Latin American Journal of Aquatic Research
E-ISSN: 0718-560X
lajar@ucv.cl
Pontificia Universidad Católica de Valparaíso
Chile

Sanches, Alexandra; Galetti Jr., Pedro M.; Galzerani, Felipe; Derazo, Janeth; Cutilak-Bianchi, Beatriz; Hatanaka, Terumi

Genetic population structure of two migratory freshwater fish species (Brycon orthotaenia and Prochilodus argenteus) from the São Francisco River in Brazil and its significance for conservation

Latin American Journal of Aquatic Research, vol. 40, núm. 1, 2012, pp. 177-186
Pontificia Universidad Católica de Valparaíso
Valparaiso, Chile

Lat. Am. J. Aquat. Res., 40(1): 177-186, 2012
DOI: 10.3856/vol40-issue1-fulltext-17

Research Article

Genetic population structure of two migratory freshwater fish species (*Brycon orthotaenia* and *Prochilodus argenteus*) from the São Francisco River in Brazil and its significance for conservation

Alexandra Sanches[1], Pedro M. Galetti Jr.[1], Felipe Galzerani[1], Janeth Derazo[1] Beatriz Cutilak-Bianchi[1] & Terumi Hatanaka[1]
[1]Departamento de Genética e Evolução, Universidade Federal de São Carlos
P.O. Box 676, 13565-905 São Carlos, SP, Brazil

ABSTRACT. Previous genetic studies conducted with migratory fish populations from downstream of the Três Marias dam in the São Francisco River Basin (Brazil) have documented the occurrence of population structuring, as reported for *Brycon orthotaenia* and *Prochilodus argenteus*, two commercially important species in this basin. We revisited the genetic structure of these species using microsatellites. *B. orthotaenia* was sampled during the spawning season and was analyzed using five heterologous microsatellites. *P. argenteus* was collected in the non-reproductive season and genetic analysis was conducted using ten species-specific microsatellites. For both species, genetic diversity between collection sites was similar. Considering *B. orthotaenia*, F_{ST} and R_{ST} estimates and the Bayesian analysis demonstrated significant differences between sites. Two well-defined populations were identified in the study area, indicating population structuring for this species. No significant differences were found for *P. argenteus*. These data provide information for knowledge regarding genetic structure of migratory fish species, which may contribute toward the conservation besides the understanding the biology and ecology of these important fishery resources.

Keywords: migratory fish, freshwater fish, genetic structure, Characidae, Prochilodontidae, microsatallite, Brazil.

Estructura genética poblacional de dos especies de peces migratorios de agua dulce (*Brycon orthotaenia y Prochilodus argenteus*) en la cuenca del Río San Francisco (Brasil) y su importancia para la conservación

RESUMEN. Estudios genéticos anteriores realizados con poblaciones de peces migratorios de aguas abajo de la represa de las Tres Marías en la cuenca del río San Francisco (Brasil) han documentado casos de estructuración genética, como se ha descrito para *Brycon orthotaenia* y *Prochilodus argenteus*, dos especies de importancia comercial. Se revisó la estructura genética de estas especies utilizando microsatélites. Se obtuvieron muestras de *B. orthotaenia* durante la temporada de desove y se analizaron mediante cinco microsatélites heterólogos. Muestras de *P. argenteus* fueron recogidas en la temporada no reproductiva y el análisis genético se realizó utilizando diez microsatélites específicos para *P. argenteus*. Para ambas especies, la diversidad genética entre los sitios de recolección fue similar. Considerando a *B. orthotaenia*, las estimaciones F_{ST} y R_{ST} y el análisis Bayesiano demostraron diferencias significativas entre los sitios. Se identificaron dos poblaciones bien definidas en el área de estudio, indicando una estructuración de la población de esta especie. No se encontraron diferencias significativas para *P. argenteus*. Estos datos proporcionan información para el conocimiento sobre la estructura genética de las especies de peces migratorios, que puede contribuir a la conservación, además de la comprensión de la biología y ecología de estos importantes recursos pesqueros.

Palabras clave: peces migratorios, peces de agua dulce, estructura genética, Characidae, Prochilodontidae, microsatélite, Brasil.

Corresponding author: Alexandra Sanches (lelesanches@gmail.com)

INTRODUCTION

In recent years, a number of genetic investigations have revealed that Neotropical freshwater migratory fish can exhibit population structuring, with different genetic populations within a single hydrographic system (Wasko & Galetti, 2002; Hatanaka *et al.*, 2006; Sanches & Galetti, 2007). It has been claimed that during the spawning season fish schools may exhibit behavior that enables the maintenance of the genetic integrity of such populations (Hatanaka *et al.*, 2006; Sanches & Galetti, 2007). Knowledge on the genetic diversity within and between wild populations is crucial to the conservation of species (Haig, 1998). The maintenance of this genetic variation is the main goal of conservation, as it allows the potential for local adaptation and the life history evolution of species (Narum *et al.*, 2004).

Cases of fish population reduction have been reported in a number of hydrographical systems in South America (Agostinho *et al.*, 2005). Brazil has approximately 2000 freshwater fish species catalogued, accounting for 20% of all freshwater fish species in the world (Buckup & Menezes, 2003). A total of 134 species are considered endangered (Agostinho *et al.*, 2005). This threat is mainly the result of anthropogenic impact on aquatic continental ecosystems, including pollution, eutrophication, silting, the construction of dams and flood control, fisheries and the introduction of exotic species (Agostinho *et al.*, 2005).

