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SSR Marker-Based Genetic Resource Assessment of the Rainbow Clam *Moerella iridescens* Along the Coasts of China: Implications for Strategy of Conservation Management

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This study aims to determine the genetic structure of rainbow clam *Moerella iridescens* in different sea areas of China. Seventeen pairs of microsatellite primers (SSR) were used to amplify the SSRs of rainbow clam in Lianyungang of Haizhou Bay, Chongming of Shanghai, Ningde of Fujian, Daishan of Zhoushan, and Cixi and Wenzhou of Zhejiang. A total of 1,146 alleles were detected in 310 individuals from the 17 SSR loci. The average observed heterozygosity of six populations was 0.4381−0.6139, the average expected heterozygosity was 0.5897−0.7325, and the average Shannon diversity index was 1.2655−1.7998. The clams exhibited rich genetic diversity, and the F_{ST} of the genetic differentiation index of the six populations was 0.0470, indicating low genetic differentiation among the populations. The results indicated that rainbow clams along the coasts of China exhibited high diversity and low population differentiation.

Keywords: ***Moerella iridescens*, SSR, genetic diversity, China coasts, conservation management**

HIGHLIGHTS

- This study aims to determine the genetic structure and diversity of rainbow clam *Moerella iridescens* in different sea areas of China.
- The clams exhibited rich genetic diversity and the F_{ST} of the genetic differentiation index of the six populations was 0.0470, indicating low genetic differentiation amongst the populations.
- The results indicated that rainbow clam along China coasts exhibited high diversity and low population differentiation.

INTRODUCTION

The rainbow clam *Moerella iridescens* is an economically important small-sized marine clam due to its delicious taste and high nutritional value. The clam is mainly distributed on the West Pacific coast and northern Australia. In recent years, the rainbow clam has been increasingly used as high-valued seafood and is preferred by consumers of China, and the output of the rainbow

TABLE 1 | Sampling time, place, and sample size of rainbow clam.

Stock	LYG	CM	ND	ZS	CX	WZ
Sampling time	2012·10	2012·10	2012·10	2012·10	2012·10	2012·10
Number of samples	50	50	50	50	50	50
Sampling site	Sanyang harbor, Haizhou Bay	East beach, Chongming Island	Xiabaishi, Sansha Bay in Fu'an	Datian Bay, Daishan, Zhoushan	Cixi Sanbei shoal, Hangzhou Bay	Simon Island, Yueqing Bay
GPS	E119.2835 N34.7996	E122.0058 N31.5316	E119.6263 N26.7948	E122.2095 N30.2888	E121.4337 N30.2998	E121.2031 N28.3229

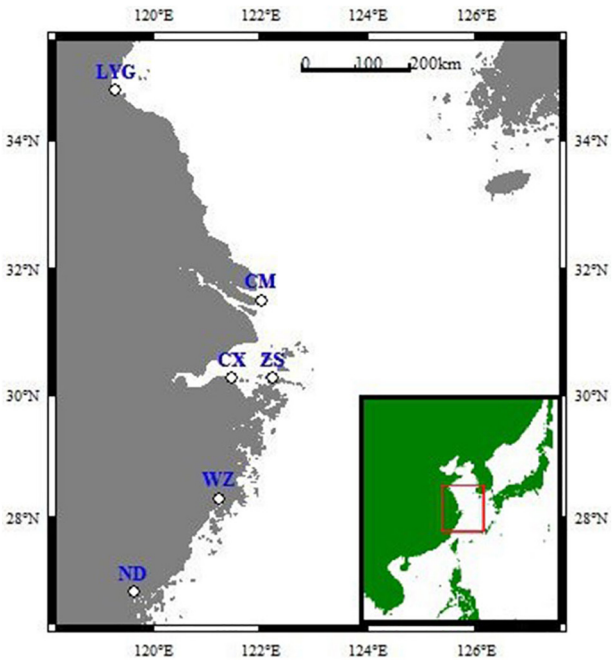

FIGURE 1 | Sample collection map of rainbow clams in coastal China.

clams mainly comes from marine fishing. However, in the past 2 years, the development of the shrimp and crab aquaculture industry and the overfishing of wild rainbow clams have exacerbated the destruction of the habitat of rainbow clams. Moreover, the status of genetic diversity of the germplasm resources of rainbow clams remains unclear. Thus, the protection of rainbow clam resources is of great importance. Several authors have explored the biological characteristics and morphological differences of rainbow clams (Ji et al., 2007; Lv et al., 2012). To date, however, reports on the genetic diversity and relationships among clam populations are limited (Xu et al., 2016).

