

## RESEARCH ARTICLE

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

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### SUMMARY

The mud crab (*Scylla paramamosain*) is a carnivorous portunid crab, mainly distributed along the southeastern coast of China. Mitochondrial DNA analysis in a previous study indicated a high level of genetic diversity and a low level of genetic differentiation. In this study, population genetic diversity and differentiation of *S. paramamosain* were investigated using nine microsatellite markers. In total, 397 wild specimens from 11 locations on the southeastern coast of China were sampled and genotyped. A high level of genetic diversity was observed, with the number of alleles, and the observed and expected heterozygosity per location in the range 7.8–9.6, 0.62–0.77 and 0.66–0.76, respectively. AMOVA analysis indicated a low level of genetic differentiation among the 11 locations, despite the fact that a statistically significant fixation index ( $F_{ST}$ ) 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 for one between Sanmen and Shantou locations. No significant deficiency of heterozygotes (inbreeding coefficient  $F_{IS}=0.0007$ ,  $P>0.05$ ) was detected for all locations except Sanmen and Zhanjiang. Cluster analysis using UPGMA showed that all locations fell into one group except Sanmen. Significant association was found between genetic differentiation in terms of  $F_{ST}/(1-F_{ST})$  and the 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 proposal of high genetic diversity and low genetic differentiation in *S. paramamosain* along the southeastern coast of China.

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

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### INTRODUCTION

The mud crab (*Scylla paramamosain* Estampador 1949), mainly distributed along the southeastern coast of China, is a commercially important crab resource for fisheries and aquaculture. Records of *S. paramamosain* aquaculture 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 the gravid females generally migrate offshore to spawn (Perrine, 1979). Because of over-exploitation and environmental deterioration, numbers in the wild have decreased quickly. In order to conserve and sustainably harvest this important crab resource, genetic studies are necessary as they enable a better understanding of genetic diversity and structure (Dickerson et al., 2010), allow investigation of phylogenetic and evolutionary history (Gvozdík et al., 2010; Van Syoc et al., 2010), and also provide constructive guidance for resource conservation and management (Ortega-Villaizán Romo et al., 2006). A mitochondrial DNA (mtDNA) study of *S. paramamosain* indicated a genetically homogeneous population structure and a recent population expansion event (He et al., 2010). Moreover, again using mtDNA, a high level of genetic diversity and low genetic differentiation at different locations were observed in *S. paramamosain* inhabiting the southeastern coast of China (Lu et al., 2009; Ma et al., 2011a).

Microsatellites are nuclear molecular markers characterized by a 1–6 bp length repeat motif, high polymorphism and co-dominant 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 quantitative trait loci (Zhang et al., 2011). To date, microsatellite markers have been isolated in *S. paramamosain* (Takano et al., 2005; Ma et al., 2010; Ma et al., 2011c; Cui et al., 2011), but no information about population genetic diversity and differentiation has been reported for this important crab species.

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

### MATERIALS AND METHODS

#### Sample collection and DNA extraction

A total of 397 wild specimens of *S. paramamosain* were collected from the following locations along the southeastern coast 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; Table 1). Each specimen was killed by a lethal dose of MS-222. Genomic DNA was extracted from muscle tissue using traditional

