Journal of Mammalogy, 91(6):1449–1458, 2010

Phylogeography of *Loxodontomys micropus* with comments on the alpha taxonomy of *Loxodontomys* (Cricetidae: Sigmodontinae)

Carola Cañón, Guillermo D'Elía,* Ulyses F. J. Pardiñas, and Enrique P. Lessa

Programa de Magíster en Ciencias, mención Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, casilla 160-C, Concepción, Chile (CC)
Instituto de Ecología y Evolución, Facultad de Ciencias, Universidad Austral de Chile, Valdivia and Centro de Investigación en Ecosistemas de la Patagonia, Coyhaique, Chile (GD)
Unidad de Investigación Diversidad, Sistemática y Evolución, Centro Nacional Patagónico, CC 128, 9120, Puerto Madryn, Chubut, Argentina (UFJP)
Laboratorio de Evolución, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay (EPL)

* *Correspondent: guillermo@fcien.edu.uy*

Loxodontomys micropus is a rodent that is widely distributed in Andean and Patagonian Argentina and Chile. This range covers a heterogeneous area that has been influenced by geologic and palaeoclimatic events, such as the glaciations during the Neogene. To investigate the genetic structure, phylogeographic pattern, and biogeographic history of this sigmodontine rodent we analyzed a 801-base-pair fragment of the mitochondrial genome (cytochrome-*b* gene) of 87 specimens from 24 localities from Argentina and Chile. Results indicate that *L. micropus* has a shallow genealogy that is geographically structured and is a taxon characterized by an historical population expansion. We discuss the distribution of the genetic variation of *L. micropus* in relation to population history and the concordance with other codistributed sigmodontine rodents. On the basis of molecular evidence, we suggest that the *L. pikumche*, corresponding to the second extant species of the genus, could be a junior synonym of *L. micropus*. DOI: 10.1644/10-MAMM-A-027.1.

Key words: Andes, cytochrome b, genetic structure, *Loxodontomys pikumche*, Patagonia, phylogeography

Current species distributions are, in part, a result of changes and fluctuations in historical processes. The nature of these processes can be inferred from the study of current patterns of genetic variation within and among populations (Avise 2000). In this sense, southern South America is a geographic area of great interest because during the Neogene, it experienced a complex palaeoclimatic and geomorphological history. These include the uplift of the southern Andes, starting at the beginning of the Miocene (~23 million years ago [mya]) and continuing to the present (Ramos 1989), and several glaciations during the Pliocene–Pleistocene (~5 mya to 18,000 years ago) that produced multiple landscape and sea-level shifts. Geological evidence suggests that during the last glacial maximum (LGM) glaciers covered most of southern Chile and adjacent Argentina (Rabassa 2008), and extended to the north as a single continuous unit along the higher elevations of the Andes to approximately 38°S (Hulton et al. 2002). These events would have promoted the genetic differentiation of the biota of southern Argentina and Chile (Allnutt et al. 2003; Hinojosa and Villagrán 1997; Lessa et al. 2010; Morando et al. 2007; Palma et al. 2005; Rodríguez-Serrano et al. 2006; Ruzzante et al. 2008).

Loxodontomys micropus (Waterhouse 1837), the southern pericote, is a medium-size sigmodontine rodent that occurs in forest, shrubs, and forest–steppe shrub ecotonal areas along the Andes of Argentina and Chile from about 35°S to the Strait of Magellan, including Chiloé Island (Pardiñas et al. 2008; Teta et al. 2009; Fig. 1). Most of this distribution is in the Patagonian region. In the last decade owl pellet analyses that have uncovered its presence in isolated populations to the east of the main range have been found in the Patagonian steppes of the Argentinean provinces of Chubut, Río Negro, and Santa Cruz (Teta et al. 2002; Udrizar Sauthier 2009; Udrizar Sauthier et al. 2008). Additionally, fossils have been found in archeological deposits of the Pleistocene and Holocene in Argentinean and Chilean Patagonia (Pardiñas 1998; Pearson

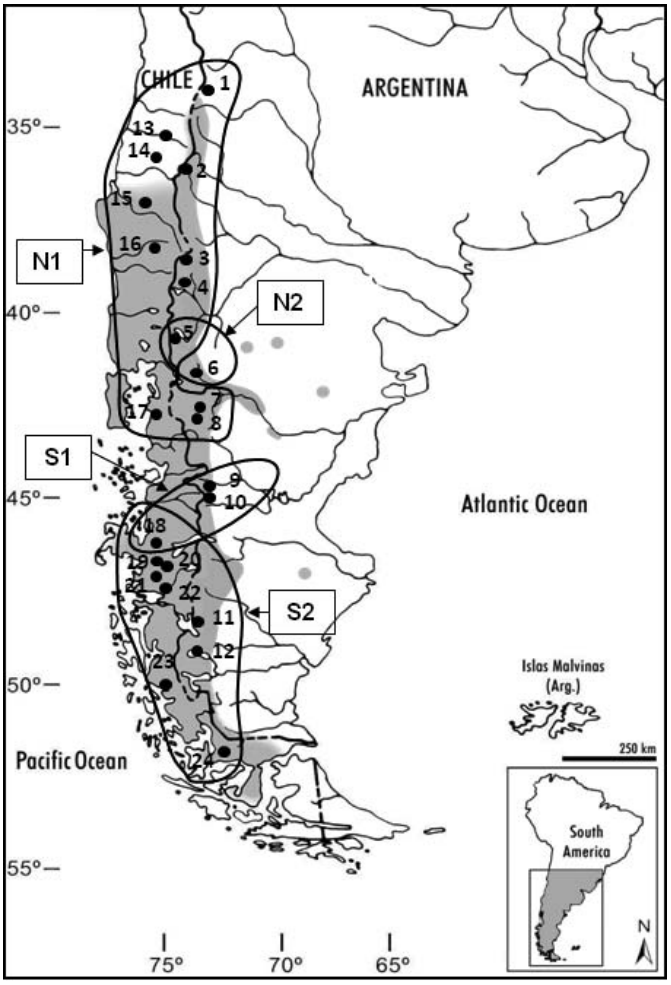

Fig. 1.—Geographic distribution (gray area) of *Loxodontomys micropus* (modified from Teta et al. 2009) showing the collecting localities of the specimens analyzed in this study. Locality numbers follow those of Appendix I. Polygons indicate the 4 main clades found in the genealogical analysis (Fig. 2).

and Pearson 1993; Teta et al. 2005; Udrizar Sauthier 2009). Some of these fossil localities lie outside of the current range of the species, showing that the distribution of *L. micropus* has fluctuated greatly in the recent past, including regional extinctions over more than 300 km (Pardiñas 1999; Udrizar Sauthier 2009).

Previous studies of codistributed sigmodontines, such as *Abrothrix olivaceus* (Rodríguez-Serrano et al. 2006; Smith et al. 2001), *A. longipilis* (Lessa et al. 2010), *Oligoryzomys longicaudatus* (Belmar-Lucero et al. 2009; Palma et al. 2005), and *Phyllotis xanthopygus* (Kim et al. 1998), showed that only *A. longipilis* and *P. xanthopygus* were structured phylogeographically. In contrast, low genetic divergence and geographical homogeneity was found within *A. olivaceus* and *O. longicaudatus* across Patagonia.

