Research Article

Phylogeny of Gobiidae and identification of gobiid lineages

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The teleost family Gobiidae includes at least 1120 described species of fishes, distributed worldwide in both tropical and temperate habitats. The majority of gobies inhabit marine environments, in particular Old World coral reefs. However, a radiation of gobies inhabits the rivers and near-shore habitats of Europe and Asia, and a variety of genera are also found in the seas of the New World. This study builds on previous work in which gobids were placed among their gobioid relatives by adding additional taxa as well as additional markers, providing a much more comprehensive portrait of gobiid intrarelationships and including all major lineages of gobies. We used DNA sequences from both mitochondrial (ND1, ND2, COI) and nuclear (RAG2, Rhodopsin, RNF213) genes to infer phylogeny among 127 representatives of 100 species of gobies, using two gobionellid species as outgroups. We delineated 13 lineages within Gobiidae, including one clade of shrimp-associated gobies represented by the genera Cryptocentrus, Mahidolia and Stonogobiops and a second separate shrimp-associated goby clade including Amblyeleotris, Cenogobiops and Vanderhorstia. The Mediterranean, Ponto-Caspian and Eastern Atlantic gobies are resolved in a clade along with two genera known from the Western Indian Ocean. Invasion of the New World is shown to have occurred multiple times among the sampled taxa, in the American seven-spined gobies, the Coryphopterus, Lophogobius and Rhinogobiops radiation (sister to Fusigobius) and separately in the wormfishes Cerdale and Microdesmus, resolved in a clade alongside the Indo-Pacific Gunnelichthys, Ptereleotris and Nemateleotris. The cosmopolitan genera Bathygobius and Priolepis represent further separate radiations, and Lythrypnus shows complex relationships with both Priolepis and Trimma.

Key words: Atlantic, biogeography, Caribbean, goby, Indo-Pacific, Mediterranean, Ponto-Caspian, systematics

Introduction

Gobiidae is one of the largest families of acanthomorph fishes, with at least 1120 species described in 170 genera and many more yet to be described (Thacker, 2011). Gobies are distributed worldwide, in marine, estuarine and freshwater habitats. They are generally benthic, and may occupy various niches in the substrate including the bodies or burrows of invertebrates. Gobies attain a small body size (often less than 50 mm), and most bear pelvic fins wholly or partially joined ventrally into a disc. Most species have separate spinous and rayed dorsal fins, and colouration ranges from drab to a variety of bright patterns. Gobies constitute a large portion of the fishes in both tropical and temperate near-shore marine, brackish and freshwater environments, including prominent representation on coral reefs, where they are estimated to constitute 35% of the total number of fishes and 20% of the species diversity (Winterbottom et al., 2011). Small-bodied reef-dwellers such as Eviotia species mature rapidly, experience short lifespans (59–99 days), and so have rapid generation times (up to 7.4 generations per year; Depczynski & Bellwood, 2005, 2006). However, due to their small size and often cryptic ecologies, the full extent of gobiid diversity often goes unnoticed. These same characteristics have also hindered evolutionary and taxonomic studies of the group, but advances in molecular phylogenetics have provided a novel avenue for untangling gobioid relationships (Thacker, 2003, 2009; Thacker & Hardman, 2005).

Here we seek to determine the relationships among gobiid gobies, and to identify the lineage diversity in this speciose family. Gobiidae is used in the restricted monophyletic sense proposed in Thacker (2009), a clade composed of the former gobioid families and subfamilies Gobiinae, Microdesmidae, Ptereleotridae, Kraemeriidae and Schindleriidae. This group is sister to the clade Gobionellidae, which similarly includes the taxa Gobionellinae, Sicydinae, Amblyopinae and Oxudercinae. These taxa were formally named in Thacker (2009), but were first delineated in an earlier study (Thacker, 2003) that was...
based on a smaller dataset and used different analytical methods. The previous molecular phylogenetic studies of Gobiiformes that included more than a few representatives of Gobiidae (Thacker, 2003, 2009) have provided several insights into relationships among gobids, but discerning the evolution of lineages within Gobiidae was not their primary focus. In both those earlier studies, relationships among lineages within Gobiidae were poorly resolved, with weak support for relationships at the base of Gobiidae. Here we build on the gobiid sampling of those studies with additional taxa, representing all the common gobiid lineages and including the American seven-spined and Pontoco- Caspian, Mediterranean and Eastern Atlantic radiations. We also augment a previously exclusively mitochondrial dataset with sequence from three nuclear genes. With this expanded taxon and marker dataset, we are able to provide a much more comprehensive accounting of gobiid relationships and evolution than previously, discerning a variety of lineages within Gobiidae and representing the entire geographic range of the family. Many of the lineages include taxa previously postulated to be related based on various explicitly quantified morphological characters, and also on more qualitative characteristics including overall shape, behaviour or ecology. We then review the genera not examined in our phylogeny, and based on previous systematic studies, seek to place each genus into one of the phylogenetically identified lineages. The delineation of these lineages reveals biogeographic and evolutionary patterns that span the diversity of the family, as well as providing identification of subsections of the remarkable diversity of gobies. We provide an updated (relative to Thacker, 2011), and categorized genus list that may be used to guide further studies of diversity and phylogeny, as well as facilitate communication in wider-ranging studies of ecology or evolution.

Materials and methods

This study combines DNA sequence data from an earlier, more widely focused phylogenetic analysis of Gobiiformes (Thacker, 2009) with additional data. New taxa were sequenced for three mitochondrial genes, and three nuclear gene fragments were added for the entire taxon set. All specimens are vouchered, most in the Natural History Museum of Los Angeles County Ichthyology Tissue Collection. Species examined, GenBank accession numbers for sequences, and tissue catalogue information for vouchers are listed in Appendix 1 (see supplementary material, which is available on the Supplementary Content table of the article’s online page at http://dx.doi.org/10.1080/14772000.2011.629011).

Extraction of DNA from tissue and PCR were performed as described in Thacker et al. (2011) for 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). An 840 bp fragment from the protein-coding region of RNF213 was initially amplified using the primer pair C17 F3150/C17 R4096 (Li et al., 2009). Amplification of the target fragment was successful for approximately half of the taxa; those products were sequenced and aligned using MUSCLE (Edgar, 2004). Internal, gobiid-specific, internal primers were then designed by eye. Those primers were used to amplify and sequence a fragment approximately 675 bp in length, and have the following sequences: 5′-GGG AAA CNG AGC CNC ATC CNT AC-3′ (forward) and 5′-TYA GCN CGK ACT CTG TAT CC-3′ (reverse). PCR products were visualized, extracted and sequenced, and aligned as described in Thacker et al. (2011).

Bayesian phylogenetic analyses were performed in MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The appropriate model for base substitution frequencies was determined by jModelTest (Posada, 2008), using the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC), for each gene partition as well as for the complete six-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 10,000,000 generations with two replicates, each with four simultaneous chains, and trees sampled every 10,000 generations.

Phylogenetic analyses were also performed using the maximum likelihood optimality criterion, implemented in the software package TREEFINDER (Jobb et al., 2004). Searches were performed with a single model applied to the entire concatenated dataset (unpartitioned) as well as with separate models applied to each individual gene (partitioned). Support for both the partitioned and unpartitioned analyses was assessed by performance of 1000 bootstrap replications; the proportion of these replications supporting the figured hypothesis is shown on each node, except nodes supported by fewer than 50% of replicates, which are unlabelled. The alignment used in all phylogenetic analysis is available as listed in Appendix 2 (see supplementary material, which is available on the Supplementary Content table of the article’s online page at http://dx.doi.org/10.1080/14772000.2011.629011).

Our phylogeny includes 58 genera of Gobiidae, or just over one third of the total 170. Using the lineages identified in our molecular phylogeny, we then review the 112 genera not sampled, in an attempt to postulate placement of the unsampled diversity among our phylogenetic lineages. Placements were made based on shared morphological characters, similarity of shape, distribution, habitat, ecology, behaviour, or any other characteristics identified or relationships advanced by the many workers that have treated all the various gobies. We present the hypothesized lineage groups in Table 1; throughout, vertebral counts are given in
Table 1. Generic groups within Gobiidae, inferred based on molecular and morphological data. Thirteen gobiid lineages are listed here, as identified in the phylogenetic hypothesis given in Fig. 1; the Mediterranean, Ponto-Caspian and Eastern Atlantic gobies are subdivided into their three component regions. Genera sequenced in this study are indicated in bold; genera not sequenced are placed into groups based on morphological similarity with sequenced genera. The sand gobies are listed but indicated with an asterisk because this group is likely part of Gobionellidae, not Gobiidae. This table includes 169 of the 170 valid genera of Gobiidae: Austrolethops is not placed in a lineage and is incertae sedis within Gobiidae.

