GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Simple sequence repeat-based analysis of the genetic diversity and population genetic structure of populations of *Siniperca chuatsi*

C.F. Sun, X. Ye, Y.Y. Tian and J.J. Dong

Key Laboratory of Tropical and Subtropical Fisheries Resource Application and Cultivation of China Ministry of Agriculture, Pearl River Fisheries Institute, Chinese Academy of Fishery Sciences, Guangzhou, China

Corresponding author: X. Ye
E-mail: gzyexing@163.com

Genet. Mol. Res. 14 (3): 9343-9352 (2015)
Received January 8, 2015
Accepted May 11, 2015
Published August 10, 2015
DOI http://dx.doi.org/10.4238/2015.August.10.15

ABSTRACT. In order to provide genetic information for the selective breeding of *Siniperca chuatsi*, 14 microsatellite DNA loci were used to evaluate the genetic diversity and structure of four farmed populations and one wild population in China. The four cultivated populations were Foshan (FS), Jiangmen (JM), Nanjing (NJ), and Hongze Lake (HZL), and the wild population was collected from the Hubei HuangGang section of the Yangtze River (HG). All five populations exhibited high genetic diversity (H_E values of between 0.608 and 0.633); the highest was found in the wild population (H_E = 0.633). Genetic differentiation within the populations was relatively low (F_{ST} < 0.15); 5.44% of the genetic variation was between the populations and 94.56% was within the populations. The greatest genetic distance was between JM and HG (0.1894), which had the lowest genetic identity (0.8725). NJ and HG had the shortest genetic distance (0.0365) and the highest genetic identity (0.9641). A phylogenetic analysis revealed that FS, JM, and

HZL were clustered into one group, while NJ and HG were in another group, suggesting that the wild and NJ populations were closely related. Our results demonstrate that although the farmed populations have maintained a relatively high genetic diversity, they exhibit lower genetic diversity and higher genetic differentiation than the wild population. These results provide evidence that wild resources should be used for breeding, in order to maintain genetic diversity and ensure sustainable *S. chuatsi* farming.

Key words: Microsatellite; *Siniperca chuatsi*; Genetic diversity; Genetic structure; Genetic similarity; Genetic distance

INTRODUCTION

Siniperca chuatsi (Basilewsky), commonly known as the Mandarin fish, Aukua, or season flower fish, is widely distributed in major Chinese river and lake systems (Fang and Chong, 1932; Zhou et al., 1988). It is an important freshwater fish species in China, and is in high demand by domestic and international markets (Zhao et al., 2008; Chen et al., 2012). *S. chuatsi* has been farmed since the early 1970s in several provinces in China, including Guangdong, Jiangsu, Hubei, and Jianxi. Its annual yield in China is about 300,000 tons; Guangdong Province alone produces 100,000 tons. Recently, there has been a decline in the growth rate of pond-cultured *S. chuatsi*, a shortened period of sexual maturity, and reduced anti-disease ability, which seriously affect the quality and safety of the product (He et al., 2002). It is thought that this is due to germplasm degeneration, which results in decreased disease resilience in the offspring. Therefore, understanding the genetic diversity of the existing farmed populations of *S. chuatsi* can provide a scientific basis for selective breeding and germplasm resource protection.

DNA-based molecular markers can accurately identify genetic traits and information, and have greatly assisted in the development of plant and animal genetic breeding (Kumar et al., 2009). Microsatellite DNA, also known as simple sequence repeats (SSRs) or simple tandem repeats (STRs), is one of the most widely used markers (Powell et al., 1996). Microsatellite DNA is widely used in germplasm protection and genetic and quantitative trait locus mapping because of its wide distribution in the genome, high polymorphism rate, and co-dominant inheritance, and is easy to analyze (Zane et al., 2002; Liu and Cordes, 2004; Chauhan and Rajiv, 2010).

Recently, studies on the genetic diversity of *S. chuatsi* have been conducted. For example, using the random amplified polymorphic DNA (RAPD) technique, Fang et al. (2005) demonstrated that a wild population of *S. chuatsi* had high genetic diversity, while genetic diversity in a farmed population was significantly lower. Yu et al. (2012) analyzed sequences of mitochondrial DNA and found that the genetic diversity of farmed populations in Hunan and Hubei were higher than that of a Guangdong farmed population. Mei et al. (2010) used 20 microsatellite loci to analyze a Hunan original population and a Xiang River farmed population. The results revealed that the original population had higher genetic diversity than the farmed population. Tian et al. (2013) examined the genetic structure and diversity of five populations of *S. chuatsi* from the middle reaches of the Yangtze River using mitochondrial cytochrome b sequences and microsatellite markers. They found that the populations had high genetic diversity and low genetic differentiation (Tian et al., 2013).

