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Population Structure and Historical Demography of the Whiskered Velvet Shrimp (*Metapenaeopsis barbata*) off China and Taiwan Inferred from the Mitochondrial Control Region

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Ta-Jen Chu, Daryi Wang, Hsien-Lu Huang, Feng-Jiau Lin, and Tzong-Der Tzeng (2012) Population structure and historical demography of the whiskered velvet shrimp (*Metapenaeopsis barbata*) off China and Taiwan inferred from the mitochondrial control region. *Zoological Studies* **51**(1): 99-107. Sequence analyses of a 344-base-pair segment of the mitochondrial control region were conducted to elucidate the population structure and historical demography of the whiskered velvet shrimp (*Metapenaeopsis barbata*) off China and Taiwan. Six populations including 187 individuals were separately collected from the northern East China Sea (ECS), waters off Kagoshima (KS, Japan), Taichung (TC, west-central Taiwan), Cheding (CD, southwestern Taiwan), Xiamen (XM, southern China) and Hong Kong (HK). The haplotype diversity (*h*) was high for all populations (96.95%), with values ranging 89.1% (CD) to 98.9% (KS). Nucleotide diversity (π) of all populations was 1.524%, with values ranging 0.714% (TC) to 1.554% (ECS). All F_{ST} values among the 6 populations were significant except for the ones from the pairs TC-CD, XM-HK, KS-XM, and KS-HK. The haplotype network was divided into 2 clades: clade I included individuals from all populations but YZR, and clade II did not include specimens from CD or TC. Neutrality tests and mismatch distribution analyses both suggested that this species had experienced a population expansion. Three distinct groups were yielded by the AMOVA tests, pair-wise F_{ST} analyses, and the UPGMA tree of the 6 sampled areas. The 1st group included the ECS, the 2nd contained TC and CD, and the 3rd included the KS, XM, and HK populations. Based on the continuity of the geographic position and gene flow, the 2nd and 3rd groups should be considered a single population.
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Key words: *Metapenaeopsis barbata*, Mitochondrial DNA, Population structure, Population expansion.

Understanding the population genetic structure is an important component of successful and sustainable long-term management of fishery resources (Hillis et al. 1996), and recent advances in population genetic (Excoffier et al. 1992) and historical demographic analyses (Rogers and Harpending 1992, Fu 1997) can be very helpful in providing valuable and complementary information to catch- and age-composition data (Pauly et al. 2002).

Analyses of population genetic structures of marine biota have frequently revealed that organisms with a high dispersal capacity have little genetic distinction over large geographic scales (Hellberg 1996). Such studies suggest that there are high levels of gene flow among

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marine populations. However, there is growing evidence that widespread marine organisms are more genetically structured than expected given their high dispersal potential and apparent lack of barriers to dispersal in the ocean (Palumbi 1997, Benzie 1999, Briggs 1999). Thus, there may be limits to the actual dispersal of marine organisms with high dispersal potential (Benzie and Williams 1997). Those limits widely vary with species, habitats, local ocean conditions, and historical events, and they may produce sufficient chances for genetic distinction (Palumbi 1994).

The whiskered velvet shrimp (*Metapenaeopsis barbata*) is a small penaeid species with a hard integument. It can be found at 20-70 m in depth on rocky, sandy, and muddy bottoms (Holthuis 1980, Yu and Chan 1986). This shrimp is distributed throughout the Indo-Pacific region: Japan, the Korean Peninsula, Hong Kong, Taiwan, Thailand, the Malay Archipelago (including Indonesia), the southwestern coast of India, and the Red Sea (Holthuis 1980, Miquel 1984). The whiskered velvet shrimp also plays an important role in capture fisheries of China, Taiwan, and other Asian countries (Wu 1984 1985, Holthuis 1980). The life history of the whiskered velvet shrimp, with an offshore planktonic larval phase, estuarine post-larval and juvenile phases, and offshore adult and spawning phases (Dall et al. 1990), may allow moderate gene flow among populations.

