



## Phylogenetic placement of the European sand gobies in Gobionellidae and characterization of gobionellid lineages (Gobiiformes: Gobioidei)

CHRISTINE E. THACKER

Section of Vertebrates - Ichthyology Natural History Museum of Los Angeles County 900 Exposition Blvd. Los Angeles CA 90007.  
E-mail [thacker@nhm.org](mailto:thacker@nhm.org)

### Abstract

The Mediterranean, northeastern Atlantic, and inland freshwaters of Europe and the Ponto-Caspian region host a distinct fauna of gobiiform fishes, including the sand gobies (*Pomatoschistus* Gill and related genera), all of which have been classified in the most diverse goby group, the family Gobiidae. Recent molecular phylogenetic analyses have suggested that the sand gobies are not gobiids, and are instead part of their sister clade Gobionellidae (Thacker and Roje 2011). Phylogenetic analysis of *Pomatoschistus* in the context of both gobiid and gobionellid taxa indicates that *Pomatoschistus* is part of Gobionellidae, specifically the *Mugilogobius* lineage. Gobionellidae includes 93 genera, which are arrayed into four lineages (*Stenogobius*, *Mugilogobius*, *Periophthalmus* and Northern Pacific). These lineages exhibit variation in characters of the jaw and suspensorium, including the shapes and relative positions of the palatine, quadrate, and ectopterygoid. The observations of the palatopterygoid complex in Gobionellidae of Harrison (1989) and Larson (2001) are supported and augmented. Gobionellidae generally exhibit suspensoria that are overall more elongated and gracile than those of gobiids: the palatine/ectopterygoid pair features a very short (*Periophthalmus* lineage) or elongate, pointed palatine (*Mugilogobius*, Northern Pacific, and *Stenogobius* lineages), with a relatively slender ectopterygoid and a short quadrate articulation. In Gobiidae, the palatine extends about halfway along the length of the ectopterygoid, and the ectopterygoid generally features a large, flat articulation with the quadrate. Suspensoria of *Pomatoschistus* and relatives are similar to those of other taxa in the *Mugilogobius* lineage. Placement of *Pomatoschistus* and relatives in Gobionellidae rather than Gobiidae is significant in that it indicates that sand gobies are not closely related to other European gobies, and has implications for any comparative evolutionary or biogeographic studies.

**Key words:** Gobiidae, Gobionellidae, phylogenetics, *Pomatoschistus*, *Economidichthys*, *Knipowitschia*, *Gobiusculus*

### Introduction

*Pomatoschistus* Gill is a genus of twelve species that inhabits the coastal waters and estuaries of the Eastern Atlantic and Mediterranean Sea (Miller 1986, 2004). *Pomatoschistus* and four additional genera, *Gobiusculus* Duncker (monotypic, distributed in the Eastern North Atlantic), *Knipowitschia* Iljin (17 species from the Ponto-Caspian region), *Economidichthys* Bianco, Bullock, Miller & Roubal (two species restricted to freshwater springs and streams in western Greece), and *Hyrnanogobius* Iljin (monotypic and restricted to the Caspian Sea), are collectively denoted the sand gobies, due to their frequent (but not exclusive) preference for sand substrates (Economidis and Miller 1990; McKay and Miller 1997; Huyse *et al.* 2004). The systematics of these genera has been thoroughly studied, and phylogenetic analyses have placed representatives of sand gobies as a distinct clade, sister to various Indo-Pacific (McKay and Miller 1997) or Mediterranean (Penzo *et al.* 1998; Huyse *et al.* 2004; Larmuseau *et al.* 2010; Vanhove *et al.* 2012) gobiids. One molecular phylogenetic analysis, focusing on Ponto-Caspian gobies but including *Pomatoschistus*, placed *Pomatoschistus* variably in a gobiid clade or a clade containing a mix of gobiid and gobionellid taxa, but those analyses were based on limited datasets and most nodes outside the focal ingroup were poorly supported (Neilson and Stepien 2009). McKay and Miller's (1997) discussion of sand goby relationships identified a diagnostic character for the group (loss of the postmaxillary process of the premaxilla) and advanced the possibility that they might be closely related to gobionellids based on pore and papillae patterns and vertebral counts. Their cluster analysis of allozyme variation as well as parsimony

analysis of a small morphological dataset additionally suggested a close relationship between sand gobies and the Mediterranean gobiids *Buenia* Iljin, *Lebetus* Winther, and *Deltentosteus* Gill, although the sampling was very limited (McKay and Miller, 1997). *Pomatoschistus* exhibits elevated vertebral counts as compared to Gobiidae, as well as a dorsal fin pterygiophore insertion pattern more typical of Gobionellidae (3-12210 rather than 3-22110, as discussed in Birdsong, Murdy, and Pezold 1988). In contrast, the number of epurals in the caudal skeleton (one rather than two), and the presence of a single interorbital pore (rather than two) is more typical of Gobiidae, although neither of these characters are uniquely restricted to that family (Pezold, 1993; Thacker 2009).

The family Gobionellidae was identified by Thacker (2003; there denoted the “expanded monophyletic Gobionellinae”) and separated from Gobiidae (Thacker 2009) as part of broad molecular phylogenetic analyses of the gobioid fishes (Order Gobiiformes). That analysis delineated a Gobionellidae composed of inshore, shallow-dwelling fishes, most of which are euryhaline; it includes stream gobies, mudskippers, and eel gobies, among others. Many inhabit muddy or silty habitats such as estuaries and rivers; the atypical *Gnatholepis* Bleeker is found on coral reefs (Keith and Lord 2011; Murdy 2011a, 2011b; Pezold 2011). Gobionellids are usually drably colored, with posteroventrally, obliquely oriented mouths, large lips, and prominent fused pelvic fin discs. They may also have reduced eyes, enlarged jaws, posteriorly displaced (and sometimes very reduced) dorsal fins, and elongate bodies, with vertebral counts ranging from 26-42, often higher than the 26 or 27 vertebrae exhibited by most Gobiidae (Birdsong *et al.* 1988). The highest diversity of gobionellids is found throughout the Indo-West Pacific; fewer gobionellids inhabit the Western Indian Ocean, Atlantic, or Eastern Pacific. A previous survey indicated that this widespread family is distributed throughout tropical and temperate waters around the world, with the exception of the northeastern Atlantic, Mediterranean Sea and Ponto-Caspian region (Pezold 2011). However, this distributional lacuna may simply be due to taxonomic misplacement; molecular phylogenetic analysis of Gobiidae has raised the possibility that the sand goby radiation (*Pomatoschistus*, *Knipowitschia*, *Economidichthys*, *Gobiusculus*, and *Hyracogobius*) is part of Gobionellidae rather than Gobiidae (Thacker and Roje 2011). Sand gobies inhabit coastal waters of the Eastern North Atlantic, the Mediterranean, and the Black and Caspian Seas and are found in marine, estuarine, and freshwaters. The analysis of Thacker and Roje (2011) showed relationships for *Pomatoschistus* outside all other gobiids sampled, but too few gobionellids were included to conclusively determine placement. In this study, *Pomatoschistus* is analyzed in the context of both gobiid and gobionellid taxa, to investigate its position among Gobiidae and Gobionellidae. Placement of *Pomatoschistus* and its sand goby relatives within Gobionellidae would increase the number of gobionellid genera to 93 and would indicate that Gobionellidae is distributed in all tropical and temperate waters worldwide.

