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Genetic diversity analysis of oriental river prawn, *Macrobrachium nipponense*, in Yellow River using microsatellite marker

H. Qiao[1], D. Lv[2], S.F. Jiang[1], S.M. Sun[1], Y.S. Gong[1], Y.W. Xiong[1], S.B. Jin[1] and H.T. Fu[1]

[1]Key Laboratory of Freshwater Fisheries and Germplasm Resources Utilization, Ministry of Agriculture, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi, China
[2]Wuxi Fishery College, Nanjing Agricultural University, Wuxi, China

Corresponding author: H.T. Fu
E-mail: fuht@ffrc.cn

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ABSTRACT. To assess the genetic status of this species, the genetic diversity of wild *Macrobrachium nipponense* from seven geographic locations in the Yellow River basin were investigated using 20 polymorphic microsatellite DNA loci. The genetic diversity between populations was indicated by the mean number of alleles per locus and mean observed heterozygosity (H) and the expected H, which was arranged from 2 to 10, from 0.4705 to 0.5731, and from 0.5174 to 0.6146, respectively. Hardy-Weinberg equilibrium analysis indicated that a deficiency of heterozygotes existed in all seven populations. Both the F_{ST} and AMOVA analyses showed that there is significant difference on population differentiation among populations. The UPGMA clustering tree demonstrated that their close relationship is consistent with their geographic proximity. The

data suggest that this Yellow River population has a wide genetic base that is suitable for breeding.

Key words: *Macrobrachium nipponense*; Genetic diversity; Yellow river; Microsatellite marker

INTRODUCTION

Oriental river prawn (*Macrobrachium nipponense*) is widely distributed in Southeast Asian countries (Yu and Miyake, 1972; Cai and Ng, 2002). As an indigenous species, it has a wide range of distribution from the southern to the northern parts of China, including rivers, lakes, reservoirs, and ditches. In recent years, the farming production of *M. nipponense* has increased quickly and reached 20.5 x 10^5 metric tons in 2008 (Bureau of Fishery et al., 2009). The increasingly economic importance of oriental river prawn culture in China prompted the current study to assess the relative levels of its genetic diversity.

The genetic definition of stocks has been a major concern in understanding natural resources to ensure the sustainability of shrimp resources. Because different broodstocks or seedstocks might differ in growth rate, or disease resistance, among other characteristics (Klinbunga et al., 2001), the identification of wild shrimp stocks is also important to provide an available source of wild genetic diversity in domestication and selective breeding programs (Brooker et al., 2000; Klinbunga et al., 2001). Currently, relative studies on *M. nipponense* have mainly focused on the development of molecular markers (Feng and Li, 2008; Zhao et al., 2010; Qiao et al., 2011; Song and Kim, 2011) and genetic diversity of the wild populations from the south rivers, mainly in the Yangtze River Basin and Qiantang River (Fu et al., 2010; Feng et al., 2011; Ge et al., 2011; Ma et al., 2011; Su et al., 2011). Previous studies on the genetic diversity of wild *M. nipponense* were mainly focused on the geographic populations of Yangtze River, Qiantang River, Taihu Lake, Poyang Lake, and Hongze Lake. Considering the fact that the Yellow River is second to the combined Ganges/Brahmaputra river system in suspended sediment load, with an estimated 1.6 billion tons of sediment annually (Milliman and Meade, 1983), research knowledge of the genetic structure and diversity of the natural crustacean populations in the Yellow River is limited. Among the molecular markers available in population genetics, microsatellite DNA markers are a useful tool for evaluating the genetic diversity and structure of various species, owing to their high variability, abundance, neutrality, co-dominance, and unambiguous scoring of alleles (Brooker et al., 2000). Previous studies have revealed the presence of subtle genetic structures at small and large geographic scales in the crustacean, such as *Penaeus monodon* (Brooker et al., 2000; Supungul et al., 2000).

