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Pronounced genetic structure in a highly mobile coral reef fish, *Caesio cuning*, in the Coral Triangle

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ABSTRACT: The redbelly yellowtail fusilier *Caesio cuning* has a tropical Indo-West Pacific range that straddles the Coral Triangle, a region of dynamic geological history and the highest marine biodiversity on the planet. Previous genetic studies in the Coral Triangle indicate the presence of multiple limits to connectivity. However, these studies have focused almost exclusively on benthic, reef-dwelling species. Schooling, reef-associated fusiliers (Perciformes: Caesionidae) account for a sizable portion of the annual reef catch in the Coral Triangle, yet to date, there have been no in-depth studies on the population structure of fusiliers or other mid-water, reef-associated planktivores across this region. We evaluated the genetic population structure of *C. cuning* using a 382 bp segment of the mitochondrial control region amplified from over 620 fish sampled from 33 localities across the Philippines and Indonesia. Phylogeographic analysis showed that individuals sampled from sites in western Sumatra belong to a distinct Indian Ocean lineage, resulting in pronounced regional structure between western Sumatra and the rest of the Coral Triangle (Φ_{CT} = 0.4796, $p < 0.004$). We found additional significant population structure between central Southeast Asia and eastern Indonesia (Φ_{CT} = 0.0450, $p < 0.001$). These data in conjunction with spatial analyses indicate that there are 2 major lineages of *C. cuning* and at least 3 distinct management units across the region. The location of genetic breaks as well as the distribution of divergent haplotypes across our sampling range suggests that current oceanographic patterns could be contributing to observed patterns of structure.

KEY WORDS: Connectivity · Gene flow · Isolation by distance · Coral reef fish · Artisanal fisheries · Coral Triangle

INTRODUCTION

The concentration of marine biodiversity in the Coral Triangle poses both biogeographical questions and management challenges. Straddling the Indo-Malay-Philippine Archipelago and extending eastward to the Solomon Islands, the Coral Triangle is home to the highest diversity of marine organisms in the world (Briggs 1995, Carpenter & Springer 2005, Veron et al. 2009). Coral reef habitat in this region is extensive and complex, rivaling the Great Barrier Reef in area and spanning well over 25 000 islands.

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During the Pleistocene epoch (~2.5 million to 12 thousand years ago), repeated glaciations caused radical changes to the regional geography as the Sunda and Sahul Shelves repeatedly rose above and fell below the surface of the water (Voris 2000). The exposure of these shelves significantly narrowed the gateway between the tropical Indian and Pacific Oceans, and sea level fluctuations during this epoch have been implicated in numerous studies as a driver of regional population differentiation and speciation across this region (e.g. Springer & Williams 1990, Mcmillan & Palumbi 1995, Barber et al. 2006, Crandall et al. 2008a,b, Vogler et al. 2008). At more recent timescales, oceanographic processes have been implicated in creating and maintaining genetic structure within this region. In particular, the Mindanao and Halmahera eddies, created at the convergence point of the Northern Equatorial Current and the New Guinea Coastal Current, have been hypothesized to limit larval dispersal and isolate populations across the Maluku sea (Barber et al. 2006, 2011, Kool et al. 2011).

Identifying regions of limited connectivity in species that span the Coral Triangle can lead to insights into the stock structure of fisheries for management as well as mechanisms promoting lineage divergence in this region. Molecular techniques are particularly useful in highlighting regions where gene exchange does not occur (Hedgecock et al. 2007). Recent reviews indicate the presence of several genetic breaks shared by multiple species across this region, demonstrating that distinct geophysical processes can promote population structure and even lineage divergence within the Coral Triangle (Barber et al. 2011, Carpenter et al. 2011). However, to date the vast majority of reef species showing pronounced genetic structure across the Coral Triangle have been demersal, such as clams, stomatopods, seastars, gastropods and clownfish (Barber et al. 2006, Crandall et al. 2008a,b, Deboer et al. 2008, Timm & Kochzius 2008, Nuryanto & Kochzius 2009). In contrast, relatively understudied near-shore pelagics give mixed results. The round scad mackerel *Decapterus macrosoma* shows very little genetic structure (Borsa 2003), while its congener *D. russelli* shows up to 3 genetically structured populations (Rohfritsch & Borsa 2005).

Unfortunately, the diversity that makes the Coral Triangle an area of evolutionary and biogeographic interest is vulnerable. The region is a hotspot for coral reef threats (Roberts et al. 2002, Nañola et al. 2011). As the human population in this region increases annually by an estimated 1 to 2% (US Census Bureau 2011), anthropogenic pressures on coral reef resources continue to rise. Coastal reefs are easily exploitable resources, and reef fish and invertebrates are important sources of food and livelihood in the coastal communities of Southeast Asia (McManus et al. 1992, McManus 1997). Informed management of coral reef ecosystems is a priority for the conservation and sustainability of coral reef resources in the coming decades.

The most accepted strategy for improving the biomass and abundance of reef organisms is the establishment of marine reserves (Roberts & Polunin 1991, Russ & Alcala 1996, Sale 2006). Because dispersive larvae are the primary means of demographic and genetic connectivity among most populations, understanding patterns of larval dispersal has been identified as one of the most critical components in developing effective reserve networks (Sale et al. 2005). Although genetic connectivity is not equivalent to demographic connectivity, genetic methods can be of use in guiding conservation planning in marine ecosystems (Palumbi 2003). By identifying regions that are genetically and demographically independent, conservation planners can partition large marine ecosystems into smaller, more tractable management areas for which networks of marine reserves can be designed (Green & Mous 2008). This approach has been specifically proposed as a management mechanism in the Coral Triangle (Carpenter et al. 2011).

Schooling, reef-associated fusiliers (Perciformes: Caesionidae) are planktivores found feeding at the reef face and account for a sizable portion of harvested reef species in the Coral Triangle. They are caught via a variety of gear, including hand-lines, fish traps, trawls, drive-in nets and gill nets (Carpenter 1988). In the Philippines alone, the annual catch of caesionids in commercial and municipal fisheries is ~22 000 t (BAS 2010), but given the artisanal nature of most reef fisheries in this region, these catch data are likely greatly underestimated (Alcala & Russ 2002).

