



Support for a ‘Center of Origin’ in the Coral Triangle: Cryptic diversity, recent speciation, and local endemism in a diverse lineage of reef fishes (Gobiidae: *Eviota*)



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ABSTRACT

The Coral Triangle is widely regarded as the richest marine biodiversity hot-spot in the world. One factor that has been proposed to explain elevated species-richness within the Coral Triangle is a high rate of *in situ* speciation within the region itself. Dwarf gobies (Gobiidae: *Eviota*) are a diverse genus of diminutive cryptobenthic reef fishes with limited dispersal ability, and life histories and ecologies that increase potential for speciation. We use molecular phylogenetic and biogeographic data from two clades of *Eviota* species to examine patterns, processes and timing associated with species origination within the Coral Triangle. Sequence data from mitochondrial and nuclear DNA were used to generate molecular phylogenies and median-joining haplotype networks for the genus *Eviota*, with emphasis on the *E. nigriventris* and *E. bifasciata* complexes – two species groups with distributions centered in the Coral Triangle. The *E. nigriventris* and *E. bifasciata* complexes both contain multiple genetically distinct, geographically restricted color morphs indicative of recently-diverged species originating within the Coral Triangle. Relaxed molecular-clock dating estimates indicate that most speciation events occurred within the Pleistocene, and the geographic pattern of genetic breaks between species corresponds well with similar breaks in other marine fishes and sessile invertebrates. Regional isolation due to sea-level fluctuations may explain some speciation events in these species groups, yet other species formed with no evidence of physical isolation. The timing of diversification events and present day distributions of *Eviota* species within the Coral Triangle suggest that both allopatric speciation (driven by ephemeral and/or ‘soft’ physical barriers to gene flow) and sympatric speciation (driven by niche partitioning and assortative mating) may be driving diversification at local scales within the Coral Triangle. The presence of multiple young, highly-endemic cryptic species of *Eviota* within the Coral Triangle suggests that (i) the Coral Triangle is indeed a ‘‘cradle’’ of reef fish biodiversity and that (ii) our current approximations of reef fish diversity in the region may be significantly underestimated.

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1. Introduction

1.1. Biodiversity within the Coral Triangle: origin, accumulation or overlap?

The world’s richest marine biodiversity hot-spot is located in a region known as the Coral Triangle (Allen and Werner, 2002; Veron et al., 2009; Carpenter et al., 2011). The Coral Triangle (CT), also known as the Indo-Malay Archipelago or the Indo-Australian Archipelago, is a region of islands in the Western Pacific that

includes the Philippines, Indonesia, Malaysian Borneo, Timor-Leste, Papua New Guinea and the Solomon Islands (Veron et al., 2009). The area is home to over 600 species of corals and over 2600 species of reef fishes (Veron et al., 2009; Allen and Erdmann, 2012). Species richness across a wide array of marine organisms declines with distance (both in latitude and longitude) from the CT (Stehli and Wells, 1971; Veron, 1995; Briggs, 1999a; Mora et al., 2003). Many hypotheses have been proposed to explain the exceptional species-richness within this region, including: (1) the center of origin hypothesis (Ekman, 1953; Briggs, 1999a, 1999b), which proposes that most species originate within the CT, likely due to elevated rates of speciation within the region, and that the abundance of new species originating within the CT

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provides a source of biodiversity for surrounding areas; (2) the center of accumulation hypothesis, which states that speciation occurs primarily in multiple peripheral regions, and fauna accumulate in the CT via dispersal of individual species (Ladd, 1960) or of entire assemblages following tectonic events (Remington, 1968; McKenna, 1973; Santini and Winterbottom, 2002); and (3) the center of overlap hypothesis (Woodland, 1983), which proposes that high biodiversity in the CT is due to its position straddling the boundary between the Indian Ocean and the Pacific Ocean, with each ocean having its own distinct fauna forming via vicariance as the two basins were separated, and fauna subsequently overlap in the CT as species expanded their ranges.

Empirical evidence in support of each of these hypotheses has begun to emerge over the last two decades, most recently in the form of molecular phylogeography studies (see Carpenter et al., 2011; Briggs and Bowen, 2013; Gaither and Rocha, 2013 for reviews). However, no single hypothesis has stood out as a front-runner in explaining most of the biodiversity in the CT. Instead, the processes proposed above are not mutually exclusive and may act in concert to produce exceptional biodiversity (Mironov, 2006; Halas and Winterbottom, 2009; Barber, 2009; Bowen et al., 2013). Species richness in the CT can also be explained in part by the availability of stable coral reef habitat in this region, especially during periods of quaternary glacial cycles, thus serving as an evolutionary refuge for species (Bellwood and Hughes, 2001; Pellissier et al., 2014). Pellissier et al. (2014) demonstrated a strong correlation between present day reef fish diversity and historical proximity to coral reef refugia during glacial cycles. Thus, in periods of dramatic climate change and habitat loss, the CT may still function as both a cradle of speciation as well as a repository for peripheral species.

The thousands of species of fishes that contribute to the diversity within the CT exhibit a variety of mating systems, locomotive behaviors, larval morphologies and dispersal capabilities, habitat requirements, life spans, reproductive output, and parental care regimes (Sale, 1993, 2002; Deloach, 1999; Leis and Carson-Ewart, 2000; Hixon, 2011). Each of these factors directly effects the potential of a lineage to diversify in a given place and time. Thus, one should not reasonably expect a single model to fit well when comparing a broad range of reef fish families and genera that differ dramatically in their ability to respond to a particular driver of speciation. On the other hand, one may expect that species that share similar ecological and life history characteristics would show similar patterns of evolution and biogeography, even if they belong to vastly different phylogenetic lineages. Thus, rather than searching for a single hypothesis explaining diversity across all organisms in the CT, it may be more informative to ask whether a given hypothesis is more likely to explain biogeographic patterns for a particular suite of organisms that possess certain ecological and life history characteristics. Conversely, by studying organisms exhibiting life history traits conducive to a particular mode or pattern of speciation we can examine evolutionary dynamics associated with the speciation of clades and ultimately the factors contributing to species richness within a region. Here we offer a model group of coral reef fishes for studying the processes and events associated with the origination of species within the CT.

1.2. Gobies and speciation within a center of origin

Empirical evidence suggests that sympatric, parapatric, and allopatric speciation are all responsible for the diversity within the CT (Briggs, 1999c, 2005; Bowen et al., 2013). If the CT indeed produces species at elevated rates relative to peripheral regions, these processes must be operating within very small geographic scales and within rapid timeframes. Thus, evidence for the center of origin hypothesis is likely to come from species with characteristics that facilitate allopatric speciation even within a localized

region where barriers to gene flow are ephemeral or 'soft' (e.g. periodic sea-level fluctuations due to glaciations; major oceanographic surface currents). Characteristics of such species would include extremely limited adult mobility, limited larval dispersal ability, fragmented populations and short life cycles with high turnover, enabling them to form distinct species after only short periods of physical reproductive isolation. On the other hand, speciation in sympatry is thought to occur very rapidly, perhaps even faster than what is required for allopatric populations (Landry et al., 2003; Coyne and Orr, 2004). Species that have increased potential for rapid, ecologically-driven sympatric speciation within the CT would have characteristics that facilitate the co-existence of closely related species via the exploitation of underutilized niches in a complex, heterogeneous reef environment, often in the face of intense competition from other distantly related reef-associated species. These characteristics facilitating both allopatric and/or sympatric speciation are present in the family Gobiidae (gobies), making them an excellent model group for elucidating processes contributing to speciation *within* the CT.

