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# Contrasting demographic history and phylogeographical patterns in two Indo-Pacific gastropods

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## Abstract

**Marine species with ranges that span the Indo-Australian Archipelago (IAA) exhibit a range of phylogeographical patterns, most of which are interpreted in the context of vicariance between Indian and Pacific Ocean populations during Pliocene and Pleistocene low sea-level stands. However, patterns often vary among ecologically similar taxa, sometimes even within genera. This study compares phylogeographical patterns in two species of highly dispersive neritid gastropod, *Nerita albicilla* and *Nerita plicata*, with nearly sympatric ranges that span the Indo-Pacific. Mitochondrial COI sequences from > 1000 individuals from 97 sites reveal similar phylogenies in both species (two divergent clades differing by 3.2% and 2.3%, for *N. albicilla* and *N. plicata*, respectively). However, despite ecological similarity and congeneric status, the two species exhibit phylogeographical discordance. *N. albicilla* has maintained reciprocal monophyly of Indian and Pacific Ocean populations, while *N. plicata* is panmictic between oceans, but displays a genetic cline in the Central Pacific. Although this difference might be explained by qualitatively different demographic histories, parameter estimates from three coalescent models indicate that both species have high levels of gene flow between demes ($2N_em > 75$), and share a common history of population expansion that is likely associated with cyclical flooding of continental shelves and island lagoons following low sea-level stands. Results indicate that ecologically similar, codistributed species may respond very differently to shared environmental processes, suggesting that relatively minor differences in traits such as pelagic larval duration or microhabitat association may profoundly impact phylogeographical structure.**

*Keywords*: demography, Indo-Pacific, mtDNA, *Nerita*, nonequilibrium, phylogeography, vicariance

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## Introduction

Pliocene and Pleistocene glaciations have strongly influenced patterns of species and population level diversity on a global level. In the temperate zones, glacial cycles resulted in species range contractions into lower latitudes followed by range expansions during interglacial periods (Hewitt 1996, 2000). In many terrestrial and freshwater species, these fluctuations resulted in a characteristic pattern of reduced genetic diversity within higher latitude populations and reciprocal monophyly or narrow zones of secondary contact among formerly allopatric populations (Avise 1992; Hewitt 1996; Bernatchez & Wilson 1998). Within this general pattern, qualitatively different demographic histories have led to a variety of distinct subpatterns that are unique to geographical regions (reviewed in Avise 2000; Hewitt 2000).

In the ocean, many temperate marine species also experienced latitudinal range contractions as a result of Plio–Pleistocene glaciations (see Wares 2002 for review). However, for tropical marine species, one of the primary impacts was the effects of sea levels dropping to 130 m below present during glacial maxima (Porter 1989; Voris 2000). These impacts were particularly strong in tropical areas like the Indo-Australian Archipelago (IAA), which are characterized by broad, shallow continental shelves that become exposed during low sea-level stands. In the IAA, Pleistocene sea-level fluctuations closed the Torres, Sunda and Malacca straits

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more than 10 times over the past million years (Pillans *et al*. 1998), and on six different occasions during the past 250 000 years alone, amounting to about half of this time period (Voris 2000).

Many marine species possess highly dispersive planktonic larvae (Thorson 1950) that should enhance genetic connectivity at large spatial scales (Lester & Ruttenberg 2005). However, the restrictions to the seaways of the IAA resulting from lowered sea levels combined with freshwater discharge from large river drainages (Voris 2000) and cold-water upwelling (Fleminger 1986) may have resulted in allopatric barriers, promoting regional lineage diversification. Many pairs of geminate species with ranges that abut at the IAA (Springer & Williams 1990; Randall 1998), along with intraspecific phylogeographical studies (below), uniformly implicate Pleistocene vicariance in promoting regional divergence in taxa that span the IAA.

Marine species vary broadly in the nature and magnitude of their apparent genetic response to increased isolation across the IAA during low sea levels, but three qualitative patterns emerge (cf. categories I, II and IV of Avise 2000). First, despite contemporary sea levels and physical oceanography that should promote mixing between the Pacific and Indian Oceans, many taxa consist of two or more reciprocally monophyletic clades corresponding to these formerly allopatric ocean basins, with only a small amount of admixture due to recent gene flow or hybridization. Examples include butterflyfish of the *Chaetodon punctatofasciatus* and *C. rhomobochaetodon* species complexes (McMillan & Palumbi 1995), black tiger prawns *Penaeus monodon* (Duda & Palumbi 1999b; Benzie *et al*. 2002), the urchin *Diadema paucispinum* (Lessios *et al*. 2001), stomatopods *Gonodactylellus viridis*, *Haptosquilla pulchella*, and *H. glytocercus* (Barber *et al*. 2006), seahorses *Hippocampus kuda* and *H. trimaculatus* (Lourie *et al*. 2005), and the gastropod *Echinolittorina trochoides* A (Reid *et al*. 2006). Although there is little concordance in the exact location of phylogeographical breaks, and divergence dates range from the late Pleistocene to early Miocene (Kirkendale & Meyer 2004; Barber & Bellwood 2005; Lourie *et al*. 2005), most of these studies implicate vicariance during glacial cycles as a force in creating the observed patterns.

Second, many taxa retain two divergent clades that occur in sympatry. While the degree of admixture differs among species, differences in the relative frequencies of the two lineages usually result in moderate genetic structuring between ocean basins. Proposed examples include the seastar *Linckia laevigata* (Williams & Benzie 1998), the snapping shrimp *Alpheus lottini* (Williams *et al*. 2002), the tasselfish *Polynemus sheridani* (Chenoweth & Hughes 2003), the barramundi *Lates calcarifer* (Chenoweth *et al*. 1998), and the pelagic scad *Decapterus russelli* (Perrin & Borsa 2001). The depth of divergence among these clades suggests that they arose from historical allopatry, particularly due to Pleistocene sea-level fluctuations.

Finally, despite the profound effects of sea level fluctuations, some taxa show no phylogeographical evidence of historical isolation between ocean basins. Examples include the urchins *Eucidaris metularia* (Lessios *et al*. 1999), *Diadema savignyi* (Lessios *et al*. 2001) and *Tripneustes gratilla + depressus* (Lessios *et al*. 2003), the trumpetfish *Aulostomus chinensis* (Bowen *et al*. 2001), and the gastropod *Echinolittorina reticulata* (Reid *et al*. 2006). Regional genetic structure in these taxa is usually correspondingly low (but not always, see Lessios *et al*. 2003). The absence of phylogeographical structure in these taxa could result from continued dispersal through the IAA during low sea level stands as the waterways of the IAA were restricted, but not closed (Voris 2000). Alternatively, the lack of observed divergence could result if one of the two divergent lineages were lost due to local extinction or a selective sweep (Grant & Bowen 1998) or if the ranges of these species did not span the IAA during Plio-Pleistocene sea level fluctuations.

The diversity and complexity of phylogeographical patterns exhibited by species whose ranges presently span the IAA suggest that taxa respond differently to sea-level fluctuations at the IAA. Moreover, discordances among congeneric taxa (e.g. *Diadema* spp. and *Echinolittorina* spp. cited above) imply that potentially subtle taxon-specific variation in characteristics such as dispersal ability, biotic interactions or habitat restrictions along with historical differences in distribution, gene flow, or effective population size may contribute to phylogeographical patterns across the IAA. It is difficult, however, to untangle the effects of taxon-specific traits from contingency in historical demography.

*Nerita albicilla* and *Nerita plicata* (Neritopsina: Neritidae) are intertidal gastropods with long-lived planktotrophic larvae (Kano 2006) and largely sympatric ranges that span the IAA. While both species extend to the shores of East Africa in the west, *N. albicilla* reaches its eastern boundary at the Cook Islands, while *N. plicata* extends all the way to Easter Island. Pelagic larval duration of *Nerita* species may be up to 6 months (Underwood 1975); however, there are no precise estimates of pelagic larval duration for either *N. albicilla* or *N. plicata*. Nevertheless, the observed differences in range size between these species suggest that the larvae of *N. plicata* may spend a longer period in the plankton, since Indo-Pacific range sizes may be correlated with estimates of pelagic larval duration in both fish and gastropod taxa (Lester & Ruttenberg 2005; Paulay & Meyer 2006). The two species are broadly similar in their adult ecology, but differ subtly in their habitat associations. While *N. plicata* lives in the high intertidal, generally on any unbroken rocky substrate above the waterline, *N. albicilla* primarily inhabits rubble and cobble fields with low wave energy that are often associated with inner-reef flats (Hughes 1971; Vermeij 1971).

Given their similarities in adult ecology and broadly sympatric geographical distributions that span the IAA,

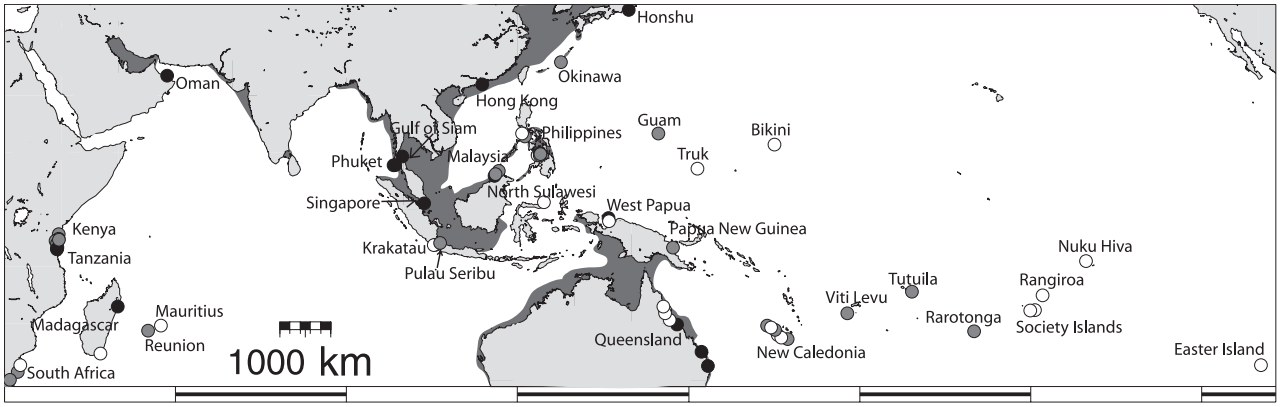


**Fig. 1** Map of the Indo-Pacific showing localities sampled. Dark grey shading delineates the approximate 100 m continental depth contour for continental regions (Smith & Sandwell 1997; Voris 2000). Sea level was at or below this depth for ~25% of the last 250 000, closing most of the major seaways between the Indian and Pacific Oceans. Black circles mark localities where only *Nerita albicilla* was sampled, white circles mark localities where only *N. plicata* was sampled and grey circles mark localities where both were sampled. Sampling of only one species does not necessarily mean that the other species was absent from this locality.

we predict that these congeneric taxa should have been similarly impacted by the numerous Plio–Pleistocene sea-level fluctuations that periodically increased isolation of Pacific and Indian Ocean populations. To test this hypothesis, we characterize phylogeographical patterns in these two congeners using cytochrome oxidase I (COI) mtDNA sequences comprehensively sampled from populations throughout the ranges of both species. We then reconstruct the demographic history of each species using several different coalescent models to determine whether qualitative differences in demographic history or species-specific traits may impact the observed phylogeographical patterns.

