Neotropical Ichthyology, 11(3):625-636, 2013

The roles of marginal lagoons in the maintenance of genetic diversity in the Brazilian migratory fishes *Prochilodus argenteus* and *P. costatus*

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The rio São Francisco basin contains many endemic species, such as *Prochilodus argenteus* and *P. costatus*, which have great commercial importance. However, information about the main recruitment sites and genetic studies containing extensive sampling of these species are scarce. To investigate the roles of the marginal lagoons in the maintenance of genetic variability and in the population structure, we analyzed six microsatellite *loci* in nine sampling groups of *P. argenteus* and five sampling groups of *P. costatus*. Our results showed high levels of genetic variability and low values of genetic differentiation for *P. argenteus* (F_{ST} = 0.008, P < 0.05) and for *P. costatus* (F_{ST} = 0.031, P < 0.05). In addition, high values of gene flow combined with a small genetic distance suggest the presence of a single population for each species in the middle rio São Francisco basin. Moreover, putative migration routes involving marginal lagoons during the reproductive season could be detected, confirming the importance of these nurseries in the lifecycle of these species. Our results also indicate the necessity of adequate management of the fish resources and the conservation of the floodplains in the rio São Francisco basin.

A bacia do rio São Francisco contém muitas espécies endêmicas, tais como *Prochilodus argenteus* e *P. costatus*, os quais têm grande importância comercial. Entretanto, informações sobre as principais áreas de recrutamento e estudos genéticos contendo uma extensa amostragem dessas espécies no rio São Francisco são escassas. Para investigar o papel das lagoas marginais na manutenção da variabilidade genética e na estruturação populacional dessas espécies, nós analisamos seis *loci* microssatélites em nove grupos amostrais de *P. argenteus* e cinco grupos amostrais de *P. costatus*. Nossos resultados revelaram altos níveis de variabilidade genética para ambas as espécies e valores baixos de diferenciação genética para *P. argenteus* (F_{ST} = 0.008, P < 0.05) e *P. costatus* (F_{ST} = 0.031, P < 0.05). Adicionalmente, valores altos de fluxo gênico combinados com a distância genética baixa sugerem a presença de uma única população para cada espécie no médio rio São Francisco. Possíveis rotas migratórias envolvendo lagoas marginais durante o período reprodutivo puderam ser detectadas, confirmando a importância das lagoas marginais no ciclo de vida dessas espécies. Nossos resultados também indicaram a necessidade de um manejo adequado dos recursos pesqueiros e a conservação das várzeas na bacia do rio São Francisco.

Key words: Conservation genetics, Fish migration, Microsatellite, Nursery area, Rio São Francisco basin.

Introduction

The rio São Francisco basin constitutes an area that covers 619,543 km², *ca.* 7.5% of Brazil, with ecological domains ranging from Atlantic rainforest to Cerrado and Caatinga. In recent years, human impact, including the destruction of wetlands and marginal lagoons for agriculture and the construction of large dams, such as the Três Marias dam (TMD), for hydroelectric power generation, has affected the basin (Sato *et al.*, 1987; Menezes, 1996). The central portion of the rio São Francisco basin, including Três Marias region, has greater investment in the fishery (Camargo & Petrere, 2001) and it is characterized by the presence of many floodplains and marginal lagoons. These marginal lagoons are important nursery habitats for migratory species recruitment because they provide an ideal habitat for the growth of juveniles with abundant food and relatively high temperatures (Moojen, 1940; Pompeu & Godinho, 2003). Unfortunately, important aspects of the fish migrations in the

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rio São Francisco, such as feeding and reproductive habitats, shoal composition and structure, homing and distances traveled between habitats, remain poorly studied (Sato & Godinho, 2003).

Prochilodontids occur abundantly in the major drainages of South America and are considered one of the most important components of the commercial and subsistence fisheries in Neotropical freshwater environments (Lowe-McConnell, 1975; Goulding, 1981; Vari, 1983; Castro & Vari, 2004). The family Prochilodontidae comprises three genera (*Ichthyoelephas*, *Prochilodus*, and *Semaprochilodus*) with highly restructured lips, teeth and jaws; these characteristics distinguish this family externally from all other characiforms (Castro & Vari, 2004). *Prochilodus argenteus*, an endemic species from the rio São Francisco basin, nowadays introduced in other Neotropical drainages (Castro & Vari, 2004), is the largest member of the Prochilodontidae family and is among the most important recreational and commercial fish species in the basin (Camargo & Petrere, 2001; Godinho *et al.*, 2003). Another endemic species, *P. costatus*, has an importance in the subsistence fishery, as one of the most captured species in that region (Camargo & Petrere, 2001).

Genetic studies using variable markers such as microsatellites and mitochondrial DNA have been conducted to understand the population structure and dynamics of a considerable number of animal species (Zhang & Hewitt, 2003). Microsatellites, or simple sequence repeats (SSRs), are codominant nuclear markers with Mendelian inheritance. Microsatellites are abundantly distributed along genomes and demonstrate high levels of allelic polymorphism (DeWoody & Avise, 2000). The molecular structure and genetic variability of microsatellites are extensively exploited in evolutionary studies of a wide variety of fish species (see Chistiakov *et al.*, 2006), including some from Neotropical freshwater (Hrbek *et al.*, 2007; Abreu *et al.*, 2009; Calcagnotto & DeSalle, 2009; Matsumoto & Hilsdorf, 2009; Pereira *et al.*, 2009; Sanches *et al.*, 2012).

Population genetic studies have been performed with both *Prochilodus* species from the rio São Francisco basin using Random Amplified Polymorphic DNA (RAPD) technique (Hatanaka & Galetti, 2003) and microsatellites (Hatanaka *et al.*, 2006; Carvalho-Costa *et al.* 2008; Sanches *et al.*, 2012; Barroca *et al.*, 2012). However, there is no knowledge about the main nursery areas of the populations of either species or the importance of the marginal lagoons in the maintenance of the genetic variability of these or other migratory species. Considering the threatened marginal regions of the middle rio São Francisco basin and the ecological and economic importance of these species, the goals of this study were to access the main recruitment sites of both species and provide a robust dataset regarding the population genetic structure of these species.

