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Development and characterization of novel SSR markers in *Siniperca kneri* Garman

M. Yang[1,2]*, H.Z. Zheng[1,2]*, X.-F. Liang[1,2], C.X. Tian[1,2], Y.Q. Dou[1,2], K.C. Zhu[1,2] and Y.C. Yuan[1,2]

[1]Key Lab of Freshwater Animal Breeding, College of Fisheries, Huazhong Agricultural University, Ministry of Agriculture, Wuhan, Hubei, China
[2]Hubei Collaborative Innovation Center for Freshwater Aquaculture, Wuhan, Hubei, China

*These authors contributed equally to this study.
Corresponding author: X.F. Liang
E-mail: xufang_liang@hotmail.com

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ABSTRACT. In this study, 37 transcriptome-derived simple sequence repeat (SSR) markers and 18 genomic SSR markers were developed and characterized in the Chinese perch, *Siniperca kneri* Garman. The average allele number per locus was 5.1 (range: 2-8) for transcriptome-derived SSRs and 3.8 (range: 2-5) for genomic SSRs. The average observed and expected heterozygosities were 0.666 (range: 0.000-1.000) and 0.692 (range: 0.230-0.857) for transcriptome-derived SSRs, respectively. These values were 0.380 (range: 0.000-1.000) and 0.527 (range: 0.201-0.799) for genomic SSRs, respectively. The average polymorphic information content was 0.638 (range: 0.215-0.824) for transcriptome-derived SSRs and 0.477 (range: 0.183-0.752) for genomic SSRs. Seven of these loci exhibited departure from Hardy-Weinberg equilibrium after sequential Bonferroni's correction for multiple tests, and no significant deviation was observed for the linkage disequilibrium. These developed and characterized markers are anticipated to be useful for studies on population genetics,

conservation genetics, and the fishery management of this species.

Key words: *Siniperca kneri* Garman; Genome; Simple sequence repeat; Transcriptome

INTRODUCTION

The Chinese perch, *Siniperca kneri* Garman, is an endemic species to East Asia, and is primarily distributed in the Yangtze River drainage system of China. This fish species is one of the most economically and geographically important species in the genus *Siniperca* (Zhou et al., 1988). Unfortunately, the wild stock is declining because of excessive exploitation and environmental pollution (Liang, 1996). Therefore, the rational use of natural resource is urgently required to maintain, and possibly enhance, the quality of brood stock.

SSR markers are used extensively in molecular ecology and conservation genetics, as well as in stock assignment and assessments of genetic diversity for commercial fish (Perez et al., 1999; Hansen et al., 2001a,b). SSR markers have been developed for some *Siniperca* species, such as *Siniperca chuatsi* (Kuang et al., 2009; Liu et al., 2011) and *Siniperca scherzeri* Steindachne (Qu et al., 2012; Yang et al., 2012). However, polymorphic SSRs have not been developed for *S. kneri* because of a lack of sequence information. Thus, sequence information, particularly transcriptomes, of closely related species could be used to reveal the polymorphisms of *S. kneri*.

Transcriptome sequencing, which is the DNA sequencing of the mRNA pool of a given tissue, has allowed sequencing efforts to focus on the protein-coding portion of the genome. As a result, this technique has enabled large numbers of molecular markers to be developed for non-model organisms, both quickly and at relatively low cost (Bouck and Vision, 2007). Recently, the transcriptome assemblies of the F_1 interspecies hybrids between *S. chuatsi* (♀) and *S. scherzeri* Steindachne (♂) were generated using Illumina paired-end sequencing technology (He et al., 2013). In this study, we developed 37 SSR markers from this previously developed transcriptome database. In addition, a further 18 genomic SSRs were also derived from SSR-enriched genomic libraries of *S. kneri*. This study is the first to report the successful use of transcriptome sequences and repeat enriched genomic libraries for SSR marker development in *S. kneri*. These SSR primers are anticipated to be useful for studies on population genetics, conservation genetics, fishery management, and for the construction of genetic linkage maps of *S. kneri.*

MATERIAL AND METHODS

Collection and DNA extraction

Thirty-two wild *S. kneri* individuals were collected from 4 sites (Guangzhou: 23°04'N113°28'E, Nanchang: 23°04'N113°28'E, Wuhan: 30°35'N114°17'E, Changsha 28°13'N112°56'E), and 8 individuals from each site. The distance among them were: Wuhan to Changsha, 290.8 km; Wuhan to Nanchang, 262 km; Changsha to Nanchang, 293 km; Wuhan to Guangzhou, 856.9 km; Changsha to Guangzhou, 563 km; Nanchang to Guangzhou, 705 km. Total genomic DNA was extracted from fin clips using the TIANamp Genomic DNA Kit (Tiangen, Beijing, China), according to manufacturer protocols. The DNA was adjusted to 100 ng/μL and then stored at -20°C until use.

SSR mining from transcriptome

The transcriptome assemblies of the F_1 interspecies hybrids between *S. chuatsi* (♀) and *S. scherzeri* (♂) were generated using the Illumina paired-end sequencing technology. Potential SSR markers were detected among the unigenes of this transcriptome using the BatchPrimer3v1.0 software (You et al., 2008). The parameters were adjusted for the identification of perfect di-, tri-, tetra-, penta-, and hexa-nucleotide motifs, with a minimum of 6, 5, 3, 3, and 3 repeats, respectively. Mononucleotide repeats were excluded, because it was difficult to distinguish genuine mononucleotide repeats from polyadenylation products and single nucleotide stretch errors generated by sequencing. The primers for these SSR loci were designed using NCBI/Primer-BLAST (http://www.Ncbi.nlm.Nih.gov/tools/primer-blast/index.cgi?LINKLOC=BlastHome).

Isolation of genomic SSR

SSRs were isolated using a hybridization-based capture methodology, following the protocol described by Billotte et al. (1999). Briefly, the extracted genomic DNA was digested with the restriction enzyme *Mse*I (BioLabs). DNA fragments of 300-1000 bp were selected using electrophoresis on agarose gel, and the excised gel was purified using a PBZ0202-1 DNA purification kit (Sangon Biotech, Shanghai, China). Specific adapters (5'-GACGATGAGTCCTGAG-3' and 5'-TACTCAGGACTCAT-3') were then ligated to the digested DNA. Approximately amplified DNA fragments were hybridized with 5'-biotin-labeled oligonucleotides $(GA)_{20}$ and $(CCT)_{15}$. Then, streptavidin magnetic beads (Sangon Biotech) were used to capture the target fragments. The captured DNA fragments were eluted from the beads-probe DNA mixture, by treating it with T-Elution buffer at 95°C for 5 min. The enriched DNAs were cloned into the pGEM-T plasmid vector (Promega Beijing Biotech, Beijing, China), and were transformed into competent *Escherichia coli* strain DH-5α (Promega Beijing Biotech). White colonies were randomly selected from the primary transformation plates, and the isolated Plasmid DNA was sequenced by an ABI 3730 Genetic Analyzer (Applied Biosystems). SSR clones were identified by screening with the SSRHUNTER software (Li and Wan, 2005). Then, sequences containing SSRs with 5 or more repeats were selected for primer design using the PRIMER PREMIER 5.0 program (PREMIER Biosoft International, USA).

