

ORIGINAL ARTICLE

# Phylogeography of the mud crab (*Scylla serrata*) in the Indo-West Pacific reappraised from mitochondrial molecular and oceanographic clues: transoceanic dispersal and coastal sequential colonization

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## Keywords

Coalescent simulation; colonization history; cytochrome oxidase I; Indo-West Pacific; paleo-oceanic circulations; *Scylla serrata*.

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## Abstract

The widespread mud crab, *Scylla serrata*, of the Indo-West Pacific is an excellent model species to demonstrate how the colonization history of a species can be influenced by complex oceanographic conditions. Through the combination of ecological data (fossil records and paleo-oceanographic conditions) and molecular data (coalescent simulations, network analysis, and nucleotide diversity tests), the phylogeographic history of *S. serrata* was re-analyzed. Based on the analysis of mtDNA cytochrome oxidase I sequences, two major clades were identified for *S. serrata*, including a widespread clade (Clade I) with three disjunct geographic clusters (IA, IB and IC) and an endemic Northwest Australian clade (Clade II). Moreover, a significant phylogeographic structure corresponding to four subpopulations was revealed: Northwest Australia, West Indian Ocean, Red Sea-South China Sea and West Pacific. A colonization history of a Northwest Australia origin for *S. serrata* followed by westward transmarine dispersal across the Indian Ocean for Clade I and sequential colonization from the West Indian Ocean to Red Sea-South China Sea and West Pacific was corroborated. The Pleistocene fluctuations of paleo-oceanographic conditions including surface circulations and physical topography in the Indo-West Pacific might be responsible for the wide distribution, colonization history and genetic divergence of this species.

## Introduction

Estuarine mud crabs (genus *Scylla*) are an economically important group of crabs that are widely distributed throughout the Indo-West Pacific (IWP). The genus contains at least four distinct non-hybridizing species, *Scylla olivacea*, *Scylla serrata*, *Scylla tranquebarica* and *Scylla paramamosain* (Keenan *et al.* 1998). Of the four species, *S. serrata* is the most widespread, occurring throughout the IWP in the coastal areas of East Africa, India, the Indo-Malaysia archipelago, Australia, and

other various islands of the West Pacific. Based on the distribution of cytochrome oxidase subunit I gene (COI) haplotypes throughout the species range, two major clades of *S. serrata* with unique phylogeographic patterns have been identified (Gopurenko *et al.* 1999; Fratini & Vannini 2002; Gopurenko & Hughes 2002). One clade (Clade I) is distributed widely across the entire IWP, whereas the other clade (Clade II) is confined to Western and Northern Australia.

Gopurenko *et al.* (1999) proposed two hypotheses explaining the unique phylogeographic patterns of these

two clades. In their first hypothesis, Clade II was suggested to be the remnant of a formerly widespread population in the Indian Ocean that became isolated from the Pacific population of Clade I, and the present wide distribution of Clade I resulted from a subsequent rapid radiation of *S. serrata* from the West Pacific back into the Indian Ocean. This scenario is based on a hypothesis of vicariance where the Indian Ocean and Pacific Ocean biogeographic provinces are isolated by the Indonesian Archipelago. Although this center-of-overlap of several biogeographic provinces (Pacific Ocean, Indian Ocean and Indonesian Seas) in the Indo-Malayan region has been suggested to explain high biodiversity in the East Indies Triangle and intraspecific phylogenetic breaks between the Indian and Pacific Oceans (Ekman 1953; Lavery *et al.* 1996; Williams *et al.* 2002; Teske *et al.* 2005), this hypothesis is not supported by the inter- or intraspecific biogeographic patterns of several species, including genus *Echinometra* spp. (Echinoida: Echinometridae) (Palumbi 1996), *Alpheus lottini* (Decapoda: Caridea: Alpheidae) (Williams *et al.* 2002), *Echinolittorina reticulata* (Littorinimorpha: Littorinoidea: Littorinidae) (Reid *et al.* 2006) and *Nerita plicata* (Neritoida: Neritoidae: Neritidae) (Crandall *et al.* 2008). These taxa have panmictic geographic distributions without genetic differentiation between the Indian and West Pacific Oceans. In other cases, different phylogeographic patterns have been observed for some ecologically similar species due to different responses to shared environmental processes (Crandall *et al.* 2008). Therefore, the inference of the historical phylogenetic break between Clades I and II from the phylogeographic patterns of unrelated species may be unreliable. Furthermore, the expansion hypothesis of a West Pacific origin for Clade I is also problematic due to the fact that the minimum spanning tree presented by Gopurenko *et al.* (1999) was forced to connect the common West Pacific haplotype A of Clade I to Clade II without considering alternative ambiguous connections between Clades I and II. Therefore, the Indian Ocean haplotypes were artificially treated as terminal haplotypes. Thus, the network of evolutionary relationships for *S. serrata* given by Gopurenko *et al.* (1999) could not accurately estimate the relative age of the haplotypes to test a hypothesis regarding the evolutionary origin of Clade I.

The second scenario proposed by Gopurenko *et al.* (1999) considers Clade II to have evolved *in situ*, potentially in the Northern Australian biogeographic province of Briggs (1995). This scenario proposes a North Australia origin for Clade II based on two lines of evidence. First, Clade II is confined to Northwest Australia. Secondly, high endemism is common in many Northern Australian taxa of this province (Norman *et al.* 1994; Chenoweth *et al.* 1998). A long period of vicariance is believed to

have caused the divergence between the two clades (I and II) followed by the rapid range expansion of Clade I around the IWP. However, this hypothesis still does not explain the wide distribution and evolutionary origin of Clade I because the detailed evolutionary history, especially for the colonization pathway of Clade I, still remains largely unclear.

In this study, all currently available molecular data, fossil evidence as well as past and present oceanographic data are combined to infer the evolutionary history of *S. serrata* in the Indo-West Pacific region using coalescent simulations. The ancestral origin of *S. serrata* and three competing colonization hypotheses for Clade I (West Indian Ocean origin, Red Sea-South China Sea origin and West Pacific origin) were investigated using the combined approach proposed in this study.

