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Phylogeography and genetic variation in the South American rodent *Tympanoctomys barrerae* (Rodentia: Octodontidae)

AGUSTINA A. OJEDA*

Grupo de Investigaciones de Biodiversidad (GIB), Instituto Argentino de Zonas Áridas (IADIZA), CONICET. Centro de Ciencia y Técnica Mendoza (CCT Mendoza), Avenida Ruiz Leal s/n Parque General San Martín, Mendoza, Argentina, CP 5500, CC 507

* *Correspondent: agustinao@mendoza-conicet.gov.ar*

The red viscacha rat, *Tympanoctomys barrerae*, is an octodontid rodent endemic to the arid west-central and southern regions of Argentina. It is solitary, lives in complex burrows built in soft soil, and occurs at low population densities in patches associated with salt basins and sand dunes in lowland habitats of the Monte and Patagonia deserts. The purpose of this study was to investigate the genetic structure and biogeography of this desert specialist. To assess genetic variation an 800-base pair fragment of the mitochondrial control region was sequenced for 60 individuals from 8 localities across the species' range. Relationships among haplotypes were inferred from phylogenetic analyses (maximum parsimony, Bayesian, and networks). Genetic structure and demographic history were analyzed with descriptive statistics, mismatch distributions, neutrality tests (Tajima's and Fu's), and analyses of molecular variance (AMOVAs). In total 26 haplotypes were found, most restricted to single populations. The presence of unshared haplotypes was consistent with low migration rates. Within the distribution (between 29°S and 39°S) southern and northern populations showed higher genetic diversity values than central populations. Populations of *T. barrerae* showed moderate to high genetic differentiation on the basis of haplotypes of central populations. AMOVA analyses indicated a moderate level of geographic structure for all populations. Low haplotype and nucleotide diversities in central populations suggest a possible bottleneck associated with Pleistocene glaciations or volcanic activity in this part of the range of the viscacha rat. Phylogeographic structure was moderate, and the analyses recovered 2 principal clades: A (with central and a part of the southern distribution) and B (with northern and another part of the southern distribution). Most populations were polyphyletic, indicating that they have not been isolated long enough to reach reciprocal monophyly. Demographic analyses conducted for clades A and B suggest a recent history of population expansion. DOI: 10.1644/09-MAMM-A-177.1.

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The red viscacha rat, *Tympanoctomys barrerae*, is an octodontid rodent endemic to the arid region of central and southwestern Argentina. Octodontid rodents represent one of the most characteristic groups in the arid lands of southern South America. The major diversification of Octodontidae is estimated to have occurred in the Pliocene–Pleistocene (Opazo 2005), which coincided with landscape changes and habitat fragmentation (Contreras et al. 1987; Honeycutt et al. 2003; Mares 1985). Within this family the split between *T. barrerae* and its sister taxon *Pipanacoctomys aureus* occurred near the Pliocene–Pleistocene boundary 1.74 million years ago (mya—Opazo 2005).

Tympanoctomys barrerae is a ground-dwelling solitary species that lives in complex burrow systems built in soft soil in areas bordering salt flats (Mares et al. 1997a; Torres et al. 2003). It occurs at low population densities in patches associated with salt basins, locally known as salares, and sand dunes in the lowland habitats of the Monte and Patagonia deserts. The red viscacha rat specializes in halophytic vegetation (chenopods such as *Atriplex*, *Heterostachys*, and *Suaeda*—Ojeda et al. 1996; Torres-Mura et al. 1989) and has remarkable ecomorphological adaptations for life in xeric habitats (Berman 2003; Díaz et al. 2000; Mares et al. 1997a, 1997b; Ojeda et al. 1996). The presence of a stiff bundle of hairs behind the upper incisors helps to remove the salt excess from chenopod leaves, reducing their salt content (Berman

2003; Mares et al. 1997b). *T. barrerae* has a specialized kidney with an elongated renal papilla that is able to concentrate urine at a maximum concentration of 7,080 mosm/l, a level similar to that observed in the North American desert rodent *Dipodomys microps* and the African rodent *Psammomys obesus* (Díaz and Ojeda 1999; Ojeda et al. 1999). Due to characteristics like its narrow geographic range, patchy distribution, habitat specialization, and low population density, *T. barrerae* has been classified as a threatened species (Díaz and Ojeda 2000; International Union for the Conservation of Nature 2008; Ojeda and Díaz 1997).

The red viscacha rat is an interesting species not only because of its natural history but also due to its unique genomic characteristics. Its chromosome complement ($2n = 102$) is one of the highest among mammals (Contreras et al. 1990), and its genome size (16.8 pg of DNA) is double that of its close relatives (Gallardo et al. 1999). Saltational increase in diploid number associated with genome size duplication suggests that *T. barrerae* may be a tetraploid (Gallardo et al. 1999, 2004), but this interpretation is still a matter of intense debate (Gallardo et al. 2006; Svartman et al. 2005).

Populations of red viscacha rats are known from 11 localities along the west-central and southern regions of Argentina in San Juan, Mendoza, Neuquén, La Pampa, and Chubut provinces (between 29°S and 43°S) within the Monte Desert and Patagonian biomes (Díaz et al. 2000; Gallardo et al. 2009; Ojeda et al. 2007). Their isolated patchy populations are, in some cases, separated by hundreds of kilometers. A new record of *T. barrerae* in the Patagonian steppe (Chubut province) extended its previously known range 550 km southward (Gallardo et al. 2009). Fossil remains of *Tympanoctomys* from the Pleistocene in Mar del Plata, a locality on the east coast of Argentina (Verzi et al. 2002), and from the late Holocene in Chubut province (Udrizar Sauthier et al. 2009) suggest dynamic distribution shifts in the recent past.

In recent years several studies have investigated the biology of *T. barrerae*, including its ecology (Mares et al. 1997a, 1997b; Ojeda et al. 1996, 1999; Torres-Mura et al. 1989), behavior (Berman 2003; Giannoni et al. 2000), physiology (Díaz and Ojeda 1999), and genetics (Gallardo et al. 1999, 2006). However, no studies have addressed levels of genetic differentiation among its isolated populations. To clarify aspects of population dynamics and differentiation it is critical to elucidate the historical and geographical context in which the evolution of the red viscacha rat took place. Phylogeographic analysis is an excellent method with which to investigate the historical factors that have shaped current diversity patterns and to document the evolution and biogeography of this unusual lineage.

The purpose of this study was to examine the genetic structure and patterns of genetic variation of *T. barrerae* across its distribution range, using sequences of the mitochondrial DNA (mtDNA) control region. The primary aims were to quantify levels of genetic variation and document the phylogeographic structure of *T. barrerae*, and to assess the demographic history of its populations.

Materials and Methods

Study area and sample collection.—Sixty animals were collected from 8 localities along the west-central portion of Argentina, including the Monte and Patagonian biomes (between 29°S and 39°S). Sample localities cover almost the entire range of the species across its northern, central, and southern regions (Fig. 1). Voucher specimens and tissue samples (Appendix 1) are housed in the mammal collections of the Instituto Argentino de Zonas Aridas (IADIZA), Centro de Ciencia y Técnica Mendoza (CCT), Mendoza, Argentina, and the Instituto de Ecología y Evolución, Universidad Austral, Valdivia, Chile. Animals collected during this study were treated following procedures approved by the American Society of Mammalogists (Gannon et al. 2007).

DNA extraction, polymerase chain (PCR) reaction amplification, and sequencing.—Genomic DNA was extracted from liver tissues with a standard phenol–chloroform method (Sambrook et al. 1989). An 800-base pair (bp) fragment of the mitochondrial control region was amplified by PCR from 60 individuals using primers that I designed: CYTB-RC-SENSE (5′-GAAAACAAACTCCTCAAATGAAG-3′) and RC-INT-TYMP (5′-TGGGTAGGGTAGGCGTAAAATT-3′). Amplifications were performed using a thermal cycler (Perkin Elmer) with the following parameters: initial denaturation at 94°C for 3 min; 33 cycles of 94°C (45 s), 51°C (1 min), and 72°C (1 min); and a final extension at 72°C (5 min). Double-stranded PCR products were purified with Wizard SV Gel and PCR Clean-Up System (Promega) and sequenced by Macrogen Inc. (Seoul, South Korea; www.macrogen.com). Sequencing reactions were carried out under BigDyeTM terminator cycling conditions and an ABI PRISM 3700 DNA automatic analyzer (PE Applied Biosystems, Foster City, California).

