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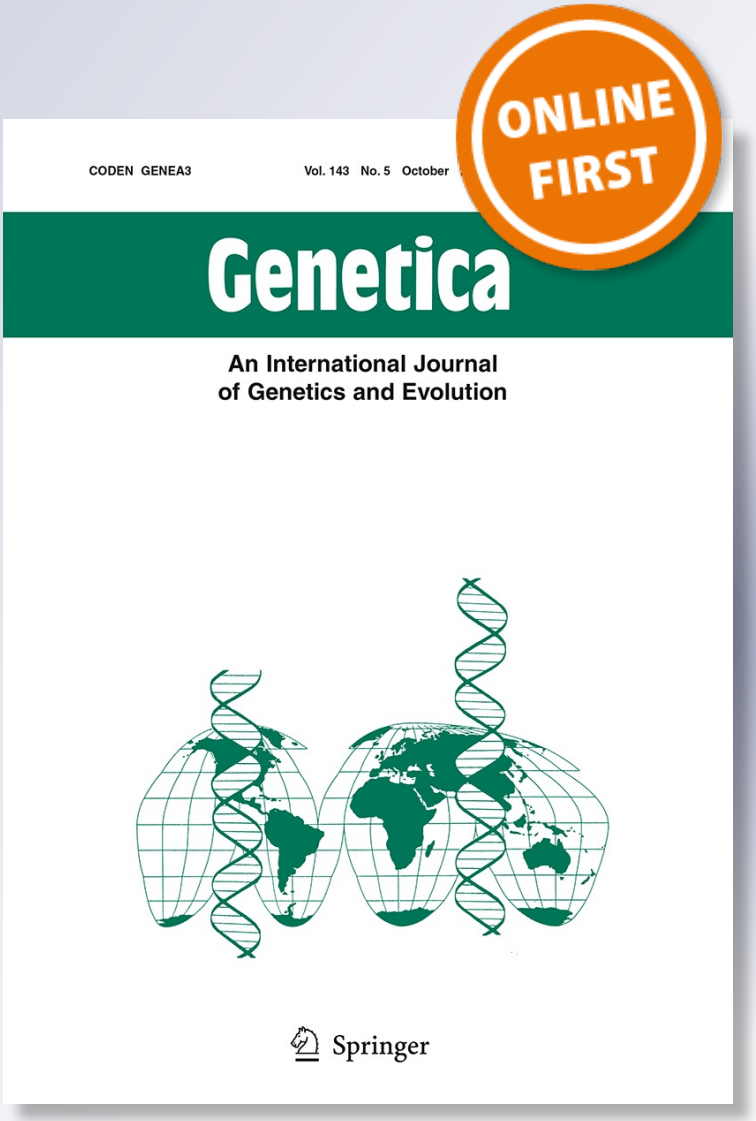

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The role of river drainages in shaping the genetic structure of capybara populations

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Abstract The capybara, *Hydrochoerus hydrochaeris*, is an herbivorous rodent widely distributed throughout most of South American wetlands that lives closely associated with aquatic environments. In this work, we studied the genetic structure of the capybara throughout part of its geographic range in Argentina using a DNA fragment of the mitochondrial control region. Haplotypes obtained were compared with those available for populations from Paraguay and Venezuela. We found 22 haplotypes in 303 individuals. Hierarchical AMOVAs were performed to evaluate the role of river drainages in shaping the genetic structure of capybara populations at the regional and basin scales. In addition, two landscape genetic models, isolation by distance and isolation by resistance, were used to test whether genetic distance was associated with Euclidean distance (i.e. isolation by distance) or river corridor distance (i.e. isolation by resistance) at the basin scale. At the regional scale, the results of the AMOVA grouping populations by mayor river basins showed significant differences between them. At the basin scale, we also found significant differences between sub-basins in Paraguay, together with a significant correlation between genetic and river corridor distance. For Argentina and Venezuela, results were not significant. These results suggest that in Paraguay, the current genetic structure of capybaras is associated with the lack of dispersion corridors through permanent rivers. In contrast, limited structuring in Argentina and Venezuela is likely the result of periodic flooding facilitating dispersion.

Keywords *Hydrochoerus hydrochaeris* · Mitochondrial DNA · HVRI · River basins · South America

Introduction

Population genetic theory predicts that high levels of gene flow among populations lead to homogeneity of gene frequencies, while limited dispersal produces isolation and

