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Redescription and phylogenetic position of *Ctenomys dorsalis* Thomas 1900, an enigmatic tuco tuco (Rodentia, Ctenomyidae) from the Paraguayan Chaco

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Abstract: *Ctenomys dorsalis* is known only from its type specimen, a female preserved as skin without skull (except for the upper incisors) from an imprecise locality in the "Northern Chaco of Paraguay". Here, we report additional individuals of this species housed, since the 1940s, at the Field Museum of Natural History (Chicago, USA). Based on these specimens, which fully match the original description of this rodent, we provide novel information regarding its phylogenetic position, external and cranial morphology, and distribution. The analysis of mtDNA sequences supports the distinctiveness of this taxon and suggests its placement within the *boliviensis* group of *Ctenomys*. Our study highlights once more the importance of museum collections as repositories of biodiversity.

Keywords: ancient DNA; Caviomorpha; Dry Chaco; Octodontoidea; Paraguay.

Introduction

The subterranean rodent genus *Ctenomys* Blainville 1826, with 63 recognized species, is the most speciose genus within the Neotropical radiation of Caviomorpha (Bidau 2015). However, despite its impressive diversity, our current understanding of its alpha taxonomy and the phylogenetic relationships among species is far from complete (D'Elia et al 1999, Leite and Patton 2002, Parada et al. 2011, Bidau 2015). In many cases, our knowledge is so limited that some species are only known from the type specimen or series, sometimes from collections carried out more than 100 years ago (e.g. Bidau and Dias de Avila-Pires 2009, Fernández et al. 2012, Tatiana Sánchez et al. 2018).

Among the poorest known species of *Ctenomys* is *Ctenomys dorsalis*, a taxon supposedly endemic to the Paraguayan Chaco and one of the four species of the genus currently known from that country (cf. de la Sancha et al. 2017a). Thomas (1900) erected this species based on a skin without skull (except for the upper incisors), supposedly from the "Northern Chaco of Paraguay". In the original description, Thomas (1900) characterized the overall size of this rodent as "about as in *Ctenomys talarum*" with a "Coloration very much as in the large *Ctenomys boliviensis*". Subsequent authors restricted its type locality or mapped it for different areas within the Dry and Humid Chaco of Argentina, Bolivia, Brazil and Paraguay (see the review in Contreras and Roig 1992), confusing the already nebulous panorama. Contreras and Roig (1992) produced a detailed account on this species, summarizing the available data about its type locality, date of collection and references in the literature, suggesting a possible relationship between *C. dorsalis* and *Ctenomys argentinus* Contreras and Berry 1982, based on some supposed similarities. Despite these references, no additional specimens of *C. dorsalis* were reported (Bidau 2015).

Here, we redescribe *Ctenomys dorsalis*, based on a sample of 16 specimens housed at the Field Museum of Natural History (Chicago, USA). These animals were collected in the latter half of 1945 at two localities in the Dry Chaco of Paraguay and closely match the original description of this rodent. In order to evaluate the taxonomic status of this species, we sequenced its mtDNA from skin snippets and provide a phylogenetic hypothesis for its relationships to other *Ctenomys* species. In addition, we assembled an emended morphological diagnosis and

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complete descriptions of its external appearance and skull morphology.

Materials and methods

Specimens examined

We examined 17 specimens from two localities: Paraguay: Boquerón: Colonia Fernheim [five females (FMNH 54389, FMNH 54390, FMNH 54396, FMNH 54397, FMNH 54398) and five males (FMNH 54391, FMNH 54392, FMNH 54393, FMNH 54394, FMNH 54395)]; Orloff [six females (FMNH 54346, FMNH 54347, FMNH 54348, FMNH 63867, FMNH 63868, FMNH 63870) and one male (FMNH 63869)]. Approximate coordinates (in decimal degrees) for these localities are –22.3534, –60.1877 and –22.3165, –59.9049, respectively.

Morphological descriptions

Standard external measurements recorded for each individual were taken from labels and included: TL: total body length; T: tail length; HF: hind foot length (including the claw). Skull measurements and character definitions follow Contreras and Contreras (1984) and include the following: total length of the skull (TLS); condylo-incisive length (CIL); nasal length; nasal width; rostral width; interorbital constriction; greatest zygomatic breadth; braincase breadth; bimeatal breadth; mastoid breadth; infraorbital foramen height; upper incisors width; upper diastema length; palatal length; upper fourth premolar length; upper toothrow length; tympanic bulla length; tympanic bulla width; mandible length and lower toothrow length. Anatomical terminology used to describe skull characters follows Langguth and Abella (1970), Contreras and Berry (1982), and Gardner et al. (2014).

DNA sampling

In this study, seven specimens labeled as *Ctenomys dorsalis* were examined for the presence of dried tissues adhering to skulls ("crusties"). In addition, dried skin snippets were taken along the ventral incision of three study skins. Extractions and amplifications were successful only for samples originating as snippets, yielding three sequences for FMNH 54391, FMNH 54393, and FMNH 63869 (Genbank accession numbers MH332900, MH332901, and MH332902, respectively). For phylogenetic analysis, 43 complete and seven partial mitochondrial gene sequences encoding for cytochrome-b (*CYTB*) protein were retrieved from GenBank from species of *Ctenomys* documented in previous studies (accession numbers are specified in Supplementary Table S1). This yielded a total of 50 sequences representing 46 species of *Ctenomys*, the outgroup consisted of a single species, *Tympanoctomys barrerae*, representing Octodontidae, the sister family of Ctenomyidae. For the study of intraspecific variation, 79 additional *Ctenomys* sequences from some better-studied species were retrieved from GenBank; accession numbers are specified in Supplementary Table S1.

