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Molecular phylogenetics and population demographic history of *Amphioctopus fangsiao*, inferred from mitochondrial and microsatellite DNA markers

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Abstract

Amphioctopus fangsiao (Cephalopoda: Octopodidae) is an important commercial species in the coastal waters of China. In recent years, however, the resource of *A. fangsiao* have declined because of habitat destruction and overfishing. To analyze the genetic variations of *A. fangsiao* caused by the fluctuation of resources, the population genetic structure of nine sampling locations collected from the Bohai Sea to the South China Sea were investigated, using mtDNA *COI* fragments and microsatellite DNA. The results of *F*-statistics, AMOVA, STRUCTURE and PCA analyses showed three phylogeographic clades (Clades A, B and C), revealing limited genetic exchange between north and south populations. These clades diverged in 2.23 (Clades A and B) and 3.67 (Clades A, B and C) million years ago, during the dramatic environmental fluctuations, such as sea level and temperature changes, have exerted great influence on the survival distribution pattern of global organisms. Our results for low genetic connectivity among *A. fangsiao* populations provide insights into the development of management strategies, that is, to manage this species as separate management unit.

Key words: genetic diversity, population genetic structure, *Amphioctopus fangsiao*, mitochondrial DNA, microsatellite DNA

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1 Introduction

Investigating the genetic structure of a species, thereby revealing its population structure, is a fundamental step toward drawing up reasonable and practical management policies (Botsford et al., 2009; Prentis et al., 2009). The generation of genetic structure in marine organisms can be mainly attributed to biological characteristics and marine environment. Due to spawning, feeding, and wintering, some marine organisms migrate large distances every year (Lin et al., 2011). Moreover, the pelagic eggs of some species are dispersed by the ocean current, resulting in gene flow among populations (Charrier et al., 2007). These factors may be responsible for the complex genetic structure of species. Besides, understanding the population demographic history of a commercial species is also very important for inferring the origin of its genetic structure (Liu et al., 2007). Phylogeographic patterns are the result of species dispersal and geological events. It is generally believed that the major geohistorical events, such as Pleistocene climate oscillations, have exerted profound influences on the population structure and evolutionary processes (Liu et al., 2006). Therefore, it is essential to understand the genetic structure and population demographic history of economically important marine organisms for resource management and conservation.

Unlike most marine fishes with low genetic differentiation, cephalopods had more complex genetic structures due to divergent migratory capability and extensive distribution (Zheng et al., 2009). Knowledge of the phylogeographic pattern of Cephalopoda is far from being completed but has significantly advanced in recent years, especially by using molecular markers (Fadhlaoui et al., 2012; Liu et al., 2019; Muhammad et al., 2020; Tang et al., 2020). *Amphioctopus fangsiao* is also known as the synonym name of *Octopus ocellatus*, which is one of the most important species of cephalopods (Jiang et al., 2020b). It is widely distributed across Northwest Pacific and is an important commercial species (Segawa and Nomoto, 2002; FAO, 1984). *Amphioctopus fangsiao* is a benthic and neritic octopus, and it is very popular among consumers owing to its rich nutritional value and fresh flavor (Zheng et al., 2022, 2023). However, *A. fangsiao* resource has declined in recent years because of overfishing for meeting the growing market demand (Jiang et al., 2020a). Although small-scale octopus farming is already possible due to the breakthrough in technical constraints of culture, indoor tank-cultured of *A. fangsiao* is still in early stages compared with the mature culture techniques of other mollusks, such as oyster (Jiang et al., 2020a). Therefore, the wild *A. fangsiao* is still the main source to meet consumer demand. In recent years, numerous studies have focused on aquaculture, biological characteristics, and ethology of *A. fangsiao* (Tziouveli and Yokoyama, 2017; Lee et al., 2017; Pang et al., 2020), yet the population genetics of this species has seldom been thoroughly studied. Relatively few studies have

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been conducted to address the population genetic aspects of *A. fangsiao*. Gao et al. (2002) investigated the genetic variation of *A. fangsiao* on the northern coast of China based on allozyme and showed a certain degree of genetic differentiation between populations, which was also supported by the results based on amplified fragment length polymorphism (AFLP) markers (Zhang et al., 2009). Lv et al. (2010) and Faiz et al. (2019) used mitochondrial DNA fragments to detect the genetic structure of *A. fangsiao* in the coastal waters of China, revealing very deep phylogeographic divergence between northern and southern populations. *Amphioctopus fangsiao* has a complex genetic structure due to the complexity of its habitat, larval dispersal and local adaptation (De Luca et al., 2014; Faiz et al., 2019). And its genetic structure is dynamic due to the resource change caused by the environment and fishing pressure. The current genetic structure of *A. fangsiao* is the basis for the formulation and implementation of management policy. Therefore, more studies on the population genetics of *A. fangsiao* are still needed to develop more reasonable conservation policies.