Various approaches have been adopted to examine population structures of marine organisms, including studies of the distribution and abundance of various life-history stages, marking and tagging, morphological characters, allozymes, and DNA markers. Because each technique has its merits and disadvantages, integrating the results of several methods in a multi-pronged approach to stock identification may maximize the likelihood of correctly defining stocks (Pawson and Jenning 1996). Several studies on the fishery biology of the whiskered velvet shrimp in the East China Sea (ECS) and Taiwan Strait (TS) were conducted (Wu 1984, Tzeng and Yeh 1995, Tzeng et al. 2005), but only 1 paper analyzed morphological characters in an attempt to determine the stock structure of this species (Tzeng et al. 2001). Two morphologically distinguishable populations of whiskered velvet shrimp in the ECS and TS were discriminated. However, variations in morphological characters can be affected by both genetic and environmental factors, so that discrimination of populations based on morphological variations must be verified by genetic evidence to confirm that the variations reflect the true degree of reproductive isolation rather than environmental isolation (Pepin and Carr 1992).

Mitochondrial (mt)DNA has many attributes that make it particularly suitable for population genetic studies, including its rapid rate of evolution, a lack of recombination, and its maternal inheritance (Hoelzel et al. 1991). Since the control region of mtDNA was shown to be the most variable region in both vertebrates and invertebrates, this region is an ideal marker for characterizing geographical patterns of genetic variations within and among prawn populations (Simon 1991). In this paper, sequence analyses of a segment of the mitochondrial control region were conducted to elucidate the population genetic structure and historical demography of the whiskered velvet shrimp in adjacent waters of China and Taiwan.

MATERIALS AND METHODS

Sampling

Six whiskered velvet shrimp populations including 187 specimens were collected from commercial shrimp trawlers (Fig. 1, Table 1). They were separately sampled from the northern ECS, waters off Kagoshima (KS, Japan), Taichung (TC, west-central Taiwan), Cheding (CD, southwestern Taiwan), Xiamen (XM, southern China), and Hong Kong (HK). Specimens were immediately iced or frozen after capture and later kept at -75°C before DNA extraction.

DNA extraction, amplification, and sequencing

Total DNA was extracted from frozen muscle tissues using a standard DNA proteinase K digestion/phenol-chloroform extraction procedure (Kocher et al. 1989). The complete control region was amplified using the primers P30 (5'-GATC TTTAGGGGAATGGTGTAATTCCATTG-3') and P24 (5'-GTGTAACAGGGTATCTAATCCTGG-3'), which respectively bind to the $tRNA^{Met}$ and 12S rRNA genes. Thermal cycling was performed in a GeneAmp 2400 thermal cycler (Perkin-Elmer, Norwalk, CT, USA), and polymerase chain reaction (PCR) conditions consisted of initial denaturation at 95°C for 5 min; then 39 cycles of denaturation at 95°C for 50 s, annealing at 50°C for 1 min, and extension at 72°C for 1.5 min; followed by

a final extension at 72°C for 10 min. Amplified DNA was separated through electrophoresis on 1.5% agarose gels and purified with the Gene Clean II kit (Bio101, La Vista, CA, USA). Double-stranded DNA was sequenced on an ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA) with the P24 primer.

Sequence analyses

DNA sequences were aligned with ClustalX, vers. 1.83 (Thompson et al. 1997), then subsequently optimized by eye in BIOEDIT, vers. 7.0.5.3 (Hall 1999). Control region sequences were confirmed by comparing them with the complete

