Evolution of microhabitat association and morphology in a diverse group of cryptobenthic coral reef fishes (Teleostei: Gobiidae: Eviota)

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to the marine environment, creating both ecological and spatial opportunities for subsequent speciation in a new, expansive and complex environment (Thacker, 2009). A more recent analysis of the Gobiidae sensu Thacker (2009) revealed it to comprise 13 distinct lineages, each united by a combination of morphological, molecular, biogeographic, ecological, or behavioral characteristics (i.e. coral gobies, lagoon gobies, reef-associated shrimp gobies, etc.; Thacker and Roje, 2011).

Ties between ecological shifts and species diversification are obvious within several subgroups of gobies. Neogobines in the subfamily Benthiophilinae (Ponto-Caspian & Mediterranean lineage of Thacker and Roje (2011)) achieved remarkable diversity via a shift from saltwater to freshwater, promoting subsequent allopatric speciation by isolation of freshwater basins (Neilson and Stepien, 2009). Speciation within mudskippers (genera Boleophthalmus, Oxuderces, Periophthalmodon, Periophthalmus, Scartelaous and Zappa) was facilitated by differentiation into several ecological guilds characterized by differences in salinity, water quality and habitat terrestriality (Polgar et al., 2010). Interstitial gobies of Japan (genus Luciogobius) underwent adaptive radiation and morphological diversification driven largely by ecological differentiation into interstitial habitats with differing sediment properties (Yamada et al., 2009). Recent speciation within the neon gobies of the Caribbean genus Elacatinus occurred in distinct stages, with several stages being driven in part by ecology. Early in their evolutionary history, neon gobies first segregated into groups occurring on sponge versus coral microhabitat, followed by differentiation into different feeding strategies, colors and morphologies (Taylor and Hellberg, 2005; Colin, 2010). Thus, studies of diversification within and among goby genera suggest that ecological plasticity in gobies has occurred throughout the evolutionary history of the group and is not limited to ancient timelines.

1.2. Indo-pacific dwarfgobies

The coral gobies (Thacker and Roje, 2011) comprise 13 genera, most of which occur on coral reefs and have obligate or facultative relationships with live coral (Herler, 2007; Herler et al., 2009; Thacker and Roje, 2011). While most genera of coral gobies include fewer than 20 species, a notable exception is the genus Eviota, commonly known as dwarfgobies. There are over 60 valid species of Eviota and many more species have yet to be described (Gill and Jewett, 2004; Shibukawa and Suzuki, 2005; Herler et al., 2009; Froese and Pauly, 2012). In addition to their exceptional species richness, the rapid life-cycle, high fecundity and overall abundance of Eviota make them an important component of the trophic webs on coral reefs (Dempzynski and Bellwood, 2003, 2005, 2006). Past studies on coral goby habitat associations suggested that, in comparison to several closely related genera of obligate coral dwelling species (Gobiodon, Pleurosicya), some species of Eviota have less stringent associations with live coral and do not possess species-specific coral associations (Herler and Hilgers, 2005). Prior studies featured only a few Eviota species, and little is known about the ecology of the vast majority of the genus.

Eviota species are miniscule in size (typically 10–20 mm in standard length) and reliable diagnostic morphological characters are scarce, likely due to the high degree of reduction, simplification or loss associated with miniaturization. The presence or absence of branching pectoral fin rays and sensory pore patterns on the head are frequently used to diagnose species of Eviota (Lachner and Karnella, 1980). Pectoral fin morphology is highly specialized in gobiids, as these fins are used primarily for propulsion (Adriaens et al., 1993). In addition to swimming, many benthic fishes also utilize pectoral fin rays for maintaining position against a current, or gripping and manipulating the substrate (Webb, 1989; Brandstätter et al., 1990; Taft, 2011). Thus, the presence of branched versus unbranched pectoral rays may alter the way Eviota species perform these actions and could potentially relate to the microhabitat with which they associate. Sensory pores of gobies are an extension of the lateral line system, which has long been known to be adapted to specific habitats or lifestyles of fishes (Coombs et al., 1988). Accessory lateral-line systems (sensory pores, sensory papillae) in gobies have been widely used as taxonomic characters, however variation (often via reduction) in these systems both within and between species may be related to differences in microhabitat characteristics such as turbidity, flow and location in the water column (Ahnelt, 1995; Ahnelt et al., 1995, 2004; Ahnelt and Scottolin, 2003; Ahnelt and Bohacek, 2004; Stelbrink and Freyhof, 2006; Asaoka et al., 2011).

