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Genetic diversity and population structure of the ark shell *Scapharca broughtonii* along the coast of China based on microsatellites

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A B S T R A C T

As a commercially important species in East Asia, the natural resources of *Scapharca broughtonii* have been suffering from severe population decline across its main habitats. In China, recovery efforts for *S. broughtonii* are in progress. To provide scientific bases for fisheries management and conservation program, genetic diversity and population structure of seven wild populations of *S. broughtonii* from the northern China coast was assessed using seven microsatellite loci in this study. High genetic diversity was present in all the seven populations, as observed in mean allelic richness per locus (11.3–12.5), and average expected heterozygosity (0.835–0.867). No significant difference in allelic richness or expected heterozygosity was observed among the seven populations. Pairwise F_{ST} estimates and NJ tree topologies based on D_C distances indicated that the seven populations fell into two groups, showing a clear division between the populations from the south and north of the Shandong Peninsula. Genetic differentiation was further analyzed using AMOVA and assignation tests. Genetic barrier analysis using Monmonier algorithm also confirmed that the Shandong Peninsula was the putative barrier separating the northern and southern populations. In addition, marine currents probably play an important role in high gene flow among three populations from the same marine gyre.

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1. Introduction

The ark shell *Scapharca broughtonii*, a cold-tolerant species inhabiting along the northwestern Pacific coast, is an important commercially marine shellfish in Asian countries (Li and Li, 2008). In the past two decades, the natural resources of *S. broughtonii* have precipitously declined across its main habitats, including Japanese, Korea and Chinese coasts (Cho et al., 2007; Li and Li, 2008; Sekino et al., 2010) because of many factors (eg.: over-exploitation, the deterioration of environmental conditions). There is a growing concern about the resource status of the ark shell. In China, *S. broughtonii* is mainly distributed in the north Yellow Sea, and was one of the dominant species in the coasts of Shandong and Liaoning provinces in the past (Wang and Wang, 2008). In 1980s, the average density of *S. broughtonii* in Rongcheng waters (Shandong province) was 0.476/m^2, while it decreased to 0.01/m^2 in 2010 (Song and Guo, 2010). The decline of *S. broughtonii* has prompted several restoration strategies including artificial breeding program, establishing marine protected area, construction of artificial reefs,

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and so on (Li and Li, 2008; Li et al., 2013). Although a well understanding of the genetic structure and gene flow among populations is needed for developing appropriate policies for management and conservation of *S. broughtonii* resources, there is few fully understanding of the genetic variation and population structure of the wild *S. broughtonii* populations along the coast of China to date.

Among the molecular markers available for analysis of population genetics, microsatellite markers are considered as an essential tool for examining genetic structure of populations, with characteristics of abundance, neutrality, codominance, unambiguous scoring of alleles, and easily amplified by PCR with small tissues (Tautz, 1989; Weber and May, 1989). Microsatellites have been widely used to assess genetic variation in many marine bivalves (Li et al., 2006; Yu et al., 2008; Brown and Stepien, 2010). In recent years, more than one hundred microsatellite markers have been developed for *S. broughtonii* (Sekino et al., 2010; Tian et al., 2012; Li et al., 2012), which provides enough candidate markers for investigation of population structure and genetic diversity in *S. broughtonii.*

In this study, seven polymorphic microsatellite markers were used to evaluate the genetic structure of seven wild *S. broughtonii* populations along the northern coast of China and estimate levels of gene flow among the populations, in order to provide useful information for designing suitable management guidelines and artificial breeding programs for *S. broughtonii*.

2. Materials and methods

2.1. Sampling and DNA extraction

Seven wild populations of *S. broughtonii* were collected from the northern coast of China in 2011, with 60 individuals per population. Five populations were obtained from the Yellow Sea, including Jimo, Lianyuangang, Rizhao, Weihai and Zhuanghe; the other two populations were sampled from the Bohai Sea (Fig. 1). Sample sites were chosen to cover a wide portion of the species, based on the accessibility, biogeography of the ark shell and oceanography of sampling sites. Specimens were dissected and the adductor muscle was preserved in 95% ethanol until DNA extraction.