Our hypothesis is that *M. nipponense* could be genetically structured along its latitudinal range of distribution owing to geographic heterogeneity. In this study, seven wild populations of *M. nipponense* were collected in or nearby the main stream of Yellow River from upstream to downstream. Twenty microsatellite markers previously developed by our laboratory were used to reveal the genetic structure and variability of wild *M. nipponense* in the Yellow River, to supply necessary information about genetic variations in different populations for setting up breeding and resource conservation programs of *M. nipponense*.

MATERIAL AND METHODS

Sample collection

Seven wild populations of oriental river prawn were collected from four provinces through which the Yellow River flows, to represent the wild genetic resources in the Yellow River, with 39 individuals from each area. Three sampling sites from downstream (Table 1) were Dongying (DY), Jinan (JN), and Dongming (DM) in Shandong Province. Three sampling sites from midstream were Jiyuan (JY) and Sanmenxia (SM) in Henan Province, and Pianguan (PG) in Shanxi Province. The only sampling site from upstream was Yinchuan (YC) in Ningxia Province, because the sediment concentration and turbulent waters of most upstream sites were not suited for survival.

Table 1. Collection sites of seven *Macrobrachium nipponense* populations in Yellow River.

Location	Sampling date	No. specimens	Sites
Dongying (DY)	October 2010	39	37°36'N, 118°45'E
Jinan (JN)	November 2010	39	36°39'N, 116°48'E
Dongming (DM)	November 2010	39	35°23'N, 115°06'E
Jiyuan (JY)	December 2010	39	34°55'N, 112°26'E
Sanmenxia (SM)	December 2010	39	34°36'N, 111°10'E
Pianguan (PG)	July 2011	39	39°19'N, 111°24'E
Yinchuan (YC)	July 2011	39	38°22'N, 106°25'E

DNA extraction and PCR

Samples from each site were stored separately in 95% ethanol in a -20°C until DNA extraction. Genomic DNA was extracted from muscle using the traditional proteinase-K digestion and phenol-chloroform extraction protocol (Sambrook and Russell, 2001).

Twenty polymorphic microsatellite loci (Qiao et al., 2011) with high expected heterozygosity values were used to investigate the genetic relationships among the seven wild oriental river prawn populations, distributed in Yellow River. The primer sequences and characteristics are listed in Table 2. PCR amplification was carried out in a 25μL reaction volume, containing 1X PCR buffer (Tiangen, Beijing, China), 30 to 50 ng genomic DNA, 0.25 μM each primer, 150 μM dNTPs, 1.5 mM $MgCl_2$, and 0.5 U *Taq* DNA polymerase (Tiangen). The PCR conditions were as follows: the DNA was first denatured at 94°C for 3 min, followed by 30 cycles including denaturation at 94°C for 30 s, annealing at 50-64°C (Table 2) for 30 s, and elongation at 72°C for 40 s, and a final elongation at 72°C for 10 min. The PCR products were size-fractionated on 6% polyacrylamide gels with silver staining. The pBR322 DNA/*Bsu*RI (*Hae*III) marker (Fermentas, Shenzhen, China) was used to determine the allele sizes.

Statistical analysis

The probability of the occurrence of null alleles and scoring error were tested using Micro-Checker (Van Oosterhout et al., 2004). The PopGen32 software (version 1.31; available at http://www.ualberta.ca/~fyeh/) was used to analyze allele frequencies, observed (H_O) and expected heterozygosities (H_E) for each locus, genetic distance (D), genetic identity (I) (Nei, 1978), statistical significance of Hardy-Weinberg equilibrium (HWE), and cluster analysis. Population

pairwise F_{ST} values based on the number of different alleles and allele frequencies were calculated using analysis of molecular variance (AMOVA) with GenAlEx6 (Peakall and Smouse, 2006; available at http://www.anu.edu.au/BoZo/GenAlEx/). The polymorphism information content (PIC), a measure of locus polymorphism, was estimated using the formula of Botstein et al., (1980). F-Statistics were calculated and their significance was tested using FSTAT version 2.9.3 (Goudet, 1995). Significance values for pairwise comparisons of population differentiation were Bonferroni corrected to account for multiple comparisons. Clustering analyses were performed using the MEGA software (version 4.1; available at http://www.megasoftware.net/mega4/mega41.html) to calculate the genetic similarity matrices, and the dendrograms were constructed by the unweighted pair-group method of arithmetic averages (UPGMA).