The purpose of the present study is to investigate the evolution of microhabitat association in Eviota species. Additionally, we examine the evolution of sensory pores and pectoral fin-ray branching to explore the relationship between morphology and microhabitat use, and ultimately evaluate the relative role of both microhabitat and morphology in the diversification of Eviota. We infer phylogenetic relationships of 18 species of Eviota collected from three regions of the Pacific Ocean and the Red Sea using molecular data from mitochondrial and nuclear DNA. The phylogenetic structure will serve as a framework to explore the association between species habitats and morphology across the evolutionary history of the group. Through this approach, we will be able to investigate the following questions: (i) are Eviota species that occur in similar habitats closely related to one another, or has the use of specific microhabitats evolved several times independently; (ii) are patterns of pectoral fin-ray branching and sensory pores homologous in Eviota; (iii) is the evolution of microhabitat association correlated with the evolution of pectoral fin-ray branching and sensory pore patterns; (iv) and did Eviota diversification occur in “stages”, similar to that of the Atlantic neon gobies Elacatinus, with each stage of speciation being characterized either by a unique ecological shift or a morphological change (Streelman and Danley, 2003; Taylor and Hellberg, 2005).

2. Methods

2.1. Collecting specimens and characterizing habitat association

Fishes were collected from four regions throughout the geographic range of Eviota species: Saudi Arabia (Red Sea), French Polynesia (Moorea), Indonesia (Hoga, southeast Sulawesi), and Micronesia (Pohnpei). All collections were from shallow fringing and patch reefs (<10 m). Past studies on habitat specificity of coral gobies demonstrated that unlike species of Gobiobodon, Bryaninops and Pleurosicya, the few Eviota species observed did not show obvious species-specific coral preferences (Herler and Hilgers, 2005; Herler, 2007). Therefore, our collections did not target specific species of coral, but instead focused on three broad microhabitats of the reef: sand, rubble and all hard coral. We sampled 106 hard coral, 57 rubble, and 63 sand microhabitats for a total of 229 sample events; 64 from Indonesia, 84 from Moorea, 63 from Pohnpei, and 18 from the Red Sea. Microhabitats were sampled using a circular fine mesh net (1 m diameter) covered with a plastic sheet weighted on the edges. The plastic sheet was used to contain fish anesthetic, a 100 ml mixture of a four parts ethanol to one part clove oil. Anesthetic was dispersed within the sample area using two 60 ml syringes. The anesthetic remained in the net for 1 min, then the plastic sheet and net were slowly removed and divers collected anesthetized fishes for 5–10 min. Fishes in crevices and holes were dislodged with forceps and a dental pick. Specimens were placed in ice slurry to preserve pigmentation for photographs to help in taxonomic identification. Specimens were then preserved whole in 95% ethanol.
A species was determined to be associated with a specific habitat type when at least 60% of the total number of specimens of that species were collected from that habitat. If species were not observed to have associations to one microhabitat type, than they were considered associates of multiple microhabitats and categorized accordingly.

2.2. Specimen identification and morphological character coding

Specimens of *Eviota* are notoriously difficult to identify partially because of their diminutive size (typically 10–20 mm), the large number of undescribed species, and the lack of a comprehensive published dichotomous key. As mentioned above, there are a very limited number of morphological characters useful for diagnosing species. An unpublished draft of a dichotomous key for all valid *Eviota* species plus several undescribed species was graciously provided by Richard Winterbottom and Dave Greenfield to supplement original species descriptions and assist in our identifications. Nevertheless, some species in our phylogeny did not perfectly fit the current species descriptions and may represent additional undescribed species. All specimens in this study are listed in Table 1 along with comments on identifications. GenBank Accession numbers and museum catalog numbers are also provided.

The morphology of pectoral fin-rays in *Eviota* fall into two general categories; branched and unbranched. Branched fin-rays typically begin as a single rigid ray at the base of the fin and bifurcate into two or more branches towards the distal tips of the ray. The number of branched rays is variable among species, and most branched rays are located in the central portion of the fin, whereas branched rays are located in the central portion of the fin, whereas

### Table 1

Specimens examined. Acronyms for catalog numbers: AMNH, American Museum of Natural History; CAS, California Academy of Sciences; MZB Museum Zoologicum Bogoriense. DII/A = number of second dorsal and anal fin rays.

