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PRIMER NOTE

Polymorphic microsatellite loci from the Southeast Asian cyprinid, *Barbodes gonionotus* (Bleeker)

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Abstract

The cyprinid *Barbodes gonionotus* (Bleeker) is a commercially important fish in both capture fisheries and aquaculture in Southeast Asia. Five polymorphic microsatellite loci from *B. gonionotus* are described. Four are highly variable, with 9–30 alleles observed per locus in four populations sampled from Thailand (H_O = 0.694–0.808). These will be of use in studies of population genetic structure and in pedigree analyses.

Keywords: *Barbodes* spp., cyprinid, genetic markers, microsatellites, silver barb

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Barbodes gonionotus (Bleeker, 1850) is a cyprinid fish, common in large river systems in Thailand, Laos, Cambodia, Vietnam, Sumatra and Java. It has also been widely introduced outside this range. In Thailand, the species occurs throughout the country, but is most abundant in major rivers of the central region, such as the ChaoPhaya, MaeKlong, NakornNayok and PaSak (Smith 1945). It comprises approximately 20% of the total inland fishery (25 000 metric tons; Anonymous 1996) and since 1960, the Thai Department of Fisheries has successfully used induced spawning to produce *B. gonionotus* fingerlings for restocking and aquaculture. Here we describe five microsatellite loci developed from *B. gonionotus*.

B. gonionotus samples were obtained either directly from the rivers or from local fishermen during April to August 1994. A muscle tissue sample was collected from each fish and preserved in 100% ethanol. Nuclear DNA was extracted using a standard phenol extraction procedure. Genomic DNA from a single *B. gonionotus* (50 µg) was digested with the restriction endonucleases; *Pal*I, *Rsa*I, *Hin*cII and *Alu*I (Pharmacia). Genomic fragments of 300–700 bp were recovered from a 1% agarose gel using a phenol freeze fracture procedure (Qian & Wilkinson 1991). The size selected DNA was ligated to *Sma*I-digested and dephosphorylated pUC18 vector (Pharmacia) and transformed into MAX efficiency ΔH5α™ competent cells (BRL). Transformed cells were plated on 2× YT medium (1.6% Bactotryptone, 1% yeast extract, 0.1 M NaCl) containing 100 µg/mL of ampicillin, and grown out at 37 °C overnight. Colonies were lifted onto Hybond-N nylon filters (Amersham).

A hybridization probe was prepared by end-labelling 100 pM of a synthetic oligonucleotide [$(GT)_{15}$]. The filters were prehybridized in [6.25 mL 20× SSPE pH 7.6 (174 g NaCl, 27.6 g $NaH_2PO_4 \cdot H_2O$, 7.4 g EDTA and ddH_2O to a total volume of 1.0 litre), 2.5 mL 50× Denhardt's solution (5 g Ficoll-Type 400, 5 g Polyvinyl pyrrolidone, 5 g BSA and ddH_2O to a total volume of 500 mL), 0.5% SDS (w/v), 100 µL/mL tRNA] at 65 °C for 1 h prior to addition of heat denatured (70 °C for 5 min) labelled probe. Hybridization was carried out at 60 °C for 12 h. The filters were washed three times; 2× SSC, 0.1% SDS for 15 min at room temperature, 1× SSC, 0.1% SDS for 15 min at room temperature, and 1× SSC, 0.1% SDS for 15 min at 60 °C and exposed to autoradiographic film.

Eighty-eight positive colonies were identified and sequenced. Thirty-nine clones contained microsatellites, five of which contained two or more microsatellite loci (separated by three or more bases). Three clones contained tetranucleotide microsatellites and two contained trinucleotide microsatellites. Thirty-six clones contained dinucleotide microsatellites, 23 of which were $(AC/GT)_n$ repeats (26.1%). Most of the *B. gonionotus* microsatellites found in this study were perfect repeats (73.2%), four clones

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Table 1 Details of five microsatellite loci developed from *Barbodes gonionotus*. H_O, observed heterozygosity; H_E, expected heterozygosity; T_a, annealing temperature

