PRIMER NOTE

Characterization of microsatellite loci for the Amazonian rummy-nose tetra, *Hemigrammus bleheri* (Teleostei, Characidae)

L. B. BEHEREGARAY,* J. CHAE,* N. L. CHAO† and A. CACCONE‡
*Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia, †Departamento de Ciências Pesqueiras, Universidade Federal do Amazonas, Manaus, AM 69700-000, Brazil, ‡Department of Ecology and Evolutionary Biology and Yale Institute of Biospheric Studies, Yale University, New Haven, CT 06520-8106, USA

Abstract

The rummy-nose tetra, *Hemigrammus bleheri*, is a small fish found in forest streams of the Rio Negro floodplain, central Amazonia. This species is popular in aquaria and used as an ornamental fishery resource by the riverine people from middle Rio Negro. Here we describe eight microsatellite DNA loci for rummy-nose tetras. Number of alleles and heterozygosity per locus in a sample of 30 fish ranged from five to 22 and from 0.52 to 0.96, respectively. These highly variable genetic markers provide important tools for investigating population history and identifying conservation units in rummy-nose tetras.

Keywords: Amazon rainforest, Characidae, conservation genetics, *Hemigrammus bleheri*, microsatellites, phylogeography

Received 14 January 2005; revision accepted 22 February 2005

The world's richest diversity of freshwater fishes is found in the lowland forests of Amazonia. Attempts to explain high species diversity in Amazonia will likely benefit by the integration of molecular comparative studies with traditional biogeographical data. We are combining DNA data with biogeographical information to investigate the history of population diversification of codistributed fish species from central Amazonia (e.g. Beheregaray *et al*. 2004a, b). One of our study species is the rummy-nose tetra *Hemigrammus bleheri*, a small fish found in flooded forest streams of the Rio Negro floodplain (Géry & Mahnert 1986). Rummy-nose tetra is a popular aquarium fish and is part of a multispecies ornamental fishery that provides about 60% of the income of the riverine people from middle Rio Negro (Chao *et al*. 2001). Here we report the isolation and characterization of a set of polymorphic microsatellite DNA markers for rummy-nose tetras. These genetic markers provide important tools for studies of population structure, population history and conservation management.

Microsatellite loci were isolated using a modified enrichment technique (Fischer & Bachmann 1998). Genomic DNA of the rummy-nose tetra 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 30 putative positive clones were sequenced on an ABI PRISM 377 automated DNA sequencer (PE Applied Biosystems) using dye terminator chemistry. Primers flanking 10 loci were designed using PRIMER 3 (Rozen & Skaletsky 1997).

Genotypic variation at each microsatellite locus was assessed by PCR using a 10-μL radiolabelled reaction containing *c*. 50–100 ng of template DNA, 12 pmol of each primer, 0.5 U of *Taq* DNA polymerase (Promega), 200 μM of dCTP, dGTP and dTTP, 20 μM of dATP, 2–2.5 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. PCR amplifications consisted of 94 °C for 3 min, followed by 32 cycles 'touchdown' (94 °C/20 s; 63 °C down to 55 °C until

Correspondence: Luciano B. Beheregaray, Fax: 61 (2) 9850 8245; E-mail: luciano.beheregaray@bio.mq.edu.au

© 2005 Blackwell Publishing Ltd

Table 1 Primer sequences and characteristics of eight rummy-nose tetra (*Hemigrammus bleheri*) microsatellite loci. Number of alleles (N_a), observed (H_O) and expected (H_E) heterozygosities are based on a sample of 30 individuals

