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PRIMER NOTE

Thirteen polymorphic microsatellite loci in the Neotropical fish *Prochilodus argenteus* (Characiformes, Prochilodontidae)

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Abstract

***Prochilodus* species inhabit the main river systems of South America and usually present commercial value to inland fishing. In the present study, we describe the isolation and characterization of 13 novel microsatellite loci in *Prochilodus argenteus*. The number of alleles per polymorphic locus varied from four to 22 and the observed heterozygosity ranged from 0.333 to 0.893. Additionally, cross-species amplification was successful in two other *Prochilodus* species. These loci will be useful for studies of the population genetic structure in this fish group.**

Keywords: conservation genetics, cross-species amplification, enrichment libraries, microsatellite, *Prochilodus*

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The family Prochilodontidae comprises species with an important role in the conservation of Neotropical rivers (Flecker 1996) and displays economical relevance for commercial and subsistence fishing (Britski 1972). The genus *Prochilodus* includes 13 described species (Castro & Vari 2003), with body sizes ranging from medium to large, occasionally reaching 50 cm in length (Britski *et al*. 1988). In the present study, we report the isolation and characterization of 13 polymorphic microsatellite loci in *Prochilodus argenteus*, contributing to further genetic population studies of this fish group.

For construction of a partial library enriched for tetranucleotide loci, total genomic DNA was extracted from liver tissue of one *P. argenteus* individual using the phenol–chloroform method (Sambrook *et al*. 1989). The DNA was digested with *Rsa*I and *Bstu*I, and DNA fragments ranging from 300 to 1200 bp were excised from the gel and purified using Wizard SV Gel and polymerase chain reaction (PCR) Clean-Up System kit (Promega). Enrichment was performed using eight biotinylated oligonucleotides [$(AAAC)_6$, $(AAAG)_6$, $(AATC)_6$, $(AATG)_6$, $(ACCT)_6$, $(ACAG)_6$, $(ACTC)_6$, $(ACTG)_6$] and streptavidin-coated magnetic beads (Streptavidin Magnesphere Paramagnetic Particles, Promega) following the protocols of Hamilton *et al*. (1999). Clones containing inserts were sequenced using DYEnamic ET Terminator Cycle Sequencing Kit (GE Healthcare), and products were resolved on an ABI PRISM 377 automated sequencer (Applied Biosystems). The microsatellite-like sequences were analysed using the TANDEM REPEATS FINDER (Benson 1999) and flanking primers were then designed using PRIMER 3 software (Rozen & Skaletsky 2000).

Variation in these loci was examined through PCR for up to 30 individuals from two collection sites on the Sao Francisco River (Brazil). All loci were amplified following a PCR method described by Schuelke (2000). In this methodology, instead of a 5′ dye-labelled primer, an M13F(–21) sequence is added to the 5′ end of each forward primer, and a labelled M13F(–21) (6-FAM or NED) primer is added to the reaction to produce labelled DNA fragments. Initially, PCR amplifications were conducted in a Mastercycler Gradient thermal cycler (Eppendorf) in order to verify the optimum primer annealing temperature. PCRs were carried out in 10 μL containing 100 ng of DNA, 200 μM dNTPs, 1× PCR buffer (20 mM Tris-HCl, pH 8.4,

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Table 1 Characterization of microsatellite loci in *Prochilodus argenteus*

