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# Population genetic diversity of mud crab (*Scylla paramamosain*) in Hainan Island of China based on mitochondrial DNA

Hongyu Ma, Chunyan Ma, Lingbo Ma*

*Key Lab of Marine and Estuarine Fisheries Resources and Ecology, Ministry of Agriculture, East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Jungong Road 300#, Shanghai 200090, China*

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ABSTRACT

In this paper, we analyzed population genetic diversity of *Scylla paramamosain* in Hainan Island of China based on COI gene sequence. Totally 92 individuals were collected from six localities: Haikou, Wenchang, Wanning, Sanya, Dongfang and Danzhou. A 761 bp fragment was sequenced, defining 32 different haplotypes. H4 was the most frequent haplotype, existing in all six localities, while the majority of haplotypes were rare ones, existing in only one or two individuals. Haplotype diversity ($h$) and nucleotide diversity ($\pi$) ranged from 0.625 to 0.914 and from 0.001 to 0.003, with an average of 0.841 and 0.002, respectively. Genetic distance ranged from 0.001 to 0.003 within localities and from 0.002 to 0.003 between localities. The AMOVA analysis indicated a low level of genetic differentiation among six localities ($F_{ST} = 0.0176$, $P > 0.05$). Neutrality tests and mismatch analysis implied a potential population expansion event for this crab species.



## 1. Introduction

Genetic studies are of crucial importance for better of understanding genetic diversity (Ma et al., 2009), phylogenetic relationships (Keskin and Can, 2009), evolutionary history (He et al., 2010), and can also provide constructive guidance for resource conservation and management (Ortega-Villaizan Romo et al., 2006), and development of breeding programs (Fuji et al., 2007; Zhan et al., 2009). Mitochondrial DNA is maternally inherited and does not undergo DNA recombination, so it is considered as an effective approach for genetic studies on molecular phylogeography (Gvozkik et al., 2010) and population genetic diversity (Yu et al., 2010; Ma et al., 2010a).

Mud crab (*Scylla paramamosain*) is distributed along the southeastern coastal regions of China and is a commercially important crab resource for fisheries and aquaculture. Records of mud crab aquaculture may date back more than 100 years in China (Shen and Lai, 1994). In ocean, adult crabs mate inshore and gravid females generally migrate offshore where they spawn their eggs (Perrine, 1979). The females then return inshore and can spawn up to three times without needing to mate again (Davis, 2003).

Hainan Island (E 108° 37′–111° 03′, N 18° 10′–20° 10′) is located at the South China Sea with a coastline of 1, 528 km. Due to over-fishing and sea water pollution, *S. paramamosain* resource has decreased significantly. In order to conserve and sustainably exploit this crab resource, population genetics research is necessary. Previous studies based on mtDNA suggested a low level of genetic diversity of *S. paramamosain* from Hainan Island (the $h$ and $\pi$ were 0.2857 and 0.0001 for COI, and 0.5714 and 0.0001 for 16S rRNA respectively, He et al., 2010).

* Corresponding author. Tel.: +86 21 65809298.
*E-mail address:* malingbo@vip.sina.com (L. Ma).

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In the present study, we amplified and sequenced a portion of the cytochrome *c* oxidase subunit I gene, and analyzed the population genetic diversity of *S. paramamosian* from six different localities in Hainan Island of China. This study should be useful for understanding genetic diversity and phylogenetic relationship of *S. paramamosain*.

## 2. Materials and methods

### 2.1. Sample collection and DNA extraction

A total of 92 individuals of *S. paramamosain* were collected from six different localities in Hainan Island of China: Haikou (HK, $N = 16$), Wenchang (WC, $N = 16$), Wanning (WN, $N = 14$), Sanya (SY, $N = 15$), Dongfang (DF, $N = 15$) and Danzhou (DZ, $N = 16$) (Fig. 1). Genomic DNA was extracted from muscle tissue using traditional proteinase K and phenol-chloroform extraction protocols as described by Ma et al. (2009). The DNA was adjusted to 100 ng/μl concentration and stored at −20 °C until being used.