The redbelly yellowtail fusilier *Caesio cuning* (Bloch 1791) is a caesionid commonly found in local markets across the Coral Triangle. It is a conspicuous mid-water member of Indo-Pacific reef ecosystems with a distribution that ranges from southern Japan to northern Australia and from Vanuatu to Sri Lanka. *C. cuning* are schooling, broadcast spawners, but beyond this, little is known about the larval ecology of the species. There are no known differences between the life-history strategies of males and females to suggest sex-biased dispersal. The closest relative with a known pelagic larval duration (PLD) is

Pterocaesio chrysozona with an estimated PLD of 37 to 47 d (Doherty et al. 1995), and there is no evidence to suggest strong larval behavior, such as homing (Leis & Carson-Ewart 2003), that may limit dispersal potential. As adults, *C. cuning* are highly mobile members of the coral reef ecosystem. While they can also be captured in trawls over soft-bottom environments (Carpenter 1988), the extent of their movement remains unknown. *C. cuning* and other fusiliers have been observed sleeping in crevices and holes in the reef structure; however, their level of fidelity to such shelter sites and individual reefs is unclear. The mobility of larval *C. cuning*, coupled with their dependence on reef structure for shelter and undefined movement as adults, suggests a varied spectrum of dispersal potential.

The purpose of the present study is to assess the regional genetic connectivity and lineage divergence in *Caesio cuning* to address 2 questions: (1) are midwater, reef-associated planktivores impacted by the same barriers we see in demersal species or do they exhibit the panmixia found in near-shore pelagics, and (2) if limitations to dispersal in *C. cuning* are present, can we identify distinct geographic stocks to aid in the management of fusiliers?

MATERIALS AND METHODS

We collected 630 *Caesio cuning* samples from fish markets or by spear while SCUBA diving or snorkeling from 33 localities in the Coral Triangle (Fig. 1). Only samples that were confirmed as being caught on nearby reefs were collected from local markets. Tissue samples were taken from the pectoral or caudal fin base and preserved in 95% ethanol.

DNA amplification and sequencing reactions were conducted at Boston University, the University of the Philippines Marine Science Institute, De La Salle University and Udayana University. Whole genomic DNA was extracted using a 10% Chelex (Biorad) solution (Walsh et al. 1991). A 382 bp region of the mitochondrial D-loop was amplified via PCR using the forward and reverse primers CR-A and CR-E (Lee et al. 1995). PCR reactions were conducted in a 25 µl reaction consisting of 1 µl DNA extraction, 2.5 µl of 10× buffer, 2 µl $MgCl_2$ (25 mM), 2.5 µl dNTPs (8 mM), 1.25 µl of each 10 µM primer, 1 µl of template and 0.625 U of AmpliTaq (Applied Biosystems). Manual hot-start thermocycling parameters were employed as follows: initial hold at 80°C, denaturation 94°C (1 min), main cycle 94°C (30 s), either 50 or

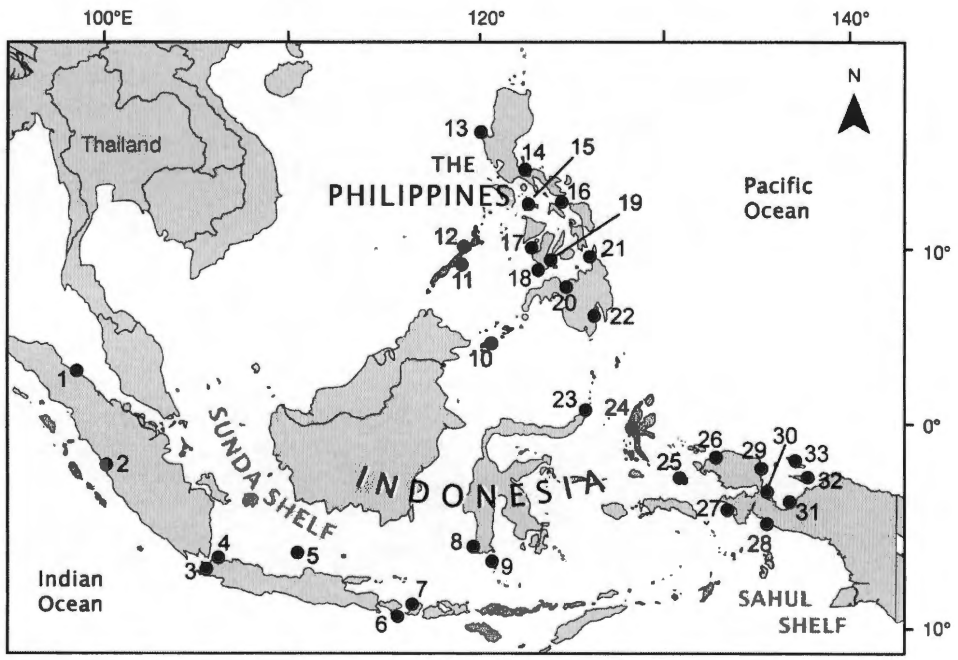

Fig. 1. Sampling localities of the present study: Medan (1), Padang (2), Anyer (3), Seribu (4), Karimunjawa (5), Bali (6), Lombok (7), Makassar (8), Selayar (9), Tawi Tawi (10), Honda Bay (11), Ulugan Bay (12), Bolinao (13), Perez (14), Romblon (15), Sorsogon (16), Guimaras (17), Negros Occidental (18), Negros Oriental (19), Balingasag (20), Dinagat (21), Davao (22), Manado (23), Halmahera (24), Raja Ampat (25), Sorong (26), Fak Fak (27), Kaimana (28), Manokwari (29), Windesi (30), Karei (31), Yapen (32), Biak (33)

52°C (30 s) and 72°C (40 s) for 39 cycles, then a final extension of 72°C (7 to 10 min).

PCR products were electrophoresed on a 1% agarose gel and visualized with ethidium bromide or SYBR® Green staining. Successful PCR reactions were enzymatically prepared for sequencing by mixing 5 µl of PCR product with 0.5 U of Shrimp Alkaline Phosphatase and 5 U of Exonuclease and incubating for 30 min at 37°C followed by 15 min at 80°C. Forward and reverse sequencing reactions were performed with Big Dye terminator chemistry and run on an ABI 3730 automated DNA Sequencer (Applied Biosystems). Forward and reverse sequences were proofread in Sequencher™ v4.7 (Gene Codes), and all resulting 382 bp fragments were aligned with ClustalX v2.0.12. The online toolkit FaBox (Villesen 2007) was used to reduce our final alignment to unique haplotypes and create an input file for the population genetics data-analysis program Arlequin v3.5.12 (Excoffier & Lischer 2010).

