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Pronounced Genetic Structure in a Highly Mobile Coral Reef Fish, Caesio Cuning, in the Coral Triangle

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Running head: Genetic structure in *Caesio cuning*

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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 regional limits to connectivity across this region. However, these 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 382bp 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 < 0.0043). We measured additional significant population structure between central Southeast Asia and eastern Indonesia (Φ_{CT} = 0.0450, p < 0.0002). These data in conjunction with spatial analyses indicate that there are two major lineages of *C. cuning* and at least three 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.

**Keywords:** 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 and 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. During the Pleistocene epoch, repeated glaciations caused radical changes to the regional geography as the Sunda and Sahul Shelves 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 (Springer and Williams 1990; Mcmillan and Palumbi 1995; Randall 1998; Lessios et al. 2001; Barber et al. 2006; Crandall et al. 2008a,b; Vogler et al. 2008). At more recent timescales, oceanographic processes have also 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 2002, 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 diversification 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 diversification within the Coral Triangle (Carpenter et al. 2011, Barber 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. 2002, 2006; Crandall et al. 2008a,b; Deboer et al. 2008; Timm and Kochzius 2008; Nuryanto and Kochzius 2009). In contrast, relatively understudied near-shore pelagics give mixed results. The round scad mackerel, Decapterus macrosoma, show very little genetic structure (Borsa 2003), while its congener Decapterus russelli shows up to three genetically structured populations (Rohfritsch and 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 1-2% (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 marine reserves (Roberts and Polunin 1991; Russ and Alcala 1996;
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 gaps 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 and Mous 2004). 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 methods including hand-line, 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 approximately 22,000 metric tons (BAS 2010), but given the artisanal nature of most reef fisheries in this region, these catch data are likely greatly underestimated (Alcala and Russ 2002).

The red belly yellowtail fusilier, *Caesio cuning* (Bloch 1791), is a caesionid commonly found in local markets across the Coral Triangle. It is a conspicuous midwater member of Indo-Pacific reef ecosystems with a distribution that ranges from southern Japan to northern Australia and from Vanuatu to Sri Lanka (Figure 1a). *C. cuning* are schooling, broadcast spawners so there is no reason to suspect sex-biased
dispersal, but beyond this, little is known about the larval ecology of *C. cuning*. The closest relative with a known pelagic larval duration (PLD) is *Pterocaesio chrysozona* with an estimated PLD of 37-47 days (Doherty et al. 1995), and there is no evidence to suggest strong larval behavior such as homing (Leis and 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 *C. cuning* as pelagic larvae coupled with their dependence on reef structure for shelter and undefined movement as adults suggests a varied spectrum of dispersal potential.

The purpose of this study is to assess regional genetic connectivity and lineage diversification in *Caesio cuning* in order to address two questions: (1) are mid-water, 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?

**Methods**

We collected 630 *Caesio cuning* samples from fish markets or by spear while SCUBA or skin diving from 33 localities in the Coral Triangle (Figure 1b). 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 polymerase chain reaction (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, 25 µL reactions of 2.5 µL of 10x buffer, 2 µL MgCl2 (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 (1min), main cycle 94°C (30 s), 50-52°C (30 s) and 72°C (40 s) for 39 cycles, then a final extension of 72°C (7-10 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 digesting 5 ul of PCR product in 0.5 U of Shrimp Alkaline Phosphatase and 5U of Exonuclease for 30 minutes at 37°C followed by 15 minutes 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™ 4.7 (Gene Codes Corporation, Ann Arbor, Michigan) and all resulting 383-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 3.5.12 (Excoffier and Lischer 2010).

The species identity of our sampled haplotypes was confirmed with a neighbor-joining tree run in PAUP* (Swofford 2003) that included the three most closely related sister 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).

