

Short Note

Isolation and Characterization of 46 Novel Polymorphic EST-Simple Sequence Repeats (SSR) Markers in Two Sinipercine Fishes (*Siniperca*) and Cross-Species Amplification

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Abstract: With the development of next generation sequencing technologies, transcriptome level sequence collections are emerging as prominent resources for the discovery of gene-based molecular markers. In this study, we described the isolation and characterization of 46 novel polymorphic microsatellite loci for *Siniperca chuatsi* and *Siniperca scherzeri* from the transcriptome of their F₁ interspecies hybrids. Forty-three of these loci were polymorphic in *S. chuatsi*, and 20 were polymorphic in *S. scherzeri*. In *S. chuatsi*, the number of alleles per locus ranged from 2 to 8, and the observed and expected heterozygosities varied from 0.13 to 1.00 and from 0.33 to 0.85, respectively. In *S. scherzeri*, the number of alleles per locus ranged from 3 to 9, and the observed and expected heterozygosities varied from 0.19 to 1.00 and from 0.28 to 0.88, respectively. We also evaluated the cross-amplification of 46 polymorphic loci in four species of sinipercine fishes: *Siniperca kneri*, *Siniperca undulata*, *Siniperca obscura*, and *Coreoperca whiteheadi*. The interspecies cross-amplification rate was very high, totaling 94% of the 184 locus/taxon combinations tested. These markers will be a valuable resource for population genetic studies in sinipercine fishes.

Keywords: *Siniperca chuatsi*; *Siniperca scherzeri*; EST-SSRs; transcriptome; cross-species amplification

1. Introduction

Mandarin fish (*Siniperca chuatsi*), an economically important species in China, has a relatively high market value, and is wide cultured throughout the country [1,2]. It has a fast growth rate, but is susceptible to diseases. Compared with *S. chuatsi*, Golden mandarin fish (*Siniperca scherzeri*) has a great disease resistance, but grows slowly. Recently, outbreaks of diseases caused by parasites, bacteria and viruses have caused severe economic losses to the aquaculture industry [3]. In addition, because of overfishing, drought and especially water pollution, the wild stock of *S. chuatsi* is declining [4]. Therefore, breeding a disease-resistant and faster growing strain and preserving fish germplasm are becoming urgent aims in China.

Microsatellites or simple sequence repeats (SSRs) have become a useful tool to assess genetic diversity and develop molecular breeding techniques in fish due to their co-dominance, ubiquitous distribution within genomes, high reproducibility, and transferability across species [5,6]. However, the development of microsatellite markers has been limited by the labor and time required to construct, enrich, and sequence genomic libraries [7]. Fortunately, 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. Since EST-SSRs derived from transcriptome exist in the transcribed region of the genome, they can lead to the development of gene-based maps which help to identify candidate function genes and increase the efficiency of marker-assisted selection (MAS) [8]. Furthermore, EST-SSRs show a higher level of transferability to closely related species than non-EST-SSRs [9].

Although a few microsatellite markers were developed for *S. chuatsi* [10–14] and *S. scherzeri* [15], the number of available SSRs is grossly inadequate for genetic and mapping studies. Here, we describe the isolation and characterization of 46 novel polymorphic microsatellite loci for the *S. chuatsi* and *S. scherzeri*. We also test the transferability of these markers in other four species of sinipercine fishes: *Siniperca kneri*, *Siniperca undulata*, *Siniperca obscura*, and *Coreoperca whiteheadi*.

2. Results and Discussion

As shown in Table 1, a total of 46 polymorphic EST-SSR markers were newly developed. Forty-three of these loci were polymorphic in *S. chuatsi*, and 20 were polymorphic in *S. scherzeri*. Concerning *S. chuatsi*, the number of alleles per locus ranged from 2 to 8, with an average of 4.3 alleles per locus. The observed (H_O) and expected heterozygosities (H_E) ranged from 0.13 to 1.00 (average of 0.55) and from 0.33 to 0.85 (average of 0.63), respectively. In *S. scherzeri*, the number of alleles per locus ranged from 3 to 9, with an average of 5.5 alleles per locus. The observed (H_O) and expected heterozygosities (H_E) ranged from 0.19 to 1.00 (average of 0.74) and from 0.28 to 0.88 (average of 0.72), respectively. Five loci (Sin134 in *S. chuatsi*, Sin118, Sin122, Sin158 and Sin159 in *S. scherzeri*) showed significant deviation from the Hardy-Weinberg equilibrium (HWE) after Bonferroni correction (adjusted p -value = 0.0012 for *S. chuatsi* and 0.0026 for *S. scherzeri*), which may be due to the small sample size ($n = 32$) or the excess of heterozygotes. Another possible explanation for the departure from HWE is the dramatic contemporary decline in spawning populations, and consequent non-random mating and genetic bottlenecks [14]. No evidence for allelic dropout was found in these loci.

