Molecular Ecology Resources (2008) **8**, 864–866 doi: 10.1111/j.1755-0998.2008.02092.x

PERMANENT GENETIC RESOURCES

Isolation and characterization of polymorphic microsatellite loci in yellowtail catfish, *Pangasius pangasius* (Hamilton, 1822)

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Abstract

A total of nine polymorphic microsatellite loci were obtained from a genomic library of *Pangasius pangasius* (order Siluriformes, family Pangasiidae). Samples from rivers Bhagirathi (n = 22) and Mahanadi (n = 20) were genotyped for each of the nine microsatellite loci to determine genetic variation. The mean number of alleles per locus was 5.22 in Bhagirathi and 5.78 in Mahanadi; and expected heterozygosity ranged from 0.567 (Bhagirathi) to 0.578 (Bhagirathi). Significant deviation ($P < 0.003$) from Hardy–Weinberg expectations was evident at three loci, Ppa2 (Bhagirathi), Ppa14 (Mahanadi) and Ppa28 (Bhagirathi and Mahanadi). The identified microsatellite loci were found to be promising for population genetics studies of *P. pangasius*.

Keywords: genetic variation, microsatellite, null allele, *Pangasius pangasius*, polymorphic

Received 20 July 2007; revision accepted 3 December 2007

Pangasius pangasius or yellowtail catfish is an important freshwater food and game fish (Talwar & Jhingran 1991) and is found in large rivers of the Indian subcontinent and Myanmar (Roberts & Vidthayanon 1991). *P. pangasius* is considered as an endangered species due to the decline in its natural abundance (Sarkar & Ponniah 2000). Therefore, conservation strategies including propagation-assisted rehabilitation of natural population are being considered as a priority. Knowledge of population structure of *P. pangasius* derived through microsatellite markers will be useful in the formulation and planning of 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 (QIAGEN 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 cycles (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 fragments 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 hybridized DNA was recovered from the membranes using 100 μL 50 mM KOH/0.01% SDS, followed by 100 μL 50 mM Tris-HCl pH 7.5/0.01% SDS. The recovered DNA was again amplified through polymerase chain reaction (PCR) 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 BigDye terminator kit with M13 forward primer (MegaBACE, Amersham Biosciences).

A total of 28 repeat sequences (Accession nos DQ835618–DQ835645) were obtained and 25 primer pairs were designed (PRIMER SELECT, version 5.07, DNASTAR). PCR amplification was performed in a 25-μL reaction using PTC200 thermocycler (MJ Research) as follows: 5 min denaturation at 95 °C; 25 cycles of 30 s at 95 °C; 30 s at the

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Table 1 Characteristics of *Pangasius pangasius* microsatellite loci including: F (forward primer); R (reverse primer); T_a (annealing temperature); N_a (alleles observed); H_E (expected heterozygosity); H_O (observed heterozygosity); River: Bha (Bhaghirathi), Mah (Mahanadi); P_{HW} (probability of conformity to HW expectations; *significant $P < 0.003$; †possibility of null alleles); P_G (probability of genetic homogeneity between the samples; ‡significant $P < 0.05$)

