Phylogeny and evolution of Indo-Pacific shrimp-associated gobies (Gobiiformes: Gobiidae)

Christine E. Thacker [a,*], Andrew R. Thompson [b], Dawn M. Roje [a,1]

[a] Section of Vertebrates – Ichthyology, Natural History Museum of Los Angeles County, 900 Exposition Blvd., Los Angeles, CA 90007, United States
[b] NOAA Fisheries Service, Southwest Fisheries Science Center, 8604 La Jolla Shores Drive, La Jolla, CA 92037, United States

ARTICLE INFO

ABSTRACT

Despite the ubiquity of obligate mutualisms on coral reef ecosystems, little is known about the evolution of many participating species. The shrimp gobies, known primarily from the coral reef habitats of the Indo-Pacific, are small benthic fishes that participate in a remarkable mutualism with alpheid shrimp. In this mutualism, the shrimp build and maintain a burrow that is guarded by the goby, and the shrimp and goby engage in an intricate tactile communication system. The mutualism is obligate for most shrimp gobies as participating species are highly vulnerable to predation when separated from a shrimp partner. We use phylogenetic analysis of nuclear and mitochondrial DNA sequence data to infer evolutionary relationships among shrimp gobies, and between shrimp gobies and their non-mutualistic gobiid relatives. We show that the mutualist shrimp association has arisen twice among gobies, once in a clade composed of *Amblyeleotris*, *Ctenogobiops*, and *Vanderhorstia*, and a second time in a clade including *Cryptocentrus*, *Mahidolia*, *Tomiamichthys* and *Stonogobiops*. We then compare the evolution of traits within each shrimp goby clade and consider their intrarelationships. We document cryptic diversity among shrimp gobies, with three distinct clades delineated among *Mahidolia mysticina* specimens captured at the same locality, paired with the same shrimp species. *Mahidolia* is placed as sister to the *Cryptocentrus* species *Cryptocentrus cinctus*; both exhibit pronounced dichromatism, occurring in both brown and yellow (xanthic) forms. We additionally clarify species identities within *Amblyeleotris*, confirming that widespread similar species *Amblyeleotris fasciata*, *Amblyeleotris steinitzi* and *Amblyeleotris wheeleri* are all distinct. We hypothesize that the flexibility of gobiid gobies and alpheid shrimp to interact with mutualist partners, as well as the apparently highly beneficial nature of mutualism between them, has contributed to the dual evolution of shrimp-association among Indo-Pacific gobies.

© 2011 Elsevier Inc. All rights reserved.

1. Introduction

In both marine and terrestrial ecosystems, positive interspecific interactions are commonly exhibited by a variety of species (Boucher et al., 1982; Sachs et al., 2004; Thompson et al., 2006). On coral reefs, both symbiotic (participants are intimately associated throughout their lives) and nonsymbiotic (participants are both free-living) mutualisms are pervasive, and include participants from all major animal groups. Examples range from nonsymbiotic, facultative interactions between cnidarians and fishes (Fautin and Allen, 1997) to symbiotic, obligate associations between corals and dinoflagellates that make up the reef substrate (Trench, 1993).

Despite the ubiquity of mutualism, little is known about the evolutionary history of many of the participating species, including whether or not the mutualism has evolved once or multiple times among participant groups. Theoretically, a mutualism between particular groups is more likely to evolve and be stable if a partnership results in a high degree of fitness feedback to both participants (Connor, 1995; Sachs et al., 2004; Foster and Wenseleers, 2006). Such a beneficial mutualism would be expected to have evolved multiple times. In contrast, if there are potential fitness costs that go along with the benefits, then the mutualism is expected to evolve only very rarely (Kawakita and Kato, 2009). In addition to the fitness consequences of an interaction, morphological, behavioral, or physiological constraints of interacting partners may affect the evolution of mutualisms. Partner taxa with highly specialized mutualistic traits are often monophyletic whereas less specialized mutualisms are expected to have arisen more frequently (Pellmyr, 1997; Pellmyr and Kren, 2002; Schultz and Brady, 2008; Santini and Polacco, 2006; Kawakita and Kato, 2009).

Fishes of the family Gobiidae (gobies) participate in a variety of mutualistic associations on coral reefs, including interactions between gobies and sessile invertebrates such as corals and sponges, as well as free-living invertebrates including crustaceans and

echinoderms (Myers, 1999; Allen et al., 2003). These associations involve gobies utilizing their mutualistic or commensal partner either directly as a habitat or shelter, or indirectly in that the partner creates a shelter that both animals occupy. Some gobies also engage in mutualistic cleaning behaviors with other, larger fishes (Böhlke and Robins, 1968; Cote, 2000). These mutualisms are all characterized by an association between participants that is not as intimate as that found between more involved symbioses such as those occurring between hosts and their parasites. Instead, each participant may disassociate with its partner, although survivorship of a lone mutualist may be greatly impaired (Thompson, 2005; Karplus and Thompson, 2011).

Among gobies, obligate mutualistic association with burrow-dwelling shrimp is documented from approximately 120 species in twelve genera (Karplus and Thompson, 2011) and is common throughout the tropics in near-reef environments, generally in sandy substrate with coral or rock rubble, but also in silty habitats (Harada, 1969; Polunin and Lubbock, 1977; Syms and Jones, 2004). In this mutualism, one or a pair of gobies occupies a burrow built and maintained by one or two (typically two) shrimp in the genus *Alpheus*. The shrimp use their chelae to excavate the burrow, and then must continually work to keep the burrow clear of sand or silt. At night, the mutualists retreat into the burrow and the shrimp seal the opening; each day, the shrimp open the burrow by clearing the entrance. The shrimp has very weak eyesight, and the fish function as sentinels, stationed at the burrow entrance and warning the shrimp of approaching danger through a tactile system of tail flicks detected by the shrimp's elongated antennae (Karplus et al., 1981; Karplus, 1987; Karplus and Thompson, 2011). Depending on the particular species involved in the interaction, the tactile communication system can be highly intricate (Karplus et al., 1979). This communication system is a hallmark of the goby-shrimp association and is one of the only known examples of interspecific communication where the survival of a one species depends almost entirely on signals produced by a partner species (Goodale et al., 2010).

Shrimp gobies provide an opportunity to examine the distribution of mutualist associations in a phylogenetic context. Previous phylogenetic analyses (Thacker, 2003, 2009) demonstrated the monophyly of the family Gobiidae (in the restricted sense of Thacker, 2009) and provided some indication of relationships among gobiid gobies. In this study, we greatly expand the sampling of mutualist gobies in the phylogeny, augment the markers used to include nuclear gene sequence as well as mitochondrial, and focus exclusively on relationships within Gobiidae. We include individuals of seven shrimp goby genera together with nineteen non-mutualistic goby genera, representatives of major tropical goby lineages throughout the old and new world oceans, in order to evaluate relationships within and among mutualistic shrimp gobies.

We focus on the Indo-Pacific shrimp gobies, with samples from the genera *Amblyeleotris*, *Ctenogobiops*, *Cryptocentrus*, *Mahidolia*, *Stonogobiops*, *Tomiyamichthys*, and *Vanderhorstia*. These seven genera encompass the bulk of the Indo-Pacific species diversity for shrimp gobies; species of *Flabelligobius*, *Lotilia*, and *Myersina* are also associated with alpheid shrimp in the Indo-Pacific but were not considered in this study. All of the goby species participating in mutualisms with alpheid shrimp are classified in the order Gobiiformes, suborder Gobioidei, family Gobiidae. Large-scale molecular phylogenies of gobioid fishes (Thacker, 2003, 2009) have suggested that shrimp gobies are not monophyletic, but instead fall into at least two clades. However, neither of those previous studies focused on Gobiidae, a family that includes a great diversity of both mutualistic and non-mutualistic taxa.