With the rapid development of sequencing techniques, many molecular markers have been brought to the study of marine organisms (Meng et al., 2003; Olivares-Paz et al., 2006; Sekino and Hara, 2001). The use of mitochondrial DNA (mtDNA) in population genetics has increased rapidly over the past several decades as clear mechanisms, simple structures and low molecular weights (Moritz et al.,1987; Wirgin et al., 2000; Tokuyama et al., 2020). Also, it has been proven to be a very effective and reusable molecular marker (Simons et al., 2001). Additionally, microsatellite DNA (SSR) has also become one of the most commonly used molecular markers in population genetics (Parida et al., 2009; Song et al., 2018) due to the characteristics of codominant inheritance, high genomic coverage and high variability (Gupta et al., 1999; Zane et al., 2002; Shabani et al., 2013; Simbine et al., 2014). It could be relatively accurate to calculate the genetic parameters among different populations and then detect the population genetic structure, genetic diversity, and historical dynamics (Simons et al., 2001; Song et al., 2014). In the present study, fragments of *Cytochrome C oxidase subunit I* (*COI*) fragments and ten microsatellite DNA loci have been used as molecular markers to analyze genetically *A. fangsiao*. This study aims to result in a more comprehensive molecular phylogenetics analysis, including genetic diversity, genetic structure and population demographic history, thereby helping to develop more reasonable resource conservation strategies.

2 Materials and methods

2.1 *Study area and sampling*

Samples from nine locations across the coastal waters of China were used in the present study (Fig. 1, Table 1). During sampling, we were approved by the local fishermen. The muscle tissue of these individuals was stored in alcohol for total genomic DNA extraction using phenol/chloroform method (Sambrook et al., 1989). Samples were collected by local fishermen with small boats.

2.2 *PCR and sequencing*

The primers designed to amplify the fragments of the *COI* were *cox1*-F-GGTCAACAAATCATAAAGATATTGG and *cox1*-R-ATGGGGAGCAACCACAAGAA. The PCR was performed in A300 Fast Thermal Cycler (LongGene Scientific Instruments, Co. Ltd., China). The PCR amplifications were carried out in volume of 25 μL,

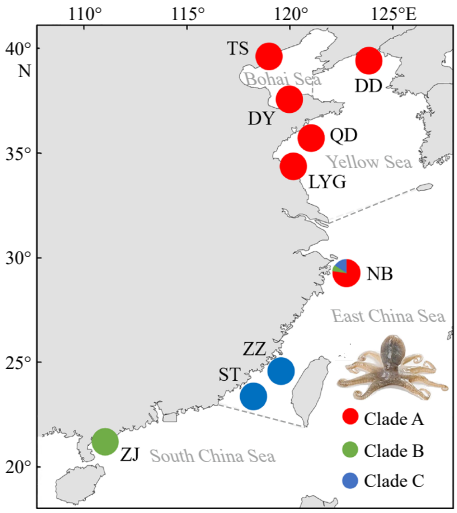

Fig. 1. The sampling sites of *Amphioctopus fangsiao* in this study. Pie charts show the frequencies of lineages A, B and C based on *COI* gene (see results for determining the two lineages). Gray dotted line mean demarcation line among sea area.

Table 1. *Amphioctopus fangsiao* sampling information and genetic diversity parameter of *COI* gene

Sample	Code	Sample number (N)	Haplotype number	Haplotype diversity (h)	Nucleotide diversity (π)
Tangshan	TS	22	4	0.454 5	0.001 2
Dandong	DD	13	5	0.692 3	0.018 3
Dongying	DY	10	4	0.644 4	0.001 6
Qingdao	QD	32	6	0.645 5	0.001 4
Lianyungang	LYG	32	6	0.606 9	0.001 6
Ningbo	NB	31	4	0.187 1	0.003 0
Zhangzhou	ZZ	8	2	0.250 0	0.000 3
Shantou	ST	16	7	0.691 7	0.025 8
Zhanjiang	ZJ	27	7	0.661 0	0.001 4
Clade A	/	142	17	0.691 9	0.006 6
Clade B	/	31	7	0.735 5	0.010 2
Clade C	/	18	3	0.215 7	0.002 9
Total	/	191	27	0.798 9	0.011 8

the reaction system containing deionized water (17.35 μL), dNTPs (2 μL), 10×PCR Buffer (2.5 μL), forward and reverse primers (1 μL), Taq polymerase (0.15 μL), DNA template (1 μL). The amplification conditions were as follows: 5 min denaturation at 94℃, 38 alternating cycles of 45 s at pre-denaturation 5 min (95℃), denaturation 45 s (94℃), annealing 45 s (50℃), extension 45 s (72℃), 38 cycles and a final extension of 10 min (72℃). The amplification products were detected by 1% agarose gel electrophoresis. The products were sequenced by Sangon Biotech (Shanghai) Co., Ltd. All samples were sequenced in both directions to ensure the accuracy of these fragments. The sequencing primers were the same as the PCR primers.

Besides, a total of 10 microsatellite loci developed by Feng et al. (2017) were selected to study the population genetics of *A. fangsiao* in the present study (Table 2). The PCR reaction system and amplification conditions were carried out as described above. The annealing temperature (T_a) of each locus is shown in Table 2. The amplification products detected by 1% agarose gel electrophoresis were sent to Sangon Biotech (Shanghai) Co., Ltd., for genotyping of microsatellites DNA.