The São Francisco River basin is one of the main hydrographic systems in Brazil and has a large biomass and diversity of freshwater fish, harboring 152 species (ANA/GEF/PNUMA/OEA, 2004). The basin has an area about 630.000 km^2, occupying approximately 7% of the Brazilian territory (Paiva, 1983), and flows 3.160 km, mostly northward (Kohler, 2003). This extensive and complex basin crosses different biomes, such as the Atlantic Rainforest, neotropical savanna and *caatinga* (scrubland).

Intense environmental changes have occurred due to the construction of hydroelectric dams along the São Francisco River. Such changes include the diminished speed, oxygenation and temperature of the waters, which can cause perturbation to various features of fish biology. It has been found that some migratory fish species downstream from the Três Marias dam are smaller and exhibit immature gonads during the spawning season. (Y. Sato, personal communication).

Brycon orthotaenia Günther, 1864 (= *Brycon lundii*) (Characidae, Characiformes) is a migratory fish species endemic to the São Francisco hydrographic basin that is considered vulnerable by the IUCN (2010). *Prochilodus argenteus* Spix & Agassiz, 1829 (Prochilodontidae, Characiformes) is also migratory and suffers with the overexploitation due to its commercial importance as local food source (Sato & Godinho, 2004).

B. orthotaenia and *P. argenteus* has been the subject of previous genetic analyses, in which significant differentiation was detected between populations sampled during the spawning season (Wasko & Galetti, 2002; Hatanaka & Galetti, 2003; Hatanaka *et al.*, 2006). These authors hypothesized that these migratory fish may constitute different populations in this one hydrographic system, coexisting and co-migrating along the main river channel. Homing instinct in *P. argenteus* was claimed to explain the maintenance of this structuring pattern (Hatanaka & Galetti, 2003).

The present study employed new sets of microsatellites to assay the genetic population structure of these two migratory fish species. The goal of this survey was to determine whether the population structuring pattern is confirmed and thereby increase and improve of the genetic data for knowledge on the biology and ecology of these fish as well as contribute toward their conservation.

MATERIALS AND METHODS

Sample collection

Fish collection was carried out at three sites located downstream from the Três Marias dam on the São Francisco River in southeastern Brazil (18°13'05"S, 45°15'54"W) (Fig. 1): the region immediately downstream from the dam (Region A); a second region about 10 km downstream from site A (Region B); and a third region approximately 40 km from site A, downstream from the confluence of the São Francisco and Abaeté Rivers (Region C).

A total of 44 DNA samples of *B. orthotaenia* available from the tissue bank of our laboratory were used. These samples were originally collected at all the sites during a single spawning season (Wasko & Galetti, 2002). *P. argenteus* specimens (n = 32) were collected during a single non-reproductive season at sites A and C.

Laboratory procedures

DNA extractions from *B. orthotaenia* were carried out based on the procedures described by Wasko & Galetti (2002). Genetic diversity was analyzed using four microsatellite loci described for *B. hilarii*: Bh5, Bh6, Bh16 and Bh17 (Sanches & Galetti, 2006) as well as one additional unpublished locus, Bh14 (TTA)

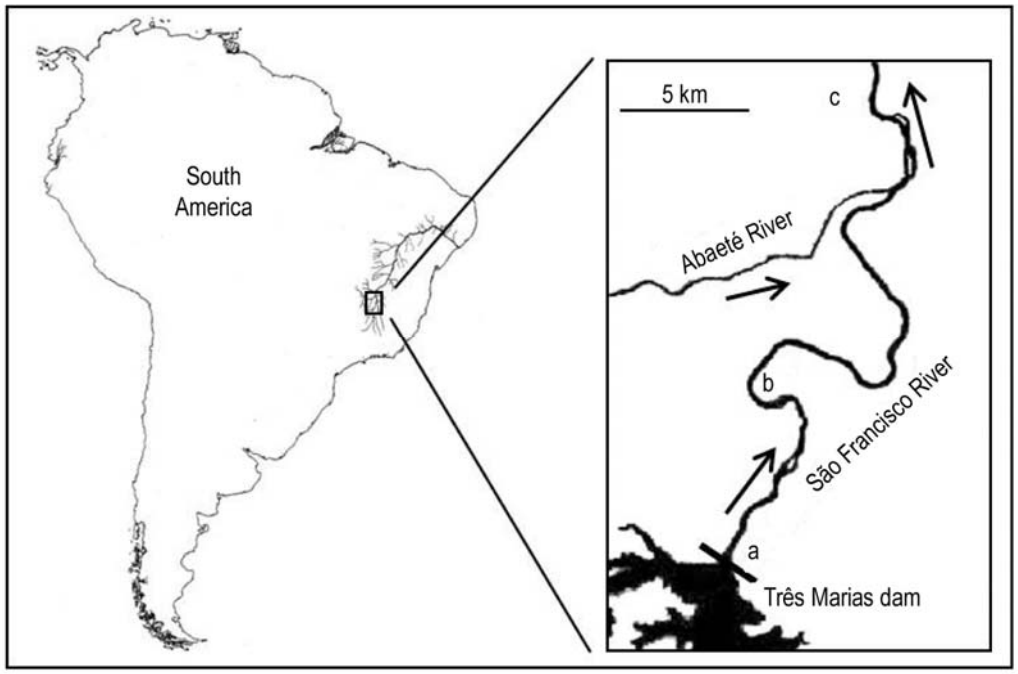

Figure 1. Collection sites in the São Francisco River Basin, southeastern Brazil. a) region immediately below the dam, b) region 10 km from the dam to the confluence of Abaeté and São Francisco Rivers, c) a stretch of approximately 20 km downstream from this confluence. Arrows indicate the direction of flow of the river.