Microsatellites, also known as simple repetitive sequences (SSRs), present the advantages of co-dominance, single locus, high polymorphism, and easy operation and can be screened from different populations to complete genetic diversity studies (Cui et al., 2011). Microsatellite marker technology has been widely used in the structural analysis of the population genetics of aquatic animals, e.g., the rainbow trout (Zhao et al., 2010), the scallop *Patinopecten yessoensis* (Chang et al., 2007), and the triangle pearl *Hyriopsis cummingii* (Bai et al., 2015). So far, few reports are available on the genetic diversity of the population of rainbow clams. In the present study, the genetic diversity of six populations of rainbow clams in Lianyungang, Chongming Island, Ningde, Zhoushan, Cixi, and Wenzhou was analyzed by using microsatellite markers. The results obtained can provide a scientific basis for assessing germplasm resources and genetic diversity protection of rainbow clams.

MATERIALS AND METHODS

Materials

Rainbow clams were collected from the six coastal mudflats, namely, Lianyungang Haizhou Bay (LYG), Chongming Island Dongtan (CM), Ningde Fu'an (ND), Zhoushan Daishan (ZS), Hangzhou Bay Cixi (CX), and Wenzhou Yueqing Bay (WZ). Fifty samples were randomly obtained from each population. Information on the samples collected is presented in **Table 1** and **Figure 1**. The sampled rainbow clams were stored at −70°C until analysis.

Microsatellite Primer

The M13 (−21) universal primer sequence of 5′-TGTAAAACGACGGCCAGT-3′ reported by Schuelke (2000) was adopted in this study to elongate short primers for economic consideration in genotyping. We used two fluorescent labels, FAM and HEX, for forwarding primes, which were abbreviated as FAM-M13 and HEX-M13. Nineteen SSRs were used for PCR, and the information of these primers is shown in **Table 2**.

Method

Total DNA was extracted with the sodium dodecyl sulphate (SDS) phenol-chloroform method and according to the detailed protocol for the DNA extraction as previously described by Li et al. (2020). The PCR system and program in this study was done as previously described by Zhang et al. (2019). Specifically, the used PCR system consisted of 1 μl of template DNA (about 30 ng), 2 μl of primer mix, 1 μl of universal fluorescent primers, 10 μl of 2 × Taq PCR Master Mix (TaKaRa, Dalian, China), and 6 μl of ddH_2O to form a total volume of 20 μl. The PCR program was as follows: pre-denaturation at 90°C for 5 min, 30 cycles, denaturation at 94°C for 3 min, annealing at 53°C for 1 min

TABLE 2 | Microsatellite primer information.