Guangdong Province is the most important area for *S. chuatsi* farming, as well as for fry production, and provides many provinces around the country with Mandarin fish and fry. However, selectively breeding *S. chuatsi* for a better growth performance or anti-disease ability has not occurred. Guangdong and Jiangsu provinces produce most of the farmed Mandarin fish in China, and account for nearly half of the country's production. HuangGang contains most of the wild Mandarin fish in China. Therefore, it is necessary to investigate the genetic diversity of *S. chuatsi* farmed in Guangdong as well as in other main farming areas, in order to provide a scientific basis for selective breeding. In this study, using microsatellite markers we analyzed the genetic structure and diversity of four farmed populations of *S. chuatsi* from Guangdong Province and other areas, including Foshan (FS), Jiangmen (JM), Nanjing (NJ), and Hongze Lake (HZL), and one wild population from the Huanggang (HG) section of the Yangtze River.

MATERIAL AND METHODS

S. chuatsi samples

Farmed *S. chuatsi* were obtained from FS, JM, NJ, and HZL. Wild *S. chuatsi* were obtained from the Yangtze River in HG (Figure 1). Fin tissues from each fish were placed in 95% ethanol for DNA isolation.

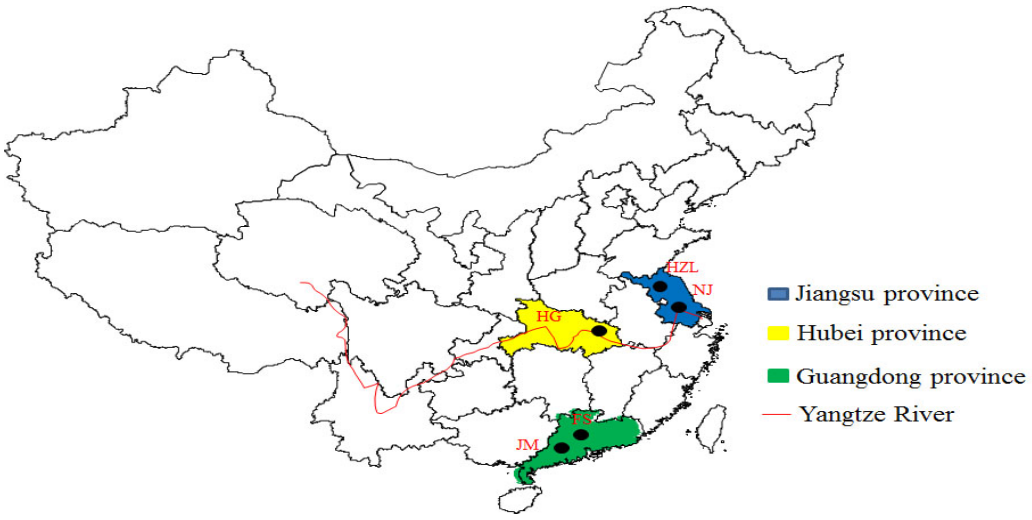

Figure 1. Sampling locations of *Siniperca chuatsi* populations in China. Foshan (FS), Jiangmen (JM), Nanjing (NJ), Hongze Lake (HZL), Yangtze River in Huanggang segment (HG).

Selection of microsatellite primers and DNA isolation

Based on the microsatellite DNA sequences of *S. chuatsi* registered in GenBank, we chose 30 pairs of primers and selected 14 pairs with high specificity, reproducibility, and polymorphisms (Table 1) (Liu et al., 2011; Qu et al., 2012). Each primer was labeled with a florescence probe (FAM, ROX, or HEX) in order to estimate the size of the amplified frag-

ments. Total DNA was isolated using a Tiangen Genomic DNA Extraction Kit (Tiangen, Beijing, China). Quality and quantity of DNA were analyzed by agarose gel electrophoresis and a spectrophotometer.

Table 1. Primer sequences and annealing temperatures for microsatellite marker amplification.