The species identity of our sampled haplotypes was confirmed with a neighbor-joining tree run in PAUP* (Swofford 2003) that included the 3 most closely related species found across our sampling range as outgroups: *Caesio lunaris*, *Caesio teres* and *Caesio xanthonota*. We examined the frequencies and phylogenetic relatedness of haplotypes in our dataset with a median-joining minimum spanning tree generated in NETWORK v4.6 (Bandelt et al. 1999).

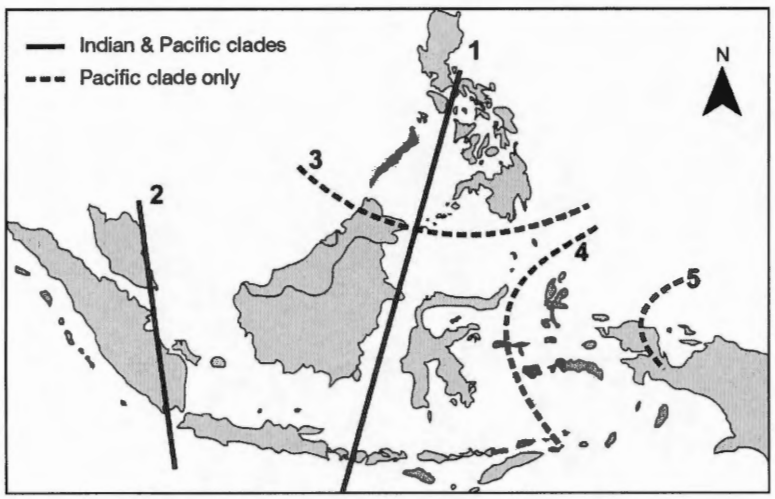

Fig. 2. AMOVA hypotheses. Lines indicate the approximate locations of regional genetic breaks found in the mtDNA of other well-sampled coral reef and near-reef species across the Coral Triangle (see Table 2). Solid lines indicate partitions tested with a hierarchical AMOVA that included sites from both the Indian and Pacific clades; dashed lines indicate partitions tested within the Pacific clade only

For each locality, we used Arlequin and DnaSP v5 (Librado & Rozas 2009) to calculate standard genetic diversity indices and tested the null hypothesis of neutrality in the mitochondrial control region using Fu's F_S and Fu and Li's D^* tests, with significance determined by 1000 simulations of a neutral coalescent model. We employed the latter 2 statistics to evaluate the potential effects of selection and demographic processes, such as population expansion, on our data (Fu 1997).

To investigate the presence of barriers to dispersal and gene flow, we employed both *a priori* and post hoc analyses. We first performed an analysis of molecular variance (AMOVA) and examined population pairwise Φ_{ST} in Arlequin. We then used the AMOVA framework to group sampling localities and test for hierarchical population structure within our dataset following *a priori* hypotheses based on previously measured phylogeographic breaks (Fig. 2, see Table 2) as follows: absence of genetic structure, restricted gene flow east and west of the Makassar strait, a Sunda Shelf break at western Sumatra, the Philippines vs. Indonesia, east vs. west of the Maluku Sea, and a break at Cenderawasih Bay in Papua. All AMOVAs were run using sites with $n \geq 15$ and employed the Tamura & Nei (1993) model of evolution, which was the model in Arlequin most equivalent to the best model for our dataset determined by jModelTest v1.0 (Posada 2008, Guindon & Gascuel 2003), the general time-reversible model (Tavaré 1986). The significance of pairwise Φ_{ST} as well as among- and within-population variance in the AMOVA framework was calculated using >30 000 random permutations of the dataset. The p-values for multiple pairwise comparisons were corrected using Benjamini & Hochberg's (1995) false discovery rate.

In addition, we employed a post hoc spatial analysis of the pairwise Φ_{ST} matrix generated in Arlequin using the program BARRIER v2.2 (Manni et al. 2004). BARRIER characterizes the spatial relationship of sites from their GPS coordinates using Voronoi tessellation and Delaunay triangulation and applies Monmonier's maximum difference algorithm to a matrix of genetic distances (Φ_{ST} in this case) to identify genetic barriers across geographic space. We tested the robustness of barriers by

resampling individuals within populations with replacement using Excel and creating 100 bootstrapped replicates of our pairwise Φ_{ST} matrix in Arlequin.

Since discrete genetic breaks can bias the results of analyses of isolation by distance (IBD) and the presence of IBD can generate false positives in analyses of hierarchical structure (AMOVA) (Meirmans 2012), we employed partial Mantel tests that controlled for both optimal AMOVA clusters and geographic distance using the 'vegan' package for R (Oksanen et al. 2012, R Development Core Team 2012). Pairwise genetic distances (Φ_{ST}) among localities with $n > 15$ were imported from Arlequin, and negative pairwise Φ_{ST} values, a result of within-population variance exceeding among population variance, were set to zero. A geographic distance matrix was generated using a previously developed Python script that calculates shortest distance over water from the GPS points of sample sites (Etherington 2011) in ArcGIS 9.3. We created a third distance matrix that reflected the hierarchical structure of our best AMOVA grouping by using 0 to code for localities within the same group and 1 to code for localities in different groups. We first tested for significant correlations between genetic and geographic distance using AMOVA group membership as a covariate. We then tested the correlation between genetic distance and AMOVA grouping using geographic distance as a covariate. Significance was tested with 10 000 random permutations, and the relationships among distances and clusters were plotted.

RESULTS

A total of 625 fish were successfully sequenced at the mitochondrial control region, representing 20 study sites across Indonesia and 13 study sites in the Philippines. When aligned, 129 sites over the amplified 382 bp were polymorphic. There were 393 haplotypes, 308 of which were unique to a single individual. The haplotype present at the highest frequency was shared by 18 individuals.

Phylogenetic relatedness

The unweighted mean pairwise difference between haplotypes in our minimum spanning tree was 11.090 bp. All haplotypes from Medan and Padang, with the exception of a single individual from Padang, fell within a divergent clade separated from all other haplotypes by 8 mutational steps (Fig. 3a,b). A single individual sampled at Makassar, Sulawesi, also fell within this divergent Indian Ocean clade. Regional clustering within the Pacific lineage showed some evidence that the distribution of haplotypes was non-random.

