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PRIMARY RESEARCH PAPER

The influence of paleoclimate on the distribution of genetic variability and demography of fishes in a large and highly fragmented neotropical river

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Abstract The present study sought to identify general patterns of genetic variability and structure of fish stocks (migratory and non-migratory species) along one of the largest Brazilian rivers, the São Francisco. Given that genetic variability of populations of all organisms is governed by both present and past influences, herein we investigate both these aspects by evaluating the current genetic differences between populations of five species (*Leporinus piau*, *Megaleporinus reinhardti*, *Pimelodus maculatus*, *Prochilodus argenteus*, and *Pygocentrus piraya*) along the entire extension of the river, as well as their demographic history. Analyses were done through sequences of two mitochondrial fragments and microsatellite data. In general, the data showed no support for recent fragmentation of stocks by the dams present in this river, and that all species show signs of past population expansion. We discuss the possible reasons for the common patterns found between these species, including the influence of the river's topography and history.

Keywords Population genetic structure · Historical demography · Genetic variability · River fish

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Introduction

São Francisco is one of the most important rivers of Brazil connecting the Southeastern and Northeastern regions of the country. It runs for about 2700 km and its basin covers an area of approximately 631,000 km^2, or 7.4% of the Brazilian territory (Carolsfeld et al., 2003). The river formation dates to mid Cretaceous (Valadão & Dominquéz, 1994), and the tectonic depression where it flows may have changed several times (Potter, 1997). At present, the different longitudinal zones exhibit distinct patterns of stability and vulnerability mostly related to the introduction of impoundments (Sato et al., 2003).

The hydrographic basin of the São Francisco has a peculiar physiography, mostly formed by upland rivers (Tucci et al., 2001). It represents a unique case

of a very extensive Neotropical river that is subdivided into several segments by anthropogenic dams not only upland, near the headwaters, as in most rivers, but also along the river channel, and near the delta, which makes it one of the most significant generators of hydroelectric power in the country. In fact, the São Francisco River has five major hydropower plants in its course, which represents about 10% of all electrical energy produced in Brazil (ANEEL, 2008).

Although hydropower plants are very important for the maintenance of the electrical energy production in the country, providing 66% of the total electrical production in the country (ANEEL, 2016), the dams may act as a large barrier for fishes and other organisms by physically separating their populations (Hanson et al., 1990; Zwick, 1992; Fahrig & Merriam, 1994). As such, they also influence patterns of migration (Gosset et al., 2006), alter faunal assemblage (Loureiro-Crippa & Hahn, 2006), as well as the richness, diversity, and system's equitability (Mol et al., 2007). The interruption of a river's course, caused by the construction of such dams, can also affect the availability of food, diet composition, and, in the worst scenario, the food chain as a whole (Mérona et al., 2001, 2003; Loureiro-Crippa & Hahn, 2006). Barriers are also responsible for reducing population sizes and altering gene flow, ultimately leading to differentiation of isolated demes (Meldgaard et al., 2003). Particularly, the dams constructed in the São Francisco River are an extreme case of disruption of natural dispersal pathways since they do not allow downstream to upstream connectivity between stretches of the river due to the lack of translocation features, such as fish ladders.

The distribution of genetic diversity within and among populations of fish species within this riverbed should help understand the extent of the impact of anthropogenic (i.e., hydroelectric power plants) as well as non-anthropogenic (i.e., climatic and geological processes) in large Neotropical rivers. While the relatively recent fragmentation of the river by dams could produce genetically structured populations, we predict that the present genetic distribution may also reflect more long-term influences relative to river physiography and historical climate changes. The climatic oscillation of the Pleistocene, which included periods of very dry weather, most likely had an impact on the hydrological flow regimes of the São Francisco (Domingues, 1948; Tricart, 1974). A drier climate implies contraction of the available habitat to fish, which could drastically influence their populations. Similarly, the topography of a river may affect how the genetic variability of fish populations is distributed along its course.

Herein, using a combination of mitochondrial DNA and microsatellite markers from five fish species with dissimilar levels of vagility (see Table 1), we test the following: (1) the phylogeography of fish fauna of different migratory behaviors, (2) the influence of dams in gene flow, and (3) the historical demography of the studied species.

