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

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

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 ($\Phi_{CT} = 0.4796$, $p < 0.0043$). We measured additional significant population structure between central Southeast Asia and eastern Indonesia ($\Phi_{CT} = 0.0450$, $p < 0.0002$). 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

69 particularly useful in highlighting regions where gene exchange does not occur
70 (Hedgecock et al. 2007). Recent reviews indicate the presence of several genetic breaks
71 shared by multiple species across this region, demonstrating that distinct geophysical
72 processes can promote population structure and even lineage diversification within in the
73 Coral Triangle (Carpenter et al. 2011, Barber et al 2011). However, to date the vast
74 majority of reef species showing pronounced genetic structure across the Coral Triangle
75 have been demersal, such as clams, stomatopods, seastars, gastropods and clownfish
76 (Barber et al. 2002, 2006; Crandall et al. 2008a,b; Deboer et al. 2008; Timm and
77 Kochzius 2008; Nuryanto and Kochzius 2009). In contrast, relatively understudied near-
78 shore pelagics give mixed results. The round scad mackeral, *Decapterus macrosoma*,
79 show very little genetic structure (Borsa 2003), while its congener *Decapterus russelli*
80 shows up to three genetically structured populations (Rohfritsch and Borsa 2005).
81 Unfortunately the diversity that makes the Coral Triangle an area of evolutionary
82 and biogeographic interest is vulnerable. The region is a hotspot for coral reef threats
83 (Roberts et al. 2002; Nañola et al. 2011). As the human population in this region
84 increases annually by an estimated 1-2% (US Census Bureau 2011), anthropogenic
85 pressures on coral reef resources continue to rise. Coastal reefs are easily exploitable
86 resources, and reef fish and invertebrates are important sources of food and livelihood in
87 the coastal communities of Southeast Asia (McManus et al. 1992; McManus 1997).
88 Informed management of coral reef ecosystems is a priority for the conservation and
89 sustainability of coral reef resources in the coming decades.
90 The most accepted strategy for improving the biomass and abundance of reef
91 organisms is marine reserves (Roberts and Polunin 1991; Russ and Alcala 1996;

92 Johannes 2002; Sale 2006). Because dispersive larvae are the primary means of
93 demographic and genetic connectivity among most populations, understanding patterns of
94 larval dispersal has been identified as one of the most critical gaps in developing effective
95 reserve networks (Sale et al. 2005). Although genetic connectivity is not equivalent to
96 demographic connectivity, genetic methods can be of use in guiding conservation
97 planning in marine ecosystems (Palumbi 2003). By identifying regions that are
98 genetically and demographically independent, conservation planners can partition large
99 marine ecosystems into smaller, more tractable management areas for which networks of
100 marine reserves can be designed (Green and Mous 2004). This approach has been
101 specifically proposed as a management mechanism in the Coral Triangle (Carpenter et al.
102 2011).

103 Schooling, reef-associated fusiliers (Perciformes: Caesionidae) are planktivores
104 found feeding at the reef face and account for a sizable portion of harvested reef species
105 in the Coral Triangle. They are caught via a variety of methods including hand-line, fish
106 traps, trawls, drive-in nets and gill nets (Carpenter 1988). In the Philippines alone, the
107 annual catch of caesionids in commercial and municipal fisheries is approximately
108 22,000 metric tons (BAS 2010), but given the artisanal nature of most reef fisheries in
109 this region, these catch data are likely greatly underestimated (Alcala and Russ 2002).

110 The red belly yellowtail fusilier, *Caesio cuning* (Bloch 1791), is a caesionid
111 commonly found in local markets across the Coral Triangle. It is a conspicuous mid-
112 water member of Indo-Pacific reef ecosystems with a distribution that ranges from
113 southern Japan to northern Australia and from Vanuatu to Sri Lanka (Figure 1a). *C.*
114 *cuning* are schooling, broadcast spawners so there is no reason to suspect sex-biased

115 dispersal, but beyond this, little is known about the larval ecology of *C. cuning.* The
116 closest relative with a known pelagic larval duration (PLD) is *Pterocaesio chrysozona*
117 with an estimated PLD of 37-47 days (Doherty et al. 1995), and there is no evidence to
118 suggest strong larval behavior such as homing (Leis and Carson-Ewart 2003) that may
119 limit dispersal potential. As adults, *C. cuning* are highly mobile members of the coral reef
120 ecosystem. While they can also be captured in trawls over soft bottom environments
121 (Carpenter 1988) the extent of their movement remains unknown. *C. cuning* and other
122 fusiliers have been observed sleeping in crevices and holes in the reef structure, however,
123 their level of fidelity to such shelter sites and individual reefs is unclear. The mobility of
124 *C. cuning* as pelagic larvae coupled with their dependence on reef structure for shelter
125 and undefined movement as adults suggests a varied spectrum of dispersal potential.
126 The purpose of this study is to assess regional genetic connectivity and lineage
127 diversification in *Caesio cuning* in order to address two questions: (1) are mid-water,
128 reef-associated planktivores impacted by the same barriers we see in demersal species or
129 do they exhibit the panmixia found in near-shore pelagics and (2) if limitations to
130 dispersal in *C. cuning* are present, can we identify distinct geographic stocks to aid in the
131 management of fusiliers?
132
133 **Methods**
134 We collected 630 *Caesio cuning* samples from fish markets or by spear while
135 SCUBA or skin diving from 33 localities in the Coral Triangle (Figure 1b). Only samples
136 that were confirmed as being caught on nearby reefs were collected from local markets.