Population structure

Haplotype diversity was high, measuring at or near 1 for all localities (Table 1). Our 2 sites from Sumatra—Medan and Padang—had lower nucleotide diversity (both 0.017 ± 0.009) compared to all other sites, which had nucleotide diversities ranging from 0.0242 to 0.0356. While high haplotype diversity and low nucleotide diversity could be an indication of recent population expansion, neither of these sites had significantly negative values for Fu's F_S (Table 1). Across all sampled localities, there were only 2 significant values for Fu and Li's D^*, which is more sensitive to the effects of background selection (Fu 1997). However, Fu's F_S, which is more sensitive to signatures of demographic expansion and genetic hitchhiking, was significantly negative at 11 of 13 sites in the Philippines and 14 of 20 sites in Indonesia, indicating that the departures from neutrality can be ascribed to 1 of these 2 processes (Fu 1997).

The results of a non-hierarchical AMOVA show significant genetic structuring in *Caesio cuning* across the Coral Triangle (Table 2; $\Phi_{ST} = 0.1421$, $p < 0.001$). Pairwise Φ_{ST} values calculated for each pair of sampling localities indicate that Medan and Padang are significantly different from all sites but each other, and sites from eastern Indonesia appear more genetically similar to each other than most other sites (see Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/m480p185_supp.xlsx).

A spatial analysis of our pairwise Φ_{ST} matrix in BARRIER indicates that the genetic variance among sites can be partitioned geographically. Bootstrapping analyses reached their highest confidence values when parameters were set to 4 barriers across the entire dataset (where $n \geq 15$). A barrier between the polygon space of Medan and Padang and all other sites is always the first to be placed by BARRIER and carries unanimous bootstrap support (1.00) regardless of number of designated barriers (Fig. 4; Barrier a). The second barrier is found in the region of Halmahera and the Maluku Sea, which carries the next highest confidence values (0.78 to 0.80; Fig. 4; Barrier b). The third barrier was complex and found in the Philippines, with the most supported divisions

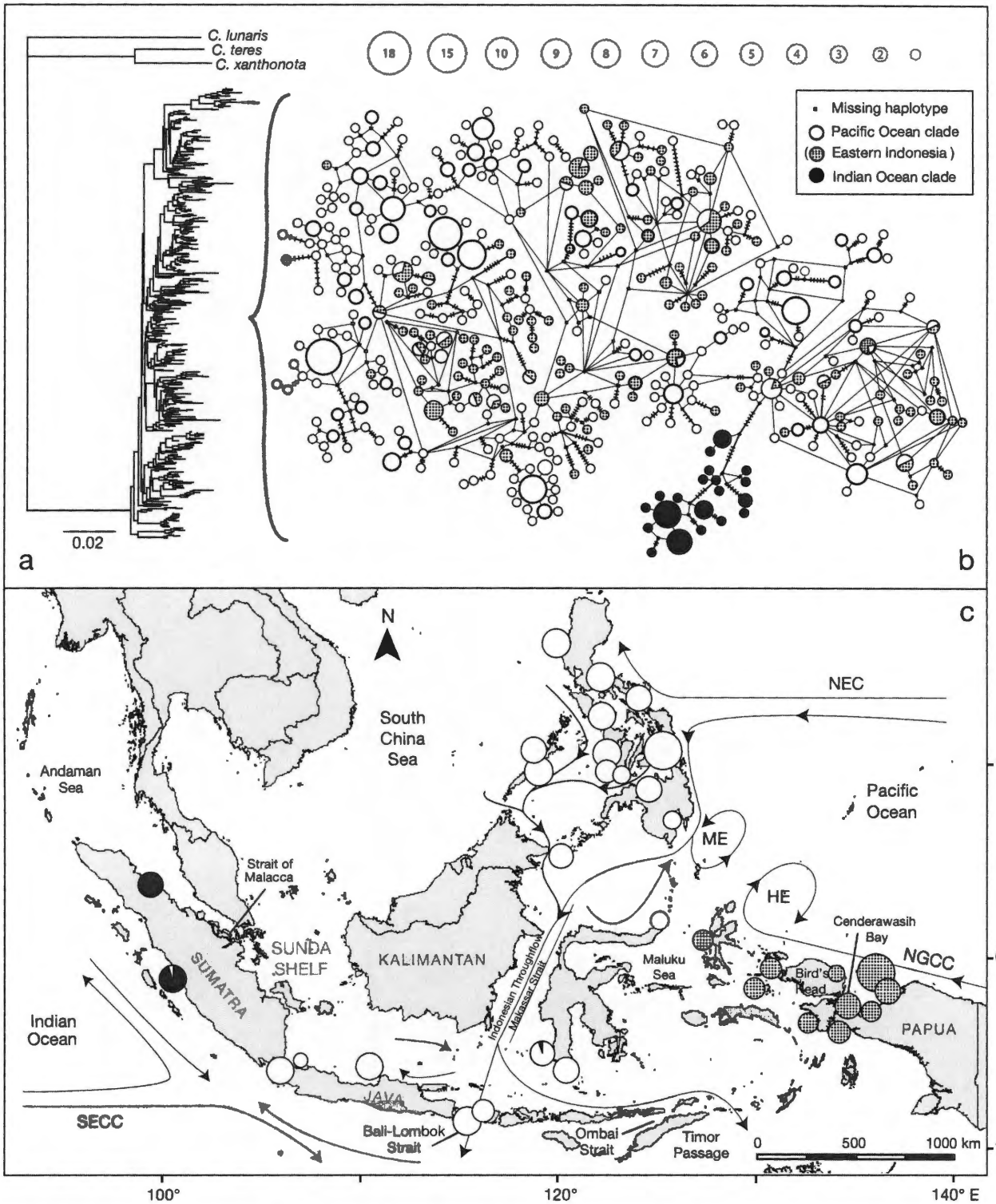

Fig. 3. (a) Neighbor-joining analysis depicting the relationship of our sampled *Caesio cuning* haplotypes to the 3 most closely related *Caesio* spp. in the region. (b) Minimum spanning tree for mitochondrial control region haplotypes of *Caesio cuning*. Eastern Indonesian sites within the Pacific clade are highlighted; uncorrected pairwise Φ_{ST}s and optimal AMOVA partitioning indicate they are significantly different from other sites in this clade. (c) Geographic distribution of regional genetic structure. Area of circles is relative to total number of individuals sampled at each site; sizes range from n = 46 (Dinagat, Philippines) to n = 7 (Pulau Seribu, Indonesia). Major oceanographic features are labeled, including the Northern Equatorial Current (NEC), the New Guinea Coastal Current (NGCC), the Halmahera Eddy (HE), Mindanao Eddy (ME) and the Southern Equatorial Countercurrent (SECC)