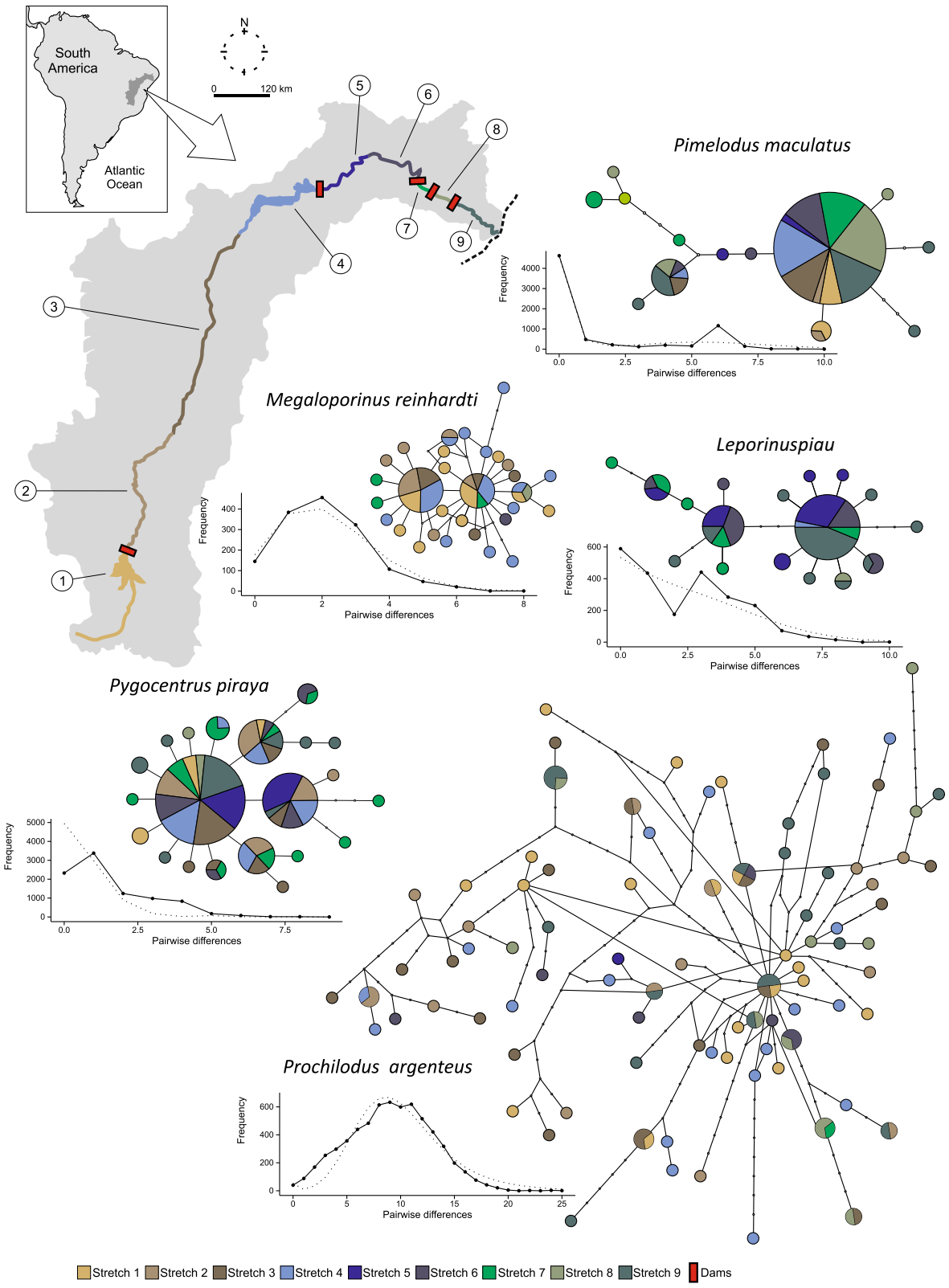
Noteworthy is that we consider nine stretches of the river as different fragments that encompass its entire length, most of them separated by dams (Fig. 1),

Fig. 1 Map of the São Francisco River within the São Francisco basin in Brazil, and each species' mismatch distribution (MD) graph and haplotype network. In the river map, *each color* represents a different stretch, based on collection points, represented by the *numbers*. In the MD graphs, the *black circles* represent observed pairwise values, the *black line* is the expected distribution, and the *gray lines* are the 95% confidence interval. In the haplotype networks, *circle size* is proportional to the number of individuals with the corresponding haplotype. *Colors* represent the river stretch from which individuals with the haplotype are derived ▶

Table 1 Space distribution and life-history traits of the fish species used in the present study

Species	Migration	Distribution	Swimming speed (BS/s)
Leporinus piau	Migratory	Country endemic	–
Megaloporinus reinhardti	Migratory	River endemic	12.85
Pimelodus maculatus	Migratory	Country endemic	6.63
Prochilodus argenteus	Migratory; reophily	Continental	5.85 (*Pr. costatus*)
Pygocentrus piraya	Non-migratory	River endemic	–

Migration indicates whether a species is migratory, non-migratory and if it presents reophily. Distribution indicates its extension. Swimming speed indicates the maximum prolonged swim speed measured as body size per second (BS/s) (see Santos et al., 2012). The swimming speed presented for *Pr. argenteus* refers to that of a congeneric, *Pr. costatus*


South America
Atlantic Ocean
N
0 120 km
1
2
3
4
5
6
7
8
9
Pimelodus maculatus
Megaloporinus reinhardti
Leporinuspiau
Pygocentrus piraya
Prochilodus argenteus
Frequency
Pairwise differences
Stretch 1
Stretch 2
Stretch 3
Stretch 4
Stretch 5
Stretch 6
Stretch 7
Stretch 8
Stretch 9
Dams

which were constructed between 1962 and 1994. With generation times between 1 year (*Pygocentrus cariba*) and 3.5 years (*Leporinus alternus*), based on data available for congeneric species (Froese & Pauly, 2008), around 15–54 generations have passed since construction of the first dam. Thus, we discuss different temporal depths that might influence the current geographic distribution of genetic diversity based on recent (dams) and long-term (physiography and climate) characteristics.

In general, we find that all species show signs of population expansion at different times and discuss the possible reasons for the common patterns found between these species, including the influence of the river's physiography and history.

Material and methods

Studied species

We studied five species that comprise a range of ecological niches, varying in morphology, distribution range, and behavior (Table 1). *Pimelodus maculatus* Lacepède, 1803 inhabits the South American rivers of the São Francisco and Paraná Basins. It is a bento-pelagic species that migrates long distances (Agostinho et al., 2007; Froese & Pauly, 2008). *Prochilodus argenteus* Spix and Agassiz, 1829 is also a migratory species, broadly distributed in South America, occurring from the La Plata Basin to the Magdalena River, in Colombia. Its abundance and widespread distribution make it an important fishery resource (Ortí et al. 2005). *Pygocentrus piraya* (Cuvier, 1819), the São Francisco piranha, is a non-migratory species endemic to the São Francisco River. It is considered the biggest piranha species, reaching 34 cm in length and weighing up to 3 kg (Fink, 1993). Of the two Anostomidae species, *Megaleporinus reinhardti* (Lütken, 1875) is endemic to the São Francisco, occurring throughout the entire basin, and *Leporinus piau* Fowler, 1941, is a migratory fish that is widely distributed in the semi-arid hydrographic basins of northeastern Brazil. Both are economically important species (Gomes & Verani, 2003; Alves & Pompeu, 2005).