	Primer name	Repeat sequence	Primer sequence	Production size	Tm (°C)	No. of alleles	Accession No.
1	Sin112	$(AC)_{15}$	F: FAM-ATCGGCACCTGAGGCAAAAG R: GCCATCCATAGAGCCACGTC	132-166	54.5	6	JQ804768
2	Sin116	$(TG)_{14}(AG)_{7}$	F: FAM-ACAATCCCAGCCCTCCTTCT R: GCAAGGTCCCTTTACATGCAG	212-265	54.5	6	JQ804771
3	Sin130	$(GTGA)_{7}N_{7}(TG)_{8}$	F: FAM-CTCGCAGGCTTTTCTCTGCT R: AGCCATCAGTTCTGTTCTTTCTT	282-300	54.5	2	JQ804785
4	Sin134	$(TG)_{14}$	F: HEX-GCCCCCTTCTCAACCCACTA R: TGCTTTCCAAAGCGAACCGT	106-120	54.5	6	JQ804789
5	Sin143	$(GTT)_{7}$	F: HEX-AAAGCAGGCCAAACAACACC R: AGGACGGGGAGGCTTTTGAT	198-246	54.5	5	JQ804797
6	Sin147	$(TCC)_{9}$	F: FAM-AGATCAGACACCAGGAGGACC R: AAGACGGAGGCAAAGAACGAC	174-232	53.5	5	JQ804801
7	Sin152	$(AG)_{8}A(AG)_{13}$	F: HEX-TGCGCCACTTTACTGATGGG R: GCATTAACCAAACCCCGCGA	185-240	54.5	8	JQ804806
8	Sin156	$(AC)_{13}$	F: FAM-TAGGAGGCTTTACAACCGGC R: ATGACCAGCCTCAGGTGTCT	188-205	53.5	2	JQ804810
9	EST1	$(CA)_{10}$	F: FAM-CCAGCCAACAACCATAAAG R: CCCAGGTAGAAGACCGTGA	200-240	58.0	6	GR477481
10	EST-6	$(TG)_{11}(GT)_{6}$	F: HEX-TCCCAGTAGCATTCAAAC R: TGCATACATACACCCACA	135-198	61.0	8	GR477337
11	EST-19	$(CT)_{7}(CT)_{11}(TG)_{14}$	F: GACAGTACAAGTAAGGCACA R: GTCGCATAAATATCACAGAA	285-336	61.0	7	GR476867
12	EST-21	$(CA)_{15}$	F: ROX-AGTGAGGTGGAGGGGTGA R: TACGTTGCCGATGAAAGC	128-230	63.0	17	GR476843
13	EST-33	$(AC)_{13}$	F: ROX-CACTGTGCTCAACGTACT R: GTGACATTTAGCCCATAA	126-144	63.0	4	GR476286
14	Mar9	$(GT)_{21}$	F: HEX-GACATCACCAATACCTCCTGACACG R:TACACACGCATGGAGTATCTGGATC	232-397	52.0	19	GU324513

Polymerase chain reaction (PCR) amplification

The total volume (20 µL) contained 2.0 µL 10X buffer, 0.8 µL 25 mM $MgCl_2$, 0.4 µL 10 µM dNTP, 0.4 µL 20 µM of each primer, 40 ng genomic DNA, and 1 U *Taq* enzyme. The PCR consisted of 5 min of pre-denaturing at 94°C, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a final extension for 10 min at 72°C. STRs were analyzed based on the sizes of the amplified fragment, and STR genotyping was conducted by Sangon Biotech (Shanghai, China). The STR sequences were analyzed using electrophoresis (Hai Qite Analytical Instruments Co. Ltd.), a gel imaging system (Gene Genius Co. Ltd.), and a 3730XL sequence analyzer (ABI, USA). The genotype of each *S. chuatsi* individual was determined according to the size of each amplified fragment.

Data analysis

The genotype of each fish was determined by STR according to the size of the band (allele). Alleles were sorted in descending alphabetical order and analyzed with POPGENE 32. Each population of *S. chuatsi* was analyzed for number of alleles (N_A), effective num-

ber of alleles (N_E), observed heterozygosity (H_O), expected heterozygosity (H_E), the genetic similarity index (I), genetic distance between populations (D_S), and genetic similarity (S). The FSTAT software was used to calculate the genetic differentiation index (F_{ST}), and a phylogenetic tree was constructed by MEGA5 using Nei's genetic distance (Tamura et al., 2011). The CERVUS 3.0 software was used to calculate the polymorphism information content (PIC) (Kalinowski et al., 2007), and F_{ST} analysis and an analysis of molecular variance (AMOVA) were performed using ARLEQUIN 3.1 (Excoffier and Lischer, 2010; Takeda et al., 2013). The population genetic structure was analyzed by the STRUCTURE 2.3 software, and the optimal K value (number of clusters) was analyzed using the maximum likelihood method (Evanno et al., 2005; Qin et al., 2014).

