Phylogeny and taxonomy of sculpins, sandfishes, and snailfishes (Perciformes: Cottoidei) with comments on the phylogenetic significance of their early-life-history specializations

W. Leo Smith [a,*], Morgan S. Busby [b]

[a] Biodiversity Institute and Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045, USA
[b] Alaska Fisheries Science Center, National Marine Fisheries Service, 7600 Sand Point Way NE, Seattle, WA 98115, USA

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ABSTRACT

Despite recent progress on the higher-level relationships of the Cottoidei and its familial components, phylogenetic conflict and uncertainty remain within the Cottoidea. We analyzed a dataset composed of 4518 molecular (mitochondrial 12S, tRNA-Val, 16S, and cytochrome *b* and nuclear TMO-4c4, Histone H3, and 28S) and 72 morphological characters for 69 terminals to address cottoid intrarelationships. The resulting well-resolved phylogeny was used to produce a revised taxonomy that is consistent with the available molecular and morphological data and recognizes six families: Agonidae, Cottidae, Jordaniidae, Psychrolutidae, Rhamphocottidae, and Scorpaenichthyidae. The traditional Agonidae was expanded to include traditional hemitripterids and *Hemilepidotus*. The traditional Cottidae was restricted to *Leptocottus, Trachidermus*, and the riverine, lacustrine, and Lake Baikal freshwater cottoids. Jordaniidae (*Jordania* and *Paricelinus*) was separated from the traditional cottids; Psychrolutidae was expanded from the traditional grouping to include nearly all traditional marine cottids and the single species of bathylutichthyid. Rhamphocottidae was expanded to include the traditional ereuniids, and Scorpaenichthyidae separated *Scorpaenichthys* from the traditional cottids. The importance of early-life-history characters to the resulting phylogeny and taxonomy were highlighted.

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1. Introduction

1.1. Cottoid background

The cottoid fishes (sculpins, sandfishes, and snailfishes) are one of the largest and most morphologically diverse teleostean suborders with more than 825 species classified in 7–20 families (Jordan, 1923; Washington et al., 1984; Yabe, 1985; Smith and Wheeler, 2004; Eschmeyer, 2014). Despite progress on cottoid inter- and intrarelationships during the last 30 years following the ground-breaking work of Yabe (1985), problems with their taxonomy remain (Hunt et al., 1997; Jackson, 2003; Kontula et al., 2003; Smith and Wheeler, 2004; Crow et al., 2004; Imamura et al., 2005; Kinziger et al., 2005; Smith, 2005; Knope, 2013). These studies have variously refuted the monophyly of the Abyssocottidae (Hunt et al., 1997; Kontula et al., 2003; Kinziger et al., 2005),

Agonidae (Smith and Wheeler, 2004), Cottocomephoridae (Hunt et al., 1997; Kontula et al., 2003; Kinziger et al., 2005), Cottidae (Hunt et al., 1997; Jackson, 2003; Kontula et al., 2003; Smith and Wheeler, 2004; Crow et al., 2004; Kinziger et al., 2005; Smith, 2005; Knope, 2013), and Hemitripteridae (Smith and Wheeler, 2004; Smith, 2005; Knope, 2013), and they have often recovered the Bathylutichthyidae (Smith, 2005), Comephoridae (Hunt et al., 1997; Kontula et al., 2003; Smith and Wheeler, 2004; Kinziger et al., 2005; Smith, 2005), Cyclopteridae (Jackson, 2003; Smith and Wheeler, 2004), Liparidae (Jackson, 2003; Smith and Wheeler, 2004), and Psychrolutidae (Jackson, 2003; Smith and Wheeler, 2004; Smith, 2005; Knope, 2013) nested within the Cottidae. In short, the limits of the various cottoid families need revision.

Biologically, the cottoid radiation is perhaps best known for its diverse reproductive modes and early-life-history specializations (Breder and Rosen, 1966; Washington et al., 1984; Abe and Munehara, 2009; Muñoz, 2010). Typically, cottoids and their close allies in the Zaniolepididae and Hexagrammidae spawn demersal eggs (Matarese et al., 1989). Further, many sculpins provide parental care or deposit their eggs in concealed spaces or cavities (e.g.,

* Corresponding author. Address: Biodiversity Institute, 1345 Jayhawk Blvd., University of Kansas, Lawrence, KS 66045, USA.
  E-mail addresses: leosmith@ku.edu (W.L. Smith), morgan.busby@noaa.gov (M.S. Busby).

within the tissues of various invertebrates; Marliave, 1978; Munehara et al., 1991; Busby et al., 2012). Along with these behavioral modifications, cottoids have developed reproductive modifications that allow for specializations ranging from live birth in some Lake Baikal sculpins (*Comephorus*), to copulation, to internal gametic association with external fertilization in some sculpins, sea ravens, and poachers (e.g., Munehara et al., 1991, 1997; Petersen et al., 2004, 2005; Muñoz, 2010). Internal gametic association involves the joining of eggs and spermatozoa in the ovary with fertilization occurring after the eggs are deposited in seawater (Munehara et al., 1991, 1997; Busby et al., 2012). Once fertilized, cottoid young often have heavy spination, the development of armor, and precocious development in which flexion occurs within the egg (Matarese et al., 1989; Busby and Ambrose, 1993; Busby, 1998). These morphological and behavioral modifications are all strategies that have the potential to increase survivorship of the eggs, larvae, and juveniles.

