Mammalian Biology 96 (2019) 14–22

Contents lists available at ScienceDirect

Mammalian Biology

journal homepage: www.elsevier.com/locate/mambio

Original investigation

Population genetics of the capybara, *Hydrochoerus hydrochaeris*, in the Chaco-pampean region

Soledad Byrne[a,*], Rubén D. Quintana[b,c,d], María L. Bolkovic[e], Juan I. Túnez[a]

[a] Grupo de Investigación en Ecología Molecular (GIEM), Departamento de Ciencias Básicas e Instituto de Ecología y Desarrollo Sustentable (INEDES-UNLu-CONICET), Universidad Nacional de Luján, Ruta 5 y Avenida Constitución, s/n (6700), Luján, Buenos Aires, Argentina
[b] Laboratorio de Biodiversidad, Limnología y Biología de la Conservación, Instituto de Investigación e Ingeniería Ambiental (3iA), UNSAM, Av. 25 de Mayo y Francia, (B1650HMP), General San Martín, Buenos Aires, Argentina
[c] GIEH, Departamento de Ecología, Genética y Evolución, FCEyN, UBA, Intendente Güiraldes 2160, (C14288EGA), Buenos Aires, Argentina
[d] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917, (1033AAJ), Buenos Aires, Argentina
[e] Dirección Nacional de Biodiversidad, Ministerio de Ambiente y Desarrollo Sustentable, San Martín 451, (C1004AAI), Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 17 December 2018
Accepted 21 March 2019
Available online 23 March 2019

Handled by Laura Iacolina

Keywords:
Hydrochoerus hydrochaeris
Mitochondrial DNA control region
Population genetic structure
Historical population dynamics

ABSTRACT

Hydrochoerus hydrochaeris is an herbivorous rodent that inhabits most wetlands of South America. It is a well-known species, but studies about their population genetics are scarce. The aim of this study was to analyze the genetic diversity, population genetic structure and historical population dynamics of the species in the Chaco-pampean region, using mitochondrial DNA control region sequences and a non-invasive sampling design. Our results showed the existence of four haplogroups in the study area. Haplogroup I is composed by individuals from most of the study area, while the remaining include individuals from some sites in Paraguay (haplogroups III and IV) or Argentina (haplogroup II). The genetic diversity was low in haplogroups I, III and IV. Our results suggest that the Paraná and Paraguay rivers would be acting as a migration corridor for the species. Historical population dynamics analyses and haplotype network showed past population expansions and secondary contact between haplogroups I–II and I–IV, which could be related to past climatic events such as the Iberá Wetlands formation. These results must be taken as working hypotheses for future studies about the population genetics of capybara and related species inhabiting the region.

Introduction

Genetic diversity is one of the most important attributes of a population, since those species that exhibit higher levels of genetic diversity can better adapt to changing environments and are less susceptible to extinction (Frankham et al., 2010; Allendorf et al., 2013). Reducing population size can cause loss of genetic diversity within populations and the emergence of negative genetic effects. Small and isolated populations may suffer from the effects of inbreeding, resulting in decreased reproductive success and increased probability of extinction (Luck et al., 2003; Freeland, 2005). This phenomenon has been repeatedly mentioned as an impediment to the growth of mammal populations that are found in low densities (Mills and Smouse, 1994; Lacy, 1997; Avise, 2004; O'Grady et al., 2006). Early detection of potentially negative genetic changes maximizes our ability to implement management strategies to limit or reverse negative genetic effects before they become substantial or irreversible.

The increase of population subdivision directly promotes an intensification of genetic drift, which acts by fixing different allelic variants in each subpopulation depending of their effective population size (Hartl and Clark, 1997; Hamilton, 2009). The knowledge of how a species is divided in different genetic units is fundamental for the conservation of the biodiversity. Thus, one of the main objectives in conservation is to preserve the evolutionary potential of the species, sustaining the diversity that is found in genetic units. Consequently, the knowledge of the structure of these units allows a more adequate definition of management policies (Haig, 1998).

Historical, environmental or anthropogenic factors such as population reductions or expansions, changes in habitat configuration related to glaciations, topographic characteristics or hunting can affect the spatial structure of populations and act over the genetic diversity patterns of a species. Knowledge of these patterns of differentiation at molecular level may reveal valuable information about the underlying evolutionary processes and past demographic events (Milá et al., 2000). In particular, historical events, gene flow,

* Corresponding author.
E-mail address: solebyrne@gmail.com (S. Byrne).

https://doi.org/10.1016/j.mambio.2019.03.008
1616-5047/

and genetic drift are the major evolutionary factors that explain the geographic distribution of neutral genetic diversity within species (Dutech et al., 2004). Under constant gene flow and demographic equilibrium, spatial genetic structure can be predicted by different theoretical models (*e.g.* Wright, 1931; Slatkin, 1993). However, historical events, such as bottlenecks or a sudden demographic expansion can be predicted by the genetic patterns observed.

