Fig. 4), 7 February 2017, 8 February 2017, 9 February 2017, 10 February 2017, 11 February 2017, 12 February 2017, 13 February 2017, 17 February 2017, 6 March 2017, 22 April 2017, 16 May 2017, 17 May 2017, 18 May 2017, and 22 May 2017; CMSNA Camera Site 3 in Cinco-de-Mayo Canyon on 17 May 2016, 10 November 2016, 15 December 2016, 16 December 2016, 24 December 2016, 26 December 2016, 29 December 2016, 4 February 2017, 5 February 2017,

Figure 3. Voucher photograph of *Canis latrans* taken at CMSNA Camera Site 2 in San Antonio Canyon on 10 February 2017.

Figure 4. Voucher photograph of *Urocyon cinereoargenteus* taken at CMSNA Camera Site 2 in San Antonio Canyon on 6 February 2017.

15 February 2017, 28 February 2017, 17 April 2017, and 18 May 2017; CMSNA Camera Site 4 at Pelillos Arroyo Waterfall on 12 May 2017, 20 May 2017, 26 May 2017, and 27 May 2017. These results suggest that the common gray fox is more abundant and widespread in CMSNA than previously reported. *Urocyon cinereoargenteus* has been recorded throughout all types of habitats in neighboring Mexico, and is listed as a species of least concern by the IUCN (Servin and Chacón, 2014).

Lynx rufus
Bobcat

The bobcat has not previously been reported as an inhabitant of CMSNA (Jones et al. 2011). During this study this cat was photo-verified at CMSNA Camera Site 1 at La Ciénega on 26 February 2016, 9 May 2016, and 18 May 2016; CMSNA Camera Site 2 in San Antonio Canyon on 2 October 2016, 28 December 2016, 7 January 2017, 9 February 2017, 20 February 2017, 25 February 2017, 2 March 2017, 24 March 2017 (Fig. 5), 4 April 2017, 3 June 2017, and 17 June 2017; CMSNA Camera Site 3 in Cinco-de-Mayo Canyon on 27 December 2016 and 26 March 2017. Although not reported previously from CMSNA, it appears that the bobcat is somewhat common in CMSNA. *Lynx rufus* is known from the arid scrublands, oak forests, grasslands, and riparian habitats in neighboring Mexico (Bárcenas and Romero R. 2014).

Figure 5. Voucher photograph of *Lynx rufus* taken at CMSNA Camera Site 2 in San Antonio Canyon on 24 March 2017.

Puma concolor
Mountain Lion

Jones et al. (2011) included *Puma concolor* as a component of the mammalian fauna at CMSNA based only on a single observation of fresh tracks in the San Antonio Cabin area. During this study this large cat was photo-verified at CMSNA Camera Site 2 in San Antonio Canyon on 21 August 2016, 27 September 2016, 29 September 2016 (Fig. 6), 25 November 2016, and 6 August 2017; CMSNA Camera Site 3 in Cinco-de-Mayo Canyon on 21 January 2016, 1 May 2016, 7 September 2016, 21 October 2016, 1 November 2016, 24 November 2016, 23 December 2016, and 25 December 2016; CMSNA Camera Site 4 at Pelillos Arroyo Waterfall on 30 April 2017 and 3 May 2017. Although not taken or visually observed during their study, Jones et al. (2011) speculated the mountain lion may be quite common at CMSNA; data from this study support that contention. The mountain lion occurs in all types of habitats in nearby Mexico where it is classified as a species under special protection (Tovar and Ceballos 2014). There is no special provision for the species in Texas.

Figure 6. Voucher photograph of *Puma concolor* taken at CMSNA Camera Site 2 in San Antonio Canyon on 29 September 2016.

Conepatus leuconotus
Hog-nosed Skunk

Jones et al. (2011) did not include the hog-nosed skunk as a species of occurrence at CMSNA. During this study, this species was photo-verified at CMSNA Camera Site 2 in San Antonio Canyon on 13 December 2016 (Fig. 7). As this is the only record of the hog-nosed skunk in CMSNA, it should be regarded as rare at the natural area. Although *Conepatus leuconotus* is relatively abundant in Mexico in both agricultural and undisturbed habitats (Durán and Ceballos 2014), in Texas its populations are considered to be declining in many areas (Schmidly and Bradley 2016).

Figure 7. Voucher photograph of *Conepatus leuconotus* taken at CMSNA Camera Site 2 in San Antonio Canyon on 13 December 2016.

Mephitis mephitis
Striped Skunk

The striped skunk was included as an inhabitant of CMSNA by Jones et al. (2011) based upon a single close-range sighting, as well the detection of skunk odors on several occasions. *Mephitis mephitis* was photo-verified at CMSNA Camera Site 2 in San Antonio Canyon on 20 February 2017, 19 March 2017 (Fig. 8), and 19 June 2017; CMSNA Camera Site 3 in Cinco-de-Mayo Canyon on 24 March 2016 and 6 March 2017. Striped skunks do not seem to be uncommon in CMSNA, but do not appear to be as abundant as in other nearby areas in the Big Bend region and northern Mexico, where the species is reported to be common (Yancey 1996, 1997; Pacheco 2014).

Figure 8. Voucher photograph of *Mephitis mephitis* taken at CMSNA Camera Site 2 in San Antonio Canyon on 19 March 2017.

Spilogale gracilis
Western Spotted Skunk

The western spotted skunk was not reported as a member of the mammalian fauna of CMSNA by Jones et al. (2011). On 20 March 2016, an individual that had drowned in a stock tank near the road into San Antonio Canyon was collected. The specific locality for this specimen is: CMSNA, UTM: 13 0548968E 3303206N. The skull was salvaged and cataloged into the NSRL (TTU-M 135934). This is the first record of this rare skunk in CMSNA. *Spilogale gracilis* was somewhat recently added to the mammalian fauna of nearby Big Bend Ranch State Park, as well (Jones and Lockwood 2008). As populations of this species appear to be declining in Texas and neighboring Mexico (Romero 2014; Schmidly and Bradley 2016), this species should be monitored at CMSNA.

Bassariscus astutus
Ringtail

Jones et al. (2011) did not include the ringtail in the mammalian fauna of CMSNA. During this study, this species was photo-verified at CMSNA Camera Site 3 in Cinco-de-Mayo Canyon on 15 January 2016 (Fig. 9), 28 January 2016, and 6 February 2017. *Bassariscus astutus* probably is more common in CMSNA than these results suggest, as Camera Site 1 and Camera Site 2 both lack the rocky terrain that these animals prefer (Schmidly and Bradley 2016). Additional sampling in

other rocky habitats likely would result in additional records within CMSNA.

Figure 9. Voucher photograph of *Bassariscus astutus* taken at CMSNA Camera Site 3 in Cinco-de-Mayo Canyon on 15 January 2016.

Procyon lotor
Northern Raccoon

The northern raccoon was not detected by Jones et al. (2011), and previously was not known from CMSNA. During this study, this species was photo-verified at CMSNA Camera Site 2 in San Antonio Canyon on 1 March 2017; CMSNA Camera Site 3 in Cinco-de-Mayo Canyon on 15 January 2016; CMSNA Camera Site 4 at Pelillos Arroyo Waterfall on 11 May 2017 (Fig. 10). At CMSNA, *Procyon lotor* probably occurs near most water-associated areas, this species' preferred habitat in Texas (Schmidly and Bradley 2016) and Mexico (Galván 2014).

Figure 10. Voucher photograph of *Procyon lotor* taken at CMSNA Camera Site 4 at Pelillos Arroyo Waterfall on 11 May 2017.

Ursus americanus
American Black Bear

The American black bear previously was unknown from CMSNA. It was not reported by Jones et al. in 2011, and there were no specimen or literature accounts of this species in the Chinati Mountains prior to this study. During this study, *Ursus americanus* was photo-verified at CMSNA Camera Site 2 in San Antonio Canyon on 7 June 2017 (Fig. 11), 3 July 2017, and 7 July 2017. See Yancey and Lockwood (in press) for details of this first record of the American black bear in the Chinati Mountains.

Figure 11. Voucher photograph of *Ursus americanus* taken at CMSNA Camera Site 2 in San Antonio Canyon on 7 June 2017.

Ammotragus lervia
Barbary Sheep or Aoudad

Jones et al. (2011) reported the aoudad from CMSNA based on a single visual observation of a small group of these animals near the north end of the natural area boundary. During this study, aoudads were photo-verified at CMSNA Camera Site 2 in San Antonio Canyon on 18 March 2016, 19 March 2016, 27 April 2017 (Fig. 12), and 16 June 2017; CMSNA Camera Site 3 in Cinco-de-Mayo Canyon on 5 October 2016, 27 February 2017, 20 May 2017, 7 June 2017, 20 June 2017, and 5 July 2017; CMSNA Camera Site

4 at Pelillos Arroyo Waterfall on 26 April 2017, 27 April 2017, 3 May 2017, 4 May 2017, 6 May 2017, 11 May 2017, 12 May 2017, 16 May 2017, and 27 May 2017. Jones et al. (2011) suggested that *Ammotragus lervia* may be more common in CMSNA than their single sighting might indicate, and data from this study support that contention. There is some evidence that aoudad compete for browse with mule deer where the two species co-occur (Schmidly and Bradley 2016), therefore monitoring of this species and its environmental impacts within CMSNA is warranted.

Figure 12. Voucher photograph of *Ammotragus lervia* taken at CMSNA Camera Site 2 in San Antonio Canyon on 27 April 2017.

Taurotragus oryx
Common Eland

The common eland was not known to occur at CMSNA (Jones et al. 2011). During this study, this species was photo-verified at CMSNA Camera Site 2 in San Antonio Canyon on 7 January 2016 (Fig. 13). This African native was introduced onto private game ranches in Texas for hunting purposes, and now has established feral populations in many parts of the state (Schmidly and Bradley 2016). The provenance of the photographed animal, and whether it is a lone individual or part of a feral population in CMSNA, are unknown.

Figure 13. Voucher photograph of *Taurotragus oryx* taken at CMSNA Camera Site 2 in San Antonio Canyon on 7 January 2016.

SUMMARY

This study resulted in the addition of 13 species of mammals (11 native and two introduced) to the verified mammalian fauna of CMSNA. Of these 13 species, five (*C. latrans*, *U. cinereoargenteus*, *M. mephitis*, *P. concolor*, and *A. lervia*) previously have been reported based upon nonverifiable means (Jones et al. 2011). The remaining eight species have not previously been reported from CMSNA, including seven native species (*N. crawfordi*, *L. rufus*, *B. astutus*, *P. lotor*, *C. leuconotus*, *S. gracilis*, and *U. americanus*), and one introduced species (*T. oryx*). Continued research using all appropriate methods available will provide valuable information related to distribution, relative abundance, and habitat affinities of the mammals of CMSNA. Because of the documentation of previously unreported species to the mammalian fauna of CMSNA, as well as recent changes in the taxonomy and nomenclature of mammals previously reported to occur at the site, an updated checklist of mammals is presented herein.

CHECKLIST OF MAMMALS OF CMSNA

The following checklist presents all species of mammals known to occur on CMSNA. Accounts are based on previous reports (Jones et al. 2011), as well as new additions from this study. Both the binomial (for species) and the trinomial (for subspecies if applicable) for native mammalian taxa known to occur in CNSNA are listed. An asterisk (*) indicates a free-ranging non-native species.

ORDER LAGOMORPHA – Pikas, Hares, and Rabbits

Family Leporidae – Hares and Rabbits

Lepus californicus Gray 1837 – Black-tailed Jackrabbit

L. c. texianus Waterhouse 1848

Syvilagus audubonii (Baird 1858) – Desert Cottontail

S. a. neomexicanus (Nelson 1907)

ORDER SORICOMORPHA – Shrews and Moles

Family Soricidae

Notiosorex crawfordi (Coues 1877) – Crawford's Desert Shrew

N. c. crawfordi (Coues 1877)

ORDER CHIROPTERA – Bats

Family Molossidae – Free-tailed Bats

Tadarida brasiliensis (I. Geoffroy St. Hilaire 1824) – Brazilian Free-tailed Bat

T. b. mexicana (Saussure 1860)

Family Mormoopidae – Leaf-chinned Bats

Mormoops megalophylla Peters 1864 – Ghost-faced Bat

M. m. megalophylla Peters 1864

Family Phyllostomidae – New World Leaf-nosed Bats

Leptonycteris nivalis (Saussure 1860) – Mexican Long-nosed Bat

This is a monotypic species

Family Vespertilionidae – Vesper Bats

Aeorestes cinereus (Palisot de Beauvois 1796) – Hoary Bat

A. c. cinereus (Palisot de Beauvois 1796)

Antrozous pallidus (Le Conte 1856) – Pallid Bat

A. p. pallidus (Le Conte 1856)

Corynorhinus townsendii (Cooper 1837) – Townsend's Big-eared Bat

C. t. australis (Handley 1955)

Eptesicus fuscus (Palisot de Beauvois 1796) – Big Brown Bat

E. f. pallidus Young 1908

Myotis californicus (Audubon and Bachman 1842) – California Myotis

M. c. californicus (Audubon and Bachman 1842)

Myotis thysanodes G. S. Miller 1897– Fringed Myotis

M. t. thysanodes G. S. Miller 1897

Myotis velifer (J. A. Allen 1890) – Cave Myotis

M. v. incautus (J. A. Allen 1890)

Myotis volans (H. Allen 1866)

M. v. interior (Miller 1914)

Parastrellus hesperus (H. Allen 1864) – American Parastrelle

P. h. maximus (Hatfield 1936)

ORDER CARNIVORA – Carnivores

Family Canidae – Dogs, Foxes, and Wolves

Canis latrans Say 1823 – Coyote

C. l. texensis V. Bailey 1905

Urocyon cinereoargenteus (Schreber 1775) – Common Gray Fox

U. c. scottii Mearns 1891

Family Felidae – Cats

Lynx rufus (Schreber 1777) – Bobcat

L. r. texensis J. A. Allen 1895

Puma concolor (Linnaeus 1771) – Mountain Lion

P. c. stanleyana Goldman 1936

Family Mephitidae – Skunks

Conepatus leuconotus (Lichtenstein 1832) – Hog-nosed Skunk

C. l. leuconotus (Lichtenstein 1832)

Mephitis mephitis (Schreber 1776) – Striped Skunk

M. m. varians Gray 1837

Spilogale gracilis Merriam 1890– Western Spotted Skunk

S. g. leucoparia Merriam 1890

Family Procyonidae – Raccoons, Ringtails, and Coatis

Bassariscus astutus (Lichtenstein 1830) – Ringtail

B. a. flavus Rhoads 1894

Procyon lotor (Linnaeus 1758) – Northern Raccoon

P. l. mexicanus Baird 1858

Family Ursidae – Bears

Ursus americanus Pallas 1790 – American Black Bear

U. a. amblyceps Baird 1859

ORDER ARTIODACTYLA – Even-toed Ungulates

Family Bovidae – Cattle, Antelope, Sheep, and Goats

*Ammotragus lervia** – Barbary Sheep or Aoudad

*Taurotragus oryx** – Common Eland

Family Cervidae – Deer and Allies

Odocoileus hemionus Rafinesque 1817 – Mule Deer

O. h. crooki (Mearns 1897)

Odocoileus virginianus (Zimmerman 1780) – White-tailed Deer

O. v. texana (Mearns 1898)

Family Tayassuidae – Peccaries

Pecari tajacu (Linnaeus 1758) – Collared Peccary

P. t. angulatus (Cope 1889)

ORDER RODENTIA – Rodents

Family Cricetidae – New World Mice, Rats, and Voles

Neotoma leucodon Merriam 1894 – White-toothed Woodrat

N. l. robusta Blair 1939

Neotoma mexicana Baird 1855 – Mexican Woodrat

N. m. mexicana Baird 1855

Neotoma micropus Baird 1855 – Southern Plains Woodrat

N. m. canescens J. A. Allen 1897

Onychomys arenicola Mearns 1896 – Chihuahuan Grasshopper Mouse

O. a. arenicola Mearns 1896

Peromyscus boylii (Baird 1855) – Brush Deermouse

P. b. rowleyi (J. A. Allen 1893)

Peromyscus eremicus (Baird 1858) – Cactus Deermouse

P. e. eremicus (Baird 1858)

Peromyscus laceianus V. Bailey 1906 – Lacey's White-ankled Deermouse

This is a monotypic species

Peromyscus leucopus (Rafinesque 1818) – White-footed Deermouse

P. l. tornillo Mearns 1896

Peromyscus maniculatus (Wagner 1845) – North American Deermouse

P. m. blandus Osgood 1904

Reithrodontomys fulvescens J. A. Allen 1894 – Fulvous Harvest Mouse

R. f. canus Benson 1939

Reithrodontomys megalotis (Baird 1858) – Western Harvest Mouse

R. m. megalotis (Baird 1858)

Sigmodon hispidus Say and Ord 1825 – Hispid Cotton Rat

S. h. berlandieri Baird 1855

Sigmodon ochrognathus Bailey 1902 – Yellow-nosed Cotton Rat

This is a monotypic species

Family Erethizontidae – New World Porcupine

Erethizon dorsatum (Linnaeus 1758) – North American Porcupine

E. d. couesi Mearns 1897

Family Geomyidae – Pocket Gophers

Cratogeomys castanops (Baird 1852) – Yellow-faced Pocket Gopher

C. c. clarki (Baird 1855)

Family Heteromyidae – Pocket Mice and Kangaroo Rats

Chaetodipus eremicus (Mearns 1898) – Chihuahuan Desert Pocket Mouse

This is a monotypic species

Chaetodipus intermedius Merriam 1889 – Rock Pocket Mouse

C. i. intermedius Merriam 1889

Chaetodipus nelsoni Merriam 1894 – Nelson's Pocket Mouse

C. n. canescens Merriam 1894

Dipodomys merriami Mearns 1890 – Merriam's Kangaroo Rat

D. m. ambiguus Merriam 1890

Perognathus flavus Baird 1855 – Silky Pocket Mouse

P. f. flavus Baird 1855

Family Sciuridae – Squirrels and Allies

Ammospermophilus interpres (Merriam 1890) – Texas Antelope Squirrel

This is a monotypic species

Otospermophilus variegatus (Erxleben 1877) – Rock Squirrel

O. v. grammurus (Say 1823)

Xerospermophilus spilosoma (Bennett 1833) – Spotted Ground Squirrel

X. s. marginatus V. Bailey 1890

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LITERATURE CITED

Bárcenas, H. V., and F. Romero R. 2014. Bobcat. Pp. 503–504 in Mammals of Mexico (G. Ceballos, ed.). Johns Hopkins University Press, Baltimore, Maryland.

Carraway, L. N. 2014. Crawford's gray or desert shrew. Pp. 469–470 in Mammals of Mexico (G. Ceballos, ed.). Johns Hopkins University Press, Baltimore, Maryland.

Durán, A. M., and G. Ceballos. 2014. Hog-nosed skunk. Pp. 551–552 in Mammals of Mexico (G. Ceballos, ed.). Johns Hopkins University Press, Baltimore, Maryland.

Galván, D. V. 2014. Common raccoon. Pp. 571–573 in Mammals of Mexico (G. Ceballos, ed.). Johns Hopkins University Press, Baltimore, Maryland.

Jones, C., and M. W. Lockwood. 2008. Additions to the mammalian fauna of Big Bend Ranch State Park, Texas. Occasional Papers, Museum of Texas Tech University 282:1–3.

Jones, C., M.W. Lockwood, T.R. Mollhagen, F.D. Yancey, II, and M.A. Bogan. 2011. Mammals of the Chinati Mountains State Natural Area, Texas. Occasional Papers, Museum of Texas Tech University 300:1–32.

Pacheco, R. J. 2014. Striped skunk. Pp. 555–556 in Mammals of Mexico (G. Ceballos, ed.). Johns Hopkins University Press, Baltimore, Maryland.

Romero, R. F. 2014. Spotted skunk. Pp. 558–559 in Mammals of Mexico (G. Ceballos, ed.). Johns Hopkins University Press, Baltimore, Maryland.

Schmidly, D. J. 1977. The mammals of Trans-Pecos Texas including Big Bend National Park and Guadalupe Mountains National Park. Texas A&M University Press, College Station.

Schmidly, D. J., and R. D. Bradley. 2016. The Mammals of Texas, 7th Edition. University of Texas Press, Austin.

Servin, J., and E. Chacón. 2014. Gray Fox. Pp. 514–515 in Mammals of Mexico (G. Ceballos, ed.). Johns Hopkins University Press, Baltimore, Maryland.

Servin, J., E. Chacón, and R. List. 2014. Coyote. Pp. 510–511 in Mammals of Mexico (G. Ceballos, ed.). Johns Hopkins University Press, Baltimore, Maryland.

Tovar, C. C., and G. Ceballos. 2014. Puma. Pp. 505–507 in Mammals of Mexico (G. Ceballos, ed.). Johns Hopkins University Press, Baltimore, Maryland.

Yancey, F. D., II. 1996. Diversity, distribution, and natural history of the mammals of Big Bend Ranch State Park, Texas. Ph.D. dissertation, Texas Tech University, Lubbock.

Yancey, F. D., II. 1997. The mammals of Big Bend Ranch State Park, Texas. Special Publications, Museum of Texas Tech University 39:1–210.

Yancey, F. D., II, and M. W. Lockwood. First record of the American Black Bear (*Ursus americanus*) from the Chinati Moutains of western Texas. The Southwestern Naturalist, in press.

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Mammals of Barataria Preserve, Jean Lafitte National Historical Park and Preserve, Louisiana: Biodiversity, Distribution, and Habitat use Pre- and Post-Katrina

Craig S. Hood and Lauren Nolfo-Clements

Abstract

This paper describes the first systematic survey of the mammals inhabiting the Barataria Preserve of Jean Lafitte National Historical Park & Preserve in Marrero, Louisiana (south of New Orleans). Initial field work was conducted in 2003–2005, ending just prior to the impacts of Hurricanes Katrina and Rita in August–September of 2005. In 2011–2012, a re-survey (post-Katrina) assessed the status of mammal diversity, abundance, and habitat distribution. These biodiversity assessments made full use of many new methods for documenting mammals, including camera traps (for medium–large taxa), electronic bat detectors, and radio frequency PIT tags for live-captured mammals. The generation of photographic, electronic, and other forms of virtual (digital) vouchers builds upon the scholarship of Texas Tech Museum publications and NSRL museology, including papers on digital data. Although the original surveys could not have anticipated that the Summer of 2005 would be an historic year of Gulf Coastal tropical storms and hurricanes (more than 24 named storms), it did provide an opportunity to collect pre-disturbance data. Twenty-six species of mammals were documented as occurring in the marsh, swamp, bottomland hardwood forests, and on the natural and man-made spoil banks of waterways (bayous and canals) during the pre-Katrina study, and only one species diversity change occurred post-Katrina—the appearance and establishment of a resident population of feral hogs, *Sus scrofa*, in all major habitats in the Preserve.

Key words: bat detectors, biodiversity, camera trapping, distribution, mammals, mark recapture, remote sensing, survey

Introduction

This paper describes the first systematic survey of the mammals inhabiting the Barataria Preserve, a unit of Jean Lafitte National Historical Park & Preserve (JLNHPP) in Marrero, Louisiana (south of New Orleans). Initial field work was conducted in 2003–2005, ending just prior to the impacts of Hurricanes Katrina and Rita in August–September 2005. In 2011–2012, a re-survey (post-Katrina) assessed the status of mammal diversity, abundance, and habitat distribution, with studies continuing to the present. Although the original surveys could not have anticipated that the Summer of 2005 would be an historic year of Gulf Coastal tropical storms and hurricanes (more than 24 named storms), it did provide an opportunity to collect pre-disturbance data.

The park unit of Barataria Preserve is found within the Barataria Basin, which historically derived its freshwater from the Mississippi River at its northern edge (see Fig. 1). The Barataria Preserve includes diverse, complex, and productive ecosystems. The wetland habitats include unique ecological assemblages, including flotant (floating) marshes dominated by variety of species, including *Sagittaria lancifolia*, *Typha* spp., *Schoenoplectus americanus*, and *Eleocharis* spp. as described by Nolfo-Clements (2006). Swamp zones are found both west and east of Bayou des Familles. The bottomland hardwood forests of the more upland areas adjacent to and east of Bayou des Familles include some of the last remnant forests of the Barataria Basin south and west of New Orleans and the Mississippi

River. For a full description of the vegetative communities of the preserve, see White et al. (1983).

A unique aspect of this park unit is that public hunting, trapping, and fishing are permitted and incorporated into the park management. The extensive marsh systems that comprise the western two-thirds of the Barataria Preserve support populations of waterfowl, furbearers, fish, and shellfish that can be accessed and harvested (under appropriate permits) by the public.

The mammalian fauna of southeastern Louisiana is rather poorly known. George Lowery's (1974) *Mammals of Louisiana and Adjacent Waters* remains the most comprehensive professional reference, although it is now nearly 40 years out of date. In 1994, Choate et al. published *Handbook of Mammals of the South-Central States*, which provided an updated, but very general, reference to the mammals of the south including Louisiana. This publication includes distribution maps, but lacks voucher specimen lists or locality symbols to document distribution and status of mammal species. Few distributional studies have been published since Lowery (1974), although several dissertations have focused on specific taxa (Moncrief 1993; Lance 1999; Lance et al. 1996, 2001). Additional recent publications on mammals of southeastern Louisiana include studies of mammals that are deposited at the Tulane Museum of Natural History (TMNH) (e.g., Jones 1967; Jones 1975; Suttkus and Jones 1991; Suttkus and Jones 1999).

Prior to the pre-Katrina study, which reported a two-year mammal inventory with field work from 2003 to 2005 (Hood 2006), only two superficial mammal inventories had been conducted in the Barataria Preserve (Smalley 1982; Demastes and Rossman 1989) and both are considered incomplete. Smalley's (1982) study was part of a general survey of the fauna of the forested areas of the Barataria Preserve and did not undertake a collection protocol that would have documented mammal species with voucher specimens. Demastes and Rossman's (1989) study was a more systematic effort, which included study of Lowery's (1974) specimen and species distribution lists, as well as confirmation of specimens housed at the LSU Museum of Natural Science. That survey did include some trapping and observational protocols, but yielded only a limited number of voucher specimens.

METHODS

Documenting bat species.—Methods to document bat species included both capture and observational methods (Jones et al. 1996; Kunz et al. 1996; ASM 1998; Kunz and Kurta 1988). Electronic detection of bats as a method for identification of species to document their occurrence and relative abundance has developed rapidly in recent years (for reviews see Anderson and Miller 1977; Fenton 1988; Kunz et al. 1996). These methods record the ultrasonic echolocation calls of bats and, provided that call libraries exist for the species encountered at the study site, the calls can be identified by their quantitative bioacoustic signature patterns (O'Farrell and Gannon 1999; O'Farrell et al. 1999; Parsons et al. 2000). Numerous published field and laboratory studies have demonstrated both the utility and the limitations of electronic detection for species identification of bats (Lance et al. 1996; Britzke et al. 1999; Murray et al. 1999; O'Farrell and Gannon 1999; O'Farrell et al. 1999; Parsons et al. 2000). Electronic monitoring of bat calls at Barataria Preserve were coordinated with mist-netting to maximize efficiency of effort. Two to six ANABAT bat detectors and field crews were used simultaneously in different areas at a field site to allow data to be collected in different areas of the park. Captured bats were identified to species, GPS data was collected, individuals were PIT tagged (see below), and calls were recorded for a call library, if needed. In addition to development of call libraries from bats collected on site, call libraries constructed by Hood (2006) included confirmed species from Barataria and from bats collected from nearby locations. Figure 1 was created from GPS coordinates following Padgham et al. (2017) Pebesma et al. (2018), R Core Team (2018), and Tennekes (2018).

Documenting small mammals.—Rodents (including native and introduced mice, rats, and squirrels), lagomorphs, opossums, and small carnivores

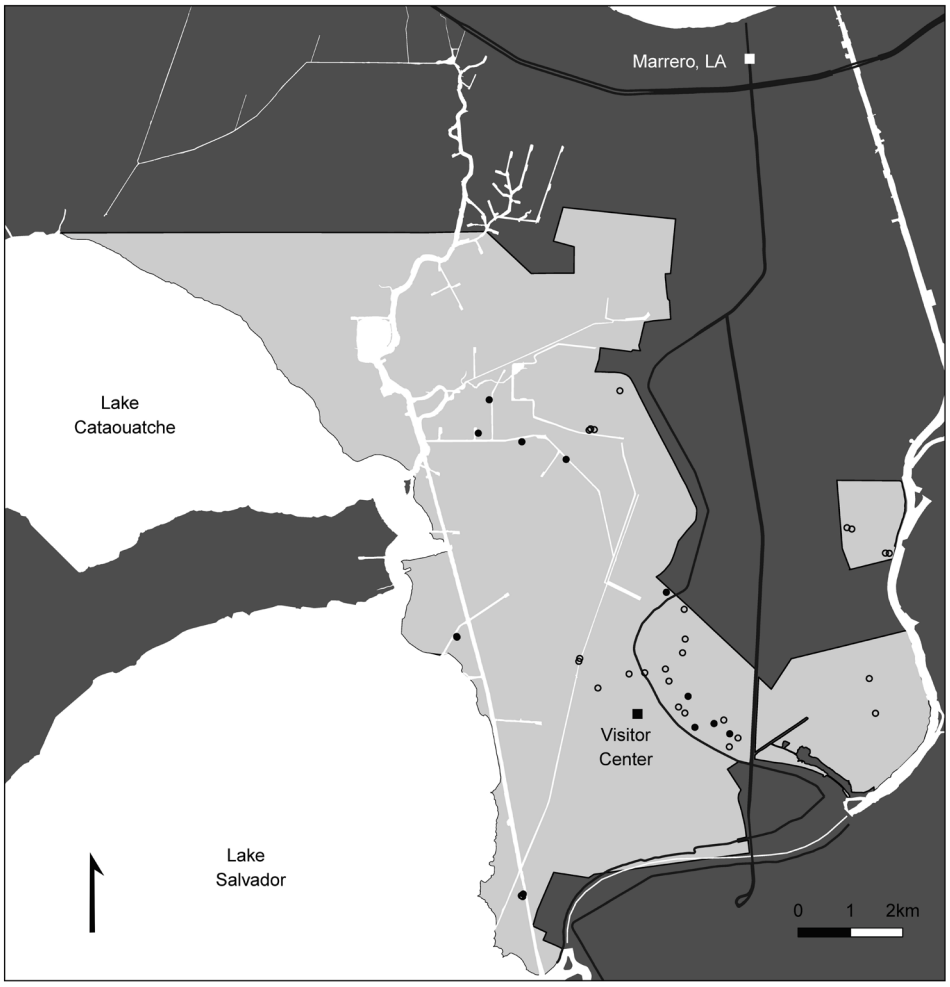

Figure 1. Site map of the Barataria Preserve of Jean Lafitte National Historical Park and Preserve, Louisiana (light gray shading indicates current Preserve boundaries). Circles indicate the locations of 39 camera-trap sites used in the post-Katrina study (Hood 2012); eleven pre-Katrina camera-trap sites that were co-located with the post-Katrina sites are indicated by solid circles. The Barataria Visitor Center (square) is located at 29°47'4"N, 90°6'56"W.

were documented by live-trapping and observation (identification of sign, visual observations, road-killed specimens, and use of motion-activated still cameras). In the post-Katrina study, live-trapping transects were located in the two localities previously studied by Hood (2006) within the Barataria Preserve. Sherman live traps (small rodents), Havahart #1 traps (squirrels, small carnivores), Havahart #2 traps (squirrels to opossums) were utilized. Live traps were monitored nightly and closed or removed when not attended. GPS location data, environmental data, habitat description, and field identification of species were recorded for each capture. All captured individuals were PIT-tagged for subsequent identification if re-captured. Only individuals representing a newly documented species were sacrificed and saved as voucher specimens (with all data, including GPS location data; see Appendix for GPS localities).

Documenting large mammals.—Large mammal species were documented by observation (identification of sign, visual observation, road kill specimens, and use

of remote still cameras). The walking trails and roads that course through the bottomland hardwood forest habitats are major routes of movement for large mammals in the Preserve, as are the natural levee and spoil banks found throughout the swamp and marsh habitats. Wildlife monitors (motion-activated still cameras) were placed in major habitats within the Barataria Preserve.

In the pre-Katrina study, motion-activated SLR (film-based) camera traps were used at 11 sites (Fig. 1). These camera-traps had a short battery life (30–45 days) and used 35 mm film that limited the system to 24–36 photographs per deployment. In the post-Katrina study, a combination of two digital camera-trap systems were utilized at the same 11 sites in the pre-Katrina study and at 28 additional sites (Fig. 1). The protocol for deployment and use of the cameras generally followed that of TEAM Network (2008) for terrestrial vertebrate monitoring. Cuddeback Capture Game Scouting cameras with 3.0 megapixel images were used at 34 sites. Cuddeback cameras have been reviewed as being reliable and affordable for mammal surveys (Swann et al. 2011; Meeks 2012). Reconyx HC600 Hyperfire cameras were used at six sites (both camera systems were utilized at two sites; see Appendix and Hood 2012). Reconyx cameras have higher fidelity, have long deployment battery life (up to 6 months), and can take photographs at shorter re-setting trigger times.

Marking captured mammals with PIT tags.—To document the occurrence and relative abundance of mammals at the Barataria Preserve, all live-captured mammals were marked with PIT tags for identification of individuals upon recapture. Mark/recapture protocols allow multiple observations of individuals and also allow for estimates of species richness, distribution, and abundance by application of established sampling and statistical designs (Fagerstone and Johns 1987; Camper and Dixon 1988; Ball et al. 1991; Germano and Williams 1993; Schooley et al. 1993; Nichols and Conroy 1996; Williams et al. 1997).

Count-per-unit-effort (CPUE).—In mammalian ecological and biodiversity studies, a common method of comparing samples is to calculate count-per-unit-effort (CPUE), which is simply the observation or data recorded adjusted per sampling unit or design. CPUE can be calculated by sampling unit (e.g., at a given site), for some period of time (e.g., per year or study), or by a given method (e.g., comparing different specific methods). In the comparison of pre- and post-Katrina results, CPUE was calculated for all mammal data as follows: For bats using mist-netting and electronic detection protocols, CPUE is the number of bats collected per bat net-night or detector-night; for live-trapped mammals, CPUE is the number of animals live-captured per trap-night; and for camera-trap protocols, CPUE is the number of animals photographed (trigger events) per camera-day.

Voucher specimens.—Traditional voucher specimens are essential for positive identification and documentation of species. Representative specimens encountered during live-trapping and mist-netting activities were sacrificed and prepared as standard museum study specimens (Jones et al. 1996). Only specimens representing new records for Barataria Preserve were collected. These voucher specimens were deposited in the Mammal Collections of the Tulane Museum of Natural History (museum acronym TU), which have since been transferred to the LSU Museum of Natural Science. The studies also generated electronic bat detector files and digital photographs of thousands of records. These are deposited with the National Park Service. These digital voucher specimens align with modern views of what constitutes non-physical voucher specimens as discussed and argued by Monk and Baker (2001) and Kageyama et al. (2007).

RESULTS

A total of 26 mammal species were documented as occurring within the Barataria Preserve in the pre- and post-Katrina studies (Hood 2006, 2012).

Bats.—In the pre-Katrina study, five species of bats were collected by mist nets—*Perimyotis subflavus*, *Nycticeius humeralis*, *Myotis austroriparius*, *Corynorhinus rafinesquii*, and *Tadarida brasiliensis*. Electronic bat detectors were used at nine site localities for 44 detector nights and resulted in the documentation of 1,624 bat pass sequences and 46,745 bat calls for these five species, as well as for two additional species

that were not captured in mist nets, *Lasiurus borealis* (eastern red bat) and *Dasypterus intermedius* (northern yellow bat) (Table 1). The most frequent species encountered was *P. subflavus*, which comprised nearly 35% of the bat pass sequences, with *N. humeralis* being the second most common (> 18%).

In the post-Katrina study, a total of seven site localities and 18 mist net-nights resulted in the capture of four *N. humeralis*. The maternity colony of *N. humeralis* discovered in the bridge supports of the Kenta Canal/Bayou Coquille bridge by Hood (2006) remains intact and is being used by this species. Overall netting success was very low, and of four individuals captured, none were recaptures from the pre-Katrina study and none were re-captured post-Katrina. Electronic bat detectors at 10 site localities for 63 detector nights resulted in the documentation of 1,809 bat pass sequences and 20,041 bat calls (Table 2). Of the bat pass sequences recorded, 94% were positively identified to species. The remaining 6% were documented as bat calls but were unidentifiable.

Overall, *P. subflavus* was the most commonly recorded species (45% of total detector captures) in the post-Katrina study, with *N. humeralis* also being commonly encountered (23% of total detector captures). Southeastern myotis, *Myotis austroriparius*, were regularly recorded, although the species is not commonly encountered in southeastern Louisiana. Recordings of bats at 38–40 mHz that had the characteristic signature of *L. borealis* and/or *Lasiurus seminolus* were relatively commonly recorded. Neither of these species of *Lasiurus* have been captured by mist-nets and therefore future studies should focus on clarifying which species (or both species) occurs in Barataria. Although much less frequently recorded, electronic recordings of *Lasiurus cinereus*, *Corynorhinus rafesinquii*, and *T. brasiliensis* were made at a number of sites.

Partitioning the data by major habitat type reveals that although *P. subflavus* is the most common hardwood forest species, it is less common in swamp/swamp transition habitats (Coquille Trail viewing platforms) and along waterways (Bayou des Familles and Kenta Canal); *N. humeralis* was most common in those habitats. Because there were more detector-nights at the hardwood forest sites (49 detector-nights) compared with the swamp (6 detector-nights) and bayou (6 detector-nights) sites, catch-per-unit-effort (CPUE) was compared. These revealed that despite the much larger data set for hardwood forest sites, *P. subflavus* was not only the most common species in that habitat, but was most common each night.

Small and medium-sized mammals.—In the pre-Katrina study, several traplines where set on flotant marsh. Hood (2006) Site 3 (Tarpaper Canal Flotant Marsh) reported three traplines set for 685 trap-nights. This resulted in 23 *Oryzomys palustris* captures and 21 recaptures for a total of 44 captures, yielding a CPUE of 0.050. Two *Sigmodon hispidus* were captured and marked/released during this trapping interval.

Similarly, several traplines were placed within flotant marsh during the post-Katrina study. Trapping conducted at the WWL Radio Towers Marsh Site consisted of three traplines set for 800 trap-nights that resulted in 54 *O. palustris* captures and seven recaptures for a total of 61 captures, yielding a CPUE of 0.070. There were 32 males and 22 females captured; all seven re-captures were males.

There was a high number of juvenile/subadult *O. palustris* captured (30% of captures), indicating that the population was actively reproducing (trapping was conducted 20–24 February 2012), and the high number of males encountered and recaptured (32 of 54 captures of individuals, all seven recaptures being male) is consistent with the higher activity patterns of subadult male rodents in winter. CPUE in 2012 was 40% higher (0.070 vs. 0.050), as was trapping success rate (6.63% vs. 4.97%). Re-capture rate was high, but much lower than reported in Hood (2006; 15.09% vs. 91.3%). The capture of a large number of juvenile/subadult individuals in the present study provides evidence of a healthy, reproductively active population of *O. palustris* in the Preserve.

The bottomland hardwood forest results of the pre-Katrina study were from Site 4 (E. Plantation Trail Forest). On two traplines, set for a total of 1,596 trap-nights, seven *Peromyscus leucopus* were captured and there were 11 recaptures for a total of 19 captures, yielding a CPUE of 0.012. One *Rattus rattus* and one *Mus musculus* were captured adjacent to the Twin Canals boat launch along the edge of a forested habitat.

Table 1. Summary of Hood (2006) electronic bat detection results by species from a mammal survey of Jean Lafitte National Historical Park and Preserve conducted 2003–2005.

Species	Number of sequences	Percent of sequences	Number of calls	Frequency minimum range (kHz)
Perimyotis subflavus	567	34.9%	18,650	42–44
Nycticeius humeralis	300	18.4%	6,526	35–37
Myotis austroriparius	123	7.6%	3,465	45–47
Corynorhinus rafinesquii	33	2.0%	458	47
Tadarida brasiliensis	21	1.4%	409	27
Lasiurus borealis/seminolus	153	9.4%	4,785	35–40
Dasypterus intermedius	3	0.2%	33	26
Multiple	154	9.5%	9,112	
Unidentified	270	16.6%	3,307	
Total	1,624		46,745	

Table 2. Summary of electronic bat detection results from a mammal re-survey of Jean Lafitte National Historical Park and Preserve conducted 2011–2012.

Species	Number of sequences	Percent of sequences	Number of calls	Frequency minimum range (kHz)
Perimyotis subflavus	819	45.3 %	11,257	42–44
Nycticeius humeralis	413	22.8 %	4,889	35–37
Myotis austroriparius	187	10.3 %	1,809	45–47
Corynorhinus rafinesquii	34	2.9 %	122	47
Tadarida brasiliensis	10	0.6 %	25	27
Lasiurus borealis/seminolis	225	12.4 %	1,709	35–40
Dasypterus intermedius	5	0.3 %	22	26
Unidentified	116	6.4 %	208	
Total	1,809		20,041	

The bottomland hardwood forest results of the post-Katrina study were from the Plantation Trail Forest Site. Three traplines, set for 1,836 trap-nights, resulted in 37 *P. leucopus* (and no recaptures), yielding a CPUE of 0.020. There were 25 males, 10 females, and two un-sexed mice.

The post-Katrina study revealed similar results, with *P. leucopus* being captured. CPUE in 2012 was twice as high (0.020 vs. 0.012). Re-capture rate in the post-Katrina study was zero. The capture of many more mice, together with high numbers of juvenile/subadult individuals and no re-captures, in the post-Katrina study provides evidence of a healthy, reproductively active population.

Large mammals.—In the pre-Katrina study, Hood (2006) reported results of camera-trap data for 11 site localities, with 1,462 camera-days resulting in 438 photographs of mammals (with some repeated photographs of individuals) representing nine species—*Didelphis virginiana*, *Dasypus novemcinctus*,

Sylvilagus aquaticus, *Sciurus carolinensis*, *Myocastor coypus*, *Canis latrans*, *Procyon lotor*, *Felis catus*, and *Odocoileus virginianus*.

In the post-Katrina study, Hood (2012) reported results of a total of 39 site localities and 5,627 camera-days resulting in 6,149 photographs of mammals (with repeated photographs of some individuals) representing 12 mammal species—*D. virginiana*, *D. novemcinctus*, *S. aquaticus*, *S. carolinensis*, unidentified rodents, *C. latrans*, *P. lotor*, *Lontra canadensis*, *Felis rufus, F. catus*, *Odocoileus virginianus*, and *Sus scrofa.* Note that the 11 pre-Katrina sites were re-studied along with 28 new sites. Photographs of large-antlered *O. virginianus* and *S. scrofa* included dozens of different individuals recorded at a given site.

Table 3 summarizes the post-Katrina records expressed as CPUE by species to allow comparison across sites in bottomland hardwood and swamp forests. Cuddeback Camera Sites 1–8 and 20 had the highest mammal activity. Sites 1–8 are in bottomland hardwood forest, whereas Site 20 is in a hardwood/swamp transition near Bayou des Familles. Sites 9, 10, and 12 have a heavy palmetto understory, and although they recorded fewer mammals, large white-tailed deer and feral hogs were recorded in that dense habitat. Sites 11–17 are forested habitats that are near roads and park trails and may have had less mammal species activity due to human use. Cuddeback Camera Sites 30, 32, and 33–37 are in swamp or hardwood/swamp transition areas and recorded deer, coyotes, bobcats, raccoons, and medium-sized mammals, but no feral hogs. Reconyx Camera Sites 1, 20, and 31 had high mammal activity, including many white-tailed deer, feral hogs, rabbits, and raccoons. Reconyx cameras also captured rarer species in the bottomland harwood and swamp forests, including coyotes, bobcats, opossums, and rodents.

Table 3 also summarizes post-Katrina records in marsh and adjacent spoil bank habitats (Cuddeback Camera Sites 21–29 and 38–39). Cuddeback Camera Sites 24 and 25 had the highest mammal activity. At Site 24, feral hogs (38 photographs of large, adult animals) were common, with the highest CPUE for this species of any site post-Katrina. This was the only marsh site that recorded feral hogs. Site 25 recorded coyotes, bobcats, raccoons, rabbits, and an otter. Reconyx Camera Sites 23 and 26 recorded white-tailed deer, raccoons, armadillos, bobcats, rodents, and some birds. As in the hardwood and forest habitats, Reconyx cameras at the marsh and spoil bank sites consistently captured rarer species than did the Cuddeback cameras, including coyotes, bobcats, opossums, otter, and rodents.

Comparison of pre- and post-Katrina results.—A comparison of 11 co-located camera trap sites is shown in Table 4. A summary of the comparisons reveals a number of interesting findings. Post-Katrina, there is a large, resident population of feral hogs (*S. scrofa*) in hardwood/swamp forests (Sites 1–10, 12, 14, 17, 20, and RECONYX 20 and 33) and one marsh/spoil bank (Site 24); this species was entirely absent in the pre-Katrina study. Hogs were not found in Bayou aux Carpes nor in any marsh areas other than Site 24. Post-Katrina data provided the first camera records of bobcats (*F. rufus*). These were in hardwood/swamp (Sites 2, 4, and 33) and marsh/spoil bank (Sites 25 and RECONYX 26) habitats. Post-Katrina data also provided the first camera record of *L. canadensis*. This was in marsh/spoil bank (Site 25). Post-Katrina cameras recorded fewer *C. latrans* in hardwood/swamp habitats (Sites 4, 7, 10, 20, 32, and 36) and marsh/spoil bank (Sites 21, 24, 25, and RECONYX 26) habitats than pre-Katrina. The large number of *C. latrans* photographed pre-Katrina came from two sites (located at current Sites 25, 33) that were baited with coyote urine scent, thus potentially biasing the results. Lastly, *D. novemcinctus* were commonly captured by camera-traps pre-Katrina, but were much less commonly encountered post-Katrina.

Table 3. Summary of Post-Katrina (Hood 2012) camera trapping records excluding repeated photos of the same individuals. Most sites were monitored using Cuddeback cameras; RECON indicates a deployed Reconyx camera site. Records are photograph images of individual mammals; some photographs included multiple individuals. Recoynx records are TRIGGER EVENTS, with a burst of 10 photographs per trigger event. The same individual is not reported more than once in these records, unless captured by subsequent trigger events. Column headings are as follows: DEER = *Odocoileus virginianus*; HOG = *Sus scrofa*; RAC = *Procyon lotor*; ARM = *Dasypus novemcinctus*; RAB = *Sylvilagus aquaticus*; COYOTE = *Canis latrans*; BOBCAT = *Lynx rufus*; OPOS = *Didelphis virginiana*; SQUIR = *Sciurus carolinensis*; OTHER = domestic cats, humans, river otter, rodents, birds; CPUE = Catch-per-unit-effort (captures/camera days).

Camera Site	DEER	HOG	RAC	ARM	RAB	COYOTE	BOBCAT	OPOS	SQUIR	OTHER	Total Captures	Camera Days	CPUE
1	22	22	9	11	4				15	2	85	188	0.45
2	164	1									165	233	0.71
3	343	39		1			1			2	386	245	1.58
4	87	38	87		7	2				3	224	246	0.91
5	984	41	3	1						4	1,033	335	3.08
6	293	4								9	306	292	1.05
7	333	7	3	4		1				2	350	297	1.18
8	151	12							3	1	167	278	0.60
9	26	4								2	32	320	0.10
10	31	1	2			1				3	38	252	0.15
11	5										5	22	0.23
12	2	1									3	22	0.14
13	13									9	22	143	0.15
14	5	1									6	54	0.11
15											0	19	0
16			2							2	4	19	0.21
17	34	1								1	36	99	0.36
18	40										40	134	0.30
19	4										4	45	0.09
20	110	9	6	3	2	4	3			1	138	113	1.229
21	30					2				1	33	146	0.23
22	2										2	146	0.014
24	8	38				4				3	53	146	0.36

Table 3. (cont.)

Camera Site	DEER	HOG	RAC	ARM	RAB	COYOTE	BOBCAT	OPOS	SQUIR	OTHER	Total Captures	Camera Days	CPUE
25			20	2	16	2	3			3	46	146	0.32
27	4										4	122	0.03
28	8									4	12	162	0.07
30	5								10		15	117	0.13
32	2					1				1	4	117	0.03
33	5		1	11		1	2	1		4	25	117	0.21
34	3										3	94	0.03
35										1	1	83	0.01
36	7		10	1		3				2	23	79	0.29
37	4		2								6	91	0.07
RECON01	223	26	25	40	13	2		7	77	13	426	148	2.88
RECON20	347	36	136	29	64	12	1	37	51	13	726	170	4.27
RECON23	71		8	3	29				1	10	122	101	1.21
RECON26	4		20	1	18		2			15	60	126	0.48
RECON29	3				4						7	24	0.29
RECON31	12		25	9	59	3	1	7	23	14	153	136	1.12
TOTAL	3,385	281	359	116	216	38	13	52	180	125	4,765	5,627	0.84

Table 4. Comparison of Hood (2012) and Hood (2006) camera trapping records at the 11 sites common to the two study periods. Records exclude repeated photos of the same individuals. Most sites were monitored using Cuddeback cameras; RECON indicates a deployed Reconyx camera. Records are photograph images of individual mammals; some photographs included multiple individuals. Reconyx records are TRIGGER EVENTS, with a burst of 10 photographs per trigger event. The same individual is not reported more than once in these records, unless captured by subsequent trigger events. Column headings are as follows: DEER = *Odocoileus virginianus*; HOG = *Sus scrofa*; RAC = *Procyon lotor*; ARM = *Dasypus novemcinctus*; RAB = *Sylvilagus aquaticus*; COYOTE = *Canis latrans*; BOBCAT = *Lynx rufus*; OPOS = *Didelphis virginiana*; SQUIR = *Sciurus carolinensis*; OTHER = domestic cats, humans, river otter, rodents, birds; CPUE = Catch-per-unit-effort (captures/camera days).

Camera Site	Year	DEER	HOG	RAC	ARM	RAB	COYOTE	BOBCAT	OPOS	SQUIR	OTHER	Total	Camera Days	CPUE
SITE 2	2012	22	22	9	11	4				15	2	85	188	0.45
	2006	44		1	49	2	5					101	292	0.35
SITE 5	2011	984	41	3	1						4	1033	335	3.08
	2006	17		1	33	1	2		6	15		75	188	0.40
SITE 6	2012	293	4								9	306	292	1.05
	2006	2		2	2					3		9	90	0.10
SITE 11	2012	5										5	22	0.23
	2006	37			2					2	1	42	101	0.42
SITE 20	2012	110	9	6	3	2	4	3			1	138	113	1.22
	2006	13		4	1	8	2			1		29	80	0.36
SITE 21	2012	30	0				2				1	33	146	0.23
	2006	7		3								10	45	0.22
SITE 22	2012	2										2	146	0.01
	2006			1		2						3	75	0.04
RECON23	2012	71		8	3	29				1	10	122	101	1.21
	2006	31				5	11			1		48	99	0.48

Table 4. (cont.)

Camera Site	Year	DEER	HOG	RAC	ARM	RAB	COYOTE	BOBCAT	OPOS	SQUIR	OTHER	Total	Camera Days	CPUE
SITE 24	2012	8	38				4				3	53	146	0.36
	2006	20		4	2	5				2		33	60	0.55
SITE 25	2012			20	2	16	2	3			3	46	146	0.32
	2006	1		20		3	37					61	284	0.22
SITE 33	2012	5		1	11		1	2	1		4	25	117	0.21
	2006	5		7	5	2	36		3	2		60	78	0.77
TOTALS	2012	1,530	114	47	31	51	13	8	1	16	37	1,848	1,752	1.06
	2006	177	0	43	94	28	93	0	9	26	1	471	1,392	0.34

SPECIES ACCOUNTS

The following accounts provide assignments of park status, voucher/observation status, and additional comments for the mammal species documented. Species marked with an asterisk (*) are introduced or domesticated.

ORDER MARSUPIALIA
Family Didelphidae
***Didelphis virginiana* (Kerr 1792)**
Virginia Opossum

Preserve status.—Common. Occurs in swamp, bottomland hardwood forest, natural levees/canal spoil banks, and marshes throughout the Barataria Preserve.

Vouchers/observations.—Hood (2006) reported voucher photographs of live captured specimens and numerous photographic and personal observations of tracks and live individuals. Hood (2012) documented camera-trap records. Four voucher specimens (TU 9407, 9408, 9409, and 9410) and 61 photographs document this species.

Comments.—Virginia opossums were observed in all major habitats of the Barataria Preserve with motion-activated cameras, live-trapping, and observations of sign. A native of wetland habitats of southeastern Louisiana, they are an expected resident species of the Barataria Preserve. JLNHPP hunting records dating back to the mid 1980s record opossums being occasionally taken as "by-catch" to nutria-trapping activities in swamp and marsh habitats. Activity patterns by month show opossum activity being highest in winter months (January–February) with few records in the spring and summer months. During nights of field work to capture and detect bats, opossums were sighted by field workers at Bayou des Familles Bridge, along Christmas Road Trail, and Coquille Trail. Visitors to the park in the early evening report seeing them along roads and trails.

ORDER XENARTHRA
Family Dasypodidae
***Dasypus novemcinctus* (Linnaeus 1758)**
Nine-banded Armadillo

Preserve status.—Common. Occurs in swamp, bottomland hardwood forest, natural levees/canal spoilbanks, and marshes.

Vouchers/observations.—Pre-Katrina, Hood (2006) generated a voucher specimen, photographs of live captured specimens, and photographic and personal observations of burrows, tracks, and live individuals. Post-Katrina, Hood (2012) documented additional camera-trap records. Four voucher specimens (TU 9403, 9404, 9405, and 9406) and 210 photographs document this species.

Comments.—*Dasypus novemcinctus* have recently extended their range from northern Mexico into western Louisiana in the 1920s. The occurrence of *D. novemcinctus* in southeastern Louisiana and in the vicinity of the Barataria Preserve is as recent as the 1970s. Hood (2006) reported that they are among the most common mammals sighted in daylight and early evening hours in the bottomland hardwood forests and swamps east of Bayou des Familles and along all public trails of the park. *Dasypus novemcinctus* were observed in all major habitats of the Barataria Preserve with motion-activated cameras and observations of sign. Post-Katrina, *D. novemcinctus* were photographed at 14 sites in all habitats (Table 3). It is noteworthy that sign (tracks, digging) also was very commonly observed on all natural levee and canal spoil banks throughout the park, including spoil banks extending far into the marsh.

In comparison of 11 co-located camera trap sites pre- and post-Katrina, the same presence/absence was recorded at six of the sites; at four sites, *D. novemcinctus* was recorded pre-Katrina but was not documented in the present study; at one site this species was undocumented pre-Katrina but was recorded post-Katrina (Table 3). For these 11 co-located sites, the total number of *D. novemcinctus* recorded post-Katrina was much lower (CPUE 0.079) than that reported in 2006 (CPUE 0.031).

Activity patterns by month show *D. novemcinctus* activity being highest in fall and winter months (September–February) with few records in the spring and summer months. Hourly records show that *D. novemcinctus* can be active throughout most hours of a 24 hour period, but the highest activity is in the early and later evening hours. The activities of *D. novemcinctus* (digging for food and constructing burrows) in the bottomland hardwood forests and swamps are likely contributing various ecological services to the forest floor ecosystem.

ORDER LAGOMORPHA
Family Leporidae
***Sylvilagus aquaticus* (Bachman 1837)**
Swamp Rabbit

Preserve status.—Common. Occurs in swamp, hardwood forest, natural levees/canal spoilbanks, and marshes.

Vouchers/observations.—Hood (2006) generated voucher photographs and personal observations of burrows, tracks, and live individuals. Hood (2012) documented numerous additional camera-trap records. Four voucher specimens (TU 9465, 9466, 9467, and 9468) and 244 photographs document this species.

Comments.—Swamp rabbits were observed in all major habitats of the Barataria Preserve with motion-activated cameras, live-trapping, and observations of sign (scat, tracks) and live individuals. In southeast ern Louisiana and along the Mississippi Gulf Coast, they are one of the most common mammals of coastal wetlands, including both marsh and swamp habitats. Hood (2006) reported that *S. aquaticus* are active and sighted in early morning and early evening hours in the bottomland hardwood forests and swamps east of Bayou des Familles, along public trails of the park, on natural levees and canal spoil banks, and especially in marsh habitats. Examination of owl pellets collected from *Tyto alba* (barn owls) and *Strix varia* (barred owls) on spoil banks adjacent to marsh habitats contain fragments of *S. aquaticus* bones. *Canis latrans* scats examined throughout the park also contain *S. aquaticus* fur and bones. Thus, *S. aquaticus* represent a major food resource for the raptors and carnivores inhabiting the Barataria Preserve. JLNHPP hunting and trapping records dating back to the late 1980s record *S. aquaticus* being regularly taken as "by-catch" to nutria-trapping in swamp and marsh habitats.

In the post-Katrina study, *S. aquaticus* were photographed at 16 sites in all habitats (Table 3). It is noteworthy that sign (scat) also was very commonly observed on park trails, within bottomland hardwood forests, and on natural levee and canal spoil banks

throughout the park, including spoil banks extending far into the marsh.

In comparison of 11 co-located camera trap sites between the pre- and post-Katrina studies (Hood 2006, 2012), the same presence/absence was recorded at six of the sites; at four sites, *S. aquaticus* were recorded in 2006 but were not documented in the post-Katrina study; at one site they were undocumented in 2006 but were recorded post-Katrina (Table 4). However, overall, *S. aquaticus* were commonly encountered and their populations appear to be stable.

Activity patterns by month show *S. aquaticus* activity being highest in winter months (December–February) with few records in the spring, summer, and fall months. Hourly records show that *S. aquaticus* are strongly nocturnal.

ORDER CHIROPTERA
Family Vespertilionidae
***Myotis austroriparius* (Rhoads 1897)**
Southeastern Myotis

Preserve status.—Uncommon. Occurs in swamps, bottomland hardwood forests, and along waterways. May be locally common in bottomland hardwood forests and swamps, but population size is likely low.

Vouchers/observations.—Hood (2006) reported voucher specimens, photographs of live captured specimens, and electronic detection of calls from the bottomland hardwood forests east of Bayou des Familles and in swamps and along waterways adjacent to the Coquille trail (paralleling Bayou Coquille) and Kenta Canal. Post-Katrina records include many electronic calls. Three voucher specimens (TU 9421, 9422, and 9423) and 301 echolocation recordings document this species.

Comments.—Southeastern myotis are uncommonly and rarely encountered throughout their range in the southern United States. In southeastern Louisiana and along the Mississippi Gulf Coast, they have been most commonly found inhabiting bridges and occasionally roosting in hollow trees of oaks, hickories (*Carya* spp.), and tupelos. The live-trapping of one individual by Hood (2006) and the frequent electronic recording of the species at the study localities throughout the evening hours suggest that the species is resident in the Barataria Preserve and is likely using the bottomland hardwood forest (and swamp) trees as roost sites.

Post-Katrina, *Myotis austroriparius* was the third most commonly recorded bat with electronic bat detectors. Southeastern myotis were recorded at bottomland hardwood forest, bayou/canals, and in swamp habitats. They are not one of the early emerging species, but passive bat detectors deployed at Sites 7 and 8 showed they are active throughout the night until near sunrise. The population status of this species should be studied further at the Barataria Preserve, especially their use of roost tree species. The Louisiana Natural Heritage Program does not list southeastern myotis as a species of concern, but does recommend the need for study to understand its status in the state. Other states and some National Wildlife Refuges list it as a species of concern. Given that southeastern myotis are relatively commonly encountered, Barataria Preserve may be an important population site for the species in Louisiana.

***Perimyotis subflavus* (F. Cuvier 1832)**
American Perimyotis

Preserve status.—Common. Occurs in swamps, bottomland hardwood forests, and along waterways.

Vouchers/observations.—Hood (2006) reported voucher specimens, photographs of live captured specimens, and electronic detection of calls from the hardwood forests east of Bayou des Familles and in swamps and along waterways adjacent to the Coquille trail (paralleling Bayou Coquille) and Kenta Canal. Post-Katrina records include many additional electronic calls. Six voucher specimens (TU 9450–9454 and 9489) and 1,386 electronic recordings document this species.

Comments.—American perimyotis are one of the most commonly encountered bat species at the Barataria Preserve. They are common throughout their range in the southern United States. In southeastern Louisiana and along the Mississippi Gulf Coast, they have been most commonly found inhabiting man-made structures, buildings, and occasionally roosting in trees (hardwoods and pines). The live capture of three individuals pre-Katrina by Hood (2006), as well as the

frequent electronic recording of the species at all localities in that study throughout the evening hours seasons, suggest that the species is resident in the Barataria Preserve and is likely using the bottomland hardwood forest (and swamp) trees as roost sites. As has been found in other studies of the American perimyotis, this species was found to be first species to emerge in the very early evening hours. The documentation of this species as being resident throughout the year was an important new finding by Hood (2006).

Post-Katrina, *P. subflavus* was the most commonly recorded bat with electronic bat detectors considering all sites. American perimyotis were recorded at bottomland hardwood forest, bayou/canals, and in swamp habitats, however they are most common in hardwood forests and along bayous and canals. At Sites 9, 10, and 11 (along Coquille Trail at observation platforms), only a single bat pass sequence (bat flying in range of one detector) was recorded, whereas *N. humeralis* and *M. austroriparius* were commonly captured electronically.

In previous published Louisiana studies (Jones and Pagels 1968; Jones and Suttkus 1973), *P. subflavus* from southeastern Louisiana migrate north during the winter/spring to establish maternity colonies. The population status of this species should be studied further at the Barataria Preserve, especially their use of roost tree species.

***Lasiurus borealis* (Müller 1776)**
Eastern Red Bat
and
***Lasiurus seminolis* (Rhoads 1895)**
Seminole Bat

These two species are presented together because mist netting did not capture any individuals of either species (Hood 2006, 2012). However, bat echolocation recordings that match both species are identified as *L. borealis* is presented as documentation evidencing their occurrence at the Barataria Preserve. The echolocation calls of red bats are considered readily identified compared with other bat species with a characteristic frequency sweep that terminates between 35 and 40 kHz. The echolocation calls of *L. seminolus* are not well established in North America,and is is the only other lasiurine bat species that could overlap with *L. borealis* and they are known to terminate at this range.

Preserve status.—Uncommon. Occurs in swamps, bottomland hardwood forests, and along waterways. May be locally common (in bottomland hardwood forests and swamps), but population size is likely low.

Vouchers/observations.—Hood (2006) reported voucher electronic recordings of echolocation calls from the hardwood forests east of Bayou des Familles and in swamps and along waterways adjacent to the Coquille trail (paralleling Bayou Coquille) and Kenta Canal. Post-Katrina records include electronic calls. Three voucher specimens of *L. borealis* (TU 9411, 9412, and 9413) and 278 electronic recordings (of *L. borealis* and possibly *L. seminolus*) document these species.

Comment.—*Lasiurus borealis* and *L. seminolus* are commonly encountered throughout their ranges in the eastern and southern United States. In southeastern Louisiana and along the Mississippi Gulf Coast, both species have been most often found roosting among Spanish moss-covered hardwoods, in swamps, and in coastal marshes. Based on distributional records in southeastern Louisiana, *L. borealis* and *seminolus* were expected to occur in the Barataria Preserve. Post-Katrina, numerous recordings of bats at 35–40 kHz were made with electronic bat detectors at bottomland hardwood forest, bayou/canals, and in swamp habitats; however, they were most common in hardwood forests and along bayous and canals. These bats are likely either *L. borealis* or *L. seminolis*. The population status of this species should be studied further at the Barataria Preserve.

***Dasypterus intermedius* (H. Allen 1862)**
Northern Yellow Bat

Preserve status.—Uncommon. Occurs in swamps, bottomland hardwood forests, and along waterways. May be locally common (in hardwood forests and swamps), but population size is likely low.

Vouchers/observations.— Hood (2006) reported voucher electronic recordings of echolocation calls from the bottomland hardwood forests east of Bayou des Familles and in swamps and along waterways adjacent to the Coquille trail (paralleling Bayou Coquille) and Kenta Canal. No *D. intermedius* were captured in mist nets. Post-Katrina records include electronic calls.

Two voucher specimens (TU 9414 and 9415) and eight electronic recordings document this species.

Comments.—Northern yellow bats are uncommonly encountered throughout their range in the eastern United States. In southeastern Louisiana and along the Mississippi Gulf Coast, they have been most often found roosting among Spanish moss-covered hardwoods and in association with palmettos. Based on distributional records, yellow bats could possibly be encountered at the Barataria Preserve. However, mist netting did not capture any individuals pre- or post-Katrina (Hood 2006, 2012). Calls recorded by Hood (2006) that were identified as *D. intermedius* are presented as documentation evidencing their occurrence at the Barataria Preserve. The calls of *D. intermedius* are considered readily identifiable compared with other bat species, with a characteristic frequency sweep that terminates at about 25 kHz. No other bats in this area have calls that have echolocation characteristics that overlap with this species.

Post-Katrina, more electronic records were obtained at Sites 1 (Bayou des Familles Bridge), 2 (Bayou des Familes Canoe Launch), 10 (Coquille Trail Platform # 2), and 13 (Kenta Canal/Coquille Bridge). Therefore although rare, *D. intermedius* were recorded at bottomland hardwood forest, bayou/canals and in swamp habitats. The population status of this species should be studied further at the Barataria Preserve.

Nycticeius humeralis (Rafinesque 1818)
Evening Bat

Preserve status.—Uncommon. Occurs in swamps, bottomland hardwood forests, and along waterways. May be locally common (in bottomland hardwood forests and swamps), but population size is likely low.

Vouchers/observations.—Hood (2006) collected voucher specimens, photographs of live captured specimens, and electronic detection of calls from the bottomland hardwood forests east of Bayou des Familles and in swamps and along waterways adjacent to the Coquille trail (paralleling Bayou Coquille) and Kenta Canal. The Post-Katrina records include mist-net and bat detection records (Hood 2012). Six voucher specimens (TU 9425–9428, 9483, and 9484) and 713 electronic recordings document this species.

Comments.—Evening Bats are commonly encountered throughout their range in the eastern United States. In southeastern Louisiana and along the Mississippi Gulf Coast, they have been most often found roosting in buildings, other man-made structures, and in hollow trees (hardwoods and pines) in uplands and swamps. Hood (2006) discovered a maternity colony at the bridge supports at Kenta Canal/Bayou Coquille, live-captured 25 individuals, and frequently electronically recording the species at all study localities throughout the evening hours.

Post-Katrina, four *N. humeralis* were caught in mist nets at Site 13 (the Kenta Canal/Coquille Bridge site) and they were very commonly recorded with electronic bat detectors at all sites. Evening Bats were recorded at bottomland hardwood forest, bayou/canals and in swamp habitats, however, they are most common along bayous and canals and in swamp habitats. Evening Bats are likely using the bottomland hardwood forest (and swamp) trees as roost sites, in addition to man-made structures. Given the discovery of a maternity colony location, the population status and breeding biology of this species should be studied further at the Barataria Preserve.

Corynorhinus rafinesquii (Lesson 1827)
Rafinesque's Big-eared Bat

Preserve status.—Uncommon. Occurs in swamps, bottomland hardwood forests, and along waterways. May be locally common (in bottomland hardwood forests and swamps), but population size is likely low.

Vouchers/observations.—Hood (2006) reported voucher specimens, photographs of live captured specimens, and electronic detection of calls from the hardwood forests east of Bayou des Familles, and in swamps and along waterways adjacent to the Coquille trail (paralleling Bayou Coquille) and Kenta Canal. The post-Katrina study documented bat detection records. Five voucher specimens (TU 9398–9402) and 77 electronic recordings document this species.

Comments.—Rafinesque's big-eared bats are uncommonly encountered throughout their range in the southern United States. In southeastern Louisiana and along the Mississippi Gulf Coast, they have been most often found in maternity or mixed sex colonies

inhabiting bridges, buildings, and occasionally roosting in hollow trees (hardwoods). Hood (2006) mist netted two individuals and made electronic recordings of the species at the study localities early in the evening, suggesting that the species is resident in the Barataria Preserve and is likely using the bottomland hardwood forest trees as roost sites. Post-Katrina, *C. rafinesquii* were electronically recorded at nearly every site and were found in bottomland hardwood forests, along bayous/canals, and in swamp habitats. They are not one of the early emerging species, but passive bat detectors deployed at some sites showed they are active throughout the night until near sunrise. Although not common, there appears to be clear evidence that *C. rafinesquii* have and are maintaining a resident population in Barataria Preserve. The population status of this species should be studied further at the Barataria Preserve, especially their use of roost tree species. The Louisiana Natural Heritage Program does not list *C. rafinesquii* as a species of concern, but does recommend the need for study to understand its status in the state. Other states and some National Wildlife Refuges list it as a species of concern.

Family Molossidae
***Tadarida brasiliensis* I. Geoffroy 1824**
Brazilian Free-tailed Bat

Preserve status.—Uncommon. Occurs in swamps, bottomland hardwood forests, and along waterways. May be locally common (in bottomland hardwood forests and swamps), but population size is likely low.

Vouchers/observations.—Hood (2006) reported voucher specimens, photographs of live captured specimens, and electronic detection of calls from the hardwood forests east of Bayou des Familles, and in swamps and along waterways adjacent to the Coquille trail (paralleling Bayou Coquille) and Kenta Canal. The post-Katrina study documented bat detection records. Three voucher specimens (TU 9469–9471) and 31 electronic recordings document this species.

Comments.—*Tadarida brasiliensis* are commonly encountered throughout their range in North America. Along the Mississippi Gulf Coast they have been most often found roosting in buildings and other man-made structures. *Tadarida brasiliensis* form very large colonies (thousands of individuals), usually occupying large cave systems or abandoned buildings. Based on their natural history and distributional records in southeastern Louisiana, *T. brasiliensis* was expected to be encountered at the Barataria Preserve as a non-resident bat, foraging at the park from nearby urban areas. Hood (2006) mist-netted four individuals and made electronic recordings of the species at Kenta Canal and Bayou des Familles late in the evening, suggesting that the species is not a resident in Barataria and is likely foraging in the park habitats.

Post-Katrina, no *T. brasiliensis* were captured by mist nets, but a few electronic recordings were made that were very similar to the pre-Katrina results. The passive bat detectors at forest sites had but two pass sequences at about 5–6 hrs after sunset (after midnight). These results are consistent with other records to suggest that *T. brasiliensis* may well be roosting outside of Barataria. The status of this species should be studied further at the Barataria Preserve, especially to establish their residency, population status, and use of the park.

ORDER CARNIVORA
Family Canidae
***Canis latrans* (Say 1823)**
Coyote

Preserve status.—Common. Occurs in all park habitats—swamp, bottomland hardwood forest, natural levees/canal spoil banks, and marshes.

Vouchers/observations.—Hood (2006) generated voucher photographs and personal observations of tracks, scat, and live individuals. The post-Katrina study documented camera-trap records and personal observations of tracks and scat. Six voucher specimens (TU 9392–9397) and 131 photographs document the species.

Comments.—*Canis latrans* were observed in all major habitats of the Barataria Preserve with motion-activated cameras and observations of sign. They are non-native to Louisiana and to the Barataria Preserve, having extended their range from the western United States into western Louisiana in the 1940s. The first occurrence of *C. latrans* in southeastern Louisiana and

in the vicinity of the Barataria Preserve is as recent as the 1950s. The first confirmed observational record of *C. latrans* at the Barataria Preserve was in 1987 (naturalist record). Today, *C. latrans* are among the common large mammals in all habitats of the park. Hood (2006) reported that motion-activated cameras captured more than 90 photographs of *C. latrans*, many of which appear to be different individuals. *Canis latrans* sign (scat, tracks, digging) was commonly observed on natural levees and canal spoil banks throughout the park. The sound of howling (by groups of individuals) was commonly heard in the early evenings from the Marsh Overlook platform on the Bayou Coquille trail. Scats include hair and bone fragments of *S. aquaticus* and *S. carolinensis*.

Post-Katrina, *C. latrans* were photographed at 13 sites in all habitats (Table 3). It is noteworthy that sign (scat) also was very commonly observed on park trails, within bottomland hardwood forests, and on natural levee and canal spoil banks throughout the park, including spoil banks extending far into the marsh.

In comparison of 11 co-located camera trap sites with pre-Katrina studies (Hood 2006), the same presence/absence was recorded at six of the sites; at three sites, *C. latrans* recorded in 2006 were not documented in the present study; at two sites they were undocumented in 2006 but recorded in the present study (Table 4). An important difference in methodology was that camera-traps were "baited" with *C. latrans* urine in the earlier study but were not baited in the later study.

Activity patterns by month show *C. latrans* are encountered throughout the year, with the highest activity in winter months (December–January). Hourly records show that *C. latrans* can be active throughout most hours of a 24 hour period, but the highest activity is in evening hours.

The status of this species should be studied further at the Barataria Preserve, especially to establish its population size, dynamics, and impact on the overall ecology of the natural systems. Given the camera-trap records and sightings from the present study, *C. latrans* have established a resident population at Barataria.

Family Procyonidae
***Procyon lotor* (Linnaeus 1758)**
Raccoon

Preserve status.—Common. Occurs in swamp, bottomland hardwood forest, natural levees/canal spoil banks, and marshes, i.e., occurs in all habitats.

Vouchers/observations.—Hood (2006) generated voucher photographs and personal observations of tracks, scat, and live individuals. The post-Katrina study documented camera-trap records and personal observations of tracks, scat, and live individuals. Five voucher specimens (TU 9455–9459) and 402 photographs document this species.

Comments.—*Procyon lotor* were observed in all major habitats of the Barataria Preserve with motion-activated cameras and observations of sign and live individuals. They are adaptable, wide ranging generalists that can live in many different habitats and settings. Today, they are among the most common medium-large mammals in all habitats of the park. Hood (2006) reported that motion-activated cameras captured many photographs of *P. lotor*, some of which appear to be different individuals. *Procyon lotor* sign (scat, tracks) was commonly observed on park trails, in forests, in swamps, and on natural levees and canal spoil banks.

In the post-Katrina study, *P. lotor* were photographed at 13 sites in all habitats (Table 3). It is noteworthy that sign (scat) also was very commonly observed on park trails, within bottomland hardwood forests, and on natural levee and canal spoil banks throughout the park, including spoil banks extending far into the marsh. In many instances, camera-traps captured two or three *P. lotor* traveling together; this was especially common using the Reconyx cameras that capture 10 photographs per triggering event with a 10-second camera re-set.

In comparison of 11 co-located camera trap sites between pre- and post-Katrina, the same presence/absence was recorded at six of the sites; at three sites *P. lotor* were recorded in 2006 but were not documented in the present study; at two sites they were undocumented in 2006 but were recorded in the post-Katrina study

(Table 4). Activity patterns by month show *P. lotor* are active in fall and winter months, with the highest activity in winter months (December–February).

Family Mustelidae (mustelids)
***Mustela vison* (Schreber 1777)**
American Mink

Preserve status.—Uncommon. Occurs in marshes and swamps. May be locally common, but population size is likely low.

Vouchers/observations.—Hood (2006) reported one road-killed voucher specimen (TU 9474). No additional records were documented in the present study.

Comments.—*Mustela vision* are not common at the Barataria Preserve, but observations and records indicate that they inhabit marsh habitats. None were documented by motion-activated cameras, trapping, or observation of sign by Hood (2006). One *M. vision* was found dead on LA HWY 45 within the park boundaries. JLNHPP hunting and trapping records dating back to the mid-1980s record *M. vision* as being occasionally taken as "by-catch" to nutria-trapping in swamp and marsh habitats. The most recent of these records are from 1992, although trapping focus has been on *Myocastor coypus* this past decade. Post-Katrina, no *M. vision* were live-captured or found as road-killed specimens. The status of this species should be studied further at the Barataria Preserve, especially to increase knowledge of their residency, population status, and use of the park.

***Lontra canadensis* (Schreber 1777)**
River Otter

Preserve status.—Uncommon. Occurs in marshes, swamps, along waterways, and in bottomland hardwood forests. May be locally common, but population size is likely low.

Vouchers/observations.—Hood (2006) generated a voucher specimen (road-killed), a voucher photograph, and personal observations of tracks, scat, and live individuals. Hood (2012) documented a camera-trap record and a second road-killed individual. Two voucher specimens (TU 9416, 9417), and one photograph document this species.

Comments.—*Lontra canadensis* are not common at the Barataria Preserve, but regular observations and records indicate that they are residents of the park. None were documented by motion-activated cameras, trapping, or observation of sign by Hood (2006). Two *L. canadensis* were found dead on the Lafitte-LaRose HWY (LA HWY 3134) at the northern boundary park. NPS staff and visitors report visual observations of *L. canadensis* along park trails (Plantation trail and the Bayou Coquille trail), in waterways (Twin Canals, Kenta Canal, Bayou des Familles), and on natural levees and canal banks within the marsh habitats of the park about five to six times per year. JLNHPP hunting and trapping records dating back to the mid-1980s record *L. canadensis* as being occasionally taken as "by-catch" to nutria-trapping in swamp and marsh habitats.

Post-Katrina, a *L. canadensis* was found dead on Barataria Boulevard 1 mi N of the NPS boundary and one was photographed at Site 25 on the Oak Chenier spoil bank. Park visitors and NPS staff regularly make sightings of *L. canadensis* along NPS trails, especially on Coquille Trail. The status of this species should be studied further at the Barataria Preserve, especially to increase knowledge of their residency, population status, and use of the park. Given the camera-trap records and sightings from the present study, *L. canadensis* have established a resident population at Barataria.

Family Felidae (cats)
***Lynx rufus* (Schreber 1777)**
Bobcat

Preserve status.—Uncommon. May be locally common.

Vouchers/observations.—Hood (2006) generated a voucher photograph of tracks at a scent station. The post-Katrina study documented camera-trap records at multiple sites. Two voucher specimens (TU 9418 and 9464) and 13 photographs document this species.

Comments.—The distribution records of *L. rufus* and their natural history suggests that they should be expected to occur at the Barataria Preserve. No documented records existed prior to the study by Hood (2006), although a visitor sighting (on one of the Plantation trails) was reported in 2000. A set of well-preserved, fresh tracks were observed at a scent station

that was established on the Oak Chenier site locality. The station had been baited with *L. rufus* urine scent. The tracks were photographed and a motion-activated camera placed at the site. Unfortunately, no additional tracks or photographs of live individuals were captured thereafter during that study.

In the post-Katrina study, *L. rufus* were photographed at seven sites in bottomland hardwood forests, swamps, swamp/forest transition areas, and on spoil banks adjacent to marshes (Table 3). Visitors and NPS staff have reported sightings of *L .rufus* fairly regularly, and litters of kittens have been observed along Palmetto Trail, beginning in early summer 2012.

In comparison of 11 co-located camera trap sites pre- and post-Katrina, the same presence/absence was recorded at eight of the sites (no *L. rufus* records); at three sites they were undocumented in 2006 but recorded in the present study (Table 3). An important difference in methodology was that in Hood (2006) camera-traps were "baited" with *C. latrans* or *L. rufus* urine and that in the post-Katrina study they were not "baited"; therefore, the overall *L. rufus* activity records suggest a resident population.

Activity patterns by month show *L. rufus* can be encountered throughout the year, with the highest activity in winter months (December–February). Although sample sizes were low, the highest number of *L. rufus* were recorded in December (with 10 records from three localities). The status of this species should be studied further at the Barataria Preserve, especially to establish their residency, population status, and use of the park. Given the camera-trap records from Post-Katrina, it appears that *L. rufus* have established a resident population at Barataria.

ORDER ARTIODACTYLA
Family Cervidae
***Odocoileus virginianus* (Zimmermann 1780)**
White-tailed Deer

Preserve status.—Common. Occurs in swamp, bottomland hardwood forest, natural levees/canal spoil banks, and marshes—ie., occurs in all habitats.

Vouchers/observations.—Hood (2006) generated a voucher specimen, voucher photographs, and personal observations of tracks, scat, and live individuals. The post-Katrina study documented numerous camera-trap records. Nine voucher specimens (TU 9429–9437) and 3,562 photographs document this species.

Comments.—*Odocoileus virginianus* were recorded in all major habitats of the Barataria Preserve with motion-activated cameras as well as observations of sign. They are the most common large mammals in all habitats of the park. Visitors can observe *O. virginianus* or their sign along park trails, and visitors walking on the Plantation trails (in bottomland hardwood forest) in early mornings or evenings are likely to see or hear *O. virginianus* moving through the forest. Hood (2006) reported motion-activated camera data of more than 150 photographs, many of which appear to be different individuals. Many of these records come from natural levees and canal spoil banks adjacent to marsh habitats. *Odocoileus virginianus* populations are clearly quite large for the size and nature of the habitat at the Barataria Preserve. The impacts of *O. virginianus* populations on the park's ecology should be studied, especially as recent housing developments immediately north of the park have converted forested areas into residential subdivisions.

In the post-Katrina study, *O. virginianus* were photographed at 35 sites in all habitats (Table 3). Camera-trap photographs document antlered *O. virginianus* throughout much of the year, with most fawns being recorded from early summer into the early winter. In many instances, camera-traps captured two to three *O. virginianus* traveling together, especially using the Reconyx cameras that capture 10-photographs per triggering event with a 10-second camera re-set for the next triggering event.

In comparison of 11 co-located camera trap sites between the previous study (Hood 2006) and the post-Katrina study, the same presence/absence was recorded at nine of the sites; at one site *O. virginianus* was recorded in 2006 but was not documented in the post-Katrina study; and at one site they were undocumented in 2006 but were recorded in the post-Katrina study (Table 4).

Activity patterns by month show *O. virginianus* are present throughout the year, but have the highest activity in fall and winter months (October–January).

