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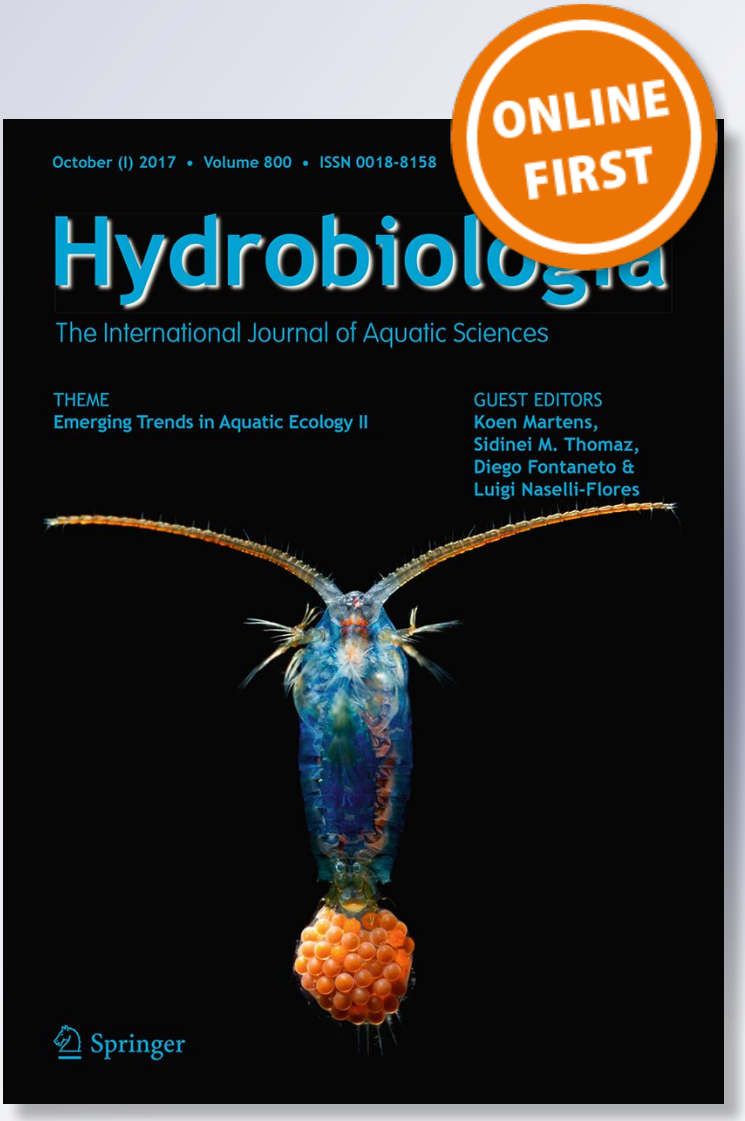
Hydrobiologia
The International Journal of Aquatic Sciences

ISSN 0018-8158

Hydrobiologia
DOI 10.1007/s10750-018-3643-7



Springer

Hydrobiologia
https://doi.org/10.1007/s10750-018-3643-7

PRIMARY RESEARCH PAPER

Species assignment and population genetic studies of Gran Paraná pejerrey (*Odontesthes* sp., Atheriniformes, Atherinopsidae) from La Plata Basin in South America

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Received: 21 November 2017 / Revised: 7 May 2018 / Accepted: 8 May 2018

Abstract Pejerrey is the common name given to *Odontesthes* species from South America. Every year, individuals of pejerrey called "Gran (Big) Paraná" appear during the low-temperature season at rivers in the southern section of the La Plata Basin. Gran Paraná fishes are highly appreciated for fishing, and present some biological features different from other well-characterized *Odontesthes* fish, such as bigger size and migratory behavior. Regulations for the management of pejerrey fisheries within La Plata Basin have not been implemented yet. The aims of the present work were to characterize the Gran Paraná pejerrey species by molecular methods and carry out the first population genetic study of Gran Parana pejerrey from the La Plata Basin. All Gran Paraná specimens were classified as *O. bonariensis*, based on both morphology and microsatellite *loci*. Genetic differentiation was observed between Gran Paraná pejerrey and *O. bonariensis* pejerrey sampled at Chascomús lagoon. In addition, temporal genetic differentiation was observed, suggesting the presence of different cohorts migrating along the basin. The knowledge of population dynamics and differentiation will contribute to figure out fisheries models and to design protective areas for this species under commercial exploitation.

Keywords Molecular markers · River · Estuary · Genetics · Sustainability · Fish · *Odontesthes bonariensis*

Handling editor: Christian Sturmbauer

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10750-018-3643-7) contains supplementary material, which is available to authorized users.

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Published online: 17 May 2018

Introduction

Knowledge about the genetic component of biodiversity is the baseline for conservation efforts and legislation. Estuaries are dynamic ecosystems with highly variable salinities and temperatures, inhabited by a poorly described diversity of fish populations, without geographic barriers. The Rio de la Plata estuary is on the South Atlantic coast (35–36°S) and is part of the La Plata Basin, the second largest basin in South America. The Rio de la Plata estuary receives freshwater from the Paraná River and the Uruguay River, and pours its waters into the Argentinean Sea (Fig. 1). The Paraná River, in the lowest section, becomes a flood plain called "Delta" between 32°04′°S–60°39′W and 34°22′S–58°27′W that covers over 17,500 km^2 on the final 300 km of the river. This Delta is a completely freshwater environment (Baigún et al., 2008) and is considered a wetland macro system (Neiff & Malvárez, 2004) in which the Paraná River splits into several streams, creating a network of islands and intermittent interfluvial (flood pulses) wetlands, shallow lakes, and lagoons. The Paraná Delta is a migratory exchange route which facilitates the penetration of temperate marine fish from the Atlantic Ocean into a freshwater region, and where potamodromous fish species reach the Río de la Plata estuary (Kandus, 2006).

Every year, fishermen report the appearance of "Gran (Big) Paraná" pejerrey in the Lower Paraná and Uruguay Rivers and the inner Río de la Plata estuary, during the low-temperature season (i.e., from April to September) and their "disappearance" during the spring–summer seasons (i.e., from October to March).