Table 1. *Caesio cuning*. Molecular diversity indices. n: number of samples; hap: number of unique haplotypes; *h*: haplotype diversity; Π: nucleotide diversity; θ_s: theta estimated using the number of segregating sites; Fu's F_S and Fu and Li's D^* are 2 neutrality statistics. *significant values of Fu's F_S and Fu and Li's D^* ($\alpha = 0.05$)

Sampling locality		n	hap	*h*	Π	θ_s	F_S	D^*
1	Medan	20	12	0.921 ± 0.039	0.017 ± 0.009	6.765	−1.641	−1.118
2	Padang	22	13	0.918 ± 0.036	0.017 ± 0.009	8.778	−2.168	−2.081*
3	Anyer	22	19	0.983 ± 0.021	0.026 ± 0.014	10.973	−7.154*	−0.322
4	Seribu	7	7	1.000 ± 0.076	0.024 ± 0.016	9.796	−1.725	−0.565
5	Karimunjawa	20	20	1.000 ± 0.016	0.036 ± 0.018	15.503	−10.469*	−0.072
6	Bali	26	22	0.982 ± 0.018	0.028 ± 0.015	10.482	−8.891*	−0.239
7	Lombok	16	15	0.992 ± 0.025	0.029 ± 0.016	11.452	−5.286*	−0.481
8	Makassar	18	18	1.000 ± 0.019	0.027 ± 0.016	13.665	−10.237*	−0.993
9	Selayar	20	15	0.942 ± 0.043	0.025 ± 0.014	10.429	−3.034	−0.794
10	Tawi Tawi	17	13	0.963 ± 0.033	0.028 ± 0.015	10.944	−1.984	−0.644
11	Honda Bay	26	23	0.991 ± 0.013	0.030 ± 0.016	11.793	−10.162*	−0.349
12	Ulugan Bay	21	19	0.991 ± 0.018	0.027 ± 0.015	10.562	−8.230*	−0.047
13	Bolinao	24	24	1.000 ± 0.012	0.028 ± 0.015	10.712	−16.723*	−0.527
14	Perez	25	24	0.997 ± 0.013	0.027 ± 0.014	11.388	−15.200*	−0.415
15	Romblon	17	17	1.000 ± 0.020	0.030 ± 0.016	10.649	−9.056*	−0.237
16	Sorsogon	19	18	0.994 ± 0.019	0.026 ± 0.014	10.872	−9.019*	−0.369
17	Negros Occidental	15	14	0.991 ± 0.028	0.027 ± 0.015	10.457	−5.352*	−0.767
18	Guimaras	26	25	0.997 ± 0.012	0.029 ± 0.015	12.579	−15.492*	−1.044
19	Negros Oriental	8	8	1.000 ± 0.063	0.031 ± 0.018	12.342	−1.933	−0.609
20	Balingasag	21	19	0.990 ± 0.017	0.029 ± 0.015	11.952	−8.981*	−0.446
21	Dinagat	46	44	0.998 ± 0.005	0.027 ± 0.014	13.197	−43.847*	−1.489
22	Davao	9	9	1.000 ± 0.052	0.026 ± 0.015	10.302	−2.911	−0.533
23	Manado	9	8	0.972 ± 0.052	0.026 ± 0.015	10.670	−1.157	−0.849
24	Halmahera	12	11	0.985 ± 0.040	0.029 ± 0.016	9.934	−2.627	0.494
25	Raja Ampat	13	10	0.949 ± 0.051	0.027 ± 0.015	10.312	−0.918	−0.633
27	Fak Fak	11	11	1.000 ± 0.039	0.023 ± 0.014	10.584	−4.636*	−0.797
28	Sorong	14	14	1.000 ± 0.027	0.025 ± 0.014	9.434	−6.906*	−0.409
28	Kaimana	16	16	1.000 ± 0.022	0.026 ± 0.014	9.644	−8.432*	0.037
29	Manokwari	8	8	1.000 ± 0.063	0.031 ± 0.018	12.727	−1.853	−0.436
30	Windesi	20	19	0.995 ± 0.018	0.026 ± 0.014	10.429	−9.444*	−0.591
31	Karei	13	13	1.000 ± 0.030	0.024 ± 0.013	10.634	−6.112*	−0.765
32	Yapen	21	19	0.991 ± 0.018	0.026 ± 0.014	9.728	−8.330*	−0.293
33	Biak	43	36	0.991 ±0.007	0.028 ± 0.014	13.174	−24.146*	−1.963*

between the southern Philippines and eastern Indonesia (0.49 to 0.60; Fig. 4; Barrier c). The fourth barrier divided the Philippines from central Indonesia but was supported by less than half of our bootstrap replicates (0.44; Fig. 4; Barrier d). While the third and fourth barriers partition more variance in our dataset, neither carries strong enough bootstrap support to be viewed with any confidence.

Hierarchical AMOVAs based on previously detected genetic clusters in other marine organisms showed concordance with our pairwise Φ_{ST} values and BARRIER results. Grouping all sites east and west of the Makassar Strait (partition 1, Fig. 2) did not account for a significant portion of the genetic variance among groups measured at this locus, whereas grouping our 2 western Sumatra sites separately from all others (partition 2, Fig. 2) accounted for 47.96% of the genetic variance ($\Phi_{CT} = 0.0258$, $p < 0.086$ vs. $\Phi_{CT} = 0.4796$, $p < 0.004$). Since the variance generated by spatially explicit, divergent clades can overwhelm signatures of structure within a dataset, we removed Medan and Padang from further AMOVA analyses. When the remaining sites from the Pacific Clade were split into a Philippines' group and an Indonesian group (partition 3, Fig. 2), the Φ_{CT} was significant but only explained 0.09% of the variance between groups ($\Phi_{CT} = 0.0091$, $p < 0.022$). Splitting sites east and west of the Maluku Sea (partition 4, Fig. 2) gave us our optimal partition and accounted for 4.50% of the variance between groups ($\Phi_{CT} = 0.0450$, $p < 0.001$). When this partition was shifted to Cenderawasih Bay (partition 5, Fig. 2), it remained significant, accounting for slightly less variance between groups ($\Phi_{CT} = 0.0420$, $p < 0.001$). Of the 5 tested breaks across the Coral Triangle, *Caesio cuning* exhibits 2 commonly found in reef-associated,