## Materials and methods

### *Sampling and sequencing*

We collected *Nerita albicilla* ($n$ = 529, 60 localities) and *Nerita plicata* ($n$ = 653, 73 localities) from a total of 97 localities throughout the Indo-Pacific region from 2000 until 2005 (Fig. 1) and preserved specimens in 95% ethanol or 20% DMSO.

DNA amplification and sequencing was conducted at Boston University and the University of California, Davis (UC Davis). We amplified a 658-bp region of the mitochondrial cytochrome oxidase subunit-I gene (COI) using primers HC0-2198 and LCO-1490 (Folmer *et al.* 1994). At Boston University, DNA was extracted in a 10% Chelex (Biorad) solution (Walsh *et al.* 1991). Polymerase chain reaction (PCR) occurred in 25 L reactions with 2.5 L of 10x buffer, 2 L $MgCl_2$ (25 mM), 2.5 L DNTPs (8 mM), 1.25 L of each 10 mM primer, 1 L of template, and 0.625 U of Ampli*Taq* (Applied Biosystems). At UC Davis, DNA was extracted with a Puregene kit (Gentra) and PCR-amplified in 50 L volumes, with 5 L of 10× buffer, 5 L $MgCl_2$ (25 mM), 5 L DNTPs (1 mM), 0.5 L of Bovine Serum Albumin, 2.5 L of each 10 mM primer, 2 L of template, and 1 U of Ampli*Taq*. Thermocycling conditions were: initial denaturation 94 °C (15 s), main cycle 94 °C (30 s), 50 °C (30 s) and 72 °C (30–40 s) for 35–39 cycles, then a final extension of 72 °C (3–10 min).

Following ethidium-bromide visualization on a 1% agarose gel, we cleaned PCR products by adding 0.5 U of Shrimp Alkaline Phosphatase (Biotech Pharmacon) and 5 U of Exonuclease I (GE Healthcare) to 5 L of PCR product and incubating at 37 °C for 30 min and 80 °C for 15 min. Sequencing reactions were performed for both forward and reverse strands using BigDye (Applied Biosystems) terminator chemistry, and run on an ABI 377 or ABI 3730 (Applied Biosystems) automated DNA sequencer. Complementary strands for each sample were proofread and aligned in SEQUENCHER, and translations confirmed using MACCLADE 4.05 (Maddison & Maddison 2002).

### *Phylogeographical analyses*

We characterized phylogeographical patterns iteratively. First, we calculated pairwise $\Phi_{ST}$ values among sampling localities with ARLEQUIN 2.0 (Schneider *et al.* 2000), and tested significance of these values with 10 000 random replicates and applied a standard Bonferroni correction for multiple tests. To improve statistical power, we then pooled localities that showed no pairwise structure (i.e. those localities with a pairwise $\Phi_{ST}$ that did not significantly differ from 0) into demes defined by country and region, and recalculated pairwise $\Phi_{ST}$ as well as pairwise Wright's $F_{ST}$. We excluded demes with fewer than five individuals from all population-level analyses due to lack of statistical

power. Second, we used analysis of molecular variance (AMOVA), implemented in ARLEQUIN 2.0, to characterize regional patterns of genetic differentiation and to calculate haplotype and nucleotide diversity indices. Initially, we defined a priori regional groupings comprised of Pacific and Indian Ocean demes; however, for *N. plicata*, we also ran analyses with Rarotonga, Rangiroa, and Society Island localities in one group and all other localities in the other, corresponding to the observed phylogeographical structure. For *N. albicilla*, we included Krakatau and Pulau Seribu with Pacific Ocean demes, due to their extremely high proportion of Pacific clade haplotypes. For *N. plicata*, in which distinct mitochondrial clades were geographically mixed, we analysed the data with highly divergent clades (those differing by more than 10 steps) included and excluded so that results were not driven by admixture.

Minimum-spanning trees were calculated in ARLEQUIN based on pairwise sequence differences and hand-drawn with Adobe Illustrator. Alternative connections were evaluated for any possible significant changes in topology. The frequencies of clades exceeding 10 steps divergence were summarized in pie diagrams and plotted onto a map of the Indo-Pacific region. For each tip clade that qualitatively resembled a star polytomy, we calculated the mean and variance of the mutational distance to the central haplotype as an unbiased indicator of the age of the clade relative to others, assuming the correct topology and a panmictic population (Saillard *et al*. 2000).

### *Coalescent models of demographic history*

Phylogeographical analyses suggested strong departures from neutrality and gene flow-drift equilibrium, which can be caused either by selection or changes in effective population size. We addressed this issue with analyses using four different coalescent approaches. The first of these uses summary statistics to identify departures from neutrality, while the other three use Markov chain Monte Carlo (MCMC) methods to estimate demographic parameters of three different but complementary models.

We first tested the data against a neutral Wright-Fisher model using Fu's $F_S$ (Fu 1997) which aims to identify an excess of recent substitution events caused by population growth, genetic hitchhiking, or background selection. To further evaluate the relative contributions of these processes to genetic patterns apparent in the data, we also calculated D* of Fu & Li (1993), which considers the difference between two estimates of θ, one of which incorporates only substitutions on the external branches. Fu (1997) found that $F_S$ has more power to detect population growth and genetic hitchhiking than D*, while the opposite is true for background selection. Thus, the two statistics can be used in conjunction to evaluate the relative importance of background selection vs. hitchhiking or population growth (Fu 1997). We performed all of these tests in DNASP 4.10.7 (Rozas *et al*. 2003). Significance of $F_S$ was determined by 1000 coalescent simulations of the neutral model, while that of D* was determined by the critical values presented in Fu & Li (1993), where $p$ must be less than 0.02 to be significant due to the non-normal distribution of the statistic.

Second, because departures from neutrality are often caused by changes in effective population size, we used a Bayesian skyline plot to fit demographic models to the data from each species for comparative purposes. The Bayesian skyline plot (Drummond *et al*. 2005) is an extension of the generalized skyline plot (Strimmer & Pybus 2001), which constructs a model of demographic history based on how the number of coalescent events over a given interval differs from that expected under a neutral model for a panmictic population, where optimal intervals are chosen using an Akaike information criterion. While the generalized skyline plot uses a single genealogy, the Bayesian method, as implemented in BEAST version. 1.4.1 (Drummond *et al*. 2002; Drummond 2003), summarizes over all possible genealogies and provides confidence intervals for all parameters in the model. Because the model assumes a single panmictic population, we analysed the two clades of both species separately, using randomized subsets of 100 sequences for each clade. To check for convergence, we ran each subset twice for 50 million steps under an HKY + G model of mutation (chosen using hLRTs in MODELTEST 3.7, Posada & Crandall 1998) with parameters estimated from the data, a constant skyline model with five groups, and uniform priors. We checked replicate runs from each subset against each other in the adjunct program, Tracer. We combined the log files from both runs using LogCombiner to check effective sample size values, but report results from only one of the replicate runs due to computer memory constraints. We also used BEAST to estimate the time to most recent common ancestor (TMRCA) for each clade as well as for the combined data set.

Third, because AMOVA tests revealed genetic breaks between geographical regions in both species, we used the program IM to fit a coalescent model of isolation with migration (Hey & Nielsen 2004) to both of these breaks. This model allows us to estimate the coalescent parameter, θ, for two divergent populations and their common ancestor, as well as the migration rate between them, $m$, and the time, $t$, since they split. For *N. albicilla*, the data set included 200 randomly selected sequences composed of 100 sequences from each of the Indian and Pacific Ocean demes, as defined above. We created a similar data set for *N. plicata* with sequences drawn from Rarotonga, the Society Islands and Rangiroa vs. the remaining Indo-Pacific. Following several initial runs of ~1 million steps, the maximum values for priors were set for *N. albicilla* as θ12 = 2500, $\theta_A$ = 500, $t$ = 5, and $m_{12}$ = 5 and for *N. plicata* as as θ12 = 20 000, $\theta_A$ = 2000, $t$ = 5, and $m_{12}$ = 20. To check for convergence, the *N. albicilla*

data set was run three times for more than 30 million steps (and a burn-in of 150 000 steps), while the *N. plicata* data set was run once with 20 million steps, and then three more times with a heated burn-in, and between four and seven heated metropolis-coupled chains that were each run for between 3 and 7 million steps.

Fourth, to compare dispersal potential in both species, we estimated gene flow in the Pacific under a structured model of the coalescent available in the Bayesian implementation of LAMARC 2.0.2 (Kuhner *et al*. 2005; Kuhner 2006). Gene flow was estimated between demes on New Caledonia, Fiji, American Samoa and Rarotonga under a nonequilibrium island model (θ, *m* and growth rate (*g*) all constrained to be equal between demes). These demes are separated by an average of ~2000 km of deep water that requires long distance dispersal of larvae for successful gene flow to occur. We ran the analyses under an F84 mutation model with the observed ti:tv ratios of 10.29 and 20.56, and gamma shape parameters of 1.26 and 1.51 for *N. albicilla* and *N. plicata*, respectively, as determined by PAUP* 4.b10 (Swofford 2002) on a neighbour-joining tree. Uniform, linear priors for migration and growth rate were from –500 – 4000 and 1 – 2000, respectively, and a logarithmic prior for θ ranged from 0.001 to 100. We ran three replicate runs for each species, one of 4 million steps and 2 of 2 million steps each, with a sampling increment of 20, and 200 000 steps discarded for the burn-in. Because we found a sharp cline in clade frequency in this region for *N. plicata*, we also ran three replicate runs on a data set with clade B removed. Ancestral θ was calculated using the formula $\theta_A = \theta e^{-gt}$ where *t* is any time in the past divided by a 3-year generation time. We chose a *t* equal to the mean TMRCA estimated by BEAST for clade B in *N. albicilla* and clade A in *N. plicata* (Table 6, Fig. 4).