The capybara, *Hydrochoerus hydrochaeris*, is a semiaquatic herbivorous rodent widely distributed throughout most of South American wetlands. A year-round standing water source such as ponds, lakes, marshes or swamps is essential for its presence in a given area (Herrera and Macdonald, 1989; Quintana, 1999). At international level, the species is catalogued as "Least Concern" (IUCN, 2016). However, information about its distribution and current population status varies considerably between countries and regions. In Brazil, the species is considered a local pest because of the damage it causes to crops (Paschoaletto et al., 2003). In the Paraguayan Chaco-pampean region the conversion of forest into pastured land has allowed capybaras to expand beyond their native range (Campos-Krauer and Wisely, 2010), but the current economic situation threatens to increase deforestation and overgrazing in these region (Bucher and Huszar, 1999). In the Chaco-pampean region of Argentina, the species was classified as near threatened (SAREM, 2012) and is protected in eight National Parks (Álvarez and Kravetz, 2004). In this country, most of the woodland and grassland that comprise the Chaco-pampean region have been converted to agricultural use and capybaras have a long history of commercial exploitation and poaching (Bolkovic et al., 2006; Schivo et al., 2015), so the probability of occurrence of local extinctions is very high (Schivo et al., 2015).

Due to the high level of polymorphism detected in comparison with other mitochondrial markers and the relative ease of amplification from samples obtained by non-invasive methods, the sequence of the mitochondrial control region has been used to analyze the genetic diversity and structure of capybara populations (Campos-Krauer and Wisely, 2010; Borges-Landáez et al., 2012; Ruíz-García et al., 2016) and to study how genetic structure is modeled by the spatial structure of the hydrographic networks (Byrne et al., 2015). However, there are still no genetic studies on these topics throughout the entire Chaco-Pampean region. Thus, the aim of this study was to analyze the genetic diversity, population genetic structure and historical population dynamics of the capybara in the Chaco-pampean region, using sequences from the Hypervariable Region I (HVRI) of the mitochondrial DNA control region.

Material and methods

Sample collection

A total of 54 samples (n = 27 tissue and n = 27 fecal) were collected from 5 sampling sites in the Argentinean Chaco-Pampean region (Fig. 1B; Table 1), preserved in ethanol 96% during field work and stored at −20 °C until DNA extraction was performed. Tissue samples were obtained from dead individual, while fecal ones were collected in the field from different mounds separated from more than 50 m, in order to decrease the probability of re-sampling the same individual.

Mitochondrial DNA extraction and PCR amplification

Different DNA extraction protocols were used depending on sample source. Tissues samples were incubated overnight at 37 °C in extraction buffer containing 10 μl of proteinase K, 10 mg/ml; 5 μl of RNase, 20 mg/ml and 10% SDS. After incubation, DNA was isolated from the sample by phenol–chloroform extraction and alcohol precipitation. For fecal samples we followed the protocol designed by J.M. Campos-Krauer (pers. comm). The amplification protocol used depended on the sample source and the quality of the DNA obtained, more degraded in fecal samples. For tissue samples, each PCR had a final reaction volume of 20 μl and contained 1X GoTaq DNA Polymerase Buffer (Promega), 0.2 mM of each deoxynucleotide triphosphate, 1 μM of forward and reverse primers, 1 U of GoTaq DNA Polymerase (Promega), 3 μl of 5 ng/μl DNA and water to reach the final reaction volume. For fecal samples each PCR was carried out in a final reaction volume of 10 μl and contained 1X Pegasus Taq DNA Polymerase Buffer (EmbioTec), 0.2 mM of each deoxynucleotide triphosphate, 0.2 μM of forward and reverse primers; 1.5 mM of $MgCl_2$; 1.5 μg/μl BSA 10X (New England Bio Lab), 0.5 U of Pegasus Taq DNA Polymerase; 3–5 μl of DNA extract and water to reach the final volume. The primer pair used was Capy-D2R (5′-TAATGCATGTCCCCATGAAC-3′) and Capy-D5 F (5′-TTCCCCATGAATATTTAGCATGT-3′), developed by Campos-Krauer and Wisely (2010) and designed to amplify a 292 bp region of the HVR1 of the mitochondrial control region. We chose this short DNA fragment due to the difficulty to amplify longer fragments from fecal samples and because it contains the HVRI of the mtDNA which includes most of the variable sites of the capybara control region. The amplification protocol for DNA obtained from all samples, consisted of a single denaturation step at 95 °C for 10 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 56 °C for 45 s, and extension at 72 °C for 45 s, and a final extension at 72 °C for 8 min. Amplification reactions were carried out in a MyCycler (BIORAD) or a MasterCycler (Eppendorf) thermal cycler. Seven microliters of each PCR product were purified using the enzymatic method EXO/SAP (Werle et al., 1994) and sent to an external laboratory (Biotechnology Institute of INTA Castelar, Argentina) for direct sequencing using the same oligonucleotide primers.

Fourteen additional mtDNA control region sequences from 13 populations located in the Gran Chaco ecosystem of Paraguay were obtained from the GenBank (Table 1; Fig. 1A). These haplotypes had a length of 386 bp and were obtained from 110 individuals (Campos-Krauer and Wisely, 2010; GenBank Access numbers GU456363-376). All these sequences include the HVRI fragment amplified for the Argentinean samples.

Data analyses

Haplotype distribution, genetic diversity and population structure

All sequences were aligned and analyzed for polymorphic sites using MEGA 7 (Kumar et al., 2016). Aligned sequences were manually edited using Chromas v2.23 (Technelysium Ltd.). Haplotype frequencies and haplotype (H) and nucleotide (π) diversities (Tajima, 1983; Nei, 1987) were computed using Arlequin 3.5.2 (Excoffier and Lischer, 2010).