137 Tissue samples were taken from the pectoral or caudal fin base and preserved in 95%
138 ethanol.
139 DNA amplification and sequencing reactions were conducted at Boston
140 University, the University of the Philippines Marine Science Institute, De La Salle
141 University and Udayana University. Whole genomic DNA was extracted using a 10%
142 Chelex (Biorad) solution (Walsh et al. 1991). A 382-bp region of the mitochondrial d-
143 Loop was amplified via polymerase chain reaction (PCR) using the forward and reverse
144 primers CR-A and CR-E (Lee et al. 1995). PCR reactions were conducted in a 25 uL
145 reaction consisting of 1 uL DNA extraction, 25 µL reactions of 2.5 µL of 10x buffer, 2
146 µL MgCl2 (25 mM), 2.5 µL dNTPs (8 mM), 1.25 µL of each 10 uM primer, 1 µL of
147 template, and 0.625 U of AmpliTaq (Applied Biosystems). Manual hot start
148 thermocycling parameters were employed as follows: initial hold at 80°C, denaturation
149 94 °C (1min), main cycle 94°C (30 s), 50-52°C (30 s) and 72°C (40 s) for 39 cycles, then
150 a final extension of 72°C (7-10 min).
151 PCR products were electrophoresed on a 1% agarose gel and visualized with
152 ethidium bromide or SYBR® Green staining. Successful PCR reactions were
153 enzymatically prepared for sequencing by digesting 5ul of PCR product in 0.5 U of
154 Shrimp Alkaline Phosphatase and 5U of Exonuclease for 30 minutes at 37°C followed by
155 15 minutes at 80°C. Forward and reverse sequencing reactions were performed with Big
156 Dye terminator chemistry and run on an ABI 3730 automated DNA Sequencer (Applied
157 Biosystems). Forward and reverse sequences were proofread in Sequencher™ 4.7 (Gene
158 Codes Corporation, Ann Arbor, Michigan) and all resulting 383-bp fragments were
159 aligned with ClustalX v2.0.12. The online tookit FaBox (Villesen 2007) was used to

160 reduce our final alignment to unique haplotypes and create an input file for the population
161 genetics data analysis program Arlequin 3.5.12 (Excoffier and Lischer 2010).
162 The species identity of our sampled haplotypes was confirmed with a neighbor-
163 joining tree run in PAUP* (Swofford 2003) that included the three most closely related
164 sister species found across our sampling range as outgroups– *Caesio lunaris*, *Caesio teres*
165 and *Caesio xanthonota*. We examined the frequencies and phylogenetic relatedness of
166 haplotypes in our dataset with a median-joining minimum spanning tree generated in
167 NETWORK v4.6 (Bandelt et al. 1999).
168 For each locality we used DnaSP v5 (Librado and Rozas 2009) to calculate
169 standard genetic diversity indices and tested the null hypothesis of neutrality in the
170 mitochondrial control region using Fu's F_S and Fu and Li's D* tests, with significance
171 determined by 1000 simulations of a neutral coalescent model. We employed the latter
172 two statistics to evaluate the potential effects of selection and demographic processes
173 such as population expansion on our data (Fu 1997).
174 To investigate the presence of barriers to dispersal and gene flow, we employed
175 both *a priori* and *post hoc* analyses. We first used examined population pairwise Φ_{ST}, and
176 performed an analysis of molecular variance (AMOVA) in Arlequin. For the AMOVA
177 analysis, we grouped sampling localities to test for hierarchical population structure
178 within our dataset following *a priori* hypotheses based on previously measured
179 phylogeographic breaks (Figure 3; Table 2) as follows: absence of genetic structure,
180 restricted gene flow east and west of the Makassar strait, a Sunda Shelf break at western
181 Sumatra, the Philippines vs. Indonesia, east vs. west of the Maluku Sea, and a break at
182 Cenderawasih Bay in Papua. All AMOVAs were run using sites with $n \geq 15$ and

183 employed the Tamura and Nei model of evolution, which was the model in Arlequin
184 most equivalent to the best model for our dataset determined by jModelTest v1.0 (Posada
185 2008; Guindon and Gascuel 2003). The significance of pairwise Φ_{ST} as well as among and
186 within population variance in the AMOVA framework was calculated using 30,000+
187 random permutations of the dataset. The p values for multiple pairwise comparisons were
188 adjusted using Bonferroni as well as Benjamini and Hochberg's (1995) false discovery
189 rate to reduce Type II error associated with the former method (Narum 2006).
190 In addition we employed a *post hoc* spatial analysis of the pairwise Φ_{ST} matrix
191 generated in Arlequin using the program BARRIER version 2.2 (Manni et al. 2004).
192 BARRIER characterizes the spatial relationship of sites from their GPS coordinates using
193 Voronoi tessellation and Delaunay triangulation and applies Monmonier's maximum
194 difference algorithm to a matrix of genetic distances (Φ_{ST} in this case) to identify genetic
195 barriers across geographic space. We tested the robustness of barriers by resampling
196 individuals within populations with replacement using Excel and creating 100
197 bootstrapped replicates of our pairwise Φ_{ST} matrix in Arlequin.
198 Since discrete genetic breaks can bias the results of analyses of Isolation by
199 Distance (IBD) and the presence of isolation by distance can generate false positives in
200 analyses of hierarchical structure (AMOVA) (Meirmans 2012), we employed partial
201 Mantel tests that controlled for both optimal AMOVA clusters and geographic distance
202 using the 'vegan' package for R (Oksanen et al. 2012; R Core Team 2012). Pairwise
203 genetic distances (Φ_{ST}) among localities with n > 15 were imported from Arlequin, and
204 negative pairwise Φ_{ST} values, a result of within population variance exceeding among
205 population variance, were set to zero. Our geographic distance matrix was generated