Because the latter three models give parameters that are scaled by a mutation rate, we converted the estimates to demographic units assuming a generation time of 3 years (Underwood 1975; Y. Kano, personal communication) and a per-site lineage mutation rate of 0.5% per million years (myr) to make all estimates comparable to each other and intuitive with respect to the history of the region (Fig. 4). This lineage mutation rate is equal to a 1%/myr divergence rate, which falls in the middle of a range of previously calculated fossil-calibrated divergence rates based on molluscan COI (Marko 2002; 0.7–1.2%/myr).

## Results

We sequenced a total of 658 bp of mitochondrial COI from 653 individual *Nerita plicata* and 529 *Nerita albicilla*, yielding 479 and 355 unique haplotypes, respectively (GenBank Accession nos EU252619–EU253452). All sequences aligned easily and translated without stop codons. Six different nonsynonymous substitutions occurred in single individuals in *N. plicata*, whereas all substitutions were synonymous in *N. albicilla.*

Haplotype diversity was extremely high in all demes for both species ($h > 0.94$ in *N. albicilla* and $h > 0.99$ in *N. plicata*). Nucleotide diversity ranged from 0.006 to 0.016 in *N. albicilla* and 0.009–0.023 in *N. plicata* (Tables 1 and 2). In *N. albicilla,* 49 (13.9%) haplotypes were shared between more than one individual, with 40 shared among demes and nine private to a single deme. Similarly, in *N. plicata*, 76 (16.2%) haplotypes occurred multiple times, with 64 shared with at least one other deme, and the remainder being private.

### *Phylogeographical analyses*

Minimum-spanning trees (MSTs) for both species exhibit similar phylogenetic patterns, but differ markedly in their phylogeographical structure (Figs 2a and 3a). Both trees have two clades (A and B) separated by a large genetic break of 21 steps (3.2% sequence divergence, 4.1% average divergence, 3.4% net divergence) for *N. albicilla* and 15 steps (2.3% sequence divergence, 3.4% average divergence, 2.6% net divergence) for *N. plicata*. In *N. albicilla*, the two clades largely correspond to the Indian Ocean (clade A) and Pacific Ocean (clade B), with minor mixing occurring in Singapore, Krakatau, Pulau Seribu, New Caledonia, and Rarotonga (Fig. 2b). Additionally, a single divergent individual from South Africa fell out 18 steps from the Pacific clade. This individual is monophyletic with *N. albicilla* when placed on a larger phylogeny of *Nerita* (M. Frey, unpublished data), but was removed from coalescent analyses. In contrast to the regional concordance in *N. albicilla*, both clades of *N. plicata* are distributed across the entire range of the species (Fig. 3b). However, clade B is much less frequent than clade A, representing 10% of total haplotypes, and has a proportionally larger representation of Central Pacific haplotypes (63% come from the Society Islands, Rarotonga, Rangiroa, Marquesas, American Samoa, and Fiji).

The general topology of both MSTs consists of multiple 'star' polytomies, with central high frequency haplotypes separated by one or two base differences from multiple singleton haplotypes that lack discernible geographical clustering. For example, in *N. plicata* the most common haplotype appeared 28 times in 17 out of 27 demes, from South Africa to Easter Island. Of the rarer haplotypes, three co-occur in the Marquesas and South Africa, two appear in the Society Islands and Tanzania, and two are shared between both Easter Island and Kenyan samples. In *N. albicilla*, there is a similar lack of geographical association for haplotypes within both the Indian and Pacific Ocean clades, but only one haplotype is shared between oceans. The mean mutational distance to the central haplotype in star-like tip clades ranged from 0.58 to 1.83 in *N. albicilla*, corresponding to 177 000–557 000 years before present (BP). In *N. plicata*, this distance ranged from 1.08 to 2.08, or 330 000–633 000 BP.

**Table 1** Summary statistics and neutrality test statistics for *Nerita albicilla*. Haplotype diversity ($h$) and nucleotide diversity ($\pi$) calculated in ARLEQUIN (Schneider *et al*. 2000). $F_s$ and D*, measure departure of the data from neutrality (Fu 1997) and were calculated in DNASP (Rozas *et al*. 2003). Indian Ocean demes are in bold type

| Country — region | $n$ | $h$ | $\pi$ | $F_S$ | D* |
|---|---|---|---|---|---|
| American Samoa — Tutuila | 9 | 1 | 0.012 | –3.44 (NS) | –0.71 (NS) |
| Australia — Queensland | 30 | 0.99 | 0.009 | –22.26* | –2.95* |
| Fiji — Viti Levu | 40 | 0.98 | 0.009 | –12.52* | –3.19* |
| Guam | 5 | 1 | 0.009 | –1.06 (NS) | –0.78 (NS) |
| Hong Kong | 30 | 0.98 | 0.008 | –17.33* | –2.48 (NS) |
| Indonesia — West Papua | 19 | 0.99 | 0.009 | –14.40* | –1.07 (NS) |
| Indonesia — Krakatau | 18 | 1 | 0.016 | –9.77* | –0.42 (NS) |
| Indonesia — Pulau Seribu | 19 | 0.99 | 0.015 | –6.55* | –0.38 (NS) |
| Japan – Honshu | 20 | 1 | 0.010 | –16.62* | –1.65 (NS) |
| Japan — Okinawa | 21 | 0.99 | 0.010 | –11.28* | –1.82 (NS) |
| **Kenya** | 39 | 0.99 | 0.008 | –33.53* | –4.45* |
| **Madagascar** | 2 | 1 | — | — | — |
| Malaysia — Borneo | 29 | 0.94 | 0.008 | –12.70* | –3.06* |
| **Mauritius** | 9 | 0.97 | 0.006 | –3.34 (NS) | –0.84 (NS) |
| New Caledonia | 30 | 0.99 | 0.012 | –12.27* | –2.46 (NS) |
| **Oman** | 12 | 0.98 | 0.007 | –5.79* | –0.96 (NS) |
| Papua New Guinea | 10 | 0.97 | 0.009 | –3.07 (NS) | –0.74 (NS) |
| Philippines — Bohol | 18 | 1 | 0.011 | –12.17* | –1.90 (NS) |
| Philippines — Mindoro | 14 | 1 | 0.010 | –8.65* | –1.10 (NS) |
| Rarotonga | 30 | 0.98 | 0.011 | –10.46* | –3.57** |
| Singapore | 10 | 1 | 0.015 | –3.52 (NS) | –1.52 (NS) |
| **South Africa — Durban** | 20 | 0.99 | 0.011 | –9.51* | –2.88* |
| **South Africa — Mission Rocks** | 10 | 1 | 0.008 | –5.79* | –1.08 (NS) |
| **Tanzania** | 65 | 0.98 | 0.007 | –71.89* | –3.92* |
| Thailand — Gulf of Siam | 10 | 0.97 | 0.009 | –5.43* | –1.47 (NS) |
| **Thailand — Phuket** | 10 | 1 | 0.009 | –2.21 (NS) | –1.54 (NS) |
| Clade A — Indian Ocean | 171 | 0.985 | 0.008 | –250.60* | –5.43* |
| Clade B — Pacific Ocean | 358 | 0.983 | 0.008 | –430.85* | –5.31* |
| Overall | 529 | 0.989 | 0.021 | — | — |

NS, not significant.

AMOVA showed strong genetic structure between regions dominated by each clade, and weak or nonexistent structure within regions (Table 3). In *N. albicilla*, the partition between the Indian and Pacific Oceans explains 77% of observed nucleotide variation ($\Phi_{CT} = 0.77$, $P < 0.0001$) and structure within regions was small but significant ($\Phi_{SC} = 0.011$, $P < 0.0001$). In contrast, *N. plicata* showed no evidence of a split between the Indian and Pacific Oceans ($\Phi_{CT} = 0.010$, $P = 0.15$). However, the partition between the Society Islands, Rarotonga, Rangiroa, and the rest of the Indo-Pacific explained 20.7% of the variance ($\Phi_{CT} = 0.207$; $P < 0.00066$); the remaining variance was distributed within demes and there was no detectable intraregional structure ($\Phi_{SC} = 0.0$; $P = 0$). When clade B was removed from the analysis, $\Phi_{CT}$ was not significant across the entire range of *N. plicata*. After Bonferroni correction, the only significant pairwise $\Phi_{ST}$ or $F_{ST}$ values in either species were between demes on either side of the breaks identified by AMOVA, with one exception: a pairwise $F_{ST}$ of 0.005 between American Samoa and Queensland found in *N. plicata*.

### *Coalescent models of demographic history*

The two tests of neutrality yielded contrasting results. Fu's $F_S$ rejected neutrality ($P < 0.05$) for all demes in both species with $n > 10$. However, Fu and Li's D* was significant ($P < 0.02$) for only 10 out of 23 regional demes tested in *N. plicata* and seven out of 26 demes tested in *N. albicilla* (Tables 1 and 2). Both tests were significant when samples were grouped by ocean basin.

