Conservation Genet Resour (2011) 3:425–427
DOI 10.1007/s12686-010-9371-6

TECHNICAL NOTE

Isolation and characterization of eight novel microsatellite loci in whisker sheatfish (*Micronema bleekeri* Günther, 1864)

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Received: 1 December 2010 / Accepted: 6 December 2010 / Published online: 19 December 2010

Abstract We describe the isolation and characterization of eight microsatellite loci from whisker sheatfish (*Micronema bleekeri* Günther, 1864). Genetic variability was assessed using 35 individuals. The number of alleles per locus ranged from two to four. The observed heterozygosity (H_o) ranged from 0.072 to 0.637, whereas the expected heterozygosity (H_e) ranged from 0.174 to 0.725. All loci conformed to the Hardy–Weinberg expectation (HWE). Linkage disequilibrium was not significant for any pair of loci. These loci should prove useful for population genetic studies of whisker sheatfish and other siluriforme species.

Keywords Whisker sheatfish · Microsatellite marker · Genetic conservation

The whisker sheatfish (*Micronema bleekeri* Günther, 1864) is an economic freshwater fish species which is caught from natural freshwater sources (Rainboth 1996). This species is distributed in the main rivers of Thailand, as well as their tributaries (Coates et al. 2003; Na-Mahasarakarm 2007). The decline in the distribution of whisker sheatfish populations is due to habitat degradation, overexploitation and geographical barriers during the dry season. In Thailand, genetic diversity and population structure have been studied in other commercial freshwater fish species, such as the walking catfish (Na-Nakorn et al. 2004) and the giant catfish (Na-Nakorn et al. 2006; Ngamsiri et al. 2006), but not for the whisker sheatfish.

The current study developed microsatellite markers from whisker sheatfish for evaluation of the genetic diversity of whisker sheatfish. The enrichment of a microsatellite library was undertaken using the methodology described by Zane et al. (2002). High quality genomic DNA was fragmented using a restriction enzyme, *Mse*I. The fragmented DNAs were ligated to specific adapters (5′-GATGAGTCCTGA GTAAC-3′ and 5′-TACTCAGGACTCATCA-3′). The polymerase chain reaction (PCR) products were size-selected to preferentially obtain small fragments (300-1,000 bp), which were hybridized to four streptavidin-biotinylated oligo simple sequence repeat (SSR) complexes: $(CA)_{15}$, $(GA)_{15}$, $(ACC)_{10}$, $(CCT)_{10}$. The enriched DNAs were ligated into pGEM®-T easy Vector (Promega, USA) and transformed into competent *E. coli* JM109 cells (Promega, USA). To confirm the presence of microsatellite sequences, the positive clones were subjected to Southern hybridization using repeat-containing probes and a North2South® Chemiluminescent Hybridization and Detection Kit (PIERCE, USA). The recombinant clones were directly sequenced and flanking regions were recovered to design primers for the amplification of each microsatellite sequence.

In total, 570 positive clones were obtained through the enrichment method, but only 54 sequences were confirmed to carry microsatellite sequences. Of the microsatellite sequences, 8 polymorphic markers were selected and used

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Table 1 Characteristics of eight microsatellite loci isolated from whisker sheatfish