Data analyses.—Electropherograms were scored using PROSEQ version 2.91 (Filatov 2002) and aligned using the default parameters of CLUSTAL X (Thompson et al. 1997). Genetic diversity within populations was estimated as haplotype (h) and nucleotide diversity (π) using DNAsp 4.10 (Rozas et al. 2003). A file that included haplotypes and polymorphic sites was generated in the same program. Genetic distances between and within populations were examined using MEGA version 3.1 (Kumar et al. 2004). I chose a simple model of evolution, the K2P substitution model, which accommodates different transition and transversion probabilities (Kimura 1980). This model is used widely in mammal studies and so is useful for comparing divergence levels.

Phylogenetic relationships among haplotypes were reconstructed using maximum parsimony (MP) as executed in PAUP 4.0b10 (Swofford 2002) and Bayesian analyses (Rannala and Yang 1996), performed in MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck 2003). Trees were rooted with outgroup sequences from *Octodon degus* and *Octomys mimax*, both close relatives of *T. barrerae* (Wilson and Reeder 2005). MP trees were inferred with 200 replicates of a heuristic search with random addition sequences and tree–bisection–reconnection branch swapping. Node stability was tested using 1,000 bootstrap replicates. The best-fit substitution model of

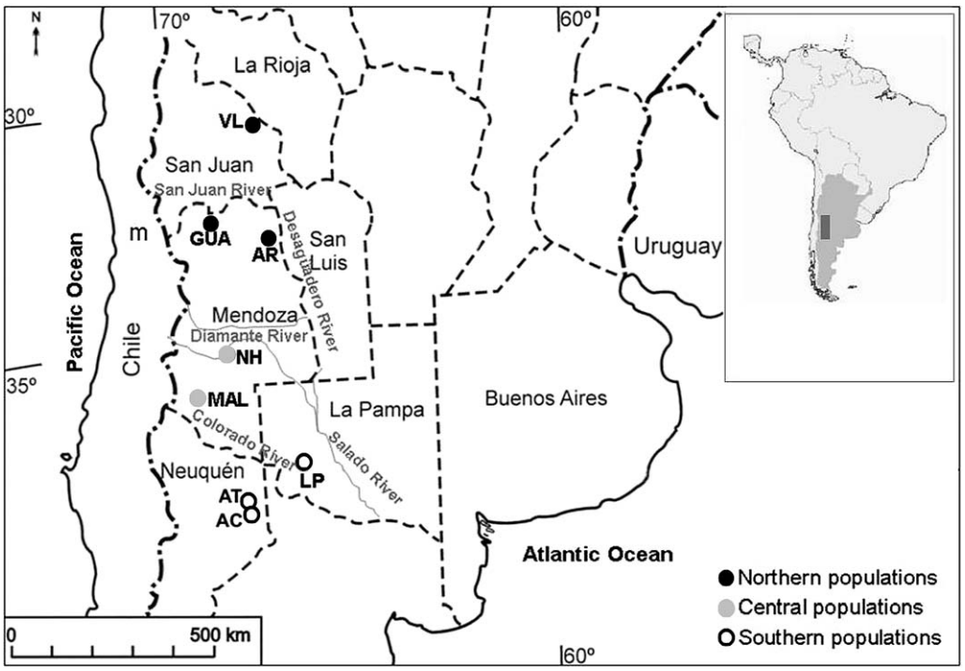

Fig. 1.—Geographical distribution of *Tympanoctomys barrerae* in Argentina. Dots indicate localities sampled for *T. barrerae*: VL: Valle de la Luna (30°5′S, 67°56′W), GUA: Guanacache (32°9′S, 68°12′W), AR: Arroyito (32°49′S, 67°17′W), MAL: Malargüe (35°41′S, 69°28′W), NH: Nihuil (35°1′S, 68°40′W), AT: Añelo Tero (38°10′S, 68°53′W), AC: Añelo Castillo (38°14′S, 68°57′W), LP: La Pampa (37°23′S, 67°12′W).

sequence evolution was identified with Modeltest 3.06 (Posada and Crandall 1998). The selected model under the Akaike information criterion (Akaike 1974) was Hasegawa, Kishino, Yano (Hasegawa et al. 1985) + invariable sites + gamma-distributed rate variation among sites (HKY + I + G) with base frequencies A = 0.3275, C = 0.2402, G = 0.1527, T = 0.2796. The proportion of invariable sites was I = 0.8751, and the gamma distribution shape parameter was G = 0.8042. Bayesian analysis was conducted under the best-fit model of evolution obtained with Modeltest and performed with 2 independent runs, each with 3 heated and 1 cold Markov chains. The analysis was run for 1,000,000 generations, sampling every 100 generations with a burn-in of 2,500 generations. To check that each run converged on a stable log-likelihood value I 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 estimates. Relationships among haplotypes also were examined in a haplotype network. The network was built using the median-joining approach implemented in Network 4.1.1.2 software (v. 4.200; http://www.fluxus-technology.com). The median-joining method uses a maximum parsimony approach to search for the shortest phylogenetic trees from a given data set (Bandelt et al. 1999).

To assess demographic history (expansion, stability) of the populations, I calculated Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997) values. ARLEQUIN software (Schneider et al. 2000) was used to conduct these tests and calculate the corresponding P-values. Significance of both tests was examined using 1,000 permutations. These neutrality tests assume that the population has been in mutation–drift balance for a long period of evolutionary time (Nei and Kumar 2000). When the population is not under mutation–drift equilibrium due to sudden expansion, these indices tend to have significantly negative values. I also conducted a ''mismatch distribution'' analysis. When a population has undergone rapid expansion it is expected to have a unimodal distribution, whereas a population that either is subdivided or in demographic equilibrium is expected to exhibit a multimodal distribution (Rogers and Harpending 1992). The test is based on 3 parameters: $\theta_0 = 2\mu N_0$, $\theta_1 = 2\mu N_1$, and τ; μ is the mutation rate, N_0 is the population size before expansion, N_1 is the population size after expansion, and τ is the time since expansion in mutational units (Rogers and Harpending 1992). I also used 1,000 coalescent simulations under the sudden expansion model to test the significance of the raggedness statistic (rg) for each mismatch distribution. Populations that have undergone a large expansion are expected to exhibit smooth, unimodal mismatch distributions and low raggedness values. More ragged mismatch distributions tend to result from large stable populations (Harpending 1994). Subsequently, after 1,000 replicates, the R_2 statistic and its associated probability (Ramos-Onsins and Rozas 2002) were used to assess the significance of the distribution's fit to that of an expanding population. R_2 is based on the differences between number of singleton mutations and mean number of nucleotide differences within a population (Ramos-Onsis and Rozas 2002), and is considered the best statistical test for detecting population growth with small sample sizes. Those tests were conducted using DNAsp 4.10 (Rozas et al. 2003).

A hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) was performed to investigate levels of population structure. Different analyses were conducted using ARLEQUIN, taking into account genetic distance between haplotypes and their frequencies. Individuals were grouped into populations, and populations into 3 different groups according to geographical location: northern group (VL: Valle de la Luna, GUA: Guanacache, and AR: Arroyito); central group (MAL: Malargüe and NH: Nihuil); southern group (AC: Añelo Castillo, AT: Añelo Tero, and LP: La Pampa). I then tested the effect of natural barriers (rivers) on the partitioning of genetic variance. I took into account 4 main rivers (Colorado, Salado, San Juan, and Diamante) that bisect the distribution of *T. barrerae*. The populations were separated into 5 groups defined us follows: AC: Añelo Castillo and AT: Añelo Tero; LP: La Pampa; MAL: Malargüe and NH: Nihuil; GUA: Guanacache and AR: Arroyito; and VL: Valle de la Luna. Finally, I conducted an AMOVA taking account of the major clades obtained from the phylogenetic analyses (see Fig. 1 for definition of the groups and rivers).