RESULTS

Genetic diversity across 20 loci and 7 populations

All of the microsatellite loci showed sufficient polymorphism for evaluating the genetic diversity of *M. nipponense*. Null alleles were not detected from each locus through the analysis by Micro-Checker. A total of 103 alleles were detected at the 20 microsatellite loci assayed in 7 wild *M. nipponense* populations, and the average number of alleles per locus was 5.15, with the range of 2 to10. The values of H_O ranged from 0.2644 to 0.7949, with a mean of 0.5081. The values of H_E ranged from 0.2783 to 0.7885, with a mean of 0.6003. The average observed heterozygosity was less than the expected (0.5081 ± 0.1619 and 0.6003 ± 0.1773, respectively). The mean PIC was 0.5575, ranging from 0.2811 to 0.7885 (Table 2). Apparently, the above-mentioned data showed a relatively higher genetic diversity of wild oriental river prawn in the Yellow River.

The mean number of alleles (MNE) was different among populations. The MNE in the seven populations varied from 3.5500 to 4.7000. The SM and DM populations were the most diverse populations, with the highest allelic richness at 4.7 and 4.65 respectively. H_O and H_E were slightly different amongst the populations from different sites. SM had the highest H_O (0.5731) and H_E (0.6146). YC and PG had relatively lower H_O values (0.4718 and 0.4705, respectively). YC had the lowest H_E (0.5174) amongst the populations (Table 3).

Of 140 population-locus cases (7 populations x 20 loci) examined by Hardy-Weinberg tests, 91 (65.0%) were in HWE ($P > 0.05$), whereas the other 48 (34.3%) showed significant deviation ($P < 0.05$). An exact P-value test indicated that most loci significantly deviated from HWE in the DY and JN populations with the number of 10 loci, respectively ($P < 0.05$). The deviations of HWE were also detected in the other 5 populations with the number of 7 in DM, 6 in JY, 7 in SM, 7 in PG, and 8 in YC. The F_{IS} value verified that most loci presented heterozygote deficiencies ($F_{IS} > 0$, Table 4).

Population genetic differentiation

The population differentiation was revealed by a pairwise F_{ST} coefficients matrix. According to the values of the matrix, the F_{ST} coefficients ranged from 0.0172 (between DY and DM) to 0.0791(between PG and JY). The P-values for any other two geographic populations ($P < 0.05$) indicated a genetic differentiation between any of these two populations (Table 5). AMOVA for the seven populations based on 20 microsatellite loci showed 83.61% of the total

genetic variation within the individuals. The genetic variation among populations and between individuals among populations was 6.04 and 9.42%, respectively. Statistical analysis showed a significant difference for the three variations (a = 0.01, Table 6).

Table 2. Summary statistics of 20 loci for seven *Macrobrachium nipponense* populations.

