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PRIMER NOTE

Isolation and characterization of polymorphic microsatellite markers in the migratory freshwater fish *Prochilodus costatus*

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Abstract

We isolated six polymorphic microsatellite loci in the migratory freshwater fish *Prochilodus costatus*, which is an endemic species and important fisheries resource from São Francisco river basin, Brazil. We have evaluated the applicability of these loci to study genetic variation in wild population of this fish. Thus, based on the genotypes of 32–48 individuals, we detected two to 21 alleles per locus, observed and expected heterozygosities ranging from 0.19 to 0.89 and from 0.17 to 0.92, respectively. These polymorphic markers should provide efficient tools to study population genetic structure of this fish.

Keywords: genetic variation, microsatellites, polymorphism, *Prochilodus costatus*

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'Curimatá-pióa', or *Prochilodus costatus* (= *Prochilodus affinis* Valenciennes, 1850), is an endemic species from São Francisco river basin (southeastern and northeastern Brazil) (Froese & Pauly 2005). It is characterized as a migratory fish as well as an important fisheries resource of that basin (Paiva 1983).

A good way to study population genetic variation is through the use of specific molecular markers. Among them, microsatellites (short tandemly repeated base-pair sequences) are the most useful due to the high variability caused by changes in their repeat number (Schlötterer & Pemberton 1998).

To date, there are no available isolated microsatellite loci for *P. costatus*. Here, we present the isolation and characterization of polymorphic microsatellite loci for this species, with the intention to use them in order to characterize the genetic variation of wild populations of this fish.

To obtain microsatellite loci, we generated a plasmid library containing genomic inserts previously enriched for eight tetranucleotide repeats via hybridization capture procedure, which is a modification of the protocol presented by Hamilton *et al.* (1999). Clones containing inserts were sequenced using kit DYEnamic ET Terminator Cycle Sequencing kit (GE Healthcare) and products were resolved on an ABI 377 (Applied Biosystems) automated sequencer.

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The sequences were screened through GENERUNNER 3.05 software (Hastings Software) and microsatellite-like sequences were found using TANDEM REPEATS FINDER (Benson 1999). Primers were designed based on the sequences flanking the repeat in each clone using the software PRIMER 3 (Rozen & Skaletsky 2000). Polymorphisms were analysed by polymerase chain reaction (PCR) for a pool of individuals from the São Francisco River.

DNA extractions from liver samples followed Aljanabi & Martinez (1997). PCRs were initially performed in Mastercycler Gradient thermal cycler (Eppendorf) in order to verify the optimal primer annealing temperature. Posteriorly, PCRs were carried out in a PTC-100 thermal cycler (MJ Research) using 10 μL reactions containing 100 ηg of DNA, 0.2 mM of dNTPs, 1× PCR buffer (200 mM Tris-HCl, pH 8.4 and 500 mM KCl; Invitrogen Life Technologies), 10 pmol/μL of each primer, 1.5 mM $MgCl_2$ and 1 U of *Taq* DNA polymerase. The thermal cycler was programmed for an initial denaturing step at 95 °C for 5 min, followed by 35 cycles consisting of 30 s at 94 °C, 30 s at the primer annealing temperature and 30 s at 72 °C, followed by 20 min of final extension at 72 °C. Amplification products were first resolved in 1% agarose gel, under 100 V, using molecular weight marker of 100 bp (Invitrogen Life Technologies). We assessed the number of alleles per locus by genotyping the fragments in an automated sequencer using the forward primer of each locus with a M13 tail, reverse primer and M13 + NED/FAM

fluorescently labelled primer (Schuelke 2000). Allele sizes were scored against an internal GeneScan-350 (ROX) size standard (PE Applied Biosystems) through GENESCAN 3.1 and GENOTYPER 2.5 software (ABI).

Of 124 clones analysed, 42 (33.9%) contained microsatellite motifs, of which we were able to design primers to 20. Of these, we could verify the best primer annealing temperature in 13 (Table 1). At last, only six (46%) showed polymorphism (Table 1).

The number of alleles ranged from two to 21, while the observed and expected heterozygosities ranged from 0.19 to 0.89 and from 0.17 to 0.92, respectively. GENEPOP (version 3.4, Raymond & Rousset 1995) analysis revealed no evidence of linkage disequilibrium among pairs of loci for our sample sizes. The analysis indicated that Pcos03, Pcos04, Pcos17 and Pcos18 loci showed significant deviations from Hardy–Weinberg equilibrium (HWE), suggesting significant heterozygote deficit for these loci. This is probably due to our sampling strategy, which included individuals from different collection sites in the analysed pool. If they represent subpopulations, such pattern of heterozygote deficit may arise due to the Wahlund effect. In contrast, in the case of a single population it may be due to null alleles. However, only future population studies will be able to clarify this question.

We evaluated the applicability of these loci to other taxa, in an attempt to amplify similar size products in two other *Prochilodus* species, *Prochilodus argenteus* and *Prochilodus lineatus*, using the same PCR conditions. There was cross amplification of all loci.

These loci provide efficient tools to study genetic variation of *P. costatus* and should contribute to the establishment of future management plans for these important ecological and fisheries resources.

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Table 1 Locus assignment/GenBank Accession nos, nature of the repeat motif, primer annealing temperature (T_a), sample size (n), size range of alleles in base pairs, number of alleles, observed (H_O) and expected (H_E) heterozygosities for six microsatellite loci isolated from *Prochilodus costatus*

Locus	Repeat motif	Primer sequence	T_a (°C)	n	Size range (bp)	No. of alleles	H_O	H_E
Pcos03* DQ267693	$(CTGT)_5$	F: CTGGAGTAGCATGAGGAG R: GTTTAACAGCATAGTGATGG	55.6	48	212–228	06	0.38	0.54
Pcos04* DQ269986	$(GT)_{11}(GA)_7(GTGA)_2$	F: GCCTTTTATATGGCAGTATC R: GTTCTGTTTTCCAGTTAGTG	59.8	34	152–180	07	0.35	0.68
Pcos14 DQ267694	$(TC)_{49}$	F: CGTGAATGTGCTTTATATGC R: AATGCCATTTCTGATTAAGG	47.0	47	204–262	21	0.89	0.92
Pcos17* DQ267696	$(CAAA)_{13}$	F: ACGGTAATGATGGGTATTTG R: CGTTGTTTAACCCTTCTCAG	51.3	46	158–242	20	0.57	0.80
Pcos18* DQ267697	$(GT)_{20}$	F: TCTCTTCTCACACACCTTCC R: TGATTACCAGCAACAGTTTG	55.6	48	208–250	10	0.31	0.73
Pcos20 DQ267698	$(GACA)_6$	F: GTGTACATTGGCCTGTTTATC R: AGGGCAGTTAGTGTCACATC	55.6	32	170–186	02	0.19	0.17

*Loci that displayed significant deviations from HWE.