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Pelagic Larval Dispersal Habits Shape the Weak Population Structure of *Thais clavigera* in the Coastal Areas of China Sea

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ABSTRACT

Thais clavigera (T. clavigera) is an ecologically and economically important gastropod species in the coastal regions of China. Compared to other molluscs, *T. clavigera* has a long planktonic larval period (i.e., ~ two months). In order to identify the relevant factors affecting the genetic structure of *T. clavigera*, a total of 147 *T. clavigera* individuals distributed along the Chinese coast from 9 populations were analysed genetically on the bases of cytochrome oxidase I (COI) gene. Analysis of the COI genetics indicated a high level of genetic diversity among *T. clavigera*. Our analysis of population genetic and demographic (AMOVA, haplotype networks and mismatch distribution) revealed a single genealogical branch and indicated undifferentiation of *T. clavigera* in the China Sea. Migration dynamic analysis showed that gene flow was asymmetrical and QD as the source population. Additional, canonical correspondence analysis (CCA) analysis between environmental factors (SKT, TOP, SLP, and SUND) and haplotypes indicated that SLP and SUND carried highest influence on the haplotype distribution. SKT and total precipitation TOP were highly correlated with haplotype formation. Combine life story of *T. clavigera* revealed that environmental factors contribute to the nuances of population genetic in different regions. Understanding the genetic variation and population structure of *T. clavigera* populations along the coast of China Sea obtained from this study will support the aquaculture management and conservation of *T. clavigera* in China.

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YY and CY conceived and designed the study. YY, CY, JM and JF performed the collections and provided samples. CY performed laboratory work and the data analyses. All authors participated in the interpretation of the data and gave final approval for the publication of the article.

Key words
***Thais clavigera*, COI, Population genetics, Genetic diversity, Environmental factors**

INTRODUCTION

Compared to freshwater organisms, differentiation and speciation of marine organisms is limited. In the marine environment, genetic structure of marine species is affected by multiple and complex factors such as dynamic oceanographic features, larval behaviour, spawning period and post-settlement mortality (Lambeck *et al*., 2002; Wares, 2002; Liu *et al*., 2007; Xu *et al*., 2009; Shen *et al*., 2011; Liu *et al*., 2012a; Guo *et al*., 2015; Mcveigh *et al*., 2017). Most marine organisms exhibit a planktonic stage and during this stage, these organisms disperse several meters to a long distance from their original location (He *et al*., 2019; Hou *et al*.,2020). Owing to the small size and weak swimming capabilities of most marine larva, the dispersal potential is primarily determined by the length of their pleagic stage (Scheltema, 1971; Grantham *et al*., 2003; Weersing and Toonan, 2007; Koga *et al*., 2016). Population genetic structure of marine organisms carrying a long planktonic larval stage can result in increased gene flow, and consequently decreased levels of population differentiation (Selkoe *et al*., 2011; Ye *et al*., 2015). Taylor and Hellberg (2003) upon exploring the relationship between planktonic period and population differentiation, it has been revealed that a significant genetic differentiation occurs in fishes of 21 days of their planktonic age. The planktonic period of some marine fishes impose a great influence on the genetic differentiation of their populations. In this regard, eight species of reef fishes were analysed by mtDNA RFLP by Shulman and Bermingham (1995). They have found one out of eight species has a significant genetic structure with shorter planktonic periods. Siegel *et al*. (2003) have discovered that the mean absolute dispersal

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distance (estimated using a population-genetic modelling approach) show a strong relevance with the length of the pelagic larval phase (Siegel *et al*., 2003). However, others have reported that population genetic exceptions which have decreased subdivision in species with long length of the planktonic larval stage (Todd, 1998; Taylor *et al*., 2003; Rocha *et al*., 2005; Baums *et al*., 2006; Bowen *et al*., 2006). Therefore, these complex relationships between the gene flow and the pleagic larval stage need to be studied further in greater details (Galarza *et al*., 2009).