A recent multispecies survey of the phylogeography of Patagonian sigmodontines (Lessa et al. 2010) included a few specimens of *L. micropus* and failed to detect significant geographical structure. We documented the pattern of geographical structure and demographic history of the species on the basis of a more complete coverage of its geographical distribution. Additionally, we assessed the recent evolutionary history of *L. micropus* with respect to variation in other codistributed sigmodontines. We also suggest the need of taxonomic revision for the genus *Loxodontomys* Osgood, 1947.

Materials and Methods

Sampling.—Geographic coverage spans most of the species range with samples from 87 specimens of *L. micropus* collected at 24 localities in Argentina and Chile (Fig. 1; Appendix I). Animal care and use procedures followed guidelines approved by the American Society of Mammalogists (Gannon et al. 2007). Sequences of two specimens were retrieved from GenBank (AF108690 and AY275122). Ten specimens from the northern part of the Argentinean and Chilean range currently are assigned to *L. pikumche* Spotorno et al., 1998, the other extant species in the genus.

Laboratory procedures.—We extracted genomic DNA using the protocol of Wizard Genomic DNA Purification System (Promega, Madison, Wisconsin). We then amplified a 801-base-pair fragment of the mitochondrial genome corresponding to the first portion of the protein-coding cytochrome-*b* gene using primers MVZ05 and MVZ16 (da Silva and Patton 1993) with the following conditions: 94°C for 3 min; 35 cycles of 94°C for 20 s, 45°C for 15 s, 72°C for 60 s, and 72°C for 7 min. All reactions included a negative control to check for contamination. Amplicons were purified and sequenced by Macrogen, Inc. (Seoul, Korea). All sequences were deposited in GenBank (GU553838–GU553922).

Alignment, descriptive, and genealogical analysis.—Sequence alignment was performed with Clustal X (Thompson et al. 1997) using the default parameter values. Haplotype and population comparisons were performed with MEGA 4.0 (Tamura et al. 2007) in the form of observed genetic distances. Haplotype and nucleotide diversity were assessed with Arlequin (Schneider et al. 2000).

Only nonredundant haplotypes previously selected with the program DNAsp (Librado and Rozas 2009) were used for genealogical reconstructions. Haplotype relationships were inferred using Bayesian inference. Genealogies were rooted using the outgroup criterion with sequences of the closely related phyllotines (D'Elía 2003) *Auliscomys pictus*, *Graomys griseoflavus*, and *Phyllotis anitae* that are on GenBank (APU03545, AY275117, and AY627299, respectively). Bayesian analysis was conducted in MrBayes 3.1 (Ronquist and Huelsenbeck 2003), with 2 independent runs, each consisting of 3 hot and 1 cold Markov chains. The model used included 6 categories of base substitution, a gamma-distributed rate parameter, and a proportion of invariant sites estimated in MrBayes. Uniform-interval priors were assumed for all parameters except base composition and generalized time-reversible parameters, which assumed a Dirichlet prior process. Runs were allowed to proceed for 6,000,000 generations with

trees sampled every 500 generations per chain. To check for convergence on a stable log-likelihood value we plotted the log-likelihood values against generation time. The first 25% of the trees were discarded as burn-in, and the remaining trees were used to compute a 50% majority rule consensus tree and obtain posterior probability (PP) estimates for each clade.

Population structure.—F_{ST} values, previously obtained in Arlequin (Schneider et al. 2000), were plotted in multidimensional space using nonmetric multidimensional scaling (MDS—Lessa 1990) performed in the XLSTAT software (Addinsoft 2009). Given the nature of the data, the analysis was implemented with the ordinal 2 model and from 3 to 2 dimensions to evaluate the distortion associated with the decrease of dimensions. A Kruskal stress value <0.1 suggests the occurrence of significant associations in the multidimensional space (Ludwig and Reynolds 1988). This analysis was used to visualize and test the existence of discrete groups as a function of genetic similarities and geographic proximity and to evaluate the concordance with main clades recovered in the genealogy.

XLSTAT software also was used to assess the relationship between F_{ST} values and geographic distances among population pairs via a Mantel test. Geographical distances were determined in kilometers from the latitudinal and longitudinal coordinates using a distance calculator available from the National Weather Service (http://www.nhc.noaa.gov/gccalc.shtml). The significance of the relationship between genetic and geographic distances was tested using a nonparametric permutation approach (Addinsoft 2009). A pattern of isolation by distance is anticipated when populations have been stable over a long period and when gene flow is higher among geographically proximate populations.

As an additional method to determine how the genetic variation was geographically structured, we performed hierarchical analyses of molecular variance (AMOVA—Excoffier et al. 1992) implemented in Arlequin (Schneider et al. 2000). Haplotypes were grouped in 4 different hierarchical schemes according to the genealogical results and geography. These analyses partition the total variance observed into covariance components that result from the variance among groups, among populations within groups, and within populations. The covariance components then are used to calculate fixation indices that are equivalent to Wright's *F* statistics (Wright 1978). The significance of the fixation indices is tested using a nonparametric permutation approach (Excoffier et al. 1992).

Molecular diversity and historical population dynamics.—To infer population demographic history we conducted several analyses in Arlequin. Frequency distributions of pairwise differences between haplotype pairs (Rogers and Harpending 1992) and the associated sum-of-square deviations (SSD) and raggedness indices were calculated. A unimodal distribution is expected when the sample is drawn from populations that have experienced recent demographic expansion, whereas samples from populations at demographic equilibrium typically show multimodal distributions. We calculated Fu's *Fs* and Tajima's *D* statistical tests of neutrality (Fu 1997; Tajima 1989), which are based on an infinite-sites model without recombination. These tests evaluate the probability of observing a random neutral sample with a number of alleles that is equal to or smaller than the observed number of alleles. Negative and significant *Fs* and *D* values are obtained from samples corresponding to populations that have undergone recent demographic expansion or show certain departures from neutrality.

Results

Mitochondrial diversity.—We analyzed sequences from 87 specimens of *L. micropus* from 24 localities (Fig. 1) and identified 72 polymorphic sites that define 68 haplotypes. Overall haplotype diversity is 0.99 ($\pm$0.009 *SD*) and nucleotide diversity of 0.011 $\pm$ 0.0056. Two haplotypes (H1 and H39) are shared among individuals from pairs of localities in Argentina and Chile. Haplotype H1 is shared among individuals collected over a large geographic area: Las Leñas, Mendoza Province, and La Angostura and Laguna Epulafquen, Neuquén Province, in Argentina; and Lircay, Región del Maule and Quilleco, Región del Bio Bio in Chile. This shared haplotype was recovered from individuals previously assigned to *L. pikumche* and specimens from the distributional range of *L. micropus*. Haplotype H39 is present in individuals collected in southern Patagonia at Estancia Ensenada, Santa Cruz Province, Argentina and El Manzano, Aysén, Chile.

Average pairwise difference among haplotypes is 1.1%. The observed divergence value among Argentinean samples is 1.12%, and Chilean samples differ, on average, by 0.97%. Intrapopulational divergence ranges between 0.08% and 1.13% for Quilleco and Lucaschewsky localities, respectively, both in Chile (Fig. 1).

