

Phylogeny of Gobioidae and Placement within Acanthomorpha, with a New Classification and Investigation of Diversification and Character Evolution

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Phylogenies based on DNA sequence data are providing a range of new insights into relationships within Acanthomorpha, particularly in cases where morphological characters have been scanty or misleading. Molecular phylogenetic analyses of the perciform suborder Gobioidae have illuminated relationships within and among groups, with recovered clades generally corresponding to groups identified based on morphological characters. This study integrates and expands previous molecular phylogenetic hypotheses to infer a comprehensive gobioid phylogeny, including a variety of outgroup taxa included to confirm sister taxon identity and position of the group among other acanthomorphs. This and other phylogenetic hypotheses indicate that Gobioidae is part of a clade that also includes Apogonidae, Pempheridae, and Kurtidae. These groups share characteristics of the skeleton, soft tissues, and reproductive ecology. A new six-family clade-based classification of Gobioidae is presented, and Gobioidae, Apogonoidei (Apogonidae + Pempheridae), and Kurtidae are placed as suborders of a resurrected Gobiiformes. Patterns of morphological character evolution among gobioids are then examined in the context of the molecular phylogeny, and their distribution is correlated with species diversification and phylogenetic imbalance. The two largest gobioid clades, Gobiidae and Gobionellidae, differ significantly in species richness, perhaps due to increased diversification in marine habitats by gobiids. The phylogeny of Gobiidae exhibits a convex plot of lineages through time, consistent with an elevation of speciation rate early in the clade's history. The γ statistic is consistent with this increase, but is not significant after correction with the Monte Carlo Constant Rates (MCCR) test.

MOLECULAR phylogenetic analyses are reshaping our view of relationships among groups within Acanthomorpha, the spiny-rayed fishes. For several decades, since the groundbreaking synthetic works of Greenwood et al. (1966), Rosen (1973), and Lauder and Liem (1983), a basic understanding of acanthomorph relationships has predominated that includes Acanthomorpha divided into Lampriformes, Polymixiiformes, Paracanthopterygii, and Acanthopterygii; Acanthopterygii composed of Atherinomorpha and Percomorpha; and within Percomorpha, Beryciformes and Stephanoberyciformes forming the sister taxa to the remainder. Sequential sister taxa outside Acanthomorpha are Myctophiformes and Aulopiformes, respectively, and those taxa plus Stomiiformes constitute the Neoteleostei. Outside Acanthomorpha, morphological studies of relationships generally agree and a good deal of character evidence may be identified; within Acanthomorpha, and particularly within Percomorpha, morphological evidence is scantier and frequently contradictory (Stiassny and Moore, 1992; Johnson and Patterson, 1993; Springer and Johnson, 2004; Springer and Orrell, 2004).

As phylogenetic systematics has become the dominant method of identifying evolutionary groupings, and naming systems have been adjusted to correspond with the clades so identified, some changes have been made in higher group membership and nomenclature. These changes have generally not been sweeping, but rather have represented only moderate adjustments to the existing classifications. A new perspective on acanthomorph relationships is provided by phylogenetic analysis of large DNA sequence datasets. A major advantage of molecular data is that they are abundant and easily comparable across all acanthomorph taxa, resolving the problem of inapplicable or missing data that may be encountered in broad morphological studies. In recent years, molecular hypotheses have become complete enough that consistent patterns have begun to emerge, and

it is now possible to evaluate how well the molecular hypotheses agree with one another, and with the traditional classification.

The most remarkable result for the various comprehensive molecular phylogenies put forth for Neoteleostei (Stomiiformes, Aulopiformes, Myctophiformes, and Acanthomorpha) or subgroups thereof is how well they agree with one another. Studies based on protein-coding or ribosomal mitochondrial or nuclear genes, singly or in combination, yield a consistent picture that features Stomiiformes, Aulopiformes, some combination of Myctophiformes, Atelepodiformes, and Lampriformes, then an altered Paracanthopterygii, and Berycomorpha and Percomorpha. The traditionally recognized Paracanthopterygii, consisting of Percopsiformes, Gadiformes, Batrachoidiformes, Lophiiformes, and Ophidiiformes (Greenwood et al., 1966 [included Gobiesociformes]; Fraser, 1972a [excluded Ophidiiformes]; Patterson and Rosen, 1989), is not monophyletic (as predicted by most authors who have considered it), with several molecular studies demonstrating that Lophiiformes and Batrachoidiformes are nested within a Percomorpha that also includes Ophidiiformes as sister to the remainder (Wiley et al., 2000; Chen et al., 2003; Miya et al., 2003, 2005; Smith and Wheeler, 2006; Mabuchi et al., 2007). Molecular data have also shown that the beryciform families Berycidae and Holocentridae form a clade with the stephanoberyciform families (Miya et al., 2003, 2005; Smith and Wheeler, 2006), rather than grouping with other Percomorpha, as suggested by Moore (1993). Both Beryciformes and Stephanoberyciformes are placed sister to the remainder of Percomorpha, consistent with the classification of Johnson and Patterson (1993). Relationships among families within Percomorpha, and particularly within Percoidei, have rarely been considered with morphological data, although the monophyly of many families is well established (reviewed by Johnson, 1993). It is in delineating groups of families

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within Percomorpha that large molecular phylogenies provide the most significant advances (Miya et al., 2003, 2005; Smith and Craig, 2007).

The most diverse taxon within Percomorpha is the order Perciformes, including 20 suborders, 160 families, and more than ten thousand species (Nelson, 2006). Other percormorph orders have been delineated based on shared specializations (Gasterosteiformes, Dactylopteriformes, Scorpaeniformes) and/or if they are considered to be "advanced" relative to other taxa (Pleuronectiformes, Tetraodontiformes; Johnson and Patterson, 1993). Molecular phylogenies indicate that these distinctions are artificial (Smith and Craig, 2007), and that the orders listed above are all interspersed among perciform taxa, with the Scorpaeniformes in particular having a complex history (Smith and Wheeler, 2004, 2006). Within Perciformes, relationships based on morphological data have been difficult to assemble due to the diversity of the group, the paucity of informative character data, and the homoplasy present in many morphological characters that are identified.