For each locality we used DnaSP v5 (Librado and Rozas 2009) to calculate standard genetic diversity indices and tested the null hypothesis of neutrality in the mitochondrial control region using Fu’s Fs and Fu and Li’s D* tests, with significance determined by 1000 simulations of a neutral coalescent model. We employed the latter two 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 used examined population pairwise $\Phi_{ST}$, and performed an analysis of molecular variance (AMOVA) in Arlequin. For the AMOVA analysis, we grouped sampling localities to test for hierarchical population structure within our dataset following a priori hypotheses based on previously measured phylogeographic breaks (Figure 3; 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 and Nei 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 and Gascuel 2003). The significance of pairwise $\phi_{st}$ 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 adjusted using Bonferroni as well as Benjamini and Hochberg’s (1995) false discovery rate to reduce Type II error associated with the former method (Narum 2006).

In addition we employed a post hoc spatial analysis of the pairwise $\phi_{st}$ matrix generated in Arlequin using the program BARRIER version 2.2 (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 ($\phi_{st}$ 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 $\phi_{st}$ matrix in Arlequin.

Since discrete genetic breaks can bias the results of analyses of Isolation by Distance (IBD) and the presence of isolation by distance 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 Core Team 2012). Pairwise genetic distances ($\phi_{st}$) among localities with $n > 15$ were imported from Arlequin, and negative pairwise $\phi_{st}$ values, a result of within population variance exceeding among population variance, were set to zero. Our 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 a zero to code for localities within the same group and a one 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 highest frequency haplotype 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 (Figure 2a,b). A single individual sampled at Makassar, Sulawesi also fell within this divergent Indian Ocean clade. Regional clustering within
the Pacific lineage shows some evidence that the distribution of haplotypes is non-random.

**Population Structure**

Haplotype diversity was high, measuring at or near 1 for all localities (Table 1). Our two sites from Sumatra - Medan and Padang - had slightly lower nucleotide diversity (0.0171 and 0.0169, respectively) 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 two 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 one of these two processes (Fu 1997).

The results of our AMOVA analyses indicate significant genetic structuring in *Caesio cuning* across the Coral Triangle (Table 2; $\Phi_{ST} = 0.1421$, $p < 0.00001$). Grouping sites east and west of the Makassar Strait accounted for a non-significant portion of the genetic variance between groups measured at this locus whereas grouping our two western Sumatra sites separately from all others accounted for 47.96% of the genetic variance ($\Phi_{CT} = 0.0258$, $p < 0.08554$ vs. $\Phi_{CT} = 0.4796$, $p < 0.00426$). 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, the $\Phi_{CT}$ was significant but only explained 0.09% of the variance
between groups ($\Phi_{CT} = 0.0091, p < 0.02246$). Splitting sites east and west of the Maluku
Sea gave us our optimal partition and accounted for 4.50% of the variance between
groups ($\Phi_{CT} = 0.0450, p < 0.00023$). When this partition was shifted to Cenderawasih
Bay, it remained significant accounting for slightly less variance between groups ($\Phi_{CT} =
0.0420, p < 0.00083$). These patterns of genetic structure were echoed in the pairwise $\Phi_{ST}$
values calculated for each pair of sampling localities (tables attached as supplemental).
Of the five tested breaks across the Coral Triangle, *C. cuning* exhibits two commonly
found in reef-associated, demersal species: a Sunda Shelf break at western Sumatra
(partition 2, Figure 3) and a break near the Maluku Sea in eastern Indonesia (partition 4,
Figure 3).
Spatial analysis of our pairwise $\Phi_{ST}$ matrix showed good agreement with our *a
priori* AMOVA results. Bootstrapping analyses reached their highest confidence values
when parameters were set to four 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 (Figure 4a). The second barrier is found in the region of
Halmahera and the Maluku Sea, which carries the next highest confidence values (0.78-
0.80; Figure 4b). The third barrier was complex and found in the Philippines with the
most supported divisions between the southern Philippines and eastern Indonesia (0.49-
0.60; Figure 4c). The fourth barrier divided the Philippines from central Indonesia, but
was supported by less than half of our bootstrap replicates (0.44; Figure 4d). While the third and fourth barriers partition more variance in our dataset, neither carries strong enough bootstrap support to be viewed with any confidence.

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 (Figure 5a). To avoid bias arising from their uniquely divergent lineage coupled with their location on the edge of our sampling range, these two 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 (Figure 5b, dashed line). We measured a Z of 8964.2023 and a correlation coefficient (r) of 0.4216 with a corresponding p-value of less than 0.0001.