Genealogical reconstruction.—The recovered genealogy is shallow, but geographically structured (Fig. 2). Four main, but weakly supported, clades replace each other latitudinally. The first clade (North 1: N1; 0.58 PP) contains 17 haplotypes from the northern Argentinean (Chubut, Mendoza, and Neuquén provinces) and Chilean (Araucanía, Bio Bio, Maule, and Los Lagos regions) distributional range. Haplotypes from specimens collected at populations currently assigned to *L. pikumche* are in this clade. The second clade (North 2: N2; 0.94 PP) consists of 10 haplotypes recovered from specimens collected in northern Argentinean Patagonia (Chubut and Río Negro Provinces). The third clade (South 1: S1; 0.8 PP) includes 11 haplotypes from specimens collected in central Argentinean (southern Chubut) and Chilean (Aysén) Patagonia. The fourth clade (South 2: S2; 0.8 PP) includes 30 haplotypes recovered from specimens from southern Argentinean (Santa Cruz) and Chilean (Aysén and Magallanes) Patagonia. Clades N1 and N2 overlap at El Maitén in northern Chubut Province. Similarly, clades S1 and S2 overlap at El Manzano, Aysén. Intraclade average divergence is low and similar in the four clades, ranging from 0.31% in clade N1 to 0.73% for clade S2 (Table 1).

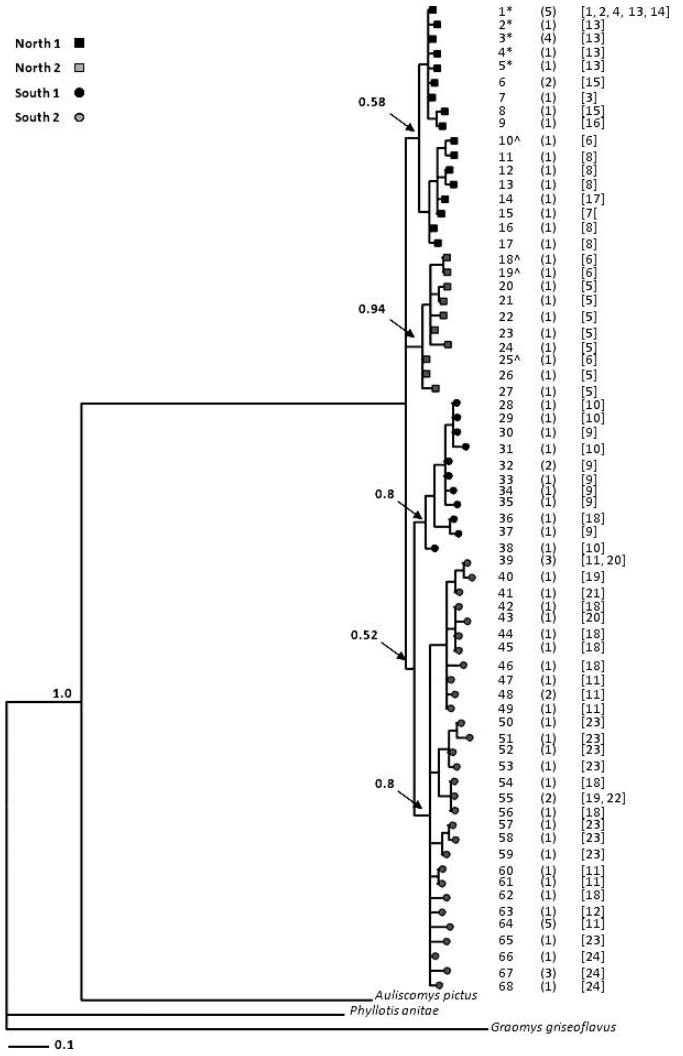

Fig. 2.—Genealogical relationship of 68 haplotypes of *Loxodontomys micropus*. Bayesian posterior probabilities (>0.50) indicating nodal support of the main clades are shown above branch lines. * indicates haplotypes recovered from specimens previously assigned to *L. pikumche* (see text). ^ indicates haplotypes recovered from topotypic specimens of *L. m. alsus*. Numbers within parentheses indicate the number of specimens sharing each haplotype. Numbers within brackets indicate localities from where haplotypes were recovered (see Appendix I and Fig. 1).

Relationships among the four clades are poorly resolved. At the base of the *Lodoxontomys* clade is a trichotomy formed by clades N1, N2, and a clade (0.52 PP) formed by clades S1 and S2. Divergence among clades is also low and ranges from 1.13% for the pair N1–N2 to 1.3% for the comparison between S1 and S2 (Table 2). Maximum parsimony analysis recovers the same four main clades, but relationships among them are slightly different: ([{S1, S2} N1] N2) and poorly supported. Values of haplotype (Hd) and nucleotide diversity (π) for each of the main clades identified in the phylogenetic analysis are presented in Table 1.

Geographic structure and demography.—Multidimensional scaling recovers 2 nonoverlapping populations from dimensions 1 and 3 (Fig. 3). One group includes populations from the northern half of the range that in the genealogical analysis form clades N1 and N2. The second group includes those southern populations that form clades S1 and S2. Populations in which clades N1 and N2 (El Maitén, Chubut Province) and clades S1 and S2 (El Manzano, XI region) overlap fall in an intermediate position within their respective groups. Populations from each of the four main clades tend to group together, agreeing with the genealogy.

The Mantel test indicates no correlation between genetic and geographical distances among populations of *L. micropus* ($r = 0.059$, $n = 24$, $P = 0.256$). Therefore, these populations do not reflect a pattern of isolation by distance.

Results of 4 different geographical arrangements of AMOVA (Table 2) show that the variation among groups is maximized (58.8%) when localities are grouped according to the 4 main clades recovered in the genealogical analysis (i.e., N1 versus N2 versus S1 versus S2). This component has a minimum value (0.63%) when localities are grouped into an Argentinean and a Chilean group (i.e., eastern and western groups). When localities are grouped into 2 (i.e., [N1 + N2] versus [S1 + S2]) or 3 (i.e, N1 versus N2 versus [S1 + S2]) groups, variation explained by differences among groups has intermediate values (37.43% and 47.34%, respectively). Furthermore, percentage of variation explained by differences among populations within groups is minimized (9.94%) when the localities are grouped into the 4 main genealogical groups and maximized (58.06%) when populations are pooled into an Argentinean and a Chilean group.

Mismatch distributions (Fig. 4) for groups N1, N2, and S2 are unimodal, suggesting recent historical population growth.

Table 1.—Genetic variation of cytochrome *b* for the 4 main clades of *Loxodontomys micropus* found in the genealogical analysis (Fig. 2). Locality numbers are those of Fig. 1 and Appendix 1. Sample size (*n*), number of haplotypes found (h), nucleotide diversity (π), haplotype diversity (Hd), and within- and between-clade observed genetic distances (p) for each clade.

						Genetic distance			
							Between groups		
Clade	Localities	*n*	h	π ($\pm SD$)	Hd ($\pm SD$)	Within groups	N1	N2	S1
N1	1, 2, 3, 4, 6, 7, 8, 13, 14, 15, 16, 17	25	17	1.000 ± 0.0113	0.0031 ± 0.00193	0.31			
N2	5, 6	10	10	1.000 ± 0.0447	0.0038 ± 0.00249	0.39	1.13		
S1	9, 10, 18	12	11	1.000 ± 0.0340	0.0038 ± 0.00240	0.38	1.17	1.34	
S2	11, 12, 18, 19, 20, 21, 22, 23, 24	40	30	1.000 ± 0.0056	0.0073 ± 0.00395	0.73	1.37	1.50	1.30

TABLE 2.—Results of 4 analyses of molecular variance (AMOVA) with different arrangements of populations into groups.