Gobies are one of the most diverse families of vertebrates in the world. With more than 1700 species spread across more than 200 genera, gobies make up a dominant fraction of the total fish diversity in a broad range of ecosystems including coral reefs (Murdy, 2011a, 2011b; Keith and Lord, 2011; Patzner et al., 2011; Pezold, 2011; Thacker, 2011; Eschmeyer, 2014; Thacker and Roje, 2011; Herler et al., 2011; Ahmadiya et al., 2012).

One of the most diverse clades of coral-associated gobies is the genus *Eviota*. Commonly referred to as dwarfgobies, species of *Eviota* are miniscule in size (frequently less than 20 mm total length) and occur on a broad range of microhabitats including a variety of hard corals, coral rock, rubble, and sand (Herler and Hilgers, 2005; Herler, 2007; Herler et al., 2011; Ahmadiya et al., 2012; Tornabene et al., 2013a). With over 90 valid species of *Eviota* described to date, dwarfgobies represent one of the most evolutionarily successful lineages of coral reef fishes. Their remarkable diversity may be explained by repeated habitat shifts from a strict obligate coral-dwelling lifestyle into sand or rubble habitats, promoting subsequent speciation in novel and underutilized niches (Tornabene et al., 2013a).

Evolutionary time scales may also be accelerated in dwarfgobies relative to other reef fish. The maximum life span of *Eviota sigillata* is 59 days and is the shortest known life cycle of any vertebrate (Depczynski and Bellwood, 2005, 2006). Short generation times (47–74 days) and high mortality result in rapid turnover of populations (Depczynski and Bellwood, 2005, 2006). Reproduction in dwarfgobies also likely plays a role in their evolutionary potential. Mating in *Eviota* occurs in pairs and is characterized by a series of well-choreographed, species-specific spawning interactions prior to deposition and fertilization of eggs (Sunobe, 1998; Sunobe and Nakazono, 1999). Females often chose males with stronger secondary sexual traits (e.g. dorsal fin length), and males spend significant effort guarding nests after fertilization (Sunobe and Nakazono, 1999; Sekiya and Karino, 2004; Karino and Arai, 2006). As a result, home ranges for mating pairs are very small, and thus their small adult size, localized spawning, and short pelagic larval duration of 24–26 days produce populations that may be genetically and demographically isolated. The combination of isolation and high turnover may increase the rates at which dwarfgoby populations respond to natural selection and are subjected to genetic drift.

1.3. Tips of the *Eviota* tree: the *E. nigriventris* and *E. bifasciata* species complexes

The *Eviota nigriventris* species complex is an ideal group for studying recent speciation within the CT. For 78 years *E. nigriventris*

was considered to be a single species until recent studies indicated the presence of a complex containing at least three species with distinct coloration (Greenfield and Randall, 2011; Greenfield and Tornabene, 2014). Similarly, *E. bifasciata* was considered a single species until studies suggested the presence of a species complex that includes at least two other taxa from the CT (Allen, 2001; Allen et al., 2013). The distributions of both of these species complexes are centered in the CT and do not extend considerably into the Indian Ocean or Central Pacific Ocean. Additional un-named color morphs in both complexes exist in the CT and peripheral regions. Therefore, these species complexes represent two independent instances where recent speciation events may have occurred within the CT, thus making them excellent groups to examine the factors generating biodiversity within the region. Furthermore, a comprehensive genetic survey of dwarf gobies in this region may reveal additional cryptic species in other clades of *Eviota*, ultimately resulting in a better understanding of the breadth of the diversity in one of the world's most diverse lineages of marine fishes.

The present study uses a molecular phylogenetic approach to study diversification at the tips of the dwarfgoby phylogeny. Here we present a time-calibrated molecular phylogeny for 133 specimens of *Eviota* from within the Coral Triangle, as well as external localities in Fiji, Pohnpei, Moorea, the Red Sea, Saipan, and Seychelles. By focusing our taxon sampling efforts on the *E. nigriventris* and *E. bifasciata* species complexes, we test several hypotheses regarding speciation in *Eviota* and diversification in the CT, including: (i) regional color morphs within the *E. nigriventris* and *E. bifasciata* complexes reflect genetic differences associated with distinct species; (ii) the timing of speciation events and the present day biogeography of the *E. nigriventris* and *E. bifasciata* complexes within the CT are consistent with the center of origin hypothesis; and (iii) contemporary species boundaries in *Eviota* correspond to well-documented genetic breaks found in other species within the CT.

2. Methods

We collected specimens of *Eviota* from throughout the Indo-Pacific region and additional samples were utilized from museum collections (Fig. 1; Supplementary Tables S1). Most specimens were photographed underwater or immediately following collection to help confirm identification and record color patterns. Fish were collected with hand-nets and a solution of clove oil anesthetic or ichthyocide. Specimens were stored whole in 95% ethanol prior to DNA extraction with a Qiagen[®] DNAeasy Blood and Tissue Kit (Qiagen, Valencia, California). Mitochondrial cytochrome c oxidase I (COI), and the nuclear gene protease III (Ptr) were amplified via PCR using primers from Thacker (2003; GOBYL6468,

GOBYH7696), Ward et al. (2005; FishF1-5, FishR1-5), and Yamada et al. (2009; PtrF2, PtrR2), with GoTaq[®] Hotstart Master Mix (Promega, Madison, Wisconsin). The thermal profile consisted of 2 min at 95 °C, followed by 35 cycles of 40 s at 95 °C, 40 s at 52–55 °C, and 90 s at 72 °C. Purification of PCR products and sequencing was performed by Beckman Coulter Genomics (Danvers, Massachusetts). The nuclear gene Ptr was included to help resolve relationships towards the root of the phylogeny, and very few phylogenetically informative sites were found between closely-related species at this locus. Sequences were assembled and aligned in Geneious R6 (Biomatters Ltd., available at <http://www.geneious.com>), and alignments were double checked by eye. Several specimens used in this study were sequenced in previous studies using similar protocols (Tornabene et al., 2013a, 2013b; Greenfield and Tornabene, 2014). Nexus files for sequence alignments are provided as Supplementary material.