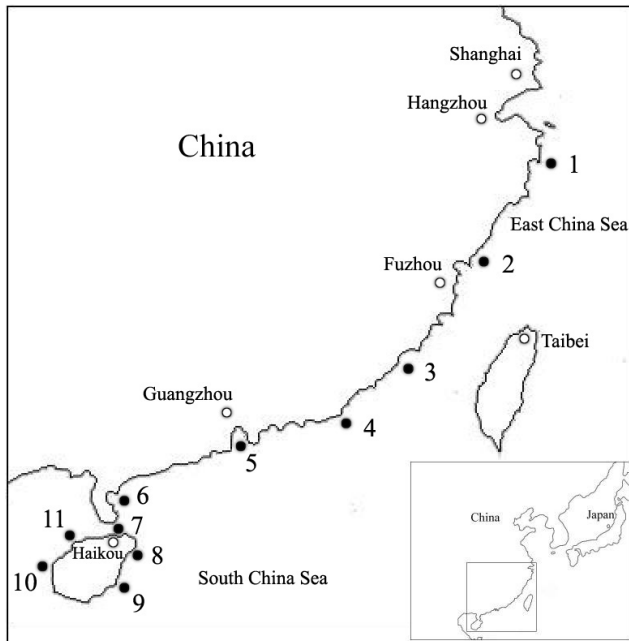


Fig. 1. Geographic map of southeastern coast of China. Filled circles, sampling location; 1, Sanmen (SM); 2, Ningde (ND); 3, Zhangzhou (ZZ); 4, Shantou (ST); 5, Shenzhen (SZ); 6, Zhanjiang (ZJ); 7, Haikou (HK); 8, Wenchang (WC); 9, Wanning (WN); 10, Dongfang (DF); and 11, Danzhou (DZ).

proteinase K and phenol–chloroform extraction protocols as described previously (Ma et al., 2009). The DNA was adjusted to  $100\text{ ng}\mu\text{l}^{-1}$  and stored at  $-20^\circ\text{C}$  until use.

#### Microsatellite genotyping

Nine polymorphic microsatellite loci were selected for genotyping, of which eight were developed using the 5' anchored PCR method (Cui et al., 2011), and the remaining one was developed using PCR-based isolation of microsatellite arrays (PIMA) (Ma et al., 2010) in our laboratory (Table 2). The criteria for selection were as follows: annealing temperature of  $50\text{--}63^\circ\text{C}$ , expected product size between 110 and 320 bp, observed heterozygosity value  $>0.5$  and no stuttering bands. PCR reactions were conducted in a total volume of  $25\mu\text{l}$  and included  $0.4\mu\text{mol l}^{-1}$  each primer,  $0.2\text{ mmol l}^{-1}$  each dNTP,  $1\times$  PCR reaction buffer,  $1.5\text{ mmol l}^{-1}$   $\text{MgCl}_2$ ,  $0.75\text{ U}$  *Taq* polymerase and approximately 100 ng template DNA, under the following conditions: one cycle of denaturation at  $94^\circ\text{C}$  for 4 min; 30 cycles

of 30 s at  $94^\circ\text{C}$ , 50 s at a primer-specific annealing temperature (Table 2), and 50 s at  $72^\circ\text{C}$ . As a final step, products were extended for 7 min at  $72^\circ\text{C}$ .

Several methods, including agarose gel electrophoresis, denaturing polyacrylamide gel electrophoresis and automated DNA sequencing, were employed for detecting differences in nucleotide sequence, of which the second 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: a high resolution (about 1 bp) and large output (100 samples each), low expense and easily mastered. In this study, the PCR products were separated on 6% denaturing polyacrylamide gels as described previously (Ma, 2009). The microsatellite fragments were visualized by silver staining, which was performed as follows. The gel was soaked in 1.01 staining solution ( $1.5\text{ g AgNO}_3$ ) for about 10 min; this solution was then removed and the gel was washed in  $\text{ddH}_2\text{O}$  for 5 s. Then the gel was soaked in 1.01 coloured solution ( $20\text{ g NaOH}$  and  $4\text{ ml}$  formaldehyde) for about 10 min. Finally, the gel was cleaned with  $\text{ddH}_2\text{O}$ . The size of alleles was 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 obtained 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 10,000 permutations for LD and by 1000 permutations for fixation index ( $F_{ST}$ ) 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 the 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.  $N_a$  ranged from six (Scypa1) to 16 (Scypa8 and Scypa03) per locus and from 7.8 (ST) to 9.6 (WC) per location.  $H_O$  and  $H_E$  ranged from 0.32 to 1.00 and from 0.31 to 0.93 per locus–location combination, and from 0.62 (SM) to 0.77 (HK) and from 0.66 (ST) to 0.76 (ND and DZ) per location, respectively.  $F_{IS}$  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 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 Scypa03 (in SM, SZ, ZJ and WC). Two loci (Scypa5 and Scypa11) were in keeping with HWE in all locations. Probability tests of genotypic LD for all pairs of loci within each location suggested significant non-random associations in only one of 396 possible pairwise comparisons after sequential Bonferroni correction

