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PRIMER NOTE

Polymorphic microsatellite markers isolated from partially enriched genomic library of *Chitala chitala*

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Abstract

A total eight polymorphic microsatellite loci were obtained from genomic library of Indian feather back, *Chitala chitala* (order Osteoglossiformes, family Notopteridae) and the 46 samples were analysed to determine genetic variation. The mean number of alleles per locus ranged from 4.50 to 5.25, and expected heterozygosity ranged from 0.124 to 0.852. Deviation from Hardy–Weinberg equilibrium expectations ($P < 0.002$) was observed at loci *Cch2*, *Cch9* (Bhaghirathi) and *Cch9* (Brahmaputra). The identified microsatellite loci were found promising for population genetics studies of *C. chitala* and related species *Notopterus notopterus* (family Notopteridae).

Keywords: *Chitala chitala*, microsatellite, *Notopterus notopterus*, null allele, polymorphic, population genetics

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Chitala chitala, is widely distributed in freshwater bodies of the Indian subcontinent (Froese & Pauly 2003). *C. chitala* is commercially important as ornamental and food fish. Conservation strategies including propagation-assisted rehabilitation of natural population are necessary in view of the decline in the species' abundance (Sarkar *et al*. 2006). Population structure of *C. chitala* derived through microsatellite markers will be useful in planning conservation strategies. A microsatellite-enriched genomic library was constructed following the method of Fleischer & Loew (1995) and M. Hamilton and R. Fleischer (personal communication). Genomic DNA was extracted from blood and digested with Sau3Al restriction enzyme. Genomic DNA fragments of 300–800 bp were gel-purified (QUIGEN kit) and ligated to SAULA (CGGTACCCGGGAAGCTTGG) and SAULB (ATCCCAAGCTTCCCGGGTACCGC) linkers. The fragments were amplified using SAULA as primer in a 50-μL reaction [95 °C, 5 min; 30 × (95 °C, 40 s, 60 °C, 1 min, 72 °C, 1 min); 72 °C, 5 min].

Nylon membranes (5 mm^2) saturated with CA/GT, GA/CT and CAGA/GTCT target repeats were hybridized overnight to amplified DNA fragment in DIG Easy Hyb solution (Roche) at 50 °C for dinucleotide and 60 °C for tetranucleotide repeats. The membranes were washed to remove unbound DNA and the hybridized DNA was recovered from the membrane using 100 μL 50 mM KOH/0.01% SDS, followed by 100 μL 50 mM Tris-HCI pH 7.5/0.01% SDS. The recovered DNA was again polymerase chain reaction (PCR)-amplified using SAULA primer and digested with Sau3Al to remove linkers. The DNA fragments were ligated to *Bam*HI digested pUC18 vector and transformed into DH5α competent cells (Invitrogen). Inserts were sequenced using the Big Dye terminator kit with M13 forward primer (MegaBACE, Amersam Biosciences).

A total of 40 repeat sequences were obtained and 29 primer pairs were designed (PRIMER SELECT, version 5.07, DNA Star). PCR amplification was performed in 25 μL reaction on thermocycler (PTC 200, MJ Research) as follows: 5 min denaturation at 95 °C; 25 cycles of 30 s at 95 °C; 30 s at specific annealing temperature (Table 1) and 60 s at 72 °C with a final extension of 10 min at 72 °C. The PCR consisted of 50 ng of DNA, 1× PCR buffer (10 mM Tris-HCl, pH 9.0; 50 mM KCl; 0.01% gelatin), 5 pmol of each primer, 15 mM $MgCl_2$, 2 μL of 2.5 mM dNTPs and 1.5 U *Taq* polymerase (Genei). Amplified products were resolved on polyacrylamide gel followed by silver staining. Out of 29 microsatellite loci amplified, 8 were polymorphic, 14 were monomorphic and 7 yielded unspecified products. Genotype data at each polymorphic microsatellite locus for samples collected from Satluj ($n = 16$), Bhaghirathi ($n = 15$) and Brahmaputra ($n = 15$) rivers were analysed (GENETIX version 4.05, Belkhir

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Table 1 Characteristics of *Chitala chitala* microsatellites including; F (forward primer), R (reverse primer), T_a (annealing temperature), N_a (alleles observed), H_E (expected heterozygosity), H_O (observed heterozygosity), Sat (Satluj), Bha (Bhaghirathi), Bhm (Bhramputra), P (agreement to HW expectations)

