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## Molecular Phylogenetics of the Clingfishes (Teleostei: Gobiesocidae)— Implications for Classification

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**Gobiesocidae are a moderate-sized family (currently 182 species, 51 genera) of predominantly coastal marine fishes, commonly referred to as clingfishes. Depending on the classification adopted, the species and genera of clingfishes are organized either across ten subfamilies, based on a classification scheme introduced in the 1950s (“traditional” classification, comprising Aspasminae, Cheilobranchinae, Chorisochisminae, Diademichthyinae, Diplocrepinae, Gobiesocinae, Haplocylicinae, Lepadogastrinae, Protogobiesocinae, and Trachelochisminae), or just two subfamilies, in a classification scheme adopted only recently (“reduced” classification, comprising Cheilobranchinae and Gobiesocinae). We investigated the phylogenetic relationships among members of the family Gobiesocidae using both mitochondrial and nuclear DNA sequence data to assess whether the alternative classification schemes (traditional and reduced) are compatible with inferred evolutionary relationships. Phylogenetic hypotheses are derived from maximum-likelihood and Bayesian analyses of a seven-gene concatenated dataset (2 mitochondrial and 5 nuclear markers; 4,857 bp) compiled from individuals representing 82 (of 182) species, 42 (of 51) genera, and 10 (of 10) subfamilies of the Gobiesocidae. Although our investigation provides strong support for the monophyly of the Gobiesocidae, multiple subfamilies of the traditional classification (Aspasminae, Diademichthyinae, Diplocrepinae, Gobiesocinae, and Trachelochisminae), one subfamily of the reduced classification (Gobiesocinae), and multiple genera (*Aspasmichthys*, *Cochleocephs*, *Lepadogaster*, and *Lepadichthys*) are resolved as non-monophyletic groups. Based on our results and the results of previous studies, we recommend a systematic reassignment of genera between subfamilies, of which we recognize nine: Cheilobranchinae, Chorisochisminae, Diademichthyinae, Diplocrepinae, Haplocylicinae, Gobiesocinae, Lepadogastrinae, Protogobiesocinae, and Trachelochisminae. Membership of the Lepadogastrinae is unchanged from previous usage; the Cheilobranchinae are expanded to contain additional genera from southern Australia, including those placed previously in the Aspasminae (*Nettorhamphos* and *Posidonichthys*) and the Diplocrepinae (*Barryichthys*, *Cochleocephs*, and *Parvicrepis*); the Aspasminae are placed in the synonymy of the Diademichthyinae and all genera placed in the former (excluding *Modicus* and *Posidonichthys*) are transferred to the latter; the Diplocrepinae are restricted to *Diplocrepis*; *Eckloniaichthys scylliorhiniceps* is transferred from the Gobiesocinae to the Chorisochisminae; Gobiesocinae are restricted to the New World members of this group (*Acyrtops*, *Acyrtus*, *Arcos*, *Derillissus*, *Gobiesox*, *Rimicola*, *Sicyases*, and *Tomicodon*); the Haplocylicinae are expanded to include additional genera from New Zealand (*Gastrocyathus*, *Gastrocymba*, and *Gastroscyphus*); the Protogobiesocinae are expanded to accommodate three genera of deep water**

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**taxa (*Gymnoscyphus*, *Kopua*, and *Protogobiesox*); and the Trachelochisminae are restricted to *Dellichthys* and *Trachelochismus*. Four genera (*Aspasmogaster*, *Conidens*, *Creocele*, and *Modicus*) of uncertain placement are not assigned to any subfamily herein and are considered *incertae sedis* within the Gobiesocidae.**

**C**LINGFISHES (family Gobiesocidae) are predominantly marine fishes, distributed worldwide in tropical and temperate regions of the Atlantic, Pacific, and Indian Oceans (Briggs, 1955). Though most commonly encountered in the intertidal and subtidal zones, some species reach greater depths (down to 560 m; Hastings and Conway, 2017), and seven species are found in freshwaters in Central and northern South America and adjacent Pacific and Caribbean islands (Briggs and Miller, 1960; Conway et al., 2017a). All clingfishes, except those in the genus *Alabes*, have a well-developed ventral adhesive disc. This is a complex structure, which is made up of elements of both the pectoral and pelvic girdles, with connection to the skull (Guitel, 1888). Clingfish discs can generate adhesive forces 80–1,200 times the body weight of an individual and allow gobiesocids to adhere to even irregular and heavily fouled substrates in high energy zones (Wainwright et al., 2013; Ditsche et al., 2014).

Though these fishes are commonly considered small, drab inhabitants of the intertidal zone, in reality, they are remarkably variable and exhibit a stunning range of morphological and ecological diversity (Fig. 1). The smallest clingfishes reach only 10–13 mm standard length (SL) as adults (e.g., *Derillissus*; Briggs, 1969; Sparks and Gruber, 2012), whereas the largest are 30 times larger at up to 300 mm SL, such as the South American *Sicyases sanguineus* and the South African Rocksucker, *Chorisochismus dentex* (Briggs, 1955). The adhesive disc can be a prominent structure that completely spans the belly, comparatively small, or even absent (Springer and Fraser, 1976). It varies also in complexity and may comprise either a single complete circular structure (single disc of Briggs, 1955) or an incomplete arc with a small, inner disc (double disc of Briggs, 1955). Males of many species have prominent genital papillae, and in one species (*Eckloniaichthys scylliorhiniceps*), this papilla is used during internal fertilization (Smith, 1943; Wilkes, 2013). Clingfishes are voracious predators of small invertebrates and fishes. Some specialize in feeding on tightly attached molluscs like limpets (Stobbs, 1980), others feed on echinoderms or their parts (Sakashita, 1992; Conway et al., 2018), whereas others feed almost exclusively on the eggs of other fishes (Hirayama et al., 2005). In nearshore habitats, clingfishes can be found under rocks or rubble, within oyster beds or fields of maerl, under urchins or between their spines, or attached to the blades and stipes of macroalgae (Patzner, 1999; Hofrichter and Patzner, 2000). Others are known only from seagrass beds (Gould, 1965; Hutchins, 1983, 1991, 2008); whereas, members of the genus *Discotrema* are obligate inhabitants of the arms of crinoids (Briggs, 1976; Randall, 2005; Craig and Randall, 2008). Specimens trawled from deeper water come from rubble bottoms and likely live in the interstices of coral rubble, cobble, and shell hash (Böhlke and Robins, 1970; Hastings and Conway, 2017). Seven members of the genus *Gobiesox* are also found exclusively in freshwater as adults and exhibit an amphidromous lifestyle (Conway et al., 2017a; Frotté et al., 2020).

The 182 species and 51 genera of clingfishes have traditionally been divided into eight subfamilies (Aspaminae, Chorisochisminae, Diademichthyinae, Diplocrepinae, Gobie-

socinae, Haplocyclicinae, Lepadogastrinae, and Trachelochisminae; Table 1; Fig. 2A) solely based on a three-character classification system introduced by Briggs (1955). This “traditional” classification distributes species between subfamilies based on the type of adhesive disc present (single or double), the number of gill arches with gill filaments (three or four), and the arrangement of the gill membranes (united with or free from the isthmus). For example, following Briggs (1955), a clingfish with gill filaments on three arches, the gill membranes attached to the isthmus, and a “single” adhesive disc would automatically be considered a member of the Diademichthyinae (Briggs, 1955: 10). Subsequent to Briggs (1955), Springer and Fraser (1976) transferred the discless Australian shore eels of the genus *Alabes* (previously placed in the family Cheilobranchidae) to the Gobiesocidae. *Alabes* did not fit into the three-character system of Briggs (1955) and were placed in a new, ninth subfamily Cheilobranchinae (Briggs, 1993). Recently, Fricke et al. (2017) erected a tenth subfamily, Protogobiesocinae, based on perceived asymmetry in body plan. This subfamily included two taxa, the newly described *Protogobiesox asymmetricus* and *Lepadicyathus mendeleevi*, which had earlier been placed in the Aspaminae (Prokofiev, 2005).

With the continued discovery of new species of clingfishes (19 since 2015; Fujiwara and Motomura, 2020a, in this volume), it has become increasingly difficult to place new taxa into the subfamilies defined by Briggs (1955), the composition of many of which have been criticized (e.g., Smith, 1957; Böhlke and Robins, 1970; Briggs, 1993; Almada et al., 2008). Other morphological characteristics beyond those used by Briggs have often been disregarded when new taxa are added to an existing subfamily, and, in many cases, these new additions differ markedly from the other members of a group. For example, *Modicus* was placed in the Aspaminae by Hardy (1983), but it differs notably from all other members of this group by the number of gill rakers and pectoral-fin rays and features of oral dentition (Hardy, 1983). On other occasions, the decision to place a new taxon within a particular subfamily appears to have been arbitrary. For example, *Derillissus* was added to the Gobiesocinae (Briggs, 1969) even though it exhibits the characters of the Diademichthyinae (Böhlke and Robins, 1970). Even Briggs (1993) recognized the weakness of his traditional scheme in the placement of *Posidonichthys* in the Aspaminae, when he asserted that although it exhibits the three character states of the subfamily, it had many other characters that suggested it should be in the Diplocrepinae, despite lacking the free isthmus (Briggs, 1993). Several recently described genera (*Barryichthys*, *Flexor*, and *Nettorhamphos*; Conway et al., 2017b, 2018, 2019) were intentionally not assigned to any available subfamily, yet these have been subsequently assigned without comment to either the Aspaminae or Diplocrepinae by Fricke et al. (2020).

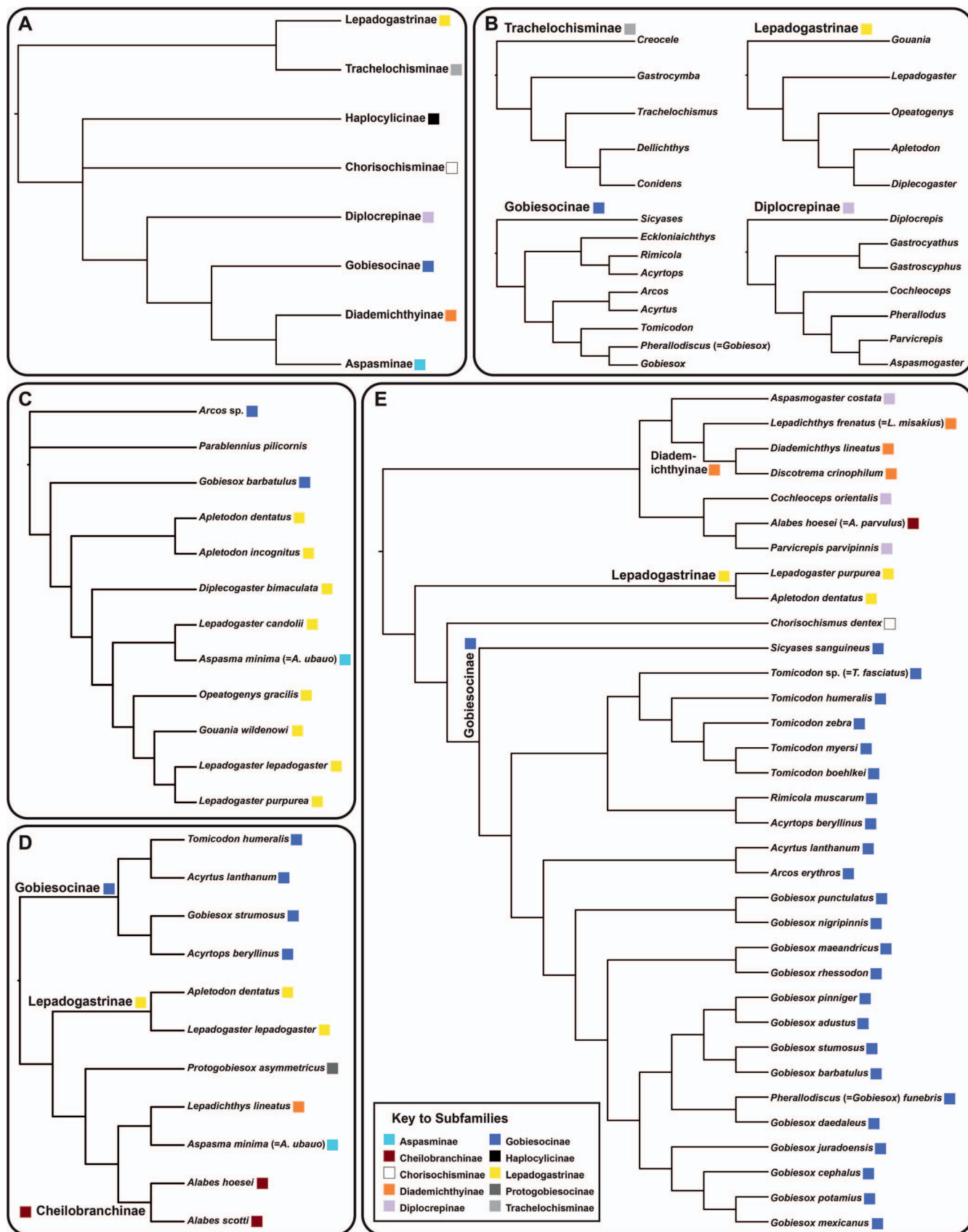
An alternative classification scheme for the Gobiesocidae comprising two subfamilies only has been used by a few authors (e.g., Eschmeyer, 1998 [since 2015, Fricke et al., 2020 have used the more traditional classification scheme of Briggs, 1955]; Van der Laan et al., 2014; Nelson et al., 2016). This alternative (“reduced”) classification (Table 1) divides



