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Disentangling the complex alpha taxonomy of Andean populations of *Ctenomys* (Rodentia: Ctenomyidae) from northern Patagonia: the need for extensive sampling in heterogeneous landscapes

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In the Andean portion of northern Patagonia, populations of *Ctenomys* are found from low-elevation plains to high-elevation meadows and valleys. Despite their prevalence, the taxonomy of these subterranean rodents remains poorly resolved. Using genetic and morphological data obtained from museum specimens and animals collected in the field, we examined the taxonomy of *Ctenomys* from southwestern Mendoza Province, Argentina. Our analyses suggest the presence of at least five species of *Ctenomys* within the study area. The highest, innermost portion of the Andes is occupied by *C. maulinus*. To the east, the mountains and foothills are inhabited by two forms associated with the "*mendocinus*" and the "*magellanicus*" lineages, respectively. The former appears to be a local variant of *C. emilianus*, while the latter is sister to *C. pontifex*. Although *C. pontifex* was not encountered during our field sampling, it remains a valid species that likely is restricted to the isolated Valle Hermoso in westernmost Mendoza Province. In addition, we report an undescribed form from Las Leñas Valley that is associated with the "*mendocinus*" lineage. This complex alpha taxonomic scenario occurs within less than one degree of latitude, thereby highlighting the need to conduct detailed field collections to improve our knowledge of the systematics of *Ctenomys*.

Key words: Andes, Argentina, Chile, *Ctenomys emilianus*, *Ctenomys pontifex*, cytochrome *b*, phylogenetics, systematics, tuco-tuco, Volcán Peteroa

En la parte andina del norte de la Patagonia, las poblaciones de *Ctenomys* se encuentran desde llanuras de baja elevación hasta prados y valles de gran altitud. A pesar de ser frecuentes, la taxonomía de estos roedores subterráneos permanece sin resolverse. Utilizando datos genéticos y morfológicos obtenidos de especímenes de museos y animales recolectados en el campo, examinamos la taxonomía de *Ctenomys* del suroeste de la Provincia de Mendoza, Argentina. Nuestros análisis sugieren la presencia de al menos cinco especies de *Ctenomys* en el área de estudio. La porción más alta e interna de los Andes está ocupada por *C. maulinus*. Al este, las montañas y estribaciones están habitadas por dos formas asociadas con los linajes "*mendocinus*" y "*magellanicus*", respectivamente. La primera parece ser una variante local de *C. emilianus*, mientras que la última es hermana de *C. pontifex*. Aunque no pudimos encontrar a *C. pontifex* durante nuestro muestreo de campo, esta sigue siendo una especie válida que probablemente esté restringida a Valle Hermoso en los Andes más occidentales de Mendoza.

Además, reportamos una forma no descrita asociada con el linaje "*mendocinus*" del Valle de Las Leñas. Este complejo escenario de taxonomía-alfa que sucede en menos de un grado de latitud resalta la necesidad de realizar muestreos de campo detallados para mejorar nuestro conocimiento de la sistemática de *Ctenomys*.

Palabras clave: Andes, Argentina, Chile, citocromo *b*, *Ctenomys emilianus*, *Ctenomys pontifex*, filogenética, sistemática, tuco-tuco, Volcán Peteroa

Characterizing systematic relationships among organisms is essential to understanding their biology, including identifying relevant processes of evolutionary divergence as well as preserving current taxonomic diversity (Jones and Safi 2011; Theodoridis et al. 2020). Resolving systematic relationships and the associated taxonomies typically requires multiple data sets, particularly for lineages that have undergone rapid evolutionary diversification (Sistrom et al. 2013; Argolo et al. 2020). For many mammals, these efforts require additional field sampling to delineate the geographic distributions of distinct taxonomic units.

Despite extensive effort, the taxonomy of the caviomorph genus *Ctenomys* remains poorly resolved for southern Argentina and Chile. Having apparently undergone a rapid burst of speciation, ca. 70 species of *Ctenomys* currently are recognized as valid (Bidau 2015; Freitas 2016; Mammal Diversity Database 2020), with new species accounts continuing to appear (e.g., Teta and D'Elía 2020). The Patagonian region (latitudes 35°–55° south) is home to more than 15 nominal forms, many of which were described during the late 19th and early 20th centuries (Bidau 2015; Freitas 2016). The topographic complexity of this region (large distances, rugged landscapes, complex arrays of geological and climatic conditions), together with the lack of detailed locality data for historical specimens of some species (e.g., Allen 1903; Thomas 1918; Pearson and Lagiglia 1992; Christie and Pardiñas 2016), have made it challenging to resolve the systematics of these animals, with the result that several taxonomic hypotheses that have been proposed require verification (Teta and D'Elía 2020; Teta et al. 2020). To accomplish this, a thorough field sampling effort is necessary to gain an accurate picture of potential geographic variation and species diversity within *Ctenomys*.

To address these challenges and to begin a comprehensive revision of *Ctenomys* in northern Patagonia, we combine analyses of newly collected materials and existing type specimens from Mendoza and Neuquén Provinces, Argentina. This region (latitudes 35°–37° south) includes the type localities for three poorly known species: *C. maulinus*, *C. emilianus*, and *C. pontifex*—the latter two of which are known only from historical museum specimens and have never been included in phylogenetic analyses of the genus. In addition, the study area contains numerous extant populations variously referred to as *C. maulinus*, *C. mendocinus*, or *C. haigi* (Chebez et al. 2014) for which the taxonomic status and systematics remain unknown. Using molecular, morphological, and geographic data, we evaluate the taxonomy of the field-collected and museum specimens examined and we propose a biogeographic framework for understanding the diversity of *Ctenomys* in this region. In addition to generating new insights into extant diversity in this genus, our analyses lay the foundation for future studies aimed at exploring patterns and processes of evolutionary diversification among Patagonian species of *Ctenomys*.

Materials and Methods

Background.—The data used to advance taxonomic hypotheses for *Ctenomys* in northern Patagonia consist primarily of specimens collected during the late 19th and early 20th centuries (Thomas 1918, 1927a; Thomas and Saint Leger 1926). Analyses generally were based on a limited number of individuals, some of which lacked robust locality data. For example, *C. pontifex* was described from a single specimen, originally collected by Thomas Bridges in ca. 1840, that was reportedly obtained on the "east side of the Andes near Fort San Rafael, Province of Mendoza" (Thomas 1918, 40). No further analyses of this species were carried out until the 1980s, when the type locality was questioned by Pearson and Christie (1985). Modern populations of *Ctenomys* from the vicinity of Fort San Rafael appear to belong to *C. mendocinus*, which differs markedly from *C. pontifex* in bullar morphology, leading Pearson and Lagiglia (1992) to argue that the holotype for *C. pontifex* must have been collected at a different location. Based on several lines of ancillary evidence, these authors concluded that the terra typica of *C. pontifex* more likely was located near Volcán Peteroa in Mendoza Province, along the border between Argentina and Chile. At present, the type locality for *C. pontifex* remains uncertain, as does the taxonomic status of the species, which has been variably treated as a junior synonym of *C. maulinus* (Pearson and Lagiglia 1992) or as a valid species (Bidau 2015).

The systematics of extant populations of *Ctenomys* from northern Patagonia also are uncertain. These animals have been referred to as *C. haigi*, *C. maulinus*, and *C. mendocinus*, as well as various combinations of these names (Thomas 1918, 1927a; Thomas and Saint Leger 1926; Yepes 1935; Sage et al. 1986; Tiranti 1996; Pardiñas et al. 2008). This diversity suggests a potentially complex distribution of species in this region, in which the southern "*magellanicus*" and more northern "*mendocinus*" lineages (sensu Parada et al. 2011) may come into contact. The latter is characterized by a complex pattern of morphological and genetic differentiation of populations within Mendoza (Rosi et al. 1992, 2002; Mapelli et al. 2017), suggesting potentially substantial variation among closely related forms for which species boundaries remain undetermined. In addition, this region also is occupied by *C. emilianus*, a large-bodied form erected by Thomas and Saint Leger (1926) based on specimens collected near Chos Malal in Neuquén Province.

Field work and specimens examined.—Most of the specimens included in this study were collected by the authors

during multiple field trips to northern Patagonia, specifically southwestern Mendoza and northwestern Neuquén Provinces, Argentina. This region is dominated by the Andes, which are divided by numerous large rivers and deep valleys. Our sampling program was designed to encompass different altitudinal bands from Monte desert in the eastern foothills of the Andes to alpine portions of the high Andes along the border with Chile (Cabrera 1971). In March 2011, we sampled in the vicinity of Volcán Domuyo and Auca Mahuida in northern Neuquén Province. In February 2015, we sampled at El Nihuil, near San Rafael in Central Mendoza Province. During October 2018 and July 2019, we completed four trips to the upper Río Grande valley and surrounding areas in southwestern Mendoza; this included sampling along the border between Argentina and Chile at two localities: Paso del Planchón and Paso Pehuenche. A list of all sampling localities and their geographic locations is provided in Table 1 and Fig. 1.

Animals were captured using unbaited traps constructed from a length of PVC pipe (30 cm × 7.5 cm) that had been fitted with an acrylic drop door. Traps were set by opening a recently plugged burrow entrance and inserting the trap into the tunnel leading away from that entrance. Traps were checked every 2 h. Captured animals were removed from traps as soon as they were detected, after which individuals were weighed and their sex and reproductive status were determined. In general, the animals captured were euthanized via overdose with isoflurane, after which samples of liver tissue were collected and museum specimens were prepared; a subset of individuals from the upper Río Grande were released at the point of capture after a nondestructive tissue sample had been collected following the procedures of Lacey (2001) and Cutrera et al. (2005). All tissue samples were preserved in 0.5 M EDTA-$DMSO_4$ buffer at ambient temperature until analysis. Specimens collected as part of this study were deposited in the Colección de Mamíferos del Centro Nacional Patagonico (CNP), Chubut, Argentina (Table 1). All procedures involving live animals were consistent with the guidelines of the American Society of Mammalogists (Sikes et al. 2016) for the use of wild mammals in research and were carried out under the auspices of permits issued by the wildlife authorities (Dirección de Fauna) for the provinces of Mendoza and Neuquén.

In addition to specimens collected during this study, we examined historical specimens attributed to *C. pontifex* and *C. emilianus* housed in the Natural History Museum (NHM, London, United Kingdom). The specimens examined included the holotype for each species (NHMUK ZD 1860.1.5.2 and NHMUK ZD 1926.10.11.54, respectively) as well as one additional individual per species (NHMUK ZD 1860.1.5.1 and NHMUK ZD 1926.10.11.61, respectively) belonging to the same original series of specimens. Morphological data (see below) were collected from these specimens using high-quality digital images provided by the NHM. In addition, the NHM provided small samples of dried skeletal muscle (osteocrusts) from two specimens (NHMUK ZD 1860.1.5.1 and NHMUK ZD 1926.10.11.61) for use in molecular genetic analyses.

Genetic analyses.—To place samples collected during this study into a phylogenetic context, we sequenced these materials for the entire 1,140 bp mitochondrial cytochrome b (*Cytb*) locus. Sequencing of the same locus was completed for historical specimens for *C. emilianus* and *C. pontifex*; no previous molecular phylogenies of *Ctenomys* have included these species. In addition, to help resolve taxonomic and systematic relationships for *Ctenomys* from central Neuquén Province, we sequenced fresh tissue from a specimen collected at Laguna Blanca National Park (39°05′S, 70°19′W) that was provided by Richard D. Sage.

Modern specimens.—Genomic DNA was obtained from modern tissue samples (n = 39) using the AccuPrep Genomic DNA Extraction Kit (Bioneer Inc., Korea), following the manufacturer's protocol for mammalian tissue. PCR amplification of the *Cytb* locus was undertaken using two pairs of primers: MVZ05-MVZ16 and MVZ127-MVZ108 (Smith and Patton 1993; Lessa and Cook 1998). Master mix contents and thermocycling conditions were as described by Tammone et al. (2016). DNA extractions and PCR amplifications were carried out in the Laboratory of Genetic Amplification at INIBIOMA (CONICET) at the Universidad Nacional del Comahue, in Bariloche, Argentina.