Table 2. The sequence information of 10 microsatellite loci

Locus	Repeat motif	Primer sequence (5′–3′)	Size	T_a/℃
DS-106	(CA)5	F: GTCACTACGACCACTCTTCCA	184–198	55
		R: GTCCACCTCTACCTAAAAATCTG		
DS-116	(AT)6	F: AACCACAGGTCGGTCAACG	108–130	62
		R: GCCAGGGAACGCAACTAAA		
DS-132	(GT)7	F: ACGGACAATGGCGTTTAC	160–178	52
		R: GGATTTGGGACATAGAAGAA		
DS-135	(TAC)7	F: CCTGTCTGGCGACTATTG	258–286	50
		R: GGTTTCTGTTGCTACTTCG		
DS-137	(AAC)8	F: CTCATACTACCAGCTTACCTT	234–260	48
		R: TTGATGCCACATATTATACAC		
DS-150	(AC)13	F: GGACAGACTCTTTAGGCATT	152–192	51
		R: CTCCCAACTGAACTCAACTC		
DS-152	(GAT)5	F: GACAGCAATGACCGATAGG	172–214	53
		R: TGTGAGTCCAACACCCAGT		
DS-210	(TA)6(TA)10	F: GATGGCTACGACACTCCTACTGA	220–240	48
		R: CAATGCCCCTCCCCTTTT		
DS-216	(CT)7	F: TGCGGCAAGGACTTTCAC	254–280	50
		R: AGAGGCGAGGCGATTAGG		
DS-226	(TCC)6(TCC)5(TCC)5	F: GATGGTTGCTGTATGTGCTGC	187–211	50
		R: GGGGGTGTTCCAATGTCTTC		

Note: T_a: the annealing temperature.

2.3 *Data analysis*

DNASTAR software was used to align and edit all sequences (DNASTAR Inc., Madison, WI). Genetic diversity indices such as polymorphic sites, nucleotide diversity (π), and haplotype diversity (h) (Nei, 1987) were calculated using ARLEQUIN v.3.0 (Excoffier et al., 2007). The phylogenetic relationships based on haplotypes were analyzed by the Maximum Likelihood (ML) method (Tamura et al., 2011) and using TIM+F+G4 (*COI*), as a substitution model calculated by ModelFinder plugin (Kalyaanamoorthy et al., 2017) integrated into IQTREE v1.6.12 (Nguyen et al., 2015). The ML analysis was performed in IQ-TREE v1.6.12 with 1 000 bootstraps replicates. Popart v.1.7 with default settings was used to construct the haplotype networks. And then, the haplotype network was visualized and manually adjusted. *F*-statistics (F_{st}) and molecular variance (AMOVA) with Kimura-2-parameters model of substitution were calculated in ARLEQUIN v.3.0 to evaluate population structure (Kimura, 1980; Weir and Cockerham, 1984; Excoffier et al., 1992). The genetic distances among clades based on TIM+F+G4 model were calculated in MEGA v.5.0 (Tamura et al., 2011). The Bayesian skyline plot (BSP) was generated with BEAST v.2.3.0 (Bouckaert et al., 2014) and Tracer v.1.7.1 (Rambaut et al., 2018). The strict molecular clock and stepwise skyline were selected as a model.

Genemarker v.1.91 was used to calculate microsatellite alleles (Hulce et al., 2011). Genetic diversity parameters such as allelic abundance, allelic richness (A_R), polymorphic information content (PIC), expected heterozygosity (H_e), and observed heterozygosity (H_o) were calculated using Excel Microsatellite Toolkit (MS-tools) (Park, 2001). The value of F_{st} was obtained using Fstat v.2.9 (Goudet, 1995). Population v.1.2 was used to calculate the $(\delta\mu)^2$ genetic distance (Raymond and Rousset, 1995; Page, 1996). Principal component analysis (PCA) which was analyzed by the software of Genetix v.4.5.0 and R 3.2.2 was used to examine the genetic relationships among *A. fangsiao* populations (Belkhir et al., 2004, R. R Development Core Team, 2006). STRUCTURE v.2.2 was used to detect the cryptic population structure of *A. fangsiao* (Pritchard et al., 2000). The parameters of Markov Chain Monte Carlo (MCMC) were set as follows: 100 000 burn-in iterations, followed by 1 000 000 iterations. The clusters *K* value (the maximum number of clusters) estimated with the admixture model ranged from 1 to 9 (total sites) (Evanno et al., 2005). To verify the results, each cluster *K* value was run independently. To confirm the consistency of analysis, we carried out ten independent runs for each specific *K* value. The most appropriate number of *K* values (the optimum number of ancestral groups that explain the genotypic distribution) was estimated based on the ad hoc estimated likelihood of *K*. The Mantel test was carried out by IBDWS (Bohonak, 2002; Jensen et al., 2005).

3 Results

3.1 *Mitochondrial DNA analysis*

3.1.1 *Sequence variation and genetic differentiation*

A total of 581 bp of *COI* sequences were aligned for 191 samples from 9 sampling locations. There were 57 polymorphic sites, which defined 56 substitutions consisting of 46 transitions and 10 transversions. The average base composition content was 29.09% for A, 17.61% for C, 14.38% for G, 38.92% for T. The number of haplotypes was 27 (Table 1). The diversity parameters like haplotype diversity (h) and nucleotide diversity (π) of each locality are shown in Table 1.