A Bayesian Analysis of Population Structure (BAPS) was conducted using BAPS 6.0 software (Corander et al., 2003; Corander and Tang, 2007; Corander et al., 2008) in order to group genetically similar individuals into panmictic genetic clusters. Calculations were performed with the number of K clusters varying from 2 to 20. For each k value, five replicates were performed. After that, an Analysis of Molecular Variance (AMOVA; Excoffier et al., 1992) was performed on the four BAPS clusters identified (see Results) using Arlequin 3.5.2. The significance of the observed Φ-statistics was tested using the null distribution generated from 10,000 non-parametric random permutations of the data matrix variables. Differences in alleles frequencies (FST-like) were used to compute the distance matrix for Φ*ST*. Population pairwise Φ*ST* values were also calculated using Arlequin 3.5.2 software.

The median-joining network method (Bandelt et al., 1999) implemented in PopART 1.7 (http://popart.otago.ac.nz) was

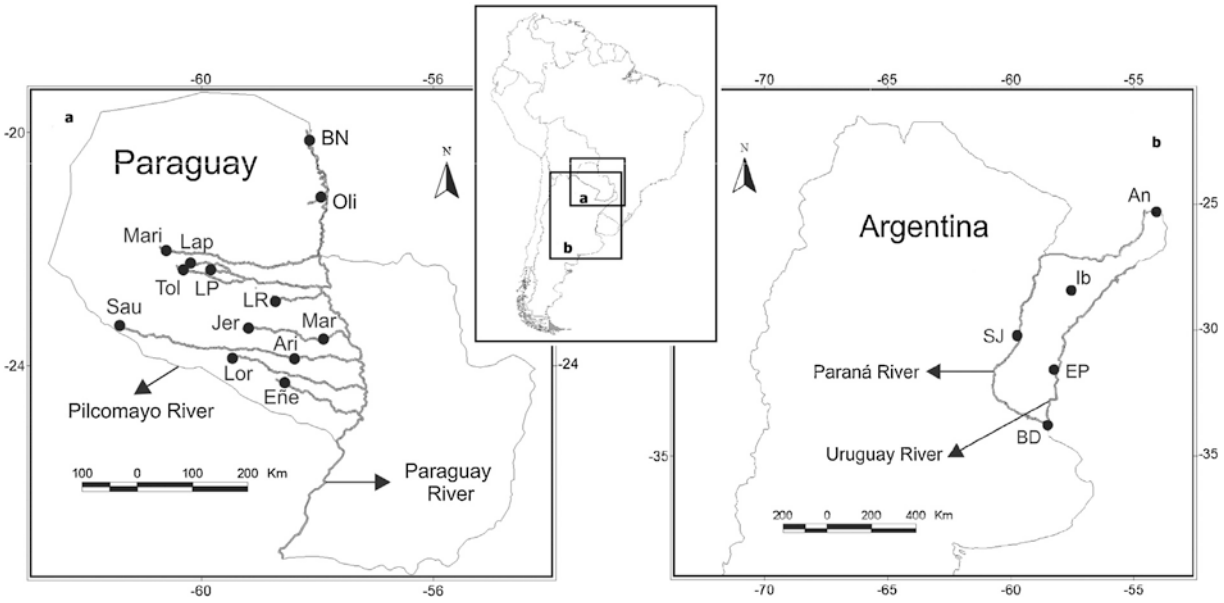

Fig. 1. Map showing the location of capybara sampling sites in Paraguay (A) and Argentina (B). Abbreviations of sampling sites correspond to those described in Table 1.

Table 1
Origin of capybara samples used in this study. References: N and n (sample size), T (tissue sample), F (fecal sample).

Sampling site	Location	N	Source
Argentina (n = 54)			
Andresito (An)	25°40' S, 54°02' W	3 (F)	
Iberá (Ib)	28°01' S, 57°14' W	20 (18 T, 2 F)	
San Javier (SJ)	30°35' S, 59°57' W	10 (5 T, 5 F)	This work
El Palmar (EP)	31°54' S, 58°15' W	4 (F)	
Bajo Delta (BD)	34°06' S, 58°22' W	17 (4 T, 13 F)	
Paraguay (n = 110)			
Toledo (Tol)	22°21' S, 60°19' W	13	
Mariscal (Mari)	22°01' S, 60°37' W	3	
Lapacho (Lap)	22°13' S, 60°11' W	5	
Loma Plata (LP)	22°23' S, 59°50' W	10	
Laguna Rey (LR)	22°54' S, 58°42' W	4	
Jerovia (Jer)	23°23' S, 59°12' W	8	Campos-Krauer and Wisely (2010)
Arizona (Ari)	23°53' S, 58°24' W	10	GenBank Access numbers
Maroma (Mar)	23°32' S, 57°54' W	6	GU456363-376
Eñe (Eñe)	24°17' S, 58°34' W	8	
Loreto (Lor)	23°52' S, 59°27' W	11	
Sauces (Sau)	23°18' S, 61°25' W	11	
Olimpo (Oli)	21°09' S, 57°56' W	12	
Bahía Negra (BN)	20°09' S, 58°08' W	9	

applied to our dataset in order to estimate the phylogeographic structure of haplotypes and haplogroups, taking into account the results of BAPS. This method, using a parsimony criterion, combines the minimum-spanning trees (MSTs) with a single network, allowing more detailed population information than do strictly bifurcating trees (Posada and Crandall, 1998).