**Fig. 1.** Representatives of the Gobiesocidae. (A) *Trachelochismus pinnulatus*, Trachelochisminae (photograph by P. Caiger); (B) *Dellichthys morelandi*, Trachelochisminae (P. Caiger); (C) *Kopua minima*, Trachelochisminae (K. Fujiwara); (D) *Haplocylix littoreus*, Haplocylicaenae (K. Conway); (E) *Lepadogaster candolii*, Lepadogastrinae (M. Wagner); (F) *Gouania willdenowi*, Lepadogastrinae (S. Iglésias); (G) *Chorisochismus dentex*, Chorisochisminae (M. Nieuwoudt); (H) *Diplocrepis puniceus*, Diplocrepinae (D. Hoffmann); (I) *Gastrocyathus gracilis*, Diplocrepinae (K. Conway); (J) *Gobiesox cephalus*, Gobiesocinae (K. Conway); (K) *Eckloniaichthys scylliorhiniceps*, Gobiesocinae (G. Zsilavec); (L) *Aspasma ubauo*, Aspasminae (S. Yamamoto); (M) *Discotrema crinophilum*, Diademichthyinae (M. Erdmann); (N) *Diademichthys lineatus*, Diademichthyinae (M. Erdmann); (O) *Alabes parvula*, Cheilobranchinae (B. Hutchins).

**Table 1.** Subfamilies and genera of the Gobiessocidae under the "Reduced" and "Traditional" classification schemes, and a revised scheme based on the present study. An asterisk (\*) accompanies those genera not represented in the molecular data set of the present study under the Revised scheme. Generic names listed in quotation marks under the Revised scheme are those found to be non-monophyletic in the results of the present study.

"Reduced"		"Traditional"		Revised
Subfamily	Genera	Subfamily	Genera	Subfamily
		Aspsaminae	<i>Aspasma</i> , <i>Aspasmichthys</i> , <i>Aspasmodes</i> , <i>Briggsia</i> , <i>Liobranchia</i> , <i>Lissonanchus</i> , <i>Modicus</i> , <i>Nettorhamphos</i> , <i>Pherallodichthys</i> , <i>Posidonichthys</i>	
Cheilobranchinae	<i>Alabes</i>	Cheilobranchinae	<i>Alabes</i>	Cheilobranchinae
		Chorisoichisminae	<i>Chorisoichismus</i>	
		Diademichthyinae	<i>Diademichthys</i> , <i>Discoatrema</i> , <i>Lepadichthys</i> , <i>Unguitrema</i>	Chorisoichisminae Diademichthyinae
		Diplocrepinae	<i>Aspasmogaster</i> , <i>Baryichthys</i> , <i>Cochleocephs</i> , <i>Diplocrepis</i> , <i>Flexor</i> , <i>Gastrocyathus</i> , <i>Gastrocymba</i> , <i>Gastroscyphus</i> , <i>Parvicrepis</i> , <i>Pherallodus</i> , <i>Propherallodus</i>	
Gobiesocinae	<i>Acyrtops</i> , <i>Acyrtus</i> , <i>Arcos</i> , <i>Apletodon</i> , <i>Aspasma</i> , <i>Aspasmichthys</i> , <i>Aspasmodes</i> , <i>Aspasmogaster</i> , <i>Briggsia</i> , <i>Chorisoichismus</i> , <i>Cochleocephs</i> , <i>Conidens</i> , <i>Creocele</i> , <i>Dellichthys</i> , <i>Derilissus</i> , <i>Diademichthys</i> , <i>Diplecogaster</i> , <i>Diplocrepis</i> , <i>Discoatrema</i> , <i>Eckloniaichthys</i> , <i>Gastrocyathus</i> , <i>Gastrocymba</i> , <i>Gobiesox</i> , <i>Gouania</i> , <i>Gymnoscyphus</i> , <i>Haplocylix</i> , <i>Kopua</i> , <i>Lepadichthys</i> , <i>Lepadicyathus</i> , <i>Lepadogaster</i> , <i>Lecanogaster</i> , <i>Liobranchia</i> , <i>Lissonanchus</i> , <i>Modicus</i> , <i>Opeatogenys</i> , <i>Parvicrepis</i> , <i>Pherallodus</i> , <i>Pherallodichthys</i> , <i>Posidonichthys</i> , <i>Propherallodus</i> , <i>Protogobiesox</i> , <i>Rimicola</i> , <i>Sicyases</i> , <i>Tomiconodon</i> , <i>Trachelochismus</i> , <i>Unguitrema</i>	Gobiesocinae	<i>Acyrtops</i> , <i>Acyrtus</i> , <i>Arcos</i> , <i>Derilissus</i> , <i>Eckloniaichthys</i> , <i>Gobiesox</i> , <i>Rimicola</i> , <i>Sicyases</i> , <i>Tomiconodon</i>	Gobiesocinae
		Haplocydicinae	<i>Haplocylix</i> , <i>Gymnoscyphus</i>	Haplocydicinae
		Lepadogastrinae	<i>Apletodon</i> , <i>Diplecogaster</i> , <i>Gouania</i> , <i>Lepadogaster</i> , <i>Lecanogaster</i> , <i>Opeatogenys</i>	Lepadogastrinae
		Protogobiesocinae	<i>Lepadicyathus</i> , <i>Protogobiesox</i>	Protogobiesocinae
		Trachelochisminae	<i>Conidens</i> , <i>Creocele</i> , <i>Dellichthys</i> , <i>Kopua</i> , <i>Trachelochismus</i>	Trachelochisminae Incertae sedis
				<i>Alabes</i> , <i>Baryichthys</i> , <i>"Cochleocephs"</i> , <i>Nettorhamphos*</i> , <i>Parvicrepis</i> , <i>Posidonichthys</i> , undescribed Genus A, undescribed Genus C <i>Chorisoichismus</i> , <i>Eckloniaichthys</i> <i>Aspasma</i> , <i>"Aspasmichthys"</i> , <i>Aspasmodes*</i> , <i>Briggsia*</i> , <i>Diademichthys</i> , <i>Discoatrema</i> , <i>Flexor</i> , <i>"Lepadichthys"</i> , <i>Lepadicyathus*</i> , <i>Liobranchia*</i> , <i>Lissonanchus*</i> , <i>Pherallodus</i> , <i>Pherallodichthys</i> , <i>Propherallodus*</i> , <i>Unguitrema*</i> <i>Diplocrepis</i>



**Fig. 2.** Previously published hypotheses of gobiesocid relationships. (A) Dendrogram of subfamilial relationships provided by Briggs (1955: 144, dendrogram 1). (B) Relationships as depicted in dendrograms 2–5 from Briggs (1955: 146, 148) representing relationships within subfamilies Trachelochisminae, Lepadogastrinae, Gobiesocinae, and Diplocrepinae. (C) Topology of lepadogastrine clingfishes derived from Bayesian analysis of mitochondrial genes (12S and 16S) by Almada et al. (2008: fig. 1). (D) Topology derived from maximum-likelihood analysis of six-gene concatenated dataset by Fricke et al. (2017: fig. 1). (E) Topology derived from Bayesian analysis of six-gene concatenated dataset by Conway et al. (2017a: fig. 1). Original branch support values associated with topologies in C–E omitted. Subfamily membership indicated by colored boxes (explained in key).

gobiesocids between the Cheilobranchinae (including the discless Australian shore eels of genus *Alabes*) and the Gobiesocinae (non-Briggs; including all remaining genera), but it is problematic because previous phylogenetic studies of gobiesocids have not recovered *Alabes* as the sister taxon to all remaining gobiesocids (Conway et al., 2017a; Fricke et al., 2017; Fig. 2D, E). A large-scale phylogenetic study of the Gobiesocidae is overdue and urgently needed to better understand the intrarelationships of these fishes given both the rate at which new taxa continue to be described and also the growing interest in the structure and function of the clingfish adhesive system from the broader scientific community (Ditsche et al., 2014, 2017; Ditsche and Summers, 2019; Sandoval et al., 2019, 2020).

In this study, we use a multi-locus approach to investigate the phylogenetic relationships of 82 species of clingfishes, representing 42 genera and all 10 recognized subfamilies of the Gobiesocidae. Our main objective was to assess whether the two alternative classification schemes currently used ([1] the traditional classification with ten subfamilies based upon Briggs, 1955; and [2] the reduced classification with two subfamilies only; Table 1) are compatible with the evolutionary relationships of the group, as inferred from our dataset. We also provide a brief review of the systematic literature on the subfamilies of the Gobiesocidae, with emphasis on generic membership, and introduce a revised systematic assignment of genera (Table 1) that we hope will serve as a useful starting point for future phylogenetic investigations and taxonomic revisions of this interesting and highly specialized group of fishes. A phylogenetic analysis based on morphological characters, though highly desirable, is not currently available. We hope that our proposed classification may spark morphological studies that will test our molecular hypothesis and provide character evidence for or against the phylogenetic relationships and subfamily assignments hypothesized here.

## MATERIALS AND METHODS

**Taxon sampling.**—The ingroup comprises 82 species (4 undescribed), representing 42 genera (2 undescribed) and all 10 recognized subfamilies of the Gobiesocidae (Table S1; see Data Accessibility). Three members of the Blenniidae (*Ophioblennius atlanticus*, *Salarius fasciatus*, and *Entomacrodus nigricans*), one member of the Grammatidae (*Gramma loreto*), and one member of the Pseudochromidae (*Labracinus cyclophthalmus*) were used as outgroup taxa (following Conway et al., 2017a). We note here that our taxon sampling is not designed to investigate the sister group relationships of the Gobiesocidae, and the reader is referred to other works for an overview of this problem (e.g., Gosline, 1970; Allen, 1984; Winterbottom, 1993; Gill, 1996; Breining and Britz, 2000; Chen et al., 2003; Hastings and Springer, 2009; Wiley and Johnson, 2010; Datovo et al., 2014). Museum collection abbreviations used herein follow Sabaj (2020).