## Material and Methods

### Data acquisition

Mitochondrial cytochrome oxidase subunit I gene sequence data for *Scylla serrata* were downloaded from GenBank (Table 1). In total, 439 sequences from 24 locations throughout the Indo-West Pacific were included in this study. Collection localities, sampling size and GenBank accession numbers were summarized in Table 1 and shown in Fig. 1. All sequences were aligned in CLUSTALX (Thompson *et al.* 1997) using the default parameters. The evolutionary model (TVM + I) was determined using MODELTEST (Posada & Crandall 1998) from the Akaike Information Criterion (AIC).

### Phylogenetic analyses

Phylogenetic analyses using Bayesian inference (BI) were performed in MRBAYES3.1 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) with a six-parameter model (GTR + I) similar to TVM + I. These parameters were estimated in the program using the following settings: ngen = 4,500,000; samplefreq = 100; burnin = 11,250; Nchains = 4; Nruns = 2. The convergence of independent runs was achieved when white noise was seen in the overlay plot of generation *versus* the log probability for both runs with the potential scale reduction factor (PSRF) approaching 1 and low standard deviation of split frequencies ( $0.007224 < 0.01$ ) after 4,500,000 generations.

Due to the weak statistical capability of traditional phylogenetic methods for minimally differing intraspecific 'taxa' (*i.e.* haplotypes) (Templeton *et al.* 1992; Posada & Crandall 2001), a median-joining (MJ) network (Bandelt

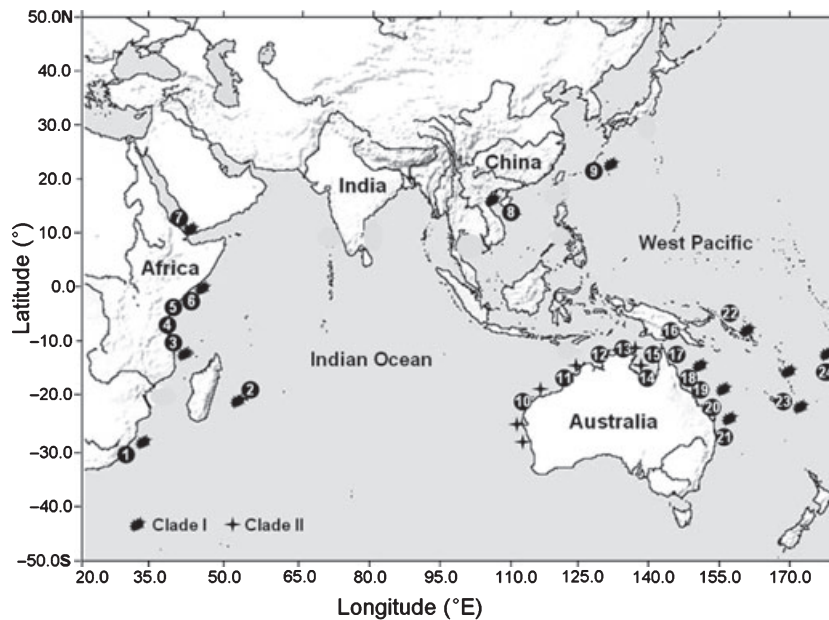
**Table 1.** Geographic distribution of the haplotypes, sample size and GenBank accession numbers in 24 sampling localities.

ID	Location	Coordinates	Number of individuals	Accession No.-Haplotypes (frequency)
1	Durban, South Africa	29°50' S 30°56' E	11	AF097013-SsSAFR(11)
2	Mauritius, Indian Ocean	20°10' S 57°30' E	5	AF097012-SsMAUR(5)
3	Zanzibar, Tanzania	6°9' S 39°22' E	20	AF097013-SsSAFR(12), AF097012-SsMAUR(4), AF279330-SsAP22(1), AF279331-SsAP23(2), AF279332-SsAP24(1)
4	Gazi Bay, Kenya	4°20' S 39°34' E	18	AF097013-SsSAFR(9), AF279310-SsAPL2(1), AF279311-SsAPL3(1), AF279312-SsAPL4(1), AF279313-SsAPL5(1), AF279314-SsAPL6(1), AF279315-SsAPL7(1), AF279316-SsAPL8(1), AF279317-SsAPL9 (2)
5	Mida Creek, Kenya	3°23' S 39°58' E	19	AF097013-SsSAFR(7), AF279318-SsAP10(2), AF097012-SsMAUR(3), AF279320-SsAP12(1), AF279321-SsAP13(1), AF279322-SsAP14(1), AF279323-SsAP15(1), AF279324-SsAP16(1), AF279325-SsAP17(1), AF279326-SsAP18(1)
6	Lamu, Kenya	2°16' S 40°54' E	20	AF097013-SsSAFR(16), AF097012-SsMAUR(1), AF279327-SsAP19(1), AF279328-SsAP20(1), AF279329-SsAP21(1)
7	Jeddah, Red Sea	21°28' N 39°10' E	5	AF097011-SsRSEA(5)
8	Lingshui, Hainan, China	18°24' N 109°53' E	7	AF097002-Ss-1A(2), AF097011-SsRSEA(1), EF203943-Ss01(1), EF203945-Ss05(1), EF203946-Ss06(1), EF203947-Ss07(1)
9	Okinawa, Ryukyu Islands	26°23' N 127°51' E	5	AF097002-Ss-1A(5)
10	Exmouth, WAU	22°15' S 114°15' E	24	AF097002-Ss-1A(3), AY373341-Ss-2J(18), AY373349-Ss-2K(2), AY373350-Ss-2L(1)
11	Broome, WAU	18°0' S 122°15' E	8	AF097002-Ss-1A(1), AY373341-Ss-2J(5), AY373349-Ss-2K(2)
12	Adelaide River, NAU	12°14' S 131°16' E	16	AF097002-Ss-1A(1), AF097016-Ss-2A(10), AF097017-Ss-2B(2), AF097014-Ss-2F(3)
13	Roper River, NAU	14°43' S 135°27' E	31	AF097002-Ss-1A(3), AF097016-Ss-2A(20), AF097017-Ss-2B(5), AF097018-Ss-2C(1), AF097019-Ss-2D(1), AY373346-Ss-2E(1)
14	Karumba, NAU	17°31' S 140°50' E	14	AF097016-Ss-2A(4), AF097014-Ss-2F(9), AF097015-Ss-2G(1)
15	Weipa, NAU	12°45' S 141°30' E	35	AF097002-Ss-1A(1), AF097016-Ss-2A(9), AF097014-Ss-2F(18), AF097015-Ss-2G(3), AY373347-Ss-2H(3), AY373348-Ss-2I(1)
16	Fly River, PNG	7°43' S 143°56' E	3	AF097002-Ss-1A(3)
17	Cape Grenville, EAU	11°57' S 143°12' E	22	AF097002-Ss-1A(1), AY373345-Ss-1J(21)
18	Hinchinbrook, EAU	18°32' S 146°45' E	21	AF097002-Ss-1A(19), AF097004-Ss-1C(1), AF097007-Ss-1F(1)
19	Mackay, EAU	21°36' S 148°39' E	34	AF097002-Ss-1A(27), AF097004-Ss-1C(1), AF097005-Ss-1D(4), AY373342-Ss-1G(1), AY373344-Ss-1I(1)
20	Rockhampton, EAU	23°30' S 150°50' E	22	AF097002-Ss-1A(19), AY373342-Ss-1G(2), AY373343-Ss-1H(1)
21	Morepton Bay, EAU	27°27' S 153°09' E	79	AF097002-Ss-1A(65), AF097003-Ss-1B(2), AF097004-Ss-1C(3), AF097005-Ss-1D(4), AF097006-Ss-1E(1), AF097007-Ss-1F(4)
22	Solomon Islands, West Pacific	8°51' S 158°13' E	7	AF097008-SsSolo(7)
23	New Caledonia, West Pacific	22°11' S 166°22' E	6	AF097010-SsNCAL(6)
24	Fiji, West Pacific	18°06' S 178°38' E	7	AF097009-SsFiji(7)