This study also seeks to determine the specific placement of *Pomatoschistus* among gobionellid lineages. Assignment of *Pomatoschistus* and the sand gobies to a lineage of related genera will enable accurate classification, as well as indicate appropriate comparative taxa for evolutionary and biogeographic analyses. As a complement to the molecular phylogenetic analysis, elements of the jaw and suspensorium are examined for each of the gobionellid lineages, and compared to the condition in *Pomatoschistus* and other sand gobies. A subset of genera within Gobionellidae have recently been divided into three groups: the Northern Pacific lineage, the *Mugilogobius* lineage, and the *Stenogobius* lineage, each possessing common characteristics of the cutaneous pores and axial skeleton (Larson 2001; Pezold 2011). Pezold (2004) provided a phylogenetic analysis and augmented generic diagnoses for several *Stenogobius* lineage genera, and Harrison (1989) postulated a close relationship among several *Stenogobius* lineage genera and the stream gobies based on characters of the head skeleton. Thacker (2003, 2009) showed using molecular phylogenetics that the mudskippers (traditionally Oxudercinae), eel gobies (traditionally Amblyopinae), and stream gobies (traditionally Sicydiinae), previously placed as separate from other gobionellids, are nested within Gobionellidae. An updated, phylogenetic group breakdown for Gobionellidae is used here, with the stream gobies included in the *Stenogobius* lineage and a fourth lineage designated for the mudskippers and eel gobies, the *Periophthalmus* lineage (Table 1). This list updates and expands the breakdown given by Pezold (2011).

In tropical Indo-Pacific habitats, gobionellids of the *Mugilogobius* and *Periophthalmus* lineages predominate, with some *Stenogobius* lineage members, particularly in freshwater streams. *Mugilogobius* lineage genera are common in estuarine and freshwater, and include the speciose *Pseudogobius* Popta and *Rhinogobius* Gill in addition to *Mugilogobius*. Many *Mugilogobius* lineage genera are notable for attaining very small adult sizes (*Brachygobius* Bleeker, *Gobiopterus* Bleeker, *Pandaka* Herre, *Redigobius* Herre) and sometimes also a reduced, paedomorphic morphology (*Mistichthys* Smith, *Paedogobius* Iwata, Hosoya & Larson; Iwata *et al.* 2001).

*Periophthalmus* lineage genera are distinctive; the mudskippers inhabit intertidal mudflats and mangrove swamps, both in and out of the water and in some cases will occupy burrows in the substrate (Murdy 1989, 2011a). Eel gobies, also known from muddy habitats, are burrowers, and their eyes and ventral fins are typically very reduced (Murdy 2011b).

*Stenogobius* lineage genera are the only Gobionellidae that inhabit the Atlantic and Eastern Tropical Pacific except for one mudskipper known from West Africa (Murdy 1989, 2011a). The *Stenogobius* lineage genera *Awaous* Valenciennes, *Cotylopus* Guichenot, *Ctenogobius* Gill, *Evermannia* Jordan, *Evorthodus* Gill, *Gnatholepis*, *Gobioides* Lacépède, *Gobionellus* Girard, and *Sicydium* are all known from the New World as well as Old; *Evermannia* and *Evorthodus* are exclusively New World. Stream gobies (traditional subfamily Sicydiinae) are part of this lineage; the genera *Akihito* Watson, Keith & Marquet, *Cotylopus*, *Lentipes* Günther, *Parasicydium* Risch, *Sicydium* Bleeker, *Sicyopterus* Gill, *Sicyopus* Gill and *Stiphodon* Weber comprise the stream gobies (Keith and Lord 2011). These fishes are amphidromous: the adults reproduce in streams, and then the larvae are washed out to sea. Juveniles return to live out their lives in freshwater. Stream gobies possess thick, fleshy pelvic fin discs, which they use in their upstream migrations. Their mouths are adapted to scrape algae off river rocks, with thickened, robust premaxillae, often bearing multiple tiny simple teeth, and fleshy lips. Teeth on the dentaries and fifth ceratobranchials may also be numerous and elongate (Parenti and Thomas 1998).

As the name indicates, the Northern Pacific lineage inhabits the temperate Pacific coastlines of Asia and North America, and this group contains the gobionellids of the Eastern Temperate Pacific, known as the Bay gobies and including *Clevelandia* Eigenmann, *Eucyclogobius* Gill, *Gillichthys* Cooper, *Ilypnus* Jordan, *Lepidogobius* Gill, *Lethops* Hubbs, *Quietula* Jordan & Evermann, and *Typhlogobius* Steindachner (Miller and Lea 1972). It also includes *Acanthogobius* Gill and *Tridentiger* Gill, two Asian genera that have also been inadvertently introduced into the coastal waters of California (Miller and Lea 1972). This lineage is most diverse in the coastal waters of Japan, and features a radiation of small, elongate, reduced-eyed gobionellids (genera *Astrabe* Jordan & Snyder, *Clariger* Jordan & Snyder, *Luciogobius* Gill, and *Inu* Snyder; Yamada *et al.* 2009) as well as the more speciose *Chaenogobius*. An interesting and unanswered question is whether or not the Eastern Temperate Pacific Bay gobies are monophyletic. It is not known whether they are the result of a single invasion from the Northwestern Pacific, or multiple invasions. One possibility is that the burrowing, reduced-eyed genera *Typhlogobius* and *Lethops* are part of a clade that also contains the Japanese *Astrabe*, *Clariger*, *Luciogobius*, and *Inu*, while the remaining, more typical Eastern Temperate Pacific gobionellids (*Eucyclogobius*, *Ilypnus*, *Lepidogobius*, *Gillichthys* and *Quietula*) comprise a separate invasion and radiation.

Harrison (1989) described a variety of characters in the suspensorium (bones of the cheek and operculum) shared by subgroups of Gobionellidae (the classification of families within Gobioidae has changed since that study was performed; here the new names for families and lineages, as given in Table 1, are used). In particular, the length of the palatine, either very long or very short, the position of the ectopterygoid/quadrangle articulation, and the shape of the quadrangle were identified as being variable and potentially phylogenetically informative. Harrison's (1989) survey suggested that the stream gobies (part of the *Stenogobius* lineage), and the genus *Awaous* were close relatives; those taxa possess an elongate palatine and an ectopterygoid that articulates at the lower anterior corner of the quadrangle, near the jaw articulation. He additionally delineated and discussed a "*Ctenogobius* lineage", also characterized by a long palatine that meets or nearly meets the quadrangle and a narrow ectopterygoid that articulates with the quadrangle's lower anterior corner. This group included the genera *Ctenogobius*, *Evorthodus*, *Gnatholepis*, *Gobionellus*, and *Oligolepis* Bleeker. *Gobioides* was discussed separately but has a similar suspensorium arrangement, and the long palatine character was also present in the representatives of *Awaous*, and *Stenogobius* Bleeker, examined, as well as one species of *Sicydium*. All these genera are now placed in the *Stenogobius* lineage; molecular phylogenetic analyses indicate that the stream gobies are monophyletic and nested among the other *Stenogobius* lineage genera (Thacker 2009). The configurations described and figured for the stream gobies in Harrison (1989) are similar to those of other *Stenogobius* group genera, although the shapes of the quadrangles differ slightly. The long palatine condition as coded by Harrison (1989) is present in several other gobioids; it is characteristic of *Stenogobius* lineage genera, but is intermittently more widespread within Gobioidae.

**TABLE 1.** Lineage groups within the family Gobionellidae. The five sand goby genera *Economidichthys*, *Gobiusculus*, *Hyrceanogobius*, *Knipowitschia*, and *Pomatoschistus* are listed within the *Mugilogobius* lineage.

