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High genetic diversity and low differentiation in mud crab (*Scylla paramamosain*) along southeastern coast of China revealed by microsatellite markers

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Abstract: Mud crab (*Scylla paramamosain*) is a carnivorous portunid crab, mainly distributed along southeastern coast of China. Mitochondrial DNA analysis in previous study indicated a high level of genetic diversity and low differentiation of it. In this study, population genetic diversity and differentiation of *S. paramamosain* were investigated using nine microsatellite markers. In total, 397 wild specimens of 11 locations from southeastern coast of China were sampled and genotyped. A high level of genetic diversity was observed, with N_a, H_O and H_E per location ranging from 7.8 to 9.6, from 0.62 to 0.77 and from 0.66 to 0.76, respectively. AMOVA analysis indicated a low level of genetic differentiation among 11 locations, despite that a statistically significant F_{ST} value was found (F_{ST}=0.0183, P<0.05). Out of 55 pairwise location comparisons, 39 showed significant F_{ST} values (P<0.05), but all of them were lower than 0.05， except one between SM and ST locations. No significant deficiency of heterozygotes (F_{IS}=0.0007, P>0.05) was detected for all locations except SM and ZJ. Cluster analysis using UPGMA showed that all locations fell into one group except SM. Significant association was found between genetic differentiation in terms of $F_{ST}/(1-F_{ST})$ and natural logarithm of geographical distance (r^2=0.1139, P=0.02), indicating that the genetic variation pattern closely resembled an isolation by distance model. This study supports the viewpoint of high genetic diversity and low differentiation in *S. paramamosain* along southeastern coast of China.

Key words: *Scylla paramamosain*, microsatellites, genetic diversity, genetic differentiation

Running title: Genetic diversity of *Scylla paramamosain*

Introduction

Mud crab (*Scylla paramamosain*), mainly distributed along southeastern coast of China, is a commercially important crab resource for fisheries and aquaculture. Records of *S. paramamosain* aquaculture can date back more than 100 years in China (Shen and Lai, 1994) and more than 30 years in other Asian countries (Keenan and Blackshaw, 1999). In wild environments, adults mate inshore and then gravid females generally migrate offshore and spawn their eggs (Perrine, 1979). Due to over-exploitation and environmental deterioration, the wild resource decreased quickly. In order to conserve and sustainably exploit this important crab resource, genetic studies are necessary, as they are helpful for better understanding genetic diversity and structure (Dickerson et al., 2010), investigation of phylogenetic and evolutionary history (Gvozdik et al., 2010; Van Syoc et al., 2010), and can also provide constructive guidance for resource conservation and management (Ortega-Villaizan et al., 2006). For *S. paramamosain*, mtDNA has been studied and the result suggested a genetically homogeneous population structure and a recent population expansion event (He et al., 2010). Moreover, a high level of genetic diversity and low differentiation of different locations were observed in *S. paramamosain* inhabited along southeastern coast of China using mtDNA also (Lu et al., 2009; Ma et al., 2011a).

Microsatellites are nuclear molecular markers with the characteristics of 1 to 6 bp length repeat motif, high polymorphism and codominant inheritance. Microsatellite markers have been widely used for investigation of genetic diversity (Dudaniec et al., 2010), determination of pedigree (Li et al., 2009a), construction of genetic maps (Ma et al., 2011b) and mapping of QTLs (Zhang et al., 2011). To date, microsatellite markers have been isolated in *S.*

paramamosain (Takano et al., 2005; Ma et al., 2010, 2011c; Cui et al., 2011), but no references about population genetic diversity and differentiation are reported for this important crab species.

In this study, a total of 397 wild specimens of 11 locations from southeastern coast of China were sampled and genotyped using nine microsatellite markers. The purpose is to investigate the level of population genetic diversity and differentiation in *S. paramamosain* across these regions to provide valuable information for conservation, exploitation and management of this important fishery resource.

Materials and Methods

Sample collection and DNA extraction

A total of 397 wild specimens of *S. paramamosain* were collected from 11 different locationsalong southeastern coastal regions of China: Sanmen (SM, *N*=38), Ningde (ND, *N*=35), Zhangzhou (ZZ, *N*=32), Shantou (ST, *N*=25), Shenzhen (SZ, *N*=40), Zhanjiang (ZJ, *N*=41), Haikou (HK, *N*=37), Wenchang (WC, *N*=51), Wanning (WN, *N*=35), Dongfang (DF, *N*=30) and Danzhou (DZ, *N*=33) (Fig. 1 and Table 1). Each specimen was euthanized by administering a lethal dose of MS-222. Genomic DNA was extracted from muscle tissue using traditional proteinase K and phenol-chloroform extraction protocols as described by Ma et al. (2009a). The DNA was adjusted to 100 ng/μl concentration and stored at -20℃ until use.

Microsatellite genotyping

Nine polymorphic microsatellite loci were selected for genotyping, of which eight were developed using 5’ anchored PCR method (Cui et al., 2011), while the other one was

developed using PCR-based isolation of microsatellite arrays (PIMA) (Ma et al., 2010) in our laboratory (Table 2). The criteria for selection are as follows: the annealing temperature within 50–63 ℃, the expected product size between 110 and 320 bp, the observed heterozygosity value > 0.5, and no stuttering bands. PCR reactions were conducted in a total volume of 25 μl and 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℃ for 4 min; 30 cycles of 30s at 94℃, 50s at a primer-specific annealing temperature (Table 2), and 50s at 72℃. As a final step, products were extended for seven min at 72℃.