To examine the level of subdivision among localities I used the Φ_{ST} statistics developed for sequence data by Excoffier et al. (1992) using ARLEQUIN software and F_{ST} statistics based on sequence data (Hudson et al. 1995) using DNAsp 4.10 (Rozas et al. 2003). Estimates of the effective number of migrants (*Nm*) between pairs of populations were obtained in two ways: $<Nm>_F$ estimates migration from F_{ST} values, taking into account only haplotype frequencies; and $<Nm>_\Phi$ estimates migration from Φ_{ST}, taking into account haplotype frequencies and sequence divergence. The relationships between geographical distance and migration rate among populations were used to examine patterns of isolation by distance (Slatkin 1993) using ARLEQUIN. This program estimates the effective number of migrants between 2 populations as $<Nm>_F = (1 - \Phi_{ST})/(2 - \Phi_{ST})$ and correlates $<Nm>_F$ values with geographic distances. The significance of the correlation was assessed with Mantel's (1967) nonparametric test. Geographical distances were obtained using the program ''How far is it?'' (http://www.indo.com/distance/).

Results

Nucleotide diversity and haplotype distribution.—An 800-bp fragment of the mitochondrial control region was sequenced from 60 *T. barrerae* individuals. Alignment of the sequences showed 34 variable sites, including 13 singletons and 21 parsimony informative sites, which determined 26 haplotypes (GenBank accession numbers from GQ168691 to GQ168716; Table 1). No gaps (insertions–deletions) were needed in the alignment. For the total sample the haplotype diversity (*h*) was 0.92 and the nucleotide diversity (π) was 0.0086, indicating high haplotype and relatively moderate nucleotide diversity. At the population level, haplotype diversity ranged from 0.0 (for the central locality of Nihuil) to 1.0 (for the southern locality of La Pampa), whereas nucleotide diversity ranged from 0.0 (in Nihuil) to 0.0076 (in Añelo-Tero, a southern locality; Table 2). Among regional population groups central populations presented the lowest values of genetic diversity, whereas southern and northern populations displayed the highest values (see Table 2). No haplotype was present in all populations; only 4 haplotypes (H1, H3, H7, and H24) were shared among 2 or 4 populations (Table 1). The most frequent haplotype (H24) was found in 13 individuals and was present only at central localities (Malargüe and Nihuil). The 2nd-most-frequent haplotype (H3), which differs by 11 mutational steps from H24, was represented in 9 individuals from Añelo and La Pampa populations (southern distribution) and from Arroyito (northern distribution). I also found a high number (13) of unique haplotypes that differed in an elevated number of nucleotide changes from the most frequent haplotype (Table 1). Sequence divergences among localities indicated a close genetic similarity among central populations (0% divergence between Malargüe and Nihuil) but greater distances between these populations and northern or southern populations (1.1% to 1.5%). Northern versus southern populations presented intermediate distance values from 0.3% to 1%.

Phylogenetic relationships among haplotypes.—MP and Bayesian phylogenetic analyses produced trees that had similar overall topologies. I therefore present only the MP tree, which illustrates the relationships among the 26 haplotypes of *T. barrerae*. Several clades had bootstrap values in the range of 53% to 69% (Fig. 2). The analyses recovered 2 principal clades with low bootstrap support: clade A (central and southern distribution) and clade B (northern and southern distribution). The B clade contains nearly all of the haplotypes (20 of the total 26) and spans most of the geographic range. The haplotype network (Fig. 3) showed a similar topology, with 2 well-differentiated main haplotype groups separated by 7 mutational steps. The average divergence between haplotypes in clades A and B was 1.4% (Fig. 3).

Geographic genetic variation.—Global F_{ST} estimates among all localities presented a relatively high value (F_{ST} = 0.58) and a very low migration rate (*Nm* = 0.36). Moreover, pairwise estimates of gene flow showed values ranging from 0.02 between Arroyito and La Pampa localities to 0.94 between Nihuil and Añelo-Castillo localities. Overall, pairwise F_{ST} values indicated a moderate to high differentiation among subpopulations; highest differentiation was found between central versus northern and southern populations, with values ranging from 0.62 to 0.94 and $Nm < 1$. Pairwise F_{ST} comparisons between northern and southern populations showed a lower differentiation, with values ranging from 0.02 to 0.52 and $Nm > 1$ for most of the comparisons. The Mantel test did not show a significant correlation between pairwise estimates of log *Nm* (on the basis of F_{ST}; see ''Materials and Methods'') and log geographical distances ($r = -0.16$; $P > 0.05$; Fig. 4), suggesting no isolation by distance.

The AMOVA performed for localities grouped into 3 geographic regions (southern, central, and northern) showed a significant apportionment of genetic variance among regional groups. The among-group component of variance was

TABLE 1.—Polymorphic sites in the control region of mitochondrial DNA of *Tympanoctomys barrerae* from Argentina. Haplotype number, distribution of each haplotype by locality, and GenBank accession numbers are shown. See Fig. 1 for abbreviation of localities.

Haplotype no.	Position of polymorphic sites																																	Distribution of haplotypes by locality							GenBank accession no.
	7	24	28	29	51	55	116	148	168	169	188	222	228	255	258	285	296	303	307	308	312	316	329	333	363	368	386	421	422	424	567	619	622	640	VL	GUA	AR	NH	MAL	AC	AT	LP	
H1	T	T	G	C	A	C	G	C	C	A	A	T	C	T	A	C	A	T	A	G	T	A	T	G	T	G	C	C	T	C	A	A	C	G						2	2		GQ168694
H2	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.							1		GQ168692
H3	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			2			1	5	1	GQ168702
H4	.	.	A	T	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.								1	GQ168706
H5	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.						1			GQ168716
H6	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	.							1		GQ168695
H7	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.						3	1		GQ168698
H8	C	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.								1	GQ168705
H9	.	.	A	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.							1		GQ168693
H10	.	.	A	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.								1	GQ168707
H11	.	.	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.		2							GQ168715
H12	.	.	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			2						GQ168711
H13	.	.	A	.	.	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.								1	GQ168703
H14	.	.	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.								1	GQ168704
H15	.	.	A	.	T	.	T	.	.	.	.	T	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			1						GQ168713
H16	.	.	A	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	A	.	G	.	.	.	.	.	.	.	A	.	.	.	.	.	1								GQ168700
H17	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.	A	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.			1						GQ168712
H18	.	.	A	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	A	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.		2							GQ168714
H19	.	.	A	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	C	.	T	.	.	.	G	.	.	.	.	3								GQ168701
H20	.	.	A	.	.	.	.	.	G	.	.	T	.	.	T	.	C	.	.	.	.	C	A	C	.	T	.	.	.	G	.	.	.	.	1								GQ168699
H21	.	C	A	.	.	.	.	.	.	.	.	C	T	.	.	.	C	.	A	.	G	.	.	C	.	T	.	.	.	.	G	T	T								3		GQ168691
H22	.	C	A	.	.	.	.	.	.	.	.	C	T	.	T	.	C	.	A	.	G	.	.	C	.	T	.	.	.	.	G	T	T								1		GQ168697
H23	.	C	A	.	.	.	.	.	.	.	.	.	T	.	.	.	C	.	A	.	G	.	A	C	.	T	.	.	.	.	G	T	T								1		GQ168696
H24	.	C	A	.	.	.	T	.	.	.	.	T	.	.	.	.	C	.	A	.	G	.	.	C	.	T	.	.	.	.	G	T	T					4	9				GQ168708
H25	.	C	A	.	.	.	T	.	.	.	.	T	.	.	.	.	C	.	A	.	G	.	.	C	.	T	T	.	.	.	G	T	T						2				GQ168709
H26	.	C	A	.	.	.	T	.	.	.	.	T	.	.	.	G	C	.	A	.	G	.	.	C	.	T	.	.	.	.	G	T	T						1				GQ168710

Table 2.—Number of sequences, polymorphic sites, haplotype and nucleotide diversity, and values of Tajima's *D* and Fu's F_S tests from each locality of *Tympanoctomys barrerae*. See Fig. 1 for abbreviation of localities.

