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research paper

Concordant phylogenetic patterns inferred from mitochondrial and microsatellite DNA in the giant clam *Tridacna crocea*

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ABSTRACT.—The boring giant clam, *Tridacna crocea* Lamarck, 1819, is a CITES-listed bivalve that is declining due to overharvest and environmental degradation. Previous molecular studies in the Coral Triangle using mitochondrial DNA indicated the presence of deep phylogenetic divergence and strong phylogeographic structure across this region, suggesting the possibility of multiple cryptic species. In the present study, we compare data from non-recombining mitochondrial (mtDNA; cytochrome oxidase subunit 1, COI) and eight microsatellite loci to better understand patterns of genetic structure and species boundaries in *T. crocea* populations across Indonesia and the Philippines. Microsatellite loci and mtDNA data from 618 individuals representing 27 populations revealed highly concordant phylogeographic patterns and identified three genetically distinct regions: (1) Western Indonesia, (2) Philippines and Central Indonesia, and (3) Eastern Indonesia. Both marker types also showed evidence of isolation by distance. These results build on previous studies and confirm the presence of only three genetic partitions and the genetic isolation of Western Indonesia and Eastern Indonesia. However, individual admixture analyses based on microsatellite data show that the mtDNA clade that defines a phylogeographic province spanning the Philippines and Central Indonesia is a mixture of unique genetic clusters from the Philippines/Central Indonesia and Eastern Indonesia. The admixture of nuclear loci from individuals with regionally distinct mtDNA genomes suggests that despite deep genetic divisions, the three mitochondrial lineages are likely not distinct species and that some populations in Central Indonesia may be a sink for genetic diversity accumulated from populations to the north and east. While microsatellite data refined our understanding of the biology and evolutionary history of *T. crocea,* the broad concordance between these markers highlights the continued utility of mtDNA, particularly in developing biodiversity-rich countries where resources to support biodiversity science are limited.

The Coral Triangle, which includes all or parts of Malaysia, the Philippines, Indonesia, Papua New Guinea, East Timor, and the Solomon Islands, is the global epicenter of marine biodiversity (Roberts et al. 2002). Phylogeographic methods have been increasingly applied to marine species in this region driven by an interest in understanding the evolution of the Coral Triangle biodiversity hotspot (see Connolly et al. 2003, Bellwood and Meyer 2008, Barber 2009), as well as the need to guide conservation planning in this unique and highly threatened ecosystem (Barber et al. 2006, DeBoer et al. 2008). Such studies variously implicate Pleistocene sea level fluctuations (Lavery et al. 1996, Benzie and Williams 1998, Benzie 1999), physical oceanography (e.g., Barber et al. 2006, 2011), and habitat differences (Lourie and Vincent 2004, Williams and Reid 2004, Lourie et al. 2005, Reid et al. 2006) in contributing to the evolution of biodiversity in this region (for reviews see Barber 2009, Barber et al. 2011, Carpenter et al. 2011). Phylogeographic patterns have been used to infer that connectivity among reefs in this region must be low in many species (Meyer et al. 2005, Barber et al. 2006, DeBoer et al. 2008, Kochzius and Nuryanto 2008, Timm et al. 2008), providing information useful in determining the spatial scale of management units in this area.

The vast majority phylogeographic studies in the Coral Triangle and elsewhere have relied on data from non-recombining mitochondrial DNA (mtDNA) because of the ease of obtaining mtDNA sequence data, and because maternal inheritance decreases the effective population size, reducing the time required for lineage sorting to reveal phylogeographic patterns (see Avise 2000 for review). While mtDNA as a population marker is still supported by many researchers (see Zink and Barrowclough 2008, Bowen et al. 2014), reliance solely on mtDNA is frequently criticized (e.g., Galtier et al. 2009). The primary limitation noted for mtDNA is that it is effectively a single locus and therefore reveals only part of the evolutionary history of a species (Zhang and Hewitt 2003). Moreover, mtDNA may not always be selectively neutral resulting in selective sweeps and can in some cases undergo recombination (see Galtier et al. 2009 for review), violating critical assumptions required for common inferences based on genetic data.

The alternative to mtDNA markers is biparentally inherited nuclear markers that allow for recombination, thereby integrating additional genealogical processes (e.g., Heuertz et al. 2004). Microsatellites have been a popular nuclear marker because of their highly polymorphic nature, codominant transmission, ease of detection by polymerase chain reaction (PCR), relative abundance, extensive genome coverage, and requirement for only a small amount of starting DNA (Powell et al. 1996). As a result, microsatellites have become widely used in both plant and animal non-model organisms for population genetics, demographic history, and paternity testing among others. Yet microsatellites also have significant limitations as genetic markers including size fragment homoplasy (Garza and Freimer 1996) due to constraints on allele size (Garza et al. 1995), lack of selective neutrality (Santucci et al. 2007), and null alleles (Chapuis and Estoup 2007). The development of microsatellites can be difficult, requiring a significant investment of time and money, limiting the utility of these markers in developing countries with limited resources to support biodiversity research (Barber et al. 2014).

While the marine environments of the Coral Triangle are the most biodiverse in the world, they are also among the most threatened, resulting in substantial losses of coral reef habitat (Burke et al. 2002, 2011) and population declines in many marine

taxa. For example, five of eight known giant clam species of the genus *Tridacna* occur in the Coral Triangle (Lucas 1988, Othman et al. 2010); although giant clam populations were once abundant across the Coral Triangle, overharvest for food and the aquarium trade combined with environmental stressors has resulted in the decline and/or functional extinction of *Tridacna* across this region (Othman et al. 2010). As a result, IUCN has Red-Listed seven of eight species of *Tridacna*.

The boring giant clam, *Tridacna crocea* Lamarck, 1819, is the smallest of all the *Tridacna* and ranges from Thailand in the eastern Indian Ocean across the Indo-Malay Archipelago and Northern Australia to Southern Japan and New Caledonia in the western Pacific (Othman et al 2010). It reproduces by broadcast spawning (Lucas 1988), and larvae are competent to settle in approximately 10 d (Copland and Lucas 1988). After settlement, *T. crocea* bores into the reef, where it remains attached and sessile.

Previous studies of phylogeographic structure of *T. crocea* in the Coral Triangle using mtDNA cytochrome-*c* oxidase I (COI) revealed three highly divergent clades and pronounced phylogeographic structure separating populations from Western Papua, Central Indonesia, and Western Sumatra (DeBoer et al. 2008), although Kochzius and Nuryanto (2008) indicate the presence of eight clades across Indonesia. Broad phylogeographic patterns in *T. crocea* are broadly concordant with previous phylogeographic studies from this region from a wide variety of taxa, including stomatopods (Barber et al. 2000, 2006, 2011), fish (Timm et al. 2008, Ackiss et al. 2013), echinoderms (Crandall et al. 2008), and other *Tridacna* (Nuryanto and Kochzius 2009), suggesting that these patterns result from a common response to a shared physical environment (Schneider et al. 1998, Avise 2000, Argoblast and Kenagy 2001). However, these previous studies were based on a single mtDNA locus and left questions as to how many regional genetic partitions exist in *T. crocea,* how patterns in Indonesia compare to other regions of the Coral Triangle, and whether *T. crocea* is really a complex of cryptic species. Furthermore, while previous studies showed clear limits to gene flow and connectivity among populations of *T. crocea* in Indonesia, both studies also indicated admixture of divergent mtDNA lineages in Indonesia.

In the present study, we employ a multilocus approach to better understand patterns of population genetic structure and admixture in *T. crocea* populations across the Coral Triangle. Toward this end, we expand previous sampling efforts in Indonesia to include the Philippines, a major region of the Coral Triangle biodiversity hotspot. Using an expanded mtDNA data set complemented with new data from eight highly variable microsatellite markers, we test whether patterns of population structure from nuclear microsatellite markers correspond to those recovered from analysis of mtDNA. Specifically, we test (1) how many genetic partitions exist within *T. crocea* across Indonesia and the Philippines and the geographic distribution of those partitions, and (2) whether deeply divergent mitochondrial lineages in *T. crocea* may represent cryptic species. Lastly, through comparing mtDNA and microsatellite results, we highlight the different insights provided by these different markers, and the utility of these results for conservation.

Methods

Sample Collection and Genetic Data.—We collected a small piece of mantle tissue from 618 individual *T. crocea* from 27 populations in Indonesia and the

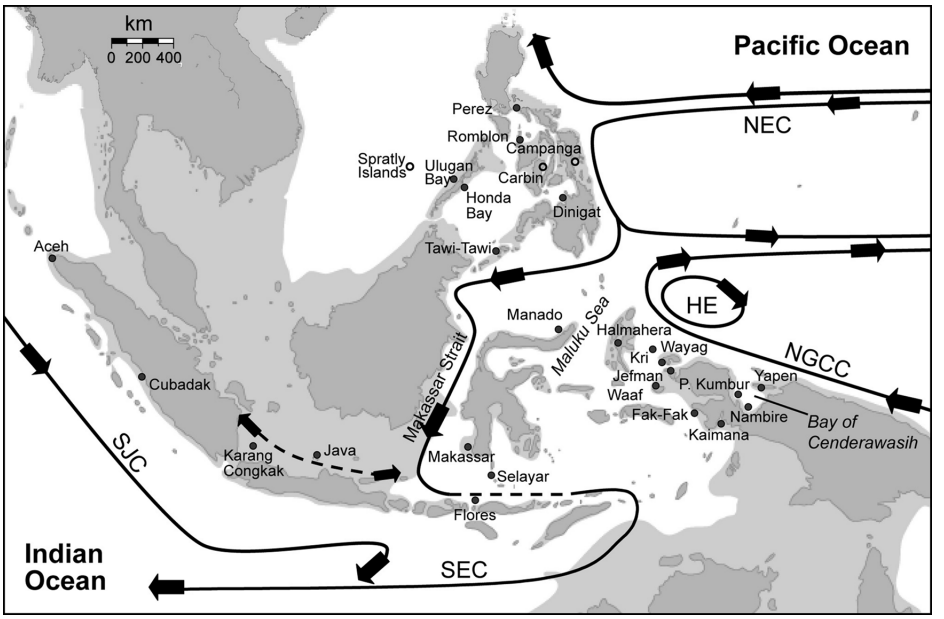

Figure 1. Map of the study area showing the location of 27 sampled populations of *Tridacna crocea* across the Coral Triangle. Open circles indicate populations without mtDNA COI data. Closed circles indicate populations with microsatellite and mtDNA COI data. Dominant (solid lines) and seasonally reversing currents (dashed lines) are shown including the North Equatorial Current (NEC), New Guinea Coastal Current (NGCC), Halmahera Eddy (HE), Makassar Strait (MS, the main passageway of the Indonesian Throughflow), the South Equatorial Current (SEC), and the South Java Current (SJC). Light gray shading indicates coastal margins during Pleistocene low sea level stands, after Voris (2000). Exact sampling locations are given in Table 1.