Hourly records show that *O. virginianus* can be active at all times, but their highest activity is in crepuscular hours near dawn and sunset.

Chamberlain and Nyman (2006), reported the results of a series of studies (see their appendices 1–5; Bernatas 2003, 2004; Nyman 2004; Scognamillo and Nyman 2004; Bordelon 2005) using analyses of hunting reports, aerial photography, thermal aerial photography, and browse surveys to address *O. virginianus* population size, distribution, and impacts in major habitats of the park. Their overall findings included: a) the documentation of a substantial *O. virginianus* population in all major habitats; b) some, but limited, impact on understory vegetation browsed within the hardwood bottomland forests of the Big Woods; c) application of thermal imaging for *O. virginianus* census; and d) recommendations for hunting management.

Post-Katrina, camera-traps provided complementary data that are consistent with their results—*O. virginianus* populations through the major habitats of the park are very substantial. The camera-traps provide data on mammals in especially dense habitats (e.g., in hardwood forest Sites 1–12 and swamp Sites 31–33) that are difficult to census with aerial photography or line transect methods. The camera-trap data documented the timing of activity (date, time), status of individual *O. virginianus* (fawn, not antlered, in velvet, antlered, spike buck, large point buck, group size), as well as behavior (travel, browse, agonistic behaviors, fighting).

*Family Suidae (pigs)
******Sus scrofa* **(Linnaeus 1758)**
Wild Boar

Preserve status.—Common post-Katrina, absent previously. A small number of *S. scrofa* were hunted and extirpated from the park in the early 1980s. The species now occurs in swamps and/or bottomland hardwood forests and is expanding into marsh habitats along spoil bank corridors. *Sus scrofa* have emerged as a resident population.

Vouchers/observations.—Pre-Katrina, Hood (2006) did not find any evidence of *S. scrofa* at Barataria Preserve. Post-Katrina studies documented numerous camera-trap records, observations, tracks, and scat. No voucher specimens and available but 281 photographs document the species.

Comments.—*Sus scrofa* is a moderately-common, non-native invasive species of forests and agricultural areas in much of the eastern United States. They are common in forested areas of southeastern Louisiana. Large populations of *S. scrofa* can be found in bottomland hardwood forests and swamps adjacent to the Mississippi River levee and in forests near Belle Chasse, Louisiana (5 miles east of Barataria Preserve). Given their distributional records, they were expected to occur in the park and to be a serious management concern. A small number of *S. scrofa* were hunted and extirpated from the park in the early 1980s (pers. comm., David Muth, Chief of Planning and Resource Stewardship). None were documented by motion-triggered cameras, trapping, or observations of sign by Hood (2006). The lack of observations then suggested that *S. scrofa* did not occur in the park at that time. In February 2006, following fieldwork for that earlier study, several visitor reports were made of *S. scrofa* in the area of south Plantation trail. Post-Katrina, *S. scrofa* began to establish a large, resident population in most forested areas; the presence of this species represents a significant natural resource management concern.

In the post-Katrina study, *S. scrofa* were photographed at 17 sites in all major habitats (Table 3). It was noteworthy that only at Site 24 (former spoil bank on Tarpaper Canal) were *S. scrofa* photographed in a marsh habitat. Large adult *S. scrofa* (black and multicolored) were regularly photographed in bottomland hardwood sites in the Big Woods area (Sites 1–12), as were piglets. In many instances, camera-traps captured two to three large adult males and females traveling together, as well as sequences of 4–5 piglets traveling with adults. This was especially documented by using the Reconyx cameras that capture 10-photographs per triggering event with a 10-second camera re-set for the next triggering event.

In comparison of 11 co-located camera trap sites pre- and post-Katrina, the same presence/absence was recorded at five of the sites (all with no *S. scrofa*, as none were recorded in pre-Katrina); at six sites they were undocumented in pre-Katrina but recorded post-Katrina (Table 4). Activity patterns by month show *S. scrofa* are present throughout the year, but they have

the highest activity in fall and winter months (September–February).

To date, no hogs have been recorded in camera-trap sites in Bayou aux Carpes, nor in most marsh locations, so special efforts should be made to monitor and control their spread into these areas. The number of individuals and the physical evidence of impact (destructive rooting) make the management and control of *S. scrofa* the most important natural resource issue at Barataria Preserve.

ORDER RODENTIA
Family Sciuridae
***Sciurus carolinensis* (Gmelin 1788)**
Eastern Gray Squirrel

Preserve status.—Common. Occurs in swamps, bottomland hardwood forests, and natural levees/canal spoil banks.

Vouchers/observations.—Hood (2006) generated a voucher specimen, photographs of live captured specimens, and photographic and personal observations of tracks, scat and live individuals. The post-Katrina study documented camera-trap records. Three voucher specimens (TU 9461–9463) and 206 photographs document this species.

Comments.—*Sciurus carolinensis* were observed in all major habitats of the Barataria Preserve, except for marshes, with motion-activated cameras, live-trapping, and observations of sign (scat, tracks) and live individuals. In southeastern Louisiana and along the Mississippi Gulf Coast, they are a common squirrel of wooded areas, especially near cities and towns. Hood (2006) reported that *S. carolinensis* are active and sighted in daylight hours in the bottomland hardwood forests and swamps east of Bayou des Familles, along public trails of the park, on natural levees and canal spoil banks. Owl pellets collected from *S. varia* on spoil banks adjacent to marsh habitats can contain fragments of *S. carolinensis* bones. *Canis latrans* scats examined throughout the park can include *S. carolinensis* fur and bones. Thus, *S. carolinensis* represent a food resource for the raptors and carnivores inhabiting the Barataria Preserve. JLNHPP hunting and trapping records dating back to the mid-1980s record *S. carolinensis* as being taken regularly during the fall hunting season. During the 2002–2004 hunting seasons, several hundred were taken by hunters annually (pers. comm., Leigh Zahm, NPS Law Enforcement officer). In 2006, enforcement officers and park naturalists report that in reviewing hunting takes of squirrels, all were *S. carolinensis*, as opposed to *Sciurus niger* (fox squirrels).

In the post-Katrina study, *S. carolinensis* were photographed at eight sites in hardwood forest and swamp habitats (Table 3). As *S. carolinensis* are small mammals, the ability of motion-triggered camera traps to detect them can be questioned. At Sites 1 and 20, where both Cuddeback and Reconyx camera traps were simultaneously deployed for several months, the Reconyx camera photographed many more individuals than did the Cuddeback.

In comparison of 11 co-located camera trap sites between the pre- and post-Katrina studies (Hood 2006, 2012), the same presence/absence was recorded at one of the sites; at one site *S. carolinensis* was recorded in 2006 but were not documented in the post-Katrina study; at the remaining nine sites they were undocumented in 2006 but were recorded post-Katrina (Table 4). These data may indicate that *S. carolinensis* activity and populations are greater now than in 2006. However, the ability of the digital camera-traps used in the post-Katrina study may also explain a higher number of records.

Family Cricetidae
***Oryzomys palustris* (Harlan 1837)**
Marsh Rice Rat

Preserve status.—Common. Occurs in marsh and swamp habitats.

Vouchers/observations.—Hood (2006) reported voucher specimens, photographs of live captured specimens, and photographic and personal observations of live individuals. Post-Katrina, *O. palustris* were commonly live-captured and re-captured in flotant marsh. One voucher specimen (TU 9441) and 77 live-capture/release records document this species.

Comments.—*Oryzomys palustris* are the most common rodent inhabiting marsh habitats in the Barataria Preserve. Hood (2006) reported a total of 23 individuals live-trapped within marsh habitat and

an additional 21 recaptures were made. However, of more than 400 trap-nights on spoil banks adjacent to marsh habitats, no *O. palustris* were captured. This suggested that they do not frequently use spoil bank habitats. Additionally, of more than 5,000 trap nights in bottomland hardwood forests and swamps, no *O. palustris* were captured. Owl pellets collected from barn owls and barred owls on spoil banks adjacent to marsh habitats predominantly contained fragments of *O. palustris* bones.

Post-Katrina, *O. palustris* were live-captured and re-captured at the Marsh Site near Horseshoe Canal during 800 trap-nights in February 2012. Of these individuals, two-thirds were males and nearly half were juveniles/subadults.

Comparisons with a comparable trapping site from Hood (2006), Tarpaper Canal Marsh, showed very comparable results, although the CPUE and capture rates were higher and the re-capture rates lower post-Katrina. These results suggest healthy populations of *O. palustris* inhabit the flotant marshes of Barataria.

Camera-traps on spoil banks at Sites 26 (adjacent to marsh habitat) and 31 (adjacent to swamp habitat) recorded large rodents that could not be identified, but could have been large, adult *Orzyomys palustris*. Site 26, adjacent to a flotant marsh, is a plausible location for marsh rice rats to utilize spoil banks.

***Peromyscus leucopus* (Rafinesque 1818)**
White-footed Mouse

Preserve status.—Common. Occurs in bottomland hardwood forests and swamp habitats.

Vouchers/observations.—Hood (2006) reported voucher specimens and photographic and personal observations of live individuals. Post-Katrina, *Peromyscus leucopus* were commonly live-captured in bottomland hardwood forest. Eight voucher specimens (TU 9442–9449) and 44 live-capture/release records document this species.

Comments.—*Peromyscus leucopus* is one of the common rodents inhabiting forested areas and riparian habitats in the eastern United States. Given distributional and previous voucher records, it was not surprising that it is the most common rodent inhabiting forested habitats in the Barataria Preserve. Hood (2006) reported 29 individuals live-trapped within hardwood forests and swamps and an additional 18 recaptures were made. However, of more than 400 trap-nights on spoil banks adjacent to marsh habitats, no *P. leucopus* were captured. Likewise, owl pellets collected from barn owls and barred owls on spoil banks adjacent to marsh habitats did not contain any fragments of *P. leucopus* bones. This suggests that P. *leucopus* do not frequently use spoil bank habitats deep within the marsh.

Post-Katrina, 37 *P. leucopus* were live-captured (none re-captured) at the Hardwood Forest Site N of Plantation Trail during 1,836 trap-nights in January–February 2012. Of these individuals, two-thirds were males and one-quarter were juveniles/subadults.

Comparisons with a co-located trapping site from Hood (2006), Original Site 4, showed very comparable results, although the CPUE and capture rates were much higher and the re-capture rates lower (zero vs. 91%) in the post-Katrina study. These results suggest healthy populations of *P. leucopus* inhabit the bottomland hardwood forests of Barataria.

In southeastern Louisiana and the Mississippi Gulf Coast, white-footed mice can be found sympatrically with a congener, the cotton mouse (*Peromyscus gossypinus*). To date, *P. gossypinus* have not been documented to occur in the Barataria Preserve.

***Sigmodon hispidus* (Say or Ord 1825)**
Hispid Cotton Rat

Preserve status.—Uncommon. Only recorded from marsh habitats. May be locally common (in marsh and swamp habitats), but population size is likely low.

Vouchers/observations.—Hood (2006) reported no voucher specimens, but two individuals were live captured and marked/released. No additional records were documented in the post-Katrina study. No voucher specimens are available; three live-capture/release records document this species.

Comments.—*Sigmodon hispidus* is a common rodent inhabiting many grassland, wetland, forest, and

riparian habitats, as well as agricultural land in the United States, including Louisiana. Given its distributional records, hispid cotton rats were expected to be found in some or all of the habitats in the Barataria Preserve. Hood (2006) reported three individuals live-trapped and these were captured (and re-captured multiple times) within a *Sagittaria*-dominated marsh. These individuals were marked and released and no others captured thereafter. More than 400 trap-nights on spoil banks adjacent to marsh habitats did not result in any captures. None of the owl pellets collected from barn owls and barred owls on spoil banks adjacent to marsh habitats contained fragments of *S. hispidus* bones. *Sigmodon hispidus* were not captured in more than 5,000 trap-nights of collecting in forests and swamps. Despite the lack of additional captures or observations, the documentation of *S. hispidus* within a marsh site far from other habitats suggests that there is a resident population at the Barataria Preserve.

Post-Katrina, no hispid cotton rats were live-captured and re-captured at the Marsh Site near Horseshoe Canal during 800 trap-nights in February 2012. The status of this species should be studied further at the Barataria Preserve, especially to establish their residency, population status, and use of the park.

***Ondatra zibethicus* (Linnaeus 1766)**
Muskrat

Preserve status.—Uncommon. Occurs in marsh habitats. May be locally common (in marsh habitats), but population size is likely low.

Vouchers/observations.—Hood (2006) reported a voucher specimen (skull), photographs of an active *O. zibethicus* mound, and personal observations of tracks and scat. No additional voucher specimens were documented in the present study, however, areas of marsh damage ("muskrat eatouts") have been observed in flotant marshes in the northern end of Barataria. Two voucher specimens (TU 9439 and 9440) document this species.

Comments.—Historically, muskrats were the most important fur-bearing mammal in coastal Louisiana. However, significant declines in muskrat populations have been noted during the past several decades. Some resource managers attribute this to population expansion by *Myocastor coypus*. Apparently, *O. zibethicus* have not been abundant in Barataria for many decades. Hood (2006) reported that at that time they appeared to be restricted to *Scirpus* spp.-dominated marsh habitats, which are uncommon and patchy in Barataria. JLNHPP hunting and trapping records dating back to the mid-1980s record muskrats being rarely taken as "by-catch" to nutria-trapping in swamp and marsh habitats.

Post-Katrina, *O. zibethicus* mounds and large areas of marsh damage by them (and perhaps nutria) were discovered by NPS resource managers in marshes at the far northern portions of the Preserve adjacent to Bayou Segnette Waterway. These areas were recently added to the park boundaries. Given the historical and cultural significance of *O. zibethicus* to the region, the population status of this species should be studied further at the Barataria Preserve, especially to increase knowledge of their residency and population status. Study of the ecological relationship between *O. zibethicus* and *M. coypus* (which are highly abundant in some locations) is another potentially important area of future research

*Family Muridae
****Rattus rattus* (Linnaeus 1758)**
Black Rat

Preserve status.—Uncommon. Occurs in near NPS buildings, public facilities, and in nearby forested areas.

Vouchers/observations.—Hood (2006) reported one voucher specimen (TU 9460) and personal observations of live individuals. No additional records were documented post-Katrina.

Comments.—*Rattus rattus* is an introduced species commonly associated with buildings and urban areas. At the Barataria Preserve, they are pests that occasionally occupy NPS buildings and public facilities. Hood (2006) reported one individual trapped at the Twin Canals forest trapping locality, which was within 200 meters of the Twin Canals public boat launch. With no other specimens captured in more than 7,500 trap-nights of effort, it is likely that *R. rattus* do not occur in natural habitats of the park. An effort should be made to control the population of *R. rattus* that live near NPS facilities to ensure that their populations do

not grow and expand into natural areas. No *R. rattus* were live-captured in a total of 2,636 trap nights in hardwood forest and marsh habitats. Camera-traps on spoil banks at Sites 26 (adjacent to marsh habitat) and 31 (adjacent to swamp habitat) recorded large rodents that could not be identified, but could have been *Rattus rattus*.

***Mus musculus* (Linnaus 1758)**
House Mouse

Preserve status.—Uncommon. Occurs in near NPS buildings, public facilities, and in nearby forested areas.

Vouchers/observations.—Hood (2006) reported one voucher specimen (TU 9418) and personal observations of live individuals. No additional records were documented in the post-Katrina study.

Comments.—*Mus musculus* is an introduced species commonly associated with buildings and urban areas. At the Barataria Preserve, they are pests that occasionally occupy NPS buildings and public facilities. Hood (2006) reported one individual was trapped at the Twin Canals forest trapping locality, which was within 200 meters of the Twin Canals public boat launch. With no other specimens captured in more than 7,500 trap-nights of effort, it is likely that *M. musculus* do not occur in most natural habitats of the park. As in the account of *R. rattus*, an effort should be made to control their populations at NPS facilities so that their populations do not grow and expand into natural areas. Post-Katrina, no *M. musculus* were live-captured in a total of 2,636 trap nights in hardwood forest and marsh habitats.

*Family Myocastoridae (myocastorids)
***Myocastor coypus* (Molina 1782)**
Nutria

Preserve status.—Common. Occurs in marsh and swamp habitats.

Vouchers/observations.—Hood (2006) reported photographs and personal observations of tracks and scat. No additional records were documented in the post-Katrina study. One voucher specimen (TU 9416) and seven photographs document this species.

Comments.—*Myocastor coypus* are an introduced species that became established in Louisiana in the 1920s and dramatically expanded their distributional range and population numbers. Numbers of *M. coypus* within the marshes of the Barataria Preserve are so great that active management programs have been in place for many years. Several important studies concerning population biology have been conducted in the park during the past few years. Nolfo-Clements (2009, 2012) documents their home range, fine scaled habitat use (to plant species), and movement in the flotant marsh. *Myocastor coypus* are usually active and can be sighted in early morning and early evening hours in swamps, along public trails of the park, on natural levees and canal spoil banks, and especially in marsh habitats. They can be seen on trails within the forests east of Bayou des Familles, but are less common there.

Post-Katrina, it was surprising that no *M. coypus* were recorded with camera-traps that included more than 5,000 camera-days and 6,000 photographs of mammals. The camera traps at marsh (and adjacent spoil bank) locations were expected to capture some *M. coypus* activity, but did not.

Given the historical and cultural significance of trapping to the region, the status of this species should be studied further at the Barataria Preserve, especially to develop management strategies (their residency and population status is well established). A study of the ecological relationship between *O. zibethicus* and *M. coypus* is another potentially important area of future research.

Accounts of Species Reported but Undocumented or no Longer Present

The following accounts provide comments on the status of species that have been reported (in previous NPS inventories, public hunting and trapping records, and naturalist reports) or have been observed by NPS staff as occurring within the Barataria Preserve in the past (before 1990). Species with asterisk (*) are introduced or domesticated.

ORDER CARNIVORA
Family Canidae
***Vulpes vulpes* (Linnaeus 1758)**
Red Fox

Preserve status.—Unconfirmed. This species, if it occurs in the Preserve, would be expected to occur in swamps, marshes, natural levees and canal spoil banks, and/or bottomland hardwood forests.

Vouchers/observations.—None verified.

Comments.—*Vulpes vulpes* are moderately-common inhabitants of forests and agricultural areas in northern and central Louisiana. Historically, they were targeted as furbearers. They are not common in southeastern Louisiana, although they have been observed in habitats on higher ground in the region over the past 20–30 years. Given their distributional records, they would not be expected to occur in the park. Hood (2006) did not document any *V. vulpes* by motion-activated cameras, scent stations (baited with *V. vulpes* urine), trapping, or observations of sign in the present study. JLNHPP hunting and trapping records dating back to the mid-1980s do not record any taken as "by-catch", although annotations of "fox" occasionally are noted.

Post-Katrina, no *V. vulpes* were documented with camera-traps or with confirmed observations, despite deployment of 39 camera sites resulting in more than 5,000 camera-days of observation. *Canis latrans* and *L. rufus* were captured with these same camera traps, but no *V. vulpes* have been documented to date.

***Urocyon cinereoargenteus* (Schreber 1775)**
Gray Fox

Preserve status.—Unconfirmed. This species, if it occurs in the Preserve, would be expected to occur in swamps, marshes, natural levees and canal spoil banks, and/or bottomland hardwood forests.

Vouchers/observations.—None verified.

Comments.—*Urocyon cinereoargenteus* are moderately-common inhabitants of forests and agricultural areas in northern and central Louisiana. Historically, they were targeted as furbearers. They are not common in southeastern Louisiana, although they have been observed in habitats on higher ground in the region in recent years. Hood (2006) did not document any *U. cinereoargenteus* by motion-activated cameras, scent stations (baited with *V. vulpes* and *C. latrans* urine), trapping, or observations of sign in the post-Katrina study. JLNHPP hunting and trapping records dating back to the mid-1980s do not record any taken as "by-catch", although annotations of "fox" occasionally are noted. Several visitor reports have been made of "gray fox" on park trails, however field identification of *U. cinereoargenteus* and small (young adult) *C. latrans* is difficult.

Post-Katrina, no *U. cinereoargenteus* were documented with camera-traps or with confirmed observations, despite deployment of 39 camera sites resulting in more than 5,000 camera-days of observation. *Canis latrans* and *L. rufus* were captured with these same camera traps, but no *U. cinereoargenteus* have been documented to date.

ORDER RODENTIA
Family Sciuridae
***Glaucomys volans* (Linnaeus 1758)**
Southern Flying Squirrel

Preserve status.—Unconfirmed. This species, if it occurs in the Preserve, would be expected to occur in swamps and/or bottomland hardwood forests.

Vouchers/observations.—None verified.

Comments.—*Glaucomys volans* are common inhabitants of hardwood forests in the Southern United States, especially those dominated by oaks and hickories. Given their distributional records, and the large number of large oak and other hardwood tree species in forested areas of the Barataria Preserve, *G. volans* would be expected to occur in the park. Hood (2006) used live traps (10 stations) positioned on trees (at 2 m height) within the north and south Old Barataria trail trapping sites for 66 trap-nights, but failed to capture any individuals. Motion-triggered cameras in this area also did not photograph any *G. volans*. The bat field work included more than 20 nights of work in these areas, and another 20 nights of field observations did

not lead to observations of *G. volans*. There does not seem to be any particular reason why *G. volans* would not occur at the Barataria Preserve, aside from their evading observation given their secretive nature.

Post-Katrina, no *G. volans* were live-captured or documented with confirmed observations. NPS staff and visitors have sporadically reported possible sightings. The status of this species should be studied further at the Barataria Preserve, especially to increase knowledge of their residency, population status, and use of the park.

Family Castoridae
***Castor canadensis* (Kuhl 1820)**
American Beaver

Preserve status.—Historical. A beaver dam was constructed in the 1980s and was occupied for a short time, but there has been no reported beaver activity since then. This species, if it occurs in the Preserve, would be expected to occur in swamps and/or bottomland hardwood forests.

Vouchers/observations.—No vouchers. A beaver dam was constructed in the 1980s and was occupied for a short time.

Comments.—*Castor canadensis* are moderately-common species of forested wetlands and waterways in much of eastern United States. They are not common in southeastern Louisiana. Given their distributional records, they could be expected to occur in the preserve. A beaver dam was observed to be constructed north of Kenta Canal in the early 1980s (pers. comm., David Muth, Chief of Planning and Resource Stewardship). It was not maintained for more than a couple of years and the *C. canadensis* was presumed to have left. No *C. canadensis* were documented by motion-triggered cameras, trapping, or observations of sign in the post-Katrina study, although a special effort was made to determine if they were present in the Preserve. The lack of observations suggests that they do not presently occur there. This species should be studied further at the Barataria Preserve, especially to establish their residency, population status, and use of the park.

Family Cricetidae (native mice and rats)
***Neotoma floridana* (Ord 1818)**
Eastern Woodrat

Preserve status.—Unconfirmed. This species, if it occurs in the Preserve, would be expected to occur in swamps and/or bottomland hardwood forests.

Vouchers/observations.—None verified.

Comments.—*Neotoma floridana* are common inhabitants of hardwood forests in the eastern U.S., especially those dominated by hardwoods. They are known to create stick middens ("woodrat middens") throughout their range and have been recorded from hardwood forests and marsh habitats in southeastern Louisiana. Given their distributional records and the large number of large oak and other hardwood tree species in forested areas of the Barataria Preserve, they would be expected to occur in the park. To date, no evidence of *N. floridana* have been documented through the pre-Katrina and post-Katrina field work of Hood (2006, 2012). Care was taken to search for *N. floridana* middens and to trap in areas that included large amounts of woody debris on the forest floor (more than 8,000 trap nights pre-Katrina and 1,800 trap nights post-Katrina). The status of this species should be studied further at the Barataria Preserve, especially to increase knowledge of their residency, population status, and use of the park

DISCUSSION

This paper reports the first professional, systematic survey of the mammals inhabiting the Barataria Preserve of Jean Lafitte National Historical Park & Preserve in Marrero, Louisiana. Mammal inventories immediately pre- and post-Katrina (Hood 2006, 2012) have provided significant new knowledge about the occurrence, distribution, habitat use, and status of mammals at the Barataria Preserve. The 25 species of mammals that have been documented to inhabit (or use) the Barataria Preserve include common mammals found in southeastern Louisiana and areas adjacent to the northern Gulf of Mexico. However, they extend the

documented ranges of many of these species compared with Lowery (1974) and Choate et al. (1994), which previous to the current study were the most recent descriptions of mammal distribution for this region.

No federally-listed endangered or threatened species were documented. Several species of bats (*M. austroriparius*, *C. rafinesquii*, and *P. subflavus*) were discovered to be resident in forest and swamp habitats and are important records to add to the North American Bat Monitoring Program database. Other bat species were previously very poorly documented in southeastern Louisiana and these records are important new findings for North American bat conservation biology. The commonly encountered rodents were *O. palustris* (in marsh habitats) and *P. leucopus* (in bottomland hardwood forest habitats), and these rodents undoubtedly are important prey for owls, raptors, and snakes that are common throughout the Preserve.

White-tailed deer were well known to inhabit the Preserve, with historic local hunting being allowed under special permits. The authors' studies using camera-traps reported herein complement well the hunting and aerial surveys that were conducted in the mid-2000s (Nyman 2004; Scognamillo and Nyman 2004; Chamberlain and Nyman 2006) and demonstrate that there is a very large deer population in all habitats within the Preserve.

In addition to white-tailed deer, coyotes and bobcats were documented to co-occur in all major habitats throughout the Barataria Preserve. Because motion-activated cameras could be a primary research protocol for some studies, there can be an economy of scale in monitoring design and implementation to study all of these species. The use by large mammals of marsh habitats and the landscape features of the park (i.e., spoil banks that traverse a large area of marsh) is unique and allows for a number of research projects in landscape ecology.

The impact of *S. scrofa* on plant community ecology in all habitats of the park is a serious, significant management concern. Active management through hunting and removal by trapping have been underway, but continued and expanded efforts to control or eradicate *S. scrofa* continues to be a priority. Elsey et al. (2012) report significant impacts by *S. scrofa* on *Alligator mississipiensis* nests in western Louisiana. Their study reports 2011 surveys of *A. mississipiensis* nest predation and destruction statewide, for which there were no records of these impacts in Jefferson Parish.

Nutria, *M. coypus*, remain an invasive mammal species of management concern at the Barataria Preserve. Their populations fluctuate annually, however their numbers are clearly significant based on the fact that the limited public trapping season generates several thousand nutria taken each year. Studies by Nolfo-Clements (2009, 2012) have provided important baseline data on *M. coypus* ecology at Barataria.

The absence, or lack of detection, of some expected (or possible) mammal species, including red fox, fox squirrels, southern flying squirrels, and several rodent species, reinforces their generally undocumented status south of Lake Pontchartrain by Lowery (1974) and Choate et al. (1994). Nonetheless, continued studies to document these and other mammals, as well as to monitor population abundance, are warranted.

The only change in the mammal alpha diversity after impacts of Hurricane Katrina/Rita during the summer of 2005, is the appearance of feral hogs (*S. scrofa*) in the Preserve and the rapid establishment of their populations, as well as the destructive ecological impacts of their activities throughout the Preserve.

The summer of 2005 did not just bring Hurricane Katrina to the region, but 24 other named tropical storms or hurricanes. Several of these caused inundation of the bottomland forests with high water for extended periods of time. Of these many storms, Hurricane Rita (with impacts to the Preserve three weeks following Katrina) had the most significant impact on the Barataria Preserve with respect to wind damage (i.e., causing disturbance of forest canopies in bottomland forests) and subsequent growth of weedy vegetation and invasive plant species. The results presented in this paper indicate that no loss of mammal species diversity occurred (one species, *S. scrofa* was added), and that indeed several species of medium-large mammals (*O. virginianus, C. latrans, L. rufus*) expanded their distribution and did not suffer significant population declines.

Rapid residential development of most forested areas immediately north of the Barataria Preserve may place significant new environmental pressures on the park. Immediately pre-Katrina (2003–2005), most of the undeveloped land north of the Barataria Preserve boundary had been identified for residential development, and housing tract development is now underway. The increased need for housing following Hurricane Katrina accelerated this development. Post-Katrina, levees north of Barataria have been raised and extended significantly. This could well generate new environmental challenges, especially as the natural hydrology of Barataria Preserve is likely to be impacted. It is possible that mammals inhabiting areas immediately adjacent to the Preserve that are capable of dispersal (especially *O. virginianus* and *C. latrans*) could take refuge in the Preserve. This could lead to a number of ecological consequences that would transform or reshape the ecosystem functions of the park. Of special concern would be a significant increase in *O. virginianus* populations leading to over-browsing and various other impacts on plant communities. Non-native mammals such as *S. scrofa*, murid rodents, and feral dogs and cats also may increase. Most of the species mentioned here are known to have significant impacts on native flora and fauna. Monitoring activities that target these and other species will become an important management strategy in the near future.

It is likely that visitor traffic to the Barataria Preserve will increase significantly as urbanization accelerates immediately adjacent to the Preserve. Increased visitor traffic on trails (and especially off-trail where people will wander) will likely increase impacts on park ecosystems. The expansion of urban areas to the boundaries of the park also will likely place increased pressures on the park to manage mosquitoes due to concerns about the nuisance of insect bites and West Nile Virus. Thus, the Preserve will need to integrate its natural resource management and its public information strategies.

In conclusion, these pre- and post-Katrina studies not only document mammal biodiversity, distribution, and habitat use in the Barataria Preserve, they demonstrate the remarkable ecological resilience of the mammalian fauna. This is a reminder that what humans experience in major storm events is shared with mammals living in a human-dominated landscape.

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LITERATURE CITED

Animal Care and Use Committee. 1998. Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. Journal of Mammalogy 79:1416–1431.

Andersen, B. B., and L. A. Miller. 1977. A portable ultrasonic detection system for recording bat cries in the field. Journal of Mammalogy 58:226–229.

Ball, D. J., et al. 1991. Evaluation of a microchip implant system used for animal identification in rats. Laboratory Animal Science 41:185–186.

Barras, J. A., P. E. Bourgeois, and L. R. Handley. 1994. Land loss in coastal Louisiana 1956–90. National Biological Survey, National Wetlands Research Center Open File Report 94-01.

Bernatas, S. 2003. Infrared deer survey of Jean Lafitte National Historical Park and Preserve. Report to National Park Service. [Included as Appendix 3 in Chamberlain and Nyman 2006]

Bernatas, S. 2004. Aerial survey of deer using Forward Looking Infrared (FLIR) videography in the Jean Lafitte National Historical Park and Preserve, Report to National Park Service. [Included as Appendix 4 in Chamberlain and Nyman 2006]

Bordelon, S. 2005. Effects of white-tailed deer herbivory on the growth and survival of seedlings in a coastal wetland forest. Report to National Park Service. [Included as Appendix 2 in Chamberlain and Nyman 2006]

Britzke, E. R., D. W. Bossi, B. M. Hadley, and L. W. Robbins. 1999. The acoustic identification of bats in Missouri. Bat Research News 39:158-159.

Camper, J. D., and J. R. Dixon. 1988. Evaluation of a microchip marking system for amphibians and reptiles. Texas Parks & Wildlife Department, Research Publication, 7100, 159:1-22.

Chamberlain, M. J., and J. A. Nyman. 2006. Final report: quantify deer population and develop a hunting management plan for the Barataria Preserve. National Park Service.

Choate, J. R., J. K. Jones, Jr., and C. Jones. 1994. Handbook of mammals of the south-central states. Louisiana State University Press, Baton Rouge, Louisiana.

Demastes, J. W., and D. A. Rossman. 1989. Inventory of the amphibians, reptiles and mammals of the Jean Lafitte National Park. Final report to Jean Lafitte National Historical Park and Preserve. New Orleans, Louisiana.

Elsey, R. M., E. C. Mouton Jr.., and N. Kinler. 2012. Effects of feral swine (*Sus scrofa*) on alligator (*Alligator mississippiensis*) in Louisiana. Southeastern Naturalist 11:205–218.

Fagerstone, K. A., and B. E. Johns. 1987. Transponders as permanent identification markers for domestic ferrets, black-footed ferrets and other wildlife. Journal of Wildlife Management 51:294–297.

Fenton, M. B. 1988. Detecting, recording, and analyzing vocalizations of bats. Pages 91–104 in Ecological and behavioral methods in the study of bats (T. H. Kunz, ed.). Smithsonian Institution Press, Washington, DC.

Fry, R. C. 1988. An examination of autogenic factors influencing plant composition during secondary succession on spoilbanks at Jean Lafitte National Park, Louisiana. Final report to Jean Lafitte National Historical Park & Preserve. New Orleans, Louisiana.

Germano, D. J., and D. F. Williams. 1993. Field evaluation of using passive integrated transponder (PIT) tags to permanently mark lizards. Herpetological Review 24:54–56.

Hood, C. S. 2006. Final Report. Inventory of the mammals of Jean Lafitte National Historical Park and Preserve, Barataria Preserve and Chalmette Battlefield. Report to National Park Service (Cooperative Agreement H21150300017).

Hood, C. S. 2012. Final Report. Re-survey and inventory of the mammals of Jean Lafitte National Historical Park and Preserve, Barataria Preserve. Report to National Park Service (Cooperative Agreement H7530100057).

Jones, C. 1967. Growth, development, and wing loading in the evening bat, *Nycticeius humeralis* (Rafinesque). Journal of Mammalogy 48:1–19.

Jones, C. 1975. Notes on the natural history of *Plecotus rafinesquii*. Occasional Papers, Museum of Zoology, Louisiana State University, Baton Rouge, Louisiana. 47:1–14.

Jones, C., W. J. McShea, M. J. Conroy, and T. H. Kunz. 1996. Capturing mammals. Pages 115–156 in Measuring and monitoring biological diversity – standard methods for mammals (D. E. Wilson, F. R. Cole, J. D. Nichols, R. Rudran, and M. S. Foster, ed.). Smithsonian Institution Press, Washington, DC.

Jones, C., and J. Pagels. 1968. Notes on a population of *Pipistellus subflavus* in southern Louisiana. Journal of Mammalogy 49:134–139.

Jones, C., and R. D. Suttkus. 1973. Colony structure and organization of *Pipistellus subflavus* in southern Louisiana. Journal of Mammalogy 54:962.

Kageyama, M., R. R. Monk, R. D. Bradley, G. F. Edson, and R. J. Baker. 2007. The changing significance and definition of the biological voucher. Pp. 257–264 in Museum studies: Perspectives and innovations (S. L. Williams and C. A. Hawks, eds.). Society for the Preservation of Natural History Collections, Washington, DC.

Kunz, T. H., and A. Kurta. 1988. Capture methods and holding devices. Pages 1–30 in Ecological and behavioral methods in the study of bats (T. H. Kunz, ed.). Smithsonian Institution Press, Washington, DC.

Kunz, T. H., D. W. Thomas, G. C. Richards, C. R. Tidemann, E. D. Pierson, and P. A. Racey. 1996. Observational techniques for bats. Pages 105–114 in Measuring and monitoring biological diversity – standard methods for mammals (D. E. Wilson, F. R. Cole, J. D. Nichols, R. Rudran, and M. S. Foster, eds.). Smithsonian Institution Press, Washington, DC.

Lance, R. F. 1999. Ecological and population genetic studies of forest-dwelling bats. Unpublished Ph.D. dissertation, University of Southwestern Louisiana, Lafayette, Louisiana.

Lance, R.F., B. Bollich, C.L. Callahan, and P.L. Leberg. 1996. Surveying forest-bat communities with Anabat dectectors. Pages 175–184 in Bats and Forest Symposium (R.M.R. Barclay and R.M. Brigham, eds.). October 19–21, 1995, Victoria, British Columbia, Canada. Paper #23. Res. Board, B.C. Mining Forestry, Victoria, BC, Canada.

Lance, R. F., B. T. Hardcastle, A.Talley, and P. L. Leberg. 2001. Day-roost selection by Rafinesque's big-eared bats (*Corynorhinus rafinesquii*) in Louisiana forests. Journal of Mammalogy 82:166–172.

Lance, R F. and D. L. Rogowski. 1999. Noteworthy record of the silver-haired bat in Louisiana (Mammalia: Chiroptera: *Lasionycteris noctivagans*). The Southwestern Naturalist 44:241–242.

Lowery, G. H., Jr. 1974. The mammals of Louisiana and adjacent waters. Louisiana State University Press, Baton Rouge, Louisiana.

Meeks, P. D. 2012. Refining and improving the use of camera trap technology for wildlife management and research in Australia and New Zealand. Report to The Winston Churchill Memorial Trust of Australia, Canberra, Australian Capital Territory.

Michot, T. C. 1984. A marsh vegetation study of Jean Lafitte National Historical Park. Final report to Jean Lafitte National Historical Park & Preserve. New Orleans, Louisiana.

Michot, T. C. 1985. Habitat evaluation procedures analysis and vegetation transect update of Jean Lafitte National Historical Park. Final report to Jean Lafitte National Historical Park & Preserve. New Orleans, Louisiana.

Moncrief, N. D. 1993. Geographic variation in morphology and allozymes within tree squirrels, *Sciurus niger* and *S. carolinensis*, of the Lower Mississippi River Valley. Journal of Mammalogy 74:547–576.

Monk, R. R., and R. J. Baker. 2001. e-Vouchers and the use of digital imagery in natural history collections. Museology, Museum of Texas Tech University 10:1–8.

Murray, K. L., E. R. Britzke, B. M. Hadley, and L. W. Robbins. 1999. Surveying bat communities: a comparison between mist nets and the Anabat II bat detector system. Acta Chiropterologica 1:105–112.

Muth, D., C. Swarzenski, T. Doyle, and T. Hargis. 2000. Ecology of floating peat marshes of Jean Lafitte National Historical Park and Preserve in coastal Louisiana. P. 446 in Quebec 2000: Millennium wetland event program with abstracts (A. Crowe and L. Rochefort, eds.). Quebec, Canada, August 6–12, 2000.

Nichols, J. D., and M. J. Conroy. 1996. Techniques for estimating abundance and species richness. Pages 177–234 in Measuring and monitoring biological diversity – standard methods for mammals (D. E. Wilson, F. R. Cole, J. D. Nichols, R. Rudran, and M. S. Foster, eds.). Smithsonian Institution Press, Washington, DC.

Nolfo-Clements, L. E. 2006. Vegetative survey of wetland habitats at Jean Lafitte National Historical Park and Preserve in southeastern Louisiana. Southeastern Naturalist 5:499–514.

Nolfo-Clements, L. E. 2009. Nutria survivorship, movement patterns, and home ranges. Southeastern Naturalist 8:399–410.

Nolfo-Clements, L. E. 2012. Habitat selection by nutria in a freshwater Louisiana marsh. Southeastern Naturalist 11:183–204.

Nyman, J. A. 2004. Aldous deer browse survey at Barataria Preserve, Jean Lafitte National Park and Historic Preserve. Report to National Park Service. [Included as Appendix 1 in Chamberlain and Nyman 2006]

O'Farrell, M. J., and W. L. Gannon. 1999. A comparison of acoustic versus capture techniques for the inventory of bats. Journal of Mammalogy 80:24–30.

O'Farrell, M. J., B. W. Miller, and W. L. Gannon. 1999. Qualitative identification of free-flying bats using the Anabat detector. Journal of Mammalogy 80:11–13.

Padgham, M., B. Rudis, R. Lovelace, and M. Salmon. 2017. Osmdata Journal of Open Source Software 2(14). URL https://doi.org/10.21105/joss.00305.

Parsons, S., A. M. Boonman, and M. K. Obrist. 2000. Advantages and disadvantages of techniques for transforming and analyzing chiropteran echolocation calls. Journal of Mammalogy 81:927–938.

Pebesma, E. 2018. Simple features for R: standardized support for spatial vector data. The R Journal. URL https://journal.r-project.org/archive/2018/RJ-2018-009.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Schooley, R. L., B. Van Horne, and K. P. Burnham. 1993. Passive integrated transponders for marking free-ranging Townsend's ground squirrels. Journal of Mammalogy 74:480–484.

Scognamillo, D. G., and J. A. Nyman. 2004. Report: Habitat use by white-tailed deer survey in the Jean Lafitte Historic Park during February 2004. Report to National Park Service. [Included as Appendix 5 in Chamberlain and Nyman 2006]

Smalley, A. E. 1982. Faunistic inventory of the core area -- Jean Lafitte National Historical Park. Final report to Jean Lafitte National Historical Park & Preserve. New Orleans, Louisiana.

Suttkus, R. D., and C. Jones. 1991. Observations on winter and spring reproduction in *Peromyscus leucopus* (Rodentia, Muridae) in southern Louisiana. Texas Journal of Science 43:179–189.

Suttkus, R. D. 1999. Observations of the nine-banded armadillo, *Dasypus novemcinctus*, in southern Louisiana. Tulane Studies Zoology and Botany 31:1–22.

Swann, D. E., K. Kawanishi, and J. Palmer. 2011. Evaluating types and features of camera traps in ecological studies: guide for researchers. Pp. 27–44 in Camera traps in animal ecology: methods and analyses (A. F. O'Connell, J. D. Nichols, and J. Karanth, eds.). Springer-Verlag, New York.

TEAM Network. 2008. Terrestrial vertebrate (Camera Trap) monitoring protocol implementation manual. Version 3.0. Tropical Ecology, Assessment and Monitoring Network, Center for Applied Biodiversity Science, Conservation International, Arlington, Virginia.

Tennekes, M. 2018. "tmap: Thematic Maps in R." Journal of Statistical Software 84(6):1–39. doi: 10.18637/jss.v084.i06.

White, D. A., S. P. Darwin, and L. Thien. 1983. Plants and plant communities of Jean Lafitte National Historical Park. Tulane Studies Zoology and Botany 24:101–129.

Williams, D. F., W. Tordoff III, and D. J. Germano. 1997. Evaluation of methods for permanently marking kangaroo rats (*Dipodomys*: Heteromyidae). Pages 259–271 in Life among the muses: papers in honor of James S. Findley (T. L. Yates, W. L. Gannon, and D. E. Wilson, eds.). Special Publications of the Museum of Southwestern Biology, No. 3. University of New Mexico, Albuquerque.

Padgham, M., B. Rudis, R. Lovelace, and M. Salmon. 2017. Osmdata Journal of Open Source Software 2(14). URL https://doi.org/10.21105/joss.00305.

Pebesma, E. 2018. Simple features for R: standardized support for spatial vector data. The R Journal. URL https://journal.r-project.org/archive/2018/RJ-2018-009/.

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APPENDIX

GPS coordinates of localities for camera-trap sites, bat mist nets and electronic detection, and live-trapping. Detailed descriptions of sites can be found in Hood (2012). Camera-trap sites compared pre- and post-Katrina are indicated by an asterisk (*). Sites where a Reconyx camera was employed are indicated by R.

Site	Habitat	Latitude	Longitude
	Camera-traps		
Camera Site 01 R	Hardwood forest	29° 47' 07"N	90° 6' 20"W
Camera Site 02 *	Hardwood forest	29° 46' 59"N	90° 6' 16"W
Camera Site 03	Hardwood forest	29° 46' 56"N	90° 6' 10"W
Camera Site 04	Hardwood forest	29° 46' 51"N	90° 6' 16"W
Camera Site 05 *	Hardwood forest	29° 47' 05"N	90° 6' 27"W
Camera Site 06 *	Hardwood forest	29° 47' 22"N	90° 6' 46"W
Camera Site 07	Hardwood forest	29° 47' 31"N	90° 6' 59"W
Camera Site 08	Hardwood forest	29° 47' 39"N	90° 7' 02"W
Camera Site 09	Hardwood forest	29° 47' 58"N	90° 6' 48"W
Camera Site 10	Hardwood forest	29° 47' 49"N	90° 6' 50"W
Camera Site 11 *	Hardwood forest	29° 48' 27"N	90° 7' 01"W
Camera Site 12	Hardwood forest	29° 48' 16"N	90° 6' 48"W
Camera Site 13	Hardwood forest	29° 47' 37"N	90° 7' 16"W
Camera Site 14	Hardwood forest	29° 47' 36"N	90° 7' 28"W
Camera Site 15	Hardwood forest	29° 47' 27"N	90° 7' 50"W
Camera Site 16	Hardwood forest	29° 47' 44"N	90° 8' 04"W
Camera Site 17	Hardwood forest	29° 47' 46"N	90° 8' 03"W
Camera Site 18	Hardwood forest	29° 47' 12"N	90° 6' 48"W
Camera Site 19	Hardwood forest	29° 47' 15"N	90° 6' 52"W
Camera Site 20 * R	Hardwood/swamp	29° 47' 03"N	90° 6' 41"W
Camera Site 21 *	Marsh	29° 50' 05"N	90° 9' 16"W
Camera Site 22 *	Marsh	29° 50' 26"N	90° 9' 08"W
Camera Site 23 * R	Marsh	29° 50' 00"N	90° 8' 44"W
Camera Site 24 *	Marsh	29° 49' 49"N	90° 8' 13"W
Camera Site 25 *	Marsh	29° 47' 59"N	90° 9' 31"W
Camera Site 26 R	Marsh	29° 47' 59"N	90° 9' 31"W
Camera Site 27	Marsh	29° 50' 08"N	90° 7' 53"W
Camera Site 28	Marsh	29° 50' 08"N	90° 7' 55"W
Camera Site 29 R	Marsh	29° 50' 07"N	90° 7' 56"W
Camera Site 30	Swamp	29° 50' 32"N	90° 7' 34"W
Camera Site 31 R	Swamp	29° 45' 18"N	90° 8' 44"W
Camera Site 32	Swamp	29° 45' 18"N	90° 8' 45"W
Camera Site 33 *	Swamp	29° 45' 19"N	90° 8' 43"W

Site		Latitude	Longitude
Camera Site 34	Swamp	29° 47' 11"N	90° 4' 32"W
Camera Site 35	Swamp	29° 47' 33"N	90° 4' 36"W
Camera Site 36	Swamp	29° 48' 51"N	90° 4' 22"W
Camera Site 37	Swamp	29° 48' 51"N	90° 4' 24"W
Camera Site 38	Marsh	29° 49' 06"N	90° 4' 48"W
Camera Site 39	Marsh	29° 49' 07"N	90° 4' 52"W

Site	Latitude	Longitude
Bat mist-net/detecting localities		
Bottomland Forest Batnet 1	29° 47' 04"N	90° 6' 32"W
Bottomland Forest Batnet 2	29° 47' 02"N	90° 6' 27"W
Bottomland Forest Batnet 3	29° 46' 59"N	90° 6' 23"W
Bottomland Forest Batnet 4	29° 47' 10"N	90° 6' 32"W
Bottomland Forest Bridge	29° 47' 03"N	90° 6' 43"W
Bottomland Forest Canoe	29° 47' 05"N	90° 6' 46"W
Bottomland Forest Education Center	29° 47' 09"N	90° 6' 42"W
Bottomland Forest Pump	29° 47' 07"N	90° 6' 42"W
Swamp Coquille Bridge	29° 47' 30"N	90° 7' 53"W
Swamp Coquille Trail 1	29° 47' 31"N	90° 7' 36"W
Swamp Coquille Trail 2	29° 47' 31"N	90° 7' 40"W
Swamp Coquille Trail 3	29° 47' 29"N	90° 7' 42"W
Swamp Batnet	29° 47' 26"N	90° 7' 50"W
Live-trapping localities		
Bottomland Forest Trapline H1A	29° 47' 13"N	90° 6' 37"W
Bottomland Forest Trapline H1B	29° 47' 14"N	90° 6' 37"W
Bottomland Forest Trapline H2A	29° 47' 13"N	90° 6' 36"W
Bottomland Forest Trapline H2B	29° 47' 13"N	90° 6' 36"W
Bottomland Forest Trapline 1	29° 47' 13"N	90° 6' 37"W
Bottomland Forest Trapline 2	29° 47' 13"N	90° 6' 38"W
Marsh Trapline 1	29° 50' 08"N	90° 7' 53"W
Marsh Trapline 2	29° 50' 09"N	90° 7' 52"W
Marsh Trapline 3	29° 50' 08"N	90° 7' 53"W

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Recent Appraisal of the Mexican Mammals on Deposit at The Natural History Museum (London), United Kingdom

Joaquín Arroyo-Cabrales, Livia León-Paniagua, and Noé González Ruiz

Abstract

Data repatriation from scientific specimens is an important issue in all developing countries. Herein, Mexican mammals that are on deposit at The Natural History Museum (London), United Kingdom [BM(NH)] are documented. Mexican specimens have been recorded at the BM(NH) since 1829, with 76 specimens being holotypes. At least 1,666 specimens were found and identifications were confirmed. This initial report sets the stage for a forthcoming Catalog of Type Specimens of Mexican Mammals.

Key words: data repatriation, Mammalia, Mexico, Natural History Museum (London), systematics, taxonomy, type specimens

Resúmen

La repatriación de la información es una cuestión importante para los zoólogos en el presente. Una de dichas iniciativas fue la documentación de todos los mamíferos mexicanos que están bajo depósito en el Museo de Historia Natural (Londres), Reino Unido. Los ejemplares mexicanos fueron catalogados desde 1829 y, varios de ellos fueron descritos como holotipos (76). Al menos 1,666 ejemplares fueron documentados y su identificación verificada. Este reporte inicial establece las bases para el próximo Catálogo de Ejemplares Tipo de Mamíferos Mexicanos.

Palabras clave: especímenes tipo, Mammalia, México, Natural History Museum (Londres), repatriación de datos, sistemática, taxonomía

Introduction

Mexico is one of the world's megadiverse countries, meaning that it has at least 10% of the world's known biological diversity living inside the country's boundaries (Mittermeier et al. 1997). After Indonesia, it is the second most diverse country for mammals (Ceballos et al. 2014). Documenting the Mexican biological diversity that was collected in the past by naturalists and zoologists from other countries would be a starting point to develop the best conservation efforts for such a highly diverse country.

An example of what has been called data repatriation is the efforts of Ricardo López-Wilchis and assistants from the Metropolitan Autonomous University, Campus Iztapalapa, in developing the Base de Datos de Mamíferos Mexicanos bajo depósito en Colecciones de Estados Unidos de América y Canadá (Mexican Mammal Database in U.S. and Canadian collections). Their objectives included a complete catalog of mammal collections in the USA and Canada holding Mexican specimens, their status, and making the information accessible to the Mexican mammalogical community, either students or professionals. Such a large project was accomplished through the publication of three major compendia (López-Wilchis and López Jardines 1998, 1999, 2000), as well as a synthesis study summarizing the results of the project (López-Wilchis et al. 1998). More recently, Lorenzo et al. (2012), based on

Internet-available databases, such as the Global Biodiversity Information Facility (www.gbif.org), updated the data on Mexican mammals on deposit in U.S. and Canadian collections.

Similar to those mammalogy collections found in North America north of Mexico, where a large number of Mexican mammals were deposited during the end of the 19th century and the first half of the 20th century, many specimens were collected by European naturalists who visited Mexico during most of the 17th to 19th centuries (Koleff et al. 2004). In fact, little is known about those specimens in the European scientific collections, although many species were described based on those specimens. Their current state of conservation is unknown, including those materials studied by the great naturalist Carolus Linnaeus (Thomas 1911). Few catalogs have reported the current status of the specimens on deposit in various European collections; some of those include the type catalog of mammals at Muséum d´Historie Naturelle de Genève (Genève, Switzerland) (Baud 1977), the holotypes of Neotropical bats in some European museums (Carter and Dolan 1978), and the chiropteran holotypes at the Muséum National d'Histoire Naturelle (Paris, France) (Rode 1941).

A project focusing on the vertebrate zoological collections at the The Natural History Museum (London) was initiated 20 years ago by personnel of the Zoology Museum "Alfonso L. Herrera", Universidad Nacional Autónoma de México, directed by Dr. Adolfo Navarro-Sigüenza. It was initially supported by the Museum´s Department of Vertebrate Zoology and later by the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO). The initial project was focused primarily on the birds, and here mammals are treated. Specimens collected in Mexico and deposited in The Natural History Museum (London) date back to the first half of the 1800s. Although those specimens are few in comparison with the number of Mexican specimens on deposit world-wide (> 600,000), they are important for historical and nomenclatural reasons because several of those were the basis for describing either species or subspecies new to science (Napier 1976; Jenkins and Knutson 1983).

Based on the above, a catalog of specimens collected in Mexico and under the care of The Natural History Museum (London) was prepared, with an initial visit in March 1997 for three weeks (Arroyo-Cabrales and León-Paniagua 2010). The first task was preparing an electronic listing of the specimens collected in Mexico and reported in the Museum Registrar's Book, beginning from the start of the book in 1837 and up to the present. Such an effort resulted in a list of 1,772 specimens. It took several visits to check each one of the listed specimens, and further search for documents that dated the collections arrival at specific periods. Finally, the most important holdings from Mexico, holotypes, were studied, measured, photographed, and their current condition of conservation was documented. A database of all mammal specimens on deposit at The Natural History Museum (London) was prepared, verifying the identification of each specimen.

DATABASE ORGANIZATION

Database fields recorded included Registrar Number (corresponding to the current catalog number), Order, Family, Genus, Species, Subspecies, Collector name, Collector field number, Collecting date (day, month, year), Country, State, Specific locality, Preparation type, and Remarks, including information on available measurements or photographs for each specimen. Nomenclature initially was based on that proposed by Wilson and Reeder (2005), but it was updated by the latest listing by the American Society of Mammalogists (Burgin et al. 2018). For identification, Hall (1981) was followed, and for bats the keys by both Álvarez et al. (1994) and Medellín et al. (1997) were utilized. For rodents, several cranial and body measurements were taken with a digital Vernier Ultra-Cal III; Registrar's original names and the corresponding current name are provided in Appendix I.

For type specimens, a copy of each publication containing the description of a Mexican mammal was obtained (see Appendix II). Each type specimen was measured, its conservation status evaluated, and photographs of skin and skull were taken.

ANALYSIS

The database containing the information for Mexican mammal specimens on deposit at The Natural History Museum (London) contains 1,666 specimens that were examined to confirm identification. This number is fewer than the documented records shown in the Registrar´s Book (1,772). The difference is due to the decay of some specimens, as well as destruction from fires, mostly in the early 1900s. Mexican mammals housed there date to 1829, but there is missing information on the arrival dates for the oldest specimens, which were not recorded in the Registrar's Book until after 1937. Specimens were grouped by decade, by the year when they were registered in the Registrar´s Book (Fig. 1). A major increase peaked in the last two decades of the 19th century and the initial decade of the 20th century.

In addition, a list of collectors (83 names) who participated either in the field expeditions or preparing specimens that arrived in the museum was compiled. Among those names are: I. T. Sanderson, with a large expedition into southeastern Mexico (in the states of Yucatán and Chiapas among others); H. Gadow in Veracruz and Oaxaca; G. S. Miller, Jr., and W. W. Price, explored the Peninsula of Baja California; O. Salvin and F. D. Godman, explored almost the entire country; A. C. Buller, collected mammals in Jalisco and Zacatecas; and P. O. Simons in Sinaloa. Among the scientists, some were outstanding due to their taxonomic studies, such as Thomas, Gray, Kerr, etc.; for example, Thomas has 31 new species described based on Mexican specimens on deposit at The Natural History Museum (London).

At The Natural History Museum (London) there are specimens from at least 26 Mexican states (246 localities), including some islands, ranging from Hidalgo with only two specimens to Sinaloa with 203

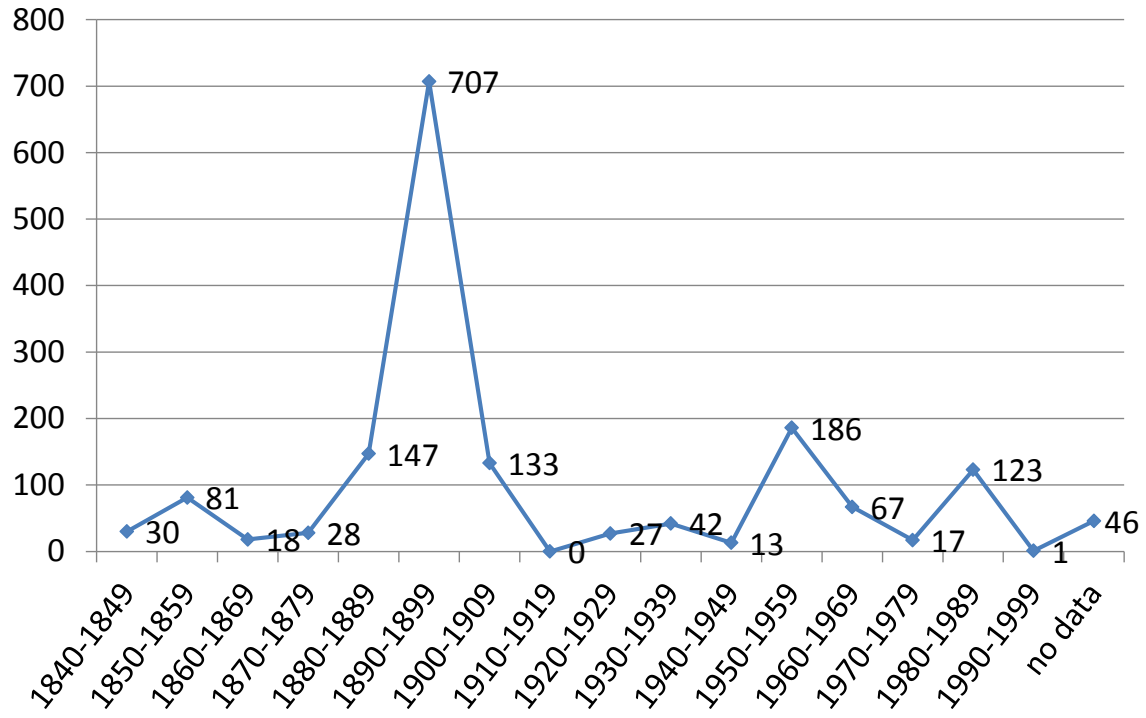

Figure 1. Specimens collected in Mexico on deposit at The Natural History Museum (London), according to the Registrar's Book. Numbers indicate the specimens registered by decade.

specimens (Table 1). The larger numbers are from Baja California, Jalisco, Veracruz, and Yucatán, but 174 records lack provenance data and some only state the country, Mexico.

Table 1. Mexican mammal specimens on deposit at The Natural History Museum (London) by collecting state locality.

Mexican State	Number of Records
Baja California	168
Baja California Sur	95
Chiapas	65
Chihuahua	7
Coahuila	5
Colima	24
Distrito Federal	39
Durango	30
Estado de Mexico	13
Guerrero	25
Hidalgo	2
Jalisco	161
Michoacan	3
Nayarit	44
Nuevo Leon	4
Oaxaca	92
Puebla	4
Quintana Roo	10
San Luis Potosi	6
Sinaloa	203
Sonora	86
Tabasco	22
Tamaulipas	4
Veracruz	195
Yucatan	172
Zacatecas	11
No data	175

Currently, Mexico has more than 500 terrestrial mammal species (Ceballos et al. 2014), while The Natural History Museum (London) contains 229 Mexican species representing 113 genera and 10 orders (Table 2). The largest number of specimens and species are from the orders Rodentia and Chiroptera and only 24 are from Carnivora. Most bat specimens, about a fifth of the holdings (20.8%), were well identified, reflecting the care taken by a former curator, Dr. John Edwards Hill. That contrasted with rodents, especially cricetids, which were ~60% of documented specimens and required much work.

Table 2. Mexican mammal specimens on deposit at The Natural History Museum (London) by nomenclatural status.

Order	Species	Specimens
Artiodactyla	6	19
Carnivora	24	117
Chiroptera	65	329
Didelphimorphia	6	39
Eulipotiphla	15	34
Lagomorpha	9	67
Rodentia	100	1,048
Cingulata	1	8
Pilosa	2	4
Perisodactyla	1	1

It is important to point out that 76 specimens from the Mexican mammal collection represent type specimens, for both species and subspecies (Appendix II). A catalog dedicated to those type specimens and any available information is in preparation.

The complete digital database is on deposit at CONABIO, www.conabio.gob.mx, and it is available for consultation upon request.

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LITERATURE CITED

Álvarez, T., S. T. Álvarez-Castañeda, and J. C. López-Vidal. 1994. Claves para murciélagos mexicanos. Centro de Investigaciones Biológicas del Noroeste, S. C. and Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México.

Arroyo-Cabrales, J., and L. León-Paniagua. 2010. Catálogo de los mamíferos de México en resguardo de The Natural History Museum (London), Inglaterra. Instituto Nacional de Antropología e Historia, Universidad Nacional Autónoma de México. Final Report SNIB-CONABIO project DE020, México.

Bailey, V. 1906. Identity of *Thomomys umbrinus* (Richardson). Proceedings of the Biological Society of Washington 19:3–6.

Baud, F. 1977. Catalogue des types de mammifères et d´oiseaux du Muséum d´Histoire naturrelle de Genève. Revie suisse de Zoologie 84:201–220.

Burgin, C. J., J. P. Colella, P. L. Kahn, and N. S. Upham. 2018. How many species of mammals are there? Journal of Mammalogy 99:1–14.

Carter, D. C., and P. G. Dolan. 1978. Catalogue of type specimens of Neotropical bats in selected European museums. Special Publications, Museum of Texas Tech University 15:1–136.

Ceballos, G., J. Arroyo-Cabrales, R. A. Medellín, L. Medrano González, and G. Oliva. 2014. Diversity and conservation. Pp. 1–44 in Mammals of Mexico (G. Ceballos, ed.). Johns Hopkins University Press, Baltimore.

Hall, E. R. 1981. The mammals of North America. 2nd ed. John Wiley & Sons, New York.

Jenkins, P. D., and L. Knutson. 1983. A catalogue of the type specimens of Monotremata and Marsupialia in the British Museum (Natural History). British Museum (Natural History), London.

Kellogg, R., and E. A. Goldman. 1944. Review of the spider monkeys. Proceedings of the U. S. National Museum, 96:1–45.

Koleff, P., C. Fernández, J. M. Martínez, and E. Moreno. 2004. Información sobre la biodiversidad de México en el extranjero. Biodiversitas 54:1–7.

López-Wilchis, R., and J. López Jardines. 1998. Los mamíferos de México depositados en colecciones de los Estados Unidos y Canadá. Volumen 1. Universidad Autónoma Metropolitana, Unidad Iztapalapa, México.

López-Wilchis, R., and J. López Jardines. 1999. Los mamíferos de México depositados en colecciones de los Estados Unidos y Canadá. Volumen 2. Universidad Autónoma Metropolitana, Unidad Iztapalapa, México.

López-Wilchis, R., and J. López Jardines. 2000. Los mamíferos de México depositados en colecciones de los Estados Unidos y Canadá. Volumen 3. Universidad Autónoma Metropolitana, Unidad Iztapalapa, México.

López-Wilchis, R., J. López Jardínes, and M. G. Sánchez Hernández. 1998. Specimens of mammals from México in collections in the United States and Canada. Journal of Mammalogy 79:1029–1037.

Lorenzo, C., et al. 2012. Los mamíferos de México en las colecciones científicas de Norteamérica. Therya 3:239–262.

Medellín, R. A., H. T. Arita, and O. Sánchez H. 1997. Identificación de los murciélagos de México. Claves de campo. Publicación Especial, Asociación Mexicana de Mastozoología 2:1–83.

Mittermeier, R. A., P. Robles G., and C. Goettsch de M. 1997. Megadiversidad. Los países biológicamente más ricos del mundo. Agrupación Sierra Madre, S. C., and CEMEX, México, D.F.

Napier, P. H. 1976. Catalogue of Primates in the British Museum (Natural History). Part I: Families Callitrichidae and Cebidae. British Museum (Natural History), London.

Thomas, O. 1911. The mammals of the Tenth Edition of Linnaeus: an attempt to fix the types of the genera and exact bases and localities of the species. Proceedings of the Zoological Society of London 1911:120–158.

Rode, P. 1941. Catalogue des types de mammifères du Muséum National d'Histoire Naturelle. II. Ordre des Chiroptères. Bulletin du Muséum National d'Histoire Naturelle 13:227–252.

Wilson, D. E., and D. M. Reeder (eds.). 2005. Mammal species of the World: a taxonomic and geographic reference. Third edition. Johns Hopkins University Press, Baltimore.

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APPENDIX I

List of taxonomic names that were originally applied to the Mexican mammals on deposit at The Natural History Museum (London). First three columns are the names as recorded in the Registrar's Book, and the last two are the current names following Burgin et al. (2018) with updates.

Taxonomy from The Natural History Museum (London) Registrar´s Book			Current Taxonomy	
Genus	Species	Subspecies	Genus	Species
Didelphis	*marsupialis*	*californica*	*Didelphis*	*virginiana*
Didelphis	*marsupialis*		*Didelphis*	*marsupialis*
Didelphys	*breviceps*		*Didelphis*	*virginiana*
Didelphys	*lanigera*		*Caluromys*	*derbianus*
Didelphys	*marsupialis*		*Didelphis*	*virginiana*
Didelphys	*murina*		*Tlacuatzin*	*canescens*
Didelphys	*murina*		*Marmosa*	*mexicana*
Didelphys	*opposum*		*Philander*	
Didelphys	*virginiana*		*Didelphis*	*virginiana*
Marmosa	*murina*	*mexicana*	*Marmosa*	*mexicana*
Marmosa	*sinaloae*		*Tlacuatzin*	*sinaloae*
Marmosa			*Marmosa*	*mexicana*
Metachirops	*opposum*	*pallidus*	*Philander*	
Metachirus	*opposum*		*Philander*	
Philander (*Didelphys*)	*laniger* (*lanigera*)	*aztecus*	*Caluromys*	*derbianus*
Dasypus	*niger*		*Dasypus*	*novemcinctus*
Dasypus	*peba*?		*Dasypus*	*novemcinctus*
Tatusia	*novemcincta*		*Dasypus*	*novemcinctus*
Cyclothurus	*didactylus*		*Cyclopes*	*didactylus*
Tamandua	*tetradactyla*		*Tamandua*	*mexicana*
Blarina	*alticola*		*Cryptotis*	*alticola*
Blarina	*fossor*		*Cryptotis*	*goldmani*
Blarina	*goldmani*		*Cryptotis*	*mexicanus*
Blarina	*mexicana*		*Cryptotis*	*parvus*
Blarina	*mexicana*		*Cryptotis*	*mexicanus*
Blarina	*mexicana*	*peregrina*	*Cryptotis*	*peregrina*
Blarina	*nelsoni*		*Cryptotis*	*nelsoni*
Blarina	*soricina*		*Cryptotis*	*parvus*
Blarina			*Cryptotis*	*parvus*
Blarina (*Soriciscus*)			*Cryptotis*	*mexicanus*
Notiosorex	*crawfordi*		*Notiosorex*	*crawfordi*
Notiosorex	*crawfordi*	*evotis*	*Notiosorex*	*evotis*

Taxonomy from The Natural History Museum (London) Registrar´s Book			Current Taxonomy	
Genus	Species	Subspecies	Genus	Species
Sorex	*caudatus*		*Sorex*	*veraepacis*
Sorex	*godmani*		*Sorex*	*cristobalensis*
Sorex	*macrodon*		*Sorex*	*macrodon*
Sorex	*obscurus*	*ventralis*	*Sorex*	*ventralis*
Sorex	*oreopolus*		*Sorex*	*emarginatus*
Sorex	*oreopolus*	*ventralis*	*Sorex*	*ventralis*
Sorex	*orizabae*		*Sorex*	*emarginatus*
Sorex	*parvus*		*Cryptotis*	*mexicanus*
Sorex	*sclateri*		*Sorex*	*sclateri*
Sorex			*Cryptotis*	*parvus*
Sorex			*Notiosorex*	*crawfordi*
Sorex			*Sorex*	*saussurei*
Sorex (*Blarina*) (*Notiosorex*)	*micrura*		*Cryptotis*	*goodwini*
Balantiopteryx	*plicata*	*plicata*	*Balantiopteryx*	*plicata*
Rhynchiscus	*naso*		*Rhynchonycteris*	*naso*
Saccopteryx	*bilineata*	*centralis*	*Saccopteryx*	*bilineata*
Saccopteryx	*plicata*		*Balantiopteryx*	*plicata*
Vespertilio			*Balantiopteryx*	*plicata*
Vespertilio			*Noctilio*	*leporinus*
Chilonycteris	*davyi*	*fulvus*	*Pteronotus*	*fulvus*
Chilonycteris	*mexicana*		*Pteronotus*	*mexicanus*
Chilonycteris	*parnellii*	*mexicana*	*Pteronotus*	*mexicanus*
Chilonycteris	*psilotis*		*Pteronotus*	*psilotis*
Chilonycteris	*rubiginosa*	*fusca*	*Pteronotus*	*mexicanus*
Chilonycteris	*rubiginosa*		*Pteronotus*	*mexicanus*
Mormoops	*megalophylla*	*senicula*	*Mormoops*	*megalophylla*
Pteronotus	*davyi*	*fulvus*	*Pteronotus*	*fulvus*
Pteronotus	*parnellii*	*rubiginosus*	*Pteronotus*	*mexicanus*
Vespertilio			*Pteronotus*	*psilotis*
Anoura			*Glossophaga*	*soricina*
Anura	*geoffroyi*		*Anoura*	*geoffroyi*
Arctibeus			*Artibeus*	*jamaicensis*
Artibeus	*cinereus*	*phaeotis*	*Dermanura*	*phaeotis*
Artibeus	*cinereus*		*Dermanura*	*tolteca*
Artibeus	*cinereus*		*Dermanura*	*azteca*
Artibeus	*jamaicensis*	*triomylus*	*Artibeus*	*jamaicensis*
Artibeus	*jamaicensis*	*palmarum*	*Artibeus*	*lituratus*

Taxonomy from The Natural History Museum (London) Registrar´s Book			Current Taxonomy	
Genus	Species	Subspecies	Genus	Species
Artibeus	*lituratus*	*palmarum*	*Artibeus*	*lituratus*
Artibeus	*lituratus*	*intermedius*	*Artibeus*	*lituratus*
Artibeus	*nanus*		*Dermanura*	*phaeotis*
Artibeus	*nanus* (*quadrivittatus*)		*Dermanura*	*phaeotis*
Artibeus	*perspicillatus*		*Artibeus*	*jamaicensis*
Artibeus	*toltecus*		*Dermanura*	*tolteca*
Artibeus	*turpis* (*cinereus*)		*Dermanura*	*phaeotis*
Carollia	*brevicaudata*		*Carollia*	*sowelli*
Carollia	*perspicillata*		*Carollia*	*sowelli*
Carollia	*subrufa*		*Carollia*	*subrufa*
Chiroderma	*salvini*		*Chiroderma*	*villosum*
Choeronycteris	*mexicana*		*Choeronycteris*	*mexicana*
Desmodus	*rotundus*	*murinus*	*Desmodus*	*rotundus*
Desmodus	*rufus*		*Desmodus*	*rotundus*
Desmodus			*Desmodus*	*rotundus*
Glossophaga	*soricina*	*leachii*	*Glossophaga*	*soricina*
Glossophaga	*soricina*		*Glossophaga*	*soricina*
Leptonycteris	*nivalis*		*Leptonycteris*	*curasoae*
Leptonycteris	*nivalis*	*nivalis*	*Leptonycteris*	*yerbabuenae*
Leptonycteris	*nivalis*		*Leptonycteris*	*yerbabuenae*
Macrotus	*bulleri*		*Macrotus*	*waterhousii*
Macrotus	*californicus*		*Macrotus*	*californicus*
Macrotus	*mexicanus*	*mexicanus*	*Macrotus*	*waterhousii*
Macrotus	*waterhousii*		*Macrotus*	*waterhousii*
Micronycteris	*megalotis*	*mexicana*	*Micronycteris*	*microtis*
Mimon	*bennetii*		*Mimon*	*cozumelae*
Musonycteris	*harrisoni*		*Musonycteris*	*harrisoni*
Otopterus	*californicus*		*Macrotus*	*californicus*
Phyllostoma	*verrucosum*		*Phyllostomus*	*discolor*
Saelus			*Glossophaga*	*soricina*
Sturnira	*lilium*		*Sturnira*	*hondurensis*
Sturnira	*lilium*		*Sturnira*	*parvidens*
Trachyops	*cirrhosus*		*Trachops*	*cirrhosus*
Vespertilio (*Hemiderma*)	*brevicauda*		*Carollia*	*sowelli*
Natalus	*mexicana*		*Natalus*	*mexicanus*
Natalus	*mexicanus*		*Natalus*	*mexicanus*
Natalus	*stramineus*		*Natalus*	*mexicanus*

Taxonomy from The Natural History Museum (London) Registrar´s Book			Current Taxonomy	
Genus	Species	Subspecies	Genus	Species
Antrozous	*pallidus*	*pacificus*	*Antrozous*	*pallidus*
Atalapha	*borealis*		*Lasiurus*	*frantzii*
Atalapha	*cinerea*		*Aeorestes*	*cinereus*
Atalapha	*noveboracensis*		*Lasiurus*	*borealis*
Atalapha	*noveboracensis*		*Lasiurus*	*frantzii*
Corynorhinus	*macrotis*		*Corynorhinus*	*mexicanus*
Dasypterus	*ega*		*Dasypterus*	*ega*
Dasypterus	*ega*	*xanthinus*	*Dasypterus*	*xanthinus*
Dasypterus	*ega*		*Dasypterus*	*xanthinus*
Dasypterus	*intermedius*		*Dasypterus*	*intermedius*
Eptesicus	*fuscus*		*Eptesicus*	*fuscus*
Myotis	*albescens*		*Myotis*	*albescens*
Myotis	*californicus*		*Myotis*	*californicus*
Myotis	*fortidens*		*Myotis*	*fortidens*
Myotis	*lucifugus*	*fortidens*	*Myotis*	*fortidens*
Myotis	*parvulus*		*Myotis*	*nigricans*
Myotis	*peninsularis*		*Myotis*	*velifer*
Myotis	*thysanodes*		*Myotis*	*thysanodes*
Myotis	*velifer*		*Myotis*	*velifer*
Myotis			*Myotis*	*thysanodes*
Noctulina			*Eptesicus*	*fuscus*
Nycticeus	*crepuscularis*		*Nycticeius*	*humeralis*
Pipistrellus	*hesperus*		*Parastrellus*	*hesperus*
Pizonyx	*vivesi*		*Myotis*	*vivesi*
Plecotus	*macrotis*		*Corynorhinus*	*mexicanus*
Rhogeessa	*alleni*		*Rhogeessa*	*alleni*
Rhogeessa	*parvula*		*Rhogeessa*	*parvula*
Rhogeessa	*parvula*		*Rhogeessa*	*aeneus*
Rhogeessa	*tumida*		*Rhogeessa*	*aeneus*
Vespertilio	*fuscus*	*peninsulae*	*Eptesicus*	*fuscus*
Vespertilio	*leucogaster*		*Myotis*	*thysanodes*
Vespertilio	*nigricans*		*Myotis*	*californicus*
Vespertilio	*nigricans*		*Myotis*	*nigricans*
Vespertilio			*Myotis*	*thysanodes*
Vespertilio			*Myotis*	*yumanensis*
Vespertilio			*Myotis*	*velifer*
Vespertilio			*Myotis*	*californicus*

Taxonomy from The Natural History Museum (London) Registrar´s Book			Current Taxonomy	
Genus	Species	Subspecies	Genus	Species
Vespertilionidae			*Tadarida*	*brasiliensis*
Vesperugo	*parvulus*		*Rhogeessa*	*parvula*
Eumops	*glaucinus*		*Eumops*	*ferox*
Lasiurus			*Tadarida*	*brasiliensis*
Molossus	*abrasus*		*Eumops*	*auripendulus*
Molossus	*glaucinus*		*Eumops*	*ferox*
Molossus	*nigricans*		*Promops*	*centralis*
Molossus	*obscurus*		*Molossus*	*molossus*
Molossus	*rufus*		*Glossophaga*	*soricina*
Molossus	*rufus*		*Molossus*	*rufus*
Molossus	*rufus*		*Promops*	*centralis*
Nyctinomus	*brasiliensis*		*Tadarida*	*brasiliensis*
Nyctinomus	*femorosaccus*		*Nyctinomops*	*femorosaccus*
Nyctinomus	*gracilis*		*Nyctinomops*	*laticaudatus*
Nyctinomus	*mexicanus*		*Tadarida*	*brasiliensis*
Promops	*centralis*		*Promops*	*centralis*
Tadarida	*aurispinosa*		*Nyctinomops*	*aurispinosus*
Tadarida	*brasiliensis*		*Tadarida*	*brasiliensis*
Tadarida	*laticaudata*	*ferruginea*	*Nyctinomops*	*laticaudatus*
Canis	*clepticus*		*Canis*	*latrans*
Canis	*latrans*		*Canis*	*latrans*
Urocyon	*cinereoargentatus*		*Urocyon*	*cinereoargenteus*
Urocyon	*cinereoargentatus*	*scotti*	*Urocyon*	*cinereoargenteus*
Vulpes	*cinereoargentata*		*Urocyon*	*cinereoargenteus*
Vulpes	*virginiana*		*Urocyon*	*cinereoargenteus*
Vulpes	*virginianus*		*Urocyon*	*cinereoargenteus*
Felis	*concolor*		*Puma*	*concolor*
Felis	*macroura*		*Leopardus*	*wiedii*
Felis	*pardalis*		*Leopardus*	*wiedii*
Felis	*pardalis*		*Leopardus*	*pardalis*
Felis	*pardalis*		*Leopardus*	*pardalis*
Felis	*tigrina*		*Leopardus*	*wiedii*
Herpailurus (*Felis*)	*yaguaroundi*	*tolteca*	*Herpailurus*	*yagouaroundi*
Leopardus	*hernandesii*		*Panthera*	*onca*
Leopardus	*onca?*		*Panthera*	*onca*
Leopardus (*Felis*)	*wiedii* (*tigrina*)	*glauculus* (*glaucula*)	*Leopardus*	*wiedii*

Taxonomy from The Natural History Museum (London) Registrar´s Book			Current Taxonomy	
Genus	Species	Subspecies	Genus	Species
Lynchus	*macularis*		*Lynx*	*rufus*
Lynchus	*macularus*		*Lynx*	*rufus*
Lyncus (*Lynchus*)	*macularus* (*rufa*)		*Lynx*	*rufus*
Lynx	*rufus*	*peninsularis*	*Lynx*	*rufus*
Panthera	*onca*	*goldmani*	*Panthera*	*onca*
Conepatus	*mapurito*		*Conepatus*	*leuconotus*
Conepatus	*nasutus*		*Conepatus*	*leuconotus*
Conepatus	*semistriatus*	*conepatl*	*Conepatus*	*semistriatus*
Galera	*barbara*	*senex*	*Eira*	*barbara*
Lutra	*annectens*	*major*	*Lontra*	*longicaudis*
Lutra	*felina*		*Lontra*	*longicaudis*
Meles	*taxus*		*Taxidea*	*taxus*
Mephites	*macroura*		*Mephitis*	*macroura*
Mephites	*macrourus*		*Mephitis*	*macroura*
Mephites			*Mephitis*	*macroura*
Mephitis	*macroura*		*Mephitis*	*macroura*
Mephitis	*macrura*		*Mephitis*	*macroura*
Mephitis	*mexicana*		*Mephitis*	*macroura*
Mephitis	*vittata*	*var. concolor*	*Mephitis*	*macroura*
Mephitis	*vittata*		*Mephitis*	*macroura*
Mustela	*brasiliensis*		*Mustela*	*frenata*
Mustela	*frenata*		*Mustela*	*frenata*
Mustela			*Mustela*	*frenata*
Putorius	*brasiliensis*		*Mustela*	*frenata*
Spilogale	*lucasana*		*Spilogale*	*gracilis*
Spilogale	*pygmaea*		*Spilogale*	*pygmaea*
Taxidea	*taxus*	*infusca*	*Taxidea*	*taxus*
Basaris	*astuta*		*Bassariscus*	*astutus*
Basaris	*astutus*		*Bassariscus*	*astutus*
Bassaris	*astuta*		*Bassariscus*	*astutus*
Bassaris	*astuto*		*Bassariscus*	*astutus*
Bassaris			*Bassariscus*	*astutus*
Bassariscus	*astutus*		*Bassariscus*	*astutus*
Cercoleptes	*caudivolvolus*	*aztecus*	*Potos*	*flavus*
Nasua	*narica*	*narica*	*Nasua*	*narica*
Nasua	*nelsoni*		*Nasua*	*narica*

Taxonomy from The Natural History Museum (London) Registrar´s Book			Current Taxonomy	
Genus	Species	Subspecies	Genus	Species
Nasua			*Nasua*	*narica*
Nasua Thersites (*Nasua*)	*nelsoni*		*Nasua*	*narica*
Procyon	*cancrivorous*		*Procyon*	*lotor*
Procyon	*lotor*		*Procyon*	*lotor*
Dicotyles	*tajacu*		*Pecari*	*tajacu*
Dicotyles	*tayassu*		*Pecari*	*tajacu*
Pecari	*tajacu*	*nelsoni*	*Pecari*	*tajacu*
Cariacus	*virginians*		*Odocoileus*	*virginianus*
Cariacus (*Coassus*)	*rufinus*		*Mazama*	*temama*
Coassus	*mexicanus*		*Odocoileus*	*virginianus*
Coassus	*rufinus*		*Mazama*	*temama*
Coassus (*Mazama*)	*tema*		*Odocoileus*	*virginianus*
Dorcelaphus	*americanus*	*mexicanus*	*Odocoileus*	*virginianus*
Dorcelaphus	*hemionus*	*peninsulae*	*Odocoileus*	*hemionus*
Dorcelaphus	*americanus*	*mexicanus*	*Odocoileus*	*virginianus*
Antilocapra	*americana*		*Antilocapra*	*americana*
Ovis	*canadensis*	*nelsoni*	*Ovis*	*canadensis*
Ovis	*canadensis*	*gaillardi*	*Ovis*	*canadensis*
Ovis	*canadensis*	*mexicana*	*Ovis*	*canadensis*
Ovis	*mexicanus*		*Ovis*	*canadensis*
Ammospermophilus	*leucurus*	*peninsulae*	*Ammospermophilus*	*leucurus*
Citellus	*annulatus*	*annulatus*	*Notocitellus*	*annulatus*
Citellus	*annulatus*	*goldmani*	*Notocitellus*	*annulatus*
Citellus	*beecheyi*	*fisheri*	*Otospermophilus*	*beecheyi*
Citellus	*leucurus*	*peninsulae*	*Ammospermophilus*	*leucurus*
Citellus	*mexicanus*		*Ictidomys*	*mexicanus*
Citellus	*spilosoma*		*Xerospermophilus*	*spilosoma*
Citellus	*variegatus*	*grammurus*	*Otospermophilus*	*variegatus*
Citellus	*variegatus*	*fisheri*	*Notocitellus*	*beecheyi*
Macroxus	*leucops*		*Sciurus*	*aureogaster*
Otospermophilus	*grammurus*	*fisheri*	*Notocitellus*	*beecheyi*
Sciurus	*aberti*	*durangi*	*Sciurus*	*aberti*
Sciurus	*albipes*	*nemoralis*	*Sciurus*	*aureogaster*
Sciurus	*albipes*	*quercinus*	*Sciurus*	*aureogaster*
Sciurus	*alleni*		*Sciurus*	*alleni*
Sciurus	*aureogaster*	*poliopus*	*Sciurus*	*aureogaster*