206 using a previously developed Python script that calculates shortest distance over water
207 from the GPS points of sample sites (Etherington 2011) in ArcGIS 9.3. We created a
208 third distance matrix that reflected the hierarchical structure of our best AMOVA
209 grouping by using a zero to code for localities within the same group and a one to code
210 for localities in different groups. We first tested for significant correlations between
211 genetic and geographic distance, using AMOVA group membership as a covariate. We
212 then tested the correlation between genetic distance and AMOVA grouping, using
213 geographic distance as a covariate. Significance was tested with 10,000 random
214 permutations, and the relationships among distances and clusters were plotted.
215
216 **Results**
217 A total of 625 fish were successfully sequenced at the mitochondrial control
218 region, representing 20 study sites across Indonesia and 13 study sites in the Philippines.
219 When aligned, 129 sites over the amplified 382 bp were polymorphic. There were 393
220 haplotypes, 308 of which were unique to a single individual. The highest frequency
221 haplotype was shared by 18 individuals.
222
223 *Phylogenetic Relatedness*
224 The unweighted mean pairwise difference between haplotypes in our minimum
225 spanning tree was 11.090 bp. All haplotypes from Medan and Padang, with the exception
226 of a single individual from Padang, fell within a divergent clade separated from all other
227 haplotypes by 8 mutational steps (Figure 2a,b). A single individual sampled at Makassar,
228 Sulawesi also fell within this divergent Indian Ocean clade. Regional clustering within

229 the Pacific lineage shows some evidence that the distribution of haplotypes is non-
230 random.
231
232 *Population Structure*
233 Haplotype diversity was high, measuring at or near 1 for all localities (Table 1).
234 Our two sites from Sumatra - Medan and Padang - had slightly lower nucleotide diversity
235 (0.0171 and 0.0169, respectively) compared to all other sites, which had nucleotide
236 diversities ranging from 0.0242 to 0.0356. While high haplotype diversity and low
237 nucleotide diversity could be an indication of recent population expansion, neither of
238 these sites had significantly negative values for Fu's F_s (Table 1). Across all sampled
239 localities, there were only two significant values for Fu and Li's D* which is more
240 sensitive to the effects of background selection (Fu 1997). However Fu's F_S, which is
241 more sensitive to signatures of demographic expansion and genetic hitchhiking, was
242 significantly negative at 11 of 13 sites in the Philippines and 14 of 20 sites in Indonesia,
243 indicating that the departures from neutrality can be ascribed to one of these two
244 processes (Fu 1997).
245 The results of our AMOVA analyses indicate significant genetic structuring in
246 *Caesio cuning* across the Coral Triangle (Table 2; $\Phi_{ST} = 0.1421$, $p < 0.00001$). Grouping
247 sites east and west of the Makassar Strait accounted for a non-significant portion of the
248 genetic variance between groups measured at this locus whereas grouping our two
249 western Sumatra sites separately from all others accounted for 47.96% of the genetic
250 variance ($\Phi_{CT} = 0.0258$, $p < 0.08554$ vs. $\Phi_{CT} = 0.4796$, $p < 0.00426$). Since the variance
251 generated by spatially explicit, divergent clades can overwhelm signatures of structure

252 within a dataset, we removed Medan and Padang from further AMOVA analyses. When
253 the remaining sites from the Pacific Clade were split into a Philippines' group and an
254 Indonesian group, the Φ_{CT} was significant but only explained 0.09% of the variance
255 between groups ($\Phi_{CT} = 0.0091$, $p < 0.02246$). Splitting sites east and west of the Maluku
256 Sea gave us our optimal partition and accounted for 4.50% of the variance between
257 groups ($\Phi_{CT} = 0.0450$, $p < 0.00023$). When this partition was shifted to Cenderawasih
258 Bay, it remained significant accounting for slightly less variance between groups ($\Phi_{CT} =$
259 0.0420, $p < 0.00083$). These patterns of genetic structure were echoed in the pairwise Φ_{ST}
260 values calculated for each pair of sampling localities (tables attached as supplemental).
261 Of the five tested breaks across the Coral Triangle, *C. cuning* exhibits two commonly
262 found in reef-associated, demersal species: a Sunda Shelf break at western Sumatra
263 (partition 2, Figure 3) and a break near the Maluku Sea in eastern Indonesia (partition 4,
264 Figure 3).
265 Spatial analysis of our pairwise Φ_{ST} matrix showed good agreement with our *a*
266 *priori* AMOVA results. Bootstrapping analyses reached their highest confidence values
267 when parameters were set to four barriers across the entire dataset (where $n \geq 15$). A
268 barrier between the polygon space of Medan and Padang and all other sites is always the
269 first to be placed by BARRIER and carries unanimous bootstrap support (1.00) regardless
270 of number of designated barriers (Figure 4a). The second barrier is found in the region of
271 Halmahera and the Maluku Sea, which carries the next highest confidence values (0.78-
272 0.80; Figure 4b). The third barrier was complex and found in the Philippines with the
273 most supported divisions between the southern Philippines and eastern Indonesia (0.49-
274 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 ($n \geq 15$) 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 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 ($p < 0.0657$). A partial Mantel test examining the correlation of pairwise Φ_{ST} to the location of sites within one or the other of our two optimal sites while accounting for geographic distance

