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A NEW SPECIES OF *ORYZOMYS* (RODENTIA: MURIDAE) FROM AN ISOLATED POCKET OF CERRADO IN EASTERN BOLIVIA

DANIEL M. BROOKS, ROBERT J. BAKER, R. JULIETA VARGAS M.,
TERESA TARIFA, HUGO ARANIBAR, AND JOSÉ MANUEL ROJAS

Reliable characterization of a species is an essential step toward eventual reconstruction of phylogenetic alliances among related taxa (Musser et al. 1998). Although characterization of species within the genus *Oryzomys* has met with some confusion in the past, significant work has taken place to help better define specific limits within this group (Musser et al. 1998; Bonvicino and Moreira 2001; Langguth and Bonvicino 2002).

In spite of several recent surveys performed in the eastern Bolivian Panhandle (Emmons 1993; Taber et al. 1997; Brooks et al. 2002), our knowledge of the mammalian fauna in this region is still incomplete, and further studies are warranted. For example, of 1,259 collecting localities in Bolivia analyzed by Anderson (1997), less than two percent are from the eastern panhandle of Santa Cruz Department. Thus, this region constitutes a priority for mammalian exploration and conservation.

In mid April 1999, during an expedition to the eastern Bolivian panhandle (Brooks et al. 2002), we collected a single specimen of the genus *Oryzomys* that could not be assigned to any known species previously reported for the region in former studies (e.g., Anderson 1993, 1997). Extensive morphological comparisons with deposited voucher specimens revealed that this specimen may represent an undescribed species most closely related to the *O. subflavus* group (Guy Musser, pers. comm.). To confirm this taxonomic hypothesis, molecular analyses using a portion of the mitochondrial cytochrome-*b* gene were performed to establish phylogenetic relationships. Molecular data supported our conclusion that this specimen represents a new taxon within the genus. In this study, we describe a new form of *Oryzomys* from the Department of Santa Cruz, Bolivia.

METHODS

The Cerrado represents a semi-deciduous savannah, comprised of a mosaic of open grassland and dry forest (Redford and da Fonseca 1986). The vegetation of this region is characteristic of a semi-humid

climate (Ibisch et al. 2002). Low cover is comprised of grasses and small shrubs. Tree species rarely reach high densities, and often have adaptations to water stress, such as ‘twisted’ trunks, deep roots, and hard,

leathery leaves (Redford and da Fonseca 1986; Ibish et al. 2002). Although Cerrado is found predominately in central Brazil, there is an isolated patch in southeastern Bolivia, acting as an island that likely promotes isolating mechanisms of speciation.

Small mammals were collected at two sites in the study region (both localities denoted with an "X" in Fig. 1), with GPS readings taken at each area. Collecting effort varied from site to site: the Las Conchas site ($17^{\circ}33'58.3''S$; $59^{\circ}28'17.1''W$) included Riverine Cerrado (40 trap-nights) and typical Cerrado (100 trap-nights) habitats; the Pozo Mario site ($17^{\circ}35'46.9''S$; $59^{\circ}30'20.5''W$) was comprised of a mosaic of Cerrado and savannah (260 trap-nights) vegetation. Sherman, Victor, and Museum Special traps were used to inventory small mammals. Traps were set on the ground in pairs along linear transects (trap lines), with a distance of 10 m between traps, for one to three nights in each selected microhabitat. Dry oatmeal flakes, vanilla, tuna or sardines, and peanut butter were used as bait.

Voucher specimens were deposited at Colección Boliviana de Fauna (CBF), La Paz, Bolivia.