PCR amplification and genotyping

PCRs were performed in a final volume of 25 µL containing 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, Beijing, China). PCR amplifications were conducted under the following conditions: 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 45 s at a primer-specific annealing temperature (Table 1), 1 min 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 were visualized by silver staining. The denatured pBR322 DNA/*Msp*I molecular weight marker (Tiangen, Beijing, China) was used as size standard to identify alleles.

Table 1. Characterization of the polymorphic microsatellite markers in a sample of 32 *Siniperca kneri* Garman individuals including locus name, repeat motif, primer sequences, allele size range, annealing temperature (Ta), locus type, number of observed alleles (N_A), observed (H_O) and expected heterozygosities (H_E), polymorphism information contect (PIC), chi-square tests for Hardy-Weinberg equilibrium (HWE) after Bonferroni's correction (adjusted P value = 0.0009) and Genbank accession number.

Locus name	Repeat motif	Primer sequence (5'-3')	Size range (bp)	Ta (°C)	Locus type	N_A	H_O	H_E	PIC	P_{HWE}	GenBank accession No.
Transcriptome-derived SSR											
SK472	$(TG)_6N(GTGTGA)$	F: GTATTTGGCAGGCTTTTAG R: TAGACTCGTCTCCCTGACA	268-373	57	I	4	0.781	0.734	0.674	0.090	JX503195
SK473	$(CA)_{28}$	F: TTCATCCTGTCTCACCGC R: CACTGCCACAGCTAGGATCA	232-289	55	P	5	1.000	0.796	0.749	0.060	JX503194
SK483	$(AC)_8N(AC)_6$	F: AGGTTGGATTTTGGGTCAAT R: AAGGCACTTCGGCTAATG	221-309	55	I	5	0.719	0.810	0.765	0.170	JX503184
SK490	$(GT)_{25}N(GA)_7N(GAT)_4$	F: CAGCAGGAATTGGGATGAAA R: CAGATGCGGCCAATACAAGA	247-306	55	I	7	0.813	0.830	0.794	0.553	JX503177
SK491	$(GT)_{12}$	F: GCTCTTGCTCCCTTTTACTT R: TAGCCGTGGAGATGGGAATA	245-266	55	P	5	1.000	0.770	0.720	0.000*	JX503176
SK492	$(GT)_6N(AC)_9$	F: GTGCCAACCGCTAAAAACAT R: AGCGAGGCACTTACACAATC	192-252	55	I	4	0.125	0.675	0.607	0.064	JX503175
SK494	$(TG)_{16}$	F: TGATCTCGTGGTGATGTTTC R: GAGAGGGGTGAGAAGAGTTA	300-340	55	P	5	0.500	0.720	0.658	0.058	JX503173
SK498	$(GT)_{17}$	F: CTTCTCCTTCGACCCACAAC R: GTTGGAGGGGATCTATATGG	228-298	55	P	6	0.750	0.796	0.750	0.052	JX503169
SK509	$(CA)_{15}$	F: AGCACGAAGATAGACCTGTC R: AGTTTGGTTCAGCTCAGCTC	262-323	55	P	6	1.000	0.715	0.662	0.000*	JX503158
SK516	$(GT)_{20}$	F: TTTATTAAGTCTTGTGTTAGC R: ATGTGGCTTCGTTTCTCAGA	260-344	55	P	4	0.844	0.668	0.602	0.098	JX503151
SK519	$(TG)_{20}$	F: TACAGCAGGCAATCAATG R: GGGTGTGCTGTCAGTCAA	245-314	60	P	8	0.969	0.683	0.616	0.022	JX503372
SK524	$(TC)_6N(TC)_9N(TG)_6$	F: GCTTTCATCACCGCTTCT R: GACGCCATTATTGATGCT	213-306	55	I	5	0.500	0.692	0.623	0.107	JX503367
SK530	$(AC)_{12}$	F: CTGAAGACAAAGACCCGCTA R: CCTTGTGACAGTGTTTCAGTTC	159-246	58	P	8	0.469	0.759	0.722	0.349	JX503361
SK532	$(TG)_{12}$	F: CAACACGGAGAGGAAGGT R: ATCATCGACTATCTGGAGCAC	187-238	55	P	8	1.000	0.842	0.807	0.004	JX503359
SK533	$(TG)_{17}$	F: GCTGGTCTGGCAGGATACA R: GATCCAGGTCACTGACTGTTTG	240-309	60	P	4	0.813	0.743	0.683	0.056	JX503358
SK534	$(TG)_6$	F: TGAATGTACTGCCTTGTCTG R: GCGTGGTAGAGTAAGGTGAA	213-254	58	P	6	0.781	0.809	0.767	0.615	JX503357
SK538	$(CA)_{11}$	F: AGACCTGGGGAAGAATAAGT R: GCGATTACAGCACTATCATC	197-240	58	P	6	1.000	0.741	0.687	0.324	JX503353
SK541	$(GT)_7N(AC)_{10}N(AC)_6$	F: AGCCGAACTACATCAACAA R: TCTTCCAACCTCAGAGATAAC	285-314	55	I	3	0.000	0.514	0.450	0.163	JX503350
SK543	$(CTC)_6$	F: TGCCTGTAGTTGCTGTTGCT R: CGGTGTGAAAACTGAAGGT	282-310	58	P	3	0.313	0.518	0.457	0.381	JX503348
SK544	$(CA)_{15}$	F: TGACGAGGAAGACAGAGACG R: GCAGCAAAGTGGATTGTAGC	170-233	60	P	7	1.000	0.826	0.787	0.003	JX503347
SK546	$(AAT)_6N(AAAT)_3$	F: CTGAGGCTGAGCTGGATT R: GAAGGTGTTGTACCAGATGTG	206-258	58	I	4	0.594	0.515	0.468	0.575	JX503345
SK559	$(GT)_9$	F: GTTCGTTCTTCCCTGATGCT R: AGTTGCTGCCAATCAAACCA	200-236	58	P	4	0.469	0.709	0.640	0.236	JX503332
SK560	$(GT)_{15}$	F: GTAATACTGTTGCACTTCGT R: GTAGGCATCAAGTGAAGC	270-320	55	P	3	0.375	0.372	0.309	0.546	JX503331
SK565	$(GT)_{12}$	F: TAGACGAGGGTATATGTGGA R: GAGGGAAATGATGGACTACTAC	171-226	58	P	3	0.031	0.372	0.309	0.381	JX503326
SK567	$(TA)_8N(CTT)_4$	F: AGCACCCACCTCATTTCAGT R: AGGATTTGCTGTGTTCACATAG	282-305	58	I	3	0.938	0.600	0.503	0.000*	JX503324
SK569	$(TC)_{14}$	F: TCTCCTCTTCTTCGTCGTCC R: CGAGATTAGCGGTGAATTGA	262-303	60	P	5	1.000	0.782	0.732	0.133	JX503322
SK574	$(AC)_8N(AC)_7N(AGG)_4N(GCAC)_3$	F: CAGCAAGATCCGTAACGC R: GTCGCTACACCTACCTGGAG	300-345	60	I	6	1.000	0.806	0.001	0.761	JX503317

Continued on next page

Table 1. Continued.