298 resulted in an r of 0.5907 ($p < 0.0002$), indicating the hierarchical clustering of our sites
299 explains a significant percentage of the variance in our dataset while isolation by distance
300 does not. This is further supported by a Mantel test of only sites within the Philippines
301 and central Indonesia cluster (we were unable to run a Mantel test on the eastern
302 Indonesia cluster since all pairwise $\Phi_{ST} = 0$). We measured a Z of 2093.5389 and a
303 correlation coefficient (r) of 0.1258 with a non-significant p-value of 0.1306 (Figure 5b,
304 dotted line).
305
306 **Discussion**
307 *Patterns of genetic structure in a mid-water planktivore*
308 Hierarchical genetic analyses revealed two significant regions of genetic structure
309 across the Coral Triangle in the coral reef fish, *Caesio cuning*. A sharp genetic break was
310 observed across the Sunda Shelf barrier, echoing patterns reported from a diversity of
311 reef taxa including groupers, giant clams, crown-of-thorns seastars, damselfishes,
312 surgeonfish and snappers (Craig et al. 2007; Timm et al. 2008; Vogler et al. 2008; Drew
313 and Barber 2009; Eble et al. 2010; Gaither et al. 2010). Such population divergence
314 across the Sunda shelf is frequently attributed to historical vicariance between Pacific and
315 Indian Ocean populations during Pleistocene low sea level stands (e.g. Barber et al. 2000;
316 Rohfritsch and Borsa 2005; Deboer et al. 2008). In addition, significant departures from
317 neutrality, as measured by Fu's F_S, indicate the lingering effects of a Pleistocene
318 population expansion onto the Sunda and Sahul Shelves as sea levels rose during the Last
319 Glacial Maximum. Similar departures have been seen in every species examined in this
320 region so far (see Crandall et al. 2012). Shared phylogeographic patterns such as these

321 result from broadly acting physical processes that shape genetic patterns in codistributed
322 taxa (Avise 2000). However, the maintenance of these patterns in modern times, despite
323 the lack of physical isolation, likely results from oceanographic currents or reproductive
324 isolation between the two lineages.

325 During the northeast monsoon, the Southern Equatorial Counter Current (SECC)
326 bifurcates off the coast of southern Sumatra (Schott and McCreary 2001). During the
327 southwest monsoon, this reverses, and where Sumatra meets Java, a southeastern flow
328 hits a northwesterly flowing current that is driven by the Indonesian Throughflow. Both
329 monsoonal patterns have the potential to create a barrier to continuous gene flow at the
330 site of bifurcation and conjunction (Figure 2c), potentially reinforcing isolation during
331 periods of lowered sea levels. Support for this hypothesis comes from a recent
332 quantitative analysis using biophysical models coupled with matrix projection (Kool et al.
333 2011) that predicts the genetic isolation of populations in the Andaman Sea and western
334 Sumatra.

335 While studies of many reef organisms indicate divergence between Pacific and
336 Indian Ocean populations, only a few have sampled at a scale fine enough to illuminate
337 the extent and location of overlap between these divergent lineages (e.g. Barber et al.
338 2002, 2006; Crandall et al. 2008a,b; Deboer et al. 2008; Nuryanto and Kochzius 2009;
339 Gaither et al. 2011). The overlap between divergent Indian and Pacific Ocean lineages in
340 *Caesio cuning* is surprisingly small for such a potentially mobile fish. Haplotype
341 distributions from our minimum spanning tree indicate very limited gene flow between
342 the northern tip of Java and equatorial Sumatra – a distance of just over 800 km. No
343 landmass or geographical feature separates the waters of Padang (Sumatra) from the two

344 closest sample sites on Java, Anyer and Kepulauan Seribu, yet only a single individual
345 unites the maternal lineages of Padang to these two sites (Figure 2c). While regional
346 oceanographic patterns could be limiting the genetic connectivity in *C. cuning* across this
347 region, it is notable that across the same geographic range, the anenomefish *Amphiprion*
348 *ocellaris* shows greater admixture of Indian and Pacific maternal lineages in the Java Sea
349 (Timm and Kochzius 2008), and anenomefishes have a larval dispersal period of only 8-
350 12 days (Fautin and Allen 1992) and larvae exhibit natal homing (Jones et al. 2005).
351 Given the limited overlap of our two lineages, reproductive isolation between the clades
352 cannot be ruled out as a possible explanation for the absence of gene flow in this region.
353 In addition to the phylogeographic break observed at the Sunda shelf, significant
354 limits to genetic exchange were also seen in eastern Indonesia. At first pass, a significant
355 correlation between genetic distance and over-water distance suggests that limits to gene
356 flow in this region might be due a stepping-stone model of gene flow in which nearby
357 localities exchange more migrants than they do with distant localities (Figure 5b).
358 However, our partial Mantel tests clearly show that this appearance of isolation-by-
359 distance is actually an artifact of hierarchical structure between the two regions delimited
360 by BARRIER and AMOVA analysis (Figures 3 & 4; Table 2).
361 This genetic structuring across the Maluku Sea mirrors genetic structure and even
362 pronounced phylogeographic breaks east and west of Halmahera found in two species of
363 giant clam (Deboer et al. 2008; Nuryanto and Kochzius 2009) and 14 species of
364 stomatopods (Barber et al. 2006; Barber et al. 2011), suggesting this region may be
365 important for lineage diversification. While *Caesio cuning* populations on either side of
366 Halmahera are not characterized by distinct clades as is seen in western Indonesia, the

367 minimum spanning tree indicates some non-random, regional clustering of haplotypes.
368 Frequency differences among related haplotypes within the Pacific Ocean clade may be
369 caused by isolation facilitated by two eddies generated at the convergence point of the
370 Northern Equatorial Current and the New Guinea Coastal Current, the Mindanao Eddy
371 and the Halmahera Eddy (Figure 2c). The Halmahera Eddy has previously been
372 suggested as important for driving lineage diversification in the region of the Maluku Sea
373 (Barber et al 2002, 2006, 2011), however, both eddies direct a significant amount of flow
374 back into the Pacific Ocean, so both may be contributing to genetic isolation observed in
375 population genetic and computer modeling studies (Kool et al. 2011) conducted in this
376 region.

