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Comparative phylogeography of two seastars and their ectosymbionts within the Coral Triangle

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Abstract

Repeated exposure and flooding of the Sunda and Sahul shelves during Pleistocene sea level fluctuations is thought to have contributed to the isolation and diversification of sea basin populations within the Coral Triangle. This hypothesis has been tested in numerous phylogeographic studies, recovering an assortment of genetic patterns that the authors have generally attributed to differences in larval dispersal capability or adult habitat specificity. This study compares phylogeographic patterns from mitochondrial COI sequences among two co-distributed seastars that differ in their adult habitat and dispersal ability, and two seastar ectosymbionts that differ in their degree of host specificity. Of these, only the seastar *Linckia laevigata* displayed a classic pattern of Indian-Pacific divergence, but with only moderate genetic structure ($\Phi_{CT} = 0.067$). In contrast, the seastar *Protoreaster nodosus* exhibited strong structure ($\Phi_{CT} = 0.23$) between Teluk Cenderawasih and the remainder of Indonesia, a pattern of regional structure that was echoed in *L. laevigata* ($\Phi_{CT} = 0.03$) as well as its obligate gastropod parasite *Thyca crystallina* ($\Phi_{CT} = 0.04$). The generalist commensal shrimp, *Periclimenes soror* showed little genetic structuring across the Coral Triangle. Despite species-specific phylogeographic patterns, all four species showed departures from neutrality that are consistent with massive range expansions onto the continental shelves as sea levels rose, and date within the Pleistocene epoch. Our results suggest that habitat differences may affect the manner in which species responded to Pleistocene sea level fluctuations, shaping contemporary patterns of genetic structure and diversity.

Introduction

Comparative phylogeography is an important tool for evaluating the effects of shared historical biogeographic processes in driving the evolution and regional distribution of biodiversity (Avise 2000; Bermingham & Moritz 1998). Concordant phylogeographic patterns across multiple co-distributed species strongly suggest that the patterns arose from the action of a shared physical process (Arbogast & Kenagy 2001; Schneider *et al.* 1998; Walker & Avise 1998). Conversely, discordant patterns can downplay the importance of shared historical processes, highlighting the role of unique colonization events or refugial habitats (Carstens *et al.* 2005; Taberlet *et al.* 1998; Wares & Cunningham 2001), differences in dispersal characteristics (Dawson *et al.* 2002), or species-specific ecological requirements (e.g. congeners Reid *et al.* 2006) in driving individual phylogeographic patterns.

Similarly, phylogeographic comparisons among taxa with strong symbiotic interactions such as mutualists (DeChaine & Martin 2006; Thompson *et al.* 2005), commensals (Obst *et al.* 2005; Richards *et al.* 2007), and host-parasite systems (Criscione & Blouin 2007; Nieberding *et al.* 2004) generates, *a priori*, an hypothesis of common history (reviewed in Nieberding & Olivieri 2007; Whiteman & Parker 2005). While this expectation of phylogeographic congruence is particularly true for vertically-transmitted symbionts (Funk *et al.* 2000; LaJeunesse *et al.* 2004), it also holds for symbionts with a free-living stage in their life history (Nieberding *et al.* 2004; Thompson *et al.* 2005), and even for those with an intermediate host (Criscione & Blouin 2007). Departures from this expectation highlight ecological differences that may influence the co-evolution of the host and parasite.

In addition to having the highest levels of marine biodiversity in the world (Carpenter & Springer 2005; Roberts *et al.* 2002) the Coral Triangle has a complex geological history (Hall 2002), creating a dynamic evolutionary environment for marine taxa distributed across the region. In particular, sea level fluctuations of up to 130m repeatedly exposed the Sunda and Sahul continental shelves during the Pliocene and Pleistocene (Pillans *et al.* 1998; Voris 2000), resulting in a vicariant barrier that has been hypothesized to have caused genetic divergence between Indian and Pacific Ocean populations of many marine taxa (e.g. Benzie *et al.* 2002; Duda & Palumbi 1999b; Lavery *et al.* 1996; Vogler *et al.* 2008). However, species from this region that have been studied in a phylogeographic context show a variety of genetic patterns. Patterns from single species studies range from regional mosaics of strongly divergent clades (e.g. Barber *et al.* 2002b; Meyer *et al.* 2005) to limited or no evidence of structure (e.g. Bowen *et al.* 2001; see Crandall *et al.* 2008; Williams *et al.* 2002 for review). Similarly, the small number of comparative phylogeographic studies in this region (Barber *et al.* 2006; Crandall *et al.* 2008; Lourie *et al.* 2005; Reid *et al.* 2006) have often found discordant patterns of genetic structure among closely related species, which are generally ascribed to differences in larval dispersal potential or adult ecology. However, a complicating factor in these studies is that while species may be co-distributed, they may not experience the same environmental parameters during sea level fluctuations. An ideal test of the effects of Plio-Pleistocene sea level fluctuations on phylogeographic patterns would include host-parasite or host-commensal pairs, ensuring that species have experienced the same environment.

Linckia laevigata (Valvatida: Ophidiasteridae) is a common seastar found in shallow waters throughout most of the Indo-Pacific. A classic example of Pacific-Indian Ocean vicariance (Williams & Benzie 1993; Williams & Benzie 1997; Williams & Benzie 1998; Williams *et al.* 2002), *L. laevigata* populations are predominantly blue in the Pacific and predominantly orange in the Indian Ocean. Nothing is known about patterns of genetic structure within the Indonesian Archipelago, the transition zone between these ocean basins.

The seastar *Protoreaster nodosus* (Valvatida: Oreasteridae) is co-distributed with *L. laevigata*, though with a more restricted range (Table 1). Although commonly found in the same reef systems, *P. nodosus* is found more on sandy substrates, often in shallow back-reef seagrass meadows, while *L. laevigata* is most common in the coral environs of the fore reef. The two species also differ in several larval characteristics: *P. nodosus* spawns larger eggs (~200 µm in diameter vs. ~140 µm in *L. laevigata*), which develop into larvae that remain in the water column for half as long (10-14 days) as *L. laevigata* (22-28 days). *P. nodosus* larvae also exhibit strong positive geotaxis that might keep them out of surface currents (Table 1, Yamaguchi 1973; Yamaguchi 1977) while *L. laevigata* stay high in the water column (Yamaguchi 1973). With seemingly more limited dispersal potential, it is expected that *P. nodosus* should exhibit more pronounced genetic structure across the Coral Triangle than *L. laevigata.*

The snail *Thyca crystallina* (Sorbeoconcha: Eulimidae) and shrimp *Periclimenes soror* (Decapoda: Pontoniinae) are ectosymbionts found on the oral surfaces of one or both of the above seastars. *T. crystallina* is an obligate parasite of *Linckia* spp. that fuses permanently to its host and feeds on the hemal and perihemal fluids (Egloff *et al.* 1988). Infection rates in a *Linckia* subpopulation can range from complete absence to over 60%, and infected seastars typically have 1-5 *T. crystallina* (Elder 1979, and personal observation). Although larval duration is not known for this species, the veliger larvae have multi-spiral protoconchs (Warén 1980), which are diagnostic of planktotrophy in gastropods (Jablonski & Lutz 1983), suggesting a relatively long period in the plankton.

Unlike *Thyca crystallina*, which parasitizes only *Linckia* species, *Periclimenes soror* is a symbiont found on more than 25 species of seastar throughout the tropical Pacific Ocean, although the exact relationship with their host is undetermined (Bruce 1976, AJ Bruce, personal communication). This species is found on both *P. nodosus* and *L. laevigata* (approximately 20% of seastars with 1-5 shrimp/seastar), where they match the color of their hosts. Reproduction presumably occurs between shrimp on the same host, and planktotrophic zoeae are released into the water column (Wear 1976) although the larval duration of this species is unknown. The generalist habit of *P. soror* should reduce the amount of genetic structure measured across the Coral Triangle relative to the more host-specific *T. crystallina*.