Environmental variables are key factors for survival of marine organisms with a complex life cycle, influencing larval stages and therefore, indirectly affecting later benthic stages (Seguel *et al*., 2019; Bueno *et al*., 2021). A typical example is the effect of gene exchanging between populations to varying degrees caused by rivers dilute water. Zhao and colleagues have studied the *Cyclina sinensis* population genetic structure, and concluded that the dilute water of the Yangtze River may be a barrier to the gene exchange of species in the area (Zhao *et al*., 2007). Similar results were show in gastropod *Cellana toreuma* by Su *et al*. (2005), in bivalve *Cyclina sinensis* by Zhao *et al*. (2009), and in two varieties from *Sargassum* by Cheang *et al*. (2010). This universal phenomenon was attributed to the salinity effects on embryos and larval development. Temperature is also one of the most important environmental factors, especially in the coast of China in the temperate zone, which exhibit a significant temperature fluctuation (warm waters in summer and cold waters in winter) (Pörtner and Gutt, 2016). Thus, pelagic larval stage of marine organisms is adapt to environmental changes which are not otherwise suitable for larval and embryo development (Seguel *et al*., 2019). Additional factors such as light intensity (Hogman, 1968; Higgins and Talbot, 1985; Swift, 1995; Sakai *et al*., 2020) and substrate (Walne, 1965) are not yet investigated for their impact on the population genetic structure and such studies are required to better study the formation of genetic structure of marine species.

The *Thais clavigera (T. clavigera)*, a member of family Muricidae, is living in the middle and lower tidal areas of the intertidal zone. It is a eurythmic benthic species commonly found in the coastal areas of China, Japan and Korea (Zhu *et al*., 2008; You *et al*., 2010). Similar to most of the marine species, *T. clavigera* carry a planktonic larval period which lasts only for two months (Ewers *et al*., 2019). During this stage, *T. clavigera* disperse over larger distances mediated through tides and ocean currents. Depending upon different life history, different species are influenced by different factors of genetic pattern. Guo *et al*. (2015) have proposed that the Yangtze River dilution water was a subtle factor to the *T. clavigera* genetic structure formation in the past coastal areas of China and Janpan sea. However, the Bohai sea area has not been included in their studies. The study conducted by Xu (1997) indicated that the boundary was not an insurmountable challenge for some bivalve shellfish which have broad temperature tolerance because they harbour the ability to cross this boundary and disperse widely.

Cytochrome oxidase I (COI) gene possesses special characteristics which make it suitable as a molecular marker for evolutionary studies because it carries highly conserved and variable regions which provide useful insights into evolutionary studies (Cerutti *et al*., 2012; Fernando *et al*., 2020). Based on these features, the mitochondrial COI gene of *T. clavigera* individuals (n=147) from 9 sites in coastal areas of China were collected, sequenced and characterized to assess population genetic structure of *T. clavigera* in China coastal area. The long planktonic larval stage, and environmental factors were used to investigate the population genetic pattern. Compared to other marine organisms with diffusion of ocean currents in the larval stage, it remains to be determined those factor that influence the formation of *T. clavigera* genetic structure. Additionally, it is also not known if environmental factors are related to the formation of genetic pattern or not? These studies highlight the importance of the molecular markers that guide the genetic patterns on marine species.

MATERIALS AND METHODS

Sampling and sequencing

A total of 147 individuals of *T. clavigera* were collected from 9 geographic locations in the coastal area of the China (Fig. 1). Whole organism samples were frozen and shipped to Zhejiang Ocean University. Muscle samples were obtained and preserved in 95% ethanol or frozen for subsequent DNA extraction. The genomic DNA was extracted followed salting-out method (Folmer *et al*., 1994) from muscles, then stored at -20°C refrigerator in the National Engineering Research Center for Marine Aquaculture, Zhejiang Ocean University until use.