Several methods such as agarose gel electrophoresis, denaturing polyacrylamide gel electrophoresis, and automated DNA sequencing were often employed for detecting differences of nucleotide acid, of which the second one is a very effective and practical technique for genotyping of microsatellites and has been used in a wide range of organisms, as it has many advantages: the high resolution (about 1 bp) and large outputs (100 samples for one time), the low expenses, and can be easily mastered. In this study, the PCR products were separated on 6% denaturing polyacrylamide gel as described by Ma (2009b). The microsatellite fragments were visualized by silver-staining which was performed as follows: the gel was soaked in 1.0 L staining solution (1.5g $AgNO_3$) for about 10 min. After dropping the solution, the gel was washed by ddH_2O for 5 s. Then the gel was soaked in 1.0 L coloured solution (20 g NaOH and 4 ml formaldehyde) for about 10 min. Finally, the gel was cleaned by ddH_2O. The sizes of alleles were estimated according to the pBR322/*Msp* I marker.

Data analysis

Observed and expected heterozygosity, departure from Hardy-Weinberg equilibrium (HWE), linkage disequilibrium (LD) and inbreeding coefficient (F_{IS}) were performed using ARLEQUIN version 3.01 software (Excoffier et al., 2005). Genetic differentiation among locations was estimated using the analysis of molecular variance (AMOVA) approach by GENAlEX version 6.41 software (Peakall and Smouse, 2006). The significance levels were tested by 10000 permutations for LD and by 1000 permutations for F_{ST} values. Observed number of alleles (N_a), effective number of alleles (N_e) and genetic distance were estimated using POPGENE version 1.31 software (Yeh et al., 1999). An unweighted pair-group mean analysis (UPGMA) tree was constructed based on Nei's genetic distance (Nei, 1978) of pairwise locations using MEGA version 4.0 software (Tamura et al., 2007). The association between genetic differentiation and geographic distance (isolation by distance) among locations was estimated by Mantel test (Mantel, 1967) with 1000 permutations.

Results

All nine microsatellite loci used in this study were polymorphic in each location, showing a high level of genetic diversity (Table 3). In total, 104 alleles were detected from 397 individuals in 11 locations across nine loci. Observed number of alleles (N_a) ranged from six (Scypa1) to 16 (Scypa8 and Scpa03) per locus and from 7.8 (ST) to 9.6 (WC) per location. Observed and expected heterozygosity (H_O and H_E) ranged from 0.32 to 1.00 and from 0.31 to 0.93 per locus-location combination, while from 0.62 (SM) to 0.77 (HK) and from 0.66 (ST) to 0.76 (ND and DZ) per location, respectively. The inbreeding coefficient (F_{IS}) ranged from -0.278 to 0.440 per locus-location combination and from -0.137 (ST) to 0.136 (SM) per

location, with an average of 0.001 as a whole.

An exact probability test of Hardy-Weinberg equilibrium (HWE) was performed among 99 locus-location combinations, and it revealed a significant deviation at 19 loci (P <0.05). These 19 loci were Scypa1 (in ZZ and HK), Scypa2 (in SM and ZJ), Scypa3 (in ND and ST), Scypa4 (in DF), Scypa8 (in ND, ZZ, HK and WN), Scypa13 (in SM, ST, ZJ and WN) and Scpa03 (in SM, SZ, ZJ and WC), respectively. Two loci (Scypa5 and Scypa11) were in keeping with HWE in all locations. Probability tests of genotypic linkage disequilibrium (LD) for all pairs of loci within each location suggested significant nonrandom associations in only one of 396 possible pairwise comparisons after sequential Bonferroni correction (Scypa2 and Scypa13 in DF, P<0.00139) (Rice, 1989). When each location was analyzed separately, there was no evidence for stuttering and large allelic dropout in any of the loci confirmed by MICRO-CHECKER version 2.2.3 software (Van Oosterhout et al., 2004).

The analysis of molecular variance (AMOVA) showed that genetic variation existed mainly within locations, rather than among locations, as the percentage of variance was 98.17% within locations, while 1.83% among locations. Although the overall F_{ST} value over all locations and loci was statistically significant (F_{ST}=0.0183, P<0.05), the genetic differentiation was still low, because the F_{ST} values was much lower than 0.05 (Table 4 and Table 5). Multilocus estimates of F_{ST} for all possible pairwise locations ranged from 0.002 (ZJ and DZ) to 0.067 (SM and ST). The highest differentiation was between SM and ST (F_{ST}=0.067), and the lowest differentiation was between ZJ and DZ (Table 5). Thirty-nine out of 55 pairwise locations showed significant differentiation (P<0.05). Nei's genetic distances between pairwise locations ranged from 0.0121 (ZZ and SZ) to 0.2036 (SM and ST), and they

were lower than 0.1 between 43 out of total 55 pairwise locations. Among 11 locations, SM was the most distinctive one, as it showed significant differentiation to all other 10 locations (F_{ST} values ranged from 0.024 to 0.067). In contrast, DZ was the most representative one as it significantly differed only to four locations (F_{ST} values ranged from 0.012 to 0.029).