NAU = Northern Australia; WAU = Western Australia; EAU = Eastern Australia; PNG = Papua New Guinea.

*et al.* 1999) was used to clarify the evolutionary relationship of *S. serrata* in NETWORK (version 4.5.1.0; fluxus-engineering, 2008). To simplify ambiguous reticulations

in the network, character weighting was applied [*e.g.* the most weight (90) was set to each of the characters with the minimum number of mutations (1), and downweighting



**Fig. 1.** The sampling localities of *Scylla serrata* throughout the Indo-West Pacific from a combination of several data sources: 1, 2, 7, 9–24 from Gopurenko *et al.* (1999) and Gopurenko & Hughes (2002), 3–6 from Fratini & Vannini (2002), and 8 from Lin *et al.* (2007). The distributional ranges for the two clades (I and II) of *S. serrata* are distinguished by different stars (● = Clade I, + = Clade II). The sites are numbered in Table 1.

(9) was set to the hypervariable sites with 10 mutations]. To infer the evolutionary polarity and genealogical age of those haplotypes in Clade I, Clade II was used as the closest outgrouping (Castelloe & Templeton 1994).

To approximate the time of divergence of the two *S. serrata* clades (I and II), the assumption of a molecular clock was applied. First, the rate constancy of *S. serrata* was assessed using a closely related outgroup (*Scylla tranquebarica*) in RRTREE (Robinson-Rechavi & Huchon 2000). Then, pairwise mutation rates for COI segments (1.66–2.33% per Myr) from other brachyuran crabs (genus *Sesarma*) calculated by Schubart *et al.* (1998), were applied to the net genetic distance of Kimura-2 parameter (K2P) between the two clades estimated in MEGA4 (Tamura *et al.* 2007).

#### Demographic history and population structure

The population dynamics of Clades I, II, and the three clusters of Clade I (IA, IB and IC) were inferred from the following three statistics in ARLEQUIN version 3.1 (Excoffier *et al.* 2005): the mismatch distribution of pairwise nucleotide differences between haplotypes and two neutrality tests including Tajima's D (Tajima 1989) and Fu's  $F_s$  (Fu 1997). A unimodal pattern of pairwise differences is expected for a population having passed through a recent demographic or range expansion (Rogers & Harpending 1992). The validity of the expansion model was tested using the sum of square deviations (SSD) between

the observed and expected mismatch to infer the significance with the parameter bootstrap approach (1000 replicates). Tajima's D and Fu's  $F_s$  statistic are both sensitive to population demographic expansions and a significant negative value generally indicates population expansion in the absence of selection (Tajima 1989; Fu 1997). Significance of the neutrality tests was assessed by 1000 permutations. Selective neutrality was tested by the McDonald–Kreitman test (McDonald & Kreitman 1991) in DNASP version 4.0 (Rozas *et al.* 2003). This test is based on comparison of the replacement/synonymous substitutions ratio between clades (I and II for *S. serrata*) and within clades.

Analyses of molecular variance (AMOVA) (Excoffier *et al.* 1992) based on K2P distance was performed in ARLEQUIN to assess the degree of genetic differentiation among individuals, sampling sites and geographic groups with statistical significance assessed by permutation. The haplotype (H) and nucleotide diversity ( $\Pi$ ) were also calculated in ARLEQUIN for each clade (I and II), cluster (IA, IB and IC) and inferred four main geographic subpopulations including the Northwest Australia, West Indian Ocean, the Red Sea–South China Sea and the West Pacific (Fig. 1, Table 2).

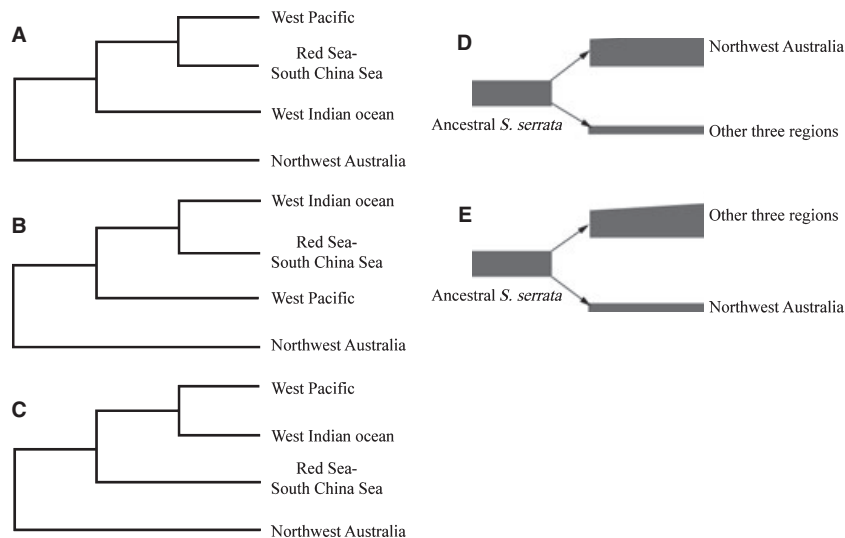
#### Tests of assumptions

The ancestral origin of *Scylla serrata* and three opposing hypotheses for the colonization history of Clade I were

**Table 2.** Summary statistics for genetic diversity and population dynamics by clades (or clusters) and geographic groups of *Scylla serrata*.

