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Phylogeny of the gudgeons (Teleostei: Cyprinidae: Gobioninae)

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ABSTRACT

The members of the cyprinid subfamily Gobioninae, commonly called gudgeons, form one of the most well-established assemblages in the family Cyprinidae. The subfamily is a species-rich group of fishes, these fishes display diverse life histories, appearances, and behavior. The phylogenetic relationships of Gobioninae are examined using sequence data from four loci: cytochrome *b*, cytochrome *c* oxidase I, opsin, and recombination activating gene 1. This investigation produced a data matrix of 4114 bp for 162 taxa that was analyzed using parsimony, maximum likelihood, and Bayesian inference methods. The phylogenies our analyses recovered corroborate recent studies on the group. The subfamily Gobioninae is monophyletic and composed of three major lineages. We find evidence for a *Hemibarbus–Squalidus* group, and the tribes Gobionini and Sarcocheilichthyini, with the *Hemibarbus–Squalidus* group sister to a clade of Gobionini–Sarcocheilichthyini. The *Hemibarbus–Squalidus* group includes those two genera; the tribe Sarcocheilichthyini includes *Coreius*, *Coreoleuciscus*, *Gnathopogon*, *Gobiocypris*, *Ladislavia*, *Paracanthobrama*, *Pseudorasbora*, *Pseudopungtungia*, *Pungtungia*, *Rhinogobio*, and *Sarcocheilichthys*; the tribe Gobionini includes *Abbottina*, *Biwia*, *Gobio*, *Gobiobotia*, *Huigobio*, *Microphysogobio*, *Platysmacheilus*, *Pseudogobio*, *Romanogobio*, *Saurogobio*, and *Xenophysogobio*. The monotypic *Acanthogobio* is placed into the synonymy of *Gobio*. We tentatively assign *Belligobio* to the *Hemibarbus–Squalidus* group and *Mesogobio* to Gobionini; *Paraleucogobio* and *Parasqualidus* remain *incertae sedis*. Based on the topologies presented, the evolution of swim bladder specializations, a distinctive feature among cyprinids, has occurred more than once within the subfamily.

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1. Introduction

Fishes of the cyprinid subfamily Gobioninae (Teleostei: Ostariophysi: Cypriniformes), commonly called gudgeons, are distributed across Europe and Asia, displaying a Palearctic distribution. Only two genera (*Gobio* and *Romanogobio*) occur natively in Europe, with the remaining genera concentrated in Asia, mostly in China, Japan, and Korea (Bănărescu and Coad, 1991; Howes, 1991; Eschmeyer, 2010). However, some species have been introduced elsewhere: *Pseudorasbora parva*, a native of eastern Asia,

has established itself as an invasive pest species in many parts of Europe and Central Eurasia (e.g. Gozlan et al., 2002; Ekmekçi and Kirankaya, 2006; Pollux and Korosi, 2006; Britton et al., 2009) and it has even been reported from north Africa (Perdices and Doadrio, 1992). The subfamily includes 29 genera (Rainboth, 1991; Bănărescu, 1992; Nelson, 2006; Yang et al., 2006; Eschmeyer, 2010; Liu et al., 2010), with approximately 200 species (Eschmeyer, 2010). Fishes of this subfamily are generally small- to medium-sized (<200 mm SL) though species of some genera (e.g. *Hemibarbus*, *Sarcocheilichthys*) can grow to larger sizes (Froese and Pauly, 2010). These fishes are predominantly freshwater, with a few species that enter into brackish environments. They specialize on a diet of aquatic invertebrates or vegetation

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(Bănărescu and Nalbant, 1973). Gudgeons display a variety of life histories, some like *Abbottina* and *Biwia* are found in turbid, stagnant or low flow waters, whereas others like *Gnathopogon caeruleus* are pelagic and lacustrine (Bănărescu and Nalbant, 1973; Hosoya, 1986). However, most gobiionines are benthic, occurring over sand/cobble bottoms, displaying modifications associated with their benthic lifestyle (e.g. inferior mouths with barbels, papillae on the lips and chin, reduction/modification of the swim bladder, modified fins), with some found in fast flowing water (Bănărescu and Nalbant, 1973; Bănărescu and Coad, 1991). Because of their diversity in ecology and habitat, gudgeons show a wide range of body shapes; some are slender and elongate, specialized for a rheophilic lifestyle (e.g. *Gobiobotia*, *Saurogobio*), whereas others are deeper bodied with a more generalized minnow form (e.g. *Gnathopogon*, *Sarcocheilichthys*). Their reproductive biology is also variable, most gobiionines lay their eggs on aquatic vegetation or the substrate; some have pelagic eggs (e.g. *Gnathopogon strigatus*, *Saurogobio dabryi*); others (e.g. most *Sarcocheilichthys* spp.) lay their eggs inside freshwater mussels using specialized ovipositors (Nikolskii, 1954; Bănărescu and Nalbant, 1973; Bănărescu and Coad, 1991). Some species display parental care through nest building and/or egg-guarding behavior (e.g. *Abbottina rivularis*, *P. parva*; Nikolskii, 1954).

1.1. Taxonomic history

The subfamily Gobioninae has been one of the more stable and well-established within Cyprinidae. The subfamily was first erected by Bleeker (1863), who proposed the name Gobiones for a subgroup of Leuciscini, itself a subfamilial group of unspecified rank within his family Cyprinoidei. His Gobiones included two genera, *Gobio* and *Sarcocheilichthys*. The terms “Gobioni” and “Gobiones” do appear earlier in Bonaparte (1839, 1845), but in the sense of a genus-group name in plural form and not as a suprageneric taxon (Art. 11.7.1.2; ICZN, 1999). Günther (1868) did not follow Bleeker's (1863) classification for Gobiones, instead placing *Gobio* and *Pseudogobio* (with *Sarcocheilichthys* as a junior synonym) in his Cyprina, a subgroup of his family Cyprinidae. Jordan and Fowler (1903) recognized the group as Gobioninae, providing a standardized family-group suffix, and included *Leucogobio* [= *Gnathopogon*], *Pseudogobio*, *Sarcocheilichthys*, *Abbottina*, and *Zezer* [= *Pungtungia*] in the subfamily. Several genera which are currently classified as members of Gobioninae were also mentioned, but were treated as members of other subfamilies: *Gnathopogon* in Rhodeinae; *Hemibarbus* in Barbinae; *Biwia*, *Pseudorasbora*, and *Otakia* [= *Gnathopogon*] in Leuciscinae (Jordan and Fowler, 1903). Jordan and Hubbs (1925) later synonymized *Zezer* with *Pungtungia*.

Rendahl (1928) recognized a subfamily Gobioninae that included *Gobio*, *Paraleucogobio*, *Coripareius* [= *Coreius*], *Pseudogobio*, *Rhinogobio*, *Saurogobio*, *Chilogobio* [= *Sarcocheilichthys*], *Agenigobio* [= *Ochetobius*], *Sarcocheilichthys*, *Gobiobotia*, and *Pseudorasbora*. Nichols (1930) postulated that gudgeons made for a “convenient subfamily” but did not refer to them as Gobioninae until later (Nichols, 1938), when he classified them as one of eight subfamilies in Cyprinidae, with *Gnathopogon*, *Gobio*, *Pseudogobio*, and *Saurogobio*, among others as its constituent taxa (not all gobiionine genera were listed). One genus, *Gobiobotia*, puzzled Nichols, who noted that it combined features of both gudgeons and loaches (Nichols, 1930) and he tentatively placed them with the loaches in the cobitid subfamily Homalopterinae [= Balitoridae] (Nichols, 1938). Tchang (1931) converged on a similar classification for these species, though he referred to them as Gobionina, a subgroup of his subfamily Cyprinines. His Gobionina included *Gobio*, *Pseudogobio*, *Sarcocheilichthys*, *Pseudorasbora*, *Coreius*, *Gnathopogon*, *Megagobio* [= *Rhinogobio*], *Rhinogobio*, and *Saurogobio*. Tchang also reached a similar conclusion as Nichols regarding *Gobiobotia*, placing it with

the loaches, although Tchang treated loaches as a cyprinid subfamily, Cobitidines.

The history of *Gobiobotia* has been uneven, with the enigmatic genus often placed in a separate subfamily or family of its own. Mori (1933) first proposed Gobiobotinae [= Gobiobotiinae] as a separate subfamily of Cyprinidae for *Gobiobotia* and its allies (*Saurogobio* and *Microphysogobio*) on the basis of an encapsulated swim bladder, a feature that is also present in some loaches (Ramaswami, 1955). Mori (1934) maintained Gobiobotiinae as a cyprinid subfamily and also recognized a tribe Gobionini within the subfamily Cyprininae which included *Paraleucogobio*, *Gobio*, *Gnathopogon*, *Pseudogobio*, and *Pseudorasbora*; *Hemibarbus* was placed in the Barbini. Liu (1940) subsequently recognized Gobiobotiinae at the rank of family as Gobiobotidae [= Gobiobotiidae]. Other workers (e.g. Berg, 1940; Kryzhanovsky, 1947) followed Mori's recognition of this group as distinct from Gobioninae, though not always at the family rank *sensu* Liu (1940). Some continued to recognize *Gobiobotia* as distinct from other gudgeons but most later workers placed *Gobiobotia* in Gobioninae.

In Lin's (1933, 1934) studies of Chinese cyprinids, he placed *Fustis* [= *Luciocyprinus*], *Gobiobotia*, *Discogobio*, *Ptychidio*, *Pseudorasbora*, *Sarcocheilichthys*, *Chilogobio* [= *Sarcocheilichthys*], *Gobio*, *Paraleucogobio*, *Coreius*, *Pseudogobio*, *Rhinogobio*, and *Saurogobio* in Gobioninae. In his study of Chinese cyprinids, Chu (1935) ascribed *Hemibarbus* and *Paracanthobrama* to Gobioninae. He recognized Gobioninae as a cyprinid subfamily comprising *Gobio*, *Abbottina*, *Pseudogobio*, *Saurogobio*, *Coreius*, *Rhinogobio*, *Pseudorasbora*, *Sinigobio* [= *Squalidus*], *Gnathopogon*, *Paraleucogobio*, *Chilogobio*, *Sarcocheilichthys*, *Hemibarbus*, and *Paracanthobrama*. Chu identified *Ptychidio* as a member of Cyprininae, not Gobioninae. Based on his observations, he noted striking similarities in key scale characters of Gobioninae and Acheilognathinae. Chu's examination of gobiionine pharyngeal arches revealed the prevalence of two tooth rows in most gobiionines, with four genera (*Abbottina*, *Saurogobio*, *Coreius*, and *Pseudorasbora*) having what he described as a more derived condition, which was the reduction in tooth rows to only a single row. Only *Hemibarbus* was observed to retain the primitive condition of three tooth rows seen in other cyprinids, a condition that has been cited as a reason for aligning *Hemibarbus* with cyprinines (e.g. Nikolskii, 1954; Bănărescu and Nalbant, 1965). However, Chu commented that the outermost (third) tooth row was occupied by only a single tooth which was greatly reduced and weak. Mori (1935) described two new genera, *Coreoleuciscus* and *Pseudopungtungia*, which he placed in Cyprininae. *Coreoleuciscus* was associated with *Leuciscus* with no mention of any link to gudgeon species. *Pseudopungtungia* was described as being closely related to *Pungtungia*, presumably placing *Pseudopungtungia* in the cyprinine tribe Gobionini where *Pungtungia* had been classified earlier (Mori, 1934), though this was not explicitly stated.

Liu (1940) examined the structure of the air bladder in 13 genera and 16 species of gobiionine fishes: *Abbottina fukiensis* [= *Microphysogobio fukiensis*], *A. obtusirostris*, *Chilogobio nigripinnis* [= *Sarcocheilichthys nigripinnis*], *Coreius cetopsis*, *C. zeni* [= *C. guichenoti*], *Discogobio tetrabarbus*, *Fustis vivus* [= *Luciocyprinus langsoni*], *Gobio wolterstorffi* [= *S. wolterstorffi*], *Gobiobotia abbreviata*, *Hemibarbus maculatus*, *Leucogobio taeniatus* [= *Gnathopogon imberbis*], *P. parva*, *Rhinogobio typus*, *Rhinogobio ventralis*, *Sarcocheilichthys sinensis*, and *S. dabryi*. The presence of a reduced and encapsulated swim bladder was noted as a distinguishing feature of several genera of putative gobiionines (*Rhinogobio*, *Coreius* [in part], *Discogobio*, *Abbottina*, *Gobiobotia*, and *Saurogobio*). Although this condition is also found in Cobitidae, Liu (1940) did not believe that these taxa belonged in that family because they lacked a number of cobitid features. For this reason, Liu (1940) recognized Mori's (1933) subfamily Gobiobotiinae as a separate family, Gobiobotiidae, placing it intermediate between Cyprinidae and Cobitidae.

Liu stated that membership in this newly elevated family would be for any cyprinid fish with an encased air bladder, whether bony and/or membranous in nature.

Kryzhanovsky (1947) divided his subfamily Gobionini into three groups: Gobionina, Sarcocheilichthyna, and Armatogobionina. His Sarcocheilichthyna included only *Sarcocheilichthys*, whereas Gobionina and Armatogobionina were each subdivided into two subgroups (Gobionina: Gobioninae and Pseudogobioninae; Armatogobionina: Armatogobioninae and Gobiobotiinae). His Gobioninae consisted of *Hemibarbus* and *Gobio*, his Pseudogobioninae included only *Pseudogobio*. *Gobiobotia* comprised Gobiobotiinae, *Armatogobio* [= *Saurogobio*] and *Rostrogobio* [= *Microphysogobio*] formed Armatogobioninae. Nikolskii (1954) did not recognize all of the subdivisions of Kryzhanovsky (1947) but did include *Gobiobotia* and *Saurogobio* as members of the subfamily Gobioninae. *Hemibarbus* was treated as a member of the subfamily Barbiinae, with a comment noting its intermediate position between the barbels and the gudgeons.

Ramaswami (1955) examined 12 gobionine genera (*Gobio*, *Gobiobotia*, *Abbottina*, *Saurogobio*, *Pseudorasbora*, *Chilogobio* [= *Sarcocheilichthys*], *Hemibarbus*, *Sarcocheilichthys*, *Pseudogobio*, *Gnathopogon*, *Leucogobio*, and *Coreius*) and posited that there were no more than 14–15 genera total. Based on observations of their skeletal features, Ramaswami felt that the subfamily Gobioninae could be divided into two groups, one of which included *Saurogobio*, *Pseudogobio*, and *Abbottina*, the other comprising the remainder of the taxa (mostly for convenience), with *Gobiobotia* forming a third group by itself. He identified one character which differentiated *Gobiobotia*, *Saurogobio*, *Pseudogobio*, and *Abbottina* from all other members of Gobioninae: occipital canal passes through the supraoccipital in addition to the parietal (canal passes through parietal only in other gobionines). This character is cited as one of the reasons Ramaswami included *Gobiobotia* in Gobioninae; another was that *Gobiobotia* lacks the distinguishing characters of Cobitidae, ruling it out as a loach, though its swim bladder is similar to that of nemacheiline loaches, which he speculated was a result of convergence. He also noted that although both *Gobiobotia* and *Saurogobio* have a bony capsule enclosing their gas bladders, the capsules actually differed in the details of their structure.