Genomic DNA was extracted from adductor muscle according to the protocol described by Li et al. (2002). The concentration of DNA was measured with an Ultrospec 2100 pro UV/visible spectrophotometer (Amersham Biosciences) for absorption at 260 nm.

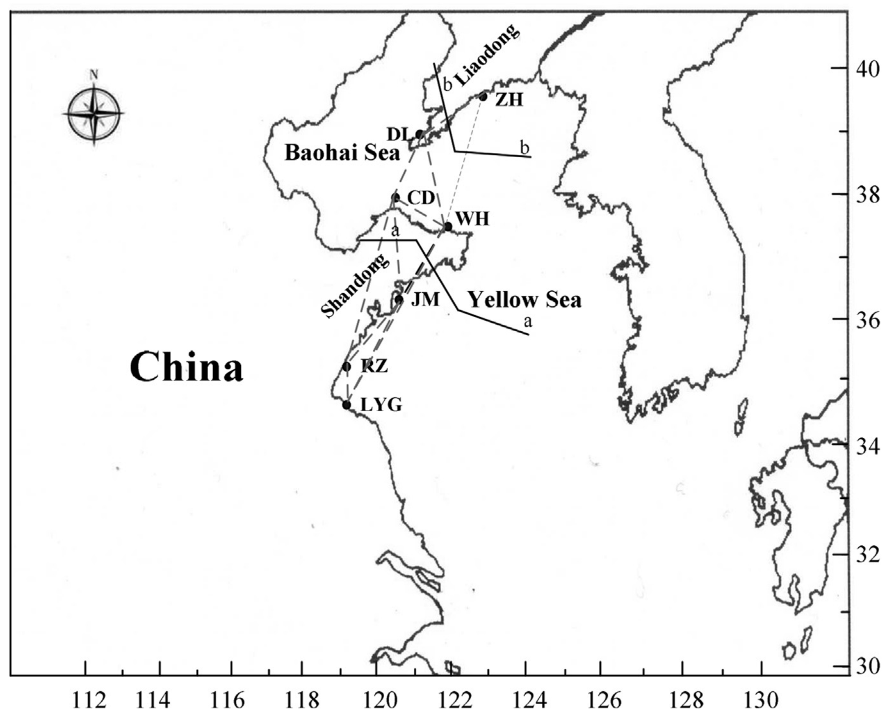

Fig. 1. Map showing locations and abbreviated names for seven *Scapharca broughtonii* populations (●). Areas of limited gene flow identified by barrier analysis were shown on the map (Full lines = main barriers to gene flow. Dotted lines = Delaunay triangulation).

Table 1
Number of alleles (N), allele richness (A_R), observed and expected heterozygosity (H_O, H_E), and probability value of Hardy–Weinberg equilibrium (P) at each locus for each population.