AMOVA	N1 versus N2 versus S1 versus S2		(N1 + N2) versus (S1 versus S2)		N1 versus N2 versus (S1 + S2)		Argentina versus Chile	
	Variance components	Percentage explained	Variance components	Percentage explained	Variance components	Percentage explained	Variance components	Percentage explained
Among groups	3.18	58.81	2.07	37.43	2.66	47.34	0.02845	0.63
Among populations within groups	0.537	9.94	1.77	32.03	1.27	22.65	2.63939	58.06
Within populations	1.69	31.25	1.69	30.53	1.69	30.01	1.87823	41.32
F_{ST}	0.687*		0.694*		0.699*		0.587*	

* indicates $P < 0.0001$

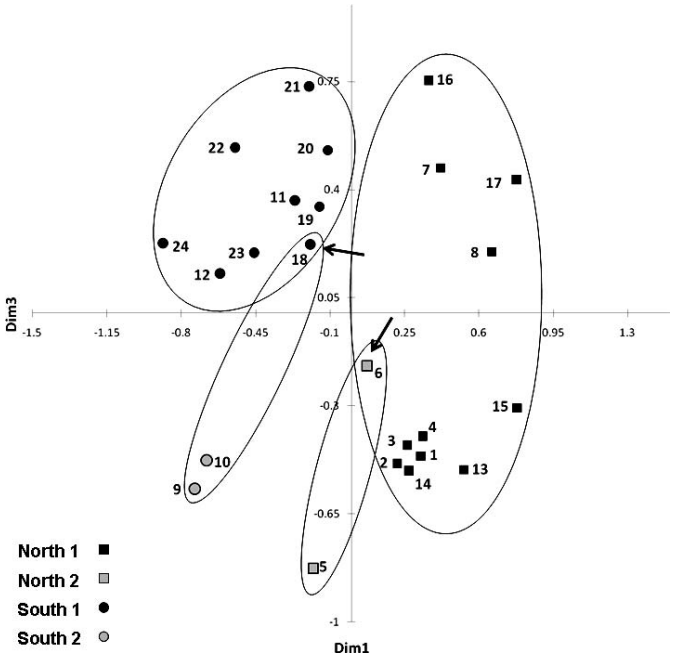

FIG. 3.—Nonmetric multidimensional scaling analysis on the basis of F_{ST} values among 24 localities of *Loxodontomys micropus* (Kruskal stress value = 0.184). Solid lines delimit the geographic groups discussed in the text. Populations marked by arrows are those in the genealogical analysis (Fig. 2) that fall in 2 different clades of the 4 main clades that were recovered. Locality numbers are those of Appendix I.

Although the S1 group appears slightly bimodal, the tests of the observed SSD and raggedness index failed to reject the hypothesis of recent population expansion. Similarly, Tajima's and Fu's neutrality test values are negative and, in the latter case, significant (Table 3), suggesting a population expansion for *L. micropus* along the entire distributional range.

DISCUSSION

Phylogeographic structure.—Loxodontomys micropus is a phyllotine rodent widely distributed along the central and southern Andes of Argentina and Chile. As also reported by Lessa et al. (2010), limited divergence exists among haplotypes of this species. However, we document haplotypes from 4 main clades that are latitudinally differentiated (Figs. 1 and 2). The phylogeny shows a monophyletic southern group, which is composed of 2 main clades (S1, S2) and 2 clades (N1, N2) restricted to the northern part of the distribution. Relationships among S1–S2, N1, and N2 are unresolved. Of these 4 clades only clade N2 is restricted to 1 side of the Andes in the eastern Argentinean provinces of Río Negro and Chubut. The 3 others clades (N1, S1, S2) are distributed across the Andes in both Argentinean and Chilean localities. Results of an AMOVA, in which haplotypes and localities were grouped into eastern and western groups (Table 2), indicate that differences between both groups is minimal (0.63%) and as such, most of the variation lies among populations within the groups (58.06%), followed by differences within localities

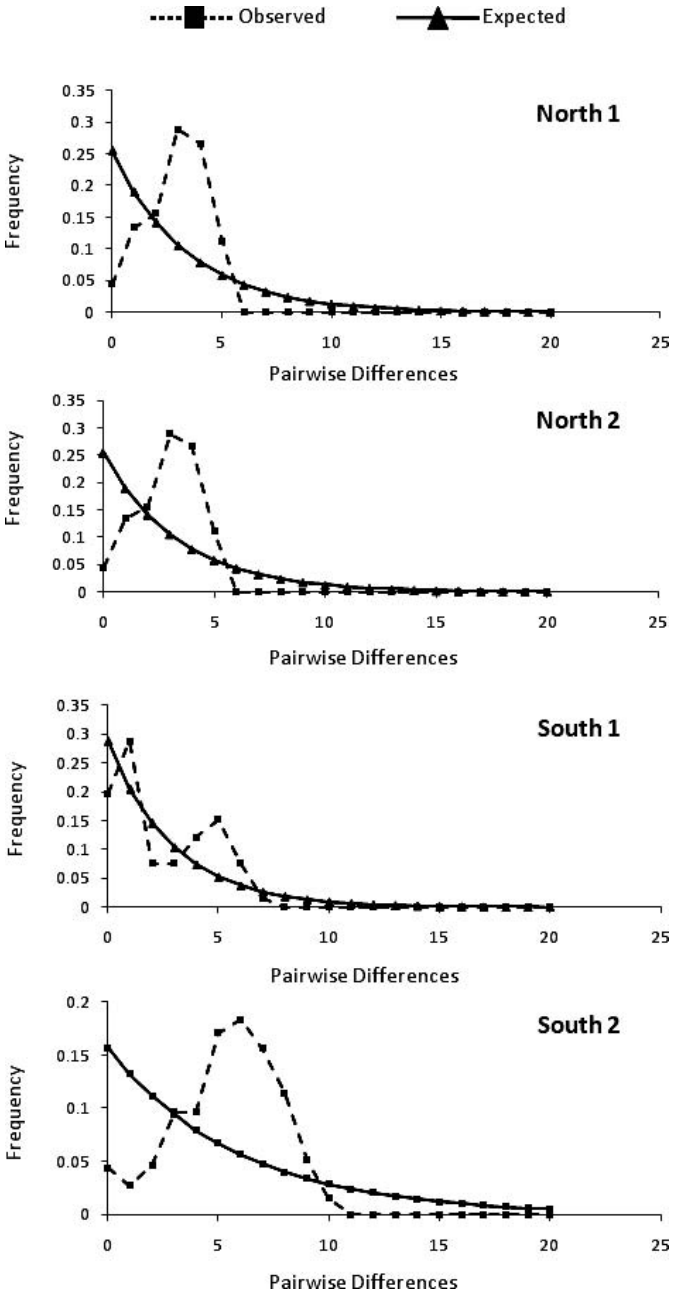

Fig. 4.—Observed and expected patterns of the mismatch distribution of the 4 main clades of *Loxodontomys micropus* (Fig. 2). The y axis shows the frequency values of the number of nucleotide differences within haplotype pairs. For additional details see Table 3.

(41.32%). Thus, the Andean mountain range does not play a relevant role in structuring the genetic variation of *L. micropus*. Similar results have been found in the codistributed sigmodontines *Abrothrix longipilis* (Lessa et al. 2010), *A. olivaceus* (Rodríguez-Serrano et al. 2006; Smith et al. 2001), and *O. longicaudatus* (Palma et al. 2005).