<b>Mugilogobius Lineage</b>	<b>Periophthalmus Lineage</b>
<i>Brachygobius</i>	<i>Amblyotrypauchen</i>
<i>Caecogobius</i>	<i>Apocryptes</i>
<i>Chlamydogobius</i>	<i>Apocryptodon</i>
<i>Economidichthys</i>	<i>Boleophthalmus</i>
<i>Eugnathogobius</i>	<i>Brachamblyopus</i>
<i>Gobiopterus</i>	<i>Caragobius</i>
<i>Gobiusculus</i>	<i>Ctenotrypauchen</i>
<i>Hemigobius</i>	<i>Gymnoamblyopus</i>
<i>Hyrceanogobius</i>	<i>Karsten</i>
<i>Knipowitschia</i>	<i>Odontamblyopus</i>
<i>Mistichthys</i>	<i>Oxuderces</i>
<i>Mugilogobius</i>	<i>Parapocryptes</i>
<i>Nesogobius</i>	<i>Paratrypauchen</i>
<i>Paedogobius</i>	<i>Periophthalmodon</i>
<i>Pandaka</i>	<i>Periophthalmus</i>
<i>Papuligobius</i>	<i>Pseudapocryptes</i>
<i>Pomatoschistus</i>	<i>Pseudotrypauchen</i>
<i>Pseudogobiopsis</i>	<i>Scartelaos</i>
<i>Pseudogobius</i>	<i>Taenioides</i>
<i>Redigobius</i>	<i>Trypauchen</i>
<i>Rhinogobius</i>	<i>Trypauchenichthys</i>
<i>Schismatogobius</i>	<i>Trypauchenopsis</i>
<i>Stigmatogobius</i>	<i>Zappa</i>
<i>Tamanka</i>	
<i>Tasmanogobius</i>	
<b>Northern Pacific Lineage</b>	<b>Stenogobius Lineage</b>
<i>Acanthogobius</i>	<i>Akihito</i>
<i>Amblychaeturichthys</i>	<i>Awaous</i>
<i>Astrabe</i>	<i>Cotylopus</i>
<i>Chaenogobius</i>	<i>Ctenogobius</i>
<i>Chaeturichthys</i>	<i>Evermannia</i>
<i>Clariger</i>	<i>Evorthodus</i>
<i>Clevelandia</i>	<i>Gnatholepis</i>
<i>Eucyclogobius</i>	<i>Gobioides</i>
<i>Eutaeniichthys</i>	<i>Gobionellus</i>
<i>Gillichthys</i>	<i>Lentipes</i>
<i>Gymnogobius</i>	<i>Oligolepis</i>
<i>Ilypnus</i>	<i>Oxyurichthys</i>
<i>Inu</i>	<i>Parasicydium</i>
<i>Lepidogobius</i>	<i>Parawaous</i>
<i>Lethops</i>	<i>Sicydium</i>
<i>Leucopsarion</i>	<i>Sicyopterus</i>
<i>Lophiogobius</i>	<i>Sicyopus</i>
<i>Luciogobius</i>	<i>Stenogobius</i>
<i>Polyspondylogobius</i>	<i>Stiphodon</i>
<i>Pterogobius</i>	
<i>Quietula</i>	
<i>Sagamia</i>	
<i>Suruga</i>	
<i>Siphonogobius</i>	
<i>Tridentiger</i>	
<i>Typhlogobius</i>	

Harrison (1989) also documented distinct suspensorial characteristics in the *Periophthalmus* lineage, including a short stubby palatine and small ectopterygoid that articulates with the dorsal edge of the quadrate. The ectopterygoid was triangular in the mudskippers he examined, and rectangular in the eel gobies. Although

Harrison's (1989) focus was on *Stenogobius* and *Periophthalmus* lineage genera, representative genera of the Northern Pacific and *Mugilogobius* lineages were examined and coded for his suspensorial characters, and the Northern Pacific group genera *Clariger* and *Luciogobius* were also illustrated and discussed. The suspensorial configuration in those lineages was generally not considered to be derived by Harrison (1989), but the illustrated Northern Pacific genera are distinct in their degree of suspensorial elongation and dorsoventral depression.

The suspensoria of numerous *Mugilogobius* lineage genera were documented and illustrated by Larson (2001). In her comprehensive, detailed revision of *Mugilogobius* and its relatives, she showed that *Mugilogobius* Smitt and 13 related genera (most of the *Mugilogobius* lineage genera listed here and in Pezold, 2011) are distinguished from several *Stenogobius* lineage genera by the presence of head villi, a modally longitudinal pattern of head papillae, and absence of the anterior nasal pore. Additionally, in *Mugilogobius* lineage genera the infraorbital pores are always present, a plesiomorphic condition among gobionellids. She also indicates that several, but not all, *Mugilogobius* lineage genera exhibit the condition of an elongate palatine, extending nearly to or contacting the quadrate. The character of an elongate palatine is the same as used by Harrison (1989), and reported by him as occurring in some *Mugilogobius* lineage genera as well as his focal taxa in the *Stenogobius* lineage.

Here Harrison's (1989) and Larson's (2001) characters are evaluated in a broader context, consistent with the new classification of Gobionellidae and its component lineages. Characters of the palatine, ectopterygoid, quadrate, and metapterygoid are examined in representatives of the four gobionellid lineages as well as species of eleven of the thirteen lineages of Gobiidae as identified by Thacker and Roje (2011). Observations of cleared and stained skeletal specimens are combined with those detailed in the illustrations of Harrison (1989) and Larson (2001). Suspensoria of two *Pomatoschistus* species are also examined, and compared with descriptions and illustrations of suspensoria for *Economidichthys* and *Knipowitschia* (Economidis and Miller 1990), as well as with other gobioids to assess whether or not those morphological characters are consistent with the placement of sand gobies based on molecular phylogenetic analysis.

## Materials and methods

Sequence data used for phylogenetic analysis were combined from the studies of Thacker (2009) and Thacker and Roje (2011); all voucher and GenBank accession numbers are listed in Table 2. For this analysis, *Pomatoschistus minutus* was the only sand goby taxon examined, analyzed in the context of an additional 14 Gobionellidae and 17 Gobiidae; the hypothesis was rooted with the outgroup *Oxyeleotris lineolata* (Butidae). For this analysis, the aim was to place *Pomatoschistus* in one of the gobiid or gobionellid lineages. The dataset used here was assembled from the broader sampling focused on Gobiidae in Thacker and Roje (2011), and on family-level relationships in Thacker (2009). Representatives of most (11 out of 13) gobiid and all (four) gobionellid lineages were included, along with the data for *P. minutus*. Sequence from the mitochondrial COI (1,236 base pairs), ND1 (975 base pairs), and ND2 (1,047 base pairs) genes were assembled for this analysis, resulting in a matrix of 3,258 aligned base pairs. Alignment was performed by MUSCLE (Edgar, 2004), as implemented by Geneious (Biomatters Ltd., Auckland, New Zealand). Alignments were unambiguous and correlated with the translated amino acid sequence; no gaps were inserted. Aligned gene fragments were concatenated into a single matrix using Mesquite (Maddison, W. P. and D. R. Maddison, version 2.73, available at mesquiteproject.org). Phylogenetic analysis was performed using TREEFINDER (Jobb *et al.* 2004) for likelihood analysis, and with MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) for Bayesian analysis. Both analyses were partitioned by gene fragment using models suggested by TREEFINDER under the Akaike Information Criterion; those models were directly implemented in the likelihood analysis, and support for nodes was assessed with one thousand bootstrap replicates. The closest available MrBayes models were used in the Bayesian analysis, which was run for 3,000,000 generations with two replicates, each with four simultaneous chains. Trees were sampled every 1,000 generations, and the first 1,500 trees (half of the total) were discarded as burn-in. A 50% majority-rule consensus tree was constructed of the post burn-in trees, and clade support was assessed with the posterior probability values for each node.

Suspensorial morphology was examined for the species listed in Table 3, augmented with the illustrations of Harrison (1989) and Larson (2001). Within Gobionellidae, each of the four lineages outlined here was represented by several species, including most of the taxa used in the phylogenetic analysis, plus additional representatives of

the Northern Pacific and *Mugilogobius* lineages. To allow clear visualization of the suspensorial elements, specimens were cleared and stained using a modification of the method of Pothoff (1984) and stored in glycerin. Jaws and suspensoria of the right side were dissected away and photographed using a Samsung NX10 digital camera mounted on a Zeiss SteREO Discovery v8 dissecting microscope. All suspensorial elements were examined and compared, but particular attention was paid to areas highlighted as variable by Harrison (1989) and Larson (2001): the palatine, ectopterygoid, quadrate and metapterygoid bones.

**TABLE 2.** Species of gobioid fishes considered in this study, voucher numbers, and GenBank accession numbers for sequences. Tissue vouchers with numbers beginning with T are catalogued in the fish tissue collection, Natural History Museum of Los Angeles County. Those beginning with KU are catalogued in the fish tissue collection of the University of Kansas Natural History Museum, those beginning with CAS are from the collection of the California Academy of Sciences, and those beginning with PU are from the collections of Peter Unmack.

