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Phylogenetic evidence for recent diversification of obligate coral-dwelling gobies compared with their host corals ☆

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ABSTRACT

The rich diversity of coral reef organisms is supported, at least in part, by the diversity of coral reef habitat. Some of the most habitat specialised fishes on coral reefs are obligate coral-dwelling gobies of the genus *Gobiodon* that inhabit a range of coral species, mostly of the genus *Acropora*. However, the role of this specialised pattern of habitat use in the evolution of coral-dwelling gobies is not well understood. Diversification of coral-dwelling gobies may be driven by the diversification of their host corals (cospeciation), or alternatively, diversification of these fishes may have occurred independently of the diversification of host corals. The cospeciation hypothesis assumes similar timing in evolution of the gobies and their host corals. We used four genes for each group and the available fossil records to reconstruct and date phylogenies for 20 species of *Gobiodon* from the Indo-Pacific and the Red Sea, and for 28 species of the coral genus *Acropora*. Our results indicate that *Gobiodon* diversified mostly in the last ~5 My, whereas *Acropora* corals have consistently diversified since the Eocene, making the hypothesis of cospeciation untenable. The fully resolved molecular phylogeny of the genus *Gobiodon* is in part at odds with previous analyses incorporating morphological data and indicates that some morphological traits form paraphyletic clades within *Gobiodon*. Our phylogeny supports a hypothesis in which *Gobiodon* diversified in the Indo-Pacific Ocean and then radiated recently, with multiple new variants found in the Red Sea.

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1. Introduction

Species interactions can influence evolution and result in coevolved systems (Thompson, 2009). If interactions between species are close enough, the organisms involved may speciate at similar evolutionary times, so a reconstruction of their evolutionary histories would show congruent events of speciation; a pattern known as cospeciation (Paterson and Banks, 2001). Phylogenetic inferences have been used to study cospeciation in closely interacting groups of organisms, mainly from parasitic and mutualistic associations (e.g. fish and their parasites, Huyse and Volckaert, 2005; figs and fig wasps, Weiblen and Bush, 2002; salamanders and their viruses, Storfer et al., 2007; echinoderms and annelids, Lanterbecq et al., 2010; yucca plants and yucca moths, Althoff et al., 2011). Although evidence for cospeciation events is most often supported, the assumption of similar timing of evolution of interacting groups is rarely tested (Paterson and Banks, 2001). Current techniques to date evolutionary events provide a tool to test this assumption robustly and may give better insight into the prevalence of cospeciation.

Interactions between reef fishes and corals are important to reef ecosystems both ecologically and evolutionarily (Jones et al., 2004; Graham et al., 2006; Cowman and Bellwood, 2011), therefore it is important to understand the history of their associations (Bellwood and Wainwright, 2002; Rocha and Bowen, 2008; Kiessling et al., 2010). Reef fishes and reef building corals are both known for their extraordinary diversity; however, the evolutionary links between these two groups of coral reef organisms is not fully understood. Some of the most habitat specialized fishes on coral reefs are from the genus *Gobiodon*, which are obligate coral-dwelling gobies that mostly inhabit coral colonies from the genus *Acropora* (Munday et al., 1997, 1999). *Gobiodon* species are highly selective among coral species (Munday et al., 1997; Munday, 2004a; Dirnwoeber and Herler, 2007) and they compete strongly

for access to preferred coral species, both within and between species (Munday et al., 2001; Hobbs and Munday, 2004). The fitness of coral gobies depends on the availability of coral host species (Munday, 2001; Caley and Munday, 2003; Herler et al., 2011), which indicates that the association has evolutionary ramifications for the fish. Given that the genus comprises at least 20 species, many of which remain faithful to their preferred coral host across reef systems (Munday, 2000, 2002), their evolution and interaction with *Acropora* provides an intriguing case study of reef fish evolution.

Ecological aspects of the association between *Gobiodon* species and *Acropora* corals may suggest a linked evolutionary history. Coral-dwelling gobies depend on coral colonies as a source of shelter, food and breeding sites (Munday et al., 2001; Hobbs and Munday, 2004; Brooker et al., 2010). Most species of *Gobiodon* have specific preferences and inhabit a limited number of the *Acropora* coral species present on coral reefs (Munday et al., 1999, 2004). Furthermore, habitat use of some *Gobiodon* species varies little across geographic regions in the Indo-West-Pacific (Munday, 2002), indicating that habitat choice can be a highly constrained trait. *Gobiodon* also provide benefits to their host corals; for instance, chemical cues released by *Acropora* corals that have come into contact with toxic algae trigger the gobies to feed on the algae and prevent it overgrowing the coral (Dickson and Hay, 2012). Similarly, gobies may protect the coral from predation by butterflyfishes and other corallivorous fishes (Dirnwoeber and Herler, 2012). This close association between *Gobiodon* and *Acropora* corals is suggestive of a linked evolutionary history.