SSR	Repeat motif	Sequence (5′–3′)*	Size of original fragment/bp	(Tm)/°C
HGZ2	(AC)17(CA)11	F: TGAGGTGGAATGAGTTAC R: TAAGTTCGGATGACAAAG	124	45
HGZ3	(GT)22	F: ATGGGAGACAACTCGCTAC R: CTGTCACAACGGCAATCT	353	52
HGZ6	(TG)4	F: GGGCCAAATCAGGGAATG R: AGCAGGAAACGCAGCACA	103	58
HGZ9	(AC)9(AC)7(CA)7	F: CAGCCTGGGCAACATAGT R: TAGGACCACAGGTAAGCATC	116	53
HGZ10	(GT)6(GT)8(GT)6	F: AGGTAGGGCGTGAAGGAA R: GCAAAATCGACCCTACTACATA	193	55
HGZ14	(CA)6	F: ACTAGTACGTGAAGATTAGCCAA R: GAGGCGATACTCATAATGTTCA	338	55
HGZ17	(AC)26(CA)21	F: ATGAGAGAGCGACAGAATG R: TAGAGGCTCCCTAAATGG	150	50
HGZ22	(GA)19(AG)12	F: TTTCCACTTCGCACATTG R: CTCGCACAAACAAATGAAC	196	52
Mir8	(GT)15	F: GTAGGTTTGGCATGGCTTTGTAGC R: ACCATTGAGGGCTCGTCTGAATTAT	124	64
Mir12	(AG)16	F: TCACCAGAAAGGAGACCGTAAAAGT R: CTACGGATTTCGCAGTGAAAATGT	120	63
Mir13	(AC)15	F: GCAACACAACGAGAGTG R: ACAACAAACAACAAAGAAAT	116	47
Mir14	(CT)4	F: ATCGTTGGGGCATTCTAGTTTTCT R: GGGTATAATAATTTTGAAACGCAGC	83	62
MH19A	(TG)28	F: GTGAGCAGGAATCAAAGGTG R: CTCCGCTCTGTTTGCCTAT	105–145	55
Mi44A	(TG)61	F: CCTCGGAGACCATTCGCTAC R: TGCTTTTCTATGACAACCCT	85–101	52
MW33A	(TG)13TA(TG)5(CG)2TGCG	F: TTCCTATCCTTACCCTTG R: CTGACTGGAAACTCAACAC	111–171	48
MW15A	(CA)24	F: GATCAAAATTGACAAGGCT R: AAGACAAACACGGATGGT	88–150	46
MX39C	TGTT(TG)6(GG)4GT	F: CCCAACCAGAATAATACCA R: TCCAACAAAGGAATACGATA	200–220	48
MY36B	(TG)7(GG)3	F: CCGTTGGTAAAGACGATAT R: TGGTTGCGAGTTGGACAC	251–283	58
MZT46B	(TG)75	F: GACATAAAGGTTGTAGGGA R: ATGGTAGTGATGATGCTTG	151–283	46

**Primers of forward sequence tailed with universal M13 (–21) sequences (5′-TGTAAAACGACGGCCAGT-3′) at their 5′ ends.*

and at 72°C for 30 s, and a final extension at 72°C for 10 min at the end of the cycle. The PCR products were subjected to capillary electrophoresis, and the electrophoresis patterns were genotyped by GeneMapper 3.7 and Peak Scanner software.

Data Processing

Pop32 software was utilized to calculate the number of effective alleles (*Ne*), expected heterozygosity (*He*), observed heterozygosity (*Ho*), and gene flow ($Nm = 1- F_{ST}/4 F_{ST}$, where the F_{ST} represent genetic differentiation index), genetic distance (*Ds*), and Shannon diversity index (Raymond and Rousset, 1995). Population clustering was analyzed using the unweighted pair-population method with arithmetic means (UPGMA) of the MEGA 3.0 software. Analysis of molecular variance (AMOVA) was performed using Arlequin 3.11software, and the genetic diversity and genetic differentiation index (F_{ST}) were computationally analyzed.

RESULTS

Genetic Diversity of the Rainbow Clam

There were 1,146 alleles that were successfully detected from the genomes of rainbow clam population *via* 17 SSR loci scanning in the six populations. Despite this, the HGZ22 in the Wenzhou population failed to be amplified by PCR. Except for the alleles detected at the two loci of HGZ2 and HGZ14, the remaining alleles were highly polymorphic loci, which indicated that the 17 microsatellite loci could be used to study the population genetics of rainbow clam (**Table 3**).

The average number of alleles among the 17 loci in the six populations was 5.5000–22.5000 (17 loci average, 11.9039),

TABLE 3 | Genetic diversity of six populations of rainbow clams.