Locus		CD	DL	WH	ZH	JM	LYG	RZ
ScBr03	N	6	7	8	7	6	7	5
	A_R	5.8	7	7.8	6.8	6	6.8	5
	H_O	0.567	0.55	0.55	0.65	0.55	0.583	0.583
	H_E	0.619	0.624	0.717	0.655	0.726	0.658	0.661
	P	0.377	0.041	0.026	0.007	0.002	0.027	0.413
ScBr20	N	15	14	13	16	14	15	15
	A_R	14.8	13.8	12.8	15.8	13.7	14.9	14.6
	H_O	0.75	0.55	0.817	0.722	0.717	0.75	0.85
	H_E	0.918	0.917	0.91	0.879	0.885	0.91	0.911
	P	0.000*	0.000*	0.004	0.000*	0.034	0.007	0.032
ScBr14	N	11	11	14	12	13	12	12
	A_R	10.8	10.8	13.8	11.7	12.7	11.6	11.8
	H_O	0.617	0.533	0.667	0.55	0.6	0.7	0.617
	H_E	0.870	0.840	0.905	0.801	0.830	0.824	0.856
	P	0.000*	0.000*	0.000*	0.000*	0.001*	0.107	0.000*
ScBr04	N	12	12	12	13	13	13	15
	A_R	11.8	11.8	11.9	12.8	12.3	12.6	14.6
	H_O	0.783	0.85	0.917	0.9	0.733	0.883	0.9
	H_E	0.859	0.862	0.883	0.875	0.853	0.867	0.876
	P	0.616	0.760	0.414	0.480	0.140	0.021	0.396
ScBr05	N	13	13	14	12	13	14	13
	A_R	12.9	12.9	13.9	11.6	12.8	13.7	13
	H_O	0.912	0.818	0.83	0.783	0.727	0.85	0.82
	H_E	0.902	0.891	0.898	0.878	0.896	0.901	0.906
	P	0.766	0.133	0.580	0.119	0.004	0.007	0.015
ScBr09	N	13	13	15	16	15	15	16
	A_R	12.9	12.7	14.3	15.2	14.6	14.3	15.7
	H_O	0.904	0.86	0.9	0.817	0.862	0.867	0.857
	H_E	0.898	0.899	0.899	0.896	0.904	0.896	0.909
	P	0.814	0.112	0.768	0.218	0.224	0.096	0.638
SB6	N	12	10	10	12	12	14	13
	A_R	11.7	9.8	9.8	11.8	11.6	13.6	12.8
	H_O	0.661	0.712	0.750	0.707	0.750	0.833	0.759
	H_E	0.863	0.876	0.854	0.861	0.859	0.887	0.860
	P	0.003	0.035	0.000*	0.007	0.004	0.179	0.037
Mean	N	11.7	11.4	12.3	12.6	12.3	12.9	12.7
	A_R	11.5	11.3	12	12.2	11.9	12.5	12.5
	H_O	0.742	0.696	0.776	0.733	0.706	0.781	0.769
	H_E	0.847	0.844	0.867	0.835	0.850	0.849	0.854

* Significant after Bonferroni correction ($P < 0.01/7$).

2.2. Microsatellite analysis

Seven polymorphic microsatellite markers for *S. broughtonii* were analyzed: SB6 (Li and Li, 2008), ScBr03, ScBr20, ScBr14, ScBr04, ScBr05, ScBr09 (Sekino et al., 2010). PCRs were performed in a volume of 10 μL containing 0.25 U *Taq* DNA polymerase (Takara), 1× PCR buffer, 0.2 mM dNTP mix, 1.5 mM $MgCl_2$, 1 μM of each primer set, and 50 ng of genomic DNA. The PCR amplification conditions were as follows: 3 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at the optimal annealing temperature, and 1 min at 72 °C, followed by a final extension of 5 min at 72 °C. Amplification products were separated by electrophoresis through a 6% denaturing polyacrylamide gel, and visualized by silver staining. Allele sizes were determined by using a 10 bp DNA ladder (Invitrogen). A control DNA sample was included in each set of samples for each gel to avoid inaccuracy in scoring due to differences in gels.

2.3. Statistical analysis

For each locus and population, the number of alleles per locus (N), expected heterozygosity (H_E) and observed heterozygosity (H_O) were estimated using the program MICROSATELLITE ANALYSER (MSA) (Dieringer and Schlötterer, 2003). Allelic richness (A_R), a standard index for measuring the number of allele independent of sample size, was calculated for each population using FSTAT (http://www.unil.ch/izea/softwares/fstat.html). A nonparametric analysis of variance was performed to test for differences in allelic richness among populations with the Kruskal–Wallis test (Sokal and Rohlf, 1995). Exact tests for deviations from Hardy–Weinberg equilibrium (HWE) were performed using the exact test (Guo and Thompson, 1992) implemented in GENEPOP software (Raymond and Rousset, 1995). Tests for linkage disequilibrium between pairs of loci were performed using the same program. Due to large allele dropout and stuttering, expected null alleles and scoring errors for each locus per population were checked with the software MICRO-CHECKER (Van Oosterhout et al., 2004).

