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Phylogeography and population genetic structure of the Talas tuco-tuco (*Ctenomys talarum*): integrating demographic and habitat histories

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We examined the phylogeography of the South American subterranean rodent *Ctenomys talarum* (Talas tuco-tuco) using mitochondrial DNA control region (D-loop) sequences. This species is an herbivorous rodent endemic to the province of Buenos Aires, Argentina, that lives in natural grasslands in coastal sand dune habitats and in some fragmented inland populations. In this study we assessed the genetic relationship among populations of *C. talarum* across its entire distributional range and analyzed how the geological history of the habitat has affected the genetic structure and demographic history of these populations. A complex network of haplotypes in conjunction with analysis of molecular variance results showed high genetic subdivision and a strong phylogeographic pattern among populations of *C. talarum*. Pairwise F_{ST}-values showed significant differentiation among all populations studied. The overall pattern was similar to that expected under the isolation-by-distance model, suggesting equilibrium between gene flow and local genetic drift. Major geographical barriers (e.g., rivers and unsuitable habitat) in the area, in conjunction with population isolation, appeared to be associated with strong genetic differentiation among the different geographical groups. Local mismatch distributions and tests of neutrality suggest contrasting histories for different groups of populations; although some populations appeared to be characterized by demographic stability and no significant departures from neutrality, others showed departures from strict neutrality consistent with a recent demographic expansion. Finally, a close association seems to exist between the major climatic changes that occurred during the late Pleistocene and Holocene in the central region of Argentina and the main historical demographic changes inferred from *C. talarum*. Current populations of *C. talarum* appear to be relicts of a more extended historical distribution along the Argentinean Pampas. These historical extinctions, however, have not erased the signature of long-term stability and geographical structure in this species along the coastal and inland distribution ranges.

Key words: *Ctenomys talarum*, historical demography, phylogeography, sand dune habitats, subterranean rodents

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Population genetic structure is determined by a complex interaction of ecological (e.g., migration rate and population size) and evolutionary (e.g., genetic drift and natural selection) processes (Aars et al. 1998; Hutchison and Templeton 1999; Mora et al. 2007). Different organisms display diverse types of demographic and population characteristics, which result in different levels of population genetic structure (Opazo et al. 2008; Pavlova et al. 2005; Steinberg and Patton 2000). In this context, demographic history and biogeography have an essential role in shaping the genetic variation in natural populations (Michaux et al. 2005; Milá et al. 2000). Thus, long periods of population stability, or, alternatively, the recent colonization of new habitats have contrasting effects on the pattern of spatial apportionment of genetic variation at local and regional levels (Matocq et al. 2000; Slatkin 1993; Wlasiuk et al. 2003).

Many small mammal species occur in naturally fragmented environments (e.g., *Ctenomys lami* [El Jundi and de Freitas 2004]; *Ctenomys flamarioni* [Fernández-Stolz et al. 2007]; *Hylomyscus denniae*, *Hybomys lunaris*, and *Lophuromys woosnami* [Huhndorf et al. 2007]; *Ctenomys sociabilis* [Lacey 2000]; *Ctenomys porteousi* [Mapelli and Kittlein 2009]; *Ctenomys australis* [Mora et al. 2006]; *Spalacopus cyanus* [Opazo et al. 2008]; *Ochotona princeps* [Peacock et al. 2002]; *Pteromys volans* [Selonen and Hanski 2004]; *Tamiasciurus hudsonicus* [Wilson et al. 2005]; and *Ctenomys rionegrensis* [Wlasiuk et al. 2003]). When their dispersal abilities are restricted in relation to the spatial scale of the available habitat, they usually occur in small genetic units in which genetic variation is low, and interpopulation divergence is high (Lacey et al. 1999; Steinberg and Patton 2000). This is the case of the South American rodents of the genus *Ctenomys* (tuco-tucos), which is the most speciose subterranean rodent genus (Castillo et al. 2005; Cook and Lessa 1998; Lessa and Cook 1998; Mora et al. 2007; Reig et al. 1990). *Ctenomys* shares with other subterranean rodents limited dispersal capabilities, high territoriality, small local effective population size, and in some cases socially structured mating systems (Lacey 2000; Reig et al. 1990). Populations of *Ctenomys* are composed of semi-isolated genetic units occupying patches of suitable habitat where soil hardness and other characteristics provide appropriate conditions for burrowing activities (Busch et al. 2000; Lessa and Thaeler 1989). Species-specific habitat requirements, such as those related to body size differences (see Lessa and Thaeler 1989) and other factors intrinsically linked with the species' biology (i.e., pelage crypticity—Krupa and Geluso 2000), strongly influence the type of soil that subterranean rodents use to build their burrows. The distribution and abundance of subterranean rodents in a fragmented landscape, and connectivity among local populations, ultimately depend upon both intrinsic features of the patches (e.g., soil hardness, granulometry, permeability, degree of vegetal cover, and terrain elevation [see Kittlein and Gaggiotti 2008]) and dispersal capabilities of the species (Mora et al. 2010).

Here, we report results of a phylogeographic study of the Talas tuco-tuco (*C. talarum*—Thomas 1898, 1912), a species associated with coastal habitats. This species mainly occurs along the Atlantic coast of Buenos Aires Province, Argentina (Contreras and Reig 1965), between the cities of Magdalena (35°1′S, 57°31′W) and Santa Clara (37°49′S, 57°29′W; northern distribution), and between Necochea (38°37′S, 58°50′W) and Quequén Salado River (38°54′S, 60°30′W; southern distribution), with an important gap of 150 km within its coastal distribution between Santa Clara and Necochea (Mora et al. 2007; Fig. 1A). In addition, there are some small inland and fragmented populations far from the coast in a system of hills called Sierra de la Ventana (approximately 100 km from the coast), and in the towns of Coronel Suarez, Saladillo, and El Cazón (Mora et al. 2007; Quintana 2004; Fig. 1A). On the other hand, Mora et al. (2007) and Mora (2008), using mitochondrial genome data, showed that the coastal parapatric populations located between the Arroyo Sauce Grande and Punta Alta (approximately 80 km along the coast), near Bahia Blanca (see Fig. 1A), might not belong to *C. talarum*, despite these populations having been originally assumed to be this species by other authors (Contreras and Reig 1965).

As with most species of this genus, the Talas tuco-tuco shows high habitat specificity (Busch et al. 2000), occupying both sand interdune and inland habitats with soft, humid, and vegetated soils (Malizia et al. 1991; Mora et al. 2007; Vassallo 1993, 1998), and in some cases on hard soils up to approximately 10 km from the shoreline. Along the coast, the distribution of *C. talarum* is linear: this 1-dimensional distribution restricts gene flow among populations of *C. talarum* (Mora 2008; Mora et al. 2007). In addition, the coastal distribution of *C. talarum* is interrupted by some potential barriers, such as rivers and, in recent times, urbanization, forestation over the natural sand dune habitat (historically characterized by low plant cover), and agricultural activities (Isla et al. 2001; Mora et al. 2007), and it is yet to be known whether these groups of populations currently are or historically were connected by gene flow.

Mora et al. (2007), using mitochondrial genome data, reported very low effective population sizes in some parts of the southern distribution of this species. Furthermore, the inland populations are naturally fragmented (with lower individual densities than the coastal populations [M. S. Mora, pers. obs.]) and, in recent years, the degree of fragmentation has increased with the expansion of soybean cultivation in the region (Mapelli and Kittlein 2009). Thus, *C. talarum* has a limited and disjunctive distribution characterized by isolated and fragmented populations (Mora 2008).

The geological history of the habitat of the inland populations of *C. talarum* in the Pampas region has a relatively ancient origin: soils constitute remnants from late Pleistocene aeolian deposits (Quattrocchio et al. 2008). However, the largest part of the distribution of this species is restricted to the coast, where *C. talarum* is associated with more recent sand dune habitat.

The goal of our study was to examine the genetic structure and geographical patterns of genetic variation in *C. talarum*. We sequenced the mitochondrial DNA (mtDNA) control region from specimens of this species from coastal and inland populations across an extensive range of its distribution and analyzed them from a phylogeographic and population genetic perspective. We addressed the following questions. To what extent does the predominantly 1-dimensional distribution of this species on the coast, coupled with limited dispersal, impose an isolation-by-distance pattern of genetic variation? In what way has habitat history affected the genetic structure and demographic stability of these populations? What is the historical relationship between the inland and coastal populations?

In addition, we compared our results to data on Pearson's tuco-tuco (*C. pearsoni*), a species that lives along the Atlantic coast of Uruguay and, like *C. talarum*, occupies both interdune and inland habitats (Tomasco and Lessa 2007), and with the