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divergence as a result of the action of genetic drift and/or natural selection (Wright 1931; Slatkin 1985). The degree of connectivity among populations may be influenced by many environmental or biological factors, such as climate, dispersion barriers, philopatry or dispersal ability, limiting generalizations across taxa, habitats, or life histories. In the case of faunal species restricted to freshwater environments, the shared constraint of habitat structure imposed on all species simplifies the task (Hughes et al. 2009). Gene flow in these species is expected to depend on the spatial structure of river networks, which determine the dispersal distance and connectivity between populations. The hierarchical structure of stream networks and drainage basins provides a natural spatial framework for considering the effects of dispersal and resulting gene flow in resident species (Meffe and Vrijenhoek 1988; Grant et al. 2007). During the last decades, numerous studies have used these ideas to infer levels of dispersal from measured differentiation in gene frequencies among populations of freshwater fishes, insects and aquatic or semi-aquatic mammals (Jackson and Resh 1992; Hanfling and Brandl 1998; Hurwood and Hughes 1998; Hughes et al. 1999; Waters et al. 2001; Banguera-Hinestroza et al. 2002; Vignieri 2005; Burridge et al. 2008; Hughes et al. 2009; Pujolar et al. 2011; Pickles et al. 2012), and several models have been proposed to predict how populations of taxa with different life-history traits and dispersal capabilities interact within structured freshwater habitats (see Finn et al. 2007; Hughes et al. 2009 for review). For aquatic or semi-aquatic organisms gene flow typically occurs primarily within rather than among drainage basins (the Stream Hierarchy Model, Meffe and Vrijenhoek 1988). Under this scenario, population genetic variance is expected to be significantly partitioned among drainage basins at any spatial scale at which basins can be defined within the stream network (Hughes et al. 2009). Moreover, if dispersal is confined to water presence, the dispersal distance between two sites might be better defined by the length of the watercourse that connects two points (i.e. the river corridor distance) (Chaput-Bardy et al. 2009; Brown and Swan 2010) than by the Euclidean distance. In this context, a better correlation between genetic and river corridor distances (i.e. an isolation by resistance model) is expected compared to that between the genetic and Euclidean distance (i.e. an isolation by distance model).

The capybara, *Hydrochoerus hydrochaeris,* is a semi-aquatic herbivore rodent widely distributed throughout most of South American wetlands. The species is closely associated with aquatic environments. Water is a vital resource for capybaras and a year-round standing water source as ponds, lakes, marshes or swamps is essential for capybara to be present in a given area (Herrera and Macdonald 1989; Quintana 1999). Water is used not only for drinking, but also to control their body temperature and as an escape from predators. Capybaras usually mate in the water and, as semi aquatic grazers, most of their food is found near or within water bodies (Barreto and Quintana 2012). Resting occurs both in land and on tall and rooted aquatic plants, which are trampled in order to build beds (Quintana 1999). Capybaras live in social groups with sizes typically ranging from 6 to 16 adult members (Herrera et al. 2011). Until now, only a few ecological studies have described dispersal patterns of the species (Herrera 1992; Salas 1999; Congdon 2007). Results of these studies suggest that, in addition to differences in density, variation in dispersal patterns and distances would be related to the spatial distribution of water, a key territory component (Herrera et al. 2011).

Two previous studies analyzed the genetic diversity and population structure of the species in natural populations; however, none have assessed the role that structured freshwater habitats play on capybaras genetic structure. Using a 386 bp segment of the mitochondrial control region, Campos-Krauer and Wisely (2010) performed a phylogeographic analysis of the species using non-invasive samples collected in 13 populations located in the Gran Chaco ecosystem of Paraguay. They found genetic signals of recent population range expansion that were related to deforestation and cattle ranching. In a second study, Borges-Landaez et al. (2012) assessed the genetic variability and population structure of five managed capybara populations from the seasonally flooded savanna of Venezuelan Llanos. Using a 545 bp segment of the mitochondrial control region, the authors found significant genetic structuring and a correlation between genetic and geographic distance, suggesting widespread gene flow with isolation by distance.

The study of genetic variability and population structure, especially from the mtDNA coding regions, is limited by the availability of a relatively small number of polymorphisms in the sequences (Torroni et al. 1996; Wallace et al. 1999). Alternatively, sequences from the first hypervariable region (HVRI) of the rapidly evolving non-coding control region have been extensively used to assess genetic structure and phylogeography in mammals (Lucchini et al. 2004; Trujillo et al. 2004; Piaggio and Perkins 2005; Hoffman et al. 2006; Jalil et al. 2008; Marín et al. 2008; Campos-Krauer and Wisely 2010; Tunez et al. 2010, 2013) due to its high levels of polymorphism and relative ease amplification from samples obtained by non-invasive methods.

In this paper, we studied the genetic diversity and population genetic structure of *H. hydrochaeris* throughout part of its geographical distribution in Argentina, amplifying the HVRI of the mitochondrial control region from tissue and faecal samples. Data were compared with

available sequences from capybara populations of Paraguay and Venezuela in order to evaluate, at different ecological scales, the role of river drainages in shaping the genetic structure currently found. If, as previously suggested, gene flow between capybara populations occurs mainly associated with the spatial distribution of water streams (i.e. across river networks), we expect: (1) low genetic differentiation between populations within the same river or river basin and high levels of genetic differentiation between populations located in different basins, and (2) that genetic distance between populations is better correlated with river corridor distance (i.e. isolation by resistance hypothesis) than with Euclidean distance between geographic locations (i.e. isolation by distance hypothesis).

Materials and methods

Sample collection

Forty tissue (n = 9) or fecal (n = 31) samples from the Argentinean capybara population were collected in eight sampling sites located along the Paraná and Uruguay River basins (Fig. 1c; Table 1), preserved in ethanol 96 % during field work and stored at −20 °C until DNA extraction was performed. Fecal samples were collected in the field from different mounds, to decrease the probability of re-sampling the same individual.

Mitochondrial DNA extraction and PCR amplification

Depending on sample source, different DNA extraction protocols were used. Tissue 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 described in Reed et al. (1997) with one modification; in the last step of the Wizard SV Gel and PCR Clean-Up System kit (Promega) protocol, centrifugation time was changed from 1 to 5 min in order to increase the amount of purified DNA.