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

Location	Code	Sample size	Latitude (N)	Longitude (E)
Sanmen	SM	38	$29^\circ06''$	$122^\circ04''$
Ningde	ND	35	$26^\circ60''$	$120^\circ15''$
Zhangzhou	ZZ	32	$24^\circ27''$	$118^\circ17''$
Shantou	ST	25	$23^\circ16''$	$116^\circ84''$
Shenzhen	SZ	40	$22^\circ45''$	$113^\circ84''$
Zhanjiang	ZJ	41	$21^\circ04''$	$110^\circ58''$
Haikou	HK	37	$20^\circ12''$	$110^\circ34''$
Wenchang	WC	51	$19^\circ47''$	$110^\circ85''$
Wanning	WN	35	$18^\circ72''$	$110^\circ23''$
Dongfang	DF	30	$19^\circ26''$	$108^\circ30''$
Danzhou	DZ	33	$19^\circ81''$	$108^\circ87''$

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

Locus	Repeat motifs	Primer sequence (5'–3')	$T_a$ (°C)	GenBank accession no.	References
Scypa1	(CTC) <sub>4</sub> TTC(CTC) <sub>2</sub>	CCCTACCTACCATTACACCC TATTACAAAGGACAGCCAGACA	54	HM623189	Cui et al., 2011
Scypa2	(GCA) <sub>13</sub>	TCTGTAATCAGACCAAGGAGGT CAAAATAGCCATACTGGAAGC	53	HM623190	Cui et al., 2011
Scypa3	(AGT) <sub>8</sub>	GCGGTTTCATTTGCTTCG GAGACTGGGTTGTCCTTA	53	HM623191	Cui et al., 2011
Scypa4	(TCC) <sub>8</sub> N <sub>26</sub> (CTG) <sub>5</sub>	CTCCTGCCATCCTCATT AGCGGCATCTTTGTC	58	HM623192	Cui et al., 2011
Scypa5	(TAG) <sub>6</sub> TTG(TAG) <sub>2</sub>	ATAGTTGCTGGTTGATGAAG GGTCTGCGGCGAAT	54	HM623193	Cui et al., 2011
Scypa8	(CT) <sub>10</sub>	ACGAGACAGAGGGGAGGC GGGTTTCGAGATACAAGAT	63	HM623196	Cui et al., 2011
Scypa11	(CA) <sub>17</sub> N <sub>110</sub> (GTA) <sub>5</sub>	AACGCTACATCACTACTGC CTGTTGCTATTTCTGCTT	50	HM623199	Cui et al., 2011
Scypa13	(AGG) <sub>8</sub> N <sub>10</sub> (AGG) <sub>4</sub> N <sub>3</sub> (AGG) <sub>3</sub>	CGTCTGTCCACCCTTAG CTTTCCACAACTCGTAT	61	HM623201	Cui et al., 2011
Scpa03	(TGTA) <sub>2</sub> N <sub>5</sub> (AT) <sub>4</sub>	CTGTAACACCCCAAAACAT GCCCAGGTACTCTCCAATC	52	GU182883	Ma et al., 2010

$T_a$ , annealing temperature.

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

Locus	SM	ND	ZZ	ST	SZ	ZJ	HK	WC	WN	DF	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
$P_{H-W}$	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
$P_{H-W}$	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
$P_{H-W}$	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
$P_{H-W}$	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
$P_{H-W}$	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
$P_{H-W}$	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
$P_{H-W}$	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
$P_{H-W}$	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
$P_{H-W}$	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
Mean											
$N_a/N_e$	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

For location names, see Table 1.

$N_a$ , observed number of alleles;  $N_e$ , effective number of alleles;  $H_o$ , observed heterozygosity;  $H_e$ , expected heterozygosity;  $P_{H-W}$ ,  $P$ -values for Hardy–Weinberg equilibrium;  $F_{IS}$ , inbreeding coefficient.