Sequence acquisition

All steps were performed in a UV-sterilized hood to avoid contamination. As museum specimens are handled often, it was necessary to perform washes before extractions to remove foreign DNA. All these additional steps were performed using Rauri Bowie's modification of the Qiagen DNA easy protocol for contamination control as follows: (1) we placed skin into 1 ml 95–100% EtOH and vortexed at high speed for 30 s; (2) we removed fluid, added 1 ml 70% EtOH and vortexed at high speed for 30 s; (3) we removed the fluid, added 1 ml dH2O and vortexed for 30 s; (4) we removed the fluid, added 1 ml dH2O, and finally we soaked the tissue for 30–45 min (Velazco and Patterson 2013). After rinsing, the DNeasy Blood & Tissue Kit (QIAGEN, Venlo, Netherlands) protocol was followed per manufacturer's instructions for DNA extractions. Because these specimens were more than 70 years old, their DNA was degraded, and it was not possible to amplify complete *CYTB* sequences at once. Amplifications were performed using internal primers, to successfully amplify short fragments of DNA ranging from approximately 200 base pairs (bp) to 400 bp. In total, four overlapping fragments were successfully amplified with four pairs of primers. Primers used were MVZ05 and MVZ16 (Smith and Patton 1993), TUCO23, TUCO37 and TUCO14a (Lessa and Cook 1998), *CYTB* 245 (GATGCTCCGTTGGCATGTA), *CYTB* 761 (GTTGGCAGGGGTGTAGTTGT) and *CYTB* 936 (GGTCGGAATGATATACTGCGT). All primers followed a thermal profile that consisted of an initial denaturing step at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing temperature 47–53°C for 30 s and extension at 72°C for 1 min and a final extension step at 72°C for 10 min. Modified PCR annealing temperatures were made as required for each particular primer. For the new primers, annealing temperatures were performed at 50°C for 30 s for

CYTB 245, a gradient between 43.2 and 49.7°C for 30 s for *CYTB* 761 and 49.7°C for 30 s for *CYTB* 936. Every PCR had to be reamplified (replacing DNA for PCR product) probably due to low concentration of DNA. Cleanup of PCRs was performed by adding 2 µl ExoSAP-IT to each sample and incubating it for 15 min at 37°C and 15 min at 80°C. Cycle-sequencing was carried out for each of the products in both forward and reverse directions using ABI PRISM Big Dye v. 3.1 (Applied Biosystems, Foster City, CA, USA) with a cycling protocol using an initial denaturation step at 96°C for 60 s, followed by 25 cycles of denaturation at 96°C for 10 s, annealing at 50°C for 5 s, and extension at 60°C for 4 min. Cycle-sequenced products were purified through an EtOH–EDTA precipitation protocol and run on an ABI PRISM 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Viewing and editing of sequences was accomplished using Geneious R10 (10.3.2) (http://www.geneious.com, Kearse et al. 2012).

Phylogenetic analyses

We performed five different alignments using the Geneious alignment tool, Muscle (Edgar 2004): in the first alignment, we included all four fragments of each *Ctenomys dorsalis* specimen; in the second one, only the first fragment from each specimen was used; in the third one, only the second fragment was used; in the fourth one, only the third, and in the fifth, only the fourth fragment was employed. This protocol sought to verify that all four fragments yielded the same results, evaluating the possibility of cross contamination or presence of numts. *CYTB* alignments were examined in Geneious for premature stop codons and were used in maximum likelihood (ML) and Bayesian Inference (BI) analyses, missing bases were specified as unknown characters. The best-fitting partition scheme and DNA substitution model was evaluated in PartitionFinder2 (Lanfear et al. 2017), our *CYTB* matrix was partitioned by codon position and MrBayes DNAsubstitution models were evaluated using the *greedy* search algorithm under the Bayesian Information Criterion (Lanfear et al. 2012). Four individual ML search replicate tests with bootstrap supports (BS) of 1000 pseudo-replicated data sets were performed in Garli-2.01-OSX (Zwickl 2006). The best tree topology was chosen and then summarized with SUMTREES v. 4.3.0 (Sukumaran and Holder 2010). We ran four different ML analyses, one for each fragment of *CYTB* aligned individually. For each of the four alignments, we also made four individual ML search reps using Garli-2.01-OSX and selected the one with the best score. We evaluated BS of 100 pseudoreplicated data sets with the same parameters as the four individual searches and finally summarized the best ML tree with the BS values on SUMTREES v. 4.3.0. Bayesian analysis was implemented in MrBayes v3.2.6 (Ronquist et al. 2012). We ran two independent Markov chain Monte Carlo (MCMC) analyses for 10,000,000 generations each, sampling every 1000 generations, including one cold chain and three heated chains. To evaluate convergence, MCMC runs were inspected in TRACER v. 1.7 (Rambaut et al. 2018). The first 10% of trees were discarded as burn-in and the remaining trees were summarized in a Maximum Clade Credibility Tree using TreeAnnotator v. 1.8.4 (Rambaut and Drummond 2016). Both ML and BI analyses were performed through the CIPRES Science Gateway portal (Miller et al. 2010).

Finally, uncorrected *p*-distances were calculated in MEGA7 (Kumar et al. 2015) for species of *Ctenomys* with adequate intraspecific sampling. In total, 81 nucleotide sequences for six species (*C. ibicuiensis*, *C. haigi*, *C. pearsoni*, *C. rionegrensis*, *C. torquatus* and *C. dorsalis*) were analyzed for all 1st + 2nd + 3rd codon positions. There were a total of 1006 positions in the distance dataset.

