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# A polymorphic, telomeric-like sequence microsatellite in the Neotropical fish *Prochilodus*

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**Abstract.** A microsatellite locus from the Neotropical fish genus *Prochilodus* was isolated using PCR-based isolation of microsatellite arrays. Of 470 positive clones, 15 were sequenced, and 10 of them showed an $(AATTT)_n$ repeat. Primers were designed, and analysis of polymorphism revealed 11 alleles in three *Prochilodus* species. Fluorescence in situ hybridization analysis showed signals predominantly in the telomeric regions of several chromosomes. The description of this microsatellite may contribute to studies of the population structure of this fish group.



Microsatellites are tandemly repeated tracts of DNA composed of 1- to 6-bp-long units randomly distributed along the chromosomes. These markers are extremely polymorphic both within and among populations and, therefore, can offer a source of information for a more thorough understanding of population structure. *Prochilodus* is considered a dominant element in the structure of fish communities, with important implications in the conservation of Neotropical streams (Flecker, 1996). Thirteen *Prochilodus* species have been described (Castro, 1990), most of which are highly valuable commercially in inland fishing. In view of the wealth of Neotropical ichthyofauna and the previous description of microsatellites for a single species, *Piaractus mesopotamicus* (Calcagnotto et al., 2001), the purpose of the present study was to isolate the microsatellite of *Prochilodus*, correlate the findings with population studies in this fish group, and chromosomally localize the microsatellite by means of fluorescence in situ hybridization (FISH).

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## Materials and methods

Three Brazilian species of *Prochilodus* were studied, *P. marggravii* and *P. affinis* from the São Francisco River (Minas Gerais) and *P. lineatus* from the Mogi-Guaçu River (São Paulo). Mitotic chromosome preparations were obtained from a cell suspension of the anterior kidney (Bertollo et al., 1978). Genomic DNA was extracted from liver using phenol-chloroform (Sambrook et al., 1989). The microsatellite and its flanking regions were isolated using PCR-based isolation of microsatellite arrays (PIMA), as described by Lunt et al. (1999). RAPD fragments were amplified by PCR, using the primers OPP-7 (5′ GTC CAA TGC CA 3′) and OPP-9 (5′ GTG GTC CGC A 3′) (Operon Technologies) and DNA samples from one *P. marggravii* (male) specimen and one *P. lineatus* (female) specimen in individual reactions. The PCR products were purified using a Wizard-PCR spin column (Promega) and cloned into pGEM-T (Promega). Altogether, 470 recombinant colonies were analyzed in PCR reactions using the primers M13 Forward and M13 Reverse, plus a repeat-specific primer, 5′ TGT GGC GGC $GC(TG)_8$ V 3′.

A total of 15 clones presented an extra amplification product, which were sequenced on an automated DNA sequencer (Applied Biosystems model 377) using a ABI Prism BigDye Terminator Cycle Sequencing kit (Applied Biosystems). Sequence analysis revealed 10 clones with short tandem repeats; all containing the same repetitive unit (AATTT), although two distinct types of alleles were observed $(AATTT)_{10}$ and $(AATTT)_{12}$, indicating that the individual (*P. lineatus*) from which this microsatellite was isolated was heterozygous for this locus.

PCR primers were designed from the flanking sequence for clone *pl1* (Accession No. AY176774), and the variability of this locus was examined using DNA samples from *P. marggravii*, *P. lineatus*, and *P. affinis*. Microsatellite PCR amplification was conducted in a 25-µl volume containing 100 ng template DNA, 200 µM dNTP, 0.4 µM of each primer, 1 × *Taq* buffer, and 1 U *Taq* DNA polymerase. After an initial 4-min denaturation at 94 °C, 35 cycles of PCR (1 min at 94 °C, 1 min at 56 °C, and 1 min at 72 °C) were performed, followed by 5 min at 72 °C. PCR products were resolved on 6 %

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polyacrylamide denaturing gels containing 7 M urea. Gels were run at 55 W for 3 h and then stained with silver nitrate as described by Comincini et al. (1995). The number of alleles was identified using a 10-bp ladder (Invitrogen).

