

PRIMER NOTE

Microsatellites loci isolated in the freshwater fish *Brycon hilarii*

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We describe seven microsatellite loci, including tetra-, tri- and dinucleotides, isolated from *Brycon hilarii*, which is a migratory fish inhabiting the Paraguay River basin (Brazil) and is highly valued for its meat quality. Three to eight alleles per locus were detected and the expected heterozygosity ranged from 0.31 to 0.81. Positive results were obtained with cross-amplification in five other species of *Brycon*. These microsatellites provide a potential tool for wild population and aquaculture studies of *B. hilarii* and other species of the genus.

Keywords: Bryconinae, Characidae, microsatellites, population genetics

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Brycon comprises a fish group of great importance for fishing and aquaculture (Mendonça 1994). It is the main representative of the subfamily Bryconinae (Characidae) and occurs in the large hydrographic basins of the Neotropical region (Lima 2003). It is valued for its superior meat quality. Some species of this group are suffering a populational decline mostly related to unregulated human activities. Six species are endangered in various Brazilian hydrographic basins (MMA 2004). *Brycon hilarii* (Valenciennes, 1850) is a migratory fish that occurs throughout the Paraguay River basin. In this study, we report the isolation and characterization of microsatellite loci from *B. hilarii* which will allow the analysis of the genetic variability of wild and captive populations.

Total DNA was extracted from blood samples (Lahiri & Nurnberger 1991). We used the random amplified polymorphic DNA (RAPD)-based PIMA (polymerase chain reaction isolation of microsatellite arrays) methodology (Lunt *et al.* 1999). RAPD reactions (Welsh & McClelland 1990; Williams *et al.* 1990) were performed using 13 10-mer primers. RAPD fragments were purified by the Wizard PCR Purification kit (Promega), ligated into pGEM-T vectors (Promega) and then used to transform DH5 α *Escherichia coli* cells.

PCRs from 1056 recombinant colonies were performed using the standard M13 primers plus the repeat-specific [5'-TGTGGCGGCCGC(TG)₈V-3'] primer. Eighty-six of the recombinant colonies (0.08%) presented an extra amplification product, indicating a repetitive element in the insert. These were sequenced using the DYEnamic ET Terminator Cycle Sequencing kit (GE Healthcare) on an ABI PRISM 377 automatic sequencer (Applied Biosystems). Forty-one per cent of the sequenced clones presented repetitive motifs.

Primers for 17 loci were designed using the GENERUNNER 3.05 software (Hastings Software Inc.). Preliminary assessment of polymorphism was performed on a few individuals. Reactions were performed in a total volume of 15 μ L containing 100 ng DNA, 1 \times PCR buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl), 1.5 mM MgCl₂, 5 pmol of each primer, 0.2 mM of each dNTP and 1 U *Taq* DNA polymerase. The PCR profile consisted of 5 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 56 °C, 1 min at 72 °C, and a final extension at 72 °C for 20 min. PCR products were separated by electrophoresis on a 6% polyacrylamide gel and visualized by the silver staining method (Comincini *et al.* 1995). Seven loci (Table 1) produced consistent and polymorphic results and were selected for the genotyping of 30 individuals collected at the Miranda River sub-basin (Paraguay River Basin, Brazil).

Genotyping was performed by fluorescent dye labelling of the PCR fragments in one reaction (Schuelke 2000) using

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Table 1 Characterization of seven microsatellite loci isolated from *Brycon hilarii*. Number of alleles (n_a), observed (H_O) and expected (H_E) heterozygosities based on a sample of 30 individuals. GenBank Accession nos. DQ408242–DQ408248

Locus	Primer sequence (5'–3')	Repeat motif	Size range n_a (bp)	H_O/H_E	P value	Cross-amplification/allele range				
						<i>Brycon orthotaenia</i>	<i>Brycon orbignyana</i>	<i>Brycon falcatus</i>	<i>Brycon cephalus</i>	<i>Brycon insignis</i>
Bh5	F: CTTCCACTCATACCCGGCACT R: ACATCTGGCATTAGGCATAG	(AC) ₁₃	7 204–220	0.76/0.81	0.63	214–220	204–208	—	216–218	218
Bh6	F: GCGTTCGCTGTGTATGT TAA R: AGAGGTGTCCACAAAGTTTT	(GT) ₁₄	6 160–184	0.57/0.67	0.03*	164–180	172	—	180–184	172–208
Bh8	F: CCATGGCTCAACACAGATAT R: TGTACGAATCCTGAAATGCT	(GAT) ₅	8 127–196	0.77/0.76	0.21	185	187	176	189–198	186–189
Bh13	F: AGCAATTTAAGCAAGTGAAG R: GCGTCGGAGCAGTAGTTATA	(AT) ₇	5 120–160	0.83/0.78	0.65	135–143	148–152	168	158	158
Bh15	F: GAGAGCATGTGTCAGGATTTA R: ACTAATGACTGCTACTGCGG	(ATTT) ₅	3 130–142	0.53/0.53	1	138	138	—	130–150	130
Bh16	F: CCTCCAATGAAAACAGTGGC R: ACGACTTAGCCACCCACCT	(TAA) ₈	3 141–147	0.27/0.31	0.10	144–153	144–150	144	141	141
Bh17	F: GTCAGCACTCAGCACATAGC R: AGAGAGCCTGAAAGTGAGTC	(GTTT) ₄ (GGTTT) ₃	7 152–212	0.83/0.81	0.82	—	226	105	220–228	214–219

*Significant P value < 0.05.

the reverse microsatellite primer, the forward primer with an M13(–21) tail at its 5' end, and the universal fluorescent-labelled M13(–21) primer. 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). Hardy–Weinberg and linkage disequilibrium tests were carried out using GENEPOP 3.4 (Raymond & Rousset 1995). Values of significance were estimated by the Markov chain method with 10 000 batches.

Three to eight alleles per locus were detected and the expected heterozygosity ranged from 0.31 to 0.81 (Table 1). All but one (Bh6) conformed to the Hardy–Weinberg expectations (Table 1). The Bh6 locus presented heterozygote deficit, probably indicating the occurrence of null alleles, non-random sampling, inbreeding or mating systems. Two out of 21 pairwise tests of genotypic disequilibrium (Bh8 × Bh13, Bh6 × Bh17) were significant (P value 0.032 and 0.007, respectively) and may indicate that these loci are linked.

Cross-amplification was successful in five other *Brycon* species — *B. orthotaenia* (= *B. lundii*), *B. orbignyana*, *B. falcatus* (= *B. brevicauda*), *B. cephalus* and *B. insignis* (Table 1). The present data introduce a set of primers as a powerful tool to study populations of *Brycon hilarii* and other species of this genus.

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