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Short Communication

Evidence of lack of population substructuring in the Brazilian freshwater fish *Prochilodus costatus*

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Abstract

Curimbatá-pióa (*Prochilodus costatus* Valenciennes, 1850) is an endemic species from the São Francisco River Basin with migratory habits, important ecological role in the ecosystem and relevant fishing importance. The present study aimed to assess the genetic variation in *P. costatus* in order to investigate its population genetic structure. Genetic variation was studied at three sites downstream the Três Marias dam (Upper-middle São Francisco River, State of Minas Gerais) through six specific microsatellite loci. Fish from the three sites had quite similar genetic diversity levels and no genetic differentiation was detected, suggesting that *P. costatus* might represent a single reproductive unit in the studied area. Alternatively, the present study was not able to detect putative coexistent and comigrating populations along the main channel river. Our findings could be very helpful for the management and conservation of this fish.

Key words: Prochilodontidae, Curimbatá, microsatellites, genetic variation.

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The São Francisco River is one of the main Brazilian rivers located in an area inhabited by approximately 13 million people (Cappio *et al.*, 1995). The river was effectively fragmented into two parts since the Três Marias dam was built in the upper-middle region of the basin (Sato and Godinho, 2004). Although fish from 152 species inhabits the river basin, and some of them migrate in the reproduction season, no mechanism for fish passage exists at the Três Marias dam (Sato and Godinho, 2004).

One of these species is the endemic *Prochilodus costatus* (= *P. affinis*, Valenciennes, 1850), the "curimbatá-pióa", a long distance migratory species (Froese and Pauly, 2006) and an important resource for artisanal fishing communities, representing one of the commonly landed species of the region (Camargo and Petrere Jr, 2001). Moreover, this species can play an important ecological role considering its detritivorous habit that involves the processing of sediments (Flecker, 1996), representing a key-species for conservation programs. Little is known on population genetics of this fish. Previous molecular studies in some other migratory fish species inhabiting the upper-middle São Francisco River revealed the occurrence of population substructuring, and posed the question whether this is a common pattern in all migratory Neotropical freshwater fish (Wasko and Galetti Jr., 2002; Hatanaka and Galetti Jr., 2003; Hatanaka *et al.*, 2006). Confronted with this scenario, the goals of this study were to assess the genetic diversity of *Prochilodus costatus*, and to investigate its population genetic structure downstream of the Três Marias dam.

The specimens were collected during the reproductive seasons of 2003/2004 downstream of the Três Marias dam near the municipality of Três Marias (State of Minas Gerais, southeastern Brazil). Fish were sampled at three sampling sites (Figure 1): A) the main channel of the São Francisco River immediately downstream of the dam; B) the Abaeté River; and C) the main channel of the São Francisco River downstream of the junction between the Abaeté and São Francisco Rivers (approximately 40 km downstream of the Três Marias dam).

DNA was extracted from liver tissues stored in 95% ethanol following the method of Aljanabi and Martinez (1997). Six previously isolated species-specific microsatellite loci were used to assess genetic variation following the protocol of Carvalho-Costa *et al.* (2006). The number of alleles per locus was assessed by genotyping the fragments in an automated sequencer using the protocol developed by Schuelke (2000). Allele sizes were scored against the internal size standard GeneScan-350-Rox (PE Applied Biosystems) through the Genescan 3.1 and Genotyper 2.5 softwares (ABI).

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Allele frequencies, expected (*He*) and observed (*Ho*) heterozygosities, departures from Hardy-Weinberg expectations (HWE) and linkage disequilibrium were obtained in the program Genepop 3.3 (Raymond and Rousset, 1995). Population differentiation was estimated through Wright's fixation indices (Wright, 1978) according to the variance method of Weir and Cockerham (1984) implemented in the program Fstat 2.9.3.2 (Goudet, 2001). Allelic richness was also calculated in the program Fstat 2.9.3.2 and tested nonparametrically: Kruskal-Wallis and Mann-Whitney (for independent samples) and Wilcoxon (for related samples). These tests were also used to compare the observed heterozygosities among the sampling sites. All these tests were performed in Bioestat 3.0 (Ayres *et al.*, 2003).