Locus (accession No.)	Sequence (5' to 3')	Ta (°C)	Product size (bp)	No. of alleles	H_O	H_E	PIC
WXM01 (GU189600)	F: TTCCCCACGCACCTCAAT R: GACATCCATGCAAAGCAACAG	54	96-100	3	0.2967	0.4241	0.3826
WXM02 (GU189601)	F: GCCATTTTCTCATAAGGGT R: ACGGTGGTATTCAGGGAT	52	172-220	10	0.7546	0.7892	0.7634
WXM03 (GU189602)	F: AGAGGCAATTGTAGCCGAGA R: TGGCACGATAGGAAGGAGTT	54	192-245	4	0.5897	0.6601	0.5993
WXM05 (GU189604)	F: ATCCTGCGAAGATCATACGG R: TGCATTTGCAATCCACTCAT	50	158-185	8	0.6703	0.8156	0.7885
WXM06 (GU189605)	F: TTGGCAAGTCTCGTCTGATG R: CGAGGA AACGCCTGCTAC	54	132-172	6	0.7216	0.8127	0.7833
WXM07 (GU189606)	F: CGACGAGGCAACAGAATA R: TGATAATGCGAGGGAGTAA	50	234-267	5	0.5604	0.6370	0.5631
WXM09 (GU189608)	F: GTCACTGACTATGAACAATAACA R: GGTTTGATCTGGAAGTTTAG	46	116-145	4	0.4066	0.5184	0.4503
WXM13 (GU189611)	F: ATGAAGGTAAGTGGCTGAGA R: TCACACGTAAAGTTTTTGGA	54	165-206	7	0.6923	0.7860	0.7512
WXM14 (GU189612)	F: GCAGCAGTAAAGCAAATGAGG R: TTGAATTCCTTGCCTCTTCC	54	115-140	5	0.5128	0.7111	0.6586
WXM16 (GU189614)	F: AATGTAAGAATCAGGGGAGA R: TCGGCAGTTTGTGGGT	54	185-225	5	0.5531	0.6601	0.6142
WXM17 (GU189615)	F: GAAAGGAACTTCAAGAGGC R: GGACAGTGAGCAAAGCATC	54	210-235	4	0.2784	0.3062	0.2811
WXM18 (GU189616)	F: TCGATGGCGACTTGCA R: CCTCTGCCCTGACTTGAA	54	115-160	5	0.3370	0.3683	0.3526
WXM20 (GU189618)	F: TCCCGATGAAAGGCACA R: TAAAGGCGGTGATGAT	52	145-188	5	0.5018	0.6130	0.5684
WXM21 (GU189619)	F: CCAACAGGTCCCATCCAAC R: GGTCGAGATTACCGATTTCAT	50	108-118	2	0.4359	0.4415	0.3436
WXM28 (GU189624)	F: ACGGCGGTAAAGAATC R: AACAGGGCAGAAAGAAA	54	223-242	4	0.3846	0.3927	0.3660
WXM31 (GU189627)	F: TCTTATCCCGTAACCTTTCC R: GCCTCATTTGCATGTCGT	52	180-265	7	0.7949	0.8055	0.7747
WXM32 (GU189628)	F: TTGTGACGCCAGAGTG R: GCTTGTAGAGCGCCTAT	52	188-216	4	0.4799	0.6005	0.5518
WXM34 (GU189630)	F: AGTAAGTAAGAGTTGGATGG R: AACAAACAACGAGGTCA	52	229-293	5	0.2711	0.2783	0.2644
WXM36 (GU189632)	F: CAGTGCTGCTTCAAGAG R: ACCGAGCATCTAACCTAC	52	145-180	5	0.3626	0.6416	0.5963
WXM37 (GU189633)	F: CTTGTGCGGTTTTCTAT R: TGGATGAAGTTTATGCC	54	180-210	5	0.5568	0.7449	0.6975
Average				5.1500	0.5081	0.6003	0.5575

Table 3. Summary statistics amongst seven *Macrobrachium nipponense* populations.

Population	*MNE*	H_O	H_E
YC	3.5500	0.4718	0.5174
PG	3.7000	0.4705	0.5438
SM	4.7000	0.5731	0.6146
JY	4.3000	0.4833	0.5440
DM	4.6500	0.4987	0.5626
JN	4.5000	0.5218	0.5735
DY	4.4500	0.5372	0.5701
Average	4.2643	0.5081	0.5609

Table 4. P value of chi-square test for Hardy-Weinberg equilibrium and F_{IS}.