\*SAND GOBIES
Economidichthys, Gobiulusculus, Hyrcanogobius, Knipowitschia, Pomatoschistus

MEDITERRANEAN, PONTO-CASPIAN AND EASTERN ATLANTIC GOBIES

PONTO-CASPIAN GOBIES
Anatrostrum, Babka, Benthophilus, Benthophiloides, Castosoma, Mesogobius, Neogobius, Padogobius, Ponticola, Proterorhinus

MEDITERRANEAN AND NORTHEAST ATLANTIC GOBIES
Aphia, Buania, Chromogobius, Corycygobius, Crystallogobius, Deltentosteus, Didagobius, Gammagobius, Gobius, Gobiusculus, Lebetus, Lesuergobius, Mauligobius, Millierigobius, Odondebuena, Pseudaphya, Speleogobius, Thorogobius, Vanneaugobius, Zebrus, Zosterisessor

AFRICAN GOBIES (SOUTHEAST ATLANTIC AND WESTERN INDIAN OCEAN)
Caffrogobius, Corygobius, Crotilia, Ehomegobius, Gorogobius, Heterereotris, Nematogobius, Porogobius, Safflogobius, Wheeleigobius

AMERICAN SEVEN-SPINED GOBIES (EASTERN PACIFIC AND WESTERN ATLANTIC)
Akkra, Aruma, Barbarilfer, Bolmlannia, Chriolepis, Elacatinus, Eleotrica, Enypnias, Evermannichthys, Ginsburgellus, Gobiosoma, Gobius, Gymneleotris, Microgobius, Nes, Ophiogobius, Palatogobius, Parrella, Pariah, Psilotratis, Pycnomma, Risor, Robinsichthys, Tigrigobius, Varicus, Vomergobius

LAGOON GOBIES
Acentrogobius, Afuracargobius, Amaya, Ancistrogobius, Arcygobius, Arenigobius, Aulopareia, Cahillus, Echinogobius, Exyrias, Favonigobius, Gladiogobius, Hazeus, Heteroplomopus, Istigobius, Macrodonotogobius, Nocogobius, Oplopus, Oplopatogobius, Paracheturichthys, Silhouettea, Yoneigobius

SILT SHRIMP GOBIES
Cryptocentrus, Cryptocentroides, Flabelligobius, Lotilia, Mahidolia, Myersina, Psilogobius, Stonogobiops, Tomyiamichthys

REEF SHRIMP GOBIES
Amblyeleotris, Asterropteryx, Ctenogobiops, Vanderhorstia

SAND DIVERS
Kraemeria, Gobitrichonotus, Parakraemeria

FLAPHEADED GOBIES
Barbuligobius, Callogobius, Discordipinna, Drombus, Feia, Gobiopsis, Mangarinus, Palutrus, Phoxacromion, Platygobiopsis

BURROWING PAIRED GOBIES
Amblygobius, Signigobius, Valenciennnea

CORAL GOBIES
Bryaninops, Eviota, Gobiodon, Kelloggella, Larsonella, Lobulogobius, Lubricogobius, Lupsicya, Minisicya, Paragobiodon, Phyllogobius, Pleuroiscya, Sueviota

TINY BANDED GOBIES
Egglestonichthys, Ego, Lythrurus, Obliquogobius, Paratrimma, Priolepis, Trimma, Trimmatom, Tryssogobius

CRESTED GOBIES
Coryphopterus, Cristatogobius, Fusigobius, Lophogobius, Rhinogobiops

WORMFISHES AND DARTFISHES
Aiolops, Cerdale, Clarksichthys, Gunnellichthys, Microdesmus, Navigobius, Nematoleotris, Oxymetopon, Paragunnellichthys, Parioglossus, Ptereleotris, Pterocerdale, Schindleria

INSHORE GOBIES
Bathygobius, Glossogobius, Grallenia, Psammogobiops

the formula precaudal + caudal = total, and dorsal spine pterygiophore insertion patterns in the formula of Birdsong et al. (1988). In this formula, the first digit indicates the interneural space in which the spinous dorsal fin begins, followed by a hyphen and then the number of pterygiophores in each successive interneural space. Thus, 3-2211 indicates a total of six dorsal spines, two in the third and fourth interneural spaces, and one each in the fifth and sixth.
Results

A total of 127 individuals representing 102 species in 60 genera (100 Gobiidae and two outgroup Gobionellidae sensu Thacker, 2009) were included in the analysis. The matrix consisted of 5278 aligned base pairs (bp): 978 bp of ND1, 945 bp of ND2, 1191 bp of COI, 780 bp of RAG2, 618 bp of RNF213 and 766 bp of rhodopsin.

The appropriate base substitution model for the complete dataset was determined to be GTR + I + G; the same model applied to the individual gene datasets ND1 and COI. For ND2, the GTR + G model obtained; for RAG2 and RNF213 HKY + G was selected, and for Rao, HKY + I + G. Bayesian phylogenetic analyses were run for 10 million generations (approximately 30 days) and failed to approach convergence in both the partitioned and unpartitioned analyses. Likelihood analyses for both the partitioned and unpartitioned datasets yielded hypotheses that differed only slightly, and only in the relative positions of larger subclades within Gobiidae. Bootstrap analysis of the likelihood results revealed that there was little support for relationships at the backbone of the Gobiidae tree, and confirmed that the evidence for that part of the phylogeny was scanty enough that Bayesian sampling strategies could not resolve a single optimal region of tree space. However, the lineages identified within Gobiidae are generally well-supported, and were consistently present in both partitioned and unpartitioned likelihood analyses.

The hypothesis resulting from the partitioned likelihood analysis is shown in Fig. 1. In several cases, more than one individual for a given taxon was sequenced, which validates placement of that species as well as providing evidence for species monophyly. In all but one case, multiple individuals identified as the same species grouped together, generally with very little sequence difference between them. The exception is Valenciennesia longipinnis, a widespread Pacific species. One of the two V. longipinnis individuals sampled grouped more closely to an individual of V. strigata, a species with an even larger range, extending throughout the Pacific and Indian Oceans. Potential causes for this result are numerous, but postulation of biological explanations is premature given the possibility of simple phylogenetic uncertainty. The node supporting the pairing has an estimated bootstrap support of 90%, and in particular the V. strigata branch is notably longer than those of V. longipinnis, potentially causing a spurious result due to uncertain resolution of a highly autapomorphic taxon.

Although recovered resolution was poor among the basal nodes of Gobiidae, our phylogeny clarifies relationships within and among smaller subgroups of gobies, and reveals lineages consisting of related genera. With this evolutionary framework, these lineages may be examined and interpreted. Thirteen separate lineages were identified among the Gobiidae sampled; 11 consisting of multiple genera, plus the single-genus lineages Kraemeria and Callogobius. Relationships among the lineages are generally poorly supported and should be considered preliminary. Many of the lineage clades identified here are supported by bootstrap percentages of 80% or above, but a few are not. In particular, the deeper splits among the lagoon gobies (including placements of Oplopomus oplopomus, Arenogobius bifrenatus, Cabillus tongarevae and Acentrogobius pflaumii), the wormfishes and dartfishes (placement of Ptereleotris species and Nemateleotris magnifica) and the American seven-spined gobies (resolution of Microgobius microlepis) have short internodes and low bootstrap support. In each of these cases, the poorly supported basal members are included in the described lineages, in concordance with other morphological or ecological information as discussed in the individual lineage sections. These lineages represent all the familiar groups of gobies, including Indo-Pacific shrimp gobies, coral-associated gobies, burrowing monogamous gobies, near-shore lagoon gobies, the American seven-spined gobies, the Ponto-Caspian/Mediterranean radiation, the wormfishes and dartfishes, Coryphopterus and its relatives, and the circumglobal Bathygobius and Priolepis lineages. Shrimp gobies were recovered in two separate clades, in accordance with another recent phylogenetic analysis (Thacker et al., 2011). Invasion of New World waters has occurred multiple independent times on this hypothesis, among the American seven-spined gobies, the wormfishes (Microdesmus and Cerdale), in Rhinogobiops, Lophogobius and some Coryphopterus, and some species of the widespread genera Bathygobius and Priolepis. Lythrypnus also inhabits the western Atlantic and eastern Pacific, and is recovered as closely related to Priolepis and Trimma, species of which are all very diminutive and usually schooling or cryptobenthic in tropical reef environments.