No significant linkage disequilibrium (LD) was detected across all loci following Bonferroni correction (adjusted p -value = 0.0001 for *S. chuatsi* and 0.0003 for *S. scherzeri*).

Overall, a high level of cross-species amplification was observed across the four species (Table 2). Forty-five of 46 polymorphic loci (97.8%) were amplified successfully in *S. undulate* and *S. obscura*, 44 (95.7%) in *S. kneri*, and 39 (84.8%) in *C. whiteheadi*. These results were expected because of the taxonomical relationships of the families [16]. *S. kneri*, *S. undulata*, *S. obscura* are closely related to *S. chuatsi* and *S. scherzeri*, and all species belong to *Siniperca*, whereas *C. whiteheadi* is from *Coreoperca* which is sister genera to *Siniperca*. As transcriptome sequences are typically conserved relative to nontranscribed regions, SSRs residing in transcriptome sequences typically benefit from higher amplification rates and higher levels of cross-species transferability [17,18]. The high level of cross-species amplification tested here indicated not only the potential utility of transcriptome sequences for the identification and characterization of large numbers of gene-based SSR loci across species for which limited marker resources were available, but also the potential usefulness of the developed markers for a broader range of evolutionary, conservation and management studies in sinipercine fishes.

3. Experimental Section

De novo transcriptome sequencing of F₁ hybrids between *S. chuatsi* (♀) and *S. scherzeri* (♂) was performed and a total of 118,218 unigenes were identified. The processes of library preparation for transcriptome analysis and sequence assembly were as described in [19]. This unigene set was used for mining EST-SSR markers using the default parameters of the BatchPrimer3 v1.0 software [20]. In this study, a subset of 62 EST-SSR markers was screened on 32 *S. chuatsi* (Chibi, Hubei Province, China) and 32 *S. scherzeri* (Fengcheng, Liaoning Province, China), respectively. The primers for these SSR loci were designed using NCBI/Primer-BLAST [21].

Total genomic DNA was extracted from fin clips using the TIANamp Genomic DNA Kit (Tiangen) following the manufacturer's instructions. Polymerase chain reaction (PCR) conditions were optimized for each pair of primers. PCRs were performed in 25 µL reaction volumes containing 2.5 µL of 10× PCR buffer, 1.0–3.0 mM MgCl₂, 50 µM dNTPs, 0.4 µM of each primer, 1 U *Taq* polymerase (Takara) and 50 ng genomic DNA. PCR conditions were as follows: initial denaturation at 94 °C for 3 min followed by 35 cycles at 94 °C for 30 s, the optimized annealing temperature (Table 1) for 30 s, 72 °C for 30 s, and then a final extension step at 72 °C for 10 min. PCR products were separated on a 8% non-denaturing polyacrylamide gel electrophoresis and visualized by silver staining. A denatured pBR322 DNA/MspI molecular weight marker (Tiangen) was used as a size standard to identify alleles.

Table 1. Characterization of 46 polymorphic EST-simple sequence repeats (SSR) markers in *S. chuatsi* and *S. scherzeri*.