Several studies have investigated the taxonomy of shrimp gobies, but higher-level systematics has not been examined with morphology. In most cases, the genera are diagnosable, or at least

identifiable based on a combination of morphological characters (Hoese and Steene, 1978; Hoese and Randall, 1982; Hoese and Lubbock, 1982; Akihito and Meguro, 1983; Chen et al., 1998; Winterbottom, 2001; Mohlmann and Randall, 2002; Hoese and Larson, 2004). The genera *Amblyeleotris*, *Cryptocentrus*, *Myersina*, and *Stonogobiops* may be distinguished from the remaining six by their cheek papillae pattern (transverse rows present) and pattern of gill raker ossification. The genera *Ctenogobiops*, *Flabelligobius*, *Mahidolia*, *Tomiyamichthys*, and *Vanderhorstia*, lack transverse papillae rows on the cheek (Smith, 1956; Lachner and McKinney, 1980; Iwata et al., 2000; Winterbottom, 2001; Satapoomin and Winterbottom, 2002), and may also be distinguished from one another by various other morphological characteristics (Klausewitz, 1960; Chen and Fang, 2003; Randall et al., 2003; Shibukawa and Suzuki, 2004). We use our phylogeny to evaluate whether or not the presence or absence of transverse cheek papillae is a useful diagnostic character for larger groups of shrimp gobies. We also evaluate monophyly of each shrimp goby genus and gauge how well the current taxonomy accords with lineages recovered in the phylogeny. Species may be over or under-named; an over-named species has more than one name applied to a single historical entity, while an under-named species includes cryptic species diversity, with more than one lineage subsumed under the same species name. To address these possibilities, we include multiple individuals of species wherever possible, so that potential cryptic diversity or unrecognized synonymy may be revealed in the phylogenetic analysis.

2. Material and methods

Gobies sequenced in this study were obtained from several sources. The majority were captured at Kimbe Bay, New Britain, Papua New Guinea and Kenting, Taiwan in 2005 in a related study that quantified pairing patterns and habitat associations of shrimp and goby mutualists. Gobies were captured with the aid of spear-guns fitted with trident tips. To capture the fish, a burrow was observed until a goby left the entrance and settled on the nearby substrate. Then, a speargun was shot into the burrow opening, blocking access to the burrow and preventing the goby from retreating (modification of the protocol of Karplus and Vercheson, 1978). The stranded goby could then be pursued and captured with anesthetic and hand nets. Additional specimens (*Amblyeleotris fasciata* and *Vanderhorstia ornatissima*) were collected at Moorea, Society Islands, French Polynesia in August, 2000, and Rarotonga, Cook Islands, in September, 2001. Specimens of *Amblyeleotris wheeleri* were collected at the Mamanuca and Yasawa Island groups adjacent to Viti Levu, Fiji (September, 2001), and at Nusa Penida, Bali, Indonesia (October, 2000). Some samples of *Amblyeleotris steinitzi*, *A. wheeleri*, *Cryptocentrus cinctus*, and *Ctenogobiops tongaensis* were collected by Phil Munday at Lizard Island, Great Barrier Reef, Australia, in December of 2004. Gobies were identified with standard Indo-Pacific fish reference guides (Myers, 1999; Allen et al., 2003), as well as a specialist guide to gobies (Hayashi and Shiratori, 2003). None of the fishes collected were of uncertain identity; *Cryptocentrus* sp. A is that of Myers (1999). Individuals of *Cr. cinctus* were captured that exhibited both brown and yellow (xanthic) coloration, and the condition was recorded. All species of gobies were digitally photographed following capture, and all specimens collected for this study are vouchered in the Natural History Museum of Los Angeles County Ichthyology Tissue Collection.

Nonmutualistic gobiid taxa *Amblygobius nocturnus*, *Amblygobius phaelaena*, *Asterropteryx ensifera*, *Asterropteryx semipunctata*, *Barbulifer ceuthoecus*, *Bathygobius cocosensis*, *Bathygobius curacao*, *Callogobius bifasciatus*, *Callogobius sclateri*, *Cerdale floridana*, *Gobiodon*

histrio, *Gobiosoma macrodon*, *Gunnellichthys monostigma*, *Istigobius rigillus*, *Microdesmus bahianus*, *Microdesmus longipinnis*, *Nemateleotris magnifica*, *Oplopomus oplopomus*, *Ptereleotris microlepis*, *Ptereleotris monoptera*, *Ptereleotris zebra*, *Risor ruber*, *Signigobius biocellatus*, *Trimmatom eviotops*, *Valenciennea longipinnis*, *Valenciennea puellaris*, and *Valenciennea strigata* were also included in the analysis in order to place the shrimp gobies among other lineages of gobiids. *Gnatholepis anjerensis*, a member of the sister family to gobiid gobies (Gobionellidae; Thacker, 2009) was included and used as an outgroup and to root the tree. A list of taxa included and voucher catalog information is given in Table 1.

Total genomic DNA was extracted from approximately 25 mg of skeletal muscle or fin tissue using the animal tissue protocol from the Dneasy tissue extraction kit (QIAGEN Inc., Chatsworth, CA). Aliquots of genomic DNA (except those few fragments that were re-amplified to increase yield) were used as template in all polymerase chain reactions (PCR) to amplify double-stranded DNA product from three mitochondrial genes, cytochrome oxidase subunit 1 (CO1), NADH dehydrogenase subunits 1 (ND1) and 2 (ND2), and two nuclear genes, recombination activating gene 2 (RAG2) and rhodopsin (Rho). All PCR reactions were carried out using Applied Biosystems (Foster City, CA) Geneamp ABI Thermal Cyclers (9700), and each had a total volume of 25 μl with the following concentrations of reagents: 1.5–3.0 μl of genomic DNA isolate, 1.0 μl each of forward and reverse 10 μM primer, 2.5 μl of 10X buffer, 0.5 μl of 50 mM $MgCl_2$, 0.75 μl of 8 mM premixed deoxynucleotide triphosphates, and 1.0 unit of BIOLASE REDTaq® DNA Polymerase (Bioline Co., Taunton, MA).

An approximately 1.2 kb fragment of the mitochondrial protein coding gene CO1 was amplified using the primer pair GOBYL6468/GOBYH7696 (Thacker and Hardman, 2005), with the following protocol: an initial denaturation step at 94 °C for 3 min followed by 35 cycles of PCR. The cycles included denaturation at 94 °C for 30 s, annealing at 48–55 °C for 45 s, and extension at 72 °C for 60 s. After a final extension step at 73 °C for 10 min, all samples were held at 4 °C. The same protocol was used with the primer pair GOBYL3543/GOBYH4937 (Thacker and Hardman, 2005) to amplify ND1 in its entirety. ND2 was also amplified in its entirety, but with the primer pair GOBYL4919/GOBYH6064 and Palumbi's (1996) Touchdown Procedure.

For amplification of the protein coding nuclear gene RAG2, taxon specific primers were designed by obtaining sequences for the following goby taxa from GenBank: *Chaenogobius gulosus* (AB504194), *Eutaeniichthys gilli* (AB504198), *Gymnogobius isoza* (AB504196), *Leucopsarion petersii* (AB504199), and *Luciogobius guttatus* (AB504181), aligning them using MUSCLE (multiple sequence comparison by log-expectation; Edgar, 2004) and designing primers in conserved 5′ and 3′ ends of the fragment. The forward primer has the sequence 5′-GTT TGC CGC CTT GAC CCT TAT GAY GG-3′ and the reverse has the sequence 5′-CTT CAT CTG AGC TGT YTT CCA RTT C-3′. Using this primer pair, an 825 base pair (bp) fragment was amplified with the same protocol used for CO1 and ND1. That protocol was also used to amplify a 770 base pair fragment of the protein-coding region of Rho, using the primer pair Rh193/Rh1039r (Chen et al., 2003).

All PCR products were visualized under UV light on 1.5% agarose gels stained with ethidium bromide; bands of the target fragment length were then cut directly from the gel and cleaned using the QIAquick Gel Extraction kit (QIAGEN Inc., Chatsworth, CA). Forward and reverse sequences were generated at the High-Throughput Genomics Unit (HTGU), Department of Genome Sciences, University of Washington, Seattle. All chromatographs were aligned in Geneious (Biomatters Ltd., Auckland, New Zealand) and checked against their complement to verify base pair calls. All instances of heterozygosity were determined (overlapping peaks of equal strength) for the nuclear markers and those sites were then coded according to the IUPAC ambiguity code.