Museum specimens.—Genomic DNA was extracted from samples of dried skeletal muscle (n = 2) following protocols for samples containing low concentrations of DNA (Gilbert et al. 2005; Mullen and Hoekstra 2008). These extractions were carried out in a room dedicated to this purpose (i.e., free of vertebrate PCR products) maintained by the Laboratorio Ecotono at the Universidad Nacional del Comahue. For each historical specimen, a 25 mg sample of muscle was frozen with liquid nitrogen, then ground with a mortar and pestle. The resulting powder was placed in 1 ml of 1X STE buffer (sodium chloride–Tris–EDTA); an extraction blank (no tissue) was included to allow detection of contamination of extraction reagents or equipment. After 3 h, the STE was removed and each sample was rinsed with Milli Q water, after which DNA was extracted using the same AccuPrep Genomic DNA Extraction Kit and mammalian tissue protocol employed for fresh samples, with the addition of 1.25 times the recommended amount of Proteinase K.

To minimize the risk of contamination while working with historical samples, laboratory instruments and work surfaces were treated with 10% bleach and Milli Q water prior to each use. Per-sample PCR master-mixes were prepared in a UV-sterilized hood; pipettes, tips and tubes were irradiated prior to use. Due to the typically degraded nature of historical DNA, amplification of the *Cytb* locus was undertaken using multiple primer pairs, including primers designed specifically for *Ctenomys*; the primers used were MVZ05-Tuco300R, Tuco86F-Tuco04, Tuco23-MVZ16, and MVZ129-Tuco1042R (Lessa and Cook 1998; Tammone et al. 2016). PCR master mix composition and thermocycling conditions followed Tammone et al. (2016). All reactions included an extraction blank and a negative control.

Table 1.—List of specimens examined

No.	Species	Voucher	Collector	Locality	Locality description	Age–sex	GenBank
1	*C. mendocinus*	CNP 6535	MNT072	Nihuil	Mendoza: El Nihuil, 3.2 km S del embalse	Adult–female	MT787196
2	*C. mendocinus*	CNP 6475	MNT071	Nihuil	Mendoza: El Nihuil, 3.2 km S del embalse	Adult–female	MT787196
3	*Ctenomys* sp. 3	CNP 6537	MNT148	Leñas	Mendoza: RP22, 5.8 km N de Las Leñas	Adult–female	MT787188
4	*Ctenomys* sp. 3	CNP 6538	MNT149	Leñas	Mendoza: RP22, 5.8 km N de Las Leñas	Adult–male	MT787188
5	*C. pontifex*	NHMUK 1860.1.5.1[a]		pontifex	Mendoza: "East side of the Andes near Fort San Rafael"	Adult–unknown	MT787199
6	*C. pontifex*	NHMUK 1860.1.5.2[a]		pontifex	Mendoza: "East side of the Andes near Fort San Rafael"	Adult–female	
7	*C. maulinus*	CNP 6539[a]	MNT144	Valenzuela	Mendoza: Arroyo Valenzuela, 2.7 km NW Arroyo Tordillo	Young–female	MT787184
8	*Ctenomys* sp. 1	CNP 6540[a]	MNT145	Calquenque	Mendoza: Valle Noble, RP226	Adult–male	MT787185
9	*Ctenomys* sp. 1	CNP 6541[a]	MNT125	Calquenque	Mendoza: RP226. 52 km N Las Loicas	Adult–female	MT787193
10	*Ctenomys* sp. 1	CNP 6542	MNT146	Calquenque	Mendoza: 9 km W de Castillos de Pincheira	Young–female	MT787186
11	*Ctenomys* sp. 1	CNP 6543[a]	MNT140	Calquenque	Mendoza: Portillo Calquenque	Adult–male	MT787181
12	*Ctenomys* sp. 2	CNP 6544[a]	MNT151	Pincheira	Mendoza: Pincheira, 6.5 km E Malargüe	Adult–male	MT787190
13	*Ctenomys* sp. 2	CNP 6545[a]	MNT150	Pincheira	Mendoza: Pincheira, 6.5 km E Malargüe	Adult–female	MT787189
14	*Ctenomys* sp. 1	CNP 6546[a]	MNT141	Calquenque	Mendoza: La Valenciana	Adult–female	MT787182
15	*Ctenomys* sp. 1	CNP 6547[a]	MNT142	Calquenque	Mendoza: La Valenciana	Adult–female	MT787181
16	*Ctenomys* sp. 1	CNP 6548	MNT143	Calquenque	Mendoza: Arroyo Toscoso	Young–female	MT787183
17	*Ctenomys* sp. 1	CNP 6549[a]	MNT124	Loicas	Mendoza: RP226, 14 km N Las Loicas	Adult–male	MT787192
18	*Ctenomys* sp. 1	LA13[b]		Loicas	Mendoza: RP226, 9 km ENE Las Loicas	Adult–male	MT787177
19	*Ctenomys* sp. 1	LA8[b]		Loicas	Mendoza: RP226, 9 km ENE Las Loicas	Adult–female	MT787191
20	*Ctenomys* sp. 1	CNP 6550[a]	MNT123	Loicas	Mendoza: RP226, 9 km ENE Las Loicas	Adult–female	MT787191
21	*Ctenomys* sp. 1	CNP 6551[a]	MNT122	Loicas	Mendoza: RP226, 9 km ENE Las Loicas	Adult–male	MT787192
22	*Ctenomys* sp. 1	LA3[b]		Loicas	Mendoza: RP226, 9 km ENE Las Loicas	Young–female	MT787191
23	*Ctenomys* sp. 1	LA14[b]		Loicas	Mendoza: RP226, 9 km ENE Las Loicas	Adult–male	MT787173
24	*Ctenomys* sp. 1	CNP 6552[a]	MNT128	Loicas	Mendoza: RP226, 9 km ENE Las Loicas	Adult–female	MT787177
25	*Ctenomys* sp. 1	CNP 6553	MNT126	Loicas	Mendoza: RP226, 9 km ENE Las Loicas	Adult–female	MT787177
26	*Ctenomys* sp. 1	CNP 6554[a]	MNT121	Loicas	Mendoza: RP226, 9 km ENE Las Loicas	Adult–male	MT787177
27	*Ctenomys* sp. 1	CNP 6555[a]	MNT127	Loicas	Mendoza: RP226, 9 km ENE Las Loicas	Adult–male	MT787177
28	*Ctenomys* sp. 1	LA21[b]		Loicas	Mendoza: RP226, 4.3 km E de Las Loicas	Adult–female	MT787179
29	*Ctenomys* sp. 1	CNP 6556[a]	MNT139	Loicas	Mendoza: RP226, 4.3 km E de Las Loicas	Adult–male	MT787180
30	*Ctenomys* sp. 2	CNP 6557[a]	MNT147	Agua Botada	Mendoza: RN40, 15 km NE Bardas Blancas	Adult–female	MT787187
31	*Ctenomys* sp. 2	CNP 6558[a]	MNT129	Agua Botada	Mendoza: RN40, 15 km NE Bardas Blancas	Adult–female	MT787171
32	*Ctenomys* sp. 1	LA16[b]		Loicas	Mendoza: Portezuelo, 10 km ESE Las Loicas	Young–female	MT787174
33	*Ctenomys* sp. 1	CNP 6559[a]	MNT134	Loicas	Mendoza: Minacar, mallin, 3.4 km S RN145	Adult–female	MT787176
34	*Ctenomys* sp. 1	CNP 6560	MNT133	Loicas	Mendoza: Minacar, mallin, 3.4 km S RN145	Young–male	MT787176
35	*Ctenomys* sp. 2	CNP 6561	MNT135	Bardas Blancas	Mendoza: RN40, 8 km S de Bardas Blancas	Young–male	MT787178
36	*Ctenomys* sp. 2	CNP 6562	MNT136	Bardas Blancas	Mendoza: RN40, 8 km S de Bardas Blancas	Young–female	MT787175
37	*Ctenomys* sp. 2	CNP 6563[a]	MNT137	Bardas Blancas	Mendoza: RN40, 8 km S de Bardas Blancas	Adult–male	MT787175
38	*C. maulinus*	CNP 6564[a]	MNT130	Pehuenche	Mendoza: RN145, 17 km E Paso Pehuenche	Adult–female	MT787172
39	*C. maulinus*	CNP 3621[a]	MNT042	Domuyo	Neuquén: Volcán Domuyo, 13.1 km NE Varvarco	Adult–male	
40	*C. maulinus*	CNP 4798[c]	MNT040	Domuyo	Neuquén: Volcán Domuyo, 10.5 km NE Varvarco	Adult–male	MT787195
41	*C. maulinus*	CNP 3620	MNT041	Domuyo	Neuquén: Volcán Domuyo, 10.5 km NE Varvarco	Young–female	
42	*C. emilianus*	NHMUK 1926.10.11.54[c]		emilianus	Neuquén: "Chos Malal"	Adult–male	
43	*C. emilianus*	NHMUK 1926.10.11.61[a]		emilianus	Neuquén: "Chos Malal"	Adult–female	MT787198
44	*Ctenomys* sp. 4	CNP 3623	MNT039	Auca Mahuida	Neuquén: Auca Mahuida, 2 km NE casa de Guardaparques	Adult–male	MT787194
45	*Ctenomys* sp. 5	RDS 18307		Laguna Blanca	Neuquén: SW Cerro Mellizo Sur. PN Laguna Blanca	Adult–male	MT787197

For each individual, the number of the voucher specimen is given, as is the collection locality, age class, and sex. For individuals collected in this study, the collector number is given. Column "No." refers to the sampling locality numbers in Figure 1. GenBank accession numbers are provided for unique haplotypes generated as part of this study. CNP, Colección de Mamíferos del Centro Nacional Patagonico; NHMUK, Natural History Museum, United Kingdom.
[a]Specimens used in qualitative and quantitative morphological analyses. [b]Non-destructive tissue sample; animal released after capture. [c]Specimens used in qualitative morphological analyses.

Cytb *sequencing.*—For both modern and historical samples, the sizes of PCR products were confirmed via visualization on agarose gels that also contained a known size standard. PCR products in the appropriate size range for a given primer pair were sequenced by Macrogen Inc. (Seoul, South Korea). Amplicons from fresh tissues were sequenced in the forward direction; amplicons from historical specimens were sequenced both in the forward and reverse directions. For historical specimens, replicate amplicons from the same sample were sequenced to provide additional verification of the accuracy of the resulting *Cytb* data.

Molecular phylogenetic analyses.—In addition to the *Cytb* sequences generated as part of this study (27 unique haplotypes, GenBank MT787171–MT787199), we downloaded 51

Fig. 1.—Map depicting the geographic locations of the specimens examined. The location of the study area in northern Patagonia is shown in the upper panel (inset: location in South America). The lower panel provides a more detailed map of the sites sampled as part of this study. Each numbered locality is described in Table 1.

(801–1,140 bp) *Cytb* haplotypes for *Ctenomys* from GenBank. We also downloaded three *Cytb* haplotypes for members of the family Octodontidae (Supplementary Data SD1), which served as outgroups in our phylogenetic analyses. The resulting data set (n = 81 sequences) was used to evaluate phylogenetic relationships among *Ctenomys* from northern Patagonia. To include sequences from historical specimens of *C. emilianus* (540 bp) and *C. pontifex* (821 bp) in these analyses, *Cytb* sequences were trimmed to match the partial sequence obtained from *C. emilianus*, after which the resulting data set (n = 78 sequences) was subjected to phylogenetic analysis; three haplotypes used in our first analysis were excluded from this second analysis due to their failure to align with historical sequences. All *Cytb* sequences were aligned and trimmed using Sequencher (version 3.1.1, Gene Code Corporation, Ann Arbor, Michigan).