### 2.2. COI gene amplification and sequencing

A pair of primers (COI-s: 5′ - TTGACCCTGCTGGCGGTGG - 3′ and COI-a: 5′ - CAATTGAGGAGGGTAAAAATGGAGTAA - 3′) were designed successfully based on the mtDNA sequence (Accession number: FJ827761) from GenBank database. Polymerase chain reaction (PCR) was performed on a Peltier Thermal Cycler (PTC-200) in 25 μl total volume that included 0.4 μM each primer, 0.2 mM each dNTP, 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.75 unit *Taq* polymerase, and approximately 100 ng template DNA under the following conditions: one cycle of denaturation at 94 °C for 4 min; 37 cycles of 30 s at 94 °C, 50 s at 54 °C, and 50 s at 72 °C. As a final step, products were extended for 7 min at 72 °C. The PCR products were separated on 1.5% agarose gels. After recovered and purified, the PCR products were directly sequenced in both directions using ABI Prism 3730 automated DNA sequencer (PE Corporation).

### 2.3. Data analysis

Sequences were edited and spliced using software DNAstar version 7.1 (DNAstar, Madison, WI, USA) and manually revised. The variable and parsimonious sites were identified using software MEGA 4.0 (Tamura et al., 2007). Haplotypes were identified using software Dna SP version 4.1 (Rozas et al., 2003) and then deposited into GenBank database using software Sequin (the accession numbers were listed in Table 1). A median-joining network of phylogenetic relationships among haplotypes was constructed using software Network version 4.51 (Polzin and Daneshmand, 2003).

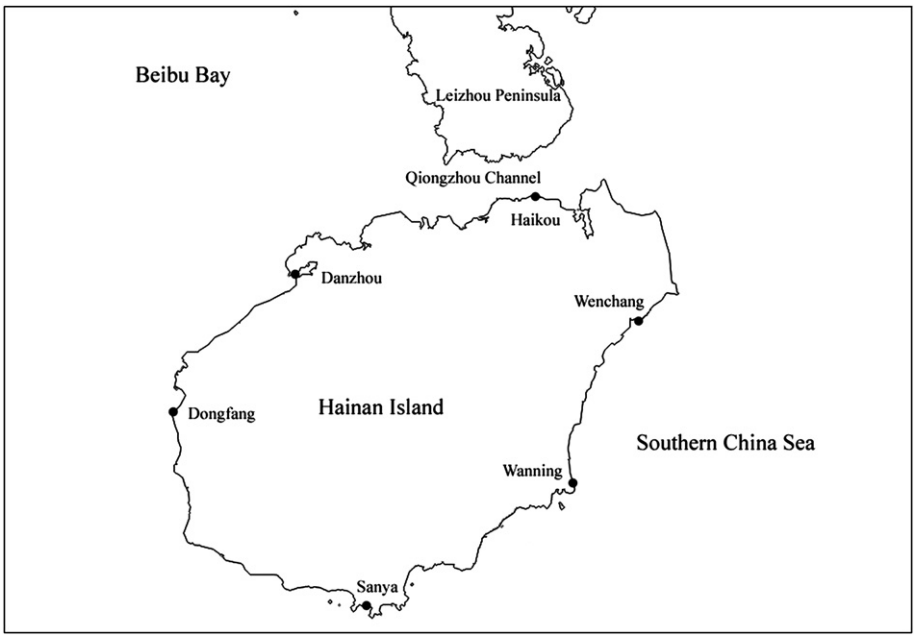


**Fig. 1.** Geographic map of Hainan Island of China. The six sampling localities were Haikou (HK), Wenchang (WC), Wanning (WN), Sanya (SY), Dongfang (DF) and Danzhou (DZ).

**Table 1**
Polymorphic sites among 32 mitochondrial haplotypes of *S. paramamosain* in Hainan Island of China (H1 to H32). Haplotypes are compared with consensus haplotype H1; "·" indicates identical nucleotides; "*N*" indicates number of haplotype in all localities; "AA1 and AA2" indicates amino acids translated by haplotype H1 and by other haplotypes respectively after mutation.