Aliquots of total DNA were used as templates in Polymerase Chain Reaction (PCR) to amplify a double-stranded DNA product from the capybara's HVR1 control region. Each PCR had a reaction volume of 20 µl and contained 1X Pfu DNA Polymerase buffer (200 mM Tris–HCl, pH 8.8, 100 mM KCl, 100 mM $NH4_2S0_4$, 20 mM $MgS0_4$, 1 % Triton X-100 and 1 mg/ml nuclease-free BSA), 0.2 mM of each deoxynucleotide triphosphate, 1 µM of forward and reverse primers, 1U of Pfu DNA Polymerase (Promega), 1 µg/µl BSA (New England Biolabs), 0.5–4 µl of the DNA extract and water to reach the final volume reaction. The primer pair used included the Capy D2 (5′-TAATGCATGTCCCCATGAAC-3′) and Capy D3R (5′-TGGTGCATGTCTAACGATGG-3′) primers developed by Campos-Krauer and Wisely (2010) and designed to amplify a 245 bp DNA fragment of the mtDNA 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 include most of the variable sites of the capybara control region. Amplification protocol consisted in a single denaturation step at 95 °C for 10 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 52–54 °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 thermal cycler (BIORAD). PCR products were resolved in 1.5 % agarose gel electrophoresis and visualized under UV light. Bands of expected size were cut from the gel and purified using the Wizard SV Gel and PCR Clean-Up System kit (Promega), following manufacturers' protocol with the following modification: in the DNA elution step, we changed the centrifugation time from 1 to 5 min and used 30 µl of nuclease-free water instead of 50 µl in order to concentrate the DNA prior sequencing. The purified DNA products were sent to an external laboratory (Biotechnology Institute of INTA Castelar, Argentina) for direct sequencing using the same oligonucleotide primers.

Twenty-four additional mtDNA control region sequences were obtained from the GenBank, including 10 haplotypes from 5 populations in Venezuela and 14 haplotypes from 13 populations in Paraguay (Fig. 1; Table 1). Haplotypes from Venezuela had a length of 545 bp and were obtained from 153 individuals (Borges-Landaez et al. 2012; GenBank Access numbers: EU149767-776), while haplotypes from Paraguay 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 included the HVRI fragment amplified for the Argentinean samples.

Data analyses

Sequences were aligned and analyzed for polymorphic sites using ClustalX v2.0.11 (Larkin et al. 2007). Aligned sequences were manually edited using Chromas v2.23 (Technelysium Ltd.). Absolute and relative frequencies of haplotypes within sampling sites and pairwise comparisons of percent sequence divergence were computed using ARLEQUIN v3.11 (Excoffier et al. 2005). Sequences from Argentina were shorter than those from Paraguay and