Studies on *A. olivaceus* (Rodríguez-Serrano et al. 2006; Smith et al. 2001) and *O. longicaudatus* (Palma et al. 2005) have shown that these species have reduced genetic divergence lacking phylogeographic structure, suggesting that they originated from a single refugium. Their current distributional area was covered by ice during the LGM (Hulton et al. 2002). In the case of *A. olivaceus* the refuge was suggested to be located in central Chile, and the species would have dispersed south and east of the Andes after the ice shield melted (Smith et al. 2001). For *O. longicaudatus* Palma et al. (2005) suggested a scenario where the species would have crossed the Andes at southern latitudes from Argentina to Chile with a latter expansion to the north; however, the evidence for this is not conclusive. In contrast, genetic variation of *L. micropus* is geographically structured. Therefore, it is possible to suggest that *L. micropus* colonized its current geographical range from more than 1 refuge. The number of refuges to be invoked would vary depending on whether contact zones between clades N1 and N2 and between clades S1 and S2 are considered areas of primary or secondary contact. Currently, it is not possible to distinguish between these 2 scenarios.

Table 3.—Demographic parameters for the 4 main clades of haplotypes of *Loxodontomys micropus* recovered in the genealogical analysis (Fig. 2), including estimates of Tajima's *D* (Tajima 1989), Fu's *Fs* (Fu 1997) neutrality tests, and parameters of the mismatch distributions: sum-of-square deviations (SSD), Harpending's raggedness index, and *P* (value of the simulated raggedness $\geq$ observed raggedness) of goodness-of-fit test (Rogers and Harpending 1992; Schneider and Excoffier 1999).

Clade	Tajima *D*	Fu's *Fs*	SSD	Harpending's raggedness index	*P*
N1	−1.14927	−26.6472*	0.00863	0.02493	0.95
N2	−1.20399	−8.12019*	0.01046	0.04938	0.74
S1	−1.23917	−11.21354*	0.14265	0.02617	1.00
S2	−1.14939	−25.31466*	0.00534	0.01354	0.40

* $P < 0.05$.

The geographic placement of refugia of *L. micropus* remains unclear; however, several lines of evidence suggest that refugia might have been outside of our sampling area. First, much of the range that we sampled was mostly covered by ice during the LGM (Hulton et al. 2002; Rabassa 2008), although recent studies indicate that the ice sheet was not continuous, allowing the existence of refugia surrounded by ice (Xu et al. 2009). Second, the 4 main clades show signs of recent population expansion (Table 3, Fig. 4), suggesting that the sampled populations are of recent origin. Third, the fossil record (Pardiñas and Teta 2008) shows that *L. micropus* has experienced shifts in its range during the Pleistocene and Holocene, previously occupying areas that now are locally extinct. Similarly, isolated populations in the Argentinean Patagonian steppe (Teta et al. 2009) may be considered relicts of a past, more extensive eastern distribution. In the area of Chacays, a vast basaltic plateau in central Patagonia, a single recording locality of *L. micropus* is about 250 km east of the present continuous range (Udrizar Sauthier et al. 2008).

On the basis of the information just described, we suggest the following scenario for the recent history of *L. micropus*. During the LGM the range of *L. micropus* was fragmented into at least 2, and possibly 4, areas. The location of these isolates

is uncertain, but they likely were outside the area surveyed in this study, probably in the Argentinean Patagonian steppe to the east of the current range. However, the possibility of a refugium in central Chile, north of the sampled area, cannot be disregarded. Later, *L. micropus* colonized its current range after the ice retreated. Under this scenario, current steppe isolates (Teta et al. 2009; Fig. 1), which were not sampled in this study because they are based only on remains recovered from owl pellets, are interpreted as relicts of a past distribution that as a whole was more to the east than the current distribution. If this is the case, these isolates are expected to be more structured and show less or no evidence of demographic expansion, relative to populations in recently colonized areas. Therefore, the inclusion of these isolates would constitute a partial test of this biogeographic scenario. This, together with the analysis of unlinked loci, would help to further our understanding of the effect of the Neogene climatic fluctuations on *L. micropus* and, by extension, of the Patagonian mammal assemblage.

Taxonomic implications.—Results of the present study have several implications relative to the alpha diversity of *Loxodontomys*. The first relates to the distinctiveness and validity of *L. pikumche*, and the second concerns the 3 currently recognized subspecies of the southern pericote.

Loxodontomys pikumche was described by Spotorno et al. (1998) mostly on the basis of presumably fixed karyotypic differences with *L. micropus*. Although *L. micropus* has a diploid complement of 2n = 34 and a fundamental number of FN = 36, *L. pikumche* has 2n = 32 and FN = 34. Morphological differences between both forms are subtle; Spotorno et al. (1998) identified a few dental traits (e.g., incisor orientation varying from hyperopisthodont to opisthodont, upper incisor dentine fissure long or comma-shaped) as differing in both taxa. However, cranial and dental variability within populations of *Loxodontomys* is high, as noted by Hershkovitz (1962), Pearson (1995), and Spotorno et al. (1998). Several populations assigned to *L. micropus* have individuals with character states used to diagnose *L. pikumche*. For example, populations of *L. micropus* studied by Pearson (1995) include specimens with molar root numbers considered to be diagnostic of *L. pikumche*. Both forms are distributed parapatrically in central and southern Chile; however, their geographic limits have not been clearly established. They supposedly replace each other at about the latitude of Chillan (~35.5°S) in central Chile (Spotorno et al. 1998). Recently, Novillo et al. (2009) reported the first putative Argentinean record of *L. pikumche*. Specimens were collected in Las Leñas, Mendoza province. They have a third karyomorph, 2n = 32 and FN = 32, but were referred to *L. pikumche* on the basis of morphological characters. However, none of the dental features used by Spotorno et al. (1998) to diagnose *L. pikumche* was discussed. In addition, the character state added by Novillo et al. (2009) to discriminate between *L. pikumche* and *L. micropus* was based on the examination of 1 specimen of each taxon and did not take into account intrapopulation variation (e.g., zygomatic plate morphology—Hershkovitz 1962).

Our sampling includes 10 specimens assignable to *L. pikumche* from 1 Argentinean (n = 1: Las Leñas, Mendoza) and 2 Chilean (n = 1: Las Trancas, VIII Region; n = 8: Lircay, VII Region) localities. The Argentinean specimen is one used by Novillo et al. (2009) to cite the species for Argentina. Among these 10 specimens 5 haplotypes were recovered. One of these haplotypes was recovered not only from specimens from the three localities of *L. pikumche* but also from individuals morphologically recognized as *L. micropus* from the Argentinean localities of Laguna Epulafquen, and La Angostura localities, in the Neuquén Province. This widely distributed haplotype falls in clade N1 and is remarkably similar (0.05%) to the other haplotypes assignable to *L. pikumche* and to those of *L. micropus* forming the same clade. We cannot rule out that haplotype sharing among taxa may be the result of a selective process or introgression after hybridization. However, given the marked morphological similarities between *L. pikumche* and *L. micropus*, we suggest that both taxa might represent the same biological species and as such the former may constitute a junior synonym of the latter. Under this scenario, *L. micropus* would be an example of another known karyotypically variable sigmodontine species in addition to, among others, *Akodon cursor* (Geise et al. 1998; Nogueira and Fagundes 2008), *Holochilus brasiliensis* (Nachman 1992), and *O. flavescens* (Weksler and Bonvicino 2005).