Pejerrey is the common name that includes several fish species of the order Atheriniformes (FISHBASE ver. 06, 2016). *Odontesthes* (Atheriniformes: Atherinopsidae) is a monophyletic group (Campanella et al., 2015) that encompasses 19 recognized species distributed across marine, coastal, estuarine, and freshwater environments of temperate South America (Dyer, 1993, 2006). The name "Gran Paraná" is due to the bigger size of these fishes (up to 50.0 cm of Standard length (SL) and 1 kg in weight) compared to those usually found at lagoon environments (18.16 cm mean SL; Mancini & Grosman, 2001). Gran Paraná pejerrey annual appearance is first reported at the Río de la Plata estuary (35°S), and later on at lower latitudes in Paraná and Uruguay Rivers (31°S) (Fig. 1). Ripe males and females are found at Paraná Delta lagoons and streams during the low-temperature season, which suggests spawning and reproduction following upstream migration. Pejerrey commercial and sport fishing takes place during this period. Gran Paraná pejerrey distribution during the spring–summer seasons is unknown because of the lack of representative capture records. However, the presence of this pejerrey in the outer section of the Río de la Plata estuary and Argentinean Sea has been suggested by Sr:Ca analyses in otoliths (Avigliano & Volpedo, 2013).

Fig. 1 Sampling localities. Pejerrey *O. bonariensis* and Gran Paraná´s sampling localities are shown in black at the map. PR, Paraná River; UR, Uruguay River; iRLP and cRLP, Rio de la Plata estuary: inner and central zone; CHL, Chascomús Lagoon; Oa, *O. argentinensis*, Oi, *O. incisa*; and Os, *O. smitii* samples were taken from Atlantic coast, and sampling localities are shown in white

"Gran Paraná" pejerrey is highly appreciated for sport, and commercial fishing and is included among the most exported freshwater fish species from the La Plata Basin (5th place, 351 Tn in 2012; Iwaskiw & Lacoste, 2011). However, regulations for the management of pejerrey fisheries inside the basin have not been implemented yet.

Six pejerrey species have been reported at the South of the La Plata Basin, including the estuary: *Odontesthes perugiae* Evermann & Kendall, 1906, and *Odontesthes orientalis* de Buen, 1950, both belonging to the *O. perugiae* species complex that occurs in freshwater environments (Beheregaray et al., 2002; Cuello et al., 2010; Wingert et al., 2017); *Odontesthes humensis* de Buen, 1953, and *Odontesthes retropinnis,* (de Buen, 1953), both poorly studied species described at the Uruguay River (Bemvenuti, 2006); *Odontesthes bonariensis* (Valenciennes, 1835) an euryhaline species described at freshwater lagoons and rivers at the temperate region; and *Odontesthes argentinensis* (Valenciennes, 1835) a western Atlantic coastal marine and estuarine species, distributed from the State of Sao Paulo (25°S), Brazil, to south of Chubut (45°S), Argentina. According to data on species distribution, *O. argentinensis* and *O. bonariensis* coexist in the brackish water of the Río de la Plata estuary near the Argentinean Sea. Intermediate forms, since a morphometric and meristic point of view, can be found in environments where both species coexist, as was reported at Mar Chiquita coastal lagoon

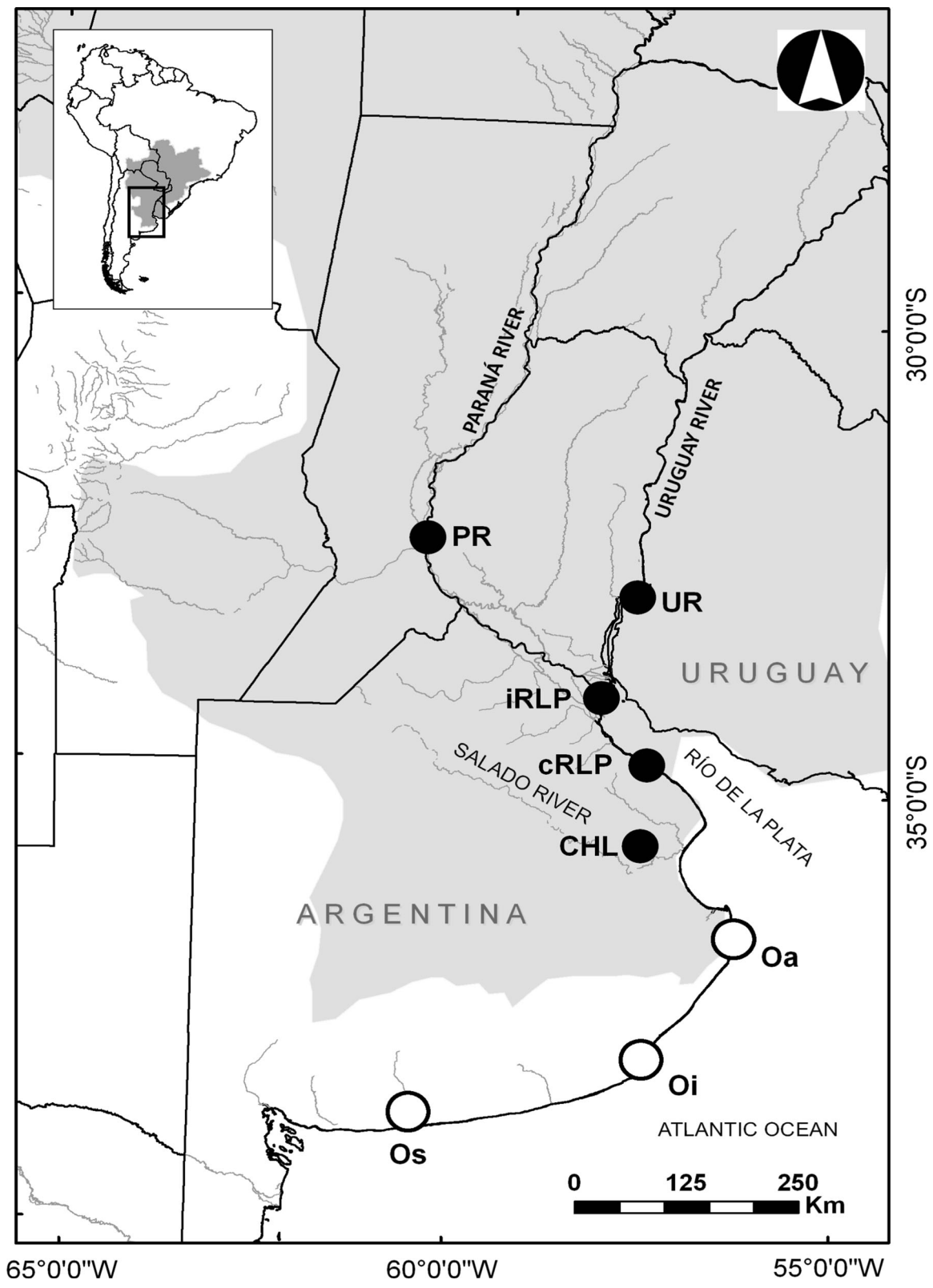

Table 1 Summary of collection sites, sample sizes, and geographic coordinates

Group	Collection site	Map code	Geographic coordinates	Year of collection	N
Gran Paraná pejerrey	Paraná River	PR	− 32.952745, − 60.610641	2011	49
				2013	12
	Uruguay River	UR	− 33.111153, − 58.377609	2011	16
	Rio de la Plata estuary	iRLP	− 34.290231, − 58.367794	2011	5
				2012	43
				2013	15
		cRLP	− 35.432886, − 57.081772	2011	13
				2012	23
O. bonariensis	Chascomús Lagoon	CHL	− 35.432886, − 57.081772	2012	20
O. argentinensis	Atlantic Coast	Oa	− 35.432886, − 57.081772	2012	42
O. incisa		Oi	− 36.543001, − 56.671672	2012	14
O. smitii		Os	− 38.600546, − 58.718499	2012	14

All samples were collected between 2011 and 2013

N number of sampled specimens

(González-Castro et al., 2016). In addition, interbreeding between both species has been mentioned by Tejedor (2001).