Material and Methods

Sampling sites

Migratory fishes reproduce in the mainstream or tributaries of the rio São Francisco basin, and their eggs and larvae are carried out downstream reaching marginal lagoons (Moojen, 1940) that stay unconnected of the mainstream in the dry season. Thus, samples collected in marginal lagoons were originated from spawning sites located upstream to the lagoons (*e.g.*, samples collected in marginal lagoons from rio Paracatu was derived from eggs spawned only in upstream of the rio Paracatu). Thus, we consider samples from different lagoons of the same tributary as a single sampling group (Fig. 1, Table 1). We divided samples from the mainstream of rio São Francisco in the Três Marias region in three sampling groups to test previous ecological and genetic hypotheses that suggested a population differentiation among them (Godinho & Kynard, 2006; Hatanaka *et al.*, 2006). Additionally, individuals from Três Marias dam were subdivided in two different units (dry and rainy seasons) trying to check the presence of different migrant shoals inhabiting the same spawning site.

A total of 273 individuals, that were subdivided into nine sampling groups of *Prochilodus argenteus*, were collected between July 2008 and February 2010 along the middle rio São Francisco basin. Five sampling groups were from the marginal lagoons of their tributaries, rio Carinhanha (CAR, n = 25), rio Jequitaí (JEQ, n = 33), rio Paracatu (PAR, n = 33), rio Urucuia (URU, n = 33) and rio das Velhas (VEL, n = 28); one sampling group was from the marginal lagoons of the

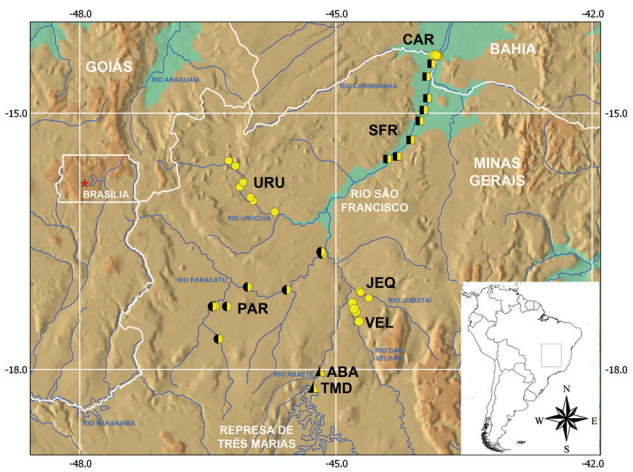

Fig. 1. Map showing the central portion of the rio São Francisco basin and the distribution of the samples of *Prochilodus argenteus* (yellow) and of *P. costatus* (black). The circles represent marginal lagoons from tributaries, squares represent marginal lagoons from the rio São Francisco, and triangles represent places in the mainstream rio São Francisco in the Três Marias region. ABA = rio Abaeté; CAR = rio Carinhanha lagoons; JEQ = rio Jequitaí lagoons; PAR = rio Paracatu lagoons; SFR = rio São Francisco lagoons; URU = rio Urucuia lagoons; VEL = rio das Velhas lagoons; TMD = Três Marias Dam.

Table 1. Samples of *Prochilodus argenteus* and *P. costatus* from the rio São Francisco basin collected between July/2008 and July/2010. Samples from TMDr and ABAr were collected during the rainy season (November to February), and samples from TMDd were collected during the dry season (March to October). ABAr = rio Abaeté at rainy season; CAR = rio Carinhanha lagoons; JEQ = rio Jequitaí lagoons; PAR = rio Paracatu lagoons; SFR = rio São Francisco lagoons; URU = rio Urucuia lagoons; VEL = rio das Velhas lagoons; TMDd = Três Marias Dam at dry season; TMDr = Três Marias Dam at rainy season.

Locality	Sampling	Geographical coordinates	Abbreviation	*P. argenteus* (n)	*P. costatus* (n)
Três Marias Region	Downstream to	18º12'32"S 45º15'41"W	TMDd	33 (dry)	33 (dry)
	Três Marias Dam		TMDr	22 (rainy)	26 (rainy)
	Downstream to rio Abaeté	18º01'01"S 45º10'56"W	ABAr	33 (rainy)	31 (rainy)
Rio São Francisco Lagoons	Lagoa Lapinha	14º57'45"S 43º58'10"W	SFR	33	33
	Lagoa Grande	15º30'27"S 44º17'04"W			
	Lagoa Beirada	14º34'19"S 43º56'15"W			
	Lagoa Lavagem	14º49'30"S 43º55'46"W			
	Lagoa Maris	14º25'17"S 43º52'42"W			
	Lagoa Ipueira	15º32'11"S 44º23'44"W			
	Lagoa Cajueiro	15º05'29"S 44º01'07"W			
	Lagoa Banguê	15º18'46"S 44º07'30"W			
Rio Das Velhas Lagoons	Lagoa Periperi	17º26'14"S 44º43'40"W	VEL	28	-
	Lagoa Tiririca	17º13'33"S 44º48'27"W			
	Lagoa Capivara	17º18'50"S 44º47'16"W			
	Lagoa Maria Joana	17º19'30"S 44º45'57"W			
	Lagoa do Saco	17º17'21"S 44º47'08"W			
Rio Jequitaí Lagoons	Lagoa Cascalho	17º09'55"S 44º36'54"W	JEQ	33	-
	Lagoa Lagoão	17º04'39"S 44º43'30"W			
	Lagoa Tapera	17º05'46"S 44º42'40"W			
Rio Paracatu Lagoons	Lagoa Piranhas	16º38'54"S 45º09'39"W	PAR	33	33
	Lagoa Faz. Sertaneja	17º04'07"S 45º34'42"W			
	Lagoa Ferradura	17º02'03"S 46º02'03"W			
	Lagoa Redonda	17º15'43"S 46°16'38"W			
	Lagoa Neves	17º38'33"S 46º22'38"W			
	Lagoa Água Limpa	16º37'24"S 45º10'20"W			
	Lagoa Názara	17º15'43"S 46º26'38"W			
Rio Urucuia Lagoons	Lagoa Cinquenta	16º09'18"S 45º43'01"W	URU	33	-
	Lagoa Sucupira	15º37'01"S 46º10'33"W			
	Lagoa Encantada	16º01'34"S 45º58'15"W			
	Lagoa Silvério	15º59'06"S 46º00'07"W			
	Lagoa Taboada	15º33'22"S 46º15'30"W			
	Lagoa Capão Grosso	15º51'47"S 46º07'36"W			
	Lagoa Piranhas	15º48'32"S 46°05'14"W			
Rio Carinhanha Lagoons	Lagoa Água Branca	14º19'08"S 43º51'18"W	CAR	25	-
	Lagoa Peixe Gordo	14º20'09"S 43º47'48"W			
	Lagoa Pau Branco	14º19'28"S 43º48'28"W			
	Lagoa Morrinhos	14º19'39"S 43º48'55"W			
	Lagoa Rio Velho	14º19'22"S 43º49'35"W			
Total				273	156