Table 2. *Caesio cuning*. Unstandardized results of AMOVA tests with localities where n ≥ 15 using >30 000 random permutations. Tested partitions are labeled 1 to 5 corresponding to illustrations in Fig. 2. The first 3 analyses include both lineages, while the lower 3 analyses examine genetic structure within the Pacific Clade. *k*-values give the number of groupings tested; p-values ≤ 0.05 indicate significant statistics, and optimal partitions for each group of analyses are in **bold**. The last column lists pelagic and demersal species that exhibit phylogeographic breaks in mtDNA on which our hypotheses for partitioning are based

	Hypothesis	Sites	Statistic		p	% var	Partition examples
Both clades (Indian & Pacific)							
	$k = 1$	23	–	–	–	–	*Decapterus macrosoma* (Borsa 2003)
			–	–	–	–	
			Φ_{ST}	0.1421	0.001	14.21	
1	$k = 2$; east vs. west of the Makassar Strait	23	Φ_{CT}	0.0258	0.086	2.58	*Decapterus russelli* (Rohfristch & Borsa 2009)
			Φ_{SC}	0.1312	0.001	12.78	
			Φ_{ST}	0.1537	0.001	84.64	
2	$k = 2$; Western Sumatra vs. all other sites	23	Φ_{CT}	**0.4796**	0.004	**47.96**	*Dascyllus trimaculatus* (Leray et al. 2010),
			Φ_{SC}	0.0189	0.001	0.98	*Acanthaster planci* (Vogler et al. 2008),
			Φ_{ST}	0.4894	0.001	51.06	*Tridacna crocea* (DeBoer et al. 2008),
							Nerita albicilla (Crandall et al. 2008b)
Pacific Clade							
3	$k = 2$; Philippines vs. Indonesia	21	Φ_{CT}	0.0091	0.022	0.091	*Hippocampus kuda* (Lourie et al. 2005)
			Φ_{SC}	0.0140	0.001	1.39	
			Φ_{ST}	0.0229	0.001	97.71	
4	$k = 2$; central CT vs. eastern Indonesia at Halmahera	21	Φ_{CT}	**0.0450**	0.001	**4.50**	*Tridacna crocea* (DeBoer et al. 2008),
			Φ_{SC}	0.00260	0.273	0.25	*Haptosquilla glyptocercus* (Barber et al. 2006)
			Φ_{ST}	0.0474	0.001	95.25	
5	$k = 2$; central CT vs. eastern Indonesia at Cenderawasih Bay	21	Φ_{CT}	0.0420	0.001	4.20	*Haptosquilla pulchella* (Barber et al. 2006),
			Φ_{SC}	0.0056	0.110	0.54	*Tridacna maxima* (Nuryanto & Kochzius 2009)
			Φ_{ST}	0.0473	0.001	95.26	*Protoreaster nodosus* (Crandall et al. 2008a)

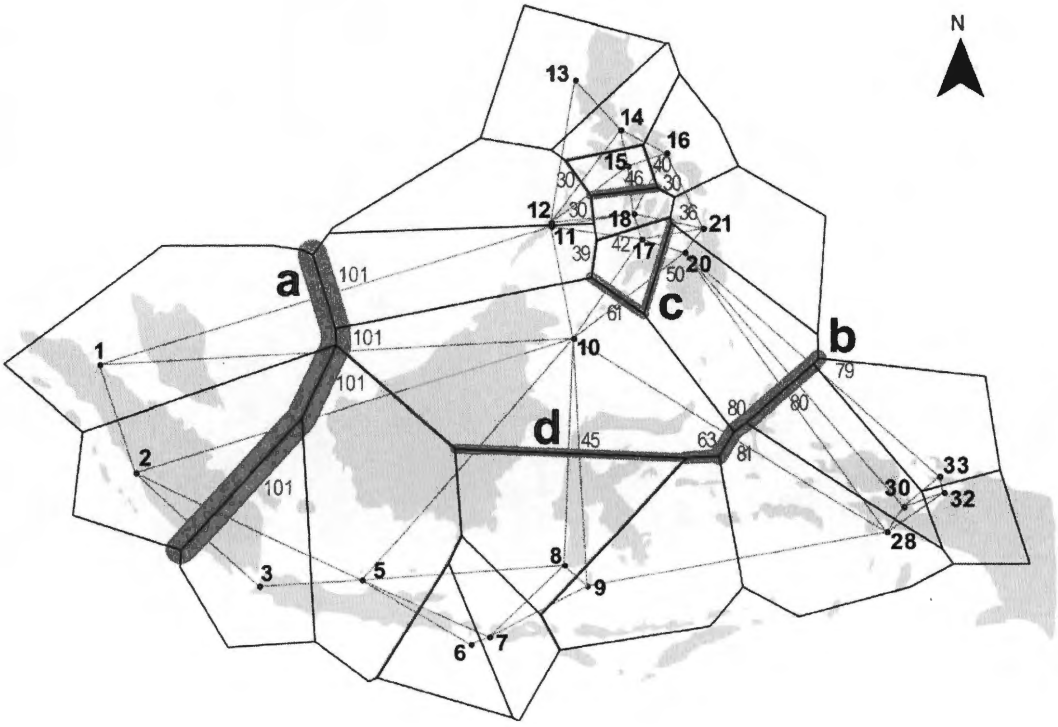

Fig. 4. *Caesio cuning*. BARRIER analysis. Spatial analysis of sites (n ≥ 15) with 4 barriers designated (results labeled a to d) and corresponding confidence values labeled in gray (100 bootstrap replicates +1). Black polygons indicate Voronoi tessellation; gray lines indicate Delaunay triangulation. Thickness of barriers is relative to bootstrap support

demersal species: a Sunda Shelf break at western Sumatra and a break near the Maluku Sea in eastern Indonesia.

Isolation by distance

When all localities (n ≥ 15) were included in our IBD analysis, points associated with the western Sumatran sites Medan and Padang clustered separately from other sites (Fig. 5a). To avoid bias arising from their uniquely divergent lineage coupled with their location on the edge of our sampling range, these 2 localities were excluded from further IBD analyses. When we ran a Mantel test of only the localities within the Pacific lineage, our results showed that there is a significant indication of IBD within this Pacific lineage (Fig. 5b, dashed line, r = 0.4216, p < 0.001). We measured a *Z* of 8964.2023.