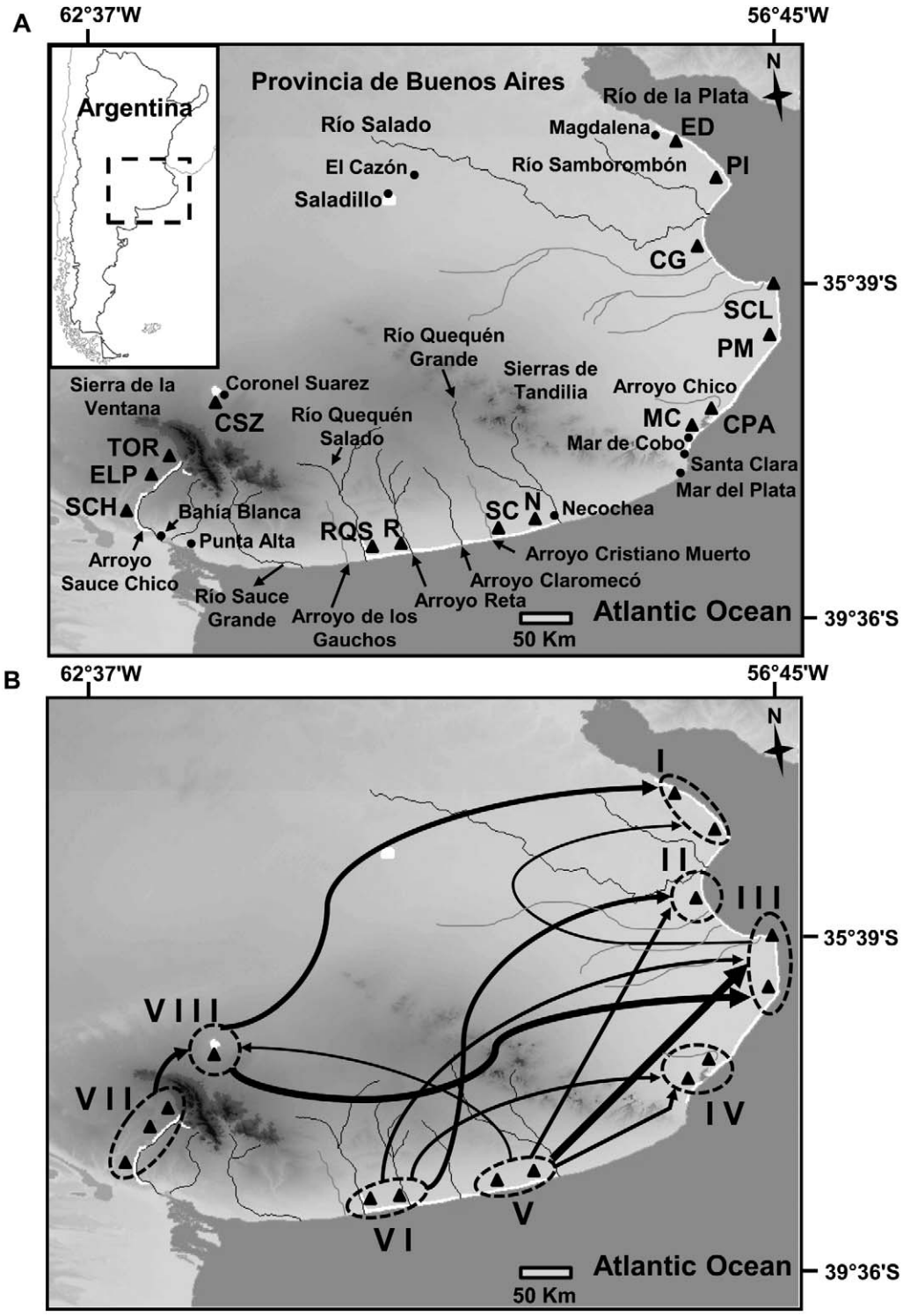

Fig. 1.—A) Geographical distribution of the sampled populations of *Ctenomys talarum* in Buenos Aires Province, Argentina, with the most important rivers and streams in the area shown. The areas in white represent the current distribution of *C. talarum* (see Mora et al. 2007). Abbreviations and coordinates of sampling localities (in black triangles) are indicated as follows: ED, El Destino (35°08′S, 57°23′W); PI, Punta Indio (35°20′S, 57°11′W); CG, Cerro de la Gloria (35°51′S, 57°26′W); SCL, San Clemente (36°19′S, 56°45′W); PM, Punta Médanos (36°52′S, 56°42′W); CPA, Estación Celpa (37°43′S, 57°25′W); MC, Mar de Cobo (37°46′S, 57°26′W); N, Necochea (38°37′S, 58°50′W); SC, Balneario San Cayetano (38°43′S, 59°26′W); R, Reta (38°53′S, 60°19′W); RQS, Río Quequén Salado (38°54′S, 60°28′W); SCH, Arroyo Sauce Chico (38°19′S, 62°34′W); ELP, Estancia La Paloma (38°09′S, 62°23′W); TOR, Tornquist (38°03′S, 62°15′W); and CSZ, Coronel Suarez (37°27′S, 61°52′W). B) The most important historical gene flow among different regions inferred from MIGRATE 3.0.3 is shown by black arrows (the width of arrows is proportional to the magnitude of gene flow). Roman numerals (from I to VIII) correspond to regional groups described in the analyses of molecular variance (see Table 2).

southern tuco-tuco (*C. australis*), a coastal species that is restricted to Holocene sand dunes in the southern part of Buenos Aires Province (Mora et al. 2006).

Materials and Methods

Sample collections.—We obtained tissue samples (toe snips, preserved in 95% ethanol for subsequent DNA extraction) from a total of 206 individuals of *C. talarum*, which were livetrapped with Oneida Victor N° 0 snap-traps (Oneida Victor, Inc., Ltd., Eastlake, Ohio), with a rubber cover to avoid injuring the animals (see Mora et al. 2006). Upon completion of these procedures, the animals were immediately released into the burrows from which they were captured. All parts of the study involving live animals followed guidelines of the American Society of Mammalogists (Sikes et al. 2011). This study included both of the 51 mtDNA control region sequences reported previously by Mora et al. (2007—GenBank accession numbers EF531719–EF531721 and EF531736–EF531750) from the southern coastal distribution of *C. talarum* (from Necochea to Rio Quequén Salado; Fig. 1A), and 155 additional mitochondrial sequences from individuals sampled in this study, including 7 different populations from Magdalena to Mar de Cobo localities along the Atlantic coast (Fig. 1A), 3 populations from Sierra de la Ventana, 1 population from Coronel Suarez (a new cited inland locality reported in this study; see Fig. 1A and Supporting Information S1, DOI: 10.1644/11-MAMM-A-242.1S). The distinct sequences reported in this paper were deposited in GenBank under accession numbers JX297345–JX297372 (see Supporting Information S1). A total of 206 mitochondrial sequences from 15 different populations (between 5 and 24 individuals per population; see Table 1) were included both in the phylogeographic and phylogenetic analyses.

Extraction of DNA, polymerase chain reaction amplification, and sequencing.—The DNA extractions were performed using a sodium dodecyl sulfate–proteinase K–NaCl–alcohol precipitation method (modified from Miller et al. 1988). A fragment of the control region of the mitochondrial genome was amplified by polymerase chain reaction from all specimens of *C. talarum* using the primers TucoPro (5′-TTC TAA TTA AAC TAT TTC TTG-3′—Tomasco and Lessa 2007) and TDKD (5′-CCT GAA GTA GGA ACC AGA TG-3′—Kocher et al. 1989). Amplification was carried out in a total volume of 12.5 μl containing the following components: 6.25 μl of DNA (4 μg/ml) used as a template and 0.5 units of Taq DNA polymerase, and the following reagents and final concentrations: 1X Taq polymerase buffer, deoxynucleoside triphosphates at 0.2 mM each, primers at 0.2 μM each, and $MgCl_2$ at 4 mM. Polymerase chain reaction amplifications were performed in a Rapidcycler (Idaho Technology, Salt Lake City, Utah) with a program that consisted of an initial denaturation of 1 min at 94°C, followed by a touchdown of 30 cycles consisting of in 5 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 52°C, and 1 min of extension at 72°C, followed by 5 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 50°C, and 1 min of extension at 72°C, followed by 20 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 48.5°C, and 1 min of extension at 72°C, and a final extension of 5 min at 72°C. Aliquots of amplification products were checked on 0.8% agarose gel. The remaining polymerase chain reaction products were purified and sequenced at MACROGEN, Inc., Seoul, Korea, with the same primers used in polymerase chain reaction. Forward and reverse sequences were aligned and cross-checked to resolve ambiguities. DNA extractions of *C. talarum* were deposited in the collection of the Laboratorio de Evolución, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay. The final 206 sequences of *C. talarum* were 438 base pairs in length.

Data analysis.—Electropherograms were scored and analyzed using ProSeq version 3.0 (Filatov 2002) and

Table 1.—Number of sequences (S), haplotypes (Ha), polymorphic sites by population (PS), haplotype diversity (*HaDi*), and nucleotide diversity (*NuDi*) in *Ctenomys talarum* from Argentina are shown. The total and overall values of these parameters and the corresponding standard deviations (*SD*s) also are given. Abbreviations for locations are defined in Fig. 1.

Sample locations	S (*n*)	Ha (*n*)	PS (*n*)	*HaDi*	*SD*	*NuDi*	*SD*
ED	9	4	12	0.75	0.112	0.0136	0.005
PI	24	3	2	0.58	0.076	0.0021	0.001
CG	18	4	8	0.63	0.073	0.0077	0.002
SCL	18	4	2	0.54	0.123	0.0015	0.001
PM	18	1	0	0	0	0	0
CPA	5	4	3	0.9	0.161	0.0032	0.002
MC	22	7	8	0.73	0.088	0.0039	0.002
N	13	5	7	0.7	0.114	0.0041	0.003
SC	9	5	6	0.72	0.159	0.003	0.003
R	22	7	8	0.76	0.065	0.0046	0.003
RQS	7	3	4	0.52	0.209	0.0026	0.002
SCH	5	1	0	0	0	0	0
ELP	11	2	2	0.18	0.144	0.0004	0.001
TOR	13	3	3	0.3	0.156	0.0010	0.001
CSZ	12	3	3	0.44	0.158	0.0017	0.001
Total	206	61	65				
Overall				0.52		0.004	

aligned using CLUSTAL X (Thompson et al. 1997). Analyses were performed across populations, which corresponded to sampling localities (El Destino, ED; Punta Indio, PI; Cerro de la Gloria, CG; San Clemente, SCL; Punta Médanos, PM; Estación Celpa, CPA; Mar de Cobo, MC; Necochea, N; Balneario San Cayetano, SC; Reta, R; Río Quequén Salado, RQS; Arroyo Sauce Chico, SCH; Estancia La Paloma, ELP; Tornquist, TOR; and Coronel Suarez, CSZ); across regions, delimited by major geographical barriers (Region I: ED and PI; Region II: CG; Region III: SCL and PM; Region IV: CPA and MC; Region V: N and SC; Region VI: R and RQS; Region VII: SCH, ELP, and TOR; and Region VIII: CSZ; Fig. 1B); across macroregions (Northern coastal group: I, II, III, and IV; Southern coastal group: V and VI; and Western group: VII and VIII); and globally, which included all samples. For the subdivisions into macroregions, both the pronounced distance among groups of populations and the most important landscape discontinuities in the distribution of *C. talarum* were taken into consideration. Between the Northern and the Southern coastal groups, there was a significant gap of ~130 km in the distribution of *C. talarum* (Fig. 1A).

Nucleotide variation within populations was estimated as π (the average number of pairwise differences between haplotypes, which estimates $\theta = 2N_{ef}\mu$, where θ is genetic diversity, N_{ef} is the female effective population size, and μ is the mutation rate per locus per generation) and θ_w, an estimate of θ based on the number of segregating sites in the sample (Watterson 1975) using MEGA software version 4.0 (Tamura et al. 2007). Arlequin version 3.0 (Excoffier et al. 2005) and DnaSP version 5.0 (Librado and Rozas 2009) were used for computing estimates of genetic variation within and among populations, and to calculate global and pairwise estimates of gene flow (Hudson et al. 1992). Differentiation between all pairs of populations was assessed using an exact test of differentiation described by Raymond and Rousset (1995) and implemented in Arlequin 3.0 (Excoffier et al. 2005). The minimum spanning network of haplotypes was generated using NETWORK 4.5.1 (Brandelt et al. 1999). For this haplotype network reconstruction we used the median-joining algorithm defined by Brandelt et al. (1999), which essentially uses a maximum-parsimony approach to search for the shortest, least complex network from a given data set.

In order to infer hierarchical population structure, analyses of molecular variance (AMOVAs) were performed considering both genetic distances between haplotypes and their frequencies, using Arlequin version 3.0 (Excoffier et al. 1992, 2005; Weir and Cockerham 1984). AMOVAs were performed across regions, to test the effect of natural barriers (e.g., rivers and highlands) and geographical proximity on the partitioning of the genetic variance; across sampling localities; and across macroregions.

Pairwise estimates of gene flow and linear geographical distances were used to examine patterns of isolation by distance within macroregions and globally across populations (Slatkin 1987, 1993; Slatkin and Hudson 1991; Wright 1943). A Mantel test (Mantel 1967) was used to assess the significance of the correlation between gene flow estimates and geographical distances using 1,000 permutations. The mean number of migrants per generation (Nm) was estimated as $Nm = (1 - \Phi_{ST})/(2\Phi_{ST})$, and the geographical distance matrix was calculated using the software IDRISI32 (Eastman 1999).

We used MIGRATE 3.0.3 (Beerli and Felsenstein 2001) to obtain maximum-likelihood estimates of effective population sizes from theta ($\theta = 2N_{ef}\mu$), and rates of migration ($2N_{ef}m$) across regions, where N_{ef} is the effective population size of females, μ is the mutation rate per site per generation, and $N_{ef}m$ is the number of migrating females per generation. Thus, in order to infer the magnitude and direction of gene flow between regions and their effective population sizes, we used the same grouping levels considered in the AMOVAs (see Table 2). The maximum-likelihood method uses a Markov chain Monte Carlo coalescent approach to explore across possible genealogies. It relaxes the assumptions of the populations having the same size and symmetric migration rates, which are used in Φ_{ST}-derived estimates of Nm, but assumes no large variation in population size over time. Therefore, we ran MIGRATE grouping the 15 sampling localities (see Fig. 1B) into 8 clusters based on geographical proximity and major natural barriers among them. We used the following genealogy searching settings: 10 short Markov chains each consisting of 50,000 iterations with sampling every 500 trees, followed by 3 long Markov chains with 100,000 iterations, with sampling every 500 trees. At the beginning of each chain 10,000 trees were discarded so that the next chain was not biased toward the parameters estimated for the previous chain. We used the Felsenstein's 84 mutation model as implemented in this program, without site rate variation. Base frequencies were estimated from the data, assuming a transition/transversion ratio of 2. MIGRATE was run 5 times, with the Φ_{ST}-based starting parameters and different random seed numbers. Profile likelihoods for all parameters were evaluated at 0.025 and 0.975 percentiles. Migration estimates among groups of populations were included in the analyses, if more than 3 estimate values were observed to be greater than 0 from the 5 independent runs. Thus, estimates of migration rates represented the average values among these different runs.

