

Population differentiation in the barnacle *Chthamalus malayensis*: postglacial colonization and recent connectivity across the Pacific and Indian Oceans

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ABSTRACT: *Chthamalus malayensis* is a common intertidal acorn barnacle widely distributed in the Indo-West Pacific. Analysis of the mitochondrial cytochrome *c* oxidase subunit I (COI) sequences of samples from its distribution range revealed 3 genetically differentiated clades: the South China Sea, Indo-Malay and Taiwan clades. The clades have an allopatric distribution and differed by ~7.5 to 14 % in COI. They also differed in zonation patterns and abundances within vertical ranges. The genetic and ecological differences suggest that the 3 clades probably represent distinct species. There were signatures of postglacial demographic expansion, yet the timing of expansion varied among clades, which is attributable to the differences in their geographical distributions. The Indian Ocean population of the Indo-Malay clade apparently attained its present range by postglacial re-colonization from the Pacific and, as a result, genetic differentiation among populations in the 2 oceans is low ($\Phi_{CT} = -0.01$, $p = 0.49$). There were differences in cohort structure between populations in India and the Malay Peninsula. Together, this suggests a considerable level of contemporary gene flow over an evolutionary, but relatively restricted dispersal on an ecological time scale. Cohort structure also varied among Malaysia and Singapore, the South China Sea and Taiwan waters, indicating distinct larval supplies among the 3 clades, possibly determined by different ocean current systems. This physical dispersal of larvae interacts with local biological factors in determining the on-shore distribution and genetic structure of the barnacle populations. Our findings highlight the importance of combining ecological and genetic data to understand factors that mould biodiversity patterns.

KEY WORDS: Population connectivity · Demographic expansion · Cryptic species · Glaciation · Indo-West Pacific · Chthamalidae

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INTRODUCTION

The diversity and genetic structure of species reflect long histories of speciation, driven by past tectonic events and recent dispersal and colonization processes after the last ice age (Avice 2000, Hewitt 2000). The

extraordinarily high marine biodiversity in the Indo-West Pacific (IWP) is well known (Briggs 1999, Hughes et al. 2002) and has attracted the interests of evolutionary biologists and ecologists. Ancient tectonic events and more recent fluctuating sea levels during the Quaternary are believed to have promoted allopatric

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diversification in this region (Briggs 1999). As a result, there is a phylogeographic break between the Pacific and Indian Oceans in an array of widely distributed taxa (Benzie 1999). Genetic differentiation is believed to result from the repeated lowering of sea level during the Pleistocene glaciation, which restricted dispersal between the 2 oceans (Benzie 1999, Voris 2000). Yet, some taxa are highly genetically homogeneous across the 2 oceans (e.g. Lessios et al. 2003, Craig et al. 2007), which may be attributed to postglacial colonization of the Indian Ocean by Pacific Ocean populations (Lessios et al. 2003) or to high levels of present-day gene flow (Craig et al. 2007). Thus, taxon-specific ecological characteristics and the current connectivity among populations also contribute to determining the genetic structure and distribution pattern of a species.

On the other hand, molecular phylogeographic studies have also discovered a large number of cryptic species in the IWP (e.g. Meyer et al. 2005, Chan et al. 2007a). Although the IWP is already famous for its high biodiversity and endemism, diversity appears to be far higher than currently documented. This has important implications for conservation planning and evolutionary theory (Bickford et al. 2007), prompting more intensive studies in the region. Our recent studies revealed that the common intertidal acorn barnacle *Tetraclita squamosa*, which was once considered to be widely distributed in the IWP, is actually a species complex in which each biological species has a specific, restricted distribution in comparison to the whole complex (Chan et al. 2007a,b). Barnacles are major space occupiers and play important roles in community dynamics on rocky shores (Reimer 1976, Bertness et al. 1998). A poor understanding of species identities will hamper ecological studies of community-level interactions. Elucidating the distribution and genetic structures of species like barnacles and the processes governing observable patterns will greatly enhance knowledge of intertidal biogeography and community ecology.

Chthamalus malayensis Pilsbry 1916 is a common intertidal barnacle widely distributed throughout the IWP (Southward & Newman 2003). The species is common in Indo-Malay and Australian waters and is also found in the Indian Ocean and western Pacific region; it varies little morphologically over its wide geographic range (Southward & Newman 2003). However, given what we now know of the *Tetraclita squamosa* complex, it is possible that cryptic species are also present within the range of '*C. malayensis*'. There have, for instance, been reports of differences in reproductive season among populations of *C. malayensis* (Koh et al. 2005, Yan et al. 2006). While *C. malayensis* in Singapore is reproductively active year round (Koh et al. 2005), individuals in Hong Kong only spawn during

the summer (Yan et al. 2006). These differences have been attributed to climatic differences between the 2 areas, as populations in tropical Singapore experience only slight seasonal variation in contrast to those inhabiting the seasonal climate of Hong Kong (Yan et al. 2006). An alternative hypothesis, however, would be that the 2 populations represent 2 cryptic species with different life-history traits. To test this, detailed molecular and ecological studies are needed.

The wide distribution range of *Chthamalus malayensis* allows investigation of past and present-day connectivity of intertidal organisms in the IWP. Lowered sea levels during the Pleistocene glaciations exposed a large part of the Indo-Malay region (Voris 2000), where *C. malayensis* is now the most common barnacle. This retreat of the seas would have reduced habitat availability for intertidal species, leading to population bottlenecks and vicariance among fragmented populations. Following the retreat of glaciers, however, species were able to undergo range expansion, enabling reconnection of isolated populations. The level of contemporary connectivity among populations will determine whether any detectable genetic signature of isolation could be maintained and will also have important effects on population dynamics and ranges of different species (e.g. Pannacciulli et al. 1997, Craig et al. 2007). There is an increasing recognition that dispersal capacity alone is not a good indicator of connectivity (Taylor & Hellberg 2003), promoting the need for more studies to elucidate the causal factors affecting population dynamics in the marine realm. Most studies to date have focused mainly on fishes, especially coral reef fishes (reviewed in Mora & Sale 2002). There have been no large-scale studies on intertidal organisms, and *C. malayensis* provides a good opportunity to investigate connectivity among oceans.