Locus name	Repeat motif	Primer sequence (5'-3')	Size range (bp)	Ta (°C)	Locus type	N_A	H_O	H_E	PIC	P_{HWE}	GenBank accession No.
Transcriptome-derived SSR											
SK578	$(TG)_{23}$	F: AGTCTCTGGGCGAAGTGT R: GGATCTGCTAACCTGTAACTGTC	196-250	58	P	6	0.969	0.835	0.797	0.017	JX503313
SK580	$(ACTA)_5$	F: TCATCAGCAGTGTTGGTAAT R: GCCATTGTATAAGAAGAACACAG	318-394	55	P	6	0.781	0.819	0.778	0.619	JX503311
SK592	$(TG)_{12}$	F: AGCAACCCAATGTTACTCTT R: AACAAAGCCATTAGATCGTC	191-270	55	P	5	0.250	0.801	0.755	0.035	JX503299
SK603	$(GT)_{13}$	F: CCACTTGGTCAATGAAATGT R: GCCCTGTGTCATAACTCAATC	187-240	58	P	7	0.875	0.807	0.765	0.840	JX503288
SK607	$(ACA)_5$	F: CAAGAACCCACCGAGAAAC R: TGCCACCTTTAGATTTCAGC	195-231	58	P	3	0.125	0.230	0.215	0.821	JX503284
SK608	$(GT)_{34}$	F: TGGGTAGGCTTCATGTGGTA R: CACTCCACTGAATTGAATGTAGG	125-205	58	P	5	0.469	0.753	0.698	0.076	JX503283
SK609	$(GAG)_5$	F: GCATCAGAAGGTGAAGAGA R: AACCTCCTCAATGTTTGTC	183-294	55	P	6	0.938	0.765	0.711	0.248	JX503282
SK613	$(AC)_{12}$	F: ACTGCCTTGTCAATAGCGGT R: GGTGATGATGGAGAGAAGTGTAG	146-211	60	P	8	0.969	0.857	0.824	0.041	JX503278
SK616	$(GAG)_5$	F: GAAGGAGGAGGCGTGTCAT R: GCCAACAACATCGTCAGAGA	189-205	60	P	3	0.000	0.564	0.456	0.032	JX503275
SK624	$(AT)_{10}N$ $(TGTT)_3$	F: AGCTCAGTTTCACCTGTCAC R: GCTTGCGGTATAATCCAGTC	172-205	60	I	2	0.500	0.381	0.305	0.081	JX503267
Mean						5.1	0.666	0.692	0.638		
Genomic SSR											
FC0572	$(TG)_{14}N$ $(TG)_7$	F: CTGTTGGGAGGATTTCAGTA R: AACATACCTTCATAACGGTC	129-160	55	I	5	0.469	0.521	0.597	0.000*	JX449064
FC0580	$(GT)_{24}$	F: CTCGTCAGGAAACGGTAAA R: ATTTGAATGTATGAATGAAT	140-154	55	P	2	0.000	0.347	0.674	0.000*	JX449065
FC0661	$(GT)_{21}$	F: AGCCTTGTGTTTATCAGACC R: TAATCCAAACATGCTCACAA	156-199	55	I	4	0.375	0.721	0.248	0.000*	JX449071
FC070	$(CTC)_6N$ $(TCC)_4$	F: ATCTGACACGATAAACCCTC R: GCTGATGCTGAGGAGGAAAT	210-246	58	I	4	0.281	0.511	0.631	0.012	JX449076
FC076	$(GGA)_9$	F: TACCCCAGTCGTGTCCCTT R: CTTTCCTTATTTATTGACTTC	142-217	55	P	3	0.250	0.587	0.481	0.008	JX449082
FC077	$(CCT)_{10}$	F: CAAGACCGACTGAATCCTGA R: ATCCGAACAGACTTTCCATT	203-273	58	P	5	0.406	0.399	0.283	0.021	JX449083
FC0791	$(TG)_{22}$	F: GGTATCCATCCAGGTCTAAT R: CTCCTCTGAGCCTGTTCTCC	176-211	60	P	5	0.406	0.487	0.661	0.188	JX449085
FC0820	$(TG)_{29}N$ $(TG)_6$	F: CACCACCAGGCTACCTCAGT R: CACTGGGGAGATACACTACT	234-291	60	I	5	1.000	0.748	0.462	0.578	JX449087
FC084	$(GAG)_8$	F: TTTGTGCTCCTCTGCTTGTC R: TTTCAGGGTCAAGAGGTCAG	210-224	60	P	4	0.188	0.701	0.501	0.167	JX449088
FC086	$(CTC)_7$	F: GGAAGAAGACCCACAACATC R: GGACCAACGCAACCCAGCAT	109-216	62	P	4	0.531	0.539	0.369	0.722	JX449090
FC093	$(TG)_{14}N(CTC)_6N$ $(TGTGA)_3$	F: GCCGTGATGTATCCACTCTG R: CGTCCACACACCCATCACAT	190-207	62	I	2	0.000	0.347	0.452	0.013	JX449095
FC094	$(GT)_{16}$	F: TCCCGCATAGAGGAGTCTGT R: AACTCAACGCAAGCAGGC	126-153	58	P	3	0.031	0.201	0.690	0.010	JX449096
FC095	$(CTC)_{10}$	F: TCTGACTACAGTTCAACAGG R: ATCCCAAGAAATATGGAGGC	149-265	58	P	3	0.063	0.228	0.635	0.257	JX449097
FC096	$(TCC)_6$	F: CACGCCTGTTTATCTCTTTG R: CTCAAGAGTCCTACCATCCA	162-224	58	P	5	1.000	0.799	0.481	0.379	JX449098
FC102	$(TCC)_9$	F: GTTTGTGTCGTATATGACGG R: CTCCTCCTTGGTCTTGAGAT	121-189	58	P	4	0.563	0.653	0.283	0.245	JX449104
FC104	$(GGA)_5N$ $(GAG)_5N$ $(AGC)_5N$ $(AGA)_5$	F: CCAGTTCAGGAGGTGGCG R: TGCAGAAGAGCTATGTAAGG	182-238	58	I	5	0.656	0.729	0.183	0.321	JX449106
FC105	$(CTC)_5N$ $(CCT)_4$	F: CAGTTCAACAGGACTATGGG R: GGAGGCGTTGAAGGAATAAT	141-225	58	I	3	0.063	0.276	0.210	0.000*	JX449107
FC108	$(TCC)_7$	F: TAGTGGCAATCAGGATGAAA R: CGTCTTTTTAGATTCCTCGC	207-288	55	P	4	0.563	0.696	0.753	0.140	JX449109
Mean						3.8	0.380	0.527	0.477		

P = pure; I = interrupted. *Show significant deviation from HWE after Bonferroni's correction (P < 0.0009).

Data analysis

The number of alleles (N_A), observed heterozygosities (H_O), and expected heterozygosities (H_E) were computed by the POPGENE software (Version 3.2) (Yeh and Boyle, 1997). The polymorphic information content (PIC) was calculated by the formula:

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

where *qi* and *qj* is the i^{th} and j^{th} allele frequency, respectively, while *n* is the number of alleles (Botstein et al., 1980). The exact test for genotypic linkage disequilibrium and the exact tests for conformation to Hardy-Weinberg equilibrium (HWE) at each locus were performed using the GENEPOP version 1.2 program (Raymond and Rousset, 1995). The SSR markers were classified by Weber's rules (1990). Null alleles among loci were detected by the Micro-Checker V.2.2.3 software (Van Oosterhout et al., 2004). All results were adjusted for multiple simultaneous comparisons using sequential Bonferroni's correction (Holm, 1979).