Philippines (Fig. 1, Table 1), adding 204 additional samples from the Philippines and Indonesia to the data published in DeBoer et al. (2008). Samples were collected in situ to minimize clam mortality and preserved in 95% ethanol. We extracted whole genomic DNA using 10% Chelex (Biorad) solution (Walsh et al. 1991), and collected 485 bp of mtDNA COI sequence data following the methods of DeBoer et al. (2008). For microsatellites, we amplified eight highly variable loci using primers and thermocycling parameters described in DeBoer and Barber (2010). Briefly, PCR amplification of microsatellite loci was carried out in 10 ml reactions containing 1 ml DNA template, 0.25 U Taq Gold polymerase (Applied Biosystems), 1 ml of 10x PCR Buffer, 1 ml of dNTPs (8mM), 0.8 ml $MgCl_2$ (25mM), and 0.5 ml of each primer (10mM). For loci Tc04 and Tc160 the amount of $MgCl_2$ was doubled and the water reduced to a final reaction volume of 10 ml. Thermocycling parameters were: 1 × 94 °C (10 min), 30 × (30 s at 94 °C, 30 s T_a °C, 40 s at 72 °C), and 1 × 72 °C (60 min). Annealing temperatures (T_a °C) were 47.1 °C (Tc04, Tc160), 50 °C (Tc40, Tc50, Tc157, Tc92), and 57.9 °C (Tc59, Tc74, Tc161). Microsatellite primers with the same annealing temperatures were multiplexed in the PCR except for loci Tc157 and Tc92, which were run in separate reactions to increase amplification success. Resulting PCR products were screened on an ABI 3730 sequencer using Genscan v3.1.2 (Applied Biosystems) and LIZ500 ladder as a size standard. Alleles were scored using STRand (Locke et al. 2000, Toonen 2007) and coded as total fragment size in base pairs.

Table 1. List of *Tridacna crocea* collection localities with coordinates in decimal degrees and sample sizes for microsatellite and mitochondrial cytochrome oxidase *c* (subunit 1) DNA sequence data. 2008 data refers to previously published data from DeBoer et al 2008.

Map	Population	Latitude	Longitude	Msat data (*n*)	COI data 2013 (*n*)	COI data 2008 (*n*)
1	Pulau Weh, Aceh	5.61642	95.69967	20	31	21
2	Cubadak, Sumatra	–1.22167	100.38368	14	32	16
3	Karang Congkak, Pulau Seribu	–5.70878	106.57227	21	26	21
4	Karimunjawa, Java	–5.83495	110.59833	17	21	17
5	Sabolan Kecil, Flores	–8.39158	119.79933	20	20	22
6	Barang Lompo, Makassar	–5.04806	119.32889	14	36	14
7	Selayar, South Sulawesi	–6.20754	120.39877	15	35	15
8	Halmahera, Maluku	0.8	127.6	20	33	21
9	Manado, North Sulawesi	1.45720	124.76225	16	18	17
10	Waaf, West Papua	–2.13804	130.20823	17	20	17
11	Fak-Fak, West Papua	–3.93647	132.83217	20	20	19
12	Kaimana, West Papua	–3.81558	133.92687	18	18	18
13	Jefman Is., West Papua	–0.92735	131.12347	17	20	20
14	Wayag, West Papua	0.17593	130.05942	19	20	20
15	Kri, West Papua	–0.55652	130.69028	17	25	17
16	Perez, Quezon	14.18577	121.91132	29	31	0
17	Romblon, MIMAROPA	12.57450	122.24823	24	34	0
18	Carbin, Visaya	10.98150	123.46700	19	0	0
19	Camanga, E. Samar	11.08935	125.61942	16	0	0
20	Dinigat, Dingat	9.99988	125.50080	24	25	0
21	Tawi-Tawi, Mindanao	4.97026	119.76220	24	25	0
22	Spratly Islands	10.20000	114.40000	14	0	0
23	Ulugan Bay, Palawan	10.08149	118.81101	24	30	0
24	Honda Bay, Palawan	9.87876	118.76191	22	25	0
25	Pulau Kumbur, Teluk Cenderawasih	–2.99778	135.05562	21	22	22
26	Nambire, Teluk Cenderawasih	–2.94975	135.67070	21	22	21
27	Yapen, Teluk Cenderawasih	–1.96062	136.32053	21	29	21

Data Analysis.—To determine the phylogenetic relationships of new mitochondrial haplotypes to the three clades identified by DeBoer et al. (2008), we created a Neighbor-Joining tree using uncorrected p-distance. We examined structure in the mitochondrial data set using AMOVA (Excoffier et al. 1992) as implemented in Arlequin 3.11 with significance tested using 10,000 randomized replicates. Initial AMOVA analyses were run assuming no regional genetic structure and again enforcing regional partitions according to groups identified by the distribution of unique clades recovered.

For each microsatellite locus, we calculated the observed number of alleles, observed heterozygosity, and expected heterozygosity for each population. We used Arlequin 3.11 (Excoffier et al. 2005) to test Hardy-Weinberg equilibrium and to test for linkage disequilibrium with likelihood-ratio tests. Significance was determined using sequential Bonferroni correction. We tested for scoring errors and null alleles at each locus using MicroChecker using the Brookfield 1 correction (van Oosterhout et al. 2004). Because evidence for null alleles was present (see Results), we completed all further analyses in three ways: (1) using all uncorrected data, (2) using only six

loci with null allele frequencies <20%, (3) using only four loci with null allele frequencies <10%, and (4) using a data set corrected for null alleles using FreeNA (Chapuis and Estoup 2007).

Genetic structure among populations was assessed in multiple ways. First, we used individual-based assignment with Structure 2.3.1 (Pritchard et al. 2000) using the admixture model with allele frequencies not correlated among populations and no prior information on population origin. This assignment method was performed using 200,000 iterations, the first 20,000 of which were discarded as burn-in. *K* values from 2 to 10 were each tested three times to check for convergence. To test the total number of genetic partitions, the *Delta K* method of Evanno et al. (2005) was used to determine the most appropriate *K* value. We also used Structure to estimate individual admixture proportions, *q* (i.e., the estimated proportion of an individual's genotype originating from each of the *K* populations), and their 90% posterior probability intervals. We repeated all analyses using the recessive alleles model in Structure to account for the possibility of null alleles in the dataset (Pritchard et al. 2009).

Second, we examined genetic structure using AMOVA (Excoffier et al. 1992) as implemented in Arlequin 3.11 with significance tested using 10,000 randomized replicates. AMOVA analyses were run assuming no regional genetic structure and again enforcing regional partitions according to groups identified by Structure.

Third, we calculated pairwise F_{ST} values in FSTAT 2.9.3.2 (Goudet 1995). Because high heterozygosities constrain F_{ST} values from achieving their theoretical maximum of 1 (Hedrick 1999), we also calculated G'_{ST} (Hedrick 2005), using SMOGD (Crawford 2009), which standardizes G_{ST} to the maximum possible value given the level of genetic diversity in the sample (Hedrick 2005). All subsequent analyses are performed with and without standardized measures of pairwise genetic differentiation.

Lastly, we tested for isolation by distance (IBD) across all populations and within each region identified by Structure. We compared matrices of pairwise genetic distance (F_{ST}, $F_{ST\text{-}ENA}$, G_{ST}, and G'_{ST} as described above) and geographic distance (km) via Mantel tests using Isolation by Distance Web Service 3.144 (Jensen et al. 2005) with significance determined from 10,000 random permutations. Geographic distance was measured as the shortest distance by sea between each pair of sampled locations using the ruler tool in Google Earth (http://earth.google.com/).

Because *T. crocea* can self-fertilize under laboratory conditions (Murakoshi and Hirata 1993) and self-fertilization could impact analyses of genetic structure, we investigated the possibility of self-fertilization in natural populations using the program RMES (David et al. 2007). We calculated the maximum likelihood estimate of the selfing rate, *s,* and compared this value to the corresponding value calculated from a constrained analysis ($s = 0$). The resulting test statistic [2*(lnl(unconstrained) – lnl(constrained)] was compared to the chi-squared distribution with one degree of freedom to determine significance (David et al. 2007). As a second estimate of inbreeding, we calculated F_{IS} across all loci and Weir and Cockerham's (1984) estimate of F_{IS} using FSTAT (Goudet 1995).

We directly compared estimates of genetic differentiation based on microsatellite and mtDNA COI data to (1) determine the degree of correspondence in results based on the two types of markers; and (2) determine the impact null alleles might have on analyses of the microsatellite dataset. Correlation between population pairwise values calculated from mtDNA sequence data (F_{ST}) and microsatellite loci (F_{ST}, $F_{ST\text{-}ENA}$, G_{ST}, and G'_{ST}) were assessed using a Mantel test with 1,000 permutations with all

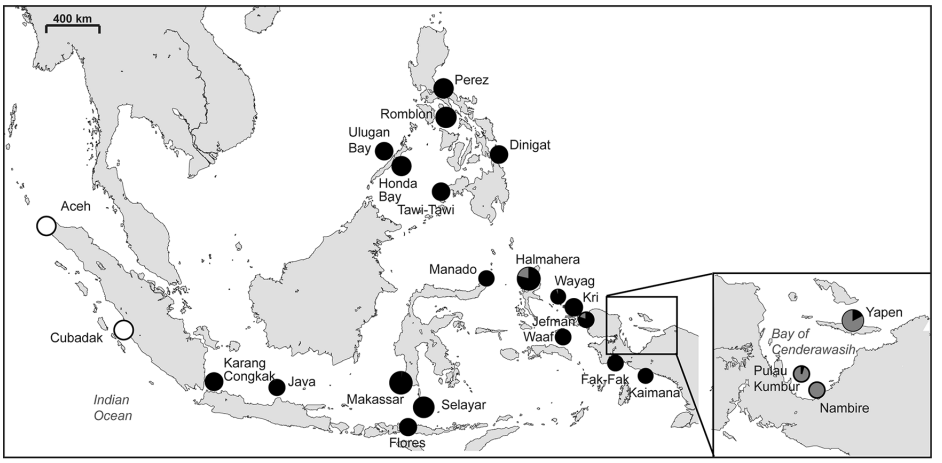

Figure 2. Map of the study area with pie diagrams representing the proportion of each mtDNA COI clade at each sampling site. The size of each circle reflects the number of individuals sampled from a population (e.g., 18 and 36 individuals in Manado and Makassar, respectively). Circle color corresponds to the percentage of each mtDNA COI clade found in the population (named black, grey, and white following DeBoer et al. 2008).

negative genetic distance values converted to zero. Only populations for which we had both mtDNA sequence data and microsatellite data were included in the analysis (n = 24).