**DNA extraction, PCR amplification, and sequencing.**—Total genomic DNA was extracted from fin clip or muscle tissue (see Table S1 for source of tissues; see Data Accessibility) with a DNeasy Blood and Tissue Extraction Kit (QIAGEN, Valencia, CA) following the manufacturer's protocol. Two mitochondrial genes (12S ribosomal RNA [12S] and cytochrome *c* oxidase subunit I [CO1]) were amplified using

primers obtained from Kocher et al. (1989), Folmer et al. (1994), and/or Ward et al. (2005). An additional five protein-coding nuclear loci (zic family member 1 [ZIC1]; SH3 and PX domain containing 3 gene [SH3PX3]; glycosyltransferase [GLYT]; ectodermal-neural cortex 1 [ENC1]; and cardiac muscle myosin heavy chain 6 alpha [MYH6]) were amplified with primers obtained from Li et al. (2007). PCR reactions were conducted in a 25  $\mu$ l reaction containing 12.5  $\mu$ l of EmeraldAmp® GT PCR Master Mix (Takara Bio USA Inc., Mountain View, CA), 0.4  $\mu$ l of each primer (10 mM), 10.7  $\mu$ l of ddH<sub>2</sub>O, and 1  $\mu$ l of DNA template. The mitochondrial loci were amplified using the reaction conditions described in Conway et al. (2017a). The nested PCR method and reaction conditions described in Li et al. (2007) were used for amplification of the nuclear loci. PCR products were purified and sequenced using high-throughput sequencing facilities at Beckman Coulter Genomics (Danvers, MA) or the Genetics Core Lab of Texas A&M University—Corpus Christi (Corpus Christi, TX). Usable sequences were obtained for all seven genes for the majority of samples. We were unable to obtain the full complement of nuclear genes for a small number of the included samples (Table S1; see Data Accessibility). Only mitochondrial (mtDNA) sequences could be obtained for the samples of *Kopua minima* and *Aspasma ubauo* available to us.

**Sequence alignment and phylogenetic analyses.**—Sequences were assembled in Geneious v.10.2.3 (Kearse et al., 2012) and manually checked for accuracy. Sequences of protein-coding genes were translated into amino acids to check for frame shifts and premature stop codons prior to alignment. Individual gene alignments were conducted in MACSE (Ranwez et al., 2011) for protein-coding genes or MAFFT v.7.0 (Katoh and Standley, 2013) for the non-coding gene 12S. Resulting alignments were checked for accuracy manually in Mesquite v.3.2.0 (Maddison and Maddison, 2011). Mitochondrial sequences for *P. asymmetricus* made available from Fricke et al. (2017) were obtained from GenBank and added to our dataset. The seven individual gene alignments were combined into a single concatenated alignment (4,857 bp) using Mesquite v.3.2.0. This concatenated dataset was 94% complete at the level of locus, with coverage for individual genes varying between 28–100% for included taxa (three taxa are represented by mitochondrial genes only). Summary information for the concatenated dataset and the individual gene datasets are provided in Table S2 (see Data Accessibility).

The best-fit sequence substitution models and partitioning scheme for use in subsequent maximum-likelihood (ML) and Bayesian analyses were determined using PartitionFinder v.1.0.1 (Lanfear et al., 2012). PartitionFinder was run separately for the RAxML and MrBayes analyses with the following settings: models = raxml or mrbayes; model\_selection = BIC; search = greedy. ML analyses of the seven-gene concatenated dataset were conducted with RAxML v.7.3.4 (Stamatakis, 2014) implementing the GTRGAMMA model for each of the partitions as identified by PartitionFinder (Table S3; see Data Accessibility) under the -f a setting, which simultaneously conducts rapid bootstrap analysis (1,000 replicates) and searches for the best-scoring ML tree (using ten distinct randomized maximum-parsimony trees as a starting point). We ran RAxML analyses three times and chose the phylogram resulting from the run with the highest log-likelihood score as our final topology. Bayesian analyses

were conducted with MrBayes v.3.2.3 (Ronquist et al., 2012) implementing the partitions and models as identified by PartitionFinder (Table S3; see Data Accessibility). We performed two independent runs of 40 million generations with four chains, sampling trees every 4,000 generations. Convergence was assessed using potential scale reduction factor (PSRF) values in MrBayes. The resulting tree samples were used to construct a 50% majority-rule consensus tree after discarding burn in. Final topologies resulting from the ML and Bayesian analyses were viewed and rooted (using *Labracinus cyclophthalmus*) in FigTree v1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree>).

## RESULTS

The results of the different analyses (RAxML and MrBayes) of the concatenated dataset are shown in Table S3 (see Data Accessibility). We found relationships between taxa in the topologies resulting from the ML (Fig. 3) and Bayesian (Fig. 4) analyses of the concatenated dataset to be largely congruent with minor differences relating to the placement of those species that have low or moderate branch support. Topologies resulting from each of these analyses include a monophyletic Gobiesocidae (supported by high branch support values; 100% bootstrap [BS] in ML phylogram [Fig. 3], 1.0 posterior probability [PP] in Bayesian topology [Fig. 4]). The interrelationships among the major clades of the family are only poorly supported in terms of branch support values in each of these topologies, with many relationships receiving lower than 75% BS (Fig. 3) or 0.75 PP (Fig. 4) that are equivalent to a large polytomy at the base of the ingroup.

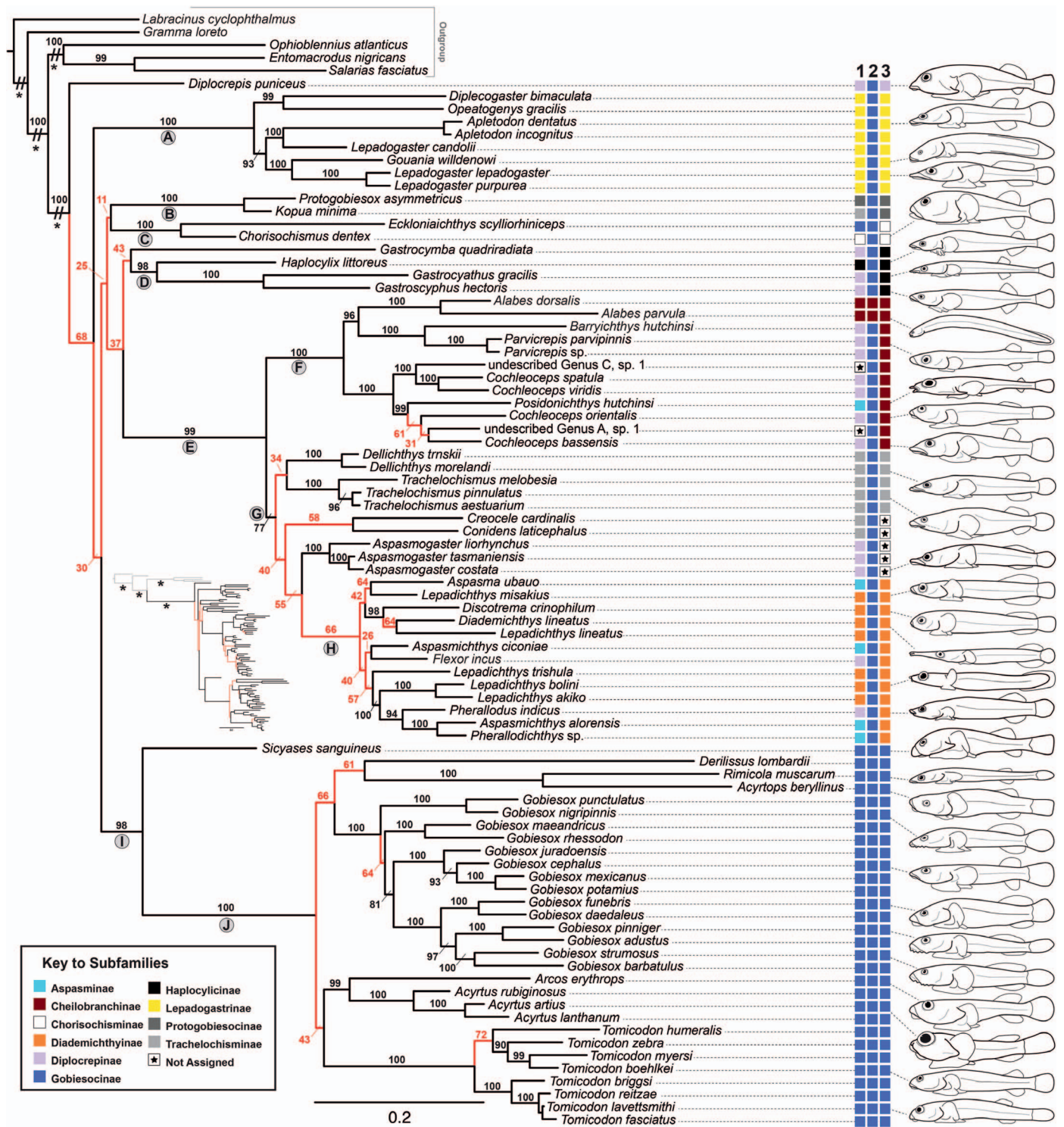
In the topology resulting from both the ML (Fig. 3) and Bayesian analysis (Fig. 4), the New Zealand endemic Orange Clingfish *Diplocrepis puniceus* (a member of the Diplocrepinae in the traditional classification) is the sister taxon to the remainder of the Gobiesocidae included in the dataset. The clade including all other gobiesocids (less *D. puniceus*) is poorly supported in terms of branch support values (BS 68% in the ML topology [Fig. 3], 0.89 PP in the Bayesian topology [Fig. 4]) but contains six smaller clades (labeled A–E, I in Figs. 3, 4) that generally received high branch support values. Clade A (BS 100% in the ML topology [Fig. 3], 1.0 PP in the Bayesian topology [Fig. 4]) is equivalent to the Lepadogastriinae of Briggs (1955), including members of the genera *Apletodon*, *Diplecogaster*, *Gouania*, *Lepadogaster*, and *Opeatogynys*. Within Clade A, members of *Lepadogaster* are not recovered as a monophyletic group, with *L. candollei* recovered in a sister-group relationship with *Apletodon*, and *L. purpurea* + *L. lepadogaster* recovered in a sister-group relationship with *Gouania* (Figs. 3, 4). Clade B (BS 100% in the ML topology, 1.0 PP in the Bayesian topology) comprises the deeper dwelling clingfishes *Protogobiesox asymmetricus* (Protogobiesocinae) and *Kopua minima* (Trachelochisminae) in a sister-group relationship (Figs. 3, 4). Clade C (BS 100% in the ML topology [Fig. 3], 1.0 PP in the Bayesian topology [Fig. 4]) comprises the southern African endemic clingfishes *Chorisochismus dentex* (Chorisochisminae) and *Eckloniaichthys scylliorhiniceps* (Gobiesocinae) in a sister-group relationship. Clade D (BS 98% in the ML topology [Fig. 3], 1.0 PP in the Bayesian topology [Fig. 4]) comprises three New Zealand endemic genera of macroalgae-dwelling clingfishes. In this clade, *Haplocylix* (Haplocylicinae) is the sister group to *Gastrocyathus* + *Gastroscyphus* (Diplocrepinae). Another New

Zealand endemic genus of macroalgae-dwelling clingfish (*Gastrocytha quadriradiata*) is the sister taxon to Clade D in the ML topology, but this relationship received low branch support values (BS 43%) and is not present in the topology resulting from the Bayesian analysis (in which *G. quadriradiata* is placed as the sister taxon to Clade B, with low branch support; 0.65 PP [Fig. 4]).

Clade E (BS 99% in the ML topology [Fig. 3], 1.0 PP in the Bayesian topology [Fig. 4]) is a large clade comprising predominantly Indo-Pacific and Australasian taxa that are currently placed in the Aspasminae (*Aspasma*, *Aspasmichthys*, *Pherallodichthys*, and *Posidonichthys*), Cheilobranchinae (*Alabes*), Diademichthyinae (*Diademichthys*, *Discotrema*, and *Lepadichthys*), Diplocrepinae (*Aspasmogaster*, *Barryichthys*, *Cochleoceps*, *Flexor*, and *Parvicrepis*), and Trachelochisminae (*Conidens*, *Creocele*, *Dellichthys*, and *Trachelochismus*), and taxa that have not previously been assigned to any subfamily (undescribed Genus A and Genus C). The members of Clade E are divided between two smaller clades: Clade F and Clade G. Clade F (BS 100% in the ML topology [Fig. 3], 1.0 PP in the Bayesian topology [Fig. 4]) comprises exclusively genera endemic to southern Australia that are currently assigned to multiple subfamilies. This includes members of the genus *Alabes* (Cheilobranchinae), *Aspasmogaster*, *Barryichthys*, *Cochleoceps*, *Parvicrepis* (Diplocrepinae), *Posidonichthys* (Aspasminae), and the undescribed Genus A and Genus C (which have yet to be assigned to subfamily). In this clade, the discless Australian shore eels of the genus *Alabes* are recovered in a strongly supported sister-group relationship (BS 96%, 0.99 PP) to a clade that comprises *Barryichthys* + *Parvicrepis* (Figs. 3, 4). The sister group to the clade comprising *Alabes* + (*Barryichthys* + *Parvicrepis*) is also strongly supported (BS 100%, 1.0 PP) and includes members of *Cochleoceps*, *Posidonichthys*, and the undescribed Genus A and Genus C. Within this clade, the four members of *Cochleoceps* included in the dataset are not recovered as monophyletic due to the placement of *Posidonichthys hutchinsi* and the undescribed taxa.