| Haplotype | Polymorphic nucleotide position | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | *N* | Genbank accession no. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 6 | 9 | 1 | 2 | 2 | 2 | 2 | 3 | 3 | 4 | 4 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 7 | 7 | 7 | |
| | 2 | 7 | 3 | 0 | 6 | 6 | 0 | 0 | 1 | 9 | 0 | 6 | 2 | 3 | 6 | 0 | 1 | 2 | 3 | 5 | 6 | 9 | 0 | 2 | 3 | 4 | 6 | 7 | 8 | 0 | 0 | 5 | |
| | | | | | | 8 | 1 | 7 | 0 | 7 | 3 | 6 | 0 | 5 | 5 | 7 | 0 | 3 | 1 | 5 | 4 | 4 | 0 | 1 | 0 | 5 | 3 | 5 | 0 | 3 | 8 | 3 | | |
| H1 | A | C | T | A | G | G | C | G | C | T | T | T | C | C | A | T | G | T | A | C | T | T | A | T | A | C | A | T | C | G | T | T | 1 | HQ687226 |
| H2 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | 9 | HQ687227 |
| H3 | G | · | · | · | · | · | · | A | T | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | 3 | HQ687228 |
| H4 | G | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | 35 | HQ687229 |
| H5 | · | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | 4 | HQ687230 |
| H6 | · | · | · | · | · | · | · | · | T | · | · | · | · | · | · | C | T | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | 1 | HQ687231 |
| H7 | G | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | T | · | · | T | · | · | · | · | · | · | · | · | · | · | · | · | 1 | HQ687232 |
| H8 | G | · | · | · | · | · | · | · | T | · | · | C | T | · | · | · | T | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | 2 | HQ687233 |
| H9 | G | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | 2 | HQ687234 |
| H10 | G | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | · | · | · | C | · | · | · | · | 1 | HQ687235 |
| H11 | G | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | T | · | T | · | · | · | · | · | · | · | · | · | · | · | · | · | 4 | HQ687236 |
| H12 | G | · | · | · | · | A | · | · | T | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | 1 | HQ687237 |
| H13 | G | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | · | · | · | · | · | · | · | C | 1 | HQ687238 |
| H14 | G | · | · | · | · | · | · | · | T | · | · | C | · | · | · | · | T | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | 6 | HQ687239 |
| H15 | G | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | · | · | · | · | · | A | · | · | 2 | HQ687240 |
| H16 | G | · | C | · | · | · | · | · | T | · | · | · | · | · | · | · | T | · | · | · | · | · | G | · | · | · | · | · | · | · | · | · | 1 | HQ687241 |
| H17 | G | · | · | · | · | · | · | · | T | · | · | C | · | · | · | · | T | · | · | · | · | C | · | · | · | · | · | · | · | · | · | · | 1 | HQ687242 |
| H18 | G | T | · | · | · | · | · | · | T | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | 1 | HQ687243 |
| H19 | G | · | · | G | · | · | · | · | T | · | · | · | | | | C | T | · | · | · | · | · | · | · | · | · | G | · | · | · | · | · | 2 | HQ687244 |
| H20 | G | · | · | · | · | · | A | · | T | · | C | · | · | · | · | · | T | C | · | · | · | · | · | · | · | · | · | · | · | · | · | · | 1 | HQ687245 |
| H21 | · | · | · | · | · | · | · | · | T | C | · | · | · | · | · | · | T | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | 1 | HQ687246 |
| H22 | G | · | · | · | · | · | · | · | T | · | C | · | · | A | · | · | T | · | · | · | · | · | · | · | · | T | · | · | · | · | · | · | 1 | HQ687247 |
| H23 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | G | · | · | · | · | · | · | · | 1 | HQ687248 |
| H24 | G | · | · | · | · | · | · | · | T | · | · | · | · | · | · | C | T | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | 1 | HQ687249 |
| H25 | G | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | T | · | · | · | C | · | · | · | · | · | · | · | · | · | · | · | 1 | HQ687250 |
| H26 | G | · | · | · | · | · | · | · | T | · | C | · | · | · | · | · | T | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | 1 | HQ687251 |
| H27 | G | · | · | · | · | · | · | · | T | C | · | · | · | · | · | · | T | · | · | · | · | · | · | C | · | · | · | · | · | · | · | · | 1 | HQ687252 |
| H28 | G | · | · | · | · | · | · | · | T | · | C | · | T | A | · | · | T | · | · | · | · | · | · | · | · | T | · | · | · | · | · | · | 1 | HQ687253 |
| H29 | G | · | · | · | A | · | · | · | T | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | 1 | HQ687254 |
| H30 | G | · | · | · | · | · | · | · | T | · | · | · | · | · | G | · | T | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | 2 | HQ687255 |
| H31 | G | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | · | · | · | · | · | · | C | · | 1 | HQ687256 |
| H32 | G | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | T | · | · | · | · | · | · | · | · | · | · | · | A | · | · | · | 1 | HQ687257 |
| Codon position | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 1 | 3 | 3 | | |
| AA1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | S | V | | | | |
| AA2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Y | I | | | | |