Bănărescu and Nalbant (1965) produced a major revision of the subfamily, in which they recognized Gobioninae as a valid subfamily with the following genera: *Pseudorasbora*, *Pungtungia*, *Coreoleuciscus*, *Ladislavia*, *Sarcocheilichthys*, *Pseudopungtungia*, *Gnathopogon*, *Squalidus*, *Gobio*, *Rhinogobio*, *Acanthogobio*, *Coreius*, *Gobiobotia*, *Pseudogobio*, *Abbottina*, *Biwia*, *Microphysogobio*, and *Saurogobio*. They removed *Hemibarbus* from Gobioninae, placing it in Cyprininae. Bănărescu and Nalbant (1965) noted that although both *Hemibarbus* and *Acanthogobio* possess a spinous dorsal ray, it is the third dorsal ray that is ossified in *Hemibarbus* whereas it is the second dorsal ray in *Acanthogobio*, and therefore it is not an indicator of close relationship. On the basis of its upwardly directed mouth and small scales, they also removed *Fustis* [= *Luciocyprinus*] to the subfamily Danioninae (a group which had variably been called the Danioinae, Bariliinae, or Rasborinae). According to Chen et al. (1984) and Cui and Chu (1986), *Luciocyprinus* is a member of Barbiinae, whereas Rainboth (1991) considered it a member of Oreini, both groups that are generally subsumed in the large subfamily Cyprininae. Bănărescu and Nalbant included *Coreoleuciscus* in Gobioninae, removing it from Leuciscinae where, according to them, it had been placed by Mori (1935); Mori actually classified it in Cyprininae, remarking that it was most closely allied with *Leuciscus*. Mori (1935) also described the genus *Pseudopungtungia* in the subfamily Cyprininae and, as the name suggests, placed it near *Pungtungia*, which was a member of the cyprinine tribe Gobionini (Mori, 1934). Bănărescu and Nalbant classified *Pseudopungtungia* in their Gobioninae. Bănărescu and Nalbant disagreed with Lin

(1933) on the placement of *Ptychidio* and *Discogobio*, removing both genera from Gobioninae to Cyprininae, hypothesizing that they are closely related to *Garra*. This affiliation with *Garra* and/or other labeonins within the subfamily Cyprininae has been corroborated by several recent molecular studies (Kong et al., 2007; Wang et al., 2007; Li et al., 2008; Tang et al., 2009; Yang and Mayden, 2010; Zheng et al., 2010). Bănărescu and Nalbant reported the presence of an encapsulated air bladder in *Microphysogobio*, a genus not examined by Ramaswami (1955), which they thought was more closely related to *Pseudogobio* and *Abbottina*, genera with free swim bladders, than *Saurogobio*, a genus with an encapsulated swim bladder. They also reported an encapsulated air bladder in *Rhinogobio* and one species of *Coreius* (*C. guichenoti*). Bănărescu and Nalbant observed that encapsulation and reduction of the gas bladder was a specialization that had occurred independently multiple times in the subfamily.

Within Gobioninae, Bănărescu and Nalbant (1965) recognized several “phyletic series” which they felt represented natural groups but stopped short of formally recognizing them as tribes. In their grouping scheme, *Pseudorasbora* and *Pungtungia* represented a “primitive” series, united by a number of shared characters, with *Pseudorasbora elongata*, which lacks barbels and possesses a superior mouth as in *Pseudorasbora*, but has the body shape and single longitudinal stripe of *Pungtungia*, as an intermediate form between the two genera. *Ladislavia*, *Sarcocheilichthys*, and *Pseudopungtungia* represented another series, with *Ladislavia* as the intermediate taxon between the other two, dissimilar genera. *Coreoleuciscus* appeared to them to be an independent offshoot that is isolated within the subfamily. They posited that *Gnathopogon*, *Squalidus*, *Gobio*, *Rhinogobio*, and *Acanthogobio* formed a natural group, representing a phyletic series grading from most primitive (*Gnathopogon*) to most specialized (*Rhinogobio* and *Acanthogobio*). Among those genera, *Rhinogobio* is the only one with an encapsulated swim bladder. They separated *Coreius* into its own series and considered it to be an aberrant genus because of features like small eyes, long barbels, smooth lips, and molariform teeth; the condition of the swim bladder also varied, with some (e.g. *C. heterodon*) possessing a free swim bladder and others (e.g. *C. guichenoti*) possessing an encapsulated one. Possible ties to *Rhinogobio* and *Acanthogobio* were mentioned, which they stated would point to *Coreius* being a specialized member of the *Gobio* group. *Gobiobotia* was deemed another unique offshoot within the gobionines due to its various specializations (e.g. number of barbels), isolated from the remainder of the subfamily, although with some similarities to *Gobio*, but they acknowledged the possibility that those similarities were the result of convergence. Finally, their last phyletic series included the remaining five genera: *Pseudogobio*, *Abbottina*, *Saurogobio*, *Microphysogobio* (including *Huigobio*), and *Biwia*. The members of this group share several osteological characters (e.g. reduced or absent supraorbitals). The swim bladder is large and free in the “three more primitive genera” (i.e. *Abbottina*, *Biwia*, and *Pseudogobio*), whereas it is reduced and the anterior chamber is encapsulated by either a fibrous (*Microphysogobio*) or bony (*Saurogobio*) capsule in the other two genera.

Bănărescu and Nalbant (1973) expanded on their previous work (Bănărescu and Nalbant, 1965). The most notable change was the return of *Hemibarbus* to Gobioninae, a reversal of their earlier decision to move it to Cyprininae. However, they still expressed some doubt about the status of not only *Hemibarbus* but also *Coreoleuciscus* among the gobionines, *Coreoleuciscus* because of its earlier placement with Leuciscinae (Mori, 1935), and *Hemibarbus* because of its three rows of pharyngeal teeth (two or fewer in other gobionines) and ossified last dorsal ray, features which originally prompted them to remove *Hemibarbus* from Gobioninae (Bănărescu and Nalbant, 1965). Their classification recognized 20 genera with 84 species, divided amongst eight groups, with an organization

similar to that of Bănărescu and Nalbant (1965). *Hemibarbus* was now placed in its own phyletic group, with mention of the possibility that it is an offshoot of *Gobio*. *Pseudogobio*, *Abbottina*, *Saurogobio*, *Microphysogobio*, and *Biwia* were again recognized as a natural group. Among these five genera, *Pseudogobio* and *Abbottina* were understood to be the most primitive, members of both possessing free swim bladders; *Biwia*, also with a free swim bladder, was considered close to *Abbottina*; the remaining two genera have encapsulated swim bladders, *Microphysogobio* is hypothesized to have arisen from either *Pseudogobio* or the *Abbottina-Biwia* group, with certain shared similarities to *Biwia*; no relationships were suggested for *Saurogobio*, only a statement on its status as the most derived genus within this group. *Belligobio* and *Paracanthobrama* were treated as subgenera of *Hemibarbus*; *Rheogobio* [= *Romanogobio*] and *Romanogobio* were classified as subgenera of *Gobio*. Bănărescu and Nalbant (1973) described a new genus, *Mesogobio*, said to be intermediate between *Gobio*, *Gobiobotia*, and the *Pseudogobio* group, sharing similarities with each. They speculated that it was derived from *Gobio* or something *Gobio*-like, and its ancestor may have been close to *Gobiobotia*, with any similarities between *Mesogobio* and *Microphysogobio* arising from convergent evolution. Bănărescu and Nalbant also discussed the evolution of Gobioninae, describing what they saw as clear evolutionary trends within the subfamily: reduction of tooth rows from two to one; modification of body shape from compressed to cylindrical, with accompanying changes in shape of ventral surface and position of pectoral fins; reduction and encapsulation of swim bladder; modification of lips and jaws, with development of papillae on lips, mental barbels, and/or horny sheath on jaws; shift of mouth from terminal to inferior position; advancement of dorsal fin and vent. With the exception of the first change listed, these were all features associated with a rheophilic/benthic lifestyle, a lifestyle displayed by what Bănărescu and Nalbant considered to be the most specialized gudgeons (*Microphysogobio*, *Saurogobio*, and *Gobiobotia*). They were unsure of how the subfamily was related to other cyprinids. The intermediate condition of *Hemibarbus* suggested a relationship with Barbinae [=Cyprininae], but they were uncertain if *Hemibarbus* was representative of a basal gobionine lineage because the gudgeons most similar in appearance to *Hemibarbus* (*Gobio* and *Acanthogobio*) appeared to be relatively derived. Turning their attention to more generalized gobionines (e.g. *Gnathopogon*), Bănărescu and Nalbant noted that the gross morphology and overall appearance of these less specialized members of the subfamily indicated a connection with either Danioninae or Leuciscinae.

Arai (1982) proposed a classification of Cyprinidae based on chromosome number and morphological characters. Arai (1982) followed Günther (1868) as a general guideline, stating that Gobioninae represented genera 30–43 of Günther's Cyprinina, which would have included *Aulopyge*, *Gobio*, *Pseudogobio*, *Ceratichtys* [= *Nocomis*], *Bungia* [= *Gobio*], *Pimephales*, *Hyborhynchus* [= *Pimephales*], *Campostoma*, *Hybognathus*, *Ericymba*, *Pseudorasbora*, *Cochlognathus* [= *Pimephales*], *Exoglossum*, and *Rhinichthys*. Of the ones with available chromosome data, Arai (1982) placed *Aulopyge*, *Gnathopogon*, *Gobio*, *Hemibarbus*, *Pseudogobio*, *Pseudorasbora*, *Pungtungia*, and *Sarcocheilichthys* in his Gobioninae, and recognized Gobiobotiinae with *Gobiobotia* and *Microphysogobio*. Arai noted that the monotypic *Aulopyge* differs from other gobionines in several key features and speculated that this variation may be the result of polyploidy in *Aulopyge* ($2n = 100$ versus $2n = 50$ or 52 for other gobionines); he remarks that *Aulopyge* exhibits a mosaic of barbinae and gobionine characters. These irregularities were explained a few years later when Howes (1987) classified *Aulopyge huegelii* as a member of Cyprininae. During his studies of *Barbus* and other cyprinine fishes, Howes found no evidence supporting the assignment of *Aulopyge* to the subfamily Gobioninae. Based on his observations, Howes hypothesized that *Aulopyge*

is most closely related to *Barbus*, though their exact relationships remained unclear to him. Howes (1987: Fig. 20; 190–192) proposed two alternate hypotheses that placed *Aulopyge* either as a member of *Barbus sensu stricto* (Eurasian *Barbus* spp.) or as the sister group of *Barbus sensu lato* (Eurasian plus African *Barbus* spp.). Recent molecular studies (e.g. Tsigenopoulos and Berrebi, 2000; Machordom and Doadrio, 2001; Tsigenopoulos et al., 2003) have provided additional evidence corroborating Howes (1987). Arai (1982) ascribed Gobiobotia and *Microphysogobio* to a distinct subfamily Gobiobotiinae, but he did observe that the diploid chromosome number of both genera matched that found in gobionines, prompting him to suggest that gobiobotiines were derived from gobiiniines.

Hosoya (1986) made a major advance in the study of gobionine relationships by producing the first phylogeny of the subfamily based on a cladistic approach, identifying three synapomorphies uniting the subfamily Gobioninae, which he restricted to only 12 genera: *Hemibarbus*, *Squalidus*, *Gobio*, *Mesogobio*, *Acanthogobio*, *Gobiobotia*, *Pseudogobio*, *Abbottina*, *Saurogobio*, *Rhinogobio*, *Microphysogobio*, and *Biwia*. The other eight genera from earlier classifications (*Gnathopogon*, *Pseudorasbora*, *Pungtungia*, *Pseudopungtungia*, *Coreoleuciscus*, *Sarcocheilichthys*, *Ladislavia*, and *Coreius*; Bănărescu and Nalbant, 1973) were excluded from the subfamily, because he was unable to find synapomorphies linking these taxa to his Gobioninae *sensu stricto*. Hosoya noted that traditional diagnostic characters used to identify Gobioninae (e.g. short anal fin with six branched soft rays) were not apomorphic based on his results. Within Gobioninae, Hosoya (1986) recovered two main lineages, one composed of *Hemibarbus*, *Squalidus*, *Gobio*, and *Mesogobio*, the other of *Gobiobotia*, *Pseudogobio*, *Saurogobio*, *Microphysogobio*, and *Biwia*, which he called "true bottom dwellers." His findings also reinforced earlier studies (e.g. Taranetz, 1938; Kryzhanovsky, 1947; Nikolskii, 1954; Ramaswami, 1955; Bănărescu and Nalbant, 1965) that aligned *Gobiobotia* with the subfamily Gobioninae and rejected the recognition of a distinct subfamily or family as proposed by Mori (1933, 1934) and Liu (1940). His topology did recover a clade that included *Gobiobotia*, *Saurogobio*, and *Microphysogobio*, which matches Mori's (1933) Gobiobotiinae, but that clade, which he called the "second phyletic line," also included *Abbottina*, *Biwia*, and *Pseudogobio* and was part of his Gobioninae *sensu stricto* (Hosoya, 1986: fig. 14). For higher level relationships, Hosoya questioned some of Chen et al.'s (1984) interpretations of synapomorphies for Acheilognathinae and therefore did not accept their hypothesis of that subfamily as the sister group to Gobioninae, instead Hosoya agreed with the results presented by Arai (1982), which united Cyprininae with Gobioninae.

Rainboth (1991) attempted a compromise between the classifications of Bănărescu and Nalbant (1973) and Hosoya (1986). Rainboth divided Gobioninae into two tribes: Gobionini and Sarcocheilichthyini. Rainboth ascribed 12 genera to the tribe Gobionini: *Abbottina*, *Acanthogobio*, *Belligobio*, *Gobio*, *Gobiobotia*, *Hemibarbus*, *Microphysogobio*, *Paracanthobrama*, *Pseudogobio*, *Rhinogobio*, *Saurogobio*, and *Squalidus*. This tribe was intended to reflect the revised Gobioninae *sensu* Hosoya (1986) as these 12 genera largely match Hosoya's Gobioninae (Rainboth recognized *Belligobio* and *Paracanthobrama* instead of *Biwia* and *Mesogobio* of Hosoya). Sarcocheilichthyini included *Coreius*, *Gnathopogon*, *Pseudorasbora*, and *Sarcocheilichthys*, four genera Hosoya (1986) had removed from the subfamily. Rainboth (1991), noting Hosoya's (1986) results and the lack of synapomorphies uniting this group to Gobionini, acknowledged that Sarcocheilichthyini may not be monophyletic.

In Bănărescu (1992), the Gobioninae included the same 20 genera as in Bănărescu and Nalbant (1973): *Pseudorasbora*, *Coreoleuciscus*, *Pungtungia*, *Pseudopungtungia*, *Ladislavia*, *Sarcocheilichthys*, *Gnathopogon*, *Coreius*, *Hemibarbus* (subgenera *Belligobio* and

Paracanthobrama), *Squalidus*, *Gobio* (subgenera *Rheogobio* and *Romanogobio*), *Mesogobio*, *Acanthogobio*, *Rhinogobio*, *Gobiobotia*, *Pseudogobio*, *Abbottina*, *Saurogobio*, *Biwia*, and *Microphysogobio* (subgenera *Huigobio*, *Platysmacheilus*, and *Rostrigobio*). However, Bănărescu acknowledged the limitations of such a classification in light of the phylogeny presented by Hosoya (1986). He accepted that Hosoya's revised Gobioninae probably represented a monophyletic group, but maintained Gobioninae *sensu lato* because he concluded that the excluded taxa were most likely more closely related to Gobioninae *sensu* Hosoya (1986) than any other cyprinid subfamily, citing unpublished karyotype data linking *Pseudorasbora* to *Gobio* (Bănărescu, 1992: 308). Bănărescu divided up the subfamily into four groups: an aberrant group with terminal mouths that included *Pseudorasbora*, *Coreoleuciscus*, *Pungtungia*, *Pseudopungtungia*, *Ladislavia*, *Sarcocheilichthys*, and *Gnathopogon*; another aberrant group for *Coreius* only; a *Hemibarbus*–*Gobio* group, which also included *Squalidus*, *Mesogobio*, *Acanthogobio*, and *Rhinogobio*; a *Gobiobotia*–*Pseudogobio* group, which also included *Abbottina*, *Saurogobio*, *Biwia*, and *Microphysogobio*. Bănărescu did not consider *Paraleucogobio* to be monophyletic and therefore did not recognize it as a valid genus.