As discussed above, evidence for vicariance due to sea level fluctuations within the Coral Triangle is mixed, and a species' susceptibility to population fragmentation in this region may depend on the ecology of the adult, or the dispersal characteristics of the larval stage (Reid *et al.* 2006; Crandall *et al.* 2008). Although the long-distance dispersal capabilities of marine larvae can diffuse the signal of shared vicariance in the ocean (Bay *et al.* 2006; Rocha *et al.* 2007), the strong ecological ties between hosts and symbionts in this study should result in similar patterns of genetic structure among these species. Furthermore, we would expect to see an excess of recent mutations in all four species resulting from range expansions as shelf habitat re-flooded. To test these hypotheses, we compared phylogeographic patterns and demographic history reconstructed from mitochondrial COI sequences for samples of all four taxa collected across the Coral Triangle.

Materials and Methods

Sampling and Sequencing

We collected a small piece (~2 cm) of arm tissue from *Linckia laevigata* (n=504, 24 localities) and *Protoreaster nodosus* (n=320, 22 localities) populations from across Indonesia and preserved them in 95% ethanol (Fig. 1). Because of the possibility that sympatric populations of blue and orange color morphs in *L. laevigata* may be genetically structured (Williams & Benzie 1998), we opted to take samples only from slate blue and royal blue morphs. *Thyca crystallina* (n=289, 24 localities) and *Periclimenes soror* (n=297, 25 localities) were collected from the same localities when present. Samples of *P. soror* from different host seastars (mostly *Linckia, Protoreaster* and *Culcita* spp.) were kept separated due to the possibility of local host adaptation (Sotka 2005).

We extracted DNA from tube feet or muscle tissues using a 10% Chelex™ (Biorad) solution (Walsh *et al.* 1991). For all species except *P. nodosus* 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) and previously published PCR protocols (Barber *et al.* 2006). For *P. nodosus* we constructed primers tRNAasn42F (5'- AACGGCCAATYGCCTTTCCATTAGG-3') and ValvaCOI-770R (5'- TATACYTCKGGGTGGCCAAAGAATC-3') from an alignment of mitochondrial sequences for the order Valvatida (Hart *et al.* 1997; Waters *et al.* 2004; Williams 2000). These primers amplify an 866-bp region including the region amplified by the Folmer primers and extend in the 5' direction to encompass a portion of the Asparagine tRNA region. PCR reaction mixture was the same as for the other species, and cycling parameters for these primers were: initial denaturation 94^{o}C (2 min.), main cycle 94^{o}C (30s), 60^{o}C (30s) and 72^{o}C (60s) for 39 cycles, then a final extension of 72^{o}C (3 min.). We cleaned 5µl of PCR products with 0.5 units of Shrimp Alkaline Phosphatase (Biotech Pharmacon) and 5 units of Exonuclease I (GE Healthcare), and incubated them at 37^{o}C for 30 minutes and 80^{o}C for 15 minutes. We sequenced forward and reverse directions of double-stranded PCR products with Big Dye™3.1 (Applied Biosystems Inc.) terminator chemistry on an ABI 377 sequencer. Chromatograms were assembled, aligned and proofread in Sequencher™4.5, and amino acid translations confirmed using MacClade 4.05 (Maddison & Maddison 2002).

Data Analysis

We investigated the relationship and geographic distributions of individual haplotypes through several methods. First, we constructed minimum spanning trees based on pairwise differences in Arlequin 3.1 (Excoffier *et al.* 2005) and drew them in Adobe Illustrator™. To depict genetic structure in the two seastars in a geographic context, we summarized the frequencies of haplotype clusters that diverged by five or more mutational steps in *L. laevigata* and plotted these onto a map of the study region (Fig. 4). Because of limited genetic variation in *P. nodosus* we lowered this arbitrary threshold to two steps for this species.

To examine patterns of genetic structure in each species we used Arlequin to calculate pairwise Φ_{ST} between sampling localities, and between samples from different host species for *P. soror*. To improve statistical power, and because sampling localities differed slightly between species, we grouped localities that showed no significant pairwise structure for any species ($p > 0.10$ from 10,000 random permutations of haplotype distribution) into regional sites that could be compared across species (Fig. 1). We then estimated pairwise Φ_{ST} and net divergence, d_A (Nei & Li 1979) for these new groupings, with p-values determined by 10,000 random permutations of the data and significance determined for a false discovery rate of 0.05 (Benjamini & Hochberg 1995) using Pairwise Multiple Tests 1.0 (Watkins 2005) . We also estimated standard genetic diversity indices within each site (Appendix I, Table 2). Because admixture of divergent Pacific and Indian Ocean clades could give misleading results in *L. laevigata*, pairwise Φ_{ST} analyses were also run using only Clade A (Fig. 2a), as well as with standard F_{ST} measures that ignore genetic distance between haplotypes.

Regional genetic structure was then examined using AMOVA as implemented in Arlequin. We tested the hypothesis of Pleistocene vicariance by imposing a structure consisting of sites that border the Indian Ocean (sites 1-7, Fig. 1) and Pacific Ocean sites (sites 8-18, Fig. 1). We also tested an alternative hypothesis of vicariance, that compared western Indonesian populations (sites 1 & 2) to the remaining populations, following patterns previously observed in *Linckia laevigata* and the crown-of-thorns seastar, *Acanthaster plancii* (Benzie 1999), and from patterns observed in the pairwise Φ_{ST} values.

We conducted further AMOVA analyses that focused on divergence between Teluk Cenderawasih and the rest of the Coral Triangle, following the observation of multiple significant pairwise Φ_{ST} values in multiple species and the distinct phylogeographic pattern exhibited by *Protoreaster nodosus* in this region. As above, to account for the effects of admixture of Pacific and Indian Ocean clades, AMOVA analyses for *L. laevigata* were run using all data and Clade A only. We did not run AMOVA with standard F_{ST} because high haplotype diversity within sites can greatly reduce the maximum value of this statistic (Hedrick 2005). Significance of AMOVA analyses was tested using 10,000 randomized replicates.

To compare demographic histories of mtDNA associated with each species, we calculated Fu's F_s (Fu 1997) to test each site for departures from the neutral model due to positive selection, background selection or population growth. We also estimated a Bayesian skyline plot for each species using BEAST v.1.4.3 (Drummond 2003; Drummond *et al.* 2002) that estimates effective population size scaled by generation length ($N_e\tau$), based on departures from coalescent expectations for a neutral model over a series of discrete time intervals (Drummond *et al.* 2005) running back to the T_{MRCA} of the sample. Due to computational limits on large datasets, we constructed trimmed datasets of sequences for each species comprising 100 randomly selected sequences. We excluded two sequences from the divergent clade in *T. crystallina,* which likely immigrated from a divergent population. Each sub-sampled dataset was run twice for 50 million steps under an HKY+I+G model of mutation and a stepwise skyline model with five separate time intervals and uniform priors. We inspected logfiles and treefiles from replicate runs for convergence in Tracer and then combined them using LogCombiner, and generated a skyline plot in Tracer 1.4 (Rambaut & Drummond 2007).

To convert BEAST estimates into more intuitive units of time and effective population size, we used a lineage mutation rate of 0.5% per million years as a heuristic (corresponding to a divergence rate of 1% per million years). This heuristic was used because divergence rates are not well known for taxa in this study, but likely differ among taxa (ranging from 0.7% - 1.59% in mollusks, crustaceans and echinoderms Hickerson *et al.* 2006; Knowlton & Weigt 1998; Marko 2002), and may be much faster over the relatively recent timescales in question (Ho *et al.* 2005). Our use of this heuristic value does not reflect an acceptance of equal mutation rates among the studied taxa. Because we were interested in whether expansion events occurred within the Pleistocene, the use of this conservative divergence rate ensures that ages are consistently overestimated.

Results

We obtained sequence data from mitochondrial COI for 1,419 individuals from all four species. All sequence data aligned without indels and translated without stop codons.

Sequence data from 504 *Linckia laevigata* yielded 250 unique haplotypes (h = 0.983, π = 0.013) that were partitioned into three clades separated by five or more steps (A, B, C; Fig. 2a). Clade A corresponds to the Indian Ocean clade recovered by Williams (2000) and Clades B and C are subsets of her Pacific Ocean clade. Clade A has its highest frequency in Western Indonesia, while Clades B and C have a higher frequency in Eastern Indonesia (Fig. 4a).