1.2. Cottoid interrelationships

Historically, cottoid fishes and their close relatives in the Hexagrammoidei, Zaniolepidoidei, and Anoplopomatoidei have been classified with the scorpionfishes and allies in the Scorpaeniformes (Greenwood et al., 1966; Washington et al., 1984; Imamura and Shinohara, 1998; Nelson, 2006). However, recent work (Yabe and Uyeno, 1996; Imamura and Yabe, 2002; Chen et al., 2003; Miya et al., 2003; Smith and Wheeler, 2004, 2006; Smith and Craig, 2007; Lautredou et al., 2013) has demonstrated that the eelpouts (Zoarcoidei) or sticklebacks (Gasterosteioidei), not the scorpaenoid lineage, are the closest relatives to the Cottoid Lineage (reviewed in Smith and Wheeler (2004) and Lautredou et al. (2013)). Further, Smith and Wheeler (2004), Imamura et al. (2005) and Smith (2005) have provided morphological and molecular evidence or both to support the placement of the Trichodontidae in an expanded Cottoidei. Recent studies now consistently recover this expanded Cottoid Lineage among the scorpionfishes and seabasses (Scorpaenoidei), perches and icefishes (Percoidei), sticklebacks (Gasterosteioidei) and eelpouts (Zoarcoidei) in a clade that is most similar to an expanded Scorpaeniformes (Smith and Craig, 2007; Near et al., 2012, 2013; Lautredou et al., 2013; Wainwright et al., 2012; Betancur-R et al., 2014).

Washington et al. (1984) were the first to recognize a "cottoid" assemblage equivalent to the Cottoid Lineage (Anoplopomatoidei, Cottoidei, Hexagrammoidei, and Zaniolepidoidei); however, they did not provide any synapomorphies to unite the group. Shinohara (1994) proposed seven diagnostic characters for this clade (several of which built upon the pre-cladistic work of Regan (1913) and Quast (1965)): a parasphenoid–pterosphenoid junction, six branchiostegal rays, absence of a third epibranchial tooth plate, dorsal pterygiophores arranged singly in each interneural space, absence of accessory spine on head of cleithrum, absence of supraneurals (his predorsals), and the absence of anal spines with robust pterygiophores. Shinohara (1994) also provided morphological evidence for the monophyly and relationships among the Cottoid Lineage suborders (Fig. 1A). These hypotheses have generally been supported in subsequent molecular or morphological studies (Crow et al., 2004; Smith and Wheeler, 2004; Smith, 2005). Washington et al. (1984), Yabe (1985), Shinohara (1994) and Jackson (2003) all provided evidence for the monophyly of the Cottoidei, including the pleural ribs being absent or restricted to the posterior three abdominal vertebrae, lack of a basihyal, and the loss of the third *levator externus*. The monophyly of this suborder was supported by Crow et al. (2004), Smith and Wheeler (2004), Imamura et al. (2005) and Smith (2005), but was minimally contradicted by Lautredou et al. (2013) by the unusual placement of the psychrolutid, *Ebinania*, in their study.

1.3. Cottoid intrarelationships

Cottoidei is the largest suborder of traditional mail-cheeked fishes. This group has approximately 833 species (Eschmeyer, 2014) spread among 139 genera that are currently classified in nine families: Agonidae (46 spp.), Cottidae (289 spp.), Cyclopteridae (27 spp.), Ereuniidae (3 spp.), Hemitripteridae (8 spp.), Liparidae (416 spp.), Psychrolutidae (41 spp.), Rhamphocottidae (1 sp.), and Trichodontidae (2 spp.). This classification follows Yabe (1985) with the following recommendations by Smith and Wheeler (2004) and/or Smith (2005): inclusion of trichodontids (previously in the Trachinoidei) in Cottoidei, inclusion of the Bathylutichthyidae in Psychrolutidae, and the inclusion of abyssocottids, comephorids, and cottocomephorids in Cottidae. Regan (1913) first united this cottoid assemblage (less Trichodontidae) as the Cottiformes, an order of his Scleroparei. He used the reduction in size of the endopterygoid (his mesopterygoid) and intercalar (his opisthotic) and the loss of the basisphenoid to differentiate this taxon from other mail-cheeked fishes. Later, Jordan (1923), Berg (1940), Matsubara (1943), Quast (1965) and Greenwood et al. (1966) retained this assemblage in their classifications looking at various aspects of teleostean phylogeny.