Figura 1. Sítios de colecta en la cuenca del Río Sao Francisco, en el sur del Brazil. a) region inmediatamente abajo a la presa, b) region a 10 km de la represa hasta la confluencia de los ríos Abaeté y Sao Francisco, c) un tramo de aproximadamente 20 km aguas abajo de esta confluencia. Las flechas indican la dirección del flujo del río.

(Table 1) which has not produced reliable amplifications in *B. hilarii*, the original species used for the isolation of microsatellite loci. The sequence of this locus is now available in the GenBank (Accession Number: FJ844396). PCRs were carried out following the conditions proposed by Sanches & Galetti (2006). PCRs were carried out at a final volume of 10 µL, containing 100 ng of DNA, 200 µM of each deoxynucleotide triphosphate, 1.5 mM of $MgCl_2$, 1x reaction buffer (20 mM Tris-HCl pH 8; 50 mM KCl) 0.5 U of *Taq* polymerase (Fermentas Life Sciences) and 5 pmol of each primer (Fermentas Life Sciences). Thermal cycling conditions were as follows: 5 min at 95ºC, 35 cycles of 1 min at 94ºC, 1 min at 56ºC (for all loci), 1 min at 72ºC, and a final extension of 20 min at 72ºC. Amplified products were resolved on 7% silver-stained denaturing polyacrylamide gel (Comincini *et al.*, 1995). Alleles were scored by comparison with a 10 bp DNA ladder (Fermentas Life Sciences). Each sample was genotyped twice and only confirmed results were considered.

Total genomic DNA from *P. argenteus* was extracted from liver tissue through the saline solution method described by Aljanabi & Martinez (1997). Genetic diversity was analyzed using ten specific microsatellite loci: Par12, Par14, Par15, Par21, Par35 (Barbosa *et al.*, 2006), Par66, Par69, Par80, Par82 and Par85 (Barbosa *et al.*, 2008) (Table 1). PCRs were performed using the method described by Schuelke (2000) and were carried out in a final volume of 10 µL, containing 100 ng of DNA, 200 µM of dNTPs, 1 x PCR buffer (20 mM Tris-HCl, pH 8.4 and 50 mM KCl; LGC Biotecnologia), 4 pmol of each reverse and 6-FAM or NED M13 (-21) primers as well as 1 pmol of the forward primer, 1.5 mM $MgCl_2$ and 1 U of *Taq* DNA Polymerase (LGC Biotecnologia). PCR conditions were as follows: 1 cycle at 95ºC (5 min), 30 cycles at 94ºC (30 s), 45 s at the annealing temperature (Table 1) and 72ºC (45 s), followed by 8 cycles at 94ºC (30 s), 53ºC (45 s), 72ºC (45 s) and a final extension at 72ºC for 10 min. The microsatellite loci were analyzed on an ABI 377 automated sequencer (Applied Biosystems) and the alleles were scored with the Genescan and Genotyper 2.5 software programs (Applied Biosystems).

Data analysis

The genetic diversity of each population was quantified as the number of alleles (N_A) and number of private alleles as well as both observed (H_O) and

Table 1. Information on the microsatellites loci used for the genetic analyses of *Brycon orthotaenia* and *Prochilodus argenteus*. Annealing temperature (Ta).

Tabla 1. Información sobre los loci de microsatélites para el análisis genético de *Brycon orthotaenia* y *Prochilodus argenteus*. Temperatura de alineamiento (Ta).

Species	*Locus* name	Motif	Primer sequences (5'- 3')	Ta (°C)
Brycon orthotaenia	Bh5[1]	$(AC)_{13}$	CTTCCACTCATACCGGCACT ACATCTGGCATTAGGCATAG	56
	Bh6[1]	$(GT)_{14}$	GCGTTGCGTGTGTATGTTAA AGAGGTGTCCACAAAGTTTT	56
	Bh14	$(TTA)_9$	GTATCACCACCAACAGTAAT ATCAATGGTGGAAGAAGGAG	56
	Bh16[1]	$(TAA)_8$	CCTCCAATGAAAACAGTGCG ACGACTTAGCCACCCACCCT	56
	Bh17[1]	$(GTTT)_4(GGTTT)_3$	GTCAGCACTCAGCACATAGC AGAGAGCCTGAAAGTGAGTC	56
Prochilodus argenteus	Par12[2]	$(AAAC)_7$	CGAGCTGGTACCGTCACATA AGCATGATGCAAAGGATCTG	56
	Par14[2]	$(TGTC)_5$	GTATTAGGGGAGAGAATTTG TCTCATCAGTTATCACCAAC	48
	Par15[2]	$(CT)_{19}(GTCT)_{10}(GT)_2$	AGTTGGTTACACCTAACATC TCTTAATATGGGTCCACTAC	47
	Par21[2]	$(ATGA)_6$	CAAAAGGATAAGTAGCTCAG TAGCTCTGTTTATGATGACC	47
	Par35[2]	$(GA)_3(GTGA)_6(GAAA)_2(GA)_6$	AGCCAGAGGAGACCTGAACA CCTCCCTCCTCCAGATCTTT	62
	Par66[3]	$(AACA)_{12}$	TCTATAACTGTGGTCGTATG GAGGTTTTGAGATCAGTTG	47
	Par69[3]	$(TTAT)_7(TCAT)_6$	AATCTTTTCTAGGCTGTAGG GGGAAGTAGAAAGAAGAAAC	56
	Par80[3]	$(CT)_{37}$	CTAACCTACAAACCTCATTC CTGTAAAAGCTCCACTTATC	51
	Par82[3]	$(CT)_{27}$	CTCTAACAAGGTGAAACAAC TTTAAACTGTAGGCACAGAC	51
	Par85[3]			51