We investigated genetic and ecological variation of *Chthamalus malayensis* in the IWP. We examined genetic differentiation using the DNA sequences of mitochondrial cytochrome *c* oxidase subunit I (COI) among *C. malayensis* populations; COI has proved to be informative in phylogeographic studies of barnacles (e.g. Chan et al. 2007a, Tsang et al. 2007). We integrated these data with those on vertical zonation and cohort structure of *C. malayensis* in different regions. These molecular and ecological analyses specifically addressed the following questions: (1) Are *C. malayensis* populations genetically differentiated, perhaps into cryptic species? (2) Does *C. malayensis* have any genetic signature caused by the Pleistocene glaciations? (3) What is the level of connectivity among populations between and within the Pacific and Indian Oceans, and does this affect the genetic structure of the barnacle?

MATERIALS AND METHODS

Sample collection. *Chthamalus malayensis* specimens were collected from 11 localities in the Indo-West Pacific, either through our own site visits or by local collaborators (Table 1, Fig. 1). During the site visits, we also conducted vertical zonation and cohort structure investigations at 8 of the localities: 2 in Taiwan (Hualien and Northeast Coast), 3 along the coast of southern China (Hong Kong and Hoi Ling Island in the Guangdong Province and Sanya in the Hainan Province), and 1 each in Malaysia (Mersing), Singapore (Sentosa Island) and India (Tranquebar). Ecological surveys were also conducted at Mahabalipuram (MM, 12° 37' N, 80° 12' E) in India, but these specimens were not included in the genetic study. Ecological studies were not conducted at the other 3 sites included in the genetic analyses, i.e. Sri Chang Island in Thailand (TH), Langkawi Island (LK) in Malaysia, and south Sri Lanka (SL).

Vertical zonation and cohort structure. At each ecological sampling site, a 20 m stretch of shoreline was chosen. Six 20 m transects were set parallel to the shore at ~2.5 m and 2.25 m (high shore), ~2 m and 1.75 m (mid shore), ~1.5 m and 1.25 m (low shore) above Chart Datum (CD), as judged from predicted tide tables. Maximum tidal range for all sites was ~2.5 m; therefore, the high, mid and low shore levels of all sites were comparable. At each level, digital photographs of 10 randomly positioned 15 × 15 cm

quadrats were taken. The density and percentage cover of *Chthamalus malayensis* in each quadrat was scored on each photograph. Spatial variation in cohort structure of *C. malayensis* was compared among Mahabalipuram and Tranquebar in India, Sentosa Island in Singapore, Mersing in Malaysia, Hong Kong and Hoi Ling Island in Guangdong, and Hualien and NE Coast in Taiwan, as these sites were all sampled from January to March 2007. Data from Sanya in Hainan were not included in the comparison, as sam-

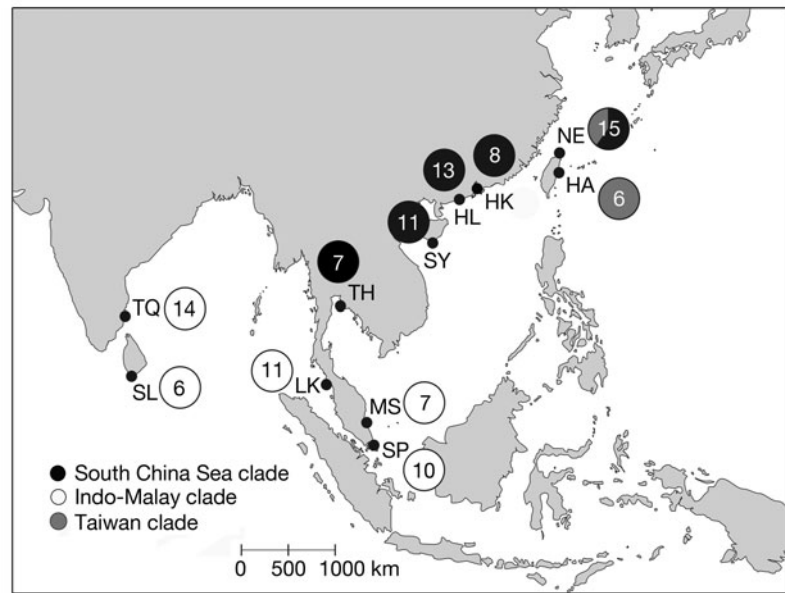


Fig. 1. *Chthamalus malayensis*. Sampling localities (capital letters). Clade distribution based on mitochondrial COI is shown inside each pie chart. Numbers within the pie charts indicate sample size. See Table 1 for abbreviations of localities

Table 1. *Chthamalus malayensis*. Sample localities, abbreviations, sample sizes (n), and genetic diversity of COI (mitochondrial cytochrome c oxidase subunit I) for each population: number of haplotypes, haplotype diversity ($h \pm SD$), nucleotide diversity ($\pi \pm SD$), Tajima's *D* and Fu's F_S . * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Population	Abbreviation	Lat./Long.	n	No. of haplotypes	$h \pm SD$	$\pi \pm SD$	Tajima's <i>D</i>	Fu's F_S
Pacific Ocean								
Northeast Coast, Taiwan	NE	24° 58' N, 121° 55' E	15	13				
Taiwan clade			6	4	0.800 ± 0.172	0.007 ± 0.004	-1.22	0.85
South China Sea clade			9	9	1.000 ± 0.052	0.013 ± 0.008	-0.76	-3.26*
Hualien, Taiwan	HA	23° 28' N, 121° 29' E	6	6	1.000 ± 0.096	0.007 ± 0.004	-0.94	-2.59*
Hong Kong, China	HK	22° 12' N, 114° 13' E	8	8	1.000 ± 0.063	0.013 ± 0.008	-0.64	-2.74*
Hoi Ling Is., China	HL	21° 36' N, 111° 54' E	13	13	1.000 ± 0.030	0.015 ± 0.009	-0.69	-6.09**
Sanya, China	SY	18° 19' N, 109° 45' E	11	10	0.982 ± 0.046	0.010 ± 0.006	-1.22	-3.54*
Sri Chang Is., Thailand	TH	13° 08' N, 100° 48' E	7	7	1.000 ± 0.076	0.014 ± 0.009	-0.67	-1.75
Mersing, Malaysia	MS	2° 26' N, 103° 50' E	7	7	1.000 ± 0.076	0.004 ± 0.003	-1.58*	-5.28***
Sentosa Is., Singapore	SP	1° 14' N, 103° 50' E	10	7	0.867 ± 0.107	0.004 ± 0.003	-0.92	-2.24
Indian Ocean								
Langkawi Is., Malaysia	LK	6° 25' N, 99° 40' E	11	7	0.873 ± 0.089	0.002 ± 0.002	-1.65*	-4.05**
Tranquebar, India	TQ	11° 02' N, 79° 51' E	14	7	0.802 ± 0.094	0.004 ± 0.002	-1.15	-1.76
South Sri Lanka	SL	6° 02' N, 80° 14' E	6	6	1.000 ± 0.096	0.005 ± 0.004	-1.43*	-3.03*

pling at this site was conducted in October 2006. The rostral–carinal basal diameter (R–C basal diameter) of *C. malayensis* in each quadrat was measured from the calibrated photographs using Sigma Scan Pro 5 (see Chan et al. 2008). Cohort structures were analysed using electronic length frequency analysis (ICLARM, see Chan & Williams 2004).