All sequences were initially aligned using MUSCLE (Edgar, 2004) implemented by Geneious and refined by eye according to the translated amino acid sequences. To check the reading frame, the mitochondrial sequences generated for this study were aligned with the existing GenBank sequences as references. RAG2 was aligned using the same sequences from the primer design described above as references; these sequences were subsequently removed from the alignment. All sequences, including those generated for this study as well as existing sequences for the individuals sampled, are available in GenBank; their accession numbers and corresponding whole specimen vouchers are listed in Table 1.

Phylogenetic analyses were performed in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). The appropriate model for base substitution frequencies was determined by jModelTest (Posada, 2008), using the Aikake Information Criterion (AIC) and Bayesian Information Criterion (BIC), for each gene partition as well as for the complete five gene concatenated dataset. MrBayes searches were run for the concatenated dataset as well as a partitioned analysis run with each gene evaluated under its appropriate model. In both cases, the search consisted of 3,000,000 generations with two replicates, each with four simultaneous chains. Trees were sampled every 1000 generations, and the first 2000 trees were discarded as burnin. A 50% majority-rule consensus tree was constructed of the post-burnin trees, and clade support was assessed with the posterior probability values for each node.

3. Results

A total of 103 individuals representing 55 species (54 species of Gobiidae including 27 shrimp gobies, as well as one outgroup taxon, *Gn. anjerensis*, from the sister family Gobionellidae) were represented in the phylogeny. The aligned matrix was composed of 4691 bp: 975 bp of ND1, 945 bp of ND2, 1207 bp of COI, 800 bp of RAG2, and 764 bp of Rho (GenBank accession numbers for all sequences are given in Table 1). jModeltest (Posada, 2008) indicated that the best-fit nucleotide substitution model for the combined dataset (all five genes combined) is GTR+I+G, based on both the AIC and BIC. Individually, the datasets fitted different models: GTR+I+G for COI and ND1, GTR+G for ND2 and RAG2, and HKY+I+G for Rho. The combined and partitioned Bayesian analyses yielded topologies that were nearly identical, with the partitioned analysis exhibiting less resolution among deeper nodes. In order to present a conservative view of the analyses, the phylogeny based on the partitioned analysis is shown in Fig. 1 and discussed below. Support values (posterior probabilities) for shallower nodes in the hypothesis (within genera and among smaller groups of genera) were strong, ranging from 95% to 100%, with most nodes supported at the 100% level. Two distinct clades of shrimp-associated gobies were recovered, with 100% support for each, as well as strong support for relationships within them. All shrimp goby genera were found to be monophyletic, with the exception of one *Cryptocentrus* species, *Cr. cinctus*, which grouped with the taxon *Mahidolia mysticina* as sister to the remainder of *Cryptocentrus*. Deeper in the hypothesis, among larger groups of genera, support values fell markedly. Thus, this hypothesis should be interpreted cautiously for those relationships. Little phylogeographic structure or cryptic diversity was observed in cases where many individuals were present for a species. The prominent exception to this generalization is *Ma. mysticina*, within which three widely separated, strongly supported clades were revealed. All other species identifications based on morphology corresponded with the groups delineated in the molecular phylogeny.

Table 1

Species of gobiid fishes considered in this study, voucher numbers, ecology, and GenBank accession numbers for sequences. Tissue voucher numbers beginning in T are catalogued in the fish tissue collection, Natural History Museum of Los Angeles County.