Naseka (1996) devised a classification system based on variation in the vertebral column of gobionines, which did not agree with either Hosoya (1986) or Rainboth (1991). His Gobioninae included the genera excluded by Hosoya (1986) but did not find them grouped in the two tribes of Rainboth (1991). He divided Gobioninae into two main assemblages, each further divided into two subgroups. The four genera displaying the primitive vertebral condition (*Pseudorasbora*, *Gnathopogon*, *Pungtungia*, and *Pseudopungtungia*) plus another ten genera (*Coreoleuciscus*, *Sarcocheilichthys*, *Ladislavia*, *Acanthogobio*, *Coreius*, *Abbottina*, *Hemibarbus*, *Gobio*, *Megagobio*, *Mesogobio*) formed the first assemblage. Within this assemblage, Naseka grouped all but *Megagobio* and *Mesogobio* together into one group, with *Megagobio* and *Mesogobio* forming the second group of the first assemblage. In doing so, Naseka formally recognized *Megagobio* as a genus distinct from *Rhinogobio*, a decision later reversed by Bănărescu (1997) and Yue et al. (1998). The remaining genera (*Romanogobio*, *Biwia*, *Pseudogobio*, *Microphysogobio*, *Gobiobotia*, *Rhinogobio*, *Saurogobio*) were placed into the second assemblage. The first group of this second assemblage consisted of *Biwia*, *Microphysogobio*, *Pseudogobio*, *Gobiobotia*, *Romanogobio*, with only *Rhinogobio* and *Saurogobio* forming the second group. This classification elevated *Romanogobio*, previously a subgenus of *Gobio*, to generic status. Although he examined some species of *Squalidus*, limited material examined caused Naseka to omit it from his classification of the subfamily. Böhme (2007) split Gobioninae into two tribes, *Sarcocheilichthyini* and *Gobionini*, though *Sarcocheilichthyini* was not monophyletic, instead appearing as a paraphyletic grade leading up to a monophyletic *Gobionini*.

1.2. Molecular systematics

With the rise of molecular techniques, a number of studies produced phylogenies which could address the issue of gobionine monophyly and relationships. However, many only dealt with Gobioninae in a limited, tangential manner because these studies were not focused solely on Gobioninae and/or due to restricted taxon sampling. Among the first were Briolay et al. (1998), who used cytochrome *b* sequences to infer cyprinid relationships. Their taxon sampling was not extensive, but they did resolve Gobioninae as a distinct group when the two gobionines they examined (*Gobio gobio* and *P. parva*) were recovered together. However, they were unaware of this conclusion because they acknowledged only one representative of Gobioninae for their study, recognizing *Pseudorasbora* as a member of Rasborinae [=Danioninae], likely following Howes (1991) who included it in his Rasborinae. Their *Gobio*–*Pseu-*

dorasbora clade (i.e. Gobioninae) was recovered with Leuciscinae *sensu lato*. Using cytochrome *b* data, Zardoya and Doadrio (1999) and Zardoya et al. (1999) investigated European cyprinid relationships and their gobionine representatives, *Gobio* spp. and *P. parva*, formed a distinct group but their nearest relatives remained unresolved. Gilles et al. (2001) only included *Gobio* in their analyses, which they consistently recovered as the sister group to Leuciscinae. Cunha et al. (2002) recovered *Gobio* and *Pseudorasbora* together but not all members of Gobioninae formed a group, because *Abbottina* and *Gobiobotia* were recovered elsewhere. Based on the studies that have come since, these results are somewhat odd. Cunha et al. (2002) found *Abbottina* with *Sinocyclocheilus*, nested within Cyprininae, and they found *Gobiobotia* with *Raiamas guttatus*, a member of Danioninae. This *Gobiobotia*–*Raiamas* group was found with *Xenocypris* (in part), within a group that included members of the subfamilies Acheilognathinae and Xenocyprininae. Rüber et al. (2007) used two gobionine sequences from Cunha et al. (2002) for their analyses, *A. rivularis* was recovered within Gobioninae in the newer analysis, whereas *G. abbreviata* (*G. ichangensis* and *G. longibarba* not included) was again found with *Xenocypris*. Rüber et al. (2007) recovered a different species of *Gobiobotia* (*Gobiobotia meridionalis*; not from Cunha et al., 2002) as part of Gobioninae. With the exception of the aberrant *G. abbreviata* sequence from Cunha et al. (2002), Rüber et al. (2007) were able to resolve a monophyletic Gobioninae, but could not determine the gobionine sister group.

Subsequent papers touched on the Gobioninae in similar ways, with the inclusion of some representative taxa but not with Gobioninae as the primary focus of the study. In almost all cases, the members of Gobioninae were recovered as monophyletic, but there was little consensus on the identity of its sister group. The subfamily Acheilognathinae (bitterlings) was the most common result (Chen et al., 2008; Mayden et al., 2008; Yang and Mayden, 2010; Tang et al., 2010). Liu and Chen (2003) found a monophyletic Gobioninae in two of the three topologies they presented and in those two cases where its monophyly was resolved, the subfamily Leuciscinae was determined to be the sister group. He et al. (2004) found Gobioninae (including *Gobiobotiinae*) to be the sister group of all other cyprinids, minus Cyprininae and Danioninae. Saitoh et al. (2006) recovered a Tincinae–Leuciscinae clade as the sister taxon. Wang et al. (2007) found a *Tanichthys*–Acheilognathinae clade to be sister to Gobioninae; their recovery of *Discogobio* and *Ptychidio* nested within the tribe Labeonini supported the removal of these two genera from Gobioninae and their placement in Cyprininae by previous workers (Chu, 1935; Bănărescu and Nalbant, 1965). He et al. (2008) did not find much resolution within Cyprinidae which extended to an inability to identify the gobionine sister group, but they did recover a monophyletic Gobioninae that included *Gobiobotiinae*. Li et al. (2008) found *Gobio* and *Gobiobotia* sister to Leuciscinae. Mayden et al. (2009) presented several different topologies, almost all of which found Gobioninae to be monophyletic, but with the sister group varying between Acheilognathinae and Leuciscinae.

Amid this surge in molecular phylogenies, Yang et al. (2006) produced the first molecular phylogeny focused on the relationships within Gobioninae. Utilizing the ubiquitous cytochrome *b* gene, they reconstructed the phylogeny of the subfamily based on 49 species representing 24 of the 29 gobionine genera, lacking *Coreoleuciscus*, *Ladislavia*, *Paraleucogobio* (their representative of this genus, *Paraleucogobio strigatus*, is a member of *Gnathopogon*; Kottelat, 2006), *Parasqualidus*, and *Pseudopungtungia*. They also recognized *Rostrigobio* as a distinct genus, which is currently in the synonymy of *Microphysogobio*, as a result of the assignment of its type species, *R. amurensis*, to *Microphysogobio* (Kottelat, 2006; Bogutskaya et al., 2008). Yang et al. (2006) recovered a monophyletic Gobioninae which included *Gobiobotia* and *Xenophysogobio*. They divided the subfamily into four major groups: the *Hemibarbus*

group with *Belligobio*, *Hemibarbus*, and *Squalidus*, sister to the remaining gobionines; the *Sarcocheilichthys* group (not monophyletic in their maximum likelihood tree) with *Coreius*, *Gnathopogon*, *Gobiocypris*, *Paracanthobrama*, *Paraleucogobio* [= *Gnathopogon*], *Pseudorasbora*, *Pungtungia*, *Rhinogobio*, and *Sarcocheilichthys*, sister to the remaining two groups; and the *Gobio* (*Acanthogobio*, *Gobio*, *Mesogobio*, and *Romanogobio*) and *Pseudogobio* (*Biwia*, *Gobiobotia*, *Huigobio*, *Microphysogobio*, *Platysmacheilus*, *Pseudogobio*, *Rostrogobio* [= *Microphysogobio*], *Saurogobio*, and *Xenophysogobio*) groups forming the crown clade. There was disagreement between the parsimony and likelihood topologies over the composition of the last two groups, with *Abbottina* recovered as the sister group of the *Gobio* group in the parsimony tree whereas it is found in the *Pseudogobio* group (sister to *Saurogobio*) in the likelihood tree, thus rendering the *Pseudogobio* group non-monophyletic in the parsimony tree. Yang et al. (2006) stated that their tree supported a closer relationship between Gobioninae and Leuciscinae than with Cyprininae. Although this statement is technically correct, it is difficult to evaluate because their data set included only two cyprinines, which were also the outgroups. Their topology found Gobioninae sister to a large clade that included Leuciscinae as well as members of the subfamilies Acheilognathinae, Cultrinae, and Xenocypridinae. Kim et al. (2009), using whole mitochondrial genome sequences, found relationships among gobionines that were generally similar to those of Yang et al. (2006), albeit with greatly reduced taxon sampling, examining only ten gobionines. Despite the limited taxa, Kim et al. (2009) found an overall framework of relationships that matched what was reported by Yang et al. (2006), where *Hemibarbus* was sister to the remaining gobionines, with a group corresponding to Yang et al.'s (2006) *Sarcocheilichthys* group sister to a clade of corresponding to Yang et al.'s (2006) *Gobio* + *Pseudogobio* groups. More recently, Liu et al. (2010) expanded on Yang et al. (2006), adding their own data and some from Zhang et al. (2008). The Liu et al. (2010) study focused on rates of speciation and evolution within the subfamily, finding gobionine relationships congruent with those reported by Yang et al. (2006), an unsurprising result given the overlap in sequence data.

1.3. Goals

The purpose of this study is to expand on these earlier molecular studies, particularly Yang et al. (2006), using more sequence data to resolve the relationships among the members of the subfamily Gobioninae and to illuminate the sister-group relationships of the subfamily. We present a molecular phylogeny of these fishes and use this phylogeny as a framework for an updated classification of the Gobioninae, one which reflects the relationships within the subfamily. This study is a preliminary assessment of the taxonomic composition of the group, presenting new information on the systematics of Gobioninae as well as highlighting areas of the tree that require further study.

2. Materials and methods

In this study, we examined 162 taxa, consisting of 141 cyprinids (94 putative gobionines and 47 other cyprinids) and 21 non-cyprinid ostariophysan outgroups, representing 93 genera, including 27 putative gobionine genera. We were unable to obtain material from two monotypic gobionine genera, *Paraleucogobio* and *Parasqualidus*. In order to test the limits of the subfamily Gobioninae, we included taxa like *Discogobio* and *Ptychidio*, which have been removed from the subfamily (Bănărescu and Nalbant, 1965), but were classified with Gobioninae in the past (e.g. Lin, 1933; Liu, 1940). Sequences for some gobionine species were obtained from GenBank, mainly from data originally published by Yang et al.

(2006), Liu et al. (2010), and Tang et al. (2010). Sequence data from the recently described *Biwia yodoensis* were published in Kawase and Hosoya (2010). Within Cypriniformes, we selected a representative sampling of the major cyprinid lineages (Acheilognathinae, Alburninae, Cultrinae, Cyprininae, Danioninae, Leptobarbinae, Leuciscinae, Tincinae) and other cypriniform families (Balitoridae, Botiidae, Catostomidae, Cobitidae, Ellopostomatidae, Gyriinocheilidae, Nemacheilidae, Psilorhynchidae, and Vaillantellidae). These were chosen based on availability of sequences through GenBank, primarily published in Saitoh et al. (2006) and Tang et al. (2010). Outgroup taxa included additional ostariophysan diversity, with *Chanos chanos* serving as the root. A full list of taxa examined with corresponding GenBank accession numbers is provided in Table 1. Nomenclature, type information, and synonymies follow Eschmeyer (2010), unless otherwise indicated.

Target loci for sequencing and analysis followed the strategy presented by Tang et al. (2010), focusing on the same same four loci used therein: cytochrome *b* (*cyt b*), cytochrome *c* oxidase I (COI), exon 3 of recombination activating gene 1 (RAG1), and opsin (rhodopsin). This suite of loci proved capable of resolving the relationships within the subfamily Danioninae as well as across the family Cyprinidae (Tang et al., 2010), so it appeared to be well suited to resolve the relationships within another cyprinid subfamily, Gobioninae. Amplification and sequencing used the PCR primers and laboratory protocols detailed in Tang et al. (2010). All novel sequences generated for this study were deposited in GenBank (Table 1). Sequences were concatenated and aligned according to codon positions in a NEXUS file prior to conversion by Mesquite 2.6 (Maddison and Maddison, 2009) into the appropriate file format necessary for each tree search application.

Tree search analyses were performed under maximum likelihood and parsimony optimality criteria and Bayesian inference. Maximum likelihood searches were executed in the parallel version of RAxML 7.2.6 (Stamatakis, 2006) as implemented by CIPRES Portal 2.2 (Miller et al., 2009), for 100 independent searches. The GTR + I + Γ model of nucleotide substitution was applied to the data. The topology with the best likelihood score was retained. Bootstrap values were calculated from 1000 replicates generated by RAxML and GTR + CAT approximation for rapid bootstrapping (Stamatakis et al., 2008). Parsimony searches used search strategies modified from those outlined in Tang et al. (2010) for TNT 1.1 (Goloboff et al., 2008). Tree searches used the “xmult” command with 10 replicates, each with 20 iterations of tree drifting (default settings), sectorial searches (constrained, exclusive, random; default settings), 20 iterations of tree fusing (default settings), and 20 iterations of ratcheting (default settings) implemented. Searches continued until the most-parsimonious tree length was hit independently 20 separate times. The resulting trees were then subjected to another round of tree fusing (50 iterations), followed by tree bisection-reconnection (TBR) branch swapping of all trees retained in memory. All unique, most-parsimonious topologies were then used to calculate the consensus tree. Bootstrap support (Felsenstein, 1985) was calculated from 1000 replicates and Bremer support (Bremer, 1988) was calculated using the “pbsup.run” script written by Peña et al. (2006), available from the TNT website: <http://www.zmuc.dk/public/phylogeny/TNT/scripts/>. Bayesian analyses were executed in the MPI version of MrBayes 3.1.2 (Altekar et al., 2004; Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) by CIPRES. Prior to analysis, the sequence data were partitioned by gene and then by codon position, resulting in 12 partitions. MrModelTest 2.2 (Nylander, 2004) and PAUP* 4.0b10 (Swofford, 2002) were used to conduct hierarchical likelihood ratio tests (hLRT) to determine the best model of nucleotide substitution for each partition. Two independent Bayesian searches were conducted, with 4 chains each. Both searches ran for 10,000,000 generations, sampling every 1000

Table 1

Taxa examined for this study, with GenBank accession numbers. Institutional abbreviations as follows: AMS = Australian Museum; CBM-ZF = Natural History Museum and Institute, Chiba; IHB = Institute of Hydrobiology, Chinese Academy of Sciences; STL = Saint Louis University; UAIC = University of Alabama Ichthyological Collection; USNM = United States National Museum.