Data from 320 *Protoreaster nodosus* yielded only 87 haplotypes (h = 0.773, π = 0.003) that grouped into three star-like clusters, each separated by two steps; there were no phylogenetic divergences greater than three steps (Fig. 2b). The haplotype at the center of Cluster A was shared by 150 individuals and was distributed throughout the range of the species, although at lower frequency in Teluk Cenderawasih (sites 14-17). Of 20 total non-synonymous substitutions, one occurs along the branch leading to the second most common haplotype, and defines haplotype cluster C, which is found with highest frequency in the region of Teluk Cenderawasih (Fig. 4b).

Sequences from 289 *Thyca crystallina* contained 93 unique haplotypes (h = 0.958, π = 0.006). The minimum spanning tree contained five star-like polytomies (Fig. 3a). Two individuals, from Sebesi and Raja Ampat (sites 3 and 13), had haplotypes that were seven steps divergent from other Indonesian samples, and were closely related to *T. crystallina* haplotypes sequenced from Fiji (E. Crandall, unpublished data).

We recovered 175 unique haplotypes from 297 COI sequences of *Periclimenes soror* (h = 0.972, π = 0.007). These sequences showed no evidence of phylogenetic divergence, with five major "star" polytomies separated by one step, but several tip haplotypes separated by four or five mutational steps (Fig. 3b). We found no evidence of genetic divergence or structuring between shrimp taken from any of the three host seastars. However, eleven individuals taken from *P. nodosus* in Raja Ampat (site 13) and nine taken from Anak Krakatau (site 2, host unrecorded, but no *P. nodosus* were found at this locality), fell into a second, highly divergent clade (~17% uncorrected p-distance, with 10 non-synonymous substitutions). These individuals were not morphologically distinguishable from other *P. soror* (A.J. Bruce, personal communication), but are likely a cryptic species. These divergent haplotypes were excluded from subsequent phylogeographic analyses.

Genetic structure

Pairwise Φ_{ST} values for *Linckia laevigata* indicated genetic structure between sites in Aceh and Krakatau and the rest of Indonesia (Table S1). Much of this structure remained significant when Clades B and C were removed from the analysis (Table S2). Under both analyses, there was significant genetic variation partitioned between the islands of Krakatau (site 2) and the islands of Sebesi, Sebuku and Sangiang (site 3). In *P. nodosus*, we found sites in mainland Teluk Cenderawasih, Numfor, and Sabolo Kecil were significantly structured with other sites (Table S3). Due to a high frequency of haplotypes from Cluster C, the site at Sabolo Kecil was strongly structured with its neighboring sites in Southern Indonesia ($\Phi_{ST} = 0.21$) despite only 13 km of separation from the nearest locality in its neighboring site. Neither *Thyca crystallina* nor *Periclimenes soror* showed significant pairwise structure after correction for type I errors. Tables of pairwise Φ_{ST} and d_A values can be found in the Supplementary Material.

We found limited evidence of genetic structure between Indian and Pacific Ocean as defined in the original AMOVA analysis. *Linckia laevigata* exhibited weak structure ($\Phi_{CT} = 0.024$, $p < 0.05$; Table 3), but this structure was not significant when only Clade A was included, indicating that it results from admixture of clades. Structure increased when Indian Ocean populations were defined as Aceh and the islands of Krakatau and compared to all other populations to the north and east (Clade A $\Phi_{CT} = 0.067$, $p < 0.01$; Table 3). No other species exhibited any evidence of Indo-Pacific divergence.

The strongest patterns of regional structure were observed in *Protoreaster nodosus,* which showed strong evidence of isolation between Teluk Cenderawasih, an isolated bay in West Papua (sites 14-17), and sites in the remainder of the Coral Triangle ($\Phi_{CT} = 0.23$, $p < 0.01$; Table 3). Because of this result, we tested for isolation in the three remaining species and found significant, but weaker concordant structure in *Linckia laevigata* Clade A in the same location ($\Phi_{CT} = 0.03$, $p < 0.05$; Table 3). This region also contained significant genetic structuring for *Thyca crystallina*, although it was found between localities on the mainland of Teluk Cenderawasih (site 14), and the bay's islands ($\Phi_{CT} = 0.04$, $p < 0.05$; Table 3). *P. soror* had no significant structure in this region.

Demographic History

Fu's F_s was significantly negative at most sites ($p < 0.02$) for all species, indicating departures from neutral expectations for the number of recent mutations (Table 2). F_s values for a few *L. laevigata* sites in eastern Indonesia were not significant, most likely due to the admixture of three divergent clades. Bayesian skyline plots obtained from BEAST revealed exponential growth in effective population size in all four species. Although the timing of these expansions was not concurrent, estimated times for these expansions all fall well within the Pleistocene (0.15 – 0.75 mya), even when considering a very slow substitution rate (Fig. 4). *L. laevigata* commenced growth earliest and at a slower rate than the other species, and is followed in chronological order by *P. soror, T. crystallina*, and *P. nodosus*. Two replicate runs for each dataset produced highly similar parameter estimates for each species, and combined effective sample sizes (ESS) were greater than 200.

Discussion

Vicariance during Plio-Pleistocene sea level fluctuations is commonly invoked as a major force in driving genetic divergence between Pacific and Indian Ocean populations (e.g. Barber *et al.* 2000; McMillan & Palumbi 1995; Williams & Benzie 1998). Although the similar distribution of two sea stars and the tight ecological associations with their symbionts/parasites could reasonably lead to the observance of concordant patterns of genetic structure, results instead reveal a complex response to this common environment. All four species retain the genetic imprint of recent demographic expansions dating to the Pleistocene, consistent with a range expansion following periods of lowered sea levels. However, besides *Linckia laevigata,* a species where Pacific-Indian Ocean vicariance has been well established through broad-scale sampling across these basins (Williams & Benzie 1998, Williams *et al.* 2002), none of the other species exhibit a strong signal of Indo-Pacific vicariance within the Coral Triangle.

Instead, phylogeographic patterns vary considerably among these closely associated taxa. Strongest patterns of regional structure were observed in *P. nodosus*, where populations in Teluk Cenderawasih were divergent from the remainder of Indonesian populations. Subtle evidence of structure in this region was also seen in *Linckia laevigata* and *Thyca crystallina,* although Indonesia was largely a region of admixture of Pacific and Indian Ocean clades in *L. laevigata. Periclimenes soror* showed no evidence of structure across the Coral Triangle. Results indicate that even when species experience the same environment by virtue of physical association, their overall genetic diversity and structure are strongly affected by their individual ecologies.

Structure of *Linckia laevigata* in the Coral Triangle

Multiple studies have established that Indian and Pacific ocean populations of *Linckia laevigata* were separated at some point in the Pliocene or Pleistocene, likely due to sea-level fluctuations (Williams & Benzie 1998; Williams *et al.* 2002). These results are corroborated by the predominance of blue color morphs in the Pacific and orange color morphs in the Indian Ocean. Although we did not sample orange morphs, individuals previously sampled from Indian Ocean populations all fall within Clade A (Fig. 2a, Williams 2000). Together with its predominance in the west, it seems likely that this clade was the ancestral Indian Ocean clade, while clades B and C may have developed in the Pacific. Extensive mixing of Indian and Pacific mtDNA lineages within the islands of Indonesia contrasts with other species that show sharp genetic breaks between Indian and Pacific Ocean lineages on small spatial scales within the Coral Triangle (Barber *et al*. 2000, Crandall *et al.* 2008).

Admixture between formerly allopatric lineages has been inferred in several Indo-Pacific species (Chenoweth *et al.* 1998; Crandall *et al.* 2008; Perrin & Borsa 2001; Williams *et al.* 2002), a result that can dramatically impact inferences of genetic structure. Although obscured by secondary admixture of Indian and Pacific clades, significant genetic structure is still observed in *L. laevigata* populations in the Sunda Strait and Teluk Cenderawasih excluding all but clade A. For example, if we compare *L. laevigata* from Teluk Cenderawasih to remaining populations, we find no significant structure when all clades are included, but there is significant structure when only clade A is considered ($\Phi_{CT} = 0.03$, $p < 0.05$), highlighting the importance of investigating patterns within individual lineages if admixture is observed.