The suborder Gobioidae comprises a significant fraction of perciform diversity. Estimates of the total number of gobioid species range from 1,590 (calculated from an unpublished list of gobioid species compiled by Edward Murdy, augmented with data from the online version of the Catalog of Fishes [Eschmeyer, 2008]) to 2,211 (Nelson, 2006). Gobioidae is traditionally divided into nine families: Rhyacichthyidae, Odontobutidae, Xenisthmidae, Eleotridae, Gobiidae, Microdesmidae, Ptereleotridae, Kraemeriidae, and Schindleriidae (Miller, 1973; Springer, 1983; Hoese, 1984; Hoese and Gill, 1993; Johnson and Brothers, 1993; Thacker, 2000). Of these families, the two largest (Eleotridae and Gobiidae) contain the bulk of the species and include several subfamilies. Eleotridae are divided into Eleotrinae and Butinae (Hoese and Gill, 1993), and Gobiidae include Gobiinae, Gobionellinae, Sicydiinae, Oxudercinae, and Amblyopinae (Hoese, 1984). Abundant morphological character evidence indicates that Gobioidae is monophyletic (Winterbottom, 1993), and, as with Neoteleostei and its subgroups, molecular phylogenies have served to confirm many hypothesized groupings within Gobioidae. A primary difference between morphological and molecular hypotheses of gobioid relationships is that molecular hypotheses (Thacker, 2003; Thacker and Hardman, 2005) have confirmed that most of the smaller families and subfamilies (Sicydiinae, Oxudercinae, Amblyopinae, Xenisthmidae, Microdesmidae, Ptereleotridae, Kraemeriidae, Schindleriidae) are nested within the larger ones (Gobiidae, Eleotridae). Identification of the gobioid sister taxon has been addressed with both morphological (Miller, 1973; Winterbottom, 1993) and molecular (Thacker and Hardman, 2005) characters, with the consensus being that the family Apogonidae is a close relative. Beyond this, the placement of Gobioidae among all the other perciform lineages has been hinted at in comprehensive molecular phylogenies; when gobioids are sampled, the group is placed as part of a deep split in Percomorpha, closely related to Apogonidae, Kurtidae, Dactylopteridae, and several other percormorph families (Miya et al., 2003, 2005; Smith and Wheeler, 2006).

The purpose of this study is to assemble and analyze molecular data for Gobioidae and an assortment of acanthomorph taxa, in order to resolve relationships within and among gobioid groups as well as confirm placement among outgroups. The data of Thacker (2003) and Thacker and

Hardman (2005) are combined and augmented to produce a comprehensive phylogenetic hypothesis for the group. A new six-family clade-based classification is presented, in accordance with the phylogenetic relationships. A new, higher classification scheme for Gobioidae is also proposed. In the context of the new gobioid phylogeny, the distribution of various morphological characters, as well as habitat type (fresh, brackish, or marine) is examined. Measures of tree imbalance are assessed in order to explore the history of gobioid diversification, and to compare diversification among gobioid clades.

MATERIALS AND METHODS

This study combines DNA sequence data from previous studies (Miya et al., 2003; Thacker, 2003; Thacker and Hardman, 2005) with new data. Specimens sequenced here are vouchered at various institutions; abbreviations are as listed at <http://www.asih.org/codons.pdf>. The taxa in Thacker (2003) were sequenced for three mitochondrial genes (ND1, ND2, and COI), while those in Thacker and Hardman (2005) utilized those genes plus an additional mitochondrial gene, cytochrome *b* (*cytb*). To effectively combine these datasets, sequence for *cytb* was obtained for most of the taxa of Thacker (2003), and sequence for all four genes was added for an additional six taxa: *Acentrogobius pflaumii*, *Callogobius bifasciatus*, *Cryptocentroides cristatus*, *Trimma caesiura*, *Trimmatom eviotops*, and two individuals of an undescribed species of *Microphilypnus* collected from the Rio Negro, Venezuela (ANSP 180643). Tissues of *A. pflaumii* and *C. cristatus* were obtained from the Australian Museum's tissue holdings (*A. pflaumii*: AMS I.40841005, Glebe Is., NSW; *C. cristatus*: AMS I.41283021, north of Evans River mouth, NSW). *Callogobius bifasciatus* was collected at Abu Dhabi, Ghasha Is., (LACM T-000242: entire specimen vouchered as tissue), and *T. caesiura* and *T. eviotops* were obtained from the University of Kansas Natural History Museum fish tissue collection (*T. caesiura* [KU 5683: entire specimen vouchered as tissue], Saipan, Lau Lau Beach; *T. eviotops* [KU 5591: entire specimen vouchered as tissue], Saipan, Obyan Reef). Sequences were obtained following the methods of Thacker and Hardman (2005). GenBank accession numbers for sequences of the six newly sequenced taxa are EU380993–9 (ND1); EU381015–9 (ND2); EU381035–41 (COI); EU380964–7, EU380975–7 (*cytb*); accession numbers for new *cytb* sequences for the taxa of Thacker (2003) are EU380907–EU380957.

Sequences for all four genes were additionally obtained from taxa thought to be closely related to Gobioidae. Nine individuals from four genera in the family Apogonidae (*Archamia biguttata* [KU 4048: specimen voucher KU 32567], *Archamia fucata* [KU 6960: specimen voucher RUSI 78070, KU 6961: specimen voucher RUSI 78070], *Apogon doderleini* [LACM T-000974: entire specimen vouchered as tissue], *Apogon maculatus* [LACM T-000968: entire specimen vouchered as tissue], *Apogon exostigma* [KU 4061: specimen voucher CAS 217459], *Cheilodipterus macrodon* [KU 6821: specimen voucher RUSI 77936], and *Fowleria aurita* [KU 7123: specimen voucher RUSI 76469]), two individuals from the family Pempheridae (both *Pempheris vanicolensis* [KU 6927: specimen voucher RUSI 77856; KU 7031: specimen voucher RUSI 77805]), one individual of the family Kurtidae (*Kurtus gulliveri* [KU 1850: field number Norris 97]), and three representatives of three genera in the family Leiognathidae (*Leiognathus equulus* [Taiwan: UMMZ-WLS14],