Taxonomy from The Natural History Museum (London) Registrar´s Book			Current Taxonomy	
Genus	Species	Subspecies	Genus	Species
Sciurus	*aureogaster*	*hypopyrrhus*	*Sciurus*	*aureogaster*
Sciurus	*aureogaster*	*frumentor*	*Sciurus*	*aureogaster*
Sciurus	*carolinensis*		*Sciurus*	*colliaei*
Sciurus	*carolinensis*		*Sciurus*	*nayaritensis*
Sciurus	*cervicalis*		*Sciurus*	*aureogaster*
Sciurus	*colliaei*	*colliaei*	*Sciurus*	*colliaei*
Sciurus	*deppei*		*Sciurus*	*deppei*
Sciurus	*durangae*		*Sciurus*	*aberti*
Sciurus	*goldmani*		*Sciurus*	*variegatoides*
Sciurus	*hypopyrrhus*	*colliaei*	*Sciurus*	*yucatanensis*
Sciurus	*hypopyrrhus*	*colliaei*	*Sciurus*	*colliaei*
Sciurus	*hypopyrrhus*	*melanonotus*	*Sciurus*	*aureogaster*
Sciurus	*intermedius?*		*Sciurus*	*aureogaster*
Sciurus	*maurus*		*Sciurus*	*aureogaster*
Sciurus	*nayaritensis*		*Sciurus*	*nayaritensis*
Sciurus	*nelsoni*		*Sciurus*	*aureogaster*
Sciurus	*niger*	*ludovicianus*	*Sciurus*	*nayaritensis*
Sciurus	*niger*	*melanonotus*	*Sciurus*	*oculatus*
Sciurus	*niger*	*nayaritensis*	*Sciurus*	*nayaritensis*
Sciurus	*sinaloensis*		*Sciurus*	*colliaei*
Sciurus	*socialis*	*cocos*	*Sciurus*	*aureogaster*
Sciurus	*tephrogaster*		*Sciurus*	*deppei*
Sciurus	*variegatus*	*leucops*	*Sciurus*	*aureogaster*
Sciurus	*variegatus*		*Sciurus*	*variegatus*
Sciurus	*yucatanensis*	*yucatanensis*	*Sciurus*	*yucatanensis*
Sciurus			*Sciurus*	*griseus*
Sciurus			*Sciurus*	*deppei*
Sciurus			*Sciurus*	*aureogaster*
Spermophilus	*grammurus*		*Sciurus*	*variegatus*
Spermophilus	*palustris*		*Ammospermophilus*	*leucurus*
Spermophilus	*lewisi*		*Notocitellus*	*annulatus*
Spermophilus	*macrourus*		*Otospermophilus*	*variegatus*
Spermophilus	*mexicanus*		*Ictidomys*	*mexicanus*
Spermophilus	*spilosoma*		*Xerospermophilus*	*spilosoma*
Spermophilus			*Otospermophilus*	*variegatus*
Spermophilus			*Ammospermophilus*	*leucurus*

Taxonomy from The Natural History Museum (London) Registrar´s Book			Current Taxonomy	
Genus	Species	Subspecies	Genus	Species
Spermophilus			*Otospermophilus*	*variegatus*
Tamias	*asiaticus*	*quadrivittatus*	*Otospermophilus*	*variegatus*
Tamias	*asiaticus*	*quadrivittatus*	*Neotamias*	*dorsalis*
Tamias	*asiaticus*	*dorsalis*	*Neotamias*	*dorsalis*
Tamias	*asiaticus*	*bulleri*	*Neotamias*	*dorsalis*
Tamias	*dorsalis*		*Neotamias*	*dorsalis*
Tamias	*harrisi*		*Ammospermophilus*	*leucurus*
Tamias	*leucurus*	*peninsulae*	*Ammospermophilus*	*leucurus*
Tamias	*quadrivittatus*		*Neotamias*	*dorsalis*
Geomys	*bulleri*		*Pappogeomys*	*bulleri*
Geomys	*bursarius*		*Cratogeomys*	*merriami*
Geomys	*estor*		*Cratogeomys*	*merriami*
Geomys	*hispidus*		*Heterogeomys*	*hispidus*
Geomys	*mexicanus*		*Cratogeomys*	*merriami*
Geomys	*umbrinus*		*Thomomys*	*umbrinus*
Geomys			*Heterogeomys*	*hispidus*
Geomys (*Orthogeomys*)	*nelsoni*		*Heterogeomys*	*hispidus*
Geomys Scalops (*Thomomys*)			*Orthogeomys*	*grandis*
Orthogeomys	*grandis*		*Orthogeomys*	*grandis*
Orthogeomys	*hispidus*		*Heterogeomys*	*hispidus*
Saccophorus	*mexicanus*		*Cratogeomys*	*merriami*
Saccophorus (=*Geomys*)	*hispidus*		*Heterogeomys*	*hispidus*
Saccophorus (=*Geomys*)	*quachil*		*Heterogeomys*	*hispidus*
Thomomys	*atrovarius*		*Thomomys*	*umbrinus*
Thomomys	*bottae*	*camoae*	*Thomomys*	*bottae*
Thomomys	*fulvus*		*Thomomys*	*bottae*
Thomomys	*fulvus*	*anitae*	*Thomomys*	*bottae*
Thomomys	*fulvus*	*alticolus*	*Thomomys*	*bottae*
Thomomys	*talpoides*	*umbrinus*	*Thomomys*	*umbrinus*
Zygogeomys	*trichopus*		*Zygogeomys*	*trichopus*
Cricetodipus	*flavus*		*Perognathus*	*flavus*
Dipodomys	*merriami*	*mayensis*	*Dipodomys*	*merriami*
Dipodomys	*merriami*	*merriami*	*Dipodomys*	*merriami*
Dipodomys	*merriami*	*melanurus*	*Dipodomys*	*merriami*
Dipodomys	*phillipsii*		*Dipodomys*	*phillipsii*
Dipodomys	*spectabilis*	*spectabilis*	*Dipodomys*	*spectabilis*

Taxonomy from The Natural History Museum (London) Registrar´s Book			Current Taxonomy	
Genus	Species	Subspecies	Genus	Species
Dipodomys			*Dipodomys*	*merriami*
Dipodomys			*Dipodomys*	*simulans*
Dipodomys (*Dipodops*)			*Dipodomys*	*merriami*
Heteromys	*albolimbatus*		*Heteromys*	*irroratus*
Heteromys	*bulleri*		*Heteromys*	*irroratus*
Heteromys	*desmarestianus*		*Heteromys*	*gaumeri*
Heteromys	*gaumeri*		*Heteromys*	*gaumeri*
Heteromys	*gaumeri*		*Heteromys*	*pictus*
Heteromys	*goldmani*		*Heteromys*	*desmarestianus*
Heteromys	*hispidus*		*Heteromys*	*pictus*
Heteromys	*irroratus*		*Heteromys*	*irroratus*
Heteromys	*longicaudatus*		*Heteromys*	*desmarestianus*
Heteromys	*pictus*	*obscurus*	*Heteromys*	*pictus*
Heteromys			*Heteromys*	*desmarestianus*
Heteromys			*Heteromys*	*pictus*
Liomys	*pictus*	*sonoranus*	*Heteromys*	*pictus*
Liomys	*pictus*		*Heteromys*	*pictus*
Paradipus			*Dipodomys*	*simulans*
Perodipus	*agilis*		*Dipodomys*	*simulans*
Perognathus	*californicus*		*Chaetodipus*	*californicus*
Perognathus	*flavus*		*Perognathus*	*flavus*
Perognathus	*goldmani*		*Chaetodipus*	*goldmani*
Perognathus	*penicillatus*	*angustirostris*	*Chaetodipus*	*penicillatus*
Perognathus	*pernix*		*Chaetodipus*	*pernix*
Perognathus	*spinatus*	*spinatus*	*Chaetodipus*	*spinatus*
Perognathus	*spinatus*	*peninsulae*	*Chaetodipus*	*spinatus*
Arvicola	*mexicanus*		*Microtus*	*mexicanus*
Arvicola	*riparius*		*Microtus*	*pennsylvanicus*
Baiomys	*taylori*		*Baiomys*	*taylori*
Cricetus	*palustris*		*Sigmodon*	*hispidus*
Gerbillus (*Hesperomys*)	*(couesi*)		*Oryzomys*	*couesi*
Hesperomys	*aureolus*		*Oligoryzomys*	*fulvescens*
Hesperomys	*couesi*		*Oryzomys*	*couesi*
Hesperomys	*couesi*		*Peromyscus*	*simulus*
Hesperomys	*leucopus*		*Handleyomys*	*rostratus*
Hesperomys	*leucopus*		*Peromyscus*	*leucopus*

Taxonomy from The Natural History Museum (London) Registrar´s Book			Current Taxonomy	
Genus	Species	Subspecies	Genus	Species
Hesperomys	*leucopus*		*Peromyscus*	*mexicanus*
Hesperomys	*leucopus*		*Peromyscus*	*maniculatus*
Hesperomys	*leucopus*		*Peromyscus*	*levipes*
Hesperomys	*leucopus*		*Peromyscus*	*leucopus*
Hesperomys	*maculipes*		*Oryzomys*	*couesi*
Hesperomys	*sumichrasti*		*Nyctomys*	*sumichrasti*
Hesperomys			*Oryzomys*	*couesi*
Hodomys	*alleni*	*alleni*	*Hodomys*	*alleni*
Hodomys	*vetulus*	*elatturus*	*Hodomys*	*alleni*
Microtus	*fulviventer*		*Microtus*	*mexicanus*
Microtus	*mexicanus*		*Microtus*	*mexicanus*
Microtus	*quasiater*		*Microtus*	*quasiater*
Microtus	*umbrosus*		*Microtus*	*umbrosus*
Mus	*alexandrinus*		*Rattus*	*rattus*
Mus	*leucopus*	*sonorensis*	*Peromyscus*	*leucopus*
Mus	*maculipes*		*Oryzomys*	*couesi*
Mus	*musculus*	*jalapae*	*Mus*	*musculus*
Mus	*rattus*		*Rattus*	*rattus*
Mus			*Mus*	*musculus*
Mus			*Rattus*	*norvegicus*
Nelsonia	*neotomodon*		*Nelsonia*	*neotomodon*
Neotoma	*alleni*		*Hodomys*	*alleni*
Neotoma	*arenacea*		*Neotoma*	*lepida*
Neotoma	*brevicauda*		*Neotoma*	*mexicana*
Neotoma	*bryanti*		*Neotoma*	*bryanti*
Neotoma	*floridana*		*Neotoma*	*mexicana*
Neotoma	*fuscipes*	*macrotis*	*Neotoma*	*macrotis*
Neotoma	*fuscipes*		*Hodomys*	*alleni*
Neotoma	*mexicana*	*sinaloae*	*Neotoma*	*albigula*
Neotoma	*mexicana*	*chamula*	*Neotoma*	*mexicana*
Neotoma	*phenax*		*Neotoma*	*phenax*
Neotoma	*sinaloae*		*Neotoma*	*mexicana*
Neotoma	*tenuicauda*		*Neotoma*	*mexicana*
Neotoma			*Neotoma*	*albigula*
Neotoma			*Neotoma*	*lepida*
Neotomodon	*alstoni*		*Neotomodon*	*alstoni*

Taxonomy from The Natural History Museum (London) Registrar´s Book			Current Taxonomy	
Genus	Species	Subspecies	Genus	Species
Nyctomys	*sumichrasti*	*colimensis*	*Nyctomys*	*sumichrasti*
Ochetodon	*humulis*		*Reithrodontomys*	*fulvescens*
Ochetodon	*mexicanus*		*Reithrodontomys*	*mexicanus*
Ochetodon	*mexicanus*		*Reithrodontomys*	*zacatecae*
Ochetodon	*mexicanus*		*Reithrodontomys*	*fulvescens*
Onychomys	*torridus*	*yakiensis*	*Onychomys*	*torridus*
Oryzomys	*chapmani*		*Handleyomys*	*chapmani*
Oryzomys	*fulgens*		*Oryzomys*	*couesi*
Oryzomys	*fulvescens*		*Oligoryzomys*	*fulvescens*
Oryzomys	*jalapae*		*Oryzomys*	*couesi*
Oryzomys	*maculipes*		*Oryzomys*	*couesi*
Oryzomys	*melanotis*	*colimensis*	*Handleyomys*	*melanotis*
Oryzomys	*melanotis*	*chapmani*	*Handleyomys*	*chapmani*
Oryzomys	*mexicanus*		*Oryzomys*	*couesi*
Oryzomys	*peninsulae*		*Oryzomys*	*couesi*
Oryzomys	*rostratus*		*Handleyomys*	*rostratus*
Oryzomys			*Peromyscus*	*yucatanicus*
Oryzomys			*Oligoryzomys*	*fulvescens*
Oryzomys			*Oryzomys*	*couesi*
Oryzomys			*Handleyomys*	*rostratus*
Oryzomys			*Oligoryzomys*	*fulvescens*
Oryzomys			*Oryzomys*	*couesi*
Otonyctomys	*hatti*		*Otonyctomys*	*hatti*
Ototylomys	*brevirostris*	*affinis*	*Ototylomys*	*phyllotis*
Ototylomys	*phyllotis*	*guatemalae*	*Ototylomys*	*phyllotis*
Peromyscus	*auritus*		*Peromyscus*	*megalops*
Peromyscus	*beatae*		*Peromyscus*	*beatae*
Peromyscus	*boylii*	*spicilegus*	*Peromyscus*	*simulus*
Peromyscus	*boylii*		*Peromyscus*	*aztecus*
Peromyscus	*cecilii*		*Peromyscus*	*melanotis*
Peromyscus	*comptus*		*Peromyscus*	*megalops*
Peromyscus	*cristobalensis*		*Peromyscus*	*zarhynchus*
Peromyscus	*difficilis*		*Neotomodon*	*alstoni*
Peromyscus	*difficilis*		*Peromyscus*	*difficilis*
Peromyscus	*eremicus*	*eva*	*Peromyscus*	*eva*
Peromyscus	*eva*		*Peromyscus*	*eva*

Taxonomy from The Natural History Museum (London) Registrar´s Book			Current Taxonomy	
Genus	Species	Subspecies	Genus	Species
Peromyscus	*felipensis*		*Peromyscus*	*difficilis*
Peromyscus	*fraterculus*		*Peromyscus*	*fraterculus*
Peromyscus	*furvus*		*Peromyscus*	*furvus*
Peromyscus	*gratus*		*Peromyscus*	*gratus*
Peromyscus	*guatemalensis*		*Peromyscus*	*guatemalensis*
Peromyscus	*leucopus*	*coolidgei*	*Peromyscus*	*maniculatus*
Peromyscus	*leucurus S*	*gadovii*	*Peromyscus*	*melanophrys*
Peromyscus	*madrensis*		*Peromyscus*	*madrensis*
Peromyscus	*maniculatus*		*Peromyscus*	*maniculatus*
Peromyscus	*martirensis*		*Peromyscus*	*truei*
Peromyscus	*megalops*		*Peromyscus*	*megalops*
Peromyscus	*melanotis*		*Peromyscus*	*melanotis*
Peromyscus	*mexicanus*		*Peromyscus*	*mexicanus*
Peromyscus	*mexicanus*	*orizabae*	*Peromyscus*	*mexicanus*
Peromyscus	*musculoides*		*Peromyscus*	*leucopus*
Peromyscus	*musculus*		*Mus*	*musculus*
Peromyscus	*musculus*	*brunneus*	*Baiomys*	*musculus*
Peromyscus	*musculus*		*Baiomys*	*taylori*
Peromyscus	*spicilegus*		*Peromyscus*	*simulus*
Peromyscus	*tehuantepecus*		*Peromyscus*	*mexicanus*
Peromyscus	*thomasi*		*Megadontomys*	*thomasi*
Peromyscus	*totontepecus*		*Peromyscus*	*mexicanus*
Peromyscus	*truei*	*ssp.*	*Peromyscus*	*gratus*
Peromyscus	*truei*	*ssp.*	*Peromyscus*	*maniculatus*
Peromyscus	*umbrosus*		*Microtus*	*umbrosus*
Peromyscus	*yucatanensis*	*yucatanensis*	*Peromyscus*	*yucatanicus*
Peromyscus	*yucatanensis*		*Osgoodomys*	*banderanus*
Peromyscus	*zarrynchus*		*Peromyscus*	*zarhynchus*
Peromyscus			*Peromyscus*	*yucatanicus*
Peromyscus			*Peromyscus*	*levipes*
Peromyscus			*Peromyscus*	*mexicanus*
Peromyscus			*Osgoodomys*	*banderanus*
Peromyscus			*Onychomys*	*torridus*
Pitymys			*Microtus*	*quasiater*
Rattus	*norvegicus*		*Rattus*	*norvegicus*
Rattus	*rattus*		*Rattus*	*rattus*

Taxonomy from The Natural History Museum (London) Registrar´s Book			Current Taxonomy	
Genus	Species	Subspecies	Genus	Species
Reithrodontomys	*megalotis*		*Reithrodontomys*	*megalotis*
Reithrodontomys	*mexicanus*		*Reithrodontomys*	*fulvescens*
Reithrodontomys	*mexicanus*		*Reithrodontomys*	*megalotis*
Reithrodontomys	*mexicanus*		*Reithrodontomys*	*fulvescens*
Reithrodontomys	*rufescens*		*Reithrodontomys*	*sumichrasti*
Reithrodontomys	*saturatus*		*Reithrodontomys*	*megalotis*
Reithrodontomys	*tenuis*		*Reithrodontomys*	*fulvescens*
Reithrodontomys			*Reithrodontomys*	*fulvescens*
Sigmodon	*colimae*		*Sigmodon*	*mascotensis*
Sigmodon	*hispidus*	*microdon*	*Sigmodon*	*hispidus*
Sigmodon	*hispidus*	*major*	*Sigmodon*	*arizonae*
Sigmodon	*hispidus*	*microdon*	*Sigmodon*	*toltecus*
Sigmodon	*hispidus*		*Sigmodon*	*toltecus*
Sigmodon	*hispidus*	*toltecus*	*Sigmodon*	*toltecus*
Sigmodon	*hispidus*		*Sigmodon*	*hispidus*
Sigmodon	*inexoratus*		*Sigmodon*	*mascotensis*
Sigmodon	*mascotensis*		*Sigmodon*	*mascotensis*
Sitomys			*Peromyscus*	*spicilegus*
Sitomys			*Peromyscus*	*difficilis*
Teanopus	*phenax*		*Neotoma*	*phenax*
Cercolabes	*mexicanus*		*Coendou*	*mexicanus*
Coendou	*mexicanus*	*yucataniae*	*Coendou*	*mexicanus*
Coendou	*mexicanus*		*Coendou*	*mexicanus*
Synethiris	*mexicanus*		*Coendou*	*mexicanus*
Dasyprocta	*mexicana*		*Dasyprocta*	*mexicana*
Dasyprocta	*punctata*		*Dasyprocta*	*punctata*
Coelogenys	*paca*		*Cuniculus*	*paca*
Lepus	*californicus*	*xanti*	*Lepus*	*californicus*
Lepus	*callotis*		*Lepus*	*callotis*
Lepus	*flavigularis*		*Lepus*	*flavigularis*
Lepus	*graysoni*		*Sylvilagus*	*graysoni*
Lepus	*nigricaudatus*		*Lepus*	*callotis*
Lepus	*palustris*		*Sylvilagus*	*cunicularius*
Lepus	*palustris*		*Sylvilagus*	*floridanus*
Lepus	*peninsularis*		*Sylvilagus*	*bachmani*
Lepus	*sylvaticus*		*Sylvilagus*	*floridanus*

Taxonomy from The Natural History Museum (London) Registrar´s Book			Current Taxonomy	
Genus	Species	Subspecies	Genus	Species
Lepus	*sylvaticus*		*Sylvilagus*	*cunicularius*
Lepus	*verae-crucis*		*Sylvilagus*	*cunicularius*
Lepus			*Lepus*	
Lepus			*Sylvilagus*	*cunicularius*
Lepus			*Sylvilagus*	*floridanus*
Lepus			*Lepus*	*alleni*
Romerolagus	*diazi*		*Romerolagus*	*diazi*
Romerolagus	*diazi*	*nelsoni*	*Romerolagus*	*diazi*
Romerolagus	*nelsoni*		*Romerolagus*	*diazi*
Sylvilagus	*floridanus*	*yucatanicus*	*Sylvilagus*	*floridanus*
Sylvilagus			*Sylvilagus*	*graysoni*
Sylvilagus			*Sylvilagus*	*cunicularius*

APPENDIX II

Catalog of the holotypes of Mexican mammals housed in The Natural History Museum (London). The entries are arranged as follows: catalog number of the holotype, the taxon name as used at the time of publication, locality, preparation type, and external measurements. Abbreviations for specimen preparation types are as follows: AL – specimen stored in alcohol; SA – skull and alcoholic skin; SK – skull only; SN – skin, skull, and skeleton; SO – skin only; SS – skin and skull. External measurements are arranged in the following order: total length, length of tail, length of hind foot, and length of ear. In the measurements, x = no data available.

Allen, J. A. 1898. Description of new mammals from western Mexico and Lower California. Bulletin of the American Museum of Natural History 10:143–158.

BM(NH) 98.3.2.161 *Marmosa sinaloae* Tatemales, State of Sinaloa SS 242-130-16-22

BM(NH) 98.3.1.165 *Lepus peninsularis* Santa Anita; Lower California SS 324-20-73-61

BM(NH) 98.3.1.125 *Thomomys fulvus anitae* Santa Anita; Lower California SS 250-83-34-9

BM(NH) 98.3.2.102 *Thomomys atrovarius* Tatemales, State of Sinaloa SS 210-65-26-7

BM(NH) 98.3.2.126 *Perognathus pernix* Rosario, State of Sinaloa SS 165-90-22-9

BM(NH) 98.3.2.88 *Neotoma sinaloae* Tatameles, State of Sinaloa SS 332-160-32-27

BM(NH) 98.3.1.112 *Neotoma arenacea* San José del Cabo, Lower California SS 349-167-35-31

Allen, J. A. 1899. Descriptions of five new American rodents. Bulletin of the American Museum of Natural History 12:11–17.

BM(NH) 98.3.1.176 *Thomomys fulvus alticolus* Sierra Laguna, 7,000 ft, Lower California SS 225-61-30-9.5

BM(NH) 98.3.2.167 *Reithrodontomys tenuis* Rosario, Sinaloa SS 152-81-20-15

Andersen, K. 1906. Brief diagnoses of a new genus and ten new forms of stenodermatouse bats. Annals and Magazine of Natural History, series 7 18:419–423.

BM(NH) 88.8.8.29 *Artibeus turpis* Teapa, Tabasco, S. Mexico SA x-x-14.18-17.39

BM(NH) 89.1.30.5 *Artibeus nanus* Tierra Colorada, Sierra Madre del Sur, Guerrero, Mexico SA x-x-8.53-12.75

Bennett, E. T. 1833. Characters of new species of Mammalia from California. Proceedings of the Zoological Society of London, part 1:39–42.

BM(NH) 57.12.14.5 *Didelphys breviceps* Unknown SS x-x-56-x

BM(NH) 55.12.24.360 *Spermophilus spilosoma* Unknown SS x-x-30-9

BM(NH) 55.12.24.123 *Spermophilus macrourus* Unknown SS 500-200-61-25

BM(NH) 53.8.29.37 *Lepus nigricaudatus* Unknown SS x-x-116.59-110.1

Dobson, G. E. 1878. Catalogue of the Chiroptera in the collection of the British Museum. British Museum (Natural History), London 567 + 30 pp.

BM(NH) 50.8.29.3 *Chilonycteris psilotis* Unknown SA 56-14-11-15

BM(NH) 50.8.29.4 *Chilonycteris psilotis* Unknown AL 61-14-10-15

Gray, J. E. 1841. A new genus of Mexican glirine Mammalia. Annals and Magazine of Natural History, series 1 7:521–522.

BMNH 45.1580 *Dipodomys phillipii* Mexico, near Real del Monte SO x-x-40.38-x

Gray, J. E. 1858. Notice of a new species of jaguar from Mazatlan living in the gardens of the Zoological Society. Proceedings of the Zoological Society of London 1857:278.

BM(NH) 67.4.5.1 *Leopardus Hernandesii* Mazatlan SN x-x-x-x

Gray, J. E. 1865. Revision of the genera and species of Mustelidae contained in the British Museum. Proceedings of the Zoological Society of London 1865:100–154.

BM(NH) 53.5.7.17 *Mephitis vittata* var. *concolor* Mexico SS 488-260-55.14-22.84

Gray, J. E. 1866. Notice of some new species of spider monkeys (*Ateles*) in the collection of the British Museum. Proceedings of the Zoological Society of London for 1865:732–733.

BM(NH) 43.9.14.3 *Ateles vellerosus* Brazil? SS x-x-x-x

[*Note*: The actual type locality was restricted to Mirador, 15 mi NE Huatusco, Veracruz, Mexico, altitude 2000 ft, by Kellogg and Goldman (1944).]

Gray, J. E. 1867. Synopsis of the species of American squirrels in the collection of the British Museum. Annals and Magazine of Natural History, series 3 20:415–434.

BM(NH) 59.11.1.4 *Macroxus maurus* Oaxaca, Mexico SS x-x-62.01-24.09

BM(NH) 59.11.1.5 *Macroxus maurus* Oaxaca, Mexico SS x-x-57-29

BM(NH) 58.10.22.4 *Macroxus leucops* Oaxaca, Mexico SS x-x-55.89-x

BM(NH) 56.8.1.11 *Macroxus tephrogaster* Mexico SS x-x-49-22

Gray, J. E. 1868. Synopsis of the species of Saccomyinae, or pouched mice, in the collection of the British Museum. Proceedings of the Zoological Society of London 1868:199–206.

BM(NH) 56.8.1.13 *Heteromys longicaudatus* Mexico (Sallé) SS x-x-x-x

BM(NH) 59.7.10.2 *Heteromys irroratus* State of Oaxaca, Mexico SS x-x-x-x

BM(NH) 61.11.14.9 *Heteromys albolimbatus* Mexico? La Parda SS x-x-x-x

BM(NH) 61.11.14.10 *Heteromys albolimbatus* Mexico? La Parda SS x-x-x-x

Gray, J. E. 1869. Catalogue of carnivorous, pachydermatous, and edentate Mammalia in the British Museum. British Museum (Natural History), London 398 pp.

BM(NH) 43.9.27.9 *Mephitis vittata* var. *intermedia* Mexico SO 679-317-60.59-16.29

BM(NH) 48.9.12.14 *Mephitis vittata* var. *intermedia* Mexico SK 679-317-60.59-16.29

Horsfield, T., and N. A. Vigors. 1829. Observations on some of the Mammalia contained in the collection of the Zoological Society of London. The Zoological Journal 4:380–384.

BM(NH) 55.12.24.275 *Felis maculata* Mexico SS 836-146-61.66-60.35

Laurie, E. M. O. 1953. Rodents from British Honduras, Mexico, Trinidad, Haiti and Jamaica collected by Mr. I. T. Sanderson. Annals and Magazine of Natural History, series 12 6:382–394.

BM(NH) 1952.306 *Ototylomys brevirostris affinis* Chichen-Itza, Yucatan, Mexico SS x-146-25-22

BM(NH) 1952.324 *Nyctomys sumichrasti colimensis* Juarez, Colima, Mexico SS x-104-21-19

Lydekker, R. 1898. On a new mule deer. Proceedings of the Zoological Society of London for 1897 4:899–900.

BM(NH) 98.3.1.172 *Mazama* (*Dorcelaphus*) *hemionus peninsulae* Sierra Laguna, Lower California SS x-x-x-x

Major, C. J. F. 1897. Der centralamerikanische Fischotter und seine nächsten Verwandten. Zoologischer Anzeiger (Leipzig) 20:136–142.

BM(NH) 92.3.17.8 *Lutra annectens* Central America SK x-x-x-x

Miller, G. S., Jr. 1898. Description of a new bat from Lower California. Annals and Magazine of Natural History, series 7, 2:124–125.

BM(NH) 98.3.1.59 *Myotis peninsularis* San José del Cabo, Lower California SS 91-34-10-15

Miller, G. S., Jr., and G. M. Allen. 1928. The American bats of the genera *Myotis* and *Pizonyx*. Bulletin of the U. S. National Museum 144: I–VIII + 1–218.

BM(NH) 88.8.8.18 *Myotis lucifugus fortidens* Teapa, Tabasco, Mexico SA 78-35-8-13

BM(NH) 58.6.2.3. *Myotis thysanodes aztecus* San Antonio, Oaxaca, Mexico SS x-x-x-x

Richardson, J. 1829. Fauna Boreali–Americana; or the zoology of the northern parts of British America: containing descriptions of the objects of the natural history collected on the late northern land expeditions, under command of captain Sir John Franklin, R. N. John Murray, Albemarle–Street, London, 1:XLVI + 1–300.

BM(NH) 55.12.24.205 *Geomys umbrinus* Cadadaguios, SW Louisiana SS x-x-x-x

[*Note*: The actual locality was restricted to southern México, probably in the vicinity of Boca de Monte, Veracruz, by Bailey (1906).]

Thomas, O. 1890. On a collection of mammals from central Veracruz, Mexico. Proceedings of the Zoological Society of London 11:71–76.

BM(NH) 89.12.7.8 *Sciurus niger melanonotus* Las Vigas, Jalapa SS x-x-65-28

BM(NH) 89.12.7.19 *Lepus veræ-crucis* Las Vigas, Jalapa SS x-x-101.75-82.89

Thomas, O. 1892a. Note on Mexican examples of *Chilonycteris Davyi* Gray. Annals and Magazine of Natural History, series 6 10:410.

BM(NH) 93.2.5.24 *Chilonycteris Davyi fulvus* Las Peñas, west coast of Jalisco SS x-x-x-x

Thomas, O. 1892b. Diagnosis of a new Mexican *Geomys*. Annals and Magazine of Natural History, series 6 10:196–197.

BM(NH) 92.10.7.16 *Geomys Bulleri* Talpa, Mascota, Jalisco, 8,500 ft SA x-62.14-25.76-x

Thomas, O. 1892(1893). Description of a new Mexican bat. Annals and Magazine of Natural History, series 6 10:477–478.

BM(NH) 93.2.5.25 *Rhogeessa Alleni* Santa Rosalia, near Autlan, Jalisco, Mexico SA 79-39-7-13

Thomas, O. 1893a. Notes on some Mexican *Oryzomys*. Annals and Magazine of Natural History, series 6 11:402–405.

BM(NH) 70.6.20.3 *Oryzomys fulgens* México SS x-x-x-x

BM(NH) 93.3.6.25 *Oryzomys melanotis* Mineral San Sebastian, Jalisco, Mexico SS x-x-28-19

Thomas, O. 1893b. Description of two new "pocket–mice" of the genus *Heteromys*. Annals and Magazine of Natural History, series 6 11:329–332.

BM(NH) 93.3.6.39 *Heteromys Bulleri* La Laguna, Sierra de Juanacatlan, Jalisco, Mexico, 7,000 ft SA x-121.7-31.48-14.53

Thomas, O. 1893c. On two new members of the genus *Heteromys* and two of *Neotoma*. Annals and Magazine of Natural History, series 6 12:233–235.

BM(NH) 93.8.12.2 *Heteromys pictus* Mineral San Sebastian, Jalisco, Mexico, 4,300 ft SS x-x-x-x

Thomas, O. 1893d. On some of the larger species of *Geomys*. Annals and Magazine of Natural History, series 6 12:269–273.

BM(NH) 70.6.20.2 *Geomys Merriami* S. Mexico SS 380-89-44.98-7.16

Thomas, O. 1893e. On the Mexican representative of *Sciurus Aberti*. Annals and Magazine of Natural History, series 6 11:49–50.

BM(NH) 82.3.20.16 *Sciurus Aberti durangi* Ciudad, Durango, central Mexico, 8,100 ft SS x-x-67-40

Thomas, O. 1894a. On two new neotropical mammals. Annals and Magazine of Natural History, series 6 13:436–439.

BM(NH) 79.1.6.2 *Geomys scalops* Tehuantepec SS 352-82-45.08-6

Thomas, O. 1894b. Description of some new neotropical Muridae. Annals and Magazine of Natural History, series 6 14:346–366

BM(NH) 79.1.6.3 *Peromyscus leucurus* Tehuantepec SS x-x-x-x

Thomas, O. 1897. Descriptions of new bats and rodents from America. Annals and Magazine of Natural History, series 6 20:544–553.

BM(NH) 98.3.1.14 *Dasypterus ega xanthinus* Sierra Laguna, Lower California SS 116-48-10-16

BM(NH) 98.3.1.107 *Oryzomys peninsulae* Santa Anita, Lower California SS 298-190-34-18

Thomas, O. 1898a. On new mammals from western Mexico and Lower California. Annals and Magazine of Natural History, series 7 1:40–46.

BM(NH) 98.3.2.17 *Felis yaguarondi tolteca* Tatemales, Sinaloa SS 1030-460-138-57

BM(NH) 98.3.1.51 *Lynx rufus peninsularis* Santa Anita, Lower California SS 761-154-160-87

BM(NH) 98.3.1.3 *Vespertilio fuscus peninsulae* Sierra Laguna, Lower California SS 95-34-10-14

BM(NH) 98.3.1.88 *Peromyscus eva* San José del Cabo, Lower California SS 196-108-21-17

BM(NH) 98.3.1.75 *Peromyscus leucopus Coolidgei* Santa Anita, Cape region of Lower California SS 167-76-22-20

BM(NH) 98.3.1.169 *Lepus californicus Xanti* Santa Anita, Lower California SS 540-63-120-125

Thomas, O. 1898b. On indigenous Muridae in the West Indies; with the description of a new Mexican *Oryzomys*. Annals and Magazine of Natural History, series 7 1:176–180.

BM(NH) 97.9.9.30 *Oryzomys Chapmani* Jalapa, Mexico SS x-116-24-19

Thomas, O. 1897 (1898c). *Taxidea taxus infusca*, a new subspecies from Lower California. Proceedings of the Zoological Society of London for 1897 p. 899.

BM(NH) 98.3.1.56 *Taxidea taxus infusca* Santa Anita, Lower California SS 702-123-94-50

Thomas, O. 1897 (1898d). Of a remarkably small skunk of the genus *Spilogale* which had been received in a collection made by Mr. P. O. Simons in western Mexico. Proceedings of the Zoological Society of London for 1897 pp. 898–899.

BM(NH) 98.3.2.24 *Spilogale pygmaea* Rosario, Sinaloa, W. Mexico SS 250-68-64-23

Thomas, O. 1900. The geographical races of the tayra (*Galictis barbara*), with notes on abnormally colored individuals. Annals and Magazine of Natural History, series 7 5:145–148.

BM(NH) 89.12.7.4 *Galictis barbara senex* Hacienda Tortugas, Jalapa, Vera Cruz, 190 m SS 690-385-110-30.34

Thomas, O. 1901. New insular forms of *Nasua* and *Dasyprocta*. Annals and Magazine of Natural History, series 7 8:271–273.

BM(NH) 86.10.8.1 *Nasua thersites* Cozumel Island, off the coast of Yucatan SS 888-364-76-28

Thomas, O. 1902a. On the geographical races of kinkajou. Annals and Magazine of Natural History, series 7 9:266–270.

BM(NH) 1888.8.8.1 *Potos flavus aztecus* Atoyac, Vera Cruz SO 1005-314-89.23-38

BM(NH) 1888.8.8.2 *Potos flavus aztecus* Atoyac, Vera Cruz SK 1005-314-89.23-38

Thomas, O. 1902b. New forms of *Saimiri*, *Oryzomys*, *Phyllotis*, *Coendou*, and *Cyclopes*. Annals and Magazine of Natural History, series 7 9:246–250.

BM(NH) 91.3.24.1. *Coendou mexicanus yucataniae* Yucatán (probably near Izamal) SS x-x-62-x

Thomas, O. 1903a. On three new forms of *Peromyscus* obtained by Dr. Hans Gadow, F.R.S. and Mrs. Gadow in Mexico. Annals and Magazine of Natural History, series 7 11:484–487.

BM(NH) 1903.3.4.55 *Peromyscus leucurus Gadovii* San Carlos Yantepec, Oaxaca, Mexico, 2,250 m (bet. Oaxaca and Tehuantepec) SS x-150-27-25

BM(NH) 1903.3.4.21 *Peromyscus Beatae* Xometla camp, Mt. Orizaba, 8,500 ft SS 118-21-19-28.3

BM(NH) 1903.3.4.23 *Peromyscus Cecilii* Santa Barbara camp, southern slope Mt. Orizaba, 12,500 ft SS x-75-21-17

Thomas, O. 1903b. Notes on neotropical mammals of the genera *Felis*, *Hapale*, *Oryzomys*, *Akodon*, and *Ctenomys*, with descriptions of new species. Annals and Magazine of Natural History, series 7 12:234–243.

BM(NH) 90.1.4.1 *Felis glaucula* Beltran, Jalisco, Mexico SS 974-330-108-42

Thomas, O. 1904. New forms of *Saimiri*, *Saccopteryx*, *Balantiopteryx*, and *Thrichomys* from the Neotropical Region. Annals and Magazine of Natural History, series 7 13:250–255.

BM(NH) 88.8.8.20 *Saccopteryx bilineata centralis* Teapa, Tabasco, S.E. Mexico SA 62-10-10-14

Thomas, O. 1913c. The geographical races of the woolly opossum (*Philander laniger*). Annals and Magazine of Natural History, series 8 12:358–361.

BM(NH) 94.12.18.28 *Philander laniger aztecus* San Juan de la Punta, Vera Cruz, Mexico SS x-x-x-x

Thomas, O. 1915. On bats of the genus *Promops*. Annals and Magazine of Natural History, series 8 16:61–64.

BM(NH) 94.2.5.4 *Promops centralis* N. Yucatan, Central America SS x-x-11.53-13.9

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New Karyotypic Information for Small Mammals from Botswana with Implications for Regional Biogeography

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Abstract

Recent genetic studies demonstrate that small mammals from Botswana have a unique phylogenetic history shaped by Pleistocene climate fluctuations, indicating patterns of isolation and population expansion. Karyotypic descriptions are useful for characterizing chromosomal rearrangements that may have led to genetic isolation and speciation. Yet, most of the karyotypic descriptions for southern African mammals lack descriptions from Botswana. Given that many species within southern Africa exhibit chromosomal polymorphisms or distinct cytotypes at the population level, new information is examined from Botswana to fill in geographic gaps in the current knowledge. Here, karyotypes are reported for non-volant small mammals from Botswana, including representative species of Eulipotyphla (N = 1), Macroscelidea (N = 2), and Rodentia (N = 14). Additionally, mitochondrial sequence data are provided for a subset of individuals to support taxonomic implications. Noteworthy results include: further evidence for chromosomal variation in the *Saccostomus campestris* species complex and for *Micaelamys namaquensis*; the first karyotypic description for *Steatomys parvus*; and new karyotypic information for currently unrecognized species of gerbils.

Key words: Africa, cytochrome-*b*, cytogenetics, rodents, shrews

Introduction

Descriptions of mammalian karyotypes serve an important role for characterizing chromosomal rearrangements, which provide information on genetic barriers to gene flow and ultimately on the processes involved in speciation. Southern Africa has long been a geographic hub for studies of mammalian chromosomes, especially for studies of rodents (Kingdon et al. 2013). Of particular interest is the high number of species exhibiting chromosomal variability within this region. Taylor (2000) summarized karyotypic trends within ten southern African rodent species, with examples that include: ecologically distinct chromosome races within the Southern African vlei rat (*Otomys irroratus*); interspecific chromosomal variation in sympatric species of multimammate mice (*Mastomys*); and high intraspecific variation in two species of tree rats (*Thallomys*). Likewise, African pygmy mice (*Nannomys*) exhibit a high degree of chromosomal variation, including sex-autosome translocations (Veyrunes et al. 2010) and a case of sex reversal (Veyrunes et al. 2013).

To date, most of the karyological descriptions of small mammals from southern Africa lack descriptions from Botswana, a country which is centrally located within the southern African subregion that includes the Kalahari Desert, Okavango Delta, and vast savanna woodland habitats. Recent phylogenetic studies have revealed that during the Pleistocene, this geographic region underwent periods of climate fluctuations that altered the distributions and demographic histories for several widespread small mammal species (McDonough et al. 2013, 2015; Mazoch et al. 2017). Understanding the chromosomal variation for small mammals from this region will contribute towards a deeper understanding of the processes that have led to genetic isolation and speciation of taxa.

In this study, new karyotypic information is described for small mammals collected in 2008, 2009, and 2011 in Botswana. Diploid and fundamental numbers are described and mitochondrial sequence

data were used to place a subset of taxa within a phylogenetic context. By assigning DNA sequence data to karyotyped individuals, this study aimed to provide a robust assessment of the true taxonomic diversity of this understudied region and set a framework for future taxonomic decisions using integrative approaches.

MATERIALS AND METHODS

Sampling of specimens and voucher preparations.—Samples were collected during three field expeditions to Botswana, carried out in the years 2008, 2009, and 2011. Specimens were collected from natural populations in multiple sites across the country (Fig. 1; Table 1) using Sherman live-traps and pitfall traps (karyotyped individuals), and Museum Special snap traps (non-karyotyped specimens). Permits were obtained from Botswana Ministry of Environment, Wildlife, and Tourism, and euthanasia and animal handling protocols followed the guidelines from the Animal Care and Use Committee of the American Society of Mammalogists (Gannon et al. 2007; Sikes et al. 2016).

Identification of rodent specimens was confirmed by DNA sequencing (see below), and for non-rodents, identification was based on assessment of external morphology, standard external measurements, and cranial and dental characters, following comparison to available data present in region-specific literature (Smithers 1971; Skinner and Chimimba 2005). Voucher specimens (skins with complete skeletons, skulls only, or whole bodies in alcohol) were deposited at the Natural Science Research Laboratory at the Museum of Texas Tech University (NSRL-TTU) or the Botswana National Museum (BNM), Gaborone, Botswana (Table 1). Tissue samples, preserved in 95% ethanol, lysis buf-

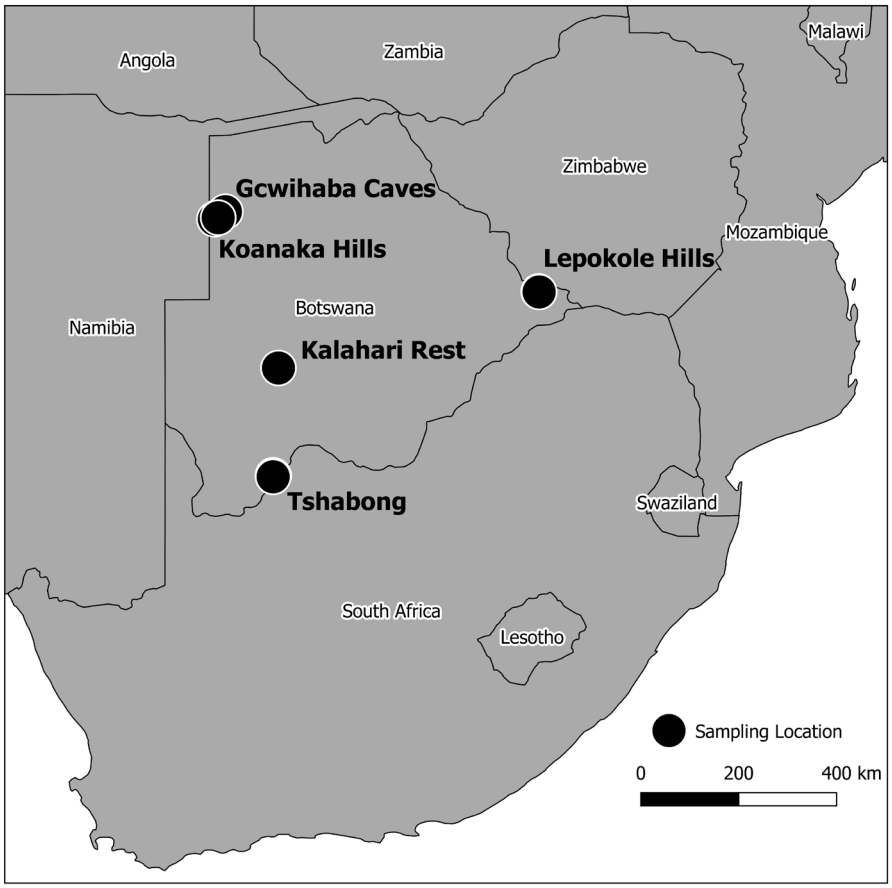

Figure 1. Botswana collecting localities (June 2008, July 2009, and August 2011).

Table 1. List of small mammal species karyotyped and sequenced from Botswana, including tissue and cell suspension identifier (TK number), GenBank accession number, specific collection locality, and the museum where the voucher specimen is deposited.

Species	Tissue/ Karyotype No.	GenBank No.	*Cytb*	Diploid number	Specific locality within Botswana	Latitude	Longitude	Specimen deposited
Crocidura hirta	TK164648		No	2n = 50	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau), Koanaka South	-20.15995622	21.19364721	BNM
Crocidura hirta	TK164821		No	2n = 50	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau), main camp	-20.15828517	21.19301357	NSRL
Crocidura hirta	TK172843		No	2n = 50	Kalahari Rest, 16 km N, 25 km W Kang	-23.53498	22.54607	NSRL
Elephantulus intufi	TK164770		No	2n = 26	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau), dry pan area	-20.14363452	21.19527285	NSRL
Elephantulus intufi	TK172837		No	2n = 26	Kalahari Rest, 16 km N, 25 km W Kang	-23.53498	22.54607	NSRL
Elephantulus myurus	TK172788		No	2n = 30	Lepokole Hills, 3.6 km S, 4.9 km E Lepokole Village	-21.82653	28.39898	NSRL
Fukomys damarensis	TK164780		No	2n = 80	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau), Koanaka North	-20.14174869	21.20859915	NSRL
Dendromus melanotis	TK154629	MK879613	Yes		Koanaka Hills (Nkumsta Hills), 150 km W of Tsao	-20.159992	21.193695	BNM
Dendromus melanotis	TK154656	MK879614	Yes		Koanaka Hills (Nkumsta Hills), 150 km W of Tsao	-20.159992	21.193695	BNM
Dendromus melanotis	TK172842		No	2n = 42	Kalahari Rest, 16 km N, 25 km W Kang	-23.53498	22.54607	NSRL
Saccostomus campestris	TK154618	MK879606	Yes		Koanaka Hills (Nkumsta Hills), 150 km W of Tsao	-20.148023	21.197891	NSRL
Saccostomus campestris	TK154623	MK879611	Yes		Koanaka Hills (Nkumsta Hills), 150 km W of Tsao	-20.148638	21.198111	BNM
Saccostomus campestris	TK154628	MK879610	Yes		Koanaka Hills (Nkumsta Hills), 150 km W of Tsao	-20.148638	21.198111	BNM
Saccostomus campestris	TK154641	MK879609	Yes		Koanaka Hills (Nkumsta Hills), 150 km W of Tsao	-20.148638	21.198111	NSRL
Saccostomus campestris	TK154651	MK879608	Yes		Koanaka Hills (Nkumsta Hills), 150 km W of Tsao	-20.148638	21.198111	BNM

Table 1. (cont.)

Species	Tissue/ Karyotype No.	GenBank No.	*Cyt*b	Diploid number	Specific locality within Botswana	Latitude	Longitude	Specimen deposited
Saccostomus campestris	TK164766	MK879605	Yes	2n = 36	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau), dry pan area	-20.14374287	21.19534953	BNM
Saccostomus campestris	TK164767		No	2n = 36	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau), dry pan area	-20.14374287	21.19534953	NSRL
Steatomys parvus	TK164993	MK879612	Yes	2n = 70	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau), Koanaka South	-20.15842879	21.19389413	NSRL
Acomys spinosissimus	TK172813		No	2n = 60	Lepokole Hills, 3.6 km S, 4.9 km E Lepokole Village	-21.826653	28.39898	NSRL
Aethomys chrysophilus	TK164834	KY965370	Yes	2n = 44	Lepokole Hills, 3.6 km S, 4.9 km E Lepokole Village	-20.14844795	21.19798678	NSRL
Aethomys chrysophilus	TK164852	MK879604	Yes	2n = 44	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau), waterhole	-20.19307591	21.10824377	NSRL
Aethomys chrysophilus	TK172790	KY965371	Yes	2n = 50	Lepokole Hills, 3.6 km S, 4.9 km E Lepokole Village	-21.82653	28.39898	NSRL
Lemniscomys rosalia	TK164822	MK879618	Yes	2n = 48	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau)	-20.15843931	21.19253529	NSRL
Lemniscomys rosalia	TK164823	MK879617	Yes	2n = 48	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau)	-20.15843931	21.19253529	BNM
Lemniscomys rosalia	TK164833	MK879616	Yes		Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau)	-20.14877338	21.19788193	BNM
Lemniscomys rosalia	TK164853	MK879615	Yes		Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau), waterhole	-20.19386197	21.10842617	NSRL
Mastomys coucha	TK164618	MK879620	Yes	2n = 36	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau)	-20.15842042	21.19328168	BNM
Mastomys coucha	TK164995	MK879619	Yes		Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau), Koanaka South	-20.15861031	21.19316708	BNM
Mastomys coucha	TK172786		No	2n = 36	Lepokole Hills, 2.2 km S, 4 km E Lepokole Village	-21.81518	28.38802	BNM
Micaelamys namaquensis	TK164610	MK879603	Yes	2n = 24	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau)	-20.15858295	21.19339671	NSRL
Micaelamys namaquensis	TK164611	MK879602	Yes	2n = 24	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau)	-20.15858295	21.19339671	BNM

Table 1. (cont.)

Species	Tissue/ Karyotype No.	GenBank No.	*Cyt*b	Diploid number	Specific locality within Botswana	Latitude	Longitude	Specimen deposited
Micaelamys namaquensis	TK164612	MK879601	Yes	2n = 24	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau)	-20.15858295	21.19339671	NSRL
Micaelamys namaquensis	TK164616	MK879600	Yes		Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau)	-20.15858295	21.19339671	NSRL
Micaelamys namaquensis	TK164617	MK879599	Yes	2n = 24	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau)	-20.15858295	21.19339671	BNM
Micaelamys namaquensis	TK164968		No	2n = 24	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau), Koanaka South	-20.15868121	21.19443989	NSRL
Micaelamys namaquensis	TK164972		No	2n = 24	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau), Koanaka South	-20.15868121	21.19443989	NSRL
Micaelamys namaquensis	TK164973		No	2n = 24	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau), Koanaka South	-20.15868121	21.19443989	NSRL
Micaelamys namaquensis	TK164981		No	2n = 24	Gcwihaba Caves, 18.8 km N, 114.2 km W Tsao (Tsau) (Tsau)	-20.02369728	21.35487113	NSRL
Micaelamys namaquensis	TK164982		No	2n = 24	Gcwihaba Caves, 18.8 km N, 114.2 km W Tsao (Tsau) (Tsau)	-20.02369728	21.35487113	NSRL
Micaelamys namaquensis	TK164989		No	2n = 24	Gcwihaba Caves, 18.8 km N, 114.2 km W Tsao (Tsau) (Tsau)	-20.02369728	21.35487113	NSRL
Micaelamys namaquensis	TK164996		No	2n = 24	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau), Koanaka South	-20.15861031	21.19316708	NSRL
Micaelamys namaquensis	TK164997		No	2n = 24	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau), Koanaka South	-20.15861031	21.19316708	NSRL
Micaelamys namaquensis	TK172787		No	2n = 24	Lepokole Hills, 3.6 km S, 4.9 km E Lepokole Village	-21.82653	28.39898	BNM
Micaelamys namaquensis	TK172789		No	2n = 24	Lepokole Hills, 2.2 km S, 4 km E Lepokole Village	-21.81518	28.38802	NSRL
Rhabdomys pumilio	TK172841		No	2n = 48	Kalahari Rest, 16 km N, 25 km W Kang	-23.53498	22.54607	NSRL
Desmodillus auricularis	TK170612	MK879631	Yes	2n = 50	4.6 km N, 2.1 km E Tsabong	-25.97822	22.42691	NSRL
Desmodillus auricularis	TK170618	MK879632	Yes		4.6 km N, 2.1 km E Tsabong	-25.97822	22.42691	NSRL

Table 1. (cont.)

Species	Tissue/ Karyotype No.	GenBank No.	*Cyt*b	Diploid number	Specific locality within Botswana	Latitude	Longitude	Specimen deposited
Gerbillurus paeba	TK164646		No	2n = 36	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau)	-20.15811903	21.19616167	NSRL
Gerbillurus paeba	TK164818	MK879629	Yes		Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau)	-20.15998351	21.19348456	BNM
Gerbillurus paeba	TK164819	MK879630	Yes		Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau)	-20.15998351	21.19348456	NSRL
Gerbillurus paeba	TK170599	MK879625	Yes		Berry Bush Farm, 8 km N, 2 km E Tsabong (Tshabong)	-25.94283	22.42405	NSRL
Gerbillurus paeba	TK170607	MK879626	Yes		7.3 km N, 3.3 km E Tsabong	-25.95404	22.43919	NSRL
Gerbillurus paeba	TK170608	MK879623	Yes		7.3 km N, 3.3 km E Tsabong	-25.95404	22.43919	NSRL
Gerbillurus paeba	TK170609	MK879627	Yes		7.3 km N, 3.3 km E Tsabong	-25.95404	22.43919	NSRL
Gerbillurus paeba	TK170610	MK879628	Yes		7.3 km N, 3.3 km E Tsabong	-25.95404	22.43919	NSRL
Gerbillurus paeba	TK172811	MK879624	Yes		20 km N, 20 km W Kang	-23.49900	22.59889	BNM
Gerbilliscus cf. *grique*	TK164575		No	2n = 44	Koanaka Hills (Ncqumtsa Hills), 150 km W Tsao (Tsau)	-20.15842042	21.19328168	NSRL
Gerbilliscus cf. *grique*	TK170613	MK879622	Yes		7.3 km N, 3.3 km E Tsabong	-25.95404	22.43919	NSRL
Gerbilliscus cf. *grique*	TK170614	MK879621	Yes		7.3 km N, 3.3 km E Tsabong	-25.95404	22.43919	NSRL
Gerbilliscus leucogaster	TK170593	KM454042	Yes	2n = 40	Kalahari Rest, 16 km N, 25 km W Kang	-23.53498	22.54607	NSRL
Gerbilliscus leucogaster	TK170594	KM454043	Yes	2n = 40	Kalahari Rest, 16 km N, 25 km W Kang	-23.53498	22.54607	NSRL
Gerbilliscus leucogaster	TK172838	KM454052	Yes	2n = 40	Kalahari Rest, 16 km N, 25 km W Kang	-23.53498	22.54607	NSRL
Gerbilliscus leucogaster	TK172839	KM454053	Yes	2n = 40	Kalahari Rest, 16 km N, 25 km W Kang	-23.53498	22.54607	NSRL

fer, or liquid nitrogen, as well as karyotypes preserved in Carnoy's fixative (3:1, methanol:acetic acid), are deposited at the NSRL-TTU.

Karyotyping in the field and chromosome analysis.—Cell suspensions enriched for mitotic metaphases were obtained via bone marrow extraction and fixation following the methods described in Baker et al. (2003). Slides were stained with a 2% Giemsa solution in phosphate buffer to allow bright-field visualization and analysis of metaphases in an Olympus BX51 microscope. Images were photographed using the GENUS SYSTEM version 3.7 (Applied Imaging Systems, San Jose, California), and karyotypes were arranged according to reference karyotypes for the species when available, including those in the Atlas of mammalian chromosomes (O'Brien et al. 2006), or using previous karyotype descriptions of closely related species (on a case by case basis) as reference for numbering system. Diploid number (2n) and fundamental number of autosomes (FNa; which does not include the count of arms of sex chromosomes) were reported, as well as the sex chromosome morphology and size in comparison to what has been previously described for each taxon.

DNA sequencing and phylogenetic analysis.—Genomic DNA was extracted from rodent tissue using a DNeasy Blood and Tissue Kit (Qiagen Inc., Chatsworth, California). The complete cytochrome-*b* gene (*Cyt*b, 1,140 nucleotides) was amplified following methods outlined in Veyrunes et al. (2010). Cycle sequencing reactions were performed with BigDye terminator version 3.1 and were electrophoresed on an ABI 3100-*Avant* (Applied Biosystems, Foster City, California). Sequences were edited and aligned using Geneious version 10.0.9 (https://www.geneious.com). Novel sequences (GenBank accession numbers MK879599–MK879649) were visually aligned with previously published sequences deposited on GenBank. Phylogenies were estimated using the Maximum-likelihood method with the program RAxML-HPC2 on XSEEDE in the CIPRES Science Gateway (Miller et al. 2010) with 1,000 bootstrap replicates.

Results and Discussion

ORDER EULIPOTYPHLA Waddell et al., 1999
Family Soricidae G. Fischer, 1814
Crocidura Wagler, 1832
***Crocidura hirta* Peters, 1852**
Lesser Red Musk Shrew

Based on geographic range, habitat, and body size measurements, the Botswana soricids were tentatively assigned to *C. hirta* until further molecular and morphological analyses confirm the species identification. The karyotype of the lesser red musk shrew from Botswana consists of 2n = 50 chromosomes. A total of seven clearly biarmed (meta-submetacentric) chromosome pairs, 10 subtelocentric, and seven acrocentric autosomes is shared in the karyotypes of two males (TK164821 and TK172843) and one female (TK164648) analyzed in this report. The autosomal fundamental number is FNa = 62, not including the short arms of the subtelocentric chromosomes. The X is the largest biarmed chromosome in the complement, whereas the Y is a small, most likely acrocentric, chromosome (Fig. 2).

Approximately 105 species of *Crocidura* are estimated to occur in Africa (Churchfield 2013), but only half of them have karyotypic descriptions (Primus et al. 2006). Nevertheless, considerable chromosomal variation is found, with diploid numbers ranging from 36 to 68 among African species (Zima et al. 1998; Primus et al. 2006), which is within the range (2n = 22 to 2n = 68) reported for the rest the genus (Maddalena and Ruedi 1994; Schlitter et al. 1999). The karyotype of *C. hirta* described here shares similarities to those of other Afrotropical *Crocidura*. According to Dubey et al. (2008), species within the *C. olivieri* group, which includes *C. hirta*, share the unique chromosomal formula of 2n = 50, FNa = 62 (e.g., *C. olivieri*, *C. viaria*, *C. greenwoodi*, and *C. attila*) or FNa = 70 (e.g. *C. flavescens*) (Maddalena et al. 1987; Schlitter et al. 1999).

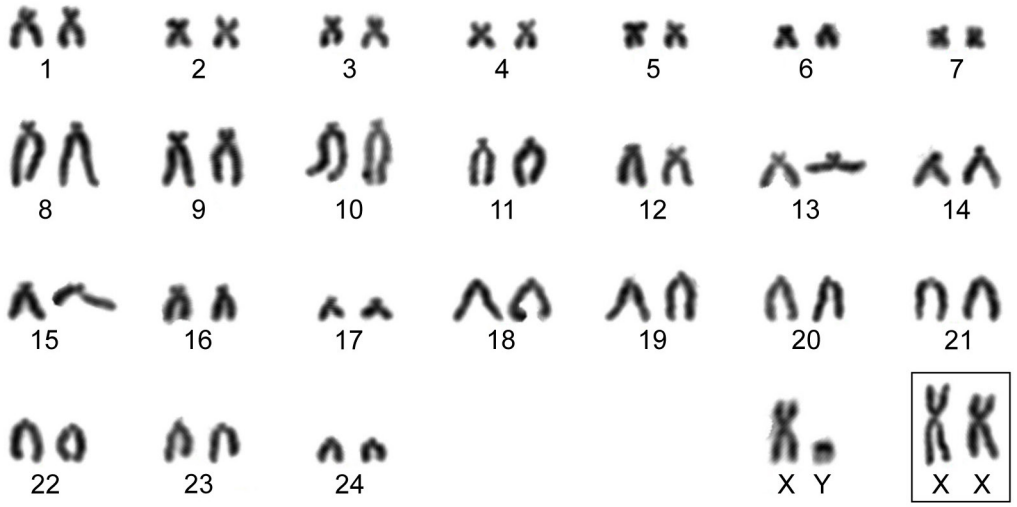

Figure 2. Karyotype of a male *Crocidura hirta* (lesser red musk shrew) from Koanaka Hills, Botswana. The X chromosomes from a female are shown in the inset.

ORDER MACROSCELIDEA Butler, 1956
Family Macroscelididae Bonaparte, 1838
Elephantulus Thomas and Schwann, 1906
***Elephantulus intufi* A. Smith, 1834**
Bushveld Sengi

All autosomes of *E. intufi* are biarmed, and together with a small submetacentric X and a small acrocentric Y, exhibit the chromosomal formula of 2n = 26 and FNa = 48 (TK64770 and TK172837; Fig. 3a). This karyotypic constitution has been reported by Tolliver et al. (1989) for *E. intufi* from Namibia, as well as the former Cape and Transvaal provinces in South Africa. At least five other species of sengis from southern Africa have a conserved 2n = 26 karyotype (i.e., *E. intufi*, *E. rupestris*, *E. edwardi*, *E. brachyrhychus*, *Macroscelides proboscideus*), with slight morphological variations accounted for differential amount of heterochromatin (Tolliver et al. 1989; Robinson et al. 2004; O'Brien et al. 2006; Smit et al. 2011)

***Elephantulus myurus* Thomas and Schwann, 1906**
Eastern Rock Sengi

The eastern rock sengi from Botswana (n = 1) had a karyotype with 2n = 30 and FNa = 48 (TK172788; Fig. 3b). These results are in agreement with other studies that report the same diploid and fundamental numbers in specimens from western South Africa (Ford and Hamerton 1956; Tolliver et al. 1989). The karyotype with 2n = 30 is autapomorphic in *E. myurus* and was hypothesized to be derived from the ancestral karyotype with 2n = 26 by two independent fissions (Smit et al. 2011). *Elephantulus myurus* is the only *Elephantulus* species that exhibits a chromosomal constitution distinct from the 2n = 26 karyotype. Within other elephant shrews; however, only *Petrosaltator rozeti* (formerly, *Elephantulus rozeti*) and *Petrodromus tetradactylus* have a distinct karyotype with 2n = 28, which, based on molecular phylogenies, are sister taxa that potentially shared a common ancestor with 28 chromosomes (Wenhold and Robinson 1987; Smit et al. 2008; Dumbacher et al. 2016).

ORDER RODENTIA Bowdich, 1821
Family Bathyergidae Waterhouse, 1841
Fukomys Kock et al., 2006
***Fukomys damarensis* (Ogilby, 1838)**
Damara Mole Rat

The karyotype of one male individual of *Fukomys damarensis* (formerly *Cryptomys damarensis*) consisted of 2n = 80 and FNa = 92. Most of the chromosomes had an acrocentric morphology, except four medium-sized metacentric (9–12), and 3 smaller biarmed (28–30) chromosome pairs. The X is a large metacentric, whereas the Y is a small acrocentric chromosome (TK164780; Fig. 4).

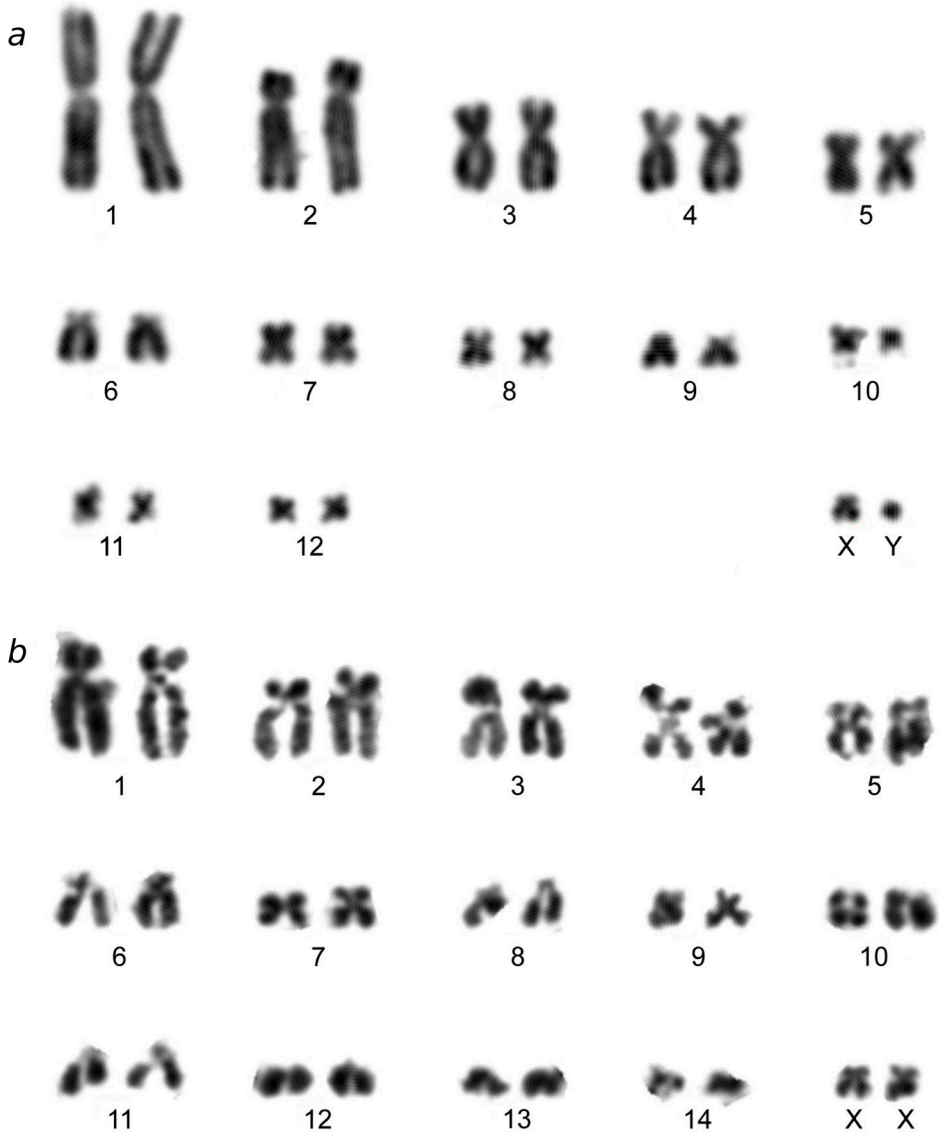

Figure 3. Karyotypes of elephant shrews. a) Female *Elephanthulus intufi*, bushveld sengi; b) Male *E. myurus*, eastern rock sengi.

Mole-rats in the family Bathyergidae possess a considerable degree of karyotypic diversity. Distinct trends are seen among different monophyletic lineages, including conservative versus karyotypically variable clades. As for *F. damarensis*, karyotypes have been useful tools for providing support for their phylogenetic affinities and taxonomic delimitation (Aguilar 1993; Faulkes et al. 1997).

Nevertheless, there are many discrepancies in karyotypic descriptions of mole-rat species, and it is unclear how much geographic karyotypic variation exists in species within the family Bathyergidae (Deuve et al. 2008). Three diploid numbers have been reported so far for *F. damarensis*: 2n = 80 in 10 specimens from South Africa and seven from Namibia (Deuve et al. 2008), and 2n = 74 and 2n = 78 were reported by Nevo et al.

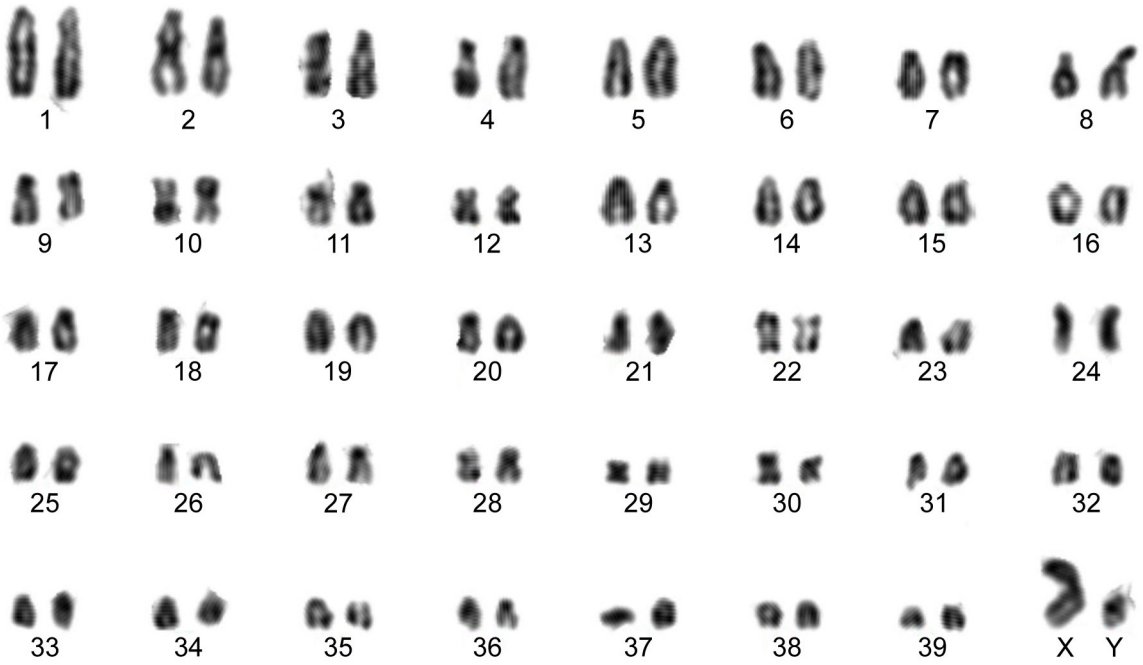

Figure 4. Karyotype of *Fukomys damarensis* (Damara mole rat).

(1986) in individuals from Namibia and South Africa, respectively. In addition, 2n = 78 was reported for Zambian specimens (Van Daele et al. 2004); however, with a distinct FNa (116), as opposed to a previously described fundamental number of FNa = 92, which also was present in our specimen. Therefore, further karyotypic information coupled with molecular data is paramount to a better understanding of the trends and evolutionary shifts in the karyotypes of lineages, as well as to refine their relationship, taxonomic status, and geographical distribution.

Family Nesomyidae Major, 1897
Dendromus A. Smith, 1829
***Dendromus melanotis* (Smith, 1834)**
Gray Climbing Mouse

The karyotype of one specimen of *D. melanotis* had 2n = 42, with 12 biarmed and 8 acrocentric autosomes (FNa = 64; Fig. 5). The sex chromosomes could not be assigned with certainty because there was a single female for analysis; however, the X chromosome was assumed as a large biarmed element, from comparative analyses to available karyotype images from other studies (Matthey 1970; Solano et al. 2014).

The karyotype and mitochondrial sequences (Fig. 7) reported here for a single individual collected from the Kalahari region from Botswana most closely resemble that described by Solano et al. (2014) from South Africa, with the same diploid number (2n = 42); however, a higher number of biarmed elements was observed (TK172842; Fig. 5). In addition, different karyotypes have been reported for *D. melanotis* from Ethiopia (2n = 44; Bulatova et al. 1995), central Africa (2n = 36 and FNa = 58; Matthey 1970), South Africa (2n = 42, FNa = 56; Solano et al. 2014) and South African Kalahari (2n = 52, FNa = 62; Dippenaar et al. 1983).

Saccostomus Peters, 1846
***Saccostomus campestris* Peters, 1846**
Pouched Mouse

Saccostomus campestris is debatably referred to as a complex of species, very tolerant to chromosome fusions (Maputla et al. 2011; Mikula et al. 2016). The karyotypic variation reported ranges from 2n = 28 to 46, and two groups of cytotypes (2n = 28–38 in western southern Africa and 2n = 46 in eastern southern Africa) are considered distinct species (Gordon and Rautenbach 1980; Gordon and Watson 1986). Nevertheless, labora-

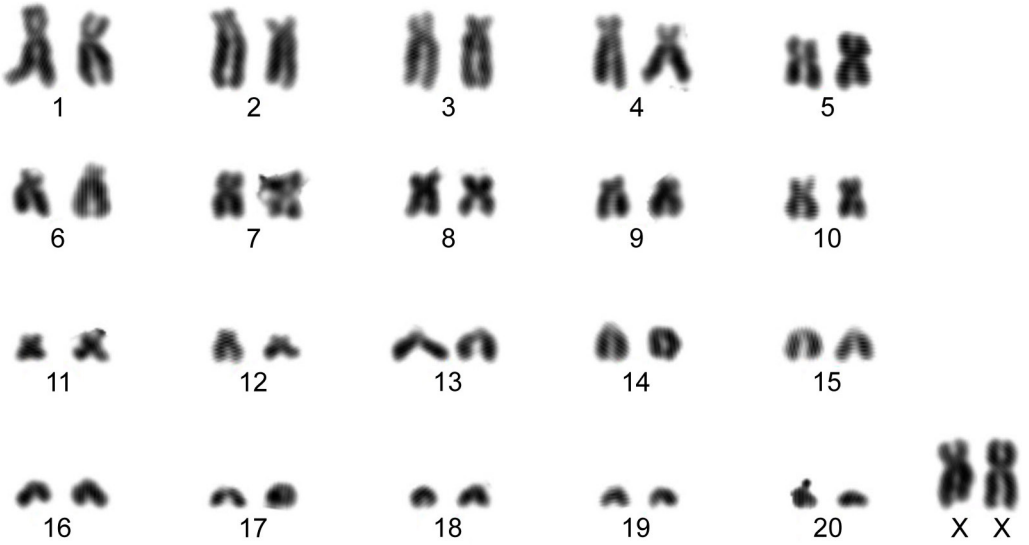

Figure 5. Karyotype of a female *Dendromus melanotis* from Kalahari Rest, Botswana.

tory hybridization experiments from parentals with 2n = 32 and 2n = 46 and their offspring (F1 to F3) have been shown to be both viable and fertile (Maputla et al. 2011). Interestingly, in the karyotypic descriptions of *S. campestris* it is common to find chromosomes lacking identical homologues. Hence, those chromosomes appear as unpaired in the standard karyotype. The variation includes up to 17 different cytotypes and several chromosomes of various sizes and morphology that cannot be paired (Gordon and Rautenbach 1980; Gordon and Watson 1986; Maputla 2007; Maputla et al. 2011).

The two male individuals analyzed here had the same karyotype, with 2n = 36, which included at least four (to six) unpaired chromosomes (TK164766 and TK164767; Fig. 6). Individual TK164766 clusters phylogenetically with individuals from South Africa with the 2n = 46 cytotype (Fig. 7), which would correspond to the proposed *S. c. mashonae* as defined by Mikula et al. (2016). These results provide further evidence of chromosome variation within this species complex, and indirect evidence of tolerance to hybrid crossings and viability in the wild, beyond controlled laboratory crossing experiments.

Steatomys Peters, 1846
***Steatomys parvus* Rhoads, 1896**
Tiny Fat Mouse

This is the first report of the karyotype of *S. parvus*. Due to the highly condensed metaphases obtained, it was difficult to determine the morphology of all *S. parvus* chromosomes. The best estimate using the material here is a karyotype with at least 14 biarmed and up to 20 acrocentric autosome pairs, totaling a 2n = 70 and therefore a FNa count of approximately 96 (TK164993; Fig. 8). Both sex chromosomes are biarmed and the X is the largest metacentric chromosome in the karyotype.

The only available karyotypic information for *Steatomys* was reported by Matthey (1954) for *S. pratensis* from an unreported collection locality. *Steatomys pratensis* karyotype comprises 2n = 68 with the X and Y as the largest chromosomes in the complement. Both sex chromosomes are biarmed, but the X is a metacentric and the Y has a submetacentric morphology. The FNa was reported as between 74 and 78.

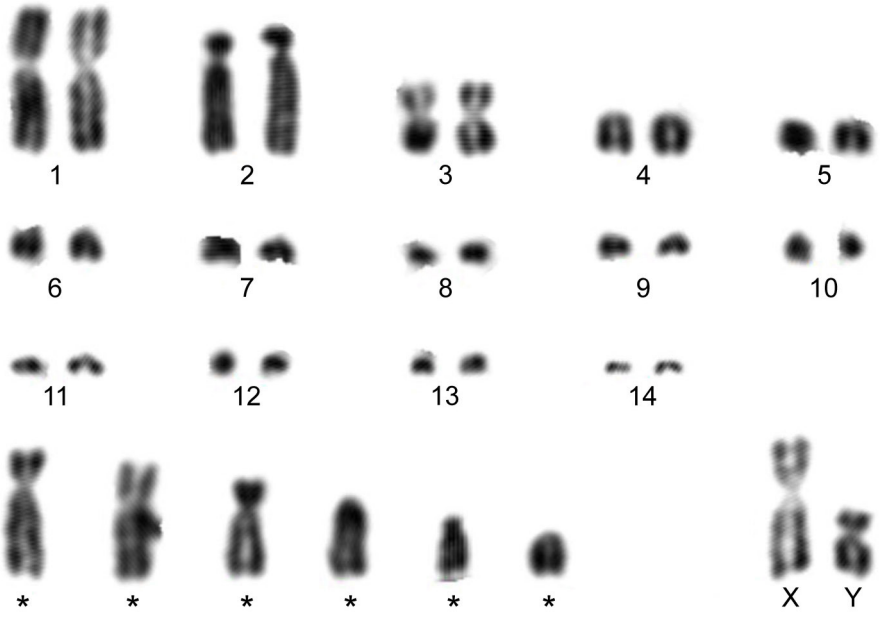

Figure 6. Karyotype of a male *Saccostomus campestris* (pouched mouse) with 2n =36. To best assign chromosome pairs and tentatively determine the sex chromosomes, the reported karyotype with 2n = 30 (Maputla 2007) was used as a guide. Specimens from Botswana have at least four unpaired chromosomes (six in this image, denoted by *), which is a common feature in karyotypic descriptions of the species.

Family Muridae Illiger, 1811
Subfamily Deomyinae Thomas, 1888
Acomys I. Geoffroy, 1838
***Acomys spinosissimus* Peters, 1852**
Southern African Spiny Mouse

The karyotype of *Acomys spinosissimus* from Botswana consists of 2n = 60 and FNa = 68 (TK172813; Fig. 9). Although only a single female from the Lepokole Hills was analyzed, comparative analyses to other described *Acomys* karyotypes (see references below) allowed the inference of the X chromosome size and morphology. Namely, the X chromosome is the largest submetacentric chromosome "pair" of the examined specimen. In addition, the karyotype consists of five bi-armed chromosome pairs, including one submetacentric and four metacentric, and is identical to the karyotype described by Dippenaar and Rautenbach (1986) for specimens from Transvaal province in South Africa. This karyotype is consistent with the species diagnosis presented in several other cytotaxonomic studies, and also agrees with previously reported data on the distributional range (parts of central and southern Africa, more specifically between Zambezi and Limpopo rivers) of the species (Castiglia et al. 2007; Verheyen et al. 2011; Petružela et al. 2018). The exception is the FNa = 70 reported for some individuals from Tanzania, which have a small metacentric pair (not observed in specimens outside that locality), as well as acrocentric X chromosomes (Barome et al. 2001; Corti et al. 2005; Lavrenchenko et al. 2011; Petružela et al. 2018).