Pop	SSR	MH19A	Mi44A	MW15A	MX39C	MY36B	MZT46B	HGZ3	HGZ6	HGZ9	Mir8	Mir12	Mir14	HGZ10	HGZ17	HGZ22	Mir13	MW33A	Mean
ZS	Sample No.	96	100	100	98	84	96	100	100	100	96	96	94	96	92	10	14	34	83
	Na	17	7	20	18	27	6	19	3	10	10	21	21	5	7	5	9	13	12.8235
	Ne	7.5789	3.3003	4.1391	3.2035	17.9086	2.3226	8.7260	1.1064	5.6243	5.8701	7.1888	4.3829	1.6317	2.0564	3.8462	6.5333	9.6333	5.5913
	Ho	0.8542	0.9600	0.6200	0.8980	0.2619	0.8958	0.0400	0.0600	0.8800	0.8125	0.9583	0.8085	0.0000	0.2174	0.2000	0.4286	0.1765	0.5336
	He	0.8772	0.7040	0.7661	0.6949	0.9555	0.5754	0.8943	0.0972	0.8305	0.8384	0.8700	0.7801	0.3912	0.5194	0.8222	0.9121	0.9234	0.7325
	I	2.3251	1.3946	1.9772	1.8728	3.0815	1.0159	2.5069	0.2322	1.9486	1.9286	2.4370	2.0440	0.8046	1.0947	1.4708	2.0449	2.4172	1.7998
ND	Sample No.	78	92	96	98	78	86	98	98	98	100	96	94	100	96	54	50	50	86
	Na	18	8	30	10	15	24	8	14	2	8	9	10	21	5	5	7	7	9
	Ne	11.7000	2.9782	11.1036	2.7805	18.2156	2.3012	5.5514	1.0416	5.2082	3.6928	3.3932	3.8518	1.3951	1.4908	4.7032	4.4326	5.6306	5.2630
	Ho	0.7692	1.0000	0.8333	0.8163	0.4615	0.9767	0.0204	0.0408	1.0000	0.9800	0.9583	0.7447	0.0000	0.1042	0.0000	0.0000	0.0000	0.5121
	He	0.9264	0.6715	0.9195	0.6470	0.9574	0.5721	0.8283	0.0404	0.8163	0.7366	0.7127	0.7483	0.2861	0.3327	0.8022	0.7902	0.8392	0.6839
	I	2.6503	1.3328	2.8777	1.6638	3.0326	1.0217	2.0495	0.0996	1.7543	1.5876	1.4903	2.0490	0.6114	0.7219	1.7032	1.6550	1.9249	1.6603
CX	Sample No.	90	100	100	96	82	88	98	100	100	94	100	98	100	94	58	96	26	89
	Na	22	5	30	18	28	5	11	4	15	11	6	18	7	5	14	8	9	12.7059
	Ne	11.3764	4.0750	6.7843	5.2364	15.0089	1.9979	2.4984	1.3369	6.8871	7.1143	2.9656	3.0703	1.8997	2.0771	8.7604	2.7560	6.1455	5.2935
	Ho	0.9111	1.0000	0.9400	0.9167	0.6585	0.7045	0.7755	0.0000	0.9400	0.9362	0.9600	0.6735	0.0600	0.4255	0.1034	0.3542	0.0769	0.6139
	He	0.9223	0.7622	0.8612	0.8175	0.9449	0.5052	0.6059	0.2545	0.8634	0.8687	0.6695	0.6813	0.4784	0.5241	0.9014	0.6439	0.8708	0.7162
	I	2.7154	1.4715	2.5566	2.2264	2.9907	0.8671	1.3413	0.5388	2.2126	2.0765	1.2420	1.7969	0.9429	1.0515	2.3665	1.2693	2.0008	1.7451
CM	Sample No.	108	120	114	112	90	116	116	120	120	120	118	92	110	118	36	48	28	99
	Na	21	5	17	15	25	9	11	5	11	8	22	10	4	4	10	17	9	11.9412
	Ne	12.7615	2.3614	4.3995	2.1261	8.8621	2.3168	1.4771	1.4682	3.9067	5.1173	6.0592	1.6067	2.0481	1.6927	7.0435	14.0488	7.6863	4.9989
	Ho	0.9815	1.0000	0.3333	0.4464	0.4667	0.5690	0.1207	0.3500	0.9167	0.9500	0.7797	0.0870	0.0000	0.0678	0.1111	0.1250	0.1429	0.4381
	He	0.9303	0.5814	0.7795	0.5344	0.8971	0.5733	0.3258	0.3216	0.7503	0.8113	0.8421	0.3817	0.5164	0.4127	0.8825	0.9486	0.9021	0.6701
	I	2.7482	0.9969	1.9305	1.4246	2.6834	1.1756	0.8329	0.6585	1.6460	1.7885	2.3283	0.9337	0.9241	0.7746	2.1073	2.7358	2.1138	1.6355
WZ	Sample No.	100	98	96	56	82	76	96	100	100	100	100	56	96	94	–	12	12	85
	Na	23	12	18	10	9	8	2	5	8	9	10	3	4	2	–	5	5	12.3648
	Ne	11.5207	3.6051	7.1331	1.6049	1.4038	2.3442	1.6528	1.5494	4.3365	5.3022	3.1250	1.1979	2.0907	1.2605	–	4.5000	4.8000	3.8135
	Ho	1.0000	1.0000	0.8333	0.3214	0.1220	1.0000	0.0000	0.3200	0.9600	0.9000	0.9600	0.0357	0.0000	0.0638	–	0.0000	0.1667	0.5102
	He	0.9224	0.7301	0.8689	0.3838	0.2912	0.5811	0.3991	0.3582	0.7772	0.8196	0.6869	0.1682	0.5272	0.2089	–	0.8485	0.8636	0.6266
	I	2.7197	1.6343	2.2915	0.9778	0.7424	1.0546	0.5841	0.7232	1.6415	1.7577	1.3815	0.3456	0.8837	0.3609	–	1.5607	1.5890	1.6135
LYG	Sample No.	98	100	94	100	72	86	94	100	100	92	100	100	100	90	48	88	30	88
	Na	21	5	20	16	29	5	4	2	17	18	4	20	3	10	13	13	14	12.5882
	Ne	12.0957	3.9777	4.6456	2.0080	18.6475	1.9091	1.5314	1.0202	10.0402	10.2470	2.5316	2.7397	2.0358	3.1395	7.0675	2.7172	11.8421	5.7762
	Ho	0.8571	1.0000	0.8085	0.5200	0.8056	0.3488	0.3830	0.0200	0.7000	0.6957	0.9600	0.6600	0.0000	0.2000	0.0417	0.2727	0.1333	0.4945

