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Isolation and characterization of twenty-five polymorphic microsatellite markers in *Siniperca scherzeri* Steindachner and cross-species amplification

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Introduction

The golden mandarin fish (*Siniperca scherzeri* Steindachner) is one of the commercially important freshwater species mainly distributed in east Asia (Deng *et al.* 2010). Unfortunately, the wild *S. scherzeri* population is declining due to overexploitation and environmental pollution in their habitat (Wang *et al.* 2006; Luo *et al.* 2011), which could also cause the decline of genetic diversity. Artificial reproduction and selective breeding programmes have been undertaken to meet market demand of *S. scherzeri* (Yang *et al.* 2007; Mi *et al.* 2010), but the genetic diversity of cultured population tends to be lower than wild population, particularly for those under selective breeding (Sunden and Davis 1991; Wang *et al.* 2012). Understanding of the population structure is important to test hypotheses about diversification and speciation (McDonald 2003) and to find appropriate strategies for the conservation of biodiversity (Cegelski *et al.* 2003). Molecular markers are an important tool for evaluating levels and patterns of genetic diversity and have been used to study genetic diversity in a number of cultured fish species (Liu and Cordes 2004). Among the various molecular markers, the most informative and polymorphic are microsatellite markers (simple sequence repeats), which have been extensively used to evaluate genetic diversity (Serrano *et al.* 2009; Missohou *et al.* 2011). However, there is still limited number of available microsatellite markers (SSRs) for *S. scherzeri* (Yang *et al.* 2012).

Transcriptome assemblies of the F_1 interspecies hybrids between *S. chuatsi* (♀) × *S. scherzeri* (♂) were generated using Illumina sequencing. In this study, 25 novel polymorphic SSR markers were developed for *S. scherzeri* from transcriptome of F_1 interspecies hybrids. Additionally, cross-species transferability of these markers were tested in five additional species within the genus *Siniperca* (*S. chuatsi* (Basilewsky), *S. kneri* Garman, *S. undulata* Fang & Chong, *S. obscura* Nichols) and genus *Coreoperca* (*C. whiteheadi* Boulenger).

Materials and methods

Illumina sequencing was performed for the F_1 interspecies hybrids between *S. chuatsi* (♀) × *S. scherzeri* (♂). A total of 1,18,218 unigenes were generated after *de novo* assembly. In the present study, we selected 76 unigenes containing 85 SSR motif loci, from which 85 primer pairs were designed using NCBI/Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome). Those primer pairs were then characterized using 32 specimens from a wild *S. scherzeri* population (Chongyang, Hubei, China).

Total genomic DNA was extracted from fin clips using the TIANamp Genomic DNA kit (Tiangen, Beijing, China) following the manufacturer's instructions. Polymerase chain reaction (PCR) conditions were optimized for each pair of primers, and PCR was conducted in a total volume of 25 μL including 2.5 μL of 10× PCR buffer, 1.0–3.0 mM $MgCl_2$, 50 μM dNTPs, 0.4 μM of each primer, 1 U *Taq* polymerase (Takara, Tokyo, Japan) and 50 ng genomic DNA. PCR thermal conditions were as follows: an initial denaturation step of 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at locus-specific annealing temperature (see table 1), 30 s at 72°C, followed by a final extension at 72°C for 10 min. PCR products were separated on a 8% nondenaturing polyacrylamide gel electrophoresis and visualized by silver staining. A denatured pBR322 DNA/*Msp*I molecular weight marker (Tiangen) was used as a size standard to identify alleles.

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Table 1. Characteristics of 25 polymorphic SSR markers in *S. scherzeri*.