Secutor megalolepis [Australia: AMNH-WLS20], and *Gazza squamiventralis* [AMNH 120341]) were newly sequenced following the methods of Thacker and Hardman, (2005). GenBank accession numbers for these sequences are EU380978–92 (ND1); EU380100–14 (ND2); EU381020–381034 (COI); EU380958–63, EU380968–74 (*cytb*). Finally, sequences from 28 individuals representing Polymixiiformes (*Polymixia japonica* [AB034826], *P. lowei* [AP002927]), Dactylopteriformes (*Dactyloptena peterseni* [AP002947], *D. tiltoni* [AP004440]), Perciformes: Moronoidei (*Cheimarrichthys fosteri* [AY722258, AY722321, AY722119, AY722190], *Pterocassio tile* [AP004447]), Perciformes: Cottoidei (*Aptocyclus ventricosus* [AP004443], *Arctoscopus japonicus* [AP003090], *Cottus reinii* [AP004442]), Perciformes: Scorpaenoidei (*Satyricichthys amiscus* [AP004441]), Ophidiiformes (*Bassozetes zenkevitchi* [AP004405], *Cataetys rubirostris* [AP004407]), Stephanoberyciformes (*Cetostoma regani* [AP004423], *Danacetichthys galathenus* [AP002936], *Poromitra oscitans* [AP002935], *Rondeletia loricata* [AP002937], *Scopelogadus mizolepis* [AP002934]), and Beryciformes (*Anomalops katoptron* [AP004428], *Anoplogaster cornuta* [AP004425], *Beryx splendens* [AP002939], *B. decadactylus* [AP004430], *Diretmoides veriginae* [AP004426], *Diretmus argenteus* [AP004427], *Hoplostethus japonicus* [AP002938], *Monocentrus japonicus* [AP004429], *Myripristis berndti* [AP002940], *Ostichthys japonicus* [AP004431], *Sargocentron rubrum* [AP004432]) was obtained from GenBank (accession numbers for each sequence are given in parentheses), deposited from the studies of Miya et al. (2003, 2005) and Thacker and Hardman (2005).

Alignments were constructed by translating sequences into their amino acid residues and evaluating agreement in the amino acid sequences using Sequencher 4.1.2 (GeneCodes Corp., Ann Arbor, MI). No gaps were present due to insertions or deletions; all gaps in the final alignment were due to missing data. Aligned sequence files were exported as NEXUS files for phylogenetic analysis. Phylogenetic analyses were performed using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Bayesian analyses were conducted by first determining the appropriate model for base and substitution frequencies with the likelihood-ratio test (LRT) and Akaike Information Criteria (AIC), as implemented in MrModeltest 2.0 (J. Nylander, 2004). The MrBayes search was run for 1,500,000 generations with two replicates, each with four simultaneous chains. This length of search ensured that the runs converged and that stationarity was achieved, as indicated by the standard deviation of split frequencies and examination of a graph of posterior probability vs. replicates. Trees were sampled every 3,000 generations; the trees generated before stationarity was attained (250 trees representing 750,000 generations) were discarded as burn-in. The Bayesian estimates of posterior probabilities were included to indicate support for clades in a 50% majority rule consensus tree.

Morphological character states were recorded for three characters potentially useful for diagnosing groups within Gobioidae. These characters are the number of branchiostegal rays (five or six), the number of epurals (one, two, or three), and whether or not the pelvic fins are joined to form a disc. Additionally, the habitat (freshwater, brackish, or marine) for each taxon was examined. These characters were largely scored from literature reports, primarily the comprehensive character surveys of McAllister (1968), Fraser

(1972b), Zehren (1979), and Birdsong et al. (1988); habitat data were obtained from FishBase (Froese and Pauly, 2008). In some cases conditions were recorded for a genus only, and if only one member of that genus was present in the phylogeny, the range of states for a genus as a whole was recorded. The advantage of this is that a more complete picture of character evolution is obtained; the disadvantage is that it assumes monophyly of the genera. Although the monophyly of many genera has not been evaluated, this strategy was followed with the aim of presenting a broad-scale picture of gobioid character evolution.

Characters were tabulated and mapped using Mesquite 2.0 (Maddison and Maddison, 2007). Clade diversities were compared using the χ^2 test, implemented in Statistica 4.1 (Statsoft, Inc., Tulsa, OK). The tempo of speciation was investigated using the tree statistic γ (Pybus and Harvey, 2000). Negative values of γ indicate that speciation has slowed towards the recent; positive values are consistent with an increase in speciation as the clade has evolved. The MCCR test accounts for incomplete sampling in the phylogeny by recalculating γ for each of a set of simulated phylogenies with the same sampling fraction (in this case, 40 taxa out of 954) and providing an adjusted p-value. Calculation of γ and MCCR test were done with the R package laser (R Development Core Team, R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2007. <http://cran.r-project.org>) with trees simulated using PhyloGen version 1.1 (Rambaut, 2002). Trees were manipulated and, when necessary, made ultrametric (using the nonparametric rate smoothing method of Sanderson, 1997) with TreeEdit (Rambaut and Charleston, 2002).

RESULTS

Sequences from 196 individuals representing 148 species were included in the analysis, comprising 107 gobioids and 41 outgroups. MrModeltest indicated that the GTR+I+G model was most appropriate for these data, based on both the LRT and AIC; the Bayesian phylogeny, rooted with the polymixiiforms *Polymixia japonica* and *P. lowei*, is shown in Figure 1. This phylogeny is a 50% majority-rule consensus, with the Bayesian posterior probabilities shown at each node. By and large the phylogeny is well resolved, the exceptions being the node including *Ratsirakea legendrei*, *Tateurndina ocellicauda* and the clade containing *Ophieleotris* plus *Mogurnda*; the node subtending *Milyeringa veritas*, *Rhyacichthys aspro*, and *Percottus* plus *Odontobutis* is also unresolved. Two additional polytomies (less than 50% support in the consensus) are found among some of the gobioid taxa. In all cases except *Milyeringa*, *Rhyacichthys*, and *Odontobutidae*, the nodes nearby are also relatively poorly supported, indicating a generally weak level of support in those areas of the tree.

