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TECHNICAL NOTE

Isolation and characterization of 23 microsatellite loci in the Chinese sucker (*Myxocyprinus asiaticus*)

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Abstract Chinese sucker (*Myxocyprinus asiaticus*) is a second class state-protected animal in China. In this study, we developed twenty-three polymorphic microsatellite loci in Chinese sucker. The observed and expected heterozygosity ranged from 0.292–0.958 to 0.423–0.900, respectively. The polymorphic information content ranged from 0.356 to 0.869, with a mean of 0.710. These microsatellite loci are expected to be useful for further studies of genetic diversity, population genetic structure, and assessments of the artificial propagation release effect of Chinese sucker.

Keywords Chinese sucker · Microsatellite · *Myxocyprinus asiaticus* · Yangtze River

The Chinese sucker, *Myxocyprinus asiaticus,* is an endemic freshwater fish in China and the only representative of the Catostomidae family in Asia (Nelson 1976). It is distributed in the Yangtze River and Minjiang River (Fujian Province), and has been an economically important fish historically. However, the natural numbers of Chinese sucker have declined sharply in recent years (Yu et al. 2005) due to over-fishing, water pollution, and dam construction (Zhang et al. 2000). Recently, the Chinese sucker has been artificially propagated successfully and juveniles were released in the Yangtze River to enhance the local stock. Genetic information is useful for monitoring the genetic diversity of natural populations and breeding stocks. The mitochondrial DNA variation of the Chinese sucker has been reported (Sun et al. 2004) and 14 microsatellite markers were developed by Chen et al. (2010). In this study, we developed 23 novel polymorphic microsatellite loci for the Chinese sucker that can be used to study its genetic diversity and for paternity identification.

The total genomic DNA was extracted using the standard proteinase K/SDS extraction method. A microsatellite enriched genomic library for the repeat motifs (CA)n and (CT)n was constructed essentially by following the Fast Isolation by Amplified Fragment Length Polymorphism (AFLP) of Sequences Containing Repeats (FIASCO) protocol (Zane et al. 2002). Briefly, the genomic DNA was digested using the *Mse*I restriction enzyme (New England Biolabs) and fractions measuring 400–1,000 bp were recovered using a 1.5 % agarose gel with a Gel Extraction Kit (Omega, Canada). The fractions digested were ligated to *Mse*I adapters (Vos et al. 1995) using T4 DNA ligase (TaKaRa) for 12 h at 16 °C. The CA and CT microsatellites were enriched using biotin-labeled probes containing the repeat motif $(CA)_{15}$ and $(CT)_{15}$, and streptavidin-coated magnetic beads (Promega). The enriched microsatellite fragments were ligated into the pMD18-T vector (TaKaRa) and propagated in *Escherichia coli* Trans1-T1 Competent Cells (Tansgene). We designed 69 primer pairs using Primer Premier 5.0 based on the flanking sequence of the repeat motifs. The polymorphisms of the loci were analyzed using a population of 24 individuals collected from a Chinese sucker farm in Wanzhou, Chongqing, China. The

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Table 1 Characterization of 23 polymorphic microsatellite loci in the Chinese sucker (*Myxocyprinus asiaticus*)

