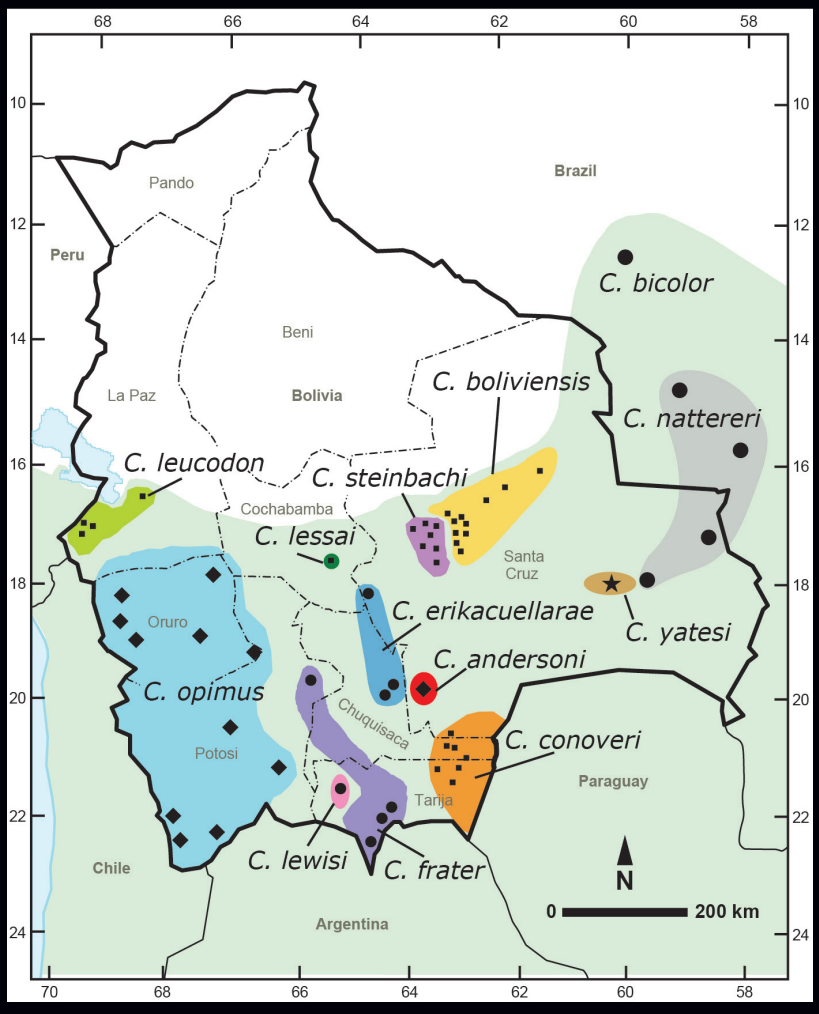
TEXAS TECH UNIVERSITY
Natural Science Research Laboratory

Special Publications

Museum of Texas Tech University
Number 62 17 June 2014

New Species of *Ctenomys* Blainville 1826 (Rodentia: Ctenomyidae) from the Lowlands and Central Valleys of Bolivia



Scott L. Gardner, Jorge Salazar-Bravo, and Joseph A. Cook

Front cover: Collection localities and estimated ranges for 12 Bolivian species of *Ctenomys*: *C. boliviensis*, *C. conoveri*, *C. frater*, *C. leucodon*, *C. lewisi*, *C. nattereri*, *C. opimus*, *C. steinbachi*, and the four new species described herein, *C. andersoni* n. sp., *C. erikacuellarae* n. sp., *C. lessai* n. sp., and *C. yatesi* n. sp. Shown also is a single locality for *C. bicolor*. Figure modified from Gardner (1991).

Special Publications

Museum of Texas Tech University

Number 62

New Species of *Ctenomys* Blainville 1826 (Rodentia: Ctenomyidae) from the Lowlands and Central Valleys of Bolivia

Scott L. Gardner, Jorge Salazar-Bravo, and Joseph A. Cook

Layout and Design: Lisa Bradley
Cover Design: Scott L. Gardner
Production Editor: Lisa Bradley

This book was set in Times New Roman and printed on acid-free paper that meets the guidelines for permanence and durability of the Committee on Production Guidelines for Book Longevity of the Council on Library Resources.

Printed: 17 June 2014

Library of Congress Cataloging-in-Publication Data

Special Publications, Museum of Texas Tech University, Number 62
Series Editor: Robert J. Baker

New Species of *Ctenomys* Blainville 1826 (Rodentia: Ctenomyidae) from the Lowlands and Central Valleys of Bolivia

Scott L. Gardner, Jorge Salazar-Bravo, and Joseph A Cook

ISSN 0149-1768
ISBN 1-929330-26-X
ISBN13 978-1-929330-26-3

Museum of Texas Tech University
Lubbock, TX 79409-3191 USA
(806) 742-2442

New Species of *Ctenomys* Blainville 1826 (Rodentia: Ctenomyidae) from the Lowlands and Central Valleys of Bolivia

Scott L. Gardner, Jorge Salazar-Bravo, and Joseph A. Cook

Abstract

The genus *Ctenomys* Blainville 1826 is one of the most diverse of South American hystricognath rodents. Currently, nine species of tuco-tucos are reported from Bolivia, four at elevations above 2,000 m and five inhabiting the lowlands (< 1,000 m). In the present paper, morphology, karyology, and phylogenetic analyses of DNA sequences for a mitochondrial locus were used to assess the taxonomic status of specimens of *Ctenomys* from localities beyond the previously known ranges of these rodents in the departments of Chuquisaca, Cochabamba, Santa Cruz, and Tarija. Based on these analyses, we describe four new species in the genus *Ctenomys*, all apparently endemic to the country. In addition, we place *Ctenomys goodfellowi* Thomas 1921 in synonymy under *C. boliviensis* Waterhouse 1848 and confirm the presence of *C. nattereri* Wagner 1848 as a denizen of the eastern lowlands; therefore the total number of documented extant species of *Ctenomys* in Bolivia is now 12.

Key words: Bolivia, *Ctenomys*, cytochrome-*b*, inter-Andean valleys, subterranean rodents, tuco-tuco, Yungas

Resumen

El género *Ctenomys* Blainville 1826 es uno de los géneros de roedores histricognatos más diversos de América del Sur. Nueve especies de tuco-tuco se han reportado en Bolivia, cuatro en elevaciones mayores a 2,000 m y cinco que habitan en las llanura tropicales y chaqueñas (<1,000 m). En el presente trabajo utilizamos caracteres morfológicos y cariológicos, los que combinados con los resultados de análisis filogenéticos de marcadores moleculares (secuencias de ADN para un locus mitocondrial) nos sirvieron para evaluar el estatus taxonómico de especímenes de *Ctenomys* provenientes de localidades en los departamentos de Chuquisaca, Cochabamba, Santa Cruz y Tarija y que hasta el momento no habían sido analizados en detalle. Sobre la base de estos análisis, se describen cuatro especies nuevas en el género *Ctenomys*, todas aparentemente endémicas del país. Además, estos análisis nos permiten sinonimizar *Ctenomys goodfellowi* bajo *C. boliviensis* Waterhouse 1848 y confirmar la presencia de *C. nattereri* Wagner 1848 como un habitante de las tierras bajas del oriente; por lo tanto, en este momento existen 12 especies documentadas de *Ctenomys* en Bolivia.

Palabras claves: Bolivia, citocromo-*b*, *Ctenomys*, roedores subterráneos, tuco-tuco, Valles Interandinos, Yungas

Online supplemental documentation available at http://digitalcommons.unl.edu/parasitologyfacpubs/722.

INTRODUCTION

Tuco-tucos are subterranean rodents in the genus *Ctenomys* Blainville 1826 (Rodentia: Ctenomyidae). These rodents are endemic to South America and occur in well-drained, friable soils from about latitude 12°S in southern Peru (Sanborn and Pearson 1947), south to Tierra del Fuego, and from the highlands of Peru and Bolivia to the lowlands of southeastern Brazil and northeastern Uruguay (Pearson and Christie 1985; Wood and Kilpatrick 2005; Cueto et al. 2008). Many aspects of the biology of ctenomyids remain unstudied, and in many cases distributional limits of species are poorly defined. Estimates of the number of species in the genus range from 38 to more than 60 (Woods and Kilpatrick 2005).

Early studies on the systematics and taxonomy of *Ctenomys* relied on color of pelage and general morphological analyses of skull and other characters of the body. Later, chromosomes, allozymes, and DNA sequences were used to examine the systematics and biogeography of these rodents (Cook 1990; Gardner 1991; Cook and Yates 1994; Lessa and Cook 1998; Cook and Lessa 1998; Mascheretti et al. 2000; Cook and Salazar-Bravo 2004; Castillo et al. 2005; Freitas 2006; Parada et al. 2011; Freitas et al. 2012).

In lowland Bolivia, these rodents reach a northernmost limit of about 16°S in the department of Santa Cruz. At higher elevations, in mountains and on the altiplano of western Bolivia, tuco-tucos occur south of Lake Titicaca (ca. 3,800 m.). Anderson et al. (1987) showed that four species occur in the eastern lowlands of Bolivia including *C. boliviensis* Waterhouse 1848, *C. steinbachi* Thomas 1907 (see Thomas 1907, Wagner 1948, and Waterhouse 1848), *C. conoveri* Osgood 1946 (see Osgood 1946), and a species that was referred to as *C. minutus* Nehring 1887. Cook et al. (1990) showed that four species of *Ctenomys* occurred at high elevations (> 2,000 m) including: *Ctenomys opimus* Wagner 1848, *C. leucodon* Waterhouse 1848, *C. lewisi* Thomas 1926, and *C. frater* Thomas 1902 (see Thomas 1902, 1926). *Ctenomys goodfellowi* Thomas 1921 was considered a species different from *C. boliviensis* by Cook and Yates (1994), a conclusion accepted by Anderson (1997).

Ctenomyids occupying habitats in intermediate elevations, including the Andean valleys of central Bolivia, have not been studied in detail (Anderson 1997). In the present paper, we examined all species of *Ctenomys* in and around Bolivia, first using mitochondrial DNA sequences to define species limits and relationships and then analyzing patterns of morphometric, morphological, and karyological variation.

Species of *Ctenomys* have mostly allopatric distributions (Anderson 1997) and are highly variable in morphological and chromosomal characters. To identify species, we used the principles of concordance (Avise 2004) and reciprocal monophyly. Therefore, we recognize species as the most exclusive hierarchical taxa where agreement among independent data define diagnosable and reciprocally monophyletic lineages.

MATERIALS AND METHODS

Field collecting, specimens, and cytogenetics.—Animals were collected using traps set in burrows following guidelines approved by the American Society of Mammalogists (Sikes et al. 2011). Individuals were measured, weighed, determined to sex, and prepared as museum specimens (Yates et al. 1996) with tissue samples and karyotypes frozen in liquid nitrogen (Cook 1990). All arthropod and helminth parasites were archived following standard protocols (e.g., Gardner 1996; Gardner and Jiménez-Ruiz 2009). Representative individuals from most populations were karyotyped in the field using the standard bone marrow technique (Baker et al. 1982), as modified by Anderson et al. (1987). At least 10 metaphase cells were photographed and scored to determine diploid and fundamental numbers. Fundamental number (FN) of chromosomal arms follows the "autosomal number" convention (Gardner and Patton 1976). For each individual specimen studied, fur and teeth color were characterized under natural light. For both descriptions and comparisons of species, we followed the color nomenclature of Ridgway (1912).

Specimens examined are archived in these museum collections: Division of Mammals, Museum of Southwestern Biology (MSB), Albuquerque, New Mexico, USA; Department of Mammalogy, American Museum of Natural History (AMNH), New York City; Colección Boliviana de Fauna (CBF), La Paz, Bolivia; and Museo "Noel Kempff Mercado" (MNKM), Santa Cruz, Bolivia. Frozen tissues, cell suspensions, and slides with stained chromosome spreads are deposited in the Division of Genomic Resources (DGR) of the MSB. Any parasites collected during our field-expeditions are archived in the Bolivian Mammal Parasite Collection in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska State Museum Lincoln, Nebraska, USA.

Standard external measurements recorded for each individual at time of collection include: TL: total body length; T: tail length; HF: hind foot length (including the claw); EL: ear length; and W: weight. Skull measurements and character definitions (Fig. 1) follow Anderson et al. (1987) and Gardner (1991) and included: condylobasilar root length (CBR), condyloincisive length (CIL), palatal root length (PLR), alveolar molar length (MOL), palatal breadth, minimum (PAB), dental span breadth (DSB), zygomatic breadth (ZYB), lambdoidal breadth (LAB), interorbital breadth (IOB), and height of skull (SHT). Additional qualitative characters included: curvature of zygomatic process (CZP), jugal process of zygomatic arch (PZA), incisive foramen (IF), frontal parietal foramen (FPF), articulation surface of condyloid process (AS), and occipital crest (OC). Nomenclature used to describe skull characters follows Langguth and Abella (1970) and Wahlert (1974, 1985).

Taxonomic names follow Woods and Kilpatrick (2005) unless modified by more recent taxonomic changes (e.g., Freitas et al. 2012, Stolz et al. 2013). Informal names associated with specimens analyzed by previous authors are included in the synonymies.

Morphological and morphometric analyses.—Morphological comparisons and morphometric analyses of the *Ctenomys* species were guided by phylogenetic analyses of molecular data (see below). Specimens were allocated into three age groups following Langguth and Abella (1970) and Altuna and Lessa (1985). Pooled (male and female) measurements of skull, weight, and external dimensions are given in Table 1. Measurements of all individuals included in this study are given for each species in Table-S1 in online supplemental documentation.

SAS 9.2 software was used for all multivariate statistical analyses. Because the frequency distributions of some characters deviated slightly from normality, all data were log transformed ($\log_{10}$) prior to analysis. For the morphometric variables listed above, stepwise discriminant function analysis (STEPDISC) was employed to explore the data-set and attempt to identify diagnostic mensural characters. Canonical discriminant function analysis (CANDISC) was also used to establish a linear combination of morphological variables that best delineated species that were defined *a priori* on the basis of morphological comparisons, chromosomes, and phylogenetic analyses. These variables were then used to highlight the similarities and differences between and among species of *Ctenomys* that we studied.

Methods for DNA extraction, amplification, and sequencing were as given in Lessa and Cook (1998), Parada et al. (2011), and Freitas et al. (2012). Each PCR fragment was sequenced in both directions; sequences were free of indels, premature stop codons, and ambiguities in forward and reverse directions, providing support for a mitochondrial origin (Triant and DeWoody 2007). Sequence data that we generated for five individuals included in this paper have been deposited in GenBank. All species names used in our study, along with specific field-catalog, museum-catalog, and (or) GenBank accession numbers are given in Table-S2 in the online supplemental documents.

DNA sequence analyses.—Genetic comparisons and phylogenetic analyses were based on complete cytochrome-*b* gene (*cyt*b) sequences consisting of 1,140 base pairs (bp), with the exception of 13 sequences (e.g. *C. bicolor*) which ranged in length from 806 to 1,127 nucleotides. Our dataset differs from that of Freitas et al. (2012), the most inclusive analysis of *cyt*b sequence data to date, in that we included five additional Bolivian samples and representatives of both *C. bicolor* and *C. nattereri* from Brazil. In the present paper, we generated a matrix of 75 terminal taxa representing approximately 50 currently recognized species.

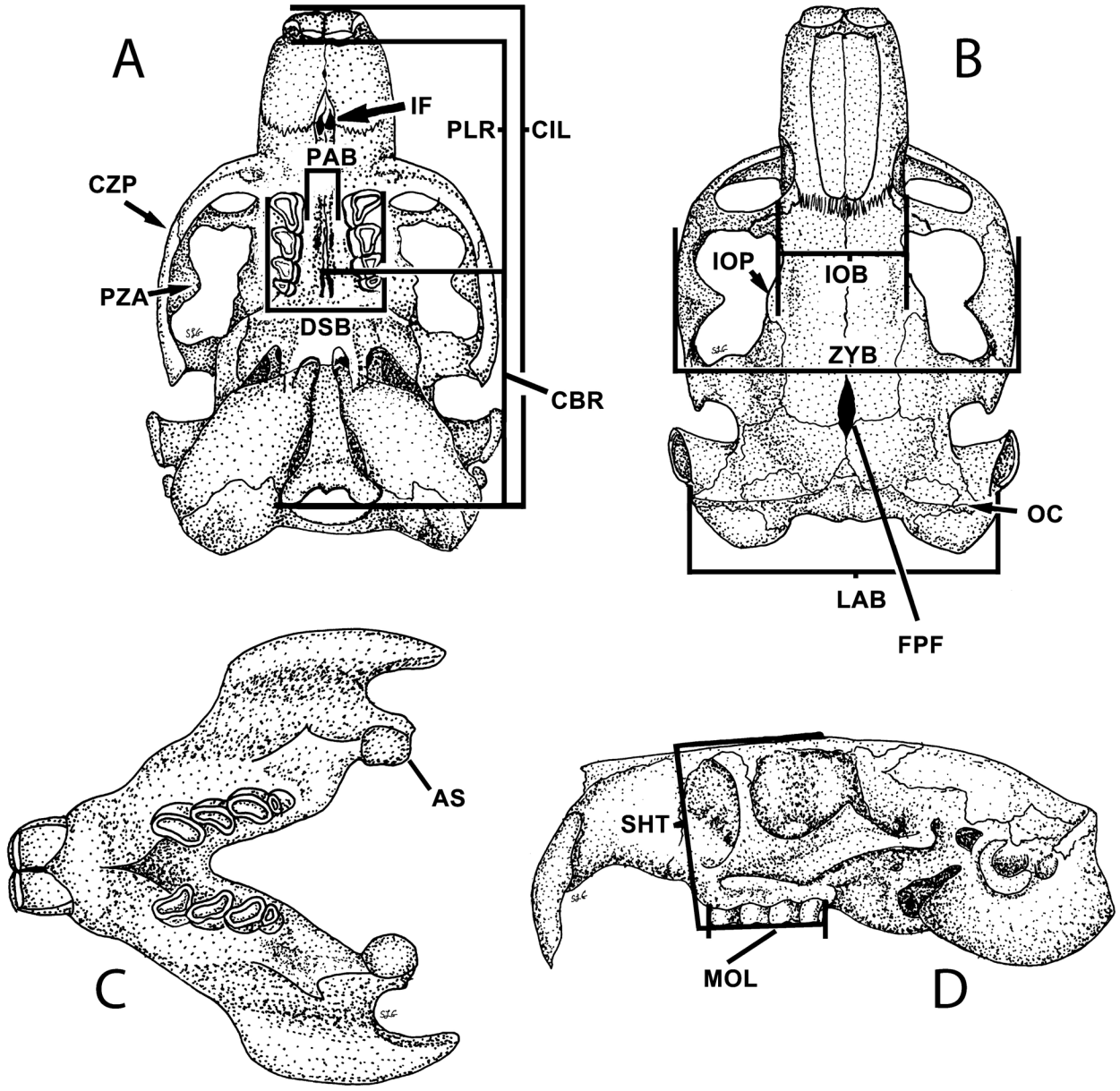


Figure 1. Dimensions of the cranium and mandible used in the descriptions and morphometric analyses of species of *Ctenomys* included in this study (for additional definitions, see Gardner 1991 and Gardner and Anderson 2001). Measurements include: condylobasilar root length (CBR), condyloincisive length (CIL), palatal root length (PLR), alveolar molar length (MOL), palatal breadth minimum (PAB), dental span breadth (DSB), zygomatic breadth (ZYB), lambdoidal breadth (LAB), interorbital breadth (IOB), and height of skull (SHT). Additional qualitative characters that were studied included curvature of zygomatic process (CZP), jugal process of zygomatic arch (PZA), incisive foramen (IF), frontal parietal foramen (FPF), articulation surface of condyloid process (AS), occipital crest (OC). Labels on figure are: A) ventral view of cranium, B) dorsal view of cranium, C) dorsal view of mandible, D) left lateral view of skull.