Historical population dynamics

We used several methods to determine the demographic history for the two genetic clusters which presented more than one haplotype (haplogroups I and II, see Results). First, Tajima's D (Tajima, 1989) and Fu's FS (Fu, 1997) neutrality tests were carried out using Arlequin 3.5.2 software. Then, a mismatch distribution analysis was performed using both Arlequin 3.5.2 and DnaSP v.5 software (Librado and Rozas, 2009). This method, based on an assumed stepwise growth model (Rogers and Harpending, 1992), was used to evaluate: (1) whether there was signature of population expansion, and (2) the timing of demographic expansion measured in units of mutational time. Approximate confidence intervals for growth parameters were obtained by a parametric bootstrap approach (1000 replicates). The validity of the estimated stepwise expansion model was tested using the same parametric bootstrap approach. A goodness-of-fit test between the observed and simulated distributions of pairwise differences was performed. To quantify the smoothness of the observed haplotype frequency distribution, Harpending's raggedness index (Harpending, 1994) was applied. Statistical significance of the index was tested by a coalescent simulation (10,000 permutations) of the random expected value, as implemented in DnaSPv.5. If the raggedness statistic is significantly more similar to the expected curve for population expansion than to the expected curve for a constant size population, the population expansion is considered to be significant. Moreover, the tau value (τ), obtained in the mismatch distribution analysis provides a rough estimate of the time when the sudden population expansion started (Rogers and Harpending, 1992; Rogers, 1995). The substitution rate suggested by Ruíz-García et al. (2016) for the

Table 2
Distribution of *Hydrochoerus hydrochaeris* control region mitochondrial DNA haplotypes by locality and total number of individuals per haplotype (Nh). Sample sizes are given at the final of the Table (Nseq). Abbreviations of populations are shown in Table 1.

Hapl.	Distribution																	Total (Nh)	
	Argentina					Paraguay													
	An	Ib	SJ	EP	BD	Tol	Mari	Lap	LP	LR	Jer	Ari	Mar	Eñe	Lor	Sau	Oli	BN	
A	3	8	10		11	4	3		5		8	4	6	8	3	1	2	1	77
B		2		1	2														5
C		9		3	1														13
D		1																	1
E				2														2	
F				1														1	
G					3		5	5	3		6			8	10	1		41	
H																9	2	11	
I					6													6	
J																	3	3	
K																	1	1	
L																	1	1	
M																	1	1	
N									1									1	
Total (Nseq)	3	20	10	4	17	13	3	5	10	4	8	10	6	8	11	11	12	9	164

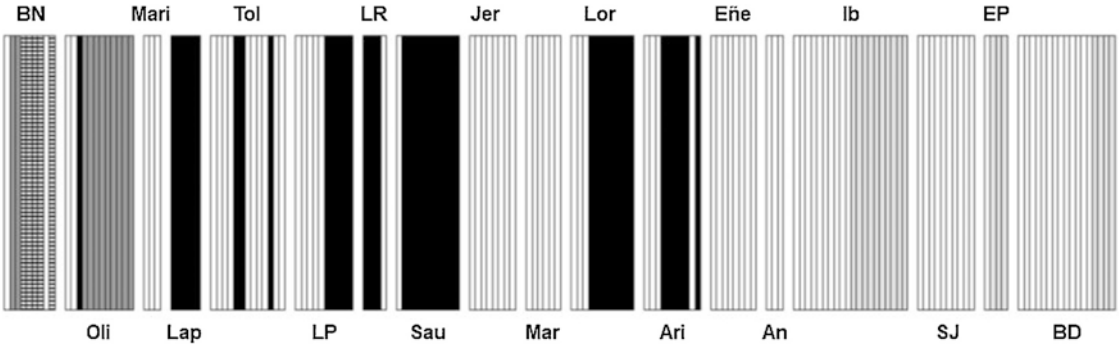

Fig. 2. Bayesian assignment analysis of control region sequences. Each vertical bar represents an individual and its associated probability of belonging to one of the four haplogroups detected. References: haplogroup I (white), haplogroup II (light grey), haplogroup III (dark grey), haplogroup IV (black), Non-assigned individuals (horizontal bars). Abbreviations of sampling sites correspond to those described in Table 1.

capybara mitochondrial control region (2.57×10^{-1} substitutions/site/My) was used to convert mutational time (τ) into real time according to equation: $\tau = 2\mu t$, where t is the time in years and μ is the substitution rate per generation. The generational time used was 3 years (Colin, 1991; Nowak, 1991).

Furthermore, Beast 1.6 (Drummond et al., 2012) was employed to infer historical demography through a Bayesian coalescent approach that improves recovery of the historical signal within DNA sequences. By using the Bayesian skyline plot (BSP) analysis, we estimated changes in effective population size over time for haplogroups I and II. This method, that uses standard MCMC sampling procedures, provides a powerful framework for estimating effective population size through time (Ho and Shapiro, 2011). The parameters and priors used were the Bayesian skyline plot tree prior, the HKY substitution model, as indicated by JModelTest (Posada, 2008), a strict molecular clock set to a lognormal distribution with a substitution rate of 2.57×10^{-1} substitutions/site/My (Ruíz-García et al., 2016), and five grouped intervals. Three replicates of 4×10^7 MCMC steps each were run and sampled every 1000 steps. The first 10% of each run was discarded as burn-in. Tracer 1.6.2 (Drummond and Rambaut, 2007) was used to assess convergence of runs through the effective sample size (ESS) of each parameter. In order to obtain an adequate ESS-value (>200), the three independent runs performed for each simulation were combined using the LogCombiner v. 1.6.2 utility of the Beast package. The resulting file was used to estimate population size changes through time, which was visualized by the Bayesian skyline plot obtained with the utility Tracer v. 1.6.2.

