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research paper

Concordance between phylogeographic and biogeographic boundaries in the Coral Triangle: conservation implications based on comparative analyses of multiple giant clam species

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ABSTRACT.—Marine habitats are in decline worldwide, precipitating a strong interest in marine conservation. The use of biogeographic data to designate ecoregions has had significant impacts on terrestrial conservation efforts. However, classification of marine environments into ecoregions has only become available in the last several years, based on biogeographic data supplemented by geomorphology, ocean currents, and water temperatures. Here we use a comparative phylogeographic approach to test for concordant phylogeographic patterns in three closely related species of *Tridacna* giant clams across the Coral Triangle, the most biodiverse marine region in the world and one of the most threatened. Data from a 450 base pair fragment of mitochondrial cytochrome-*c* oxidase subunit one DNA from 1739 giant clams across Indonesia and the Philippines show strong concordance between phylogeographic patterns in three species of giant clams as well as evidence for potentially undescribed species within the genus. Phylogeographic patterns correspond broadly to marine ecoregions proposed by Spalding et al. (2007), indicating that processes contributing to biogeographic boundaries likely also limit genetic connectivity across this region. These data can assist with designing more effective networks of marine protected areas by ensuring that unique biogeographic and phylogeographic regions are represented in regional conservation planning.

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Driven by a wide range of stressors, including over-harvesting (Jackson et al. 2001, Pandolfi et al. 2003), destructive fishing practices (McManus 1997), pollution (Williams et al. 2002, McCulloch et al. 2003), disease (Harvell et al. 2002), and climate change (Wilkinson 2002, Gardner et al. 2003, Hughes et al. 2003), among others, many marine habitats are in steep decline worldwide. While these declines have precipitated a strong interest in conservation, marine environments are vast and relatively difficult for humans to observe, creating a unique challenge in comparison to terrestrial conservation efforts. In addition, the majority of marine species have a bipartite life history where adults are completely or largely sedentary, but dispersal and population connectivity are achieved through small dispersive larvae. These features of marine ecosystems make defining the appropriate scale of marine management units, and developing effective networks of marine reserves, particularly challenging (Sale et al. 2005).

To address large-scale challenges in the terrestrial realm, conservation practitioners have turned to "ecoregions," areas of relatively homogenous species composition, clearly distinct from adjacent regions (sensu Spalding et al. 2007). Based largely on biogeographic and environmental data, this ecoregion approach has had significant impacts on terrestrial conservation efforts (Chape et al. 2003, Hazen and Anthamatten 2004, Hoekstra et al. 2005, Burgess et al. 2006, Lamoreux et al. 2006). Building on these successes, a hierarchical classification of marine ecoregions has been proposed to help facilitate marine conservation planning (Spalding et al. 2007). This classification system is largely based on biogeographic data (e.g., species range discontinuities, distribution of habitats types) augmented with information on geomorphology, ocean currents, and ocean temperature. Noticeably absent in the designation of ecoregions, however, is information on phylogeography (the geographic distribution of genetic diversity) or gene flow, information that can provide valuable insights for conservation prioritization (Berger et al. 2014).

While genetic data are very powerful tools for highlighting boundaries in marine environments (Avise 1994, Palumbi 1996, Hedgecock et al. 2007), such data were neglected in defining the marine ecoregions of Spalding et al. (2007). However, biogeographic and phylogeographic studies are very similar in scope. Both focus on understanding the geographical distribution of diversity and the processes responsible for these patterns. The primary difference is that biogeography focuses on the distribution of species and communities, while phylogeographic studies are typically concerned with the distribution of intraspecific genetic diversity (Avise 2000). Early work in phylogeography (Avise 1992, 1994) considered that genetic boundaries in widespread species might coincide with biogeographic boundaries, as the same physical processes that limit species distributions may act as filters to gene flow, creating regional genetic structure. However, few studies have followed up on testing this idea (but see Burton 1998, Lee and Johnson 2009, Kelly and Palumbi 2010, Toonen et al. 2011).

The Coral Triangle, a region comprised of Indonesia, Malaysia, the Philippines, East Timor, Papua New Guinea, and the Solomon Islands, supports the highest marine biodiversity in the world (Roberts et al. 2002, Carpenter and Springer 2005, Bellwood and Meyer 2008); it is also one of the most threatened (Burke et al. 2002, 2011). This combination of diversity and threats to diversity have made the Coral Triangle an area of intense conservation planning efforts by local governments and international non-profit organizations. Consequently, defining the scale of conservation units for

marine species in the area has become a topic of active research, largely through biogeographic methods (Green and Mous 2004, but see Berger et al. 2014). However, there is a large amount of genetic data that can also be brought to bear.

Understanding the evolutionary processes responsible for the Coral Triangle biodiversity hotspot has been a subject of intense interest for decades (see Bellwood and Meyer 2008, Barber 2009, Barber et al. 2011, Carpenter et al. 2011), precipitating a plethora of phylogeographic studies (McMillan and Palumbi 1995, Lavery et al. 1996, Benzie 1999, Barber et al. 2000, 2006, Crandall et al. 2008, DeBoer et al. 2008, Kochzius and Nuryanto 2008, Ackiss et al. 2013, DeBoer et al. 2014, Jackson et al. 2014). While studies indicate a role of Pleistocene vicariance (e.g., Lavery et al. 1996, Duda and Palumbi 1999, Benzie et al. 2002, Vogler et al. 2008), physical oceanography (e.g., Barber et al. 2006, 2011), and habitat type (Lourie and Vincent 2004a, Williams and Reid 2004, Lourie et al. 2005, Reid et al. 2006) in shaping biodiversity in the Coral Triangle, general patterns have yet to be elucidated (Barber 2009). The few comparative phylogeographic studies conducted in this region have often found discordant patterns of genetic structure between species, which are generally ascribed to differences in larval dispersal potential or adult ecology (Lourie et al. 2005, Reid et al. 2006, Crandall et al. 2008, but see Barber et al. 2006). The growing number of genetic studies in this area on a wide variety of taxa could be extremely valuable for defining conservation (Berger et al. 2014). However, variation in sampling strategies and objectives of these studies make it difficult to elucidate generalities from these studies.

Seven of the eight species of giant clams have been assessed under IUCN Red List criteria in categories ranging from Least Concern to Vulnerable to Extinction (Mollusk Specialist Group 1996a). Five of the *Tridacna* species occur in sympatry in the Coral Triangle (Lucas 1988). Based on molecular and morphological phylogenetic analyses (Maruyama et al. 1998, Schneider and Foighil 1999), the three smallest species form a closely-related group with *Tridacna crocea* Lamarck, 1819 and *Tridacna squamosa* Lamarck, 1819 as sister species and *Tridacna maxima* (Röding, 1798) sister to that clade. These species are ecologically very similar. All species inhabit coral reefs and occur at relatively shallow depths (Lucas 1988), although *T. crocea* and *T. maxima* are typically found at more shallow depths than *T. squamosa* (0–3 vs 2–10 m; Lucas 1988). All three have similar larval durations of 12 (*T. squamosa*), 14 (*T. maxima*), and 11 (*T. crocea*) days from fertilization to settlement (Lucas 1988), and all three species establish symbiosis with photosynthetic algae (zooxanthellae) from environmental pools shortly after metamorphosis (Fitt and Trench 1981).

Previous phylogeographic studies on *T. crocea* (DeBoer et al. 2008, Kochzius and Nuryanto 2008, DeBoer et al. 2014) and *T. maxima* (Nuryanto and Kochzius 2009) have revealed strong patterns of phylogeographic structure, indicating limited dispersal among regions of the Coral Triangle, particularly among western, central, and eastern Indonesian populations. However, lack of concordant sampling precludes the use of new powerful tools for comparative spatial analyses (Manni et al. 2004), limiting our ability to test for concordant patterns of regional isolation.

In the present study, we focus on two objectives. First, we assess phylogeographic patterns in mtDNA COI for three sympatric, ecologically similar sister species to determine if putative barriers to dispersal are congruent across species. Concordant phylogeographic patterns across multiple sympatric sister-species would strongly suggest the action of broadly acting physical processes (Schneider et al. 1998, Walker

and Avise 1998, Argoblast and Kenagy 2001). Second, we compare the location of phylogeographic barriers to dispersal with the boundaries of the marine ecoregions defined by Spalding et al. (2007). Concordance between phylogeographic and biogeographic patterns could indicate that similar and/or complementary processes (e.g., currents impact larval dispersal, but temperature variation associated with currents shapes adult distributions) act to shape both genetic connectivity and community level patterns of biodiversity, further highlighting the utility of genetics in the utility of genetics in facilitating marine conservation planning.

Methods

Sampling and Sequencing.—We collected a small piece of mantle tissue from *T. crocea* (n = 796, 39 localities), *T. maxima* (n = 530, 34 localities), and *T. squamosa* (n = 413, 32 localities) populations across Indonesia and the Philippines and preserved them in 95% ethanol (Fig. 1). Clams were identified to species in the field based on the morphology of shells and the incurrent and excurrent siphons. However, because small individuals can sometimes be difficult to distinguish in the field, we also sequenced 16S and beta-tubulin genes for a subset of individuals and assigned final species identity based on molecular phylogeny, rather than field identifications (see Results).

We extracted whole genomic DNA using 10% Chelex (Biorad) solution (Walsh et al. 1991), then amplified an approximately 450-bp fragment of the mitochondrial cytochrome oxidase subunit-I gene (COI) following previously published protocols (DeBoer et al. 2008). For *T. crocea*, amplifications used primers *Tridacna* 1F and *Tridacna* 3R (DeBoer et al. 2008). For *T. maxima*, we used Maxima F3 (5′–GTT TAG RGT RAT AAT YCG AAC AG–3′) and universal primer HCO-2198 (Folmer et al. 1994). For *T. squamosa* we used SQUA-F3 (5′–CAT CGT TTA GAG TAA TAA TTC G–3′) and SQUA-R1 (5′–ATG TAT AAA CAA AAC AGG ATC–3′). 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 or ABI 3730 sequencer. Chromatograms were assembled, proofread, and aligned using Sequencher (Gene Codes Corp.), and amino acid translation was confirmed using MacClade 4.05 (Maddison and Maddison 2002).