[1]Sanches & Galetti, 2006; [2] Barbosa *et al.*, 2006; [3] Barbosa *et al.*, 2008

expected (H_E) heterozygosity. Allelic richness (Petit *et al.*, 1998) and the inbreeding coefficient (F_{IS}) were also obtained using the Fstat software (Goudet, 1995). Significant differences in heterozygosity, gene diversity and allelic richness were evaluated between sites using the Kruskal-Wallis or Mann-Whitney in test the Bioestat software (Ayres *et al.*, 2003). Departure from Hardy-Weinberg expectations was calculated using a test analogous to Fisher's exact test (Guo & Thompson, 1992), estimated with a Markov Chain Monte Carlo series of permutations (10.000 batches/1000 iterations), implemented in Genepop (Raymond & Rousset, 1995). Tests for linkage disequilibrium between all pairs of loci were also performed with the Markov Chain Monte Carlo method in Genepop. Significance values were adjusted by the sequential Bonferroni correction (Rice, 1989). The Micro-Checker (Van Oosterhout *et al.*, 2004) was used to identify the presence of null alleles.

Genetic differences between populations were estimated with F_{ST} (Weir & Cockerham, 1984), based on differences in allele frequencies using Fstat. The R_{ST} statistic, based on differences in the allele size using the R_{ST} Calc (Goodman, 1997) was also used.

All significance values of multiple tests were adjusted by the sequential Bonferroni correction (Rice, 1989). The Mantel test was conducted using Genepop (Raymond & Rousset, 1995) to determine the significance of the relationship between the genetic distance (F_{ST}) and geographic distance (km) for all population pairs. Population structure was assessed using a model-based Bayesian procedure implemented on the STRUCTURE 2.1 program (Pritchard *et al.*, 2000). This analysis was carried out assuming the admixture model and correlated allele frequencies. Three individual repetitions of each K estimate (1-5) were run (500.000 iterations and a burn-in of 200.000 iterations).

RESULTS

Brycon orthotaenia

A total of 39 alleles were found in all loci throughout all populations. The number of alleles per locus found in each sampling site ranged from two (Bh14, region A and B) to twelve (Bh6, region B) and the mean ranged from 5.6 to 7.2 (Table 2). Three private alleles were found: two at site B (locus Bh6) and one at site C (locus Bh14). Null alleles were identified in the locus Bh17 (0.412). After the Bonferroni correction, one pair of genotypes presented linkage disequilibrium (Bh5 x Bh16, $P = 0.03$).

All sites exhibited departure from the Hardy-Weinberg expectations for at least one locus, with a deficit or excess of heterozygotes revealed by positive or negative F_{IS} values, respectively. The mean observed heterozygosity ranged from 0.65 to 0.73 and expected heterozygosity ranged from 0.63 to 0.75. Mean allelic richness ranged from 5.48 to 6.44. The fish from site A had the smallest mean value of all these genetic diversity parameters, but differences between sites were non-significant (Kruskal-Wallis test, $P > 0.05$).

Both pairwise F_{ST} and R_{ST} estimates demonstrated low values, although there were significant differences between sites (Table 3). The fish from the site A (immediately downstream from the dam) were significantly different from the other sites. The same differentiation pattern was achieved through Bayesian analysis, which identified two populations ($K = 2$, estimated –ln probability of data = -680.7; $P\ (K/X) = 1.00$). Most of fish from site A were assigned to one cluster (dark gray), while the fish from sites B and C predominantly represent another cluster (light gray) (Fig. 2). Mantel tests demonstrated no correlation between geographic and genetic distances, suggesting no isolation by distance. The locus Bh 17 was not used in these analyses, since it presents null allele.

Prochilodus argenteus

All loci produced a total of 115 alleles ranging from three (Par35, site A) to 20 (Par85, site C) alleles per locus per population. Specimens from site A exhibited fewer alleles (82, mean number of 8.2 alleles/locus) than the fish from site B (105, mean of 10.5 alleles/locus). Among the total of alleles, 43 were considered private alleles – ten in site A and 33 in site B. No significant linkage disequilibrium ($P > 0.05$) was observed. Locus Par15 exhibited a significant deviation from the Hardy-Weinberg equilibrium only at site B (Table 4). Null alleles were identified in the loci Par 15 (0.0677), Par 21 (0.1706) e Par 80 (0.0852). The genetic diversity demonstrated by allelic richness, observed and expected heterozygosities was similar in all sites (Mann-Whitney test, $P > 0.05$).

There were no significant differences between samples ($F_{ST} = 0.002$, $P = 0.400$; $R_{ST} = -0.021$, $P = 0.954$). Bayesian analysis also identified the two samples belonging to a single population ($K = 1$; estimated – ln probability of data = 1600.3; $P > 0.99$).

DISCUSSION

In recent years, the number of genetic studies on Neotropical freshwater fish have indicated structuring of their populations within a same hydrographic basin (for a review, see Piorski *et al.*, 2008), including migratory species (Machado *et al.*, 2005; Hatanaka *et al*,. 2006; Sanches & Galetti, 2007). The present study revealed different results for both migratory fish species studied. Unlike *P. argenteus*, significant genetic differentiations were found among populations of *B. orthotaenia* in the small region studied.