Locus	DY		JN		DM		JY		SM		PG		YC	
	P	F_{IS}	P	F_{IS}	P	F_{IS}	P	F_{IS}	P	F_{IS}	P	F_{IS}	P	F_{IS}
WXM01	0.011395*	0.2884	0.031460*	0.3725	0.768794	0.0476	0.008962*	0.3329	0.284145	-0.2549	0.755655	0.0824	0.000000*	0.9100
WXM02	0.019364*	-0.1007	0.973042	-0.0798	0.563482	-0.0047	0.151738	-0.0858	0.793878	-0.0560	0.002570*	0.2524	0.430096	-0.0719
WXM03	0.000021*	-0.1421	0.006446*	0.1136	0.113934	0.0806	0.009807*	-0.2021	0.019317*	0.0334	0.017358*	0.1273	0.000000*	0.4138
WXM05	0.001112*	0.1518	0.000311*	-0.0699	0.019039*	0.0300	0.429141	0.1262	0.742849	0.0317	0.160681	0.0852	0.236046	0.1141
WXM06	0.111620	0.0362	0.202285	0.2076	0.182693	0.0155	0.071638	0.1251	0.961964	0.0076	0.727981	-0.0669	0.311795	-0.0149
WXM07	0.102233	-0.3027	0.526434	0.0880	0.000092*	0.4367	0.004355*	0.1482	0.436077	0.0626	0.099753	-0.0344	0.000000*	-0.1220
WXM09	0.419557	-0.2022	0.964320	-0.1406	0.999592	-0.0196	0.000007*	0.3393	0.039555*	0.0348	0.000187*	-0.1196	0.823063	0.0226
WXM13	0.294135	0.0475	0.399439	-0.0573	0.289799	-0.0455	0.120809	0.0946	0.193580	0.0518	0.444832	0.1596	0.005983*	0.1683
WXM14	0.003708*	0.3062	0.000000*	0.1762	0.004169*	0.2628	0.045325*	0.0196	0.046161*	0.2920	0.038065*	0.1423	0.074161	0.2213
WXM16	0.005948*	0.0578	0.801991	-0.1000	0.832192	-0.0107	0.379581	0.1857	0.268233	0.1767	0.000119*	0.2435	0.519962	0.0899
WXM17	0.973793	-0.0612	0.868949	-0.0093	0.096697	0.3413	0.973793	-0.0612	0.201983	-0.3118	0.985995	-0.0569	0.703753	-0.0685
WXM18	0.003259*	-0.0706	0.999649	-0.1103	0.002152*	0.0127	0.978377	-0.1318	0.882848	-0.1809	0.065824	0.3731	0.002039*	0.2928
WXM20	0.267717	-0.0461	0.059230	0.0795	0.108968	0.0762	0.286522	0.1746	0.043787*	0.1723	0.008083*	0.3085	0.122982	0.1113
WXM21	0.573151	0.0769	0.873169	-0.0385	0.263727	-0.1917	0.823989	0.0222	0.360076	0.1333	0.194302	0.1931	0.263727	-0.1917
WXM28	0.811653	-0.1128	0.120822	0.2435	0.723267	0.1281	0.870246	0.0189	0.345777	-0.0331	0.349771	-0.2799	0.980274	-0.1027
WXM31	0.039114*	-0.0477	0.440920	-0.0205	0.910649	-0.0091	0.624602	-0.1245	0.665195	-0.0017	0.086247	-0.1387	0.092882	-0.0526
WXM32	0.431495	0.0104	0.311873	0.1048	0.009521*	0.0447	0.239005	0.2454	0.192205	0.1443	0.000732*	0.3591	0.000038*	-0.0923
WXM34	0.987602	-0.1245	0.956007	-0.0769	0.874372	-0.0950	0.270756	-0.0363	0.776296	-0.0578	1.000000	-0.0130	-	-
WXM36	0.000000*	0.5290	0.000017*	0.3224	0.000000*	0.4684	0.000108*	0.4406	0.000000*	0.4701	0.293050	0.0757	0.970883	0.0294
WXM37	0.000001*	0.2012	0.000058*	0.3662	0.028288*	0.2615	0.223520	0.1038	0.000000*	0.0492	0.000031*	0.3738	0.422740	0.0557