DNA extraction, PCR and sequencing. Total genomic DNA was extracted from whole soft tissues of individual barnacles using the commercial QIAamp tissue kit (QIAGEN). Partial sequences of mitochondrial COI were amplified using the universal primers LCO1490 and HCO2198 (Folmer et al. 1994). The amplifications were conducted in a reaction mix containing 1 µl of template DNA, 1 × PCR reaction buffer, 2 mM MgCl₂, 200 nM of each primer, 200 µM dNTPs, 1.5 units of *Taq* polymerase (Amersham) and double-distilled H₂O to a total volume of 50 µl. The PCR profile was as follows: 3 min at 94°C for initial denaturation, then 33 cycles of 30 s at 94°C, 30 s at 47°C, 1 min at 72°C with a final extension for 3 min at 72°C. The PCR products were then purified using the QIAquick gel purification kit (QIAGEN) according to the manufacturer's instructions. Sequences were generated using the same set of primers and an Applied Biosystems (ABI) 3100 automated sequencer using the ABI big-dye ready-reaction mix kit, following the standard cycle sequencing protocol.

Phylogenetic analysis. Nucleotide sequences were aligned using CLUSTAL W (Thompson et al. 1994), and alignments were confirmed by translating sequences into amino acid sequences. Phylogenetic relationships between individuals were determined by neighbour-joining (NJ), based on analysis of Kimura 2-parameter (K2P) distance and using PAUP* v4.0 b10 (Swofford 2000) with *Chthamalus moro* and *C. challenger* (collected from Hualien, Taiwan and Wakayama, Japan, respectively) as outgroups. The support for individual nodes was evaluated by 1000 bootstrap replicates. To determine the genealogical relationships among haplotypes, a haplotype network was constructed using the 95% parsimony criterion as implemented by the program TCS 1.13 (Clement et al. 2000).

Population genetic analysis. Haplotype diversity (*h*) and nucleotide diversity (π) were calculated for each population and clade using ARLEQUIN 3.0 (Excoffier et al. 2005). Pairwise Φ_{ST} values were calculated to estimate the level of gene flow among populations within each clade. To test the hypothesis of a phylogeographic break and reduced gene flow between the Indian and Pacific Ocean populations, genetic differentiation between populations from the 2 oceans was examined by an analysis of molecular variance (AMOVA, Excoffier et al. 1992) as implemented in the

same program with K2P distance and 10 000 random permutations to test for statistical significance. This analysis provided insight into the proportion of genetic variation attributable to within-population (Φ_{ST}), within-group (Φ_{SC}), and among-group (Φ_{CT}) differences.

Tajima's *D* test (Tajima 1989) and Fu's *F_S* test (Fu 1997) in ARLEQUIN 3.0 were used to test for departures from mutation and drift equilibria expected for a selectively neutral marker. Negative *D* and/or *F_S* values indicate an excess of low-frequency haplotypes, which may be an indication of rapid demographic expansion. Mismatch distribution analysis (Rogers & Harpending 1992) was conducted with the same software. When a signature of rapid expansion (a unimodal distribution) was detected, we computed the parameter τ , which was used to estimate the approximate timing of population expansion (Schneider & Excoffier 1999). A divergence rate of 3.1% per million years in the COI locus between lineages was calibrated for a trans-isthmian *Euraphia* sister species pair; *Euraphia* is a genus closely related to *Chthamalus* (Wares 2001). As heterogeneity in substitution rate among lineages might lead to bias in the molecular clock, the expansion time obtained should be interpreted with caution and only provides evidence of a causal role of geological events on observed demographic changes.

RESULTS

Vertical zonation

Abundance and vertical zonation varied among *Chthamalus malayensis* populations from the 3 geographic regions: Indo-Malaysia (MM, TQ, MS and SP), southern China (HK, HL and SY) and Taiwan (HA and NE) (Fig. 2). In India, Malaysia and Singapore, *C. malayensis* was the major space occupier, with >50% coverage on the shore and high abundance on the mid to low shore (Fig. 2). At the 3 sites in southern China, *C. malayensis* was abundant only on the high shore, with ~50% coverage (Fig. 2). In Taiwan, *C. malayensis* was found from high to low shores, but abundance was low, with <10% coverage (Fig. 2).

Cohort structure

Cohort structure varied among sites in India, Malaysia and Singapore, southern China and Taiwan. In India, the *Chthamalus malayensis* population comprised a single cohort, with a mean R–C basal diameter of 3 to 4 min (Fig. 3). In Malaysia and Singapore,