Discussion

Our phylogenetic hypothesis (Fig. 1) resolves all but one of the gobids sampled into a clade with shallow internodes separating the various gobiid lineages. Within Gobiidae, our hypothesis resolves three subclades, although the internodes between them are very short and poorly supported. One clade includes several Indo-Pacific genera, some of the shrimp-associated gobies, and the inshore gobies Bathygobius and Psammogobius; another small subgroup comprises the American seven-spined gobies. The third and largest subclade contains the majority of the genera, including the remainder of the New World taxa, a clade of Mediterranean, Ponto-Caspian and Eastern Atlantic taxa, and many Indo-Pacific lineages including a second clade of shrimp gobies. Overall within Gobiidae, 11 multi-genus lineages were recovered, along with isolated genera Callogobius and Kraemeria. Identification of these 13 lineages, particularly those that constitute the highly diverse Indo-Pacific reef gobies (generally, the Priolepis group of Birdsong et al., 1988), allows interpretation of evolution and biogeography, as well as assessment of morphological patterns that could not
Fig. 1. Phylogenetic analysis of 100 species of Gobiidae and two outgroup Gobionellidae, based on partitioned Maximum Likelihood analysis. Numbers on nodes are bootstrap support values, shown as percentages of 1,000 replications. To the right, each larger clade is given a lineage name and the constituent genera in the hypothesis are listed.
previously be understood phylogenetically. In Fig. 1 and in the text, each clade is denoted with a lineage name that reflects common ecology, distribution or morphology for the included taxa. The names are provided for ease of communication and wherever possible, incorporate informal names already applied to lineages or their members; they are not intended to indicate that the named characteristics are unique to certain clades. Thus, the tiny banded gobies are not the only diminutive gobies with a banded colour pattern, and the burrowing paired gobies are not the only gobies who inhabit burrows. Similarly, not all lagoon gobies inhabit lagoons, not all crested gobies bear a crest, and not all coral gobies live in coral. Simply, these names enable communication and comparison better than numbers, letters or other acronyms. Given the phylogenetically identified lineages, it is also possible to postulate placement of other gobii genera not sampled in this study, based on morphological commonalities with the sampled taxa. A list of the 13 lineages recovered in our analysis, with hypothesized placements for all gobii genera, is given in Table 1; this table updates, expands and categorizes the list of gobii genera given in Thacker (2011). These lineages represent reviews of character evidence and relationships hypothesized up to this point; they provide a convenient summary of knowledge, as well as facilitating meaningful communication and discussion of lineages.

Mediterranean, Ponto-Caspian and African Gobiidae

This large clade includes all the Ponto-Caspian and Mediterranean taxa sampled (except Pomatoschistus), with the clade of Ponto-Caspian taxa resolved as sister to the Mediterranean Gobius and Zosterisessor. Outside them, arrayed as a grade, are two genera that inhabit the inshore waters of Africa, Caffrogobius and Corygobalops. The entire large clade (Fig. 1, Table 1) is divided into its three constituent areas, in which sequenced genera along with all the additional genera postulated to be close relatives are listed. All of these Old World Gobiidae possess elevated vertebral counts compared with those of most Indo-Pacific Gobiidae (usually 10 + 16 = 26). The African genera have 27 (rarely 28) vertebrae, the Mediterranean and North Atlantic gobies usually exhibit a vertebral count of 11 + 17 = 28, and the Ponto-Caspian species counts are even higher, ranging from 31–35 (Hoese, 1986; Birdsong et al., 1988; Simonovic et al., 1996; Simonovic, 1999).

Sand gobies

The one putatively gobii taxon placed outside all the other gobii genera sampled is the Mediterranean/Eastern Atlantic Pomatoschistus minutus, which forms a basal grade along with outgroups Gnatholepis anjerensis and Sicyopterus taenius (Gobionellidae). Pomatoschistus and the four similar genera Knipowitschia, Gobiusculus, Hyrcanogobius, and Economidichthys are collectively known as the sand gobies. They inhabit the coastal waters of the eastern North Atlantic, the Mediterranean and the Black Sea. Salinity tolerances for species of sand goby genera range through marine (Gobiusculus), inshore marine/estuarine (Pomatoschistus), estuarine/freshwater (Knipowitschia) and fully freshwater (Economidichthys). Hyrcanogobius is endemic to the Caspian Sea and is known from fresh, brackish, and marine water. The biology of these genera has been fairly well-studied, with visual pigment evolution (Larmuseau et al., 2010), ecology and biogeography (Penzo et al., 1998; Huyse et al., 2004), allozyme polymorphism (Webb, 1980; Wallis & Beardmore, 1984; Miller et al., 1994; McKay & Miller, 1997), karyotype and soft tissue morphology (Webb, 1980), and skeletal characters (McKay & Miller, 1997; Vasil’eva & Kuga, 2001) all having been described and analysed. Pomatoschistus is unusual among gobii in that it possesses a dorsal fin pterygiophore insertion pattern more typical of gobionellids (3-122100 or 3-221100 rather than 3-22110 or 3-22111) as well as vertebral counts that are elevated compared with most Gobiidae (Birdsong et al., 1988). There is a single epural in the caudal skeleton, a condition more typical of Gobiidae, but also present among Gobionellidae (McKay & Miller, 1997; Larson, 2001; Pezold, 2004; Thacker, 2009). Allozyme-based phylogenetic hypotheses show that Pomatoschistus and the other sand gobies are distinct from other gobii, including Ponto-Caspian species. Those comparisons did not include any representatives of Gobionellidae. Similarly, in phylogenies based on analysis of DNA sequence, the sand gobies are distinct from other Gobiidae, but are not compared to Gobionellidae, unsurprisingly given the scale of comparisons considered in those studies (Penzo et al., 1998; Huyse et al., 2004; Larmuseau et al., 2010). McKay and Miller (1997) hypothesize and discuss morphological character evidence that the sand gobies are related to Gobionellidae, and provide a cladistic analysis of morphology showing the sand gobies and several other gobii genera nested within a gobionellid clade. Neilson & Stepien’s (2009) study of Ponto-Caspian goby relationships included molecular phylogenetic analyses with Pomatoschistus and Knipowitschia in addition to a variety of other gobii outgroups. Their hypothesis places those sand gobies among other Gobiidae, not with Gobionellidae, although support values outside the Ponto-Caspian clade are low. It is possible that Pomatoschistus is a gobionellid, and that due to the paucity of gobionellids included here, our phylogenetic analysis may have yielded a paraphyletic reconstruction of Gobionellidae, a pattern seen in comparisons of previous analyses of gobii phylogeny (Thacker, 2003; Thacker & Hardman, 2005). Gobionellidae lacks a diagnostic morphological character, and instead the clade is identified by combinations of characters such as dorsal fin formula (3-12210), epural number (2), interorbital pore configuration...
Benthophilus includes only one exemplar for each, and the relationships of Ponto-Caspian gobies inhabiting the Ponto-Caspian region, are part of a single clade. The Ponto-Caspian gobies (Babka, Benthophilus, Mesogobius, Neogobius, Ponticola, Proterorhinos) are a radiation of hardy freshwater species native to the Aral, Azov, Black and Caspian Seas and their drainages (Simonovic, 1999). Several of these species have invaded freshwater ecosystems in Europe, and the species Neogobius melanostomus and Proterorhinos semilunaris have been introduced into the American Great Lakes system by ballast transport. A detailed study of Ponto-Caspian goby relationships has demonstrated that the lineage is monophyletic, and comprises three subgroups or tribes (Neilson & Stepien, 2009). The same tribes were recovered in our phylogeny, but with slightly different interrelationships. Both studies are based on analysis of both mitochondrial and nuclear DNA sequences, but the markers are non-overlapping with the exception of mitochondrial COI. The largest tribe, Ponticolini, includes the majority of Ponto-Caspian gobies: Babka, Mesogobius, Ponticola and Proterorhinos. Our study includes only one exemplar for each, and the relationships recovered are the same as in Neilson & Stepien (2009). In that study, the tadpole gobies Benthophilus and Caspiosoma (tribe Benthophilini) are sister to the Ponticolini, with Neogobius (tribe Neogobiini) as the sister to them both. Our study included a single Benthophilus species, which was placed outside a Neogobiini/Ponticolini clade, but in both hypotheses these deeper relationships are not well-supported, so the relationships effectively remain unresolved.

Mediterranean and Northeast Atlantic gobies

Our hypothesis and that of Neilson & Stepien (2009) agree that the wider ranging Gobius and Zosterisessor, known from the Eastern Atlantic and Mediterranean as well as the Black Sea, are grouped together as sister to the Ponto-Caspian species. Neilson & Stepien (2009) also include another Mediterranean gobiid genus in the outgroups, Chromogobius, that was not represented in our study. Separation of the Ponto-Caspian species as the sister clade, but distinct from Northeast Atlantic/Mediterranean gobiid lineages, is consistent with geological history. Well after the closure of the Tethys sea and isolation of Mediterranean and Indian Oceans, the Messinian salinity crisis (approximately 5.3–6.0 million years ago) resulted in the Mediterranean experiencing cycles of desiccation, leaving relict lakes and inland seas throughout the current areas of the Mediterranean, Black, Caspian and Aral basins. Separate refugia formed in the east (Paratethys: Central Europe/Asia) and west (Mediterranean basin), isolating various euryhaline gobioic lineages. Eventually, the Mediterranean was re-filled gradually due to increased freshwater input, followed by seawater flooding, and reintroduction of marine taxa, from the Atlantic through the Strait of Gibraltar (Penzo et al., 1998; Simonovic, 1999; Neilson & Stepien, 2009).