Cluster analysis of 11 locations using UPGMA approach revealed two groups: one contained 10 locations and the other contained only one location (SM) (Fig. 2). Mantel tests for isolation by distance among locations detected a significant positive correlation between pairwise $F_{ST}/(1- F_{ST})$ and the natural logarithm of geographic distance (km) (r^2=0.1139, P=0.02), while no significant correlation between pairwise F_{ST} and geographic distance (km) (r^2=0.1230, P=0.06) (Fig. 3)

Discussion

This study suggested a high level of population genetic diversity of *S. paramamosain* along southeastern coast of China (N_a, H_O and H_E ranged from 7.8 to 9.6, from 0.62 to 0.77 and from 0.66 to 0.76 per location respectively) thatis in accord with the previous studies which showed a high level of mtDNA genetic diversity in *S. paramamosain* (Lu et al., 2009; Ma et al., 2011a). High population genetic diversity also has been observed in other marine animals, such as scallop (*Chlamys farreri*) (Zhao et al., 2009), Atlantic salmon (*Salmo salar*) (Karlsson et al., 2010) and silver pomfret (*Pampus argenteus*) (Zhao et al., 2011). Three factors including the life history characteristics, environmental heterogeneity and large population sizes may help to maintain a high level of genetic diversity (Perrine, 1979; Nei, 1987; Avise, 1998). On the whole, the level of genetic diversity of *S. paramamosain* from the southern

regions was higher than that from the northern regions (Table 3) that may be due to the different inhabitation environments. A similar finding was observed in previous study which indicated a reduction trend for genetic diversity of *S. paramamosain* from south to north step by step using mtDNA (Lu et al., 2009).

Generally, marine fishes are considered to have low level of genetic differentiation among different geographic populations due to the high dispersal capabilities, large population sizes and relatively small barriers in the marine environment (Beheregaray and Sunnucks, 2001). For fish *Nibea albiflora*, little population genetic structure between Yellow Sea and East China Sea was observed using mtDNA (Han et al., 2008). For shrimp *Feneropenaeus chinensis*, no significant population genetic differentiation between Yellow Sea and Bohai Sea was found using both microsatellite DNA and mtDNA (Liu et al., 2004; Li et al., 2009b). For the crab *S. paramamosain*, a genetically homogeneous population structure with high gene flow was observed among most localities along the coasts of East China Sea and South China Sea using mtDNA (He et al., 2010). In this study, statistically significant genetic differentiation was detected among 11 locations along southeastern coast of China (F_{ST}=0.0183, P<0.05), but the F_{ST} value was still low (<0.05), suggesting a low level of genetic differentiation (Wright, 1978). A similar finding was reported in earlier studies which suggested a low differentiation in *S. paramamosain* by mtDNA (Lu et al., 2009; He et al., 2010; Ma et al., 2011a). The above information indicated that all locations of *S. paramamosain* should be a single genetically homogeneous population. Low F_{ST} value indicated the relatively high gene flow among locations. There are three most likely explanations: the unique reproductive habit that adult and juvenile migrate between ocean

basins and adjacent continental margins, the high dispersal capabilities of larvae, and the small limits of physical barriers in the marine environment.

Among these 11 locations, SM was the most genetically distinctive one in two main ways: (1) it has the lowest genetic diversity (the overall H_O was 0.62) and the highest F_{ST} values (the ranges between 0.024 and 0.067) compared with other locations; (2) it has the greatest overall F_{IS} value (F_{IS}=0.136, P<0.05) compared with other locations. These findings indicated that the gene exchange is relatively lower between SM and other locations than that between other location-pairs. Naturally, the optimum temperature range of this crab is from 18 to 27℃ for growth, and the relatively higher temperature is needed for spawning. However, SM is the most northern one among these locations, so the seawater temperature is the lowest in the same period. Low temperature may limit the effective population size and the high dispersal capabilities of *S. paramamosain*. Moreover, the over-fishing of human may be another potentially reason. The significant positive correlation between genetic differentiation and geographic distance was found, suggesting an isolation by distance model of genetic variation.

In conclusion, a high level of population genetic diversity and low differentiation were found in mud crab (*Scylla paramamosain*) from 11 locations along southeastern coastal regions of China by microsatellites analysis that showed a genetically homogeneous population structure of these 11 locations of *S. paramamosain*. In the future, more population genetic studies should be done fully in this crab species. The findings in this study will provide valuable information for conservation, exploitation, and artificial selective breeding of this important fishery resource.

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References

Avise, J. (1998). Phylogeography. Harvard University Press. Cambridge, MA.

Beheregaray, L.B. and Sunnucks, P. (2001). Fine-sclae genetic structure, estuarine colonization and incipient speciation in marine silverside fish *Odontesthes argentinensis*. *Mol. Ecol.* **10**, 2849-2866.

Cui, H.Y., Ma, H.Y., Ma, L.B., Ma, C.Y. and Ma, Q.Q. (2011). Development of eighteen polymorphic microsatellite markers in *Scylla paramamosain* by 5' anchored PCR technique. *Mol. Biol. Rep.* **38** (8), 4999-5002.

Dickerson, B.R., Ream, R.R., Vignieri, S.N. and Bentzen, P. (2010). Population structure as revealed by mtDNA and microsatellites in northern fur seals, *Callorhinus ursinus*, throughout their range. *Plos One* **5** (5), 10671-10679.

Dudaniec, R.Y., Storfer, A., Spear, S.F. and Richardson, J.S. (2010). New microsatellite markers for examining genetic variation in peripheral and core populations of the coastal giant salamander (*Dicamptodon tenebrosus*). *Plos One* **5** (12), 14333-14336.

Excoffier, L., Laval, G. and Schneider, S. (2005). Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol. Bioinform.* **1**, 47-50.

Gvozdik, V., Moravec, J., Klutsch, C. and Kotlik, P. (2010). Phylogeography of the Middle Eastern tree frogs (Hyla, Hylidae, Amphibia) as inferred from nuclear and mitochondrial DNA variation, with a description of a new species. *Mol. Phylogenet. Evol.* **55**, 1146-1166.

