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PRIMER NOTE

Isolation and characterization of polymorphic microsatellite loci in Wuchang bream (*Megalobrama amblycephala*)

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Abstract

Wuchang bream (*Megalobrama amblycephala*) is an economically important fish in China. From a $(GT)_{13}$-enriched genomic library, 20 microsatellites were developed. Nine of these 20 loci were polymorphic in a test population with allele numbers ranging from two to four, and the observed and expected heterozygosities ranging from 0.2609 to 0.7826 and from 0.3739 to 0.7546, respectively. In the cross-species amplifications, six of these nine loci were also polymorphic in white amur bream (*Parabramis pekinensis*). These polymorphic microsatellite loci are potentially useful for population genetics of Wuchang bream and its closely related species.

Keywords: cross-species amplification, genomic library, *Megalobrama amblycephala*, microsatellite, *Parabramis pekinensis*, polymorphism

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The Wuchang bream, or blunt snout bream (*Megalobrama amblycephala*), is an herbivorous cyprinid fish endemic to limited lakes around Wuhan (Ke 1965). As one of the most economically important species in the Chinese freshwater polyculture system, Wuchang bream has been intensively cultured in ponds and cages in China since the 1960s (Ke 1965). In 2001, the total production of Wuchang bream reached 541 115 tons (CAFS 2001). As a result of fast domestication and over-fishing, however, the natural populations of this species have declined in recent decades in China (Li 1996). For the purposes of conservation and sustainable exploitation for fishery resources of Wuchang bream, population genetic studies are necessary. Intraspecific variations and population diversity of Wuchang bream have been investigated by using enzyme and (random amplified polymorphic DNA) RAPD markers (Zhang 2001; Li *et al*. 2002). Microsatellites or simple sequence repeats (SSRs) have been widely used as DNA markers in population genetic studies, parentage and kinship analyses because of their high level of polymorphism and codominant Mendelian inheritance (O'Connell & Wright 1997). Some microsatellites from genomic library were recently reported in Wuchang bream but their polymorphism was not assessed (Li *et al*. 2006). Here we report the isolation and characterization of a batch of novel polymorphic microsatellites from a genomic library of Wuchang bream which are potentially useful in future genetic studies.

Genomic DNA of one adult Wuchang bream was extracted from muscles by using traditional proteinase-K digestion and phenol–chloroform protocol with RNase treatment, and then an enriched partial genomic library for the repeat motif $(GT)_{13}$ was constructed essentially following the (fast isolation by AFLP of sequences containing repeats) FIASCO protocol (Zane *et al*. 2002) with some modifications (Zhu *et al*. 2005). Enriched fragments were ligated into pMD18-T vector (TaKaRa) and then transformed into *Escherichia coli* DH5α cells. Grown overnight in LB solid medium with ampicillin, clones were subject to the tests of polymerase chain reaction (PCR) amplifications using M13 forward and reverse primers. After PCR confirmation, 28 positive clones were sequenced using BigDye termination kit (PerkinElmer Applied Biosystems) and the products were resolved on an ABI 3730 sequencer. Sequences were analysed for the repeat region using software TANDEM REPEATS FINDER (Benson 1999). The software PRIMER 3 (Rozen & Skaletsky 2000) was then used to design 28 pairs of primers flanking the repeat regions of interest.

Adult Wuchang bream (23 individuals) and white amur bream (*Parabramis pekinensis*) (10 individuals) were sampled

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Table 1 Characterization of nine polymorphic microsatellites inWuchang bream and cross-species amplification in white amur bream

Locus & GenBank Accession numbers	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	N	Size range (bp)	H_O	H_E	Cross-species amplification in white amur bream		
								T_a (°C)	N	Size range (bp)
Mam02 DQ996292	$(TG)_{13}$	F: TTCGGTTCTGCCTTCACTCT R: AAGACGCATGCTCAACAACA	65	4	226–249	0.7391	0.7546		Mono	
Mam03 DQ996293	$(CA)_{18}$	F: TTGCAGGTACTGTGGGAAAA R: AGCAACATGCAAACATCAAA	60	4	235–252	0.7389	0.6812		Mono	
Mam04 DQ996294	$(TG)_{14}(GT)_7$	F: TTGCAGTTTTACCCACATGC R: TGCGCTGATCTAACCACTGA	65	4	227–244	0.3913	0.5836		Mono	
Mam05 DQ996295	$(CA)_{20}$	F: AACAACAAGGGGGCAAGAC R: GACTCAAGCCATTCCCTTCA	55	2	172–185	0.7826	0.4870	50	3	167–190
Mam10 DQ996299	$(TG)_{13}CG(TG)_{14}$	F: GAACGGTATGAGAGCGGAGA R: GGAAGTGTCCGCATAAACCA	62	3	166–177	0.6957	0.5874	50	3	175–189
Mam19 DQ996304	$(CG)_5(TG)_{11}$ $(AG)_3(TG)_4$	F: GAGCAGCGACAGGTTTATCA R: GAGGGCGATGACGACATACT	57	2	211–229	0.6522	0.4918	50	2	220–234
Mam25 DQ996307	$(AC)_5(AC)_{14}$	F: TCACACCAACAACACCGAAT R: CCTTGTTTTCTCCAGGCATC	62	2	176–188	0.5652	0.5111	50	8	150–217
Mam27 DQ996309	$(TC)_{27}(GA)_{14}$	F: GCAAAACATGGTCAGGTC R: CAGGGGATGAAAGGTGAAAG	58	3	310–335	0.2609	0.3739	50	7	299–342
Mam28 DQ996310	$(TG)_{51}(TG)_{12}$	F: CTGTAGTTTTATCATTTGAC R: CAGGGAACATGGACACTCTC	57	2	198–210	0.6087	0.5111	50	4	175–215

T_a, annealing temperature; N, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; Mono, monomorphic.

from Wuhan, Hubei Province of China. Genomic DNA for a panel of Wuchang bream (n = 23) and a panel of white amur bream (n = 10) were extracted using a high salt protocol (http://sciencepark.mdanderson.org/mbcore/protocols.html), and these DNA samples were used for the test and characterization of polymorphism and cross-species amplification of the isolated microsatellite loci.