To evaluate the levels of genetic differentiation among populations, F_{ST} (Weir and Cockerham, 1984) was calculated using the software MSA. The Cavalli-Sforza and Edwards (1967) chord distance D_C was calculated, and an unrooted neighbor-joining tree (NJ tree) was constructed with the software POPULATIONS (http://www.cnrs-gif.fr/pge) based on D_C. Nodal support was assessed by bootstrapping with 1000 replicates. A multi-locus analysis of molecular variance (AMOVA), assessed with exact tests based on 10,000 permutations, was conducted to check for hierarchical structure of variability, using ARLEQUIN (Excoffier and Lischer, 2010). The Bayesian approach of population assignment test (Cornuet et al., 1999) implemented in the GENECLASS program (Piry et al., 2004) was used to estimate the likelihood of an individual's multilocus genotype to be assigned to the population from which it was sampled. Tests for geographical frontier were performed using the program BARRIER (Manni et al., 2004) for identifying potential physical barriers. A network, generated by Delauney triangulation with the geographical coordinates of each site, connects all populations in present study. Also, for identifying putative barriers, Monmonier's maximum distance algorithm was used. The Bonferroni correction (Rice, 1989) was used to adjust for all the multiple significance tests.

3. Results

3.1. Genetic diversity within populations

Number of alleles, observed (H_O) and expected (H_E) heterozygosities for the seven microsatellite loci are summarized in Table 1. All the seven microsatellite loci were highly polymorphic and a total of 106 alleles were detected. Allele number of each locus ranged from 5 to 16, and allelic richness per locus varied from 5 to 15.8. Mean allelic richness per locus was in the following order: DL (11.3), CD (11.5), JM (11.9), WH (12), ZH (12.2), LYG (12.5) and RZ (12.5). There was no significant difference in average allelic richness among the seven populations ($P = 0.9995$).

Mean H_O and H_E values ranged from 0.696 to 0.781 and from 0.835 to 0.854, respectively. H_O values were slightly lower than H_E values for all the populations. On average, the WH population had the highest H_E value (0.867) and the ZH population showed the lowest (0.835). No linkage disequilibrium was detected among loci ($P > 0.05$ in each case).

Ten of the 49 locus–population combinations were out of Hardy–Weinberg equilibrium after Bonferroni correction ($P < 0.01/7$). Locus ScBr14 had the most departures within six populations. Analysis from MICRO-CHECKER suggested that the observed heterozygote deficits were caused by null alleles. Null alleles were detected across all the populations. No evidence of stuttering or dropout was found for all the loci across populations.

3.2. Genetic differentiation among populations

Genetic structure among the seven populations of *S. broughtonii* was moderate but significant (Global $F_{ST} = 0.020$, $P = 0.001$), indicating the population heterogeneity. Pairwise multilocus F_{ST} across the seven populations ranged from 0.0016 to 0.0436 (Table 2). Within the populations from the south of the Shandong Peninsula (JM, LYG and RZ), no significant pairwise F_{ST} were observed, and there is also no significant divergence within the populations from the north of the Shandong Peninsula (CD, DL, WH, and ZH). The highest estimate was between CD and LYG populations. Analysis of data corrected for null alleles, displayed similar results and not shown here.

Genetic distances (D_C) between populations are shown in Table 2. D_C values between populations closely reflected pairwise F_{ST} values (Table 2). D_C genetic distance was smallest between CD and DL (0.188), while the largest distance was between CD and LYG (0.287). The NJ tree generated from values of D_C is shown in Fig. 2. The seven populations were separated into two groups, one group including four northern populations (CD, DL, WH and ZH), and the other consisting of JM, RZ and LYG populations.

A hierarchical AMOVA test for groups of populations, defined according to the NJ tree topologies, was carried out for levels of genetic differentiation between populations among groups, among populations within groups, and between individuals within populations. The results showed that a significant variation between groups accounted for 2.7% of total variation. Variation between populations within groups contributed the least (0.9%) while most of the variance was distributed within populations (96.4%).

Table 2
Pairwise F_{ST} averaged over seven loci for the seven *Scapharca broughtonii* populations and geneic distances between the ten populations (above diagonal, D_C; below diagonal, F_{ST}).

	CD	DL	WH	ZH	JM	LYG	RZ
CD	–	0.188	0.207	0.239	0.253	0.287	0.286
DL	0.0019	–	0.224	0.236	0.254	0.269	0.273
WH	0.0073	0.0107	–	0.244	0.244	0.259	0.253
ZH	0.0123	0.0105	0.0171	–	0.230	0.236	0.270
JM	0.0264*	0.0271*	0.0149*	0.0226*	–	0.220	0.238
LYG	0.0436*	0.0390*	0.0199*	0.0340*	0.0042	–	0.218
RZ	0.0381*	0.0371*	0.0162*	0.0351*	0.0062	0.0016	–

*Significant after Bonferroni correction ($P < 0.01/21$).