We examined the demographic history of populations using a mismatch distribution analysis to distinguish between populations that have been stable over time (according to an "equilibrium model" with constant long-term N_e) from those that have experienced recent demographic or range expansion, or both (or departures from strict neutrality or a combination of these factors—Harpending et al. 1998; Ramos-Onsins and Rozas 2002; Rogers and Harpending 1992; Schneider and Excoffier 1999; Slatkin and Hudson 1991). Although mismatch distributions from populations of constant size are characteristically ragged and erratic (e.g., Matocq et al. 2000; Slatkin and Hudson 1991), mismatch distributions are typically unimodal in populations that have gone through a recent demographic expansion (Excoffier 2004; Harpending and Rogers 2000; Michaux et al. 2005; Rogers and Harpending 1992). We employed parametric bootstrapping as implemented

Table 2.—Hierarchical analysis of molecular variance, using a square matrix of pairwise genetic distances between haplotypes. The fixation indexes (Φ-statistics) are shown. Φ-statistics and significance of variance component (*P*) were tested by 16,000 permutations according to Excoffier et al. (1992). The respective levels of subdivision of each population are shown in square brackets. Abbreviations for locations are defined in Fig. 1.

Source of variation	Level of subdivision for populations of *C. talarum*	Φ_{CT}	*P*	Φ_{ST}	*P*
Eight regions limited by major natural barriers (major rivers, streams, and highlands)	[ED, PI] [CG] [SCL, PM] [CPA, MC] [N, SC] [R, RQS] [SCH, ELP, TOR] [CSZ]	0.62	<0.001	0.78	<0.001
No clustering of subpopulations into regions	[ED] [PI] [CG] [SCL] [PM] [CPA] [MC] [N] [SC] [R] [RQS] [SCH] [ELP] [TOR] [CSZ]			0.77	<0.001
Among 3 macroregions	[ED, PI, CG, SCL, PM, CPA, MC] [N, SC, R, RQS] [SCH, ELP, TOR, CSZ]	0.033	0.08	0.47	<0.001

in Arlequin version 3.0 to test the goodness of fit of the observed mismatch distribution to that expected under the sudden expansion model using the sum of squared deviations (*SSD*) statistic. We also used the raggedness index defined by Harpending (1994), implemented in Arlequin version 3.0. This index takes lower values for unimodal and smoother distributions, distinctive of expanding populations, than for multimodal distributions commonly found in a stationary population (see Excoffier 2004). Its significance was tested in the same way as that of *SSD*. Mismatch distributions were built globally, for each macroregion, and for each region.

Tajima's *D* (Tajima 1989) and Fu's F_S (Fu 1997) tests of neutrality were performed globally, across macroregions, and across regions using 1,000 iterations as performed in Arlequin version 3.0. Significant negative values of Tajima's *D* and Fu's F_S are indicative of an excess of low-frequency mutations, relative to expectations under the standard neutral model (strict neutrality of variants, constant population size, and lack of subdivision and gene flow), and are consistent with demographic expansion or purifying selection, or both—unlikely at the mitochondrial control region. Tajima's *D* and Fu's F_S statistics and mismatch distributions are able to provide insights about population expansions, but they are not able to provide information about the detailed population changes over time. Therefore, to estimate the shape of population change through time we constructed Bayesian skyline plots as implemented in BEAST 1.5.4 (Drummond and Rambaut 2007). This Bayesian approach incorporates the uncertainty in the genealogy by using Markov chain Monte Carlo integration under a coalescent model, providing information about effective population sizes through time (Drummond et al. 2005). The program was run for 2.5×10^7 iterations and sampled every 1,000 steps under a relaxed lognormal molecular clock with uniformly distributed priors. In our analyses, the first 10% of the iterations were discarded to allow for burn-in. The best-fit substitution model for the data was estimated in jModelTest (Posada 2008). To assess the robustness of parameter estimates, 4 independent chains were run with identical settings. Log-files were analyzed in Tracer 1.4.8 (Drummond and Rambaut 2007), and effective sample sizes were used to evaluate Markov chain Monte Carlo convergence within chains. These analyses were performed globally and for each of the 3 macroregions defined above.

To obtain estimates of absolute times of demographic events suggested by Bayesian skyline plots, we estimated a specific control region mtDNA substitution rate (and node ages) for the genus *Ctenomys*, as follows. We constructed a Bayesian phylogenetic tree using BEAST 1.5.4 (Drummond and Rambaut 2007), which employs a Bayesian Markov chain Monte Carlo approach. As out-groups we used control region sequences from 3 species of the family Octodontidae (*S. cyanus*, *Tympanoctomys barrerae*, and *Octodon degus*; GenBank accession numbers HM544133.1, HM544132.1, and HM544134.1, respectively), and 5 species from the family Ctenomyidae (*C. sociabilis*, *C. australis*, *C. leucodon*, *C. pearsoni*, and *C. torquatus*), all of them pertaining to the infraorder Hystricognathi (GenBank accession numbers HM544129.1, DQ416740.1, HM544131.1, AY755457.1, and AY755459.1, respectively). These species of *Ctenomys* represent some of the most divergent species of this genus (D'Elía et al. 1999; Lessa and Cook 1998). The same fragment length of the control region was considered both in *C. talarum* and in the 8 out-groups. We used the GTR substitution model with 4 gamma categories, using a Yule branching rate prior, with rate variation across branches assumed to be uncorrelated and lognormally distributed (Drummond et al. 2006). Each Markov chain Monte Carlo chain was run for 10^7 iterations (burn-in 20,000), with parameters sampled every 1,000 steps. For comparison, 3 independent Markov chain Monte Carlo runs were performed to estimate the rate of substitution of the genus *Ctenomys*. Examination of Markov chain Monte Carlo samples using Tracer 1.4.8 (Rambaut and Drummond 2007) suggested that the independent chains were each adequately sampling the same probability distribution and effective sample sizes for all parameters of interest were greater than 500, conditions suggested by the authors for proper functioning of the analysis. Two direct fossil calibrations were used and treated as having a translated normal distribution: these data are based on the appearance of the 1st fossil of each clade or group, as follows. Superfamily Octodontoidea is almost as old as Caviomorpha, and the early differentiation of the families Ctenomyidae and Octodontidae is at least 9.13 million years ago (mya—Verzi 1999; Vucetich et al. 1999). We used 9.3 mya as the upper limit for the radiation of Octodontidae, and 3.5 mya for the radiation of the genus *Ctenomys* (Verzi et al. 2010).

A phylogenetic tree also was constructed using a maximum-likelihood approach implemented in PhyML 3.0 (Guindon et al. 2010; Guindon and Gascuel 2003), considering the same out-groups used in the Bayesian phylogenetic inference. The program implements simultaneous nearest neighbor interchanges to improve a reasonable topology of the starting tree. We ran PhyML considering the best substitution model inferred by jModelTest (Posada 2008), where both the transitions/transversions ratio and gamma distribution parameter were empirically estimated. Consistency for internal branch and nodes was assessed using the standard bootstrapping method (sample with replacement and 1,000 bootstrap replicates) implemented in PhyML.

Results

Variation among control region haplotypes.—Sequences of *C. talarum* yielded a total of 47 variable sites, of which 16 were singletons and 31 were parsimony-informative, defining 46 haplotypes in the sample of 206 sequences analyzed (Supporting Information S1). The high number of polymorphic sites within this species resulted in high average haplotype and nucleotide diversity values (Table 1).

Genealogical relationships among haplotypes and genetic variation within populations.—Most of the 46 haplotypes (78%) were limited to single populations, and most populations maintained large numbers of haplotypes (between 3 and 7, with the exception of PM, ELP, and SCH populations; abbreviations of populations are shown in Fig. 1A). Overall haplotype diversity was 0.52 (Table 1).

The Western group of populations (SCH, ELP, TOR, and CSZ) exhibited the lowest haplotype and nucleotide diversity. Except for PM in the north, the Northern (ED, PI, CG, SCL, CPA, and MC) and Southern (N, SC, R, and RQS) coastal groups of populations showed moderate to high haplotype and nucleotide diversity (Table 1). Of these populations, the CPA and R populations showed the highest values of haplotype diversity, whereas ED presented the highest value of nucleotide diversity. Two populations, PM and SCH, were invariant (Table 1). The minimum spanning network showed high genetic subdivision and a strong phylogeographic pattern among populations of *C. talarum* (Fig. 2). A complex network of haplotypes was obtained, and a high portion of genetic variation was attributable to differences among regions. Almost all of the populations were reciprocal monophyletic groups of haplotypes (Figs. 2 and 3). The Bayesian phylogenetic tree showed high posterior probabilities for most groups of populations, giving strong support to the conformation of the regional groups (e.g., ELP, TOR, and SCH within group VII; Fig. 3). Similarly to the Bayesian inference, the maximum-likelihood phylogenetic tree showed high support of the nodes for most groups of populations within the regional groups (Fig. 3). Except for CG, the northernmost populations (ED, PI, SCL, and PM) were included in a clade that exhibited high phylogenetic support, showing strong differentiation with other populations of *C. talarum* (Fig. 3). Overall, most of the haplotypes had restricted distributions, and shared haplotypes were not found in populations separated by distances greater than 150 km (with the exception of R and MC; Figs. 2 and 3).

Genetic variation among regions.—A hierarchical analysis of variance (AMOVA) showed significant genetic differentiation among all populations studied. Rivers as well as hills (e.g., Sierra de la Ventana and Sierras de Tandilia; Fig. 1A) seem to have an important effect on the genetic differentiation among regions ($\Phi_{CT} = 0.62$, $P < 0.001$; Table 2). The interpopulation divergence (Φ_{ST}) without any a priori subdivision of sampling localities into regions was 0.77 ($P < 0.001$; Table 2). Variation among populations within regions and within populations was relatively low (12.34% and 19.43% of total variation, respectively) relative to the among-group variance component. Subdivision into macroregions explained a small portion of total genetic variation (Table 2).

Results of the global test of differentiation among all populations based on haplotype frequencies were highly significant ($P < 0.001$—Raymond and Rousset 1995). Furthermore, significant pairwise Φ_{ST}-values (Table 3) between sampling localities ranged from 0.15 (PM–SCL) to 1. Interestingly, most pairwise comparisons between western populations and coastal populations had the highest Φ_{ST}-values and were highly significant. In general, these results suggest low levels of historical gene flow among these macroregions, supporting a scenario of historical isolation between them.

Equilibrium and nonequilibrium populations and patterns of gene flow.—Genetic differentiation in *C. talarum* (across all 15 populations) was consistent with a weak but significant pattern of isolation by distance, suggesting an equilibrium between gene flow and local genetic drift across the entire distribution of *C. talarum* ($r = 0.31$, $P < 0.05$, Mantel test). Similarly, the populations composed of the Northern coastal and Western macroregions (ED, PI, CG, SCL, PM, CPA, MC, ELP, TOR, SCH, and CSZ) exhibited a significant isolation-by-distance pattern ($r = 0.27$, $P < 0.05$). Two additional Mantel tests were performed considering only the Northern (ED, PI, CG, SCL, PM, CPA, and MC) and Southwestern (N, SC, R, RQS, ELP, TOR, SCH, and CSZ) groups of localities of this species. Neither the Northern ($r = 0.45$, $P = 0.086$) nor the Southwestern ($r = 0.19$, $P = 0.148$) distributions fit an isolation-by-distance model. One final Mantel test was performed in order to assess a model of isolation by distance in a 1-dimensional distribution on the coast, considering all populations belonging to Northern and Southern groups (see Fig. 1A). Results of this latter Mantel test were not significant ($r = 0.12$, $P = 0.233$), suggesting a lack of equilibrium between gene flow and local genetic drift among coastal populations.

