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Development and characterization of twenty-nine novel polymorphic microsatellite loci in the mandarin fish *Siniperca chuatsi*

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Introduction

Mandarin fish *Siniperca chuatsi* (Basilewsky), mainly distributed in the Yangtze and Pearl rivers, is an important commercial freshwater fish species in China (Liang 1996). It has a fast growth rate, but is susceptible to diseases. Compared with *S. chuatsi*, golden mandarin fish *S. scherzeri* (Steindachner) has great disease resistance, but grows slowly. Since the hybrid retained desired characteristics such as fast growth rate and great disease resistance from their parents (Mi *et al.* 2009), breeding a disease-resistant and faster growing strain has been attracting more and more attention from researchers. However, the research mainly focussed on the morphology of the hybrid (Zhao *et al.* 2008; Mi *et al.* 2009), very little was known about its genetic variation.

Microsatellites, also known as simple sequence repeats (SSRs), have become a useful tool to assess genetic diversity and develop molecular breeding technique in fish because of their codominant nature and high allelic polymorphism (Walter and Epperson 2001; Chen *et al.* 2005). Nevertheless, only a few microsatellite markers are available for *S. chuatsi* (Zhang *et al*. 2006; Kuang *et al*. 2007a, b, 2009; Liu *et al*. 2011; Qu *et al*. 2012) and *S. scherzeri* (Qu *et al.* 2012; Yang *et al.* 2012). The number of available SSRs is grossly inadequate for genetic and mapping studies. These research areas have long suffered from one of the challenges of systematic biology studies, namely the lack of genomic resources such as genome or transcriptome sequences. Hence, there is a need to enhance such resources. With the advent of next-generation sequencing technologies, transcriptome sequencing is emerging as a rapid and efficient means for gene discovery and genetic marker development. As hybrid gene expression is under the influence of two different and divergent genomes, gene expression in hybrids may reveal information about interactions of two parental alleles and their impacts. So, we hybridized *S. chuatsi* (♀) and *S. scherzeri* (♂), and *de novo* transcriptome sequencing for their F_1 interspecies hybrids was performed. Here, we describe the isolation and characterization of 29 novel polymorphic SSR markers for *S. chuatsi* from this transcriptome and test these markers in *S. scherzeri*.

Materials and methods

Using high-throughput Illumina RNA-seq (BGI, Guangdong, China), the transcriptome from RNA of F_1 interspecies hybrids was analysed and 118,218 unigenes were obtained. This unigene set was used for mining genic-SSR markers using BatchPrimer3 v. 1.0 software and the parameters were left at the default settings (You *et al.* 2008). A total of 22,418 SSRs were found. We selected a subset of 54 SSR markers from 52 unigene sequences for validation. All the relevant sequences have been deposited in GenBank (JQ686834–JQ686884) (table 1). Primers for these SSR loci were designed using NCBI/Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/).

The polymorphism of microsatellite loci was evaluated on 39 individuals from a wild *S. chuatsi* population. 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) amplification and SSR genotyping were performed using reagents and protocols described in our previous study (Qu *et al.* 2012).

The genetic diversity indexes, such as the number of alleles (N_a), expected (H_E) and observed heterozygosities (H_O) were calculated using POPGENE v. 3.2 (Yeh and

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Table 1. Characteristics of 29 polymorphic SSR markers of *S. chuatsi*.