Phylogeographic Structure.—We investigated the relationship between haplotypes and their geographic distributions through several methods. First, we constructed minimum spanning trees based on pairwise differences in Arlequin 3.1 (Excoffier et al. 1992) to examine phylogenetic structure. Then, to investigate geographical partitioning of this structure in the three giant clam species, we summarized the frequencies of haplotype clusters and plotted these onto a map of the study region.

Regional genetic structure was examined using analysis of molecular variance (AMOVA) as implemented in Arlequin with significance tested using 10,000 randomized replicates. AMOVA was run initially with no a priori assumptions. Then, populations were grouped into regions to maximize the percent of variation explained by regions using several strategies. First, populations were grouped following the phylogeographic structure based on the distribution of divergent clades within each species. Second, we grouped populations based on marine ecoregion

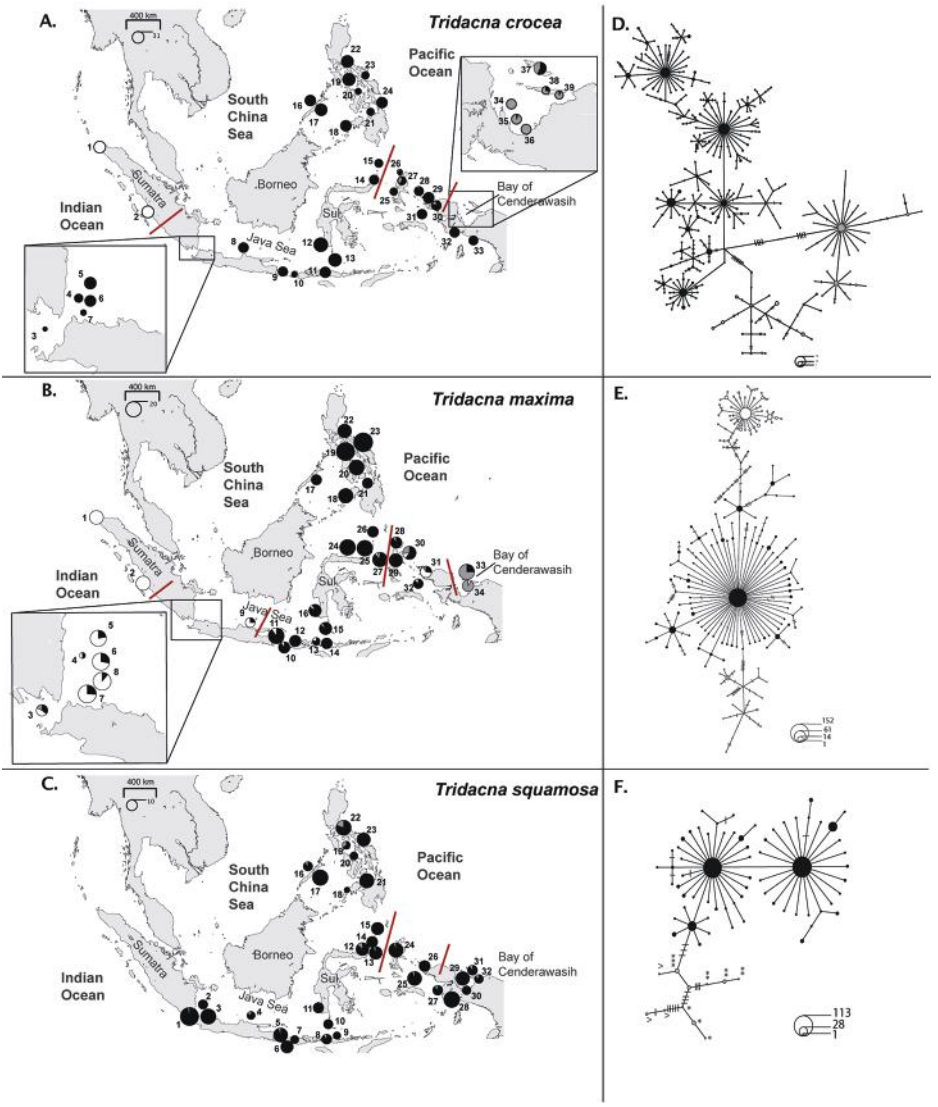

Figure 1. Map of the study area for (A) *Tridacna crocea*, (B) *Tridacna maxima*, and (C) *Tridacna squamosa* with pie diagrams representing the frequencies of each clade in each sampling site listed in Table 1. Red lines indicate putative barriers to dispersal identified based on maximizing F_{CT} in AMOVA analyses for each species (Table 2). Intraspecific clades were defined based on arranging haplotypes into minimum spanning trees in (D) *T. crocea*, (E) *T. maxima*, and (F) *T. squamosa*. All haplotypes are separated by one mutational step unless denoted by a higher number of hash marks. For *T. squamosa* (F), gray haplotypes are marked according to area of collection as follows: southern Indonesia (***), Philippines (**), Fiji and Solomon Islands (*), and eastern Indonesia (^). In (D–F), labeled circles indicate sample sizes.

boundaries, as defined by Spalding et al. (2007). Briefly, the hierarchical classification system proposed by Spalding et al. (2007) includes three levels of biogeographic boundaries: realms, provinces, and ecoregions. Realms are the largest spatial units and the entire Coral Triangle region is included in the Central Indo-Pacific realm. Provinces are nested within realms and are defined by the presence of distinct biotas

that have some cohesion over evolutionary time; Spalding et al. (2007) breaks the Coral Triangle into the eastern and western Coral Triangle provinces. Finally, ecoregions are the smallest units in the classification system and are nested within provinces. There are eight ecoregions in the western Coral Triangle province and four in the eastern province; the province and ecoregion of each sampling locality is given in Table 1.

To detect potential barriers to dispersal without the a priori assumptions required by AMOVA, we used Barrier v2.2 (Manni et al. 2004) to identify common barriers among populations of all three species in the study area. Barrier v2.2 (Manni et al. 2004) is an analytical approach based on computational geometry that identifies population edges associated with the highest rate of change in a given distance measure. Pairwise estimates of F_{ST} (K2P) were mapped onto a matrix of sampling locality geographic coordinates (latitude and longitude in decimal degrees), their spatial organization was modeled by Voronoi tessellation (Voronoi 1908) and a Monmonier (Monmonier 1973) maximum-difference algorithm identified borders between neighboring sites that exhibited the highest levels of genetic differences and ranked them accordingly (Manni et al. 2004). Genetic barriers designate sites with greater F_{ST} values than would be expected from their geographical proximity. The analyses require common sampling localities for each species, so we used only data from the 23 sites for which we had data from all three species (Table 1). Distance matrices for all three species were input into the program and support for each barrier was determined by counting the number of species for which each boundary was included.

Demographic Analyses.—To compare demographic histories of mtDNA associated with each species, we calculated Fu's F_S (Fu 1997) using Arlequin to test each site for departures from the neutral model due to positive selection, background selection, or population growth. In Fu's F_S test, h is estimated as the observed number of pairwise differences between the sampled haplotypes, and the F_S statistic is defined as the log of the probability to observe k or more haplotypes conditional on the sampled haplotypes (Schneider et al. 2000). F_S tends to be negative if there is a significant excess of rare haplotypes, and a significant negative departure of F_S from 0 is often taken as evidence of recent demographic expansions or population bottlenecks in situations where no selective advantage among haplotypes exists (Fu 1997). Significance of F_S values was estimated with the simulated distribution of random samples (1000 steps) using a coalescence algorithm assuming neutrality and population equilibrium (Hudson 1990). For the F_S test, $P = 0.02$ is considered to be significant at the $\alpha = 0.05$ level (Fu 1997, Schneider et al. 2000).

Phylogenetics.—Because DNA sequencing resulted in the recovery of two cryptic lineages within the three sampled *Tridacna* species, additional DNA sequence data were obtained for a subset of samples for 16S following the methods of DeBoer et al. (2008) to compare with published sequences from Genbank for all known *Tridacna* species. To determine whether mtDNA clades were observed in the nuclear genome, we also sequenced beta-tubulin by modifying primers TUB 3.1 and TUB 4.1 (Duda and Palumbi 2004). Tubulin 41F (5′–CCT TTT GGA CAG ATT TTC AGA CC–3′) and Tubulin 250R (5′–TGT TCC CAT ACC AGA TCC AG–3′) amplified approximately 200 bp of the Beta-tubulin gene. Bayesian phylogenetic analysis was conducted using Mr. Bayes (Huelsenbeck and Ronquist 2001) using model parameters determined by ModelTest (Posada and Crandall 1998). Bayesian run parameters

were NST = 6 with gamma rate variation. The analysis was run for 5 × 10E6 generations with a sampling frequency of 1000. Posterior probability estimates of node support were summarized from the resulting 5000 trees with a burn-in of 1250 trees. Outgroups from Genbank were included for 16S analyses, but outgroup sequences were not available for beta-tubulin.

Results

We obtained mtDNA COI sequence data from 1325 individuals in three *Tridacna* species, yielding a total data set of 1739 individuals (Table 1) when combined with the data from DeBoer et al. (2008). All sequences aligned without indels and translated without stop codons. Sequence data from 796 *T. crocea* yielded 301 unique haplotypes (h = 0.9749, π = 0.0254; Table 1) from 39 sampling localities throughout the study area (Fig. 1A–C). For *T. maxima*, we sequenced 530 individuals and found 193 unique haplotypes (h = 0.9064, π = 0.0220) at 34 sampling localities. Data from 413 *T. squamosa* yielded only 80 haplotypes (h = 0.8235, π = 0.0047) from 32 sampling localities. New sequences for *T. crocea* (KF446283–KF446328) and *T. maxima* and *T. squamosa* (KF446329–KF446591) are available in Genbank.

