



Original investigation

Population genetic structure and historical dispersal patterns in the subterranean rodent *Ctenomys* “*chasiquensis*” from the southeastern Pampas region, Argentina

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ABSTRACT

Gene flow plays an essential role in the evolutionary history of the organisms and helps to identify those historical landscape features that most likely had affected the dispersal patterns. In this work, we assess, using mitochondrial control region DNA sequences, the historical migration patterns and population structure in *Ctenomys* “*chasiquensis*”, a highly, vulnerable and endemic subterranean rodent distributed in a very small area from the central part of Argentina. We used Bayesian and maximum likelihood approaches to evaluate the effects of historical gene flow among populations. Moreover, we used Bayesian skyline plots, tests of neutrality and mismatch distributions to assess the potential changes in population size through time. Our analyses show that populations of *C. “chasiquensis”* are moderate structured at regional level and this population pattern is probably the result of an asymmetric historical gene flow essentially from the South-West to the North-West, further of a recent demographic population expansion in the North-West, in conjunction with an important degree of isolation in some populations over its eastern geographical range. Evidently, historical gene flow seems to have been more frequently on the West. Finally, a close relationship appears to exist between the major climatic episodes occurred during the Late Quaternary in the central region of Argentina and the main historical demographic changes inferred for *C. “chasiquensis”*. The current distribution of *C. “chasiquensis”* appear to be a relicts of a more extended historical distribution in the Argentinean Pampas in the Late Pleistocene, with a perceptible population decline at the beginning of the Holocene. In this context, Bayesian demographic inferences showed a small but constant increment of population expansion of this species from approximately 90,000 to 11,000 years BP, after which a period of decrease in population size (that started in the early Holocene and continues nowadays) was observed.

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Introduction

Genetic variation within and among populations is shaped by the interplay of microevolutionary forces as well as their history

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and current ecological conditions (Shikano et al., 2010). Natural populations occur in landscapes in which environmental features may constrain or promote movements of individuals and genes affecting, therefore, migration patterns among local populations and the resultant population structure (Manel et al., 2005). Even though dispersal has significant population level consequences, it originates as a behavioral trait at the level of individuals (Kittlein and Gaggiotti, 2008).

In general, the subterranean rodents are highly specialists to a particular habitat, which makes them a very interesting case to study population genetic structure (Mora et al., 2013). They present restricted dispersal abilities and often occupies naturally fragmented environments into demes of low effective population

sizes. This is the case of the genus *Ctenomys* (Lessa, 2000; Mapelli et al., 2012a; Mirol et al., 2010; Mora et al., 2006, 2007) which is distributed throughout the southern cone of South America and constitutes the most speciose group of all subterranean rodents (Lessa, 2000; Reig et al., 1990).

The genus *Ctenomys* is currently composed by at least eight phylogenetic groups of species described by Parada et al. (2011). One of them is the “*mendocinus* phylogenetic group”, which conform a monophyletic group currently distributed from the western portion of Argentina to the southeast of Brazil, and they are composed by seven recognized species of tuco-tucos: *Ctenomys australis*, *Ctenomys azarae*, *Ctenomys “chasicuensis”*, *Ctenomys flamarioni*, *Ctenomys mendocinus*, *Ctenomys porteوسي*, and *Ctenomys rionegrensis* (D’Elía et al., 1999; Massarini et al., 1991; Massarini and de Freitas, 2005; Parada et al., 2011; Slamovits et al., 2001). These species occupies well-drained sandy soils, mostly associated to the recent Quaternary sand dunes on the coastal plains of southeastern of Brazil, southwestern of Uruguay and eastern of Argentina in the case of *C. flamarioni*, *C. rionegrensis* and *C. australis* (Fernández-Stolz et al., 2007; Kittlein and Gaggiotti, 2008; Mora et al., 2006; Wlasiuk et al., 2003), or are related to the Pleistocene continental dunes and paleo-dunes from central Argentina, in the case of *C. azarae*, *C. mendocinus*, *C. porteوسي* and *Ctenomys. “chasicuensis”* (D’Elía et al., 1999; Mapelli and Kittlein, 2009; Mapelli et al., 2012a; Massarini et al., 1991).

In particular, *C. “chasicuensis”* (or Chasicó’s tuco-tuco), is a form of uncertain status, initially reported only for the Chasicó Lake (Buenos Aires Province) by Contreras and Maceiras (1970) and then by Contreras (1973), but without proper taxonomic description, which makes this designation a “nomen nudum”. Although the close phylogenetic relationship among all species belonging to the “*mendocinus* phylogenetic group”, we consider the populations of this study as *C. “chasicuensis”*, using the same terminology of nominal species adopted by Contreras and Maceiras (1970). Thus, the proposed distribution for this taxonomic group includes the sand-dunes that extend from the Southwest of Buenos Aires to the Southeast of La Pampa in Argentina. As other species of tuco-tucos, the Chasicó’s tuco-tuco is strictly related with sandy soils, on dunes with low vegetation cover and some elevation, eluding low and flood areas. Particularly, Chasicó Lake is the biggest body water in the region, and divides the sand dune rearrangement in conspicuous sandy diagonals (Fig. 1).

The landscape is also interrupted by the presence of some small inland salt flats such as Salina Chica, Salina Colorada Chica, Salina Colorada Grande, Salinas de Jacinto Aráuz and some streams as Arroyo Chasicó, from which the homonymous lagoon arise. These topographic features constitute natural important barriers to dispersal and essentially divide the distribution of this species in three evident sandy diagonals (Fig. 1), two at the West and one at the East relative to the Chasicó Lake. The sand dunes inhabited by the Chasicó’s tuco-tuco are located along the side valleys with NE-SW direction. Beyond this, there are some important differences in the eastern and western parts of the distribution of this species. The East part comprise sandy, well-drain and friable soils that occur in the higher topographic positions surrounding by few lagoons. On the contrary, the distribution of this species becomes more continuous and homogeneous westward.

In current days the natural configuration of this landscape is being lost to forestry, urban development, and the progressive advance of farming (e.g., soybean) in the region; these factors associated with the particular biological attributes of this group, might have caused a negative impact on their populations. Thus, the viability of this endemic species is also associated to the protection of the whole sand-dune environment. Considering this scenario, it is essential to know the population dynamics and dispersion pat-

terns in species with particular restrictions in highly fragmented habitats.

Here we use a mitochondrial data to characterize the population genetic structure and historical migration patterns of *C. “chasicuensis”*, assessing the effects of habitat configuration on the genetic structure of this endemic rodent. Thus, one of the main objectives of this work is to determine whether areas with greater environmental connectivity (located on the West of the species distribution) have shown higher levels of gene flow between populations than the most isolated populations (located in the East, where the human activity produced a strong impact over the borders of this area in current days). Alternatively, we assess the connection between the demographic changes in population sizes through time and the most important climatic changes occurred during the Late Quaternary.

Material and methods

Sampling design and habitat characteristics

Typical sand dune habitat of this species, where sampling of populations was conducted, are characterized by three major sand dunes diagonals disposed in NE-SW direction, from Lihuel Calel (in the Southwest of La Pampa) to Salitral de la Vidriera, nearby Bahía Blanca (Southeast of Buenos Aires; see Fig. 1). Some important landscape features (e.g., depressions of Salinas Grandes, Chasicó Lake and the Chasicó stream) break the nearly continuous distribution of this species on these sandy diagonals (Fig. 1). Population densities of *C. “chasicuensis”* are higher on these sandy diagonals compared to the surrounding environment, which are composed by a highly patchy landscape (pers. obs.). The sampling sites were chosen over this area (sediments in the diagonals are conformed by slime-sand material of eolian origin), between 20 and 40 km of distance between locations (Fig. 1). Mean annual rainfalls around the study area are ~600–700 mm with no pronounced seasonality (Zech et al., 2009).