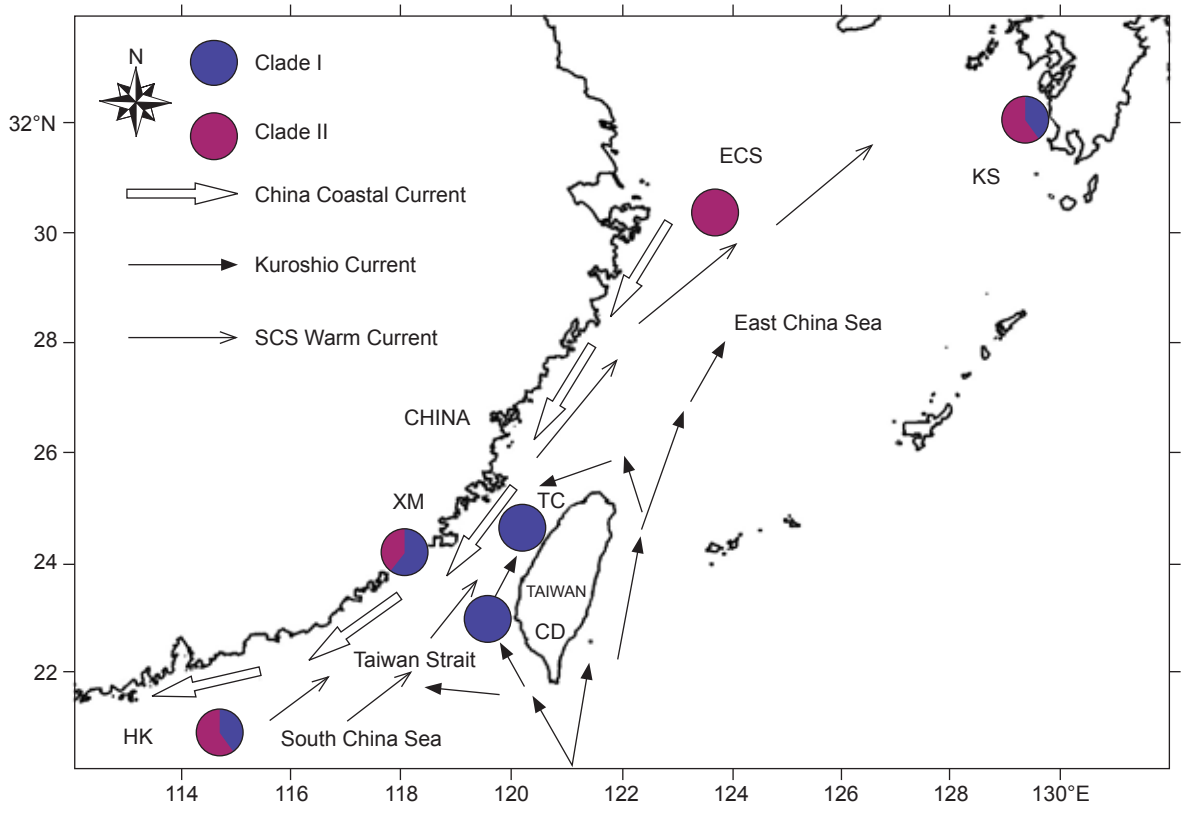

Fig. 1. Sampling localities and haplotype frequencies of whiskered velvet shrimp off China and Taiwan. Numbers of clades I and II in each sampling site are shown in table 1.

Table 1. Codes of sampling sites, sample size (n), number of haplotypes (n_h), number of clades, gene diversity (h), and nucleotide diversity (π) with the 95% confidence interval (CI), Tajima's D, and Fu's Fs statistics in 6 whiskered velvet shrimp populations

Code	Sampling site	n	n_h	No. of clade I	No. of clade II	h (%)	π (%)	Tajima's D	Fu's Fs
ECS	East China Sea	30	25	0	30	98.2 ± 1.600	1.554 ± 0.138	-1.14700^{ns}	-18.99674**
XM	Waters off Xiamen	39	24	22	17	90.4 ± 4.200	1.170 ± 0.104	-0.17860^{ns}	-15.46402**
TC	Waters off Taichung	34	19	34	0	93.2 ± 2.600	0.714 ± 0.099	-1.69561*	-14.24433**
CD	Waters off Cheding	45	23	45	0	89.1 ± 3.800	0.727 ± 0.109	-2.04709**	-18.54332**
HK	Waters off Hongkong	25	18	10	15	97.0 ± 2.500	1.484 ± 0.108	0.05348^{ns}	- 8.61357**
KS	Waters off Kagoshima	14	13	6	8	98.9 ± 3.100	1.348 ± 0.114	-0.06866^{ns}	- 8.18931**
Clade I		115	50			92.8 ± 1.500	0.746 ± 0.061	-2.11381**	-26.79920**
Clade II		72	52			98.2 ± 0.700	1.362 ± 0.090	-1.49631*	-25.65157**
Total		187	101			96.95 ± 0.65	1.524 ± 0.068	-1.70788*	-25.14003**

* $p < 0.05$; ** $p < 0.01$, ^{ns}; not significant ($p > 0.05$).

published mtDNA sequence of *Penaeus monodon* (Wilson et al. 2000). Subsequent analyses were based on a segment of the control region sequence obtained from 187 individuals. The nucleotide composition and numbers of variable sites were assessed with MEGA3 (Kumar et al. 2004). The genetic diversity (*h*), nucleotide diversity (π), number of polymorphic sites (S), and average number of nucleotide differences (K) (Nei 1987) in each sample were calculated using DnaSP vers. 4.10 (Rozas et al. 2003).