Population structuring of *B. orthotaenia* was revealed by the F_{ST} and R_{ST} statistics. The structuring pattern was also clearly demonstrated in the Bayesian analysis, in which two populations were identified. Individuals from site A represent one population, whereas most fish from sites B and C belong to the other genetic population. This result corroborates a previous study in which a significant divergence was found between sites, as revealed by a diagnostic RAPD pattern that was present in 100% of the fish from site A and 27% of the fish from site C (Wasko & Galetti, 2002). The authors hypothesized that the animals collected from site A could represent a unique population, whereas those from site C would comprise a mix of different populations.

Population structuring has also been found in *B. hilarii* from the Paraguay River Basin (Sanches & Galetti, 2007). Significant genetic differences were

Table 2. Information on the genetic diversity of *Brycon orthotaenia* collected at three sites downstream from the Três Marias dam on the São Francisco River (Brazil). Sample size (n) per site, number of alleles (N_A), number of private alleles (N_{AP}), allelic richness (R_A), observed heterozygosity (H_O), expected heterozygosity (H_E), *P*-value for departures from Hardy-Weinberg expectations already corrected by the sequential Bonferroni correction (P_{HW}) and inbreeding coefficient (F_{IS}).

Tabla 2. Información sobre la diversidad genética de *Brycon orthotaenia* recogidos en tres sítios aguas abajo de la represa de las Tres Marias en el Río Sao Francisco (Brazil). Tamaño de la muestra (n), número de alelos (N_A), número de alelos privados (N_{AP}), riqueza alélica (R_A), heterocigosidad observada (H_O), heterocigosidad esperada (H_E), valores de *P* para el análisis de las expectativas de Hardy-Weinberg ya ajustados por la corrección de Bonferroni secuencial (P_{HW}) y coeficiente de endogamia (F_{IS}).

Sites	Statistics	Loci					All loci
		Bh5	Bh6	Bh14	Bh16	Bh17	
A (n = 12)	N_A	7	11	2	5	3	5.6
	N_{AP}	0	0	0	0	0	0
	R_A	6.91	10.57	2.00	4.91	2.99	5.48
	H_O	0.83	0.83	0.58	1.00	0.00	0.65
	H_E	0.85	0.89	0.42	0.70	0.30	0.63
	P_{HW}	0.43	0.18	0.48	0.09	0.03*	0.03*
	F_{IS}	0.02	0.07	-0.43	-0.46	1.00	-0.03
B (n = 17)	N_A	11	12	2	6	5	7.20
	N_{AP}	0	2	0	0	0	2
	R_A	9.79	10.63	2.00	5.25	4.52	6.44
	H_O	1.00	0.86	0.71	1.00	0.06	0.73
	H_E	0.90	0.90	0.47	0.73	0.69	0.74
	P_{HW}	0.001*	0.85	0.49	0.30	0.00*	0.00*
	F_{IS}	-0.11	0.05	-0.52	-0.38	0.92	0.02
C (n = 15)	N_A	8	11	3	5	5	6.40
	N_{AP}	0	0	1	0	0	1
	R_A	7.14	10.49	3.00	4.86	4.93	6.08
	H_O	1.00	0.75	0.60	1.00	0.00	0.67
	H_E	0.83	0.88	0.64	0.69	0.73	0.75
	P_{HW}	0.004*	0.24	0.03*	0.47	0.00*	0.00*
	F_{IS}	-0.22	0.15	0.07	-0.46	1.00	0.12

*significant $P < 0.05$

detected between populations and the authors assumed that the spawning schools may organize themselves in such way as to maintain the integrity of each subunit residing in the system. Recent studies with *B. insignis* and *B. opalinus* revealed this same genetic structuring pattern of different populations from Paraíba do Sul River Basin (Barroso *et al.*, 2005; Matsumoto & Hilsdorf, 2009).

A previous study revealed genetic differentiation between populations of *Prochilodus argenteus* collected during the reproduction season in the same sites as those of the present work (Hatanaka & Galetti, 2003; Hatanaka *et al.*, 2006). The authors claim that this migratory fish may constitute different populations in this hydrographic system, coexisting and comigrating along the main river channel.

Unlike the above-mentioned studies, no differentiation was found in the present investigation regarding the non-reproductive season for *P. argenteus*. The occurrence of weak population genetic differentiation or no differentiation is common in fish (Wirth & Bernatchez, 2001; Laikre *et al.*, 2005; Santos *et al.*, 2007), especially for species that exhibit high vagility, abundance and wide distribution, with

Table 3. Pairwise F_{ST} and R_{ST} estimates of *Brycon orthotaenia*. The significance levels in parentheses are already adjusted by the sequential Bonferroni correction.

Tabla 3. Estimaciones de F_{ST} y R_{ST} de pares de poblaciones de *Brycon orthotaenia*. Los níveles de significación entre parenthesis ya están ajustados por la corrección de Bonferroni secuencial.

	F_{ST} (*P*)	R_{ST} (*P*)
A x B	0.090 (0.05*)	0.017 (0.05*)
A x C	0.112 (0.048*)	0.068 (0.046*)
B x C	0.005 (0.327)	-0.013 (0.493)

*Significant $P < 0.05$

no visible barriers to gene flow (Jorgensen *et al.*, 2005). Alternatively, the lack of genetic differentiation along a hydrographic system also could be due to overlapping discrete populations during the non-reproductive season, reflecting in non-significant F_{ST} values even when comparing sites that are long distances apart.