Locus	Accession number	Repeat motif	Primer sequence (5′–3′)	Size range (bp)	T_a (°C)	N_a	H_O	H_E	PIC	P	N_a for *S. scherzeri**
SC01	JQ686834	$(TCA)_{12}$	F: TTTTAAAGACGGGGCAGCGG R: ACCAACGTTTGGCGTAAAGC	211–309	55.5	13	0.7949	0.8615	0.8366	0.2734	12
SC02	JQ686835	$(GAT)_{13}$	F: GGCACTTGTGTAAAGGTTGGAT R:TAAATTAAGCCAGGAGCAAAGC	252–321	59	5	1.000	0.6767	0.6090	0.0000*	8
SC03	JQ686836	$(CA)_7A(AC)_6AA(AC)_{13}(CACT)_{14}$	F: AACTCCGCAACCTCATCTGG R:TCTGTAAAGTGCAGCCTCAGC	258–291	55.5	3	0.5641	0.6550	0.5739	0.0357	0
SC04	JQ686837	$(TGCT)_3N(AC)_{70}$	F: AATGAGGCCCCTTTTTCCCC R:TCCTCTGAATCATCAGTGTGTGG	192–286	58	10	1.000	0.8603	0.8396	0.0088	12
SC05	JQ686838	$(ATAG)_{13}$	F: AATCTATGCCTTGGCTTTTGG R: CCGGACTATCAGTACCCCACT	174–265	59	5	0.1053	0.1536	0.2078	0.1615	0
SC09	JQ686842	$(GCCTGA)_7$	F: CACGAACTACTCGCCCTCAC R: TCTGTGGGAACCGTGGTGAT	199–247	58.5	7	0.6316	0.7895	0.7928	0.0052	10
SC10	JQ686843	$(TTTC)_{10}$	F: CAGGGAACGGGGAAAAAGCA R:AGAATTCCTCCGTACCTGTCTG	222–277	53.5	6	0.0526	0.1522	0.5935	0.0265	6
SC11	JQ686844	$(GAG)_{10}$	F:GCATAAAATCTGAGGGGAGCGT R: TTGGGCTCCACTGCCTGTAT	206–228	55.5	4	0.1500	0.3872	0.4027	0.0319	7
SC12	JQ686845	$(TG)_{17}N(GAGG)_3$	F: GCTCCCTGCTTTTCCCTCTCA R: GCTCCCTGCTTTTCCCTCTCA	132–186	55.5	4	1.000	0.7244	0.5555	0.0007*	6
SC13	JQ686846	$(AC)_{46}AT(AC)_7$	F:TCAAGGGAGTGTTTAGTTTAGTGT R: ACTGGCACATTTGGTAGACAGG	226–276	53.5	6	1.000	0.7256	0.6849	0.0602	8
SC14	JQ686847	$(TAA)_{12}$	F: AGGCATTGTGTCACTTTGAGC R: TGCTGGTGTTACCCACCTGA	182–254	54.5	5	0.9744	0.6613	0.5801	0.0202	0
SC15	JQ686848	$(TAA)_{10}(AAC)_5$	F: ACAACACCCGTCCCAATGTC R:GGGTGCTCACACTAGTAAGTGG	220–290	54.5	5	0.8718	0.7483	0.6914	0.3517	10
SC16	JQ686849	$(CTT)_5$	F: AACGTCTCCGTTTCCATCAGG R: TCTGCTGCCCGAGAGACTTT	244–298	54.5	5	0.3500	0.3897	0.4989	0.3817	9
SC23	JQ686856	$(GT)_{38}$	F: ACATGACAGCAGGCGACAAT R: TTGCTGCTAGAAGAGATCCTCG	263–392	54.5	9	1.0000	0.8846	0.8483	0.1891	12
SC24	JQ686857	$(CA)_{42}$	F: ACGGAGAGCAGAGTCTTACGTC R: ACTCTCCAGCTGCCGTCACA	202–284	54.5	8	1.0000	0.8397	0.8157	0.0132	8
SC26	JQ686859	$(TGA)_{10}$	F: TATGAGCAGGTAGACGGCACA R: TGCAGACCTGTCAGCTGTTTC	212–271	54.5	5	1.0000	0.6415	0.6505	0.0004*	5
SC28	JQ686861	$(GACA)_9$	F: TCGGATGAACGCTTGTTGAGA R: GTGCCATAGAGCTCCGTTGT	270–318	54.5	5	0.3684	0.6871	0.6208	0.0693	0