Similarly, structure in Clade A is observed between Western Indonesia sites in Aceh and Krakatau (sites 1 and 2), when compared with sites to the east, echoing the deep vicariance among Pacific and Indian Ocean clades (Williams *et al.* 2002). Interestingly, this break occurs between Krakatau and sites in Sebuku and Sebesi that are only 10-15 km to the north ($\Phi_{CT} = 0.03$, $p < 0.01$). All of these sites were recently re-colonized following the 1883 volcanic explosion of Krakatau that sterilized the seafloor for a radius of at 15 kilometers, and covered much of the Sunda Strait with a meter-thick blanket of ash (Barber *et al.* 2002a; Carey *et al.* 1996; Mandeville *et al.* 1996). The modern genetic affinity of the islands of Krakatau with Aceh suggests that a large proportion of the larvae that re-colonized the islands came from Indian Ocean sites, while islands further inside the strait were likely resettled from reefs on the Sunda Shelf.

Given that all orange morphs in Williams (2000) fall out in the Indian Ocean clade (Clade A), the exclusion of orange individuals in our sampling likely decreases the Indian Ocean signature in our data set. However, orange individuals were extremely rare except at sites 1-3, where they were still outnumbered by blue morphs. Given that Clade A was the dominant clade throughout Indonesia, despite sampling only blue individuals, it is unlikely that the inclusion of a small number of orange individuals from sites 1-3 would affect our results. However, inclusion of orange morphs may have increased the relatively weak genetic structure observed between the Indian and Pacific oceans ($\Phi_{CT} = 0.02$, $p < 0.05$) by virtue of including more Indian Ocean haplotypes. The recovery of mostly Indian Ocean (clade A) haplotypes from blue individuals confirms and supports the notion of Williams (2000) that mixing between Indian and Pacific populations has "smudged" the formerly distinct boundaries in this species.

Structure of *Protoreaster nodosus* in the Coral Triangle

While *L. laevigata* exhibits weak genetic structure across the Coral Triangle ($\Phi_{\mathbf{ST}} = 0.068$, $p < 0.0001$, genetic structure is more pronounced in *P. nodosus* ($\Phi_{\mathbf{ST}} = 0.166$, $p < 0.0001$). However, this pattern is driven by the high percentage (23%) of variation sequestered between Teluk Cenderawasih and the rest of the Coral Triangle (Table 4, Fig. 4b), indicating very little gene flow among these regions since the selective sweep or range expansion reflected by its mtDNA genealogy. In addition, the site at Sabolo Kecil near Flores had a radically different genetic composition than its neighbor at Sebayur, showing that fine scale structure can occur over distances as little as the 13km of coastal ocean between these two populations, highlighting the stochasticity in the system.

The more pronounced pattern of genetic structure in *P. nodosus* may result from the shorter pelagic duration of its larvae as there is some evidence for an inverse relationship between pelagic larval duration and genetic structure (but see Bay *et al.* 2006; Bohonak 1999; Bowen *et al.* 2006; Shulman & Bermingham 1995). However, *Protoreaster nodosus* shows a much higher degree of genetic structure across the Coral Triangle than the abalone *Haliotis asinina*, which has an even shorter PLD (4-10 days, Imron *et al.* 2007). This contrast suggests that the observed genetic structure could potentially be influenced by larval behavior, such as the positive geotaxis observed in *P. nodosus* larvae (Yamaguchi 1977). The few studies that have explicitly considered differences in larval behavior have found significantly greater genetic structure in species with larval behaviors that favor local retention (Gerlach *et al.* 2007; Riginos & Victor 2001).

Interestingly, *Protoreaster nodosus* had the most pronounced phylogeographic patterns despite having levels of nucleotide diversity approximately five times lower than those in *L. laevigata* (Table 2), a patterns than may result from habitat differences. *P. nodosus* is a lagoonal species while *L. laevigata* inhabits the reef, including the reef slope. During lowered sea levels lagoonal habitat would be lost, resulting in local extinctions and a loss of genetic diversity. In contrast reef dwelling species like *L. laevigata* could simply by migrate down the reef slope (figure 2 in Paulay 1990) and retain much more genetic diversity, and thereby a deeper genetic history. Additional support for this hypothesis comes from the failure to find any *P. nodosus* along the western shores of Sumatra, a region characterized by a steep continental shelf and little back reef or lagoonal environments. Thacker (2004) and Fauvelot *et al*. (2003) made similar inferences for lagoonal fish species that showed reduced genetic diversity in comparison to species that can survive on the reef slope.

Genetic Structure in Ectosymbionts

Although no significant structure was observed between Pacific and Indian Ocean demes of *T. crystallina*, a subtle but significant pattern of structure emerges between Teluk Cenderawasih and the rest of the Coral Triangle ($\Phi_{ST} = 0.04$, $p < 0.05$). Structure in this region is also observed in clade A of its host, *L. laevigata* ($\Phi_{ST} = 0.03$ $p < 0.05$), although the exact boundaries vary slightly. In contrast, there was no structure at all observed in *Periclimenes soror*. The only evidence of differentiation in *P. soror* was the recovery of a highly divergent lineage (17% uncorrected pair-wise divergence) in Raja Ampat and on the island of Anak Krakatau. While morphologically indistinguishable from *P. soror*, the depth of divergence is much greater than the 2-5% divergence that is found between sister species in the Caridean genus *Alpheus* (Mathews *et al.* 2002), suggesting that these divergent sequences are likely cryptic species.

One potential explanation for the unique patterns of the ectosymbionts is the nature of their commensalism. While both *T. crystallina* and *P. soror* are obligate symbionts of seastars, *P. soror* is much more of a generalist, occurring on more than 25 species of Indo-Pacific Asteroid, while *T. crystallina* only occurs on species of *Linckia*. The plethora of seastar species that host *P. soror* live in a wide variety of habitats ranging from deep sand flats to coral reefs to shallow lagoons. Thus, while larvae of *T. crystallina* must find a species of *Linckia* before they can settle and mature, the larvae of *P. soror* can find suitable habitat wherever any of their host species is present. This generalist nature may facilitate gene flow among *P. soror* populations, and may help explain its large effective population size, and negligible genetic structure across the Coral Triangle.

A Shared History of Sea Level Change in the Coral Triangle

Although these species did not exhibit concordant patterns of divergence across the Coral Triangle, there is a strong signal of a shared demographic history. The strongly negative Fu's F_s values (Table 2), together with multiple star-polytomies (Figs. 3 and 4) indicate either a recovery from a selective sweep or a recent range expansion. While selective sweeps due to adaptive variation in the mitochondrial genome (e.g. Rawson & Burton 2006) cannot be excluded, multiple independent selective sweeps in unrelated taxa seems unlikely. Alternatively, eustatic reductions in sea level of over 130 meters during glacial periods of the Pliocene and Pleistocene eras would have exposed the Sunda and Sahul shelves (Voris 2000), causing local extinctions of shelf populations followed by flooding and recolonization during inter-glacial periods. Bayesian skyline plots indicate expansions in all taxa and date the expansions in each species to well within the Pleistocene (Fig. 5), even when using a conservative mutation rate, suggesting a more plausible explanation for departures from neutrality.

In addition, there is some evidence of shared isolation during periods of lowered sea levels. Although they differ in the exact location and magnitude of differentiation (Table 4), both seastar species, together with *T. crystallina,* show evidence of genetic structure in Teluk Cenderawasih. The submerged portion of the Biak-Yapen terrane forms a sill 10m-200m in depth that stretches nearly across the bay (Hall 2002; USGS 2007, Figure 1). Much of this sill would have been exposed during low sea level stands, likely constricted water flow and thus larval dispersal into the bay. Similar patterns of isolation in Teluk Cenderawasih are seen in stomatopods and giant clams (Barber *et al*. 2006, DeBoer *et al*. 2008).