Locus	Accession number	Repeat motif	Primer sequence (5′–3′)	Size range (bp)	T_a (°C)	N_a	H_O	H_E	PIC	*P* value
SS181	JX294968	$(GAG)_5(GAT)_8$	F: CAGAATGGCAAATCAAACA R: TGGCAGAGGTCCGCTTA	124–144	53.5	4	0.3125	0.4980	0.4545	0.9715
SS182	JX294969	$(AC)_{12}$	F: GAAGAACGGGCTGACTGGAC R: GCACTCATATAGGAAGCCTGACA	273–280	55	2	0.0000	0.4365	0.3374	1.0000
SS189	JX294970	$(TGAA)_6$	F: ACCAAGCATCTACCCAGCAG R: AAGTGTTGTTTTGAGGACCACCT	180–206	55	5	0.2500	0.7396	0.6888	1.0000
SS190	JX294971	$(AC)_{12}$	F: TGCTGATTGGTCGTCTTCTGTC R: CACACACTAGGACAATCCACCTG	169–209	55	3	0.6250	0.5650	0.4806	0.0909
SS201	JX294972	$(GT)_{11}(TG)_{12}$	F: GTCCAGAGAGACTGTGGGGG R: GTGGACTCGAGCAGCTTTGT	156–204	55	7	0.9688	0.8398	0.8035	0.0835
SS204	JX294973	$(AC)_{12}$	F: GGCCTACTGTGTAAGGCAGTG R: TGCCATGGAAACAAGCAGGT	243–276	55	6	0.9375	0.8051	0.7628	0.2042
SS206	JX294974	$(TCA)_8$	F: CGAACCGTCTCACTTCGTCC R: AAACAAACTGGCGTGTGGGT	84–108	52.5	7	0.4375	0.7728	0.7255	1.0000
SS211	JX294975	$(TAC)_8$	F: AAAGTGGGACATTTGTGCAGGT R: CTGGTGACGCTGGTGAATGT	72–76	55	2	0.1875	0.1726	0.1556	0.7716
SS213	JX294976	$(CAG)_8$	F: CCGCAGCTCCTCCTGAAAAC R: CTTCATCTCCACTCCGGCTC	147–159	55	5	0.0625	0.7346	0.6745	1.0000
SS214	JX294977	$(CA)_{12}$	F: GCAGCTCCACGGTGAAACCC R: AGAAGAGCGCAGCCAGACTC	157–197	53.5	7	0.5625	0.7857	0.7425	0.9602
SS220	JX294978	$(AC)_7(GCAC)_5$	F: TCCTAGCATGCTGCCCTGTA R: TGGACTGCGTCTTTTCGAGG	145–230	55	9	0.9688	0.8690	0.8388	0.1547
SS221	JX294979	$(ATA)_8$	F: AAAACGGCTGCTTAGCTCTG R: ATGGCCAAAAGTATGTGGAC	120–155	55	9	0.5938	0.8413	0.8059	0.991
SS222	JX294980	$(GAT)_8$	F: GATCTGGGACGGGGTAGGAC R: ATATTGGACGCGCTGGATGG	146–192	55	4	0.4688	0.6682	0.5868	0.9481
SS224	JX294981	$(TCC)_8$	F: TTGTTCCCGGGTGTCCCTTA R: TTGTCTCGAGCTGTTGCGG	199–239	55	6	0.5938	0.6017	0.5644	0.9547
SS229	JX294982	$(TC)_8C(CT)_{11}(CA)_{12}$	F: TGTGAGTTTGCAGTCCGAGC R: CCAAACTGTCCACAACACGTTC	200–254	55	8	0.8438	0.8378	0.8025	0.4929
SS230	JX294983	$(GAG)_8$	F: TAACCAGGGCGGAATGGGAA R: AAAACAACCTCGCCTGACCC	226–254	55	3	0.5938	0.5580	0.4696	0.2502
SS236	JX294984	$(GT)_6GCGA(GT)_{12}$	F: CAGTTCTGGTCCGTCGGTTA R: CGGTATTCGGACACGACAGA	154–208	55	7	0.9375	0.8383	0.8010	0.1019
SS240	JX294985	$(CAC)_8$	F: CGGTATCTGAGCCCCACAAG R: CGGTCTGAGTAGAGCTGCTT	214–235	55	4	0.0938	0.2584	0.2439	1.0000
SS243	JX294986	$(TG)_{11}$	F: CTGTTGTTGGGGCTGACTGA R: ACTGTACTGCACACACTGTCC	120–136	55.5	5	0.5000	0.6990	0.6441	0.9751
SS250	JX294987	$(AC)_{11}$	F: TTTACACACACAGCCCCACC R: CTGCCTTGAGTGCCAGAACA	159–192	55	5	1.0000	0.7783	0.7276	0.0004

Table 1 (*contd.*)

Locus	Accession number	Repeat motif	Primer sequence (5′–3′)	Size range (bp)	T_a (°C)	N_a	H_O	H_E	PIC	*P* value
SS257	JX294988	$(AC)_{11}$	F: ACTAGTAGTGTGTGCAAGTAGA R: AGGCATATCACCTCCTCCCT	188–247	55	8	0.875	0.8185	0.7804	0.2352
SS259	JX294989	$(AC)_{11}$	F: GCATGCTTTATGTGAAAATGGAGG R: GCTTTGGGAAAGTTCCACCG	170–232	55	9	0.9375	0.8596	0.8279	0.1119
SS261	JX294990	$(GT)_{11}$	F: TGGCTGCATGGACATTCCTA R: CAGAAACCAGTGGATTACGTT	177–225	55	6	0.8125	0.8070	0.7639	0.8637
SS264	JX294991	$(TG)_{11}A(GT)_6$	F: CTCAGCTCTGGCTGTCCAAG R: AGGCTTGTTCACTAACTTACACTG	165–219	55	7	1.0000	0.8279	0.7919	0.0019
SS265	JX294992	$(AC)_{11}$	F: GCGCCTATGTTGGCCAGTAA R: GGTGTCATGATCTCCACGGC	257–287	55	6	1.0000	0.7004	0.6504	0.0000

T_a, annealing temperature; N_a, number of alleles; H_O/H_E, observed/expected heterozygosities; PIC, polymorphic information content; *P* value, the test for deviation from Hardy–Weinberg expectations.