The four migratory species (i.e., *Pi. maculatus*, *Pr. argenteus*, *L. piau*, and *M. reinhardti*) present different levels of vagility. Santos et al. (2012) compared the vagility ability of three of these four genera to find that *M. reinhardti* is the fastest swimmer, in terms of body length per second, followed by *Pi. maculatus* and *Pr. costatus*.

Sampling

Tissue samples were collected from approximately 20 individuals of each studied species per location. There were nine sampling sites spanning the entire length of the São Francisco River. Sampling locations are represented in Fig. 1 and described in Online Resource Table 1. The first point is located at the Três Marias Reservoir, upstream of the first large hydroelectric Brazilian dam on the São Francisco River. Noteworthy here is that, except between locations 2–4, all other sites are intercalated by river dams (Fig. 1). Because collections were opportunistic, not every species was sampled at every site (see Table 2). Tissue samples were preserved in salt-saturated DMSO (Seutin et al., 1991). Representative specimens were deposited in the Zoology Museum at the University of São Paulo (MZUSP), Brazil (http://www.mz.usp.br; access codes 102695–102702; 118349–118375).

DNA isolation and amplification

We extracted DNA from a total of 379 tissue samples with an iPrep^{TM} (Invitrogen) robot using the iPrep^{TM} ChargeSwitch$^{®}$ gDNA Tissue kit and following standard protocol. Mitochondrial DNA (mtDNA) D-loop region was amplified using primers H16498 (Meyer et al., 1990) and L15774M (Prioli et al., 2002) for all species, except *Pr. argenteus*. A specific set of primers was developed for this particular species (XiraF1—5′-ACTCCCACCACTAACTCCAAA-3′ and XiraR1—5′-ARCAKTTGGTGGTCTCTTACTA-3′). A fragment of the cytochrome b (CYTB) region was amplified in *L. piau* and *M. reinhardti* with primers GLUDG.L (Palumbi, 1996) and H16460 (Perdices et al., 2002). Additionally, because morphological distinction between species of *Prochilodus* is considered unreliable (Castro & Vari, 2004; Ortí et al. 2005), a fragment of the cytochrome c oxidase I (COI) gene was amplified for specimens of *Prochilodus* spp. with the intent of confirming taxonomic identification, using primers FR1d and FF2d (Ivanova et al., 2007). A

Table 2 Summary statistics for all 9 sites and species for D-loop and concatenated data when applicable (*L. piau* and *M. reinhardti*)

	1	2	3	4	5	6	7	8	9
D-loop									
L. piau									
n	–	–	–	1	20	17	6	1	23
H				1	6	6	3	1	8
π				0	0.002	0.002	0.001	0	0.001
$D = -1.3$, $F_S = -29.68$*									
M. reinhardti									
n	15	10	6	18	–	2	3	1	–
H	9	6	2	10		1	1	1	
π	0.002	0.003	0.001	0.003		0	0	0	
$D = -2.21$*, $F_S = -27.83$*									
Pi. maculatus									
n	9	3	14	17	3	14	17	23	20
H	3	2	3	2	2	4	4	3	4
π	0.001	0.001	0.003	0.001	0.002	0.003	0.004	0.002	0.004
$D = -1.31$, $F_S = -28.08$*, $\theta = 0.007$, $g = 347.25$									
Pr. argenteus									
n	19	17	20	18	1	7	1	12	18
H	19	15	19	18	1	6	1	11	15
π	0.018	0.024	0.027	0.027	0	0.019	0	0.02	0.023
$D = -1.62$*, $F_S = -24.54$*, $\theta = 0.76$, $g = 240.16$									
Py. piraya									
n	6	19	19	20	19	13	16	3	20
H	3	5	7	5	2	5	10	2	8
π	0.002	0.004	0.003	0.003	0.001	0.003	0.006	0.001	0.002
$D = -0.08$*, $F_S = -28.29$*, $\theta = 0.019$, $g = 952.41$									
Concatenated data									
L. piau									
n	–	–	–	1	20	17	6	1	23
H				1	8	8	4	1	11
π				0	0.002	0.002	0.002	0	0.001
$D = -1.47$*, $F_S = -27.0$*, $\theta = 0.009$, $g = 934.67$									
M. reinhardti									
n	15	10	6	18	–	2	3	1	–
H	13	7	6	12		1	1	1	
π	0.002	0.001	0.001	0.002		0	0	0	
$D = -2.36$*, $F_S = -27.13$*, $\theta = 0.02$, $g = 977.25$									

n number of individuals, *H* haplotype number, π nucleotide diversity, *D* Tajima's *D*, F_S Fu's F_S

* Statistically significant ($P < 0.05$)

detailed description of the DNA barcoding protocol is given below.

Polymerase Chain Reactions (PCR) were conducted in 25 µl reactions, containing 1 µM of each primer, 0.4 mM dNTP (Biotools, Jupiter, Florida, USA), 3 mM MgCl, 2.5 U Taq polymerase (Invitrogen, Carlsbad, California, USA), 1× buffer, 0.06 µg/µl BSA and approximately 2 ng/µl of DNA. Amplification conditions were as follows: 4 min at 95°C; followed by 35 cycles of 45 s at 94°C, 45 s at 59°C (except COI at 68°C), 60 s at 72°C; and a final extension of 5 min at 72°C. Following amplification, PCR products were electrophoresed in 1.5% agarose gels stained with ethidium bromide and visualized

under ultra-violet light. PCR products were purified using a Microcon PCR Filter Unit kit (Millipore©), following the manufacturer protocol. Subsequent sequencing reactions were conducted using Big Dye v3.1 (Applied Biosystems®) following instructions of the manufacturer. These reactions were done with the same PCR primer pairs for all species, except *L. piau* and *M. reinhardti*, for which we developed the following sequencing primers: dloopWF11 (5′-GAGG TATACCAGTAGAAGACCCCTTT-3′) and dloop WR678 (5′-CTGACCTATCAAGGACCGTGT-3′). Sequencing reactions were purified using Sephadex™ G-50 medium (GE Healthcare Bio-Sciences AB).