RESULTS

Microsatellite polymorphisms and genetic diversity

All 14 selected primers amplified fragments from the DNA isolated from 149 individual fish belonging to the five populations. These fragments exhibited different levels of polymorphism (Table 2). N_A ranged from 3 to 15, N_E from 1.28 to 5.51, the Shannon Index (SI) from 0.392 to 2.078, H_O from 0.128 to 0.98, H_E from 0.220 to 0.822, and the PIC from 0.396 to 0.778. Except for the locus sin156 that had a PIC of 0.396 and was moderately polymorphic ($0.25 < PIC < 0.5$), the loci exhibited high levels of polymorphism ($PIC > 0.5$).

Table 2. Number of alleles, heterozygosity and polymorphic information content of in *Siniperca chuatsi* 14 microsatellite loci.

Locus	Number of allele (N_A)	Effective number of alleles (N_E)	Shannon index (SI)	Observed heterozygosity (H_O)	Expected heterozygosity (H_E)	Polymorphic information content (PIC)
EST1	6	4.83	1.646	0.541	0.796	0.597
EST6	5	2.37	1.000	0.980	0.581	0.579
EST19	5	2.56	1.087	0.226	0.611	0.720
EST21	8	5.05	1.702	0.980	0.805	0.617
EST23	4	2.43	1.022	0.569	0.590	0.735
Mar9	7	3.71	1.514	0.356	0.733	0.701
sin112	4	3.24	1.232	0.963	0.694	0.775
sin116	8	3.85	1.595	0.696	0.743	0.708
sin130	4	1.76	0.807	0.401	0.433	0.702
sin134	7	2.66	1.308	0.128	0.626	0.535
sin143	3	1.28	0.392	0.237	0.220	0.672
sin147	6	3.21	1.248	0.705	0.691	0.778
sin152	15	5.51	2.078	0.784	0.822	0.743
sin156	3	2.07	0.811	0.483	0.518	0.396
Average value	6.07	3.18	1.246	0.575	0.633	0.661

The genetic diversity of the five populations is shown in Table 3. The HG wild population had the highest N_A (5.29) and N_E (3.23), while the JM population had the lowest N_A (4.21) and N_E (2.78). The HG wild population had the highest SI value (1.197), while the HZL population had the lowest (1.096). The FS population had the highest H_O (0.596), while the NJ population had the lowest (0.494). The highest H_E was in the HG wild population (0.633), and the lowest was in the FS population (0.608). The highest PIC was in the HG wild population (0.574), while the lowest was in the JM population (0.544). These results suggest that all five

populations have relatively high genetic diversity, with the greatest genetic diversity in the wild population.

Table 3. Genetic diversity of five *Siniperca chuatsi* population.

Genetic diversity index	Foshan (FS)	Jiangmen (JM)	Nanjing (NJ)	Hongze Lake (HZL)	Huanggang (HG)
N_A	5.07	4.21	4.79	4.36	5.29
N_E	2.84	2.78	2.96	2.91	3.23
I	1.143	1.110	1.112	1.096	1.197
H_O	0.596	0.576	0.494	0.566	0.536
H_E	0.608	0.624	0.626	0.616	0.633
PIC	0.550	0.544	0.563	0.551	0.574

Inter-population genetic differentiation and analysis of genetic distance

F_{ST} values ranged between 0.0027 and 0.0814 (Table 4), suggesting that there was moderate or low differentiation ($F_{ST} < 0.05$). The lowest genetic differentiation was between the HG wild population and the NJ population ($F_{ST} = 0.0027$). The highest genetic differentiation was between the HG wild population and the JM population ($F_{ST} = 0.0814$), but was still only at a moderate level ($0.05 < F_{ST} < 0.15$) (Table 4). The results of the AMOVA indicate that only 5.44% of the genetic variation was between the populations, while 94.56% was within the populations. These results suggest that genetic variation between individual *S. chuatsi* was higher than that between populations (Table 5).

Table 4. Fixation index (F_{ST}) for five *Siniperca chuatsi* populations.

