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TECHNICAL NOTE

Isolation and characterization of polymorphic microsatellite loci from Yellowcheek (*Elopichthys bambusa*)

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Abstract Nine yellowcheek (*Elopichthys bambusa*) microsatellite loci were isolated using the fast isolation by AFLP of sequences containing repeats (FIASCO) protocol. Three to eight alleles per locus were detected in 29 samples collected from five populations of *E. bambusa*. The mean number of alleles was 5.6 ± 1.9 and the level of observed heterozygosities ranged from 0.415 to 0.843. These are the first microsatellite loci characterized from *E. bambusa* that can be used for estimating genetic diversity, population structure and parentage analysis.

Keywords *Elopichthys bambusa* · FIASCO · Microsatellites

Yellowcheek (*Elopichthys bambusa*) is a large carnivorous pelagic fish of high meat quality (Zhu and Chen 1959). This species is confined to Asia, particularly, Chinese mainland (Xiao et al. 2001), Russia (Bogutskaya and Naseka 1996; Pietsch et al. 2000; Reshetnikov et al. 1997) and Vietnam (Rainboth 1991; Kottelat 2001). In China, *E. bambusa* is widely distributed from north to south especially in the Yangtze, Pearl and Heilong (Amur) River. Recently, because of isolation of rivers and lakes, construction of irrigation works and environmental depravation of aquatic ecosystems, the natural populations of *E. bambusa* has declined rapidly. Now, it is largely restricted to the Yangtze River and the lakes connected with the river. The species can scarcely be found from great mass of rivers and lakes (Li et al. 2005). There is dire need to resurrect the stocks of the species through conservation and management. It is prerequisite to define the systematic position and population structure of a species for accurate management and interpretation of ecological studies (Ferguson and Mason 1981). The development of molecular genetic markers seems to be valuable for the goal. Microsatellite DNA markers are powerful tools for the investigations of genetic structure of fish, but till now no species specific microsatellite primers have been developed for the *E. bambusa*.

In this study totally nine informative microsatellite loci were isolated and the initial characterization of them was also studied. Microsatellites were isolated using the fast isolation by AFLP of sequences containing repeats (FIASCO) protocol (Zane et al. 2002) with slight modifications. A total of 250 ng genomic DNA was completely digested with 3 U of *Mse*I (BioLabs) in a 25 μl volume, and then 15 μl of digested DNA was ligated to *Mse*I AFLP adaptor (5′-GACGATGAGTCCTGAG-3′/5′-TACTCAGG ACTCAT-3′) using 1 U of T4 DNA ligase (BioLabs) in a 30 μl volume at 20°C for 3 h. The digestion-ligation mixture was diluted (1:10), and directly amplified using *Mse*I adaptor-specific primers (5′-GATGAGTCCTGAG TAAN-3′) in 20 μl with 0.9 μM *Mse*I-N, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 1 U of *Taq* DNA polymerase (Tiangen) and 5 μl diluted digestion-ligation DNA. The PCR was performed using a program of 94°C 30 s, 53°C 1 min, 72°C 1 min for 20 cycles. Approximately 1,000 ng amplified DNA fragments were hybridized with 200 pmol of 5′-biotinylated $(AC)_8$ probe in a total volume of 250 μl of SSC 4.2× and 0.07% SDS, by denaturing DNA for 5 min at 95°C and incubating at 60°C for 2 h. The hybridized DNA was then