Results

MtDNA Results.—We sequenced approximately 485 bp of COI from 618 individuals (251 unique haplotypes, GenBank Accession numbers HM187782–HM188392) in 24 populations across the Coral Triangle. All sequences aligned unambiguously without gaps and translated into protein. Neighbor-Joining analyses showed that all COI haplotypes from Indonesia and the Philippines unambiguously grouped into one of three highly divergent clades identified by DeBoer et al. (2008). The mtDNA clades defined three distinct phylogeographic regions: (1) Indian Ocean, (2) Central Indonesia and the Philippines, and (3) Bay of Cenderawasih (Fig. 2). Phylogenetic analyses showed the Bay of Cenderawasih was ancestral to Western Indonesia and Philippine/Central Indonesian populations (data not shown) and uncorrected sequence variation between these clades was 3.8% and 3.0%, respectively.

AMOVA analyses with no a priori groupings imposed showed strong genetic structure (Table 2); Φ_{ST} = 0.57 (P < 0.0001) AMOVA analyses of mtDNA COI indicate that regional variation is maximized by assigning populations to the three aforementioned regions (Φ_{CT} = 0.746, P < 0.00001). The mtDNA COI data show evidence of isolation by distance across all populations (r = 0.3866, P = 0.002) and within the central Indonesia and Philippines region (r = 0.3548, P < 0.001), but not within the Bay of Cenderawasih (r = 0.5073, P = 0.332), although limited numbers of populations in the later limit power to detect a significant relationship.

Microsatellite Genetic Diversity.—We genotyped 524 individuals at eight microsatellite loci from 27 populations across Indonesia and the Philippines (Fig. 1). The number of alleles per locus ranged from a minimum of three at Cubadak,

Table 2. Results from AMOVA analyses from mitochondrial COI DNA sequence data and six microsatellite loci. Analyses were run imposing no a priori population groupings and by enforcing three regional population groupings as described in the text. * $P < 0.00001$.

	Among populations within regions				Among regions				Within populations			
AMOVA analysis	df	Var	% Var	Φ_{ST}	df	Var	% Var	Φ_{CT}	df	Var	% Var	Φ_{SC}
mtDNA, no structure	23	3.18	57.39	0.570*	na	na	na	na	580	1,370.10	42.61	na
mtDNA, 3 groups	21	0.23	2.17	0.770*	2	7.85	74.62	0.750*	580	2.44	23.21	0.085*
msats, no structure	26	0.11	4.50	0.045*	na	na	na	na	1,021	2.43	95.50	na
msats 3 groups	24	0.06	2.09	0.088*	2	0.18	6.68	0.067*	1,021	2.43	91.23	0.022*

Sumatra for Locus Tc59 to a maximum of 20 alleles at Romblon, Philippines for Locus Tc92 (Table 3). There was little evidence of linkage disequilibrium between loci or within populations, with only 20 of 756 tests significant after sequential Bonferroni correction. All 20 significant comparisons involved locus Tc92, but the other putative "linked" locus varied among populations and no two loci were linked in a majority of localities.

Two loci showed significant departures from Hardy-Weinberg equilibrium (HWE; 25 populations for Tc04 and 26 populations for Tc92; Table 3) with an excess of homozygotes indicated in the majority of populations. These loci also had higher levels of missing data (9.2% and 5.7% missing data for Tc04 and Tc92, respectively), consistent with the possibility of null alleles. Frequency of null alleles (supplemental materials) was assessed using FreeNA (Chapuis and Estoup 2007). To determine the effect of null alleles data were analyzed using: (1) the entire data set including all eight loci, (2) excluding loci Tc04 and Tc92 where null allele frequency exceeded 20% (six total loci included), (3) excluding loci Tc04, Tc92, Tc074, and Tc160 where null allele frequencies exceeded 10% (four total loci include), and (4) on the entire data set corrected for null alleles using FreeNA (eight total loci included).

Comparison of Corrected and Uncorrected Microsatellite Data.—Pairwise F_{ST} values from the uncorrected data set were higher than the data set corrected for null alleles after converting negative genetic distances to zero (paired *t*-test: $P = 4.1E\text{-}19$; mean uncorrected $F_{ST} = 0.040$, mean corrected $F_{ST} = 0.036$ after converting negative values to zero). However, F_{ST} values from both data sets were highly correlated ($r = 0.994$, $P < 0.001$). Pairwise F_{ST} and standardized G'_{ST} were also highly correlated ($r = 0.958$, $P < 0.001$). As expected, standardized G'_{ST} values were greater than pairwise F_{ST} values (paired *t*-test: $P = 2.8E\text{-}49$; mean $G'_{ST} = 0.175$, mean $F_{ST} = 0.040$ after converting negative values to zero).

Population Genetic Structure and Individual Admixture from Microsatellite Data.—The *Delta K* test in Structure (Pritchard et al. 2009) indicated that $K = 3$ is the best supported number of genetic clusters to fit the data whether eight, six, or four loci are used or whether all eight loci are corrected for the presence of null alleles. Results indicate three groups corresponding to the Indian Ocean, Central Indonesia and Philippines, and the Bay of Cenderawasih (Fig. 3), consistent with results based on mtDNA COI sequence analyses. In contrast, the data were a poor fit to $K = 8$, a value predicted from Kochzius and Nuryanto (2008). Identical results were obtained from (1) all loci, (2) six loci, (3) four loci, and (3) from all loci corrected for null alleles were incorporated into the model employed

Table 3. Genetic diversity measures in 27 populations of *Tridacna crocea* at eight microsatellite loci, including number of alleles (A), observed heterozygosity (H_O), expected heterozygosity (H_E), *p*-value to reject Hardy-Weinberg equilibrium (significant values following sequential Bonferroni correction in **bold**; 216 comparisons, family-wise alpha = 0.05), and presence of null alleles as indicated by a departure from HWE.

		Locus								
Map	Population (*n*)	Tc04	Tc40	Tc50	Tc59	Tc74	Tc92	Tc160	Tc161	Mean
1	Aceh (20)									
	A	7	8	12	5	10	10	9	9	8.750
	Ho	0.0000	0.6000	0.8500	0.2000	0.3160	0.2630	0.6000	0.6500	0.435
	He	0.8700	0.8270	0.8600	0.4310	0.8580	0.8890	0.8180	0.7450	0.787
	P	**0.0000**	0.0028	0.0982	0.0023	**0.0000**	**0.0000**	0.0030	0.1191	
	null alleles	yes	yes	no	yes	yes	yes	yes	no	
2	Cubadak (14)									
	A	5	6	11	3	11	8	8	7	7.375
	Ho	0.0000	0.5710	0.8570	0.0000	0.6430	0.3080	0.4290	0.7860	0.449
	He	0.7160	0.7880	0.8940	0.2650	0.8570	0.8490	0.7800	0.8520	0.750
	P	**0.0000**	0.1386	0.3086	0.0013	0.0275	**0.0000**	0.0028	0.3802	
	null alleles	yes	no	no	yes	no	yes	yes	no	
3	Karang Congkak (21)									
	A	8	7	13	9	12	13	11	12	10.625
	Ho	0.4210	0.4290	0.7500	0.7140	0.5000	0.3330	0.6190	0.6670	0.554
	He	0.7620	0.5080	0.8920	0.8360	0.9000	0.9010	0.8850	0.9090	0.824
	P	**0.0000**	0.0918	0.0635	0.2346	**0.0000**	**0.0000**	0.0377	0.0115	
	null alleles	yes	no	no	no	yes	yes	yes	yes	
4	Java (17)									
	A	6	6	11	6	11	15	10	12	9.625
	Ho	0.3530	0.4710	0.8240	0.5880	0.5000	0.4380	0.6470	0.8240	0.580
	He	0.6560	0.5990	0.9140	0.7610	0.8690	0.9520	0.8650	0.9180	0.817
	P	**0.0002**	0.0489	0.4417	0.3331	**0.0000**	**0.0000**	0.0213	0.0495	
	null alleles	yes	no	no	no	yes	yes	yes	no	
5	Flores (20)									
	A	9	4	11	8	12	14	12	13	10.375
	Ho	0.2500	0.4500	0.9440	0.7370	0.7890	0.3500	0.7000	1.0000	0.653
	He	0.7620	0.6500	0.8840	0.8580	0.8960	0.9230	0.9170	0.9090	0.850
	P	**0.0000**	0.0934	0.1663	0.0255	0.2501	**0.0000**	0.0043	0.7501	
	null alleles	yes	no	no	no	no	yes	yes	no	
6	Makassar (14)									
	A	8	5	12	7	11	13	9	11	9.500
	Ho	0.3080	0.8570	0.8570	0.7860	0.4620	0.4290	0.5710	0.7860	0.632
	He	0.7940	0.7350	0.9050	0.8360	0.8980	0.9150	0.8920	0.8940	0.859
	P	**0.0001**	0.1245	0.2173	0.1058	0.0022	**0.0000**	0.0043	0.0500	
	null alleles	yes	no	no	no	yes	yes	yes	no	
7	Selayar (15)									
	A	9	5	10	8	12	12	11	12	9.875
	Ho	0.2670	0.6000	0.8570	0.8000	0.6670	0.0670	0.8670	0.9330	0.632
	He	0.7330	0.6370	0.8780	0.8570	0.9310	0.9310	0.8760	0.9240	0.846
	P	**0.0000**	0.5994	0.3397	0.3376	0.0246	**0.0000**	0.3800	0.9424	
	null alleles	yes	no	no	no	yes	yes	no	no	

Table 3. Continued.

