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*Phylogeography of Cnesterodon
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Argentina: ancient versus recent patterns in
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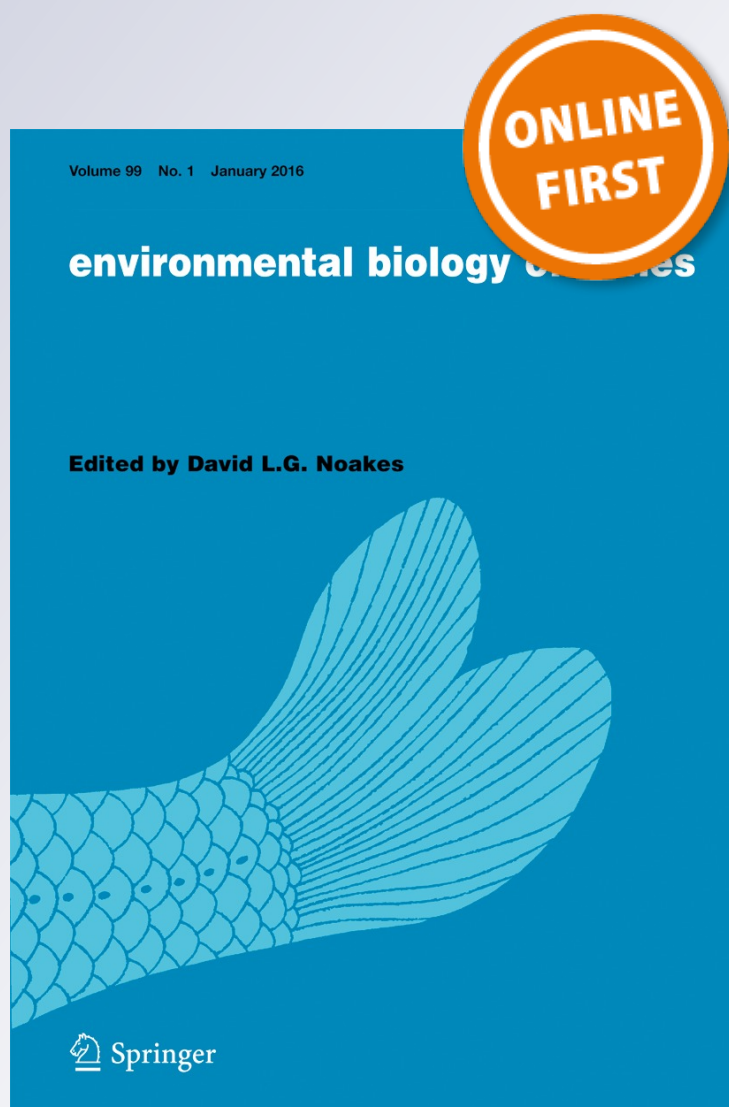
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Phylogeography of *Cnesterodon decemmaculatus* (Cyprinodontiformes: poeciliidae) in Southern Pampas, Argentina: ancient versus recent patterns in freshwater fishes

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Abstract The Southern Pampean Area includes the southernmost limit of the Brazilian subregion and represents an interesting model to study how evolution of the landscape has affected the distribution of freshwater fishes in time and space. The hydrogeographic pattern of the region results from a combination of factors including climatic and sea level changes occurred since the Miocene and extending into the Quaternary. The hydrologic basins of this region are isolated, and have a similar ichthyological composition among them. Here we use a phylogeographic approach to infer how paleoclimatic changes affected the distribution of genetic variation in the populations of freshwater fishes. We analyzed the control region of mtDNA of *Cnesterodon decemmaculatus* and compared it with published data

for the species *Jenynsia multidentata* and *Corydoras paleatus* in the same area. *Cnesterodon decemmaculatus* showed two main haplotypes with scarce divergence and wide geographical distribution within the area. The low divergence found between the *Cnesterodon decemmaculatus* haplotypes and the results obtained in the demographic analyses could suggest that the presence of this species in the area have a recent demographic history. These results are in contrast with the pattern reported for *Jenynsia multidentata* and *Corydoras paleatus*, where data suggest an ancient history in the area. The incongruence in the observed phylogeographical patterns could be due to the different ecological requirements of each species, and to the various responses of them to the environmental conditions resulting from geologic and paleoclimatic changes occurred in the region during the Late Quaternary.

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Keywords Southern Pampean Area · Brazilian subregion · Demographic history · Comparative phylogeography

Introduction

Phylogeography analyses gene genealogies to study the evolution of populations, and allows drawing inferences regarding the sequence of colonization, diversification and extinction events of gene lineages in given areas (Lanteri and Confalonieri 2003). Comparative phylogeography seeks topological congruence in the phylogeographic patterns of species that share their

geographical distributions, and contrasts these patterns with geographic, paleoecological or biogeographic information (Bermingham and Avise 1986; Zink 1991; Joseph et al. 1995; Avise 2000; Arbogast and Kenagy 2001; Zink 2002; Lapointe and Rissler 2005; Carstens and Richards 2007; Garrick et al. 2008; Castoe et al. 2009; Arbeláez-Cortés 2012).

Faced with severe historical perturbations of landscape configuration, several species may present similar demographic responses, thus generating topologically congruent phylogeographic patterns (Avise 2000). A well-known example is the pattern of retraction into peninsular refugia in Europe during the Quaternary advancement of glaciers (Avise 2000; Hewitt 2000). However, under less severe and/or more gradual changes, the demographic responses of given species may be more influenced by their life history traits, thus resulting in topological incongruence among the respective phylogeographic patterns. Accounting for the incongruence presents the challenge of identifying the reason behind the differences found between species (Arbeláez-Cortés 2012). This would allow predicting possible responses of these populations to future environmental perturbations, which may be of special interest in a context of environmental change.

In South America, the extent of glaciation was notably lower than in North America and Europe (Clapperton 1993). Although environmental conditions also changed notoriously in nonglaciated regions (Quattrocchio et al. 2008), their effects on the South American fauna and flora are poorly known (Lessa et al. 2003; Ruzzante et al. 2008).