377 The recovery of multiple regions of significant genetic structure in *Caesio cuning*
378 is somewhat surprising because the high mobility potential of adults could result in
379 genetic admixture, such as the signal of secondary contact seen in migratory *Decapterus*
380 *macrosoma* (Borsa 2003). However, the concordance of our data to phylogeographic
381 patterns of demersal reef species with larval dispersal as well as to biophysical models of
382 larval dispersal (Kool et al. 2011) suggests that adult *C. cuning* are site-attached, and that
383 the major avenue of genetic connectivity in *C. cuning* is via larval dispersal. If adults are
384 truly site-attached, *C. cuning* would be dependent on larval dispersal to maintain gene
385 flow among populations across its range.

386

387 *Implications for management*

388 As a significant artisanal fishery in the Coral Triangle, *Caesio cuning* is subject to
389 anthropogenic population declines. A study of Sumilon Island in the Philippines

390 documented changes in reef fish density after protective management was removed for a
391 quarter of the island's reefs. Alcala and Russ (1990) measured a 64% decrease in
392 caesionid density after an eighteen-month period of fishing by approximately 100 local
393 fishermen from an adjacent island using hand-paddled canoes. Given that artisanal
394 fishing of caesionids has been shown to cause precipitous drops in local abundance, a
395 better understanding of stock structure is particularly important for the management of *C.*
396 *cuning*.

397 The results of this study suggest that *Caesio cuning* populations in the Philippine
398 and Indonesian portions of the Coral Triangle should be best viewed as at least three
399 stocks. However, managing a reef fishery at this scale is complex because these stocks do
400 not conform to national borders. We saw no significant genetic divergence across sites in
401 the Philippines and central Indonesia that are nearly 3000 km apart (see pairwise Φ_{ST}
402 table, supplemental material). This connectivity is likely facilitated by the Indonesian
403 Throughflow, a strong current originating in the Western Pacific that flows between
404 Kalimantan and Sulawesi and empties into the Indian Ocean via three major
405 "chokepoints" – the Bali-Lombok Strait, the Ombai Strait and the Timor Passage (Figure
406 2c). Dispersal simulations have predicted a net flow of larvae north to south via this
407 pathway (Kool et al. 2011). The boundaries among stocks in western, central and eastern
408 Indonesia all occur within Indonesian national borders, which potentially simplifies
409 management planning and authority. However, the absence of genetic structure between
410 the Philippines and central Indonesia implies that the diversity and abundance of larvae
411 produced from Philippine reefs could have an important impact on the sustainability and
412 genetic diversity of reefs of central Indonesia. This interdependence between countries

413 within the Coral Triangle emphasizes the importance of developing informed,
414 multinational management plans such as the Coral Triangle Initiative
415 (www.coraltriangleinitiative.org).

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

431

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449

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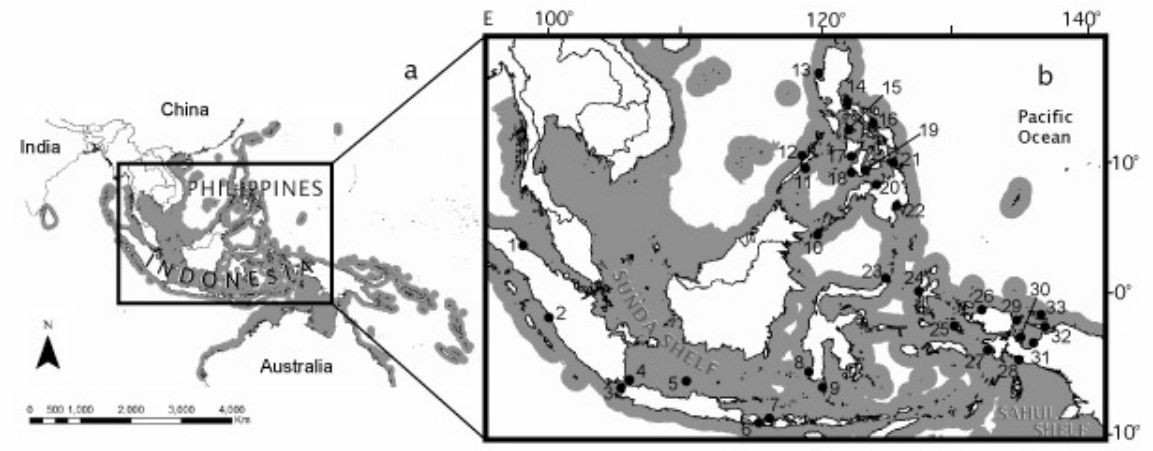
639

640 **Figures**



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642

643 **Figure 1 a.** The distribution of *Caesio cuning*. **b.** Inset. Sampling localities of this study:
644 Medan (1), Padang (2), Anyer (3), Seribu (4), Karimunjawa (5), Bali (6), Lombok (7),
645 Makassar (8), Selayar (9), Tawi Tawi (10), Honda Bay (11), Ulugan Bay (12), Bolinao
646 (13), Perez (14), Romblon (15), Sorsogon (16), Guimaras (17), Negros Occidental (18),
647 Negros Oriental (19), Balingasag (20), Dinagat (21), Davao (22), Manado (23),
648 Halmahera (24), Raja Ampat (25), Sorong (26), Fak Fak (27), Kaimana (28), Manokwari
649 (29), Windesi – Teluk Cenderawasi (30), Karei – Teluk Cenderawasi (31), Yapen (32),
650 Biak (33).