Phylogeny of *Eviota* was inferred using Bayesian methods on the concatenated dataset partitioned by gene. Prior to concatenation, COI and Ptr alignments were analyzed in *jModeltest ver.0.1.1* (Posada, 2008) to determine the best fitting substitution models based on Akaike Information Criterion (AIC) scores. The COI and Ptr alignments were then concatenated and phylogeny was then inferred using *MrBayes ver.3.2*, with each gene receiving its own partition and substitution model. For each MrBayes analysis, two parallel Metropolis-coupled Markov Chain Monte Carlo runs were generated for 10,000,000 iterations with a sampling frequency of 1000 iterations. Stationarity and adequate mixing of each MCMC run was determined using the program *Tracer ver.1.5* (Rambaut and Drummond, 2007). Branch length priors for MrBayes runs were set to an unconstrained-exponential prior with a mean of 0.01 (default mean in MrBayes is 0.1) to remedy the common problem of MCMC chains converging on local optima with unrealistically long branch lengths (Brown et al., 2010; Marshall, 2010). Outgroups included the two Coral Goby genera *Gobiodon* and *Bryaninops*, and the more distantly related *Asterropteryx*. Within the *E. nigriventris* and *E. bifasciata* complexes, *Network ver. 4.6.1.2* (available at <http://www.fluxus-engineering.com/sharenet.htm>) was used to construct median-joining haplotype networks from the COI datasets (Bandelt et al., 1999). Alignments for median-joining analysis were truncated both in sequence length and in numbers of samples (individuals with large amounts of missing data were excluded) resulting in 1094 bp for the *E. nigriventris* group and 577 bp for the *E. bifasciata* group. Genetic distance matrices for these datasets are listed in Supplementary Tables S2 and S3.

Divergence times were estimated from the COI dataset using Bayesian methods in the program *BEAST ver.1.7* (Drummond et al., 2012). The fossil record for gobiids is depauperate and accurately identified fossils that would be appropriate for dating our tree do not exist. Therefore, our phylogeny was calibrated using

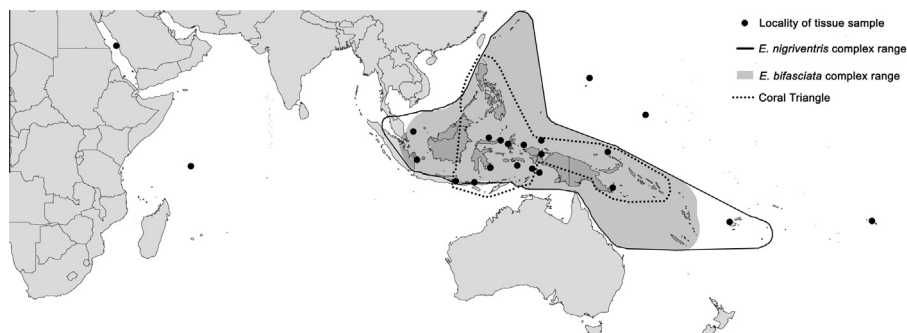


Fig. 1. Localities of samples used in this study. The range of the *E. nigriventris* complex is outlined in a solid line, and the range of the *E. bifasciata* species complex is shaded. The Coral Triangle, as delineated by Veron et al. (2009), is outlined by a dotted line.

COI mutation rate estimates calculated from the divergence between *Evorthodus lyricus* and *Evorthodus minutus* (approximately 0.048 mutations/site per 10^6 years), a geminate-pair of gobies separated by the Isthmus of Panama approximately 2.8 Ma. *Evorthodus* sequences used for estimating mutation rate were public data at the Barcode of Life Data System (Ratnasingham and Hebert, 2013; <http://www.boldsystems.org/>, accessed 10 April 2014). An uncorrelated lognormal relaxed molecular-clock prior was used for the analysis, as our data deviated significantly from a strict global molecular clock model ($p < 0.05$; likelihood ratio test). Mutation rates were drawn from a prior with a normal distribution centered on a mean of 0.05 with a standard deviation of 0.01. The topology of the *MrBayes* multi-locus tree was used as starting tree for the *BEAST* analysis.

3. Results

The monophyly of the *Eviota nigriventris* complex and the *E. bifasciata* complex were both well-supported, with the former being sister to the *E. lachdebereri* complex, and the latter being sister to a clade containing an undescribed species from Saipan and Pohnpei (Figs. 2 and 3). The precision of age estimations decreased towards the root of the phylogeny, where the median age estimate of the genus *Eviota* was 10.63 Ma with a wide 95% credible interval of 6.5–16.8 Ma. Ranges for age estimates were considerably narrower towards the tips of the phylogeny. The *E. nigriventris* complex and *E. bifasciata* complex are both less than 2.5 million years old, and most species on our tree have origins from the late Miocene through the late Pliocene (Fig. 3). However, in groups where taxon sampling was more robust, divergence times between recently-evolved lineages are of Pleistocene origin

(<0.5 Ma in the *E. nigriventris* complex, <1.5 Ma in the *E. bifasciata* complex).

The *Eviota nigriventris* complex includes six reciprocally monophyletic lineages (Fig. 4); *E. nigriventris*, from the Banda Sea, Sulawesi, Halmahera, Cendrawasih Bay, and Raja Ampat; *E. dorsogilva*, from the type locality in Fiji; *E. brahmi*, from the type locality in New Hanover, Papua New Guinea; *E. dorsopurpurea*, from the type locality in Milne Bay, Papua New Guinea; and two lineages that are similar to *E. dorsogilva* in morphology but are genetically distinct and have unique coloration, from Ferguson Island and New Hanover Island, respectively. The first split within this complex occurs around 1.25 Ma (0.71–2.08 Ma 95% HPD interval) with the divergence of a clade that includes *E. dorsopurpurea* and *E. brahmi*, the two of which then separate within 60,000–230,000 years ago. Subsequent splits within the *E. nigriventris* group occurred around 830 Ka (460 Ka–1.37 Ma), 330 Ka (170–560 Ka) and 210 Ka (90–380 Ka).

The *Eviota bifasciata* complex also contains six lineages (Fig. 5); *E. pamae*, from the type locality in the Kei Islands; *E. raja*, from the type locality in Raja Ampat; three lineages that are identified as *E. bifasciata*, with one from Cendrawasih Bay, a second from Bali and Anambas, and a third from Milne Bay, Papua New Guinea; and a unique eastern Indonesian color morph from the Banda Sea, Sulawesi, Halmahera and Raja Ampat. The five major speciation events within this complex occurred in rapid succession within a very narrow time frame (approximately 1 million years). The rooted phylogeny depicts the eastern Indonesia/Palau clade as the first lineage to diverge from the common ancestor. However, the median-joining haplotype network depicts six missing haplotypes at the center of the network that differ by less than 10 mutations. It is from these central haplotypes that each of the six major lineages diverge dramatically (12–37 mutations).

