Molecular Ecology Notes (2004) **4**, 330–332 doi: 10.1111/j.1471-8286.2004.00686.x

PRIMER NOTE

Microsatellite markers for the cardinal tetra *Paracheirodon axelrodi*, a commercially important fish from central Amazonia

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Abstract

The cardinal tetra *Paracheirodon axelrodi* is a very popular aquarium fish and the most important ornamental fishery resource for riverine communities inhabiting the Rio Negro floodplain in central Amazonia. Here we describe the isolation and characterization of 14 microsatellite DNA loci for cardinal tetras. Number of alleles and heterozygosity per locus in a sample of 30 fish ranged from 2 to 22 and from 0.49 to 0.92, respectively. These markers provide powerful tools for studies on conservation and management of cardinal tetra resources and for investigating evolutionary processes underlying population diversification in Amazonian flooded forest fishes.

Keywords: Amazon rain forest, Characidae, conservation genetics, microsatellites, *Paracheirodon axelrodi*, phylogeography

Received 20 December 2003; revision accepted 7 April 2004

The Amazon basin contains the world's highest diversity of freshwater fishes. A considerable fraction of this diversity is represented by small-sized species inhabiting flooded forests and their streams, such as those found in the Rio Negro floodplain (RNF). The RNF is located in central Amazonia and covers an area of 0.75 million km^2 of largely undisturbed primary forests. The unspoiled status of the RNF can be partly attributed to a thriving ornamental fishery that provides about 60% of the income of local riverine people (Chao *et al*. 2001). Although over 100 ornamental fish species are traded along the RNF, a single resource, the cardinal tetra *Paracheirodon axelrodi*, constitutes more than 80% of the total catch (over 30 million cardinal tetras are exported from the RNF every year) (Chao *et al*. 2001). This study is part of a large project on conservation genetics and comparative phylogeography of ornamental fish species from the RNF (e.g. Beheregaray *et al*. 2004). We are combining nuclear and mitochondrial DNA data with biogeographical information to investigate spatial and temporal patterns of population diversification in Amazonian flooded forest fishes. From a conservation standpoint, genetic data will be used in conjunction with fishery and social-economical information to propose strategies for protecting the integrity and the evolutionary potential of cardinal tetra populations and maintaining the Rio Negro ornamental fishery at commercially feasible and ecologically sustainable levels.

Here we report the isolation and characterization of 14 microsatellite DNA markers for cardinal tetras. Loci were isolated using an enrichment technique (Fischer & Bachmann 1998) modified as in Saltonstall (2003). In summary, genomic DNA was digested with *Rsa*I and *Hae*III and fragments ligated to two oligo adaptors. Two biotinylated oligo probes (dGA_{10} and dGT_{10}) were hybridized to the digested DNA and selectively retained 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. The plasmid DNA was purified