Name	Tissue voucher	Mutualist?	ND1	ND2	RAG2	Rho	COI
Amblyeleotris fasciata	T-10094	Y	HQ536754	HQ536780	–	HQ536957	HQ536682
Amblyeleotris fasciata	T-10095	Y	HQ536755	HQ536781	HQ536867	HQ536958	HQ536683
Amblyeleotris fasciata	T-10096	Y	HQ536756	HQ536782	HQ536868	HQ536959	HQ536684
Amblyeleotris fasciata	T-10097	Y	HQ536757	HQ536783	HQ536869	HQ536960	HQ536685
Amblyeleotris fasciata	T-10098	Y	HQ536758	HQ536784	HQ536870	HQ536961	HQ536686
Amblyeleotris guttata	T-001025	Y	HQ536711	FJ796083	HQ536816	HQ536912	HQ536637
Amblyeleotris guttata	T-001076	Y	HQ536741	FJ796082	HQ536852	–	HQ536670
Amblyeleotris gymnocephalus	T-001083	Y	HQ536746	FJ796084	HQ536859	–	HQ536676
Amblyeleotris periopthalmus	T-001045	Y	HQ536723	FJ796085	HQ536832	HQ536926	HQ536651
Ambyleleotris randalli	T-001075	Y	–	FJ796086	HQ536851	HQ536944	HQ536669
Amblyeleotris steinitzi	T-001029	Y	HQ536713	FJ796125	HQ536821	HQ536915	HQ536642
Amblyeleotris steinitzi	T-001034	Y	HQ536717	FJ796095	HQ536825	HQ536919	HQ536644
Amblyeleotris steinitzi	T-001035	Y	HQ536718	FJ796088	HQ536826	HQ536920	HQ536645
Amblyeleotris steinitzi	T-001043	Y	HQ536721	FJ796089	HQ536830	HQ536924	HQ536649
Amblyeleotris steinitzi	T-001044	Y	HQ536722	FJ796090	HQ536831	HQ536925	HQ536650
Amblyeleotris wheeleri	T-001021	Y	HQ536708	FJ796091	HQ536811	HQ536907	HQ536708
Amblyeleotris wheeleri	T-001022	Y	HQ536709	FJ796092	HQ536812	HQ536908	HQ536633
Amblyeleotris wheeleri	T-001032	Y	HQ536715	FJ796093	HQ536823	HQ536917	-
Amblyeleotris wheeleri	T-001033	Y	HQ536716	FJ796095	HQ536824	HQ536918	HQ536643
Amblyeleotris wheeleri	T-001092	Y	HQ536752	–	HQ536865	HQ536955	HQ536680
Amblyeleotris wheeleri	T-001093	Y	HQ536753	HQ536779	HQ536866	HQ536956	HQ536681
Amblyeleotris yanoi	T-001048	Y	HQ536726	FJ796097	HQ536835	HQ536929	HQ536654
Amblygobius nocturnus	T-001069	N	HQ536735	FJ796156	HQ536845	HQ536939	HQ536663
Amblygobius phaelaena	T-000868	N	AF391441	AF391513	HQ536801	HQ536897	AF391369
Amblygobius phaelaena	T-000905	N	AF391450	AF391522	HQ536803	–	AF391378
Asterropteryx ensifera	T-001050	N	–	FJ796099	HQ536837	HQ536931	HQ536656
Asterropteryx semipunctata	T-000899	N	AF391449	AF391521	HQ536802	HQ536899	AF391377
Barbulifer ceuthoecus	T-000197	N	AF391425	AF391497	HQ536795	HQ536891	AF391353
Bathygobius cocosensis	T-000929	N	AF391460	AF391532	HQ536806	HQ536902	AF391388
Bathygobius curacao	T-000193	N	AF391426	AF391498	HQ536796	HQ536892	AF391354
Callogobius bifasciatus	AUST-R	N	EU380995	EU381016	HQ536808	HQ536904	EU381037
Callogobius sclateri	T-000172	N	AF391462	AF391534	HQ536807	HQ536903	AF391390
Cerdale floridana	T-000604	N	AF391409	AF391481	–	HQ536887	AF391337
Cryptocentrus albidorsus	T-001105	Y	HQ536712	FJ796101	HQ536818	HQ536913	HQ536639
Cryptocentrus cinctus	T-001038	Y	HQ536719	FJ796103	HQ536827	HQ536921	HQ536646
Cryptocentrus cinctus	T-001040	Y	HQ536720	FJ796105	HQ536828	HQ536922	HQ536647
Cryptocentrus cinctus	T-001046	Y	HQ536724	FJ796106	HQ536833	HQ536927	HQ536652
Cryptocentrus cinctus	T-001073	Y	HQ536739	FJ796107	HQ536849	HQ536942	HQ536667
Cryptocentrus cinctus	T-001085	Y	–	FJ796108	–	HQ536976	HQ536700
Cryptocentrus cinctus	T-001086	Y	HQ536748	FJ796109	HQ536861	HQ536952	HQ536678
Cryptocentrus inexplicatus	T-001081	Y	–	FJ796111	HQ536857	HQ536949	HQ536674
Cryptocentrus inexplicatus	T-001082	Y	HQ536745	FJ796112	HQ536858	HQ536950	HQ536675
Cryptocentrus inexplicatus	T-001084	Y	HQ536747	FJ796113	HQ536860	HQ536951	HQ536677
Cryptocentrus leptocephalus	T-001057	Y	HQ536767	FJ796114	HQ536880	HQ536970	HQ536695
Cryptocentrus leptocephalus	T-001065	Y	HQ536731	FJ796116	HQ536841	HQ536935	HQ536660
Cryptocentrus leptocephalus	T-001090	Y	HQ536750	HQ536777	HQ536863	HQ536953	HQ909444
Cryptocentrus leptocephalus	LACM C221	Y	HQ536772	FJ796115	–	HQ536977	HQ536701
Cryptocentrus lutheri	T-001088	Y	HQ536776	HQ536791	–	HQ536982	HQ536706
Cryptocentrus nigroocellatus	T-001030	Y	HQ536714	FJ796120	HQ536822	HQ536916	-
Cryptocentrus sp. A.	T-001071	Y	HQ536737	FJ796122	HQ536847	HQ536940	HQ536665
Cryptocentrus sp. A.	T-001074	Y	HQ536740	FJ796121	HQ536850	HQ536943	HQ536668
Cryptocentrus sp. A.	T-001080	Y	HQ536744	FJ796123	HQ536856	HQ536948	-
Cryptocentrus strigilliceps	T-001026	Y	–	FJ796124	HQ536817	–	HQ536638
Ctenogobiops aurocingulus	T-001023	Y	GU187238	FJ796133	HQ536813	HQ536909	HQ536634
Ctenogobiops aurocingulus	T-001027	Y	GU187240	FJ796134	HQ536819	HQ536914	HQ536640
Ctenogobiops crocineus	T-001028	Y	GU187222	FJ796125	HQ536820	–	HQ536641
Ctenogobiops formosa	T-001024	Y	GU187229	FJ796128	HQ536815	HQ536911	HQ536636
Ctenogobiops mitodes	T-001072	Y	GU187227	FJ796126	–	HQ536978	HQ536702
Ctenogobiops mitodes	T-001077	Y	GU187228	FJ796127	HQ536853	HQ536945	HQ536671
Ctenogobiops tangaroai	KU 5712	Y	–	GU187254	HQ536810	HQ536906	HQ536632
Ctenogobiops tongaensis	T-001047	Y	HQ536725	FJ796131	HQ536834	HQ536928	HQ536653
Ctenogobiops tongaensis	LACM C210	Y	HQ536738	FJ796132	HQ536848	HQ536941	HQ536666
Gnatholepis anjerensis	T-000632	N	AF391447	AF391519	HQ536877	–	AF391375
Gobiodon histrio	T-000238	N	AF391432	AF391504	HQ536799	HQ536895	AF391360
Gobiosoma macrodon	T-000247	N	AF391420	AF391492	HQ536793	HQ536889	AF391348
Gunnellichthys monostigma	T-000641	N	AF391445	AF391517	–	HQ536898	AF391373
Istigobius rigillus	T-001078	N	HQ536742	FJ796137	HQ536854	HQ536946	HQ536672
Mahidolia mysticina	T-001052	Y	HQ536728	FJ796138	HQ536838	HQ536932	HQ536657
Mahidolia mysticina	T-001053	Y	HQ536729	FJ796140	HQ536839	HQ536933	HQ536658
Mahidolia mysticina	T-001054	Y	HQ536730	FJ796141	HQ536840	HQ536934	HQ536659
Mahidolia mysticina	T-001055	Y	HQ536773	FJ796142	–	HQ536979	HQ536703
Mahidolia mysticina	T-001056	Y	HQ536774	FJ796143	–	HQ536980	HQ536704
Mahidolia mysticina	T-001058	Y	HQ536768	FJ796118	HQ536881	HQ536971	HQ536696

Table 1 (continued)

Name	Tissue voucher	Mutualist?	ND1	ND2	RAG2	Rho	COI
Mahidolia mysticina	T-001059	Y	HQ536766	FJ796139	HQ536879	HQ536969	HQ536694
Mahidolia mysticina	T-001060	Y	HQ536769	FJ796144	HQ536882	HQ536972	HQ536697
Mahidolia mysticina	T-001061	Y	HQ536765	FJ796145	HQ536878	HQ536968	HQ536693
Mahidolia mysticina	T-001062	Y	HQ536771	FJ796146	HQ536884	HQ536974	HQ536699
Mahidolia mysticina	T-001063	Y	HQ536775	FJ796147	–	HQ536981	HQ536705
Mahidolia mysticina	T-001064	Y	HQ536770	FJ796119	HQ536883	HQ536973	HQ536698
Mahidolia mysticina	T-001070	Y	HQ536736	FJ796148	HQ536846	–	HQ536664
Microdesmus bahianus	T-000611	N	AF391419	AF391491	–	HQ536888	AF391347
Microdesmus longipinnis	T-000628	N	AF391413	AF391485	–	HQ536885	AF391341
Nemateleotris magnifica	T-000591	N	AF391399	AF391471	HQ536792	HQ536886	AF391327
Oplopomus oplopomus	T-001066	N	HQ536732	FJ796151	HQ536842	HQ536936	HQ536661
Ptereleotris microlepis	T-000882	N	–	AF391524	–	HQ536975	AF391380
Ptereleotris monoptera	T-000593	N	AF391429	AF391501	HQ536797	HQ536893	AF391357
Ptereleotris zebra	T-000594	N	AF391430	AF391502	HQ536798	HQ536894	AF391358
Risor ruber	T-000187	N	AF391423	AF391495	HQ536794	HQ536890	AF391351
Signigobius biocellatus	T-001079	N	HQ536743	FJ796149	HQ536855	HQ536947	HQ536673
Stonogobiops xanthorhinica	T-001049	Y	HQ536727	FJ796152	HQ536836	HQ536930	HQ536655
Trimmatom eviotops	KU 5591	N	EU380996	EU381017	HQ536809	HQ536905	EU381038
Tomiyamichthys oni	T-001089	Y	HQ536749	FJ796153	HQ536862	–	–
Valenciennea longipinnis	T-001041	N	–	FJ796154	HQ536829	HQ536923	HQ536648
Valenciennea puellaris	LACM C133	N	HQ536710	FJ796157	HQ536814	HQ536910	HQ536635
Valenciennea strigata	T-000898	N	AF391456	AF391528	HQ536804	HQ536900	AF391384
Vanderhorstia lanceolata	T-001067	Y	HQ536733	FJ796158	HQ536843	HQ536937	HQ536662
Vanderhorstia lanceolata	T-001068	Y	HQ536734	FJ796159	HQ536844	HQ536938	-
Vanderhorstia ornatissima	T-001099	Y	HQ536759	HQ536785	HQ536871	HQ536962	HQ536687
Vanderhorstia ornatissima	T-001100	Y	HQ536760	HQ536786	HQ536872	HQ536963	HQ536688
Vanderhorstia ornatissima	T-001101	Y	HQ536761	HQ536787	HQ536873	HQ536964	HQ536689
Vanderhorstia ornatissima	T-001102	Y	HQ536762	HQ536788	HQ536874	HQ536965	HQ536690
Vanderhorstia ornatissima	T-001103	Y	HQ536763	HQ536789	HQ536875	HQ536966	HQ536691
Vanderhorstia ornatissima	T-001104	Y	HQ536764	HQ536790	HQ536876	HQ536967	-

4. Discussion

The phylogeny indicates that mutualistic associations with alpheid shrimp have evolved twice among the gobiid fishes sampled, with two clades of shrimp-associated gobiid genera nested amongst non-mutualistic gobiid relatives (Fig. 1). Studies on the evolution of positive interactions theorize that stable mutualistic interactions are more likely to form if a high increase in fitness (high benefit to cost ratio) for both participants results from the interaction (Kawakita and Kato, 2009; Sachs et al., 2004). The goby-shrimp mutualism likely benefits both partners over a wide range of conditions because both gobies and shrimp receive protection from a diverse array of predators present in most near-reef environments (Karplus and Thompson, 2011). Indeed, field and laboratory experiments demonstrate that gobies incur high rates of mortality when separated from shrimp burrows (Thompson, 2005) and shrimp curtail burrowing activity and thus grow at lower rates when separated from gobies (Karplus and Thompson, 2011). This evidence indicates that benefit to cost ratios are high for both gobies and shrimp over a wide range of ecological contexts, thereby suggesting conditions that could allow the mutualism to evolve multiple times.