Clade G (BS 77% in the ML topology [Fig. 3], 0.99 PP in the Bayesian topology [Fig. 4]) comprises Indo-Pacific and temperate Australasian species that are currently assigned to multiple subfamilies. This includes members of the genus *Aspasma*, *Aspasmichthys*, and *Pherallodichthys* (Aspasminae), *Diademichthys*, *Discotrema*, and *Lepadichthys* (Diademichthyinae), *Aspasmogaster*, *Flexor*, and *Pherallodus* (Diplocrepinae), and *Conidens*, *Creocele*, *Dellichthys*, and *Trachelochismus* (Trachelochisminae). In Clade G, the members of Aspasminae, Diademichthyinae, and Trachelochisminae are not monophyletic groups but are dispersed throughout the clade. The basal nodes within Clade G are poorly supported and are occupied by members of the trachelochismine genera, *Trachelochismus*, *Dellichthys*, *Conidens*, and *Creocele*, and the aspasmine genus *Aspasmogaster*. The relationships between the basal members of Clade G differ in the topologies resulting from the different analyses largely due to the placement of *Creocele*, which is placed as the sister taxon to *Conidens* in the topology resulting from the ML analysis (Fig. 3) or as the sister taxon to a larger clade comprised exclusively of Indo-Pacific taxa (Clade H) in the topology resulting from the Bayesian analysis (Fig. 4). Both alternatives are poorly supported in terms of branch support. Clade H (BS 66% in the ML topology [Fig. 3], 1.0 PP in the Bayesian topology [Fig. 4]) unites members of *Aspasma*, *Aspasmichthys*, and *Pherallodichthys* (Aspasminae), *Diademichthys*, *Discotre-*

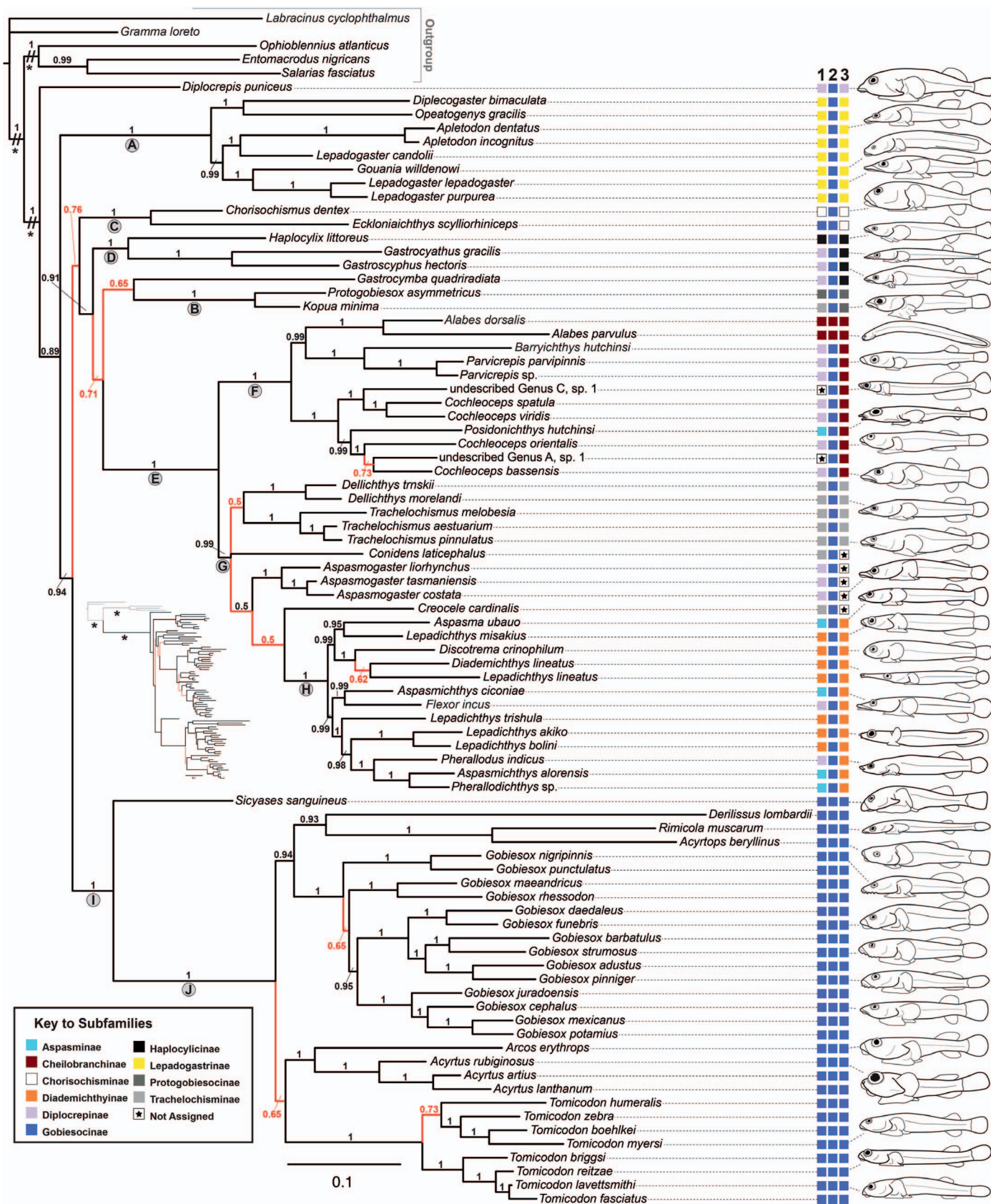




**Fig. 3.** Maximum-likelihood phylogram obtained from RAxML analysis of the concatenated seven-gene dataset. Branches marked with an asterisk (\*) have been shortened to facilitate viewing (see inset topology for original branch lengths). Numbers above branches represent bootstrap (BS) support values. Red branches are those downstream of nodes with BS values  $\leq 75\%$ . Letters (A–J) in gray circles are clades discussed in text. Rows of colored boxes to right side of image represent subfamily designations (see corresponding key) within the following three classification schemes: (1) “traditional” classification with ten subfamilies; (2) alternative “reduced” classification with two subfamilies; and (3) revised classification with ten subfamilies. See Data Accessibility for tree file.

ma, and *Lepadichthys* (Diademichthyinae), *Flexor* and *Pherallodus* (Diplocrepinae). Within Clade H, members of *Lepadichthys* are not recovered as a monophyletic group, but they are, instead, dispersed throughout the clade and placed in poorly supported sister-group relationships with

members of other genera. A similar result was found for *Aspasmichthys*: *A. cicionae* is placed in a sister-group relationship with *Flexor incus* (BS 26%, 0.99 PP), and *A. alorensis* is placed in a strongly supported sister-group relationship with an undescribed species, *Pherallodichthys* sp. (BS 100%, 1.0 PP).



**Fig. 4.** Topology resulting from Bayesian analysis of the concatenated seven-gene dataset. Branches marked with an asterisk (\*) have been shortened to facilitate viewing (see inset topology for original branch lengths). Numbers above branches represent posterior probabilities (PP). Red branches are those downstream of nodes with PP values ≤ 0.75. Letters (A–J) in gray circles are clades discussed in text. Rows of colored boxes to right side of image represent subfamily designations (see corresponding key) within the following three classification schemes: (1) “traditional” classification with ten subfamilies; (2) alternative “reduced” classification with two subfamilies; and (3) revised classification with ten subfamilies. See Data Accessibility for tree file.

Clade I (BS 98%, 1.0 PP) is a large clade comprised exclusively of New World taxa, including members of *Acyrtops*, *Acyrtus*, *Arcos*, *Derilissus*, *Gobiesox*, *Rimicola*, *Sicyases*, and *Tomicodon* (Figs. 3, 4). This clade is equivalent to the Gobiesocinae in the traditional classification (Briggs, 1955) less *Eckloniaichthys*. In this clade, the South American clingfish *Sicyases sanguineus* is placed as the sister taxon to the remaining taxa (Clade J in Figs. 3, 4). Within Clade J, all polytypic genera represented by more than one taxon (*Acyrtus*, *Gobiesox*, and *Tomicodon*) are recovered as monophyletic with strong branch support (BS 100%, 1.0 PP). The relationships among these genera are poorly supported except for the relationship between *Acyrtus* and *Arcos* (BS 99%, 1.0 PP) and *Acyrtops* and *Rimicola* (BS 100%, 1.0 PP). The deepwater species *Derilissus lombardii* is placed as the sister taxon to the clade comprising *Acyrtops* + *Rimicola* in the topologies resulting from both the ML and Bayesian analyses, though this relationship received only low branch support (BS 61%, 0.93 PP).

## DISCUSSION

Depending on the taxonomic scheme (Table 1), gobiesocids have been classified previously across two subfamilies (reduced classification) or up to as many as ten (traditional classification). Though several authors have raised concerns over the composition of multiple subfamilies in both the traditional (e.g., Smith, 1957; Böhlke and Robins, 1970; Briggs, 1993; Almada et al., 2008; Conway et al., 2017a, 2018) and the reduced classification schemes (e.g., Conway et al., 2017a), studies have not been designed to adequately test the monophyly of individual subfamilies and were limited in their ability to assess alternative classification schemes. Non-overlapping taxon-sampling schemes among the prior work has contributed to conflicting results. For example, in their investigation of the eastern Atlantic lepadogastrine clingfishes, Almada et al. (2008; Fig. 2C) had representatives of three subfamilies (Aspasminae, Gobiesocinae, and Lepadogastrinae) and obtained two (Gobiesocinae and Lepadogastrinae) as non-monophyletic. Fricke et al. (2017) included representatives of six subfamilies (Fig. 2D), including three represented by multiple species (Cheilobranchinae, Gobiesocinae, and Lepadogastrinae), all of which appeared monophyletic. In their molecular phylogenetic investigation of the New World clingfishes, Conway et al. (2017a) noted problems with each of the alternative classification schemes, including the non-monophyly of the Gobiesocinae (non-Briggs) and the non-monophyly of at least the Diplocrepinae (Fig. 2E), but they lacked representatives of several polytypic subfamilies (Aspasminae, Haplocylicinae, Protogobiesocinae, and Trachelochisminae) and could not comment on the monophyly or relationships of these taxa.

Our taxon sampling exceeds that of previous studies (82 species vs. 32 in Conway et al., 2017a and 11 in Fricke et al., 2017) and allows us to comment on the composition of several subfamilies in the traditional classification that have been represented in previous studies only by a single taxon (e.g., Aspasminae) or missing entirely (e.g., Haplocylicinae or Trachelochisminae). It also allows us to critically assess the monophyly of other subfamilies. In the following sections, we discuss the results of our study in relation to the subfamilies included in the traditional and reduced classification schemes. We end our discussion by proposing a

revised classification scheme, comprising ten subfamilies, derived largely from the results of this molecular study, but also taking into account previous morphological investigations (e.g., Briggs, 1955; Springer and Fraser, 1976; Hayashi et al., 1986; Conway et al., 2015, 2017b, 2018, 2019).