Population	N° Sequences	Polymorphic sites	Haplotype diversity	Nucleotide diversity	Tajima's *D*		Fu's *Fs*	
					D	*P* ($D_S < D_O$)	*Fs*	*P* ($F_S < F_O$)
North	15	19	0.93	0.0070	−0.15	0.46	−0.75	0.33
Central	16	2	0.34	0.0004	−1.03	0.17	−0.97	0.08
South	29	23	0.91	0.0060	−0.60	0.30	−3.71	0.06
VL	5	13	0.70	0.0067	−0.97	0.19	2.46	0.88
GUA	4	7	0.66	0.0058	2.17	0.99	3.85	0.94
AR	6	8	0.86	0.0039	−0.62	0.34	0.31	0.50
NH	4	0	0.00	0.0000	0.00	0.00	0.00	0.00
MAL	12	2	0.43	0.0006	−0.84	0.25	−0.72	0.09
AT	16	18	0.88	0.0076	0.50	0.72	−0.24	0.44
AC	7	3	0.81	0.0016	0.40	0.68	−0.91	0.14
LP	6	9	1.0	0.0042	−0.81	0.24	−2.98	0.01

50.87%, and the remaining percentage of variation was partitioned as follows: 38.61% within populations and 10.53% among populations within groups (Table 3). An AMOVA performed on localities assembled into groups separated by rivers showed a significant apportionment of genetic variance among groups. The among-group component of variance was 54.69% and the remaining percentage of variation was partitioned as follows: 41.19% within populations and 4.11% among populations within groups (Table 3). Finally, the AMOVA taking into account the 2 major clades obtained from the phylogenetic analyses showed an among-group variance component of 72.65%, with the remaining percentage of variation partitioned as follows: 16.92% within populations and 10.43% among populations within groups (Table 3).

Population demographic history.—For the entire sample both Tajima's and Fu's neutrality tests were negative but not significant ($D = -0.16$, $P = 0.49$; $F_S = -6.27$, $P = 0.06$), and the R_2 test was nonsignificant ($R_2 = 0.096$; $P = 0.49$). Some of the populations presented negative values for these statistics, but only the outcome for the southern LP was significant (Table 2). Among regional groups the values for these statistics were in all cases negative and nonsignificant (Table 2). Clade A showed negative values for both tests, but they were not significant ($D = -0.93$, $P = 0.18$; $F_S = -1.70$, $P = 0.09$), and the R_2 test was nonsignificant ($R_2 = 0.094$; $P = 0.075$). Clade B showed negative values for both tests, but only F_S was significant ($D = -1.39$, $P = 0.07$; $Fs = -9.60$, $P < 0.001$), and the R_2 test was significant ($R_2 = 0.063$; $P = 0.045$). The mismatch distribution analysis for the whole

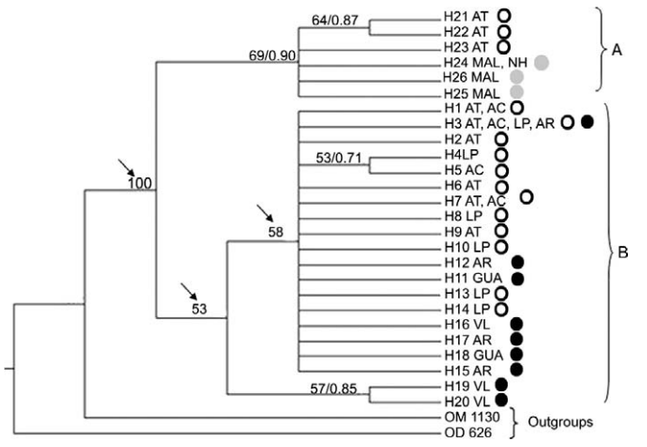

Fig. 2.—Maximum-parsimony tree of 26 mitochondrial DNA haplotypes for *T. barrerae*. *Octodon degus* (GenBank accession number GQ168717) and *Octomys mimax* (GenBank accession number GQ168718) were used as outgroups. Numbers on branches represent bootstrap support after 1,000 iterations (left of the diagonal) and Bayesian posterior probabilities (right of the diagonal). Arrows indicate nodes that were not recovered in the Bayesian analysis. Dots indicate geographic sample locations: black, northern populations; gray, central populations; and white with black border, southern populations. A and B refer to clades discussed in the text.

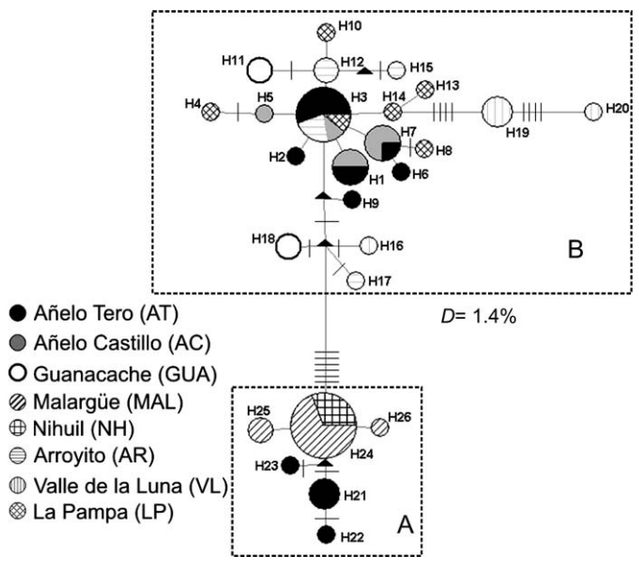

Fig. 3.—Haplotype network of all 26 haplotypes recovered from 8 populations of *T. barrerae*. The sizes of circles are related positively to haplotype frequency. Shading indicates populations. Each bar through the solid line represents 1 nucleotide difference between haplotypes. *D* indicates the average percentage divergence between haplotypes in clades A and B.

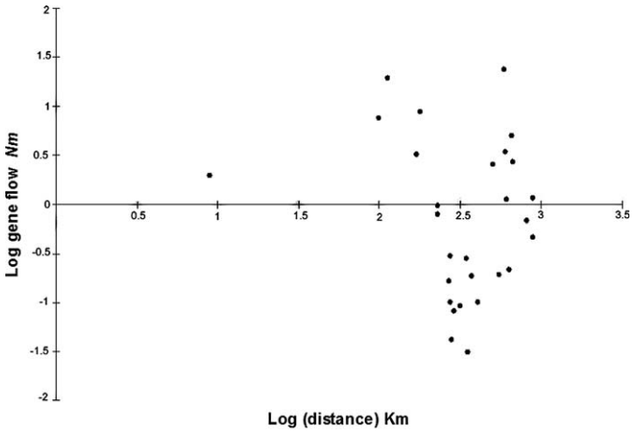

FIG. 4.—Relationship between pairwise geographical distances and estimates of gene flow *Nm* for *T. barrerae*, on the basis of F_{ST} from mitochondrial control region sequences.

sample suggests a stable demographic history, indicated by a bimodal distribution and a high average number of pairwise differences ($k = 6.929$; Fig. 5a). However, the distribution was not significantly different from one generated under a null model of population expansion. The raggedness value was not significant ($rg = 0.024$, $P > 0.05$) and failed to reject the hypotheses of population expansion. However, the mismatch distributions for clades A and B individually suggest population expansions, indicated by a unimodal distribution and a low number of pairwise differences ($k_{\text{clade A}} = 1.17$ and $k_{\text{clade B}} = 3.77$; Figs. 5b and c). The raggedness value was not significant for clade A ($rg = 0.075$, $P > 0.05$) and was approximately that expected under an expansion model. However, clade B displayed a significantly low raggedness value ($rg = 0.01$, $P < 0.05$) and poor fit to a model of recent expansion. The τ parameter, which describes the relative time since expansion (in mutational units) of the geographic range, was smaller in clade A than in clade B ($\tau_{\text{clade A}} = 0.870$; $\tau_{\text{clade B}} = 1.975$).