Despite the correlation between genetic and geographic distance, our plot indicated that there were still sites nearly 3000 km apart within the Pacific lineage that exhibited no measurable genetic differences. Since our AMOVA analyses indicate the presence of hierarchical structure, we ran partial Mantel tests to determine the nature of the significant correlation we measured. A partial Mantel test examining the correlation of geographic distance to pairwise Φ_{ST} while accounting for our optimal AMOVA clusters (central Indonesia and the Philippines vs. sites in the Bird's Head region of Papua) resulted in a non-significant correlation coefficient (r) of 0.1642 (p < 0.066). A partial Mantel test examining the correlation of pairwise Φ_{ST} to the location of sites within one or the other of our 2 optimal sites while accounting for geographic distance resulted in an r of 0.5907 (p < 0.001), indicating the hierarchical clustering of our sites explains a significant percentage of the variance in our dataset while isolation by distance does not. This is further supported by a Mantel test of only sites within the Philippines and central Indonesia cluster (we were unable to run a Mantel test on the eastern Indonesia cluster since all pairwise $\Phi_{ST} = 0$). We measured a *Z* of 2093.5389 (Fig. 5b, dotted line, r = 0.1258, p = 0.131).

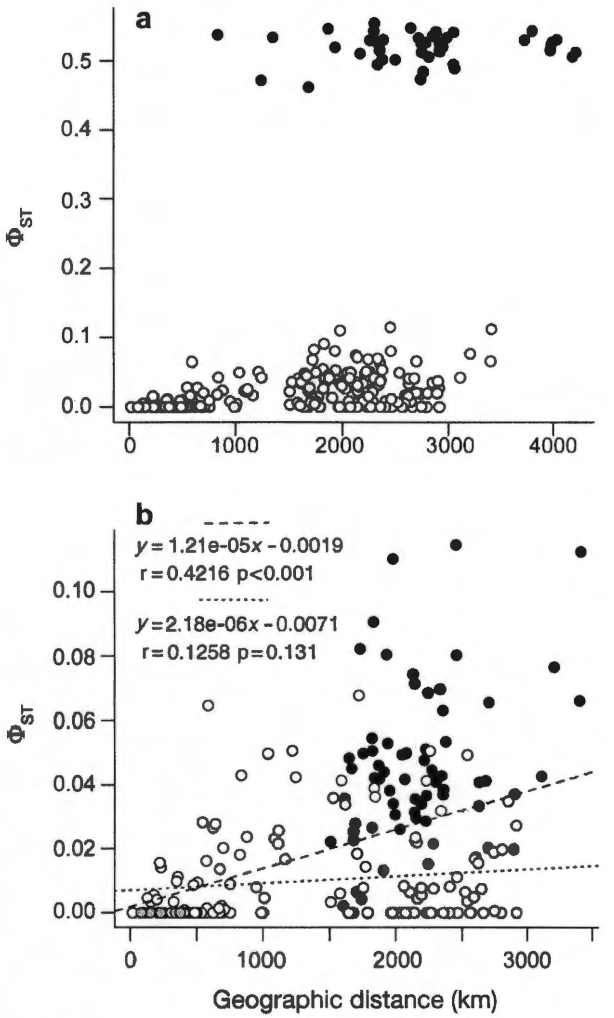

Fig. 5. *Caesio cuning*. Isolation by distance. Comparison of pairwise Φ_{ST} to geographic distance for (a) all sites with n > 15, showing clustering of Φ_{ST} Medan and Padang (black dots) associated with their spatial orientation and divergent clade, and (b) Pacific clade only. Black dots are pairwise comparisons between sites belonging to different AMOVA clusters, white dots are comparisons between sites within the Philippines and central Indonesia cluster, gray dots are comparisons between sites within the eastern Indonesia cluster (all $\Phi_{ST} = 0$). The dashed line is the regression for all sites in the Pacific clade (significant due to presence of hierarchical structure), and the dotted line is the regression for only sites across the Philippines and central Indonesia (white dots only; non-significant)

DISCUSSION

Patterns of genetic structure in a mid-water planktivore

Hierarchical genetic analyses revealed 2 significant regions of genetic structure across the Coral Triangle in the coral reef fish *Caesio cuning*. A sharp genetic break was observed across the Sunda Shelf barrier, echoing patterns reported from a diversity of reef taxa including crown-of-thorns seastars, damselfishes, snappers and snails (Crandall et al. 2008b, Vogler et al. 2008, Drew & Barber 2009, Gaither et al. 2010). Such population divergence across the Sunda

shelf is frequently attributed to historical vicariance between Pacific and Indian Ocean populations during Pleistocene low sea level stands (e.g. Barber et al. 2000, Rohfritsch & Borsa 2005, Deboer et al. 2008). In addition, significant departures from neutrality, as measured by Fu's F_S, might indicate the lingering effects of a Pleistocene population expansion onto the Sunda and Sahul Shelves as sea levels rose during the Last Glacial Maximum. Similar departures have been seen in every species examined in this region so far (see Crandall et al. 2012). Shared phylogeographic patterns such as these result from broadly acting physical processes that shape genetic patterns in codistributed taxa (Avise 2000). However, the maintenance of these patterns in modern times, despite the lack of physical isolation, likely results from oceanographic currents or reproductive isolation between the 2 lineages.

During the northeast monsoon, the Southern Equatorial Counter Current (SECC) bifurcates off the coast of southern Sumatra (Schott & McCreary 2001). During the southwest monsoon, this reverses, and where Sumatra meets Java, a southeastern flow hits a northwesterly flowing current that is driven by the Indonesian Throughflow. Both monsoonal patterns have the potential to create a barrier to continuous gene flow at the site of bifurcation and conjunction (Fig. 3c), potentially reinforcing isolation during periods of lowered sea levels. Support for this hypothesis comes from a recent quantitative analysis using biophysical models coupled with matrix projection (Kool et al. 2011) that predicts the genetic isolation of populations in the Andaman Sea and western Sumatra.