Included in this lineage are all of the gobid genera that inhabit the Mediterranean and Northeast Atlantic; a few species distributions extend south to the Southeast Atlantic (Miller, 1973, 1988) but generally, the lineage is geographically cohesive. With the exception of Gobius, the other 20 genera in this lineage each contain only one to three species; some of the genera are exclusively Mediterranean (Gammogobius, Millirigobius, Odondehuenia, Speleogobiini), and some exclusively Atlantic (Gobiusculus, Mauligoebius, Vanneaugobius), but the majority are widespread throughout both areas. Similarly, most species in this lineage are cryptobenthic, with a colour pattern that features few (3–7) dark lateral bands along the body (Ahnelt, 1991; Ahnelt et al., 1994; Brito & Miller, 2001; Schliewen & Kovacic, 2008). Exceptions include the genera Aphia, Crystallogobius, Gobiusculus and Pseudaphya, all diminutive and wholly or partially transparent, with the habit of forming pelagic schools. Lesserigobius and Thorogobius may attain a much larger size, inhabit muddy or silty habitats, and have spotted colour patterns (Miller, 1988; Ahnelt & Kovacic, 1997; Ahnelt & Dorda, 2004). Some Didogobius are commensal with shrimp, in this case the axiid Axiops serratifrons (Schliwenn & Kovacic, 2008); a different group of shrimp than the alfheids that engage in most mutualistic relationships with Gobiidae (Thacker et al., 2011).

African gobies (Eastern Central Atlantic; Southeast Atlantic and Western Indian Ocean)

Our Ponto-Caspian plus Northeast Atlantic/Mediterranean clade is rooted with two genera that inhabit the Western Indian Ocean and Southeast Atlantic: Corygalops and Caffrogobius. Placement of these taxa as a grade basal to the Ponto-Caspian and Atlantic/Mediterranean species is also consistent with tectonic history; the Mediterranean and Indian Ocean basins were contiguous as part of the
Tethys Sea until the Oligocene/Miocene transition, with the final closure occurring approximately 20 million years ago (Hrbek & Meyer, 2003). In Table 1, we include nine additional genera in the African Goby lineage, several of them monotypic, and all restricted to the waters of sub-Saharan Africa (Smith, 1959; Hoese, 1986) except Heteroleotris. That genus includes at least 17 species (Pascua is a synonym) and although it has its greatest species diversity in the Western Indian Ocean, there are also Heteroleotris species that inhabit the Western and South Pacific (Hoese & Larson, 2005).

Caffrogobius inhabits near-shore shallow habitats such as tidepools and estuaries, and was postulated by Goren (1996) to be related to Coryogalops, Heteroleotris, Gorogobius and Nematogobius due to similarity in cephalic papillae pattern and vertebral number. These genera, as well as the others grouped with the African gobies, all share with Bathy gobius, Glossogobius and Lesueurigobius a \( 10 + 17 = 27 \) (more rarely, \( 11 + 16 = 27 \), and very rarely \( 28 \) vertebral count (Hoese, 1986, Miller, 1988). These elevated \( 27 \)–\( 35 \) vertebrae, in different proportions (this lineage comprises the Gobiosoma and Microgobius groups of Birdsong et al., 1988). As with a great many of the genera within Gobiidae, 21 of the genera included in this lineage contain fewer than five species; nine are monotypic. The more diverse genera in this lineage are Chirolepis (10 species), Bollmannia (13 species), Microgobius (14 species), Gobiosoma (16 species) and Elacatinus (32 species; Froese & Pauly, 2011). The seven-spined gobies dominate tropical reef and near-reef environments, inhabiting live corals, sponges, sea urchins, mangroves, mudflats and seagrass beds (Böhler & Chaplin, 1993). They include the neon gobies (Elacatinus), some of which engage in cleaner mutualisms with larger fishes (Colin, 1975). The monotypic Nes longus is commensal with the snapping shrimp Alpheus floridanus, and has an antennal/tail flick communication system similar to that seen in Indo-Pacific shrimp gobies and their shrimp (Karplus, 1992; Thacker et al., 2011). The evolution of this diverse clade has been studied in the context of inferring patterns of marine speciation and ecological specialization (Rüber et al., 2003; Taylor & Hellberg, 2005), as well as showing that local larval retention can contribute to species differentiation (Taylor & Hellberg, 2003).

Another trait that may contribute to isolation and diversification by facilitating exploitation of patchy microhabitats and tolerance of low population densities is functional hermaphroditism, the ability to change sex in response to environmental cues. One species examined, Tigri gobius multifasciatus, exhibits such hermaphroditism in a protogynous pattern, beginning as female and switching to male if needed. Curiously, five additional species of American seven-spined gobies that do not express a hermaphroditic pattern as adults do exhibit a transient hermaphroditic gonad as juveniles, consistent with retention of vestiges of a functional hermaphroditic gonad, inferred to be present earlier in the clade’s evolutionary history (Cole, 2008).

Our hypothesis includes representatives of ten American seven-spined gobies, including the genera Microgobius, Nes, Barbulifer, Gobiosoma, Risor, Ophiogobius, Tigri gobius and Elacatinus. Microgobius is sister to a clade containing the remainder of the genera, a placement consistent with Birdsong et al.’s (1988) separation of Bollmannia, Microgobius, Palatogobius and Parrella (as the Microgobius group) from the remainder of the Gobiosomatini (Gobiosoma group); the Microgobius group shares vertebral and median fin meristic characters with the other American seven-spined gobies, but lacks the complete fusion of all hyural elements with the terminal vertebral element. The placement of Microgobius among the American seven-spined gobies is poorly supported in our molecular phylogeny, but it is retained here due to those shared meristic characters as well as concordant placement in another molecular phylogenetic analysis (Rüber et al., 2003). Previous phylogenetic hypotheses of the American seven-spined gobies include two studies linking species diversification to

New World Gobiidae: American seven-spined gobies

This large lineage is the only clade identified in our hypothesis that exclusively inhabits the waters of the New World: the Eastern Pacific, Western Atlantic and Caribbean. The American seven-spined gobies, or Gobiosomatini, includes 26 genera that represent 40% of New World gobiid genera and 50% of the species (Birdsong & Robins, 1995; Rüber et al., 2003). As the name indicates, members bear seven spines (rather than the more common condition of six spines) in the first dorsal fin, have a variety of pterygiophore insertion patterns (most commonly 3-221110), and possess
habitats, one wide-ranging (using mitochondrial ribosomal genes) and one more *Elacatinus*-focused (based on both mitochondrial and nuclear genes; Rüber et al., 2003; Taylor & Hellberg, 2005). Where the taxon sampling of those studies overlaps with ours, the relationships recovered are the same, with one small exception: both those studies recovered the part of *Tigrigobius* containing *T. macrodon* as sister to *Elacatinus*, to the exclusion of *Risor*. Our hypothesis differs slightly in that *Tigrigobius*, *Risor* and *Ophiogobius* form a clade sister to *Elacatinus*, but this result is comparatively weakly supported, and likely due to uncertainty caused by inequivalent sampling, as neither of those previous studies included *Ophiogobius*.

One additional genus is placed with this lineage, the deeper water (30–42 m) *Vomerogobius*, also known from the Western Atlantic. This genus, containing only *V. flavus*, is unusual in that it possesses 11 + 16 = 27 vertebrae, but only six weak dorsal spines (Gilbert, 1971). In the original description, Gilbert suggested that although no close relative was clear, he considered it more likely that *Vomerogobius* was related to the six-spined Indo-Pacific Gobiidae such as *Trimma*. Radiographs of *V. flavus* (ANSP 109589 [1], ANSP 138127 [4], ANSP 150228 [1] and ANSP 150383 [4]) confirm the vertebral counts, as well as the presence of six dorsal spines arrayed as 3-2211000. It is placed here provisionally on the assumption that the loss of a dorsal spine is more likely to have occurred than the gain of a vertebra.

**Pacific and Indian Ocean near-shore Gobiidae**

The tropical reef environments of the Indian and Pacific oceans are home to the greatest diversity of gobiid fishes. Our hypothesis delineates several lineages of goby genera inhabiting these areas. Lineages included here encompass the majority of well-known gobiid taxa, such as the common near-reef benthic *Istigobius*, *Oplopomus* and *Asterropteryx*, the coral-dwelling *Gobiodon*, *Paragobidon*, *Bryaninops* and *Eviota*, the larger-bodied hovering *Ambygobius* and *Valenciennesia*, the tiny, secretive *Priolepis* and *Trinma*, the circumglobal fringe-finned *Bathygobius*, and the shrimp-associated *Amblyeleotris*, *Ctenogobiops*, *Cryptocentrus*, *Mahidolia* and *Vandermorsia*. The majority of these clades include or are completely composed of species and genera that are widely distributed across the Indo-Pacific. It is among Indo-Pacific reef Gobiidae that phylogenetic relationships have traditionally been most difficult to discern. Birdsong et al. (1988) placed most of these genera in his single largest group, the *Priolepis* group, distinguished by possession of a dorsal fin pterygiophore formula of 3-22110, a single epural, and (with rare exceptions) a 10 + 16 = 26 vertebral count. These Gobiidae are diverse, yet exhibit few meaningful morphological characters that can be used to delineate subgroups within the family. Previous molecular phylogenetic studies of Gobiidae have confirmed that the family contains a variety of distinct lineages, with subgroups that had been demarcated from the remainder found to be nested within the more typical, *Priolepis* group members (Thacker, 2009, 2011). This hypothesis increases sampling greatly for these gobids, allowing clades within Gobiidae to be identified.