Each species contained multiple divergent clades, based on mtDNA COI. For *T. crocea* we identified three clades separated by eight mutational steps each (black, white, gray; Fig. 1D). Maximum uncorrected sequence variation within these clades was 1.1% in the black and white clades, while variation among these clades ranged from a minimum of 3.7% between the black and white clades to a maximum of 7.1% between the white and gray clades. We also identified three divergent clades in *T. maxima* with two clades (gray and black) separated by eight mutational steps. A third clade (white) was diagnosed by the presence of a common haplotype found in 61 individuals; the white clade was separated from the black by three steps (Fig. 1E). Maximum uncorrected sequence variation within these clades was 1.4% in the gray clade while variation among these clades ranged from a minimum of 2.1% between clades A and B to a maximum of 9.1% between the gray and white clades. Genetic diversity in *T. squamosa* grouped into two star-like clusters separated by only one step (Fig. 1F). Both central haplotypes (black) occur throughout the study area (data not shown). A second gray clade is separated from the black clade by three steps (Fig. 1F). Some gray clade haplotypes wewre highly divergent, separated by as many as five steps, but these were represented by a single color due to their low frequency. Maximum variation within these clusters was 2.5% and minimum variation between them was 0.2%. Two cryptic divergent lineages did not form a monophyletic group with any of the three focal taxa (below). Maximum variation within these two lineages ranged from 0.9% to 3.0%, but minimum variation between these clades and other *Tridacna* species was 9.2%. As such, despite being field identified as one of the three focal taxa, these samples were excluded from further population analyses.

In all three species, clades were distributed in an east to west pattern across the study area. For *T. crocea*, the white clade was restricted to the Indian Ocean; the gray clade was restricted to eastern Indonesia (Fig. 1A); the largest clade, black, occurred throughout central Indonesia, confirming previous results (DeBoer et al. 2008) and also extended into the Philippines (Fig. 1A). For *T. maxima* the white clade occurred in Indian Ocean populations, but also infrequently in southern Indonesia and Raja Ampat (Fig. 1B). The gray clade was largely restricted to eastern Indonesia, but was

Table 1. Summary statistics and neutrality test results for each site shown in Figure 1. Province (Prov.) and ecoregion (E.R.) numbers refer to regions identified in Spalding et al. (2007). Map numbers refer to localities numbered on Figures 1A, 1B, and 1C. Haplotype diversity (h), nucleotide diversity (π), and Fu's F_S (Fu 1997) calculated in Arlequin 3.1 (Excoffier et al. 2005). Bold values are significant at $P < 0.02$, which is equivalent to an alpha value of 0.05 for Fu's F_S. For *Tridacna crocea,* all sequence data were previously published (DeBoer et al. 2008, 2013) except for n indicated in parentheses with unique haplotypes in Genbank as KF446283–KF446328. All *Tridacna maxima and Tridacna squamosa* COI sequences are published for the first time here (KF446329–KF446591).

		T. crocea						*T. maxima*						*T. squamosa*					
Prov.	E.R.	Map	Locality	n	π	h	F_S	Map	Locality	n	π	h	F_S	Map	Locality	n	π	h	F_S
24	111	1	Aceh	31	0.0110	0.95	**−5.50**	1	Aceh	16	0.0039	0.89	—	—					
—	—	—	—	—	—	—	−2.38	2	Cubadak	17	0.0037	0.83	**−4.99**						
27	119	3	Krakatau	4 (2)	0.0084	1.00	−0.95	3	Krakatau	7	0.0194	1.00	−1.99	1	Krakatau	30	0.0045	0.88	**−7.01**
27	119	4	Alam Kotok	15 (2)	0.0116	0.80	0.77	4	Alam Kotok	4	0.0195	0.83	2.35						
27	119	5	Belat	30 (5)	0.0116	0.82	1.20	5	Belat	22	0.0134	0.84	−1.77	2	Belat	10	0.0055	0.53	0.99
27	119	6	Karang Congkak	26	0.0101	0.82	−2.01	6	Karang Congkak	25	0.0158	0.97	**−7.76**	3	Pulau Seribu	21	0.0019	0.48	−0.36
27	119	7	Pramuka/Semak Duan	7 (2)	0.0062	0.95	−2.71	7	Semak Duan	27	0.0094	0.80	**−4.06**						
27	119							8	Pramuka	27	0.0136	0.80	−1.39						
26	117	8	Karimunjawa	21	0.0091	0.77	−0.66	9	Karimunjawa	8	0.0140	0.93	−0.07	4	Karimunjawa	6	0.0046	0.60	1.02
30	119							10	Nusa Penida	11	0.0036	0.78	−0.94	6	Nusa Penida	14	0.0040	0.91	**−5.51**
30	117	9	Bali	19 (6)	0.0135	0.96	−4.64	11	Bali	21	0.0106	0.95	**−10.24**	5	Bali	18	0.0062	0.96	**−6.65**
30	132	10	Lombok	7 (5)	0.0211	0.95	−0.26	12	Lombok	11	0.0045	0.93	**−4.55**	7	Lombok	6	0.0028	0.80	−1.45
30	132	11	Flores	20 (1)	0.0109	0.92	**−6.93**	13	Flores/Komodo	5	0.0240	1.00	−1.63	9	Flores	5	0.0046	0.90	−1.01
30	128	12	Makassar	36	0.0091	0.95	**−9.87**	16	Makassar	13	0.0087	0.91	−2.67	10	Makassar	10	0.0022	0.64	0.39
30	132							14	Sebayur	10	0.0067	0.67	0.25	8	Sebayur	9	0.0075	0.92	−2.30
30	131	13	Selayar	35	0.0076	0.82	**−12.26**	15	Selayar	13	0.0116	0.87	−1.75	11	Selayar	8	0.0016	0.46	−0.44
30	128							24	Bunaken	21	0.0045	0.74	**−6.35**						
30	128	14	Manado	18	0.0085	0.91	−2.33	25	Manado/Lembeh	22	0.0047	0.70	**−2.95**	12	Manado	15	0.0047	0.75	0.25
30	128													13	Lembeh	15	0.0059	0.95	**−6.59**
30	128													14	Bangka Batu	11	0.0027	0.73	**−3.10**
30	128	15	Sangihe	14 (3)	0.0102	0.99	**−8.45**	26	Sangihe	10	0.0021	0.38	0.30	15	Sangihe	14	0.0027	0.77	**−2.52**
30	126	16	Ulugan Bay	25	0.0107	0.91	−1.92							16	Ulugan Bay	8	0.0064	0.86	−0.44
30	126	17	Honda Bay	30	0.0103	0.97	**−14.84**	17	Honda Bay	9	0.0026	0.58	−0.82	17	Honda Bay	22	0.0025	0.73	−0.82

Table 1. Continued.

		T. crocea						*T. maxima*						*T. squamosa*					
Prov.	E.R.	Map	Locality	*n*	π	*h*	F_S	Map	Locality	*n*	π	*h*	F_S	Map	Locality	*n*	π	*h*	F_S
30	126	18	Tawi−Tawi	25	0.0105	0.92	−2.00	18	Tawi−Tawi	18	0.0049	0.90	**−8.22**	18	Tawi−Tawi	3	0.0015	0.67	0.20
30	127	19	Romblon	34	0.0094	0.92	**−10.21**	19	Romblon	29	0.0136	0.86	**−4.57**	19	Romblon	6	0.0136	0.73	3.39
30	127	20	Siquijor	8 (2)	0.0065	0.79	−0.52	20	Siquijor	19	0.0038	0.67	**−3.28**	20	Siquijor	6	0.0012	0.53	0.63
30	127	21	Camiguin	12 (7)	0.0111	1.00	**−10.22**	21	Camiguin	10	0.0384	0.98	−0.68	21	Camiguin	19	0.0007	0.46	**−2.77**
30	127	22	Perez	31	0.0096	0.95	**−12.94**	22	Perez	16	0.0019	0.67	**−1.98**	22	Perez	21	0.0098	0.77	−0.42
30	127	23	Sorsogon	12 (1)	0.0034	0.44	1.55	23	Sorsogon	30	0.0077	0.84	**−7.27**	23	Sorsogon	16	0.0015	0.52	−1.21
30	127	24	Dinigat	25	0.0084	0.87	**−6.99**												
30	129	25	Western Halmahera	12	0.0086	0.97	**−4.80**	29	Halmahera	15	0.0034	0.83	**−4.25**	24	Halmahera	19	0.0040	0.91	**−4.13**
30	129	26	Pulau Doi	6	0.0224	1.00	−1.00	28	Pulau Doi	11	0.0169	0.91	−0.50						
30	129							30	T. Jerawai	15	0.0397	0.97	−0.40						
30	129	27	Kolorai	15	0.0296	0.98	−2.68	27	Mayu Island	18	0.0094	0.64	−0.59						
30	130	28	Wayag	20	0.0136	0.97	**−10.39**	31	Dampier Straight	11	0.0281	0.89	0.74	26	Dampier Straight	11	0.0046	0.93	**−4.48**
30	130	29	Kri	25	0.0105	0.95	**−10.78**												
30	130	30	Jefman Island	20	0.0237	0.97	−4.23	32	Misool	8	0.0100	0.86	0.50	25	Misool	18	0.0056	0.84	−2.86
30	130	31	Misool	20	0.0097	0.96	**−4.69**												
30	130	32	Fak Fak	20	0.0089	0.94	**−9.55**							27	Fak Fak	9	0.0082	0.83	−0.77
30	130	33	Kaimana	18	0.0048	0.89	**−7.48**							28	Kaimana	23	0.0027	0.78	−2.65
30	130	34	Tridacna Atoll	22 (6)	0.0042	0.78	**−4.64**							29	Teluk Cenderawasih	17	0.0020	0.68	−1.70
30	130	35	Pulau Kumbur	22	0.0096	0.90	−4.08												
30	130	36	Nambire	22	0.0071	0.91	−2.29							30	Nambire	7	0.0022	0.76	−0.06
30	130	37	Biak	28 (7)	0.0269	0.94	−3.51	33	Biak	20	0.0329	0.96	−2.35	31	Biak	9	0.0089	0.97	−3.47
30	130	38	Yapen-Serui	14	0.0252	0.93	−1.15	34	Yapen	11	0.0232	0.89	−0.37	32	Yapen	7	0.0053	0.71	0.13
30	130	39	Yapen-Ambai	15	0.0103	0.93	−2.51												

also found in Krakatau and Selayar. The largest clade, black, occurred throughout central and eastern Indonesia and north into the Philippines (Fig. 1B). For *T. squamosa*, the gray clade haplotypes occurred in low frequency throughout the study area (Fig. 1C). Some geographic trends were apparent in the gray clade (Fig. 1F) with closely related haplotypes clustering in southern Indonesia, the Philippines, Fiji and the Solomon Islands (n = 5, data not used in analyses), and eastern Indonesia (see Fig. 1C legend).