The Southern Pampean Area (SPA) (Fig. 1) is located in the south of Buenos Aires province, Argentina, and includes the southernmost sector of the faunal Brazilian subregion (see Area and sampling sites in Material and methods). The hydrogeographic pattern of the region is the result of a combination of tectonics, climate and sea level changes since the Miocene (Casciotta et al. 1999). The Pampean Area is a large ecotonal biome between the warm and wet northern Brazilian faunas and the cold and arid western and southern faunas prevailing in Patagonia today (Tonni et al. 1999). The Southern Pampean Area has been characterized by the alternance of arid and cold versus humid and warm conditions (glacial and interglacial periods, respectively) during the Quaternary, and even since the late Miocene (Aguirre and Farinati 1999; Aramayo et al. 2002; Quattrocchio et al. 2008). The flow of many water

courses would have been altered during this fluctuation, with the smaller ones even disappearing during the most arid periods (Nagle 2010). On the other hand, ample floodplains occupied by interconnected lakes (Aramayo et al. 2002) and anastomosing river systems would have arisen during the warmer and more humid periods (Quattrocchio et al. 1998). These processes had strong influence on the persistence and distribution of fish species, markedly altering their population dynamics (Bruno et al. 2015). For example, the fossil record in the area includes *Callichthys sp.*, a typical Brazilian taxon whose current distribution reaches the northern Buenos Aires province, and *Percichthys sp.*, an Austral taxon that currently lives in Patagonia (Casciotta et al. 1999). Fortunately, this situation is reflected in the genetic composition of populations and may be inferred at present.

Two major hypotheses have been proposed and discussed by diverse authors in order to explain the current distribution of the ichthyofauna that inhabit these aquatic environments (Cione and Barla 1997; Casciotta et al. 1999; Bruno et al. 2013, 2015).

Refugia hypothesis: the fishes survived unfavorable climate conditions at a few locations within the SPA, with one or two rivers acting as refugia for relictual populations. As climatic conditions improved during the Holocene, these populations expanded from the refugia.

Connectivity and Colonization Hypothesis: the fishes were eradicated from the SPA during the Pleistocene. When favorable climatic conditions returned, the SPA drainages became connected with each other and with basins outside of SPA (e.g., Colorado and Salado river basins) during periods of flooding, allowing movement of fish among basins. Thus, the freshwater fish ensemble of SPA is of relatively recent establishment.

If these fish populations had persisted within refugia, we would expect to find a high degree of populational differentiation due to the great genetic diversity. Such lineages would have had enough time for genetic differentiation. If, alternatively, these populations only recently colonized the area through connections to external basins, we would expect a low degree of population structure. In the latter case, genetic diversity would be low because after colonization the populations would be genetically similar, given that not enough time would have elapsed for them to become genetically differentiated.

Here, we analyze the phylogeographic pattern of *Cnesterodon decemmaculatus* in the SPA in order to

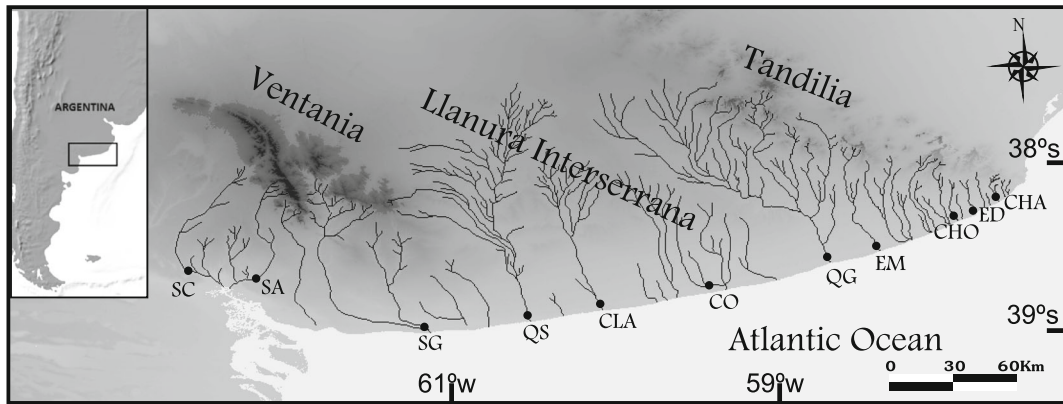


Fig. 1 Southern Pampean Area including sample localities. CHA: Chapadmalal. ED: El Durazno. CHO: Chocorí. EM: El Moro. QG: Quequén Grande. CO: Cortaderas. CLA: Claromecó. QS: Quequén Salado. SG: Sauce Grande. SA: Saladillo. SCH: Sauce Chico

determine whether their populations fit any of these hypotheses. Our results are compared with published data for other two species that also inhabit SPA, *Jenynsia multidentata* and *Corydoras paleatus* (Bruno et al. 2013, 2015) to determine the existence of topological congruence of phylogeographic patterns and understand the processes that governed the biogeographic history of the freshwater fish assemblage in SPA. The studied species are those with widest distribution and large population size within the area, which are necessary conditions for a phylogeographic approach. Likewise, these species differ in their ecological traits, an interesting fact to test the fit of different hypotheses. The goal of this comparison is to provide support to the numerous hypotheses regarding the current distribution of freshwater fishes in SPA, and to correlate this information with the major paleoclimatic events that took place during the Late Quaternary in this region.

Materials and methods

Area and sample sites

The SPA is an extensive plain grassland interrupted in the south by two northwest-southeast-trending sierra ranges (Ventania and Tandilia). The plain between both ranges is called Bonaerian Interserran Plain (Llanura Interserrana Bonaerense; Casciotta et al. 1999). The streams flow south from Tandilia, Ventania and the Llanura Interserrana Bonaerense. The drainages in the area flow to the south in a parallel pattern and are isolated from each other and they are also completely

separated from those major external basins located to the north and south (Casciotta et al. 1999; Bruno et al. 2013, 2015). In spite of this, it is worth noting that the ichthyofaunal composition is similar between the diverse water bodies of the area and also to the external basins located to the north (Casciotta et al. 1999). Despite that at least fourteen species occur in the area no more than six species have been collected together in one environment. Usually, only two to four species occur in each stream (Casciotta et al. 1999).

Sampling was carried out so that a close comparison could be made with previously published works on *J. multidentata* and *C. paleatus* (Bruno et al. 2013, 2015). Sequences of *J. multidentata* and *C. paleatus* used in this study were obtained from Bruno et al. (2015).

Ten sampling sites were chosen in the localities Chapadmalal creek (CHA), Chocorí creek (CHO), El Moro creek (EM), Quequén Grande river (QG), Cortaderas creek (CO), Claromecó creek (CLA), Quequén Salado river (QS), Sauce Grande river (SG), Sauce Chico creek (SCH) and Saladillo creek (SA). For these analyses each locality was considered as a population. Tissue samples were obtained from 94 *C. decemmaculatus* individuals. Sample sizes for each population are showed in Table 2.