Locus ID	GenBank	Repeat motif	Anneal (°C)	Primer sequences (5′ → 3′)	*A*	a_e	H_o	H_e	*P*-HWE
MB71	GQ128432	$(GT)_{12}$	58	F: GCCGTCTCTCTCACTCCATC	2	1.496	0.072	0.334	0.009^{NS}
		$N(GT)_{13}$		R: CTCCACTGAGCACAAGTCCA					
MB79	HQ660230	$(GA)_{20}$	55	F: GTTATCTGTCTTGTGTAGACG	4	3.570	0.637	0.725	0.014^{NS}
		$N(GA)_{14}$		R: AACACTCCTGTCTGTCCAGCC					
MB81	GQ128433	$(GT)_{15}$	57	F: CAGCAGCAAGAAGCAGACG	2	1.815	0.449	0.452	1.000^{NS}
				R: CAAGGTCAGTAGGGAAGTGTG					
MB153	GQ128434	$(GGA)_{12}$	53	F: TGTGTGAGAAACAGCAGGTG	2	1.257	0.203	0.206	0.441^{NS}
				R: TCAAGCGAAATCCCGTCTGC					
MB320	GQ128436	$(GAG)_{21}$	60	F: AGAAGCAGCTCGGGTCTGC	3	1.704	0.261	0.416	1.000^{NS}
				R: TCCATGGCCTGGTTGAACTC					
MB401	GQ128439	$(AG)_{13}$	61	F: TAGAGATCAAGAGACCTTTAG	3	1.781	0.406	0.441	0.051^{NS}
				R: CACTCTTTCATCTGTGCGG					
MB456	GQ128440	$(GA)_{20}$	56	F: GCTGATCTAAAGTTCATTAGC	3	1.723	0.420	0.422	0.084^{NS}
		$N(T)_{11}$		R: ACACCATACTGCAGGAGG					
MB645	HQ660229	$(GA)_{20}$	58	F: GAAGGATGTAACTATGGAAGC	3	1.209	0.188	0.174	1.000^{NS}
				R: ATACGGAGTGATGTGTTAGAG					

F forward primer, *R* reverse primer, *N* nucleotide interrupted motif sequences. a_e effective number alleles per locus. *NS* not significant
Number of alleles per locus *(A)*, observed (H_o) and expected (H_e) heterozygosity. *P-HWE P*-values for the Hardy–Wienberg Expectation test

to evaluate genetic variation of the whisker sheatfish samples.

Genomic DNAs from 35 adult whisker sheatfish samples were used for PCR amplification of eight microsatellite loci shown in Table 1. PCR reactions were carried out on a XP cycler (BIOER, China) in 12.5 μl volumes containing 50 ng of genomic DNA, 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.1 μM of each primer and 1U *Taq* DNA polymerase (Invitrogen, Norway). Details of the thermal profile for all loci consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 45 s, 53°C-61°C for 30-45 s depending on the locus and 72°C for 30-45 s and a final extension at 72°C for 5 min. The PCR products were subsequently separated on 8% (w/v) denaturing polyacrylamide gel (Bio-Rad, USA) and visualized by silver-staining, as described by Karnsomdee and Meckvichai (2002). The alleles were sized based on the PhiX 174 *Hin*fI marker (Promega, USA).

In total, 22 alleles were obtained from all loci. The number of alleles per locus ranged from 2 to 4. The effective number of alleles varied from 1.209 to 3.570. The Hardy–Weinberg expectation (HWE) and gametic disequilibrium were tested for each sub-population using Genepop (Raymond and Rousset 2004). All loci conformed to HWE after sequential Bonferroni correction ($P > 0.006$) (Holm 1979). No evidence of significant linkage disequilibrium was found among the tests for each pair of loci. The expected and observed heterozygosities were estimated for each locus using the POPGEN 32 software package (Yeh and Yang 1999). The observed heterozygosity ranged from 0.072 to 0.637, whereas the expected heterozygosity ranged from 0.174 to 0.725. The results of eight primer pairs are shown in Table 1. MICRO-CHECKER (Oosterhout et al. 2004) did not detect any other genotyping error among the loci.

These markers will be useful for genetic monitoring of whisker sheatfish. The major conservation concerns for this species are to sustain genetic diversity and to avoid genetic erosion. Evaluation of the genetic diversity and population structure of whisker sheatfish distributed in the other tributaries and basins of the Main River is in progress to provide more genetic information on this valuable fish species.

Acknowledgments This work was carried out under a grant from the Office of the Higher Education Commission (CHE490140), and the Kasetsart University Research and Development Institute (2007-2008). The authors would like to thank Professor Dr. Uthairat Na-Nakorn and Associate Professor Dr. Surin Peyachoknagul for technical suggestions.

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