Map	Population (*n*)	Locus								Mean
		Tc04	Tc40	Tc50	Tc59	Tc74	Tc92	Tc160	Tc161	
8	Halmahera (20)									
	A	11	11	14	10	14	14	8	13	11.875
	Ho	0.2940	0.6500	0.7000	0.5500	0.7370	0.3000	0.5500	0.7500	0.566
	He	0.8840	0.8820	0.8710	0.8970	0.9080	0.9240	0.8760	0.9190	0.895
	P	**0.0000**	0.0360	0.0593	**0.0000**	0.1227	**0.0000**	0.0031	0.0120	
	null alleles	yes	yes	no	yes	yes	yes	yes	no	
9	Manado (16)									
	A	7	4	11	9	11	9	11	11	9.125
	Ho	0.2500	0.7500	0.9380	0.6880	0.6880	0.2500	0.6250	0.9380	0.641
	He	0.7940	0.6230	0.8970	0.8750	0.9050	0.9090	0.8590	0.9050	0.846
	P	**0.0006**	0.4884	0.2968	0.0022	0.0010	**0.0000**	0.0023	0.4426	
	null alleles	yes	no	no	no	yes	yes	yes	no	
10	Waaf (17)									
	A	9	7	12	8	8	14	11	12	10.125
	Ho	0.2860	0.4710	0.8240	0.6470	0.7060	0.2670	0.4710	0.7650	0.554
	He	0.8650	0.6720	0.8540	0.7380	0.8470	0.9450	0.8910	0.9130	0.841
	P	**0.0000**	0.0137	0.1877	0.0120	0.0397	**0.0000**	**0.0000**	0.0017	
	null alleles	yes	no	no	no	no	yes	yes	no	
11	Fak-Fak (20)									
	A	10	5	7	8	12	19	11	13	10.625
	Ho	0.4710	0.7500	0.6320	0.8000	0.5000	0.3160	0.7500	0.7000	0.615
	He	0.7830	0.6910	0.7840	0.8670	0.9030	0.9600	0.9060	0.9010	0.849
	P	0.0050	0.9693	0.0501	0.3022	**0.0000**	**0.0000**	0.5576	0.0024	
	null alleles	yes	no	no	no	yes	yes	no	no	
12	Kaimana (18)									
	A	9	6	12	10	11	18	11	12	11.125
	Ho	0.5000	0.7220	0.9440	0.8330	0.7780	0.3330	0.5560	0.6670	0.667
	He	0.7640	0.6250	0.8950	0.8750	0.8710	0.9560	0.8560	0.9020	0.843
	P	0.0237	0.4246	0.7662	0.5929	0.5982	**0.0000**	0.0006	0.0042	
	null alleles	no	no	no	no	no	yes	yes	yes	
13	Jefman Is. (17)									
	A	9	5	11	7	12	14	13	13	10.500
	Ho	0.1330	0.4710	0.8240	0.5880	0.5880	0.3750	0.7650	0.6470	0.549
	He	0.8320	0.6200	0.8680	0.8290	0.9270	0.9380	0.8880	0.9180	0.852
	P	**0.0000**	0.1415	0.0697	0.0506	**0.0000**	**0.0000**	0.1810	0.0098	
	null alleles	yes	no	no	yes	yes	yes	no	yes	
14	Wayag (19)									
	A	9	5	10	7	10	12	12	12	9.625
	Ho	0.4440	0.3160	0.6840	0.7890	0.7220	0.6670	0.5790	0.6840	0.611
	He	0.8430	0.6190	0.7520	0.8590	0.7670	0.9170	0.8580	0.8950	0.814
	P	0.0012	0.0040	0.3385	0.5914	0.4500	0.0060	0.0030	0.0192	
	null alleles	yes	yes	no	no	no	no	yes	yes	

Table 3. Continued.

Map	Population (*n*)	Locus								Mean
		Tc04	Tc40	Tc50	Tc59	Tc74	Tc92	Tc160	Tc161	
15	Kri (17)									
	A	7	8	10	10	10	12	10	9	9.500
	Ho	0.2350	0.7060	0.8820	0.5880	0.5630	0.4000	0.5880	0.8000	0.595
	He	0.6060	0.7020	0.8630	0.8880	0.8190	0.9150	0.8790	0.8800	0.819
	P	0.0006	0.2997	0.6894	**0.0000**	**0.0000**	**0.0000**	0.0067	0.3424	
	null alleles	yes	no	no	yes	yes	yes	yes	no	
16	Perez (29)									
	A	9	7	10	10	14	18	11	14	11.625
	Ho	0.1740	0.4480	0.7930	0.6900	0.4480	0.3700	0.4480	0.7930	0.521
	He	0.5690	0.6870	0.8310	0.7910	0.9130	0.9110	0.8670	0.8930	0.808
	P	**0.0000**	0.0016	0.4780	0.0268	**0.0000**	**0.0000**	**0.0000**	0.0089	
	null alleles	yes	yes	no	no	yes	yes	yes	no	
17	Romblon (24)									
	A	11	10	13	9	13	20	14	12	12.750
	Ho	0.3750	0.7080	0.9580	0.7080	0.6670	0.3750	0.6960	0.7620	0.656
	He	0.8400	0.6990	0.8530	0.8590	0.9140	0.9530	0.9080	0.8980	0.866
	P	**0.0000**	0.5108	0.1954	0.1137	0.0240	**0.0000**	0.0196	0.3023	
	null alleles	yes	no	no	no	yes	yes	yes	no	
18	Carbin (19)									
	A	8	7	11	10	10	18	11	12	10.875
	Ho	0.2350	0.6320	0.6320	0.5790	0.6670	0.4210	0.7890	0.5560	0.564
	He	0.7500	0.7200	0.8850	0.8950	0.8700	0.9470	0.9020	0.9060	0.859
	P	**0.0000**	0.4770	0.0085	0.0043	0.0103	**0.0000**	0.0447	**0.0000**	
	null alleles	yes	no	yes	yes	no	yes	no	yes	
19	Campanga (16)									
	A	6	7	8	9	13	18	10	10	10.125
	Ho	0.3750	0.5000	0.5630	0.6880	0.7330	0.6250	0.5000	0.8670	0.606
	He	0.7220	0.6110	0.8290	0.8040	0.8870	0.9560	0.8970	0.8900	0.824
	P	0.0035	0.3250	0.3069	0.2614	0.0593	**0.0000**	0.0022	0.1786	
	null alleles	yes	no	yes	no	no	yes	yes	no	
20	Dinigat (24)									
	A	7	8	12	9	11	15	11	14	10.875
	Ho	0.2080	0.5830	0.7830	0.8330	0.5420	0.2860	0.7920	0.8750	0.613
	He	0.7420	0.6530	0.8970	0.8130	0.8690	0.9290	0.8990	0.9270	0.841
	p	**0.0000**	0.2531	0.0165	0.0647	**0.0000**	**0.0000**	0.5400	0.2530	
	null alleles	yes	no	no	no	yes	yes	no	no	
21	Tawi-Tawi (24)									
	A	4	6	9	11	9	15	11	9	9.250
	Ho	0.2500	0.6670	0.8330	0.5830	0.6000	0.3480	0.6250	0.7080	0.577
	He	0.5140	0.7080	0.8480	0.8190	0.8850	0.9290	0.8910	0.8800	0.809
	P	0.0005	0.6332	0.6346	0.0004	0.0048	**0.0000**	0.0009	0.0151	
	null alleles	yes	no	no	yes	yes	yes	yes	yes	

Table 3. Continued.

Map	Population (*n*)	Locus								Mean
		Tc04	Tc40	Tc50	Tc59	Tc74	Tc92	Tc160	Tc161	
22	Spratly (14)									
	A	8	4	12	7	11	15	10	9	9.500
	Ho	0.2860	0.5000	0.7690	0.5380	0.6150	0.5710	0.7140	0.9170	0.614
	He	0.6300	0.6800	0.8710	0.7630	0.9200	0.9520	0.8730	0.8770	0.821
	P	0.0087	0.1541	0.4595	0.0909	0.0024	**0.0000**	0.1522	0.5989	
	null alleles	yes	no	no	no	yes	yes	no	no	
23	Ulugan Bay (24)									
	A	4	5	9	10	13	18	10	13	10.250
	Ho	0.3480	0.3330	0.6520	0.7920	0.7080	0.3750	0.6520	0.9580	0.602
	He	0.4060	0.4100	0.8550	0.8430	0.8550	0.9180	0.8080	0.8970	0.749
	P	0.3760	0.6411	0.0149	0.3657	0.0623	**0.0000**	0.0297	0.8945	
	null alleles	no	no	yes	no	no	yes	no	no	
24	Honda Bay (22)									
	A	12	8	9	9	12	15	12	14	11.375
	Ho	0.3180	0.7730	0.9520	0.5910	0.6360	0.2380	0.8180	0.9090	0.654
	He	0.7900	0.7060	0.8760	0.7860	0.9110	0.9410	0.8770	0.9090	0.850
	P	**0.0000**	0.3492	0.7913	0.0500	0.0014	**0.0000**	0.4722	0.6268	
	null alleles	yes	no	no	yes	yes	yes	no	no	
25	Pulau Kumbur (21)									
	A	11	8	7	11	8	16	10	14	10.625
	Ho	0.5240	0.5240	0.9050	0.6670	0.4760	0.4000	0.7620	0.7620	0.627
	He	0.8970	0.8400	0.8150	0.8860	0.7380	0.9120	0.8660	0.9290	0.860
	P	**0.0000**	0.0055	0.8216	0.0594	0.0161	**0.0000**	0.1057	0.1106	
	null alleles	yes	yes	no	yes	yes	yes	no	yes	
26	Nambire (21)									
	A	13	7	6	9	7	18	10	12	10.250
	Ho	0.6840	0.7620	0.8570	0.5240	0.4290	0.2860	0.6670	0.8100	0.627
	He	0.9230	0.8150	0.7800	0.8690	0.7020	0.9410	0.8790	0.9110	0.852
	P	0.0152	0.5393	0.6974	0.0006	**0.0000**	**0.0000**	0.0293	0.0185	
	null alleles	yes	no	no	yes	yes	yes	yes	no	
27	Yapen (21)									
	A	14	10	9	8	7	18	6	14	10.750
	Ho	0.4760	0.8570	0.5710	0.7620	0.5000	0.2630	0.5710	0.8100	0.601
	He	0.9180	0.8500	0.8290	0.8390	0.7650	0.9540	0.8160	0.9200	0.861
	P	**0.0000**	0.3553	0.0015	0.0300	0.0015	**0.0000**	0.1272	0.3581	
	null alleles	yes	no	yes	no	yes	yes	yes	no	

by Structure. While results indicate three distinct genetic partitions, Central Indonesia and Philippine populations show some evidence of different genetic composition. Structure results indicate that one genetic cluster (blue, Fig. 3) dominates the Philippines and most of Central Indonesia, although some Central Indonesian populations have a chaotic assignment of ancestral populations. This result was not improved by rerunning analyses with only Central Indonesian and Philippines populations and a *K* value of 2 (data not shown). These results suggest the occurrence of marked genetic structuring into three regions across the study area, and relative but not complete genetic homogeneity in Central Indonesia and the Philippines.

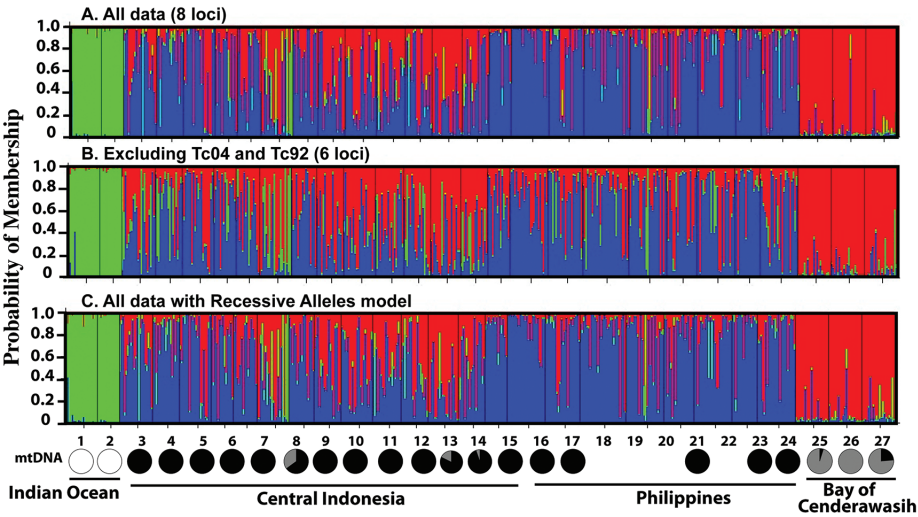

Figure 3. Bar plot showing Bayesian assignment of individuals to three ancestral clusters ($K = 3$) using Structure 2.3.1 (Pritchard et al. 2000). Each bar represents the estimated admixture coefficient (q) from each inferred population for each individual. Population numbers correspond to Table 1. Results based on three versions of the data are similar: (A) entire uncorrected dataset with eight loci, (B) dataset excluding loci Tc04 and Tc092 due to heterozygote deficiencies, and (C) entire uncorrected data set analyzed using the Recessive Alleles model in Structure to account for putative null alleles. Pie charts at bottom indicate the frequency of each of the three mtDNA clades.