A global estimate of migration rate per generation, under the assumption of an island model of population structure, was $Nm = 0.148$. The average Nm-value between populations separated by less than 100 km was 0.7 (± 1.08 *SD*), and the average Nm-value among populations separated by more than 200 km was 0.2 (± 0.23 *SD*). These results suggest a limited gene flow among populations at greater geographical scales. The maximum-likelihood estimates of female effective population

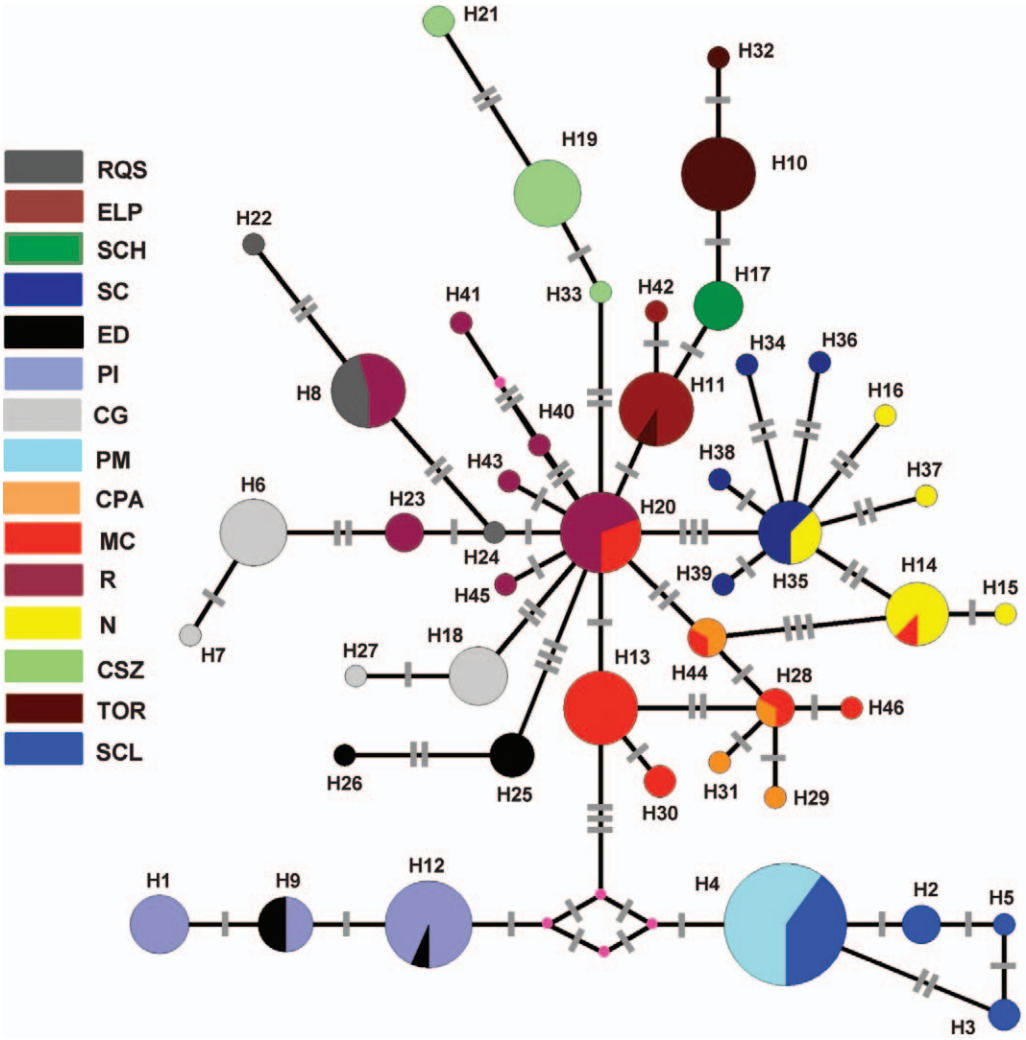

Fig. 2.—Minimum spanning tree of 46 mitochondrial DNA haplotypes of *Ctenomys talarum* is shown. Areas are proportional to haplotype frequencies, shading indicates localities, and gray small lines represent nucleotide differences between haplotypes. Haplotype numbers for *C. talarum* correspond to those shown in Supporting Information S1. Small black circles represent missing or unsampled haplotypes. Abbreviations of populations are defined in Fig. 1.

size and migration rates between regions were generally stable over multiple MIGRATE runs (Supporting Information S2, DOI: 10.1644/11-MAMM-A-242.1S). Most estimates of gene flow among populations were low or near zero (*Nm* lower than 1×10^{-8}; see Supporting Information S2).

By contrast, higher values of historical gene flow were estimated between regions belonging to different macroregions, in some cases among regions located >350 km from each other (e.g., groups I, II, and II receiving migrants from groups V and VI; see Fig. 1B), as well as a northward tendency of global historical migration, which suggested a pattern of migration from the Southern and Western groups of populations to the northern range of the distribution of *C. talarum*. All Northern coastal populations received migrants from the most southwestern populations (e.g., from V, VI, and VIII groups to the north).

Estimates of migration rates among Southern–Western macroregions suggested low levels of genetic connectivity among these groups of populations (Supporting Information S2), with a predominance of migration from south to west. The most isolated population in Coronel Suarez (CSZ, group VIII) appeared to be a source of emigration to the north, only receiving immigrants from the southern populations and by the populations from Sierra de la Ventana (Fig. 1B; Supporting Information S2).

Large effective population sizes were observed in the southernmost populations on the coast (groups V and VI) and in MC and CPA in the north (group IV); smaller values were found in groups VII and VIII in the west and III in the north (Fig. 1B; Supporting Information S2). Levels of historical gene flow appeared different between the western and northern populations. Northern coastal populations showed larger effective population numbers, in general, than western populations (Supporting Information S2). This situation suggested that Northern coastal populations of Talas tuco-tuco have been able to expand more rapidly over continuous

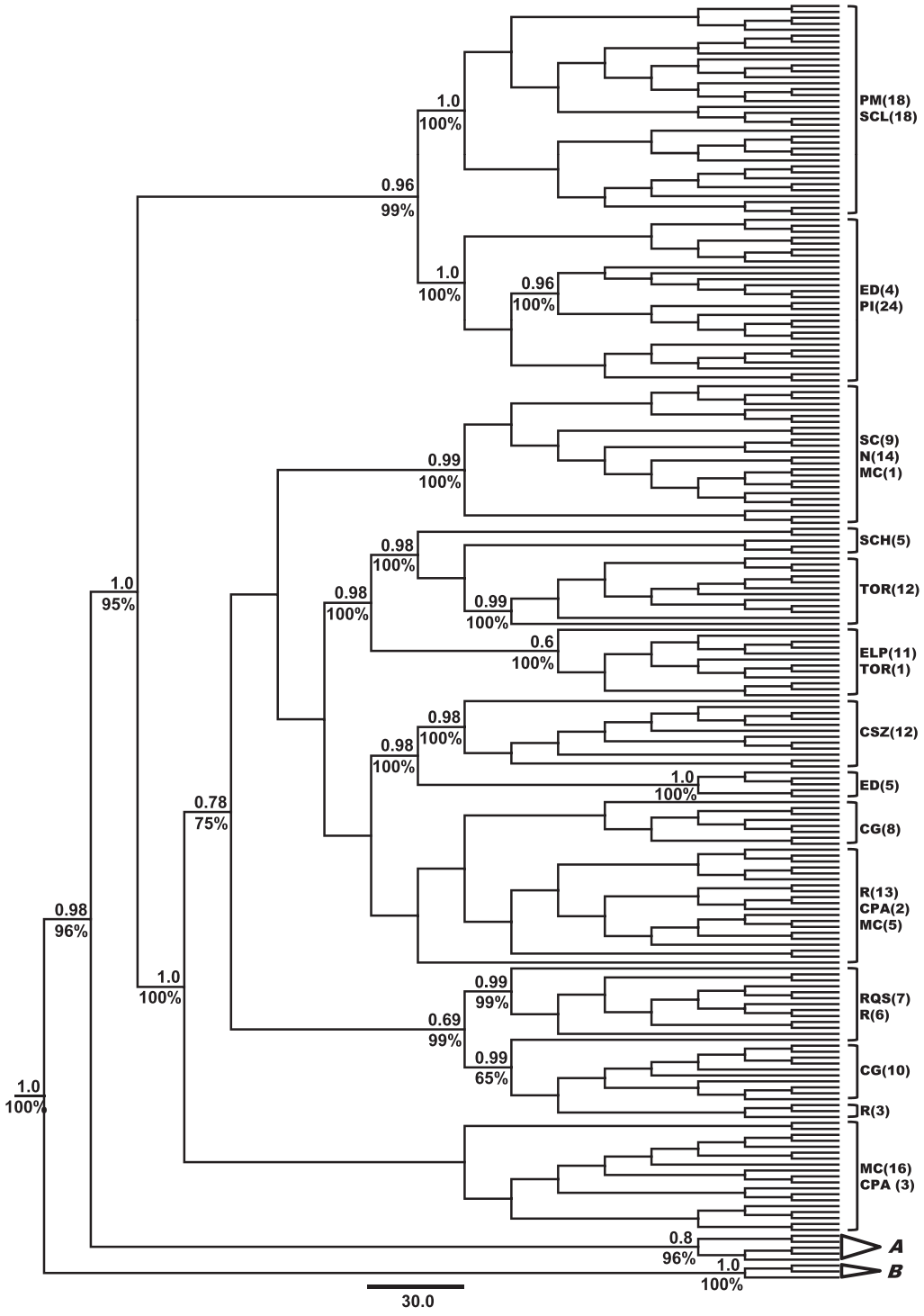

Fig. 3.—Bayesian phylogenetic (Drummond and Rambaut 2007) and maximum-likelihood (Guindon et al. 2010) tree of 46 mitochondrial DNA haplotypes for *Ctenomys talarum* ($n = 206$) from Argentina, constructed on the basis of the number of pairwise differences between haplotypes. Frequencies above branches correspond to posterior probabilities values associated with the main phylogroups of *C. talarum* (only clades with high posterior probabilities are shown). Percentages below branches correspond to bootstrap support values (≥50%) of the nodes resulting from the maximum-likelihood phylogenetic inference performed in PhyML program version 3.0 (Guindon et al. 2010; Guindon and Gascuel 2003). Abbreviations of locations are defined in Fig. 1. The total number of sequences by population is shown in parentheses. Control region sequences from 5 species from the family Ctenomyidae (*Ctenomys sociabilis*, *C. australis*, *C. leucodon*, *C. pearsoni*, and *C. torquatus*) represented by clade A, and 3 specimens of the family Octodontidae (*Spalacopus cyanus*, *Tympanoctomys barrerae*, and *Octodon degus*) represented by clade B, all of them pertaining to the infraorder Hystricognathi, are included as out-groups.

TABLE 3.—Population pairwise Φ_{ST}s for comparisons between populations of *Ctenomys talarum* from Argentina. Abbreviations for locations are defined in Fig. 1.

	ED	PI	CG	SCL	PM	CPA	MC	N	SC	R	RQS	SCH	ELP	TOR
PI	0.55**													
CG	0.39**	0.76**												
SCL	0.66**	0.84**	0.74**											
PM	0.71**	0.87**	0.78**	0.15*										
CPA	0.4**	0.88**	0.55**	0.9**	0.96**									
MC	0.41**	0.81**	0.47**	0.81**	0.85**	0.4*								
N	0.62**	0.89**	0.66**	0.9**	0.93**	0.68**	0.67**							
SC	0.57**	0.9**	0.62**	0.91**	0.95**	0.79**	0.67**	0.31*						
R	0.41**	0.81**	0.26**	0.81**	0.84**	0.57**	0.33**	0.67**	0.64**					
RQS	0.48**	0.89**	0.39**	0.90**	0.96**	0.79*	0.66**	0.79**	0.81**	0.33*				
SCH	0.48**	0.91**	0.55**	0.94**	1**	0.86*	0.63**	0.81**	0.85**	0.56**	0.86*			
ELP	0.52**	0.91**	0.53**	0.94**	0.99**	0.86**	0.57**	0.81**	0.85**	0.46**	0.86**	0.88**		
TOR	0.64**	0.92**	0.59**	0.94**	0.98**	0.88**	0.72**	0.85**	0.88**	0.65**	0.88**	0.7**	0.8**	
CSZ	0.56**	0.89**	0.63**	0.92**	0.96**	0.84**	0.7**	0.83**	0.85**	0.64**	0.85**	0.89**	0.88**	0.9**

* Significant ($P < 0.05$); ** highly significant ($P < 0.001$).

habitats (e.g., sand dunes), in contrast to the more reduced western inland populations in Sierra de la Ventana (TOR, ELP, and SCH sampling localities) and Coronel Suarez, which are restricted to small habitat patches.