\* $P < 0.05$ ; \*\* $P < 0.01$ .

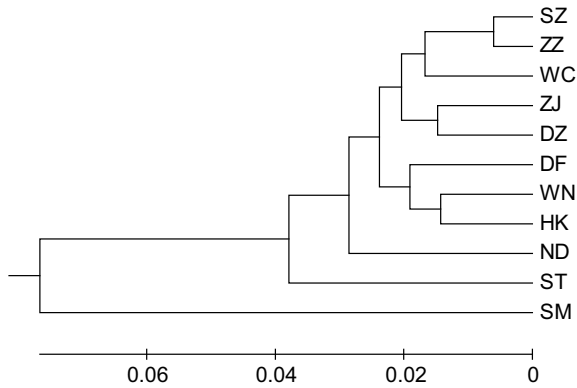


Fig. 2. The unweighted pair-group mean analysis (UPGMA) tree of 11 locations of *Scylla paramamosain*. SM, Sanmen; ND, Ningde; ZZ, Zhangzhou; ST, Shantou; SZ, Shenzhen; ZJ, Zhanjiang; HK, Haikou; WC, Wenchang; WN, Wanning; DF, Dongfang; and DZ, Danzhou.

(Scypa2 and Scypa13 in DF,  $P < 0.00139$ ) (Rice, 1989). When each location was analysed separately, there was no evidence of stuttering and large allelic dropout in any of the loci, as confirmed by MICRO-CHECKER version 2.2.3 software (Van Oosterhout et al., 2004).

The AMOVA showed that genetic variation existed mainly within locations, rather than among locations, as the percentage of variance was 98.17% within locations and 1.83% among locations. Although the overall  $F_{ST}$  value for all locations and loci was statistically significant ( $F_{ST} = 0.0183$ ,  $P < 0.05$ ), the genetic differentiation was still low, because the  $F_{ST}$  value was much lower than 0.05 (Tables 4, 5). Multi-locus 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 were lower than 0.1 in 43 out of the 55 pairwise locations. Of the 11 locations, SM was the most distinctive, as it showed significant differentiation in relation to all the other 10 locations ( $F_{ST}$  values ranged from 0.024 to 0.067). In contrast, DZ was the most representative as it significantly differed from only four locations ( $F_{ST}$  values ranged from 0.012 to 0.029).

Cluster analysis of 11 locations using the 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 there was no significant correlation between pairwise  $F_{ST}$  and geographic distance (km) ( $r^2 = 0.1230$ ,  $P = 0.06$ ) (Fig. 3)

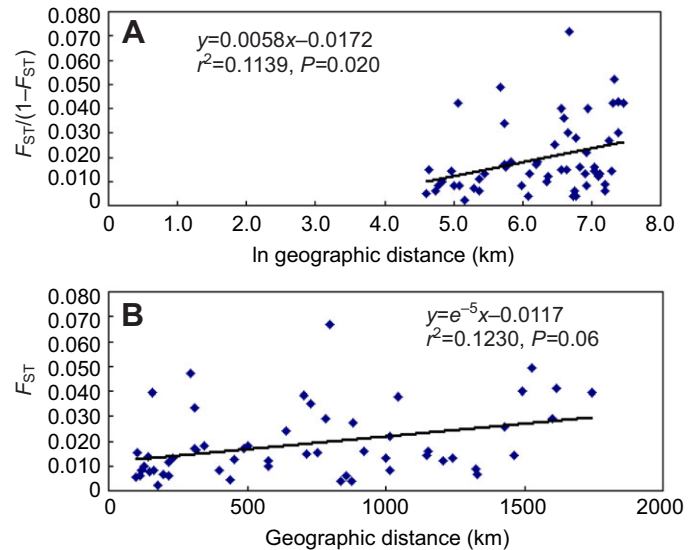


Fig. 3. Relationship between genetic differentiation and geographic distance among the 11 locations. (A) Relationship between pairwise  $F_{ST}/(1-F_{ST})$  (where  $F_{ST}$  is the fixation index) and the natural logarithm of geographic distance. (B) Relationship between pairwise  $F_{ST}$  and geographic distance.