Spatial Structure of Genetic Variation.—Using AMOVA to partition genetic variation into regions, we found evidence of strong genetic structure in all species (Table 2). Assuming no a priori structure in *T. crocea,* F_{ST} = 0.5625 (P < 0.0001). There was significant structure between Indian Ocean, central, and eastern regions in the study area (F_{CT} = 0.4306, P < 0.001). The level of structure was maximized when populations in the Bay of Cenderawasih were split into a fourth group (F_{CT} = 0.60145, P < 0.001). Grouping populations according to marine provinces (F_{CT} = 0.36192, P < 0.001) and marine ecoregions (F_{CT} = 0.2736, P < 0.001) also accounted for strong genetic structure.

Genetic structure in *T. maxima* was also strong, and similar to partitions described for *T. crocea.* Assuming no a priori structure, F_{ST} = 0.4890 (P < 0.0001). The level of genetic structure between groups was maximized with five groups: (1) Indian Ocean, (2) Pulau Seribu and Java, (3) central, (4) eastern, and (5) Bay of Cenderawasih (F_{CT} = 0.5521, P < 0.001; Fig. 1B). Grouping populations of *T. maxima* according to marine provinces (F_{CT} = 0.3104, P < 0.001) and marine ecoregions. (F_{CT} = 0.3325, P < 0.001) also explained regional genetic structure.

We were unable to locate any *T. squamosa* in the Indian Ocean localities we visited. The distribution of clades in this species differs somewhat from the strong west-central-east pattern seen in *T. crocea* and *T. maxima* (Fig. 1). Assuming no a priori structure, F_{ST} = 0.1031 (P < 0.0001). Populations of *T. squamosa* showed evidence of reduced overall genetic structure, relative to its sister species (F_{ST} = 0.1031 vs F_{ST} = 0.5625 and F_{ST} = 0.2523 in *T. crocea* and *T. maxima*, respectively). However, genetic structure was partitioned in a geographic pattern consistent with the other two species with maximal between-group variation achieved when central, eastern, and Bay of Cenderawasih populations were grouped (F_{CT} = 0.0826, P < 0.001). Organizing groups based on marine provinces (F_{CT} = −0.0108, P > 0.05) or marine ecoregions (F_{CT} = 0.0252, P = 0.065) does not result in significant structure between groups.

Barrier v2.2 identified 10 barriers among populations of all three species in the study area (Fig. 2), although Indian Ocean populations had to be excluded from this analysis because we did not have data for *T. squamosa* from this area. The 10 strongly-supported barriers (3/3 species) are labeled with Roman numerals in Figure 2. Weaker barriers, supported by only one of the species, are represented by thin lines in the figure. Strong barriers were identified isolating Krakatau from the rest of Pulau Seribu (Barrier I), between Bali and Lombok (Barrier II), between populations north and south of the Java Sea (Barriers III and IV), isolating the island of Halmahera (Barrier V), isolating the Bay of Cenderawasih (Barrier VI), between far western Philippine populations and those in northern Sulawesi (Barrier VII), between far western Philippine populations and the rest of the country (Barrier X), and between northern and southern Philippine populations (Barriers IX and VIII).

Table 2. AMOVA results showing variance components (Var), percent variation (%Var), and *F*-statistics for various putative barriers to gene flow. We also show results for groupings based on marine provinces and marine ecoregions, as defined by Spalding et al. (2007). Groups maximizing F_{CT} for each species are highlighted in bold and are shown in Figure 1. All F_{ST} $P < 0.001$. For F_{CT}, *P*-values are as follows: $P < 0.001$ (***), $P < 0.005$ (**), $P < 0.05$ (*), $P > 0.05$ (NS).

	Among groups		Among populations within groups		Within populations				F_{CT}
	Var	%Var	Var	%Var	Var	%Var	F_{ST}	F_{CT}	*P*-value
Tridacna crocea									
No barriers			3.28	56.25	2.55	43.75	0.5625		
Sumatra vs Central vs Eastern	2.97	43.06	1.43	20.78	2.50	36.21	0.6379	0.4306	***
Sumatra vs Central vs Eastern vs TC	4.35	60.14	0.38	5.30	2.50	34.55	0.6545	0.6015	***
Provinces	2.44	34.57	2.13	30.07	2.50	35.36	0.6464	0.3457	**
Ecoregions	1.59	27.36	1.72	29.62	2.50	43.02	0.5698	0.2736	**
Ecoregions + Bay	3.01	51.34	0.35	6.00	2.50	42.66	0.5734	0.5134	***
Tridacna maxima									
No barriers			2.48	48.90	2.59	51.10	0.4890		
Sumatra vs Central vs Eastern	1.43	25.23	1.74	30.78	2.49	43.99	0.5601	0.2523	**
Sumatra vs Central vs Eastern vs TC	2.74	43.11	1.13	17.71	2.49	39.18	0.6082	0.4311	***
Sumatra vs P Seribu and Java vs Central vs Eastern vs TC	3.24	55.21	0.13	2.30	2.49	42.50	0.5751	0.5521	***
Provinces	1.77	31.04	1.45	25.34	2.49	43.63	0.5637	0.3104	***
Ecoregions	1.67	33.25	0.87	17.22	2.49	49.53	0.5047	0.3325	***
Ecoregions + Bay	2.16	42.27	0.46	8.89	2.49	48.75	0.5125	0.4227	***
Tridacna squamosa									
No barriers			0.11	10.31	0.94	89.69	0.1031		
Central vs Eastern	0.06	5.86	0.08	7.44	0.93	86.70	0.1330	0.0586	***
Central vs Eastern vs TC	0.09	8.26	0.06	5.98	0.93	85.76	0.1424	0.0826	***
Provinces	0.00	−0.16	0.11	10.37	0.93	89.79	0.1021	−0.0016	NS
Ecoregions	0.03	2.52	0.08	8.04	0.93	89.35	0.1057	0.0252	NS
Ecoregions + Bay	0.06	5.37	0.06	5.43	0.93	89.20	0.1080	0.0537	**

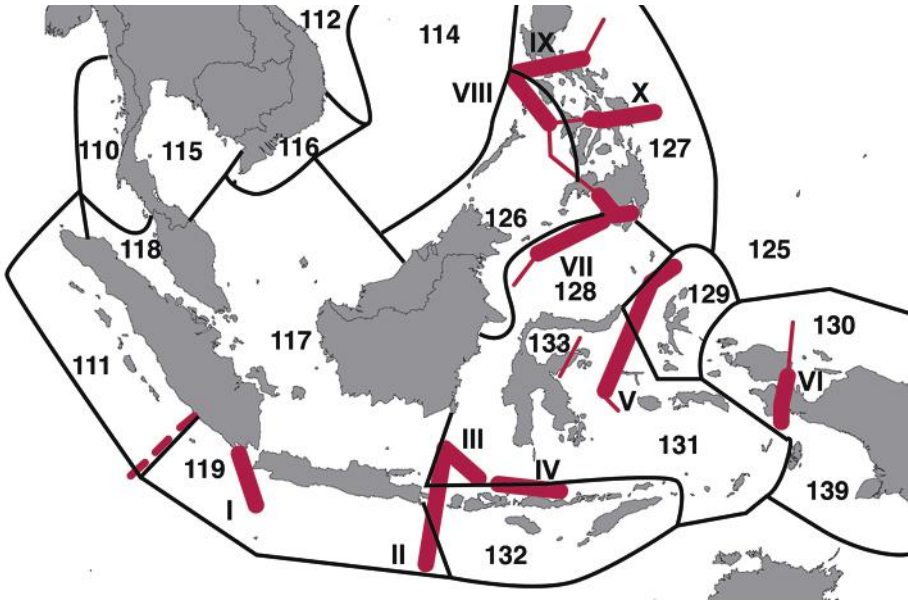

Figure 2. Marine ecoregions, with numeric labels, as defined by Spalding et al. (2007). Red lines (online version) marked by Roman numerals are major genetic boundaries as identified in Barrier v2.2 based on data from all three species at 23 common collection sites. Line thickness corresponds to the number of species that support the barrier (thick lines are supported by 3/3 species, thinner lines are supported by 2/3 species). The thin dotted line represents the common barrier between Indian Ocean populations and the rest of the study area identified in *Tridacna crocea* and *Tridacna squamosa*, which could not be investigated using Barrier because there are no samples from *T. squamosa* in this area.

Strongly supported boundaries from Barrier are largely consistent with marine ecoregion boundaries across the study area (Fig. 3A). Concordance between phylogenetic barriers identified by Barrier and ecoregion boundaries is summarized in Table 3. Six of the 10 barriers align directly with specific ecoregion boundaries. An additional barrier (Barrier I) was in close proximity to an ecoregion boundary. The final three barriers (Barriers VI, IX, and X) were located within large ecoregions without internal divisions based on the biogeographic classification. While Spalding et al. (2007) lump the geographically distinct regions of the Bay of Cenderawasih and Raja Ampat into the "Papua" ecoregion, Barrier VI shows that these regions are genetically isolated. Similarly, while the majority of the Philippines were included in the "Eastern Philippines" ecoregion, Barrier VIII separates northern and southern Philippine populations at a location consistent with the location of the bifurcation of the Northern Equatorial Current. Lastly, Barrier IX isolates a northern Philippine population, another genetic subdivision within the "Eastern Philippines" ecoregion.