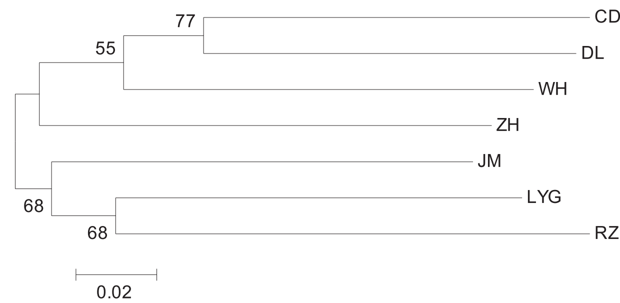

Fig. 2. A neighbor-joining tree based on D_C distances for seven *S. broughtonii* populations. The numbers refer to the percentage bootstrap values from 1000 replications of resampled loci.

Provided that populations JM, RZ and LYG are considered as one group, and ZH, CD, WH, and DL as one group, 92.5% and 90.4% of the individuals were assigned to their sampling groups.

3.3. Genetic barrier predication

Results revealed by the BARRIER analyses were corresponding to the analyses of genetic structure and the oceanography features of local sampling areas. A putative barrier, which directly separated seven populations into two groups, was detected when the number of barrier was set as only one using this program, indicating that the Shandong Peninsula might be a possible barrier hampering gene flow between the two groups (Fig. 1). Moreover, another barrier was shown up if change the number of barrier to two, isolating ZH population from the northern group, which suggested that the Liaodong Peninsula might act as another possible boundary for the gene flow.

4. Discussion

4.1. Genetic diversity and departures from HWE

In this study, seven microsatellite markers revealed a comparatively high level of genetic diversity in terms of allelic richness and expected heterozygosity in all the seven populations, indicating that the wild populations of *S. broughtonii* in China maintained considerable variations. Similar results were also detected in *S. broughtonii* populations from Korea (five populations), China (one population) and Russia (one population) by Cho et al. (2007) using COI sequences. Mass fecundity, external fertilization, and broad larval dispersal might be the cause of the high genetic diversity of *S. broughtonii*, which is a common phenomenon in many bivalves (Cassista and Hart, 2007; Lallias et al., 2010; Arias-Pérez et al., 2012). No significant difference in genetic diversity among the seven populations was detected in this study.

Heterozygote deficiencies related to HWE have been observed in many natural populations of marine mollusks using microsatellites (Prakoon et al., 2010; Arias-Pérez et al., 2012). In the present study, the population-locus cases deviated from HWE also showed heterozygote deficiencies (Table 1). As has been observed in prevenient microsatellite studies of bivalves (Li et al., 2007; Yu and Li, 2007), departure from HWE towards homozygote excess in this study could be largely caused by null alleles. In our study, some samples were failed to amplify at some loci, while easy to amplify at other loci, strongly suggesting the presence of null alleles. In addition, null alleles were also detected using MICRO-CHECKER program. In fact, microsatellites are known to suffer from extremely high levels of null alleles in marine invertebrate species (Li et al., 2003; Yu and Li, 2007). Besides, inbreeding and Wahlund effect can also result in heterozygote deficiencies. However, inbreeding can be precluded in the present study, because inbreeding would affect all loci and cause uniform heterozygote deficiencies across loci. With a period of larval duration approximately 20 days, admixture of samples from different populations across fine scale could happen. Therefore, Wahlund Effect, resulting from sampling across subdivided sites, is a likely explanation for the departures.