A portion of muscle tissue and of the pectoral fin of each specimen were extracted and used for the molecular analyses. The tissue samples were deposited in the tissue collection of the Centro Regional de Estudios Genómicos, Universidad Nacional de La Plata, Argentina (CREG-EM tissue bank). Voucher identification number is indicated in Table 1.

Table 1 Vouchers of tissue samples from *Cnesterodon decemmaculatus*. CREG-EM: Centro Regional de Estudios Genómicos

Locality	Coordinates	Accession number
Chapadmalal	38°8'1.9"S 57°44'40"W	CREG-EM-1816 CREG-EM-1827 CREG-EM-1824 CREG-EM-1830 CREG-EM-1825 CREG-EM-1833 CREG-EM-1826
Chocorí	38°16'11.5"S 58°9'26.8"W	CREG-EM-1057 CREG-EM-1064 CREG-EM-1058 CREG-EM-1065 CREG-EM-1059 CREG-EM-1066 CREG-EM-1061
El Moro	38°25'42.1"S 58°27'44"W	CREG-EM-1767 CREG-EM-1778 CREG-EM-1768 CREG-EM-1779 CREG-EM-1774 CREG-EM-1801 CREG-EM-1775 CREG-EM-1802
Quequén Grande	38°11'7.1"S 59°12'2.4"W	CREG-EM-1560 CREG-EM-1641 CREG-EM-1561 CREG-EM-1644 CREG-EM-1562 CREG-EM-1645 CREG-EM-1563 CREG-EM-1648 CREG-EM-1564 CREG-EM-1649 CREG-EM-1650
Cortaderas	38°38'16.1"S 59°12'2.4"W	CREG-EM-1104 CREG-EM-1110 CREG-EM-1105 CREG-EM-1111 CREG-EM-1107 CREG-EM-1113 CREG-EM-1108 CREG-EM-1114
Claromecó	38°42'13.7"S 60°10'10"W	CREG-EM-1181 CREG-EM-1190 CREG-EM-1186 CREG-EM-1191 CREG-EM-1187 CREG-EM-1193 CREG-EM-1188 CREG-EM-1195 CREG-EM-1189
Quequén Salado	38°44'7.4"S 60°34'20.9"W	CREG-EM-1249 CREG-EM-1297 CREG-EM-1253 CREG-EM-1298 CREG-EM-1254 CREG-EM-1299 CREG-EM-1295 CREG-EM-1301 CREG-EM-1296
Sauce Grande	38°45'44.6"S 61°42'42"W	CREG-EM-1324 CREG-EM-1329 CREG-EM-1325 CREG-EM-1330 CREG-EM-1326 CREG-EM-1334 CREG-EM-1327 CREG-EM-1335
Saladillo	38°33'42.6"S 62°33'3.2"W	CREG-EM-1517 CREG-EM-1525 CREG-EM-1520 CREG-EM-1537 CREG-EM-1521 CREG-EM-1539 CREG-EM-1522 CREG-EM-1541 CREG-EM-1524
Sauce Chico	38°29'49.9"S 62°38'45.4"W	CREG-EM-1438 CREG-EM-1445 CREG-EM-1439 CREG-EM-1447 CREG-EM-1440 CREG-EM-1448 CREG-EM-1442 CREG-EM-1449 CREG-EM-1443 CREG-EM-1450 CREG-EM-1444

Molecular analyses

Total DNA was extracted from the preserved tissue samples following the protocol of [Aljanabi and](#)

[Martínez \(1997\)](#): tissue digestion using proteinase K and lysis-buffer, protein precipitation using C1Na and subsequent precipitation of DNA using absolute ethanol. The resulting DNA was preserved in TE buffer and

refrigerated at -20°C . The polymerase chain reaction (PCR) was used to amplify the control region of mitochondrial DNA. The primers used were K (5'AGCTCAGCGCCAGAGCGCCGGTCTTGTA AAA 3') and G (5'CGTCGGATCCCATCTTCAGTG TTATGCTT 3'; Lee et al. 1995). The amplification reaction was carried out in a total volume of 25 μl , with final concentration 0.25 μl of Taq DNA polymerase, 1.5 μl of Cl_2Mg 3 mM, 0.4 μl of dinucleotids 50 mM, 0.25 μl 10 mM of each primer and 1 μl of DNA as template. The reaction was carried out under the following conditions: initial DNA denaturation at 95°C for four minutes, followed by 30 denaturalization cycles at 95°C for 30 s, annealing at 54°C for 30 s and extension at 72°C for 45 s, followed by a final extension period of five minutes at 72°C . Negative controls were performed in all cases to verify absence of contamination. Amplicons were sequenced using an ABI Prism 3100 Genetic Analyzer (Macrogen Inc., Korea). Chromatograms were edited using the software Proseq (Filatov 2002) and aligned using Clustal-W (Thompson et al. 1994).

Data analyses

Genetic diversity

Genetic variation was assessed using two estimators: nucleotide diversity (π) and haplotype diversity (h). These estimators provide evidence of demographic changes occurred within populations (Avise 2000). Values were calculated both for all samples and for each locality, using the software Arlequin v 3.5 (Excoffier and Lischer 2010).

Phylogenetic relation among control region haplotypes

To evaluate phylogenetic relationships among the mitochondrial DNA haplotypes of *C. decemmaculatus*, we used both evolutionary (Maximum Parsimony and Bayesian Inference) and genetic distances methods (Neighbour Joining). The software *JModelTest* (Posada and Buckley 2004; Posada 2008) with Akaike's information criterion (Akaike 1974) was used to select the model of nucleotide substitution that best fit the data. The resulting model was *TIM3*. The parameters obtained for the nucleotide substitution models were used to optimize the data in the phylogenetic reconstruction.

A Maximum Parsimony (MP) analysis was implemented in PAUP* 4.0b10 (Swofford 2002). A heuristic search with 1000 random stepwise additions and tree bisection and reconnection (TBR) branch swapping was made. Secondly, a Bayesian Inference (BI) analysis was performed using the software Mr. Bayes (Ronquist and Huelsenbeck 2003). Two independent runs of 10×10^6 generations were made and the first 40,000 trees were discarded as burn-in. Lastly, a genetic distance-based analysis was made using the Neighbour-Joining (NJ) algorithm implemented in PAUP* 4.0b10 (Swofford 2002). For both MP and NJ methods, 1000 bootstrap replicates (Felsenstein 1985) were conducted to assess node support values by calculating the 70 % Majority Rule Consensus Tree. The outgroup for tree rooting was the sequence of the species *Poecilia reticulata*, phylogenetically close to *C. decemmaculatus* (GenBank, accession number GQ855708.1).