Outside Gobioidae, most nodes are well supported. One node, at the split between the clade containing Leiognathidae, Kurtidae, Pempheridae, Apogonidae, and Gobioidae versus the remainder of the taxa has a posterior probability of 52% and should be considered unresolved. Sister to this group are, in sequence, Dactylopteridae, Perciformes (Moronoidei, Cottoidei, and Scorpaenoidei), Ophidiiformes, Stephanoberyciformes plus beryciform families Holocentridae and Berycidae, Beryciformes (less Holocentridae and Berycidae), and Polymixiiformes. Within Gobioidae, the major clades hypothesized are *Odontobutidae* plus *Rhyacichthy-*

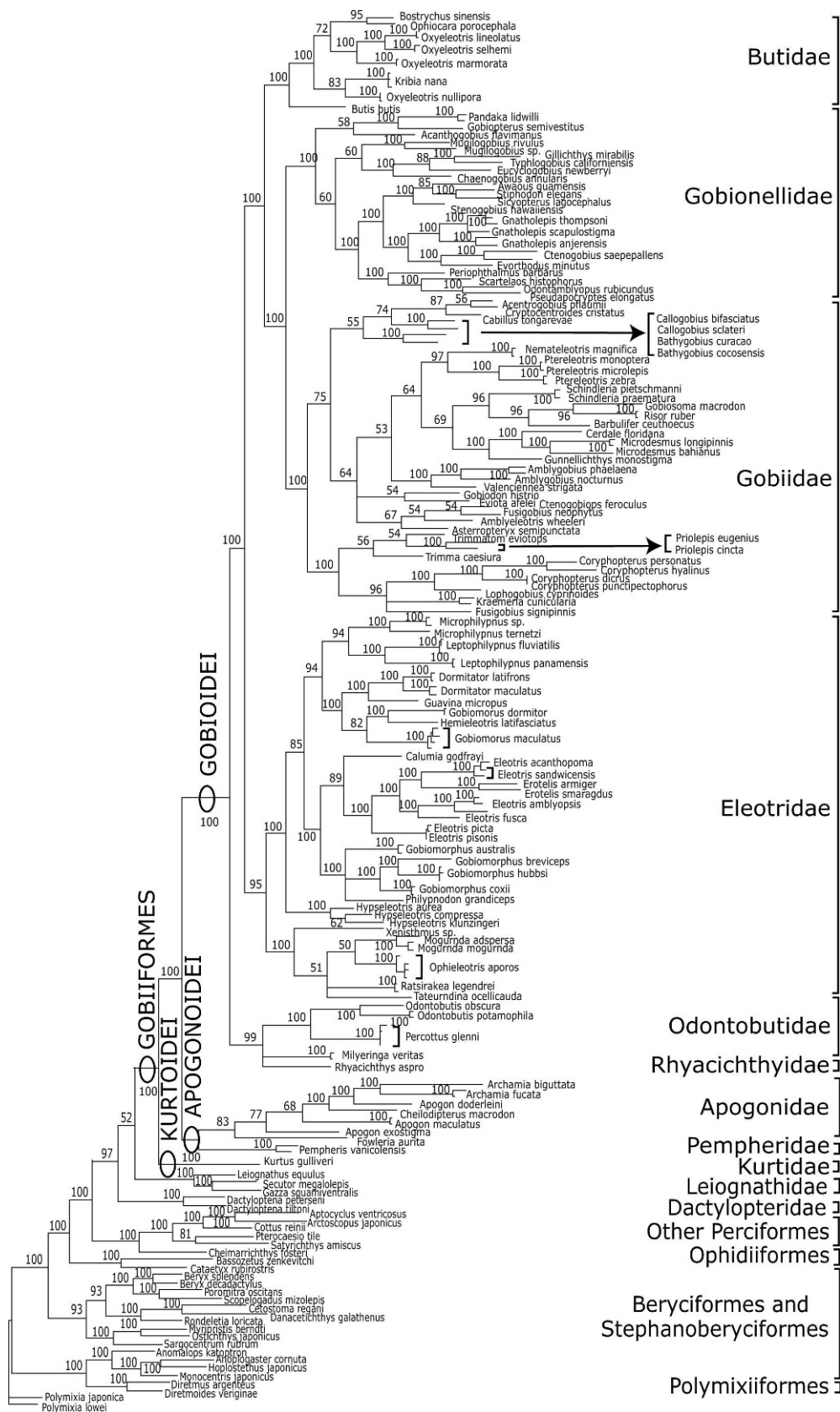


Fig. 1. Phylogeny of Gobiiformes and outgroups. This is a 50% majority-rule consensus of Bayesian trees found after stationarity was achieved. Posterior probability values are shown on nodes.

Table 1. Phylogenetic Taxonomy of Gobioidae. New names proposed herein with equivalence to older names tabulated in Thacker (2003).

Family name	Included taxa
Rhyacichthyidae	Rhyacichthyidae
Odontobutidae	Odontobutidae, <i>Milyeringa</i>
Butidae	Butinae
Eleotridae	Eleotrinae, Xenisthmidae
Gobionellidae	Gobionellinae, Oxudercinae, Amblyopinae, Sicydiinae
Gobiidae	Gobiinae, Microdesmidae, Ptereleotridae, Kraemeriidae, Schindleriidae

dae and the butine genus *Milyeringa*; Eleotrinae plus Xenisthmidae; Butinae; Gobionellinae plus Sicydiinae, Oxudercinae, and Amblyopinae; and Gobiidae plus Microdesmidae, Ptereleotridae, Kraemeriidae, and Schindleriidae. These names correspond only partially to those used in Figure 1 and Table 1; the phylogeny requires adjustments to the current classification, which are given below. Morphological characters and habitat were recorded for every terminal in the phylogeny, except a few instances where epural number was not known. The interpretation of morphological characters and habitat on the molecular phylogeny is given in Figure 2. The γ statistic of Pybus and Harvey (2000) was calculated at -3.798 , significant at $P < 0.0001$. For the MCC test, a sample of 10,000 random trees was generated using the Yule pure birth model, each with 954 terminals, of which 40 were then retained for each tree. At that level of incomplete sampling, γ takes a critical value of -5.982 for significance at $P < 0.05$, rendering the γ value for Gobiidae non-significant.