**Aspasminae.**—Briggs (1955) originally placed three genera into the Aspasminae, including *Aspasma* (type genus), *Aspasmichthys*, and *Liobranchia*. Within this group, Briggs (1955: 149) considered *Aspasma* and *Aspasmichthys* to be more closely related to each other than to *Liobranchia*. Briggs (1955: 149) may have also considered *Aspasma* and *Aspasmichthys* (but not *Liobranchia*) to be putative close relatives of his Diademichthyinae (comprising at that point in time *Diademichthys* and *Lepadichthys*) when he wrote “*Aspasma* and *Aspasmichthys* seem to be directly on the evolutionary line which extends to the Diademichthyinae.” This hypothesized relationship is also evident by the placement of the Diademichthyinae as the closest relative of the Aspasminae by Briggs in his dendrogram of subfamilial relationships (Briggs, 1955; Fig. 2A). Several additional genera have been added to the Aspasminae subsequent to Briggs (1955), including *Aspasmodes* (Smith, 1957), *Briggsia* (Craig and Randall, 2009), *Lissonanchus* (Smith, 1965), *Modicus* (Hardy, 1983), *Nettorhamphos* (Fricke et al., 2020 [discussed further below]), *Pheraliodichthys* (Shiogaki and Dotsu, 1983), and *Posidonichthys* (Briggs, 1993). Prokofiev (2005) originally placed *Lepadicyathus* in the Aspasminae, but this species exhibits a “single” disc type (not the “double” disc type characteristic of members of Aspasminae *sensu* Briggs, 1955) and should have been placed instead in the Diademichthyinae if Prokofiev (2005) had followed Briggs’s scheme. Fricke et al. (2017) recently transferred *Lepadicyathus* to their Protogobiesocinae, but based on information contained within the original description, we considered this taxon to instead belong to the Diademichthyinae (discussed below).

Only five species, representing four genera of aspasmine clingfishes, were available for our investigation. This includes *Aspasma ubauo* (mitochondrial DNA only), *Aspasmichthys alorensis*, *A. ciconiae*, *Pheraliodichthys* sp., and *Posidonichthys hutchinsi*. Regardless of our method of analysis, the five aspasmine taxa included in our dataset were never recovered as a monophyletic group. Instead, these taxa were scattered throughout a large clade of Indo-Pacific and Australasian taxa (Clade E; Figs. 3, 4) and placed in sister-group relationships with taxa currently assigned to other subfamilies in the traditional classification (including Diademichthyinae and Diplocrepinae) or not previously assigned to a subfamily. *Aspasma ubauo* (referred to incorrectly in previous studies as *A. minima*; see Fujiwara and Motomura, 2020b) was placed in a sister-group relationship with *Lepadichthys misakius* (a member of the Diademichthyinae). Fricke et al. (2017) also obtained *Aspasma ubauo* in a sister-group relationship with a species of *Lepadichthys* (in this case *L. lineatus*) based on a combination of nuclear DNA and mitochondrial DNA sequence data (Fig. 2D), whereas Almada et al. (2008) obtained this species in a poorly supported sister-group relationship with the eastern Atlantic *Lepadogaster candolii* (Lepadogastrinae) based on analyses of 12S and 16S mtDNA sequence data (Fig. 2C). This latter relationship may be an artifact of insufficient taxon sampling (Almada et al.’s study was designed to investigate the intrarelationships of lepadogastrine clingfishes only; see below).

The two species of *Aspasmichthys* were never obtained as sister taxa in our results. *Aspasmichthys ciconiae* (type species of *Aspasmichthys*), known currently from Japan and Taiwan (Hayashi, 2002; Allen and Erdmann, 2012), was obtained in a poorly supported sister-group relationship with *Flexor incus*, a species known currently only from the remote Kermadec archipelago to the northeast of New Zealand (Stewart, 2015; Conway et al., 2018). *Aspasmichthys alorensis*, known only from a handful of specimens collected recently from the Alor Strait of Indonesia (Allen and Erdmann, 2012), was obtained in a well-supported sister-group relationship with *Pheraliodichthys* sp., a putative new species from the Indo-Pacific (currently under investigation by K. Fujiwara). Allen and Erdmann (2012) tentatively assigned *Aspasmichthys alorensis* to *Aspasmichthys* based on the three-character system of Briggs (1955) and described this decision as “provisional.” Based on the results obtained herein, generic reassignment of *Aspasmichthys alorensis* to *Pheraliodichthys* or to a new genus may be warranted.

The southern Australian *Posidonichthys hutchinsi* (Briggs, 1993; Hutchins, 2008) was obtained as a member of Clade F, comprised exclusively of taxa from temperate southern Australia that are currently assigned to either the Cheilobranchinae, Diplocrepinae, or are unassigned to any subfamily. Though Briggs (1993) placed *Posidonichthys* within the Aspasminae, he alluded to a possible close relationship with unspecified members of the Diplocrepinae based on shared characteristics of the adhesive disc and general osteology that were not discussed. A close relationship between *Posidonichthys* and two other genera of southern Australian clingfishes (undescribed Genus A and *Nettorhamphos*) was also recently hypothesized by Conway et al. (2017b) based on putatively derived characteristics of the subopercle. *Nettorhamphos* was described only recently by Conway et al. (2017a), who did not assign this taxon to any of the recognized subfamilies. Without comment, Fricke et al. (2020) assigned *Nettorhamphos* to Aspasminae, likely following the three-character system of Briggs (1955), even though the number of gill arches bearing gill filaments (three in Aspasminae) has not been reported for this taxon. Though *Nettorhamphos* was not included in the current study (due to a lack of appropriately fixed samples), within Clade F, *Posidonichthys* was obtained as the sister taxon to a clade comprising the undescribed Genus A, *Cochleocephus orientalis*, and *C. bassensis* (Figs. 3, 4; the latter two lack the derived subopercular characteristic identified by Conway et al., 2017b).

Given that *Aspasma* (type genus of Aspasminae; Van der Laan et al., 2014) is embedded within a clade (Clade G) comprising members of multiple other subfamilies (Figs. 3, 4), including Diademichthyinae Whitley, 1950 (type species *Diademichthys*) which has priority over Aspasminae Briggs, 1955, we recommend that Aspasminae be placed in the synonymy of Diademichthyinae and that all genera placed in the former (excluding *Posidonichthys*, *Modicus*, and *Nettorhamphos*) now be considered members of the latter. The decision to also consider the six genera of former aspasmine clingfishes that are not represented in this study either as members of an expanded Diademichthyinae (*Aspasmodes*, *Briggsia*, *Lepadicyathus*, *Liobranchia*, and *Lissonanchus*) or *incertae sedis* (*Modicus*; see below) is provisional, and this should be investigated further with molecular (if tissues samples become available) and/or morphological data. The

composition of our expanded Diademichthyinae is outlined further below.

**Cheilobranchinae.**—The family Cheilobranchidae was placed in the synonymy of the Gobiesocidae by Springer and Fraser (1976), resulting in the addition of the elongate and discless Australian shore eels of the genus *Alabes* to the Gobiesocidae, which were subsequently assigned to the subfamily Cheilobranchinae by Briggs (1993). Only two of the 11 currently recognized species of *Alabes* (*A. dorsalis* and *A. parvula*) were included in our dataset, and, as in a previous study (Fricke et al., 2017; Fig. 2D), *Alabes* was recovered as a monophyletic group regardless of the method of analysis (Figs. 3, 4).

Springer and Fraser (1976) speculated that *Alabes* may be a close relative of *Gastrocymba quadriradiata*, a relatively elongate species of clingfish endemic to the sub-Antarctic region of New Zealand (Briggs, 1955; Stewart, 2015). This putative relationship was based on many osteological reductions and a high vertebral count in both taxa compared to other gobiesocids. A close relationship between *Alabes* and *G. quadriradiata* was not present in any of our topologies. In all cases, *Alabes* was obtained as part of a monophyletic group (Clade F; Figs. 3, 4) comprised exclusively of other temperate southern Australian taxa, including members of the Diplocrepinae (*Barryichthys*, *Cochleocephus*, and *Parvicrepis*), Aspasminae (*Posidonichthys*), and a number of undescribed taxa from southern Australia not currently assigned to any subfamily (the undescribed Genus A and Genus C *sensu* Hutchins, 1994, 2008). The placement of *Alabes* with other southern Australian taxa is congruent with the results of Conway et al. (2017a), in which *A. parvula* (referred to incorrectly as *A. hoesei*) was recovered as part of a strongly supported monophyletic group together with *Cochleocephus orientalis* and *Parvicrepis parvipinnis* (Fig. 2E) but not with those of Fricke et al. (2017), who obtained *Alabes* as the sister taxon to a clade comprising the Indo-Pacific taxa *Aspasma ubauo* and *Lepadichthys lineatus* (Fig. 2D). This incongruence likely stems from the limited taxon sampling of Fricke et al. (2017).

Within Clade F (Figs. 3, 4), *Alabes* is placed in a strongly supported sister-group relationship with a clade comprising the miniature and obligate macroalgae/seagrass-dwelling clingfishes of the genera *Barryichthys* and *Parvicrepis*. This ‘deep’ placement of *Alabes* within the Gobiesocidae causes problems not only for the application of the reduced classification scheme (because Cheilobranchinae would render Gobiesocinae [non-Briggs] paraphyletic) but also the traditional classification because *Alabes* is embedded inside a clade comprised predominantly of taxa assigned previously to the Aspasminae and Diplocrepinae or not currently assigned to any subfamily. Our solution to this problem is to expand Cheilobranchinae to include all genera included within Clade F, including *Barryichthys*, *Cochleocephus*, *Parvicrepis*, *Posidonichthys*, and the undescribed Genus A and Genus C. We have chosen this option because Cheilobranchinae (available from Günther, 1870) is the oldest name that could be applied to the monophyletic group represented by Clade F but realize that it may be unpalatable to many because at this time we are unaware of any morphological characters that could be used to diagnose the Cheilobranchinae as we have circumscribed it, in part due to the extreme morphological reductions exhibited by members of *Alabes*. The alternative option would be to use Cheilobranchinae only for *Alabes*

(i.e., status quo) and to divide the other members of Clade F across multiple new subfamilies. We have chosen not to adopt this latter option because these taxa are relatively poorly studied and further taxonomic study is needed to circumscribe the limits of both undescribed (Genus A and Genus C *sensu* Hutchins, 1994, 2008) and described genera, at least one of which (*Cochleoceps*) is not monophyletic based on the molecular data that we have analyzed (Figs. 3, 4), as suspected by Hutchins (1991) based on external appearance. We consider the changes to the membership of Cheilobranchinae that we have suggested the most conservative option for the time being but recognize that further taxonomic and morphological work is needed in this area of the clingfish tree, which may result in further changes to the classification. Though not represented in our dataset, we also tentatively assign *Nettorhamphos* to our expanded Cheilobranchinae based on a suspected close relationship among this taxon, *Posidonichthys*, and undescribed Genus A (Conway et al., 2017b).

**Chorisochisminae.**—The subfamily Chorisochisminae was erected by Briggs (1955) for the sole inclusion of the South African endemic clingfish *Chorisochismus dentex*. The relationships of *C. dentex* were considered “a problem” by Briggs (1955: 142). In his dendrogram of subfamilial relationships, Briggs (1955) placed Chorisochisminae as the closest relative to a larger group including Aspasminae, Diademichthyinae, Diplocrepinae, and Gobiesocinae (Fig. 2A) but stated, rather confusingly, that this taxon “did not arise directly from the Lepadogastrinae” but “from a more primitive stock before the origin of the Lepadogastrinae.” In Conway et al. (2017a), *C. dentex* was obtained as the sister taxon to a clade comprised exclusively of New World taxa (a relationship with only weak branch support; Fig. 2E). In the results of the present study, *C. dentex* is placed in a strongly supported sister-group relationship with *Eckloniaichthys scylliorhiniceps*, another South African endemic species (Smith, 1943) that has been previously considered a member of the Gobiesocinae (Briggs, 1955). Though the branch support values in support of the clade comprising *Chorisochismus* + *Eckloniaichthys* are strong (100% BS; 1.0 PP), the placement of this clade is incongruent in the topologies resulting from the different analyses of our dataset (Figs. 3, 4), and further investigation will be needed to clarify the phylogenetic relationships of this newly identified clade. Based on our results, we recommend the transfer of *E. scylliorhiniceps* to the Chorisochisminae, and this change is discussed further below (under Gobiesocinae).

**Diademichthyinae.**—Briggs (1955) originally included two genera in the Diademichthyinae, *Diademichthys* (type genus of Diademichthyidae) and *Lepadichthys*. Briggs (1976) later added *Discotrema* and Fricke (2014) added *Unguitrema*, which he suspected to be a close relative of *Discotrema*. Seven species, representing three of the four genera of diademichthyine taxa of previous authors (Briggs, 1955; Fricke, 2014), were available for investigation herein, including: *Diademichthys* (*D. lineatus*), *Discotrema* (*D. crinophilum*), and *Lepadichthys* (*L. akiko*, *L. bolini*, *L. lineatus*, *L. misakius*, and *L. trishula*).