Microsatellite loci were amplified exclusively for *Pi. maculatus* individuals. A total of five loci, previously developed by Paiva & Kalapothakis (2008), were amplified: Pma01, Pma05, Pma06, Pma08, and Pma11. Amplifications were performed in 10 µl reactions, containing 1× buffer, 2 mM $MgCl_2$, 0.2 mM dNPT, 0.6 µM of each primer, 0.025 U/µl Taq, and 1.5 ng/µl DNA. Amplifications were conducted as follows: 3 min at 95°C; followed by 35 cycles of 40 s at 94°C, 40 s at 67°C, and 30 s at 72°C; and a final extension of 40 min at 72°C. The forward primers of each pair were labeled with fluorescent dyes, HEX, FAM, and NED. Following amplification, microsatellite alleles of each marker were defined by electrophoresis in an ABI 3130 automated DNA sequencer (Applied Biosystems, Carlsbad, California, USA), then visualized and scored in the program GeneMapper v.3.7 (Applied Biosystems).

Barcoding of *Prochilodus* specimens

Prochilodontids are known for problematic field identification because of the few morphological distinguishing characters (Castro & Vari, 2004). Out of the 130 prochilodontids initially sequenced for the D-loop fragment, 17 showed a higher number of nucleotide differences relative to the other individuals in the alignment. In order to accurately identify species, we performed barcode amplifications for all individuals using the COI primers FF1d and FF2d (Ivanova et al., 2007). PCRs were performed in 25 µl reactions containing 2 pmol of each primer, 2.5 units of Taq polymerase, 1× buffer, 1.6 mM dNPT (Biotools, Jupiter, Florida, USA), 3 mM MgCl, and approximately 2 ng/µl of DNA. Amplification conditions were as follows: 4 min at 95°C; followed by 35 cycles of 45 s at 94°C, 45 s at 68°C, and 60 s at 72°C; and a final extension of 5 min at 72°C. Determinations of species were based on a dendrogram of nucleotide sequences constructed with the neighbor-joining criterion in the software MEGA 5 (Tamura et al., 2011). jModelTest v. 0.1.1 (Posada, 2008) was used to select the DNA evolution model. Kimura 2 parameters model was inferred as the best estimator.

Analyses

Analyses of mitochondrial DNA data

Sequences were edited and assembled with the Staden Package (Staden, 1996), aligned using ClustalW v.1.4 (Thompson et al., 1994), as implemented in BioEdit v.7.0.5.2 (Hall, 1999), and deposited in GeneBank (accession numbers MF432466–MF432554; MF4325 62–MF432695). Analyses were performed with each gene separately, as well as with concatenated data. The species for which the two mitochondrial gene regions were amplified (*L. piau* and *M. reinhardti*) showed similar results between markers (see Table 2); hence, concatenated data were used for haplotype network and demographic analyses.

Genetic diversity was estimated based on the calculation of nucleotide diversity and haplotype number using the software Arlequin v. 3.11 (Excoffier et al., 2005). Population genetic structure was tested using a hierarchical analysis of molecular variance (AMOVA). Overall and pairwise F_{ST} values were also calculated with Arlequin, using a permutation of 10,000 iterations. Significance levels were corrected with the Bonferroni method (Rice, 1989). Populations with a single individual were excluded from pairwise F_{ST} analyses. Haplotype networks were constructed for each species using the parsimony criterion in TCS v.1.21 (Clement et al., 2000).

Population historical demography was analyzed using six methods. Because of the general lack of genetic structure between sites (see "Results"), demographic analyses were performed per species, including individuals from all sites. One of the methods used was based on site mismatch distribution (Rogers & Harpending, 1992), estimated with Arlequin. Statistical significance of distributions was tested using sum of squares distances (SSD), and the degree of approximation

between the observed mismatch distribution and that expected under population growth was tested using Harpending's raggedness index, r (Harpending et al., 1993), with 10,000 bootstrap replicates. The estimated values of the tau parameter (τ) were converted into time-since-expansion (t) by solving $t = \tau/2u$ (Rogers & Harpending, 1992), where u is the cumulative probability of substitution in the sequenced region, using the Mismatch Calculator provided by Schenekar & Weiss (2011).

We also used a coalescence-based method, Bayesian Skyline Plots (Drummond et al., 2005), to infer past demographic history of populations. Four independent runs were conducted in Beast v. 1.7.1 (Drummond et al., 2012), each with 50,000,000 replicates and 5,000,000 burn-in, and the appropriate model of evolution of each species was determined with jModelTest. The D-Loop rates of evolution used in these analyses were of 0.93% per million year (My) for *Pi. maculatus*, based on the rate described for *Hypostomus* (Siluriformes) by Montoya-Burgos (2003); 0.58% per My for *Py. piraya* as described by Hubert et al. (2007a); and 0.835% per My for *Pr. argenteus*, based on Sivasundar et al. (2001). The CYTB rate used for *M. reinhardti* and *L. piau* was of 1.15% per My, based on Ramirez et al. (2017). From this rate of evolution for CYTB for this species, we calculated the rate for the D-loop region, also using Beast and the same parameters described above. The values obtained (1.65% per My for *L. piau* and 4.28% per My for *M. reinhardti*) were then used in further analyses.

Departures from the mutation-drift equilibrium were tested using the neutrality tests of Tajima (1989) and Fu (1997). The respective D and F_S statistics were calculated using Arlequin. Significantly negative values of these statistics also indicate population expansion.