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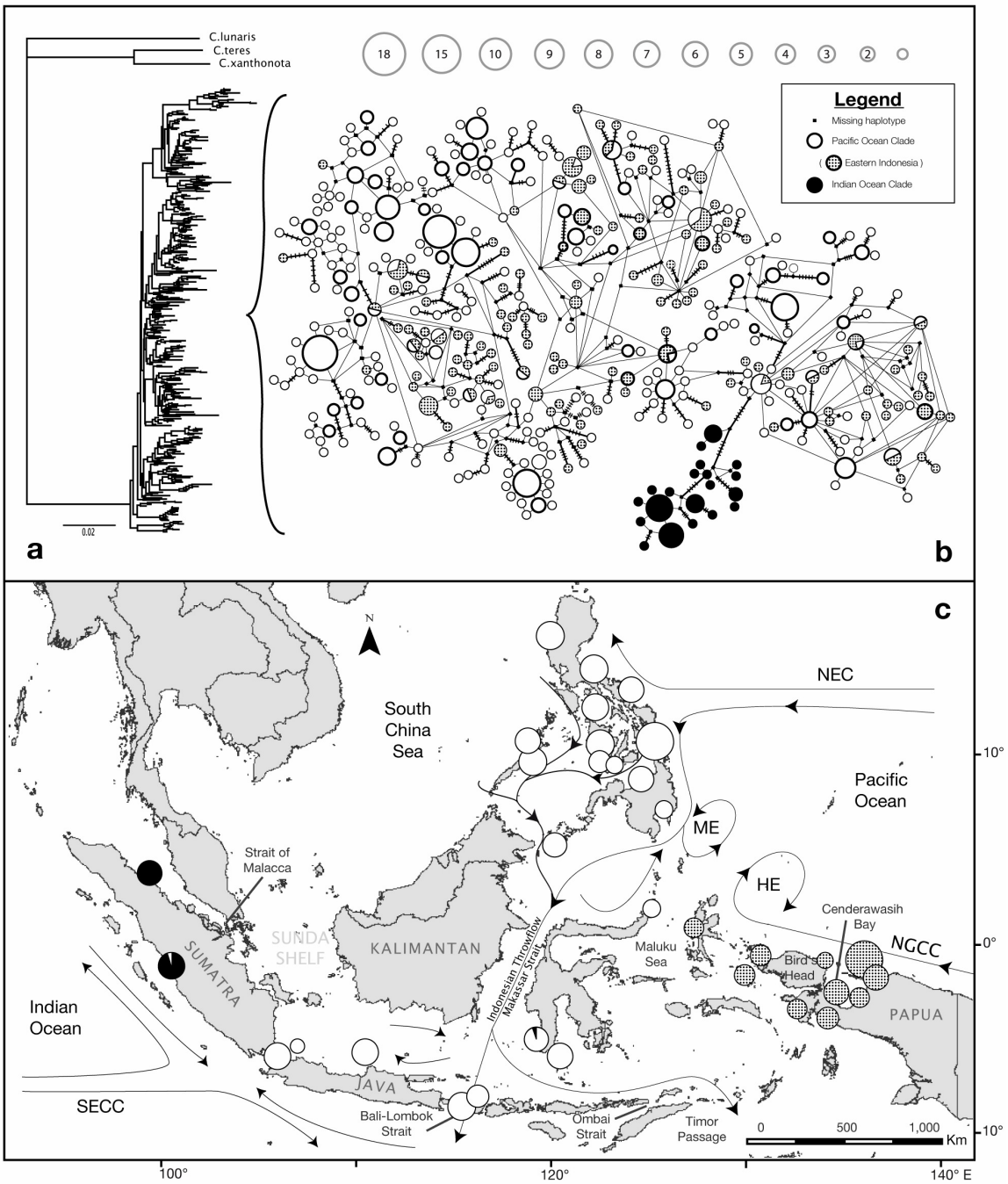


Figure 2. a. Neighbor-joining analysis depicting the relationship of our sampled *Caesio cuning* haplotypes to the three most closely related *Caesio* spp. in the region. **b.** Minimum spanning tree for mitochondrial control region haplotypes of *Caesio cuning*. Gray shading highlights the eastern Indonesian sites within the Pacific Clade, which uncorrected pairwise Φ_{ST}s 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).

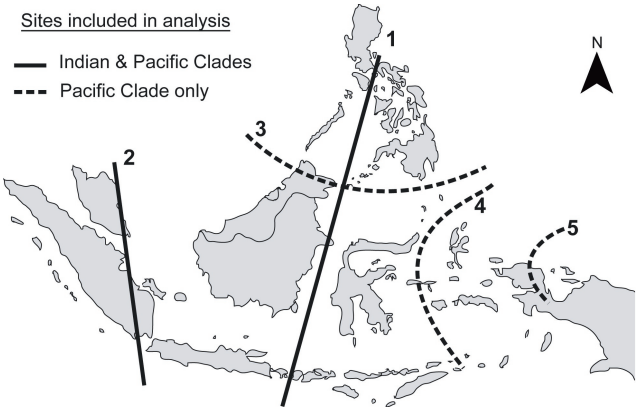
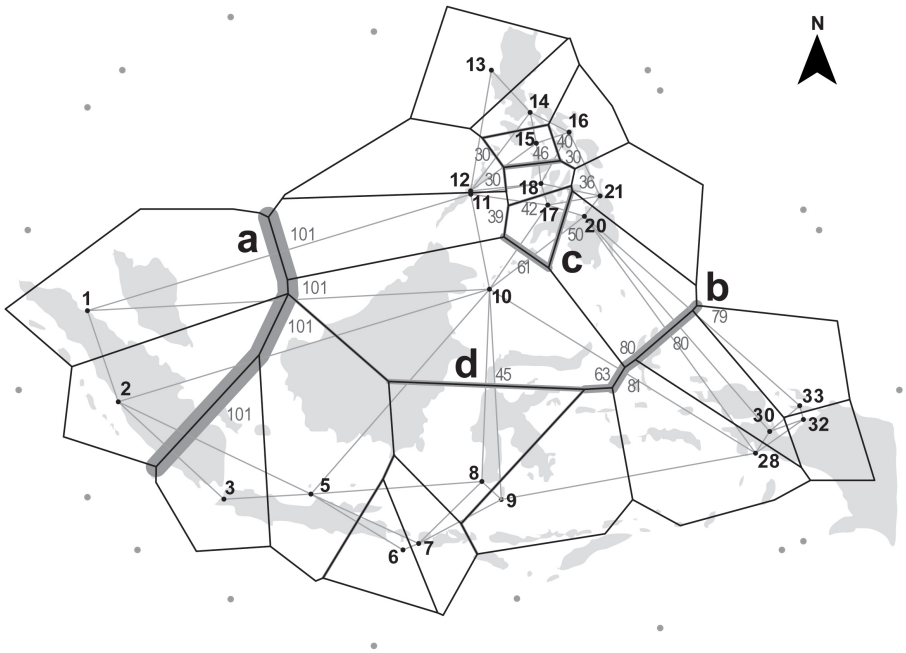