The central region of Argentina where the study area is included comprises from the Andean foothills to the Atlantic coast between latitudes 32°–40°S, and is covered by powerful and extensive Quaternary aeolian deposits (Iriando, 1999; Zárate and Tripaldi, 2012). Iriando (1999) and Zárate and Tripaldi (2012) described the sandy and silty deposits (loess) covering the Pampean Plain as the “Eolian Pampean System”. Thus, the sampling area is naturally included into this major aeolian unit.

Some natural barriers such as the Chasicó Lake and the Chasicó stream divided the distribution of *C. “chasicuensis”* between two groups of populations: the westernmost sampling sites (LCH, R1, R154, PSR, LE, LC) located at the West of Chasicó Lake, and the easternmost sampling sites (SC, LM) located at the East of this lagoon (Fig. 1). The eastern unit presents more percentage of loess and silt, and higher proportion of precipitation (with higher diversity of grassland species) in comparison to the West (Fig. 1).

It should be noted that vegetation in the eastern unit (38°38’S to 38°53’S, 63°03’W and 62°29’W) has been highly modified by clearings, cultivation, introduction of domestic livestock (especially cattle and sheep) and the occurrence of accidental and directed fires during the last century (Zárate and Tripaldi, 2012). Despite the sub humid conditions, only a tall grass steppe with some shrubs prevails, which is probably due to the intensive land use (Zech et al., 2009). In contrast, the western unit is less affected, creating a continuous sand dune habitat within the phylogeographical Argentinean regions of “Espinal” and “Monte” (Fig. 1), which constitutes a heterogeneous mosaic of landscapes, from open sand dune habitats composed by *Larrea* shrublands and dense vegetation patches of *Prosopis* woodlands to important salt flats.

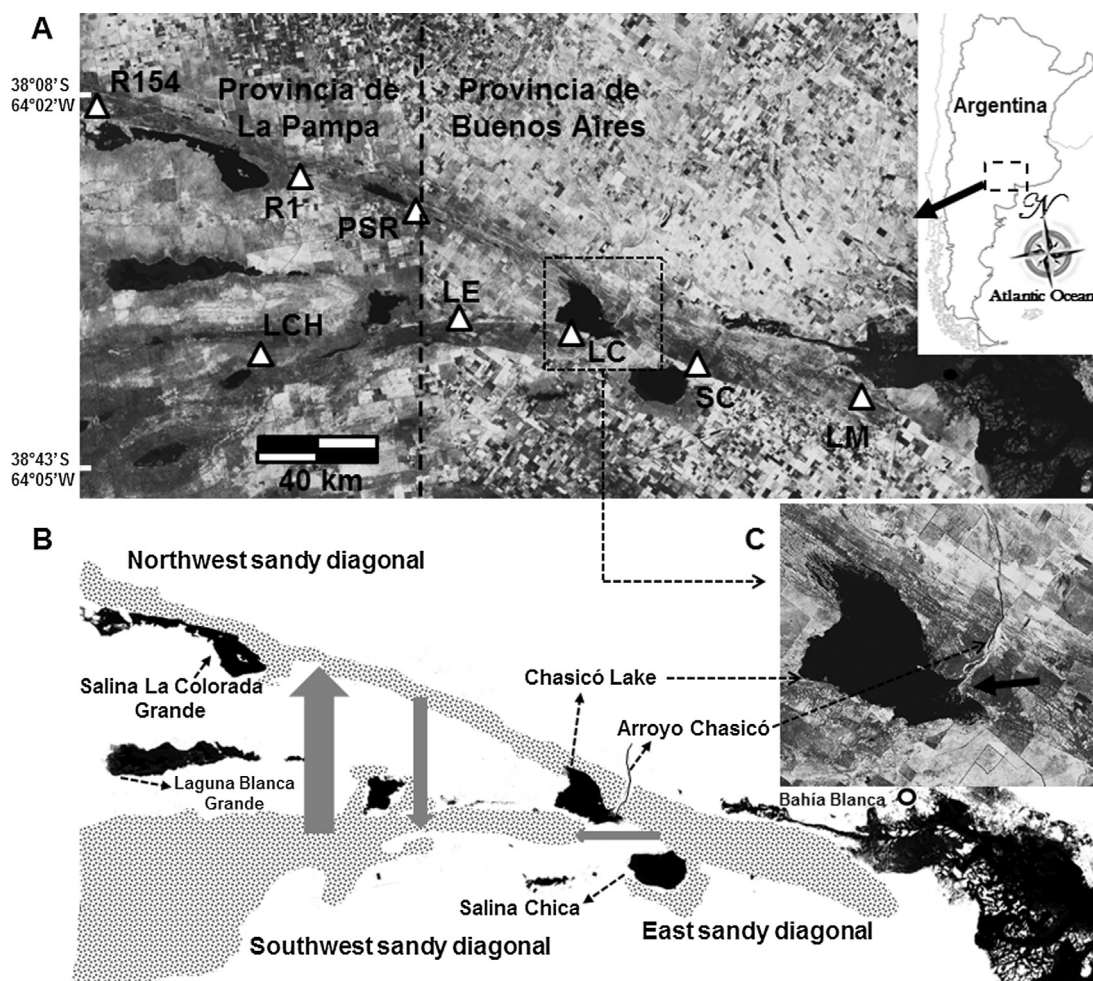


Fig. 1. (A) Distribution of *Ctenomys "chasiquensis"* from the Southwest of Buenos Aires and Southeast of La Pampa (Argentina), and geographical coordinates of sampling sites are shown as follows: Chasicó Lake (LC; 38° 39' S, 63° 5' W), La Chufra (LCH; 38° 33' S, 63° 44' W), La Equidad (LE; 38° 38' S, 63° 17' W), La Mascota (LM; 38° 47' S, 62° 38' W), Pulpería San Rosario (PSR; 38° 23' S, 63° 22' W), Ruta1 (R1; 38° 18' S, 63° 36' W), Ruta154 (R154; 38° 8' S, 64° 4' W), and Salina Chica (SC; 38° 44' S, 62° 52' W). (B) Polygons in dashed pattern represent the three sandy diagonals where this species is more probable to be found. Grey arrows designate the most important historical gene flow patterns among different sand dune diagonals inferred from MIGRATE 3.0.3 (the width of arrows is proportional to the magnitude of gene flow). Only Bayesian gene flow estimates among diagonals are given (see Appendix D in Supplementary material). (C) Enlarged image showing the intersection of the three sandy diagonals on the Chasicó Lake. The black arrow denotes the most significant disruption on these sand diagonals, produced by the Chasicó Lake and Chasicó stream.

Table 1

Number of sequences (S), haplotypes (Ha), polymorphic sites by population (PS), mean number of pairwise differences (π) haplotype diversity (*HaDi*), and nucleotide diversity (*NuDi*) in *Ctenomys "chasiquensis"* from Argentina are shown. The total and overall values of these parameters and the corresponding standard deviations (SD) are also given. Values of Ramos-Onsins and Rozas's R_2 , Tajima's *D* and Fu's F_5 tests by population are shown. The global neutrality tests are also given. NS: not significant. Global neutrality test were performed considering all populations as a whole. Abbreviations for locations are given in Fig. 1.