RESULTS

A total of 356 unique candidate sequences containing SSR motifs were selected from the transcriptome of the F_1 interspecies hybrids between *S. chuatsi* (♀) and *S. scherzeri* (♂). One hundred and seventy-two SSR-containing sequences (GenBank accession Nos. JX503150-JX503199 and JX503252-JX503373) flanked by suitable priming sites were selected for conversion into SSR markers. From the SSR-enriched gnomic libraries, 576 positive clones were selected and sequenced. Among these clones, 220 sequences were found to contain SSR motifs with 6 or more repeat nucleotide. We designed 60 primer pairs from the sequences with sufficient flanking region (GenBank accession Nos. JX449062-JX449139). One hundred and thirty-six transcriptome primer pairs and 49 genomic primer pairs produced clear amplified products by SSR-PCR amplification. These primers were further examined for polymorphism with *S. kneri* collected from 4 populations across China: Guangdong, Guangxi, Hubei, and Hunan (8 individuals from each population). Finally, 55 SSR markers (37 from transcriptome and 18 from genomic) displayed polymorphisms, while the other markers displayed monomorphisms.

The characteristics of the polymorphic and monomorphic SSR markers are shown in Tables 1 and 2, respectively. The average N_A per locus was 5.1 (range: 2-8) for transcriptome-derived SSRs and 3.8 (range: 2-5) for genomic SSRs. The average H_O and H_E were 0.666 (range: 0.000-1.000) and 0.692 (range: 0.230-0.857) for transcriptome-derived SSRs, respectively. These values were 0.380 (range: 0.000-1.000) and 0.527 (range: 0.201-0.799) for genomic SSRs, respectively. The PIC was 0.638 (range: 0.215-0.824) for transcriptome-derived SSRs and 0.477 (range: 0.183-0.752) for genomic SSRs. There were 72% of di-nucleotide repeats, 16% of tri-nucleotide and 8% of tetra-nuleotide among EST-SSR markers, and 63% of tri-nucleotide, 24% of di-nucleotide and 13% of penta-nucleotide among genomic SSR markers. Seven of these loci exhibited departure from HWE after sequential Bonferroni's correction for multiple tests, and no significant deviation was observed for the linkage disequilibrium. Null alleles were not detected among any of the loci in the analysis of the allelic inheritance mode. The results revealed no significant instances of linkage disequilibrium following the Bonferroni correction, indicating the independent behavior of all loci.

Table 2. Characterization of all microsatellite markers (polymorphic markers excluded) including locus name, Genbank accession number, repeat motif, annealing temperature (Ta), locus type, and primer sequences.

Locus name	GenBank accession No.	Repeat motif	Ta (°C)	Locus type	Primer sequence (5'-3')
Transcriptome-derived SSR					
SK468	JX503199	$(CA)_{11}$	55	P	F:AGCAGAGACAGCAGGGAATG R:ATACCGCTGCAACCCTAGTG
SK469	JX503198	$(AGG)_{7}$	53.5	P	F:GAGCATCCAGGAAGTAGACT R:ACCGAGTATCTCACCCATCT
SK470	JX503197	$(TCT)_{5}$	56	P	F:AGCAGCAGGAGTCGGAGA R:TCCTCTTCGTCCACTGATGA
SK471	JX503196	$(AAT)_{11}$	53.5	P	F:GGCGACGATAGGTGAATAAG R:CGAGCCTGTTTACTGCATAG
SK474	JX503193	$(GGA)_{6}$	53.5	P	F:CAAGAAACAGGCTGTGGT R:GGCTTCCTCATTGTATCAGA
SK475	JX503192	$(GT)_{15}(GC)_{9}$	56	C	F:AGAATCTACACACAGCAGCACA R:CGTGGAGAGCGTCTTTTCTA
SK476	JX503191	$(TCC)_{6}$	57.5	P	F:TCCCTCACAGTTGGTGTC R:GTCTGCACTACCTGGAGTC
SK477	JX503190	$(TCA)_{4}N(TCA)_{9}$	53.5	I	F:CAGAAATGGTGTGGATGCT R:TAGGCGGAACAGAGGTAATA
SK478	JX503189	$(ATG)_{9}$	53.5	P	F:AACGGACGGAAAGACAGA R:TCCAACAGGTGTAAACAGTAGA
SK479	JX503188	$(TG)_{6}N(AC)_{8}$	53.5	I	F:TACTGTTTACACCTGTTGGA R:TGAATCTCTCAGAATTGCTG
SK480	JX503187	$(GGA)_{5}$	53.5	P	F:TTCACCCAGCAATAATAGAG R:CCTCGGTTTATGTGGTAGTA
SK481	JX503186	$(GGT)_{5}$	53.5	P	F:TGAAGGCAATCTGAGGCAAC R:CCAGACGGAAGAGGAAGTGA
SK482	JX503185	$(TTG)_{11}$	53.5	P	F:CACATTTGACATGACAAGAC R:ATCCTTGGACAGCATTATAC
SK484	JX503183	$(GA)10N(TG)_{11}$	53.5	I	F:AAACAGCAGCCCACAGGAAG R:TGCCTGGAACATCACCCTGG
SK485	JX503182	$(TGTT)_{7}$	53.5	P	F:AGATAGGAGGGCAGTAAAGA R:GAATGACCTACCAAGAATGT
SK486	JX503181	$(ATT)_{5}$	55.5	P	F:CATTTCTTCCCGATGTTAGA R:CGGCAACTATTCTCATAACC
SK487	JX503180	$(TCC)_{5}N(TCC)_{5}$	55.5	I	F:TCCTCCTTTTTCACATCGG R:GAAATCTGTCAGGAGCCGTT
SK488	JX503179	$(CT)_{6}(TCAC)_{3}N(CCT)_{7}$	55.5	I	F:CCCTCTCCCGGACTGACA R:CAGAGTTTCATCCTCTCAGC
SK489	JX503178	$(AC)_{7}N(AC)_{11}$	55.5	I	F:TACTGACTGCTTAACTGTGC R:TTCCCACCAACCTCTCGCAT
SK493	JX503174	$(CTT)_{7}$	55.5	P	F:TCCACACACGAACATCACAA R:CGTCTGTCTCTCCTCATCTT
SK495	JX503172	$(TG)_{10}N(AG)_{6}$	53.5	I	F:AATCAGTAGCCACAGCGTGT R:TTTGAGATTATGGGGTGCGA
SK496	JX503171	$(GAG)_{6}$	55	P	F:GACAGGTCCCTGGTCTCAAC R:ATGGTGAAGTCAGGAGACGC
SK497	JX503170	$(CT)_{7}(CA)_{7}N(CA)_{10}$	53.5	I	F:GTGTGTAAGGCCCTACTCTC R:TTGCTCTCACTCACTCTGCT
SK499	JX503168	$(GAT)_{5}N(GAC)_{4}$	53.5	I	F:GATAAGGTGAGGCAAAACAT R:GCATCAACCTCGTCCTTACC
SK500	JX503167	$(GTT)_{6}$	55.5	P	F:ATGACGGCCACTGTTCCAAT R:GCCGACCAACCACATCTTCT
SK501	JX503166	$(GT)_{9}N(GT)_{10}$	55.5	I	F:CGAAAGATGGGAGGAGGAA R:GCATGGCTTTGATTTGACC
SK502	JX503165	$(AAG)_{5}$	55.5	P	F:TACTGCCAGGAAGGTGTTA R:CTTTGTGGTGACAGGAGTC
SK503	JX503164	$(AG)_{12}$	53.5	P	F:CCATTGTGCGAGAGATGTGC R:CTGGGTGCTGTAGGCAGTAG
SK504	JX503163	$(AATAG)_{3}$	55	P	F:CACAGGCTAATGGATAGATA R:GATTCAGCAAATGCCTTCAG
SK505	JX503162	$(TG)_{20}$	53.5	P	F:AAAGGGTTAGGGTTAGAGTT R:CCTCATCTCTGCCTCATACT