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Table 1 Primer sequences and characteristics of 14 *Paracheirodon axelrodi* 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 Accession no.
Pa 1	F: GTGGAGCACATGATCACAGC R: TGAAGACGAGGCAAGCTACA	$(CA)_7$	2.5	10	202–226	0.80/0.86	AY496014
Pa 4	F: CAGTCCCTGCTGGAGTCCTA R: TGGAACATATTGTGTCAGCAG	$(CA)_6$	2.5	5	249–291	0.55/0.58	AY496015
Pa 7	F: CTGAGGTTTACCGACACTGC R: CCGCCTTCAGATCTTTCTCA	$(GA)_{15}$	2.5	9	177–197	0.72/0.75	AY496016
Pa 8	F: ACATGACCCAGAGCCAAGTC R: TAGCCTCAAACGGTCAGACA	$(GA)_7$	2.5	2	172–174	0.48/0.49	AY496017
Pa 11	F: GCGAATGGTCTCTGATTGG R: TGTGAAAAATGAAGCGAGG	$(CA)_7$	2.5	7	161–171	0.77/0.80	AY496018
Pa 13	F: ACGAATGTGGGGACACAAAG R: AACACATCAGGCTGGTCCTC	$(GA)_{11}$	2	5	161–173	0.72/0.76	AY496019
Pa 19	F: CTGGAGGTGTGAGATGGTTG R: TGCACGGTAAGCTGATAGTTTG	$(CA)_7$	2.5	7	224–238	0.79/0.83	AY496020
Pa 26	F: TCAGGCTTGCTGTTCTCTG R: CATTAAACCCAGCGTTAGC	$(CA)_{11}$	2.5	7	201–221	0.66/0.70	AY496021
Pa 27	F: CATATCGCCCACACATGAAG R: GAAACGGAGCCTGTATTGGA	$(CA)_7$	2.5	4	233–239	0.62/0.64	AY496022
Pa 32	F: TGGTTTTGACTTACCGCTG R: CAACGATATTGTGTTCAACTC	$(GA)_{20}$	2	22	182–234	0.87/0.92	AY496023
Pa 33	F: ACAAGGAGACTAAACAAGGAGTTG R: CTGAGCCATGTAAGGCATAAGG	$(CA)_{10}$	2.5	10	208–244	0.78/0.82	AY496024
Pa 34	F: TAAGCAGGGACTGACCGAG R: ATGCTGCAACACAAAAGATAC	$(CA)_{19}$	1.5	17	230–282	0.89/0.92	AY496025
Pa 37	F: GGAGAAGGGGCGTTATTAGC R: GTATACAGGCATATGGGGCG	$(CA)_{11}$	2.5	15	194–222	0.87/0.92	AY496026
Pa 39	F: AAGGCTAGACAGACCACAGTC R: ACATCTGGCATCTTGCGTTA	$(GA)_{13}$	2	8	140–162	0.75/0.79	AY496027

and 40 putative positive clones were sequenced on an ABI 377 automated DNA sequencer (PE Applied Biosystems) using dye terminator chemistry. Primers flanking 16 loci were designed using PRIMER 3 (Rozen & Skaletsky 1997). We chose both long and short repeated loci, since these are expected to inform at different levels of divergence (e.g. Beheregaray *et al*. 2002).

Variation at each of these loci was assessed 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, 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]dATPat 1000 Ci/mmol. PCR amplifications consisted of 94 °C for 3 min, followed by a 32 cycles 'touchdown' (94 °C/20 s; 63 °C down to 55 °C until fifth cycle/45 s; 72 °C/60 s), and 72 °C for 4 min. For locus PA26 the annealing temperature of the touchdown ranged from 55 °C to 47 °C. PCR products were separated by 6% polyacrylamide gel electrophoresis and visualized by autoradiography. We used GENEPOP version 3.3 (Raymond & Rousset 1995) to estimate expected (H_E) and observed (H_O) heterozygosities and test for linkage disequilibrium.

Fourteen loci successfully amplified and were screened for variation in a sample of 30 cardinal tetras collected from Rios Tea, Cuiuni and Igarapé Arixaná (middle Rio Negro, Brazil). Loci revealed substantial levels of genetic variation, with the number of alleles per locus ranging from 2 to 22 and expected heterozygosities from 0.49 to 0.92 (Table 1). Most loci were at Hardy–Weinberg equilibrium in all populations, except for PA1, which showed significant excess of homozygotes possibly related to null alleles. No evidence for linkage disequilibrium was detected in locus-pair/population comparisons. All primer sets also amplified more than one allele per locus without additional optimization in a sample of four green neon tetras (*P. simulans*), suggesting that these markers are potentially useful for characterizing genetic variation in the three described species of *Paracheirodon*.

Acknowledgements

We thank the Ecosave program (Yale Institute of 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 the Amazon was provided by Project Piaba (Universidade Federal do Amazonas — PRONEX CNPq no. 46.6090/2001–4 and Bio-Amazonia Conservation International). LBB was supported by a Gaylord Donnelley Postdoctoral Fellowship (YIBS).

References

Beheregaray LB, Sunnucks P, Briscoe DA (2002) A rapid fish radiation associated with the last sea level changes in southern Brazil: the silverside *Odontesthes perugiae* complex. *Proceedings of the Royal Society of London B*, **269**, 65–73.

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

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.

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

Raymond M, Rousset F (1995) 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.

Saltonstall K (2003) Microsatellite variation within and among North American lineages of *Phragmites australis*. *Molecular Ecology*, **12**, 1689–1702.