We estimated θ (Theta) as a proxy for present-day effective population size, as implemented in LAMARC v.2.1.10 (Kuhner, 2006; Kuhner & Smith, 2007). $\theta = 2N_e\mu$, where N_e is the effective population size, and μ is the mutation rate per nucleotide and per generation. We used the Bayesian approach (Kuhner & Smith, 2007) and verified run convergence using Tracer v.1.5 (Rambaudt et al., Rambaut et al. 2014) by checking that EES values were higher than 200. The MCMC parameters for the short initial chains were a burn-in of 10,000, a thinning interval of 20, and a sample size of 5000. The parameters for the long run were a burn-in of 40,000, a thinning interval of 20 and a sample size of 30,000. We set the transition/transversion ratios (ti/tv) to match the ones empirically calculated with jModeltest for each species. LAMARC can simultaneously estimate exponential growth rates. The growth rate (g) presents estimates of the exponential growth or shrinkage rate. Positive values of g indicate that population has been growing, and negative values indicate that it has been shrinking (Kuhner, 2006).

Analyses of microsatellite data

Microsatellite loci were amplified for populations of *Pimelodus. maculatus.* For each locus and site, we calculated the number of alleles, expected (H_e) and observed (H_o) heterozygosity, using Arlequin. Deviations from Hardy–Weinberg equilibrium (HWE) were tested with 1,000,000 Markov Chain steps and 100,000 dememorizations, also using Arlequin. Genotypes artifacts were assessed with Microchecker v.2.2.1 (van Oosterhout et al., 2004).

Genetic differentiation was calculated by estimating Wright's F_{ST} using Arlequin, and Slatkin's R_{ST} using Fstat v. 2.9.3.2 (Goudet, 1995). The former assumes an infinite allele model, while the latter assumes a stepwise mutation model and is considered more adequate for microsatellite analyses (Slatkin, 1995). An AMOVA was used to calculate the percentage of variance distribution within and between populations using 1000 permutations in Arlequin. Significance levels were corrected with the Bonferroni method (Rice, 1989).

The Bottleneck software (Cornuet & Luikart, 1996; Piry et al., 1999) was used to test each population for evidence of a recent population bottleneck using the infinite allele model (IAM), and the Wilcoxon's test of significance. According to the authors of the software used for this purpose (Piry et al., 1999), the software can detect reductions within the past $2N_e - 4N_e$. Populations that have suffered a bottleneck are expected to show excess heterozygosity in comparison to the expected number under mutation-drift equilibrium (Piry et al., 1999).

Results

Mitochondrial data

Table 2 describes the summary statistics for all five species. Genetic diversity indices were very similar

Table 3 Analysis of molecular variance (AMOVA) for all species based on D-loop, cytochrome b and concatenated data when applicable

Species	DNA fragment	Percentage variation			
		Within population	Between populations	Overall F_{ST}	*P*
L. piau	D-loop	88.29	11.71	0.12	0.002
	Cytochrome b	85.29	14.71	0.141	0.008
	Concatenated data	86.73	13.27	0.133	0.003
M. reinhardti	D-loop	102.15	−2.15	−0.021	ns
	Cytochrome b	97.93	2.07	0.021	ns
	Concatenated data	100.67	−0.67	0.006	ns
Pi. maculatus	D-loop	101.19	−1.19	−0.012	ns
Pr. argenteus	D-loop	99.82	0.18	0.002	ns
Py. piraya	D-loop	96.69	3.31	0.033	ns

ns not significant

among species, except for *Pr. argenteus*, which showed much higher genetic variability in number of haplotypes and nucleotide diversity. Nucleotide diversity ranged from 0.018 to 0.027 in populations of *Pr. argenteus*, and from 0.001 to 0.004 among populations of all the other four species (i.e., *L. piau*, *M. reinhardti*, *Pi. maculatus*, and *Py. piraya*). Similarly, haplotype numbers ranged from 6 to 18 in *Pr. argenteus* populations, and from 2 to 13 in populations of all other species combined.

AMOVA results were also similar between species, indicating that for most species there is no genetic differentiation along the extension of the São Francisco River. Four out of the five species showed no sign of population structure, with the exception of *L. piau* (Table 3). Pairwise F_{ST} values for *L. piau* populations indicate differentiation between site 9 and site 6 (Table 4, Fig. 1 for site locations). For all species, percentage of genetic variation was much higher within populations than between them. The overall F_{ST} value for *L. piau* was 0.133, but ranged between much lower numbers in all other species, from −0.012 to 0.03 (Table 4).

As expected from the lack of genetic structure and low genetic variability of most species, haplotype networks show a considerable amount of haplotype admixture between different sites. Haplotype networks of all fish species are shown in Fig. 1. A star-like signature is also evident in haplotype networks of all species, except *Pr. argenteus*. This network shape is indicative of a recent population expansion (Slatkin & Hudson, 1991).

Table 4 Pairwise F_{ST} values for cytochrome b, D-loop, and concatenated data between sampled populations of *Leporinus piau*

	Site 9	Site 7	Site 6	Site 5
Cytochrome b				
Site 9		0.012	0.003	0.242
Site 7	0.3574		0.884	0.105
Site 6	0.2672*	0.0827		0.075
Site 5	0.0276	0.1461	0.0902	
D-loop				
Site 9		0.017	0.0003	0.156
Site 7	0.2392		0.304	0.212
Site 6	0.2363*	0.0222		0.062
Site 5	0.0221	0.0421	0.0852	
Concatenated				
Site 9		0.015	0.001	0.177
Site 7	0.3083		0.477	0.133
Site 6	0.2536*	−0.0316		0.061
Site 5	0.0248	0.0973	0.0877	

* Statistically significant ($P < 0.05$)

The haplotype networks show a clear sign of low genetic variability in *L. piau*, *M. reinhardti*, *Pi. maculatus*, and *Py. piraya*. For these four species, the haplotype networks are dominated by one or two major haplotypes that differ from the other less frequent satellite haplotypes by one or a few mutations. In *Pi. maculatus*, 77% of individuals sampled throughout the river share the same haplotype. In *L. piau* and *M. reinhardti*, there are two haplotypes that

account for over 50% of the individuals. In *Py. piraya*, 45% of fishes share the most common haplotype. The haplotype network for *Pr. argenteus*, however, depicts a different scenario. In this species, there are no more than four individuals of the same haplotype in any population. Higher genetic variability is corroborated by the total number of haplotypes ($n = 89$), and a lower admixture between river sites, where haplotype frequencies range between 0.009 and 0.035.