rio São Francisco (SFR, n = 33); two sampling groups were collected downstream of the Três Marias dam, one during rainy season (TMDr, n = 22) and one during dry season (TMDd, n = 33); and one sampling group was collected downstream rio Abaeté during the rainy season (ABAr, n = 33) (Fig. 1, Table 1). In reference to *P. costatus*, a total of 156 individuals subdivided into five sampling groups were collected along the middle rio São Francisco basin. One sampling group was collected in the marginal lagoons of rio Paracatu (PAR, n = 33); one sampling group was from marginal lagoons of the rio São Francisco basin (SFR, n = 33); two sampling groups were collected downstream of the Três Marias dam, one group was obtained during the rainy season (TMDr, n = 26) and one was obtained during the dry season (TMDd, n = 33); and one sampling group was from downstream rio Abaeté during the rainy season (ABAr, n = 31) (Fig. 1, Table 1). All of the fish used for this study were collected in accordance with Brazilian laws under a permanent scientific collection license. Fin clips or muscles were fixed and preserved in 95% ethanol and deposited in the Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista "Júlio de Mesquita Filho", câmpus de Botucatu, São Paulo, Brazil.

Molecular analyses. Genomic DNA was extracted using a saline solution with proteinase K described by Aljanabi & Martinez (1997). Amplifications were carried out using six microsatellite *loci* developed by Barbosa *et al.* (2008) for *Prochilodus argenteus*: Par66, Par69, Par71, Par76, Par83 and Par85; the same *loci* were used for *P. costatus*, exception the *locus* Par80 was used instead of Par76 (monomorphic for this species). The reactions were performed in a total volume of 12.5 µl with 7.3 µl of ultrapure H_2O, 1.25 µl of Taq DNA buffer (10X), 0.45 µl of $MgCl_2$ (50 mM), 0.4 µl of dNTP (2 mM), 0.5 µl

of each primer (10 μM), 0.1 μl of Platinum Taq DNA Polymerase enzyme (Invitrogen; www.invitrogen.com) (5 U/μl) and 2.0 μl of genomic DNA (10-50 ng). Polymerase chain reactions (PCR) consisted of an initial denaturation step (5 min at 95ºC) followed by 35 cycles of chain denaturation (30 s at 95ºC), primer hybridization (30 s at 52-54ºC) and nucleotide extension (30 s at 72ºC). After these cycles, a final extension was performed at 72ºC for 10 minutes. Microsatellite amplified fragments were subjected to electrophoresis on 6% polyacrylamide gels for approximately 12 hours at 150 v. The gels were then stained with silver nitrate. The allele lengths were identified by reference to a 10 bp ladder (Invitrogen) using Kodak Digital Science 1D software.

Population genetic analyses

Allele numbers, private allele counts and gene flow ($Nm = 0.25(1 - F_{ST})/ F_{ST}$) were obtained with Popgene 1.32 (Yeh & Boyle, 1997). To construct and export the matrices, we used GenAlex 6.1 (Peakall & Smouse, 2006). The expected and observed heterozygosity (He, Ho), inbreeding coefficient F_{IS} (Wright, 1951) were obtained with Arlequin 3.1 (Excoffier *et al.*, 2005). The software Microchecker 2.2.1 (van Oosterhout *et al.*, 2004) using the equation 2 of Brookfield (1996) was used to infer the most probable cause of HWE departures produced for null alleles, stuttering and large-allele dropout. The exact test of the Hardy-Weinberg Equilibrium (HWE) (P-value < 0.05) were calculated with 1 million generations of Marchov chain of Monte Carlo (MCMC) with 100,000 'burn-ins' using Arlequin 3.1 (Excoffier *et al.*, 2005). Significance levels for the HWE, F- and R-statistics tests were adjusted using Bonferroni corrections (Rice, 1989). The linkage disequilibrium was calculated for all *loci* using Genepop 4.0.1 online (Raymond & Rousset, 1995).

The F_{ST} (Wright, 1951) assuming the infinite allele model (IAM, Kimura & Crow, 1964), the R_{ST} (Slatkin, 1995) assuming the stepwise mutation model (SMM, Kimura & Otha, 1978) and the analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992) calculated for all *loci* and for all populations with 16,000 permutations (Guo & Thomson, 1992) were performed using the Arlequin 3.1 program (Excoffier *et al.*, 2005). Initially, the samplings of *Prochilodus argenteus* were considered to be a single group for the AMOVA. Then, the samplings were subdivided into two groups: group 1 (adult fishes collected at mainstream of Três Marias region - ABAr, TMDd, and TMDr) and group 2 (juveniles collected inside unconnected marginal lagoons - SFR, VEL, JEQ, PAR, URU, and CAR). Similarly, the populations of *P. costatus* were first considered to be a single group and then subdivided into two groups: group 1 (adult fishes collected at mainstream of Três Marias region - ABAr, TMDd, and TMDr) and group 2 (juveniles collected inside unconnected marginal lagoons - LSF and PAR). This criterion was adopted to answer where are the main recruitment areas of the fishes found in the mainstream of Três Marias region (the most fishery activity of the São Francisco basin). In addition, rates of chord genetic distance (Cavalli-Sforza & Edwards, 1967) corrected for sample size were calculated with Geneclass2 (Piry *et al.*, 2004), and these values were used to construct an unrooted neighbor-joining tree (Saitou & Nei, 1987) using the software Paup 4.0b10 (Swofford, 2003).