Figure 3. AMOVA Hypotheses Lines indicate the approximate locations of regional genetic breaks found in the mtDNA of other well-sampled coral reef and near reef species across the Coral Triangle (see Table 2). Solid lines indicate partitions tested with a hierarchical analysis of molecular variance that included sites from both the Indian and Pacific clades; dashed lines indicate partitions tested within the Pacific clade only.



683

684 **Figure 4. BARRIER Analysis** Spatial analysis of sites (n ≥ 15) with four barriers

685 designated (results labelled a-d) and corresponding confidence values labeled in gray

686 (100 bootstrap replicates +1). Black polygons indicate Voronoi tessellation, gray lines

687 indicate Delaunay triangulation. Thickness of barriers is relative to bootstrap support.

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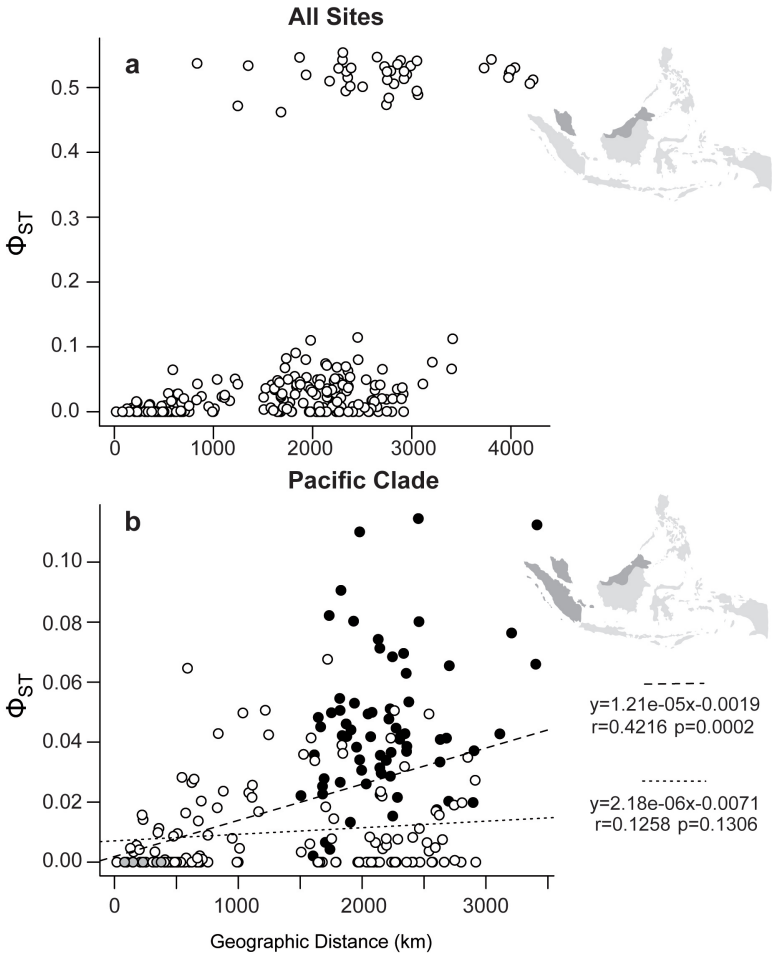

691

692 **Figure 5. Isolation By Distance graphs** Comparison of pairwise Φ_{ST} to geographic
693 distance for **a.** all sites with sample sizes greater than 15, showing clustering of Medan
694 and Padang associated with their spatial orientation and divergent clade, and **b.** Pacific
695 Clade only. Black dots are pairwise comparisons between sites belonging to different
696 AMOVA clusters, white dots are comparisons between sites within the Philippines and
697 central Indonesia cluster, and gray dots are comparisons between sites within the eastern
698 Indonesia cluster (all $\Phi_{ST} = 0$). The dashed line is the regression for all sites in the Pacific
699 Clade (significant due to presence of hierarchical structure), and the dotted line is the
700 regression for only sites across the Philippines and central Indonesia (white dots only;
701 non-significant).