Since the isolating mechanism driving the speciation of this form was believed to be a small island of Cerrado habitat in Santa Cruz, Bolivia (the only true Cerrado occurring outside of Brazil), we compared the specimen of the new species (CBF6151) to specimens of *O. subflavus* from Santa Cruz, Bolivia, as well as from the Brazilian Cerrado. Specimens examined are housed at AMNH (American Museum of Natural History), CMNH (Carnegie Museum of Natural History), CBF, FMNH (Field Museum of Natural History), MCZ (Harvard Museum of Comparative Zoology), NMNH (National Museum of Natural History), and OMNH (Oklahoma Museum of Natural History).

To further explore the specific affinity of this specimen, part of the mitochondrial cytochrome-*b* gene was sequenced. DNA was extracted from skin clip tissue using the guanidinium thiocyanate/silica extrac-



Figure 1. Map depicting location of collecting localities (X) in southeastern Bolivia.

tion technique (United States Department of the Interior, Fish and Wildlife Service, Division of Law Enforcement, National Fish and Wildlife Forensics Laboratory). The partial cytochrome-*b* gene (219 bp) was amplified using the following polymerase chain reaction (PCR) parameters modified as described by Saiki et al. (1988): 27 cycles of 95° C denaturation (1 min), 50° C annealing (1 min), 72° C extension (2 min), and 1 final 72° C extension cycle (7 min). Primers utilized in the PCR reaction were LGL765 (J. L. Patton, unpublished sequences) and Tbott191Rev (J. K. Wickliffe, unpublished sequences). The resulting PCR product was purified using the QIAquick PCR purification kit (Qiagen®, Valencia, California). The same two primers were used for cycle sequencing reactions to amplify the forward (LGL765) and reverse (Tbott191Rev) strands. Cycle sequencing was conducted using the ABI Big Dye version 3.0 ready reaction mix (PE Applied Biosystems®, Foster City, California) and samples were analyzed on an ABI Prism 310 automated sequencer (PE Applied Biosystems®, Foster City, California). Vector NTI 7.0 software (Informax, Inc., Bethesda, Maryland) was used to align and proof nucleotide sequences.

The specimen of the new taxon was compared to *Oryzomys* sequences found on GenBank (AF181274-

-AF181279, AF181281, AF251520, AF251522, AY041185, AY275124). Likelihood and parsimony models (PAUP*, Swofford 2002) were used to generate hypotheses concerning phylogenetic relationships of taxa. The variable nucleotide positions within the data set were treated as unordered, discrete characters with four possible states; A, C, G, or T. Parsimony analyses (PAUP*, Swofford 2002) were constructed using equally-weighted characters. The heuristic search option was used to obtain the most-parsimonious tree(s). All phylogenetically uninformative characters were excluded from these analyses. Bootstrap analysis (Felsenstein 1985) with 1,000 iterations was used to evaluate nodal support. Under the likelihood model, the transition/transversion ratio was set to two and rates were assumed to be equal. An optimal tree was generated using the heuristic search option in PAUP* (Swofford 2002). The Kimura 2-parameter model of evolution (Kimura 1980) was used to calculate genetic distances. These values were then used to assess levels of genetic divergence among species of *Oryzomys* following the criteria outlined in Bradley and Baker (2001).

RESULTS

The following description was prepared by Daniel M. Brooks and Robert J. Baker:

Oryzomys andersoni, new species

Holotype: CBF 6151; skull and skin of a subadult male specimen; collected by Julieta Vargas-M., field number JVM 486, on 17 April 1999.

Type Locality: Pozo Mario, Estancia Las Conchas, Santa Cruz, Bolivia; 17°35'46.9"S; 59°30'20.5"W; 220 m.

Distribution: Known only from the type locality.

Diagnosis: Externally this medium-sized species is white ventrally; dorsum brownish-gray laterally, and a darkish stripe along the top. The skull has reduced foramen anterior to the auditory bullae; post-maxillary palatine foramen present, mid-maxillary palatine foramen absent; incisive foramen crescent shaped, yet blunt at the anterior terminus; zygomatic process of the squamosal terminates in a sharp point (Figs. 2 and 3). Differences between *Oryzomys andersoni* and *O. subflavus* are highlighted in Table 1.