Overall, historical gene flow appears to have been greater than current patterns of gene flow, and suggests expansion and settlement following a south to north route followed by a progressive degradation of the typical habitats of this species in the inland areas.

Tests of neutrality, mismatch distributions, and Bayesian skyline plots.—Neutrality tests were performed to detect evidence of population range expansion. Results of the global Fu's F_S-test of neutrality were significantly negative at the global level (F_S: –24.88, $P < 0.001$), suggesting a demographic range expansion process. In contrast, the Tajima's *D*-test did not reveal a signal of demographic range expansion at the global scale (D: –0.74, $P = 0.25$; Table 4).

A signal of population range expansion was observed in the major Southern macroregion (coastal groups V and VI; F_S: –6.78, $P < 0.05$). However, neither the major Northern coastal group (I, II, III, and IV; F_S: –2.24, $P = 0.3$) nor the Western group of populations (VII and VIII; F_S: 0.368, $P = 0.62$; Table 4) showed significant departures from neutrality. Similarly, signals of population expansion were not detected in any of these macroregions using Tajima's *D*-test (Table 4). When all regions were considered separately, V was the only population group that showed deviations from strict neutrality (Table 4), suggesting, most likely, a historical population range expansion. In sum, Tajima's D and Fu's F_S indicated that the demographic histories of some regions differed, suggesting that some areas within the distribution of *C. talarum* have experienced different demographic events in the recent past: stability in the Western and Northern macroregions, and

TABLE 4.—Roman numerals (from I to VIII) correspond to regional groups described by the analyses of molecular variance (see Table 2). Total numbers of sequences are shown in parentheses. Mean number of pairwise differences (π), number of polymorphic sites (θ_w), and values of Tajima's *D*- and Fu's F_S-tests from *Ctenomys talarum* are shown. The global neutrality tests also are given. π and θ_w parameters were estimated using MEGA 4.0 (Tamura et al. 2007), whereas D and F_S neutrality statistics were estimated using Arlequin 3.0 (Excoffier et al. 2005; see "Materials and Methods").

			Tajima's *D*		Fu's F_S	
	π	θ_w	D	P	F_S	P
Regional geographic groups						
I ($n = 33$)	3.34	13	0.143	0.62	3.53	0.94
II ($n = 18$)	3.36	8	1.54	0.95	3.37	0.94
III ($n = 36$)	0.36	2	−0.49	0.32	−1.82	0.057
IV ($n = 27$)	1.99	10	−0.76	0.26	−2.46	0.075
V ($n = 22$)	1.97	13	−1.59	<0.05	−3.07	<0.05
VI ($n = 29$)	2.19	9	−0.15	0.47	−1.91	0.14
VII ($n = 29$)	1.14	4	0.32	0.66	−0.32	0.42
VIII ($n = 12$)	0.77	3	−0.73	0.27	0.18	0.43
Major geographical groups						
Northern coastal group (I, II, III, and IV; $n = 114$)	5.33	29	−0.073	0.544	−2.24	0.3
Western group (VII and VIII; $n = 41$)	2.74	9	0.88	0.8	0.368	0.62
Southern coastal group (V and VI; $n = 51$)	9.2	47	−0.57	0.32	−6.78	<0.05
Global neutrality tests considering all populations			−0.74	0.25	−24.88	<0.001

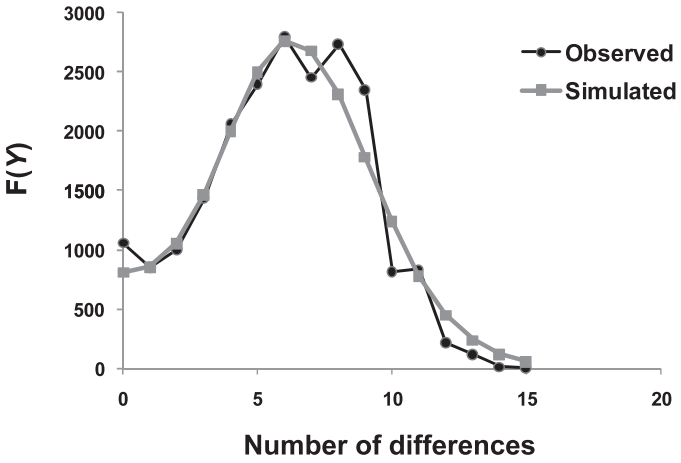

Fig. 4.—Observed and expected mismatch distributions for *Ctenomys talarum* from Argentina. Black circles represent observed distribution and gray circles represent theoretical expected distribution under a population expansion model following Schneider and Excoffier (1999). F(*Y*) indicates frequency.

expansion in 1 portion of the Southern macroregion. Purifying selection also could produce this pattern.

The mismatch distribution of all 15 populations was roughly multimodal (Fig. 4), and did not show significant departures from an equilibrium model ($SSD = 0.002$, $P = 0.62$), suggesting constant population size or sustained subdivision, or both, for a long period of time. Also, the Harpending raggedness index was not significant ($H = 0.009$, $P = 0.63$; Table 5). The average number of nucleotide differences considering all 15 populations was 5.96. Mismatch distributions performed for each region (Table 5) showed different patterns than those produced from Tajima's *D* and Fu's F_S. None of the regions, except VIII, showed evidence of a sudden expansion model using the *SSD*. Harpending raggedness index was significant only for regions II and V. Neither *SSD* nor the Harpending raggedness index showed any signals of population expansion when each macroregion was analyzed (Northern, Western, and Southern groups; Table 5).

Three absolute substitution rates (see "Materials and Methods") were estimated for the mtDNA control region for *Ctenomys*: 3.91×10^{-8}, 4.42×10^{-8}, and 4.49×10^{-8} per site per year, which were similar to 5.56×10^{-8} ($\pm 2.02 \times 10^{-8}$ *SD*) substitutions per site per year estimated by Goios et al. (2007) for the same locus in *Mus* sp. An intermediate rate of substitution (4.42×10^{-8}) was selected for the genus *Ctenomys* in the posterior Bayesian skyline plot analysis. The demographic scenario for *C. talarum* presented by the Bayesian skyline plots showed a dual pattern; a long time of constant population size followed by a short period of marked demographic changes estimated to have started about 8,000 years ago (Fig. 5). In the last 8,000 years during the early Holocene, a marked increase in population size was inferred. Population expansion reached a period of stability approximately 2,000 years ago.

Discussion

Ctenomys talarum has been studied extensively with regard to its population biology (Busch et al. 2000), mating system (Zenuto et al. 1999), and population genetics (Cutrera et al. 2005; Mora et al. 2007) for almost 25 years. However, this is the 1st study to assess the phylogeography of the Talas tuco-tuco over its entire distribution, yielding important new insights into the history of the species in relation to historical changes in its habitat, and shedding light onto the relationships among currently isolated regions. Our analyses show that populations of *C. talarum* are strongly structured genetically,

Table 5.—Values for tau, θ_0, and θ_1 (estimates parameters of the sudden expansion model), the sum of squared deviations (*SSD*—Schneider and Excoffier 1999), and Harpending's raggedness index (Harpending 1994) for regional and major geographical groups are shown. All parameters were estimated under the sudden expansion model (Excoffier 2004) using Arlequin version 3.0 (Excoffier et al. 2005). The estimates parameters θ_0, θ_1, and tau are defined as $\theta_0 = 2N_0\mu$, $\theta_1 = 2N_1\mu$, and $\text{tau} = 2T\mu$, where N_0 and N_1 are the population sizes previous to and after the sudden expansion event, μ is the mutation rate per generation at a particular site, and *T* is the time in number of generations from the expansion event in the past.

				Sum of squared deviations (*SSD*)		Harpending's Raggedness index	
	tau	θ_0	θ_1	*SSD*	*P*	*H*	*P*
Regional geographic groups							
I ($n = 33$)	1.54	0.042	3.61	0.04	0.12	0.07	0.69
II ($n = 18$)	7.43	0	3.73	0.2	0.053	0.36	<0.05
III ($n = 36$)	2.98	0	3.6	0.23	0.16	0.24	0.14
IV ($n = 27$)	2.52	0.007	5.92	0.009	0.51	0.042	0.7
V ($n = 22$)	2.58	0.004	8.22	0.047	0.08	0.18	<0.05
VI ($n = 29$)	3.24	0.002	6.04	0.05	0.1	0.13	0.1
VII ($n = 29$)	1.31	0.005	44.39	0.013	0.19	0.08	0.44
VIII ($n = 12$)	0	0	427.2	0.29	<0.001	0.26	1.00
Major geographical groups							
Northern coastal group (I, II, III, and IV; $n = 114$)	6.96	0	17.83	0.0096	0.25	0.016	0.41
Southern coastal group (V and VI; $n = 51$)	4.3	0.89	11.32	0.012	0.32	0.04	0.22
Western group (VII and VIII; $n = 41$)	5.47	0.002	4.38	0.018	0.53	0.058	0.54
Global (all populations)	7.1	0	25.4	0.002	0.62	0.009	0.63

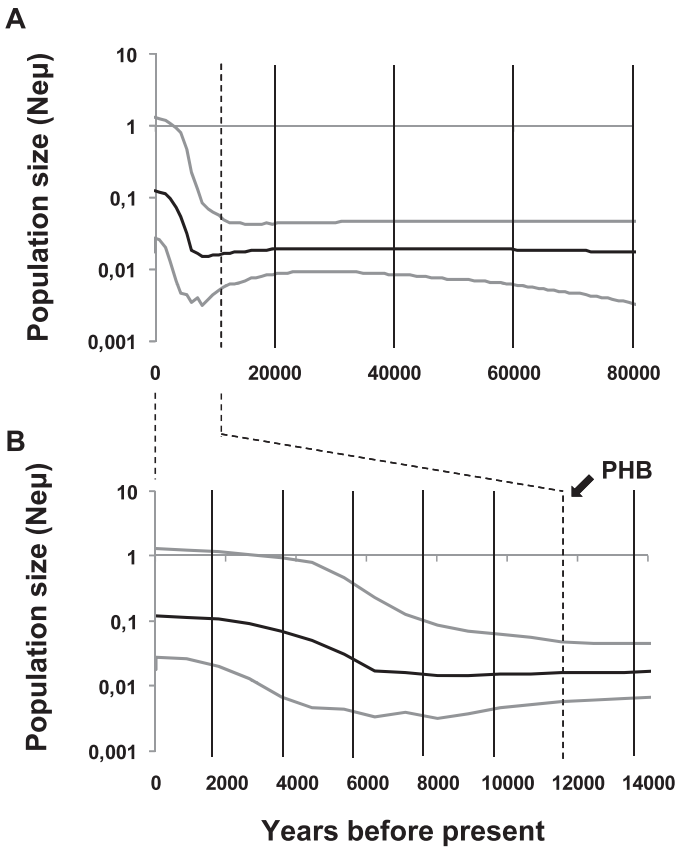

Fig. 5.—A) Bayesian skyline plot showing the complete reconstruction of female effective population size fluctuations throughout time in *Ctenomys talarum* during the late Pleistocene–Holocene period. Black lines represent median estimations and gray lines represent the upper and lower 95% credible intervals. The x-axes of these figures were estimated using a mutation rate of 4.42% calculated in this study for the genus *Ctenomys*. B) Partial demographic reconstructions (details of the last 14,000 years) showing the strong increase in population size starting in the early Holocene, at approximately 8,000 years ago. PHB denotes the Pleistocene–Holocene boundary.

as a result of long-term persistence over its geographical range and limited dispersal abilities. Even though major rivers and highlands appear to be associated with genetic differentiation, geographical distance among populations seems to be the main factor in shaping the genetic structure of the species. Based on our results, current populations of *C. talarum* appear to be relicts of a more extended historical distribution in the Argentinean Pampas, with possible local extinctions of some inland populations. These historical extinctions, however, seem not to have erased the signature of long-term stability (with the exception of the southernmost region of their distribution) and geographical structure of this species along the coastal and inland distributional range. A close association appears to exist between the major climatic changes of the late Pleistocene and Holocene in the central region of Argentina and the main historical demographic changes inferred from *C. talarum*.

