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New microsatellite loci for the mandarin fish *Siniperca chuatsi* and their application in population genetic analysis

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ABSTRACT. The mandarin fish is a popular fresh water food fish in China. Fifty-three polymorphic microsatellite markers were isolated through construction of an enriched library of genomic DNA of *Siniperca chuatsi* (Percichthyidae). We found 2 to 7 alleles per locus. The observed and expected heterozygosity values varied from 0.059 to 1.000 and from 0.305 to 0.818, respectively. The polymorphic information content value varied from 0.255 to 0.782. Twelve microsatellite loci deviated significantly from Hardy-Weinberg equilibrium after Bonferroni's correction. These markers were evaluated in five species of sinipercine fish; 98% of the 265 locus/taxon combinations tested gave cross-amplification. Eight polymorphic microsatellite markers were randomly selected for genetic characterization of three *S. chuatsi* populations. The Ganjiang River and Yuanjiang River populations had moderate levels of genetic diversity, while the Mudanjiang River population had a relatively low level genetic diversity. Genetic distance-based cluster analysis showed clustering of the Ganjiang River and Yuanjiang River

populations in a single group and the Mudanjiang River population in a separate group. Based on these results, we suggest that *S. chuatsi* from the Yangtze River watershed are distinct from the Mudanjiang River population. These SSR markers will be useful for diversity, mapping and marker assisted studies of *S. chuatsi* and other sinipercine fishes.

Key words: *Siniperca chuatsi*; Microsatellite markers; Cross-amplification; Population genetics

INTRODUCTION

The mandarin fish *Siniperca chuatsi* (Basilewsky) is an endemic freshwater fish species in East Asian countries, specifically distributed in the Yangtze River drainage in China (Liang, 1996). As one of the commercially important and peculiar freshwater fish species in China, its status now has increasingly risen in lake and reservoir fisheries (Liu and Cui, 1998). Interests in stocking and artificial breeding of this fish are developing (Xie et al., 2003). Farmed production of *S. chuatsi* from China was reported to be almost 252,622 tonnes by 2010 (FAO). However, because of the damming of rivers, water pollution and overfishing, its natural resources have been exhausted. Thus, the genetic characterization of the *S. chuatsi* wild population is urgently needed, especially in the main distribution areas (such as the Yangtze River drainage and the Heilongjiang River drainage), which has not been done for this species so far.

Genetic tools are useful to improve fishery management and exploit new fishery sources (Zhang et al., 2006). Microsatellites (also known as simple sequence repeats, SSRs) have become a useful marker system in population genetic analysis, genetic mapping and marker-assisted selection (MAS) of many kinds of fish species because of their co-dominant nature, high allelic polymorphism and high reproducibility (Hamada et al., 1982; Walter and Epperson, 2001). In previous studies, Fang et al. (2005) first developed the random amplified polymorphic DNA markers in *S. chuatsi*, and several genomic SSR DNA markers were gradually isolated from this species (Zhang et al., 2006; Kuang et al., 2009; Liu et al., 2011). These SSR markers have provided a sufficient tool to evaluate wild and cultured genetic resources in this species, but they are still lacking for SSR-based mapping studies and further MAS studies.

To aid in the investigation of the population genetic structure and MAS of *S. chuatsi*, it is important to isolate more polymorphic molecular markers. In this study, 53 polymorphic SSR markers for *S. chuatsi* were isolated and cross-amplified in five species of sinipercine fishes. Meanwhile, eight polymorphic SSR markers developed in this study were used to carry out the genetic characterization of three wild populations from two main distribution areas.

MATERIAL AND METHODS

Sample collection and DNA extraction

A total of 83 *S. chuatsi* individuals were sampled from three local populations in China: two populations from the Yangtze River drainage, Ganjiang River (GJ, N = 30) and Yuanjiang River (YJ, N = 29), and one population from Mudanjiang River, a tributary of Heilongjiang River drainage (MDJ, N = 24). Total genomic DNA was extracted from fin clips using the TIANamp Genomic DNA kit (Tiangen, Beijing, China) following manufacturer instructions. The DNA was adjusted to 100 ng/μL and stored at -20°C.