Results

Morphology

Ctenomys dorsalis is a distinctive species within the genus, characterized by its striking external coloration and by a skull with a broad and heavy rostrum. A detailed redescription of this species is as follow:

Ctenomys dorsalis Thomas 1900

Holotype

"Female. Original Number 255. Collected 7th May, 1900" by John Graham Kerr (Thomas 1900:285) (Figures 1 and 2). The specimen is housed at the British Museum [Natural History Museum, London]. The date of collection was questioned by Contreras and Roig (1992), who suggested that J. G. Kerr had captured this species during his travels in 1889–1891 or 1896–1897. Contreras and Roig (1992) argued that the time period between the date of collection (7th May, 1900) and publication (before the year 1900 ended) was too short for the specimen to have made the

Figure 1: Dorsal, ventral and lateral view of the skin of a female of *Ctenomys dorsalis* (FMNH 54396) from Colonia Fernheim, Boquerón, Paraguay.

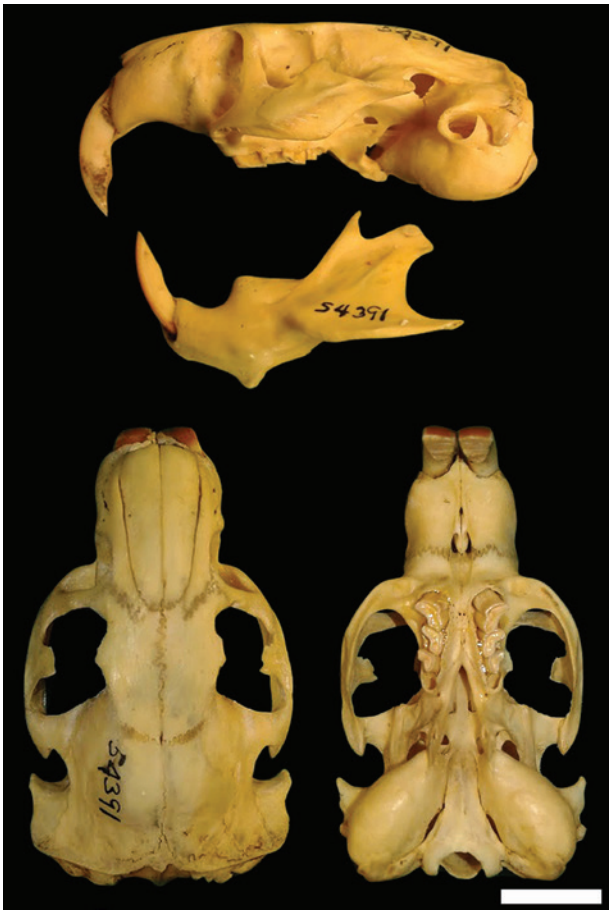

Figure 2: Lateral (above), dorsal (below, left) and ventral (below, right) views of the skull and labial view of the mandible (middle) of a male *Ctenomys dorsalis* (FMNH 54391) from Colonia Fernheim, Boquerón, Paraguay.
Scale = 5 mm.

trip from Paraguay to London. An additional fact is that J. G. Kerr was in London throughout 1900.

Type locality

Imprecisely as "Northern Chaco of Paraguay" by Thomas (1900:285) in the original description; Contreras and Roig (1992), based on the itineraries of its collector, J. G. Kerr, suggested that its type locality is presumably located at the Department of Presidente Hayes (see discussion below) (Figure 3).

Emended morphological diagnosis

A medium-sized tuco-tuco of the *boliviensis* group of *Ctenomys* with dorsal and ventral coloration well differentiated; dorsum shiny buffy fawn, bisected by a striking black dorsal band from the head to the rump; venter pale buffy with gray colored basal hairs; a well-marked pale collar is present behind cheeks and chin extending to each side of ear. Skull strongly built, with a broad

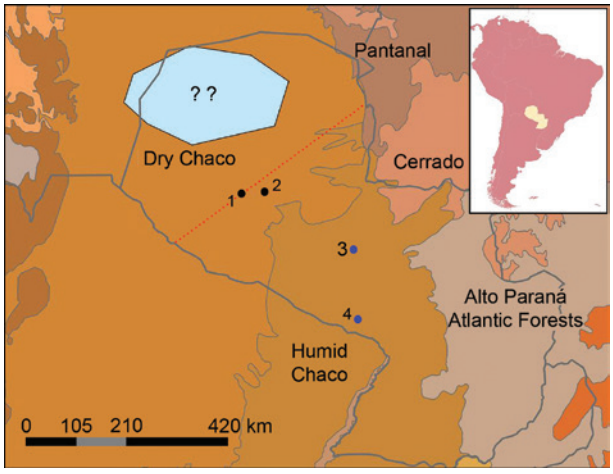

Figure 3: Map of Paraguay and surroundings, showing the newly documented localities for *Ctenomys dorsalis* (1 – Paraguay: Boqueron; Colonia Fernheim, 16 km W Filadelfia; and 2 – Paraguay: Boqueron; Colonia Mennonita, Orloff).
Localities discussed by Contreras and Roig (1992) as possible type localities of this species include: 3 – Misión Inglesa, Waikhtlatingmalyalwa and 4 – a point between Las Juntas and Fortín Page. The dotted line corresponds to the limit between Bolivia and Paraguay according to the Treaty of Benítez-Ichazo (23th November 1894). The suggested distribution for this species proposed by Bidau (2015) is indicated in light blue (? ?). Landscape cover represents the ecoregions according to Olson et al. (2001).

and heavy rostrum; incisors slightly proodont; tympanic bullae moderately inflated (Figures 1 and 2).