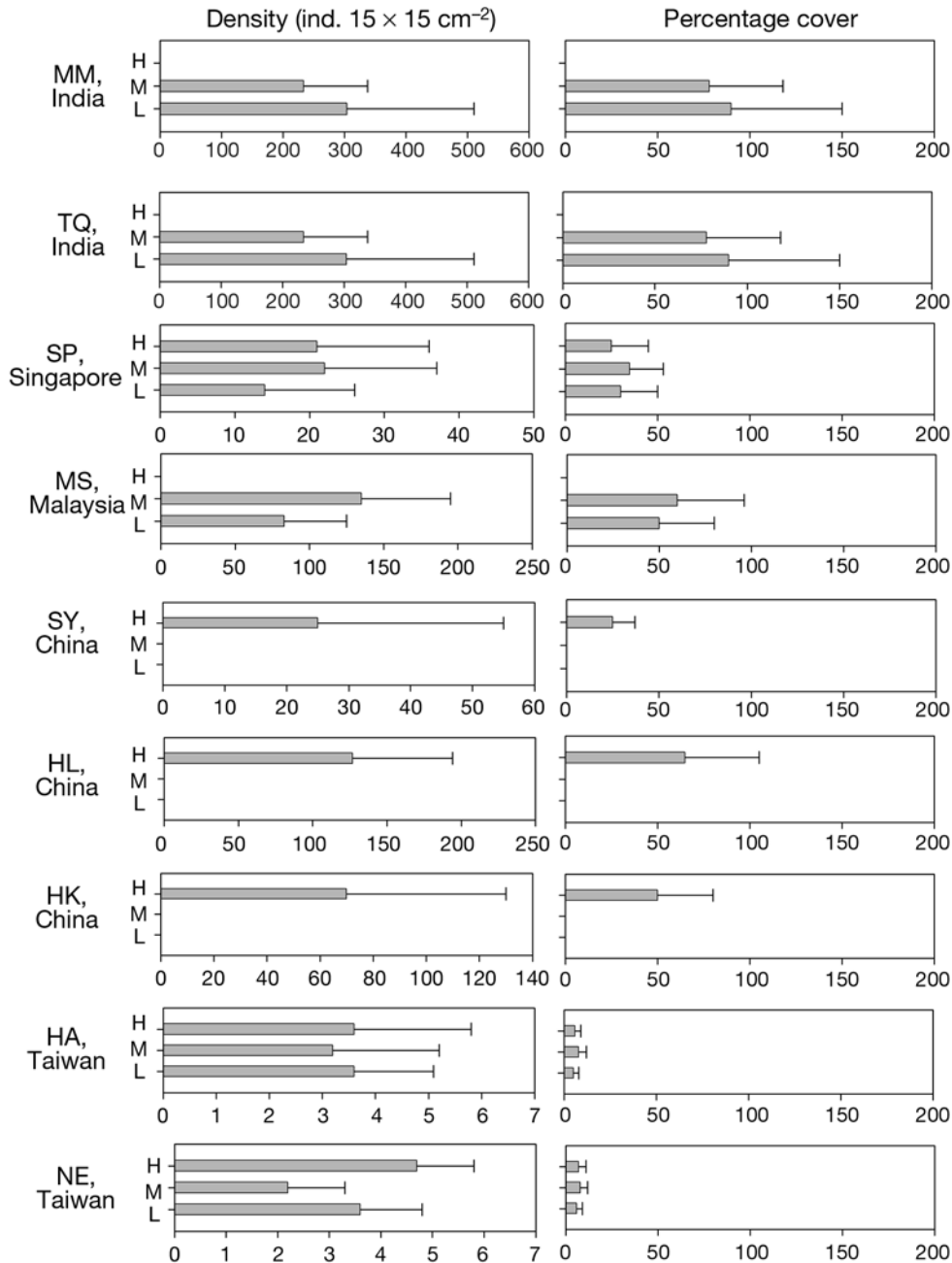


Fig. 2. *Chthamalus malayensis*. Variation in density (ind. $15 \times 15 \text{ cm}^{-2}$) and percentage cover (mean + SD, $n = 10$). MM: Mahabalipuram; see Table 1 for other abbreviations of localities

C. malayensis populations had 2 distinct cohorts, viz. a juvenile cohort with a mean R–C basal diameter of ~2 mm and an older cohort with a mean R–C basal diameter of ~6 to 8 mm. In southern China, populations were dominated by a single cohort, with a mean R–C basal diameter of ~8 mm, whilst a second, larger cohort was identified from Hong Kong (mean R–C basal diameter of 11.2 mm). Taiwan populations had 3 common cohorts, with mean R–C basal diameters of 4 mm, 7.5 mm and 10 mm.

Sequence divergence and phylogenetic analysis

The aligned COI fragments consisted of 624 bp, of which 124 of 195 polymorphic sites were parsimony informative. A total of 81 haplotypes was found among 108 sequences obtained from 12 populations of *Chthamalus malayensis* (Table 1). All representative haplotypes and sequences of the 2 outgroups *C. moro* and *C. challengerii* were deposited with GenBank (Accession nos. EU304363 - EU304448).

Neighbor-joining (NJ) analysis of the 81 haplotypes revealed significant genetic structure within *Chthamalus malayensis*. Three clades were resolved with 100% bootstrap support (Fig. 4). The first clade consisted mainly of samples from the South China Sea (hereafter, the South China Sea clade), including all individuals from the 3 sites in southern China, Thailand and some individuals from the NE coast of Taiwan. The second clade contained all samples from Singapore, Malaysia and the Indian Ocean (hereafter, the Indo-Malay clade); members of the third clade were found only in Taiwan (hereafter, the Taiwan clade). The 3 clades had an allopatric geographical distribution (Fig. 1), the NE coast of Taiwan being the only location where 2 clades (South China Sea and Taiwan clades) co-existed.

The sequence divergences among the 3 clades were high. The South China Sea clade was the most distantly related, differentiated by ~12 to 13% (range

11 to 14.4%) from the other 2 clades in the COI region. Conversely, the Indo-Malay and Taiwan clades differed by only ~7.5% (range 7 to 8.5%). These values were much greater than sequence divergences within each clade ($\leq 2.7\%$).

Population genetic analysis and demographic history of the clades

Since the divergence between clades was high, population genetic parameters were analyzed separately by clade. Haplotype and nucleotide diversity were highest for the South China Sea clade, whilst the Indo-Malay clade had the lowest genetic diversity (Tables 1 & 2). The haplotype network for the South China Sea clade showed that individual haplotypes were randomly distributed within the network and were not clustered by location (Fig. 5a).

Most haplotypes were sampled in only 1 individual. The Indo-Malay clade haplotype network was star-like, with 1 dominant haplotype (~31%) shared by 4 of 5 populations in this clade (Fig. 5b). A majority of the other haplotypes differed from the dominant haplotype by only 1 to 3 mutations (Fig. 5b). No pattern could be observed in the Taiwan clade network due to the limited number of sequences (Fig. 5c).

Most pairwise Φ_{ST} values between populations were small and non-significant within the 3 clades (Tables 3 & 4), suggesting a lack of within-clade genetic structuring. For the Taiwan clade, the value between the 2 sites was -0.0195 , suggesting that the true value was near 0. For the Indo-Malay clade distributed across the Indian and Pacific Oceans, AMOVA revealed a lack of genetic differentiation between ($\Phi_{CT} = -0.01$, $p = 0.49$) and within oceans ($\Phi_{SC} = 0.01$, $p = 0.21$). AMOVA was also performed by grouping Langkawi Island (Malaysia) in the Indian Ocean with the geographically more proximate Singapore and Mersing (Malaysia) populations instead of with Tranquebar and south Sri Lanka. This analysis also revealed little differentiation between the 2 groups ($\Phi_{CT} = -0.04$, $p = 0.97$).