Name	Tissue Voucher	COI	ND1	ND2
<b>Gobiidae</b>				
<i>Amblyeleotris wheeleri</i>	T-001022	HQ536633	HQ536709	FJ796092
<i>Amblygobius phalaena</i>	T-000905	AF391378	AF391450	AF391522
<i>Asterropteryx semipunctata</i>	T-000899	AF391377	AF391449	AF391521
<i>Barbulifer ceuthoecus</i>	T-000197	AF391353	AF391425	AF391497
<i>Bathygobius cocosensis</i>	T-000929	AF391388	AF391460	AF391532
<i>Cabillus tongarevae</i>	T-000875	AF391382	AF391454	AF391526
<i>Coryphopterus dicrus</i>	T-000582	AF391395	AF391467	AF391539
<i>Ctenogobiops feroculus</i>	T-CTENFER	AF391363	AF391435	AF391507
<i>Eviota afelei</i>	T-000907	AF391391	AF391463	AF391535
<i>Gobiodon histrio</i>	T-000238	AF391360	AF391432	AF391504
<i>Lophogobius cyprinoides</i>	T-000156	AF391362	AF391434	AF391506
<i>Nemateleotris magnifica</i>	T-000592	AF391328	AF391400	AF391472
<i>Priolepis cincta</i>	T-000903	AF391385	AF391457	AF391529
<i>Ptereleotris zebra</i>	T-000595	AF391359	AF391431	AF391503
<i>Risor ruber</i>	T-000188	AF391352	AF391424	AF391496
<i>Trimma caesiura</i>	KU 5683	EU381039	EU380997	EU381018
<i>Valenciennea strigata</i>	T-000898	AF391384	AF391456	AF391528
<b>Gobionellidae</b>				
<i>Awaous guamensis</i>	T-C25	AF391338	AF391410	AF391482
<i>Chaenogobius annularis</i>	T-000159	AF391365	AF391437	AF391509
<i>Ctenogobius saepepallens</i>	T-000300	AY077609	AY077595	AY077602
<i>Eucyclogobius newberryi</i>	CAS 86280	AF391361	AF391433	AF391505
<i>Evorthodus minutus</i>	T-000265	AY077607	AY077593	AY077600
<i>Gillichthys mirabilis</i>	T-000278	AF391340	AF391412	AF391484
<i>Gnatholepis anjerensis</i>	T-C73	AF391375	AF391436	AF504306
<i>Gobiopterus semivestitus</i>	T-000088	AF391387	AF391459	AF391531
<i>Pandaka lidwilli</i>	T-000255	AY077604	AY077590	AY077597
<i>Periophthalmus barbarus</i>	T-000272	AF391339	AF391411	AF391483
<i>Pomatoschistus minutus</i>	T-CS7	HQ909497	HQ909555	HQ909596
<i>Pseudapocryptes elongatus</i>	CAS 90433	AF391394	AF391466	AF391538
<i>Scartelaos histophorus</i>	T-000280	AF391346	AF391418	AF391490
<i>Stenogobius hawaiiensis</i>	T-000649	AF391349	AF391421	AF391493
<i>Stiphodon elegans</i>	T-000645	AF391350	AF391422	AF391424
<b>Butidae</b>				
<i>Oxyeleotris lineolata</i>	PU-01-10 OL	AY722139	AY722276	AY722340

## Results

The phylogenetic dataset was partitioned by gene fragment; TREEFINDER indicated an appropriate model of TVM+G for COI, GTR+G for ND1, and J3+G for ND2. Bayesian and likelihood analyses yielded concordant

topologies, and the resultant phylogeny is shown in Figure 1. Some nodes are weakly supported, particularly those for subgroups of Gobiidae, a phenomenon seen also in larger analyses of gobiid phylogeny (Thacker and Roje 2011). However, support for both Gobiidae and Gobionellidae is strong, and the analysis places *Pomatoschistus minutus* within Gobionellidae, specifically the *Mugilogobius* lineage.

**TABLE 3.** Species and numbers of cleared and stained individuals examined for suspensorial morphology. Species are listed in their lineage groups as delineated for Gobiidae by Thacker & Roje, 2011, and for Gobionellidae herein. CAS = California Academy of Sciences; ANSP = Academy of Natural Sciences, Philadelphia; LACM = Natural History Museum of Los Angeles County; UMMZ = University of Michigan Museum of Zoology.

---

Gobiidae	
American seven-spined gobies	
<i>Barbulifer ceuthoecus</i>	LACM 6024 (3)
<i>Chriolepis minutillus</i>	LACM 20148 (1)
<i>Gobulus crescentalis</i>	LACM 32562-49 (1)
<i>Microgobius gulosus</i>	UMMZ 158862 (4)
<i>Tigrigobius janssi</i>	LACM 32524-46 (3)
Burrowing paired gobies	
<i>Amblygobius albimaculatus</i>	LACM 39985-14 (3)
<i>Valenciennesa muralis</i>	LACM 35969-6 (1)
Coral gobies	
<i>Eviota prasina</i>	UMMZ 186039 (3)
<i>Gobiodon citrinus</i>	LACM 42491-69 (2)
Crested gobies	
<i>Coryphopterus dicrus</i>	LACM 2549 (4)
<i>Coryphopterus urosphilus</i>	LACM 6973-11 (4)
<i>Fusigobius neophytus</i>	LACM 54120-3 (2); LACM 59117-1 (2)
<i>Lophogobius cyprinoides</i>	LACM 7847 (3)
<i>Rhinogobiops nicholsii</i>	LACM 32149-8 (4)
Flapheaded gobies	
<i>Callogobius sclateri</i>	LACM 42489-61 (1)
Inshore gobies	
<i>Bathygobius lineatus</i>	LACM 43690-27 (3)
Lagoon gobies	
<i>Cabillus tongarevae</i>	LACM 54128-3 (1)
Mediterranean/Ponto-Caspian/Eastern Atlantic gobies	
<i>Neogobius fluviatilis</i>	LACM44708-1 (3)
Reef shrimp gobies	
<i>Amblyeleotris wheeleri</i>	LACM 57162-1 (1)
<i>Asterropteryx semipunctata</i>	LACM 33723-13 (3)
<i>Ctenogobiops feroculus</i>	LACM 57164-1 (3)
Tiny banded gobies	
<i>Priolepis cincta</i>	LACM 26566 (3)
<i>Trimma macrophthalmum</i>	LACM 33723-54 (1)
Wormfishes and dartfishes	
<i>Nemateleotris magnifica</i>	ANSP 130708 (1)
<i>Ptereleotris zebra</i>	LACM 26559 (2)