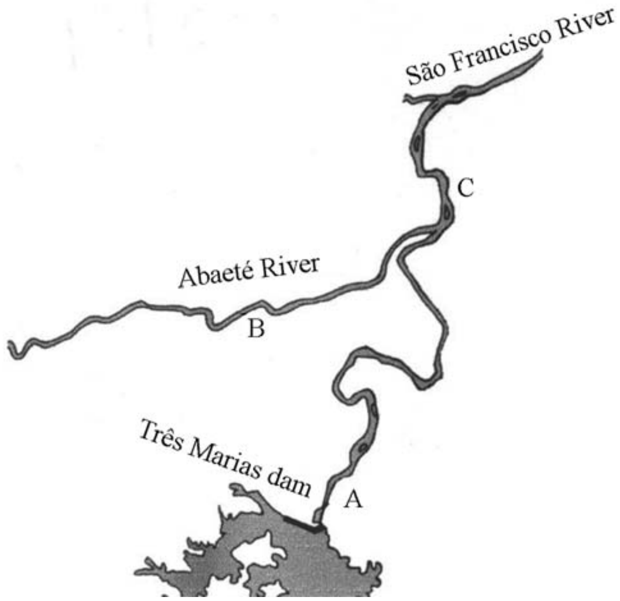

Figure 1 - Sampling sites downstream of Três Marias dam located between 18° 13' S, 45° 00' W and 17° 57' S, 45° 20' W - A: main channel of São Francisco River immediately downstream of the dam; B: Abaeté River; C: main channel of São Francisco River, downstream to the junction of the Abaeté and São Francisco Rivers.

The results for intra-population genetic variation are shown in Table 1. We found allele numbers ranging from 43 to 48, including 28 private alleles. Site A presented 18.75%, site B 18.6% and site C 21.75% of private alleles. All sites presented departures from Hardy-Weinberg expectations for some loci (A: Pcos04 and Pcos18; B: Pcos03 and Pcos18; C: Pcos17 and Pcos18) after Bonferroni correction, also shown by positive F_{IS} values (Table 1). There was no significant non-random association among the genotypes of the six loci, suggesting that the analyses could be performed assuming statistical independence of the loci.

The observed and expected heterozygosities varied from 0.160 (Pcos20, site A) to 1.000 (Pcos14, site C) and from 0.150 (Pcos20, site C) to 0.950 (Pcos14, site C), re-

Table 1 - Sample size (n) per locality and genetic diversity features of *Prochilodus costatus*. NA: number of alleles, NAp: number of private alleles, Ho: observed heterozygosity, He: expected heterozygosity, F_{IS}: inbreeding coefficient, P: value for departures of Hardy-Weinberg expectations. A = dam; B = Abaeté River and C = downstream junction of Abaeté/São Francisco rivers. *significant ($p < 0.05$).

Sampling sites	Statistics	Loci						Mean/All
		Pcos03	Pcos04	Pcos14	Pcos17	Pcos18	Pcos20	
A (n = 24)	NA	5	4	16	15	6	2	8/48
	NAp	1	1	3	3	1	0	1.5/9
	Ho	0.430	0.350	0.950	0.750	0.350	0.160	0.480
	He	0.440	0.650	0.920	0.890	0.730	0.150	0.660
	F_{IS}	0.037	0.468	-0.031	0.162	0.527	-0.063	0.214
	P	0.672	0.004*	0.441	0.169	0.000*	1.000	0.000*
B (n = 17)	NA	4	5	12	14	6	2	7.570/43
	NAp	0	1	2	3	2	0	1.3/8
	Ho	0.250	0.500	0.750	0.640	0.210	0.230	0.440
	He	0.540	0.590	0.900	0.830	0.600	0.210	0.650
	F_{IS}	0.547	0.165	0.172	0.238	0.655	-0.091	0.307
	P	0.003*	0.079	0.013	0.067	0.000*	1	0.000*
C (n = 16)	NA	4	8	15	9	7	3	7.430/46
	NAp	0	4	2	1	2	1	1.6/10
	Ho	0.380	0.530	1.000	0.270	0.320	0.270	0.420
	He	0.570	0.630	0.950	0.600	0.800	0.250	0.660
	F_{IS}	0.345	0.164	-0.055	0.564	0.615	-0.071	0.281
	P	0.133	0.143	0.713	0.001*	0.000*	1.000	0.000*