Table 1. Descriptive statistics for external and cranial measurements for four new species of Ctenomys *from Bolivia. Measurements for sexes (see Materials and Methods text and Fig.1 for abbreviations) are pooled in this table (see Table-S3 in online supplemental documentation for statistical summary and measurements of individual specimens). All measurements are given as mean ± 1 standard deviation, minimum and maximum measurements, and sample size (in parentheses).*

Character	*Ctenomys erikacuellarae* n.sp.	*Ctenomys yatesi* n.sp.	*Ctenomys andersoni* n.sp.	*Ctenomys lessai* n.sp.
TOTL	259.2 ± 28.2 184 – 331 (69)	209.5 ± 10.5 199 – 220 (3)	253 ± 27.6 188 – 310 (27)	238.3 ± 28 177 – 265 (9)
TAIL	72.9 ± 9.4 25 – 96 (68)	60.5 ± 2.5 58 – 63 (3)	69.8 ± 10 35 – 90 (27)	63.9 ± 9.9 44 – 79 (9)
HFL	36.4 ± 3.2 28 – 44 (69)	31.7 ± 2.4 30 – 35 (3)	33.8 ± 4.6 21 – 41 (27)	32.1 ± 2.9 27 – 37 (9)
EAR	7 ± 1.1 5 – 10 (69)	4.3 ± 0.9 5 – 5 (3)	6.8 ± 1.4 3 – 10 (26)	6.6 ± 1.2 4 – 8 (9)
WT	222.1 ± 66.8 82 – 390 (68)	92 ± 13 79 – 105 (3)	219.9 ± 60.8 83 – 360 (27)	175.8 ± 52 98 – 286 (9)
CBR	40.6 ± 3.6 30.8 - 48.9 (57)	31.3 ± 2 29.2 - 33.3 (3)	38.5 ± 3.9 28.6 - 46.1 (22)	36.2 ± 3.4 30.9 - 39.3 (6)
CIL	43.9 ± 4.2 32.9 - 53.5 (57)	34.9 ± 2.1 31.9 - 36.7 (3)	41.4 ± 4.4 30.6 – 50 (22)	38.7 ± 3.5 33 - 41.9 (6)
PLR	20.2 ± 2.3 14.7 - 24.7 (56)	15.2 ± 1 13.8 - 16.1 (3)	20.3 ± 5.7 13.8 - 44.5 (22)	18.3 ± 1.9 15.6 – 20 (6)
MOL	10.1 ± 0.8 8.2 - 11.5 (56)	8 ± 0.7 7.2 - 8.8 (3)	9.4 ± 0.7 7.6 - 10.8 (22)	9 ± 0.8 7.8 - 9.7 (6)
PAB	2.4 ± 0.3 1.8 - 3.1 (56)	1.9 ± 0.1 1.7 – 2 (3)	2.2 ± 0.3 1.7 - 3.1 (22)	2.3 ± 0.2 2 - 2.6 (6)
DSB	9.8 ± 0.8 8.1 - 11.2 (56)	7.8 ± 0.6 7.1 - 8.5 (3)	9.5 ± 0.8 7.8 - 10.6 (22)	9.3 ± 0.8 8 – 10 (6)
ZYB	28.6 ± 2.6 21.7 - 35.2 (56)	21.8 ± 1.5 19.7 - 22.9 (3)	26.1 ± 2.5 21.3 - 31.2 (22)	26.4 ± 1.9 23.1 – 28 (6)
LAB	27 ± 2 20.9 - 32.3 (56)	22.1 ± 1.2 20.6 - 23.6 (3)	25.2 ± 2 20.1 - 29.1 (22)	24.3 ± 2 21.4 - 26.4 (6)
IOB	11.2 ± 1 8.7 – 14.1 (56)	7.9 ± 0.6 7.1 - 8.5 (3)	9.4 ± 1 7 - 11.2 (22)	10.3 ± 0.9 9 - 11.6 (6)
SHT	15.4 ± 1.4 12.2 - 18.8 (56)	12.4 ± 1.3 10.8 – 14 (3)	14.9 ± 1.3 11.3 - 16.7 (22)	14.2 ± 1.3 12 - 15.7 (6)

Phylogenetic analysis.—Phylogenetic relationships based on *cyt*b gene sequences were assessed using maximum parsimony (MP) in PAUP* (Swofford 2003). A Maximum Likelihood (ML) tree was generated using RAxML GUI (Silvestro and Michalak 2012). PAUP* was used to conduct heuristic searches with 500 replicates random-taxon addition (RTA) and tree bisection-reconnection branch swapping (TBR) with all data considered unordered and equally weighted. Nonparametric bootstrap analyses (Felsenstein 1985; 5,000 pseudoreplicates and 10 random-sequence additions with each replicate) were run to assess support for individual nodes. Nodes with bootstrap support above 85% were considered well-supported. MrModelTest (Nylander 2004) was employed to find the evolutionary model that best fitted our *cyt*b data based on the Akaike information criteria: the General Time Reversible model of substitution, with a gamma distribution and a proportion of invariable sites (GTR + G + I), was determined to be the best fit for our *cyt*b dataset and was the model of evolution used in our RAxML runs; 2,500 ML rapid bootstrap analyses were run to estimate the level of support for different clades in our resulting trees. Pairwise genetic distances were calculated to assess within and among species differences using the "p" uncorrected distance estimator in MEGA 5 (Tamura et al. 2011).

RESULTS

Genetic distances (Table 2) for the *cyt*b gene between and among Bolivian species of *Ctenomys* averaged 8.8% and were slightly smaller when all species were included (7.2%, see Table-S3 in online supplemental documents). The smallest distance among Bolivian species was 5% and the greatest was 12.5% (Table 2); when all other species of *Ctenomys* were considered (Table-S3), these estimates were 1% and 12.8%, respectively.

Results from phylogenetic analysis using both maximum parsimony (MP) and maximum likelihood (ML) as optimality criteria of our *cyt*b data-set showed congruent topologies relative to the Bolivian species of *Ctenomys*. For the MP tree (Fig. 2) the pertinent statistics for characters were: tree length = 1192; consistency index is 0.323, retention index is 0.686. As in previous studies (e.g., Parada et al. 2011, Freitas et al. 2012) our phylogenetic analysis of all available *cyt*b data also recovered various species groups with relatively high-levels of support; several of these species and species groups occur in Bolivia. Our phylogenetic hypotheses (Figs. 2 and 3) show three undescribed Bolivian species (Fig. 2A, arrow "a") that comprise a monophyletic group. In addition, our analyses confirm *C. bicolor* Miranda Ribeiro 1914 as the sister species to *C. nattereri* Wagner 1848 which, as shown recently by Stoltz et al. (2013), is unequivocally present in Bolivia. In addition, this tree (Fig. 2B, arrow "b") indicates that the undescribed species (NK22840) shares a most recent common ancestor with *C. conoveri* and these two species are part of a well-supported clade that also includes *C. lewisi* and *C. frater* (see descriptions below).

Like the MP analysis, the tree from the ML analysis (Fig. 3, based on unique haplotypes) shows a topology congruent with previous studies, including the short branches at the base of the radiation indicating rapid diversification of species (Lessa and Cook 1998).

To assist in description and exploration of skull morphology and to provide a basis for identifying distinguishing characters of *Ctenomys*, we examined morphometric variation based on a previously defined set of quantitative characters. For the complete data-set with 11 species from Bolivia, we investigated all variables of the skull using discriminant analysis with both stepwise (STEPDISC) and canonical (CANDISC) analyses. We found that using the stepwise analysis, the characters most important in discrimination among groups are MOL, SHT, ZYB, PLR, DSB (Table 3). Results from a standard canonical discriminant analysis show that MOL, ZYB, DSB, IOB, and SHT best discriminate among species (see plot, Fig. 4, and the full SAS plot output in the file named "Multiv-Stats.pdf" in online supplemental documents.). Therefore, by combining results from both multivariate analyses, common characters of the skull that enable best discrimination among all Bolivian species of *Ctenomys* appear to include MOL, ZYB, DSB, and SHT.

Table 2. Mean pairwise uncorrected cytochrome-b p-distances (scaled as percent sequence divergence) among Bolivian species of Ctenomys *(below diagonal) and intraspecific p-distances (diagonal). Comparisons of distances of outgroup taxa with the ingroup* Ctenomys *sp. show much greater distances (>18%). A total of 1,140 positions in the final cytochrome-*b *dataset were included. See the complete matrix of genetic distances based on cytochrome-*b *in the online supplemental material.*

		1	2	3	4	5	6	7	8	9	10	11	12
1	*C. andersoni*	n/c											
2	*C. boliviensis*	6.4	**1.2**										
3	*C. conoveri*	9.5	10.0	**n/c**									
4	*C. erikacuellarae*	5.0	5.8	10.1	**1.8**								
5	*C. frater*	10.4	10.9	9.2	10.3	**1.2**							
6	*C. lessai*	10.5	10.7	6.8	10.4	8.6	**n/c**						
7	*C. leucodon*	9.0	9.1	12.5	9.0	12.1	11.6	**n/c**					
8	*C. lewisi*	10.4	10.5	8.2	10.7	5.3	8.3	11.8	**n/c**				
9	*C. nattereri*	7.0	5.6	9.7	6.3	11.0	10.1	8.8	10.1	**1.0**			
10	*C. opimus*	6.6	6.4	9.2	6.9	11.4	9.6	9.0	10.7	7.1	**0.2**		
11	*C. steinbachi*	6.3	6.5	10.0	6.2	11.0	11.2	9.9	10.7	6.8	7.5	**n/c**	
12	*C. yatesi*	5.4	6.5	9.2	5.7	10.2	10.0	9.5	9.9	6.3	6.8	6.9	**0.2**

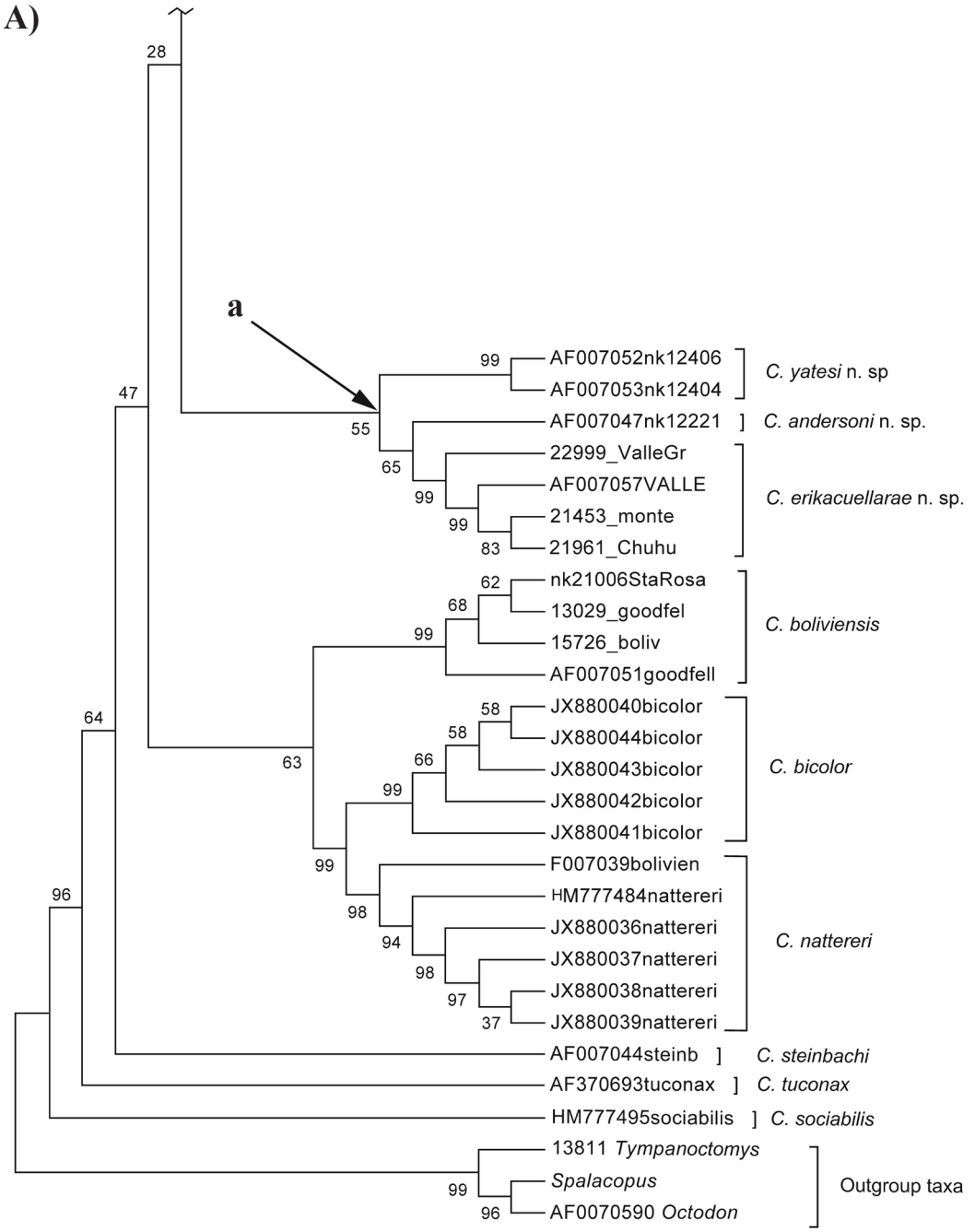


Figure 2. Results of the maximum parsimony phylogenetic analysis in PAUP* for 72 taxa of *Ctenomys* and three outgroup taxa that included the octodontids: *Tympanoctomys barrerae* (Lawrence 1941), *Octodon degus* (Molina 1782), and *Spalacopus cyanus* (Molina 1782). A) bottom half of the tree, B) top half of the tree. See text for explanation of a and b arrows. The analysis was run on 1,140 unordered, equally weighted characters of the complete mitochondrial cytochrome-*b* (*cyt*b) gene. The tree depicted is the result of a heuristic search with 500 replicates of random taxon addition and TBR and RTA in PAUP*. Nonparametric bootstrap analysis was run to assess branch support (bootstrap values are mapped).

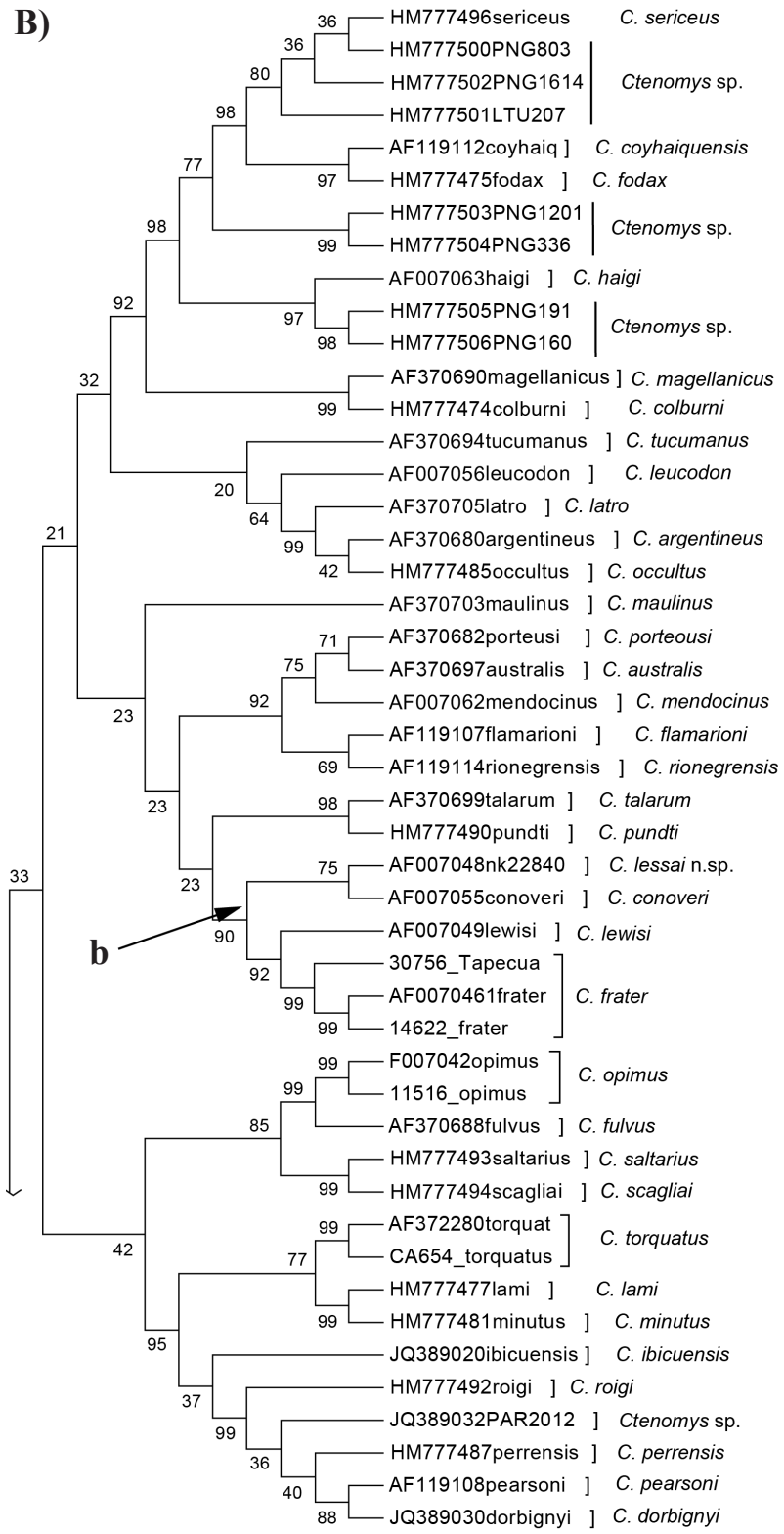


Figure 2 (continued).