The conditions for polymerase chain reaction (PCR) were optimized for each pair of primers, and the tests of polymorphism were performed in both Wuchang bream and white amur bream. The PCR amplifications were carried out in 12.5 μL volume on a PTC-100 thermocycler (MJ Research), the mixture containing 1 × PCR buffer (10 mM Tris-Cl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl), 10–50 ng genomic DNA, 0.2 μM for each primer, 120 μmol/L dNTPs and 0.5 U *Taq* DNA polymerase (Biostar). The amplification profile was: a predenaturation at 94 °C for 240 s; 35 cycles including denaturation at 94 °C for 30 s, annealing at 50–65 °C (Table 1) for 30 s and elongation at 72 °C for 40 s; and a final extension at 72 °C for 600 s. Amplified fragments were size-fractionated on 8% or 10% nondenaturing polyacrilamide gels running at 65 W for about 100 min. Products were stained using ethidium bromide and visualized with Ultraviolet Gel Document System (Tong & Liao 2005; Zhu *et al*. 2005). The analyses of polymorphism, including allele diversity, observed (H_O) and expected heterozygosities (H_E), and the exact test for Hardy–Weinberg equilibrium (HWE), were performed using ARLEQUIN software (Laurent *et al*. 2006).

Out of the 28 pairs of primers designed, eight failed to amplify scorable PCR products. In total, nine of the 20 successfully amplified microsatellite loci were polymorphic, with the number of alleles per locus ranging from two to four. For those nine polymorphic loci, H_O and H_E ranged from 0.2609 to 0.7826 and from 0.3739 to 0.7546, respectively (Table 1). The remaining 11 loci were monomorphic. The tests for HWE revealed that the majority (seven out of nine) of these polymorphic microsatellites were in HWE, and two (Mam02; Mam03) of the nine loci showed significant departure from HWE ($P < 0.0056$), which may suggest population subdivision or occurrence of null alleles as heterozygotes involving a null allele may be detected as homozygotes. These nine polymorphic microsatellite loci in Wuchang bream were further investigated for the feasibility of cross-species amplifications in white amur bream. In a test panel of 10 individuals, seven (Mam04, Mam05, Mam10, Mam19, Mam25, Mam27, and Mam28) of those nine loci were found to be also polymorphic in white amur bream, with allele numbers ranging from two to eight (Table 1). These results show that microsatellites isolated in this study have a moderate level of polymorphism. Isolations and characterization of these microsatellite loci,

including the *trans*-species amplifications, provide new candidate markers in Wuchang bream and other related bream species which are ready for studies on genetic diversity, reproductive ecology and fine-scale structure of natural populations. These microsatellites will also facilitate future studies on conservation genetics in Wuchang bream and its closely related species.

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References

Benson G (1999) TANDEM REPEATS FINDER: a program to analyze DNA sequences. *Nucleic Acids Research*, **27**, 573–580.

CAFS (2001) *Fishery Statistic Data*. Chinese Academy of Fishery Sciences, Beijing, China.

Ke HW (1965) The artificial reproduction and culture experiment of *Megalobrama amblycephala*. *Acta Hydrobiologica Sinica*, **5**, 282–283.

Laurent E, Guillaume L, Stefan S (2006) ARLEQUIN: an integrated software package for population genetics data analysis, Version 301. *Computational and Molecular Population Genetics Laboratory (CMPG)*. Institute of Zoology University of Berne, Switzerland.

Li S, Zhu Z, Zou S, Zhao J (2002) Interspecific phylogenesis and interspecific genetic differences of genus *Megalobrama* bluntnose black bream *M. amblycephala* (Guangdong black bream *M. hoffmanni*) and black bream *M. terminalia*. *Acta Zoologica Sinica*, **48** (3), 339–345.

Li SW, Chang Y, Liang L, Sun X (2006) Rapid isolation of microsatellites from genome of blunt nose black bream (*Megalobrama amblycephala*). *Journal of Fisheries of China*, **13**, 187–192.

Li S, Yang X (1996) Effects of two-way selection on biochemical genetics of blunt snout bream (*Megalobrama amblycephala*). *Journal of Fisheries of China*, **3**, 1–5.

O'Connell M, Wright M (1997) Microsatellite DNA in fishes. *Reviews in Fish Biology and Fisheries*, **7**, 331–363.

Rozen S, Skaletsky H (2000) PRIMER 3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener S), pp. 365–386. Humana Press, Totowa, New Jersey.

Tong J, Yu X, Liao X (2005) Characterization of a highly conserved microsatellite marker with utility potentials in cyprinid fishes. *Journal of Applied Ichthyology*, **21**, 232–235.

Zane L, Bargelloni L, Patarnello T (2002) Strategies for microsatellite isolation: a review. *Molecular Ecology*, **11**, 1–16.

Zhang D (2001) Study on genetic diversity of bluntnose black bream from Yunihu and Liangzihu lakes. *Journal of China Three Gorges University (Natural Sciences)*, **3**, 282–284.

Zhu B, Liao X, Shao Z, Rosenthal H, Chang J (2005) Isolation and characterization of microsatellites in Chinese sturgeon, *Acipenser sinensis*. *Molecular Ecology Notes*, **5**, 888–892.