For each population and overall population, software Arlequin version 3.11 (Excoffier et al., 2005) was employed to calculate haplotype diversity ($h$) and nucleotide diversity ($\pi$) according to Nei (1987). The index $h$ indicates the probability that two randomly chosen haplotypes were different in a single population, and $\pi$ shows mean number of differences between all pairs of haplotypes in a single population. Neutrality tests including Ewens-Watterson (Ewens, 1972; Watterson, 1978), Chakraborty (Chakraborty, 1990), Tajima $D$ (Tajima, 1989), and Fu's $Fs$ (Fu, 1997) with 1000 permutations and mismatch analysis (Rogers, 1995) with 10000 bootstrap replicates were performed using Arlequin 3.11 software.

Furthermore, molecular variance (AMOVA) analysis (Excoffier et al., 1992) and the fixation index $F_{ST}$ value were carried out to explain the genetic differentiation between and within populations. The significant level of the test was assessed using 1000 permutations of each pairwise comparison.

## 3. Results

### 3.1. Characteristics of COI sequence

A 761 bp length fragment of mtDNA COI gene was obtained from 92 individuals of *S. paramamosain*. The average nucleotide frequencies of T, C, A and G among all sequences were 38.7%, 18.2%, 28.3% and 14.8%, respectively. The A/T contents (67.0%) were significantly higher than the C/G contents (33.0%). A total of 32 variable sites were detected, of which 15 were parsimonious sites and others were singleton sites, no insertions or deletions were found. Among the variable sites, 27 contained transitions, five contained transversions, and two substitutions resulted in amino acid changes. The details about variable sites of COI sequence were listed in Table 1.

**Table 2**
Genetic diversity of *S. paramamosain* samples from six localities in Hainan Island of China. "$N_H$" indicates number of haplotypes per locality; "*N*" indicates number of individuals tested per locality; "*h*" indicates haplotype diversity; "$\pi$" indicates nucleotide diversity. "HK" indicates Haikou locality; "SY" indicates Sanya locality; "WN" indicates Wanning locality; "DF" indicates Dongfang locality; "WC" indicates Wenchang locality; "DZ" indicates Danzhou locality.

| Locality | $N_H$ | *N* | *h* | $\pi$ |
|---|---|---|---|---|
| HK | 7 | 16 | 0.625 | 0.001 |
| WC | 10 | 16 | 0.900 | 0.003 |
| WN | 8 | 14 | 0.769 | 0.003 |
| SY | 9 | 15 | 0.914 | 0.002 |
| DF | 9 | 15 | 0.905 | 0.002 |
| DZ | 10 | 16 | 0.900 | 0.003 |
| Average | 8.8 | 15.3 | 0.841 | 0.002 |

**Table 3**
Genetic distance within (diagonal) and between (below diagonal) localities, and the fixation index ($F_{ST}$) between localities (above diagonal). "HK" indicates Haikou locality; "SY" indicates Sanya locality; "WN" indicates Wanning locality; "DF" indicates Dongfang locality; "WC" indicates Wenchang locality; "DZ" indicates Danzhou locality; "$*$" indicates $P < 0.05$.

| Locality | HK | SY | WN | DF | WC | DZ |
|---|---|---|---|---|---|---|
| HK | 0.001 | −0.0026 | 0.0106 | 0.1184$*$ | −0.0029 | −0.0089 |
| SY | 0.002 | 0.002 | −0.0003 | 0.0897$*$ | −0.0076 | 0.00154 |
| WN | 0.002 | 0.003 | 0.003 | 0.0221 | 0.0123 | −0.0391 |
| DF | 0.002 | 0.003 | 0.003 | 0.002 | 0.0527 | 0.0304 |
| WC | 0.002 | 0.002 | 0.003 | 0.003 | 0.002 | −0.0083 |
| DZ | 0.002 | 0.002 | 0.003 | 0.003 | 0.003 | 0.003 |