Development of microsatellite markers

SSRs were isolated using a hybridization-based capture method, following the protocol described by Zane et al. (2002). Briefly, high-quality genomic DNA was fragmented using the restriction enzyme *Mse*I (BioLabs, USA). The fragmented DNAs were ligated to specific adapters (5'-GACGATGAGTCCTGAG-3' and 5'-TACTCAGGACTCAT-3'). The PCR products were size selected to preferentially obtain small fragments (300-1000 bp), which were hybridized with 3'-biotinylated oligoprobes for $(CA/GT)_{14}$, $(CCT/GGA)_{15}$ and $(GATA/CTAT)_5$ repeats. The enriched DNAs were cloned into the pGEM-T vector (Promega, USA) and then transformed into *Escherichia coli* DH5a competent cells (Promega). White colonies were randomly picked from the primary transformation plates, identified by PCR using *Mse*I-N and M13 primers, and the isolated Plasmid DNA was then sequenced using an ABI 3730 Genetic Analyzer (Applied Biosystems, USA). The SSRs were screened using the SSRHUNTER program (Li and Wan, 2005). For all types of SSRs, a minimum length criterion of 12 bp was selected, and only perfect SSRs were considered. Primers flanking SSRs were designed using the PRIMER PREMIER 5.0 program (PREMIER Biosoft International, USA).

Amplification and characterization of microsatellite loci

PCR amplifications were performed in a 25-µL reaction volume comprising about 50 ng genomic DNA, 2.5 µL 10X PCR buffer, 1.0-3.0 mM $MgCl_2$, 0.4 µM of each primer, 50 µM of each dNTP and 1.0 U EasyTaq™ DNA polymerase (Transgen, China). PCR amplifications were conducted under the following conditions: 4 min at 94°C followed by 30 cycles of 30 s at 94°C, 45 s at a primer-specific annealing temperature (Table 1), and 30 s at 72°C, with a final extension step of 10 min at 72°C. The PCR products were separated on a sequencing gel containing 8% polyacrylamide and visualized using silver staining. Denatured pBR322 DNA/*Msp* I molecular weight marker (Tiangen) was used as size standard to identify alleles. Screening of all the above SSR loci was carried out with 11 to 12 individuals from each of the three *S. chuatsi* populations: GJ (N = 12), YJ (N = 11), MDJ (N = 11).

Cross-species amplification of the above-developed polymorphic SSR loci was tested in five species of sinipercine fishes: *Siniperca scherzeri*, *Siniperca kneri*, *Siniperca undulata*, *Siniperca obscura*, and *Coreoperca whiteheadi*. Two individuals of each species were analyzed. The same PCR conditions were used as described above. Amplification products were visualized on 1.5% agarose gels, and fragments were sized by comparison with a 2000 DNA marker (Transgen). Primer pairs that amplified fragments with similar sizes as those observed in source species were considered a successful cross-species amplification. Next, a total of 83 adult *S. chuatsi* individuals from three populations were genotyped using the eight polymorphic SSR loci developed in this study.

Data analysis

The number of alleles per locus (N_A), observed heterozygosity (H_O) and expected heterozygosity (H_E) for each population at each locus and fixation index (F_{IS}) were calculated directly from SSR phenotypes using the Genepop 3.1 program (Raymond and Rousset, 1995). Departures from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium between pairs of loci were also estimated by Genepop 3.1. All results were adjusted for multiple simul-

taneous comparisons using a sequential Bonferroni correction (Holm, 1979). The presence of null alleles was tested at a 95% confidence interval using MICRO-CHECKER, version 2.2.3 (Van Oosterhout et al., 2004). The Arlequin 3.0 program (Excoffier et al., 2005) was employed to calculate pairwise F_{ST} values and test their significance by bootstrapping analysis (1000 replicates) for evaluating genetic differentiation between populations. The genetic distance between the populations was calculated using chord distance D_{CE} with Gendist (Cavalli-Sforza and Edwards, 1967), included in Phylip 3.5 (Felsenstein and Churchill, 1995). The distance matrices were then used to construct a UPGMA dendrogram with neighbor in Phylip 3.5.

RESULTS

Polymorphic microsatellite marker development and cross-amplification

A microsatellite-enriched library was constructed from the genomic DNA of *S. chuatsi*. A total of 450 putative recombinant clones were picked from the enriched library, sequenced, and analyzed for presence of SSRs. Sequence analysis revealed that 96 clones (21.33%) were redundant clones. Of the remaining 354 unique clones (78.67%), 324 (91.53% of the unique clones) were found to harbor SSRs (GenBank accession Nos. JN602684-JN602722, JQ723523-JQ723598, JQ804524-JQ804670, and JX027215-JX027276), and 144 could be finally used for primer design. Sequence analysis of all the SSR-containing clones indicated that dinucleotide SSRs were found to be more frequent (87%) than trinucleotide SSRs (5%). Furthermore, a small number (7%) of tetra-/penta-/hexanucleotide SSRs were also identified in the library. Among the dinucleotide SSRs, the AC/TG class of repeat motif was the most frequent (88.5% of total dinucleotide microsatellites), followed by the GA/CT class (6.3%).