Han, Z.Q., Gao, T.X., Yanagimoto, T. and Sakurai, Y. (2008). Genetic population structure of *Nibea albiflora* in the Yellow Sea and East China Sea. *Fisheries Sci.* **74**, 544-552.

He, L., Zhang, A., Weese, D., Zhu, C., Jiang, C. and Qiao, Z. (2010). Late Pleistocene population expansion of *Scylla paramamosain* along the coast of China: A population dynamic response to the Last Interglacial sea level highstand. *J. Exp. Mar. Biol. Ecol.* **385**, 20-28.

Karlsson, S., Moen, T. and Hindar, K. (2010). Contrasting patterns of gene diversity between microsatellites and mitochondrial SNPs in farm and wild Atlantic salmon. *Conserv. Genet.* **11**, 571-582.

Keenan, C. and Blackshaw, P.A. (1999). Mud crab aquaculture and biology. ACAIR Proceedings No. 78. Watson Ferguson & Co, Australia.

Li, M., Valimaki, K., Piha, M., Pakkala, T. and Merila, J. (2009a). Extrapair paternity and maternity in the three-toed woodpecker, *Picoides tridactylus*: insights from microsatellite-based parentage analysis. *Plos One* **4** (11), 7895-7900.

Li, Y.L., Kong, X.Y., Yu, Z.N., Kong, J., Ma, S. and Chen, L.M. (2009b). Genetic diversity and historical demography of Chinese shrimp *Feneropenaeus chinensis* in Yellow Sea and Bohai Sea based on mitochondrial DNA analysis. *Afr. J. Biotechnol.* **8** (7), 1193-1202.

Liu, P., Meng, X.H., Kong, J., Zhuang, Z.M., Ma, C.Y. and Wang, Q.Y. (2004). Genetic

diversity analysis of *Penaeus chinensis* by microsatellite DNA. *Pro. Nat. Sci.* **14** (3), 333-338.

Lu, X.P., Ma, L.B., Qiao, Z.G., Zhang, F.Y. and Ma, C.Y. (2009). Population genetic structure of *Scylla paramamosain* from the coast of the Southeastern China based on mtDNA COI sequences. *J. Fish. China.* **33** (1), 15-23. (In Chinese with English abstract)

Ma, H.Y., Chen, S.L., Yang, J.F., Chen, S.Q. and Liu, H.W. (2011b). Genetic linkage maps of barfin flounder (*Verasper moseri*) and spotted halibut (*Verasper variegatus*) based on AFLP and microsatellite markers. *Mol. Biol. Rep.* **38** (7), 4749-4764.

Ma, H.Y., Ma, C.Y. and Ma, L.B. (2011a). Population genetic diversity of mud crab (*Scylla paramamosain*) in Hainan Island of China based on mitochondrial DNA. *Biochem. Syst. Ecol.* **39** (4-6), 434-440.

Ma, H.Y., Ma, C.Y. and Ma, L.B. (2011c). Identification of type I microsatellite markers associated with genes and ESTs in *Scylla paramamosain*. *Biochem. Syst. Ecol.* **39** (4-6), 371-376.

Ma, H.Y., Ma, C.Y., Ma, L.B. and Cui, H.Y. (2010). Novel polymorphic microsatellite makers in *Scylla paramamosain* and cross-species amplification in related crab species. *J. Crustacean Biol.* **30** (3), 441-444.

Ma, H.Y., Yang, J.F., Su, P.Z. and Chen, S.L. (2009a). Genetic analysis of gynogenetic and common populations of *Verasper moseri* using SSR markers. *Wuhan Univ. J. Nat. Sci.* **14**, 267-273.

Ma, H.Y. (2009b). Development of sex-specific markers and SSR markers and construction of genetic linkage maps of three important cultured marine fish species. Ph. D. thesis,

Ocean University of China, Qingdao, China.

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Caner. Res.* **27**, 209-220.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583-590.

Nei, M. (1987). Molecular evolutionary genetics. Columbia University Press. New York.

Ortega-Villaizan Romo, M., Suzuki, S., Nakajima, M. and Taniguchi, N. (2006). Genetic evaluation of interindividual relatedness for broodstock management of the rare species barfin flounder *Verasper moseri* using microsatellite DNA markers. *Fisheries Sci.* **72**, 33-39.

Peakall, R. and Smouse, P.E. (2006). GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **6**, 288-295.

Perrine, D. (1979). The mangrove crab on Ponape. Marine Resources Division, Ponape Eastern Caroline Islands. 88 pp.

Rice, W.R. (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223-225.

Shen, Y. and Lai, Q. (1994). Present status of mangrove crab (*Scylla serrata* (Forskål)) culture in China. Naga, the ICLARM Quarterly, January 1994, 28-29.

Takano, M., Barinova, A., Sugaya, T., Obata, Y., Watanabe, T., Ikeda, M. and Taniguchi, N. (2005). Isolation and characterization of microsatellite DNA markers from mangrove crab, *Scylla paramamosain*. *Mol. Ecol. Notes* **5,** 794-795.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. (2007). MEGA 4: molecular evolutionary genetics anlysis (MEGA) software version 4.0. *Mol. Biol. Evol.* **24**, 1596-1599.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. and Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in mirosatellite data. *Mol. Ecol. Notes* **4**, 535-538.

Van Syoc, R.J., Fernandes, J.N., Carrison, D.A. and Grosberg, R.K. (2010). Molecular phylogenetics and biogeography of *Pollicipes* (Crustacea: Cirripedia), a Tethyan relict. *J. Exp. Mar. Biol. Ecol.* **392**, 193-199.

Wright, S. (1978). Evolution and the genetics of populations. Variability within and among natural populations. Vol 4. The University of Chicago Press, Chicago.