García et al. (2014) pointed out promiscuous and recent contact between *Odontesthes* species from southern South America with high gene flow that blurs species boundaries and hinders the taxonomic analysis and species delimitation of this fish group. In addition, several studies pointed out the capacity of pejerrey to inhabit coastal lagoons and estuaries, highlighting their potential for developing locally adapted populations with different genotypic and phenotypic characteristics (Beheregaray & Sunnucks, 2001; Bloom et al., 2013; González-Castro et al., 2016).

The aims of the present work were to characterize the Gran Paraná pejerrey population by molecular methods and carry out the first population genetic study of Gran Parana pejerrey from the La Plata Basin, in order to improve the knowledge about this group and suggest management actions for the sustainable conservation of this important resource.

Materials and methods

Sampling and taxonomic identification of *Odontesthes* specimens

A total of 266 adult specimens belonging to *Odontesthes* species were collected between 2011 and 2013 (Table 1), and classified following the morphological criteria proposed by Dyer (2006). Among them, 176 individuals of Gran Paraná (GP) pejerrey from Paraná River (PR), Uruguay River (UR), and Río de la Plata estuary (RLP) were collected during winter (June–September). Two sampling sites were located respectively at the inner RLP (iRLP) and the central RLP (cRLP) estuary (Jaureguizar et al., 2004). In addition, 20 individuals of *O. bonariensis* (Ob) from Chascomús lagoon (CHL) and 42 *O. argentinensis* (Oa) from the Atlantic coast were included in the analysis for comparison; and 14 *Odontesthes smitii* (Os) (Lahille, 1929) and 14 *Odontesthes incisa* (Oi) (Jenyns, 1841) from the Atlantic coast were included in the analysis to be used as outgroups (Fig. 1). *O. bonariensis* from CHL were considered as a reference population (Colautti et al., 2015), and *O. argentinensis* was included because it is present in the outer zone of Río de la Plata estuary. In spite of having morphologic and meristic characteristics that allow to differentiate both species, intermediate forms between *O. argentinensis and O. bonariensis* have been previously described when both species share the same habitat (González-Castro et al., 2016). Samples from *O. perugiae, O. orientalis, O. humensis,* and *O. retropinnis* were not included in the analysis because these four species that are also present in the basin can be undoubtedly distinguished from *O. bonariensis* using morphologic and meristic characteristics (Bemvenuti, 2002; Dyer, 2006; Wingert et al., 2017).

Tissues of voucher specimens were deposited in the collection of the "Laboratorio de Biotecnología Acuática" from the "Acuario del Río Paraná" Centre, Rosario, Santa Fe, Argentina. All sampling procedures and methods were in accordance with the Bioethics Commission for the Management, and the Use of Laboratory Animals from Facultad de Ciencias Bioquímicas y Farmacéuticas—Universidad Nacional de Rosario (resolution No. 302/2013).

DNA purification

Fin clips were stored in 95% ethanol at 4°C until DNA extraction. Total DNA was isolated from samples using sodium chloride protein precipitation followed by ethanol precipitation according to the modified method from Barrero (2008) or Chelex resin (BioRad, USA) procedure (Walsh et al., 1991). DNA samples were conserved at − 20°C until their further use.

Genetic characterization of pejerrey species using microsatellite markers

Nine polymorphic microsatellite loci developed for *O. bonariensis* (Villanova et al., 2013) were amplified and checked for its cross-amplification in all collected *O. argentinensis, O. smitii*, and *O. incisa* reference samples (Table S4). Later all 176 Gran Paraná samples were analyzed.

The forward primer of each pair was fluorescently labeled with 5´FAM, 5´HEX, NED or VIC. PCR amplification conditions were those reported by Villanova et al. (2013). PCR reactions were carried out in a Verity 96-Well Thermal Cycler (Applied Biosystems). Amplified fragments were genotyped using an ABI 3730 DNA Sequencer (Applied Biosystems) at the INTA DNA genotyping service (INTA-Argentina), and results were visualized using the software Peak scanner v.1 (Applied Biosystems). Alleles were scored using a GeneScan 500 LIZ Size Standard (Applied Biosystems, Inc.).

Genotyping accuracy and presence of null alleles were evaluated using Micro-Checker software (Van Oosterhout et al., 2004). Number of alleles (Na), allelic richness (Ar), expected heterozygosity corrected for sampling bias (He), observed heterozygosity (Ho), and estimated null allele frequency were calculated for each microsatellite locus at each sampling site using CERVUS version 3.0.3 (Kalinowski et al., 2007) and FSTAT version 2.9.3.2 (Goudet, 2001). Linkage disequilibrium between loci and deviations from Hardy–Weinberg equilibrium expectations for each locus were tested by a Markov chain method following the algorithm of Guo & Thompson (1992) and applying the Bonferroni correction (Dunn, 1961) for multiple comparisons ($\alpha = 0.05$; $P < 0.0055$) as implemented in GENEPOP 4.0.10 (Rousset, 2008). Exact tests for Hardy–Weinberg (HW) equilibrium per locus (test multipopulation) and population (test multilocus), and global HW tests for heterozygote excess and deficit were performed using the Markov chain method with 1000 iterations using GENEPOP 4.0.10 (Rousset, 2008). Wright's *F*-statistics (Wright, 1951) over populations and loci were calculated by FSTAT version 2.9.3.2 (Goudet, 2001) and GenAlEx v 6.5 (Peakall & Smouse, 2006). UPGMA tree based on Da distance (Nei et al., 1983) was constructed using Populations, 1.2.30 software package (Langella, 1999). The confidence of nodes was inferred with 1000 bootstrap replicates. Pairwise F_{ST} values between groups (Gran Paraná, reference *O. bonariensis* from CHL, *O. argentinensis*, *O. smitii*, and *O. incisa*) were estimated with ARLEQUIN 3.1 and their significance for genetic differentiation ($P < 0.05$) was tested with 1000 permutations (Excoffier et al., 2005).