Population	N	Nh	H	$\Pi$ ( $10^{-3}$ )	Mismatch analyses SSD (P)	Tajima's D (P)	Fu's $F_s$ (P)
I. Clades or clusters							
Clade I	320	42	0.7438	3.088	0.005992 (0.470)	-2.09129 (0.001)	-27.34206 (0.000)
Clade II	119	12	0.7683	2.382	0.004860 (0.080)	-0.79284 (0.242)	-4.35011 (0.034)
Cluster IA	80	23	0.5291	2.074	0.192407 (0.000)	-2.52351 (0.000)	-26.00995 (0.000)
Cluster IB	22	5	0.5974	1.61	0.001402 (0.800)	-0.60352 (0.314)	-1.37869 (0.085)
Cluster IC	218	14	0.5141	1.303	0.004931 (0.040)	-1.66719 (0.012)	-10.32988 (0.000)
II. Geographic group (ID)							
Northwest Australia (10–15)	128	13	0.7954	5.161			
Western Indian Ocean (1–6)	93	24	0.6339	2.712			
Red Sea-South China Sea (7, 8)	12	6	0.7576	2.274			
Western Pacific (9, 16–24)	206	13	0.5307	1.361			

N = number of individuals; Nh = number of haplotypes; H = haplotype diversity;  $\Pi$  = nucleotide diversity; SSD = sum of square deviations; P = probability.



**Fig. 2.** The competing colonization hypotheses for *Scylla serrata*: (A) West Indian Ocean origin of Clade I, (B) West Pacific origin of Clade I, (C) Red Sea-South China Sea origin of Clade I, (D) Northwest Australia origin of ancestral *S. serrata*, and (E) three-region combination origin of ancestral *S. serrata*.

inferred from the four geographic subpopulations (Fig. 2). The three hypotheses regarding the colonization history of Clade I were: (i) a model for a West Indian Ocean origin of Clade I followed by sequential coastal colonization to the West Pacific (Fig. 2A); (ii) an alternative model for a West Pacific origin of Clade I followed by westward dispersal and eventual colonization of the West Indian Ocean (Fig. 2B); and (iii) a model for the East Indies Triangle (Red Sea-South China Sea for this study) origin of Clade I followed by westward/eastward dispersal and genetic divergence between populations of the Indian and Pacific Oceans (Fig. 2C). It was also hypothesized that the ancestral origin of *S. serrata*

(Northwest Australia (Fig. 2D) or the three-region combination of Clade I (*i.e.* West Indian Ocean, Red Sea-South China Sea and West Pacific subpopulations; Fig. 2E), would have the highest founding proportion.

Due to the weak statistical resolution and uncertainty of the branching order in Clade I, traditional phylogenetic methods (NJ, ML and BI) were insufficient to resolve the intraspecific colonization history of *S. serrata*. In this study, we applied coalescent simulations based on the isolation-with-migration model (IM) to distinguish between these ambiguous colonization scenarios. Seven demographic parameters could be estimated for a pair of divergent populations using the IM software (Hey 2005, 2006):

effective population size for two descendent populations (1 and 2) and their ancestral population (A) ( $N_i = \theta_i/\mu$ ,  $i = A, 1, 2$ ), unidirectional migration between the two descendent populations ( $M_i = m_i \times \mu$ ,  $i = 1, 2$ ), population divergence time ( $T = t/\mu$ ) and proportion of ancestral population founding the first descendent population ( $S$ ). Except for the mutation rate ( $\mu$ ) for a studied DNA segment, all the parameters including  $t$ ,  $\theta_A$ ,  $\theta_1$ ,  $\theta_2$ ,  $m_1$ ,  $m_2$  and  $S$  could be directly calculated in IM. The founding parameter,  $S$ , has been shown to be informative in studying the origin of populations based on the assumption that a descendent population in an ancestral region often possesses high ancestral diversity and founding proportion ( $S$  or  $1-S$ ,  $0.5-1$ ), whereas a new derived population was founded by only few individuals from a rare event with low splitting proportion ( $S$  or  $1-S$ ,  $0-0.5$ ) (Hey 2005; Russell *et al.* 2008a).

After excluding the four-gamete violations and homoplasy signals using an online program IMgc (Woerner *et al.* 2007), three pairs of datasets from the four geographic subpopulations were obtained to test the above-mentioned hypotheses. The three datasets included the Red Sea-South China Sea/West Indian Ocean dataset (535 bp), which contained 10 individuals from the Red Sea-South China Sea and 89 individuals from the West Indian Ocean, the Red Sea-South China Sea/West Pacific dataset (524 bp), containing 11 individuals from the Red Sea-South China Sea and 197 individuals from the West Pacific, and the combined three regions/Northwest Australia dataset (524 bp), containing 291 individuals of Clade I from three geographic subpopulations (West Indian Ocean, Red Sea-South China Sea and West Pacific) and 117 individuals from the Northwest Australia subpopulation. The Hasegawa-Kishino-Yano (HKY) substitution model was used for each dataset. We aimed to determine the founding proportion ( $S$ ) of ancestral population in the Red Sea-South China Sea subpopulation for the Red Sea-South China Sea/West Indian Ocean and Red Sea-South China Sea/West Pacific datasets, respectively. The founding proportion of ancestral population *S. serrata* in a descendent population from the combined three regions of Clade I (West Indian Ocean, Red Sea-South China Sea and West Pacific) was also inferred from the three regions/Northwest Australia dataset. Initial parameters were obtained from a single run with uniform prior distributions for each dataset:  $q_1$ ,  $q_2$ ,  $q_A$ ,  $0-10$ ;  $m_1$ ,  $m_2$ ,  $0-10$ ;  $t$ ,  $0-10$  and  $s$ ,  $0-1$ . Three independent runs were subsequently repeated with identical parameters and different random number seeds for  $10^7$  steps, 3-4 chains and a burnin of  $10^5$  steps. Coalescent analysis was considered to have converged on a stationery distribution if these independent runs reached similar results or the lowest current effective sample size was over 50 (Hey 2005).