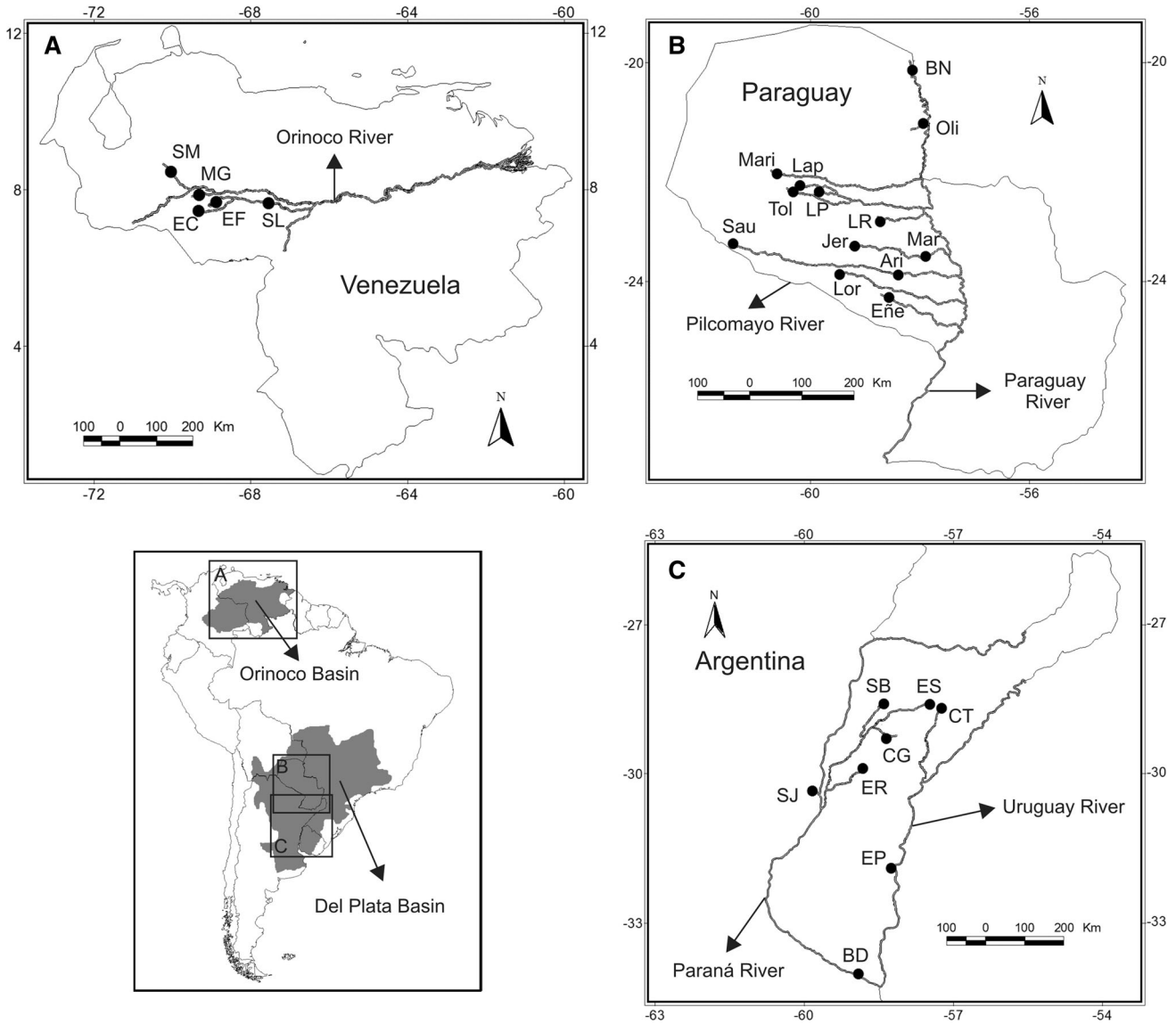

Fig. 1 Mayor river drainages and location of capybara sampling sites in Venezuela (**a**), Paraguay (**b**) and Argentina (**c**). Abbreviations of sampling sites names correspond to those described in Table 1

Venezuela. As a consequence, when all sequences were aligned, the number of haplotypes from Paraguay and Venezuela used in the AMOVA comparing all populations (see below) was reduced to 8 and 5, respectively (Table 2).

The median-joining network method (Bandelt et al. 1999) implemented in the Network v4.6 program (Fluxus Technology Inc.) was applied to our dataset in order to estimate the phylogeographic structure of haplotypes. 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).

In order to evaluate the role of river drainages in shaping the genetic structure of capybara populations, we performed three hierarchical AMOVAs, using pairwise differences between haplotypes as a measure of molecular distance. First, we tested the occurrence of genetic partitioning at a regional scale. To do that, we defined two mayor river basins, the Orinoco Basin, including populations from Venezuela, and the Del Plata Basin, including populations from Paraguay and Argentina (Fig. 1). After that, two additional hierarchical AMOVAs were performed for the populations in Paraguay and Argentina. The first one included the 13 populations from the Paraguay River Basin and was carried out using the 386 bp DNA fragments obtained from GenBank. Populations were divided in eight river basins as follows: (1) Olimpo and Bahía Negra, (2) Mariscal, (3) Toledo, Lapacho and Loma Plata, (4) Laguna Rey, (5) Jerovia and Maroma, (6) Arizona and Sauces, (7) Loreto, and (8) Eñe (Fig. 1b). The AMOVA including only

Table 1 Origin of capybara samples used in this study

Sampling sites	Location	N	Source
Venezuela (VEN, $n = 153$)			
El Cedral (EC)	7°25′N, 69°20′W	29	Borges-Landaez et al. (2012)
El Frío (EF)	7°47′N, 68°53′W	34	
Mata de Guamo (MG)	7°58′N, 69°13′W	19	
Santa Luisa (SL)	7°41′N, 67°31′W	29	
Santa María (SM)	8°19′N, 70°16′W	42	
Paraguay (PAR, $n = 110$)			
Toledo (Tol)	22°21′S, 60°19′W	13	Campos-Krauer and Wisely (2010)
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	
Arizona (Ari)	23°53′S, 58°24′W	10	
Maroma (Mar)	23°32′S, 57°54′W	6	
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	
Argentina (ARG, $n = 40$)			
Santa Bárbara (SB)	28°36′S, 58°24′W	1	This work
El Socorro (ES)	28°40′S, 57°26′W	10	
Cerro Tuna (CT)	28°41′S, 57°14′W	9	
Capi-Guarí (CG)	29°18′S, 58°21′W	1	
El Rezongo (ER)	29°54′S, 58°49′W	2	
San Javier (SJ)	30°21′S, 59°48′W	7	
El Palmar (EP)	31°54′S, 58°15′W	5	
Bajo Delta (BD)	34°02′S, 58°54′W	5	

populations from Argentina was carried out using the haplotypes obtained in this work. Populations were grouped into two river basins; the Uruguay Basin ($n = 14$), including samples from Cerro Tuna and El Palmar, and the Paraná basin ($n = 22$), including samples from El Socorro, San Javier and Bajo Delta (Fig. 1c). Samples from El Rezongo, Capi-Guarí and Santa Bárbara, three of the sampling sites in Argentina, were not included in the analyses due to its low sample size ($n \leq 2$). The analysis of population structure within the Orinoco Basin (Venezuela) could not be carried out since the authors of that work did not publish haplotype frequency information of each sampling site. In this case, we only compared the pairwise F_{ST} values informed in Borges-Landaez et al. (2012), dividing sampling sites in three river basins as follows: (1) El Cedral, El Frío, and Santa Luisa, (2) Mata de Guamo, and (3) Santa María (Fig. 1a).