To examine whether any 2 populations genetically differed from each other, pair-wise F_{ST} statistics (Wright 1965) among the 6 populations were estimated and tested using the program, ProSeq (Filatov 2002). The statistical significance of the estimate was tested using 1000 permutations. A dendrogram of the 6 sampling areas was constructed using the unweighted pair-group method with arithmetic means (UPGMA) based on F_{ST} values using MEGA3.

An analysis of molecular variance (AMOVA) implemented in ARLEQUIN vers. 3.01 (Excoffier et al. 2005) was performed to test geographic divisions among samples. Various groupings of samples were suggested by (1) the UPGMA tree of sampling areas, (2) F_{ST} values between samples, and (3) the geographic distribution. The significance of these Φ statistics was evaluated by 104 random permutations of sequences among them.

A network of haplotypes was constructed using the median-joining method (Bandelt et al. 1999) in Network vers. 4.2.0.1 available at www.fluxus-engineering.com. To check for deviations from neutrality, Tajima's *D* (Tajima 1989) and Fu's *Fs* statistical tests (Fu 1997) were run to assess evidence for population expansion using ARLEQUIN. Meanwhile, concordance of the data with the distribution underlying the expansion model was assessed. Historical demographic expansion was investigated by examining the frequency distributions of pair-wise differences between sequences (mismatched distribution) with ARLEQUIN. Rough dates of the population expansion were estimated using the formula $\tau = 2\mu T$ (Rogers and Harpending 1992), where *T* is the time since expansion, τ is the expansion time, and 2μ is μ (the mutation rate) × generation time × number of bases sequenced.

RESULTS

Amplification of whiskered velvet shrimp genomic DNA with the P30 and P24 primers produced a PCR product of approximately 1200 nucleotides base pairs long. We were able to obtain a 344-bp segment of the control region for each specimen. The nucleotide composition of the fragment was AT-rich (A, 40.57%; G, 6.91%; C, 5.21%; T, 47.31%), as is usual for this region in many invertebrate species. In total, 69 variable sites, including 28 singletons and 41 parsimoniously informative sites, were observed. The average number of nucleotide differences (K) of all populations was 5.243, with values ranging 2.455 (TC) to 5.347 (ECS). The haplotype diversity (*h*) was high for all populations (96.95%), with values ranging 89.1% (CD) to 98.2% (ECS). Nucleotide diversity (π) for all populations was 1.524%, with values ranging 0.714% (TC) to 1.554% (ECS) (Table 1).

All F_{ST} values among pairs of populations were significant except the 4 pairs of TC-CD, XM-HK, KS-XM, and KS-HK (Table 2). The UPGMA tree of these 6 sampled areas could be divided into 3 groups: 1 included the ECS, another comprised HK, XM, and KS, and the last contained TC and CD (Fig. 2).

Various groupings of samples were tested using AMOVA, but only 2 significant groupings

Table 2. F_{ST} values among 6 whiskered velvet shrimp populations. Abbreviations for the sampling locations are defined in table 1

	ECS	XM	TC	CD	HK
XM	0.2685**				
TC	0.5444**	0.3211**			
CD	0.5540**	0.3324**	0.0063^{ns}		
HK	0.1786**	0.0419^{ns}	0.3018**	0.3189**	
KS	0.1504**	-0.0113^{ns}	0.3638**	0.3772**	-0.0072^{ns}

* $p < 0.05$; ** $p < 0.01$, ^{ns}; not significant ($p > 0.05$).

were obtained (Table 3). In the 1st grouping, the AMOVA for the 6 populations included in a single group yielded a significant Φ_{ST} value of 0.3531, indicating that at least one of the pair-wise comparisons showed significant heterogeneity. None of the values of Φ_{CT} in the 2-group pattern was significant. One of Φ_{CT} values in the 3-group pattern was significant (Φ_{CT} = 0.3739, p = 0.0163). The 1st one included the ECS, the 2nd comprised TC and CD, and the 3rd contained the XM, HK, and KS populations.