Table 3
Sampling size (N) and genetic diversity measured as number of haplotypes (n), haplotype diversity (H) and nucleotide diversity (π) in each haplogroup.

	N	n	H	π
Haplogroup I	90	5	0.26	0.001
Haplogroup II	17	4	0.42	0.002
Haplogroup III	11	1	0.00	0.000
Haplogroup IV	41	1	0.00	0.000

Results

From the 54 samples analyzed for the Argentinean populations we found 6 haplotypes (Table 2) determined by 4 polymorphic sites. Haplotypes A and C were the most frequent, being found in 32 (59.26%) and 13 (24.07%) individuals, respectively (Table 2). Despite none of the haplotypes were present in all sampling sites, three of them were shared between populations.

When sequences from Argentina were pooled with the 110 homologous sequences from Paraguay, we found a total of 14 haplotypes (Table 2) determined by 16 polymorphic sites. In this case, haplotypes A and G were the most frequent, being found in 77 (46.95%) and 41 (25%) individuals, respectively (Table 2).

BAPS revealed the presence of four genetic clusters (hereafter called haplogroups I–IV) supported by the maximum probability value ($P = 1$) (Fig. 2). Haplogroup I was composed of 90 individuals from all sampling sites except Lapacho, in northern Paraguay (Fig. 3, Table 3). This haplogroup presented five haplotypes and low values of haplotype and nucleotide diversity ($H = 0.26$, $\pi = 0.001$).

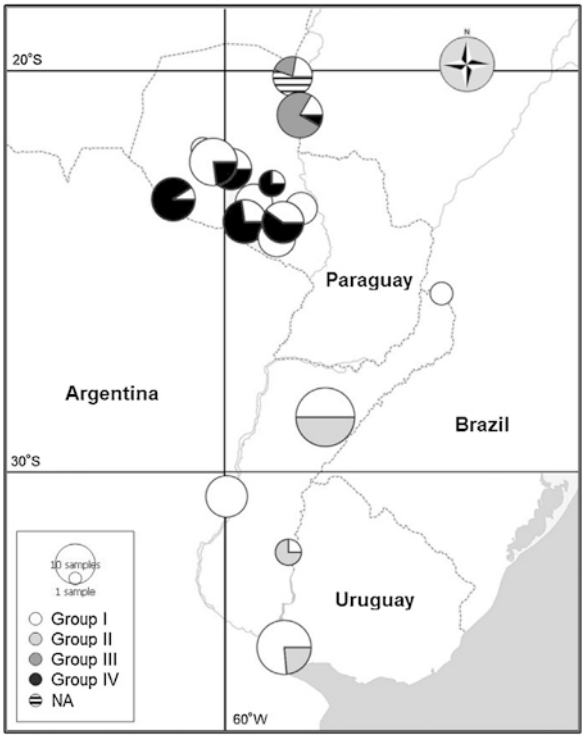

Fig. 3. Distribution of the four control region haplogroups detected in the 18 populations sampled. The size of divisions inside each pie chart is proportional to the number of individuals that belong to each haplogroup. NA: non–assigned individuals.

On the other hand, haplogroup II was composed of 17 individuals, all of them from sampling sites located at the southeast of the study area in Argentina (*i.e.* Iberá, El Palmar and Bajo Delta). It presented four haplotypes and moderate to low values of haplotype and nucleotide diversity (H = 0.42, π = 0.002). Haplogroup III, in turn, was composed by 11 individuals, all of them from the northernmost sampling sites in Paraguay (*i.e.* Bahía Negra and Olimpo). Finally, haplogroup IV was composed by 41 individuals, all of them from eight of the 13 sampling sites located at the Gran Chaco ecosystem in Paraguay. A single haplotype was observed in each of these last two haplogroups, in consequence, genetic diversity was equal zero. Five individuals from Bahía Negra, the northernmost sampling site, were not assigned to any haplogroup (Figs. 2 and 3).

Table 4
Analysis of Molecular Variance (AMOVA), using a square matrix of pairwise genetic distances between haplotypes. The fixation indices (Φ_{ST}) are shown. Φ-statistics and significance of variance components *(P)* were tested by 10,000 permutations according to Excoffier et al. (1992). Significant differences are indicated in bold.

Comparison	Fixation Index (Φ_{ST})	*P*
Among haplogroups	0.946	**0.001**
Between pairs of haplogroups		
Haplogr. I/haplogr. II	0.768	**0.001**
Haplogr. I/haplogr. III	0.965	**0.001**
Haplogr. I/haplogr. IV	0.954	**0.001**
Haplogr. II/haplogr. III	0.955	**0.001**
Haplogr. II/haplogr. IV	0.958	**0.001**
Haplogr. III/haplogr. IV	1.000	**0.001**

The genealogical relationship between haplotypes is shown in Fig. 4. It revealed the presence of two consecutively connected sub-networks, corresponding to haplogroups I and II, whose more frequent haplotypes (A and C) are separated by only one mutational step. These sub-networks are separated by 3–4 mutational steps from haplotype G, the second most frequent haplotype in the network and the only one present in haplogroup IV, which in turn is separated by other three mutational steps from haplotype H, the only one present in haplogroup III. Three other low frequency haplotypes (J, L and K), not assigned to any haplogroup, complete the network. The star-like topology found in haplogroup I suggested that haplotype A, the most frequent haplotype in the study area, represented an ancestral form. A similar pattern is observed in haplogroup II, where haplotype C seems to represent the ancestral variant from which haplotypes D–F derived. However, this haplotype may seem to be ancestral due to all the alternative links in the network exhibiting a star-like phylogeny.