---

.....continued on the next page

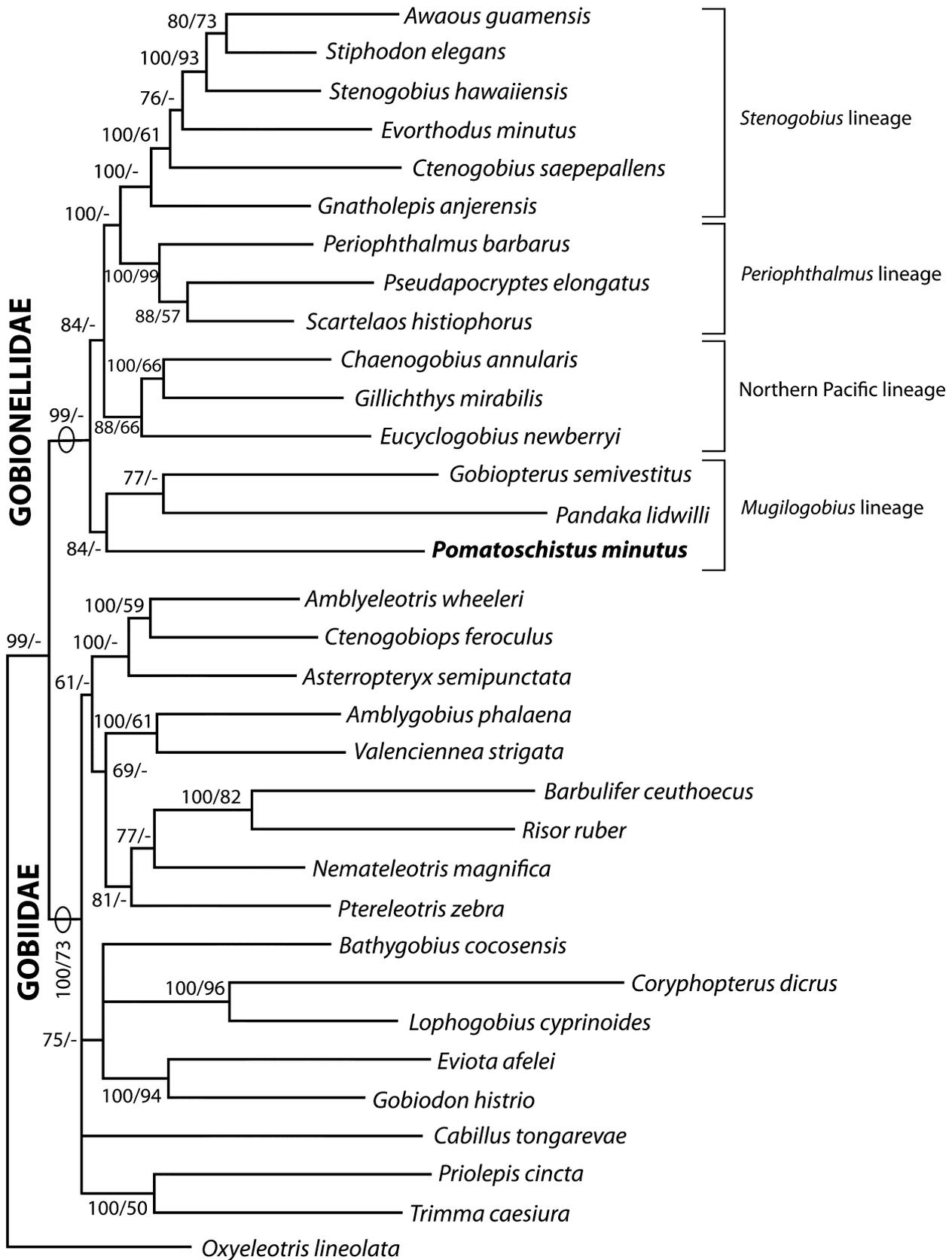
TABLE 3. (Continued)

---

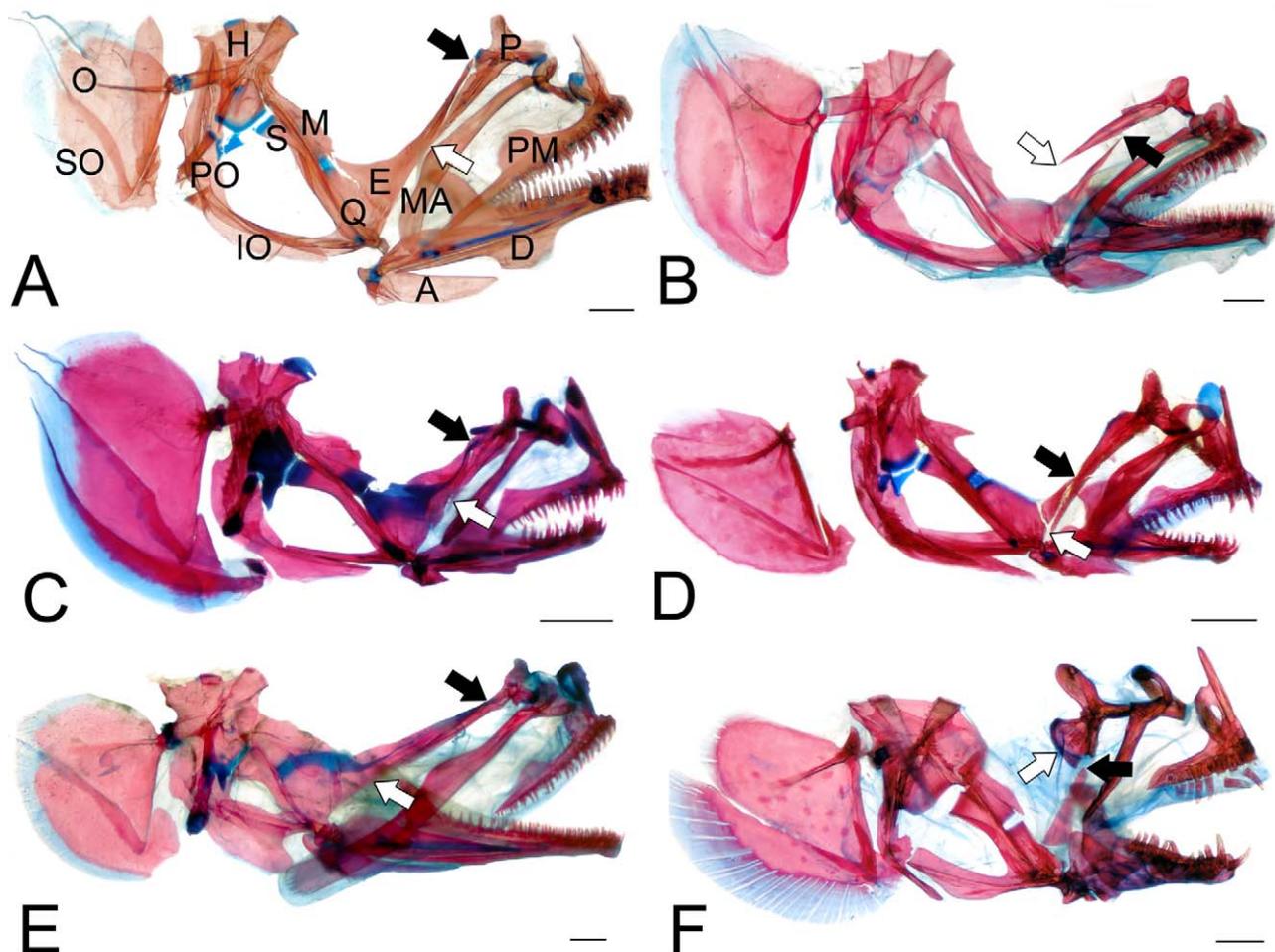
Gobionellidae	
<i>Mugilogobius</i>	
<i>Chlamydogobius ranunculus</i>	LACM 44624-1 (2)
<i>Pandaka pygmaea</i>	UMMZ 174664 (3)
<i>Pomatoschistus microps</i>	UMMZ 201313 (5)
<i>Pomatoschistus minutus</i>	LACM 38466-2 (4)
Northern Pacific	
<i>Acanthogobius flavimanus</i>	LACM 42662-5 (8)
<i>Eucyclogobius newberryi</i>	LACM 36657-3 (3)
<i>Gillichthys mirabilis</i>	LACM 42690-3 (3)
<i>Gillichthys seta</i>	LACM 30124-7 (3)
<i>Lepidogobius lepidus</i>	LACM 32168-8 (3)
<i>Tridentiger bifasciatus</i>	LACM 45686-1 (3)
<i>Typhlogobius californiensis</i>	LACM CCS 642-3 (4)
<i>Periophthalmus</i>	
<i>Periophthalmus sp.</i>	LACM 38331-1 (2)
<i>Periophthalmus argentilineatus</i>	LACM 48469-1 (3)
<i>Odontamblyopus rubicundus</i>	CAS 88635 (3)
<i>Scartelaos tenuis</i>	LACM 38141-2 (3)
<i>Stenogobius</i>	
<i>Awaous banana</i>	LACM 4786 (3)
<i>Evorthodus lyricus</i>	LACM 43423-2 (3)
<i>Gnatholepis anjerensis</i>	LACM55960-4 (3)
<i>Gnatholepis thompsoni</i>	LACM 20636 (1)
<i>Oligolepis acutipennis</i>	UMMZ 218768 (5)
<i>Sicydium multipunctatum</i>	UMMZ 171945 (5)
<i>Sicyopterus taeniurus</i>	LACM 35935-1 (1)
<i>Stiphodon elegans</i>	LACM 35511-1 (3)