Taranetz (1941) and Bolin (1947) provided the first detailed morphological surveys of the cottoids. Taranetz (1941) examined species across the Cottoidei and provided a wealth of anatomical data to justify his classification. Unfortunately, his study suffered from the use of inexplicit methods and a proliferation of family-level names that has often complicated cottid taxonomy. Bolin (1947; Fig. 1B) provided a "phylogenetic tree" of the cottids of California that utilized his own evolutionary methods. Bolin's hypothesis shares many similarities with recent phylogenetic hypotheses (Yabe, 1985; Smith and Wheeler, 2004), but its global taxonomic conclusions were hampered by its restriction to species in California and its lack of key cottoid groups (e.g., Agonidae, Psychrolutidae).

Washington et al. (1984; Fig. 1C) provided the first phylogenetic analysis of the Cottoidei. Their study, which briefly summarizes the hypothesized apomorphic states of 47 characters, was based on an unpublished manuscript by Washington and Richardson. This phylogeny separated their Cyclopteridae (Cyclopteridae + Liparidae) from the remaining families in the Cottoidea. Within the Cottoidea, Rhamphocottidae was sister to all other groups, followed by *Scorpaenichthys* + *Hemilepidotus*. The remainder of the Cottoidea was left unresolved as five clades plus a clade comprising Hemitripteridae and Agonidae. Shortly thereafter, Yabe (1985) published a more taxon-rich phylogeny of the Cottoidea building upon his earlier work (Yabe, 1981). The phylogeny and classification presented in Yabe (1985) is the basis of the current cottoid classification (e.g., Nelson, 2006). Yabe's (1985: Fig. 1D) study shared the sister-group placement of Rhamphocottidae to all other cottoids and a sister-group relationship between the Hemitripteridae and Agonidae with Washington et al. (1984). His published cladogram was more resolved than the work of Washington et al. (1984), but some of this resolution was not supported in the strict consensus phylogeny resulting from later computer-aided analyses of his matrix (see Jackson, 2003; Smith and Wheeler, 2004). In contrast to Washington et al. (1984) and Yabe (1985), the phylogenetic analysis presented in Jackson (2003) recovered *Scorpaenichthys* at the base of the Cottoidei. Jackson (2003) recovered *Jordania* and *Hemilepidotus* as subsequent splitting events leading toward a polytomy that included the Agonidae, Hemitripteridae, Rhamphocottidae, Cyclopteridae, Liparidae, Psychrolutidae, and the remainder of the Cottidae. One of the most important findings presented in Jackson (2003) was a cottid placement for the Psychrolutidae with *Artediellus* recovered as the sister group of the Psychrolutidae. The consensus of these studies is that there are some similarities
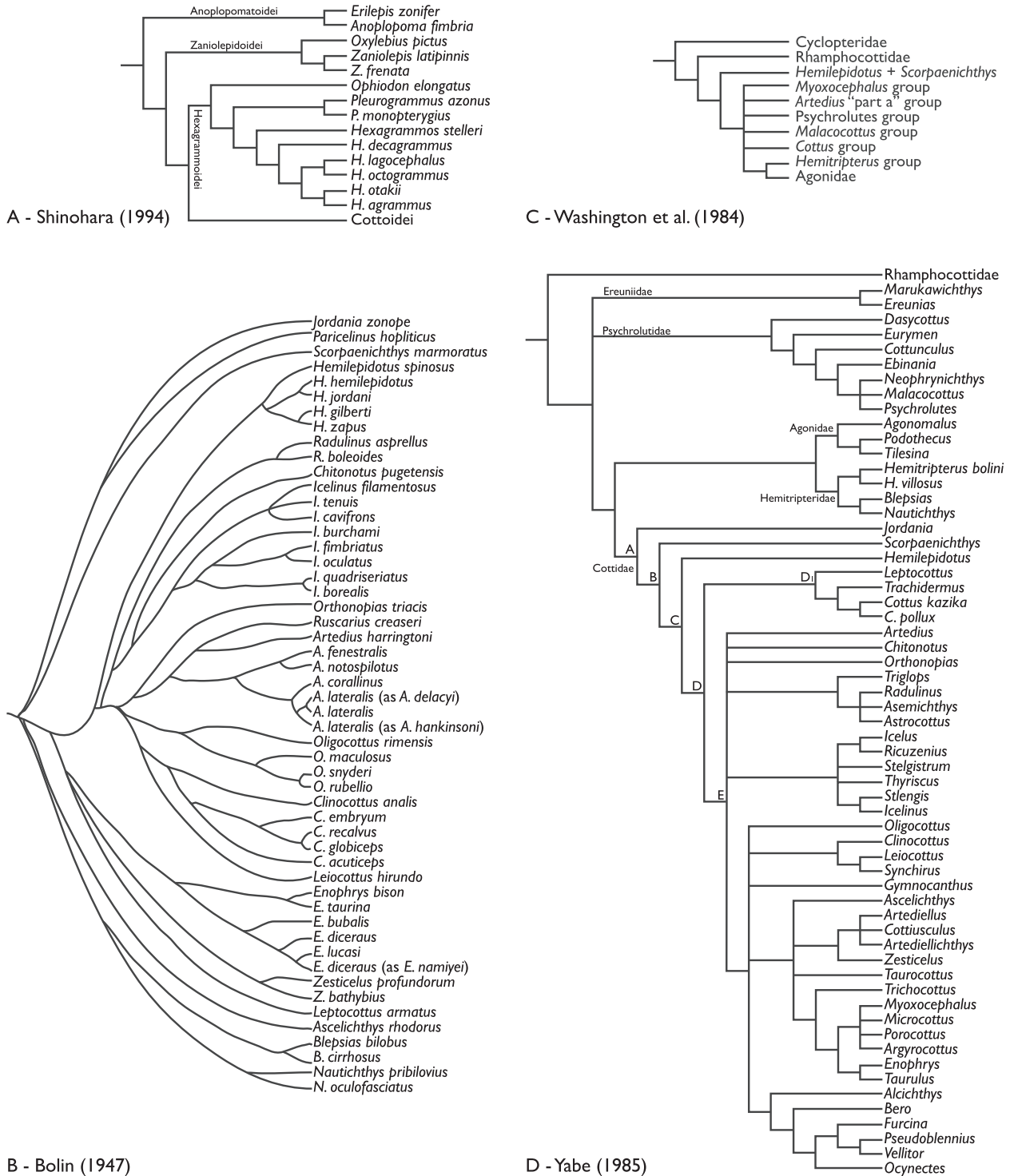
Fig. 1. Hypotheses of subordinal, superfamilial, and familial relationships of the cottoid lineage: (A) Cottoid Lineage (Shinohara, 1994); (B) California Cottidae (Bolin, 1947); (C) "cottoids" (Washington et al., 1984); and (D) Cottoidea (Yabe, 1985).