Locus Accession no.	Primer (5′–3′)	Tm (°C)	Size range (bp)	Na	PIC	H_O	H_E	*P*-HWE
Mas1	CCGACATGGAATGGATAGA	45	180–210	7	0.685	0.500	0.745	0.505
JX855137	TAGCTCCTCCTCACTGGAC							
Mas2	ATTCCGAACATAGCCAGAG	42	350–400	6	0.759	0.542	0.808	0.001
JX855138	AAGGACAGAGCGTCTACCA							
Mas3	AGACCAGACCCACCTTTAC	50	249–280	12	0.869	0.750	0.900	0.941
JX855139	GAACTGCTGAATCACCCTC							
Mas4	CCGACTTACAGCTACAAA	51	220–250	3	0.581	0.750	0.669	0.244
JX855140	GTCAAATAACGCGGGACT							
Mas5	GAAGTGAATAGTAGCAGGTG	50	140–160	8	0.742	0.542	0.786	0.037
JX855141	AAGCAAGATAAATGGAGA							
Mas6	CTGCCAGGAAACTCTAAA	45	100–120	8	0.818	0.833	0.856	0.299
JX855142	TTCTTACTGCATAGTCTTTA							
Mas7	TAGCGTCTGCCCATTTAGC	52	230–300	9	0.812	0.875	0.851	0.019
JX855143	TACGAGCCGTTCACCACTT							
Mas8	ACAATGAAAGCCCACAGAG	51	205–260	7	0.723	0.875	0.769	0.554
JX855144	TGGTAGTTACAAGGCAGAATA							
Mas9	GAGTAATAACAAGGAGGGC	50	165–220	7	0.692	0.875	0.745	0.048
JX855145	TGTAAGTGGCAACATCTAA							
Mas10	CCTGAGTAACTGATGCCCTAA	50	230–270	10	0.858	0.833	0.890	0.456
JX855146	ATGTAGCCGTCTGAAGCAA							
Mas11	AACCACCAGACTCAAACA	49	240–270	9	0.806	0.833	0.846	0.074
JX855147	GGTTATGCCCTCCTGAAA							
Mas12	GCAGCCATTGAACAAGTAC	59	450–500	4	0.541	0.667	0.618	0.623
JX855148	AATCGTGCCAGGGTTAGAC							
Mas13	ATGAATAGTTTGACAAGCAG	50	190–275	11	0.846	0.917	0.879	0.823
JX855149	GGGGAAACAATAACAATAA							
Mas14	ACTTCTAACTTCCAACTACA	50	200–250	5	0.749	0.958	0.801	0.073
JX855150	TCTGGCCTGAAACCTCAT							
Mas15	AGACATTTGTGCCGAAGT	49	285–370	6	0.659	0.708	0.723	0.766
JX855151	TCAGTGGAAAGAGGGAAG							
Mas16	AAAATGGAAGAGGGAGAT	51	270–300	3	0.356	0.458	0.423	0.005
JX855152	ACCGAGGTGGTACTAAAA							
Mas17	GTTATCGTACAAGCGGAGAC	51	290–340	5	0.706	0.292	0.762	0.000
JX855153	TATGCAACCACAAGAGGG							
Mas18	ATCCCAAAGACCACAATG	50	380–410	4	0.617	0.792	0.684	0.000
JX855154	TTCCGGGTTCAATACAAG							
Mas19	ATCAACGCACCAAATACA	49	270–285	4	0.493	0.625	0.566	0.553
JX855155	TGGAAGGCAGCATAAGTC							
Mas20	GTTAGGTTTAGGAGTAGGGTTA	42	200–250	7	0.631	0.458	0.681	0.007
JX855156	CCTGAATGTGGAGGGTAG							
Mas21	GAGTAACTGATGCCCTAA	50	220–270	7	0.763	0.750	0.810	0.438
JX855157	AGCCGTCTGAAGCAATAT							
Mas22	AAATCCCAAAGACCACAA	51	230–270	10	0.838	0.882	0.879	0.881
JX855154	GTCGTATTACCACGCACA							
Mas23	GAGTAACAGCACAGGAAC	50	200–250	8	0.781	0.824	0.831	0.834
JX855158	ATCACAGAAACGGTCATC							

Tm, annealing temperature; Size range (bp), allele range; Na, observed number of alleles; PIC, polymorphic information content; H_O, observed heterozygosity; H_E, expected heterozygosity; *P*-HWE, *P* value the test for deviation from Hardy–Weinberg equilibrium

PCR amplification was carried out in a 25 μL volume that contained 1 × PCR buffer (TaKaRa), 50–100 ng genomic DNA, 0.25 μM of each primer, 150 μM dNTPs, 1.5 mM $MgCl_2$, and 0.25 U Taq DNA polymerase (TaKaRa). The thermal cycle comprised an initial denaturation at 94 °C for 4 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at the appropriate temperature (Table 1) for 40 s, and extension at 72 °C for 1 min. The PCR products were separated on 8 % non-denaturing polyacrylamide gel and visualized by silver staining (Liu et al. 2004). A 50-bp DNA ladder molecular weight marker (TaKaRa) was used as the standard to determine the size of the alleles.

The polymorphic parameters, including the number of alleles (Na), observed heterozygosity (H_O), expected heterozygosity (H_E), and Hardy–Weinberg equilibrium (HWE) test, for each locus were performed using Popgen32. The polymorphic information content (PIC) was calculated using PIC_CALC. In this study, we developed 23 novel polymorphic microsatellite loci for the Chinese sucker. There were 3–12 alleles per locus (Table 1). The observed and expected heterozygosity were 0.292–0.958 and 0.423–0.900, respectively (Table 1). The polymorphic information content (PIC) were 0.356–0.869 (Table 1). Five loci (Mas2, Mas16, Mas17, Mas18, and Mas20) deviated significantly from the Hardy–Weinberg equilibrium ($P < 0.01$; Table 1).

In conclusion, the microsatellite loci described here are expected to be useful for further studies of genetic diversity and for paternity identification in the Chinese sucker.

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