Sample locations	S (n)	Ha (n)	PS (n)	π	<i>HaDi</i>	SD	<i>NuDi</i>	SD	Tajima's <i>D</i>		Fu's F_5		Ramos-Onsins and Rozas's R_2			
									<i>D</i>	<i>P</i>	F_5	<i>P</i>	R_2	<i>P</i>		
LM	14	3	3	0.79	0.48	0.14	0.0018	0.001	-0.49	NS	0.38	NS	0.093	0.53		
PSR	12	6	11	4.55	0.88	0.06	0.011	0.004	1.03	NS	0.76	NS	0.092	0.54		
R154	15	6	10	2.5	0.84	0.061	0.0058	0.003	-0.69	NS	-0.2	NS	0.09	0.57		
LC	13	5	10	4.45	0.83	0.06	0.01	0.004	1.52	NS	2.09	NS	0.093	0.51		
LCH	13	5	6	1.9	0.81	0.066	0.0044	0.002	-0.04	NS	-0.2	NS	0.09	0.57		
LE	13	3	16	5.16	0.59	0.12	0.013	0.005	0.44	NS	6.57	NS	0.092	0.55		
R1	12	6	9	3.6	0.85	0.074	0.0084	0.003	0.87	NS	0.18	NS	0.091	0.55		
SC	11	3	2	0.84	0.57	0.14	0.0019	0.001	0.68	NS	0.24	NS	0.093	0.52		
Total	103															
Overall		29	24	4.48	0.95	0.07	0.01	0.003	Global neutrality tests		-0.08	NS	-10.24	<0.05	0.093	0.53

Sample collection and DNA extraction

Tissue samples of *C. "chasiquensis"* were obtained from a total of 103 individuals from mtDNA analyses in the 8 sampling sites distributed across the study area (see Fig. 1). Individuals were live

trapped with Oneida Victor N°0 snap traps, with a rubber cover to avoid injuring animals. Experience indicates that this procedure neither affects survival nor digging performance of the individuals (Mapelli et al., 2012a; Mora et al., 2006). After collection of tissue samples for genetic analyses, animals were immediately released

back within the same burrow system where they had been originally captured. Sampling was conducted during 2009 and 2010. Geographical position of captures was recorded with a GPS.

Tissue samples (first phalange of the toe) were preserved in 95% ethanol at -70°C . Tissue vouchers were prepared and deposited in the collection of the Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” (MACN). Genomic DNA was isolated following a protocol modified from Miller et al. (1988), involving treatment with sodium-dodecyl-sulphate (SDS) and digestion with proteinase-K, NaCl precipitation of proteins, and subsequent isopropyl alcohol precipitation of DNA, as described in Mora et al. (2006, 2007).

PCR amplification and sequencing of mitochondrial DNA

A fragment of 433 bp of the 5' hypervariable portion of the mitochondrial DNA control region was amplified by polymerase chain reaction (PCR) from all specimens 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 following Mora et al. (2006, 2007). Amplicons were purified with shrimp alkaline phosphatase and exonuclease I (Amersham Biosciences). These final PCR products were sequenced with a capillary sequencer ABI3100 (Macrogen Inc., Korea), with the primers used in PCR. Electropherograms were scored and analyzed using Chromas 2.01 (Technelysium, Helensvale, Qld, Australia) and aligned using CLUSTALX (Thompson et al., 1997). GenBank accession numbers of this study were included in the Appendix A (KU577534-KU577562) and Appendix B in Supplementary material (KU577563-KU577570).

Population genetic structure and gene flow

Haplotype and nucleotide diversity were calculated to estimate DNA polymorphisms in each sampling site using DnaSP version 5.0 (Librado and Rozas, 2009). We constructed a haplotype network using the median-joining algorithm defined by Brandelt et al. (1999) in NETWORK 4.5.1, which essentially uses a maximum-parsimony approach to search for the shortest, least complex network from a given data set.

For examining the overall level of genetic divergence among localities we used the F_{ST} statistics based on sequence data (Φ_{ST} , Hudson et al., 1992). Significance of these values was evaluated using 1000 permutations in ARLEQUIN 3.0 (Excoffier et al., 2005). Pairwise estimates of F_{ST} and linear geographic distances among localities were used to examine the fit of the data to an isolation by distance pattern (Slatkin, 1993), using PASSaGE version 2 (Rosenberg and Anderson, 2011). This program uses a nonparametric test to assess the significance of this correlation, using 1000 permutations of the original matrices (Mantel, 1967). The mean number of migrants per generation (Nm) was estimated as $Nm = (1 - \Phi_{ST}) / (2\Phi_{ST})$. 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).

In order to infer the partitioning of genetic variance within and among populations, Analyses of Molecular Variance (AMOVAs) were performed considering both genetic distances between haplotypes and their frequencies, using ARLEQUIN 3.0 (Excoffier et al., 2005; Weir and Cockerham, 1984). Therefore, three AMOVAs were performed considering (1) the eight sampling sites as independent units, (2) the differentiation between the western and eastern sampling sites (defined on the base of the geographic position of these populations relative to the Chasicó Lake), and (3) considering the three sandy diagonals of suitable habitat for this species clearly visible in our study area. Western and eastern sampling sites (or East

and West, see Appendix C in Supplementary material) are divided in current days by the Chasicó Lake and Chasicó stream.

We used MIGRATE 3.0.3 (Beerli and Felsenstein, 2001) to obtain maximum-likelihood and Bayesian estimates of effective population sizes from theta ($\theta = 2N_e\mu$), and rates of migration ($2N_e m$) across the major sand dune diagonals, where N_e is the effective population size of females, μ is the mutation rate per site per generation, and $N_e m$ is the number of migrating females per generation. Thus, in order to infer the magnitude and direction of gene flow across these diagonals and their effective population sizes, we grouped the populations considering the three major sand diagonal in the system, two on the West (composed by R154/R1/PSR and LCH/LE/LC respectively), and one in the East of the species distribution (composed by SC/LM; see AMOVA above). 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. We used the following genealogy searching settings for both methods: 10 short Markov chains each consisting of 50,000 iterations with sampling every 50 trees, followed by 3 long Markov chains with 500,000 iterations, with sampling every 50 trees. At the beginning of each chain 100,000 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.

Demography

First, we examined the demographic history of populations using a mismatch distribution analysis, which allow to differentiate 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, departures from strict neutrality or a combination of these factors (Harpending et al., 1998; Ramos-Onsins and Rozas, 2002; Schneider and Excoffier, 1999). We employed parametric bootstrapping as implemented in ARLEQUIN 3.0 to test the goodness of fit of the observed mismatch distribution to that expected under the sudden and spatial expansion model using the sum of squared deviations (SSD) statistic. Mismatch distributions were performed including all populations globally, the West and East sampling units, and considering the three sandy diagonals independently.

Tajima's D (Tajima, 1989) and Fu's F_S (Fu, 1997) tests of neutrality were performed globally, from each sampling site, from the West and East sampling units, and separately from the three sandy diagonals using 1000 iterations as performed in ARLEQUIN 3.0 (Excoffier et al., 2005). 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 and/or purifying selection (unlikely at the mitochondrial control region).

In addition, an evidence of population range expansion was also inferred from the Ramos-Onsins and Rozas' R_2 (Ramos-Onsins and Rozas, 2002) test of neutrality using DnaSP version 5.0 (Librado and Rozas, 2009). This test is based on the difference between the number of singleton mutations and the average number of nucleotide differences, and showed to be more robust to small sample sizes

than Fu's F_S and better estimator than Tajima's D (Ramos-Onsins and Rozas, 2002). For this purpose, the same hierarchical rearrangements of populations used in the previous neutrality tests were considered.

Although Ramos-Onsins and Rozas' R_2 , Tajima's D and Fu's F_S statistics and mismatch distributions are able to provide insights about population expansions, these approaches are not able to provide information about the population changes over time. Therefore, to estimate the shape of population change through time we performed a Bayesian skyline plot (BSP) 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 1000 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 (considering all populations as a whole). To convert coalescence times to years from the substitutions per site estimated by BEAST, we used a mutation rate of $4.42\% \text{ Myr}^{-1}$ estimated for the mitochondrial control region of the genus *Ctenomys* by Mora et al. (2013).