Studies on the movement of *P. argenteus* (Godinho & Kynard, 2006) using telemetry have reported the existence of different population units determined by a spawning-site homing, corroborating previous genetic analyses on this migratory freshwater fish (Hatanaka & Galetti, 2003). Moreover, a similar study with *Pseudoplatystoma corruscans* revealed that the spatial distribution of the fish was greater during the non-spawning season than during the spawning season (Godinho *et al.*, 2007), demonstrating that the fish are more dispersed in the former season. Thus, the probability of detecting genetic differentiation during the non-reproductive season is much lower, which corroborates the idea of coexisting populations. The spatial distribution of fish from different population units may be wider and consequently overlap during the non-reproductive season, which has no relevance to the genetic population structure (Laikre *et al.*, 2005).

Therefore, we must be cautious in reaching conclusions on a lack of differentiation for migratory fish species. It is important to consider the sampling strategies of these studies, as fish exhibit different behavior at different times of their life cycle. From the standpoint of management and conservation, it is dangerous to consider the existence of a single population, because different genetic populations can be cohabitating a particular space. Considering different genetic population as only one population can result in the depletion of genetic variation (Laikre *et al.*, 2005) and consequently reduce population viability (Allendorf & Ryman, 2002).

Knowledge on population genetic structure is essential for fisheries management and the conservation of fish species (Moritz, 1994; Paetkau, 1999). Without such information, unsuitable management could result in the overexploitation of some population units or segment of populations (Laikre *et al.*, 2005), causing the loss of entire gene pools (population units) or genetic diversity within populations (Ryman & Utter, 1987; Allendorf & Ryman, 2002).

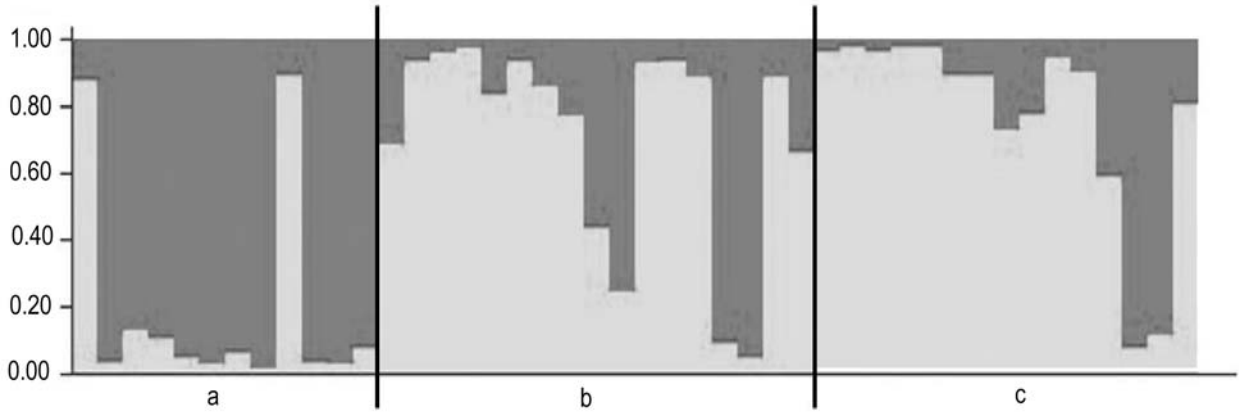

Figure 2. Estimated population structure for *Brycon orthotaenia*. Plot of the highest probability run at $K = 2$ (two clusters). Each individual is represented by a vertical line in which shading (light or dark gray) indicates the individual's estimated membership (scale at left) to alternative clusters. Black vertical lines separate the sites, the names of which are indicated above.

Figura 2. Estructura poblacional estimada para *Brycon orthotaenia*. Gráfico con más alta probabilidad, para K = 2 (dos grupos). Cada individuo es representado por una barra vertical, el sombreado (gris claro o oscuro) representa los grupos alternativos a que los individuos pueden pertenecer. Líneas negras separan los sítios.

Table 4. Information on the genetic diversity of *Prochilodus argenteus* collected from two regions downstream from the Três Marias dam on the São Francisco River. Sample size (n) per site, number of alleles (N_A), number of private alleles (N_{AP}), allelic richness (R_A), observed heterozygosity (H_O), expected heterozygosity (H_E), *P*-value for departures from Hardy-Weinberg expectations already corrected by the sequential Bonferroni correction (P_{HW}) and inbreeding coefficient (F_{IS}).

Tabla 4. Información sobre la diversidad genetic de *Prochilodus argenteus* recogidos en dos regiones aguas abajo de la represa de las Tres Marias en el Río Sao Francisco. Tamaño de la muestra (n), número de alelos (N_A), número de alelos privados (N_{AP}), riqueza alélica (R_A), heterocigosidad observada (H_O), heterocigosidad esperada (H_E), valores de *P* para el análisis de las expectativas de Hardy-Weinberg ya ajustados por la corrección de Bonferroni secuencial (P_{HW}) y coeficiente de endogamia (F_{IS}).