4.2. Population structure and gene flow among populations

Pairwise F_{ST} were significant between populations from northern (CD, DL, WH, and ZH) and southern (JM, LYG and RZ) groups, but not significant between the populations within the groups. The NJ tree topologies also supported a division between the northern and southern populations. The AMOVA analysis identified a significant among-group component. In addition, assignation tests correctly assigned high percentages (>90%) of individuals to their sampling groups further demonstrated a clear division between northern and southern populations. A strong barrier (the Shandong Peninsula) to gene flow was detected between northern and southern groups, suggesting that peninsulas may act as restriction for the gene flow. Moreover, the three southern populations were collected from the same marine gyre (Su and Yuan, 2005). Larval dispersal driven by marine currents may accelerate gene flow and depress the differentiation between these, and also may block gene flow between the three populations with other populations. Similar gene flow in this area was also found in other bivalves,

such as *Chlamys farreri* and *Mactra chinensis* (Zhan et al., 2009; Ni's et al., 2011). The Bohai Sea is characterized as having comparatively low rates of water exchange with the Yellow Sea (Wu et al., 2013). However, the populations CD and DL sampled from the Bohai Sea with weak divergence ($F_{ST} = 0.0019$) forming a cluster, did not showed significant differentiation from other two populations (ZH and WH) from the Yellow Sea. It is probably because the locations of CD and DL populations were near the boundary of the Bohai Sea and the Yellow Sea, and the currents flowing into the Bohai Sea may drive gene flow between the two populations and the other two populations (ZH and WH).

In conclusion, our results suggest that *S. broughtonii* populations along the northern China coast should be subdivided into two groups. There is no significant divergence within the groups, but significant population differentiations were detected between the two groups. Marine currents and the Shandong Peninsula may play important roles in population structuring of *S. broughtonii*. The results obtained from this study can provide useful information for fishery management as well as aquaculture industry and conservation activities.