Given the large sea level changes in the Sunda area, it is surprising that little evidence of Pleistocene vicariance among Pacific and Indian Ocean populations was found. Some evidence of Indian-Pacific vicariance was seen in *Linckia laevigata* with significant structure among western Sumatra population and populations to the west, a result consistent with this hypothesis but different from predictions based purely our definition of Indian Ocean populations (e.g. islands that border the Indian Ocean). Given that admixture of *L. laevigata* within the Coral Triangle only becomes apparent when compared to the results of broad spatial sampling conducted by Williams and Benzie (1998) and Williams *et al*. (2002), the absence of broad-scale samples in the remaining three species may limit our ability detect Indian-Pacific Ocean vicariance. Further data from more peripheral populations of these species may help to resolve patterns within the Coral Triangle, as observed with *L. laevigata*.

Conclusions

Despite predictions for concordant divergence across the Coral Triangle two species of seastar and their ectosymbionts show unique patterns of structure across this region. Although sea level fluctuations have likely shaped patterns of genetic diversity and structure in all species, species-specific differences appear to have led to different phylogeographic responses to this shared environment, despite their close physical and ecological associations.

One key difference may be the vulnerability of a species' adult habitat to past climate change. *Linckia laevigata*, which can live on outer reef slopes, has maintained an effective population size large enough to retain two divergent mitochondrial lineages. The genetic diversity of *Protoreaster nodosus*, on the other hand, with lower dispersal abilities and vulnerable adult habitat, appears to have been greatly lowered by local extinctions resulting from sea level change. A similar contrast can be made among the ectosymbionts. *Periclimenes soror,* whose host-generalist habit provides it with the widest array of potential adult habitat, shows almost no structuring across the entire Coral Triangle, while genetic structure in *Thyca crystallina* echoes that in its host, *L. laevigata*. Although more work will be required to test this hypothesis, phylogeographic patterns at the Coral Triangle should not be viewed simply in terms of vicariance. While the isolating effects of lowered sea levels cannot be denied, species-specific habitat differences, and how these differences affect the ability of local populations to endure the effects of sea-level change in this region may also play a role in shaping patterns of genetic structure and diversity in this region.

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Figure Legends

Figure 1. Map of localities sampled in Indonesia. Dark grey shading depicts the 100m continental depth (USGS 2007; Voris 2000). Sea level was at or below this depth for ~25% of the last 250 kya, closing most of the major seaways between the Indian and Pacific Oceans. Open circles indicate localities where one or more species were sampled. Numbers indicate sites into which sampling localities were grouped for comparison (see Table 2 for site names).

Figure 2. Minimum spanning trees for both seastars. Light grey haplotypes were found in Teluk Cenderawasih, and dark grey haplotypes were found in Aceh and Krakatau (*L. laevigata* only). Circles

are sized proportionally to the frequency of occurrence, ranging from 1-44 in *L. laevigata* and 1-19 in *P. nodosus.* The most frequent haplotype for *P. nodosus* was found in 150 individuals and is not shown to scale. Dotted lines indicate the relationships of overlapping COI sequences for *L. laevigata* from Williams (2000). These sequences were not used in any analyses. All haplotypes are separated by one mutational step unless denoted by a higher number of hatch marks, or a number. White asterisks denote the most probable root haplotype found by TCS (Clement *et al.* 2000). Non-synonymous mutations found in more than three individuals are marked with a white rectangle. For tip clades, the mean mutational distance to the central haplotype is given with a standard deviation.

Figure 3. Minimum spanning trees for both ectosymbionts. Grey haplotypes were found in mainland Teluk Cenderawasih (*T. crystallina* only). Circles are sized proportionally to the frequency of occurrence, ranging from 1-25 in *T. crystallina* and 1-33 in *P. soror.* All haplotypes are separated by one mutational step unless denoted by a higher number of hatch marks. White asterisks denote the most probable root haplotype found by TCS (Clement *et al.* 2000). Non-synonymous mutations found in more than three individuals are marked with a white rectangle. For tip clades, the mean mutational distance to the central haplotype is given with a standard deviation.

Figure 4. Maps of the study area for a) *Linckia laevigata* and b) *Protoreaster nodosus* with pie diagrams representing relative frequencies each clade/haplotype cluster in each site listed in table 2.

Figure 5. Bayesian skyline plots of effective population size scaled by generation time ($N_e\tau$) for mtDNA in all four species. The plots run from the present to their mean time to most recent common ancestor (T_{MRCA}). Grey lines represent 95% C.I. for $N_e\tau$. Parameter estimates were converted from mutational units using a slow per site mutation rate of 0.5% per million years, solely as a heuristic.

Table 1. Dispersal, range and habitat characteristics for the four species compared in this study.

Species	Adult Habitat	Range	Larval Type/Behavior	Pelagic Larval Duration	Genbank Accession #
Linckia laevigata	Coral reef to 30m	South Africa to Cook Islands[1]	Planktotrophic/ Negatively geotaxic[1]	22-28 days[1]	COI - 658 bp XXXXXX-XXXXXX
Protoreaster nodosus	Lagoon/seagrass meadow to 5m	Sri Lanka to New Caledonia[2]	Planktotrophic/ Positively geotaxic[2]	10-14 days[2]	COI - 658 bp XXXXXX-XXXXXX
Thyca crystallina	Oral surface of *Linckia* spp.[3]	Samoa to Madagascar[3]	Planktotrophic[4]/ Unknown	Unknown	COI - 658 bp XXXXXX-XXXXXX
Periclimenes soror	Oral surface of > 25 host seastars[5]	Africa to South America[6]	Planktotrophic[7]/ Unknown	?? 14-34 days in *P. sagittifer*[8]	COI - 866 bp XXXXXX-XXXXXX

[1] Yamaguchi 1973
[2] Yamaguchi 1977
[3] Warén 1980
[4] Multi-spiral protoconch described in Warén 1980 is diagnostic of planktotrophy Jablonski & Lutz 1983
[5] A.J. Bruce, personal communication
[6] Li & Bruce 2006
[7] Wear 1976
[8] dos Santos *et al.* 2004

Table 2. Summary statistics and neutrality test statistics for each of 21 sites shown in Figure 1. Haplotype diversity (h), nucleotide diversity (π) and F_s (Fu, 1997) calculated in Arlequin 3.1 (Excoffier *et al*. 2005).

Site	*Linckia laevigata*				*Protoreaster nodosus*				*Thyca crystallina*				*Periclimenes soror*			
	n	**π**	**h**	**F_S**	**n**	**π**	**h**	**F_S**	**n**	**π**	**h**	**F_S**	**n**	**π**	**h**	**F_S**
1. Aceh	15	0.010	0.98	**-5.19**									19	0.007	0.97	**-7.90**
2. Krakatau	49	0.010	0.99	**-25.21**									8	0.008	0.93	-1.72
3. Sebesi/Sebuku/Sangiang	47	0.013	0.98	**-24.77**					17	0.007	0.93	**-7.08**				
4. Pulau Seribu	79	0.013	0.99	**-24.75**					17	0.006	0.94	**-5.20**	7	0.008	1.00	**-2.94**
5. Karimunjawa					38	0.002	0.72	**-10.95**								
6. Nusa Tenggara (Lesser Sundas)	23	0.015	0.99	**-10.04**	24	0.002	0.74	**-3.44**	21	0.006	0.97	**-9.73**	47	0.005	0.97	**-26.19**
7. Sabolo Kecil, Flores					9	0.003	0.69	1.25								
8. South Sulawesi	7	0.014	0.95	0.01	31	0.003	0.84	**-6.17**	11	0.005	0.95	**-4.13**	26	0.005	0.98	**-23.38**
9. Manado	77	0.013	0.98	**-24.70**	29	0.002	0.68	**-5.06**	34	0.007	0.96	**-14.54**	18	0.006	0.85	-2.23
10. Lembeh Strait	20	0.015	0.97	-2.72									17	0.006	0.99	**-11.37**
11. Sangihe	12	0.010	0.98	**-4.21**					9	0.006	1.00	**-5.66**	14	0.008	1.00	**-10.29**
12. Halmahera	75	0.013	0.96	**-24.69**	66	0.002	0.77	**-27.34**	76	0.006	0.96	**-25.89**	54	0.005	0.95	**-26.05**
13. Raja Ampat	31	0.013	0.98	**-12.22**	55	0.001	0.55	**-12.90**	42	0.007	0.94	**-17.53**	32	0.004	0.95	**-17.89**
14. Teluk Cenderawasih (mainland)	22	0.016	0.99	**-6.73**	7	0.002	0.71	-0.13	26	0.007	0.96	**-13.00**	20	0.007	0.98	**-13.48**
15. Numfor					15	0.003	0.79	-0.09								
16. Biak	7	0.018	1.00	-1.25	23	0.003	0.79	**-3.19**	19	0.006	0.98	**-9.10**	17	0.007	0.99	**-12.26**
17. Yapen	19	0.009	0.91	-1.35	22	0.003	0.86	**-3.14**	12	0.005	0.95	**-3.54**	18	0.007	0.99	**-11.19**
18. Jayapura	19	0.012	0.98	**-4.48**	1	n/a	n/a	n/a								

Table 3. AMOVA results showing variance components (Var. Comp.), % Variation (% Var.) and F-statistics for a hypothesis of Pacific vs. Indian Ocean vicariance, as well as for isolation within Teluk Cenderawasih. We also show results for an alternative hypothesis that Krakatau (site 2) is more similar to Aceh (site 1), while Sebesi, Sebuku and Sangiang (site 3) are more closely related to Pacific sites. AMOVA was run on the full dataset as well as a dataset with haplotypes from Clade A. Comparisons in bold had p-values < 0.05.