<table>
<thead>
<tr>
<th>Species</th>
<th>Country</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Genbank Accession Numbers</th>
<th>Catalog number</th>
<th>Notes on identification</th>
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<td><em>Eviota albolineata</em></td>
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<td>5°28′28.04″S</td>
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<td>JX483989, JX483966</td>
<td>AMNH 256486</td>
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<td><em>Eviota cf. spilota</em></td>
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<td>6°57′55.79″N</td>
<td>158°18′22.74″E</td>
<td>JX483984, JX483970</td>
<td>CAS 234536</td>
<td>9/8 rays in DII/A; 5th pelvic ray ≈ 40% of 4th ray; pore pattern 2; pectoral rays unbranched; but lacks diagnostic spot on pectoral base present in <em>E. spilota</em> pattern 1; pectoral rays unbranched; possesses pore pattern 2.</td>
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<tr>
<td><em>Eviota Red Sea sp. 1</em></td>
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<td>39°8′34.43″E</td>
<td>JX483977, JX483972</td>
<td>CAS 234525</td>
<td>8/9 elements in DII/A; 5th pelvic ray ≈ 10% of 4th ray; pore pattern 1; pectoral rays branched</td>
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<td>39°8′34.43″E</td>
<td>JX483997, JX483975</td>
<td>CAS 234526</td>
<td>9/9 rays in DII/A; 5th pelvic ray ≈ 10% of 4th ray; pore pattern 1; pectoral rays branched</td>
</tr>
<tr>
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<td>39°8′34.43″E</td>
<td>JX483978, JX483995</td>
<td>CAS 234527</td>
<td>9/9 rays in DII/A; 5th pelvic ray ≈ 20–30% of 4th ray; pore pattern 3; pectoral rays heavily damaged</td>
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<td><em>Eviota cf. guttata</em></td>
<td>Saudi Arabia (Red Sea)</td>
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<td>39°8′34.43″E</td>
<td>JX483993, JX483963</td>
<td>CAS 234528</td>
<td>Resembles <em>E. guttata</em> in all counts, measurements, and pigment patterns but possesses pore pattern 3 vs. pattern 1 as in <em>E. guttata</em> 9/8 rays in DII/A; typically pore pattern 2 (sometimes pattern 1); pectoral rays branched; resembles <em>E. zonaru</em> but lacks prominent spots on pectoral fin base</td>
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<td>123°45′29.84″E</td>
<td>JX483953, JX483968</td>
<td>CAS 234535</td>
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<td>123°45′29.84″E</td>
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<td>MZB 29011</td>
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<td>JX483987, JX483974</td>
<td>MZB 20917</td>
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<td>158°14′30.36″E</td>
<td>JX483980, JX483973</td>
<td>CAS 234531</td>
<td>9/9 elements in DII/A; pelvic fin broken; pore pattern 2; pectoral rays branched; prominent spot on caudal peduncle; no vertical bars on cheek</td>
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<td>JX483999, JX483960</td>
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<td>158°14′30.36″E</td>
<td>JX483982, JX483964</td>
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<td><em>Bryaninops ridens</em></td>
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<td>JX483991, JX483957</td>
<td>AMNH 256488</td>
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<td><em>Gobiodon unicolor</em></td>
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<td>JX483995, JX483958</td>
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the rays on the dorsal and ventral portions of the rays usually show less branching. For our analysis, species were coded as “branched” if any of the rays on the fin were branched, or “unbranched” if all rays were unbranched. Coding of sensory pore patterns follows terminology of Lachner and Karnella (1980), with pattern 1 being a “complete” pattern with all head pores present, and all other patterns having one or several pores absent or fused (Fig. 1).