## Results

### Intraspecific phylogenetic trees and branching order

An aligned dataset including 53 haplotypes (535 bp) was obtained. Based on the Akaike informative criterion (AIC), the best evolutionary model, the TVM + I model was selected: proportion of invariable sites, pinvar = 0.8234; base frequencies,  $f_A = 0.2802$ ,  $f_C = 0.1681$ ,  $f_G = 0.1655$ ,  $f_T = 0.3862$ ; rate matrix,  $r(AC) = 1950252.6250$ ,  $r(AG) = 23797776.0000$ ,  $r(AT) = 274716.2812$ ,  $r(CG) = 3276340.5000$ ,  $r(CT) = 23797776.0000$ ,  $r(GT) = 1.0000$ .

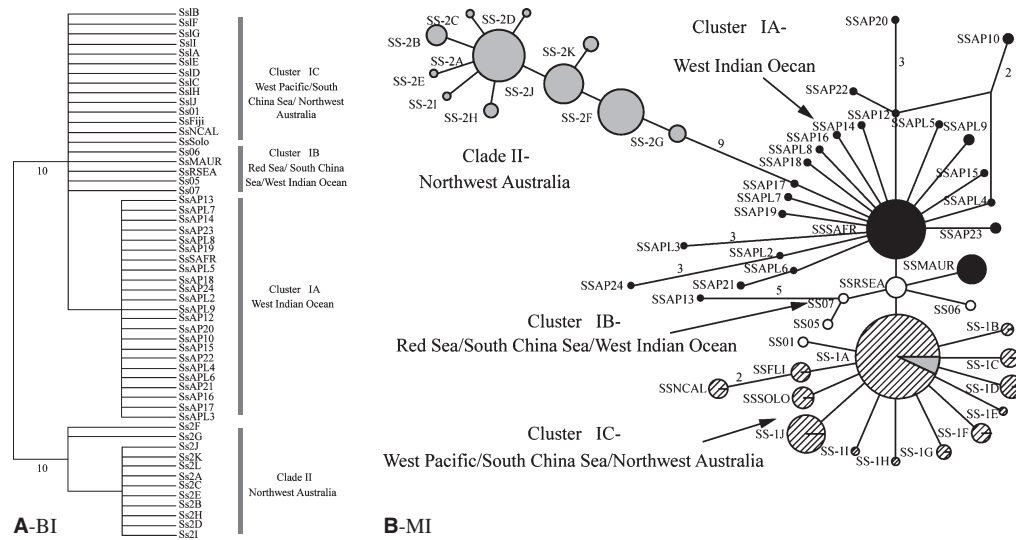
Two clearly distinguished clades (Clades I and II) with 2% divergence (K2P) were recovered for *Scylla serrata* with strong statistical support (Fig. 3A). Two main clusters (IA and IB + C) were also identified for Clade I without strong nodal support from the BI tree (Fig. 3A). The evolutionary network recovered an intermediate cluster (IB) between these clusters (IA and IC) of Clade I (Fig. 3B). The four clusters had disjunct distributions roughly corresponding to four geographic regions: Northwest Australia (II), West Indian Ocean (IA), Red Sea-South China Sea (IB) and West Pacific (IC). Furthermore, unlike the uncertain branching order recovered using traditional phylogenetic methods (e.g. BI in Fig. 3A), Clade I was rooted at SSAP17, based on the closely related outgroup (Clade II) in the MJ network (Fig. 3B). Thus, Cluster IA was inferred to be the oldest cluster in Clade I, with Cluster IC being considered the most derived cluster.

The evolutionary rate test between the two clades (I and II) showed no significant rate difference ( $P > 0.05$ ), implying that the molecular clock used in this study was appropriate. An early Pleistocene (1.2-0.86 Ma) divergence was indicated for the separation of Clades I and II (2% K2P distance) based on the molecular clock of 1.66-2.33% per Myr from other brachyuran crabs (genus *Sesarma*).

### Population demographic history and genetic structure

No significant deviation from selective neutrality was detected for *Scylla serrata* by the McDonald-Kreitman test (Neutrality index = 0.7, Fisher test  $P = 1.0$ ). Therefore, a significant population demographic expansion could be inferred for Clade I and the two clusters IA and IC (see Table 2 for the tests of mismatch distribution, Tajima's  $D$  and Fu's  $F_s$ ).

From the results of the AMOVA (Table 3), genetic population structure with significant components of variance within and among four geographic groups was detected from the 24 localities ( $\Phi_{SC} = 0.2838$ , %V = 5.27,  $P = 0$  and  $\Phi_{CT} = 0.8145$ , %V = 81.44,  $P = 0$ ): a West Indian Ocean group including Mauritius and the Eastern African coast (sites 1-6), a Red Sea-South China Sea



**Fig. 3.** The phylogenetic relationship of haplotypes for *Scylla serrata* (clades I and II): BI (A), MJ network (B), numbers indicated nodal support values (>95%) for the BI tree, and steps of over two substitutions between the haplotypes for MJ network. The distributional regions of haplotypes in networks (B) are distinguished using different patterns and colors with gray for Northwest Australia, backward diagonal for West Pacific, white for Red Sea-South China Sea, and black for West Indian Ocean.

group (sites 7 and 8), a Northwest Australia group (sites 10–15), and a West Pacific group (sites 9, 16–24) (Fig. 1, Table 2), which is consistent with the phylogeographic structure inferred from the phylogenetic trees/network and geographic distributions of the haplotypes. Nucleotide diversities were in a descending order of Clade II, cluster IA, IB and IC. Furthermore, for the comparisons between geographic subpopulations, nucleotide diversity estimates were also in descending order of Northwest Australia, West Indian Ocean, Red Sea-South China Sea, and West Pacific groups (Table 2).