Yeh, F.C., Yang, R.C. and Boyle, T. (1999). POPGENE version 1.31. Microsoft window-based freeware for population genetic analysis. Available: (www.ualberta.ca/~fyeh/). University of Alberta and the Centre for International Forestry Research.

Zhang, Y., Xu, P., Lu, C., Kuang, Y., Zhang, X., Cao, D., Li, C., Chang, Y., Hou, N., Li, H., Wang, S. and Sun, X. (2011). Genetic linkage mapping and analysis of muscle fiber-related QTLs in common carp (*Cyprinus carpio* L.). *Mar. Biotechnol.* **13**, 376-392.

Zhao, C., Li, Q. and Kong, L. (2009). Inheritance of AFLP markers and their use for genetic diversity analysis in wild and farmed scallop (*Chlamys farreri*). *Aquaculture* **287**, 67-74.

Zhao, F., Dong, Y., Zhuang, P., Zhang, T., Zhang, L. and Shi, Z. (2011). Genetic diversity of silver pomfret (*Pampus argenteus*) in the Southern Yellow and East China Seas. *Biochem. Syst. Ecol.* **39**, 145-150.

Figures Legend:

Figure 1: Geographic map of southeastern coast of China. •, sampling location; 1, Sanmen (SM); 2, Ningde (ND); 3, Zhangzhou (ZZ); 4, Shantou (ST); 5, Shenzhen (SZ); 6, Zhanjiang (ZJ); 7, Haikou (HK); 8, Wenchang (WC); 9, Wanning (WN); 10, Dongfang (DF) and 11, Danzhou (DZ).

Figure 2: The UPGMA tree of 11 locations of *Scylla paramamosain*. SM, Sanmen; ND, Ningde; ZZ, Zhangzhou; ST, Shantou; SZ, Shenzhen; ZJ, Zhanjiang; HK, Haikou; WC, Wenchang; WN, Wanning; DF, Dongfang and DZ, Danzhou.

Figure 3: Relationship between genetic differentiation and geographic distance (km) among 11 locations. (a) relationship between pairwise $F_{ST}/(1- F_{ST})$ and the natural logarithm of geographic distance (km). (b) relationship between pairwise F_{ST} and geographic distance (km).

Tables Caption:

Table 1: Characteristics of 11 locations of *Scylla paramamosain*

Table 2: Characterization of nine microsatellite markers used in this study.

Table 3: Summary statistics of nine microsatellite markers in 11 locations of *Scylla paramamosain*.

N_a, observed number of alleles; N_e, effective number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; $P_{H\text{-}W}$, *P* values for Hardy-Weinberg equilibrium; *, Significant *P* value <0.05; **, Significant *P* value <0.01.

Table 4: AMOVA design and results for 11 locations of *Scylla paramamosain*.

Table 5: Pairwise F_{ST} (below diagonal) and genetic distance (above diagonal) among 11 locations of *Scylla paramamosain*.

*, Significant *P* value <0.05; **, Significant *P* value <0.01.

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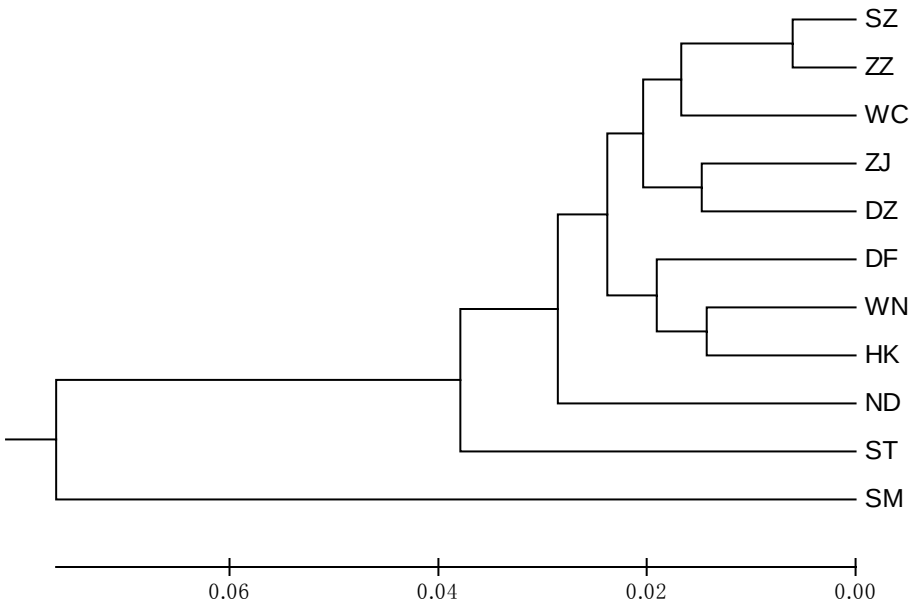
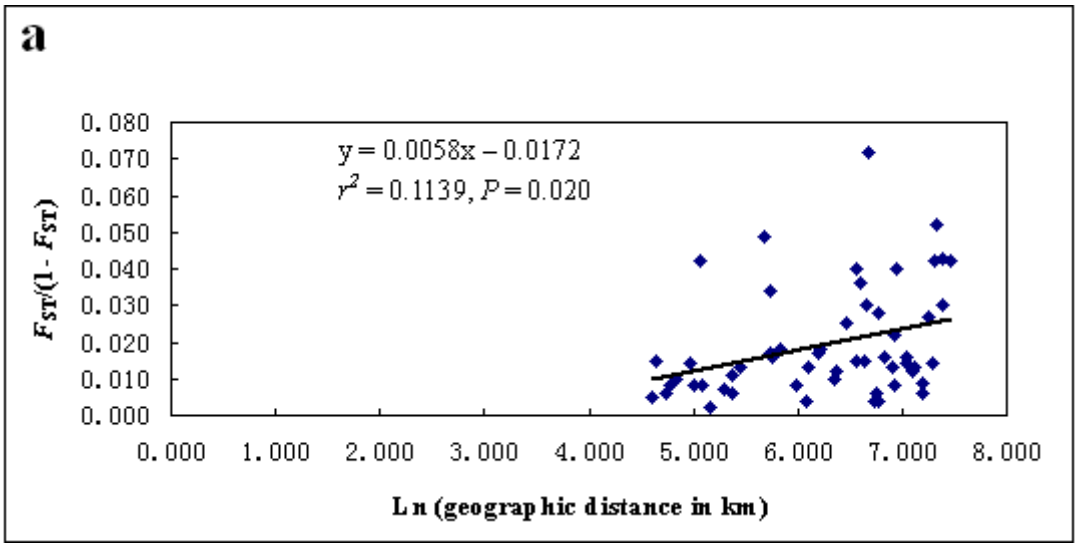


