Biochemical Systematics and Ecology 66 (2016) 129–136

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Population genetics of the common long-armed octopus *Octopus minor* (Sasaki, 1920) (Cephalopoda: Octopoda) in Chinese waters based on microsatellite analysis

Xiaolei Gao [a, b], Xiaodong Zheng [a, b, *], Qikang Bo [a], Qi Li [a]

[a] Laboratory of Genetics and Breeding of Molluscs, Fisheries College, Ocean University of China, Qingdao, China
[b] Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao, China

ARTICLE INFO

Article history:
Received 21 August 2015
Received in revised form 13 March 2016
Accepted 19 March 2016

Keywords:
Octopus minor
Microsatellite DNA
Population genetic structure
Current

ABSTRACT

Octopus minor (Sasaki, 1920) is a commercially important cephalopod in Chinese waters. To provide a theoretical basis for resource protection and sustainable management, we investigated genetic structure of ten *O. minor* populations in Chinese waters using microsatellite DNA markers. Eight microsatellite loci revealed high allelic diversity with 11–26 alleles per locus. Observed and expected heterozygosity varied from 0.412 to 0.900 and from 0.337 to 0.845, respectively. The overall F_{ST} value was 0.198, indicating great genetic differentiation among populations. The F_{ST} value between Yilan and other populations reached more than 0.3 that may be indicative of subspecies rank. Mantel test showed significant correlations between genetic and geographic distance ($R = 0.383$, $P = 0.004$) indicating that genetic differentiation of *O. minor* conformed to a pattern of isolation-by-distance. Using the Neighbor-joining method, cluster analysis divided nine populations into three groups and divided ten populations into two groups wherein Yilan was distinguished from the other populations. Analysis based on F_{ST}, D_c values and clustering highlighted the heterogeneity of Yilan and the relative homogeneity between Yilan and Ganyu. The significant population genetic structure of *O. minor* is related to the combined effects of geographical barriers, current features and life history characteristics.

1. Introduction

Octopus minor (Sasaki, 1920) (Cephalopoda: Octopoda: Octopodidae) is distributed mainly in warm temperate coastal waters of China, Korea and Japan. Specifically, its distribution in Chinese waters ranges over a wide area covering the Bohai Sea, Yellow Sea, East China Sea and South China Sea (Dong, 1988). Due to its high nutritional value, it is very popular with consumers (Qian et al., 2010). As a commercial species, *Octopus minor* has attracted widespread concern among many researchers due to the over-exploitation of wild populations in recent years. It is therefore important to understand whether the genetic diversity of this species has been impacted through the overfishing.

Octopus minor produces large eggs and its life cycle lacks a pelagic larval period implying its dispersal potential is limited (Villanueva and Norman, 2008; Zheng et al., 2014), therefore, population differentiation might be caused by geographic isolation. Mechanisms for geographic differentiation and population structuring have not been clarified explicitly for this

* Corresponding author. Laboratory of Genetics and Breeding of Molluscs, Fisheries College, Ocean University of China, Qingdao, China.
E-mail address: xdzheng@ouc.edu.cn (X. Zheng).

http://dx.doi.org/10.1016/j.bse.2016.03.014
0305-1978/

species. To date, several reports exploring the population genetic structure of *O. minor* have been published based on different molecular markers such as allozymes (Gao et al., 2009), COI (Sun et al., 2010), 16S rRNA (Li et al., 2010), 12S rRNA and CO III (Xu et al., 2011), ISSR (Guo et al., 2011), AFLP and COII (Lü et al., 2013), and SSR (Kang et al., 2012). All these studies showed substantial differentiation among geographical populations at different molecular levels. These studies did not encompass the entire distributional range of *O. minor* in Chinese waters, however. Additionally, sample numbers were limited and genetic variability was relatively low. At present, there are still few systematic reports concerning population genetic diversity and genetic structure of *O. minor* in Chinese waters.

Microsatellite markers are considered ideal molecular markers in analyzing of genetic diversity and population genetic structure because of their high polymorphism, co-dominance, neutrality, abundance and unambiguous scoring of alleles (Tautz, 1989; Weber and May 1989). Microsatellite markers have been successfully applied in population genetic studies of some cephalopods and with meaningful results (Reichow and Smith, 2001; Pérez-Losada et al., 2002; Cabranes et al., 2008; Zheng et al., 2009). By 2013, microsatellite markers have been isolated for more than 30 species of cephalopod according to NCBI official statistics. Zuo et al. (2011) first developed 12 polymorphic SSR loci in *O. minor*.

In the present study, we collected *O. minor* from ten widely distributed geographic locations in the Bohai Sea, Yellow Sea, East China Sea as well as Taiwan, and used eight of the polymorphic microsatellite loci developed by Zuo et al. (2011) to analyze the population genetic structure of this species.