Locus	Primer sequence	Core sequence	T_a (°C)	River	N_a	Size range (bp)	H_E	H_O	P_{HW}	P_G
Ppa01	F: CTCCATCTGCTAGAGCAAAGC	$(CA)_{15}$	55	Bha	3	135–145	0.210	0.231	1.000	0.9631
DQ 835618	R: TTTTTGGATTACGTCTTTGTTCTG			Mah	5	135–145	0.281	0.313	1.0000	
Ppa02	F: AACTACACGGTACGCGAAAAA	$(GT)_{21}$	55	Bha	6	109–123	0.590	0.500	0.032	0.007‡
DQ 835619	R: TGTCAAACCCTGCATTGGTA			Mah	9	111–135	0.839	0.471	< 0.001*†	
Ppa05	F: TGGGGAAATTACCACCTTCA	$(TC)_{12}TG(TC)_7$	50	Bha	2	130–134	0.482	0.429	0.660	0.029‡
DQ 835622	R: CCAGCACAGACAATCTGCAT			Mah	3	130–134	0.553	0.474	0.197	
Ppa14	F: GTAAATGCGCGAATGGATG	$(CA)_{12}GAGATT$	55	Bha	8	136–152	0.790	0.550	0.001*†	0.072
DQ 835631	R: TGAACTTTTAATTGTCTACTCCGACT	$(GA)_{14}(GA)_3CAGC(GA)_{12}$		Mah	7	136–150	0.750	0.444	0.004†	
Ppa17	F: GCAAACGCTTTTCTGAACATT		50	Bha	7	113–131	0.626	0.441	0.007	0.311
DQ 835634	R: TCATCTGTGAGCTCGTGGAG			Mah	7	111–127	0.476	0.412	0.042	
Ppa18	F: GCTGGGTGTGTTGTGTGAGT	$(TG)_{18}$	50	Bha	5	110–122	0.370	0.449	0.666	0.406
DQ 835635	R: GGATTGTTTTGGTCACATTCC			Mah	5	112–124	0.381	0.368	0.666	
Ppa22	F: AGTCCCAAAGCAGACAGAGG	$(GA)_{17}AA(GA)_5$	55	Bha	4	112–120	0.611	0.564	0.027	0.066
DQ 835639	R: ATTTTCGTTCCGTGCTGTTC			Mah	3	112–120	0.550	0.450	0.161	
Ppa23	F: TGATAAAGGCAGGAGCCAGA	$(AC)_{13}$	50	Bha	6	095–125	0.636	0.646	0.008†	0.836
DQ 835640	R: CCCTCCGTGTTATGTTGTGC			Mah	6	103–129	0.364	0.600	0.820	
Ppa28	F: CCAGAAGGATACTGGTATCTGAGG	$(GA)_{18}$	55	Bha	6	163–177	0.789	0.680	< 0.001*†	< 0.001‡
DQ 835645	R: GCTTGCACTCTGCACACAAT			Mah	7	161–173	0.471	0.412	0.003*†	

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 pM of each primer, 1.5 mM $MgCl_2$, 2 μL of 0.2 mM dNTPs and 1.5 U *Taq* DNA Polymerase (Genei). Amplified products were resolved on polyacrylamide gel followed by silver staining. Out of 25 microsatellite loci amplified, nine were polymorphic (Table 1), three were monomorphic and 13 yielded unspecified products. Genotype data at each of the nine polymorphic microsatellite loci for samples collected from rivers, Bhaghirathi ($n = 22$, Farakka, West Bengal, 24°05′N; 88°06′E) and Mahanadi ($n = 20$, Cuttack, Orrisa, 21°58′N, 86°07′E), were analysed using the software GENETIX version 4.05, Belkhir *et al*. 2004) to determine parameters of genetic variation (Table 1). Tests for linkage disequilibrium (LD), conformity to Hardy–Weinberg equilibrium and genotypic differentiation were performed using GENEPOP version 3.4 (Raymond & Rousset 1995). The mean number of alleles per locus was 5.22 (Bhagirathi) and 5.78 (Mahanadi). Expected heterozygosities were 0.567 and 0.578 and observed heterozygosities were 0.439 and 0.438, respectively, for Bhagirathi and Mahanadi samples. After sequential Bonferroni (SB) correction (Lessios 1992), LD was not detected for any pair of loci ($P > 0.005$) in individual sample or over all samples. There was evidence of significant deviation from Hardy–Weinberg expectations ($P > 0.003$) at three loci, Ppa2 (Mahanadi), Ppa14 (Bhagirathi) and Ppa28 (Bhagirathi and Mahanadi) after the probability level was corrected for SB correction. Assessment with the software MICRO-CHECKER (Van Oosterhout *et al*. 2004) using Bonferroni confidence interval revealed the possible signs of null alleles at these loci that could be responsible for the observed excess of homozygotes ($+F_{IS}$). Null alleles were also indicated (Table 1) at loci Ppa14 (Mahanadi) and Ppa23 (Bhagirathi). Significant genetic heterogeneity ($P < 0.05$) was evident at three loci, Ppa02, Ppa05 and Ppa28.

In conclusion, the study identified microsatellite loci that can be useful to determine genetic variation in wild populations of *P. pangasius*; however, cautious interpretation due to possible presence of null alleles at some of the loci is suggested.

Acknowledgement

This work was done as a part of the project, ICAR-NBFGR Project no. DNA-34B.

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