Results

Prochilodus argenteus

Genotypes of 273 specimens of nine sampling groups were analyzed. All microsatellite *loci* were highly polymorphic. A total of 99 alleles were detected for all *loci* in all populations. The number of alleles per *locus* ranged from three (Par76) to 24 (Par85), with an overall average of 9.6 alleles (Table 2). Nineteen private alleles were found in low frequency (≤ 0.05) with the allele 239 (Par85 - URU), presenting a frequency of 0.052. Ho and He ranged from 0.379 (Par69) to 0.937 (Par71) and 0.473 (Par69) to 0.951 (Par85), respectively (Table 2). From the 54 pairwise F_{IS} estimates, 41 showed positive values (heterozygote deficiency), and only 13 showed negative values (an excess of heterozygotes). Positive F_{IS} values indicating heterozygote deficiency was predominant in all *loci* with a positive overall average ($F_{IS} = 0.123$, $P < 0.008$) (Table 2). Only six out of 54 HWE tests performed for *P. argenteus* (6 microsatellite *loci* in 9 sampling groups) were significant. In all departure occurrences, the significant values of disequilibrium were associated with the presence of null alleles (Table 2). Null alleles were detected in 14 estimates and stutters were verified only in Par85 in the population TMDr. No evidence of linkage disequilibrium was detected in any *loci* ($P > 0.05$).

Low indices of F_{ST} obtained by means of pairwise values among local populations of *Prochilodus argenteus* for all *loci* were significantly detected ($F_{ST} = 0.008$, $P < 0.05$), with pairwise F_{ST} for all *loci* ranging from -0.054 (ABA - JEQ) to 0.007 (ABAr - TMDd) (Table 3, above diagonal), clearly indicating an absence of population structure. Additionally, the overall R_{ST} also showed significant lower values ($R_{ST} = 0.059$, $P < 0.05$), ranging from -0.460 (ABAr - PAR) to 0.184 (TMDr - PAR) (Table 3, bellow diagonal). When we considered all specimens to be a single group (see Material and Methods), the AMOVA values (Table 4) revealed that only 0.82% of the total genetic variance was due to differences among populations ($F_{ST} = 0.008$, $P < 0.05$; $R_{ST} = 0.069$, $P < 0.05$) and 99.18% of the genetic variation within populations. When we considered the putative existence of two groups (see Material and Methods), the hierarchical AMOVA also revealed that 99.13% of the total variance was found within populations (values are shown in the Table 4).

The gene flow parameter Nm was calculated from the mean F_{ST} value. The mean value obtained was Nm = 9.318,

Table 2. Summary of the six microsatellite *loci* for each analyzed sampling of *Prochilodus argenteus*. *N,* number of individuals; A, number of alleles; P, number of private alleles; Ho, observed heterozygosity; He, expected heterozygosity; F_{IS}, inbreeding coefficient; HWE, probability test for deviation from expected Hardy-Weinberg proportions, *, *P-value* = 0.05 (adjustment Bonferroni correction $P \leq 0{,}008$; $K = 6$); *r*, null alleles frequency per *loci*. ABAr = rio Abaeté at rainy season; CAR = rio Carinhanha lagoons; JEQ = rio Jequitaí lagoons; PAR = rio Paracatu lagoons; LSF = rio São Francisco lagoons; URU = rio Urucuia lagoons; VEL = rio das Velhas lagoons; TMDd = Três Marias Dam at dry season; TMDr = Três Marias Dam at rainy season.

Sampling	*Loci* Par66	Par69	Par71	Par76	Par83	Par85
ABAr						
N	30	32	22	30	31	21
A/P	09/1	5/0	10/0	5/0	12/0	15/1
Ho	0.733	0.656	0.682	0.666	0.677	0.428
He	0.787	0.641	0.860	0.732	0.844	0.927
F_{IS}	0.069	-0.023	0.211	0.091	0.200	0.543
HWE	0.473	0.026	0.018	0.565	0.132	0.000*
r	-	-	0.515	-	0.195	0.641
TMDd						
N	30	31	19	32	26	28
A/P	8/0	07/1	12/2	4/0	16/1	18/0
Ho	0.833	0.451	0.789	0.687	0.769	0.750
He	0.799	0.568	0.901	0.688	0.904	0.947
F_{IS}	-0.043	0.208	0.127	0.001	0.151	0.211
HWE	0.488	0.079	0.079	0.533	0.056	0.002*
r	-	-	-	-	-	0.309
TMDr						
N	20	19	19	22	22	19
A/P	7/0	4/0	10/0	3/0	13/1	16/0
Ho	0.800	0.526	0.737	0.727	0.818	0.737
He	0.774	0.621	0.850	0.669	0.860	0.939
F_{IS}	-0.034	0.156	0.137	-0.089	0.050	0.219
HWE	0.640	0.033	0.426	0.326	0.211	0.048
r	-	-	-	-	-	0.292
LSF						
N	27	29	26	27	28	30
A/P	7/0	6/0	12/0	4/0	13/0	20/0
Ho	0.778	0.379	0.807	0.518	0.785	0.933
He	0.746	0.646	0.890	0.740	0.865	0.951
F_{IS}	-0.042	0.417	0.094	0.303	0.093	0.019
HWE	0.834	0.009	0.099	0.038	0.831	0.672
r	-	0.356	-	0.392	-	-
VEL						
N	26	17	25	25	14	20
A/P	7/0	06/1	8/0	4/0	9/0	17/0
Ho	0.807	0.588	0.760	0.640	0.643	0.650
He	0.767	0.693	0.851	0.703	0.830	0.939
F_{IS}	-0.054	0.155	0.109	0.091	0.232	0.313
HWE	0.469	0.230	0.697	0.664	0.092	0.000*
r	-	-	-	-	-	0.495