The complete mitochondrial genome data of *T. clavigera* (NC_010090) were utilized for the COI sequencing search. Primer Premier v6.0 (Singh *et al*., 1998) was used to design the COI primers (COI-F: 5'-TTATGATAGAACAAGTAAACG-3' and COI-R: 5'-AGAATAGCATAAATCATAGGC-3'). Each polymerase chain reaction (PCR) was carried out in 25 µL volumes containing 0.5 µl of template DNA, 1 µl of each of the primer, 10 µl of dH_2O, 12.5 µl San *Taq* Fast PCR Master Mix (with blue Dye) (Sangon Biotech, Shanghai, China). The amplification conditions were initial denaturation at 94°C for 3 min, the course of reaction was 35 cycles at 94°C for 30s, 52°C for 30s, 72°C for 1 min, and final elongation at 72°C for 7 min. The products

were checked in the electrophoresis on 1% agarose gels. All of these products were sequenced in both directions by Sangon Biotech, Shanghai, China. All the obtained sequences were deposited in GenBank with accession numbers MW279153-MW279181.

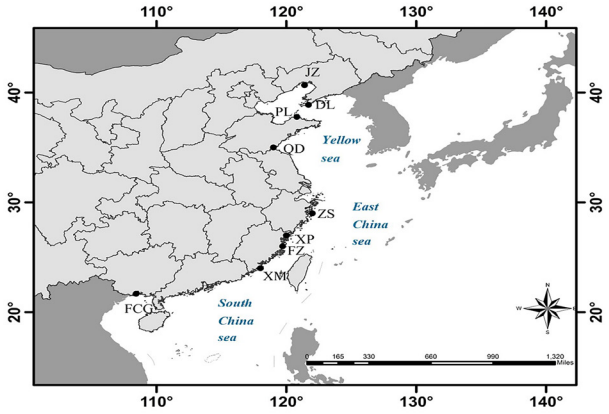

Fig. 1. Map showing the sampling locations along the coast of China.

Data analysis

The DNA sequences were examined and aligned using Bioedit and MEGA (Excoffier *et al*., 2010). Molecular diversity indices including the number of haplotype diversity (Hd), nucleotide diversity (p), and the mean number of pairwise differences (k) were obtained using ARLEQUIN v2.0 (Librado and Rozas, 2009) and DnaSP (Excoffier *et al*., 1992). The historical demographic patterns of *T. clavigera* were examined by Tajima's D, Fu's Fs, which were used to test neutrality (Fu, 1997; Bandelt *et al*., 1999). Significant negative statistics were interpreted as signatures of population expansion. The significance levels of Tajima's D and Fu's Fs were evaluated under 10,000 permutations, and both mismatch analysis and neutrality tests were performed in ARLEQUIN.

Population genetic structure was evaluated with F_{ST} statistics, and analysis of molecular variation (Tamura and Nei, 1993) was performed by ARLEQUIN. Genetic distances between haplotypes were determined using the model of Tamura and Nei (Tajima, 1989). The significance of the F_{ST} was tested by 10,000 permutations and was calculated the gene flow (N_m) by ARLEQUIN. Population structure of *T. clavigera* was further investigated using the molecular variance software package in ARLEQUIN. Network 5.0 (Sundqvist *et al*., 2016) software constructed haplotype Network diagram to analyze the corresponding relationship between each haplotype and the group. The web-based software divMigrate-online (Nei, 1973) was used to infer the directional relative migration patterns using the F_{ST} statistic (Ting *et al*., 2018) as a measure of genetic differentiation. Implementation approaches in divMigrate-online is based on supposing a hypothetical pool of migrants for a pair of given populations and estimating a properly measure of genetic differentiation between each of the two populations. Directed genetic differentiation was used to evaluate the relative level of migration between two populations. Larger relative migrations values indicate that the population is most likely the source population, whereas the smaller of the two values indicate the population is most likely to be the sink populations (Sheik *et al*., 2012; Ting *et al*., 2018).

Total precipitation (TOP), mean sea level pressure (SLP), sunshine duration (SUND), mean air temperature (SKT) and data from the European Meteorological Center (ECMWF)-ERA Interim (https://apps.ecmwf.int/datasets/data/interim-full-mnth/levtype=sfc/), were selected, downloaded and the average value over the past 20 years was calculated. The correlation between haplotype, population and environmental factors of COI in nine populations was analysed. The Canoco5 was used to carry out canonical correspondence analysis (CCA) of the acquired environmental factors and hadic data (Ter *et al*., 2012).