Comparison	Species	Among Regions				Among Sites Within Regions				Among All Sites			
		Var. Comp.	% Var.	Φ_{CT}	P-value	Var. Comp.	% Var.	Φ_{SC}	P-value	Var. Comp.	% Var.	Φ_{ST}	P-value
Indian Ocean (1-7) vs. Pacific Ocean (8-18)	*L. laevigata* All Clades	**0.11**	**2.40**	**0.02**	**< 0.05**	**0.25**	**5.41**	**0.06**	**< 0.0001**	**4.21**	**92.20**	**0.08**	**< 0.0001**
	L. laevigata Clade A	0.03	1.11	0.01	0.13	**0.07**	**2.96**	**0.03**	**< 0.0001**	**2.30**	**95.94**	**0.041**	**< 0.0001**
	P. nodosus	-0.01	-1.35	0.00	0.29	**0.2**	**17.28**	**0.17**	**< 0.0001**	**1.15**	**84.07**	**0.16**	**< 0.0001**
	T. crystallina	-0.01	1.24	0.00	0.7	0.03	1.24	0.012	0.065	2.1	99.27	0.01	0.09
	P. soror	-0.01	-0.20	0.00	0.48	**0.04**	**1.68**	**0.02**	**< 0.01**	**2.08**	**98.52**	**0.01**	**< 0.01**
Aceh+Krakatau (1,2) vs. All Other Sites (3-18)	*L. laevigata* All Clades	0.33	6.88	0.07	0.087	**0.23**	**4.78**	**0.05**	**< 0.0001**	**4.22**	**88.35**	**0.13**	**< 0.0001**
	L. laevigata Clade A	**0.17**	**6.72**	**0.07**	**< 0.01**	**0.03**	**1.35**	**0.02**	**< 0.01**	**2.31**	**91.93**	**0.08**	**< 0.0001**
	P. soror	0.00	0.33	0.00	0.23	**0.03**	**1.53**	**0.02**	**< 0.01**	**2.07**	**98.15**	**0.02**	**< 0.01**
Teluk Cenderawasih (14-17) vs. Rest of Coral Triangle (1-13,18)	*P. nodosus*	**0.32**	**23.38**	**0.23**	**< 0.01**	**0.07**	**5.35**	**0.07**	**< 0.0001**	**0.97**	**71.27**	**0.29**	**< 0.0001**
	L. laevigata All Data	-0.02	-0.49	0	NA	**0.31**	**6.91**	**0.07**	**< 0.0001**	**4.22**	**93.58**	**0.06**	**< 0.0001**
	L. laevigata Clade A	**0.08**	**3.34**	**0.03**	**< 0.05**	**0.072**	**2.91**	**0.03**	**< 0.0001**	**2.31**	**93.75**	**0.06**	**< 0.0001**
Mainland Teluk Cenderawasih (14) vs. Rest of Coral Triangle (1-13, 14-18)	*T. crystallina*	**0.089**	**4.09**	**0.04**	**< 0.05**	0.011	0.5	0.005	0.25	2.09	95.41	0.046	0.07

Figure 1.

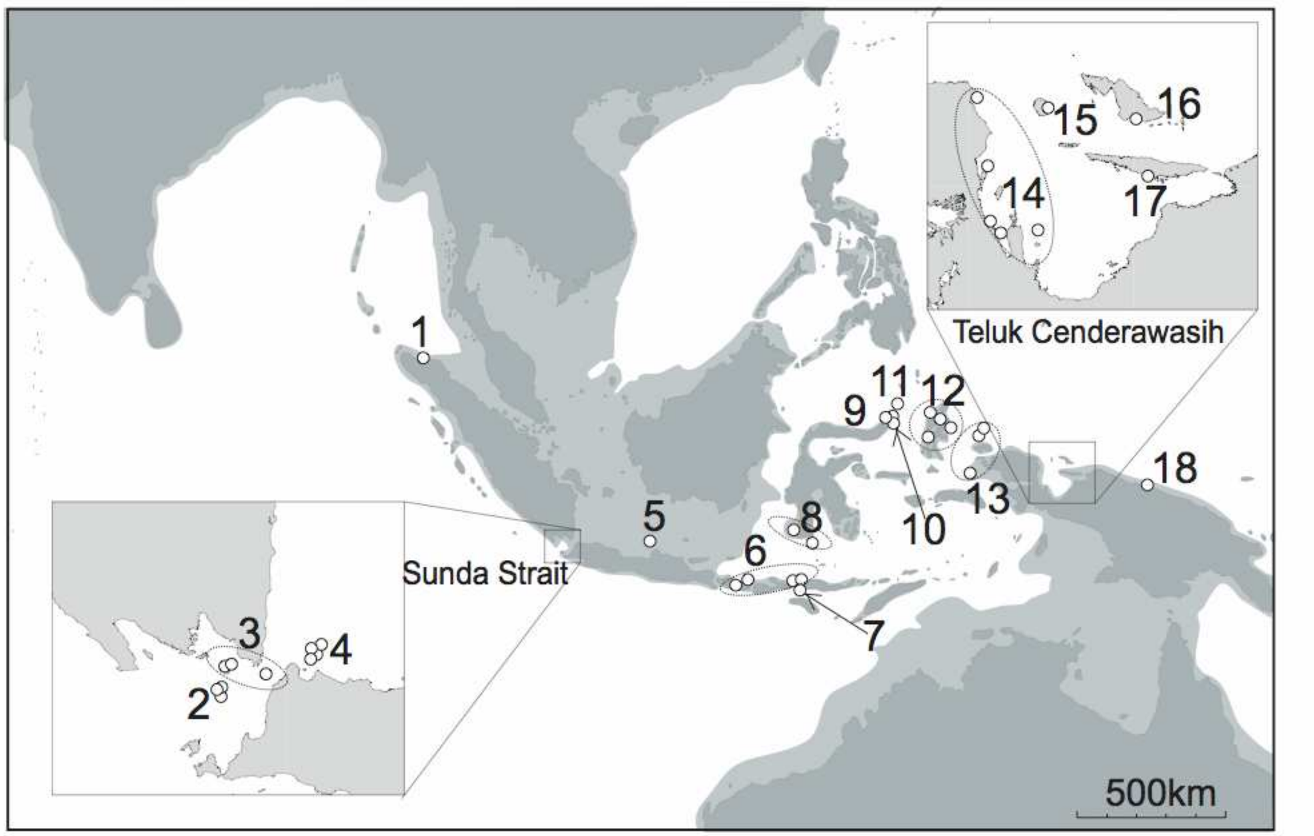
Teluk Cenderawasih
Sunda Strait
500km
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

Figure 2.