Genetic characterization of pejerrey species using mtDNA markers

Mitochondrial DNA (mtDNA) markers amplification was performed in 38 individuals selected by chance taking into account their origin, including 32 Gran Paraná from 3 sampling sites (PR, UR, and iRLP) and 6 *O. bonariensis* from CHL (Table 1). Cytochrome Oxidase subunit I (COI) and Control Region (CR) mtDNA sequences were amplified by polymerase chain reactions (PCR) using the primer sets described by Ivanova et al. (2007) for COI and Sivasundar et al. (2001) for CR. COI PCR reactions were performed with a master mix of 0.4 µM of each primer (VF2-t1, FishF2-t1, FishR2-t1, FR1d-t1; Ivanova et al., 2007), 2.0 mM $MgCl_2$ (Invitrogen, Brazil), 0.2 mM of dNTPs (Promega, USA), 1 U of Taq DNA polymerase (Invitrogen, Brazil), 50–100 ng of total DNA, and 5 µl of 10 × Taq buffer (Invitrogen, Brazil) in 50 µl final reaction volume. Cycling conditions were as follows: denaturation step at 94°C for 5 min followed by 35

cycles of denaturation at 94°C for 1 min, annealing at 54°C for 50 s, and extension at 72°C for 1 min with a final 10 min extension step at 72°C. CR PCR reactions were performed with a master mix of 0.4 µM of each primer (FTTF, F12R; Sivasundar et al., 2001), 2.5 mM $MgCl_2$ (Invitrogen, Brazil), 0.2 mM of dNTPs (Promega, USA), 1 U of Taq DNA polymerase (Invitrogen, Brazil), 50–100 ng of total DNA, and 5 µl of 10 × Taq buffer (Invitrogen, Brazil) in 50 µl reaction volume. Cycling conditions were as follows: denaturation step at 95°C for 2 min, 97°C for 30 s, followed by 38 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 40 s, and extension at 72°C for 1 min 10 s, with a final 10 min extension step at 72°C.

PCR products were checked in 1% agarose gels and purified using the GFXTM kit (GE Healthcare, Germany). Sequencing was performed from both ends for accuracy by Macrogen service (Maryland, USA) on an ABI PRISM 3730 xl Genetic Analyzer (Applied Biosystems, Foster City, CA).

The quality of the sequences and the presence of variable sites were checked by hand with SeqScape v2.5 (Applied Biosystem, Foster City, CA) taking the complete mitochondrial genome of *Odontesthes* sp. (GenBank Accession number NC_011175.1) as reference, and alternatively with BioEdit v7.0 (Hall, 1999). Then, sequences were aligned with ClustalW (Higgins et al., 1994) as implemented in Mega 5.1 (Tamura et al., 2011). Haplotype identification was performed with DnaSP v. 5.10.01 (Librado & Rozas, 2009). Resulting haplotypes were submitted to Genbank (Accession numbers for COI: KF254405 to KF254416; for CR: KF254417 to KF254440).

Available Sequences in GenBank database for COI (Table S1) (Heras & Roldan, 2011; Mabragaña et al., 2011; García et al., 2014) and CR (Table S2) (Beheregaray & Sunnucks, 2001; Beheregaray et al., 2002, Heras & Roldan, 2011) from *Odontesthes* species were used for comparison with Gran Paraná pejerrey. Only records that belong to published works were used in the analysis in order to have reliable sequences for comparisons. Plots of accumulated transitions (ts) and transversions (tv) *vs*. Tamura and Nei genetic distance were employed to test nucleotide saturation using the program DAMBE v4.5.61 (Xia et al., 2003). Tajima's D (Tajima, 1989) and Fu's Fs (Fu & Li, 1993) neutrality tests were performed using the program DnaSP v.5.10.01 (Librado & Rozas, 2009). Saturation tests performed to evaluate the phylogenetic information (Schneider, 2003) for both mtDNA markers revealed that both transitions and transversions changes remained informative. The Tajima's D and Fu's Fs tests were not significant ($P > 0.05$) for all sampling sites, supporting neutrality of mtDNA variation.

Phylogenetic relationships were inferred by Maximum-likelihood (ML) approximation using Mega version 5.1 (Tamura et al., 2011). The robustness of nodes was inferred with 1000 bootstrap replicates. For each molecular marker, the applied model of nucleotide substitution was estimated using the "best fit substitution model" tool (Nei & Kumar, 2000) implemented in MEGA 5.1. For COI marker, the Hasegawa Kishino Yano (HKY) model (Hasegawa et al., 1985) with a gamma distribution of variable sites (*ts*/*tv* value = 6.81, $\alpha = 0.36$ and $f(A) = 0.235$, $f(T) = 0.308$, $f(C) = 0.292$ and $f(G) = 0.165$) was the best fitted to data. For CR marker, the Tamura-3 parameter (Tamura, 1992) with a T92 + G variable sites model (*ts*/*tv* bias = 4.87, $\alpha = 0.13$, $f(A) = f(T) = 0.337$, and $f(C) = f(G) = 0.163$) was the best fitted to data. Finally, Median-Joining networks (Bandelt et al., 1999) were built using the program NETWORK 4.5.1.0 (http://www.fluxus-engineering.com/sharenet.htm).

Population genetics analysis of Gran Paraná using microsatellite markers

Since all the 176 Gran Parana individuals were classified as *O. bonariensis* (see Results section), population genetic analyses were performed using all samples collected at PR, UR, iRLP, cRLP, and CHL sampling sites.

Genetic parameters were estimated as described previously. Pairwise F_{ST} values among populations were estimated with ARLEQUIN 3.1 and their significance for genetic differentiation ($P = 0.05$) was tested with 1000 permutations (Excoffier et al., 2005). Taking into account that Gran Paraná seems to be a nonresident fish, the temporal component was also investigated as a factor of variability. Temporal differentiation of samples collected in 2011, 2012 and 2013 was estimated by the Wright's F_{ST} index. Further, an AMOVA (Analysis of Molecular Variance) approach was conducted using the program ARLEQUIN 3.1 to know the relative percentages of

temporal (F_{SC}) and spatial (F_{CT}) variations. In all cases of multiple tests, table-wide significance levels were applied using the sequential Bonferroni correction (Rice 1989).

A factorial correspondence analysis (FCA) was performed using the software program GENETIX (Belkhir et al., 1996) to graphically represent the temporal and spatial distribution of genetic variability in a multidimensional space.