2. Materials and methods

2.1. Sample collection and DNA extraction

A total of 334 *O. minor* specimens were collected from ten locations in Chinese waters including the Bohai Sea, Yellow Sea, East China Sea as well as Taiwan from 2012 to 2013 (Table 1, Fig. 1). The Bohai Sea population was sampled in Laizhou (LZ). Four sample localities were distributed across the Yellow Sea: Dalian (DL), Yantai (YT), Rongcheng (RC), Ganyu (GY). Five other sample localities were distributed across the East China Sea: Shengsi (SS), Nanji Island (NJD), Lianjiang (LJ), Quangang (QG) and Yilan (YL) of Taiwan. The maximum and minimum geographic distances between populations are approximately 1623 km (DL-YL) and 98 km (YT-RC), respectively. Approximately 1 cm^3 of mantle muscle was removed from fresh specimens and frozen in −80°C prior to DNA preparation. Genomic DNA was extracted from mantle muscle by using a CTAB method modified from Winnepenninckx et al. (1993).

2.2. Microsatellite analysis

Individuals were genotyped at eight polymorphic microsatellite loci (Table 2): *OM01*, *OM02*, *OM03*, *OM05*, *OM07*, *OM08*, *OM11* and *OM12* (Zuo et al., 2011). The polymerase chain reactions (PCRs) were performed in 10-μl volumes containing 0.25 U *Taq* DNA polymerase (Takara, Japan), 1 × PCR buffer, 0.2 mM dNTP mix, 1.5 mM $MgCl_2$, 1 μm of each primer set, and 100 ng of genomic DNA. PCR was performed on a thermal cycler (GeneAmp System 9700) as follows: 3 min at 94 °C, 35 cycles of 45 s at 94 °C, 45 s at the optimal annealing temperature (Ni et al., 2010), and 45 s at 72 °C, followed by a final extension of 5 min at 72 °C. Amplification products were resolved via 6% denaturing polyacrylamide gel, and visualized by silver staining. For allele size determination, a 10-bp DNA ladder (Invitrogen USA) was used as a reference.

2.3. Data analysis

Null alleles and scoring errors due to large allele dropout and stuttering were checked with the software MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004). Number of alleles (N), expected heterozygosity (H_E), observed heterozygosity (H_O) and allelic size range (R) were calculated using MICROSATELLITE ANALYSER software (MSA; Dieringer and Schlötterer, 2003). Allelic richness (A_R) as a standardized measure of the number of alleles per locus, independent of the sample size, was calculated using FSTAT version 2.9.3 (Goudet, 2001). The Kruskal–Wallis test (Sokal and Rohlf, 1995), a nonparametric

Table 1
Sample details for *Octopus minor* in Chinese waters.

Population	Abbreviation	Geographic coordinates	Date of collection	No. of specimens	Mean total length (mm) ± SD
Dalian	DL	121°44′E, 39°01′N	2011.10	28	–
Laizhou	LZ	119°76′E, 37°14′N	2013.10	36	630.55 ± 98.07
Yantai	YT	121°39′E, 37°52′N	2012.04	24	–
Rongcheng	RC	122°42′E, 37°17′N	2012.10	40	–
Ganyu	GY	119°19′E, 34°83′N	2013.11	32	641.48 ± 117.05
Shengsi	SS	122°45′E, 30°72′N	2012.04	28	667.44 ± 101.65
Nanji Island	NJD	121°05′E, 27°27′N	2012.04	49	679.24 ± 107.36
Lianjiang	LJ	119°53′E, 26°20′N	2012.07	24	836.32 ± 178.90
Quangang	QG	118°58′E, 24°93′N	2012.05	38	682.27 ± 106.41
Yilan	YL	121°72′E, 24°69′N	2013.08	18	424.29 ± 102.24*