Phylogenetic relationship among species of the "mendocinus phylogenetic group"

In order to assess the phylogenetic relationship among the identified mtDNA haplotypes of *C. "chasiquensis"* and the other species of the "mendocinus phylogenetic group" we constructed a Bayesian phylogenetic tree (BA) using BEAST 1.5.4 (Drummond and Rambaut, 2007), which employs a Bayesian Markov chain Monte Carlo (MCMC) procedure. The program was run for 1×10^7 iterations and sampled every 1000 steps under a relaxed lognormal molecular clock with uniformly distributed priors. This analysis yielded 10,000 final trees; 2000 from these trees were discarded as burn-in to compute the 50% majority rule consensus tree; inferences were finalized with the last 8000 trees. To assess the robustness of parameter estimates, four independent chains were run with the same settings. Log-files were examined in Tracer v1.6 (Rambaut et al., 2014) to assess Markov Chain Monte Carlo convergence within chains. The best nucleotide substitution model was estimated in jModelTest (Posada, 2008) using Akaike's Information Criterion. We used the GTR+I+G substitution model with four gamma categories, using a Yule branching rate prior, with rate variation across branches (Drummond and Rambaut, 2007).

A phylogenetic tree was also performed using a Maximum-Likelihood (ML) approach implemented in PhyML 3.0 (Guindon and Gascuel, 2003; Guindon et al., 2010) considering the same out-group used in the BA phylogenetic inference. The program implements simultaneous Nearest Neighbor Interchanges (NNIs) to recover a reasonable topology of the starting tree. We ran PhyML using 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, 1000 bootstrap replicates) implemented in PhyML.

Finally, a Maximum Parsimony (MP) tree was constructed using MEGA6 (Tamura et al., 2013). We used the Subtree-Pruning-

Regrafting (SPR) search method with 10 initial trees (random addition). As the previous phylogenetic analyses, the best substitution model was inferred by jModelTest (Posada, 2008). Consistency for internal branch and nodes was assessed using the standard bootstrapping method (sample with replacement and 1000 bootstrap replicates) implemented in MEGA6.

The topology of the phylogram was taken from BA analysis and was drawn with FigTree v1.4 (Rambaut, 2012). We used sequences of the five recognized Argentinean species belonging to the "mendocinus group" (four sequences of each species, Appendix B in Supplementary material). As out-group for all phylogenetic reconstructions we also used three control region sequences of *Ctenomys talarum* and one sequence of *Ctenomys magellanicus* (Appendix B in Supplementary material). All phylogenetic analyses were performed using the same fragment length than the previous analysis (433 bp of the 5' hypervariable portion of the mtDNA control region). The sequences of each species are representative of their overall intraspecific genetic variability. GenBank accession numbers and characteristics of these sequences are shown in Appendix B (Supplementary material). The type locality of each species of the "mendocinus group" is also included.

Results

Haplotype and nucleotide diversity

Sequences of *C. "chasiquensis"* (433 bp) yielded a total of 24 variable sites, of which 2 were singletons and 22 were parsimony-informative, defining 29 haplotypes out of 103 sequences analyzed (Table 1). The moderate number of polymorphic sites within this species resulted in high average nucleotide and haplotype diversity values (Table 1).

Most of the haplotypes (79.3%) were limited to single populations, and most populations maintained large numbers of haplotypes (between 3 and 6). LM and SC showed the lowest haplotype and nucleotide diversities among all populations. Overall haplotype diversity was 0.95 (Table 1). LM was the only populations that not share any haplotype with other populations.

Phylogenetic relationships between haplotypes

The median-joining network of haplotypes (Fig. 2) was complex, denoting a limited geographical subdivision and showing shared haplotypes among some populations. Even though most of the populations analyzed were polyphyletic, suggesting that the populations have not been isolated long enough to reach reciprocal monophyly, some populations showed unique haplotypes (e.g., LM), while others showed substantial haplotype diversity (e.g., R154, R1, LCH and LC). Overall, the median-joining network do not showed a markedly restricted geographic structure.

Population structure and gene flow

All populations were significantly different based on the exact test of sample differentiation (Raymond and Rousset, 1995).

Overall level of genetic divergence among localities was estimated by the F_{ST} statistics based on sequence data (Φ_{ST} , Hudson et al., 1992; Table 2). In general, pairwise Φ_{ST} values were higher comparing LM and SC relative to the other localities (Table 2), showing some degree of historical isolation from these latter populations. In addition, pairwise gene-flow estimates showed values ranging from 0.03 between PSR and LC to 0.74 between LCH and SC. Overall, pairwise Φ_{ST} values between subpopulations indicate from moderate to high genetic differentiation between subpopulations (Hudson et al., 1992). Global population subdivision among all localities yielded a moderate to high value: ($\Phi_{ST} = 0.29$).

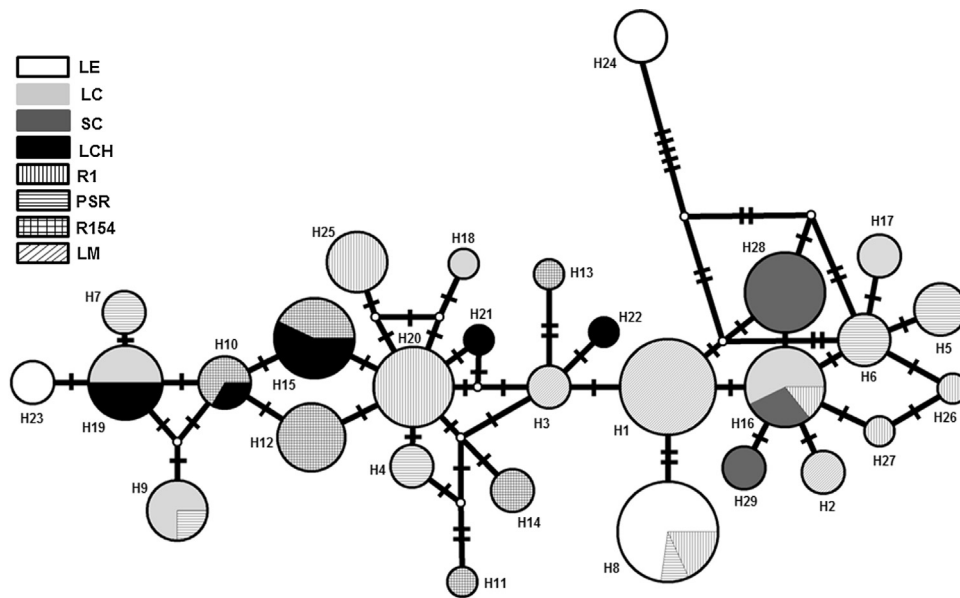


Fig. 2. Haplotype network of 29 mitochondrial DNA (mtDNA) haplotypes of *C. “chasiquensis”* from Argentina was constructed using the median-joining algorithm defined by Brandelt et al. (1999). Areas are proportional to haplotype frequencies, shading indicates localities, and cross hatches represent nucleotide differences between haplotypes. Abbreviations of populations are shown in Fig. 1.

Table 2
Pairwise F_{ST} estimates (Φ_{ST}), showing the genetic differentiation in *C. “chasiquensis”* among sampling sites from mtDNA.

	LM	PSR	R154	LC	LCH	LE	R1	SC
LM	–							
PSR	0.34**	–						
R154	0.6**	0.28**	–					
LC	0.4**	0.03	0.22*	–				
LCH	0.67**	0.32**	0.04	0.22*	–			
LE	0.25**	0.21*	0.4**	0.28*	0.43**	–		
R1	0.37**	0.18*	0.22*	0.18*	0.22*	0.23*	–	
SC	0.59**	0.38**	0.7**	0.39**	0.74**	0.36**	0.5**	–

Abbreviations for locations are defined in Fig. 1.