Because traditional methods of phylogenetic analysis have not been designed to be applied at the intraspecific level (Posada and Crandall 2001), evolutionary relationships between the haplotype variants were also analyzed by constructing a haplotype network. This was done using the Median Joining algorithm (Bandelt et al. 1999, implemented in the software Network 4.5.1 (<http://www.fluxus-engineering.com>)).

Historical demography

Historical demography was assessed using Fu's (1997) and Tajima's (1989) neutrality tests. These statistics evaluate deviation from neutrality (mutation-drift equilibrium) through significant excess of low frequency haplotypes. Significant and negative values are expected in populations that have undergone recent expansion when analyzing a neutral molecular marker such as the control region. These analyses were made both globally for all samples, as well as for each locality. Significance of these tests was assessed using 1000 replicates of coalescent simulations in Arlequin v 3.5 (Excoffier and Lischer 2010).

In addition, mismatch distribution analysis were made for the different populations and for the total samples using the software Arlequin v 3.5 (Excoffier and Lischer 2010). An erratic or multimodal curve is expected in populations that have remained in demographic equilibrium; in contrast, populations that have undergone recent demographic expansion are expected to show an unimodal curve (Rogers and Harpending

1992). We employed the sum of squared deviations (SSD) statistic and the raggedness index (Rg) defined by Harpending (1994) to test the goodness of fit of the observed mismatch distribution to that expected under the sudden expansion model. These analyses were made using the software Arlequin v 3.5 (Excoffier and Lischer 2010).

Genetic structure

A description of the genetic structure of the populations was obtained from an Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) using Arlequin v 3.5 (Excoffier and Lischer 2010). This analysis was made using two approaches. For the first one, three hierarchical population groupings were established on the basis of the geological province where each water course is located. Three groups were considered: an Eastern or Tandilia group, a Central or Bonaerian Intersierran Plain group, and a Western or Ventania group. For the second approach, all the populations were pooled in a single group. Alternatively, pairwise Φ_{ST} were calculated using the same software as a measurement of genetic distances that allow establishing genetic differentiation between populations. The statistical significance of these indicators was assessed using 1000 permutations. Sequential Bonferroni procedure was used to maintain type I probability error at $\alpha = 0.05$ (Rice 1989).

In order to assess the existence of migration-genetic drift equilibrium at regional scale, we used the approach proposed by Hutchison and Templeton (1999). Under equilibrium conditions, genetic distances are expected to increase with the increase of geographic distance between populations, in a pattern known as isolation by distance (Wright 1942). The absence or weakness of this relationship would indicate lack of regional equilibrium. When gene flow is stronger than genetic drift, the expected pattern would reflect panmixia, with no relationship between the genetic and geographic distances and little variation in the estimations of genetic divergence. On the other hand, when genetic drift is strong compared to gene flow, the expected pattern includes high population divergence irrespective of geographic distances between populations. The relationship between Euclidean geographic (Km) and genetic distances (Φ_{ST}) was evaluated by means of a Mantel test

(Mantel 1967). Statistical significance was assessed using 1000 permutations.

Results

Genetic diversity

An 828 base-pair (bp) fragment from the mitochondrial DNA control region was obtained from 94 individuals. Eleven polymorphic sites were identified, of which four were transitions and seven indels, which were considered for the diverse analyses as a fifth state. Twelve haplotypes were defined on the basis of these polymorphic sites (GenBank acc number: KT 183387-KT183398).

The populations of *C. decemmaculatus* presented two widely distributed haplotypes: haplotype 1, present in seven populations and the only one in the Chapadmalal creek population, and haplotype 4, which occurred in six populations (Table 2).

The analysis of genetic diversity for all samples showed low nucleotide diversity ($\pi = 0.0038 \pm 0.002$) and high haplotype diversity ($h = 0.83 \pm 0.019$); this pattern agrees with the expectations for a process of sudden population expansion. Genetic diversity values for each population are shown in Table 2.

Table 2 Genetic diversity of *Cnesterodon decemmaculatus* in the Southern Pampean Area. N = number of individuals

Locality	N	NH	S	$h \pm SD$	$\pi \pm SD$
CHA	7	1	-	-	-
CHO	7	1	-	-	-
EM	10	5	7	0.844 ± 0.079	0.003 ± 0.002
QG	12	2	1	0.530 ± 0.076	0.0006 ± 0.0006
CO	8	2	1	0.428 ± 0.168	0.0005 ± 0.0006
CLA	9	3	6	0.416 ± 0.190	0.002 ± 0.001
QS	9	2	1	0.555 ± 0.090	0.0006 ± 0.0007
SG	10	4	6	0.533 ± 0.180	0.002 ± 0.001
SA	9	5	6	0.888 ± 0.071	0.0037 ± 0.002
SCH	13	2	5	0.153 ± 0.126	0.0009 ± 0.0008
Global	94	12	11	0.835 ± 0.019	0.0038 ± 0.002

S = number of polymorphic sites. NH = number of haplotypes. $h \pm SD$ = haplotype diversity \pm standard deviation. $\pi \pm SD$ = nucleotide diversity \pm standard deviation

Phylogenetic relation among control region haplotypes

The patterns of phylogenetic relationships among haplotypes obtained by different methods used here were highly concordant. Geographic structure is not clear in the ingroup sequences (NJ, MP and BI analysis). The phylogenetic reconstruction methods (Fig. 2) showed two scarcely divergent clades (only four mutational steps between them, see haplotype network, Fig. 3) with moderate bootstrap support. Most phylogenetic relationships between haplotypes are shown as polytomies. In general, the geographic structure among localities is weak, and almost all populations were recovered as polyphyletic units.