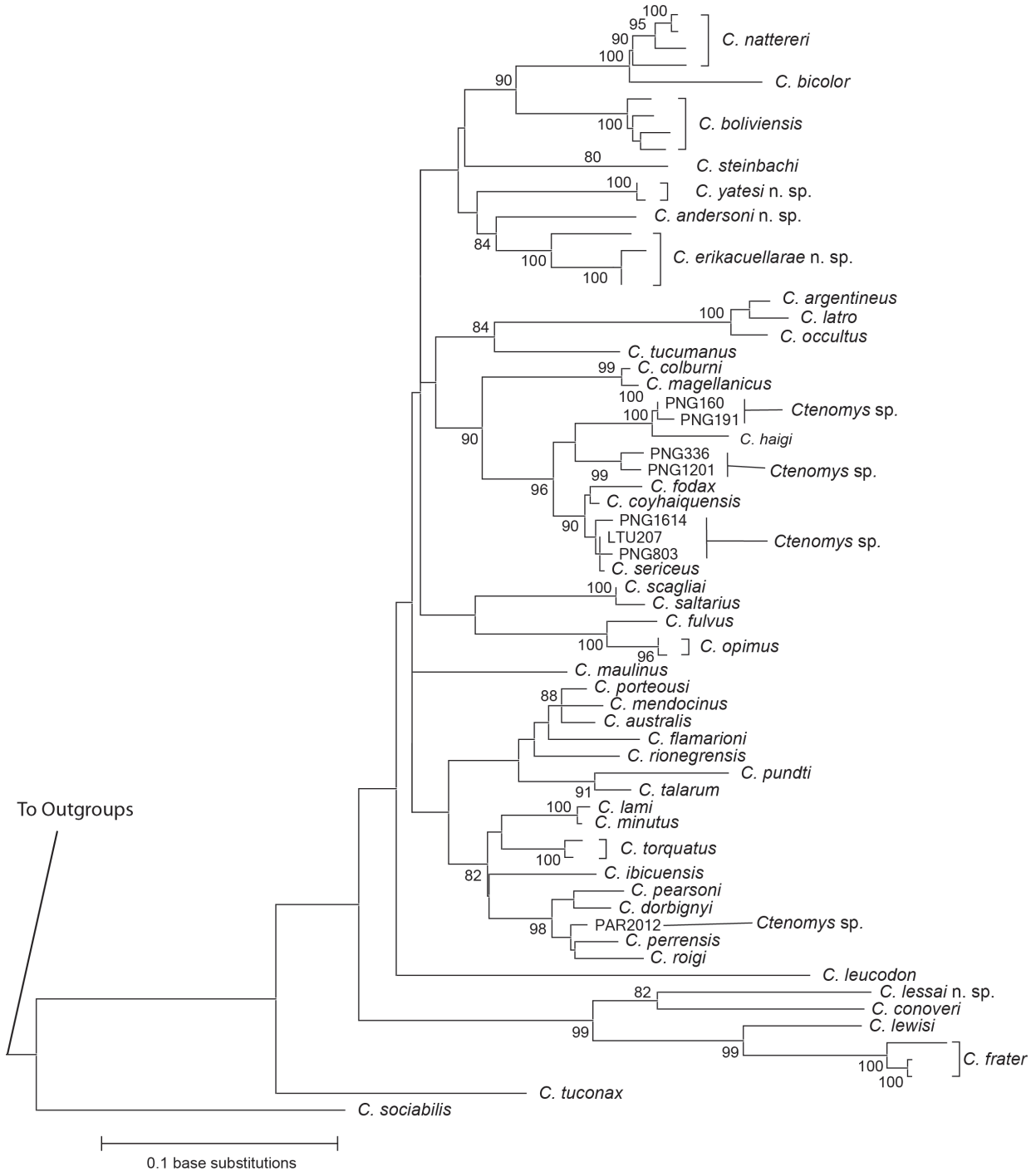


Figure 3. Results of a run with RAxML of 2,500 maximum likelihood rapid bootstrap analyses of our *cty*b dataset. The general topological groups seen in Figure 2 are evident in this tree. Numbers on the tree represent bootstrap values.

Table 3. Results of a stepwise discriminant analysis of log_{10} transformed variables for 11 species of Ctenomys *from Bolivia (including* C. erikacuellarae *n. sp.* C. andersoni *n. sp.,* C. lessai *n. sp.,* C. yatesi *n. sp.,* C. frater, C. boliviensis, C. opimus, C. lewisi, C. conoveri, C. leucodon, *and* C. steinbachi*) (individuals of* C. nattereri *not included here). The most important characters discriminating among the species included in this analysis are listed. Those with the greatest F-value (the first 8 characters) appear most important in the canonical discrimination among species.*

Step	Character Entered	Partial R-Square	F Value	Pr > F	Wilks' Lambda	Pr < Lambda
1	MOL	0.7932	80.17	<0.0001	0.20678130	<0.0001
2	SHT	0.2998	8.91	<0.0001	0.14478849	<0.0001
3	ZYB	0.4662	18.08	<0.0001	0.07728911	<0.0001
4	PLR	0.2899	8.41	<0.0001	0.05488471	<0.0001
5	DSB	0.2128	5.54	<0.0001	0.04320292	<0.0001
6	CIL	0.1914	4.83	<0.0001	0.03493257	<0.0001
7	CBR	0.0983	2.21	0.0184	0.03149903	<0.0001
8	LAB	0.0961	2.15	0.0225	0.02847223	<0.0001
9	PAB	0.0800	1.75	0.0726	0.02619546	<0.0001

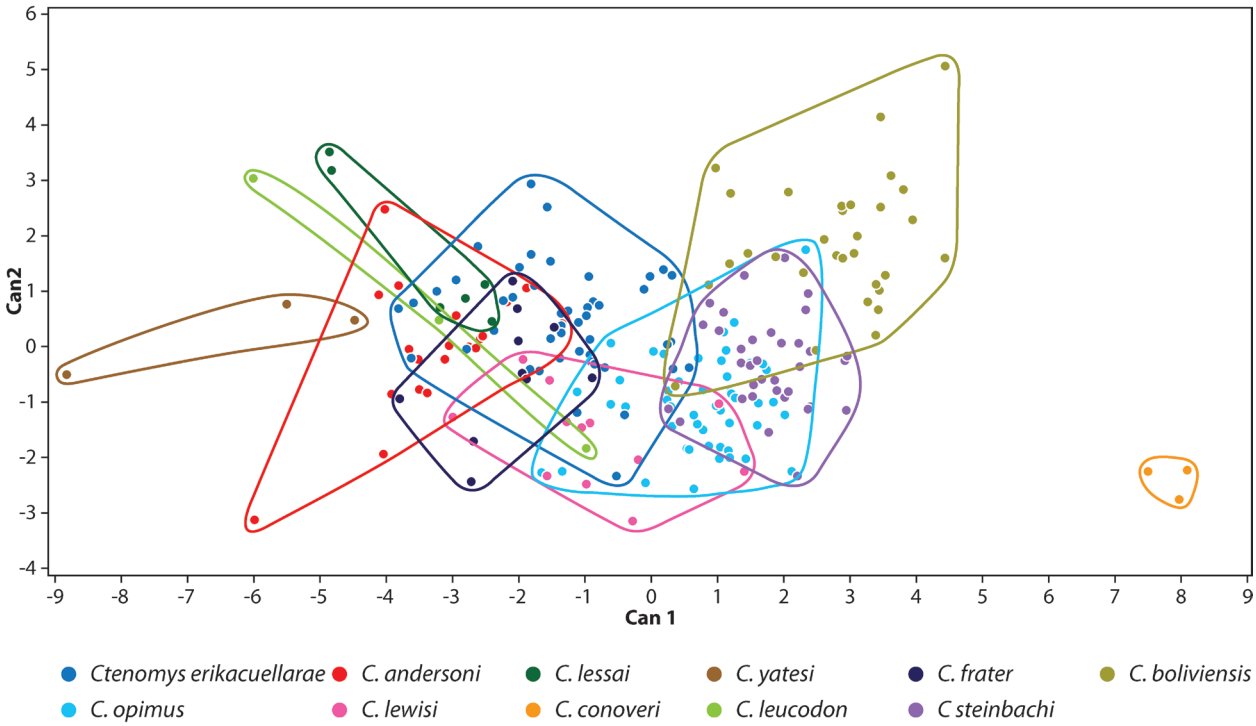


Figure 4. Plot of canonical scores of mensural data of cranial measurements for 11 species of *Ctenomys* from Bolivia. The analysis was run using canonical discriminant analysis (CANDISC) SAS 9.3. Minimum polygons represent the plots of individual canonical scores for axes I and II. The first two axes account for 79.63% of the variation in the data set. Zygomatic breadth and interorbital breadth contribute most to discrimination between and among species. See online supplemental documentation (Multiv-Stats.pdf) for complete data set and results of the CANDISC analysis.

TAXONOMY AND SPECIES DESCRIPTIONS

Based on the patterns of phylogenetic, karyotypic, and morphological variation in specimens collected from several localities in the valleys and lowlands of Bolivia, four new species of *Ctenomys* (Rodentia Bowdich 1821: Ctenomyidae Lesson 1842) are herein described.

Ctenomys erikacuellarae, new species

Erika's Tuco-Tuco, El Tuco-Tuco de Erika
Figs. 5, 6, 7, 8 (map), Table 1

Ctenomys sp. (part): Anderson (1997:499)
Ctenomys "monte": Lessa and Cook (1998:89)
Ctenomys "mont": Cook and Lessa (1998:1,524)
Ctenomys "Monte": Castillo et al. (2005:172)
Ctenomys "monte": Parada et al. (2011:676)
C. sp. MONTE: Freitas et al. (2012:1,367)

Holotype.—MSB63391 (Museum of Southwestern Biology), adult male. Skin, skull and axial skeleton collected 7 August 1990 prepared by Sydney Anderson, field number SA10348. At the time of collection, the liver, kidney, and heart tissue were frozen in liquid nitrogen and subsequently stored at -80°C in the Division of Genomic Resources, Museum of Southwestern Biology, NK21901.

Type locality.—Bolivia, Department of Chuquisaca, 2 km E Chuhuayaco, 19°43'S, 63°51'W, elevation 1,200 m.

Measurements of holotype.—TOTL, 287; TAIL, 80; HFL, 41; EAR, 8; WT, 370; CBR, 47.09; CIL, 50.52; PLR, 23.87; MOL, 11.33; DSB, 10.95; PAB, 2.5; ZYB, 32.08; IOB, 12.76; LAB, 28.4; SHT, 17.8.

Specimens examined.—Sixty-eight additional specimens from the type locality and two additional localities in the Departments of Santa Cruz and Chuquisaca. Besides the holotype, the rest of the series of specimens collected from the type locality are designated paratypes as follows: nine AMNH (263922–263930), nine CBF (1098, 1099, 1102, 1105, 1160, 1164, 2655–2657), and six MSB (63391, 63393, 63394, 63507, 63508, 63510). The rest of the specimens studied (but not designated as types) include the following: Twenty-seven individuals from 2 km SW of Monteagudo, 1,130 m elevation, Department of Chuquisaca, Bolivia (19°49'S, 63°58'W): 11 AMNH (263943–263953), nine CBF (1093, 1094, 1097, 1100, 1101, 1108, 1109, 1182, 2654), seven MSB (63381–63384, 63392, 63393, 63510) and trapped in July 1990; and 25 additional specimens, trapped in June 1991 from near the Río Las Ciénegas, at 5.5 km NNE of Vallegrande, 1,800 m elevation, Department of Santa Cruz, Bolivia (18°28'S, 64°08'W): five MNK (620, 1729, 1733–1735), nine AMNH (264548-264556), 11 MSB (67103–67105, 67362, 67413, 67416–67418, 67421, 67422, and 67424). Pooled measurements of skulls and external dimensions of both sexes are summarized in Table 1 (see also Table-S1 in online supplemental documents for complete data-set and measurements of individual specimens).

Diagnosis.—A medium-sized tuco-tuco of the "*boliviensis*" group of *Ctenomys* with dorsal and ventral coloration well differentiated; dorsum mostly Ochraceous Orange except on the upper surface of head and muzzle which is blackish brown; venter Drab Brown or Buffy Brown displaying extensive white or Light Buff markings on inguinal, axillary and/or pectoral regions. Skull strongly built, with strongly curved zygomatic arches, orthodont incisors, rostrum widest at the tip of premaxillaries, a ribbon-like alisphenoid-presphenoid bridge even in young individuals, and a karyotype with 2N=24 and FN=40.

Description.—Pelage dense, fine, soft, about 15-16 mm long over back and rump; dorsum with fur ranging from Ochraceous Orange to Buckthorn Brown, hairs dark colored, except last 2.5 or 3 mm, which are distinctly lighter. Most individuals with dark cap (Fuscous Black) on head, ca. 1.5 to 2 cm wide, running from just above nose to at least neck; mid-dorsal stripe of same color running along back, and sometimes reaching rump. Color of ventral pelage more sharply set off from dorsal in some individuals (e.g. AM263922) than in others (e.g. AM264549); deep Neutral Gray basally, with superficial (1 to 2 mm) wash of Drab Brown or Buffy Brown. "Collar" of light fur extending ventrad from pinnae to gular region (e.g. AM263947). Fur of fore and hind limbs colored like dorsum and rump, except internal sides of both (axillary areas) which are White to Light Buff; in some individuals, inguinal area

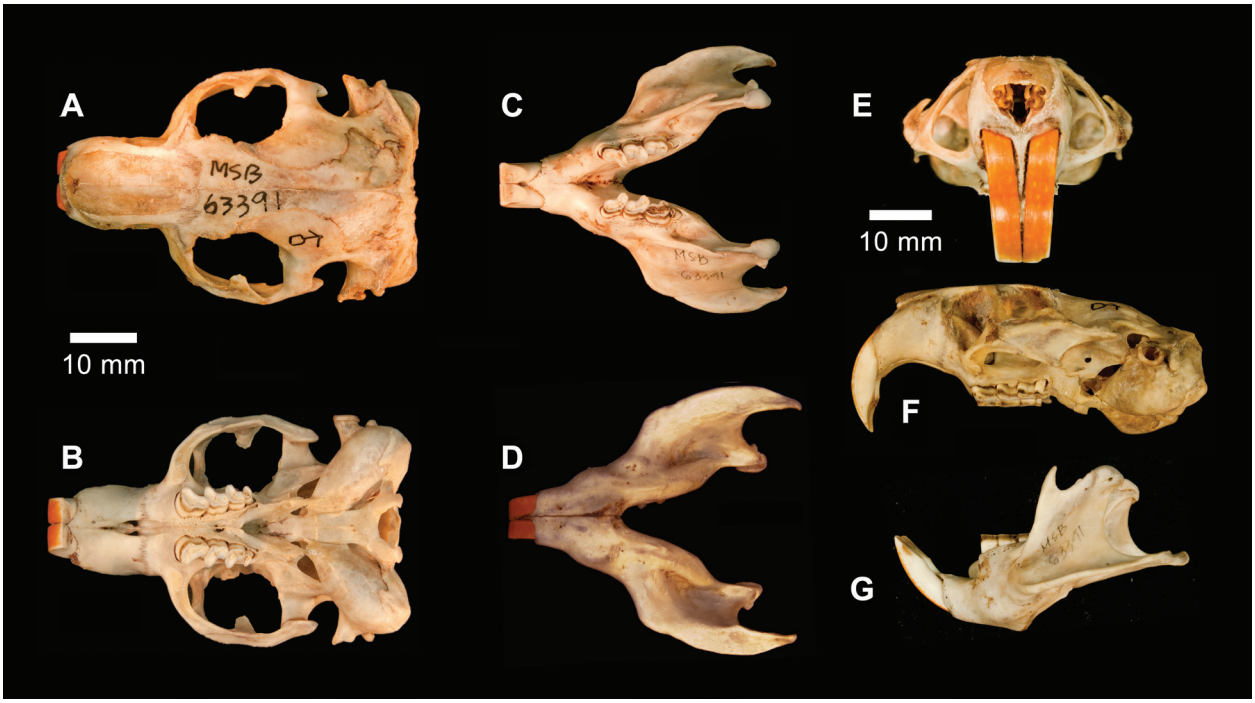


Figure 5. Views of skull and mandible of holotype (MSB63391) of *Ctenomys erikacuellarae* n. sp. A) dorsal view of skull, B) ventral view of skull, C) dorsal view of mandible, D) ventral view of mandible, E) en face view of skull showing dark orange color of upper incisors, F) left lateral view of skull, G) left lateral view of mandible.