**Population splitting parameters from coalescent simulations**

Through coalescent simulations for the pairs of neighboring geographic subpopulations, higher founding proportions were revealed for the West Indian Ocean (Red Sea-South China Sea *versus* West Indian Ocean, average

1–S = 0.9972) and Red Sea-South China Sea (Red Sea-South China Sea *versus* West Pacific, average S = 0.9992) subpopulations, respectively (Fig. 4A, B). Furthermore, higher ancestral diversity was retained in Northwest Australia (average 1–S = 0.9995) through the comparison of three-region combination (West Indian Ocean, Red Sea-South China Sea and West Pacific) *versus* Northwest Australia (Fig. 4C). Therefore, an ancestral origin of *S. serrata* in Northwest Australia (Fig. 2D), and a subsequent sequential colonization pathway of Clade I originating from the West Indian Ocean (Fig. 2A) through the Red Sea-South China Sea to the West Pacific could be inferred based on these validated simulations.

**Discussion**

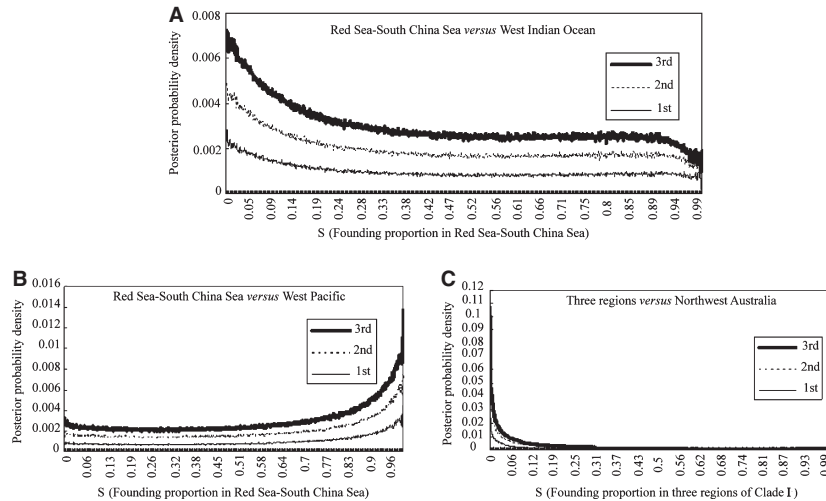
**Transoceanic dispersal and sequential colonization history of *Scylla serrata***

Despite some degree of error associated with applying the IM model to real-world scenarios (*i.e.* poor estimates of ancestral population size and gene flow due to incorrect model of nucleotide substitutions), the IM model estimates proved to be quite robust for other parameters (Strasburg & Rieseberg 2010). Furthermore, there is no evidence that the IM model estimated the population splitting parameters (S) incorrectly. As studied in other species (Hey 2005; Russell *et al.* 2008a,b), coalescent simulations of the IM model has shown powerful discriminative ability to reveal the dispersal origin and colonization

**Table 3.** Results of hierarchical AMOVA from four subpopulations for *Scylla serrata*.

Comparison	DF	SSD	VC	%V	Φ
Among groups	3	1002.66	3.46434	81.44	F <sub>CT</sub> = 0.81445
Among populations within groups	20	88.829	0.22398	5.27	F <sub>SC</sub> = 0.28378
Whin populations	415	234.598	0.56530	13.29	F <sub>ST</sub> = 0.86710

DF = degrees of freedom; SSD = sum of squares; VC = Variance component; %V = percent of variance, for all values of Φ are significant (P = 0.00).



**Fig. 4.** Founding proportion for three pairs of geographic subpopulations (subpopulation 1 versus subpopulation 2) of *Scylla serrata* through coalescent simulations in IM: (A), Red Sea-South China Sea versus West Indian Ocean, (B) Red Sea-South China Sea versus West Pacific, (C) combination of three geographic regions versus Northwest Australia.

direction for *S. serrata*, despite the ambiguous branching order and incomplete lineage sorting for this species. A novel ancestral origin of *S. serrata* from Northwest Australia (Fig. 2D), followed by sequential colonization of Clade I dispersing from the West Indian Ocean to the Red Sea, South China Sea and West Pacific (Fig. 2A), was corroborated based on the founding proportions simulated in IM. Furthermore, this inferred colonization pathway was also confirmed by other cross-validated evidence including lineage age in networks, fossil records, and genetic diversity tests for each cluster/geographic group.

First, the rooted network of Clade I, using Clade II as an outgroup, revealed a descending order in the cluster ages from cluster IA (from West Indian Ocean) to IB (mainly from the Red Sea and South China Sea) and then IC (mainly from the West Pacific) (Fig. 3B), which indicated that the earliest colonization occurred in the West Indian Ocean (Fig. 2A) and eventually arrived in the West Pacific for Clade I.

Secondly, fossil records provide evidence for the West Indian Ocean origin hypotheses of Clade I (Fig. 2A). Several fragmented chela fossils of *S. serrata* have been reported for the first time from the late Pleistocene deposit of Guam (Schweitzer *et al.* 2002). However, a larger number of fossils of *S. serrata* have been recorded from the early Pleistocene mudstone of South Africa (Cooper & Kensley 1991). These fossils indicate the earliest known colonization ages of *S. serrata* along the coast of the West Indian Ocean and West Pacific. The occurrence of early Pleistocene fossils from South Africa is consistent with our estimated divergence time (1.2–0.86 Ma) between Clade I and Clade II. Therefore, the fact that