Phylogenetic trees were generated using both Maximum Likelihood (RAxML v.8, Stamatakis 2014) and Bayesian inference (MrBayes v. 3.2.7a; Ronquist et al. 2012) algorithms, as implemented in the CIPRES Getaway portal (Miller et al. 2010). Using MrModeltest 2.3 (Nylander 2004) and the Akaike information criterion (AIC), we identified the GTR+I+G model of DNA evolution as the best fit for our data set; this model was used in all subsequent phylogenetic analyses. The maximum likelihood (ML) analysis was run 1,000 times using the rapid Bootstrap protocol, followed by identification of the tree with the best ML score. The Bayesian inference (BI) analysis was run for 10 million generations with sampling every 1,000 generations using one cold and three hot chains. Twenty-five percent of sampled trees were removed as a conservative measure of burn-in, which was assessed using Tracer 1.7 (Rambaut et al. 2018). This analysis was repeated four times using different numbers of initial seeds, after which the convergence of the resulting trees was assessed. Statistical support for nodes was evaluated using posterior probabilities (PPs) for BI reconstructions and bootstrap values (maximum likelihood bootstrap [MLB]) for ML analyses. Values for these metrics are shown on the final Bayesian 50% majority-rule consensus trees for all branches for which the same topology was recovered for both BI and ML trees (see results). We considered clades with PP ≥ 0.95 and MLB ≥ 75% to be well supported (Achmadi et al. 2013; Díaz-Nieto et al. 2016). In contrast, clades with PP < 0.90 and MLB < 70% were considered poorly supported, with intermediate values for these metrics considered indicative of moderate support. Once supported nodes had been identified, we calculated percent sequence divergence (d_{xy}; Nei 1987) among clades and nucleotide diversity (π; Nei 1987) within clades using DnaSP 5.00 (Libardo and Rozas 2009).

Morphometric analyses.—To examine morphometric variation among the putative taxonomic units identified by our phylogenetic analyses (see Results section), we quantified multiple cranial traits for specimens of adult *Ctenomy*s collected in the upper Río Grande (Table 1). As part of efforts to evaluate the taxonomic status of *C. emilianus* and *C. pontifex*, specimens for these species also were included in our morphometric analyses. For individuals collected as part of this study, adults were identified in the field based on body weight and, for females, reproductive status (e.g., perforate vaginal opening). Adult status was confirmed via subsequent examination of the skulls of these individuals, specifically the complete fusion of the sutures between the basioccipital-exoccipital and the exoccipital-supraoccipital bones (Daly and Patton 1986; Verzi et al. 2010). Fontanelles in the frontal and/or parietal bones as well as incompletely fused sutures between the basioccipital–basisphenoid and the basisphenoid–presphenoid bones—typically thought to be indicative of subadults—were found in individuals with larger basilar lengths (i.e., animals typically considered adults; Gardner and Anderson 2001; García Esponda et al. 2009; Verzi et al. 2010), suggesting that these skull features were not reliable indicators of relative age in *Ctenomys*. Accordingly, we focused on the degree of fusion of the occipital bones when using crania to assess the ages of individuals.

A total of 44 measurements were taken from the skull (cranium and dentaries) of each individual examined. Measurements were made using digital calipers with a precision of 0.01 mm. Characters that were bilaterally symmetrical were measured from the left side of the skull unless damage to a specimen required that the right side be used. The variables measured were as follows: upper incisor procumbency (Proc); total length of skull (TLS); basilar length (BL); condyle-basilar length (CBL); nasal length (NL); anterior nasal width (NWa); posterior nasal width (NWp); zygomatic breadth (ZB); mastoid breadth (MB); interorbital breadth (IB); rostral width (RW); braincase width (BW); preorbital foramen breadth (PFB); condyle-PM^4 length (CPM^4L); palatal length (PL); diastema length (DL); bullar length (BulL); bullar width (BulW); bullar height (BulH); incisor width (IW); incisors width (IsW); PM^4 length (PM^4L); upper alveolar length (UAL); skull height (SH); length of frontal bones (LF); length of parietal bones (LP); upper tooth row length (UTL); PM^4-premaxillary length (PM^4PML); basioccipital length (BaL); basioccipital width (BaW); zygomatic length (ZL); length of postglenoid articular space (PgL); mandibular width (MW); mandibular length (ML); mandibular height to pm_4 (MH); mandibular height to alveolus (MH′); lower alveolar length (LAL); lower diastema length (LDL); pm_4 length (pm_4L); condyle-mandibular foramen length (CmfL); condyle length (CL); angular notch length (AnL); coronoid-condyle length (CCL); and angular height (AW). The upper incisor procumbency, as defined by Thomas (1919), was measured from digital images of crania taken in lateral view (Supplementary Data SD2). Descriptions of all measures are given in Supplementary Data SD3. For *C. emilianus* and *C. pontifex*, a subset of these measurements could not be taken with confidence from digital images (see Supplementary Data SD3). In addition, the bullae and occipital bones of the original specimens of *C. pontifex* were damaged; as a result, measurements associated with these cranial elements were not recorded.

Descriptive statistics for a subset of skull characters (Reig et al. 1965; Contreras and Ch. de Contreras 1984) as well as two estimates of body size (body weight [W] and total body

Table 2.—Summary of values for 23 morphological traits (mean ± *SD*, min.–max.) measured from specimens in each of the three clades of *Ctenomys* identified in the upper Río Grande valley, as well as for specimens of *C. emilianus* and *C. pontifex*

Variable	Eastern (5)	Central (14)	Western (3)	*C. pontifex* (2)	*C. emilianus* (2)
W	132.40 ± 25.50	185.64 ± 12.66	170.00 ± 7.93		
	90–230	114–270	155–182		
TL	221.20 ± 8.22	260.42 ± 6.08	252.00 ± 5.13	260.00	286.50 ± 15.50
	206–253	220–300	245–262		271–302
Proc	97.20 ± 0.48	98.32 ± 0.82	103.76 ± 0.37	101.25 ± 1.25	97.50 ± 4.00
	95.50–98.50	94.50–103.80	103.30–104.50	100.00–102.50	93.50–101.50
TLS	39.64 ± 1.23	44.31 ± 0.66	44.64 ± 0.79		47.25 ± 6.75
	37.91–44.43	40.76–48.93	43.18–45.93		40.50–54.00
BL	37.74 ± 1.20	43.03 ± 0.71	42.49 ± 0.83		46.75 ± 6.95
	36.26–42.54	39.72–48.26	41.26–44.09		39.80–53.70
NL	13.15 ± 0.45	15.53 ± 0.32	15.78 ± 0.30	14.75 ± 0.75	17.20 ± 2.70
	12.37–14.87	13.35–17.08	15.31–16.36	14.00–15.50	14.50–19.90
NWa	5.19 ± 0.28	6.72 ± 0.13	6.38 ± 0.36	6.35 ± 0.05	6.95 ± 1.05
	4.67–6.27	5.91–7.62	5.67–6.82	6.30–6.40	5.90–8.00
NWp	3.60 ± 0.23	4.99 ± 0.18	4.54 ± 0.24	5.20 ± 0.20	3.05 ± 0.05
	3.05–4.41	4.05–6.93	4.17–5.01	5.00–5.40	3.00–3.10
ZB	23.04 ± 0.85	26.39 ± 0.30	25.70 ± 0.58	24.10 ± 0.70	28.80 ± 4.60
	21.89–26.42	24.46–28.31	24.56–26.52	23.40–24.80	24.20–33.40
MB	23.68 ± 0.43	26.14 ± 0.28	22.65 ± 0.78		27.70 ± 3.30
	23.03–25.42	24.46–28.34	21.09–23.50		24.40–31.00
IB	7.71 ± 0.21	9.29 ± 0.16	9.10 ± 0.14	8.80 ± 0.00	9.70 ± 1.40
	7.39–8.56	8.51–10.11	8.96–9.39	8.80–8.80	8.30–11.10
RW	8.73 ± 0.27	10.47 ± 0.18	10.49 ± 0.35	10.00 ± 0.10	11.10 ± 1.80
	8.21–9.70	9.44–11.55	10.00–11.17	9.90–10.10	9.30–12.90
BW	16.29 ± 0.44	17.55 ± 0.14	17.65 ± 0.51	16.90 ± 0.10	17.60 ± 1.40
	15.19–17.58	16.88–18.72	17.06–18.69	16.80–17.00	16.20–19.00
PL	16.79 ± 0.52	19.27 ± 0.37	19.62 ± 0.20	19.35 ± 0.65	20.75 ± 3.15
	15.7–18.76	17.24–21.89	19.34–20.03	18.70–20.00	17.60–23.90
DL	9.81 ± 0.45	11.34 ± 0.29	11.80 ± 0.18	11.95 ± 0.15	12.75 ± 2.25
	8.99–11.49	9.87–13.32	11.44–12.00	11.80–12.10	10.50–15.00
BulL	15.36 ± 0.47	16.17 ± 0.24	13.84 ± 0.40		17.45 ± 2.45
	14.63–17.25	14.34–17.8	13.05–14.39		15.00–19.90
BulW	7.64 ± 0.23	7.69 ± 0.10	6.87 ± 0.11	6.35 ± 0.25	8.65 ± 0.65
	6.88–8.32	7.19–8.57	6.72–7.09	6.10–6.60	8.00–9.30
PM^4L	3.06 ± 0.08	3.53 ± 0.03	3.48 ± 0.09	3.05 ± 0.05	3.50 ± 0.40
	2.89–3.38	3.29–3.82	3.38–3.67	3.00–3.10	3.10–3.90
UAL	8.85 ± 0.22	10.01 ± 0.11	10.35 ± 0.16	10.10 ± 0.10	10.32 ± 1.17
	8.25–9.65	9.27–10.88	10.06–10.62	10.00–10.20	9.15–11.50
BaL	6.38 ± 0.14	7.80 ± 0.14	7.38 ± 0.07		7.90 ± 1.20
	6.42–7.32	7.00–8.81	7.29–7.53		6.70–9.10
BaW	1.93 ± 0.20	2.70 ± 0.09	2.50 ± 0.11	2.00 ± 0.10	2.10 ± 0.00
	1.37–2.64	2.00–3.24	2.30–2.71	1.90–2.10	2.10–2.10
PgL	4.21 ± 0.17	4.86 ± 0.20	4.93 ± 0.18		
	3.65–4.65	2.60–5.82	4.63–5.27		
MW	29.38 ± 1.08	32.16 ± 0.49	29.56 ± 0.13		36.70 ± 4.80
	27.65–33.39	29.29–35.93	29.37–29.83		31.90–41.50

For each clade, the number of specimens examined is given in parentheses. A description of each morphological variable is given in the text. All measurements are in millimeters except for mass (W), which is in grams, and procumbency (Proc), which is in degrees. Variables are defined in "Materials and Methods."

length [TL]) are provided in Table 2. Given that sexual dimorphism in body size was evident among individuals collected at the same locality (males were larger than females) and given that the holotype for *C. pontifex* was a female, analyses that included this specimen were restricted to data from adult females. Although the sex of the second specimen of *C. pontifex* was unknown, the size was not noticeably different from that of the holotype. This specimen was included in analyses of females due to the paucity of material available for this species. In contrast, the holotype for *C. emilianus* was a large-bodied male; this specimen was not included in our morphometric analyses. Instead, morphometric data for this species were obtained from the second specimen examined, which was an adult female.

Morphometric variation was assessed using principal component analysis (PCA) with the clades identified by our phylogenetic analyses as grouping variables. All morphological measurements were log-10 transformed prior to PCA analyses to capture information regarding allometric relationships among traits in the first principal component (Strauss 2010; Klingenberg 2016). To maximize the geographic and taxonomic coverage of these analyses, PCAs were run using two different variance–covariance matrices: one matrix for the complete set of morphological variables examined (44 variables, 21 specimens representing both sexes, *C. emilianus* and *C. pontifex* excluded) and one matrix restricted to the 32 variables that were measurable from digital images of specimens of *C. pontifex* and *C. emilianus* (16 specimens, females only,

C. emilianus and *C. pontifex* included). PCA analyses were run using the computer package PAST 4.02 (Hammer et al. 2001).