Etymology: This species is named in honor of Sydney K. Anderson for his strong contributions to Bolivian Mammalogy. Dr. Anderson's studies of Bolivian mammals have spanned over three decades.

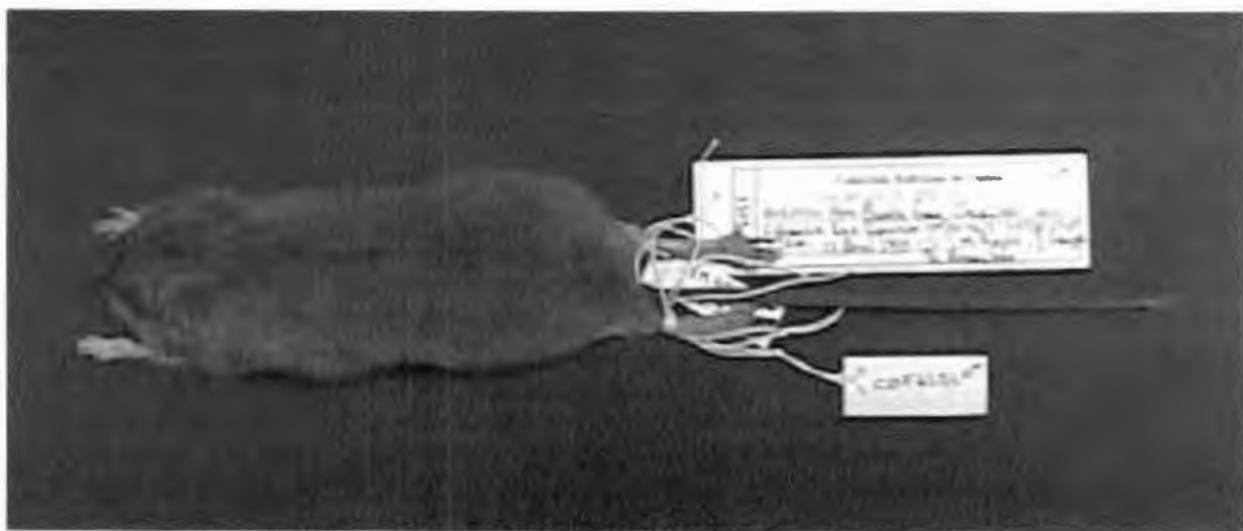


Figure 2. Study skin of the holotype of *Oryzomys andersoni* (CBF 6151).

DISCUSSION

DESCRIPTION AND COMPARISONS

Pelage – *Oryzomys andersoni* is a medium-sized species within the genus and overall color is brownish-gray; dorsum has a darker brown, diffuse stripe from the rostrum to the tail, becoming increasingly dark towards the posterior end; ventrum is white; bases of the hairs are dark charcoal throughout dorsal and ventral regions; head has same color as dorsum; feet are covered with sparse white hair and are lighter colored; dorsal side of the tail is a dark, charcoal color above; ventral side of the tail is lighter, becoming increasingly darker towards the distal tip, which has few hairs; ears are darker brown exteriorly (matching the dorsal stripe), and lighter grayish-brown interiorly (matching the sides, and overall dorsal pelage) (Fig. 2).

Oryzomys andersoni can be distinguished externally from *O. subflavus* by the following characters: *O. subflavus* from Santa Cruz Bolivia tend to have more tawny sides, whereas *O. subflavus* from the Brazilian Cerrado tend to be tawny throughout (*O. andersoni* is brownish-gray overall). Additionally, *O. subflavus* from Santa Cruz, Bolivia tend to have a tawny ventrum (*O. andersoni* has a whitish ventrum).

External Measurements – Measurements of holotype skin taken during preparation are as follows

(mm): total length = 233, tail length = 122, pes = 30, ear = 17; weight (g) = 37.