In this research, among the successfully amplified primer pairs, 53 loci (36.8% of the designed primers) showed polymorphism in the 34 individuals (Table 1), while the others were monomorphic. The N_A, H_O and H_E per locus in 34 individuals ranged from 2 to 7, 0.059 to 1.000, and 0.305 to 0.818, respectively. Polymorphic information content varied from 0.255 to 0.782. Twelve SSR loci deviated significantly from HWE ($P < 0.00097$) after Bonferroni's correction (Table 1). Analysis with MICROCHECKER indicated the possible occurrence of null alleles at seven of the SSRs (CB02, CB54, PY01, PY05, PY08, PY25, and MDJ477).

Overall, a high level of cross-species amplification was observed across the five species (Table 2). All 53 polymorphic loci (100%) were successfully amplified in *S. scherzeri*, *S. kneri*, and *S. undulata*, 51 (96.2%) in *S. obscura*, and 50 (94.3%) in *C. whiteheadi*.

Population genetic variation

A total of 83 adult *S. chuatsi* individuals collected from three local populations in China were genotyped using the eight SSR loci developed in this study. Of the eight SSR loci screened, six were found to be highly polymorphic, while two loci, MDJ847 and MDJ825, were found to be weakly polymorphic (Table 3) in the GJ and YJ populations. While in the MDJ population, only one locus (PY45) was found to be highly polymorphic, five loci weakly polymorphic, one locus (PY27) monomorphic, and one locus (CD273) had no PCR products. In 83 individuals from the three populations, 91 alleles were observed and N_A was 3.77 (ranging from 2.43 to 4.63). The average number of alleles per population per locus varied from 3 to 8. Among the three populations, the lowest mean number of alleles per locus (2.43) was observed in the MDJ population, while the highest (4.63) was found in the GJ population.

Table 1. Characteristics of 53 polymorphic microsatellite loci in *Siniperca chuatsi* in a sample of 34 individuals.

Locus	GenBank	Repeat motif	Primer sequence (5'-3')	N_A (size range, bp)	Ta (°C)	H_O	H_E	PIC	P-HWE
PY01	JQ804524	$(TG)_{22}$	F: GCCGTCTGACACCACCAT R: CCGCAGCAGGAGGGAAAA	5 (181-233)	56	0.133	0.764	0.711	0.000*
PY02	JQ804529	$(GAG)_6$	F: AAAAGTAGCCCACTGACA R: AGGGAGGTTGTCATTGTT	2 (155-168)	52	0.167	0.305	0.255	0.033
PY05	JQ804536	$(CA)_{28}$	F: AGTCACCCTCAAACAGCC R: AAGAAAGTGCCACAAGGA	3 (184-234)	57	0.333	0.581	0.499	0.002
PY08	JQ804547	$(GT)_3$	F: TTGGACAGGGATGGAGAA R: CCTGAACACCACCTGAAA	5 (75-184)	55	0.467	0.747	0.689	0.002
PY11	JQ804560	$(CA)_5N(AC)_{22}$	F: CCAATCACGGTAGAGGAC R: TGTATGTGACTGTCTTTCG	6 (158-230)	55	1.000	0.815	0.773	0.002
PY12	JQ804563	$(AC)_{20}$	F: TGCGAGAAACTGGCTACA R: GTTAGCAGATGAAAGGGA	4 (184-289)	57	0.767	0.725	0.659	0.614
PY16	JQ804578	$(TG)_{36}$	F: GAAGAGGAGGAAATGTGA R: GCAATGCTATCGCCTCTG	4 (238-453)	57	0.800	0.729	0.666	0.233
PY21	JQ804603	$(AC)_{19}$	F: ACCAACCCGCTGTAATGT R: CACCTCAGCCCTTATCTC	3 (112-160)	55	0.667	0.533	0.442	0.027
PY22	JQ804604	$(AC)_{26}$	F: TGCGTAAGCATAAATCTC R: TACAAACTCAGGCTCACA	4 (311-347)	50	0.400	0.708	0.637	0.003
PY25	JQ804619	$(AGAC)_7$	F: GCACGATACACACAAAAAC R: TGTTTGCGTCCATCCTTT	5 (170-260)	50	0.533	0.799	0.752	0.000*
PY27	JQ804621	$(TG)_{26}$	F: TCATACTGCCTGAAGCCA R: AAATGGAGGTCAAGTGGG	4 (200-262)	57	0.767	0.671	0.601	0.906
PY28	JQ804622	$(GA)_{19}$	F: GAGCCACATCAGAAATCG R: TTAGCCAGTAGATAACGCAC	3 (189-270)	55	0.533	0.576	0.499	0.038
PY39	JQ804634	$(CT)_{15}(CA)_{24}$	F: GCATTAGGGTCCTTTCCG R: TGGGCTTTGTCTAACTCTTTGC	3 (194-241)	55	0.900	0.662	0.576	0.010
PY42	JQ804656	$(TCA)_{16}$	F: TGGCAACATTGGCATTTC R: GGTGGAACTGAAGTAAAACG	3 (367-557)	55	0.900	0.611	0.523	0.012
PY45	JQ804638	$(TAGA)_{25}$	F: CCTGTTGCTTCCTCCATC R: AACTCCTCACCTGCCATA	7 (120-244)	54	0.800	0.815	0.776	0.287
PY46	JQ804639	$(GT)_{15}N(GA)_{27}$	F: CGCCGTTTTTGTAAGACC R: TTCTTTACCCCGTCTCTG	6 (188-275)	54	1.000	0.732	0.675	0.000*
PY48	JQ804643	$(GT)_{27}$	F: GCCAGTTTTATGCCTTGT R: CTTCATCTGCCTGCTTTT	5 (297-485)	52	1.000	0.753	0.699	0.000*
PY55	JQ804651	$(CAG)_5$	F: GTGGTTTCTACATTTGGGTC R: CAGTCAACATTACAGAACCTCA	2 (156-158)	55	0.367	0.305	0.255	0.326
CB01	JN602684	$(AC)_2AT(AC)_2AT$ $(AC)_{19}$	F: GTTTGAAGCAGGTGGAGG R: GGGAACCCTCCTTCTTGT	3 (314-356)	58	0.978	0.653	0.573	0.708
CB02	JN602685	$(TG)_{18}$	F: CCGCAAGGATGGAGTAAC R: GCCGAAACGGCAGAGTAGA	4 (138-165)	57	0.978	0.654	0.581	0.006