Another condition that is theorized to affect the evolution of mutualism is whether an interaction requires a complex morphological or behavioral precursor (Pellmyr et al., 1996; Kawakita and Kato, 2009). Specifically, if the mutualism cannot occur unless some highly specialized trait occurs in at least one of the partners, then it is unlikely that the mutualism would evolve on multiple occasions. In the goby-shrimp mutualism, tactile communication based on tail flicks is a trait unique to this system, and all of the gobies examined were observed conducting this behavior (Thompson and Thacker, personal observation). Sophisticated communication systems between different species (or even conspecifics) are rare in nature (Bradbury and Vehrencamp, 1998), and the goby-shrimp system is one of the only known examples in which interspecific communication serves such a vital role for both participants

(Goodale et al., 2010). However, unlike the key innovations that characterize some mutualisms, a lack of complex communication does not appear to prevent initial pairing among gobies and alpheid shrimp, and it has been hypothesized that complex communication between gobies and shrimp evolved from initial interactions where a goby merely occupied a shrimp burrow (Karplus, 1992). Among west Atlantic shrimp goby species (*Bat. curacao*, *Ctenogobius saepepallens*, and *Nes longus*: all pair with the shrimp *Alpheus floridanus*), a gradient of communication complexity has been detected. Here, *N. longus* produces multiple tail flick signals similar to Indo-Pacific shrimp gobies, *C. saepepallens* exhibits limited tail flicks, and *Bat. curacao* only warns *A. floridanus* of danger through head-first entry into a burrow (Karplus, 1992; Randall et al., 2005; Kramer et al., 2009). The presence of this variation in interactions demonstrates that complex communication is not a prerequisite for the establishment of goby-shrimp mutualism and potentially provides insight towards the inception of this mutualism.

Observations of goby ecology indicate that they exhibit considerable flexibility in their ability to reside commensally with other living organisms. Gobies are often found associated with invertebrates, including stony corals (*Gobiodon*, *Eviota*, *Gobiosoma*, many others), whip corals (*Bryaninops*), soft corals (*Lobulogobius*, *Pleurosicya*), sponges (*Evermannichthys*, *Pleurosicya*), sea urchins (*Lythrypnus*), and ghost shrimp (*Typhlogobius*; Allen et al., 2003; Miller and Lea, 1972; Myers, 1999). In most cases, these associations are not obligate and may be very casual; gobies are frequently quite small (many are less than 30 mm in length) and may simply be inhabiting a complex living substrate, rather than participating in a mutualistic or coevolved relationship. In the case of shrimp gobies, the partnership is clearly a mutualism, with each partner deriving benefit from the association (shelter for the goby, protection for the shrimp). Presence of the complex behavioral communication system and demonstration of adverse consequences for individuals not partnered both indicate that the mutualists have evolved in response to this association. However, it is
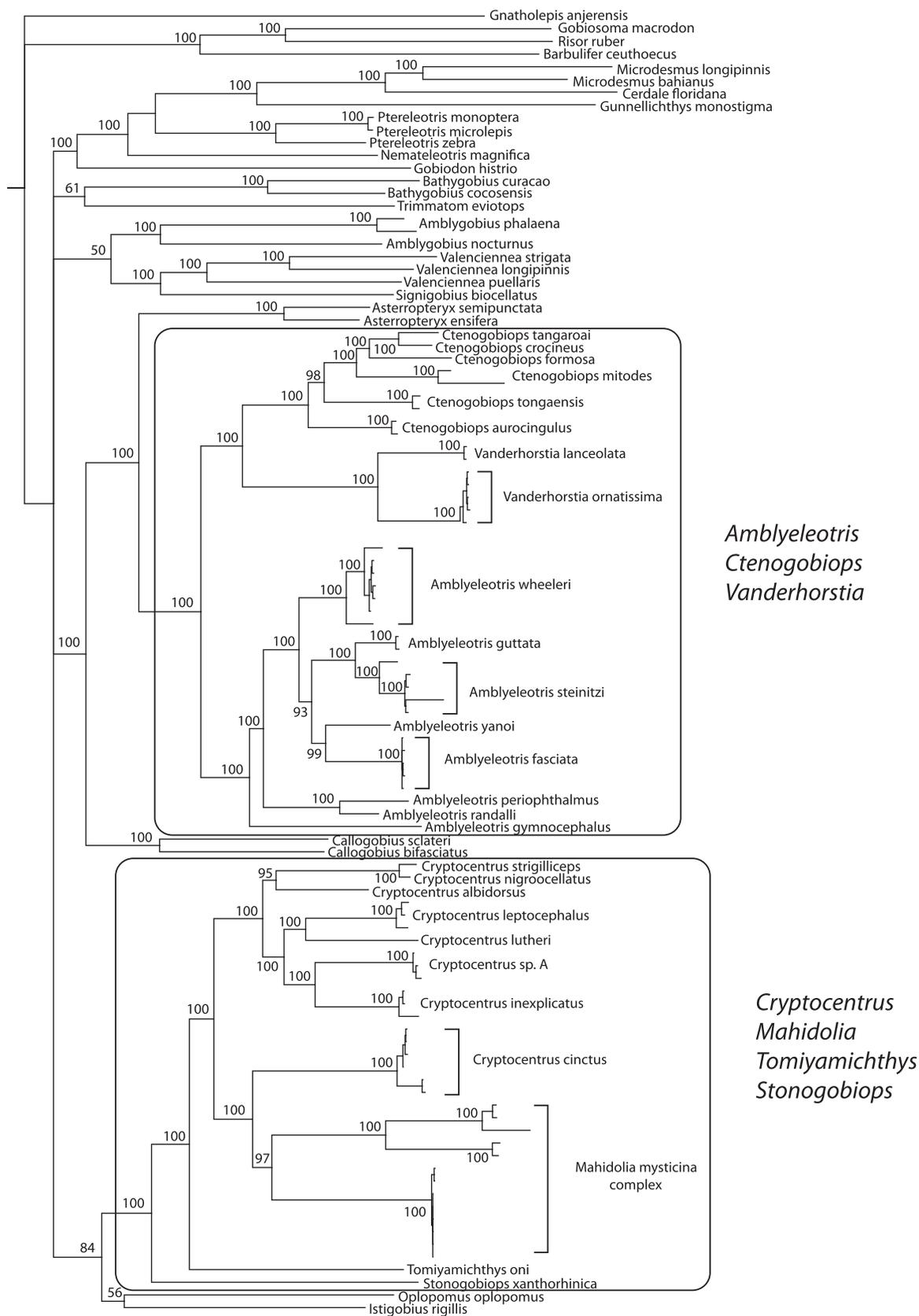
Fig. 1. Partitioned Bayesian analysis (50% majority-rule consensus of post-burnin trees) of phylogeny for gobiid gobies. The two clades of mutualist shrimp gobies are boxed and their component genera listed. Numbers at nodes are posterior probability values. Here and in the text, the species *Vanderhorstia lanceolata* is retained in *Vanderhorstia*, rather than included in *Tomiyamichthys* as proposed by Shibukawa and Suzuki (2004).

clear that the specific pairings of mutualists are not intimate; gobies and shrimp are only associated throughout part of their life cycle, and if a shrimp burrow is abandoned, another goby may occupy it (Thompson, 2005). Unlike obligate endosymbiotic mutualisms, gobies and shrimp are associated in only the adult phases of their lifetimes, and intra and interspecific partner switching has been documented in multiple species of shrimp gobies(Cummins, 1979; Thompson, 2005). Thus, although the presence of one partner is beneficial to the other, the specific identity of the partners is not as important in many documented cases, as long as they are paired.

