Genetics and Molecular Research

# Isolation and characterization of polymorphic EST-SSR and genomic SSR markers in spotted mandarin fish (*Siniperca scherzeri* Steindachne)

**Y.Q. Dou, X.F. Liang, M. Yang, C.X. Tian, S. He and W.J. Guo**

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

Corresponding author: X.F. Liang
E-mail: xufang_liang@hotmail.com

Genet. Mol. Res. 14 (4): 19317-19322 (2015)
Received August 3, 2015
Accepted October 29, 2015
Published December 29, 2015
DOI http://dx.doi.org/10.4238/2015.December.29.41

**ABSTRACT.** Spotted mandarin fish (*Siniperca scherzeri* Steindachne) feed solely on live fry of other fish species once the fry start feeding in the wild. In the present study, 26 polymorphic transcriptome-derived simple sequence repeat (SSR) markers and 14 genomic SSR markers were developed and characterized in *S. scherzeri* Steindachne by combining a biotin-enrichment protocol and transcriptome of $F_1$ interspecies hybrids between *S. chuatsi* (♀) and *S. scherzeri* (♂). These 40 polymorphic SSRs amplified 168 alleles (mean 4.2). The number of alleles, observed heterozygosity, expected heterozygosity, and polymorphic information content per locus were in the range of 2 to 7 (mean 4.3), 0.1111 to 1.000 (mean 0.6718), 0.3118 to 0.8276 (mean 0.6901), and 0.2735 to 0.7902 (mean 0.6298), respectively. Ten of these microsatellite loci deviated significantly from Hardy-Weinberg equilibrium ($P < 0.00125$) after Bonferroni correction for multiple tests and no significant linkage disequilibrium ($P < 0.00006$) was

 

observed. The microsatellite markers characterized from *S. scherzeri* could be a valuable tool in genetic evaluation for conservation and for assessment of the mechanism associated with unique food preference of *S. scherzeri* from a genetic point of view.

**Key words:** Polymorphic microsatellite; *Siniperca scherzeri*; EST-SSR; Genomic SSR

## INTRODUCTION

Spotted mandarin fish (*Siniperca scherzeri* Steindachne) is a commercially important freshwater fish in China (Zhou et al., 1988). It has unique food preference. In the wild, once the fry starts feeding, it feeds solely on live fry of other fish species (Liang, 1998). Among major species of Sinipercinae fishes, *S. scherzeri* is more likely to accept dead prey fish compared to another mandarin fish, *S. chuatsi* (He et al., 2013). In recent years, genetic diversity of the wild *S. scherzeri* is continuously decreasing because of overexploitation and anthropogenic interference (Luo et al., 2011).

Microsatellite (also known as single sequence repeat, SSR), a codominant and comprehensive molecular marker, has been extensively used to evaluate genetic diversity in a number of fish species (Liu and Cordes, 2004). Genomic library enrichment and transcriptome sequencing techniques have given rise to the identification of SSR markers (Li et al., 2004). SSRs are broadly classified into expressed sequence tag (EST)-SSRs, derived from transcriptome sequences and genomic SSRs (G-SSRs), derived from genomic libraries. Although the SSR loci of *S. scherzeri* were isolated and characterized in earlier studies, it is still insufficient for studying the genetic diversity and molecular marker-assisted breeding of *S. scherzeri*. Molecular marker-assisted selection (MAS) plays an important role in fish breeding. Therefore, it is desirable to develop highly informative and reliable SSR markers in *S. scherzeri.* In the present study, we isolated and characterized 26 polymorphic EST-SSR makers and 14 G-SSR markers. Moreover, we found the mean values of number of alleles ($N_A$), observed heterozygosity ($H_O$), expected heterozygosity ($H_E$), and polymorphic information content (PIC) of the G-SSRs to be higher than those of the EST-SSRs. These SSR markers could play important roles in studies on conservation genetics, genetic linkage map, and molecular breeding in *S. scherzeri*.