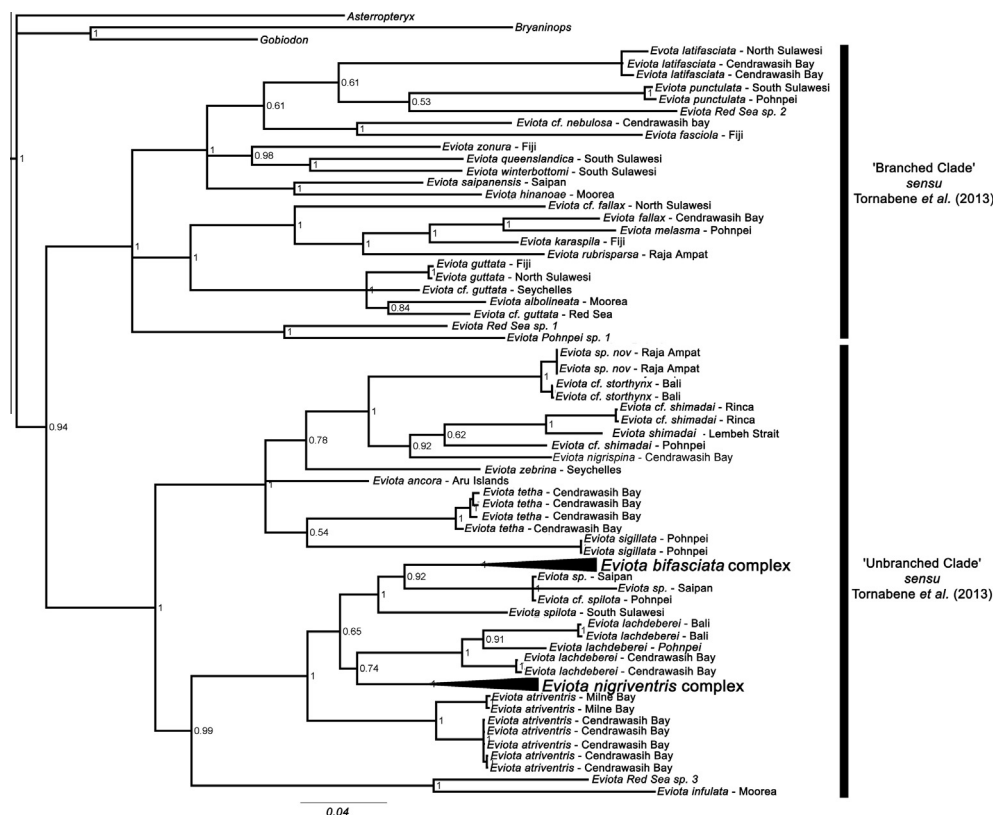


Fig. 2. Bayesian phylogeny of *Eviota* based on concatenated COI and Ptr datasets. Node values are Bayesian posterior probabilities. Nodes with values less than 0.50 are collapsed into polytomies. *Eviota nigriventris* and *E. bifasciata* complexes are collapsed as black triangles.

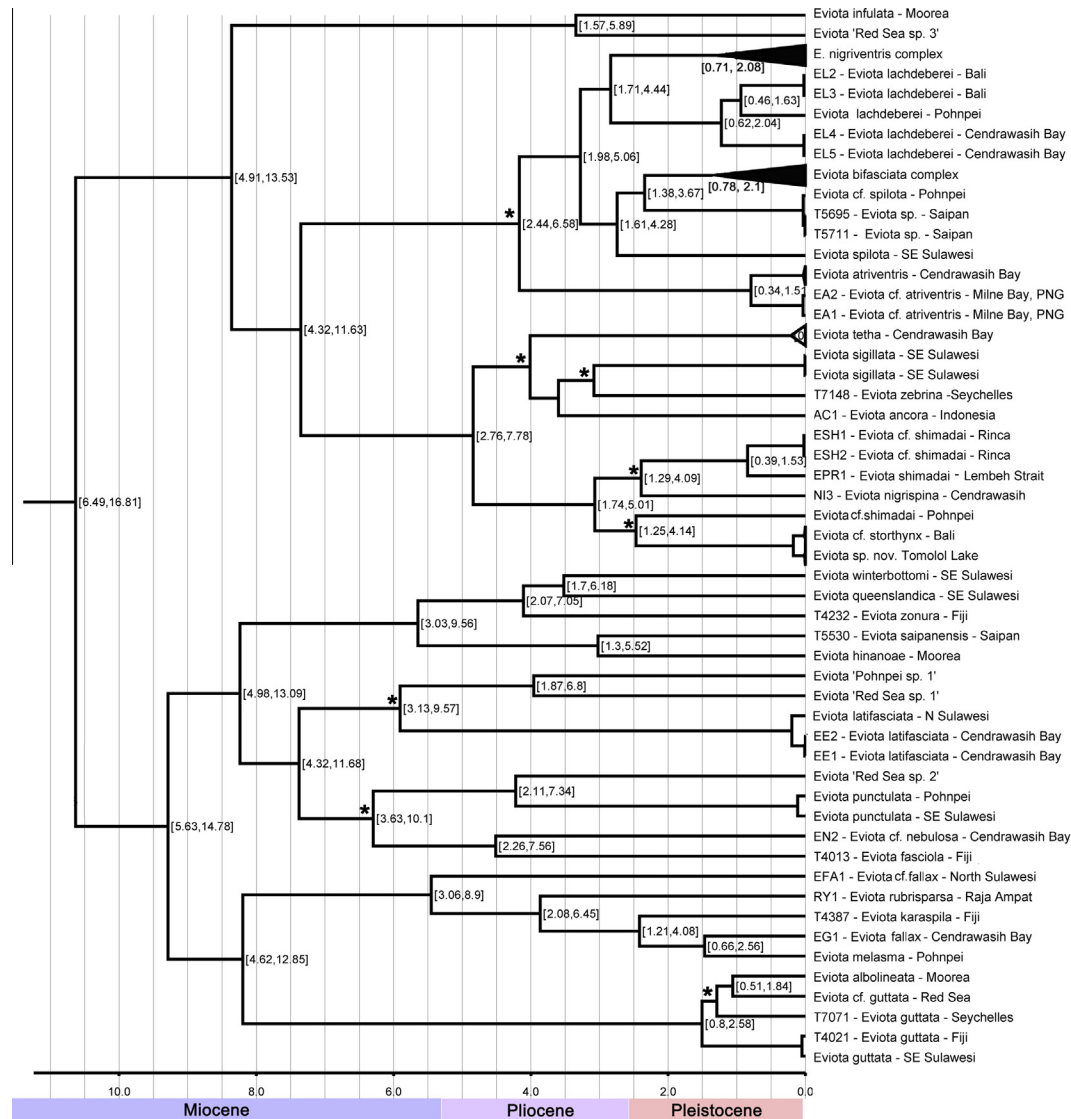


Fig. 3. Time-calibrated phylogeny of *Eviota* based on COI dataset. Units for the x-axis are 10^6 years. Node values represent the minimum and maximum values of the 95% highest-posterior-density intervals (credible intervals) of age estimates. Tips of the *E. bifasciata* and *E. nigriventris* complexes are collapsed (black triangles). Asterisks indicate relationships that are not supported in concatenated dataset.