Sampling	*Loci* Par66	Par69	Par71	Par76	Par83	Par85
JEQ						
N	32	33	26	14	18	28
A/P	7/0	7/0	11/0	4/0	10/1	19/1
Ho	0.875	0.636	0.807	0.643	0.611	0.750
He	0.789	0.624	0.865	0.717	0.839	0.946
F_{IS}	-0.110	-0.018	0.068	0.106	0.278	0.210
HWE	0.771	0.881	0.009	0.262	0.088	0.012
r	-	-	-	-	0.652	0.308
PAR						
N	33	32	25	29	24	27
A/P	08/1	7/0	15/1	4/0	9/0	21/3
Ho	0.848	0.656	0.840	0.483	0.708	0.740
He	0.823	0.693	0.916	0.678	0.834	0.951
F_{IS}	-0.030	0.054	0.085	0.291	0.153	0.224
HWE	0.051	0.258	0.125	0.094	0.048	0.000*
r	-	-	-	0.319	-	0.349
URU						
N	33	29	16	17	31	29
A/P	7/0	5/0	8/0	4/0	15/1	16/1
Ho	0.818	0.379	0.937	0.706	0.838	0.482
He	0.823	0.565	0.863	0.693	0.890	0.923
F_{IS}	0.006	0.333	-0.089	-0.018	0.059	0.481
HWE	0.341	0.001*	0.636	0.545	0.019	0.000*
r	-	0.334	-	-	-	0.385
CAR						
N	23	21	21	24	21	21
A/P	9/0	5/0	12/2	4/0	13/0	16/0
Ho	0.869	0.523	0.714	0.583	0.762	0.809
He	0.793	0.473	0.877	0.703	0.888	0.933
F_{IS}	-0.098	-0.108	0.189	0.173	0.145	0.136
HWE	0.482	0.807	0.031	0.159	0.019	0.053
r	-	-	-	-	-	-

Table 3. Pairwise F_{ST} (above diagonal) and pairwise R_{ST} (bellow diagonal) values for *Prochilodus argenteus*. * $P < 0.05$, after Bonferroni correction.ABAr = rio Abaeté at rainy season; CAR = rio Carinhanha lagoons; JEQ = rio Jequitaí lagoons; PAR = rio Paracatu lagoons; SFR = rio São Francisco lagoons; URU = rio Urucuia lagoons; VEL = rio das Velhas lagoons; TMDd = Três Marias Dam at dry season; TMDr = Três Marias Dam at rainy season.

	ABAr	TMDd	TMDr	SFR	VEL	JEQ	PAR	URU	CAR
ABAr	-	0.007	-0.000	-0.005	-0.038	-0.054	0.005	-0.015	0.001
TMDd	0.079	-	-0.007	-0.004	-0.021	-0.042	-0.003	-0.018	-0.003
TMDr	-0.006	-0.007	-	0.002	-0.037	-0.052	-0.005	-0.025	-0.001
SFR	-0.012	0.024	-0.040	-	-0.037	-0.038	-0.002	-0.021	-0.001
VEL	-0.107	0.059	-0.201	-0.031	-	-0.040	-0.013	-0.062	-0.018
JEQ	-0.101	0.114	-0.117	0.006	0.031	-	-0.019	-0.027	-0.051
PAR	-0.460	0.184*	0.013	0.117*	0.015	0.138*	-	-0.023	0.002
URU	-0.032	0.020	-0.005	-0.005	-0.127	-0.032	-0.000	-	-0.028
CAR	-0.015	0.064	-0.026	0.007	-0.090	-0.013	0.016	-0.017	-

Table 4. Analysis of Molecular Variance (AMOVA) among sampling groups of *Prochilodus argenteus*. Structure tested: All samplings as a single group and divided in two groups: group 1 (Mainstream ABAr, TMDd and TMDr) and group 2 (Marginal lagoons of CAR, JEQ, PAR, SFR, URU and VEL).

Hierarchical model	Source of variation	Variance components	Percentage of variation	*F*- statistics	*P*-value
One group	Among populations	0.019	0.824	$F_{ST} = 0.008$	$P < 0.05$
	Within populations	2.395	99.175	-	-
Two groups	Among groups	0.002	0.105	$F_{CT} = 0.001$	$P > 0.05$
	Among populations within groups	0.018	0.766	$F_{SC} = 0.007$	$P < 0.05$
	Within populations	2.395	99.127	-	-

indicating an intense gene exchange occurring among all sampling groups. The gene flow values within each group (group 1: ABAr, TMDd, TMDr; and group 2: CAR, JEQ, PAR, SFR, URU, VEL) were Nm = 12.065 and Nm = 10.331, respectively. The pairwise values of chord distance were estimated and ranged from 24.0% (JEQ - PAR) to 35.3% (ABAr - URU) (Table 5), and the dendrogram of genetic distance showed a low genetic differentiation among sampling groups analyzed (Fig. 2).

Prochilodus costatus

Five sampling groups with a total of 156 specimens were analyzed. All *loci* used in the analysis were highly polymorphic with a total of 112 alleles for all *loci* in all sampling groups. The number of alleles per *locus* ranged from four (Par69) to 29 (Par85), with an overall average of 12.9 alleles (Table 6). Nineteen private alleles were found at low frequency (d" 0.04). The observed heterozygosity ranged from 0.231 (Par69) to 0.958 (Par71), and the expected heterozygosity ranged from 0.580 (Par69) to 0.960 (Par85). The F_{IS} indexes showed 24 comparisons with positive values (heterozygote deficiency) and six comparisons with negative values (excess of heterozygotes). The deficiency of heterozygotes was predominant in all *loci* with a positive overall average ($F_{IS} = 0.113$, $P < 0.008$) (Table 6). Only seven out of 30 HWE tests performed for *P. costatus* (6 microsatellite *loci* in 5 sampling groups) were significant, and of these, six estimates were associated with the presence of null alleles (Table 6). Presence of stutters was verified in the Par71 *loci* on PAR. No linkage disequilibrium was detected ($P > 0.05$), and the allelic variation was treated independently.

Our results indicate a significant absence of the population structure based on $F_{ST} = 0.031$, $P < 0.05$ and $R_{ST} = 0.044$, $P < 0.05$. In addition, pairwise F_{ST} values do not indicated structuring in all estimatives, ranging from -0.005 to 0.049. Median R_{ST} values were detected between SFR and TMDd ($R_{ST} = 0.113$, $P < 0.05$) (Table 7). Considering no predefined hierarchical models, the AMOVA showed that 96.82% of the genetic variance was found within

Table 5. Chord genetic distance among sampling of *Prochilodus argenteus*. Values in %. ABAr = rio Abaeté at rainy season; CAR = rio Carinhanha lagoons; JEQ = rio Jequitaí lagoons; PAR = rio Paracatu lagoons; SFR = rio São Francisco lagoons; URU = rio Urucuia lagoons; VEL = rio das Velhas lagoons; TMDd = Três Marias Dam at dry season; TMDr = Três Marias Dam at rainy season.