Figure 2. The UPGMA tree of 11 locations of *Scylla paramamosain*. SM, Sanmen; ND, Ningde; ZZ, Zhangzhou; ST, Shantou; SZ, Shenzhen; ZJ, Zhanjiang; HK, Haikou; WC, Wenchang; WN, Wanning; DF, Dongfang and DZ, Danzhou.



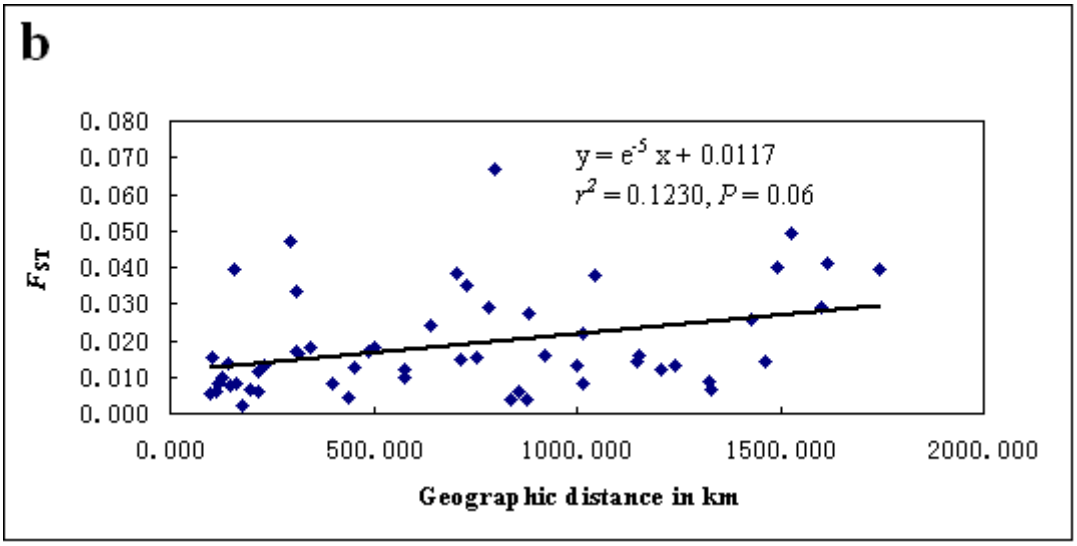


Figure 3. Relationship between genetic differentiation and geographic distance (km) among 11 locations. (a) relationship between pairwise $F_{ST}/(1- F_{ST})$ and the natural logarithm of geographic distance (km). (b) relationship between pairwise F_{ST} and geographic distance (km).

Table 1. Characteristics of 11 locations of *Scylla paramamosain*

Location	Code	Sample size	Latitude (N)	Longitude (E)
Sanmen	SM	38	29° 06′	122° 04′
Ningde	ND	35	26° 60′	120° 15′
Zhangzhou	ZZ	32	24° 27′	118° 17′
Shantou	ST	25	23° 16′	116° 84′
Shenzhen	SZ	40	22° 45′	113° 84′
Zhanjiang	ZJ	41	21° 04′	110° 58′
Haikou	HK	37	20° 12′	110° 34′
Wenchang	WC	51	19° 47′	110° 85′
Wanning	WN	35	18° 72′	110° 23′
Dongfang	DF	30	19° 26′	108° 30′
Danzhou	DZ	33	19° 81′	108° 87′

Table 2. Characterization of nine microsatellite markers used in this study.

Locus	Repeat motifs	Primer sequences (5' – 3')	T_m (℃)	GenBank accession no.	References
Scypa1	$(CTC)_4TTC(CTC)_2$	CCCTACCTACCATTACACCC TATTACAAAGGACAGCCAGACA	54	HM623189	Cui et al., (2010)
Scypa2	$(GCA)_{13}$	TCTGTAATCAGACCAAGGAGGT CAAAATAGCCATACTGGAAGC	53	HM623190	
Scypa3	$(AGT)_8$	GCGGTTCATTTGCTTCG GAGACTGGGTTGTCCTTA	53	HM623191	
Scypa4	$(TCC)_8N_{26}(CTG)_5$	CTCCTGCCATCCTCATT AGCGGCATCTTTGTC	58	HM623192	
Scypa5	$(TAG)_6TTG(TAG)_2$	ATAGTTGCTGGTTGATGAAG GGTCTGCGGCGAAT	54	HM623193	
Scypa8	$(CT)_{10}$	ACGAGACAGAGGGAGGC GGGTTCGAGATACAAGAT	63	HM623196	
Scypa11	$(CA)_{17}N_{110}(GTA)_5$	AACGCTACATCATACTGC CTGTTGCTATTTCTGCTT	50	HM623199	
Scypa13	$(AGG)_8N_{10}(AGG)_4N_3(AGG)_3$	CGTCTGTCCACCCTTAG CTTTCCCACAACCTCGTAT	61	HM623201	
Scpa03	$(TGTA)_2N_5(AT)_4$	CTGTAACACCCCAAAACAT GCCCAGGTACTCTCCACTC	52	GU182883	Ma et al., (2010)

418 **Table 3.** Summary statistics of nine microsatellite markers in 11 locations of *Scylla paramamosain*.