Mismatch distributions for all 3 clades were unimodal (Fig. 6), suggesting a rapid demographic expansion. This was

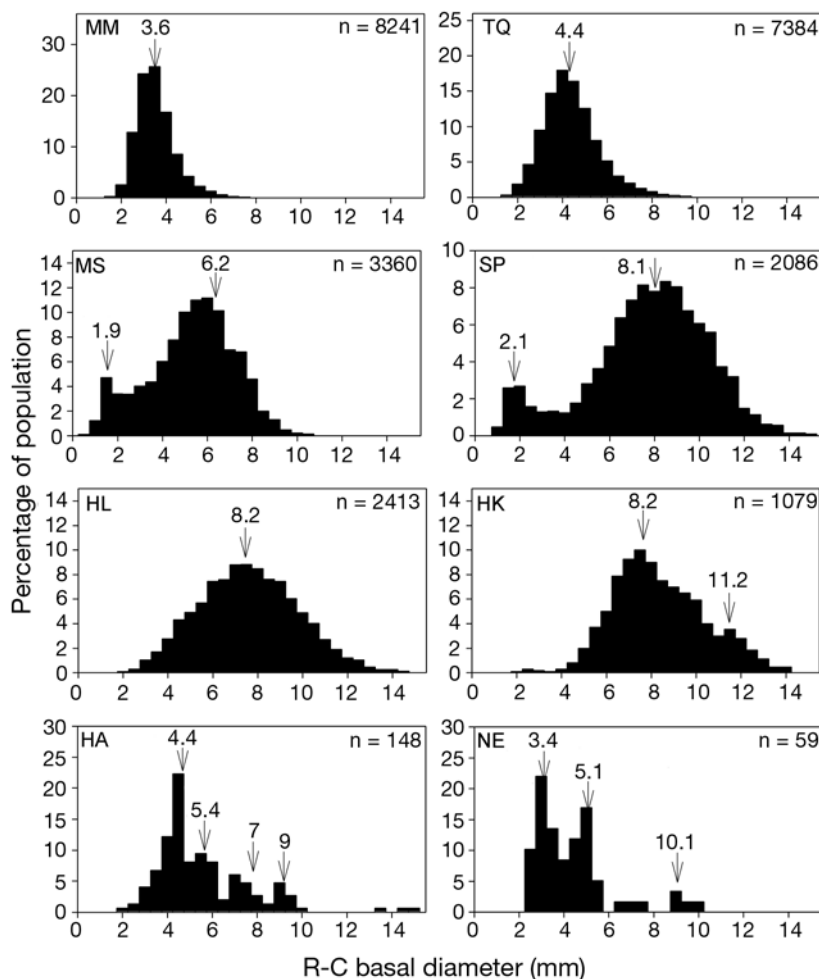
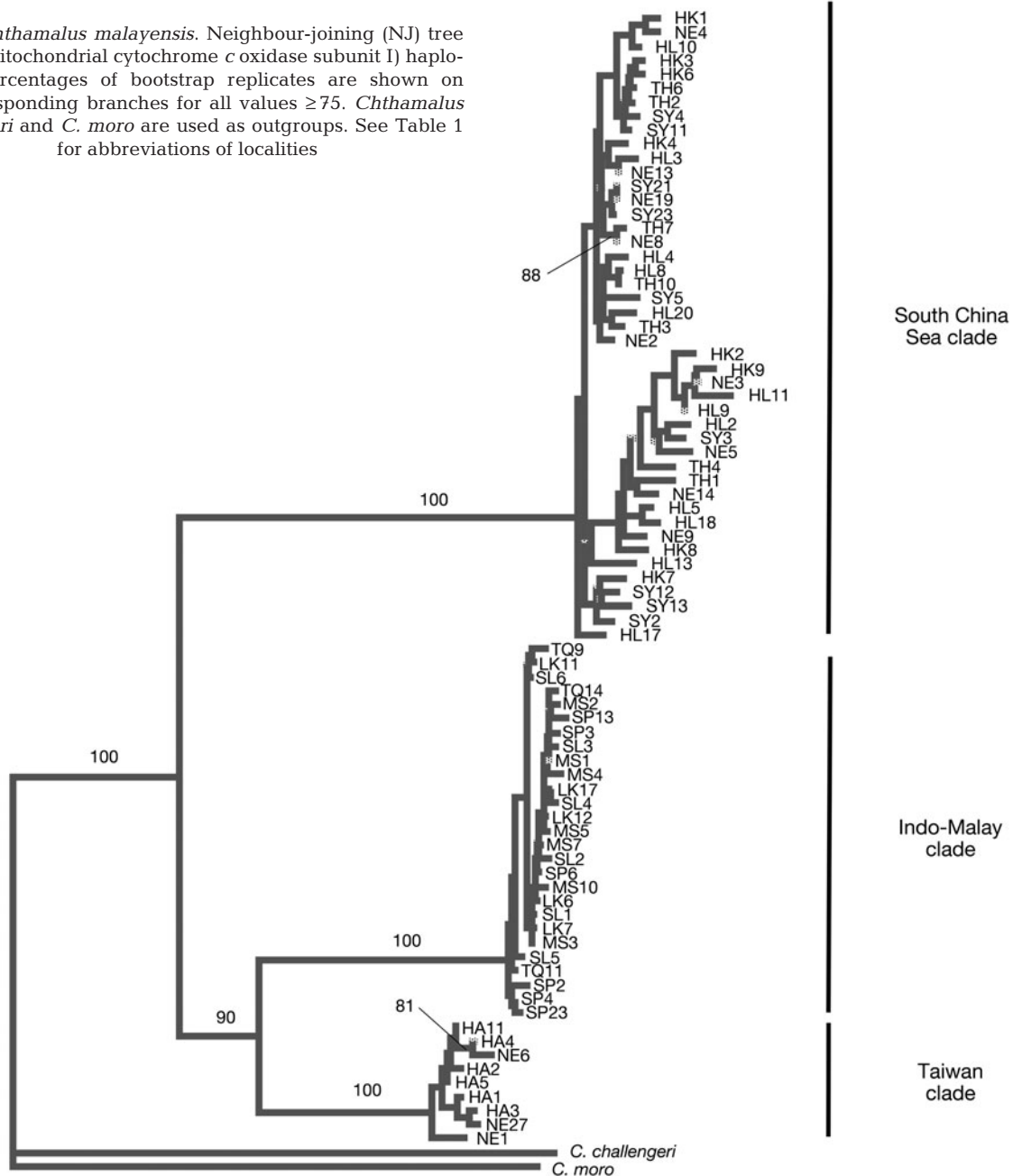