Demographic analyses, which were performed per species because of the general lack of genetic structure, showed signs of past population expansion for all species at different times (Figs. 1, 2). Negative values of Tajima's D and/or Fu's F_S also indicated populations expansion for all species. Furthermore, growth rate (g) values corroborate with the indications of population expansion in all species (Table 2). θ values are described in Table 2.

The graphics of mismatch distributions (MD) for these studied species suggest complex demographic histories (Fig. 1). While most species present signal of recent expansion, likely following a bottleneck event, some also depict a bimodal distribution of pairwise comparisons. Thus, for *Pi. maculatus*, *Py. piraya*, and *L. piau*, there are evidences of population expansion subsequent to a bottleneck event associated with secondary contact. The pattern of the MD graphic for *Pr. argenteus* reveals a unique demographic history, suggesting expansion but with no signal of bottleneck or secondary contact as the other species. Calculations of time since expansion indicate that species started expanding at different times, from around 1.4 Mya (*Pr. argenteus*) to 33 kyr ago (*M. reinhardti*) (Online Resource Table 2). Reconstruction of effective population size through time with the Bayesian Skyline Plots (BSP) supports the general demographic processes revealed by the MD analysis (Fig. 2).

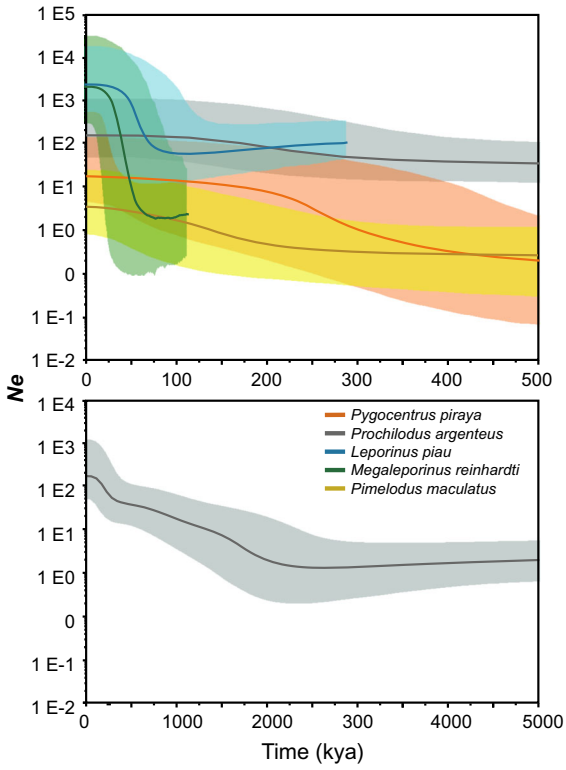


Fig. 2 Bayesian Skyline Plots (BSP) showing changes in population size through time (in thousand years). *Each color* represents a different species according to the legend. The *second panel* is a wider view of the BSP of *Pr. argenteus*

Microsatellite data

We analyzed 147 individuals of *Pi. maculatus* in total. Number of alleles per locus varied from 5 (Pm08) to 42 (Pm05 and Pm06). Observed and expected heterozygosity varied from 0.44 to 1.00 and from 0.44 to 0.97, respectively (Table 5). None of the populations showed significant deviation from Hardy–Weinberg expectations, or the presence of null alleles.

Congruently with mitochondrial data, microsatellite analyses of F_{ST}, R_{ST}, and AMOVA indicate no genetic structure between the nine sites sampled. Pairwise F_{ST} and R_{ST} values were low and ranged from −0.03 to 0.02, and −0.11 to 0.08, respectively and are not significant (Online Resource Table 3). AMOVA revealed that all variation is found within individuals (109.17%). Bottleneck test indicates excess of heterozygosity (Wilcoxon test, $P < 0.05$),

Table 5 Summary statistics for microsatellite data of *Pimelodus maculatus* for all 9 sites sampled

	n	A	H_o	H_e
1	19	15.4	0.84	0.83
2	6	7.2	0.83	0.80
3	19	15.0	0.86	0.85
4	20	16.0	0.83	0.82
5	6	6.3	0.72	0.75
6	18	15.0	0.86	0.82
7	20	15.6	0.86	0.83
8	20	16.6	0.87	0.87
9	20	15.0	0.85	0.82

n number of individuals, A number of alleles, H_o observed heterozygosity, H_e expected heterozygosity

indicating that the species was subjected to an event of sudden population size contraction.

Discussion

We found common genetic and historical patterns in fish populations of the São Francisco River: four out of the five species present low genetic variability; most species do not show any evidence of genetic structure associated with fragmentation by river dams; and all species show signs of historical population expansion—four with evidence of bottleneck events and secondary contact of formerly allopatric populations. These finds are discussed in detail below.

Low genetic variability in fishes of the São Francisco River

The summary statistics values found in four of the species in this study (*L. piau*, *M. reinhardti*, *Pi. maculatus*, and *Py. piraya*) reveal a comparatively low genetic variability of fishes along the São Francisco River. These species lineages have very different biogeographic and phylogeographic histories, having different origin times and locality (Hubert & Renno, 2006; Hubert et al. 2007a, b; Lundberg et al., 2011), and yet all four have similar low genetic variability in the São Francisco River.

Both mtDNA and microsatellite markers (the latter for *Pi. maculatus*) corroborate with the low genetic variability. Because the D-loop region is known for its high diversity in fish populations (e.g., Bay et al., 2004; Terencio et al., 2012), the low diversity found here is surprising. Martins et al. (2003), for instance, found high values of genetic variability in *L. elongatus* in several tributaries of the Paraná River in Brazil based on the same molecular marker. Other studies also found indication of higher genetic variability with other markers for fishes of the same families studied here. For instance, Ramos et al. (2012) found high genetic variability in populations of *L. elongatus* in the Paranapanema River and Batista & Alves-Gomes (2006) found high levels of haplotype diversity in populations of *Brachyplatystoma rousseauxii* in the Amazon Basin, which belongs to the same family of the catfish species studied here (Pimelodidae).

