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ONLINE RESOURCES

Identification of novel polymorphic microsatellite loci in the endangered Chinese sucker (*Myxocyprinus asiaticus*)

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Introduction

Chinese sucker (*Myxocyprinus asiaticus* Bleeker), an endangered freshwater fish in China and the only representative of the family Catostomidae in Asia (Nelson 1976), is distributed mainly in the upper reaches of the Yangtze river. It is important in studies of systematics and zoogeography, and has significant economic value for its delicious taste. However, wild populations of *M. asiaticus* have declined dramatically since the 1970s due to overfishing, damming and other anthropogenic influences (Zhang *et al.* 2000). *M. asiaticus* was listed as second class national protected species and vulnerable in the China red data book of endangered animals (Wang 1998). Studies on artificial propagation of *M. asiaticus* have been carried out since the 1970s and a release programme in the Yangtze river on a large scale was first conducted in 2005. However, no genetic information was used in any of these conservation programmes, which may have led to unintended consequences. Although a few sets of microsatellite loci in *M. asiaticus* have been identified in recent years (Chen *et al.* 2010; Cheng *et al.* 2012; Li *et al.* 2012), more valid loci are needed to provide a broad knowledge of genetic structure in future studies. This paper reports characterization of 10 novel polymorphic microsatellite loci that show simple four-base-pair motifs.

Materials and methods

DNA for constructing a microsatellite-enriched library was extracted from fin clips of *M. asiaticus* using a traditional proteinase-K digestion and phenol–chloroform protocol with RNase treatment. Enriched partial genomic library for the repeat motif $(AAAG)_n$ was obtained, essentially following the fast isolation by amplified fragment length polymorphism of sequences containing repeats (FIASCO) (Zane *et al.* 2002) with a few modifications (Zhu *et al.* 2005; Yang *et al.* 2009). One hundred and thirty-three clones were sequenced using BigDye termination (PerkinElmer Applied Biosystems, Foster City, USA) with the products being resolved on an ABI Prism 3730 automated DNA sequencer (Applied Biosystems, Foster City, USA). Sequences obtained were analysed for the repeat region using software Tandem Repeats Finder 4.0 (Benson 1999). Sixty-six sequences with simple sequence repeat motifs were identified and 59 primer pairs flanking the repeat region of interest were created using the software Primer Premier 5.0 (Zhang and Gao 2004). Loci were tested for polymorphism in 54 samples of *M. asiaticus* broodstocks in Wanzhou Fisheries Research Institute, Chongqing, China. Amplification reactions were conducted in 10 μL mixture comprising 20–40 ng genomic DNA, 10 mM Tris-HCl, 50 mM KCl, 0.5 U *Taq* DNA polymerase (Takara, Dalian, China), 1 μM of each microsatellite primer, 1.5 mM $MgCl_2$ and 0.2 mM dNTPs (Biomed, Wuhan, China). PCRs were performed on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, USA) with the following conditions: an initial denaturing step at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 40 s, annealing at 52–62°C (table 1) for 40 s, extension at 72°C for 50 s and a final extension at 72°C for 10 min. One or two reamplifications were performed in case of PCR failure. PCR products were electrophoresed with Applied Biosystems GeneScan LIZ-500 size standard on an ABI 3730 DNA Analyser and then analysed with GeneMapper 4.0 software (Applied Biosystems, Foster City, USA). Exact tests for Hardy–Weinberg equilibrium (HWE) and tests for linkage disequilibrium (LD) were conducted using

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Table 1. Characteristics of 10 polymorphic microsatellite loci in *M. asiaticus*.