* 0.001 < P < 0.05.
** P < 0.001.

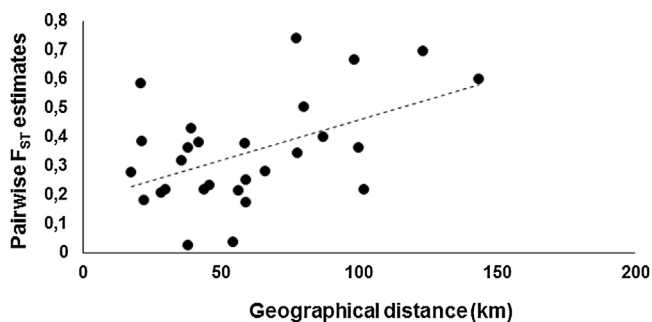


Fig. 3. Relationship between pairwise geographical distances and genetic differentiation among sampling sites measured by pairwise F_{ST} estimates (Φ_{ST}) from mtDNA.

Mitochondrial DNA was consistent with a simple IBD pattern, showing a weak but significant association between pairwise estimates of Φ_{ST} and linear geographic distances ($R=0.49$, $P<0.05$, Fig. 3). This scenario agrees with the prevalence of a historical equilibrium between genetic drift and gene flow.

On one hand, the AMOVA performed on localities grouped in the three sandy diagonals did not show significant differences relative to the apportionment of the genetic variance ($F_{ST}=0.062$, $p=0.21$, see Table 3). On the other hand, the AMOVA performed on local-

ities assembled in the East and West groups showed significant differentiation ($F_{ST}=0.18$, $p=0.036$). These groups of populations were opportunely defined by their geographic location, considering the two major barriers that may influence the gene flow between the East and West in current days: the Chasicó Lake and the Chasicó stream. It should be noted, however, the major differentiation was observed without clustering of subpopulations into major hierarchical units ($F_{ST}=0.3$, $p<0.001$; Table 3). Therefore, genetic variation seems to be distributed at local level, without exhibiting a clear regional pattern.

MIGRATE 3.0.3 (Beerli and Felsenstein, 2001) was used to infer the effective population sizes and migration patterns considering as the unit of analysis the three sandy diagonals in where the populations were sampled. Both maximum-likelihood and Bayesian estimates of effective population sizes showed higher level of variability (denoted by $\theta=2N_e\mu$) in the western diagonals, while the lowest estimates of effective population sizes was observed in the eastern one (Appendix D in Supplementary material). On the other hand, both maximum-likelihood and Bayesian inferences of migration patterns showed the highest values of asymmetric gene flow predominantly in direction SW-NW, from the unit III (LCH/LE/LC) to unit II (R154/R1/PSR). The unit I (composed by SC/LM) showed the lowest values of historical gene flow relative to the observed between the other sand dune diagonals (Appendix D in Supplementary material).

Tests of neutrality and mismatch distributions

Even though Tajima’s D ($D=-0.08$, $P=0.47$) and R_2 statistics ($R_2=0.093$, $P=0.53$) were not significant, Fu’s F_S ($F_S=-10.24$, $P<0.05$) suggests a demographic expansion of these populations in their recent past, further supporting a weakly geographical subdivision of this species (Table 1). The global mismatch distribution of *C. “chasiquensis”* was not clearly multimodal, suggesting a lack of constant population size and/or sustained subdivision for a long period of time, such that the SSD between observed and expected mismatch distributions did not show significant departures from a sudden ($SSD=0.0031$, $P=0.61$) or spatial ($SSD=0.0045$, $P=0.31$) expansion model (Fig. 4). Likewise, Harpending’s Raggedness index (HRI) did not presented significant departures neither

Table 3
Hierarchical analysis of molecular variance (AMOVA), using a square matrix of pairwise genetic distances between haplotypes. The fixation indices (Φ -statistics) are shown. Φ -statistics and significance of variance component (P) were tested by 100,000 permutations according to Excoffier et al. (2005). The respective levels of subdivision of each population are shown in brackets. Abbreviations for locations are given in Fig. 1.

Source of variation	Level of subdivision for <i>C. "chasiquensis"</i> populations	Φ_{CT}	P	Φ_{ST}	P
No clustering of subpopulations into groups	[LCH] [R1] [R154] [PSR] [LE] [LC] [SC] [LM]			0.3	<0.001
Among two groups defined by their geographic location relative to the Chasicó Lake	[LCH, R1, R154, PSR, LE, LC] [SC, LM]	0.18	<0.05		
Among three groups defined by the major continuous sandy diagonals	[PSR, R1, R154] [LCH, LE, LC] [SC, LM]	0.062	0.21		

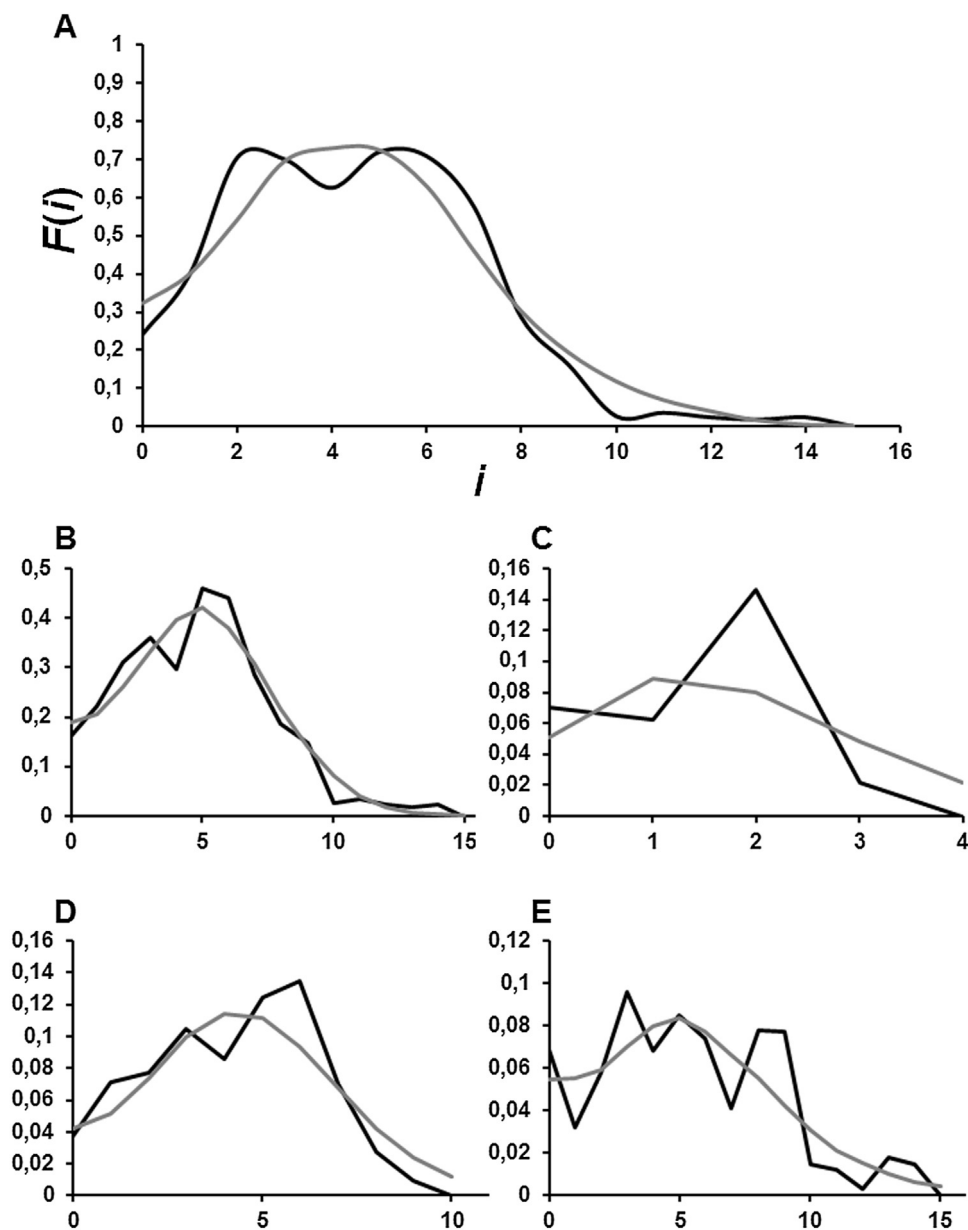


Fig. 4. Observed and expected mismatch distributions for *C. "chasiquensis"* from Argentina from the whole study area (A), from the West area (B), from the East area (C), from the Northwest diagonal (D), and from the Southwest diagonal (E, see details in Appendix C Supplementary material). Black line represent observed distribution; gray line represent theoretical expected distribution under a sudden population expansion model, following Schneider and Excoffier (1999). $F(i)$ indicates frequency, i denotes pairwise nucleotide differences.