## MATERIAL AND METHODS

Thirty-six wild *S. scherzeri* individuals were collected from the Yangtze River. Total genomic DNA was extracted from fin clips using the TIANamp Genomic DNA Kit (Tiangen, Beijing, China) following the manufacturer instructions and DNA concentration was adjusted to 100 ng/μL and stored at -20°C.

Transcriptome sequencing of $F_1$ interspecies hybrids (from Guangdong Freshwater Fish Farm) of *S. chuatsi* (♀) x *S. schezeri* (♂) was performed (He et al., 2013). A total of 118,218 unigenes were identified during the sequencing. Potential EST-SSR markers were selected among the unigenes employing the default parameters of BatchPrimer3 v1.0 software and corresponding primer pairs were designed using NCBI/Primer-BLAST (available online: http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome; accessed on June 18, 2012).

A microsatellite-enriched genomic library was constructed using the fast isolation by amplified fragment length polymorphism of sequences containing repeats (FIASCO) protocol adapted from Zane (Zane et al., 2002). The total genomic DNA was extracted from fin clips with

standard phenol-chloroform method and fragmented with the restriction enzyme, *Mse*I. Specific adapters (5'-GACGATGAGTCCTGAG-3' and 5'-TACTCAGGACTCAT-3') were ligated to the fragmented DNA. Polymerase chain reaction (PCR) products were size selected to preferentially obtain small fragments (300-1000 bp), which were hybridized with 5'-biotin-labeled oligonucleotides $(GA/CT)_{20}$ and $(CCT/GGA)_{15.}$ The enriched DNA were cloned into the pGEM-T vector (Promega, Madison, WI, USA) , and transformed into competent DH5α strain (Promega, USA). White colonies were randomly picked from the primary transformation plates and the isolated plasmid DNA were subsequently sequenced using ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Identification of SSR clones was performed using SSRHUNTER software (Li and Wan, 2005). The resulting sequences were first screened for microsatellite (minimum five repeats) and flanking sequences and then PCR primers were designed for selected sequences using the PRIMER PREMIER 5.0 program (PREMIER Biosoft International, Palo Alto, CA, USA).

PCRs were performed in 25 µL reaction volumes containing 2.5 µL 10X PCR buffer, 1.0-3.0 mM $MgCl_2$, 50 µM dNTPs, 0.4 µM each primer, 1 U Taq polymerase (Takara, Dalian, China) and 50 ng genomic DNA. PCR conditions were as follows: initial denaturation at 94°C for 3 min followed by 30 cycles at 94°C for 30 s, the respective optimized annealing temperature (Table 1) for 30 s, 72°C for 30 s, and a final extension step at 72°C for 10 min. PCR products were separated on a 8% non-denaturing polyacrylamide gel and visualized by silver staining. Denatured pBR322 DNA/*Msp*I molecular weight marker (Tiangen, Beijing, China) was used as the size standard, to identify the alleles.

The $N_A$, $H_O$, and $H_E$ were computed by the POPGENE software (version 3.2). PIC was calculated using the formula (1), where *qi*, *qj* are the *i*th and *j*th allele frequencies, respectively, while *n* is the number of alleles (Botstein et al., 1980). Exact tests for genotypic linkage disequilibrium and for conformance to Hardy-Weinberg equilibrium (HWE) at each locus were performed using the GENEPOP 1.2 program (Raymond and Rousset, 1995). The SSR markers were classified by Weber's rules (Weber and May, 1989). All the results were adjusted for multiple simultaneous comparisons using a sequential Bonferroni correction (Holm, 1979).

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

## RESULTS

A microsatellite-enriched library was constructed from the genomic DNA of *S. scherzeri*. Eighty-four putative recombinant clones from the enriched library were sequenced using ABI 3730 Genetic Analyzer (Applied Biosystems). We observed putative SSR loci in 74 (88.1%) unique clones. A subset of 50 (67.6% of the unique clones) G-SSR markers (GenBank accession No.: KC920593-KC920642) were amplified for the polymorphism examination. In addition, we examined 98 EST-SSR markers (GenBank accession No.: JX503104-JX503149, JX503200-JX503251). Fourteen G-SSRs (28.0%) and 26 EST-SSRs (26.5%) were polymorphic in the 36 wild *S. scherzeri* individuals.