Qualitative morphological analyses.—We carried out qualitative comparisons of cranial features among the clades revealed by our phylogenetic analyses. Specifically, we examined the following nine cranial features or regions that have been used previously to distinguish species of *Ctenomys* (Thomas 1918; Reig et al. 1965; Contreras and Berry 1982; Pearson and Christie 1985): (i) *upper incisor procumbency*: estimated through the "angle of Thomas", scored as 1 = angle < 100° and 2 = angle > 100°; (ii) *postglenoid articular space* (Pgs): degree to which the auditory capsule is hidden by the Pgs when the cranium is viewed ventrally, scored as 1 = Pgs visible and 2 = Pgs partially or fully hidden; (iii) *fronto-parietal fenestra*: presence or absence of a fenestra at the junction of the frontal and parietal bones, scored as 1 = present and 2 = absent; (iv) *dorsal profile of the cranium*: degree of downward inflection of the parietal-occipital region when the cranium is viewed laterally, scored as 1 = sloping and 2 = flat; (v) *expansion of auditory capsule below auditory meatus*: degree of expansion of the auditory capsule (ectotympanic) bellow the auditory meatus when the cranium is viewed laterally, scored as 1 = minimal and 2 = noticeable; (vi) *development of zygomatic arch*: degree of dorsoventral development of the zygomatic arch close to the suture between the maxillary and the jugal when the cranium is viewed laterally, scored as 1 = broad and 2 = slender; (vii) *maxillary-jugal suture*: shape of this suture, scored as 1 = straight, 2 = irregular, and 3 = acute; (viii) *postorbital process of jugal*: shape of the tip of the postorbital process, scored as 1 = inflected backwards and 2 = not inflected; and (ix) *premaxillary-maxillary suture*: shape of this suture when the cranium is viewed ventrally, scored as 1 = oblique, 2 = straight, and 3 = irregular. Detailed descriptions and illustrations of all qualitative characters are provided in Supplementary Data SD2.

Results

Phylogenetic analyses of Ctenomys from the upper Río Grande.—Complete *Cytb* sequences generated for 39 individuals reveal 27 distinct haplotypes. The complete data set (n = 81 haplotypes) encompasses 326 polymorphic sites, of which 265 are parsimony-informative. Both ML and BI analyses indicate that animals from the upper Río Grande (n = 23 haplotypes) form three clearly distinct clades that we refer to as the western, central, and eastern clades (Fig. 2), characterized as follows:

1. Western clade: specimens from Pehuenche, Valenzuela, and Domuyo form a well-supported clade (PP = 0.98; MLB = 75). Mean nucleotide diversity among western haplotypes is 0.70%; these haplotypes are monophyletic with respect to sequences assigned to *C. maulinus* from Chile (PP = 1; MLB = 100).
2. Central clade: specimens from Loicas and Calquenque form a second highly supported monophyletic clade (PP = 1, MLB = 98) that is associated with the "*mendocinus*" lineage of *Ctenomys* (sensu Parada et al. 2011). Mean nucleotide diversity among sequences from the central clade is 0.54%.
3. Eastern clade: specimens from Bardas Blancas, Agua Botada, and Pincheira, constitute a third, well-supported monophyletic group (PP = 1; MLB = 100) that is part of the "*magellanicus*" lineage (sensu Parada et al. 2011) of *Ctenomys* (PP = 1; MLB = 86). Mean nucleotide diversity among sequences from members of the eastern clade is 0.75%.

The remaining four haplotypes generated as part of this study (localities of El Nihuil, Auca Mahuida, Las Leñas, and Laguna Blanca) each occur in different parts of the tree; all of these sequences fall outside the three clades from upper Río Grande described above. The haplotype recovered from El Nihuil clusters in a polytomy with topotypes of *C. mendocinus* (PP = 0.90; MLB = 64). The mean nucleotide divergence between this haplotype and those of the *C. mendocinus* is 0.59%. The haplotype from Auca Mahuida (Neuquén) is basal (PP = 0.99; MLB = 64) to the subclade containing both *C. mendocinus* and its sister suite of species (*C. australis*, *C. flamarioni*, and *C. porteousi*; D'Elía et al. 1999; Parada et al. 2011) but clearly is most closely associated with the "*mendocinus*" lineage. The haplotype from Las Leñas also appears to be associated with the "*mendocinus*" lineage, although this placement should be considered tentative as it reflects a basal polytomy for this lineage (PP = 0.76; MLB = 43) and it is possible that the Las Leñas population instead represents an undescribed species (see also Mapelli et al. 2017). The sample from Laguna Blanca in Neuquén Province is basal (PP = 1; MLB = 98) to a subclade containing our eastern clade and the newly described *C. bidaui* (Teta and D'Elía 2020) from Península Valdéz (Chubut); these haplotypes are included in the "*magellanicus*" lineage" (PP = 1; MLB = 82).

Estimates of percent sequence divergence (p-distances) between each of the clades identified here and the recognized species of *Ctenomys* with which they are associated range from 0.59% for the Nihuil haplotype versus *C. mendocinus* to > 1% for all other pairwise comparisons (Table 3).

Phylogenetic position of C. pontifex and C. emilianus.—When shorter (540 bp) *Cytb* fragments are considered, 80 distinct haplotypes are recovered; these include 220 polymorphic sites, of which 174 are parsimony-informative. The topologies of phylogenetic trees generated using 540 bp segments of *Cytb* are similar to those obtained from complete *Cytb* haplotypes (Fig. 3). In particular, analyses of partial *Cytb* haplotypes recover the same monophyletic eastern, central, and western clades identified during analyses of complete haplotypes. However, shorter *Cytb* sequences result in reduced resolution of some nodes (e.g., placement of C. *talarum* within the "*mendocinus*" lineage), indicating that shorter fragments are less effective at resolving deeper phylogenetic relationships.

The haplotype obtained for *C. pontifex* (NHMUK ZD 1860.1.5.1) appears to be associated with the eastern clade, albeit it without strong support (PP = 0.56; MLB = 29; Fig. 3). The majority of the trees generated suggest that the *C. pontifex* haplotype is associated with the "*magellanicus*" lineage (PP = 1; MLB = 69; Fig. 3). Mean percent sequence divergence

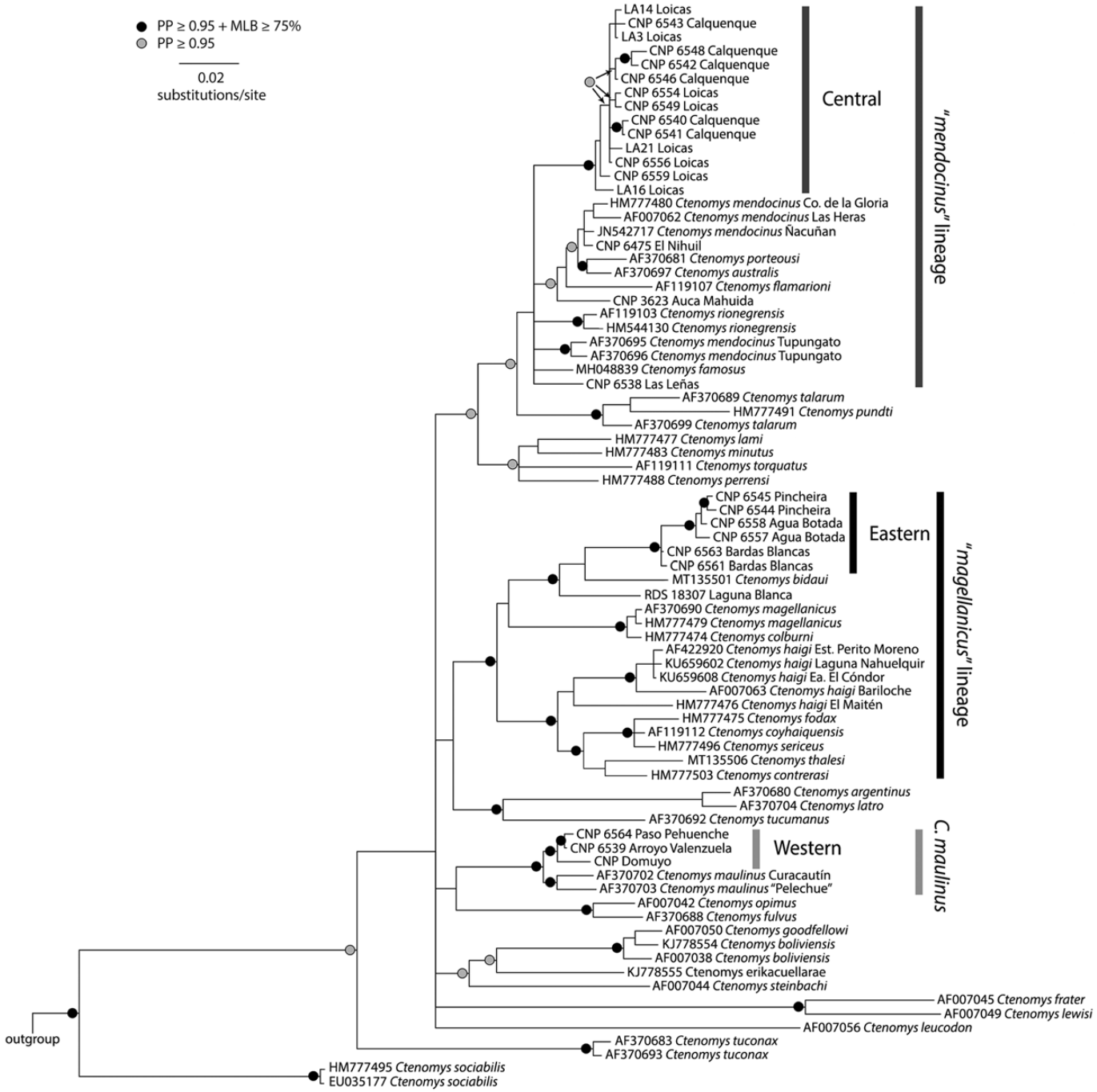

Fig. 2.—Phylogenetic relationships among the *Ctenomys* sampled based on Bayesian inference analyses of *Cytb* haplotypes (801–1,140 bp). Branch support values are posterior probabilities (Bayesian inference analysis) and bootstrap values (maximum likelihood analysis), respectively. Outer vertical bars denote previously identified lineages of *Ctenomys* (sensu Parada et al., 2011). Inner vertical bars indicate the western, central, and eastern clades from the upper Río Grande valley identified in this study.

between *C. pontifex* and the other haplotypes (n = 6) in the eastern clade is 2.5%, corresponding to an average of 13.5 nucleotide differences between pairs of haplotypes. Mean percent divergence decreases to 1.6% when this comparison is repeated using the full 827 bp fragment generated for *C. pontifex*. In contrast, the haplotype obtained for *C. emilianus* (NHMUK ZD 1926.10.11.61) falls within the central clade, with strong support for this placement (PP = 1; MLB = 95; Fig. 3). Mean percent sequence divergence between *C. emilianus* and the other haplotypes (n = 14) in the central clade is 0.7%, corresponding to an average of 4.3 nucleotide differences between pairs of haplotypes. Thus, our analyses indicate that both *C. pontifex* and *C. emilianus* are associated with populations of *Ctenomys* from the upper Río Grande, although placement of

these species differs with respect to the three clades identified by our phylogenetic analyses.

Quantitative analyses of morphological variation.—PCA analyses of all skull measurements (n = 21 adult specimens, 10 males and 11 females) produces clusters of individuals that are consistent with the clades revealed by phylogenetic analyses of *Cytb* haplotypes. Specimens assigned to the eastern, central, and western genetic clades plot separately in morphospace (Fig. 4), providing additional evidence that these subsets of specimens are distinct. The total percentage of variation accounted for by the first two components of the PCA is 80.0%. PC1 (74.0% of total variation) is positively associated with all variables (Table 4). Ordination of specimens along PC1 indicates that members of the eastern clade are typically smaller than those from the other two clades identified (Table 2). Examination of loading coefficients reveals that two variables—basioccipital width and posterior nasal width—are the primary contributors to the ordination of samples along PC1 (Table 4). Ordination of specimens along PC2 (6.0% of total variation) is due primarily to five variables: bullar length, height and width, condyle-mandibular foramen length, and mastoid breadth (Table 4). Each of these variables loads positively on PC2. Ordination along PC2 reveals that relative to the eastern and central clades, members of the western clade are characterized by smaller bullar dimensions and smaller values for other traits associated with the bullae (e.g., smaller mastoid breadth; Table 2; Fig. 4).