Among the 187 individuals studied, 101 haplotypes were defined. The median-joining network of these haplotypes revealed 2 clades (Fig. 3). These 2 clades appeared to have a geographic structure. Specimens from TC and CD were not found in clade II, whereas individuals from the ECS did not appear in clade I. Individuals from HK, XM, and KS were found in each clade. Haplotype diversities (h) for clades I and II were 92.8% and 98.2%, respectively. Nucleotide diversities (π) for clades I and II were 0.746% and 1.362%, respectively (Table 1).

Tajima's D and Fu's Fs statistical tests were performed to determine departure from neutrality. Significant Tajima's D values were obtained for TC and CD, but not for the other populations. Fu's Fs tests were significant for all sampling locations (Table 1). The model of population expansion could not be rejected using Tajima's D and Fu's Fs statistical tests when all populations were combined. The mismatch distribution including both clades was bimodal (Fig. 4), with 1 mode corresponding to the number of differences within the clades, and the other to differences between the 2 clades. Separate analyses of clades I and II in both cases yielded a unimodal distribution, which did not significantly differ (as measured by the sum of the squared deviation; $p > 0.05$) from that predicted by the growth expansion model (Fig. 4). Both Tajima's D and Fu's Fs statistical tests of the 2 clades were negative and highly significant, which indicated population demographic expansion (Table 1).

τ values of clades I and II were 2.346/2u (95% confidence interval (CI), 1.814-2.930) and 4.338 (95% CI, 3.512-5.115) generations. Because of the shrimp's short lifespan, a generation time of 1 yr was used (Tzeng and Yeh 1995). McMillen-Jackon and Bert (2003) roughly estimated a

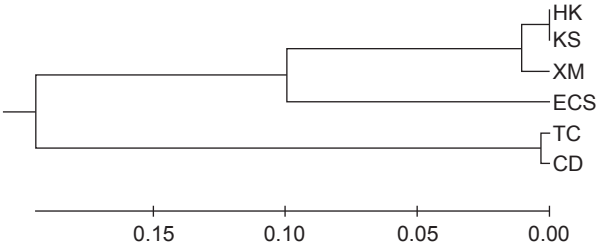

Fig. 2. UPGMA tree showing relationships among the 6 sampled areas.

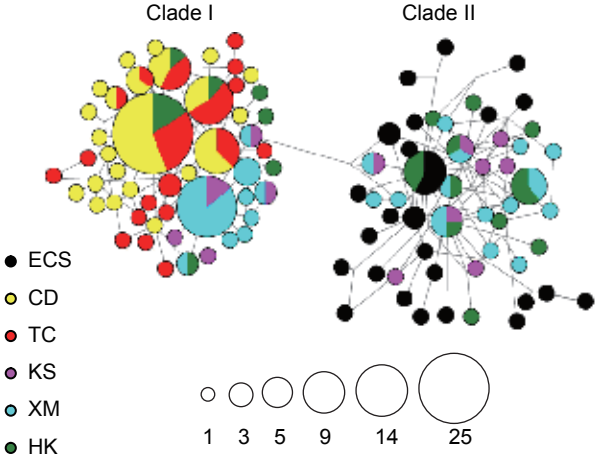

Fig. 3. Network of haplotypes for whiskered velvet shrimp from 6 populations. The sizes of the circles are proportional to the haplotype frequency.

Table 3. Results of the AMOVA. Abbreviations for sampling locations are given in table 1

Grouping	Source of variation	*d.f.*	Variance component	Φ-statistics	*p*
One group for all locations					
Group 1 {ECS, XM, CD, TC, HK, KS}	Among populations	5	1.2093	Φ_{ST} = 0.3531	0.0000
	Within populations	181	2.2154		
Three groups					
Group 1 {ECS}	Among groups	2	1.1422	Φ_{CT} = 0.3739	0.0163
Group 2 {TC, CD}	Among populations within groups	3	0.0289	Φ_{SC} = 0.0151	0.1178
Group 3 {XM, HK, KS}	Within populations	181	1.8838	Φ_{ST} = 0.3833	0.0000

mutation rate of 19%/10^6 yr (MY) for the mtDNA control region of brown shrimp *Farfantepenaeus aztecus* and white shrimp *Litopenaeus setiferus*. Using this rate, the estimated time of expansion for clade I was 17,946 (95% CI, 13,877-22,414) yr ago, and for clade II, was 33,185 (95% CI, 26,867-39,129) yr ago.