### 3.2. Haplotypes and genetic diversity of S. paramamosain

Among the total 92 sequences, 32 different haplotypes were defined, of which seven were from HK, 10 from WC, eight from WN, nine from SY, nine from DF and 10 from DZ. Haplotype H4 was the most frequent haplotype (38.0% of all individuals) and was present in all six localities, the majority of haplotypes (28) were rare ones present in only one or two individuals. The average *h* of all localities was 0.841, ranging from 0.625 (HK) to 0.914 (SY). The average $\pi$ was 0.002, ranging from 0.001 (HK) to 0.003 (WC, WN and DZ) (Table 2).

### 3.3. Genetic differentiation and phylogeny of S. paramamosain

Pairwise genetic distances ranged from 0.001 to 0.003 within localities and from 0.002 to 0.003 between localities (Table 3). The molecular variance analysis (AMOVA) indicated that 98.2% of the total genetic variation was contributed by within-localities variation and only 1.8% was contributed by among-localities variation ($F_{ST} = 0.0176$, $P > 0.05$) (Table 4). No significant genetic differentiation was observed among six localities except between DF and HK ($F_{ST} = 0.1184$, $P < 0.05$), and DF and SY ($F_{ST} = 0.0897$, $P < 0.05$). All above analysis indicated a low level of genetic differentiation among six localities, suggesting a limited genetic structure of *S. paramamosain* in Hainan Island. In addition, a star-like topology with a high ratio of unique haplotype (65.6%) was observed (Fig. 2), implying a historical population expansion event. Haplotype H4 was present in the centre of this topology, and was closely related to the majority of the haplotypes, suggesting it is the ancestral haplotype.

### 3.4. Neutrality tests and mismatch analysis

All neutrality tests except Ewens-Watterson ($F_O = 0.17$ and $F_E = 0.06$, $P > 0.05$) provided consistent results that suggested a significant deviation from mutation–drift equilibrium (Table 5). In Tajima *D* and Fu's $F_S$ tests, the negative values were detected in all localities, with the total values of −2.22 ($P < 0.01$) and −27.76 ($P < 0.01$), respectively. These statistical findings implied that *S. paramamosain* may undergo population selection or expansion events.

The demographic parameters of mismatch distribution for each locality are shown in Table 6. The estimated effective population size after population growth was significantly larger than that before population growth for each locality

**Table 4**
Analysis of molecular variance (AMOVA) of mtDNA COI sequence of *S. paramamosain* in Hainan Island of China.

| Source of variation | df | Sum of squares | Variance components | Percentage of variation | $F_{ST}$ | *P* |
|---|---|---|---|---|---|---|
| Among localities | 5 | 5.603 | 0.01572Va | 1.76 | 0.0176 | 0.107 |
| Within localities | 86 | 75.646 | 0.87961Vb | 98.14 | | |
| Total | 91 | 81.249 | | | | |