Demographic Analyses.—As an additional test of whether these species have similar evolutionary histories that would support the finding of concordant phylogeographic patterns, we calculated Fu's F_S to determine if there was any evidence of a shared demographic history. Fu's F_S was significantly negative ($P < 0.02$), indicating population expansion, in less than half of localities for all species (Table 1). Patterns seen in each species were unique. In *T. crocea*, the majority of populations in central Indonesia showed evidence of expansion. Populations in eastern Indonesia (including

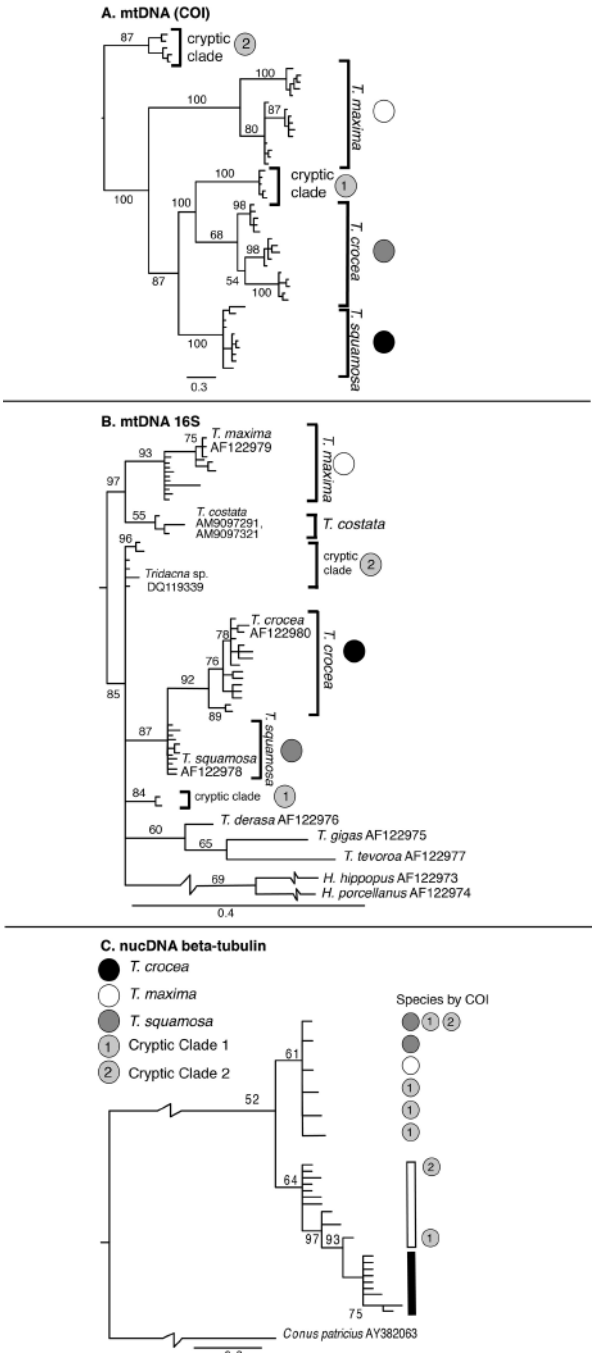

Figure 3. Bayesian phylogenetic trees of *Tridacna maxima, Tridacna crocea,* and *Tridacna squamosa* unique haplotypes of (A) mtDNA cytochrome oxidase c subunit I (COI), (B) mtDNA 16S, and (C) nuclear beta-tubulin sequences with outgroups from GenBank where available for *Tridacna coastata* Roa-Quiaoit, Kochzius, Jantzen, Zibdah, Richter, 2008; *Tridacna derasa* (Röding, 1798); *Tridacna gigas* (Linnaeus, 1758); *Tridacna tevoroa* Lucas, Ledua and Braley, 1990; *Hippopus hippopus* (Linnaeus, 1758); *Hippopus porcellanus* Rosewater, 1982; and *Conus patricius* Hinds, 1843. Posterior probabilities for each node are given above branches.

Table 3. Concordance between phylogenetic divisions identified by Barrier v2.2 and marine ecoregions designated by Spalding et al. (2007). Barrier and ecoregion labels and coincide with Figure 1.

Barrier	Location	Coincident with ecoregion boundary?	Ecoregions
I	Bali vs Lombok	Yes	119 vs 132, 117 vs 128
II	Java Sea: north vs south	Yes	131 vs 132
III	Halmahera	Yes	128 vs 129, 129 vs 131
IV	Philippines vs Indonesia	Yes	128 vs 127
V	Far western Philippines vs Philippines	Yes	126 vs 127
VI	Bay of Cenderawasih	No	Divides 130
VII	Philippines: north vs south	No	Divides 127
VIII	Philippines: north vs south	No	Divides 127
IX	Krakatau vs Pula Seribu	Close	Divides 119, but near 117 vs 119
X	Java Sea: north vs south	Yes	128 vs 132

most of the Bay of Cenderawasih) and western Indonesia (including Sumatra) do not show evidence of expansion. Populations in the Philippines were split, with approximately half showing evidence of expansion. In *T. maxima* populations, western and southern Indonesian localities showed evidence of expansion. In contrast, none of the eastern Indonesian populations showed evidence of expansion. As in *T. crocea*, Philippine populations of *T. maxima* gave mixed results for population expansion. In *T. squamosa*, many of the central and southern Indonesian populations showed evidence of expansion. However, the majority of Philippine populations did not.

Phylogenetic Analysis.—A total of 450 bp of CO1, 500 bp of 16S, and 390 bp of beta tubulin sequence data was analyzed from a subset of samples, representing 15 *T. crocea,* 16 *T. maxima* and 20 samples that were morphologically identified as *T. squamosa* in the field. Bayesian analyses recovered well-resolved trees, showing three distinct clades for *T. crocea, T. maxima,* and *T. squamosa.* However, the CO1 and 16S data also recovered two independent lineages within samples identified as *T. squamosa* in the field, although the phylogenetic placement of these two distinct clades was not well defined (Fig. 3A,B). Genetic variation was minimal in beta-tubulin and consequently the cryptic lineages were not recovered, nor were three distinct clades for the three focal taxa (Fig. 3C).

Discussion

Genetic data from three species of giant clams (genus *Tridacna*) show strong patterns of phylogeographic structure across the Coral Triangle; each species consists of multiple highly divergent clades and this genetic variation is distributed nonrandomly across the study area from west to east. Significant regional partitions common in all three species are (1) central Indonesia and the Philippines, (2) eastern Indonesia, and (3) Cenderawasih Bay, confirming previous work on *T. crocea* (DeBoer et al. 2008, 2014, Kochzius and Nuryanto 2008), *T. maxima* (Nuryanto and Kochzius 2009), and many other marine species from the Coral Triangle (reviewed in Barber et al. 2011, Carpenter et al. 2011). In addition, Indian Ocean populations form a distinct region in *T. crocea* and *T. maxima,* the two species for which we have data. Concordant phylogeographic patterns across multiple codistributed species strongly suggests that the patterns arose from the action of a shared physical process (Schneider et al. 1998, Walker and Avise 1998, Argoblast and Kenagy 2001) and these boundaries have strong similarities to the marine ecoregions proposed by Spalding

et al. (2007) indicating that processes shaping genetic structure may also be influencing biogeographic patterns.

Genetic Structure of *Tridacna* Species Across the Coral Triangle.—The strongest patterns of regional structure were seen in *T. crocea* (F_{CT} = 0.6015, P < 0.001; Table 2) and *T. maxima* (F_{CT} = 0.5521, P < 0.001; Table 2), both of which contain three highly divergent mtDNA COI clades (Fig. 1D,E) and show strong regional divergence among populations in (1) western Sumatra, (2) central Indonesia and the Philippines, (3) eastern Indonesia, and (4) Cenderawasih Bay. In contrast, *T. squamosa* only contains two minimally distinct haplogroups, rather than three. However, this is likely a sampling artifact. The third clade in *T. crocea* and *T. maxima* is restricted to western Sumatra, but no *T. squamosa* could be found in these regions. Given that *T. squamosa* is known from the Indian Ocean (Lucas 1988) further sampling may uncover a third Indian Ocean clade. Although levels of structure were lower in *T. squamosa,* patterns were still very similar to *T. crocea* and *T. maxima*, with regional subdivisions in (1) central Indonesia and the Philippines, (2) eastern Indonesia, and (3) Cenderawasih Bay (F_{CT} = 0.0826, P < 0.005; Table 2).

Divergence between Indian and Pacific ocean populations likely results from allopatry during Pleistocene low sea level stands, as suggested for other species (e.g., Lavery et al. 1996, Duda and Palumbi 1999, Barber et al. 2000, Benzie et al. 2002, Vogler et al. 2008), including *T. crocea* (DeBoer et al. 2008). During this time, the Sunda and Sahul shelves were exposed, resulting in constricted water flow between the Pacific and Indian oceans (Voris 2000). As sea levels rose, populations recolonized the shelves leaving genetic signals of population range expansion (Crandall et al. 2011). Not only do phylogeographic breaks in *Tridacna* demonstrate isolation across the Sunda Shelf, but results of Fu's F_S neutrality tests provide evidence of range expansion in many populations, particularly in the western parts of Indonesia near the Sunda Shelf (Table 1). Changes in population size could be explained by the reduction of habitat during sea level low stands and recolonization of new habitats after sea levels rose. This hypothesized impact of the Quaternary ice ages has been implicated in the genetic population structure of numerous Coral Triangle species (e.g., Barber et al. 2000, 2002, Fauvelot et al. 2003, Lourie and Vincent 2004a, Crandall et al. 2008). Given the concordant genetic patterns we found, this mechanism may be common to other tridacnid species in the area. However, future studies should be able to differentiate statistically between "single-event" and "multiple-event" biogeographic hypotheses by evaluating genetic divergence across a large number of independent loci in each species (Edwards and Beerli 2000).

Divergence between populations from West Papua and those from the Philippines and Central Indonesia is likely the result of physical oceanographic processes such as the Halmahera Eddy (Barber et al. 2006, 2011) that strongly limits water transport in the area (Nof 1995, Morey et al. 1999). Patterns in all three species mirror results from larval dispersal simulations in the Coral Triangle (Kool et al. 2011), suggesting that contemporary oceanography contributes to the observed regional divergences or may reinforce historical barriers. However, regional differences in the distribution of symbiotic *Symbiodidium* clades in *Tridacna* from Indonesia (DeBoer et al. 2012) suggest that environmental variables could also contribute to the observed patterns.

The phylogeographic patterns above were confirmed by analyses in Barrier, a method that does not require a priori assumptions for regional partitions. Using data

on genetic and geographic distance, Barrier identified 10 partitions within the study area where gene flow is limited. Although concordance among species appears somewhat limited using traditional phylogeographic approaches (Fig. 1), the high percentage of barriers common in all three species (thick lines in Fig. 2) demonstrates that structure in all three species is highly similar. This result emphasizes the importance of using comparative phylogeographic analyses to identify broad patterns.