Subfamily Murinae Illiger, 1811
Genus *Aethomys* Thomas, 1915
***Aethomys chrysophilus* (de Winton, 1896)**
Red Rock Rat

Aethomys chrysophilus has been considered a species complex, widely distributed in southern Africa, but with a pronounced phylogeographic structure, correlated with well-described biogeographical patterns (Mazoch et al. 2017). Two distinct clades and karyotypes were recovered for *A. chrysophilus* sensu Mazoch et al. 2017 (Fig. 10). One karyotype (Fig. 11a) consists of 50 chromosomes with a tentative FNa = 56 (which might be 58 if the small satellite of pair 10 is

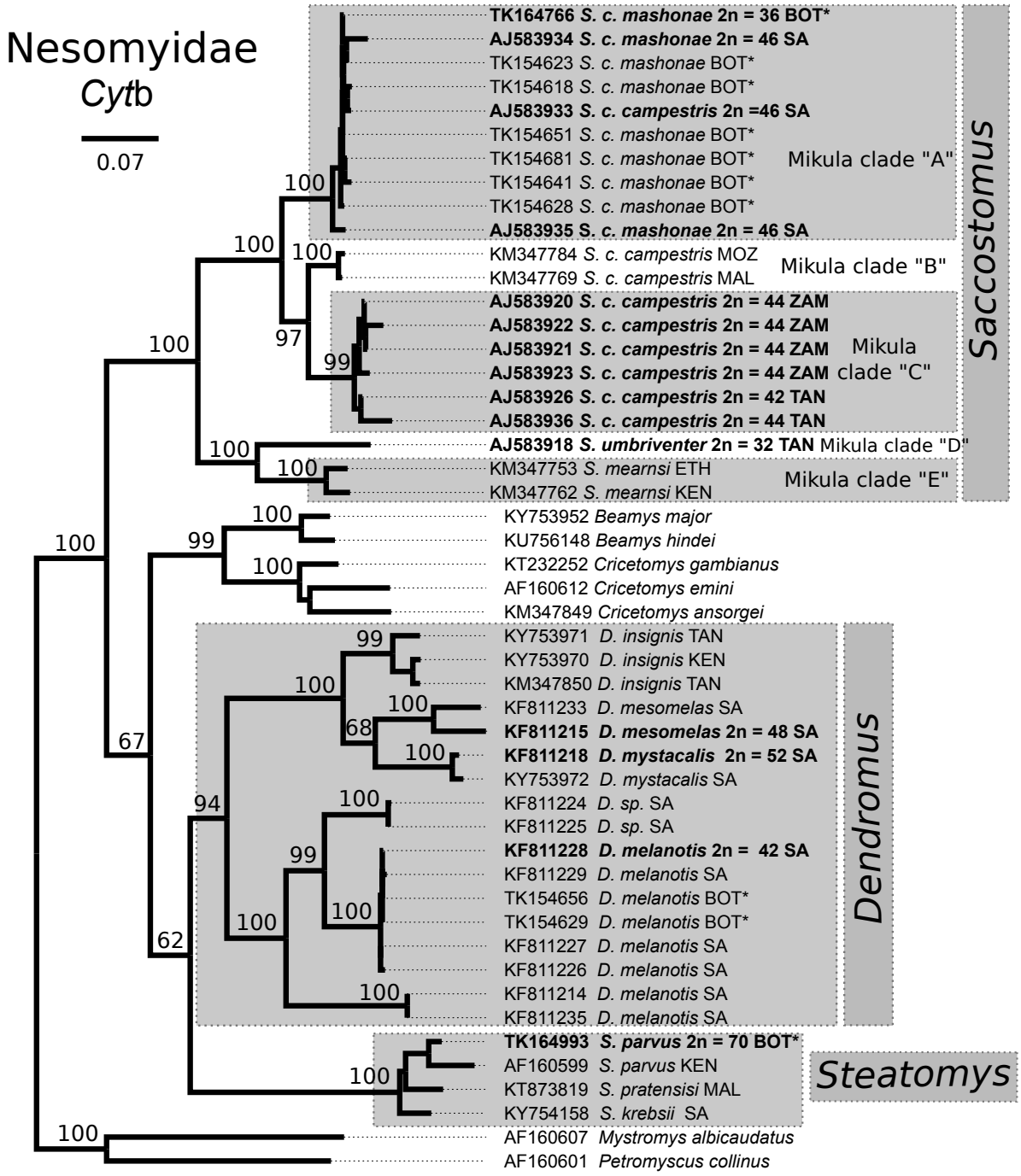

Figure 7. Maximum-likelihood phylogram estimating the phylogenetic relationships of the family Nesomyidae using 1,140 base pairs of the *Cyt*b gene. Nodal support estimated using 1,000 bootstrap replicates. Karyotyped individuals in bold and individuals from Botswana shown with an asterisk.

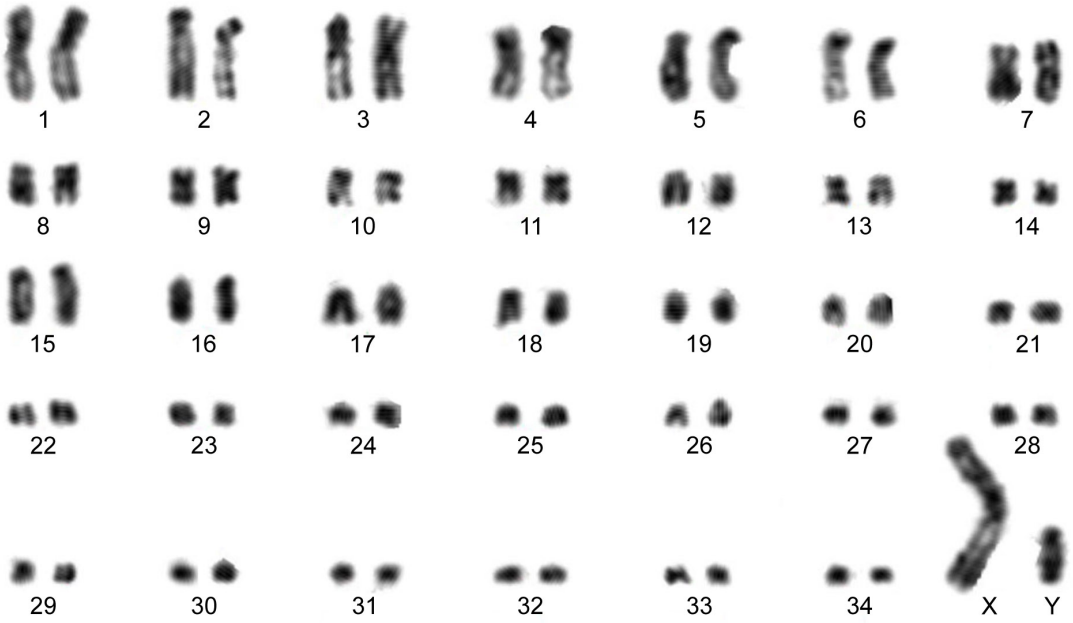

Figure 8. Karyotype of a male *Steatomys parvus* captured in Koanaka Hills, Botswana.

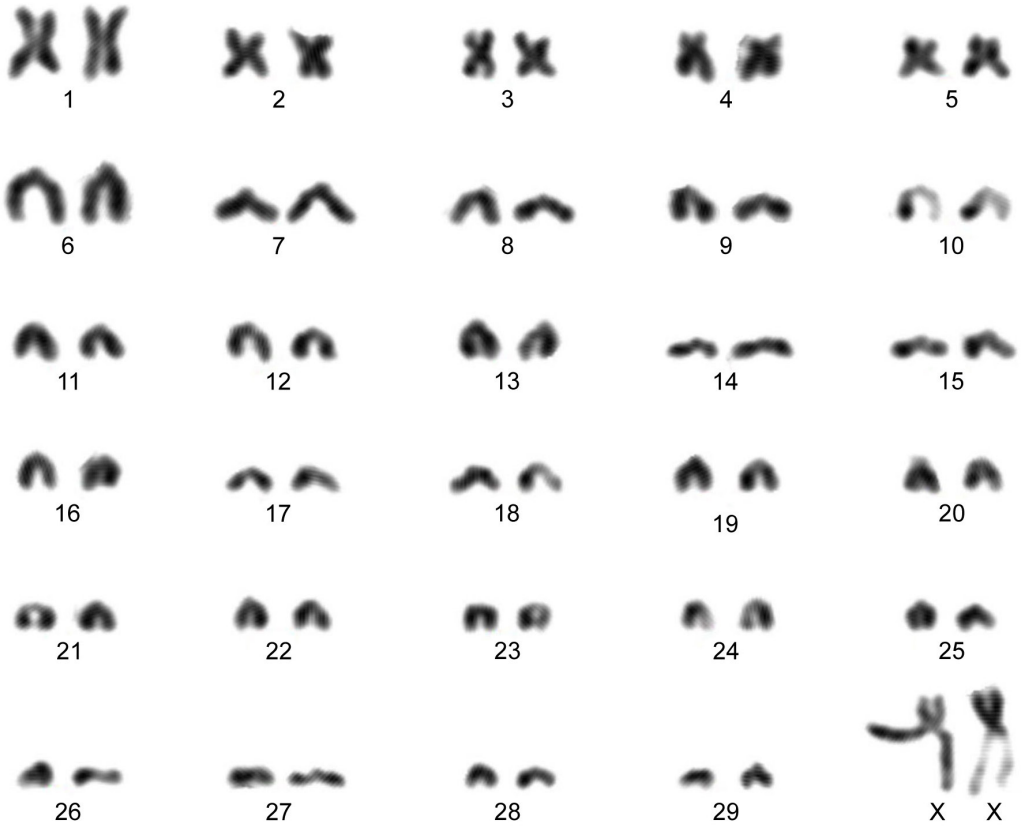

Figure 9. The karyotype of a female southern African spiny mouse, *Acomys spinosissimus*. The X chromosome size and morphology was inferred based on comparison with published data from Dippenaar and Rautenbach (1986).

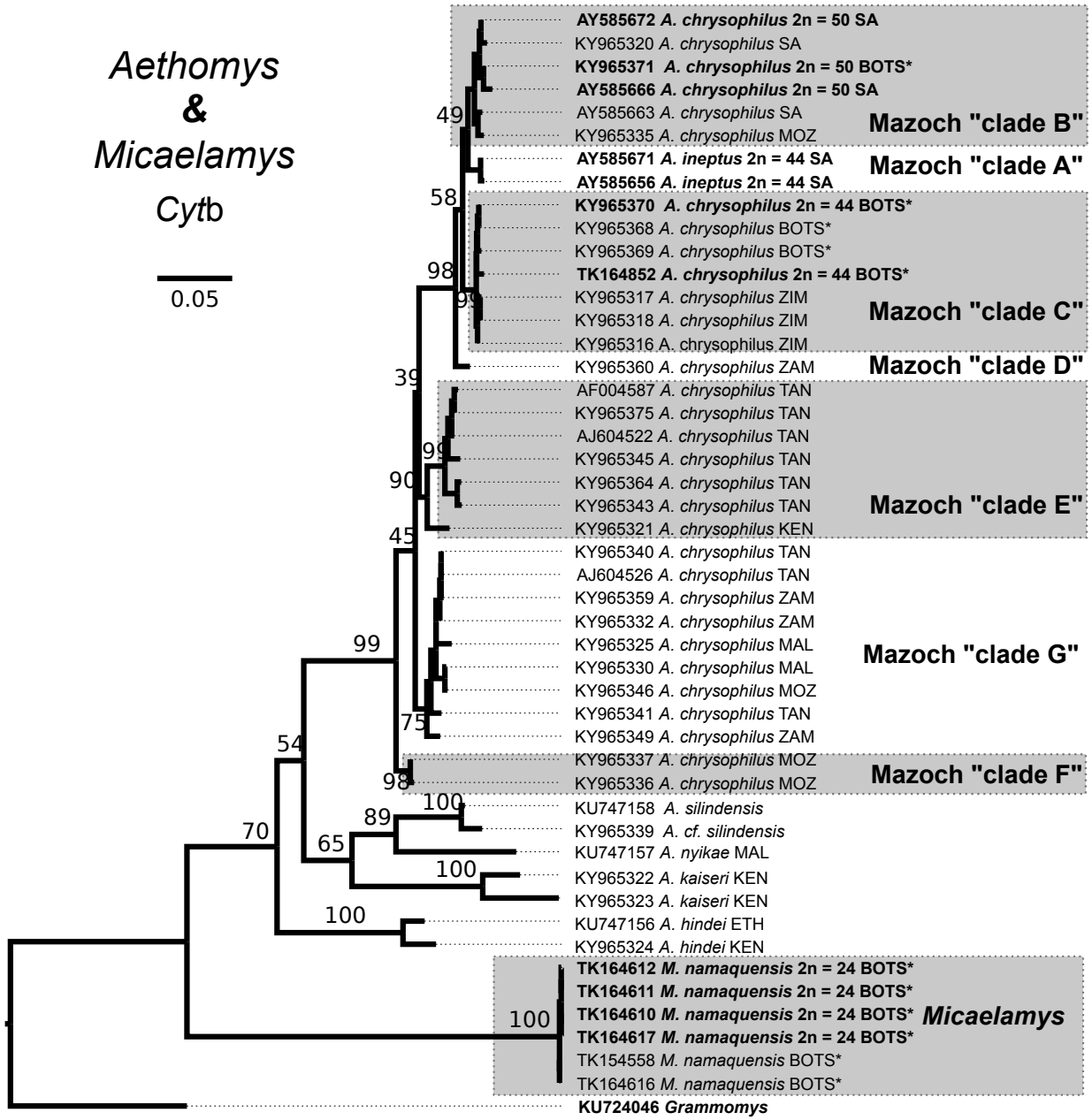

Figure 10. Maximum-likelihood phylogram estimating the phylogenetic relationships of *Aethomys* and *Micaelamys* using 1,140 base pairs of the *Cyt*b gene. Nodal support estimated using 1,000 bootstrap replicates. Karyotyped individuals in bold and individuals from Botswana shown with an asterisk.

taken into account) and with *Cyt*b sequences that cluster phylogenetically within the Mazoch et al. (2017) "clade B". This is consistent with the reported distributional range for this lineage, which includes individuals from the southernmost occurrence of the species (below the lower Zambezi-Kafue river system), such as South Africa and Mozambique (TK172790; Fig. 11a). Individuals from "clade B" appear as sister to the "clade A" corresponding to populations of *A. ineptus* sensu stricto, whose specific status is still debated (Chimimba and Bennett 2005; Mazoch et al. 2017).

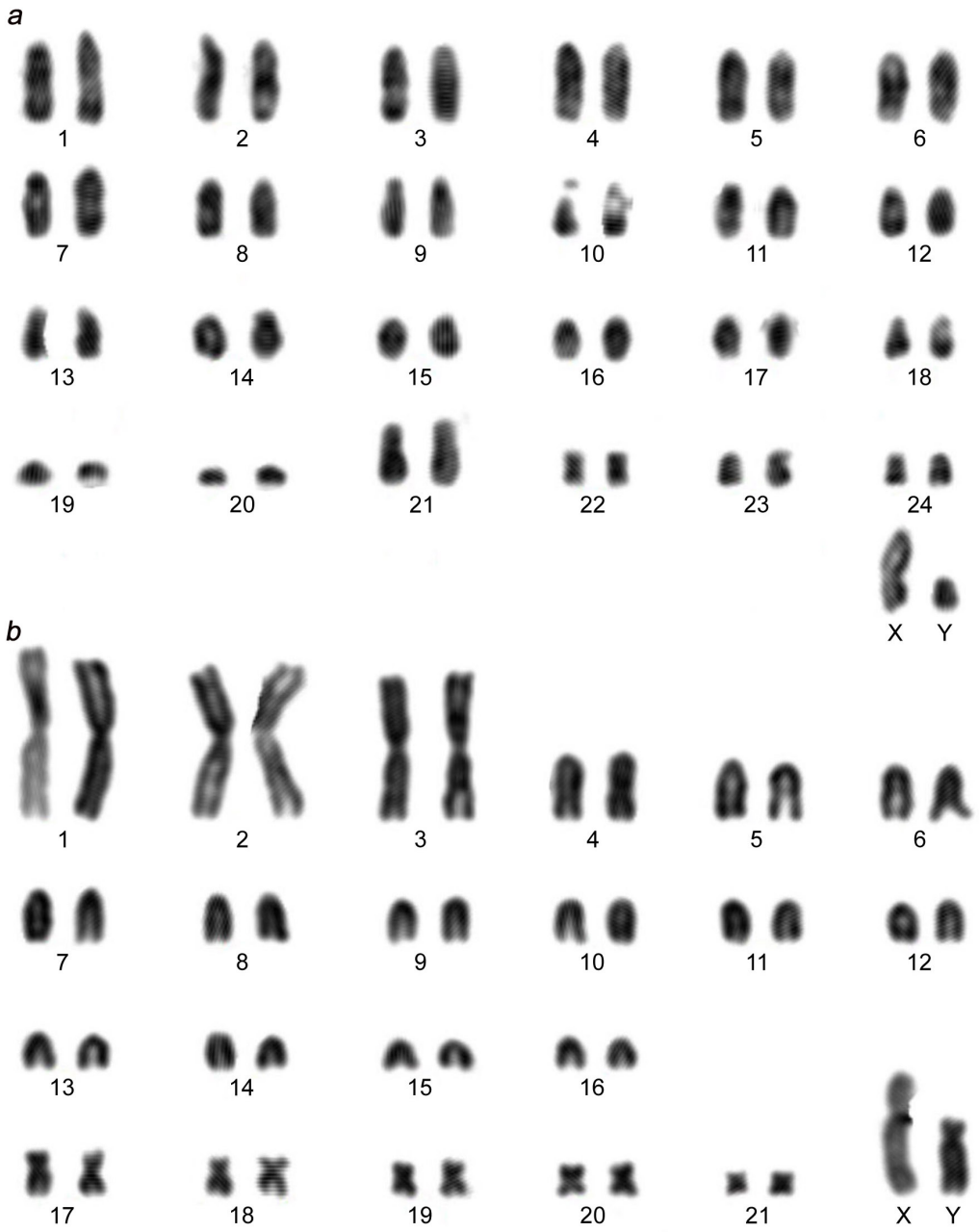

Figure 11. Distinct chromosome numbers in *Aethomys chrysophilus* (red rock rat) from Botswana. a) karyotype with 2n = 50 (TK172790); b) representative karyotype (TK164852) with 2n = 44 of male individuals.

A second karyotype was recovered with 2n = 44 and FNa = 58 (Fig. 11b); the two males analyzed here exhibited identical karyotypes, with three large and five small biarmed, and 13 acrocentric autosome pairs. The X chromosome is a medium-to-large-sized submetacentric, whereas the Y chromosome is a medium subtelocentric element. Interestingly, the Botswana 2n = 44 individuals cluster phylogenetically with Mazoch et al. (2017) "lineage C". There is no available information on the chromosomal number of

"clade C" individuals (from western Zimbabwe and Botswana and Namibia borders).

Overall, variation in diploid number within *A. chrysophilus* complex seems to be restricted to the 2n = 44 forms found in *A. ineptus* lineage and our Botswana samples that cluster with "clade C". The 2n = 50, FNa = 58 karyotype seems to be widespread throughout the species distributional range, including specimens from Zimbabwe (Gordon and Rautenbach 1980), South Africa (Visser and Robinson 1986; Baker et al. 1988), and Tanzania (Fadda et al. 2001; Castiglia et al. 2003; Denys et al. 2011). *Aethomys chrysophilus* is one of the southern African species with the broadest distributional range, and yet there is a significant gap of karyotypic descriptions across its range. It cannot be ruled out that *A. chrysophyllus* might contain hidden karyotypic diversity, useful to the distinction of taxa from different clades recovered by molecular data. Due to its wide distribution in Africa, *A. chrysophilus* is therefore a group that deserves attention and further cytotaxonomic and systematics studies.

Lemniscomys Trouessart, 1881
***Lemniscomys rosalia* (Thomas, 1904)**
Single-striped Grass Mouse

The karyotype of *L. rosalia* from Botswana consists of 48 chromosomes and FNa = 62 (one male TK164822 and one female TK164823 were analyzed; Fig. 12). Out of the eight biarmed autosomes, four had a subtelocentric morphology (pairs 1, 3, 4, and 7). The remaining 15 autosome pairs are acrocentric. The X is a large submeta-subtelocentric, whereas the Y is a small meta-submetacentric chromosome. This karyotype is identical to that described by Ducroz et al. (1999) from female specimens from eastern South Africa and, likewise, the Botswana specimens cluster with a South African sequence in the *Cyt*b phylogeny (Fig. 13). Analysis of our male specimen confirmed the X chromosome assignment previously provided (Ducroz et al. 1999) using interspecific comparison of banding patterns of female individuals only.

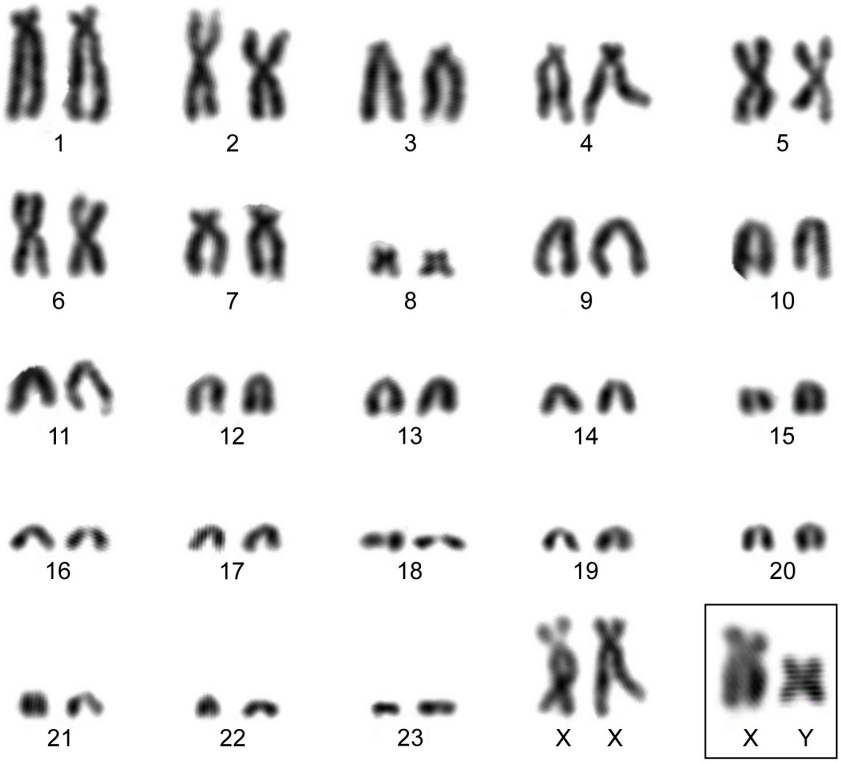

Figure 12. Karyotype of a female *Lemniscomys rosalia* (TK164823) from Koanaka Hills. The sex chromosomes of a male individual from the same locality (TK164822) are shown in the inset.

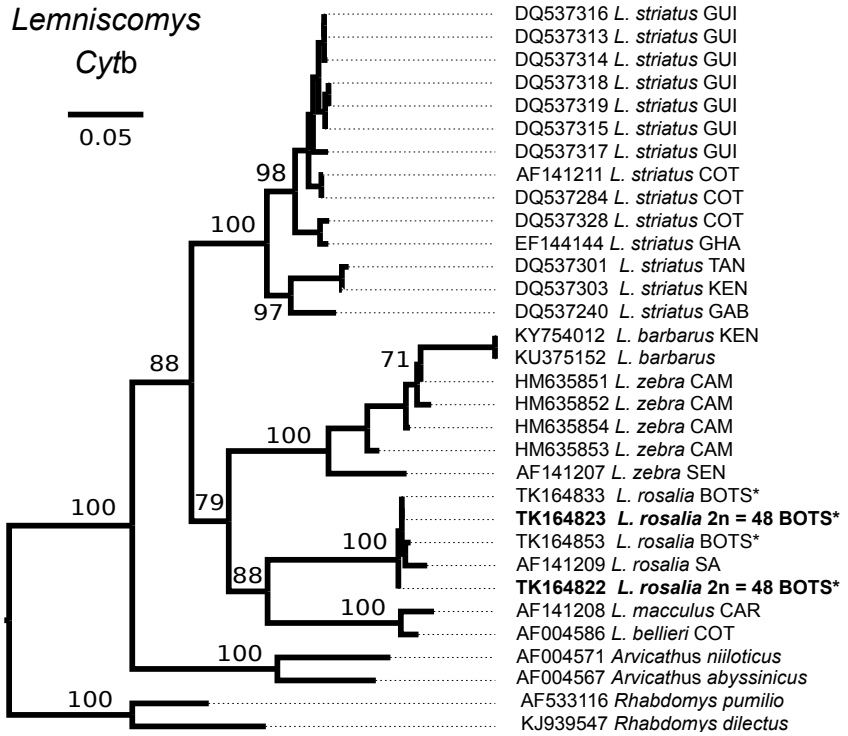

Figure 13. Maximum-likelihood phylogram estimating the phylogenetic relationships of *Lemniscomys* using 1,140 base pairs of the *Cyt*b gene. Nodal support estimated using 1,000 bootstrap replicates. Karyotyped individuals in bold and individuals from Botswana shown with an asterisk.

The karyotype described here differs from that reported for *L. rosalia* specimens from Tanzania, which consisted of 54 chromosomes and FNa = 64 (Fadda et al. 2001; Castiglia et al. 2002) and following the recommendation of Castiglia et al. (2002) could correspond to a separate taxon, probably with equivalent status as the 2n = 48 South African specimens (Ducroz et al. 1999).

Mastomys Thomas, 1915
***Mastomys coucha* (Smith, 1834)**
Southern Multimammate Mouse

The karyotype of a single female individual was similar to that described previously for animals from South Africa and Zimbabwe (Green et al. 1980; Lyons et al. 1980; Britton-Davidian et al. 1995) with a total of 36 chromosomes, 20 of each biarmed, apart from a metacentric pair assigned here as the putative X (see references in Britton-Davidian et al. 1995 and Corti et al. 2005). Likewise, the *Cyt*b sequences from Botswana specimens clustered with *M. coucha* individuals from South Africa (Fig. 14). One of the analyzed specimens was heterozygous for an inversion of pair 10, probably due to a pericentric inversion. In addition, the short arms of the subtelocentric chromosome 8 were visible (TK172786; Fig. 15), and were considered for the inference of a FNa = 53. The second individual analyzed did not exhibit the inversion of pair 10 and therefore had a FNa = 54 (considering the short arms of pair 8), similar to that described by Venturi et al. (2003) for South African specimens.

The genus *Mastomys* is characterized by high karyotypic diversity between species as well as the presence of several intraspecific chromosomal polymorphisms, usually due to pericentric inversions (Britton-Davidian et al. 1995; Volobouev et al. 2001; Corti et al. 2005) and B chromosomes (Dobigny et al. 2010). Due to extreme morphological similarities between sibling species and their occurrence in sympatry, karyotypes have been regarded as a useful tool to aid in their taxonomic distinction (Volobouev et al. 2002; Dobigny et al. 2008).

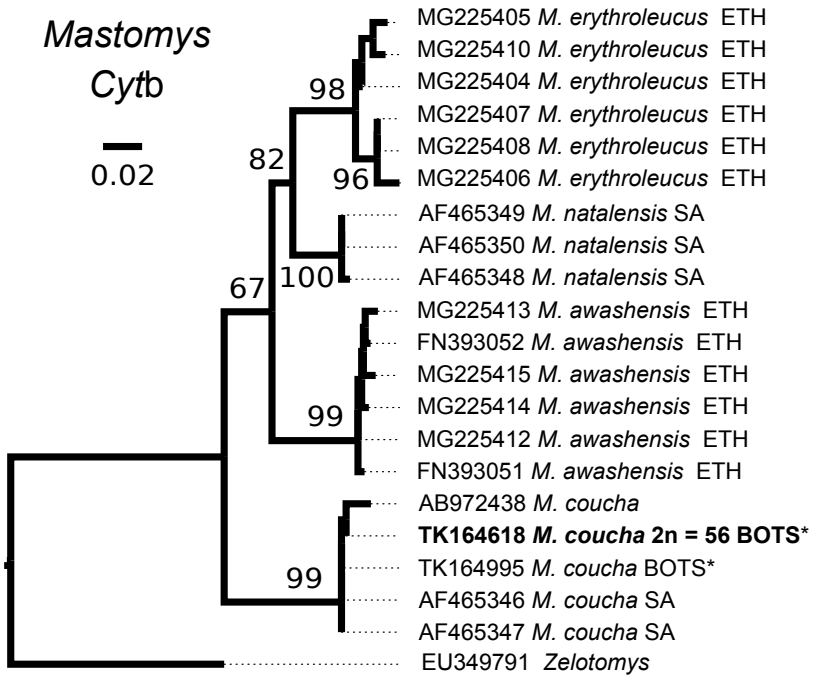

Figure 14. Maximum-likelihood phylogram estimating the phylogenetic relationships of *Mastomys* using 1,140 base pairs of the *Cyt*b gene. Nodal support estimated using 1,000 bootstrap replicates. Karyotyped individuals in bold and individuals from Botswana shown with an asterisk.

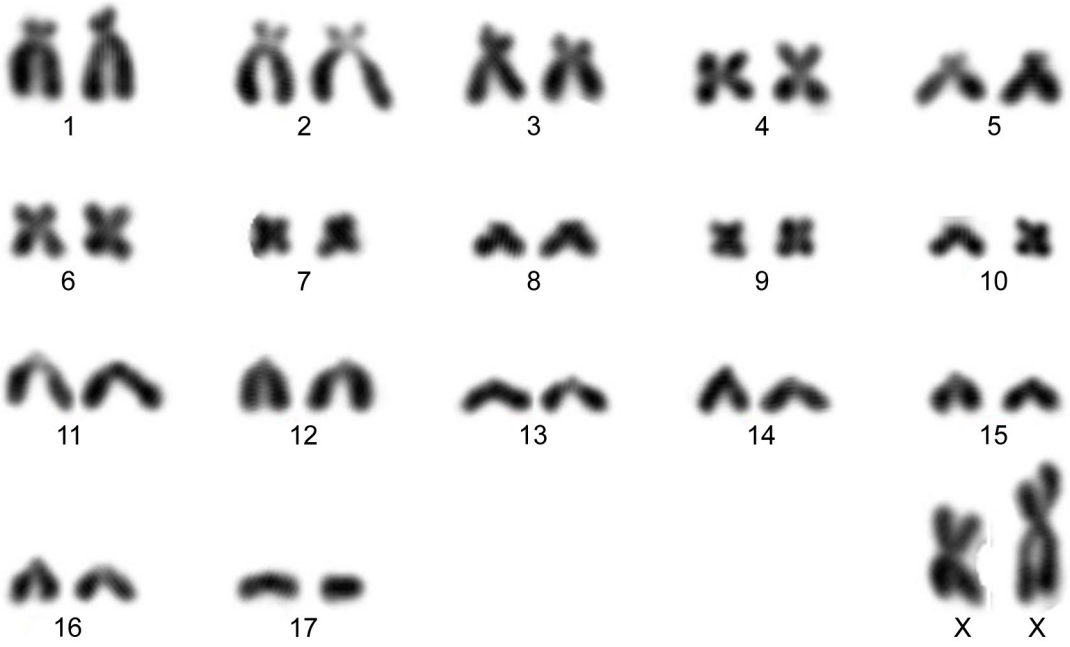

Figure 15. Karyotype of a female *Mastomys coucha* (TK164618) from Koanaka Hills, Botswana. Note heterozygous inversion of pair 10 and short arms of pair 8.

Micaelamys (Wroughton 1908)
***Micaelamys namaquensis* (A. Smith, 1834)**
Namaqua Rock Rat

Formerly grouped within *Aethomys*, the genus *Micaelamys* is karyotypically distinguishable by their lower diploid numbers (24 or 32), thought to have originated from a series of tandem and centric fusions since the divergence of the two genera (Baker et al. 1988). Karyotypic information was critical for elevating *Micaelamys* as distinct from *Aethomys* (Visser and Robinson 1986; Baker et al. 1988; Ducroz et al. 2001; Castiglia et al. 2003). All 14 individuals of *M. namaquensis* analyzed here exhibited a 2n = 24, FNa = 32 karyotype (Fig. 16), as well as low genetic diversity based on *Cyt*b sequence data (Fig. 10). In addition, three of the females were presumed to be heterozygous for a pericentric inversion of pair 5, which was originally metacentric and resulted in a subtelocentric chromosome. None of the specimens analyzed had both homologs as subtelocentric chromosomes and to our knowledge, no other chromosome polymorphism has been described for the species (Visser and Robinson 1986; Baker et al. 1988; Corti et al. 2005).

Genus *Rhabdomys* Thomas, 1916
***Rhabdomys pumilio* (Sparrman, 1784)**
Four-striped Grass Mouse

The male *R. pumilio* specimen analyzed here had a 2n = 48 and FNa = 60 (TK172841; Fig. 17). The karyotype consisted of seven biarmed and 16 acrocentric chromosomes; however, the largest acrocentric pair assigned here can also show small short arms, depending on the degree of chromosome condensation.

Rambau et al. (2003) demonstrated that two *R. pumilio* clades, with distributions corresponding to the xeric and mesic biotic zones of southern Africa, respectively, exhibit two trends in chromosomal numbers: specimens from the xeric region are exclusively found to have 2n = 48 chromosomes, whereas the individuals from the mesic clade can present 2n = 46 or 2n = 48. No intermediate karyotypes or polymorphisms have been observed for the latter group, and thus was assigned to a distinct taxon with two subspecies *R. dilectus dilectus* (2n = 46) and *R. d. chakae* (2n = 48). Later studies found additional variation within *R. dilectus*, with the report of a 2n = 38 karyotype in high altitude individu-

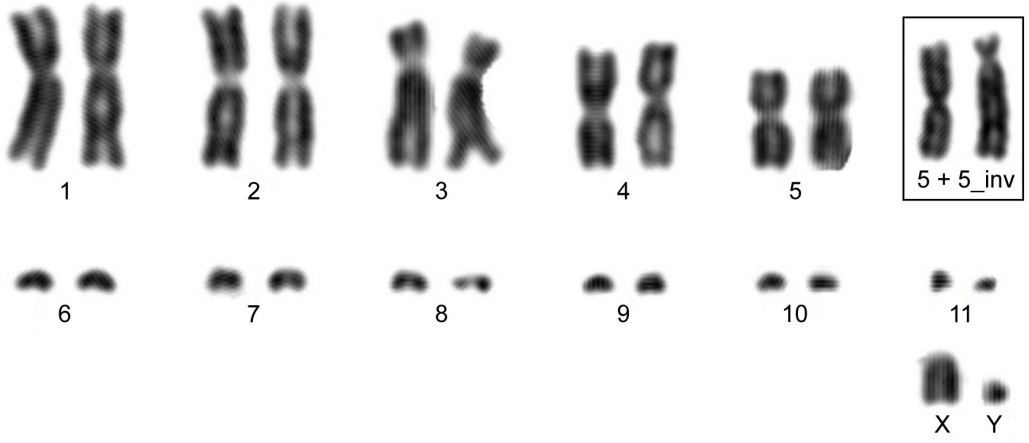

Figure 16. Representative karyotype of *M. namaquensis* based on 14 individuals from diverse collecting sites in Botswana. The pericentric inversion of pair 5 observed in three females (TK164968, TK164972, and TK164997) is displayed in the inset (top right).

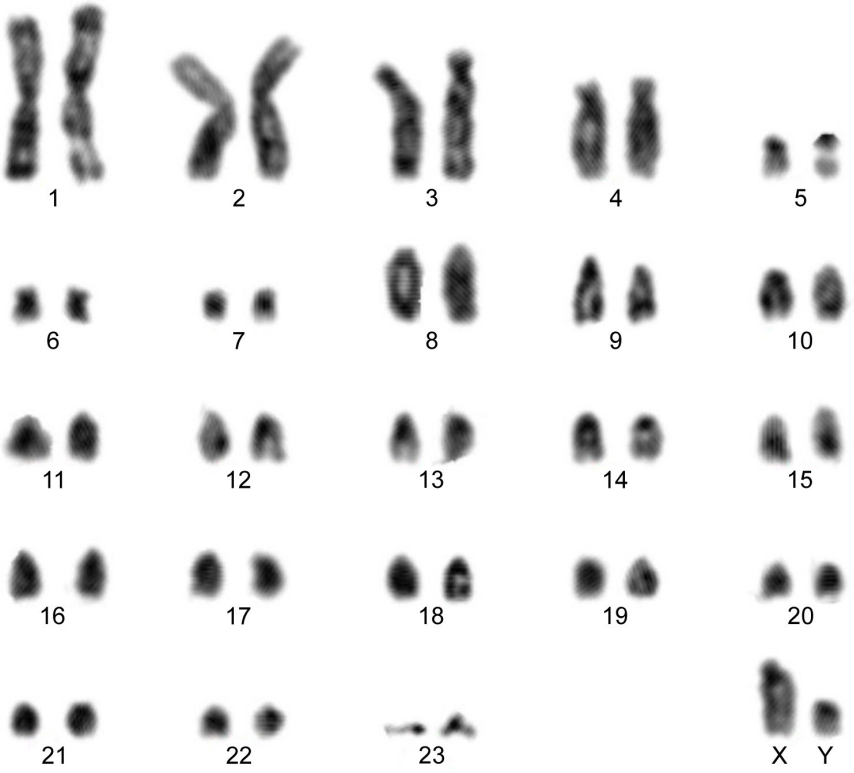

Figure 17. Karyotype of a male four-striped grass mouse, *Rhabdomys pumilio*.

als from Tanzania (Castiglia et al. 2011). It was not possible to sequence the individual from Botswana; however, given that this individual was collected from the xeric, Kalahari region, it likely corresponds to the more dry adapted lineage noted above.

Subfamily Gerbillinae Gray, 1825
***Desmodillus auricularis* (Smith, 1834)**
Cape Short-eared Gerbil

The single male specimen of *Desmodillus* analyzed from Botswana had a diploid number of 2n = 50, with 14 meta-submetacentric and 10 acrocentric pairs of chromosomes (FNa = 76; Fig. 18). Some homologs were different in size from one another (e.g. pair 1), which suggests the occurrence of heterochromatin additions or translocation variations. The X chromosome was a large submetacentric, whereas the Y was a small to medium submetacentric equivalent in size to pair 16 (TK170612; Fig. 18).

This karyotype is similar to that of specimens from the Northern Cape of South Africa (Knight et al. 2013); however, it is different from the previously reported 2n = 52, FNa = 78 from Namibian (central) and South African (Cape) specimens (Qumsiyeh 1986) and 2n = 52, FNa = 70 (location not provided) described in Matthey (1954). Given the low genetic diversity (Fig. 19) between 2n = 52 and 2n = 50 individuals, we hypothesize that the monotypic genus *Desmodillus* represents another example of intraspecific karyotypic variation in southern Africa. Nevertheless, both the karyotypic and *Cyt*b data (Fig. 19) agree with previous studies, which show *Desmodillus* as the sister clade to *Gerbilliscus* and *Gerbillurus* (Colangelo et al. 2007; Granjon et al. 2012).

Desmodillus auricularis exhibits one of the highest diploid numbers reported for southern African gerbils (surpassed only by *Gerbillurus setzeri* and *G. vallinus*, with 2n = 60 in Namibia). This karyotype

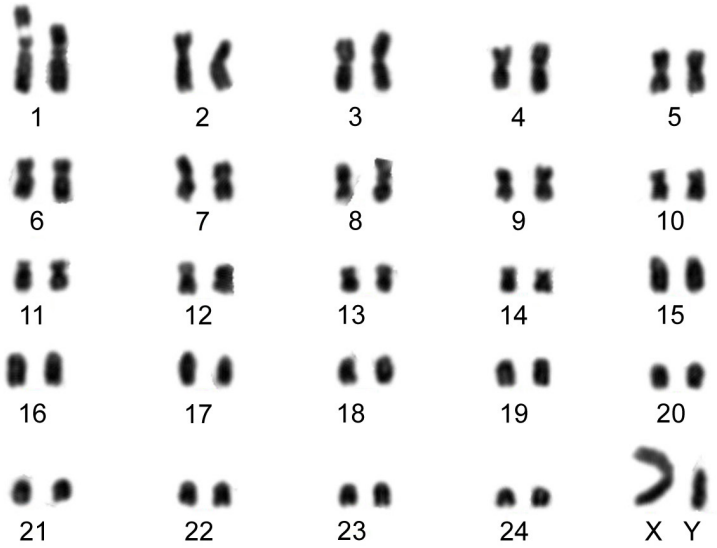

Figure 18. Karyotype of *Desmodillus auricularis* from Tsabong, Botswana.

is thought to have formed from four Robertsonian rearrangements and five inversions from the ancestral karyotype (2n = 56) of its last common ancestor with *Gerbilliscus* (Qumsiyeh et al. 1991; Knight et al. 2013).

Gerbillurus Shortridge, 1942
***Gerbillurus paeba* (A. Smith, 1836)**
Hairy-footed Gerbil

A single individual of *G. paeba* was examined from Botswana and found to have a total of 36 chromosomes, all biarmed, including the sex chromosomes (FNa = 68; Fig. 20). Karyotypes identical to that of the Botswanan specimen have been reported for South Africa and Namibia, and thus far, there is no record of chromosomal number variation along the distributional range for the species (Matthey 1958; Schlitter et al. 1984). Nevertheless, the species has been shown to vary in the amount and location of heterochromatin, which indicates that there might be ongoing expansion of repetitive elements currently shaping the chromosomal architecture in *G. paeba* populations, especially near centromeres of specific chromosome pairs (Qumsiyeh 1986, 1988; Qumsiyeh et al. 1991). Whole chromosome probes from *G. paeba* have been used to better understand the chromosomal evolution of Gerbillinae and confirmed that the molecular defined sister clade *G. paeba* + *G. tytonis* shares identical karyotypes. The combination of chromosomal, morphological, ecological, and DNA sequence data suggests that these species should be reevaluated and revised, as they might represent a single taxon or at least correspond to a case of very recent divergence (Knight et al. 2013).

Gerbilliscus Thomas, 1897
***Gerbilliscus brantsii* (Smith, 1836)**
Highveld Gerbil

Qumsiyeh (1986) and Qumsiyeh et al. (1987) described the karyotype for *G. brantsii* (formerly *Tatera brantsii*) of specimens from South Africa as 2n = 44 and FNa = 66. The karyotype recovered from Botswana (TK164575; Fig. 21) was similar to the Qumsiyeh (1986) description; however, based on *Cyt*b sequences from specimens collected at the same locality (Fig. 19), this clade represents a currently unrecognized taxon assigned here as G. cf. grique (Wroughton 1906).

***Gerbilliscus leucogaster* (Peters, 1852)**
Bushveld Gerbil

Four individuals of *G. leucogaster* from Botswana had 2n = 40 and FNa = 66 (Fig. 22). There were a total of 14 meta-submetacentric autosomal pairs and five acrocentric autosomes in the karyotypes of three females analyzed, and it was assumed the male (TK172839) had the same chromosomal composition, despite the poor quality of the material and few (n =

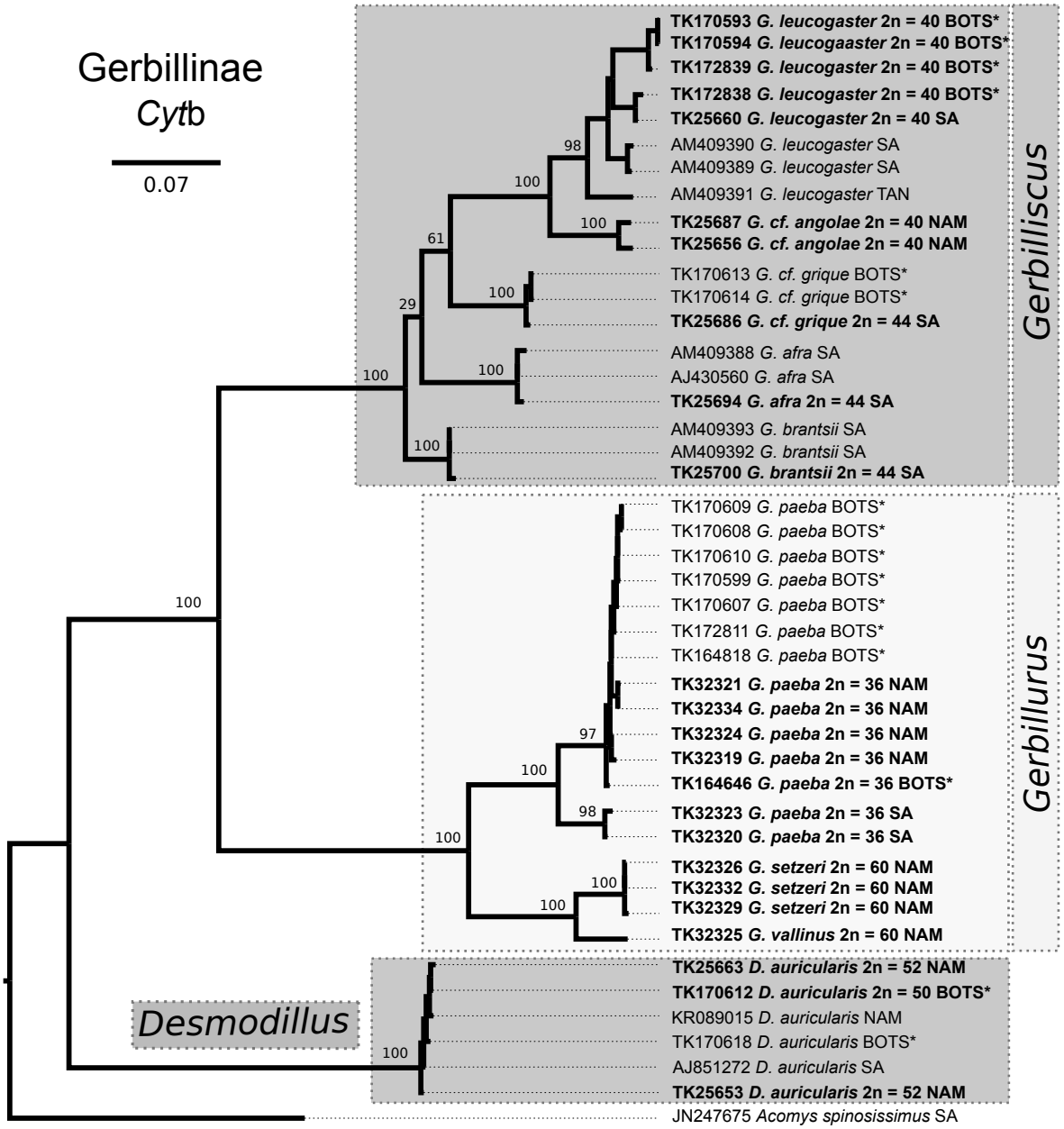

Figure 19. Maximum-likelihood phylogram estimating the phylogenetic relationships with the subfamily Gerbillinae using 1,140 base pairs of the *Cyt*b gene. Nodal support estimated using 1,000 bootstrap replicates. Karyotyped individuals in bold and individuals from Botswana shown with an asterisk.

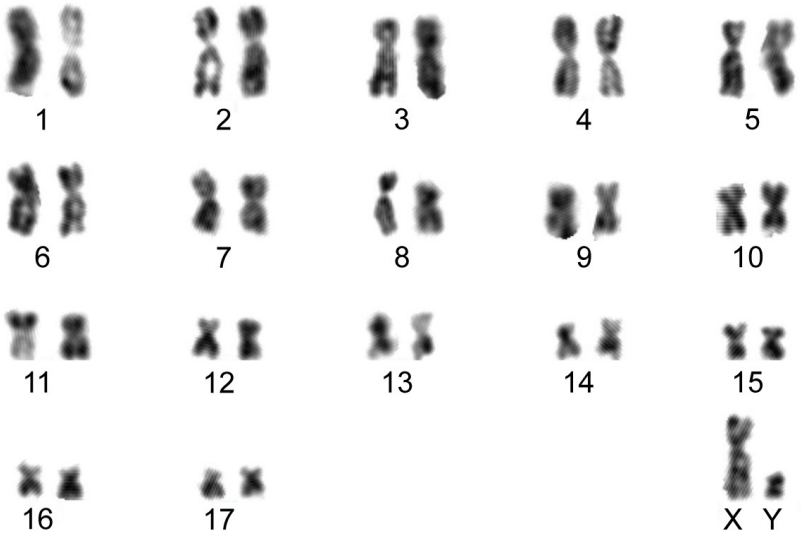

Figure 20. Karyotype of *Gerbillurus paeba*.

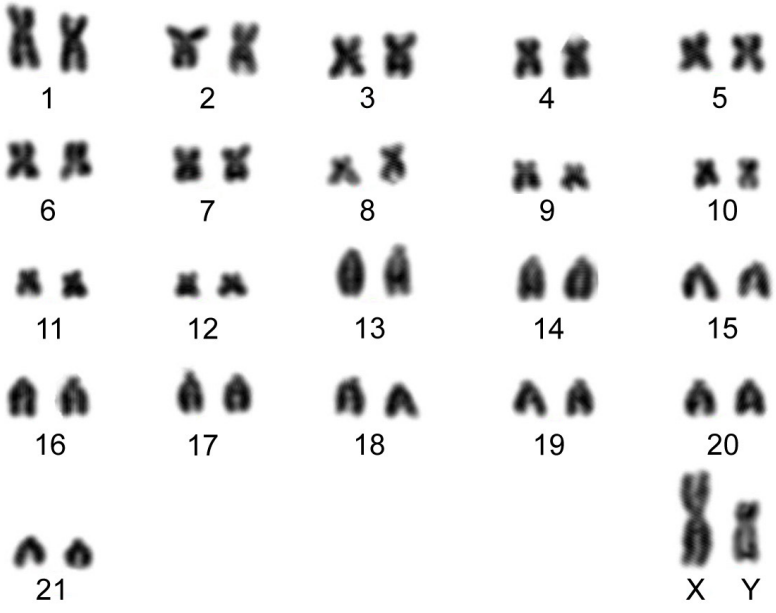

Figure 21. Karyotype of *Gerbilliscus* cf. *grique*.

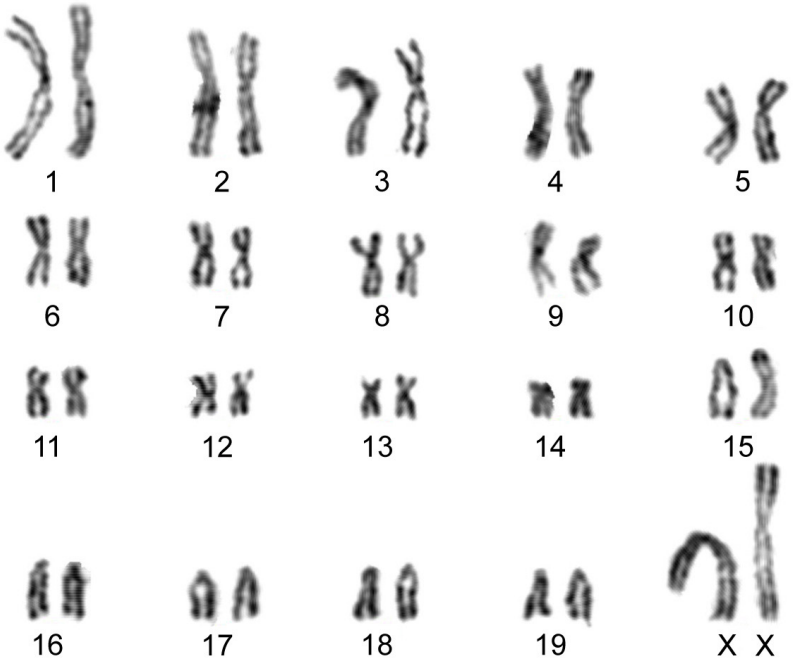

Figure 22. Representative karyotype of *Gerbilliscus leucogaster*.

3) metaphases analyzed. Using previously published karyotypes for comparison (see references below) it was possible to assign the X chromosome as one of the largest biarmed chromosome pairs.

Gerbilliscus leucogaster has a widespread distribution in southern Africa, but few studies have dealt with chromosomal characterization over the entire geographic range of the species. Karyotypes identical to those described for specimens from Zimbabwe, Namibia, and South Africa (Gordon and Rautenbach 1980; Qumsiyeh 1986; Qumsiyeh 1987), as well as different regions in Tanzania (Colangelo et al. 2005; Denys et al. 2011) were recovered in Botswana. However, Matthey (1958) described the karyotypes of individuals from a South African population that had the same sex chromosome morphology described herein, but a total of 42 chromosomes in their complement. Despite the lack of a thorough geographic study of chromosomal variation within the species, available data suggest that the karyotype of *G. leucogaster* is stable and was used by Qumsiyeh (1986) to define the standard numbering system of chromosomal arms for Gerbillinae comparative cytogenetic studies. In addition, the species was also included in a study dealing with full chromosome homology comparison to other African gerbils using chromosome painting (Knight et al. 2013).

Additional sequence data also was included (Fig. 19) for two individuals from Namibia previously karyotyped by Qumsiyeh (1986) and Qumsiyeh et al. (1987) that were classified as *G. leucogaster*. This genetically divergent lineage shares the 2n = 40, FNa = 66 karyotype and represents a currently unrecognized taxon that was tentatively called here as *G. cf. angolae* (Wroughton 1906). Although the karyotypic formula with 2n = 40 is not unique to a single species of the *G. leucogaster* lineage, it will still be a useful tool to distinguish members of the clade occurring in sympatry with other gerbil species. For example, *G. leucogaster* and *G. brantsii* occur in sympatry in regions of southern Africa, but can be distinguished by their different chromosome numbers, 2n = 40 and 2n = 44, respectively (Taylor 2000).

CONCLUSION

Here, new karyotypic information and genetic sequence data were provided for 63 individuals representing 17 species of small mammals from Botswana. This region is considered a biogeographical crossroads between the Namib and Kalahari Deserts, as well as savanna woodlands. Its position is critical for describing phylogeographic patterns of small mammal species, as it connects east and west African regions with different bioclimatic features. These results build upon previous descriptive studies and will help to establish hypotheses for future studies related to species delimitation and phylogeography. Some of the noteworthy findings from our study include: further evidence for chromosomal variation in the *S. campestris* species complex; the first karyotypic description for *S. parvus*; and new karyotypic information and mitochondrial sequences for currently unrecognized species of gerbils.

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LITERATURE CITED

Aguilar, G. H. 1993. The karyotype and taxonomic status of *Cryptomys hottentotus darlingi* (Rodentia, Bathyergidae). South African Journal of Zoology 28:201–204.

Baker, R. J., M. J. Hamilton, and D. A. Parish. 2003. Preparations of mammalian karyotypes under field conditions. Occasional Papers Museum of Texas Tech University 228:1–8.

Baker, R. J., M. B. Qumsiyeh, and I. L. Rautenbach. 1988. Evidence for eight tandem and five centric fusions in the evolution of the karyotype of *Aethomys namaquensis* A. Smith (Rodentia: Muridae). Genetica 76:1661–1669.

Barome, P. O., et al. 2001. Phylogeny of *Acomys spinosissimus* (Rodentia, Muridae) from north Malawi and Tanzania: evidence from morphological and molecular analysis. Biological Journal of the Linnean Society 73:321–340.

Britton-Davidian, J., J. Catalan, L. Granjon, and J. M. Duplantier. 1995. Chromosomal phylogeny and evolution in the genus *Mastomys* (Mammalia, Rodentia). Journal of Mammalogy 76:248–262.

Bulatova, N. S., V. N. Orlov, and M. I. Baskevich. 1995. Karyological comments to the list of Ethiopian mammals. Pp. 32–58 in Theriological Investigations in Ethiopia (V. E. Sokolov, ed.). Nauka, Moscow.

Castiglia, R., et al. 2003. Chromosomal and molecular characterization of *Aethomys kaiseri* from Zambia and *Aetho-*

mys chrysophilus from Tanzania (Rodentia, Muridae). Hereditas 139:81-89.

Castiglia, R., C. Fadda, M. Corti, A. Scanzani, W. Verheyen, and E. Capanna. 2002. Chromosomal evolution in the African Arvicanthine rats (Murinae, Rodentia): comparative cytogenetics of *Lemniscomys* (*L. zebra*, *L. rosalia*, *L. striatus*) and *Arvicanthis dembeensis*. Journal of Zoological Systematics and Evolutionary Research 40:223–231.

Castiglia, R., R. Makundi, and M. Corti. 2007. The origin of an unusual sex chromosome constitution in Acomys sp.(Rodentia, Muridae) from Tanzania. Genetica 131:201–207.

Castiglia, R., E. Solano, R. H. Makundi, J. Hulselmans, E. Verheyen, and P. Colangelo. 2011.Rapid chromosomal evolution in the mesic four-striped grass rat *Rhabdomys dilectus* (Rodentia, Muridae) revealed by mtDNA phylogeographic analysis. Journal of Zoological Systematics and Evolutionary Research 50:165–172.

Chimimba, C. T., and N. Bennett. 2005. Order: Rodentia. Pp. 156–163 in The mammals of the southern African subregion, 3rd edition (J. D. Skinner and C. T Chimimba, eds.). Cambridge University Press, Cambridge, Massachusetts.

Churchfield, S. 2013. Family Soricidae shrews. Pp. 43–50 in Mammals of Africa (vol. 4) Hedgehogs, shrews and bats (M. Happold and D. Happold, eds.). Bloomsbury Natural History, London.

Colangelo, P., et al. 2005. Mitochondrial phylogeny reveals differential modes of chromosomal evolution in the genus *Tatera* (Rodentia: Gerbillinae) in Africa. Molecular Phylogenetics and Evolution 35:556–568.

Colangelo, P., L. Granjon, P. J. Taylor, and M. Corti. 2007. Evolutionary systematics in African gerbilline rodents of the genus *Gerbilliscus*: inference from mitochondrial genes. Molecular Phylogenetics and Evolution 42:797–806.

Corti, M., et al. 2005. Cytotaxonomy of rodent species from Ethiopia, Kenya, Tanzania and Zambia. Belgian Journal of Zoology 135:197–216.

Denys, C., et al. 2011. A faunal survey in Kingu Pira (south Tanzania), with new karyotypes of several small mammals and the description of a new Murid species (Mammalia, Rodentia). Zoosystema 33:5–48.

Deuve, J. L., N. C. Bennett, J. Britton-Davidian, and T. J. Robinson. 2008. Chromosomal phylogeny and evolution of the African mole-rats (Bathyergidae). Chromosome Research 16:57–74.

Dippenaar, N. J., J. Meester, I. L. Rautenbach, and D. A. Wolhuter. 1983. The status of Southern African mammal taxonomy. In Proceedings of the 3rd international colloquium on ecology and taxonomy of African small mammals, Antwerp (E. van der Staeten, W. N. Verheien, F. De Vree, eds.). Annals of the Museum of the Royal African Center, Sc Zool. 237:1–227.

Dippenaar, N. J., and I. L. Rautenbach. 1986. Morphometrics and karyology of the southern African species of the genus *Acomys* I. Geoffroy Saint-Hilaire, 1838 (Rodentia: Muridae). Annals of the Transvaal Museum 34:129–183.

Dobigny, G., et al. 2008. An update on the taxonomy and geographic distribution of the cryptic species *Mastomys kollmannspergeri* (Muridae, Murinae) using combined cytogenetic and molecular data. Journal of Zoology 276:368–374.

Dobigny, G., et al. 2010.Geographic patterns of inversion polymorphisms in a wild African rodent, *Mastomys erythroleucus*. Heredity 104:378.

Dubey, S., K. Koyasu, R. Parapanov, M. Ribi, R. Hutterer and P. Vogel. 2008. Molecular phylogenetics reveals Messinian, Pliocene, and Pleistocene colonizations of islands by North African shrews. Molecular Phylogenetics Evolution 47:877–882.

Ducroz, J. F., L. Granjon, M. Lombard, and V. Volobouev. 1999. Comparative chromosome analysis (R-and C-bands) of two South African murid species, *Lemniscomys rosalia* and *Rhabdomys pumilio* (Rodentia, Murinae). Cytogenetic and Genome Research 87:69–74.

Ducroz, J. F., V. Volobouev, and L. Granjon. 2001. An assessment of the systematics of arvicanthine rodents using mitochondrial DNA sequences: evolutionary and biogeographical implications. Journal of mammalian Evolution 8:173–206.

Dumbacher, J. P., E. J. Carlen, and G. B. Rathbun. 2016. *Petrosaltator* gen. nov., a new genus replacement for the North African sengi *Elephantulus rozeti* (Macroscelidea; Macroscelididae). Zootaxa 4136:567–579.

Fadda, C., et al. 2001. The rodent fauna of Tanzania: a cytotaxonomic report from the Maasai Steppe (1999). Rendiconti Lincei 12:29–49.

Faulkes, C. G., N. C. Bennett, M. W. Bruford, H. P. O'Brien, G. H. Aguilar, and J. U. M. Jarvis. 1997. Ecological constraints drive social evolution in the African mole-rats. Proceedings of the Royal Society of London B: Biological Sciences 264:1619–1627.

Ford, C. E., and J. L. Hamerton. 1956. Chromosomes of five rodent species. Nature 177:140.

Gannon, W. L., R. S. Sikes, and the Animal Care and Use Committee of the American Society of Mammalogists. 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 88:809–823.

Gordon, D.H., and I. L. Rautenbach. 1980. Species complexes in medically important rodents-chromosome-studies of *Aethomys*, *Tatera* and *Saccostomus* (Rodentia, Muridae, Cricetidae). South African Journal of Science 76:559–561.

Gordon, D. H., and C. R. B. Watson. 1986. Identification of cryptic species of rodents (*Mastomys*, *Aethomys*, *Saccostomus*) in the Kruger National Park. South African Journal of Zoology 21:95–99.

Granjon, L., P. Colangelo, C. Tatard, M. Colyn, G. Dobigny, and V. Nicolas. 2012. Intrageneric relationships within Gerbilliscus (Rodentia, Muridae, Gerbillinae), with characterization of an additional West African species. Zootaxa 3325:1–25.

Green, C. A., H. Keogh, D. H. Gordon, M. Pinto, and E. K. Hartwig. 1980. The distribution, identification, and naming of the *Mastomys natalensis* species complex in southern Africa (Rodentia: Muridae). Journal of Zoology 192:17–23.

Kingdon, J., D. Happold, T. Butynski, M. Hoffmann, M. Happold, and J. Kalina. 2013. Mammals of Africa. Bloomsbury Natural History, London.

Knight, L. I., B. L. Ng, W. Cheng, B. Fu, F. Yang, and R. V. Rambau. 2013. Tracking chromosome evolution in southern African gerbils using flow-sorted chromosome paints. Cytogenetic and Genome Research 139:267–275.

Lavrenchenko, L.A., R.S. Nadjafova, and N.S. Bulatova. 2011. Three new karyotypes extend a Robertsonian fan in Ethiopian spiny mice of the genus *Acomys* I. Geoffroy, 1838 (Mammalia, Rodentia). Comparative Cytogenetics 5:423.

Lyons, N. F., D. H. Gordon, and C. A. Green. 1980. G-banding chromosome analysis of species a of the *Mastomys natalensis* complex (Smith, 1834) (Rodentia, Muridae). Genetica 54:209–212.

Maddalena, T., A. M. Mehmeti, G. Bronner, and P. Vogel. 1987. The karyotype of *Crocidura flavescens* (Mammalia, Insectivora) in South Africa. Zeitschrift für Säugetierkunde, 52:129-132.

Maddalena, T., and M. Ruedi. 1994. Chromosomal evolution in the genus *Crocidura* (Soricidae, Insectivora). Pp. 335–344 in Advances in the biology of Shrews (J. F. Merritt, G. L. Kirkland, Jr. and R. K. Rose, eds.). Carnegie Museum of Natural History Special Publication 18.

Maputla, N.W. 2007. Taxonomic status of *Saccostomus campestris* (Rodentia: Cricetomyinae) from southern Africa: a multidisciplinary approach (Doctoral dissertation, University of Pretoria).

Maputla, N. W., E. R. Dempster, J. Raman and J. W. H. Ferguson. 2011. Strong hybrid viability between two widely divergent chromosomal forms of the pouched mouse. Journal of Zoology 285:180–187.

Matthey, R. 1954. Nouvelles Recherches sur les Chromosomes des Muridae, Caryologia: International Journal of Cytology. Cytosystematics and Cytogenetics 6:1–44.

Matthey, R. 1958. Les chromosomes et la position systematique de quelques Murinae africains (Mammalia-Rodentia). Acta Tropica 15:97–117.

Matthey, R. 1970. Caryotypes de mürides et de dendromurides originaires de republiqüe centrafricaine. Mammalia 34:459–466.

Mazoch, V., et al. 2017. Phylogeography of a widespread sub-Saharan murid rodent *Aethomys chrysophilus*: the role of geographic barriers and paleoclimate in the Zambezian bioregion. Mammalia 82:373–387.

McDonough, M.M., C. G. Sotero-Caio, A. W. Ferguson, P. J. Lewis, M. Tswiio, and M. L. Thies. 2013. Mitochondrial DNA and karyotypic data confirm the presence of *Mus indutus* and *Mus minutoides* (Mammalia, Rodentia, Muridae, Nannomys) in Botswana. ZooKeys 359:35.

McDonough, M.M., R. Šumbera, V. Mazoch, A. W. Ferguson, C. D. Phillips, and J. Bryja. 2015. Multilocus phylogeography of a widespread savanna–woodland adapted rodent reveals the influence of Pleistocene geomorphology and climate change in Africa's Zambezi region. Molecular Ecology 24:5248–5266.

Mikula, O., et al. 2016. Evolutionary history and species diversity of African pouched mice (Rodentia: Nesomyidae: *Saccostomus*). Zoologica Scripta 45:595–617.

Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. URL http://www.phylo.org [accessed on December 2018].

Nevo, E., E. Capanna, M. Corti, J. U. M. Jarvis, and G. C. Hickman. 1986. Karyotype differentiation in the endemic subterranean mole-rats of South Africa (Rodentia, Bathyergidae). Zeitschrift fur Saugetierkunde 51:36-49.

O'Brien, S. J., J. C. Menninger, and W. G. Nash. 2006. Atlas of mammalian chromosomes. John Wiley & Sons, Hoboken, New Jersey.

Petružela, J., et al. 2018. Spiny mice of the Zambezian bioregion–phylogeny, biogeography and ecological differentiation within the *Acomys spinosissimus* complex. Mammalian Biology 91:79–90.

Primus, A., et al. 2006. Karyology and chromosomal evolution of some small mammals inhabiting the rainforest of the Rabi oilfield, Gabon. Gamba, Gabon: biodiversity of an equatorial African rainforest. Bulletin of the Biological Society of Washington 12:1807–1813.

Qumsiyeh, M. B. 1986. Phylogenetic studies of the rodent family Gerbillidae: I. Chromosomal evolution in the southern African complex. Journal of Mammalogy 67:680–692.

Qumsiyeh, M. B. 1988. Pattern of heterochromatic variation and phylogeny in the rodent family Gerbillidae. Texas Journal of Science 40:63–70.

Qumsiyeh, M. B., M. J. Hamilton, E. R. Dempster, and R. J. Baker. 1991. Cytogenetics and systematics of the rodent genus Gerbillurus. Journal of Mammalogy 72:89–96.

Qumsiyeh, M. B., M. J. Hamilton, and D. A. Schlitter. 1987. Problems in using Robertsonian rearrangements in determining monophyly: examples from the genera *Tatera* and *Gerbillurus*. Cytogenetic and Genome Research 44:198–208.

Rambau, R.V., T. J. Robinson, and R. Stanyon. 2003. Molecular genetics of *Rhabdomys pumilio* subspecies boundaries: mtDNA phylogeography and karyotypic analysis by fluorescence in situ hybridization. Molecular Phylogenetics and Evolution 28:564–575.

Robinson, T. J., B. Fu, M. A. Ferguson–Smith, and F. Yang. 2004. Cross-species chromosome painting in the golden mole and elephant-shrew: support for the mammalian clades Afrotheria and Afroinsectiphillia but not Afroinsectivora. Proceedings of the Royal Society of London B: Biological Sciences 271:1477–1484.

Schlitter, D. A., R. Hutterer, T. Maddalena, and L. W. Robbins. 1999. New karyotypes of shrews (Mammalia: Soricidae) from Cameroon and Somalia. Annals of Carnegie Museum 68:1–13.

Schlitter, D. A., I. L. Rautenbach, and C. G. Coetzee. 1984. Karyotypes of southern African gerbils, genus *Gerbillurus* Shortridge, 1942 (Rodentia: Cricetidae). Carnegie Museum of Natural History.

Sikes, R. S., and Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy 97:663–688.

Skinner, J. D., and C. T. Chimimba. 2005. The mammals of the southern African sub-region. Cambridge University Press.

Smit, H.A., B. Jansen van Vuuren, P. C. M. O'Brien, M. Ferguson Smith, F. Yang, and T. J. Robinson. 2011. Phylogenetic relationships of elephant shrews (Afrotheria, Macroscelididae). Journal of Zoology 284:133–143.

Smit, H. A., T. J. Robinson, J. Watson, and B. J. van Vuuren. 2008. A new species of elephant-shrew (Afrotheria: Macroscelidea: *Elephantulus*) from South Africa. Journal of Mammalogy 89:1257–1269.

Smithers, R. H. N. 1971. The mammals of Botswana. Doctoral dissertation, University of Pretoria.

Solano, E., P. J. Taylor, A. Rautenbach, A. Ropiquet, and R. Castiglia. 2014. Cryptic speciation and chromosomal repatterning in the South African climbing mice *Dendromus* (Rodentia, Nesomyidae). PloS one 9:p.e88799.

Taylor, P. J. 2000. Patterns of chromosomal variation in southern African rodents. Journal of Mammalogy 81:317–331.

Tolliver, D. K., L. W. Robbins, I. L. Rautenbach, D. A. Schlitter and C. G. Coetzee. 1989. Biochemical systematics of elephant shrews from southern Africa. Biochemical Systematics and Ecology 17:345–355.

Van Daele, P. A. A. G., P. Damman, J. L. Meier, M. Kawalika, C. Van De Woestijne, and H. Burda. 2004. Chromosomal diversity in mole–rats of the genus *Cryptomys* (Rodentia: Bathyergidae) from the Zambezian region: with descriptions of new karyotypes. Journal of Zoology, London 264:317–326.

Venturi, F. P., C. T. Chimimba, R. J. Van Aarde, and N. Fairall. 2003. The distribution of *Mastomys natalensis* and *M. coucha* (Rodentia: Muridae) in South Africa. ACIAR Monograph Series 96:529–530.

Verheyen W., J. Hulselmans, W. Wendelen, H. Leirs, M. Corti, and E. Verheyen. 2011. Contribution to the systematics and zoogeography of the East African *Acomys spinosissimus* Peters 1852 species complex and the description of two new species (Rodentia: Muridae). Zootaxa 3059:1–35.

Veyrunes F., et al. 2010. Mitochondrial and chromosomal insights into karyotypic evolution of the pygmy mouse, *Mus minutoides*, in South Africa. Chromosome Research 18:563–574.

Veyrunes F., J. Perez, S. N. C. Paintsil, E. Fichet-Calvet, and J. Britton-Davidian. 2013. Insights into the evolutionary history of the X-linked sex reversal mutation in *Mus minutoides*: clues from sequence analyses of the Y-linked Sry gene. Sexual Development 7:244–252.

Visser, D. S. and T. J. Robinson. 1986. Cytosystematics of the South African *Aethomys* (Rodentia: Muridae). African Zoology 21:264-268.

Volobouev, V.T., V. M. Aniskin, E. Lecompte, and J. F. Ducroz. 2002. Patterns of karyotype evolution in complexes of sibling species within three genera of African murid rodents inferred from the comparison of cytogenetic and molecular data. Cytogenetic and Genome Research 96:261–275.

Volobouev, V. T., A. Hoffmann, B. Sicard, and L. Granjon. 2001. Polymorphism and polytypy for pericentric inversions in 38-chromosome *Mastomys* (Rodentia, Murinae) and possible taxonomic implications. Cytogenetic and Genome Research 92:237–242.

Wenhold, B. A., and T. J. Robinson. 1987. Comparative karyology of three species of elephant-shrew (Insectivora: Macroscelididae). Zeitschrift für Säugetierkunde 52:1–9.

Wroughton, R. C. 1906. Notes on the genus *Tatera*, with descriptions of new species. Journal of Natural History 17:474–499.

Zima, J., L. Lukácová, and M. Macholán. 1998. Chromosomal evolution in shrews. In: Evolution of shrews (J. M. Wójcik and M. Wolsan, eds.). Polish Academy of Sciences, Bialowieza, Poland.

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Distribution and Expression of Ribosomal DNA in the Composite Genomes of Unisexual Lizards of Hybrid Origin (Genus *Aspidoscelis*)

Calvin A. Porter, Oscar G. Ward, Charles J. Cole, and Robert J. Baker

Abstract

The teiid lizard genus *Aspidoscelis* includes bisexual and unisexual species. Each parthenogenetic unisexual species was formed by a past hybridization event and has a composite genome derived from two or three bisexual species. The presence (by *in situ* hybridization) and activity (by silver staining) of ribosomal gene sequences (rDNA) were examined in unisexual and bisexual species. In the diploid unisexual species that were examined, both *in situ* hybridization and silver staining indicated nucleolar inactivity and reduction of rDNA in one of the ancestral genomes. These findings are noteworthy in documenting reduction or elimination of rDNA in the composite genome of parthenogenetic lineages. In the four triploid unisexual species examined, there was a range of expression and reduction of rDNA. In *A. uniparens*, rDNA is retained in all three ancestral genomes (albeit reduced in two of the genomes) but expressed in no more than two. In *A. exsanguis*, rDNA is present and expressed in one ancestral genome, and usually eliminated from the other two. *Aspidoscelis sonorae* and *A. velox* were found to have patterns of rDNA reduction and expression intermediate to that seen in *A. uniparens* and *A. exsanguis*. These results are consistent with selective inactivation of some NORs to ensure homogeneity of the ribosomes, followed by the selective elimination of the inactivated gene sequences.

Key words: *Aspidoscelis*, hybridization, nucleolar organizing regions, parthenogenesis, ribosomal DNA, unisexual lizards, whiptail lizards

Introduction

The North American whiptail lizards (genus *Aspidoscelis*) of the family Teiidae include both bisexual and unisexual (all-female) species, which previously were included in the genus *Cnemidophorus* (see Reeder et al. 2002). As is typical among vertebrates, the bisexual species reproduce by fusion of male and female gametes to form a recombined diploid genome with a unique mixture of parental genes. In contrast, the naturally-occurring unisexual species (either diploid or triploid) reproduce asexually by parthenogenesis, with daughters developing from unfertilized eggs. The diploid parthenogenetic species were formed by interspecific hybridization between various combinations of two bisexual species so that each of these unisexual forms possesses one haploid genome from each of its two ancestral species. Triploidy in *Aspidoscelis* originated when a member of a diploid unisexual lineage subsequently hybridized with a lizard of a bisexual species (either backcrossing with one of the original ancestors or hybridizing with a third species). The parthenogenetic forms are natural clones that pass their conjoined genome to the next generation without recombination.

Karyotypic and genetic evidence strongly support a natural hybrid origin for each of the parthenogenetic lineages of *Aspidoscelis* (reviewed by Reeder et al. 2002). Whiptail lizards seem particularly prone to forming parthenogenetic hybrid clones (Vrijenhoek et al. 1989; Reeder et al. 2002). However, laboratory attempts to replicate interspecific hybridization among the natural bisexual species have produced rare apparently-sterile hybrids, but no new parthenogenetic lineages (Cole et al. 2010). This may suggest that

natural hybridization is rare and that it is even more uncommon that interspecific hybrids clone themselves and form a unisexual population. However, laboratory hybridization between triploid parthenogens and males of bisexual species has produced numerous but similar independently-generated tetraploid clonal lineages (Lutes et al. 2011; Cole et al. 2014, 2017).

The parthenogenetic lizards have an atypical genetic environment in which alleles from disparate origins are propagated to the offspring without sexual recombination (Dessauer and Cole 1986, 1989). This hybrid genetic condition is maintained in each lizard and in each generation of the resulting populations, although mutation and selection continue to operate on the genome.

Whiptail lizards and similar parthenogenetic hybrids offer unique insights into genome evolution and gene regulation. Natural parthenogenetic species of hybrid origin and sterile laboratory hybrids both express allozymic alleles characteristic of their ancestral genomes (Neaves and Gerald 1968; Neaves 1969; Dessauer and Cole 1984, 1989; Taylor et al. 2001; Cole et al. 2007, 2010). However, Ward and Cole (1986) found that rDNA (i.e., the repeated sequences coding for ribosomal RNA) of parthenogenetic whiptails is often transcriptionally active in only one of the lizard's haploid genomes. They reported that the active nucleolar organizing regions revealed by silver staining (AgNORs) corresponded to secondary constrictions that are visible in standard geimsa-stained karyotypes. In both of the bisexual species *Aspidoscelis inornatus* and *A. tigris*, the AgNORs were documented near the telomeric end of a single large biarmed chromosome pair and appeared to be codominant within each species (Ward and Cole 1986). Each of these two species has a NOR-bearing chromosome with distinctive morphology and rDNA. It is therefore possible to identify the origin of NOR-bearing chromosomes in unisexual species that have a composite genome derived from these species.

In unisexual species, Ward and Cole (1986) found that the dissimilar AgNORs usually did not exhibit codominant activity. They reported that the diploid *A. neomexicanus* (possessing a hybrid genome derived from *A. tigris* and *A. inornatus*) commonly had only one active AgNOR. In *A. neomexicanus*, the *A. inornatus* AgNOR was incompletely dominant over that of *A. tigris*, meaning that the ribosomal genes derived from *A. tigris* were often not transcribed, whereas those of *A. inornatus* always were expressed (Ward and Cole 1986).

Aspidoscelis tesselatus is a diploid unisexual clone possessing a hybrid (*A. tigris* × *A. septemvittatus*) genome. Ward and Cole (1986) studied this species and determined that the *A. tigris*-derived AgNOR was dominant over that of *A. septemvittatus*. They also found evidence for nucleolar dominance in the triploid species *A. exsanguis*, *A. uniparens*, and *A. sonorae*. None of the triploids examined by Ward and Cole (1986) expressed three AgNORs, indicating that ribosomal gene expression was inactivated in one or two of their component genomes.

The silver-staining method employed by Ward and Cole (1986) identified the location of ribosomal genes being actively transcribed but could not test for the actual presence of those repetitive ribosomal gene sequences. Because they had tested only for rDNA activity, Ward and Cole (1986) conservatively assumed the continued presence of ancestral rDNA in the hybrid unisexual species. As a result, they interpreted the absence of an AgNOR as inactivity of gene sequences (and thus dominance of the active AgNOR), rather than the possible deletion of the apparently inactive AgNOR from the hybrid genome.

In the present study, silver staining was again used to visualize transcriptionally active regions of tandemly repeated rDNA (AgNORs), and fluorescence *in situ* hybridization (FISH) with an rDNA probe was used to detect the presence and location of ribosomal DNA sequences, including those that may be inactivated or suppressed in the presence of a "dominant" NOR derived from a different ancestral species. The purpose of the study was to test whether the rDNA is present, absent, or apparently reduced in cases where AgNORs were not observed on the chromosomes where they would have been expected.

The use of specific and subspecific names in *Aspidoscelis* has long been in a state of flux. See the last two paragraphs in Methods for an explanation of nomenclature in this paper, and the Appendix for a list of the specimens used for this report.

METHODS

Representatives of ten species were collected from natural populations and most specimens were examined by both i*n situ* rDNA and silver-staining methods (see Appendix). AgNOR data from *A. burti* and *A. costatus griseocephalus* were included to confirm that these bisexual species have the same AgNORs as the other species of the *A. sexlineatus* species group (including *A. inornatus*, *A. septemvittatus*, and *A. stictogrammus*).

Flame-dried chromosome preparations were made from bone marrow using the methods of Cole (1979) or Porter et al. (1991). AgNORs were visualized using the methods described by Ward and Cole (1986).

For *in situ* hybridization, chromosomes were treated with a biotin-labeled molecular probe. The probe sequence was Arnheim's (1979) I-19 probe, which was isolated from the 28S ribosomal gene of the laboratory mouse (*Mus musculus*). The probe has been shown to be sufficiently sensitive to detect repetitive rDNA sequences of at least 30 copies in chromosomes of widely divergent vertebrate species (Baker et al. 1992; Porter et al. 1994). Methods of labeling, probing, and visualizing were as described by Porter et al. (1991, 1994). For photomicroscopy, the chromosome preparations were illuminated at 436 nm, with regions of hybridization identified with a yellow color and the remainder of the chromosome counterstained in orange.

Tucker et al. (2016) was followed in using masculine specific epithets with *Aspidoscelis*. Following Dessauer et al. (2000), *A. tigris marmoratus* was treated as a subspecies, rather than a species separate from *A. tigris*. Ancestral genomes within unisexual species of hybrid origin follow Reeder et al. (2002), which reviews in detail alternative hypotheses, in part owing to different opinions in taxonomy.

Consistent with the taxonomy at the time, Reeder et al. (2002) recognized subspecies of *A. inornatus*, including *A. i. arizonae* and *A. i. pai*, which are extremely similar genetically (see also Dessauer and Cole 1989). Ward and Cole (1986) examined *A. i. arizonae*, which is not recognized as a subspecies today, whereas *A. i. pai*, which has similar NORs, was used in this study. Reeder et al. (2002) also treated the genetically similar (Dessauer and Cole 1989) *A. burti burti* and *A. burti stictogrammus* as subspecies, but today these are treated as separate species (Walker and Cordes 2011). Specimens of *A. stictogrammus* processed for this study did not produce results, so the closely related *A. burti* was used as a surrogate in this report. Also, genetically very similar to *A. stictogrammus* are members of the *A. costatus* complex (see also Dessauer and Cole 1989), such as *A. costatus griseocephalus*, which were used in this study, and *A. c. barrancorum*, the latter of which has yet to be extensively compared genetically with other members of the *A. costatus* complex (but see Fig. 3 in Barley et al. 2019, which associates *A. barrancorum* with the *A. burti* complex).

RESULTS

Bisexual species.—For both of the bisexual species (*A. inornatus* and *A. tigris*) examined by FISH techniques, hybridization was observed on both homologues of a large metacentric pair (Table 1). The NOR-bearing chromosomes of *A. tigris* can be distinguished from those of *A. inornatus* (and other members of the *A. sexlineatus* group) by the location of the rDNA. In *A. tigris*, hybridization occurred on the terminal end of both homologues of the second largest metacentric pair (as previously reported by Porter et al. 1991). In *A. inornatus*, the probe hybridized subterminally on the largest (metacentric) chromosome pair (Fig. 1). This corresponds to the location of secondary constrictions and AgNORs found by Ward and Cole (1986). One individual of *A. tigris* (MSB: Herp: 72571) examined for AgNOR activity consistently showed only one AgNOR, indicating that in that individual, transcriptional activity was suppressed in one homologue (Table 1). Ribosomal DNA was present on both homologues in this individual (Table 1, Fig. 1). *Aspidoscelis burti* and *A. costatus* were examined for AgNOR activity, and both of these diploid bisexual species exhibited two AgNORs (Table 1).