2.3. DNA sequencing

Total genomic DNA was extracted from ethanol-preserved tissue samples using the Qiagen®DNAeasy Blood and Tissue Kit (Qiagen, Valencia, California). Partial mitochondrial cytochrome oxidase I (COI) sequences were targeted using the primers GQB-YL6468 (5’GCTCAGCCATTTTACCTGTC3’; Thacker, 2003) and GQB-YH7696 (5’AGGCCTTAGGAATTGAGGGAAAG3’; Thacker, 2003). A segment of the nuclear gene protease III (Ptr) was targeted using the primers PtrF2 (5’TGCCTCATGGATTTCACAAAT3’, Yamada et al., 2009) and PtrR2 (5’GGATGACGAGTTGCTCCAGAG3’, Yamada et al., 2009). Heterozygous sites in the nuclear data were coded with IUPAC ambiguity codes. Segments of COI were amplified via PCR using GoTaq® Hotstart Master Mix (Promega, Madison, Wisconsin) with the following thermal profile: 2 min at 95 °C, followed by 35 cycles of 40 s at 95 °C, 40 s at 52–54 °C, and 90 s at 72 °C, followed by a single extra extension period of 5 min at 72 °C. Thermal profile for PCR of the Ptr gene was identical to that of the COI with the exception of an annealing temperature of 55 °C. Amplified PCR products were verified via gel electrophoresis using 2% agarose gel stained with SYBR® Green fluorescent dye. Purification of PCR products and DNA sequencing was performed by Molecular Cloning Labs (MCLABS, San Francisco, California). Sequences were assembled and aligned using the program Sequencher ver.4.8 (Gene Codes, Ann Arbor, Michigan) and alignments were checked by eye. Three outgroups were used in the analysis: Bryaninops ridens, Gobiodon unicolor, and Asterropteryx ensifera. Both Bryaninops and Gobiodon belong to a clade of coral reef gobies hypothesized to be sister to Eviota (Herler et al., 2009; Thacker and Roje, 2011). Asterropteryx is more distantly related to Eviota but belongs to the same major gobiid clade (Gobiidae sensu Thacker, 2009; Gobiinae sensu Pezold, 1993).

2.4. Phylogenetic analysis

Best fitting substitution models were determined for each gene using the program jModelTest ver.0.1.1 (Posada, 2008) based on

![Fig. 1. Typical arrangement of sensory pore pattern I. Pore pattern 2 is missing the IT pore. Pore pattern 3 is missing the IT and PITO pores, and has an enlarged or paired AITO pore. Pore pattern 4 is missing the IT pore and the NA pores. For definition of abbreviations, see Lachner and Karnella (1980). Figure modified from Lachner and Karnella (1980).](image1)

![Fig. 2. Bayesian phylogeny of Eviota based on the concatenated dataset. Support values at nodes are Bayesian posterior probabilities. Scale bar units = expected substitutions/site.](image2)
Akaike Information Criterion (AIC) scores. Phylogeny was inferred for each dataset independently as well as a concatenated matrix using Bayesian methods in the program MrBayes ver.3.2 (Ronquist et al., 2012). For each 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 of each MCMC run was assessed using the program Tracer ver. 1.5 (Rambaut and Drummond, 2007), and the parallel MCMCs were considered to have reached convergence when the average standard deviation of split frequencies for each analysis approached 0.01. Preliminary MrBayes analyses indicated that the default branch length priors implemented in MrBayes caused MCMCs to fail, with runs frequently converging on local optima sampling trees with unrealistically long branches – an increasingly common phenomenon observed in Bayesian phylogenetics (Brown et al., 2010; Marshall, 2010). This was remedied by setting the branch length prior to an unconstrained-exponential prior with a mean of 0.01 (default mean in MrBayes is 0.1; Marshall, 2010). To further confirm that each parallel MCMC run converged on trees with similar topologies, a maximum-clade-credibility tree was generated from each set of post-burnin trees using the program TreeAnnotator (available at http://beast.bio.ed.ac.uk/TreeAnnotator) and compared to one another. Once it was visually confirmed that each run converged on a similar tree topology, the trees from both parallel runs were combined and a maximum-clade-credibility tree was generated from the total sample of post-burnin trees (Fig. 2).