The characteristics of polymorphic SSR markers are shown in Table 1. These 40 polymorphic SSRs amplified 168 alleles (mean 4.2). The $N_A$, $H_O$, $H_E$, and PIC of all the loci were in the range of 2 to 7 (mean 4.3), 0.1111 to 1.000 (mean 0.6718), 0.3118 to 0.8276 (mean 0.6901), and 0.2735 to 0.7902 (mean 0.6298), respectively. After Bonferroni correction, 10 microsatellite loci significantly deviated from HWE ($P < 0.00125$), which might be due to the limited sample size, sampling strategy, heterozygote deficiency, and null alleles (Miao et al., 2011). No significant linkage disequilibrium ($P < 0.00006$) was detected among all of the loci by analyzing the allelic inheritance with the MICROCHECKER V.2.2.3 software (Van Oosterhout et al., 2004).

**Table 1.** Characterization of 40 polymorphic microsatellite markers in a sample of 36 *Siniperca scherzeri* individuals.

| Locus | GenBank | Repeat motif | Primer sequence (5'-3') | Size range ( bp) | TA (°C) | $N_A$ | $H_O$ | $H_E$ | PIC |
|---|---|---|---|---|---|---|---|---|---|
| EST-derived SSR | | | | | | | | | |
| ESTAP31-04 | JX503248 | $(TG)_5$…$(GGC)_5$ | F: GTTCCCCTCTGCAATCCCTC<br>R: CACAAACACACTCTCCTCTC | 177-204 | 61 | 2 | 0.4118 | 0.3319 | 0.2735 |
| ESTAP31-06 | JX503246 | $(TG)_5$…$(CA)_9$ | F: GCCGAACGGGTCTTGATGTG<br>R: GTTACTCCCGGAGATTTACA | 124-139 | 61 | 2 | 0.4444 | 0.4820 | 0.3624 |
| ESTAP31-13 | JX503239 | $(TCA)_6$ | F: GCTGGCGTTGTTGAAGAGTC<br>R: AACCCCTGCACATACTTTTG | 238-280 | 61 | 3 | 0.3611 | 0.3118 | 0.2792 |
| ESTAP31-14 | JX503238 | $(AC)_{20}$ | F: AGAGTTCCTGGAGCTGATAC<br>R: AAAACTCAGAAATGCCAAGC | 210-250 | 58 | 5 | 0.8889 | 0.7750 | 0.7258* |
| ESTAP31-15 | JX503237 | $(ATT)_5(GTT)_5$ | F: AGACAAGAGCTGAGCACGGT<br>R: AAACATCCGCCGTCTAATAG | 179-220 | 61 | 5 | 0.9714 | 0.7963 | 0.7498* |
| ESTAP31-23 | JX503229 | $(CA)_{18}$ | F: ATTTGCTAAAGGACTGGACC<br>R: AAGCAGTGCAAGAAATTCCA | 179-199 | 58 | 3 | 1.0000 | 0.6501 | 0.5645 |
| ESTAP31-41 | JX503211 | $(GA)_5$…$(GA)_6$ | F: GAACTCAATCCTGGAG<br>R: CCAGTTCTTCCAGTTCAGTT | 181-205 | 56 | 3 | 0.2571 | 0.6348 | 0.5500 |
| ESTAP31-44 | JX503208 | $(CCT)_5$ | F: CCTCTGGAATAGCGTGGAAC<br>R: GGCTCTTCCTGTCCAGTCTG | 221-247 | 62 | 4 | 0.1111 | 0.6745 | 0.6181 |
| ESTAP31-45 | JX503207 | $(TG)_{15}$ | F: AAGCGTTGTTTCTAGTTGAC<br>R: GTTTGCATGTCCTCTGAGTT | 226-251 | 56 | 4 | 0.9722 | 0.7218 | 0.6590* |
| ESTAP31-49 | JX503203 | $(CA)_{10}$ | F: TGTCTTAACACCTCACATGG<br>R: AACAGCTCACTAACATTGCC | 162-186 | 59 | 5 | 0.7500 | 0.7602 | 0.7128 |
| ESTAP31-51 | JX503201 | $(CA)_{24}$ | F: GTTCAGACCCACATTTTACA<br>R: AAGGAGATCCGGTGAGCTGA | 183-193 | 59 | 3 | 0.8857 | 0.5710 | 0.4659* |