spectively. Fish from the dam (A) were the ones that presented the largest mean *Ho* (0.480). Altogether, the average *Ho* was 0.45, a value comparable to that of other freshwater fish (*Ho* = 0.46, DeWoody and Avise, 2000). No significant *Ho* differences between sampling sites were observed by Kruskal-Wallis (H = 0.390, p = 0.823), Mann-Whitney (AxB: p = 0.689; BxC: p = 0.522; AxC: p = 0.749) and Wilcoxon tests (AxB: p = 0.249; BxC: p = 0.345; AxC: p = 0.753). Similarly, the allelic richness was not significantly different (Kruskal-Wallis: H = 0.246, p = 0.884; Mann-Whitney: AxB: p = 0.936, BxC: p = 0.631 and AxC: p = 0.522; Wilcoxon: AxB: p = 0.655, BxC: p = 0.5 and AxC: p = 0.5).

These results show similar levels of genetic variability for the three sites, differing from the results previously reported for the congeneric *P. argenteus* (Hatanaka and Galetti Jr., 2003; Hatanaka *et al.*, 2006) and *Brycon orthotaenia* (Wasko and Galetti Jr., 2002). In both of the latter species, significant heterozygosity differences were observed when the downstream population nearest the dam (comparable to sample site A) was compared with the population further downstream (equal to site C), revealing population divergence. In all cases, the lower heterozygosity levels of the populations nearest the dam were correlated to the degraded environmental condition of this area (Wasko and Galetti Jr., 2002; Hatanaka and Galetti Jr., 2003).

The estimated pairwise F_{ST} values show no significant differences between the three sites (Dam x Abaeté River = -0.009, p = 0.6; Dam x Downstream junction of Abaeté/São Francisco Rivers = 0.004, p = 0.6; Abaeté River x Downstream junction of Abaeté/São Francisco Rivers = 0.006, p = 0.52). This pattern was also observed when loci Pcos17 and Pcos18 (loci showing departure from HWE) were eliminated from the analyses (data not presented).

Although sampling error could affect the accuracy of the estimated genetic population parameters, we raise two hypotheses to explain our results. The first one states that this species may constitute a single panmictic population in the Três Marias region, as was already reported for some fishes such as *Prochilodus lineatus* (Revaldaves *et al.*, 1997; Sivasundar *et al.*, 2001). This pattern may arise from the annual reproductive migration acting as a homogenizing agent of different gene pools. Combined with large effective population sizes (Turner *et al.*, 2004) and strong gene flow, it could minimize the erosive effect of genetic drift on within-population genetic variation, thus homogenizing inter-population diversity.

On the other hand, we might not be able to detect a significant signal for population substructuring, considering previous evidence indicating that different genetic populations of other reophilic fishes may coexist and comigrate along the main river channel (Wasko and Galetti Jr., 2002; Hatanaka and Galetti Jr., 2003; Hatanaka *et al.*, 2006). If this is true for *P. costatus*, hidden substructuring may explain the heterozygote deficit found in some loci (Wahlund effect).

With the use of microsatellites we were able to perform the first diagnosis on the genetic structure of this important endemic fish, which could be valuable for management and conservation purposes. Further analyses with a larger sample size, more sampling sites and temporal replicates are encouraged. Comparing the genetic diversity between fish from both up and downstream sites of the Três Marias dam would help to evaluate the impact of the dam on the genetic structure and the diversity of this fish.

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