DISCUSSION

Phylogeny and classification.—The phylogeny depicted in Figure 1 is an integration and expansion of those given in Thacker (2003; focusing on Gobiidae) and Thacker and Hardman (2005; focusing on Eleotridae, Odontobutidae, Rhyacichthyidae, and related outgroup taxa). In both of those previous molecular phylogenies, restricted sampling among groups that were not the focus of the study yielded some paraphyly among the less-sampled taxa. In the case of Thacker (2003), few eleotrids were sampled, and they formed a paraphyletic grade outside of Gobiidae. In Thacker and Hardman (2005) the complementary situation obtained: the few gobiids sampled were nested within the butines. This study provides a complete overview of gobioid phylogeny; with this more comprehensive sampling for the entire group, several clades are revealed that concur in many respects with the traditional taxonomy, but require some adjustments to the classification. A new classification is proposed in Table 1; the goal of this rearrangement was to preserve names and their meanings as much as possible to facilitate understanding and communication, with adjustments made such that the classification names only monophyletic groups. The major alteration proposed is that the largest subfamilies (Eleotrinae, Butinae, Gobiinae, and Gobionellinae) are all elevated to family rank, and other families and subfamilies are subsumed within them. In the case of Eleotridae and Gobiidae, this means that the phylogenetic usage proposed here replaces more inclusive

usages. The families Gobiidae and Eleotridae include their type genera (*Gobius* Linnaeus, 1758 and *Eleotris* Bloch and Schneider, 1801), and Gobionellidae and Butidae are recognized as separate families. This is particularly appropriate in the case of Butidae, formerly a subfamily of Eleotridae. Butidae are sister to Gobionellidae plus Gobiidae and thus, inclusion of this taxon within the family Eleotridae would render Eleotridae paraphyletic.

Gobiidae and Gobionellidae *sensu* this study coincide with the “expanded monophyletic Gobiinae” and “expanded monophyletic Gobionellinae” of Thacker (2003), with one exception. The traditional family Kraemeriidae, represented here by two individuals of *Krameria cunicularia*, is nested within Gobiidae (not Gobionellidae). Overall, placement of the other smaller gobioid groups concurs with earlier molecular studies, and this expanded sampling yields results that are more congruent with morphological characters as well. The former Sicydiinae (here represented by *Stiphodon* and *Sicyopterus*) are recovered within Gobionellidae along with *Awaous* and *Stenogobius*, as in Thacker (2003) and as postulated based on morphology by Harrison (1989), Parenti and Thomas (1998), and Larson (2001). The former Oxudercinae (mudskippers: *Periophthalmus*, *Scartelaos*, and *Pseudapocryptes* sampled here) are paraphyletic with respect to the former Amblyopinae (represented by *Odontamblyopus*), and the relationships of former oxudercine genera, unlike in Thacker (2003), are more congruent with those presented in the cladistic analysis of morphology given in Murdy (1989). That hypothesis showed the relationships (*Pseudapocryptes* (*Scartelaos*, *Periophthalmus*)); the relationships presented here are simply those rerooted, with *Odontamblyopus* added as sister to *Pseudapocryptes*. This expanded mudskipper clade is also nested within Gobionellidae. Other gobionellid relationships postulated here are largely congruent with those of Thacker (2003); the genera *Gnatholepis*, *Evorthodus*, and *Ctenogobius* are grouped with *Awaous*, *Stenogobius*, and the former Sicydiinae, Oxudercinae, and Amblyopinae. The remaining gobionellid genera form a paraphyletic grade in this hypothesis versus a clade in Thacker (2003); however, the support for those nodes is notably low.

Within Gobiidae, placement and relationships of smaller former families also concurs better with previous studies than in Thacker (2003). In that hypothesis, the former Microdesmidae were split, with *Cerdale* and *Microdesmus* resolving apart from *Gunnellichthys*. Thacker (2003) also postulated a nonmonophyletic former Ptereleotridae. In this comprehensive study, *Cerdale*, *Microdesmus*, and *Gunnellichthys* are recovered together, as are former ptereleotrids *Nemateleotris* and *Ptereleotris*; both these results are congruent with the morphological phylogeny of Thacker (2000). Both those clades are resolved as relatives to the tropical Indo-Pacific *Schindleria*, as well as the New World *Gobiosoma*, *Risor*, and *Barbulifer*. Sister to that larger clade are the Indo-Pacific *Amblygobius* and *Valenciennesa*, followed by *Gobiodon*, *Eviota*, *Asterropteryx*, *Amblyeleotris*, *Ctenogobius*, and one species of *Fusigobius*, *F. neophytus*. While the shallower groupings in this part of the phylogeny are well supported, the deeper nodes are not, and thus the exact relationships among groups of genera presented here must be interpreted cautiously. The results of this study also include several additional gobiid genera, making comparisons with Thacker (2003) difficult. Both studies agree that the species-rich *Priolepis* group of Birdsong et al. (1988), containing most of



Fig. 2. Characters optimized on the phylogenetic hypothesis. (A) Number of branchiostegals; (B) number of epurals; (C) presence or absence of fused pelvic fins; (D) habitat. Outgroups are not shown, but were included in the character optimization; the state shown at the root of each phylogeny is that optimized for Gobioidae in comparison to outgroups.

the reef-dwelling Indo-Pacific gobiid genera, is paraphyletic with respect to the *Bathygobius* and *Gobiosoma* groups, as well as the former Microdesmidae, Ptereleotridae, Kraemeriidae, and Schindleriidae. One prominent difference is that the current, larger hypothesis does not recover a single clade of New World taxa; instead, three different clades are distributed in the New World. They are *Cerdale* plus *Microdesmus*; *Gobiosoma*, *Risor*, and *Barbulifer*; and *Coryphopterus* plus *Lophogobius*.