2.5. Habitat and character mapping

To visualize branch lengths in units of time, an ultrametric phylogeny was generated using BEAST ver. 1.7.0 (Drummond et al., 2012). Molecular data were partitioned by gene with each gene receiving its own substitution model as determined by the results of the model test. Outgroups were removed from the alignment for the BEAST analysis. Each partition was assigned an uncorrelated-lognormal relaxed molecular clock model and a yule speciation prior was used for the analysis. The tree root height prior was arbitrarily set to 1.0 so that the units of time across the tree were relative to the age of the entire tree rather than absolute years. The BEAST analysis was run for 10,000,000 iterations with a sampling frequency of 1000 generations. Output from the MCMC was analyzed using Tracer to determine that stationarity was reached, insure proper mixing, and to assess burnin. Two additional BEAST runs were performed to confirm that analyses converged on similar trees. A maximum-clade-credibility tree was generated from our post-burnin trees using TreeAnnotator.

Ancestral state reconstructions for habitat associations were estimated for nodes on the ultrametric phylogeny using maximum-likelihood in the software language R (R Development Core Team, 2012) through the function “ace” in the APE package (Paradis et al., 2004). The same method was used to estimate the likelihood of ancestral character states for pectoral fin branching and sensory pore patterns. For all ancestral state estimations, three different models of character state change were fit to our phylogenetic data: an equal-rates model, where all rates of change between states are equal; a symmetrical model, where the rate of change from state $1 \rightarrow 2 = 2 \rightarrow 1$, from $1 \rightarrow 3 = 3 \rightarrow 1$, and from $2 \rightarrow 3 = 3 \rightarrow 2$; and a model where all rates of character state changes were different. The fit of each model was evaluated using AIC scores and models were compared using ANOVA.

Fig. 3. Chronogram of Eviota species showing distribution of microhabitat association. Pies at nodes are probabilities of ancestral character states. Scale bar is in units of time relative to the root age.
3. Results

3.1. Phylogenetic analysis

We successfully sequenced 1089 bp of COI and 607 bp of Ptr for a total of 1696 bp. The MCMCs for all Bayesian analyses reached stationarity by 300,000 iterations. The single-gene analysis of the Ptr dataset yielded a topology identical to the concatenated phylogeny (Fig. 2). Ancestral relationships on COI gene tree were poorly resolved with many long branches forming a polytomy at the base of the phylogeny, however the few relationships that were well supported in the COI tree were identical to those of the Ptr gene tree and the concatenated phylogeny (see Supplementary material for individual gene trees). The Bayesian phylogeny of the concatenated dataset (Fig. 2) showed strong support for the monophyly of Eviota. Species within the genus were divided into two well-supported deeply divergent clades with each clade containing species from all four collection localities. Bayesian posterior probabilities at nodes were generally high across the tree with one exception; the position of the branch containing Eviota Pohnpei sp. 1 + Eviota Red Sea sp. 1 was poorly resolved. The inclusion of this lineage within the larger of the two clades of Eviota is well-supported, however its position within this group is either sister to all other species in this clade, or sister to a clade containing E. melasma, E. guttata, E. cf. guttata, and E. albolineata (as shown in the ultrametric phylogenies, Figs. 3–5). Relationships within the smaller of the two major Eviota clades are fully resolved with high posterior probability support values.

3.2. Microhabitat association and morphological character evolution

We collected a total of 374 specimens of Eviota comprising 18 species from 229 sampling events across our four localities (Table 2). Habitat associations of species from the Red Sea were based on only one individual for each species and additional collections are obviously needed to corroborate their associations with hard coral. Nevertheless, most species in our study demonstrated a strong preference for one microhabitat type. The exceptions are E. sigillata and E. shimadai, which were evenly distributed across both rubble and sand microhabitats, and therefore they were deemed to be associated with both habitats. Myers (1999) also notes that E. sigillata is commonly found over sand. When collected over rubble, both species were more common when there was also some sand present amongst the rubble as opposed to when there was only a deep layer of rubble (Ahmadia pers. observation). We therefore coded the combination of rubble and sand as a third microhabitat type.