Morphological description

The pelage is dense, fine, soft, ~15 mm long over back and rump; the dorsum is shiny buffy fawn; individual hairs dark gray, except for the apical 2–3 mm, which are distinctly paler. A well marked black dorsal band runs from the head just above the nose to the rump; this band is ~10 mm anteriorly, becoming wider (15–20 mm) between shoulders and more diffuse (sometimes even disappearing) near the rump. The cheek and flanks are paler, without a clear separation from the venter. The color of ventral pelage is pale, faint buffy; individual hairs are slaty gray at their base and paler towards its tip. Below each ear there is a patch of pale brownish hairs, which continues to the gular region, forming a "collar". The ears are sparsely covered with short, brownish hairs. The mystacial and superciliary vibrissae are abundant and whitish. The tail is short and brownish, slightly darker above than below. The fore and hind limbs are covered by whitish hairs. The manus and pes are broad and all digits have ungual tufts of stiff bristles and strong claws (Figure 1).

The skull is broadly built and the zygomatic arches are robust. The rostrum is wide, strong and blunt. The nasal bones are short and broad, slightly broader distally. The premaxillary bones are visible in dorsal view and slightly projected behind the posterior end of the nasals. The frontals are planar and wide, with a well-developed postorbital apophysis; the interorbital region has squared margins. The postorbital apophyses of frontals are almost as wide as the rostrum, with the frontals constricting at the level of the sutures of frontals and parietals. The parietals are planar, with their narrowest point at the level of the attachment of the squamosal root of the zygomatic arch. The interparietal is completely fused or extremely small and indistinct in older individuals. The lambdoidal ridges are usually present and well developed. The supraoccipital crest is strongly developed in adults. The zygomatic arch is strongly built, with a well developed postorbital process of the jugal and a moderately developed mandibular apophysis of the jugal. The interpremaxillary foramen is small but conspicuous. The incisive foramina are recessed in a common fossa and separated by a weak bony septum. The palate is narrow, with two palatine foramina at about the level of M1. The mesopterygoid fossa is "V" shaped and extends anteriorly to the level of M3. The posterolateral palatal pits are small. The alisphenoid-presphenoid bridge is flat and ribbon-like. The roof of the mesopterygoid fossa is incompletely ossified, with moderately large sphenopalatine vacuities. The auditory bullae are moderately inflated and pyriform, with salient auditory tubes. The upper incisors are large, slightly proodont and covered with orange enamel. The maxillary tooth rows are posteriorly divergent. The mandible is robust, with the coronoid process falciform and not strongly angled backwards. The condyloid process is strong and squared in lateral view and bears a modestly developed articulation flange (Figure 2).

External and cranial measurements for individual examples are provided in Supplementary Table S2.

Variation

Four adults present whitish vertical belts along the thorax on one (n = 2) or both sides (n = 2). A young individual has a paler coloration with the hairs on the venter entirely whitish and with two (right flank) and one (left) belts of whitish hairs around its thorax (see Supplementary Figure S1).

Karyology and sperm morphology

Unknown.

Morphological comparisons

Ctenomys dorsalis can be recognized as distinct from other species of *Ctenomys* known from western Paraguay and its environs by the following combination of morphological traits: *C. dorsalis* is much smaller (head and body length = 152–200 mm) than *Ctenomys conoveri* (head and body length = 265–568 mm; Anderson et al. 1987) and has smooth pelage compared to the shaggy coat of this later species. *Ctenomys dorsalis* differs from *Ctenomys argentinus*, a species occurring south in the Dry and Humid Chaco of Argentina, by its smaller overall size (mean total body length = 230 mm vs. 259 mm; mean tail length = 64 mm vs. 81 mm; mean TLS = 39.77 mm vs. 43.85 mm) and by a better developed mandibular apophysis of the jugal (Contreras and Berry 1982). *Ctenomys dorsalis* differs from the Bolivian species *Ctenomys andersoni*, *Ctenomys erikacuellarae* and *Ctenomys yatesi* by having a marked black dorsal band from the head to the rump. *Ctenomys andersoni* (CIL = 30.6–50 mm; IOB = 7–11.2 mm) has a brown dorsal coloration, with an almost indistinct olive brown dorsal band, no cap of dark hairs on head or dark collar in gular region. *Ctenomys erikacuellarae* (CIL = 32.9–53.5 mm;

IOB = 8.7–14.1 mm) has well contrasted dorsal and ventral colorations with the dorsum mostly ochraceous orange, except on the upper surface of head and muzzle which is blackish brown; its venter is drab to buffy brown and displays extensive white or light buff markings on inguinal, axillary and/or pectoral regions. Finally, *C. yatesi* (CIL = 31.9–36.7 mm; IOB = 7.1–8.5 mm) is dorsally pale brown, and even paler ventrally, without markings on fur in the gular region.

Comments

Rusconi (1928) restricted the type locality of this species to Resistencia, Chaco province, Argentina. However, as explained by Contreras and Roig (1992), there are no reasons to accept this restriction. Moojen (1952) mapped this rodent in the present-day Chaco Boreal (which was partially claimed by Bolivia at the time the holotype was collected) and suggested it occurred in adjacent areas of Brazil. Other authors produced variations around these references (e.g. Cabrera 1961, Mares and Ojeda 1982, Woods and Kilpatrick 2005). Redford and Eisenberg (1992) depicted two localities in western Paraguay, although without documentation. In their checklist of Paraguayan mammals, Gamarra de Fox and Martin (1996:558) mentioned two localities for *Ctenomys dorsalis*, "Dpto. Boquerón, Colonia Fernheim, 16 km O de Filadelfia" and "Dpto. Boquerón, Colonia Mennonita Orloff", but without giving any other details or reference to specimens or literature. The localities match the ones presented in this work and probably correspond to the same specimens. Bidau (2015) mapped the general area as specified in Thomas' description, although with respect to the modern-day borders of Paraguay. The two localities represented by the FMNH series lie 200 km S of this range (see Figure 3), but still fall within the Dry Chaco of northern Paraguay (see further comments below).