Locus	Sanmen (SM)	Ningde (ND)	Zhangzhou (ZZ)	Shantou (ST)	Shenzhen (SZ)	Zhanjiang (ZJ)	Haikou (HK)	Wenchang (WC)	Wanning (WN)	Dongfang (DF)	Danzhou (DZ)
Scypa1											
N_a/N_e	6.0/2.7	6.0/2.6	5.0/2.4	4.0/2.5	6.0/2.5	5.0/2.5	5.0/2.4	6.0/2.3	5.0/2.9	6.0/3.3	6.0/2.7
H_O/H_E	0.54/0.64	0.63/0.63	0.47/0.60	0.64/0.61	0.65/0.62	0.65/0.61	0.67/0.59	0.57/0.58	0.59/0.66	0.70/0.71	0.67/0.63
$P_{H\text{-}W}$	0.376	0.718	0.005**	0.652	0.157	0.310	0.046*	0.289	0.230	0.079	0.281
F_{IS}	0.157	0.001	0.217	-0.052	-0.050	-0.060	-0.126	0.017	0.102	0.011	-0.052
Scypa2											
N_a/N_e	7.0/3.4	5.0/3.5	5.0/2.8	4.0/2.2	6.0/3.9	8.0/3.2	8.0/2.9	8.0/3.4	5.0/3.4	5.0/3.8	8.0/3.4
H_O/H_E	0.47/0.72	0.71/0.73	0.56/0.66	0.72/0.57	0.68/0.75	0.54/0.70	0.74/0.67	0.68/0.71	0.79/0.72	0.92/0.75	0.76/0.72
$P_{H\text{-}W}$	0.000**	0.068	0.405	0.507	0.331	0.007**	0.861	0.462	0.905	0.628	0.324
F_{IS}	0.345	0.028	0.149	-0.278	0.093	0.233	-0.118	0.041	-0.106	-0.225	-0.059
Scypa3											
N_a/N_e	9.0/3.8	7.0/4.2	8.0/4.1	8.0/5.1	7.0/4.0	8.0/4.5	7.0/3.5	8.0/4.1	7.0/3.8	8.0/3.6	7.0/4.4
H_O/H_E	0.59/0.75	0.60/0.77	0.63/0.77	0.82/0.82	0.83/0.76	0.78/0.79	0.60/0.72	0.78/0.76	0.63/0.75	0.76/0.73	0.84/0.79
$P_{H\text{-}W}$	0.068	0.027*	0.129	0.025*	0.729	0.159	0.069	0.755	0.062	0.588	0.955
F_{IS}	0.209	0.227	0.191	0.008	-0.094	0.018	0.173	-0.028	0.171	-0.034	-0.074
Scypa4											
N_a/N_e	8.0/4.0	4.0/3.4	7.0/4.6	6.0/1.6	7.0/4.3	6.0/4.8	7.0/3.3	7.0/4.0	7.0/3.6	6.0/3.1	7.0/4.9
H_O/H_E	0.73/0.76	0.86/0.71	0.81/0.80	0.33/0.40	0.67/0.78	0.78/0.80	0.75/0.71	0.68/0.76	0.68/0.73	0.44/0.69	0.78/0.81
$P_{H\text{-}W}$	0.641	0.672	0.446	0.313	0.060	0.659	0.404	0.828	0.272	0.007**	0.827
F_{IS}	0.039	-0.205	-0.014	0.173	0.143	0.032	-0.062	0.104	0.075	0.357	0.034
Scypa5											
N_a/N_e	8.0/3.1	7.0/2.3	7.0/2.8	4.0/1.4	9.0/2.4	9.0/2.6	11.0/2.7	10.0/3.0	7.0/2.0	10.0/2.7	8.0/2.3
H_O/H_E	0.61/0.69	0.65/0.57	0.66/0.65	0.32/0.31	0.59/0.59	0.68/0.62	0.69/0.64	0.72/0.67	0.60/0.50	0.61/0.64	0.52/0.57
$P_{H\text{-}W}$	0.233	0.538	0.922	0.331	0.352	0.379	0.737	0.820	0.861	0.139	0.456
F_{IS}	0.121	-0.137	-0.015	-0.021	-0.006	-0.102	-0.084	-0.077	-0.188	0.057	0.099
Scypa8											
N_a/N_e	12.0/5.9	13.0/7.2	10.0/5.2	14.0/7.0	11.0/5.6	12.0/6.2	11.0/5.4	13.0/6.1	11.0/6.6	9.0/5.0	15.0/7.3