702

703

704

Tables

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

	Sampling Locality	n	hap	*h*	*Π*	θ_s	F_S	D*
1	*Medan*	20	12	0.921	0.017	6.765	-1.641	-1.118
2	*Padang*	22	13	0.918	0.017	8.778	-2.168	-2.081*
3	*Anyer*	22	19	0.983	0.026	10.973	-7.154*	-0.322
4	*Seribu*	7	7	1	0.024	9.796	-1.725	-0.565
5	*Karimunjawa*	20	20	1	0.034	15.503	-10.469*	-0.072
6	*Bali*	26	22	0.982	0.026	10.482	-8.891*	-0.239
7	*Lombok*	16	15	0.992	0.029	11.452	-5.286*	-0.481
8	*Makassar*	18	18	1	0.027	13.665	-10.237*	-0.993
9	*Selayar*	20	15	0.942	0.025	10.429	-3.034	-0.794
10	*Tawi Tawi*	17	13	0.963	0.027	10.944	-1.984	-0.644
11	*Honda Bay*	26	23	0.991	0.028	11.793	-10.162*	-0.349
12	*Ulugan Bay*	21	19	0.991	0.026	10.562	-8.230*	-0.047
13	*Bolinao*	24	24	1	0.027	10.712	-16.723*	-0.527
14	*Perez*	25	24	0.997	0.026	11.388	-15.200*	-0.415
15	*Romblon*	17	17	1	0.028	10.649	-9.056*	-0.237
16	*Sorsogon*	19	18	0.994	0.025	10.872	-9.019*	-0.369
17	*Negros Occidental*	15	14	0.991	0.025	10.457	-5.352*	-0.767
18	*Guimaras*	26	25	0.997	0.027	12.579	-15.492*	-1.044
19	*Negros Oriental*	8	8	1	0.030	12.342	-1.933	-0.609
20	*Balingasag*	21	19	0.990	0.024	11.952	-8.981*	-0.446
21	*Dinagat*	46	44	0.998	0.024	13.197	-43.847*	-1.489
22	*Davao*	9	9	1	0.025	10.302	-2.911	-0.533
23	*Manado*	9	8	0.972	0.025	10.670	-1.157	-0.849
24	*Halmahera*	12	11	0.985	0.029	9.934	-2.627	0.494
25	*Raja Ampat*	13	10	0.949	0.026	10.312	-0.918	-0.633
27	*Fak Fak*	11	11	1	0.023	10.584	-4.636*	-0.797
28	*Sorong*	14	14	1	0.025	9.434	-6.906*	-0.409
28	*Kaimana*	16	16	1	0.026	9.644	-8.432*	0.037
29	*Manokwari*	8	8	1	0.031	12.727	-1.853	-0.436
30	*Windesi - Teluk Cenderwasi*	20	19	0.995	0.026	10.429	-9.444*	-0.591
31	*Karei - Teluk Cenderwasi*	13	13	1	0.024	10.634	-6.112*	-0.765
32	*Yapen*	21	19	0.991	0.025	9.728	-8.330*	-0.293
33	*Biak*	43	36	0.991	0.024	13.174	-24.146*	-1.963*

* denotes significant values of Fu's Fs and Fu and Li's D* (α=0.05).

713

714 **Table 2. AMOVA Summary**. Unstandardized results of AMOVA tests with localities
715 where n ≥ 15 using 30,000+ random permutations. Tested partitions are labeled 1-5
716 corresponding to illustrations in Figure 3. The first three analyses include both lineages,
717 while the lower three analyses examine genetic structure within the Pacific Clade. K
718 values give the number of groupings tested. P-values ≤ 0.05 indicate significant statistics,
719 and optimal partitions for each group of analyses are bolded. The last column "**e.g.**" lists
720 pelagic and demersal species that exhibit phylogeographic breaks in mtDNA on which
721 our hypotheses for partitioning are based.

	Hypothesis	Sites	Statistic		p	% var	e.g.
Both Clades (Indian & Pacific)							
	k = 1	**23**	-	-	-	-	*Decapterus macrosoma* (Borsa 2003)
			-	-	-	-	
			Φ_{ST}	0.1421	0.00001	14.21	
1	*k = 2; east vs. west of the Makassar Strait*	**23**	**Φ_{CT}**	0.0258	0.08554	2.58	*Decapterus russelli* (Rofristch and Borsa 2009)
			Φ_{SC}	0.1312	0.00001	12.78	
			Φ_{ST}	0.1537	0.00001	84.64	
2	*k = 2; Western Sumatra vs. all other sites*	**23**	**Φ_{CT}**	**0.4796**	0.00426	**47.96**	*Dascyllus trimaculatus* (Leray et al. 2010) *Acanthaster planci* (Vogler et al. 2008) *Tridacna crocea* (Deboer et al. 2008) *Nerita albicilla* (Crandall et al. 2008b)
			Φ_{SC}	0.0189	0.00003	0.98	
			Φ_{ST}	0.4894	0.00001	51.06	
Pacific Clade							
3	*k = 2; Philippines vs. Indonesia*	**21**	**Φ_{CT}**	0.0091	0.02246	0.091	*Hippocampus kuda* (Lourie et al. 2005)
			Φ_{SC}	0.0140	0.00136	1.39	
			Φ_{ST}	0.0229	0.00007	97.71	
4	*k = 2; central CT vs. eastern Indonesia at Halmahera*	**21**	**Φ_{CT}**	**0.0450**	0.00023	**4.50**	*Tridacna crocea* (Deboer et al. 2008) *Haptosquilla glyptocercus* (Barber et al. 2006)
			Φ_{SC}	0.0026	0.27264	0.25	
			Φ_{ST}	0.0474	0.00003	95.25	
5	*k = 2; central CT vs. eastern Indonesia at Cenderawasih Bay*	**21**	**Φ_{CT}**	0.0420	0.00083	4.20	*Haptosquilla pulchella* (Barber et al. 2006) *Tridacna maxima* (Nuryanto and Kochzius 2009) *Protoreaster nodosus* (Crandall et al. 2008a)
			Φ_{SC}	0.0056	0.11032	0.54	
			Φ_{ST}	0.0473	0.00003	95.26	

722