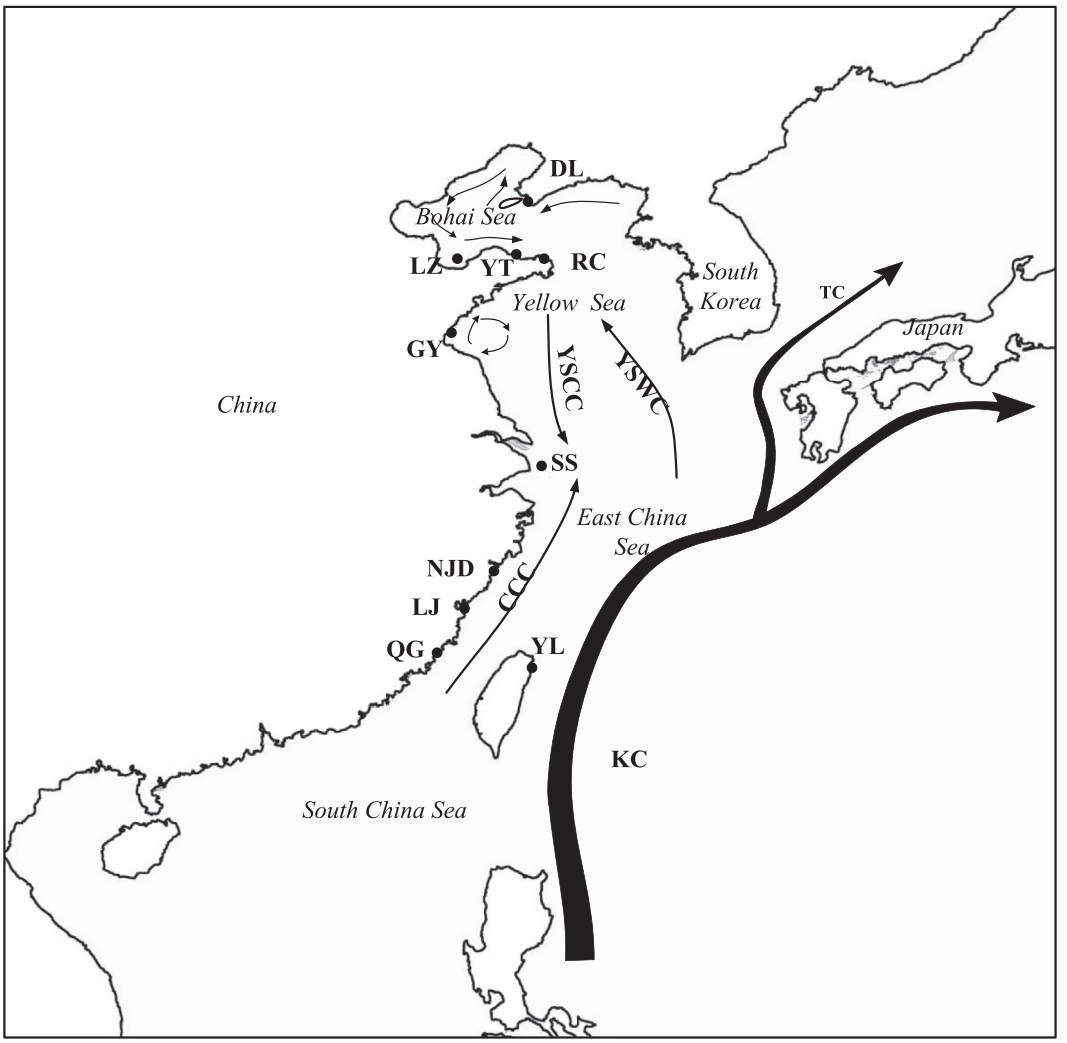

Fig. 1. Map of the sample locations and ocean currents along Chinese waters. DL, Dalian; LZ, Laizhou; YT, Yantai; RC, Rongcheng; GY, Ganyu; SS, Shengsi; NJD, Nanji Island; LJ, Lianjiang; QG, Quangang; YL, Yilan. KC, Kuroshio Current; TC, Tsushima Current; YSWC, Yellow Sea Warm Current; YSCC, Yellow Sea Coastal Current; CCC, China Coastal Current. (Su and Yuan, 2005).

analysis of variance, was performed to test for differences in allelic richness among populations. Exact tests for deviations from Hardy–Weinberg equilibrium (HWE) were performed using GENEPOP 3.4 (Raymond and Rousset, 1995). The same software was used to test for genotypic linkage disequilibrium for each pair of loci in each population. Significance levels for multiple comparisons were adjusted with a sequential Bonferroni correction (Rice, 1989).

Weir and Cockerham's (1984) F_{ST} (θ) was calculated with GENEPOP 3.4 to estimate pairwise genetic differentiation between populations. 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 1.2.30 (Langella, 1999). Nodal support was assessed by bootstrapping with 1000 replicates. The tree was visualized in TREE EXPLORER 2.12 (Tamura, 1997). The Bayesian clustering analysis was implemented in STRUCTURE v 2.3 (Pritchard et al., 2000). The value of K is the number of groups ranging from 1 to 13 (population number plus 3). The optimal value of K was selected with the online software Structure Harvester v 0.6.92 (Earl and vonHoldt, 2012) by calculating the statistical value ΔK (Evanno et al., 2005). Multilocus analysis of molecular variance (AMOVA) was conducted to check for hierarchical structure of variability, using ARLEQUIN 3.5 (Excoffier and Lischer, 2010). Molecular variance was assessed with exact tests based on 10,000 permutations. Isolation by distance (IBD), a model in which genetic differentiation increases with geographic distance, has been suggested as robust for investigating gene flow of marine species (Palumbi, 2003). A Mantel test implemented in GENEPOP 3.4 was performed to test the IBD model by correlating geographic distance (ln of the shortest distance)) to genetic distance [$F_{ST}/(1-F_{ST})$; Rousset 1997]. Significance of the IBD pattern was determined with 10,000 permutations.

Table 2
Characteristics of the eight-microsatellite markers for *Octopus minor*.

Locus	Repeat motif	Primer sequence (5′-3′)	T_a (°C)	Number of alleles	Size range (bp)
OM01	$(TG)_4N_{32}(GT)_8N_{36}(TG)_4$	ACGTGGTGGTGGGCTGTG ATACCCTCATTCTCCATCACTAA	54	2	249–259
0M02	$(TG)_5N_{52}(TG)_5T(TG)_4N_{29}(GT)_4$	TGTCAGTGAACTTTGTTTGCAT TCATCCCCGTAACCTCCG	54	3	398–418
OM03	$(GT)_5N_{36}(GT)_{11}N_{23}(GA)_4$	AAGTATAGGCTAGGAAAGGT CTACTTCTCCCTACCCTCT	60	8	209–223
OM05	$(CA)_4N_{16}(CA)_4\ N_4(CA)_6$	TGAGGTTTGTTTCTTTTATATT TAATCTTGTCTAAGCGATAAAT	50	4	322–394
OM07	$(AC)_6N_7(CA)_5$	CTACCTTCCCTAACCTCTCACTA CTAGGAGTCTGAACAACGTCAA	64	5	262–282
OM08	$(AT)_4(GT)_5GC(GT)_5$	GATCATCCTCCTCACCTAGC CGTAAACAAACCGACTCCT	64	11	241–265
OM11	$(TA)_{10}(TG)_4$	CGACCACTCCACATCAGAC ACACACCTAACACCTATACCCA	60	5	284–292
OM12	$(TG)_4(GT)_9N_{107}(GT)_6$	GACGAGACAGATTATTGTGACAGT TACACCACCGCTCTGATTTC	58	5	263–277