Three subspecies are currently recognized within *L. micropus* (Teta et al. 2009). The type locality of *L. m. micropus* (Waterhouse 1837) was recorded as ''Interior plains of Patagonia in lat. 50°, near the banks of the Santa Cruz'' (Waterhouse 1839), Santa Cruz Province, Argentina. However, uncertainty remains about the exact location where Charles Darwin collected the holotype (Teta et al. 2009). The second subspecies is *L. m. alsus* (Thomas 1919), and the holotype comes from ''Maitén, W. Chubut. 700 meters'' (Thomas 1919), Chubut Province, Argentina (=El Maitén; 42°03′S, 71°10′W). Finally, *L. m. fumipes* (Osgood 1943) was described from ''Quellón, Chiloé Island, Chile'' (Osgood 1943—43°07′S, 73°35′W, Región de Los Lagos). Differences among these 3 forms related mostly to pelage color, and their degree of differentiation has not been assessed with contemporary approaches. Similarly, the geographic distribution of these forms is unclear.

Our sampling includes haplotypes from topotypic specimens of *L. micropus alsus* and from near the type locality of *L. m. micropus* (i.e., Estancia Ensenada and Lago Viedma localities, Santa Cruz). Unfortunately, we were unable to include specimens from Chiloé Island. Haplotypes assignable to each nominal form fall in different clades. Those of *L. m. alsus* fall in clades N1 and N2 of the northern group, and those of *L. m. micropus* belong to clade S2 of the southern group. Therefore, our molecular data indicate that *L. m. micropus* and *L. m. alsus* could represent distinct evolutionary units within the main lineage of *L. micropus* that might be considered subspecies. Under this taxonomic arrangement *L. pikumche* is a synonym of *L. m. alsus*.

Final considerations.—Additional studies combining broader geographic coverage (especially Chiloé Island, central Chile and isolated eastern populations) and the analysis of nuclear loci, karyotypes, and morphological characters are needed. Such investigations will be important in determining the validity of the biogeographic and taxonomic hypotheses presented in this study.

Resumen

Loxodontomys micropus es un roedor sigmodontino de tamaño medio, ampliamente distribuido en los Andes y Patagonia de Argentina y Chile. Se caracteriza por habitar una región altamente heterogénea, que ha sido históricamente afectada por procesos geológicos y paleoclimáticos de distinta envergadura como los períodos glaciales del Neógeno. Con el objetivo de evaluar el grado de variación genética, patrón filogeográfico e historia biogeográfica de la especie, analizamos un fragmento de 801 pb del genoma mitocondrial (gen que codifica para citocromo b) de 87 individuos colectados en 24 localidades de Argentina y Chile. Los resultados muestran que *L. micropus* presenta una genealogía llana y estructurada geográficamente en sentido latitudinal y que sus clados principales tienen señal de expansión poblacional reciente. Discutimos el patrón de variación genética de *L. micropus* en relación a la historia poblacional y la concordancia con otros sigmodontinos codistribuidos. Finalmente, sugerimos que la otra especie viviente del género, *L. pikumche*, podría constituir un sinónimo junior de *L. micropus*.

Acknowledgments

We thank the following people and institutions for providing tissue samples: Agustina Novillo and Agustina Ojeda (CRICYT), Joe Cook (MSB), Jonathan Guzmán (UdeC), Milton Gallardo (IEEUACH), Richard Sage (MVZ), and Robert Baker (TTU). Assistance during fieldwork was provided by Daniel Udrizar Sauthier, Jonathan Guzman, Dario Podestá, Marta Lizarralde and her team, and Sebastian Cirignoli. The suggestions of 2 anonymous reviewers contributed to the improvement of this study. Financial support was given by NGS 7813-05, CONICET PIP 6179, Agencia PICT 2008-0547, and FONDECYT 11070157.

Literature Cited

Addinsoft. 2009. XLSTAT 2009. Addinsoft SARL, New York.

Allnutt, T. R., A. C. Newton, A. Premoli, and A. Lara. 2003. Genetic variation in the threatened South American conifer *Pilgerodendron uviferum* (Cupressaceae), detected using RAPD markers. Biological Conservation 114:245–253.

Avise, J. C. 2000. Phylogeography: the history and formation of species. Harvard University Press, Cambridge, Massachusetts.

Belmar-Lucero, S., P. Godoy, M. Ferrés, P. Vial, and R. E. Palma. 2009. Range expansion of *Oligoryzomys longicaudatus* (Rodentia, Sigmodontinae) in Patagonian Chile, and first record of hantavirus in the region. Revista Chilena de Historia Natural 82:265–275.

da Silva, M. N., and J. L. Patton. 1993. Amazonian phylogeography: mtDNA sequence variation in arboreal echimyid rodents (Caviomorpha). Molecular Phylogenetics and Evolution 2:243–255.

D'Elía, G. 2003. Phylogenetics of Sigmodontinae (Rodentia, Muroidea, Cricetidae), with special reference to the akodont group, and with additional comments on historical biogeography. Cladistics 19:307–323.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes—application to human mitochondrial-DNA restriction data. Genetics 131:479–491.

Fu, Y. X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925.

Gannon, W. L., R. S. Sikes, and the Animal Care and Use Committee of the American Society of Mammalogists. 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 88:809–823.

Geise, L., F. C. Canavez, and H. N. Seuánez. 1998. Comparative karyology in *Akodon* (Rodentia, Sigmodontinae) from Southeastern Brazil. Journal of Heredity 89:158–163.

Hershkovitz, P. 1962. Evolution of Neotropical cricetine rodents (Muridae), with special reference to the phyllotine group. Fieldiana: Zoology 46:1–524.

Hinojosa, L., and C. Villagrán. 1997. Historia de los bosques del sur de Sudámerica. I: antecedentes paleobotánicos, geológicos y climáticos del Terciario del cono sur de América. Revista Chilena de Historia Natural 70:225–239.

Hulton, N., R. Purves, R. McCulloch, D. Sudgen, and M. J. Bentley. 2002. The Last Glacial Maximum and deglaciation in southern South America. Quaternary Science Reviews 21:233–241.

Kim, I., et al. 1998. Habitat islands, genetic diversity, and gene flow in a Patagonian rodent. Molecular Ecology 7:667–678.

Lessa, E. P. 1990. Multidimensional analysis of geographic genetic structure. Systematic Zoology 39:242–252.

Lessa, E. P., G. D'Elía, and U. F. J. Pardiñas. 2010. Genetic footprints of late Quaternary climate change in the diversity of Patagonian-Fueguian rodents. Molecular Ecology 19:3031–3037.

Librado, P., and J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452.

Ludwig, J. A., and J. F. Reynolds. 1988. Statistical ecology: a primer on methods and computing. John Wiley & Sons, Inc., New York.

Morando, M., L. J. Ávila, C. R. Turner, and J. W. Sites, Jr. 2007. Molecular evidence for a species complex in the Patagonian lizard *Liolaemus bibronii* and phylogeography of the closely related *Liolaemus gracilis* (Squamata: Liolaemini). Molecular Phylogenetics and Evolution. 43:952–973.

Nachman, M. W. 1992. Geographic patterns of chromosomal variation in South American marsh rats, *Holochilus brasiliensis* and *H. vulpinus*. Cytogenetics and Cell Genetics 61:10–16.