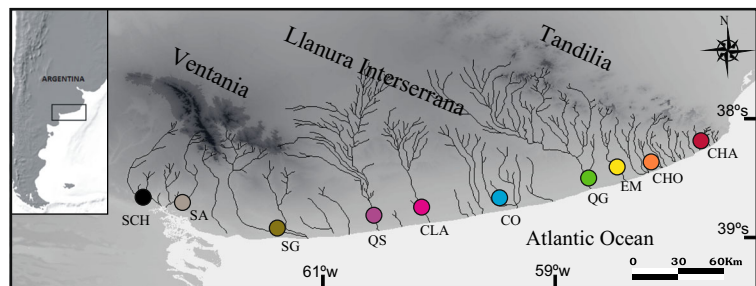
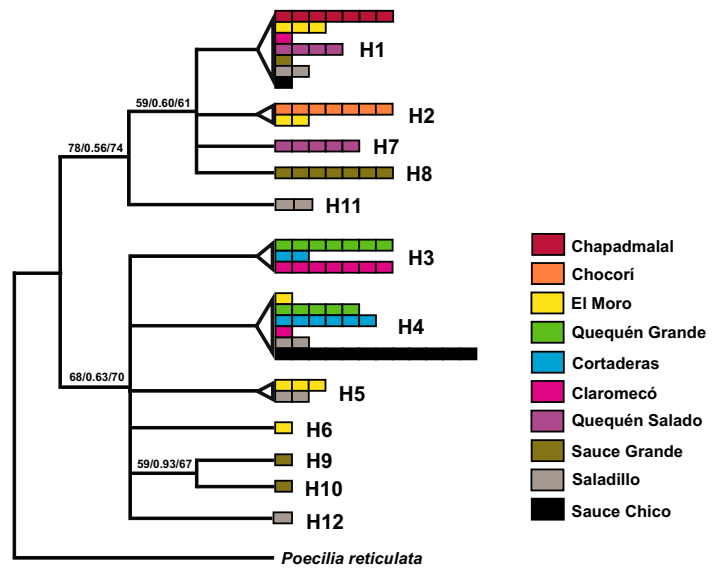
The topology of the haplotype network (Fig. 3), like the methods of phylogenetic reconstruction, shows weak geographic structure among the populations. Again, the two clades recovered in the phylogenetic analyses are present, separated by only four mutational steps. Within each clade, the

haplotypes present one or a few nucleotide substitutions resulting in a star-like topology of the haplotype network. Such a topology would be consistent with a pattern of demographic expansion.

Historical demography

The mismatch distribution for the pooled samples showed a bimodal curve, with both modes at low values of nucleotide differences (Fig. 4). The first mode is located around one nucleotide difference and indicates all the paired haplotype comparisons within the same clade. The other mode, located at 5–6 nucleotide differences, corresponds to all the paired haplotype comparisons between clades. The goodness of fit tests yielded low, although not significant, values (Table 3), indicating that the total samples do not deviate from the pattern expected under a process of population expansion. Fu's and Tajima's neutrality tests were negative although non-significant for the pooled samples

Fig. 2 Phylogenetic strict consensus tree obtained by Maximum Parsimony based on mitochondrial DNA control region of *Cnesterodon decemmaculatus*. Numbers on the nodes represents the Bootstrap values. The length branches are proportional to mutations per site. H1-H12: Haplotypes. Each square corresponds to one individual



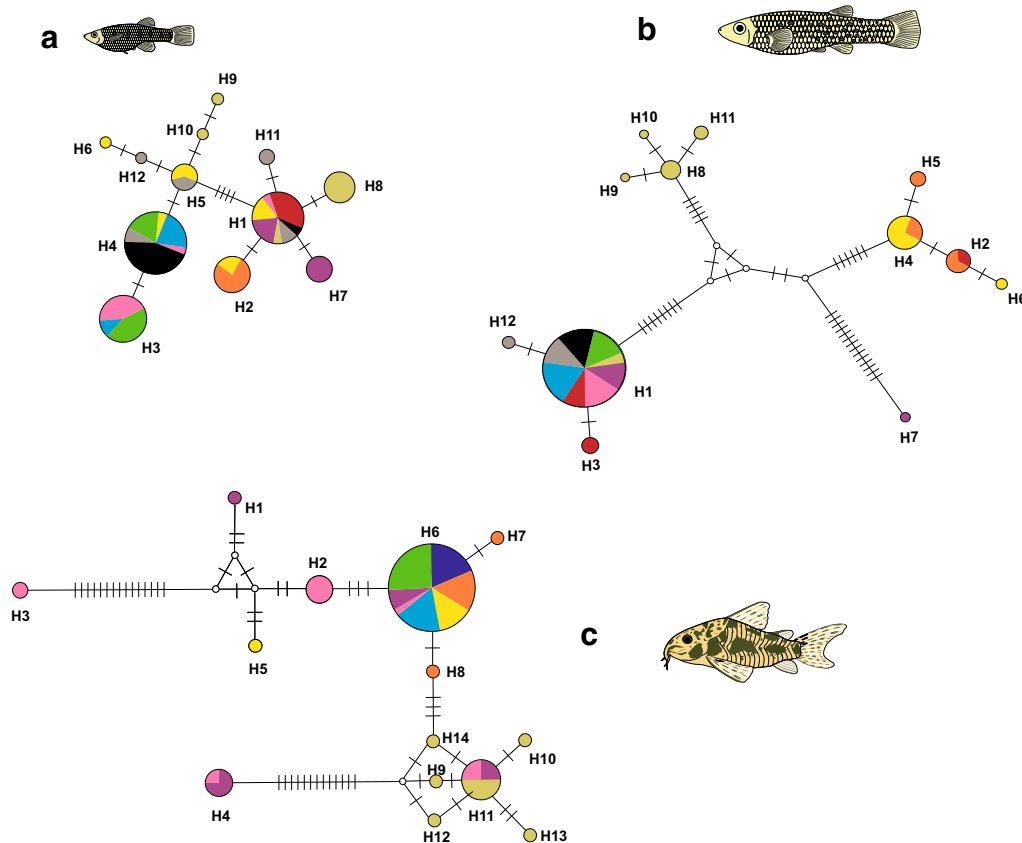
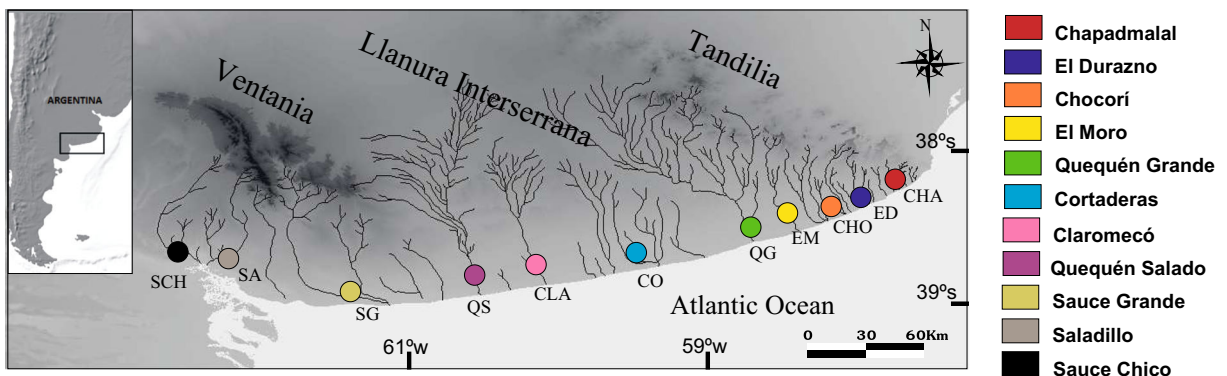