F_{IS} = inbreeding coefficient; P = probability of significant deviation from Hardy-Weinberg equilibrium; *$P < 0.05$.

Table 5. Genetic differentiations in seven geographic populations of *Macrobrachium nipponense*.

Population	DY	JN	DM	JY	SM	PG	YC
DY							
JN	0.0177*	-					
DM	0.0172*	0.0203*	-				
JY	0.0418*	0.0488*	0.0525*	-			
SM	0.0260*	0.0298*	0.0237*	0.0393*	-		
PG	0.0538*	0.0572*	0.0644*	0.0791*	0.0518*	-	
YC	0.0559*	0.0641*	0.0650*	0.0706*	0.0517*	0.0316*	-

F_{ST} values are below the main diagonal and *P < 0.05.

Table 6. Analysis of molecular variance (AMOVA) for 7 geographic populations based on 20 microsatellite loci.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variance
Among populations	6	68842.694	120.814	6.04%*
Between individuals among populations	266	545368.128	188.547	9.42%*
Within individuals	273	456773.500	1673.163	83.61%*
Total	191	1070984.322	18.669	-

*a = 0.01.

Population genetic relationships

The Nei unbiased genetic distances (Nei, 1978) between each two different populations was from 0.0455 to 0.2223 (Table 7). The data of genetic distance matrix (DSA) showed that populations DM and DY had the nearest genetic distance, whereas the farthest genetic distance was between populations PG and JY. The genetic relationships between populations were further confirmed by using the UPGMA in PopGen32 (Figure 1). As this phylogenetic tree shows the relationship between DM and DY was the closest. JN was clustered with DM and DY and then clustered with SM. PG and YC were also close. These four populations (DM, DY, JN, and SM) were clustered together and then clustered with JY and finally with PG and YC.

Table 7. Inter-population genetic identification (up triangle) and genetic distances (down triangle) between 7 geographic populations.

Population	DY	JN	DM	JY	SM	PG	YC
DY	*****	0.9532	0.9555	0.8938	0.9261	0.8616	0.8640
JN	0.0480	*****	0.9471	0.8742	0.9141	0.8511	0.8417
DM	0.0455	0.0543	*****	0.8667	0.9343	0.8346	0.8423
JY	0.1123	0.1345	0.1431	*****	0.8937	0.8007	0.8330
SM	0.0767	0.0898	0.0680	0.1124	*****	0.8570	0.8670
PG	0.1490	0.1612	0.1808	0.2223	0.1543	*****	0.9285
YC	0.1462	0.1724	0.1717	0.1827	0.1428	0.0742	*****

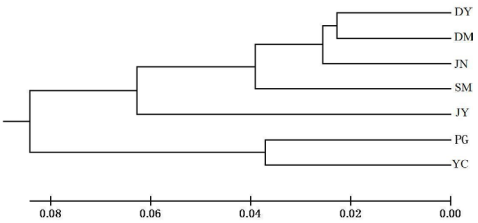

Figure 1. Cluster analysis result among seven geographic populations. Unweighted pair group method with arithmetic mean (UPGMA) in MEGA software (version 4.1) were used for this analysis.