| ESTAP31-52 | JX503200 | $(GT)_{20}$…$(CT)_5$ | F: ACGGCAACGGTAGGTGAGAT<br>R: GACAGGAAACACATTTGAAC | 199-227 | 59 | 3 | 1.0000 | 0.5775 | 0.4767 |
| ESTAP33-06 | JX503144 | $(GT)_{10}$ | F: AGGGAGAAAAAGAGAGCGTG<br>R: TTTGATGTCTGCTGTCAGGA | 153-165 | 57 | 3 | 0.4286 | 0.4634 | 0.4039 |
| ESTAP33-07 | JX503143 | $(CA)_{10}$ | F: TTTGAGAGCAGTTGGAACAC<br>R: ACAGGTCATTTCATCGTCTT | 167-214 | 55 | 5 | 0.8571 | 0.6683 | 0.6018 |
| ESTAP33-09 | JX503141 | $(TG)_5$…$(TA)_8$ | F: TCCCTCATTCACTCCCTCCA<br>R: TTTCAGCCCCTGCCAAACAT | 175-210 | 62 | 4 | 0.5000 | 0.7289 | 0.6692 |
| ESTAP33-12 | JX503138 | $(CA)_5AG(CA)_5$ | F: CCCAAGGACGACCTCTTA<br>R: GTGTGGGACTGTTTGAACAA | 169-190 | 56 | 4 | 0.4167 | 0.7171 | 0.6524* |
| ESTAP33-14 | JX503136 | $(AC)_{10}$ | F: AGACATTTGGATTCAGCC<br>R: GGGATGTTCTGCTGAACT | 200-225 | 56 | 4 | 0.3143 | 0.7437 | 0.6830 |
| ESTAP33-16 | JX503134 | $(TC)_5$…$(CT)_{16}$ | F: ATGGCAGAGGCAGTGTTTGT<br>R: AGAGAGGAAGGCACGAGTTG | 245-282 | 62 | 4 | 0.8485 | 0.7408 | 0.6789 |
| ESTAP33-18 | JX503132 | $(TG)_{10}$ | F: GCTTTAGTGTCACCCTTCCT<br>R: CGAACCTGTTGTTGTTCTTG | 214-241 | 59 | 3 | 0.2778 | 0.5305 | 0.4652 |
| ESTAP33-20 | JX503130 | $(GT)_{11}$ | F: GATGGTGGAATGTGACATCAC<br>R: TGGATTCAGACTGTTGTGTCA | 257-302 | 56 | 5 | 0.5278 | 0.7766 | 0.7263 |
| ESTAP33-22 | JX503128 | $(TC)_9$…$(TC)_5$ | F: GCTGTGGGTCTAAATGTCTC<br>R: GGAAGAAGAGGAGAAGAACA | 183-246 | 59 | 6 | 0.8286 | 0.7710 | 0.7238 |
| ESTAP33-30 | JX503120 | $(GAT)_6$…$(TA)_7$ | F: CGCCAAGAGATGAACTGCTA | 290-351 | 62 | 5 | 0.7778 | 0.7746 | 0.7254* |
| ESTAP33-35 | JX503115 | $(GT)_{19}$ | F: CGTGACATTATGATTACAGG<br>R: CTGTTTTGGTTTCTCCTTAC | 228-264 | 56 | 4 | 0.9714 | 0.6894 | 0.6268 |
| ESTAP33-37 | JX503113 | $(AGA)_6$…$(AGA)_7$…$(ATA)_5$ | F: GTCGCTGACTGACAGAACTA<br>R: CAGCAGAGAAACACTCACAG | 199-241 | 59 | 4 | 0.5556 | 0.7038 | 0.6440 |
| ESTAP33-39 | JX503111 | $(TG)_{10}$ | F: AAGTCCATCAGCAGTCACAGA<br>R: GGCAGTGGAACAGGAGAGAA | 217-261 | 59 | 4 | 0.6111 | 0.7242 | 0.6623* |
| ESTAP33-40 | JX503110 | $(CA)_{10}$ | F: TAAGGACACCTGCGGATT<br>R: CACGGTCCTCACAAAGATAG | 239-287 | 56 | 5 | 0.5278 | 0.7062 | 0.6613 |
| Mean | | | | | | 3.9 | 0.6345 | 0.6548 | 0.5908 |
| G-derived SSR | | | | | | | | | |
| FC01 | KC920593 | $(TG)_5$...$(TG)_{33}$ | F: GAGCAGAGCAGCAGAGGTTT<br>R: CGGCACTGGGGAGTAATGAT | 266-306 | 59 | 3 | 0.6333 | 0.6435 | 0.5615 |
| FC02 | KC920594 | $(TG)_{15}$…$(GT)_{16}$…$(GT)_5$ | F: TTCCCCAAGAGAAGTCCTCC<br>R: GAAAGGTCAAATGCTCTGTG | 214-276 | 61 | 6 | 0.9722 | 0.7883 | 0.7458* |
| FC13 | KC920605 | $(GAG)_{12}$ | F: CATCGCCTCAGCCCACTACA<br>R: CTTTGCTATCATCCTGTGGG | 217-252 | 61 | 4 | 0.7429 | 0.6770 | 0.6166 |
| FC14 | KC920606 | $(AGG)_{11}$ | F: GTTACACTCGTTTCTCTCTT<br>R: TCAGGCGGCAGATTCAGAC | 263-301 | 59 | 5 | 0.0882 | 0.7283 | 0.6824 |

