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., 2 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.

**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
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 pelagic 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 Japan.

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 dH₂O, 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 ($N_{e}$) 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), 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.

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

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

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Variance components</th>
<th>Percentage of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>COI Among populations</td>
<td>8</td>
<td>5.449</td>
<td>0.00357 Va</td>
<td>0.57</td>
</tr>
<tr>
<td>Within populations</td>
<td>138</td>
<td>85.993</td>
<td>0.62314 Vb</td>
<td>99.43</td>
</tr>
<tr>
<td>Total</td>
<td>146</td>
<td>91.442</td>
<td>0.62671</td>
<td></td>
</tr>
</tbody>
</table>

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

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 others.
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Table III. F$_{ST}$ and Nm of *T. clavigera* based on COI gene.

<table>
<thead>
<tr>
<th></th>
<th>JZ</th>
<th>DL</th>
<th>PL</th>
<th>QD</th>
<th>ZS</th>
<th>XP</th>
<th>FZ</th>
<th>XM</th>
<th>FCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>F$_{ST}$</td>
<td>0</td>
<td>Inf</td>
<td>0.993</td>
<td>0.003</td>
<td>0.007</td>
<td>0.031</td>
<td>0.006</td>
<td>0.006</td>
<td>0.031</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>COI Statistics</th>
<th>JZ</th>
<th>DL</th>
<th>PL</th>
<th>QD</th>
<th>ZS</th>
<th>XP</th>
<th>FZ</th>
<th>XM</th>
<th>FCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>19</td>
<td>20</td>
<td>16</td>
<td>17</td>
<td>20</td>
<td>15</td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Tajima's D test</td>
<td>1.39181</td>
<td>1.06842</td>
<td>2.22500</td>
<td>0.69118</td>
<td>1.15789</td>
<td>1.65714</td>
<td>1.40000</td>
<td>0.75556</td>
<td>0.92632</td>
</tr>
<tr>
<td>Tajima's D p-value</td>
<td>-1.60306</td>
<td>-1.77344</td>
<td>-1.46972</td>
<td>-1.71874</td>
<td>-1.63971</td>
<td>-1.5529</td>
<td>-1.83913</td>
<td>-1.03446</td>
<td>-1.44134</td>
</tr>
<tr>
<td>No. of alleles</td>
<td>9</td>
<td>7</td>
<td>9</td>
<td>5</td>
<td>10</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Theta_pi</td>
<td>1.39181</td>
<td>1.06842</td>
<td>2.22500</td>
<td>0.69118</td>
<td>1.15789</td>
<td>1.65714</td>
<td>1.40000</td>
<td>0.75556</td>
<td>0.92632</td>
</tr>
<tr>
<td>FS p-value</td>
<td>0.00030</td>
<td>0.00050</td>
<td>0.00950</td>
<td>0.01140</td>
<td>0.00000</td>
<td>0.00020</td>
<td>0.00850</td>
<td>0.39950</td>
<td>0.00120</td>
</tr>
</tbody>
</table>

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).
Environmental factors

Canonical correspondence analysis (CCA) was performed between environmental factors and haplotypes of *T. clavigera* (Addison et al., 2004). In CCA analysis, the longer arrow of environmental factors indicates a greater degree of influence, and closer indicate a stronger correlation.

**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, *Fst* 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 mitiquata*, *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 position 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.

REFERENCES


Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**: 915-925. [https://doi.org/10.1093/genetics/147.2.915](https://doi.org/10.1093/genetics/147.2.915)


Pires, A. C., Nolasco, R., Rocha, A., Ramos, A. M. and Dubert, J., 2015. Climate change in the Iberian


C. Yan et al.


