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Short Communication

Genetic Structure of *Octopus minor* (Sasaki, 1920) (Cephalopoda: Octopoda) from Chinese Waters using Mitochondrial ATPase 6 Gene

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Authors' Contributions
ZL designed and supervised the work. FM performed experimental work and wrote the paper. XD collected samples and extracted DNA. CD helped in data analysis. LG helped in experiments. MS helped in paper writing.

Key words
Cephalopods, *Octopus minor,* ATPase 6, Genetic structure, Haplotype networking.

ABSTRACT

Genetic variability and structure in *Octopus minor* was investigated from 154 individuals using mtDNA ATPase 6, sampled from Dalian, Dongshan, Nantong, Qingdao, Shanghai, Wenzhou, Xiamen, and Zhoushan China. Based on 672 bp fragment of Mitochondrial DNA subunit ATPase 6, thirty haplotypes and 140 polymorphic sites were noted, whereas (0.740, 0.048) high and low haplotype and nucleotide diversity observed. The data was further tested with AMOVA that validates 91.24% variation among populations and 8.76% within population. Genetic fixation index was also calculated as 0.91243*. The neighbor-joining phylogenetic analysis revealed three lineages. The Dongshan population distinctly has shown separate lineage. The haplotype networking has shown that Hap1 contributed 48.051% and appeared in six populations whereas, Hap6, Hap11, Hap 16 and Hap17 were also shared by some populations, rest of the haplotypes were unique. This study solely provide species specific information on its genetic structure and population variability in Chinese waters.

Octopus minor (Sasaki, 1920) has profound commercial and medicinal importance (Qian *et al*., 2010). It is distributed in Chinese waters from Bohai Sea to South China Sea (Dong, 1988, 1991), including Korean Peninsula and Japanese archipelago (Okutani *et al*., 1987; Lu *et al*., 2012). It has also synonymized as *O. variabilis* (Yamamoto, 1942; Dong, 1988; Lu *et al*., 2012, 2013). Moreover, propagation efficiency of marine organisms immensely influence the genetic structure (Daniels *et al*., 2002; Liu *et al*., 2007). Increased distribution capabilities causes no regional divergence in gene frequency (Gyllensten, 1985; Liu *et al*., 2007), whereas concise dispersal competence isolate gene flow (Riginos and Victor, 2001; Han *et al*., 2008). *O. minor* has little dispersal capacity and weak migratory potential; male adults are slow mover or remain sessile crawling away on the seabed or burrowing deep in mud. The juvenile grow spontaneously without a planktonic larval stage leading to low gene flow and genetic differentiation (Dong, 1988, 1991).

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An extensive range of investigations has been conducted in population genetics of marine organisms including cephalopod (Shaw, 2003; Gao *et al*., 2016; Chang *et al*., 2010; Kang *et al*., 2012). The genetic studies play a fundamental role in the development and management of resources and contribute immensely in searching population history, genetic diversity and geographical partitioning throughout natural dispersal range of targeted organisms. Due to the recent advancement in population genetics these attributes can directly be analyzed by using genetic markers (Hutchings, 2000). ATPase 6 (mtDNA marker) gene has been considered as fast evolving and useful genetic marker for assessment of population structure (Ovender and Street, 2003). The present investigation was designed to probe genetic variability and structure in *O. minor* using ATPase 6 gene from eight sampling locations of China.

Materials and methods

Specimens of *Octopus minor* were collected from commercial trawl catches from eight different geographical locations, Dalian, Donghsan, Nantong, Qingdao, Shanghai, Wenzhou, Xiamen, and Zhoushan (Supplementary Fig.

S1). Samples were preserved following standard procedure at the site, and were transported to the laboratory in 95% ethyl alcohol and placed in refrigerator until further investigation. Total genomic DNA was isolated from muscle tissues (Liu *et al*., 2017), extracted DNA was electrophoresed and concentrated DNA was further diluted with DEPC water to reach working concentration (20ng/µl) for PCR reactions.

The complete ATPase 6 gene was amplified by PCR, using primers ATP6F2 5′ACATGCCACAATTATCACCA 3′ andATP6R 5′ AGCTTAATAAGAGCGTAACG 3′. The pair of primers were designed from mtDNA gene sequence taken from NCBI accession number AB158363. The PCR mixture consisted of 0.2µM of each primer, 5.0µL of 10XTaq Plus polymerase buffer, 0.2 mM of dNTPs, 2 units of Taq Plus DNA polymerase, and 1 µL of DNA template. The PCR conditions were as follows: denaturation at 94 ◦C for 5 min, 35 cycles at 94 ◦C for 30 s, annealing at 55 ◦C for 30 s, and extension at 72 ◦C for 30s, and the final extension at 72 ◦C for 10 min. The PCR products were electrophoresed on a 1.5% agarose gel to check integrity, and visualized, using Molecular Imager Gel Doc XR system (Bio- Rad USA). The PCR products were sequenced, using ABI 3730 automated sequencer for which the same PCR primers were used. The DNA sequences were aligned using CLUSTALW and sequence composition was estimated using software MEGA 6 (Tamura *et al*., 2013). Analysis of genetic differentiation, AMOVA, molecular diversity indices, genetic differentiation values and Fst values were determined with software ARLEQUIN (Excoffier and Lischer, 2010). Haplotype and nucleotide diversity was estimated, using DnaSP (Librado and Rozas, 2009). The neighbor joining tree was constructed to evaluate genetic relationship between the populations using MEGA 6 (Tamura *et al*., 2013). The haplotype networking was created, using NETWORK software version 5.0.0.1 (Bandelt *et al*., 1999).