We applied a Principal Coordinate Analysis (PCoA) to the pairwise F_{ST} matrices in order to test the hypotheses of isolation by distance (IBD) or isolation by resistance (IBR). To carry out these analyses, we retained the first axes of the PCoA based on a Kaiser-Guttman criterion. Specifically, we retained the first axis for the analysis in Argentina and the first two axes for the analyses in Paraguay and Venezuela. Then we used these axes as a response matrix in a series of Canonical Correspondence Analyses (CCA) (ter Braak 1988). To test the IBD hypothesis we used eigenvectors obtained from matrices of Euclidean geographic distances between populations, while to test the alternative hypothesis of IBR we used eigenvectors obtained from matrices of distances between population following possible capybara routes of dispersion throughout permanent rivers as independent variables. Distance measures were made over a digital georeferenced map of the study area, containing a digital cover of permanent rivers and the location of sampling sites. Distance throughout permanent rivers was measured using the tool Distance, included in the Spatial Analyst extension of ArcMap 10 software. To transform geographic space in raw data (i.e. variables by population,

Table 2 Distribution of the HVRI haplotypes across sampling sites

Haplot.	N	Distribution									
		VEN	PAR	ARG							
				SB	ES	CT	CG	ER	SJ	EP	BD
H01	11					7 (0.78)			4 (0.57)		
H02	1					1 (0.11)					
H03	1					1 (0.11)					
H04	1				1 (0.10)						
H05	2				1 (0.10)			1 (0.50)			
H06	7				5 (0.50)					2 (0.40)	
H07	1				1 (0.10)						
H08	1									1 (0.20)	
H09	1				1 (0.10)						
H10	48		34 (0.31)	1 (1.00)	1 (0.10)		1 (1.00)	1 (0.50)	3 (0.43)	2 (0.40)	5 (1.00)
H11	41		41 (0.37)								
H12	18		19 (0.17)								
H13	8		8 (0.07)								
H14	3		3 (0.03)								
H15	3		3 (0.03)								
H16	1		1 (0.01)								
H17	1		1 (0.01)								
H18	38	38 (0.25)									
H19	102	102 (0.67)									
H20	6	6 (0.04)									
H21	5	5 (0.03)									
H22	2	2 (0.01)									
		153	110	1	10	9	1	2	7	5	5

Absolute and (relative) frequencies are shown. Abbreviations of sampling sites names correspond to those described in Table 1

instead of a distance matrix) we applied eigenfunction analyses of geographic distances to obtain "eigenvector maps", expressing spatial relationships among populations (Diniz-Filho et al. 2013). The idea of the method is that when PCoA axes are correlated with these eigenvectors, some of them will tend to describe the spatial patterns in genetic variation. Thus, we modeled simultaneously the different axes from the PCoA of F_{ST} matrices using a CCA, following a multidimensional approach. One of the main difficulties with this approach is to decide which spatial eigenvectors shall be used in the analyses, and several criteria can be applied. Here, we used a forward selection approach to select spatial eigenvectors, following Blanchet et al. (2008).

Results

From the 40 samples analyzed for the Argentinean populations we found 10 haplotypes determined by 8 polymorphic sites (Table 2). Haplotypes 10 and 01 were the most frequent, being found in 14 (35.0 %) and 11 (27.5 %) individuals, respectively. Despite none of the haplotypes were present in all sampling sites, some haplotypes were shared between populations: Cerro Tuna and San Javier shared haplotype 01; El Socorro shared haplotype 05 with El Rezongo and haplotype 06 with El Palmar, and all sampling sites except Cerro Tuna shared haplotype 10.

When sequences from Argentina were pooled with the homologous sequences from Paraguay and Venezuela, we found a total of 22 haplotypes and 26 polymorphic sites (Table 2). In this case, haplotype 19 was the most frequent, being found in 102 of the 303 individuals (33.7 %), all of them from Venezuela. In addition, other three haplotypes were found in high frequencies in the sample. Haplotype 10 was found in 48 individuals (15.8 %), belonging to Paraguay and all the sampling sites in Argentina with the exception of Cerro Tuna. Haplotype 11 was found in 41 individuals (13.5 %), all of them from Paraguay, and haplotype 18 was found in 38 individuals (12.5 %), all of them from Venezuela. From the remaining 18 haplotypes, 15 were private from Paraguay ($n = 6$), Venezuela

($n = 3$), El Socorro ($n = 3$), Cerro Tuna ($n = 2$) or El Palmar ($n = 1$) (Table 2).

The genealogical relationship between haplotypes is illustrated in Fig. 2. It shows a pattern of phylogeographic structure with three main groups, and three haplotypes (16, 17 and 20) that differ markedly with respect to the main groups. The first main group (haplotypes 18, 19, 21 and 22) is composed exclusively by individuals from the Orinoco Basin (Venezuela), the second one (haplotypes 11, 13, 14 and 15) is composed exclusively by individuals from the Paraguay Basin (Paraguay), while the third one is composed by haplotypes from the Paraguay, Uruguay and Paraná basins (Paraguay and Argentina). These results suggest that capybaras from Venezuela are isolated from those in Paraguay and Argentina and that some degree of gene flow occurs between Paraguay and Argentina.

The AMOVA analysis grouping populations by mayor river basins showed significant differences among individuals within sites ($F_{ST} = 0.761$; $P < 0.0001$), among populations within the same basin ($F_{SC} = 0.557$; $P < 0.0001$) and among basins ($F_{CT} = 0.460$; $P < 0.0001$) (Table 3). The greatest source of variation (46.02 %) was found at the among basin level. Pairwise differences of F_{ST} values among capybara populations showed that the populations from the Orinoco Basin (Venezuela) were genetically different from those of the Del Plata Basin (Paraguay and Argentina) ($F_{ST} > 0.600$; $P < 0.0001$), while within Del Plata Basin some populations were genetically similar ($F_{ST} < 0.706$; $P > 0.054$).