4. Discussion

Renema et al. (2008) demonstrated the CT as a marine hotspot formed approximately 23 Ma with the collision of Australia with the Pacific arc and Southeast Asian margin. They summarize data from the fossil record and from dated molecular phylogenies to suggest that many groups that exist in the CT today have origins that predate the origin of the current CT hotspot, and that most of the recent diversification within the region pre-dates Pleistocene sea-level fluctuations (occurring mainly in the Pliocene or earlier). Our analysis of *Eviota* reveals that the genus as a whole is young, forming in the mid- to late Miocene after the CT hotspot was established. Most of the branching events on the phylogeny are in the late Miocene to early Pliocene, which is in agreement with the synopsis of Renema et al. (2008). However, species groups that have been sampled from multiple localities (e.g. *E. nigriventris* complex, *E. bifasciata* complex, *E. guttata* complex, *E. lachdeberiei*, *E. atriventris*) reveal multiple isolated genetic lineages (species) corresponding to regional color morphs that diverged more recently in late Pliocene through the Pleistocene. This pattern suggests that the lineages originating in the Miocene to Pliocene may have stem

ages that are old, but likely represent cryptic species complexes comprised of recently-diverged lineages that have yet to be sequenced throughout their range, thus inflating the estimated ages of individual species. This would mirror the pattern found in the gobiid genus *Trimma*, where nearly all morphologically defined 'species', when sequenced from multiple locations, represent complexes of cryptic lineages (Winterbottom et al., 2014). If this pattern of widespread cryptic diversity holds true in *Eviota*, the number of species in the genus may be well over 120–130 species, considering there are currently 91 described species (most based on morphology or color alone) and perhaps a dozen others that await description (authors' unpublished data; David Greenfield, pers. comm.).

The present-day distributions of the *E. bifasciata* and *E. nigriventris* complexes are centered in the CT (Fig. 1). Both species groups extend slightly beyond the CT in the north to the Ryukyu Islands and Palau, as well as to the east off Australia, Vanuatu, New Caledonia and Fiji (*E. nigriventris* complex only). Thus, the ranges of the groups themselves suggest an origin within the CT, as neither group occurs well into the Indian Ocean or the Central Pacific Ocean. Within each species group, there is strong agreement

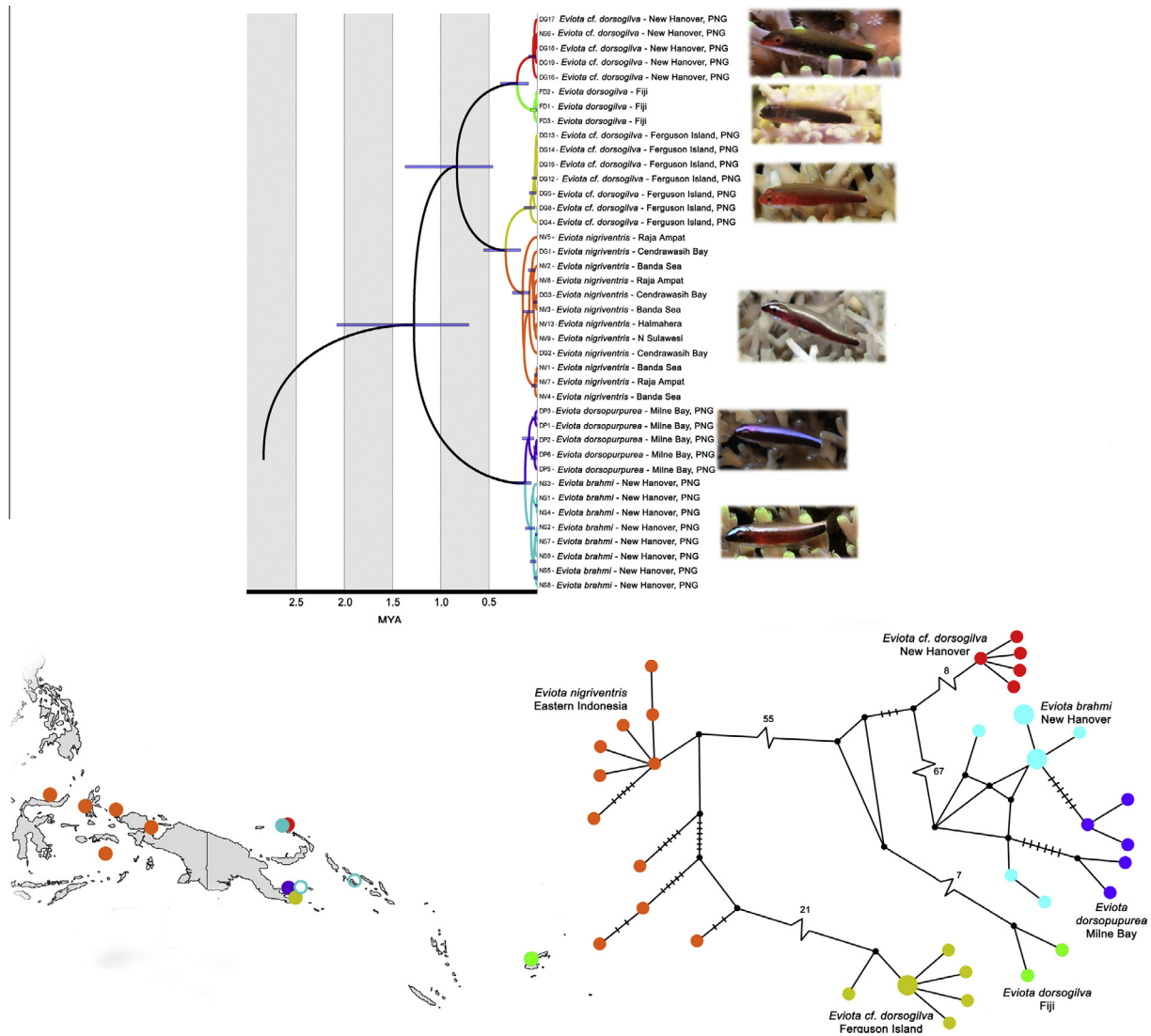


Fig. 4. Time-calibrated phylogeny and median-joining haplotype network of the *Eviota nigriventris* complex based on COI dataset. Blue bars at nodes are 95% highest-posterior-density intervals (credible intervals) for age estimates. Closed circles on the map are localities where samples from each lineage were collected. Open circles represent localities where a particular lineage/color morph was observed but not collected. Size of circles on haplotype network represents the number of individuals with that haplotype. Black circles represent missing haplotypes. Lines connecting haplotypes are one mutational step unless otherwise denoted by tick marks or numbers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

between genetic divergence, coloration and geography. Each group comprises at least six genetic lineages, with most lineages possessing unique geographic distributions and color morphs. These data indicate the presence of recent speciation occurring both within the CT, and between the CT and bordering localities.

4.1. *Eviota nigriventris* complex

The most recent speciation even in the *Eviota nigriventris* complex is between *E. brahmi* and *E. dorsopurpurea*. *Eviota brahmi* occurs in the Bismarck Archipelago, the Solomon Islands, and Milne Bay (Papua New Guinea), although tissues were only available from the Bismarck Archipelago. *Eviota brahmi* co-occurs with its sister species *E. dorsopurpurea* in Milne Bay, where the latter species is endemic. The sea-level fluctuations during the Pleistocene are unlikely to have caused the split between this species pair, as the waterways surrounding Papua New Guinea, the Bismarck Archipelago, and the Solomon Islands remained open during that period. The relatively widespread occurrence of *E. brahmi* suggests that ocean currents such as the South Equatorial Current/New