	Foshan (FS)	Jiangmen (JM)	Nanjing (NJ)	Hongze Lake (HZL)	Huanggang (HG)
Foshan (FS)	****	-	-	-	-
Jiangmen (JM)	0.0147	****	-	-	-
Nanjing (NJ)	0.0731	0.0814	****	-	-
Hongze Lake (HZL)	0.0302	0.0556	0.0759	****	-
Huanggang (HG)	0.0709	0.0714	0.0027	0.0681	****

Table 5. AMOVA analysis among five *Siniperca chuatsi* populations.

Source of variation	d.f.	Sum of squares	Variance components	Percentage variation
Among populations	4	72.020	0.24422	5.44065
Within populations	287	1214.182	4.24454	94.55935**
Total	291	1286.202	4.48876	

**Highly significant ($P < 0.01$) after 1023 permutation tests.

The inter-population Nei's genetic distance (D_A) ranged between 0.0365 and 0.1894 and S ranged between 0.8275 and 0.9641 (Table 6). The lowest D_A (0.0365) and the highest S (0.9641) were between the NJ population and the HG wild population. In contrast, the highest D_A (0.1894) and lowest S (0.8275) were between the JM population and the HG wild population. Phylogenetic analysis using the unweighted pair group method with arithmetic mean (UPGMA) revealed that the five populations could be divided into two groups, one group containing the NJ and HG populations and the other containing the FS, JM, and HZL populations (Figure 2).

Table 6. Nei's genetic distance (below diagonal) and genetic similarity (above diagonal) of five *Siniperca chuatsi* populations.

	Foshan (FS)	Jiangmen (JM)	Nanjing (NJ)	Hongze Lake (HZL)	Huanggang (HG)
Foshan (FS)	****	0.9451	0.8610	0.9325	0.8605
Jiangmen (JM)	0.0565	****	0.8275	0.8735	0.8371
Nanjing (NJ)	0.1496	0.1894	****	0.8533	0.9641
Hongze Lake (HZL)	0.0699	0.1353	0.1587	****	0.8607
Huanggang (HG)	0.1503	0.1778	0.0365	0.1501	****

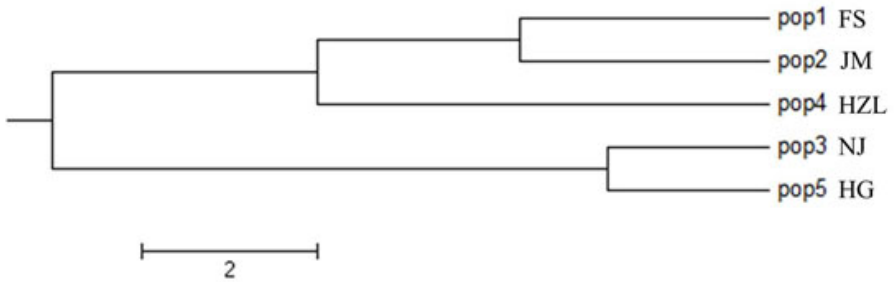

Figure 2. A UPGMA dendrograrn of 5 mandarin fish populations based on Nei's genetic distance.

Population genetic structure analysis

Clustering was conducted using the STRUCTURE software, with a hypothetical K value of between 2 and 5, using 10 replicates. Bayesian analysis, based on the parameters corresponding to each hypothetical K value, revealed that a K value of 2 occurred in the plateau phase, suggesting that all of the *S. chuatsi* included in this study could be divided into two clusters. Figure 3 shows that the FS, JM, and HZL populations formed one group and the HG and NJ populations formed another.

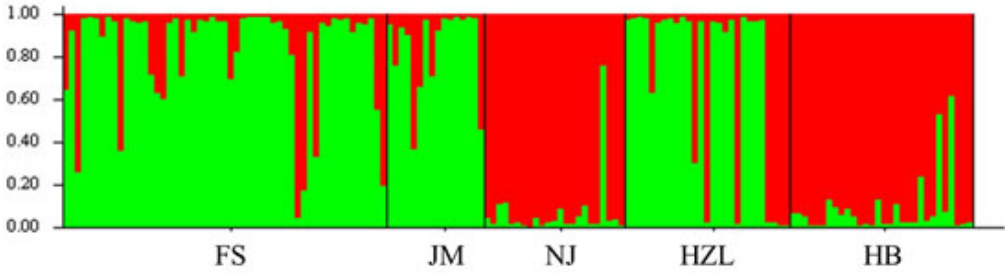

Figure 3. Cluster analysis based on 14 microsatellite loci for the *Siniperca chuatsi* specimens from STRUCTURE (K = 2).