The PCA based exclusively on adult females (n = 14), including specimens of *C. emilianus* and *C. pontifex*, does not clearly associate these nominal forms with any of the clades revealed by our phylogenetic analyses (Fig. 4). Although the number of variables included in this PCA is smaller than that used in the PCA described above, both data sets reveal the same morphometrically distinct clusters of individuals corresponding to the eastern, central, and western genetic clades. The total variation explained by the first two components (74.7%) is slightly less than that for the PCA analysis based on the larger morphological data set. PC1 (61.6% of total variation) is positively associated with all variables except bullar width, suggesting that the smaller individuals depicted in the scatterplot for this analysis have relatively larger bullae than do the larger individuals depicted in this plot. Ordination along PC1 is determined by the same variables as in our first PCA analysis, namely basioccipital width and posterior nasal width. Ordination along PC2 (13.1% of total variation) reveals a contrast between *C. pontifex* and the other specimens examined with respect to bullar width, basioccipital width, postglenoid articular space, and condyle length (Table 4), all of which are associated with the degree of inflation of the auditory capsule (Thomas 1918; Verzi and Olivares 2006; Morgan et al. 2017).

Qualitative analyses of morphological variation.—The clades revealed by our phylogenetic analyses also are distinguishable morphologically based on a suite of qualitative cranial traits (Table 5). Specifically, members of the western clade are distinguished by procumbency angles > 100°, a postglenoid articular space that is visible in ventral view, a posteriorly sloping dorsal cranial profile, minimal inflation of the auditory

Table 3.—Mean sequence divergence (%) among cytochrome *b* haplotypes (1,140 bp) sampled during this study as well as haplotypes downloaded from GenBank for known species of *Ctenomys*

	Eastern clade	Central clade	Western clade	Nihuil	Auca Mahuida	Las Leñas	Laguna Blanca	Tupungato	*C. mendocinus*	*C. maulinus*	*C. bidaui*	*C. haigi*	*C. magellanicus*
Central clade	7.60												
Western clade	6.14	5.82											
Nihuil	6.98	3.05	5.11										
Auca Mahuida	7.16	3.43	5.11	1.75									
Las Leñas	6.46	2.89	5.05	2.28	2.63								
Laguna Blanca	4.51	6.62	5.67	5.70	6.05	5.87							
Tupungato	6.81	2.98	5.17	2.36	2.54	2.36	5.70						
C. mendocinus	7.08	3.32	5.27	0.59	1.89	2.55	5.78	2.43					
C. maulinus	6.12	5.92	1.75	5.35	5.26	5.26	5.92	5.35	5.54				
C. bidaui	4.23	5.89	6.11	5.74	5.73	5.48	3.74	5.29	5.73	6.17			
C. haigi	6.51	6.55	5.91	6.59	6.81	6.38	6.01	6.15	6.13	6.72	5.10		
C. magellanicus	5.81	6.57	6.43	6.31	6.31	5.96	4.91	6.14	6.31	6.34	3.99	5.57	
C. sociabilis	11.00	11.40	10.20	10.80	11.10	11.30	10.30	10.40	10.48	10.80	10.78	11.30	11.10

Haplotypes from Tupungato were analyzed separately from the other haplotypes for C. *mendocinus*, as these are suggested to represent a species distinct from C. *mendocinus* (Parada et al. 2011; Mapelli et al. 2017).

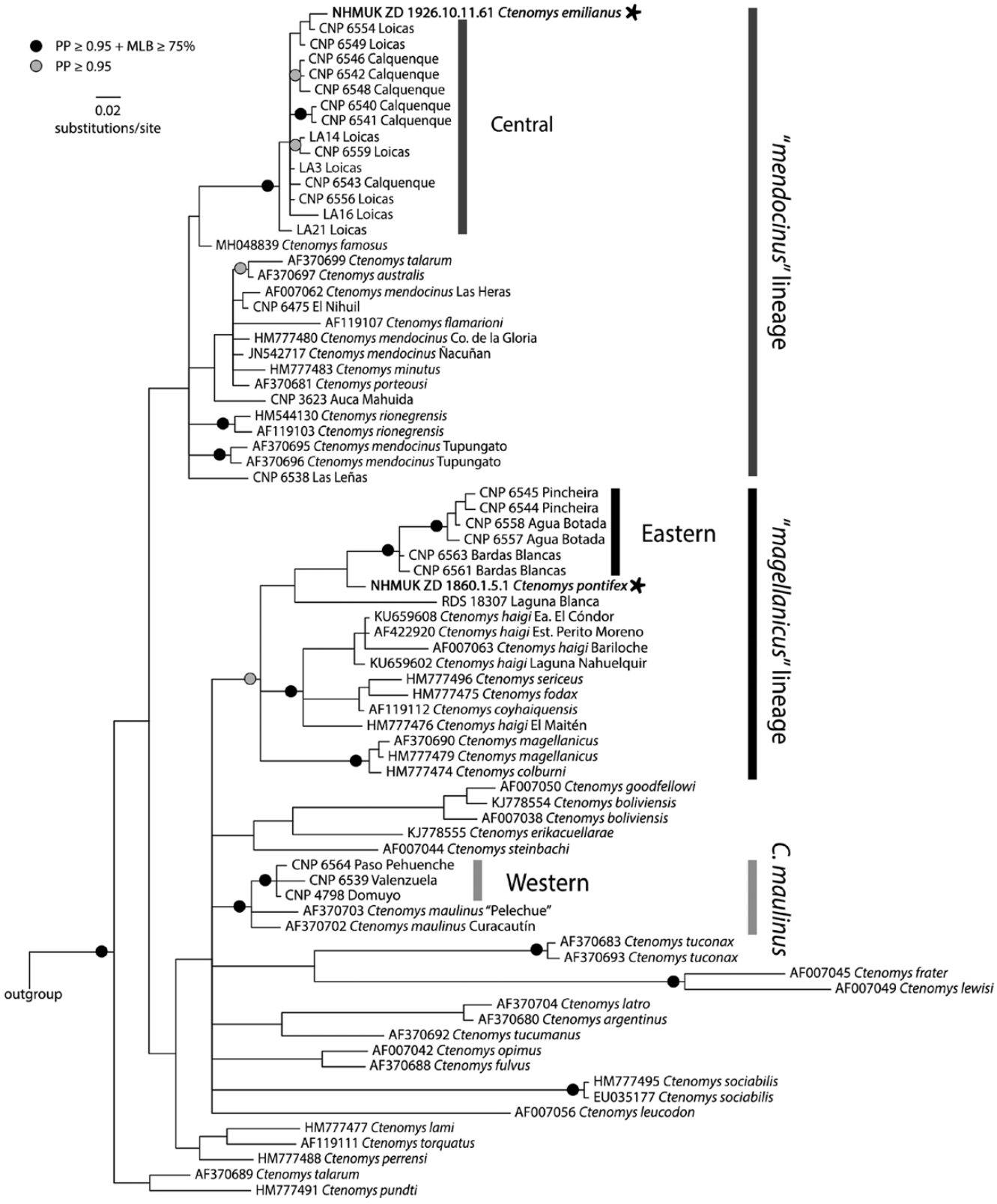

Fig. 3.—Phylogenetic relationships among the *Ctenomys* sampled based on Bayesian inference analyses of partial *Cytb* sequences (540 bp); sequence data were trimmed to match the partial sequences obtained from *C. emilianus*. Branch support values (posterior probability for Bayesian inference analyses and bootstrap values for maximum likelihood analyses, respectively) are indicated for branches containing sequences for *C. emilianus* and *C. pontifex* (denoted in bold "*"). Outer vertical bars denote previously identified lineages of *Ctenomys* (sensu Parada et al., 2011). Inner vertical bats indicate the western, central, and eastern clades from the upper Río Grande valley identified in this study.

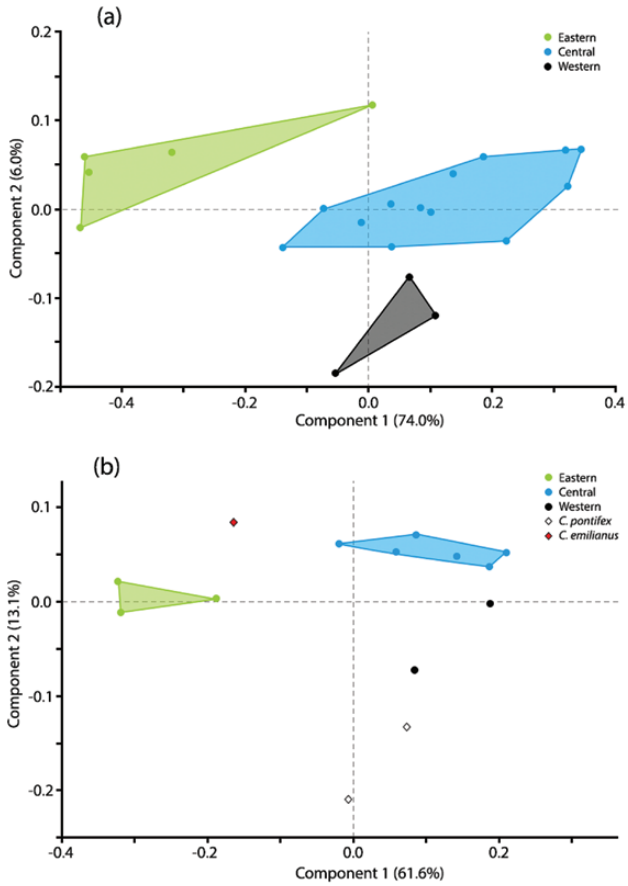

Fig. 4.—Scatterplots of first and second principal component scores for individuals assigned to the western, central, and eastern clades of *Ctenomys* from the upper Río Grande valley. In (a), scores are shown for 21 adult males and females based on analyses of 44 skull variables. In (b), scores are shown for 14 adult females, including specimens of *C. emilianus* and *C. pontifex*, based on analyses of 32 skull variables that could be measured from digital images of museum specimens.

capsule, a straight maxillary-jugal suture, and a backwards-inflected postorbital process of the jugal. In contrast, members of the central clade are distinguished by an acutely shaped maxillary-jugal suture whereas all members of the eastern clade are distinguished by a slender zygomatic arch and an irregularly shaped maxillary-jugal suture. These traits provide clear criteria for assigning individual specimens to one of the genetic clades identified by our phylogenetic analyses.

The characters examined indicate that although *C. pontifex* and *C. emilianus* share some attributes with members of the genetic clades to which they are assigned, each of these named forms shows distinctive qualitative morphological traits. For *C. pontifex*, these distinctive features include a fenestra at the fronto-parietal suture that persists into adulthood as well as a premaxillary-maxillary suture that is obliquely shaped in ventral view (Fig. 5). As noted above, although the two specimens of *C. emilianus* examined were determined to be adults, they vary markedly in size (Fig. 6). These two specimens are consistent, however, with regard to seven of the nine qualitative cranial traits considered (Table 5). In particular, these specimens possess a maxillary-jugal suture that is straight, which contrasts with the acute suture displayed by the other members of the central clade (Fig. 6).