Table 1. (*Continued.*)

Locus	Accession number	Repeat motif	Primer sequence (5′–3′)	Size range (bp)	T_a (°C)	N_a	H_O	H_E	PIC	P	N_a for *S. scherzeri**
SC29	JQ686862	$(AGTGT)_8$	F: TGCCGTTGGAGGAAGTCAGA R: CGGTTGTGCCTGGAGGTATC	246–277	54.5	4	0.0500	0.2731	0.1960	0.0007*	8
SC36	JQ686868	$(GT)_{35}$	F: ATGACTCTGGCACCAACGC R:AACAACAGCTAACTGAACACAAG	222–257	55	6	0.9744	0.7989	0.7556	0.0040	11
SC38	JQ686870	$(TG)_{27}$	F: AATTAGCATGAGCCAGGGACC R: GCAGCCTTGAACACACTTGA	180–253	55	7	1.0000	0.7949	0.7744	0.3233	11
SC41	JQ686873	$(TG)_{25}$	F: CTGCACACAAACCATCAGACT R: GGGAGACACCTGCTCTTACT	298–420	55	9	1.0000	0.8137	0.8563	0.0490	0
SC43	JQ686875	$(TG)_{25}$	F: GTGATTGTCTTGCTCCACCTG R:ATCCTTCCATAGTGTTCTACAGT	210–323	55	8	1.0000	0.7639	0.7564	0.0955	13
SC44	JQ686876	$(CA)_{22}$	F: CAGACCAAAGGACTGCACTG R: AGGGGCTGGGTTGGCATTAT	205–265	55	7	1.0000	0.7816	0.7352	0.0505	11
SC45	JQ686877	$(TG)_{27}$	F: AGATTAGGCCTGCAGGTGAC R:ACACACATTTCTTACAGTTTGGT	175–251	55	8	0.9744	0.7602	0.7092	0.0032	13
SC46	JQ686878	$(CA)_{27}$	F: AGAGGGGCCTTGAATCTGGG R: TCTGGCTTCAGCCAAACGTG	204–307	55	10	1.0000	0.8492	0.7816	0.0821	10
SC49	JQ686880	$(CTC)_{10}$	F: TTGGTGGTGGCTGATCCCAGG R: GACGGGCAGAAAACATCCTCC	99–106	55	2	0.4000	0.4923	0.3690	0.0391	8
SC51	JQ686882	$(CA)_{19}$	F:CTGTGCTTCGGAAGTTGAATGG R:ACCTTTACAGTTTCAGTGTCCATGT	249–320	55.5	6	1.0000	0.6730	0.6051	0.0000*	0
SC52	JQ686883	$(TG)_{19}$	F: GAATGCCATGTGTCGTTGCG R: CCGGCCTGACATCCCTAAGA	165–211	55.5	5	0.5128	0.7746	0.7260	0.0255	9
SC53	JQ686884	$(CA)_{19}$	F: GTCGGACTTGGTTCAGCTACA R: GTCCTACTCTACCTGCCCCG	224–276	55.5	6	0.9744	0.6980	0.6318	0.0658	10

*N_a of 0 indicates no amplification or smear; T_a, annealing temperature; N_a, number of alleles; H_O/H_E, observed and expected heterozygosities; PIC, polymorphism information content; P, the test for deviation from Hardy–Weinberg expectation; *, significant deviation from HWE after Bonferroni correction ($P < 0.0017$). The last column shows the number of alleles per loci for *S. scherzeri*.

Boyle 1997). Deviations from Hardy–Weinberg equilibrium (HWE) for each locus, and genotypic linkage disequilibrium (LD) between all loci were tested using the online version of GENEPOP (http://genepop.curtin.edu.au/) (Raymond and Rousset 1995). All results were adjusted for multiple simultaneous comparisons using a sequential Bonferroni correction (Rice 1989).