Guinean Coastal Current (SEC/NGCC), which passes between Milne Bay and the Bismarck Archipelago, may not be a barrier of dispersal between these localities. Thus, the recent divergence of *E. dorsopurpurea* represents a potential case of sympatric speciation within Milne Bay. In Milne Bay, both species have been observed in close proximity, but in distinct groups segregating between different species of coral (Greenfield and Randall, 2011). Thus, a combination of microhabitat partitioning and assortative mating between *E. brahmi* and *E. dorsopurpurea* may be responsible for creating and/or maintaining species boundaries. Habitat partitioning has also been observed between other closely related sympatric species of *Eviota* (e.g. *E. rubra* and *E. susanae* in Hawaii: Greenfield and Randall, 1999), as well as in sympatric pairs of Caribbean neon gobies that possess identical coloration (Colin, 1975), and in sympatric sister-species of coral-dwelling *Gobiodon* (Munday et al., 2004), with the latter example representing one of the strongest cases for sympatric speciation in the sea. The shallow genetic divergence between the *E. brahmi* and *E. dorsopurpurea* is striking given the dramatic color differences between the two, and is even less than the intraspecific variation seen in other *Eviota*

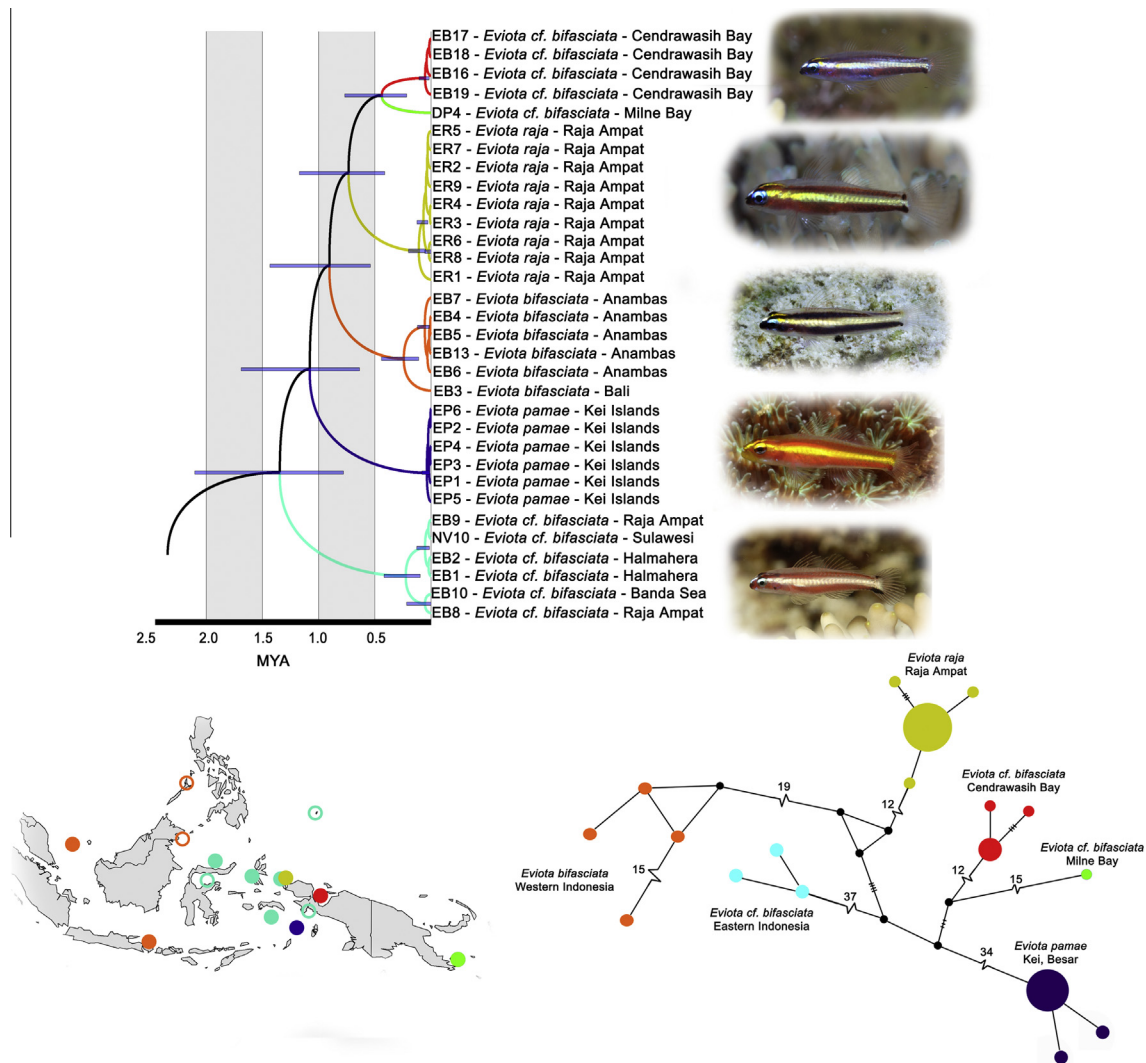


Fig. 5. Time-calibrated phylogeny and median-joining haplotype network of the *Eviota bifasciata* complex based on COI dataset. Blue bars at nodes are 95% highest-posterior-density intervals (credible intervals) for age estimates. Closed circles on the map are localities where samples from each lineage were collected. Open circles represent localities where a particular lineage/color morph was observed but not collected. Size of circles on haplotype network represents the number of individuals with that haplotype. Black circles represent missing haplotypes. Lines connecting haplotypes are one mutational step unless otherwise denoted by tick marks or numbers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

lineages. Nonetheless, the rooted phylogeny recovers the species as reciprocally monophyletic, and this appears to be a situation where divergence in color pattern evolved faster than mutations could accumulate in the mitochondrial genome – a pattern also observed in *Centropyge* (Gaither et al., 2014).

The divergence between *Eviota nigriventris* and a genetically-distinct undescribed color morph from southeast Papua New Guinea (*Eviota cf. dorsogilva*, Ferguson Island) corresponds with similar genetic breaks between Papua New Guinea and eastern Indonesia populations of nautilus, giant clams, stomatopods, and anemonefish (Wray et al., 1995; Benzie and Williams, 1997; Barber et al., 2002, 2006; Timm et al., 2008; Huelsenken et al., 2013). Concordant genetic breaks in this region may be maintained by the eastward reflection of the SEC/NGCC in the region north of the Bird's Head, West Papua that forms the Halmahera Eddy, an oceanographic feature that may present a barrier for dispersal of pelagic larvae. The last split within this group separates *E. dorsogilva* (Fiji) from a genetically distinct color morph in New Hanover, Papua New Guinea. This split has also been observed in five species of reef fish, *Pomacentrus moluccensis*, *Amphiprion melanopus*, *Chrysiptera talboti*, *Cirrhitilabrus punctatus* and *Labroides dimidiatus* (Drew et al., 2008).

Four of those species also possess distinct multiple color morphs that correspond with a Fiji/Indo-West Pacific genetic break.