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References

Arias-Pérez, A., Fernández-Tajes, J., Gaspar, M.B., Méndez, J., 2012. Isolation of microsatellite markers and analysis of genetic diversity among east Atlantic populations of the sword razor shell *Ensis siliqua*: a tool for population management. Biochem. Genet. 50, 397–415.
Brown, J.E., Stepien, C.A., 2010. Population genetic history of the dreissenid mussel invasions: expansion patterns across North America. Biol. Invasions 12, 3687–3710.
Cassista, M.C., Hart, M.W., 2007. Spatial and temporal genetic homogeneity in the Arctic surfclam (*Mactromeris polynyma*). Mar. Biol. 152, 569–579.
Cho, E.S., Jung, C.G., Sohn, S.G., Kim, C.W., Han, S.J., 2007. Population genetic structure of the ark shell *Scapharca broughtonii* Schrenck from Korea, China, and Russia based on COI gene sequences. Mar. Biotechnol. 9, 203–216.
Cornuet, J.M., Piry, S., Luikart, G., Estoup, A., Solignac, M., 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153, 1989–2000.
Dieringer, D., Schlötterer, C., 2003. Microsatellite Analyser (MSA): a platform independent analysis tool for large microsatellite data sets. Mol. Ecol. Notes 3, 167–169.
Excoffier, L., Lischer, H., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10, 564–567.
Guo, S.W., Thompson, E.A., 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48, 361–372.
Lallias, D., Boudry, P., Lapègue, S., King, J.W., Beaumont, A.R., 2010. Strategies for the retention of high genetic variability in European flat oyster (*Ostrea edulis*) restoration programmes. Conserv. Genet. 11, 1899–1910.
Li, G., Hubert, S., Bucklin, K., Ribes, V., Hedgecock, D., 2003. Characterization of 79 microsatellite DNA markers in the Pacific oyster *Crassostrea gigas*. Mol. Ecol. Notes 3, 228–232.
Li, J., Li, Q., 2008. Isolation and characterization of twelve novel microsatellite loci in the ark shell *Scapharca broughtonii*. Conserv. Genet. 9, 1055–1057.
Li, M., Zhu, L., Zhou, C.Y., Lin, L., Fan, Y.J., Zhuang, Z.M., 2012. Development and characterization of EST-SSR markers from *Scapharca broughtonii* and their transferability in *Scapharca subcrenata* and *Tegillarca granosa*. Molecules 17, 10716–10723.
Li, Q., Park, C., Kijima, A., 2002. Isolation and characterization of microsatellite loci in the Pacific abalone, *Haliotis discus hannai*. J. Shellfish Res. 21, 811–815.
Li, Q., Xu, K., Yu, R., 2007. Genetic variation in Chinese hatchery populations of the Japanese scallop (*Patinopecten yessoensis*) inferred from microsatellite data. Aquaculture 269, 11–219.
Li, Q., Yu, H., Yu, R., 2006. Genetic variability assessed by microsatellites in cultured populations of the Pacific oyster (*Crassostrea gigas*) in China. Aquaculture 259, 95–102.
Li, R., Li, Q., Wang, C., 2013. Sibship reconstruction and effective population size estimation in mass spawning ark shell, *Scapharca broughtonii* based on microsatellite analysis. Genes. Genom. 35, 703–708.
Manni, F., Guérard, E., Heyer, E., 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by 'Monmonier's algorithm'. Hum. Biol. 76, 173–190.
Ni, L., Li, Q., Kong, L., 2011. Microsatellites reveal fine-scale genetic structure of the Chinese surf clam *Mactra chinensis* (Mollusca, Bivalvia, Mactridae) in Northern China. Mar. Ecol. 32, 488–497.
Piry, S., Alapetite, A., Cornuet, J.M., Paetkau, D., Baudouin, L., Estoup, A., 2004. GeneClass2: a software for genetic assignment and first-generation migrant detection. J. Hered. 95, 536–539.
Prakoon, W., Tunkijjanukij, S., Nguyen, T.T., Na-Nakorn, U., 2010. Spatial and temporal genetic variation of green mussel, *Perna viridis* in the gulf of Thailand and implication for aquaculture. Mar. Biotechnol. 12, 506–515.
Raymond, M., Rousset, F., 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86, 248–249.
Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223–225.
Sekino, M., Kurokawa, T., Sasaki, K., 2010. Multiplex PCR panels of novel microsatellites for the ark shell *Scapharca broughtonii* (Pteriomorphia, Arcoida). Conserv. Genet. Resour. 2, 39–42.
Sokal, R.R., Rohlf, F.J., 1995. Biometry: the Principles and Practice of Statistics in Biological Research, third ed. Freeman, New York.
Song, Y., Guo, X., 2010. The investigation of the feasibility of the artifical enhacement and aquaculture of *Scapharca broughtonii* in Rongcheng. Shandong Fish. 27, 53–54.
Su, J.L., Yuan, Y.L., 2005. Hydrology of China Seas. Ocean Press, Beijing, pp. 172–192 (in Chinese).
Tautz, D., 1989. Hypervariability of simple sequences as a general source for polymorphic markers. Nucleic. Acids Res. 17, 6463–6471.
Tian, J.T., Liu, Z.H., Zhou, L.Q., Wu, B., Liu, P., Yang, A., 2012. Isolation and characterization of 48 polymorphic microsatellite markers for the blood clam *Scapharca broughtonii* (Arcidae). Genet. Mol. Res. 11, 4501–4507.
Van Oosterhout, C., Hutchinson, W.F., Wills, D., Shipley, P., 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4, 535–538.
Wang, R., Wang, Z., 2008. Science of Marine Shellfish Culture. Ocean University of China Press, Qingdao.

Weber, J.L., May, P.E., 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am. J. Hum. Genet. 44, 388–396.
Weir, B., Cockerham, C., 1984. Estimating F-statistics for the analysis of population structure. Evolution 38, 1358–1370.
Wu, Z., Yu, Z., Song, X., Yuan, Y., Cao, X., Liang, Y., 2013. Application of an integrated methodology for eutrophication assessment: a case study in the Bohai Sea. Chin. J. Ocean. Limnol. 31, 1064–1078.
Yu, H., Li, Q., 2007. Genetic variation of wild and hatchery populations of the Pacific oyster *Crassostrea gigas* assessed by microsatellite markers. J. Genet. Genom. 34, 1114–1122.
Yu, H., Li, Q., Yu, R., 2008. Genetic differentiation between Zhe oyster *Crassostrea plicatula* and Pacific oyster *Crassostrea gigas* populations in China assessed by microsatellite analysis. Fish. Sci. 74, 88–97.
Zhan, A., Hu, J., Hu, X., Zhou, Z., Hui, M., Wang, S., Peng, W., Wang, M., Bao, Z., 2009. Fine-scale population genetic structure of Zhikong scallop (*Chlamys farreri*): do local marine currents drive geographical differentiation? Mar. Biotechnol. 11, 223–235.