3.1.2 *Phylogenetic relationships*

Clustering analysis of *COI* haplotypes was conducted by ML method. Three clades were found in topologies. In *COI* tree, the localities from the north of Ningbo (the Bohai Sea, the Yellow Sea and the northern East China Sea) clustered in Clade A, while Clade B mainly included the localities ZJ (the South China Sea), Clade C mainly included the localities ZZ and ST (the East China Sea). Clade B haplotypes were separated by 7 mutational steps from Clade A haplotypes, and Clade C haplotypes were separated by 26 mutational steps from Clade A haplotypes. The individuals from Ningbo belonged to the three clades which showed

complex genetic structures. The median-joining network of the *COI* gene revealed that only three haplotypes were shared by Clades A, B and C (Fig. 2). This result suggests low gene flow and hence the genetic differentiation among three clades.

3.1.3 *Population structure*

Subsequently, the above results led to an analysis of the possible genetic difference between three clades using F_{st} and AMOVA analyses. Significant genetic differentiation caused by geographical isolation was detected among three clades using *COI* genes, supporting the results of phylogenetic relationships (Fig. 3).

AMOVA analysis demonstrated that the genetic variation among sampling locations was 65.57%, while the 34.42% variation was detected within populations when all populations were considered as one gene pool. When these populations were divided into two groups (Clade A and Clades B, C), the genetic variation among groups was 45.02%, which was all greater than the variation within population of 29.26% (Table 3). These populations were finally divided into three groups according to the phylogenetic results (Clades A, B, C). This result showed that most variation was detected among groups, revealing significant population structure existed throughout the examined range of *A. fangsiao*.

3.1.4 *Population demographic history*

The Bayesian skyline plots demonstrated that there was a recent demographic expansion in Clade A but no significant expansion event was found in Clades B and C (Fig. 4). The different population demographic history among clades may be an im-

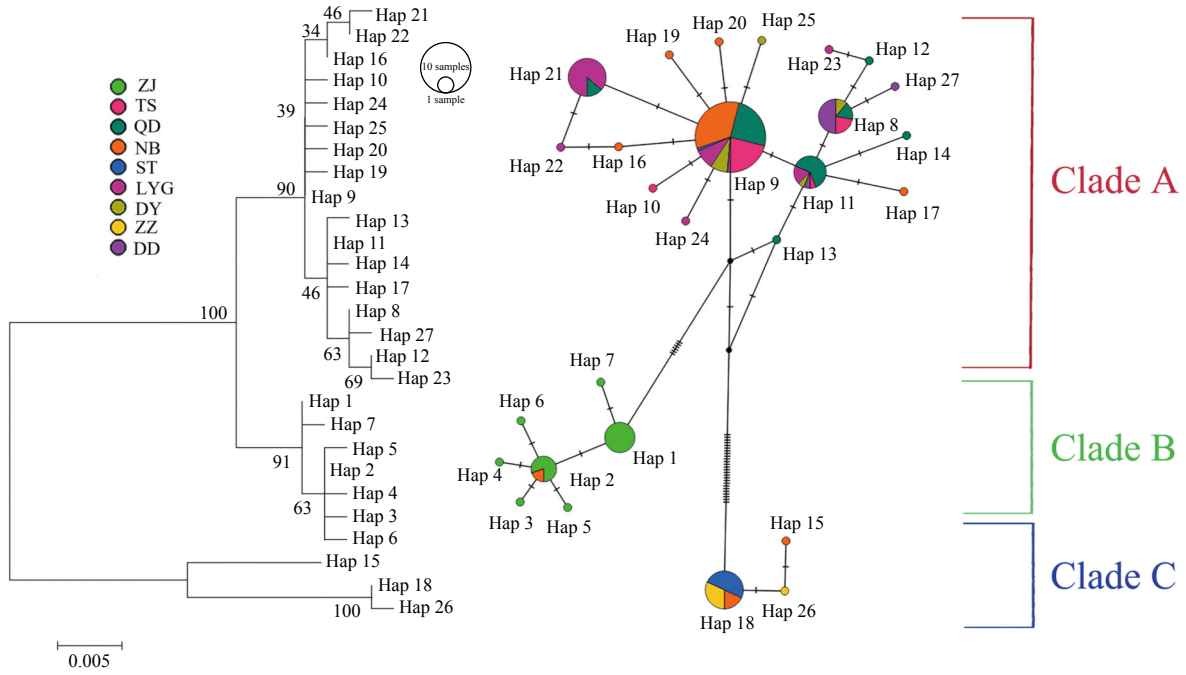

Fig. 2. The phylogenetic analyzes for *Amphioctopus fangsiao* investigated using Maximum Likelihood tree (left) and haplotype network (right).