Although only a few variable nucleotide sites divide haplogroups in the haplotype network, results of AMOVA analysis showed significant differences between haplogroups (Φ_{ST} = 0.946, *P* = 0.001; Table 4). The greatest source of variation (94.60%) was found between haplogroups. Pairwise comparisons of Φ_{ST} values showed significant differences between the four haplogroups (Table 4).

Results of Tajima neutrality test showed negative but non-significant values for both haplogroups

(haplogroup I: *D* = -1.31, *P* = 0.06; haplogroup II: *D*: -1.38, *P* = 0.08), while results of Fu test showed negative and significant values (haplogroup I: *Fs* = -3.26, *P* = 0.01; haplogroup II: *D*: -1.94, *P* = 0.01), suggesting a population expansion process.

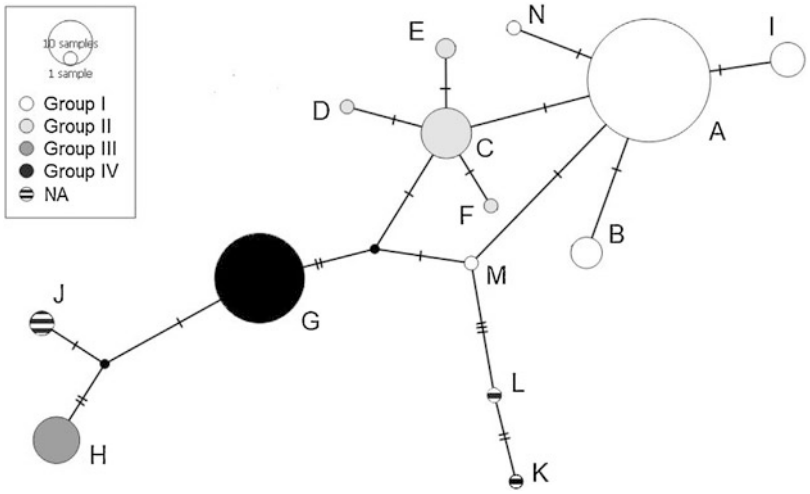

Fig. 4. Genealogical relationships of the 14 capybara haplotypes analyzed. Circle areas are proportional to haplotype frequencies and their colors indicate the belonging of each haplotype to one of the haplogroups. Each small bar on the branches represents one mutational step. Haplotype names correspond to those described in Table 2.

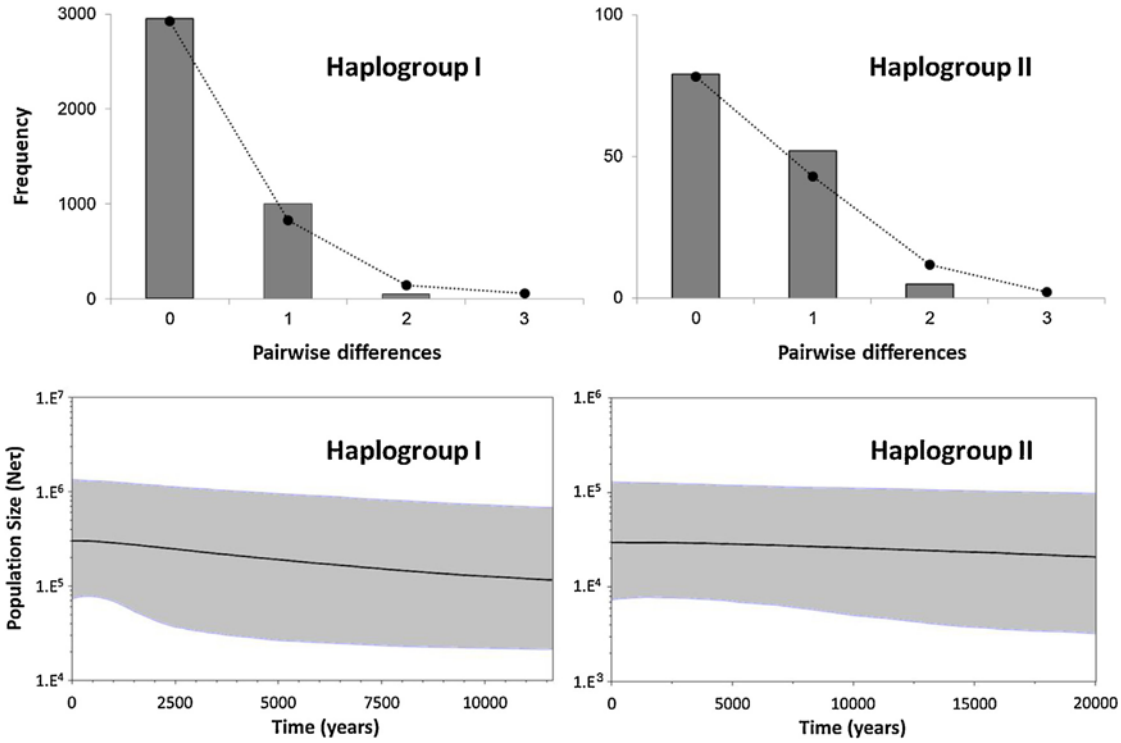

Fig. 5. Demographic history based on the control region sequences from haplogroups I and II. Mismatch distributions (top panel): Bars are observed distributions, the lines shows the distribution simulated under a sudden expansion model. Bayesian skyline plots (bottom panel): The black line represents the estimated median and the grey zone represents the 95% highest posterior density (HPD) intervals.