DISCUSSION

Genetic diversity is an important basis of germplasm resources assessment, and high genetic diversity indicates good survival capability and breeding potential (O'Connell and Wright, 1997). The mean allele number and heterozygosity are used as estimators to evaluate genetic characteristics and diversity. In the present study, the mean observed heterozygosity of Yellow River populations were from 0.4705 to 0.5731, which was higher than that of Qiantang River (0.56 to 0.66) and Poyang Lake (0.54 to 0.67) populations, and lower than Taihu Lake wild populations (0.683 to 0.835) (Feng et al., 2011; Ma et al., 2011; Su et al., 2011), suggesting that the Yellow River populations in this study represented higher molecular diversity compared with the Qiantang River and Poyang Lake populations but had less diversity than the Taihu Lake populations. In the present study, the observed mean heterozygosity was lower than the expected mean heterozygosity for all populations, and the values within the population differentiation were positive, indicating the validity of inbreeding within those populations. Compared with previous reports on genetic diversity analysis of *M. nipponense* populations in Yangtze River, Poyang Lake, and Taihu Lake, respectively, similar results were observed in this study, in that population-locus cases exhibited departures according to the statistical significance of HWE. It is very likely that random variation and/or the small sample size might account for the HWE departures. If a population is in HWE, the value of H_E is close to H_O, and the population deviates from HWE as a result of the excess and/or deficit of heterozygotes reflected by the inbreeding coefficient (F_{IS}) (Quan et al., 2006). Considering the fact that H_E was apparently higher than H_O, heterozygote deficiency seems to be the strongest evidence for all these deviations from HWE. There is reasonable explanation that heterozygote deficiency may include natural selection, phenotypic assortative mating, and inbreeding. Such phenomena are worth paying enough attention to in germplasm conservation because it might be a potential risk for *M. nipponense* to rapidly lose genetic diversity and suffer further inbreeding depression. Through comparison of the H_O and HWE, it may be concluded that *M. nipponense* of the Yellow River is in a better condition, suggesting that the wild *M. nipponense* populations in the Yellow River have retained considerable amounts of genetic diversity within individuals. These baseline data can be effectively used in further aquaculture breeding of *M. nipponense*.

In this study, the pairwise F_{ST} coefficients showed that the Pianguan and Yinchuan populations in the middle and upper reaches of the Yellow River presented a significant difference to other populations in the lower reaches. Such a phenomenon may be attributed to the special geologic conditions of the middle and upper reaches of the Yellow River. Both the F_{ST} and AMOVA analyses showed that there was significant difference in population differentiation among the seven populations, which was consistent with some earlier reports on the genetic diversity studies of wild *M. nipponense* populations in different geographic locations (Fu et al., 2010; Feng et al., 2010; Ma et al., 2011). Taking our results together with literature evidence, we found that population differentiation was higher in rivers than in lakes according to the comparison of F_{ST} and AMOVA analyses. This could be explained by the higher capability of dispersal in lake environments. The UPGMA clustering tree based on genetic distances demonstrated that their close relationship is consistent with their geographic proximity. The pairwise genetic distances were directly related to the geographic distances, suggesting that dispersal is spatially limited owing to the geographic distance and the tendency of individuals to find mates from nearby populations rather than from distant populations.

Genetic variations are critically important for genetic improvement of farmed populations (Gjedrem and Baranski, 2009). So far, our laboratory has reported that the breeding work of *M. nipponense* is mainly in southern China and made some progress on interspecific hybridization, which is an available way to create new germplasms and enrich breeding materials (Fu et al., 2004), but many new different breeding materials and schemes are still urgently needed to respond to the problems arising in aquaculture. The wild populations of *M. nipponense* in the Yellow River studied here could be good starting materials for aquaculture and for initiating a selective breeding program for genetic improvement.

In conclusion, microsatellite markers were effective in assessing the genetic variability within genotypes of *M. nipponense*, given the large number of markers identified and the high polymorphisms found, and the data suggest that this Yellow River population has a wide genetic base that is suitable for breeding. It also reminds us of the urgent need to conserve the abundant resources of *M. nipponense* in the Yellow River.

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