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Table 1. Continued.

Locus	GenBank	Repeat motif	Primer sequence (5'-3')	N_A (size range, bp)	Ta (°C)	H_O	H_E	PIC	P-HWE
CB05	JN602687	$(CA)_{36}$	F: CACAGACAGCAGCCAAGC R: CCCTCTTCCCTAACTCCC	4 (239-267)	59	0.696	0.657	0.584	0.239
CB13	JN602691	$(GT)_{13}N(GC)_4(GT)_{12}N(GA)_4(GACA)_6$	F: GTGGGTAGACCTTTGTTA R: CTTTCAACCTACCGCTCA	2 (329-361)	55	0.633	0.465	0.354	0.015
CB27	JN602701	$(GT)_9N(TG)_{19}$	F: CTCTACCATCCTTGCCAGTC R: AAGAAACGGACGGTAGGG	4 (366-445)	58	0.874	0.691	0.635	0.012
CB36	JN602709	$(TG)_{12}TCTC(TG)_4$	F: TCGCCACCATTACCTCCC R: GCTGCTGCTCTTCACTCG	6 (171-202)	59	0.543	0.622	0.586	0.797
CB37	JN602710	$(TG)_{28}$	F: AAAGGGAGGCAAGGAGGA R: TTTCCGTCCAGTTGTTTG	4 (134-180)	55	0.588	0.705	0.632	0.411
CB54	JN602719	$(CA)_{23}$	F: AGCAGTTGGCATTGTTGG R: GGGAACCCTCCTTCTTGT	3 (309-415)	56	1.000	0.635	0.561	0.000*
CB58	JN602722	$(CA)_{11}N(AC)_8$	F: GCCCACTGATTATCCCAACC R: ATTTGTCTGAGCGTATGT	3 (178-204)	55	0.978	0.613	0.716	0.006
CD07	JQ723529	$(TG)_{26}$	F: GGTCAGAGATGGATGAAATGAG R: TTTGTCTCAACACCAGTGCAG	4 (209-244)	62	1.000	0.717	0.653	0.000*
CD29	JQ723547	$(TG)_{24}$	F: TGACTTGGAACATGGAAT R: GCATCCAGCTGTTTGTTTACC	5 (246-401)	56	0.941	0.789	0.741	0.004
CD33	JQ723551	$(TG)_{21}$	F: CCAGTGTCACCTATTGTGT R: TTTGTCTCAACACCAGTGCAG	4 (199-277)	59	0.588	0.691	0.622	0.555
CD34	JQ723552	$(AGAA)_3$	F: AGGAGAGTGTGTGTGGTGGAC R: AATGCTTTTCCCGGTCTTGTA	3 (221-288)	63	0.559	0.676	0.592	0.895
CD39	JQ723557	$(GT)_{36}$	F: GGAGGAGAGGAAATGAAATGC R: CCACATTGCCCAATTACAACT	4 (194-295)	61	0.706	0.676	0.596	0.233
CD72	JQ723582	$(CA)_{18}$	F: ATGCAGCAGAGAAACGACATT R: CTGCTCCGTCTTTTCTTCCTT	4 (203-269)	65	1.000	0.729	0.667	0.000*
CD75	JQ723585	$(TG)_{30}$	F: TCTTGACTGTTTGTGCGACTG R: ACAGGCTCAGTGTGATTTTGC	3 (200-263)	66	0.059	0.536	0.416	0.992
CD77	JQ723587	$(GT)_{25}$	F: GTTTATGTTGCATGGCAGTGA R: TTTGACTCTGGGGACAGAAAG	3 (219-282)	61	0.893	0.607	0.519	0.007
CD90	JQ723596	$(TG)_{22}$	F: GAACATAGAGCCGCAAAGGTA R: CACACAAACACACACAGAGCA	3 (198-286)	62	0.618	0.673	0.589	0.562
CD92	JQ723598	$(AGGG)_3N(AC)_{24}$	F: GTGGAGGAGAGGAGAAGGCTA R: GATGGCAGTGAACACCTGAGT	4 (213-304)	65	0.588	0.739	0.679	0.972
CD201	JQ804657	$(TG)_{27}$	F: CTTCAAACAGCTCCTACAG R: ACTGAAGTCCTACTTATCTCAA	6 (222-307)	58	0.946	0.799	0.759	0.072
CD203	JQ804659	$(GT)_{58}$	F: AAAATGCAGGCAGAAAAG R: AGACTTGTGTATGGATGTGTAT	5 (108-172)	55	0.622	0.650	0.588	0.000*
CD215	JQ804671	$(TG)_{26}$	F: AATCACCAACTAAATCCCTA R: AGGCTGTACATAATTGATTG	7 (105-184)	54	0.892	0.818	0.782	0.227