Locus	Accession number	Repeat motif	Primer sequence(5'-3')	Size range (bp)	Ta (°C)	Na	H _O	H _E	PIC	p-Value
Sin109	JQ804765	(AG) ₁₅	F: GGACACTGGACACTCAAACAT	220–270	54.5	4	0.2500	0.6339	0.5747	1.0000
			R: AGAGGATCAAAATTGTGCTTGAA	246–285	54.5	6	0.6875	0.8170	0.7756	0.9510
Sin110	JQ804766	(AC) ₁₅	F: TGCTGTTTCCTCAAAACCCCT	177–244	54.5	6	0.7188	0.8194	0.7798	0.9933
			R: AATCCAAGTGACAGGACGCC	—	—	—	—	—	—	—
Sin112	JQ804768	(AC) ₁₅	F: ATCGGCACCTGAGGCAAAAG	132–166	54.5	6	0.9688	0.7897	0.7444	0.0030
			R: GCCATCCATAGAGCCACGTC	129–198	54.5	9	1.0000	0.8770	0.8479	0.0090
Sin113	JQ804769	(TG) ₁₅	F: TCCCCATATCTGCCCTGACC	90–126	54.5	6	0.8125	0.7907	0.7439	0.9514
			R: GTGCACATGTCGAGTCAGTA	—	—	—	—	—	—	—
Sin114	JQ804770	(AC) ₁₄	F: AAGAGACAAGACACCACCGC	185–209	54.5	5	0.4375	0.6736	0.6012	0.9347
			R: ATGGTTTGACGGGAGACAGC	194–243	54.5	7	1.0000	0.8418	0.8064	0.0039
Sin116	JQ804771	(TG) ₁₄ (AG) ₇	F: ACAATCCCAGCCCTCCTTCT	212–265	54.5	6	0.5625	0.8219	0.7813	0.9999
			R: GCAAGGTCCCTTTACATGCAG	219–259	54.5	5	0.8750	0.7877	0.7393	0.0798
Sin117	JQ804772	(GT) ₁₄	F: GGGCGGAAGACCAACTATGT	268–291	54.5	3	0.4062	0.5332	0.4697	0.9871
			R: TTTCTGTCCTTTTTCCTCTCGC	—	—	—	—	—	—	—
Sin118	JQ804773	(GT) ₁₄	F: AGGCCACACTTTAGTCACATC	163–192	54.5	4	0.8750	0.7376	0.6754	0.0393
			R: ACCCACTCCAGCATTTCCTCC	157–189	54.5	3	1.0000	0.6225	0.5378	0.0000 *
Sin119	JQ804774	(CA) ₁₄	F: AACAACTTTTTACCGCCAGCC	180–226	54.5	4	0.8438	0.7282	0.6652	0.1123
			R: ACCTCTGCTGCACAGCTAATC	—	—	—	—	—	—	—
Sin120	JQ804775	(TTTG) ₇	F: CCATCCCTCCGACCTTCAGT	119–134	54.5	4	0.5312	0.6900	0.6209	0.9418
			R: TTTAGGAACCCGACTCCGCT	—	—	—	—	—	—	—
Sin122	JQ804777	(TG) ₁₄ TAG(GT) ₇	F: TGCACTCACACACCTGTCTC	—	—	—	—	—	—	—
			R: AGCAGGATGCTTCATGCACTT	205–246	54.5	5	1.0000	0.6667	0.5927	0.0000 *
Sin123	JQ804778	(AC) ₁₄	F: GATGGTGGTGAAACACTGGCT	249–307	54.5	6	0.7812	0.7773	0.7352	0.7478
			R: GTGTTGAGAGGGTCCTGGTG	198–214	56.0	3	0.5000	0.5397	0.4683	0.9912

Table 1. Cont.

Locus	Accession number	Repeat motif	Primer sequence(5'-3')	Size range (bp)	Ta (°C)	Na	Ho	He	PIC	p-Value
Sin124	JQ804779	(CA) ₁₄	F: TCAAACACCACCCACCCCTG R: ACCGGGACAGGATGGGAGTC	248–281 —	54.5 —	4 —	0.8750 —	0.7297 —	0.6667 —	0.0180 —
Sin125	JQ804780	(CA) ₁₄	F: ACCCTCTGTGTGGCGAATGT R: CGGGACAGGATGGGAGTCG	277–311 —	54.5 —	3 —	0.6250 —	0.6265 —	0.5474 —	0.5368 —
Sin127	JQ804782	(TG) ₁₄	F: AGACGTAGCCCAGGCTCAAA R: TGTGGGGTTCCTACAGGGT	215–251 —	54.5 —	3 —	0.5938 —	0.5055 —	0.4213 —	0.0303 —
Sin128	JQ804783	(AC) ₁₄	F: CTGTGCCTCAGTGTGCTGC R: ACTTGTAATGGGCAAATTGTCACT	225–257 —	54.5 —	3 —	0.4062 —	0.6394 —	0.5572 —	0.9995 —
Sin129	JQ804784	(CA) ₁₄	F: ACGCTGCGAGGTGTGATATG R: CTGGCCCTCGTTAGTGCTTG	131–164 185–217	54.5 54.5	5 8	0.6250 1.0000	0.7500 0.8457	0.6971 0.8103	0.9857 0.0046
Sin130	JQ804785	(GTGA) ₇ N ₇ (TG) ₈	F: CTCGCAGGCTTTTCTCTGCT R: AGCCATCAGTTCTGTTCTTTCTT	282–300 252–280	54.5 54.5	2 5	0.3438 0.7188	0.3963 0.7485	0.3140 0.6982	0.8902 0.3062
Sin131	JQ804785	(ATGG) ₇	F: GGAGGAAAATAATTTCAATTTGGGAT R: GTCATTGCATTCAAAAGTTAGGCT	180–200 —	54.5 —	3 —	0.1250 —	0.4107 —	0.3665 —	0.9998 —
Sin134	JQ804789	(TG) ₁₄	F: GCCCCCTTCTCAACCCACTA R: TGCTTTCCAAAGCGAACCGT	106–120 108–134	54.5 54.5	6 8	1.0000 0.9688	0.8075 0.8621	0.7645 0.8299	0.0008 * 0.0451
Sin135	JQ804790	(TG) ₁₄	F: GTGATATCTCCTCCTGACGGC R: ACATTCTGAATTGCAAAGGCTCA	273–302 —	54.5 —	4 —	0.5938 —	0.5585 —	0.4900 —	0.3484 —
Sin136	JQ804791	(TG) ₁₄	F: AACTGAAATGTGTGGTGAAGTGA R: GTGTCTCCCAACAAGTGGCA	138–164 —	56.8 —	5 —	0.5000 —	0.7202 —	0.6585 —	0.9243 —
Sin137	JQ804792	(TCA) ₉	F: AGCGTCTACTGAGGGTCAAAGT R: GGTGGACTGACCAGCAAGGA	234–280 —	54.5 —	5 —	0.4688 —	0.7207 —	0.6700 —	0.9960 —
Sin138	JQ804793	(ATC) ₉	F: TCATCTGAGGACGACTCGCT R: AACTTAACTTCCTGCTGTCCCT	229–253 —	52.5 —	3 —	0.4375 —	0.5997 —	0.5025 —	0.9772 —