Figure 6. Museum study-skin of holotype (MSB63391) of *Ctenomys erikacuellarae* n. sp. A) dorsal view, B) ventral view, C) left lateral view.

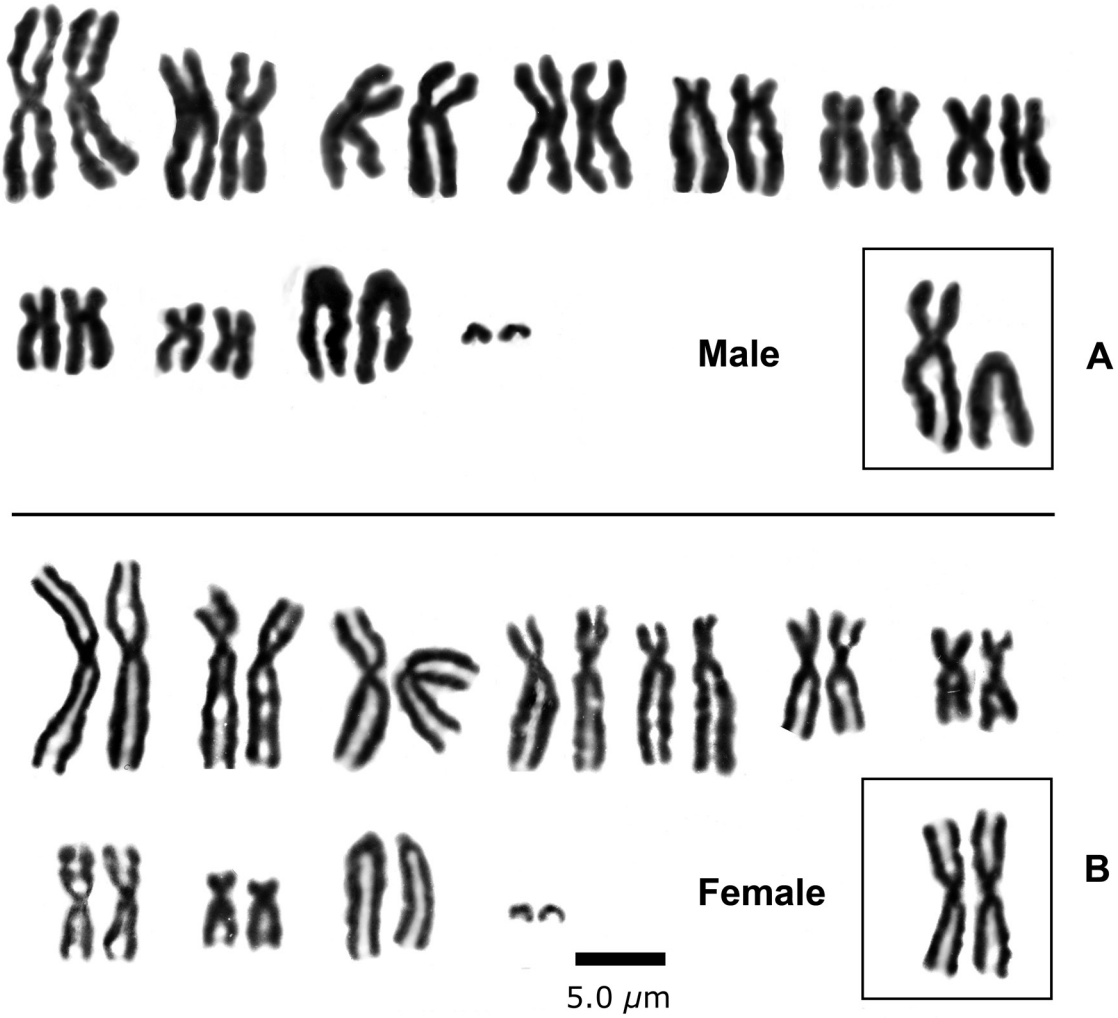


Figure 7. Standard giemsa-stained karyotype preparations of *Ctenomys erikacuellarae* n. sp. [2N=24; FN=40]. A) holotype male (MSB63391), showing metacentric X and acrocentric Y chromosome distinctive of this species; B) female, showing the bi-armed metacentric XX chromosomes.

colored White to Light Buff (e.g. AM264552), and in others, with a pectoral shield of White or Light Buff hairs. Top of feet covered with agouti hairs to base of metacarpals and then replaced by longer, self-colored hairs. Mystacial vibrissae extending to the base of the pinnae when laid back alongside head; superciliary vibrissae sparse, extending to the dorsal edge of the pinnae when laid back along side of head; genal-one present, interramal present.

Ears sparsely covered with short, brownish hairs, not contrasting conspicuously with color of head. Opening to auditory canals protected by small cteniform, self-colored bristles; bundles of stiff hairs posterior to upper and lower incisors. Pes broad, all digits with ungal tufts of stiff bristles (e.g. cteniform), and strong claws. Forefoot with a well-developed pollex bearing a short open nail, other four digits bearing basally closed claws. Tail short, strong, teretiform in cross section, darker above than below in most specimens, sparsely covered with dark hairs, except for terminal 5–7 mm, which are covered with whitish hairs. Six mammae in inguinal, abdominal, and postaxial pairs, postaxial pair enlarged even in very young

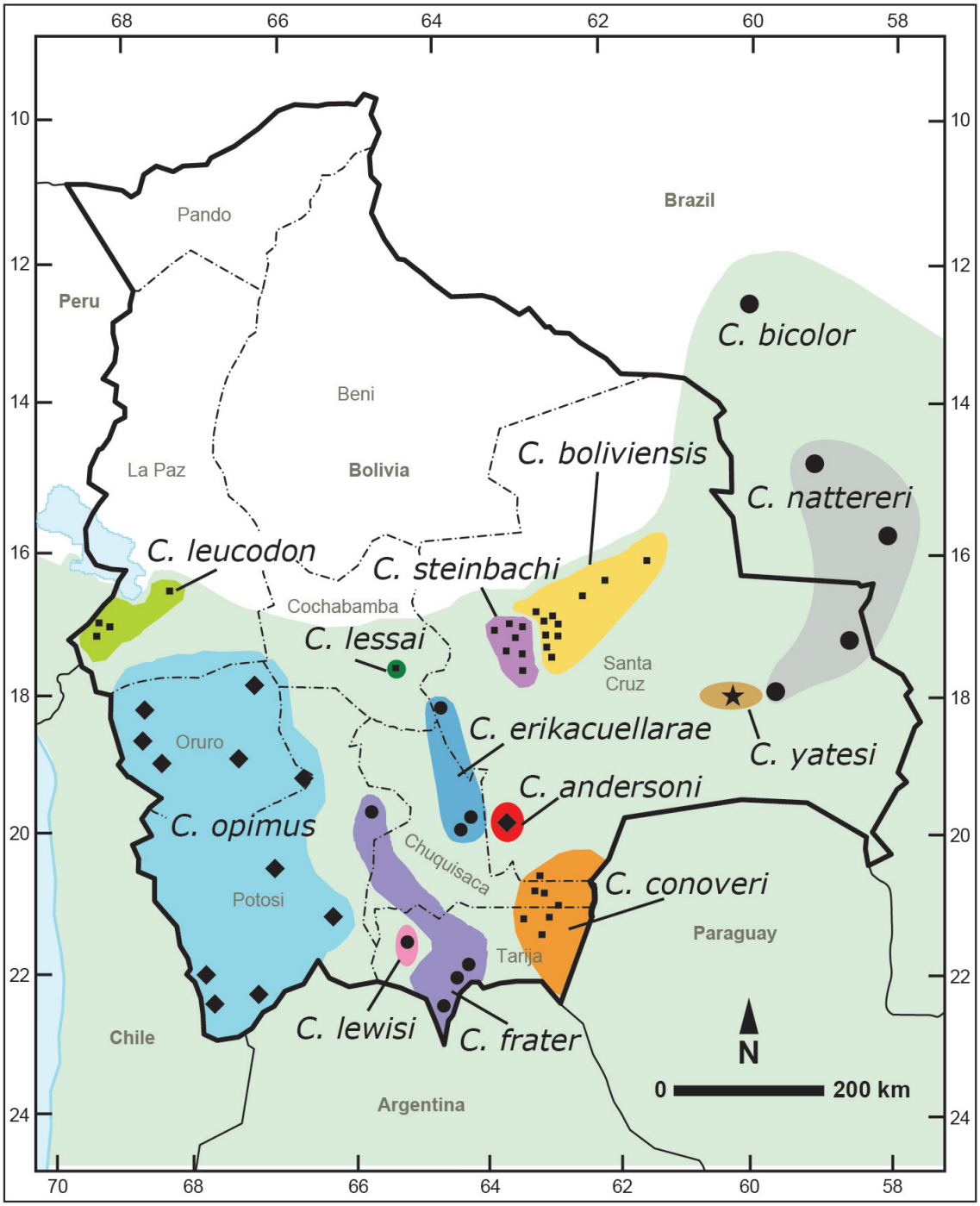


Figure 8. Map of Bolivia (modified from Gardner 1991) with collection localities of *Ctenomys* species that we collected from 1984 to 1996. Continuously shaded area through the southern half of Bolivia and into Brazil indicates approximate range of rodents of the genus *Ctenomys*. Included are approximate collection localities and estimated ranges in Bolivia for *C. opimus*, *C. leucodon*, *C. lessai* n. sp., *C. lewisi*, *C. frater*, *C. erikacuellarae* n. sp., *C. steinbachi*, *C. boliviensis*, *C. yatesi* n. sp., *C. conoveri*, and *C. andersoni* n. sp. Shown also are localities and estimated range for *C. nattereri* (from Bolivia and Brazil, see text) and for a single locality for *C. bicolor* (skull specimen from AMNH that was examined by us).

females. Glans penis 4 to 5 times longer than wide and cylindrical, externally covered with coarse spines (well developed in adults only).

Skull robust; interorbital region with squared margins; zygomatic arches broad; auditory bullae inflated, pyrifom, auditory tubes salient. Nasal bones broadest anteriorly with flat dorsal edges; nasals short, premaxillary clearly visible when viewed from dorsal aspect. Frontals widest at level of connection of zygomatic arches, lateral to posterior anterior sutures of frontals, nasals, and maxillaries. Interorbital processes of frontals almost as wide as widest part of frontals, frontals constricting to their narrowest at level of sutures of frontals and parietals; parietals narrowest at site of attachment of squamosal root of zygomatic arch. Squamosal appearing to expand with age and thus in young individuals parietal and back of frontals are about same width. In older individuals, interparietal completely fused or extremely small and indistinct. Temporal foramen at junction of squamosal and parietal always present and not elongated. Lambdoidal ridge always present, even in young individuals. Supraoccipital crest strongly developed in adults. Zygomatic arch strongly built, with massive processes extending both dorsad and ventrad (jugal extended dorso-ventrally); incisive foramina recessed in a common fossa and incompletely separated by a bony septum (or if present, septum is weakly developed); in most individuals, interpremaxillary foramen conspicuous; palatal bridge with two major palatine foramina at about level of M1. Mesopterygoid fossae, an inverted "V" shape, reaching anteriorly to level of M2. Posteropalatal pits present, but not enlarged. Alisphenoid-presphenoid bridge flat and ribbon-like, even in younger individuals. Bony roof of mesopterygoid fossa usually completely ossified (sometimes with tiny perforations but never large sphenopalatine vacuities); buccinator-masticatory foramen enlarged and undivided in many individuals (including holotype), but divided into two or sometimes three small foramina in other individuals. Upper incisors large, robust, Orange in color, and opisthodont. Maxillary tooth rows slightly divergent posteriad.

Mandible robust, with coronoid process falciform, not strongly angled backwards; condyloid process strong; bearing an articulation flange, not well developed.

Karyology.—2N=24 and FN=40. Karyotypes were examined from seven individuals from 2 km SW of Monteagudo (one male, MSB 63510 and six females, MSB 63383; AMNH 263943–263944; CBF 1100–1101, 2654), six specimens from 2 km E of Chuhuayaco (one male, MSB63391 and five females, AMNH 263924–263925; CBF 1098, 1102, 1164) and eight animals from 5.5 km NNE of Vallegrande (one male and seven females). Typical karyotype (Fig. 7) showing nine pairs of bi-armed autosomes, and two pairs of acrocentric autosomes of which one is medium-sized and distinctive and the other is a pair that is extremely small. Of the biarmed autosomes, six pairs are submetacentric, and three pairs are metacentric, large acrocentric pair, distinctive. The X chromosome is large and metacentric, the Y chromosome is acrocentric and about half the size of the X chromosome (Fig. 7).

Distribution and habitat.—Erika's tuco-tuco is known only from three localities (Fig. 8) situated on the eastern flanks of the Cordillera Oriental of the Andes (between 810 and 1,800 meters altitude) of south-central Bolivia in the Departments of Chuquisaca and Santa Cruz. A minimum geographic-area polygon connecting all three localities encompasses an area slightly larger than 1,000 km² (see Fig. F-1 in online supplemental documents).

All three known localities are part of the ecological zone known as Bosques Secos Interandinos (Ibisch et al. 2003) or Andean dry valleys (Lopez 2003). The locality designated "2 km SW of Monteagudo" was on an agricultural experimental station. Vegetation was less disturbed inside the fenced station and was typical of the lower, eastern escarpments of the Andes in southern Bolivia (Ibisch et al. 2003).

At the collection locality "5.5 km NNE of Vallegrande" on the Río Ciénega, the hillsides were covered with mesquite (*Prosopis* L.), columnar cacti (e.g. *Trichocereus* sp.), acacia (*Acacia sp.*), and *Ximenia* sp.. The Río Ciénega runs through the valley and is surrounded by dense stands of shrubs and trees (*Prosopis*, etc.). The area was converted to cropland and is grazed heavily by livestock. The collecting locality at "2 km East of Chuhuayaco" was similar to that of Vallegrande.

Individual tucos from Vallegrande have a distinct coloration; some show white markings behind the ears, and all had frontoparietal fontanelles or fenestrae. Elsewhere, the percentage of animals with fenestrae ranges from 78% near Monteagudo to 92% in Chuhuayaco (Gardner and Anderson 2001).

Etymology.—We name this species in honor of our friend and colleague Erika Cuéllar, a Bolivian conservation biologist who has worked tirelessly to protect the flora and fauna of the Gran Chaco region of South America by empowering the people in local communities. In addition, Erika participated in the field-expeditions that secured some of the earliest specimens of this poorly known species of tuco-tuco; we fondly remember her enthusiasm, positive attitude, and inquisitive mind, and hail these as examples to follow. The species name is a patronym in the genitive singular.

Comparisons.—Ctenomys erikacuellarae n. sp. can be recognized as distinct from species of *Ctenomys* known from in and around Bolivia by the following: *Ctenomys erikacuellarae* differs from *C. steinbachi,* a species occurring in lowland Bolivia near the city of Santa Cruz de la Sierra, by having no collar of light colored fur, by having fur that is lighter and more Ochraceous in color and by having a diploid fundamental chromosome number of 2N=24, FN=40 versus 2N=10, FN=16 in *C. steinbachi* (see Anderson et al. 1987).

Ctenomys erikacuellarae is smaller than *C. conoveri* and has fewer chromosomes (2N=48, FN=70 in *C. conoveri*, see Anderson et al. 1987) and a smooth pelage relative to the shaggy coat of *C. conoveri*.

Ctenomys erikacuellarae can be differentiated from *C. boliviensis* by lacking a collar through the gular region, a karyotype of 2N=24 and FN=40 versus 2N=42–46 and FN=64 in known populations of *C. boliviensis* (see Anderson et al. 1987), smaller overall skull sizes of adults, and high prevalence of fenestrae at the frontal parietal suture versus very low prevalence to none in *C. boliviensis* (see Gardner and Anderson 2001).

Ctenomys erikacuellarae can be distinguished from *C. frater* by having rough, bicolored, Ochraceous fur dorsally, while *C. frater* has fur that is very soft and Slate Gray in color, and a much different (lower) chromosome number versus a 2N=48 and FN=90 in *C. frater* (see Cook et al. 1990).

Finally, *C. erikacuellarae* can be differentiated from Lewis' tuco-tuco (*C. lewisi*) by having Ochraceous fur (versus very dark fur in *C. lewisi*) and a different karyotype (*C. lewisi* has a 2N=56, FN=74; Cook et al. 1990). In dimensions of the skull, *C. erikacuellarae* differs from *C. lewisi* in mean skull-length (condyloincisive length), in inter-orbital breadth, and mean skull height (Table 1).

***Ctenomys yatesi*, new species**

Yates' Tuco-Tuco, el tuquito de Yates, Yates' tuquito
Figs. 8 (map), 9, 10, Table 1

Ctenomys minimus: Anderson (1985:14) (misspelling of *minutus*)
Ctenomys minutus: Olds et al. (1987:17)
Ctenomys minutus: Anderson et al. (1987:1)
Ctenomys minutus: Anderson (1997:496)
Ctenomys sp. ("minut"): Lessa and Cook (1998:89)
*Ctenomys "*min*"* : Cook and Lessa (1998:1,524)
Ctenomys sp. ("minut"): Slamovits et al. (2001:1,709)
Ctenomys minutus: Gardner and Anderson (2001:5)
C. sp. "Minut": Castillo et al. (2005:172)
Ctenomys sp. MINUT: Parada et al. (2011:682)
C. sp. MINUT: Freitas et al. (2012:1,367)

Holotype.—AMNH 260835 (American Museum of Natural History), adult male. Skin, skull, and axial skeleton collected 9 October 1984, prepared by Sydney Anderson field number SA8407. At the time of collection, tissues of the liver, kidney, and heart were frozen in liquid nitrogen, and subsequently stored at -80˚C in the Division of Genomic Resources, Museum of Southwestern Biology, NK12406.