Nogueira, C. D. A., and V. Fagundes. 2008. *Akodon cursor* Winge, 1887 (Rodentia: Sigmodontinae): one or two species? New evidences based on molecular data. Zootaxa 1768:41–51.

Novillo, A., A. Ojeda, and R. Ojeda. 2009. *Loxodontomys pikumche* (Rodentia, Cricetidae): a new species for Argentina. Mastozoología Neotropical 16:239–242.

Osgood, W. H. 1943. The mammals of Chile. Field Museum of Natural History, Zoology Series 30:1–268.

Osgood, W. H. 1947. Cricetine rodents allied to *Phyllotis*. Journal of Mammalogy 28:165–174.

Palma, E., et al. 2005. Phylogeography of *Oligoryzomys longicaudatus* (Rodentia: Sigmodontinae) in temperate South America. Journal of Mammalogy 86:191–200.

PARDIÑAS, U. F. J. 1998. Roedores holocénicos del sitio Cerro Casa de Piedra 5 (Santa Cruz, Argentina): tafonomía y paleoambientes. Palimpsesto 5:66–90.

PARDIÑAS, U. F. J. 1999. Los roedores muroideos del Pleistoceno tardío-Holoceno en la región pampeana (sector este) y Patagonia (República Argentina): aspectos taxonómicos, importancia bioestratigráfica y significación paleoambiental. Tesis doctoral, Facultad de Ciencias Naturales y Museo, Universidad Nacional La Plata, La Plata, Argentina.

PARDIÑAS, U. F. J., AND P. TETA. 2008. Small mammals and paleoenvironments around the Pleistocene–Holocene boundary in Patagonia. Current Research in the Pleistocene 25:186–188.

PARDIÑAS, U. F. J., P. TETA, AND D. UDRIZAR SAUTHIER. 2008. Mammalia, Didelphimorpia and Rodentia, southwest of the province of Mendoza, Argentina. Check List 4:218–225.

PEARSON, O. P. 1995. Annotated keys for identitifying mammals living in or near Nahuel Huapi National Park or Lanin National Park, Southern Argentina. Mastozoología Neotropical 2:99–148.

PEARSON, O. P., AND A. K. PEARSON. 1993. La fauna de mamíferos pequeños de Cueva Traful I. Argentina: pasado y presente. Præhistoria 1:73–89.

RABASSA, J. 2008. Late Cenozoic glaciations in Patagonia and Tierra del Fuego. Pp. 151–204 in The Late Cenozoic in Patagonia and Tierra del Fuego (J. Rabassa, ed.). Developments in Quaternary Sciences 11.

RAMOS, V. 1989. The birth of southern South America. American Scientist 77:444–450.

RODRÍGUEZ-SERRANO, E., R. A. CANCINO, AND E. PALMA. 2006. Molecular phylogeography of *Abrothrix olivaceus* (Rodentia: Sigmodontinae) in Chile. Journal of Mammalogy 87:971–980.

ROGERS, A. R., AND H. HARPENDING. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9:552–569.

RONQUIST, F., AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

RUZZANTE, D. E., ET AL. 2008. Climate control on ancestral population dynamics: insight from Patagonian fish phylogeography. Molecular Ecology 17:2234–2244.

SCHNEIDER, S., AND L. EXCOFFIER. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics 152:1079–1089.

SCHNEIDER, S., D. ROESSLI, AND L. EXCOFFIER. 2000. Arlequin, version 2.000: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

SMITH, M. F., D. A. KELT, AND J. L. PATTON. 2001. Testing models of diversification in mice in the *Abrothrix olivaceus/xanthorhinus* complex in Chile and Argentina. Molecular Ecology 10:397–405.

SPOTORNO, A., H. COFRÉ, G. MANRÍQUEZ, Y. VILINA, P. MARQUET, AND L. WALKER. 1998. Una nueva especie de *Loxodontomys*, otro mamífero filotino en los Andes de Chile central. Revista Chilena de Historia Natural 71:359–373.

TAJIMA, F. 1989. The effect of change in population size on DNA polymorphism. Genetics 123:597–601.

TAMURA, K., J. DUDLEY, M. NEI, AND S. KUMAR. 2007. MEGA4. Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. Molecular Biology and Evolution 24:1596–1599.

TETA, P., A. ANDRADE, AND U. F. J. PARDIÑAS. 2002. Novedosos registros de roedores sigmodontinos (Rodentia: Muridae) en la Patagonia central argentina. Mastozoología Neotropical 9:79–84.

TETA, P., A. ANDRADE, AND U. F. J. PARDIÑAS. 2005. Micromamíferos (Didelphimorphia y Rodentia) y paleoambientes del Holoceno tardío en la Patagonia noroccidental extra-andina (Argentina). Archaeofauna 14:183–197.

TETA, P., U. F. J. PARDIÑAS, D. E. UDRIZAR SAUTHIER, AND G. D'ELÍA. 2009. *Loxodontomys micropus* (Rodentia: Cricetidae). Mammalian Species 837:1–11.

THOMAS, O. 1919. On small mammals collected by E. Budin in northwestern Patagonia. Annals and Magazine of Natural History. Series 9, 3:199–212.

THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAK, F. JEANMOUGIN, AND D. G. HIGGINS. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24:4876–4882.

UDRIZAR SAUTHIER, D., P. TETA, P. WALLACE, AND U. F. J. PARDIÑAS. 2008. Mammalia, Rodentia, Sigmodontinae, *Loxodontomys micropus*: new locality records. Check List 4:171–173.

UDRIZAR SAUTHIER, D. E. 2009. Los micromamíferos y la evolución ambiental durante el Holoceno en el río Chubut (Chubut, Argentina). Mastozoología Neotropical 16:521–523.

WATERHOUSE, G. R. 1837. Characters of new species of the genus *Mus*, from the collection of Mr. Darwin. Proceedings of the Zoological Society of London 1837:15–21, 27–32.

WATERHOUSE, G. R. 1839. The zoology of the voyage of H. M. S. Beagle, under the command of Captain Fitzroy, R. N., during the years 1832 to 1836. Part II. Mammalia. Smith, Elder and Co., London, United Kingdom.

WEKSLER, M., AND C. R. BONVICINO. 2005. Taxonomy of pigmy rice rats genus *Oligoryzomys* Bangs, 1900 (Rodentia, Sigmodontinae) of the Brazilian Cerrado, with the description of two new species. Arquivos do Museu Nacional, Rio de Janeiro 63:113–130.

WRIGHT, S. 1978. Evolution and the genetics of populations. Vol. IV. Variability within and among natural populations. University of Chicago Press, Chicago, Illinois.

XU, J. W., M. PÉREZ-LOSADA, C. G. JARA, AND K. A. CRANDALL. 2009. Pleistocene glaciation leaves deep signature on the freshwater crab *Aegla alacalufi* in Chilean Patagonia. Molecular Ecology 18:904–918.

Submitted 26 January 2010. Accepted 14 June 2010.

Associate Editor was Burton K. Lim.