Locus GenBank Accession no.	Repeat motif	Primers (5′–3′)	T_a (°C)	Size range (bp)	N_a	H_O	H_E	HWE test
Par10 DQ367227	$(TC)_{11}$	F: TGATACGGTCAGCTTTGCAG R: CTCTGTGGCCAGATGCTAGA	56	178–208	7	0.500	0.792	< 0.001
Par12 DQ367228	$(AAAC)_7$	F: CGAGCTGGTACCGTCACATA R: AGCATGATGCAAAGGATCTG	56	192–232	8	0.655	0.610	NS
Par13 DQ367229	$(GT)_{24}$	F: CATCTCACCCATTCAATTCCA R: AACACCCCACTTAGATACACCA	56	242–290	22	0.620	0.955	< 0.001
Par14 DQ367230	$(TGTC)_5$	F: GTATTAGGGGAGAGAATTTG R: TCTCATCAGTTATCACCAAC	48	232–278	8	0.516	0.529	NS
Par15 DQ367231	$(CT)_{19}(GTCT)_{10}(GT)_2$	F: AGTTGGTTACACCTAACATC R: TCTTAATATGGGTCCACTAC	47.2	144–196	20	0.888	0.926	NS
Par21 DQ367233	$(ATGA)_6$	F: CAAAAGGATAAGTAGCTCAG R: TAGCTCTGTTTATGATGACC	47.2	165–193	8	0.742	0.654	NS
Par26 DQ367234	$(GA)_3(CAGA)_9$	F: AGGTATGCAATGTTCACTC R: CTCTCTATCTGCCTTTCTTC	47.2	256–266	5	0.793	0.730	0.009
Par31 DQ367235	$(GAGT)_4(GA)_5(GGGA)_3(GA)_4$	F: GAGATGTTCCTACCTTTTTG R: TAGCCTCTCATTGTCTAGTG	56	267–279	6	0.621	0.698	0.028
Par34 DQ367240*	$(CA)_8$	F: AAGGCCTAGCTAGCAGAATCAA R: TTCCCTGTGTTCAGGTCTCC	47.2	350–384	4	0.563	0.677	NS
Par35 DQ367240*	$(GA)_3(GTGA)_6(GAAA)_2(GA)_6$	F: AGCCAGAGGAGACCTGAACA R: CCTCCCTCCTCCAGATCTTT	61.6	252–274	6	0.333	0.305	NS
Par43 DQ367237	$(GA)_6(CA)_2(CAGA)_4(GA)_{21}$	F: GGCGTCTGACTCGTTACCTC R: AACCTCATTCCCTCAAGTGC	50	241–299	17	0.893	0.904	NS
Par53 DQ367238	$(GTT)_2A(GTTT)_6$	F: ACGAATAACTGGCTGGCTGT R: CAGCCAATCATGGACTCAGA	48	164–192	8	0.714	0.723	NS
Par54 DQ367239	$(GT)_{44}$	F: GCTGTTGTTGTAGAGTGAAG R: AGAATCTGTTCTCACCAAG	48	214–262	21	0.866	0.939	0.042

T_a, annealing temperature; bp, base pairs; N_a, total number of different alleles observed; H_O, observed heterozygosity; H_E, expected heterozygosity; HWE test, P values for HWE test; NS, not significant ($P > 0.05$); *same GenBank Accession no.

and 50 mM KCl; LGC Biotecnologia), 4 pmol of each reverse and 6-FAM or NED M13(–21) primers and 1 pmol of the forward primer, 1.5 mM $MgCl_2$ and 1 U of *Taq* DNA Polymerase (LGC Biotecnologia). Conditions of the PCR amplification were as follows: 95 °C (5 min), then 35 cycles at 94 °C (30 s), 30 s at the annealing temperature (Table 1) and 72 °C (30 s), followed by 8 cycles 94 °C (30 s), 53 °C (45 s), 72 °C (45 s), and final extension at 72 °C for 10 min. For genotyping, the PCR products were submitted to electrophoresis along with GENESCAN ROX 350 (Applied Biosystems) internal size standards on an ABI 377 automated sequencer (Applied Biosystems). Allele sizes were assigned using GENESCAN and GENOTYPER version 2.5 software (Applied Biosystems). The Hardy–Weinberg equilibrium (HWE), observed and expected heterozygosities and linkage disequilibrium (LD) tests were performed using GENEPOP version 3.3 software (Raymond & Rousset 1995).

Of the 180 clones sequenced, 42 (23.33%) contained microsatellite sequences and 15 of these loci were successfully amplified. All except two (Par16 and Par42) showed high levels of polymorphism. The number of alleles per polymorphic locus varied from four (Par34) to 22 (Par13), and values of observed and expected heterozygosities ranged from 0.333 (Par35) to 0.893 (Par43) and from 0.305 (Par35) to 0.904 (Par43), respectively. The characteristics of each locus are displayed in Table 1. Cross-species amplification for three individuals of two *Prochilodus* species (*P. costatus* and *P. lineatus*) was successful for all loci, with the exception of Par34, which presented a faint band in *P. lineatus*. Significant deviations from the HWE were found in the Par10, Par13, Par26, Par31 and Par54 loci ($P < 0.05$), which can be explained by the presence of null alleles, mating systems, cryptic population structure and nonrandom sampling. According to the genotypic LD test, no two loci appeared to be linked ($P > 0.05$ in all cases). Although two loci (Par 34 and Par35) were found in the same sequence read, genotypic LD was not significant ($P = 0.64$). The microsatellite loci developed in the present work will provide useful markers to allow a better understanding of the *Prochilodus* population structure through the identification of natural stocks and, thus, contribute to conservation programs regarding this fish group.

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