Sites	Statistics	Loci										All Loci
		Par12	Par14	Par15	Par21	Par35	Par66	Par69	Par80	Par82	Par85	
A (n = 14)	N_A	7	6	12	7	3	5	5	11	9	17	82
	N_{AP}	1	2	0	0	1	0	0	1	2	3	10
	R_A	6.14	5.49	12.0	6.10	2.83	4.78	4.69	9.81	8.62	15.1	7.56
	H_O	0.71	0.36	1.00	0.78	0.08	0.71	0.46	0.57	0.92	1.00	0.66
	H_E	0.75	0.61	0.94	0.64	0.22	0.76	0.51	0.87	0.87	0.95	0.71
	P_{HW}	0.69	0.31	1.00	0.79	0.32	0.98	0.65	0.30	0.48	1.00	0.26
	F_{IS}	0.04	0.42	-0.78	-0.24	0.66	0.06	0.10	0.35	-0.06	-0.05	0.07
C (n = 32)	N_A	9	5	19	8	5	9	6	12	12	20	105
	N_{AP}	3	1	7	1	3	4	1	2	5	6	33
	R_A	6.15	3.39	11.2	6.08	2.83	6.06	4.58	8.59	7.76	13.6	7.03
	H_O	0.65	0.41	0.77	0.47	0.18	0.78	0.58	0.71	0.84	0.91	0.63
	H_E	0.74	0.37	0.90	0.67	0.18	0.78	0.60	0.85	0.83	0.95	0.69
	P_{HW}	0.49	0.45	0.00*	0.16	1.00	0.45	0.87	0.21	0.34	0.12	0.00*
	F_{IS}	0.11	-0.09	0.14	0.30	-0.04	0.00	0.04	0.17	-0.01	0.04	0.08

* Significant $P < 0.05$

In the present study, population structuring was identified for *B. orthotaenia* during the reproductive season, corroborating a previous study (Wasko & Galetti, 2002), whereas no structuring pattern was found for *P. argenteus* during the non-reproductive season. However, the idea of population structuring should not be discounted, as fish from different genetic populations units can be spread and mixed in the main channel of the river during the non-reproductive season. Thus, further studies are needed, including more collection sites and different seasons over the course of several years, in order to reach a more faithful conclusion regarding the population genetic structure of these migratory fish.

ACKNOWLEDGMENTS

The authors are thankful to the Instituto Florestal de Minas Gerais for authorizing the capture of specimens. We also thank Dr. Yoshimi Sato and CEMIG/CODEVASF for collecting the samples and Prof. Dr. Flávio Henrique da Silva for facilitating the use of the automated sequencer. This study was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

REFERENCES

Agostinho, A.A., S.M. Thomaz & L.C. Gomes. 2005. Conservação da biodiversidade em águas continentais do Brasil. Megadiversidade, 1: 70-78.

Aljanabi, S.M. & I. Martinez. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res., 25: 4692-4693.

Allendorf, F.W. & N. Ryman. 2002. The role of genetics in population viability analysis. In: R.S. Bessinger & D.R. McCullough (eds.). Population viability analysis. University of Chicago Press, Chicago, pp 50-85.

Agência Nacional de Águas (ANA), Fundo Mundial para o Meio Ambiente (GEF), Programa das Nações Unidas para o Meio Ambiente (PNUMA) & Organização dos Estados Americanos (OEA). 2004. Plano decenal de recursos hídricos da bacia hidrográfica do Rio São Francisco (2004-2013) – Diagnóstico da bacia e cenários de desenvolvimento. Brasília, 217 pp.

Ayres, M., M. Ayres Jr., D.L. Ayres & A.S.S. Santos. 2003. BioEstat 3.0: aplicações estatísticas nas áreas das ciências bio-médicas. CNPq/MCT, Belém.

Barbosa, A.C.D.R., T.C. Corrêa, F. Galzerani, P.M. Galetti Jr. & T. Hatanaka. 2006. Thirteen polymorphic microsatellite loci in the Neotropical fish *Prochilodus argenteus* (Characiformes, Prochiodontidae). Mol. Ecol. Notes, 6: 936-938.

Barbosa, A.C.D.R., T.C. Corrêa, F. Galzerani, P.M. Galetti Jr. & T. Hatanaka. 2008. Description of novel microsatellite loci in the Neotropical fish *Prochilodus argenteus* and cross-amplification in two other species. Gen. Mol. Biol., 31(Suppl.): 357-360.

Barroso, R.M., A.W.S. Hilsdorf, H.L.M. Moreira, P.H. Cabello & Y.M. Traub-Cseko. 2005. Genetic diversity of wild and cultured populations of *Brycon opalinus* (Cuvier, 1819) (Characiforme, Characidae, Bryconiae) using microsatellites. Aquaculture, 247: 51-65.

Buckup, P.A. & N.A. Menezes. 2003. Catálogo dos peixes marinhos e de água doce do Brasil. Disponível em http://www.mnrj.ufrj.br/catalogo.

Comincini, S., P. Leone, L. Redaelli, L. Giuli, Y. Zhang & L. Ferretti. 1995. Characterization of bovine microsatellites by silver staining. J. Anim. Breed. Genet., 112: 415-420.

Godinho, A.L. & B. Kynard. 2006. Migration and spawning of radio-tagged zulega *Prochilodus argenteus* in a dammed Brazilian River. Trans. Am. Fish. Soc., 135: 811-824.

Godinho, A.L., B. Kynard & H.P. Godinho. 2007. Migration and spawning of female surubim (*Pseudoplatystoma corruscans*, Pimelodidae) in the São Francisco river, Brazil. Environ. Biol. Fish., 80: 421-433.

Goodman, S.J. 1997. RST CALC: a collection of computer programs for calculating unbiased estimates of genetic differentiation and determining their significance for microsatellite data. Mol. Ecol., 6: 881-885.