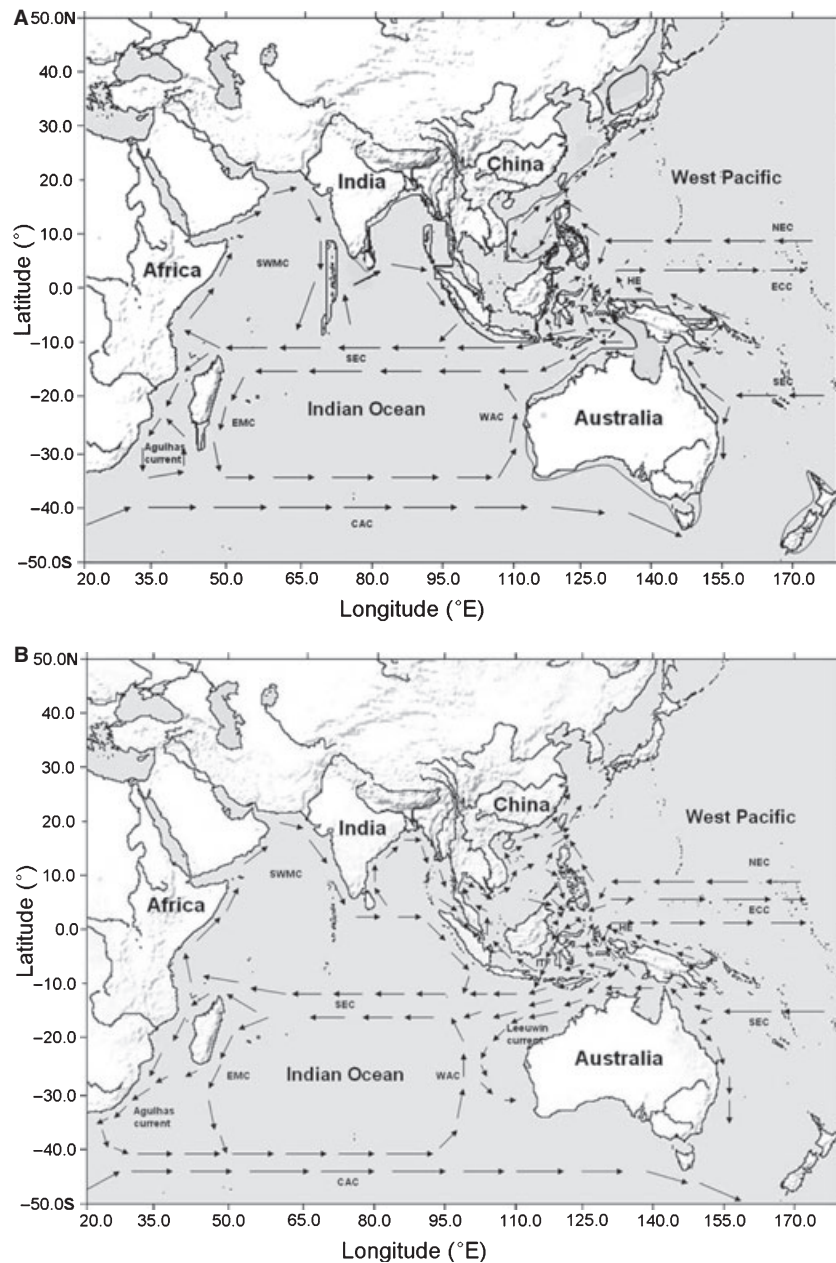
*S. serrata* appeared earlier and was more dominant in the species composition of South Africa than in the West Pacific (Guam) indicates that the coast of the West Indian Ocean may have served as the ancestral origin of Clade I, which subsequently dispersed to the West Pacific.

Thirdly, genetic diversity within each cluster or geographic subpopulation indicates a transmarine dispersal and sequential colonization history for Clade I (Table 2). A descending nucleotide diversity pattern was detected for the four clusters (in the order II, IA, IB and IC), and also for the four geographic subpopulations (Northwest Australia, West Indian Ocean, Red Sea-South China Sea and West Pacific). The origin of dispersal generally possesses the highest genetic diversity, and due to the founder effect, newly colonized regions often possess lower genetic diversity (Hewitt 1996, 1999). As inferred from this criterion, Clade II and Northwest Australia could have served as older, ancestral lineages and the original region for *S. serrata* (Fig. 2D), and Cluster IA and the West Indian Ocean might have also served as ancestral lineages and origins of dispersal for Clade I (Fig. 2A). Hence, the founding population of Clade I in the West Indian Ocean would have derived from the older ancestral population in Northwest Australia through a transoceanic dispersal event in early Pleistocene.

#### Paleo-oceanographic conditions and phylogeographic history of *Scylla serrata*

The divergence time between Clade I and II (1.2–0.86 Ma) implies an early Pleistocene separation event. The glacial topology of Australia and oceanic circulation patterns





**Fig. 5.** Oceanic circulation patterns during the month of August in the Indo-West Pacific for glacial (A) and present (B) times. The arrows show the circulation direction (after Prell *et al.* 1980; Pollock 1993; Gordon & Fine 1996; Kuhnt *et al.* 2004), and the  $-200$  m contour is illustrated by the black line in (A) (after Pollock 1993; Voris 2000) (SEC = South Equatorial Current; NEC = North Equatorial Current; ECC = Equatorial Counter Current; WAC = West Australia Current; EMC = East Madagascar Current; CAC = Circum-Antarctic Current; SWMC = Southwest Monsoon Wind Current; HE = Halmahera Eddy; ITF = Indonesian Throughflow).

could have facilitated the transmarine dispersal of *S. serrata* across the Indian Ocean from Northwest Australia. Large numbers of larvae from Clade II individuals might have been passively dispersed to East Africa by the glacial coastal West Australia Current and westward South Equator Current from Northwest Australia (Fig. 5A). Transoceanic dispersal patterns have commonly been observed in some

freshwater and terrestrial species, such as Pacific freshwater shrimp (genus *Paratya*) (Page *et al.* 2005), freshwater fish (*Galaxias maculatus*) (Waters *et al.* 2000), Atlantic *Mabuya* lizards (Mausfeld *et al.* 2002; Carranza & Arnold 2003), Indian Ocean chameleons (Raxworthy *et al.* 2002), geckos (Austin *et al.* 2004), coastal *Cryptoblepharus* lizards (Rocha *et al.* 2006), and even frogs in the genera *Ptychadena*, *Man-*

*tidactylus* and *Boopis* (Vences *et al.* 2003, 2004). Moreover, this long-distance transmarine dispersal across the Indian Ocean has also been observed in two widespread Indian Ocean species of spiny lobsters, *Panulirus homarus homarus* and *Panulirus longipes longipes* (Pollock 1990, 1993), both of which show extensive population mixture between the west (East Africa) and east (Indonesia and Northwest Australia) margins of the Indian Ocean.