Correspondence between Phylogeographic and Biogeographic Patterns in the Coral Triangle.—Spalding et al. (2007) defined marine ecoregions using a global hierarchical classification system of coastal and shelf marine regions, which was based on a broad array of source information including species range discontinuities, dominant habitats, geomorphologic features, currents, and temperatures, but not intraspecific genetic diversity. Despite the fact that genetic data were not used to define marine ecoregions, the phylogenetic patterns identified in this study are strikingly similar to biogeographic patterns represented by the boundaries of marine ecoregions (Spalding et al. 2007, Fig. 2). Ecoregion boundaries explained 27% and 32% of the genetic variation present in *T. crocea* and *T. maxima*, respectively (although only 3% for *T. squamosa*) and genetic barriers common to all 3 species were found at most ecoregion boundaries (Fig. 2). Specifically, genetic data support the ecoregion divisions between Bali and Lombok (Barrier II); areas in the Java Sea and those south of the islands (Barriers III and IV); western Halmahera (V); far eastern Philippine waters (Barrier X); and the separation between the Philippines and Indonesia (Barrier VII). Genetic data from *T. crocea* and *T. maxima* also support the ecoregion division between western Sumatra and the rest of the study area (dashed line in Fig. 2), which would presumably have been identified by Barrier if we had data from *T. squamosa* for that area.

This strong concordance of biogeographic and phylogeographic data indicates that similar processes may shape both genetic connectivity and community level patterns of biodiversity, as suggested by Avise (1992, 1994). Although it cannot be ruled out that different processes acting on larval dispersal and adult survival could result in the concordant biogeographic and phylogeographic patterns, the recovery of similar phylogeographic patterns across benthic (e.g., Timm and Kochzius 2008), midwater (e.g., Ackiss et al. 2013), and pelagic fishes (e.g., Jackson et al. 2014), as well as benthic marine invertebrates (see Barber et al. 2011 and Carpenter et al. 2011 for reviews) argues for a single process as it would be improbable that different processes would act so similarly across such taxonomic and ecological diversity. Regardless of the specific process(es) involved, the recovery of strong phylogeographic structure indicates an absence of connectivity among geographic regions (Hedgecock et al. 2007), providing additional genetic support for the independence of the ecoregions defined by Spalding et al. (2007).

The strong agreement between biogeographic and genetic methods for defining ecoregions is heartening given the difficulty and costs of conducting genetic studies in the Coral Triangle and the need to include a broad diversity of taxa in conservation planning. However, it is important to note the areas of discord where genetic data identified breaks within a single ecoregion. First, the Bay of Cenderawasih in eastern Indonesia is identified as a genetically unique area for all three *Tridacna* species (Fig. 2, Barrier VI) and their *Symbiodidium* symbionts (DeBoer et al. 2012), as well as numerous other Coral Triangle species (e.g., Barber et al. 2006, 2011, Crandall

et al. 2008), a result that highlights the genetic and demographic independence of this region. Thus, this area may warrant reclassification as a unique area within the ecoregion, if not as a separate ecoregion itself. A second location where we identified a genetic break, but no ecoregion boundary, is within the Philippine islands (Barriers IX and X). Our results and others (Malay et al. 2002, Juinio-Menez et al. 2003, Magsino 2004, Magsino and Juinio-Menez 2008, Ravago-Gotanco et al. 2007) provide evidence of limits to genetic connectivity within the Philippines, suggesting this area may warrant further division into multiple ecoregions to manage divergent areas as well.

Potentially Undiscovered Taxa.—Giant clams of the genus *Tridacna* are a conspicuous and well-known taxon from Indo-Pacific coral reefs. The majority (five of eight) of *Tridacna* clams were described nearly 200 yrs ago in the 18th and 19th centuries, although the most recent species description was *T. costata* in 2008 (Richter et al. 2008), a species restricted to the Red Sea, a peripheral area on the very edge of the Indo-Pacific region. It is therefore surprising that our phylogenetic analysis revealed the presence of two highly differentiated lineages that do not fall clearly within one of the described species known from the Coral Triangle. Although results from the nuclear beta tubulin gene were inconclusive, COI and 16S sequence data strongly indicate that these lineages are evolutionarily independent (Fig. 3). Maximum variation within these two lineages ranged from 0.9% to 3.0% while being a minimum of 9.2% divergent from the most closely related taxa, a level (Hebert et al. 2003) that suggests these are likely new *Tridacna* species. While DeBoer et al. (2014) found a lack of concordance between nuclear and mitochondrial partitions in *T. crocea,* the depth of divergence of these two cryptic clades is substantially higher. Similarly, even within clades of *T. maxima,* levels of variation between clades reached 9.1%, suggesting the possibility of cryptic taxa within this species as well. Unfortunately, the non-destructive sampling techniques employed in our study included leaving clams intact on the reef, thereby precluding further morphological analysis, but the majority of samples with these divergent lineages were sampled on the islands of Krakatau, suggesting the need to conduct further work in this region.

Conservation Implications.—Understanding the evolutionary history of a species can help inform conservation planning efforts in many ways, including through the identification of cryptic biodiversity, genetically unique populations, and demographically separate regions, among others (reviewed in Feral 2002), and can be directly incorporated into conservation prioritization algorithms (Beger et al. 2014). Genetic analyses of three nominal species of *Tridacna* identified multiple, distinct clades that likely represent unique evolutionarily significant units (ESUs, Moritz 1994) that are restricted to different regions of the Coral Triangle. Results also uncovered potentially undescribed species. Populations of *Tridacna* across the Coral Triangle are either functionally extinct or in sharp decline due to overharvest (for food or sale into aquarium trade) and/or environmental stressors (Othman et al. 2010). Although all *Tridacna* are internationally protected by the Convention on International Trade in Endangered Species (CITES) and appear on the IUCN Red List of Threatened Species (Mollusk Specialist Group 1996b), the presence of multiple, highly divergent lineages in each species means that individually each lineage is more endangered than previously believed.

Extensive information on a single taxonomic group is valuable to conservation efforts, but our finding of concordance between phylogeography and marine ecoregions provides evidence to validate the general model of dividing the ocean into multiple management units. While we did not sample all of the 12 ecoregions in the Coral Triangle, those that we did sample aligned closely with breaks in the genetic data. This result suggests that such an approach is generally applicable across taxa. The ability to generalize across multiple species is important because, while genetic data are very powerful for highlighting boundaries in marine environments (Avise 1994, Palumbi 1996, Hedgecock et al. 2007), such data are still relatively uncommon in marine species throughout the entirety of the world's oceans. Our finding that the limits to genetic connectivity are concordant with transitions from one ecoregion to another suggests that the ecoregion classification system may be a powerful tool for identifying common patterns shaping biodiversity and regional limits to connectivity in the Coral Triangle. For conservation areas to be "representative" requires protection on a full range of biodiversity at multiple levels including genes, species, communities, evolutionary patterns, and ecological processes that create and sustain biodiversity (reviewed in Spalding et al. 2007). Biogeographic classifications, therefore, provide a crucial foundation for the assessment of this representativeness (Olson and Dinerstein 2002, Lourie and Vincent 2004b); concordance of genetic data with this biogeographic classification ensures that an additional, critical level of biodiversity will also be protected.

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Literature Cited

Ackiss AS, Pardede S, Crandall ED, Ambariyanto, Ablan-Lagman MCA, Romena N, Barber PH, Carpenter KE. 2013. Pronounced genetic structure in a highly mobile coral reef fish, *Caesio cuning,* in the Coral Triangle. Mar Ecol Prog Ser. 480:185–197. http://dx.doi.org/10.3354/meps10199

Argoblast BS, Kenagy GJ. 2001. Comparative phylogeography as an integrative approach to historical biogeography. J Biogeogr. 28:819–825.

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. http://dx.doi.org/10.2307/3545516

Avise JC. 1994. Molecular Markers, Natural History, and Evolution. New York: Chapman and Hall. http://dx.doi.org/10.1007/978-1-4615-2381-9

Avise JC. 2000. Phylogeography—the history and formation of species. Cambridge, MA: Harvard University Press.

Barber P, Boyce SL. 2006. Estimating diversity of Indo-Pacific coral reef stomatopods through DNA barcoding of stomatopod larvae. Proc RSoc B-Biol Sci. 273(1597):2053–2061. PMid:16846913. PMCid:PMC1635474. http://dx.doi.org/10.1098/rspb.2006.3540

Barber PH. 2009. The challenge of understanding the Coral Triangle biodiversity hotspot. J Biogeogr. 26(10):1845–1846. http://dx.doi.org/10.1111/j.1365-2699.2009.02198.x

Barber PH, Cheng SH, Erdmann MV, Tengardjaja K, Ambariyanto. 2011. Evolution and conservation of marine biodiversity in the Coral Triangle: insights from stomatopod Crustacea. Crustacean Issues. 19:129–156. http://dx.doi.org/10.1201/b11113-9

Barber PH, Erdmann MV, Palumbi SR. 2006. Comparative phylogeography of three codistributed stomatopods: origins and timing of regional lineage diversification in the coral triangle. Evolution. 60(9):1825–1839. PMid:17089967.

Barber PH, Palumbi SR, Erdmann MV, Moosa MK. 2000. Biogeography—a marine Wallace's line? Nature. 406(6797):692–693. PMid:10963585. http://dx.doi.org/10.1038/35021135

Barber PH, Palumbi SR, Erdmann MV, Moosa MK. 2002. Sharp genetic breaks among populations of *Haptosquilla pulchella* (Stomatopoda) indicate limits to larval transport: patterns, causes, and consequences. Mol Ecol. 11(4):659–674. PMid:11972755. http://dx.doi.org/10.1046/j.1365-294X.2002.01468.x

Beger M, Selkoe K, Treml E, Barber PH, von der Heyden S, Crandall ED, Toonen RJ, Riginos C. 2014. Evolving coral reef conservation with genetic information. Bull Mar Sci. 90:159–185. http://dx.doi.org/10.5343/bms.2012.1106

Bellwood DR, Meyer CP. 2008. Searching for heat in a marine biodiversity hotspot. J Biogeogr. 36(4):569–576. http://dx.doi.org/10.1111/j.1365-2699.2008.02029.x

Benzie JAH. 1999. Genetic structure of coral reef organisms: ghosts of dispersal past. Am Zool. 39:131–145.

