

Short Communication

Primers from the orders Osteoglossiform and Siluriform detect polymorphic microsatellite loci in sun-catfish, *Horabagrus brachysoma* (Teleostei: Bagridae)

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Summary

Horabagrus brachysoma (sun-catfish, Bagridae, Siluriformes) is a valuable ornamental and food fish. The stock structure of *H. brachysoma*, necessary to conserve its declining natural populations, is not known. Twenty-five primers developed for four fish species belonging to the orders Siluriform (3) and Osteoglossiform (1) were tested and eight primers amplified microsatellite loci in *H. brachysoma*. The results demonstrate that cross-priming between fish species belonging to different families and even to different orders can yield microsatellite loci. Five of eight primers each amplified two loci. However, the loci that had repeat motifs after sequencing were considered only for genotyping. Finally, eight loci were polymorphic with three to seven alleles. Individual fish genotype data ($n = 42$; 21 each in two rivers) at each locus was analysed. Significant genetic heterogeneity was detected at six loci. The identified loci exhibited potential for use in population genetics application in *H. brachysoma*.

Horabagrus brachysoma is endemic to the rivers of Western Ghats in Peninsular India. The fish is endangered through exploitation as an ornamental and food fish, which has led to a decline in natural abundance (Gopalakrishnan and Ponniah, 2000). Microsatellites are among the most useful markers for detecting genetic variation and provide options of using the primers developed for one species to amplify loci in other related species (review by Scribner and Pearce, 2000). A survey of 161 species (http://www.abs-c.usgs.gov/research/genetics/heterologous_primers.htm) across taxonomic groups indicates limited reports on use of primers between families (31, polymorphism = 17) and orders (4, polymorphism = 1). Microsatellite loci conserved between families and orders can be useful in evolutionary studies and in generating population genetics data for a wide range of species.

Primers developed for four fish species (resource species) from the orders Siluriform (3) and Osteoglossiform (1) were examined to amplify microsatellite loci in sun-catfish (Table 1). The study aim was to identify polymorphic microsatellite loci and assess their suitability for population structure analysis of *H. brachysoma*.

Specimens of yellow catfish *H. brachysoma* were obtained from commercial catches of the Chalakkudy and Nethravati rivers. The blood samples, extracted through caudal puncture, were stored in 95% ethanol. Total DNA was extracted following the procedure of Ruzzante et al., 1996. A cross-priming experiment was performed on eight specimens

($n = 4$ /river). Procedures for PCR reaction, electrophoresis and genotyping were followed as described in Lal et al. (2004). The optimum annealing temperature to obtain a scorable band pattern was determined through experimental standardization for each primer pair.

Eight primer pairs exhibited amplification in *H. brachysoma* (Table 1). The optimum annealing temperature to obtain scorable band patterns in *H. brachysoma* differed from that reported for the respective primer pair in resource species (Table 2). The study demonstrated successful cross-priming of microsatellite loci between fish species that are distantly or not related. Certain sequences flanking the tandem repeats could be conserved between the various families of the order Siluriform. Interestingly, some microsatellite sequences from the primitive order osteoglossiform have remained conserved in the order Siluriform, of relatively later evolutionary origin.

The eight primers amplified 13 scorable loci (Table 2). The additional five loci came from the primers Phy07, Cma4, Cga06, D33, and D38 that amplified two loci each. Before using for genotyping, it was essential to confirm whether both loci for these primers had repeat motifs. Following the sequence observations, the eight loci (Phy01, Phy05, Phy07-1, Cma3, Cma4, Cga06-1, D33-2 and D38-1) that confirmed the presence of repeat motifs were used for genotyping (Table 2); these loci were polymorphic, with three to seven alleles. To assess genetic variation, 42 samples ($n = 21$ /river) were individually genotyped at each locus. Genetix 4.05 (Belkhir et al., 1997) was used to obtain observed (H_o) and expected (H_e) heterozygosities at each locus, mean overall loci, and mean number of alleles per locus for the two sample sets (Table 3). Deviations from the Hardy–Weinberg Equilibrium and linkage disequilibrium were tested using Markov chain approximation in GENEPOP 3.4 (Raymond and Rousset, 1995). The probability of conformity to the Hardy–Weinberg expectations indicated significant deviation ($P < 0.05$) in both samples after the Bonferroni correction was applied to the probability levels (Table 2). Positive F_{is} values at these loci revealed the heterozygote deficiency. There was no evidence of linkage disequilibrium.

Genetic homogeneity was tested based on genotype (GENEPOP 3.4; Raymond and Rousset, 1995) and the combined probability over all loci indicated significant divergence between the two sample sets. With sequential Bonferroni correction made to the probability levels, six loci exhibited significant ($P < 0.0063$) heterogeneity (Table 3). G_{st} for small sample size (Genetix 4.05; Belkhir et al., 1997) was 0.0752.