DISCUSSION

The scattered ''patchy'' populations of the red viscacha rat, *T. barrerae*, show different levels of genetic variation across their geographic range (29°S to 39°S). The lowest genetic diversity was recorded for the central populations (i.e., Malargüe and Nihuil). Only a single mtDNA haplotype was found in Nihuil locality, and it was shared with the population of Malargüe, at a distance of 100 km. However, most haplotypes recorded for the southern and northern sites were unique to those populations, resulting in high haplotype diversity. In total, 26 haplotypes were found for 8 populations of *T. barrerae*. The number of haplotypes per population (3.25) was comparable with the value (3) reported for the subterranean tuco-tuco *Ctenomys australis* (Mora et al. 2006). However, these values were much higher than those reported for the fossorial octodontid *Spalacopus cyanus* (1.86—Opazo et al. 2008), the subterranean *Ctenomys pearsoni* (1.91—Tomasco and Lessa 2007), and *Ctenomys rionegrensis* (1.89—Wlasiuk et al. 2003). Each haplotype of *T. barrerae* occurred in an average of 1.9 individuals in the southern populations and 1.6 individuals in the northern populations. In contrast, central populations lacked variation, and each haplotype occurred in an average of 5.3 individuals. A similar pattern of haplotype diversity was found in the Stephens' kangaroo rat (*Dipodomys stephensi*), an endangered species with a restricted range in the interior coastal valleys of Southern California (Metcalf et al. 2001).

In contrast to the high levels of haplotype variation, nucleotide diversity in *T. barrerae* was low to moderate. Among regional population groups southern and northern populations had >10 times higher nucleotide diversity than the central group (Table 2). Nucleotide diversity values reported in this study were similar to those reported for *S. cyanus* (Opazo et al. 2008) and the fossorial tuco-tuco *C. australis* (Mora et al. 2006). This pattern of low nucleotide diversity and high haplotype diversity indicates that the populations are composed of many closely related haplotypes. The statistical parsimony network corroborated this pattern; 22 of 26 haplotypes were separated by 1–2 mutational steps, and the remaining 4 haplotypes were separated by 5 or 7 mutational steps. Grant and Bowen (1998) suggested that patterns of low nucleotide diversity and high haplotype diversity are indicative of a low effective population size followed by expansion.

Although central populations (i.e., Malargüe and Nihuil) showed the lowest genetic variability, they also displayed the highest genetic differentiation when compared with northern

TABLE 3.—Hierarchical analyses of molecular variance (AMOVAs) for 3 geographical groups: northern group [VL, GUA, and AR], central group [MAL and NH], southern group [AC, AT, and LP], and for 5 regions limited by rivers. Significance of variance component (*P*) was tested by permutation according to Excoffier et al. (1992). See Fig. 1 for abbreviation of localities.

Source of variation	% of variation	Fixation indices (Φ-statistics)	*P*
Among regional groups: [north] [central] [south]	50.87	0.508	0.005
Among populations within groups	10.53	0.214	0.009
Within populations	38.61	0.613	<0.001
Among 5 regions limited by rivers: [AC-AT] [LP] [MAL-NH] [AR-GUA] [VL]	54.69	0.546	0.02
Among populations within groups	4.11	0.090	<0.005
Within populations	41.19	0.588	<0.001
Among 8 populations without hierarchical levels: [AC] [AT] [LP] [MAL] [NH] [AR] [GUA] [VL]	55.87	0.558	<0.001

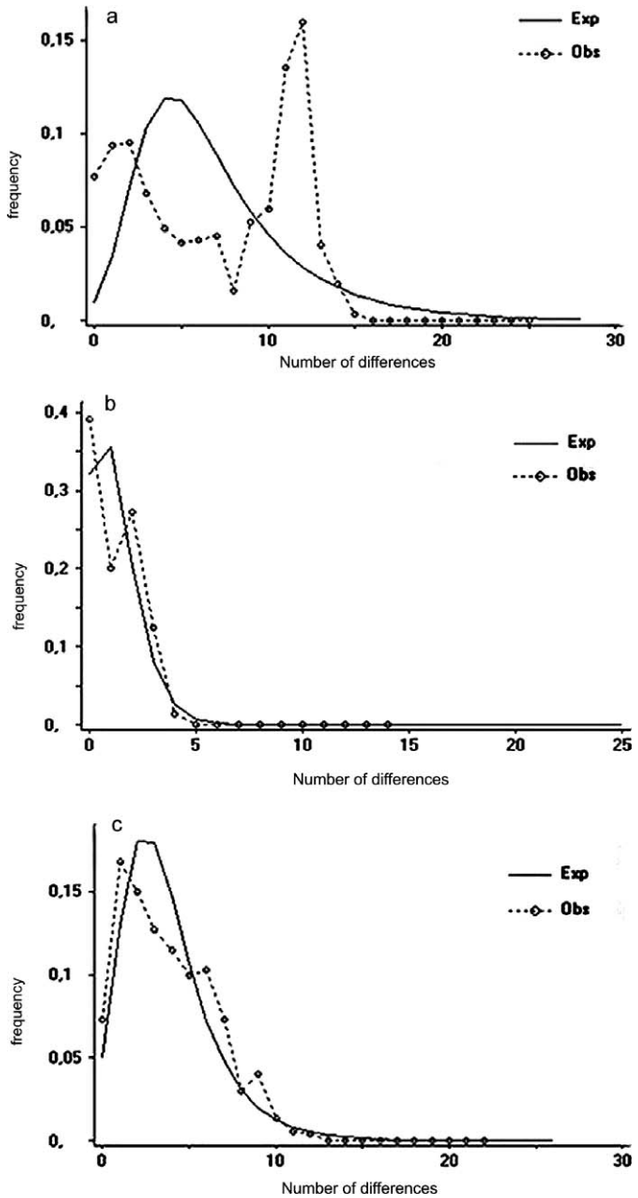

Fig. 5.—Mismatch distributions (Obs) for a) the entire sample, b) clade A, and c) clade B of *T. barrerae*. The expected (Exp) frequency is based on a population expansion model with θ_0 initial = 0, θ_1 final = 1.000, and τ = 0.870 and 1.975 for clades A and B, respectively.

and southern populations, on the basis of divergence between haplotypes. This pattern of genetic differentiation was corroborated by the AMOVAs. Central populations differed from northern and southern ones in haplotype frequency and composition and were critical population units in determining the significance of differences among all 3 regions. When I excluded the central populations from the AMOVA analyses, the difference between southern and northern regions became insignificant (2.46%; $P = 0.18$). The northern population of Valle de La Luna also showed high values of genetic differentiation compared with other populations, but was not significant in determining any differences among regions. When I excluded the Valle de La Luna population from the AMOVA, differences among regions were still highly significant (59.24%; $P = 0.01$).

Genetic differentiation commonly is observed in widely distributed species (Avise et al. 1987). Narrowly distributed species are generally expected to have lower levels of genetic differentiation across their range (Frankham 1996). Although the geographic range of *T. barrerae* lies between 29°S and 43°S, populations within that range have a patchy distribution (Diaz et al. 2000; Ojeda et al. 2007). In this study the levels of genetic differentiation in *T. barrerae* were moderate to high. The overall F_{ST} value is indicative of substantial population structure. Comparisons between central localities (Malargüe and Nihuil) versus northern and southern localities yielded the highest F_{ST} values. Estimates of migration rates *Nm* calculated from F_{ST} values for these populations were low ($Nm < 1$), reflecting an absence of gene flow between them. Furthermore, the absence of a pattern of isolation by distance, shown by an insignificant correlation between pairwise estimates of *Nm* and geographical distances (Fig. 4), suggests that the species is not at equilibrium between gene flow and genetic drift. Moreover, failure to detect a pattern of isolation by distance when relatively large values of *Nm* are found suggests that a species recently has colonized parts of the area it currently occupies. No pattern of isolation by distance with only low values of *Nm* would suggest that ongoing gene flow does not exist (Slatkin 1993). Lack of a pattern of isolation by distance in the red viscacha rat could be influenced by recent expansion into new areas and by interrupted gene flow, especially between central and northern or southern populations. Consistent with this, most genetic variance in red viscacha rats was among regions. Also, significant differentiation among regional groups indicates that natural barriers (e.g., rivers) might be preventing dispersion among localities.