Locus	Primer sequence	Core sequence	T_a	River	N_a	Size range (bp)	H_E	H_O	HW (P)	Genic (P) homogeneity
Cch1	F: CGGAGATGAAGAGCAGCAGTA	$(GA)_{19}(GT)_9$	55	Sat	4	216–232	0.428	0.400	0.718	0.0011**
(DQ525389)	R: TGTGTTCCGTGTTTCCTCCTA	$(GA)_8$		Bha	6	220–250	0.785	0.642	0.244	
				Bhm	6	216–238	0.751	0.733	0.194	
Cch2	F: ACCCAAGCCATGTTAAGTGGTC	$(GA)_{19}GTT$	55	Sat	4	202–228	0.551	0.466	0.155	0.5375
(DQ525390)	R: GGCGAGTCCACGATTTCAAG	$(TG)_9$		Bha	5	194–228	0.617	0.333	0.001**	
				Bhm	5	194–228	0.533	0.600	0.650	
Cch4	F: AGAGATTCCAGCCGCACCACT	$(GA)_{29}$	60	Sat	7	177–205	0.724	0.571	0.071	0.3252
(DQ525391)	R: AGACGCAGCGCGAACTATCACAG			Bha	7	177–205	0.804	0.461	0.003*	
				Bhm	7	177–205	0.770	0.500	0.031*	
Cch6	F: ATTTCCAGCTTCTAACCGCACACC	$(CA)_{13}$	57	Sat	3	83–87	0.460	0.466	0.769	0.0229*
(DQ525392)	R: AGTTGGGGATGCCGCTGTC			Bha	3	83–87	0.636	0.538	0.229	
				Bhm	4	77–87	0.637	0.466	0.048*	
Cch9	F: TGGTGTTGGAGTGTGAGTGCTTAG	$(TC)_{12}(AC)_{14}$	60	Sat	3	222–236	0.526	0.333	0.008*	0.0580
(DQ525393)	R: ATATGCAGTGGCACGACAGGT			Bha	3	222–236	0.571	0.200	< 0.001**	
				Bhm	3	222–236	0.660	0.133	0.000**	
Cch10	F: TCGTTATTTCTGACATTCAAGTGC	$(GT)_{21}$	53	Sat	5	114–132	0.742	0.500	0.015*	0.0108*
(DQ525394)	R: TACAAGCTCCATGCACAATTACAA			Bha	6	114–132	0.783	0.857	0.312	
				Bhm	6	114–132	0.762	0.800	0.623	
Cch13	F: AAGGGTACTGATGAGTGAATGAGC	$(CT)_{14}$	55	Sat	2	220–228	0.191	0.214	1.000	0.9347
(DQ525395)	R: TCATAACAGGCTGTTTATTGTCCA			Bha	2	220–228	0.142	0.000	0.040*	
				Bhm	2	220–228	0.124	0.000	0.034*	
Cch15	F: AACACTGAGCGAAAAGCAACA	$(GA)_5[CA(GA)_3]_4$	55	Sat	8	130–198	0.760	0.615	0.370	0.0004**
(DQ525396)	R: GATAAACGGGTGAGAGCAAGTG	$[CA(GA)_2]_3CA$		Bha	7	130–146	0.816	0.714	0.092	
		$(GACA)_{11}$		Bhm	10	138–184	0.852	0.571	0.006*	
Mean				Sat	4.50	—	0.548	0.568	1	0.0001**
over all loci				Bha	4.87	—	0.636	0.672	1	
				Bhm	5.25	—	0.636	0.475	1	

*Significant ($P < 0.05$); **significant after sequential Bonferroni correction ($P < 0.002$); Accession no. given below the locus name.

et al. 1997) to determine parameters of genetic variation (Table 1). Tests for linkage disequilibrium, genotypic differentiation, and Hardy–Weinberg equilibrium were performed using GENEPOP version 3.4 (Raymond & Rousset 1995). The mean number of allele per locus in Satluj, Bhaghirathi and Brahmaputra were 4.50, 4.87 and 5.25, respectively. Expected heterozygosities ranged from 0.124 to 0.852 and observed heterozygosities from 0.00 to 0.857. After sequential Bonferroni correction, linkage disequilibrium was not detected for any pair of loci ($P > 0.2$) in any sample. Nonconformity to Hardy–Weinberg expectations ($P < 0.002$) was observed on loci *Cch2*, *Cch9* (Bhaghirathi) and *Cch9* (Brahmaputra). The observed heterozygosity deficiency (+ F_{IS}) at these loci might be due to the presence of null allele and/or small sample size. The data were corrected for the possible null alleles (software FREENA, CHAPUIS MP and ESTOUP A) and the corrected data did not exhibited heterozygote deficiency. Significant genetic heterogeneity ($P < 0.002$) was evident at loci *Cch1*, *Cch15* and at the overall loci.

Amplification with five individuals of *Notopterus notopterus* revealed that the loci *Cch1*, *Cch2* and the two loci found monomorphic in *C. chitala* (*Cch18* and *Cch20*) were polymorphic in *notopterus* (Table 2) at annealing temperature of 55 °C. In conclusion, the study identified the microsatellite loci that can be useful in population studies of *C. chitala* and

Table 2 Cross-amplification of primers developed from library of *Chitala chitala* in *Notopterus notopterus*

Locus	Polymorphic	Size range	No. of alleles
Cch1	Yes	180–182	2
Cch2	Yes	216–230	4
Cch4	No	147	1
Cch18	Yes	130–152	4
Cch20	Yes	180–184	2

Cch18 (Accession no. DQ525397; primer forward TGTAGGAGCCGGAGTCGGAGAA, reverse CTGCAGCAGTAGGCCTGTGAGTG).
Cch20 (Accession no. DQ525298; primer forward GGAGGGATGCTGTGCACTATAAAG, reverse CCGTTGGGTCTGTCTGTATATCTG).

N. notopterus; however, cautious interpretation is suggested due to possible presence of null alleles at some of the loci.

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