| Classification | Taxon | Catalog no. | Source | RAG1 | Rh | Cyt <i>b</i> | COI |
|---|-----------------------------------|-----------------|------------------------------------|----------|----------|--------------|----------|
| Gonorynchiformes | <i>Chanos chanos</i> | N/A | GenBank | AY430207 | FJ197072 | AB054133 | AB054133 |
| | <i>Gonorynchus greyi</i> | AMS-1.33768-001 | New South Wales, Australia | EU409606 | EU409632 | AB054134 | AB054134 |
| Siluriformes | <i>Ictalurus punctatus</i> | N/A | GenBank | DQ492511 | AF028016 | AF482987 | AF482987 |
| Characiformes | <i>Phenacogrammus interruptus</i> | N/A | GenBank | FJ197124 | FJ197073 | AB054129 | AB054129 |
| Cypriniformes Cobitoidea Balitoridae | <i>Homaloptera leonardi</i> | N/A | Moon River, Kaeng Saphue, Thailand | EU711130 | FJ197027 | AB242165 | AB242165 |
| | <i>Sewellia lineolata</i> | CBM-ZF-11315 | Aquarium | HM224068 | EU409635 | AP011292 | AP011292 |
| Botiidae | <i>Chromobotia macracantha</i> | CBM-ZF-11438 | Aquarium | EU711137 | FJ197037 | AB242163 | AB242163 |
| | <i>Parabotia mantschurica</i> | CBM-ZF-11432 | K. Saitoh | EU711138 | FJ197038 | AB242170 | AB242170 |
| Catostomidae Catostominae | <i>Catostomus commersonii</i> | STL 814.04 | Illinois, USA | EU409612 | FJ197032 | AB127394 | AB127394 |
| | <i>Hypentelium nigricans</i> | UAIC 12136.04 | Youghiogheny River, Maryland, USA | EU711134 | FJ197033 | AB242169 | AB242169 |
| Cycleptinae | <i>Cycleptus elongatus</i> | UAIC 12497.21 | Duck River, Tennessee, USA | EU409613 | FJ197035 | AB126082 | AB126082 |
| | <i>Myxocyprinus asiaticus</i> | N/A | Aquarium | EU711136 | FJ197036 | AB223007 | AB223007 |
| Cobitidae | <i>Acantopsis choirorhynchos</i> | STL uncataloged | Aquarium | EU711139 | FJ197039 | AB242161 | AB242161 |
| | <i>Cobitis striata</i> | CBM-ZF-10606 | Lake Biwa, Japan | EF458303 | HM223938 | AB054125 | AB054125 |
| Ellopostomatidae | <i>Ellopostoma mystax</i> | UAIC 14301.02 | Tapi River Basin, Thailand | FJ650417 | FJ650477 | JN003323 | JN003347 |
| Gyrinocheilidae | <i>Gyrinocheilus aymonieri</i> | UAIC 12928.03 | N/A | EU292682 | FJ197071 | AB242164 | AB242164 |
| Nemacheilidae | <i>Barbatula toni</i> | CBM-ZF-11288 | Hokkaido, Japan | EU711133 | FJ197030 | N/A | N/A |
| | <i>Lefua echigonia</i> | N/A | Ussuri River, Russia | N/A | N/A | AB242162 | AB242162 |
| Vaillantellidae | <i>Lefua echigonia</i> | N/A | Hino, Shiga, Japan | EF458305 | FJ197028 | AB054126 | AB054126 |
| | <i>Vaillantella maassi</i> | CBM-ZF-11437 | Aquarium | EU711132 | FJ197031 | AB242173 | AB242173 |
| Cyprinoidea Cyprinidae Acheilognathinae | <i>Acheilognathus typus</i> | CBM-ZF-11423 | Hanamaki, Iwate, Japan | EU292688 | FJ197042 | AB239602 | AB239602 |
| | <i>Rhodeus ocellatus</i> | N/A | Yao, Osaka, Japan | EU711142 | FJ197043 | AB070205 | AB070205 |
| | <i>Tanakia limbata</i> | CBM-ZF-11178 | Okayama, Japan | HM224070 | HM223952 | HM224309 | HM224190 |
| Alburninae | <i>Alburnus alburnus</i> | N/A | Saône River, Lyon, France | EU711143 | FJ197044 | AB239593 | AB239593 |
| Cultrinae | <i>Aphyocypris chinensis</i> | CBM-ZF-11424 | Tanushimaru, Fukuoka, Japan | EU292692 | FJ197066 | AB218688 | AB218688 |
| | <i>Chanodichthys mongolicus</i> | UAIC 14382.01 | N/A | EU711145 | FJ197047 | AP009060 | AP009060 |
| | <i>Ctenopharyngodon idella</i> | IHB 0411070 | Wuhan, China | EF178284 | HM223939 | EU391390 | EU391390 |
| | <i>Hypophthalmichthys nobilis</i> | IHB 0411071 | Wuhan, China | HM224058 | HM223941 | EU343733 | EU343733 |
| | <i>Ischikauia steenackeri</i> | N/A | Lake Biwa, Japan | EU292687 | EU409648 | AB239601 | AB239601 |

(continued on next page)

Table 1 (continued)

| Classification | Taxon | Catalog no. | Source | RAG1 | Rh | Cyt b | COI |
|----------------|-------------------------------------|---------------|--------------------------------------|-------------|-------------|-------------|-------------|
| | <i>Macrochirichthys macrochirus</i> | CBM-ZF-11207 | Kandal, Cambodia | EU409630 | EU409659 | AP011234 | AP011234 |
| | <i>Megalobrama amblycephala</i> | CBM-ZF-11345 | Aquarium | EU409620 | EU409647 | N/A | N/A |
| | <i>Opsariichthys uncirostris</i> | N/A | GenBank | N/A | N/A | EU434747 | EU434747 |
| | | CBM-ZF-11177 | Fukuoka, Japan | EF452847 | EF452916 | EF452748 | EF452894 |
| | <i>Oxygaster anomalura</i> | USNM 394000 | Kalimantan Selatan, Indonesia | Unpublished | Unpublished | Unpublished | Unpublished |
| | <i>Zacco platypus</i> | CBM-ZF-11160 | Okayama, Japan | EF452848 | EF452917 | EF452749 | EF452896 |
| Cyprininae | <i>Aulopyge huegelii</i> | N/A | GenBank | N/A | N/A | AF112133 | N/A |
| | <i>Barbonymus gonionotus</i> | CBM-ZF-11230 | Kampong Chhnang, Cambodia | EU711146 | FJ531344 | N/A | N/A |
| | | N/A | Moon River, Kaeng Saphue, Thailand | N/A | N/A | AB238966 | AB238966 |
| | <i>Barbus barbuis</i> | N/A | Danube River, Lutzmannsburg, Austria | EU711147 | FJ197049 | AB238965 | AB238965 |
| | <i>Barbus trimaculatus</i> | N/A | Maputo, Mozambique | EU711148 | FJ197050 | AB239600 | AB239600 |
| | <i>Carassius auratus</i> | N/A | GenBank | DQ196520 | L11863 | AB006953 | AB111951 |
| | <i>Cyprinus carpio</i> | N/A | GenBank | AY787040 | U02475 | X61010 | X61010 |
| | <i>Discogobio tetrabarbatus</i> | | Rong'an, Guangxi, China | GQ913455 | GQ913507 | AY953022 | Unpublished |
| | <i>Gymnocypris przewalskii</i> | N/A | Qinghai Lake, Qinghai, China | EU711149 | FJ197051 | AB239595 | AB239595 |
| | <i>Labeo senegalensis</i> | N/A | Ouémé and Iguidi Rivers, Benin | EU711151 | FJ197053 | AB238968 | AB238968 |
| | <i>Ptychidio jordani</i> | | Tian'e, Guangxi, China | GQ913487 | GQ913538 | Unpublished | Unpublished |
| | <i>Puntius ticto</i> | N/A | GenBank | EU711152 | FJ197054 | AB238969 | AB238969 |
| Danioninae | <i>Amblypharyngodon mola</i> | CBM-ZF-11790 | Koshi Barrage, Nepal | HM224019 | HM223899 | HM224256 | HM224137 |
| | <i>Barilius vagra</i> | UAIC 14179.07 | Aquarium | HM224022 | HM223901 | HM224259 | HM224140 |
| | <i>Danio rerio</i> | N/A | GenBank | U71093 | L11014 | AC024175 | AC024175 |
| | <i>Danionella dracula</i> | UAIC 14169.47 | Aquarium | EF452841 | HM223913 | EF452741 | EF452887 |
| | <i>Devario auropurpureus</i> | CBM-ZF-11324 | Aquarium | EU292708 | HM223915 | HM224275 | HM224156 |
| | <i>Esomus danricus</i> | UAIC 14169.04 | Aquarium | HM224044 | HM223926 | HM224287 | HM224168 |
| | <i>Luciosoma setigerum</i> | CBM-ZF-11273 | Aquarium | EU292704 | FJ531352 | AP011423 | AP011423 |
| | <i>Microrasbora rubescens</i> | UAIC 14167.06 | Aquarium | EF452844 | EF452913 | EF452744 | EF452890 |
| | <i>Paedocypris carbunculus</i> | UAIC 14180.63 | Aquarium | GQ365218 | GQ365226 | HM224326 | HM224209 |
| | <i>Raiamas guttatus</i> | CBM-ZF-11363 | Aquarium | HM224092 | HM223977 | AP011222 | AP011222 |
| | <i>Rasbora cephalotaenia</i> | CBM-ZF-11443 | Aquarium | HM224099 | HM223984 | AP011430 | AP011430 |
| | <i>Sundadanio axelrodi</i> | UAIC 14300.01 | Aquarium | EU292711 | GQ365228 | HM224383 | HM224253 |
| Gobioninae | <i>Abbottina liaoningensis</i> | N/A | GenBank | N/A | N/A | EU934498 | N/A |
| | <i>Abbottina rivularis</i> | CBM-ZF-11181 | Fukuoka, Kyushu, Japan | EU711102 | JN003285 | AF051856 | JN003353 |
| | <i>Acanthogobio guentheri</i> | N/A | GenBank | N/A | N/A | AY953003 | N/A |
| | <i>Belligobio nummifer</i> | | | N/A | JN003293 | AY952987 | JN003342 |
| | <i>Biwia springeri</i> | CBM-ZF-11346 | Aquarium | JN003249 | JN003300 | AP011360 | AP011360 |
| | <i>Biwia yodoensis</i> | N/A | GenBank | N/A | N/A | AB499046 | N/A |
| | <i>Biwia zezera</i> | CBM-ZF-11393 | | EU409626 | EU409654 | JN003331 | JN003368 |
| | <i>Coreius guichenoti</i> | N/A | GenBank | N/A | N/A | AY953001 | N/A |
| | <i>Coreius heterodon</i> | N/A | GenBank | N/A | N/A | AY953000 | N/A |
| | <i>Coreoleuciscus splendidus</i> | CBM-ZF-11283 | Aquarium | EU711114 | JN003301 | N/A | N/A |
| | | N/A | GenBank | N/A | N/A | DQ267433 | DQ347951 |
| | <i>Gnathopogon elongatus</i> | CBM-ZF-11430 | Lake Biwa, Japan | EU711153 | FJ197055 | AB218687 | AB218687 |
| | <i>Gnathopogon herzensteini</i> | IHB 0400131 | | JN003259 | JN003296 | JN003334 | JN003349 |

Table 1 (continued)

| Classification | Taxon | Catalog no. | Source | RAG1 | Rh | Cyt b | COI |
|----------------|--------------------------------------|---------------|---------------------------|----------|----------|----------|----------|
| | <i>Gnathopogon imberbis</i> | N/A | GenBank | N/A | N/A | AY952998 | N/A |
| | <i>Gnathopogon nicholsi</i> | N/A | GenBank | N/A | N/A | AY952997 | N/A |
| | <i>Gnathopogon strigatus</i> | CBM-ZF-11352 | Aquarium | JN003260 | JN003295 | AP011361 | AP011361 |
| | <i>Gobio coriparoides</i> | IHB 0411036 | | JN003256 | JN003309 | JN003326 | JN003367 |
| | <i>Gobio cynocephalus</i> | UAIC 14387.01 | | JN003258 | JN003279 | JN003328 | JN003364 |
| | <i>Gobio gobio</i> | N/A | Planá, Czech Republic | EU292689 | FJ197056 | AB239596 | AB239596 |
| | <i>Gobio huanghensis</i> | N/A | GenBank | N/A | N/A | FJ904648 | N/A |
| | <i>Gobio macrocephalus</i> | N/A | GenBank | N/A | N/A | AY953006 | N/A |
| | <i>Gobio soldatovi</i> | N/A | GenBank | N/A | N/A | EU934491 | N/A |
| | <i>Gobiobotia filifer</i> | N/A | GenBank | N/A | N/A | AY953002 | N/A |
| | <i>Gobiobotia meridionalis</i> | | | N/A | JN003312 | AF375867 | JN003344 |
| | <i>Gobiobotia pappenheimi</i> | UAIC 14388.01 | | JN003274 | JN003277 | N/A | N/A |
| | <i>Gobiocypris rarus</i> | N/A | GenBank | N/A | N/A | AF309083 | AY879113 |
| | <i>Hemibarbus barbuis</i> | N/A | Sarugaishi River, Japan | EU711154 | FJ197057 | AB070241 | AB070241 |
| | <i>Hemibarbus labeo</i> | UAIC 14389.01 | | JN003271 | JN003280 | DQ347953 | DQ347953 |
| | <i>Hemibarbus longirostris</i> | N/A | GenBank | N/A | N/A | DQ347952 | DQ347952 |
| | <i>Hemibarbus maculatus</i> | UAIC 14390.01 | | JN003270 | JN003281 | JN003322 | JN003360 |
| | <i>Hemibarbus medius</i> | N/A | GenBank | N/A | N/A | AY952989 | N/A |
| | <i>Hemibarbus mylodon</i> | N/A | GenBank | N/A | N/A | DQ345787 | DQ345787 |
| | <i>Hemibarbus mylodon</i> | CBM-ZF-11451 | Aquarium | JN003253 | JN003286 | AP011414 | AP011414 |
| | <i>Hemibarbus cf. umbrifer</i> | CBM-ZF-11630 | Lang Son, Vietnam | JN003275 | JN003294 | AP011415 | AP011415 |
| | <i>Hemibarbus umbrifer</i> | N/A | GenBank | N/A | N/A | EU934486 | N/A |
| | <i>Huigobio chinssuensis</i> | N/A | GenBank | N/A | N/A | AY953016 | N/A |
| | <i>Ladislavia taczanowskii</i> | UAIC 14392.01 | | JN003255 | JN003311 | JN003315 | JN003350 |
| | <i>Mesogobio tumenensis</i> | N/A | GenBank | N/A | N/A | AY953008 | N/A |
| | <i>Microphysogobio amurensis</i> | UAIC 14397.01 | | JN003248 | JN003283 | JN003335 | JN003356 |
| | <i>Microphysogobio elongatus</i> | IHB 0411042 | | JN003251 | JN003299 | JN003341 | JN003348 |
| | <i>Microphysogobio fukiensis</i> | N/A | GenBank | N/A | N/A | AY953014 | N/A |
| | <i>Microphysogobio liaohensis</i> | N/A | GenBank | N/A | N/A | AY953012 | N/A |
| | <i>Microphysogobio longidorsalis</i> | CBM-ZF-11551 | Aquarium | JN003250 | JN003297 | AP011394 | AP011394 |
| | <i>Microphysogobio sp.</i> | CBM-ZF-11935 | Lang Son, Vietnam | N/A | N/A | JN003339 | JN003361 |
| | <i>Microphysogobio tungtingensis</i> | N/A | GenBank | N/A | N/A | AY953013 | N/A |
| | <i>Paracanthobrama guichenoti</i> | N/A | GenBank | N/A | N/A | AY952994 | N/A |
| | <i>Platysmacheilus exiguus</i> | N/A | GenBank | N/A | N/A | AY953015 | N/A |
| | <i>Platysmacheilus longibarbus</i> | N/A | GenBank | N/A | N/A | AY953017 | N/A |
| | <i>Platysmacheilus sp.</i> | CBM-ZF-11739 | Lang Son, Vietnam | JN003246 | JN003290 | JN003330 | JN003362 |
| | <i>Pseudogobio esocinus</i> | | | JN003265 | JN003303 | JN003317 | JN003371 |
| | <i>Pseudogobio guilinensis</i> | | | N/A | JN003302 | AY953018 | JN003343 |
| | <i>Pseudogobio vaillanti</i> | IHB 0307021 | | JN003266 | JN003298 | JN003316 | JN003372 |
| | <i>Pseudopungtungia nigra</i> | N/A | GenBank | N/A | N/A | EU597300 | EU597300 |
| | <i>Pseudorasbora elongata</i> | N/A | GenBank | N/A | N/A | AY952996 | N/A |
| | <i>Pseudorasbora elongata</i> | N/A | GenBank | N/A | N/A | EU934505 | N/A |
| | <i>Pseudorasbora parva</i> | CBM-ZF-11176 | Shimane, Japan | HM224064 | HM223947 | HM224302 | HM224184 |
| | <i>Pseudorasbora pumila</i> | N/A | Kashimadai, Miyagi, Japan | EU711155 | FJ197058 | AB239599 | AB239599 |
| | <i>Pungtungia herzi</i> | | | EU711156 | JN003313 | JN003333 | JN003352 |
| | <i>Rhinogobio cylindricus</i> | N/A | GenBank | N/A | N/A | AY952992 | N/A |
| | <i>Rhinogobio hunanensis</i> | N/A | GenBank | N/A | N/A | AY952993 | N/A |
| | <i>Rhinogobio typus</i> | IHB 0426236 | | JN003268 | JN003288 | JN003329 | JN003357 |
| | <i>Romanogobio banaticus</i> | N/A | GenBank | N/A | N/A | AY952329 | N/A |
| | <i>Romanogobio ciscaucasicus</i> | UAIC 14403.01 | | EU409624 | JN003308 | JN003325 | JN003365 |
| | <i>Romanogobio elimeius</i> | N/A | GenBank | N/A | N/A | AF090751 | N/A |
| | <i>Romanogobio kesslerii</i> | N/A | GenBank | N/A | N/A | AY952328 | N/A |
| | <i>Romanogobio macropterus</i> | N/A | GenBank | N/A | N/A | AY952332 | N/A |
| | <i>Romanogobio tanaiticus</i> | UAIC 14404.01 | | JN003257 | JN003278 | JN003324 | JN003366 |
| | <i>Romanogobio tenuicorpus</i> | N/A | GenBank | N/A | N/A | AY953004 | N/A |