RESULTS

Genetic diversity of T. clavigera

A total of 147 COI sequences from 9 populations were examined in the coastal of China. In the analysed data a total of 17 variation sites and 29 haplotypes were obtained (Fig. 1). The Hd ranged from 0.37778 to 0.85833, the nucleotide diversity (Pi) ranged from 0.00100 to 0.00323, and the average number of nucleotide differences (K) ranged from 0.69118 to 2.22500. The haplotype diversity (Hd), nucleotide diversity (Pi) and nucleotide difference (K) of PL population were the highest, and those of XM were the lowest. The estimated mean Hd was 0.69826, Pi was 0.00182, and K was 1.25263 (Table I). These results reveal that the genetic diversity of XM population is the lowest among the 9 populations, and PL population is the highest.

Population genetic structure

The results indicated a 99.43% of the genetic variation within populations, whereas 0.57% of the variation were found between populations (Table II). These finding highlight that COI gene was less differentiated between populations and the level of genetic differentiation between populations is low. The pairwise F_{ST} values between nine populations ranged from -0.03102 to 0.06702 (Table III). Most of which were non-significant and indicate undifferentiation of *T. clavigera*. However, statistically highly significant variations were found among FCG and

Table I. Sampling information of *T. clavigera* including sample name, sample size, sample abbreviate (ID) and data of collection. Several diversity indices were also indicated. H, haplotypes numbers; s, mutation sites; Hd, haplotype diversity; Pi, nucleotide diversity; K, average number of pairwise divergences.

Population		Latitude, longitude	Size	h	s	Hd	Pi	K
Jinzhou	JZ	40°67′N, 121°40′E	19	9	9	0.81287	0.00202	1.39181
Dalian	DL	38°88′N, 121°70′E	20	7	8	0.58421	0.00155	1.06842
Penglai	PL	37°78′N, 120°81′E	16	9	12	0.85833	0.00323	2.22500
Qingdao	QD	35°01′N, 119°01′E	17	5	5	0.50735	0.00100	0.69118
Zhoushan	ZS	29°01′N, 122°01′E	20	10	8	0.75789	0.00168	1.15789
Xiapu	XP	27°01′N, 120°81′E	15	9	10	0.84762	0.00241	1.65714
Fuzhou	FZ	26°01′N, 119°71′E	10	6	7	0.77778	0.00203	1.40000
Xiamen	XM	24°01′N, 118°01′E	10	3	3	0.37778	0.00110	0.75556
Fangchenggang	FCG	21°68′N, 108°43′E	20	7	6	0.63684	0.00135	0.92632
Total Data Estimates			147	29	17	0.69826	0.00182	1.25263

Table II. AMOVA analysis of 9 *T. clavigera* population.

	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
COI	Among populations	8	5.449	0.00357 Va	0.57
	Within populations	138	85.993	0.62314 Vb	99.43
	Total	146	91.442	0.62671	

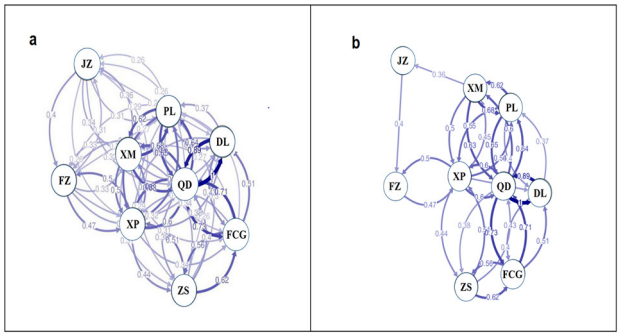

Fig. 2. Directional relative migration networks of *T. clavigera* populations constructed with divMigrate using F_{ST} Values above 0.25 (a) and 0.35 (b) are shown.

two populations (DL and PL). Migration dynamic analysis showed that the gene flow was asymmetrical from QD to DL and PL, and was stronger than that from QD to ZS, XP and XM (Fig. 2). In addition, gene flow between JZ and FZ was weak. The results of this migration pattern showed a higher level of gene exchange between QD population and others. Haplotype analysis based on COI gene showed a total of 29 haplotypes in 9 geographical populations. Similar interpretation were also made from haplotype Network (Fig. 3) and Bayes tree (Fig. 4) analysis.