Prochilodus argenteus, contrastingly, showed a much higher genetic diversity compared to the other species (Table 2). Previous studies suggest that this is the norm for species of *Prochilodus* (Sivasundar et al., 2001; Rueda et al., 2013), and, therefore, the higher genetic diversity found here is likely associated to inherent characteristics of the fish species that can affect diversity, such as reophily, high migration habits, and vagility (e.g., Ramella et al., 2006; Oliveira et al., 2009). Ortí et al. (2005), for example, found similarly high values of genetic diversity in four species of *Prochilodus* from three river basins in South America: Magdalena, Amazonas and Paraná. *Prochilodus* species typically undertake long-distance migrations, swimming up to 2000 km along the river from the feeding grounds to the spawning areas (Boncompagni-Junior et al., 2013). Several studies have shown the effects of ecological and life-history traits, including migration and mobility on the variation in the genetic diversity among species on a wide range of animal and plant species (e.g., Bazin et al., 2006; Leffler et al., 2012; Romiguier et al., 2014). Recently, Dalongeville et al. (2016) have found that microsatellite and mitochondrial DNA diversity are lower for less mobile species of marine fishes and this genetic diversity increased with the degree of mobility of the species.

Lack of genetic structure between populations separated by dams

Despite the different genetic markers used as well as the differences in migratory potential and in the genetic diversity of the species studied, most showed no sign of genetic structuring along the river, which spans over 2700 km. Analyses of microsatellite data (from *Pi. maculatus*) also attest to the lack of genetic differentiation along the river. The lack of differentiation in such a long distance is particularly interesting for *Py. piraya*, which is a non-migratory species (Fink, 1993; Gomes & Verani, 2003). The absence of genetic structure is usually interpreted as an indication of high gene flow (Slatkin, 1987), and a review of conservation genetics and biodiversity of Neotropical freshwater fishes (Piorski et al., 2008) indicates that most studies of long-distance migration have shown weak differentiations between populations. Noteworthy is that in the case of the present study, populations are physically separated by several dams along the river, all of which allow no connectivity between fragments of river in the downstream–upstream

direction, since they are not equipped with fish ladders or any other type of structure or equipment for translocation.

The lack of genetic structure in rivers that have isolated sections due to the construction of dams is not unique to the São Francisco. Also in Brazil, Almeida et al. (2003) found no genetic structure in the population of *Pi. maculatus* using RAPD from the Tiête River, which has nine dams along its length. Similarly, Ribolli et al. (2012) found no structure for the same species in the Uruguay River based on microsatellite data. Ramos et al. (2012) found no evidence of genetic structure between populations of *Leporinus elongatus* in the Canoas Hydrological Power Complex in the Paranapanema River.

However, this lack of genetic differentiation is not a consensus in river dam systems. For example, Sanches et al. (2012) found populations of *Brycon orthotaenia* to be genetically differentiated in fragments of the São Francisco River downstream from the Três Marias dam. Around the world, other authors have also found evidence of population structure in rivers with impoundments associated with hydropower systems (e.g., Neraas & Spruell, 2001; Meldgaard et al., 2003; Laroche & Durand, 2004; Nguyen, 2008). Notably, in most of these examples, the fragment sizes between dams are much smaller compared to the ones in this study.

Several studies have also used a combination of mtDNA and microsatellites to assess the genetic structure of fish populations that are separated by dams. Ferreira et al. (2017) found no evidence of genetic structuring in populations of *Prochilodus lineatus* along the Brazilian Paraná River; Carlsson et al. (2004) used both markers in bluefin tuna populations of the Mediterranean Sea and they corroborate in the detection of population structure, among many others (e.g., Ramstad et al., 2007; Bryja et al., 2010; da Silva et al., 2015). However, we highlight the relatively short period since the construction of the dams and the influence this may have on our results.

Influence of river topography on the geographic distribution of genetic diversity

Every dam causes unique environmental changes and, therefore, the nature and/or existence of its consequence to the ichthyofauna can be highly site-specific. It is logically reasonable to expect that isolation should result in genetic drift and differentiation between fragments along the river. This, however, was not observed for the majority of the studied species in the São Francisco. A tentative explanation is strongly based on the physiography of this river, which differs from the general patterns observed for large rivers elsewhere. In most watersheds, the heads of the rivers are located in highlands, where dams are usually concentrated due to the high hydropower potential, and subsequently run on the lowlands, often for long distances, before reaching the ocean. Similarly, the São Francisco River has a relatively short portion in highlands, located above the Três Marias hydroelectric power plant, but contrary to the pattern described