Phylogeny

Our phylogenetic analysis involved 50 mitochondrial sequences representing 47 species, there were a total of 1140 positions in the final dataset. Three sequences, each obtained from four individual fragments, from three individuals (FMNH 54391, FMNH 54393 and FMNH 63869) identified as *Ctenomys dorsalis* were successfully amplified. After trimming, positions for each fragment ranged from 1 to 403, 404 to 675, 676 to 884 and 885 to 1140 for a total of 403 bp for the first fragment, 272 bp for the second, 219 bp for the third one, and 256 bp for the fourth fragment. Final sequences lengths were 1140 bp for FMNH 54391 and FMNH 63869; the sequence for FMNH 54393 had a 14 bp reading gap (positions 885–898) due to low sequence quality. The scheme that best-fitted our data corresponded to three different partitions (i.e. by codon position). The nucleotide substitution model was (Tavaré 1986) GTR + I + Γ for the three codon positions.

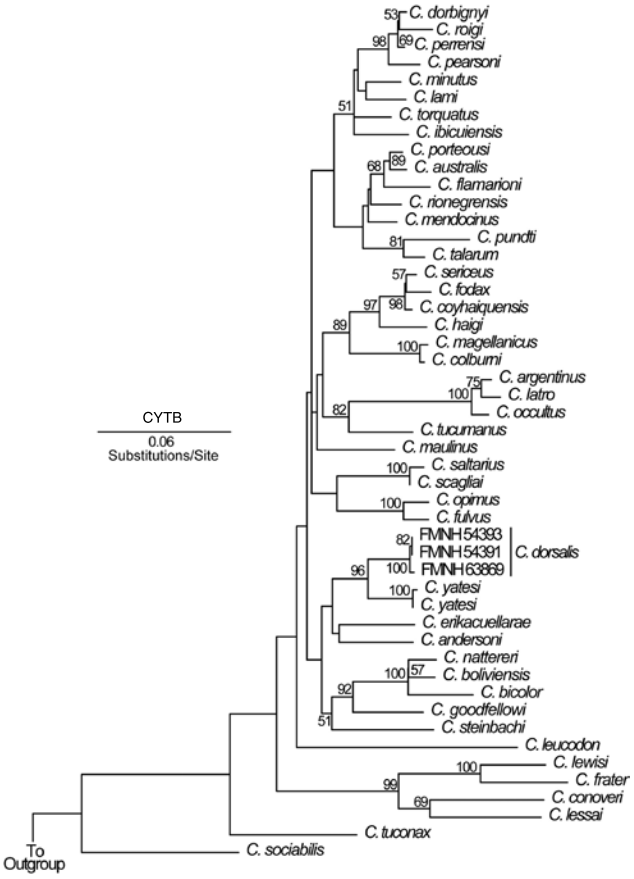

Figure 4: Garli tree inferred from the maximum likelihood (ML) analysis of the 1140 bp Cytb dataset, partitioned by first, second and third codon position, under the GTR + G4 + I model for the three codon positions of sequence evolution.
Node values represent Garli1000 bootstrap replicate percentages (BPs).

Maximum likelihood (Figure 4) and Bayesian (Supplementary Figure S2) analyses of *CYTB* sequence data recovered almost identical topologies, with minor differences in some supporting values and in individual taxon positions. For example, in the BI analysis, *Ctenomys minutus* was not recovered within the *torquatus* group as in the ML topology; instead, it was placed within the *mendocinus* group. The first branching event separates *Ctenomys sociabilis* from the all the other species. *Ctenomys tuconax*, *Ctenomys leucodon*, and *Ctenomys maulinus* remain as unresolved species or species that were

not allied with previously described groups. Broadly, we recovered the same eight *Ctenomys* species groups described by previous authors (Lessa and Cook 1998, Slamovits et al. 2001, Castillo et al. 2005, Parada et al. 2011, Freitas et al. 2012). For the ML analysis, the *boliviensis*, *mendocinus*, *opimus* and *torquatus* groups were recovered with low BS support (<51%), whereas the remaining groups had higher support values: *frater* (99%), *magellanicus* (89%), *talarum* (81%) and *tucumanus* (82%). In the BI analysis, all of the groups were recovered with high support (PP > 0.95) except for the *boliviensis* (0.85) and the *torquatus* (0.22) group. Most of the relationships between terminals have strong support, but internal nodes remain unstable and with low support.

Ctenomys yatesi was recovered as the sister species to *Ctenomys dorsalis* with high confidence values (BS = 96%; PP = 1) and together the two species form a group with *Ctenomys erikacuellarae* and *Ctenomys andersoni*. These four species either compose a new group or belong to the *boliviensis* group; both relationships are poorly supported (BS < 50%; PP = 0.85). All three specimens of *C. dorsalis* were grouped as monophyletic with BS = 100% and PP = 1.

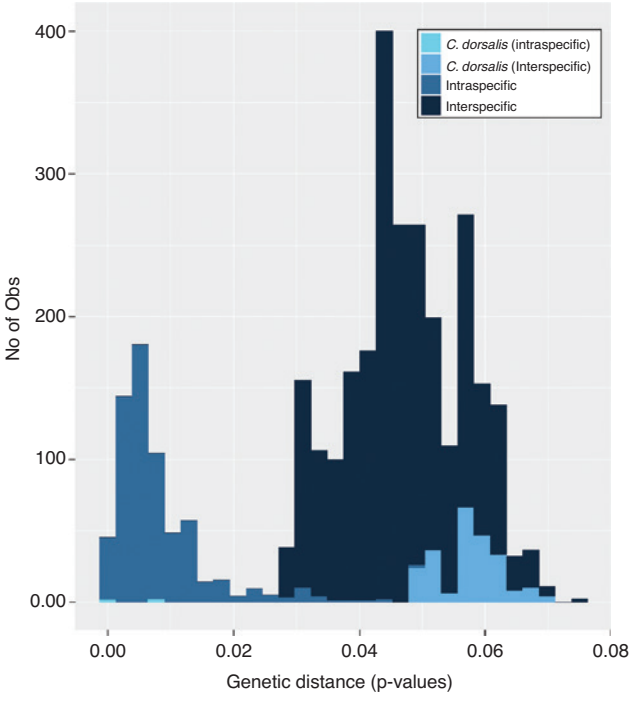

Figure 5: Pairwise distance values for divergence within the genus *Ctenomys*, including interspecific (range 0.029–0.077, with an average of 0.046) and intraspecific (range = 0–0.049, with an average of 0.008) ranges and values for divergence within *C. dorsalis* and between *C. dorsalis* and various other members of the genus.