	ABAr	TMDd	TMDr	SFR	VEL	JEQ	PAR	URU	CAR
ABAr	-								
TMDd	32.7	-							
TMDr	28.4	27.3	-						
SFR	29.4	23.8	27.2	-					
VEL	31.0	31.9	29.0	25.2	-				
JEQ	26.7	30.0	27.9	24.7	27.8	-			
PAR	31.2	29.1	29.2	28.7	28.4	24.0	-		
URU	35.3	29.0	32.0	31.1	32.4	31.5	30.7	-	
CAR	29.7	28.4	28.2	27.6	26.9	29.3	29.8	31.5	-

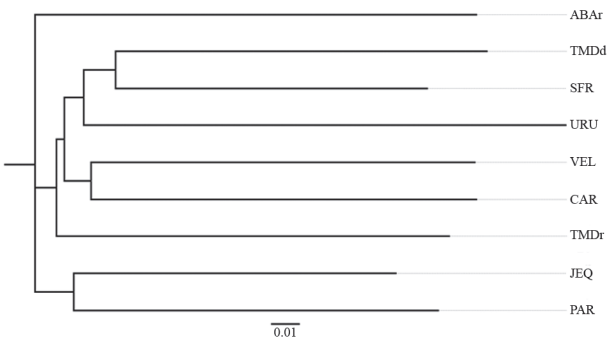

Fig. 2. Dendrogram representing the chord genetic distance among sampling groups of *Prochilodus argenteus*. ABAr = rio Abaeté at rainy season; CAR = rio Carinhanha lagoons; JEQ = rio Jequitaí lagoons; PAR = rio Paracatu lagoons; SFR = rio São Francisco lagoons; URU = rio Urucuia lagoons; VEL = rio das Velhas lagoons; TMDd = Três Marias Dam at dry season; TMDr = Três Marias Dam at rainy season.

Table 6. Summary of the six microsatellite *loci* for each analyzed sampling of *Prochilodus costatus*. *N,* number of individuals; A, number of alleles; P, number of private alleles; Ho, observed heterozygosity; He, expected heterozygosity; F_{IS}, inbreeding coefficient; HWE, probability test for deviation from expected Hardy-Weinberg proportions, *, *P-value* = 0.05 (adjustment Bonferroni correction $P \leq 0.008$; $K = 6$); *r*, null alleles frequency per *loci*. ABAr = rio Abaeté at rainy season; PAR = rio Paracatu lagoons; LSF = rio São Francisco lagoons; TMDd = Três Marias Dam at dry season; TMDr = Três Marias Dam at rainy season.

Sampling	*Loci*					
	Par66	Par69	Par71	Par80	Par83	Par85
ABAr						
N	30	28	29	30	26	31
A/P	6/0	7/0	16/1	13/0	15/0	26/5
Ho	0.833	0.535	0.827	0.600	0.692	0.935
He	0.741	0.731	0.915	0.710	0.904	0.960
F_{IS}	-0.126	0.270	0.097	0.158	0.238	0.026
HWE	0.320	0.001*	0.044	0.077	0.016	0.717
r	-	0.278	-	-	0.335	-
TMDd						
N	33	26	31	33	26	32
A/P	7/1	5/1	16/1	16/2	12/0	22/0
Ho	0.788	0.231	0.742	0.909	0.731	0.906
He	0.803	0.615	0.915	0.912	0.879	0.946
F_{IS}	0.020	0.629	0.192	0.003	0.171	0.043
HWE	0.388	0.000*	0.000*	0.367	0.087	0.019
r	-	0.511	0.187	-	-	-
TMDr						
N	25	26	24	26	25	26
A/P	7/0	4/0	16/1	10/1	16/0	17/0
Ho	0.720	0.577	0.958	0.500	0.880	0.846
He	0.734	0.580	0.930	0.843	0.908	0.939
F_{IS}	0.020	0.006	-0.031	0.411	0.032	0.101
HWE	0.332	0.607	0.000*	0.000*	0.292	0.129
r	-	-	-	0.178	-	-
LSF						
N	28	18	25	27	22	25
A/P	7/0	6/0	16/0	17/1	13/0	18/1
Ho	0.750	0.666	0.680	0.889	0.909	0.800
He	0.782	0.647	0.903	0.865	0.889	0.936
F_{IS}	0.042	-0.030	0.251	-0.028	-0.023	0.148
HWE	0.050	0.612	0.002*	0.210	0.489	0.109
r	-	-	0.431	-	-	-
PAR						
N	29	31	29	31	19	28
A/P	7/0	6/0	14/0	16/1	17/1	21/2
Ho	0.724	0.677	0.620	0.774	0.947	0.786
He	0.812	0.730	0.914	0.874	0.927	0.942
F_{IS}	0.110	0.073	0.325	0.116	-0.022	0.168
HWE	0.215	0.656	0.000*	0.074	0.988	0.057
r	-	-	0.322	-	-	0.290

populations ($F_{ST} = 0.031$, $P < 0.05$; $R_{ST} = 0.044$, $P < 0.05$). When we considered the putative existence of two groups (see Material and Methods) the estimates remain almost unchanged with the most of the variation within the populations (96.66%) with $F_{ST} = 0.033$, $P < 0.05$ (values are shown in Table 8).

The mean value of gene flow obtained for *Prochilodus costatus* was Nm = 5.911, and the values within each group (group 1: ABAr, TMDd, TMDr; and group 2: PAR, SFR) were Nm = 6.698 and Nm = 13.010, respectively, showing a higher number of migrants per generation. The chord distance ranged from 32.6% (TMDr - PAR) to 39.6% (SFR - ABAr) (Table 9). The SFR sample group has more discrepant indexes, which is shown in the dendrogram (Fig. 3).

Discussion

Genetic diversity and lack of genetic structuring

In the present study, we did not find any genetic evidence of the existence of population structure in the species *Prochilodus argenteus* or *P. costatus* from the middle rio São Francisco basin. The population differentiation indexes F_{ST} and R_{ST}, the chord genetic distance associated to a high gene flow and previous studies (Carvalho-Costa *et al.*, 2008; Sanches *et al.*, 2012) corroborate the panmictic unit model for both species. The migration behavior, which is common in prochilodontids (Castro & Vari, 2004) likely plays an important role in the continuous gene flow in these species, keeping them as a single unit in the studied area.