Bayesian skyline plots generated from BEAST reveal concurrent histories of exponential population growth between clades in both species (Fig. 4). Based on a conservative heuristic mutation rate of 0.5%/myr, growth commenced at approximately 500 000 BP in *N. albicilla* and 650 000 BP in *N. plicata*. These dates correspond to the earliest dates inferred from mutational depths of the tip clades in each species. Figure 4 shows estimates of current effective population size of each clade, with 95% confidence intervals. Ancestral population size was estimated from the last interval of the skyline plot and is presented as the sum of the population

**Table 2** Summary statistics and neutrality test statistics for *Nerita plicata*. Haplotype diversity ($h$) and nucleotide diversity ($\pi$) calculated in ARLEQUIN (Schneider *et al*. 2000). $F_S$ and D* measure departure of the data from neutrality (Fu 1997) and were calculated in DNASP (Rozas *et al*. 2003). Indian Ocean demes are in bold type

| Country —region | $n$ | $h$ | $\pi$ | Clade B percentage | $F_S$ | D* |
|---|---|---|---|---|---|---|
| American Samoa — Tutuila | 38 | 0.99 | 0.014 | 7.89% | –17.83* | –1.27 (NS) |
| Australia — Queensland | 40 | 0.99 | 0.012 | 5.00% | –36.99* | –2.72* |
| Easter Island | 4 | 1 | 0.020 | 25.00% | — | — |
| Fiji – Viti Levu | 40 | 0.99 | 0.016 | 10.00% | –34.83* | –2.24 (NS) |
| Guam | 25 | 0.99 | 0.020 | 4.00% | –12.27* | –3.03* |
| Indonesia — West Papua | 19 | 0.99 | 0.012 | 5.26% | –7.98* | –2.43* |
| Indonesia — Krakatau | 17 | 0.99 | 0.015 | 5.88% | –6.88* | –2.47* |
| Indonesia — North Sulawesi | 16 | 1 | 0.011 | 0.00% | –10.45* | –2.15 (NS) |
| Indonesia — Pulau Seribu | 19 | 1 | 0.013 | 0.00% | –12.56* | –2.07 (NS) |
| Japan — Okinawa | 30 | 1 | 0.010 | 0.00% | –31.19* | –3.79* |
| **Kenya** | 50 | 0.99 | 0.011 | 2.00% | –33.80* | –3.77* |
| **Madagascar** | 2 | 1 | — | 0.00% | — | — |
| Malaysia — Borneo | 10 | 1 | 0.017 | 10.00% | –3.16* | –1.50 (NS) |
| Marquesas — Nuku Hiva | 20 | 0.99 | 0.017 | 15.00% | –8.61* | 0.81 (NS) |
| Marshall Islands — Bikini | 2 | 1 | — | 0.00% | — | — |
| **Mauritius** | 6 | 1 | 0.009 | 0.00% | –1.91(NS) | –0.87 (NS) |
| New Caledonia | 40 | 0.99 | 0.012 | 2.50% | –33.51* | –2.76965* |
| Papua New Guinea | 27 | 1 | 0.015 | 7.41% | –32.60* | –2.06 (NS) |
| Philippines | 32 | 0.99 | 0.013 | 6.25% | –20.96* | –1.68 (NS) |
| Rangiroa | 18 | 0.99 | 0.022 | 44.44% | –23.17* | –0.43 (NS) |
| Rarotonga | 40 | 0.99 | 0.022 | 35.00% | –5.55* | –2.24 (NS) |
| **Reunion** | 8 | 1 | 0.010 | 0.00% | –18.85* | –1.37 (NS) |
| Society Islands | 48 | 0.99 | 0.023 | 43.75% | –3.21* | –2.53* |
| **South Africa** | 40 | 0.99 | 0.012 | 0.00% | –20.56* | –3.11* |
| **Tanzania** | 59 | 0.99 | 0.012 | 3.39% | –28.12* | –2.68* |
| Micronesia — Truk | 3 | 1 | — | 0.00% | — | — |
| Clade A | 586 | 0.995 | 0.010 | 0.00% | –33.75* | –3.09* |
| Clade B | 67 | 0.979 | 0.007 | 100.00% | –47.34* | –3.11* |
| Overall | 653 | 0.995 | 0.01396 | 10.26% | — | — |

NS, not significant.

sizes from both clades, since this interval falls within the confidence interval for the TMRCA of each clade (Table 4). Replicate runs from two random subsets of each clade produced highly similar parameter estimates in all cases, and all combined effective sample sizes were greater than 200.

Parameter estimates for an isolation with migration (IM) model for *N. albicilla* (Table 5) were equal or very similar across all three replicates. All effective sample size values were greater than 100, and plots of parameter trends indicated sufficient mixing among chains. Both descendant populations (Pacific and Indian) have effective population sizes more than an order of magnitude larger than the ancestral population. The heuristic estimate for $t$, the time of population splitting, is 634 000 years ago (95% CI, 530 000–798 000 BP), corresponding to a time just prior to the period of population expansion estimated by BEAST for *N. albicilla*, and approximately the same time as the population expansion in *N. plicata*. Estimates of gene flow are low ($2N_em = 0.79$ into the Indian Ocean and 0.36 into the Pacific); and, since the tails of the posterior distributions were not complete, these estimates are not significantly greater than 0 (Nielsen & Wakeley 2001). The estimates for current and ancestral effective population size are generally lower than those produced by BEAST, but fall within BEAST's 95% confidence intervals (Fig. 4).

The IM model did not similarly converge for the genetic break in the Central Pacific in *N. plicata*. For example, replicate estimates for effective population size for the Central Pacific island demes ranged from 29 million to 700 million and population migration rates ($2N_em$) ranged from 350 to 17 000. None of the effective sample size values rose above 50, and parameter plots showed multiple peaks — further evidence that the chains were not mixing. Three different two-step heating schemes did not improve on this result. The only parameter that did not vary across runs was the TMRCA, which remained stable at about 3.5 million years ago (95% CI 2.0–4.5 million BP).

Results from the nonequilibrium island model of the structured coalescent examined in LAMARC indicate that while *N. albicilla* has a higher migration rate ($m/$ ) than

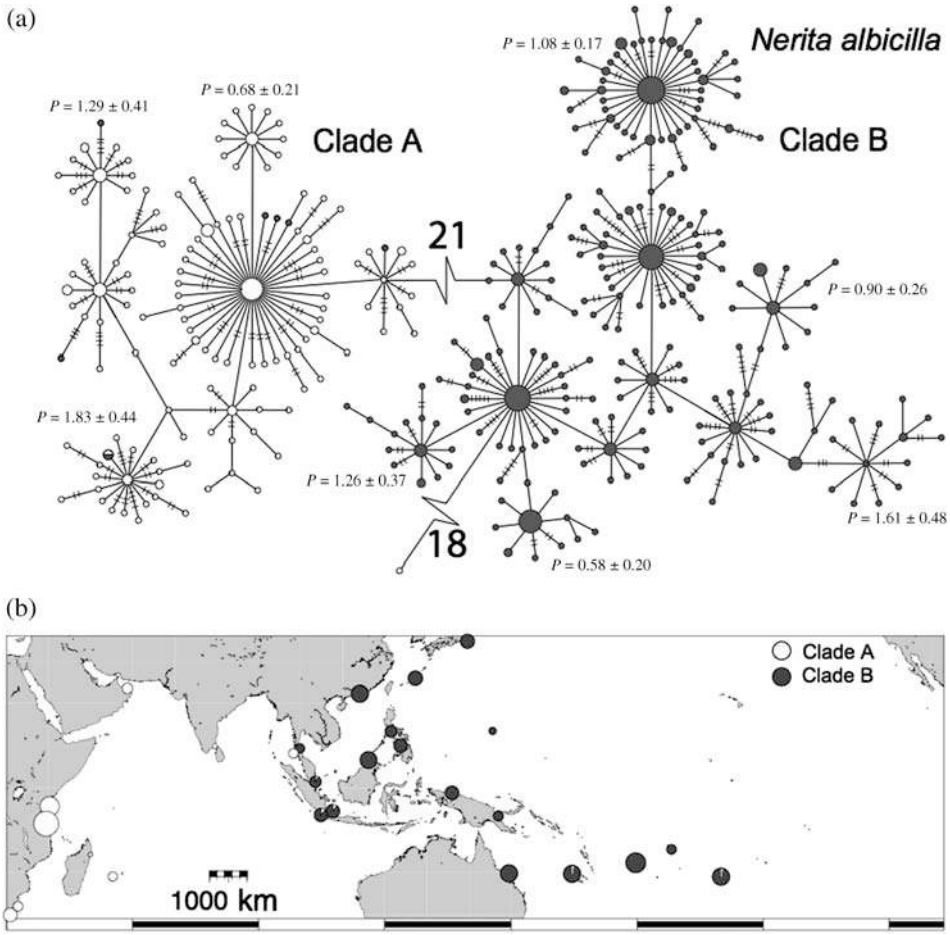


**Fig. 2** Phylogeography of *Nerita albicilla*: (a) unrooted minimum-spanning tree showing the relationships between 467 unique CO1 haplotypes. White haplotypes were found in Indian Ocean localities and grey haplotypes were found in Pacific Ocean localities. Circles are sized proportionally to the frequency of occurrence, ranging from 1 to 30. All haplotypes are separated by one mutational step unless denoted by a higher number of hatch marks or number. For tip clades, the mean mutational distance to the central haplotype is given with a standard deviation. (b) Map of the Indo-Pacific with pie diagrams showing the relative frequency of each clade at each deme listed in Table 1. Clade A haplotypes are coloured white and clade B haplotypes are grey. The size of each pie diagram is proportional to the number of individuals sampled from each deme, ranging from two in Madagascar to 65 in Tanzania.

*N. plicata* in the Central Pacific, it also had a smaller θ, making the population migration rate ($2N_em$) about equal in the two species (Table 6). *N. albicilla* had a faster growth rate ($g$) than *N. plicata*, which is consistent with the Bayesian skyline plots. Estimates of current and ancestral $N_e$ are in approximate agreement with what was found by BEAST and IM (Fig. 4). A similar analysis that was run on an *N. plicata* data set with clade B removed gave the same the same values for $2N_em$, although migration rate was slightly higher, and θ was slightly lower (Table 6). Multiple runs converged on the same posterior distributions.

## Discussion

Despite having similar ecologies and broadly sympatric distributions spanning the Indian and Pacific Oceans, the congeneric taxa *Nerita albicilla* and *Nerita plicata* have responded uniquely to the dynamic environments of the IAA. Although both species have two reciprocally monophyletic mtDNA lineages and experienced demographic expansions that date broadly to the Pleistocene, their phylogeographical patterns differ sharply. In *N. albicilla*, the two lineages have allopatric distributions in the Pacific and Indian Oceans, corresponding to a classic pattern of Indo-Pacific vicariance reported in many marine species (Lavery *et al*. 1996; Duda & Palumbi 1999b; Barber *et al*. 2000). In contrast, *N. plicata* has a genetic break between demes in the Central Pacific and Pacific/Indian Ocean populations lying to the west.