**Lagoon gobies**

The genera *Acentrogobius*, *Afurcagobius*, *Favonigobius* and *Papillogobius* are resolved together (although the placement of *Acentrogobius* is poorly supported), and all are known from the Indo-Pacific; *Acentrogobius*, *Favonigobius* and *Papillogobius* are widespread, and *Afurcagobius* is known from the temperate Australian coast and Tasmania. *Acentrogobius*, *Favonigobius* and *Papillogobius* are euryhaline and inhabit brackish as well as marine waters; *Afurcagobius* is not found in marine waters, and is known only from estuaries and rivers (Gill, 1993). *Acentrogobius* currently includes more than 20 species, and studies by both Hoese (1983) and Gill and Miller (1990) have suggested that *Acentrogobius* may contain more than one distinct lineage. Indeed, *Acentrogobius* has a generalized gobiid morphology and a complex taxonomic history, with many species having been reassigned to other genera, including *Exyrias*, *Macrodontogobius*, *Istigobius*, *Amoya*, *Yongeichthys* and *Favonigobius* (Hoese, 1983). *Favonigobius* and *Papillogobius* have been synonymized in some accounts (Hoese, 1986), but in our hypothesis, *Afurcagobius* is resolved as sister to *Favonigobius*, in accordance with the revisions of Gill and Miller (1990) and Gill (1993). *Papillogobius* and *Acentrogobius* may be distinguished from *Favonigobius* on the basis of skeletal characters, meristic counts and cephalic lateral line pore configurations (Gill & Miller, 1990; Gill, 1993). The differences among them are slight, but the species examined here are placed into distinct genera, simply because our sampling is incomplete. Even though all the species of *Afurcagobius*, *Favonigobius* and *Papillogobius* examined here form a clade, and thus could arguably be subsumed under *Favonigobius*, the names are retained here because increased sampling could yield a more complicated picture.

Our hypothesis indicates that the genera *Arenigobius*, *Cabiulus*, *Oplopomus* and a clade containing both *Exyrias* and *Istigobius* are sister to the *Acentrogobius* clade, although the relationships among these genera are poorly supported. This lineage is denoted the Lagoon gobies (Fig. 1, Table 1), although as mentioned previously, not all the constituent taxa inhabit lagoons. *Arenigobius* is a genus of three species, all restricted to Australia and New Caledonia. It is also euryhaline and recorded from coastal waters and estuaries. *Oplopomus*, *Cabiulus*, *Exyrias* and *Istigobius* are marine, inhabit sand or mud substrates, and are widespread throughout the Indo-Pacific. *Oplopomus* is a comparatively large goby, roughly cylindrical in shape and frequently occupying burrows; it is also distinguished...
by possession of one to three small spines on the predorsal list. In contrast, Cabillus is tiny and cryptobenthic, with a more dorsoventrally flattened aspect and dorsally placed eyes (Myers, 1999). A close relationship between Exyrias and Istigobius is well-supported in our hypothesis, and accords well with hypothesized placement based on morphology. Both genera are benthic, and feed by sifting sand through their gill rakers, filtering out the infauna (Myers, 1999). Exyrias is both mud and reef dwelling, estuarine and marine, and was anticipated by Murdy in his review of the genus to be related to Istigobius, Acentrogobius and Favonigobius, as confirmed here (Murdy, 1985). Exyrias is similar to Istigobius in pigmentation, with which it shares a ‘dash-dot’ lateral pattern of spots; the two differ in that Istigobius bears lighter scolation on the opercles and cheeks. Istigobius is also generally found over sand substrates, or areas of reef rubble, but some species may also inhabit mud, silt or mangrove habitats (Murdy & Hoese, 1985). Collectively, the lagoon gobies share a common overall squat shape and blunt head, a benthic, usually mud-dwelling ecology, and many of the lagoon gobies also bear a medio-lateral pigmentation pattern of alternating small and larger spots, denoted the “dot-dash” pattern above (Smith, 1959; Myers, 1999). Some of the genera are distinguished by the presence of opercular spines (also known from Asterropteryx), comparatively stout spines in the dorsal fin, and cutaneous toxin secretions (Iwata et al., 1998; Myers, 1999; Shibukawa & Allen, 2007; Shibukawa et al., 2010b).

Silt and reef shrimp gobies

Shrimp-associated gobies are a prominent component of the Indo-Pacific fish fauna. Our new gobiid hypothesis is consistent with the earlier study of Thacker et al. (2011), which demonstrated that the complex mutualistic association seen between several genera of gobiid gobies and alpheid shrimp has evolved twice. One clade, including Cryptocentrus, Mahidolia and Stonogobiops, was resolved separately from a second containing Amblyeleotris, Ctenogobiops and Vanderhorstia, as well as Asterropteryx, a free-living genus not associated with shrimp. In the current hypothesis, the placement of Stonogobiops with the silt shrimp gobies is comparatively poorly supported; it is more confidently placed there by Thacker et al. (2011). Within the shrimp goby clades, relationships echo those inferred in Thacker et al. (2011), with slightly fewer species sampled. The markers selected for that study and this one are nearly identical; our new hypothesis includes an additional nuclear gene, RNF213. Thus, the congruence of the interrelationships inferred is not surprising. This study also differs from the previous one in that far more non-mutualistic gobiid taxa are sampled, and so the more significant result is that the double evolution of the shrimp mutualism, in distantly related clades, is confirmed.

Identification of distinct diagnostic morphological characteristics for each shrimp-associated goby clade is difficult. One character hypothesized to be useful in diagnosing lineages of shrimp-associated gobies is the pattern of cheek papillae (presence or absence of transverse rows); however, the distribution of that character is not in accord with the two clades identified in this and the previous, more extensive phylogeny (Thacker et al., 2011). Many shrimp-associated gobies share an overall congruence of shape and appearance, likely related to convergence given their similar, specialized ecologies. In particular, the most speciose genera, Amblyeleotris and Cryptocentrus, are quite similar, although distinguishable based on subtle meristic and shape characteristics (Chen et al., 1998; Hoese & Larson, 2004). Also, species of Cryptocentrus exhibit pelvic fins fused into a robust, round disc, whereas Amblyeleotris species frequently bear unfused or weakly fused, posteriorly pointed pelvic fins. Sister taxa to Amblyeleotris (Ctenogobiops and Vanderhorstia) have fused pelvic fins, but the close non-mutualist relative Asterropteryx has separate pelvic fins. Hoese & Larson (2004) note that while Amblyeleotris species are associated with shrimp in sandy habitats near coral reefs (as are Ctenogobiops and Vanderhorstia), many Cryptocentrus are known from more silty habitats. Although not an exclusive characteristic, as Cryptocentrus may also inhabit sandy near-reef areas, a preference for silt and fine sandy habitats is common to many Cryptocentrus species as well as related genera Mahidolia, Tomiyamichthys and Stonogobiops, and also similar genera Myersina and Flabelligobius (Hoese & Randall, 1982; Iwata et al., 2000; Winterbottom, 2002; Chen & Fang, 2003; Thacker et al., 2011). Accordingly, the clade consisting of Amblyeleotris, Ctenogobiops and Vanderhorstia (as well as the non-mutualist starry goby Asterropteryx) is named the reef shrimp gobies (Table 1), and the clade of Cryptocentrus and related shrimp-associated genera is denoted the silt shrimp gobies.

Sanddivers

Kraemeria is an unusual, sand-dwelling gobiid, classified either in its own family (with one additional genus, Gobitrichonotus) or among Gobiidae (Akihito et al., 1988), and as a distinct morphological group in Birdsong et al. (1988). Those genera are identified as the Sanddivers (Fig. 1, Table 1). Species of Kraemeria inhabit near shore sandy habitats throughout the Indo-Pacific, and are characterized by several reductions in elements of the pectoral and caudal skeletons (Matsubara & Iwai, 1959). Parkraemeria shares with both Kraemeria and Gobitrichonotus a similar body shape, sensory pore pattern, and fin configuration, although the head is much less pointed and the eyes are not as reduced (Akihito et al., 1998). A previous study (Thacker, 2003) placed the species K. cunicularia among Gobionellidae, but it was resolved in Gobiidae by both Thacker (2009) and this study. This changed placement represented the major discrepancy between those earlier analyses; however,
some differences are to be expected considering the use of different samples and analytical methods among the various studies. The hypothesis of Thacker (2003) was inferred using parsimony analysis, while model-based inferences and much larger datasets characterize Thacker (2009; Bayesian inference) and this study (maximum likelihood inference and a matrix including nuclear as well as mitochondrial markers). In this study, the addition of two individuals of K. bryani in addition to the two K. cunicularia examined in Thacker (2009) has confirmed the monophyly of Kraemeria and that it is placed within Gobiidae. However, the uncertainty in both these hypotheses still precludes definitive identification of the sister taxon. The Kraemeria lineage is distinct from other clades in Gobiidae, either because no closer sister taxon exists, or because its sister taxon has simply not been sampled yet.