Continued on next page

Table 1. Continued.

Locus	GenBank	Repeat motif	Primer sequence (5'-3')	N_A (size range, bp)	Ta (°C)	H_O	H_E	PIC	P-HWE
CD226	JQ804680	$(TG)_{10}G(GT)_{29}$	F: AGACCAACCACTAATCACTAC R: ATGGAGACAGACATAGACATA	4 (189-248)	59	1.000	0.714	0.648	0.000*
CD256	JQ804700	$(GT)_{39}$	F: CTCCTTCACCCTCCATCC R: TTGGCTTATTTGCTTCTG	5 (270-466)	59	0.919	0.761	0.71	0.443
CD260	JQ804702	$(TG)_{45}$	F: GTCAGAAGGGTTATATTGTATG R: ATGATAACTTTGGCTTGTG	4 (186-244)	55	0.919	0.712	0.648	0.017
CD214	JQ804670	$(TG)_{31}$	F: GAGGGTTTAGACTTGGGATA R: CTTTGTAAGGAGGAGGGA	5 (186-269)	56	0.973	0.679	0.608	0.006
CD288	JQ804722	$(CA)_{35}$	F: ACATTCATGCATTCTCTCT R: TTGCCATAGAGGTCAAGTGT	5 (93-141)	55	0.973	0.722	0.666	0.000*
CD273	JQ804713	$(CA)_{32}$	F: TAAATGCACGACTTCTATACTC R: CACCTTGCATAGCTCAAT	5 (166-273)	55	0.973	0.738	0.682	0.000*
MDJ471	JX027216	$(TG)_{23}N(GT)_5$	F: TGAGCATTTATTTCCGTGTC R: GCACAAAGAGACTACAAGAGAAG	5 (201-268)	55	0.972	0.772	0.719	0.578
MDJ477	JX027217	$(AG)_6N(GA)_{15}N(GA)_6N(GA)_{20}$	F: AGCATCAGCCGCAAAGTG R: AGCCTGAGGACCTGGAAA	3 (209-254)	56	1.000	0.656	0.573	0.000*
MDJ820	JX027228	$(AC)_{30}$	F: ACCAGGTTATCCCAGTCC R: CAGAAGGAACAGAAGAGCAC	5 (129-194)	51	0.417	0.627	0.571	0.177
MDJ821	JX027229	$(GT)_{11}$	F: TCGCATCTCCTGTTTGTT R: TACACGCACTGACAAGCA	7 (124-252)	51	0.417	0.670	0.610	0.113
MDJ825	JX027232	$(GAG)_9$	F: TAGGGTCAAGATGATGGG R: TACATCTATCCACCAAATCG	3 (186-226)	48	0.333	0.604	0.521	0.018
MDJ847	JX027246	$(GAG)_7$	F: GTGTCAGGAAACGGCTCAT R: CTCTGTGGTTTCCGTGCT	5 (159-239)	54	0.306	0.460	0.427	0.054
MDJ879	JX027264	$(CTC)_8$	F: TTACCACCTCTCCAGCCC R: CAGATGCTTAGCGGAACG	7 (115-174)	55	0.583	0.714	0.673	0.050