between the various morphological hypotheses, but more work is still required to reach stability.

In addition to the variation in morphological hypotheses, molecular phylogenies are further refining our understanding of the limits and relationships of cottoid families (Hunt et al., 1997; Kontula et al., 2003; Crow et al., 2004; Smith and Wheeler, 2004; Kinziger et al., 2005; Knope, 2013). Knope (2013; Fig. 2), in particular, provides a thorough analysis of the traditional Cottidae with one mitochondrial and one nuclear marker. This study, like Bolin (1947), lacks a few key groups for resolving the limits and

relationships of the family-level phylogeny of the Cottoidei, but it does include robust sampling for the traditional marine sculpins. These molecular studies corroborate several morphological hypotheses, but they variously and inconsistently refute several of the morphologically diagnosed clades. Despite these differences, the independent morphological and molecular datasets often support similar clades (e.g., *Myoxocephalus* + *Microcottus,* Lake Baikal cottoids nested within *Cottus,* a close relationship between hemitripterids and agonids, *Artediellus* as sister to Psychrolutidae).
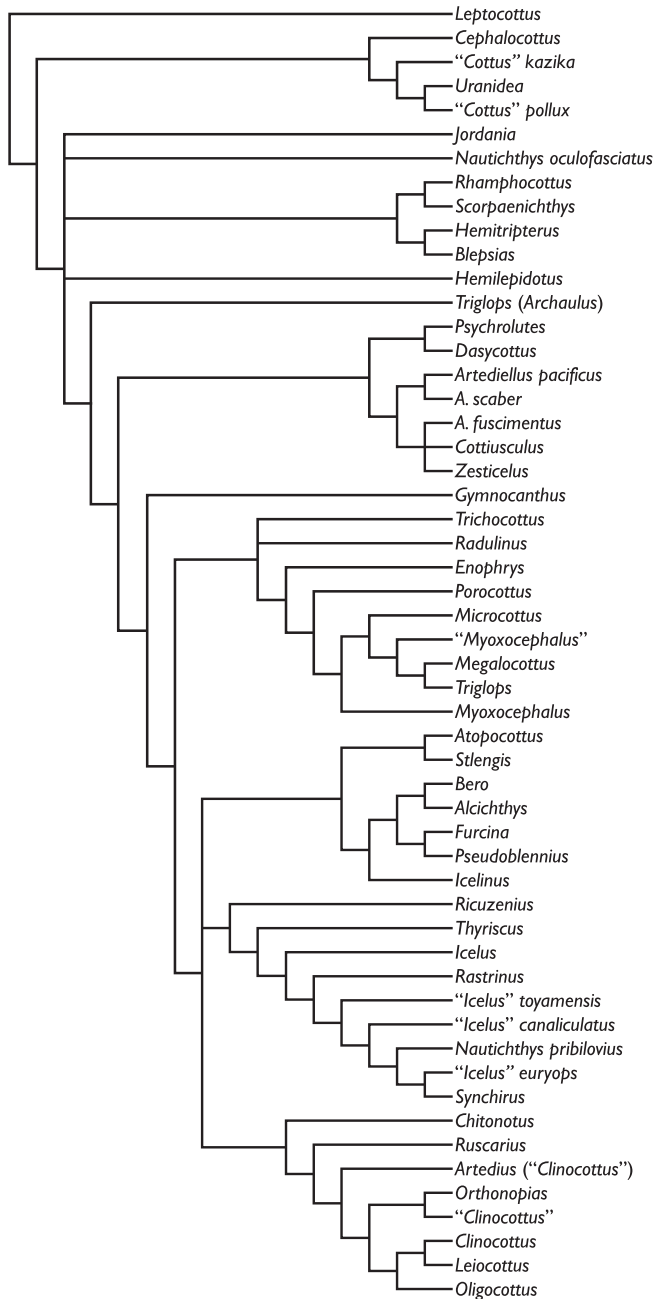
Fig. 2. Hypotheses of subordinal, superfamilial, and familial relationships of the Cottoid Lineage: from Eastern Pacific Cottidae (modified from Knope, 2013). Relative to the figure from Knope (2013), monophyletic genera are illustrated as a single genus-level terminal and polyphyletic genera have the non-name bearing components labeled with quotation marks. If monotypic genera were recovered within otherwise monophyletic genera, those paraphyly-inducing genera are listed in parentheses.

Building upon the wealth of published morphological data and the few published molecular phylogenies, we present the results of a simultaneous analysis of morphological and molecular data that aims to resolve the family-level phylogeny and taxonomy of cottoids. Our analysis includes representatives of all cottoid families. The resulting hypothesis of relationships is based upon the simultaneous analysis of nucleotide characters from four mitochondrial loci: the small ribosomal subunit (12S), the complete tRNA-Valine (tRNA-Val), the large ribosomal subunit (16S), and cytochrome *b* (Cyt*b*); three nuclear loci: the large ribosomal subunit (28S), histone H3 (H3), and TMO-4c4 (TMO); and 72