Results

ATPase 6 gene was sequenced from 154 individuals of eight populations. Only 672 bp fragment of ATPase 6 of *O. minor* was used for data analyses. The average nucleotide composition of T, C, A, and G were 42.5%, 18.4%, 31.9% and 7.2%, respectively. A total of 30 haplotypes, 140 polymorphic sites were identified based on 154 individuals from eight populations. The haplotype diversity was higher (0.740) whereas nucleotide diversity was lower (0.04845) in the investigated populations of *O. minor*. The average number of nucleotide variations, "K" was 32.028. The details of each population are given in Table I. The high haplotype diversity was observed in Zhoushan following Xiamen, Nantong, Qingdao, Wenzhou, Dongsha, Dalian, and Shanghai, respectively.

Table I.- Nucleotide and haplotype diversities for different populations.

Location	n	P. sites	Hap. (h)	Hap. div. (Hd)	Nucl. div. (π)	Avg. diff. (K)
DL	18	2	2	0.209	0.00063	0.418
DS	24	109	4	0.239	0.01386	9.159
NT	28	7	7	0.576	0.00121	0.798
Q	16	15	5	0.450	0.00286	1.875
SH	10	0	1	0.00000	0.00000	0.000
W	18	1	2	0.424	0.00064	0.424
X	28	25	12	0.78307	0.00947	6.256
Z	12	7	7	0.86364	0.00309	2.045

n, sample number; P. sites, number of polymorphic sites; Hap., Haplotype; Hap. div., Haplotype diversity; Nucl. div., Nucleotide diversity; Avg. diff., average number of differences; DL, Dalian; DS, Dongshan; NT, Nantong; Q, Qingdao; SH, Shanghai; W, Wenzhou; X, Xiamen; Z, Zhoushan.

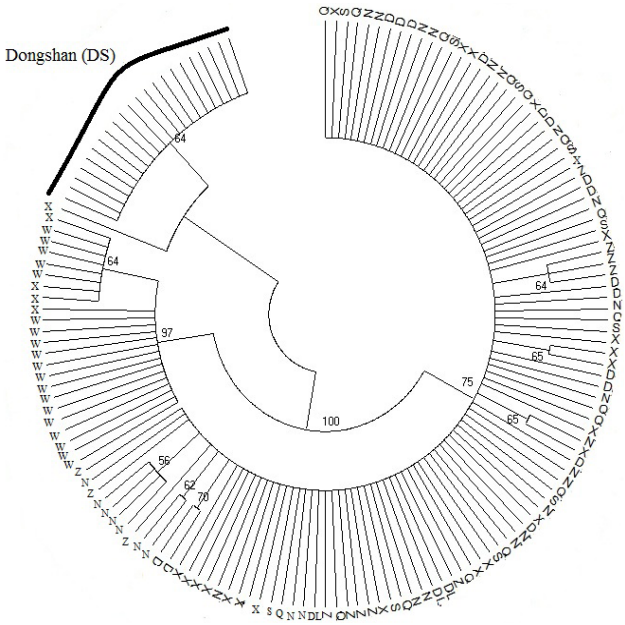

Fig. 1. Neighbor joining tree from eight populations of *O. minor*. The abbreviations in tree are given as: DL, Dalian; DS, Dongshan; N, Nantong; Q, Qingdao; S, Shanghai; X, Xiamen; W, Wenzhou; Z, Zhoushan.

The AMOVA test validates 8.76% within population and 91.24% among populations (Supplementary Table I). The population structure illustrated high and significant F_{ST} value of 0.912* at $P < 0.05$. The Tajima's D test defined significant negative values ranging (-0.684 - -2.728). The detailed description of Tajima's D, Fu's Fs, corresponding

P values and mismatch distribution parameter estimates are shown in Supplementary Table II, whereas the pairwise F_{ST} between populations of *O. minor* are described in Table II. The phylogenetic analysis revealed three lineages. The lineage one clustered five populations. Second lineage included the Xiamen and Wenzhou populations while Lineage three distinctly distinguishes Dongshan population (Fig. 1).