Table 1
Primers of microsatellite loci tested for cross-priming in *Horabagrus brachysoma*

Species	Tested (no.)	Loci	GenBank accession no.	Reference	Amplified in <i>H. brachysoma</i> no.(%)
Order Siluriform <i>Pangasius hypophthalmus</i> (Pangasidae)	4	Phy 01, 03, 05, 07	AJ131380 AJ131381 AJ131382 AJ131383	Volchaert et al., 1999	3 (75.00)
<i>Clarias macrocephalus</i> (Clariidae)	4	Cma 01,02,03,04	–	Na-Nakorn et al., 1999	2 (50.00)
<i>Clarias gariepinus</i> (Clariidae)	7	Cga 01, 02,03,05,06,09,10	–	Galbusera et al., 1996	1 (14.20)
Order Osteoglossiform <i>Scleropages formosus</i> (Osteoglossidae)	10	D11,13,14,16,33,35,37,38,42,72	AF219953 AF219954 AF219955 AF219957 AF219961 AF219962 AF219963 AF219964 AF219965 AF219966	Yue et al., 2000	2 (20.00)
Total	25				8 (32.00)

Table 2
Annealing Temp. (Ta°C) in resource species (Res. sp.) and optimized for *H. brachysoma*; Parameters of genetic variability at individual and mean over all loci in Chalakkudi (Ch) and Nethravati (Ne) samples; agreement to HW expectations (P) and genic homogeneity (P), Significant *(P < 0.05) **(After Sequential Bonferroni correction, Lessios, 1992)

Locus	Res. sp. <i>H. brachysoma</i>	Ta°C	River	Size range (bp)	No. of alleles/locus	Ho	He	HW (P)	Genic homogeneity (P)
Phy01	65	56	Ch	162–196	7	0.909	0.793	0.0836	<0.0001**
			Ne	162–190	6	0.619	0.632	<0.0830*	
Phy05	60	55	Ch	146–170	6	0.667	0.774	0.0743	0.0015**
			Ne	146–166	5	0.714	0.703	0.1381	
Phy07-1	68	55	Ch	270–285	4	0.191	0.588	0.0003**	0.0360
			Ne	270–285	4	0.143	0.761	0.0001**	
Cma3	50	45	Ch	147–170	7	0.571	0.744	0.0070*	<0.0001**
			Ne	147–166	6	0.524	0.747	0.0030**	
Cma4-2	48	56	Ch	172–182	4	0.286	0.516	0.0250*	0.0380*
			Ne	172–182	4	0.238	0.398	0.0465*	
Cga06	...	57	Ch	218–244	5	0.524	0.641	0.0380*	<0.0001**
			Ne	218–244	5	0.567	0.634	0.0080*	
D33-2	55	53	Ch	192–212	3	0.238	0.289	0.4483	0.0011**
			Ne	200–212	3	0.667	0.559	0.0146*	
D38-1	50	55	Ch	252–310	4	0.905	0.653	0.0070*	0.0000**
			Ne	252–295	3	0.810	0.602	0.4733	
Mean	–	–	Ch	–	4.875	0.536	0.614	–	<0.0001**
overall loci	–	–	Ne	–	4.375	0.589	0.623	–	

The various estimates provided evidence that genetic variation detected at the identified microsatellite loci can be significant in determining the population structure of *H. brachysoma*.

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References

- Belkhir, K.; Borsa, P.; Goudet, J.; Chikhi, L.; Bonhomme, F., 1997: GENETIX vers. 4.05, Genetics logiciel sous windows pour la génétique des populations. <http://www.univ-montp2.fr/~genetix/genetix.htm>
- Galbusera, P.; Volckert F.A.; Hellemans B.; Ollevier F., 1996: Isolation and characterisation of microsatellite markers in the African catfish *Clarial gariepinus* (Burchell, 1822) Mol. Ecol. **5**, 703–705.
- Gopalakrishnan, A.; Ponniah, A.G., 2000: Cultivable, food, sport and ornamental fish species endemic to Peninsular India with special reference to Western Ghats. In: Endemic fish diversity of Western Ghats. A. G. Ponniah and A. Gopalakrishnan (Eds.). NBFGR–NATP Publ. No. 1, NBFGR, Lucknow, India, pp. 13–32.
- Lal, K. K.; Chauhan, T.; Mandal, A.; Singh, R. K.; Khulbe, L.; Ponniah, A. G.; Mohindra, V., 2004: Identification of microsatellite DNA markers for population structure analysis in Indian major carp, *Cirrhinus mrigala* (Hamilton-Buchanan, 1882). J. Appl. Ichthyol. **20**, 87–91.
- Lessios, H. A., 1992: Testing electrophoretic data for agreement with Hardy Weinberg expectations. Mar. Biol. **112**, 517–523.
- Na-Nakorn, U.; Taniguchi, N.; Nugruho, E.; Seki, S.; Kamourat, W., 1999: Isolation and characterisation of microsatellite loci of

- Clarias macrocephalus* and their application to genetic diversity study. *Fish. Sci.* **65**, 520–526.
- Raymond, M.; Rousset, F., 1995: GENEPOP (vers. 1.2): a population genetics software for exact test and ecumenicism. *J. Hered.* **86**, 248–249, vers. 3.4, <http://www.cefe.cnrs-mop.fr>.
- Ruzzante, D. E.; Taggart, C. T.; Cook, D.; Goddard, S., 1996: Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: microsatellite DNA variation and antifreeze level. *Can. J. Fish. Aquat. Sci.* **53**, 634–645.
- Scribner, K. T.; Pearce, J. M., 2000: Microsatellites: evolutionary and methodological background and empirical applications at individual, population, and phylogenetic levels. In: *Molecular methods in ecology*. A. Baker (Ed.). Blackwell Science Limited, England, pp. 235–271.
- Volckaert, F.A.M.; Hellemans, B.; Poyard, L. 1999: Nine polymorphic microsatellite markers in the SE Asian Catfishes *Pangasius hypophthalmus* and *Clarias batrachus*. *Anim. Genet.* **30**, 383–384.
- Yue, G.M.; Chen, F.; Orban, L., 2000: Rapid isolation and characterization of microsatellites from the genome of Asian arowana (*Scleropages formosus*, Osteoglossidae, Pisces). *Mol. Ecol.* **9**, 1007–1009.
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