phenotypic features (a combination of variable characters from Yabe (1985) and Kido (1988), previously documented characters from Washington et al. (1984), and new early-life-history specializations). These gene fragments alone and in combination with other morphological or molecular data have shown efficacy in studies of similar scope (e.g., Smith et al., 2009; Chakrabarty et al., 2011; Li et al., 2011). The objectives of this study are to use these characters to (1) test the monophyly and interrelationships of the Hexagrammoidei, Zaniolepidoidei, Cottoidei, Cyclopteroidea, and Cottoidea; (2) test the monophyly of the cottoid families; (3) resolve relationships among the cottoid families; and (4) make family-level taxonomic changes to reflect a monophyletic classification of the Cottoidea.

2. Materials and methods

2.1. Taxon sampling

Sixty-nine taxa were analyzed in the current study. To provide a robust test of cottoid monophyly, one anoplopomatid, both zaniolepidid genera, and all three hexagrammid genera were included as outgroups. The topology was rooted with *Anoplopoma*. The 63 cottoid terminals analyzed in this study included all nine cottoid families, 54 of 147 genera in Cottoidei, and 45 of 114 genera in Cottoidea (where all familial taxonomic conflict lies (Smith and Wheeler, 2004; Kinziger et al., 2005; Smith, 2005; Knope, 2013)).

2.2. Character sampling

A total of 4590 morphological and molecular characters were analyzed. These data included 4518 aligned nucleotides from four mitochondrial and three nuclear loci. The molecular terminals analyzed in the present study and GenBank accession numbers corresponding to the gene fragments sequenced are listed in Table 1. For the analyses, the 208 novel DNA sequences were combined with previously published DNA sequences from the following sources: Streelman and Karl (1997), Kinziger and Wood (2003), Kontula and Vainola (2003), Kontula et al. (2003), Miya et al. (2003), Near et al. (2003), Smith and Wheeler (2004), Kinziger et al. (2005), Yokoyama and Goto (2005), Knudsen et al. (2007), Mabuchi et al. (2007), Ramon and Knope (2008), Mandic et al. (2009) and Yamazaki et al. (2013). The molecular matrix was 91% complete at the amplicon level (Table 1) and 88% complete at the cell or individual-base-pair level. These molecular data were simultaneously analyzed with a morphological dataset (Table 2) composed of 72 characters that was built from multiple sources, but focused on the work of Yabe (1985). Details about additional sources of morphological data are listed along with all character descriptions in Appendix A. The morphological matrix was 81% complete at the cell or individual character level (Table 2).