AMOVA analyses could not be performed including loci Tc04 and Tc92 due to the level of missing data (>5%; Excoffier et al. 2005); therefore, all AMOVA analyses are based on the uncorrected data set excluding Tc04 and Tc92. AMOVA analysis with no a priori regional structure indicates weak but significant genetic partitioning in the data set with $F_{ST} = 0.045$ ($P < 0.00001$). Enforcing the regional partitions (Indian Ocean, Central Indonesia and the Philippines, and the Bay of Cenderawasih) from Structure and mtDNA COI yielded $F_{CT} = 0.067$ ($P < 0.00001$) and 6.7% of the variation explained by variation among groups, 2.1% of the variation among populations within groups and 91.2% of the variation within populations.

To test for additional genetic structuring within each of the three regions above, we examined pairwise F_{ST} values calculated from the uncorrected and corrected microsatellite data. There was no evidence of genetic differentiation between the two Indian Ocean populations, nor between the three populations in the Bay of Cenderawasih (Table 4). In contrast, several Philippine populations are significantly differentiated from those in Central Indonesia. Within the Philippines, the far western populations of Honda Bay and Ulugan Bay are significantly differentiated from each other, but no other populations within the country show evidence of differentiation (Table 4). Results based on pairwise G'_{ST} were similar (significance determined by examining the lower bound of 99% confidence intervals, Table 4).

There is a significant pattern of Isolation by Distance (IBD) over all populations ($r = 0.5902$, $P < 0.001$) for all eight loci. Significant IBD was also observed in the central Indonesian and Philippine region ($r = 0.3808$, $P < 0.001$; Fig. 4) and within the Bay of Cenderawasih region ($r = 0.5883$, $P < 0.001$). Analyses excluding microstatellite loci with >20% or >10% null alleles and or including all eight alleles corrected for

Table 4. Pairwise F_{ST} (top) and G'_{ST} (bottom) values based on all eight microsatellite loci. Values significantly different than zero are in **bold** following Bonferroni correction (F_{ST} based on 351 comparisons, family-wise alpha = 0.05) or 99% confidence intervals (G'_{ST}).

	Indian Ocean		Central region (central Indonesia and Philippines)																					Bay of Cenderawasih		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1. Aceh	-	0.023	**0.118**	**0.110**	**0.108**	**0.102**	**0.099**	**0.118**	**0.135**	**0.170**	**0.108**	**0.135**	**0.104**	**0.107**	**0.092**	**0.122**	**0.110**	**0.081**	**0.121**	**0.110**	**0.087**	**0.100**	**0.106**	**0.107**	**0.096**	**0.105**	**0.092**
2. Cubadak	0.058	-	**0.136**	**0.123**	**0.131**	**0.128**	**0.109**	**0.135**	**0.162**	**0.202**	**0.131**	**0.156**	**0.129**	**0.134**	**0.111**	**0.141**	**0.128**	**0.105**	**0.152**	**0.146**	**0.108**	**0.130**	**0.130**	**0.123**	**0.123**	**0.124**	**0.115**
3. Karang Congkak	**0.552**	**0.467**	-	0.003	0.023	0.012	0.021	0.015	0.011	**0.070**	**0.041**	**0.023**	0.047	0.025	0.051	0.036	0.041	0.024	**0.032**	0.028	0.009	0.008	**0.021**	0.020	**0.061**	**0.062**	**0.064**
4. Java	**0.583**	**0.501**	**0.050**	-	0.023	0.004	0.019	0.014	0.011	**0.064**	0.021	0.011	0.042	0.019	**0.045**	0.026	0.030	**0.035**	**0.038**	0.027	0.014	0.011	0.016	0.012	**0.074**	**0.078**	**0.067**
5. Flores	**0.578**	**0.598**	**0.113**	**0.125**	-	0.000	0.001	0.008	0.011	0.030	0.013	0.007	0.017	0.000	0.012	0.021	0.012	0.021	0.022	0.011	0.002	0.010	0.002	0.013	**0.058**	**0.051**	**0.047**
6. Selayar	**0.566**	**0.622**	0.075	0.022	0.001	-	0.000	0.003	0.003	0.026	0.010	0.001	0.013	0.000	0.010	0.015	0.016	0.013	0.010	0.004	0.000	0.002	0.000	0.007	**0.043**	**0.043**	**0.038**
7. Makassar	**0.568**	**0.534**	**0.085**	**0.133**	0.022	0.000	-	0.013	0.012	**0.048**	0.007	0.011	0.013	0.000	0.011	0.016	0.012	0.015	0.031	0.024	0.003	0.022	0.005	0.011	**0.056**	**0.050**	**0.051**
8. Manado	**0.663**	**0.623**	**0.082**	0.069	0.038	0.019	**0.099**	-	0.011	0.047	0.016	0.010	0.022	0.002	0.027	0.030	0.018	**0.023**	0.029	0.026	0.004	0.004	0.002	0.002	**0.054**	**0.058**	**0.050**
9. Spratly	**0.678**	**0.699**	0.049	**0.077**	0.060	0.009	**0.068**	**0.087**	-	0.026	0.015	0.000	0.025	0.002	0.021	0.011	0.011	0.034	0.043	0.022	0.011	0.007	0.006	0.024	**0.066**	**0.070**	**0.069**
10. Ulugan Bay	**0.615**	**0.718**	**0.152**	**0.114**	0.066	0.029	**0.170**	**0.108**	0.045	-	**0.024**	0.022	0.027	0.017	0.016	0.025	0.020	**0.073**	**0.074**	**0.057**	0.053	0.052	0.037	**0.069**	**0.108**	**0.112**	**0.101**
11. Honda Bay	**0.634**	**0.699**	**0.165**	0.037	**0.073**	0.023	0.032	**0.072**	**0.082**	0.077	-	0.008	0.001	0.000	0.000	0.009	0.000	**0.027**	0.047	0.038	**0.030**	0.018	0.013	0.023	**0.063**	**0.062**	**0.053**
12. Tawi-Tawi	**0.637**	**0.618**	0.088	0.044	0.037	0.019	0.059	**0.061**	0.011	0.046	0.038	-	0.021	0.000	0.020	0.015	0.009	0.028	0.033	**0.023**	0.017	0.011	0.002	0.016	**0.066**	**0.070**	**0.066**
13. Romblon	**0.577**	**0.632**	**0.135**	**0.114**	0.069	0.007	0.063	0.080	**0.085**	0.073	0.008	0.065	-	0.001	0.007	0.019	0.003	**0.026**	0.039	0.040	0.024	0.020	0.008	0.035	**0.052**	**0.050**	**0.043**
14. Carbin	**0.640**	**0.695**	0.084	0.059	0.007	0.005	0.012	0.014	0.005	0.036	0.000	0.003	0.018	-	0.000	0.003	0.000	0.016	0.034	0.016	0.014	0.005	0.001	0.009	**0.049**	**0.049**	**0.043**
15. Dinigat	**0.475**	**0.505**	**0.136**	0.105	0.026	0.009	0.031	0.076	**0.073**	0.032	0.013	**0.079**	0.041	0.003	-	0.009	0.000	**0.039**	**0.052**	0.025	0.025	0.025	0.014	0.032	**0.064**	**0.066**	**0.055**
16. Perez	**0.586**	**0.628**	**0.129**	**0.102**	**0.101**	0.054	0.081	**0.130**	0.026	**0.091**	0.044	0.049	0.065	0.016	**0.062**	-	0.002	**0.050**	**0.059**	0.026	**0.034**	0.028	0.012	0.030	**0.075**	**0.077**	**0.070**
17. Campanga	**0.567**	**0.579**	0.099	0.054	**0.079**	**0.088**	0.049	0.058	0.039	0.069	0.002	0.037	0.027	0.000	0.009	0.010	-	**0.045**	0.055	0.043	0.031	0.019	0.018	0.036	**0.075**	**0.074**	**0.068**
18. Halmahera	**0.485**	**0.541**	**0.127**	0.188	**0.107**	0.045	**0.108**	**0.146**	**0.225**	**0.152**	**0.159**	**0.086**	**0.126**	0.068	**0.164**	**0.262**	**0.248**	-	0.022	0.025	0.007	0.015	0.011	0.014	0.017	0.014	**0.022**
19. Wayag	**0.558**	**0.647**	**0.175**	**0.170**	0.078	0.047	**0.147**	**0.112**	**0.260**	**0.193**	**0.192**	**0.189**	**0.097**	**0.146**	**0.154**	**0.304**	**0.252**	**0.140**	-	0.024	0.008	0.010	0.007	0.021	**0.037**	0.024	0.024
20. Kri	**0.489**	**0.618**	**0.115**	0.110	0.040	0.017	0.071	0.099	**0.123**	0.110	**0.169**	**0.094**	0.098	0.051	0.025	**0.111**	**0.152**	**0.097**	0.064	-	0.006	0.006	0.001	0.011	**0.052**	**0.061**	**0.048**
21. Jefman	**0.442**	**0.456**	**0.082**	**0.119**	**0.038**	0.002	**0.056**	0.038	0.061	0.067	**0.136**	**0.111**	0.033	0.069	0.026	**0.176**	**0.108**	**0.077**	0.045	0.037	-	0.000	0.000	0.003	**0.038**	**0.035**	0.031
22. Waaf	**0.504**	**0.572**	**0.037**	**0.051**	0.071	0.015	0.109	0.034	**0.054**	**0.132**	**0.100**	0.055	0.056	0.041	**0.050**	**0.092**	**0.062**	0.108	**0.079**	0.010	0.018	-	0.000	0.010	0.032	0.034	0.031
23. Fak-Fak	**0.567**	**0.617**	0.129	0.079	**0.029**	0.001	**0.054**	0.030	0.057	**0.111**	**0.101**	**0.016**	0.010	0.018	0.046	0.063	0.110	**0.056**	**0.046**	0.014	0.002	0.001	--	0.000	**0.038**	**0.035**	0.027
24. Kaimana	**0.585**	**0.509**	**0.143**	**0.083**	**0.074**	0.047	0.061	0.007	**0.112**	**0.180**	**0.069**	**0.060**	**0.100**	0.026	0.048	**0.150**	**0.139**	**0.075**	0.060	0.031	0.028	0.054	**0.003**		**0.048**	**0.054**	**0.047**
25. Pulau Kumbur	**0.343**	**0.443**	**0.245**	**0.340**	**0.285**	**0.152**	**0.271**	**0.255**	**0.348**	**0.328**	**0.306**	**0.260**	**0.227**	**0.213**	**0.241**	**0.256**	**0.321**	**0.102**	**0.199**	**0.173**	**0.174**	**0.095**	0.168	**0.153**	-	0.000	0.000
26. Nambire	**0.424**	**0.536**	**0.311**	**0.349**	**0.219**	**0.174**	**0.212**	**0.350**	**0.294**	**0.311**	**0.269**	**0.223**	**0.205**	**0.160**	**0.223**	**0.242**	**0.269**	**0.091**	**0.134**	**0.200**	**0.184**	**0.167**	**0.151**	**0.234**	0.012	-	0.000
27. Yapen	**0.437**	**0.551**	**0.400**	**0.284**	**0.243**	**0.172**	**0.297**	**0.301**	**0.342**	**0.316**	**0.259**	**0.262**	**0.204**	**0.201**	**0.239**	**0.294**	**0.335**	**0.186**	**0.128**	**0.176**	**0.193**	**0.160**	**0.093**	**0.184**	0.013	0.000	-