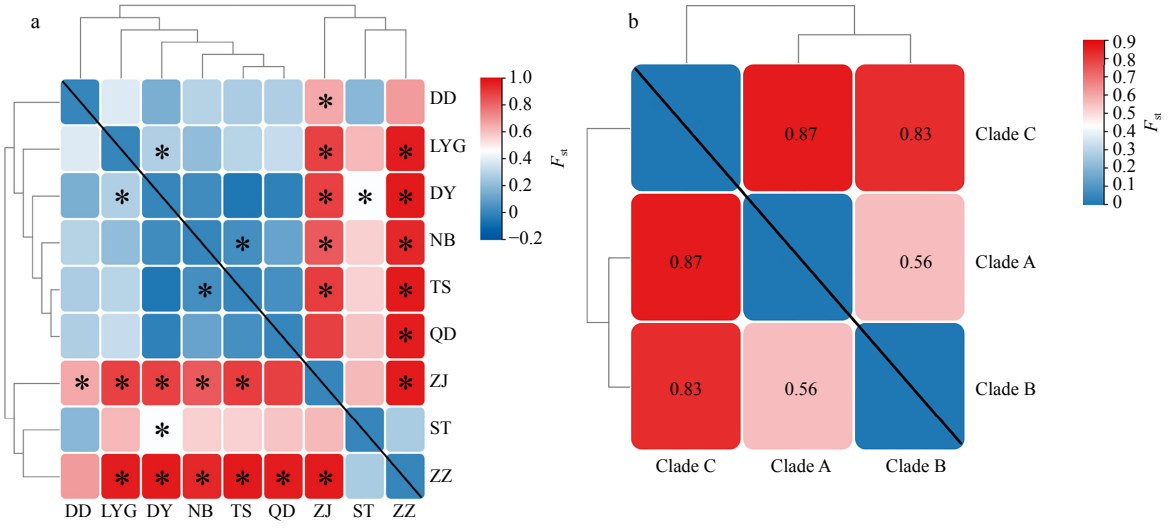

Fig. 3. *F*-statistics (F_{st}) of *Amphioctopus fangsiao* in sampling locations. * means significant genetic differentiation ($p \leqslant 0.05$).

Table 3. Molecular variance of *Amphioctopus fangsiao* populations of different locations

	Source of variation	Variance components	Percentage variation	*P*
One gene pool (all samples)	among populations	2.841 0	65.57	<0.001
	within populations	1.491 2	34.42	<0.001
Two gene pool (north) (south)	among groups	2.611 3	45.02	<0.001
	among populations within groups	1.491 2	25.71	<0.001
	within populations	1.697 2	29.26	<0.001
Three gene pool (Clade A) (Clade B) (Clade C)	among groups	6.613 5	76.10	0.032 2
	among populations within groups	0.415 63	4.78	0.032 2
	within populations	1.661 79	19.12	0.032 2

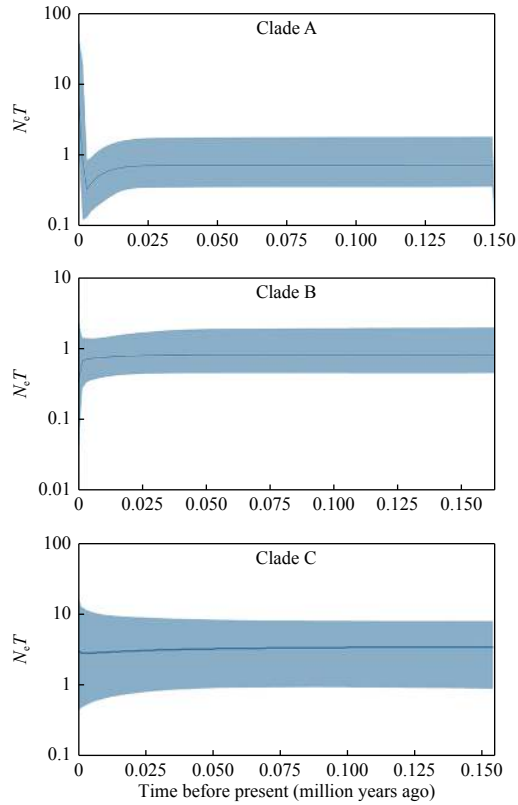

Fig. 4. Bayesian skyline plots for *Amphioctopus fangsiao* populations based on *COI* fragment. N_e: effective population size; T: generation time.

portant reason of the generation of genetic structure.

The divergence time between three clades was calculated based on the *COI* sequences. The equation $T=D/(2\alpha)$, where T was divergence time, D was genetic distance and α was the substitutions rate, was used in this study (Amor et al., 2014). The genetic distance calculated in this study among clades was 0.017 (Clade A and Clade B), 0.048 (Clade A and Clade C) and 0.048 (Clade B and Clade C), and about 0.381% substitutions per site per million years of *COI* gene for octopods was used based on the previous studies (Strugnell et al., 2012). Therefore, the divergence time among clades was about 2.23 MYA (million years ago, between Clade A and Clade B) and 3.67 MYA (between Clade A and Clade C, and between Clade B and Clade C).

3.2 *Microsatellite analysis*

3.2.1 *Genetic diversity of A. fangsiao populations*

Summary statistics of genetic diversity parameters are shown in Tables 2 and 4. A total of 90 alleles (A) were detected in 9 sampling locations, and the number of alleles (A) per loci ranged from 8 to 22. The highest average allele richness was found in localities NB (12.146), while the lowest was in localities ZJ (9.598). The range of average observed heterozygosity was 0.902 (ZJ) to 0.940 (NB), and the average polymorphic information content ranged from 0.855 (ZZ) to 0.899 (NB), revealing high genetic diversity of *A. fangsiao* in different sampling locations (PIC>0.5).

3.2.2 *Genetic structure and differentiation*

The genetic structure of *A. fangsiao* in different sampling locations was investigated using pairwise F_{st}. Consistent with mitochondrial DNA, significant genetic differentiation was detected among three clades (Fig. 5a). The UPGMA tree was also constructed based on genetic distance $(\delta\mu)^2$ (Fig. 5b). The result also indicated that there were three clusters, localities NB TS, DD, DY, LYG and QD formed one cluster; locality ZZ, ST formed another cluster; the locality ZJ was clustered separately.