3. Results

3.1. Genetic diversity within populations

All the microsatellite loci were successfully amplified except *OM02* locus of YL, which had only one allele and differed greatly from other populations. All the microsatellite loci were highly polymorphic, while the degree of diversity was different at each locus. Genetic diversity indices for the ten *Octopus minor* populations are summarized in Table S1. Of the samples, loci *OM08*, *OM05*, *OM07* exhibited the highest variation with 25, 19, and 19 alleles, respectively and *OM12* the lowest (11 alleles). The number of alleles per locus in each population varied from 2 (*OM01*) to 16 (*OM08*), and allelic richness per locus ranged from 2.0 (*OM01*) to 15.1 (*OM08*) for each sample. The average observed and expected heterozygosity per locus ranged from 0.412 (*OM01*) to 0.900 (*OM03*) and from 0.337 (*OM01*) to 0.845 (*OM03*), respectively.

Among all loci, the average number of alleles for each population varied from 4.5 to 8.1. Specifically, the lowest average number of alleles (only 4.5) was detected in the YL population, whereas LJ (8.1), NJD (7.9) and GY (7.9) had the highest. Similarly, the YL population had the lowest average allelic richness (4.5) and LJ had the highest (7.7). There was no significant difference in the average allelic richness among these populations (Kruskal–Wallis test). The average observed and expected heterozygosity per population ranged from 0.516 (LZ) to 0.862 (LJ) and from 0.580 (DL) to 0.760 (LJ), respectively. No linkage disequilibrium was detected in locus pairs, suggesting that the loci can be treated as independent variables. Twenty of the 80 locus–population combinations were out of Hardy–Weinberg equilibrium ($P < 0.05/9$) after Bonferroni correction, of which *OM08* and *OM11* had the most departures. Every locus was detected to deviate significantly from HWE and no locus was detected to deviate significantly from HWE across all populations. Most deviated-populations of *OM03*, *OM05*, *OM07*, *OM08*, *OM12* loci showed loss of heterozygosity.

3.2. Genetic differentiation and genetic distance among populations

The global multilocus F_{ST} value was significant (0.198, $P < 0.01$), suggesting the genetic differentiation among the ten populations was large. Pairwise F_{ST} values across all samples ranged from 0.0354 to 0.3594 (Table 3). All 40 pairwise F_{ST} tests were significant after Bonferroni correction, indicating that all ten *O. minor* populations were significantly different from one another. The lowest F_{ST} value was observed between the RC and YT populations, which are separated by the least geographic distance while the highest F_{ST} value was observed between the DL and YL populations, which are separated by the greatest geographic distance. Genetic differentiation among the YT, DL, RC populations was relatively low while the differentiation between LZ and these three populations had higher F_{ST} values. The F_{ST} values between YL and the other populations were up to more than 0.3, indicating the large genetic differentiation between this population and the others.

D_C values based on Cavalli-Sforza and Edwards chord distance between populations are shown in Table 3. Similar to F_{ST} values, the lowest D_C value was detected between YT and RC (0.2427), while the highest was found between DL and YL (0.7183). Consistent with the F_{ST} values, the Dc values between YL and other populations were large while GY was the population which differed least from YL.

3.3. Clustering analysis and molecular variance analysis

D_C is suggested as one of the most efficient distance measures from which to obtain tree topology (Takezaki and Nei, 1996). In this study, two neighbor-joining trees were constructed with the D_C genetic distance based on nine populations (not including YL) and ten populations. According to the Neighbor-joining tree (Fig. 2), the nine populations were subdivided into

Table 3
Pairwise F_{st} values (below diagonal) and Cavalli-Sforza and Edwards chord distance (D_c, above diagonal) among ten *Octopus minor* populations.