Table 1. Number of rDNA sites (determined by fluorescence *in situ* hybridization) and AgNOR activity (determined by silver staining) for four diploid bisexual species of *Aspidoscelis*. *Aspidoscelis burti* serves as a surrogate to the genetically similar ancestral species *A. stictogrammus*.

Species	Specimen	# rDNA sites (cells examined)	# AgNORs (cells examined)
A. burti (BUR)	AMNH R-131435	--	2 (10)
A. costatus (COS)	AMNH R-131440	--	2 (2)
A. inornatus (INO)	AMNH R-136830	2 (1)	--
A. tigris (TIG)	MSB:Herp:72572	2 (1)	--
	MSB:Herp:72571	2 (3)	1 (13)
	MSB:Herp:72570	2 (1)	--
	TNHC H-88142	2 (1)	--

Diploid unisexual species.—The diploid unisexual *A. neomexicanus* includes one haploid genome derived from *A. tigris*, and one derived from *A. inornatus*. rDNA was observed on both the *A. tigris* chromosome (in a terminal location) and the *A. inornatus* chromosome (in a subterminal location) in *A. neomexicanus* (Table 2, Fig. 1). However, in all four individuals, the NOR site on the *A. tigris* chromosome hybridized more strongly to the probe than did the site on the *A. inornatus* chromosome. Silver staining of *A. neomexicanus* (one individual) revealed that the subterminal *A. inornatus* NOR site was consistently transcribed and the *A. tigris* site also was active in a minority of cells (Table 2, Fig. 1).

Aspidoscelis tesselatus is a diploid unisexual with an *A. tigris* × *A. septemvittatus* genome. In *A. tesselatus*, the rDNA probe hybridized more strongly to the *A. septemvittatus* chromosome than to the *A. tigris* chromosome (Table 2, Fig. 1). Silver staining consistently showed gene expression for only the rDNA on the *A. septemvittatus* chromosome (Fig. 1).

Triploid unisexual species.—Four unisexual triploid species of *Aspidoscelis* were examined (Table 3). Based on AgNOR data, *A. exsangis* and *A. velox* consistently showed rDNA transcription activity in only one of the three chromosomes expected to carry rDNA (Table 3, Fig. 2). FISH revealed that these species showed one strong chromosomal location of rDNA. In a cell from one individual (AMNH R-136873) of *A. exsanguis*, two additional sites showed weak hybridization to the rDNA probe (Table 3). In the triploid *A. velox*, the rDNA probe showed a strong signal in one chromosome, but also hybridized weakly to one (rarely two) additional chromosomes (Table 3, Fig. 2). Because the NOR-bearing chromosomes of the triploid species are indistinguishable, it cannot be determined which ancestral chromosome is active.

In all individuals of *A. sonorae* and *A. uniparens* examined, two AgNORs were observed in a subterminal location (Table 3, Fig. 2). In three individuals of *A. uniparens*, the rDNA probe located strong rDNA sites on one large biarmed chromosome, weaker hybridization on another large biarmed chromosome, and even more faint (but consistent) hybridization on a third (Table 3, Fig. 2). One specimen (UTEP H-13700) showed rDNA on just one chromosome (Table 3).

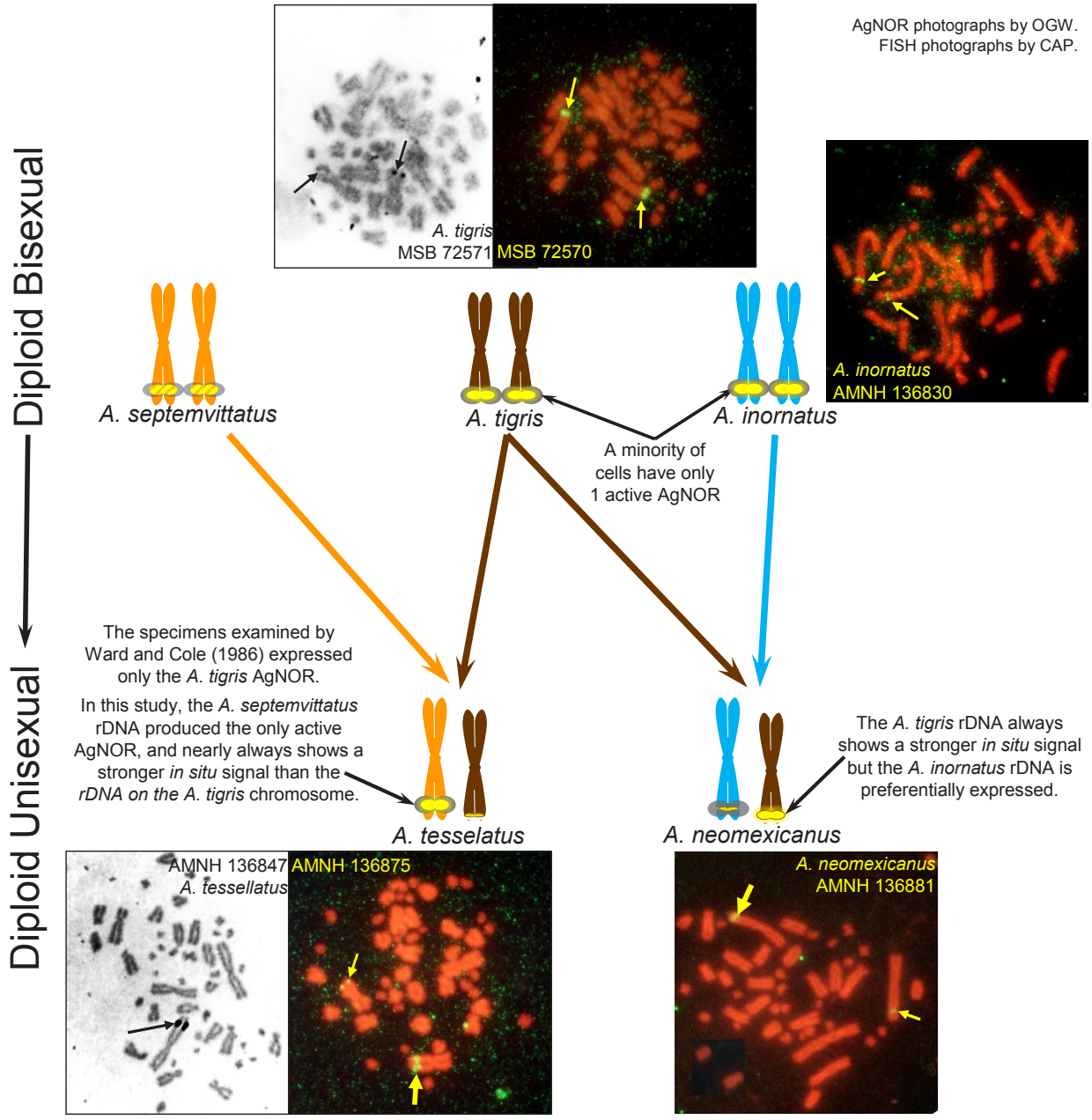

Figure 1. Derivation of NOR-bearing chromosomes in diploid hybrid-derived species of *Aspidoscelis*, with data on presence and activity of ribosomal DNA from this study and Ward and Cole (1986). The colors of the chromosome drawings are intended to suggest typical coloration of the ancestral lizard species. The silver-gray represents the common locations of an actively-transcribed NOR as revealed by silver staining. Solid yellow represents the presence of rDNA as revealed by FISH. A narrow dark yellow site represents weak hybridization. Yellow with diagonal hatching represents the inferred position of rDNA in cases where *in situ* hybridization has not been performed. Activity of rDNA in *A. septemvittatus* has not been directly tested with silver staining but is inferred from results in other species of the *A. sexlineatus* group and from results in *A. tesselatus*. In the photographs, AgNORs (dark spots) and sites of rDNA hybridization (yellow) are indicated by arrows. A thick arrow indicates a site that consistently shows a comparatively stronger signal. For both *A. tesselatus* and *A. neomexicanus*, the maternal parent of the original F1 hybrid was *A. tigris marmoratus*.

Table 2. Number of rDNA sites (determined by fluorescence *in situ* hybridization) and AgNOR activity (determined by silver staining) for diploid unisexual species of *Aspidoscelis*. *Aspidoscelis neomexicanus* has one haploid genome of *A. tigris* (TIG) and one of *A. inornatus* (INO). *Aspidoscelis tesselatus* has one haploid genome of *A. tigris* and one of *A. septemvittatus* (SEP).

		rDNA		AgNORs	
Species	Specimen	# rDNA sites (cells examined)	Chromosome of strongest hybridization	# AgNORs (cells examined)	Chromosome of active AgNOR
A. neomexicanus (TIG × INO)	AMNH R-136881	2 (5)	TIG	1 (2)	INO (2 cells)
				2 (1)	both (1 cell)
	AMNH R-136882	2 (1)	TIG	--	--
	AMNH R-136884	2 (1)	TIG	--	--
	AMNH R-136885	2 (1)	TIG	--	--
A. tesselatus (pattern class C) (TIG × SEP)	AMNH R-136875	2 (2)	SEP	1 (1)	SEP
	AMNH R-136876	2 (1)	SEP	1 (15)	SEP
	AMNH R-136877	2 (2)	SEP	--	--
	AMNH R-136878	2 (3)	SEP	1 (15)	SEP
	AMNH R-136879	2 (4)	SEP	1 (15)	SEP
A. tesselatus (pattern class D) (TIG × SEP)	AMNH R-136880	2 (3)	SEP (2 cells) equal (1 cell)	1 (15)	SEP
A. tesselatus (pattern class E) (TIG × SEP)	AMNH R-136845	2 (3)	SEP	1 (15)	SEP
	AMNH R-136846	2 (2)	SEP	1 (15)	SEP
	AMNH R-136847	2 (3)	SEP	1 (25)	SEP

Table 3. Number of rDNA sites (determined by fluorescence *in situ* hybridization) and activity (determined by silver staining) for triploid unisexual species of *Aspidoscelis*. *Aspidoscelis exsanguis* has one haploid genome each from *A. inornatus* (INO), *A. stictogrammus* (STI), and *A. scalaris* (SCA). Genomes present in the other triploid species are indicated by the same abbreviations. See Reeder et al. (2002) for discussions of alternative hypotheses for ancestry of these triploid species.

		Number of rDNA sites			
Species	Specimen	Strong hybridization	Weak hybridization	Cells examined	# AgNORs (cells examined)
A. exsanguis (STI × INO × SCA)	AMNH R-136831	1	--	3	1 (15)
	AMNH R-136832	1	--	2	1 (5)
	AMNH R-136833	1	--	3	1 (6)
	AMNH R-136834	1	--	3	1 (1)
	AMNH R-136873	1	--	4	1 (20)
		1	2	1	
A. sonorae (INO × STI × STI)	AMNH R-136837	2	--	3	2 (2)
	AMNH R-136838	2	--	2	2 (17)
A. uniparens (INO × STI × INO)	AMNH R-136888	1	2	1	--
	AMNH R-136890	1	2	1	2 (2)
	AMNH R-136892	1	2	2	2 (3)
	UTEP H-13700	1	--	2	--
A. velox (INO × STI × INO)	AMNH R-136820	1	1	3	1 (1)
	AMNH R-136827	1	2	1	--
	AMNH R-136839	1	1	1	1 (20)
		1	--	1	
	AMNH R-136840	1	1	1	1 (6)
	AMNH R-136841	1	1	3	--
	AMNH R-136842	1	1	2	--
	AMNH R-136852	1	1	1	1 (13)
		1	2	1	

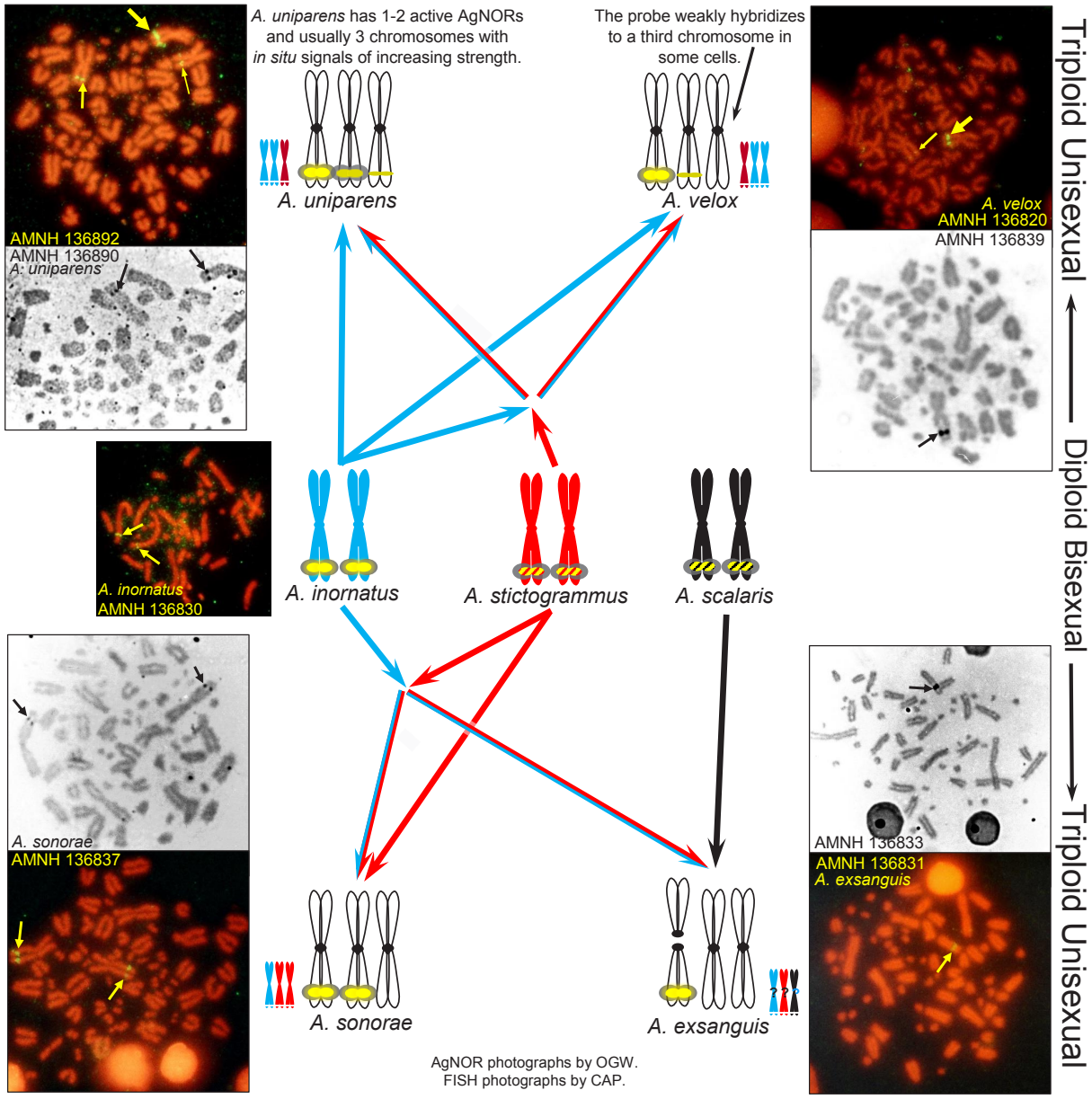

Figure 2. Derivation of NOR-bearing chromosomes in triploid unisexual hybrid-derived species of *Aspidoscelis*, with data on presence and activity of ribosomal DNA from this study and from Ward and Cole (1986). Drawings of NOR-bearing chromosomes of the ancestral bisexual species are in the center, with the triploid unisexual hybrids above and below. *A. stictogrammus* is an ancestral species of the triploid unisexual forms. The AgNORs and rDNA were not examined in *A. stictogrammus* or *A. scalaris*, but *A. burti* and *A. costatus* are expected to be genetically similar, and plausible surrogates suggesting the expected chromosomal locations in *A. stictogrammus*. Colors are as described in Figure 1. The small colored chromosomes indicate the derivation of the ancestral genomes. One NOR-bearing chromosome in *A. exsanguis* has undergone a centric fission, but the species origin of the chromosome is undetermined. Ribosomal data are illustrated on colorless chromosomes because the species derivation of a chromosome with a particular ribosomal pattern cannot be determined from current data. AgNOR data for *A. inornatus* are from Ward and Cole (1986). The illustrations assume that AgNORs are found on the same chromosome as the strongest rDNA signal, but that has not been directly tested. See Reeder et al. (2002) for discussions of alternative hypotheses for ancestry of these triploid species.

DISCUSSION

Distribution and expression of rDNA in hybrid-derived unisexual lineages of Aspidoscelis.—Ward and Cole (1986) examined specimens of *A. tesselatus* (pattern class E) from the Trans-Pecos region of Texas and observed active expression only in the terminal (*A. tigris*) NOR. The *A. septemvittatus* NOR was inactive in both specimens they examined. Ward and Cole (1986) attributed this inactivity to nucleolar dominance, that is the suppression of transcription in one chromosome when a dominant NOR is present on another chromosome.

The AgNOR data from this study differ from Ward and Cole (1986) in that the subterminal *A. septemvittatus* NOR is consistently the only one expressed in the genome of *A. tesselatus*. This was the case in all eight specimens examined, sampled from populations in northern New Mexico, including pattern classes C, D, and E (see Taylor et al. 2003 for discussion of pattern classes in *A. tesselatus*). The difference in expression of ribosomal genes between specimens of *A. tesselatus* from Texas and from New Mexico may represent postformational divergence between geographic populations of a single clone (see Densmore et al. 1989 and Taylor et al. 2003 for discussion of *A. tesselatus* origins) or variation in expression within populations. However, distinct hybrid origins resulting in lineages of *A. tesselatus* with different patterns of rDNA expression cannot be ruled out.

The FISH analysis of *A. tesselatus* from New Mexico documented rDNA on two chromosomes, but (excepting one cell) with a stronger signal on the *A. septemvittatus* chromosome, in the same location as the expressed AgNOR (Table 2, Fig. 1). This would suggest that the expression of a single NOR in *A. tesselatus* is not solely the result of suppression of transcription but also may result from a reduction of rDNA copies in the *A. tigris* genome within the hybrid genome of *A. tesselatus*. The I-19 rDNA probe was derived from a rodent and is known to hybridize across a wide variety of vertebrate taxa (Baker et al. 1992), including squamate reptiles (Porter et al. 1994). It would be expected that extreme sequence divergence (perhaps to the point of rendering the ribosomes nonfunctional) would be required for the probe to fail to identify ribosomal gene sequences. However, a reduction of gene copy number would be expected to cause a weaker hybridization signal such as was observed in the transcriptionally inactive chromosome of *A. tesselatus*.

In *A. neomexicanus*, rDNA was found in both ancestral genomes but more frequently expressed only in the *A. inornatus* chromosome (Table 2, Fig. 1). These observations are consistent with Ward and Cole (1986) in showing incomplete dominance in the expression of *A. inornatus* over *A. tigris* NORs in this species. Paradoxically, the NOR that is consistently active (see Table 2 and Ward and Cole 1986) is on the chromosome with reduced rDNA repeats, whereas the NOR with a greater number of repeats is often inactive. The frequent absence of an *A. tigris* AgNOR in *A. neomexicanus* appears to be strictly at the transcriptional level as proposed by Ward and Cole, rather than the result of a reduction in the DNA repeats.

Different patterns of rDNA distribution and expression were found in the four triploid unisexual species examined (Fig. 2). Each of the triploid species is presumed to have originated having three chromosomes with repeated rDNA sequences. In three of four specimens examined, *A. uniparens* has retained sufficient rDNA on all three chromosomes to consistently detect with the I-19 probe. The three NOR-bearing chromosomes of *A. uniparens* each show progressively weaker rDNA signals (Fig. 2). In one specimen, only one chromosome showed evidence of rDNA. In this study, two AgNORs were observed (Table 3), whereas Ward and Cole (1986) found one active NOR in 91% of cells in their specimens of *A. uniparens* and no active NORs in the remaining cells.

Aspidoscelis velox has the same chromosomal complements as *A. uniparens* (i.e., two haploid genomes of *A. inornatus* and one of *A. stictogrammus*). However, this triploid parthenogenetic form has only one AgNOR and one chromosome showing a strong *in situ* signal (Fig. 2). One and occasionally two additional chromosomes weakly hybridize to the rDNA probe (Table 3, Fig. 2). The one chromosome that shows the AgNOR is likely to be the same chromosome with a strong rDNA signal. But since silver staining

and *in situ* hybridization were not performed on the same cells, it cannot be determined if that is actually the case, and the nucleolar activity of *A. neomexicanus* indicates the potential shortcoming in making such a presumption.

Aspidoscelis sonorae expresses two AgNORs on two metacentric chromosomes that have equally strong hybridization to the rDNA probe (Table 3, Fig. 2). This suggests that the rDNA sequences have been eliminated from one of the three ancestral genomes of *A. sonorae* and are fully expressed in the other two.

Only one AgNOR and one rDNA site are consistently seen in *A. exsanguis* (Table 3, Fig. 2). Both appear on an acrocentric chromosome that is derived from one of the three NOR-bearing chromosomes of the ancestral species (Cole 1979). Ward and Cole (1986) reported an AgNOR on the same chromosome. *Aspidoscelis exsanguis* includes chromosomes from three ancestral species, but it has not been determined which chromosome has undergone the centric fission that produced the acrocentric, and hence it is not known which ancestral rDNA sequences are retained in this species.

Evolution of repetitive DNA in hybrid-derived unisexual species.—The process of concerted evolution acts to ensure that repetitive DNA elements maintain sequence homogeneity, rather than each gene copy diverging from the others as they accumulate different mutations (Hillis et al. 1991). In diploid bisexual species, two genetically dissimilar haploid genomes are combined with the formation of a zygote. However, since concerted evolution has already been acting on all the repetitive elements in the population, the repeated copies combined in a diploid bisexual species are expected to be identical, or nearly so.

The situation is very different in the case of hybrid-derived species, where disparate families of gene elements are abruptly brought together within a composite "permanent hybrid" genome. This often results in mechanisms such as biased gene conversion, gene inactivation, or gene elimination (Ward and Cole 1986; Hillis et al. 1991; Porter 1994; Elder and Turner 1995; Kovaric et al. 2005; Zozomová-Lihová et al. 2014) that result in a gradual and progressive increase in the effective uniformity of repetitive genes or their products. Selection seems to favor uniformity in the RNA components of ribosomes, and this process often begins within a few generations of the hybridization event, possibly starting immediately after hybridization (Kovaric et al. 2005; Zozomová-Lihová et al. 2014).

In the case of *Aspidoscelis*, various degrees of this process can be observed. In both of the diploid unisexual species that were examined, rDNA transcription was limited to an AgNOR on one chromosome, thus ensuring uniformity of the rRNA used in synthesizing ribosomes. In the triploid species where three NORs would be expected, only two (in *A. sonorae* and *A. uniparens*) or one (in *A. exsanguis* and *A. velox*) were found to be transcribed and expressed. In the case of the two triploid species that express two AgNORs, those NORs plausibly could be on chromosomes originating from a single ancestral species.

The results of this study document that homogeneity of ribosomal elements can result from elimination of gene copies and that the pattern of AgNOR expression cannot be explained solely in terms of nucleolar dominance. In all unisexual hybrid forms that were examined, the ribosomal genes showed various degrees of elimination, having one or two rDNA sites reduced or eliminated. A reasonable interpretation of these data is that selection is favoring the inactivation of some NORs, perhaps to ensure homogeneity of the ribosomes. Once the process of inactivation proceeds, selection would also favor elimination of the inactivated gene sequences. In *A. exsanguis*, this process appears to have gone to an expected conclusion, with rDNA from only one of the ancestral species remaining in the genome, and only a single AgNOR being expressed on the same acrocentric chromosome.

It should be noted also that there is variation in expression (strong versus weak hybridization) of the rDNA sites, especially in *A. uniparens* and *A. velox* (Table 3, Fig. 2). This fits expectations for lineages of unisexual *Aspidoscelis*. When a non-lethal mutation occurs in a germ cell, it results in a new clonal lineage, which is not affected by random assortment and recombination. Mutations may accumulate, but in each clonal lineage they occur differently and independently from other clones of the same ancestry going back to the original F_1 hybrid, resulting in genetic and phenotypic differences such as seen in the pattern classes of *A. tesselatus*.

It is possible that selection favors the rDNA of one ancestral genome over another (as in the parthenogenetic gecko *Heteronotia binoei*, Hillis et al. 1991). Alternatively, it may be that uniformity of the rDNA is favored in *Aspidoscelis*, but that random factors determine which rDNA sequences are inactivated or eliminated in each clone. The latter case could result in a geographic mosaic of different NORs being active in different clones and different populations.

Future studies should determine if unisexual populations have continued the trend of eliminating inactive rDNA repeats, and if this process is adaptive or stochastic. Studies of large samples representing widespread localities of individual unisexual species should test whether different clones show different degrees or patterns of inactivation and elimination of rDNA.

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LITERATURE CITED

Arnheim, N. 1979. Characterization of mouse ribosomal gene fragments purified by molecular cloning. Gene 7:83–96.

Baker, R. J., M. Maltbie, J. G. Owen, M. J. Hamilton, and R. D. Bradley. 1992. Reduced number of ribosomal sites in bats: evidence for a mechanism to contain genome size. Journal of Mammalogy 73:847–858.

Barley, A. J., et al. 2019. Complex patterns of hybridization and introgression across evolutionary timescales in Mexican whiptail lizards (*Aspidoscelis*). Molecular Phylogenetics and Evolution 132:284–295.

Cole, C. J. 1979. Chromosome inheritance in parthenogenetic lizards and evolution of allopolyploidy in reptiles. Journal of Heredity 70:95–102.

Cole, C. J., L. M. Hardy, H. C. Dessauer, H. L. Taylor, and C. R. Townsend. 2010. Laboratory hybridization among North American whiptail lizards, including *Aspidoscelis inornata arizonae* × *A. tigris marmorata* (Squamata: Teiidae), ancestors of unisexual clones in nature. American Museum Novitates 3698:1–43.

Cole, C. J., C. W. Painter, H. C. Dessauer, and H. L. Taylor. 2007. Hybridization between the endangered unisexual Gray-Checkered Whiptail Lizard (*Aspidoscelis dixoni*) and the bisexual Western Whiptail Lizard (*Aspidoscelis tigris*) in southwestern New Mexico. American Museum Novitates 3555:1–31.

Cole, C. J., H. L. Taylor, D. P. Baumann, and P. Baumann. 2014. Neaves' Whiptail Lizard: The first known tetraploid parthenogenetic tetrapod (Reptilia: Squamata: Teiidae). Breviora 539:1–19.

Cole, C. J., et al. 2017. The second known tetraploid species of parthenogenetic tetrapod (Reptilia: Squamata: Teiidae): Description, reproduction, comparisons with ancestral taxa, and origins of multiple clones. Bulletin of the Museum of Comparative Zoology 161:285–321.

Densmore, L. D., III, J. W. Wright, and W. M. Brown. 1989. Mitochondrial-DNA analyses and the origin and relative age of parthenogenetic lizards (Genus *Cnemidophorus*). II. *C. neomexicanus* and the *C. tesselatus* complex. Evolution 43:943–957.

Dessauer, H. C. and C. J. Cole. 1984. Influence of gene dosage on electrophoretic phenotypes of proteins from lizards of the genus *Cnemidophorus*. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 77:181–189.

Dessauer, H. C., and C. J. Cole. 1986. Clonal inheritance in parthenogenetic whiptail lizards: Biochemical evidence. Journal of Heredity 77:8–12.

Dessauer, H. C. and C. J. Cole. 1989. Diversity between and within nominal forms of unisexual teiid lizards. Pp. 49–71 in Evolution and ecology of unisexual vertebrates (R. M. Dawley and J. P. Bogart, eds.). Bulletin 466, New York State Museum, Albany, New York.

Dessauer, H. C., C. J. Cole, and C. R. Townsend. 2000. Hybridization among Western Whiptail Lizards (*Cnemidophorus tigris*) in southwestern New Mexico: Population genetics, morphology, and ecology in three contact zones. Bulletin of the American Museum of Natural History 246:1–148.

Elder, J. F., Jr. and B. J. Turner. 1995. Concerted evolution of repetitive DNA sequences in eukaryotes. Quarterly Review of Biology 70:297–320.

Hillis, D. M., C. Moritz, C. A. Porter, and R. J. Baker. 1991. Evidence for biased gene conversion in concerted evolution of ribosomal DNA. Science 251:308–310.

Kovarik, A., et al. 2005. Rapid concerted evolution of nuclear ribosomal DNA in two Tragopogon allopolyploids of recent and recurrent origin. Genetics 169:931–944.

Lutes, A. A., D. P. Baumann, W. B. Neaves, and P. Baumann. 2011. Laboratory synthesis of an independently reproducing vertebrate species. Proceedings of the National Academy of Sciences of the United States of America 108:9910–9915.

Neaves, W. B. 1969. Adenosine deaminase phenotypes among sexual and parthenogenetic lizards in the genus *Cnemidophorus* (Teiidae). Journal of Experimental Zoology 171:175–183.

Neaves, W. B., and P. S. Gerald. 1968. Lactate dehydrogenase isozymes in parthenogenetic teiid lizards (*Cnemidophorus*). Science 160:1004–1005.

Porter, C. A. 1994. Organization and chromosomal location of repetitive DNA sequences in three species of squamate reptiles. Chromosome Research 2:263–273.

Porter, C. A., M. W. Haiduk, and K. de Queiroz. 1994. Evolution and phylogenetic significance of ribosomal gene location in chromosomes of squamate reptiles. Copeia 1994:302–313.

Porter, C. A., M. J. Hamilton, J. W. Sites, Jr., and R. J. Baker. 1991. Location of ribosomal DNA in chromosomes of squamate reptiles: systematic and evolutionary implications. Herpetologica 47:271–280.

Reeder, T. W., C. J. Cole, and H. C. Dessauer. 2002. Phylogenetic relationships of Whiptail Lizards of the Genus *Cnemidophorus* (Squamata: Teiidae): A test of monophyly, reevaluation of karyotypic evolution, and review of hybrid origins. American Museum Novitates 3365:1–61

Taylor, H. L., C. J. Cole, H. C. Dessauer, and E. D. Parker, Jr. 2003. Congruent patterns of genetic and morphological variation in the parthenogenetic lizard *Aspidoscelis tesselata* (Squamata: Teiidae) and the origins of color pattern classes and genotypic clones in eastern New Mexico. American Museum Novitates 3424:1–40.

Taylor, H. L., et al. 2001. Natural hybridization between the teiid lizards *Cnemidophorus tesselatus* (parthenogenetic) and *C. tigris marmoratus* (bisexual): Assessment of evolutionary alternatives. American Museum Novitates 3345:1–64.

Tucker, D. B., et al. 2016. Methodological congruence in phylogenomic analyses with morphological support for teiid lizards (Sauria: Teiidae). Molecular Phylogenetics and Evolution 103:75–84.

Vrijenhoek, R. C., R. M. Dawley, C. J. Cole, and J. P. Bogart. 1989. A list of the known unisexual vertebrates. Pp. 19-23 in Evolution and ecology of unisexual vertebrates (R. M. Dawley and J. P. Bogart, eds.). Bulletin 466, New York State Museum, Albany, New York.

Walker, J. M. and J. E. Cordes. 2011. Taxonomic implications of color pattern and meristic variation in *Aspidoscelis burti burti*, a Mexican whiptail lizard. Herpetological Review 42:33–39.

Ward, O. G. and C. J. Cole. 1986. Nucleolar dominance in diploid and triploid parthenogenetic lizards of hybrid origin. Cytogenetics and Cell Genetics 42:177–182.

Zozomová-Lihová, J., et al. 2014. When fathers are instant losers: homogenization of rDNA loci in recently formed *Cardamine* × *schulzii* trigenomic allopolyploid. New Phytologist 203:1096–1108.

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APPENDIX

Voucher specimens are cited by their individual catalog numbers as follows: AMNH (American Museum of Natural History); MSB (Museum of Southwestern Biology, University of New Mexico); TNHC (Texas Natural History Collections, University of Texas at Austin); and UTEP (University of Texas at El Paso Biodiversity Collections).

A. burti burti (1).—MEXICO: Sonora (AMNH R-131435).

A. costatus griseocephalus (1).—MEXICO: Sonora (AMNH R-131440).

A. exsanguis (4).—USA: New Mexico, Guadalupe County (AMNH R-136873); Arizona, Cochise County (AMNH R-136831–136834).

A. inornatus pai (1).—USA: Arizona, Coconino County (AMNH R-136830).

A. neomexicanus (4).—USA: New Mexico, Hidalgo County (AMNH R-136882, 136884–136885); New Mexico, San Miguel County (AMNH R-136881).

A. sonorae (2).—USA: Arizona, Cochise County (AMNH R-136837–136838).

A. tesselatus pattern class C (5).—USA: New Mexico, San Miguel County (AMNH R-136875–136879).

A. tesselatus pattern class D (1).—USA: New Mexico, San Miguel County (AMNH R-136880).

A. tesselatus pattern class E (3).—USA: New Mexico, Cibola County (AMNH R-136845–136847).

A. tigris marmoratus (1).—USA: New Mexico, Doña Ana County (TNHC H-88142).

A. tigris punctilinealis (3).—USA: New Mexico, Hidalgo County (MSB:Herp:72570-72572).

A. uniparens (4).—USA: Arizona, Graham County (UTEP H-13700); New Mexico, Hidalgo County (AMNH R-136888, 136890, 136892).

A. velox (7).—USA: Arizona, Navajo County (AMNH R-136820); Arizona, Coconino County (AMNH R-136827); New Mexico, Cibola County (AMNH R-136839-136842); New Mexico, McKinley County (AMNH R-136852).

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Systems-level Change in an Educational Institution Impacts a Community and its Students and Families

Anette J. Carlisle and Russell Lowery-Hart

"Nothing in biology makes sense except in the light of evolution" ~Theodosius Dobzhansky

"Nothing in life makes sense except in the light of evolution." ~J. Taylor Carlisle, M.D.

Abstract

Herein, two educators, one professionally employed and a second operating as a "volunteer", discuss problems and solutions faced by students and educational institutions in mid-sized communities. A hypothetical student, "Maria", is used to represent the day-to-day difficulties confronting a typical student in such a setting. A case study is presented to serve as a model for addressing these issues. One of the authors attributes her problem-solving abilities to early life training in evolutionary biology.

Key words: collective impact, community college, demographics, educational attainment, poverty

Introduction

This contribution to the Robert J. Baker memorial volume demonstrates how training in evolutionary biology can lead to research at a systems-level in educational institutions in mid-sized communities, rather than just in laboratory or field work. One of us (AJC) trained in Dr. Robert J. Baker's research laboratory as an undergraduate and then a graduate student, with Dr. Baker serving as the Chair for my Master of Science degree. Years later, when evaluating the community of Amarillo and the region of the Panhandle, I applied the skills learned from laboratory and field-based research, along with the cultural expectation of questioning the *status quo*, of exploration, and of problem-solving, that I gained from Dr. Baker and fellow grad students. With co-author RLH and others, a community coalition was formed to address significant social, cultural, and economic challenges that, although not unique to the Texas Panhandle, provide a community petri dish of innovative approaches that could be replicable across the state and nation. Modifications of behaviors within and among existing educational institutions generated a systems-level change that improved success for a variety of individuals. The efforts implemented at Amarillo College are highlighted as a case study.

The emphasis for this project stemmed from the fact that declining indicators of community growth (based on data from the United States Census Bureau) concerned some local Amarillo citizens. Spearheading an initiative that became Panhandle Twenty/20, we worked with others to pull together a loose coalition of concerned community leaders and citizens, beginning with the question, "Who is planning for the future of Texas?" It was evident that most educational institutions and organizations were operating as silos, with no one examining the long-term and interconnected well-being of the region or the state. At the community launch of Panhandle Twenty/20 in 2003, Dr. Steve Murdock, State Demographer, projected that the region would become poorer and less educated, based on current trends (Murdock 2013). Local participants worked together to create a plan of action, and many of those recommendations are still in play today, rippling through new and varied partnerships, internal changes at institutions, and cultural shifts in the way educational organizations operate.

Panhandle Twenty/20 pulled together diverse entities to address a common goal in an attempt to

create large-scale social change around root causes of issues. The use of data drove much of the work, so research (Panhandle Twenty/20 2014) was a big part of creating community support for change. We began working under the umbrella of the Amarillo Area Foundation in 2005.

The first item tackled was educational attainment. This metric was low for Texas, and numbers in the Panhandle were lower (Table 1). Higher levels of educational attainment would provide a better workforce for the community, which in turn would attract and retain higher paying jobs to build a stronger economy for all residents. Our first community study launched in 2006, with RLH serving as facilitator for dozens of community study workshops and AJC serving as study coordinator and program director. Involving hundreds of dedicated community participants and organizations, we released our first report in 2007, *The Panhandle Imperative: Economic Implications of Educational Attainment* (Carlisle et al. 2007).

A number of actions were embraced from this initial report, including: the establishment of the Texas Panhandle P-16 Council, to better align all educational institutions in the region to improve access and success for all students; internships for students; career-focused courses in middle schools, to enable students to expand their options and to better plan coursework; and an increased level of interaction among the various stakeholders that did not exist before. Bringing together educators, workforce, elected officials, the business community, and nonprofits created the opportunity for new collaborations, better understanding of each other's challenges and roles, and stronger and more deliberate interactions to move more students through the system to success.

As one of the biggest barriers to educational attainment, poverty became a focus of Panhandle Twenty/20 in 2010. Poverty expert Dr. Donna Beegle was engaged to educate our community about the challenges faced by individuals living in the warzone of poverty. Bus tours of our neighborhoods, followed by a community summit, and numerous poverty trainings for individuals, institutions, churches, elected officials, and educators created an awareness and understanding that allowed us to better meet the needs of our low-income students and families. Ultimately, this allowed us to help students move through our educational systems more successfully and thereby out of poverty.

As the community continued to focus on the issues of educational attainment and poverty, Amarillo was selected as one of two sites in Texas to receive the Bill and Melinda Gates Partners for Postsecondary Success grant. Over time, this partnership effort became our No Limits No Excuses initiative that maintains a systems approach to moving families into and through education by removing poverty and access barriers. Perhaps no single institution has more successfully done this than Amarillo College.

Table 1. Percent educational attainment levels in Amarillo, Potter, and Randall counties, and the Texas Panhandle, as compared to state and national data (2000 U.S. Census data).

Educational attainment	Nation	Texas	Panhandle region	Potter County	Randall County	Amarillo
<9th Grade	7.5	11.5	10.0	10.8	2.5	7.5
9th to 12th	12.1	12.8	14.2	18.1	8.0	13.2
High School	28.6	24.9	28.2	29.3	23.3	25.6
Bachelor's	15.5	15.6	12.2	8.7	19.6	13.7
Graduate	8.9	7.6	5.5	4.8	9.3	6.8

Case Study—Amarillo College

After years of work with Panhandle Twenty/20 on educational attainment, Amarillo College (AC) knew it must respond systemically and culturally. In 2014, Amarillo College used community demographic data, student secret shoppers, and student success data to develop the AC Culture of Caring. The AC Culture of Caring focuses on structured accelerated learning systems and systemic student support approaches and embraces systems change by integrating accelerated learning, predictive-analytics, and wrap-around social services to overcome student poverty barriers. Through student secret shoppers, focus groups, and interviews, AC asked our students to redesign the college for them, fitting their needs, not our own.

This initiative became the core of AC's No Excuses 2020 strategic plan and the central focus of AC's student success agenda. The data were overwhelming: our community was becoming less educated, and without significant improvements, would become poorer as a result. By listening to our students and joining with Panhandle Twenty/20, AC understood that improving educational attainment and reducing poverty would require significant cultural change based on powerful relationships with students and the use of predictive analytics to build student support systems. By learning who our students actually are, listening to their educational dreams, and understanding the reality of their educational attainment and completion journey, we transformed our institution and ourselves. We believe college completion and career readiness drive our efforts at AC, regardless of job descriptions or reporting structures. We love the student we have, rather than the student we thought or wished we had enrolled. By establishing caring systems to address poverty barriers, students would succeed.

When our students identified the Top 10 Barriers to their completion, none were based in academics. Each barrier was life-related rather than academic. Initially, we were so focused on academic success that we failed to realize the more powerful and debilitating barrier—poverty. In response, we developed our theory of change—if AC removes poverty barriers in an accelerated learning platform, within a relational/loving culture, students will succeed academically and graduate with the skills and credentials needed to earn a living wage.

Amarillo College implemented a cohesive system designed to address the Top 10 Barriers to Student Success. The Advocacy & Resource Center (ARC) is the hub of AC's system designed to eradicate poverty barriers. The ARC now collaborates with more than 60 local non-profits, who have federal/state/private funds, to help our students meet life's basic needs. Through these partnerships, students have access to funds for transportation, housing, utilities, and childcare.

Today, AC has evolved from a theory of change to an action-oriented initiative focused on three key components: data analytics and predictive modeling; creating systems supported by on-campus programs and community agencies to eradicate student poverty barriers; and accelerated learning systems that meet a student's academic and non-academic needs. When we love our students to success, they graduate, transfer, and/or enter the workplace.

A Need for a Systems Change

Once we learned who our students "were", we had a better picture of how AC could respond to their needs. Throughout this case-study, we refer to "Maria" who is representative of our typical student at AC (Fig. 1). "Maria" is female, attends part-time, receives financial aid, requires developmental education, and is a first-generation student. She is full Pell eligible and works two part-time jobs, must take out a loan for her education, and is still unable to pay for her living expenses and the cost of a college education. To succeed and ultimately graduate, Maria will have to go hungry on some days, forgo some books for classes, potentially drop a course and prolong her time-to-degree, and ration her transportation. Maria must hope and pray her child does not get sick, her car does not break down, gas prices do not surge, and she keeps her fingers crossed that her utilities and rent do not increase.

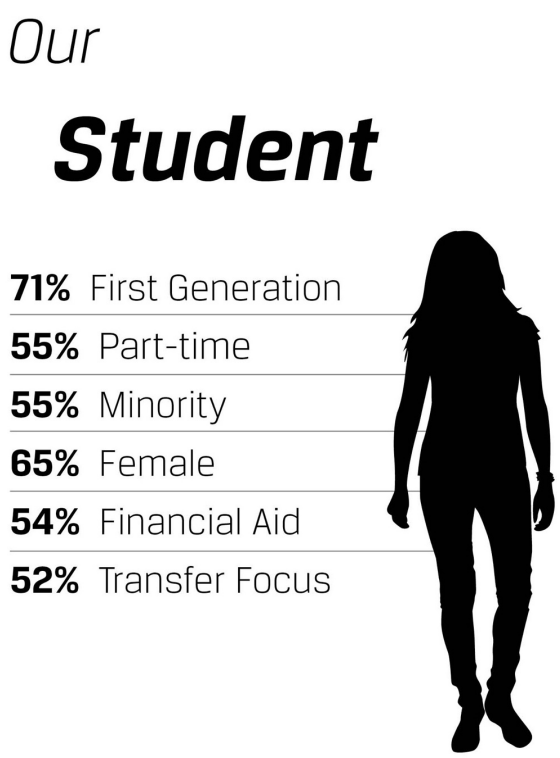

Figure 1. Typical Amarillo College student "Maria".

In 2015, AC established a dynamic five-year strategic plan, No Excuses 2020, with a clear vision of 70% completion. We restructured our entire college experience around our students' needs and removing the barriers to their success. We asked the students to write the No Excuses 2020 college values, reflecting our resolve to being a higher education institution focused on meeting students' needs, facilitating student success and completion, and creating a "data ecosystem" to drive change.

For AC, the most powerful No Excuses impact on students is two-fold: culture shift and relationship. Students told us what their "ideal" college looked and felt like. As a college, we knew we needed to embrace a culture of good service and intense caring. Consequently, students were asked to identify values, with a focus on articulating the first-generation college student experience.

The new AC Culture of Caring values—Caring through WOW, Fun, Innovation, Family, and Yes—are not typical "higher education" lingo. These values are written into every employee job description and merit pay evaluation. The first week of classes, we put these values on significant display by placing employees all over our campuses—from parking lots to classrooms—to ensure that our students have someone to walk them to their class, take them to the bookstore, and guide them through advising. We host monthly No Excuses communication meetings, open to all college employees, to discuss the status of our No Excuses 2020 strategic plan, retention initiatives, data discussion, etc. No Excuses meetings are live-streamed to all campus sites, allowing faculty and staff in our regional campuses to participate. No Excuses 2020 uses key performance indicators to gauge the success of AC. We track performance data for all students and then aggregate student data based on demographic indicators. AC also tracks student participation in student success intervention activities via a scanning system.

What We Learned

Data analytics and predictive modeling are the drivers of our No Excuses poverty initiative. Predictive modeling led AC to create an at-risk profile for all incoming first-time-in-college (FTIC) students in fall 2017. Students most at-risk (level 3 and higher) were connected to institutional systems designed to eradicate poverty barriers, increase student learning and completion, and provide non-academic student support by faculty. Students in the pilot had an increased retention rate of nearly 10% over previous cohorts. Further, AC's data ecosystem was the driving component of the AC Culture of Caring. To rethink higher education, AC created a robust data-centric environment focusing on transparency and using data to improve, not penalize. We believe data should be reviewed consistently so employees have an accurate picture of how AC is performing and fulfilling its mission.

Over a five-year span, AC noted a 75% growth rate in first-generation students graduating with a degree/certificate (and a 21% growth rate in students transferring to complete a higher degree), as well as

a 64% growth rate in Pell students graduating (29% growth rate in transfers). Amarillo College course success rates (pass rates) increased 110% for Hispanic students, 112% for African-American students, and 108% all students. The AC Culture of Caring illustrated that by addressing student poverty barriers, students will complete and graduate from AC or transfer to another four-year university. By changing our perspectives regarding higher education, AC has seen a 60% growth rate increase in our student attainment/transfer rates over the last five years (from 30% to 48%; Table 2).

In 2016, AC opened the ARC and saw a 205% increase in student visits during the second year and a 149% increase the following year. Through intensive Achieving the Dream (ATD) coaching, AC was able to scale the poverty interventions and social services systems for impact. Because of our ATD experience, our poverty work moved from a boutique intervention to a college-wide, scaled initiative that includes all employees and more than 50% of our entire student body (Fig. 2). The retention rate for students accessing ARC services is on par with all AC students and

Table 2. Student number and graduation rate and growth increase, by total number, first generation, and Pell recipient status.

3-Year Graduation Rate	Total Number Grads	% Total Grads	% Growth Increase	% First Gen Grads	% Growth Increase	% Pell Grads	% Growth Increase
Fall 2011 Cohort	1,702	13%	--	12%	--	14%	--
Fall 2012 Cohort	1,661	13%	--	13%	--	14%	--
Fall 2013 Cohort	1,607	15%	--	14%	--	15%	--
Fall 2014 Cohort	1,529	20%	--	19%	--	20%	--
Fall 2015 Cohort	1,393	22%	69%	21%	75%	23%	64%

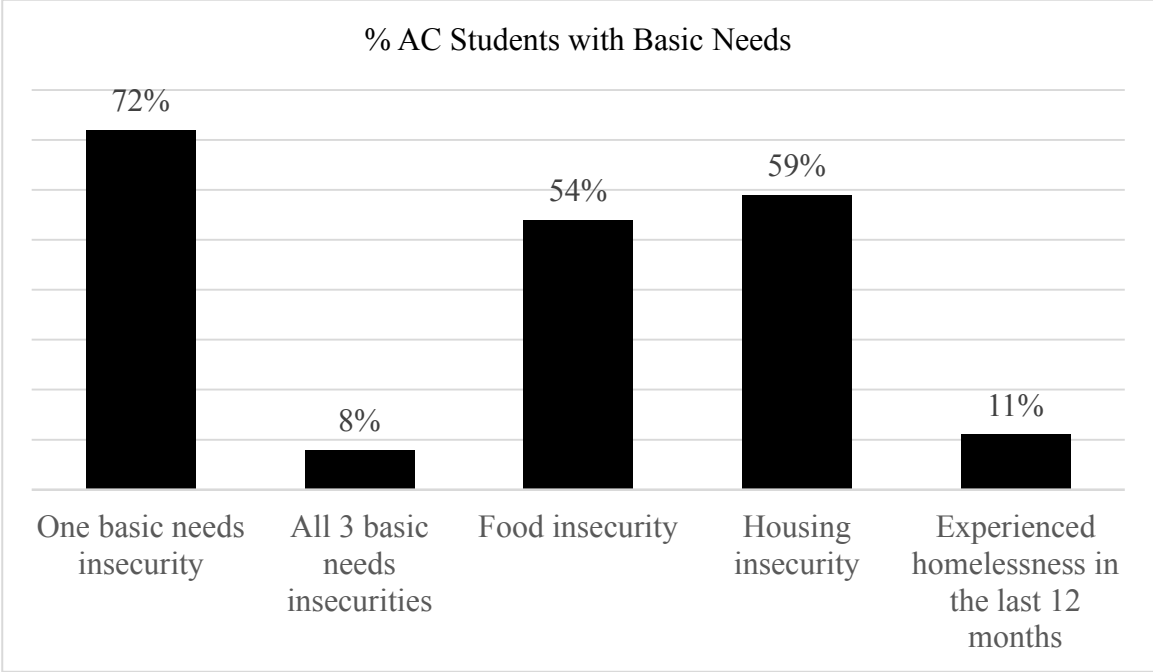

Figure 2. Percentage of Amarillo College students experiencing food and housing insecurity.

slightly below the value for all students for fall-to-fall persistence. In 2017–2018, AC provided ARC services to 21% of our non-dual credit enrollment. Yet, we know we need to provide social services to a minimum of 50% of our enrollment as our first-generation and Pell recipient students increase.

In 2018, Amarillo College was highlighted in a case study by Sara Goldrick-Rab and Clare Cady (Goldrick-Rab and Cady 2018), in *The Atlantic* (Bombardieri 2018), and by the Lumina Foundation (Lumina Foundation 2018). The case study led by Dr. Goldrick-Rab and the Hope Center at Temple University in fall 2018 demonstrated that systemically addressing poverty barriers increases academic success and educational attainment. Funded by The Trellis Foundation, this study analyzed and developed a playbook for other institutions by focusing on two key components of AC's No Excuses poverty initiative: 1) eradicating a student's poverty barriers leads to academic success; and 2) education is the path to economic revitalization.

Further, we learned that data from the AC Culture of Caring opened the doors for faculty, staff, and students to engage in meaningful dialogue, programs, and support services to ensure student success. Amarillo College's environment does not shame individuals living in poverty; instead, we acknowledge the struggles of these students and work to assist them with overcoming barriers. Consequently, AC hosted a Poverty Summit in June 2018, for more than 40 institutions across the country, and two summits in May 2019, with 65 institutions attending. The AC Poverty Summits mentored other schools to identify appropriate data, incorporate their students' voices, and design their own systems to address life barriers.

Finally, AC publishes an annual "No Excuses 2020 Report Card" which describes the typical AC student, "Maria", and how AC should strive to address "Maria's" needs as a student. Amarillo College continuously improves our processes, procedures, and policies to support "Maria" and all students in fulfilling their educational goals. Amarillo College employees never expected to become advocates for removing poverty barriers; however, our systemic approach to addressing poverty is driving the success of all our students and changing their lives.

Concluding Remarks

When we address poverty barriers for students, we are equalizing the attainment of a college degree or university transfer for all students. A student's socioeconomic status should never hinder their educational dreams. Unfortunately, the financial barriers of life do not prioritize the completion of a degree. The longer "Maria" takes to complete her degree, the more costly it becomes, and the less likely "she" is to graduate. With an intentional system to graduate every student in three years, AC innovatively accelerates "Maria's" learning.

This project started as a collective impact focus by Panhandle Twenty/20, creating innovative approaches to facing community realities to better understand our Amarillo, and beyond. When institutions across communities integrate their missions, efforts, resources, and work, those communities succeed. The success of AC students is a direct example of why collective impact efforts are so critical—and effective. In addition, as with problem solving in evolutionary biology, educators should know their research subject, reject long-standing dogma, construct hypotheses based on observations, and trust the data.

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REFERENCES AND SOURCES

Bombardieri, M. 2018. Colleges are no match for American poverty. The Atlantic, 30 May 2018. https://www.theatlantic.com/education/archive/2018/05/college-poor-students/560972/

Carlisle, A., R. Bara, and A. O'Brien, for Panhandle Twenty/20. 2007. The Panhandle Imperative: Economic Implications of Educational Attainment in Amarillo and the Texas Panhandle. http://www.panhandle2020.org/reports/

Goldrick-Rab, S., and C. Cady. 2018. Supporting community college completion with a culture of caring: A case study of Amarillo College. Wisconsin Hope Lab, University of Wisconsin-Madison. https://hope4college.com/wp-content/uploads/2018/09/wisconsin-hope-lab-case-study-amarillo-college.pdf

Lumina Foundation. 2018. Amarillo College accepts 'no excuses' in pursuing its anti-poverty mission. Focus, Lumina Foundation, Fall 2018. https://focus.luminafoundation.org/amarillo-college-accepts-no-excuses-in-pursuing-its-anti-poverty-mission/

Murdock, S. H. 2013. Population change in Texas and the Panhandle: Implications for current and future socioeconomic development. Panhandle Twenty/20 presentation, Amarillo, Texas, 17 September 2013. http://pbs.bento.storage.s3.amazonaws.com/hostedbento-prod/filer_public/kacv/PDF/P2020_Presentation_091713_PDF.pdf

Panhandle Twenty/20. 2014. Includes links to various data used in analyses over time, including Dr. Murdock's relevant data. http://www.panhandle2020.org/data/

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Robert J. Baker's Commitment to and Influence on Athletics: Fan, Donor, and Faculty Athletics Representative of Texas Tech University

Jennifer Brashear, Meredith J. Hamilton, Judi Henry, Ronald Phillips, and David J. Schmidly

Abstract

During Robert Baker's nearly 50 years at Texas Tech University, he contributed in many ways and at many levels to Texas Tech's mission as a public research university. A portion of his contributions, in both time and money, were focused on the Athletic Department through his role as a fan, a donor, and Texas Tech's Faculty Athletics Representative (FAR) to the Big 12 Conference and the National Collegiate Athletic Association. As the FAR, his role was to be the eyes and ears of the university president. He also was a driver and enforcer of athletic-related compliance policy, a mentor to other FARs, and an advocate for student-athlete welfare.

Key words: athletics, Faculty Athletics Representative, sports fan, Texas Tech University

Introduction

Robert J. Baker arrived at Texas Tech University (TTU) in 1967. He was young for a new faculty member, just 25 years old. He wasted no time in taking on his role as a teacher and top-notch researcher. As time progressed he began contributing to the university in leadership and administrative roles, and perhaps one of his favorite roles involved the Athletics Department at Texas Tech University. Robert was not just a fan, he was a passionate, vocal fan, and that big voice of his carried a long way across a basketball court. He also supported Texas Tech athletics by becoming a donor, and in 2001 President David Schmidly appointed him as Texas Tech's Faculty Athletics Representative (FAR), making him the liaison between the university president and the Athletics Department. He held that position until 2008. During Robert's tenure as FAR, he was an active participant in formulating critical compliance policy for both the university and the Big 12 Conference.

The Lady-Raider Fan

In 1992, two of Robert's former Ph.D. students, Meredith Hamilton and Ron Van Den Bussche, returned to Lubbock to work in Robert's laboratory as postdoctoral fellows. They were quickly persuaded to purchase Lady Raider basketball tickets, and they celebrated each victory with Robert and Robert's wife, Laura. In 1993, Robert and Laura traveled to the Women's Final Four tournament in Atlanta, leaving their son Bobby, who was five years old at the time, in the joint care of Meredith, Ron, and Dr. Cynthia (Cindy) Dunn. Bobby spent most of this time with Cindy, but he did watch the championship game with Meredith and Ron. Bobby was a serious Lady Raider fan and at times could be just as loud as Robert. When the game was over, Meredith and Ron took Bobby to campus—primarily because they wanted to see what a campus looked like when a team wins a national championship, but also because they wanted Bobby to experience it as well. It was a bit rowdy for a five-year-old, but he hung in there for 20 minutes or so. A couple of days after their return to Lubbock, Robert and Laura invited Cindy, Meredith, and Ron to their house to watch the DVR tape of the championship game. At one point during the game, Cindy yelled "freeze the tape, back up!" Sheryl Swoopes had just scored, and the camera switched from the court to the crowd. There was Robert, in his Final Four t-shirt, fist pumping after the made basket. Coach Sharp could not have asked for a more loyal fan than Robert.

There was rarely a time when Robert sat quietly during a Lady Raider basketball game. Remembered by all, Robert sat right behind the team's bench, cheering them on at every home game and at as many away games as he could. His reputation at Lady Raider basketball games will live on as being passionate, rowdy, animated, and very vocal toward the officials, particularly if he believed they made an unjustified call against the Lady Raiders. Meredith remembered Robert used to call the refs squirrel bait (nuts), until 6-year-old Bobby started doing it.

At times, Dr. Judi Henry thought the coaches feared what the officials might do with the noise that was coming from behind their bench. At the time, Robert was serving as FAR, and Judi was the Administrator for women's basketball. Judi remembered her role sometimes was to keep Robert in line as they sat behind the team bench during road games. Judi said, "He was a great man, very supportive, but was just very intense, but we had some great road trips and a bunch of great wins."

Coach Sharp recalled how he loved to voice his opinions, especially to the officials regularly during basketball games. She said, "I always thought it was interesting, because when an official at our home games would make a bad call, I really never had to say anything because he had my back, and he was making enough noise for all of us right behind our bench." Coach Sharp mentioned that "he was just a great friend of the program and went with us through thick and thin, and it was always interesting to hear his perspective and how he viewed things as a fan and those kinds of things." Coach Sharp was always interested to hear his perspective on a game, and the things that intrigued him the most about it were things that the average fan wouldn't even be interested in. Robert was always very interested in the motivation of the players and how the coaches and coaching staff attempted to get them all on the same page and how they tried to impact them as people; he was always a big part of the discussions.

Coach Sharp said he was brilliant, and the things that he worked on around the world were pretty amazing. He was passionate about sharing his biology experiences with everyone in athletics. Coach Sharp always recruited in the summer, and Robert would regularly tell her to put recruitment on hold for a little bit and travel to Brazil with him. He wanted to show her the caves and the bats that lived in them. Coach Sharp said he always thought it would be really cool to show her how the bats lived and roosted at the top of the caves. He just wanted to share his world with everyone, and they were fine with that. Coach Sharp said Robert and she had a lot of those kinds of discussions that would go from bats and rats to how a coach can motivate a kid to play 40 minutes of a basketball game, and everything in between.

Coach Sharp recalled another story that will always endear him to her. They were in Norman, Oklahoma, on their way to the OU arena to play a game. Everyone on the bus was getting game ready. It was quiet, and the mood was serious. Coach Sharp said she was "probably the world's worst. I was really focused and ready, and I didn't want a lot of distractions to my thought process." She wanted to set the tone of being in game mode on the way to the arena. So, as they were driving along, only a quarter mile away from the arena, Robert tapped her on the shoulder and said, "Did you know, at this moment we are going over the biggest highway of mice migrating from Canada to Mexico? This is the road that they take, and they are probably right below us as this very moment." And, all of a sudden, Coach Sharp was thinking, "Oh my god!", and she couldn't get that image of traveling mice out of her head. Obviously, it was a joke to lighten the serious mood.

"We lived it—Lady Raider basketball—with him," said Coach Sharp. Dr. Schmidly added, "He was very proud of the Lady Raiders, and I don't think they ever had a bigger fan." After the Lady Raiders won the national championship in 1993, Robert called Coach Sharp and invited her to go to lunch to talk about his memories of the playoffs that year, the games that the team played that season, and how excited he was about the program. Coach Sharp said, "He was hooked. I think it's safe to say at that point, he wanted to be inside our program as much as he could."

Who Robert J. Baker was to Texas Tech Athletics

NCAA major infractions case and appointment as FAR—The NCAA began an investigation of Texas Tech Athletics in the spring of 1997. The case mainly involved the improper certification of student-athlete eligibility. In August 1998, Texas Tech's Athletic Department was put on a four-year probation for violating National Collegiate Athletics Association (NCAA) rules. The institution had to forfeit wins, scholarships, awards, and money given by the NCAA.

Dr. Donald Haragan retired from the presidency during the period of probation. Dr. David Schmidly became the new president and appointed Robert to be the FAR for Texas Tech in 2001. Dr. Schmidly was not only a professional colleague of Robert's, but they also were really great friends, and he trusted that Robert would make a great FAR for the university and Athletic Department.

Dr. Schmidly had decided, as he watched the NCAA investigation unfold, that as President, he was going to have somebody in the FAR position that he knew well, that knew him, and that wouldn't mince words or keep him in the dark if he saw something wrong. Dr. Schmidly had known Robert since 1967 when Robert had interviewed for a job at Texas Tech. Dr. Schmidly said he knew, "Robert was cantankerous enough to not be browbeat down." He knew Robert might ruffle a few feathers, because he had ruffled Dr. Schmidly's, but he knew that if Robert found something out at 10 o'clock on a Sunday night, that Robert would call him as soon as possible, and Dr. Schmidly would answer because he knew it was important. Dr. Schmidly said he developed a huge measure of comfort in knowing that Robert was there looking out for TTU's interests.

Robert was asked to be FAR when he was at the pinnacle of his research and scholarly publication career, and Dr. Schmidly knew he didn't have time to take on the new challenge. Robert initially told Dr. Schmidly, "Oh Jesus! I just don't know." Dr. Schmidly replied, "Robert, look—nobody around here loves athletics more than you do. Sometimes you just have to step up and do something for the institution." Dr. Schmidly said that Robert thought about it overnight and came back the next day and then told Dr. Schmidly something he will never forget. Robert informed him bluntly, "I'll do it. But by God, you better not ever cut my feet out from under me, and you're going to have to support me."

Dr. Schmidly promised to support him and told him that he wanted Robert to sit down and work with the athletic staff and coaches, and that if he saw something wrong, Robert was to let him know immediately. Then he emphasized, "If we know something is wrong, then we are going to deal with it. We don't want to end up in the same situation that Don [Haragan] was in."

Dr. Schmidly and Robert worked together as President and FAR until Dr. Schmidly's departure from Texas Tech in 2003. To Dr. Schmidly's knowledge, he doesn't recall any major infractions during his tenure as President or during Dr. Baker's tenure as FAR. Dr. Schmidly did note that Robert had some run-ins with certain coaches, and that Robert would call him complaining about what those certain coaches were doing. Dr. Schmidly also recalls getting calls from coaches frustrated with Dr. Baker and that as President, he was always stuck in the cross-fire.

"But, nonetheless," Dr. Schmidly added, "I thought Robert was a great choice for the position because I knew he loved Texas Tech, and he was totally devoted to it. He loved the athletic program and he was obstinate enough to never be intimidated."

Gerald Myers, Texas Tech Athletics Director at the time Robert was FAR, thinks he was the most involved FAR the Athletic Department has ever had. He said that Robert visited the department at least twice a week or more. Robert worked with Jennifer Brashear, Senior Associate Athletic Director–Compliance and Strategic Initiatives, to make sure Texas Tech had one of the best compliance programs in collegiate athletics. Gerald believes Robert and Jennifer were able to develop a model program that is second to none as far as compliance is concerned.

Jennifer Brashear worked closely with Robert from the time he became FAR until he resigned from the position in late 2008. During the time that Jennifer worked with Robert, Texas Tech had numerous

legendary, dominating, and passionate coaches who were tough, but who could also be a handful at times. So, strategically, Jennifer and Robert had to find a way to work around these coaches' personalities to get their work done and make sure Texas Tech was following all NCAA compliance rules.

"With Coach Bobby Knight, Coach Marsha Sharp, and Coach Mike Leach, we had a few different situations, and whether it was a review, a possible rules violation, or a question about the eligibility of a student-athlete, I always knew that Dr. Baker had my back. He respected and protected the relationship I needed to have on a daily basis with these coaches. There's no one I'd rather be in a foxhole with," Jennifer said.

In addition to working with these legendary coaches, Jennifer and Robert also worked together on a number of high-level policies, both inside and outside of athletics. Jennifer and Robert developed or significantly revised enforcement and eligibility certification policies and had an opportunity to have a voice at the university level, as the academic council reviewed the definition of good academic standing for the university. There were also a few lunches with Dr. Whitmore, former TTU president, in which they discussed the TOEFFL (English proficiency) requirements for international admissions, and the frustration Texas Tech coaches sometimes had when recruiting international student-athletes. Jennifer said, "There are just times you're facing something for the first time and you look to guidelines or parameters and you don't have any, so you're in an uncharted territory. So, we would work through whatever was in front of us the best we could, and then we made sure to use that experience to put guidelines in place so that the next time there was a similar question, we wouldn't be starting from scratch."

Jennifer remembers one day she walked into work in the Athletic Department and got a call from the Athletic Director's office. There was an article published on the website of the Fort Worth Star Telegram accusing one of the Texas Tech coaches of giving cash to a student-athlete. She called Dr. Baker and they immediately started an investigation. Then, over the next several weeks, they worked around the clock with Ronald Phillips and Victor Mellinger in the President's office conducting interviews, and trying to determine the facts and whether there was any truth to the allegations. Jennifer said, "I just couldn't imagine not having Dr. Baker by my side and working in the best interest of Texas Tech during that time."

A policy driver—Gerald observed that Robert was involved with other organizations outside of Texas Tech, but his main focus was Tech. "He made sure that we had policies and procedures in place," added Gerald. Gerald recalls a policy that Robert worked hard on creating and getting passed. Gerald said, "I remember one issue particularly after he became FAR. We had an issue with some faculty members because our athletes missed a lot of class time when they traveled to events —especially a team like baseball. You know they may be gone for half the week, and sometimes 4–5 days, on trips. Robert met with the faculty governing body to discuss an attendance policy." Gerald does not recall the particular details of the procedure by which the policy was developed, but notably an attendance policy was established that not only covered student-athletes but all students from all programs. When students traveled, either for athletic competition or an academic-related event in which they were representing Texas Tech, the new policy permitted them to make up the missed work. "I don't think we had a concrete policy on that previously. There were some faculty members who felt like some student-athletes might be trying to take advantage of being able to come back and make up work or make up a test that they missed while they were on school trips," Gerald recalled.

A leader in the Big 12—Ronald Phillips, Chief of Compliance and Security for Texas Tech and former Chief of Staff, said, "When the NCAA was developing the position of Faculty Athletics Representative, I believe they had Robert Baker in mind. He possessed every characteristic that we think of when identifying what makes an individual a good FAR. His character and integrity were astonishing. Doing the right thing was simply a part of who he was. He never hesitated to think of what difficulties might arise from making tough decisions to do the right thing."

Being the FAR for Texas Tech Athletics meant he was a representative in the Big 12 Conference, as well. Gerald said Robert was a leader. He said, "Everything he was involved in, he became a leader, and he was definitely one of the leaders in the Big 12 FAR Committee." During Robert's time as FAR, Gerald

and Robert had joint meetings with the other FARs in the conference and Gerald could tell Robert was highly respected by the other FARs. Gerald recalls that those faculty representatives went to Robert for advice, guidance, and leadership.

Robert was known as a guy who always seemed to have an opinion about everything that came up at the Big 12 conference meetings. He was very vocal in expressing his concerns and ideas and always made sure Texas Tech was well represented in any meeting he attended. He made certain that people knew Texas Tech was a leader in the conference.

Don Green, a former FAR and an Emeritus Distinguished Professor of Chemical and Petroleum Engineering for the University of Kansas, said, "The Big 12 Conference had a rule, called the intraconference transfer rule (which is still in effect). If a student-athlete transferred from one school to another within the conference, then he/she not only had to sit out for a year, as required by NCAA rules, but also lost a year of eligibility. I opposed this rule as being very unfair to the students. Bob, however, strongly favored the rule as he felt such transfers could damage the conference by causing bad feelings between conference members. We had several lively 'discussions' with Bob and me on opposing sides, but they were always very professional. In the end his side won the day, in part due to his persuasive arguments."

Another former Big 12 FAR, Mike Holen, the Emeritus Dean of Education at Kansas State, observed: "I had the privilege of serving with Robert as a Big 12 FAR for about a decade. He was an especially valued colleague—always down to earth, frank, blunt, and on point, while showing a keen humor and a deep sense of caring for the interests of the student-athletes of our conference's institutions. Beyond our 'official' responsibilities, Robert was a favorite for all of us at informal, often wine-infused gatherings. Knowing his expertise and interest, my wife and I once told him we wished we had more bats for insect control around our home in Manhattan, Kansas. Robert indicated that was no problem—he would simply send us a container of bat guano to smear on bat houses to attract and hold them. We actually never took him up on the offer, but the ensuing banter about the underlying psychology of someone who chose to become an international expert on bats and could access his collection of bat excrement has always invoked fond thoughts of our friend."

Connie Dillion, who served as FAR for the University of Oklahoma when Robert was the Texas Tech FAR, particularly remembers Robert's many passionate arguments about very important issues during their FAR meetings. Although she said she can't recall the specific issues, she said, "I do know there were many, including the causes of a few athletes whose names we recognize today who got their chance because Robert took up their cause." Connie also said, "I'll never forget Robert's commitment to our kids and to our integrity."

A Mentor and Leader—When Robert retired from the FAR position, Brian Shannon was appointed to step in for Robert following Robert's recommendation to then-President Whitmore. It was a unique situation for Brian, because he had the opportunity to shadow Robert for several months as Robert was transitioning out of the position. During this period, Brian shadowed Robert in a wide array of meetings, and was able to get to know and begin working with Jennifer and her staff. Brian said, "It was great getting to follow Robert and to be able to see first-hand his passion and unyielding support for student-athletes." Brian remembers how great it was to learn from Robert, and to recognize that he was all about doing the right thing. Brian was grateful for all of the tips that Robert passed on to him. One of his tips was simply to ask a lot of questions, but a tip that Brian might not have known about had it not been for Robert's emphasis, was "to let Jennifer and her counterpart in academics know that if they needed to have the FAR as a foil in making a hard decision, to be free to simply state that the FAR said we have to do it this way."

During this period of transition, and just as school was starting in the fall of 2008, they had to review a matter involving eligibility of a high-profile, talented student-athlete. Brian noted that there some "very interested individuals at high levels within the university" who were aware of the review, and that they had to work thoroughly and quickly to gather necessary information. Brian added that with Robert's leadership, they were successful in gathering the needed information to be confident to conclude that the individual had not violated any rules and would be eligible to participate.

Being able to watch Dr. Baker in that process dealing with high-level campus administrators, along with a highly passionate coach, was a valuable learning experience for Brian.

Brian also remembers attending the NCAA Division I FAR annual meeting with Robert in September 2008, shortly before Brian was going to officially succeed Robert as the FAR for Texas Tech. Brian said, "It was great to see the kind of respect and warm friendship that FAR peers from the Big 12, and even beyond, had for Robert." Brian got to experience first-hand Roberts' views and comments about numerous items, including a couple of Big 12 waiver hearings via conference call. Brian said, "It was a great opportunity to learn the process, but also to see Robert's passion in action. What a role model!"

Brian also recalled that when he was trying to decide whether or not to accept the FAR position, Robert had significantly underestimated or even misrepresented the amount of time necessary to do a good job in the position, but Brian has found it to be a worthwhile and rewarding experience and believes that Robert undoubtedly felt the same way.

Brian also emphasized how strong of a campus leader Robert was, and fondly remembers having had the opportunity to observe him work through the process of handling policies and challenges within the university and through the NCAA. Even after Robert retired from being FAR, Brian would call him for advice when he encountered issues that were new to him, and Robert was always gracious about sharing his time with Brian.

Gerald said, "He was a natural leader; he always emerged in a leadership role in about any group or organization that he got involved with. I remember also in our capital campaign, which was the university's capital campaign as well as an athletic campaign to raise funds to build and improve facilities, Robert's job was to raise a certain amount of money for Chancellor Montford. And, Robert did it! He went out and raised what I recall was close to one million dollars for that particular project across campus that Montford had assigned to him. He raised a lot of that money from faculty and staff on campus, as well as through other support from donors. He is one of only a few people who could have got that done like he did."

A friend—Ronald Phillips described Robert as "an interesting mix of personality traits. He was hardworking and expected those he was working with to keep pace with him, which was no easy task. He demanded quality work from himself and those working with him. He was hardened steel and made those around him stronger at the same time. I have never met a more loving and caring person. He could be blustering at you one minute, then hugging you the next, and both with the same level of emotion and devotion. Dr. Baker was an incredibly intellectual person. He knew so much about so many different things. As intelligent and accomplished as he was, he was a humble man. His humility was entertaining in that he could make fun of himself without any reservation."

Connie Dillion recalled many great memories of Robert outside of athletics. For example, she remembered many conversations Robert would have with Dave, her spouse, about bats, other mammals, hunting, fishing, and coaches. One particular story she remembered from Robert was about him taking Bobby Knight on a hunting trip. Connie said, "Robert and Knight stopped for lunch at a burger place in a tiny, rural West Texas town. Knight went to the counter and ordered a malt. The young girl recognized with awe the figure before her and somewhat anxiously took his order. A few minutes later, she brought out the malt and the imposing coach took a sip. He gave the youngster a bit of a surly look and asked, 'Did you make this malt, young lady?' Taken aback, she said that, yes, she had made the malt. The coach replied, 'Well, young lady, that is the BEST malt I ever tasted.' Robert beamed as he described the young girl's reaction and said, 'That youngster will remember that moment for the rest of her life!' As we know, Robert wasn't afraid to stand up to anyone, but lest we forget, neither was he afraid to see the good in everyone."

Jo Potuto, the long-time FAR for Nebraska said, "Robert was one of the funniest people I have ever known. Certainly, that was the case when he knew he was being funny. But often he was funny when he wasn't trying. He always spoke with such passion, and such conviction. His appreciation of rules was epic, so much so that he never saw a good reason for waiving one, especially the rule for intraconference transfers. Don Green (KU FAR at the time), by contrast, never met an intraconference transfer of which he disapproved. His exchanges with Robert were wonderful.

Neither missed an opportunity to argue for his position. Good natured of course, but protracted and vigorous. Every intraconference transfer request the FARs considered triggered yet another battle between Robert and Don. If anyone wondered why FAR meetings typically ran longer than the ADs and SWAs, Robert and Don were part of the reason."

Jo also recalled a time when Robert had a diabetic episode and passed out in the lobby of a hotel at which the FARs were all staying for a Big 12 Conference meeting. Jo noted, "Gerald Lage, the former FAR at Oklahoma State, was walking by. At the time, Gerald was being treated for the cancer that ultimately killed him. Gerald looked down at Robert, lying on the hotel floor; he did not express sympathy, did not express empathy, and did not offer his help. Instead, he opined that Robert's fainting episode was interesting, given that Gerald was the one with the terminal disease. (Yes, we FARs are a morbid crew)."

Jo, however, mentioned, "perhaps my favorite Robert Baker story was a conversation I had with him about a year after Bobby Knight was hired as the Head Men's Basketball Coach at Texas Tech. At the time, Mike Leach was the Head Football Coach. All FARs, indeed all athletic administrators, know that it sometimes can be difficult working with head coaches. They understandably are focused and centered on their programs. They often, particularly in season, have difficulty stepping back and seeing the bigger picture. They are accustomed to being in charge and setting the agenda in their programs. Whether their reputations are deserved or not, Leach and Knight stand out among head coaches as "difficult." I told Robert it must be a challenge being a FAR with two such head coaches to work with. Robert responded that Coach Marsha Sharp was more difficult than the other two. I told Robert I marveled at his stamina! I loved working with Robert. I loved getting to know him. He was a real presence, and certainly memorable. I miss him."

Don Green recalled a time where he was not involved but heard a story from Robert. Don said, "During the time that Coach Knight was coach of your basketball team, he and Bob became friends, at least Bob thought so. He had Coach Knight out to his ranch to fish and hunt as I recall, and so they interacted a fair amount. And then a possible violation of NCAA rules involving basketball was reported. (I do not recall the specific issues of the possible violation.) Bob had the responsibility of looking into it and felt it required that he talk to Coach Knight and his staff. This, to put it mildly, upset the coach to the point that serious disagreements arose between the two men. I think the friendship ended. I do not know how the issue was resolved, but hearing about it led me to respect Bob even more than before. He was determined to do his job even if it required confronting a friend and a person with considerable power at the university. He was a man of integrity."

Robert owned a ranch in Patton Springs, and Gerald Myers remembers all of the times he and Robert spent together, fishing and hunting, at his ranch and all of the times Robert made Gerald feel welcome there. Gerald said, "He was good with a shotgun; he shot dove and quail and was very good."

Robert was not only a leader, mentor, friend, husband, and dad, he was also a golden retriever breeder. Jennifer said Robert had a rule about the puppies that his dogs produced. She said, "Any females that were born in each litter would be given only to those who were special to Robert." Robert would have a request list and depending on how many females were born in each litter determined how many on the list got a dog from him. Jennifer heard about that list and asked if she could get on it. So, she did, and that litter had two females and Jennifer and her family received a golden retriever puppy from Robert. Jennifer and her family named their dog Golden, and she said Golden is still with them today.

"Robert is a man who was dear to my heart, both personally and professionally, and he taught me so many things," Jennifer said. Jennifer said Robert gave her a lot of tips and advice that molded, shaped, and helped her become who she is today. She would always soak up his words of wisdom and if he didn't offer advice, Jennifer would ask Robert what he thought about a certain situation she encountered and what she could have done better. She said he was just incredible, and he shared his experiences and knew exactly how they were going to handle a situation, what they weren't going to do, how they were going to talk to the people they needed to, and how to leverage relationships so that when they went in to get their business

done, it was already handled. Jennifer said when the two of them were finished with work, they would meet at a Mexican restaurant, and he would always have a chili relleno and a large water, and she would always have the same because she trusted his judgment about good Mexican food and she knew he wouldn't steer her wrong, just like he never did professionally.

"You know, I think he had about as many loyal friends and good friends as anyone I've ever known. I know he taught a lot of doctoral students that thought a great deal of him, and his colleagues respected him and appreciated what he had done in his field of research. He was an unassuming guy, and I think his unselfishness and willingness to help others and to serve the university was really important to him. And, he certainly did that with athletics. He was just a good friend," Gerald said.

John Anderson, a former Associate Athletics Director for Academic Services, described Robert as being a great man, someone who wasn't afraid to stand up to coaches, someone who was always thinking of the best ways to help student-athletes, and someone who always thought of others before himself. John said Robert wanted the best for Texas Tech and made it a better place. John also talked about how Robert was a Texas Tech fan through and through, even when his health wasn't the greatest. Robert still persevered through everything and worked just as hard.

Coach Sharp said, "Robert just really wanted to impact his world a lot, and he had such a great mind." Coach Sharp talked about how she was always so amazed when around him and when she would listen to his conversations. She was amazed by what he would think about and all of the different kinds of things he worked on all the time. Coach Sharp described their relationship as pretty special.

Judi Henry described Robert as being such a bright guy. She talked about how much he cared about the university's student-athletes, the success in the department, and the people in the department. Judi remembered some fun stories about Robert having a glass of wine and a good Cuban cigar.

Ronald Phillips said, "Robert had a profound impact on so many people's lives. He would challenge you to be the best that you can be, and he would support and encourage you to become that person. Dr. Baker is one of those individuals that comes into your life and you are a better person for it. I respected and admired this man. For me, he was a mentor, a colleague, and most importantly my friend."

A FINAL WORD

The quote below is from the introduction to *Cotton Candy on a Rainy Day*—one of Nikki Giovanni's books of poetry. Often times in Robert's lab the conversations veered away from research, grant writing, athletics, and lectures, and one of the favorite topics was to talk about music and poetry. Nikki Giovanni was one of Robert's favorites. He so loved this quote that Meredith Hamilton reproduced it in a piece of embroidery work that was framed and placed on the lab wall by the door. When Robert exited the lab, he often paused, read the quote, straightened the frame, and then headed home. If you knew Robert, you can see the similarities in how he approached life—whether it was in the research lab or the world of athletics.

> "There is always something to do. There are hungry people to feed, naked people to clothe, sick people to comfort and make well. And while I don't expect you to save the world I do think it's not asking too much for you to love those with whom you sleep, share the happiness of those whom you call friend, engage those among you who are visionary and remove from your life those who offer you depression, despair and disrespect." — Nikki Giovanni

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What to Teach? A Course for Undergraduates Majoring Outside of Science

James J. Bull

Abstract

Universities are actively addressing alternative teaching methods. Compared to the question of *how* to teach, less thought is going into *what* should be taught, a burgeoning problem that is stemming from the advances in science and technology and the increased access to information provided by social media and the internet. We cannot teach it all. This paper describes the rationale behind and brief content of a course that the author and a colleague have taught to undergraduate students for the last 30 years. The course is designed for students whose majors lie outside of science. The conceptual foundation of the course is a broad interpretation of the scientific method as a way of solving everyday problems. From a scientist's perspective, this approach to problem solving is the familiar one of merely letting the data inform decisions—evidence-based reasoning. This paper describes a brief structure of the material for the course, with examples, and explains the nature of external assignments for the students. Some challenges with the material are noted. The author attributes his interest in teaching and much of his inspiration to Robert Baker, in whose memory this set of papers is offered.