Skull – Skull elongate and slightly convex; bullae small and smooth; zygomatic process of the squamosal terminates in a sharp point; incisive foramen is crescent shaped, and blunt at the anterior terminus; vomerine process well developed; post-maxillary palatine foramen present; reduced foramen anterior to the auditory bullae.

Skull Measurements – Measurements of holotype skull taken as follows (mm): greatest length of skull = 29, zygomatic breadth = 15, length of nasals = 11, length of upper toothrow = 4.6, length of incisive foramen = 5.6 (Fig. 3).

Genetics – Based on comparisons of the first 219 bp of the mitochondrial cytochrome-*b* gene, this specimen groups with *O. subflavus* and is closest aligned to an unidentified specimen from Brazil (*O. sp. nov.* 3, AF181277). These clades were present in parsimony, likelihood, and neighbor-joining analyses, although the position of specimens within the clades differed. The parsimony tree with bootstrap values is shown in Figure 4. Percent sequence divergence values ranged from 0.92% to 16.05% (Table 2) within this group. Following the criteria and principles outlined in Bradley and Baker (2001), as they pertain to

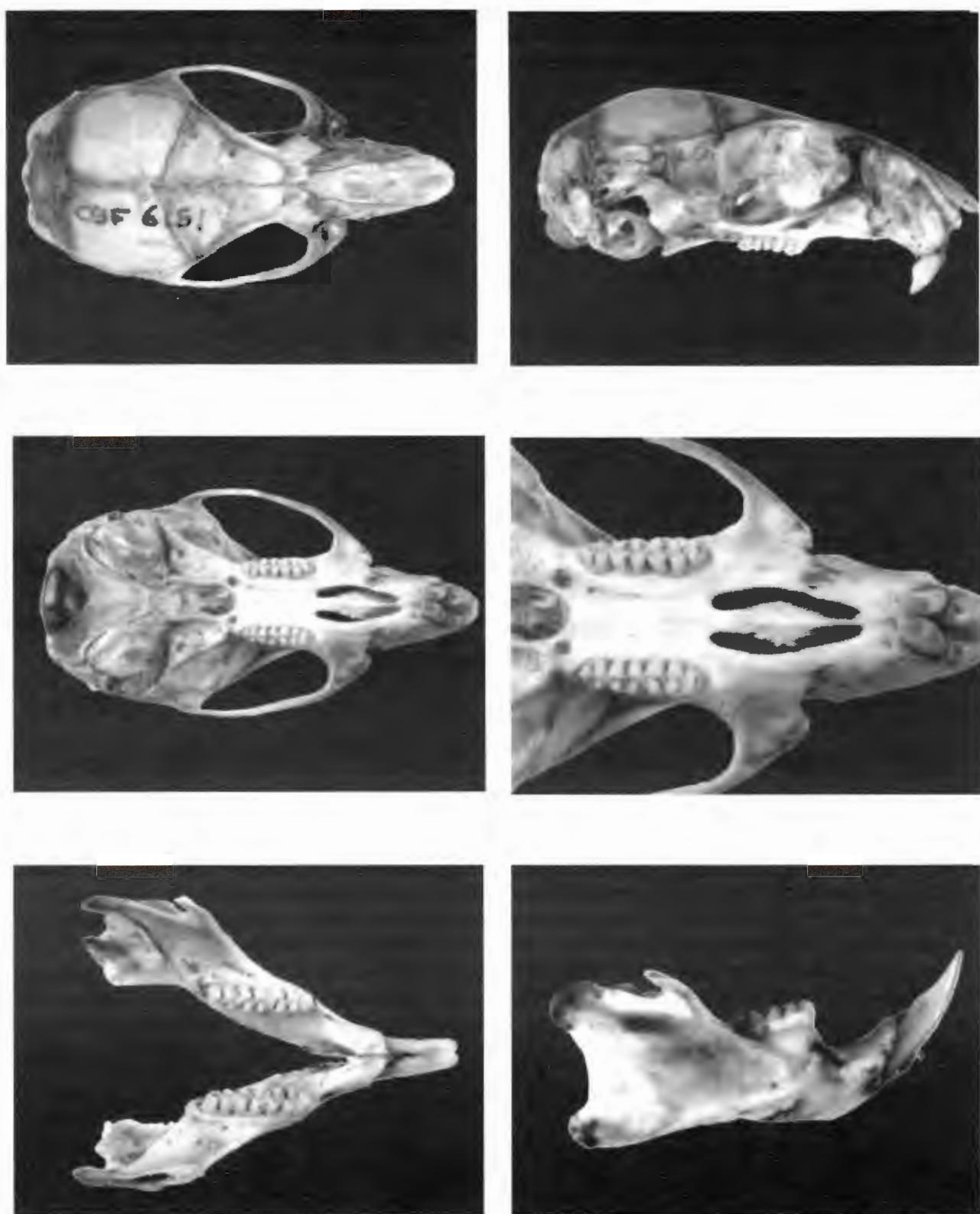


Figure 3. Skull of the holotype of *Oryzomys andersoni* (CBF 6151). Photographs are at different levels of enlargement. Relative size can be obtained by the following measurements: greatest length of skull 29; zygomatic breadth 15; length of nasal 11; length of upper toothrow 4.6; and length of incisive foramen 5.6. Photos by Mark Mauthner.

Table 1 – Character differences between *Oryzomys andersoni* and *O. subflavus*.

	<i>O. andersoni</i>	<i>O. subflavus</i>
Foramen anterior to auditory bullae	reduced	tends to be prominent
Post-maxillary palatine foramen	present	tends to be reduced or absent
Mid-maxillary palatine foramen	absent	tends to be present, but reduced
Incisive foramen	more crescent shaped	straighter, with more pointed tips
Zygomatic process of squamosal	terminates in sharper point	more rounded at termination
Ventrum	white	tends to be more tawny
Dorsum	brownish-gray sides	tawny sides

the genetic species concept (Dobzhansky 1950), the holotype of *O. andersoni* exhibits a genetic divergence from the *O. subflavus* clade, similar to those that separate other currently recognized species of rodents (Bradley and Baker 2001). Although we sequenced only 219 base pairs of the cytochrome-*b* gene, the addition

of the sequence values for the remainder of the gene will undoubtedly change the distance values slightly. The distance values distinguishing *O. andersoni* from *O. subflavus* are sufficiently high that slight changes in distance values will not alter conclusions relative to the specific recognition based on the genetic species concept.