The AMOVA carried out with sequences from Paraguay showed significant differences among individuals within sites ($F_{ST} = 0.359$; $P = 0.0001$), among populations within the same river basin ($F_{SC} = 0.223$; $P < 0.0001$) and among groups of populations belonging to different basins ($F_{CT} = 0.176$; $P < 0.01$) (Table 3). The greatest source of variation (64.01 %) was found at the intrapopulation level. The two-dimensional PCoA plot (Fig. 3a) showed that the first two principal coordinates account for 56.48 and 17.98 % of total variation, respectively. CCA revealed no evidence of isolation by distance. None eigenvector of the nine considered in the CCA was retained by forward selection procedure as a significant predictor of genetic structure (IBD model: $P > 0.15$). However, when the first two axes derived from the PCoA matrix were correlated with eigenvectors obtained from de IBR model, all nine eigenvectors were retained by forward selection as significant predictors of genetic structure. The model accounted for 54.44 % of total variation and showed a highly significant relationship (IBR model: $P = 0.0001$; Table 4) between genetic distance and geographic distance measured throughout rivers connecting populations.

In Argentina, the AMOVA also showed significant genetic differences among individuals within sites ($F_{ST} = 0.353$; $P < 0.0001$) and among populations within the same basin ($F_{SC} = 0.427$; $P < 0.0001$). Non significant differences were found among groups of populations belonging to different river basins ($F_{CT} = -0.129$; $P = 0.703$) (Table 3). The greatest source of variation (64.74 %) was found at the intrapopulation level. The PCoA (Fig. 3b) showed that the first two principal coordinates account for 65.65 and 24.24 % of total variation, respectively. CCA revealed no evidence of isolation by distance or isolation by resistance. For both models, none eigenvector of the four considered in the CCA was retained by forward selection as a significant predictor of genetic structure (both IBD and IBR models: $P > 0.21$).

Finally, the analysis of the pairwise F_{ST} values obtained by Borges-Landaez et al. (2012) for populations in

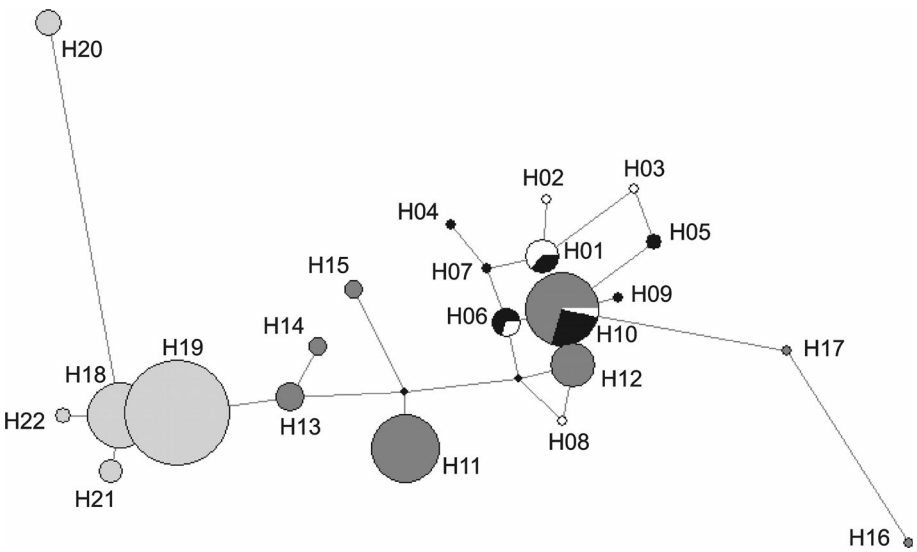

Fig. 2 Genealogical relationships of the 22 capybara haplotypes analyzed. *Circle areas* are proportional to haplotype frequencies and length of the branches to the number of mutational changes between haplotypes. Haplotype *numbers* correspond to those described in Table 2. References: *Light grey* Orinoco Basin (Venezuela), *Dark grey* Paraguay Basin (Paraguay), *Black* Paraná Basin (Argentina), *White* Uruguay Basin (Argentina)

Table 3 AMOVA results for the analysis of population structure at the regional and basin scales

Populations grouping	Variance component	% of total variance	ϕ Statistics	*P*
1. By mayor river basins	Among basins	46.02	$F_{CT} = 0.460$	<0.0001
	Among populations within basins	30.08	$F_{SC} = 0.557$	<0.0001
	Among individuals within sites	23.90	$F_{ST} = 0.761$	<0.0001
2. By river basin-Paraguay	Among basins	17.59	$F_{CT} = 0.176$	<0.01
	Among populations within basins	18.40	$F_{SC} = 0.223$	<0.0001
	Among individuals within sites	64.01	$F_{ST} = 0.359$	<0.0001
3. By river basin-Argentina	Among basins	−12.94	$F_{CT} = -0.129$	0.703
	Among populations within basins	48.20	$F_{SC} = 0.427$	<0.0001
	Among individuals within sites	64.74	$F_{ST} = 0.353$	<0.0001

Venezuela showed significant genetic differences between most of them, regardless of the basin to which they belong, while El Cedral showed no genetic differences with Santa María, a population located in a different basin. The PCoA (Fig. 3c) shows that the first two principal coordinates account for 60.87 and 37.34 % of total variation, respectively. As occurred in Argentina, CCA revealed no evidence of isolation by distance or isolation by resistance. For both models, none eigenvector of the four considered in the CCA was retained by forward selection as a significant predictor of genetic structure (IBD model: $P > 0.13$; IBR model: $P > 0.43$).