DISCUSSION

The PIC value indicates the genetic variation and polymorphism of an allele in a population (Sanjay et al., 2000). In this study, the PIC of 14 microsatellite loci ranged from 0.396 to 0.778. According to Botstein et al. (1980), a locus is classified as highly polymorphic

when the PIC is higher than 0.5, moderately polymorphic when the PIC is higher than 0.25 but lower than 0.5, and weakly polymorphic when the PIC is lower than 0.25. Our results indicate that 13 loci were highly polymorphic and one (sin156) was moderately polymorphic.

Heterozygosity is an important indicator of population genetic variation. H_O is strongly affected by sample size, and H_E more accurately reflects population genetic diversity (Bao et al., 2007). In this study, the average H_E ranged between 0.608 and 0.633, and the highest H_E was in the HG wild population (0.633). The H_O and H_E results demonstrate that all five populations have high genetic diversity, indicating that they have the genetic basis for further breeding. It should be noted that genetic diversity in the wild population was higher than that in the four farmed populations, suggesting that the wild population has greater potential for breeding. These results are consistent with those from previous studies that found that a wild population in Poyang Lake exhibited higher levels of genetic polymorphism than a farmed population (Fang et al., 2005; Yu et al., 2012). Our results also indicate that long-term farming reduces the genetic diversity of *S. chuatsi*. To avoid inbreeding, more wild populations should be used as parents during the breeding process, in order to ensure a high genetic diversity of the breeding population.

Genetic distance and similarity are parameters that describe the genetic relationship between two populations (Cui et al., 2012). Our results show that the NJ population had the shortest genetic distance (D_A = 0.0339) and the highest genetic similarity (S = 0.9667) to the HG wild population, indicating that the closest relationship was between the NJ population and the wild population. The FS population had the greatest genetic distance (D_A = 0.1778) and the lowest genetic similarity (S = 0.8371) to the HG wild population, indicating that the weakest relationship was between the FS population and the wild population. The F_{ST} analysis obtained similar results, and the phylogenetic tree clearly demonstrates this relationship. The NJ population may have been introduced from the Yangtze River, and therefore had a similar genetic structure to the HG wild population, which was collected from the middle reaches of the river. Recently, inbreeding has become a concern. Some fish breeding companies catch wild *S. chuatsi* fry from the Yangtze River and breed them in ponds. Sexually mature adults are then chosen as parents for breeding, in order to enhance the genetic diversity of *S. chuatsi*.

The STRUCTURE software performs population simulation analysis based on an individual's genetic composition. It is not affected by the number of individuals in a population, and is an ideal tool to analyze population genetic structure (Kalinowski et al., 2007; Fu et al., 2013). All of the individual *S. chuatsi* in this study could be divided into two geographical groups, confirming the UPGMA clustering results. The genetic structure results are also consistent with the genetic differentiation results. The HG wild population had a similar genetic structure to the NJ population, and the two farmed populations in Guangdong had similar genetic structures to the HZL population. Gene flow may have occurred between the farmed populations and the wild population. These results also suggest that breeding companies should use wild *S. chuatsi* as parents, in order to increase the genetic diversity of farmed populations.

In conclusion, this study provides evidence that farmed *S. chuatsi* populations have lower genetic diversity than wild populations. However, the farmed populations have maintained a relatively high genetic diversity, suggesting that they have the genetic basis for further breeding. Genetic differentiation between the farmed and wild populations was high, indicating that long-term artificial propagation can reduce genetic diversity in farmed populations. Therefore, wild *S. chuatsi* resources should be introduced during the breeding process to

maintain the genetic diversity of farmed populations. In addition, *S. chuatsi* selective breeding should be developed in order to obtain novel varieties that exhibit rapid growth and anti-disease characteristics, to ensure the sustainable development of the *S. chuatsi* aquaculture industry.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by grants from the Science and Technology Project of Guangzhou (#20134411115) and the State Administration of Foreign Experts Affairs (#20140326016).

REFERENCES

Bao WB, Shu JT, Xu SH and Li HF (2007). Effects of sample size and sex ratio on various genetic diversity measures with microsatellite markers. *Chin. J. Anim. Sci*. 43: 6-9.