APPENDIX I

List of specimens of *Loxodontomys micropus* used in the present study, including: collection locality, identification number, GenBank accession number (those 2 retrieved from GenBank are indicated by **), haplotype number (see Fig. 2), and geographic groups (N1: North 1; N2: North 2; S1: South 1; S2: South 2). Locality numbers correspond to those of Fig. 1. Museum collection acronyms and personal field numbers are as follows. Argentina: Colección de Mamíferos del Centro Nacional Patagónico, Puerto Madryn (CNP); Agustina Novillo (AN, vouchers will be deposited at Colección Mastozoológica del Instituto Argentino de Zonas Áridas, IADIZA). Chile: Instituto de Ecología y Evolución, Universidad Austral de Chile, Valdivia (IEEUACH); Guillermo D'Elía (GD, vouchers will be deposited at Museo de Zoología, Universidad de Concepción); Jonathan Guzmán (JG, voucher will be deposited at Museo de Zoología, Universidad de Concepción). United States: Museum of Southwestern Biology, Albuquerque (NK: tissue accession prefix);

The Museum, Texas Tech University, Lubbock (TK: tissue accession prefix); Richard D. Sage (RDS, vouchers will be deposited at the Museum of Vertebrate Zoology, Berkeley).

ARGENTINA: 1) Mendoza Province, Las Leñas, 35°06′S, 70°05′W (AN 60: GU553838: 1: N1). 2) Neuquén Province, Laguna Epulafquen, 36°49′S, 71°04′W (CNP 1478: AY275122**: 1: N1). 3) Neuquén Province, Alumine, 3.8 km W, 0.7 km N Quillén, 39°22′S, 70.95°W (RDS 18031: GU553839: 7: N1). 4) Neuquén Province, Los Lagos, 12.2 km N, 4.8 km W, Villa La Angostura, 40°39′S, 71°42′W (RDS 18113: GU553840: 1: N1). 5) Río Negro Province, Bariloche, 41°14′S, 71°32′W (MVZ 182661: AF108690**: 22: N2; RDS 17829: GU553841: 26: N2; RDS 17830: GU553842: 27: N2; RDS 17834: GU553843: 20: N2; RDS 17839: GU553844: 21: N2; RDS 18129: GU553845: 23: N2; RDS 18140: GU553846: 24: N2). 6) Chubut Province, Estancia El Maitén, 42°03′S, 71°09′W (CNP 1980: GU553847: 18: N2; CNP 1981: GU553848: 25: N2; CNP 1982: GU553849: 10: N1; CNP 1983: GU553850: 19: N2). 7) Chubut Province, Laguna Larga, 42°53″S, 71°34″W (CNP 1046: GU553851: 15: N1). 8) Chubut Province, Futaleufú, 5.5 km S and 2.8 km W Villa Futalaufquén, 42°56′S, 71°34′W (RDS 17861: GU553852: 12: N1; RDS 17866: GU553853: 16: N1; RDS 17894: GU553854: 11: N1; RDS 18069: GU553855: 17: N1; RDS 18078: GU553856: 13: N1). 9) Chubut Province, Lago Blanco, W-SW end, 45°55′S, 71°19′W (CNP 299: GU553857: 32: S1; CNP 312: GU553858: 35: S1; CNP 317: GU553859: 32: S1; CNP 398: GU553860: 30: S1; CNP 419: GU553861: 34: S1; CNP 1221: GU553862: 37: S1; CNP 1984: GU553863: 33: S1). 10) Chubut Province, Lago Blanco, Estancia Valle Huemules, 45°56′S, 71°31′W (CNP 399: GU553864: 29: S1; CNP 482: GU553865: 28: S1; CNP 1165: GU553866: 38: S1; CNP 1585: GU553867: 31: S1). 11) Santa Cruz Province, Estancia La Ensenada, 48°21′S, 72°05′W (CNP 536: GU553868: 64: S2; CNP 1985: GU553869: 47: S2; CNP 1986: GU553870: 60: S2; CNP 1987: GU553871: 61: S2; CNP 1988: GU553872: 48: S2; CNP 1989: GU553873: 64: S2; CNP 1990: GU553874: 48: S2; CNP 1991: GU553875: 39: S2; CNP 1992: GU553876: 64: S2; CNP 1993: GU553877: 64: S2; CNP 1994: GU553878: 39: S2; CNP 1995: GU553879: 49: S2; CNP 1996: GU553880: 64: S2). 12) Santa Cruz Province, Lago Viedma, Bahía Túnel, 49°12′S, 72°58′W (CNP 1997: GU553881: 63: S2).

CHILE: 13) VII región del Maule, Altos del Lircay, 35°35′S, 71°02′W (GD 1301: GU553882: 2: N1; GD 1302: GU553883: 3: N1, GD 1303: GU553884: 3: N1; GD 1304: GU553885: 3: N1; GD 1305: GU553886: 4: N1; GD 1328: GU553887: 3: N1; GD 1329: GU553888: 5: N1; GD 1330: GU553889: 1: N1). 14) VIII región del Bio Bío, Chillán, Las Trancas, 36°55′S, 71°30′W (GD 1300: GU553890: 1: N1). 15) VIII región del Bío Bío, Quilleco, Hacienda San Lorenzo, 37°32′S, 71°27′W (NK 120134: GU553891: 6: N1; NK 120138: GU553892: 6: N1; NK 120139: GU553893: 8: N1). 16) IX región de la Araucanía, Curacautín, Piedra Santa, Malalcahuello, 38°28′S, 71°35′W (IEEUACH 4897: GU553894: 9: N1). 17) X región de los Lagos, Palena, El Encuentro, 43°37′S, 71°45′W (NK 129189: GU553895: 14: N1). 18) XI región de Aysén del General Carlos Ibáñez del Campo, Aysén, El Manzano, 47°09′S, 72°39′W (GD 928: GU553896: 36: S1; GD 929: GU553897: 62: S2; GD 930: GU553898: 42: S2; GD 944: GU553899: 54: S2; GD 997: GU553900: 44: S2; GD 998: GU553901: 56: S2; GD 1006: GU553902: 45: S2; JG 085: GU553903: 46: S2). 19) XI región de Aysén del General Carlos Ibáñez del Campo, Aysén, Predio Lucaschewsky, 47°11′S, 72°44′W (JG 023: GU553904: 40: S2; JG 086: GU553905: 55: S2). 20) XI región de Aysén del General Carlos Ibáñez del Campo, Aysén, Sur de Cochrane, 47°19′S, 72°36′W (GD 963: GU553906: 43: S2; GD 1000: GU553907: 39: S2). 21) XI región de Aysén del General Carlos Ibáñez del Campo, Aysén, Confluencias ríos Colonia-Baker, 47°18′S, 72°50′W (JG 084: GU553908: 41: S2). 22) XI región de Aysén del General Carlos Ibáñez del Campo, Aysén, Sector Barrancoso, 47°29′S, 72°48′W (GD 967: GU553909: 55: S2). 23) XII región de Magallanes, Torres del Paine, 50°56′S, 72°58′W (TK 110006: GU553910: 52: S2; TK 110007: GU553911: 59: S2; TK 110009: GU553912: 58: S2; TK 110027: GU553913: 50: S2; TK 110028: GU553914: 53: S2; TK 110033: GU553915: 65: S2; TK 110037: GU553916: 57: S2; TK 110042: GU553917: 51: S2). 24) XII región de Magallanes, Punta Arenas, Puerto del Hambre, 53°36′S, 70°56′W (NK 129278: GU553918: 66: S2; NK 129295: GU553919: 67: S2; NK 129297: GU553920: 67: S2; NK 129298: GU553921: 68: S2; NK 129308: GU553922: 67: S2).