Table 4.—Loading scores for skull traits included in principal component analyses

Variable	Analysis 1 (n = 21)		Analysis 2 (n = 14)	
	PC 1	PC 2	PC 1	PC 2
Proc	0.008	–0.091	0.058	–0.066
TLS	0.125	0.004		
BL	0.139	0.020		
CBL	0.131	0.023		
NL	0.166	–0.041	0.184	0.011
NWa	0.217	–0.051	0.281	0.034
NWp	0.262	–0.124	0.381	–0.371
ZB	0.134	0.036	0.144	0.088
MB	0.091	0.257		
IB	0.164	–0.063	0.176	–0.020
RW	0.170	–0.054	0.169	–0.071
BW	0.063	–0.005	0.114	0.013
PFB	0.139	–0.116	0.166	–0.081
CPM^{4}L	0.113	0.078		
PL	0.150	–0.047	0.127	–0.136
DL	0.182	–0.027	0.152	–0.259
BulL	0.080	0.365		
BulW	0.034	0.316	–0.022	0.324
BulH	0.046	0.271		
IW	0.186	–0.161		
IsW	0.182	–0.099		
PM^{4}L	0.110	–0.112	0.137	0.202
UAL	0.110	–0.151	0.119	–0.104
SH	0.175	–0.048	0.189	0.010
LF	0.117	–0.117	0.102	–0.054
LP	0.017	–0.019	0.011	–0.186
UTL	0.153	–0.037	0.147	–0.132
PM^{4}PML	0.209	–0.092	0.263	0.142
BaL	0.127	0.053		
BaW	0.339	0.029	0.414	0.298
ZL	0.113	0.145	0.091	0.081
PgL	0.161	–0.069	0.122	0.297
MW	0.110	0.181		
ML	0.171	–0.016	0.203	–0.090
MH	0.161	0.013	0.123	0.043
MH′	0.148	0.041	0.127	0.101
LAL	0.118	–0.145	0.150	–0.005
LDL	0.156	0.159	0.104	–0.256
Pm$_4$L	0.080	–0.158	0.119	0.197
CmfL	0.098	0.484		
CL	0.172	–0.164	0.135	–0.362
AnL	0.116	0.193	0.049	0.167
CCL	0.183	0.176	0.227	0.188
AW	0.165	0.071	0.146	0.135
Eigenvalue	0.062	0.005	0.033	0.007
% variance	74.0	6.0	61.6	13.1

Analysis 1 included all variables measured and individuals of both sexes. Analysis 2 included only those variables that could be measured for *C. emilianus* and *C. pontifex*, with analyses restricted to data from females. The eigenvalue and percent variance for the first two principal components are indicated. Sample size for each analysis is shown in parentheses. Variables are defined in "Materials and Methods" and in "Supplementary Data SD3."

Discussion

Our analyses indicate that *Ctenomys* populations from the upper Río Grande in northern Patagonia comprise three distinct clades, each of which is associated with a previously identified lineage within this genus. Genetic and morphological

Table 5.—Occurrence of qualitative cranial features among members of the western, central, and eastern clades of *Ctenomys* identified here as well as in *C. emilianus* and *C. pontifex*

Character/state	Western (n = 4)	Central (n = 13)	Eastern (n = 5)	*C. emilianus* (n = 2)	*C. pontifex* (n = 2)
Upper incisor procumbency					
> 100°	1 (4)	0.3 (4)	0	0.5 (1)	0.5 (1)
< 100°	0	0.7 (9)	1 (5)	0.5 (1)	0.5 (1)
Postgleniod articular space					
Visible	1 (4)	0.1 (1)	0	0	1 (2)
Partially or fully hidden	0	0.9 (12)	1 (5)	1 (2)	0
Fronto-parietal fenestra					
Present	0.5 (2)	0.1 (1)	0.4 (2)	0	1 (2)
Absent	0.5 (2)	0.9 (12)	0.6 (3)	1 (2)	0
Dorsal profile of the cranium					
Sloping	1 (4)	0	0	0	0
Flat	0	1 (13)	1 (5)	1 (2)	1 (2)
Expansion of auditory capsule below auditory meatus					
Minimal	1 (4)	0	0	0	0.5 (1)
Noticeable	0	1 (13)	1 (5)	1 (2)	0
Development of zygomatic arch					
Broad	1 (4)	0.9 (12)	0	1 (2)	1 (2)
Slender	0	0.1 (1)	1 (5)	0	0
Maxillary-jugal suture					
Straight	1 (4)	0.2 (2)	0	1 (2)	1 (2)
Irregular	0	0	1 (5)	0	0
Acute	0	0.8 (11)	0	0	0
Postorbital process of jugal					
Inflected backwards	1 (4)	0.4 (5)	0	0	1 (2)
Not inflected	0	0.6 (8)	1 (5)	1 (2)	0
Premaxillary-maxillary suture					
Oblique	0	0	0	0	1 (2)
Straight	1 (4)	0.7 (9)	0.6 (3)	0.5 (1)	0
Irregular	0	0.3 (4)	0.4 (2)	0.5 (1)	0

For each clade or species, the number of individuals examined is shown in parentheses. Values shown represent frequency and number of individuals in parentheses. Descriptions of each trait are given in the text.

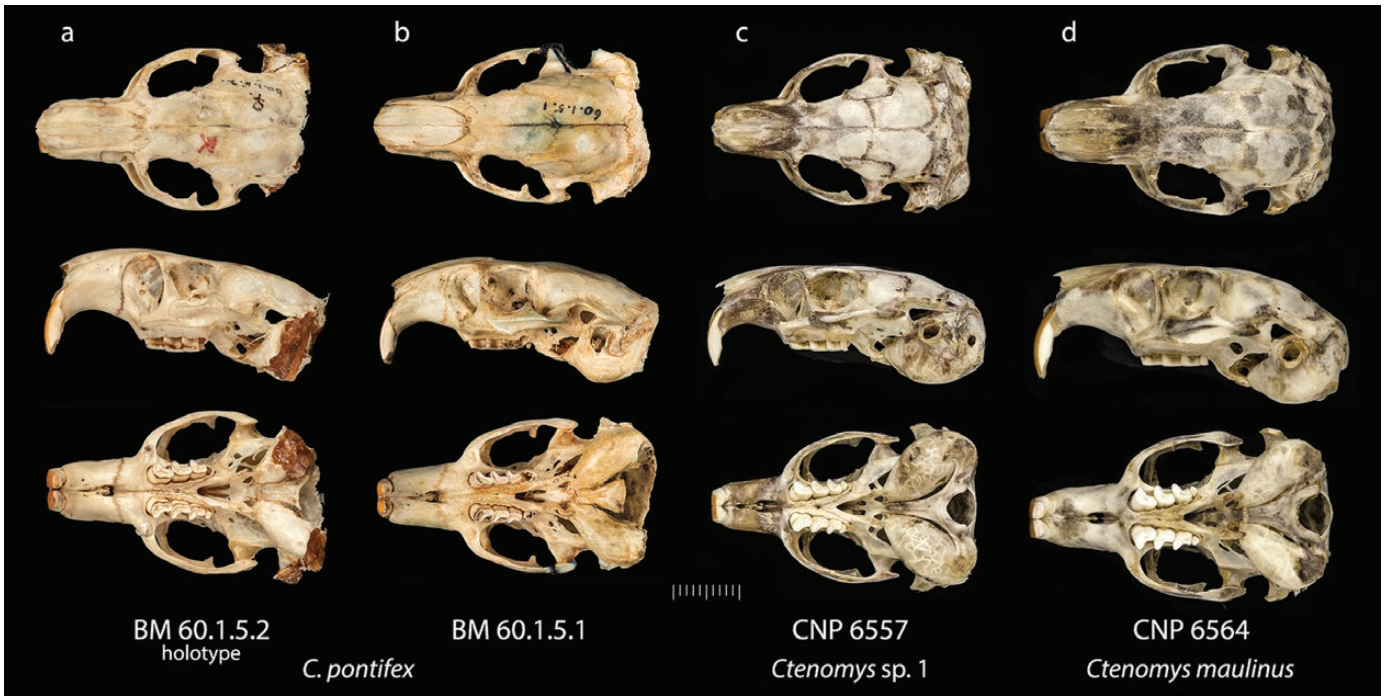

Fig. 5.—Crania from specimens of *Ctenomys pontifex* (a, b) as well as representative specimens from the eastern (c) and western clades (d) identified in this study. Each cranium is shown in dorsal, lateral left, and ventral view. The specimen number for each individual is indicated. Scale bar equals 10 mm.

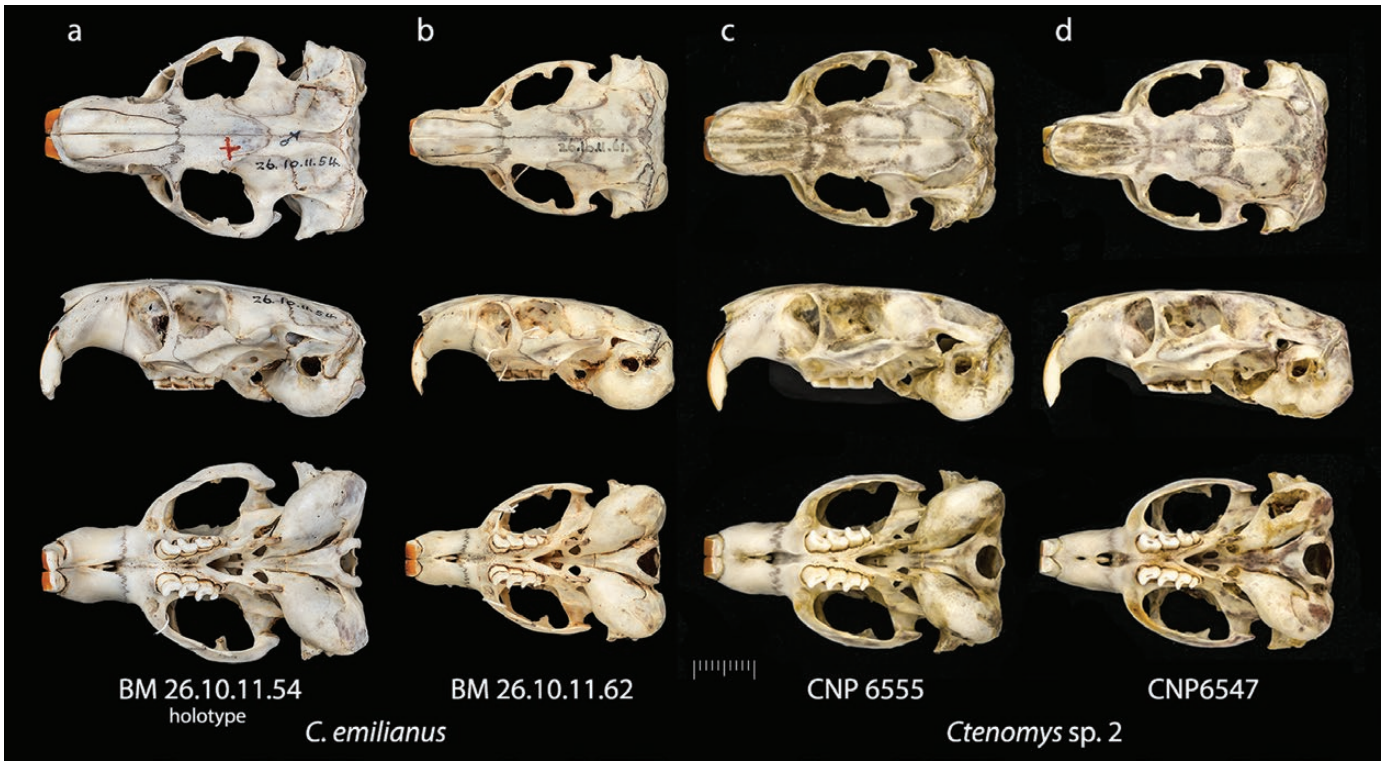

Fig. 6.—Crania from specimens of *Ctenomys emilianus* (a, b) as well as representative specimens from the central clade (c, d) identified in this study. Each cranium is shown in dorsal, lateral left, and ventral view. The specimen number for each individual is indicated. Scale bar depicts 10 mm.

analyses of specimens collected as part of this study were consistent in suggesting that western samples are associated with *C. maulinus*, while central and eastern samples are associated with the "*mendocinus*" and "*magellanicus*" lineages, respectively. Further, by incorporating historical specimens for the poorly known species *C. pontifex* and *C. emilianus* into this phylogenetic framework, our analyses provide important insights into the placement of these taxa within *Ctenomys*.

Systematics of Ctenomys *in southern Mendoza.*—Our analyses of molecular and morphological (quantitative and qualitative) evidence from populations of *Ctenomys* in the upper Río Grande valley and surrounding areas consistently support the occurrence of at least three distinct taxonomic units. To determine if these units correspond to named species or represent new, as of yet undescribed forms, it is necessary to examine previous taxonomic hypotheses advanced for *Ctenomys* in the Andean region between 34° and 40° S.