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mixed with 600 µl of Streptavidin MagneSphere Paramagnetic Particles (Promega) which had been treated three times with 150 µl of TEN100 (10 mM Tris–HCl, 1 mM EDTA, 100 mM NaCl, pH 7.5), allowing a selective binding at room temperature for 30 min. The beads-probe-DNA complex was separated by a magnetic field. After removing nonspecific DNA fragments by non-stringent washes (10 mM Tris–HCl, 1 mM EDTA, 1 mM NaCl, pH 7.5) and stringent washes (SSC 0.2× and 0.1% SDS) for three times each, the target DNA was released from the bead-probes with 50 µl TE (Tris–HCl 10 mM, EDTA 1.0 mM, pH 8.0) at 95°C for 5 min, and transferred as soon as possible. DNA containing repeats were amplified for 30 cycles with *Mse*I-N primers and the same program mentioned above was used. Fragments ranging from 400 to 1,000 bp were isolated and purified (Omega Biotek). They were then ligated into the pMD18-T plasmid vector (TaKaRa) and were transformed into competent *Escherichia coli* cells DH-5α. Positive clones were identified by blue/white selection, then were amplified using M13 universal primers and visualized by agarose gel electrophoresis. Eighty clones with different insert fragments were sequenced, 85% of which contained simple sequence repeats. Subsequently, 19 primer pairs were developed using the software PRIMER 3 (Rozen and Skaletsky 2000) from simple sequence repeats containing seven or more repeats with suitable flanking sequences. All of the 19 pairs of primers were tested using 29 *E. bambusa* individuals sampled from five population located in Hubei (Eastern lake, five samples; Dan River, five samples), Hunan (Dongting lake, four samples), Jiangxi (Poyang lake, ten samples) and Jiansu (Tai lake, five samples) province, central and eastern China. PCR reactions were performed in a 10 µl volume containing approximately 20 ng DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 µM each primer, 1× Taq buffer and 0.5 U *Taq* polymerase (Tiangen). The PCR profiles included an initial denaturation at 94°C for 5 min, followed by 35 cycles of 50 s at 94°C, 50 s at annealing temperature, 90 s at 72°C and then 10 min at 72°C. Amplified products were electrophoresized in 6% denaturation polyacrylamide gel and visualized by silver staining. A 25 bp DNA ladder (Promega) was used to identify alleles. Among 19 microsatellite primers synthesized, four primers did not produce an amplified product and six primers were monomorphic. The last nine primers produced polymorphic DNA products (Table 1).

The polymorphic nine loci had their allelic diversity ranging from three to eight. The mean number of alleles was 5.6 ± 1.9 and their observed heterozygosities ranged from 0.415 to 0.843. Deviation from Hardy–Weinberg equilibrium was tested using Fisher's exact test in GENEPOP version 3.4 (Raymond and Rousset 1995). The test was corrected by a sequential Bonferroni correction (Rice 1989). There was no evidence showed that any locus deviated significantly from Hardy–Weinberg equilibrium ($P = 0.05$), and also no evidence for linkage disequilibrium among loci at a 5% significance level.

Table 1 Primer sequences, PCR conditions and characteristics for nine microsatellite loci in *Elopichthys bambusa*

Locus	Reapt motifs	Primer sequences (5′–3′)	Ta (°C)	$MgCl_2$ (mM)	Size range (bp)	A	He	Ho
YC01	$(GA)_{15}$	F: ACTGACTGCCCTCCCTTCTC	58	2.5	143–175	8	0.597	0.474
		R: CCCCTCTTTCTTTGTCCTTT						
YC02	$(AC)_{12}$	F: TTCAGCTAGGGATGTATTG	54	2.5	190–214	5	0.368	0.460
		R: TGGAAGTTTGGAATTGGT						
YC03	$(AC)_7…(AC)_7…(AC)_7$	F: CGCTACACACTGGCTACATCCT	59	1.5	251–272	3	0.382	0.415
		R: TGGCATGTGTAGCGTTTGAAT						
YC04	$(TG)_9…(GT)_5…(GT)_8…(GA)_6$ …$(GT)_8GAGATA(GT)_8…(GT)_7$	F: TCGTAAATACAAACTTCCCAGG	56	1.5	189–240	6	0.792	0.675
		R: TCGCTCTTACACAAAAACACAA						
YC05	$(GA)_{15}$	F: AGCACTGACTGCCCTCC	55	2.5	169–197	8	0.866	0.843
		R: TTATACTTCATGTTCCATCC						
YC06	$(TG)_{18}…(TG)_9…(TG)_{15}…(TG)_5$	F: TCAGCAATGTGACGTTTAGC	57	2.5	252–344	4	0.724	0.703
		R: TCCATTCAAGCATTCTCACTC						
YC07	$(TC)_{14}$	F: TCCATTCAAGCATTCTCACTC	53	2.5	168–194	6	0.438	0.542
		R: TGTTTCCGTATTCCTCTGTCA						
YC08	$(TG)_8G(GT)_{15}G(GT)_7$	F: AGGAGGAAGGAGAAAGAACTG	53	2.5	155–183	7	0.739	0.690
		R: GACAACGCAAGCCACCTA						
YC09	$(AC)_5CCAG(AC)_8$	F: TCTGCTGTGCCACCTTCATCTT	54	1.5	105–129	3	0.652	0.683
		R: CTGTGATTCCGTCCTCATTTGTC						

Ta annealing temperature, *He* expected heterozygosity, *Ho* observed heterozygosity

These polymorphic microsatellite DNA markers may be used as powerful tools for investigating genetic diversity within populations and genetic structure of *E. bambusa*.

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