Locus	Primer sequences (5′–3′)	Repeat structure	Mg^{2+} (mM)	N_a	Size range (bp)	H_O/H_E	GenBank Accesion no.
Hb3	AACCATAAACATGAGCAGAGACTG AAAGTTCATTAATATAGTTGCATGT	$(GT)_{13}$	2.5	5	180–188	0.68/0.70	AY775337
Hb8	TTTCTTTTTGGAGCTCAGTGTG CGTTTGTTGTTTCAGGCAAAG	$(CA)_8$	2.5	22	246–300	0.98/0.96	AY775338
Hb13	TCCACACCGCTGTAAAAGTG CTGAGTTTTTCTGCTCTGGACA	$(GA)_{12}$	3.0	12	216–248	0.74/0.73	AY775339
Hb15	GAATTATTCACACTGTTTGCAGAGC CGCTCTCTCATCCTCACCAC	$(GT)_{15}$	2.5	7	188–200	0.64/0.62	AY775340
Hb19	TTGGGTTTCAGTGTTCCAAAG TGCATGGGTGTTAGTTCTGC	$(CA)_{18}$	2.5	22	136–234	0.92/0.94	AY775341
Hb21	GCAGTGGGTGGAGGGTAGTAG GTGGTCAGCGGTAGTCCTG	$(GA)_{13}$	2.5	6	146–160	0.58/0.62	AY775342
Hb29	AGCGACCGTGTTCTTACAGG CCGGAGTTAGTGCAAGGAAC	$(GT)_{17}$	3.0	7	122–144	0.70/0.66	AY775343
Hb33	ACACGAGGATGTTCCAGGAG TTTGTAAGCTATAGGATATGGATGC	$(GT)_{20}$	3.0	5	200–220	0.40/0.52	AY775344

the fifth cycle/45 s; 72 °C/60 s) and 72 °C for 4 min. The products of PCRs 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.

Eight dinucleotide loci amplified successfully and were screened for variation in a sample of 30 rummy-nose tetras collected from Rios Tea, Cuiuni and Igarapé Arixaná (middle Rio Negro, Brazil). These loci revealed high levels of genetic variability, with the number of alleles per locus ranging from five to 22 and H_E from 0.52 to 0.96 (Table 1). No evidence for LD was detected in locus-pair/population comparisons. The majority of the eight loci were at Hardy–Weinberg equilibrium in all populations, except for Hb 33, which showed marked excess of homozygotes possibly related to null alleles.

Acknowledgements

We thank the Ecosave program (Yale Institute for Biospheric Studies, YIBS) and the Macquarie University Early Career Research Scheme for funding this research. We are also grateful to Kristin Saltonstall for the modified enrichment protocol. Logistic support and field assistance in Amazonia was provided by Project Piaba (Universidade Federal do Amazonas — PRONEX CNPq no. 46.6090/2001-4 and Bio-Amazonia Conservation International).

References

Beheregaray LB, Möller LM, Schwartz TS, Chao NL, Caccone G (2004a) Microsatellite markers for the cardinal tetra *Paracheirodon axelrodi*, a commercially important fish from central Amazonia. *Molecular Ecology Notes*, **4**, 330–332.

Beheregaray LB, Schwartz TS, Möller LM, Call D, Chao NL, Caccone G (2004b) A set of microsatellite DNA markers for the one-lined pencilfish *Nannostomus unifasciatus*, an Amazonian flooded forest fish. *Molecular Ecology Notes*, **4**, 333–335.

Chao NL, Petry P, Prang G, Sonneschien L, Tlusty M (2001) *Conservation and Management of Ornamental Fish Resources of the Rio Negro Basin, Amazonia, Brazil. (Project Piaba)*. Universidade do Amazonas Press, Manaus, Amazonas.

Edwards KJ, Barker JHA, Daly A, Jones C, Karp A (1996) Microsatellite libraries enriched for several microsatellite sequences in plants. *BioTechniques*, **20**, 758–760.

Fischer D, Bachmann K (1998) Microsatellite enrichment in organisms with large genomes (*Allium cepa* L.). *BioTechniques*, **24**, 796–802.

Géry J, Mahnert V (1986) A new rummy-nose tetra from the Rio Negro, Brazil: *Hemigrammus bleheri* n. sp. (Characidae, Tetragonopterinae), with comments on *Paracheirodon*. *Tropical Fish Hobbyist*, **37**, 40–41.

Raymond M, Rousset F (1995) GENEPOP (version 3.3): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rozen S, Skaletsky HJ (1997) PRIMER 3. Whitehead Institute for Biomedical Research. Code available at http://www.genome.wi.mit.edu/genome_software/other/primer3.html