Sister to the Gobiidae plus Gobionellidae clade is the family Butidae, formerly a subfamily of Eleotridae. A close relationship between these groups was also revealed in Thacker and Hardman (2005); in that hypothesis, Gobiidae was nested within Butidae. This study disagrees with Thacker and Hardman (2005) in some rearrangements among groups of genera, most notably the placements of *Philypnodon*, *Microphilypnus*, and *Leptophilypnus*. The hypothesis of Thacker and Hardman (2005) featured these three genera forming a clade, with *Microphilypnus* and *Philypnodon* placed as sister taxa. In the new hypothesis, two individuals of an undescribed species of *Microphilypnus* from Venezuela are included; here *Microphilypnus* and *Leptophilypnus* are sister taxa, and that clade is grouped with the exclusively neotropical genera *Guavina*, *Gobiomorus*, and *Hemieleotris*, as well as the primarily neotropical *Dormitator*. All of the other eleotrid species examined here occur in the Indo-Pacific, with the exception of some species of *Eleotris* and *Erotelis*. These two genera are grouped together, and represent a second radiation into the Neotropics and West Africa, as an extension of a widespread group rather than as an exclusively neotropical clade. *Philypnodon*, an Australian endemic, is placed as sister to *Gobiomorphus* (known only from Australia and New Zealand). Other genera primarily known from Australia and New Guinea are *Hypseleotris* and *Mogurnda*; these genera are resolved separately from each other, and from *Philypnodon*/*Gobiomorphus*.

A clade composed of Rhyacichthyidae and Odontobutidae is sister to the remainder of Gobioidae, and in both this hypothesis and that of Thacker and Hardman (2005), the blind, troglodytic genus *Milyeringa* is included in that clade (rather than Butidae). In the current hypothesis, a polytomy is obtained among *Milyeringa*, *Rhyacichthys*, and *Odontobutidae*; *Milyeringa* is classified as part of Odontobutidae (Table 1) in accordance with Thacker and Hardman (2005).

This study expands greatly the outgroup sampling of Thacker and Hardman (2005). In particular, the families Kurtidae and Pempheridae were included, and were resolved as close relatives to Gobioidae and Apogonidae. Sister to this large group is Leognathidae and Dactylopteridae, followed by the remainder of Perciformes sampled (suborders Moronoidae, Cottoidei, and Scorpaenoidei). Ophidiiformes forms the sister to the included perciform taxa, with Beryciformes and Stephanoberyciformes forming a grade outside that clade. In the current hypothesis, there is strong support for the monophyly of Gobioidae, of its included families and higher-level groupings, and for the relationships within and among most of the outgroup taxa. The exception is the node subtending Leognathidae and the clade including Kurtidae, Pempheridae, Apogonidae, and Gobioidae, as discussed above.

The resolution of a clade including Gobioidae, Apogonidae, Kurtidae, and Pempheridae as distinct from the remainder of Perciformes and Scorpaeniformes confirms a variety of postulated relationships among these groups. The

sister group relationship between Gobioidae and Apogonidae was put forth by Thacker and Hardman (2005) based on molecular data, as well as morphological similarities discussed by Miller (1973), Johnson (1993), and Winterbottom (1993). Smith and Craig (2007) recovered Apogonidae and Kurtidae as sister taxa in their study of perciform and scorpaeniform relationships, and Smith and Wheeler (2006) included Kurtidae, Eleotridae, Odontobutidae, and Apogonidae in their study of 165 families of neoteleost fishes and confirmed that Apogonidae and Gobioidae are sisters, with Kurtidae sister to that clade. Morphologically, Kurtidae also shares with Apogonidae and Gobioidae the presence of sensory papillae rows on the head and body, and those three plus Pempheridae share a configuration of the dorsal gill-arch elements in which the second epibranchial and second pharyngobranchial do not articulate (Johnson, 1993; Glaucosomatidae also shares this character and Johnson recommended it be included as a subfamily of Pempheridae). Springer (1983) discussed the ventral intercleithral cartilage, present in gobioids and some pempherids (*Parapriacanthus ransonetti* but not *Pempheris*). Tominaga (1968) considered Kurtidae to be similar to both Berycidae and Pempheridae, exhibiting characters intermediate between *Beryx* and *Pempheris*. Finally, the recent study of Prokofiev (2006) described a genus, *Kurtamia*, which he placed in Apogonidae and postulated is closely related to *Arhamia*, but demonstrated that it shares several characters (expanded pleural ribs, modified anterior anal pterygiophores, fusion of hypurals one and two) with Kurtidae. The pleural ribs of *Kurtamia* bear expansions that form a bony sheath around the swimbladder, a characteristic also shared by *Kurtus*. Pempheridae, Leptobramidae, and Glaucosomatidae also have bony sheaths on the swimbladder derived from vertebrae, but involving expansions of the parapophyses rather than pleural ribs (Tominaga, 1968; Carpenter et al., 2004; Prokofiev, 2006). Smith and Wheeler (2006) and Smith and Craig (2007) both presented evidence that Leptobramidae is part of Carangoidei, and not a close relative to these groups. *Kurtamia* does not possess the distinctive cephalic hook of Kurtidae, but does feature a supraoccipital crest that is extended into a spine, similar to the underlying structure of the kurtid hook; Prokofiev (2006) suggested that *Kurtus* and *Kurtamia* both be included in Apogonidae.