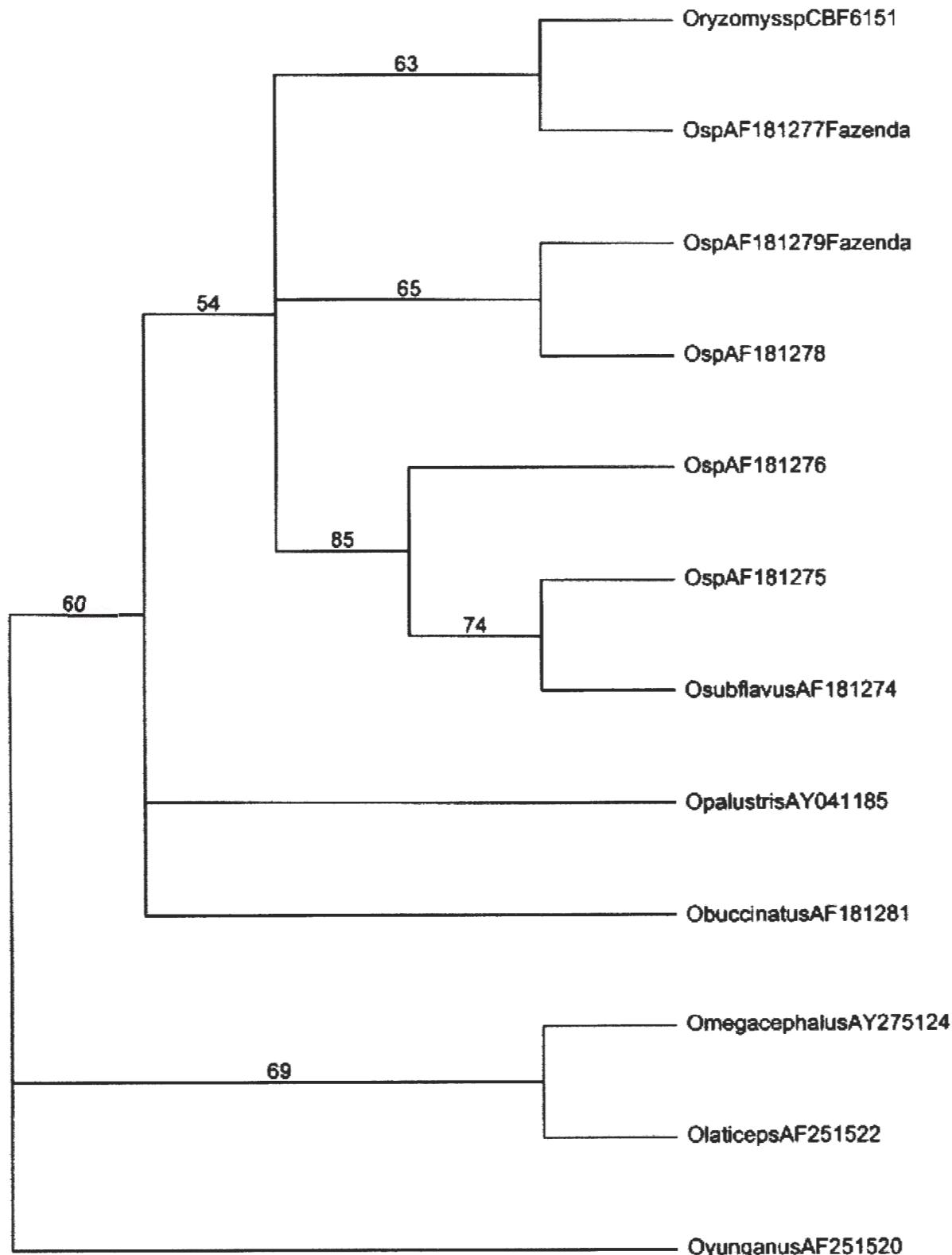
ECOLOGY

Habitat – This holotype was captured in Cerrado (see general description above), in a transitional mosaic between dense and high savannah, with open forest. The ground cover contained some dead leaves and 30-40% gramineae cover.

Sympatric Mammals – Other species of small mammals trapped at the site include singletons of

Monodelphis domestica and *Proechimys longicaudatus*. Larger rodents observed in the region included *Sciurus spadiceus*, *Galea spixii* and *Dasyprocta punctata*. Other species of mammals that were common included *Dasyurus novemcinctus*, *Cerdocyon thous*, *Mazama gouazoubira* and *M. americana* (Brooks et al. 2002).

Bootstrap



*Table 2 – Percent sequence divergence values between *O. andersoni* (CBF6151), *O. subflavus*, and five unidentified specimens from Brazil. The Kimura 2-parameter model of evolution (Kimura 1980) was used to calculate genetic distances.*