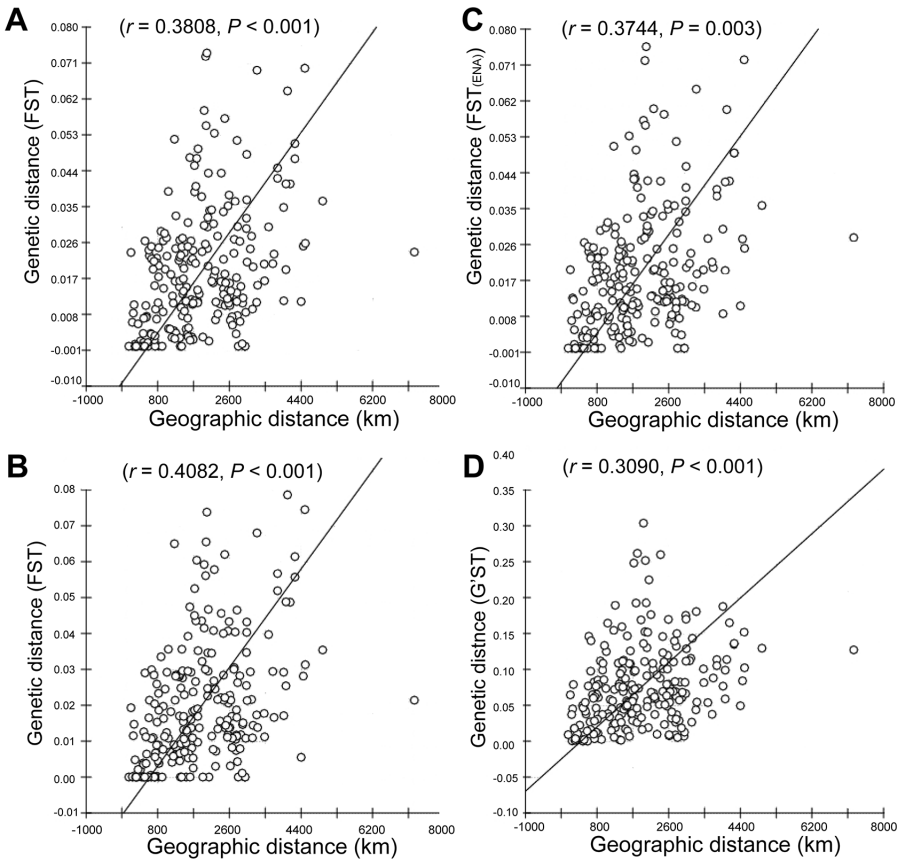

Figure 4. Isolation by distance results for central Indonesia and Philippine populations (n = 22) based on (A) F_{ST} calculated from all eight microsatellite loci, (B) F_{ST} calculated from dataset excluding loci Tc04 and Tc92 because of putative null alleles, (C) $F_{ST(ENA)}$ calculated from the dataset corrected for putative null alleles, and (D) G'_{ST} calculated from the entire uncorrected data set. All data partitions show evidence of isolation by distance (all $P < 0.003$).

null alleles gave equivalent results (results not shown). Results from G'_{ST} showed IBD over all populations (r = 0.6136, $P < 0.001$) and within the central Indonesian and Philippine region (r = 0.3090, $P < 0.001$), but the pattern was not significant for the Bay of Cenderawasih region (r = −0.3155, P = 0.470). These results are consistent with those of the Structure analysis, which did not consistently assign individuals from central region populations to a single ancestral population (see below).

Individual admixture analysis indicates the absence of admixture in Indian Ocean and Bay of Cenderawasih populations (see Online Appendices, Fig. 3), similar to regional mtDNA patterns. However, individuals with "black clade" mtDNA showed high levels of nuclear admixture with the "grey clade" populations. Gene flow appears unidirectional in this case, flowing from eastern into central areas, but not vice versa, as grey clade individuals in the Bay of Cenderawasih do not appear admixed. Individuals with "white clade" mtDNA haplotypes show no evidence of admixture in nuclear loci (Fig. 3). Using only individuals from populations where "black" and "grey" mtDNA clades occur in sympatry (Halmahera, Wayag, Jefman Island, Pulau

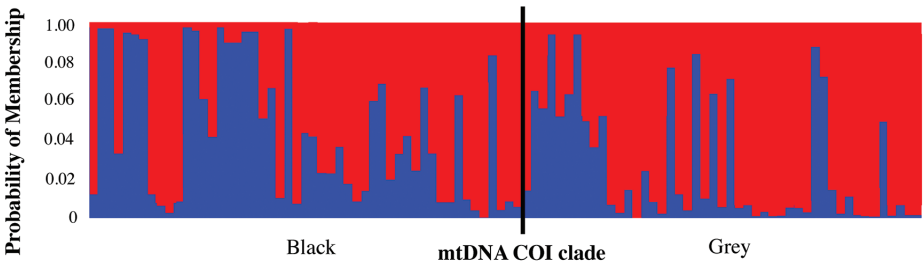

Figure 5. Bar plots showing Bayesian assignment of individuals from populations where multiple mtDNA COI clades occur in sympatry (Halmahera, Wayag, Jefman, Pulau Kumbur, and Yapen) using Structure 2.3.1 and two clusters ($K = 2$).

Kumbur, and Yapen), Structure analyses revealed that individual microsatellite genotypes do not correspond directly to mtDNA clade (Fig. 5).

Comparison of mtDNA and Microsatellite Data.—Pairwise F_{ST} values calculated from mtDNA COI sequence data were substantially greater (Table 5) than those calculated using microsatellite loci (F_{ST}, $_{FST(ENA)}$, G_{ST}, and G'_{ST}; paired t-test: all $P < 1.9E\text{-}24$), but were significantly positively correlated (F_{ST}, $_{FST(ENA)}$, G_{ST}, and G'_{ST}, all $r > 0.72$, all $P < 0.001$), suggesting that these metrics are recovering similar patterns of population differentiation. The highest correlation was between COI mtDNA F_{ST} and the standardized microsatellite distance measure, G'_{ST} ($r = 0.7732$).

Inbreeding.—Because high rates of self-fertilization can result in heterozygote deficiencies independent of the presence of null alleles, we did not use the corrected data set in estimates of selfing rate. Results indicate that only Makassar, Spratly Islands, and Yapen populations showed significant levels of selfing ($\chi^2_{crit,0.05} = 3.841$; all $\chi^2 > 6.21$, all $P < 0.05$; Table 6) with s estimated at 0.24, 0.26, and 0.27 in each population, respectively. We repeated these analyses excluding loci Tc04 and Tc92 with similar results: four populations showing selfing rates that differed significantly from zero [$0.16 < s < 0.26$ for Makassar, Spratly, Fak-Fak, and Yapen; all $\chi^2 > 5.12$, all $P < 0.05$]. Estimates of F_{IS} for each population were moderate, averaging 0.295, ranging from a minimum of 0.199 in Ulugan Bay to a maximum of 0.456 in Aceh, when all loci are included.

Discussion

Population Structure and Cryptic Species.—Data from mtDNA COI indicate pronounced regional structure ($F_{CT} = 0.746$, $P < 0.00001$) in *T. crocea* across Indonesia and the Philippines, expanding previous studies by DeBoer et al. (2008) and Kochzius and Nuryanto (2008). Results confirm the presence of three highly differentiated phylogeographic regions corresponding to populations from Western Indonesia, Central Indonesia and Eastern Indonesia (DeBoer et al. 2008), and similar genetic partitioning of marine populations across the Coral Triangle has been reported in stomatopods (Barber et al. 2000, 2006, 2011), seastars (Crandall et al. 2008), snails (Reid et al. 2006), a variety of fish (Perrin and Borsa 2001, Lourie et al. 2005, Timm et al. 2008, Drew and Barber 2009, Ackiss et al. 2013, Jackson et al. 2014), and other marine species (see Barber et al. 2011, Carpenter et al. 2011 for

Table 5. Pairwise F_{ST} values based on all mtDNA cytochrome oxidase 1 C (subunit 1) sequences. Values significantly different than zero are in **bold** following Bonferroni correction (276 comparisons, family-wise alpha = 0.05).