Pop	DL	LZ	YT	RC	GY	SS	NJD	LJ	QG	YL
DL	–	0.4610	0.3510	0.3271	0.5581	0.6014	0.5295	0.5515	0.5346	0.7183
LZ	0.1881[a]	–	0.5092	0.4696	0.5254	0.6333	0.5722	0.5775	0.6190	0.7090
YT	0.0843[a]	0.1581[a]	–	0.2427	0.4991	0.5773	0.5207	0.5792	0.5045	0.6940
RC	0.0781[a]	0.1671[a]	0.0354[a]	–	0.5445	0.6026	0.5032	0.5856	0.5069	0.7145
GY	0.2133[a]	0.2048[a]	0.1561[a]	0.2123[a]	–	0.6349	0.5416	0.5655	0.5336	0.6043
SS	0.2086[a]	0.2551[a]	0.1849[a]	0.2193[a]	0.2406[a]	–	0.5278	0.4733	0.5002	0.7019
NJD	0.1740[a]	0.2106[a]	0.1624[a]	0.1622[a]	0.1797[a]	0.1659[a]	–	0.5841	0.5020	0.6584
LJ	0.1599[a]	0.1981[a]	0.1603[a]	0.1745[a]	0.1996[a]	0.1168[a]	0.1672[a]	–	0.5070	0.6535
QG	0.1903[a]	0.2682[a]	0.1674[a]	0.1987[a]	0.2129[a]	0.1792[a]	0.1609[a]	0.1765[a]	–	0.6470
YL	0.3594[a]	0.3540[a]	0.3232[a]	0.3532[a]	0.2447[a]	0.2924[a]	0.2900[a]	0.2665[a]	0.3324[a]	–

[a] Significant difference after Bonferroni correction.

three groups: one group consisting of four populations (DL, YT, RC and LZ), one group containing another four populations (SS, LJ, QG and NJD) and with the population of GY isolated. As shown in Fig. 3, after adding the YL population, the ten populations were divided into two groups: YL, formed a separate group, distinguished from the other groups. Bootstrap support values for the NJ tree were not high for most groups. The tree topology was not highly compatible with the geographic locations of sample sites. Based on the STRUCTURE software, the greatest division between groups appears when $K = 2$ (value of ΔK is greatest). YL was classified with northern populations (shown in Fig. S1).

The AMOVA analysis based on groups determined by the Neighbor-joining tree (YL as a group and the other as another group) showed that variation between individuals within populations accounted for most of the variation (70.0%) and identified significant variation among groups, explaining 14.6% of the total variance (Table S2). The variance component among populations within groups was also significant (15.4%), indicating that populations within groups should not be pooled together due to their genetic heterogeneity.

3.4. Isolation by distance (IBD)

Although the correlation coefficient of genetic distances [F_{ST}/(1-F_{ST})] and geographic distances (converted to log values) for all the *Octopus minor* samples was low (R = 0.383), it was significant ($P = 0.004$, 10,000 permutations) (as shown in Fig. S2, $R^2 = 0.148$, $P = 0.004$), displaying an isolation-by-distance (IBD) pattern of gene flow. The IBD pattern remained significant ($P < 0.05$) after excluding any single locus from the analysis, indicating that the correlation was not severely affected by any particular locus.

4. Discussion

4.1. Genetic diversity and Hardy–Weinberg equilibrium

The microsatellite markers in this study were richly polymorphic, exhibiting from 11 to 25 alleles. Loci *OM08* (25 alleles detected), *OM05* (20 alleles detected), *OM07* (19 alleles detected) have the most alleles, and are therefore desirable loci in the population analysis. Most of the populations showed a high level of heterozygosity indicating there is a high level of genetic variation in the wild.

Twenty significant deviations from Hardy–Weinberg equilibrium were found in the eighty (8×10) combinations of microsatellite loci and populations. In particular, deviations were marked for the loci *OM08* and *OM11*. Loci *OM03*, *OM05*, *OM07*, *OM08*, and *OM12* exhibited a relative heterozygote deficiency, which may have resulted from inbreeding, the Wahlund effect, selection or existence of null alleles (Astanei et al., 2005). In this study selection can be excluded as an explanation as satellite markers are neutral markers. The Wahlund effect is likely to be the reason for the heterozygote deficiency through geographic barriers to gene flow and genetic drift in the subpopulations. In previous population studies of octopuses (Cabranes et al., 2008; Doubleday et al., 2009; Juárez et al., 2010; Moreira et al., 2011), heterozygote deficits have been detected and considered to be largely caused by null alleles. In this study, the existence of null alleles in some deviated loci was detected using Micro-Checker, suggesting that this may also be a reason for departures from HWE. According to Kang et al. (2012), inbreeding may be also an important factor due to the restricted habitat and geographic isolation of octopuses.

4.2. Population genetic structure

In this study, genetic divergence was significant in the *Octopus minor* populations located in Chinese waters. Wright (1978) defined the relationship between the F_{ST} values and degree of differentiation: F_{ST} 0–0.05 indicating small differentiation among populations; F_{ST} 0.05–0.15 meaning moderate differentiation; F_{ST}0.15–0.25 showing great differentiation and over 0.25 indicating extremely great differentiation. Although RC and YT populations were separated by the least geographic distance (100 km), and the genetic differentiation between them was small ($F_{ST} = 0.0354$), this difference was significant.

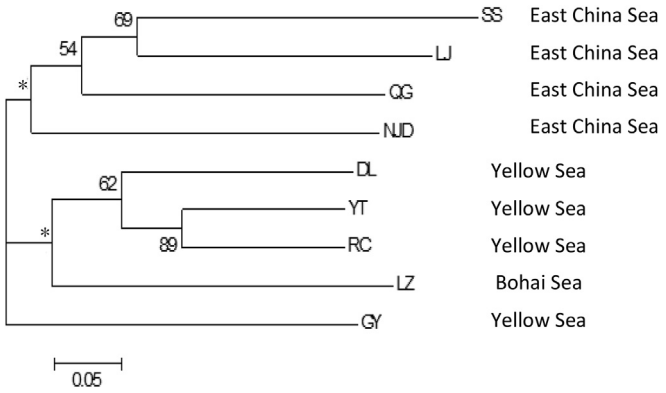

Fig. 2. Unrooted neighbor-joining tree among nine populations of *O. minor* based on Dc values. Numbers on branches indicates bootstrap support values. (* indicates bootstrap support values < 50%).