(continued on next page)

Table 1 (continued)

| Classification | Taxon | Catalog no. | Source | RAG1 | Rh | Cyt b | COI |
|-----------------|---------------------------------------|-----------------------------|---------------------------------------|----------|----------|----------|----------|
| | <i>Romanogobio tenuicorpus</i> | UAIC 15500.01 | | JN003264 | JN003310 | JN003327 | JN003363 |
| | <i>Romanogobio uranoscopus</i> | N/A | GenBank | N/A | N/A | AY952331 | N/A |
| | <i>Sarcocheilichthys czerskii</i> | N/A | GenBank | N/A | N/A | EF193433 | N/A |
| | <i>Sarcocheilichthys hainanensis</i> | N/A | GenBank | JN003263 | N/A | JN003314 | JN003354 |
| | <i>Sarcocheilichthys kiangsiensis</i> | N/A | GenBank | N/A | N/A | AY952984 | N/A |
| | <i>Sarcocheilichthys lacustris</i> | N/A | GenBank | N/A | N/A | EF193411 | N/A |
| | <i>Sarcocheilichthys nigripinnis</i> | IHB | | JN003261 | JN003291 | JN003319 | JN003369 |
| | <i>Sarcocheilichthys parvus</i> | 0411030 CBM-ZF- 11299 | Aquarium | EU409625 | EU409653 | AP011332 | AP011332 |
| | <i>Sarcocheilichthys sinensis</i> | UAIC 14406.01 | | JN003247 | JN003284 | JN003332 | JN003355 |
| | <i>Sarcocheilichthys soldatovi</i> | UAIC 14407.01 | | JN003262 | JN003292 | JN003337 | JN003358 |
| | <i>Sarcocheilichthys variegatus</i> | CBM-ZF- 10604 | Lake Biwa, Japan | EU711157 | FJ197060 | AB054124 | AB054124 |
| | <i>Saurogobio dabryi</i> | UAIC 14408.01 | | JN003273 | JN003282 | JN003336 | JN003370 |
| | <i>Saurogobio gracilicaudatus</i> | N/A | GenBank | N/A | N/A | EU934510 | N/A |
| | <i>Saurogobio gymnocheilus</i> | N/A | GenBank | N/A | N/A | AY953009 | N/A |
| | <i>Saurogobio immaculatus</i> | N/A | GenBank | N/A | N/A | AY953010 | N/A |
| | <i>Saurogobio cf. immaculatus</i> | CBM-ZF- 11551 | Hanoi, Vietnam | JN003272 | JN003289 | JN003340 | JN003359 |
| | <i>Saurogobio xiangjiangensis</i> | N/A | GenBank | N/A | N/A | EU934511 | N/A |
| | <i>Squalidus argentatus</i> | N/A | GenBank | N/A | JN003305 | AY952985 | JN003345 |
| | <i>Squalidus atromaculatus</i> | N/A | GenBank | N/A | N/A | AY952333 | N/A |
| | <i>Squalidus chankaensis</i> | CBM-ZF- 11402 | | FJ531252 | FJ531358 | JN003321 | JN003374 |
| | <i>Squalidus gracilis</i> | CBM-ZF- 11179 | River Yabe, Fukuoka, Kyushu, Japan | JN003254 | JN003287 | JN003318 | JN003351 |
| | <i>Squalidus japonicus</i> | CBM-ZF- 11263 | | JN003267 | JN003304 | JN003320 | JN003375 |
| | <i>Squalidus nitens</i> | N/A | GenBank | N/A | N/A | AY952986 | N/A |
| | <i>Squalidus sp.</i> | N/A | GenBank | JN003269 | JN003276 | JN003338 | JN003373 |
| | <i>Squalidus wolterstorffi</i> | N/A | GenBank | N/A | JN003306 | N/A | JN003346 |
| | <i>Squalidus wolterstorffi</i> | CBM-ZF- 11354 | Aquarium | JN003252 | JN003307 | AP011392 | AP011392 |
| Leptobarbinae | <i>Xenophysogobio boulengeri</i> | N/A | GenBank | N/A | N/A | AF375868 | N/A |
| | <i>Leptobarbus hoevenii</i> | CBM-ZF- 11225 | Kampong Chhnang, Cambodia | FJ531249 | FJ531351 | AP011286 | AP011286 |
| Leuciscinae | <i>Cyprinella lutrensis</i> | UAIC 11405.07 | Frio River, Texas, USA | EU711158 | FJ197061 | AB070206 | AB070206 |
| | <i>Notemigonus crysoleucas</i> | N/A | St. Catharines, Ontario, Canada | EF452831 | FJ197062 | U01318 | EF452854 |
| | <i>Notropis atherinoides</i> | UAIC 10485.06 | Wisconsin, USA | HM224059 | HM223942 | HM224297 | HM224179 |
| | <i>Pelecus cultratus</i> | N/A | Lake Balaton, Hungary | EU711144 | FJ197045 | AB239597 | AB239597 |
| | <i>Pteronotropis hypselopterus</i> | UAIC 12730.02 | Alabama, USA | HM224065 | HM223948 | HM224303 | HM224185 |
| | <i>Semotilus atromaculatus</i> | UAIC 10875.13 | Alabama, USA | EU409629 | EU409658 | HM224307 | HM224188 |
| Tincinae | <i>Tanichthys albonubes</i> | CBM-ZF- 11334 | Aquarium | FJ531253 | FJ531359 | AP011397 | AP011397 |
| | <i>Tanichthys micagemmae</i> | UAIC 14167.47 | Aquarium | HM224136 | HM224017 | HM224384 | HM224254 |
| | <i>Tinca tinca</i> | N/A | Saône River, Fareins, France | EU711162 | FJ197070 | AB218686 | AB218686 |
| Psilorhynchidae | <i>Psilorhynchus homaloptera</i> | IHB uncataloged | Aquarium | FJ531250 | FJ531354 | DQ026436 | DQ026436 |
| | <i>Psilorhynchus sucatio</i> | CBM-ZF- 11322 | Aquarium | FJ531251 | FJ531355 | AP011288 | AP011288 |

generations. The distribution of log likelihood scores was used to determine burn-in times for each analysis; the sampled tree statistics were visualized with AWTY (Nylander et al., 2008). Trees retained after burn-in were used to construct the 50% majority-rule consensus in PAUP* and to calculate the clade credibility scores for each node.

Because many sequences were downloaded from GenBank (45 gobionines and one outgroup), and most consisted of only cyt b (four also had COI data), we addressed issues that may arise due to the inclusion of incomplete data. To that end, we conducted additional searches with a reduced data matrix where taxa represented only by data from GenBank were deleted. These deletions

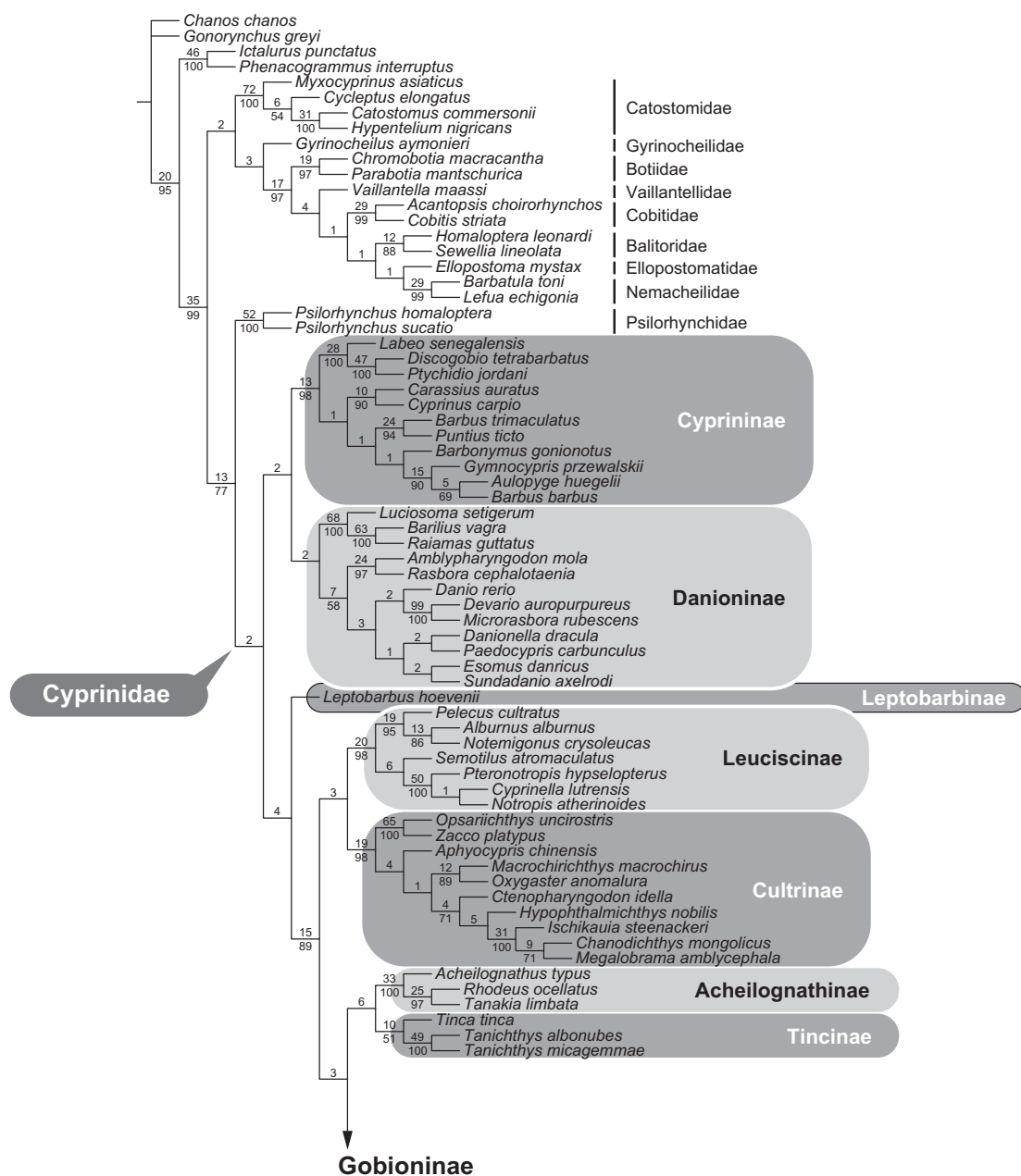


Fig. 1. The phylogenetic relationships of the subfamily Gobioninae (Teleostei: Cypriniformes: Cyprinidae), as represented by the strict consensus of six most-parsimonious trees (length = 29,216 steps; CI = 0.140; RI = 0.472). Bremer (above) and bootstrap (below) support values are displayed at each node; bootstrap values below 50% are not shown. A monophyletic Gobioninae *sensu stricto* includes the following genera: *Abbottina*, *Belligobio*, *Biwia*, *Coreius*, *Coreoleuciscus*, *Gnathopogon*, *Gobio* (including *Acanthogobio*), *Gobiobotia*, *Gobiocypris*, *Hemibarbus*, *Huigobio*, *Ladislavia*, *Mesogobio*, *Microphysogobio*, *Paracanthobrama*, *Platysmacheilus*, *Pseudogobio*, *Pseudopungtungia*, *Pseudorasbora*, *Pungtungia*, *Rhinogobio*, *Romanogobio*, *Sarcocheilichthys*, *Saurogobio*, *Squalidus*, and *Xenophysogobio*. The subfamily is divided into three major lineages: *Hemibarbus*–*Squalidus* group, tribe *Sarcocheilichthyini*, and tribe *Gobionini*.

reduced the number of terminals to 116, the number of characters per taxon remained the same. The settings for these searches using this abridged data set were identical to what was reported above for the analyses of the full data set, except that we did not calculate Bremer decay indices for the parsimony tree generated from the reduced data matrix.

The use of rhodopsin has gained popularity as a molecular marker in recent phylogenetic studies (e.g. Chen et al., 2003; Dettai and Lecointre, 2005; Taylor and Hellberg, 2005; Mayden et al., 2007; Schönhuth et al., 2008). However, other recent studies have demonstrated positive selection on opsin genes in various fish groups (Sugawara et al., 2002; Dann et al., 2004; Spady et al., 2005;

Larmuseau et al., 2010). The effects of such selection may confound phylogenetic reconstructions that rely on this gene, leading some (e.g. Larmuseau et al., 2010) to warn against the use of rhodopsin in phylogenetic studies. To examine this phenomenon in our data, we performed a one-tailed Z-test for positive selection as implemented in MEGA 5.04 (Tamura et al., in press). The number of synonymous (d_S) and non-synonymous (d_N) substitutions were determined for pairwise comparisons among all taxa with rhodopsin data (114 of 162). The variances of d_S and d_N were calculated from 1000 bootstrap replicates. These values were used to test the null hypothesis (neutral selection; $H_0: d_N = d_S$) versus the alternative hypothesis (positive selection; $H_A: d_N > d_S$).

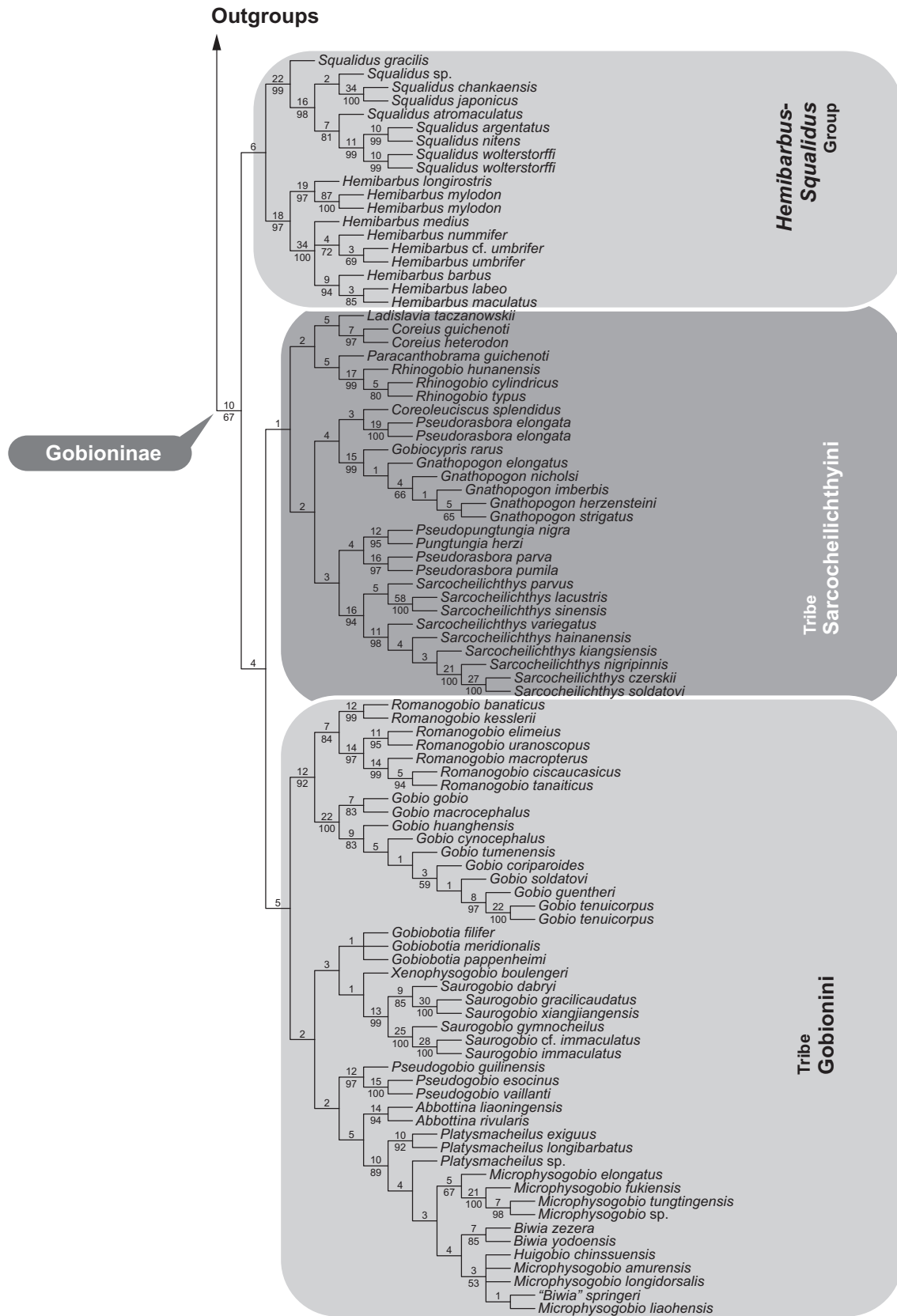


Fig. 1 (continued)



Fig. 2. The phylogenetic relationships of the subfamily Gobioninae, as represented by the tree topology with the best log likelihood score ($\ln L = -122975.541$) recovered by maximum likelihood analysis from 100 independent searches. Bootstrap values are reported at each node (values below 50% are not shown).