Continued on next page

Table 2. Continued.

Locus name	GenBank accession No.	Repeat motif	Ta (°C)	Locus type	Primer sequence (5'-3')
Transcriptome-derived SSR					
SK506	JX503161	$(TGG)_6$	54	P	F:CAGCGTGTGTTGTTCCGTAG R:GAACCTCTGCCTACTCCTGC
SK507	JX503160	$(AGG)_5$	54	P	F:CCTCTGGAAAAGGCAAAGCA R:AGCCGTCTTCTGTTTACTTC
SK508	JX503159	$(TTC)_5$	55	P	F:TCGGCGATGCTGAGAAACCA R:AACAGGAAGTGACAGAGGAG
SK510	JX503157	$(CAG)_5$	55.5	P	F:GAGAACTGGGAGGAGAGATG R:TCACAGACACCTCCAGGGAT
SK511	JX503156	$(CA)_{26}$	55	P	F:GAAGCCCAGCAAAGAGAACA R:CACCTACATCTACATTTTGA
SK512	JX503155	$(TCC)_{14}$	54.5	P	F:CTGTGAGGCTTCAAACCGTC R:ACTCACAATGCTACGACAAG
SK513	JX503154	$(CTG)_4$	54	P	F:TCCATAGGGTTCGTAGCGTC R:TTTGAAGGTGCTTGACTCGT
SK514	JX503153	$(ATTT)_5$	55.5	P	F:TCAGACAGAAGCTCAGCAAT R:GGAAGTGACAAACCAAATC
SK515	JX503152	$(CAG)_6N(CAG)_4$	58	I	F:GCTGCTCTGGTCCAACAACA R:CGCCTGTCGTTCTCTCTCCT
SK517	JX503150	$(GAG)_5$	53.5	P	F:TCGTGTGGAGATGTCAACAG R:ACGGCTCCTGCTGTGGCTA
SK518	JX503373	$(TG)_{11}$	60	P	F:AAGAAGACGCAAGTTGGGAG R:ACCCTGCCATTAGCCATTAG
SK520	JX503371	$(CT)_{12}$	58	P	F:AACAATGACTCAATCCTTCCC R:ACACGTCAGAGTCAGGCAG
SK521	JX503370	$(AC)_{24}$	58	P	F:CTGCCAACACTAACCTCTGA R:GCAAAGCCAGTACAGCCA
SK522	JX503369	$(CTC)_5$	58	P	F:TCCACCTCACCGATATAAGT R:AGAGTATGTGTGGAGGTGAA
SK523	JX503368	$(GAG)_5$	58	P	F:TCACAGTGAGGAGGTGCT R:TATTCCTGCTGACACTGC
SK525	JX503366	$(AC)_{12}$	55	P	F:CACTGCATTGTAACTTCTTG R:ATGGACTATTGATGATGTACTG
SK526	JX503365	$(GT)_7$	55	P	F:GACCATTCCTCCAGTCAT R:TGCACCCTTGCTACTCTA
SK527	JX503364	$(GTT)_5N(TGT)_4$	55	I	F:GTACGACTCCTGCTGTCCT R:TACCCACAACAACAACAGA
SK528	JX503363	$(GCT)_7N(TGT)_3$	55	I	F:TTGGCAGGCATCATAGGG R:GTCGGGGAGCAGTTTCTACC
SK529	JX503362	$(CCT)_5$	58	P	F:CTACCCTCCCTCTCATCACC R:TCTGCCAGATTCAGTAATGC
SK531	JX503360	$(AAAC)_5$	60	P	F:CCTCCAGCCCTGTACTTCTA R:CTTTTGGACTCTGGACTCTG
SK535	JX503356	$(TC)_6N(CA)_7$	58	I	F:CTCCACATAGCACCTTCAAA R:GCATGACACACAAGGTTACG
SK536	JX503355	$(GT)_7$	58	P	F:CAGAGGGAACCCATTCTACT R:AAACTCCCCAGAGCAGACAC
SK537	JX503354	$(CTG)_4N(CTG)_5N(CTC)_6$	55	I	F:CCTCTTTGTTTCCTCCTCACG R:GAAGAGAGGAAGCGGTTAGAA
SK539	JX503352	$(TCC)_4$	58	P	F:AGGCATCCAGATGACGAA R:AATGTCAGACACCAAGCAG
SK540	JX503351	$(AC)_7$	58	P	F:TCACTTGGTGTTGATGAGGA R:ATCTTACTGAAGCCGATGAG
SK542	JX503349	$(AC)_{11}(CA)_6$	58	C	F:AACACACTGGTCGTTAATGC R:GAGGACATGACTCAGGTGTAC
SK545	JX503346	$(GAG)_5$	60	P	F:CCTACAGCAAGTTCCAACAC R:GACTTGACCTTGCCACATT
SK547	JX503344	$(GCA)_8$	60	P	F:CAGTGACTCTGATGTGCCC R:GCCACACAGAGTGAAGAGTT
SK548	JX503343	$(GTCT)_5$	62	P	F:TCATGCCGTAACAGAAGTG R:TATGGGGAGAGAGCTGACA
SK549	JX503342	$(CA)_7$	55	P	F:ATCACTTGTGGTCCACTTAT R:CTCGTCAATGGAAGACTAGA

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Table 2. Continued.