(Continued)

TABLE 3 | (Continued)

Pop	SSR	MH19A	Mi44A	MW15A	MX39C	MY36B	MZT46B	HGZ3	HGZ6	HGZ9	Mir8	Mir12	Mir14	HGZ10	HGZ17	HGZ22	Mir13	MW33A	Mean
	He	0.9268	0.7562	0.7932	0.5071	0.9597	0.4818	0.3507	0.0200	0.9095	0.9123	0.6111	0.6414	0.5139	0.6891	0.8768	0.6392	0.9471	0.6786
	I	2.7247	1.4155	2.0706	1.3773	3.1140	0.9262	0.6279	0.0560	2.5136	2.5504	1.0406	1.7503	0.8030	1.4816	2.2518	1.5439	2.5520	1.6941
Mean	Sample No.	95	101.7	100.0	93.3	81.3	91.3	100.3	103.0	103.0	100.3	101.7	89.0	100.3	97.3	41.2	51.3	30.0	88.3
	Na	20.3333	7.0000	22.5000	14.5000	22.1667	9.5000	9.1667	5.5000	10.5000	10.6667	12.0000	13.6667	7.3333	5.5000	9.4000	9.8333	9.5000	11.9039
	Ne	11.1722	3.3830	6.3675	2.8266	13.3411	2.1986	3.5729	1.2538	6.0005	6.2240	4.2106	2.8082	1.8502	1.9528	6.2842	5.8313	7.6230	5.1287
	Ho	0.8955	0.9933	0.7281	0.6531	0.4627	0.7491	0.2233	0.1318	0.8995	0.8791	0.9294	0.5016	0.0100	0.1798	0.0912	0.1968	0.1161	0.5171
	He	0.9176	0.7009	0.8314	0.5975	0.8343	0.5482	0.5674	0.1820	0.8245	0.8312	0.7321	0.5668	0.4522	0.4478	0.8570	0.7971	0.8910	0.6847
	I	2.6472	1.3743	2.2840	1.5905	2.6074	1.0102	1.3238	0.3847	1.9528	1.9482	1.6533	1.4866	0.8283	0.9142	1.9799	1.8016	2.0996	1.6914

Ne, the number of effective alleles; Na, observed number of alleles; Ho, observed heterozygosity; He, expected heterozygosity; I, Shannon's Information index.

the *Ne* was 1.2538−13.3411 (average, 5.1287), the *Ho* was 0.0100−0.9933 (average, 0.5171), the *He* was 0.1820−0.9176 (average, 0.6847), and the Shannon diversity index was 0.3847−2.6472 (average, 1.6914).