Fig. 3 Median-Joining haplotype network for a: *Cnesterodon decemmaculatus*. b: *Jenynsia multidentata* (Bruno et al. 2015). c: *Corydoras paleatus* (Bruno et al. 2015). Circle sizes are

proportional to haplotype frequencies. The crossed marks are nucleotide substitutions inferred in that branch. White circles represent hypothetical haplotypes. H1-H14: haplotypes

(Table 3). The values for each population are shown in Table 3. Regarding the mismatch distribution analyses by locality (data not shown), those localities with haplotypes that were only part of one of the clades (Quequén Grande, Cortaderas and Quequén Salado, Fig. 3) presented unimodal

curves with very low values of nucleotide differences, a pattern consistent with processes of population expansion. Those populations with haplotypes belonging to both clades (El Moro, Claromecó, Sauce Grande, Saladillo and Sauce Chico) showed two modes in the mismatch

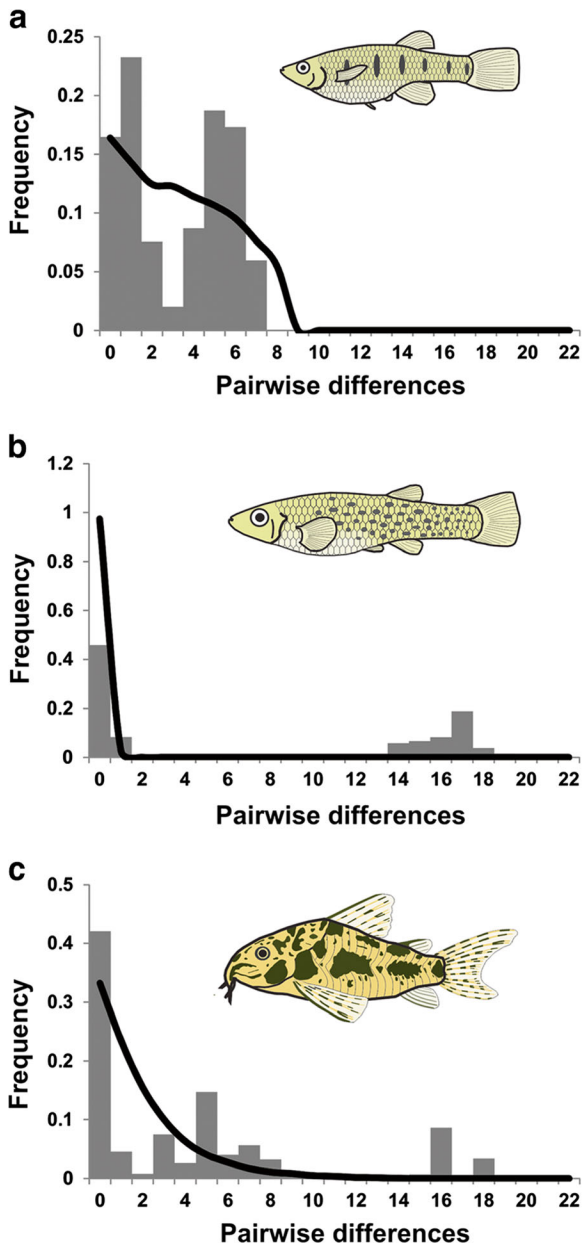


Fig. 4 Mismatch distribution of mitochondrial DNA control region for **a:** *Cnesterodon decemmaculatus*. **b:** *Jenynsia multidentata* (Bruno et al. 2015). **c:** *Corydoras paleatus* (Bruno et al. 2015). Bars: observed distribution. Line: expected distribution under a sudden population expansion model

distribution analysis; although in the populations from Claromecó, Sauce Grande, Saladillo and Sauce Chico the frequency of the second mode is much lower than that of the first one. The goodness of fit tests were non-significant for most populations except the one from Sauce Grande,

which presented high and significant value of the sum of squared deviations, thus corroborating that it does not fit a pattern of population expansion.

Genetic structure

The genetic differentiation between localities was highly significant ($\Phi_{ST} = 0.64$ $P < 0.000$). Almost all the paired comparisons between populations also showed significant values for Φ_{ST} (Table 4), even for very near localities (Chapadmalal versus Chocorí, with a value of 1). These two populations were also the ones with greatest divergence with respect to the rest (see Table 4). On the other hand, the low and non-significant values of paired Φ_{ST} for the populations Quequén Grande, Cortaderas and Claromecó indicate a lack of population structure in the central region of the sampling area. At regional level, the populations from El Moro and Saladillo were the ones with least divergence in the paired comparisons. The Mantel test resulted in a low, non-significant correlation coefficient (-0.103 $P = 0.70$) showing that there was no correlation between genetic and geographic distance (Fig. 5).

The results from the AMOVA showed that most of the molecular variance occurs between populations (59.94 %). In addition, grouping the localities on the basis of geologic provinces does not capture a significant portion of the genetic variation (4.06 %). Lastly, the percent variation within populations was 36 %.