Results

Gran Paraná pejerrey genetic characterization using nuclear markers

All the specimens from PR, UR, cRLP, and iRLP were classified as *O. bonariensis* according to morphological criteria although they showed some differences in pigmentation compared with reference specimens from CHL, previously identified as *O. bonariensis*. Their color body pattern was yellowed-brown dorsally, silvery ventrally with a broad silvery band with dark blue upper margin running from the pectoral fin base to the caudal fin base along the middle-body, and the upper surface of head, pectoral, and caudal fins were yellow. *O. bonariensis* from the CHL showed a bluish brown dorsal region of their bodies and a blackish upper-head, pectoral, and caudal fins (Fig. 2).

The nine polymorphic microsatellite loci developed for *O. bonariensis* (Villanova et al., 2013) amplified and were informative in all *O. argentinensis, O. smitii*, and *O. incisa* samples (Table S1). Thus, all these markers were used to analyze genetic distance between species and species assignment.

The UPGMA tree based on Nei´s genetic distance matrix detected four main clusters belonging to each included species (i.e., *O. argentinensis*, *O. smitii*, *O. incisa*, and *O. bonariensis*), with Gran Paraná individuals found in the *O. bonariensis* group (Fig. 3).

Pairwise F_{ST} values among Gran Paraná pejerrey, *O. bonariensis*, *O. argentinensis*, *O. smitii*, and *O. incisa* groups (Table S2) confirmed the closer relationship between Gran Paraná pejerrey and *O. bonariensis* (F_{ST} = 0.06702) than when compared with *O. argentinensis* (F_{ST} = 0.36040), *O. smitii* (F_{ST} = 0.44160), and *O. incisa* (F_{ST} = 0.48633).

Gran Paraná pejerrey genetic characterization using mitochondrial markers

Molecular analyses using mtDNA markers were also conducted to support whether Gran Paraná specimens belong to *O. bonariensis*. Sequence alignment of mtDNA markers revealed lower variation at COI than at CR, as expected when coding and noncoding regions are compared. No insertions or deletions (indels) were found in the analyzed regions. Ten different haplotypes were observed for COI and 21 for CR (Table S3).

Fig. 2 *Odontesthes bonariensis* and Gran Paraná pejerrey. Representative specimens of pejerrey *O. bonariensis,* from CHL, and Gran Paraná pejerrey from UR and PR. Bars = 10 cm

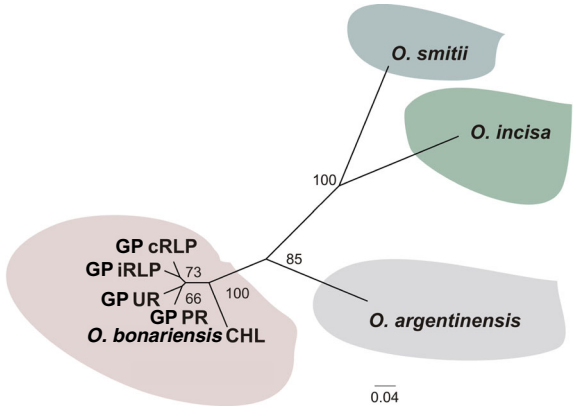

Fig. 3 UPGMA tree based on Nei's distance (Da) displaying the relative relationships among *O. argentinensis, O. bonariensis*, *O. smitii, O. incisa*, and Gran Paraná (GP) pejerrey. Boostrap support was generated from 1000 replicates. PR, Paraná River; UR, Uruguay River; iRLP, inner Río de la Plata estuary; cRLP, central Rio de la Plata estuary; CHL, Chascomús Lagoon