Table 1. Cont.

Locus	Accession number	Repeat motif	Primer sequence(5'-3')	Size range (bp)	T _a (°C)	N _a	H _O	H _E	PIC	p-Value
Sin139	JQ804794	(CTC) ₉	F: GTGACTGCATCCAGGTGTCG R: GGCCGAGGTCGGTTGTTATC	— 189–207	— 54.5	— 3	— 0.1875	— 0.2803	— 0.2584	— 0.9947
Sin140	JQ804795	(TCA) ₉	F: TGTGGTTCTCCTCTCCCACA R: AGAGGTTGGTGCAGGAGACTT	253–304 —	53.2 —	5 —	0.4375 —	0.7336 —	0.6759 —	0.9985 —
Sin142	JQ804797	(CTT) ₉	F: CATCAACGCAATGCAAGGGT R: CTGGAGCCGGACTTGAGGAA	150–180 181–226	54.5 54.5	2 6	0.1562 0.3438	0.3289 0.6994	0.2713 0.6492	0.9997 0.9998
Sin143	JQ804797	(GTT) ₇	F: AAAGCAGGCCAAACAACACC R: AGGACGGGGAGGCTTTTGAT	198–246 —	54.5 —	5 —	0.4062 —	0.7733 —	0.7206 —	1.0000 —
Sin146	JQ804800	(GAG) ₆ N ₅ (AAG) ₉	F: GTAATCGACACGGACAGCGA R: CACACACATTCTCCTCAGCGT	370–452 —	54.5 —	4 —	0.5938 —	0.6781 —	0.6069 —	0.8240 —
Sin147	JQ804801	(TCC) ₉	F: AGATCAGACACCAGGAGGACC R: AAGACGGAGGCAAAGAACGAC	174–232 192–225	53.5 54.5	5 4	0.3125 0.5625	0.7242 0.7614	0.6576 0.7026	0.9971 0.9964
Sin148	JQ804802	(CAT) ₉	F: CGAGGCCAGGAGTGAACCAA R: GCACAGCTGGAGGTGTTTCG	255–303 —	53.5 —	3 —	0.6875 —	0.4866 —	0.4009 —	0.0027 —
Sin151	JQ804805	(GT) ₁₃	F: GTGCAAGGCCTTAGTCTCTCC R: GCCCACCAGATCTACCGAGT	170–221 —	55.5 —	4 —	0.6875 —	0.6434 —	0.5761 —	0.8897 —
Sin152	JQ804806	(AG) ₈ A(AG) ₁₃	F: TGCGCCACTTTACTGATGGG R: GCATTAACCAAACCCCGCGA	173–242 185–240	53.5 54.5	6 8	0.6562 0.9688	0.8105 0.8522	0.7677 0.8193	0.9164 0.1740
Sin153	JQ804807	(AG) ₁₃	F: GCACAGGTTTTTCTAAACATTGCT R: TGTTGTTATTGTCAGTGTGTTTCT	155–208 177–215	53.5 54.5	5 4	0.1875 0.2500	0.3795 0.6806	0.3538 0.6160	0.9662 1.0000
Sin154	JQ804808	(GT) ₁₃	F: ACTGGTTTGTGGTTTGGAGGT R: ATGATTTTCTTGCCTTCGTGT	211–229 —	53.5 —	2 —	0.5625 —	0.4107 —	0.3225 —	0.0356 —
Sin155	JQ804809	(AC) ₁₃	F: GAATGGTGTGTTGCACAGCG R: CATTCTAGCATGTGCGAGGC	157–190 160–201	53.5 54.5	3 7	0.2188 0.6875	0.3894 0.8021	0.3473 0.7600	0.9939 0.9004