N_A = observed number of alleles; Ta = annealing temperature; H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphic information content. *Indicates significant deviation from Hardy-Weinberg equilibrium (HWE) after Bonferroni's correction (adjusted P value = 0.00097).

Table 2. Cross-species amplification for the 53 polymorphic SSR markers in five species (*Siniperca* or *Coreoperca*) of sinipercine fishes.

Locus	Species				
	S. scherzeri	*S. kneri*	*S. obscura*	*S. undulata*	*C. whiteheadi*
CB01	58.5	58.5	58.5	58.5	58.5
CB02	56.5	56.5	56.5	56.5	56.5
CB05	58.5	58.5	58.5	58.5	58.5
CB13	55	55	55	-	55
CB27	58.5	58.5	58.5	58.5	58.5
CB36	58.5	58.5	58.5	58.5	58.5
CB37	55	55	55	55	55
CB54	56.5	56.5	56.5	56.5	56.5
CB58	55	55	55	55	55
CD07	63	63	63	63	63
CD29	56.5	56.5	56.5	56.5	56.5
CD33	58.5	58.5	58.5	58.5	58.5
CD34	63	63	63	63	63
CD39	63	63	63	63	63
CD72	63	63	63	63	63
CD75	63	63	63	63	63
CD77	63	63	63	63	63
CD90	63	63	63	63	63
CD92	63	63	63	63	63
CD201	58.5	58.5	58.5	58.5	58.5
CD203	55	55	55	55	55
CD214	56.5	56.5	56.5	56.5	56.5
CD215	55	55	55	55	55
CD226	58.5	58.5	58.5	58.5	58.5
CD256	58.5	58.5	58.5	58.5	58.5
CD260	55	55	55	55	55
CD273	55	55	55	55	-
CD288	55	55	55	55	55
MDJ471	55	55	55	55	55
MDJ477	56.5	56.5	56.5	56.5	-
MDJ820	50	50	50	50	50
MDJ821	55	55	55	55	-
MDJ879	50	50	50	50	50
MDJ847	55	55	55	55	55
PY01	56.5	56.5	56.5	56.5	56.5
PY02	50	50	50	50	50
PY05	56.5	56.5	56.5	56.5	56.5
PY08	55	55	55	55	55
PY11	55	55	55	55	55
PY12	56.5	56.5	56.5	56.5	56.5
PY16	56.5	56.5	56.5	56.5	56.5
PY21	55	55	55	55	55
PY22	50	50	50	50	50
PY25	50	50	50	50	50
PY27	56.5	56.5	56.5	56.5	56.5
PY28	55	55	55	-	55
PY39	55	55	55	55	55
PY42	55	55	55	55	55
PY45	55	55	55	55	55
PY46	55	55	55	55	55
PY48	50	50	50	50	50
PY55	55	55	55	55	55

The annealing temperature for each locus was shown. Unsuccessful amplification of PCR products for each locus is denoted by '-'.

H_E ranged from 0.44 to 0.63. The lowest H_E was in the MDJ population (0.44), and the highest in the GJ population (0.63). The lowest H_O was in the MDJ population (0.57), whereas the highest in the YJ population (0.76) (Table 3). H_O of all the populations at loci

CD39, CD215, CD273, PY16, PY27, and PY45 was higher than H_E, while H_O at loci MDJ847 and MDJ825 was lower than that expected.

Table 3. Genetic variability of eight microsatellite loci in three populations for *Siniperca chuatsi*.