Key words: course content, non-science majors, teaching methods, undergraduates, universities, what to teach

Our Current Academic Climate

The last couple of decades have been challenging and dynamic ones for US universities. Undergraduate degrees in many disciplines no longer hold the promise of rewarding careers. Increasing costs of tuition and the availability of online courses have reduced enrollments at many institutions. Many universities increasingly employ adjunct faculty to teach and thus offset the high cost of research professors.

More recently, universities have actively started embracing new teaching methods and increased assessment. There is increasing recognition of which types of instruction best contribute to learning (Kuh and Schneider 2008). The lecture style of old is being replaced with 'flipped classrooms' and active or experiential learning, and electronic classroom response systems now allow students to use their phones to answer questions in real time so that instructors can know immediately what is misunderstood (Mazur 1996). The emphasis on how to teach is on the heels of a long-term trend away from various types of 'hands-on' teaching in which labs and field courses have been reduced or eliminated because of expense.

Despite a strong focus on how to teach, on assessment, and on new technologies and methods that facilitate instruction and student learning (Kuh and Schneider 2008), one dimension to undergraduate education that has escaped much introspection is *what* we teach. As the knowledge base or technology of a discipline expands, it seems obvious that curricula should keep abreast of the new. The question of what to teach is being asked in some disciplines (e.g., genetics, Redfield 2012), but not much. But therein lies a dilemma. To prevent degree requirements from ever-expanding, a course must be deleted for every course added. And with the profound increase in understanding the foundations of biology, as a prime example, it is not obvious what to delete—certainly not the foundations on which understanding 'the new' depends. There is so much

happening that the curriculum can cover only a fraction of what might be relevant. The question has no simple answer, and the problem grows. In many cases, degree plans 'bloated' to the point that 4-year degrees became 5-year degrees (solved at Texas state universities by the Texas Higher Education Coordinating Board mandating a 120 credit-hour cap on degrees).

The teaching of science to students who are not science majors presents a unique case of science education, one that does not face the usual dilemma. Texas mandates two natural science and technology courses in the same discipline for all undergraduate degrees; other states have similar requirements. The standard approach, and the path of least resistance, has been to offer special science courses for these 'non-majors' that consist of thinned versions of the courses for majors. This path is followed without much forethought or much rationale. There is no expectation that the curriculum of students majoring outside of science will continue building on the knowledge they receive in their few science courses, so the standard approach is a dead end, leaving them with little information they can apply elsewhere. This reality does mean there is a freedom to choose the content of these courses unlike that of most other courses in degree plans.

A Non-traditional Course for Non-majors

For the last two and a half decades, I have taught a biology course for 'non-science' majors that differs from most other science courses taught at my university. The course was originally developed with a colleague, Craig Pease, who in 1998 moved the course to Vermont Law School (and modified it to be suitable for graduate students), while I maintained it at the University of Texas. The goal has been to teach students how to use evidence to draw conclusions and to understand the limitations of those conclusions—to teach them a structured framework for how to think, not what. The material lends itself to conceptual analysis and problem-solving far more than to memorization, and the examples come from everyday life rather than formal science. There is no public documentation of the content of this course, so below, I offer an introduction to its substance.

The audience has included majors in Liberal Arts, Business, and Law. This is an important group of students to educate about the basics of how to use evidence when informing decisions, because they will assume careers outside of science with important roles in society. They can make the difference between a society that understands and uses science or one that ignores and abhors it. A populace that understood this material would be far less vulnerable to the emotional appeals and scams that so easily sway attitudes these days. Craig Pease and I thus share a common interest with efforts to educate the public about pseudoscience (Shermer and Gould 2002; Hecht et al. 2018), but our emphasis on why one might want to use the method is substantially different.

Scientific Method Basics

The core material is tethered to a broad interpretation of the scientific method, which is introduced to the class as a sophisticated version of trial-and-error. The method is explained to be an ongoing process with five elements: Goals, Models, Data, Evaluation, and Revision.

These elements are tied to a figure illustrating the flow (Fig. 1; reprinted with permission of Bull and Pease). This scientific method structure lends itself to illustration by countless examples familiar to anyone: cooking a meal, writing a term paper, decoding a computer program, improving your team's performance, or trying to figure out why your car won't start. Our intent is to have students recognize the formal process as something already familiar to them and thus not be intimidated by it. Furthermore, we try to use familiar examples for illustration. When trying to teach the inner workings of a process, the learning should be easier if students are already familiar with the factual material used to illustrate and explain the process, so they are not trying to learn the process at the same time they are trying to learn what the process is being applied to.

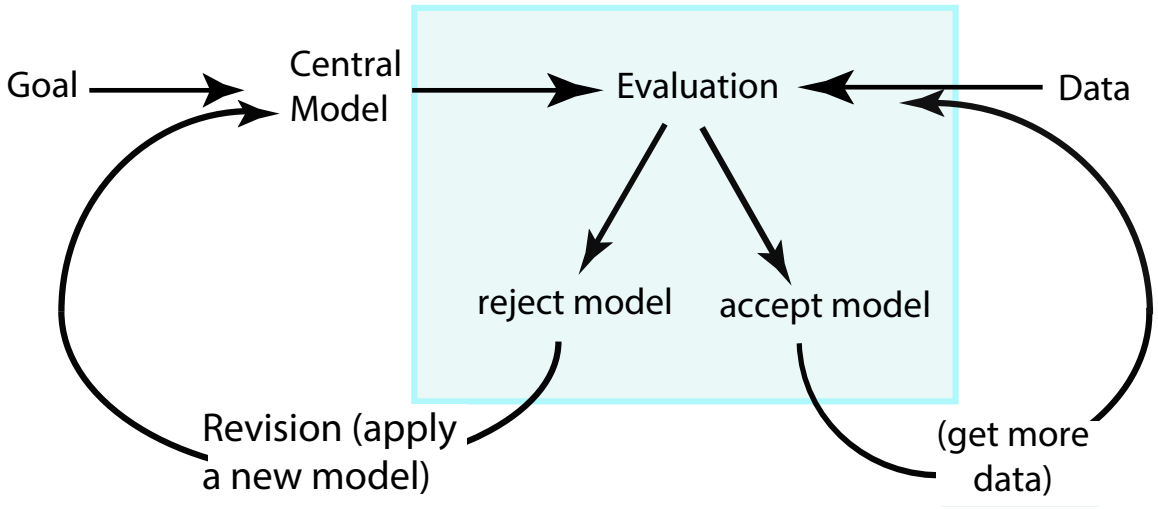

Figure 1. Diagrammatic illustration of the scientific method. Five key elements are recognized: goals, models, data/evidence, evaluation, and revision. For almost any goal, the rest of the process is continual, and different iterations may go through the revision loop or the acceptance loop. Models are used more widely than depicted here, but there is always one or several 'central' models at the core of any implementation.

Not only are students not taught what they should think, but they also are made aware that evidence-based reasoning cannot be applied to everything; it is not a basis for some types of decisions and conclusions. Foremost, ethical and moral decisions should not come from scientific reasoning. The current climate in the US seems to adhere to this view, but there have been notable exceptions in the past here and abroad (e.g., social Darwinism, eugenics). Additionally, evidence-based reasoning does not apply to mathematics. Mathematics is internally logical and relies on proofs and theorems. Science has no proof; it is sloppy and relies on often temporary judgement calls. What we think today may change tomorrow.

The course material is a progression of topics with illustrations throughout the semester. That progression follows the list above, although only three of the five elements are covered in depth: models, data, and evaluation. The concept of Goals is obvious and doesn't need attention; Revision is a natural subset of, and is included in, Evaluation. The elements are intrinsically modular, and exams have been tailored to single elements. An introduction to each element is followed by examples. For anyone teaching this framework, the choice of examples is highly flexible—they can be chosen to reflect the instructor's personal knowledge and background or the students' interests. Homework has been designed to allow the students to apply their knowledge to topics and articles of their choice (see below). The next few pages explain how these three modules work and how each contributes to an understanding through the scientific method. Equally, the goal is to understand the limits of scientific reasoning and how we can go wrong.

MODELS

The concept of models is familiar to students, although they don't immediately realize it, nor do they realize the full ramifications of what they already know. A model may be an idea/hypothesis/theory/assumption, an organism, a formula, a scaled up or down plastic structure, and more. What is less obvious to students (and even to many scientists) is that models are used at countless levels in everything we do—they are used so commonly that we tend to take them for granted and ignore their role. The challenges for the course are

in getting students to realize that models sit at many levels in any decision we make, and in getting students to understand how models may make the difference between good and bad judgements.

Models are little more than substitutes for 'the real thing.' And because they are substitutes, they are imperfect. Their imperfections may be a reason for a (partly) wrong outcome or conclusion. As one example of a model flaw with important social ramifications, consider the composition of patients in early clinical trials. Clinical trials provide the ultimate model of humans for FDA approval of new drugs and treatments. For decades, clinical trials were confined to white males on the assumption that one human was an appropriate model of all other humans. We eventually discovered that white males were not representative of women or males of other races in important ways (Dresser 1992). The clinical trial approval process now requires inclusion of both genders and of multiple races, or at least requires justification for omission of these groups.

Topics abound for models that appeal to students: DWI testing, condom testing, food safety inspections, water quality, résumés, how to study for exams. One résumé example I have used is about an 1800s cowboy named Remus Reid, a distant and long-dead relative of the former Senate Majority Leader Harry Reid. Harry Reid's 'spin doctors' wrote a flattering and technically accurate résumé of Remus Reid, who in reality was a bandit. The contrast of the two descriptions of the same person provides students with a reminder of the different ways their résumés—models of themselves—might be written. Many layers of models can be found in countless examples, and indeed, their ubiquity likely hampers students' grasp of the topic. But models can be a weak link in the scientific method, and one that is rarely appreciated, so we have consistently emphasized the topic.

Example: DWI testing. For illustration here, a particularly simple demonstration of models appropriate for students is the way we test for alcohol-impaired driving (DWI). The social goal is to identify impaired drivers, but some methods (models) in use do not measure driving performance at all—such as blood alcohol concentration (BAC, Table 1). BAC is used because it is convenient and can be applied uniformly, but different people will be differentially impaired at the same BAC. The method that would be most relevant to driving performance—a road test—could never be used because it is too risky. This example is easily used to illustrate many of the key issues about models—they all have limitations, and we make do with those limitations until something better comes along. Formal science operates the same way.

DATA AND DATA ANALYSIS

The topic of data—evidence and observations—is a more familiar topic for students than is models. Most non-scientists have a grasp of data as our measure of nature's reality. Students also arrive with some comprehension of data quality issues, such as sample size and bias, but they don't have a deep understanding of where problems arise and how to fix the problems. The most important goal for data, however, is to have them realize that everything rests on the evidence, and that any suspicious conclusion should first be questioned for the evidence. Asking for the evidence is the one easy weapon anyone has to confront a wild claim. Beyond that, understanding how the evidence was obtained is important to know whether it's trustworthy: data can have flaws.

Most of my emphasis on data has been on data quality. The basic issues are easy to grasp; data can deviate from the truth for several reasons: sampling error, bias, rounding, and human or technical error. All of these data problems are easy to demonstrate in the classroom, and the fixes are likewise easy to explain and illustrate.

Example: Forensics. The most compelling examples I have for the importance of data quality come from forensic science. In criminal trials where there is a victim, forensic methods are usually used to match something—a sample from a crime with a sample taken from a suspect. The quantitative issues in forensics are thus relatively simple—do the samples match and

Table 1. Evaluation of different (possible) types of models for testing driver impairment.

Model	How useful	Limitations as a measure of driving ability for each individual
Blood alcohol concentration of 0.08%	Easy to obtain accurate reading. Is an objective criterion. Is correlated with behaviors important to driving.	Does not measure actual driving performance. One threshold does not produce the same level of impairment in all people. Is not measured at time of arrest.
Standardized field sobriety test (walk and turn, etc.)	Performance is measured for behaviors somewhat directly relevant to driving impairment. Easy to administer.	Performance is affected by many factors unrelated to driving. Scoring is partly subjective (lack of uniformity). No baseline data exist for each person.
Road test (not used)	Measures exactly what we care about—driving performance.	Impractical, although might become more practical with on-site video-game capability. Risky—the test might endanger lives.

with what odds? There is a near-century history of supposed scientific methods used in US courts that were later shown to be flawed (Fisher 2008). We even have the benefit of the Innocence Project to provide faces and details to hundreds of wrongful convictions that stemmed from flawed methods (Scheck et al. 2000). The forensic errors that have pervaded our criminal justice system ultimately come down to poor data quality and analysis, a lack of safeguards to ensure quality, and lack of an effort to measure the uncertainty. It is relatively easy to use this material to convey the importance of many features of 'ideal' data: how blind testing of samples would prevent many types of errors (such as deliberate falsification of lab results), how replication of sample testing would detect accidental mistakes, and why a database of population characteristics is needed to measure the statistical significance of a match (as opposed to an expert merely testifying that, in his/her experience, accidental matches do not occur).

EVALUATION

This part of the scientific method is perhaps the most satisfying to teach, in that it comprises a logical nexus between the nature of data and the conclusions drawn about which models are supported. Evaluation refers to the process by which one compares data to models and decides whether to accept those models or reject them. Most students have some of the basics but just a rudimentary understanding of the ramifications and how it all fits together. It feels rewarding to assemble the parts for them.

As Craig and I developed it, this material starts with the basic and useful point that correlation does not imply causation: one should not attempt to infer that two variables are causally related simply because they are correlated. The media is filled with countless examples that violate this principle, so there is no shortage of examples. One of my favorites is the claim, by a CDC-funded study, that increasing taxes on beer will reduce sexually transmitted diseases (Staras et al. 2014). A more insidious one is the blanket recommendation by advisors at a US university that all undergraduates should be coerced into taking 15 or more credit hours each semester because national data showed higher graduation rates for students taking at least 15 hours.

From there, we explain why correlation does not imply causation and what one can do about it. As

is well known, the culprit is 'hidden variables.' You observe a correlation between number of hours enrolled per semester and graduation rate, but the true cause of graduation rate may not be number of hours taken per semester, it might be extra-curricular; for example, students taking fewer hours per semester could have family and financial obligations that keep them from continuing their degree in a timely fashion. Thus, advisors that force students to take at least 15 hours per semester could worsen the graduation rate for students with these challenges. Problems such as these allow the class to comprehend not only that correlation may not reflect causation but that the correlation may even go in the opposite direction as causation (Simpson's paradox).

Another illustration that is more entertaining was repeated to me by Holly Wichman: a positive correlation between the number of bars and churches in the different cities and towns of a state. To assume the number of churches and bars is causally related lets the imagination run wild, but it is easy to appreciate that the hidden variable is the size of the town—larger towns have more of everything. (When Robert Baker arrived at Texas Tech, Lubbock would have been an outlier in this correlation—lots of churches and no bars. Lubbock was a dry city in the heart of the Bible belt.) The church/bar correlation is apparently often invoked in statistics classes as an easy illustration.

Upon demonstrating that third variables are the problem, how does one get to causation? That question is a segue into controls and experiments. The reason for a randomized assignment of patients to control versus treatment groups in a clinical trial becomes obvious—it destroys all possible correlations between hidden variables and the treatment variable, so the only possible causal variable is the treatment.

Example: Facilitated Communication. The most compelling example of an experiment I have is one underlying the dismantling of a technique once used indiscriminately on people with severe autism—facilitated communication. This method used an adult to hold the autistic client's hand over a keyboard as the client typed. The typed messages were interpreted as coming from the client. But were they? The typing might have been controlled by the facilitator.

A simple experiment was used to test the origin of the messages. The client was shown one object, the facilitator something else; each was blinded to what the other saw. The client was then asked to type what was shown. In a great many studies, the facilitator was invariably the source of the typing—the typing *never* matched what the client was shown. The example is great for class; it comes with a Frontline video (Prisoners of Silence 1993) and has a strong emotional element that draws them in. And the experiment is so simple that everyone understands it and understands why it is needed. The experiments also illustrate the benefit of controls.

PUTTING IT TOGETHER

Ultimately, my hope is that students will assimilate the class material and be able to apply the understanding in the real world. Our world is increasingly one of a plethora of information mixed with misinformation and disinformation. It helps to know the difference.

After finishing 'Evaluation,' the end of semester is approaching, and there is little time to cover a lot of ground in explaining how to detect bogus information and advice. Even so, there are now a few thoroughly documented case histories of intentional, documented scams that span the research planning phase to the media coverage of the work (Abramson 2008; Godoy 2015). These examples can help students understand the process from the inception of a study to its eventual delivery to the public and how deception can be arranged. But the message about intentional deception is also somewhat negative.

DELIVERY

In bridging the material to everyday examples, the course allows opportunities that would not accrue to most other courses. In addition to standard exams, our course used homework assignments that required the students to find articles of their choice and then apply templates of class material to those articles. This allowed students to connect their interests and individual majors to the course material.

The reverse approach also lent itself to online quizzes: have the student read articles or watch videos chosen by the instructor and answer questions that rely on an understanding of class material. I felt the quizzes and homework assignments were perhaps the most important intellectual exercises for the students, and I would have preferred the entire grade came from these activities. But there would be no way to ensure that all students did their own work. Indeed, I actually wanted students to collaborate on the quizzes—to help them reason together—though I could not really advertise my preference without inciting an avalanche of highly asymmetric 'collaborations.'

CHALLENGES

Development of a new course that lies outside the standard curriculum faces several challenges. Foremost, with no precedent for such a course, there is bureaucratic inertia against it. Fortunately, the University of Texas was supportive of our efforts and became increasingly so over the years. The course started out as an unofficial variation of a standard non-majors course in biology but then acquired its own number and designation. After a title change from 'Process and Politics of Biology' to 'Biology for Business, Law, and Liberal Arts,' enrollment doubled and was henceforth limited only by classroom size.

Not long after writing our book for the class (and long before the feasibility of allowing for downloads of pdfs), Craig and I approached several publishers with the material. Getting our book aggressively marketed would be the most effective way to disseminate the course. We were visited by several representatives of established publishers, some very enthusiastic about our material. Yet within weeks of the visits, the editorial enthusiasm invariably gave way to the reality that there was no market for the material. Other universities had no such courses, so no one other than Pease and Bull would have courses using the book.

An important question that remains is the extent to which the students benefit from the course. Many colleagues and adults outside the university see the benefit to society of teaching non-scientists this type of material instead of having them memorize the stages of meiosis, Hardy Weinberg equilibrium, cell biology, or annelid anatomy. There is, after all, a lot of weird belief out there that many would like to change (Shermer and Gould 2002). And many core degree requirements are meant to broaden a student's understanding, which this course certainly strives to do.

Yet for a course to truly succeed, the students need to feel that they are learning something useful to their futures. I confess to being somewhat unsure of how much our course satisfies this goal. Student evaluation comments often mention this feature of the class, and I think that assignments and quizzes that draw on current articles help connect the course material to everyday life. In this respect, the course looks good relative to the core alternatives that do not so obviously connect to current events. Even so, I think that the material could be rendered more relevant to their careers and interests, and this is one area that I will strive to improve.

For the last nearly three decades, this type of material has not been mainstream, and indeed, has been rarely taught. Whether the material serves the student audience well has thus not been formally evaluated or debated on a wide scale. It is indeed possible that most students will feel little need for critical thinking in their lives, hence the course should have a more carefully chosen audience, although I would argue that the need is increasing with the onslaught of unvalidated internet sources of advice, information, and disinformation. And the community at large does not necessarily support this type of education—the 2012 Texas Republican Platform briefly opposed the teaching of critical think-

ing (Platform 2012), although that position has since reversed. But any evaluation of whether this kind of alternative material should be taught is unlikely to happen until it starts being adopted on a wider scale.

The ongoing development of the material for this course was largely at the expense of testing different methods for *how* to teach. With no alternative textbooks or courses that closely matched our material (that we knew of), we were constantly in pursuit of new examples and clarifying the scientific method elements and their connections in ways that would appeal to the 'non-majors.' As more of a curriculum develops around this type of material, an obvious step is to undertake comparative studies to determine the most effective methods and materials for student learning.

My Debt to Robert Baker

The volume in which this paper is published is in honor of Robert Baker. As Robert and I were close friends for half a century, and he both inspired my entry into academia and had a continuing major influence on my career, I use this closing section to reflect on and acknowledge his contribution to my thinking.

Robert and I both arrived at Texas Technological College in 1967, him as a young assistant professor, me as a freshman. Within a year or so, and into spring of 1971, I was working under his supervision on my first research projects and also as his assistant in some of his courses, a large freshman biology course and histology. He was my first in-depth introduction to the excitement and intellectual freedom of being a professor. His energy toward research, supervising students, and teaching seemed to know no bounds. Work I started with him (on sex chromosomes) led to my first major research direction that spanned 25 years.

Of my many collaborators and mentors at different institutions, Robert stands out as one of two who valued and enjoyed teaching large undergraduate courses (John Legler of Utah being the other). Even after formally retiring, Robert continued his freshman course until the logistics became too much for him. I attribute my early exposure to his attitude in fostering my interest in developing a course that enrolled large numbers of undergraduates. I have not kept track of numbers, but I estimate that I have taught 5,000–8,000 undergraduates in the last 35 years. Robert's career total may be similar.

Despite recent changes in academia and increasing public scrutiny of our activities, universities remain one of the last bastions of intellectual freedom in the world (even if freedom of speech seems to be increasingly under attack within universities). They are the breeding grounds of new ideas and technologies. Research is part of that. So is teaching. Robert Baker recognized and embraced the role professors have in upholding this responsibility. One might speculate that his upbringing in a financially poor environment in rural Arkansas gifted him with a sense of duty and appreciation for how privileged are our academic positions. In my formative days as an undergraduate, Robert inspired those around him to accept a duty in serving society. Adopting that attitude certainly has made it easy to accept teaching responsibilities and interesting to do so.

Academia remains in transition, and some aspects of its future are not clear. But even as new teaching methods come available, and as the classroom environment and the nature of courses change, universities and their faculties remain in control of deciding what knowledge should be passed onto the next generation. With a vastly increasing information base, it is becoming increasingly obvious that information is not knowledge. Universities still have a major role in filtering that information to create social knowledge. Choosing the knowledge that will form the roots of public education deserves to be front and center in our jobs. More than anyone else in my career, Robert Baker inspired me to think about that perspective.

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LITERATURE CITED

Abramson, J. 2008. Overdosed America: the broken promise of American medicine. 3rd edition. Harper Perennial, New York.

Dresser, R. 1992. Wanted: single, white male for medical research. The Hastings Center Report 22(1):24–29. https://doi.org/10.2307/3562720.

Fisher, J. 2008. Forensics under fire: are bad science and dueling experts corrupting criminal justice? Rutgers University Press, New Brunswick, New Jersey.

Godoy, M. 2015. Why a journalist scammed the media into spreading bad chocolate science. NPR, 28 May 2015. https://www.npr.org/sections/thesalt/2015/05/28/410313446/why-a-journalist-scammed-the-media-into-spreading-bad-chocolate-science.

Hecht, D. K., et al. 2018. Pseudoscience: the conspiracy against science (A. B. Kaufman and J. C. Kaufman, eds.). The MIT Press, Cambridge, Massachusetts.

Kuh, G. D., and C. Geary Schneider. 2008. High-impact educational practices: what they are, who has access to them, and why they matter. Association of American Colleges, Washington, DC.

Mazur, E. 1996. Peer instruction: a user's manual. 1st edition. Pearson, Upper Saddle River, New Jersey.

Platform. 2012. 2012 State Republican Party Platform. https://www.empowertexans.com/wp-content/uploads/2012/07/2012-GOP-Platform-Final.pdf.

Prisoners of Silence. 1993. FRONTLINE | S12 E2 |PBS. 1993. https://www.pbs.org/wgbh/frontline/film/prisoners-of-silence/.

Redfield, R. J. 2012. Why do we have to learn this stuff?—A new genetics for 21st Century students. PLOS Biology 10(7):e1001356. https://doi.org/10.1371/journal.pbio.1001356.

Scheck, B., P. Neufeld, and J. Dwyer. 2000. Actual innocence: five days to execution, and other dispatches from the wrongly convicted. 1st edition. Doubleday, New York.

Shermer, M., and S. J. Gould. 2002. Why people believe weird things: pseudoscience, superstition, and other confusions of our time. Revised & Enlarged edition. Holt Paperbacks, New York.

Staras, S. A. S., M. D. Livingston, A. M. Christou, D. H, Jernigan, and A. C. Wagenaar. 2014. Heterogeneous population effects of an alcohol excise tax increase on sexually transmitted infections morbidity. Addiction (Abingdon, England) 109(6):904–12. https://doi.org/10.1111/add.12493.

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On Being a Graduate Student of Robert J. Baker: Prospects, Perils, and Philosophies—Lessons Learned

Robert D. Bradley

Abstract

Dr. Robert J. Baker mentored 98 graduate students during his 48-year career at Texas Tech University. Herein, an attempt is made to dissect his *modus operandi* and philosophical positions concerning graduate education. An overview is provided from the perspective of a former student and faculty colleague.

Key words: Robert J. Baker, graduate student, mentor, MS, PhD

Introduction

In almost every parameter by which we measure academic and scholastic success (accolades, publications, notoriety, grants, etc.), Dr. Robert J. Baker (Robert or simply Baker from here on) would be at the top of most lists. Most of us can name several extremely productive researchers who have published as many, or nearly as many, scientific papers as did Robert (see Genoways et al. 2018 and Schmidly et al. this volume for a list of Robert's publications and their relevance to the scientific community); however, it would be next to impossible to name an individual who mentored as many graduate students as did Robert. During his 48 years at Texas Tech University, Robert mentored 48 PhD and 50 MS/MA students (see Genoways et al. 2018 and Schmidly et al. this volume for a complete listing). During his career, Robert averaged 2.09 students graduated per year. For relevance, I compared the top graduate student generating faculty currently active in the Department of Biological Sciences here at Texas Tech University (Table 1), and several observations became apparent. First, no single faculty member approaches Robert's number for total number of graduate students. In fact, it takes a combination of the top three faculty members to exceed Robert's total number of 98. The top three faculty members produced 99 graduate students; however, it took the faculty 89 years to accomplish that—Robert did it in 48 years. Second, the top five faculty members generated an average of 1.14 (range 1.1 to 1.33) students per year, compared to Robert's average of 2.09. Finally, in almost all categories examined, it would take the three best faculty members to replace Robert as a graduate mentor; although the output would be lower and the number of years to accomplish those endpoints would be greater (see averages reported in Table 1).

Obviously, this extraordinary number of students required a remarkable amount of time, energy, tolerance, molding, reshaping, patience, pressure, reassurance, consoling, planning, adjustments, soul searching, etc. Just imagine the time Robert spent editing proposals, theses, and dissertations; not to mention manuscripts and grant applications prepared by his students! Imagine the effort that went into writing letters of recommendation. Those of you that have mentored graduate students know the drill; you can appreciate the energy and commitment required to see a student to a successful completion—now multiply that by 98! Further, as a mentor, you know that there is no "how-to book" for directing graduate students and you know that no two students are cut from the same cloth. Each student responds differently to being mentored and each requires his or her own operation manual and method of advising. At the completion of each successful student/mentor relationship, as a mentor you realize that it has been a mixture of trial and error, a series of "pats-on-the-back" (perhaps more than a few "kicks-in-the-butt"), a large dose of perseverance and endurance (by both mentor and mentee), and, in some cases, a substantial amount of luck.

Table 1. Relevant data for overall number of graduate students produced by Robert J. Baker and the rate at which they graduated (students per year) compared to the five top graduate student generating faculty currently residing in the Department of Biological Sciences at Texas Tech University. For anonymity reasons, names of those faculty members are listed as Professors A–E.

Professor	Years on Faculty	Number of Master Students	Number of PhD Students	Total Students	Students/Year
Professor A	33	15	18	33	1.00
Professor B	32	22	12	34	1.06
Professor C	24	21	11	32	1.33
Professor D	24	9	17	26	1.08
Professor E	20	15	11	26	1.30
Total	133	82	69	151	5.77
Average	26.6	16.4	13.8	30.2	1.15
Average x 3 Professors	79.8	49.2	41.4	90.6	1.14
Baker	47	50	48	98	2.09

So just how did Robert successfully pull off this incredible feat? With 98 completed students, there had to have been more to it than just blind luck. What was his secret formula, and how and where did he find all of those students in the first place? Did he recruit only the best and the brightest, or did he take average students and work to develop them into outstanding graduates? How did he shepherd them to completion? How did he continually turn out students that were competitive with the best in the country? To answer these questions, I will draw upon my own experiences and observations garnered from 5.33 years spent in the Baker program (as a PhD student) and over a quarter century as a colleague, 6th floor neighbor, and personal friend. Turns out, I either over-lapped with, or was on the faculty at Tech, for 66 of Robert's 98 graduate students. Further, Robert served on the committees of hundreds of other Tech students, including many of my own students; so, I was able to experience first-hand and then observe from a distance as to: how did Robert attract students to Tech; how did he train and motivate students to succeed; what was it like to work with him; what were his philosophies on graduate education; how did he stay energized and relevant throughout a long career; what worked for him and what did not work; and how did he interact with his students. Given the variance of these topics, I organized my observations and thoughts into three general sections—Prospects, Perils, and Philosophies.

PROSPECTS

I titled this section "Prospects" because undertaking a graduate degree is all about the opportunities that said degree will convey to the recipient. Seeking a graduate degree is not a decision to be made lightly—it becomes a commitment by the student who has hopes of receiving a positive return from that investment somewhere in his or her future. For most MS students in the biological sciences, the average time commitment is approximately 2.5 years for obtaining a degree, and PhD students generally complete their degree within 5.5 years. As a consequence, the student must weigh the cost (financial, time, hardship, etc.) of obtaining the degree versus the possibility that something beneficial comes of this endeavor. For example, will the degree lead to employment? Will it be a good job? Will it be the job that you desire? In hindsight, we can justify our decisions based on the outcomes we accrued along the way; but as a freshly graduated BS or MS

student thinking about joining the Baker program (or Tech)—what made us pack up and head for Lubbock? What convinced us that this career choice (i.e., working with Baker) was going to be a good one? Was it the right one?

Why did we choose the Baker Lab?—What were the factors that led 98 students to agree to place their futures in the hands of Robert J. Baker? What was it about Robert or his research program that influenced these 98 students to say—"sign me up, this is what I want to do"? I think there were three aspects that made this work so well. First, I think Robert's dynamic personality convinced most students that only good things were on their horizon. Robert had the ability to talk a good talk—he did not blow smoke, because as usual he had the data to back up his statements. But he did have that ability to cast such a positive spin on things that you were convinced that you would never fail to achieve your dreams. He always made potential students feel special and then insisted that they should go to Tech for their next degree. His aura was infectious; he made it easy to sign up for the full tour! Second, you could not argue with the successes (placement in academic and other professional employment) of his former students (see Genoways et al. 2018 and Schmidly et al. this volume for a list of Baker former students and some of the institutions that employed them). I had heard more than once—"if you work with Baker, you can write your own ticket!" Third, Robert actively recruited graduate students. He was always on the lookout for "quality" students, especially at scientific meetings. He would attend paper sessions and then actively visit with a student following their presentation. He would do his best to convince you that Tech was the place to be!

Robert was attracted to any student that seemed excited about graduate education or anyone that showed an interest in pursuing a higher degree or was willing to work hard. Robert's opinion was always that the world is better off when someone becomes educated (earns an advanced degree), so it did not matter to him if his students took employment outside of academia. Over the years, he gave several students the opportunity to complete a MS degree—many of these students would not have gone down that path without Robert's encouragement and nudging. As a side note, Robert's active recruitment helped attract some outstanding students to TTU, ones that might not have come here otherwise. Each newly recruited student brought a new personality to the Baker lab, a new person with which to engage, and new research endeavors to pursue. I think each of us, in our own way, rejuvenated Robert. I am not implying that presented a challenge against which he had to prove himself as a mentor; instead we represented a new chapter, a new set of colleagues, and a new round of ideas. We were young, ambitious, and ready to charge forward; that helped keep Robert young and motivated to continue as a mentor.

Getting recruited my ownself.—Robert began recruiting me as a graduate student in early 1985. At the time, I was working on my MS degree with Dr. David J. Schmidly at Texas A&M University (TAMU). Dave was a close friend of Robert's and was a "Tech" graduate, having completed BS and MS degrees in the late 1960's. In fact, Dave was a graduate student at Tech when Robert applied for and accepted his faculty position. My recollection was that during the Christmas break in 1984, Dave was visiting family in Levelland, Texas, and that he stopped by Tech to visit with Robert. During their visit, Robert mentioned that he was looking for a couple of new PhD students. His current crop of PhD's (Craig S. Hood and Mazin B. Qumsiyeh) were going to be finishing soon and Robert was looking for replacements. Dave told Robert that I should be finishing my MS degree soon and that I would be a good match for him and Tech. Soon thereafter, Robert began contacting me about moving to Tech and working on a PhD with him. I was flattered, excited, and worried at the same time. As any student interested in mammalian systematics and evolution during the 1980s, I knew who Robert J. Baker was. He was an icon in the field of cytogenetics, systematics, and mammalian evolution! Baker's influence in mammalogy seemed to be everywhere you looked—for example, at the time, two of his former PhD students, Ira F. Greenbaum and John W. Bickham, were on the faulty at TAMU, Terry L. Yates was on the faculty at University of New Mexico, and Rodney L. Honeycutt was at Harvard University. Several other former students were either faculty members at smaller universities or were completing their PhDs in other programs. In those days, if you attended the annual meetings of the Texas Society of Mammalogists, Southwestern Association of Naturalists, or the American Society of Mammalogists (ASM), you encountered former and current Baker students. Obviously, Baker's program

was recognized as one of the very best places in the country for mammal-based research and it was at the top of the list for many graduate students. As I began to learn who Baker's former students were, I was truly amazed at their successes; they all seemed exceptional or were employed in good positions!

So, on one hand I was very excited about the possibilities of going to Tech and working with an icon; however, self-doubt and reality surfaced and I really worried—was I "Baker quality material"? Another look at the success of Robert's former students begged the question—did Robert recruit only the best students, or did he develop and mold average students into outstanding students? I figured if Robert attracted only the brightest students, I was going to be in for a difficult ride at Tech—but if Robert's *modus operandi* was one of developing students, then I had a chance. Consequently, I contacted several of Robert's current and former students, and asked them for advice—could I make it? Some of these students I had met at scientific meetings, others were complete strangers except in reputation and name. All said something along the line that Robert would work closely with me and that failure was not going to be an option; and to a person, they encouraged me to take advantage of this opportunity. A look back on this interaction among Robert's former students and myself speaks volumes about their admiration and loyalty to their former mentor (more on this under *Being in the Baker Family*), as they without hesitation, whole-heartedly recommended Robert's program to me. In fact, several of Robert's former and then current students actually helped recruit me to Tech; and I am so glad they did.

PERILS

Obviously, being a Baker student was not all sunshine and roses. Each of us experienced a few gustnados and sandburs during our association with Robert; so "Perils" seemed an apropos subtitle. However, as I wrote this section of the manuscript it occurred to me that the "perils" of being a Baker student did not necessarily end with the completion of your tenure in Lubbock; rather the "perils" usually followed most of us to wherever we finally called home. My goal here is not to cast stones at Robert or his methods, but instead to emphasize that despite some "perils" here and there (that were well known in advance by most of us), 98 of us still signed up for (and completed!) the "Baker experience."

The Baker persona.—Robert was: successful, unrelenting, focused, uncompromising, driven, 10 feet tall and bullet proof, loyal (stayed at Tech for 48 years although he could have left many times), tireless, scheming, brilliant, crotchety, supportive, emotional, prima donna, fatherly, gregarious, romantic (hundreds of pounds of chocolates distributed to the office ladies, graduate students, and friends every Valentine's Day), spontaneous, unorganized, pain-in-the-ass, demanding, unpredictable, complimentary, etc. As a Baker student you encountered these personality traits (sometimes several within a few minutes of each other) and you quickly learned to avoid Robert during the "Category 5 gustnado and sandbur ridden moments." Many-a-morning, one or more of us would watch for Robert's approach to the Biology building from the parking lot. This exercise served as a "barometer" for the remainder of his graduate students. Normally you could predict Robert's mood by his approach as he exited his surburban (truck in later years) and approached the biology building—a slow, nonchalant walk meant it was "safe" to be in the lab or his office; a hurried/determined/head down/rapid approach meant "danger, danger" (per former student Meredith J. Hamilton) and it was best to "scurry for cover." I am sure each cohort of Baker students had similar experiences and appropriate litmus tests and lookouts. Schmidly et al. (this volume) discusses Robert and the linkage to "type A and D" personalities—and the traits that accompany each type (I wish this knowledge had been available to me back in "the day"….).

One of the most interesting parts of Robert's demeanor was his habit of yelling **to you** about something that someone else had done. I bolded "to you" in order to emphasize that when he blew up about something, he grabbed the closest person and "voiced" his dis-

pleasure. During these yelling bouts, about 99% of the time he was not angry with the person standing in his presence—they were just handy…. Once, Rodney L. Honeycutt did something to earn Baker's displeasure and Ronald A. Van Den Bussche, Meredith J. Hamilton, Calvin A. Porter, and I (lab mates at Tech at the time) "were handy" because Rodney was on the faculty at Harvard and therefore was "not handy." After about five days of being yelled at, because of Rodney's minor infraction, we (Ron, Meredith, Calvin, and I) voted to call Rodney and get him to either make up with Robert or move to Lubbock so that he could be "handy" instead of us!

Yes, Robert could get extremely angry, but he would get back to normal just as fast. He could be thermonuclear one minute and completely over it 10 minutes later. In fact, he would act like it never happened. One morning Robert and I had a pretty severe confrontation and I was so angry with him that I was looking for someone "handy"… About 30 minutes later Robert came into my office and said "let's go to lunch"; like nothing had ever happened between us! I was still mad at him and replied no, I need to stay mad at you for a couple of days, then maybe lunch would be appropriate. I often wish I could have let go of things as quickly as he could. With Robert, once the mushroom cloud settled, it was over (typically buried forever) and time to get back to work.

Diabetes.—It was no secret that Robert was a diabetic. If you did not know of it prior to meeting Robert—sooner or later you were sure to witness either an insulin shot or a blood sugar test. Robert had no qualms about stopping in mid-sentence and pricking his finger or raising his shirt and injecting himself in the stomach; often to the chagrin of the observer! One story has Robert injecting himself with insulin during a plane flight which led to one very confused and frightened passenger reporting to the flight attendant that Robert was a drug addict! As one of his students, you got used to the needles and you got good at watching for the tell-tale sign of low blood sugar. You learned to intercede and insist that he stop and take an insulin shot or eat something.

No doubt, Laura Baker was a godsend to Robert. She kept him alive more years than he could ever have pulled off on his own! She would prepare the "ditty bag" which contained cokes (regular and unleaded), candy bars, and crackers. This was a really nice plan, except Robert usually left the bag in his truck—a reality that was realized when Robert was the greatest distance one could possibly be from the truck and exactly when he was not sure which planet he was on. The long walk back to Baker's truck usually provided ample time for musing about why you were making the walk for him. A few years ago, former student, O. James (Jim) Reichman, tongue-in-cheek, apologized to a gathering of Baker and his former students (informal lunch at an ASM meeting, as I remember). Jim recalled that during a collecting trip Baker had his normal blood sugar crash and Jim was sent back to the vehicle for the candy bar. Jim recalled that Baker was in pretty bad shape and that only Reichman's timely action would mean the difference between Baker's life and death. Then Jim apologized profusely (to those of us who came after him) for retrieving the candy bar… and we had a good laugh because most of us had made the same decision!

You would think that after spending his entire adult life as a diabetic, Robert would have been smart enough to carry food in his pocket. I pointed this out to him one afternoon, and his reply was classic Baker…. he said that I had been a graduate student with him for a couple of years and that meant that I should have been smart enough to know that he would forget to take food for himself —therefore it was my lack of intelligence for not having the foresight to carry food, not his. Lesson learned, from then on, I always carried a candy bar in my pocket for him (so that I did not have to walk back to the truck).

No doubt, diabetes kicked Robert's butt on a daily basis, but he persevered, over-compensated, and plowed straight ahead. In 1985 or so, when Robert was recruiting me to Tech, he told me that he was a diabetic and that he was not expected to live past his late 40s. He said he figured to live long enough to get me and another contemporary student or two finished before his time was up. When I graduated in 1991, I was PhD student number 18; that means 30 PhD students (and 27 MS students) followed…and approximately 57% of his publications (approximately 250) remained unwritten. On one hand, diabetes slowed Robert down but on the other hand it motivated him to not waste his remaining days. Consequently, diabetes provided the emphasis for more students, more project, and more manuscripts.

Anything worth doing is worth overdoing.—This saying was Robert's mantra (actually inscribed on his gravestone in Afton, Texas) and fit him to the "T." When it came to work or play, he never skimped; it was full throttle and damn the torpedoes. I am reminded of Robert when I see the baseball t-shirt slogan "Play Hard or Go Home." Although the mantra was quintessential Robert, his gregarious personality guaranteed that you would be swept up by the tornado that resulted from whatever activity was on the agenda. As a graduate student, you constantly were challenged to keep up with the boss (most of us will freely admit that we could not). Most papers and grant proposals were written between 8:00 pm and 1:00 am, and as a student you were expected to be in attendance; as well as back to work early the next morning. You were "on call" 24/7/365. Robert had strange and inconsistent work hours; partly because of his numerous duties and meetings across campus and partly because he only required about 4.5 hours of sleep. Since you were expected to be on hand when he was in the office or lab, you essentially lived in the Biology building. Clearly, Robert was driven to succeed and demanded excellence from those around him, particularly his students, which he saw as an extension of himself and to be held to the same standards.

2nd law of thermodynamics.—This was one of Robert's favorite sayings—actually it was an excuse offered when his office, truck, garage, etc., got so disorganized, messy, out of control, that it resembled, in his words, a "thousand miles of bombed-out-runway." He said it took energy to maintain order (hence, the 2nd law of thermodynamics being quoted on a routine basis) and that the energy required to clean and organize his office, for example, was time better spent on writing a paper or on a hunting trip. Eventually, the procrastination would approach "critical mass" and everything and everyone shut down for a major overhaul and battle with entropy. Over the years, I argued that a little energy spent upfront (i.e., daily or weekly cleanings) would save a ton of energy in the long run—another argument that I never won.

In 1985, during my "recruitment phase," I made a pilgrimage to Tech to visit Baker and his lab. On the drive from College Station to Lubbock I had plenty of time to envision the grandiose lab I was about to see. Given Robert's success and icon status, I imagined an expansive research facility that was spotless, well-equipped, professional, state-of-the-art, etc. Needless-to-say when I walked into Robert's office and lab I received my first exposure to the phrase "thousand miles of bombed-out-runway." Although I was around Robert for nearly 30 years, I could never understand how he managed to function, much less flourish, amid such chaos, disrepair, and pandemonium!

Cluttered mind.—Not only was Baker's lab and office a mess, so was Robert's mind. I say this with the upmost respect and bewilderment! Robert's inability to maintain focus was legendary. That does not mean he did not focus—it just means that about 50 things were running through his mind at any given time and no one knew which one was going to surface or when. When you wrote a paper with Robert it was a side-by-side event that made Homer's Odyssey seem simple and uneventful. You might write a couple of sentences in the introduction, leap ahead to write a paragraph for the discussion, stop and draw a figure, have an idea for a table, have an idea for a grant proposal, come back to the introduction, outline an idea for another paper, repeat the preceding multiple times, leave the office, go train dogs for an hour, grab a hamburger, look for geese at Buddy Holly Park, and then return to office and write on the discussion until 1:00 am (see Bradley 2005 for a description of a typical paper writing ordeal). This was the norm….

Robert never did anything until "it was time." You could not schedule a productive manuscript writing session with Robert—it just happened. During my association with Robert, I learned that eventually "all things would come to pass," or in other words, 10:30 pm some night your phone would ring and he would say he was ready to write on your paper. In the late 1980s, I nearly dropped out of his research program. I had completed very good drafts of three manuscripts and was trying to get them submitted. Robert was a co-author on all three manuscripts and I needed his help in finishing them, but I simply could not get him to help. I took it personally that he was not interested in helping me get these papers finished. I was pretty upset that I was "being neglected" during a critical juncture in my academic development. Then about 10:00 pm one night came the phone call from Robert; apparently and unbeknownst to me, I had three manuscripts that I

needed to hurry up and finish! My academic career desperately needed those papers completed, so get to the NSRL and let's get started! So off we went, full speed ahead! We finished all three in a 3–4 week period and all three manuscripts were published in 1991. Later, I was to learn that Robert was not ignoring me, it was that my papers were just not ready to write themselves, yet. The stars had to align themselves before Robert would be ready and there was little you could do to force the issue. Another thing I came to learn on this topic was that Robert needed time to "write the paper in his own mind." He was not ignoring you, he just needed to think and chew on it for a while.

Although the "perils" were always present, we graduate students learned how to navigate the mine field. We kept watch on the mood barometer, kept a steady supply of candy bars around, and learned how to change topics to avoid the type A and D personality that was rolled into one human being. I often wonder how productive Robert would have been if he were a normal person—non-diabetic, non-procrastinating, and organized. Perhaps he would have been less productive; perhaps he thrived on the chaos and unpredictability. At least it was never mundane or predictable in the Baker lab; every day was an adventure!

PHILOSOPHIES (OR, WHAT ROBERT "PREACHED" TO HIS GRADUATE STUDENTS)

Good science, the scientific method, and search for the truth.—Robert's philosophy about research and education was pretty straightforward—always use the scientific method and accept only statistically supported results. Frequently, Robert had us run blind experiments. For example, he would have us generate a bunch of G-banded karyotypes or have us sequence some gene—but he would withhold the taxon name, locality, sex, or other relevant data. That way there was no anticipation of what the results might look like; in other words, there was no way we would bias the results. These blind experiments and the occasional resampling (redoing some subset of the dataset to see if you obtained consistent results) were standards for most of his students. Robert always preached about searching for the truth. It did not matter if your preconceived expectations or hypotheses were "shattered" by your results—only the "truth" mattered. He often said it is very simple, you either accept or reject—there is no in-between, no fudging, and no ignoring any inconvenient data. Typically, that statement was followed by the comment that hypothesis testing was like being pregnant—"either you are or you are not."

Nothing manifested Robert's view of academic honesty more than his own actions when he and his colleagues discovered an error in estimating mutation rates for a portion of the Chernobyl research project. As soon as they verified and reverified the error, Robert's team published a retraction and disclosed that the error was theirs. That took a lot of courage on their part and it took a major toll on Robert. We all make mistakes, but it takes a special person to step up and admit "it was my fault."

Another example of Robert's devotion to good science was his ability to stay relevant to new methods. Just think of the methodological changes in systematics and evolutionary biology during Robert's lifetime. In his MS days at Oklahoma State University, Robert's thesis involved standard skin/skull morphologic studies; during his PhD research at the University of Arizona he switched to chromosomes and became one of the leaders in mammalian karyology; during the 1970–1980s, his lab was well known for chromosome and allozyme research; in the late 1980s he took a developmental leave to go to Harvard and work with former PhD student Rodney Honeycutt and retool in molecular biology; and finally during the later stages of his career he was enamored with chromosome painting, next generation sequencing, and the promises that genomics held for evolutionary biology. As he was known to say—"to remain competitive, you must stay cutting edge!"

Show me the data.—If only I had a dollar for every time that statement was uttered…. I think it is safe to say that those words dominated every Baker thought process, research project, and argument (friendly or not). Robert challenged his students to document their ideas and posits with data. He would not let you get by saying "some study showed x, y, and z"; you better be able to quote "chapter and verse" and give a thorough synopsis of your supporting data! He encouraged you

to challenge him or to disagree with him—but you best have some overpowering data on your side. It was his way of "fact checking." It took a lot of effort to win an argument with Robert—generally he already had been thinking about whatever you brought to the table, so he was prepared. I think Robert's insistence on "show me the data" helped his students to formulate a well-constructed answer rather than shooting from the hip.

Amplify your strengths and cover your weaknesses.—Another pearl from Robert. The statement is simple, but Robert made it almost procedural. Robert collaborated with hundreds of researchers (see Schmidly et al. this volume, for more details), partly because of his gregarious personality but mostly because he wanted to do "better science." That meant designing more elaborate studies, using state-of-the-art technology, and incorporating the most recent data analyses. Robert excelled at designing studies—that may have been one of his greatest academic attributes—but he was not good at data analyses, and the lack of resources and infrastructure at Tech often meant we were behind in the arms race for the latest and greatest technologies. The latter two scenarios would have hindered or slowed down most researchers, but not Robert. He would figure out the best researchers who were doing what he wanted to do (but could not for whatever reasons) and invite them to collaborate. This way, he catered to his strengths and received quality assistance in areas where he was not an expert. By focusing on the things he did exceptionally well (design experiments and work with students) and letting others cover "his weaknesses," Robert was not only able to maintain a high level of productivity, where others would have been bogged down, but through these collaborations, he was able to always be at the "cutting edge."

Another method Robert often employed was shipping his graduate students off to another researcher's laboratory so that his students could learn the newest techniques. His opinion was to get the new techniques up and running as quickly as possible so that his students would be as competitive as those from the "big schools." So, many of us had the opportunity to travel to other labs and work with the experts and learn new methods. We were expected to learn the new methods, bring the technology to Tech, and then teach our lab mates the new skills.

Each thesis or dissertation was customized to place a student in the best position of being competitive for their "dream job or career path." That attention to detail placed students in the position of being well qualified for a particular job. In other words, our competitiveness (strength) was amplified before we ever interviewed for a position. Robert made sure that we gave a few guest lectures and were placed into the appropriate teaching assistant slots so that we could claim experience and expertise in relevant teaching areas. Also, he made us identify our "dream job" and then figure out what we needed to accomplish to be competitive for said job. He always placed an emphasis on having our CVs indicate that we were the perfect candidate.

Surround yourself with the brightest people possible, use all of your brains, and borrow theirs.—Perhaps a corollary to *Amplify your strengths and cover your weaknesses*… It explains Robert's desire to collaborate and to work closely with students and peers while writing manuscripts. He always said his ability to do good science was enhanced when he could talk to and learn from others. Schmidly et al. (this volume) point out how few manuscripts Robert authored alone. He valued input from others and gladly shared the credit, as evidenced by the multiple author lines on his papers.

Dress for success.—Every few years Robert had us read *Malloy's Live for Success* (Malloy 1981). It was an exercise meant to teach us the importance of first impressions. I think all of us hated this phase of the Baker education process… but looking back there was wisdom in the exercise. Robert never specifically told us how to dress or behave. The few times that he actually weighed in on the topic was in parsing out advice for job interviews. He would tell us that someone had invited us to come to their university or place of business and to become part of their team and that they had paid for plane ticket and hotel bill—so we damn well better look like we appreciated it. One other thing he always imparted was not to drink any alcohol during the interview, because someone will be watching to see how much you drink. He would say "stay sharp and alert, there will be time for drinking when they offer you the job." Words of advice for a successful first appearance seemed strange coming from the flip-flop wearin', hillbilly from Arkansas! In Robert's later

years, he always commented that he had made many mistakes on this topic and that once he learned to wear the coat and tie at the appropriate time, he was better accepted by the administration and dignitaries.

Seminar time!!!!!—Quintessential Baker. This was Robert's main teaching tool for his graduate program—although you may not have known it at the time. All Baker students were expected to enroll in his "seminar course." This course officially meant one hour per week; although they seldom ended on time. It was a combination lab meeting, journal club, brainstorming session, practice session for presentations at scientific meetings and job interviews, bitch session, celebration (birthdays, paper acceptance, successful qualifying exams, etc.), and whatever else showed up on the agenda. It could be focused, chaotic, purposeful, or simply serve as a distraction from a bad day at the office. However, it was the most useful "course" I ever took. We learned the skills for good presentations, thinking on our feet, how to be successful, how to interview well, how science works, ideas for grant proposals, ideas for manuscripts, ideas for new projects, how departments work, what makes for a good faculty member, what do you need to do to get tenure, how to navigate departmental politics, which faculty members should you emulate (and those you should not), life lessons, and much, much more. I think many of us former students have tried to incorporate something similar into our own graduate programs.

Publish, publish, publish.—Each new graduate student was taught from day one the importance of publishing your work. Your job, careers, promotions, grants, etc., would depend on your publication record; best to have a really competitive CV. Robert taught it, he encouraged it, he demanded it, he exemplified it—enough said…

Publishable unit.—This may have been one of Robert's greatest assets. He knew exactly how many samples, how many taxa, and how many localities were needed to make for a robust study! The boundaries of a study were well thought out in advance and the critical pieces aligned. Consequently, we did not waste a lot of time and effort in last-minute redesigns of the sampling scheme or a need to suddenly include additional samples. As important as the premonition of knowing what was necessary to include in a study was Robert's uncanny ability to have a sense of what not to include. He was exceptional in not including extraneous data, samples, or words unless they pertained to the manuscript in hand; those could wait until the next publishable unit.

A good graduate program runs itself, or, learn from your lab mates.—For lack of a better term, I am going to use the phrase "Vicariant Success" to describe a Baker *modus operandi*. He always said the more his graduate students accomplished the better he looked, and by extension, the more he accomplished the better his students would be regarded. Further, he said the better a cohort of students performs, the more prestigious it will be for those that followed in their footsteps. For many of us, the prestige bar was set by our predecessors or contemporaries. Imagine being across the lab bench from Ira F. Greenbaum, John W. Bickham, or Terry L. Yates. Imagine matching wits with Ronald A. Van Den Bussche, Kateryna Dmytrivna Makova, Jeffery K. Wickliffe, or Peter A. Larsen (to name only a few). The standards were set by the established students and the expectations were made clear to the newcomers. As "newcomers" to the Baker Lab, we saw how the "old timers" worked in the lab, wrote papers and grant proposals, taught their lab sections, and prepared for scientific meetings. We saw the commitment, the competitiveness, and the desire to succeed. We learned to do as our predecessors did, and then we set the standards for the next generation.

Although it kind of ran itself, Robert's graduate program took its inspiration from the top. Robert led by example. He probably worked 60+ hours per week; and did so for his entire career. He worked essentially every evening and at least some of the weekends (even during pheasant season!) and that set the expectations for his graduate students. He stayed current in the literature and up to date in methodologies and techniques and insisted we do likewise. He was never satisfied with where he was as a scientist. He always wanted to grow and to improve, and he drug us along with him.

"Not all synapomorphies are created equally," and cultural and gender diversity.—Robert argued, vehemently in some instances, that synapomorphies came in different degrees of strengths. For example, he would reason that more mutations, genetic changes, and other evolutionary forces were responsible for one of

his "beloved chromosomal rearrangements" than would be required for the generation of a new allozymically detectable allele (maybe a single nucleotide substitution at a charged amino acid). One could write a book on the debates that took place in Robert's office, relative to the search for synapomorphies, but that is not the focus of this section. I always liked that phrase and wanted to use it to segue into a more important topic—the diversity of Robert's graduate students. In Robert's world, students, unlike synapomorphies, were equal, or at least they were afforded the same opportunities.

Robert's graduate students were an eclectic group to the say the least. Amongst us were Jordanians, Mexicans, Canadians, a South Korean, a Zimbabwean, a Uruguayan, Ukrainians, an Argentinian, Ecuadorians, a Brazilian, a Malaysian, a Bolivian, and a Peruvian. He had big city kids, farm and ranch kids, offspring of PhDs, first generation graduate schoolers, welders, ex-teachers, veterans (including a Bronze-Star recipient), and the son of an Olympian gold-medalist. Some were pseudo-geniuses, and some of us were, let's say, less academically gifted. We were a diverse group, united by the desire and motivation to learn, and that was enough for Robert.

In addition to geographic and cultural diversity, Robert was a pioneer in allowing women to work in his research laboratory. Several of Robert's early female graduate students indicated that he gave them a chance when other faculty advisors denied them opportunities simply because they were female. Forty of Robert's 98 graduate students were women, including 28 of his last 50 students. We do not know how many female undergraduates worked in Robert's laboratory (he did not keep track of the number), but it had to be more than 40 based on memories of those of us that were around his program. Many of those female undergraduates went on to medical school and a few chose to go the graduate school route. Regardless, through Robert's efforts many young women were afforded an opportunity for higher education when others closed the door.

CONCLUSIONS

At the start of this manuscript, I had hoped to outline who Robert J. Baker was in terms of a graduate mentor. What did he do that worked so well? How did he shepherd such a diverse group of individuals through his graduate program? How did he maintain a highly successful graduate research program for such a long period of time? Hopefully, through my personal observations as both a former student and later as a colleague, I have provided some insight on these topics. In a nutshell, my assessment is that Robert's secret formula was simply to recruit the best students possible and make all of them better, no matter what their starting level was. It did not matter what your GRE scores were, what your academic pedigree was, or even what your previous exposure to evolutionary biology might have been. All of this improved significantly under his tutelage. It was his investment in us—his time, his energy, and his desire to see us succeed. Some of his students would have been successful in any graduate program at any university in the country (O. James Reichman and John W. Bickham, for example). Others, like Rodney L. Honeycutt and myself, were provisionally accepted to Tech only after Baker argued to the selection committee that there was more to being a graduate student than was indicated by one's GRE scores. Fortunately, for me and many of my academic brethren, Robert had the ability to take average kids and turn them into something worthwhile (see Rodney L. Honeycutt's letter in the Schmidly et al. chapter, this volume).

Another factor that may have played a role in Robert's success as a mentor was his constant interaction with his students. Because he spent so much time in the lab, he was always a continual presence in our daily lives. Further, he worked side-by-side with his students. His way of writing a paper or grant proposal was to gather his students and have everyone involved at some level. We often sat around a work table and wrote or edited as a group. This made his students interact, argue, debate, defend, agree, etc., through different iterations of the exercise. I think it helped us learn to question some ideas and to reinforce others. Further, it made us more comfortable in defending our position on a topic; perhaps it helped us become more confident in speaking on a professional level.

Did all 98 of Robert's graduate students achieve their desired outcomes following the completion of their degrees? That answer is beyond the scope of this paper—but I would assume the answer is probably no. However, I think most of us are pleased with the journey we took with Robert, what we learned, what we accomplished, and where we eventually landed. Although some of us followed Robert's footsteps and entered academia, others became employed at federal agencies, in professional health, in teaching positions, at biotech companies, etc. No matter where we wound up, I know Robert was proud of each of us and as he always said—"my students are my biggest success." I think it is safe to say that many doors were opened that would not have been available if it were not for Robert. Also, I suspect most of us would "do it all over again," with few modifications—maybe at the end of the day that becomes the greatest compliment we could give him.

EPILOGUE: WHAT IT MEANS TO BE IN THE BAKER LAB/FAMILY

Robert genuinely cared for his students, both as individuals and colleagues, and he treated us as such. He worked incessantly to make sure each student received the best possible training for our individual futures and success. He tested us daily and made us better scientists and citizens by constantly pushing the boundaries of our knowledge, abilities, and thoughts. He got us to think outside of our comfort zone. He treated us like peers, which resulted in us having to step-it-up a notch every day so that we remained his "equal." You worked "with" Robert, not "for" Robert. He formed a personal bond with most of us, a relationship that remained after we left Tech and pursued our own paths and endeavors.

Robert was never one to hold back, pull punches, or let a perceived injustice go unnoticed. Whether it was departmental politics or reclassifying the phyllostomid bats, there was always a controversy brewing on the horizon. Robert was no angel, and he never pretended to be, but as his student, you could be drawn into a fray by simply being part of the Baker lab. Consequently, there was a chance that you were guilty of some "crime" that you did not even know had been committed. Robert's baggage could be heavy and at various times in his career, I dare say, our grant proposals and manuscripts were scrutinized a little more intensely than they should have been…

However, being a member of the Baker family has had many advantages. First, there is no doubt that Robert's prestige and scholastic reputation helped many of us as we applied for additional degrees, postdocs, and jobs. He knew how to prepare us for interviews and he knew how to help market us. I do not mean to insinuate that we rode Robert's coat-tails, but I do think being a "Baker product" meant a little something extra —just like being a protegé of James L. Patton at the University of California at Berkeley, James H. Brown at the University of New Mexico, or E. Raymond Hall from the old days at the University of Kansas.

Second, each of Robert's former students know the "Baker program" and how students were trained within that system. We understand what was expected of our academic brothers and sisters and we know that each of us adopted a little of the "Bakerian Method." This becomes quite advantageous when recruiting graduate students ourselves. Several of my own students have come from programs run by former Baker students; consequently, I know how those students were trained and the standards to which they were held. In other words, I know what kind of student I will get. In reality, a great student is just a phone call away. Over the years, there have been several "pipelines" running from Lubbock to College Station, Stillwater, San Angelo, and many other places. Often the road runs both directions as a student earns a MS at one institution and then is "shipped off" to another for his or her PhD or Postdoc.

In Figure 1, I quickly outlined a version of my academic pedigree and that of some of my students and academic siblings. Obviously, several ancestral/descendent lines cross, double back, and circle around…. My academic pedigree involves two academic "uncles" (Ira F. Greenbaum and John W. Bickham both served on my MS committee) who later became "brothers"; a "nephew of sorts" (James J. Bull) who served as a postdoctoral mentor; and a "brother" (Rodney L. Hon-

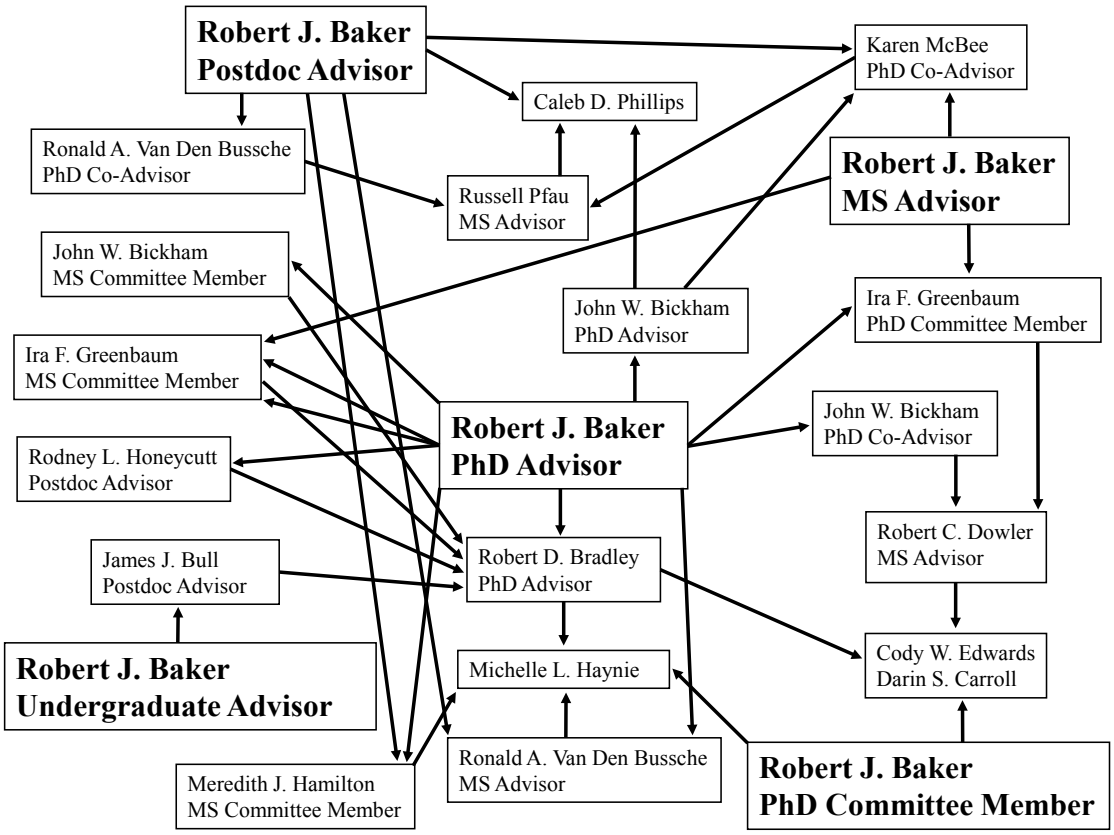

Figure 1. Diagram illustrating levels of academic relatedness of Robert J. Baker to some of his academic progeny. Bolded text reflects various academic roles played by Robert J. Baker. Arrows flow from "mentor" to student.

eycutt) who later served as another of my postdoctoral mentors. According to quick calculations using pathways of relatedness methodology and traditional population genetic values (parent-offspring = 0.5, grandchild = 0.125, etc.), Dr. Michelle L. Haynie has the distinct honor of having the highest relatedness value (2.125), just edging out Caleb D. Phillips (see Fig. 1). I use this illustration of Michelle's academic lineage as fondly as possible—as she represents the "knowing what you will get scenario." She received her MS degree at Oklahoma State University with Ronald A. Van Den Bussche (my academic brother; PhD with Baker), with Meredith J. Hamilton (academic sister; PhD with Baker) serving on her advisory committee—by the way, Ron and Meredith had both undertaken postdoctoral stints with Baker. Ron recommended that I recruit Michelle as a PhD student—so it was an easy choice… if my academic brother and sister said she was a good student, then she must be. Once Michelle set up her PhD advisory committee (with me as Chair), she added Baker as a member (her academic grandfather), thus helping complete the inbreeding web. Some would call this academic inbreeding; however, as my own ranching-educated son points out, "if it works, it is called line breeding, and that can be a very good thing!"

Finally, there is the comradery that exists among "survivors"! By pedigree and reputation, we know who our academic brothers and sisters are and many of us have become or have remained close friends throughout our careers. Because Robert had so many students and many of those students overlapped with each other, there has been a connection from one generation to the next. We know the stories of those that were here prior to arrivals and we share our stories and experiences with those that came after. We trade our favorite Baker stories when we encounter each other at scientific meetings. Everyone had to go find the candy bar and

everyone had to "scurry for cover"—so we have places to start a conversation and a thread to connect the first group of Baker students to his very last. We belonged to something great and there is a general "sense of family" shared amongst us.

Systematic mammalogy and evolutionary biology has lost a great mind and ambassador. However, we former students lost a great mentor, teacher, collaborator, surrogate father, and friend. Although Robert received numerous awards and honors, he always said his greatest success was his students—all 98 of them. In my eulogy and encomia, I mentioned that the loss of Robert had left some big shoes to fill—but a colleague reminded me "he gave you everything you need to fill them." He taught us a lot and he gave a lot of himself; in his absence, I realize that more and more each day. No doubt, Robert was one of a kind; but he lives a little in each of his academic sons and daughters (n = 98), grandchildren (n = at least 445), and great grandchildren (n = at least 549); a number totaling at least 1,092 (and growing).

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Literature Cited

Bradley, R. D. 2005. What the old dogs said: perspectives from a pup. Pp. 285–289 in Going Afield (Clyde Jones and Carleton J. Phillips, eds.). Museum of Texas Tech University, Lubbock.

Genoways, H. H., et al. 2018. Obituary: Robert James Baker (1942–2018). Journal of Mammalogy 99:983–1012.

Malloy, J. T. 1981. Molloy's Live for Success. William Morrow and Company, Inc., New York.

Schmidly, D. J., R. D. Bradley, E. K. Roberts, L. C. Bradley, and H. H. Genoways. 2019. A scientometric appreciation of Robert James Baker's contributions to science and mammalogy. Pages 1–38 in From field to laboratory: a memorial volume in honor of Robert J. Baker (R. D. Bradley, H. H. Genoways, D. J. Schmidly, and L. C. Bradley, eds.). Special Publications, Museum of Texas Tech University 71:xi+1–911.

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Just What Is a Naturalist? Thoughts on Natural History in the Early 21st Century

" I was born a naturalist." Charles Darwin

Robert C. Dowler

Abstract

The concept of a naturalist as one who studies natural history has cycled through multiple phases over the last few centuries. The 20th century in particular saw this field decrease in acceptance in the eyes of many scientists because of the increasing emphasis on research in cell and molecular biology. Today there has been a resurgence in the importance of natural history, yet still there is confusion about who is a naturalist or by what standards we should recognize someone's contributions in natural history. Despite this debate, it is clear that scientific naturalists make an essential contribution to documenting and understanding biodiversity on Earth. The general public is playing an increasing role with the advent of technological advances that allow them to assist in natural history studies. The participation of citizen scientists in natural history studies has resulted in a new awareness and appreciation for organismal biology. During the last two decades, there have been remarkable shifts in the way the public views the natural world. Scientific naturalists will continue to contribute to our understanding of nature in new and important ways, but public participation in natural history research will play an important part in gathering data for these scientists.

Key words: citizen science, eBird, iNaturalist, naturalist, natural history, organismal biology

Introduction

The concept of natural history and those who study it, i.e. naturalists, dates back as far as written language. William Beebe (1944) even suggested that the early cave dwellers of France and Spain were actually naturalists at least in some sense, based on their depictions of animals on the cave walls. Beebe also proposed, that since the advent of written language, Aristotle was the founder of natural history and the greatest naturalist of all time. Aristotle's written work, History of Animals, based largely on his observations on the island of Lesbos in the 4th century BCE, stood as the authoritative view of the natural world for 2000 years. With the scientific revolution around the middle of the 16th century came a growing interest in geology and the study of plants and animals. The earliest focus was on studying nature to improve human lives through an understanding of foods and potential medicinal uses of plants and, to a lesser degree, animals. Early herbals, books about plants, such as John Gerard's *The Herbal or General History of Plants* published in 1597, described the plants that were useful in curing ailments or enhancing health through better diets and often were well illustrated. These descriptions led to the first botanical gardens (e.g. Jardin du Roi in Paris, 1635), usually at the behest of royalty in western Europe, and these efforts allowed research into the benefits of particular plants and information to be shared across the continent. As the wealthy became more interested in natural history, the first cabinets of curiosity began to take shape and people began to collect and display objects found in nature. The growth of such collections ultimately resulted in the first public museums, such as the Ashmolean Museum in Oxford, England, dating to 1683. As this interest spread across Western Europe, and exploration of the world expanded the knowledge of the diversity of life, scientists began

to fill in the details about what was going to become geology and biology. Carl von Linné provided a universal naming system for both plants and animals and a system of classification that accelerated research on natural history. By the early 19th century, evidence was accumulating that fossils in the geological strata represented a history of past life (Cuvier and Brongniart 1811) and that history involved transmutation, change in lifeforms through time (Lamarck 1809). Charles Darwin's (1859) revelation of the mechanism behind the change brought new meaning to all of the descriptive studies before and since. Darwin was certainly not the first naturalist, but *On the Origin of Species* literally changed biology forever.

During the Victorian era of the 19th century, there was no question about what a naturalist was (Barber 1980). Everyone knew those who sought to better understand the natural world were included under this title. Throughout this time in England, natural history became all the rage. Individuals would become serious pursuers of natural history, gathering everything from fossils to mosses, ferns, flowers, butterflies, and birds. Clubs promoting specific aspects of nature sprouted up across the country. Many of these people were amateur naturalists hoping to make a contribution to science. Others were serious scientists who published results throughout their careers. Unfortunately, the study of natural history was not a lucrative career, and many had a difficult time finding a paid position, even in academic circles at universities and museums.

Perhaps the most famous naturalist of this time was Charles Darwin. Darwin considered himself a naturalist, writing in notes for his autobiography: "I was born a naturalist" (Colp 1980). From an early age he was outdoors hunting and collecting, and in general exploring the natural world near his home. This continued into his teenage years, much to the disappointment of his father, who as a physician, had higher hopes for his son. In Darwin's autobiography he singled out his father's opinion of him with a line recollected as: "You care for nothing but shooting, dogs, and rat-catching, and you will be a disgrace to yourself and all your family" (Darwin 1892). Darwin's career, after a degree in divinity from Christ's College at Cambridge University, was an immersion in natural history from the voyage of the H. M. S. *Beagle* to his self-funded career as a naturalist. He published works in geology, zoology, and botany, as well as his most recognized works on evolution by natural selection, sexual selection, and the descent of man—publications that are recognized as some of the most important biological contributions in history. Others in this time frame included Alfred Russel Wallace, Thomas Henry Huxley, and Darwin's colleagues and friends, Joseph Hooker, Director of Kew Gardens, and Charles Lyell, a prominent geologist. By this time the field of natural history, which once linked together geology, botany, and zoology, was being divided into the separate sciences of geology and biology.

By the late 1800s, natural history in both Europe and North America was starting to be taught in schools, but this may have removed some of the romance that the public had with the field (Barber 1980). As with many subjects taught in school, natural history became viewed as dull and the public's interest in learning scientific names and building collections waned. The writings of John Muir in the United States promoted the preservation of nature and played a critical role in communicating that natural areas were important and deserved protection. His interactions with another naturalist, Theodore Roosevelt, ensured that the U.S. national park system expanded to protect some of America's natural treasures. Most know of Roosevelt's commitment to expansion of the national parks and forests of the U.S., but few realize that his life both before and after the presidency was dedicated to natural history through exploration, field studies, and collection of scientific specimens that contributed greatly to the understanding of biology (Lunde 2016).

The 20th Century Decline in Natural History

The 20th century saw an increased emphasis on experimentation in biology, especially in the new field of genetics. This led to the further acceptance of a reductionist view in science—one that proposed that almost all questions in biology could be answered by delving into the cellular and ultimately the chemical nature of organisms, rather than understanding them as parts of communities and ecosystems. Reductionism

was in contrast to what some considered a holistic or Darwinian approach (Dobzhansky 1966). In the middle of the 20th century, with a newly acquired understanding of DNA and the true beginning of molecular biology, this movement away from natural history accelerated. Universities followed this trend and restructured departments to reflect the shift toward what was perceived as modern science and left behind the "old fashioned" field-based natural history research (Dobzhansky 1966; Futuyma 1998; Schmidly 2005). In addition to changes to university programs, funding for research moved toward cell and molecular studies, and soon the public's attention was drawn away from natural history and into the biotechnology era. To be fair, many scientists agreed with Dobzhansky (1966) that a union of what he called reductionist and compositionist or holistic approaches was essential for a real understanding of the biological world. This consolidation of ideas had occurred earlier in the century to form the Modern Synthesis (Huxley 1942), the idea that all science, including genetics, systematics, and paleontology, was in agreement that evolution has shaped our world and Darwinian natural selection plays an important role in that process. But as the 21st century approached it was clear that the computer age, technological advances in the way science could be conducted, and ultimately in the distribution of information and ability to communicate almost instantly brought on by the internet were having an impact on research in natural history. The increasing perception was that naturalists and studies of natural history were indeed "old fashioned" and there was the fear that the days were numbered for researchers who chose that career path in natural history (Noss 1996). A similar concern was expressed by Michael Mares (2002) about field naturalists of the 20th century and rigors of field studies that will continue to provide data for future researchers who never leave the confines of their research labs and offices.

> "All of our work in the Chaco in 1976 yielded fewer than 100 animals. The two trips in the 1990's brought in another 300 museum specimens… In the future some researcher sitting in a comfortable laboratory in an air-conditioned building will examine them, compare them to others, and make scientific decisions based on the animals that we collected. Given current trends that researcher may never have been in the field, his or her computer providing much of the information as to what is or is not a species. When they handle the animals, even if for just a moment, will they feel the stifling heat, the howling wind, the choking dust, and the vicious thorns? Will they feel the biting insects, see the desperately poor Indians, and taste the hot, salty drinking water? …I hope that somehow they will appreciate that these specimens were collected by field biologists, a diminishing group of researchers willing to go into nature to seek out new life forms and learn new facts about animals in their native habitats. Although many of the specimens are of common species, I want them to know that nothing about those specimens is common." (Mares 2002)

Natural History in the Recent Past

Several biologists near the end of the century addressed this trend of the decline of naturalists. In an editorial piece in *Conservation Biology* titled "The Naturalists are Dying Off", Reed Noss (1996) lamented that the cause of much of this shift away from natural history was "our increasing separation from Nature." He pointed to changes in biology curricula that no longer emphasized field aspects of biology and genuine field experiences for students. His plea, in part, was to resist what he called the trend toward "indoor biology." Both Douglas Futuyma (1998) and David Schmidly (2005) echoed this concern that university courses should not move away from presenting an understanding of whole organisms and the biodiversity of the world, but rather should balance these offerings against the conceptual and theoretical approaches that dominate many biology programs today. All three of these authors presented the case that natural history research has as much or more value and importance to modern biology as it ever has. Ecological modeling, for example, requires field-based data on species, and those data, for the majority of species on Earth, still do not exist. We, as biologists, should be encouraging students to study natural history, to become the next generation of naturalists who provide the data necessary to understand large-scale changes in the biosphere

and propose shifts in the conceptual understanding of biological phenomena. The field of natural history is in the process of rebirth rather than being viewed by many as a quaint artifact of the past.

The Amateur Naturalist Versus the Scientific Naturalist

It is clear that academic training for naturalists was rare through much of history. Georges-Louis Leclerc, Compte de Buffon studied mathematics, Charles Darwin's degree was in divinity, and Alfred Russel Wallace was self-trained. Toward the end of the 19th century and into the 20th, universities began to have degree programs that emphasized natural history. But in the past 100 years, the word naturalist took on a pejorative context that implied an amateur field biologist, rather than a true scientist (Futuyma 1998; Schmidly 2005). Futuyma (1998) in particular, argued that the term naturalist has been and continues to connote someone who is anything but an amateur:

> "I think of a scientific naturalist as a person with a deep and broad familiarity with one or more groups of organisms or ecological communities, who can draw on her knowledge of systematics, distribution, life histories, behavior, and perhaps physiology and morphology to inspire ideas, to evaluate hypotheses, to intelligently design research with an awareness of organisms' special peculiarities." (Futuyma 1998)

Amateur naturalists have often contributed much to scientific endeavors, but Bates (1950) distinguished between what he called amateur naturalists and nature lovers. His view was that many amateur naturalists have contributed to our basic knowledge (and some have rivaled scientists in this regard), but nature lovers are those that let emotion trump their objective recording or reporting of observations in natural history. He pointed out that low salaries in the field of natural history have caused some to seek alternate employment through their lives, but these often contribute their free time to scientific study of natural history (Bates 1950).

Systematic Collections and Natural History

Parallel to the perceived decline in natural history was a related view that the systematic collections of the world were less and less important with the growing wave of molecular studies. Historically, those who collected specimens and deposited them in collections, as well as the systematists who used the specimens, were considered naturalists. Over the past century, systematic collections grew considerably, but recently active collecting has declined. Part of this certainly has to do with the increasing level of difficulty in securing permits required as well as the public's negative perception of collecting. The number of collections grew through most of the 20th century but, in general, has leveled off recently. The consolidation of existing collections and decrease in establishment of new collections has contributed to this trend, although there have been an increased number of collections in some developing countries. The reduced growth in collections has coincided with the decline in natural history studies and the increased emphasis on molecular research. Data showing the decline in collections are documented for herbaria and vertebrate collections (Tewksbury et al. 2014) and mammal collections (Dunnum et al. 2018).

The decline in systematic collections is especially alarming in that the need to understand and document biodiversity are increasingly important in light of the high rate at which human-caused extinction is occurring (Ceballos et al. 2015). The reliance of the fields of disease ecology, molecular systematics, and climate change, among many others, on specimens and tissues residing in systematic collections is further evidence of the increasing need for continued collecting and long-term archival deposits in museums (Funk 2018; Hope et al. 2018; Schindel and Cook 2018). In the same way, historic collections of specimens are being used in ways never imagined, adding unanticipated value to specimens collected decades before. Included in this list are disease screening, 3D imaging, distribution modeling, stable isotope analysis for food habits (e.g. Blight et al. 2015), and extraction of DNA from historic specimens (e.g. McDonough et al. 2018). In

contrast to the increasing use of scientific specimens is the perception by some that scientific collecting is no longer essential and can have negative effects on rare species (Minteer et al. 2014), although this view was contested by more than 100 authors (Rocha et al. 2014). There is consensus among systematists that collecting of vouchers and archival preservation of specimens is still an essential part of documenting the Earth's biodiversity.

The Citizen Scientist Movement

As mentioned, amateur naturalists have played a significant role in contributions to and the popularity of natural history research over at least 2 ½ centuries. The idea of bird watching, in contrast to bird collecting, was proposed in the 1880s by, among others, Florence Merriam in *Birds Through an Opera Glass* (Merriam 1889). The first Christmas Bird Census was proposed by Frank M. Chapman in 1900 to counter the Christmas "Side Hunt" that had been popular for years. This was a tradition where hunters would choose sides and see which team could come back with the largest pile of birds and mammals. The Christmas Bird Census, by the then recently-formed Audubon Society, was one of the early efforts to involve the general public, alongside scientists, in collecting data that became valuable additions to our knowledge of the natural history of animal species. In the 118th year of what is now called the Christmas Bird Count, 2,585 individual counts were conducted by 76,987 participants and 59,242,067 birds were documented (https://www.audubon.org/news/the-118th-christmas-bird-count-summary). Although the public has had an interest in natural history to varying degrees throughout the last century, a change that has brought natural history back to the attention of the public is usually termed citizen science. It is now clear that members of the public who might be called amateur naturalists have made striking contributions to science.

Over the past two decades, millions of people across the globe have been involved in citizen science projects that have contributed worthwhile data to natural history studies. The results of these public efforts have increasingly changed our scientific view of the natural world. Much of this activity has become possible because of several major shifts in technology. The first is the availability of digital photography. Unconstrained by the use of film for photographic images, the cost of photography declined to the point that anyone could take literally thousands of photographs inexpensively. This, coupled with the development of a camera on mobile phones, gave an unprecedented ability to take an image of something in nature at the time it was observed. Much of the world's population now has the ability to take a digital image almost anytime and anywhere. GPS applications on phones also have simplified the task of providing a geographic tag to each image. Lastly, the development of apps on mobile phones and other devices made reporting data and images easy for anyone to do. There are many examples of projects that have benefited from these new "naturalists" but I will use three examples to illustrate the potential impact that citizens can now have in the field of natural history. These three are iNaturalist (inaturalist.org), eBird (ebird.org), and Snapshot Serengeti, a project administered through Zooniverse (www.zooniverse.org/projects/zooniverse/snapshot-serengeti).