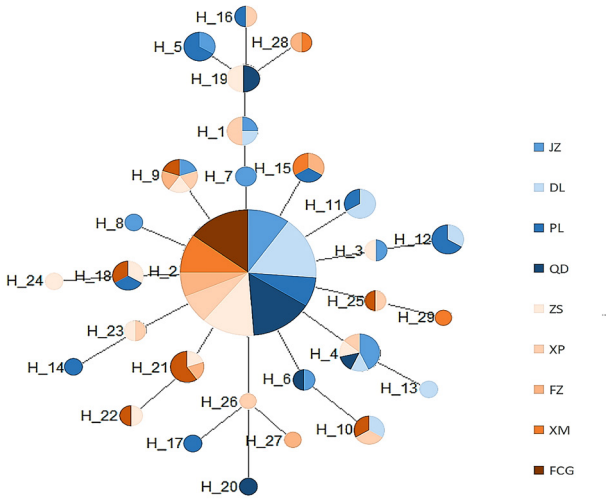

Fig. 3. Networks of *T. clavigera* developed using COI data. Color representation is showing the population frequency. Numbers are representing the haplotype numbers.

Demographic analysis

The topology of the Bayes tree of *T. clavigera* was shallow and there were no significant genealogical branches or clusters of samples corresponding to sampling locations. A dominant haplotype H2 was found in all populations. The JZ, DL, QD, ZS, and XP, contributed to

Table III. F_{ST} and Nm of *T. clavigera* based on COI gene.

FST	JZ	DL	PL	QD	ZS	XP	FZ	XM	FCG
JZ	0	Inf	252.27	Inf	32.862	Inf	Inf	7.9709	4.6024
DL	-0.01809	0	Inf	Inf	40.6665	Inf	Inf	24.4048	6.6256
PL	0.00099	-0.00112	0	18.7758	26.4024	Inf	Inf	69.5824	3.4802
QD	-0.00329	-0.00899	0.01314	0	Inf	Inf	Inf	9.3065	6.5269
ZS	0.00755	0.00611	0.00938	-0.00332	0	Inf	Inf	7.8249	Inf
XP	-0.03102	-0.0059	-0.00246	-0.02612	-0.00237	0	Inf	10.4155	6.456
FZ	-0.01604	-0.00481	-0.01516	-0.02661	-0.04453	-0.03989	0	Inf	Inf
XM	0.03041	0.01014	0.00358	0.02616	0.03096	0.02344	-0.01677	0	4.8803
FCG	0.05152	0.03636	0.06702	0.03689	-0.01175	0.03728	-0.0102	0.04873	0

Table IV. Based on the sequences of COI, the neutral test was conducted for the 9 populations of *T. clavigera*.

COI	Statistics	JZ	DL	PL	QD	ZS	XP	FZ	XM	FCG	Mean	s.d.
	Sample size	19	20	16	17	20	15	10	10	20	16.3333	4.031
Taji-ma's D test	S	9	8	12	5	8	10	7	3	6	7.5556	2.69774
	Pi	1.39181	1.06842	2.22500	0.69118	1.15789	1.65714	1.40000	0.75556	0.92632	1.25259	0.48243
	Tajima's D	-1.60306	-1.77344	-1.46972	-1.71874	-1.63971	-1.75529	-1.83913	-1.03446	-1.44134	-1.58610	0.24681
	Tajima's D p-value	0.03830	0.02190	0.06090	0.02110	0.03030	0.02650	0.01270	0.21600	0.06030	0.05422	0.06296
	No. of alleles	9	7	9	5	10	9	6	3	7	7.2222	2.27913
	Theta_pi	1.39181	1.06842	2.22500	0.69118	1.15789	1.65714	1.40000	0.75556	0.92632	1.25259	0.48243
Fu's FS test	Exp. no. of alleles	4.25926	3.73288	5.15442	2.82679	3.90436	4.32272	3.43070	2.56892	3.44806	3.73868	0.79239
	FS	-5.17399	-3.29864	-3.72262	-2.30804	-7.69624	-5.36891	-2.82716	-0.04647	-3.84083	-3.80921	2.15202
	FS p-value	0.00030	0.00650	0.00950	0.01140	0.00000	0.00020	0.00850	0.39950	0.00120	0.04857	0.13168