Regardless of the method of analysis, the aforementioned taxa were never obtained together as a monophyletic group but instead were consistently recovered as more closely related to taxa that have been previously placed in Aspasminae or Diplocrepinae throughout Clade H (Figs. 3, 4). As

discussed above, our solution to this problem is to expand Diademichthyinae to include all members of Aspasminae (excluding *Modicus*, *Nettorhamphos*, and *Posidonichthys*) and also the members of Diplocrepinae that are included in our dataset and obtained as members of Clade G. Our expanded Diademichthyinae include *Aspasma*, *Aspasmichthys*, *Aspasmodes*\* (not represented in our dataset), *Briggsia*\*, *Diademichthys*, *Discotrema*, *Flexor*, *Lepadichthys*, *Lepadicyathus*\*, *Liobranchia*\*, *Lissonanchus*\*, *Pheralldichthys*, *Pheralldodus*, *Propheralldodus*\*, and *Unguitrema*\*. A subset of these genera (*Aspasmichthys*, *Diademichthys*, *Flexor*, *Lepadichthys*, *Pheralldichthys*, and *Pheralldodus*) were identified as a putative monophyletic group by Conway et al. (2018) based on three derived morphological characters, including: (1) a unique type of laterally compressed incisiviform tooth with a hooked tip; (2) an oval opening (variable in size) in the upper jaw, formed by a semicircular indentation along the medial edge of each premaxilla; and (3) a complex articulation between the posterior tip of the basiptyergium and the anteromedial edge of the ventral postcleithrum. We note here that all of the genera listed above (excluding *Briggsia* and *Lissonanchus* for which osteological information is not available) possess character 3 and this character may represent a synapomorphy for our expanded Diademichthyinae. Though overlooked by Conway et al. (2018), *Lepadicyathus* also exhibits characters 1 and 2 (Fujiwara, pers. obs.), and these characters may also represent synapomorphies in support of our expanded Diademichthyinae or a subset of these taxa (a scenario that requires the absence of characters 1 and 2 in some taxa to be the result of secondary loss).

In our expanded Diademichthyinae, *Aspasmichthys* (discussed above) and *Lepadichthys* are both polyphyletic as currently defined based on our results (Figs. 3, 4). Of the five species of *Lepadichthys* included in our dataset, only two (*L. akiko* and *L. bolini*) formed a monophyletic group, with the remaining three species scattered throughout Clade H. *Lepadichthys trishula* was placed as the sister group to a clade including the aforementioned sister-group pair of *Lepadichthys*, plus *Aspasmichthys alorensis*, *Pheralldichthys* sp., and *Pheralldodus indicus*. *Lepadichthys misakius* was placed as the sister taxon to *Aspasma ubauo*. *Lepadichthys lineatus* was placed as the sister taxon to *Diademichthys lineatus*. The rampant polyphyly of *Lepadichthys* is an unexpected result, but we are unable to recommend nomenclatural changes to clarify this problem at this time because our dataset does not include the type species of the genus, *Lepadichthys frenatus*. Based on the topologies obtained herein, and as previously noted by Craig and Randall (2008) and Fujiwara and Motomura (2018a), the erection of new genera or redefinition of existing genera may be necessary to accommodate several of the species currently assigned to *Lepadichthys*.

**Diplocrepinae.**—Briggs (1955) originally included seven genera in the subfamily Diplocrepinae (Fig. 2B): *Aspasmogaster*, *Cochleoceps*, *Diplocrepis* (type genus), *Gastrocyathus*, *Gastroscyphus*, *Parvicrepis*, and *Pheralldodus*. Shiogaki and Dotsu (1983) later added *Propheralldodus* to this group. Though *Gastrocymba* was considered to be a member of the Trachelochisminae by Briggs (1955), Springer and Fraser (1976) reassigned this taxon to the Diplocrepinae based on the number of gill arches bearing gill filaments (three instead of the four reported by Briggs, 1955). Fourteen species, representing eight of the nine genera considered to be

diplocrepines by previous authors (Briggs, 1955; Springer and Fraser, 1976), were available for investigation herein: *Aspasmogaster* (*A. costata*, *A. liorhynchus*, and *A. tasmaniensis*), *Cochleocephalus* (*C. bassensis*, *C. orientalis*, *C. spatula*, and *C. viridis*), *Diplocrepis* (*D. puniceus*), *Gastrocyathus* (*G. gracilis*), *Gastrocymba* (*G. quadriradiata*), *Gastroscyphus* (*G. hectoris*), *Parvicrepis* (*P. parvipinnis* and *P. sp.*), and *Pheralloodus* (*P. indicus*).

Regardless of the method of analysis, the diplocrepine taxa represented in our dataset were never obtained as a monophyletic group but were instead distributed across the entire gobiesocid tree. The monotypic New Zealand endemic genus *Diplocrepis* (type genus of Diplocrepinae) was recovered as the sister taxon to the remainder of the Gobiesocidae in the topology resulting from both the ML (Fig. 3) and Bayesian analysis (Fig. 4). The remaining diplocrepine genera were obtained as members of clades D, F, and G, and as more closely related to members of other subfamilies (Figs. 3, 4). In order to correct the issue of diplocrepine non-monophyly, we recommend restricting the Diplocrepinae to *Diplocrepis*. The remaining ex-diplocrepine genera are referred to other subfamilies, including Cheilobranchinae (*Cochleocephalus* and *Parvicrepis*; see above), Diademichthyinae (*Pheralloodus*; see above), and Haplocylicinae (*Gastrocyathus*, *Gastrocymba*, and *Gastroscyphus*; see below) or are considered *incertae sedis* (*Aspasmogaster*) pending further investigation (see below). *Propheralloodus* is not represented in our dataset, but based on information from Shioyaki and Dotsu (1983: fig. 3C) and Fujiwara and Motomura (2018b: fig. 4), the members of this genus exhibit a complex articulation between the posterior tip of the basipterygium and the anteromedial edge of the ventral postcleithrum similar to that found in members of the Diademichthyinae (see Conway et al., 2018). Based on this character, we also tentatively consider *Propheralloodus* as a member of this subfamily.

The clade comprising Gobiesocidae (less *Diplocrepis puniceus*) is only poorly supported in terms of branch support statistics (68% BS/0.85 PP), and we are aware of no derived morphological characters that would potentially provide support for a clade comprising all gobiesocids to the exclusion of *Diplocrepis*. A detailed morphological study of *Diplocrepis puniceus* is not currently available but could be insightful, and, in our opinion, it is warranted, given the potential key position of *Diplocrepis*.

**Gobiesocinae (sensu Briggs).**—Briggs (1955) originally included *Acyrtops*, *Acyrtus*, *Arcos*, *Eckloniaichthys*, *Gobiesox*, *Pheralloodus* (recently placed in the synonymy of *Gobiesox* by Conway et al., 2017a), *Rimicola*, *Sicyases*, and *Tomicodon* in his Gobiesocinae. Briggs (1969) later added *Derilissus* to his Gobiesocinae. Thirty-two species, representing all nine genera of the Gobiesocinae (sensu Briggs, 1955), were available for investigation herein.

Though Briggs (1955) considered the southern African endemic *Eckloniaichthys scylliorhiniceps* to be a close relative of *Acyrtops* and *Rimicola* (Fig. 2B) based on characters of oral-jaw dentition, Conway et al. (2015) concluded that *Eckloniaichthys* was not a close relative of these New World taxa and speculated that it may instead be more closely related to “several Indo-Pacific members of the Aspasminae, Diplocrepinae, and all of the Diademichthyinae [traditional usage].” Conway et al. (2015) also concluded that all New World genera of the Gobiesocinae likely represented a monophy-

letic group (referred to as the New World clingfishes) based on derived characteristics of oral-jaw dentition. An equivalent monophyletic group has appeared in the topologies resulting from subsequent molecular phylogenetic studies using different combinations of genes and taxa (Conway et al., 2017a; Fricke et al., 2017; Figs. 2D, E), though neither including representatives of all New World gobiesocine genera. A monophyletic group of New World taxa was not obtained in the molecular phylogenetic study of Almada et al. (2008; Fig. 2C), but this is likely an artifact of limited taxon sampling and/or a limited character set, comprising mitochondrial genes only.

Our investigation is the first to include members of all New World genera of gobiesocine taxa and also *E. scylliorhiniceps*. As in the majority of previous molecular phylogenetic investigations, the topologies resulting from other analyses also include a strongly supported monophyletic group comprised solely of New World taxa (Clade I; Figs. 3, 4). *Eckloniaichthys scylliorhiniceps* is neither a member of nor sister group to this clade and is placed instead in a strongly supported sister-group relationship with another southern African endemic, *Chorisochismus dentex* (Clade C; Figs. 3, 4). This placement of *Eckloniaichthys* renders the Gobiesocinae (sensu Briggs) a non-monophyletic group, and, based on our results, we recommend transfer of this genus to the Chorisochisminae and the restriction of the Gobiesocinae so that it includes only the New World genera placed in this group by Briggs (1955). We recognize that *Eckloniaichthys* is morphologically quite different from *Chorisochismus* (the only other member of the Chorisochisminae), as evidenced by Briggs's decision to originally place these taxa in different subfamilies, and future morphological work will be needed to rediagnose the Chorisochisminae in light of our revision to its membership.

The intrarelations among gobiesocine taxa obtained herein mirror some, but not all, of the relationships reported previously among these taxa by Conway et al. (2017a). In that study (Fig. 2E) and in ours, the South American clingfish *Sicyases sanguineus* is placed as the sister taxon to the remaining members of the group (Clade J in Figs. 3, 4). In Conway et al. (2017a), the sister clade to *Sicyases* (equivalent to our Clade J) is divided into two subgroups, one including *Acyrtops*, *Rimicola*, and *Tomicodon* and the other including *Acyrtus*, *Arcos*, and *Gobiesox*. Conway et al. (2017a) commented that the presence of a paired patch of papillae in region C of the adhesive disc may represent a “putative morphological synapomorphy” in support of a monophyletic group including *Acyrtus*, *Arcos*, and *Gobiesox* but made no comment on potential morphological evidence in support of a group comprised of *Acyrtops*, *Rimicola*, and *Tomicodon*. In the topologies resulting from our analyses, the taxa that exhibit a paired patch of papillae in region C of the adhesive disc (viz. *Acyrtus*, *Arcos*, and *Gobiesox*) do not represent a monophyletic group. Instead, *Gobiesox* is placed as the sister taxon to a clade comprising *Derilissus*, *Acyrtops*, and *Rimicola*, whereas *Acyrtus* + *Arcos* is placed as the sister taxon to *Tomicodon* (Figs. 3, 4). Though the relationships between genera in our Clade J have in general only poor branch support, the differences between the relationships in the current analysis and those of Conway et al. (2017a) are particularly troublesome given that we have used essentially the same sequences and taxa, with the addition of one nuclear gene (ENC1). Though it is important to highlight the

incongruence in relationships between the results of different studies, it is equally important to report congruence, and we note here that two monophyletic groups are common between the topologies reported by Conway et al. (2017a) and us. This includes: (1) *Rimicola* + *Acyrtops*; and (2) *Acyrtus* + *Arcos*. Each of these monophyletic groups is strongly supported in terms of branch support statistics in both studies and corroborate relationships that were initially proposed by Briggs (1955), though without character evidence, in his dendrogram of relationships of gobiesocine taxa (Fig. 2B).