The development of iNaturalist in 2008 began as a final project by Nate Agrin, Jessica Kline, and Kenichi Ueda for the Master's degree in the University of California, Berkeley's School of Information (Agrin et al. 2008). The iNaturalist site allows anyone to upload observations in the form of images of organisms with specific georeferenced data and have the public and authorities provide identification for the taxon. In 2014, iNaturalist merged with the California Academy of Science (CAS) and in that year recorded its one-millionth observation. Since then, iNaturalist, now sponsored by CAS and the National Geographic Society, has become one of most successful platforms in the world for natural history observations. As of January 2019, more than 1,135,000 people have registered to use iNaturalist and more than 15.5 million observations have been posted of more than 195,000 species of organisms. The growth of iNaturalist over the past 11 years, both in users and observations reported, has been rapid and consistent (Fig. 1). Although not all images

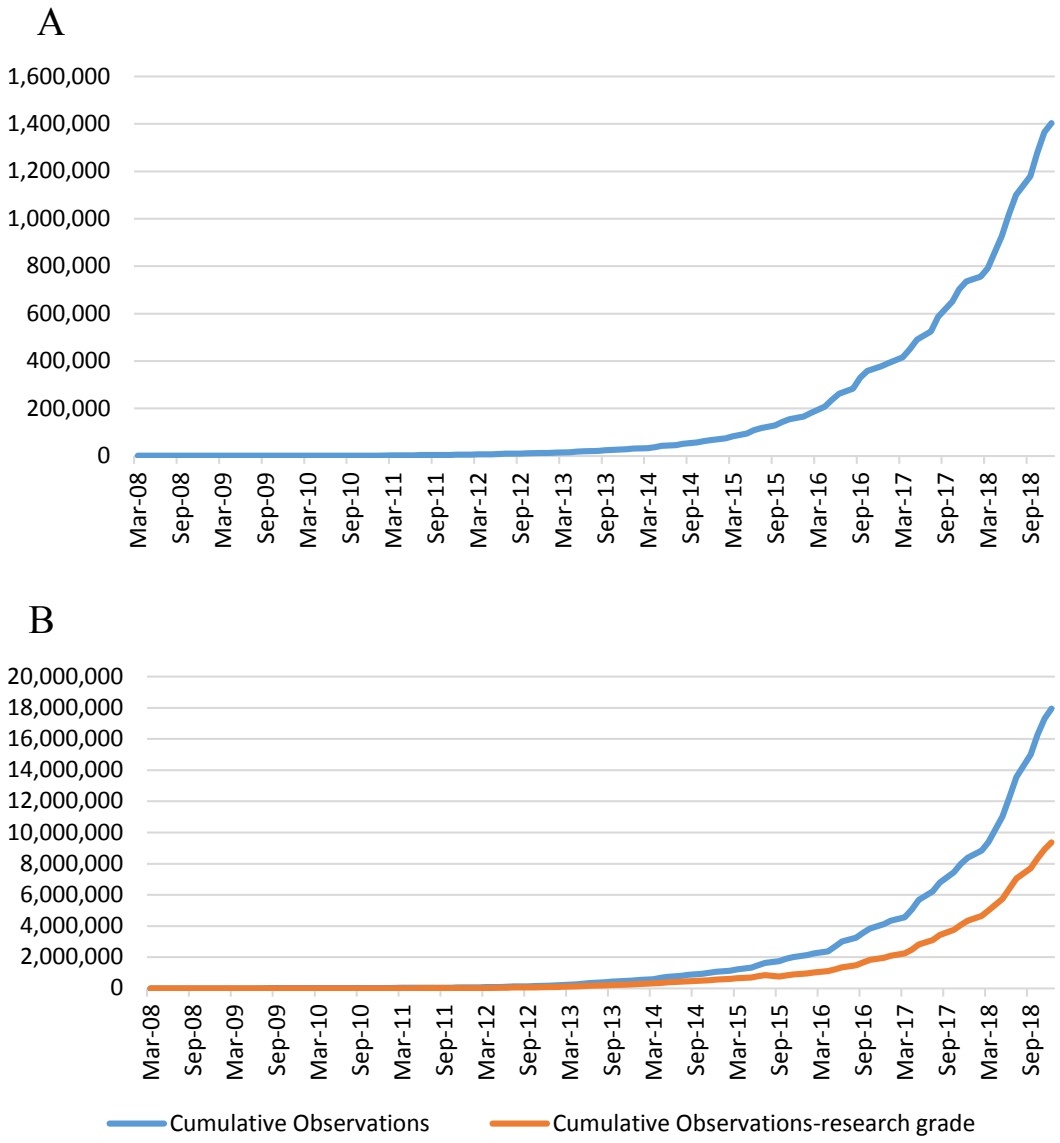

Figure 1. Cumulative number of iNaturalist users (A) and observations (B) from inception of iNaturalist in 2008 through 2018. Research grade observations are those that have GPS coordinates and have been identified by two or more reviewers. Data courtesy of iNaturalist.

can be georeferenced or identified to species, millions have been and are designated research grade, meaning that multiple identifications are in consensus. These observations have been used to better understand distributions of animals, plants, fungi, and other organisms, as well as allow further understanding of the biology of thousands of species. These data also can be used in predictive models, including shifts in distribution as a result of climate change (e.g. Fourcade 2016).

Another very successful platform for reporting observations is eBird (Sullivan et al. 2009), started in 2002 by the Cornell Lab of Ornithology. The original idea was to enable birdwatchers to report data in the form of lists from specific areas at a specified time period and maintain the data across the world. This has been boosted by collaboration with hundreds of partner organizations. Since its inception, it has grown to the largest biodiversity-related citizen science project on

Earth with more than 100 million observations made each year. By making a mobile device app, now in 27 languages, available at no cost, hundreds of thousands of people participate in recording birds and these cumulative sightings provide a remarkable data set of patterns of bird occupation and movement across the world (e.g. Fink et al. 2013). The eBird organization stores and archives the data and makes it available to anyone. The current eBird web site provides assistance to birders on identification and works to maintain data quality by providing lists of species that birders are likely to encounter. The mobile device app flags those reports that are deemed rare by authorities and requires additional information on those unusual sightings. All images and recordings submitted through eBird are archived in the Macaulay Library of the Cornell Lab of Ornithology, a collection that now is approaching 10 million photographic images and has more than 415 thousand sound recordings (https://search.macaulaylibrary.org/catalog). The result has been an immense and growing data set that has had major impacts on ornithological research and conservation of birds (e.g. Amano et al. 2016).

Just as iNaturalist and eBird have benefited from the public's use of digital images of organisms to verify identifications, the field of wildlife biology has benefited from the shift from film to digital images generated from remote cameras used in camera-trap studies. Although the use of cameras in natural history studies dates probably to the 1890s, their consistent use in wildlife studies exploded in the late 1980s and 90s with the development of infrared triggers of motion that could be captured on film (Cutler and Swann 1999). As high resolution digital cameras became commercially available during the first decade of the new century, camera trapping technology rapidly followed, and with it a rebirth in wildlife studies that relied on this methodology; however, no longer was a camera restricted to a roll of 36 slides or negatives. The number of images was limited only by SD card size and battery life. Soon camera-trapping studies increased to provide cost-effective ways to address even more kinds of wildlife research than before, such as behavioral studies (e.g., nest defense, seed dispersal, and activity budgets in birds), density estimates, and occupancy modeling (O'Brien and Kinnaird 2008; De Bondi et al. 2010).

One large-scale study in Tanzania utilized 225 cameras within a 1,125 km^2 grid in Serengeti National Park (Swanson et al. 2015). From June 2010 through May 2013, the camera trap grid yielded 1.2 million image sets (1 to 3 images taken in rapid succession). To address the huge task of identifying and quantifying the species on the images, the researchers devised an innovative solution that utilized citizen scientists through a platform called Zooniverse (www.zooniverse.org). A website was established and volunteers, after following a simple tutorial, were asked to identify and count animals in the images (and identify images in which there were no animals) as well as record behaviors. Remarkably, after only three days, volunteers had submitted one million species classifications and removed an 18-month backlog of data (Swanson et al. 2015). Over the course of the study, more than 28,000 registered users provided 10.8 million classifications. The same image was provided to multiple users to establish consensus and a subset of images was evaluated by experts. When identifications were analyzed for accuracy by comparison with those made by experts, the overall species identification accuracy by citizen scientists was a remarkable 97.9% (Swanson et al. 2015).

The Potential Decline of Future Naturalists

I have a deep concern that young people today do not have the exposure to nature that was so typical for many naturalists in their childhood. Many have expressed similar concerns that in the digital age, fewer and fewer children are spending time outdoors (Louv 2005). As a child, I spent a considerable amount of time exploring the fields, forests, and waterways of northeastern Ohio. I observed organisms on every outing and that piqued my curiosity. That led to trips to the library to find out more about what was known about the animals and plants of my area. I cannot remember a time that my parents were actively involved in these explorations; rather, it was friends and I who took off to explore somewhere that we had never been. Those friends with similar interests and I started collecting animals and I learned a lot about wildlife by capturing

animals in the wilds near my home. Growing up, I shared another thing with many present and past naturalists. Bates (1950) wrote "The commonest first sign of a developing naturalist is the collecting habit." I had the collecting "gene" and found myself assembling my own cabinet of curiosities that included rocks and fossils, skulls, birds' nests, and bird wings from road-killed birds. Most of my collecting, however, was live animals from our adventures in nature. At one time or another we kept frogs, snakes, turtles, rats, squirrels, raccoons, a woodchuck, muskrat, red fox, and great horned owls. All this came to an unpleasant end when our "zoo" was visited by area game wardens and they explained the state and federal laws to young teenagers and my parents.

Many naturalists have suggested that instilling the wonder of nature in the youth is the key to an informed public on the topic of natural history. That, as it turns out, is a tall order. Competing with the lure of nature is perhaps the equally appealing lure of the digital world. When I was young, television was the competition for going outdoors. Some television actually stimulated our draw to nature. For many, television shows like Wild Kingdom that aired between 1963 and 1988 introduced the natural world to America. This served to interest young and old in the natural world. Today, many television programs have done the same thing for another generation. PBS series like Nova and Nature have grabbed the attention of many young people and drawn them into the idea of at least considering careers in natural history. Their popularity led to entire networks devoted to nature filming such as Animal Planet and the Discovery Channel. Unfortunately, experiencing nature vicariously through television or a computer screen is usually an insufficient incentive to go out into nature. This hesitancy to actually experience the natural world is compounded by often-irrational fears of risks associated with animals and field studies (Louv 2005; Hafner 2007)

Despite the potentially positive influences, with the advent of the internet, YouTube, Google, Facebook, and video games, the idea of getting out in nature has become far less enticing than when I was growing up. If there is any chance to reverse this dearth of experience in the natural world—what Robert Pyle called the extinction of experience (Pyle 2001)—perhaps it is in promoting activities that continue to give students an experience in nature. School programs that require students to be outside and collect data on natural environments nearby have proven to be a positive influence on student learning (Louv 2005), but these programs are rare across the United States. Many have now pointed out that an increasing problem will be that we are no longer training the career naturalists who can inspire the next generation of naturalists (Noss 1996; Futuyma 1998; Wilcove and Eisner 2000; Pyle 2001; Schmidly 2005). Other ways to get young people involved and outdoors include volunteer programs at nature centers, natural history museums, and zoos. Training opportunities for the public, like the Master Naturalist programs now in almost every state, are another positive step. As an example, the Texas Master Naturalist Program has now trained almost 10,000 people who have volunteered an estimated 2.8 million hours of service in nature activities (https://txmn.org/about/want-to-know-more/). However, those of us who have taught classes in the program can attest to the fact that these classes are dominated by retired people, not the youth of Texas.

CONCLUSIONS

So what makes someone a naturalist? Many have described characteristics of a naturalist but here is my view. A naturalist appreciates the natural world in a way that generally goes beyond that of the "normal public". Does that make one abnormal? ….absolutely. The normal person does not ask why the animals and plants around them are there, why they have the characteristics they do, and how they interact with the other organisms and the abiotic environment around them. Naturalists have a boundless curiosity about life. It drives them to make observations that would be passed up by many. These observations cause them to ask questions and look for answers—the very essence of scientific research.

Most naturalists have a specific area of expertise but are well versed in many areas of natural history. Their interests often go well beyond the area on which they may have focused their training. Naturalists also communicate to others their findings. Some of this may be formal scientific publications or presentations before other scientists, or it may take the form of less formal presentations that educate the public. In today's world, that includes blogs or podcasts that are followed sometimes by thousands of people.

And what of citizen scientists? Is everyone who participates in citizen science projects a naturalist? Certainly not—but the opportunity to participate brings the interested public closer to the process of research and that, in itself, has long-term benefits for the continuation of natural history. The knowledge in the public's eye that natural history data have value is one critical step for continued appreciation in many areas of research and reason for support of increased funding for it. Scientific naturalists who contribute to our wealth of knowledge continue to be an essential part of the scientific understanding of our world, and the public's participation in the process will continue to be an important part of that research.

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Robert J. Baker was a naturalist. It was my privilege to have known him for over 40 years. He exuded an enthusiasm for life, both in academia and in the natural world as a field biologist. His mentoring of students also resulted in another generation of naturalists who have had an impact across the world. In addition to Robert, I would like to thank other mentors of mine who all played a critical role at times over my career. These include Peter Dalby, Hugh H. Genoways, Jerry R. Choate, John W. Bickham, David J. Schmidly, and Terry C. Maxwell. I would also like to thank a cadre of students, a few who worked with me at Fordham University and many in my 30 years as a professor at Angelo State University. I would like to think that I have instilled in at least some of them the joy of being a naturalist. Lastly, my wife Paula has supported my career as a naturalist in ways that go beyond description. Thank you to her for facilitating the opportunity to do what I do.

The topic of this manuscript began as an oral presentation I gave as out-going President of the Southwestern Association of Naturalists. Further discussions with colleagues, students, and friends about our mutual concern for the field of natural history convinced me that it would be appropriate as a written paper. Thanks to all of you who have expressed opinions. Thanks also to Loren K. Ammerman, Michael A. Mares, and an anonymous reviewer who made improvements to an earlier version of the manuscript.

LITERATURE CITED

Agrin, N., J. Kline, and K. Ueda. 2008. iNaturalist.org: final project write-up. School of Information, University of California, Berkeley.

Amano, T., J. D. L. Lamming, and W. J. Sutherland. 2016. Spatial gaps in global biodiversity information and the role of citizen science. BioScience 66:393–400.

Barber, L. 1980. The heyday of natural history 1820–1870. Doubleday and Company, Inc., Garden City, New York.

Bates, M. 1950. The nature of natural history. Charles Scribner's Sons, New York.

Beebe, W. 1944. The book of naturalists: an anthology of the best natural history. Alfred A. Knopf, Inc., New York.

Blight, L. K., K. A. Hobson, T. K. Kyser, and P. Arcese. 2015. Changing gull diet in a changing world: a 150-year stable isotope (δ13C, δ15N) record from feathers collected in the Pacific Northwest of North America. Global Change Biology 21:1497–1507.

Ceballos, G., P. R. Ehrlich, A. D. Barnosky, A García, R. M. Pringle, and T. M. Palmer. 2015. Accelerated modern human–induced species losses: entering the sixth mass extinction. Science Advances 1(5):e1400253

Colp, R., Jr. 1980. "I was born a naturalist": Charles Darwin's 1838 notes about himself. Journal of the History of Medicine and Allied Sciences 35:8–39.

Cutler, T. L., and D. E. Swann. 1999. Using remote photography in wildlife ecology: a review. Wildlife Society Bulletin 27:571–581.

Cuvier, G., and A. Brongniart. 1811. Essais sur la géographie minéralogique des environs de Paris, avec une carte géognostique et des coupes de terrain. Boudouin, imprimeur de l'Institut Impérial de France, Paris.

Darwin, C. 1859. On the origin of species by means of natural selection or the preservation of favoured races in the struggle for life. John Murray, London.

Darwin, F. 1892. Charles Darwin: his life told in an autobiographical chapter, and in a selected series of his published letters. John Murray, London

De Bondi, N., J. G. White, M. Stevens, and R. Cooke. 2010. A comparison of the effectiveness of camera trapping and live trapping for sampling terrestrial small-mammal communities. Wildlife Research 37:456–465.

Dobzhansky, T. 1966. Are naturalists old-fashioned? American Naturalist 100:541–550.

Dunnum, J. L., B. S. McLean, R. C. Dowler, and the Systematic Collections Committee of the American Society of Mammalogists. 2018. Mammal collections of the Western Hemisphere: a survey and directory of collections. Journal of Mammalogy 99:1307–1322.

Fink, D., T. Damalous, and J. Dave. 2013. Adaptive spatio-temporal exploratory models: hemisphere-wide species distributions from massively crowdsourced eBird data. Proceedings of the Twenty-Seventh AAAI Conference on Artificial Intelligence: 1284–1290.

Fourcade, Y. 2016. Comparing species distributions modelled from occurrence data and from expert-based range maps: implication for predicting range shifts with climate change. Ecological Informatics 36:8–14.

Funk, V. A. 2018. Collections-based science in the 21st Century. Journal of Systematics and Evolution 56:175–193.

Futuyma, D. J. 1998. Wherefore and whither the naturalist? American Naturalist 151:1–6.

Hafner, M. S. 2007. Field research in mammalogy: an enterprise in peril. Journal of Mammalogy 88:1119–1128.

Hope, A. G., B. K. Sandercock, and J. L. Malaney. 2018. Collection of scientific specimens:benefits for biodiversity sciences and limited impacts on communities of small mammals. BioScience 68:35–42.

Huxley, J. 1942. Evolution: the modern synthesis. George Allen and Unwin, London.

Lamarck, J. B. 1809 [1984]. Zoological philosophy: an exposition with regard to the natural history of animals. Translated by H. Elliot. The University of Chicago Press, Chicago, Illinois.

Louv, R. 2005. Last child in the woods: saving our children from nature-deficit disorder. Algonquin Books, Chapel Hill, North Carolina.

Lunde, D. 2016. The naturalist: Theodore Roosevelt, a lifetime of exploration, and the triumph of American natural history. Crown Publishers, New York.

Mares, M. A. 2002. A desert calling: life in a forbidding landscape. Harvard University Press, Cambridge, Massachusetts.

McDonough, M. M., L. D. Parker, N. R. McInerney, M. G. Campana, and J. E. Maldonado. 2018. Performance of commonly requested destructive museum samples for mammalian genomic studies. Journal of Mammalogy 99:789–802.

Merriam, F. A. 1889. Birds through an opera glass. Riverside Press, Cambridge, Massachusetts.

Minteer B. A., J. P. Collins, K. E. Love, and R. Puschendorf. 2014. Avoiding (re)extinction. Science 344: 260–261.

Noss, R. F. 1996. The naturalists are dying off. Conservation Biology 10:1–3.

O'Brien, T. G., and M. F. Kinnaird. 2008. A picture is worth a thousand words: the application of camera trapping to the study of birds. Bird Conservation International 18:S144–S162.

Pyle, R. M. 2001. The rise and fall of natural history: how a science grew that eclipsed direct experience. Orion Autumn 2001:16–23.

Rocha, L. A. et al. 2014. Specimen collection: an essential tool. Science 344:814–815.

Schindel, D. E., and J. A. Cook. 2018. The next generation of natural history collections. PLoS Biology 16(7): e2006125. (https://doi.org/10.1371/journal.pbio.2006125)

Schmidly, D. J. 2005. What it means to be a naturalist and the future of natural history at American universities. Journal of Mammalogy 86:449–456.

Sullivan, B. L., C. L. Wood, M. J. Iliff, R. E. Bonney, D. Fink, and S. Kelling. 2009. eBird: a citizen-based bird observation network in the biological sciences. Biological Conservation 142:2282–2292.

Swanson, A., M. Kosmala, C. Lintott, R. Simpson, A. Smith, and C. Packer. 2015. Snapshot Serengeti, high-frequency annotated camera trap images of 40 mammalian species in an African savanna. Scientific Data 2:150026.

Tewksbury, J. J., et al. 2014. Natural history's place in science and society. BioScience 64:300–310.

Wilcove, D. S., and T. Eisner. 2000. The impending extinction of natural history. The Chronicle of Higher Education 47(3):B24.

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In Appreciation of Robert J. Baker

Barry Lopez

I did not know Robert Baker well. He and I saw each other only rarely, once or twice a year during my regular visits to the Texas Tech campus. We quickly developed, however, an enthusiastic personal relationship. We respected each other's professional pursuits, mine as a writer, his as a biologist and academic. He made a great and positive impression on me over the years as a dogged researcher and educator. I can easily understand why he was revered by his colleagues, and why he was held in such high regard by students, faculty, and administrators at Tech. Looking back, what stands out most for me, though, is his concern for the future of his students.

I arrived at the university in 2003, a Visiting Scholar with an idealized view of how life in a university setting worked. Robert didn't disparage those innocent ideals of mine, but he instructed me more fully in how to manage in a sea of university politics. He also encouraged me to develop more completely the sense of responsibility I already felt toward the students at Tech. He spoke of this commitment as though it were the *sine qua non* of teaching in higher education. He was a Horn Professor in Biology and I was a writer who occasionally addressed subjects in his field; but we each regarded the obligation to teach impressionable young students well and carefully as not only crucial but daunting.

I traveled to Tech every year, once in the spring and once in the fall, for ten days each time, for fifteen years. Bob and I almost always managed to have dinner together. Occasionally a few graduate students joined us. We had spirited conversations about research questions in biology and about university and local politics. I was periodically in over my head with him when it came to things like molecular phylogenetics and gene sequencing, but I always found these late-night discussions invigorating and thought provoking because they bore on the fate of the larger world, not just intellectual life on a university campus. After dinner, Bob and I usually wandered off to enjoy a couple of Cuban cigars together. His treat.

It was mostly during those long evenings together, at his home and in Lubbock restaurants, that I came to appreciate how deep Bob's passion was for empirical science and how serious he was about education. The professional work each of us did, I thought, was vaguely similar, preparing sometimes complex material for the edification of students and readers. And those dinner sessions inspired me not only to get better in the classroom but to push into new territory of my own, and to remain as cognizant of the reader's needs as Bob, in his world, was of his students' needs.

Robert once invited me to speak to a freshman class he taught every year—Biology for Non-Biology Majors. He had prepped me on that day's particular topics and, grounding my presentation in my own field experience, I hoped to lead a discussion with the students that would meet with Bob's approval. As I recall, he passed rather perfunctorily over my classroom pedagogy—"that was good," he said—and moved straight on to another subject: he wanted to tell me that, in his view, I had related very well to the generalized life experience of incoming freshmen, understanding their anxieties, their hesitation to speak in class, their misconceptions about college, their naiveté about the world, their aspirations. He told me, "You have to get to know them. You have to care what happens to them, and you have to convey to them that you care."

I understand passion, enthusiasm, and long-term commitment to something outside one's self. That's why I loved Robert Baker. As important and as impressive as those 449 papers he published are, and as deserving as he was of all the honors, the awards, and the accolades, it was seeing him that day with his students—a senior professor who insisted on still teaching freshmen, specifically those who were not going to go on to make a career in his chosen field—that for me went to the heart of Robert Baker's greatness. He cared what happened to them. He believed a college education would give every one of them a better chance to find their way in the world. I believe he actually measured his own success by the success of his students.

He was, for me, someone who understood what he was on Earth to do.

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Encomia

In the closing section of this memorial volume, we have included 54 encomia. The word encomium is Latin based on the Greek word *enkōmion*, meaning *en*, in + *kōmos*, revel or celebrate. Traditionally these have been thought of as poetic speeches or songs to honor someone. The Greeks used them as songs for champions of the Olympic Games, sung at a celebration of victory. They can also be given at funerals, a eulogy, to praise the person who has passed away. The 54 small songs/speeches of praise that follow were written as very personal messages from those that knew Robert best.

Robert James Baker (1942–2018)

Photograph courtesy of Southwest Collection/Special Collections Library, Texas Tech University. Reprinted from: Genoways, H. H., et al. 2018. [Obituary] Robert James Baker (1942–2018). Journal of Mammalogy 99:983–1012.

SERGIO TICUL ALVAREZ

I met Robert Baker at the ASM Congress in 1990 just when I had finished my bachelor studies in biology, and he was already a highly renowned professor. The first day (social event), I remember I felt as a *Neotoma* in a Sherman, overwhelmed with so many personalities but at the same time trying to set myself free from those visible and invisible barriers that tightly surround someone new in a community. Of course, it increased with my not very good use of English, which at that time was worse than it is now. One of the people who got close to on check the *Neotoma* who was standing in the corner and didn't know whether to run or not, was Robert Baker. He approached kindly to start a conversation possibly because it was a new and off-centered face in the corner.

We started chatting for a while, in my case making an effort to understand, and he was trying hard so I could understand him. He then found out who I was and told me that he had personally met my father. During the rest of the congress, we met more frequently and together with Knox Jones, they both adopted me and were my support. Thanks to them I have been coming back to ASM congresses periodically.

My relationship with Robert was one that can be considered strange. There was much empathy between us, and he occasionally had some time to share with me in all the congresses or events where we had the opportunity to meet, and at least we had a beer together. I never had the fortune of sharing time with him in a class, field trip, or in a publication. The closest experience was in 2003 when I had the intention of taking my sabbatical with him in Texas Tech. In fact, I visted him in his laboratory and had a maginificent dinner at Carleton Phillips' home, but when I got back to Mexico, due to different policies of my institution, I was not allowed to take my sabbatical and lost one of the best academic opportunities of my life.

Why did I mention that our relationship was considered strange? He and my father both had diabetes, that disease that little by little kills people, so he knew I understood him in many aspects of the disease and that I knew what it caused, as well as its ups and downs. Robert also understood that the disease could cause him similar symptoms to those my father experienced, so he would ask constantly "How did he worked it out in field? How did he do it in the laboratory? How was his life in general?"

I can assure you that a great empathy existed between them although they did not show it directly because they happened to meet very few times, and I was the bridge that linked them together. Besides we always talked about college football; he was taken with the Texas Tech "Red Raiders," and personally I have always been a football fan more at college than professional level. Consequently, I was always bombarding him with question because my access to games was very limited, including on TV. We talked about the outstanding teams and different aspects, especially about managing college teams internally.

The two ocassions we had more time to be together was in Jalapa, Veracruz in 2012, when he was granted the "M en C. Ticul Álvarez Solórzano" academic excellence award by the Asociación Mexicana de Mastozoología A. C., and in Jacksonville, Florida in 2015. At that time, we had much more time to talk because we shared another thing, unfortunately not a good one. Robert had lost his son. I had also had the same experience before, and I understood his feelings at that moment and remembered the saying "time heals all wounds," scars endure and they ocassionally open up with no reason at all.

MIKE ARNOLD

I remember vividly my first sight of Robert. I walked into his 6th floor lab and asked a guy with long hair, an unkempt beard, wearing beat-up sandals that were propped up on a desk, if Dr. Baker was in. He gave what I came to recognize as a patent Robert grin – the one that always crinkled his eyes to even smaller-than-usual slits – and said, 'that would be me'. I suspect Robert liked shocking uptight West Texas boys. It worked on me. I wrestled at times with Robert's approach to life, the universe and everything, but he was without any doubt the person who turned me into the scientist – the evolutionary biologist – I am today. Robert had an ability to ignite, or at least encourage, a passion for doing science. This ability is very, very rare. I don't have it, and I know few who do. I will always miss my mentor.

JOAQUÍN ARROYO-CABRALES

On a personal view, Robert taught us that the scientific endeavor was learned not just in the classroom, but by developing the skills that sooner or later were required as researchers. These skills included the capacity to write projects and proposals, as well as submitting those to different agencies, honesty in participating in the peer review process, either of proposals or manuscripts, the writing of texts based on the lab work done after established protocols, and the lab recording of those, all of that being done even as a student with the normal courses load. An example of

important advice to me was to write daily at least 15 minutes, for your thesis, dissertation, an essay, or a manuscript. If I correctly understood his teachings, I could say that Robert did not believe in genius moments, but on the systematic and continuous work as being responsible for the achievements. Also, Robert was a humanist that showed his solidarity as required, supporting friends, colleagues, and students in and beyond the academic world. Whenever he learned of an issue that required any support, he was willing to provide it, whether it was a congratulation letter to Ticul Álvarez at the time of being granted with a UNESCO award or a journal membership for a Cuban college. On this regard, I think Robert was a true guardian, and not just an advisor, shown as considering his students as "academic children," including myself and my "brothers" and "sisters" that have gone through his lab.

AMY BICKHAM BAIRD

I am lucky to have known Dr. Baker my whole life. He was first a family friend, who had mentored my father as a PhD student and they remained close friends and colleagues for the rest of their careers. I grew up watching these two brilliant scientists sit around our dining room table writing papers together, discussing ideas for projects, interpreting results, and telling stories of their time together in the field. It is no wonder I wanted to follow in their footsteps.

When I decided to go to Texas Tech for my bachelor's degree, Robert became my mentor. Robert treated me like his graduate students, assigning me independent research projects and requiring me to present my results at local, national, and international meetings. At first, I was terrified of public speaking, but Robert knew that challenging me to do it would be valuable for my future. Of course, he was right, and I am so thankful that he pushed me out of my comfort zone. As a sophomore, he let me travel to Chernobyl to participate in a conference and see my research sites first-hand. I did not know how unique my undergraduate research experience was at the time, I just knew that I loved it. I ended up publishing four papers and giving about 10 talks at meetings in my three years at Tech. No other mentor could get that kind of productivity from an undergraduate!

Robert continued to support me after I graduated from Tech by serving on my dissertation committee and collaborating on additional research projects. When I was in graduate school, I came home to visit my parents while Robert was there writing "Bats of Jamaica" with my dad. They asked to join them and Robert handed me a copy of the manuscript and wanted my opinion on it. I can remember that as being one of the first times I truly felt like a scientist. That Robert, someone whom I had admired and looked up to as a mentor for years, treated me like an equal was a moment I will never forget.

I decided to become a professor at a university where I could concentrate on mentoring undergraduates to pass on some of what I learned from Robert. My time as an undergraduate in Robert's lab was the most fun, challenging, and arguably the most productive period of my education/career. Without a doubt, I would not be where I am today without Robert's guidance, love, support, and fierce dedication. I especially appreciated how much he genuinely cared about his students' personal lives and would always ask about my family whenever we talked on the phone. He could also twirl a partner around a dance floor like no one I have ever seen! I will miss him greatly!

JOHN BICKHAM

I always knew this day would come, that Robert would be gone and my world and that of so many others, would never be the same. To say that I'll miss him does not begin to express my feelings. He was mentor, colleague, and family friend all rolled into one. I studied in Robert's lab from 1973 to 1976; an exciting time to be a geneticist and a systematist! He was an outstanding comparative cytogeneticist who ultimately became the greatest mammalian systematist of his generation. As I look back on my days at Tech the emotions that come to mind are enthusiasm (for the exciting research and for all I was learning), optimism (for the boundless future potential of the work), and appreciation (of the friendship and camaraderie in the lab). I developed lifelong friendships and career-long collaborations with

some of my fellow grad students and with Robert. Early in my professorial career Robert and I worked closely together including frequent visits by him to College Station and me to Lubbock. He truly became part of our family as he watched our kids grow up and provided us with three golden retrievers. Those days working with Robert and writing papers on chromosomal evolution were among the most exciting, productive, fulfilling and enjoyable of my career. I really treasure those memories! Likely because of all those visits to College Station by Robert, my daughter Amy also studied in his lab and became a mammalian molecular systematist. Besides Amy, we also "exchanged" a lot of students. Some that worked in my lab and then went on to work in his were Mike Haiduk, Jim Cathey, Jeff Wickliffe, and Caleb Phillips. How great it was that when Amy arrived at Tech as a freshman, Jeff, whom she already knew, was there and they began a project together on Chernobyl mice! Karen McBee and Cole Matson worked first for Robert, and then came to my lab. There was a lot of academic "inbreeding" back then, but the two-way highway between Lubbock and College Station worked well for all of us. Looking back, I realize that Robert inspired me and did more for me than anyone in the world outside of my immediate family. I can't find words to express the appreciation, affection and esteem I have for him.

A great thing about working in the field of science is that you get to meet many brilliant people. Some are humble, others are not. Some are fun to be with, and to work with, and others are not. Some you want to be friends with, and others you don't. Like all these successful scientists, Robert had a brilliant mind and was a deep thinker. But you might not detect it in casual conversation because he had a very down-to-earth way of talking to people. But the sharpness of his mind became apparent when you worked together on papers, or if you challenged him to any kind of serious discussion from politics to poetry. But that is not what made him great in my view. Rather it was his intelligence in combination with his tireless drive, outstanding leadership ability, and with his personal charisma that set him apart from many of the greats of our field of science. In mammalogy, he will always be a legendary figure. With his passing, he takes his place among the legends, among the people on whose shoulders we stand.

Let me just describe one of my experiences with Robert. In 1974, John Patton, Hugh Genoways, Robert, and I spent eight weeks travelling through the Caribbean collecting bats. It was the learning experience of a lifetime for John and me, seeing so many species of bats for the first time and learning so much about their biology and, importantly, how to catch them. Our first stop was Jamaica, where John and I drank from the firehose of Robert's and Hugh's vast reservoir of bat knowledge. Eventually, we caught every species but one known to the island, and we published the book "Bats of Jamaica." But one day was of special interest. We had been driving around the island for some time looking for a place to net but Robert and Hugh were not satisfied with anything we saw. Then, we passed a locality, Orange Valley, where Robert saw a tree in a pasture near a pond. The place didn't look like much to any of us, but Robert was insistent that this is where we needed to collect. I don't know what Robert saw there, but this turned out to be one of the most successful collecting nights of the trip. The tree turned out to be a fruiting fustic tree and we collected large numbers of three of the rarest bats on Jamaica: *Ariteus flavescens*, *Phyllonycteris aphylla*, and *Erophylla sezekorni*. I think we all developed a very healthy respect for Robert's knowledge of bat ecology, and his intuition for where to collect them that night. It turned out to be typical because similar success was repeated time again on Jamaica, Trinidad, and Guadeloupe where Hugh and Robert in one night collected two undescribed species. Many scientists who study the genetics of wildlife don't know a lot about the natural history of the species of which they are studying, and don't collect the samples on which they work. Robert knew as much about the bats, gophers, and mice, he studied, including where they lived, what they ate, and how to catch them as he did about their genetics. He straddled the difficult line between the old days of skin and skull mammalogy and the new era of mammalian molecular systematics, of which he was the greatest pioneer.

These pictures were taken in late 2004 or early 2005 at Bickham's home in College Station when Baker and Bickham were working on *Bats of Jamaica*. Amy Bickham (now Amy Baird) was asked to review the draft. Note that this was during Baker's "curly red-hair phase."

KEVIN BOWERS

Robert never taught just science. He also tried to impart life lessons. Not only those that would guide us to success professionally, but also those that would lead to personal well-being. While I, perhaps, listened too infrequently, the anecdotes to which I did pay attention, eventually directed me to my own yellow brick road. Lions, and tigers, and bears, and the oft repeated wicked witch? Most certainly, but Robert did get through to me that giving up is not an option.

ROBERT D. BRADLEY

I first met Robert J. Baker in 1984 at the annual meetings of the Texas Society of Mammalogists. It was my first year as a Master's student with David J. Schmidly at Texas A&M University and Robert was an icon not only in Texas mammalogy but world renowned. Meeting him was a highlight and more than slightly intimidating. By 1985, Robert was encouraging me to attend Texas Tech University and do a PhD degree with him, so throughout 1985 we had several, serious conversations about why I should move to Lubbock and attend Tech. Generally, he would end the conversations with something along the line of "I am a diabetic and they tell me that I probably won't live past my mid-forties - so my plan is to recruit a few good students, do great science, and live long enough to get you guys finished." Later in our lives, as we became close friends, hunting buddies, and colleagues, I was reminded of his upcoming death on a near daily basis. Usually the reminder occurred when I was the greatest possible distance from the vehicle that contained the Coke and Snicker's bar that eventually would stabilize his blood sugar and save his life! Eventually, I learned to carry a Coke or Snicker's bar in hunting vest or jacket pocket! Still later in life would come a series of heart attacks and other life-threatening aliments that constantly seemed to be knocking on the door. I do not know how many times over the years that I figured his days were numbered – knowing Robert's attitude and constitution, I should have known that he would defeat Death time and time again!

I knew Robert very well for over 33 years (1985–2018) and still I have no suitable words to capture what Robert meant to me, what he meant to his family, or what he meant to those who were his academic family and colleagues. I could use words like: he was caring, passionate, intense, influential, larger than life, successful, and fearless. I could say he was a friend, he was a teacher, he was a mentor, and so on. All seem hollow, inadequate, and certainly they are not sufficient to describe Robert J. Baker. He meant so much to so many. He meant so much to so many, in so many different ways. To some he was the mentor, the person who changed our lives forever and dictated what we would become professionally. For some he was that unflinching friend that was always there and always just a phone call away. For some he was the boss that took care of you and helped you move up the ladder. He was the guy that bought Valentine's candy, flowers for your birthday, and asked how your kids were doing. He listened when we needed someone to hear our problems and he gave us the advice when we needed words of wisdom. He was the guy that built an empire at a teaching college. He was the guy who helped put a university on the map. He was an ambassador for Tech. He WAS Red and Black!

Being around Robert was like being on a roller coaster ride, he wore his emotions on his sleeve, and they could change faster than our West Texas weather. He could go from Happy to Thermo-Nuclear in a nano-second, and then in the next breath ask you to go to lunch with him! That's who he was. Take him or leave him… Love him or hate him… Worship him or curse him. No pretenses and no regrets. When thinking of Robert, I am reminded of the lyrics to the *The Pilgrim - Chapter 33* written by Kris Kristofferson - "He's a walking contradiction, partly truth, and partly fiction," and later in the song…"From the rocking of the cradle to the rolling of the hearse, the going up was worth the coming down." Robert lived life, he embraced life, and he demanded life on his terms. We should be honored that we had the opportunity to be along for the ride!

The day before Robert's funeral I had to e-mail a colleague and tell her that, due to the funeral, I would be missing a scheduled meeting. My friend peripherally knew Robert and she replied with her condolences. I replied to her thanking her for thoughts... then and I added that with Robert's passing we had some big shoes to fill. The day after Robert's funeral, I saw that she had replied to my last e-mail. She had said "he gave you everything you need to fill them." There was a lot of truth and wisdom in her comment.

As I write this encomium, it has been about five months since Robert's death. A day does not go by that I am not reminded of him in some way. For those of us that shared a portion of Robert's life, certain words trigger a memory. For example, what do you think of when you hear: "Lady Raiders", "golden retriever", "diabetes", "Tech", "Dallas Cowboys", "bat", "chromosome", "Arkansas", "data", or "Diet Coke"? What will you feel when the first flight of geese pass overhead each October. Robert's influence, professionally and privately, touched many people. To say he will be missed is so inadequate and so unworthy…. The best I can do is offer a couple of lines from *Seasons in the Sun* written by Rod McKuen and Jacques Brel and sung by Terry Jacks—"Goodbye to you, my trusted friend....We had joy, we had fun, we had seasons in the sun."

JENNIFER LISLE BRASHEAR

I met Robert J. Baker (Dr. Baker) in 2001, shortly after he was appointed to the position of Faculty Athletics Representative (FAR) by Dr. David J. Schmidly, then President of Texas Tech University. I was a young staff member in the Office of Athletics Compliance, with primary responsibility for the education and monitoring of NCAA rules. I was promoted to Associate Athletics Director in 2005 and began spending considerable hours each week in meetings with Dr. Baker.

Dr. Baker once told me that Dr. Schmidly told him he "needed a son of a bitch" in the position of FAR and that he could be exactly that when he needed to be. Little did we know then that we would deal with some powerful and very angry coaches as we investigated allegations of NCAA rules violations.

Dr. Baker took me under his wing, shared past mistakes when dealing with university politics and taught me how you really get things done. He created an awareness for me about perception, details to consider when chairing a meeting including professional dress and he always without hesitation had my back.

He invited me and my family to his ranch—anytime—where we canoed, fished and camped-out. He gave one of his female golden retrievers to my oldest daughter Ellyn and told me I was lucky to make the list and what number I was in line.

I cherished the moments we worked together, lunched together, and laughed together. And, I learned each time I was around him. There is only one Dr. Baker and I'm super thankful for him.

DAN BROOKS

I knew Robert Baker ('Baker' hereafter, as he often referred to himself) through nearly three decades, first as a student when I attended TTU, and later as a scientific collaborator which evolved into friendship. He had many wonderful attributes—champion of the students, mega-fan of nature and poetry, and perhaps my favorite, an incredible story teller.

I first met Baker in 1990 in Buenos Aires at a joint meeting of the American (ASM) and Argentine (SAREM) Mammal Societies. At the time I had spent the better part of a year study behavioral ecology of Chacoan peccaries (Catagonus) in Paraguay. At the field station where my work took place there was no air conditioning, computers, TV or anything like that. The big entertainment was reading a novel by kerosene lamp at night before hitting the sack. Although the opportunities to deeply entrench myself in the study of Neotropical mammals was unrivaled in this situation, I was in my early 20's and missed my home state of Texas, and all things associated with it, terribly.

At the meeting in Argentina, Baker stood up and introduced himself as someone from Texas Tech (TTU), and proceeded to very eloquently describe newly funded research opportunities for Latin American students. He spoke with a 'deep south' accent that I was drawn to, as I needed a fix of interaction with Texas! I recall not fully understanding what grad school was at the time, but Baker wanted me to consider coming to grad school in Texas. I told him I thought of myself as an ecologist leaning towards natural history. Even though that was outside his area of specialty, he helped introduce me to several professors with similar interests at that meeting - some now deceased (John Eisenberg, Oliver Pearson), some Texans (Dave Schmidly, Robert Martin), and others (Peter Meserve, Kent Redford). What dawns on me now, is Baker cared about me as a student before his own interests, despite not even knowing me! It didn't benefit him in the least that I was interested in a topic other than systematics, he was most interested in finding me a good fit.

In the end I made my way to TTU to study mammalian ecology for my thesis. During my first semester of grad school I took Baker's course on Systematic Evolution. The way he taught, a synthesis of knowledge and examples (personal stories), made his class one of the best I ever took in my life. We gathered around a table and Baker spoke, not once having to pause to look at notes. His lectures were amazingly concise and organized. I made friends with some of his students, and at the end of the semester was invited as a passenger in his lab's van that drove to the ASM meetings in Manhattan, Kansas (1991). Among the passengers besides Baker were Robert Bradley (lead singer and driver of the Winnebago), Shelley Witte, Matt Powell, Mary Maltbie, Kevin Bowers, and Steve Williams (Collections Manager for the NSRL).

While Baker had a reputation for over-collecting, this was not always the case. When I was first learning how to erect and take down bat nets at the TTU Campus at Junction, he saw that I was erecting bat nets under a grove of oak trees. He walked over and told me that over our heads was a small nursery colony of *Antrozous*, the ladies of which were personal friends of his, and he'd appreciate me not collecting them!

One of the things Baker used to impress upon me often was how one of the greatest moments in his career was the

opportunity to describe new species unknown to science. This stayed in my mind through many years of fieldwork in Latin America, and when I was beginning to be 'tethered' to my office at my current institution (Houston Museum of Natural Science), Baker's words spoke to me louder and louder. Loud enough to the point that I began collaborating on species descriptions, half of which were for new species of tropical bats. One of my proudest moments was describing Bolivia's first endemic bat with Baker, the man who first planted that seed in my head! We named it *Micronycteris yatesi*, in honor of Baker's late student and lifelong friend, Terry Yates.

I remember when we were in the very early stages of these descriptions Baker made it a point to come visit me at my office in Houston. He literally took time out of his busy schedule to do this—he flew into Houston to have a meeting with one of his former students and asked him to stop by my office after he was picked up at the airport! He didn't need to stop by, we could have sorted things out by usual means of communication, but the fact that he made time to because it was important to him spoke volumes, and it was around this time that I began to view Baker as a friend.

As mentioned previously, one of Baker's greatest attributes in my humble opinion was his ability to tell a great story. His stories were gauged and custom fit for his audience, always thoughtfully considering what to spin depending on the listeners in the crowd. If it was a group of Latinos studying endangered mammals, Baker might tell about the time a Giant Armadillo (*Priodontes*) came wandering up to him on an Amazonian island in the middle of the night while he was netting bats, and when it began sniffing his crotch it really gave him a shock! Or Baker might tell the story of a bunch of Rastafarians who were chanting "Free the bird, mon" as he was struggling to untangle a mass of *Tadarida* that were emerging from a cave in Jamaica, until the head rasta-man got the dickens bit out of his finger with blood spurting everywhere when he decided to lead the crusade to "free the birds."

You shattered the mold into a million tiny fragments Robert. I feel honored to have known you, noble scholar and gentleman, and your absence is felt. The hundreds of people's lives you have positively impacted lives on as your legacy…

JIM BULL

Robert was a master of knowing how to read people and how to respond in a situation. Of course, he wasn't always politic in his responses, and he might not always have kept his voice to a low volume, either. But my favorite story about this side of him reveals just how capable he was of handing a potentially touchy situation with a class of students.

In the 1960s and 1970s, Texas Tech put up several new buildings on the main parcel of campus, mostly east of Flint Avenue. One of the first steps in establishing a construction site was to install a slatted but otherwise solid wooden fence at the perimeter. Such a fence would span 70-100 yards on each side of the site. Even though Tech students may have still been in the steep learning phase of their lives, it did not escape their notice that these otherwise blank and soon-to-be disposed of surfaces might be used for graffiti. And with a flat, open campus and a paucity of trees, any graffiti could be seen for hundreds of yards. One popular phrase decorating those construction sites was "the wind doesn't blow in Lubbock, it sucks."

One day, as Robert was walking to his classroom within sight of an adorned construction fence, he observed a comment that he considered might have been directed at him: "Baker sucks jolly green donkey _____," the last word of which was a non-tangential reference to part of a male donkey's anatomy. He realized that most of the students in his class would have seen the comment and, at the time, might be pondering the philosophical depths of how he was going to handle it. He arrived at class somewhat early, walked around the front obviously agitated, slamming books, and doing his best to be noticed by the class. When the period started, he paused, then spoke to his class: "You know, if there's one thing that really pisses me off, …, it's a jolly green donkey that kisses and tells."

I am so terribly envious of anyone who can be that creative in taking control of such a situation.

CIBELE G SOTERO-CAIO

I have been one of many whose life was greatly influenced by the interaction with Dr. Robert Baker. As I prepared to write this encomium, my greatest struggle was to find a way to put in words a collection of memories of my time at the Baker lab during the pursue of my PhD at Texas Tech, that would summarize what for me was a life-changing experience. I came to the conclusion that it is not possible after all and decided then, just to share some thoughts on what I think was Robert's greatest strengths as an educator and mentor. Overall, if I had to pick a quote to summarize my perception of Bakers role on his students' lives, I would go with "Education is the kindling of a flame". And I think

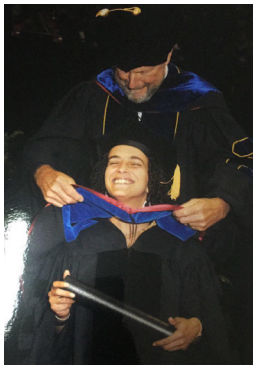
this is true, for every single one of his students came to accomplish much from the feeding of their "individual fire", and all of them with the sense of belonging to a common unit that felt more like a family than a laboratory. I am sure that we can all proudly refer to ourselves as "one of *them* Baker kids" and relate genuinely to these words.

Nowhere before I had seen a family with people from so many distinct backgrounds and life experiences than when I arrived at the Baker's lab in 2009. At the time we were around 13 students, only a couple of things uniting us all, which were the passion for science and mammals, and our major advisor, but that now feels like so much more than that. In such heterogeneous setting, the greatest challenge for a mentor is to provide an environment that would allow for our individual growth regardless of our personalities and cultural upbringings. And that is what I think Robert mastered with excellence. I remember thinking in several occasions that one of his greatest merits was the handling of people, and to know exactly how to treat you in a way that you will grow from that interaction. He would not give you tasks you cannot handle, but would push you towards that edge of a challenge to force you into accomplishing what you didn't even know you were capable of. "Cover your weaknesses by emphasizing your strengths" was something he said frequently on his speeches, and he was mostly right on what should be "lit up" in each one of us. I think being part of that family meant that each of us would have autonomy to pursue our own interests (even if distinct from his own), while having Robert's availability to trigger further our curiosity and help with whatever we needed. His trust in our ability to accomplish great things was exactly what fueled our desire to pursue them. That is evident when looking at the current work of those that took advantage of his availability and were inspired by his passion and curiosity towards nature. His joy of being the first to know something, which he would reiterate in all "Baker seminars" and not take observations for granted are examples of things that will be always remembered and linked to him, but now also a part of us.

I can imagine that although each person had their unique set of interactions with him, that feeling of belonging to the environment he created is what unites all his students. As for my personal experience, our shared interests on understanding chromosomes and bats were also key to a fruitful mentor/mentee relationship. He enjoyed spending time beside the photomicroscope with me and there was where we had the most interesting conversations and ideas for what became my PhD dissertation. I think it is also important to say that growth also came from disagreements. He made me want to cry and/or kick him several times, but I guess this is also part of the whole experience. However, I learned that banging heads at the table when something was not right was also something I could do without fear in front of him. I think that having the freedom to argue and complain was equally important in building our relationship: what I got in the end from our lively discussions and disagreements was also a sense of security from his constant and unconditional support. I can say with confidence that to him my education was always one of his priorities.

It was a pleasure to be RJ Baker's student. He was brilliant and to me a mentor on its literal sense, always pushing for my professional growth. Because of him I have a much different view of world, science, people, and life itself; and here I stand, and cannot help to think what his comments would be on every little new discovery I make. I miss him often and there is not a single week when I do not mention some random story of my time as his Ph.D. student. One that makes me smile is a bit related to education being kindling flames, which might have been taken too literally when I set his sunroom table on fire, while he was teaching me how to take biopsies for tissue culture. He looked at my horror and apprehension with his typical closed-eye-grinning, mice, newspapers, plastics and all, up in flames, and just told me that well, I was not the first person to lose an eyebrow over a burning sample.

Kyrgyzstan, 2009


Graduation, 2015

Retirement, 2015

I am glad I was able to let him know how grateful I was for the opportunity of learning from him. Robert and his family made me feel at home far away from my homeland, as well as part of something bigger. I love that crazy, brilliant old boss I had; and he will be greatly missed by many, but always remembered through his legacy.

ANETTE JOHNSON CARLISLE

Robert was a very influential mentor in my late undergrad and grad school years. He encouraged us all to explore the natural world, to question things (including him, when we felt it appropriate), and to think for ourselves. My understanding of evolutionary biology (though a challenging skill for someone who chooses to run for public office in the Texas Panhandle) and of systems change in general was largely developed under his teaching. While I've not stayed a lab researcher as anticipated, I've used this understanding to create systems-level change both in my region and beyond. Robert's wry sense of humor and his view of life lives on in those of us emboldened by his spirit. I wear a bat bracelet to this day.

On a more personal note, I remember very much enjoying his Mammalogy class, and another 'life skills' of sort for community work. But what I thoroughly enjoyed was working in his lab, the camaraderie of the group of students and friends, our ball teams of the Haploids and the Masterbatters, and the food and fun we shared together at his home or at someone else's. And maybe a few beers. ;)

My husband, Taylor, also a Mammalogy student under Baker, and I got engaged on Robert's birthday, and I remember we announced it at his party that night! And I still remember us all going dancing together as a group, and, boy, would I love to relive a few of those evenings!

But who could forget going collecting with Robert? We will all miss his charm, his charisma, and even his occasional biting comments.

It was a delight to reconnect with some lifelong friends in person at his memorial in June, and to make some new ones!

I have no idea if personalities as large as Robert's understand the influence they have on their students. His influence on my life was nothing but profound, in a good way.

With much love and a sincere feeling of loss, Anette, aka Annie

JUAN PABLO CARRERA-E

I met Dr. Robert J. Baker in March 2001. He came to Ecuador with Carleton J. Phillips and Federico Hoffmann to ultimate details for the Sowell Expedition 2001. Their mission was to visit the surveying area for the expedition and to establish some potential scientific and academic collaboration with Ecuadorian institutions. Luckily, my good friend René Fonseca (†) and I were officially invited to participate in the Sowell Expedition as Ecuadorian counterpart. At that time internet was not a common resource, as it is today, so we were not sure about who were these Texan fellows. However, we were excited to know that Dr. Robert J. Baker was the main editor of the "Biology of bats of the New World family Phyllostomatidae" (sic) series. Since their publication, these books have been a classic in bat research, and a key reference for anybody interested in Neotropical fauna. The opportunity to participate in an international expedition with renowned scientists seemed a little surreal for young Ecuadorian scientists in his early 20s and definitely changed our lives for good. During the expedition, Robert was a true leader, friendly, funny, and very knowledgeable about bats. He was also very demanding with his students, encouraging them to work hard and take advantage of the opportunity to be in a foreign country doing Mammalogy. I remember him chatting with René and me about Ecuador, science, education, and of course mammals. He fostered us to learn on how to collect tissues, to record all the fieldtrip information in the museum (TK) books, and to follow the proper standards to work in the field. At the end, the Sowell Expedition was a success and Robert invited us to be part of his team at Texas Tech University.

In 2004, I joined the Museum Science Program at Texas Tech. The experience to study abroad and to work at the Natural Science Research Laboratory of the Museum definitely changed my life forever. My experiences during my tenure at TTU were intense and exciting since Day 1 and allowed me to grow as a human being and as a professional. Thanks to Robert, I improved my education and got job experience; participated in several fieldtrips and expeditions; presented my research in scientific meetings; met a lot of interesting people; shared a plenty of seminars, meals, parties, and social events; developed ideas and participated in several projects; celebrated academic achievements (including mine); and forced myself to start and finish a doctoral program.

Robert and I went through rough and sad moments too; we faced problems and dealt with my own limitations. I remember vividly when Robert hugged me at Bobby's funeral and told me that he appreciated that I was always by his side and his family in such times. It was hard for me to see him devastated in several moments, but it was more impressive to see how he recovered from such losses, because he considered his students as family as well. All those experiences will be always important and special in my life.

I had many fond memories with Robert, but probably one of my favorite ones occurred during the Sowell Expedition 2004 to Ecuador. I was in charge of organizing and managing all the field material (TK books, tags, numbers, tubes, supplies, database, etc.). Moreover, Robert decided to train me at preparing karyotypes. I was not that excited about that task, but definitely need to try it, at the end he was my boss. As anybody can imagine, I was not the type of assistant that Robert expected. After a few days trying, he got cranky and told me basically that I sucked at that job, but on the other hand he was very impressed on how I managed the expedition's material. Robert told me that my job was very important and that I needed to keep doing that in order to have a successful expedition. He emphasized to me that in order to achieve success and joy in life, it is necessary to do what you love to do, to believe more in yourself, and to always pick your strengths and cover your weaknesses. That advice remains with me every day of my life.

Robert influenced a lot of people's lives including mine. I will always remember him as a very genuine person, a generous human being, a passionate mammologist, a good boss and mentor, a great scientist, and as somebody who believed and supported me until the end. Although, I need to recognize that his diabetes sometimes did not help to have a better communication; I am sure and positive that he was always proud and wanted the success of his people. Thank you for everything Robert! I will never have enough words to express my gratitude for the opportunity you gave me and for allowing me to be part of your academic legacy, a true honor that will stay with me forever.

JACKIE CHAVEZ

I was Dr. Baker's secretary for ten years. I was never his student, I never received a dog from him, but I learned a huge amount about, well, about a lot of different things. He was a generous man, who "pressured me like a father" to finally pursue my bachelor's degree. I honestly never would have gotten it without him or Dr. Jones. Dr. Baker did a lot for me. He gave me courage I never had before I met him. We had so many fun times. I just still have such a hard time believing he is gone. I miss him!

JOHN E. CORNELY

In early 1973, Dr. Robert J. Baker offered me the opportunity to participate in a "dream project," a survey of mammals of Guadalupe Mountains National Park in west Texas. I wanted to become a field biologist and had a strong interest in national parks. After graduating with a biology degree from Hastings College in Nebraska, the Viet Nam War had put my graduate training on hold for over four years to serve in the U.S. Air Force. Although I had a very good job as an electronics officer, I never wavered from a desire to go the graduate school as soon as possible.

I first met Dr. Baker in May, 1973, when I arrived on the Texas Tech Campus. My wife continued to work in San Antonio while I spent most of the summer in the Guadalupes. Housing had been arranged for me in a Mrs. Hatch's boarding house, known to the residents as the "Hatcheria." Shortly after getting settled Dr. Baker, Dallas Wilhelm, and I headed to Upper Dog Canyon in the Park in a TTU Museum crew cab. To enter the park you drive on a rutted dirt road through the front yard of New Mexico rancher Marion Hughes. We took a wrong turn and found ourselves being chased by a truck load of cowboys. Robert went back a bit later to visit with Mr. Hughes and that evening we set up a mist net in a stock tank on the Hughes property. Unfortunately, we had forgotten the "bat poles." Dallas and I were sent up the canyon to retrieve some 20 foot long, ¾ inch water pipe abandoned at a spring site. As we struggled dragging the pipes down the trail we had a serious discussion concerning "El Jefe's" sanity! Dr. Baker got the nets up and we caught some bats as he floated around the tank on a truck inner tube. On my first night in the field as I held a bat bag open for Robert J. to insert a Hoary Bat, the bat opened wide and sliced my hand open with a razor sharp incisor. "Now what, Doc!!" "Oh, just wash it good with soap and water, and get back to work."

Almost all of the specimens we captured were kept alive and transported back to Lubbock to be karyotyped. The largest specimen we karyotyped was a Gray Fox. I am thankful we weren't trapping Mountain Lions! One night Dallas and I were awakened by the sound of pistol fire following by the unmistakable odor of fresh skunk spray. Dr. Baker came up out of the dry creek next to our camp site in his undershorts and headlamp carrying a dead skunk and his "Buntline Special" pistol. The skunk had been examining

our live rodents in their individual oil cans, plus we needed a skunk for a voucher specimen. After his morning shot and packing some warm Coca Cola away we would go to check traps. Like many other students and colleagues, we always had a stash of candy or other sugary substances when we were in the field with Robert and, on occasion they were needed. Going to the field with Robert J. was always memorable!

The fall of 1973, Dr. Baker called all of the new grad students together to outline his expectations. He told us that we were in a very competitive field and would need to work very hard on academics and research. He expected us to be working most evenings and weekends to achieve our goals and be successful. He wanted us to start publishing our work as soon as feasible. One student quit on the spot, before beginning the fall semester! The rest of us applied his advice and have done well. Robert J. had high expectations and required hard work, but if we did those things we could count on his strong support.

Just like the military and the Fish and Wildlife Service, Robert handed out "other duties as assigned. Perhaps because I was older and had managed a large budget, he asked me to manage his grant funds and take charge in purchasing for his lab, office, and field operations. He asked Dallas Wilhelm and I to edit the "Literature Cited" for Journal of Mammalogy articles; what a great way to become familiar with the literature and gain editorial experience! Dr. Baker also hired my wife to be his secretary for his correspondence as a journal editor.

My primary interest was ecology, and despite most of Robert's students doing research in cytogenetics, he let me complete an ecological project on woodrats. I did, however, get a lot of experience karyotyping a diversity of mammals.

In early 1974, Dr. Baker told me that after I finished my MS, I should go on and seek a Ph.D. At the time I declined. I wanted to go to work for the National Park Service as soon as possible. Because of a hiring freeze on federal jobs in 1975, I ended in a doctoral program after all. I never had an academic or research position and I have spent 40 years working mostly with migratory birds. Never-the-less, I have nearly 50 publications, served on graduate committees at several universities, and collaborated with researchers the entire time. I credit Robert J. Baker with assists from other mentors at TTU and Northern Arizona University with helping me build a strong science foundation for a most rewarding and enjoyable career.

Robert J. and I also shared a passion for sports and being sportsmen. I miss him as a mentor, colleague, and friend. I know wherever he is, he is looking at us with that mischievous smirk and admonishing us to "keep our Guns Up"!!

MATTHEW A. CRONIN

I met Robert Baker at the Society for the Study of Evolution meeting at Pacific Grove, Asilomar, California in 1988. I was a graduate student at the time, and I thanked Robert for sending me reprints of his papers that I had requested. He said you're welcome, and asked me to send him some of my papers. I was amazed because this eminent biologist, with a fine Texas accent, was giving a graduate student (and a Yankee) respect. It was my first experience with Robert's kind, gentlemanly character.

Later, I was studying the potential mutagenic effects of oil spills, and Robert's fine work at Chernobyl was state-of-the-art in the field of induced mutations. We collaborated, and in 2002 published a study of K-*ras* oncogene DNA sequences in pink salmon. I was honored to be a co-author with Robert.

We continued to correspond, and had stimulating discussions about systematics and evolution whenever I visited Texas Tech. In 2010, his generous character was on display again. I took my son, Jack, and his high school friend, Chris, from our home in Alaska on a trip visiting prospective colleges. Jack was looking for a school at which to play baseball, and Chris a school at which to play football. We started in the northeast, and ended up in Lubbock. Robert graciously met with the boys, and arranged for them to meet with the Texas Tech Red Raiders head baseball Coach Dan Spencer. Two high school kids from Anchorage, Alaska, got to meet with a NCAA Division 1 head coach. They were in awe. Robert also arranged for us to attend a Red Raiders home baseball game. The boys were very impressed with the game and still talk about Robert's kindness to them.

Over my career, I've been impressed with Robert Baker's scientific achievements, but his kindness and generosity to others is, in my opinion, his most lasting legacy. I am proud to have him as a colleague and a friend.

LOU DENSMORE

I first met Robert when I was a graduate student at the Society of Systematic Zoology annual meeting in Tampa, Florida in 1979. I had heard about him of course, as he was already earning a national reputation due to his work in chromosomal evolution and mammalian systematics. In the session that he was speaking, James "Steve" Farris was

'holding court', essentially commenting on every single person's presentation (occasionally during their talks) and informing them how they had either misused parsimony analysis or not even applied it to their data sets (obviously a more grievous offense in his eyes). Robert (at that time sporting a rather long 'bowl' haircut and bangs) got up to give his talk, which was the last or second to last in the session and said something to the effect of "Steve, I sure hope that you like my analyses better than you have everyone else's." While intended as a "joke" (and getting a good laugh from the audience), it completely disarmed Farris and Robert went on to give an excellent presentation without interruption or serious question about his analysis. That was my introduction to Robert Baker.

Little did I know then how influential Robert Baker was to be in my own career. If we fast-forward 5 to 6 years, I am a postdoc in Wesley M. Brown's lab at Michigan, applying for jobs across the country. Wes and Robert were friends and when I applied for the Texas Tech job, Wes wrote me a strong letter. To make a long story short, I got an interview (my first), I got an offer (again my first) accepted the job and I have been in Lubbock for 33 years. I found out after I accepted the position that Robert had been one of my strongest advocates. He was interested in bring molecular systematic analyses of mitochondrial DNA to Texas Tech and as such was also influential in me getting $50,000 in startup (in 1985, the first time that much money had been given to an Assistant Professor).

Over the years, Robert and I developed a collegial friendship and mutual respect. That is not to say that we always agreed. When we did not, neither of us was what you might call 'quiet'. In fact, we could empty the entire 6th floor of Biology of students and even faculty closed their doors when we started 'discussing' whatever issue we disagreed on. Fortunately, that only happened about five times during the 25 odd years that our offices were adjacent to one another. Every time that it did, the next day we were back to being cordial to each other. The vast majority of the time we got along very well, as he regularly served on my student's committees and I served on his. He was genuinely interested in all of biology, even bringing me animals (including a desert massasagua rattlesnake) that he had caught on his property to include in our educational reptile collection. During my six years as department chair I regularly sought out Robert's opinion, as his knowledge of the history of the university and department were invaluable. Furthermore, I knew how much he wanted to enhance the development and reputation of both Biological Sciences and Texas Tech. Most people in the department did not know that he served as the university's athletic academic representative to the Big 12 or that he was a major player in an important effort to raise funds for Texas Tech. He had the ear of Chancellors and Presidents and was certainly one of the most influential faculty members in the university for at least 35 years. He loved Texas Tech; as I believe that Jim Bull stated during the memorial, "Robert bled red and black."

I learned many things from Robert Baker. Probably the most important was the influence he had on me regarding graduate education. Robert was simply the most prolific and insightful mentor of graduate students that I have ever known. He could handle a much larger research lab than anyone else in the department and still know what every student was doing. He taught me that it does not necessarily matter if the student's GRE score or GPA were slightly lower than might be expected (or at least desired). By putting in the time to help train his students, teaching them writing skills, developing their work ethic and then fostering in them the idea that they needed to "publish, publish and then publish some more," he could take what appeared to be marginal students and make them into stars. Robert did this so many times that it was clear that he had the uncanny ability to recognize potential and turn it into future greatness. He also was an excellent undergraduate research mentor, turning out students that became everything from physicians to university professors. Because of his willingness to cultivate the people in his lab and develop them into scientists, he will influence mammalogy and vertebrate evolutionary biology through his students and their academic progeny for many decades to come.

I feel very fortunate to have known Robert Baker. He was an excellent writer, communicator and advocate for academic research. He was also a good father and a good man that held off diabetes in part by the efforts of Laura and in part by will alone to live more than 20 years longer than his doctors had originally told him he would. He was not perfect, nor would he have ever told you that he was. However, one cannot deny that he was incredibly significant in the academic development and maturation of his university and was nothing less than a true giant in his discipline. More importantly, his colleagues, students and peers greatly admired and respected him. We should all be so lucky.

J. ANDREW DEWOODY

I was a PhD student in Dr. Robert J. Baker's laboratory from 1994 to 1997. I learned of Robert's program from my M.S. advisor (Rodney Honeycutt, himself a Baker lab alumnus). Rodney explained many of the pros and cons of working with Robert, and I found that his assessment was generally accurate. Robert was mercurial, inspiring, and he could be a lot of fun when he wasn't being an ass. Together we worked in the field, we planned experiments, we hunted/

fished together, we talked about Science *writ large*, we discussed religion, and we dissected the playbook of the Dallas Cowboys. I did not always agree with Robert and we locked horns once or twice, but we had the same ultimate goals—we both wanted to be successful, respected scientists.

That said, I probably learned more about being an advisor from Robert than I did about being a scientist. I still remember how he used to say that his job was to cover my weaknesses and develop my strengths; how he used to say that if you weren't publishing, you weren't doing Science; how he provided an immersive field experience for every student. He had a more profound influence on my own advising philosophy than did any of my other academic/scientific advisors. I always knew that when push came to shove, Robert would have my back and that gave me additional confidence to pursue my research. I also always thought I could call him on Saturday night if I somehow ended up at the police station (fortunately, an untested hypothesis).

Robert invited my wife Yssa and I into his home for dinner parties and to his ranch for fun. He gave us Loki (a golden retriever), a favorite hunting companion and wonderful pet. In short, Robert impacted our personal lives as much as he did my professional life. He was larger than life and made the world a more interesting place. He'll be missed.

SCOTT EDWARDS

I feel blessed to have known and loved Robert since 1985 when he was on sabbatical at Harvard, where I was an undergraduate. He was a father figure for me, and indeed he would often call me his son. (I could legitimately be called his academic grandson, given my undergraduate research with Robert's PhD student Rodney Honeycutt). We spent late nights in Rodney Honeycutt's lab talking science and life. Through conversations with him, I realized how fun it was to talk science with colleagues, and even occasionally impart new knowledge to him. We were both learning the ropes of mitochondrial DNA isolation and analysis, with help from Kim Nelson. I had taken a year off after sophomore year, and hence most of my undergrad buddies had already graduated. So I found someone to hang out with in Robert, and it was through him that I was first exposed to his classic studies of hybridization in *Uroderma* bats and other creatures. I was extremely impressed when Robert told me about how he had met with Ernst Mayr in the Museum of Comparative Zoology and had discussed his research on chromosome evolution and speciation. It was during that time that Robert's 1986 paper on speciation by monobrachial centric fusions was communicated by Mayr to PNAS. I vividly recall how Robert explained that Mayr would only accept the paper if Robert inserted greater emphasis on allopatric aspects of speciation, which was of course Mayr's legacy. One of the first scientific conferences where I presented my own data was the 1986 meeting of the American Society of Mammalogists, in Wisconsin, where Robert introduced me to his extensive cohort of students and associates.

I next intersected with Robert soon after I got married, in 1993. I was doing my postdoc at the University of Florida Gainesville on MHC variation, but I had continued to be fascinated with chromosomal variation in natural populations. I announced to my new wife, Elizabeth Adams, that I would visit Robert soon after our wedding (we took an extended honeymoon later the next year). So there I was, two weeks after being married, learning in Robert's backyard how to karyotype birds! By then Robert was publishing with Holly Wichman extensively on karyotype and genome organization in diverse groups of mammals. Robert's dual interest in population biology and speciation on the one hand, and repetitive elements and genome organization on the other, was very impressive to me. I have no doubt that those striking images of fluorescence in-situ hybridization on chromosomes that graced many of Robert's papers during that time made a deep impression on me, and fueled my own interest in genome evolution in birds.

It was one of the great joys of my career when Robert accepted an invitation in 2012 to come to Harvard, where I was by then a professor, to give a research seminar. The title of his seminar was "Insights into Speciation in Mammals". I was frankly a bit nervous, not so much that Robert's research would fail to impress, but more about the rather large cultural gap between Robert's rugged zoological perspective and emphasis on fieldwork and the rather effete and potentially snobbish perspective of Harvard graduate students, by then already using next-generation sequencing and other approaches that had not yet made it into Robert's research. I was also a little nervous about the fact that Robert could get away with approaching anyone and saying anything – anything – without them getting angry: but would that lackadaisical approach to social relations work at Harvard 25 years after his first visit? In the end, it was one of the most memorable visits by a seminar speaker in my 15 years at Harvard. The students absolutely loved him and, although my department doesn't have strengths in systematic mammalogy, Robert's wide ranging curiosity never failed to bridge generations and taxonomic boundaries. I have kept a voicemail he left for me once, inquiring in his gravelly voice about "parasite birds" and how one could use DNA to figure out their tactics of laying eggs in other birds' nests. Robert's mind was exceptionally wide-ranging, and although he emphasized mammalogy, his interests spanned all animals, parasites, and biodiversity in general.

It was probably around his 2012 visit to Harvard that Robert and I began discussions about collecting birds on his and Laura's "DNA Works" ranch about an hour east of Lubbock. My lab had been studying house finches and their bacterial pathogens (still does), and Robert regaled me with stories about how abundant the finches and other birds were at his ranch. We secured the necessary permits from Texas Parks and Wildlife and made our first visit in 2013. Frankly, given the challenges of finding a swath of land in Massachusetts that is free from inquiring passers-by and available for collecting with the owner's blessings, it is much easier for my lab to travel to Texas and work on Robert's ranch than to collect in Massachusetts. Thus, the ranch he owned with Laura has been a welcome source of bird specimens for my lab's research for the last 7 years. We often visit in January between terms and once visited in June before the Evolution meetings in Norman, Oklahoma. Needless to say, Robert and Laura took good care of us. He supplied us with shotguns and I couldn't help chuckle when we strolled into Walmart to buy ammunition (that would not happen in the northeast). The landscape of the DNA Works ranch, with its junipers and gently sloping aspect, is among the most beautiful I know in the United States. And the bird life is exceptional, with representatives of both the eastern (Blue Jays) and western (Scrub Jays, Black-crested Titmice) avifaunas present. Having never been a hunter, I learned a lot about hunting from the many colorful characters that passed through during our collecting trips. I was surprised to learn that someone as erudite as Jim Bull was a passionate squirrel hunter – and ate them! I guess my city-boy roots were showing through. All manner of pig-hunters, deer-hunters, zoologists, and other colorful characters passed through. It was a central meeting place in the alte plano of Texas.

The last time I saw Robert was during our last collecting trip to his ranch, in January 2017. By then he was having persistent health problems and on that trip Laura had to take him back to Lubbock early. I was always impressed how Robert lived such full life, unfettered by his diabetes and need for constant shots and monitoring of his sugar. At the end of that trip, I visited Robert in his and Laura's home and, more so than at the ranch, his health decline was showing through. But, thankfully, he was smiling constantly, and, at least outwardly, seemed happier than ever. There are very few people I know like Robert. He drew you in and showed you how to respect others and break down unnecessary boundaries preventing friendships and collaborations. His voice and words mixed wisdom and mischief, in equal parts. He gave to others much more than he took from them. His science pushed the boundaries of vertebrate zoology. He showed you how to live life to the fullest as a scientist, improving the lives of others in the process. We will all miss him very much.

HUGH GENOWAYS

Dear Robert:

This is the letter that I always intended to write, but never found the right time to do it, so now seems as good a time as any. I want to take this opportunity to thank you for all of your kindness to me and my family over the years. I arrived in Lubbock in early September in 1971 with a newly minted Ph.D. and no idea of how to survive in a fairly hostile academic environment. You took me under your wing and guided me through five interesting years. I know that there was no reason that you needed to do that because you were already a well-established faculty member. To me, it was done out of your kindness and your desire to see me succeed. This is something that I can never repay, although we did waste hours trapping *Cratogeomys* at the Old Lubbock Airport. The succeeding years were a pure joy for me with time and effort doing fieldwork with you, both foreign and domestic.

I want to thank you for being a friend and colleague for nearly 50 years, but no years were more important to me than those first five years at Texas Tech. My recollection of our first meeting was when I came to Lubbock in the fall of 1969. I was in serious need of help getting karyotypes analyzed for my *Liomys* to complete my dissertation at the University of Kansas. As I recall, your "lab" was in the old Science Building, sharing a room with the mammal research collection, and in my memory there were live animals in a small room at the back. With your time and help, those *Liomys* chromosomes were rescued from the edge of oblivion.

Fast forward two years. I am arriving in Lubbock in early September with a two-year post-doc position to work on the mammals of Nicaragua, a wife and child, and no place to call "home." It was an interesting situation with four established mammalogists—Knox, Dilford, Bob, and you—already on campus. It was clear from the very start who was at the bottom of the academic totem pole. My "office" was a small desk in the entryway to the office complex of the senior mammalogists in the basement of the recently completed building of The Museum of Texas Tech University. And I had my Nicaraguan mammals that were on loan from KU. Before the first weekend in Lubbock someone rushed through my small space and asked if I wanted to go gopher trapping on Saturday. As with any mammalogist, pocket gopher trapping was always on my agenda.

Saturday dawned cold, rainy, windy (as it turned out every day was windy in Lubbock, no matter the weather), but you arrived promptly at 9 am. We were off in your pickup truck heading down the Brownfield Highway. At some predetermined place along the highway, we came to a stop and parked along side the road. It was still cold, raining, and, of course, windy, which were not ideal conditions for trapping gophers, but we carried on. Neither one of us wanted to be first to mention how nutty it was to be out digging holes in this weather. Fortunately, the soil was sandy because we were setting some large traps that I had never seen before—the Baker-Williams live pocket gopher traps. As everyone knows, where there are sandy roadsides there are always sandburs, and this area seemed to be the capital of sandbur country. We set five or six traps and almost immediately caught two pocket gophers. After setting a few more traps, you said that we had to go and you headed for your truck with me trailing along behind. It was a short but very fast trip down Highway 62 to Meadow and the first open bar that we saw. In we went, and you ordered up a coca-cola and downed it in about two breaths. This was my introduction to a diabetes crash and the need for sugar. This incident in endless variations was played out many times in the next 50 years. Our return trip to the gopher traps was more leisurely. We had two more gophers and by mutual agreement we agreed that we had had a fine morning of trapping, considering the weather conditions, and it was time to return Lubbock. These pocket gophers ultimately were used in our description of *Geomys knoxjonesi*.

The next five years were more of the same. Never did you treat me as anything but a colleague. You were a mentor and an advisor. You taught me how to navigate the pitfalls of academia. At many points you were my defender and at others you were my advocate. You made a barely tolerable situation fun. Then there was work in Guadeloupe Mountains National Park and we were co-principal investigators. This brought Peggy O'Connell and John Cornely as graduate students to head up the field work. Soon thereafter was the NSF grant to study bats in the West Indies and a trip through the islands with "John" (Bickham) and "John" (Patton). This led us to be setting mist nets at the edge of a mangrove swamp not far from Pointe-a-Pitre on the island of Guadeloupe on the night of 29 July 1974. We went home that night with 4 specimens of 2 species that we knew were new to science. By 1976, I had a new office all of my own in the Natural Sciences Research Laboratory, an appointment as curator of mammals, an adjunct appointment in the Department of Biological Science, and a lectureship in the Museum Science Program. You had a direct hand in all of these except the last.

The hardest decision in my young career was deciding to leave dear old TTU, but we both agreed that it was necessary for me to get out on my own. It proved to be a good decision for me and it did not interfere with our chasing bats in Colombia, Suriname, and several islands in the West Indies or "running" the American Society of Mammalogists and SWAN. The final publication of our island work is included in this memorial volume. By count, we co-authored 48 papers. I was surprised at the number but we did work at it. Over the years, I tried to find a way to say "thank you" for the support but these words seem inadequate, so I have gone in search of other words that could convey a meaning closer to my feelings. The words beholden, indebted, obligated, or appreciative attracted my attention.

With Deepest Appreciation, Hugh

JIM GOETZE

I first met Dr. Robert Baker in the mid-1980's on a trip to the NSRL with Dr. Frederick Stangl, Jr. At that time, I was considering a Master's Degree in Museum Science, and Dr. Stangl took me to Lubbock so that I could learn more about that program while he took care of some research-related business with Dr. Baker.

After an interview with Dr. Clyde Jones, I remember briefly meeting Dr. Baker on the loading dock at the back of the old NSRL. He was supervising the stowing and loading of field equipment for a research trip (I don't remember where to), and, oddly enough (at least to my mind at the time), I remember that he and the students had Bob Seger songs playing on a radio on the dock. At the time, to say the least, I was a bit nervous about meeting Dr. Baker, after the stories I'd heard from Fred.

When I finally was accepted into Tech, I took the "Principles of Systematic Zoology" course with Dr. Baker in 1991 and was greatly impressed with his, to say the least, vast knowledge of systematics (much of which he helped to pioneer), his teaching techniques, and mannerisms. A vivid memory for me were the topic discussions the class would have, with Dr. Baker as the moderator. Dr. Baker would usually introduce the topic to us and next pose some questions for the class to discuss. He was always open to different points of view, as-long-as you had supporting facts or evidence to back up your statements.

Another technique that he used was to begin a two-sided debate on an issue and let the class express their opinions. One such discussion related to "assigning weights or values" to characters to be used in systematic classification

schemes. Dr. Baker asked the class, "Which do you think should be weighted the most in a systematic classification, a difference in the DNA sequence of a single gene or a difference in a "classical morphological character" such as (and I can't remember the exact example here, so I'll throw this in) total length or height?"

Most of the students enrolled in the class were Dr. Baker's graduate students and, in a sense, I was an "outsider" from the "Jones' research group". Perhaps because of their affiliation with Dr. Baker's primary research interests, most of the students immediately replied that the different DNA sequence should carry the greatest weight, not the other character. I believe that most of the students posed this answer because they thought it was what Dr. Baker wanted to hear.

However, Dr. Baker then proceeded to explain to the class the "error of their ways" in terms of morphological characters (of which he said DNA is also one) and the dangers of assigning those kinds of weights to characters without appropriate knowledge of the organisms and systems of concern. This confirmed for me that Dr. Baker didn't simply want "yes people" around. He was always genuinely interested in what you were thinking and doing as related to your research and study.

Also, I was aware of Dr. Baker's demands for the absolute highest of standards and care related to the NSRL facilities and collections. We worked closely with the Director (Dr. Baker), the other Curators, and the Collections Manager in specimen acquisition, cataloging, and curating the Mammal Collection throughout my time at Texas Tech University. Dr. Baker and the others demanded that everything in the collection have accurate data associated with it and that the entire collection was well-cared-for.

I became better acquainted with Dr. Baker when he became a member of my PhD committee after the passing of Dr. J. Knox Jones, Jr. I must say that, at this point in my graduate student career at Texas Tech, I had some difficulties with my progress toward candidacy for the PhD. Degree. I needed to replace some committee members, and someone suggested that I might ask Dr. Baker if he would serve on my committee. I honestly expected Dr. Baker to say "no" when I asked him, but his reply was the opposite.

This was a turning point for me in several ways. Along with my Chairperson, Dr. Baker was like a stabilizing force on my committee. Completion of my comprehensive exams, research, and dissertation work proceeded about as smoothly as could be expected. I really doubt that Dr. Baker will remember because some of the things that he did for me likely didn't seem like much to him, but his constant encouragement and small touches of what I call "kindness" at critical points in my progress toward completion of my studies helped in ways impossible to define. It was great to have someone of Dr. Baker's stature on my committee and know that he actually believed that "I could do it"!

Regarding another aspect of his personality, his sense of humor, I'll conclude with this example. I was searching for documents related to a research project with another colleague at the NSRL, and we were looking through some of Dr. Baker's personal, research library materials when he came in, sat, and proceeded on some paperwork.

During the course of our "rooting around" in the papers (some of which were his own research), we happened to ask Dr. Baker what led to his great interest in molecular systematics and study of mammals. His reply was as follows: "Well boys, I'll tell you, strange stuff is always exciting!"

We will all miss you, Dr. Baker.

IRA GREENBAUM

I joined Robert Baker's program in 1974 along with an outstanding cohort of nine other new Baker graduate students. Although I had done undergraduate research with Carl Phillips at Hofstra University, I had no experience in field-based mammalogy. I cannot overstate what I learned from Robert over that next five years, a lot of what to do and a little of what not to do. Three years into my Assistant Professorship at Texas A&M I recognized that I could not distinguish which of my scientific thoughts were mine and which were Robert's (I'm still not sure I know the difference). No, life with Robert wasn't always easy. He once told me that he only assigned *Peromyscus maniculatus* projects to students he really didn't like. Well, I didn't always like him either; but I loved him and recognize what an incredible impact he had on my life and professional career. Thank you, Pappy.