2.3. Acquisition of nucleotide sequences

Fish tissues were preserved in 95% ethanol prior to extraction of DNA. Nuclear and mitochondrial DNA was extracted from muscle using a DNeasy Tissue Extraction Kit (Qiagen, Valencia, CA). The polymerase chain reaction was used to amplify all gene fragments. Double-stranded amplifications were performed in a 25 µL volume containing one Ready-To-Go PCR bead (GE Healthcare, Piscataway, NJ), 1.25 µL of each primer (10 pmol), and 2–5 µL of undiluted DNA extract. Primers and primer sources are listed in Table 3. Amplifications for all novel fragments were carried out in 36 cycles using the following temperature profile: initial denaturation for 6 min at 94 °C, denaturation for 60 s at 94 °C, annealing for 60 s at 46–53 °C (see Table 3 for core annealing temperature for each

Table 1
Revised classification of species with GenBank accession numbers and voucher specimens for new sequences (KM057847–KM058054).

Taxon	Voucher	12S	tRNA-Val-16S	16Sar-br	Cytochrome *b*	Histone H3	TMO-4c4	28S
Anoplopomatoidei								
Anoplopomatidae								
Anoplopoma fimbria	KU 23726	KM057941	AY538905, AY539522	AY539010	FJ264418	AY539219	AY539422	AY539115
Zaniolepidoidei								
Zaniolepididae								
Oxylebius pictus	SIO 01-193	KM057978	KM058045	KM057868	FJ264487	KM057931	KM058015	KM057893
Zaniolepis frenata	SIO Uncat. – San Diego	KM057996	AY538908, AY539525	AY539013	Unavailable	AY539222	AY539425	AY539118
Hexagrammoidei								
Hexagrammidae								
Hexagrammos decagrammus	SIO 01-113	KM057963	AY538906, AY539523	AY539011	FJ264366	AY539220	AY539423	AY539116
Ophiodon elongatus	KU 23737	KM057976	KM058043	KM057866	AY225661	KM057930	KM058013	KM057892
Pleurogrammus azonus	SIO Uncat. – San Diego	KM057979	AY538907, AY539524	AY539012	AB087415	AY539221	AY539424	AY539117
Trichodontoidea								
Trichodontidae								
Arctoscopus japonicus	N/A	AP003090	AP003090	AP003090	AP003090	Unavailable	Unavailable	Unavailable
Trichodon trichodon	J. Orr	KM057991	AY538961, AY539578	AY539066	FJ264405	AY539275	AY539474	AY539170
Cyclopteroidea								
Cyclopteridae								
Aptocyclus ventricosus	KU 2553	KM057942	AY538937, AY539554	AY539042	NC008129	AY539251	AY539450	AY539146
Cyclopterus lumpus	G. Lecointre	KM057954	AY538938, AY539555	AY539043	EU492084	AY539252	AY539451	AY539147
Eumicrotremus orbis	KU27982	KM057957	KM058036	KM057859	KM057901	KM057923	KM058007	KM057886
Liparidae								
Careproctus melanurus	SIO 95-2	KM057949	AY538939, AY539556	AY539044	DQ082906	AY539253	AY539452	AY539148
Liparis mucosus	SIO 00-166	KM057969	AY538940, AY539557	AY539045	FJ264468	AY539254	AY539453	AY539149
Paraliparis devriesi	G. Hoffman	Unavailable	KM058046	KM057869	KM057907	KM057932	Unavailable	Unavailable
Rhinoliparis barbulifer	KU28010	Unavailable	KM058051	KM057874	EF508331	KM057937	KM058020	KM057897
Cottoidea								
Agonidae								
Blepsias cirrhosus	AMNH Uncat. – Friday Harbor	KM057948	KM058027	KM057851	EU836702	KM057916	KM057999	KM057881
Hemilepidotus jordani	KU28436	KM057959	AY538916, AY539533	AY539021	AY833367	AY539230	AY539432	AY539125
Hemilepidotus zapus	SIO 94-240	KM057960	AY538917, AY539534	AY539022	Unavailable	AY539231	Unavailable	AY539126
Hemitripterus americanus	AMNH Uncat. – Mid Atlantic Bight	KM057961	AY538929, AY539546	AY539034	KM057903	AY539243	AY539443	AY539138
Hemitripterus bolini	KU28392	KM057962	KM058039	KM057862	KM057904	KM057926	KM058010	KM057888
Nautichthys pribilovius	SIO AH-110	KM057975	AY538930, AY539547	AY539035	Unavailable	AY539244	AY539444	AY539139
Agoninae								
Agonopsis vulsa	AMNH Uncat. – Friday Harbor	KM057940	KM058023	KM057847	FJ264384	KM057912	KM057997	KM057877
Aspidophoroides monopterygius	KU28325	KM057944	KM058024	KM057848	AB126377	KM057913	Unavailable	KM057878
Bathyagonus nigripinnis	UW Uncat. – KU Tissue 2048	Unavailable	KM058025	KM057849	FJ264479	KM057914	Unavailable	KM057879
Bathyagonus pentacanthus	SIO 97-184	KM057946	KM058026	KM057850	FJ264423	KM057915	KM057998	KM057880
Hypsagonus quadricornis	SIO 94-240	KM057964	AY538931, AY539548	AY539036	FJ264435	AY539245	AY539445	AY539140
Podothecus accipenserinus	AMNH Uncat. – Friday Harbor	KM057980	KM058047	KM057870	FJ264307	KM057933	KM058016	KM057894
Sarritor frenatus	KU28041	KM057986	AY538932, AY539549	AY539037	Unavailable	AY539246	AY539446	AY539141
Stellerina xyosterna	SIO 03-93	KM057988	KM058053	AY769986	AY973043	KM057939	KM058021	Unavailable
Xeneretmus latifrons	SIO Uncat. – San Diego	KM057995	AY538933, AY539550	AY539038	KM057911	AY539247	AY539447	AY539142
Cottidae								
Asprocottus pulcher	S. Kirilchik	KM057945	AY538928, AY539545	AY539033	AY116346	AY539242	AY539442	AY539137
Batrachocottus baicalensis	J. Volff	KM057947	AY538926, AY539543	AY539031	Unavailable	AY539240	AY539440	AY539135
Cephalocottus amblystomopsis	A. Nolte	AB188182	KM058028	KM057852	AY833329	KM057917	KM058000	Unavailable