Analyses of individual fragments (see Supplementary Figure S3) for evaluating the robustness of relationships recovered with the 1140 bp alignment retrieved all eight *Ctenomys* groups, but the topologies are variable and always have low support values. In all fragment trees *Ctenomys dorsalis* is consistently placed as sister species to *Ctenomys yatesi* and together both are sister species to *Ctenomys andersoni* and *Ctenomys erikacuellarae*. Analysis for fragment 1, as an exception, recovered *C. dorsalis* and *C. yatesi* more closely related to the *frater* group, but again with low BS support. In all other three-fragment analyses, *C. dorsalis* and the Bolivian species *C. andersoni*, *C. erikacuellarae* and *C. yatesi* are recovered as sister species to the *boliviensis* group, resembling the complete alignment tree, also with low BS values.

Pairwise genetic distance values suggest the species-level rank of *Ctenomys dorsalis* (Figure 5). Intraspecific divergence of some better-documented *Ctenomys* species ranged from 0 to 0.049 with an average of 0.008; interspecific divergence among these same species ranged from 0.029 to 0.077 with an average of 0.046. Intraspecific and interspecific values involving *C. dorsalis* (plotted separately in Figure 5) both fall within these ranges of values. Intraspecific p-values for the three *C. dorsalis* were 0 between the two samples from the same locality and 0.007 between the different localities. Distances between these *C. dorsalis* and other *Ctenomys* species ranged from 0.049 to 0.071, with an average of 0.057.

Discussion

Ctenomys dorsalis is definitely known only from two localities, Colonia Fernheim and Orloff, located in the Paraguayan Chaco in the Department of Boquerón and 30 km apart, on either side of the town of Filadelfia. The entire area had been converted to cultivated fields and pasturelands by at least the 1920s, when the first Mennonite colonies were established in western Paraguay. The original vegetation corresponds to the Dry Chaco ecoregion, a landscape characterized by a mosaic of thorn tree forest, shrub-filled scrub, arboreal cacti and grassy savannas (Prado 1993, Mereles 2005).

The location of the type locality of *Ctenomys dorsalis* is still unclear. At the time of collection, the "Paraguayan Chaco" was an area considerably smaller than at present, but expanded after the war between Bolivia and Paraguay ("Guerra del Chaco" 1932–1935). Based on the itineraries described by Kerr (1950), Contreras and Roig (1992) hypothesized that the holotype was collected within the

modern department of Presidente Hayes. If their hypothesis is true that Thomas incorrectly reported the date of collection, there are at least two Chacoan localities visited by J. G. Kerr on May 7th, one in 1890 and another in 1897. During 1890, J. G. Kerr traveled between Las Juntas (ca. –24.9167, –58.2500) and Fortín Page (ca. –24.7833, –58.1667), near the modern frontier with Argentina. In 1897, J. G. Kerr worked at the Misión Inglesa in Waikhtlatingmalyalwa (ca. –23.4450, –58.3175). These two localities are ca. 325 km SE and 200 km SE, respectively, of the localities here reported. Suggestively, at this second locality J. G. Kerr collected on May 10th, 1897, the holotype of *Akodon lenguarum* (=*Necromys lenguarum*), which its *terra typica* was referred to by Thomas (1898:272) as "Waikthlatingmayalwa, *Northern Chaco of Paraguay* [italics are ours]". Another plausible line of evidence to address this issue was suggested by Contreras and Roig (1992), based on the local vernacular name "*Sumkum*", given by indigenous people to the animal that Kerr collected (cf. Thomas 1900, p. 385). These authors noted that careful examination of the vocabularies of the various tribes indigenous to the Chaco may help to elucidate its provenance. Coincidently, the word *Soomcon* was included within the vocabulary of the Enlhet people, an indigenous group from the central Chaco that lived around the Misión Inglesa (Unruh and Kalisch 1997). In sum, there are several lines of evidence which suggest that it is entirely possible that J.G. Kerr collected the type of *C. dorsalis* at or near Waikhtlatingmalyalwa, where this researcher spent most of the month of May of 1897.

Part of the series studied in this contribution was taken by Pedro Willim at Colonia Fernheim between the 3th and 8th July of 1945. At this same time and locality, this collector caught the type of *Ctenomys conoveri* (July 8, 1945). This instance agrees with the reference of Krumbiegel (1941), which noted a small tuco-tuco, never identified, in "Colonia Mennonita" (ca. 22°–23°S, 60°–61°W) in the same general area of the new reported specimens. Individuals from Orloff were collected by Jacob Unger, where *C. conoveri* is also known to occur. Ecological mechanisms of their coexistence of course remain unstudied.

Phylogenetic analysis of molecular characters suggest that *Ctenomys dorsalis* could be closely allied to *Ctenomys yatesi*, as part of the *boliviensis* group of *Ctenomys* (sensu Parada et al. 2011; =Bolivian Matogrossense group of Contreras and Bidau 1999). Species within this group are widely distributed through the eastern lowland areas of Bolivia, adjoining portions of southwestern Brazil (Anderson et al. 1987, Gardner et al. 2014) and western Paraguay (this work). This group mostly comprises small to medium-sized species with broad rostra. Although we recovered the same species groups described by previous authors, not all of them were recovered with high BS or PP values and most of the internal nodes have unstable or unresolved relationships. More extensive genomic sampling, including nuclear loci, are needed to resolve poorly supported and unstable nodes, and to have higher confidence in sister species relationships.