	Indian Ocean		Central region (central Indonesia and the Philippines)																			Bay of Cenderawasih		
	1	2	3	4	5	6	7	8	10	11	12	13	15	16	18	19	20	21	22	23	24	25	26	27
1. Aceh	-																							
2. Cubadak	0.027	-																						
3. Karang Congkak	**0.117**	**0.133**	-																					
4. Java	**0.139**	**0.154**	0.000	-																				
5. Flores	**0.065**	**0.081**	0.095	0.130	-																			
6. Selayar	**0.116**	**0.131**	**0.157**	**0.192**	0.000	-																		
7. Makassar	**0.050**	**0.065**	0.064	0.077	0.022	0.061	-																	
8. Manado	**0.071**	**0.087**	0.123	**0.148**	0.064	**0.108**	0.022	-																
10. Ulugan Bay	**0.069**	**0.085**	**0.130**	**0.154**	**0.083**	**0.129**	0.049	0.053	-															
11. Honda Bay	**0.040**	**0.055**	0.070	0.088	0.038	**0.085**	0.006	0.007	0.036	-														
12. Tawi-Tawi	**0.067**	**0.083**	0.070	0.080	0.053	0.107	0.018	0.015	0.067	0.020	-													
13. Romblon	**0.064**	**0.080**	**0.117**	**0.141**	**0.078**	**0.128**	**0.052**	0.051	**0.072**	0.007	**0.078**	-												
15. Dinigat	**0.090**	**0.106**	**0.144**	**0.179**	**0.105**	**0.154**	**0.083**	0.063	**0.091**	0.022	**0.101**	0.000	-											
16. Perez	**0.051**	**0.066**	**0.098**	**0.122**	**0.062**	**0.114**	**0.043**	0.051	**0.062**	0.004	**0.061**	0.005	0.007	-										
18. Halmahera	**0.034**	**0.050**	0.060	0.073	0.024	0.070	0.004	0.027	**0.044**	0.003	0.018	**0.042**	**0.070**	**0.028**	-									
19. Wayag	**0.042**	0.058	0.085	0.112	0.000	0.022	0.012	0.051	**0.060**	0.020	0.043	0.055	0.082	0.039	0.003	-								
20. Kri	**0.049**	**0.065**	0.064	0.085	0.000	0.025	0.005	0.047	**0.062**	0.017	0.032	**0.060**	**0.082**	0.038	0.004	0.000	-							
21. Jefman	**0.039**	**0.055**	0.066	0.082	0.031	0.078	0.005	0.023	0.042	0.007	0.012	0.052	0.075	0.035	0.000	0.004	0.009	-						
22. Waaf	**0.047**	**0.063**	0.066	0.091	0.008	0.044	0.001	0.020	0.048	0.000	0.018	0.034	0.049	0.027	0.004	0.002	0.000	0.004	-					
23. Fak-Fak	**0.055**	**0.071**	0.097	0.125	0.000	0.012	0.020	0.062	**0.073**	0.031	0.050	0.068	**0.095**	**0.052**	0.018	0.000	0.000	0.025	0.000	-				
24. Kaimana	**0.080**	**0.096**	0.019	0.017	0.047	0.093	0.029	0.093	**0.099**	0.043	0.037	**0.093**	**0.121**	0.069	0.023	0.033	0.015	0.031	0.028	0.042	-			
25. Pulau Kumbur	**0.077**	**0.093**	**0.144**	**0.168**	**0.092**	**0.143**	**0.075**	**0.098**	**0.095**	**0.065**	**0.094**	**0.090**	**0.117**	**0.076**	0.045	0.053	**0.075**	0.018	**0.073**	**0.081**	**0.107**	-		
26. Nambire	**0.071**	**0.087**	**0.139**	**0.162**	**0.085**	**0.137**	**0.069**	**0.091**	**0.089**	**0.059**	**0.087**	**0.084**	**0.111**	**0.070**	0.037	0.053	**0.069**	0.032	**0.067**	**0.075**	**0.101**	0.016	-	
27. Yapen	**0.060**	**0.076**	**0.113**	**0.134**	0.063	**0.110**	**0.051**	**0.078**	**0.078**	**0.045**	**0.070**	**0.073**	**0.099**	**0.058**	0.022	0.031	0.047	0.007	0.048	0.054	0.075	0.000	0.002	-

Table 6. Maximum likelihood estimates of selfing rate (s) and inbreeding coefficient (F_{IS}) based on all eight microsatellite loci, and with two loci excluded due to null alleles (Tc04 and Tc92). Selfing rates significantly greater than zero are in **bold** ($\chi^2_{(crit)} = 3.841$).

Population	All loci			Tc04 and Tc92 excluded		
	delta ln likelihood	s	F_{IS}	delta ln likelihood	s	F_{IS}
1. Pulau Weh, Aceh	1.556	0.109	0.456	1.529	0.109	0.312
2. Cubadak, Sumatra	–0.074	0.006	0.412	–0.079	0.009	0.354
3. Karang Congkak, Pulau Seribu	2.887	0.188	0.333	1.671	0.134	0.250
4. Karimunjawa, Java	3.277	0.183	0.296	2.571	0.166	0.225
5. Sabolan Kecil, Flores	–0.097	0.007	0.237	–0.096	0.007	0.108
6. Barang Lompo, Makassar	6.218	0.239	0.272	5.126	0.230	0.156
7. Selayar, South Sulawesi	–0.079	0.009	0.259	–0.079	0.008	0.074
8. Halmahera, Maluku	0.013	0.083	0.374	–0.090	0.027	0.270
9. Manado, North Sulawesi	–0.082	0.013	0.250	–0.082	0.008	0.075
10. Waaf, West Papua	–0.009	0.053	0.348	–0.087	0.012	0.215
11. Fak-Fak, West Papua	3.714	0.122	0.282	5.332	0.155	0.175
12. Kaimana, West Papua	2.705	0.125	0.214	2.777	0.128	0.094
13. Jefman Is., West Papua	0.063	0.039	0.364	0.088	0.010	0.236
14. Wayag, West Papua	1.074	0.116	0.255	0.957	0.106	0.217
15. Kri, West Papua	–0.089	0.011	0.280	–0.088	0.008	0.178
16. Perez, Quezon	1.942	0.124	0.360	1.171	0.090	0.274
17. Romblon, MIMAROPA	2.958	0.166	0.246	1.801	0.119	0.118
18. Carbin, Visaya	–0.068	0.026	0.350	-0.093	0.008	0.256
19. Camanga, E. Samar	–0.072	0.023	0.271	0.312	0.095	0.220
20. Dinigat, Dingat	–0.102	0.006	0.276	–0.100	0.005	0.129
21. Tawi-Tawi, Mindanao	–0.095	0.011	0.292	–0.103	0.007	0.200
22. Spratly Islands	7.289	0.257	0.259	5.503	0.240	0.196
23. Ulugan Bay, Palawan	3.282	0.148	0.199	2.385	0.126	0.132
24. Honda Bay, Palawan	2.295	0.151	0.234	1.773	0.120	0.074
25. Pulau Kumbur, Teluk Cenderawasih	1.879	0.127	0.276	1.688	0.115	0.198
26. Nambire, Teluk Cenderawasih	2.454	0.140	0.269	1.968	0.128	0.187
27. Yapen, Teluk Cenderawasih	8.614	0.266	0.307	6.839	0.257	0.197

reviews). However, our expanded data set here also reveals a strong genetic affinity between the Philippines and Central Indonesia (Fig. 2), although these populations are not genetically homogeneous (Table 5).

Bayesian cluster analyses based on new data from eight microsatellite loci showed the presence of three genetic clusters (K = 3). The geographic distribution of these three clusters mirrored results from mtDNA. Although Structure analyses were run using a variety of K values, there was no support for K = 8 as suggested by the eight defined "clades" from Kochzius and Nuryanto (2008). However, pair-wise F_{ST} and G_{ST} values indicate do indicate the presence of additional genetic structure among populations in the three phylogeographic groups, so genetic structure is not limited to that among the three phylogeographic regions. While patterns from microsatellite data mirrored mtDNA, the increased variability of the microsatellite data resulted in AMOVA values (F_{CT} = 0.067 with 6.7% variation between the three regions, P < 0.0001) that were substantially smaller than those recovered from mtDNA (above).

Congruent phylogeographic patterns obtained from mtDNA and unlinked nuclear microsatellite data indicate that regional genetic structure in *T. crocea* across Indonesia and the Philippines almost certainly results from neutral processes rather than processes like selective sweeps, which would be unlikely to affect both mtDNA and multiple nuclear loci. Similarly, the broad concordance phylogeographic patterns in *T. crocea* to a wide range of marine taxa (above) indicate that these patterns are likely the result of a common response to a shared physical environment and/or historic processes (Avise 2000, Schneider et al. 2000, Argoblast and Kenagy 2001). Divergence between Indian Ocean populations (e.g., Western Indonesia) and populations to the east are frequently hypothesized to result from Pleistocene vicariance (e.g., Lavery et al. 1996, Duda and Palumbi 1999, Barber et al. 2000, Benzie et al. 2002). Divergence between Central and Eastern Indonesia has explain either by the isolating effects of the Halmahera eddy (e.g., Barber et al. 2006), and/or habitat differences (e.g., Reid et al. 2006).

While the three phylogeographic regions observed in *T. crocea* likely result from the processes above, the genetic affinities between clam populations from the Philippines and Central Indonesia may result from the Indonesian Throughflow (e.g., Knittweis et al. 2009) or the Sulu Sea Throughflow. The Indonesian Throughflow moves up to 19 million m^3 of water per second from northeast to southwest between these two areas (Gordon and Fine 1996, fig. 1 in Gordon 2005), providing an obvious physical oceanographic mechanism to facilitate larval dispersal among these regions. Strong connectivity between the Philippines and Central Indonesia is predicted by coupled biophysical larval dispersal models (Kool et al. 2011). This model also predicts the isolation of western and eastern Indonesian reef environments, providing additional support for the role of physical oceanography in shaping the observed genetic patterns. Thus, while historical process may contribute to the observed patterns of genetic structure, they may also result or be reinforced by more recent physical oceanography.

While there is broad concordance between phylogeographic patterns observed in *T. crocea* and other co-distributed species in the Coral Triangle, there are also key differences. In *T. crocea,* Central Indonesian haplotypes extend to Western Java with Western Indonesian haplotypes occurring only in Sumatra, matching exactly the pattern seen in the red bellied fusilier, *Caesio cuning* Bloch, 1791 (Ackiss et al. 2013). In contrast, species like the stomatopod *Haptosquilla pulchella* Miers, 1880, Western Indonesia haplotypes extend as far as east as Komodo (Barber et al. 2002). These differences could result from different recolonization histories as populations re-established following the end of the last glacial maxima and flooding of the Sunda Shelf (Crandall et al. 2011). The final land bridge between the Sunda Islands and Kalimantan went through the island of Belitung off the northwest coast of Java (Sathiamurthy and Voris 2006). Species that colonized the Sunda Shelf early would have expanded their ranges from Central Indonesia towards Western Java before Indian Ocean haplotypes could arrive from the west; later colonizing species, in contrast, could recruit from the Indian Ocean. Alternatively, variation in the location of phylogeographic breaks in Western Indonesia could result from different processes acting in their formation. For example, patterns in some taxa could be driven by recolonization history, while others are driven by larval advection.

Similarly, significant variation in the location of phylogeographic breaks is also observed in Eastern Indonesia. In *T. crocea* the divergence between Eastern and

Central Indonesian populations is focus largely in Cenderawasih Bay. Populations there are dominated by one mtDNA clade and these populations also form a cluster in Structure analyses. While the isolation of populations in Cenderawasih Bay is reported in other marine species ranging from seastars (Crandall et al. 2008) to mantis shrimp (Barber et al. 2006) and other tridacnids (Nuryanto and Kochzius 2009), in other species the genetic break in Eastern Indonesia occurs around the island of Halmahera. For example, in the mantis shrimp *Siamosquilla laevecaudata* Sun and Yang, 1998, Eastern Indonesian haplotypes extend exactly to Halmahera then are replaced by Central Indonesian haplotypes (Barber et al. 2011). Coupled biophysical models (Kool et al. 2011) predict isolation of populations on either side of the Maluku Sea (e.g., between Sulawesi and Halmahera), and some mtDNA haplotpyes from Eastern Indonesian populations of *T. crocea* extend to the island of Halmahera, suggesting that physical oceanography likely plays a role in the observed patterns. However, the dominance of Eastern haplotypes in Cenderawasih Bay may result from repeated isolation over the past 5 My due to a combination of tectonic blockages of the mouth of the bay and closure during times of low sea level (Allen and Erdmann 2006). The unique signature of Cenderawasih Bay populations could also result from environmental differences. DeBoer et al. (2012) showed that *Tridacna* clams have significantly different *Symbiodinium* diversity inside and outside of Cenderawasih Bay suggesting that genetic patterns could be influenced by environmental conditions as well. More detailed studies combining genetics, physical oceanography, and environmental variation will be required to determine why phylogeographic patterns are broadly concordant yet vary in the exact location of regional genetic breaks.