Benzie JAH, Ballment E, Forbes AT, Demetriades NT, Sugama K, Haryanti, Moria S. 2002. Mitochondrial DNA variation in Indo-Pacific populations of the giant tiger prawn, *Penaeus monodon*. Mol Ecol. 11:2553–2569. PMid:12453239. http://dx.doi.org/10.1046/j.1365-294X.2002.01638.x

Burgess ND, Hales JDA, Ricketts TH, Dinerstein E. 2006. Factoring species, non-species values and threats into biodiversity prioritisation across the ecoregions of Africa and its islands. Biol Conserv. 127:383–401. http://dx.doi.org/10.1016/j.biocon.2005.08.018

Burke L, Reytar K, Spalding M, Perry A. 2011. Reefs at risk revisited. Washington, DC: Wolrd Resources Institute.

Burke L, Selig E, Spalding M. 2002. Reefs at risk in Southeast Asia. Washington, DC: World Resources Institute.

Burton RS. 1998. Intraspecific phylogeography across the Point Conception biogeographic boundary. Evolution. 52:734–745. http://dx.doi.org/10.2307/2411268

Carpenter KE, Barber PH, Crandall ED, Ablan-Lagman MCA, Ambariyanto, Mahardika GN, Manjaji-Matsumoto BM, Juinio-Meñez MA, et al. 2011. Comparative phylogeography of the Coral Triangle and implications for marine management. J Mar Biol. 2011:Article ID 396982, 14 p.

Carpenter KE, Springer VG. 2005. The center of the center of marine shorefish biodiversity: the Philippine Islands. Environ Biol Fish. 72:467–480. http://dx.doi.org/10.1007/s10641-004-3154-4

Chape S, Blyth S, Fish L, Fox P, Spalding M. 2003. United Nations list of protected areas. Gland, Switzerland: IUCN- World Conservation Union.

Crandall ED, Jones ME, Munoz MM, Akinronbi B, Erdmann MV, Barber PH. 2008. Comparative phylogeography of two sea stars and their ectosymbionts within the Coral Triangle. Mol Ecol. 17:5276–5290. PMid:19067797. http://dx.doi.org/10.1111/j.1365-294X.2008.03995.x

Crandall ED, Sbrocco EJ, DeBoer TS, Barber PH, Carpenter KE. 2011. Expansion dating: calibrating molecular clocks in marine species from expansions onto the Sunda Shelf following the last glacial maximum. Mol Biol Evol. PMid:21926069

DeBoer TS, Subia MD, Ambariyanto, Erdmann MV, Kovitvongsa K, Barber PH. 2008. Phylogeography and limited genetic connectivity in the endangered boring giant clam across the Coral Triangle. Conserv Biol. 22(5):1255–1266. PMid:18637905. http://dx.doi.org/10.1111/j.1523-1739.2008.00983.x

DeBoer TS, Ambariyanto, Baker AC, Barber PH. 2012. Patterns of *Symbiodinium* distribution in three giant clam species across the biodiverse Bird's Head region of Indonesia. Mar Ecol Prog Ser. 444:117–132. http://dx.doi.org/10.3354/meps09413

DeBoer TS, Naguit MRA, Erdmann MV, Ablan-Lagman MCA, Ambariyanto, CarpenterKE, Toha AHA, Barber PH. 2014. Concordant phylogenetic patterns inferred from mitochondrial and microsatellite DNA in the giant clam *Tridacna crocea.* Bull Mar Sci. 90:277–299. http://dx.doi.org/10.5343/bms.2013.1003

Duda TF, Palumbi SR. 1999. Population structure of the black tiger prawn, *Penaeus monodon,* among western Indian Ocean and western Pacific populations. Mar Biol. 134:705–710. http://dx.doi.org/10.1007/s002270050586

Duda TF, Palumbi SR. 2004. Gene expression and feeding ecology: evolution of piscivory in the venomous gastropod genus *Conus.* Proc R Soc Lond Ser B. 271:1165–1174. PMid:15306367. PMCid:PMC1691710. http://dx.doi.org/10.1098/rspb.2004.2708

Edwards SV, Beerli P. 2000. Variance in coalescence time and comparative phylogeography. Evolution. 54:1839–1854. http://dx.doi.org/10.1554/0014-3820(2000)054[1839:PGDPDA]2.0.CO;2

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 131:479–491. PMid:1644282. PMCid:PMC1205020.

Fauvelot C, Bernardi G, Planes S. 2003. Reduction in the mitochondrial DNA diversity of coral reef fish provides evidence of population bottlenecks resulting from Holocene sea-level changes. Evolution. 57:1571–1583. PMid:12940362.

Feral JP. 2002. How useful are the genetic markers in attempts to understand and manage marine biodiversity? J Exp Mar Biol Ecol. 268(2):121–145. http://dx.doi.org/10.1016/S0022-0981(01)00382-3

Fitt WK, Trench RK. 1981. Spawning, development and acquisition of zooxanthellae by *Tridacna squamosa* (Mollusca, Bivalvia). Biol Bull. 161(213–235):213.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol. 3:294–299. PMid:7881515.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics. 147:915–925. PMid:9335623 PMCid:PMC1208208

Gardner TA, Cote IM, Gill JA, Grant A, Watkinson AR. 2003. Long-term region-wide declines in Caribbean corals. Science. 301(5635):958–960. PMid:12869698. http://dx.doi.org/10.1126/science.1086050

Green A, Mous J. 2004. Delineating the Coral Triangle, its ecoregions and functional seascapes. Report on an expert workshop held at the Southeast Asia Center for Marine Protected Areas. Bali, Indonesia: The Nature Conservancy Southeast Asia Center for Marine Protected Areas.

Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS, Samuel MD. 2002. Climate warming and disease risks for terrestrial and marine biota. Science. 296(5576):2158–2162. PMid:12077394. http://dx.doi.org/10.1126/science.1063699

Hazen HD, Anthamatten PJ. 2004. Representations of ecological regions by protected areas at the global scale. Phys Geogr. 25:499–512. http://dx.doi.org/10.2747/0272-3646.25.6.499

Hebert PDN, Ratnasingham S, de Waard JR. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc R Soc Lond Ser B. 270:S96–S99. PMid:12952648. PMCid:PMC1698023. http://dx.doi.org/10.1098/rsbl.2003.0025

Hedgecock D, Barber PH, Edmands S. 2007. Genetic approaches to measuring connectivity. Oceanography. 20(3):70–79. http://dx.doi.org/10.5670/oceanog.2007.30

Hoekstra JM, Boucher TM, Ricketts TH, Roberts C. 2005. Confronting a biome crisis: global disparities of habitat loss and protection. Ecol Lett. 8:23–29. http://dx.doi.org/10.1111/j.1461-0248.2004.00686.x

Hudson RR. 1990. Gene genealogies and the coalescent process. *In:* Futuyma D, Antonovics J, editors. Oxford Surveys in Evolutionary Biology. New York, NY: Oxford University Press. p. 1–44. PMid:2328964.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics. 17:754–755. http://dx.doi.org/10.1093/bioinformatics/17.8.754

Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Hoegh-Guldberg O, Jackson JBC, Kleypas J, et al. 2003. Climate change, human impacts, and the resilience of coral reefs. Science. 301(5635):929–933. PMid:12920289. http://dx.doi.org/10.1126/science.1085046

Jackson JB, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH, Cooke R, Erlandson J, Estes JA, et al. 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science. 293(5535):1589–1591.

Jackson AM, Ambariyanto, Erdmann MV, Toha AH, Stevens LA, Barber PH. 2014. Comparative phylogeography of commercial tuna and mackerel in the Indonesian Archipelago. Bull Mar Sci. 90:471–492. http://dx.doi.org/10.5343/bms.2012.1097

Juinio-Menez MA, Magsino RM, Ravago RG, Yu ET. 2003. Genetic structure of *Linckia laevigata* and *Tridacna crocea* among the Palawan shelf and shoal reefs. Mar Biol. 142:717–726.

Kelly RP, Palumbi SR. 2010. Genetic structure among 50 species of the northeastern Pacific rocky intertidal community. PLoS ONE. 5(1):e8594. PMid:20062807. PMCid:PMC2799524. http://dx.doi.org/10.1371/journal.pone.0008594

Kochzius M, Nuryanto A. 2008. Strong genetic population structure in the boring giant clam, *Tridacna crocea,* across the Indo-Malay Archipelago: implications related to evolutionary processes and connectivity. Mol Ecol. 17(17):3775–3787. PMid:18662232. http://dx.doi.org/10.1111/j.1365-294X.2008.03803.x

Kool JT, Paris CB, Barber PH, Cowen RK. 2011. Connectivity and the development of population genetic structure in Indo–West Pacific coral reef communities. Global Ecol Biogeogr. 20(5):695–706. http://dx.doi.org/10.1111/j.1466-8238.2010.00637.x

Lamoreux JF, Morrison JC, Ricketts TH, Olson DM, Dinerstein E, McKnight MW, Shugart HH. 2006. Global tests of biodiversity concordance and the iportance of endemism. Nature. 440:212–214. PMid:16382239. http://dx.doi.org/10.1038/nature04291

Lavery S, Moritz C, Fielder DR. 1996. Indo-Pacific population structure and evolutionary history of the coconut crab, *Birgus latro*. Mol Ecol. 5:557–570. http://dx.doi.org/10.1111/j.1365-294X.1996.tb00347.x

Lee JB, Johnson JB. 2009. Biogeography of the livebearing fish *Poecilia gillii* in Costa Rica: are phylogeographical breaks congruent with fish community boundaries? Mol Ecol. 18:4088–4101. PMid:19735450. http://dx.doi.org/10.1111/j.1365-294X.2009.04339.x

Lourie SA, Green DM, Vincent ACJ. 2005. Dispersal, habitat differences, and comparative phylogeography of Southeast Asian seahorses (Syngnathidae: *Hippocampus*). Mol Ecol. 14:1073–1094. PMid:15773937. http://dx.doi.org/10.1111/j.1365-294X.2005.02464.x

Lourie SA, Vincent ACJ. 2004a. A marine fish follows Wallace's line: the phylogeography of the three-spot seahorse (*Hippocampus trimaculatus,* Syngnathidae, Teleostei) in Southeast Asia. J Biogeogr. 31:1975–1985. http://dx.doi.org/10.1111/j.1365-2699.2004.01153.x

Lourie SA, Vincent ACJ. 2004b. Using biogeography to help set priorities in marine consrvation. Conserv Biol. 18:1004–1020. http://dx.doi.org/10.1111/j.1523-1739.2004.00137.x

Lucas JS. 1988. Giant clams: description, distribution, and life history. *In:* Copland JW, Lucas JS, editors. Giant clams in Asia and the Pacific. Canberra: ACIAR. p. 21–32.