Table 7. Pairwise F_{ST} (above diagonal) and pairwise R_{ST} (bellow diagonal) values for *Prochilodus costatus*. * $P < 0.05$, after Bonferroni correction. ABAr = rio Abaeté at rainy season; PAR = rio Paracatu lagoons; SFR = rio São Francisco lagoons; TMDd = Três Marias Dam at dry season; TMDr = Três Marias Dam at rainy season.

	ABAr	TMDd	TMDr	SFR	PAR
ABAr	-	0.017*	0.049*	0.040*	0.039*
TMDd	0.052	-	0.031*	0.020*	0.017*
TMDr	0.001	-0.010	-	0.018*	-0.005
SFR	0.054	0.113*	0.064	-	0.005
PAR	0.012	0.062*	-0.010	0.011	-

Variability levels in microsatellites with a mean number of 9.6 alleles per *locus* in *Prochilodus argenteus* and 12.9 alleles per *locus* in *P. costatus* were similar to those reported in freshwater fish with an average of 9.1 alleles per *locus* (DeWoody & Avise, 2000) and were similar to or higher than previous studies with both *Prochilodus* species (Hatanaka *et al.*, 2006; Carvalho-Costa *et al.*, 2008; Sanches *et al.*, 2012). High genetic diversity was previously identified in *P. lineatus* (Sivasundar *et al.*, 2001), which can be explained by the capacity of *Prochilodus* species to perform great migrations and by their large occurrence along the rivers. In the rio São Francisco basin, shoals of *P. argenteus* are capable of migrating up to 1,100 km upstream to spawn (Pinheiro, 1981), while in the rio Mogi-Guaçu (upper rio Paraná basin), *P. lineatus* migrates a maximum round trip distance of 1,300 km (Godoy, 1975; Toledo *et al.*, 1986).

Significant deviations from the HWE were found in only six microsatellite *loci* of *Prochilodus argenteus*. These deviations are common in microsatellite regions (Alam & Islam, 2005; Carreras-Carbonell *et al.*, 2006; Chevolot *et al.*, 2006). Hatanaka *et al.* (2006) found populations with significant deviations from HWE in two of the four analyzed *loci*. The occurrence of null alleles and stuttering seems to be the most probable cause of these deviations because all of the deviations were associated with the occurrence of null alleles. Additionally, F_{IS} values in *P. costatus* were positive in 80% of the estimates, also observed previously in the same species (Carvalho-Costa *et al.*, 2008).

However, we found low levels of population genetic differentiation among local populations of *Prochilodus argenteus*, as indicated by all population analyses, including F_{ST} values that were lower (0.008, $P < 0.05$) than R_{ST} values (0.059, $P < 0.05$). The R_{ST} values under the SMM are expected to be greater than the F_{ST} values under the IAM (Slatkin, 1995), although the inverse has been observed in some fish species (*e.g.*, Pereira *et al.*, 2009). Low indexes of F_{ST} have been observed in other studies with characiforms, such as in *Colossoma macropomum* (Santos *et al.*, 2007), *Piaractus mesopotamicus* (Calcagnotto & DeSalle, 2009; Iervolino *et al.*, 2010), *Prochilodus argenteus* (Hatanaka *et al.*, 2006; Sanches *et al.*, 2012), *Prochilodus costatus* (Carvalho-Costa *et al.*, 2008), *Prochilodus lineatus* (Revaldaves *et al.*, 1997) and *Salminus brasiliensis* (Lopes *et al.*, 2007).

The overall F_{ST} found in *P. argenteus* (0.008, $P < 0.05$) is similar to that found for the same species ($F_{ST} = 0.008$, $P = 0.0002$, Hatanaka *et al.*, 2006). However, these authors suggested a model of structured population based on the occurrence of private alleles and high levels of heterozygosity found in one local population. Our results do not show any genetic structure (pairwise F_{ST}) among sampling groups from the Três Marias region, any differences in the heterozygosity, or the presence of exclusive alleles. Thus, we suggest the existence of only one panmictic unit of *P. argenteus* in the central portion of the rio São Francisco basin based on F_{ST}, R_{ST} and chord genetic distance.

Similarly, our results do not show the existence of a genetic structure in *Prochilodus costatus*. The F_{ST} and R_{ST} indexes found here were significantly low ($F_{ST} = 0.031$, $P < 0.05$; $R_{ST} = 0.044$, $P < 0.05$) with a high levels of gene flow (mean of Nm = 5.911), confirming the null hypothesis of the absence of a population structure. This hypothesis, evidenced by fixation rates is also supported by the homogeneous genetic distance (Table 9). These results corroborate those of Carvalho-Costa *et al.* (2008), who found low levels of pairwise structuring (F_{ST} = -0.009 and 0.006) in populations collected downstream of Três Marias dam, the rio Abaeté and the confluence among the two rivers at the reproductive season.

Migratory routes

Migratory fishes from the rio São Francisco basin spawn only in the reproductive season, which extends from

Table 8. Analysis of Molecular Variance (AMOVA) among sampling groups of *Prochilodus costatus*. Structure tested: All samplings as a single group and divided in two groups: group 1 (Mainstream ABAr, TMDd and TMDr) and group 2 (Marginal lagoons of PAR and SFR).

Structure tested	Source of variation	Variance components	Percentage of variation	*F*- statistics	*P*-value
Single group	Among populations	0.083	3.187	$F_{ST} = 0.031$	$P < 0.05$
	Within populations	2.523	96.812	-	-
Two groups	Among groups	0.010	0.383	$F_{CT} = 0.003$	$P > 0.05$
	Among populations within groups	0.077	2.953	$F_{SC} = 0.029$	$P < 0.05$
	Within populations	2.523	96.663	-	-

Table 9. Chord genetic distance among sampling of *Prochilodus costatus*. Values in %. ABAr = rio Abaeté at rainy season; PAR = rio Paracatu lagoons; SFR = rio São Francisco lagoons; TMDd = Três Marias Dam at dry season; TMDr = Três Marias Dam at rainy season.