The average number of alleles of the six populations was 8.3125−12.8235, the *Ne* was 3.8135−5.7762, and the *Ho* was 0.4381−0.6139 (average, 0.5121). Among the populations studied, the *Ho* of CX was the highest, whereas the *Ho* of CM was the lowest. The average *He* was 0.6266−0.7325 (average, 0.6847). The Zhoushan population was the highest, whereas the Wenzhou population was the lowest. The average Shannon diversity indices were 1.7998 (ZS), 1.7451 (CX), 1.6603 (ND), 1.6941 (LYG), 1.6355 (CM), and 1.2655 (WZ). These results showed that, although the genetic diversities of the six wild populations of rainbow clam were different, the overall genetic diversity was high (**Table 3**).

Phylogenetic Relationships of the Six Populations of Rainbow Clams

Analysis of Molecular Variance

Analysis of molecular variance (AMOVA) of the six different geographical populations of rainbow clams showed that 4.70% of their genetic variation could be derived from the population, while 95.30% of their variation was from within the population. The F_{ST} of the populations was 0.0470, indicating that the degree of genetic differentiation among the populations was low (**Table 4**).

Genetic Distance

The *Ds* among the six populations of rainbow clam was 0.0668−0.3020. The *Ds* between the Wenzhou and Zhoushan populations was the largest (0.3020), and their genetic relationship was far. In contrast, the *Ds* between the Lianyungang and Cixi populations was the smallest (0.0668), and their genetic relationship was relatively close (**Table 5**).

Genetic Differentiation Index and Gene Flow

Among those of the different populations, the F_{ST} between the Chongming and Zhoushan populations was the highest (0.0790), whereas that between the Cixi and Lianyungang populations was the lowest (0.0185). The *Nm* values between the Lianyungang and Cixi populations and those between the Ningde and Zhoushan populations were 13.2635 and 10.4019, respectively. The *Nm* between the Chongming and Zhoushan populations was 2.9146. The total F_{ST} between populations was 0.04702. $F_{ST} < 0.05$ indicates a low degree of genetic differentiation, which, in turn, reveals rich genetic diversity. The overall differentiation degree among populations was low (**Table 6**).

Cluster Analysis

Based on the genetic distances among the populations, a clustering map was constructed using the UPGMA method of the MEGA3.0 software. The six populations of rainbow clam could be divided into the following three branches: LYG and CX in the first branch, CM and WZ in the second branch, and ZS and ND in the third branch (**Figure 2**).

TABLE 4 | Analysis of molecular variance among six populations of rainbow clam.

Source of variation	d.f.	Sum of square	Variance component	Percentage of variation (%)
Among populations	5	100.815	0.16327 Va	4.70
Within populations	614	2031.833	3.30917 Vb	95.30
Total	619	2132.648	3.47245	
Fixation Index			F_{ST}: 0.04702 ($P < 0.05$)	

TABLE 5 | Nie's unbiased genetic distance of six populations of rainbow clams.

Populations	ZS	ND	CX	LYG	CM	WZ
ZS						
ND	0.1196					
CX	0.1680	0.1921				
LYG	0.2157	0.2540	0.0668			
CM	0.2297	0.1986	0.1160	0.1140		
WZ	0.3020	0.2292	0.2204	0.2185	0.1228	

TABLE 6 | Genetic similarity coefficient and gene flow between six populations of rainbow clams.