Microsatellite locus	Parameters	Population		
		GJ (N = 30)	YJ (N = 29)	MDJ (N = 24)
CD39	N_A	4	4	2
	H_O/H_E	1.00/0.70	1.00/0.73	1.00/0.51
	PIC	0.63	0.66	0.38
	P_{HW}	**	**	**
	F_{IS}	-0.32	-0.32	-1.00
CD215	N_A	8	4	3
	H_O/H_E	1.00/0.86	1.00/0.75	1.00/0.54
	PIC	0.83	0.69	0.43
	P_{HW}	*	**	**
	F_{IS}	-0.13	-0.32	-0.49
CD273	N_A	5	5	-
	H_O/H_E	0.87/0.78	0.90/0.75	-
	PIC	0.73	0.69	-
	P_{HW}	n.s.	n.s.	-
	F_{IS}	-0.06	-0.13	-
MDJ847	N_A	3	2	2
	H_O/H_E	0.07/0.35	0.14/0.29	0.25/0.38
	PIC	0.31	0.24	0.30
	P_{HW}	**	*	n.s.
	F_{IS}	0.84	0.54	0.36
MDJ825	N_A	3	3	2
	H_O/H_E	0.07/0.21	0.10/0.13	0.17/0.34
	PIC	0.19	0.13	0.28
	P_{HW}	**	n.s.	n.s.
	F_{IS}	0.35	0.00	0.52
PY16	N_A	3	5	2
	H_O/H_E	1.00/0.63	1.00/0.74	1.00/0.51
	PIC	0.55	0.68	0.38
	P_{HW}	**	**	**
	F_{IS}	-0.49	-0.19	-1.00
PY27	N_A	5	4	1
	H_O/H_E	1.00/0.70	1.00/0.71	0/0
	PIC	0.64	0.64	0.00
	P_{HW}	**	**	-
	F_{IS}	-0.24	-0.32	0.00
PY45	N_A	6	6	5
	H_O/H_E	0.93/0.83	0.93/0.83	0.58/0.78
	PIC	0.79	v0.79	0.73
	P_{HW}	n.s.	n.s.	n.s.
	F_{IS}	-0.11	-0.12	0.17
Mean	N_A	4.63	4.25	2.43
	H_O/H_E	0.74/0.63	0.76/0.62	0.57/0.44
	PIC	0.57	0.58	0.36
	F_{IS}	-0.16	-0.60	-1.44

GJ = Ganjiang River; YJ = Yuanjiang River; MDJ = Mudanjiang River; N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphic information content; P_{HW} = Hardy-Weinberg probability test (*P < 0.05, **P < 0.01, n.s. = non-significant); F_{IS} = fixation indices. Unsuccessful amplification of PCR products for each locus is denoted by '-'.

HWE test

Of the 24 HWE tests, 14 were significant (Table 3). The GJ, YJ and MDJ populations had six, five and three microsatellite loci, respectively, which departed from HWE. On the

basis of average F_{IS} values, it can be seen that the pattern of heterozygote excess was most pronounced in all three populations, indicating the excess of heterozygosity within populations in accordance with significant deviations from HWE ($P < 0.05$). However, the deviations of the MDJ847 and MDJ825 loci from HWE were also detected in some of the populations due to deficiency in heterozygosity in all the samples.

Population genetic differentiation and relationships between populations

All pairwise F_{ST} statistics estimated were significant ($P < 0.01$), suggesting that all three populations were significantly different from each other (Table 4). The greatest divergence was between the YJ and MDJ populations ($F_{ST} = 0.3711$), while the least divergence was between the YJ and GJ populations ($F_{ST} = 0.2519$).

On the basis of genetic distance, the UPGMA dendrogram displayed two major clusters (Figure 1). Cluster A contained the YJ and GJ populations. The remaining population formed cluster B.

Table 4. Pairwise F_{ST} values (below the diagonal) and D_{CE} distance (above the diagonal) between three *Siniperca chuatsi* populations in China based on eight microsatellite loci.

Populations	GJ	YJ	MDJ
GJ	-	0.7761	1.1449
YJ	0.2519	-	1.0862
MDJ	0.3709	0.3711	-

For population abbreviations, see Table 3.

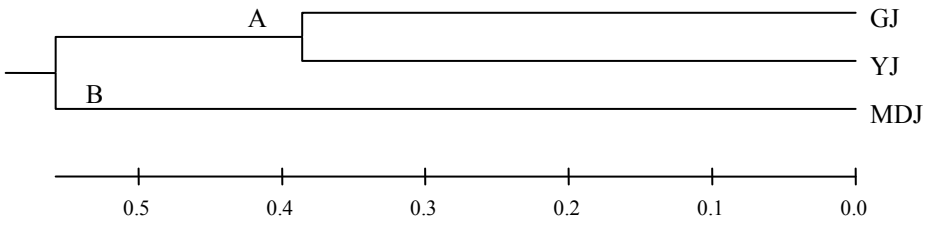

Figure 1. UPGMA dendrogram of *Siniperca chuatsi* populations based on a matrix of D_{CE} distance. Three populations were clustered into in A and B. GJ = Ganjiang River; YJ = Yuanjiang River; MDJ = Mudanjiang River.