DON HARAGAN

The evolution of excellence at any university is inevitably associated with the ideas and dedication of a few individuals who serve as role models for the institution's growth and development. These faculty and administrators have a vision for what the university can become and a willingness and determination to make that vision a reality.

Bob Baker was one of those individuals. He was an inspiring teacher at both the undergraduate and graduate levels and a respected scientist in the field of mammalogy. And he was part of a relatively small group of faculty who were singularly responsible for initiating and promoting the conversion of Texas Tech from a mostly teaching-center college to a major research university.

Bob's impact on the overall academic enterprise cannot be overstated. His contributions in teaching and research and his dedication to building excellence had a profound influence on the institution and its future.

FEDERICO HOFFMANN

My experience at Texas Tech was eerily similar to Jeffrey Wickliffe's. Once we came to Tech from our country of Uruguay, my wife, Florencia, and I became part of the extended family very quickly, and if you know me, you know the merit is all theirs. Fellow students in the department, mostly from the Baker and Bradley labs, helped me get settled. Everybody on the 6th floor in the Biology building went the extra mile to make us feel at home and to educate us at the same time. Ron Van Den Bussche, Meredith Hamilton, and Robert Bradley took me on field trips, trusted my unusual cooking style, tolerated my sense of humor, and always made me feel welcome. My academic siblings Anton Nekrutenko and Kateryna Makova, whom I met after I finished grad school, have become esteemed friends and colleagues who are always willing to lend a hand and give words of encouragement. When our first child, Guillermo, was born, Jeff, his wife Jennifer, and Laura Baker all came very quickly to check on him and his mother, and as Jeff said, I'd like to think that Robert would be proud of this.

It all had started in 1997, at an International Mammal Congress in Santiago de Compostela, in Spain, where I was with Enrique Lessa, who was my master's advisor at the time. I met Robert there for the first time, although I had read his papers before. Right after Enrique's introduction, Robert told me straightaway to apply to grad school at Texas Tech and told me he would have support for me. At that time, this was my first international meeting, so my goal was to survive these conversations more than anything else, so I did not really make any further inquiries or anything of the sort. Back in Uruguay, I started applying to grad school, and Enrique reminded me of our conversations with Robert. So, I wrote an email to Robert asking about opportunities in his lab. At that point in time, I hadn't had much luck, so I did not really have many expectations. Well, Robert replied the same morning, and he said he had it all lined up for me. On hearing this Enrique told me in clear terms that my search for a graduate program was over. I had, and still have, enormous respect for Enrique, so there and then my search was finished. Still, I did not know much about Lubbock or Tech, and I had just got married six months before and coming to Tech meant that we were going to be apart for some time, as Florencia still needed to finish her undergrad at that stage.

I arrived in Lubbock on May 31st, 1999. Monday. I had carefully planned to arrive on a working day. Little did I know, coming from Uruguay, that the last Monday of May was a holiday, Memorial Day. So much for planning. Not a good start for my Ph.D. However, it all worked out really well. I met a wonderful advisor, made excellent friends that I still have to this day, and was treated with a sense of respect and care that is hard to convey. Within the first week, I went on a field trip to catch pocket gophers and my first working project was assigned to me. In time, Robert became a surrogate father to me, a surrogate advisor to my wife, and a surrogate grandfather to my kids. I still think it is striking that my wife, Florencia, who was not a student in Robert's lab, considers him a mentor and clearly feels as a member of the 'Baker family'. Coming from a foreign country, with very different family traditions than the U.S., to have Robert embrace us was something that made up for missing Sunday lunches at our grandma's house. Florencia and I found someone to look up to, someone that came from a different world than ours, but most important, someone who showed us that giving love and respect to other people was the way to succeed. After we left Tech, Robert continued looking after us. He would often invite me back to Lubbock to work on something together, or just hang out at the ranch. My kids all loved him, and our stories about him are a sure way to capture their attention still. They all loved his hugs and attention.

During my tenure at Tech, Robert took many other South American students under his wing. Most of us were intimidated to work with one of the most prominent mammalogists of the region. Soon we all learned that Robert wanted nothing more than our success, that he was deeply committed to our endeavors. Robert had a much better understanding of where we were coming from than what we

had anticipated, and was also well aware of the challenges we would face when trying to return to our countries. He went out of his way to involve us in projects that were relevant to our home countries, help us build networks at meetings, encouraged us to collaborate among ourselves, and always insisted on the fact that we needed to pay attention to what specific qualifications we would need to get a job. A lot of the latter was discussed during his seminars. You know very well how much we all liked the seminar. Of course, with more gray hairs, I now find myself doing the same! Very much as I did with my mom, whom I told I would never do things her way when raising my children, and I now go to her for advice on how to do it, I now do many of the things Robert used to do, but I did not have the vision to appreciate in his seminars.

Robert made an indelible impact on both me and Florencia. Despite his established position, he was always looking to do more. On our field trips, he would never ask people to do what he wasn't willing to do, and was very generous with his knowledge of Neotropical bats. As an advisor, he was always focused on what he thoug ht was best for the students and their careers, even when it did not line up with his vision. To me, the people in Baker's academic family were a family in every sense. They challenged my prejudices, embraced me as one their own, challenged me to be better, sheltered me when needed, and always made me feel appreciated. Multiple times at different scientific meetings I was approached by other members of the extended 'Baker family', always asking whether I needed something, and making it clear that should the need arise I was to let them know so that they could help. I came to learn the importance of this, and I hope I can live up to this adopted family. To all of you, thank you. You have given me a great gift, and I am sure Robert would be proud of that.

Rodney Honeycutt

Robert lived his life to the fullest, and as anyone knows who has been in the field with him, he was intense and burnt the candle from both ends. I resorted to carrying candy in my pockets, just in case he had an insulin attack. Yes, he impacted all of our lives. He could be a demanding, yet concerned, academic father. For me personally, it was not all roses, but I remain forever grateful for all that he did for me. Ira said it best at the mammal meeting in Georgia. Someone was pumping us for information about what it was like to work for Robert Baker. Ira said, "Within the family, we can complain, but never to outsiders." He had such a vibrant and creative mind, and his drive was remarkable. This is what I remember most about Robert.

On the occasion of Robert's retirement from TTU in 2015, I wrote him a letter detailing his impacts on my education, my career, and my life and my deep appreciation for him as a mentor and friend. That letter is reprinted in full in the Schmidly et al. article in this volume.

Julie Isom

Dr. Baker had hundreds of undergraduate researchers under his far reaching wings. He was legendary for being a motivational mentor and inspiring teacher! Dr. Baker's high academic expectations and research standards provoked and propelled his students to work both hard and smart to meet and surpass those standards. He often helped with STEM outreach to train (and entertain) area teachers. He was engaging as a story teller, whether it was a story about the beauty of biology or a thrilling research expedition…he made discovery and learning fun and exciting! Dr. Baker was loved and appreciated by countless students and teachers alike. His bigger than life, compassionate influence and infectious humor lives on in those he trained, taught, mentored and lived and laughed with, side by side.

Lara Wiggins Johnson

I worked as an undergraduate in Robert's lab for four years. I published several papers with him, and I was privileged to get to know him well. As I have reflected on those years, I have tried to identify the most important things I learned during that time. While I did learn how to make a slide for chromosome morphology, how to do fluorescent in situ hybridization, how to present my research at a scientific meeting, and how to write a manuscript, I could have learned those things in many labs. The most important things I took from my time in Baker's lab are the lessons about mentorship, leadership, science, and academics, and I still think about them each day as I go about my work. Here are some of those lessons:

1. Everyone on the team is important and helping everyone get what they need from the work is your job as the leader.

2. Doing science is fun, and if it isn't, you are doing it wrong.

3. When you have reached the point in your career when you no longer have to do your share of the hard and thankless jobs (freshman biology for non-majors or equivalent), you should do all the more of those jobs. You will be setting a good example for your junior colleagues, and you might actually make a difference for some students in that freshman biology class.

4. Any student who has some enthusiasm and the ability to work hard can be successful. Helping them get there is hard work but is also one of the most satisfying aspects of any academic career.

I am forever grateful to have been foisted upon an unsuspecting Robert Baker as his newest undergraduate student in 1994. As I proceed along my own academic career, I try to keep all of these lessons in mind and to be a deserving part of the Robert Baker academic legacy.

KATHY MACDONALD

Although everyone may be familiar with Dr. Baker as a researcher, professor, or colleague, my favorite aspect of him may be a little bit different! I worked with Dr. Baker at the Natural Science Research Laboratory from 2002 until his retirement. He also served on my dissertation committee and was always willing to be a sounding board or provide guidance during my research. But what I loved most about Dr. Baker was the way he interacted with my daughter, Caitlin.

Dr. Baker first met Caitlin at a going away party at my house, when she was just a toddler. He took some time away from the festivities to play princess with her, and he even read her two bedtime stories. From that point forward her face lit up every time she saw him. No matter how busy he was, when she was present, he always took time to talk with her and encourage her. He would make her feel like she was the only person that mattered in those moments. At Lady Raider basketball games she was probably more excited to see Dr. Baker and get a hug than to watch the game! He offered her advice on more than one occasion. I often wonder what he would have said to her about dating, had that ever come up! Additionally, he remembered her during his annual Valentine's candy celebration usually leaving her a candy bar just for her with a note. He was always first in line to support her during Girl Scout cookie season. She always felt a little guilty since he was diabetic, but he swore he would give most of them to the students in his lab. Although they may have only seen each other a few times a year, he will always be remembered with fond memories as a light in her life growing up.

Now that Caitlin is applying for college, she was excited to learn that he went to Ouachita Baptist University since it is one of her top choices for her Theatre Arts interests. She wishes she were able to talk with him about his time there. I am sure he would have some great stories to tell as he usually did! While I have many fond memories of Dr. Baker, his role in my daughter's life will always be some of the best.

KAREN MCBEE

Robert Baker was a force of nature. I fully believe that he could warp time and bend gravity in his near vicinity. It took more than one person to live his life because it was so expansive. Because of that he invited us all into his life, the mostly good parts and some not so good. That not so good part never seemed to divert him from what he loved and pursued in life, and because we were able to watch him, I know many of us learned how to carry on when things were not so good. Robert was welcoming to all who wanted to learn and who were willing to work. This was especially important for me as a young female in the 1970s that wanted to go to graduate school and do fieldwork. I had already been told by potential advisors at other schools that they would not accept me into their programs because a woman couldn't be expected to do the types of work that were necessary. Robert never uttered a similar thought, and when I arrived in his lab I found that Peggy O'Connell, Anette Johnson, Becky Bass, and Laurie Erickson were all already there working on graduate degrees and doing fieldwork. I don't think any of us ever worried that Robert thought we wouldn't succeed because we were women, but we sure heard about it, just like the guys, when we weren't working up to the standard set by Robert. In Robert's lab I met some of my best and longest lasting friends, I learned that I really could accomplish things, learn things that others had told me I wasn't capable of doing, I found my route. Working in Robert's lab could be adventurous, challenging, certainly intellectually stimulating, and sometimes a little bit frightening, but always completely inclusive. Robert thought about and treated us like family. Robert sent so many of us on our professional routes that I think it would be near impossible to quantify his impact on young biologists, biological education, and results of research that can be traced back to Robert. Robert wasn't a pebble that sent out ripples. He was a boulder.

Editor's note: The following comment was relayed by Karen during the submission process, and the editors felt it was worthwhile to include as part of this encomium: "I remember telling Robert while we were netting bats at GTMO that it took three people just to live his life after he started telling me that he wanted me to go do something else. He just gave me the squinty-eyed look and laughed, but I was scared there for a moment. I think so many of us have such an intense sense of gratitude for the gamble that he took on us that we really can't put it into words. Without Robert's gamble on me for the master's, then pushing me on Bickham and the Carnegie guys, I'd probably be working a minimum wage job in the feed and seed store in Brownwood, Texas."

KATERYNA D. MAKOVA

The last time Dr. Robert Baker, my former PhD advisor, visited my laboratory was October 2015. His visit reminded me of ten simple rules he followed while running his highly successful laboratory. They are highly relevant to our work and everyday life. The rules will sound very familiar to many of Dr. Baker's former students, and I hope that we can transfer them to the next generation of scientists.

Ten Simple Rules for Running Your Lab

Rule 1: Amplify your strengths and cover your weaknesses. Nobody is perfect, but everyone is perfect at something.

Rule 2: Identify who your boss is and make her/him happy. It might be your advisor, it might be your wife, it might be your mother—whoever your boss is, make her happy, and you will be happy too.

Rule 3: You're gonna die soon, so live every day as your last day. Do something meaningful and enjoy.

Rule 4: Being a scientist will never bring you a lot of money, so do it only if you really like it. Otherwise—do something else.

Rule 5: Every successful relationship (including marriage) follows a 60:40 rule. If you feel like you are giving 60% and getting 40%—this is as good as it gets.

Rule 6: Whatever the truth is, embrace it. It is okay if your result is not making a sensation.

Rule 7: Be hungry for knowledge and for truth, and be around people who are just as hungry as you are.

Rule 8: A lways do your homework—before an interview, before a conference, even before a visit to another campus. It pays off to be prepared.

Rule 9: Never use red on a dark background and never make slides nobody can read from the last row.

Rule 10: The author line is never fair, but as a PI you should make it as fair as possible by discussing it early.

MARY MALTBIE

Robert had a major impact on my life in the best way. After having two rejections on getting into graduate school, I was so surprised to get a call from Robert to ask me if I wanted to join his lab.

I very much wanted to be involved in wildlife conservation and pictured myself becoming a field biologist but Robert opened my eyes to a whole new way of looking at wildlife from the lab bench. I loved everything Robert was teaching me and appreciated how he would let me take responsibility of the work and let me have at it. I found a passion for doing lab work. I still cannot explain why I enjoy lab work as much as I do but I know seeing Robert's enthusiasm for it had a great influence on me.

After being in graduate school for a couple of years and talking with other graduate students, I got to see how fortunate I was to have ended up in Robert's lab. He supported of his students in so many ways. I was far from home but I knew I was never alone with Robert around. From his morning phone calls of the Sunshine song to his late night calls seeing how everything was going. I knew that Robert would support me not only in school work but with any problem that might crop up at my time at Tech. I watched how much he supported the other undergrads and graduate students in his lab and how well these students were prepared to enter either careers or graduate school after they left his lab. Robert always put his students' futures as a priority. As a teacher, he was someone I try to emulate as much as I can.

Now onto his love of golden retrievers and his sharing his love of the breed with his students. Little did Robert know that when he gave me my first golden retriever what an impact this would have on my life. I cannot even think of what my life would be like without these dogs. Not only did this give me a whole new arena to explore of training dogs but it helped me get a lot more confidence in myself.

M. RAQUEL MARCHÁN-RIVADENEIRA

I first met Dr. Baker, as I have always called him, in 2004 while he was planning his second Sowell Expedition to Ecuador. He arrived with a crew of highly motivated and well trained scholars that were anxious to learn more about the diversity of mammals in my home country. I was an undergraduate student at the time and were lucky to be introduced to the fascinating world of bat's taxonomy and systematics by former Ph.D. students in Baker's lab.

I was first introduced to the lab by René Fonseca, a former Master student of Dr. Baker. He contributed significantly to develop my interest on fruit-eating bats, which I will later study for the rest of my academic career. René was also a huge motivation for me to choose Texas Tech University as the place to get my Master and Ph.D. degrees, under the supervision of Dr. Baker.

I was invited to visit Texas Tech in 2005. I spent a week in Lubbock testing my skills to identify bats. By the end of this visit I contrasted my species identifications with the molecular IDs developed by another student of Dr. Baker, who became a great friend and long term collaborator, Peter A. Larsen. I still remember Dr. Baker's words when he invited me to apply to Texas Tech. He said I was a "hungry student." It took me a minute to understand what he meant until he said: "you are hungry for knowledge…" This really made me feel confident about pursuing my career and the likelihood of working with someone who saw my academic potential and would be the right fit for me.

My years in Lubbock were full of novel life experiences. I found in Dr. Baker and the members of his lab a supportive group of friends and new family for me and my son. I was able to develop my skills working independently, always relying that I will have an academic advisor that will facilitate my success. The Baker's lab changed the lives of many people like me who chose to leave their home countries and navigated into the challenge of fitting into a new environment. I feel that for me it was a life changing experience, which helped me to validate the need of trusting my instincts and relying on the fact that I was lucky to have a great mentor.

MICHAEL A. MARES

I first met Robert Baker in 1970 at the Texas A&M mammal meeting—a meeting to remember. It was my first mammal meeting and I, an impressionable 24-year-old graduate student at UT Austin, came to understand that these people were unique by most standards. Where else would most of the scientific society end up fully dressed, several bleeding, in a hotel pool? They even threw into the pool a bride and groom who were trying to celebrate their wedding at a reception at the hotel! Bill Burt, in his room, and me in my room, managed to avoid getting tossed into the pool, but very few ducked the dunking. Baker and other notable mammalogists were very active in the whole activity. Alcohol may have been involved. I was a very young graduate student and Robert, while only three years older than I, had finished his PhD the same year I finished my bachelor's degree. I had only published a few papers and he was already a noteworthy mammal researcher making inroads into cytogenetics, especially of bats. So, he seemed much older to me in the ways of professional mammalogy.

I was never in the field with Robert, but got to know him at meetings and in visits to Texas Tech. I even stayed at his house a few times. He was extremely proud of his university and I doubt he ever seriously considered leaving Tech for another institution, though his publication record—more than 430 papers, articles, and books—would have made him a good catch for any university in the country. His first 10 Google Scholar citations have been cited more than 4,000 times, an excellent record.

Robert loved the American Society of Mammalogists and was generous with his time in accepting positions within the society. Eventually he served in editorial capacities, as Board member, Vice President, and President. He was an honorary member of ASM. When I was Chair of the Editorial Committee, I suddenly needed to replace an editor. I

called Robert and told him that the Society was in a tough spot. We had no editor. Did he know anyone who would be willing to serve in the very demanding position of Managing Editor? (He had served as Managing Editor a decade earlier.) He said, "Oh hell, I'll do it." It was one of the most selfless acts I had seen by someone who had nothing to gain from additional service to ASM as editor. I thought it remarkable that he would agree to this time-consuming task for at least a year. I told him I would never forget it, and I haven't.

Robert's final years were filled with personal tragedy and professional challenges. I am not surprised that he had pulled back from things at Tech, especially after his retirement and after losing his son. If you have not lost a child, you cannot know the pain and how the loss alters your entire life forever. Robert was an extraordinary mammalogist. We were lucky to have him. His research was both important and voluminous. He dedicated much of his life and fortune to the university. He produced an army of graduate students in a very diverse laboratory (22 of 48 M.S. students were women; 18 of 50 PhD students were women; many were international students). Very few of us can match this record. He had an unforgettable personality and manner, but was totally dedicated to the science of mammals and to education. His research and educational productivity were a credit to our discipline.

ROBERT E. MARTIN

I knew Robert J. Baker for almost 55 years. My first awareness of Robert was as an undergraduate assistant in the museum of Oklahoma A&M (now Oklahoma State University). The museum for vertebrates was located in the Aquatic Biology Laboratory, which housed graduate student and faculty offices, a room for aquatic research, and a classroom. Robert was a brash Master's student under my supervisor, Dr. Bryan P. Glass. My job was to take care of the vertebrate collections (refilling jars with preservative, cleaning skulls, and cataloging specimens).

In 1965, I was working a summer job in Quanah, Texas, as a "bug checker" for a company that sprayed agricultural fields. In my free time I would drive around the county at night to look for mammals. On one of those nights I found a population of the Texas kangaroo rats, *Dipodomys elator*, that had not been reported before in Hardeman County, Texas. That led to a small collection of *D. elator* that I took back to OSU for preparation. At the time, mammalogists prided themselves on how many and how well they prepared mammal study skins and how many different species of mammals those numbers represented. When I told Dr. Glass about this discovery he and Robert Baker wanted to help prepare some of the *D. elator* specimens to add to their "life list" of species prepared.

As an undergraduate student, I was in class with Robert when he took a class in Invertebrate Zoology along with some other graduate students. Later, Robert was a graduate assistant on a field trip in Mammalogy that I was taking. At that time, Dr. Glass was Director of the University Museum and not very active in fieldwork. I suggested to Robert that we take the mammalogy class to Harmon County, Oklahoma, where I had worked two summers and where in high school and college had done small mammal trapping. He readily agreed and preparations were made for the field trip. On that trip, we excavated pack rat (*Neotoma micropus*) "dens." Part of the excitement of the excavation of these dens is the possibility of discovering other species of mammals and vertebrates that often occupy these structures. On that particular field trip, we were fortunate to capture two desert shrews, *Notiosorex crawfordi*, that proved to be pregnant. Baker and a fellow Ph.D, student, Dwight L. Spencer, published a paper about that finding (1965. Late fall reproduction in the desert shrew. Journal of Mammalogy 46:380). Later, Baker and his Texas Tech students did extensive field work with *Neotoma* and *Notiosorex* in Garza County, Texas.

In 1966, at the end of my undergraduate years at OSU, I attended my first American Society of Mammalogist's (ASM) meeting in Long Beach California. I was excited to be able to attend this meeting as a new master's student under Dr. Glass. George C. Rodgers, a Ph.D. student of Dr. Glass, and I drove to Long Beach, California in the "Batmobile," a long-bed International pickup that was a grant-funded field research vehicle used by George to study the age structure of Mexican free-tailed bats (*Tadaria mexicana*) in western Oklahoma. Part of the route to Long Beach took us to Tucson, Arizona, where Robert Baker was a Ph.D. student conducting pioneering studies of bat chromosomes at the University of Arizona. Robert invited George and me to go on a field trip to Sasabee, Arizona, to net bats for his research. That was a memorable night and I learned firsthand of the intensity of Robert's research drive and my inclination to continue working with rodents was affirmed.

In the summer of1968, with wife Patty, I headed to Texas Technological College (now Texas Tech University) to begin my Ph.D. studies under Dr. Robert L. Packard. I asked Robert Baker, a young professor at Texas Tech, if I could collect mammals on my way to and from the ASM meeting in Colorado that summer since I did not have financial assistance until the fall semester. As I unfortunately remember, a number of live traps on that trip were lost due to theft but Robert just passed it off as a price for getting specimens for the museum.

All of the graduate students in mammalogy were well aware of Robert's diabetes and somewhat in awe of his refusal to let the condition interfere with his fieldwork.

In the afternoon Robert would often ask graduate students to accompany him to the student union for a snack and we presumed we were there to notify the medical professionals in case he needed help. However, we always enjoyed the attention and the stories that he was bound to spin. One such story related how he used to catch flying squirrels (*Glaucomys volans*) floundering in snow when the squirrels escaped from hollow trees after he banged on the trunk.

Following graduate studies I spent time in Chile and Chicago and occasionally saw Robert at ASM meetings. At the International Theriological Congress in Edmonton, Alberta, I was reminded how sophisticated Robert could be in the presence of international mammal experts.

One of the most interesting aspects of Robert J. was how he changed in his relationship with Robert Packard over the years. Following Packard's early death Robert followed up on a dream of Bob Packard to start a state mammal organization. Robert J. helped organize a preliminary meeting at Junction, Texas, to jumpstart this professional organization in Texas. His early support for the organization and commitment to involve his students in the Texas Society of Mammalogists was crucial for the early and continued success of this organization. He insisted from the beginning that any paper on mammals presented at the meeting be accompanied by a picture of the mammal being studied.

Robert's graduate and undergraduate students can best attest to his love for mammals and for mammalian research. Our profession has lost one of the giants of genetic research that never forgot his roots as a field biologist and instilled that love in countless students.

ELLEN ROOTS MCBRIDE

As a very young kid, I was always in love with everything about biology and conservation. It was a passion that grew into a career as I pursued a B.S. in Biology at the University of New Mexico. I took a field Mammalogy class with Department Chair Dr. Terry Yates, and became indoctrinated into the tight-knit world of mammalogists, working for awhile in the Museum of Southwestern Biology. As I became more deeply involved and interested in the biological sciences, and eventually started research with Terry that led to my undergraduate honors thesis on Bolivian pocket gophers, identifying two new species through karyology. Toward the end of the program and about the time I was starting to think about my next steps for graduate work, Terry suggested that I do a poster presentation at SWAN, the Southwestern Association of Naturalists in the spring of 1996.

Along with a number of other students giving talks and presenting posters at the meeting, we all drove with Terry from Albuquerque to southern Texas. On the day that my poster session happened, I was fielding some questions from people, when suddenly a blond man with a smile so big that his eyes almost disappeared came up to me, stuck out his hand and introduced himself as Dr. Robert Baker. Terry also appeared and we all talked briefly about my research. Shortly into the conversation, Robert said that I needed to come join his lab in Lubbock as a Master's student, to get serious and do some molecular biology. Unbeknownst to me, he and Terry had been talking about my research and Robert had already made up his mind. Much to my surprise, he made me an offer on the spot, said he had funding for a Research Associate, and to come for a visit over the summer.

Just a few short weeks later, I drove out to Lubbock to meet Robert's other graduate students, who showed me around, shared meals with me, and were not shy about talking about both the great and challenging aspects of graduate work. It was a welcome prospect, enough to make me accept. And, just like that, a couple of months later, my boyfriend and I relocated to Lubbock in the fall of 1996.

It was initially a bit intimidating joining a lab under someone with a reputation like Dr. Baker's. As a Horn Professor studying the effects of radiation fallout in Chernobyl on small mammalian genomes, he was internationally known, and his bat research and publications were prolific. There was a hitch, though – I wasn't there to work on bats or other mammals, although I would have loved to. I was there under an agricultural grant to identify genome sequences to differentiate male and female emus, as they are not sexually dimorphic when they hatch, and emu farming had become big economic business. Regardless, I soon came to know Robert as a very down-to-earth person.

After a few weeks working in the lab, Robert called me into his office. When I sat down, he said, "I'm a mammologist. I'm glad you're here to do this work, but I applied for this grant not expecting it to get funded. I'm not interested in birds and I want you to know that you're going to have to work hard to get me to pay attention to this project." I appreciated his honesty and I knew I'd have to be tenacious.

That kicked off my time in Baker's lab, which was an intense two and a half years. Robert didn't need a lot of sleep,

so he set a high standard for time logged at work. As grad students, we worked crazy long hours, sometimes pulling out the ping pong table to play in the sixth-floor hallway, while our gels, sequences, or other experiments ran late into the night. On weekends, sometimes we'd have big barbecues with mystery meat from everyone's freezers from hunting expeditions, along with jalapeño poppers to spice up the evenings. Other times, Robert would love to go grab a beer with everyone and just talk.

Some of my most inspired moments during my time there were during Monday evening seminars with Robert. He would often wax philosophical about science and life. He always wove in stories about his family, and especially his son, the things they did together, about the depth of his love for him, and how much it meant.

In my last year, I came up against two big challenges. The first was that, while I had been able to locate the gender-specific DNA sequences and was preparing the paper for submission, another researcher had made the same discovery and published their work before mine. Beyond being disappointing, it meant that I had to restructure my entire Master's thesis to find a different focus. Robert worked with me to develop a new thesis topic examining microsatellite sequences and the role that they play in ratite genome size.

One of my big take-aways from Robert was that he enjoyed mentoring. He invested a great deal of time with me talking about science, how to think through different angles and hypotheses, working in academia, networking, looking for jobs, and spending time outdoors. I appreciated that time and came to understand that this was his legacy work.

The second big hurdle came during my last summer, which was spent doing an internship at the Los Alamos National Laboratory working on my emu DNA library. When the library got shipped back to Baker's lab, it hadn't been properly packaged and the whole thing had melted, ruining it and the nearly two years of work it had taken to create it. Although I had enough data to write my thesis, if I wanted to publish it, I was going to have to create a new library, which meant dedicating another two years in the lab after graduation

on top of my new job to do so. Robert was very encouraging and committed the lab resources to make that happen.

In the fall of 1998 as I was deep in thesis writing shortly before my defense and graduation, Robert came to me one day and said, "Have you ever eaten emu meat?" When I said no, he replied, "Well, you're not graduating until you've eaten what you've spent all your time researching!" He said that a nearby farmer had an emu that he had no need for any more, and said that we could remove it. So one weekend, Robert shot it and we helped prep the meat. The rest of Baker's lab came and we each prepared a different dish to sample. For the record, emu meat is not particularly tasty, but it was an event to be remembered!

Right before graduation, we co-authored a Mammalian Species account on *Rhogeessa genowaysi,* and we eventually got my Master's work published in the Journal of Heredity in 2002. We stayed in touch over the years after I relocated, and co-authored one more Mammalian Species account on the bat *Rhogeessa parvula.*

In looking back now, I went into my graduate work as a biologist, but felt like I came out a scientist with a more profound understanding of what it meant to delve into the research, the questions, and to navigate toward the answers that the data reveal. Robert Baker was instrumental in making that happen.

GERALD MYERS

Robert was a loyal and personal friend, a tremendous fan of men's and woman's basketball, and a great Faculty Athletics Representative (FAR) to the Big 12 Conference. He was the best FAR ever and did a great job during his ten years as Tech's FAR. Bob was a strong voice in that position and made sure that Texas Tech had a prominent voice in compliance and academic issues. He was a strong advocate for the Big 12 to have fair and just academic standards. He was definitely a leader for the Big 12 FAR Committee. I certainly appreciate Bob's loyal support for athletics. He was a source person that I called on a regular basis, to get his advice concerning various university and athletic issues. Most importantly, I had the opportunity to develop a close friendship with Bob. We had some great times visiting and fishing at Bob's ranch. He was a great fan, who was a little biased about basketball officials. We appreciate the many contributions Bob made to Texas Tech Athletics, as Tech's FAR and vocal super fan at Lady Raider games.

MARGARET (PEGGY) O'CONNELL

Mentors shape our careers by challenging and supporting, setting standards by example, and opening doors. I was fortunate to have Robert Baker as my mentor. I first met Robert in 1973 when I started my Masters at Texas Tech. I had been accepted into the Department with a Teaching Fellowship, but with no major professor assigned. I formulated a general plan for a study examining diets of sympatric kangaroo rats. I then went professors and presented my ideas. Most listened politely and gave general encouragement. Robert was genuinely enthused. And so I became Robert's first female graduate student!

Both my Masters and my Doctoral research were peripheral to the main avenues of research in Robert's lab and we jokingly referred to me as "his stepchild" or to our relationship as "a marriage of convenience." What I learned from Robert was the art and science of research. He set high standards by his own example. Robert tried to make us our own best critics, often admonishing us with: "Why is there always time to do it right the second time?!" He forced us to have confidence in ourselves. Each week at least one of us would give the talk to the lab. Periodically Robert would turn off the slide projector and tell us to proceed with our talk. Years ago, I saw a speaker at a national meeting faint backwards off the podium when the projector bulb went out… I thought of Robert's training! During yesterday's lecture, the computer was not communicating with the projector and I resorted to colored markers on the white board….I thought of Robert's training!

At the time I started at TTU, I was thinking of a Masters in Biology as a route to Veterinary School. One day in early my 2nd year Robert and I were talking about my plans upon graduation. He saw my passion for teaching and research and was encouraging me to rethink my goals and look into PhD programs. The phone rang and I went back to work in the lab. Robert came in later and said that it had been John Eisenberg on the phone and he had asked Robert if he knew of any student who might be interested in going to Venezuela to study small mammals. Robert told John he knew the "perfect rat trapper" for such a job – but she's female! I studied up on tropical mammalogy and went to John's research lab at the National Zoo in DC for an interview. He greeted me with three questions: can you speak Spanish, can you climb a tree, and can you drive a 4-wheel drive stick shift? Yes to all three! After a bit more talking, but no hard questions about mammalogy, he offered me a fellowship. I later asked John why he never grilled me on mammalogy at that interview. His response was that if Robert Baker had confidence in me as a scientist that was enough for him. Robert's mentoring profoundly shaped my career and through the mentoring process we became friends for over 40 years.

MARK B. O'NEILL

I first met Robert J. Baker in 1996 as a recently transferred undergraduate student from Tarleton State University. My introduction came as part of an interview for a position in his lab as part of the Howard Hughes Undergraduate Research program at Texas Tech. While still attending Tarleton, I spoke with Dr. Herschel Garner about my opportunities in the Howard Hughes program at Tech. He told me the only person I should consider working with at Texas Tech was Robert J. Baker.

Robert selected me to be his undergraduate research student. I quickly got busy prepping DNA samples for MT-CYTB sequence analysis and becoming a member of his lab. I attended required weekly lab meetings in which discussions ranged from Mammalogy to what not to do during a future job interviews. We learned so much about life in those meetings.

Following my undergraduate graduation, Robert accepted me as a graduate student for my Master's degree in Zoology. I quickly got started completing projects started as an undergraduate and starting my main project of determining if there was species level genetic diversity in the desert shrew, *Notiosorex crawfordi*. As a graduate student, I had the privilege to present these findings of these shrew projects at the American Society of Mammalogists and the Texas Society of Mammalogists. It was not until I went to these meetings when I saw how much respect Robert had from his peers in the field of Mammalogy.

Near the end of my Master's program, I had completed all my research and needed to write and defend my thesis. At this same time, I started looking seriously at options for a Ph.D. program. An opportunity from outside academia came forward at a Biopharmaceutical company, called Lexicon Pharmaceuticals north of Houston. I approached Robert with the idea of me applying for a position at this company then completing and defending my thesis while working at this new job. Robert was not thrilled with the idea. In fact, his response drove me to completion of my thesis and defense because he told me I would not finish it if I left the university. Once I started my new position at Lexicon I made several trips back to Lubbock to work on my thesis with Robert. Over a year went by and I completed and defended my thesis. I am glad he continually pushed me to complete my thesis and Master's degree.

Outside of the lab, I got to know Robert as I was invited to his ranch to help with projects and rebuilding his fence at his house. Time spent at his ranch became cherished as fishing was simply unbelievable. Also cherished memories was time spent with Robert during that 2-week Hart, Texas pheasant hunt. Not much was completed at school during this two weeks but I will long hold the memory of Robert with his dog named PUC, which became the father of my dog that Robert gave me named Ripley (DNAs PUC Replication). She was the best dog I will ever have.

Robert, you have left a legacy. A family tree with roots that are global. Your impact at Texas Tech and universities world-wide will live on forever. I am glad to be part of this family.

DEIDRE A. PARISH

I was 14 weeks pregnant with Isabella Grace, my youngest daughter, when I joined Robert Baker's lab in January of 1999. She was born July 8, 1999. Baker immediately felt connected to her and displayed pictures of her on the filing cabinet in his office. They were buddies, bonded by picking up pecans in the backyard in Lubbock, fishing at the ranch in Afton, or riding horses at the Bozemans' in Idalou.

A few years later when I was doing my postdoctoral fellowship at The University of Texas M. D. Anderson Cancer Center, Baker had a meeting in Houston so Isabella and I picked him up at the airport. We were waiting at the curb for him to walk out of baggage claim when Isabella turned her head and spotted him. She took off towards him at a full four-year-old sprint. He happened to turn at the right time to see her running straight at him. Like in a movie, Baker dropped his bag, knelt on one knee, and opened his arms out wide. Within seconds, she was wrapped in his warm bear hug, happy to see her buddy. Such big smiles and deep belly laughs exuded from each of them. He said that he would rewind and play that scene over and over.

Through the years since, Baker told me how that moment at the airport outside of baggage claim was one of his moments that he carried in his heart to remember and experience joy. He would reiterate how important collecting the precious moments is. What I realize about the moments he collected is that the moments were usually connected to individuals. He made connections with people.

How did he make connections? Everyone was included on the author line. The entire lab was invited over for the Super Bowl Game. He collaborated with people in all 50 states and every country around the world (almost). He introduced his students to his friends and colleagues and encouraged us to network with those he did not know or like. He sent us on crazy field trips over extended periods of time with other professors and students. He invited families out to the ranch for cow branding. He worked with us side-by-side on experiments and papers. He talked about research, theories, hopes, dreams, and values.

I will forever be grateful for the people brought into my life because of and including Robert Baker. The connections with others and the moments collected while unknotting a bat net, looking in a microscope, setting rat traps, writing a paper, networking at a meeting, or visiting the Wichman lab in Idaho bring me joy.

JIM PATTON

What follows is a free-flow of thoughts and remembrances—so pardon the occasional lack of appropriate sentence structure…

Robert J. Baker—fellow student, long-time colleague, good friend, and evolutionary biologist extraordinaire...

I first met Robert (we all called him "Bob" or "Bobby" back then) sometime in the summer of 1965 when he, his then wife Jean, and toddler April arrived in Tucson to begin his PhD in the Zoology Department at the University of Arizona under Lendell Cockrum. Robert had recently completed his master's degree under Brian Glass at Oklahoma State University and had plans to pursue a study on the reproductive biology of *Leptonycteris yerbabuenae*, a summer migrant to southern Arizona under Cockrum, and in conjunction with Phil Krutzsch, then recently hired in the Department of Anatomy at the nascent University of Arizona School of Medical. I had just completed my own master's degree at the U of A under Bill Heed, a *Drosophila* ecological geneticist, and was also beginning a PhD in the same department.

Our first interactions, that I remember, were on a trip by the two of us to Buckaloo (spelling?) Cave at the northern end of the Chiricahua Mts. in southeastern Arizona sometime in the late summer of 1965. Here was a purported summer colony of some 100 to 1000 individuals of *Leptonycteris*. We teamed up, in part, because in those youthful days I was an active spelunker, and thus had both the equipment and technical knowledge of working in caves; in part, because

I had not visited this cave before; in part, because I (as a grad school transfer from anthropology to zoology the year before) was still experiencing much of the mammalogical world for the first time; and, in part, because Robert was an engaging and energetic addition to the zoology department. My recollection of this trip is that we did not find any bats, but had a great time on the several-hour drive to an from Tucson and clambering through the cave, with carbide lights on hard hats and nets in hand.

Robert and I also took several trips together to the Alamos area in southern Sonora, where I was working on cytogenetic diversity in *Chaetodipus* pocket mice and fellow graduate student Clay Mitchell was working on the reproductive biology of *Natalus*. At that time, the chromosomal complements of most mammals were unknown; I had developed a simple method to karyotype animals; and Robert became interested in chromosomal diversity within the rich bat fauna encountered in the various mines around the Alamos area, at or near the northern limit of many Neotropical taxa. We began to explore this diversity together, and jointly published a couple of the first papers on bat chromosomes. Sometime during the 1965–1966 academic year, Robert realized that a successful *Leptonycteris* study was unlikely to materialize and decided to focus his thesis research on bat chromosomes.

Also in the summer of 1965 I took a two-week cytogenetics course at Brown University in Providence, Rhode Island, where I met T. C. Hsu, one of the great pioneers in mammalian cytogenetics. T. C. had invited me to visit his lab at the M. D. Anderson Hospital complex in Houston, Texas, that fall, and I hosted him for a seminar visit to Tucson in February of 1966. It was on that occasion that Robert met T. C., a connection that would develop further as Robert delved more deeply into bat cytogenetics, and which led to the discovery of multiple sex chromosome systems in several phyllostomid species. This initial focus on bat karyology continued until the very last moments of Robert's illustrious career.

Two things stand out in my memory of our graduate days together. First, and likely unknown to most, is that Robert was then quite religious—he didn't swear or drink, and he wouldn't play football on a Sunday… several of us had formed a team in a flag football league of various graduate programs—Robert was a fleet wide receiver, but as most games were on Sundays, he didn't participate. The jury is out as to whether or not we would have won all of our games had he played! Second, he was a young man in a hurry. The zoology PhD program required 65 graduate course hours in the major (zoology) and 35 graduate hours in a minor field, normally a department outside of zoology. Somehow Robert was able to major in zoology and minor in physiology, both fields part of the Zoology Department. How he did this is unclear to me, but it meant that he could credit the same zoology courses for both his major and minor field requirements. The result was he was able to obtain his degree quickly, finishing in 1967 just two years after he began. In those days, securing an academic position was uncomplicated, and he had (if I remember correctly) two offers the year he finished, accepting the one from Texas Tech, where he joined Bob Packard as a fellow mammalogist and then spent his entire career, becoming a major force in the largest mammal-focused academic program in the country.

Our own paths diverged after Robert left Tucson, although we both pursued similar research questions (albeit on different organisms) and continued separate interests in museum-based, biodiversity science. For a reason that neither of us, in recent years, could understand, we devolved away from close friends and colleagues, becoming competitors instead. Barriers of all kinds are stupid, but this completely unnecessary one fell when, at my suggestion in 1998 to then UC Berkeley Dean of the Graduate Division, the academic unit that oversees campus Organized Research Units, of which the Museum of Vertebrate Zoology is one, to include Robert as a member of an outside review committee for the MVZ. When asked for the name of the most prominent mammalogist in the country to perform the requisite review, without hesitation I nominated Robert. From that day on, we rekindled the friendship we began so long ago, culminating for me in multiple visits to Lubbock where I had the great fortune to stay with Robert and Laura, sleeping on a water bed (a new experience for me!) in their guest room, and spending time with Robert alone at their ranch at Afton. The last time I saw Robert was at the ASM Jacksonville meetings in 2015, where he, my wife Carol (whom Robert had known since our graduate school days), and Merriam Award winner Denise Dearing, spent a most pleasant evening together over beer, dinner, and wonderful conversation, both scientific and personal.

I remember Robert for his consummate inquisitiveness, incredible drive, and innumerable scholarly achievements. But I will remember him most for just being the "Bobby" that I was fortunate to get to know long ago—I miss him dearly.

Caleb Phillips

I first met Robert in 2004 at the Texas Society of Mammalogists annual meeting in Junction, Texas. At the time I was a master's student in the lab of Russell Pfau at Tarleton State University. At TSM that year I provided an oral presentation on patterns of divergence in *Sigmodon hispidus* using a molecular technique called AFLP. Robert was pretty

excited about the potential application of the technique that Russell had put me to work on. I remember Robert sitting on the front row at the oral session with long, curly, and poufy hair (he lost a bet, I am told) bouncing up and down about the method. He subsequently invited me out to visit to consider doing a PhD with him. Thereafter my wife, Shannon, and I went out to see Lubbock and Tech, and I don't think the massive sandstorm we encountered while waiting outside for movie tickets one night during that interview in Lubbock had anything to do with our decision to not move there. All the same, I continued to see Robert when I traveled down annually from Indiana to attend TSM, and upon finishing up by PhD with John Bickham at Purdue I called Robert asking about a postdoc position. I basically told him I needed to be in Texas for family reasons, and he pretty much signed me up to his lab at that point. I can certainly say that Robert's ethos included strong value for family and the importance of encouragement and building people up. This was reflected in his willingness to find a position for me in his lab primarily based on my own personal circumstance. I spent a lot of time with Robert during those subsequent five years I was his postdoc. As a consequence, my own ethos has evolved, and I think there have been improvements in the way I view my own relationships, both personal and professional. Moreover, the academic opportunities that I found among the Texas Mammalogists are in no short part related to his own hard work and dedication over the decades to build something worthwhile. This is reflected in the careers and research that developed through his lab, as well as the immense value of the Natural Science Research Laboratory, which is difficult to overstate. I was told there is a pedigree reporting the descendants of Robert. To my own good news, I've also been told I rank among the highest in the associated academic inbreeding coefficient.

CARLETON J. PHILLIPS

I first met Robert J. Baker when we both were graduate students, way back in the mid-1960's (more decades ago than I can count). I was at The University of Kansas, studying under J. Knox Jones, Jr., and Robert was at The University of Arizona, studying under E. Lindell Cockrum. The universe of mammalogy was different back then, and communication was rarely electronic, but Baker already was well known nationally and established as a rising star. However, I remember being surprised when I heard that he had completed his Ph.D. degree in two years and had accepted a position at Texas Technological College, which seemed like a backwater in comparison to Arizona or Kansas, or the United States National Museum or the American Museum of Natural History. The latter two institutions seemed to be more suitable homes for Robert Baker's talents. At the same I need to acknowledge two things—the first being that there was some skepticism about the adequacy of Robert's preparation for a curatorial museum position and the second being his interest in such a position. Over the years Robert and I talked about this and he was consistent in saying that he felt that a museum position would not have been ideal. His interests in science, and especially the study of phyllostomid bats, went far beyond what could be done in a museum setting. Even so, he made multiple important decisions regarding the development and goals of the museum at Texas Tech University.

Robert Baker had prodigious energy and drive. Most of us who knew him when he first struggled with diabetes thought that his energy and drive were compensation for the disease. Most experts had told him to expect the worse, which included a wide assortment of challenges ranging from retinal pathology to strokes that would add up to a shortened life span. He defied the odds, and lived until shortly before his 76th birthday. Ultimately his battle with diabetes probably did catch up with him, although he also was struggling with a new challenge at the time of his death. Collectively, Robert was faced with awful health challenges that would have destroyed a lesser person at a much younger age. He almost never let diabetes hold him back, even though everyone who traveled into remote places with him ended up with their own nightmare on account of his diabetes. In the last ten years of his life, we did fieldwork together at Chernobyl, and in Ecuador and Kyrgyzstan. In each place at some point he experienced a life-threatening moment involving glucose. After being restored to normalcy, he always went right back to work.

Baker was not an easy person to work with; between his diabetic state and an intense competitive streak he could be a very difficult partner. Robert Baker's sense of competition was at times difficult to handle. He had to be "first" in everything that he did, and he did a lot of projects with a huge array of partners in the course of his career.

As one would expect, being larger than life and hugely successful in virtually every facet of his professional life, Baker was a complex person. I think that anyone who got to know him well would agree that stories about him and his life could easily fill a volume.

But as most of us know, Texas Technological College turned out to be the perfect place for Robert—the school was off the beaten path, and desperate for leadership. Robert knew this was the case, and within a few years after his arrival he had manipulated the political scene so that J. Knox Jones, Jr., was recruited to join the administration leaving Kansas just as I was completing my degree (1969). It took a while but in 1997, Robert recruited me to come to Texas

Tech University as Chairman of Biological Sciences and, ultimately as Assistant Vice President for Research. In my case he promised my wife that he would give her a horse and tack if she helped convince me to accept the offer from Texas Tech. In the meantime, he also had recruited David Schmidly as Vice President for Research and ultimately supported his elevation to the position of President of the University.

Very few if any other professors have had such a significant personal role in developing and shaping a university. Robert Baker deserves credit and recognition for his role in making Texas Tech a major research institution. Among many other examples of leadership, Baker submitted the first NSF proposal from Texas Tech. This alone was an extremely important event because it high-lighted the need for support services—and literally led to the creation of an office headed by a Vice President for Research (Knox Jones conveniently was the first). Robert loved Texas Tech, and until the last ten years of his career he still had loads of influence. It was a case of his knowing nearly everyone who had any importance from members of the Board and the Chancellor, to the President and even the Athletic Director. Whenever there was an important hiring decision at the upper stratum of any administrative area, Robert Baker would be the first faculty member invited to join the Search Committee.

Robert was the ideal faculty member. He understood his role and the expectations of his Chairman. He ran his laboratory, worked with a large number of graduate and undergraduate students, always was willing and in fact eager to teach, and accepted virtually any assignment. He was a man of great talent that set him apart from virtually everyone else. He also was a valued friend and colleague—a person so much larger than life that his presence will be felt for the rest of mine.

C. Miguel Pinto

I first met Robert J. Baker, very briefly, in 2001 when he visited Ecuador for the first Sowell expedition. However, his name—as a prominent figure of the golden generation of mammalogy—was very familiar to me and the rest of René Fonseca's gang of young Ecuadorean mammalogists. This gang was formed by enthusiastic undergraduates and recent graduates conducting research on mammals, including Pablo Jarrín, Juan P. Carrera and Carlos Carrión. In 2004, after René Fonseca's tragic death (1976–2004), Robert encouraged me to publish a new species description I was coauthoring with René and to join his lab as a PhD student.

In 2005 I declined a full-ride fellowship for a doctoral degree in Ecuador, and moved to Lubbock, Texas, to pursue my graduate studies under Robert's supervision. Soon, we started clashing because of Robert' plans about my research. He frequently told me there are two kinds of students: the ones with their own projects, and the ones that take on a Baker's idea. During my time at TTU I had difficulty convincing Baker to allow me to carry out my own projects. These disagreements resulted in me leaving TTU with a masters degree. However, my immense appreciation for Baker was intact, because of his passion for research, training scientists, and, above all, his generosity. Baker was instrumental for the graduate education at TTU of a group of Ecuadorian biologists: Juan P. Carrera, Tamara Enríquez, René Fonseca, Raquel Marchán, and I.

During my initial months at Baker's lab I felt the lab meetings were a burden. However, as time passed I grew to appreciate those meetings as one of the most important aspects of my training as a scientist. The lab meetings were a weekly pep talk, combined with firsthand knowledge accumulated during Baker's more than four decades of academic experience. My favorite meeting was when Robert asked us to answer a few questions, that I now consider key for getting to know the members of any lab: 1) What job would be ideal for you? 2) What would be expected of you in that job? 3) What areas would you need to succeed in to have a quality life? Robert also filled this questionnaire, and he ended it with the following words that have marked me deeply: "Live till I die. Work till I die. Enjoy life till I die. (Repeat daily)."

Calvin A. Porter

I first met Robert Baker in May 1984 when I enrolled in his course in field chromosome techniques at the Texas Tech Center at Junction. Robert immediately recruited me as a Ph.D student. After three weeks in Junction collecting, karyotyping, and prepping lizards, rodents, and bats, I was convinced. Two years later when I finished my M.S., I applied to study in several other labs, but I really already knew I would end up in Lubbock.

When I arrived, I moved into a garage apartment in the Tech Terrace area. Eventually, Robert bought the house and became my landlord. That was the only time in my life when a single individual had the power to have me fired, expelled, and evicted!

On a 1987 fieldtrip, we set up a *Peromyscus leucopus* grid in Oklahoma. Each member of the field crew set traps

in a parallel transect across the grid. As I remember, Robert Baker, Robert Bradley, and I were working adjacent transects. At the edge of the grid, all three traplines descended into a gully tangled with an undergrowth of vicious greenbrier. Robert Bradley was the first to fight through the aggressive vegetation. Thinking to expedite the fieldwork, and to save Robert J. the pain and effort of working through the thorny gully, Bradley turned back in the opposite direction and started checking the last few inaccessible traps of Baker's trapline. Robert was not amused. Suffice it to say that I may have heard some words used in combinations I hadn't heard before, and none of us ever checked Robert's traps again.

In Robert's nonmajors' Biology class, students were assigned seats and it was the responsibility of the course TAs to come in during lecture and mark attendance by recording unoccupied seats. One day, as I completed this task, Robert asked me to call Laura and ask her to bring insulin. Obviously, this was crucial and time-sensitive. I can't explain what happened as I rode the elevator back to the sixth floor, unless I was kidnapped by space aliens who beamed me up to their spaceship, wiped my memory, and returned me to the elevator with no recollection of needing to make the call. When Robert came upstairs after class, he asked if had called. I realized to my horror what I had done. I braced myself to hear more novel arrangements of English words, and wondered if a trip to the ER was in our immediate future. He just said "okay" and walked back to his office. Remarkably, we both survived.

In 1986, Robert and I published an *Occasional Paper* with Ron Crombie entitled *Karyotypes of five species of Cuban lizards*. I confess that the title was entirely my composition. The publication reported on—wait for it—the karyotypes of five species of Cuban lizards. For decades afterwards, Robert referenced this as his worst title. Everyone in the Baker lab who subsequently wrote better titles can thank me for providing Robert's go-to bad example. Although it may be Robert's worst title, I'm sorry to report that it isn't mine.

I often talked baseball with Robert. He took a bunch of us from the lab to Arlington Stadium to watch Nolan Ryan's 5,000th strikeout. Then, during the 1994 ASM meetings at the Smithsonian, I took Robert to an Orioles game at Camden Yards. After attending Robert's memorial service at the Museum, I recalled old times as I went over to Dan Law Field to watch the Red Raiders beat Louisville in the 2018 NCAA Regionals.

As we acclimate to a world without Robert Baker, I have come to realize how integrated he is into my life. It's surprising how often Robert passes briefly across my synapses. I might read a publication and think how he would be interested in the results. I have a story for him to hear. It would be fun to watch this ballgame with him. I would like to have him along on this fieldtrip. If Robert were eating with us, his steak would off the grill already. I have a student or colleague who should meet him. Something happened in the news or in a game, and Robert would certainly have something to say about *that*! I have a question he could answer. I might contemplate his potential input into something I am teaching, writing, or researching, or on some personal matter.

I seldom actually contacted Robert when these ideas passed through my mind. But I knew that we would have these sorts of interactions the next time we met. Now, those RJB neurotransmitters pause for a few extra milliseconds in my synapses as I realize those connections won't happen. But I had 34 years to absorb his ideas on science, biology, and life. We're all the better for the years we had.

Robert on the Baltimore Light Rail following a 1994 Orioles game. As you can see, it was Miller Lite Hat Day.

MAZIN QUMSIYEH

Sometimes I wonder if fate is a matter of luck focused on who we meet in life. I was doing my master at University of Connecticut when I realized traditional morphology was not going to help me study mammals of the Arab world (something I was keen on doing). I looked for the best person doing chromosome studies on mammals and it was Robert Baker, in a place I never heard of (TTU, Lubbock) so I wrote to him. I could have done my PhD in many universities of higher prestige, but doing it with Robert, I reasoned, was the key. I cannot in conscience say I did not hesitate. I even

hesitated the last day as I had my belongings in a UHaul coming down from Oklahoma into the seemingly boring terrain of endless plains. The move in 1982 to Lubbock turned out to be the best move in my life. Not only was I able to get a PhD and publish really good research but there is where I met my wife Jessie, where we had our son, and where I published my first book.

My wedding was held, where else, but at Baker's house! I cannot begin to tell you of all the other things Robert and his kind wife Laura did to help us. On several tough periods in my life, Robert and Laura stood by us. As a struggling student, Robert would pay me a bit of money to help around his house (many times it was clear the job was not important per se but it allowed me to feel needed and not a charitable case). When I was so ill that I thought I would never recover, Laura's connections at the hospital and her personal care were critical, but equally so was the psychological support. The same happened with the difficult birth of my son at Lubbock General Hospital (c-section of a complicated situation). We had to struggle in many ways including financially but in many other ways being away from home and family in our respective countries (Jessie from Taiwan and I from Palestine).

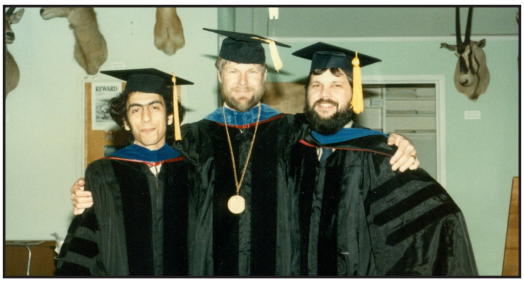
Robert's admonition that "What does not kill you only makes you stronger" was critical for example when a disgruntled ex-student tried to defame me to get back at Robert. I was so distraught then that I even considered quitting science but Robert's wisdom and advise (and the help of fellow graduate students at the time) were critical to pass through that and even come out stronger. Being away from home countries, Robert and Laura were our family. Graduate students and undergraduate students from many parts of the US and the world melded together as a close-knit "mammalogy"family. I especially remember how some graduate students with help of Robert and Laura took care of Jessie in 1985 as a new mom while I was in Kenya for two months. Families have quarrels sometimes but the love stays and family members are never forgotten.

Robert was right most of the time and when he was wrong he did not shy away from saying so. I admired that in him. For example he resisted taking one of the two choices I had for my main thesis research because he thought they would not work out. I asked to be given a chance on both and both ideas worked (one ended up as a large published paper and the thesis work on gerbils produced a series of papers).

The experience at TTU shaped our life in so many ways. I know if I had not been Robert's student, I would have never advanced to career positions at University of Tennessee, Duke, Yale, and then back home to Palistine and inspired to make a difference in people's lives. Even after I left Lubbock, I continued to consult with Robert on some things especially when dealing with tough situations. As Robert once said, the umbilical cord is never cut! I lose friends and colleagues now on a regular basis. I have lost 19 close friends since I returned to Palestine in 2008 (most killed by Israeli soldiers as they participated in non-violent resistance to the occupation). It is hard sometimes to keep going but the strength that Robert taught me is part of my character now. When Robert and Laura faced the horrific situation of losing a son, I knew that while you never get over it, they have the character to go on. Now with Robert gone, we keep his memory alive in our hearts and minds.

The best form of flattery is of course imitation and I now try to help my master students at Birzeit and Bethlehem Universities in the same way I was helped. Thus, we now have the first functioning clinical and research laboratory (cytogenetics and DNA) in the West Bank. We established masters programs in biotechnology (at Bethlehem University) and environmental studies (at Birzeit University). With volunteers and students, we also are building the first Palestine Museum of Natural History (see http://palestinenature.org). My belief is that this is the ripple effect that started in Lubbock, Texas, in 1982.

I could tell many more anecdotes/interesting reflections like the story of "critters' stew" that we students were told a must eat in Junction, Texas. I could relay the hunting stories that Robert told (I myself did not like hunting). I did write some of these experiences in reflection on my three decades in the US. The book will be titled "Joyful Participation: Reflections of a Palestinian–American on three decades in the USA" which alludes to the Buddhist admonition that we "have joyful participation in the sorrows of this world." In that book the goodness of people like Robert is highlighted. If only we could go set traps together one more time...



Mazin Qumsiyeh, Baker, and Craig Hood, 1985 graduation

EMMA K. ROBERTS

Recently, I was given an assignment in a seminar course to define what it means to have a PhD. My first thought was "someone who attains the highest degree in any discipline, with a specialization in a certain field, whether it be science, mathematics, philosophy, among others." In many ways, the PhD is the broadest degree anyone can receive. One must use the knowledge obtained from their field of study and apply it to many diverse and complex questions. Being an expert in all fields of the natural world is certainly impossible, therefore a PhD recipient must use what they know, to answer what they do not know.

In earlier times, a PhD meant knowing a little about many things, whether it be theology and religion, logic, mathematics, art, astronomy, history, politics, or even medicine! One of the earliest PhD holders was a German named Erhard Weigel, who is in Dr. Baker's (and therefore is in my) academic pedigree, and he studied mainly mathematics and astronomy (PhD, Leipzig 1652). Nowadays, having a PhD means knowing a little about many things, and also knowing everything about a specific field.

In addition to a literal definition of a PhD degree, my philosophical answer to the meaning of achieving a PhD is in the following questions: why is the sky blue and universe perceived as black, how did life begin, are we alone in the universe, what makes us human, what is consciousness, is time travel possible? All of these mind-provoking questions are at the basis of what receiving a PhD means: answering the 'potentially' unanswerable. Various scientists have dedicated their life's work to answering the aforementioned questions, and humans have made massive headway—with things like light refraction and why color is perceived in our sky, the early 'primordial soup' from which life sprang, what constitutes other planet's environments and the possible sustainment of life, human intelligence and the ability to think critically, neural circuits and how neurons synapse to allow us to receive and process information, and finally the theory of special relativity showing different time 'clocks' in space compared to on Earth's surface. Have humans answered the questions mentioned earlier? Yes and no. What we have done is worth more than any simple, or complex, clinical diagnosis (although very appreciated). We have discovered a new molecule, observed results from an experiment, developed a scientific theory, provided evidence to reject a hypothesis...and ultimately inched toward the answer to an invaluable question.

Although I did not know Dr. Robert Baker for very long, I attended his seminar series in the Fall of 2013. I was urged to do this not only because at that time he was on my PhD committee, but also because it was a good way to keep up-to-date with the latest 'Baker topic of interest,' which may or may not be on upcoming comprehensive exams.

In his weekly 1-hour seminar, I was inspired on multiple occasions by the repetitive theme of the course: 'What is Mother Nature thinking?'—she is clever, unwavering, complex, and can be merciless. Baker would always smile, knowing that somehow Mother Nature outsmarted him, and if he hadn't realized it yet, he would eventually. Even when times were unbearable for Baker and he did not wish to know Mother Nature at that moment, he continued his search for meaning. No matter what question was asked in seminar, he wanted to ponder it and give it sufficient time and space in his head. Dr. Baker would then ask cognizant and thought-provoking questions like he had already done a literature search on the topic!

One day in seminar, we went around the room and each student listed the questions that were being asked in their research—questions about bats, ecology, rats, behavior, bacteria, hybrids, taxonomic tribes, gametes, radiation, speciation, chromosomes, and conservation (these are the ones I can remember). I would notice a couple of things from Baker—his extensive knowledge about every topic (or at least his ability to BS and in Dr. Baker's words, "pontificate") and his genuine interest. He really wanted to know! Maybe Dr. Baker was a great mentor, adept at helping students learn and grow and helping students want to learn and grow, or maybe he was just addicted to learning about Mother Nature! The answer is probably both.

I was not going to write an encomium for Dr. Baker because I did not know him for very long. But when I was working on the assignment for my current seminar course, I could not help but think of him and the important impact he had on me in my "early days." I also realized that I am one of the last graduate students in the Department who had him serve (however briefly) on their committee. In my opinion, there is no 'one answer' that completely defines what a PhD degree is, so maybe the definition is a person. To me, Dr. Baker epitomizes the PhD degree and is the physical, human answer to the question: 'What does it mean to have a PhD?'

DAVID J. SCHMIDLY

Theodore "Teddy" Roosevelt (TR), the 26th President of the United States, said in a famous speech describing the finest traits of the American people: "The credit belongs to the man who is actually in the arena, whose face is marred by dust and sweat and blood, who strives valiantly, … who knows the great enthusiasms, the great devotions, who spends himself for a worthy cause; who, at the best, knows, in the end, the triumph of high achievement, and who, at the worst, if he fails, at least he fails while daring greatly, so that his place shall never be with those cold and timid souls who knew neither victory nor defeat."

Little did he know, but TR gave us the perfect description of my friend and colleague Robert J. "Bob" Baker. All of us who knew him well can recall his enthusiasm, his devotion to a worthy cause and his family and friends, his high achievement, the manner in which he held up and overcame difficulties in his life, and we most certainly knew that he was not cold or timid in either victory or defeat!

I knew Robert for more than 50 years. We first met in the Spring of 1967 when he was interviewing for a job in the Department of Biology at Texas Tech. Robert hit the department like a whirlwind. He had a small office with an equally small lab in the back that was soon filled with bright undergraduate and graduate students eager to learn this new technique of karyotyping. Right away students gravitated to him. Robert cared about his students—he didn't coddle them; in fact he was often very hard and demanding of them—but he cared that they were prepared to be successful and ready for the hard work of success as a scientist. In 1986, at the pinnacle of his publishing career, he took a leave of absence from Tech and spent a year at Harvard learning some of the new techniques of molecular biology. When I asked him why he did this, he told me it was primarily for his students. If they are going to be "cutting edge," as I tell them they must be, then I have to be as well.

I have picked out two words to describe Robert's career – legacy and legendary. He spent his entire career at one institution and that in itself is pretty remarkable in academia. I know that he had many opportunities to leave for another more prestigious institution, and one time I asked him why he didn't go. The answer I received was pretty remarkable. He told me that over the years one of his major goals became to build Texas Tech – to make it better and as good as it could be. And, he said that he also came to love Lubbock and West Texas.

In my 50 years associated with higher education, I have known only a handful of faculty with the academic accomplishments of Robert which are legendary by any measure (see the lead article of this volume). He received every major award given by the American Society of Mammalogists – the only person in the history of the Society to have accomplished this. But equally impressive to me is the fact that the number one course taught, as listed on his curriculum vitae, was "freshman biology for non-majors." It is not often that you will find a distinguished scholar teaching freshman non-major students. The impact of this to society is enormous.

I could go on and on about Robert. I consider him one of my best friends in life and he has been a life-long colleague. He devoted his career to helping the university we both love move forward, and he has dedicated his life and career to advancing the science of mammalogy and his students. He was a devoted husband to his lovely and talented wife Laura and together they raised one of the finest sons a family could ever want, and then suffered the greatest tragedy a family can endure. But they did this together with dignity and provided to all of us an example of how to cope with tragedy.

So, in conclusion, Roosevelt was right – the credit does belong to the man in the arena. And that is where Robert Baker chose to spend his life and career. A country boy from Arkansas, who lost his father in WWII and grew up reciting the Bible, and became a world-class teacher and scholar who taught us much about the natural world and life. What A Story!

FREDERICK B. STANGL, JR.

Robert Baker had a big heart, and you didn't have to be his best friend or favorite student to benefit from that generosity. Being on his "crap list" one day (or more) for some perceived error never meant that you couldn't count on him for anything. His loyalty and perceived obligations to his students didn't end at commencement, and I will always be grateful for his many courtesies and favors through the years, both personal and professional. I always considered myself fortunate to claim him as both a friend and PhD mentor.

Speaking of commencements, Robert had the firm view that animals collected were animals to be processed and prepared as specimens, and I had (and still retain) the penchant to keep small animals as pets. I remember how he grudgingly let me take one of the many antelope squirrels he collected in Utah, but I only dared ask because he forgot to show up for my graduation and hooding ceremony the night before. That little squirrel was a popular resident of my lab for the next several years.

Coming from a small school (Midwestern State University), my plans were to complete the degree in three years and return to Midwestern, coincidental with the retirement of my MS mentor, Walt Dalquest. Following in the footsteps of my immediate predecessors (the likes of John Bickham, Ira Greenbaum, Rodney Honeycutt, and Terry Yates) was pretty intimidating, but I was fortunate to be surrounded by a wonderful crop of contemporaries (Mike Arnold, Mike Haiduk, Craig Hood, Ben Koop, Lynn Robbins, Mazin Qumsiyeh) who helped me through. Robert wasn't happy that my three-year plan would mean my missing out on the meetings and group field trips that he considered so critical to a student's professional development. Nevertheless, he agreed to let me try.

Robert was a hard driver, and I pushed hard in both lab and field work. There were times of tension, but these were always forgotten by the next day (or sometimes the day after that). Things worked out—I finished the degree in three years, interviewed for the job, and landed it.

Years later, he told a mutual friend that I was one of his greatest success stories, by virtue of my getting the job that I started out seeking. Given the quality of students he put out and where they all ended up, that remains a matter of no small pride for me.

PHIL SUDMAN

Robert was my academic grandfather. I often reminded him of that fact, and in some small way he always seemed pleased. To be honest I was intimidated by him when I was a graduate student working in Ira Greenbaum's lab. I would see Robert three times a year, at TSM, SWAN, and ASM meetings, and mostly just tried to stay out of his way. However, once I graduated, it seemed that our relationship changed. He would ask my advice or impression and often go out of his way to talk to me at meetings. I distinctly remember when I was attending the TSM meetings the first year I was working as a postdoc at LSU. Back then, we used to get everyone together and all of the faculty would go around the room, introduce themselves to the new (and continuing) student members and highlight the research that was being done in their lab. When it got to me, I shrugged and looked at Robert – he said "Go ahead, you're one of us now!" To be accepted as a peer by the giants in your field is a special feeling, and I thank Robert for welcoming me into his fold.

One of my favorite memories involving Robert (RJB) involved another Robert, Robert Bradley (RDB). While at A&M working on my Ph.D., RDB invited me to come up to Lubbock to attempt to characterize the chromosomal pairing configuration of hybrid *Geomys brusarius* X *knoxjonesi* pocket gophers. First, we needed to drive out to the contact zone in eastern New Mexico, catch a bunch of gophers, and then haul back to Lubbock to prepare the chromosomal material. On one of our trips, we also set up a bat net over our cots and captured several pallid bats (*Antrozous pallidus*), which we returned to Lubbock the next day, still in their bat bags. In our haste to process the gophers, the bats were hung on a glassware drying rack in the lab, and promptly forgotten. Evidently at least one escaped overnight and was flying around the 6th floor of the Biology building when RJB arrived the next morning. By the time RDB and I arrived to pick up our gear and head back to the field, RJB was on a rant. We quietly gathered up what we needed and ducked out the door, all the time hearing RJB yelling in the background "Why the hell do you think we have a %^&# !#%& animal room?" I'm pretty sure we could still hear him as we pulled into Tiaban, NM, and headed south to the contact zone. My sincere apologies to Ron Van Den Bussche, Meredith Hamilton, Calvin Porter, and any other members of the Baker lab circa 1988 that had to endure the wrath of RJB on our behalf!

JON WHITMORE

Robert Baker may have been the single most interesting person I have met in my lifetime. This is a big statement, because I have met many complex and fascinating people, but Robert wins the "most interesting" award.

Robert was a mischievous, darkly thoughtful, lighthearted, fierce, intense, dedicated, sometimes angry, yet, mostly, loving and caring person. I learned much about a wide array of things from Robert: how Texas Tech University functioned on multiple levels, and about bats, athletics, teaching, hunting, driving badly, shot-gunning, feeding animals, cheering passionately, cooking quail and dove, caring for other people, especially students, and dealing with life's big disappointments.

Robert Baker lived life fully. More fully than anyone I know. May he rest now, content with getting three or four times more out of his life than most people do.

I confess here to having great difficulty writing about Robert Baker following his passing. All the images in my head are of passion, smiles, and forward action—pressing, pressing, pressing. That is the only way I can think of him.

HOLLY WICHMAN

Without Robert Baker's advice and steadfast support, I am not sure that I would have a career in science today. In the spring of 1990, after 18 months as an Assistant Professor at the University of Idaho, I received a 14 to 2 vote that I was *not* making adequate progress toward tenure. I had brought an NIH grant and a technician with me from my previous position, and we were doing experiments on plywood benchtops while we waited for lab renovations to be completed, but I had not yet had my first Idaho publication. My publication record was *weak*. I wanted to write big stories with all the answers, but it was pretty slow going with molecular methods at that time. I called Robert for advice. He asked what the next step in the evaluation process was, and I told him that I had a meeting with my chairman the next day. He said "Get dressed up like you are going out to dinner. Walk into his office with your chin up and say—*I understand the problem and I'll take care of it*." I did, and with Robert's help I survived to become the first tenured woman in the department. Robert taught me how to break my work down into more realistic units, and ultimately we published over a dozen papers together, but I am getting ahead of my story.

Although I had been aware of Robert Baker's work for some time, I first laid eyes on him at the annual meeting of the American Society of Mammalogists (ASM) at Humboldt State University in 1984. I think it was my first time attending these meetings, and it was certainly my first time going to a meeting alone. I did not know anyone there, but I had come to hear Rodney Honeycutt's talk because his work was closer than anyone's to the work that I was trying to do at the time. As it turned out, Rodney stayed home to be present at the birth of one of his kids. Good excuse. I had lost my voice on the plane and was having a hard time talking to anyone in that loud and enthusiastic crowd.

At one of the sessions I saw a member of the audience go after a young woman who was presenting a paper. It was a very uncomfortable situation, especially because ASM was still pretty male-dominated at the time. Even when she said she was an undergraduate, had only been working in the lab for a few months, and did not know the answer to his question, he kept pushing. It left me with a bad taste in my mouth. But later that afternoon I saw the offending "gentleman" toe-to-toe with another alpha male who was about 6 inches from his face yelling something a bit like, "Next time you should take on someone your own size instead of picking on an undergraduate"—not his exact words, of course. I wanted to cheer. I found out later that the knight in shining armor was Robert Baker, and the undergraduate was one of his students. I did not meet Robert Baker at that time, but I did meet Ira Greenbaum (one of Baker's former PhD students) and had great scientific talks with him while he provided me with a little sipping whiskey to soothe my throat. That encounter gave rise to one of those interesting but largely inaccurate urban myths, but that is another story.

The next time I met Robert Baker was at the International Theriological Congress in Edmonton Alberta in 1985. He came strolling up to my table at the banquet with an unlit cigar in his mouth and said, "Hi. I'm Robert Baker. I want to shake your hand. You're the lady that found those transposable elements in *Peromyscus*." It is a much better story with the appropriate accent, but that's a little hard to capture on paper. I was absolutely thrilled. He was referring to a retrotransposon we isolated from deer mice and named *mys*. We met again at the Evolution meetings in Bozeman, Montana in 1987, where he told my then husband "We're going to recruit your wife to Lubbock Texas." It wasn't until the Evolution meetings at Asilomar California in 1988 that we began to seriously discuss collaboration. We agreed to look at the chromosomal and phylogenetic distribution of the *Peromyscus* transposable element, and shortly after I moved to the University of Idaho in 1988 I sent him a clone of that element, *mys*. Our first paper together was published in 1990, "Retrotransposon *mys* is concentrated on the sex chromosomes: Implications for copy number containment." Thus began a long and fruitful collaboration and friendship.

Although Robert was thrilled with the opportunity to carry out fluorescent in situ hybridization using *mys* as a probe, he frequently commented that while we had a transposable element that was a 'miss,' we didn't have a 'mister.' I patiently explained to him that *mys* was not a feminist statement but rather the Greek word for mouse (which he of course knew). Nevertheless, when we isolated a new and highly active endogenous retrovirus from *Oryzomys palustris*, we called it *mys*TR.

Robert was a brilliant advocate for many women scientists—something that often mystified and was sometimes misunderstood by our male colleagues. This included many of his own students and postdocs, but like me, many of these women were not his students. He was the consummate collaborator and always a little more fair than necessary on the authorship line. He did not belittle his female collaborators, rather he promoted their ideas when they were not there and made them feel like superwomen when they were. Even now I occasionally meet a "sister" whose career was promoted by Robert and who has nothing but good things to say about him.

If you collaborated with Robert, you became part of his extended family. For more than a decade I went to Tech a couple times a year, got to know Laura and watch Bobby grow up. For many years I knew most of the students who went through his lab. Over the years I spent hours at the microscope in Robert's lab while Meredith Hamilton, Deidra

Parish, Laura Wiggins (an undergrad in Robert's lab, now Wiggins, M.D.), and Cibele Caio showed me chromosomes *in situ* hybridized to probes that we provided to Robert's lab. I also mentored some of his students. Robert Bradley spent a summer in Idaho doing bench work. Ron Van Den Bussche and Meredith Hamilton were postdocs with me.

But life is also made up of the little things. Robert was great at appreciating little things—counting the new calves, smoking a Cuban cigar, rocking a baby. I shared many such 'little thing' memories with Robert. I like to remember a spring walk at the ranch with Robert and Laura Wiggins. As we ambled along, Robert picked and identified wildflowers for us—one for her and the same one for me, one for me and the same one for her—until we each had a big bouquet where each flower was one of a kind. Life is just a bunch of memories that you stack up as you go along, and that day is one of my favorite ways to remember Robert Baker.

JEFFREY K. WICKLIFFE

Robert came into my life before he ever physically came into my life, as I think was probably the case for many. I first "met" Robert through my time with John Bickham when I was at Texas A&M University (a venerable enemy of the mighty Red Raiders unbeknownst to me at that time in my life). In so many ways that he too will never know, John laid the foundation for what would become one of the most profound and life-fulfilling experiences of my life that continues today and without question will continue for the remainder of my life.

I had what I thought was a clear plan of where my research career was going after finishing my MS with John. My time with John clearly cemented my interest in a research career and this plan of mine. However, I started my PhD at the University of Georgia where I was certain I could tackle my research interests in population genetics and environmental toxicology going forward. While a step in the process, it was but a very short step. I met some great people, learned a great deal academically and from the experience itself, but stayed true to where I was headed at the time. To remain true to my real interests, I decided I would probably have to actually move on. While at UGA, I met Mike Arnold, who it turns out had also worked with Robert Baker (see where this is going?) and also had the highest of praises for his experience with Robert. Having grown up in Texas, I never thought my research compass would ever point me to Lubbock though. Well that is what I get for thinking. One of my laboratory mates at UGA (and an old friend from TAMU), Andrew DeWoody, had done his doctoral research with Robert in Chornobyl, Ukraine. Andrew's sage advice and counseling really convinced me that this would be an ideal situation for what I wanted to do. Boy, was he right.

Robert met me at the airport for my first visit to TTU, his lab, and Lubbock, which he completely covered treating me as a valued professional from the beginning. From that point on Robert became my life coach not just an academic advisor. From our vantage in Lubbock, he introduced me to people from all over the world and to this day, I have lasting friendships with wonderful people from Ukraine, Texas, South America, and so many other places on the globe. Working with Robert and his, always, extended research family, was, and remains, priceless. Without that time and training, I would not be where I am today, an Associate Professor at Tulane University (by the way, I spoke to Robert about my Tulane offer soon after receiving it; the only person that I spoke to before Robert was my wife Jennifer). The research and academic training Robert provided are immeasureable, but what Robert really provided was so much more than what typical academic mentors provide. As with many, Robert became, a cherished friend, a second father, a second grandfather, a cold morning in the blind, a warm afternoon fishing, another glass of red wine, collecting gifts for families and friends in Kiev, oysters on the half shell, …

Robert may have left me (us) physically, but I will never be without him. Friends and families that I am close with today are because Robert was an architect of my life. Keeping close to my experience with "the Baker Lab" and not provide a truly extensive, deserving list, this includes Deidre and Bud Parish, Adam Brown, Cole and Carey Matson, Calvin and Ann Porter, Mark and Emily O'Neill, Sergey Gaschak, Ron Chesser and Brenda Rodgers, Carl Phillips, just to name a very few. Of course, my network of friends and family woven together with Robert goes far beyond the people I have included. One family in particular remains very close to my family, this again is not solely an attribute of academics but rather a tribute to Robert's ability to connect people. Federico Hoffmann and Florencia Mayer came into our lives not long after we had settled in at TTU and Lubbock. Coming from distant and foreign Uruguay, South America, we had certainly never known anyone from that small coastal country nor had we ever spent time with anyone from South America. Since that memorable day Fede arrived in Lubbock and later brought Flor to stay and pursue her graduate research career, we have spent numerous weekends and vacations together and watched each other's families, Guille, Luca, and Martina (Hoffmann family) and Justin and Lyndsey (Wickliffe family), grow up and continue to grow up. While working on scientific endeavors is something Robert would definitely be proud of, lasting relationships and friendships beyond research is also what Robert strongly fostered. We are always working on the next

experiment, manuscript, presentation, or grant application and I cannot credit Robert enough for making me feel like my scientific career is highly valuable and important to society. One thing Robert should get equal if not more credit for, is inspiring me to both become and remain an open, accepting, inclusive, and respectful global citizen.

FRANK YANCEY

It was a privilege and honor to have had Dr. Robert J. Baker serve on my Ph.D. committee, and to work under him, in part, during my postdoctoral research at Texas Tech University. His knowledge of mammalian systematics was phenomenal, and I benefited substantially from the access he provided to his lab. He always treated me with tremendous respect and kindness, and I appreciated his many words of encouragement, though often mixed with a little sarcastic humor. I often reflect, with a little laugh, on the time that Clyde Jones, my Ph.D. advisor, handed Robert a draft of my dissertation for his review, saying to Robert "Here's Frank's dissertation, over 450 pages." Robert's response: "A roll of toilet paper has over 450 pages too…doesn't make it any good."

I feel fortunate that I was able to return to Texas Tech on several occasions during recent years and visit with Robert. As busy a man as he was, he always made time to come on over to the NSRL and spend a little time catching up. Robert Baker will be missed profoundly by both the science of mammalogy and the Texas Tech family. Thank you, Robert, for all you have done.

NANCY YATES

My husband, Terry, was first introduced to Robert Baker as a possible Ph.D. candidate by David J. Schmidly. Terry was a master's student at Texas A&M University and David was his major professor. David made a call to his friend and colleague, Robert Baker, to tell him about a student of his that he should consider as a Ph. D. student. We are indebted to you, David, for seeing this perfect match! Robert agreed to accept Terry into his program.

For the next three years we would be part of the Baker Family. I could not believe how long and hard Terry worked as he made progress towards his final degree. Robert was always at the lab and I can remember his many phone calls at 2 a.m. asking Terry why he wasn't at the lab? So of course Terry would get dressed and rush to the lab. I think that's when he gleaned many of Robert's many pearls of wisdom! Robert would say, "anything worth doing is worth overdoing!" This lead to many successes and late nights! This has been fondly adopted by the Yates family as our family motto!

Robert was always there to mentor, answer questions, pose questions, teach and lead by example. I had never witnessed such an intense level of dedication from a teacher towards a student. This was the essence of Robert Baker. Robert never asked a student to do something that he hadn't done himself or wasn't willing to do right alongside them. He led by example! Robert instilled a drive and work ethic in Terry that would serve him for his entire career.

Robert's deep love and passion to work in the "field" was eagerly adopted by Terry. Terry would in turn instill this love of the "field" in his own students. This was an invaluable lesson he learned from Robert.

When Terry passed his oral comprehensive exams for his Ph. D., Robert's tradition of taking his students out to dinner after this glorious achievement resulted in my immediate labor and birth of our first born son, Brian. Robert was a big part of Brian's life as he would later share his own passion and love of hunting with Brian as he hosted and hunted with him at his ranch. He would also share his love of fishing with our son, Michael, and his daughter, Rebecca, as they made forever memories fishing at his ranch. Yes, we were a part of the Baker Family.

Robert would be a major part of a team of doctors and close friends that would oversee and guide Terry's care in his battle with cancer. Robert was a team member and author of Terry's obituary written for the *Journal of Mammalogy*. I am eternally grateful!

Before Terry's death in 2007, he had always hoped to be drawn for an elk lottery in New Mexico. He tried tirelessly and was not drawn until a week after his last radiation treatment, just a month before his passing. Robert would be the teacher, mentor, friend, and confidant to accompany Terry on this fulfilled dream.

Robert was always a big part of our lives. The desire to help and be part of our lives did not end in December 1978 when Terry graduated from Texas Tech. Robert was a true friend, a forever friend. Robert and his legacy touched my family deeply. Robert, you are missed but you are always in the hearts and fond memories of the Yates Family. Thank you, my friend, for everything!