Populations	ZS	ND	CX	LYG	CM	WZ
ZS	****	10.4019	6.0283	3.7538	2.9146	3.4532
ND	0.0235	****	4.9507	3.3291	3.6003	6.2250
CX	0.0398	0.0481	****	13.2635	6.1635	7.1116
LYG	0.0624	0.0699	0.0185	****	5.4267	5.7052
CM	0.0790	0.0649	0.0390	0.0440	****	9.2629
WZ	0.0675	0.0386	0.0340	0.0420	0.0263	****

*Genetic similarity coefficient above the diagonal, gene flow below the diagonal. The symbol **** represents blank values.*

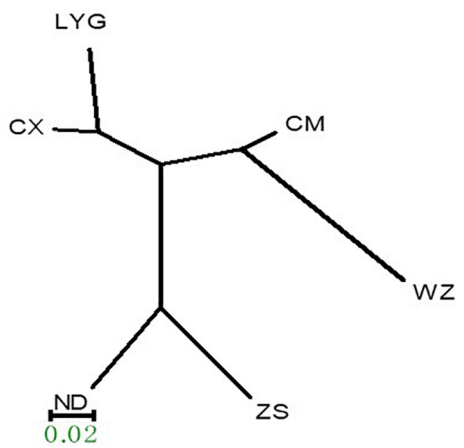

FIGURE 2 | Rainbow clam six populations of unweighted pair-population method with arithmetic means (UPGMA) trees.

DISCUSSION

Genetic differentiation index (F_{ST}) is an important parameter for measuring the degree of genetic differentiation in a population. A large F_{ST} value indicates a high degree of differentiation between populations. No differentiation exists when F_{ST} is 0−0.05, moderate differentiation is observed when F_{ST} is 0.05−0.15, high differentiation is obtained when F_{ST} is 0.15−0.25, and great differentiation is found when $F_{ST} > 0.25$ (Hartl and Clark, 1997). In this study, the F_{ST} of the six populations under study was 0.047 (<0.05). Overall, the differentiation between the populations was minimal at best. However, the F_{ST} values of the ZS−LYG, ZS−CM, ZS−WZ, LYG−ND, and CM−ND populations were in the range of 0.0624−0.0790 (0.05), which indicates that moderate genetic differentiation existed between the Zhoushan and each of the Lianyungang, Chongming, and Wenzhou populations and between the Ningde and each of the Lianyungang and Chongming populations of rainbow clams. *Nm* can indicate the degree of genetic differentiation in a population. If $Nm < 1$, genetic differentiation occurs among populations. If $Nm > 1$, genetic differentiation is relatively low. If $Nm > 4$, genetic differentiation is very low (Ratnaningrum et al., 2017). The results of AMOVA showed that the *Nm* values among the six populations of rainbow clams lay between 2.9146 and 13.2635, and the *Nm* values among the ZS−LYG, ZS−CM, ZS–WZ, LYG−ND, and CM−ND populations were greater than 1 and less than 4. These results indicate low genetic differentiation among these populations. In the present study, the sampling area of rainbow clams was distributed in the Yellow Sea and East China Seas. LYG and CM are in the Yellow Sea with a geographical distance (about 1,000 km coastline), and no differentiation between these populations was observed ($F_{ST} = 0.0040 < 0.05$). Although the geographical distance between the Chongming and Zhoushan populations is close, the Yangtze estuary is situated between them. The inflow of the Yangtze River lowers the salinity of the seawater and, hence, obstructs the passage of larvae and hinders gene communication to some extent (the *Nm* for CM−ZS is 2.9146). Therefore, moderate genetic differentiation occurs between the CM and ZS populations ($F_{ST} = 0.0790$). Xu et al. (2016) analyzed the population morphology and genetic diversity of rainbow clams from Zhejiang and revealed that the Zhoushan population has great variation from the Yueqing, Taizhou, and Wenling populations ($G_{ST} = 0.2479$). The present study also indicated that genetic differentiation occurred in the Zhoushan population ($F_{ST} = 0.2479$), which was consistent with Xu et al. (2016), who showed that the Zhoushan population of rainbow clams presents moderate genetic differentiation.