Locus name	GenBank accession No.	Repeat motif	Ta (°C)	Locus type	Primer sequence (5'-3')
Transcriptome-derived SSR					
SK550	JX503341	$(GAG)_5$	60	P	F:TTAGATGACGAGGACTTGGA R:CACAGGTTGGTCTCTCGTTC
SK551	JX503340	$(GGA)_5$	55	P	F:TCAGACCAAACGAACAGAGA R:CCTGCTTCATCCTTGTAATC
SK552	JX503339	$(CTC)_8$	55	P	F:TTACACTGACCGTGAAGAACC R:GGGAAAACCTGTCTGATGAG
SK553	JX503338	$(CA)_{16}(TC)_8$	60	C	F:CAGATGTTGTCGTCGTAGTTGA R:AGAGAGACGGGCAGGAGGA
SK554	JX503337	$(TG)_8N(TA)_6$	55	I	F:TCTGTTTGATGAATGTGCTC R:CGACGAATCTGAAATCTGAA
SK555	JX503336	$(TCC)_5$	55	P	F:CTGTCAGTCATTTTCCACCA R:GGAAACAGGGAGGTAAACAT
SK556	JX503335	$(ACA)_5$	60	P	F:CATCTCCTCCACCTGCCTC R:CGTGTCCTGTATCTTGCTGA
SK557	JX503334	$(TTG)_4N(TTG)_5N(TGTTGC)_3$	58	I	F:GGTGGGATTGATGACTGAG R:CGCTCGTCTTCACATTGTTA
SK558	JX503333	$(CTC)_5$	60	P	F:GAGAAGATGTGCTAGGGCTG R:CAACTGTCCTAATGGCTGAG
SK561	JX503330	$(CTT)_7N(TTC)_6$	58	I	F:CCAAAGGAAGGGTCAACTCT R:TGGGAAATGAAAGTGAGTTGGT
SK562	JX503329	$(CCT)_6$	60	P	F:GCTCATCACTGTCTCAGTCCAA R:CTCTGCTGCGATAGGCTGAC
SK563	JX503328	$(CCT)_7$	60	P	F:CTGCTGCTGCTCGTAATGG R:GCAGAGCATGAACGAGTACC
SK564	JX503327	$(TC)_6N(TC)_{13}$	58	I	F:GATTATCTGGTGGAGTGGTG R:CAGGGTGTTGATATTGTGAT
SK566	JX503325	$(TGG)_5$	58	P	F:GGAGCGGTACGAGTCAAT R:GCTCATTCCTCTGGTTTCAC
SK568	JX503323	$(AGCT)_5$	60	P	F:TGTAAGTGTTCACGCAAAGG R:CTCCCAGGATGATGACTTTC
SK570	JX503321	$(GAG)_6$	60	P	F:ATCCAATATCTCAGCCCACT R:TCTGTCAAGAAACCTACGAAAC
SK571	JX503320	$(TC)_6$	54	P	F:ACTGAGACACAGAGGAGGCT R:GTGGTATGATGATTACGACG
SK572	JX503319	$(GAG)_5N(AGG)_{10}N(GAG)_5$	56	I	F:AAGGCGGCACAGATAGACT R:TCTTCCTCTTCACTGGCTTC
SK573	JX503318	$(TG)_{11}N(TCA)_6$	58	I	F:CTGAGTAAACCTCTGAATTGG R:GCTTGTTGTTGAGACAGAGT
SK575	JX503316	$(GCA)_5$	55	P	F:TACCAACCATTCGGATTCTA R:CAGACAGGGCTTACGCTAGT
SK576	JX503315	$(GT)_6N(GT)_6$	60	I	F:CTCTCAGTGTGCTGCTTACC R:TGGAAACCAGTGTGAGGAAT
SK577	JX503314	$(ACC)_5$	58	P	F:CAGATGGTGGGAACAACATT R:AGAGCCCTGTGCCTGTTAAT
SK579	JX503312	$(AAT)_6$	58	P	F:AGAGGGCGAGGAATACTGTA R:GTCATTCTTGAGTGTAGTGAGTG
SK581	JX503310	$(GCT)_6$	52	P	F:GCCTACAGTGTGAGAAGCC R:TGGGAAGGTTAAGGTGGA
SK582	JX503309	$(GGA)_5N(GAG)_4$	60	I	F:GAGGAAGGCTCTGGAAAAAC R:CCACATCACCGTCTTCATCT
SK583	JX503308	$(CA)_6$	58	P	F:ACTCGTTACCAGGATGAGAC R:GGGTTTGACATAGGTGTTAGTG
SK584	JX503307	$(GT)_{11}$	58	P	F:ACTGTACTCCTCCTCTGCTGT R:GGAAAGGAGCTGAGGAAGTG
SK585	JX503306	$(CTT)_5$	55	P	F:ATCGTCCAGGTCCTCAGCA R:CAGAACAGCCAAAAGAGGTG
SK586	JX503305	$(AAG)_5$	58	P	F:ATGCCAATGGTTCTGATGTC R:GGCAGTTTATCCTTTCCAGC
SK587	JX503304	$(TGA)_5$	58	P	F:AACTGGACGGGACAGGTG R:GGAGTGAGTGGATGGTCTTTG
SK588	JX503303	$(CTAG)_6$	58	P	F:CTGCCAGACGATGAAGCC R:AAACTACGCTCGACAACACG

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Table 2. Continued.