Fig. 3. *Chthamalus malayensis*. Variation in cohort structure. MM: Mahabali-puram; see Table 1 for abbreviations of other localities. Significant cohorts identified by ELEFAN (electronic length frequency analysis) are indicated by arrows and their mean R-C (rostral-carinal) basal diameters (in mm) are given. Note different scales on y-axis. n: sample size

Fig. 4. *Chthamalus malayensis*. Neighbour-joining (NJ) tree of COI (mitochondrial cytochrome *c* oxidase subunit I) haplotypes. Percentages of bootstrap replicates are shown on the corresponding branches for all values ≥ 75 . *Chthamalus challenger* and *C. moro* are used as outgroups. See Table 1 for abbreviations of localities



also supported by negative and significant Tajima's D and Fu's F_S values for the South China Sea clade and the Indo-Malay clade (Table 2). The values of D and F_S were smaller and less significant for the Taiwan clade, which might be due to the limited number of samples. We estimated τ , which reflects the location of the crest of mismatch distribution, because we had observed a unimodal distribution for all 3 clades. τ was largest for the South China Sea clade, indicating earliest expansion in this clade (Table 2). τ values were comparable for the Taiwan and Indo-Malay clades. Using a one million year divergence rate of 3.1% between lineages (Wares 2001), the time of demographic expansion for

the South China Sea, Taiwan and Indo-Malay clades were estimated as ~450 000 BP, 126 000 BP and 88 000 BP, respectively (Table 2).

DISCUSSION

Cryptic diversity of *Chthamalus malayensis*

Among the samples from Asia and the Indian Ocean, the common intertidal acorn barnacle *Chthamalus malayensis* consists of 3 distinct, highly diverged lineages. Mean COI sequence divergences among pairs

Table 2. *Chthamalus malayensis*. Genetic diversity of the three clades. Sample size (n), number of haplotypes, haplotype diversity ($h \pm SD$), nucleotide diversity ($\pi \pm SD$), Tajima's D , Fu's F_s , the mismatch parameter τ and the estimated time of expansion for each clade are shown. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Clade	n	Number of haplotypes	$h \pm SD$	$\pi \pm SD$	Tajima's D	Fu's F_s	τ	Estimated time of expansion
South China Sea clade	48	45	0.997 ± 0.005	0.013 ± 0.007	-1.78*	-24.88***	8.711	450 000 BP
Indo-Malay clade	48	27	0.900 ± 0.039	0.004 ± 0.002	-2.15***	-26.46***	1.711	88 000 BP
Taiwan clade	12	9	0.909 ± 0.080	0.007 ± 0.004	-1.19	-2.87*	2.434	126 000 BP

of the 3 clades (~7.5 to 13%) approach the magnitude of interspecific values for other *Chthamalus* (~15%) species and another acorn barnacle genus *Euraphia* (~9%) (Wares 2001). The sequence divergence between the Taiwan and Indo-Malay clades was the lowest, (~7.5%), indicating that the 2 diverged relatively recently. This close association was unexpected, as the geographical ranges of the 2 do not overlap (based on our sampling). The degree of genetic divergence between the Taiwan and Indo-Malay clades was smaller than that between recognized species of other acorn barnacles (loc. cit.), and it remains to be seen whether these 2 clades of *C. malayensis* are reproductively isolated.

The 3 clades we identified also differed ecologically. The South China Sea clade was restricted to the high shore, whilst the Indo-Malay clade inhabited the mid to low shore. Moreover, the Indo-Malay clade was the dominant space occupant with a cover of >50% (cf. ~50% cover measured for the South China Sea clade). The Taiwan clade was very sparsely distributed on the shore. There are differences in reproductive seasonality between Hong Kong and Singapore *Chthamalus malayensis* populations (Koh et al. 2005, Yan et al. 2006). These 2 populations correspond to the South China Sea and Indo-Malay clades in our present study, suggesting that there are physiological differences between the clades as well as genetic and ecological differences. Integrating all of these observations, it is apparent that there are at least 2 species present in the currently recognized *C. malayensis*. The South China Sea clade has an independent gene pool and, therefore, should be considered as a distinct species, while the species status of the genetically less differentiated Taiwan and Indo-Malay clades requires further analyses using nuclear DNA markers and morphological features.

Postglacial expansion, colonization and recent connectivity across oceans

Both mismatch distribution analysis and neutrality tests indicated population expansion in the 3 clades. This may be attributed to range expansion following

the Pleistocene glaciation. During the Pleistocene, lowering sea levels led to changes in temperature, habitat availability and ocean current patterns that would have dramatically affected the distribution and abundance of marine organisms (Hewitt 2000). Species went extinct over large parts of their range during the glacial maxima, with only a small number of individuals surviving in refugia. Species ranges expanded again from their refugia following retreat of the glaciers (Hewitt 2000). This likely resulted in the high haplotype diversity but relatively low nucleotide diversity we observed in the 3 clades of barnacles.

However, the timing of range expansion differed among the 3 clades. The South China Sea clade was the earliest to expand, whilst the Indo-Malay clade showed the most recent expansion. These differences may be the result of differences in geographic ranges. Large areas of land were exposed in the Sunda Shelf during the Pleistocene (Voris 2000), leading to isolation and large-scale range contraction in marine organism distributions. Thus, the Indo-Malay clade in this region might have experienced a greater contraction in population size and distribution range and was only able to attain its present range after the sea level approached its present-day level. In contrast, the distribution ranges of the other 2 clades are likely to have fluctuated less (Voris 2000). Although there were also range shifts during glaciation, considerable areas of coastal habitat in the South China Sea and western Pacific regions remained in which intertidal species were able to survive. Thus, the differences in geographic range among the 3 clades seem to have resulted in different demographic histories.