from a sudden ($HRI=0.0098$, $P=0.94$) nor spatial ($HRI=0.0097$, $P=0.83$) expansion model. No single population showed signs of population expansion using R_2 , Tajima's D and Fu's F_S tests (Table 1).

The same analyses was performed separately on the three sand dune diagonals of the study area, showing a population expansion only in the Northwest sandy diagonal ($F_S = -4.95$, $P < 0.05$; $SSD_{suddenexp} = 0.0067$, $P = 0.41$; $HRI_{suddenexp} = 0.019$, $P = 0.67$;

$SSD_{spatialexp} = 0.0095$, $P = 0.24$; $HRI_{spatialexp} = 0.019$, $P = 0.73$; see Appendix C in Supplementary material). Southwest and East sand dune diagonals seem to have maintained a demographic equilibrium at historical times (Appendix C in Supplementary material). However, it should be noted that R_2 tests did not reject the constant size hypothesis for any of these sandy diagonals.

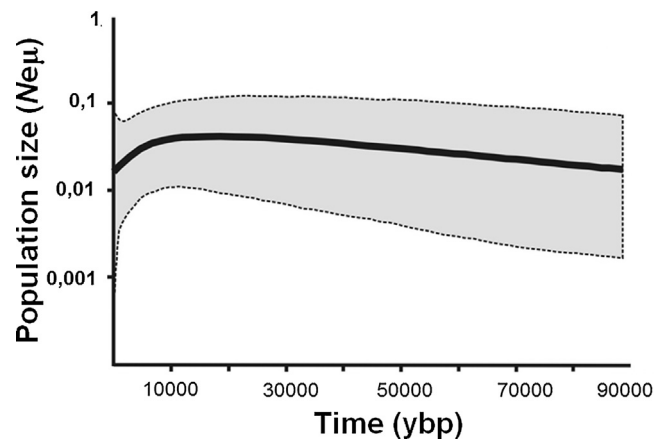


Fig. 5. Bayesian skyline plot showing the effective population size fluctuations throughout time in *C. "chasiquensis"* from Argentina. Black lines represent median estimations and the grey area represents the upper and lower 95% credible intervals. The x-axes are expressed in years before present (YBP) and was converted to time using a mutation rate of 4.42% Myr⁻¹ estimated for the mitochondrial control region of the genus *Ctenomys* (see Mora et al., 2013).

The demographic scenario for *C. "chasiquensis"* inferred by the BSP showed a dual pattern: a first period where a small demographical increment from approximately 90,000 to 11,000 years BP is perceived, and a second period where a decrease in population size (less intense than the previous period but certainly perceptible) that started in the early Holocene and continues nowadays is also detected (Fig. 5).

C. "chasiquensis" in a phylogenetic context

Here we assess the phylogenetic relationships between *C. "chasiquensis"* and the other species of the "*mendocinus* group". Phylogenetic analyses were consistent among MP, BA inference and ML consensus trees, recovering the same topology among sequences of the main recognized species of the "*mendocinus* phylogenetic group" (Fig. 6). However, some phylogenetic reconstructions did not provide strong support for some specific clades. Bayesian inference showed high posterior probabilities for all major clades among different species of the "*mendocinus* group". In general, ML reconstruction showed higher values of node support than the MP procedure. In addition, the MP method showed the lowest support of bootstrap percentage values for the species formally recognized as complete biological species. On the contrary, *C. porteusi* and *C. australis* were widely supported as complete species by all phylogenetic methods. Although, *C. azarae*, *C. mendocinus* and *C. "chasiquensis"* were species strongly supported by the Bayesian inference, these clades showed a low bootstrap support by both ML and MP methods. Basically, these results support, partially, the monophyly of the species belonging to this phylogenetic group. Two aspects must be recognized of this analysis beyond the close phylogenetic relationship among these five Argentinean species included in the "*mendocinus* group": (1) *C. "chasiquensis"* seem to be phylogenetically closer to *C. mendocinus* than the other species of this group, and (2) further studies with additional markers (mitochondrial and nuclear) are necessary to improve our knowledge about the phylogenetic relationships between the Chasicó's tuco-tuco and the other species of this phylogenetic group.

Discussion

Particularly, this is the first study to assess the population genetics and historical gene flow for *C. "chasiquensis"*, a vulnerable and endemic subterranean rodent, over its most likely entire distribution, yielding important new insights into the history of the species in relation to historical changes in its habitat.

Likewise to other species of the *mendocinus* group, this species is characterized as highly specialist of sandy habitats. It should be noted the habitat of this species is interrupted by several salt inland flats such as Salina Chica, by an important lagoon, the Chasicó Lake, and the Chasicó stream, resulting in a highly patchy landscape (essentially on the eastern sand dune diagonal; see Fig. 1A). Accordingly, within the study area gene flow seems not to be strictly restricted to the three sand dune diagonals, which are surrounded by fields where the environment is not optimal for the occupation of this species.

Our analyses show that populations of *C. "chasiquensis"* are moderate structured at regional level and this pattern is probably the result of population stability in the South, and a range expansion in the Northwest. Historical dispersal rates seem not to have been sufficient to avoid substructuring at the scale of this study. Additionally, we detected an asymmetric historical migration among the sand dune diagonals, being predominantly from the South-West to the North-West direction (Fig. 1B). Also, the demographic scenario in this species supports reasonably these conclusions.

Migration patterns and isolation by distance

Our analyses show that populations of *C. "chasiquensis"* are moderate structured at regional level and this population pattern is probably the result of uninterrupted historical gene flow in the West, and a significant degree of ancient isolation on the East (see Appendix D in Supplementary material). Major differentiation in mtDNA was observed without clustering subpopulations into regions or major hierarchical units. Thus, hierarchical subdivision of genetic variation was not observed when population groups were defined by other criteria (e.g., sandy diagonals or by the geographic position relative to the Chasicó Lake). Furthermore, population differentiation at this spatial scale seems to be, most probably, a conjunction of the geographic position of populations and their isolation. In this sense, historical migration patterns clearly showed an asymmetric and higher rate of gene flow among the sand dune diagonals on the West, mainly from the southwestern to the northwestern direction (Appendix D in Supplementary material, Fig. 1B and C).