Table 1. Cont.

Locus	Accession number	Repeat motif	Primer sequence(5'-3')	Size range (bp)	T _a (°C)	N _a	H _O	H _E	PIC	p-Value
Sin156	JQ804810	(AC) ₁₃	F: TAGGAGGCTTTACAACCGGC R: ATGACCAGCCTCAGGTGTCT	188–205 —	53.5 —	2 —	0.5625 —	0.4107 —	0.3225 —	0.0344 —
Sin157	JQ804811	(AC) ₁₃	F: CATTTGCTGGCTCTCACACC R: TGTTTAATTCATGCCTAGGTTTAGT	184–215 —	53.5 —	3 —	0.5000 —	0.4330 —	0.3477 —	0.2070 —
Sin158	JQ804812	(CA) ₁₃	F: TGAGAACTGCCTGAGCCGAG R: CTGCAGAGCCGTGGAGACTA	— 210–248	— 54.5	— 3	— 0.9062	— 0.5303	— 0.4145	— 0.0000 *
Sin159	JQ804813	(TG) ₁₃	F: CGCTGATCGCTCTGTGCTCCC R: ACACGGAAGCTGGTGAGCGG	196–234 199–233	53.5 56.0	5 5	0.6875 0.9688	0.7614 0.6379	0.7108 0.5682	0.7259 0.0000 *
Sin160	JQ804814	(TG) ₁₃	F: CCACTGGAGCCCACATGGCA R: TGAGTGGGCGCTACTGTGTGT	307–360 291–331	55.5 54.5	5 5	0.8125 0.7812	0.6711 0.7659	0.6123 0.7137	0.0151 0.5928
Sin162	JQ804815	(TA) ₁₃	F: TGCTTTGCTGGTTGGCAGGCT R: CGTGGAGGTGCGACGCGTAA	294–368 —	53.5 —	5 —	0.1875 —	0.6270 —	0.5651 —	1.0000 —
Sin163	JQ804817	(CA) ₁₃	F: ACAGCCAGGCTCCTCCACCT R: TCTTTCACAGGCAAACCACTGCT	230–269 225–273	53.5 53.5	8 6	0.6875 0.4688	0.8482 0.7803	0.8134 0.7392	0.9597 1.0000
Sin166	JQ804819	(GA) ₁₃	F: GAAATTGAAGAAGACAAGGTGATG R: CTGCTTTTGGCAGGAGCTAA	204–231 —	53.5 —	3 —	0.2500 —	0.4504 —	0.4012 —	0.9998 —
Sin169	JQ804822	(AC) ₁₃	F: TGACAAATCACTGGGTTTACTCCT R: GACATGCTGCTCTCCGATCC	214–284 —	53.5 —	5 —	0.5625 —	0.6443 —	0.5790 —	0.9164 —
Sin170	JQ804823	(GT) ₁₃	F: CTTGAGTGGTTGATTGTGCCCT R: GCAGACATTGCTGAGGGATGAA	242–270 —	55.5 —	4 —	0.7188 —	0.5491 —	0.4990 —	0.0015 —

For each locus the information in the top row refers to *S. chuatsi* and the second row refers to *S. scherzeri*. T_a corresponds to annealing temperature; N_a is number of alleles; H_O and H_E are observed and expected heterozygosity, respectively; PIC is the polymorphic information content. * indicates significant deviation from HWE after Bonferroni correction; no polymorphism for each locus is denoted by “—”.