Discussion

In this study we analyzed mitochondrial DNA sequences of *C. decemmaculatus* in a phylogeographic context to infer their historical demography in the Southern Pampean Area. We compared our data with published data of two co-distributed species, *Jenynsia multidentata* and *Corydoras paleatus* (Bruno et al. 2013, 2015), in order to evaluate topological congruence in the phylogeographic patterns. Strongly concordant phylogeographic patterns permit to infer a correlated demographic in response to the same paleoenvironmental change. On the other hand, the topological incongruence between species allows us to infer which life history traits were related to the dissimilar demographic responses. The understanding of effects of environmental changes on the demographic responses of the species in relation to their life history

Table 3 Historical demography of *Cnesterodon decemmaculatus* in the Southern Pampean Area. D_T : Tajima's D. $F_{S_{FU}}$: Fu's F. SSD: Sum of square deviations. Rg: Raggedness Index. P: probability <0.05. -: Not estimated

Locality	D_T	P (D_T sim < obs)	$F_{S_{FU}}$	P (Fssim < obs)	SSD	SSD-P	rg	rg- P
Chapadmalal	-	-	-	-	-	-	-	-
Chocorí	-	-	-	-	-	-	-	-
El Moro	-1.400	0.082	0.390	0.576	0.042	0.39	0.093	0.527
Quequén Grande	1.381	0.956	1.152	0.662	0.029	0.160	0.284	0.166
Cortaderas	0.333	0.792	0.536	0.445	0.010	0.383	0.204	0.575
Claromecó	0.156	0.745	1.152	0.741	0.049	0.190	0.255	0.607
Quequén Salado	0.00	1.000	1.015	0.642	0.037	0.249	0.320	0.190
Sauce Grande	-1.111	0.196	0.387	0.574	0.372	0.000	0.164	1.000
Saladillo	0.986	0.908	0.126	0.503	0.072	0.170	0.160	0.381
Sauce Chico	0.000	1.000	1.907	0.779	0.034	0.069	0.763	0.748
Global	-5.558	0.328	-0.118	0.495	0.036	0.166	0.063	0.339

traits is of fundamental importance in a context of global climate change.

Phylogeography and historical demography of *Cnesterodon decemmaculatus* in the SPA

The low divergence between *C. decemmaculatus* haplotypes, together with the genetic diversity values suggest recent colonization of these haplotypes within the SPA. As pointed out by Conroy and Cook (2000), the low divergence among haplotypes could result from recent radiation of the species within a given area.

The mismatch distribution analysis for all the *C. decemmaculatus* populations could be interpreted as secondary contact between divergent populations

(Frankham et al. 2004), or it could be attributed to a recent demographic expansion from two closed related haplotypes (Jalil et al. 2008). Some authors have even interpreted such a pattern as a demographically stable population (Viñas et al. 2004). Nevertheless, none of the analyses made in this work supports a scenario of demographic equilibrium for this species. Given the topology obtained in the haplotype network and the genetic diversity values, the pattern observed in the analysis of mismatch distribution could suggest a single event of demographic expansion, from two closely related haplotypes.

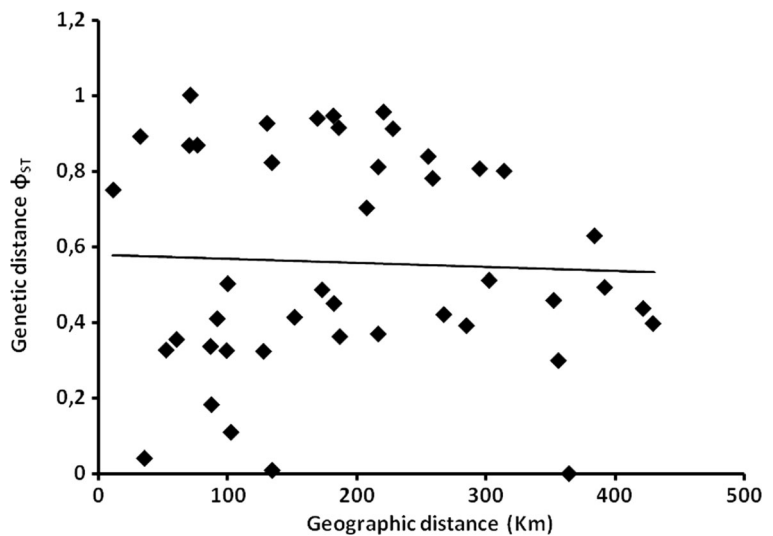
According to Hutchison and Templeton (1999), the patterns expected for individuals from regions that have not reached equilibrium may be affected both by the

Table 4 Pairwise Φ_{ST} of *Cnesterodon decemmaculatus* in the Souther Pampean Area. * = P value significant at 0.05 level. CHA: Chapadmalal. CHO: Chocorí. EM: El Moro. QG: Quequén

Grande. CO: Cortaderas. CLA: Claromecó. QS: Quequén Salado. SG: Sauce Grande. SA: Saladillo. SCH: Sauce Chico

	CHA	CHO	EM	QG	CO	CLA	QS	SG	SA	SCH
CHA	0									
CHO	1.00*	0								
EM	0.32	0.40	0							
QG	0.93*	0.92*	0.50*	0						
CO	0.95*	0.94*	0.41	0.10	0					
CLA	0.83*	0.81*	0.36	0.008	0.18	0				
QS	0.45	0.79*	0.39*	0.91*	0.91*	0.82*	0			
SG	0.43*	0.62*	0.29	0.80*	0.78*	0.70*	0.48*	0		
SA	0.40	0.50	0.08	0.51*	0.42*	0.36	0.45*	0.33	0	
SCH	0.89*	0.86*	0.35*	0.32	0.04	0.32*	0.86*	0.74*	0.36	0

Fig. 5 Relationship between geographic and genetic distances for *Cnesterodon decemmaculatus*



time elapsed since the region was colonized and by the degree of dispersal within said region. In a hypothetical recently colonized region, the diverse populations established would probably be genetically similar to one another, immediately after colonization. This reflects the recent shared evolutionary history of the new populations, with little or no relationship between genetic and geographic distance (Case II, Hutchison and Templeton 1999). It is probable that the lack of equilibrium between genetic drift and gene flow may be due to the relatively recent history of *C. decemmaculatus* in the area. Ultimately, these analyses suggest a history of recent colonization of *C. decemmaculatus* populations within SPA. In addition, the low values of genetic diversity found in certain populations suggest founder effect of these populations in those water courses (e.g. Llanura Interserrana Bonaerense). Genetic diversity values are quite homogeneous, except in water courses located near the extremes. In this context, colonization within the area may have taken place from the extremes toward the central area of the system, given that the genetic diversity decreases in that direction. Taking into account that the SPA represents the southern limit for the natural distribution of *C. decemmaculatus*, this area would have been colonized from those basins located in the northwest.