The disparity in phylogeographical patterns contrasts with the strong similarity in phylogenetic and demographic patterns. In previous marine studies at the IAA and

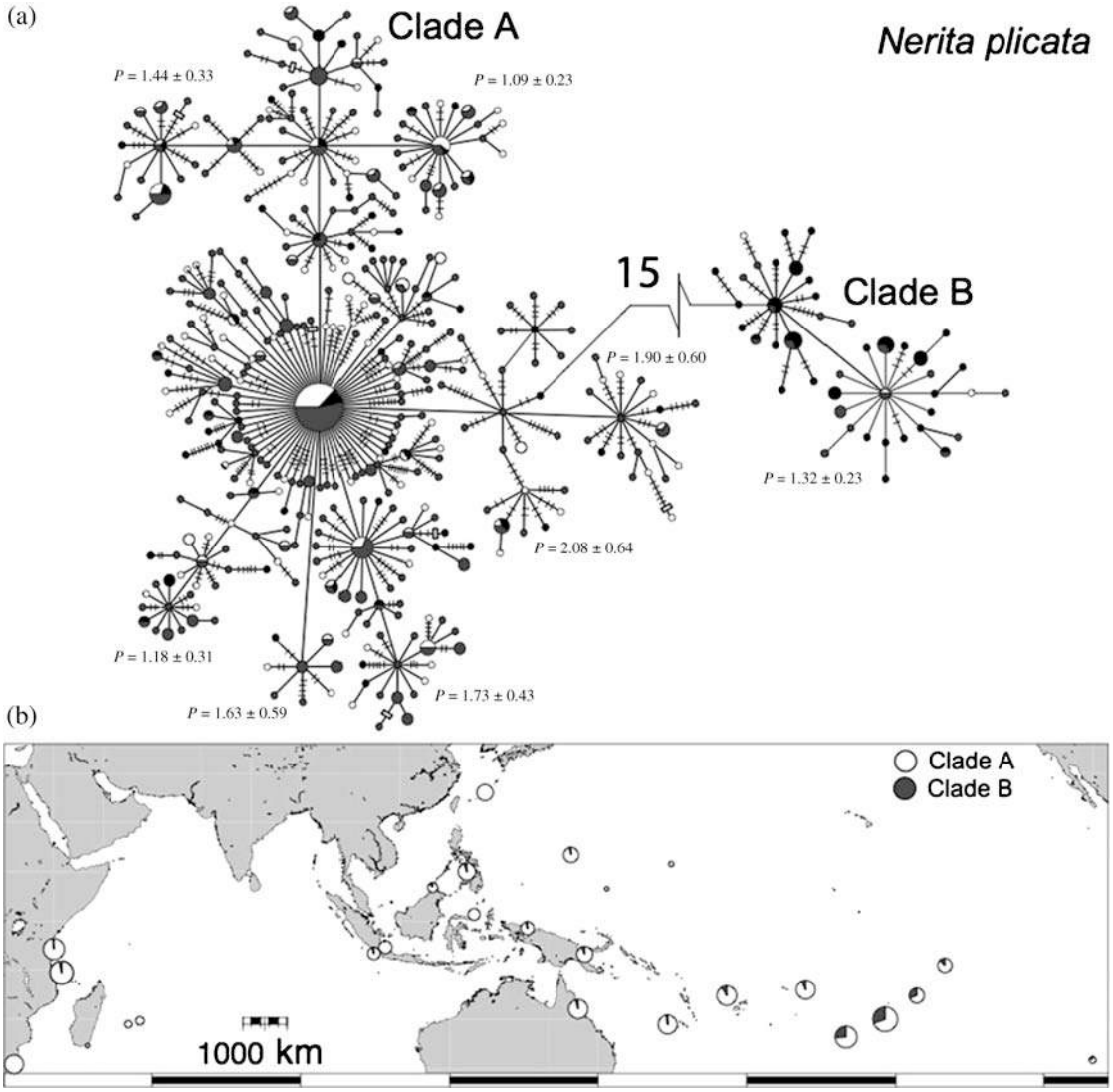


**Fig. 3** Phylogeography of *Nerita plicata*: (a) unrooted minimum-spanning tree showing the relationships between 353 unique CO1 haplotypes. White haplotypes were found in Indian Ocean localities and grey haplotypes were found in Pacific Ocean localities, except for black haplotypes which highlight haplotypes found in Rarotonga, Rangiroa, the Society Islands and Easter Island. Circles are sized proportionally to the frequency of occurrence, ranging from one to 28. All haplotypes are separated by one mutational step unless denoted by a higher number of hatch marks or a number. For tip clades, the mean mutational distance to the central haplotype is given with a standard deviation. (b) Map of the Indo-Pacific with pie diagrams showing the relative frequency of each clade at each deme listed in Table 2. Clade A haplotypes are coloured white and clade B haplotypes are grey. The size of each pie diagram is proportional to the number of individuals sampled from each deme, ranging from 2 in Madagascar and elsewhere to 59 in Tanzania. Six nonsynonymous mutations are marked with shaded rectangles.

elsewhere, such disparities between congeners have been attributed to ecological differences that allowed qualitatively different histories in which one species was able to use refugial habitat that was unavailable to others (Marko 2004; Hickerson & Cunningham 2005; Reid *et al*. 2006). While the similarity of intraspecific phylogenies and historical demography suggests similar evolutionary histories in both species, subtle differences in ecology or dispersal ability may still have played a role in creating phylogeographical discordance.

### *A shared history of population expansion*

The minimum-spanning trees recover two clades of similar divergence in both species (Figs 2 and 3). Both trees have multiple star-like radiations that strongly suggest nonequilibrium dynamics (Grosberg & Cunningham 2001). This latter result is supported by the strongly negative regional $F_S$ and D* values (Tables 1 and 2) that could indicate a demographic expansion, selective sweep, or background selection. Because D* was not significant within many demes, and

**Table 3** AMOVA results for both species. *Nerita plicata* analyses were performed for each clade individually as well as combined. Significant values (*) indicate a $P < 0.0001$ after 10 000 random permutations of the data

| | *N. albicilla* both clades | *N. plicata* both clades | *N. plicata* clade A only | *N. plicata* clade B only |
|---|---|---|---|---|
| Definition of regions | Indian Ocean vs. Pacific Ocean | Societies, Rangiroa, Rarotonga vs. all other demes | | |
| Overall $\Phi_{CT}$ (between regions) | 0.772* | 0.205* | 0.001 (NS) | 0.008 (NS) |
| Overall $\Phi_{SC}$ (within regions) | 0.011* | 0.0 (NS) | 0.002 (NS) | 0.046 (NS) |
| % variation: | | | | |
| Among regions | 77.03% | 20.68% | –0.17% | 0.84% |
| Among demes within regions | 0.26% | 0% | 0.24% | 4.62% |
| Within demes | 22.72% | 79.32% | 99.93% | 94.54% |

NS, not significant.

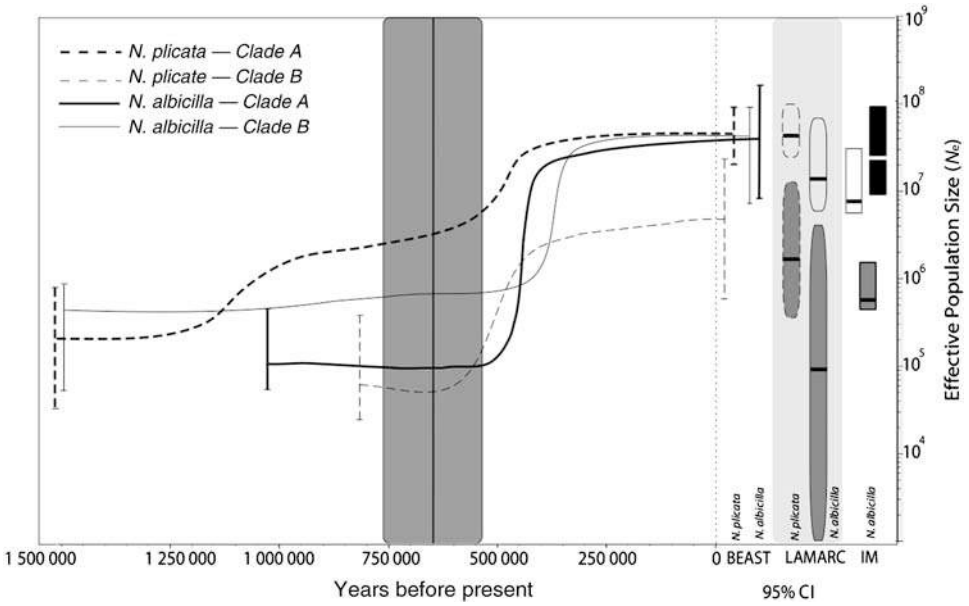


**Fig. 4** A comparison of effective population size ($N_e$) and time estimates made under the three coalescent models of demography employed in this paper. Estimates have been converted from mutational units using a heuristic divergence rate of 1% per million years (see Materials and methods). Solid lines depict results from *Nerita albicilla* while dotted lines depict results from *Nerita plicata*. Curves represent Bayesian skyline plots for $N_e$ running from the present to their mean time to most recent common ancestor (TMRCA), with 95% confidence intervals plotted at both time points. The shaded box in the centre of the figure represents 95% confidence intervals (CI) for the time of population splitting estimated for Pacific and Indian populations of *N. albicilla*. Shaded and open ovals represent 95% CI for ancestral and current $N_e$, respectively, estimated for four Pacific demes in both species by LAMARC. Boxes represent 95% CI for $N_e$ estimated by IM for *N. albicilla* only, with the Pacific population in white, the Indian population in black and the ancestral population in grey.

because it is more sensitive to background selection than $F_S$, background selection is unlikely to fully explain the lack of neutrality in these demes. It is also possible that positive selection at a linked mitochondrial locus could have produced an advantageous variant (e.g. Rawson & Burton 2006) which then swept to fixation (Gillespie 2001). However, such an event would diminish haplotype diversity, leaving a single central haplotype rather than the high diversity and multiple stars and nearly simultaneous expansions observed in both species. It seems unlikely that two taxa would experience contemporaneous selective sweeps in two separate ocean basins.