Maddison WP, Maddison DR. 2002. MacClade. Sunderland, MA: Sinauer Associates.

Magsino RM. 2004. Patterns of population genetic structuring of rabbitfishes *Siganus argenteus* (Quoy and Guimard, 1825) and *Siganus fuscescens* (Houttuyn, 1782) along the eastern Philippine coasts. [Manila]: University of the Philippines, Diliman.

Magsino RM, Juinio-Meñez MA. 2008. The influence of contrasting life history traits and oceanic processes on genetic structuring of rabbitfish populations *Signus argenteus* and *Siganus fuscescens* along the eastern Philippines. Mar Biol. 154(3):519–532. http://dx.doi.org/10.1007/s00227-008-0946-7

Malay MD, Juinio-Menez MA, Villanoy CL. 2002. Population genetic structure of the sea urchin *Tripneustes gratilla* from selected sites in western Luzon and eastern Philippines. *In:* Proceedings of the 9th International Coral Reef Symposium; 2002; Bali, Indonesia. PMCid:PMC127069.

Manni F, Guerard E, Heyer E. 2004. Goegraphic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. Human Biol. 76(2):173–190. PMid:15359530. http://dx.doi.org/10.1353/hub.2004.0034

Maruyama T, Ishikura M, Yamazaki S, Kanai S. 1998. Molecular phylogeny of the zooxanthellate bivalves. Biol Bull. 195:70–77. PMid:9739550. http://dx.doi.org/10.2307/1542777

McCulloch M, Fallon S, Wyndham T, Hendy E, Lough J, Barnes D. 2003. Coral record of increased sediment flux to the inner Great Barrier Reef since European settlement. Nature. 421(6924):705–706. PMid:12610621. http://dx.doi.org/10.1038/nature01361

McManus JW. 1997. Tropical marine fisheries and the future of coral reefs: a brief review with emphasis on Southeast Asia. Coral Reefs. 16(Suppl):S121–S127.

McMillan WO, Palumbi SR. 1995. Concordant evolutionary patterns among Indo–West Pacific butterfly fishes. Proc R Soc Lond, Ser B: Biol Sci. 260:229–236. PMid:7784441. http://dx.doi.org/10.1098/rspb.1995.0085

Mollusk Specialist Group 1996a. *Assiminea palauensis. In* IUCN 2010. IUCN Red List of Threatened Species. Version 2010.1.

Mollusk Specialist Group. 1996b. *Tridacna crocea. In:* IUCN 2006. 2006 IUCN Red List of Threatened Species.

Monmonier M. 1973. Maximum-difference barriers: an alternative numerical regionalization method. Geogr Anal. 3:245–261.

Morey SL, Shriver JF, O'Brien JJ. 1999. The effects of Halmahera on the Indonesian throughflow. J Geophys Res-Oceans. 104(C10):23281–23296. http://dx.doi.org/10.1029/1999JC900195

Moritz C. 1994. Defining evolutionarily-significant-units for conservation. Trends Ecol Evol. 9(10):373–375. http://dx.doi.org/10.1016/0169-5347(94)90057-4

Nof D. 1995. Choked flows from the Pacific to the Indian Ocean. J Phys Oceanogr. 25(6):1369–1383. http://dx.doi.org/10.1175/1520-0485(1995)025<1369:CFFTPT>2.0.CO;2

Nuryanto A, Kochzius M. 2009. Highly restricted gene flow and deep evolutionary lineages in the giant clam *Tridacna maxima*. Coral Reefs. 28(3):607–619. http://dx.doi.org/10.1007/s00338-009-0483-y

Olson DM, Dinerstein E. 2002. The Global 200: priority ecoregions for conservation. Ann Mo Bot Gard. 89:199–224. http://dx.doi.org/10.2307/3298564

Othman AS, Goh GHS, Todd, PA. 2010. The distribution and status of giant clams (family Tridacnidae)-a short review. Raffles Bull Zool. 58(1):103–111.

Palumbi SR. 1996. What can molecular genetics contribute to marine biogeography? An urcin's tale. J Exp Mar Biol Ecol. 203(1):75–92. http://dx.doi.org/10.1016/0022-0981(96)02571-3

Pandolfi JM, Bradbury RH, Sala E, Hughes TP, Bjorndal KA, Cooke RG, McArdle D, McClenachan L, Newman MJ, Paredes G, et al. 2003. Global trajectories of the long-term decline of coral reef ecosystems. Science. 302(5650):1502–1504.

Posada D, Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics. 14:817–818. http://dx.doi.org/10.1093/bioinformatics/14.9.817

Ravago-Gotanco RG, Magsino RM, Juinio-Menez MA. 2007. Influence of the North Equatorial Current on the population genetic structure of *Tridacna crocea* (Mollusca: Tridacnidae)

along the eastern Philippine seaboard. Mar Ecol Prog Ser. 336:161–168. http://dx.doi.org/10.3354/meps336161

Reid DG, Lal K, Mackenzie-Dodds J, Kaligis F, Littlewood DTJ, Williams ST. 2006. Comparative phylogeography and species boundaries in *Echinolittorina* snails in the central Indo-West Pacific. J Biogeogr. 33(6):990–1006. http://dx.doi.org/10.1111/j.1365-2699.2006.01469.x

Richter C, Roa-Quiaoit H, Jantzen C, Al-Zibdah M, Kochzius M. 2008. Collapse of a new living species of giant clam in the Red Sea. Curr Biol. 18(17):1349–1354. PMid:18760604. http://dx.doi.org/10.1016/j.cub.2008.07.060

Roberts CM, McClean CJ, Veron JEN, Hawkins JP, Allen GR, McAllister DE, Mittermeier CG, Schueler FW, Spalding M, Wells F, et al. 2002. Marine biodiversity hotspots and conservation priorities for tropical reefs. Science. 295:1280–1284. PMid:11847338. http://dx.doi.org/10.1126/science.1067728

Sale PF, Cowen RK, Danilowicz BS, Jones GP, Kritzer JP, Lindeman KC, Planes S, Polunin NVC, Russ GR, Sadovy YJ, et al. 2005. Critical science gaps impede use of no-take fishery reserves. Trends Ecol Evol. 20(2):74–80. PMid:16701346. http://dx.doi.org/10.1016/j.tree.2004.11.007

Schneider CJ, Cunningham M, Moritz C. 1998. Comparative phylogeography and the history of endemic vertebrates in the Wet Tropics rainforests of Australia. Mol Ecol. 7:487–498. http://dx.doi.org/10.1046/j.1365-294x.1998.00334.x

Schneider JA, Foighil DO. 1999. Phylogeny of giant clams (Cardiidae: Tridacninae) based on partial mitochondrial 16S rDNA gene sequences. Mol Phylogenet Evol. 13(1):59–66. PMid:10508539. http://dx.doi.org/10.1006/mpev.1999.0636

Schneider S, Roessli D, Excoffier L. 2000. Arlequin ver. 2.000: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Spalding MD, Fox HE, Allen GR, Davidson N, Ferdana ZA, Finlayson M, Halpern BS, Jorge MA, Lombana A, Lourie SA, et al. 2007. Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. BioScience. 57(7):573–583. http://dx.doi.org/10.1641/B570707

Timm J, Kochzius M. 2008. Geological history and oceanography of the Indo-Malay Archipelago shape the genetic population structure in the false clownfish (*Amphiprion ocellaris*). Mol Ecol. 17:3999–4014. PMid:19238702. http://dx.doi.org/10.1111/j.1365-294X.2008.03881.x

Toonen RJ, Andrews KR, Baums IB, Bird CE, Concepcion GT, Daly-Engel TS, Eble JA, Faucci A, Gaither MR, Iacchei M, et al. 2011. Defining boundaries for ecosystem-based management: a multispecies case study of marine connectivity across the Hawaiian Archipelago. J Mar Biol. 2011:Article ID 460173, 13 pages. http://dx.doi.org/10.1155/2011/460173

Vogler C, Benzie J, Lessios H, Barber PH, Worheide G. 2008. A threat to coral reefs multiplied? Four species of crown-of-thorns starfish. Biol Lett. 4:696–699. PMid:18832058. PMCid:PMC2614177. http://dx.doi.org/10.1098/rsbl.2008.0454

Voris HK. 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. J Biogeogr. 27(5):1153–1167. http://dx.doi.org/10.1046/j.1365-2699.2000.00489.x

Voronoi MG. 1908. Noivelles application des parametres continus a la theorie des formes quadratiques, deuxieme memoire, recherche sur le paralleloedres primitifs. Journal für die reine und angewandte Mathematik. 134:198–207.

Walker D, Avise JC. 1998. Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. Annu Rev Ecol Syst. 29:23–58. http://dx.doi.org/10.1146/annurev.ecolsys.29.1.23

Walsh PS, Metzger DA, Higuchi R. 1991. Chelex-100 as a medium for simple extraction of DNA for pcr-based typing from forensic material. Biotechniques. 10(4):506–513. PMid:1867860.

Wilkinson C. 2002. Status of coral reefs of the world. Townsville, Australia: Australian Institute of Marine Science.

Williams DM, Roth CH, Reichelt R, Ridd P, Rayment GE, Larcombe P, Brodie J, Pearson R, Wilkinson C, Talbot F, et al. 2002. The current level of scientific understanding on impacts

of terrestrial run-off on the Great Barrier Reef World Heritage Area [Internet]. CRC Reef Research Centre; [cited]. Available from: http://www.reef.crc.org.au/discover/threats/waterquality_consensus.html

Williams ST, Reid DG. 2004. Speciation and diversity on tropical rocky shores: a global phylogeny of snails of the genus *Echinolittorina.* Evolution. 58(10):2227–2251. PMid:15562687.