In this hypothesis (Fig. 1), support for two distinct clades of shrimp-associated gobies and the relationships within them is robust. One clade includes the genera *Tomiyamichthys*, *Stonogobiops*, *Mahidolia*, and *Cryptocentrus*; the second encompasses *Vanderhorstia*, *Ctenogobiops*, and *Amblyeleotris*. Representatives of one species each were included for the rarer genera *Stonogobiops* and *Tomiyamichthys*; those individuals were both placed outside a *Cryptocentrus/Mahidolia* clade, with *Stonogobiops xanthorhinica* in the deepest split, falling outside *Tomiyamichthys oni* and the remainder of the species. All of the genera in which multiple species were examined are confirmed to be monophyletic, with the exception of *Cryptocentrus*. One species in this genus, *Cr. cinctus*, is resolved apart from the remainder of *Cryptocentrus*, as the sister taxon to *Mahidolia*. In the phylogenetic analysis, all *Cr. cinctus* individuals, of both color morphs (yellow and brown/white), were grouped together. Like *Cr. cinctus*, but unlike most other *Cryptocentrus*, *Mahidolia* also exhibits pronounced dichromatism, exhibiting both a brown and white barred morph and a bright yellow morph (Myers, 1999).

Cryptic diversity was detected in only one of the mutualist gobies. Within common, wide-ranging species such as *A. steinitzi*, *A. wheeleri*, *Cryptocentrus inexplicatus*, *Cr. cinctus*, and *Van. ornatissima*, many individuals were sampled and they did not assort into different clades. The significant exception to this pattern is the structure revealed within the "species" *Ma. mysticina*. Thirteen individuals of this taxon were sampled, all collected from the shallow, nearshore, muddy coast of Kimbe Bay, Papua New Guinea. The *Ma. mysticina* individuals compose three distinct and well-supported clades, clearly distinct from one another as evidenced by the relatively long branch lengths separating them. In Fig. 1, these individuals are collectively denoted as "*Ma. mysticina* complex." All of the individuals sampled were occupying burrows with the shrimp *Alpheus brevicristatus*.

Three clades are evident within the *Ma. mysticina* complex. The largest contains eight individuals; the remaining two contain three and two individuals respectively and form a clade sister to the remainder. All thirteen of these individuals were captured in the same habitat, a nearshore area in Kimbe Bay, Papua New Guinea, with a fine, dark, silty substrate. Capture depths ranged from roughly three to ten meters. To further investigate the possibility of cryptic sympatric species within *Mahidolia*, sequenced specimens were reexamined in light of the revealed phylogenetic structure, and one coloration difference was observed: individuals in the largest clade in our study differ from the others in the possession of small light blue spots scattered over the head and dorsal surface of the body, more densely in the anterior portion. These specimens also had an overall more gray cast to their coloration, in contrast to the five other individuals which showed no apparent differences from one another and exhibited a more beige overall coloration. No clade-specific differences in meristics, scale size or distribution, or fin shape were observed, although observations were hindered by the small size (<10 mm) of several of the specimens. All specimens had been ethanol-preserved in the field, and frozen upon arrival at the museum, so some coloration was preserved that is lost in formalin-fixed individuals. While suggestive,

a sample size of thirteen is not enough to definitively identify species-level differences among three putative groups. To compare our samples with a wider geographic range of individuals, we examined preserved specimens from our collection, and those from the Bernice P. Bishop Museum (Honolulu), the Australian Museum (Sydney), and the United States National Museum (Washington, DC). Localities for the collection specimens examined extended from Fiji west to Java and their sizes ranged from 6.7 to 60 mm standard length (complete list of individuals examined, with catalog numbers, localities, and specimen sizes is given in Table 2). These museum specimens showed no clear distinctions from one another in terms of overall morphological appearance, meristics, scale configuration, or fin shape or coloration. Pigmentation was reduced as is typical in preserved specimens, with only the black or brown melanistic colors remaining. These data seem to indicate the presence of more than one species within *Mahidolia*, existing in the same habitats and differing only in subtle variations in live color pattern, however, further observations of live coloration as well as corroboration with genetic information will be required to definitively address that question.

Some species of the Indo-Pacific genera not examined for this study (*Flabelligobius*, *Lotilia*, and *Myersina*), share morphological characteristics with members of the *Cryptocentrus* clade. At least one species of *Myersina*, *M. nigrivirgata*, is found in both brown and yellow forms similar to those of *Cr. cinctus* and *Mahidolia* (Winterbottom, 2001). Sexual dimorphism is recorded from many shrimp gobies, including species of *Amblyeleotris*, *Cryptocentrus*, *Mahidolia*, and *Vanderhorstia*. Such dimorphism manifests as color variation in the body and fins, as well as differences in fin shape and jaw length (Satapoomin and Winterbottom, 2002; Larson and Lim, 2005). Similarly, *Flabelligobius* and *Tomiyamichthys* are

Table 2

Preserved individuals of *Mahidolia* examined for this study, with basic locality information, length, and number of specimens examined. Abbreviations: AMS = Australian Museum, Sydney; BPBM = Bernice P. Bishop Museum, Honolulu; USNM = United States National Museum, Washington, DC.

Catalog number	Locality	Standard length in mm (no. of specimens)
AMS I.19450-028	Lizard Is. QLD	19.9 (1)
AMS I.20995-003	Lizard Is. QLD	8.4–12.4 (2)
AMS I.21149-037	Townsville, QLD	43.7 (1)
AMS I.22070-001	Cairns, QLD	24.2 (1)
AMS I.22135-009	Palm Is. QLD	9.9 (1)
AMS I.22700-009	Daintree R. mouth, QLD	6.7 (1)
AMS I.22700-027	Daintree R. mouth, QLD	23.1 (1)
AMS I.23319-013	Townsville, QLD	33.4–47.6 (9)
AMS I.25057-001	South of Fiji	17.5 (1)
AMS I.25058-005	Fiji	10.4–17.5 (3)
AMS I.25059-003	South of Fiji	12.4–20.8 (2)
AMS I.37929-041	Vanuatu	15.4–23.8 (3)
AMS I.42912-001	Vietnam	39.6–42.7 (2)
BPBM 22051	Singapore	36 (1)
BPBM 22149	Philippines	43 (1)
BPBM 26664	Celebes	34 (1)
BPBM 31431	Palau	29 (1)
BPBM 32696	Papua New Guinea	24 (1)
BPBM 36749	Sulawesi	26 (1)
BPBM 38736	Solomon Is.	40 (1)
BPBM 39281	Fiji	56 (1)
BPBM 40522	Java	60 (1)
USNM 119614	Thailand	50.4 (1)
USNM 139362	Philippines	56 (1)
USNM 160738	Philippines	47.5–48.9 (2)
USNM 297234	Irian Jaya	48.3 (1)
USNM 297237	Papua New Guinea	14.4–31.4 (3)
USNM 346612	Bismark Archipelago	46.8 (1)
USNM 346615	Fiji	17.9–24.9 (2)
USNM 346642	Bismark Archipelago	16.9–22.2 (2)
USNM 362432	Vanuatu	17.1 (1)
USNM 378278	Solomon Is.	27.1–41.6 (2)
USNM 379516	Vanuatu	13.4–34.8 (3)

externally quite similar and have been hypothesized to be close relatives (Smith, 1956; Iwata et al., 2000) or synonyms (Randall and Chen, 2007), but may be differentiated by the presence of a small dermal flap on the anterior nostril in *Flabelligobius* species. Similarities in gill raker ossification patterns have also been noted among some species of *Cryptocentrus*, *Myersina*, and *Stonogobiops* (Akihito and Meguro, 1983).