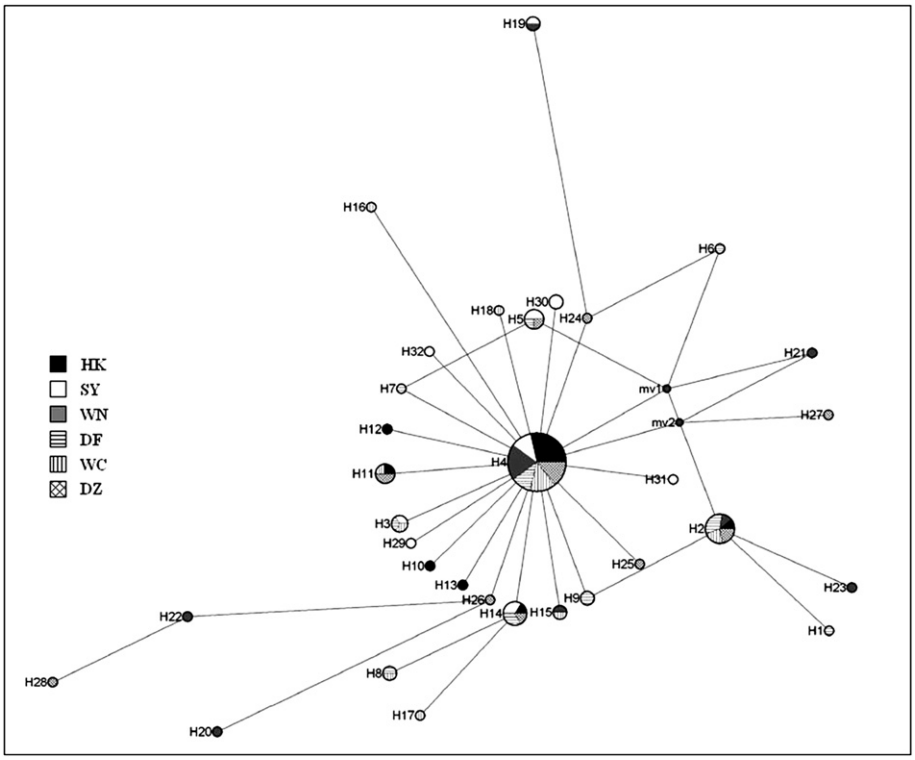


**Fig. 2.** The median-joining network of 32 haplotypes of mtDNA COI sequence of *S. paramamosain* in Hainan Island of China. "HK" indicates Haikou locality; "SY" indicates Sanya locality; "WN" indicates Wanning locality; "DF" indicates Dongfang locality; "WC" indicates Wenchang locality; "DZ" indicates Danzhou locality.

($P < 0.01$). The sum of the square deviations (SSD) per locality ranged from 0.0028 to 0.0475, with an average of 0.0195. The statistical non-significance ($P > 0.05$) of SSD between the observed and expected mismatch distributions indicated the presence of non-equilibrium and a population expansion event for *S. paramamosain*. Moreover, the raggedness indices (Rag) per locality were between 0.0724 and 0.2308, with an average of 0.1154 ($P > 0.05$), providing evidence for population expansion too.

## 4. Discussion

Many aquatic animals have been studied using mtDNA such as swimming crab (Yamauchi et al., 2003), Chinese shrimp (Shen et al., 2007; Li et al., 2009), Chinese mitten crab (Wang et al., 2008; Xu et al., 2009), northern fur seal (Dickerson et al., 2010), Eurasian otter (Ki et al., 2010) and scallop (Feng et al., 2011). In this study, the genetic diversity of *S. paramamosain* in Hainan Island was investigated using mtDNA. Thirty-two haplotypes were defined that was higher than the number in the study by Ma et al. (the haplotype number = 12, 2006), but lower than the number in the study by He et al. (the haplotype number = 40, 2010). This may mainly be due to the different sample number and different length of COI sequence. In the

**Table 5**
Neutrality tests results of *S. paramamosain* in Hainan Island of China. "$F_O$" indicates observed *F* value; "$F_E$" indicates expected *F* value; "$N_O$" indicates number of observed alleles; "$N_E$" indicates number of expected alleles; "HK" indicates Haikou locality; "SY" indicates Sanya locality; "WN" indicates Wanning locality; "DF" indicates Dongfang locality; "WC" indicates Wenchang locality; "DZ" indicates Danzhou locality; "$*$" indicates $P < 0.05$; "$**$" indicates $P < 0.01$.

| Population | Ewens-Watterson | | Chakraborty | | Tajima *D* | Fu's *Fs* |
|---|---|---|---|---|---|---|
| | $F_O$ | $F_E$ | $N_O$ | $N_E$ | | |
| HK | 0.41 | 0.23 | 7 | 3.15$^{*}$ | −2.06$^{**}$ | −4.65$^{**}$ |
| WC | 0.16 | 0.14 | 10 | 4.71 | −1.65$^{*}$ | −6.12$^{**}$ |
| WN | 0.29 | 0.18 | 8 | 4.99 | −1.74$^{*}$ | −2.80$^{*}$ |
| SY | 0.15 | 0.15 | 9 | 4.35 | −1.73$^{*}$ | −5.31$^{**}$ |
| DF | 0.16 | 0.15 | 9 | 4.55 | −0.95 | −4.90$^{**}$ |
| DZ | 0.16 | 0.14 | 10 | 4.91 | −1.68 | −5.68$^{**}$ |
| Total | 0.17 | 0.06 | 32 | 7.59$^{**}$ | −2.22$^{**}$ | −27.76$^{**}$ |