Continued on next page

**Table 1.** Continued.

| Locus | GenBank | Repeat motif | Primer sequence (5'-3') | Size range ( bp) | TA (°C) | $N_A$ | $H_O$ | $H_E$ | PIC |
|---|---|---|---|---|---|---|---|---|---|
| FC15 | KC920607 | (TCC)6 | F: CTTGCCTCATGGCTGTTGG<br>R: CACTGGCAATGGAGGTGGTA | 242-269 | 61 | 4 | 0.1667 | 0.7308 | 0.6678 |
| FC21 | KC920613 | (GC)5…(TG)13 | F: GCTACACAGACAGATAGTG<br>R: TGTGCTCGCTCTCTCTTAC | 207-238 | 56 | 5 | 1.0000 | 0.7623 | 0.7103* |
| FC23 | KC920615 | (AGG)8 | F: AATGTTCACCACTGCCGAGG<br>R: ATGAAGCAACACATACGAAT | 141-161 | 59 | 5 | 0.6571 | 0.8058 | 0.7614 |
| FC32 | KC920624 | (GT)18(TG)8 | F: CACAGAGTCACCAGATAAGG<br>R: AATGGGGCAAATGAATCAAT | 228-288 | 56 | 6 | 1.0000 | 0.8009 | 0.7567 |
| FC35 | KC920627 | (CA)18 | F: TTATGGACTATGTGATTGAC<br>R: GTATTGGGGTGTAGCCAGAA | 256-298 | 56 | 4 | 1.0000 | 0.6244 | 0.5408 |
| FC39 | KC920631 | (GT)12…(TG)14 | F: TAACTTTACGCTCCGTTTCA<br>R: CGGGACTGAAGGCTGATTGA | 280-331 | 59 | 5 | 1.0000 | 0.7672 | 0.7163 |
| FC41 | KC920633 | (GAG)12 | F: CTCTCAGCAGCAGTTCCTGT<br>R: CTCCTCTTTCTTCCTCCATT | 170-229 | 61 | 7 | 0.6774 | 0.8276 | 0.7902* |
| FC43 | KC920635 | (CCT)8 | F: GAGCGATGCTGGAGAACAAG<br>R: GCAGCGATTGTTTGAGGTAA | 309-385 | 61 | 5 | 0.7500 | 0.7876 | 0.7414 |
| FC46 | KC920638 | (GGA)7 | F: AGTTTGGGCTTGTATGTTGA<br>R: AACATTTTTATCTCCCACTC | 222-233 | 56 | 2 | 0.4062 | 0.4241 | 0.3303 |
| FC49 | KC920641 | (GT)7 | F: GCAGTTGTGTCAGTGTTTGA<br>R: AACTGTTTGGGTCTGTATTG | 180-203 | 56 | 5 | 0.8333 | 0.7887 | 0.7420 |
| Mean | | | | | | 4.7 | 0.7091 | 0.7255 | 0.6688 |
| Total mean | | | | | | 4.2 | 0.6606 | 0.6795 | 0.6181 |