Several lineages in the *Eviota nigriventris* complex have small distributions and appear to be endemic to small regions within the Coral Triangle or in the periphery. The notable exception is *Eviota nigriventris*, which occurs throughout eastern Indonesia. This species may also be more widespread than what is shown here, as the genetic identity of *Eviota nigriventris* in the NW portion of its range (Java, Sumatra, Philippines, Ryukyu Islands) is unknown. This species complex fits well with the 'peripheral budding' model proposed by Hodge et al. (2012, Fig 4c). Under this model, a large widespread parent species repeatedly produces a series of 'buds', or smaller isolated species via series of peripatric, parapatric or sympatric speciation events. These budded species can be local endemic species within the range of the larger species, or may be located on the periphery of the parent species. The phylogeographic pattern left by this process shows the widespread species as being very young on the phylogeny, despite it being the ancestral 'parent' species, as its sister species on the tree will always be the most recently formed 'bud'. In the case of the *E. nigriventris* complex, the eastern Indonesian *E. nigriventris* may have been

the parent population repeatedly ‘budding’ species to the east (and perhaps to the NW as well), the first of which led to the *E. brahmi*/*E. dorsopurpurea* lineage, the second led to the *E. dorsogilva* (Fiji)/*E. cf. dorsogilva* (New Hanover) lineage, and the last to the *E. cf. dorsogilva* (Ferguson Island) lineage.

4.2. *Eviota bifasciata* complex

The *Eviota bifasciata* complex contains six reciprocally monophyletic genetic lineages in the Coral Triangle (Fig. 5). Two of the six lineages in this complex have been described as distinct species; *E. pamae* from the Kei Islands, and *E. raja* from Raja Ampat. Another lineage occurs in western Indonesia (Bali, Anambas). This species has also been observed in Malaysia (Sabah), and near the *E. bifasciata* type locality in the Philippines, thus we tentatively consider this the ‘true’ *Eviota bifasciata*. However, this lineage possesses similar coloration to two other genetically distinct lineages labeled ‘*Eviota cf. bifasciata*’, one occurring in Cendrawasih Bay and a second in Milne Bay, the latter represented by a single specimen. The last lineage, also labeled ‘*Eviota cf. bifasciata*’, occurs in eastern Indonesia (Sulawesi, Halmahera, Banda Sea, Raja Ampat). This lineage has unique coloration and has also been observed in Palau. The five major speciation events within this complex occurred in rapid succession within a very narrow time frame (approximately 1 million years). The rooted phylogeny depicts the eastern Indonesia/Palau clade as the first lineage to diverge from the common ancestor. However, the median-joining haplotype network depicts six missing haplotypes at the center of the network that differ by less than 10 mutations. It is from these central haplotypes that each of the six major lineages diverge dramatically (12–37 mutations).

The biogeography of the *Eviota bifasciata* complex agrees well with the paleoceanographic history of the region during Pliocene–Pleistocene sea level fluctuations. During glacial periods beginning in the Pliocene, sea level in the CT dropped as polar ice caps formed. Drops in sea level reached as low as 120 m below current levels in the Pleistocene (Fig. 6; Voris, 2000). A sea level drop of 30–50 m effectively isolates the South China Sea from the Java Sea to the south via the exposure of the Sunda Shelf, and a drop of 120 m results in near-complete isolation of the South China Sea from the Celebes Sea to the east via the exposure and expansion of the Palawan Islands and Sulu Archipelago. This initial separation likely restricted gene flow and initiated speciation between the western Indonesian clade of *E. bifasciata* and populations to the east. Sea level rise in subsequent interglacial periods would allow for this species to expand its range south into the Java Sea, and east into the Celebes Sea. Interestingly, the Bali sample of *E. bifasciata* shows strong genetic divergence from the Anambas population, despite having similar coloration. It is likely that, after an initial southward range expansion, this Java Sea population was temporarily re-isolated from the South China Sea during subsequent glacial cycles with the re-emersion of the Sunda Shelf. More samples and photographs of the Java Sea population could help test this hypothesis and clarify the status of the Bali *E. bifasciata* sample. Present day gene flow between western Indonesian *E. bifasciata* and species to the east may be impeded by the Indonesian Throughflow, which passes 1–20 million m³ s⁻¹ of water through the Makassar Strait between Borneo and Sulawesi and exits the CT to the south via the Lombok Strait or to the east around the tip of Timor-Leste (Fig. 6; Godfrey, 1996; Schiller et al., 2008). This apparent genetic break between the South China Sea/western Indonesia and eastern Indonesia has been observed in the scad *Decapterus russelli*, the damselfish *Chrysiptera rex*, two species of anemonefish in the genus *Amphiprion*, and seahorses of the genus *Hippocampus* (Perrin and Borsa, 2001; Lourie et al., 2005; Timm et al., 2008; Drew et al., 2010).

A sea level drop of 40–120 m also further constricts the mouth of Cendrawasih Bay to an opening less than 100 km wide via the expansion of Yapen and Miosnum Islands (Fig. 6). This isolation may have been sufficient to restrict gene flow and result in the emergence of a unique Cendrawasih Bay haplotype. The presence of a distinct haplotype in Cendrawasih Bay is emerging as a repeated pattern in several groups of marine organisms including the sea star *Protoreaster nodosus*, anemonefishes, boring giant clams, and the stomatopod *Haptosquilla pulchella* (Barber et al., 2006; Timm et al., 2008; DeBoer et al., 2008; Crandall et al., 2008). Allen and Erdmann (2006) described an endemic wrasse from Cendrawasih Bay, moreover noting that other anomalous color morphs of reef fishes occur only within the bay. More recently, Allen and Erdmann (2012) and Greenfield and Erdmann (2014) list a total of 14 coral reef fish species (and mention a number of hard coral and mantis shrimp species) that are considered restricted range endemics of Cendrawasih Bay, including the dwarfgoby *E. tetha*. It has been hypothesized that the Tosem Block of the South Caroline Arc moved across the opening of the bay in the late Pliocene, resulting in an early period of isolation (Allen and Erdmann, 2006, 2012; Hill and Hall, 2003). The north coast of Papua receives a constant supply of surface water from eastern Papua New Guinea via the SEC/NGCC; however, flow into Cendrawasih Bay is minimal as most of the water passes north of the entrance and reflects eastward via the Halmahera Eddy (Schiller et al., 2008). Thus, it is likely that the complex history of tectonic movements, sea level fluctuations, and oceanographic currents (NGCC/Halmahera Eddy) all contribute to the isolation and observed endemism within Cendrawasih Bay. The Cendrawasih Bay haplotype is sister to a single specimen from Milne Bay, Papua New Guinea, differing by 27 mutations (Fig. 5). The Milne Bay population may show the same color pattern as the Cendrawasih Bay clade, however our only observation of Milne Bay *E. bifasciata* comes from a single photograph, and additional genetic samples and photos from Papua New Guinea are needed to help determine whether these haplotypes represent multiple species.

Eviota pamae has the most restricted range within the *E. bifasciata* complex, occurring only in the Kei Islands. The Kei Islands lie on the west side of ‘Lydekker’s Line’, which is recognized as the biogeographic divide between the Indo-Malay region and the Australia–New Guinea region (Simpson, 1977). No other member of the *E. bifasciata* complex has been observed in the Kei Islands, however the eastern Indonesian *E. cf. bifasciata* color morph was observed north of the Kei Islands at Kaimana, West Papua. One possibility is that *E. pamae* is a relic of a more widespread Sahul Shelf lineage that is now restricted to the Kei Islands after sea level rise, due to the lack of suitable coral habitat along most of the south coast of Papua. This seems unlikely as the species does not occur in the Aru Islands, which are home to reef-fish fauna typically associated with the Australia–New Guinea province (Simpson, 1977; M.V. Erdmann, pers. obs.). The mechanisms explaining the divergence of *E. pamae* and subsequent maintenance of genetic isolation remain obscure. Nevertheless, this species represents a clear example of recent speciation occurring at small geographic distances within the CT.