A perhaps more parsimonious alternative explanation of nonequilibrium dynamics comes from three separate coalescent models that strongly suggest exponential population expansion in both species. Although the demographic estimates may be upwardly biased with respect to absolute time and effective population size (due to the conservative mutation rate used in the conversion; Ho *et al*. 2005), Bayesian skyline plots suggest a broadly contemporaneous

**Table 4** Mean and 95% highest posterior density intervals for effective population size and time to most recent common ancestor for a panmictic population modelled in BEAST. Time in years to most recent common ancestor (TMRCA) was calculated for each clade individually, and for the entire mitochondrial data set in each species. Time estimates are scaled by the reciprocal of the per-site mutation rate 1/

| | *Nerita albicilla* | | | *Nerita plicata* | | |
|---|---|---|---|---|---|---|
| | È (per site) | | | | | |
| | Clade A | Clade B | Ancestral | Clade A | Clade B | Ancestral |
| **Mean** | **0.459** | **0.499** | **0.007** | **0.536** | **0.062** | **0.003** |
| 95% low | 0.113 | 0.102 | 0.002 | 0.167 | 0.008 | 0.001 |
| 95% high | 1.581 | 1.460 | 0.020 | 1.371 | 0.199 | 0.018 |
| | TMRCA | | | | | |
| | Clade A | Clade B | mtDNA | Clade A | Clade B | mtDNA |
| **Mean** | **0.0052** | **0.0074** | **0.0372** | **0.0075** | **0.0041** | **0.0246** |
| 95% low | 0.0030 | 0.0044 | 0.0327 | 0.0050 | 0.0023 | 0.0158 |
| 95% high | 0.0077 | 0.0110 | 0.0176 | 0.0106 | 0.0062 | 0.0342 |

**Table 5** Mode and 95% confidence intervals for the parameters of an isolation-with-migration model estimated by IM for Indian and Pacific Ocean populations of *Nerita albicilla*. Time estimates are scaled by the reciprocal of the per-site mutation rate, 1/ , and estimates of θ are all per site

| | θ Indian | θ Pacific | θ ancestral | *t* | TMRCA | $2N_em$ into Indian* | $2N_em$ into Pacific* |
|---|---|---|---|---|---|---|---|
| **Mode** | **0.239** | **0.111** | **0.008** | **0.0032** | **0.0204** | **0.79†** | **0.36†** |
| 95% low | 0.155 | 0.080 | 0.005 | 0.0027 | 0.0134 | 0.51 | 0.26 |
| 95% high | 0.489 | 0.185 | 0.018 | 0.0040 | 0.0301 | 30.55 | 17.63 |

*Effective migrants per generation. †Not significantly different from 0.

demographic expansion that occurred just after the population split reckoned by IM for *N. albicilla* (Fig. 4). This result, combined with the relative agreement across the different coalescent models employed in BEAST, IM and LAMARC implies a shared history of demographic expansion during the Pleistocene in both species.

Demographic expansion in these two species is expected given the geological history of the Indo-Pacific region. During low sea-level stands, the Sunda Shelf was frequently terrestrial (Voris 2000), as were the lagoons of many oceanic islands (Ladd 1960; Paulay 1990). This loss of habitat would have caused cyclical local extinction of many demes, followed by recolonization as sea levels rose. The cyclical nature of range expansion and contraction caused by sea level change is hinted at by an older population expansion detected in the skyline plot of *N. plicata* clade A (Fig. 4), as well as the differing average mutational depths of the star polytomies in all four clades (Figs 2a and 3a). The multiple star polytomies in the minimum- spanning trees may have been formed across a chronological sequence of range contractions followed by expansion events that stochastically favoured only a few haplotypes (and form the characteristic star-shape; Slatkin & Hudson 1991) during any given event. Similar patterns in other Pacific species have been attributed to range expansions into the Central Pacific islands (Thacker 2004; Thompson *et al*. 2005) and the IAA (Klanten *et al*. 2007).

### *Population divergence and the maintenance of reciprocal monophyly in* Nerita albicilla

A distinct genetic break between Indian and Pacific Ocean clades (clades A and B, respectively) occurs along the west coast of the Malay Peninsula in *N. albicilla*. This approximately matches the location of strong genetic breaks exhibited by a number of other reciprocally monophyletic taxa (Duke *et al*. 1998; Williams & Benzie 1998; Benzie 1999b; Reid *et al*. 2006), and corresponds broadly to the periodic barrier between the Indian and Pacific Oceans at the Malacca strait (Fig. 1).

A neutral estimate of divergence time based on the net divergence between clades ($d_A$; Nei & Li 1979) and a divergence rate of 1%/myr would result in a population divergence time estimate, *t*, of 3.4 million years. However, this equilibrium estimate is likely inflated because of the large ancestral effective population size ($N_e \approx 500\,000$) that

| | θ — current | Growth parameter (*g*) | θ — ancestral | $2N_em$* |
|---|---|---|---|---|
| *Nerita albicilla* | | | | |
| **Mode** | **0.193** | **1994** | **0.023†** | **164** |
| 95% low | 0.082 | 1308 | 0.002† | 93 |
| 95% high | 1.010 | 3429 | 0.254† | 519 |
| *Nerita plicata* — both clades | | | | |
| **Mode** | **0.546** | **1187** | **0.156‡** | **118** |
| 95% low | 0.241 | 849 | 0.043‡ | 77 |
| 95% high | 1.488 | 1636 | 0.607‡ | 168 |
| *Nerita plicata* — clade A only | | | | |
| **Mode** | **0.396** | **1087** | **0.126‡** | **121** |
| 95% low | 0.202 | 800 | 0.038‡ | 78 |
| 95% high | 1.349 | 1575 | 0.580‡ | 163 |

**Table 6** Mode and 95% confidence intervals for the parameters of a nonequilibrium island model of the structured coalescent estimated by LAMARC for Pacific Ocean demes of both species at New Caledonia, Fiji, American Samoa and Rarotonga. $N_e$ estimates are scaled by the reciprocal of the per-site mutation rate and generation time, 1/ τ. All estimates of θ are per site

*Effective female migrants per generation. †Estimated for *Nerita albicilla* clade B TMRCA = 0.0074/ years before present. ‡Estimated for *N. plicata* clade A TMRCA = 0.0075/ years before present.

would harbour a substantial amount of genetic polymorphism that would only sort once populations became allopatric (Edwards & Beerli 2000; Arbogast *et al*. 2002). Even the more recent estimate of $t \approx 634\,000$ years obtained in IM may be inflated given that it comes from a single locus that is almost reciprocally monophyletic (Arbogast *et al*. 2002; Carstens & Knowles 2007). Nevertheless, even with a 95% confidence interval of about 250 000 years and using the slowest rate published for molluscan CO1 (Marko 2002), this estimate still falls squarely in the Pleistocene, and immediately precedes exponential population expansion calculated independently by BEAST. Since many marine species in the Indo-Pacific may have large $N_e$, failure to take ancestral $N_e$ and population growth into account could lead to large overestimates of divergence time (Edwards & Beerli 2000) such as the fivefold error calculated for net divergence above.

The persistence of regionally concordant, nearly reciprocally monophyletic clades in *N. albicilla* could occur if larval dispersal and gene flow among regions is rare ($2N_em \leq 1$), such that gene flow is swamped by the standing populations (Cunningham & Collins 1998). However, high estimates of gene flow from LAMARC ($2N_em > 90$) across thousands of kilometres of ocean where intermediate habitats do not exist suggest that dispersal and gene flow are not intrinsically limited. These high estimates of gene flow from elsewhere suggest that while larval dispersal between oceans may be common, successful gene flow is rare due to pre- or post–zygotic barriers between the two clades (Benzie 1999a; Edmands & Burton 1999), or due to Allee effects. While the number of effective female migrants per generation ($2N_em$) estimated by LAMARC is high in evolutionary terms, it is a tiny proportion ($m$) of the total effective population size. Thus, if reproductive barriers do exist between clades, then migrants from one clade that cross to the other ocean are very unlikely to encounter members of their own clade and reproduce successfully, simply due to an Allee effect.

Waters *et al*. (2005) came to a similar conclusion for a temperate congener, *Nerita atramentosa*, that shows reciprocally monophyletic clades corresponding to two different opercular colours across a historical barrier at Wilson's Promontory in Australia. They examined > 7000 snails within a few hundred kilometres of the former barrier, using opercular colour as a proxy for mitochondrial clade, and found a cline with the frequency of each opposite clade decreasing with distance on each side of Wilson's Promontory. In the present study, we observed a similar pattern, with a low frequency of clade A haplotypes in demes that are dominated by clade B, especially along the boundary of the Indian and Pacific Oceans. Given the high gene flow estimates obtained across large distances of open ocean, post-dispersal processes may provide a better explanation for the maintenance of the phylogeographical break than rare dispersal.

### *The origin of genetic structure in* Nerita plicata

Significant $\Phi_{CT}$ values separated Rarotonga, the Society Islands and Rangiroa from *N. plicata* demes to the west. However, no structure was detected within individual clades (Table 3). This absence of detectable genetic structure ($\Phi_{SC}$ and $\Phi_{CT}$) across more than 22 000 km of ocean (from South Africa to the Marquesas) is surprising, even for a species with high dispersal potential such as *N. plicata*. This result suggests that the regional structure above likely results from admixture of clades A and B and not from restricted gene flow and population divergence in the Central Pacific. This hypothesis is supported by the failure of IM to converge

for this data set, as well as high estimates of gene flow in the Western and Central Pacific made by LAMARC for data sets that both included and excluded clade B (Table 6).

There are several hypotheses that could explain the pattern of deep divergence in *Nerita plicata*. First, the two lineages may be cryptic species. However, since both clades occur sympatrically throughout the entire Indo-Pacific region, the hypothesis of cryptic sister species seems unlikely in the absence of any evidence for ecological speciation. Without nuclear markers to delineate species boundaries, this hypothesis cannot yet be falsified.

A second possibility is that the two lineages arose sympatrically and stochastically, and represent the two deepest coalescing branches in a panmictic population with large $N_e$ (Donnelly & Tavare 1995). The persistence of multiple divergent clades is very likely given the large effective population sizes and high migration rates estimated under the coalescent models. However, the clear phylogeographical pattern with higher frequencies of clade B in the Central Pacific is unlikely to evolve stochastically under panmixia, but would instead require either significant limits to gene flow or allopatric divergence across the waters of the Central Pacific that would allow differential lineage sorting in the two geographical regions.

A third possibility, therefore, is that the two lineages of *N. plicata* sorted in allopatry in the Central Pacific where the genetic break presently occurs. Phylogeographical divergence has been observed across this region, but only in species characterized by high genetic structure that show even deeper divergence at the IAA (Bernardi *et al*. 2001; McCafferty *et al*. 2002). In contrast, *N. plicata* appears to be panmictic across 22 000 km, but only shows a genetic break in the Central Pacific. There were no major changes in the geostrophic flow of the South Equatorial Current during the last glacial maximum (Thunell *et al*. 1994) that would have separated or reunited lineages spanning this putative barrier. The absence of a clear physical filter to dispersal combined with relatively high coalescent estimates of gene flow in the region (Table 6) argues against allopatric divergence in the Central Pacific. However, it is still possible that the divergence has allopatric origins, perhaps in another geographical location.