H_O/H_E	0.75/0.84	0.74/0.87	0.74/0.82	0.87/0.88	0.77/0.83	0.74/0.85	0.62/0.82	0.88/0.85	0.69/0.86	0.74/0.82	0.82/0.88
$P_{H\text{-}W}$	0.297	0.014*	0.001**	0.756	0.292	0.206	0.000**	0.993	0.014*	0.186	0.463
F_{IS}	0.111	0.154	0.098	0.008	0.075	0.127	0.249	-0.038	0.206	0.093	0.068
Scypa11											
N_a/N_e	11.0/8.3	11.0/8.4	12.0/7.8	10.0/6.3	11.0/8.1	11.0/8.7	11.0/8.5	11.0/5.9	11.0/8.8	10.0/6.7	11.0/7.0
H_O/H_E	0.89/0.89	0.97/0.89	0.97/0.89	1.00/0.86	0.85/0.89	0.83/0.90	0.97/0.89	0.86/0.84	1.00/0.90	0.93/0.86	0.94/0.87
$P_{H\text{-}W}$	0.406	0.644	0.736	0.887	0.215	0.544	0.184	0.808	0.847	0.628	0.142
F_{IS}	-0.004	-0.085	-0.096	-0.166	0.042	0.081	-0.088	-0.024	-0.113	-0.081	-0.078
Scypa13											
N_a/N_e	6.0/1.9	8.0/3.4	7.0/1.9	7.0/2.3	9.0/2.3	9.0/1.9	8.0/4.2	9.0/3.2	7.0/2.7	9.0/2.1	8.0/3.0
H_O/H_E	0.45/0.48	0.68/0.72	0.38/0.47	0.41/0.58	0.48/0.56	0.32/0.48	0.89/0.77	0.71/0.69	0.48/0.64	0.48/0.53	0.59/0.68
$P_{H\text{-}W}$	0.048*	0.160	0.098	0.023*	0.051	0.002**	0.471	0.317	0.000**	0.282	0.189
F_{IS}	0.074	0.062	0.213	0.299	0.158	0.339	-0.160	-0.022	0.250	0.099	0.123
Scpa03											
N_a/N_e	12.0/7.9	15.0/9.2	14.0/10.7	13.0/10.5	12.0/9.7	11.0/9.2	14.0/9.4	14.0/10.1	15.0/10.2	13.0/9.1	13.0/8.9
H_O/H_E	0.50/0.89	0.91/0.91	0.89/0.92	0.75/0.93	0.72/0.91	0.68/0.90	0.95/0.91	0.82/0.91	0.89/0.92	0.92/0.91	0.94/0.90
$P_{H\text{-}W}$	0.000**	0.386	0.598	0.061	0.003**	0.009**	0.978	0.004**	0.723	0.075	0.819
F_{IS}	0.440	-0.004	0.034	0.196	0.212	0.245	-0.045	0.104	0.028	-0.018	-0.040
Average											
N_a/N_e	8.8/4.6	8.8/4.9	8.3/4.7	7.8/4.3	8.7/4.7	8.8/4.9	9.1/4.7	9.6/4.7	8.3/4.9	8.4/4.4	9.2/4.9
H_O/H_E	0.62/0.74	0.75/0.76	0.68/0.73	0.65/0.66	0.69/0.74	0.67/0.74	0.77/0.75	0.74/0.75	0.71/0.74	0.72/0.74	0.76/0.76
F_{IS}	0.136	-0.039	0.052	-0.137	0.021	0.068	-0.048	-0.009	-0.030	-0.099	-0.020

419 N_a, observed number of alleles; N_e, effective number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; $P_{H\text{-}W}$, *P* values for Hardy-Weinberg equilibrium; *, Significant *P*

420 value <0.05; **, Significant *P* value <0.01

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Table 4. AMOVA design and results for 11 locations of *Scylla paramamosain*.

Source of variation	df	Sum of squares	Variance components	Percentage of variation	F_{ST}
Among locations	10	83.706	0.064 Va	1.83	0.0183
Among individuals within locations	386	1465.290	0.388 Vb	11.18	
Within individuals	397	1199.000	3.020 Vc	86.99	
Total	793	2747.996	3.472	100	

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437
438
439 **Table 5.** Pairwise F_{ST} (below diagonal) and genetic distance (above diagonal) among 11 locations of *Scylla paramamosain*.

Location	SM	ND	ZZ	ST	SZ	ZJ	HK	WC	WN	DF	DZ
SM		0.1908	0.1058	0.2036	0.1402	0.1109	0.1954	0.1590	0.1601	0.1420	0.1245
ND	0.047**		0.0743	0.0585	0.0514	0.0675	0.0628	0.0563	0.0373	0.0583	0.0491
ZZ	0.024**	0.018*		0.0972	0.0121	0.0356	0.0671	0.0304	0.0490	0.0407	0.0472
ST	0.067**	0.018*	0.040**		0.1006	0.1051	0.0715	0.0849	0.0453	0.0557	0.0632
SZ	0.038**	0.015*	0.004	0.033**		0.0336	0.0624	0.0364	0.0395	0.0380	0.0447
ZJ	0.026**	0.014*	0.004	0.038**	0.008		0.0687	0.0425	0.0494	0.0494	0.0295
HK	0.049**	0.013*	0.016*	0.029**	0.017*	0.015*		0.0354	0.0285	0.0473	0.0516
WC	0.040**	0.012*	0.004	0.035**	0.013*	0.008	0.005		0.0335	0.0515	0.0406
WN	0.041**	0.006	0.013*	0.006	0.010*	0.011*	0.006	0.010*		0.0289	0.0371
DF	0.040**	0.014*	0.016*	0.022**	0.015*	0.016*	0.013*	0.017*	0.007		0.0407
DZ	0.029**	0.009	0.008	0.027**	0.012*	0.002	0.008	0.006	0.008	0.014*	

440 *, Significant *P* value <0.05; **, Significant *P* value <0.01