The number of alleles (N_a), the observed (H_o) and expected heterozygosities (H_e) were estimated using POPGENE version 1.32 [22]. The polymorphic information content (PIC) was calculated using 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 n is the number of alleles, and q_i , q_j is the i th and j th allele frequency, respectively [23]. Deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were tested using the online version of GENEPOP [24]. All results were adjusted for multiple simultaneous comparisons using a sequential Bonferroni correction [25]. Genotyping errors due to null alleles, stutter bands, or allele dropout were analyzed using the software Micro-checker 2.2.3 [26].

Cross-species amplification of the above-developed polymorphic SSR loci was tested in four species of sinipercine fishes: *S. kneri*, *S. undulata*, *S. obscura*, and *C. whiteheadi*. Two individuals of each species were analyzed. The same PCR conditions were used as described above except that the annealing temperature was re-optimized at each locus (Table 2). Amplification products were visualized in 1.5% agarose gels, and fragments were sized by comparison with a 2 kb DNA Marker (Trans). Primer pairs that amplified fragments with similar sizes to those observed in source species were considered as successful cross-species amplification.

Table 2. Cross-species amplification for the 46 polymorphic EST-SSR markers in four species of sinipercine fishes.

Locus	Species			
	<i>S. undulate</i>	<i>S. obscura</i>	<i>S. kneri</i>	<i>C. whiteheadi</i>
Sin109	54.5	54.5	54.5	54.5
Sin110	54.5	54.5	54.5	54.5
Sin112	54.5	54.5	54.5	54.5
Sin113	54.5	54.5	54.5	54.5
Sin114	54.5	54.5	54.5	54.5
Sin116	54.5	54.5	54.5	—
Sin117	54.5	54.5	54.5	—
Sin118	54.5	54.5	54.5	54.5
Sin119	54.5	54.5	54.5	54.5
Sin120	54.5	54.5	54.5	54.5
Sin122	54.5	54.5	54.5	54.5
Sin123	54.5	54.5	54.5	54.5
Sin124	54.5	54.5	54.5	54.5
Sin125	54.5	54.5	54.5	54.5
Sin127	54.5	54.5	54.5	54.5
Sin128	54.5	54.5	54.5	—
Sin129	54.5	54.5	—	54.5
Sin130	54.5	54.5	54.5	54.5
Sin131	54.5	54.5	54.5	54.5
Sin134	54.5	54.5	54.5	54.5
Sin135	54.5	54.5	54.5	—
Sin136	54.5	54.5	54.5	54.5
Sin137	54.5	54.5	54.5	54.5

Table 2. Cont.

Locus	Species			
	<i>S. undulate</i>	<i>S. obscura</i>	<i>S. kneri</i>	<i>C. whiteheadi</i>
Sin138	54.5	54.5	54.5	54.5
Sin139	54.5	54.5	54.5	54.5
Sin140	54.5	54.5	54.5	54.5
Sin142	54.5	54.5	54.5	54.5
Sin143	54.5	54.5	54.5	54.5
Sin146	54.5	54.5	54.5	—
Sin147	54.5	54.5	54.5	54.5
Sin148	54.5	54.5	54.5	54.5
Sin151	54.5	54.5	54.5	54.5
Sin152	54.5	54.5	54.5	54.5
Sin153	54.5	54.5	54.5	54.5
Sin154	54.5	54.5	54.5	54.5
Sin155	—	—	—	—
Sin156	54.5	54.5	54.5	54.5
Sin157	54.5	54.5	54.5	54.5
Sin158	54.5	54.5	54.5	54.5
Sin159	54.5	54.5	54.5	52.8
Sin160	54.5	54.5	54.5	—
Sin162	54.5	54.5	57.0	51.1
Sin163	54.5	54.5	54.5	52.8
Sin166	54.5	54.5	54.5	54.5
Sin169	54.5	54.5	54.5	54.5
Sin170	54.5	54.5	54.5	54.5

The annealing temperature for each locus was shown. Unsuccessful amplification of PCR products for each locus is denoted by “—”.

4. Conclusions

In summary, a total of 46 polymorphic EST-SSR markers were newly developed. Forty-three of these loci were polymorphic in *S. chuatsi*, and 20 were polymorphic in *S. scherzeri*. We only tested a small subset of the SSR loci identified in our transcriptome, but high levels of polymorphism, and high level of cross-species amplification indicate that the pairs of primers described here may be suitable for assessments of genetic diversity and population structure, the construction of high-density linkage map, conservation and molecular marker-assisted breeding in many species of siniperine fishes. Our results highlight the value of next generation transcriptome resources for the characterization and development of gene-based SSRs.

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