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 ΦST 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 < 0.0657). A partial Mantel test examining the correlation of pairwise ΦST to the location of sites within one or the other of our two optimal sites while accounting for geographic distance...
resulted in an $r$ of 0.5907 ($p < 0.0002$), 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 and a correlation coefficient ($r$) of 0.1258 with a non-significant $p$-value of 0.1306 (Figure 5b, dotted line).

**Discussion**

*Patterns of genetic structure in a mid-water planktivore*

Hierarchical genetic analyses revealed two 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 groupers, giant clams, crown-of-thorns seastars, damselfishes, surgeonfish and snappers (Craig et al. 2007; Timm et al. 2008; Vogler et al. 2008; Drew and Barber 2009; Eble et al. 2010; 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 and Borsa 2005; Deboer et al. 2008). In addition, significant departures from neutrality, as measured by Fu’s $F_S$, 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 two lineages.

During the northeast monsoon, the Southern Equatorial Counter Current (SECC)
bifurcates off the coast of southern Sumatra (Schott and 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 (Figure 2c), 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 a few have sampled at a scale fine enough to illuminate
the extent and location of overlap between these divergent lineages (e.g. Barber et al.
2002, 2006; Crandall et al. 2008a,b; Deboer et al. 2008; Nuryanto and 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 two
closest sample sites on Java, Anyer and Kepuluan Seribu, yet only a single individual unites the maternal lineages of Padang to these two sites (Figure 2c). 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 anenomefish *Amphiprion ocellaris* shows greater admixture of Indian and Pacific maternal lineages in the Java Sea (Timm and Kochzius 2008), and anenomefishes have a larval dispersal period of only 8-12 days (Fautin and Allen 1992) and larvae exhibit natal homing (Jones et al. 2005). Given the limited overlap of our two 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 than they do with distant localities (Figure 5b). However, our partial Mantel tests clearly show that this appearance of isolation-by-distance is actually an artifact of hierarchical structure between the two regions delimited by BARRIER and AMOVA analysis (Figures 3 & 4; Table 2).

This genetic structuring across the Maluku Sea mirrors genetic structure and even pronounced phylogeographic breaks east and west of Halmahera found in two species of giant clam (Deboer et al. 2008; Nuryanto and Kochzius 2009) and 14 species of stomatopods (Barber et al. 2006; Barber et al. 2011), suggesting this region may be important for lineage diversification. 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 two eddies generated at the convergence point of the Northern Equatorial Current and the New Guinea Coastal Current, the Mindanao Eddy and the Halmahera Eddy (Figure 2c). The Halmahera Eddy has previously been suggested as important for driving lineage diversification in the region of the Maluku Sea (Barber et al. 2002, 2006, 2011), however, both eddies direct a significant amount of flow back into the Pacific Ocean, so both may be contributing to 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 to phylogeographic patterns of demersal reef species with larval dispersal as well as to 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 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 and Russ (1990) measured a 64% decrease in caesionid density after an eighteen-month period of fishing by approximately 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 this study suggest that Caesio cuning populations in the Philippine and Indonesian portions of the Coral Triangle should be best viewed as at least three 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 across sites in the Philippines and central Indonesia that are nearly 3000 km apart (see pairwise $\Phi_{ST}$ table, supplemental material). 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 three major “chokepoints” – the Bali-Lombok Strait, the Ombai Strait and the Timor Passage (Figure 2c). 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 two mitochondrial clades will be key to the proper management design in

this region. Mitochondrial genetic studies do not have the power to detect reproductive

isolation with certainty, so future study should incorporate bi-parentally inherited nuclear

DNA. Multiple independent genetic markers such as microsatellites or SNPs could be

applied to extended sampling in this area to detect whether it is cryptic speciation or

barriers to genetic connectivity maintaining 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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Figure 1 a. The distribution of *Caesio cuning*. b. Inset. Sampling localities of this 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 – Teluk Cenderawasi (30), Karei – Teluk Cenderawasi (31), Yapen (32), Biak (33).
Figure 2. a. Neighbor-joining analysis depicting the relationship of our sampled *Caesio cuning* haplotypes to the three most closely related *Caesio* spp. in the region. b. Minimum spanning tree for mitochondrial control region haplotypes of *Caesio cuning*. Gray shading highlights the eastern Indonesian sites within the Pacific Clade, which uncorrected pairwise $\Phi_{ST}$ and optimal AMOVA partitioning indicate 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).