Comparison	Percent Sequence Divergence
<i>O. andersoni</i> (CBF6151)- <i>O. subflavus</i> (AF181274)	11.46%
<i>O. andersoni</i> (CBF6151)- <i>O. sp. nov. 3</i> (AF181277)	5.25%
<i>O. andersoni</i> (CBF6151)- <i>O. sp. nov. 5</i> (AF181279)	13.78%
<i>O. andersoni</i> (CBF6151)- <i>O. sp. nov. 4</i> (AF181278)	13.73%
<i>O. andersoni</i> (CBF6151)- <i>O. sp. nov. 1</i> (AF181276)	14.27%
<i>O. andersoni</i> (CBF6151)- <i>O. sp. nov. 2</i> (AF181275)	11.46%
<i>O. sp.</i> (AF181277)- <i>O. subflavus</i> (AF181274)	8.80%
<i>O. sp.</i> (AF181277)- <i>O. sp.</i> (AF181279)	12.15%
<i>O. sp.</i> (AF181277)- <i>O. sp.</i> (AF181278)	10.42%
<i>O. sp.</i> (AF181277)- <i>O. sp.</i> (AF181276)	11.49%
<i>O. sp.</i> (AF181277)- <i>O. sp.</i> (AF181275)	7.75%
<i>O. sp.</i> (AF181279)- <i>O. subflavus</i> (AF181274)	13.73%
<i>O. sp.</i> (AF181279)- <i>O. sp.</i> (AF181278)	8.33%
<i>O. sp.</i> (AF181279)- <i>O. sp.</i> (AF181276)	16.05%
<i>O. sp.</i> (AF181279)- <i>O. sp.</i> (AF181275)	12.58%
<i>O. sp.</i> (AF181278)- <i>O. subflavus</i> (AF181274)	9.26%
<i>O. sp.</i> (AF181278)- <i>O. sp.</i> (AF181276)	12.51%
<i>O. sp.</i> (AF181278)- <i>O. sp.</i> (AF181275)	9.26%
<i>O. sp.</i> (AF181276)- <i>O. subflavus</i> (AF181274)	6.21%
<i>O. sp.</i> (AF181276)- <i>O. sp.</i> (AF181275)	7.23%
<i>O. sp.</i> (AF181275)- <i>O. subflavus</i> (AF181274)	0.92%

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APPENDIX 1—SPECIMENS EXAMINED

BOLIVIA: Santa Cruz: AMNH 246934, 255954, 260378-260388, 262073, 262076, 263342-263343; CBF 6151; CMNH 4989, 5006; NMNH 390667, 390669-390672, 390674-390678, 391305, 304605; BRAZIL: Brasilia: OMNH 17581, 17582, 17584; Goias: MCZ 34053; Maranhão: FMNH 26446; Mato Grosso do Sul: OMNH 19656; Minas Gerais: NMNH 304605; Piaui: FMNH 25246

*Addresses of authors:***DANIEL M. BROOKS**

*Houston Museum of Natural Science
Department of Vertebrate Zoology
1 Hermann Circle Dr.
Houston, Texas 77030-1799, USA
e-mail: dbrooks@hmns.org*

HUGO ARANIBAR

*Sección de Mamíferos
Colección Boliviana de Fauna
Calle 26 de Cota-Cota
Casilla 8706
La Paz, Bolivia*

ROBERT J. BAKER

*Texas Tech University
Department of Biological Sciences
P.O. Box 43131
Lubbock, TX 79409, USA
e-mail: rjbaker@ttu.edu*

JOSE MANUEL ROJAS

*Colección de Mamíferos
Museo de Historia Natural "Noel Kempff Mercado"
Univ. Autonóma Gabriel René Moreno
Av. Irala #565
Casilla 3800
Santa Cruz, Bolivia*

R. JULIETA VARGAS M.

*Sección de Mamíferos
Colección Boliviana de Fauna
Calle 26 de Cota-Cota
Casilla 8706
La Paz, Bolivia*

TERESA TARIFA

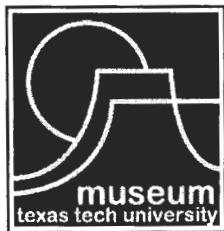
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Calle 26 de Cota-Cota
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