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PRIMER NOTE

Eleven polymorphic microsatellite loci in a coral reef fish, *Pterapogon kauderni*

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Abstract

We describe the isolation and characterization of 11 polymorphic tetranucleotide microsatellite loci from a male mouthbrooding coral reef fish, the Banggai cardinalfish *Pterapogon kauderni*. In a sample of 37 fish from a natural population, polymorphism at these loci ranged from two to 15 alleles, with expected heterozygosities ranging from 0.107 to 0.928, enabling high-resolution genetic studies of this coral reef fish.

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Despite having a very restricted geographical distribution, the Banggai cardinalfish, *Pterapogon kauderni*, is of interest to both ecologists and the fisheries (i.e. aquarium) trade. Banggai cardinalfish are ecologically interesting because they are male mouthbrooders with an extremely small clutch size (maximum of 90 eggs; Kolm & Berglund 2003). Moreover, larvae have no pelagic phase (Vagelli & Erdmann 2002). In combination with extreme site fidelity and a keen homing ability (e.g. they have been observed to return to their natal group after capture, even when separated by distances as great as 100 m; Kolm & Berglund 2003), the lack of a pelagic larval phase is probably the cause of their restricted range and suggests that they may exhibit a genetic population structure not normally observed among coral reef fish. Additionally, Banggai cardinalfish live in groups of two to 200 individuals (Kolm & Berglund 2003) and are closely associated with branching hard corals, sea urchins and anemones, which they probably use for protection (Vagelli & Erdmann 2002). However, ever since the Banggai cardinalfish was 'rediscovered' in 1994, *P. kauderni* has become a popular aquarium fish and has been heavily collected from the wild. The extraction of approximately 50 000 fish per month has caused noticeable declines in fish density (Vagelli & Erdmann 2002). Additionally, the human-mediated movements of these fish have created at least one introduced population (Vagelli & Erdmann 2002). Here we describe the isolation of 11 polymorphic microsatellite markers in the Banggai cardinalfish. These markers will help to resolve several questions in this species, including patterns of kin structure and parentage, levels of overall genetic structure and the origin of introduced populations.

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Our microsatellite enrichment protocol followed that of Jones *et al*. (2001) and is described here in brief. Genomic DNA was extracted following a standard proteinase K, phenol–chloroform procedure (Sambrook *et al*. 1989). To construct a partial genomic library enriched for GATA-motif microsatellite sequences we used a modification of a biotinylated oligonucleotide procedure originally described by Kijas *et al*. (1994). This procedure used a biotinylated $(GATA)_6$ oligonucleotide affixed to streptavidin-coated magnetic beads (Promega). The oligonucleotide was hybridized to polymerase chain reaction (PCR)-generated genomic fragments. Additionally, our hybridization and subsequent wash conditions followed the protocol of Jones *et al*. (2001). Repeat-enriched DNA was eluted, purified, PCR amplified to create double-stranded product, ligated into a plasmid cloning vector that was transformed into competent *Escherichia coli* and screened as described in Jones *et al*. (2001). Clones with microsatellite-containing inserts were isolated and the inserts were sequenced on an ABI 3100 (Applied Biosystems).

We designed PCR primer pairs for 15 sequences containing microsatellite repeats with sufficient flanking sequence

Table 1 Microsatellite primer pairs for Banggai cardinalfish (*Pterapogon kauderni*)

Locus	*n*	Sequences (5′–3′)	Annealing temp. (°C)	Repeat	Clone size (bp)	No. alleles	H_O	H_E	GenBank Accession no.
Pka03	32	*GCTTGATACAACAGTGACAGA GCGACTGGCAATTTTATAC	54	$(GATA)_8$	73	6	0.781	0.715	AY530932
Pka06	36	*AAGGTTCCACTTCCATCTACT TCGGTCTTCCACCAATAA	53.5	$(GATA)_6$	218	10	0.722	0.774	AY530933
Pka07	28	*CAAGGATTGAGCTATTAACAT AGGGAGAAGGAATTACAGA	49.5	$(GATA)_{24}$	230	14	0.786	0.883	AY530934
Pka09	29	*TGTGTGAAAATTCTAGTG GGTGAATAGAGAAAACAA	49.5	$(GATA)_{14}$	179	10	0.621	0.670	AY530935
Pka11	27	*CACACGCACTGATGTTT CGCAGTATCTTAGCTGTTC	56	$(GT)_{12}A(GATA)_5$	288	2	0.111	0.107	AY530936
Pka13	36	*AATGGCTACCTTTACAACTAC AAGGATGGAGGACAGATG	54	$(GATA)_{24}$	209	15	0.833	0.853	AY530937
Pka16	32	*GGAGCACAGGAACCCCTTTATAC CACCGCACCTGGAACAGA	51	$(GATA)_{23}$	153	12	0.656	0.873	AY530938
Pka19	37	*GGCTTGGGTTTGAGTTCT CCAGGCTGTGAGTTTGAGAC	54	$(CTAT)_{11}$	199	11	0.730	0.815	AY530939
Pka21	35	*CGCTGAACGAGACTAGATAC TTTGGTATGCTTTTGAATATC	48	$(GATA)_{15}$	185	4	0.800	0.655	AY530940
Pka24	25	*TCTCCCTGGAAATGTCTC TTCGGACACAGCCAATA	51	$(GATA)_{12}$	171	2	0.400	0.470	AY530941
Pka25	30	*ATCCCATTGTTCTGAATAA AGGGAGTTTACTGTAGTCTAAT	51	$(GATA)_{20}$	203	15	0.933	0.928	AY530942

*5′ fluorescent-labelled primer.
n, number of individuals screened from the population; H_O, observed heterozygosity; H_E, expected heterozygosity.

for primer design. The PCR was carried out in 20-μL reactions containing 1 × PCR buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100), 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.15 μM each primer with the forward primer fluorescently labelled, 0.5 U *Taq* DNA polymerase (Promega) and 2 μL (approximately 10–100 ng) DNA. After 3 min of initial denaturation, each of 35 thermocycles consisted of the following steps: 94 °C for 30 s, annealing temperature (see Table 1) for 30 s and 72 °C for 1 min, followed by a 7-min final extension. Eleven primer pairs were polymorphic in a screening of 37 individuals from a natural population. The remaining four loci amplified inconsistently or produced no product. The polymorphic loci displayed between two and 15 alleles per locus and expected heterozygosities ranged from 0.107 to 0.928 (Table 1). Microsatellite data were analysed with GENEPOP version 3.3 (Raymond & Rousset 1995) to test for Hardy–Weinberg equilibrium (Fisher's exact test) and for genotypic disequilibrium for pairs of loci within the population (Fisher's exact test). Both tests were corrected for multiple comparisons by applying a sequential Bonferroni correction (Rice 1989). Exact tests indicated that only one locus (*Pka16*) might have null alleles, as indicated by a significant deviation from Hardy–Weinberg equilibrium ($P = 0.001$) caused by a deficiency of heterozygotes. Tests for genotypic disequilibrium provided no evidence for linkage among loci at a 5% significance level. These loci provide the first set of microsatellite markers derived directly from the *P. kauderni* genome.

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