Popgene 3.2 software (Yeh and Boyle 1997) was used to calculate the number of alleles (N_a), observed (H_O) and expected (H_E) heterozygosities. We computed the polymorphic information content (PIC) using the formula:

$$\mathrm{PIC} = 1 - \left(\sum_{i=1}^{n} q_i^2\right) - \left(\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2q_i^2 q_j^2\right).$$

Where n is the number of alleles; q_i and q_j are the ith and jth allele frequencies, respectively (Botstein *et al.* 1980). The tests for deviations from Hardy–Weinberg equilibrium (HWE) as well as genotypic linkage disequilibrium (LD) between all loci were assessed by online version of Genepop (http://genepop.curtin.edu.au) (Raymond and Rousset 1995). Sequential Bonferroni correction was used to adjust the *P* values using multiple statistical comparisons for all results (Rice 1989).

Cross-species amplification of the polymorphic primer pairs was tested in five additional species. Two individuals of each species were analysed. Three annealing temperatures (53, 55, and 57°C) were conducted with the same PCR conditions as described above. PCR products were identified by agarose gel (1.5%) electrophoresis, and 1-kb DNA ladder (Invitrogen, California, USA) was used as a size standard. Primer pairs that amplified fragments of similar sizes to those observed in *S. scherzeri* were considered as successful cross-species amplification.

Results and discussion

A total of 85 primer pairs were designed, of which 67 (78.82%) primer pairs could successfully amplify fragments with expected size. And 25 (29.41%) loci were polymorphic in a test population of 32 individuals from a wild *S. scherzeri* population with number of alleles ranging from 2 to 9, and observed and expected heterozygosities from 0.0000 to 0.9688 and from 0.1726 to 0.8596, respectively. Characteristics of the 25 polymorphic loci are given in table 1. PIC ranged from 0.1556 to 0.8388 (average of 0.6450). Three microsatellite loci (SS250, SS264 and SS265) deviated significantly from HWE expectations after Bonferroni correction (adjusted *P* value = 0.0020). No significant linkage disequilibrium (LD) was detected across all loci following Bonferroni correction (adjusted *P* value = 0.0002). To date, there is still limited number of available microsatellite markers for *S. scherzeri* (Yang *et al.* 2012). Therefore, this set of microsatellites would facilitate further studies on conservation genetics, population structure and molecular marker-assisted breeding in *S. scherzeri*.

Overall, a high level of cross-species amplification was observed across the five species tested (table 2). No amplification was detected for primers SS220, SS221 and SS224 in any of the species tested. Moreover, another five pairs of primers (SS181, SS240, SS243, SS259 and SS261) failed to amplify right fragments in *C. whiteheadi*. The species of the families *S. chuatsi*, *S. kneri*, *S. undulate* and *S. obscura*

Table 2. Cross-species amplification for the 25 polymorphic microsatellite loci in five species of sinipercine fishes.

Locus	Species				
	Siniperca chuatsi	*S. undulate*	*S. obscura*	*S. kneri*	*Coreoperca whiteheadi*
SS181	53	53	53	53	–
SS182	57	55	55	55	55
SS189	55	53	55	55	55
SS190	55	55	55	55	55
SS201	55	55	55	55	55
SS204	53	53	53	53	53
SS206	55	55	55	57	55
SS211	55	55	55	55	53
SS213	55	55	55	55	55
SS214	53	53	53	53	53
SS220	–	–	–	–	–
SS221	–	–	–	–	–
SS222	55	55	55	55	55
SS224	–	–	–	–	–
SS229	55	55	55	55	55
SS230	55	55	55	55	55
SS236	55	55	55	55	55
SS240	55	55	55	55	–
SS243	57	57	57	57	–
SS250	57	57	57	55	55
SS257	55	55	55	55	55
SS 259	55	55	55	57	–
SS261	55	55	55	55	–
SS264	55	55	55	55	55
SS265	55	55	55	55	55

The annealing temperature for each locus is shown. –, Unsuccessful amplification of PCR products.

presented more successful cross-species amplification (22 loci) than species of the families *C. whiteheadi* (17 loci). These results were expected because of the taxonomical relationships of the families. *S. chuatsi*, *S. kneri*, *S. undulate*, *S. obscura* and *S. scherzeri* belong to genus *Siniperca*, whereas *C. whiteheadi* is from genus *Coreoperca*. The high level of successful cross-amplification suggests the potential usefulness of the developed markers which may be suitable for assessments of genetic diversity and population structure in sinipercine fishes. Additionally, our results highlight that cross-species amplification of SSRs is an effective option for the development of SSR markers for which limited genomic resources were available.

In conclusion, 25 polymorphic microsatellite markers were developed for *S. scherzeri* from the transcriptome sequences, and high cross-species transferability was detected in five additional species. These markers will facilitate further studies on the conservation genetics, population structure and construction of high-density linkage map. Next-generation sequencing was used for the generation of unigene data base. Our results confirm that development of SSRs from transcriptome is an efficient method to identify SSRs in transcribed regions.

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