One-dimensional distribution and limited dispersal: implications for the genetic structure of the Talas tuco-tuco.—Ecological factors and life-history traits constitute a complex set of variables whose interactions model dispersal patterns in wild populations (Schradin et al. 2010; Solomon 2003). Although data on dispersal are accessible for only a small number of species of subterranean rodents, these species are generally characterized by low dispersal capabilities (e.g., pocket gophers [Patton and Smith 1990; Steinberg and Patton 2000; Williams and Cameron 1984] and tuco-tucos [Cutrera et al. 2005, 2006; El Jundi and de Freitas 2004; Lacey 2000; Mora et al. 2010; Reig et al. 1990]). In particular for *C. talarum*, the low dispersal capability observed using direct (Malizia et al. 1995) and indirect (Cutrera et al. 2005) methods has been associated with some morphological characteristics (Vassallo 1998), the high energetic cost associated with digging (Busch et al. 2000), fragmentation of suitable habitat (Mora et al. 2010), and high levels of aboveground predation (Vassallo et al. 1994). Accordingly, our estimates of global gene flow (Nm) based on Φ_{ST} (Hudson et al. 1992), yielded lower values for *C. talarum* ($Nm = 0.148$) in comparison to other species of tuco-tucos from Buenos Aires Province, such as *C. australis* ($Nm = 1.47$—Mora et al. 2006) and *C. porteousi* ($Nm = 0.86$—Mapelli 2010).

Further, the strong association of this species to the coastal habitat may constrain the geographical genetic structure mainly to 1 dimension. Although there are a few inland populations of *C. talarum* within Buenos Aires Province (Fig. 1A), these are characterized by very low densities relative to the coastal populations. One-dimensional distributions, coupled with limited dispersal ability, often result in patterns of isolation by distance (Slatkin 1993; Slatkin and Barton 1989), which also are expected in species that have colonized and occupied their current distribution for long periods of time (Hutchison and Templeton 1999). Our results support a pattern of isolation by distance for the entire range of *C. talarum*, and suggest demographic stability and a historical equilibrium between migration and drift for this species. However, populations belonging to the nearly 1-dimensional coastal distribution showed a lack of isolation by distance.

Significant pattern of isolation by distance does not necessarily mean spatially homogeneous gene flow (Garnier et al. 2004; Lacey 2000; Steinberg and Patton 2000). Particularly, at minor spatial scales local distribution of *C. talarum* is influenced by soil, vegetation, and topographic features of the habitat; because areas of suitable soil and vegetation are patchily distributed, individuals also tend to be spatially clumped (Busch et al. 1989, 2000; Malizia et al. 1991). At greater geographical scales, the spatial distribution of this species both on the coast and in inland habitats is highly fragmented (Busch et al. 2000; Mora et al. 2007), and might not involve a strict homogeneous gene flow among populations. Furthermore, large habitat fragmentation and isolation in inland populations may result in gene flow patterns better described by a stepping-stone model (Kimura and Weiss 1964). However, because a stepping-stone model was not directly tested in this study, it was not possible to know if this alternative model may be better for describing gene flow

patterns in *C. talarum* than the isolation-by-distance model. Without denying the historical gene flow among macroregions, examination of our data suggests that gene flow has taken place more frequently between neighboring populations and might also have been limited by barriers such as rivers and highlands.

Hierarchical AMOVA showed significant population differentiation among all populations studied. The presence of barriers such as rivers and highlands seem to have had an important role in shaping the genetic differentiation among regions in the past. It should be noted, however, that the highest value of divergence (Φ_{ST}) was obtained without any a priori grouping of populations, suggesting that small local barriers such as soil characteristics (e.g., unsuitable habitat such as hard, humid soils in low terrain elevation—see Malizia et al. 1991; Mora et al. 2007; Vassallo 1993; Vassallo et al. 1994) and distance among neighboring populations also may have important implications in shaping the historical relationships between haplotypes and populations of this subterranean rodent species.

Similar results have been obtained for other species of ctenomyids, as is the case for *C. pearsoni* (Tomasco and Lessa 2007). Local populations of this species also are associated with a nearly linear, predominantly coastal distribution and show a pattern consistent with a regime of differentiation by genetic drift under limited gene flow (Tomasco and Lessa 2007). Like *C. talarum*, *C. pearsoni* occupies sandy soils on the coast of eastern Uruguay, but western inland populations also occur across a wider range of soil hardness (Tomasco and Lessa 2007). The greater flexibility in soil occupation exhibited by these 2 species of tuco-tucos may have facilitated local adjustments to Quaternary sea level changes while maintaining an overall pattern of isolation by distance, a pattern opposed to that exhibited by other species of tuco-tucos distributed in the coastal habitat of Buenos Aires Province (*C. australis*—Mora et al. 2006, 2007) and Uruguay (*C. rionegrensis*—Wlasiuk et al. 2003).

Habitat history and demographic trajectories of populations of C. talarum.—Our results add to a growing body of evidence that suggests that tuco-tucos present an interesting pattern of association with their flexibility to occupy different soils (mainly sandy soils), the origin of their coastal habitats, and their demographic and genetic structures. As mentioned above, *C. talarum* and *C. pearsoni* are characterized by a certain demographic stability and substantial geographical structure; these 2 species are primarily distributed along the coasts of the Atlantic and the Río de la Plata but are not restricted to the 1st line of dunes. In contrast, species restricted to sand dunes, such as *C. australis* (Mora et al. 2006, 2007) and *C. rionegrensis* (Wlasiuk et al. 2003), show historical demographic instability and evidence of demographic expansion.

Our analyses consistently show a history of demographic stability for *C. talarum* in most of its current distributional range. The roughly multimodal mismatch distribution and neutrality tests observed here suggest an overall scenario of long-term demographic stability (Rogers and Harpending 1992; Slatkin and Hudson 1991). These results also are associated with strong geographical subdivision. It should be noted, however, that some demographic evidence suggests a sudden expansion event in the Southern macroregion, particularly in Region V. This demographic expansion seems to be associated with major climatic changes that occurred on the coast during the Holocene (see below). This biogeographic hypothesis suggests that this species could have increased its effective population size and extended its distributional range following the establishment of a novel sand dune habitat in the southernmost portion of Buenos Aires Province.

Gene flow values from Region V to the other geographical regions could be inflated because of departure from strict neutrality. It should be noted that populations belonging to this group show a shallow geographical structuring of haplotype diversity, suggesting a scenario of a lack of equilibrium between gene flow and genetic drift among its populations. In addition to these results, this regional group seems to represent a typical starlike topology in its haplotype network. According to Harpending et al. (1998) and Slatkin and Hudson (1991), this type of starlike pattern among haplotypes found for the minimum spanning network of this geographical group most likely reflects the imprinting of a recent range expansion.

Departures of the mitochondrial genome from strict neutrality cannot be ruled out in these populations, and different selective regimes can mimic the various demographic scenarios outlined above. In particular, selective sweeps and selection against slightly deleterious mutations can result in a pattern of haplotype diversity similar to that produced by a population expansion (Harpending et al. 1998; Wlasiuk et al. 2003). As was suggested above, however, other analyses support a scenario of demographic stability at global level.

Knowledge about the major climatic changes that occurred in the Pampas region during the Pleistocene and Holocene may help understand how the demographic trajectories of populations of *C. talarum* fluctuated during this period. During most of the late Pleistocene climate in the Pampas seems to have been mostly arid and cold with short wetter periods (Quattrocchio et al. 2008; Tonni et al. 1999), where aridity and intensive aeolian activity might have prevented the spread of humid grassland habitats (Leon and Anderson 1973; Tonni et al. 1999). After that, the late Pleistocene–Holocene transition in Buenos Aires Province was characterized by increasing stability and soil formation, suggesting relative higher humidity and warmer climatic conditions compared to the previous period (Rabassa 1987). Vertebrate fossil records show the presence of species associated with more humid conditions, suggesting a markedly rising humidity and precipitation regime (Tonni et al. 1999) during the Holocene.

The geomorphologic history of the coast during the last 8,000 years differed from that inland in the Pampas region; while increased stability and pedogenesis characterized inland areas, independent cycles of dune formation occurred in coastal areas (Isla et al. 2001; Mora et al. 2006), favoring the spread of the sandy dune habitat. Palynological evidence from southeastern Buenos Aires Province dated at 8,000–6,000 years ago suggests the development of a vegetation community charac-

teristic of coastal dunes and interdune ponds with a slight marine influence (Borel et al. 2001; Quattrocchio and Borromei 1998; Quattrocchio et al. 2008).

Bayesian skyline plots showed a constant population size from the middle–late Pleistocene to the early Holocene; effective population sizes seem to have remained constant until an increase in population size approximately 8,000 years ago. This overall change in historical effective population size coincides with the important geological event of sand dune formation. During the period from 7,500 to 600 years ago, a number of independent cycles of Quaternary dune formation favored the elaboration of coastal sand dune habitats (Isla et al. 2001). A significant marine regression during this period resulted in lower water levels along the sea coast. These new sandy habitats were most likely colonized by *C. talarum* soon after they became available, resulting in an expansion of this species' range along the coast. A possible increase in migration from the southernmost region toward the northern coastal and some inner portions of the Pampean region could have characterized the period after this expansion event.

This sandy habitat is the most typical habitat currently occupied by the Talas tuco-tuco in most of the portions of its distribution, and the increase in habitat during this time period may have allowed for the increase in the distribution of this species. *C. talarum* also occupies a wide range of soil hardness, characterized by abundant plant cover and high plant biomass both above and below ground (e.g., inland populations in Fig. 1A—Busch et al. 2000; Cutrera et al. 2006), further increasing this species' ability to expand and grow in numbers during this period. Therefore, the overall increase in effective population size suggested by Bayesian skyline plots may coincide with significant climatic changes hypothesized for the region.

Both the presence of coastal grasslands and extensive areas of sandy soils could have favored the demographic expansion of *C. talarum* observed around the early and middle Holocene in the southern coastal habitat (Region V; see Fig. 1B), in concordance with evidence from Fu's F_S- and Tajima's *D*-tests (Table 4), and Harpending's raggedness index (Table 5). In fact, the strongest signal of population expansion detected by Bayesian skyline plot analyses by macroregions comes from the southern region of the distribution of *C. talarum* (these data are not shown). On the contrary, the more temperate and humid conditions that characterized the middle and late Holocene caused a retraction of the inland sand dunes around 2,000 years ago (Quattrocchio et al. 2008), most likely having an important effect on the fragmentation of habitat of *C. talarum* in that geographical area. This last historical retraction of the sand dune habitat might explain the lowest values of female effective population sizes observed from the inland regions (see Supporting Information S2).