Type locality.—Bolivia, Department of Santa Cruz, 7 km N and 38 km W of Roboré, 18°16'S, 60°07'W, 550 m elevation.

Measurements of holotype.—TOTL, 220; TAIL, 63; HFL, 30; EAR, 5; WT, 105; CBR, 33.3; CIL, 36.0; PLR, 15.7; MOL, 8.8; DSB, 8.5; PAB, 1.7; ZYB, 22.8; IOB, 8.5; LAB, 22.2; SHT, 12.4.

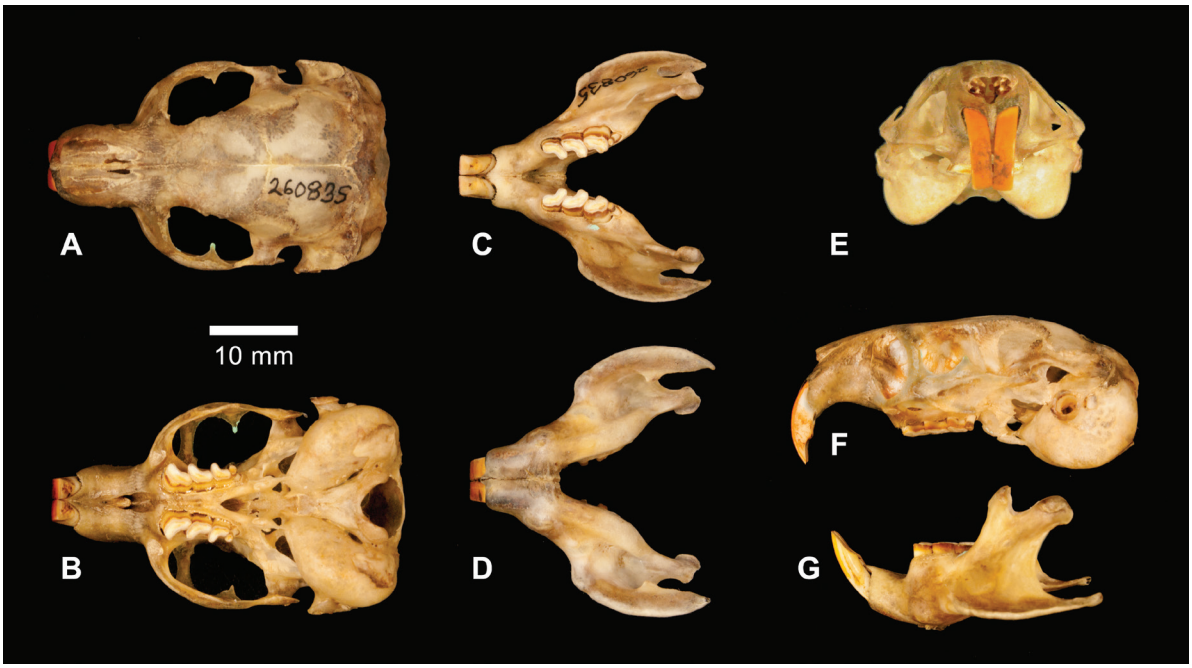


Figure 9. Views of skull and mandible of holotype (AM260835) of *Ctenomys yatesi* n. sp. A) dorsal view of skull, B) ventral view of skull, C) dorsal view of mandible, D) ventral view of mandible, E) en face view of skull showing dark orange color of upper incisors and large tympanic bullae, F) left lateral view of skull, G) left lateral view of mandible.

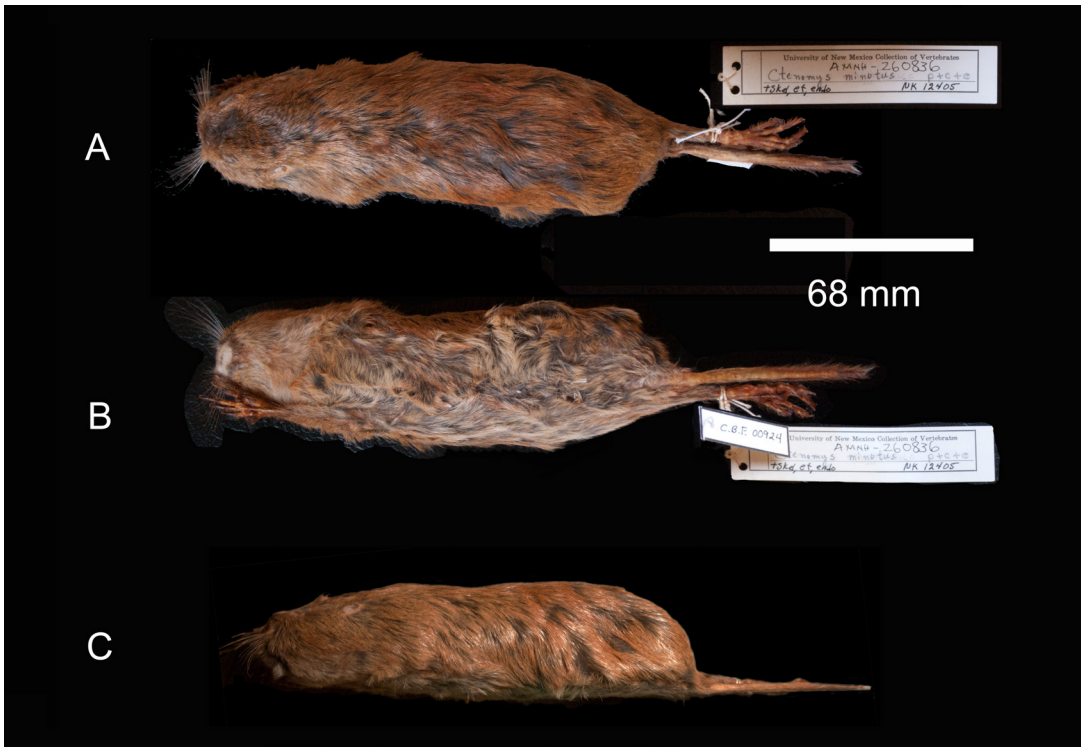


Figure 10. Museum study-skin of paratype (CBF00924) of *Ctenomys yatesi* n. sp. A) dorsal view, B) ventral view, C) left lateral view.

Specimens examined.—Two additional specimens from the type locality. Designated as paratypes: MSB55367, CBF924. Pooled (male and female) measurements of skulls, weights, and external dimensions are given in Table 1. Individual measurements used are summarized for each species in Table-S1 in online supplemental documents.

Diagnosis.—A small-sized species of *Ctenomys*, member of the "*boliviensis*" group of this genus, dorsally Pale Brown, even more pale ventrally, without a hint of dark dorsal stripe and no markings on fur in the gular region; very large tympanic bullae (Fig. 9E) and very distinctive range of morphometric variation (from 50 to 70% smaller than any other species of *Ctenomys* in Bolivia).

Description.—Pelage fine and soft, hairs about 10-12.5 mm long over back and rump, dorsal coloration near Hazel, ventral pelage with hairs Deep Neutral Gray basally, with superficial (1–2mm) wash of light colored fur (Fig. 10). Gular region having no distinctive or contrasting markings. Fur of fore and hind limbs colored like dorsum. Top of feet covered with Brown hairs to base of metacarpals then replaced by longer, self-colored hairs.

Mystacial vibrissae present with longest extending to base of pinnae when laid back alongside head; superciliary vibrissae very sparse, about 12 mm long, almost extending to dorsal edge of pinnae when laid back alongside head; genal-one vibrissae present, interramal short. Ears sparsely covered with, brownish hairs, not contrasting conspicuously with color of head. Opening to auditory canals protected by cteniform hairs; pes broad, all digits with ungal tufts of stiff cteniform bristles, and strong claws; forefoot with short pollex bearing a broad open nail and digits II–V with long, basally closed claws. Tail strong, darker above than below in most specimens; sparsely covered with dark hairs, except for terminal 4–7 mm that are covered with whitish hairs. Mammae not seen.

Glans penis cylindrical with weakly developed basal trough and rounded tip; urethral opening ventral, positioned at tip of penis; coarse spines covering glans. Os baculum 4.38 mm long (Lessa and Cook 1989).

Skull small (Fig. 9), robust, flattened in dorsal profile; interorbital region with rounded margins; zygomatic arches parallel and not outwardly bowed, strongly built, widest towards front of skull, with rounded profile in dorsal view, more evident in males due to sexual dimorphism or age or both; postorbital processes undeveloped and postorbital region posteriorly divergent; auditory bullae slightly more than 30% of skull length, with auditory tubes long and salient (Fig. 9B, E). Nasal bones broad, short. Interpremaxilary foramen very small, inconspicuous. Frontals widest just behind interorbital region, constricting just posterior to end of processes of zygomatic arch; lambdoidal ridge not well-developed, always present, even in young individuals. Supraoccipital crest strongly developed in adults. Exoccipital portion of the ectotympanic reduced. Mesopterygoid fossae with dorsal margin "V" shaped, reaching to level between M2s. Posteropalatal pits present, but not enlarged, palate grooved with deep sulci. Alisphenoid - presphenoid variable, flat and broad or thin. Very narrow sphenopalatine vacuities. Incisors strong, orange; upper incisors orthodont. Maxillary tooth rows slightly divergent posteriad. Mandible with coronoid process falciform, but not strongly angled backwards; condyloid process strong, bearing an articulation flange, not well developed.

Karyology.—No karyotypes were prepared for specimens of *C. yatesi*, all specimens were dead in the traps.

Distribution and habitat.—El tuquito de Yates is known only from the type locality in the lowlands of eastern Bolivia. The type locality is located in the southern semidecidous Chiquitano forest district of the Cerrado Biogeographic Province of eastern Bolivia (Navarro and Maldonado 2002). Individuals were collected from habitats with vegetation typical of the Cerrado that interdigitates the Lowland Chiquitano forest in this general area (see map in Cuéllar and Noss 2003:11). This Cerrado, also known as Abayoy, is characterized by the following plants: *Hymenaea stigonocarpa* (Fabaceae)*, Luehea candicans* (Tiliaceae), *Bredemeyera floribunda* (Polygalaceae)*, Terminalia argentea* and *T. fagifolia* (Combretaceae), and *Anacardium humile* (Anacardiaceae); other plant species present in the area include *Tabebuia selachidentata*

(Bignoniaceae), *Mimosa josephina* (Fabaceae), and *Centratherum cardenasii* (Asteraceae).

Etymology.—We name this species in honor of our late friend and great mentor, Dr. Terry L. Yates, former curator of the Mammal Division of the Museum of Southwestern Biology. His enthusiasm for systematics, natural history museums, and field mammalogy was - and still is - a great source of inspiration that we hope to instill in our students. Anyone who shared time in the field with Terry will recall his "remember the motto" ("come back alive" and "save the specimens") expressed equally as a demand as well as a farewell bid. (His inimitable Kentucky drawl permeates this passage in our mind's ear). The species name is a patronym in the genitive singular.

Comparisons.—Only *C. nattereri* has been collected near the type locality of *C. yatesi*. *Ctenomys yatesi* is much smaller (about 60–70% smaller) than *C. nattereri* in all measurements taken; however, the size of the tympanic bullae (relative to skull size) is larger in *C. yatesi* than in *C. nattereri*. In addition, the dorsal coloration in *C. yatesi* is lighter with weak differentiation between dorsum and venter with no collar versus a darker, Chocolate Brown above with a contrasting Yellow venter and a very distinct Yellow collar in *C. nattereri*. *Ctenomys yatesi* differs from *C. steinbachi* in being smaller, with the dorsal fur Hazel in color (rather than Fuscous Black), and ventrally Light Hazel (rather than Grizzled Whitish and Fuscous); in addition, *C. yatesi* has a relatively longer tail (compared to head and body length), and narrower interorbital breadth (Table 1).

Ctenomys yatesi differs from *C. conoveri* in being much smaller in all measurements. In addition, *C. yatesi* has a tidy pelage that is Hazel in color and differs dramatically from the shaggy Brownish pelage of *C. conoveri.*

Ctenomys yatesi can be differentiated from *C. boliviensis*, especially the populations to the south of Santa Cruz (e.g. Las Lomitas), in completely lacking a black cap and stripe that runs mid-dorsally to the rump in *C. boliviensis*. In *C. yatesi* the ventral coloration is less Ochraceous than in *C. boliviensis* and the skull is 50 to 65% smaller, with a very different shape of the tympanic bulla and rostrum. *Ctenomys* from the highlands or dry valleys of Bolivia occur in habitats very distinct from that of type locality of *C. yatesi* and all are much larger in body size.

Other species of small-bodied *Ctenomys* include *C. bergi* and *C. pundti* from Argentina (Olrog and Lucero 1981) and *C. dorsalis* from Paraguay (Thomas 1900; Redford and Eisenberg 1992). The ranges of *C. bergi* and *C. pundti* in Córdoba and San Luis provinces of north-central Argentina are far from that of *C. yatesi* and the biomes are distinctly different. Sequence divergence is 7.5% between *C. pundti* and *C. yatesi* (no comparable data exist for *C. bergi*, but a short fragment of 308 bp showed 5.1% divergence with an homologous fragment in Yates' tuco-tuco). These are values higher than those suggested by Lessa and Cook (1998) for genetic divergence between species-pairs of tuco-tucos. *Ctenomys yatesi* differs from *C. dorsalis*, a small-bodied species from the Chaco of Paraguay, in the very distinct coloration pattern of their body pelages: *C. dorsalis* has a distinct collar of light hairs behind the cheeks which extends to the ears on each side of the head; in addition, this species has a stripe of black hairs on the head, which runs down the back of the body and most of the ventral side of the body is covered with self-colored hairs or are pale buffy (Thomas 1900). *Ctenomys yatesi* is, in contrast, rather uniform in coloration, lacks the stripe of dark hairs on the back and has Buffy or in some instances whitish-tipped hairs in the venter which are otherwise slate-colored at their base.

Genetically, *C. yatesi* differs from all known species in the lowlands of Bolivia from a low of 5.4% to a high of 10.2% (Table 1).

Remarks.—Anderson et al. (1987) used the name *C. minutus* that Nehring coined in 1887 to refer to these specimens as "a reasonable working taxonomic hypothesis, although quite tentative." Anderson and collaborators justified using the name "*minutus"* because Cabrera (1961) had synonymized *C. bicolor* (Miranda Ribeiro 1914), a small bodied form from the Brazilian state of Rondônia (Bidau and Avila Pires 2009), with *C. minutus*. *Ctenomys minutus* is also a small-bodied species, originally described from southeastern Brazil (Rio Grande do Sul, Campos E of Mondo Novo). Comparisons of *C. bicolor* with other species of *Ctenomys* from Brazil by Stoltz et al. (2013) using morphological

characters, chromosomes, and genetics confirm the validity of *C. bicolor* as a distinct species that has closer affinities to *C. boliviensis* than to *C. minutus*.

Ctenomys yatesi differs in size and in coloration from *C. minutus,* and these two species also have ranges that are well-separated in geographic space. Morphologically, *C. yatesi* and *C. minutus* differ in many characters with *C. yatesi* having a shorter rostrum, larger and more inflated auditory bullae, shorter and broader nasals, and smaller paroccipital apophyses on the tympanic bullae. Finally, but significantly, we measured genetic divergence of *cyt*b sequences between these species at 5.8%.

***Ctenomys andersoni*, new species**
Anderson's Cujuchi, El cujuchi de Anderson
Figs. 8 (map), 11, 12, 13, Table 1

Ctenomys "ita": Lessa and Cook (1998:89)
Ctenomys "ita": Cook and Lessa (1998:1,524)
C. sp. "Ita": Castillo et al. (2005:172)
Ctenomys sp. ITA: Parada et al. (2011:682)

Holotype.—MSB63387 (Museum of Southwestern Biology), adult male. Skin, skull and axial skeleton collected 9 July 1990, prepared by Sydney Anderson, field number SA9971. At the time of collection, the liver, kidney, and heart tissues were frozen in liquid nitrogen and subsequently stored at -80°C in the Division of Genomic Resources, Museum of Southwestern Biology, NK21221.

Type locality.—Bolivia, Department of Santa Cruz, Cerro Itahuaticua, 19°48'S, 63°31'W, 810 m elevation (see map, Fig. 8).

Measurements of holotype.—TOTL, 271; TAIL, 73; HFL, 36; EAR, 8; WT, 292 g; CBR, 29.2; CIL, 40.8; PLR, 44.5; MOL, 9.9; DSB, 21.3; PAB, 2.4; ZYB, 10.1; IOB, 10.8; LAB, 26.0; SHT, 16.2.

Specimens examined.—Twenty-eight individual specimens, here designated as paratypes, all from the type locality: 14 AMNH (263509, 263931, 263932, 263933, 263934, 263935, 263936, 263937, 263938, 263939, 263940, 263941, 263942, 263546), seven MSB (63385, 63386, 63387, 63388, 63389, 63390, 63509), seven MNKN (604, 605, 607, 616, 622, 623, 625). Pooled measurements of skulls and external dimensions of both sexes are summarized in Table 1 (see Table-S1 in online supplemental documents for complete data-set).

Diagnosis.—A medium-sized member of the "*boliviensis*" group of tuco-tucos with brown dorsal coloration, an almost indistinct Olive Brown stripe, and no cap of dark hairs on head; venter much lighter and well-differentiated from dorsal coloration. No dark collar in gular region; small patches of self-colored hair on pectoral, inguinal, jowl and/or axillary regions. Skull with a short and narrow rostrum, very short nasals, proodont incisors, zygomatic process mostly straight, and a karyotype with 2N=46 and FN=50 of mostly acrocentric autosomes.

Description (Figs. 11, 12).—Dorsal pelage dense, buffy brown to Mummy Brown darkening to an indistinct light Olive Brown stripe starting at the head proceeding posteriad; fur relatively coarse, about 10 to 16 mm long over back and rump; hairs and fur at base a deep Mouse Gray or Neutral Gray except for the distal most part of each hair that is a distinctly lighter shade of Buffy Brown. Gular region lighter in color and with a wash of Pale Olive Buff under forelegs. Color of ventral fur lighter than dorsal fur, Pale Olive Buff with hairs a Neutral Gray or Deep Neutral Gray basally, with superficial (4–6 mm) wash of Pale Olive Buff or Buffy Brown. Inguinal area light pale Olive Buff in color, lighter than ventral fur in general. Top of feet covered with hairs to base of metacarpals replaced with cteniform hairs over each toenail and on sides of toes. Some mystacial vibrissae extending past pinnae when laid back alongside head; superciliary vibrissae sparse, extending to the bases of pinnae; genal-one present, interramal present.