Western clade.—The western clade identified here clearly is associated with the Maule tuco-tuco, *Ctenomys maulinus* Philippi, 1872. This species first was reported in Mendoza Province by Yepes (1935) under the name *C. mendocinus maulinus*; this designation follows Thomas (1927a, 1927b), who employed this trinomial to refer to populations of *Ctenomys* from northern Neuquén Province. However, subsequent accounts by Pearson (1984) and Pearson and Lagiglia (1992) regarding the status of *C. pontifex* (see below) failed to confirm the occurrence of *C. maulinus* in Mendoza. Thus, the animals collected during this study from Paso Pehuenche and Arroyo Valenzuela represent the first records for *C. maulinus* in Mendoza Province as well as the northernmost records for this species in Argentina and Chile (Bidau 2015, 848). At Paso del Planchón (near Volcán Peteroa on the border between Argentina and Chile), we observed extensive burrow systems of *Ctenomys*, including active and older, inactive tunnels. Although we were unable to capture any individuals at this site, based on the habitat, the proximity to Arroyo Valenzuela, and the morphology of two jaws recovered at this locality, the animals at Paso del Planchón most likely also are attributable to *C. maulinus*. If correct, this extends the distribution of this species to Curicó Province, Chile.

We were not able to compare specimens from the upper Río Grande or from Volcán Domuyo (Neuquén)—the western clade identified by our analyses—with specimens representing the subspecies *C. maulinus maulinus* and *C. maulinus brunneus* Osgood, 1943. The nominotypical subspecies has been reported only from its type locality, Laguna de Maule (Talca Province, Chile; Philippi 1872). In contrast, *brunneus* is more southern in distribution, occurring in Cautín and Malleco Provinces in Chile (Osgood 1943; Greer 1965). According to their original descriptions, these subspecies primarily differ with regard to pelage coloration, with *maulinus* being lighter and *brunneus* being darker brown (Osgood 1943). Given that Paso Pehuenche only is ~ 20 km east of Laguna de Maule and given that the habitat is continuous between these localities, it is probable that the Mendoza populations reported here belong to the nominotypical subspecies. This is consistent with

the taxonomic hypothesis proposed by Pearson (1984), who concluded that the pelage color of western Andean populations of *C. maulinus* more closely matches *C. m. maulinus* (see also Pearson and Christie 1985). This assignation is supported by our molecular analyses, which indicate that the "*maulinus*" group is composed of two well-supported sister clades that differ by 1.75% sequence divergence, which is only slightly greater than the mean intraspecific divergence (1.5%) suggested for the genus (Parada et al. 2011). One of these clades includes a sequence downloaded from GenBank (AF370702) that corresponds to a topotype of *brunneus* from Río Colorado, Malleco Province, Chile (Slamovits et al. 2001). The other clade contains the Argentine populations studied here and includes a quasi-topotype of *C. m. maulinus* (our specimen from Paso Pehuenche). Additional studies are needed to determine if these two monophyletic clades represent subspecies or distinct species.

Although the type locality for *C. maulinus* is in Chile (western Andes), the documented range of this species is primarily in Argentina (Chebez et al. 2014; Bidau 2015). Accordingly, occurrences of *C. m. maulinus* in Chile seem likely to represent penetrations from the eastern side of the Andes that have occurred through low-elevation valleys (e.g., Paso del Planchón, Paso Pehuenche, Paso Pino Hachado). Currently, *C. maulinus* is thought to be restricted to medium- to high-elevation locations between 35° and 40° S, where the animals inhabit a complex mosaic of environments on both side of the Andes, including *Nothofagus* and *Araucaria* forests, bare rocky patches in irrigated valleys, open prairies (southern end of distribution), and highland meadows (northern end of distribution; Bidau 2015). At Paso Pehuenche and Arroyo Valenzuela, the landscape is covered by snow for at least 4 months of the year (Aumassanne et al. 2019); at these localities, we observed subnivean burrows that had been constructed between the soil surface and the snow pack during the previous winter (Supplementary Data SD4). Similarly, Bridges (1843, 132) noted during his trip across the Andes from Curicó (Chile) to "Valle de las Cuevas" (Argentina) that "Whilst residing in the elevated valleys of the Andes, on the eastern side, I observed on the dry slopes of the mountains the labors of a rodent (probably a species of *Ctenomys* or *Poephagomys*) different from any I had previously met with; the chief difference consisted in the mouth of the cave never being left open. Its mode of burrowing is similar to *Poephagomys ater*, in being near the surface; but as I was unfortunately unprovided with traps, I could not obtain one." We suggest that what Bridges recorded was evidence of *Ctenomys*—probably *maulinus*—near Paso del Planchón, in the general vicinity of Volcán Peteroa.

Central clade.—The central clade consists of medium-sized animals that were most abundant in the upper Río Grande valley. Members of this clade construct highly visible burrow systems anywhere that soil conditions are appropriate, from Arroyo Potimalal in the south to Valle Noble in the north (Fig. 1). These animals also occur in high Andean wetlands along the Calquenque roadway that connects the Río Grande and Malargüe, as well as along road RP226 (which follows the Río Grande) to the south, toward Las Loicas (Fig. 1). To the best of our knowledge, there is no previous taxonomic reference to this form in the literature. Both our phylogenetic trees and our estimates of genetic distances strongly suggest that *Cytb* haplotypes in the central clade form a monophyletic group that appears to be associated with the "*mendocinus*" lineage (sensu Parada et al. 2011). Higher-level relationships among several species within this lineage remain unresolved (see below), possibly due to their recent divergence times (Parada et al. 2011, 2012; Mapelli et al. 2017; Sánchez et al. 2019; Leipnitz et al. 2020). Geographically, the central clade appears to be flanked to the west by *C. maulinus*, which occupies high- to mid-elevation Andean environments, and to the east by the eastern clade identified by our analyses (see below), which occurs in the precordillera of the Andes. Although the central clade may represent an undescribed form, the poorly known *C. emilianus* was described from the precordilleran region not far from the Río Grande valley (150 km). This species was identified by Thomas and Saint Leger (1926) based on animals collected by Emilio Budin in the sand dunes along the eastern edge of the Río Neuquén near Chos Malal (Neuquén Province), where it is apparently parapatric or sympatric with a smaller form referred to as *C. haigi* by Tiranti (1996; see also Thomas and Saint Leger 1926). Our phylogenetic analyses reveal a close relationship between the *Cytb* haplotype for *C. emilianus* (NHMUK ZD 1926.10.11.61) and those in the central clade, with genetic distances among these haplotypes falling within levels typical of intraspecific variation within *Ctenomys* (Freitas et al. 2012; Parada et al. 2012).

These analyses are the first to place *C. emilianus*, a species presumed to be endangered or even extinct (Chebez et al. 2014), in a phylogenetic context. If our genetically based hypothesis is correct, then the central clade in our analyses should be recognized as a Mendozan variant of this species. Although the degraded state of the DNA obtained from museum samples prevented us from generating long, high-quality *Cytb* sequences (Cooper 1994; Hofreiter et al. 2001), we found no evidence of contamination or sequencing errors that might have contributed to erroneous placement of *C. emilianus* in the central clade. While morphological data did not fully support this association, intraspecific variation in skull characters has been observed repeatedly in *Ctenomys* (e.g., Rosi et al. 1992; Freitas 2005; García Esponda et al. 2009; Teta and D'Elía 2020), sometimes with pronounced differences evident among local populations (Freitas 2005; D'Anatro and Lessa 2006). As noted above, the two specimens of *C. emilianus* examined differed markedly in skull size. This difference may be due to sex (one male, one female) and/or age (cranial features indicate that the male was older), but demonstrates morphological variation between the only two specimens recognized as *C. emilianus* (see Fig. 6). Although the alternative hypothesis that the central clade represents a novel species should be explored, available evidence leads us to propose that the members of this clade occurring in southwestern Mendoza should be referred to as *Ctenomys* cf. *emilianus*. Additional studies, particularly those involving collection of new material from the region between

the type locality for *C. emilianus* and the upper Río Grande valley should help to resolve this taxonomic conundrum.

Eastern clade.—Members of this clade are distributed along the eastern-most foothills of the Andes from northern Malargüe to southern Bardas Blancas (Fig. 7). Burrow systems are found primarily in sand dunes covered with *Panicum* grass. In addition, comparisons of specimens from the eastern clade with skulls retrieved from owl pellets collected near the western margin of Laguna Llancanelo (Pardiñas et al. 2008) suggest the occurrence of eastern clade populations in this large wetland (Fig. 1). Although Rosi et al. (1992) indicated that a population from Chihuido—geographically part of our eastern clade—can be quantitatively differentiated from *C. mendocinus*, no taxonomic hypothesis has been advanced for the Chihuido animals. Tree topologies for *Cytb* haplotypes strongly support the placement of the eastern clade within the "*magellanicus*" lineage (sensu Parada et al. 2011), although the genetic and morphological distinctiveness of this clade suggest that it may represent an undescribed species. Based solely on genetic data, both the single sample from Laguna Blanca, Neuquén, and the newly described *C. bidaui* from Península Valdéz (Chubut Province) are located sister to our eastern clade; to date, we have not been able to conduct morphological analyses of these specimens and their phylogenetic relationships with both the eastern clade and *C. magellanicus* clearly require further analysis. Thomas (1927b, 202) proposed that all *Ctenomys* from Las Lajas—north of Laguna Blanca—to El Maitén (Chubut Province) be placed under the trinomial *C. mendocinus haigi*, despite noticeable variation in pelage coloration. Our analyses contradict Thomas' hypothesis in that the sample from Laguna Blanca is not associated with typical representatives of *C. haigi* or *C. mendocinus*. Thus, the Laguna Blanca animal may represent a distinct species that requires further analysis, as may numerous populations of *Ctenomys* from Neuquén originally studied by Thomas (1927b).

The *Cytb* fragment obtained from *C. pontifex* revealed that this species is more closely related to the eastern clade and the "*magellanicus*" lineage than to the two other clades reported here for the study area. Although genetically allied with the eastern clade, *C. pontifex* departs morphologically from the former with regard to several cranial features. The most conspicuous is the development of the auditory capsule; as noted by Thomas (1918), this appears to be a diagnostic feature for the species. While it is tempting to equate the eastern clade with *C. pontifex*, several potential concerns must be considered. The first is how representative the specimen analyzed here is of *C. pontifex*. When describing the species, Thomas (1918) did not mention any individuals other than the type specimen. Although this precludes a direct link between the holotype of *C. pontifex* and the specimen sequenced here, several lines of evidence support an association between these materials. First, the catalogue of entries for the NHM (page 277) recorded two specimens referred to as *C. pontifex* during the year 1860: specimen 60.1.5.1 (sequenced as part of this study) and specimen 60.1.5.2 (designated the holotype). These individuals were registered sequentially (original numbers 1 and 2, respectively) and both are listed as coming from "Fort San Rafael." Although both animals originally were annotated as *Ctenomys braziliensis*, the epithet "*pontifex*" was added in the unmistakable handwriting of Thomas (Supplementary Data SD5). In addition, both specimens have two distinct tags attached, one of which is decidedly older and poorly preserved, the other corrected by Thomas (Supplementary Data SD6). These observations support a historical association between these specimens, raising the question of why Thomas (1918) overlooked one of them when describing *C. pontifex*. Second, our inspection