Locus name	GenBank accession No.	Repeat motif	Ta (°C)	Locus type	Primer sequence (5'-3')
Transcriptome-derived SSR					
SK589	JX503302	$(ATT)_6$	55	P	F:GGTTGTTGTATATTGTTCTGC R:CCTGGAGTAGTATTCACAAAG
SK590	JX503301	$(TC)_7$	55	P	F:CGAGGGAACGTGGGTGTAA R:ATTCCGCTCTCGTCCTATCA
SK591	JX503300	$(AC)_{19}$	55	P	F:AATCAGAAGGACAGAAAGCA R:GATTCAGTCGAGTTCATTCA
SK593	JX503298	$(GCT)_5N(TGT)_7$	58	I	F:AGTCCGTCAGCTCCTCTTCA R:CACCATCATCAAGTTCTTCCA
SK594	JX503297	$(GGA)_5$	58	P	F:AGGGTGAGAGGTAGAGACAT R:CCTTCAGTGTCAGATTCAGA
SK595	JX503296	$(TC)_{16}N(CCT)_4$	58	I	F:TCACTTGCCTCTGGTGGTAT R:GCACACAACGGAGGTGAAT
SK596	JX503295	$(AGG)_4N(GGA)_8$	58	I	F:AGGGCTGGGAGTCAAGAGT R:TGATGTCGAAGAGAATGAAGG
SK597	JX503294	$(GT)_6N(GT)_6$	58	I	F:TCCAGATTACTAGAGGCAAA R:TTGTGCTCACAGACATCACT
SK598	JX503293	$(GAG)_6(GTG)_4N(GTT)_5$	55	I	F:TTGAGAGGCAGGACAGTA R:AGTGCCAAAATAGAACAGAG
SK599	JX503292	$(TGC)_7N(TTG)_6N(TGC)_4$	55	I	F:TGTGGCTGCTGGAACTGA R:ACAGATGGCAAATATCAATCCC
SK600	JX503291	$(CA)_{19}$	58	P	F:TTGGACGGTAAGTGTAATCTC R:TGCTCAAGTTATGTGTCGTG
SK601	JX503290	$(CCT)_8$	58	P	F:GCAGGGTTTTAATCCGACAAT R:CGGAGGCTCGATGAGGAA
SK602	JX503289	$(AAG)_7(GAG)_4$	58	C	F:CGAACCAAACGCATTAGG R:GGGCAGGTAAGTTCTAGCA
SK604	JX503287	$(CAT)_{11}$	55	P	F:CACTACTGTTGCTTGGTTATAC R:TCCTCTGAGTGAAAACTGAT
SK605	JX503286	$(GT)_{31}$	58	P	F:TTGACAGTCAGATAGACAGCTC R:GGATGTCTTAACACGTCCAT
SK606	JX503285	$(CA)_6N(AC)_7$	55	I	F:GCCACTAGACTGTCAGCATC R:TGATATTCCTGTTCAGACACTC
SK610	JX503281	$(TG)_6N(TTC)_6$	58	I	F:TCTCATCATCACTGCTGCC R:CCAGAACAGCACCTGTCAC
SK611	JX503280	$(TGA)_7$	55	P	F:GTGGACACGACAAAACGA R:AAGCAACACCGTACAACAGT
SK612	JX503279	$(GAG)_7N(GAG)_4$	58	I	F:TGAAGTGTCTGAAGGAGTATGTC R:CGTGATCTCCCTGGGTGT
SK614	JX503277	$(AAG)_5$	55	P	F:GAGCAGCAAACACTGGAGG R:GTCTTTCGATGGTGGATATTCA
SK615	JX503276	$(CAC)_6$	60	P	F:CTGCTCCTCTACATGCCAAT R:CTCATCTCTGCCCTCTAGTG
SK617	JX503274	$(GTG)_5$	58	P	F:GATCTGCTGAGGTGACTCTT R:ATCAGACAGAGCAACAGAGA
SK618	JX503273	$(AC)_{15}$	55	P	F:ATACCGATTTGGAGAAAGC R:GTCGCCATTCTTTACTCTGT
SK619	JX503272	$(GTG)_4N(GGT)_6$	60	I	F:GGTAGTGGTCAGGTTTCAGG R:CTCGGTTACCACCAGCAG
SK620	JX503271	$(ATC)_5$	58	P	F:CCTGCTGGTGGAAGAAGT R:AAACCTCCCACAGACCTAGT
SK621	JX503270	$(CA)_{21}$	55	P	F:TATAGGCAAACAGAGACACA R:GCTGGGTAATTTCTGCAT
SK622	JX503269	$(TG)_{16}$	58	P	F:TGATTCACTGATGCTTTCTC R:GGTGACAAATACTGGTACGG
SK623	JX503268	$(TGTA)_5N(CTGTA)_3$	55	I	F:CAGATCACATTTCCACTACAC R:GGGTAGATAAAGGAGCACAG
SK625	JX503266	$(CA)_6N(CCA)_5$	58	I	F:AAGTCATCACTCTGCTCATC R:ACTCTGGACTCCACCTTCT
SK626	JX503265	$(AC)_{11}$	60	P	F:CCTATTTCCTTCCCTCACTT R:CACTCGTGACTCAGCTCAGA
SK627	JX503264	$(TG)_{21}$	55	P	F:AATGCTTCAATGTGTGCTCA R:CAGAGGAGGCACTGTCACTA

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Table 2. Continued.

Locus name	GenBank accession No.	Repeat motif	Ta (°C)	Locus type	Primer sequence (5'-3')
Transcriptome-derived SSR					
SK628	JX503263	$(CT)_8N(TC)_{15}$	55	I	F:GCTGCATACTCTACGTCTCC R:CTGCCAAATCTAATCACACT
SK629	JX503262	$(AGAC)_3$	55	P	F:TCCCTGACGGTGTGTGGT R:GGTTCGATACGACAGGAAGAC
SK630	JX503261	$(AC)_{15}$	55	P	F:ATAGGCTGAGACATCCGT R:ATGGACCTCTTTAGAAGTACA
SK631	JX503260	$(AC)_6N(AC)_{23}$	58	I	F:AGACGAGCACTTTGGACCAC R:TCCGAAGCAGTCATTTTACAG
SK632	JX503259	$(GAT)_8$	58	P	F:CAGAGCAAGAGGCACGTACA R:GCTCGTTCCTGCTTCTCCTT
SK633	JX503258	$(TGG)_5$	55	P	F:TCAAGTAGAGAGTCCCAAGA R:GAAGTGCCACTCTGGTCT
SK634	JX503257	$(TCC)_6$	60	P	F:TGCCACCTCGCTCTTGTCCA R:AGGGAGGCGGAGGGAGCATA
SK635	JX503256	$(GAA)_5$	60	P	F:TGTTTGCCAGAATGGTAATC R:TCTAGTGTGGTTTGTGGATCG
SK636	JX503255	$(GGA)_5$	55	P	F:CAATTTGCATCATGGTGTAG R:AGCAAACAAACACATCTCTC
SK637	JX503254	$(CAG)_5$	55	P	F:CCAGATAAGGTGAACCAGA R:GGCAAATAAGAAGTCACTCC
SK638	JX503253	$(AG)_9$	55	P	F:TCTCCAGCATTGAGTCAGAC R:GACTTCACCACAGCTTAGCC
SK639	JX503252	$(GTG)_5$	60	P	F:GAGGAAGGGTAGCGAGTGTA R:GAAACTGTCAGCCTCAGAAC
Genomic SSR					
FC055	JX449062	$(TG)_8(GT)_{14}(GT)_5N(TG)_{14}$	55	I	F:ATCAATGTGTTTTGCCTGAA R:AGAGACTGTGATTGGATTTG
FC056	JX449063	$(CCT)_{10}$	55	P	F:ACACACAAGACCGACTGAAT R:AACAGACTTTCCATTCAGGT
FC057	JX449064	$(GA)_7N(GT)_{12}$	58	I	F:ACGGGAAGAGAATCAACTAC R:CTGTCTTGTTTTCCATTCCC
FC059	JX449066	$(TCC)_9$	62	P	F:GGAGGATGAGGATGAGGATG R:CGGTTGACCTTCATTCGGAC
FC060	JX449067	$(CCT)_8$	60	P	F:GTTACAAGGAACTGGGGACC R:CTTTGTTTCCAGATGAAGGG
FC061	JX449068	$(AC)_6N(GT)_9N(CA)_7$	55	I	F:TCCAGTGTTTTTGAATGAAG R:ACTCGTGGTTGCCTCTGA
FC062	JX449069	$(CTC)_{10}$	62	P	F:CTGAGTAACGCCTTCGCTGT R:CGTGCCACCTGCTGTCTGT
FC063	JX449071	$(GGA)_7$	60	P	F:TGAGAGGAGTAGGAGGGTGT R:GTCAACTAAGGATAGGCTAC
FC064	JX449072	$(GAG)_6$	60	P	F:AAGGGCTGTGGGGATTGTAG R:GCCACCGACAAATCTGATGA
FC066	JX449073	$(GT)_{10}N(TG)_5N(TG)_5$	58	I	F:CTTCCAGGAGTGCTGACTAA R:TCACCCACTCTCTGTTATGT
FC068	JX449074	$(TG)_7N(TG)_{13}N(TG)_{12}N(TG)_{17}N(GT)_{11}$	62	I	F:CACCCATTCCCGTCTCTCTT R:GTGTTTTCCGCTCCGTCCTT
FC069	JX449075	$(TG)_8N(GT)_{14}N(TG)_{14}N(CTC)_9$	60	I	F:GTGTTGAAGGTGTGGAGGTG R:TGCTCTGATGATGGTCGTTA
FC071	JX449077	$(TG)_9N(TG)_{14}N(TG)_7$	55	I	F:ATCCTGAATAGGGCTGCTAC R:TAAAGAAATGGAGCAAAGTTAT
FC072	JX449078	$(GT)_{24}$	55	P	F:AATAGTAGTGGGGTCTGGGA R:ATCCATTGTATCTCATTGTC
FC073	JX449079	$(GAG)_7N(GAGGAT)_3$	52	I	F:TCATCTAAAAGGCAGTCT R:CTCTGCGATGCCATAAAG
FC074	JX449080	$(TCC)_7$	55	P	F:GCAGAATAGTTTGTATGTCA R:AAGAGTTTCAGGGTTTGAGA
FC075	JX449081	$(CCT)_6$	58	P	F:ACATCAACATTAGAGACCCA R:CTGACTTTCTGCTCCAGGTT
FC078	JX449084	$(TCC)_5N(TCC)_4$	55	I	F:AGTAATGTTGTGGAAGTTTG R:AACCACCTGCCTTAGCAAGT

Continued on next page

Table 2. Continued.