The other highly endemic species within the *E. bifasciata* complex is *E. raja*, which occurs in the Raja Ampat Islands. This species co-occurs with the eastern Indonesian lineage of *Eviota cf. bifasciata* throughout the Raja Ampat Islands, and both species have been observed cohabitating the same coral head (M.V. Erdmann, pers. obs.). Thus, niche partitioning via habitat preference does not appear to be occurring between these two lineages, and there are no known occurrences of intermediate color morphs that would suggest hybridization occurs in this contact zone. As with *E. pamae*, the mechanism promoting speciation of the highly endemic *E. raja*

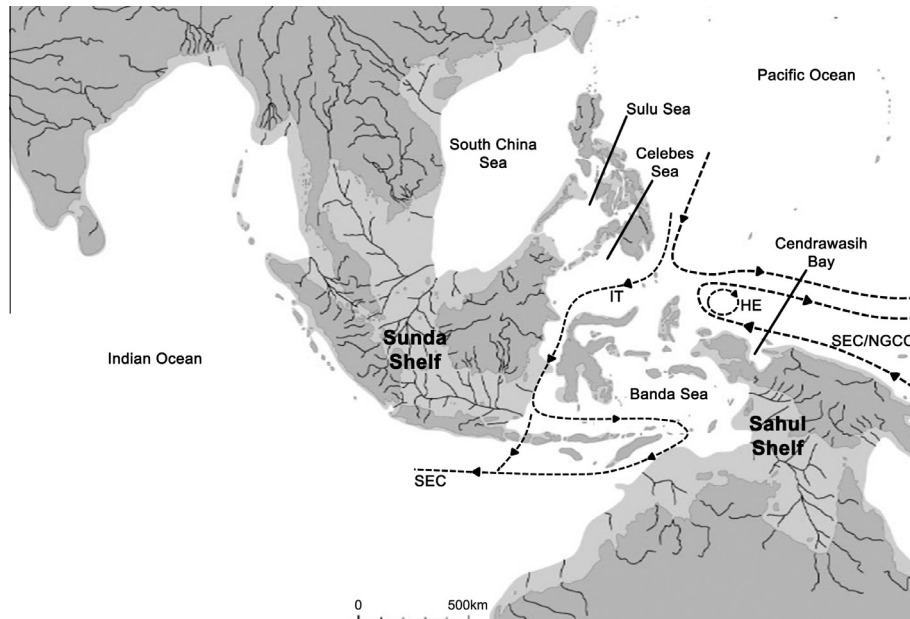


Fig. 6. Coral Triangle during glacial-maxima with sea-levels 120 m below current levels. Abbreviations: IT – Indonesian Throughflow; HE – Halmahera Eddy; SEC – South Equatorial Current; SEC/NGCC – South Equatorial Current/New Guinea Coastal Current. Sea-level map modified from Voris (2000). Currents based on Godfrey (1996) and Schiller et al. (2008).

remains elusive, but nonetheless represents an intriguing example of recent speciation and endemism within the CT.

4.3. Conclusions

In summary, concordance between color morphs, geography, and genetics indicate the presence of multiple highly-restricted endemic species these two species complexes from Milne Bay, New Hanover, Ferguson, Kei Islands, Raja Ampat, and Cendrawasih Bay. Major branching events occurred within the Pleistocene, and the patterns suggest speciation occurred in allopatry, parapatry and sympatry within the CT. On one hand, the timing of speciation events and present-day species distributions suggest that sea level fluctuations and subsequent isolation of water bodies likely played a part in the speciation process of some groups, yet other lineages appear to have diverged in the absence of hard physical barriers to gene flow. This suggests that a combination of both physical and biological factors is responsible for driving dwarfgoby speciation within the CT. The pelagic larval duration (PLD) for *Eviota* species is 24–26 days, which is not exceptionally short when compared to other fishes such as damselfishes and blennioids (Depczynski and Bellwood, 2006; Wellington and Victor, 1989; Riginos and Victor, 2001). Biophysical modeling suggests that regional self-recruitment may be high within the CT and may explain regional genetic structuring in individuals that are capable of settling after 15–30 days in the water column (Kool et al., 2011). Several studies have demonstrated that PLD is not a strong predictor of true dispersal capability, range size or levels of genetic structure (Victor and Wellington, 2000; Imron et al., 2007; Weersing and Toonen, 2009; Mora et al., 2012; Sotka, 2012; Luiz et al., 2013; but see Riginos and Victor, 2001). Instead, larval behavior, adult characteristics, or environmental traits may be more important (Woodson and McManus, 2007; Sotka, 2012; Luiz et al., 2013). Larvae that actively forage may disperse hundreds of km less than previous dispersal estimates, as species may actively avoid high-flow offshore with less primary productivity (Woodson and McManus, 2007). This may be the case for *Eviota* larvae, who exhaust yolk-sac resources in

the first two days after hatching and must immediately begin foraging or risk starvation by days three or four (Sunobe and Nakazono, 1987).

In the event of possible secondary contact between *Eviota* lineages, interspecific competition for habitat could be a strong driver of ecological partitioning (Hobbs and Munday, 2004; Munday, 2004a, 2004b; Munday et al., 2001). We see some evidence of this in the sympatric contact zone of *E. brahmi* and *E. dorsopurpurea* in Milne Bay, where closely related species show strong niche partitioning and segregate into distinct groups of conspecifics, but not in *Eviota raja* and the eastern Indonesian *E. cf. bifasciata* which frequently co-occur over a single coral head. Mating between color morphs of *Eviota* may also be prevented by their species-specific pre-mating behaviors and females that are highly selective for males with the strongest secondary sex characteristics (Sunobe, 1998; Sunobe and Nakazono, 1999; Sekiya and Karino, 2004; Karino and Arai, 2006).

Lastly, the age of speciation events in our study contradict Bellwood and Meyer's (2009) observation that CT endemics have origins dating back 4–25 Ma and likely represent the 'last stand' for previously wide-spread species that have seen their ranges shrink over time. The discrepancy between our dates and those of Bellwood and Meyer (2009) are likely due to differences in the completeness of taxon sampling, and previous studies relying mostly on taxonomically recognized species (ignoring cryptic species), potentially inflating node ages (Rocha and Bowen, 2008). Nonetheless, our findings highlight the importance of the CT for generating new biodiversity, especially in groups with limited dispersal potential, that display evidence of local niche partitioning, and that have potential for assortative mating. The presence of numerous cryptic lineages across the *Eviota* phylogeny suggests that previous estimates or species richness in *Eviota*, and of cryptobenthic reef fishes in the CT in general, may be significantly underestimated. This places increased responsibility on taxonomists to integrate this phylogeographic information into their naming and circumscribing evolutionarily distinct lineages; named species are far more likely to benefit from international conservation legislation and planning tools (Mace, 2004).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2014.09.012>.

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