Populations with low levels of haplotype and nucleotide diversity might have experienced a prolonged or severe demographic bottleneck in recent times (Avise 2000). The low levels of genetic variation in central populations suggest that they might be recovering from a catastrophic, stochastic event during their recent history. Potential causes for such a bottleneck include Pleistocene glaciations and nearby volcanism. The activity of the Andean volcanic arch in the southern part of Mendoza province, particularly near Malargüe, was intense during much of the Late Tertiary (Nullo et al. 2002; Sruoga et al. 1993) and persisted until the Holocene (10,000 years ago). Moreover, records exist of volcanic activity between 7,000 and 500 years ago (Mikkan 2004). The effect of volcanism on reducing the genetic diversity of natural populations has been shown in the fossorial tuco-tuco *Ctenomys maulinus* in east-central Patagonia (Gallardo and Köhler 1994; Gallardo et al. 1995).

Climate change and habitat loss also contribute to reductions in faunal diversity (McLaughlin et al. 2002). Good examples can be found in rodents of how species responded to major climate changes during the Holocene and how habitat

changes affected the genetic variability of populations (Chan et al. 2005; Hadly et al. 2004). Such is the case of the tuco-tuco *Ctenomys sociabilis*, restricted to a small area in Patagonia that has a complex history of environmental change associated with volcanism. Responses to that environmental change include decreased population size, range contraction, and loss of genetic diversity (Chan et al. 2005). To date, no studies have been conducted on *T. barrerae* that account for how climate change affects the genetic diversity of populations. However, a few studies related to fossil records have addressed past distributions (Udrizar Sauthier et al. 2009; Verzi et al. 2002). The fossil species *Tympanoctomys cordubensis* has been reported from Pleistocene deposits (0.9–0.78 mya) off the Atlantic coast in Argentina, where *Tympanoctomys* no longer occurs. The authors suggested that the present patchy distribution of *Tympanoctomys* may be relictual, and the peculiar habitats occupied by these rodents probably represent interglacial refuges (Verzi et al. 2002). However, recent fossil remains assignable to *T. barrerae* from 3 late Holocene localities along the Chubut River in Patagonia indicate an expanded paleodistribution and suggest local extinction of populations during the last 100 years (Udrizar Sauthier et al. 2009). Potential causes for the extirpation of *Tympanoctomys* include overgrazing by sheep, which were introduced to Patagonia at the beginning of the 20th century (Aguado 2005).

Tympanoctomys barrerae shows moderate phylogeographic structure. The MP tree (Fig. 2) and haplotype network (Fig. 3) were concordant and recognized 2 main haplotype groups: clade A, including haplotypes from central populations (Malargüe-Nihuil) and 3 haplotypes from the southern locality Añelo Tero; and clade B, including haplotypes from southern and northern populations (Añelo Tero, Añelo Castillo, and La Pampa; Valle de la Luna, Guanacache, and Arroyito). None of the populations were reciprocally monophyletic, indicating that the populations had not been isolated for a long enough time.

Demographic analyses for each clade produced unimodal mismatch distributions (Figs. 5b and c) and negative D and F_S values, all of which are associated with population expansion (Rogers and Harpending 1992). This recent population growth event is more evident in clade A, which displays a unimodal distribution, low number of pairwise differences, and a nonsignificant raggedness (*rg*) index. Small τ-values estimated for both clades (more evident in clade A) also indicate recent expansions. Population expansion in clade B seems to be older than that of clade A, as suggested by a higher number of pairwise differences and a more significant raggedness (*rg*) index. Both expansions could have taken place in southern populations, presumably in the area of Añelo-Tero. The first (clade B $\tau = 1.975$) covered a large part of *T. barrerae*'s present distribution, reaching its northern and southern limits. The second (clade A $\tau = 0.870$) was more recent and covered only the central part of the range.

I cannot discard alternatives to the demographic interpretations above. In particular, I cannot rule out departures from strict neutrality (e.g., selective sweeps and selection against slightly deleterious mutations). mtDNA has been assumed by many to be neutral, but Ballard and Kreitman (1995) concluded that this assumption follows from a series of plausibility arguments connecting features of mtDNA evolution with misconceptions about the neutral theory. Deviations from a strictly neutral model of evolution have been found for mtDNA in a variety of organisms (Nachman 1998; Rand 2001; Rand and Kann 1998). Even the noncoding control region cannot be assumed to have strictly neutral variation because of its linkage to the rest of the genome (Ballard and Kreitman 1995; Ballard and Rand 2005). When a statistically significant departure from selective neutrality is found in a gene (or a region of the genome), often two equally plausible alternative hypotheses can be offered in accord with the data, one involving natural selection and one involving one or more of the demographic or population factors (Kreitman 2000). Both selective sweeps and selection against slightly deleterious alleles can produce patterns of haplotype diversity similar to those produced by population expansion (Wlasiuk et al. 2003). These alternatives cannot be distinguished with standard statistics such as Tajima's D or Fu's F_S.

Taken as a whole, the information above suggests the following scenario for the geographic differentiation of *T. barrerae*. This species expanded from a restricted area in the recent past as indicated by the lack of equilibrium between migration and genetic drift and by signatures of sudden population expansion. The ancestral areas could be recently discovered fossil localities in Chubut (Patagonia) or other southern and northern refuges during the Pleistocene–Holocene transition. The presence of southern haplotypes in both clades suggests a possible southern origin, followed by expansion events involved in the colonization of new habitats. As noted above, one of the expansions occurred early and covered much of the current distribution (Fig. 2 clade B; Fig. 5c). The second was a more recent and sudden expansion and was restricted to the central part of the distribution (Fig. 2 clade A; Fig. 5b). When gene flow among subpopulations is limited the most geographically widespread haplotypes should be the oldest and most ancestral ones, whereas haplotypes restricted to single locations should be of more recent origin (Neigel et al. 1991). In *T. barrerae* the area comprising Añelo, La Pampa, and Arroyito could be ancestral, given that these localities share the 2nd most frequent and most geographically widespread haplotype (H3). Since the last expansion central populations have differentiated essentially in isolation, as reflected in the estimates of gene flow ($Nm < 1$) between central and northern or southern populations. High Nm estimates (>1) were obtained for the central localities of Malargüe and Nihuil. These 2 populations, separated by 100 km, seem to behave as one. However, their low genetic variability could be due to a possible bottleneck caused by a recent catastrophic event.

Several attributes of *T. barrerae* (e.g., patchy distribution, habitat and trophic specialization, low population density) increase the vulnerability of its populations (Ojeda et al. 1996). The potential loss of genetic information in these

populations should be considered in biodiversity assessments (Diaz and Ojeda 2000). In particular, the genetic distinctness of central populations could have important implications for the conservation and management of the red viscacha rat.