The second clade of shrimp gobies recovered in our analysis includes *Amblyeleotris*, *Ctenogobiops*, and *Vanderhorstia*. *Ctenogobiops* species sampled are a subset of the individuals analyzed in Thacker et al. (2010), and the relationships recovered here are congruent with those recovered in that study. The deepest split recovered is between *Ctenogobiops aurocingulus* and the remainder of the species, followed by *Ct. tongaensis*, *Ctenogobiops mitodes*, and *Ctenogobiops formosa* arrayed in a comb-like grade sister to the species pair *Ctenogobiops crocineus* and *Ctenogobiops tangaroai*. Monophyly of *Ctenogobiops* is strongly supported, and that genus is sister to *Vanderhorstia*, represented by *Vanderhorstia lanceolata* and *Van. ornatissima*. Placement of the two *Vanderhorstia* species together as a clade distinct from other shrimp gobies provides evidence for the monophyly of *Vanderhorstia*, a genus that has not been diagnosed morphologically. This placement also contradicts the proposal (based on cheek papillae patterns) that two *Vanderhorstia* species, *Van. lanceolata* and *Vanderhorstia prealta*, should be removed from *Vanderhorstia* and placed instead in *Tomiyamichthys* (Shibukawa and Suzuki, 2004). Our topology shows that *Vanderhorstia* and *Tomiyamichthys* are not closely related. *Vanderhorstia* shares meristic, skeletal, and most external morphological characters with *Ctenogobiops*, and is distinguished from that genus only on the basis of caudal fin length and coloration (Shibukawa and Suzuki, 2004). Taken together, *Ctenogobiops* and *Vanderhorstia* are placed sister to the speciose genus *Amblyeleotris*.

Eight *Amblyeleotris* species were included for this study, including multiple representatives of the common species *A. fasciata*, *A. steinitzi*, and *A. wheeleri*. In our hypothesis, the well-known species *A. fasciata*, *A. steinitzi*, and *A. wheeleri* are all distinct and well-separated from one another. *Amblyeleotris* species overlap broadly in meristic counts and all exhibit variations on a basic color pattern: white with five to seven transverse pink or red bars, often also with small gold spots over the entire body. Deviations from this pattern, some slight, are what enable distinction of separate species. In the case of *A. fasciata*, *A. steinitzi*, and *A. wheeleri*, it is the number, width and intensity of the red bars that offers the most reliable distinguishing information: in *A. steinitzi* the five bars are pale pink, in *A. fasciata* the seven bars a darker red, and in *A. wheeleri* the seven bars are deep red and thicker than in *A. fasciata*, such that the red portions are wider than the intervening white areas.

The goby molecular phylogeny does not show correlation with one morphological character proposed to delineate groups of genera: the presence or absence of transverse cheek papillae rows. If that character were diagnostic, this study should have revealed a close relationship among the genera *Amblyeleotris*, *Cryptocentrus*, and *Stonogobiops*, to the exclusion of *Ctenogobiops*, *Mahidolia*, *Tomiyamichthys*, and *Vanderhorstia*. Instead, *Amblyeleotris*, *Ctenogobiops*, and *Vanderhorstia* fall into one clade, with *Cryptocentrus*, *Mahidolia*, *Stonogobiops*, and *Tomiyamichthys* in another. However, many other morphological characters differ between *Amblyeleotris* and *Cryptocentrus*, including fin ray counts (higher in *Amblyeleotris*), pelvic fin configuration (sturdy fused disc in *Cryptocentrus*, elongate fins separate or joined only posteriorly in *Amblyeleotris*), and a variety of additional external and osteological features (Hoese and Steene, 1978; Chen et al., 1998; Hoese and Larson, 2004).

This study does provide evidence for the monophyly of the genera *Ctenogobiops*, *Amblyeleotris*, *Vanderhorstia*, and *Mahidolia*. Most *Cryptocentrus* are also recovered as monophyletic; the species *Cr. cinctus* is placed apart from the remainder of *Cryptocentrus*, as sister taxon to *Mahidolia*. In the cases of *Stonogobiops* and *Tomiyamichthys*, only one species of these rare genera was analyzed. In neither case were they nested within other genera, but this analysis does not have sufficient sampling to address confirmation of their monophyly. Overall, the phylogenetic hypothesis presented in this study corroborates generic boundaries and relationships postulated on the basis of morphological characters, with the fascinating exception of *Cr. cinctus* and the *Ma. mysticina* species complex.

Acknowledgments

References

Akihito, Meguro, K., 1983. *Myersina nigrivirgata*, a new species of shrimp goby from Okinawa Prefecture in Japan. Jpn. J. Ichthyol. 29, 343–348.

Allen, G., Steene, R., Humann, P., Deloach, N., 2003. Reef Fish Identification: Tropical Pacific. New World Publications, Jacksonville, Florida.

Böhlke, J., Robins, C., 1968. Western Atlantic seven-spined gobies, with descriptions of ten new species and a new genus, and comments on Pacific relatives. Proc. Acad. Nat. Sci. Phila. 120, 45–174.

Boucher, D.H., James, S., Keeler, K.H., 1982. The ecology of mutualism. Ann. Rev. Ecol. Syst. 13, 315–347.

Bradbury, J.W., Vehrencamp, S.L., 1998. Principles of Animal Communication. Sinauer Associates, Inc..

Chen, I.-S., Fang, L.-S., 2003. A new marine goby of genus *Flabelligobius* (Teleostei: Gobiidae) from Taiwan. Ichthyol. Res. 50, 333–338.

Chen, J.-P., Chen, I.-S., Shao, K.-T., 1998. Review of the marine gobiid genus *Amblyeleotris* (Pisces: Gobiidae) with seven new records from Taiwan. Zool. Stud. 37 (2), 111–118.

Chen, W.-J., Bonillo, C., Lecointre, G., 2003. Repeatability of clades as a criterion of reliability: a case study for molecular phylogeny of Acanthomorpha (Teleostei) with larger number of taxa. Mol. Phyl. Evol. 26, 262–288.

Connor, R.C., 1995. The benefits of mutualism: a conceptual framework. Biol. Rev. 70, 427–457.

Cote, I.M., 2000. Evolution and ecology of cleaning symbioses in the sea. Ocean. Mar. Biol. 38, 311–355.

Cummins, R.A., 1979. Ecology of Gobiid Fishes Associated with Alpheid Shrimps at One Tree Reef. Ph.D. Thesis, University of Sydney, Australia.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucl. Acid Res. 32, 1792–1797.

Fautin, D.G., Allen, G.R., 1997. Anemonefishes and their Host Sea Anemones, revised ed. Western Australian Museum, Perth, Australia.

Foster, K.R., Wenseleers, T., 2006. A general model for the evolution of mutualisms. J. Evol. Biol. 19, 1283–1293.

Goodale, E., Beauchamp, G., Magrath, R.D., Nieh, J.C., Ruxton, G.D., 2010. Interspecific information transfer influences animal community structure. Trends Ecol. Evol. 25, 354–361.

Harada, E., 1969. On the interspecific association of a snapping shrimp and gobioid fishes. Pub. Seto Mar. Biol. Lab. XXVI (5), 315–334.

Hayashi, M., Shiratori, T., 2003. Gobies of Japanese Waters. TBS Buritanica, Tokyo (in Japanese).

Hoese, D., Larson, H.K., 2004. Description of a new species of *Cryptocentrus* (Teleostei: Gobiidae) from northern Australia, with comments on the genus. Beagle, Rec. Mus. Art Gal. North. Ter. 20, 167–174.

Hoese, D., Lubbock, R., 1982. A review of the genus *Myersina* (Pisces: Gobiidae), with the description of a new species. Aust. Zool. 21 (1), 47–54.