3. Results

The aligned data matrix used for analyses consisted of 4114 base pairs (with 1894 parsimony-informative sites) for 162 taxa. Alignment of the sequences produced a complete 1140-bp sequence for *cyt b*, a 658-bp fragment of COI, a 1497-bp fragment of RAG1, and a 819-bp fragment of rhodopsin. Based on the alignment, the only indel observed in the data matrix was a unique, single-codon deletion in *cyt b* of *Ictalurus punctatus*. Parsimony analyses converged on six most-parsimonious topologies (length = 29,216 steps; CI = 0.140; RI = 0.472); the strict consensus is illustrated in Fig. 1. The optimal topology recovered by the maximum likelihood searches produced a tree score of $\ln L = -122975.541$ (Fig. 2). For the Bayesian analyses, the hLRTs conducted with MrModeltest identified GTR + I + Γ model as the best-fit model for the majority of the partitions (8 of 12), with GTR + Γ for rhodopsin 3rd positions

and for COI 2nd and 3rd positions, and F81 + I + Γ for rhodopsin 2nd positions. Trees from the first 1,000,000 generations (1001 trees) of each search were discarded as burn-in. The remaining 18,000 trees (i.e. those recovered after stationarity had been reached and combined from both searches) were used to calculate the 50% majority-rule consensus tree (Fig. 3).

With six most-parsimonious trees, the strict consensus topology is well resolved (Fig. 1). There are only three polytomies: a trichotomy within *Hemibarbus*, an unresolved clade of all three representatives of *Gobiobotia* (*Gobiobotia filifer*, *G. meridionalis*, and *Gobiobotia pappenheimi*), and an unresolved clade that includes *Biwia springeri*, *Huigobio*, and *Microphysogobio* (in part). The strict consensus tree shows support for a monophyletic subfamily Gobioninae that includes all 27 putative gobionine genera examined: *Abbottina*, *Acanthogobio*, *Belligobio*, *Biwia*, *Coreius*, *Coreoleuciscus*, *Gnathopogon*, *Gobio*, *Gobiobotia*, *Gobiocypris*, *Hemibarbus*, *Huigobio*,

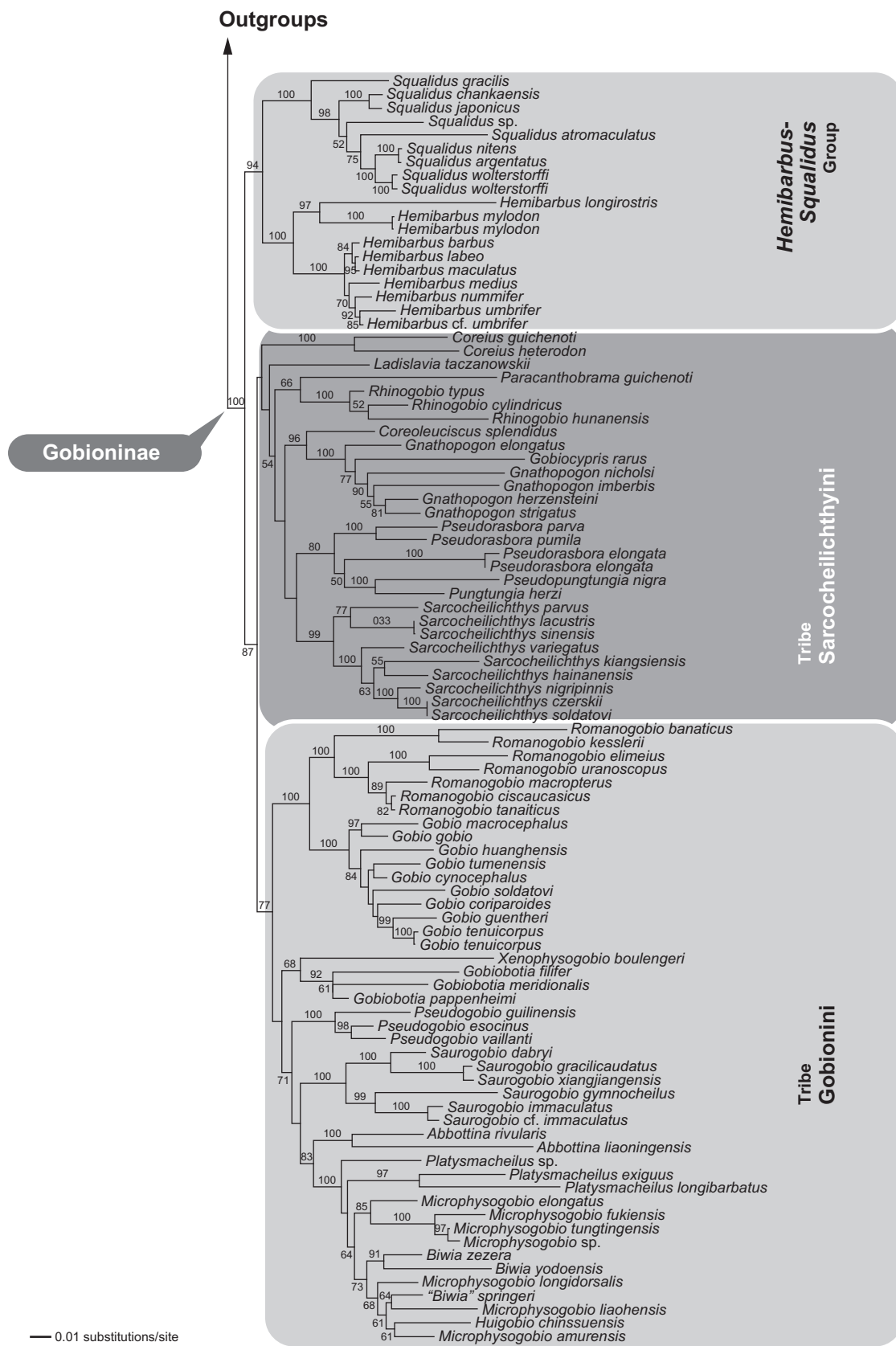


Fig. 2 (continued)

Ladislavia, *Mesogobio*, *Microphysogobio*, *Paracanthobrama*, *Platysmacheilus*, *Pseudogobio*, *Pseudopungtungia*, *Pseudorasbora*, *Pungtungia*, *Rhinogobio*, *Romanogobio*, *Sarcocheilichthys*, *Saurogobio*,

Squalidus, and *Xenophysogobio*. The relationships within Gobioninae share many similarities between the different search methods (Figs. 1–3), with the largest disparities coming in the placement

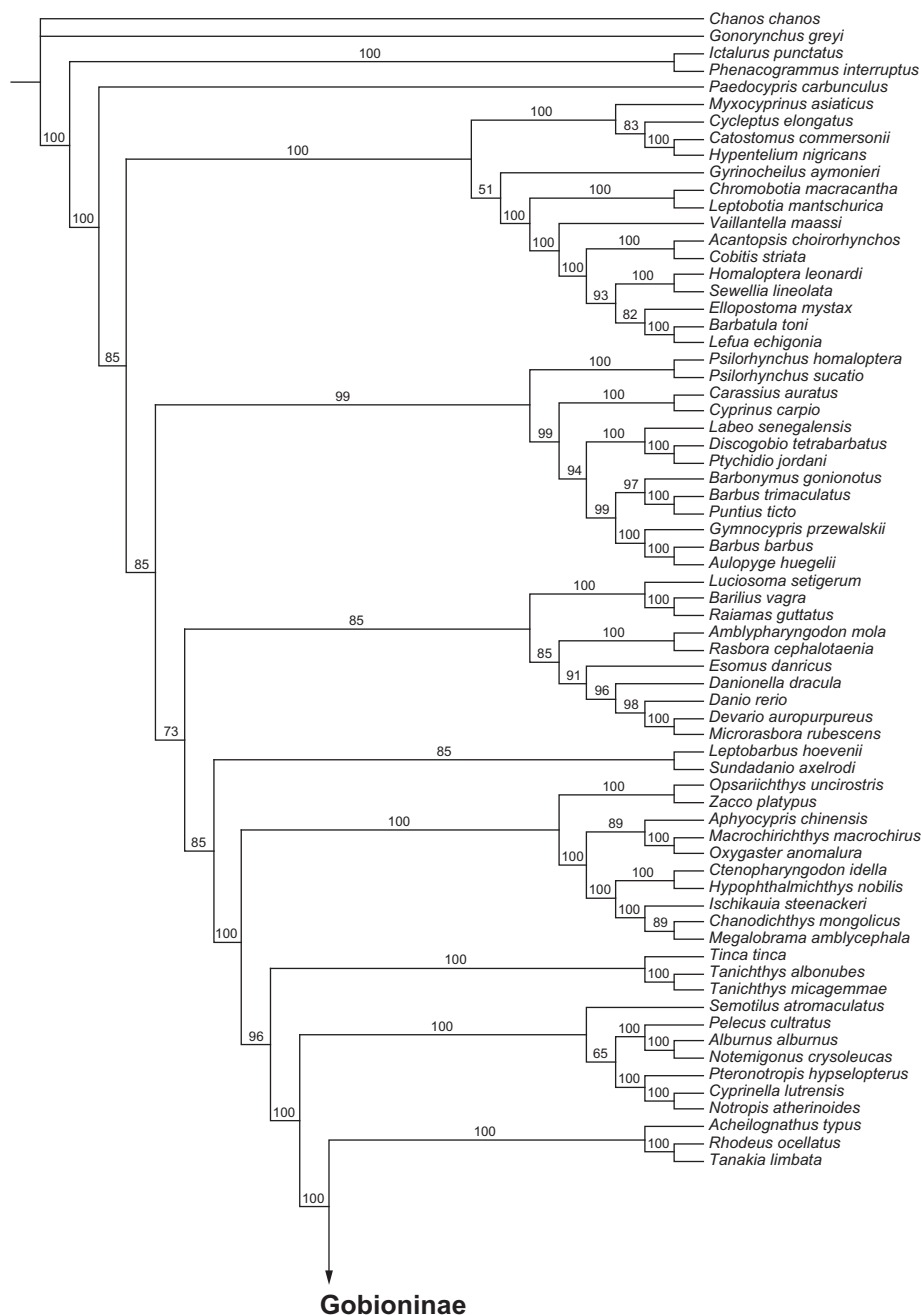


Fig. 3. The phylogenetic relationships of the subfamily Gobioninae, as represented by the 50% majority-rule consensus tree topology of 18,000 trees recovered by Bayesian inference. Clade credibility scores are reported at each node.

of *Coreoleuciscus*, *Paracanthobrama*, *P. elongata*, the genera *Coreius* and *Ladislavia*, the monophyly of the *Gobiobotia*–*Xenophysogobio* group (i.e. *Gobiobotiinae*), and the relative placements of *Pseudogobio* and *Saurogobio*. Branch support in all three trees is weak at those nodes. The Bayesian and the maximum likelihood trees are more alike than either is to the parsimony tree. These topologies show three major lineages within the subfamily: a *Hemibarbus*–*Squalidus* group, which also includes *Belligobio*; a *Sarcocheilichthys* group, with *Coreius*, *Coreoleuciscus*, *Gnathopogon*, *Gobiocypris*, *Ladislavia*, *Paracanthobrama*, *Pseudopungtungia*, *Pseudorasbora*, *Pungtungia*, *Rhinogobio*, and *Sarcocheilichthys*; and a *Gobio*–*Gobiobotia* group, with *Abbottina*, *Acanthogobio*, *Biwia*, *Gobio*, *Gobiobotia*, *Huigobio*, *Mesogobio*, *Microphysogobio*, *Platysmacheilus*, *Pseudogobio*, *Romanogobio*, *Saurogobio*, and *Xenophysogobio*. The following genera are

monophyletic: *Abbottina*, *Coreius*, *Pseudogobio*, *Rhinogobio*, *Sarcocheilichthys*, *Saurogobio*, and *Squalidus*. *Coreoleuciscus*, *Gobiocypris*, *Ladislavia*, *Paracanthobrama*, *Pungtungia*, and *Pseudopungtungia* are recovered as monophyletic either due to monotypy or the examination of only a single representative. The remaining genera are not monophyletic for various reasons (see Section 4).

The sister group of Gobioninae differs between the three trees. A clade of *Tanichthys*, *Tinca*, and the subfamily *Acheilognathinae* is the sister group in the parsimony topology (Fig. 1), whereas *Acheilognathinae* by itself is recovered as the sister group in both the Bayesian and maximum likelihood topologies (Figs. 2 and 3). In the likelihood and parsimony trees, the order *Cypriniformes* is recovered as a monophyletic group composed of two clades, a monophyletic suborder *Cobitoidea* and a monophyletic suborder

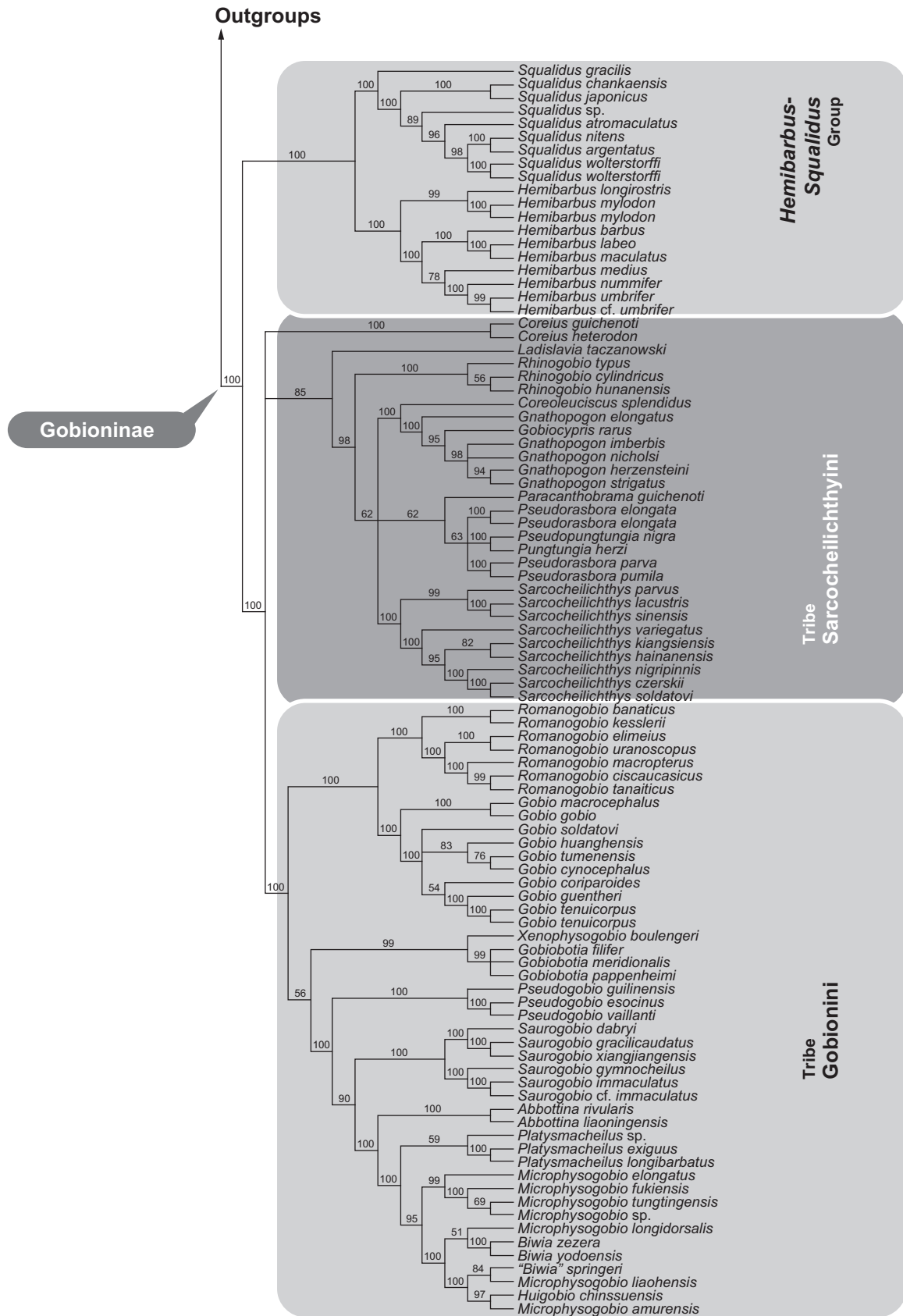


Fig. 3 (continued)