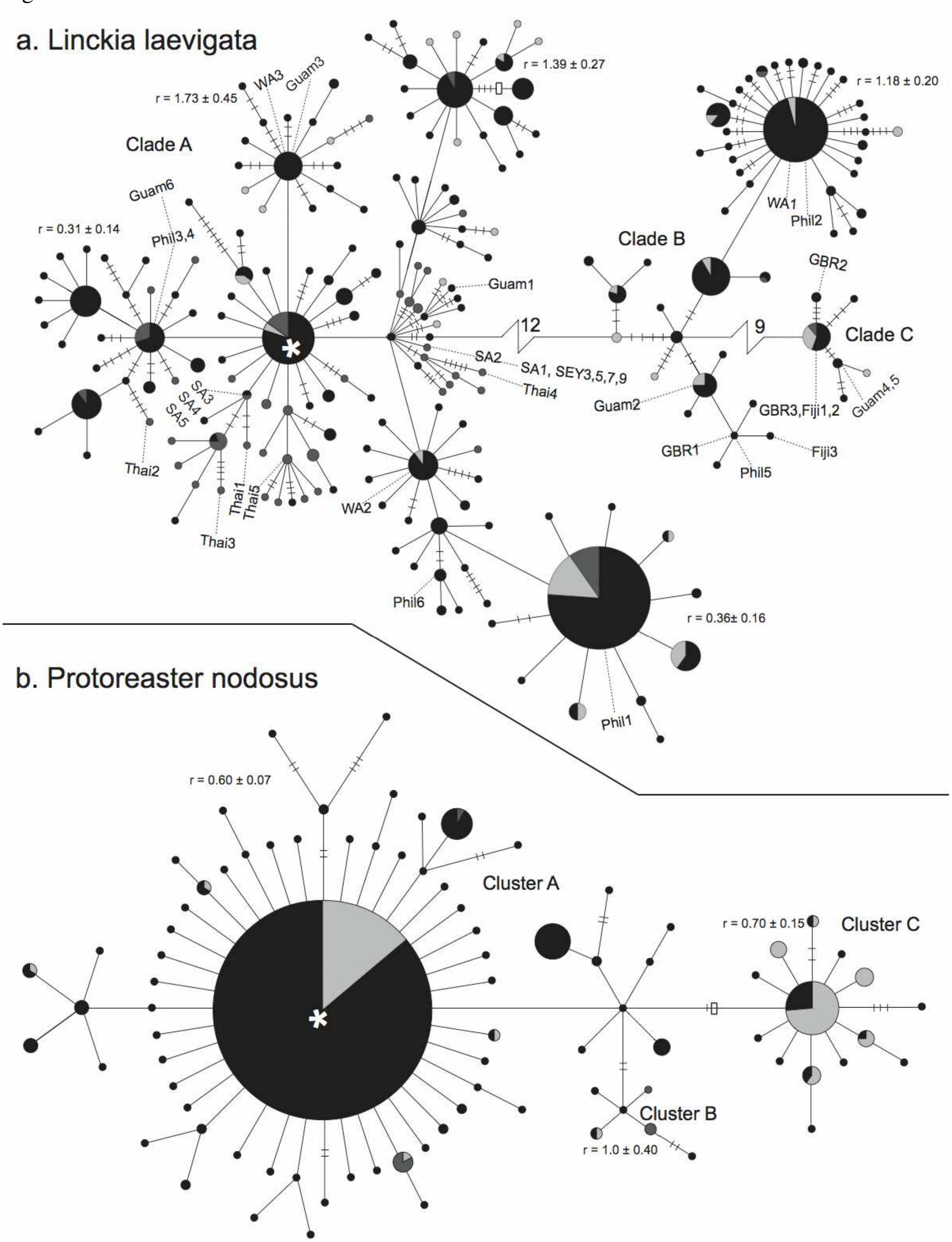
a. Linckia laevigata
Clade A
Clade B
Clade C
r = 1.73 ± 0.45
r = 1.39 ± 0.27
r = 1.18 ± 0.20
r = 0.31 ± 0.14
r = 0.36± 0.16
WA3
Guam3
Guam6
Phil3,4
Guam1
SA2
SA1, SEY3,5,7,9
Thai4
SA3
SA4
SA5
Thai2
Thai1
Thai5
Thai3
WA2
Phil6
Phil1
WA1
Phil2
GBR2
Guam2
GBR3,Fiji1,2
Guam4,5
GBR1
Fiji3
Phil5
12
9
b. Protoreaster nodosus
r = 0.60 ± 0.07
Cluster A
Cluster B
Cluster C
r = 0.70 ± 0.15
r = 1.0 ± 0.40

Figure 3.

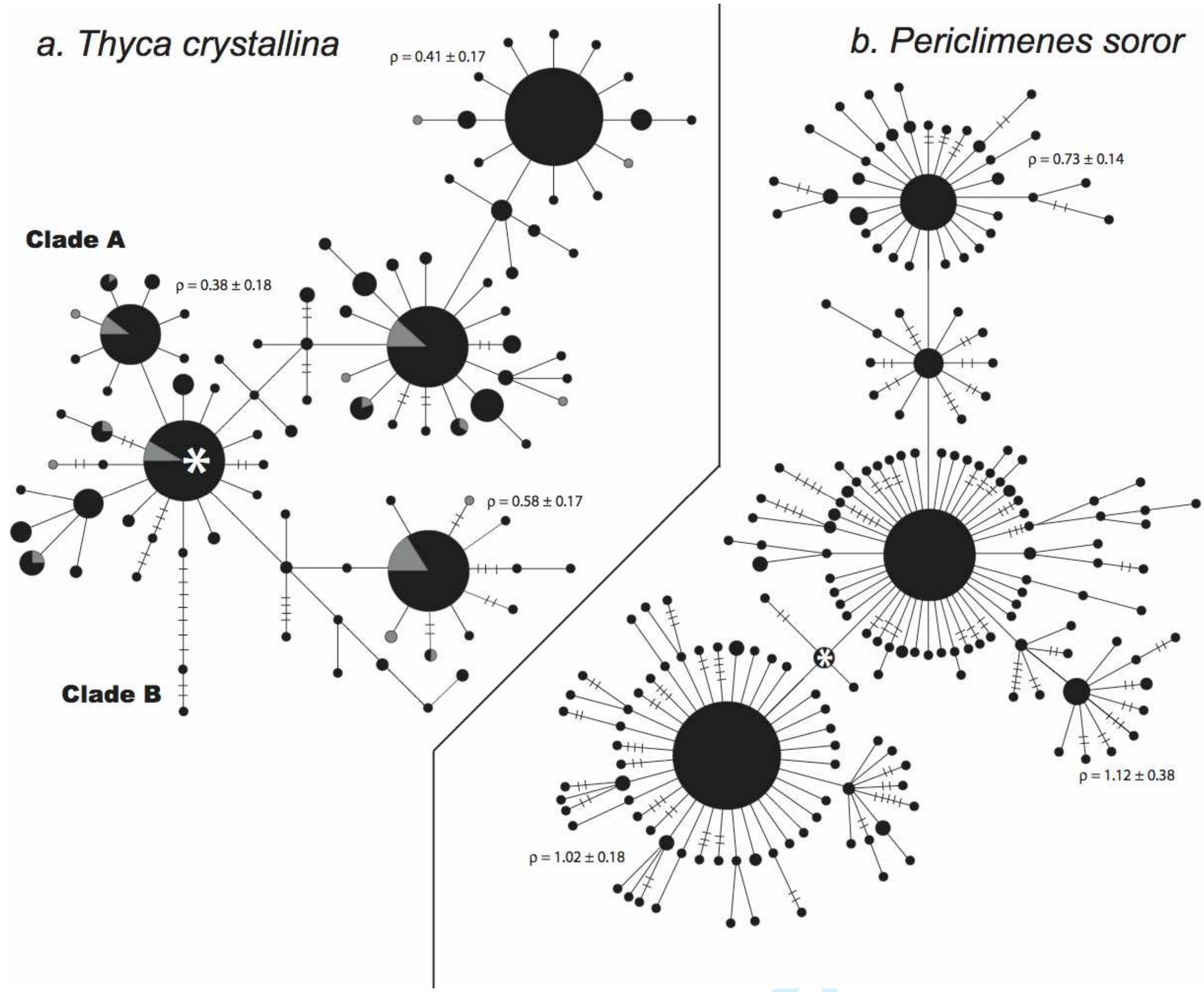
a. Thyca crystallina
ρ = 0.41 ± 0.17
Clade A
ρ = 0.38 ± 0.18
ρ = 0.58 ± 0.17
Clade B
b. Periclimenes soror
ρ = 0.73 ± 0.14
ρ = 1.12 ± 0.38
ρ = 1.02 ± 0.18

Figure 4.