**Table 6**
Mismatch distributions analysis of *S. paramamosain* in Hainan Island of China. "$\tau$" indicates units of mutational time; "$\theta_0$" indicates $\theta$ before population growth; "$\theta_1$" indicates $\theta$ after population growth; "SSD" indicates sum of the square deviations between the observed and expected mismatch; "$P_{SSD}$" indicates the probability of SSD; "Rag" indicates raggedness index; "$P_{Rag}$" indicates the probability of raggedness; "HK" indicates Haikou locality; "SY" indicates Sanya locality; "WN" indicates Wanning locality; "DF" indicates Dongfang locality; "WC" indicates Wenchang locality; "DZ" indicates Danzhou locality.

| Population | $\tau$ | $\theta_0$ | $\theta_1$ | SSD ($P_{SSD}$) | Rag ($P_{Rag}$) |
|---|---|---|---|---|---|
| HK | 0.9570 | 0.0000 | 99999.0000 | 0.0028 (0.8530) | 0.0816 (0.6586) |
| SY | 1.7461 | 0.0000 | 99999.0000 | 0.0461 (0.0611) | 0.2308 (0.0251) |
| WN | 3.3750 | 0.0053 | 5.6048 | 0.0475 (0.2047) | 0.1421 (0.2182) |
| DF | 1.9453 | 0.0000 | 99999.0000 | 0.0091 (0.4586) | 0.0889 (0.3150) |
| WC | 2.0625 | 0.0000 | 99999.0000 | 0.0064 (0.4761) | 0.0767 (0.3699) |
| DZ | 1.9297 | 0.0000 | 99999.0000 | 0.0045 (0.6795) | 0.0724 (0.4157) |
| Average | 2.0026 | 0.0008 | 83333.4341 | 0.0195 (0.4555) | 0.1154 (0.3338) |

study by Ma et al. (2006), seventy-two individuals and 597 bp length of COI sequence were used, while in the study by He et al. (2010), two hundred and eithteen individuals and 533 bp length of COI sequence were used.

A high level of genetic variation (average $h = 0.841$ and average $\pi = 0.002$) of *S. paramamosain* in Hainan Island was detected which was consistent with that in the mainland coasts of China ($h$ ranged from 0.6712 to 1.000 and $\pi$ ranged from 0.0017 to 0.0031), but higher than that from Sanya in Haihan Island in the previous study ($h$ ranged from 0.2857 to 0.2949 and $\pi$ ranged from 0.0006 to 0.0011, He et al., 2010). The high level of genetic diversity of *S. paramamosain* was confirmed by microsatellite markers too (Takano et al., 2005; Ma et al., 2010b). Further, the high genetic variation also has been observed in other sea animals, such as mud crab (*Scylla serrata*, Fratini et al., 2010), scallop (*Chlamys farreri*, Zhao et al., 2009) and Atlantic salmon (*Salmo salar*, Karlsson et al., 2010). The life history characteristics, environmental heterogeneity and large population sizes may help to maintain a high level of genetic diversity (Nei, 1987; Avise, 1998).

Low genetic differentiation ($F_{ST} = 0.0176$, $P > 0.05$) among different localities of *S. paramamosain* was detected using AMOVA analysis, suggesting a limited genetic structure. For *S. paramamosain* distributed along the mainland coasts of China, a low genetic differentiation was observed too ($F_{ST} = 0.0059$, $P > 0.05$, He et al., 2010). This may be due to adult and juvenile migrations between ocean basins and adjacent continental margins, high dispersal capabilities of larvae, and limits of physical barriers in the marine environment. Moreover, several other sea animals also showed low genetic differentiation among different geographic populations, such as *Nibea albiflora* (Han et al., 2008) and *Feneropenaeus chinensis* (Liu et al., 2004; Li et al., 2009).

In conclusion, we amplified and characterized mtDNA COI gene sequence, according to which a high level of genetic diversity and low level of differentiation of *S. paramamosain* in Hainan Island of China was found. This study should be useful for estimation of stocks resource, conservation and sustainably exploitation, and selective breeding of this important crab species.

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