Table 1 (continued)

Taxon	Voucher	12S	tRNA-Val-16S	16Sar-br	Cytochrome *b*	Histone H3	TMO-4c4	28S
Comephorus baikalensis	S. Kirilchik	KM057951	AY538927, AY539544	AY539032	AY116355	AY539241	AY539441	AY539136
Cottocomephorus inermis	S. Kirilchik	KM057952	KM058031	KM057854	AY116360	KM057920	KM058003	KM057884
Cottus gobio	A. Nolte	AB188189	KM058032	KM057855	AY116366	Unavailable	KM058004	Unavailable
"*Cottus*" *hangiongensis*	A. Nolte	AB188183	KM058033	KM057856	AY833349	Unavailable	KM058005	Unavailable
"*Cottus*" *poecilopus*	A. Nolte	AB188187	AY538915, AY539532	AY539020	AY833361	AY539229	AY539431	AY539124
Cyphocottus eurystomus	S. Kirilchik	Unavailable	KM058034	KM057857	AY116348	KM057921	Unavailable	Unavailable
Leptocottus armatus	FMNH Uncat. – Bodega Bay	KM057968	AY538920, AY539537	AY539025	EU836697	AY539234	AY539435	AY539129
Uranidea bairdi	A. Simons	KM057993	AY538913, AY539530	AY539018	AY833335	AY539227	AY539429	AY539122
Uranidea carolinae	A. Simons	KM057994	AY538914, AY539531	AY539019	AF549111	AY539228	AY539430	AY539123
Jordaniidae								
Jordania zonope	J. Marliave	KM057967	AY538919, AY539536	AY539024	EF521365	AY539233	AY539434	AY539128
Psychrolutidae								
Chitonotus pugetensis	AMNH Uncat. – Friday Harbor	Unavailable	KM058029	KM057853	EF521368	KM057918	KM058001	KM057882
Enophrys bison	AMNH Uncat. – Friday Harbor	KM057956	KM058035	KM057858	EU836693	KM057922	KM058006	KM057885
Furcina sp.	FMNH Uncat. – Pet Trade	KM057958	KM058037	KM057860	KM057902	KM057924	KM058008	Unavailable
Gymnocanthus galeatus	SIO 94-233	Unavailable	KM058038	KM057861	JQ406214	KM057925	KM058009	KM057887
Icelinus filamentosus	SIO 97-184	KM057965	AY538918, AY539535	AY539023	FJ264425	AY539232	AY539433	AY539127
Icelus spiniger	KU 27939	KM057966	KM058040	KM057863	KM057905	KM057927	KM058011	KM057889
Microcottus sellaris	KU 28353	KM057972	AY538921, AY539538	AY539026	KM057906	AY539235	Unavailable	AY539130
Myoxocephalus octodecemspinosus	AMNH Uncat. – Mid Atlantic Bight	KM057973	KM058041	KM057864	AY338279	KM057928	KM058012	KM057891
Myoxocephalus polyacanthocephalus	AMNH Uncat. – Friday Harbor	KM057974	AY538922, AY539539	AY539027	AY338280	AY539236	AY539436	AY539131
Porocottus camtschaticus	UW 044501	KM057981	KM058048	KM057871	KM057908	KM057934	KM058017	KM057895
Radulinus asprellus	KU 28230	KM057983	AY538923, AY539540	AY539028	FJ264438	AY539237	AY539437	AY539132
Rastrinus scutiger	KU 28172	KM057984	KM058050	KM057873	Unavailable	KM057936	KM058019	KM057896
Stlengis misakia	FMNH Uncat. - Pet Trade	KM057989	KM058054	KM057876	KM057910	Unavailable	KM058022	KM057899
Taurulus bubalis	G. Lecointre	KM057990	AY538924, AY539541	AY539029	EU492317	AY539238	AY539438	AY539133
Triglops scepticus	SIO 94-233	KM057992	AY538925, AY539542	AY539030	Unavailable	AY539239	AY539439	AY539134
Oligocottinae								
Artedius fenestralis	AMNH Uncat. – Friday Harbor	KM057943	AY538912, AY539529	AY539017	EU836698	AY539226	AY539428	AY539121
Clinocottus analis	FMNH Uncat. – Bodega Bay	KM057950	KM058030	AY835646	AY833327	KM057919	KM058002	KM057883