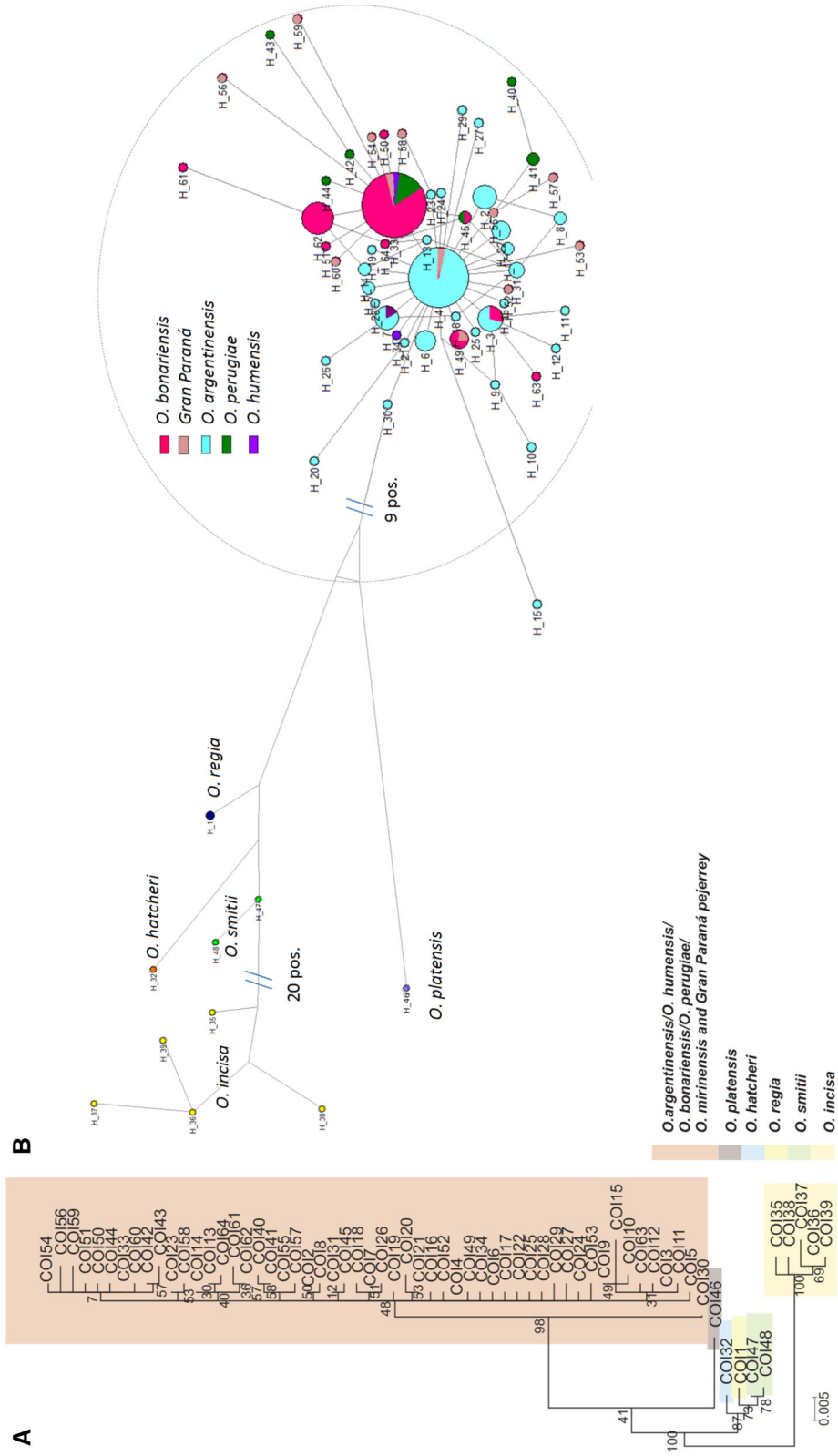

Fig. 4 Genetic analyses using COI mtDNA marker. **A** Maximum-Likelihood tree. The evolutionary history was inferred by the Maximum-Likelihood method based on the HKY + G model (α: 0.36; ts/tv: 6.81). The tree with the highest log likelihood is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. **B** Median-Joining Network. Numbers correspond to haplotypes numbers. The size of circles is proportional to the number of individuals that share a particular haplotype. Each color represents a sample site or species previously described, and it is indicated at the color reference box. Branches length is proportional to the number of differences between haplotypes

To evaluate genetic distances among Gran Paraná pejerrey and *Odontesthes* species (Fig. 4 and S1), sequences here obtained were compared to those previously reported for sympatric (*O. argentinensis, O. bonariensis, O. perugiae,* and *O. humensis*) and nonsympatric (*Odontesthes hatcheri* (Eigenmann 1909)*, Odontesthes regia* (Humboldt, 1821)*, O. incisa, Odontesthes platensis* (Berg, 1895)*, O. smitti, and Odontesthes mirinensis* Bemvenuti, 1996) species. Similar results were observed by ML tree for both mtDNA markers (Figs. 4a and S1A). *O. perugiae, O. humensis*, *O. bonariensis*, *O. argentinensis*, and Gran Paraná pejerrey were included at the same clade. COI mtDNA haplotypes were shared by Gran Paraná and *O. argentinensis* (H_4), Gran Paraná and *O. bonariensis* (H_49), and Gran Paraná with *O. humensis, O. perugiae*, and *O. bonariensis* (H_33) (Fig. 4). Haplotypes H_4 and H_33 were found in central position in the Median-Joining network suggesting their ancestral status (Fig. 4b). Additionally, there were two haplotypes shared by *O. bonariensis* and *O. argentinensis* (H_3 and H_7) and one (H_45) by *O. bonariensis* and *O. perugiae*. For CR marker, shared haplotypes were observed for Gran Paraná and *O. bonariensis* (H_126), and for *O. perugiae and O. bonariensis* (H_122) (Table S5; Fig. S1B). The Median-Joining Network (Fig. S1B) suggested the presence of three ancestral haplotypes (H_122, H_13, and H_11), H_122 shared by *O. bonariensis,* Gran Paraná and *O. perugiae,* and H_11 and H_13, only present in *O. argentinensis*.

Population genetic analysis using nuclear markers

Genetic diversity

Since all Gran Parana individuals were included in the *O. bonariensis* group, population genetic analysis was performed using all *O. bonariensis* individuals collected at PR, UR, iRLP, cRLP, and CHL. Micro-Checker analysis suggested consistent genotyping at all loci. Heterozygote deficit, and excess were detected at some loci, but not at population level. Sampling year and sampling site were considered as probable causes of differentiation in the analyses, since Gran Paraná pejerrey seems to be a nonresident group.

Diversity values per locus, sample site, and year (over all loci) were estimated (Table 2). Mean observed heterocigosity (Ho) per population and year ranged from 0.288 (iRLP2011) to 0.739 (iRLP2012); mean expected heterocigosity (He) from 0.358 (iRLP2011) to 0.641 (PR); and mean allelic richness (Ar) from 2.4 (PR2011) to 4.3 (PR2013). Significant departures from Hardy–Weinberg equilibrium (HWE) expectations after Bonferroni correction were found at specific loci in some sampling sites, and varied among years (Table 2). Global F_{IS} values suggested heterozygote deficit in PR (2011 and 2013) ($P = 0.0000$ at both sampling times) and UR (P = 0.0042).

Linkage disequilibrium analysis between pairs of loci at each temporal and sampling site showed only two deviations from random association in CHL (pair Od43–Od59) and PR2011 (pair Od54–Od11), suggesting no consistent linkage disequilibrium in the populations analyzed.

Genetic structure

Pairwise F_{ST} values among samples ranged from − 0.033 to 0.199 (Table 3). All sampling sites at rivers and estuary showed a significantly low-to-moderate genetic differentiation compared with samples collected at Chascomús lagoon (CHL). This pattern was also evident in the FCA analysis (Fig. S2).

In addition, a low-to-moderate genetic differentiation was observed among temporal samples in PR, iRLP and cRLP (Table 3; Fig. S2). The AMOVA test further supported this observation, with a significant amount of variation assigned to differences among temporal replicates within sampling site (6.59%, $F_{SC} = 0.06737$, $P = 0.000$). Moreover, pairwise F_{ST} values between PR and UR were not significant in 2011, as well as those from both sampling sites of Rio de la Plata estuary (iRLP and cRLP).

Discussion

The present study represents the first attempt to genetically characterize Gran Paraná pejerrey and to assess the genetic diversity and differentiation of this important fishery resource. This information is essential for fishery management, and conservation of pejerrey populations within the La Plata basin, taking into account that Gran Paraná pejerrey would be considered as a different stock.

Gran Paraná pejerrey fishes are captured every year between May and September at the southern La Plata Basin (between 32°S and 34°S). During these months,

Table 2 Genetic diversity based on 9 microsatellites for the *O. bonariensis* sampling sites analyzed in this study