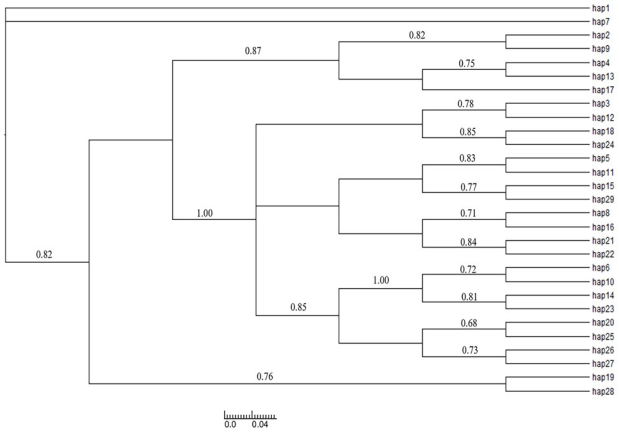

Fig. 4. Bayes haplotype evolutionary tree based on COI gene.

Hap4, JZ, ZS, XP, FZ, and FCG five groups contributed to Hap9. A dominant haplotype H2 was found in all the populations, and total 80 individuals of Hap2 indicated the ancestor haplotype covering all groups. Hap1, 5, 16, 19 and 28 were obtained in populations from JZ, DL, PL, XP, QD and ZS. Based on the above results, it is plausible that there is no obvious pattern in the distribution of haplotype, indicated a high level of gene flow of *T. clavigera* in the coastal areas of China. The Tajima's D rejected neutrality ($P < 0.05$) for all populations expected PL, XM, FCG populations (Table IV). Fu's Fs statistic analysis indicated a significantly different pattern from zero ($P < 0.05$) for all populations expect JZ and ZS. At the same time, mismatch distributions for *T. clavigera* were unimodal, and closely matching the expected distributions under the sudden expansion modal (Fig. 5).

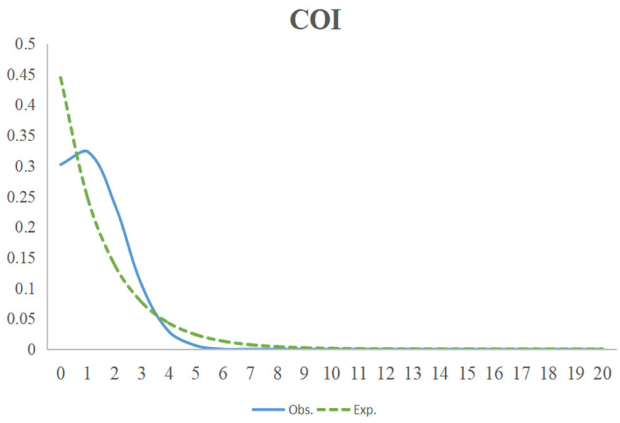

Fig. 5. Mismatch histogram-line composite graph based on COI gene.

