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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Abstract

This study aims to determine the genetic structure and diversity 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 in Fujian, Cixi and Wenzhou in Zhejiang. A total of 1146 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 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 delicious taste and high nutritional value. The clam mainly is distributed in the West Pacific coast and northern Australia. In recent years, rainbow clam has been increasingly used as high-valued seafood and as feed for shrimps and crabs in some mariculture industry of China. Deterioration of the ecological environment has led to a sharp decline in the natural resources of rainbow clams due to overfishing and increases in shrimp and crab farming. Thus, protection of rainbow clam resources is of great importance. Several authors have explored the biological characteristics and morphological differences of rainbow clam (Ji et al. 2007; Lv et al. 2012). To date, however, reports on the genetic diversity and relationships amongst clam populations are limited (Xu et al. 2016).

Microsatellites, also known as simple repetitive sequences (SSR), 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 triangle pearl *Hyriopsis cummingii* (Bai et al. 2015). So far, few reports are available on the genetic diversity of the population of rainbow clam. In the present study, the genetic diversity of six populations of rainbow clams in Lianyungang, Chongming Island, Ningde, Zhoushan, Cixi and Wenzhou was analysed by using microsatellite markers. The results obtained can provide a scientific basis for assessing germplasm resources and genetic diversity protection of rainbow clam.

Materials And Methods

Materials

Rainbow clams were collected from the six coastal mud flats, 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 obtained randomly from each population. Information on the samples collected is presented in Table 1 and Fig. 1. The sampled rainbow clams were stored at – 70°C until analysis.

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

Microsatellite Primer

The M13 (-21) universal primer sequence of 5'-TGTAACGACGGCCAGT-3' reported by Schuelke (2000) was adopted in this study to elongate short primers in for economic consideration in genotyping. We used two fluorescent labels FAM and HEX for forward primes which were abbreviated as FAM-M13 and HEX-M13. Totally, 19 SSRs were used for PCR and these primer information was shown in Table 2.

Table 2
Microsatellite primer information. *Primers of forward sequence tailed with universal M13 (– 21) sequences (5'-TGTAACGACGCGCCAGT-3') at their 5' ends.

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: CTACGGATTTTCGCAGTGAAAATGT	120	63
Mir13	(AC)15	F: GCAACACAACGAGAGTG R: ACAACAAACAACAAGAAAT	116	47
Mir14	(CT)4	F: ATCGTTGGGGCATTCTAGTTTTCT R: GGGTATAATAATTTGAAACGCAGC	83	62
MH19A	(TG)28	F: GTGAGCAGGAATCAAAGGTG R: CTCGGCTCTGTTTGCCTAT	105–145	55
Mi44A	(TG)61	F: CCTCGGAGACCATTCGCTAC R: TGCTTTTCTATGACAACCCT	85–101	52
MW33A	(TG)13TA(TG)5(CG)2TGCG	F: TTCCTATCCTTACCCTTG R: CTGACTGGAACTCAACAC	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

Method

Total DNA was extracted with SDS–phenol chloroform method. The PCR system used 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 MasterMix and 6 µL of ddH₂O to form a total volume of 20 µL. 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 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 utilised to calculate the number of effective alleles (N_e), expected heterozygosity (H_e), observed heterozygosity (H_o) and gene flow (Nm), genetic distance (D_s) and Shannon diversity index. Population clustering was analysed 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 3.11 software, and the genetic diversity and genetic differentiation index (F_{ST}) were computationally analysed.

Results

Genetic diversity of the rainbow clam

There were 1146 alleles successfully detected in the six populations through 17 SSR loci scanning the genomes of rainbow clam populations, except in the HGZ22 in the Wenzhou population failed to 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 can be used to study the population genetics of rainbow clam (Table 3).

Table 3
Analysis of Molecular Variance among Six Populations of Rainbow Clam

pop	SSR	MH19A	Mi44A	MW15A	MX39C	MY36B	MZT46B	HGZ3	HGZ6	HGZ9	Mir8	Mir12	Mir14
ZS	Sample No.	96	100	100	98	84	96	100	100	100	96	96	94
	Na	17	7	20	18	27	6	19	3	10	10	21	21
	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
	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
	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
	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
ND	Sample No.	78	92	96	98	78	86	98	98	98	100	96	94
	Na	18	8	30	10	15	24	8	14	2	8	9	10
	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
	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
	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
	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
CX	Sample No.	90	100	100	96	82	88	98	100	100	94	100	98
	Na	22	5	30	18	28	5	11	4	15	11	6	18
	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
	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
	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
	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
CM	Sample no	108	120	114	112	90	116	116	120	120	120	118	92
	Na	21	5	17	15	25	9	11	5	11	8	22	10
	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
	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
	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
	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
WZ	Sample no	100	98	96	56	82	76	96	100	100	100	100	56
	Na	23	12	18	10	9	8	2	5	8	9	10	3
	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
	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
	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
	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
LYG	Sample No.	98	100	94	100	72	86	94	100	100	92	100	100
	Na	21	5	20	16	29	5	4	2	17	18	4	20
	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
	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
	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
	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
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
	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