Discussion

Genetic structure at the regional scale

We analyzed sequence data from 23 *H. hydrochaeris* populations from Venezuela, Paraguay and Argentina in order to evaluate, at different ecological scales, the role of river drainages in shaping the genetic structure currently found in capybara populations. At the regional scale, two major hydrological drainages can be analyzed, the Orinoco Basin, which includes the sampling sites in Venezuela and the Del Plata Basin, the latter containing all sampling sites in Paraguay and Argentina. The genealogical relationships of haplotypes showed that populations from Venezuela did not share any haplotype with populations from Argentina and Paraguay, suggesting a reciprocally monophyletic state. Moreover, the results of the AMOVA grouping populations by mayor river basin, and the comparisons of pairwise F_{ST} values between populations (Online Resource 1) revealed that the capybaras population in Venezuela is genetically different from those in Paraguay and Argentina. Altogether, these results suggests that the capybara population from Venezuela would be preliminary considered as a distinct Evolutionarily Significant Unit (ESU). However, there are other requisites for the definition of ESUs, for example, to show significant differences of allele frequencies at nuclear loci and of phenotypic traits (Moritz 1994; Frankham et al. 2002). Until now, there have been no published studies analyzing the genetic structure of the species using nuclear markers, but Mones and Ojasti (1986) and Moreira et al. (2012) suggested the existence of a latitudinal cline in the species, with an increase in body size and mass as the latitude increases. Future studies including intermediate populations, nuclear markers and phenotypic traits, as skull metrics, should be conducted to validate this hypothesis.

The phylogeographic structure of haplotypes showed that capybara populations located in Paraguay and Argentina, all of them belonging to the Del Plata Basin, shared some haplotypes. In addition, results of pairwise differences of F_{ST} values among these populations showed that some of them were genetically similar (Online Resource 1). Altogether, these results suggest some degree of gene flow between populations from different countries. Other studies have demonstrated that the Paraná River is an effective route of migration for wildlife, promoting migratory routs from tropical areas to temperate zones (Kandus and Malvarez 2002; Quintana et al. 2002). Our results suggest that the same pattern of connectivity would be occurring between capybara populations.

Genetic structure at the river basin scale

The results of the AMOVAs grouping populations by river basin within countries showed that population genetic variance was partitioned significantly among drainage basins in Paraguay but not in Argentina, suggesting that in the former country gene flow is occurring primarily within, rather than between, drainage basins. This pattern is expected under the Stream Hierarchy Model (Meffe and

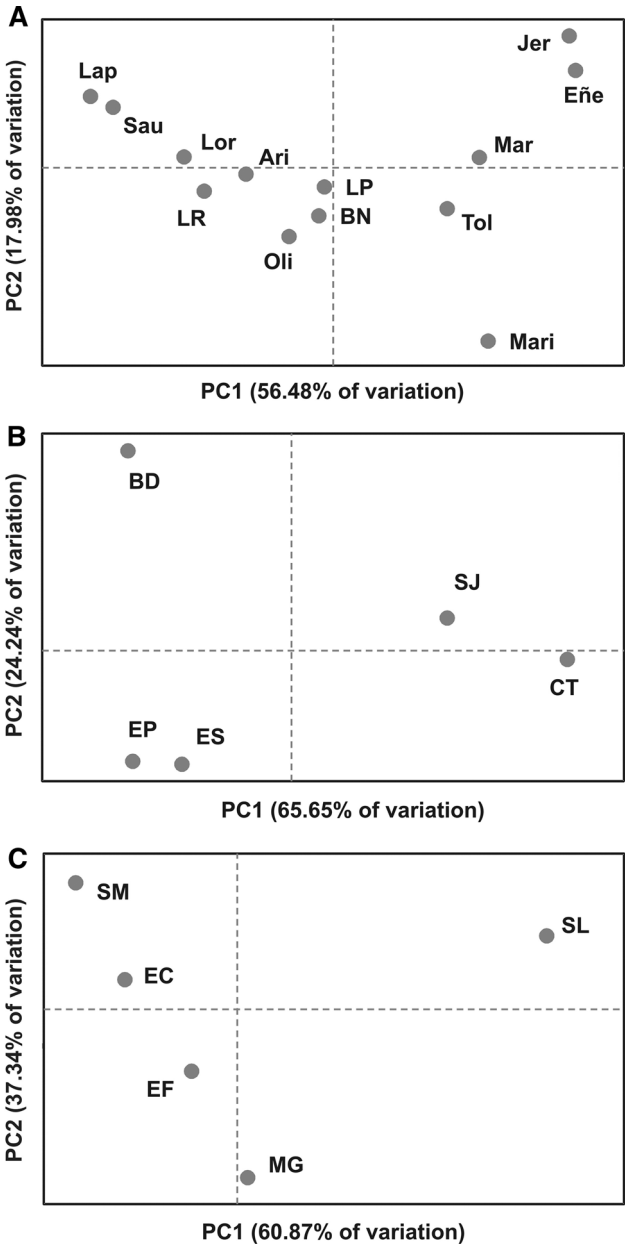

Fig. 3 Two-dimensional plots of the Principal Coordinate Analyses (PCoA) of Paraguay (**a**), Argentina (**b**) and Venezuela (**c**) populations. Abbreviations of sampling sites names correspond to those described in Table 1

Table 4 Summary statistics for the Canonical Correspondence Analysis between the first two axes derived from the PCoA matrix and the eigenvectors obtained from de isolation by resistance model in Paraguay