Phylogeographic subdivision is lacking between Pacific and Indian Ocean populations in the Indo-Malay clade of *Chthamalus malayensis*. There are 2 possible explanations for this observation. Firstly, the high level of present-day gene flow may have eroded any signature of historical isolation. However, this factor alone does not seem to be a satisfactory explanation for the pattern. If the Pacific and Indian Ocean populations were separated during the Pleistocene, and then came into secondary contact through recent gene flow, divergent haplotypes should be observed even though

hybridization occurred (Hewitt 2000). However, the genetic diversity of both the Pacific and Indian populations is low, and all haplotypes differ by only a few mutational steps, so that this hypothesis is not supported. On the other hand, the low genetic diversity suggests an alternative explanation of recent post-glacial colonization from the Pacific to the Indian Ocean. This might have been achieved by transport of a few planktonic larvae westward from the Pacific through the Indonesian seas, with final settlement in the Indian Ocean (Gordon & Fine 1996). The unimodal mismatch distribution supports this hypothesis. Similar scenarios have also been observed in other IWP taxa (e.g. Lessios et al. 2003) and in the Atlantic-Mediterranean region, where species without population structure have undergone demographic expansion following Pleistocene glaciations (Patarnello et al. 2007).

The founder effect during re-colonization may have also led to a reduction in genetic diversity and shift in haplotype frequency in the Indian Ocean populations, resulting in genetic differentiation between the Pacific and Indian Ocean populations. Moreover, the island chain of the Indo-Malaysian archipelago and Australia limits the current flow between the 2 oceans and would thus restrict larval dispersal. Yet there is clear genetic homogeneity between the 2 oceans and their populations share common haplotypes, indicating considerable contemporary gene flow. However, the populations in India have a different cohort structure from those in Singapore and Malaysia, which are similar in this respect, suggesting that the larval supply sources differ between India and the east coast of the Malay Peninsula. Hence, the connectivity of *C. malayensis* populations between the 2 oceans may be restricted on an ecological time scale, but not on an evolutionary time scale. Dispersal on an ecological time scale would not be detected by mtDNA, which is less sensitive to genetic drift, compared to more variable markers such as microsatellites (Avice 2000).

Biogeography of the western Pacific intertidal

It appears paradoxical that the Indo-Malay clade of *Chthamalus malayensis* has a relatively restricted distribution only in the western Pacific, despite its ability to disperse across the 2 oceans. Factors other than dispersal capacity seem to play a role in determining the biogeographic structure of the 3 clades. The South China Sea and the neighboring areas have a dynamic hydrology that greatly affects the dispersal and distribution of marine taxa (Gordon & Fine 1996, Guan & Fang 2006). Comparison of the distributions of the 3 clades of *C. malayensis* with those of the sympatric *Tetraclita squamosa* species complex (which

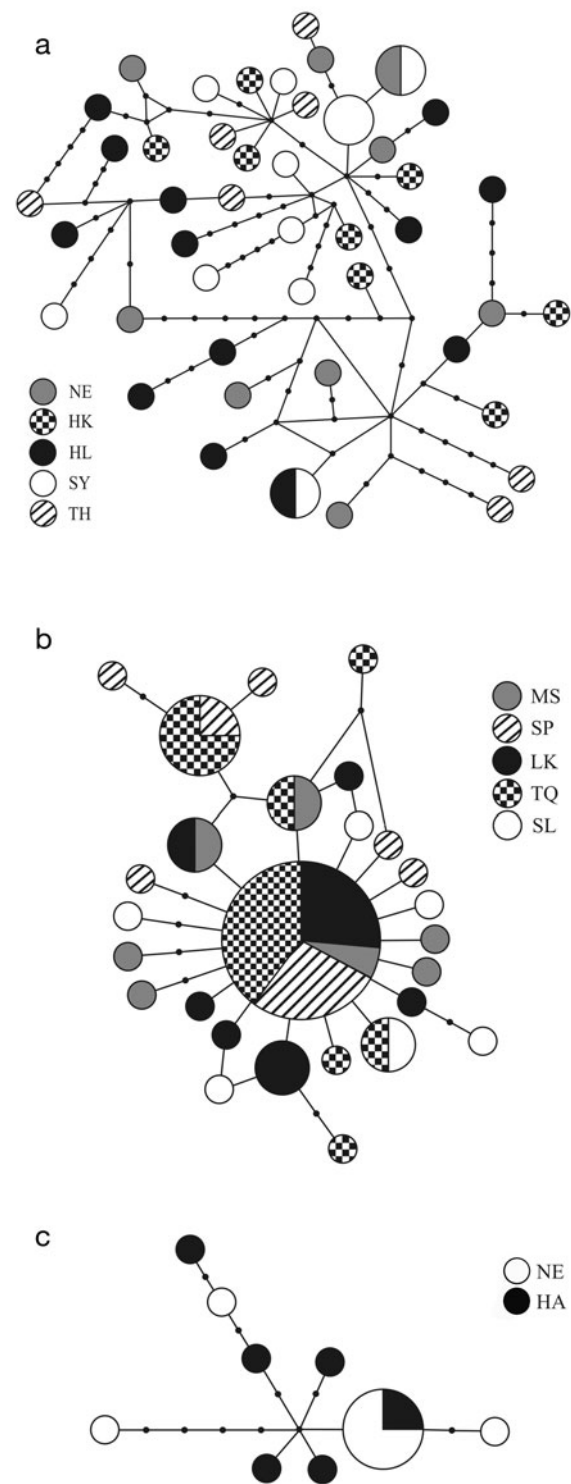


Fig. 5. *Chthamalus malayensis*. COI (mitochondrial cytochrome *c* oxidase subunit I) haplotype network of (a) South China Sea clade, (b) Indo-Malay clade, and (c) Taiwan clade. Nodes along each branch designate the number of base differences among haplotypes. Patterns within the circles denote localities (see Table 1 for abbreviations), circle areas are proportional to the number of individuals within the particular haplotype

Table 3. *Chthamalus malayensis*. Pairwise Φ_{ST} values for populations of the South China Sea clade based on COI (mitochondrial cytochrome *c* oxidase subunit I). * $p < 0.05$. See Table 1 for abbreviations of localities

Population	NE	HK	HL	SY
HK	-0.0447			
HL	-0.0194	0.0004		
SY	0.0363	0.0241	0.0897*	
TH	-0.0322	-0.0255	0.0064	-0.0105

Table 4. *Chthamalus malayensis*. Pairwise Φ_{ST} values for populations of Indo-Malay clade based on COI (mitochondrial cytochrome *c* oxidase subunit I). * $p < 0.05$. See Table 1 for abbreviations of localities