---

Examination of the suspensoria of the four lineages of Gobionellidae as well as eleven out of the thirteen lineages of Gobiidae shows notable variation in the shapes and sizes of the palatine, ectopterygoid, quadrate and metapterygoid bones. Representative cleared and stained suspensoria are depicted in Figure 2. As compared to Gobiidae, Gobionellidae often exhibit suspensoria that are overall more elongate and depressed; this is most evident in comparisons of Northern Pacific lineage genera with Gobiidae and less apparent among representatives of the other lineages, particularly the compact suspensoria of the *Periophthalmus* lineage genera, the stream gobies of the *Stenogobius* lineage, and smaller-bodied representatives of the *Mugilogobius* lineage. The observations of Harrison (1989) are supported, including that of a typically different articulation between the ectopterygoid and the quadrate in Gobionellidae as compared to Gobiidae. In most of the taxa examined here, as well as those illustrated by Harrison (1989), the ectopterygoid of Gobionellidae articulates with the lower anterior corner or edge of the quadrate, and the site of articulation is generally small. Among Gobiidae, the ectopterygoid/quadrate articulation is usually longer and placed more dorsally on the quadrate (Figure 2A). Exceptions to this generalization exist, for instance the gobionellid genus *Chlamydogobius* Whitley (*C. ranunculus* examined here, *C. eremius* illustrated by Larson 2001) possesses a broad-based ectopterygoid with a relatively dorsally placed quadrate articulation; in the *Mugilogobius* lineage the articulation is larger than in the other gobionellid lineages. Variably present among both Gobionellidae and Gobiidae are a bridge, cartilaginous or ossified, between the preopercle and symplectic at the dorsal extent of the gap displacing those two bones, and a metapterygoid flange. In some species, the metapterygoid is expanded dorsally and anteriorly into a rounded or pointed shelf, which may also extend ventrad



**FIGURE 1.** Phylogeny of Gobiidae and Gobionellidae, rooted with the outgroup *Oxyeleotris lineolata* (Butidae). The sand goby *Pomatoschistus minutus* is in boldface. This topology resulted from both Bayesian and likelihood analysis; numbers at nodes are support values, listed as posterior probability/bootstrap value. Bootstrap values less than 50% are indicated by ‘-’. Nodes with both posterior probability and bootstrap values of less than 50% are collapsed.



**FIGURE 2.** Cleared and stained suspensoria for representatives of Gobiidae and Gobionellidae. A = anguloarticular; D = dentary; E = ectopterygoid; H = hyomandibula; IO = interopercle; M = metapterygoid; MA = maxilla; O = opercle; P = palatine; PM = premaxilla; PO = preopercle; Q = quadrate; S = symplectic; SO = subopercle. Black arrows indicate dorsal extent of ectopterygoid; white arrows indicate ventral extent of palatine. A) Gobiidae: *Pomatoschistus gulosus* UMMZ 158862; B) *Mugilogobius* lineage: *Pomatoschistus minutus* LACM 38466-2; C) *Mugilogobius* lineage: *Chlamydogobius ranunculus* LACM 44624-1; D) *Stenogobius* lineage: *Gnatholepis anjerensis* LACM 55960-4; E) Northern Pacific lineage: *Gillichthys mirabilis* LACM 42690-3; F) *Periophthalmus* lineage: *Periophthalmus* sp. LACM 38331-1. Scale bars = 1mm. Note that palatine in (B) is deflected slightly dorsally away from the ectopterygoid, in order to clearly show the extents of the bones. Normally, those bones are closely apposed to one another.

to contact the quadrate. Larson (2001) discusses the distribution of metapterygoid shapes among *Mugilogobius* lineage members, including the observation that metapterygoid elaborations are sometimes sexually dimorphic.

Gobionellidae also are distinguishable from Gobiidae in the conformations of the palatine and ectopterygoid. Among Gobiidae, the palatine/ectopterygoid strut is composed of a palatine with expansions at the anterior head that articulate anteroventrally with the maxilla and dorsally with the lateral ethmoid, then narrows along its shaft to a blunt end, or, more commonly, a point. The palatine shaft extends roughly halfway along the length of the ectopterygoid, which is itself extended anteriorly nearly to the palatine head. In *Mugilogobius* lineage genera, the palatine/ectopterygoid condition most closely approximates that of Gobiidae: the palatine is pointed and in some smaller-bodied species (such as *Brachygobius*, Larson [2001]) extends only halfway to the quadrate. However, most *Mugilogobius* lineage genera feature a long palatine, extending to or approaching the anterior corner of the quadrate. The ectopterygoid is similar to that of Gobiidae in that it is anteriorly pointed, widens along its overlap with the palatine, and terminates in a broad edge along its articulation with the quadrate (Figure 2C).

The three other gobionellid lineages each exhibit palatopterygoid complexes that diverge from the common condition in varying ways. Among *Stenogobius* lineage genera the palatine is long, as it is among *Mugilogobius* lineage members, but the ectopterygoid is distinctive in that it is small to minute; small in the stream gobies and

even more reduced in the other genera (Figure 2D). *Periophthalmus* group genera exhibit a very short, blocky palatine, with a robust lateral ethmoid process. The ectopterygoid is slender and narrow, triangular in the mudskippers and oblong in the eel gobies, and does not contact the palatine (Figure 2F). Finally, Northern Pacific lineage genera feature the most elongated and depressed suspensoria, in some cases with the maxillae so elongate that the upper jaw extends lateral and posterior to the dentary. In these taxa the palatine and ectopterygoid are both very elongate and slender, and the ectopterygoid has a flat articulation with a pronounced anterior quadrate flange. The palatine extends most of the length of the ectopterygoid; the proportions of these bones are not remarkable, but the overall elongation and depression of the suspensorium is characteristic. This depression also results in an interhyal/symplectic gap that is narrower and flattened relative to the other gobiids and gobionellids examined (Figure 2E).

## Discussion

The phylogeny indicates that *Pomatoschistus minutus*, a representative of the sand gobies, is grouped among gobionellids in the *Mugilogobius* lineage (Figure 1). The recovered relationships are also consistent with the four lineages outlined in Table 1: *Mugilogobius*, Northern Pacific, *Periophthalmus*, and *Stenogobius*. This dataset is a snapshot of the relationships discussed for a bigger dataset in Thacker (2009); both depict the *Stenogobius* and *Periophthalmus* lineages as sister taxa, with the Northern Pacific and *Mugilogobius* lineages arrayed as sequential sister taxa to that clade. The current hypothesis has higher resolution and support values for the basal nodes of Gobionellidae than Thacker (2009), but it also includes fewer taxa.

Delineation of these four lineages is generally consistent with the phylogenetic results of Thacker (2009). That study sampled 19 gobionellid genera and recovered monophyletic *Stenogobius*, and *Periophthalmus* lineages, resolved as sister taxa. The *Mugilogobius* and Northern Pacific lineages were also present, but with the basal members of the lineages mixed. However, those nodes exhibited markedly low support values, and are more appropriately regarded as unresolved. These lineages are expansions of groups identified and detailed in Larson (2001) and Pezold (2011). The group designations of Birdsong *et al.* (1988) are also partially consistent with the lineages identified here, with some adjustments. The *Stenogobius* lineage of Table 1 mostly corresponds to Birdsong *et al.*'s (1988) *Gobionellus* group plus the *Sicydium* group and *Gobioides*. Three *Gobionellus* group genera (*Mugilogobius*, *Pseudogobiopsis* Koumans and *Tamanka* Herre) are here grouped with Birdsong *et al.*'s (1988) *Gobiopterus* group as the *Mugilogobius* lineage. The *Periophthalmus* lineage is composed of the mudskipper and eel goby groups: *Boleophthalmus* Valenciennes, *Oxuderces* Eydoux, *Periophthalmus* Block, *Taenioides* Lacépède, and *Trypauchen* Valenciennes. Finally, the Northern Pacific lineage encompasses the *Acanthogobius*, *Astrabe*, and *Chasmichthys* groups, delineated by Birdsong *et al.* (1988) due to the variability in vertebral counts and axial fin positioning observed among species of those genera.