Flapheaded gobies

Similarly, the genus Callogobius is recovered in our hypothesis without a strongly supported placement within the family. Two species, C. bifaxciatus and C. sclateri, are included, and their pairing is well-supported. Callogobius is a complex genus of tiny, cryptic gobies with flattened heads bearing prominent ridges of dermal papillae and large pectoral fins. They inhabit reef and near-reef habitats throughout the western Pacific. At least 30 valid species are described, but the genus is in need of revision (Myers, 1999; Chen et al., 2006). Several additional genera are proposed to be relatives of Callogobius, and are included in the flapheaded goby lineage (Table 1). The majority of these genera share the presence of a network of raised ridges on the head which bear sensory papillae. Many also exhibit a dorsolaterally compressed body and head shape, and a pigmentation pattern featuring a few large and irregular dark splotches on the head and body, often also with relatively large pectoral fins (Smith, 1959; Hoese, 1986). Feia, Callogobius, Gobiopsis, Platgyobiopsis and Mangarinus have been postulated as relatives due to shared similar arrangements of the cranial papillose ridges (Winterbottom, 2003), and the flattened, elongate Platgyobiopsis is postulated to be either the sister to Gobiopsis or possibly nested within it (Springer & Randall, 1992). Gobiopsis is the second largest genus in this lineage, with 14 included species currently considered valid. These fishes also exhibit ridges of papillae on the head, as well as longer barbels around the mouth in some species, and the same overall shape and colour patterns of Callogobius (Lachner & McKinney, 1978, 1979.) Drombus differs from Callogobius in having fused pelvic fins, as well as a less dorsoventrally flattened shape, however, they share the presence of papillose ridges as well as similar colour patterns in some species (Smith, 1959; Hoese, 1986). Palurus and Phoxacromion share cranial sensory papillae configurations with some Drombus, although neither Palurus nor Phoxacromion exhibits those papillae on raised dermal flaps (Shibukawa et al., 2010a). Discordipinnia also lacks the cranial flaps of the flapheaded gobies, but is placed therein due to its similar overall head and body shape (Hoese & Fourmanoir, 1978). Barbuligobius bears some papillar flaps and barbels around the mouth, and additionally shares with Callogobius a similar head and body shape, blotchy colour pattern, and common fin spine and ray counts as well as dorsal fin formula (Lachner & McKinney, 1974). Callogobius is unusual in that individuals may have vertebral counts ranging from 26 to 30; others in this lineage exhibit the typical count of 26 (Hoese, 1986; Birdsong et al., 1988).

Burrowing paired gobies

Two additional clades of Indo-Pacific reef gobies were identified, and in both cases the phylogeny indicates close relationships among genera that are behaviourally and ecologically similar. The three genera Amblygobius, Signigobius and Valenciennea are recovered together (with moderate bootstrap support of 79% and 80%), as predicted by Hoese & Allen (1977), with Amblygobius outside the pairing of Signigobius plus Valenciennea. These genera are collectively called the burrowing paired gobies (Fig. 1, Table 1), due to their shared ecological and reproductive characteristics. All of these gobies are relatively large, laterally compressed, and feed by sifting sand through their gill rakers. All are benthic but are frequently seen hovering above the substrate; at night and when alarmed, they will shelter in burrows they construct. These burrows are also where egg-laying and guarding take place (Hoese & Allen, 1977; Myers, 1999). Species of all three genera participate in a mating system in which adults live as monogamous pairs and occupy and guard a territory, but are not hermaphroditic (Reavis & Barlow, 1998; Takegaki & Nakazono, 1999; Takegaki, 2000; Mazzoldi, 2001; Hernanman & Munday, 2007). They are prominent and colourful members of the reef-associated gobiid fauna, notable due to their conspicuous behaviours including a defensive posture adopted by Signigobius biocellatus in which the fish orients itself side-on to the viewer, flares its fins to display prominent large ocelli, and imitates the movements of a crab (Hoese & Allen, 1977; Myers, 1999).

Coral gobies

The final lineage of exclusively Indo-Pacific reef gobiiids is comprised of the genera Bryaninops, Eviota, Gobiodon, Kellogella, Larsonella, Lobulogobius, Lubricogobius, Luposicya, Minisicya, Paragobiodon, Phyllogobius, Pleurosi- cya and Sueviota. Individuals in these genera are associated with corals and attain a minute adult body size. The coral association is obligate in Bryaninops, Gobiodon, Paragobiodon and Pleurosi- cya, and facultative in Eviota (Herler et al., 2009). Species of Bryaninops and Pleurosi- cya hover.
near or perch in the branches of gorgonians, including deposition and guarding of egg clutches on cleared areas of the coral. *Gobiodon* and *Paragobiodon* are found in the branches of stony corals; *Acropora* (*Acroporidae*) in the case of *Gobiodon*, and *Pocillopora, Seriatopora* or *Stylophora* (*Pocilloporidae*) for *Paragobiodon* (Munday et al., 1998, 2002; Herler et al., 2009). The diverse genus *Eviota* includes species that are found perching or hovering among coral branches, as well as others that inhabit reef or rock rubble, not coral. Ongoing studies of cryptobenthic reef gobies have documented that *Eviota* species experience very short lifespans (maximum 59 days, with 40% of that time spent as larvae), rapid reproduction (estimated at 7.4 generations per year), and great abundance on Western Pacific coral reefs (Depczynski & Bellwood, 2003, 2005, 2006). Both *Bryaninops* and *Eviota* are tiny, even among gobies, with most species reaching lengths of only 15–20 mm. *Gobiodon* and *Paragobiodon* differ in that they are slightly larger (25–50 mm), and with a distinctive shape: laterally compressed, with a large head, rounded in profile, and a squat body shape (Myers, 1999; Herler et al., 2009). They exhibit a monogamous mating system, as also recorded for *Amblygobius, Sigmogobius* and *Valenciennesia* (Mazzoldi, 2001). *Gobiodon* species have a high fidelity to their host corals, so much so that some species have diversified by sympatric speciation associated with a host shift (Munday et al., 2004), and overall phylogenetic patterns within the genus parallel those of host *Acroporales* corals (Herler et al., 2009).

Several genera in this lineage are fairly speciose, including *Bryaninops* (10 species), *Gobiodon* (20 species) and *Pleurosicya* (18 species); *Eviota* is by far the most diverse, with 62 species currently described and likely many additional undescribed (Herler et al., 2009; Froese & Pauly, 2011). Other genera in this lineage contain just one to five species, and are often quite similar to one of the more speciose genera. Our molecular phylogeny includes two *Gobiodon* species and single exemplars of *Paragobiodon, Bryaninops* and *Eviota*. The hypothesis indicates that *Gobiodon* and *Paragobiodon* are sister taxa, with *Bryaninops* and then *Eviota* resolved outside that clade. This result is in accordance with the molecular phylogenetic results of Herler et al. (2009), showing distinct *Gobiodon/Paragobiodon* and *Bryaninops/Pleurosicya* clades, with *Eviota* resolved as sister to both. The diversity of coral gobies may be divided into three types: laterally compressed, round-headed species that generally inhabit stony corals (*Gobiodon, Paragobiodon, Larsonella, Lubricogobius*); elongate, delicate, pointy-snouted inhabitants of gorgonians, staghorn corals or sponges (*Bryaninops, Lobulogobius, Luposicya, Minisicya, Phyllogobius, Pleurosicya*); and elongate but blunt-snouted species not obligately associated with coral (*Eviota, Kellogella, Sueviota*). *Larsonella* and *Lubricogobius* differ from *Gobiodon* and *Paragobiodon* primarily in that they are partially (*Larsonella*) or completely (*Lubricogobius*) scaleless; *Lubricogobius* individuals have been captured from non-coraline shelters such as worm tubes, urchin tests, mollusc shells and tunicates (Randall & Senou, 2001). *Lobulogobius, Luposicya, Minisicya* and *Phyllogobius* all differ from *Pleurosicya* or *Bryaninops* in slight variations of soft tissue characters, but all share a common overall morphology and commensal ecology (primarily with gorgonians, but also with other soft corals or sponges; Larson, 1983, 1986, 1987, 2002; Goren, 1984). *Sueviota* is very similar to *Eviota*, differing only in the condition of the medial (5th) pelvic ray (Winterbottom & Hoese, 1998). *Kellogella* is included in this lineage due to its small size and general similarity to *Eviota*, although *Kellogella* species are more elongate. *Kellogella* is known from tide pools throughout the Indian and Pacific Oceans, including Hawaii and Easter Island, and is distinctive in that it has tricuspid teeth and an 11 + 15 = 26 vertebral count, a condition only very rarely observed among Gobiidae (Hoese, 1975; Birdsong et al., 1988).