In the Sierras de Tandilia, and in other areas within Buenos Aires Province (see Fig. 1A), *C. talarum* disappeared some 170 ± 60 years ago (Quintana 2004). Current inland presence of *C. talarum* has been reported in Sierra de la Ventana, Saladillo, and El Cazón (Mora et al. 2007; Quintana 2004; Fig. 1A), and in the locality Coronel Suarez, cited for the 1st time in the present study. In these localities, habitat of *C. talarum* is naturally fragmented; however, in recent years, the degree of fragmentation has markedly increased associated with the expansion of agroecosystems in the last 2 centuries (Mapelli and Kittlein 2009; Mora et al. 2007). The regional replacement of cattle-raising activities by extensive cultivation of soybeans is the major threat to the viability of inland populations of *C. talarum* (Mora et al. 2007). In fact, the Talas tuco-tuco has recently been classified as vulnerable for some populations within Buenos Aires Province, with the fixation of dunes, agriculture, and fragmentation identified as the principal disrupting factors (Mora et al. 2007).

Current inland populations appear to be relicts of a wider historical distribution of *C. talarum* in the center of Buenos Aires Province (Mora et al. 2007; Quintana 2004). Historical and current local extinctions of inland populations, however, did not erase the overall signature of long-term stability, which is observed in most of their current distribution. A pattern of isolation by distance and mismatch distributions support this hypothesis. Even though *C. talarum* exhibits a higher flexibility in soil type selection (Vassallo 1998) compared with other ctenomyids (e.g., *C. flamarioni* [Fernández-Stolz et al. 2007], *C. porteousi* [Mapelli and Kittlein 2009], and *C. australis* [Mora et al. 2006]), the extension of sandy soils, isolation of regions and macroregions, and the historical arrangement of the major drainage system in Buenos Aires Province could have been important factors shaping the demographic history of this species.

It appears that the Talas tuco-tuco has occupied its range and maintained a stable regime of differentiation by genetic drift under restricted gene flow for some time. This species may have spread along the coast, in association with cycles of dune formation during the middle and late Holocene. Considering the strong association of *C. talarum* with sandy soils with moderate plant cover, the increase in population size, proposed here to have occurred 8,000 years ago, might be correlated with the spread of sandy habitats and the expansion of coastal grasslands during this time, following a period of arid and cold conditions during the late Pleistocene.

Coastal and inland populations.—The general picture that can account for the differentiation in this species appears to be primarily the result of migration processes from inland to coastal environments, and later from the southern part of its distribution to the other regions. Our results allow us to hypothesize that inland and coastal populations might have originated from the central part of Buenos Aires Province, possibly at different times and following the riverbanks. The Sierras de Tandilia and the Río Quequén Grande might have been significant geographical barriers to the historical and current dispersal of this species (Figs. 1A and 1B). This scenario could explain the absence of *C. talarum* between the northern and southern coastal populations, from Santa Clara to the Río Quequén Grande (Fig. 1A).

At the population level, the highest values of gene flow were observed between pairs of neighboring populations and

the highest levels of connection among populations were verified for the northern coastal distribution, which also had the highest population densities (Malizia et al. 1991; Pearson et al. 1968). Interestingly, high levels of gene flow were verified between a locality in the south of the distribution, R, and another in the north, MC. Further, in general, older haplotypes are expected to have a broad geographical distribution, high frequencies, and multiple connections within a network (Freeland 2005). Considering this, our results suggest that haplotype 20, broadly distributed from R to MC, might represent an ancestral variant, possibly occupying a more continuous habitat in the past. The puzzling historical connection between these 2 currently isolated localities suggests a possible origin of the distribution of *C. talarum* in inland populations in the center of Buenos Aires Province. This ancient haplotype may have originated early, with other haplotype variants observed nowadays mainly on the coastal distribution emerging later. This hypothesis also is supported by the lack of fossils found between Río Quequén Grande and Santa Clara (Fig. 1A). On the other hand, following riverbanks as a primary mode of dispersal and colonization may be able to explain the low levels of gene flow among the populations from groups V and VI, divided by Arroyos Cristiano Muerto and Claromecó (Figs. 1A and 1B).

Overall, examination of our data suggests that historical gene flow among regions was highly asymmetric, and our data support a route of colonization mainly from the south to the north, and in minor degree to the west. Even though gene flow values were mostly low, some regions separated by more than 350 km showed high values of historical gene flow (e.g., Region I receiving migrants from Region VIII, and Region II receiving migrants from Region V and Region VI; see Supporting Information S2 and Fig. 1B). This implies a more continuous and fluid historical migration compared to the current situation, in which the coastal distributions of *C. talarum* are the most spatially continuous, whereas inland populations present a high degree of habitat fragmentation, preventing gene flow between populations.

There are few studies of *Ctenomys* comparing the patterns of population genetic structure using several genetic markers in the analyses. In general, past approaches have described patterns of genetic variation at different temporal and spatial scales, showing substantially different estimates of gene flow (see Mapelli et al. 2012a, 2012b; Wlasiuk et al. 2003). However, the comparison of this mitochondrial pattern with nuclear data sets will provide a better interpretation on which to assess the general dynamics of differentiation in *C. talarum*, including historical and current changes in levels of gene flow and isolation.

Resumen

A partir del uso de secuencias de ADN mitocondrial de la región control (D-loop), analizamos la filogeografía del roedor sudamericano *Ctenomys talarum* (tuco-tuco de los talares). Esta especie es un roedor herbívoro y endémico de la Provincia de Buenos Aires, Argentina, que habita pastizales naturales en dunas costeras y en algunas poblaciones continentales fragmentadas. En este estudio evaluamos las relaciones genéticas entre las poblaciones de *C. talarum* a través de su rango de distribución total y analizamos cómo la historia geológica de su hábitat ha afectado la estructura genética y la demografía histórica de estas poblaciones. Tanto la red de haplotipos como los análisis de la varianza molecular mostraron alta subdivisión genética y un patrón filogeográfico profundo entre las poblaciones de *C. talarum*. Los valores de F_{ST} pareados mostraron una diferenciación significativa entre todas las poblaciones estudiadas. El patrón general fue similar al esperado bajo un modelo de aislamiento por distancia, evidenciando un equilibrio entre flujo génico y deriva genética local. Las barreras más importantes en el área (e.g. ríos y hábitat poco apto para la ocupación) y el propio aislamiento poblacional se asociarían con una fuerte diferenciación genética entre los diferentes grupos geográficos. Las diferencias pareadas entre sitios nucleotídicos y los tests de neutralidad sugieren historias contrastantes para los diferentes grupos de poblaciones; mientras algunas de ellas se caracterizaron por presentar estabilidad demográfica, otras mostraron apartamientos de la neutralidad estricta, consistente con una expansión demográfica reciente. Finalmente, los cambios climáticos más importantes ocurridos durante el Pleistoceno Tardío y Holoceno en la región central de Argentina parecen estar asociados a los principales cambios demográficos históricos inferidos para *C. talarum*. Las poblaciones actuales de *C. talarum* parecen ser relictos de una distribución histórica más extendida a lo largo de la pampa Argentina. Dichas extinciones históricas, sin embargo, no han borrado la señal de estabilidad a largo término y la estructura geográfica de esta especie en los rangos de distribución costeros y continentales.

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Supporting Information

Supporting Information S1.—Appendix I

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Supporting Information S2.—Appendix II

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Literature Cited

Aars, J., R. A. Ims, H. P. Liu, M. Mulvey, and M. H. Smith. 1998. Bank voles in linear habitats show restricted gene flow as revealed by mitochondrial DNA (mtDNA). Molecular Ecology 7:1383–1389.

Abdo, Z., K. A. Crandall, and P. Joyce. 2004. Evaluating the performance of likelihood methods for detecting population structure and migration. Molecular Ecology 13:837–851.

Beerli, P., and J. Felsenstein. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. Proceedings of the National Academy of Sciences, USA 98:4563–4568.

Borel, C. M., M. V. Biachinotti, and M. E. Quattrocchio. 2001. Palinomorfos fúngicos del Pleistoceno–Holoceno en el valle del Arroyo Chasicó, Provincia de Buenos Aires. Polen 11:21–37.

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

Busch, C., A. L. Malizia, O. A. Scaglia, and O. A. Reig. 1989. Spatial distribution and attributes of a population of *Ctenomys talarum* (Rodentia: Octodontidae). Journal of Mammalogy 70:204–208.

Busch, C., et al. 2000. Population ecology of subterranean rodents. Pp. 183–226 in Life underground: the biology of subterranean rodents (E. A. Lacey, G. Cameron, and J. L. Patton, eds.). University of Chicago Press, Chicago, Illinois.

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

Contreras, J. R., and O. A. Reig. 1965. Datos sobre la distribución del género *Ctenomys* (Rodentia: Octodontidae) en la zona costera de la Provincia de Buenos Aires comprendida entre Necochea y Bahía Blanca. Physis 25:169–186.

Cook, J. A., and E. P. Lessa. 1998. Are rates of diversification in subterranean South American tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) unusually high? Evolution 52:1521–1527.

Cutrera, A. P., C. D. Antinuchi, M. S. Mora, and A. I. Vassallo. 2006. Home-range and activity patterns of the South American subterranean rodent *Ctenomys talarum*. Journal of Mammalogy 87:1183–1191.

Cutrera, A. P., E. A. Lacey, and C. Busch. 2005. Genetic structure in a solitary rodent (*Ctenomys talarum*): implications for kinship and dispersal. Molecular Ecology 14:2511–2523.

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

Drummond, A. J., S. Y. W. Ho, M. J. Phillips, and A. Rambaut. 2006. Relaxed phylogenetics and dating with confidence. PLoS Biology 4:e88.

Drummond, A. J., and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7:214.

Drummond, A. J., A. Rambaut, B. Shapiro, and O. G. Pybus. 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. Molecular Biology and Evolution 22:1185–1192.

Eastman, J. R. 1999. IDRISI 3.2. A grid based geographic and image processing system. Clark University, Graduate School of Geography, Worcester, Massachusetts.

El Jundi, T. A. R. J., and T. R. O. de Freitas. 2004. Genetic and demographic structure in a population of *Ctenomys lami* (Rodentia—Ctenomyidae). Hereditas 140:18–23.

Excoffier, L. 2004. Patterns of DNA sequence diversity and genetic structure after a range expansion: lesson from the infinite-island model. Molecular Ecology 13:853–864.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47–50.

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.

Fernández-Stolz, G. P., J. F. B. Stolz, and T. R. O. de Freitas. 2007. Bottlenecks and dispersal in the tuco-tuco das dunas, *Ctenomys flamarioni* (Rodentia: Ctenomyidae), in southern Brazil. Journal of Mammalogy 88:935–945.

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

Freeland, J. R. 2005. Molecular ecology. 1st ed. John Wiley & Sons Ltd., Chichester, United Kingdom.

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

Garnier, S., P. Alibert, P. Gaudiot, B. Prieur, and J. Y. Rasplus. 2004. Isolation by distance and sharp discontinuities in gene frequencies: implications for the phylogeography of an alpine insect species, *Carabus solieri*. Molecular Ecology 13:1883–1897.

Goios, A., L. Pereira, M. Bogue, V. Macaulay, and A. Amorim. 2007. mtDNA phylogeny and evolution of laboratory mouse strains. Genome Research 17:293–298.

Guindon, S., J. F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, and O. Gascuel. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59:307–321.

Guindon, S., and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52:696–704.

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

Harpending, H. C., M. A. Batzer, M. Gurven, L. B. Jorde, A. R. Rogers, and S. T. Sherry. 1998. Genetic traces of ancient demography. Proceedings of the National Academy of Sciences, USA 95:1961–1967.

Harpending, H. C., and A. R. Rogers. 2000. Genetic perspectives on human origins and differentiation. Annual Review of Genomics and Human Genetics 1:361–385.

Hudson, R. R., D. D. Boos, and N. L. Kaplan. 1992. A statistical test for detecting population subdivision. Molecular Biology and Evolution 9:138–151.

Huhndorf, M. H., J. C. Kerbis Peterhans, and S. S. Loew. 2007. Comparative phylogeography of three endemic rodents from the Albertine Rift, east central Africa. Molecular Ecology 16:663–674.

Hutchison, D. W., and A. R. Templeton. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution 53:1898–1914.