Figure 3. 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 analysis of molecular variance that included sites from both the Indian and Pacific clades; dashed lines indicate partitions tested within the Pacific clade only.
**Figure 4. BARRIER Analysis** Spatial analysis of sites \((n \geq 15)\) with four barriers designated (results labelled a-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.
Figure 5. Isolation By Distance graphs Comparison of pairwise $\Phi_{ST}$ to geographic distance for a. all sites with sample sizes greater than 15, showing clustering of Medan and Padang 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, and 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).
### Tables

**Table 1. Molecular diversity indices for Caesio cuning:** $n =$ number of samples, $\text{hap} =$ number of unique haplotypes, $h =$ haplotype diversity, $H =$ nucleotide diversity, $\theta_s =$ theta estimated using the number of segregating sites, and Fu’s $F_s$ and Fu and Li’s $D^* =$ two neutrality statistics.

<table>
<thead>
<tr>
<th>Sampling Locality</th>
<th>$n$</th>
<th>$\text{hap}$</th>
<th>$h$</th>
<th>$H$</th>
<th>$\theta_s$</th>
<th>$F_s$</th>
<th>$D^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medan</td>
<td>20</td>
<td>12</td>
<td>0.921</td>
<td>0.017</td>
<td>6.765</td>
<td>-1.641</td>
<td>-1.118</td>
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<td>Padang</td>
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<td>0.918</td>
<td>0.017</td>
<td>8.778</td>
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<td>-2.081*</td>
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<td>Anyer</td>
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<td>10.973</td>
<td>-7.154*</td>
<td>-0.322</td>
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<td>Seribu</td>
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<td>7</td>
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<td>0.024</td>
<td>9.796</td>
<td>-1.725</td>
<td>-0.565</td>
</tr>
<tr>
<td>Karimunjawa</td>
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<td>20</td>
<td>1</td>
<td>0.034</td>
<td>15.503</td>
<td>-10.469*</td>
<td>-0.072</td>
</tr>
<tr>
<td>Bali</td>
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<td>22</td>
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<td>10.482</td>
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<tr>
<td>Lombok</td>
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<td>15</td>
<td>0.992</td>
<td>0.029</td>
<td>11.452</td>
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<td>-0.481</td>
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<td>18</td>
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<td>13.665</td>
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<td>10.429</td>
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<td>-0.794</td>
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<tr>
<td>Tawi Tawi</td>
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<td>0.027</td>
<td>10.944</td>
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<td>-0.644</td>
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<tr>
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<tr>
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<tr>
<td>Bolinao</td>
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<td>11.952</td>
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<td>-0.446</td>
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<tr>
<td>Dinagat</td>
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<td>44</td>
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<td>13.197</td>
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<td>-1.489</td>
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<tr>
<td>Davao</td>
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<td>9</td>
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<td>0.025</td>
<td>10.302</td>
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<td>-0.533</td>
</tr>
<tr>
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<td>10.670</td>
<td>-1.157</td>
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<tr>
<td>Halmahera</td>
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<td>11</td>
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<td>10.312</td>
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<td>11</td>
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<td>0.023</td>
<td>10.584</td>
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<td>13.174</td>
<td>-24.146*</td>
<td>-1.963*</td>
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</table>