Goudet, J. 1995. FSTAT: a computer program to calculate F-statistics. J. Hered., 86: 485-486.

Guo, S.W. & E.A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions or multiple alleles. Biometrics, 48: 361-372.

Haig, S.M. 1998. Molecular contributions to conservation. Ecology, 79: 413-425.

Hatanaka, T. & P.M. Galetti Jr. 2003. RAPD markers indicate the occurrence of structured populations in migratory fish freshwater fish species. Gen. Mol. Biol., 26: 19-25.

Hatanaka, T., F. Henrique-Silva & P.M. Galetti Jr. 2006. Population substructuring in a migratory freshwater fish *Prochilodus argenteus* (Characiformes, Prochilodontidae) from the São Francisco River. Genetica, 126: 153-159.

International Union for Conservation of Nature (IUCN). 2010. IUCN Red list of threatened species. <www.iucnredlist.org>. Reviewed: on 17 February 2011.

Jorgensen, H.B.H, M.M. Hansen, D. Bekkevold, D.E. Ruzzante & V. Loeschcke. 2005. Marine landscape and population genetic structure of herring (*Clupea harengus* L.) in the Baltic Sea. Mol. Ecol., 14: 3219-3234.

Kohler, H.C. 2003. Aspectos geoecológicos da bacia hidrográfica do São Francisco. In: H.P. Godinho & S.L. Godinho (eds.). Águas, peixes e pescadores do São Francisco das Minas Gerais. Pontifica Universidade Católica de Minas, Belo Horizonte, 458 pp.

Laikre, L., S. Palm & N. Ryman. 2005. Genetic population structure of fishes: implications for coastal zone management. Ambio, 34: 11-119.

Machado, V., U.H. Schulz, L.P. Palma & J.J.S. Rodrigues. 2005. Mitochondrial DNA variation and genetic population structure of the migratory freshwater fish dourado *Salminus brasiliensis* (Characidae). Acta Biol. Leopondensia, 27: 107-113.

Matsumoto, C.K. & A.S. Hilsdorf. 2009. Microsatellite variation and population genetic structure of a Neotropical endangered Bryconinae species *Brycon insignis* Steindachner, 1877: implications for its conservation sustainable management. Neotrop. Ichthyol., 7: 395-402.

Moritz, C. 1994. Defining evolutionary significant units for conservation. Trends Ecol. Evol., 9: 373-375.

Paetkau, D. 1999. Using genetics to identify intraspecific conservation units: a critique of current methods. Conserv. Biol., 13: 1507-1509.

Narum, S.R., C. Contor, A. Talbot & M.S. Powell. 2004. Genetic divergence of sympatric resident and anadromous forms of *Oncorhynchus mykiss* in the Walla Walla River. J. Fish Biol., 65: 471-488.

Paiva, M.P. 1983. Peixes e pescas de águas interiores do Brasil. Editerra Editorial, Brasília, 158 pp.

Petit, R.J., A. El Mousadik & O. Pons. 1998. Identifying populations for conservation on the basis of genetic markers. Conserv. Biol., 12: 844-855.

Piorski, N.M., A. Sanches, L.F. Carvalho-Costa, T. Hatanaka, M. Carrillo-Avila, P.D. Freitas & P.M. Galetti Jr. 2008. Contribution of conservation genetics in assessing neotropical freshwater fish biodiversity. Brazil. J. Biol., 68 (Suppl.): 1039-1050.

Received: 5 March 2011; Accepted: 15 January 2012

Pritchard, J.K., M. Stephens & P. Donnelly. 2000. Inference of population structure using multilocus genotypic data. Genetics, 155: 945-959.

Raymond, M. & M. Rousset. 1995. Genepop, population genetics software for exact tests and ecumenicism. J. Hered., 86: 248-249.

Rice, W.R. 1989. Analysing tables of statistical tests. Evolution, 43: 223-225.

Ryman, N. & F. Utter. 1987. Population genetics and fishery management. Washington Sea Grant Publications/University of Washington Press, Seattle and London, 329 pp.

Sanches, A. & P.M. Galetti Jr. 2006. Microsatellites loci isolated in the freshwater fish *Brycon hilarii*. Mol. Ecol. Notes, 6: 1045-1046.

Sanches, A. & P.M. Galetti Jr. 2007. Genetic evidence of population substructuring in the freshwater fish *Brycon hilarii*. Braz. J. Biol., 67: 889 -895.

Santos, M.C.F., M.L. Ruffino & I.P. Farias. 2007. High levels of genetic diversity and panmixia of the tambaqui *Colossoma macropomum* (Cuvier, 1816) in the main channel of the Amazon River. J. Fish Biol., 71: 33-44.

Sato, Y. & H.P. Godinho. 2004. Migratory fishes of the São Francisco River. In: J. Carolsfeld, B. Harvey, C. Ross & A. Baer (eds.). Migratory fishes of south America: biology, fisheries and conservation status. World Fisheries Trust, IDRC, Otawa, 380 pp.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nat. Biotech., 18: 233-234.

Van Oosterhout, C., W.F. Hutchinson, D.P.M. Wills & P. Shipley. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes, 4: 535-538.

Wasko, A.P. & P.M. Galetti Jr. 2002. RAPD analysis in the Neotropical fish *Brycon lundii*: genetic diversity and its implications for the conservation of the species. Hydrobiology, 474: 131-137.

Weir, B.S. & C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution, 38: 1358-1370.

Wirth, T. & L. Bernatchez. 2001. Genetic evidence against panmixia in the European eel. Nature, 409: 1037-1040.