**Circumglobal Gobiidae clades**

Several clades identified in this study include members that inhabit both Old and New World oceans. In two cases, *Bathygobius* and *Priolepis*, a single genus contains species distributed around the world. Other lineages contain taxa classified into distinct genera in the Eastern Pacific/Western Atlantic and the Indo-Pacific. The lineages of gobies detailed below, along with the American seven-spined gobies (exclusively New World distribution), comprise all the gobiid gobies known from the Eastern Pacific, Western Atlantic and Caribbean.

**Tiny banded gobies**

Nine genera are assigned here to a lineage that is characterized by very small size (most less than 50 mm in length, and many less than 30 mm), a stocky ovoid body with a narrow caudal peduncle, and in many species, a colour pattern of several (commonly 7–12) brightly coloured bands on a pale ground. *Lythrypnus, Priolepis* and *Trimma* were sequenced for this analysis, with results indicating that *Lythrypnus* and *Priolepis* are not monophyletic. Our hypothesis resolves the Eastern Pacific species of *Lythrypnus* examined, *L. dalli* and *L. zebra*, outside the other species sampled, apart from the Caribbean/Western Atlantic *L. elasson*, itself nested within *Priolepis*. The two species of *Trimma* form a clade sister to *Priolepis* and *L. elasson*. These are the most speciose genera; *Lythrypnus* contains 20 species, *Priolepis* 34 and *Trimma* 63, with many still to be described (Winterbottom & Southcott, 2008; Froese & Pauly, 2011) and precise relationships still to be investigated. *Lythrypnus* is known from the Eastern Pacific and Western Atlantic, *Trimma* from throughout the Indo-Pacific, and *Priolepis* from around the world. All inhabit tropical marine near-shore environments,
many are cryptobenthic, and others occur swimming in schools. As with the coral gobies, the other genera in this lineage are much less diverse, and often very similar to the more speciose genera. Egglestonichthys (Indo-Pacific) encompasses three species, and Ego (Red Sea) is monotypic; species of both resemble Priolepis (Miller & Wongrat, 1979; Randall, 1995a; Larson & Hoese, 1996; Froese & Pauly, 2011). Trimmatom (seven species in the Indo-Pacific) and Paratrimma (two Southeastern Pacific species) are both close relatives of Lythrypnus, Priolepis and Trimma (Hoese & Brothers, 1976; Winterbottom, 1989; Winterbottom & Burridge, 1992). O bliquo gobi us and Try s sg obius are two genera known from deepwater (27–82 m for Try ssog obius; 99–165 for one Obliquogobius species, 394–404 m for another) in the Western Pacific. They are placed here provisionally; although they share broad similarities of shape, colouration and meristics with the other genera in this lineage, they also differ in some respects, such as possessing fully scaled heads, an unusual condition (Larson & Hoese, 2001; Larson & Chen, 2007; Shibukawa & Aonuma, 2007).

The tiny banded gobies have been the subject of a number of phylogenetic and evolutionary analyses based on osteology and external morphology, including cladistic analyses of heterochrony (Winterbottom, 1990), biogeography (Santini & Winterbottom, 2002), and evolution of cutaneous papillae patterns (Winterbottom & Burridge, 1992). The age and growth patterns of several Trimma species have also been examined, and have yielded the notable result that these cryptobenthic reef fishes, similar to Eviota, experience quite short lifespans (87–140 days in the Trimma examined, with 24–39% of that time spent as pelagic larvae; Winterbottom & Southcott, 2008; Winterbottom et al., 2011) and high mortality (2.9–6.3% per day). This level of turnover in the fish community is remarkable and indicates that these gobies are major contributors to reef biomass production and energy flow.

Crested gobies

This lineage includes five genera (Coryphopterus, Cristatogobius, Fusigobius, Lophogobius and Rhinogobiops), of which all but Cristatogobius are represented in our phylogeny. This lineage spans the Indo-Pacific (Fusigobius and Cristatogobius) as well as the Eastern Pacific and Western Atlantic (Coryphopterus, Lophogobius and Rhinogobiops). The name crested gobies is applied to this lineage because Lophogobius, Rhinogobiops and Coryphopterus species bear a fleshy nuchal crest on the dorsal midline of the head, behind the eyes and extending to or nearly to the origin of the first dorsal fin (Thacker & Cole, 2002). A similar crest is present in the Western Pacific Cristatogobius, and that genus is grouped here for that reason along with its overall similarity in shape to Lophogobius, although Akihito and Meguro (2000) list differences in tooth, scapula and papillae morphology between the two genera. A few other goboids, such as Cryptocentroides gobioide s, also display nuchal crests but lack other shared morphological similarities with the crested gobies and are not hypothesized to be related to that lineage.

Crested goby genera have been the subject of active taxonomic study, particularly Coryphopterus and Fusigobius (Smith & Tyler, 1977; Randall, 1995b, 2001); a phylogeny of crested gobies based on both morphological characters and molecular data indicated that Lophogobius was the sister taxon to most Coryphopterus, and reassigned the Eastern Pacific blackeye goby to Rhinogobiops nicholsii (Thacker & Cole, 2002). Two Fusigobius species were placed separately outside the remainder of the crested gobies. These conclusions were supported in phyloge netic studies with much more extensive sampling (Thacker, 2003, 2009). Both of those analyses indicated that Coryphopterus and Lophogobius were sister taxa, with some Fusigobius also closely related, but with others placed as distantly related in the hypothesis, a strange result also highlighted by Cole (2008). None of those earlier studies included Rhinogobiops, and the sampling of Fusigobius was also sparse. In our analysis, we include not only Coryphopterus and Lophogobius, but also Rhinogobiops and three Fusigobius species, adding second individuals of F. neophytus and F. signipinnis, as well as an exemplar of F. duospilus. This expanded sampling yields a hypothesis that is consistent with earlier efforts; where the sampling overlaps, the relationships within Coryphopterus are concordant with those depicted in Thacker and Cole (2002); note that one individual identified in that work as C. punctitectophorus was misidentified and is here corrected to C. dierus [Carole Baldwin, pers. comm.]). The new hypothesis also confirms that Coryphopterus and Lophogobius are sister taxa, and Rhinogobiops is placed outside them, as sister to that pairing. In contrast to the earlier analyses, here Fusigobius species are placed together, forming a grade outside the other three genera. However, support for the placement of F. neophytus apart from the remainder of Fusigobius is very weak, and thus, evidence for the paraphyly of this genus should not be considered conclusive.

A reproductive pattern shared by a variety of gobid genera is the ability to change sex, most commonly in a protogynous pattern. Hermaphroditic gonads have been recorded in four of the lineages outlined here: the coral gobies (Bryaninops, Eviota, Gobiodon and Paragobiodon), the tiny banded gobies (Priolepis, Trimma, Lythrypnus), the crested gobies (Coryphopterus, Rhinogobiops) and the American seven-spined gobies (Tigrigobius; Cole, 1990, 2008, 2010; Cole & Hoese, 2001). Various behavioural and gonadal characteristics are associated with the hermaphroditism, including whether or not sex change can be bidirectional, and whether both ovarian and testicular tissue are simultaneously present in the gonad. Cole (2010) examined the phylogenetic distribution of hermaphroditism among...
gobies using the phylogeny of Thacker (2009). Multiple independent origins of hermaphroditism were postulated based on the phylogeny, which differed from the current hypothesis in the placement of Fusigobius neophytus separate from the remainder of the crested gobies, which were in turn resolved in a basal polytomy with Kraemeria. Similarly, our current hypothesis features weak resolution along the backbone of the phylogeny, however, additional species and sequence data have confirmed monophyly of the crested gobies, including all Fusigobius sampled, a result that is in better concordance with the distribution of reproductive characteristics enumerated by Cole (2010).