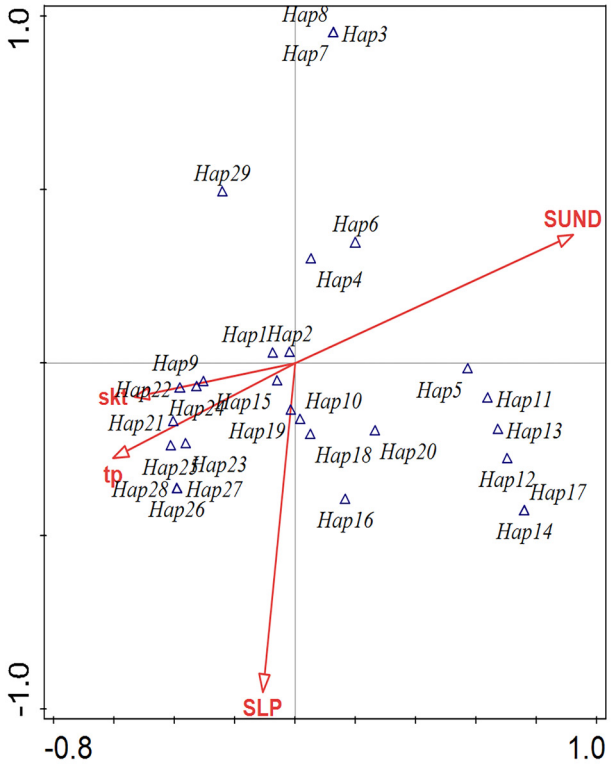

Fig. 6. The canonical correspondence analysis (CCA) on the relationship between environmental factors and haplotypes of *T. clavigera*. The longer arrow of environmental factors indicates a greater degree of influence, and closer indicate a stronger correlation.

Environmental factors

Canonical correspondence analysis (CCA) was performed between environmental factors and haplotypes (Addison *et al*., 2004). In CCA analysis, the longer arrow of environmental factors indicated a greater degree of influence, and if the haplotype is closer to environmental factors, the correlation is stronger. The formation of acute angle indicated a positive correlation while the obtuse angle indicated a negative correlation. The same acute angle between different environmental factors indicated a positive correlation and negative correlation conversely. The outcome of CCA analysis based on COI gene (Fig. 6) showed that SLP and SUND carried substantial influence on haplotype distribution where SKT and TOP were more correlated with haplotype formation. Among the environmental factors, the correlation between average temperature and total precipitation is the strongest, which is positively correlated with average sea level pressure and negatively correlated with sunshine duration. The distribution of Hap1, Hap2, Hap9, and Hap21-28 was highly correlated with mean air temperature and total precipitation. In Hap 10-20, except for Hap15 and Hap19, other haplotypes were highly correlated with mean sea level pressure and surface net solar radiation, and the duration of sunshine has a greater influence on haplotypes. Other haplotypes carried weaker correlation with environmental factors. Among the haplotypes that are highly correlated with average air temperature and total precipitation, the haplotypes of ZS, XP, FZ and FCG occupied a large proportion, while those of DL, PL, ZS and QD occupied a large proportion, which were highly correlated with mean sea level pressure and surface net solar radiation.

DISCUSSION

As common gastropod species in Chinese coastal areas, the *T. clavigera* is highly popular due to its rich nutrition and delicious taste (Ter *et al*., 2012). These features underline the rational for a sharp decrease in the resources for *T. clavigera.* In this study, we assess population genetic structure of *T. clavigera* in the coastal of China based on mitochondrial COI gene sequence and provided foundations on the role of environmental factors on the current genetic distribution of *T. clavigera* populations.

Compared to terrestrial species, marine organisms are generally considered to have low genetic diversity, as they have a higher potential for transmission at the plankton, larval or adult stage of history, and there are not many physical barriers to movement (Liu *et al*., 2012b). The length of the planktonic larval stage is a plastic life-history trait that can vary by an order of magnitude or more for some species (Toonen and Pawlik, 2001; Addison and Hart, 2004). Such variation in PLD can occur due to behaviours such as delayed metamorphosis (Pechenik, 1990) or larval responses to environmental stochasticity (Woodson and Mcmanus, 2007; Weersing and Toonen, 2007). These

studies appear to confirm the long-standing hypothesis that longer planktonic larval durations confer greater dispersal ability. Recent studies have established the existence of the planktonic phase of *T. clavigera* larvae to about two months (Tian *et al*., 2020). Considering the limited mobility of *T. clavigera* and a longer planktonic larval stage compared to other species, it is believed that the communication between the geographic populations of *T. clavigera* may mainly be through the migration caused by ocean currents in that long larval stage (Chandler *et al*., 2008).