* denotes significant values of Fu’s $F_s$ and Fu and Li’s $D^*$ ($\alpha=0.05$).
Table 2. AMOVA Summary. Unstandardized results of AMOVA tests with localities where \( n \geq 15 \) using 30,000+ random permutations. Tested partitions are labeled 1-5 corresponding to illustrations in Figure 3. The first three analyses include both lineages, while the lower three analyses examine genetic structure within the Pacific Clade. K values give the number of groupings tested. P-values \( \leq 0.05 \) indicate significant statistics, and optimal partitions for each group of analyses are bolded. The last column “e.g.” lists pelagic and demersal species that exhibit phylogeographic breaks in mtDNA on which our hypotheses for partitioning are based.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Sites</th>
<th>Statistic</th>
<th>p</th>
<th>% var</th>
<th>e.g.</th>
</tr>
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<tr>
<td>Both Clades (Indian &amp; Pacific)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( k = 1 )</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td>Decapterus macrosoma (Borsa 2003)</td>
</tr>
<tr>
<td>( \Phi_{ST} )</td>
<td>0.1421</td>
<td>0.00001</td>
<td>14.21</td>
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</tr>
<tr>
<td>1 ( k = 2; ) east vs. west of the Makassar Strait</td>
<td>23</td>
<td>( \Phi_{CT} )</td>
<td>0.0258</td>
<td>0.08554</td>
<td>2.58 Decapterus russelli (Rofristch and Borsa 2009)</td>
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<tr>
<td>( \Phi_{SC} )</td>
<td>0.1312</td>
<td>0.00001</td>
<td>12.78</td>
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<td>( \Phi_{ST} )</td>
<td>0.1537</td>
<td>0.00001</td>
<td>84.64</td>
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<tr>
<td>2 ( k = 2; ) Western Sumatra vs. all other sites</td>
<td>23</td>
<td>( \Phi_{CT} )</td>
<td>0.4796</td>
<td>0.00426</td>
<td>47.96 Dascyllus trimaculatus (Leray et al. 2010)</td>
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<tr>
<td>( \Phi_{SC} )</td>
<td>0.0189</td>
<td>0.00003</td>
<td>0.98</td>
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<td>Acanthaster planci (Vogler et al. 2008)</td>
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<td>0.4894</td>
<td>0.00001</td>
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<td>Tridacna crocea (Deboer et al. 2008)</td>
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<tr>
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<td>0.056</td>
<td>0.11032</td>
<td>0.54</td>
<td></td>
<td>Nerita albicilla (Crandall et al. 2008b)</td>
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<tr>
<td>( \Phi_{ST} )</td>
<td>0.0473</td>
<td>0.00003</td>
<td>95.26</td>
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<td></td>
</tr>
<tr>
<td>Pacific Clade</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 ( k = 2; ) Philippines vs. Indonesia</td>
<td>21</td>
<td>( \Phi_{CT} )</td>
<td>0.0091</td>
<td>0.02246</td>
<td>0.91 Hippocampus kuda (Lourie et al. 2005)</td>
</tr>
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<td>( \Phi_{SC} )</td>
<td>0.0140</td>
<td>0.00136</td>
<td>1.39</td>
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<td>( \Phi_{ST} )</td>
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<td>0.00007</td>
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<tr>
<td>4 ( k = 2; ) central CT vs. eastern Indonesia at Halmahera</td>
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<td>( \Phi_{CT} )</td>
<td>0.0450</td>
<td>0.00023</td>
<td>4.50 Tridacna crocea (Deboer et al. 2008)</td>
</tr>
<tr>
<td>( \Phi_{SC} )</td>
<td>0.0026</td>
<td>0.27264</td>
<td>0.25</td>
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<td>Haptosquilla glyptocercus (Barber et al. 2006)</td>
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<tr>
<td>( \Phi_{ST} )</td>
<td>0.0474</td>
<td>0.00003</td>
<td>95.25</td>
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<tr>
<td>5 ( k = 2; ) central CT vs. eastern Indonesia at Cenderawasih Bay</td>
<td>21</td>
<td>( \Phi_{CT} )</td>
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<td>0.00083</td>
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</tr>
<tr>
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<td>0.11032</td>
<td>0.54</td>
<td></td>
<td>Tridacna maxima (Nuryanto and Kochzius 2009)</td>
</tr>
<tr>
<td>( \Phi_{ST} )</td>
<td>0.0473</td>
<td>0.00003</td>
<td>95.26</td>
<td></td>
<td>Protoreaster nodosus (Crandall et al. 2008a)</td>
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</table>