The result of PCA showed that the contribution rates of three principal components were 37.3%, 21.79% and 12.59%, respectively, and significant population structure existed throughout the examined range of *A. fangsiao* (Fig. 6). Three groups were accurately separated by the first two principal components.

Inference of the number of genetic clusters was obtained by the program STRUCTURE. The results indicated the highest ΔK value was obtained for K=4, which can explain the clusters in a satisfactory manner (Fig. 7). *Amphioctopus fangsiao* in different sampling locations showed significant clustering trends when K=4, which supported the result of F_{st} and discriminant analysis of principal components (Fig. 8). Obvious clustering trends were observed for the nine sampling locations, the first cluster consists of localities TS, DD, DY, QD, LYG and NB, localities ZZ, ST were assigned to the second cluster, and locality ZJ was assigned to the third cluster.

4 Discussion

In this study, mitochondrial DNA *COI* fragments and microsatellite DNA loci were used to analyze the current genetic diversity status, genetic structure, and population demographic history of *A. fangsiao* populations. Three genealogical clades were found across the coastal waters of China. Strong support was found for Clade A that comprises the localities TS, DD, DY, QD and LYG, while Clade B originated from the localities ZZ and ST, and Clade C was occupied by the locality ZJ.

There was significant genetic differentiation between northern and southern. The differences in salinity, temperature and dissolved oxygen between the northern and southern seas may be the main reasons for the genetic differentiation. The Dalian of the northern seas has relatively low sea temperature with a large temperature difference (30℃) between summer and winter, while ZZ of the southern seas can maintain a high temperature (temperature difference is only 8–9℃) throughout the year (Du,

Table 4. Genetic diversity of 9 populations by 10 microsatellite loci

Locus		Population								
		TS	DD	DY	QD	LYG	NB	ZZ	ST	ZJ
DS-106	A	13	12	14	11	13	14	13	13	14
	R_S	10.713	9.831	10.662	10.193	9.320	11.794	13.000	12.052	10.950
	H_o	1	1	1	1	1	1	1	1	1
	H_e	0.930 8	0.907 3	0.926 4	0.907 3	0.915 8	0.939 2	0.947 4	0.949 3	0.928 2
	PIC	0.875 4	0.886 8	0.872 2	0.865 9	0.870 8	0.832 7	0.844 7	0.866 4	0.882 4
DS-116	A	11	11	11	12	12	10	10	11	10
	R_S	9.329	9.886	9.048	9.451	10.080	8.702	10.000	10.121	9.256
	H_o	1	1	1	1	1	1	1	1	1
	H_e	0.909	0.923 4	0.902 5	0.903 7	0.900 7	0.881	0.905 3	0.916 7	0.915 4
	PIC	0.900 0	0.869 3	0.899 6	0.868 7	0.887 8	0.898	0.892 1	0.902 6	0.897 4
DS-132	A	14	16	17	16	15	15	12	13	15
	R_S	10.291	11.804	11.079	11.774	12.322	12.958	12.000	11.766	10.954
	H_o	0.933 3	0.935 7	0.933 2	0.948 3	0.942 1	0.966 5	0.951 2	0.941 1	0.965 2
	H_e	0.915 4	0.926 9	0.922 9	0.944 7	0.941 5	0.955 0	0.947 4	0.934 8	0.917 9
	PIC	0.882 9	0.891 9	0.896	0.910 9	0.916 4	0.915 4	0.891 5	0.886 9	0.886 2
DS-135	A	10	12	10	9	11	14	9	8	10
	R_S	8.519	10.411	8.104	8.448	7.799	11.750	9.000	7.784	8.410
	H_o	0.946 5	0.903 2	0.923 1	0.942 1	0.923 1	0.932 5	0.902 3	0.895 4	0.899 8
	H_e	0.887 2	0.926 9	0.880 3	0.866 3	0.883 9	0.936 5	0.905 3	0.884 1	0.885 9
	PIC	0.850 8	0.891	0.846 7	0.822 3	0.851	0.895 1	0.844 4	0.829 4	0.848 9
DS-137	A	14	18	15	20	22	16	9	15	14
	R_S	10.085	12.504	10.147	13.617	14.455	13.504	9.000	13.679	10.283
	H_o	0.951 7	0.943 5	0.953 2	0.922 2	0.921 0	0.966 6	0.942 5	0.975 6	0.925 8
	H_e	0.902 6	0.935 8	0.906 9	0.964 3	0.950 4	0.960 3	0.915 8	0.963 8	0.914 1
	PIC	0.868 8	0.901 4	0.878	0.932 3	0.926 7	0.921 1	0.855 8	0.918 4	0.881 5
DS-150	A	12	12	11	12	12	13	11	9	13
	R_S	9.027	10.339	9.001	9.715	9.859	11.237	11.000	8.454	10.038
	H_o	1	1	1	1	1	1	1	1	1
	H_e	0.875 6	0.923 4	0.896 3	0.910 9	0.916 7	0.931 2	0.926 3	0.884 1	0.898 7
	PIC	0.838 5	0.887 1	0.865 6	0.873 1	0.888 4	0.889 4	0.868 5	0.829 7	0.864 7
DS-152	A	15	13	15	12	14	15	9	10	12
	R_S	11.685	11.368	11.728	10.459	9.685	12.408	9.000	9.611	8.985
	H_o	1	1	1	1	1	1	1	1	1
	H_e	0.935 9	0.941 2	0.942 4	0.903 7	0.918 4	0.941 8	0.810 5	0.927 5	0.864 1
	PIC	0.906	0.906 8	0.917 4	0.865	0.890 9	0.901 1	0.740 3	0.878 1	0.826 2
DS-210	A	15	15	14	17	18	14	12	10	12
	R_S	10.510	11.355	9.878	11.657	12.994	12.344	12.000	9.325	9.398
	H_o	1	1	1	1	1	1	1	1	1
	H_e	0.902 6	0.928 7	0.909 6	0.950 1	0.929 1	0.952 4	0.947 4	0.913 0	0.907 7
	PIC	0.869 4	0.893 3	0.880 6	0.916 9	0.903 1	0.912 4	0.891 5	0.861 9	0.873 8
DS-216	A	16	18	16	16	18	16	11	12	12
	R_S	11.750	13.184	11.165	11.683	12.200	13.306	11.000	11.221	9.200
	H_o	0.958 3	1	0.875	0.958 3	1	0.916 7	0.875 2	0.945 1	0.845 1
	H_e	0.935 9	0.950 1	0.923 8	0.937 6	0.928 2	0.955	0.921 1	0.938 4	0.896 2
	PIC	0.906	0.917	0.897	0.903 4	0.902 2	0.915 5	0.862 5	0.890 7	0.860 9
DS-226	A	13	13	14	15	15	16	9	10	10
	R_S	10.447	10.356	10.094	10.918	12.020	13.461	9.000	9.449	8.509
	H_o	1	1	1	1	1	1	1	1	1
	H_e	0.921 8	0.916 2	0.914 0	0.943 0	0.925 5	0.957 7	0.915 8	0.913 0	0.894 9
	PIC	0.890 2	0.879 2	0.885 7	0.909	0.898 8	0.918 4	0.856 1	0.862 3	0.859 0