Genetic differentiation in mtDNA suggested an equilibrium between genetic drift and gene flow in *C. "chasiquensis"*, supporting an immigration-drift equilibrium model as the most probably demographic scenario for this species. Although populations on the West showed higher values of gene flow among them (relative to the East), this pattern was not sufficient to breaking the equilibrium

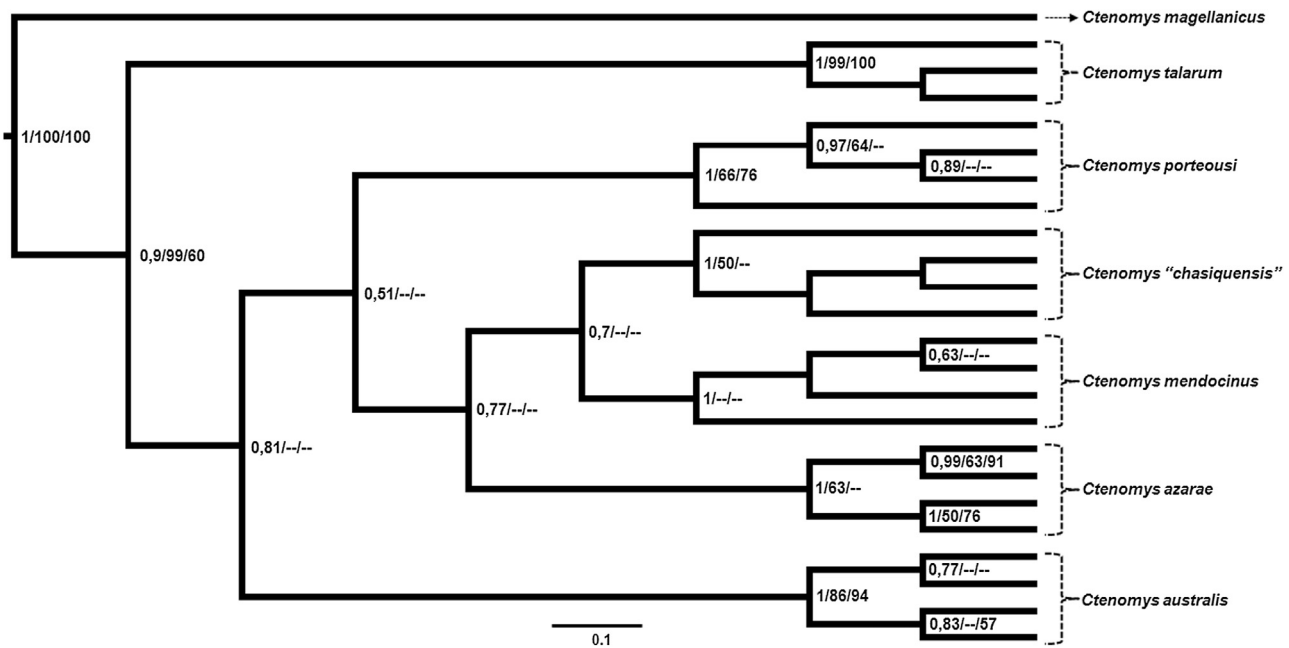


Fig. 6. Bayesian (Drummond and Rambaut, 2007), Maximum-Likelihood (Guindon et al., 2010) and Maximum Parsimony (Tamura et al., 2013) phylogenetic trees of the twenty mtDNA control region sequences from the Argentinean species belonging to the “mendocinus phylogenetic group”. Control region sequences from three individuals of *Ctenomys talarum* and from one individual of *Ctenomys magellanicus* are included as out-groups. Numbers denote the posterior probabilities for the Bayesian inference (only clades with higher posterior probabilities are shown), the values of node supports for the ML phylogenetic tree, and the values of node supports for the phylogenetic MP tree. For the ML and MP inferences only the higher percentage values of support (bootstrap values $\geq 50\%$) are shown. Characteristics of the sequences used in this phylogenetic analysis are also given in Appendix B (Supplementary material).

between gene flow and genetic drift across the entire distribution of the species. Because their isolation, populations in the East are presumed to have been more influenced by the impact of the genetic drift compared to the Western ones.

Besides the smaller extension of suitable habitat, the eastern diagonal is characterized by a patchier configuration relative to the western ones (pers. obs.). The habitat around this sand dune diagonal is strongly interrupted by flooded salt flats (e.g., Salina Chica), agricultural crops and other important human modifications like forestations and water channels (see Fig. 1A). This situation could prevent the establishment of immigrant individuals from the West. Therefore, we expect that current regional turnover of individuals will be higher in the West than in the eastern side of the study area. These results are partially supported by the asymmetry in the historical migration among diagonals (Fig. 1B).

Furthermore, there are a small number of studies of this genus comparing the patterns of genetic structure among different natural populations using several molecular markers in the analyses. Commonly, past approaches in *Ctenomys* 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; Mirol et al., 2010; Wlasiuk et al., 2003). Nevertheless, the comparison of these mitochondrial outcomes with nuclear data sets will provide a better description on which to assess the general dynamics of differentiation in *C. chasiquensis*, including historical and current changes in levels of gene flow and isolation.

Historical demography and landscape evolution

On one hand, global mismatch distribution of this species was not clearly unimodal, as would be expected from the case of a typical population expansion event. However, multiple peaks may appear in the mismatch distribution, despite population expansion, whether genetic substructuring is present in the study area, or because the expansion has not been so intense in the recent past. In

this way, both SSD and Harpending’s Raggedness indexes suggested a population expansion (both sudden and spatial) of the whole study area. Similarly, Fu’s F_5 suggested a demographic expansion of these populations at recent time, further supporting a weakly geographical subdivision of this species.

On the other hand, some statistics seem to show no departures from a condition of constant population size relative to the whole study area. Tajima’s test was negative (similarly to the Fu’s test), but not significant. Likewise, Ramos-Onsins and Rozas’ R_2 statistic did not show a departure of a population stability condition through time. Although R_2 was positive and very small, which would indicate an excess of low frequency mutations relative to expectations under the standard neutral model, this index was not significant. It should be noted, however, that these neutrality tests are based on departures of the expected neutrality, but do not show whether this departure is strictly due to population expansion, positive selection or hitchhiking among adjacent mitochondrial regions. Overall, these results support a process of population expansion for this species but, most likely, not very strong at recent times.

Inferences of demographic population changes from SSD and Harpending’s Raggedness indexes performed separately over each sandy diagonal showed both sudden and spatial demographic expansions in the northwestern diagonal; whereas populations from the eastern and southwestern diagonals seem to have maintained a demographic equilibrium at historical times. Fu’s F_5 test was the only test of neutrality that showed any signs of population expansion (or departures from the constant population size condition) in the northwestern sandy diagonal. Nevertheless, it should be noted that R_2 tests did not reject the constant population size hypothesis for any of these sandy diagonals.

Bayesian Skyline Plot (BSP) showed a close association between the major climatic changes inferred from the late Pleistocene and Holocene in the central region of Argentina and the main historical demographic changes experienced by *C. chasiquensis*. This species, like other tuco-tucos from this phylogenetic group, occurs

in a narrow range of soils and is strictly associated to sand dune habitats. Thus, historical demography of *C. "chasiquensis"* seems to have been closely related to the landscape evolution in the Pampa's region during the Late Quaternary. Effectively, this species experienced a population demographical increment, occurred from approximately 90,000 to 11,000 years BP. In the last 11,000 years during the early Holocene, however, the starting of a perceptible decrease in population size was inferred for the species.

It should be noted, however, that whether the population decline was very recent in time and not very abrupt in magnitude, is not expected that the latter demographic process can be documented by the statistical tests of neutrality. Possibly, the neutrality tests employed in this study may be reflecting the previous demographical process occurred in the Late Pleistocene when the population growth seems to have been constant and not during the last period of the population history corresponding to the Holocene. In sum, current populations of this species appear to be clearly relicts of a more extended historical distribution in the Argentinean Pampas, with a minor declining at the beginning of the Holocene.