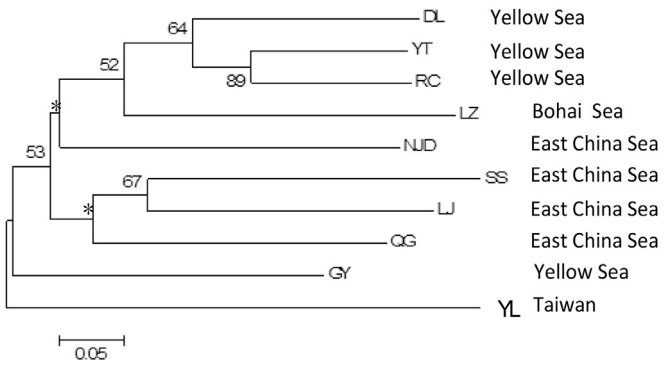

Fig. 3. Unrooted neighbor-joining tree among ten populations of *O. minor* based on Dc values. Numbers on branches indicates bootstrap support values (*indicates bootstrap support values < 50%).

Genetic divergence among the DL, YT and RC populations reached moderate levels as did that between the SS and LJ population. Genetic variation among the remaining populations was great or extremely great. It is clear that the southern populations (SS, LJ, QG and NJD) differed from the northern (DL, YT, RC and LZ) based on F_{ST} and D_C values. Given the long distance and the difference of marine ecological environment between these two regions, it was not surprising to see the divergence. The F_{ST} values between YL and other populations reached more than 0.3 and this degree of differentiation may be indicative of subspecies according to Wright (1978).

The correlation coefficient of geographic and genetic distance is not large, but it is significant, suggesting that geographic isolation can explain most of the genetic heterogeneity, although there are likely other factors that simultaneously affect genetic differentiation. Similarly, the IBD model of *O. minor* was also found by Lü et al. (2013).

4.3. Genetic differentiation affected by the dispersal ability and oceanic landscapes, especially the marine currents

According to F_{ST} and Dc values, the degree of differentiation between YL and GY populations was lower than between the YL and LJ, QG, SS, NJD populations which could not be explained by geographic isolation. Oceanographic characteristics may affect genetic differentiation in addition to geographic distance. Ocean currents are important factors affecting population genetic exchange of marine organisms, playing a very crucial role in population genetic differentiation of cephalopods (Doubleday et al., 2009). In other octopuses, marine currents and geographic isolation affecting dispersal are inferred to play a critical role in population structure (Doubleday et al., 2009; Moreira et al., 2011; Kang et al., 2012). The Yilan and Fujian groups were separated by Southern Okinawa Trough around Yilan where maximum water depth is up to 2,000 m (Diekmann et al., 2008). This certain barrier may hinder the genetic exchange between locations. The Kuroshio Current originates in northern equatorial waters and flows into the East China Sea (Su and Yuan, 2005). A branch of the Kuroshio flows to the Yellow Sea, and even to the Bohai Sea where it is termed the YSWC near the Ryukyu Islands (Fig. 1). YL is located where the Kuroshio flows and YL populations may spread to GY (Yellow Sea) generating a certain degree of genetic exchange. But the explanation is challenged by the very long distances given the restricted dispersal ability of *O. minor*. In addition, samples from the YL population were limited (only 18 individuals), so the geographic population still needs further confirmation.

The GY population differed greatly from the other four northern populations despite GY not being very far away from them geographically, and this can also be explained by the effects of ocean currents. GY is located at the junction of Shandong and Jiangsu Province where ocean eddies exist (Fig. 1), which may reduce gene exchange with other populations outside the vortex. This has been seen in studies of population differentiation of *Mactra chinensis* (Ni et al., 2011). In addition, GY is located in Jiangsu Province where a large area of beach exists. The existence of beach may limit genetic communication given the caving habit of *O. minor*. Geographic distance among the YT, RC, DL, and LZ populations is small, but there is greater genetic distance between LZ and the other three populations. This could be affected by the complex hydrology and bathymetry in Bohai Sea and Yellow Sea. LZ is located in the Bohai Sea, which is a semi-enclosed sea, so it is likely less genetic exchange takes place between the LZ and Yellow Sea populations given the limited dispersal ability. Similar findings had also been seen in the population genetic study of the other benthic marine organisms with a short pelagic larval duration (Zhan et al., 2009; Ni et al., 2011).