## Results and discussion

The results showed that the microsatellite isolated from *Prochilodus* is polymorphic, having at least 11 different alleles in the three species analyzed (Fig. 1).

The clone *pl*1 was subsequently utilized as probe for FISH in the mitotic chromosomes of *P. lineatus* and *P. marggravii*, as described by Pinkel et al. (1986). Better results were achieved, however, under low-stringency (30%) conditions (Fig. 2). The FISH data showed fluorescent signals predominantly in the telomeric regions of several chromosomes and displayed a probable genetic similarity. In higher vertebrates, telomeric DNA is composed of a variable number of repeated short units, 2–23 bp long, arranged in tandem, with a strong predominance of the TTAGGG sequence (Meyne et al., 1989), which may explain the fact that the telomeric regions hybridized with the microsatellite $(AATTT)_n$ under low-stringency conditions. The difficulty of locating this microsatellite in any chromosomal regions under higher stringency seems to be related to the inability of the FISH technique to detect very short repetitive segments (Meyne et al., 1989). In contrast, 58 microsatellites have been mapped immediately adjacent to the telomere on human chromosomes, and 38 of these microsatellites are polymorphic (Rosenberg et al., 1997). These subtelomeric sequences can contain a wide variety of repetitive DNA, ranging from interspersed repeats, where one or a few copies are found on a restricted number of chromosome ends, to highly repetitive sequences present in many subterminal regions (Cross et al., 1990; Rosenberg et al., 1997). In fish, microsatellite markers have been mapped in *Misgurnus anguillicaudatus*, with distributions on the centromeres to telomeres of their respective chromosomes (Morishima et al., 2001). Genetic maps have also been constructed using 2,000 microsatellite repeats in *Danio rerio* (Shimoda et al., 1999), and 199 in *Oncorhynchus mykiss* (Sakamoto et al., 2000). However, the construction of a genetic map for Neotropical fish is limited because only a few microsatellite markers have been characterized for any species so far.

The results of the present study are promising, highlighting the extreme importance of isolating and characterizing microsatellites in Neotropical fish. The PIMA methodology can be effectively utilized to isolate microsatellites and, being a fairly simple technique, can be applied in laboratories with a mini-

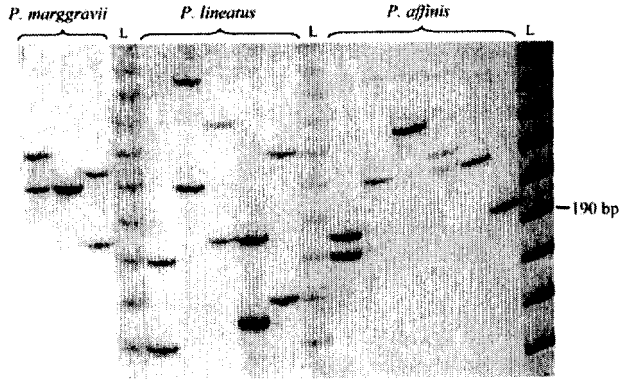


**Fig. 1.** Denaturing polyacrylamide gels showing PCR products. The *pl*1 locus was amplified from three individuals of *P. marggravii*, five individuals of *P. lineatus*, and six individuals of *P. affinis*. A 10-bp molecular weight marker is denoted in lane L.