Three species, two phylogeographic patterns

Contrary to *C. decemmaculatus*, both *J. multidentata* and *C. paleatus* showed high genetic diversity, with at

least three haplogroups greatly divergent in the SPA. Their haplotype networks presented a more complex topology and mismatch distribution analysis showed bimodal curves with, at least, one of these modes in high values of pairwise differences. These results as a whole suggest a scenario of demographic stability for *J. multidentata* and *C. paleatus* in the SPA during Late Quaternary. Moreover, restricted distribution of some haplogroups to few water courses suggest that these strongly divergent lineages would have persisted in environmental refugia during the unfavorable climatic conditions. Thereby, both species seems to fit in the Refugia hypothesis.

However, in both *J. multidentata* and *C. paleatus*, the difference in the geographic distribution of haplogroups is highly remarkable. Only one haplogroup is widely distributed for de SPA while the rest are restricted to a few water courses. The pattern of geographic distribution of widely distributed haplogroups in *J. multidentata* and *C. paleatus* resembles the phylogeographic pattern of *C. decemmaculatus*, suggesting also a recent colonization of SPA for these lineages. Therefore, as in *C. decemmaculatus*, in *J. multidentata* and *C. paleatus* at least one lineage seems to fit into the Connectivity and Colonization Hypothesis. Thus, a complex biogeographic history is suggested for *J. multidentata* and *C. paleatus* in the SPA, including the persistence of some lineages in relictual habitats and colonization events from north external basins.

To sum up, this comparative phylogeographic approach allowed us to identify two phylogeographic

patterns in the three species analyzed. One of these patterns, present in *C. decemmaculatus*, indicates a recent colonization of the region. The other one, present in *J. multidentata* and *C. paleatus*, indicates an ancient history of the species in the region, but with a recent and massive immigration of individuals. Given the similarity between the phylogeographic pattern of *C. decemmaculatus* and the pattern of the widely distributed haplogroups of *J. multidentata* and *C. paleatus*, a simultaneous event of ingression of these lineages is the most parsimonious explanation. It is worth remarking that colonization of the area by these lineages does not necessarily entail a sudden event. Colonization possibly occurred gradually, as climate conditions favorable for the arrival and survival of Brazilian freshwater fishes occurred in the area.

Evolution of SPA freshwater fishes assemblage

The glacial–interglacial cycles of Quaternary are thought to have played an important role in shaping the distribution of genetic diversity among current populations, even in the warm temperate zone, where the land was not covered with ice sheets (Aoki et al. 2008). In the central plains of Argentina, the glacial ages were correlated with an increase in extension of the arid environments (Iriondo 1999). Middle and Late Pleistocene paleoclimatic indicators in the Pampas suggest cold and dry climatic conditions (Deschamps and Toni 1992; Iriondo and García 1993; Quattrocchio et al. 2008). At about 10,000–9000 BP an important rise in temperature occurred in South America (Heusser et al. 1981). Fossil records suggest an increase in temperature and a decrease of arid conditions at the Pleistocene–Holocene transition in SPA (Borromei 1995; Grill 1997; Quattrocchio and Borromei 1998). An expansion of Brazilian fauna towards the south-west suggests a continuous increase in temperature and humidity during the Middle Holocene (Tonni 1990; Deschamps and Toni 1992). Consequently, the phylogeographic pattern of Brazilian species in central Argentina indicates a population expansion process related to this Holocene climate improvement (Poljak et al. 2010; Mora et al. 2013). On the contrary, the Patagonian-Monte lineages, related to semi-arid and arid environments, show evidence of population decline at the same time (Mapelli et al. 2012). Thereby, taking into account the information about the paleoenvironmental changes and phylogeographic pattern of others co-distributed species, we

suppose that the colonization of SPA for *C. decemmaculatus* and the widely distributed lineages of *J. multidentata* and *C. paleatus* are related to climate improvement of Holocene. The geologic and paleoclimatic characterization of the SPA during much of the Holocene includes interconnected lakes (Aramayo et al. 2002). These connected systems could have been the dispersal route by means of which a few populations underwent population expansion, colonizing most of the area. On the other hand, the geographically restricted lineages of *J. multidentata* and *C. paleatus* withstood the unfavorable climate conditions of Late Pleistocene at some locations within the SPA.

Hence, the evidence suggests the presence of lineages that have remained in the area under adverse climatic conditions in putative environmental refugia during the middle and late Pleistocene. Later on, and due to the more favorable climate, some lineages may have undergone geographic expansion with the consequent colonization of the area, as result of the formation of a modern hydrographic system.

It is interesting to consider the particular responses of species to paleoenvironmental changes of Late Quaternary, which ultimately determined their persistence and continuity within the area. Although Brazilian ichthyofauna occupies the east-central and northern Argentina, its distribution in the past appears to have been much larger and have spreading naturally to Patagonia, where environmental conditions were more favorable (Menni et al. 1996). At present, the ability of colonization of Patagonian environments by Brazilian species would be related to their tolerance to values of minimum temperature and high salinity, as the case of *C. paleatus* and *J. multidentata* which possess a southern distribution compared with *C. decemmaculatus* (Baigún et al. 2002). In this sense, it is noteworthy that even though the three species are widely distributed in the study area, they differ in some characteristics that could be significant with regards to their demographic histories. In particular, *J. multidentata* is a euryhaline species that is favorably affected by salinity (Mai et al. 2005) whereas *C. paleatus* has the higher tolerance to low temperatures, and even withstands very low concentrations of dissolved oxygen (Baigún et al. 2002). In contrast, *C. decemmaculatus* presents the lowest tolerance to low temperatures (Baigún et al. 2002). At present both *J. multidentata* and *C. paleatus* inhabit Patagonian environments where ecological conditions could be similar to those occurring during arid periods in the Late

Pleistocene. These characteristics may have been favorable for *J. multidentata* and *C. paleatus* but not so much for *C. decemmaculatus*. Due to the conditions of this area, it is probable that the SPA was not favorable for the persistence of the latter species until the climate improved in the Holocene. In fact, Baigún et al. (2002) have proposed that *J. multidentata* and *C. paleatus* could colonize northern Patagonia due to their tolerance for cold temperatures and saline conditions.

To summarize the previous discussion, codistributed species not always respond in the same manner to paleoclimatic and geological changes, showing incongruence in phylogeographic patterns. In this sense, the various ecological requirements of these species appear to have influenced the observed inconsistency. The analysis in this paper shows the need to increase the number of species under study when comparing the historical processes that have affected codistributed organisms. In short, the geologic and paleoclimatic history of the region has considerably influenced the composition of freshwater fishes assemblage of SPA, enabling colonization of the area and, in some cases, promoting population differentiation.

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