In this study, we included a large region of Chinese waters spanning a latitude of 14°32′, systematically showing genetic divergence in a broader distribution. The study herein showed significant genetic differentiation among the ten populations, shared some common views with the findings of Chang et al. (2010) and Lü et al. (2013), which found large genetic differentiation among populations from Zhoushan (south of Shengsi), Wenzhou (north of Nanji Island) and Xiamen (south of Quangang). Especially, we found the extraordinary differentiation between YL and the other populations giving some hints for taxonomic status. Overall, genetic differentiation among the ten geographical populations resulted in combined effects of geographic isolation, currents and life history characteristics. Given the complexity of the marine environment, it may not be satisfactory to try to explain the genetic differentiation of octopus populations using only geographic isolation and ocean current patterns. The complex oceanographic environment around the China Seas with many islands and gulfs makes patterns of gene flow of populations complicated. More advanced molecular markers and more wide distribution samples are expected to further the genetic progress. Nevertheless, this study provides theoretical guidance for resource protection and sustainable management of *Octopus minor* populations in Chinese waters.

Acknowledgments

We would like to thank Prof. C.C. Lu (National Chung Hsing University), Dr. Z.K. Wang, X.Z. Lin (Third Institute of Oceanography State Oceanic Administration), Mr. X.M. Tang (Hainan Academy of Fishery Sciences), Dr. S. Xiao, Dr. W.G. Liu (Nanhai Institute of the Chinese Academy of Sciences), Dr. H.T. Nie (Dalian Ocean University), Dr. Q.Z.Wang (Dalian Fisheries Research Institute), Mr. W. J. Wang (Yantai Fisheries Research Institute), Mr. Y.S. Qian, Mr. Z.S. Liu for collecting the samples. We also thank Dr. C.W. Ho of National Chung Hsing University and Dr. L. Allcock (National University of Ireland Galway) for critical revisions on the manuscript. This work was supported by National Science Funding of China (No. 31172058) and National Marine Public Welfare Research Project of China (No. 201305043).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2016.03.014.

References

Astanei, I., Gosling, E., Wilson, J., Powell, E., 2005. Genetic variability and phylogeography of the invasive zebra mussel, *Dreissena polymorpha* (Pallas). Mol. Ecol. 14, 1655–1666.

Cabranes, C., Fernandez-Rueda, P., Martínez, J.L., 2008. Genetic structure of *Octopus vulgaris* around the Iberian Peninsula and Canary Islands as indicated by microsatellite DNA variation. ICES J. Mar. Sci. 65, 12–16.

Cavalli-Sforza, L.L., Edwards, A.W.F., 1967. Phylogenetic analysis: models and estimation procedures. Evolution 32, 550–570.

Chang, K.M., Li, H., Lü, Z.M., Chi, C.F., 2010. Genetic variation in different populations of *Octopus variabilis* in China coastal waters based on the COI gene analysis. Oceanol. Limnol. Sin. 41 (3), 307–314 (in Chinese).

Diekmann, B., Hofmann, J., Henrich, R., et al., 2008. Detrital sediment supply in the southern Okinawa trough and its relation to sea-level and Kuroshio dynamics during the late quaternary. Mar. Geol. 255 (1), 83–95.

Dieringer, D., Schlötterer, C., 2003. Microsatellite ANALYSER (MSA): a platform independent analysis tool for large microsatellite data sets. Mol. Ecol. Notes 3, 167–169.

Dong, Z.Z., 1988. Fauna Sinica. Phylum Mollusca. Class Cephalopoda. Science Press, Beijing, pp. 181–182 (in Chinese).

Doubleday, Z.A., Semmens, J.M., Smolenski, A.J., Shaw, P.W., 2009. Microsatellite DNA markers and morphometrics reveal a complex population structure in a merobenthic octopus species (*Octopus maorum*) in south-east Australia and New Zealand. Mar. Biol. 156, 1183–1192.

Earl, D.A., vonHoldt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4, 359–361.

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

Excoffier, L., Lischer, H.E.L., 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.

Gao, Q., Zheng, X.D., Kong, L.F., Wang, Z.P., Wang, R.C., 2009. Biochemical genetic analysis of wild populations of *Octopus variabilis*. Period. Ocean. Univ. Chin. 39 (6), 1193–1197 (in Chinese).

Goudet, J., 2001. FSTAT: a Program to Estimate and Test Gene Diversities and Fixation Indices (Version 2.9.3). Available at: http://www2.unil.ch/popgen/softwares/fstat.htm.

Guo, B.Y., Zhou, C., Lü, Z.M., Li, J.J., Wu, C.W., 2011. Genetic diversity of different geographical populations in *Octopus variabilis* revealed by ISSR analysis. Oceanol. Limnol. Sin. 42 (6), 868–873 (in Chinese).