Ears sparsely covered with short, brownish hair, contrasting slightly with color of head. Opening to auditory canals protected by conspicuous cteniform bristles, most individuals with white patches of fur below and posterior to pinnae; bundles of stiff hair posterior to upper and lower incisors. Pes broad, all digits with ungal tufts of stiff "cteniform" bristles and strong claws; cteniform bristles on middle claw about half as long as nail. Forefoot with a well-developed pollex bearing a short nail open basally in contrast to the other four digits that bear basally closed claws. Tail

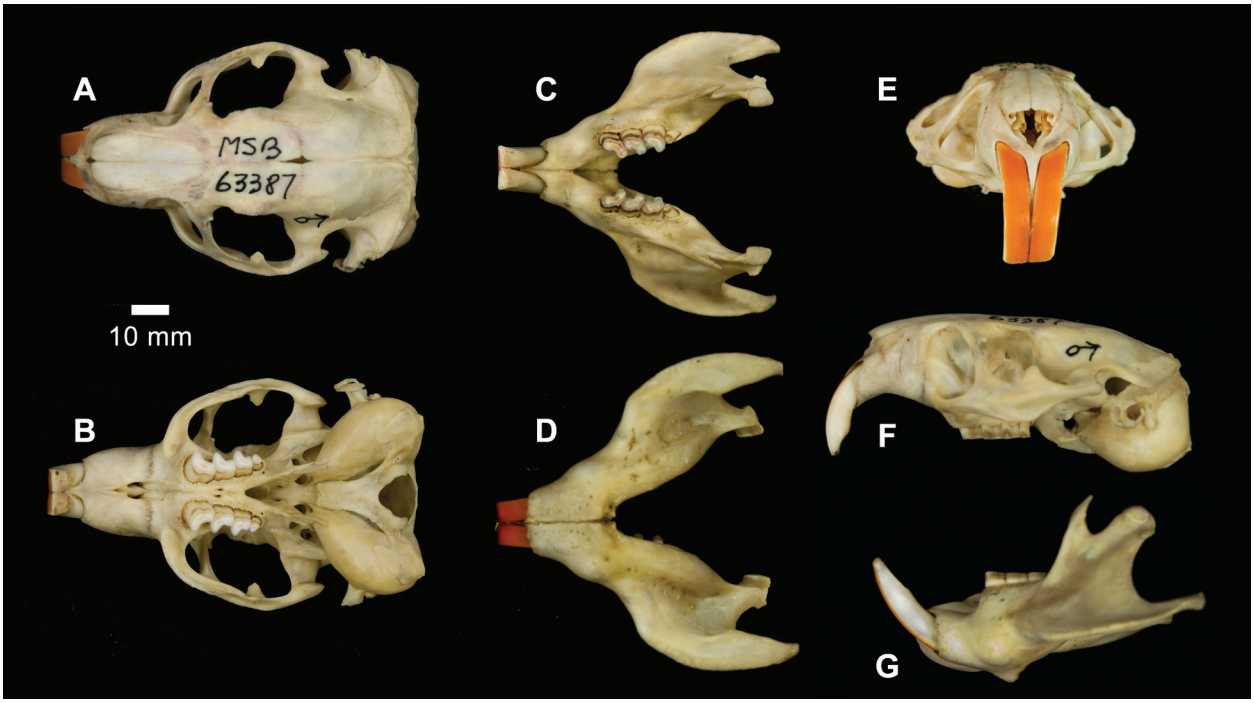


Figure 11. Skull and mandible of holotype (MSB63387) of *Ctenomys andersoni* n. sp. A) dorsal view of skull, B) ventral view of skull, C) dorsal view of mandible, D) ventral view of mandible, E) en face view of skull showing dark orange color of upper incisors, F) left lateral view of skull, G) left lateral view of mandible.

Figure 12. Museum study-skin of holotype (MSB63387) of *Ctenomys andersoni* n. sp. A) dorsal view, B) ventral view, C) left lateral view.

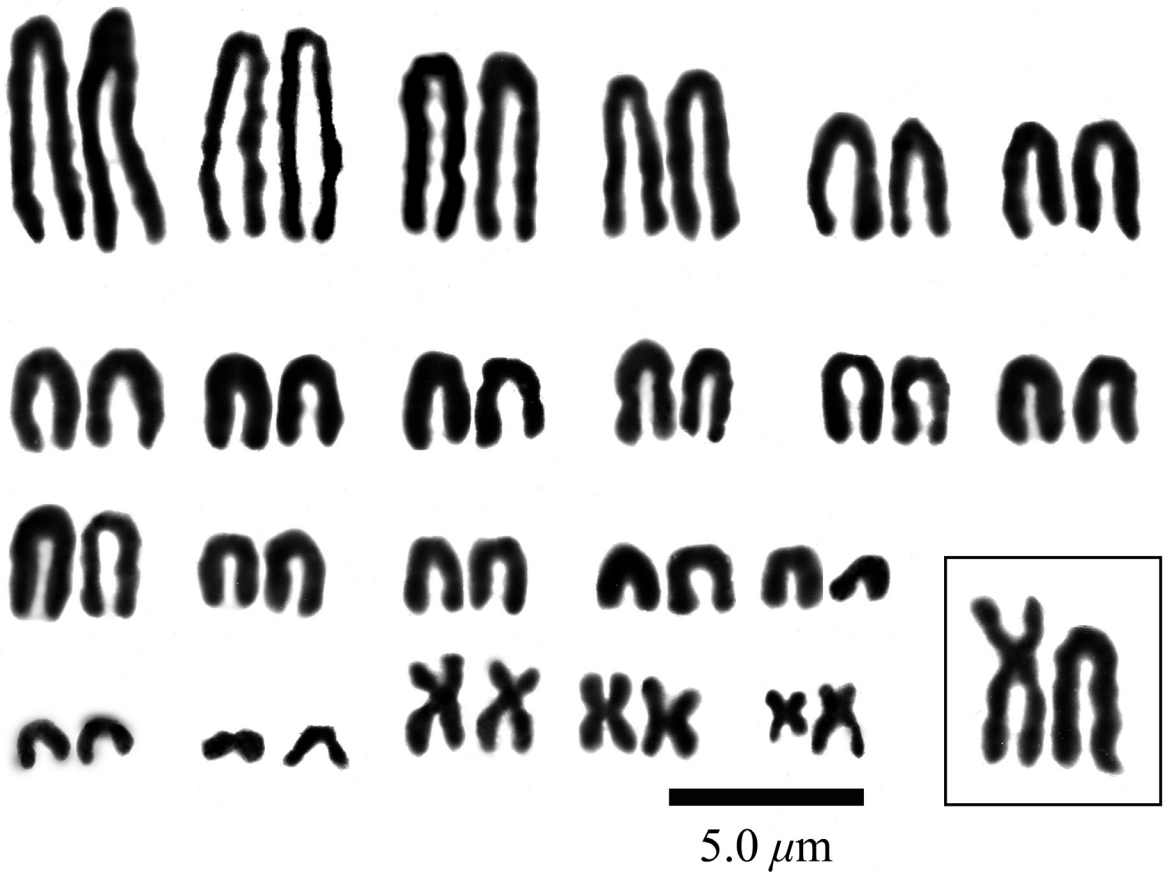


Figure 13. Image of standard giemsa-stained karyotype of *Ctenomys andersoni* n. sp. (MSB63387) consisting of 19 pairs of acrocentric autosomes, two pairs of metacentric autosomes, and one pair of submetacentric autosomes. The sex chromosomes (in box) appear as a large submetacentric X and an acrocentric Y. The 2N=46, FN=50.

short, strong, teretiform in cross-section, darker above than below in most specimens; sparsely covered with dark hairs, except for terminal 4 mm, which are covered with whitish hairs.

Skull robust (Fig. 11); interorbital region with mostly squared margins; zygomatic arches broad; auditory bullae inflated, pyriform; auditory tubes, long, salient. Nasal bones broad, broadest three-fourths anteriad with flat dorsal edges; nasal short, premaxillary visible when viewed from above. Frontals widest just at the middle of interorbital region, constricting at the same level as the attachment of the rear part of the zygomatic arch processes; parietals in older individuals narrowest at attachment of squamosal root of zygomatic arch. Squamosal evidently expanding with age; in young individuals, parietal and rear of frontals are approximately the same width. Interparietal completely fused in older individuals. Temporal foramen at junction of squamosal and parietal always present. Lambdoidal ridge always present. Most individuals with fenestra between frontals and parietals (Gardner and Anderson 2001). Supraoccipital crest strongly developed in adults. Zygomatic arch strongly built; jugals with strong dorso-ventral processes, incisive foramina recessed in a common fossa and well separated by a strongly developed bony septum; most individuals with a small interpremaxilary foramen; palatal bridge with two major palatine foramina at level of M1. Mesopterygoid fossae, an inverted "V" shape, reaching anteriorly to middle of M2s. Posteropalatal pits present, but not enlarged. Alisphenoid presphenoid bridge

narrow and with evident lateral processes. Bony roof with sphenopalatine vacuities; buccinator-masticatory foramen enlarged and undivided in many individuals, although in the holotype these foramina are separated. Upper incisors large, robust, Orange in color, proodont. Maxillary tooth rows slightly divergent posteriad.

Mandible robust, with coronoid process falciform, angled strongly backwards; condyloid process strong, bearing a well-developed articulation flange.

Karyology.—2N=46, FN=50 (Fig. 13) with 19 pairs of acrocentric autosomes, two pairs of metacentric autosomes, and one pair of submetacentric autosomes. The sex chromosomes appear as a large submetacentric X and a large acrocentric Y. A secondary constriction is evident on the largest biarmed pair of chromosomes. Karyotype slides were prepared in the field from nine specimens including three males (MSB63387, AM263941, NK21287) and six females (AM263931, MSB63386, MSB63388, MNKN616, MNKN607, and NK21259).

Distribution and habitat.—Anderson's Cujuchi is known only from the type locality at Cerro Itahuaticua. Here the vegetation was a mixture of deciduous thorny trees (Fabaceae) and several species of cacti, primarily *Opuntia* spp. Limestone outcrops were common on hillsides. This locality is in the ecological zone known as bosques secos interandino (Ibisch et al. 2003) or Andean dry valleys (Unzueta 1975; Lopez 2003).

Etymology.—This species of *Ctenomys* is named in honor of Dr. Sydney Anderson, Curator Emeritus of the Department of Mammalogy, American Museum of Natural History. Starting in 1984, Dr. Anderson lead a team of investigators, chiefly from AMNH, MSB, CBF, and MNKM, on collecting expeditions throughout Bolivia, including trips that secured these specimens. A great mentor and a dear colleague, we remember him examining the day's catch and then preparing specimens for hours on end. We are pleased to name this Bolivian endemic to honor his legacy; therefore, the name is a patronym in the genitive singular and in apposition to the generic name.

Comparisons.—Two specimens show a weak collar of pale fur extending ventrad from the pinnae, but not to the extent seen in *C. boliviensis*, which has a very well developed gular collar of light colored fur.

Ctenomys andersoni n. sp. differs from *C. steinbachi*, a species known from lowland Bolivia near Santa Cruz by having no gular collar of light colored fur, by having fur that is comparably lighter in color and by having a diploid and fundamental chromosome number of 2N=46, FN=50 versus 2N=10, FN=16 in *C. steinbachi* (see Anderson et al. 1987). In addition, the karyotype of *C. andersoni* has mostly acrocentric somatic chromosomes with only three bi-armed pairs while the chromosomes of *C. steinbachi* are all biarmed.

Ctenomys andersoni is distinct from *C. frater*, which occurs in the eastern foothills of Bolivia, by having light Mummy Brown fur instead of Dark Brown or Neutral Gray fur as in *C. frater* and by chromosome numbers (2N=48 and FN=90 in *C. frater*) see Cook et al. (1990).

Ctenomys andersoni is much smaller than *C. conoveri,* has much shorter fur, and has a diploid chromosome number of 2N=46 versus 2N=48 in *C. conoveri* (see Anderson et al. 1987).

The only other species occurring in the geographic proximity of *C. andersoni* is *C. lewisi*; specimens of *C. andersoni* were collected in much different habitat and much lower altitude than *C. lewisi*, which is known only from an area north-west of the city of Tarija at elevations above 3,400 m. In addition, the dorsal body fur of *C. andersoni* is brown in color, relative to *C. lewisi*, which is dark (Fuscous Black) dorsally. Chromosome numbers also differ between these species (2N=56, FN=74 in *C. lewisi;* Cook et al. 1990).

Finally, *C. andersoni* is genetically distinct from all other species in and around Bolivia (see Table 2) with genetic distances ranging from 5.0% in comparison with *C. erikacuellarae* to approximately 10% with both *C. lewisi* and *C. frater*. Other species of *Ctenomys* from Bolivia need not to be compared directly with *C. andersoni* as their ranges include high altitude altiplano and puna habitat (*C. opimus* and *C. leucodon*).

***Ctenomys lessai*, new species**
Lessa's Tuco-Tuco, El tuco-tuco de Lessa
Figs. 8 (map), 14, 15, 16, Table 1

Ctenomys "llathu": Lessa and Cook (1989:89)
Ctenomys "llath": Cook and Lessa (1998:1,524)
Ctenomys "llathu": Lessa and Cook (1998:89)
Ctenomys "Llathu": Castillo et al. (2005:172)
Ctenomys sp. Llathu: Parada et al. (2011:682)
C. sp. LLATHU: Freitas et al. (2012:1,367)

Holotype.—MSB67111 (Museum of Southwestern Biology), adult female. Skin, skull, and axial skeleton collected 31 May 1991, prepared by Forest W. Davis, field number FWD767. The liver, kidney, and heart tissue were frozen and archived in the Division of Genomic Resources of the MSB with number NK22870.

Type locality.—Bolivia, Department of Cochabamba, 0.5 km south of Lluthu Pampa, 17°45'S, 64°59'W, 2,700 m elevation.

Measurements of holotype.—TOTL, 255; TAIL, 64; HFL, 33.0; EAR, 6.0; WT, 170.0 g; CBR, 39.3; CIL, 41.9; PLR, 19.6; MOL, 9.4; DSB, 10.0; PAB, 2.3; ZYB, 27.3; IOB, 9.9; LAB, 25.7; SHT, 14.7.

Specimens examined.—Six specimens designated as paratypes from the type locality: Two MSB (87076, 67112), four AMNH (264557, 264558, 264559, 264560). Two, MNKN (two specimens, no museum catalog numbers available; field numbers and MSB-DGR catalog numbers NK22840, NK22869), not paratypes. Pooled measurements of skulls and external dimensions of both sexes are summarized in Table 1 (see Table-S1 in online supplemental documents for the complete data-set).

Diagnosis.—A member of the "*frater*" group of *Ctenomys*, characterized by a dorsal Olive Brown coloration with a dark cap of Clove Brown hair covering most of the head. Pectoral region colored same as dorsum, but venter covered with self-colored hairs. No collar in gular region. Skull delicate with mostly straight zygomatic arches and a flat skull profile; incisors proodont, with pale yellow enamel and a karyotype with 2N=46 and FN=64.

Description.—Pelage dense, fine, soft, about 5–20 mm long over back and rump. Color of dorsal pelage Olive Brown to Buffy Brown, ventral pelage Cinnamon Buff, some individuals with ventral fur Olive Buff. Dorsally, darkest fur a Clove Brown in central diffuse dorsal stripe, more prominent anteriorly on head and fading posteriad (Fig. 14). Small light area of Cinnamon Buff fur just posterior to and below pinnae, evident in most individuals. Collar not evident in specimens examined. Guard hairs of varying lengths approximately twice length of undercoat. Undercoat fur bicolored, lower ¾ of hairs deep Neutral Gray, distal parts with color as described above. Juveniles with consistent deep Neutral Gray color, no bicolored markings. Mystacial vibrissae of various sizes, from anterior to posteriad, initial vibrissae shorter, medial vibrissae longest in group, extending past base of pinnae when laid posteriad, distal vibrissae shorter. Genal vibrissae present, interramal present. Ears sparsely furred, same color as head, inconspicuous cteniform hairs protecting ear canal. Bundles of stiff hair on lips posterior to both upper and lower incisors. Rear legs with broad pes, all digits with ungal tufts of stiff cteniform bristles; forefoot with well-developed digits bearing basally closed claws. Tail short, covered with dark Olive Brown hair, teretiform, lighter fur ventrally, end of tail lighter in color.

Skull relatively delicate (Fig. 15), interorbital region with rounded margins; zygomatic arches wide, extending laterally almost to level of external auditory meatus. Auditory bullae pyriform; nasal bones broad tapering to a "V" shape posteriad. Frontal bones broad, constricting to narrowest point just posterior to nasal-frontal suture. Parietals narrowest at point of attachment of squamosal root of zygomatic arch. Interparietal not visible. Temporal foramen at junction of squamosal and parietal always present, ovoid. Lambdoidal ridge present, more pronounced in older individuals. Zygomatic arch strong, with massive processes extending both dorsally and ventrally. Incisive foramina recessed in a common fossa and incompletely separated by a bony septum at junction of premaxillary and maxillary bones. Small interpremaxillary foramen conspicuous; small palatine foramina, sometimes offset, sometimes parallel at level of PM1 and M1; anterior edge of mesopterygoid fossae with strongly inverted V-shaped border reaching to level of anterior part of

Figure 14. Museum study-skin of holotype (MSB67111) of *Ctenomys lessai* n. sp. A) dorsal view, B) ventral view, C) left lateral view.