Note: A: alleles; R_S: allele richness; H_o: observed heterozygosity; H_e: expected heterozygosity; PIC: polymorphic information content.

2018). Moreover, salinity gradually increases as latitude decreased across the coast of China, while dissolved oxygen has the opposite trend. Like any other octopus, *A. fangsiao* lives in benthic with weak diffusion ability, which may lead to adaptive differentiation among different sampling locations. The selection pressure from these environmental factors may lead to genetic differentiation among *A. fangsiao* (Du, 2018). Effective management of important economic species should not only consider administrative division and geographical boundaries but also the biological characteristics of the species such as mi-

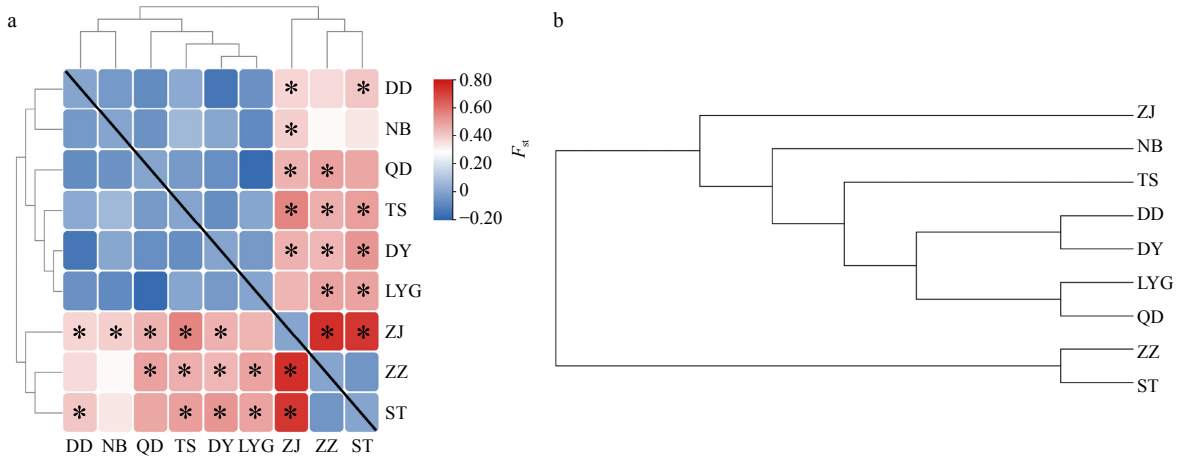

Fig. 5. Genetic structure and differentiation of *Amphioctopus fangsiao* populations. a. *F*-statistics (F_{st}) of *A. fangsiao* populations, * means significant genetic differentiation ($p \leqslant 0.05$); b. UPGMA tree based on $(\delta\mu)^2$ genetic distance.

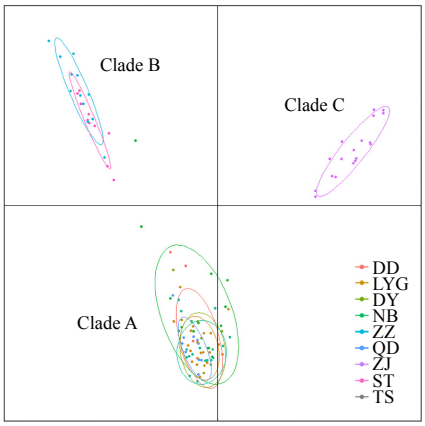

Fig. 6. Results of principal component analysis for the genetic relationships among *Amphioctopus fangsiao* populations.