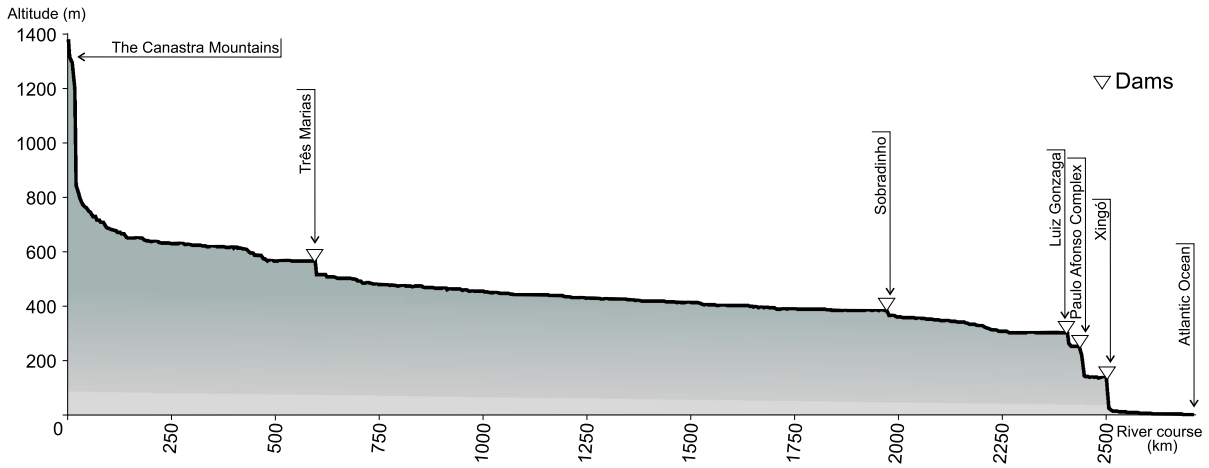


Fig. 3 Longitudinal profile of the São Francisco River, from the head of the river on the Canastra Mountains to the Atlantic Ocean. *Upside down triangles* indicate where impoundments are located

above, it flows for most of its course (about 1600 km) on a plateau and drops rapidly from about 500 m high into a short coastal plain (Fig. 3).

Older impoundments in the São Francisco River, such as Três Marias (completed in 1962) and Sobradinho (completed in 1979) (Fig. 3) resulted in upstream and downstream fragments of large size that likely housed large population of fish species (Ellstrand & Elam, 1993; Frankham, 1996; Willi et al., 2007). Despite the lack of upstream genetic flow between these fragmented populations, the size of the resulting populations in each respective fragment was apparently large enough to maintain panmixia, and delay or avoid the expected effects of random losses in the genetic profile. On the other hand, the most recent impoundments (constructions completed between 1988 and 1994), located at the end of the plateau in the high declivity portion that connects to the coastal plain (Fig. 3), resulted in smaller fragments that are apparently too recent to allow detection of any effect associated to genetic drift.

Influence of climate on historical demography

Analyses of historical demography based on mitochondrial DNA suggest that most studied species have undergone a phase of population expansion which is possibly associated with one or more bottleneck events that reduced the level of genetic variability of the populations (see Maruyama & Fuerst, 1985). We found evidence of expansion in the patterns of mismatch distribution, Bayesian Skyline Plots and star-like signature of haplotype networks, and Fu's F_S. Demographic analyses of microsatellite data from *Pi. maculatus* support a recent bottleneck signal in the São Francisco River.

The São Francisco River is thought to have originated in the mid Cretaceous after the separation of the African and South American continents (Valadão & Dominquéz, 1994; Potter, 1997); at about the same time, most of the other major rivers that compose the modern Brazilian basins were formed (Potter, 1997; Almeida, 2004; Hubert & Renno, 2006). Although the Brazilian Shield, where the São Francisco is located, has been geomorphologically stable for the past 10 My (Lundberg et al., 1998), extensive dispersal occurred among all other major Neotropical drainages (Montoya-Burgos, 2003; Pereira et al., 2013; Boeger et al., 2015). For instance, the speciation event that gave rise to *Py. piraya* and *Py. nattereri* is an example of dispersal between South American basins. The divergence occurred around 2 Mya from a lineage putatively dispersed from the Parana + Amazon basins to the São Francisco river. The latter, in turn, originated from a dispersal event from the Orinoco basin (*P. cariba*) about 7 Mya (Hubert et al., 2007a). The fish community of the São Francisco River is, thus, a complex composite of native and allochthonous lineages.

Based on the τ values from the mismatch distribution analyses, population expansion of all species date within the limits of the Pleistocene epoch (2,588,000–11,700 years ago), which spans the world's most recent period of repeated glaciations. The Pleistocene was marked by a series of climatological fluctuations that fragmented and reconnected different habitats (Hewitt, 2000). During this epoch, species of various genera suffered retractions and subsequent population expansions, such as terrestrial mammals, fishes, and arthropods (e.g., Carini & Hughes, 2004; Huey et al., 2006; Hughes et al., 2009).

We propose that fish populations of the São Francisco River were able to reestablish in larger numbers, recuperating genetic diversity, following the phase of extreme climatic instability, resulting in the population expansion signals detected herein with genetic data in fish species. The climatic oscillation of the Pleistocene, which included periods of very dry weather, could have changed the hydrological flow regimes of the São Francisco. According to Domingues (1948), the Pleistocene brought intense sedimentation to the São Francisco River, and Tricart (1974) suggested that the presence of sand dunes along the river at the time is evidence for a drier climate than present. A drier climate implies contraction of the available habitat to fishes, which could have drastically reduced the population size of the species. The evidence of secondary contact of formerly allopatric populations in both the haplotype network and Mismatch analyses strongly suggest fragmentation of the populations of studied fishes, most likely by reduction in flow during dry periods. Reestablishment of flow during wet periods would provide the opportunity of mixing of isolated populations of each species. These general patterns were, however, not observed for *Pr. argenteus*. As previously mentioned, inherent specific abilities of the species, such the great ability to overcome natural barriers, such as water falls and

rapids, could maintain genetic flow between fragments within the river channel, hiding effects derived from reduction of the river flow during dried periods.

Several studies in the Amazon basin have associated signs of population expansion of riverine ichthyofauna with the consequences of climatic oscillation of the Pleistocene (Hrbek et al., 2005; Hubert & Renno, 2006; Hubert et al., 2007a, b). Here, we show that the same applies to the São Francisco basin, which would imply that the effects of the arid climate of the epoch influenced a large part of the South American basins.

Conclusion

River physiography and past climate have dictated the evolutionary fate of the fish species in the San Francisco River. Common patterns of low genetic variability and demographic expansion show the influence of climate and river physiography. Our study did not find genetic structure in the fish populations occupying the river fragments. The absence of genetic structure could attest to the lack of influence of the river dams in the fish species studied here; however, we highlight the need of future research. The present study thus illustrates the usefulness of considering landscape structure, historical events, and inherent biological characteristics of the taxa in question to better understand processes generating the spatial patterns of genetic variability and population historical demography.

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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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