While studies of many reef organisms indicate divergence between Pacific and Indian Ocean populations, only few have sampled at a scale fine enough to illuminate the extent and location of overlap between these divergent lineages (see Barber et al. 2006, Crandall et al. 2008a,b, Deboer et al. 2008, Nuryanto & Kochzius 2009, Gaither et al. 2011). The overlap between divergent Indian and Pacific Ocean lineages in *Caesio cuning* is surprisingly small for such a potentially mobile fish. Haplotype distributions from our minimum spanning tree indicate very limited gene flow between the northern tip of Java and equatorial Sumatra, a distance of just over 800 km. No landmass or geographical feature separates the waters of Padang (Sumatra) from the 2 closest sample sites on Java, Anyer and Kepulauan Seribu, yet only a single individual unites the maternal lineages of Padang to these 2 sites (Fig. 3c). While regional oceanographic patterns could be limiting the genetic connectivity in *C. cuning* across this region, it is notable that, across the same geographic range, the anemonefish *Amphiprion ocellaris* shows greater admixture of Indian and Pacific maternal lineages in the Java Sea (Timm & Kochzius 2008), and anemonefishes have a larval dispersal period of only 8 to 12 d (Fautin & Allen 1992) and larvae exhibit natal homing (Jones et al. 2005). Given the limited overlap of our 2 lineages, reproductive isolation between the clades cannot be ruled out as a possible explanation for the absence of gene flow in this region.

In addition to the phylogeographic break observed at the Sunda shelf, significant limits to genetic exchange were also seen in eastern Indonesia. At first pass, a significant correlation between genetic distance and over-water distance suggests that limits to gene flow in this region might be due a stepping-stone model of gene flow in which nearby localities exchange more migrants with each other than they do with distant localities (Fig. 5b). However, our partial Mantel tests clearly show that this appearance of isolation-by-distance is actually an artifact of hierarchical structure between 2 genetic clusters, the junction of which is delimited by AMOVA and BARRIER (Figs. 2 & 4, Table 2).

This genetic structuring across the Maluku Sea mirrors the genetic structure and even pronounced phylogeographic breaks east and west of Halmahera found in 2 species of giant clam (Deboer et al. 2008, Nuryanto & Kochzius 2009) and 14 species of stomatopods (Barber et al. 2006, 2011), suggesting this region may be important for lineage divergence. While *Caesio cuning* populations on either side of Halmahera are not characterized by distinct clades as is seen in western Indonesia, the minimum spanning tree indicates some non-random, regional clustering of haplotypes. Frequency differences among related haplotypes within the Pacific Ocean clade may be caused by isolation facilitated by 2 eddies generated at the convergence point of the Northern Equatorial Current and the New Guinea Coastal Current, the Mindanao Eddy and the Halmahera Eddy (Fig. 3c). The Halmahera Eddy has previously been suggested as important for driving lineage divergence in the region of the Maluku Sea (Barber et al. 2006, 2011); however, both eddies direct a significant amount of flow back into the Pacific Ocean, so both may be contributing to the genetic isolation observed in population genetic and computer modeling studies (Kool et al. 2011) conducted in this region.

The recovery of multiple regions of significant genetic structure in *Caesio cuning* is somewhat surprising because the high mobility potential of adults

could result in genetic admixture, such as the signal of secondary contact seen in migratory *Decapterus macrosoma* (Borsa 2003). However, the concordance of our data with phylogeographic patterns of demersal reef species that have larval dispersal as well as with predictions from biophysical models of larval dispersal (Kool et al. 2011) suggests that adult *C. cuning* are site-attached and that the major avenue of genetic connectivity in *C. cuning* is via larval dispersal. If adults are truly site-attached, *C. cuning* would be dependent on larval dispersal to maintain gene flow among populations across its range.

Implications for management

As the target of a significant artisanal fishery in the Coral Triangle, *Caesio cuning* is subject to anthropogenic population declines. A study of Sumilon Island in the Philippines documented changes in reef fish density after protective management was removed for a quarter of the island's reefs. Alcala & Russ (1990) measured a 64% decrease in caesionid density after an 18 mo period of fishing by ~100 local fishermen from an adjacent island using hand-paddled canoes. Given that artisanal fishing of caesionids has been shown to cause precipitous drops in local abundance, a better understanding of stock structure is particularly important for the management of *C. cuning*.

The results of the present study suggest that *Caesio cuning* populations in the Philippine and Indonesian portions of the Coral Triangle should be best viewed as at least 3 stocks. However, managing a reef fishery at this scale is complex because these stocks do not conform to national borders. We saw no significant genetic divergence between sites in the Philippines and sites in central Indonesia that are nearly 3000 km apart (see pairwise Φ_{ST} table in the supplement). This connectivity is likely facilitated by the Indonesian Throughflow, a strong current originating in the Western Pacific that flows between Kalimantan and Sulawesi and empties into the Indian Ocean via 3 major 'chokepoints': the Bali-Lombok Strait, the Ombai Strait and the Timor Passage (Fig. 3c). Dispersal simulations have predicted a net flow of larvae north to south via this pathway (Kool et al. 2011). The boundaries among stocks in western, central and eastern Indonesia all occur within Indonesian national borders, which potentially simplifies management planning and authority. However, the absence of genetic structure between the Philippines and central Indonesia implies that the diversity and abundance of larvae produced from Philippine reefs could have an important impact on the sustainability and genetic diversity of reefs of central Indonesia. This interdependence between countries within the Coral Triangle emphasizes the importance of developing informed, multinational management plans, such as the Coral Triangle Initiative (www.coraltriangleinitiative.org).

Future work should focus on fine-scale sources and flow of larvae both within regions of high genetic connectivity as well as areas of restricted gene flow in order to ensure continual replenishment of coral reef resources. In the case of *Caesio cuning*, particular attention should be given to areas with evidence of severely limited gene flow, such as the junction of Sumatra and Java. Determining the nature of the limited overlap between the 2 mitochondrial clades will be key to proper management design in this region. Mitochondrial genetic studies do not have the power to detect reproductive isolation with certainty, so future studies should incorporate bi-parentally inherited nuclear DNA. Multiple independent genetic markers, such as microsatellites or single nucleotide polymorphisms, could be applied to extended sampling in this area to detect whether it is cryptic speciation or barriers to genetic connectivity that maintain this break. It is particularly important to identify whether gene flow is restricted since intense overfishing in such a region could result in temporary local extinctions. Until future research characterizes the nature and direction of genetic connectivity across these regions, our understanding of the population structure of *C. cuning* is limited to large scales.

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