The four gobionellid lineages each possess suspensoria that are distinct from the condition seen in Gobiidae (Figure 2), and the general characteristics of each are discussed above. The suspensoria of two species of *Pomatoschistus*, *P. minutus* and *P. microps*, were examined for this study, as well as illustrations of *Economidichthys pygmaeus* and *Knipowitschia caucasica* (Economidis and Miller 1990). All these sand gobies feature suspensoria that are similar to the typical *Mugilogobius* lineage condition: the elongate palatine extends nearly to the quadrate (the palatine is relatively shortest in *Knipowitschia*) and terminates in a pointed end. The ectopterygoid is similarly pointed at its narrow (anterior) end, widening to a blunt edge that articulates with the lower anterior surface of the quadrate (Figure 2B). The sand goby condition is clearly not that of the *Periophthalmus* lineage, with its short chunky palatine, nor do they have the elongate, gracile suspensoria of the Northern Pacific lineage. The sand gobies do have an elongate palatine in common with both the *Mugilogobius* and *Stenogobius* lineages, but unlike the tiny ectopterygoids of *Stenogobius* lineage genera, the ectopterygoids of sand gobies and the *Mugilogobius* lineage are relatively long, more robust than those of the Northern Pacific lineage, and terminate at the quadrate in a broad articulating edge. Two of the characters listed by Larson (2001) as characteristic of the *Mugilogobius* lineage genera she examined (absence of the anterior nasal pore and presence of the infraorbital pores) are present in *Pomatoschistus* and the sand gobies (McKay and Miller, 1997), although those characters are not exclusive to the *Mugilogobius* lineage genera. Sand gobies display a mixed transverse/longitudinal pattern of cranial papillae, not the longitudinal papillae pattern typical of *Mugilogobius* lineage

genera. Also, the specimens examined for this study do not show the head villi described by Larson (2001), although she notes that they are best observed in well-preserved specimens with an intact mucous coat, which was not present in the examined specimens. She also notes that the papillae are most prominent and dense in estuarine and mangrove species; the sand gobies are not found in those habitats.

The morphological characters reviewed and examined here are generally, but not unambiguously, consistent with placement of sand gobies in the *Mugilogobius* lineage. There is no unambiguous synapomorphy that diagnoses the lineage; many of the characteristics described for *Mugilogobius* lineage genera are variable within the lineage as well as among lineage members and other gobioids. A phylogenetic analysis of morphological data presented in McKay and Miller (1997) included several characters of the head pores and papillae, as well as the suspensorium and axial skeleton, and inferred that the sand gobies were closely related to the European gobies *Buenia*, *Lebetus* and *Deltentosteus*, as well as the southern temperate Pacific genus *Nesogobius*. However, as with other characters described here, the ones shared by sand gobies and the other genera were also more widely distributed. This is not surprising, given that relationships among gobioids generally have been difficult to discern based on morphological data alone (Thacker, 2009; Van Tassell *et al.*, 2011).

The Mediterranean *Deltentosteus* and the eastern Atlantic *Buenia* and *Lebetus* are classified in the Gobiidae, specifically the Mediterranean/Northeastern Atlantic lineage of Thacker and Roje (2011). However, placement of these genera is not unambiguous. Based on the axial skeleton, Birdsong *et al.* (1988) designated *Deltentosteus* as “unassigned”; it has 33 vertebrae, an elevated count more typical of Gobionellidae, but also the 3-2211 dorsal fin pterygiophore pattern typical of Gobiidae. Vertebral counts for *Buenia* (30-31) and *Lebetus* (27-29) are closer to the condition among most Gobiidae; *Buenia* typically has the pterygiophore pattern 3-1221 and *Lebetus* 3-2211, and all three of the genera have a single epural in the caudal skeleton (McKay and Miller, 1997). Pezold (1993), using head pore characters, placed *Deltentosteus* in Gobionellidae (then Gobionellinae) and *Buenia* in Gobiidae (then Gobiinae), and did not treat *Lebetus*. In a later review, Pezold (2011) did not include *Deltentosteus* in Gobionellidae. It is possible that additional examination of morphology, or analysis of molecular data, will reveal that *Deltentosteus*, and possibly *Buenia* and *Lebetus*, are part of Gobionellidae along with *Pomatoschistus* and the sand gobies.

Another genus with an uncertain history of placement among gobies is the temperate southern Australian endemic genus *Nesogobius*. *Nesogobius*, like *Pomatoschistus*, displays a mix of characters consistent with both Gobiidae and Gobionellidae (Hoese, 1991; Larson, 2001; Hoese and Larson, 2006). Those authors suggest it may also be related to the southern Australian *Tasmanogobius*. *Nesogobius* was placed by Thacker and Roje (2011) in the lagoon goby lineage of Gobiidae, but considered by Pezold (2011) to be in Gobionellidae. Here both *Nesogobius* and *Tasmanogobius* are listed in the *Mugilogobius* lineage of Gobionellidae, amending Thacker and Roje (2011; that work also contained a proofreading error: the genus *Gobiusculus* was listed twice in the table of lineages, once as a lagoon goby and once as a sand goby. The sand goby placement is correct).

The current analysis, using both morphological and molecular character data, supports what has been suggested previously: that the sand gobies (*Pomatoschistus*, *Gobiusculus*, *Knipowitschia*, *Economidichthys* and *Hyrnanogobius*) are part of Gobionellidae, not Gobiidae. This placement explains previous phylogenetic observations that sand gobies seem to be deeply split from Gobiidae (Huyse *et al.* 2004; Larmuseau *et al.* 2010; Vanhove *et al.* 2012), and indicates that Europe, the Mediterranean, and the Ponto-Caspian freshwater systems are inhabited by distinct radiations of both gobiids and gobiionellids. This placement also explains the relatively old divergence times calculated between sand gobies and various European gobiids (Penzo *et al.* 1988; Vanhove *et al.* 2012), as well as previous suggestions (McKay and Miller; Huyse *et al.* 2004) that the sister taxon to sand gobies could be a gobiionellid. The implications of this new placement are more than merely taxonomic; resolution of the sand gobies in Gobionellidae means that they are close relatives of neither the Ponto-Caspian radiation (including *Neogobius* Iljin and *Proterorhinus* Smitt) nor the Mediterranean marine gobies (such as *Gobius* Linnaeus and *Zosterisessor* Whitley). Studies using phylogenetic comparative methods, or those inferring divergence timing or patterns must not consider sand gobies as sister taxa to other Gobiidae in the region. Rather, their close relatives should be sought within the *Mugilogobius* lineage of the family Gobionellidae, possibly the genera *Nesogobius* and *Tasmanogobius*.

## Acknowledgments

I am grateful to Peter J. Miller for his advice and extensive published history on European gobies, and to Ian Harrison and an anonymous reviewer for providing comments during revision that greatly improved the manuscript. Daniel Geiger expertly prepared Figure 2. Grateful acknowledgement is also given to the collection managers who provided loans and permission to clear and stain specimens: Dave Catania, William Eschmeyer, William Fink, Doug Nelson, Scott Schaefer, and William Saul. This work was supported by grants from the W. M. Keck and R. M. Parsons foundations in support of the program in Molecular Systematics and Evolution at the Natural History Museum of Los Angeles County.