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

Chauhan T and Rajiv K (2010). Molecular markers and their applications in fisheries and aquaculture. *Adv. Biosci. Biotechnol*. 1: 281-291.

Chen DX, Chu WY, Liu XL, Nong XX, et al. (2012). Phylogenetic studies of three sinipercid fishes (Perciformes: Sinipercidae) based on complete mitochondrial DNA sequences. *Mitochondrial DNA* 23: 70-76.

Cui L, Xie CX, Li YH, Yu YY, et al. (2012). Analysis of genetic diversity among four different channel catfish populations by using microsatellite markers. *J. Huazhong Agr. Univ.* 31: 744-751.

Evanno G, Regnaut S and Goudet J (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol*. 14: 2611-2620.

Excoffier L and Lischer H (2010). Arlequin suite version 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* 10: 564-567.

Fang PW and Chong LT (1932). Study on the fishes referring to Siniperca of China. *Sinensia* 2: 137-200.

Fang ZQ, Chen J, Zheng WB, Wu YY, et al. (2005). RAPD analysis of wild population and cultivated population in *Siniperca chuatsi* Basilewsky. *J. Dalian Fish. Univ*. 3: 16-19.

Fu JJ, Li JL, Shen YB, Wang RQ, et al. (2013). Genetic variation analysis of wild populations of grass carp (*Ctenopharyngodon idella*) using microsatellite markers. *Hereditas* 35: 192-201.

He JG, Zeng K, Weng SP and Chan SM (2002). Experimental transmission, pathogenicity and physical chemical properties of infectious spleen and kidney necrosis virus (ISKNV). *Aquaculture* 204: 11-24.

Kalinowski T, Taper ML and Marshall TC (2007). Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment. *Mol. Ecol*. 16: 1099-1106.

Kumar P, Gupta VK, Misra AK, Modi DR, et al. (2009). Potential of molecular markers in plant biotechnology. *Plant Omics* 2: 141-162.

Liu ZJ and Cordes JF (2004). DNA marker technologies and their applications in aquaculture genetics. *Aquaculture* 5: 1-37.

Liu X, Luo W, Zeng C, Wang W, et al. (2011). Isolation of new 40 microsatellite markers in mandarin fish (*Siniperca chuatsi*). *Int. J. Mol. Sci*. 12: 4180-4189.

Mei QL, Liu Z, Zhang XW, Xie DZ, et al. (2010). Analysis of genetic diversity between protospecies and cultured populations of *Siniperca chuatsi* based on microsatellite markers. *Genome Appl. Biol*. 29: 266-272.

Powell W, Morgante M, Andre C, Hanafey M, et al. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breed.* 2: 225-238.

Qin Y, Sun DQ, Xu TJ, Liu XZ, et al. (2014). Genetic diversity and population genetic structure of the miiuy croaker, *Miichthys miiuy*, in the East China Sea by microsatellite markers. *Genet. Mol. Res*. 13: 10600-10606.

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.

Sanjay S, Hemant T and Robert CE (2000). On estimating the heterozygosity and polymorphism information content value. *Theor. Popul. Biol*. 5: 265-271.

Takeda M, Kusumi J, Mizoiri S, Aibara M, et al. (2013). Genetic structure of pelagic and littoral cichlid fishes from Lake Victoria. *PLoS One* 8: e74088.

Tamura K, Peterson D, Peterson N, Stecher G, et al. (2011). MEGA 5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol*. 28: 2731-2739.

Tian CX, Yang M, Liang XF, Cao L, et al. (2013). Population genetic structure of *Siniperca chuatsi* in the middle reach of the Yangtze River inferred from mitochondrial DNA and microsatellite loci. *Mitochondrial DNA* 8: 1-8.

Yu R, Liang XF, Yi TL, Zhang J, et al. (2012). Economic traits and genetic diversity of three cultured mandarin fish *Siniperca chuatsi* populations. *Fish. Sci.* 9: 511-515.

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

Zhao JL, Li CH, Zhao LL, Wang WW, et al. (2008). Mitochondrial diversity and phylogeography of the Chinese perch, *Siniperca chuatsi* (Perciformes: Sinipercidae). *Mol. Phylogenet. Evol.* 49: 399-404.

Zhou CW, Yang Q and Cai D (1988). On the classification and distribution of the sinipercinae fishes (Family serranidae). *Zool. Res*. 9: 113-125.