DISCUSSION

According to the results of F_{ST} tests, AMOVA tests, and the UPGMA tree of these 6 populations, 3 distinct groups appeared to exist in the studied waters. The 1st group was in the northern ECS, the 2nd was in the eastern Taiwan Strait (CD and TC), and the 3rd was in HK, XM (western TS), and KS. Two environmental factors prevailing in the studied waters here may have resulted in genetic differences between the 1st group (ECS) and the other 2 groups. First, the major source of nutrients in the northern ECS is the relatively large freshwater input from the Yangtze River (Tzeng et al. 2004). Tsang et al. (2008) indicated that the lack of suitable substratum for settlement restricted the northern distribution limit of *Tetraclita japonica* to the mouth of the Yangtze River, and this habitat discontinuity reduced the effective gene flow, resulting in genetic differentiation between different populations. Second, the mean temperature of the sea in the northern ECS is lower than those in the TS and South China Sea (SCS) (Tzeng et al. 2004). The whiskered velvet shrimp population from the northern ECS genetically differed from that from the eastern TS, and this result was in agreement with a previous outcome that 2 morphologically distinguishable stocks separately exist in the TS and ECS (Tzeng et al. 2001). Similar results were also found for kuruma shrimp (*Penaeus japonicus*) (Tzeng et al. 2004) and sword prawn (*Parapenaeopsis hardwickii*) populations (Tzeng 2007, Tzeng et al. 2008).

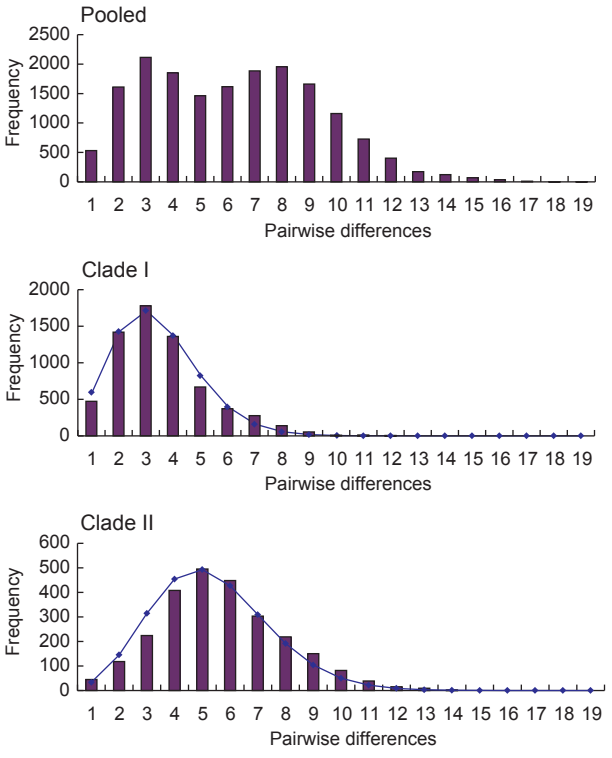

Fig. 4. Observed pair-wise differences (bars) and the expected mismatch distributions under a sudden expansion model (solid line) of mitochondrial control-region haplotypes in *M. barbata* off China and Taiwan.

During summer, the SCS Warm Current (Fig. 1) moves northwards from the SCS to the ECS (Niino and Emery 1961) and the southwesterly monsoon drives water masses from Singapore and Vietnam into the TS (Morton and Blackmore 2001). The main branch of the warm Kuroshio Current flows northward along the east coasts of the Philippines and Taiwan (Chu 1972). In winter, a small branch can be deflected by the northeasterly monsoon to flow through the eastern TS (Farris and Wimbush 1996), but this up-flowing branch is prevented from flowing further north at the Penghu Is. in the TS by deeper waters beyond Penghu (Jan et al. 2002). Multiple oceanic currents meeting in the eastern TS may thus explain significant genetic differences between populations from the eastern TS and the other groups. A similar result was also found for kuruma shrimp (*Pen. japonicus*) populations (Tsoi et al. 2007).