**Wormfishes and dartfishes**

This clade includes two groups that in older classifications were united as subfamilies in a single family, Microdesmidae (Hoese, 1984). The New World Cerdale, Clarkichthys and Microdesmus were grouped with the Indo-Pacific Gunnellichthys and Paragunnellichthys as the Microdesminae (wormfishes), with Aiolops, Nemateleotris, Oxymetopon, Parioglossus and Ptereleotris comprising the Ptereleotriinae (dartfishes; two new genera, Navigobius and Pterocerdale, have since been described [Hoese & Motomura, 2009]). Wormfishes in the New World are elongate, benthic and burrowing, with thick skin and blunt heads. They inhabit near-shore sandy and muddy habitats, including the intertidal. The Indo-Pacific Gunnellichthys and Paragunnellichthys are known from flat sandy habitats, in which they may hover or burrow. All five wormfish genera exhibit single, long dorsal fins rather than the separate spinous and rayed dorsals of most gobiotics. Dartfishes are exclusively Indo-Pacific and known from reef and near-reef habitats, where they hover above the substrate, forming monogamous pairs and feeding on zooplankton. They do not construct burrows, but will shelter in burrows or crevices when threatened (Myers, 1999; Thacker, 2000).

These lineages have also been the focus of phylogenetic investigations based both on morphological and on molecular data. A morphological phylogeny focusing just on the five wormfish genera confirmed the monophyly of each genus, and of the wormfishes as a group, but proposed that dartfishes were not closely related and assigned them to a distinct family (Thacker, 2000). Subsequent molecular phylogenies yielded divergent results; an initial study based on three mitochondrial genes and analysed with parsimony methods postulated a separation of wormfishes into distinct Old and New World lineages, with the Old World taxa placed in a clade along with the dartfishes, Schindleria, and one Fusigobius species (Thacker, 2003). A later analysis with more extensive taxon sampling and an additional mitochondrial gene, using Bayesian phylogenetic methods, yielded another rearrangement, in which the dartfishes sampled formed a clade sister to the wormfishes (Old and New World), as well as Schindleria and three members of the American seven-spined goby lineage: Risor, Barbulifer and Tigrigobius (depicted there as Gobiosoma; Thacker, 2009).

The hypotheses were consistent in that both wormfishes and dartfishes were found to be nested within Gobiidae, obviating the need for distinct family names. In each analysis, the discussion of the molecular phylogenies included the implications of the new hypothesis for morphological character interpretation. This re-evaluation of interpretations based on additional data and new hypotheses was found to be quite disturbing by some (Gill & Mooi, 2010; Mooi & Gill, 2010), who additionally scorned the possibility that another phylogenetic hypothesis would yield further reinterpretations. Amusingly, our study provides just such an opportunity; our current hypothesis represents a further increase in sampling of markers (three mitochondrial and three nuclear) and of wormfish species (Microdesmus carri and M. dipus), and we infer a third set of relationships among wormfishes and dartfishes. The groups are united into a single lineage here, without other gobiid taxa, but within this clade, dartfishes are recovered as paraphyletic with respect to wormfishes. This possibility, that the wormfishes may be nested within the dartfishes, was also put forth by Hoese & Motomura (2009), based on similarities between their newly described Pterocerdale and both dartfishes and wormfishes. However, in this as well as previous phylogenies, caution is warranted in interpreting parts of the phylogeny characterized by short internodes and low support values. Clearly, the relationships among these taxa are difficult to discern, and discrepancies among hypotheses may also be influenced by differences in both taxon and marker sampling, and of analytical methods. In any case, the homology of any character, morphological or molecular, must be inferred from a broader phylogenetic hypothesis and cannot be asserted in isolation, so reinterpretation based on new, augmented datasets is not just desirable but necessary. Characters proposed by Gill and Mooi (2010) as supporting a close relationship between dartfishes and wormfishes include some discussed previously by Thacker (2000, 2003), as well as newly identified characteristics of the cleithrum and dorsal gill arches. No phylogenetic analysis is attempted, and thus the characters identified are not homologies or synapomorphies; Gill and Mooi (2010) also discuss the occurrence of their characters in other goboid taxa. Even so, the character evidence is in accordance with the placement here of wormfishes and dartfishes in a single lineage, as well as the inclusion of Schindleria. The paedomorphic genus Schindleria is included in this lineage due to its resemblance to larval and juvenile Gunnellichthys, as well its placement with the group in earlier phylogenetic analyses (Thacker, 2003, 2009).

**Inshore gobies**

*Bathygobius* species are known from tropical regions worldwide, where they inhabit shallow sand or rocky habitats,
including tidepools. The upper pectoral fin rays are free from the fin membrane, and thus they are commonly called frillfin gobies or frillgobies (Myers, 1999; Tornabene et al., 2010). Bathygobius may be very common where they are found; the genus currently includes 29 species, with new species recently revealed through analysis of sequence diversity and phylogeny (Tornabene et al., 2010; Tornabene & Pezold, 2011). Our hypothesis includes three Bathygobius species, and they are resolved as a clade sister to Psammogobius biocellatus, the mangrove goby. The speciose genus Glossogobius, although not sequenced here, is placed in this lineage due to its close similarities with Psammogobius; Glossogobius species are distributed throughout the Indo-Pacific, inhabit brackish and freshwater, and individuals are distinguished by their flattened heads (Akihito et al., 1998; Allen et al., 2002). Species of Bathygobius, Glossogobius and Psammogobius exhibit drab overall splocheted or spotted pigmentation patterns, as well as the unusual condition of 27–30 vertebrae, also documented from Atlantic lineages including the African gobies and the American seven-spined gobies (Hoese, 1986; Birdsong et al., 1988). Glossogobius species also possess bilobed tongues (Hoese & Allen, 2009), as do some Bathygobius. One additional genus is included in this lineage, Grallenia, a western Pacific genus of two species described from sandy near-reef environments of the Western Pacific (Shibukawa & Iwata, 2007). Grallenia species have 27–28 vertebrae, and thus are tentatively postulated to be in the same lineage as Bathygobius and Glossogobius, the only western Pacific Gobiidae with that vertebral count (except some Callogobius, but Grallenia lacks the distinguishing characteristics of that lineage). The African gobies also are characterized by the presence of 27 vertebrae, and some species of Caffrogobius, Corygalops and Heteroleotris have the upper pectoral fin rays free, similar to the condition seen in Bathygobius (Hoese, 1986). This pectoral fin condition is also seen in some Atlantic Gobius and Mauligobius (Brito & Miller, 2001), but not in Glossogobius or Grallenia (Allen et al., 2002; Shibukawa & Iwata, 2007).

Only one of the 170 genera of Gobiidae was so distinctive that we were not able to postulate placement for it among the 13 lineages (Table 1). Austrolethops, a monotypic genus containing only A. wardi, is a small-eyed, laterally compressed, scaleless, pinkish fish that is widespread in the Indo-Pacific. There are no cephalic sensory canals, but sensory papillae are present on the head, and unusually for gobies, on the body along the lateral midline (Kneer et al., 2008). It was described from muddy habitats (Whitney, 1935), has been reported to inhabit coral rubble, hiding in daylight and emerging at night (Myers, 1999), and also has been recorded from seagrass beds, living commensally with the thalassinid shrimp Neanthes acanthus (Kneer et al., 2008; a third group of gobiid-associated shrimp, distinct from alpheids and axiids). Austrolethops wardi does not have fused pelvic fins, but examination of radiographs of the holotype (IA.6175, from the collection of the Australian Museum, Sydney) confirms that it has six dorsal spines arrayed in a 3-22110 pattern and 10 + 16 = 26 vertebrae, similar to the majority of Indo-Pacific Gobiidae. Austrolethops wardi also exhibits the distinctive suspensorium configuration, with the symplectic/metapterygoid strut well-separated from the preopercle that is characteristic of Gobiodei. However, other than those characters consistent with relationships somewhere among the Indo-Pacific lineages of Gobiidae, no more distinctive characters are evident that could be used to postulate a specific placement for Austrolethops.

Conclusions

Overall, the progression of phylogenetic analyses for Gobiidae has yielded a relatively consistent portrayal of lineage evolution; some lineages have been consistently identified throughout previous studies, even though marker and taxonomy sampling and analysis methods have varied (Thacker, 2003, 2009). Others have varied in their composition and interpretations, and in each case of broader gobio sampling, a notable result has been the uncertainty among backbone relationships in Gobiidae, a condition which persists in our current analysis. Gobiidae is by far the most diverse clade of Gobiodei, and this significantly greater diversity may have been due to elevated speciation rates as gobies invaded marine reef habitats (Thacker, 2009). A burst of speciation over a short time is one of the ways that backbone phylogenetic signal could be obscured. Given the uncertainty regarding the interrelationships of lineages in this analysis, we have focused instead on discussion of each lineage and postulation of other gobiid genera, unsampled here, that are likely also included in each lineage. The initial identification of lineages is phylogenetic, and the assignment of remaining gobiid genera to lineages is phenetic, based on similarity of morphology, ecology or biogeography. These lineages are identified, and additional members beyond those sampled are proposed, as a guide for ongoing phylogenetic and evolutionary studies, both of gobiods and of acanthomorphs more broadly.

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References


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WINTERBOTTOM, R. 2002. A redescription of Cryptocentrus crocatus Wongratana, a redefinition of Myersina Herre (Acanthopterygii; Gobiidae), a key to the species, and comments on relationships. Ichthyological Research 49, 69–75.


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