Locus	Accession number	Motif	Primers 5′–3′	Allele size range (bp)/ (sequenced allele size)	No. of alleles	H_O/H_E	T_a (°C)
Bgon-17	AJ291684	$(AC)_{34}$	TTACAAGGGGTTACATACTG CAGTCTCATATTTGAAAGCAG	108–182 (152)	30	0.808/0.885	49
Bgon-22	AJ291680	$(TCC)_6$	TCTTGTTGATCACACGGACG GTGACTGTATCAATGAGTCTG	73–99 (89)	2	—	49
Bgon-69	AJ291681	$(TG)_{12}$	GCAAAGGTTCTGTCAAGG GTATCCAGAAACATGTTCAG	87–103 (95)	9	0.694/0.75	49
Bgon-75	AJ291682	$(AC)_{10}$	CTGGTAAAGACTTCAGATGC GCATGCAAAATGAGAAAGGCT	96–118 (104)	11	0.729/0.759	53
Bgon-79	AJ291683	$(CA)_{12}$	GCCAGACTGGAGCGAGG GTTCGGTGAAGCCATGAGG	101–137 (109)	17	0.789/0.783	53

contained imperfect repeats (9.8%), and seven clones contained compound repeats (17%). The motif copy-number varied from four to 35 repeats, the most common size of dinucleotide motif was 12, but tri- and tetranucleotide microsatellite loci were comprised of a smaller number of repeats. The largest motif copy-number of tri- and tetranucleotides microsatellite loci found was 10, and the averages were eight and seven repeats, respectively. The polymerase chain reaction (PCR) primers were designed from the unique flanking sequences of five microsatellites (Table 1) and tested using four unrelated populations from Thailand: Chainat, Khonkaen, Phitsanulok and Surin. One primer of each pair was end-labelled with [γ^{32}]-PATP. Five microlitre PCR reactions contained 1× PCR buffer (10 mM Tris pH 8.3, 50 mM KCl, 1 mM $MgCl_2$, 0.01% gelatin), 300 μM each dNTP, 0.6 μM each of primer, 0.025 μM [γ^{32}]-PATP labelled primer, 0.25 U *Taq* polymerase and 0.05% Tween20, overlaid with mineral oil. PCR reactions with *Bgon-17* and *Bgon-69* included: 0.05 mM [γ^{32}]-PATP labelled primer, 0.6 mM forward primer and 0.075 μM reverse primer. Programme parameters were: six cycles of 1 min at 94 °C, 30 s at a primer specific annealing temperature (T_a) and 30 s at 72 °C; 39 cycles of 30 s at 90 °C, 30 s at T_a and 30 s at 72 °C. Amplification products were denatured in stop dye (99% formamide, 0.1% bromophenol blue, 0.1% xylene cyanol green, 10 mM NaOH; 95 °C for 15 min), and 2.5 μL was run on an 8% denaturing polyacrylamide gel for 2 h at 55 mV. Gels were dried and exposed overnight to an autoradiography film (Kodak XAR5) at −80 °C. Microsatellite alleles were sized relative to a sequencing ladder generated from single-stranded M13 DNA.

Four loci were highly polymorphic in the population samples surveyed with heterozygosities ranging from 0.62 to 0.9. Thirty alleles were observed at locus *Bgon-17* with sizes ranging from 108 to 182 bp, nine alleles at locus *Bgon-69* with sizes ranging from 87 to 103 bp, 11 alleles at locus *Bgon-75* with sizes ranging from 96 to 118 bp, and 17 alleles at locus *Bgon-79* with sizes ranging from 101 to 137 bp. At *Bgon-22* only two alleles were observed in the populations surveyed. All loci exhibited consecutive alleles that differed by multiple numbers of the microsatellite motif except for *Bgon-69* where one allele, *allele*92*, differed by only 1 bp (2% and 6% frequency in Chainat and Phitsanulok, respectively).

Genotypic frequencies at the four microsatellite loci; *Bgon-17*, *Bgon-69*, *Bgon-75* and *Bgon-79*, conformed to Hardy–Weinberg expectations (with sequential Bonferroni correction for multiple testing: results not shown). Allelic segregation was tested in a full-sib family consisting of 20 progeny (results not shown). The expected genotypes were observed in F_1 individuals. A significant deviation of gametic segregation from a 1:1 expectation was observed for the female parent at *Pgon-69* ($P < 0.05$) possibly due to a sampling error because only 20 offspring were studied. These polymorphic microsatellite loci will be of use both for pedigree analysis and for population genetic studies in this species.

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