Based on the limited data available, the conservation status of *Ctenomys dorsalis* cannot be addressed; it is currently listed as Data Deficient in the International Union for the Conservation of the NatureRed List (Bidau et al. 2008; see also Torres et al. 2017). However, taking into account the various regional changes due to human activities, such as agriculture and cattle grazing (e.g. Caldas et al. 2015) and the reduced area of occurrence of this species, it is possible that this rodent belongs to one of the threatened categories defined by the IUCN. Fieldwork in the Paraguayan Chaco is urgently needed, in order to assess the status of conservation of this species.

Our contribution highlights once more the importance of museum collections as repositories of biodiversity (e.g. Suarez and Tsutsui 2004, de la Sancha et al. 2017b). The samples studied in this work were collected more than 70 years ago and have since been housed at the FMNH, demonstrating with eloquence the long-term benefits and importance of maintaining voucher specimens in natural history collections.

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References

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

Bidau, C.J. 2015. Family Ctenomyidae Lesson, 1842. In: (J.L. Patton, U.F.J. Pardiñas and G. D'Elia, eds.) Mammals of South America, Volume 2 – Rodents. University of Chicago Press, Chicago, IL. pp. 818–877.

Bidau, C.J. and F. Dias de Avila-Pires. 2009. On the type locality of *Ctenomys bicolor* Miranda Ribeiro, 1914 (Rodentia: Ctenomyidae). Mastozool. Neotrop. 16: 445–447.

Bidau, C., E. Lessa and R. Ojeda. 2008. *Ctenomys dorsalis*. The IUCN Red List of Threatened Species 2008: e.T5803A11719812. Available from: http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T5803A11719812.en. [Accessed 10 January 2018].

Cabrera, A. 1961. Catálogo de los mamíferos de América del Sur. Revista del Museo Argentino de Ciencias Naturales "Bernardo Rivadavia", Instituto Nacional de Investigación Ciencias Naturales, Ciencias Zoológicas 4: 308–732.

Caldas, M.M., D. Goodin, S. Sherwood, J.M. Campos Krauer and S.M. Wisely. 2015. Land-cover change in the Paraguayan Chaco: 2000–2011. J. Land Use Sci. 10: 1–18.

Castillo, A.H., M.N. Cortinas and E.P. Lessa. 2005. Rapid diversification of South American tuco-tucos (*Ctenomys*; Rodentia; Ctenomyidae): contrasting mitochondrial and nuclear intron sequences. J. Mammal. 86: 170–179.

Contreras, J.R. and L.M. Berry. 1982. *Ctenomys argentinus*, una nueva especie de tuco-tuco procedente de la Provincia del Chaco, República Argentina (Rodentia, Octodontidae). Hist. Nat. 2: 165–173.

Contreras, J.R. and C.J. Bidau. 1999. Líneas generales del panorama evolutivo de los roedores excavadores sudamericanos del género *Ctenomys* (Mammalia, Rodentia, Caviomorpha, Ctenomyidae). Ciencia Siglo XXI. 1: 1–22.

Contreras, J.R. and A.N.C. Contreras. 1984. Craneología y craneometría del género *Ctenomys*. II. Craneometría. Hist. Nat. 4: 245–248.

Contreras, J.R. and V.G. Roig. 1992. Las especies del género *Ctenomys* (Rodentia: Octodontidae). I. *Ctenomys dorsalis* Thomas, 1900. Notulas Faun. 34: 1–4.

de la Sancha, N.U., C. López-González, G. D'Elía, P. Myers, L. Valdez and M.L. Ortiz. 2017a. An annotated checklist of the mammals of Paraguay. Therya 8: 241–260.

de la Sancha, N.U., S.A. Boyle and B.D. Patterson. 2017b. Getting back to the basics: museum collections and satellite imagery are critical to analyzing species diversity. BioScience 67: 405–406.

D'Elia, G., E.P. Lessa and J.A. Cook. 1999. Molecular phylogeny of tuco-tucos, Genus *Ctenomys* (Rodentia: Octodontidae): evaluation of the *mendocinus* species group and the evolution of asymmetric sperm. J. Mammal. Evol. 6: 19–38.

Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32: 1792–1797.

Fernández, F.A., R. Fornel and T.R.O. Freitas. 2012. *Ctenomys brasiliensis* Blainville (Rodentia: Ctenomyidae): clarifying the geographic placement of the type species of the genus *Ctenomys*. Zootaxa 3272: 57–68.

Freitas, T.R., F.A. Fernandes, R. Fornel and P.A. Roratto. 2012. An endemic new species of tuco-tuco, genus *Ctenomys* (Rodentia: Ctenomyidae), with a restricted geographic distribution in southern Brazil. J. Mammal. 93: 1355–1367.

Gamarra de Fox, I. and A.J. Martin. 1996. Mastozoología. Lista de mamíferos del Paraguay, in O. Romero Martínez, Colecciones de Flora y Fauna del Museo Nacional de Historia Natural del Paraguay. Ministerio de Agricultura y Ganadería, Asunción. pp. 469–573.

Gardner, S.L., J. Salazar-Bravo and J.A. Cook. 2014. New species of *Ctenomys* Blainville 1826 (Rodentia: Ctenomyidae) from the lowlands and central valleys of Bolivia. Spec. Publ. Mus. Texas Tech Univ. 62: 1–34.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Mentjies and A. Drummond. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649.

Kerr, J.G. 1950. A naturalist in the Gran Chaco. Cambridge University Press, Cambridge. pp. i-xv + 1-235 + 1 map + pl. I-XXIV.