## DISCUSSION

The results of this study suggest a high level of population genetic diversity of *S. paramamosain* along the southeastern coast of China ( $N_a$ ,  $H_o$  and  $H_e$  in the range 7.8–9.6, 0.62–0.77 and 0.66–0.76 per location, respectively), in accordance with previous studies that showed a high level of mtDNA genetic diversity in *S. paramamosain* (Lu et al., 2009; Ma et al., 2011a). High population genetic diversity has also 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 size 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), which may be due to the different environments. A similar finding was observed in a previous study, which indicated a trend for a reduction in 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 a low level of genetic differentiation among different geographic populations because of the high dispersal capabilities, large population sizes and relatively small barriers in the marine environment (Beheregaray and Sunnucks, 2001). For the fish *Nibea albiflora*, there was little

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

Source of variation	d.f.	Sum of squares	Variance components	% variation	$F_{ST}$
Among locations	10	83.706	0.064	1.83	0.0183
Among individuals within locations	386	1465.290	0.388	11.18	
Within individuals	397	1199.000	3.020	86.99	
Total	793	2747.996	3.472	100	

$F_{ST}$ , fixation index; AMOVA, analysis of molecular variance.



Table 5. Pairwise  $F_{ST}$  (below diagonal) and genetic distance (above diagonal) among the 11 locations of *S. 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*	

\* $P < 0.05$ ; \*\* $P < 0.01$ .

difference in the population genetic structure between the Yellow Sea and East China Sea observed using mtDNA (Han et al., 2008). For the shrimp *Fenneropenaeus chinensis*, no significant population genetic differentiation between the Yellow Sea and Bohai Sea was found using both microsatellite DNA and mtDNA (Liu et al., 2006; 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 the East China Sea and South China Sea using mtDNA (He et al., 2010). In the current study, statistically significant genetic differentiation was detected across 11 locations along the 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* using mtDNA (Lu et al., 2009; He et al., 2010; Ma et al., 2011a). The above information indicates that all locations of *S. paramamosain* should be a single genetically homogeneous population. The low  $F_{ST}$  value indicates a relatively high gene flow among locations. There are three probable explanations for this: (1) the unique reproductive habit in which adults and juveniles migrate between ocean basins and adjacent continental margins; (2) the high dispersal capabilities of larvae; and (3) the relatively small physical barriers in the marine environment.

Among these 11 locations, SM was the most genetically distinctive in two main ways: (1) it has the lowest genetic diversity (the overall  $H_O$  was 0.62) and the highest  $F_{ST}$  values (between 0.024 and 0.067) compared with other locations; and (2) it has the greatest overall  $F_{IS}$  value ( $F_{IS}=0.136$ ,  $P < 0.05$ ) compared with other locations. These findings indicate that the gene exchange is relatively low between SM and other locations compared with that between other location pairs. The optimum temperature range of this crab is 18–27°C for growth, and a higher temperature is needed for spawning. However, SM is the most northern of 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*. Over-fishing by humans may be another potential explanation. A significant positive correlation between genetic differentiation and geographic distance was found, suggesting an isolation by distance model of genetic variation.

### CONCLUSIONS

In conclusion, a high level of population genetic diversity and low differentiation were found in the mud crab (*S. paramamosain*) from 11 locations along the southeastern coastal regions of China by

microsatellite analysis, which showed a genetically homogeneous population structure for *S. paramamosain* in these 11 locations. In the future, more population genetic studies should be carried out in this crab species. The findings in this study will provide valuable information for conservation, harvesting and artificial selective breeding of this important fishery resource.

### LIST OF ABBREVIATIONS

AMOVA	analysis of molecular variance
$F_{IS}$	inbreeding coefficient
$F_{ST}$	fixation index
$H_E$	expected heterozygosity
$H_O$	observed heterozygosity
HWE	Hardy–Weinberg equilibrium
LD	linkage disequilibrium
$N_a$	observed number of alleles
$N_e$	expected number of alleles

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