Sampling location and year	Locus	Od43	Od54	Od58	Od59	Od8	Od11	Od18	Od38	Od31	Mean value
PR2011 (*N* = 49)	Na	2	4	3	3	4	7	5	23	14	7.222
	Ar	1.2	1.2	1.6	1.5	2.2	3.2	3.2	7.8	6.0	2.4
	Ho	0.041	0.143	0.125	0.061	0.375	0.469	0.415	0.771	0.857	0.3618
	He	0.040	0.191	0.120	0.099	0.393	0.587	0.584	0.941	0.861	0.4241
	pHW	1.000	**0.007**	1.000	0.1046	0.8549	0.4282	0.0459	**0.0005**	0.4203	
	*F*is	− 0.010	0.253	− 0.038	0.382	0.046	0.202	0.293	0.182	0.004	0.148*
PR2013 (*N* = 12)	Na	5	4	4	5	4	7	3	16	10	6.444
	Ar	3.5	3.05	3.4	2.9	2.8	5.0	2.4	9.1	6.7	4.3
	Ho	0.583	0.167	0.417	0.167	1.000	0.727	0.636	1.000	0.833	0.614
	He	0.612	0.435	0.583	0.377	0.601	0.805	0.481	0.979	0.902	0.6417
	pHW	0.0396	**0.0082**	0.0453	0.0102	**0.0001**	0.0316	0.6247	1.0000	0.1256	
	*F*is	0.0494	0.6271	0.2949	0.5686	− 0.7143	0.1011	− 0.3462	− 0.0227	0.075	0.044*
UR2011 (*N* = 16)	Na	1	2	1	1	3	5	3	17	11	4.880
	Ar	1.0	1.7	1.0	1.0	1.8	3.8	2.5	8.3	6.4	3.1
	Ho	0.000	0.188	0.000	0.000	0.188	0.500	0.250	0.933	0.813	0.319
	He	0.000	0.175	0.000	0.000	0.179	0.683	0.524	0.959	0.891	0.379
	pHW	–	1.0000	–	–	1.000	0.0285	0.0190	0.6715	0.3239	
	*F*is	–	− 0.071	–	–	− 0.046	0.275	0.531	0.027	0.091	0.140*
iRLP2011(*N* = 5)	Na	1	1	1	1	2	3	3	7	5	2.667
	Ar	1.0	1.0	1.0	1.0	2.0	3.0	3.0	7.0	5.0	2.7
	Ho	0.000	0.000	0.000	0.000	0.600	0.400	0.400	0.600	0.600	0.288
	He	0.000	0.000	0.000	0.000	0.467	0.644	0.378	0.911	0.822	0.3580
	pHW	–	–	–	–	1.000	0.361	1.000	0.0401	0.3428	
	*F*is	–	–	–	–	− 0.3333	0.4074	− 0.0667	0.3684	0.2941	0.212
iRLP2012 (*N* = 43)	Na	4	4	6	3	7	14	3	23	12	8.444
	Ar	2.3	2.8	2.6	1.7	2.8	5.5	2.7	7.6	6.2	3.8
	Ho	0.977	0.837	0.442	0.163	1.000	0.791	0.762	0.756	0.930	0.739
	He	0.540	0.558	0.376	0.154	0.583	0.818	0.532	0.928	0.879	0.5965
	pHW	**0.0000**	**0.0000**	0.8903	1.0000	**0.0000**	0.0194	**0.0007**	**0.0000**	0.1882	
	*F*is	− 0.827	− 0.509	− 0.1779	− 0.00576	− 0.7291	0.0335	− 0.4386	0.1872	− 0.0593	− 0.244

Table 2 continued

Sampling location and year	Locus	Od43	Od54	Od58	Od59	Od8	Od11	Od18	Od38	Od31	Mean value
iRLP2013 (*N* = 15)	Na	5	5	5	3	3	4	3	12	9	5.444
	Ar	3.4	3.6	3.0	2.0	2.3	2.6	2.6	7.2	5.5	3.6
	Ho	0.933	0.800	0.867	0.067	1.000	0.429	0.867	0.643	1.000	0.734
	He	0.662	0.609	0.598	0.191	0.549	0.418	0.559	0.926	0.848	0.5956
	pHW	**0.0003**	1.0000	0.0812	0.0335	**0.0000**	0.4110	0.0118	0.0157	0.8652	
	*F*is	− 0.430	− 0.328	− 0.473	0.658	− 0.875	− 0.026	− 0.582	0.313	− 0.186	− 0.242
cRLP2011 (*N* = 13)	Na	3	2	4	1	2	5	3	12	8	4.000
	Ar	2.9	1.8	2.6	1.0	2.0	3.6	2.6	7.2	5.5	3.3
	Ho	0.556	0.111	0.222	0.000	1.000	0.667	0.778	0.625	1.000	0.551
	He	0.660	0.111	0.399	0.000	0.538	0.732	0.621	0.908	0.817	0.5319
	pHW	0.0346	1.0000	0.0966	–	**0.0031**	0.0519	0.1044	0.0395	0.6601	
	*F*is	0.106	− 0.090	0.345	–	− 1.000	0.109.	− 0.466	0.284.	− 0.200	− 0.093
cRLP2012 (*N* = 23)	Na	2	3	7	3	5	9	3	16	12	6.667
	Ar	2.0	2.2	3.3	1.7	2.6	4.5	2.7	7.5	6.3	3.4
	Ho	0.778	0.630	0.519	0.154	0.963	0.615	0.741	0.852	0.926	0.686
	He	0.484	0.469	0.442	0.147	0.562	0.718	0.528	0.932	0.874	0.5729
	pHW	**0.0016**	**0.0000**	1.0000	1.0000	**0.0000**	0.0354	0.1075	0.2903	0.8999	
	*F*is	− 0.692	− 0.422	− 0.189	− 0.053	− 0.698	0.128	− 0.356	0.068	− 0.035	− 0.206
CHL2012 (*N* = 20)	Na	5	4	6	4	4	9	3	10	8	5.880
	Ar	3.8	2.5	3.2	2.8	2.7	5.1	2.6	6.0	5.2	3.8
	Ho	0.850	0.400	0.250	0.400	1.000	0.556	0.800	0.875	0.700	0.648
	He	0.735	0.350	0.435	0.481	0.581	0.756	0.542	0.873	0.836	0.621
	pHW	**0.0000**	1.0000	0.0072	0.0061	**0.0000**	0.1569	0.0258	0.3509	0.0902	
	*F*is	− 0.162	− 0.147	0.431	0.172	− 0.755	0.270	− 0.494	− 0.002	0.166	0.032

PR Paraná River, *UR* Uruguay River, *iRLP* inner Río de la Plata estuary, *cRLP* central Rio de la Plata estuary, *CHL*, Chascomús Lagoon, *N* number of sampled individuals, *Na* number of alleles, *Ar* allele richness, *Ho* observed heterocigocity, *He* expected heterocigocity, *pHWE* probability of Hardy–Weinberg equilibrium departure (in bold are shown significant values after Bonferroni correction $p < 0.0055$); *F*is, inbreeding coefficient estimated following Weir and Cockerham; * significant departure from HWE when H1 = heterocygotes deficit (after Bonferroni correction $p < 0.0055$)

Table 3 Pairwise F_{ST} values between sampling locations considering each sampling year