In addition to *Eckloniaichthys*, our study is also the first to include sequences of a species of the genus *Derilissus*, with some of the smallest described clingfishes to date. Their tiny maximum recorded sizes for the four described species range from 10.9–27.6 mm SL (Briggs, 1969; Fraser, 1970; Smith-Vaniz, 1971; Sparks and Gruber, 2012) with female individuals as small as 11.3 mm SL already with well-developed eggs and likely capable of reproduction (Briggs, 1969). In the original description of *Derilissus*, Briggs (1969) outlined his argument for placing this genus either in Diademichthyinae (based on his three-character system) or Gobiesocinae (based on a number of similarities shared by *D. nanus*, *Acyrtops*, *Eckloniaichthys*, and *Rimicola*) and in the end chose the Gobiesocinae. This decision ultimately opened the door to criticism of Briggs's subfamilial classification scheme for the Gobiesocidae (starting with Böhlke and Robins, 1970). Though we agree with the general criticisms articulated by Böhlke and Robins (1970) of Briggs's classification (i.e., it is based only on three characters), based on the topologies resulting from both the ML and Bayesian analyses of our dataset (which unites *Derilissus* and *Rimicola* + *Acyrtops* as sister groups), we consider Briggs's decision to 'follow his gut' on the placement of *Derilissus* within the Gobiesocinae instead of the Diademichthyinae the correct decision. The results of both previous morphological (Conway et al., 2015) and the current molecular study suggest that *Derilissus* should be grouped together with other New World gobiesocids within a restricted Gobiesocinae. The similarities between *Derilissus* and some of the taxa placed currently within the Diademichthyinae (chiefly the connection between the gill openings and the isthmus) appear, based on our results, to be the product of convergence (i.e., homoplasy).

**Haplocylicinae.**—The subfamily Haplocylicinae was erected by Briggs (1955) for the sole inclusion of the New Zealand endemic *Haplocylix littoreus*. Briggs (1955) considered his Haplocylicinae to be a relatively basal taxon within Gobiesocidae (Fig. 2A), which he differentiated from the Trachelochisminae ("the most primitive known assemblage in the family" according to Briggs, 1955: 147) based only on the form of the adhesive disc ("single" in *Haplocylix* and "double" in members of Trachelochisminae according to Briggs, 1955). Böhlke and Robins (1970) later added *Gymnoscyphus ascitus*, a deep-water-dwelling clingfish known from a small number of specimens collected via dredge in the Caribbean Sea and adjacent areas of the Atlantic (Conway and Prestridge, 2011), to the Haplocylicinae. Böhlke and Robins's (1970) decision was guided by the three-character system of Briggs (1955), but these authors also listed numerous differences between *Gymnoscyphus* and *Haplocylix*, suggesting that they were not entirely convinced of a close relationship between the two

genera ("when cleared and stained specimens of the two are compared, the differences between them are numerous" Böhlke and Robins, 1970: 8). Tissue samples of *Gymnoscyphus ascitus* were not available to us for study, but, based on morphological evidence, we suspect that this species is more closely related to members of the Protogobiesocinae than to *Haplocylix* (see below).

In the present study, *Haplocylix* is obtained in a strongly supported sister-group relationship with *Gastrocyathus* and *Gastroscyphus* (our Clade D; Figs. 3, 4), two New Zealand endemic taxa that have been placed previously in the Diplocrepinae (Briggs, 1955). Like *Haplocylix*, *Gastrocyathus* and *Gastroscyphus* are both obligate inhabitants of macroalgae in shallow coastal areas of New Zealand, from the intertidal zone to 40 meters (Paulin and Roberts, 1992; Stewart, 2015). This is also true for *Gastrocymba quadriradiata*, another diplocrepine restricted to the sub-Antarctic areas of New Zealand (Stewart, 2015). This species was placed as the sister taxon to Clade D in the topology resulting from the ML analysis but as the sister taxon to Clade B in the topology resulting from the Bayesian analysis (Figs. 3, 4). Neither of these alternative placements for *Gastrocymba* received strong branch support. In addition to being obligate inhabitants of macroalgae, these four taxa also share a number of potentially derived morphological characters, including: (1) adhesive disc of the "double" type, with the posterior inner disc well separated from the anterior disc region, and connected by a well-developed frenum along ventral midline; (2) dorsal postcleithrum a poorly ossified, narrow, vertically oriented strut of bone, with extensive fimbriae along posterior margin; and (3) principal caudal-fin rays 4+4. Briggs (1955) identified the adhesive disc of *Haplocylix littoreus* as "single" but we agree with Böhlke and Robins (1970) that the disc of this species should be considered "double." In addition to these characters, *Gastrocyathus*, *Gastrocymba*, and *Gastroscyphus* also exhibit a potentially unique (within Gobiesocidae) configuration of the pharyngeal jaws, in which the paired ceratobranchial 5 elements are in direct contact along much of their length, creating a broad plate-like structure in support of the lower pharyngeal jaw teeth (Conway, pers. obs.). This condition is not present in *Haplocylix*, in which only the anterior tips of the paired ceratobranchial 5 elements are in contact (as is the case in most gobiesocids; Springer and Fraser, 1976; for a list of materials examined, see Conway et al., 2015, 2017b, 2018).

Based on the results of this study, and the putative morphological characters listed above, we recommend transferring *Gastrocyathus* and *Gastroscyphus* from the Diplocrepinae to the Haplocylicinae. Though our results do not provide strong support for placing *Gastrocymba* as a member of our expanded Haplocylicinae, based on morphological grounds, we tentatively place this species within the Haplocylicinae pending further investigation with molecular and morphological character sets. An alternative action would be to consider *Gastrocymba* as *incertae sedis* within Gobiesocidae; however, we find the morphological similarities between *Gastrocyathus*, *Gastrocymba*, and *Gastroscyphus* compelling, and, in our opinion, strong evidence of shared ancestry.

**Lepadogastrinae.**—Briggs (1955) originally included *Apletodon*, *Diplecogaster*, *Gouania*, *Lepadogaster*, and *Opeatogenys* within his Lepadogastrinae. Shortly thereafter, Briggs (1957)

added *Lecanogaster*, which he considered to be a close relative of *Lepadogaster*. In his 1955 study, Briggs offered a dendrogram depicting his view of the relationships between the genera he had placed within the Lepadogastrinae (Fig. 2B). This scheme placed *Gouania* as the sister taxon to the remaining lepadogastrine genera, the relationships among which were: *Lepadogaster* (*Opeatogenys* (*Apletodon*, *Diplecogaster*)). Almada et al. (2008) investigated the relationships of eight lepadogastrine taxa (representing all genera except for *Lecanogaster*) using 12S and 16S mitochondrial gene sequences. The lepadogastrine taxa they included were not obtained as a monophyletic group due to the placement of *Aspasma ubauo* (treated as *A. minima* therein), which was recovered in a poorly supported sister-group relationship with *Lepadogaster candolii* (referred to as *L. candollei* by Almada et al., 2008; Fig. 2C). In Almada et al.'s (2008) study, two other species of *Lepadogaster* (*L. lepadogaster* and *L. purpurea*) were obtained in a moderately supported sister-group relationship with *Gouania* and not as close relatives of *L. candolii*. Due to the non-monophyly of *Lepadogaster*, Almada et al. (2008) recommended reclassifying *L. candolii* to the genus *Mirbelia*, but this nomenclatural act has not been widely adopted (e.g., Weitzmann and Mercader, 2012; Wagner et al., 2017; Fricke et al., 2020). Almada et al. (2008) recovered *Opeatogenys* as the sister taxon to the clade containing *Gouania*, *L. lepadogaster*, and *L. purpurea*, *Diplecogaster* as the sister taxon to a clade containing the aforementioned taxa plus *L. candolii* and *Aspasma ubauo*, and *Apletodon* as the sister taxon to all aforementioned taxa (Fig. 2C).

Our taxon coverage for the Lepadogastrinae replicates that of Almada et al. (2008). Unlike that study, we recovered all lepadogastrine taxa as members of a strongly supported monophyletic group (Clade A; Figs. 3, 4). The relationships within this group are also strongly supported and are identical in the topologies from the different analyses of our data. In all cases, we obtained *Diplecogaster* and *Opeatogenys* in a sister-group relationship, and this clade is placed as the sister group to a clade containing the remaining lepadogastrine taxa (*Apletodon*, *Gouania*, and *Lepadogaster*). As reported in Almada et al. (2008), we did not obtain the three species of *Lepadogaster* as a monophyletic group, because *L. candolii* is placed as the sister taxon to *Apletodon*, and *Gouania* is placed as the sister taxon to *L. lepadogaster* + *L. purpurea*. Based on these results, the reinstatement of *Mirbelia* (type species *Lepadogaster candolii*), currently in the synonymy of *Lepadogaster*, to accommodate *L. candolii* should be given serious consideration (as recommended by Almada et al., 2008). This nomenclatural act should take place in conjunction with rediagnosis of *Mirbelia*, which was originally introduced to include *L. candolii*, *Diplecogaster bimaculata*, and *Opeatogenys gracilis* (Canestrini, 1864). Unfortunately, we are not able to undertake this taxonomic work currently due to a lack of adequate material. The membership of the Lepadogastrinae in our revised classification is unchanged from Briggs (1955, 1957).

**Trachelochisminae.**—Briggs (1955) originally included *Conidens*, *Creocele*, *Dellichthys*, *Gastrocymba*, and *Trachelochismus* (type genus) within his Trachelochisminae (Fig. 2B), which he considered to be the “most primitive known assemblage” of the Gobiesocidae based largely on characters of oral dentition (Briggs, 1955: 143). Subsequently, Springer and Fraser (1976) recommended the transfer of *Gastrocymba*

*quadriradiata* from the Trachelochisminae to the Diplocrepinae because it lacks gill filaments on the 4<sup>th</sup> gill arch (the only character separating Trachelochisminae from Diplocrepinae in Briggs's three-character system). Hardy (1984) later added the deep water-dwelling genus *Kopua* to the Trachelochisminae, an act that he described as “provisional.”

Nine species representing all six genera (*Conidens*, *Creocele*, *Dellichthys*, *Gastrocymba*, and *Trachelochismus*) considered at some point or another to belong to the Trachelochisminae since Briggs (1955) were available herein for investigation and, regardless of the method of analysis, were never obtained as a monophyletic group. As discussed above, *Gastrocymba* was recovered either as the sister taxon to Clade B (containing *Kopua* and *Protogobiesox*) or Clade D (containing members of the Haplocylininae), and we transfer this genus to Haplocylininae in our revised classification based on morphological grounds. The deepwater clingfish genus *Kopua* (represented in our dataset only by mitochondrial genes for *K. minima*) was obtained in a strongly supported sister-group relationship with *Protogobiesox*, a member of the Protogobiesocinae (see below), within our Clade B (Figs. 3, 4). The relationships of *Kopua* are discussed further below (under Protogobiesocinae).

Though the remaining seven trachelochismine taxa included in our dataset were all obtained as members of the same clade (Clade G), they do not represent a monophyletic group within this clade and instead form a basal grade, which also involves *Aspasmogaster* (which was considered a member of the Diplocrepinae by Briggs, 1955; Figs. 3, 4). The New Zealand endemic genera *Dellichthys* and *Trachelochismus* are placed together as sister taxa, though with only weak to moderate branch support in the different analyses, and represent the sister group to the remainder of Clade G (ML analysis; Fig. 3) or are part of a basal trichotomy within this clade (Bayesian analysis; Fig. 4). The position of *Conidens* and *Creocele* also differed between the different topologies resulting from the different analyses and are placed either in a sister-group relationship (ML analysis; Fig. 3) or not (Bayesian analysis; Fig. 4). In the latter scenario, the two taxa are separated by *Aspasmogaster*, which is recovered in a sister-group relationship to a clade comprising *Creocele* + Clade H, though with only weak branch support (Fig. 4).

Based on these results, the Trachelochisminae, as currently defined (Briggs, 1955; Hardy, 1984), does not represent a monophyletic group. In order to correct this problem, we have implemented the following changes in our revised classification: (1) transfer of *Gastrocymba* to the Haplocylininae (as discussed above); (2) transfer of *Kopua* to the Protogobiesocinae (as discussed below); and (3) removal of *Conidens* and *Creocele* from the Trachelochisminae to *incertae sedis* within the Gobiesocidae (discussed below). In our revised classification, the Trachelochisminae are restricted to the New Zealand endemic genera *Trachelochismus* and *Dellichthys*.