TA, annealing temperature; $N_A$, number of alleles; $H_O$, observed heterozygosity; $H_E$, expected heterozygosity; PIC, polymorphic information content. *Indicates significant deviation from the Hardy-Weinberg equilibrium corrected for multiple comparisons using the Bonferroni correction.

## DISCUSSION

It was observed that mean values of $N_A$, $H_O$, $H_E$, and PIC of G-SSRs were higher than those of EST-SSRs, which is consistent with the earlier study in clam (*Meretrix meretrix*; Lu et al., 2011), suggesting higher polymorphism of G-SSR. EST-SSR, as type I marker, is derived from coding regions, and G-SSR, as type II marker, is derived from non-coding intergenic regions. Therefore, the non-coding sequences displayed higher levels of polymorphism, whereas coding sequences under the functional selection pressure displayed lower levels of polymorphism (Chistiakov et al., 2006).

We compared EST-SSRs and G-SSRs of *S. scherzeri* discovered in the present study with those reported in earlier studies (Yang et al., 2012; Huang et al., 2012; Huang et al., 2013; Qu et al., 2013; Table 2). It was observed that 109 polymorphic primers were derived from genome sequences (43.78%), and 29 were derived from transcriptome sequences (43.94%). The mean values of $N_A$, $H_O$, $H_E$ and PIC per locus were 5.06/4.55, 0.6271/0.8026, 0.6882/0.6896, and 0.6319/0.626 in EST-SSR/G-SSR, respectively.

**Table 2.** Summary and comparison of polymorphic EST-SSRs and G-SSRs of *Siniperca scherzeri*.

| Locus | Designed primers | % (Polymorphic/Amplified primers) | Mean $N_A$ | Mean $H_O$ | Mean $H_E$ | Mean PIC |
|---|---|---|---|---|---|---|
| EST-SSR (present study) | 98 | 33.77 (26/77) | 3.9 | 0.6345 | 0.6548 | 0.5908 |
| EST-SSR (Huang et al., 2012) | 85 | 37.31 (25/67) | 5.76 | 0.6225 | 0.6925 | 0.645 |
| EST-SSR (Huang et al., 2013) | 64 | 64.41 (38/59) | 5.07 | 0.5076 | 0.6856 | 0.6242 |
| EST-SSR (Qu et al., 2012) | 62 | 43.48 (20/46) | 5.5 | 0.7438 | 0.72 | 0.6674 |
| Total/Mean | 309 | 43.78 (109/249) | 5.06 | 0.6271 | 0.6882 | 0.6319 |
| G-SSR (present study) | 50 | 35.90 (14/39) | 4.7 | 0.7091 | 0.7255 | 0.6688 |
| G-SSR (Yang et al., 2012) | 42 | 55.56 (15/27) | 4.4 | 0.8961 | 0.6536 | 0.5831 |
| Total/Mean | 92 | 43.94 (29/66) | 4.55 | 0.8026 | 0.6896 | 0.626 |

$N_A$, number of alleles; $H_O$, observed heterozygosity; $H_E$, expected heterozygosity; PIC, polymorphic information content.

In the present study, we isolated and characterized 26 polymorphic EST-SSR and 14 G-SSR markers. The polymorphic EST-SSR and G-SSR markers developed in the study could be remarkable tools for the studies on population diversity evaluation, genetic structure analysis, high-density linkage map construction, and molecular marker-assisted selection of *S. scherzeri*.