Locus	GenBank accession no.	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	Size range (bp)	N	N_A	H_O	H_E	PIC	P
MA6	HM854867	$(AAAG)_6$	F: GCTGTAGCGCTCATGTCTGT R: CAGACCAGCAGTGTGATTCAA	58	134–149	54	3	0.315	0.346	0.295	0.052
MA7	HM854868	$(AAGA)_5$	F: AAGGAAGTGAGGAAGGAAGGA R: GTTGACAGTCGGGAACCATT	58	196–216	52	4	0.596	0.712	0.652	0.202
MA10	HM854871	$(AAGA)_6$	F: CGAAGGTAATGGCATCAATC R: TCGCACTGGAAAAGGGACT	60	117–124	53	2	0.472	0.504	0.375	0.786
MA14	HM854875	$(AGAA)_{11}$	F: TCCGACTCCTCTATGGATCTG R: TCCTATGCCTCTGCATACCC	62	96–109	54	4	0.500	0.449	0.385	0.862
MA19	HM854880	$(CTTT)_7$	F: CCCTGGATAAACTTGGTGGT R: CTTCATGGAAGCTGACCTGTT	58	149–173	54	3	0.630	0.569	0.495	0.671
MA38	HM854899	$(CTTT)_6$	F: ACAAATGGCCCTGCTGTATC R:GACAAAGTGAGAAGGAGAGAAAATG	60	227–235	54	3	0.741	0.643	0.562	0.432
MA52	HM854911	$(AAAG)_8$	F: ACGCTCAAAGTGCCATTA R: AAGTTCAGACTGCCTGCT	58	199–211	51	4	0.569	0.604	0.518	0.756
MA53	HM854912	$(CTTT)_9$	F: GATTTAGGGGACATTTAC R: TTTATTTTCAGCTACAGA	52	138–179	54	9	0.741	0.814	0.783	0.355
MA60	HM854919	$(TTTC)_9$	F: GAATGCAGTTACATCAGACTGG R: CAATACAGCAGCCATCCTCA	58	152–165	51	5	0.647	0.687	0.615	0.099
MA73	HM854927	$(TTTC)_4$	F: ATGTATGTCCTTCCCTTC R: ATAGCAGTACTTAGTGATTTAG	56	219–225	51	3	0.529	0.610	0.525	0.488
Mean						53	4	0.574	0.594	0.521	

T_a, optimized annealing temperature; N, genotyped sample size; N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity. P, P value for Hardy–Weinberg equilibrium test; PIC, polymorphism information content.

GenePop 4.0 (1000 dememorizations, 100 batches, 1000 iterations per batch) (Raymond and Rousset 1995; Rousset 2008). Numbers of alleles, expected and observed heterozygosity, polymorphism information content (PIC) and nonexclusion probability were calculated using Cervus 3.0 (Kalinowski *et al.* 2007). Scoring error due to stuttering, large-allele dropout and null alleles were tested using the software Micro-Checker 2.2 (Oosterhout *et al.* 2004).

Results and discussion

Of the 59 primer pairs tested, 56 produced discriminable PCR products and the remaining three resulted in poor or no amplification. Of the 56 loci successfully amplified, 10 loci exhibited polymorphism with two to nine alleles per locus (table 1). Six loci were highly informative (PIC > 0.5). The sampled population was in HWE at all the 10 polymorphic loci with no evidence of null alleles or genotyping error due to large-allele dropout and stuttering. None of the loci presented evidence of a significant LD. To preliminarily assess the properties of these loci for likelihood-based parentage identification in further studies, nonexclusion probabilities were calculated in Cervus. The average nonexclusion probability is the probability of not excluding a single unrelated candidate parent or parent pair from parentage of a given offspring at one locus (Kalinowski *et al.* 2007). Combined nonexclusion probability of the 10 loci was 0.09854 for one candidate parent, 0.01411 for one candidate parent given the genotype of a known parent of the opposite sex, and 0.00087 for a candidate parent pair.

The 10 novel microsatellite loci identified in this study showed stable amplification and sufficient polymorphism in the tested population. These loci will be a useful tool in assessment of genetic structure, parentage analysis and designing effective conservation programmes for *M. asiaticus* and related species.

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