An additional line of evidence supporting common ancestry for Gobioidae, Apogonidae, and Kurtidae is the reproductive behavior of egg adhesion and brooding by the male. Gobioids generally release eggs onto the substrate, where a male has deposited an adherent sperm-containing trail and then guards the clutch until hatching. The eggs themselves feature a variety of adhesive caps and filaments around the micropyle (Breder and Rosen, 1966; Takahashi, 1978; Rupé, 1984). Apogonidae are mouth-brooders, with the clutch of eggs adhered into a mass, aerated, and guarded in the buccal cavity during development (Breder and Rosen, 1966; Fraser, 1972b). Apogonid eggs are adhesive, with complex chorionic ridges and fibers that converge as they approach the micropyle (Fishelson and Gon, 2008). Kurtidae are notable for their forehead brooding, in which the eggs bear a rosette of filaments around the micropyle that twists into a single strand and adheres the eggs to each other and their gelatinous coating. The clutch of eggs is suspended from and brooded on a bony hook derived from the supraoccipital crest, present in the males (Berra and Niera,

2003; Berra et al., 2004). Berra et al. (2007) infer that during spawning, kurtid males quickly fertilize the eggs as they emerge from the female, then push the egg mass onto their forehead hook, similar to the fertilization and gathering into the mouth performed by apogonid males. Spawning and/or brooding behavior is not known for Pempheridae. Observations of pempherid species are few; when documented, spherical, non-adhesive eggs have been reported (Breder and Rosen, 1966; Leis and Carson-Ewart, 2000; Farooqi et al., 2006).

A relationship between these groups (Gobioidei, Apogonidae, Kurtidae, Pempheridae) and Leiognathidae has not been put forth previously; however, one notable feature that Leiognathidae, Apogonidae, and Pempheridae share is the presence of light organs associated with the viscera. Not all species of Apogonidae or Pempheridae possess these organs. In Pempheridae the light organ, when present, manifests as a ventral elaboration of the pyloric caecae, originating near the junction of the caecae and the stomach; some species also have small outpocketings of the intestine near the anus that are also luminescent (Mooi and Jubb, 1996). The luminescent systems of Pempheridae do not include symbiotic luminescent bacteria (Haneda and Johnson, 1962). In Apogonidae, two types of luminescent organ systems are observed. In the genus *Siphamia*, luminescent bacteria are present in a disc-shaped ventral organ connected to the intestine, as well as in bilaterally paired sacs that are elaborated from the ventral surface of the mouth (Iwai, 1958, 1960; Fishelson et al., 2005). A non-bacterial visceral light organ is found in some species of the genera *Archamia*, *Rhabdamia*, and *Apogon* (Iwai and Asano, 1958; Haneda et al., 1969). The light organ system of Leiognathidae, present in all species, is composed of elaborations of the esophagus which house luminescent bacteria, as well as a complex system of reflective and transparent tissue in the flanks, musculature, and gas bladder (Haneda, 1950; Sparks and Dunlap, 2004; Sparks et al., 2005).

Bioluminescence is not recorded in Kurtidae; however, they do possess transparent bones and musculature of the flanks, such that light may be transmitted completely through the fish (Berra, 2003; the function of these thin "windows" is hypothesized by Carpenter et al. [2004] to be related to reception of sound transmitted into the swim bladder and detected and conveyed to the inner ear by means of the lateral-line nerve). Bioluminescence has not been reported in any species of gobioid; however, it is notable that the ecology of gobioids differs qualitatively from the other four families. Gobioids are generally benthic inhabitants of streams and nearshore marine environments; leiognathids, kurtids, pempherids, and apogonids are all laterally compressed, schooling, nearshore marine and brackish water dwellers.

Based on the results of this study and the works cited above, it is proposed that the order Gobiiformes be resurrected, including three suborders: Gobioidei, Kurtoidei (currently perciform suborders), and Apogonoidei, a new suborder containing Apogonidae and Pempheridae. This suggestion is in accordance with classifications ranging from that of Günther (1880) to Freyhof (1970), and with the recent work of Wiley and Johnson (unpubl. abstract, 2007 annual ASIH meeting). This nomenclature is intended as a step towards delineation of a monophyletic Perciformes, a group that this and other studies (Miya 2003, 2005; Smith and Wheeler, 2006) have shown to be distinct from

Gobioidei and its relatives. It is anticipated that further investigations will show that additional taxa are also part of the clade Gobiiformes; the current taxonomic revision is intended as a conservative starting point. The revised higher-level taxonomy is indicated on Figure 1.

Character evolution and diversification in Gobioidei.—With a comprehensive molecular phylogeny for Gobioidei, it is possible to reexamine morphological characters that have been used to diagnose families and larger gobioid groups. Large groups may be diagnosed by the character states shown for epurals, branchiostegals, and fused pelvic fins in Figure 2. Rhyacichthyidae are easily distinguished from other gobioids by the presence of a lateral-line canal on the body and three epurals in the caudal skeleton (Miller, 1973); in other gobioids considered here, the sensory canals and pores are restricted to the head and fewer than three epurals are present. If the presence of a lateral-line canal on the body is used as a diagnostic character for Rhyacichthyidae, the *incertae sedis* genera *Protogobius* (Watson and Pöllabauer, 1998) and *Terateleotris* (Shibukawa et al., 2001) should also be placed in this family (*Terateleotris* also possesses three epurals). Odontobutidae was established by Hoese and Gill (1993); in that study they also delineated Butidae and Eleotridae (then recognized at subfamily level) and explored character distributions throughout these groups, but found that only their Eleotrinae were diagnosable.

The most notable change between this and other classifications is that Gobionellidae now includes former gobiid subfamilies Oxudercinae, Amblyopinae, and Sicydiinae, and similarly, Gobiidae now includes former gobioid families Microdesmidae, Ptereleotridae, Kraemeriidae, and Schindleriidae. Gobiidae and Gobionellidae include the bulk of gobioid diversity, and few authors have attempted to diagnose them morphologically. Figure 2 shows that the presence of fused pelvic fins is found in most members of Gobiidae + Gobionellidae, although it is not present in several groups. Gobiidae + Gobionellidae are unambiguously diagnosed (among gobioids) by a reduction from six to five branchiostegal rays. Gobiidae are diagnosed by the presence of a single epural; however, this state is found in some members of Gobionellidae as well (Fig. 2). A dorsal-fin pterygiophore insertion pattern of 3–22110 is found in most Gobiidae, and 3–12210 is common to most Gobionellidae, although there are several exceptions (Birdsong et al., 1988; the pterygiophore formula indicates the interneural space into which the first pterygiophore of the spinous dorsal-fin inserts, followed by a hyphen and then a series of digits indicating the number of pterygiophores in each successive interneural space). Pezold (1993) examined cephalic pore patterns in a range of gobioid genera and proposed a diagnostic feature found in most Gobiidae: the presence of a single, median anterior interorbital pore (rather than a pair of pores). Interpreted on the current molecular phylogenetic hypothesis, the character distribution is sporadic; several gobiids lack cephalic pores altogether, and some paired-pore species are included. In Gobionellidae, a mix of the one- and two-pored condition is found. Pezold's (2004) further work on Gobionellidae discussed characters of the vertebrae, gill rakers, and cephalic neuromasts and pores for several genera and made significant advances in generic diagnoses, providing character evidence for the monophyly of *Gobionellus*, *Ctenogobius*, *Oxyurichthys*, *Oligolepis*, and *Stenogobius*, but not for the group as a whole.