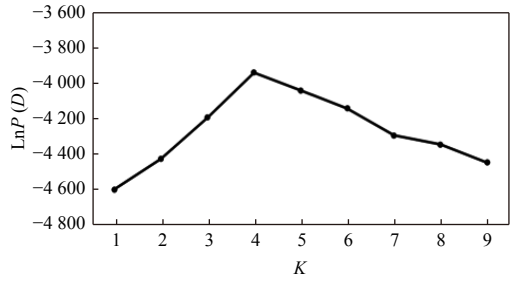

Fig. 7. The simulated *K* values ranged from 1 to 9 (total sites) estimated with the admixture model. Ln*P* (*D*): the logarithmic probability of the data.

gration and genetic structure (Ying et al., 2011; Harte et al., 2007). The analysis of population structure is the basis and prerequisite for making practical conservation strategies (Grande et al., 2004). Many studies have confirmed that it is important for fishery management to consider the spatial structure of marine organisms (Waples, 1998; Ying et al., 2011). Species with significant genetic differentiation among populations should be managed separately, and if not, they are better managed jointly (Waples, 1998). According to the findings of the present study, *A. fangsiao* in the coastal waters of China should be managed as a separate management unit.

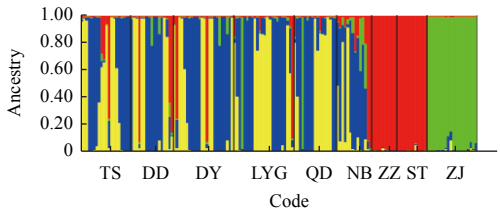

Fig. 8. STRUCTURE bar plots from twelve microsatellite loci for nine locations of *Amphioctopus fangsiao*. Different colors represent the contribution of each *K* genetic cluster to each specimen's genotype.

Geohistorical events are also hypothesized to play a role in phylogeographic patterns (Gao et al., 2002). In this study, divergence time among three clades was estimated. About 3.67 million years ago, Clade C and Clades A, B began to diverge. And then, Clades A and B began to diverge in 2.32 million years ago. This time was about in the early last ice age during the environmental fluctuations such as sea level change which have exerted great influence on the survival distribution pattern of global organisms (Herbert et al., 2001; Marret et al., 2001). During this period, the continental shelf of the coastal waters of China had frequent glacial-interglacial changes, resulting in sea-level drops and the formation of land bridges between the Asian mainland and its nearby islands (Tamaki and Honza, 1991). These land bridges were conducive to the diffusion of terrestrial organisms, yet they have blocked the gene flow of marine organisms, thereby leading to the allopatric differentiation of these marine species (Zhang, 2020). Therefore, we speculated that the genetic differentiation of *A. fangsiao* may occur due to the dramatic environmental fluctuations during this period. Still, more studies are needed to illustrate how historical-geographical events had direct effects on the phylogeographic pattern of *A. fangsiao*. Recommendations for future population genetics of *A. fangsiao* are studies based on fossil data combined with molecular markers with higher coverage and larger data sets, such as whole-genome resequencing.

More significant genetic differentiation was detected between Clade C (ZZ and ST) and Clades A, B, which was inconsistent with geographical distance among different sampling locations. This genetic pattern is also found in other marine organisms (Ni et al., 2014; Chang et al., 2017). Furthermore, Zhang (2017) found

a cryptic species named as *Amphioctopus fangsiao etchuanus* from Ningde (close to ZZ and ST) of Fujian Province. This result may be caused by the complex geographical environment of the Taiwan Strait. Taiwan Strait abuts the East Indies where is the prime hot spot for marine biodiversity, thus there is rich fish biodiversity in Taiwan Strait (Chang et al., 2017). There is different marine habitat in Taiwan Strait, including coral reefs, estuaries, and mangrove forests, resulting in a wide range of differences in water depths and water temperatures (Shao, 2009). Besides, due to the influence of the South China Sea, Kuroshio and China coast currents, specific genetic structure of marine organisms is easily generated (Chang et al., 2017). To investigate and protect genetic resources, further research on population genetics of species in Taiwan Strait is necessary.

The *A. fangsiao* in localities Ningbo in the East China Sea was distributed in all clades in this study. Previous studies have shown that *A. fangsiao* can be clearly separated from northern and southern with the Changjiang River Estuary as the boundary, which had slightly different from this study (Faiz et al., 2019). More complex genetic structure in Ningbo may be attributed to endemic branch tribes existing in the East China Sea, especially in Ningbo and Zhoushan Archipelago sea area (Hu, 1998). Special geographical environments in the coastal waters of Ningbo have resulted in the complex population structure of many species (Lv et al., 2010). Therefore, it is vital to focus on the management and conservation of endemic branch tribes found based on geographical conditions and distribution of *A. fangsiao*.

5 Conclusions

This study revealed three phylogeographic clades of *A. fangsiao* based on mtDNA *COI* fragments and microsatellite DNA, revealing limited genetic exchange between north and south populations. The historical demography showed that these clades diverged in 2.23 and 3.67 million years ago, respectively. We speculate that geohistorical events, ocean currents and biological characteristics have played an important role in shaping the contemporary phylogeographic pattern and population structure of *A. fangsiao*. According to the findings of the present study, *A. fangsiao* in the coastal waters of China should be managed as separate management unit. This study has important implications for fisheries management efforts and species with similar life history characters.

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