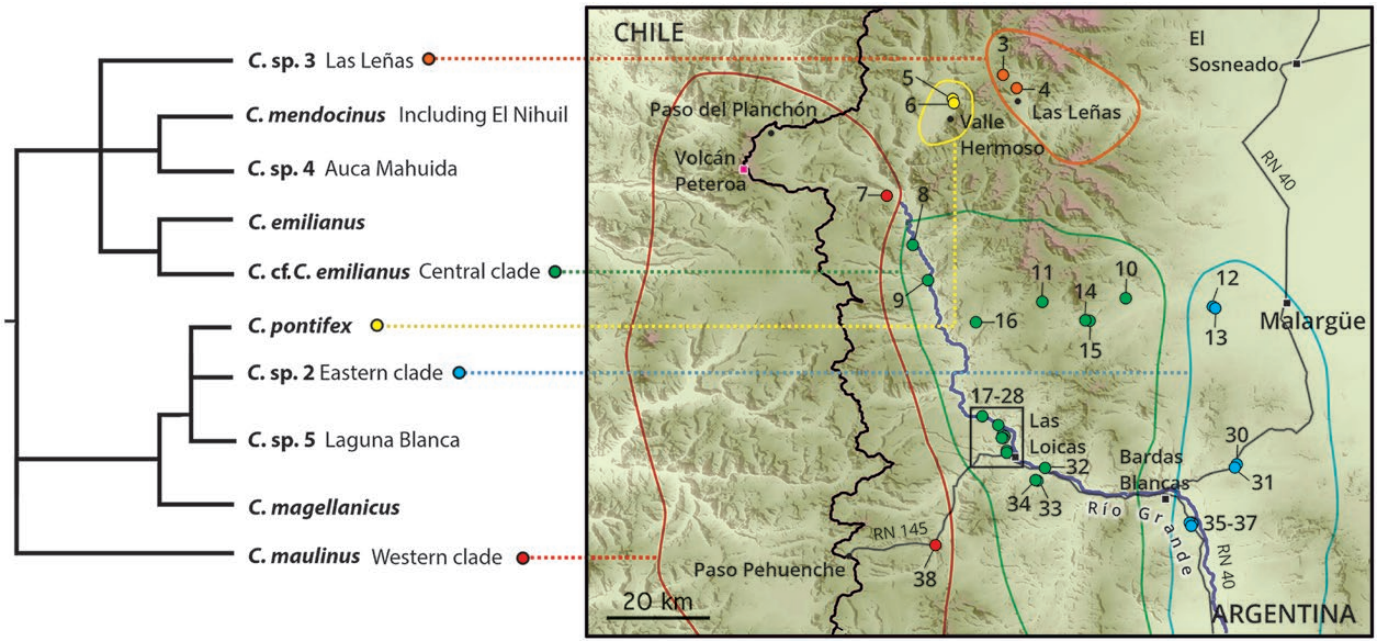

Fig. 7.—Taxonomic hypotheses for *Ctenomys* populations in southwestern Mendoza (Argentina). The map depicts the geographic range (bold line) of the phylogenetic clades identified in this study in combination with a simplified *Cytb* gene tree.

revealed significant morphological similarities between the skulls of both specimens, including the narrow condition of the auditory bullae, a feature highlighted by Thomas (1918) as diagnostic; these similarities extend to other measurements as well. Although we were not able to examine molecular data for the holotype for this species, based on the available evidence we assume that specimen 60.1.5.1 represents *C. pontifex*, with this identity being directly supported by Thomas (in schedis). Finally, the provenance of the holotype for *C. pontifex* is uncertain, with implications for the collection locality for the animal analyzed here. Because this topic was discussed extensively by Bidau (2015; but see Pearson and Lagiglia 1992), we will not reiterate the full series of arguments in detail. To summarize, Pearson and Lagiglia (1992, 38) advanced the hypothesis that the most probable provenance for *C. pontifex* is "near Volcán Peteroa." Our findings do not support this assertion. As indicated above, our analyses indicate that the *Ctenomys* located "near Volcán Peteroa" is *C. maulinus*. Areas immediately to the east of Volcán Peteroa are occupied by members of the central clade; to the north, at least in Las Leñas valley, is a different form of *Ctenomys* (Mapelli et al. 2017; see below). Neither of these forms is genetically associated with *C. pontifex*.

Despite these concerns, we can draw several conclusions regarding *C. pontifex*. First, it appears to be a morphologically diagnosable unit. Second, it is genetically associated with the "*magellanicus*" lineage and the eastern clade identified by our analyses. Although we do not know where between the Argentine–Chilean border and Fort San Rafael the original specimens were collected, our data suggest that *C. pontifex* does not occur near Volcán Peteroa and that it is not a junior synonym of *C. maulinus*, as suggested by Pearson and Lagiglia (1992). We propose that *C. pontifex* is a valid species occurring in still poorly sampled valleys in the Andes of Mendoza. If correct, this increases to five the minimum number of species of *Ctenomys* occurring within less than one degree of latitude. The heterogeneity of habitats in this region seems capable of supporting such diversity and several valleys situated between Arroyo Valenzuela and Las Leñas—including the well-known Valle Hermoso (see Ojeda et al. 2005)—remain unexplored with respect to *Ctenomys*.

An intriguing potential parallel can be found in historical accounts for *Aconaemys fuscus* (Waterhouse 1841 [1842]), which was collected by Thomas Bridges during the same trip as *C. pontifex*. The type locality for *A. fuscus* was recorded as "Valle de las Cuevas, on the eastern side of the Andes, about six leagues from the slopes of the volcano of Peteroa, at an elevation of from 5-7000 feet, in S. lat. 35°" (Bridges 1843, 130). The specific valley referred to by Bridges remains unknown; the name "Las Cuevas" likely was coined by Bridges to highlight the myriad burrow systems that he encountered there. Like *C. pontifex*, *A. fuscus* has not been collected since in this region and the original record from Bridges is the only known material from Argentina (Verzi et al. 2015). This contributes to our speculation that both rodent taxa still are present in an unsampled Andean valley. This location could be Valle Hermoso, which is 30 km (about six leagues) to the east of Volcán Peteroa. Equating one league to ~ 5 km, this distance is consistent with Valle Hermoso being the location for the Valle de las Cuevas of Bridges; unfortunately, this location was not surveyed as part of our field sampling. Alternatively, given that almost two centuries have elapsed since Bridges collected his specimens, it is possible that these taxa have gone locally extinct due to environmental changes or any of several natural catastrophes that have affected the region (e.g., the eruption of Volcan Quizapu in 1932 buried the region with over a meter of ash; Rovere et al. 2012). Clearly, increased field collection in this poorly sampled region is required.

Relationships within the "mendocinus" lineage.—Consistent with previous studies, our molecular analyses indicate that within the "*mendocinus*" lineage, relationships among putative species still require resolution (e.g., Parada et al. 2011, 2012; Mapelli et al. 2017; Sánchez et al. 2019). Tree topologies and the minimal sequence divergences (< 1%) among members of this lineage indicate that the population of *Ctenomys* sampled at El Nihuil (Mendoza) can be referred to as *C. mendocinus* Philippi, 1869. At El Nihuil, extensive populations of this form inhabit sand dunes covered with sparse Monte desert vegetation along the eastern margin of the El Nihuil dam, in close proximity to previously reported localities for *C. mendocinus* (Rosi et al. 2002, 2005; Parada et al. 2012).

Based on PP (but not MLB) support, the haplotype from Auca Mahuida appears to be sister to the clade composed of *C. australis*, *C. flamarioni*, *C. mendocinus*, and *C. porteousi*. Published analyses also include *C. azarae* in this group (Mapelli et al. 2017). Both this phylogenetic position and the degree of sequence divergence detected suggest that the Auca Mahuida population may be an unnamed species from northeastern Neuquén Province. However, given that our analyses did not include *C. azarae* (found in central La Pampa Province) and given that Auca Mahuida is only represented by a single specimen, any taxonomic conclusion regarding the latter would be premature. Based on chromosomal and molecular evidence, it has been proposed that *australis*, *azarae*, and *porteousi* should be synonymized to a single polytypic form, *C. mendocinus* (Massarini et al. 1991; Parada et al. 2012; Mapelli et al. 2017). Given this latter taxonomic arrangement and given that Thomas (1927b) remarked on the morphological similarities among *C. azarae*, *C. mendocinus*, and other *Ctenomys* from northeastern Neuquén, it is likely that the Auca Mahuida animals also belong to *C. mendocinus*. Karyotypic data from the Auca Mahuida population would provide valuable complementary information that can be used to test this hypothesis.

Although our phylogenetic analyses failed to resolve relationships at the basal node of the "*mendocinus*" lineage, it is possible that multiple populations of *Ctenomys* from the Andean portion of Mendoza represent one or more as yet undescribed species (e.g., samples from Tupungato, Arenales, and Las Leñas, as well as the members of the central clade discussed above; Parada et al. 2011; Mapelli et al. 2017). The occurrence of numerous Andean and precordilleran mountain ranges that are divided by often deep valleys lends support to this hypothesis, as these physical barriers may have contributed

to isolation of populations and allopatric speciation in this region. The specimens from Las Leñas Valley examined here provide an important potential example of these patterns; these animals were found to be morphologically and genetically divergent from the other populations of *Ctenomys* analyzed. Exploration of additional molecular makers as well as analyses of larger numbers of specimens will enhance our knowledge of the taxonomic status of these populations.

Biogeographic significance.—In addition to the systematic impacts of our analyses of taxonomic and phylogenetic relationships among *Ctenomys* from southern Mendoza Province, our results reveal a complex pattern of spatial interactions among lineages of *Ctenomys* from Patagonia and the Monte desert (Fig. 7). Although not previously documented for these rodents, this complexity is not unexpected. Southwestern Mendoza forms an ecotonal region between Patagonian and Andean domains; this interface is reflected in the diverse composition of extant communities of several vertebrate lineages (Roig 1962; Contreras and Rosi 1980; Pardiñas et al. 2008; Fernández et al. 2011). Although a thorough treatment of the biogeographic history of the region is beyond the scope of this study, several observations relevant to our analyses deserve mention. First, *Ctenomys* associated with Patagonia (i.e., "*magellanicus*" lineage) enter southern Mendoza via the precordilleran ranges. Second, the marked topographical and environmental heterogeneity of this region suggests that species limits are likely to be complex and interdigitated. Finally, multiple large and environmentally distinct tracts (e.g., the basaltic plateau of Payunia, which occupies almost all southeastern Mendoza) remain unstudied with regard to the systematics of *Ctenomys*.

Concluding remarks.—Here, we present evidence that southwestern Mendoza Province is a region of high diversity for *Ctenomys*. We suggest that this reflects the diverse array of habitats created by the Andes mountains, their foothills, and the open plains to the east. The resulting isolation of local populations, particularly those in the deep valleys of the high Andes, has likely favored allopatric speciation among these subterranean rodents. The morphological and genetic variation revealed by our analyses underscores the importance of designing field sampling strategies that emphasize locally dense efforts. Campaigns that cover large, heterogeneous regions can provide an important first assessment of relationships among *Ctenomys* (e.g., Parada et al. 2011), but more targeted sampling is required to resolve systematic issues and to explore the processes contributing to taxonomic diversification. Although our analyses suggest that the mitochondrial *Cytb* locus remains useful for systematic research, we assert that multilocus and genomic phylogenies in conjunction with morphological evidence are required to establish robust relationships within *Ctenomys* (Parada et al. 2011; Caraballo and Rosi 2018; Leipnitz et al. 2020). Two recent studies have named new species and subspecies of *Ctenomys* based primarily on monophyletic clades revealed by *Cytb* phylogenies (Gardner et al. 2014; Teta and D'Elía 2020). Patterns of diversification evident in individual gene trees, however, may not reflect actual relationships among species (Rokas and Carroll 2005; Maddison and Knowles 2006; Waters et al. 2010); this problem may be particularly acute for organisms such as *Ctenomys* that have undergone recent, rapid diversification (Parada et al. 2011; Mapelli et al. 2017). In all cases, taxonomic hypotheses should be adequately contextualized (e.g., geography, ecology) and validated using multiple data sets to avoid potentially misleading taxonomic decisions. Future studies of species-level diversity in *Ctenomys* from northern Patagonia will benefit from inclusion of phylogeographic and population genetic analyses. Improved understanding of patterns and processes of diversification in these animals should, in turn, help to inform decisions aimed at conserving this diversity.

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Supplementary Material

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—List of *Cytb* haplotypes from *Ctenomys* that were downloaded from GenBank to perform phylogenetic analyses.

Supplementary Data SD2.—Descriptions of qualitative cranio-dental traits used to examine geographical variation in *Ctenomys*.

Supplementary Data SD3.—Abbreviations and descriptions of the morphological measurements used in this study.

Supplementary Data SD4.—Subnivean burrow systems constructed by *C. maulinus* near Paso Peheunche and Valenzuela, Argentina.

Supplementary Data SD5.—Photo of the accession record for *Ctenomys pontifex*. The image is of page 277 of the accession log from the British Museum of Natural History, London.

Supplementary Data SD6.—Photos of the specimen tags from the two individuals of *Ctenomys pontifex* included in this study.

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