There was no significant difference between the fit of the equal-rates models, the symmetrical models and the all-rates-different models for any of the ancestral character state reconstructions (ANOVA, p > 0.05; delta AIC < 4), thus we used the simpler equal-rates model for our reconstructions. Ancestral state reconstruction for habitat association indicated that the common ancestor to all Eviota species was most likely a coral-associate (Fig. 3). Throughout the history of Eviota there were multiple independent habitat switches from coral to rubble or from coral to rubble/sand. There were no clear instances of reversals from rubble or sand/rubble back to coral, however several nodes have high likelihoods for...
more than one habitat association and thus the precise instance on the phylogeny of habitat transitions is ambiguous in some cases. Ancestral state reconstruction for sensory pore patterns showed that most ancestral nodes had similar likelihoods for all four pore patterns, and that changes in pore patterns were common throughout the evolutionary history of *Eviota* (Fig. 4). Analysis of pectoral fin-ray branching patterns indicated that all species with and without branched pectoral fin-rays were reciprocally monophyletic, and that this character diagnoses the two main clades of *Eviota* in our phylogeny (Fig. 5), hereafter referred to as the branched clade and the unbranched clade.

4. Discussion

4.1. Microhabitat association

Many *Eviota* species display consistent associations with a single type of microhabitat on the reef. The strength of this

![Fig. 5. Chronogram of *Eviota* species showing distribution of pectoral fin branching. Pies at nodes are probabilities of ancestral character states. Scale bar is in units of time relative to the root age.](image)
association varies between species. For example, *E. winterbottomi* was found only on hard coral, whereas *E. guttata* displayed a strong association with hard coral but was also found on rubble in one instance. Past studies report the latter species from coral rock, which we did not sample due to the scarcity of this habitat at most of our sites (Herler and Hilgers, 2005; Herler, 2007). Other species displayed strong associations for rubble, but would occasionally be found in a hard coral or sand collection (e.g., *Eviota Moorea sp. 1, E. lachdeberei*, *E. queenslandica*).

Ancestral state reconstruction of microhabitat associations revealed that the common ancestor of all *Eviota* was likely a hard coral associate. This is consistent with the fact that the sister groups to *Eviota* (*Gobiodon, Pleurosicya, Bryaninops*) are all obligate coral dwellers. Interestingly, *Eviota* species have invaded rubble habitats several times throughout their evolutionary history. As many as three independent habitat switches from coral to rubble occurred in the branched clade, the most recent of which occurred between *E. winterbottomi* and *E. queenslandica*. Within the unbranched clade both sand/rubble species likely descended from a sand/rubble common ancestor early in the history of the clade. Similarly, the rubble-associated species of unbranched clade, *E. spilota*, *E. cf. spilota* and *E. lachdeberei*, all likely descended from a rubble-associated ancestor. *Eviota influlata* and *Eviota Red Sea sp. 3* are both represented by few individuals in our samplings, but our collections indicate that both occur on hard coral and likely descend from a coral-associated ancestor.

### 4.2. Morphological character evolution

The two major clades of *Eviota* recovered in our analysis correspond with the division of species with branched versus unbranched pectoral fin-rays (Fig. 5). The maximum likelihood reconstruction of ancestral states could not unambiguously resolve the state of the common ancestor of all *Eviota*; however, if we consider that most gobies have branched pectoral fin-rays (including members of our outgroup genera *Bryaninops, Gobiodon* and other species of the “coral gobies” sensu Thacker and Roje, 2011), the most parsimonious explanation is that the ancestor of *Eviota* had branched rays. There are no examples of reversals in this character in any of the species in our study, despite multiple switches in habitat association. Sensory pore patterns, unlike pectoral fin-ray branching, displayed a large amount of homoplasy with each pattern appearing multiple times independently across the phylogeny. This is consistent with observations by Greenfield and Randall (2010), who also questioned the homology of sensory pore patterns, pointing out that *E. shimadai* and *E. sigillata* are morphologically similar and may be closely related, yet the two are placed in different “groups” based on the absence of a single pore in *E. shimadai*. Virtually all internal nodes on the phylogeny had ambiguous sensory pore character states, and there was no obvious relationship between a particular pore pattern and a specific microhabitat type.

Shifts in *Eviota* microhabitat associations occur independently of changes in morphology. Reduction of head canals has been observed in several groups of gobies that have invaded novel habitats (Miller, 2004; Kovačić, 2005; Stelbrink and Freyhof, 2006). These reductions may be associated with a release of predation pressures in the novel habitat, as predator detection is a major function of the accessory lateralis system (Stelbrink and Freyhof, 2006). The incongruence between pore reduction and shifts in microhabitat association in *Eviota* may suggest that the selective forces driving sensory pore pattern evolution act independently of the microhabitats in which *Eviota* species occur. On the other hand, sensory pore loss or fusion in *Eviota* may simply be a byproduct of overall body size reduction and associated fusion of cranial elements, thus evolving in a manner that appears to be random with respect to selective forces from different microhabitats.