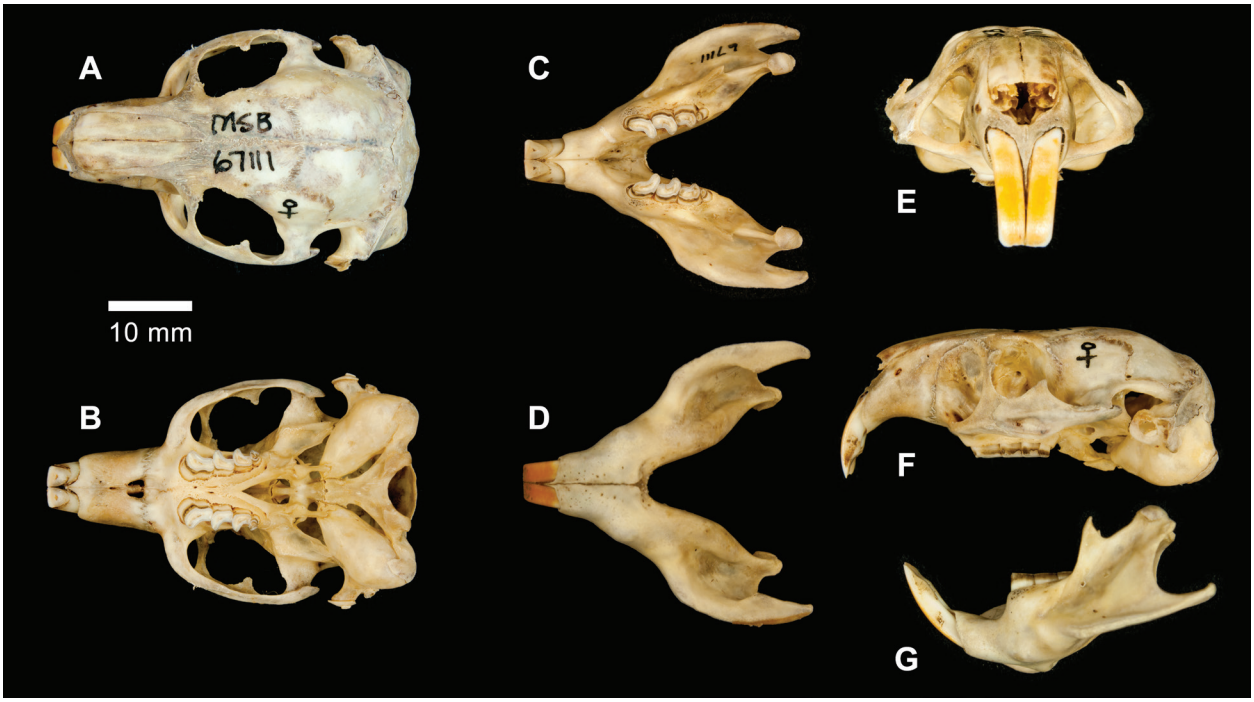


Figure 15. Views of the skull and mandible of the holotype (MSB67111) of *Ctenomys lessai* n. sp. A) dorsal view of skull, B) ventral view of skull, C) dorsal view of mandible, D) ventral view of mandible, E) en face view of skull showing yellowish color of upper incisors, F) left lateral view of skull, G) left lateral view of mandible.

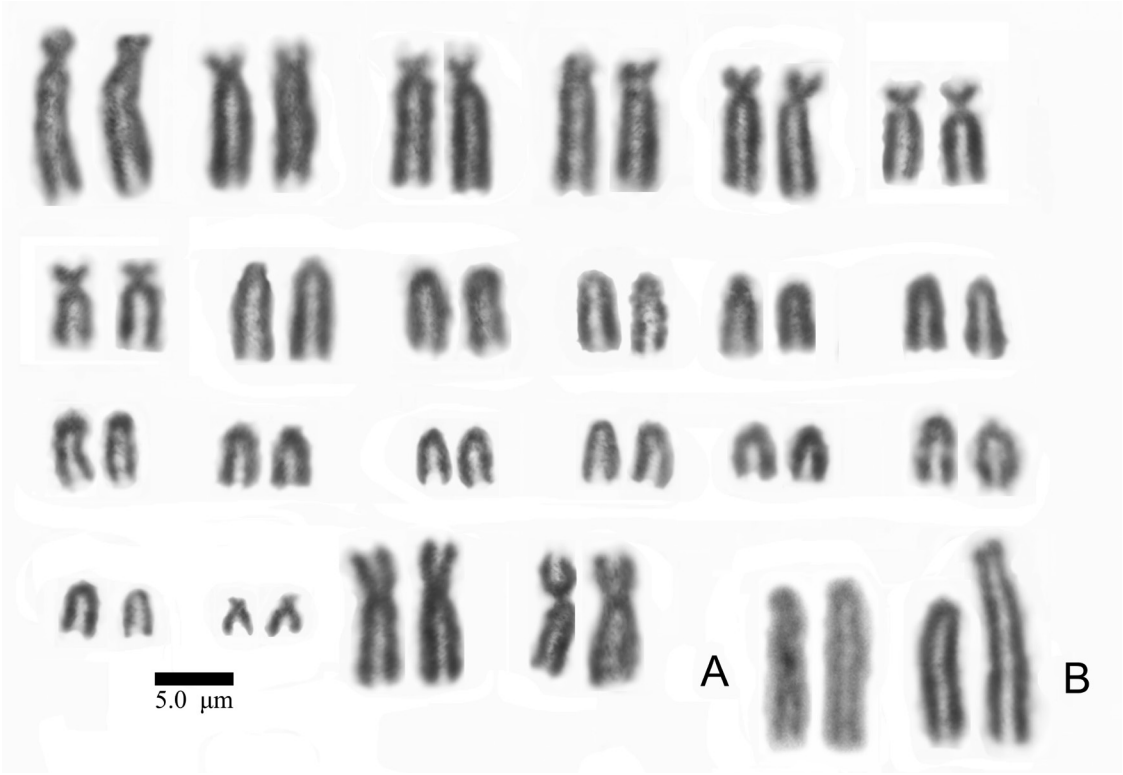


Figure 16. Image of standard giemsa-stained karyotype of *Ctenomys lessai* n. sp. consisting of one male (MSB67111) and one female (AM264558) [2N=46, FN=64], including: seven pairs of sub-telocentric, two pairs sub-metacentric, 12 pairs acrocentric, one pair of very small, distinctive, sub-telocentric, and one pair of acrocentric sex chromosomes. A = female; B = male.

M2. Suture of basioccipital and basisphenoid located at site of attachment of auditory bullae. Buccinator and masticatory foramina joined and enlarged even in young individuals. Upper incisors with enamel a light pale Yellow Ocher to Ivory Yellow color. Enamel initially white in unexposed roots, changing to Yellow Ocher with mottled yellowing slightly on frontal surfaces of exposed parts of incisors. Sometimes appearing mottled in frontal view with white showing through. Enamel of lower incisors Yellow Ocher in color. Mandible relatively delicate. Flange of mastoid process not visible.

Karyology.—Two animals from the type locality were karyotyped (one male, MSB67111 and one female AM264558) with 2N=46, FN=64 (Fig. 16). Karyotype consisting of chromosomes that include: seven pairs of sub-telocentric, two pairs sub metacentric, 12 pairs acrocentric, one pair of very small distinctive sub-telocentric, and one pair of acrocentric sex chromosomes.

Distribution and habitat.—Known only from the type locality with specimens collected at elevations ranging from 2,500 to 2,750 m. The area was an open grassland habitat near a running stream with remnant stands of trees (*Polylepis* sp.).

Etymology.—This species of *Ctenomys* is named in honor of Dr. Enrique P. Lessa, a friend, gifted mentor, and talented gaucho who has emerged as a leader in Latin American mammalogy, evolution, and in the study of the biology of tuco-tucos. The species name is thus a patronym in the genitive singular.

Comparisons.—*Ctenomys lessai* differs from *C. erikacuellarae*, the species of tuco-tuco with the closest geographic range to the southeast (see map, Fig. 8), in having pelage of a darker and more uniform color; upper incisors smaller, more procumbent, and much lighter in color than in *C. erikacuellarae*. Also, in *C. lessai*, the lateral basisphenoid foramina are conspicu-

ously enlarged but are either very small or not evident in *C. lessai* or in *C. erikacuellarae*; and the external auditory meatus is directed more anteriorly compared to *C. erikacuellarae*. Species of *Ctenomys* occurring farther east and at lower elevations, including *C. andersoni, C. boliviensis,* and *C. steinbachi*, are all larger in body size, with different pelage color, and all have different karyotypes. Finally, genetic distances based on *cyt*b range from 6.8% with *C. conoveri* to 11.6% with *C. leucodon* (Table 1).

Discussion

Recognition of Ctenomys goodfellowi *as a junior synonym of* C. boliviensis.—Originally described by Thomas (1921) from Esperanza, near Concepción in eastern Bolivia, the status of *C. goodfellowi* has historically fluctuated from being considered a subspecies of *C. boliviensis* (Cabrera 1961; Anderson et al. 1987; Gardner 1991) to being recognized as a full species (Cook and Yates 1994; Anderson 1997). Thomas (1921) identified subtle morphological differences that served to separate the single individual that he described as *C. goodfellowi* from one of *C. boliviensis* (which was then designated as a lectotype of *C. boliviensis*). Anderson et al. (1987) reported a 2N=46 for individuals assigned to *C. goodfellowi* and compared both morphological and chromosomal characters for individuals assigned to both species concluding that these may represent population level variation. In his phylogeny based only on morphology, Gardner (1991) showed that the subspecies *C. boliviensis boliviensis* Waterhouse 1848 and *C. boliviensis goodfellowi* (along with *C steinbachi*) are derived from a common ancestor, thus providing some support to hypothesis that these populations of *C. boliviensis* probably represented a single species.

Based on electromorphic variation at 21 presumptive loci, Cook and Yates (1994) compared individuals from four chromosomally polytypic populations that were assumed to all represent *C. boliviensis*; in this analysis, they found: a) minor allelic frequency variations among most samples of the species, with exception of allele SOD2-b that was fixed in the *C. boliviensis* population-1 from Las Lomitas, but importantly, b) one population (*C. boliviensis,* population-2) that was composed of individuals with a 2N=36 that was quite different and presented unique fixed alleles when compared to the remaining populations in their samples of *C. boliviensis*. Our phylogenetic analyses (Figs. 2 and 3) show that these populations, specifically, (*C. boliviensis*, population-2) of Cook and Yates (1994) belong to *C. nattereri*. At the time, Cook and Yates (1994) suggested that *C. goodfellowi* deserved species-level recognition, basing this recommendation on their study of both chromosomes (2N=46) and allozyme differences (allele frequency differences). Anderson (1997), Lessa and Cook (1998), Cook and Lessa (1998), Parada et al. (2011), Freitas et al. (2012) have since followed the conclusion of Cook and Yates (1994).

The status of *C. goodfellowi*, however, is predicated on a clearer understanding of the nature of the entire *"boliviensis*" group of *Ctenomys*, something that is only now emerging. Relevant to this discussion is the status of three species with geographic distributions mostly in Brazil (see Fig. 8) that include: *Ctenomys nattereri*, *C. rondoni,* and *C. bicolor* and are species that are poorly represented in natural history collections. Recently, Stolz et al. (2013) used geometric morphometric analyses and partial sequences of *cyt*b to validate the status of *C. bicolor* as distinct from *C. nattereri*. Opportunely, these authors sampled quasitopotypical material assigned to the latter, which in turn also provides support for the status of *C. nattereri* as a full species. With these two species more clearly defined, the nature of *C. goodfellowi* can now be better understood as follows: 1) based on the fact that individuals assigned to *C. goodfellowi* consistently are recovered as paraphyletic with respect to *C. boliviensis* in our phylogenies, 2) that their diploid chromosome number appears to be part of a cline of variation (from 2N=42 to 2N=46) in populations assigned to *C. boliviensis* (which includes the presence of at least one 2N=45 individual), and 3) very small values of genetic distances between populations assigned to *C. goodfellowi* and *C. boliviensis* (<1% in complete *cyt*b sequence data, < 5% in electrophoretic data). We therefore synonymize *C. goodfellowi* under *C. boliviensis*. We acknowledge that genetic and morphological variation among populations assigned to *C. goodfellowi* and *C. boliviensis* exist,

but suggest that these do not fulfill the criteria we set in the introduction of this report for the recognition of species in the genus.

Our phylogenetic analyses, the most inclusive to date for species in the genus *Ctenomys*, show that the *"boliviensis*" group of *Ctenomys* (*sensu* Parada et al. 2011) is only weakly supported and is composed of two groups of species: One that includes *C. bicolor*, *C. nattereri*, *C. boliviensis*, and *C. steinbachi* and one that includes three of the four species described in this report: *C. erikacuellarae, C. yatesi*, and *C. andersoni*.

The importance of the Andes as a speciation engine in Ctenomys.—The complicated orogeny of the Andes has created in Bolivia numerous ridges, valleys, and canyons stretching in a north-south orientation along its eastern flank. Part of the Yungas and the Andean dry valleys in the Departments of Cochabamba, Santa Cruz, Chuquisaca, and Tarija have a geomorphic origin co-temporal with a hypothesized rapid uplift of the central Andes between ~10 and 6 million years ago (Mya) (Horton et al. 2002; Garzione et al. 2008; Ehlers and Poulsen 2009). The Andes topography combined with slope and aspect are responsible for highly unique microclimatic and ecological conditions, which in some cases change across very short distances. A latitudinal transect (ca. 18°S) through the central plateau of the Andes shows the extreme topographic relief in the central Andean backthrust belt (see figures in McQuarrie et al. 2005 and Fig. F-3 in online supplemental documents).

The orogeny of the mountains and formation of valleys in this belt have been implicated as drivers of biodiversity in the central Neotropics (Graham et al. 2004; Hughes and Eastwood 2006). The region's characterization as a hotspot of biodiversity can be attributed partially to the combination of microclimatic variability and changing environmental conditions through the Quaternary. The multiple Yungas and Andean dry valleys and associated north-south ridges likely act as geographic barriers to movement of organisms. These barriers and microclimatic variability may especially stimulate species diversification in subterranean rodents (Nevo 1979). Ongoing formation of new ridges from active orogenic processes (McQuarrie et al. 2005) separate populations in the area, leading to a decrease in east-west gene flow while allowing north south dispersal to continue (see Figs. F-1, F-2, and F-3 in online supplemental documents).

The Octodontidae and the Ctenomyidae appear to have originated from a common ancestor about 19.1 Mya (with confidence interval between 14.3 and 23.5 Mya; Upham and Patterson 2012). Castillo et al. (2005) were the first to provide estimates of potential ages of species of *Ctenomys* based on molecular clocks of multiple genetic loci; interestingly an expanded *cyt*b dataset analyzed by those authors suggested an origin for the species of *Ctenomys* at about 3.7 Mya. This date is close to the estimate based on mitochondrial and nuclear loci provided by Upham and Patterson (2012) at ca. 4.3 Mya. In combination, these data indicate that the origin of species attributable to the genus *Ctenomys* is likely to have occurred after the end of the rapid orogeny of the Andes (Garzione et al. 2008).

All three known localities for *C. erikacuellarae* occupy the same river drainage (see Fig. F-1 in online supplemental documents) and are not separated by high elevation ridges, but geographic distance appears important in differentiation among populations of this species. Collection localities "Monteagudo" and "Chuhuayaco" are separated by only about 16 km, (see Fig. F-1 in online supplemental documents) and these two populations of *C. erikacuellarae* have a mean genetic distance of about 2.4% while comparisons of the populations of Monteagudo and Vallegrande (separated by approx. 150 km) diverge by 3.5%. *Ctenomys andersoni* shares a common ancestor with *C. erikacuellarae* (see Figs. F-1 and F-2 in online supplemental documents) and genetic distance between these two species is 5.2%. Geographic distance among localities of these two species crosses several major river valleys and ridges, but straight-line distance ranges from only 35-46 km (see Figs. F-1 and F-2 in online supplemental documents). Thus, alpha diversity in *Ctenomys* in Bolivia appears to be influenced by the complex geologic and geographic diversity of the eastern slope of the Andes.

Relative to biodiversity in the Yungas and Dry Valleys of Bolivia, we urge that additional broad scale biological surveys of these areas be conducted before anthropogenic impacts to native species are too severe. As this and investigators in other systems have shown (e.g., Gardner and Campbell 1992a; Gardner and

Campbell 1992b; Gardner and Pérez-Ponce de León 2002; Salazar-Bravo and Yates 2007; Gardner et al. 2013), there is great potential for additional species discovery in this area. This strengthens the hypothesis that the central Andes backthrust belt (i.e., the continual formation of new north-south valleys and ridges in the Yungas and valleys, cf. McQuarrie et al. 2005) in Bolivia can be considered as a "speciation engine" for biodiversity. Characterizing the extent of natural diversity in this, or any geographic area, is a necessary first step toward effective conservation of irreplaceable biotic resources.

ACKNOWLEDGMENTS

Our work on the tuco-tucos of Bolivia began in earnest in 1984 when a series of grants funded by the National Science Foundation were awarded to Sydney Anderson, Terry L. Yates, Scott L. Gardner, Joseph A. Cook, and Jorge Salazar-Bravo (BSR-8612329 to SLG; BSR-9024816 to SLG; DEB-9496263 to SLG; DEB-9631295 to SLG; DEB-0097019 to SLG; DBI-0646356 to SLG; BSR 89-20617 to TLY; BSR 90-15454 to TLY, SA, and JAC; INT-9417252 to TLY and JSB) and from NATO Collaborative Research Grant (CRG No. 920612, to SLG). Collaborating institutions in Bolivia included the Universidad Autónoma Gabriel René Moreno, the Zoológico Municipal Fauna Sudamericana (both of Santa Cruz), the Colección Boliviana de Fauna of the National Museum of Natural History, La Paz, and the Bolivian Academy of Sciences, La Paz. Noel Kempff Mercado, late director of the Zoológico Municipal Fauna Sudamericana, was instrumental in assisting our work in the lowlands in the mid-1980s. Tim Killeen of the Missouri Botanical Garden provided assistance in the 1990s, and the late Gaston Bejarano of La Paz assisted in La Paz during the early and late 1980s.