The observed distribution of pairwise differences obtained in the mismatch distribution analyses carried out for haplogroups I and II showed a clear unimodal patterns that did not differ significantly from the simulated pattern based on a sudden expansion model (haplogroup I: $P > 0.41$; haplogroup II: $P > 0.34$), supporting the demographic expansion hypothesis in both haplogroups (Fig. 5, top panel). Surprisingly, none of the two haplogroups showed significantly low raggedness values (haplogroup I: P [rg $\leq$ 0.29] = 0.45; haplogroup II: P [rg $\leq$ 0.16] = 0.14); perhaps the steep, left-skewed spike in mismatch distribution precludes a significantly low raggedness despite its unimodal shape. The time elapsed since expansion occurred was estimated at 23,726 and 4373 years before the present (YBP) for haplogroup I and II, respectively. On the other hand, results obtained from Bayesian skyline plot analyses (Fig. 5, bottom panel) appears to be inconclusive, as large confidence intervals prevent any interpretation about changes in population size.

Discussion

Bayesian assignment analysis and AMOVA revealed the presence of four haplogroups in our genetic data. Haplogroup I was the most widespread. The existence of this widespread haplogroup suggests the existence of historical gene flow between sampling sites located at the Gran Chaco ecosystem of Paraguay and the Chaco-pampean region in Argentina. Previous studies have demonstrated that the Paraná and Paraguay rivers are an effective route of migration for wildlife (Ringuelet, 1961). These rivers run in a north–south direction from tropical latitudes into subtropical–temperate ones, forming a biogeographical corridor that allows the migration of subtropical species into temperate zones (Kandus and Malvarez, 2002; Quintana et al., 2002), promoting high levels of biodiversity (Arzamendia and Guiraudo, 2012; Oakley et al., 2005). Our results suggest that, as in other wild species, the Paraná and Paraguay rivers, whose drain direction have not changed substantially in the last 3–4 million years (Brea and Zucol, 2011), would have acted as biological corridors, connecting most of the capybara populations in the Chaco-pampean region. This result agrees with those previously described in Byrne et al. (2015), who found several shared haplotypes between Paraguayan and Argentinean populations in a preliminary work in which the authors analyzed the role of river drainages in shaping the capybara genetic structure at different geographic scales.

On the other hand, haplogroup IV was only found in the Paraguayan Gran Chaco, where haplogroup I is also present. Similar results were obtained by Campos-Krauer and Wisely (2010), who performed a phylogeographic analysis using non-invasive samples collected in 13 capybara populations located in the Gran Chaco ecosystem of Paraguay, which is part of the Chaco-Pampean region. The authors described two mayor phylogroups in the study area and attributed this finding to range expansion and secondary contact. They also mentioned that the high frequency of two ancestral haplotypes for each phylogroup, and the short internal branches observed in the haplotype network, strongly suggests that descendants from two refugial populations have expanded into the Paraguayan Chaco to form sympatric populations of two ancestral groups. However, it is important to note that if descendants from both refugial populations expanded, several different haplotypes would be expected in each of our haplogroups, which is not observed for haplogroup IV. In consequence, the secondary contact proposed could have occurred as a consequence of the expansion of haplogroup I. The same pattern of two high frequency ancestral haplotypes and short internal branches is also observed for haplogroups I and II in our haplotype network, which suggests a similar process of population expansion and secondary contact for the Argentinean population. This is also supported by the results of the demographic history analyses performed (see below).

At last, haplogroup III was only found in the two northernmost sampling sites of our study area, which also include individuals belonging to haplogroups I and III. Due to its low sample size and geographic location, it is very difficult to make suggestions about its demographic history. New studies including samples from other non-sampled sites may shed light on this question.

Until now, few studies have estimated the genetic diversity of capybara populations using the HVRI of the mitochondrial control region (Campos-Krauer and Wisely, 2010; Borges-Landáez et al., 2012; Ruíz-García et al., 2016). Haplotype diversity values obtained in the present study are generally similar or higher than those obtained in Campos-Krauer and Wisely (2010) work (H = 0.00–0.20) and lower than those obtained for five managed capybara populations from the seasonally flooded savanna of Venezuelan Llanos (H = 0.44–0.77; Borges-Landáez et al., 2012) and for 78 wild capybaras sampled in Colombia, Peru, Ecuador and Brazil (H = 0.98, Ruíz-García et al., 2016). This lower genetic diversity found in the Chaco-pampean region could not be due to sampling size. For instance, our data set showed that haplogroup II, composed by only 17 individuals, presents the higher value of haplotype diversity, while haplogroup III, composed by 41 individuals presents only one haplotype. Two possible causes of the low genetic diversity found are the conversion of most woodland and grassland that comprise the Chaco-pampean region to agricultural use (Grau et al., 2015), and the long history of commercial exploitation and poaching of capybara populations in the area (Bolkovic et al., 2006; Schivo et al., 2015). However, the expected pattern of a drastic population decrease is the presence of very dissimilar haplotypes, which was not observed in our study area.