In the absence of a plausible dispersal barrier in the Central Pacific, there are limited possibilities for the location of a putative allopatric dispersal barrier. Divergence across the Eastern Pacific Barrier or in Hawaii is possible, but unlikely, given that our small sample from Easter Island reflects the same clade B frequencies (~25%) as in other Central Pacific demes, and *N. plicata* is absent from South America and rare in Hawaii (S. Godwin, personal communication). The most logical location for allopatric divergence is at the Indo-Australian Archipelago. The phylogeographical and demographic patterns in *N. plicata* could be explained by historical allopatry at the IAA, followed by the rapid expansion of clade A into the Pacific Ocean (perhaps mediated by selection). While this hypothesis is the most consistent with the remarkably similar phylogenetic and demographic histories inferred for both clades in both *Nerita* species, it is also presently untestable. Statistical methods for establishing concordance in comparative phylogeography are in their infancy (e.g. Hickerson *et al*. 2006). The development of new spatially explicit models linking genetics and dispersal in marine environments (e.g. Galindo *et al*. 2006) may provide a framework for exploring the potential outcomes of vicariance followed by range expansion.

### *Reconciling phylogeography with demographic history*

*Nerita albicilla* and *N. plicata* share a history of demographic expansion and high levels of gene flow, yet show markedly discordant phylogeographical patterns across the same geographical regions. *N. plicata* has maintained or re-established gene flow across the IAA, while *N. albicilla* has not. Although the ultimate explanation for this pattern remains to be resolved, species-specific differences in pelagic larval duration could contribute to the observed patterns. Although LAMARC revealed no interspecific differences in gene flow across large pelagic distances, *N. plicata*'s larger range size indicates that it may have a more leptokurtic dispersal kernel (Kinlan *et al*. 2005; Paulay & Meyer 2006). Thus, *N. plicata* may have sustained gene flow across the IAA during periods of lowered sea level, or re-established gene flow more quickly than *N. albicilla* once sea levels rose again.

Alternatively, the phylogeographical differences may stem from subtle, species-specific disparities in habitat requirements that have large impacts on genetic structure. Paulay (1990) found that uplifted islands such as Niue lack bivalve taxa that require inner-reef habitats. These islands approximate the condition during glacial cycles when lowered sea levels would have stranded reef flats, destroying prime habitat for *N. albicilla.* In contrast, the steep reef slope exposed during sea-level regressions could have continued to support *N. plicata*, allowing it to maintain intermediate stepping-stone populations and thus higher levels of gene flow. This would also explain the faster rate of population growth detected by both BEAST and LAMARC for *N. albicilla,* as this species would have gained more habitat area as sea levels rose again. The hypothesis that pre-existing differences in habitat requirements explain differences in response to vicariance at the IAA is similar to that of Reid *et al*. (2006), who ascribed different congeneric responses to species with 'oceanic' and 'continental' habitat requirements. Dissimilar responses to climate change within a genus have also been attributed to habitat specificity in other tropical marine species (Fauvelot *et al*. 2003; Thacker 2004; Bird *et al*. 2007), as well as temperate marine (Marko 2004; Hickerson & Cunningham 2005) and terrestrial habitats (Hewitt 2000). Indeed, habitat specificity may be as important

as the physical dispersal of larvae in contributing to contemporary genetic patterns.

## Conclusions

Despite their different purposes and assumptions, three coalescent models of demography yielded remarkably similar estimates of growth from a smaller ancestral $N_e$ to an exponentially larger $N_e$ for both species (Fig. 4; Tables 4–6). Although methods for demonstrating statistical concordance between species and among models are still being developed (e.g. Hickerson *et al*. 2006), the range expansion inferred here for both species lies well within the climatic oscillations of the Pleistocene, even when using a relatively slow molecular clock (Fig. 4). While the effects of specific glaciations cannot be detected with the current data set, both species have likely shared a history of recurring population fragmentation and expansion due to sea-level fluctuations.

Despite this similar demographic history, the discordant patterns of phylogeography in the two *Nerita* species indicate that relatively closely related taxa can respond very differently to the cyclical fluctuations in sea level at the IAA. Subtle differences in dispersal ability, and/or habitat requirements may allow higher rates of gene flow and thus maintenance or resumption of genetic connectivity. Our analyses, in addition to similar studies in this region, suggest that the IAA may represent an unusually large suture zone (Remington 1968; Benzie 1999a; Hewitt 2000), in which the genetic signature of past demography is retained by large effective population sizes, and the boundaries between highly divergent Indian and Pacific Ocean clades are defined by the rate at which they expand into newly submerged habitat following sea level rise, and their degree of admixture. Additional nuclear and mitochondrial genetic data from Indo-Pacific species will be instrumental in understanding the impacts of Pleistocene vicariance in shaping phylogeographical and demographic patterns of species spanning this dynamic region.

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## References

Arbogast BS, Edwards SV, Wakeley J, Beerli P, Slowinski JB (2002) Estimating divergence times from molecular data on phylogenetic and population genetic timescales. *Annual Review of Ecology and Systematics*, **33**, 707–740.

Avise JC (1992) Molecular population-structure and the biogeographic history of a regional fauna — a case-history with lessons for conservation biology. *Oikos*, **63**, 62–76.

Avise JC (2000) *Phylogeography — The History and Formation of Species*. Harvard University Press, Cambridge, Massachusetts.

Barber PH, Bellwood DR (2005) Biodiversity hotspots: evolutionary origins of biodiversity in wrasses (*Halichoeres*: Labridae) in the Indo-Pacific and new world tropics. *Molecular Phylogenetics and Evolution*, **35**, 235–253.

Barber PH, Erdmann MV, Palumbi SR (2006) Comparative phylogeography of three co-distributed stomatopods: origins and timing of regional lineage diversification in the coral triangle. *Evolution*, **60**, 1825–1839.

Barber PH, Palumbi SR, Erdmann MV, Moosa MK (2000) A marine Wallace's line? *Nature*, **406**, 692–693.

Benzie JAH (1999a) Genetic structure of coral reef organisms: ghosts of dispersal past. *American Zoologist*, **39**, 131–145.

Benzie JAH (1999b) Major genetic differences between crown-of-thorns starfish (*Acanthaster planci*) populations in the Indian and Pacific Oceans. *Evolution*, **53**, 1782–1795.

Benzie JAH, Ballment E, Forbes AT *et al*. (2002) Mitochondrial DNA variation in Indo-Pacific populations of the giant tiger prawn, *Penaeus monodon*. *Molecular Ecology*, **11**, 2553–2569.

Bernardi G, Holbrook SJ, Schmitt RJ (2001) Gene flow at three spatial scales in a coral reef fish, the three-spot dascyllus, *Dasyllus trimaculatus*. *Marine Biology*, **138**, 457–465.

Bernatchez L, Wilson CC (1998) Comparative phylogeography of Nearctic and Palearctic fishes. *Molecular Ecology*, **7**, 431–452.

Bird CE, Holland BS, Bowen BW, Toonen RJ (2007) Contrasting phylogeography in three endemic Hawaiian limpets (*Cellana spp*.) with similar life histories. *Molecular Ecology*. doi: 10.1111/j.1365-294X.2007.03385.x.

Bowen B, Bass AL, Rocha LA, Grant WS, Robertson DR (2001) Phylogeography of trumpetfishes (*Aulostomus*): ring species complex on a global scale. *Evolution*, **55**, 1029–1039.

Carstens BC, Knowles LL (2007) Shifting distributions and speciation: species divergence during rapid climate change. *Molecular Ecology*, **16**, 619–627.

Chenoweth SF, Hughes JM (2003) Oceanic interchange and non-equilibrium population structure in the estuarine dependent Indo-Pacific tasselfish, *Polynemus sheridani*. *Molecular Ecology*, **12**, 2387–2397.

Chenoweth SF, Hughes JM, Keenan CP, Lavery S (1998) When oceans meet: a teleost shows secondary intergradation at an

Indian–Pacific interface. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **265**, 415–420.

Cunningham CW, Collins TM (1998) Beyond area relationships: extinction and recolonization in molecular marine biogeography. In: *Molecular Approaches to Ecology and Evolution* (eds Desalle R, Shierwater B), pp. 297–321. Birkhauser-Verlag, Basel.

Donnelly P, Tavare S (1995) Coalescents and genealogical structure under neutrality. *Annual Review of Genetics*, **29**, 401–421.

Drummond AJ (2003) BEAST *Version 1.41* Available from http://beast.bio.edsac.uk/.

Drummond AJ, Nicholls GK, Rodrigo AG, Solomon W (2002) Estimating mutation parameters, population history and genealogy simultaneously from temporally spaced sequence data. *Genetics*, **161**, 1307–1320.

Drummond AJ, Rambaut A, Shapiro B, Pybus OG (2005) Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution*, **22**, 1185–1192.

Duda TF, Palumbi SR (1999b) Population structure of the black tiger prawn, *Penaeus monodon*, among western Indian Ocean and western Pacific populations. *Marine Biology*, **134**, 705–710.

Duke NC, Benzie JAH, Goodall JA, Ballment ER (1998) Genetic structure and evolution of species in the mangrove genus *Avicennia* (Avicenniaceae) in the Indo-West Pacific. *Evolution*, **52**, 1612–1626.

Edmands S, Burton RS (1999) Cytochrome *c* oxidase activity in interpopulation hybrids of a marine copepod: a test for nuclear-nuclear or nuclear-cytoplasmic coadaptation. *Evolution*, **53**, 1972–1978.

Edwards SV, Beerli P (2000) Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution*, **54**, 1839–1854.

Fauvelot C, Bernardi G, Planes S (2003) Reductions in the mitochondrial DNA diversity of coral reef fish provide evidence of population bottlenecks resulting from Holocene sea-level change. *Evolution*, **57**, 1571–1583.

Fleminger A (1986) The Pleistocene equatorial barrier between the Indian and Pacific Oceans and a likely cause for Wallace's line. In: *Pelagic Biogeography UNESCO Technical Papers in Marine Science* (ed Pierrot-Bults AC), pp. 84–97. UNESCO, Paris.