pop	SSR	MH19A	Mi44A	MW15A	MX39C	MY36B	MZT46B	HGZ3	HGZ6	HGZ9	Mir8	Mir12	Mir14
	<i>Ne</i>	11.1722	3.3830	6.3675	2.8266	13.3411	2.1986	3.5729	1.2538	6.0005	6.2240	4.2106	2.8082
	<i>Ho</i>	0.8955	0.9933	0.7281	0.6531	0.4627	0.7491	0.2233	0.1318	0.8995	0.8791	0.9294	0.5016
	<i>He</i>	0.9176	0.7009	0.8314	0.5975	0.8343	0.5482	0.5674	0.1820	0.8245	0.8312	0.7321	0.5668
	<i>I</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

The average number of alleles among the 17 loci in 6 populations was 5.5000–22.5000 (17 loci average, 11.9039), 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). Amongst 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

AMOVA

AMOVA of the six different geographical populations of rainbow clams showed that 4.70% of their genetic variation could be derived from the population, whilst 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 amongst the populations was low (Table 4).

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 populaions	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	<i>F</i> _{ST} :	0.04702	P=0.05	

Genetic Distance

The *Ds* amongst 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. By contrast, the *Ds* between the Lianyungang and Cixi populations was the smallest (0.0668), and their genetic relationship was relatively close (Table 5).

Table. 5 Nie’s unbiased genetic distance of 6 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 6 populations of rainbow clams Genetic similarity coefficient above the diagonal, gene flow below the diagonal.

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 Differentiation Index And Gene Flow

Amongst 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 that 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 amongst populations was low.

Cluster Analysis

On the basis of the genetic distances amongst the populations, a clustering map was constructed using the UPGMA method of 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.

Discussion

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 amongst 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 amongst the six populations of rainbow clams lay between 2.9146 and 13.2635, and the Nm values amongst 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 amongst these populations. In the present study, the sampling area of rainbow clam was distributed in Yellow Sea and East China Seas. LYG and CM are in Yellow Sea, with a geographical distance (about 1000 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. Inflow of the Yangtze River lowers the salinity of the sea water 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 (2016) analysed the population morphology and genetic diversity of rainbow clams from Zhejiang and revealed that the Zhoushan population have a great variation from the Yueqing, Taizhou and Wenling populations ($G_{ST} = 0.2479$). Present study also indicated genetic differentiation occurred in the Zhoushan population ($F_{ST} = 0.2479$) which was consistent with Xu (2016), who showed that the Zhoushan population of rainbow clam 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 relative far away, the F_{ST} value between the LYG and CX populations of rainbow was very low, only 0.0180 (< 0.05), which indicated a lack of genetic differentiation between the two populations. Gene exchange between the two populations occurred very frequently ($Nm = 13.2635 > 4$). The inconsistency of the relationship between degree of genetic differentiation and geographical distance has previously been observed in different populations of *Coelomactra antiquata*. Meng (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 obvious basing on the results of ITS2 and 16SrRNA 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 still were 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 a 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 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* 0.37–0.55. Chang et al. (2007) analysed 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 average *He* of 0.3620–0.4595 for the five populations. Tian et al. (2013) used 20 microsatellite markers to determine the *Ho* and *He* of four populations of *Tetranymphus quinquefasciatus* found ranges of 0.667–0.9667 and 0.6198–0.9318, respectively. An et al. (2012) studied the genetic structure of five wild-type populations of *C. philippinarum* in two sea areas of Korea by using seven SSR markers and noted high genetic diversity amongst 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 the *He* was 0.6266–0.7325 and 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 genetic diversity is similar to the *He* of four populations of clams *T. quinquefasciatus* and lower than that of five populations of clam *C. philippinarum*. Generally, in present study *He* (0.7325) and Shannon diversity index (1.7998) were the highest in the Zhoushan population which indicated the Zhoushan population has the richest genetic diversity amongst the clam populations studied.

Conclusions

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

Declarations

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Compliance with ethical standards

Conflict of interest

All authors declare that they have no conflict of interest in the publication.

Ethical approval

All applicable international, national and/or institutional guidelines for the care and use of animals (invertebrates) were followed.

Sampling and field studies

The necessary permit for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements.

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Figures

Figure 1

Sample collection map of rainbow clam in coastal China.

Figure 2

Rainbow clam 6 populations of UPGMA trees