Most subterranean rodent species undergo important physiological and behavioral selective pressures in order to maintain the subterranean lifestyle (Mapelli and Kittlein, 2009). Habitat specific features such as geographic location of sand dunes impose significant restrictions on the occurrence of fossorial rodents like tuco-tucos (Kittlein and Gaggiotti, 2008). Particularly, *Ctenomys*'s species are frequently found in open, arid or semiarid habitats associated to soft and permeable soils where underground life is favored (Mapelli and Kittlein, 2009). Similarly to *C. "chasiquensis"*, a close association between habitat occupancy and the evolution of sand dune landscapes, mostly during the Late Pleistocene and Holocene, was reported in several species of *Ctenomys* (Fernández-Stolz et al., 2007; Mora et al., 2006, 2007, 2013; Wlasiuk et al., 2003). This feedback has eventually promoted the pulses of population expansion or contraction of several species of tuco-tucos (see Mapelli et al., 2012a; Mora et al., 2006, 2007, 2013; Wlasiuk et al., 2003).

Particularly, climatic conditions during the Quaternary glaciations strongly affected the landscape in the eastern and central portions of Argentina (Quattrocchio et al., 2008; Tonni et al., 1999), including our study area. In general, during the Quaternary glacial periods, cooler and drier conditions than current climatic environment were inferred, while the interglacials were characterized by an increase in the temperature and humidity (Iriando, 1999; Quattrocchio et al., 2008).

Predominantly, the last Pleistocene glaciation gave rise to the Pampean Sand Sea, which is a large sandy cover shaped by the latter desert and semi-desert climatic episodes (Iriando, 1999; Quattrocchio et al., 2008). During the Last Glacial Maximum (LGM, 18 ka BP), this area was covered by desert vegetation growing on sand dunes and loess fields (Prieto, 2000), and aeolian sedimentation identified by loess accumulation during the LGM was evident across all Pampean region (Zárate and Tripaldi, 2012).

During the Late Pleistocene-Early Holocene from 14 ka BP to 8.5 ka BP a glacial advance occurred (Iriando, 1999), with a dry climate characterized by strong westerly winds in the Pampa (see also Zech et al., 2009). After 8.5 ka BP to the present (Middle to Late Holocene), climatic phases were characterized, in general, by an increase in humidity and temperature relative to the previous times during the LGM. Instead, the central and eastern areas of the Pampean region have, in the present century, been subject to pedogenesis under humid and sub-humid regimes (Iriando, 1999). Nowadays, the Pampean Sand Sea is characterized by sandy mantles and dunefields that originated during the Pleistocene, and constitutes a surface of prairie and steppe vegetation in most of the Pampean region (Prieto, 2000; Zárate and Tripaldi, 2012). These deposits are the parent material of presently cultivated soils in the region, which are deeply modified by agriculture (Zarate and Tri-

paldi, 2012). Under this scenario of glacial and interglacial events, the area of occurrence of *C. "chasiquensis"* has suffered a gradual decline after the Late Pleistocene (where the Pampean Sand Sea reached its highest surface) to Late Holocene. This habitat reduction, in conjunction with contemporaneous habitat degradation by rural activities, has differentially impacted the eastern and western populations of this species.

As was reported by Tonni et al. (1999), Mapelli et al. (2012b), and Mora et al. (2013), the extreme climatic changes inferred from the Late Pleistocene/Early Holocene, combined with the large variations in rainfalls throughout the Holocene, have affected the dynamics of occupation of tuco-tucos and many other small mammals of the Argentinean Pampas. Major important rodent assemblages observed nowadays in North Patagonia and the Pampas region were basically shaped during the Late Holocene (Teta et al., 2014; Tonni et al., 1999). In this context, the species richness of micromammals present in the Late Holocene (approx. 0.5 ka BP) was very different than the observed in current days. Ecological attributes for many species (specialists vs. opportunistic) has had strong implications for the faunistic configuration in the Pampean and Patagonian regions (Teta et al., 2014). As was suggested by these authors, significant variations on the micromammal assemblages have occurred in southern South America, mainly in the last 500 years. These changes included the population demographic expansions (with or without geographical expansions) and contractions (and inclusive regional extinctions) of some specialized species. Furthermore, these authors suggest that strong changes produced by human alterations (e.g., agriculture and livestock) have produced an extensive uniformity of habitat which was favorable for several opportunistic rodent taxa like *Calomys* spp. or *Oligoryzomys longicaudatus*, allowing their dispersion and population expansion. Possibly, population demography of *C. "chasiquensis"*, a highly specialist of sand dune habitat, was also highly affected by human modifications during the past 0.4 ka years BP, accelerating their population decline.

Based on our results, current populations of this species appear to be clearly relicts of a more extended historical distribution in the Argentinean Pampas, with a perceptible decline at the beginning of the Holocene, approximately 11 ka years BP. Interestingly, *C. porteousi*, another species of the "*mendocinus* group" distributed closely in the same sand dune habitat configuration than *C. "chasiquensis"*, showed a similar pattern of population declining from the Middle Holocene (11 ka years BP) to the present, in association to a progressive habitat reduction (Mapelli et al., 2012a). This highly habitat specialist has also suffered a current population decline due to human alterations in the region in the last 100 years.

Phylogenetic implications

The "*mendocinus* phylogenetic group" (sensu Massarini et al., 1991) includes at least four Linnaean species distributed in central Argentina: *Ctenomys azarae* Thomas 1903, *Ctenomys mendocinus* Philippi 1869, *Ctenomys porteousi* Thomas 1916 and *Ctenomys australis* Rusconi 1934; together with one form of uncertain taxonomic status, *Ctenomys "chasiquensis"*. Contreras and Maceiras (1970) and then Contreras (1973) mentioned this form in the Chasicó Lake and in the surrounding areas, but without proper taxonomic description, since there is no formal designation of a holotype or an appropriate diagnosis. In this work we designated this species as *Ctenomys "chasiquensis"* (or nomen nudum), relying on the reciprocal monophyly observed in the phylogenetic tree. Although this species seem to be phylogenetically closer to *C. mendocinus* than the other species of this group, further studies with additional molecular markers are necessary to improve our interpretation about the phylogenetic relationships of these species. It should be considered that *C. "chasiquensis"* inhabits sandy soils around the Chasicó

Lake, in the ecotonal zone among Pampas grasslands and Espinal, in southwestern Buenos Aires Province, and possibly extends its distributional range to the West of our study area (see Fig. 1). Additional studies will help to define more precisely the taxonomic status and the distributional limits of this nominal species.

Conclusions

Here, we characterize the historical gene flow patterns and population structure in the highly, vulnerable and endemic subterranean rodent *C. chasiquensis* from Argentina. Our results indicate moderate genetic structure in *C. chasiquensis*. The general patterns of dispersion in this species show that the most isolated and differentiated populations are located in the East, where the human activity caused a strong impact over the borders of this area in current days. On the other hand, western populations were historically situated on a more homogeneous sand dune environment, allowing a more continuous gene flow. The asymmetry in gene flow among different populations of this study is not sufficient to break the equilibrium between gene flow and genetic drift, leading to a situation where the nearest populations are the most similar at the genetic level. Hence, population differentiation of the eastern populations appears to be associated with a combination of isolation and the major natural discontinuities like Chasicó Lake and Chasicó stream, which divided their distribution in three sand dune diagonals. Finally, the sandy landscape evolution in this region during the Late Pleistocene/Early Holocene appears to have had a great impact on how the populations are currently connected by gene flow. Based on data of habitat declining during the Holocene and inferences of how the effective population size of this species had changed over time (BSP), current populations of this species appear to be relicts of a more extended historical distribution in the Argentinean Pampas, with a perceptible decline at the beginning of the Holocene, where the climatic conditions became progressively warmer and wetter. Overall, we reported a gradually population expansion in this species during the Late Pleistocene, followed by a progressive (but not very strong) decline in its effective population size from the beginning of the Holocene to the present.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.mambio.2016.02.008>.

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