The fact that *Eviota* species have been able to repeatedly invade novel habitats in the absence of significant changes to pectoral fin-ray morphology indicates that the shape of individual rays is less important in dwarfgobies than in other cryptobenthic fishes. Gobies primarily use their specialized pectoral fins for swimming (Adriaens et al., 1993). A pectoral fin with highly-branched rays could potentially increase propulsion by increasing the surface area of the fin, however most branching in many *Eviota* occurs mainly at the distal tips of the median fin rays and the increase in surface area may not be significant enough to gain any appreciable locomotive benefit. Other cryptobenthic fishes, such as some blennies and sculpins, use specialized unbranched pectoral fin-rays to grip or manipulate the substrate and support the weight of the body (Webb, 1989; Brandstätter et al., 1990; Taft, 2011). This may not be the case in *Eviota*. If the simple, unbranched fin rays in *Eviota* were adaptations for specific substrates, we would expect the form of rays to vary in species from different microhabitats. Furthermore, the structural adaptations in goby pectoral girdles that allow for strong fin adduction also limit maneuvering and manipulating the fin itself, making it unlikely that unbranched rays of *Eviota* are adaptations for interacting with the substrate (Adriaens et al., 1993). Species of *Eviota* also possess highly modified pelvic fins relative to other coral gobies, which also contact the substrate. Among species of *Eviota*, there are differences in the shape, size, and branching of pelvic fin rays, and it is possible that variation in this character may also affect microhabitat association. However, there is substantial variation within species in these pelvic fin-ray characters, and incorporating and summarizing this within-species variation in a way that facilitates a meaningful comparison across the phylogeny is problematic.

### 4.3. Diversification in stages

Taylor and Hellberg (2005) suggested that adaptive radiations occurred in stages in the reef gobies *Elacatinus* and *Tigrigobius/Ridge*or. In Atlantic *Elacatinus*, initial radiations resulted in a clade of sponge-dwelling gobies and a clade of cleaner-gobies (Taylor and Hellberg, 2005). A subsequent stage of speciation within each clade was driven largely by color differences and allopatric speciation. Additionally, segregation in habitat depth may have also played a part in speciation within sponge-dwelling *Elacatinus* by facilitating reproductive isolation (Colin, 1975, 2010). We see similar evidence of staged radiations in *Eviota*. However, unlike *Elacatinus*, *Eviota* species appear to have initially diversified in morphology first, forming distinct clades with branched versus unbranched pectoral fin-rays. Subsequent stages of divergence have been ecologically driven. In the unbranched clade, species are sorted by habitats forming clades of sand/rubble, coral, and rubble species early in the evolutionary history of the clade, whereas shifts from coral to rubble in the branched clade occurred multiple times throughout the history of the clade.

Allopatric speciation cannot be rejected as an explanation for speciation near the tips of the phylogeny. Although some pairs of closely related species within each clade currently have partially overlapping distributions (i.e. *E. shimadai* and *E. sigillata* both are reported from Pohnpei and Indonesia; Greenfield and Randall, 2010), the ages of divergence between many of these species pairs are not recent and we cannot reject the combination of initial reproductive isolation and subsequent secondary contact (via range expansions) as the most parsimonious explanation for these distribution patterns. It is worth noting that the sympatric or parapatric species pairs with similar habitat associations (i.e. *E. shimadai* and *E. sigillata*) were not collected together at any site in our study. This may be the result of one species being locally
displaced due to interspecific competition, including possible bathymetric partitioning of habitat – a pattern observed in putative Hawaiian sister species *E. susanae* and *E. rubra* (Greenfield and Randall, 1999) as well as in *Gobiodon* sp. 1 and *G. reticulatus* on host-coral *Acropora samoensis* (Dirnöwer and Herler, 2007). Alternatively, this could also be the result of a local competitive-lottery model scenario (Sale, 1978; Munday, 2004a).