Table II.- Pairwise F_{ST} between eight populations of *O. minor*.

	DL	DS	NT	Q	SH	W	X	Z
DL	0.000							
DS	0.947	0.000						
NT	0.055	0.955	0.000					
Q	0.014	0.938	0.036	0.000				
SH	0.012	0.938	0.004	-0.032	0.000			
W	0.975	0.949	0.963	0.921	0.921	0.000		
X	0.092	0.927	0.120	0.079	0.079	0.739	0.000	
Z	0.067	0.9322	0.0823	0.015	0.021	0.925	0.078	0.000

For abbreviations, see Table I.

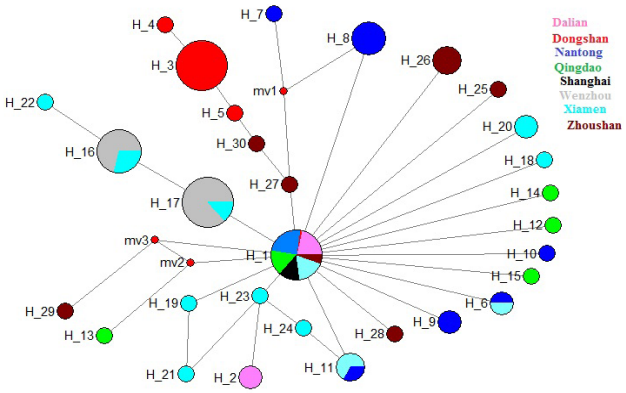

Fig. 2. Median joining networks for the ATPase 6 haplotypes in *O. minor* populations.

The phylogenetic relationship illustrated, using neighbor-joining (NJ) method which exhibit three lineages. The lineage one is extended group that describes populations from six location. However Dongshan population was distinctly different from rest of populations. The Lineage II is supported with a bootstrap value of 64% whereas the Lineage III supported with a bootstrap value of 100%. Lineage II included the individuals of Xiamen and Wenzhou populations, and Lineage III distinctly describes the Donghshan population (Fig. 2).

The haplotype network (Fig. 2) revealed that haplotype 1 (Hap1) contributed 48.051% of total *O. minor* individuals and appeared in six populations. Hap6 and Hap 11 shared by Nantong and Xiamen populations, whereas the Hap16 and Hap 17 was shared by Wenzhou and individuals of Xiamen population. Rest of the haplotypes are unique in their respective populations.

Discussion

The genetic structure and relationship of *O. minor* populations in the present study was analyzed using ATPase 6 gene which is essential for successful recovery and long-term conservation strategies of important cephalopod species. The genetic diversity ensures evolutionary potential and even a small decrease or increase in genetic diversity causes significant threat to wild populations inhabiting variable environment (Yang *et al*., 2008). Therefore, such studies play major role in collection information on the conservation genetics of a species (Crandall *et al*., 1999; Divya *et al*., 2015).

In this investigation, one hundred fifty four individuals from eight different localities of Chinese coastal waters were investigated. The results revealed 140 polymorphic sites, 30 haplotypes and high Hd values and low nucleotide diversity. AMOVA analysis of ATPase 6 sequences revealed genetic variation in *O. minor*. These variations are similar to findings reported by earlier workers in a number of Cephalopod species (Chang *et al*., 2010; Guo *et al*., 2011; Li *et al*., 2013; Lü *et al*., 2011).

Wright (1978) described relationship between F_{ST} values and degree of differentiation: FST 0-0.05 denotes little differentiation among populations; F_{ST} 0.05-0.15 indicate moderate differentiation, whereas F_{ST} 0.15-0.25 means high distinction and above 0.25 demonstrate very high differentiation (Gao *et al*., 2016).

The pairwise F_{ST} values shows highest F_{ST} value in Wenzhou population then Donghsan population, other populations have comparatively lower F_{ST} values. The phylogenetic tree and pairwise distance results may suggest a sub-species of Donghsan population. The morphological analysis also suspected this population as a sub-species.

The genetic diversity among populations or marine organisms influenced by certain factors, such as geographic segregation, habitat differences, and human activities (Hedrick, 1999; Chang *et al*., 2010). The lifestyle differences make it hard to carry out suitable gene exchange; as a result, variations revealed different geographical clades. Such general rules are also applied to target species because long armed octopus *O. minor* also has meager dispersal potential and it is adapted to benthic environment. Adult *O. minor* are sessile and juveniles develop directly, benthic eggs without a planktonic larval stage (Dong, 1988, 1991). Therefore, *O. minor* fishery require special care for exploitation and management of future resource along the coast of China. Lü *et al*. (2013) reported that long armed octopus fishery needs a special care for exploitation when many populations involved over a wide range of geographic distance.

This study would solely provide species specific information on its genetic structure and population variability in Chinese waters.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/2019.51.1.sc5

Statement of conflict of interest

Authors declared no conflict of interest.

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