Through COI gene analysis, we were unable to detect significant genetic differentiation. Additionally, the COI gene-based haplotype network also showed no obvious genetic structure. Meanwhile, AMOVA analysis indicated that the major source of the genetic variation was variations within populations instead of between areas. This suggested that there were no significant genetic differences between different geographic populations. However, in this study, F_{ST} between FCG, JZ and PL was significant. Notably, not all populations of the South China Sea and East China Sea show obvious lineage formation, and the gene flow between FZ and XP populations revealed a high level, with the northern populations contrary to the above conclusion. This conclusion was similar to previous studies where *Atrina pectinata, Coelomactya cmtiquata, Rapana venosa, Eriocheir sensu stricto* (Xu and Oda, 1999; Chandler *et al*., 2008; Kong and Li, 2009; Liu *et al*., 2012) and other species showed no significant differentiation. Migration dynamic analysis showed an asymmetrical gene flow in the coast of China and indicated that QD as the source population. It is plausible that certain factors may slightly affect genetic differentiation of *T. clavigera* which are hidden below the appearance of frequent gene exchange. Therefore, to identify the influential factors, we choose the environment factors in this study.

Combined with traditional population genetic methods and seascape genome research, it was identified that there are significant correlations between population genetic structure and environmental factors in many marine species (Bueno *et al*., 2012; Pespeni *et al*., 2013; Pires *et al*., 2015). Li *et al*. (2009) have studied the effects of environmental parameters on the immune capacity of *Mytilus galloprovincialis* and found that temperature had a positive effect on the expression of 28SrRNA, lysozyme and mussel embryo. Sarver and Bushak (1993) have found that the distribution of mussel population was closely related to temperature and salinity. A considerable emphasis has been given to environmental factors and their effects on the growth, development and reproduction of organisms, and as factors affecting the evolution of species (Schneider *et al*., 2010; Nardon *et al*., 2005). vis-à-vis *T. clavigera*, Tian *et al*. (2020) have studied its growth and development, and identified that larva of *T. clavigera* has metamorphosis after 10 d of sediment adhesion at 27 ~ 28°C, whereas the oviposition temperature was mainly concentrated at 22 ~ 28°C. The average temperature in the southern East China Sea and South China Sea is around 25°C. Life history illustrates a suitable living environment for *T. clavigera* planktonic larvae for its transformation into a juvenile snail after 10 days of attachment to the bottom at 27~28°C. In this study, haplotypes in the lower latitude locations (southern East China Sea, South China Sea) are more affected by temperature. Therefore, we argued that the formation of specific haplotype in the southeast sea population may be attributed to the influence of temperature. Temperature fluctuations significantly affect multiple aspects include timing of metamorphosis, enzyme activity, immune function indirectly affects the life history and distribution pattern of *T. clavigera*. Compared to the whole coastal area of China, the South China Sea with higher average temperature carried more advantages in the development of the bottom of *T. clavigera*. A similar conclusion was drawn in *Babylonia areolata* and *Polinices pulchellus* (Kingsley *et al*., 2005; Huang *et al*. 2010). In addition to temperature, climate is one of the most important factors limiting species distribution. Chen has found that the ability of rainfall to affect shellfish is higher, and the ability of microbial enrichment is stronger with higher precipitation (Chen, 2019). Wang *et al*. (2015) have found that nutrient concentration is one of the main factors affecting the spatial and temporal distribution of shellfish, and precipitation can effectively alleviate the silicate limit in the sea area, and precipitation can directly affect the air humidity and water vapor pressures. Based on the positive correlation between precipitation and haplotype in this study, we believe that higher precipitation can alleviate the silicate limit in the sea area and provide a favourable environment for the growth and reproduction of *T. clavigera* larvae.

CONCLUSION

We investigated population genetic structure of *T. clavigera* in the coast of China using mitochondrial COI gene and determined the environmental factors influencing the population genetic structure. We conclude that a long planktonic larval stage can make an extensive contribution to high level of gene flow in *T. clavigera* population. Additionally, environmental factors such as temperature and precipitation can slightly affect genetic differentiation in *T. clavigera* population.

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Statement of conflict of interest

The authors declare that they have no conflict of interest.

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