Past phylogenetic analyses of the genus *Gobiodon* have been based on morphological and mitochondrial genetic data (12S and 16S rRNA; Harold et al., 2008). The genus is monophyletic (Harold et al., 2008; Herler et al., 2009), although previous phylogenetic analyses using maximum parsimony resulted in low resolution for the internal nodes (Harold et al., 2008). Morphological features like body shape and osteological structures have been used in attempts to improve this resolution (Harold et al., 2008). Nevertheless, there remains considerable uncertainty about the phylogenetic relationships among *Gobiodon* species and there is some discordance between morphological and molecular results (Harold et al., 2008). The weak resolution of phylogenetic relationships in previous analyses means that they have limited power for testing evolutionary hypotheses about diversification in this group of fishes. The timing of diversification of the genus *Gobiodon* has been estimated in a previous study, suggesting that the group started diversifying around 10 Mya (Herler et al., 2009). However, this study used a subset of just eight species, used only one mitochondrial gene, and assumed a molecular clock, precluding a robust comparison with the timing of diversification in their host corals from the genus *Acropora*.

The diversification of scleratinian corals extends to the last 200 My (Simpson et al., 2011). To date, the timing of evolutionary events in the genus *Acropora* has been hypothesized utilizing phylogenies based on cladistic and maximum-likelihood analyses (Wallace, 1999; van Oppen et al., 2001). Most of the diversification of the *Acropora* genus was thought to have occurred in the Pliocene and Pleistocene (Wallace, 1999; Van Oppen et al., 2001); however, recent fossil findings suggest that the *Cervicornis* species group was already present in the Lutetian (~45 Mya; Wallace and Rosen, 2006; Wallace, 2008). Fossils from the *Hyacinthus* and the *Aspera* species groups also suggest an earlier divergence of the genus (Wallace, 2008). The rate of molecular evolution of *Acropora* corals is slow and there are mechanisms in place that retard it (Van Oppen et al., 1999). These mechanisms have been suggested to cause unusually extended longevity, and include asexual reproduction, slow growth and the lack of a mortal soma (Hellberg, 2006). Similarly, *Acropora* corals are likely to undergo hybridization and intro-

gression, which may also lead to a slower rate of molecular substitutions and reduce the rate of extinction (Willis et al., 2006; Richards et al., 2008).

Studies on the evolutionary history of reef fishes have suggested that the interaction between fishes and coral reefs became common soon after the Cretaceous-Tertiary (K/Pg) boundary (Cowman and Bellwood, 2011). However, little is known about the evolutionary history of the interaction between gobies and corals. The present study undertakes an evolutionary analysis of the association between *Gobiodon* and *Acropora* by independently estimating the dates of diversification for both groups. Specifically, we test if the diversification times of *Gobiodon* and *Acropora* overlap, which is a necessary assumption of the cospeciation hypothesis that is rarely tested (Paterson and Banks, 2001). We constructed the most complete genetic dataset for *Gobiodon* to date, using mitochondrial and nuclear markers for 20 species from the Indo-Pacific Ocean and the Red Sea. Similarly, we compiled data for four markers of *Acropora* of mitochondrial and nuclear origin, including 12 of the species most commonly inhabited by *Gobiodon*. Using available fossil data we inferred a phylogeny of *Gobiodon* and estimate the timing of first appearance of *Gobiodon* and *Acropora* to test for co-incidental dates of evolution. To gain further insight into the evolutionary dynamics of the two groups, we use a Bayesian approach to test the plausibility of evolutionary models of pure speciation (Yule process) against a model including both speciation and extinction (birth–death process). Comparing the dates and dynamics of evolution of these two groups is instrumental for future studies of the diversification of *Gobiodon* and the origins of their association with *Acropora*.

2. Materials and methods

2.1. Taxon sampling

Our phylogenetic analysis comprised twenty *Gobiodon* species (Harold et al., 2008), including 6 recognized, but as yet undescribed species (*G.* sp. A, B, C, D; Munday et al., 2004, 1999; and *G.* sp. 1 and 2; Herler et al., 2009). Additionally, we included samples from the Red Sea of three species that are widespread in the Pacific Ocean (*G. rivulatus, G. histrio* and *G. citrinus*; Harold et al., 2008; Munday et al., 1999; Herler et al., 2009). *Amblyeleotris* sp., *Ctenogobiops* sp., and *Paragobiodon xanthosomus* were used as outgroup species (Table 1). Specimens of Indo-Pacific species were collected by PLM at Lizard Island on the Great Barrier Reef, Australia, and in Kimbe Bay, Papua New Guinea (Table 1). Specimens of Red Sea species were collected by JH from the Gulf of Aqaba (Dahab, Egypt), the northern Red Sea (Marsa Alam, Egypt), and the southern Red Sea (Dahlak Archipelago, Eritrea). Specimens were collected following anaesthetization with dilute solution of clove oil (Munday and Wilson, 1997) and preserved in 80% alcohol prior to molecular analyses.

2.2. Laboratory procedures

Total DNA was extracted from *Gobiodon* tissues using standard salt-chloroform and proteinase K digestion extraction procedures (Sambrook and Russell, 2001). Four loci, three mitochondrial (12SrRNA, 16SrRNA, cytochrome *b*) and one nuclear, S7 ribosomal protein gene Intron1 (S7I1), a gene required for assembling RNA (Chow and Hazama, 1998; Maguire and Zimmermann, 2001), were sequenced. In addition to the four markers utilized in this study, we designed specific *Gobiodon* primer sequences for cytochrome *b* (Supporting Information Table 1). The *Gobiodon* primers were used interchangeably with published cyt *b* primers either replacing L14841 or H15149 (Kocher et al., 1989) in PCR reactions. Each 20 μl PCR reaction volume contained 2.5 mM Tris–Cl (pH 8.7), 5 mM