Krumbiegel, I. 1941. Die Säugetiere der Südamerika-Expeditionen Prof. Dr. Kriegs. 13. Kammratten (*Ctenomys*). Zool. Anz. 135: 125–133.

Kumar, S., G. Stecher and K. Tamura. 2015. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. Mol. Biol. Evol. 33: 1870–1874.

Lanfear, R., B. Calcott, S.Y.W. Ho and S. Guindon. 2012. Combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29: 1695–1701.

Lanfear, R., P.B. Frandsen, A.M. Wright, T. Senfeld and B. Calcott. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. 34: 772–773.

Langguth, A. and A. Abella. 1970. Las especies uruguayas del género *Ctenomys*. Comun. Zool. Mus. Hist. Nat. Montevideo 10: 1–20.

Leite, Y.L. and J.L. Patton. 2002. Evolution of South American spiny rats (Rodentia, Echimyidae): the star-phylogeny hypothesis revisited. Mol. Phylogenet. Evol. 25: 455–464.

Lessa, E.P. and J.A. Cook. 1998. The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of speciation. Mol. Phylogenet. Evol. 9: 88–99.

Mares, M.A. and R.A. Ojeda. 1982. Patterns of diversity and adaptation in South American hystricognath rodents. In: (M.A. Mares and H.H. Genoways, eds.) Mammalian biology in South America. Pymatuning Laboratory of Ecology, University of Pittsburgh, Pittsburgh. pp. 393–432.

Mereles, M.F. 2005. Una aproximación al conocimiento de las formaciones vegetales del Chaco Boreal, Paraguay. Rojasiana 6: 5–48.

Miller, M.A., W. Pfeiffer and T. Schwartz. 2010. "Creating the CIPRES Science Gateway for inference of large phylogenetic trees". In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA. pp. 1–8.

Moojen, J. 1952. Os roedores do Brasil. Instituto Nacional do Livro Rio de Janeiro. pp. 1–214, pl. 1–35.

Olson, D.M., E. Dinerstein, E.D. Wikramanayake, N.D. Burgess, G.V.N. Powell, E.C. Underwood, J.A. D'Amico, I. Itoua, H.E. Strand, J.C. Morrison, C.J. Loucks, T.F. Allnutt, T.H. Ricketts, Y. Kura, J.F. Lamoreux, W.W. Wettengel, P. Hedao and K.R. Kassem. 2001. Terrestrial ecoregions of the world: a new map of life on Earth. BioScience 51: 933–938.

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

Prado, D. 1993. What is the Gran Chaco vegetation in South America? I. A review. Contribution to the study of flora and vegetation of the Chaco. V. Candollea 48: 145–172.

Rambaut, A., A.J. Drummond, D. Xie, G. Baele and M.A. Suchard. 2018. Tracer v1.7. Available from: http://beast.community/tracer.

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

Ronquist, F., M. Teslenko, P. van der Mark, D.L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M.A. Suchard and J.P. Huelsenbeck. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61: 539–542.

Rusconi, C. 1928. Dispersión geográfica de los tuco-tucos vivientes (*Ctenomys*) en la Region neotropical. An. Soc. Argent. Estud. Geog. GAEA 3: 233–250.

Slamovits, C.H., J.A. Cook, E.P. Lessa and M.S. Rossi. 2001. Recurrent amplification and deletions of satellite DNA accompanied chromosomal diversification in South American tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae): a phylogenetic approach. Mol. Biol. Evol. 18: 1708–1719.

Smith, M.F. and J.L. Patton. 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. Biol. J. Linn. Soc. 50: 149–177.

Suarez, A.V. and N.D. Tsutsui. 2004. The value of museum collections for research and society. BioScience 54: 66–74.

Sukumaran, J. and M.T. Holder. 2010. DendroPy: a Python library for phylogenetic computing. Bioinformatics 26: 1569–1571.

Tavaré, S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. Lectures Math. Life Sci. Amer. Math. Soc. 17: 57–86.

Tatiana Sánchez, R., Ivanna Tomasco, H., M. Mónica Díaz and R.M. Barquez . 2018. Contribution to the knowledge of the rare "Famatina tuco-tuco", *Ctenomys famosus* Thomas 1920 (Rodentia: Ctenomyidae). Mammalia. doi:10.1515/mammalia-2017-0131.

Thomas, O. 1898. Descriptions of new mammals from South America. Ann. Mag. Nat. Hist., ser. 7, 2: 265–275.

Thomas, O. 1900. Descriptions of new rodents from western South America. Ann. Mag. Nat. Hist., ser. 7, 6: 383–387.

Torres, J., S.D. Ríos, J. Sánchez, N.U. de la Sancha and P. Teta. 2017. Rodentia: los roedores. En: Libro Rojo de Mamíferos del Paraguay. Asociación Paraguaya de Mastozoología y Secretaría de Ambiente. pp. 105–118.

Unruh, E. and H. Kalisch. 1997. Moya'ansaeclha' Nengelpayvaam Nengeltomha Enlhet. Biblioteca Paraguaya de Antropología, Vol. XXVII. Ya'alve-Saanga: Comunidad Enlhet. pp. 903.

Velazco, P.M. and B.D. Patterson. 2013. Diversification of the Yellow-shouldered bats, Genus Sturnira (Chiroptera, Phyllostomidae) in the New World tropics. Mol. Phylogenet. Evol. 68: 683–698.

Woods, C.A. and C.W. Kilpatrick. 2005. Infraorder Hystricognathi Brandt, 1855. In: (D.E. Wilson and D.M. Reeder, eds.) Mammal species of the world: a taxonomic and geographic reference, 3rd ed. Johns Hopkins University Press, Baltimore. pp. 1538–1600.

Zwickl, D.J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, University of Texas at Austin.

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