Population	MS	SP	LK	TQ
SP	-0.0043			
LK	-0.0057	0.0531		
TQ	-0.0022	-0.0430	0.0291	
SL	0.0049	0.0688*	-0.0286	0.0681

also consists of 3 species with overlapping ranges, Chan et al. 2007a,b), reveals a certain degree of concordance between the 2 groups. The coast of southern China is dominated by *T. squamosa* and the South China Sea clade of *C. malayensis*, while *T. kuroshioensis* and the Taiwan clade of *C. malayensis* are the major inhabitants in eastern Taiwan (Chan et al. 2007a). The southern China coast is under the influence of the South China Sea Current, while the Kuroshio Current is prevalent in east Taiwan (Guan & Fang 2006). Variation in larval supply, driven by the distinct ocean current systems, may contribute to the observed distributions. Further south, towards tropical SE Asia, these species are replaced by *T. singaporensis* and the Indo-Malay clade of *C. malayensis* in Singapore (Chan et al. 2007b). This genetic transition also occurs in the wrasse *Thalassoma hardwicki* and is attributable to complex local circulation patterns that block gene flow between the northern and southern parts of the South China Sea (Chen et al. 2004). Thus, the ocean current configuration may play an important role in determining the genetic structures and species ranges in the western Pacific intertidal. Restrictions in water currents and gene flows between the ocean systems may result in the variation in barnacle cohort structure (see Jenkins et al. 2001). Cohort structure of *C. malayensis* differs among (1) Malaysia and Singapore, (2) southern China and (3) Taiwan waters, but within each of these regions there is similarity in cohort structure, suggesting that the patterns of larval supply and gene flow (which are related to ocean currents) differ among regions.

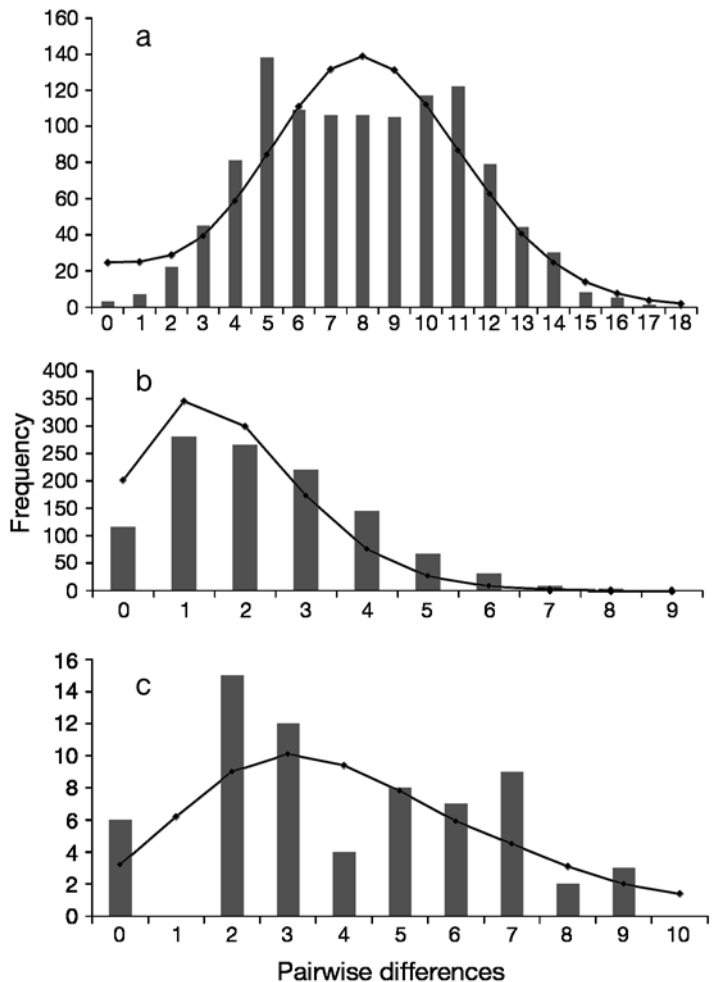


Fig. 6. *Chthamalus malayensis*. Observed (bars) and expected (line) mismatch distributions under a model of sudden demographic expansion for COI (mitochondrial cytochrome *c* oxidase subunit I) haplotypes: (a) South China Sea clade, (b) Indo-Malay clade, and (c) Taiwan clade. Harpending's raggedness indices: $r = 0.008$ ($p = 0.70$) for the South China Sea clade, $r = 0.034$ ($p = 0.76$) for the Indo-Malay clade, and $r = 0.095$ ($p = 0.15$) for the Taiwan clade

In addition to the physical dispersal pattern, the interactions of local physical and biological factors will contribute to maintaining allopatric distribution of the species. The 3 clades of *Chthamalus malayensis* have different vertical zonations and abundances in the intertidal zone, suggesting that they may respond differently to environmental factors. The major factors known to determine the upper shore limit of barnacles are post-settlement mortality, induced by heat stress (Southward 1962, Chan et al. 2006), and competition with other species (Connell 1961). In Indo-Malay waters, the Indo-Malay clade of *C. malayensis* is the most common barnacle and the major space occupant on the mid shore; *Tetraclita* is much less abundant.

Along the coast of southern China, the mid shore is dominated by *T. squamosa* and *T. japonica japonica*, while the South China Sea clade of *C. malayensis* is restricted to the high shore. This distribution pattern may be related to the higher tolerance of this clade to heat stress, or avoidance of competition with *Tetraclita* species. In Taiwan, the abundance of *C. malayensis* (the Taiwan clade) was the lowest among the areas studied, and individuals were sparsely distributed within the *Tetraclita* zone in the mid and high shore. Such variations in zonation pattern among the 3 clades of *C. malayensis* also occur in *Tetraclita* (Chan et al. 2008). In Singapore, only *T. singaporensis* has been recorded and it occurs in the mid shore (B. Chan pers. obs.). In Hong Kong, 2 *Tetraclita* species have a distinct vertical separation, with *T. squamosa* on the low shore and *T. japonica* on the mid shore. In Taiwan, *T. kuroshioensis* and *T. japonica formosana* occur together on the mid shore (Chan et al. 2008). Thus, biological interaction appears to be a factor affecting the geographical ranges of barnacles, and probably the ranges of other fauna.

Our study indicates that there is likely a high level of hidden biodiversity within the IWP intertidal biota awaiting discovery and investigation. The interactions between biological attributes among lineages and environmental factors shape genetic structure and distribution across the range of the barnacle species complex. Thus, studies which focus solely on genetic differentiation among populations in relation to physical factors such as ocean current pattern will not fully elucidate the origin of high levels of biodiversity in the IWP. Information on the ecology of individual taxa should also be considered in order to interpret and understand the biogeographic patterns observed.

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