Larval duration seems to be a restricting factor and is closely correlated with the dispersal capability of many marine organisms (Paulay & Meyer 2006), but this has been successfully overcome by some crustacean species. For example, some planktonic larvae of lobsters can travel long distances with oceanic circulation by delaying their metamorphosis until encountering physical and chemical cues similar to their native environment (Pollock 1990). A similar phenomenon has been observed in many other crustacean species including mud crabs, where the egg incubation period exponentially increases with decreasing temperature (Wear 1974; Heasman & Fielder 1983). Generally, mud crabs are believed to be able to stay in the larval stage for 3–4 weeks; however, the incubation period of *Scylla paramamosain* has been shown to last for 60 days at 16.7 °C (Hamasaki 2002), and the longest larval development of *S. serrata* has been recorded as 62 days at 25 °C (Nurdiani & Zeng 2007). Therefore, it is likely for *S. serrata* to have been transported westward across the Indian Ocean by the South Equator Current during this long planktonic period.

Continuous migration between the two edges of the Indian Ocean could have inhibited genetic differentiation, but the colonization of *S. serrata* may have been a pulsed process. Sea-level fluctuation during the Pleistocene (Voris 2000) could have interrupted the continuous transmarine dispersal of *S. serrata* across the Indian Ocean. The intensified poleward Leeuwin Current during interglacial periods may have caused the equatorward West Australia Current to shift 1000 km away from the coast (Prell *et al.* 1980) (Fig. 5B) and, as a result, westward dispersal would have been interrupted, with the retention of larvae on the West Australia coast (Pollock 1993). If dispersal frequency decreases with time, the established West Indian Ocean population could have become genetically isolated and differentiated from native populations in Northwest Australia (Paulay & Meyer 2002), which may have been responsible for the vicariance divergence between Clades I and II of *S. serrata*.

After successful colonization, the ancestral population of Clade I may have experienced a subsequent demographic expansion (Table 2) and expanded northward to the Red Sea, drifting with the Southwest Monsoon Wind Current (SMWC), and occupied empty niches along the eastern coast of Africa (Fig. 5). The divergence between

the Red Sea–South China Sea subpopulation and the West Indian Ocean subpopulation could be attributed to the glacially restricted circulation patterns in the northwest and southwest of the Indian Ocean (Fig. 5A). Similarly, the weaker glacial surface circulation in the northwest Indian Ocean (Somali/Arabian basin) and the Agulhas Current around the southeast coast of South Africa and tip of South Madagascar caused the allopatric speciation of distinct lobster subspecies *P. homarus megasculptus* and *P. homarus rubellus* in the north and south, respectively (Pollock 1993).

The sharing of haplotypes and clusters between the Red Sea and South China Sea may be a result of recent or ongoing migration process (range dispersal or gene flow). Because of the closure of the Sunda shelf to South China Sea during glaciations (Voris 2000) (Fig. 5A), dispersal could only occur during interglacial periods. Furthermore, the proposed direction of colonization (from the Red Sea to the South China Sea) is consistent with the interglacial August clockwise Southwest Monsoon Circulation in the North Indian Ocean (Fig. 5B), and the eastward flow through the Strait of Malacca to the South China Sea could further transport larvae to the Chinese coast.

Larvae in the South China Sea could be carried easily to Okinawa and Japan in the West Pacific by the eastward outflow through the Taiwan Strait and Luzon Strait (Fig. 5B). Nevertheless, the exchange between the South China Sea and other West Pacific localities is complex because of the influence of the Indonesian throughflow (ITF) and the Indonesia Seas. In the winter, northwest monsoons can drive the colder South China Sea water to the Java Sea and inhibit the southward throughflow in the Makassar Strait, or even cause a northward throughflow to the West Pacific (Gordon *et al.* 2003). Another exit of South China Sea waters to the West Pacific is the Sulu Sea. South China Sea waters can flow through the Mindoro Strait into the Sulu Sea and contribute to the ITF and West Pacific (Fang *et al.* 2005). The larvae from South China Sea may have been passively transported to the Indonesia Seas and West Pacific, and then the newly founding population (Cluster IC) experienced rapid population growth and range expansion (Table 2).

Shared common haplotypes (Ss-1A) and Cluster IC between the South China Sea and West Pacific (Table 1, Fig. 2B) might be attributed to the complicated circulation patterns in the East Indies Triangle region (Fig. 5B). The ITF within the Makassar Strait can account for the entire modern Pacific to Indian interoceanic transport (Gordon *et al.* 1999). The present (interglacial) North Pacific water is dominant in the upper thermocline of the Makassar Strait (Gordon & Fine 1996), whereas most of the South Equator Current is reflected in the Halmahera eddy

as the Equator Counter Current, and only some lower thermocline water from South Pacific enters the eastern Indonesia Seas (Hautala *et al.* 1996; Ilahude & Gordon 1996). South Pacific waters only dominated the ITF during the climate conditions of El Niño, glaciation and pre-Pliocene times (Cane & Molnar 2001; Kuhnt *et al.* 2004). As a result, it would be difficult for the individuals of Cluster IC from the West Pacific to freely enter the Indonesian Seas and South China Sea during times of deglaciations with some exceptions of occasional El Niño events. Therefore, separation by the ITF could have caused genetic divergence and recent asymmetric migration between Red Sea-South China Sea and West Pacific subpopulations.

## Summary

Based on molecular evidence, including coalescent simulations, network analysis, nucleotide diversity tests, fossil records and paleo-oceanographic conditions, we conclude that Northwest Australia served as the ancestral origin of *Scylla serrata* followed by transoceanic dispersal and sequential colonization of Clade I from the West Indian Ocean through the Red Sea-South China Sea to the West Pacific. The fluctuating paleo-oceanographic conditions (surface circulations and oceanic topography) probably accelerated the migration and physical isolation of *S. serrata*.

The insufficient sampling in the region of the Red Sea-South China Sea (North Indian Ocean and East Indies Triangle) does not seem to have influenced our analyses, based on the following facts. First, the intermediate cluster IB (from the Red Sea-South China Sea) is the closest sister cluster to IA (from the West Indian Ocean) and IC (mainly from the West Pacific), with only one nucleotide substitution between them. Thus, it is unlikely that other clusters closer to the West Indian Ocean Cluster IA and West Pacific Cluster IC remain unsampled from the Red Sea-South China Sea. Secondly, the geographic subpopulation of the Red Sea-South China Sea retains a higher ancestral founding proportion and nucleotide diversity relatively to the sufficiently sampled subpopulation of the West Pacific, which shows that sampling size had no effect on parameter estimation in IM. We emphasize that this study reveals only the matrilineal colonization history of *S. serrata*. Future studies including more sampling localities and multilocus analyses are needed to test the population history of *S. serrata*.

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