Cryptic Species.—Beginning with the application of genetics to the study of marine species and populations, studies have increasingly identified cryptic taxa (Knowlton 1993, 2000), including studies within the Coral Triangle (Barber and Boyce 2005). Based on the deep divergences among mtDNA lineages in *T. crocea,* DeBoer et al. (2008) suggested that this species could be comprised of three cryptic taxa, a result that would have major impacts on our view of the conservation status of this taxon. While our extended mtDNA analyses and new microsatellite analyses both confirmed the presence of only three genetic partitions in both data sets, these partitions were not completely concordant. Both markers indicated that *T. crocea* populations from Western Indonesia (Sumatra) and Eastern Indonesia (Cenderawasih Bay) form unique genetic partitions. However, while results from mtDNA showed only a single mtDNA clade in populations from the Philippines and Indonesia, microsatellite data instead showed these populations are an admixture of individuals from representing two different genetic clusters, one from Central Indonesia and the Philippines (blue cluster, Fig. 3), and the other from Eastern Indonesia (red cluster, Fig. 3). Additionally, a few individuals with Central Indonesia/Philippines haplotypes had a nuclear genotype that assigned to Western Indonesia. It is possible that these patterns could result from incomplete lineage sorting in a species with high effective population size, minimizing the loss of genetic diversity due to genetic drift. However, a more likely explanation is that while the phylogeographic groupings highlighted by mtDNA indicate that lineage divergence is taking place across the range of *T. crocea,* this divergence has not yet resulted in speciation and the formation of reproductive barriers that isolate these lineages. Regional admixture is discussed in more detail below.

Novel Insights from Microsatellite Data.—Phylogeographic patterns from mtDNA and multilocus nuclear microsatellite loci were highly concordant. AMOVA analyses on both markers partition genetic variation from into three groups across the study area, although *F*-statistics calculated from microsatellites were substantially smaller than those recovered from mtDNA. Pairwise genetic distance estimates based on both marker types are significantly positively correlated (COI F_{ST} vs microsatellite G'_{ST}: $r = 0.739$, $P = 1.8E\text{-}43$) and both data sets show evidence of IBD across all populations and within the central Indonesian/Philippine region (all populations: COI $r = 0.3866$, microsatellite $r = 0.6136$; central populations: COI $r = 0.3548$, microsatellite $r = 0.3090$; all $P < 0.003$). These results indicate that both markers are effective at recovering patterns of population differentiation, although mtDNA had more power to detect population structure. While this result reaffirms the utility of mtDNA as an effective marker of population history, microsatellite data do provide some novel insights not apparent from mtDNA alone, particularly in detecting admixture.

Data from mtDNA COI indicates that Philippine and Indonesian populations are genetically similar, being comprised of haplotypes from a single clade but show modest structure between these regions (average pairwise $F_{ST} = 0.103$) indicating that they are not homogeneous. Analyses from Structure provide additional insights into this pattern by showing that populations from the Philippine and parts of Central Indonesia contain almost exclusively individuals with a "central" signature (blue cluster, Fig. 3), but many Central Indonesian populations exhibit a high degree of admixture between the central (blue) and eastern (red) clusters (Fig. 3). The directional movement of the Indonesian Throughflow from the Philippines to Indonesia may allow genetic diversity from the Philippines to accumulate in populations in Central Indonesia, while maintaining a relatively pure genetic signature in the Philippines. The presence of Eastern Indonesian genotypes in Central Indonesian populations may result from transport from Eastern Indonesia into central or due to incomplete lineage sorting. Nearly 90% of Indonesian Throughflow waters originate from the North Equatorial Current and the Philippines (Nof 1995), but the remainder come from the New Guinea Coastal Current, which travels north and west along the island of Papua, toward the center of Indonesia (Fig. 1), potentially accounting for the presence of eastern (red) genotypes in the central region.

Self-fertilization and Effects of Heterozygote Deficiencies.—Most studies employing microsatellite loci check their data for deviations from HWE (reviewed in Selkoe and Toonen 2006) and heterozygote deficits are common in marine populations (Ayer and Hughes 2000). Although reported for a wide range of taxa, some groups have a particularly high frequency of departures from HWE including marine mollusks (Li et al. 2003, Astanei et al. 2005) and hard corals (Ayer and Hughes 2000, Gilmour 2002, Mackenzie et al. 2004, Whitaker 2004, Maier et al. 2005, Nishikawa and Sakai 2005, Underwood et al. 2007). The exact mechanism(s) generating these deficits is unknown (Zouros and Foltz 1984), although common explanations include null alleles, Wahlund effects, or self-fertilization.

Multilocus microsatellite data from *T. crocea* populations across Indonesia and the Philippines revealed a strong deviation from HWE expectations with an excess of homozygotes. Analysis in FreeNA and MICROCHECKER suggested the presence of null alleles. There was also evidence for a significant proportion of self-fertilization

(24%–27%) in three populations (Makassar, Spratly, and Yapen); self-fertilization was known previously only to occur when forced in laboratory conditions (Murakoshi and Hirata 1993) because giant clams have sequential release of gametes (Lucas 1988), reducing opportunity for self-fertilization.

While deviation from HWE expectations are frequently interpreted as evidence of problems with the microsatellite loci, deviations from HWE may be more likely to represent real biological phenomena in marine ecosystems. The sessile nature of breeding adults and highly restricted dispersal of many marine species can legitimately lead to non-random mating (e.g., Underwood et al. 2007, Ridgway et al. 2008) resulting in heterozygote deficiencies. Other HWE assumptions, including that of non-overlapping generations, are routinely violated in many long-lived species, including *T. crocea*. Sampling individuals from overlapping generations results in the inadvertent grouping of individuals whose allele distributions represent different generations and therefore the derived population allele frequencies should not necessarily be expected to conform to HWE.

While we found significant heterozygote deficiencies in two of eight microsatellite loci in *T. crocea* as well as evidence for self-fertilization, analyses performed with and without the loci showing departures from HWE produced similar results. Similarly analyses that explicitly corrected for putative null alleles also yielded the same results. Combined these results indicate that HWE departures did not qualitatively affect our results. Further, the concordant patterns in microsatellite and mtDNA provide strong evidence that the observed heterozygote deficiencies are likely not the result of selection, mutation, or linkage as these datasets represent two independent genomes. Instead, the deficiencies likely reflect the fact that sessile broadcast spawners are most likely to mate with close neighbors, resulting in a legitimate departure from the HWE assumption of complete random mating. Because the vast majority of marine animals exhibit a bipartite life history consisting of a sessile adult phase and a dispersive pelagic larval phase (Scheltema 1986) this may be widespread phenomenon in marine taxa.

Conservation Implications.—Understanding connectivity is critical to marine conservation planning because identifying routes of larval dispersal, or barriers to dispersal, enables better reserve network design (Sale et al. 2005). Results from both mtDNA and microsatellites show strong genetic structure among populations of *T. crocea* populations from Western Indonesia, Central Indonesia/Philippines, and Eastern Indonesia, indicating minimal genetic connectivity among these regions. While limited connectivity in *T. crocea* may result from sea level fluctuations, physical oceanography or environmental differences, the lack of genetic connectivity among these regions demonstrates a clear lack of demographic connectivity (Hedgecock et al. 2007). As such the three phylogeographic regions have to be assumed to be both evolutionarily and demographically independent and should be managed as independent units.

The significant F_{ST} and G_{ST} values and patterns of isolation by distance indicate that even within the three phylogeographic regions, genetic connectivity—and therefore ecological connectivity (Hedgecock et al. 2007)—are limited DeBoer et al. (2008) compared the slope of isolation by distance to simulations by Palumbi (2003) to estimate that the average dispersal distance in *T. crocea* was only 50 km. A similar

comparison based on the microsatellite isolation by distance plot in this study suggests that the average dispersal distance may be as low as 25 km.

While our results highlight the importance of isolation among the three primary phylogeographic regions, examining F_{ST} and Allelic Diversity measures of this data set in a Marxan framework (Beger et al. 2014) resulted in conservation prioritization that is more evenly distributed around the sampling region. In particular, this analysis highlighted additional regional conservation priorities in the Central Philippines, the Sangihe Talaud archipelago, and Indian Ocean. This result shows the versatility genetic data in spatially explicit conservation prioritization models.

Genetic patterns also highlight the need for international cooperation in management planning in the Coral Triangle. Admixture observed in many Central Indonesian populations indicate that Philippine populations of *T. crocea* may be an important source of genetic diversity while some Central Indonesian populations may be genetic diversity sinks. As genetic diversity is essential to adaptive potential, the resilience of Central Indonesian reefs may depend both on the demographic contributions as well as the accumulation of diversity from the Philippines and Eastern Indonesia, highlighting the need for cooperative management planning. Expanding sampling into other regions of the Coral Triangle may indicate even more need for such international cooperation.

Another important result for conservation comes from the comparison of patterns in mtDNA and microsatellites. As new molecular markers become available, it becomes fashionable to highlight the limitations of existing tools (see Bowen et al. 2014 for review). While mtDNA does have limitations as a single locus molecular marker, more often than not mtDNA and multilocus nDNA studies obtain the same results (see Zink and Barrowclough 2008), although microsatellites data can provide unique insights as in the present study. However, from a conservation perspective, it is important to note that mtDNA was able to provide most of the critical information about regional differentiation at a fraction of the time and cost.

Most of the world's biodiversity exists in developing countries that cannot afford the high costs of developing microsatellites, SNPs, or next generation sequencing methods (Willette et al. 2014). However, due to increasing restrictions on research in biodiversity rich countries (Pethiyagoda 2004), biodiversity research in regions like the Coral Triangle will fall increasingly on local scientists using local capacity and local funding (see Barber et al. 2014). Studies that suggest that mtDNA is "the worst marker" by selectively highlighting exceptional cases of mtDNA evolution while ignoring the thousands of studies where mtDNA provides powerful insights into the evolutionary process (Galtier et al. 2009) have promoted the belief that results from mtDNA alone cannot be trusted. As such, many journal editorial policies now preclude publication of mtDNA only data sets, disincentivizing molecular ecology research by scientists in countries that are biodiversity rich but infrastructure and funding limited. However, when applied properly mtDNA is still a very valuable tool for phylogeogeographic studies, particularly in developing biodiversity rich countries and for large-scale comparative studies (Bowen et al. 2014) and can be very valuable in supporting conservation planning. Given the growing biodiversity crisis (Ehrlich and Pringle 2008, Butchart et al. 2010) conservation goals may often be better served by data obtained from cheap, fast, and easy methods like mtDNA sequencing rather than using the most expensive and sophisticated tools available.

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