Resumen

Tympanoctomys barrerae o rata vizcacha colorada, es un roedor octodontido endémico de las regiones áridas del centro-oeste y sur de Argentina. Es una especie solitaria que habita en cuevas complejas, construidas en suelos blandos. Las poblaciones de *T. barrerae* se distribuyen a bajas densidades y en parches asociados a cuencas salinas y dunas en los desiertos de tierras bajas del Monte y Patagonia. El objetivo de este estudio fue investigar la estructura genética y biogeografía de este roedor octodontido especialista del desierto. Para ello se evaluó la variación genética, secuenciando un fragmento de 800 pb de la región control del ADN mitocondrial (ADNmt) a partir de 60 muestras de individuos de 8 de localidades a lo largo del rango de distribución de la especie. Las relaciones entre los haplotipos fueron inferidas a partir de análisis filogenéticos (máxima parsimonia, bayesianos y redes). Se estudio la estructura genética e historia demográfica a través de diferentes estadísticos, distribuciones pareadas, pruebas de neutralidad (tests de Tajima y Fu) y AMOVAs. Se encontraron 26 haplotipos, la mayoría de ellos restringidos a poblaciones únicas. La presencia de haplotipos no compartidos fue consistente con las bajas tasas de migración. A lo largo del rango de distribución (entre 29 ° S y 39 ° S), las poblaciones sur y norte presentaron los valores más altos de diversidad genética en relación a las poblaciones del centro. Las poblaciones de *T. barrerae* mostraron de moderada a alta diferenciación genética, siendo los haplotipos de las poblaciones centrales los que determinaron en mayor medida dicho grado de diferenciación. Los análisis de AMOVA indicaron un nivel moderado de estructura geográfica de las poblaciones. La baja diversidad haplotípica y nucleotídica presentada en las poblaciones centrales, podría sugerir un posible cuello de botella asociado a las glaciaciones del pleistoceno o a una intensa actividad volcánica en esta parte central de la distribución. La estructura filogeográfica fue moderada, los análisis recobraron 2 clados principales: A (con distribución centro y sur) y B (con distribución norte y sur). La mayoría de las poblaciones fueron polifiléticas, sugiriendo que no habrían permanecido aisladas durante un tiempo suficiente como para alcanzar la monofília recíproca. Análisis demográficos realizados en los clados (A y B) sugieren una reciente historia de expansión poblacional.

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Literature Cited

Aguado, A. 2005. La colonización del oeste de la Patagonia central. Departamento Río Senguer, Chubut. 1890–1919. Fondo Editorial Provincial, Gobierno de Chubut 1–175.

Akaike, H. 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 19:716–723.

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

Avise, J. C., et al. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18:489–522.

Ballard, J. W. O., and M. Kreitman. 1995. Is mitochondrial DNA a strictly neutral marker? Trends in Ecology and Evolution 10:485–488.

Ballard, J. W. O., and D. M. Rand. 2005. The population biology of mitochondrial DNA and its phylogenetic implications. Annual Review of Ecology and Systematics 36:621–42. 4

Bandelt, H. J., P. Forster, and A. Röhl. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16: 37–48.

Berman, S. L. 2003. A desert octodontid rodent, *Tympanoctomys barrerae*, uses modified hairs for stripping epidermal tissue from leaves of halophytic plants. Journal of Morphology 257:53–61.

Chan, Y. L., E. A. Lacey, O. P. Pearson, and E. A. Hadly. 2005. Ancient DNA reveals Holocene loss of genetic diversity in a South American rodent. Biology Letters 1:423–426.

Contreras, L. C., J. C. Torres-Mura, and A. E. Spotorno. 1990. The largest known chromosome number for a mammal in a South American desert rodent. Experientia 46:506–509.

Contreras, L. C., J. C. Torres-Mura, and J. L. Yáñez. 1987. Biogeography of octodontid rodents: an eco-evolutionary hypothesis. Pp. 401–411 in Studies in neotropical mammalogy: essays in honor of P. Hershkovitz (B. D. Patterson and R. E. Timm, eds.). Fieldiana: Zoology, new series 39.

Díaz, G. D., and R. A. Ojeda. 1999. Kidney structure of Argentine desert rodents. Journal of Arid Environments 41:453–461.

Díaz, G. D., and R. A. Ojeda. 2000. Libro rojo de los mamíferos amenazados de Argentina, SAREM (Sociedad Argentina para el Estudio de los Mamíferos). Mendoza, Argentina.

Díaz, G. D., R. A. Ojeda, M. H. Gallardo, and S. M. Giannoni. 2000. *Tympanoctomys barrerae*. Mammalian Species 646:1–4.

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.

Filatov, D. 2002. A software for preparation and evolutionary analysis of DNA sequence data sets. Molecular Ecology Notes 2:621–624.

FRANKHAM, R. 1996. Relationship of genetic variation to population size. Conservation Biology 10:1500–1508.

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

GALLARDO, M. H., J. W. BICKHAM, R. L. HONEYCUTT, R. A. OJEDA, AND N. KÖHLER. 1999. Discovery of tetraploidy in a mammal. Nature 401:341.

GALLARDO, M. H., C. A. GONZÁLEZ, AND I. CEBRIÁN. 2006. Molecular cytogenetics and allotetraploidy in the red vizcacha rat, *Tympanoctomys barrerae* (Rodentia, Octodontidae). Genomics 88:214–221.

GALLARDO, M. H., AND N. KÖHLER. 1994. Demographic changes and genetic losses in populations of a subterranean rodent (*Ctenomys maulinus brunneus*) affected by a natural catastrophe. Zeitschrift für Säugetierkunde 59:358–365.

GALLARDO, M. H., N. KÖHLER, AND C. ARANEDA. 1995. Bottleneck effects in local populations of fossorial *Ctenomys* affected by vulcanism. Heredity 74:638–646.

GALLARDO, M. H., ET AL. 2004. Whole-genome duplications in South American desert rodents (Octodontidae). Biological Journal of the Linnean Society 82:443–451.

GALLARDO, M. H., D. E. UDRIZAR SAUTHIER, A. A. OJEDA, AND U. F. J. PARDIÑAS. 2009. Discovery of desert-adapted *Tympanoctomys barrerae* in central Patagonia, Argentina. Mammalia 73:158–161.

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.

GIANNONI, S. M., C. E. BORGHI, AND R. A. OJEDA. 2000. Foraging ecology of *Tympanoctomys barrerae*. Journal of Arid Environments 46:117–121.

GRANT, W. S., AND B. W. BOWEN. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. Genetics 89:415–426.

HADLY, E. A., ET AL. 2004. Genetic response to climatic change: insights from ancient DNA and phylochronology. Public Library of Science Biology 2:1600–1609.

HARPENDING, H. C. 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Human Biology 66:591–600.

HASEGAWA, M., K. KISHINO, AND T. YANO. 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22:160–174.

HONEYCUTT, R. L., D. L. ROWE, AND M. H. GALLARDO. 2003. Molecular systematics of the South American Caviomorph rodents: relationships among species and genera in the family Octodontidae. Molecular Phylogenetics and Evolution 26:476–489.

HUDSON, R. R., M. SLATKIN, AND W. P. MADDISON. 1992. Estimation of levels of gene flow from DNA sequence data. Genetics 132:583–589.

INTERNATIONAL UNION FOR THE CONSERVATION OF NATURE. 2008. IUCN red list of threatened animals. International Union for the Conservation of Nature, Gland, Switzerland.

KIMURA, M. 1980. A simple model for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111–120.

KREITMAN, M. 2000. Methods to detect selection in populations with applications to the human. Annual Reviews of Genomics and Human Genetics 1:539–59.

KUMAR, S., K. TAMURA, AND M. NEI. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics 5:150–163.

MANTEL, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:209–220.

MARES, M. A. 1985. Mammal faunas of xeric habitats and the great American interchange. Pp. 489–520 in The great American biotic interchange (F. G. Stehli and S. D. Webb, eds.). Plenum Publishing Corporation, New York.

MARES, M. A., J. K. BRAUN, AND R. CHANNELL. 1997a. Ecological observations on the octodontid rodent, *Tympanoctomys barrerae*, in Argentina. Southwestern Naturalist 42:488–504.

MARES, M. A., R. A. OJEDA, C. E. BORGHI, S. M. GIANNONI, G. B. DÍAZ, AND J. K. BRAUN. 1997b. A desert rodent uses hair as a tool to overcome halophytic plant defenses. BioScience 47:699–704.

MCLAUGHLIN, J. F., J. J. HELLMANN, C. L. BOGGS, AND P. R. EHRLICH. 2002. Climate change hastens population extinctions. Proceedings of National Academy of Science 99:6070–6074.

METCALF, A. E., L. NUNNEY, AND B. C. HYMAN. 2001. Geographic patterns of genetic differentiation within the restricted range of the endangered Stephens' kangaroo rat *Dipodomys stephensi*. Evolution 55:1233–1244.

MIKKAN, R. 2004. Payunia está activa. Micro Semanario de Educación, Ciencia y Tecnología (Educyt). Facultad de Ciencias Exactas y Naturales-UBA. Año 15. Numero 518.