Cyprinoidea. The Bayesian results agree on a monophyletic Cypriniformes, but recover a putative cyprinid (*Paedocypris*) as the sister group to all other cypriniform fishes. The position of *Psilorhynchus*

varies; this enigmatic genus is found either as the sister group of the family Cyprinidae (parsimony; Fig. 1) or within Cyprinidae, as the sister group of the subfamily Cyprininae (maximum

likelihood, Bayesian; Figs. 2 and 3). The relationships between the subfamilies Cyprininae (with or without *Psilorhynchus*) and Danioninae are different in each tree: with both subfamilies as sister groups in the parsimony tree; with Danioninae as the sister group to the rest of Cyprinidae (including *Psilorhynchus*) in the likelihood tree; and with Cyprininae (plus *Psilorhynchus*) as the sister group to the rest of Cyprinidae in the Bayesian tree. *Aulopyge*, *Discogobio*, and *Ptychidio* are recovered outside of Gobioninae, within the Cyprininae, in all three topologies.

The parsimony searches using the abridged data matrix yielded seven most-parsimonious trees (length = 26,620 steps; CI = 0.152; RI = 0.446); see [Supplementary content](#) for the strict consensus topology. Although relationships in this tree are generally congruent with those presented in Fig. 1, the removal of the GenBank taxa caused a noticeable disruption in the relationships of the *Sarcocheilichthys* group. *Ladislavia taczanowskii* and *Rhinogobio typus* are displaced into the *Gobio-Gobiobotia* clade, which collapses into a large polytomy that differs from the analysis of the full data matrix (Fig. 1). The likelihood searches yielded an optimal topology with a $\ln L = -112536.810$ ([Supplementary content](#)). Unlike the two parsimony trees, the topology of the abridged likelihood tree corresponds exactly to what was shown in Fig. 2 if the deleted taxa were pruned from the larger tree. The abridged Bayesian topology ([Supplementary content](#)) is more resolved than the full Bayesian tree, but is otherwise completely congruent with the topology illustrated in Fig. 3.

The Z-test for positive selection indicated that the null hypothesis (neutral selection; $H_0: d_N = d_S$) could not be rejected for any pairwise comparison. In fact, all values of $P = 1$ (results not shown). This result indicates that there is no positive selection pressure on the rhodopsin gene in this particular group of fishes, the gudgeons.

4. Discussion

The recovery of a monophyletic Gobioninae is consistent with its long history and the relative stability of its constituent taxa over that time. The composition of the subfamily Gobioninae matches what has been proposed by previous workers (e.g. Bănărescu and Nalbant, 1973; Bănărescu, 1992; Yang et al., 2006; Liu et al., 2010), though our results contradict Hosoya (1986), who restricted Gobioninae to a smaller subset of genera than what we currently recognize, and Naseka (1996), whose classification divided the subfamily into four groups that do not match the groups we recovered. Within Gobioninae, we found evidence for three primary clades. This division into three groups corresponds with the results presented by previous molecular studies (Yang et al., 2006; Kim et al., 2009; Liu et al., 2010). A revised classification of the subfamily Gobioninae and its member tribes based on our results is provided in Table 2.

Of the genera that were not recovered as monophyletic, they fall into two broad categories. Some are non-monophyletic either because of lack of resolution or because of an easily resolved taxonomic issue; these include: *Hemibarbus* (inclusion of *Belligobio*), *Gnathopogon* (not monophyletic in the likelihood and Bayesian trees with inclusion of *Gobiocypris*), *Gobio* (inclusion of *Acanthogobio*, *Mesogobio*, and *Romanogobio tenuicarpus*), *Gobiobotia* (unresolved polytomy with *Xenophysogobio* in the parsimony tree), *Romanogobio* (*R. tenuicarpus* recovered within *Gobio*). The other non-monophyletic genera are more problematic. They are broadly polyphyletic and represent areas of concern which will require additional study to resolve. These problem taxa include: *Biwia*, *Microphysogobio* (inclusion of *Biwia* and *Huigobio*; status of *Rostrogobio*), *Platysmacheilus* (paraphyletic), *Pseudorasbora* (placement of *Pseudorasbora elongata*).

Table 2

Revised classification of the subfamily Gobioninae (Teleostei: Cypriniformes: Cyprinidae).

| Family Cyprinidae |
|---|
| Subfamily Gobioninae Bleeker 1863 |
| <i>Hemibarbus-Squalidus</i> group |
| <i>Belligobio</i> ^a |
| <i>Hemibarbus</i> |
| <i>Squalidus</i> |
| Tribe Gobionini Bleeker 1863 |
| <i>Abbottina</i> |
| <i>Biwia</i> |
| <i>Gobio</i> [includes <i>Acanthogobio</i>] |
| <i>Gobiobotia</i> |
| <i>Huigobio</i> |
| <i>Mesogobio</i> ^a |
| <i>Microphysogobio</i> [includes <i>Rostrogobio</i>] |
| <i>Platysmacheilus</i> |
| <i>Pseudogobio</i> |
| <i>Romanogobio</i> |
| <i>Saurogobio</i> |
| <i>Xenophysogobio</i> |
| Tribe Sarcocheilichthyini Kryzhanovsky 1947 |
| <i>Coreius</i> |
| <i>Coreoleuciscus</i> |
| <i>Gnathopogon</i> |
| <i>Gobiocypris</i> |
| <i>Ladislavia</i> |
| <i>Paracanthobrama</i> |
| <i>Pseudopungtungia</i> |
| <i>Pseudorasbora</i> |
| <i>Pungtungia</i> |
| <i>Rhinogobio</i> |
| <i>Sarcocheilichthys</i> |
| <i>Incertae sedis</i> |
| <i>Paraleucogobio</i> ^b |
| <i>Parasqualidus</i> ^b |

^a Type species of this genus was not examined herein, its placement and status are tentative and based on previous literature.

^b No putative species of this genus were examined herein.

The relationships among the species of gudgeons indicate that the evolution of swim bladder specializations (encapsulation, change in shape, reduction in size) has occurred more than once within the subfamily. This conclusion would match observations by Bănărescu and Nalbant (1965), who noted the distribution of modified swim bladders among these fishes and did not find close links between those species which possessed such swim bladders, leading them to speculate that the condition had arisen multiple times within the group. Of the taxa with specialized swim bladders, we found some in the *Sarcocheilichthys* group and the others in the *Gobio-Gobiobotia* group. Within the *Sarcocheilichthys* group, *Coreius guichenoti* and the species of *Rhinogobio* are not closely related. Similarly, the genera with reduced and/or encapsulated air bladders in the *Gobio-Gobiobotia* group (*Gobiobotia-Xenophysogobio*, *Microphysogobio-Huigobio-Platysmacheilus*, and *Saurogobio*) do not form a monophyletic group. Liu (1940) originally recorded the presence of the specialized type of air bladder in *Abbottina*, which would contradict Bănărescu and Nalbant (1965). However, Liu (1940) examined *A. fukiensis*, which is currently recognized as *M. fukiensis*, and *A. obtusirostris*, which was classified as *M. obtusirostris* by Bănărescu and Nalbant (1966).

Our results support the exclusion of *Aulopyge*, *Discogobio*, and *Ptychidio* from Gobioninae. The placement of *Aulopyge* within the subfamily Cyprininae confirms the removal of *Aulopyge* from Gobioninae by Howes (1987). Of the two alternative hypotheses proposed by Howes (1987), our results point to the non-monophyly of *Barbus sensu lato* because *Barbus barbus* (a European species) and *B. trimaculatus* (an African species) are not sister taxa, with *A. huegelii* sister to *B. barbus*. The status of *Barbus* is beyond the scope of this study, please refer to recent molecular phylogenies

for more detailed examinations of the relationships of *Aulopyge* and the monophyly (or lack thereof) of *Barbus sensu lato* (e.g. Machordom and Doadrio, 2001; Tsigenopoulos et al., 2003). The resolution of *Discogobio* and *Ptychidio* in the subfamily Cyprininae is consistent with Chu (1935) and Bănărescu and Nalbant (1965), as well as more recent studies (e.g. Kong et al., 2007; Wang et al., 2007; Li et al., 2008; Tang et al., 2009; Yang and Mayden, 2010; Zheng et al., 2010). Although *Luciocyprinus* was not examined, its treatment in the literature (e.g. Bănărescu and Nalbant, 1965; Chen et al., 1984; Cui and Chu, 1986; Rainboth, 1991) calls for its exclusion from Gobioninae.

4.1. *Hemibarbus–Squalidus* group

The first gobionine lineage is identified only as the “*Hemibarbus–Squalidus* group” herein because there appears to be no family-group name based on *Belligobio*, *Hemibarbus*, *Squalidus*, or any of their junior synonyms. Describing a new family-group name for this clade is beyond the scope of this study. The composition of the clade and its place in the tree match the “*Hemibarbus* group” originally reported by Yang et al. (2006). Although the recognition of *Hemibarbus* as a member of Gobioninae has been questioned historically (e.g. Jordan and Fowler, 1903; Mori, 1934; Nikolskii, 1954; Bănărescu and Nalbant, 1965), the genus is a member of Gobioninae, as indicated by the majority of studies on this subfamily (e.g. Chu, 1935; Liu, 1940; Kryzhanovskiy, 1947; Ramaswami, 1955; Bănărescu and Nalbant, 1973; Luo et al., 1977; Chen et al., 1984; Kim, 1984; Hosoya, 1986; Bănărescu, 1992; Yue et al., 1998; Yang et al., 2006; Liu et al., 2010). *Hemibarbus* is sister to *Squalidus*, which indicates that either the third tooth row was secondarily gained in *Hemibarbus*, or that reduction to two tooth rows evolved twice within Gobioninae (in *Squalidus* and in the clade sister to the *Hemibarbus–Squalidus* group). Based on its phylogenetic position, *Hemibarbus barbatus* may be distinct from *H. labeo*, and not a synonym as previously thought (e.g. Kottelat, 2006; Bogutskaya et al., 2008). This would disagree with Kim et al. (2009), who found *H. barbatus* and *H. labeo* as sister taxa, though they were unable to examine *H. maculatus*, which we found as the sister species of *H. labeo* (Figs. 1 and 2). *Hemibarbus barbatus* (Temminck and Schlegel, 1846) became a secondary homonym (Art. 57.3.1; ICZN, 1999) of *B. barbatus* (Linnaeus 1758), when Günther (1868) placed both species in *Barbus* (Kottelat, 2006; Eschmeyer, 2010). Günther (1868) proposed *B. schlegelii* as a replacement name (Kottelat, 2006), but because the replacement name is not in use, *H. barbatus* would be retained if it were to be resurrected (Art. 59.3; ICZN, 1999). Additional study at the species-level within *Hemibarbus* is needed to determine the status of *H. barbatus*.

The phylogenetic position of *Belligobio nummifer* renders *Hemibarbus* paraphyletic. The relationships of *B. nummifer* to the species of *Hemibarbus* match those presented in Yang et al. (2006), except they found *Belligobio* as the sister group to *Hemibarbus* because they were unable to examine the *Hemibarbus* species (e.g. *Hemibarbus longirostris*, *Hemibarbus mylodon*) which are responsible for our placement of *B. nummifer* inside *Hemibarbus* (Figs. 1–3). Based on our results and its location in our tree, we assign *B. nummifer* to *Hemibarbus* as *H. nummifer*, allowing for a monophyletic *Hemibarbus*. Our results and those of Yang et al. (2006) suggest that *Belligobio* is a junior synonym of *Hemibarbus*, which would corroborate earlier treatments of *Belligobio* as a subgenus of *Hemibarbus* (e.g. Bănărescu and Nalbant, 1973; Bănărescu, 1992). However, without examining the type species of *Belligobio* (*B. eristigma*), the status of *Belligobio* cannot be determined. Given this situation, we are provisionally assigning *Belligobio* to the “*Hemibarbus–Squalidus* group” based on its classification in previous studies, pending investigation of the phylogenetic placement of *B. eristigma*.

4.2. Tribe *Sarcocheilichthyini*

We recognize the second lineage as the tribe *Sarcocheilichthyini* Kryzhanovskiy 1947, which includes *Coreius*, *Coreoleuciscus*, *Gnathopogon*, *Gobiocypris*, *Ladislavia*, *Paracanthobrama*, *Pseudorasbora*, *Pseudopungtungia*, *Pungtungia*, *Rhinogobio*, and *Sarcocheilichthys*. With the exception *Rhinogobio*, these genera were removed from Gobioninae *sensu* Hosoya (1986), an exclusion that our results do not support. Instead, our results corroborate Rainboth (1991) and subsequent workers (e.g. Bănărescu, 1992; Naseka, 1996) who restored these taxa to Gobioninae. Of those studies, the composition of our *Sarcocheilichthyini* most closely corresponds with that of Bănărescu (1992), who identified one lineage of gudgeons as an “aberrant group of genera (with terminal mouths)” that included *Pseudorasbora*, *Coreoleuciscus*, *Pungtungia*, *Pseudopungtungia*, *Ladislavia*, *Sarcocheilichthys*, and *Gnathopogon*. Three sarcocheilichthyin genera (as recognized herein) were not included in his “aberrant” group: *Coreius*, which Bănărescu classified as an “[i]solated aberrant genus” in its own group; *Gobiocypris*, which was considered a danionine at the time (see below); and *Paracanthobrama*, which was classified as a subgenus of *Hemibarbus*. Rainboth (1991) included only *Coreius*, *Gnathopogon*, *Pseudorasbora*, and *Sarcocheilichthys* in his *Sarcocheilichthyini*, with *Paracanthobrama* and *Rhinogobio* in his Gobionini. Without examining *Rhinogobio nasutus* (type species of *Megagobio*), we are unable to comment on its status as a distinct genus as proposed by Naseka (1996), a decision that later workers reversed, returning *Megagobio* to the synonymy of *Rhinogobio* (Bănărescu, 1997; Yue et al., 1998). The relationships within *Sarcocheilichthys* match the pattern of relationships found by Zhang et al. (2008), in the areas where this study and that one overlapped in taxon sampling.