Results and discussion

In total, 29 of the 54 loci showed moderate to high levels of polymorphism. The remaining 25 loci were either monomorphic or failed to amplify. Characteristics of these 29 loci are given in table 1. The number of alleles per locus ranged from 2 to 13, with an average of 6.3 alleles per locus. The observed heterozygosity (H_O) varied from 0.0500 to 1.0000 (average 0.7500), while the expected heterozygosity (H_E) varied from 0.1522 to 0.8846 (average 0.6659). Five loci (SC02, SC12, SC26, SC29 and SC51) showed significant deviation from the HWE after Bonferroni correction (adjusted $P = 0.0017$). It may be the result of nonrandom mating, genetic bottlenecks and a large heterozygote excess. The presence of null alleles was checked by Micro-Checker v. 2.2.3 software (Van Oosterhout *et al.* 2004), but no evidence for allelic dropout was found in these loci. No significant LD was detected across all loci following Bonferroni correction (adjusted $P = 0.00013$).

To determine the function of genes associated with the SSR markers, BLASTx searches were conducted for all unigenes with polymorphic SSRs using cut-off *E*-value <1.00E-10 (Sha *et al.* 2011). Of the 29 SSR-containing unigenes, six had significant similarity to known genes, including mediator of RNA polymerase II transcription subunit 30, transcription elongation factor B polypeptide 3, lysosomal protective protein, transcription initiation factor IIA subunit 2, metabotropic glutamate receptor 5 and double C2-like-domain-containing protein beta-like isoform 2 (table 2). These markers will be useful for molecular marker-assisted breeding.

To evaluate cross-species amplification, we tested the 29 primer pairs described here in a related species, *S. scherzeri*. Thirty individuals were screened. Of the 29 SSR makers, 27 primer pairs successfully cross-amplified in *S. scherzeri* and 23 of these were polymorphic (table 1). These results were anticipated because the transcriptome was from F_1 interspecies hybrids of *S. chuatsi* and *S. scherzeri*. It may reveal information about interactions of two parental alleles and their impacts. Another explanation is that these EST-SSRs are derived from transcribed regions of the DNA that generally are more conserved across species, therefore such SSR markers may have a higher rate of transferability than SSRs derived from nontranscribed regions (Barbara *et al.* 2007). The high rate of successful cross-species amplification indicated substantial cross-species transferability of these and probably other EST-SSR markers developed from this transcriptome. Further, the markers described here may be suitable for assessments of genetic diversity and population structure in the *Siniperca* genus.

In conclusion, a total of 29 polymorphic microsatellite markers were developed using transcriptome sequencing. We only tested a small subset of the SSR loci identified in this transcriptome. Be that as it may, high levels of polymorphism and high rate of successful cross-species amplification indicated that the transcriptome of F_1 interspecies hybrids could be a rich source of candidate molecular markers for the *Siniperca* genus. These novel markers will facilitate further studies on genetic diversity evaluation, conservation genetics, the construction of high-density linkage map and molecular-marker-assisted breeding of *S. chuatsi* and its related species.

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Table 2. Annotation of gene-associated SSRs by BLASTx analysis.

Locus	BLASTx similarity match	*E* value	Species	Accession number
SC3-2	Mediator of RNA polymerase II transcription subunit 30	1E-45	*Salmo salar*	ACM08366.1
SC9	Transcription elongation factor B polypeptide 3	2E-43	*Danio rerio*	NP_998620.1
SC29	PREDICTED: Lysosomal protective protein	0	*D. rerio*	XP001331905.3
SC36	Transcription initiation factor IIA subunit 2	3E-45	*Esox lucius*	ACO13378.1
SC46	Metabotropic glutamate receptor 5	1E-90	*Cricetulus griseus*	EGW01814.1
SC51	PREDICTED: Double C2-like domain-containing protein beta-like isoform2	0	*Oreochromis niloticus*	XP_003454262.1

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