MORA, M. S., E. P. LESSA, M. J. KITTLEIN, AND A. I. VASALLO. 2006. Phylogeography of the subterranean rodent *Ctenomys australis* in sand-dune habitats: evidence of population expansion. Journal of Mammalogy 87:1192–1203.

NACHMAN, M. W. 1998. Deleterious mutations in animal mitochondrial DNA. Genetica 102/103:61–69.

NEI, M., AND S. KUMAR. 2000. Molecular evolution and phylogenetics. Oxford University Press, New York.

NEIGEL, J. E., R. M. BALL, JR., AND J. C. AVISE. 1991. Estimation of single generation dispersal migration distances from geographic variation in animal mitochondrial DNA. Evolution 45:423–432.

NULLO, F. E., G. C. STEPHENS, J. OTAMENDI, AND P. E. BALDAUF. 2002. El volcanismo del Terciario superior del sur de Mendoza. Revista de la Asociación Geológica Argentina 57:119–132.

OJEDA, A. A., M. H. GALLARDO, F. MONDACA, AND R. A. OJEDA. 2007. Nuevos registros de *Tympanoctomys barrerae* (Rodentia, Octodontidae). Mastozoología Neotropical 14:267–270.

OJEDA, R. A., C. E. BORGHI, G. B. DÍAZ, S. M. GIANNONI, M. A. MARES, AND J. K. BRAUN. 1999. Evolutionary convergence of the highly adapted desert rodent *Tympanoctomys barrerae* (Octodontidae). Journal of Arid Environments 41:443–452.

OJEDA, R. A., AND G. B. DÍAZ. 1997. La categorización de los mamíferos de Argentina. Pp. 73–154, en Libro rojo de mamíferos y aves amenazados de Argentina (J. G. Fernandez, R. A. Ojeda, R. Fraga, G. B. Diaz y Baigún, eds.). Administración de Parques Nacionales, Buenos Aires, Argentina.

OJEDA, R. A., J. GONNET, C. BORGHI, S. GIANONNI, C. CAMPOS, AND G. DÍAZ. 1996. Ecological observations of the red vizcacha rat *Tympanoctomys barrerae* in two desert habitats of Argentina. Mastozoología Neotropical 3:183–191.

OPAZO, J. C. 2005. A molecular timescale for caviomorph rodents (Mammalia, Hystricognathi). Molecular Phylogenetics and Evolution 37:932–937.

OPAZO, J. C., M. P. BUGUEÑO, M. J. CARTER, R. E. PALMA, AND F. BOZINOVIC. 2008. Phylogeography of the subterranean rodent *Spalacopus cyanus* (Caviomorpha, Octodontidae). Journal of Mammalogy 89:837–844.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.

RAMOS-ONSINS, S. E., AND J. ROZAS. 2002. Statistical properties of new neutrality tests against population growth. Molecular Biology and Evolution 19:2092–2100.

RAND, D. M. 2001. The units of selection on mitochondrial DNA. Annual Reviews of Ecology and Systematics 32:415–48.

RAND, D. M., AND L. M. KANN. 1998. Mutation and selection at silent and replacement sites in the evolution of animal mitochondrial DNA. Genetica 102/103:393–407.

RANNALA, B., AND Z. YANG. 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. Journal of Molecular Evolution 43:304–311.

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.

ROZAS, J., J. C. SÁNCHEZ-DELBARRIO, X. MESSEGUER, AND R. ROZAS. 2003. DnaSP. DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497.

SAMBROOK, J., E. F. FRITSCH, AND T. MANIATIS. 1989. Molecular cloning: a laboratory manual. Vol. 1. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

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

SLATKIN, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264–279.

SRUOGA, P., P. GUERSTEIN, AND A. BERMUDEZ. 1993. Riesgo volcánico. Pp. 659–667 en Geología y recursos naturales de Mendoza, relatorio XII Congreso Geológico Argentino (V. Ramos, ed.). Mendoza, Argentina.

SVARTMAN, M., G. STONE, AND R. STANYON. 2005. Molecular cytogenetics discards polyploidy in mammals. Genomics 5:425–430.

SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

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

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 25:4876–4882.

TOMASCO, I., AND E. P. LESSA. 2007. Phylogeography of the tuco-tuco *Ctenomys pearsoni*: mtDNA variation and its implication for chromosomal differentiation. Pp. 859–882 in The quintessential naturalist: honoring the life and legacy of Oliver P. Pearson (D. A. Kelt, E. Lessa, J. A. Salazar-Bravo, and J. L. Patton, eds.). University of California Publications in Zoology Series, Berkeley, California.

TORRES, M. R., C. A. BORGHI, S. M. GIANNONI, AND A. PATTINI. 2003. Portal orientation and architecture of burrows in *Tympanoctomys barrerae* (Rodentia, Octodontidae). Journal of Mammalogy 84:541–546.

TORRES-MURA, J. C., M. I. LEMUS, AND L. C. CONTRERAS. 1989. Herbivorous specialization of the South American desert rodent *Tympanoctomys barrerae*. Journal of Mammalogy 70:646–648.

UDRIZAR SAUTHIER, D. E., U. F. J. PARDIÑAS, AND E. P. TONNI. 2009. *Tympanoctomys* (Mammalia: Rodentia) en el Holoceno de Patagonia, Argentina. Ameghiniana 46:203–207.

VERZI, D. H., E. P. TONNY, O. A. SCAGLIA, AND J. O. SAN CRISTOBAL. 2002. The fossil record of the desert-adapted South American rodent *Tympanoctomys* (Rodentia, Octodontidae). Paleoenvironmental and biogeographic significance. Palaeogeography, Palaeoclimatology, Palaeoecology 179:149–158.

WILSON, D. E., AND D. A. M. REEDER. 2005. Mammal species of the world. A taxonomic and geographic reference. 3rd ed. Johns Hopkins University Press, Baltimore, Maryland.

WLASIUK, G., J. C. GARZA, AND E. P. LESSA. 2003. Genetic and geographic differentiation in the Rio Negro tuco-tuco (*Ctenomys rionegrensis*): inferring the roles of migration and drift from multiple genetic markers. Evolution 57:913–926.

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APPENDIX I

Original number and corresponding haplotype of 60 individuals of *Tympanoctomys barrerae* analyzed in this study. Acronyms MHG, AO, and DR refer to tissue catalogue numbers: MHG: Milton H Gallardo, Instituto de Ecología y Evolución, Universidad Austral, Valdivia, Chile; AO: Agustina Ojeda; and DR: Daniela Rodrguiguez, Grupo de Investigaciones de Biodiversidad (GIB), Instituto Argentino de Zonas Áridas (IADIZA), CONICET and Centro de Ciencia y Técnica Mendoza (CCT Mendoza), Argentina.

H1: MHG1652, MHG1730, MHG 1760, MHG 1761; **H2:** MHG1655; **H3:** MHG1700, MHG1704, MHG1707, MHG1710 (m), MHG1710 (2), MHG 1716, MHG1784, AO73 (E2), AO73; **H4:** MHG1713; **H5:** MHG1633; **H6:** MHG1656; **H7:** MHG 1606, MHG 1762, MHG1763, MHG1785; **H8:** MHG1715; **H9:** MHG1729; **H10:** MHG1717; **H11:** DR24, DR24 (E1); **H12:** AO74, AO77; **H13:** MHG 1714; **H14:** MHG1718; **H15:** MHG1609; **H16:** MHG 1676; **H17:** AO76; **H18:** DR23, DR23 (E1); **H19:** AO61(m), AO61(E), MHG1677; **H20:** MHG1675; **H21:** MHG1651, MHG1705, MHG1711; **H22:** MHG1692; **H23:** MHG1701; **H24:** MHG1621, MHG1624, MHG1625, MHG1627, MHG1766, MHG1768, MHG1769, MHG1770, MHG1772, MHG1774, MHG1775, MHG1776, MHG1777; **H25:** MHG1771, MHG1773; **H26:** MHG1767.

Authors Queries

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