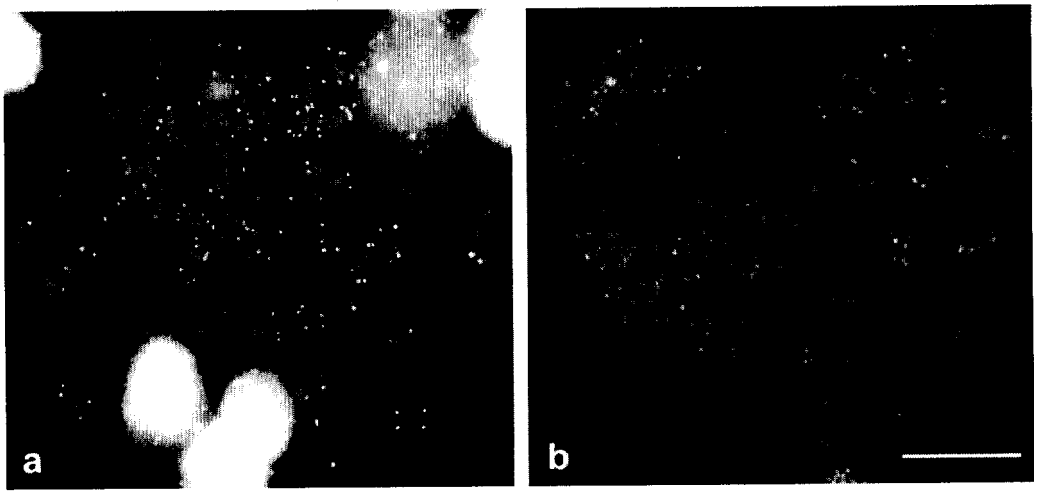


**Fig. 2.** Analysis by FISH with the *pl*1 probe in metaphase chromosomes of *P. lineatus* (**a**) and *P. marggravii* (**b**). Bar = 5 μm.

mum of available resources, dispensing with the need for radioactive materials and the construction of genomic libraries of the organism of interest. Moreover, the description of this first microsatellite in *Prochilodus* may contribute effectively to studies on the population structure of this fish group.


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## References


Bertollo LAC, Takahashi CS, Moreira-Filho O: Cytotaxonomic consideration on *Hoplias lacerdae* (Pisces, Erythrinidae). Rev bras Genet 1:103–120 (1978).

Calcagnotto D, Russello M, DeSalle R: Isolation and characterization of microsatellite loci in *Piaractus mesopotamicus* and their applicability in other Serrasalminae fish. Mol Ecol Notes 1:245–247 (2001).

Castro RMC: Revisão taxonômica da família Prochilodontidae (Ostriophysi, Characiformes). PhD thesis, Universidade de São Paulo, Ribeirão Preto, Brazil (1990).

Comincini S, Leone P, Redaelli L, De Giuli L, Zhang Y, Ferreti L: Characterization of bovine microsatellites by silver staining. J anim Breed Genet 112:415–420 (1995).

Cross S, Lindsey J, Fantes J, McKay S, McGill N, Cooke H: The structure of a subterminal repeated sequence present on many human chromosomes. Nucl Acids Res 18:6649–6657 (1990).

Flecker AS: Ecosystem engineering by a dominant detritivore in a diverse tropical stream. Ecology 77: 1845–1854 (1996).

Lunt DH, Hutchinson WF, Carvalho GR: An efficient method for PCR-based isolation of microsatellite arrays (PIMA). Mol Ecol 8:891–894 (1999).

Meyne J, Ratliff RL, Moyzis RK: Conservation of the human telomere sequence $(TTAGGG)_n$ among vertebrates. Proc natl Acad Sci, USA 86:7049–7053 (1989).

Morishima K, Nakayama I, Arai K: Microsatellite-centromere mapping in the loach, *Misgurnus anguillicaudatus*. Genetica 111:59–69 (2001).

Pinkel D, Straume T, Gray JW: Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA 83:2934–2938 (1986).

Rosenberg M, Hui L, Ma J, Nusbaum HC, Clark K, Robinson L, Dziadzio L, Swain PM, Keith T, Hudson TJ, Biesecker LG, Flint J: Characterization of short tandem repeats from thirty-one humam telomeres. Genome Res 7:917–923 (1997).

Sakamoto T, Danzmann RG, Gharbi K, Howard P, Ozaki A, Khoo SK, Woram RA, Okamoto N, Ferguson MM, Holm LE, Guyomard R, Hoyheim B: A microsatellite linkage map of rainbow trout *(Oncorhynchus mykiss)* characterized by large sex-specific differences in recombination rates. Genetics 155:1331–1345 (2000).

Sambrook J, Fritish EF, Maniatis T: Molecular Cloning: A Laboratory Manual, 2nd Ed (Cold Spring Harbor Laboratory Press, Cold Spring Harbor 1989).

Shimoda N, Knapik EW, Ziniti J, Sim C, Yamada E, Kaplan S, Jackson D, Sauvage F, Jacob H, Fishman MC: Zebrafish genetic map with 2000 microsatellite markers. Genomics 58:219–232 (1999).