Locus name	GenBank accession No.	Repeat motif	Ta (°C)	Locus type	Primer sequence (5'-3')
Genomic SSR					
FC079	JX449085	$(AGG)_9$	58	P	F:CGACGACCTGGACTTCTACA R:CCAACTGAGTAATAAACAGC
FC081	JX449086	$(GT)_{21}N(GT)_{10}N(TG)_6$	60	I	F:CCCTACATACACACAAACAC R:AGCCTAATCCAGCAGCCACC
FC087	JX449091	$(CTC)_6$	60	P	F:GAAATAATCAGTCCTGGAGT R:GTAGAAGGACAGAGTGCCAG
FC088	JX449092	$(CTC)_6$	60	P	F:AGGAGACTCTGTAGAAGGAC R:GTCTGGACTGCTCTGATGGT
FC089	JX449093	$(TCC)_8$	55	P	F:TGATAATACACTCCCAAATG R:AAGATTCTGTTGGCACAAAG
FC090	JX449094	$(GAG)_6$	55	P	F:AAGACAAAAATGGACAGGAG R:ATTTTGCTGATGGCTTATTG
FC097	JX449099	$(TG)_{22}N(AGG)_9$	58	I	F:CTGAGTTGTAGGAATCTGTG R:ATGATACGAGAAGAGGAAGC
FC098	JX449100	$(CTC)_8$	60	P	F:GCATCTGTGAGCGTATCTA R:TTCAGAGTGTCCCAGAGCGT
FC099	JX449101	$(TCC)_{11}$	58	P	F:CCTCTGCTGCTGCTCTGA R:GAGGAGCAATAGCACAATGT
FC100	JX449102	$(TG)_{29}N(TG)_6$	62	I	F:GCTTCTTCCACACCTCCACC R:ACTCTACTGCTTCCTCCTGC
FC101	JX449103	$(AGG)_7$	58	P	F:CGCTGTGTCTAAATGAGATG R:TGTTGCTATACTGAGGGACG
FC104	JX449109	$(AC)_{15}$	55	P	F:TAGTGGCAATCAGGATGAAA R:CGTCTTTTTAGATTCCTCGC
FC122	JX449118	$(GAG)_4N(GGA)_5$	58	I	F:AGGCTATCTGTGTTTTTCCA R:TTGACTTCTACCCTCCCCGT
FC126	JX449123	$(GAG)_{11}$	58	P	F:TCTTATTCTGAGGAGCCACA R:GGGGCTAAGGAAAGCATTAT
FC127	JX449124	$(GGA)_5N(GAG)_5N(AGC)_5$	58	I	F:TAAAGACTGGAAACAGGGGG R:TTTGACCTGTTACACATCAC
FC127	JX449124	$(ACA)_4N(AAAT)_3$	55	I	F:TGTGATGTGTAACAGGTCAA R:ACAAATGGGGGTTATTAGCG
FC133	JX449126	$(TCC)_7$	62	P	F:GAGTCAGCAGAAGGGAACCA R:GGGACTGGGACTAACACTTC
FC134	JX449127	$(CCT)_{10}$	58	P	F:ATCTGTGGATTAGACGCTCC R:TGATACGAGAAGAGGAAGCA
FC135	JX449128	$(TCC)_7$	58	P	F:CTCTGTCTGGCACAATAACA R:GTCCACATACACTGCTGCTC
FC136	JX449129	$(GAG)_8$	55	P	F:ATTGAGGACTCTTTGGGAAC R:AAATATCAAAAACAT
FC142	JX449134	$(GAG)_4$	58	P	F:ACTCCCTCCTTTTTTTGTGC R:AAGGATGGAAATGACAGTGG
FC143	JX449135	$(TG)_{20}$	60	P	F:CTGTGGGAGGTAGAGAAGGG R:TGGACCTGGACAAAGAACAT
FC148	JX449137	$(GA)_{25}N(GT)_8N(GT)_{35}N(GT)_6$	58	I	F:CCCGCAGGAGGAGAAACAGA R:GAATCTTCTTCACCTCTG
FC150	JX449139	$(CTT)_5$	55	P	F:CCCCAGAGGAGAAGATTC R:GGGCTTGAAGTACATTGT

P = pure; I = interrupted; C = compound.

DISCUSSION AND CONCLUSIONS

These loci departure from HWE might have been caused by the recent dramatic decline in spawning populations, and consequent non-random mating and genetic bottlenecks (Zhang and Zhao, 1999). The genomic SSRs of many aquatic species show generally more polymorphism compared to transcriptome-derived SSRs (Zhan et al., 2009; Li et al., 2010). In contrast, in this study, transcriptome-derived SSRs displayed a greater mean PIC value (0.638) compared to genomic SSRs (0.477) among the individuals sampled. One possible explanation

for this difference is that tri-nucleotide repeats were the most abundant (63%) in genomic SSRs, whereas di-nucleotide repeats were the most abundant (72%) in EST-SSR. SSR markers with di-nucleotide repeats generally had higher polymorphism compared to those with tri-nucleotide repeats (Blair et al., 1999; Celton et al., 2009). Because we only used a small sample size from each population, it was difficult to obtain accurate data about genetic difference or genetic structure among populations. SSR markers have been extensively used to evaluate the genetic diversity and structure of farmed food fish species, such as salmon (Norris et al., 1999), rainbow trout (Thrower et al., 2004), and tilapia (Rutten et al., 2004). However, relevant reports about *Siniperca* species remain limited (Wang et al., 2006; Yang et al., 2010), particularly for *S. kneri*. Therefore, we intend to focus future research on *S. kneri* in these areas.

The 55 loci (37 transcriptome-derived SSRs and 18 genomic SSRs) developed and characterized by this study for *S. kneri* are the first on record. The transcriptome data provide an excellent source for the mining and development of SSR markers. Moreover, the transcriptome-derived SSRs directly reflect the variation in gene transcriptional regions, which are closely associated with phenotypic, physiological, and biochemical indices, as well as with metabolic features (Song et al., 2012). Furthermore, utilization of the SSR markers developed from various sources may be more precise and objective for the construction of genetic linkage maps, QTL analysis of phenotypic traits, high-throughput genotyping of marker-assisted selection, and association genetics.

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