	ABAr	TMDd	TMDr	SFR	PAR
ABAr	-				
TMDd	33.6	-			
TMDr	36.2	35.2	-		
SFR	39.6	37.5	38.7	-	
PAR	34.8	33.5	32.6	35.9	-

November to February and coincides with the occurrence of rain and flooding, higher temperatures, and long photoperiods (Sato *et al.*, 1996; Sato & Godinho, 2003). After total spawning in the mainstream river or in tributaries, eggs and larvae of the migratory fishes are carried downstream by water flow reaching marginal lagoons, which provide essential habitats for juveniles with an abundant food and relatively high temperatures (Moojen, 1940). During the dry season, the marginal lagoons become isolated from the main channel. At the next rainy season, flooding provides water connections, and juveniles are ready to return to the main river (Sato & Godinho, 2003). Migrations to spawn can explain the existence of a higher number of migrants per generation observed in our analysis (*Prochilodus argenteus*, Nm = 9.318; *P. costatus*, Nm = 5.911). Wright (1931) suggests that values greater than one migrant per generation prevent the substantial local differentiation by genetic drift. In fact, the existence of a single panmictic population for each species associated with the highly migratory behavior support the hypothesis of extensive gene flow among shoals.

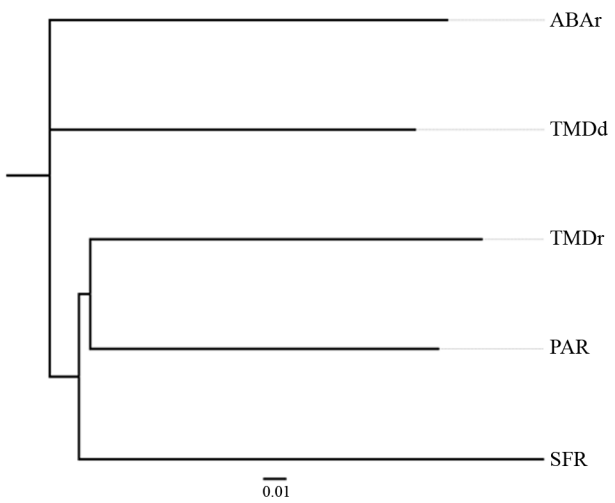

Fig. 3. Dendrogram representing the chord genetic distance among sampling groups of *Prochilodus costatus*. ABAr = rio Abaeté at rainy season; PAR = rio Paracatu lagoons; SFR = rio São Francisco lagoons; TMDd = Três Marias Dam at dry season; TMDr = Três Marias Dam at rainy season.

The convergence between rio São Francisco and rio Abaeté is considered the most important spawning site of *Prochilodus argenteus* (Godinho & Kynard, 2006), mainly because of the limnological conditions offered by the junction of the two rivers, such as water flow above 600 m³/s, water temperature over 24ºC and concentrations of dissolved oxygen above 5 mg/l (Sato *et al.*, 2005). As suggested by Hatanaka & Galetti (2003), one fraction completes its migration toward the dam during the reproductive period, and the majority possibly migrates to locations with environmental conditions more favorable for reproduction. Sato *et al.* (2005) found individuals with greater body size and weight and with better reproductive conditions downstream of the rio Abaeté compared with individuals that were collected upstream under the influence of the Três Marias dam. Observations made over the past 25 years as well as reports from local fishermen also relate the downstream confluence of the rio Abaeté and rio São Francisco as a preferential spawning site of other migratory fishes from this basin, such as *Brycon orthotaenia*, *Conorhynchos conirostris*, *Pseudoplatystoma corruscans*, and *Salminus franciscanus* (Sato *et al.*, 2005).

Considering two hypothetic scenarios for *Prochilodus argenteus*, first as a single population and second as divided into two groups, the degree of variation detected by AMOVA among populations was minimal (F_{ST} = 0.008, $P < 0.05$), and variation among the groups was not detected (F_{CT} = 0.001, $P > 0.05$). These results show that the samples belonging to the Três Marias region (group 1) are not isolated from those recruited in the marginal lagoons (group 2). Based on these data, we can suggest that the populations of *P. argenteus*, which are recruited in the tributary lagoons, migrate preferentially to the mainstream of the rio São Francisco in the feeding season and then migrate to the Três Marias region or randomly return to the headwaters of tributaries during the reproductive season. In addition, the F_{ST}, R_{ST}, AMOVA and chord distance could not detect a population genetically distinct from the other populations that possessed similarity with individuals collected downstream rio Abaeté during the rainy season. Thus, this important area (downstream of the rio Abaeté) does not receive individuals of *P. argenteus* from one exclusive region but instead receives individuals from many regions along the middle rio São Francisco basin, that it must lead to a population homogenization.

Contributions of the marginal lagoons for migratory fishes

The floodplain area from the central rio São Francisco basin was estimated to be about 2,000 km² (Welcomme, 1990). The importance of the marginal lagoons in the recruitment of fishes was previously estimated by Sato *et al.* (1987), identifying 37 juveniles including migratory species such as *Prochilodus argenteus* (< 70 g) and *P. costatus* (< 80 g).

Our results indicate a high gene flow with low genetic differentiation among samples from both marginal lagoons and mainstream rio São Francisco, suggesting a direct connectivity among these shoals.

The continuous and rapid advancement of agriculture and the subsequent sediment deposition in some of the important tributaries from the central basin (Sato & Godinho, 2003) are factors that directly affect the conservation of the lagoons. Notwithstanding, Pompeu & Godinho (2006) observed a gradual reduction in fish richness and an abundance of lagoons that did not receive annual flooding because of the water flow control by the Três Marias hydroelectric dam. Local extinction with a reduction of almost 70% of the native fish fauna was also observed in a lagoon from the rio São Francisco basin (Pompeu & Alves, 2003). Considering these fish nurseries in the rio São Francisco basin (Sato & Godinho, 2003) and upper rio Paraná (Agostinho *et al.*, 2000), the Brazilian conservation policy should include these marginal lagoons in the floodplains as a priority for the maintenance of the genetic variability in migratory fishes.

Acknowledgments

This paper benefited from comments and suggestions from Fábio F. Roxo, Fernando F. Mendonça and Luiz H. G. Pereira. We are grateful to CODEVASF-MG and IBAMA-MG for field collection and financial support (YS) and also to Daniel C. Carvalho (PUC-MG) for loan samples. This research was also supported by the Brazilian agencies FAPESP (BFM., FF., and CO), CNPq (FF, CO), and CAPES (B.F.M.). This work was developed as Master's dissertation in Biological Sciences with emphasis in Genetics by the first author.

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Submitted December 14, 2012
Accepted June 5, 2013 by Alexandre Hilsdorf
Published September 30, 2013