	PR2011	PR2013	UR2011	iRLP2011	iRLP2012	iRLP2013	cRLP2011	cRLP2012	CHL
PR2011									
PR2013	**0.120**								
UR2011	0.003	**0.134**							
iRLP2011	− 0.033	0.075	− 0.005						
iRLP2012	**0.141**	0.008	**0.146**	**0.106**					
iRLP2013	**0.176**	0.016	**0.199**	**0.138**	**0.028**				
cRLP2011	**0.085**	0.008	**0.124**	0.056	**0.029**	**0.045**			
cRLP2012	**0.128**	0.002	**0.141**	**0.088**	− 0.003	**0.030**	0.010		
CHL	**0.166**	0.016	**0.170**	**0.118**	**0.037**	**0.034**	**0.037**	**0.041**	

PR Paraná River, *UR* Uruguay River, *iRLP* inner Río de la Plata estuary, *cRLP* central Rio de la Plata estuary, *CHL* Chascomús Lagoon. In bold are shown significant values after Bonferroni correction $p < 0.0055$)

the water temperature ranges from 10 to 17°C, and most male and female individuals captured by artisanal fishing show mature gonads suggesting spawning period (Arranz, unpublished data). All the Gran Paraná specimens for this study were collected during the June–September period and were classified as *O. bonariensis*, based on morphology. However, they showed differences in body shape and color compared with *O. bonariensis* specimens collected at Chascomús lagoon. This difference in pigmentation could be related to environmental plasticity reported for the Atherinidae family (Bamber & Henderson, 1988; Mancini et al., 2016; Alarcón-Durán et al., 2017). Moreover, analysis of nuclear markers (i.e., microsatellite loci) allowed allocating Gran Paraná individuals as *O. bonariensis*. UPGMA tree, and pairwise F_{ST} values among species, supported this result. Mitochondrial markers (COI and CR) were unable to distinguish among some *Odontesthes* species on our analyses, but most of the obtained Gran Paraná mtDNA sequences were related to a group including *O. bonariensis* in close proximity with *O. argentinensis*. COI marker has been able to differentiate the 93% of the described marine and freshwater fish species. The remaining 7% likely corresponds to recently radiated species, introgressive hybridization events, and/or misidentifications (Ward, 2009). Recently radiated species and introgressive hybridization events could be taking place in the pejerrey species (Campanella et al., 2015; Diaz et al., 2016; García et al., 2014; Strüssmann et al., 1997). A few Gran Paraná fishes have CR haplotypes related with species clearly differentiated from *O. bonariensis*, supporting hybridization between *Odontesthes* species (Strüssmann et al., 1997).

O. bonariensis is a conspicuous pejerrey of the shallow lakes, small rivers, and channels of Argentina (Liotta, 2005), but its occurrence has also been registered at Paraná and Uruguay Rivers (Cordiviola et al., 1981; Liotta, 2005) and Río de la Plata estuary (Avigliano & Volpedo, 2013; Mirande & Koerber, 2015). *O. bonariensis* is an eurihalyne species with a remarkable tolerance to high-salt concentrations but not to sea water (Tsuzuki et al., 2000; Gómez & Ferriz, 2001; Hughes et al., 2017). In Salado River lakes, where CHL is included, *O. bonariensis* population abundance varies following flood periods, becoming scarce in the flooded water body (Rosso & Quirós, 2009; Colautti et al., 2015). Complete life cycle occurs at Chascomús lagoon (Rosso, 2008), being spring the spawning season, as suggested by Elisio et al. (2014, 2015). Moreover, our results pointed out the presence of more than one *O. bonariensis* population (i.e., Gran Paraná pejerrey) at the southern La Plata Basin, with a seasonal migratory behavior.

Preservation of genetic diversity is a major issue for conservation programs in order to maintain the adaptive potential of populations (Frankham et al., 2002). Genetic diversity parameters, estimated by microsatellite loci, displayed values similar to the average observed in other 78 freshwater fish species (A = 7.5, He = 0.460) reported by DeWoody & Avise (2000). However, observed values were slightly lower than those found in a previously analyzed *O.*

bonariensis population from Uruguay lagoons (A = 9.6, He = 0.697) reported by García et al. (2014).

Regarding genetic structure analyses, results from pairwise F_{ST} values and FCA consistently point toward the existence of little-to-moderate genetic differentiation among Gran Paraná pejerrey and *O. bonariensis* from CHL. In addition, a high genetic interchange among groups is suggested specially at the estuary (iRLP and cRLP). The temporal genetic differentiation observed in PR and iRLP is reported here for the first time, and could reflect the presence of different cohorts among years. Temporal genetic differentiation has been observed in other fish species related to migration timing and reproduction, such as salmonids of the genus *Oncorhynchus* (Hendry & Day, 2005), or related to environmental changes (flooding), such as African cichlid fish *Pseudocrenilabrus multicolor* (Schöller, 1903) (Crispo & Chapman, 2010).

A hypothesis could be introduced to explain the observed genetic structure of Gran Paraná. Gran Paraná could be a migratory ecotype of *O. bonariensis* with a seasonal migration from brackish waters (possibly in the outer zone of Rio de la Plata estuary) to freshwater environments, showing an admixture of different genetic groups suggested by the deficit of heterozygous observed in Paraná (PR) and Uruguay (UR) River collections. Even though hybrids between *O. bonariensis* and *O. argentinensis* were not detected in this study, genetic interchange between estuarine/marine species cannot be discarded. In addition, Delta lagoons show characteristics suitable for *O. bonariensis* reproduction, such as still and shallow waters, with abundant vegetation. In this context, studies about the ecology of reproduction and spawning time of Gran Paraná pejerrey are necessary to contrast this hypothesis.

Gran Paraná pejerrey constitutes a diverse group harboring unique genetic diversity and adaptive potential. While a more in-depth analysis beyond the scope of this study is necessary to fully understand the evolutionary forces acting on Gran Paraná pejerrey, our data support that *O. bonariensis* Gran Paraná population from Paraná and Uruguay Rivers, and Rio de la Plata estuary, should be considered as a different ESUs to *O. bonariensis* from Chascomús lagoon, for fishery policies and conservation purposes. Further, our results suggest the necessity of managing Gran Paraná pejerrey by preserving their areas of reproduction, and by defining management actions for the sustainable conservation of this important resource.

Acknowledgements We would like to thank Alexis Grimberg, Julián Aguilar, and Diego Añaño and team for fishing assistance, Darío Colautti for the kind donation of samples, Victoria Posner for reading the manuscript, and anonymous reviewers that helped to improve our work. This work was supported by the National Agency for the Promotion of Science and Technology from Argentina (ANPCyT) and the Government of Santa Fe province [Grant Numbers: PID 020-2013 and PICT 0510-2011]; FB is a Ph. D student, JD is a postdoctoral fellow, and GVV is member of the researcher carrier from the National Council of Research from Argentina.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

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