## References

- Birdsong, R.S., Murdy E.O. & Pezold, F.L. (1988) A study of the vertebral column and median fin osteology in gobioid fishes with comments on gobioid relationships. *Bulletin of Marine Science*, 42(2), 174–214.
- Economidis, P.S. & Miller, P.J. (1990) Systematics of freshwater gobies from Greece (Teleostei: Gobiidae). *Journal of Zoology, London*, 221, 125–170. <http://dx.doi.org/10.1111/j.1469-7998.1990.tb03781.x>
- Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acid Research*, 32, 1792–1797. <http://dx.doi.org/10.1093/nar/gkh340>
- Harrison, I.J. (1989) Specialization of the gobioid palatoquadrate complex and its relevance to gobioid systematics. *Journal of Natural History*, 23, 235–353. <http://dx.doi.org/10.1080/00222938900770211>
- Hoese, D.F. (1991) A revision of the temperate Australian gobiid (Gobioidei) fish genus *Tasmanogobius* with a comment on the genus *Kimberleyeleotris*. *Memoirs of the Museum Victoria*, 52(2), 361–376.
- Hoese, D. F. & Larson, H.K. (2006) Description of two new species of *Nesogobius* (Pisces: Gobioidei: Gobiidae) from southern Australia. *Memoirs of Museum Victoria* 63(1), 7–13.
- Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, 17, 754–755. <http://dx.doi.org/10.1093/bioinformatics/17.8.754>
- Huysse, T., Van Houdt, J. & Volckaert, F.A.M. (2004) Paleoclimatic history and vicariant speciation in the “sand goby” group (Gobiidae, Teleostei). *Molecular Phylogenetics and Evolution*, 32, 324–336. <http://dx.doi.org/10.1016/j.ympev.2003.11.007>
- Iwata, A., Hosoya, S. & Larson, H.K. (2001) *Paedogobius kimurai*, a new genus and species of goby (Teleostei: Gobioidei: Gobiidae) from the west Pacific. *Records of the Australian Museum*, 53(1), 103–112. <http://dx.doi.org/10.3853/j.0067-1975.53.2001.1326>
- Jobb, G., von Haeseler, A. & Strimmer, K. (2004) TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evolutionary Biology*, 4, 18. <http://dx.doi.org/10.1186/1471-2148-4-18>
- Keith, P. & Lord, C. (2011) Systematics of Sicydiinae. In: *Biology of Gobies*. R. A. Patzner, J. L. Van Tassell, M. Kovacic, and B. G. Kapoor (eds.), Science Publishers, Inc., pgs. 119–128.
- Larmuseau, M.H.D., Huysse, T., Vancampenhout, K., Van Houdt, J.K.J. & Volckaert, F.A.M. (2010) High molecular diversity in the rhodopsin gene in closely related goby fishes: a role for visual pigments in adaptive speciation? *Molecular Phylogenetics and Evolution*, 55, 689–698. <http://dx.doi.org/10.1016/j.ympev.2009.10.007>
- Larson, H.K. (2001) A revision of the gobiid fish genus *Mugilogobius* (Teleostei: Gobioidei), and its systematic placement. *Records of the Western Australian Museum Supplement No. 62*, 233 pgs.
- McKay, S.I. and Miller, P.J. (1997) The affinities of European sand gobies (Teleostei: Gobiidae). *Journal of Natural History*, 31, 1457–1482. <http://dx.doi.org/10.1080/00222939700770791>
- Miller, D.J. & Lea, R.N. (1972) Guide to the coastal marine fishes of California. *Fish Bulletin of the Department of Fish and Game of California*, No. 157, 1–235.
- Miller, P. J. (1986) Gobiidae. In: *Fishes of the North-eastern Atlantic and the Mediterranean*. P. J. P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen, and E. Tortonese (eds.), UNESCO, Paris, pgs. 1019–1085. <http://dx.doi.org/10.1017/S0025315400057209>
- Miller, P.J. (2004) *Pomatoschistus*. In: *The Freshwater Fishes of Europe*, Vol. 8/II. P. J. Miller (ed.), AULA-Verlag, Wiebelsheim, Germany, pgs. 279–330.
- Murdy, E.O. (1989) A taxonomic revision and cladistic analysis of the oxudercine gobies (Gobiidae, Oxudercinae). *Records of the Australian Museum Supplement*, 11, 1–93. <http://dx.doi.org/10.3853/j.0812-7387.11.1989.93>
- Murdy, E.O. (2011a) Systematics of Oxudercinae. In: *Biology of Gobies*. R. A. Patzner, J. L. Van Tassell, M. Kovacic, and B. G. Kapoor (eds.), Science Publishers, Inc., pgs. 99–106.
- Murdy, E.O. (2011b) Systematics of Amblyopinae. In: *Biology of Gobies*. R. A. Patzner, J. L. Van Tassell, M. Kovacic, and B. G. Kapoor (eds.), Science Publishers, Inc., pgs. 107–118.
- Nielson, M.E. & Stepien, C.A. (2009) Escape from the Ponto-Caspian: Evolution and biogeography of an endemic goby species flock (Benthophilinae: Gobiidae: Teleostei). *Molecular Phylogenetics and Evolution*, 52, 84–102. <http://>

[dx.doi.org/10.1016/j.ympev.2008.12.023](http://dx.doi.org/10.1016/j.ympev.2008.12.023)

- Parenti, L.R. & Thomas, K.R. (1998) Pharyngeal jaw morphology and homology in sicydiine gobies (Teleostei: Gobiidae) and allies. *Journal of Morphology*, 237, 257–274. [http://dx.doi.org/10.1002/\(SICI\)1097-4687\(199809\)237:3%3C257::AID-JMOR4%3E3.0.CO;2-W](http://dx.doi.org/10.1002/(SICI)1097-4687(199809)237:3%3C257::AID-JMOR4%3E3.0.CO;2-W)
- Penzo, E., Gandolfi, G., Bargelloni, L., Colombo, L. & Patarnello, T. (1998) Messinian salinity crisis and the origin of freshwater lifestyle in Western Mediterranean gobies. *Molecular Biology and Evolution*, 15, 1472–1480. <http://dx.doi.org/10.1093/oxfordjournals.molbev.a025874>
- Pezold, F. L. (1993). Evidence for a monophyletic Gobiinae. *Copeia*, 1993, 634–643. <http://dx.doi.org/10.2307/1447224>
- Pezold, F.L. (2004) A phylogenetic analysis of the genus *Gobionellus* (Teleostei: Gobiidae). *Copeia*, 2004, 260–280. <http://dx.doi.org/10.1643/CI-02-218R3>
- Pezold, F.L. (2011) Systematics of the family Gobionellidae. In: Patzner, R.A., Van Tassell, J.L., Kovacic, M., and Kapoor, B.G., eds. *The Biology of Gobies*, Science Publishers Inc., Enfield, NH, pgs. 87–98.
- Pothoff, T. (1984) Clearing and staining techniques. In: *Ontogeny and Systematics of Fishes*. H. G. Moser (ed.) Spec. Pub. ASIH No. 1. Allen Press, Lawrence, Kansas, pgs. 35–37.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574. <http://dx.doi.org/10.1093/bioinformatics/btg180>
- Thacker, C.E. (2003) Molecular phylogeny of the gobioid fishes (Teleostei: Perciformes: Gobioidei) *Molecular Phylogenetics and Evolution*, 26, 354–368. [http://dx.doi.org/10.1016/S1055-7903\(02\)00361-5](http://dx.doi.org/10.1016/S1055-7903(02)00361-5)
- Thacker, C.E. (2009) Phylogeny of Gobioidei and placement within Acanthomorpha, with a new classification and investigation of diversification and character evolution. *Copeia*, 2009, 93–104. <http://dx.doi.org/10.1643/CI-08-004>
- Thacker, C.E. & Roje, D.M. (2011) Phylogeny of Gobiidae and identification of gobiid lineages. *Systematics and Biodiversity*, 9(4), 329–347. <http://dx.doi.org/10.1080/14772000.2011.629011>
- Vanhove, M.P.M., Economou, A.N., Zogaris, A., Larmuseau, M.H.D., Giakoumi, S., Kalogianni, E., Volckaert, F.A.M. & Huyse, T. (2012) Phylogenetics and biogeography of the Balkan ‘sand gobies’ (Teleostei: Gobiidae): vulnerable species in need of taxonomic revision. *Biological Journal of the Linnean Society*, 105, 73–91. <http://dx.doi.org/10.1111/j.1095-8312.2011.01781.x>
- Van Tassell, J. L., Tornabene, L. & Taylor, M. S. (2011) A history of gobioid morphological systematics. In: Patzner, R.A., Van Tassell, J.L., Kovacic, M., and Kapoor, B.G., eds. *The Biology of Gobies*, Science Publishers Inc., Enfield, NH, pgs. 3–22.
- Yamada, T., Sugiyama, T., Tamaki, N., Kawakita, A. & Kato, M. (2009) Adaptive radiation of gobies in the interstitial habitats of gravel beaches accompanied by body elongation and excessive vertebral segmentation. *BMC Evolutionary Biology*, 9, 145. <http://dx.doi.org/10.1186/1471-2148-9-145>