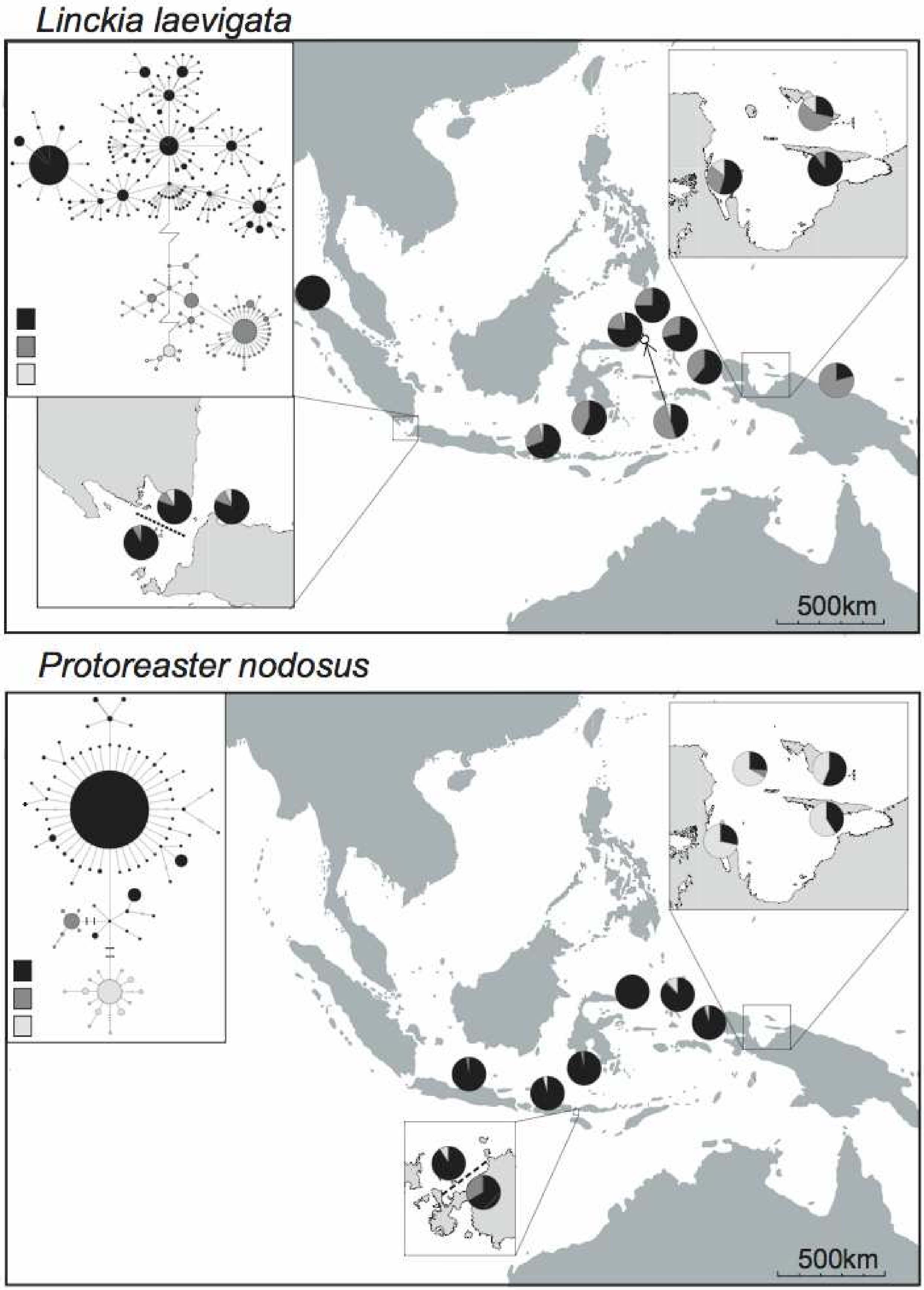

Figure 5.

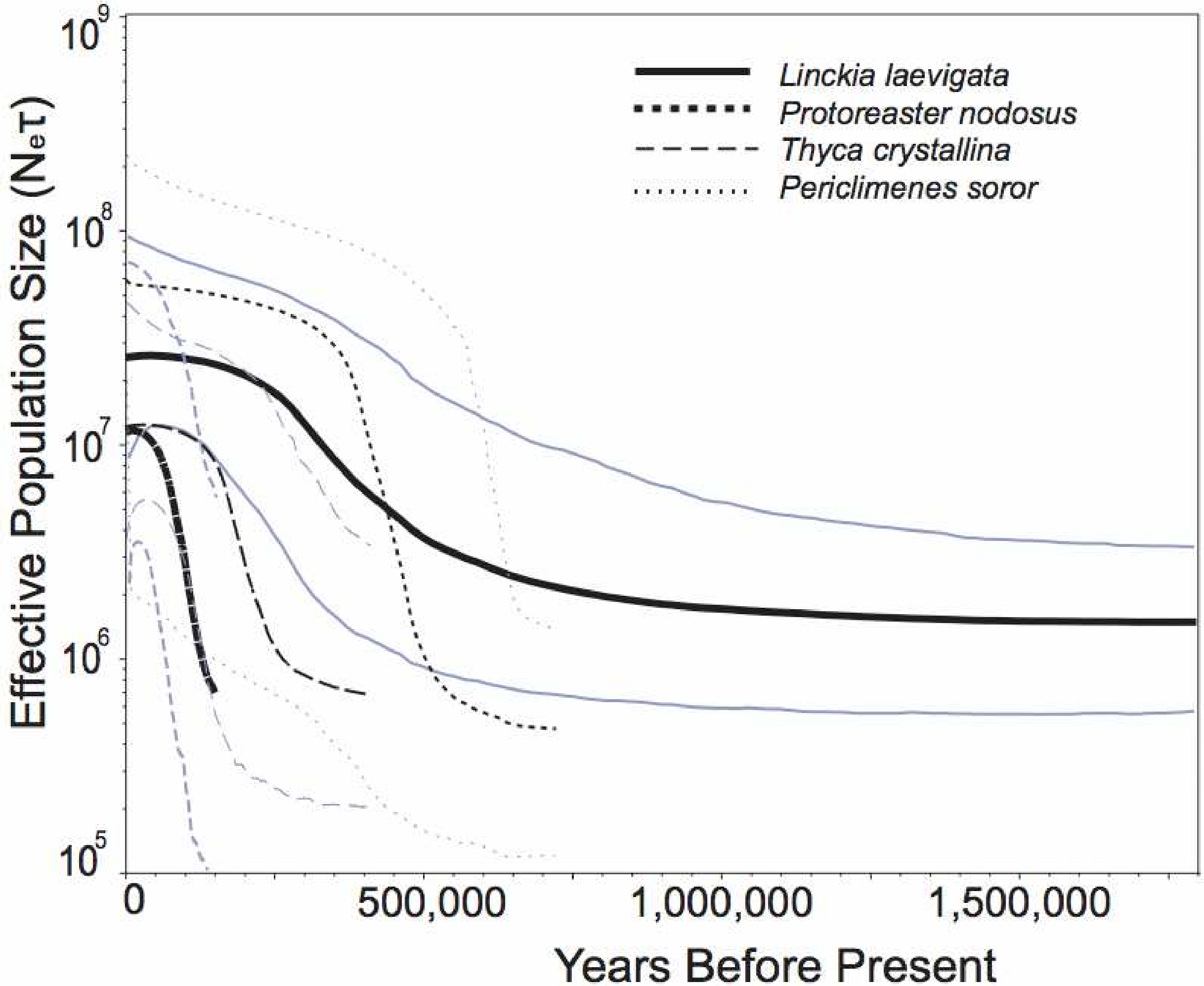
Linckia laevigata
Protoreaster nodosus
Thyca crystallina
Periclimenes soror
Effective Population Size ($N_e\tau$)
10^9
10^8
10^7
10^6
10^5
0
500,000
1,000,000
1,500,000
Years Before Present

Figure 5 (alternate).