Folmer O, Black M, Hoeh WR, Lutz R, Vrijenhoek RC (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.

Fu Y-X (1997) Statistical tests of neutrality against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.

Fu Y-X, Li W-H (1993) Statistical tests of neutrality of mutations. *Genetics*, **133**, 693–809.

Galindo HM, Olson DB, Palumbi SR (2006) Seascape genetics: a coupled oceanographic-genetic model predicts population structure of Caribbean corals. *Current Biology*, **16**, 1622–1626.

Gillespie JH (2001) Is the population size of a species relevant to its evolution. *Evolution*, **55**, 2161–2169.

Grant WS, Bowen BW (1998) Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. *Journal of Heredity*, **89**, 415–426.

Grosberg RK, Cunningham CW (2001) Genetic structure in the sea: from populations to communities. In: *Marine Community Ecology* (eds Bertness M, Gaines SD, Hay ME), pp. 61–84. Sinauer Associates, Sunderland, Massachusetts.

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247–276.

Hewitt GM (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.

Hey J, Nielsen R (2004) Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. *Genetics*, **167**, 747–760.

Hickerson MJ, Cunningham CW (2005) Contrasting Quaternary histories in an ecologically divergent sister pair of low-dispersing intertidal fish (*Xiphister*) revealed by multilocus DNA analysis. *Evolution*, **59**, 344–360.

Hickerson MJ, Stahl EA, Lessios HA (2006) Test for simultaneous divergence using approximate Bayesian computation. *Evolution*, **60**, 2435–2453.

Ho SYW, Phillips MJ, Cooper A, Drummond AJ (2005) Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. *Molecular Biology and Evolution*, **22**, 1561–1568.

Hughes RN (1971) Notes on the *Nerita* (Archaeogastropoda) populations of Aldabra Atoll, Indian Ocean. *Marine Biology*, **9**, 290–299.

Kano Y (2006) Usefulness of the opercular nucleus for inferring early development in neritomorph gastropods. *Journal of Morphology*, **267**, 1120–1136.

Kinlan BP, Gaines SD, Lester SE (2005) Propagule dispersal and the scales of marine community process. *Diversity and Distributions*, **11**, 139–148.

Kirkendale LA, Meyer CP (2004) Phylogeography of the *Patelloida profunda* group (Gastropoda: Lottidae): diversification in a dispersal-driven marine system. *Molecular Ecology*, **13**, 2749–2762.

Klanten OS, Choat JH, van Herwerden L (2007) Extreme genetic diversity and temporal rather than spatial partitioning in a widely distributed coral reef fish. *Marine Biology*, **150**, 659–670.

Kuhner MK (2006) LAMARC 2.0: maximum likelihood and Bayesian estimation of population parameters. *Bioinformatics*, **22**, 768–770.

Kuhner MK, Yamato J, Beerli P *et al*. (2005) LAMARC *Version 2.02*. University of Washington.

Ladd HS (1960) Origin of the Pacific island molluscan fauna. *American Journal of Science*, **258-A**, 137–150.

Lavery S, Moritz C, Fielder DR (1996) Indo-Pacific population structure and evolutionary history of the coconut crab *Birgus latro*. *Molecular Ecology*, **5**, 557–570.

Lessios HA, Kane J, Robertson DR (2003) Phylogeography of the pantropical sea urchin *Tripneustes*: contrasting patterns of population structure between oceans. *Evolution*, **57**, 2026–2036.

Lessios HA, Kessing BD, Pearse JS (2001) Population structure and speciation in tropical seas: global phylogeography of the sea urchin *Diadema*. *Evolution*, **55**, 955–975.

Lessios HA, Kessing BD, Robertson DR, Paulay G (1999) Phylogeography of the pantropical sea urchin *Eucidaris* in relation to land barriers and ocean currents. *Evolution*, **53**, 806–817.

Lester SE, Ruttenberg BI (2005) The relationship between pelagic larval duration and range size in tropical reef fishes: a synthetic analysis. *Proceedings of the Royal Society B: Biological Sciences*, **272**, 585–591.

Lourie SA, Green DM, Vincent CJ (2005) Dispersal, habitat differences, and comparative phylogeography of Southeast Asian seahorses (Syngnathidae: *Hippocampus*). *Molecular Ecology*, **14**, 1073–1094.

Maddison WP, Maddison DR (2002) *MacClade*. Sinauer Associates, Sunderland, Massachusetts.

Marko PB (2002) Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama. *Molecular Biology and Evolution*, **19**, 2005–2021.

Marko PB (2004) 'What's larvae got to do with it?' Disparate patterns of post-glacial population structure in two benthic marine gastropods with identical dispersal potential. *Molecular Ecology*, **13**, 597–611.

McCafferty S, Bermingham E, Quenouille B *et al*. (2002) Historical biogeography and molecular systematics of the Indo-Pacific genus *Dascyllus* (Teleostei: Pomacentridae) *Molecular Ecology*, **11**, 1377–1392.

McMillan WO, Palumbi SR (1995) Concordant evolutionary patterns among Indo-West Pacific butterflyfishes *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **260**, 229–236.

Nei M, Li W-H (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences, USA*, **76**, 5269–5273.

Nielsen R, Wakeley J (2001) Distinguishing migration from isolation: a Markov chain Monte Carlo approach. *Genetics*, **158**, 885–896.

Paulay G (1990) Effects of Late Cenozoic sea-level fluctuations on the bivalve faunas of tropical oceanic islands. *Paleobiology*, **16**, 415–434.

Paulay G, Meyer CP (2006) Dispersal and divergence across the greatest ocean region: do larvae matter? *Integrative & Comparative Biology*, **46**, 269–281.

Perrin C, Borsa P (2001) Mitochondrial DNA analysis of the geographic structure of Indian scad mackerel in the Indo-Malay archipelago. *Journal of Fish Biology*, **59**, 1421–1426.

Pillans B, Chappell J, Naish TR (1998) A review of the Milankovitch climatic beat: template for Plio-Pleistocene sea-level changes and sequence stratigraphy. *Sedimentary Geology*, **122**, 5–21.

Porter SC (1989) Some geological implications of average Quaternary glacial conditions. *Quaternary Research*, **32**, 245–261.

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Randall JE (1998) Zoogeography of shore fishes of the Indo-Pacific region. *Zoological Studies*, **37**, 227–268.

Rawson PD, Burton RS (2006) Molecular evolution at the cytochrome oxidase subunit 2 gene among divergent populations of the intertidal copepod, *Tigriopus californicus*. *Journal of Molecular Evolution*, **62**, 753–764.

Reid DG, Lal K, Mackenzie-Dodds J *et al*. (2006) Comparative phylogeography and species boundaries in *Echinolittorina* snails in the central Indo-West Pacific. *Journal of Biogeography*, **33**, 990–1006.

Remington CL (1968) Suture–zones of hybrid interaction between recently joined biotas. *Evolution Biology*, **2**, 321–428.

Rozas J, Sanchez-De I, Barrio JC, Messegyer X, Rozas R (2003) DNASP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19**, 2496–2497.

Saillard J, Forster P, Lynnerup N, Bandelt HJ, Norby S (2000) mtDNA variation among Greenland Eskimos: the edge of the Beringian expansion. *American Journal of Human Genetics*, **67**, 718–726.

Schneider SMKJDR, Excoffier L (2000) ARLEQUIN: a software for population genetic data analysis. *Genetics and Biometry Laboratory*. University of Geneva, Switzerland.

Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, **123**, 603–613.

Smith WHF, Sandwell DT (1997) Global sea floor topography from satellite altimetry and ship depth soundings. *Science*, **277**, 1956–1962.

Springer VG, Williams JT (1990) Widely distributed Pacific plate endemics and lowered sea level. *Bulletin of Marine Science*, **47**, 631–640.

Strimmer K, Pybus OG (2001) Exploring the demographic history of DNA sequences using the generalized skyline plot. *Molecular Biology and Evolution*, **18**, 2298–2305.

Swofford DL (2002) PAUP*: *Phylogenetic Analysis Using Parsimony *and Other Methods*. Sinauer Associates, Sunderland, Massachusetts.

Thacker CE (2004) Population structure in two species of the reef goby *Gnatholepis* (Teleostei: Perciformes) among four South Pacific island groups. *Coral Reefs*, **23**, 357–366.

Thompson AR, Thacker CE, Shaw EY (2005) Phylogeography of marine mutualists: parallel patterns of genetic structure between obligate goby and shrimp partners. *Molecular Ecology*, **14**, 3557–3572.

Thorson G (1950) Reproductive and larval ecology of marine bottom invertebrates. *Biological Review*, **25**, 1–45.

Thunell R, Anderson D, Gellar D, Miao Q (1994) Sea-surface temperature estimates for the tropical western Pacific during the last glaciation and their implications for the Pacific warm pool. *Quaternary Research*, **41**, 255–264.

Underwood AJ (1975) Comparative studies on the biology of *Nerita atramentosa* Reeve, *Bembicium nanum* (Lamarck) and *Cellana tramoserica* (Sowerby) (Gastropods: Prosobranchia) in S.E. Australia. *Journal of Experimental Marine Biology and Ecology*, **18**, 153–172.

Vermeij GJ (1971) Substratum relationships of some tropical Pacific intertidal gastropods. *Marine Biology*, **10**, 315–320.

Voris HK (2000) Special paper 2: maps of Pleistocene sea levels in Southeast Asia: Shorelines, river systems and time durations. *Journal of Biogeography*, **27**, 1153–1167.

Walsh P, Metzger D, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing of forensic material. *BioTechniques*, **10**.

Wares JP (2002) Community genetics in the Northwestern Atlantic intertidal. *Molecular Ecology*, **11**, 1131–1144.

Waters JM, King TM, O'Loughlin PM, Spencer HG (2005) Phylogeographical disjunction in abundant high-dispersal littoral gastropods. *Molecular Ecology*, **14**, 2789–2802.

Williams ST, Benzie JAH (1998) Evidence of a biogeographic break between populations of a high dispersal starfish: congruent regions within the Indo-West Pacific defined by color morphs, mtDNA, and allozyme data. *Evolution*, **52**, 87–99.

Williams ST, Jara J, Gomez E, Knowlton N (2002) The marine Indo-West Pacific break: contrasting the resolving power of mitochondrial and nuclear genes. *Integrative & Comparative Biology*, **42**, 941–952.

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