4.4. Dwarfgoby diversity, speciation and extinction

The repeated habitat transitions observed in *Eviota* may be a major factor contributing to the high species richness of the genus. Interspecific competition among gobies for live coral may be significantly higher than for sand or rubble habitats, as many species of gobies on coral reefs have obligatory habitat associations with specific types of coral. Entire genera may be composed of obligate coral species, including *Gobiodon*, *Pleurosicya*, and *Bryaninops* – all of which are closely related to *Eviota* (Herler, 2007; Thacker and Roje, 2011). If we parsimoniously assume that coral-association arose in the common ancestor to the aforementioned genera, then the ability to shift from a strict species-specific or morphotype-specific (e.g., branching versus massive versus foliose) coral association to a more broad, generalist coral association could be considered an ecological release. Additional ecological opportunities would exist for species that are free from coral or coral-rock obligations entirely, and can occur in rubble or sand – habitats that are typically inhabited by fewer small cryptobenthic fishes than live coral (Ahmadia et al., 2012). *Eviota* species that are found on live coral often occur on many different species of live coral as well as coral rock (Herler, 2007), suggesting that the gobies are attracted to a range of complex three-dimensional structures rather than a single organism. Although rubble (or a mixture of rubble and sand) may not be as structurally complex as coral, *Eviota* species are small enough to capitalize on smaller scale habitat complexities that characterize rubble-sand habitats and thus may be released from the need to occupy larger three-dimensional structures.

The high number of species of *Eviota* relative to other coral gobies may be explained in part by their ability to repeatedly colonize habitats that are less preferred by other reef fishes throughout their evolutionary history, thus occupying new niches on the reef and facilitating further speciation. However, the number of extant species in a group is not only a function of speciation, but is also determined by the relative rate of extinction. Extinction risk in coral reef fishes depends on several factors including their response to changes in habitat quality (Munday, 2004b; Prattchet al., 2006, 2008; Graham et al., 2011). Species with strict microhabitat requirements or narrow diets are more vulnerable to natural disturbances (Munday, 2004b; Prattchet et al., 2006). For example, obligate coral dwelling *Gobiodon* species currently experience both local and global extinction risks due to habitat loss caused primarily by coral bleaching (Munday, 2004b; Bellwood et al., 2012). Entire cryptobenthic fish assemblages can change rapidly as a result of massive coral loss, and these assemblage changes can persist for many years after corals recover (Bellwood et al., 2006, 2012). In such scenarios, *Eviota* species have actually been shown to increase in abundance (Bellwood et al., 2006). Having the ability to inhabit a variety of different types of coral or coral rock would be advantageous in bleaching events, where some types of corals are impacted more than others. Additionally, for rubble or sand associated species, habitat availability would be unaffected by most threats to coral reefs, and in some scenarios (e.g., cyclones) disturbances may even increase the amount of rubble habitat available for species. Beyond benefiting from their habitat associations, some species of *Eviota* have been shown to have very wide dietary preferences, high reproductive output, fast life-cycles, and evidence of hermaphroditism – all of which may contribute to low extinction relative to other closely related coral reef gobies (Depczynski and Bellwood, 2003, 2005, 2006; Cole, 1990).

5. Conclusions

This study represents the first phylogenetic analysis and most comprehensive ecological survey of the genus *Eviota* – one of the most diverse, yet poorly studied groups of reef fishes. Our molecular phylogeny serves as a framework to identify potential ecological drivers of this diversity, and investigate the relationship between microhabitat and morphology. This study has several important findings that increase our understanding of gobiid diversity, habitat use of cryptobenthic reef fish, and the relationships between morphological evolution and microhabitat association in reef fishes:

- Most *Eviota* species are closely associated with a particular type of habitat or substrate on reefs, although these preferences are less strict than that of other coral gobies.
- The common ancestor of all *Eviota* species was likely a coral-associated species, and habitat shifts from coral to rubble, or from coral to rubble/sand occurred many times independently throughout the evolutionary history of *Eviota*.
- Shifts in microhabitat association occurred independent of changes in pectoral fin-ray branching or sensory pore patterns, the latter of which was highly homoplasic in *Eviota*.
- The relative flexibility of coral association in *Eviota* combined with the repeated shifts into non-coral habitats may serve as an ecological release from the strict obligate coral-dwelling relationship seen in closely related species, promoting subsequent speciation in new vacant or underutilized niches. Increased speciation opportunity coupled with resilience to extinction may explain the exceptionally high species-richness in *Eviota*.

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Appendix A. Supplementary material

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