Eigenvector	Canonical coefficients	
	Axis 1	Axis 2
1	0.14	0.60
2	−0.02	0.43
3	0.43	0.02
4	0.50	0.19
5	0.16	−0.43
6	0.56	−0.01
7	0.32	−0.43
8	0.30	0.05
9	0.12	0.18
Eigenvalues	(0.99)	(0.94)
P model	0.0001	
Explained variation	54.44 %	

Vrijenhoek 1988). In addition, results of CCA revealed no evidence of IBD in Paraguay, but a highly significant relationship between genetic distance and river corridor distance was found. This suggests that an IBR model would better explain the genetic structure currently found in that country. Evidences supporting the Stream Hierarchy and IBR models expectation have also been described in other taxa, including several species of aquatic insects (Hughes et al. 1999; Wishart and Hughes 2003; Landeiro et al. 2011), fishes (McGlashan and Hughes 2002; Burridge et al. 2008; Landeiro et al. 2011; Pujolar et al. 2011; Seymour et al. 2013) and/or mammals (Banguera-Hinestroza et al. 2002; Pickles et al. 2012). In contrast, genetic variance was not significantly partitioned among drainage basins from Argentina and Venezuela, and CCA results did not showed any significant correlation between genetic and geographic distances. Only for Venezuela, Borges-Landaez et al. (2012) found a significant correlation between genetic and Euclidean geographic distances between populations (Mantel test: $R = 0.778$; $P = 0.007$), suggesting that gene flow does not necessarily depend on the spatial structure of river networks. However, in spite that only a few ecological studies have described dispersal patterns in this species (Herrera 1992; Salas 1999; Congdon 2007), their results are consistent with the idea that variation in dispersal patterns and distances are likely related to the spatial distribution of water (Herrera et al. 2011). For example, Herrera (1992) found that, at Hato El Frío, capybaras appeared to disperse predominantly north or south, possibly to increase the probability of finding a stream, and that if animals can find water and grass along their dispersion route, the cost of moving would possibly be negligible. This apparent contradiction between studies can be explained by taking into account that the Venezuelan Llanos are characterized by a marked wet-dry seasonal regime that causes widespread flooding in the wettest months, mostly in the Apure State (Herrera 1992), where most of the samples of the Borges-Landaez et al. (2012) genetic study were taken. A similar situation would occur in Argentina during El Niño events, where an increase in water supply results in extraordinary flooding (Flamenco 1998). In this context, the lack of a

genetic structuring associated with drainage basins, the lack of correlation between genetic and river corridor distances and the significant correlation found by Borges-Landaez et al. (2012) between genetic and Euclidean geographic distances may be associated with the capybara dispersal pattern during wettest seasons, when habitat do not impose constrains on dispersal directions.

Phylogeographic approaches have been used to address whether populations have been separated for a long evolutionary history, or whether there is evidence of current dispersal between them (Avise et al. 1987; Avise 2004). Populations in two adjacent drainages that present two distinct groups of haplotypes, separated by significant genetic divergence, are likely to have been separated for a long period of evolutionary time. In contrast, if populations represent groups separated by limited divergence, the separation may be more recent. Our results suggest that, at least in Paraguay, gene flow in capybaras occurs mainly associated with the spatial structure of river networks, with low genetic differentiation in the same river basin and higher levels of differentiation between basins. Although the current structure of the Del Plata Basin is several million years (Popolizio 2006) older than that detected in the Paraguay basin, our results showed limited divergence between their populations, evidenced by a limited number of nucleotide changes between haplotypes and the sharing of common haplotypes between populations. This limited divergence suggests that capybaras likely disperse between basins, possible over land or during extraordinary flooding events. Previous studies of species in which gene flow is strictly restricted to water corridors (e.g. fishes, aquatic insects and mammals) support this idea, as the genetic variance detected between basins (Banguera-Hinestroza et al. 2002; McGlashan and Hughes 2002; Wishart and Hughes 2003) was consistently higher (>40 %) than that reported in this study. In addition, our IBR model in Paraguay, although highly significant, accounted for 54.44 % of total genetic variation. Thus, the spatial structure of river networks, while important, appears not to be the only factor influencing the degree of connectivity among populations. Other environmental or biological factors would also be important. The levels of genetic differentiation reported in this study may also be an artifact of using a molecular marker with maternal inheritance, particularly in species where migration is biased toward males. In capybaras, the dispersion pattern at the local scale appears to be density dependent; at low densities, dispersal occurs in groups of both sexes, whereas at higher densities, males disperse and females are philopatric (Herrera et al. 2011). Future studies including nuclear markers (e.g. microsatellites) would contribute to a better understanding of environmental and biological factors influencing the genetic structure currently found in this species.

In summary, we investigated the population genetic structure of capybaras throughout part of its geographical distribution in Argentina and evaluated, at different ecological scales, the role of river drainages in shaping the genetic structure. At regional scale, we found that capybaras from Venezuela are genetically different from those in Paraguay and Argentina, suggesting that Venezuela would be considered as a distinct Evolutionarily Significant Unit. At river basin scale, we found a strong signature of genetic structure in Paraguay, but not in Argentinean and Venezuelan river systems, suggesting that an IBR model would explain better the genetic structure currently found in the former country. These differences between river systems have potential conservation and management implications and provide new insights into understanding the factors that may be generating and maintaining genetic structure in freshwater habitats.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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