The tribe we recovered corresponds to the “*Sarcocheilichthys* group” of Yang et al. (2006), with the additions of *Coreoleuciscus*, *Ladislavia*, and *Pseudopungtungia*. With the exception of *Gnathopogon* and *Pseudorasbora*, the genera of *Sarcocheilichthyini* are recovered as monophyletic groups, some by virtue of monotypy. In the parsimony analysis, *Gobiocypris* is found as the sister group of a monophyletic *Gnathopogon* which concurs with the results of Yang et al. (2006), whereas the likelihood and Bayesian analyses found *Gobiocypris* within *Gnathopogon* (Figs. 2 and 3). Although *Gobiocypris* was originally described in Danioninae and allied to *Aphyocypris* (Ye and Fu, 1983), it is apparent from the results of this study and others (He et al., 2004; Yang et al., 2006; Rüber et al., 2007; Liu et al., 2010; Tang et al., 2010) that *Gobiocypris* is a member of the Gobioninae. The placement of *P. elongata*, sister to *Coreoleuciscus* in the parsimony tree (Fig. 1) and sister to the *Pseudopungtungia* and *Pungtungia* clade in the likelihood tree (Fig. 2), renders *Pseudorasbora* non-monophyletic; the placement is equivocal in the Bayesian tree (Fig. 3). Expanded taxon sampling within *Pseudorasbora* and additional sequence data from *P. elongata* will be necessary to settle this issue. The lack of monophyly agrees with the results of Yang et al. (2006), who did not find a monophyletic *Pseudorasbora* either, recovering *P. elongata* with *Pungtungia* in their maximum likelihood tree and with *Paracanthobrama* in their parsimony tree. Bănărescu and Nalbant (1965) noted the similarities of *P. elongata* to *Pungtungia* (e.g. body shape, lateral stripe), divergent from other members of *Pseudorasbora*, and positioned it as an intermediate form between *Pseudorasbora* and *Pungtungia*. This appears to favor the maximum likelihood resolution, as sister to a *Pungtungia–Pseudopungtungia* clade (Fig. 2).

In the parsimony analyses, this group showed the most instability after the removal of the GenBank-only taxa. This is likely due to the number of genera (four; *Coreius*, *Gobiocypris*, *Paracanthobrama*, *Pseudopungtungia*) that were represented only by GenBank sequences, coupled with the total number of taxa from this clade that were excluded in the reduced data set (14 terminals were deleted).

We cannot explain the placement of *Ladislavia taczanowskii* (monotypic genus) and *R. typus* (lone representative of *Rhinogobio*) outside of Sarcocheilichthyini in the reduced parsimony tree (Supplementary content). Furthermore, the unexpected positions of *Ladislavia* and *Rhinogobio* destabilized the relationships of the *Gobio*–*Gobiobotia* clade, reducing much of that group to an unresolved polytomy. However, because the full parsimony tree (Fig. 1) and all other trees (Figs. 2 and 3; Supplementary content) agree on the inclusion of *Ladislavia* and *Rhinogobio*, we have classified both genera as members of the tribe Sarcocheilichthyini (Table 2).

4.3. Tribe Gobionini

The final lineage contains the remaining gobionine genera and we recognize this assemblage as the tribe Gobionini Bleeker 1863, with the following genera: *Abbottina*, *Biwia*, *Gobio*, *Gobiobotia*, *Huigobio*, *Mesogobio*, *Microphysogobio*, *Platysmacheilus*, *Pseudogobio*, *Romanogobio*, *Saurogobio*, and *Xenophysogobio*. This clade exhibits the most discrepancies between the topologies (Figs. 1–3). The likelihood (Fig. 2) and Bayesian (Fig. 3) trees are nearly identical, only differing on the monophyly of *Platysmacheilus*. Despite the areas of conflict between them, all three trees agree on the composition of this group. The tribe Gobionini corresponds to the clade formed by the “*Gobio* group” and “*Pseudogobio* group” of Yang et al. (2006). We place all of these taxa in one tribe and choose not to recognize a fourth tribe because the senior available name for the “*Pseudogobio* group” clade would be *Gobiobotiini* Mori, 1933 (see below) and, given the weak support for the position of *Gobiobotia*, applying that name would be premature. These species can be characterized as the specialized bottom-dwelling gobionine species. Many of the taxa that possess a modified swim bladder are found in this group. The presence of this type of swim bladder is a feature that has been reported by previous workers and is considered part of a suite of characters that accompanied the evolution of a rheophilic, benthic lifestyle, although not all benthic species are rheophilic or have a highly modified swim bladder (Bănărescu and Nalbant, 1973).

Many of the monophyly issues are found in this tribe. *Gobio* is not monophyletic because of the placement of putative members of *Acanthogobio* and *Mesogobio*. *Acanthogobio guentheri*, the type (and only) species of *Acanthogobio*, is found nested within *Gobio*. In order to preserve a monophyletic *Gobio* and reflect their phylogenetic relationships, we hereby place *Acanthogobio* Herzenstein 1892 in the synonymy of *Gobio* Cuvier 1816 and recognize its single species as *Gobio guentheri*. The absence of *Mesogobio lachneri* (type of *Mesogobio*) from our study precludes a decision on the status of *Mesogobio*. However, based on the position of *Mesogobio tumensis* within *Gobio*, we recommend the classification of that species as a member of *Gobio*, as *G. tumensis*. In finding *Gobio tenuicarpus* nested within *Gobio*, sister to *G. guentheri* (Figs. 1–3), our results support those who referred *R. tenuicarpus* to *Gobio* (e.g. Kottelat, 2006; Yang et al., 2006; Bogutskaya et al., 2008; Liu et al., 2010). The placement of the species identified on GenBank (AF090751) as *Gobio banarescui* (previously recognized as a distinct species by Kottelat, 1997) within *Romanogobio*, sister to *R. uranoscopus*, suggests that it is a member of the latter genus. This result would corroborate Kottelat and Freyhof (2007), who classified that species in *Romanogobio* and placed it in the synonymy of *R. elimeus*. *Gobio* and *Romanogobio* are reciprocally monophyletic following the taxonomic changes proposed above, which is compatible with Naseka's (1996) recognition of *Romanogobio* as a distinct genus. These two genera include all of the species from this subfamily that occur natively in Europe.

The other clade within Gobionini is found by all three analyses in terms of composition but with some variation in its relation-

ships (Figs. 1–3). A grouping like this one has been proposed before in the literature. Yu and Yue (1996) identified a group of “*Pseudogobio* fishes” with eight genera: *Pseudogobio*, *Saurogobio*, *Abbottina*, *Biwia*, *Rostrigobio* [= *Microphysogobio*], *Microphysogobio*, *Platysmacheilus*, and *Huigobio*. Yue et al. (1998) also recognized a “*Pseudogobionid*” group, with *Pseudogobio*, *Abbottina*, *Microphysogobio*, *Rostrigobio* [= *Microphysogobio*], *Platysmacheilus*, *Huigobio*, and *Saurogobio*. We found that *Gobiobotiinae* of Mori (1933) and others (*Gobiobotia* and *Xenophysogobio*), varies in its monophyly and placement. Although *Gobiobotia* was found to be a monophyletic (Figs. 1–3), *Gobiobotia* and *Xenophysogobio* do not form a monophyletic group in the parsimony tree, with *Xenophysogobio* appearing as the sister group of a monophyletic *Saurogobio*. However, *Gobiobotia* and *Xenophysogobio* form a clade in the likelihood and Bayesian trees, as the sister group to the remaining taxa. Recovery of a *Gobiobotia*–*Xenophysogobio* clade is consistent with previous literature. However, resolving the status and position of *Gobiobotia* and *Xenophysogobio* will require examination of more representatives of both genera. The placement of these two genera in the Gobionini is also consistent with previous work. Despite assigning *Gobiobotia* to its own distinct group within Gobioninae, Ramaswami (1955) identified a series of characters that united *Gobiobotia*, *Saurogobio*, *Pseudogobio*, and *Abbottina*, characters which Bănărescu and Nalbant (1965) also cited. Bănărescu and Nalbant (1965) recorded several additional characters in support of their phyletic lineage comprising *Pseudogobio*, *Abbottina*, *Saurogobio*, *Microphysogobio* (including *Huigobio*), and *Biwia*. Like Ramaswami (1955), Bănărescu and Nalbant (1965) separated *Gobiobotia* from other gobionines, but they did note that it shared similarities (e.g. smooth lips, two rows of hooked teeth) with *Gobio*, which they attributed to a possible case of convergence. Hosoya (1986) recognized a group of what he called “true bottom dwellers” that included *Gobiobotia* as well as *Pseudogobio*, *Saurogobio*, *Microphysogobio*, and *Biwia*. Bănărescu (1992) identified a *Gobiobotia*–*Pseudogobio* group comprising those two genera and *Abbottina*, *Saurogobio*, *Biwia*, and *Microphysogobio*.

Platysmacheilus may be paraphyletic. Although it is monophyletic in the Bayesian tree (Fig. 3), the position of *Platysmacheilus* sp. varies between the parsimony and likelihood trees, rendering *Platysmacheilus* paraphyletic in both trees (Figs. 1 and 2). The status of *Microphysogobio* is likewise problematic because its putative member species are found to be broadly polyphyletic, with *Biwia*, itself not monophyletic (see below), and *Huigobio* recovered within *Microphysogobio*. In earlier studies, *Huigobio* has been treated as a synonym (e.g. Bănărescu and Nalbant, 1966, 1973) or as a subgenus (Bănărescu, 1992; Bogutskaya et al., 2008) of *Microphysogobio*. Even though it has been synonymized with *Microphysogobio* (Kottelat, 2006; Bogutskaya et al., 2008), *Rostrigobio* does not appear to be monophyletic as a subgenus either, because *M. amurensis* and *M. liaohensis*, its two putative members, were not recovered as sister species. We follow Hosoya (1986) and Kawase and Hosoya (2010) in recognizing *Abbottina springeri* as a species of *Biwia*, contrary to its original description by Bănărescu and Nalbant (1973). Even with this taxonomic change, our results do not yield a monophyletic *Biwia*, because *B. springeri* is found apart from *B. zezera* (type of *Biwia*) and *B. yodoensis*. Restriction of *Biwia sensu stricto* to *B. zezera* and its closest relatives is the obvious solution. However, that still leaves the status of “*Biwia*” *springeri* indeterminate. A generic assignment for that species is impossible until the *Huigobio*–*Microphysogobio* situation is resolved, which is complicated by the unknown position of the type species of both genera, *H. chensienensis* and *M. hsinglungshanensis*. Those species must be placed into a phylogenetic context before the monophyly and limits of *Huigobio* and *Microphysogobio* can be addressed. Those taxonomic decisions will have repercussions for “*Biwia*” *springeri* and *Biwia sensu stricto*. Of the available generic names found in this clade,

Biwia Jordan and Fowler 1903 is the senior name and would have priority over *Huigobio* Fang 1938, *Microphysogobio* Mori 1934, and *Rostrogobio* Taranetz 1937, in case of synonymy. Given these unresolved taxonomic issues and the polyphyly of *Microphysogobio*, a revision of the genus is overdue.

Based on the position of *G. pappenheimi* (type species of *Gobiobotia*), we place *Gobiobotiini* Mori 1933 in the synonymy of *Gobionini* Bleeker 1863. Furthermore, based on the phylogenetic placement of *Pseudogobio esocinus* (type species of *Pseudogobio*) and *S. dabryi* (type species of *Armatogobio*), we place *Pseudogobionini* Kryzhanovskiy 1947 and *Armatogobionini* Kryzhanovskiy 1947 in the synonymy of *Gobionini* Bleeker 1863. These family-group names remain available to future workers if they wish to classify the diversity within the tribe with greater resolution, once gobioid intrarelationships are better understood. Based on the close relationship between *Gobiobotia*, *Pseudogobio*, and *Saurogobio*, resurrection of these junior synonyms may cause nomenclature issues. In the event of synonymy, priority is clear among these three names: *Gobiobotiini* Mori 1933 has precedence over the other two names; and, although they were described simultaneously, *Armatogobionini* Kryzhanovskiy 1947 takes precedence over *Pseudogobionini* Kryzhanovskiy 1947 because *Armatogobionini* was proposed at a higher rank (Art. 24.1; ICZN, 1999).

4.4. Sister group

The sister group of *Gobioninae* remains uncertain. The parsimony tree points to a clade of *Acheilognathinae* and *Tanichthys* + *Tinca* (Fig. 1), whereas the other two trees point to *Acheilognathinae* alone (Figs. 2 and 3). Historically, the phylogenetic placement of *Tanichthys* and *Tinca* has been difficult, with other studies finding these genera in varying positions within *Cyprinidae* (e.g. He et al., 2001; Saitoh et al., 2006; Mayden et al., 2008, 2009; Chen and Mayden, 2009; Fang et al., 2009; Tang et al., 2010). Although the taxonomic position of these taxa is not the focus of this paper, their resolution will have implications for the sister group of *Gobioninae*. With only four species total in these two genera (three in *Tanichthys* and one in *Tinca*; Eschmeyer, 2010), it is unlikely that increased taxon sampling will be an effective strategy. Despite the use of a variety of loci (mitochondrial, nuclear, both) and tree reconstruction methods (Bayesian, maximum likelihood, parsimony), previous studies have had little consensus on the relationships of these two enigmatic genera. The likelihood and Bayesian resolutions support those who have proposed *Acheilognathinae* by itself as the sister group of *Gobioninae* (e.g. Chen et al., 1984; Cavender and Coburn, 1992; Yang et al., 2006; Chen et al., 2008; Mayden et al., 2008; Tang et al., 2010), whereas the parsimony resolution supports a sister group that includes *Acheilognathinae* as part of a larger clade with *Tanichthys* (e.g. Wang et al., 2007). Both results contradict studies that have proposed *Leuciscinae* as the sister group (e.g. Gilles et al., 2001; Liu and Chen, 2003; Thai et al., 2007; Li et al., 2008; Fang et al., 2009). Similarities in scale characters (Chu, 1935) and the presence of ovipositors in some members of both subfamilies would also suggest *Acheilognathinae* as the sister group. However, Bănărescu and Coad (1991: 145) did not think that these ovipositors and the associated reproductive behavior (egg laying in mussels) were indicative of a close relationship between bitterlings and gudgeons.

5. Conclusions

The phylogenies presented in this study provide a better picture of the relationships within the subfamily *Gobioninae*. Increasing data and taxon sampling has improved our knowledge of this group, particularly in regards to the composition of the major

lineages within *Gobioninae*. There is evidence that the subfamily is divided into three monophyletic assemblages: a currently unnamed group comprising *Hemibarbus* and *Squalidus*, and possibly *Belligobio*; the tribe *Sarcocheilichthyini*; and the tribe *Gobionini*. Within the latter group, there is a clear split into two lineages: one with *Gobio* and *Romanogobio* (the only two gudgeon genera with species native to Europe), and possibly *Mesogobio*; and the other with *Abbottina*, *Biwia*, *Gobiobotia*, *Huigobio*, *Microphysogobio*, *Platysmacheilus*, *Pseudogobio*, *Saurogobio*, and *Xenophysogobio*. The results presented herein match those from Yang et al. (2006), Kim et al. (2009), and Liu et al. (2010), the previous molecular studies focusing on gobioid relationships. The distribution of specialized swim bladders indicates that that feature has evolved independently more than once within the subfamily. Likewise, the reduction of tooth rows from two to one, observed in *Abbottina*, *Coreius*, *Microphysogobio* (in part), *Pseudorasbora*, and *Saurogobio* (Chu, 1935; Bănărescu and Nalbant, 1966), shows that condition must have evolved independently multiple times.

Despite a better understanding of this group, more work needs to be done, as there are areas of weak support and conflict between the different trees. Inclusion of the putative gobioid genera that we were unable to examine, *Belligobio*, *Mesogobio*, *Paraleucogobio*, and *Parasqualidus*, is of paramount importance. Study of these taxa is necessary to establish their place within the gobioid phylogeny as well as to determine their taxonomic status. More work is necessary to resolve some of the monophyly issues surrounding several genera (e.g. *Microphysogobio*, *Platysmacheilus*, *Pseudorasbora*). These problems are most acute within the tribe *Gobionini*, among *Gobiobotia* and allies, where the monophyly of *Biwia* and *Microphysogobio*, and the status of *Huigobio*, all remain in doubt. Investigation of type species, as well as better taxon sampling overall, will be needed to sort out these outstanding issues. The evolution of the swim bladder system merits further investigation. Additional studies of the relationships among the subfamilies of *Cyprinidae* are needed to identify the sister group of *Gobioninae* with confidence.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.05.022.

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