The situation described here for gobioid systematics provides a useful perspective on the interpretation of morphological and molecular data in combination. Unambiguous, unreversed, diagnostic morphological characters are rare but overlaps of suites of characters can provide a valuable heuristic and generally effective way of identifying clades. When combined with or interpreted in the context of molecular data, a more complete view of evolution is obtained. Molecular data are not subject to the common morphological bias of perceived distinctiveness: in gobioids, the unusual groups (former Schindleriidae, Kraemeriidae, Microdesmidae, Ptereleotridae, Xenisthmidae, Oxudercinae, Sicydiinae, and Amblyopinae) were removed from the larger families and singled out, although they are in fact part of the spectrum of diversity in the families (clades) Eleotridae, Gobiidae, and Gobionellidae. This situation parallels that of acanthomorph systematics generally, where distinctive groups such as Tetraodontiformes and Pleuronectiformes have been artificially removed from Perciformes (Smith and Wheeler, 2006; Smith and Craig, 2007). With a comprehensive molecular phylogeny, reciprocal interpretation of morphological characters is possible, as well as effective investigation of the patterns and sources of species diversity.

The Gobiidae + Gobionellidae clade comprises the majority of gobioid diversity (1,410 of 1,590 valid species). This diversity is unevenly distributed, with 954 species in Gobiidae versus 456 in Gobionellidae. As these families are sister taxa and thus the same age, it is possible to evaluate if they are significantly unequally diversified. Comparing the species diversity numbers for Gobiidae and Gobionellidae, a χ^2 test indicates that the diversity imbalance between these two clades is highly significant ($\chi^2 = 90.776$, $P < 0.0001$). Phylogenetic reconstruction is a historical science; it is impossible to definitively determine causality for events in the distant past. Correlation of evolutionary patterns, however, may provide indications of the causes of differences among clades, in diversification or other attributes. In this case, the significantly greater species diversity in Gobiidae as compared to Gobionellidae may be due to a habitat shift. Figure 2 shows that at the origin of Gobiidae, a habitat switch occurred from freshwater to marine environments. Although some eleotrids and a few gobionellids inhabit marine waters, the vast majority of gobiids are marine, and the shift into marine habitats occurred at the origin of Gobiidae. This invasion into a new, complex, and extensive habitat may have allowed the tremendous diversification seen in Gobiidae as compared to its primarily fresh and brackish water-dwelling sister clades.

If the high diversity in Gobiidae is due to a shift to marine habitats early in the clade's history, it is possible that a burst of speciation during the clade's initial radiation would be detectable. Such a radiation would be expected to generate shallow and/or poorly supported deeper nodes in a phylogeny, as seen in Gobiidae (Fig. 1). It would also be expected to generate a convex lineage through time (LTT) plot; the LTT for Gobiidae given in Figure 3 exemplifies this pattern. The measure γ (Pybus and Harvey, 2000) detects acceleration or diminution of speciation rates through time; negative values indicate the speciation rate has slowed. The γ statistic for this phylogeny reveals significant deceleration of speciation in Gobiidae ($\gamma = -3.798$; $P < 0.0001$); however, the incomplete sampling in the phylogeny must be taken into account. After correction for sampling with the MCCR test, a critical value for γ of -5.982 for significance at the 5%

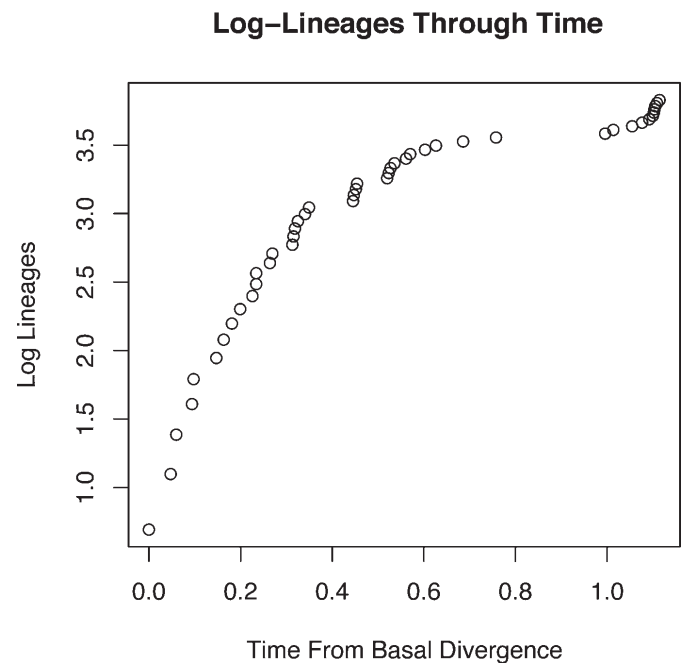


Fig. 3. Lineages through time (LTT) plot for Gobiidae. This convex pattern is indicative of an accelerated speciation rate (steady speciation over time is manifested as a straight line). The speciation appears to have been most accelerated early in the group's history, but then remains high and shows another acceleration in the recent past.

level is indicated, rendering γ non-significant. However, the switch to marine habitats is still consistent with an overall increase in diversification rate through time, yielding the greater species diversity in Gobiidae relative to its sister clade. This is additionally supported by the observation that most gobiids continue to inhabit and diversify in marine waters, with very few switches back into exclusively freshwater habitat.

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