This study also found that the degree of genetic differentiation between rainbow clam populations is not related to the geographical distance. Although the geographical distance between LYG and CX was relatively far away, the F_{ST} value between the LYG and CX populations of the rainbow was

shallow, only 0.0180 (<0.05), which indicated a lack of genetic differentiation between the two populations. The gene exchange between the two populations occurred frequently (Nm = 13.2635 > 4). The inconsistency of the relationship between the degree of genetic differentiation and geographical distance has previously been observed in different populations of *Coelomactra antiquata*. Meng et al. (2013) showed that the population genetic differentiation between the *C. antiquata* from the Southeastern Sea and that from the Yellow Sea in Lianyungang and Rizhao is not apparent based on the results of ITS2 and 16S rRNA for six populations of *C. antiquata* in the coasts of China. By contrast, the difference between the Guangxi and Fujian populations, which have a relatively close geographical distance, reached the interspecies level. The real reason may be from human activity, such as artificial farming and introduction, but more detailed reasons remain unknown so far.

Genetic diversity, including the degree of genetic variation and the genetic structure of the population, is an important basis for evaluating the status of genetic resources. A higher genetic diversity of a population results in a stronger adaptability to the living environment and a greater potential for evolution (Tian et al., 2013). The genetic diversity of a population is mainly manifested in two aspects: heterozygosity and the number of alleles (Yu et al., 2012). Many reports have explored the genetic diversity of aquatic animals by using SSR markers. Li et al. (2011) studied the population genetics of *Portunus trituberculatus* by microsatellite markers and found an average *Ho* of 0.2222−1.0000 and an average *He* of 0.4367−0.9099. Li et al. (2009) used SSR technology to measure the average *Ho* (0.32−0.49) of wild and cultured populations of scallops and found an average *He* of 0.37−0.55. Chang et al. (2007) analyzed the genetic diversity of five populations of scallops *P. yessoensis* in China and abroad and found an average *Ho* of 0.2708−0.3292 and an average *He* of 0.3620−0.4595 for the five populations. Tian et al. (2013) determine the *Ho* and *He* of four populations of *Scapharca broughtonii* by using the 20 microsatellite markers, and the results showed that the ranges of *Ho* and *He* was 0.667–0.9667 and 0.6198–0.9318, respectively. An et al. (2012) studied the genetic structure of five wild-type populations of *Ruditapes philippinarum* in two sea areas of Korea by using seven SSR markers and noted high genetic diversity among the clams (total *He* = 0.813). The results of the present study revealed that the average *Ho* of the six populations of rainbow clam was between 0.4381 and 0.6139 and that the *He* was 0.6266−0.7325. Specifically, the value of *He* was as follows: ZS (0.7325), CX (0.7162), ND (0.6839), LYG (0.6786), CM (0.6701), and WZ (0.6266). The average number of alleles of the 17 loci in the six populations ranged from 5.5000 to 22.500, and the *Ne* was between 1.2538 and 13.3411. The genetic diversity of the rainbow clams was higher than those of bay scallop *Argopecten irradians* and scallop *P. yessoensis*. This particular genetic diversity is similar to the *He* of four populations of clams *T. quinquefasciatus* and is lower than that of five populations of clam *R. philippinarum*. Generally, the *He* (0.7325) of the present study and Shannon diversity index (1.7998) were the highest in the Zhoushan population, which indicated that the Zhoushan population has the richest genetic diversity among the clam populations studied.

The population differentiation of the existing rainbow clams is not obvious, and the genetic diversity of germplasm resources is relatively high, indicating that the effective population of rainbow clams in nature is large enough. At present, it is not very urgent to establish a germplasm resource reserve and its *ex situ* culture technology. In the future, the monitoring and evaluation of the germplasm resources of rainbow clams should be strengthened.

CONCLUSION

The six populations of rainbow clams all presented high genetic diversity, which reflect the good protection and development prospects of rainbow clam resources in China. These results would be helpful to genetic breeding practice and resources management.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

All applicable International, National, and/or Institutional Guidelines for the Care and Use of Animals (invertebrates) were followed.

AUTHOR CONTRIBUTIONS

XL conceived and designed the experiments. SG performed the experiments. MZ analyzed the data. XL and ZD wrote the manuscript. All authors contributed to the article and approved the submitted version.

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