Hoese, D., Randall, J.E., 1982. Revision of the gobiid fish genus *Stonogobiops*. Indo-Pacific Fishes 1, 1–18.

Hoese, D., Steene, R., 1978. *Amblyeleotris randalli*, a new species of gobiid fish living in association with alpheid shrimps. Rec. West. Aust. Mus. 6 (4), 379–389.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Iwata, A., Ohnishi, N., Hirata, T., 2000. *Tomiyamichthys alleni*: a new species of Gobiidae from Japan and Indonesia. Copeia 2000 (3), 771–776.

Karplus, I., 1987. The association between gobiid fishes and burrowing alpheid shrimps. Oceanogr. Mar. Biol. Ann. Rev. 25, 507–562.

Karplus, I., 1992. Obligatory and facultative goby-shrimp partnerships in the western tropical Atlantic. Symbiosis 12, 275–291.

Karplus, I., Tsurnamal, M., Szlep, R., Algom, D., 1979. Film analysis of the tactile communication between *Cryptocentrus steinitzi* (Pisces, Gobiidae) and *Alpheus purpurilenticularis* (Crustaceae, Alpheidae). Z. Tierpsychol. 49, 337–351.

Karplus, I., Szlep, R., Tsurnamal, M., 1981. Goby-shrimp partner specificity I: distribution in the northern Red-Sea and partner specificity. J. Exp. Mar. Biol. Ecol. 51, 1–19.

Karplus, I., Thompson, A.R., 2011. The partnership between gobiid fishes and burrowing alpheid shrimps. In: Patzner, R.A., Van Tassell, J.L., Kovacic, M., Kapoor, B.G. (Eds.), Biology of Gobies. Science Publishers, Inc..

Karplus, I., Vercheson, A., 1978. A method for collecting live alpheid shrimp and their symbiotic gobiid fish partners. Crustaceana 34, 220–221.

Kawakita, A., Kato, M., 2009. Repeated independent evolution of obligate pollination mutualism in the Phyllantheae–*Epicephala* association. Proc. Roy. Soc. B 276, 417–426.

Klausewitz, W., 1960. Fische aus dem Roten Meer IV. Einige systematisch und ökologisch bemerkenswerte Meergrundeln (Pisces, Gobiidae). Senck. Biol. 41 (3/4), 149–162.

Kramer, A., Van Tassell, J.L., Patzner, R.A., 2009. A comparative study of two goby shrimp associations in the Caribbean Sea. Symbiosis 49, 137–141.

Lachner, E.A., McKinney, J.F., 1980. A new fish species of the genus *Vanderhorstia* (Teleostei: Gobiidae) from the Amirante Islands, Indian Ocean. Proc. Biol. Soc. Wash. 93 (4), 963–970.

Larson, H.K., Lim, K.K.P., 2005. A guide to the gobies of Singapore. Singapore Science Center, 164 p.

Miller, D.J., Lea, R.N., 1972. Guide to the Coastal Marine Fishes of California, California Department of Fish and Game Fish Bulletin 157.

Mohlmann, M.S., Randall, J.E., 2002. Three new species of gobiid fishes of the genus *Amblyeleotris* from the central and western Pacific. Raffles Bull. Zool. 50 (1), 215–226.

Myers, R.F., 1999. Micronesian Reef Fishes: A Comprehensive Guide to the Coral Reef Fishes of Micronesia, third ed. Coral Graphics, Guam.

Palumbi, S.R., 1996. Nucleic acids II: the polymerase chain reaction. In: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.), Molecular Systematics, second ed. Sinauer and Associates, Sunderland, MA.

Pellmyr, O., 1997. Why is active pollination so rare? Ecology 78, 1655–1660.

Pellmyr, O., Kren, H.W., 2002. Origin of a complex key innovation in an obligate insect–plant mutualism. PNAS 99, 5498–5502.

Pellmyr, O., Thompson, J.N., Brown, J.M., Harrison, R.G., 1996. Evolution of pollination and mutualism in the yucca moth lineage. Am. Nat. 148, 827–847.

Polunin, N.V.C., Lubbock, R., 1977. Prawn-associated gobies (Teleostei: Gobiidae) from the Seychelles, Western Indian Ocean: systematics and ecology. J. Zool. Lond. 183, 63–101.

Posada, D., 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25 (7), 1253–1256.

Randall, J.E., Lobel, P.S., Kennedy, C.W., 2005. Comparative ecology of the gobies *Nes longus* and *Ctenogobius saepepallens*, both symbiotic with the snapping shrimp *Alpheus floridanus*. Env. Biol. Fish. 74, 119–127.

Randall, J.E., Chen, I.-S., 2007. *Tomiyamichthys tanyspilus*, a new species of gobiid fish from Indonesia. Zool. Stud. 46 (6), 651–655.

Randall, J.E., Shao, K.-T., Chen, J.-P., 2003. A review of the Indo-Pacific gobiid fish genus *Ctenogobiops*, with descriptions of two new species. Zool. Stud. 42 (4), 506–515.

Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Sachs, J.L., Mueller, U.G., Wilcox, T.P., Bull, J.J., 2004. The evolution of cooperation. Q. Rev. Biol. 79, 135–160.

Santini, S., Polacco, G., 2006. Finding Nemo: Molecular phylogeny and evolution of the unusual life style of anemonefish. Gene 385, 19–27.

Satapoomin, U., Winterbottom, R., 2002. Redescription of the gobioid fish *Cryptocentrus pavoninoides* (Bleeker, 1849), with notes on sexual dichromatism in shrimp gobies. Aqua 5 (2), 53–64.

Schultz, T.R., Brady, S.G., 2008. Major evolutionary transitions in ant agriculture. PNAS 105, 5435–5440.

Shibukawa, K., Suzuki, T., 2004. *Vanderhorstia papilio*, a new shrimp-associated goby from the Ryukyu Islands, Japan (Perciformes: Gobiidae: Gobiinae), with comments on the limits of the genus. Ichthyol. Res. 51, 113–119.

Smith, J.L.B., 1956. An interesting new gobioid fish from Madagascar, with a note on *Cryptocentrus oni* Tomiyama, 1936. Ann. Mag. Nat. Hist. 9, 554–556.

Syms, C., Jones, G.P., 2004. Habitat structure, disturbance and the composition of sand-dwelling goby assemblages in a coral reef lagoon. Mar. Ecol. Prog. Ser. 268, 221–230.

Thacker, C.E., 2003. Molecular phylogeny of the gobioid fishes (Teleostei: Perciformes: Gobioidei). Mol. Phyl. Evol. 26, 354–368.

Thacker, C.E., Hardman, M.A., 2005. Molecular phylogenetics of basal gobioid fishes: Rhyacichthyidae, Odontobutidae, Xenisthmidae, Eleotridae (Teleostei: Perciformes: Gobioidei). Mol. Phyl. Evol. 37 (3), 858–871.

Thacker, C.E., 2009. Phylogeny of Gobiodei and placement within Acanthomorpha, with a new classification and investigation of diversification and character evolution. Copeia, 93–104.

Thacker, C.E., Thompson, A.R., Adam, T.C., Chen, J.-P., 2010. Phylogeny and character evolution in the Indo-Pacific genus *Ctenogobiops* (Gobiiformes: Gobiidae). Ichthy. Res. 57, 231–239.

Thompson, A.R., 2005. Dynamics of demographically open mutualists: immigration, intraspecific competition, and predation impact goby populations. Oecologia 143, 61–69.

Thompson, A.R., Nisbet, R.M., Schmitt, R.J., 2006. Dynamics of mutualist populations that are demographically open. J. Anim. Ecol. 75, 1239–1251.

Trench, R.K., 1993. Microalgal-invertebrate symbioses – a review. Endocyto. Cell. Res. 9, 135–175.

Winterbottom, R., 2001. A redescription of *Cryptocentrus crocatus* Wongratana, a redefinition of *Myersina* Herre (Acanthopterygii: Gobiidae), a key to the species, and comments on relationships. Ichthyol. Res. 49, 69–75.