## Conflicts of interest

The authors declare no conflict of interest.

## ACKNOWLEDGMENTS

Research financially supported by the National Natural Science Foundation of China (#31172420 and #31272641), the Key Projects in the National Science & Technology Pillar Program (#2012BAD25B04), and the Special Fund for Agro-Scientific Research in the Public Interest of China (#201003020).

## REFERENCES

Botstein D, White RL, Skolnick M and Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32: 314-331.

Chistiakov DA, Hellemans B and Volckaert FA (2006). Microsatellites and their genomic distribution, evolution, function and applications: A review with special reference to fish genetics. *Aquaculture* 255: 1-29.

He S, Liang XF, Sun J, Li L, et al. (2013). Insights into food preference in hybrid F1 of *Siniperca chuatsi* (♀) x *Siniperca scherzeri* (♂) mandarin fish through transcriptome analysis. *BMC Genomics* 14: 601.

Holm S (1979). A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* 6: 65-70.

Huang W, Liang XF, Qu CM, Li J, et al. (2012). Isolation and characterization of twenty-five polymorphic microsatellite markers in *Siniperca scherzeri* Steindachner and cross-species amplification. *J. Genet.* 91: e113-e117.

Huang W, Liang X, Qu C, Cao J, et al. (2013). Development and characterization of novel polymorphic microsatellite loci in *Siniperca scherzeri* Steindachner and *Siniperca chuatsi* (Basilewsky). *Mol. Biol. Rep.* 40: 751-756.

Li Q and Wan JM (2005). SSRHunter: development of a local searching software for SSR sites. *Yi Chuan.* 27: 808-810.

Li YC, Korol AB, Fahima T and Nevo E (2004). Microsatellites within genes: structure, function, and evolution. *Mol. Biol. Evol.* 21: 991-1007.

Liang XF, Kiu JK and Huang BY (1998). The role of sense organs in the feeding behaviour of Chinese perch. *J. Fish. Biol.* 52: 1058-1067.

Liu ZJ and Cordes JF (2004). DNA marker technologies and their applications in aquaculture genetics. *Aquaculture* 238: 1-37.

Lu X, Wang H, Dai P and Liu B (2011). Characterization of EST-SSR and genomic-SSR markers in the clam, *Meretrix meretrix*. *Conserv. Genet. Resour.* 3: 655-658.

Luo D, Sun JJ, Lu X, Liu LZ, et al. (2011). Comparative sperm ultrastructure of three species in *Siniperca* (Teleostei: Perciformes: Sinipercidae). *Micron* 42: 884-891.

Miao GD, Liu HW, Ma HY, Chen SL, et al. (2011). Isolation and characterization of 54 polymorphic microsatellite loci for half-smooth Tongue Sole, *Cynoglossus semilaevis*. *J. World Aquac. Soc.* 42: 462-467.

Qu CM, Liang XF, Huang W and Cao L (2012). Isolation and characterization of 46 novel polymorphic EST-simple sequence repeats (SSR) markers in two Sinipercine fishes (*Siniperca*) and cross-species amplification. *Int. J. Mol. Sci.* 13: 9534-9544.

Raymond M and Rousset F (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Heredity*. 86: 248-249.

Van Oosterhout C, Hutchinson WF, Wills DPM, and Shipley P (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes*. 4: 535-538.

Weber JL and May PE (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am. J. Hum. Genet.* 44: 388-396.

Yang M, Liang XF, Tian CX, Gul Y, et al. (2012). Isolation and characterization of fifteen novel microsatellite loci in golden mandarin fish (*Siniperca scherzeri*) Steindachne. *Conserv. Genet. Resour.* 4: 599-601.

Zane L, Bargelloni L and Patarnello T (2002). Strategies for microsatellite isolation: a review. *Mol. Ecol.* 11: 1-16.

Zhou CW, Yang Q and Cai DL (1988). On the classification and distribution of the Sinipercinae fishes (Family Serranidae). *Zool. Res.* 9: 113-125. (In Chinese).