Juárez, O.E., Rosas, C., Arena, L., 2010. Heterologous microsatellites reveal moderate genetic structure in the *Octopus maya* population. Fish. Res. 106, 209–213.
Kang, J.H., Kim, Y.K., Park, J.Y., et al., 2012. Development of microsatellite markers to genetically differentiate populations of *Octopus minor* from Korea and China. Mol. Biol. Rep. 39 (8), 8277–8286.
Langella, O., 1999. POPULATIONS 1.2.30. Available at: http//bioinformatics.org/tryphon/populations/.
Li, H., Lv, Z.M., Chang, K.M., et al., 2010. Genetic variation in different populations of *Octopus variabilis* in China coastal waters based on the 16S rRNA gene analysis. J. Zhejiang Ocean. Univ. 29 (4), 325–330 (in Chinese).
Lü, Z.M., Liu, L.Q., Li, H., et al., 2013. Deep phylogeographic break among *Octopus variabilis* populations in China: evidence from mitochondrial and nuclear DNA analyses. Biochem. Syst. Ecol. 51, 224–231.
Moreira, A.A., Tomás, A.R.G., Hilsdorf, A.W.S., 2011. Evidence for genetic differentiation of *Octopus vulgaris* (Mollusca, Cephalopoda) fishery populations from the southern coast of Brazil as revealed by microsatellites. J. Exp. Mar. Biol. Ecol. 407, 34–40.
Ni, L.H., Li, Q., Kong, L.F., 2010. Isolation and characterization of 19 microsatellite markers from the Chinese surf clam (*Mactra chinensis*). Conserv. Genet. Resour. 2, 27–30.
Ni, L.H., Li, Q., Kong, L.F., 2011. Microsatellites reveal fine-scale genetic structure of the Chinese surf clam *Mactra chinensis* (Mollusca, Bivalvia, Mactridae) in Northern China. Mar. Ecol. 32 (4), 488–497.
Palumbi, S.R., 2003. Population genetics, demographic connectivity, and the design of marine reserves. Ecol. Appl. 13, 146–158.
Pérez-Losada, M., Guerra, A., Carvalho, G.R., Sanjuan, A., Shaw, P.W., 2002. Extensive population subdivision of the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda) around the Iberian Peninsula indicated by microsatellite DNA variation. Heredity 89, 417–424.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Qian, Y.S., Zheng, X.D., Wang, P.L., Li, Q., 2010. Analysis and evaluation of nutritive composition of *Octopus minor* in Lake Swan. Mar. Sci. 34 (12), 14–18 (in Chinese).
Raymond, M., Rousset, F., 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86, 248–249.
Reichow, D., Smith, M.J., 2001. Microsatellites reveal high levels of gene flow among populations of the California squid *Loligo opalescens*. Mol. Ecol. 10 (5), 1101–1109.
Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223–225.
Rousset, F., 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145, 1219–1228.
Sokal, R.R., Rohlf, F.J., 1995. Biometry: the Principles and Practice of Statistics in Biological Research, third ed. Freeman, New York. 887.
Su, J.L., Yuan, L.Y., 2005. Hydrology of China Seas. Ocean Press, Beijing, pp. 172–192 (in Chinese).
Sun, B.C., Yang, J.M., Sun, G.H., et al., 2010. Sequence and molecular phylogeny of mitochondrial COI gene fragment in five populations of *Octopus variabilis* in China. Oceanol. Limnol. Sin. 41 (2), 259–265 (in Chinese).
Takezaki, N., Nei, M., 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144, 389–399.
Tamura, K., 1997. TreeExplorer 2.12. Available at: http://evolgen.biol.metro-u.ac.jp/TE/TE_man.html.
Tautz, D., 1989. Hypervariability of simple sequences as a general source for polymorphic markers. Nucleic Acids Res. 17, 6463–6471.
Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4, 535–538.
Villanueva, R., Norman, M.D., 2008. Biology of the planktonic stages of benthic octopuses. Oceanogr. Mar. Biol. Annu. Rev. 46, 105–202.
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.S., Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. Evolution 38, 1358–1370.
Winnepenninckx, B., Backeljau, T., De Wachter, R., 1993. Extraction of high molecular weight DNA from molluscs. Trends Genet. 9 (12), 407.
Wright, S., 1978. Evolution and the Genetics of Population, Variability within and Among Natural Populations. The University of Chicago Press, Chicago.
Xu, M.Y., Li, J.J., Guo, B.Y., Lü, Z.M., Zhou, C., Wu, C.W., 2011. Genetic diversity of seven populations of *Octopus variabilis* in China's coastal waters based on the 12S rRNA and COIII gene analysis. Oceanol. Limnol. Sin. 42 (3), 387–396 (in Chinese).
Zhan, A.B., Hu, J.J., Hu, X.L., Zhou, Z.C., Hui, M., Wang, S., Peng, W., Wang, M.L., Bao, Z.M., 2009. Fine-scale population genetic structure of Zhikong scallop (*Chlamys farreri*): do local marine currents drive geographical differentiation? Mar. Biotechnol. 11 (2), 223–235.
Zheng, X.D., Ikeda, M., Kong, L.F., Lin, X.Z., Li, Q., Taniguchi, N., 2009. Genetic diversity and population structure of the golden cuttlefish, *Sepia esculenta* (Cephalopoda: Sepiidae) indicated by microsatellite DNA variations. Mar. Ecol. 30 (4), 448–454.
Zheng, X.D., Qian, Y.S., Liu, C., Li, Q., 2014. Octopus minor. In: Iglesias, J., Fuentes, L., Villanueva, R. (Eds.), Cephalopod Culture. Springer, Netherlands, pp. 415–426.
Zuo, Z.R., Zheng, X.D., Yuan, Y., Li, Q., 2011. Development and characterization of 12 polymorphic microsatellite loci in *Octopus minor*. Conserv. Genet. Resour. 3, 489–491.