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PRIMER NOTE

Development and characterization of microsatellite markers for the Amazonian blackwing hatchetfish, *Carnegiella marthae* (Teleostei, Gasteropelecidae)

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Abstract

The blackwing hatchetfish, *Carnegiella marthae*, is a small characin species distributed in forest streams of the Negro and upper Orinoco River basins in Amazonia. Freshwater hatchetfish are popular in the aquarium trade and represent an economic resource for the riverine people from middle Rio Negro, in Brazil. We isolated and characterized seven microsatellite DNA loci for the blackwing hatchetfish. Number of alleles and heterozygosity per locus in a sample of 30 fish ranged from three to 17 and from 0.19 to 0.87, respectively. These microsatellite loci provide powerful markers for studies on taxonomy, management and phylogeographic history of Amazonian hatchetfish.

Keywords: Amazon rainforest, *Carnegiella marthae*, conservation genetics, Gasteropelecidae, microsatellites, phylogeography

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The rivers of northern South America, especially those draining the Amazonia rainforest, contain an exceptional diversity of freshwater fish. This diversity is largely understudied both from an ecological and from a biogeographic perspective. We are generating large DNA data sets using microsatellites, mitochondrial DNA and intron DNA markers to investigate population history in four codistributed fish groups from central Amazonia (e.g. Beheregaray *et al*. 2004a, b; Beheregaray *et al*. 2005). The present study adds to this effort by describing a set of microsatellite DNA markers for the fourth study species of our comparative study, the blackwing hatchetfish, *Carnegiella marthae* (Teleostei, Gasteropelecidae). This peculiarly shaped and small fish is found in forest streams throughout the Amazon basin and upper Orinoco (Géry 1977; Weitzman & Palmer 2003). Blackwing hatchetfish are popular in the aquarium trade and represent a valuable resource for ornamental fishermen from middle Rio Negro, in Brazil (Chao *et al*. 2001). We expect that the microsatellite markers described here will prove useful for studies on taxonomy, phylogeography and conservation management of Amazonian hatchetfish.

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Blackwing hatchetfish microsatellite loci were isolated using a modified enrichment technique (Fischer & Bachmann 1998). Genomic DNA was digested with *Rsa*I and *Hae*III and fragments ligated to two oligo adaptors (Edwards *et al*. 1996). Two biotinylated oligo probes (dGA_{10} and dGT_{10}) were hybridized to the digested DNA and separated using streptavidin magnetic particles (Promega). Polymerase chain reactions (PCRs) were performed on the microsatellite-enriched eluate using one of the oligo adaptors as a primer. The enriched library was purified using a gene clean kit (Qbiogene), ligated into pCR 2.1-TOPO vector (Invitrogen) and transformed into TOP10 cells (Invitrogen). The plasmid DNA was purified and 38 putative positive clones were sequenced on an ABI 377 automated DNA sequencer (PE Applied Biosystems) using dye terminator chemistry. Primers flanking eight dinucleotide microsatellite loci were designed using PRIMER 3 (Rozen & Skaletsky 1997).

We assessed allelic and genotypic variation at these eight microsatellite loci by PCR using a 10-μL radiolabelled reaction containing ~50–100 ng of template DNA, 12 pmol of each primer, 0.5 U of *Taq* DNA polymerase (Promega),

Table 1 Primer sequences and characteristics of seven blackwing hatchetfish (*Carnegiella marthae*) microsatellite loci. Number of alleles (N_a), observed (H_O) and expected (H_E) heterozygosities are based on a sample of 30 individuals. T_a is the annealing temperature(s) used in PCRs

Locus	Primer sequences (5′–3′)	Repeat structure	T_a	N_a	Size range (bp)	H_O/H_E	GenBank Accession no.
Cm3	CCTCAGGTGAGTCTGATATTAAAGG CGCGTGGACTAGAGTTCTCC	$(CA)_{21}$	63–55	8	241–277	0.57/0.47	DQ297667
Cm4	CTGCCGCTGATGAGACTGTA CTGCCGCTGATGAGACTGTA	$(CA)_7$	63–55	4	210–228	0.29/0.45	DQ297668
Cm6	AGCTGTCTGAGGCAATGGTG TGCCACCCAAAAAGAAAGTC	$(GA)_{27}$	55–47	17	240–316	0.80/0.80	DQ297669
Cm8	CAGGAGCCTCATAGGGTGAC TTTAGCACTGCTTCTTGCTGAG	$(CT)_{29}$	55–47	16	106–146	0.87/0.87	DQ297670
Cm10	CACACTCTCACACACATAGGG GAAGGAGAAGAAGCAGGCTA	$(CT)_{14}$	60	15	274–322	0.80/0.84	DQ297671
Cm20	CAGCTATGTAGAGAGTGTCAGC CACCCCCTCACTGTGTTAC	$(CA)_5$	63–55	3	123–169	0.20/0.19	DQ297672
Cm23	TGTGCACCAGTGTGTGTTTG TGACTCCTTCCTTCCAAAGC	$(GT)_{20}$	55–47	5	160–172	0.27/0.39	DQ297673

200 μM of dCTP, dGTP and dTTP, 20 μM of dATP, 2.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.1% Triton X-100 and 0.05 μL [α^{33}P]-dATP at 1000 Ci/mmol. For all loci (except Cm10), the PCR amplifications consisted of 94 °C for 3 min, followed by a 32 cycles 'touchdown' (94 °C for 20 s; annealing temperature(s) for 45 s; 72 °C for 60 s) and 72 °C for 4 min. For Cm10, the annealing temperature used was 60 °C. The PCR products were separated by 6% polyacrylamide gel electrophoresis and visualized by autoradiography. We used GENEPOP version 3.3 (Raymond & Rousset 1995) to test for linkage disequilibrium (LD) and to estimate expected (H_E) and observed (H_O) heterozygosities.

Seven out of the eight microsatellite loci amplified successfully and were screened for variation in a sample of 30 blackwing hatchetfish collected from Rios Uaupés and Jurubaxi (Rio Negro basin, central Amazonia, Brazil). We detected moderate to high levels of genetic variability with the number of alleles per locus ranging from three to 17 and expected heterozygosity ranging from 0.19 to 0.87 (Table 1). No evidence for LD was detected in locus pair/population comparisons. All loci were at Hardy–Weinberg equilibrium within each population. Extra optimization is advisable to facilitate the scoring of loci Cm4 and Cm23.

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