DISCUSSION

A number of SSR markers have been isolated in *S. chuatsi* (Zhang et al., 2006; Kuang et al., 2009; Liu et al., 2011; Qu et al., 2012) and provide a sufficient tool to evaluate its genetic resources. However, they are still lacking for SSR-based mapping studies and further MAS studies, which have not been conducted for this species so far. Fifty-three polymorphic SSR markers for *S. chuatsi* were isolated in this study, where 36.8% of the designed primers were found to be polymorphic, comparable to a value of 37.5% in two previous studies (Zhang et al., 2006; Kuang et al., 2009) but lower than 60.5% reported by Liu et al. (2011). This large difference may have been caused by the different sampling strategy. Although 11 of 53 loci

showed significant deviation from HWE after Bonferroni's correction with the plausible occurrence of a null allele at four of these loci (Miao et al., 2011), these markers can still be used for population genetic studies if analytical methods are used to correct for null alleles (Park et al., 2012). *S. chuatsi* possesses 24 haploid chromosomes (Dong et al., 2008), and some of the microsatellites undoubtedly are linked. Determination of linkage will require further mapping studies. The high levels of polymorphism and heterozygosity exhibited at these loci suggest that these loci should provide a valuable tool for population studies, mapping studies and MAS of *S. chuatsi*.

A high level of cross-species amplification was observed across five species. These results were expected because of the taxonomical relationships of the families (Liu, 1993; Qu et al., 2012). *S. scherzeri*, *S. kneri*, *S. undulata*, and *S. obscura* are closely related to *S. chuatsi*, and all species belong to Siniperca, whereas *C. whiteheadi* is from Coreoperca, which is a sister genus to Siniperca. The high level of cross-species amplification demonstrated here indicates the potential usefulness of the developed markers for a broader range of evolutionary, conservation and management studies in sinipercine fishes.

Eight polymorphic SSR markers developed in this study were successfully applied to obtain preliminary population genetic parameters for 83 *S. chuatsi* specimens from three populations. Except the MDJ population, the population genetic parameters were similar to the results of a previous study using ten SSR markers to compare genetic diversity among seven populations (Wu et al., 2010). Eight SSR loci were randomly selected in this study, so all the isolated markers may provide a valuable tool for further studies in *S. chuatsi*.

Compared to the GJ and YJ populations, the MDJ population has a relatively low genetic diversity, suggesting that genetic characteristics of *S. chuatsi* in the Yangtze River drainage are distinct compared to the Heilongjiang River drainage. It may result from inbreeding in the MDJ population during artificial propagation and neglecting the genetic diversities while carrying out artificial releasing program.

All populations deviated significantly from HWE at most of the SSR loci in which heterozygote excess was apparent. It revealed inbreeding and the bottleneck effect as the main limitations to genetic differentiation between the three populations. However, the three populations at two loci (MDJ847, MDJ825) showed deviation from HWE without heterozygosity excess at either locus, which could be explained by an excess of certain genotypes. Selection, population mixing and nonrandom mating may be the factors driving deviations from HWE (Sun et al., 2011). There were 15 private alleles found in three populations (data not shown). These private alleles could be used as population-specific markers for selection of a candidate stock in controlled breeding programs, even though more samples from each population would be needed to confirm these results (An et al., 2009; Zhuo et al., 2012).

Clustering order reflects relationships between populations. In this research, the GJ and YJ populations share the highest genetic identity among the three populations, indicating the closest genetic relationship. Populations GJ and MDJ showed the lowest genetic identity and their genetic relationship was the farthest. The population structure of freshwater organisms is primarily dependent on the distribution of river systems, as previously reported (Nagarajan et al., 2006; Zhuo et al., 2012). Genetic differentiation is primarily dependent on geographical isolation.

In conclusion, fifty-three polymorphic SSR markers for *S. chuatsi* were isolated and cross-amplified in five species of sinipercine fishes in this study. Meanwhile, the genetic characterization of three *S. chuatsi* populations was performed using eight polymorphic SSR

markers developed in this study. These SSR markers will be useful for further studies of the diversity, mapping and MAS of *S. chuatsi* and other sinipercine fishes.

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