The whiskered velvet shrimp migrates from inshore to offshore as it grows to a specific size or life stage, but migratory distances are limited (Dall et al. 1990). Thus, the dispersal of larvae is the primary source of gene flow, and ocean currents play a major role in dispersing this species. The spawning season of this shrimp lasts from June to Oct. (Sakaji et al. 1992). Along the east coast of China, larvae from the ECS may be transported and pass through the western TS and enter the SCS with the China Coastal Current (Wu 1982). This mixing of larvae results in homogeneity among the ECS and HK/XM populations, but might not be large enough to eliminate all genetic differences between them (Table 2). This also explains why the same haplotypes were found in HK, XM, and the ECS, why individuals from HK and XM were included in clade II (Fig. 3),

and why there were larger nucleotide diversities in XM and HK (1.484% in HK and 1.17% in XM) than in the eastern TS (about 0.72%) populations. Moreover, recruitment of larvae from the ECS into the western TS and SCS might partly explain genetic differences among the HK, XM, and CD/TC populations. However, larvae from the ECS could not be transported into the eastern TS, and that resulted in individuals from CD and TC not being included in clade II (Fig. 3), and the nucleotide diversity in the eastern TS population being relatively low (Table 1).

During the late spring and summer, warm water from the SCS dominates the TS and flows into the ECS (Wang and Chern 1989). Larvae from the SCS and TS may be transported into adjacent waters of KS but not into the western ECS. Thus, specimens in KS were found in 2 different clades (Fig. 3), and there was relatively larger nucleotide diversity at KS. This also explains why there was a larger geographical distance between KS and HK/XM than between KS and CD/TC, but no genetic differences between KS and HK/XM were found (Fig. 1, Table 2). Although the SCS, TS, and ECS are adjacent waters and high gene flow might occur among them, the population from the eastern TS still differed from those from different sampling locations. We considered that the genetic variation in the eastern TS population resulted from adaptation to the severe and complicated environments as described above. Based on this continuity of geographic position, gene flow, and complex oceanic currents meeting in the eastern TS (TC and CD), the 2nd and 3rd groups should be considered a single population.

The neutrality of control-region mutations was rejected on the basis of Tajima's *D* and Fu's *F* tests for the total population (Table 1). These 2 statistics are sensitive to factors such as bottlenecks and population expansions which tend to drive Tajima's *D* and Fu's *F* towards more-negative values (Tajima 1996, Martel et al. 2004). Indeed, significant negative values of these 2 indices in this study indicated that whiskered velvet shrimp off China and Taiwan had experienced a population expansion. All haplotypes were divided into 2 different clades (clades I and II) in this study. Values of Tajima's *D* and Fu's *F* of the 2 distinct clades were also significant (Table 1). Theoretical studies demonstrated that populations in long-term stable demographic equilibrium show a chaotic mismatch distribution, while recent rapid population expansions or bottlenecks translate into a unimodal (approximately Poisson) profile, with a steeper wave indicative of a smaller initial population before the expansion (Rogers and Harpending 1992, Rogers 1995). The unimodal mismatch frequency distribution pattern of each clade accorded well with the predicted distribution under a model of population expansion (Fig. 4). An analysis of the demographic history of this shrimp from the 2 distinct clades (clades I and II) seems to indicate that clade I displayed a steeper wave, which is typical of a smaller initial population prior to the expansion or bottleneck (Fig. 4) (Rogers and Harpending 1992). This picture also suggests that clade I could have experienced expansion in the more-recent past than clade II, the pair-wise distribution mode of which was more-clearly displaced to the right of the distribution pattern (Fig. 4). This was also supported by the 2 estimates of time of expansion for clades I (13,877-22,414 yr ago) and II (26,867-39,129 yr ago). Similar results were also obtained from other marine organisms in the ECS, TS, and SCS experiencing population expansions, like *Salanx ariakensis* (Hua et al. 2009) and *Chelon haematocheilus* (Liu et al. 2007).

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