Contrary to what happens in most of the study area, the southeast of Argentina, the only area where haplogroup II is located, presents a higher genetic diversity. One possible cause to explain this is that Iberá wetlands, the sampling site with more individuals representative of this haplogroup, present an optimal habitat for the species (Schivo et al., 2015), which could favor the development of large and genetically diverse populations.

Results of Fu neutrality test and mismatch distribution analyses support the hypothesis of sudden expansion for haplogroups I and II, which would have occurred about 24,000 and 4000 YPB, respectively. On the other hand, results of the Bayesian skyline plot analysis appears to be inconclusive, as large confidence intervals prevent any interpretation about changes in population size, probably as a result of low genetic resolution.

Ruíz-García et al. (2016) described different processes of population expansion for the capybara populations of the Colombian Amazon that would have occurred about 450,000-37,000 YBP and that would be related to the dynamics of the Middle and Late Pleistocene glaciations. The population expansion time obtained for haplogroup I by means of mismatch distribution analyses is located after the end of the most extensive glacial episodes that have occurred during the Late Pleistocene more than 56,000 years ago (Mercer, 1976). Campos-Krauer and Wisely (2010) do not report the population expansion times for its phylogroups, but mention that molecular clock estimated suggest that these groups diverged between 10,000 and 40,000 YBP. The authors relate their findings with regional changes in climate and hydrology that had similar effects on other semi-aquatic species near the end of the Pleistocene (Lopes et al., 2006, 2007; Marquez et al., 2006), and suggested that temporal and spatial patterns of capybaras phylogeography lend further support to the hypothesis that climatic fluctuation and the resulting ecological changes are related with historical demography of multiple members of the South American megafaunal wetland community, as it seems to happen also in our capybaras haplogroup I.

Population expansion time for haplogroup II would have occurred in much more recent times, during the Holocene Climatic Optimum (7000–3000 YBP, Giorgis et al., 2015), a period characterized by heavy rainfall. The Iberá Wetlands, the second largest wetland in South America after the Pantanal in Brazil is located in the center and north east of Corrientes Province, Argentina. These wetlands were shaped as a consequence of the migration of the Paraná River towards the northwest of the Chaco-Pampean region. The disconnection between Iberá Wetlands and the Paraná River occurred towards the end of the Pleistocene, about 10,000 YBP. The present lagoons and estuaries originated later, about 3000 YBP, as a consequence of the development of the humid climate with excessive rainfall that characterize the Holocene Climatic Optimum. This rainfall regime is nowadays a factor that favors the presence of a great diversity of biological species, among them the capybara, species for which availability of water is fundamental (Orfeo and Neiff, 2008). Thus, the population expansion observed for haplogroup II, about 4000 years ago, and the secondary contact evidenced in the haplotype network, could be explained by the formation of the Iberá Wetland. Also, an increase in predominance of herbaceous vegetation associated with these water bodies (Fernández Pacella et al., 2011), that occurred about 6000 years ago, generated a habitat with optimal conditions for capybaras (Schivo et al., 2015) that would also favor the population expansion of the species in the area.

Finally, it is interesting to note that the fact of finding considerable lower genetic diversity as well as more recent population expansion times for the Argentinean-Paraguayan capybara population than for the Colombian Amazon (Ruíz-García et al., 2016), is suggestive that the first population is derived from the second one. This is in accordance with Ruíz-Garcia et al. (2016) data. The authors suggested that the Colombian Amazon was the original capybara population from which derived Guainia and Eastern Llanos populations based on a greatest effective female number and no clear population expansion in Colombian Amazon, together with a basal position of this population ancestors in the phylogenetic trees constructed. The authors also mentioned that Western Amazon was the focus for the dispersion of many other mammalian taxa such as the lowland tapir, the jaguar, and diverse Neotropical primate genera.

In summary, the results obtained for the capybara populations in the Chaco-Pampean region showed: (1) the existence of four genetically different haplogroups, (2) that the Paraná and Paraguay rivers would be acting as biological corridors, connecting most of the capybara populations in the Chaco-pampean region, (3) past population expansions for haplogroups I and II and secondary contact between haplogroup I–II and I–IV, (4) a low value of genetic diversity in haplogroups I, III and IV, and (5) that historical population dynamics of haplogroups I and II is closely related to past climatic events such as the Iberá Wetlands formation. This preliminary conclusion must be taken as working hypotheses for future studies of potential factors that could have shaped the genetic diversity, population structure and dynamics of capybara and related species inhabiting the region. Future analyses, including nuclear markers as microsatellites, would contribute to test these hypothesis and to detect possible admixture among individuals after the split of haplogroups.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 11420100100189 to J.I.T); the

Dirección de Fauna de la Secretaria de Agricultura y Desarrollo Sustentable; the Departamento de Ciencias Básicas de la Universidad Nacional de Luján; and the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (PhD Grant to M.S.B).

We thank Ayelen Eberhardt, Natalia Fracassi, Yanina Sica, Emiliano Villegas, Mariano Tortarolo, Aristóbulo Maranta, Alexandra Craviño, Constanza Gariboldi, Guillermo Gil, Eduardo Bidart and the local people who assisted us during sample collection

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