Marcelo Zalles and Otto Carlos Jordan Camacho participated as coordinators and advisors to our Bolivian expeditions in the early 1990s. Other participants were: Justine Anderson, Sara V. Brant, Mariel L. Campbell, Michelle Blair, Suzy Peurach, Enzo Aliaga Rossel, Juan Carlos Cenzano, Nella Sanchez Cook, Rosa Lenny Cuéllar, Erika Cuéllar, Jon Dunnum, José Carlos Herrera, Jean-Pierre Hugot, Emma Luna-Pizarro, Nancy Olds, Eduardo Palma, Ronald H. Pine, C. Tom Seaton, Maria Cristina Tapia, Teresa Tarifa, Luis Fernando Terceros, and James Wood. Brandy Jacobsen generated some of the *cyt*b sequences at the University of Alaska. We are grateful for the hospitality shown us by the local people at all collecting sites, especially Sra. Wachtel at the Estancia Yatiguagua (locality designated as Cerro Itahuaticua) and officials at the experiment station of the Corporation Regional de Desarrollo de Chuquisaca just south of Monteagudo. Collecting and exportation permits were granted under the aegis of the collaborative agreements between our institutions and the Colección Boliviana de Fauna (La Paz) and the Museum "Noel Kempff Mercado" of Natural History of the Universidad "Gabriel René Moreno" in Santa Cruz de la Sierra.

Work on the final stages of this paper was facilitated by Enzo Aliaga-Rossell, Gabor Rácz, Brett Ratcliffe, A.T. Dursahinhan, and Angie Fox (for assistance with artwork). Thanks especially are extended to Sue Ann Gardner, Nella Sanchez Cook, and Jackie Miralles Salazar for support all these years.

LITERATURE CITED

Altuna, C. A., and E. Lessa. 1985. Penial morphology in Uruguayan species of *Ctenomys* (Rodentia: Octodontidae). Journal of Mammalogy 66:483–488.

Anderson, S. 1985. Lista preliminar de mamíferos Bolivianos. Cuadernos. Academia Nacional de Ciencias de Bolivia. Ciencias Naturales 6. Museo Nacional de Historia Natural 3:5–16.

Anderson, S. 1997. Mammals of Bolivia, taxonomy and distribution. Bulletin of the American Museum of Natural History 231:1–652.

Anderson, S., T. L. Yates, and J. A. Cook. 1987. Notes on Bolivian mammals, 4: The genus *Ctenomys* (Rodentia: Ctenomyidae) in the eastern lowlands. American Museum Novitates 2891:1-20.

Avise, J. C. 2004. Molecular markers, natural history, and evolution, 2. Sinauer, Sunderland, Massachusetts.

Baker, R. J., M. Haiduk, L. Robbins, A. Cadena, and B. Koop. 1982. Chromosomal studies of South American bats and their systematic implications. Pp. 303–327 in Mammalian biology in South America (M. Mares and H. Genoways, eds.). Special Publication Series, 6. Pymatuning Laboratory of Ecology, University of Pittsburgh. 539 pp.

Blainville, M. H. 1826. Sur une nouvelle espèce de Rongeur Fouisseur du Brésil. Nouveau Bulletin des Sciences par la Société Phylomatique de Paris, Zoologie, 62–64.

Bidau, C. J., and F. D. Avila Pires. 2009. On the type locality of *Ctenomys bicolor* Miranda Ribeiro, 1914 (Rodentia: Ctenomyidae). Mastozoología Neotropical 16:445–447.

Cabrera, A. 1961. Catalogo de los mamíferos de America del Sur. Revista del Museo Argentino de Ciencias "Bernardino Rivadavia" 4. 307 pp.

Castillo, A., M. Cortinas, and E. P. Lessa. 2005. Rapid diversification of South American tuco-tucos (*Ctenomys*: Rodentia: Ctenomyidae): Contrasting mitochondrial and nuclear intron sequences. Journal of Mammalogy 86:170–179.

Cook, J. A. 1990. Rapid chromosomal evolution and the systematics of the Bolivian tuco-tucos (Rodentia: Ctenomyidae). Doctoral dissertation, University of New Mexico, Albuquerque. 85 pp.

Cook, J. A., S. Anderson, and T. L. Yates. 1990. Notes on Bolivian mammals, 6: the genus *Ctenomys* (Rodentia: Ctenomyidae) in the highlands. American Museum Novitates 2980:1–27.

Cook, J. A., and T. L. Yates. 1994. Systematic relationships of the Bolivian tuco-tucos, genus *Ctenomys* (Rodentia: Ctenomyidae). Journal of Mammalogy 75:583–599.

Cook, J. A., and E. P. Lessa. 1998. Macroevolutionary patterns of diversification in subterranean rodents: The case of the South American tuco-tucos (genus *Ctenomys*: Rodentia: Octodontidae). Evolution 52:1,521–1,527.

Cook, J. A., and J. Salazar-Bravo. 2004. Heterochromatin variation among the chromosomally diverse tuco-tucos (Rodentia: Ctenomyidae) from Bolivia. Pp. 129–142 in Contribuciones Mastozoológicas en Homenaje a Bernardo Villa (V. Sánchez-Cordero and R. A. Medellín, eds.) . Instituto de Biología e Instituto de Ecología, UNAM, México.

Cuéllar, E., and A. J. Noss. 2003. Mamíferos del Chaco y la Chiquitanía de Santa Cruz, Bolivia. Editorial FAN: Santa Cruz de la Sierra, Bolivia. 202 pp.

Cueto, G. R., P. Teta, and P. De Carli. 2008. Rodents from southern Patagonian semi-arid steppes (Santa Cruz Province, Argentina). Journal of Arid Environments 72:56–61.

Ehlers, T. A., and C. J. Poulsen. 2009. Influence of Andean uplift on climate and paleoaltimetry estimates. Earth and Planetary Science Letters 281:238–248.

Felsenstein, J. 1985. Phylogenies and the comparative method. American Naturalist 125:1–15.

Freitas, T. R. O. 2006. Cytogenetics status of four *Ctenomys* species in the south of Brazil. Genetica 126:227–235.

Freitas, T. R. O., F. A. Fernandes, R. Fornel, and P. Roratto. 2012. An endemic new species of tuco-tuco, genus *Ctenomys* (Rodentia: Ctenomyidae), with a restricted geographic distribution in southern Brazil. Journal of Mammalogy 93:1,355–1,367.

Gardner, A. L., and J. L. Patton. 1976. Karyotypic variation in oryzomyine rodents (Cricetinae) with comments on chromosomal evolution in the neotropical cricetine complex. Occasional Papers of the Museum of Zoology, Louisiana State University 49:1–48.

Gardner, S. L. 1991. Phyletic coevolution between subterranean rodents of the genus *Ctenomys* (Rodentia: Hystricognathi) and nematodes of the genus *Paraspidodera* (Heterakoidea: Aspidoderidae) in the Neotropics: Temporal and evolutionary implications. Zoological Journal of the Linnean Society 102:169–201.

Gardner, S. L. 1996. Essential techniques for collection of parasites during surveys of mammals. Pp. 291–298 in Measuring and monitoring biological diversity: Standard methods for mammals (D.E. Wilson, R. Cole, J.D. Nichols, R. Rudran, and M. Foster, eds.). Smithsonian Institution Press, Washington, D.C.

Gardner, S. L., and S. Anderson. 2001. Persistent fontanelles in rodent skulls. American Museum Novitates 3327:1–15.

Gardner, S. L., and M. L. Campbell. 1992a. A new species of *Linstowia* Zschokke, 1899 (Cestoda: Anoplocephalidae) from marsupials in Bolivia with comments on the biogeography of cestodes of the genus *Linstowia*. Journal of Parasitology 78:795–799.

Gardner, S. L., and M. L. Campbell. 1992b. Parasites as probes for biodiversity. Journal of Parasitology 78:596–600.

Gardner, S. L., and G. Pérez-Ponce de León. 2002. *Yungasicola travassosi* gen. n., sp. n. (Digenea: Dicrocoeliidae: Eurytrematinae) from two species of grass mice of the genus *Akodon* Meyen (Rodentia: Muridae) from the Yungas of Bolivia. Comparative Parasitology 69:51–57.

Gardner, S. L., and F. A. Jiménez-Ruiz. 2009. Methods of endoparasite analysis. Pp. 795–805 in Ecological and behavioral methods for the study of bats (T. Kunz and S. Parsons, eds). Johns Hopkins University Press, Baltimore.

Gardner, S. L., F. A. Jiménez, and M. L. Campbell. 2013. *Pritchardia boliviensis* n. gen., n. sp. (Anoplocephalidae: Linstowinae), a tapeworm from opossums (Didelphidae) in the Yungas and lowlands of Bolivia and Atlantic Forest of Paraguay. Occasional Papers, Museum of Texas Tech University 319:1–8.

Garzione, C. N., G. D. Hoke, J. C. Libarkin, S. Withers, B. MacFadden, J. Eiler, P. Ghosh, and A. Mulch. 2008. Rise of the Andes. Science 320:1,304–1,307.

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 58:1,781–1,793.

Horton, B. K., B. A. Hampton, B. N. LaReau, and E. Baldellon. 2002. Tertiary provenance history of the northern and central Altiplano (Central Andes, Bolivia): A detrital record of plateau-margin tectonics. Journal of Sedimentary Research 72:711–726.

Hughes, C., and R. Eastwood. 2006. Island radiation on a continental scale: Exceptional rates of plant diversification after uplift of the Andes. Proceedings of the National Academy of Sciences, U.S.A. 103:10,334–10,339.

Ibisch, P., S. Beck, B. Gerkmann, and A. Carretero. 2003. Ecoregiones y ecosystemas. Pp. 47–88 in Biodiversidad: La Riqueza de Bolivia (P. Ibisch and G. Mérida, eds.). FAN: Santa Cruz, Bolivia.

Langguth, A., and A. Abella. 1970. Las species uruguayas del genero *Ctenomys* (Rodentia: Octodontidae). Comunicaciones Zoológicas del Museo de Historia Natural de Montevideo 10:1–20.

Lessa, E. P., and J. A. Cook. 1989. Interspecific variation in penial characters in the genus *Ctenomys* (Rodentia: Octodontidae). Journal of Mammalogy 70:856–860.

Lessa, E. P., and J. A. Cook. 1998. The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of speciation. Molecular Phylogenetics and Evolution 19:88–99.

Lesson, R. P. 1842. Nouveau Tableau du Règne Animal, Mammifères. Arthus-Bertrand, Paris. 204 pp.

López, R. P. 2003. Phytogeographical relations of the Andean dry valleys of Bolivia. Journal of Biogeography 30:1,659–1,668.

Mascheretti, S., P. Mirol, M. Giménez, C. Bidau, J. Contreras, and J. Searle. 2000. Phylogenetics of the speciose and chromosomally variable genus *Ctenomys* (Ctenomyidae: Octodontoidea), based on mitochondrial cytochrome-b sequences. Biological Journal of the Linnean Society 70:361–376.

McQuarrie, N., B. K. Horton, G. Zandt, S. Beck, P. G. DeCelles. 2005. Lithospheric evolution of the Andean fold-thrust belt, Bolivia, and the origin of the central Andean plateau. Tectonophysics 399:15–37.

Miranda Ribeiro, R. A. 1914. Zoologia: Comissão de Linhas Telegráphicas Estratégicas de Matto-Grosso ao Amazonas. Annexo 5, Historia Natural, publication no.17, Mamíferos. 49 pp.

Navarro, G., and M. Maldonado. 2002. Geografía Ecológica de Bolivia, Vegetación y Ambientes Acuáticos. Fundación Simón I Patiño, Cochabamba, Bolivia. 719 pp.

Nehring, A. 1887. Über eine *Ctenomys*-Art aus Rio Grande do Sul (Süd-Brasilien). Sitzungs-Beritch Gesellschaft Naturforschung Freunde zu Berlin 4:45–47.

Nevo, E. 1979. Adaptive convergence and divergence of subterranean mammals. Annual Review of Ecology, Evolution, and Systematics 10:269–308.

Nylander, J. A. A. 2004. MrModeltest v2. Evolutionary Biology Centre, Uppsala University.

Olds, N., S. Anderson, and T. L. Yates. 1987. Notes on Bolivian mammals, 3: A revised diagnosis of *Andalgalomys* (Rodentia, Muridae) and the description of a new subspecies. American Museum Novitates 2890:1–17.

Olrog, C. C., and M. M. Lucero. 1981. Guía de los mamíferos argentinos. Ministerio de Cultura y Educación, Tucumán: Fundación Miguel Lillo. 151 pp.

Osgood, W. H. 1946. A new octodont rodent from the Paraguayan Chaco. Fieldiana Zoology 31:47–49.

Parada, A. S., G. D'Elía, C. J. Bidau, and E. P. Lessa. 2011. Species groups and the evolutionary diversification of tuco-tucos, genus *Ctenomys* (Rodentia: Ctenomyidae). Journal of Mammalogy 92:671–682.

Pearson, O.P., and M. Christie. 1985. Los tuco-tucos (genero *Ctenomys*) de los parques nacionales Lanin y Nahuel Huapi, Argentina. Historia Natural 5:337–343.

Redford, K. H., and J. F. Eisenberg. 1992. Mammals of the Neotropics: The Southern Cone, 2: Chile, Argentina, Uruguay, Paraguay. University of Chicago Press, Chicago, Illinois. 460 pp.

Ridgway, R. 1912. Color standards and color nomenclature. Published by the author, A. Hoen (printer), Baltimore, Maryland. 53 colored plates.

Salazar-Bravo, J., and T. L. Yates. 2007. A new species of *Thomasomys* (Cricetidae: Sigmodontinae) from central Bolivia. Pp. 747–774 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 Publication in Zoology 134:1-981.

Sanborn, C. C., and O. P. Pearson. 1947. The tuco-tucos of Peru (genus *Ctenomys*). Proceedings of the Biological Society of Washington 60:135–137.

Sikes, 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 92:235–253.

Silvestro, D., and I. Michalak. 2012. raxmlGUI: a graphical front-end for RAxML. Organisms Diversity & Evolution 12:335–337.

Slamovits, C. H., J. A. Cook, E. P. Lessa, and M. S. Rossi. 2001. Recurrent amplifications and deletions of satellite DNA accompanied chromosomal diversification in South American tuco-tucos (genus *Ctenomys*: Rodentia: Octodontidae): A phylogenetic approach. Molecular Phylogenetics and Evolution 18:1,708–1,719.

Stolz, J., G. Gonçalves, L. Leipnitz, and T. O. Freitas. 2013. DNA-based and geometric morphometric analysis to validate species designation: A case study of the subterranean rodent *Ctenomys bicolor*. Genetics and Molecular Research 12:5,023–5,037.

Swofford, D. L. 2003. *PAUP**: Phylogenetic analysis using parsimony (*and other methods), Version 4.0b10. Sinauer, Sunderland, Massachusetts.

Tamura K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, eolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28:2,731–2,739.

Thomas, O. 1900. Descriptions of new rodents from western South America. Annals and Magazine of Natural History, ser. 7, 2:294–302.

Thomas, O. 1902. On mammals collected by Mr. Perry O. Simons in the southern part of the Bolivian plateau. Annals and Magazine of Natural History, ser. 7, 9:222–230.

Thomas, O. 1907. On Neotropical mammals of the genera *Callicebus*, *Reithrodontomys*, *Ctenomys*, *Dasypus*, and *Marmosa*. Annals and Magazine of Natural History, ser. 7, 20:161–168.

Thomas, O. 1921. A new tuco-tuco from Bolivia. Annals and Magazine of Natural History, ser. 9, 7:136–137.

Thomas, O. 1926. The Spedan Lewis South American exploration, II: On mammals collected in the Tarija Department, southern Bolivia. Annals and Magazine of Natural History, ser. 9, 17:318–328.

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.

Unzueta Q., O. 1975. Mapa Ecológico de Bolivia. Ministerio de Asuntos Campesinos y Agropecuarios, La Paz, Bolivia. 315 pp.

Upham, N. S., and B. D. Patterson. 2012. Diversification and biogeography of the Neotropical caviomorph lineage Octodontoidea (Rodentia: Hystricognathi). Molecular Phylogenetics and Evolution 63:417–429.

Wagner, A. 1848. Beiträge zur Kenntniss der Arten von *Ctenomys*. Archiv für Naturgeschichte 14:72–78.

Wahlert, J. H. 1974. The cranial foramina of protrogomorphous rodents: An anatomical and phylogenetic study. Bulletin of the Museum of Comparative Zoology, Harvard 146:363–410.

Wahlert, J. H. 1985. Skull morphology and relationships of geomyoid rodents. American Museum Novitates 2812:1–20.

Waterhouse, G. R. 1848. A natural history of the Mammalia. Rodentia, or gnawing Mammalia, 2. Hippolyte Bailliere, London. 500 pp.

Woods, C. A., and C. W. Kilpatrick 2005. Infraorder Hystricognathi. Pp. 1,560-1,570 in Mammal species of the World: A taxonomic and geographic reference, 3rd edition (D.E. Wilson and D.M. Reeder, eds.). Johns Hopkins University Press, Baltimore, Maryland.

Yates, T. L., C. Jones, and J. A. Cook. 1996. Preservation of voucher specimens. Pp. 265–274 in Measuring and monitoring biological diversity: Standard methods for mammals (D. E. Wilson, R. Cole, J. D. Nichols, R. Rudran, and M. Foster, eds.). Smithsonian Institution Press, Washington, D.C.

Addresses of authors:

SCOTT LYELL GARDNER

Harold W. Manter Laboratory
University of Nebraska State Museum and School of Biological Sciences
University of Nebraska-Lincoln
Lincoln, Nebraska, USA 68588-0547
slg@unl.edu

JOSEPH A. COOK

Department of Biology and Museum of Southwestern Biology
University of New Mexico
Albuquerque, New Mexico, USA 87131
tucojoe@gmail.com

JORGE SALAZAR-BRAVO

Department of Biological Sciences
Texas Tech University
Lubbock, Texas, USA 79409
j.salazar-bravo@ttu.edu