Isla, F. I., L. C. Cortizo, H. A. Turno Orellano. 2001. Dinámica y evolución de las barreras medanosas, Provincia de Buenos Aires, Argentina. Revista Brasileira de Geomorfología 2:73–83.

KIMURA, M., AND G. H. WEISS. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49:561–576.

KITTLEIN, M. J., AND O. E. GAGGIOTTI. 2008. Interactions between environmental factors can hide isolation by distance patterns: a case study of *Ctenomys rionegrensis* in Uruguay. Proceedings of the Royal Society, B. Biological Sciences 275:2633–2638.

KOCHER, T. D., ET AL. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences, USA 86:6196–6200.

KRUPA, J. J., AND K. N. GELUSO. 2000. Matching the color of excavated soil: cryptic coloration in the plains pocket gopher (*Geomys bursarius*). Journal of Mammalogy 81:86–96.

LACEY, E. A. 2000. Spatial and social systems of subterranean rodents. Pp. 257–296 in Life underground: the biology of subterranean rodents (E. A. Lacey, G. Cameron, and J. L. Patton, eds.). University of Chicago Press, Chicago, Illinois.

LACEY, E. A., J. E. MALDONADO, J. P. CLABAUGH, AND M. D. MATOCQ. 1999. Interspecific variation in microsatellites isolated from tuco-tucos (Rodentia: Ctenomyidae). Molecular Ecology 8:1754–1756.

LEON, R., AND D. ANDERSON. 1973. El límite occidental del pastizal pampeano. Tuexenia 3:67–82.

LESSA, E. P., AND J. A. COOK. 1998. The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of speciation. Molecular Phylogenetics and Evolution 9:88–99.

LESSA, E. P., AND C. S. THAELER. 1989. A reassessment of morphological specializations for digging in pocket gophers. Journal of Mammalogy 70:689–700.

LIBRADO, P., AND J. ROZAS. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452.

MALIZIA, A. I., A. I. VASSALLO, AND C. BUSCH. 1991. Population and habitat characteristics of two sympatric species of *Ctenomys* (Rodentia: Octodontidae). Acta Theriologica 36:87–94.

MALIZIA, A. I., R. R. ZENUTO, AND C. BUSCH. 1995. Demographic and reproductive attributes of dispersers in two populations of the subterranean rodent *Ctenomys talarum* (tuco-tuco). Canadian Journal of Zoology 73:732–738.

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

MAPELLI, F. J. 2010. Ecología y genética de metapoblaciones del roedor subterráneo *Ctenomys porteousi*. Ph.D. dissertation, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina.

MAPELLI, F. J., AND M. J. KITTLEIN. 2009. Influence of patch and landscape characteristics on the distribution of the subterranean rodent *Ctenomys porteousi*. Landscape Ecology 24:723–733.

MAPELLI, F. J., M. S. MORA, P. M. MIROL, AND M. J. KITTLEIN. 2012a. Effects of Quaternary climatic changes on the phylogeography and historical demography of the subterranean rodent *Ctenomys porteousi*. Journal of Zoology 286:48–57.

MAPELLI, F. J., M. S. MORA, P. M. MIROL, AND M. J. KITTLEIN. 2012b. Population structure and landscape genetics in the endangered subterranean rodent *Ctenomys porteousi*. Conservation Genetics 13:165–181.

MATOCQ, M. D., J. L. PATTON, AND M. N. F. DA SILVA. 2000. Population genetic structure of two ecologically distinct Amazonian spiny rats: separating history and current ecology. Evolution 54:1423–1432.

MICHAUX, J. R., R. LIBOIS, AND M. G. FILIPPUCCI. 2005. So close and so different: comparative phylogeography of two small mammal species, the yellow-necked fieldmouse (*Apodemus flavicollis*) and the woodmouse (*Apodemus sylvaticus*) in the western Palearctic region. Heredity 94:52–63.

MILÁ, B., D. J. GIRMAN, M. KIMURA, AND T. B. SMITH. 2000. Genetic evidence for the effect of a postglacial population expansion on the phylogeography of a North American songbird. Proceedings of the Royal Society of London, B. Biological Sciences 267:1033–1040.

MILLER, S. A., D. D. DYKES, AND H. F. POLESKY. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 16:1215.

MORA, M. S. 2008. Biología metapoblacional del tuco-tuco de las dunas (*Ctenomys australis*): efectos de la estructura espacial del hábitat sobre la ecología y genética de poblacional. Ph.D. dissertation, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina.

MORA, M. S., E. P. LESSA, A. P. CUTRERA, M. J. KITTLEIN, AND A. I. VASSALLO. 2007. Phylogeographic structure in the subterranean tuco-tuco *Ctenomys talarum* (Rodentia: Ctenomyidae): contrasting the demographic consequences of regional and habitat-specific histories. Molecular Ecology 16:3453–3465.

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

MORA, M. S., F. J. MAPELLI, O. E. GAGGIOTTI, M. J. KITTLEIN, AND E. P. LESSA. 2010. Dispersal and population structure at different spatial scales in the subterranean rodent *Ctenomys australis*. BMC Genetics 11:1–14.

OPAZO, J. C., M. P. BURGUEÑO, 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.

PATTON, J. L., AND M. SMITH. 1990. The evolutionary dynamics of the pocket gopher *Thomomys bottae*, with emphasis on California populations. University of California Publications in Zoology 123:1–161.

PAVLOVA, A., R. M. ZINK, AND S. ROHWER. 2005. Evolutionary history, population genetics and gene flow in the common rosefinch (*Carpodacus erythrinus*). Molecular Phylogenetics and Evolution 36:669–681.

PEACOCK, M. M., V. S. KIRCHOFF, AND S. J. MERIDETH. 2002. Identification and characterization of nine polymorphic microsatellite loci in the North American pika, *Ochotona princeps*. Molecular Ecology Notes 2:360–362.

PEARSON, O., ET AL. 1968. Estructura social, distribución espacial y composición por edades de una población de tuco-tucos (*Ctenomys talarum*). Investigaciones Zoológicas Chilenas 13:47–80.

POSADA, D. 2008. jModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25:1253–1256.

QUATTROCCHIO, M. E., AND A. M. BORROMEI. 1998. Paleovegetational and paleoclimatic changes during the Late Quaternary in southwestern Buenos Aires Province and southern Tierra del Fuego (Argentina). Palynology 22:67–82.

QUATTROCCHIO, M. E., A. M. BORROMEI, C. M. DESCHAMPS, S. C. GRILL, AND C. A. ZAVALA. 2008. Landscape evolution and climate changes in the Late Pleistocene–Holocene, southern Pampa (Argentina): Evidence from palynology, mammals and sedimentology. Quaternary International 181:123–138.

QUINTANA, C. A. 2004. El registro de *Ctenomys talarum* durante el Pleistoceno Tardío–Holoceno de las Sierras de Tandilia Oriental. Journal of Neotropical Mammalogy 11:45–53.

RABASSA, J. 1987. The Holocene of Argentina, a review. Quaternary of South America and Antarctic Peninsula 5:269–291.

RAMBAUT, A., AND A. J. DRUMMOND. 2007. Tracer v1.4. http://tree.bio.ed.ac.uk/software/tracer/. Accessed 24 January 2013.

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

RAYMOND, M., AND F. ROUSSET. 1995. An exact test for population differentiation. Evolution 49:1280–1283.

REIG, O. A., C. BUSCH, J. CONTRERAS, AND M. ORTELLS. 1990. An overview of evolution, systematics, population biology and molecular biology in *Ctenomys*. Pp. 71–96 in Biology of subterranean mammals (E. Nevo and O. A. Reig, eds.). Allan Liss, New York.

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.

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.

SCHRADIN, C., B. KÖNIG, AND N. PILLAY. 2010. Reproductive competition favors solitary living while ecological constraints impose group-living in African striped mice. Journal of Animal Ecology 79:515–521.

SELONEN, V., AND I. K. HANSKI. 2004. Young flying squirrels (*Pteromys volans*) dispersing in fragmented forests. Behavioral Ecology 15:564–571.

SIKES, R. S., W. L. GANNON, AND THE ANIMAL CARE AND USE COMMITTEE OF THE AMERICAN SOCIETY OF MAMMALOGISTS. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 92:235–253.

SLATKIN, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787–792.

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

SLATKIN, M., AND N. H. BARTON. 1989. Comparison of three indirect methods for estimating average levels of gene flow. Evolution 43:1349–1368.

SLATKIN, M., AND R. R. HUDSON. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129:555–562.

SOLOMON, N. G. 2003. A reexamination of factors influencing philopatry in rodents. Journal of Mammalogy 84:1182–1197.

STEINBERG, E. K., AND J. L. PATTON. 2000. Genetic structure and the geography of speciation in subterranean rodents: opportunities and constraints for evolutionary diversification. Pp. 301–331 in Life underground: the biology of subterranean rodents (E. A. Lacey, G. Cameron, and J. L. Patton, eds.). University of Chicago Press, Chicago, Illinois.

TAJIMA, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

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.

THOMAS, O. 1898. Descriptions of two new Argentine rodents. Annals and Magazine of Natural History, Series 7, 1:283–286.

THOMAS, O. 1912. A new genus of opossum and a new tuco-tuco. Annals and Magazine of Natural History, Series 8, 9:239–241.

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. P. Lessa, J. A. Salazar-Bravo, and J. L. Patton, eds.). University of California Publications in Zoology Series. University of California, Berkeley.

TONNI, E. P., A. L. CIONE, AND A. J. FIGINI. 1999. Predominance of arid climates indicated by mammals in the Pampas of Argentina during the late Pleistocene and Holocene. Palaeogeography, Palaeoclimatology, Palaeoecology 147:257–281.

VASSALLO, A. I. 1993. Habitat shift after experimental removal of the bigger species in sympatric *Ctenomys talarum* and *Ctenomys australis* (Rodentia: Octodontidae). Behaviour 127:247–263.

VASSALLO, A. I. 1998. Functional morphology, comparative behaviour, and adaptation in two sympatric subterranean rodents of the genus *Ctenomys* (Caviomorpha: Octodontidae). Journal of Zoology 244:415–427.

VASSALLO, A. I., M. J. KITTLEIN, AND C. BUSCH. 1994. Owl predation on two sympatric species of tuco-tucos (Rodentia, Octodontidae). Journal of Mammalogy 75:725–732.

VERZI, D. H. 1999. The dental evidence on the differentiation of the ctenomyine rodents (Caviomorpha, Octodontidae, Ctenomyinae). Acta Theriologica 44:263–282.

VERZI, D. H., A. I. OLIVARES, AND C. C. MORGAN. 2010. The oldest South American tuco-tuco (late Pliocene, northwestern Argentina) and the boundaries of the genus *Ctenomys* (Rodentia, Ctenomyidae). Mammalian Biology 75:243–252.

VUCETICH, M. G., D. H. VERZI, AND J. L. HARTENBERGER. 1999. Review and analysis of the radiation of the South American Hystricognathi (Mammalia, Rodentia). Comptes Rendus de l'Académie des Sciences II 329:763–769.

WATTERSON, G. A. 1975. On the number of segregating sites in genetic models without recombination. Theoretical Population Biology 72:256–276.

WEIR, B. S., AND C. C. COCKERHAM. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.

WILLIAMS, L. R., AND G. N. CAMERON. 1984. Demography of dispersal in Attwater's pocket gopher (*Geomys attwateri*). Journal of Mammalogy 65:67–75.

WILSON, G. M., R. A. DEN BUSSCHE, K. MCBEE, L. A. JOHNSON, AND C. A. JONES. 2005. Intraspecific phylogeography of red squirrels (*Tamiasciurus hudsonicus*) in the central Rocky Mountain region of North America. Genetica 125:141–154.

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

WRIGHT, S. 1943. Isolation by distance. Genetics 28:114–128.

ZENUTO, R. R., E. A. LACEY, AND C. BUSCH. 1999. DNA fingerprinting reveals polygyny in the subterranean rodent *Ctenomys talarum*. Molecular Ecology 8:1529–1532.

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