**Protogobiesocinae.**—Fricke et al. (2017) recently, and somewhat perplexingly, introduced the subfamily Protogobiesocinae to accommodate two species, *Protogobiesox asymmetricus* and *Lepadicyathus mendeleevi*. *Protogobiesox asymmetricus* was described as a new genus and species by Fricke et al. (2017) based on five specimens collected between 370–560 meters off the coast of West Papua. *Lepadicyathus mendeleevi* was described earlier by Prokofiev (2005), also coincidentally



based on a small number of specimens (two) collected from Papua New Guinea (though from shallower depths). These two taxa are morphologically very different and would be placed in different subfamilies following the three-character based classification scheme of Briggs (1955): *Protogobiesox* within the Aspasminae and *Lepadicyathus* within the Diademichthyinae, though originally placed in the Aspasminae by Prokofiev (2005). Despite the obvious differences in external appearance, Fricke et al. (2017) considered *Protogobiesox* and *Lepadicyathus* to be close relatives based on variable levels of purported asymmetry, ranging from “highly asymmetrical” in *Protogobiesox* to “partial lateral asymmetry” in *Lepadicyathus*. The purported “highly asymmetrical” form of *Protogobiesox* was even reported to be sexually dimorphic, with the vertebral column “strongly bent towards the left in males, towards the right in females” (Fricke et al., 2017: 54, 57). This purported asymmetry of *Protogobiesox* was further qualified by Fricke et al. (2017: 57) as “not artificial” because “all four [sic] specimens of the type series show exactly the same pattern,” despite the reported sexual dimorphism. *Lepadicyathus* was also reported to exhibit the same type of sexually dimorphic asymmetry by Fricke et al. (2017: 57), with “vertebral column bent towards left in males, towards the right in females” despite not having examined specimens of this species (only photographs and radiographs) and the fact that Prokofiev (2005: 549), based on examination of the type series, reported that “In view of very small sizes... sex of fish cannot be determined upon dissection” and that “Among the species of clingfish described nearly in one half, a pronounced sex dimorphism in the urogenital papilla structure was revealed... there are no considerable differences with respect to this character; however, note that in specimen SL 14 mm [the holotype], the papilla was slightly better developed than in specimen SL 15 mm [the paratype]. Possibly holotype and paratype are individuals of different sexes.” Male gobiesocids typically exhibit larger genital papillae than females (Briggs, 1955) and if papilla size was indicative of sex in the holotype and paratype of *L. mendeleevi*, the 14 mm holotype with the larger genital papilla would likely represent a male, despite being labeled as female by Fricke et al. (2017: fig. 6). Having examined the type material of *L. mendeleevi* (Fujiwara, pers. obs.), we concluded that the “partial lateral asymmetry” reported by these authors in this species is clearly an artifact of the fixation process and not natural, as already reported by Prokofiev (2005: 547) at least for the larger paratype specimen: “The paratype was slightly damaged (crumpled), obviously, during fixation.” We have come to the same conclusion about *Protogobiesox asymmetricus* based on the information in Fricke et al. (2017: figs. 4, 5) and via examination of photographs and radiographs of the paratype available via the MNHN website (<https://science.mnhn.fr/institution/mnhn/collection/ic/item/2016-0333>). To get to the heart of the matter, in our opinion both taxa are asymmetrical as a result of post-mortem factors.

Like Fricke et al. (2017), we have not included sequences representing *Lepadicyathus* in our dataset. However, based on examination of the type material (Fujiwara, pers. obs.), we consider this taxon to belong to our expanded Diademichthyinae, because it exhibits: (1) the three characters of the Diademichthyinae originally put forward by Briggs (1955), including a “single” adhesive disc (not “double” as originally reported by Prokofiev, 2005), only three of four gill arches

bearing filaments, and the gill membranes united with the isthmus; and (2) the unique type of incisiform tooth with the hooked tip directed slightly posteriorly (clearly visible in Prokofiev, 2005: fig. 2b) that is found otherwise only in members of this subfamily as described above.

We have not examined material of the deep dwelling *Protogobiesox asymmetricus*, but mitochondrial DNA sequences of this species (available from Fricke et al., 2017) were included in our analyses. Regardless of the method of analysis, in all cases this taxon was obtained in a strongly supported sister-group relationship with *Kopua minima* (Clade B; Figs. 3, 4). Like *Protogobiesox*, members of *Kopua* also inhabit deep coastal areas (down to 380 meters; Hardy, 1984), exhibit a color pattern comprising bright red-orange markings on a lighter background (e.g., see Moore et al., 2012: fig. 3; Shinohara and Katayama, 2015: fig. 6; Fricke et al., 2017: figs. 4, 5; Fujiwara et al., 2018: fig. 2; Fujiwara and Motomura, 2020c: fig. 3), extremely large eyes positioned high on the head and separated by a narrow interorbital width, and a large, poorly papillated adhesive disc (“double” in *Kopua* but reportedly “single” in *Protogobiesox*) in which the pelvic-fin rays are elongate and reach past the posterior margin of the disc (Hardy, 1984; Moore et al., 2012; Shinohara and Katayama, 2015; Fricke et al., 2017; Fujiwara et al., 2018; Fujiwara and Motomura, 2020c). Based on these morphological characters and the results of the present study, we consider *Kopua* and *Protogobiesox* close relatives and recommend transfer of *Kopua* from the Trachelochisminae to the Protogobiesocinae. We suspect that the Caribbean deep-water-dwelling clingfish, *Gymnoscyphus ascitus*, which exhibits all of the morphological characters listed above and has previously been considered a member of the Haplocylininae (Böhlke and Robins, 1970), also is a close relative of *Kopua* and *Protogobiesox* and it is transferred herein from the Haplocylininae to the Protogobiesocinae pending further investigation.

**A revised systematic assignment of genera to subfamilies.**—In Table 1, we present a revised systematic assignment for the genera of the Gobiesocidae. This scheme is based largely on the results of the current study and those of several previous morphological studies. Our revisions are in most cases conservative (i.e., we have chosen to lump rather than split subfamilies further) and have resulted in changes only at the level of the subfamily. Though we have identified several genera that may not be monophyletic as currently defined, we have refrained from making changes at this level because either we do not currently have access to adequate material that would allow us to diagnose new or re-diagnose available genera or because we do not consider the taxonomic coverage in some areas of our dataset to be comprehensive enough to support changes at the generic level, which could have a negative impact on nomenclatural stability (Reis et al., 2019).

As discussed above, four genera are considered to be *incertae sedis* in Gobiesocidae. These include *Aspasmogaster* (previously assigned to the Diplocrepinae by Briggs, 1955), *Creocele* and *Conidens* (previously assigned to the Trachelochisminae by Briggs, 1955), and *Modicus* (tentatively assigned to the Aspasminae by Hardy, 1983). Some may ask why we have chosen not to place these taxa within our system of subfamilies when we have done so even for genera that are not represented in our dataset (viz., *Aspasmodes*,

*Briggsia*, *Gymnoscypus*, *Lecanogaster*, *Lepadicyathus*, *Liobranchia*, *Lissonanchus*, *Modicus*, *Nettorhamphos*, *Propherallodus*, and *Unguitrema*). We have taken this approach for different reasons, which we outline here. *Aspasmogaster*, *Creocele*, and *Conidens* are placed within our Clade G, but their position within this clade is incongruent in the topologies resulting from our different analyses. These three taxa also exhibit a number of plesiomorphic traits (*Creocele* has been described as the “most primitive known clingfish”; Briggs, 1955: 145) and do not exhibit any of the morphological characters that we have proposed as potential morphological synapomorphies that may provide evidence in support of certain subfamilies in our system (i.e., *Diademichthyinae*, *Gobiesocinae*, and *Haplocyclicinae*). Though *Gastrocymba* also switched positions between the topologies resulting from our different analyses, this taxon shares morphological characters in common with other taxa that we have placed within the *Haplocyclicinae*, and we feel comfortable transferring *Gastrocymba* from the *Diplocrepinae* to the *Haplocyclicinae* based on morphological grounds. We would not be comfortable placing *Aspasmogaster*, *Conidens*, or *Creocele* in any subfamily listed in our revised scheme based either on the results of our molecular study or morphology, and so we have chosen not to do so. Unlike the three other genera that we have labeled as *incertae sedis*, *Modicus* is characterized by a number of autapomorphies, including a unique heterodont oral dentition and arrangement of the gill rakers (Hardy, 1983) that are not known from other clingfishes. This genus was not represented in our dataset and, in the absence of a detailed investigation of its morphology, we do not currently have morphological evidence to justify placing it in any subfamily group. An alternative would have been to erect a new monogeneric subfamily for *Modicus*. We do not consider the erection of an additional subfamily for *Modicus* to be justified at the present time.

In his 224-page monographic treatment of the *Gobiesocidae*, Briggs (1955) provided descriptions of 33 genera (13 new), 82 species (21 new), and 11 subspecies (four new) of *gobiesocid* fishes. Simultaneously, he introduced a classification comprising eight subfamilies (five new) to accommodate these 33 genera that relied on a simple combination of three morphological characters, including: (1) the type of adhesive disc (single or double); (2) the number of gill arches with gill filaments (3 or 4); and (3) the arrangement of the gill membranes (united with or free from the isthmus). We are aware of no taxonomic studies on clingfishes published since 1960 that have not referenced Briggs's monograph work, which attests to the value of this contribution to the systematics of clingfishes. However, Briggs's classification has not been spared from criticism, the most vocal and directed of which first appeared in Böhlke and Robins (1970: 8), who wrote that “A new look at the subfamilies within the *Gobiesociformes* may be in order.” This call for a “new look” or “revision” of the subfamilies of Briggs has become commonplace in taxonomic studies of clingfishes published over the last three decades, especially those that have introduced new genera (e.g., Shioyaki and Dotsu, 1983; Hardy, 1983, 1984; Briggs, 1993; Craig and Randall, 2009), the most recent of which have either introduced a new subfamily to accommodate the new genus (e.g., Fricke et al., 2017) or have refrained from assigning new genera to subfamilies entirely (e.g., Conway et al., 2017b, 2018, 2019).

Though 50 years have passed since Böhlke and Robins (1970) first made their call for a “new look” or further investigation of Briggs's subfamilies, we are only now in a position to do so in a critical way. Herein, we assembled and analyzed the first multi-locus phylogenetic dataset for the *Gobiesocidae* to include members of all recognized subfamilies. As detailed herein, the results of our analyses suggest that many of the subfamilies that populate the classification stemming from Briggs and also the more recently adopted classification scheme that recognizes two subfamilies only are non-monophyletic. This includes the *Aspasminae*, *Diademichthyinae*, *Diplocrepinae*, *Gobiesocinae*, *Haplocyclicinae*, and *Trachelochisminae* in the traditional classification, and the *Gobiesocinae* (non-Briggs) in the reduced classification. Our results also have identified several genera that may not represent monophyletic groups, including the genera *Aspasmichthys*, *Cochleocephs*, *Lepadichthys*, and *Lepadogaster*, as currently defined. Based on the results of our phylogenetic analyses, and, taking into account the results of previous morphological studies, we have proposed (Table 1) a revised systematic assignment in which 47 of the 51 currently described genera of the *Gobiesocidae* are accommodated across nine subfamilies. Four genera (*Aspasmogaster*, *Conidens*, *Creocele*, and *Modicus*) are considered *incertae sedis*. Though we consider this a step forward, the groups that we have proposed are far from the final word on clingfish classification.

The “elephant in the room” is of course the poorly supported backbone uniting the major clades of the *Gobiesocidae* in the topologies that we have presented herein. These short internodes have extremely low branch support and, though they have not hindered our efforts to assign or reassign current genera to different subfamilies, they hinder any attempt to investigate the evolution of character traits (morphological, ecological, or geographical). Further investigation, involving additional molecular datasets will certainly be needed to assess whether the ‘comb’ forming the backbone of our topologies is a soft (i.e., result of insufficient information in our dataset) or a hard polytomy (i.e., cannot be solved through the addition of more data and/or more taxa). Such work should be conducted concurrently with comparative morphology studies, which are urgently needed not only to further our understanding of the relationships of those taxa for which DNA data are not currently available (e.g., *Modicus*) but also to provide a more comprehensive picture of the evolutionary history of the clingfishes, their anatomy, life history, and biology in general.

#### DATA ACCESSIBILITY

All sequences generated as a result of this study have been deposited in GenBank under accession numbers MT053010–MT053045, MT541780–MT541821, and MT569619–MT569861 (Table S2). A nexus file containing the seven-gene concatenated dataset has been deposited on TreeBASE (<http://purl.org/phylo/treebase/phylo/study/TB2:S25648>). Supplementary material is available at <https://www.copeiajournal.org/ci2020054>.

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