Oligocottus snyderi	FMNH Uncat. – Bodega Bay	Unavailable	KM058042	KM057865	EU836695	KM057929	Unavailable	Unavailable
Orthonopias triacis	SIO 03-166	KM057977	KM058044	KM057867	EF521370	Unavailable	KM058014	Unavailable
Psychrolutinae								
Cottunculus thomsonii	G. Lecointre	KM057953	AY538934, AY539551	AY539039	KM057900	AY539248	AY539448	AY539143
Dasycottus setiger	AMNH Uncat. – Friday Harbor	KM057955	AY538935, AY539552	AY539040	FJ264339	AY539249	AY539449	AY539144
Malacocottus zonurus	SIO 94-225	KM057970	AY538936, AY539553	AY539041	AY116340	AY539250	Unavailable	AY539145
Psychrolutes phrictus	SIO 95-26	KM057982	KM058049	KM057872	KM057909	KM057935	KM058018	Unavailable
Rhamphocottidae								
Marukawichthys ambulator	HUMZ 181238	KM057971	AY538911, AY539528	AY539016	Unavailable	AY539225	Unavailable	KM057890
Rhamphocottus richardsonii	SIO 01-79	KM057985	AY538910, AY539527	AY539015	FJ264436	AY539224	AY539427	AY539120
Scorpaenichthyidae								
Scorpaenichthys marmoratus	AMNH Uncat. – Bodega Bay	KM057987	KM058052	KM057875	EU836694	KM057938	U71186	KM057898

locus), and extension for 75 s at 72 °C, with an additional terminal extension at 72 °C for 6 min. The double-stranded amplification products were desalted and concentrated using AMPure (Agencourt Biosciences, Beverly, MA). Both strands of the purified PCR fragments were used as templates and amplified for sequencing using the amplification primers and a Prism Dye Terminator Reaction Kit Version 1.1 (Applied Biosystems, Foster City, CA) with minor modifications to the manufacturer's protocols. The sequencing reactions were cleaned and desalted using cleanSEQ (Agencourt Biosciences, Beverly, MA). The nucleotides were sequenced and the base pairs were called on a 3730 automated DNA sequencer (Applied Biosystems, Foster City, CA). Contigs were built in Sequencher (Gene Codes, Ann Arbor, MI) using DNA sequences from the complementary heavy and light strands. Sequences were

Table 2
Matrix of phenotypic characters analyzed in the current study (characters 1–72) with the following notations for polymorphic states (*A* = 0 and 1, *B* = 0 and 2).

```
                                    1111111111222222222233333333334444444444555555555566666666667777
                                    1234567890123456789012345678901234567890123456789012345678901234567890123456789012

Anoplopoma fimbria                  10?10?1?010100011000010020000310000000000?0??10?00??000????????????1200
Oxylebius pictus                    00?10?1?011000001000010020000A01000000110020??10?000?000????????????1211
Zaniolepis frenata                  10?10?1?1001000000010020000A001000001101?0??10?000?000????????????2211
Ophiodon elongatus                  00?10?1?1101?0010000010020000A100100?10000?0??10?0??000????????????1201
Hexagrammos decagrammus             00?10?1?000100011000010120000A100100010000?0??10?010?000????????????1201
Pleurogrammus azonus                00?10?1?000101011000010120000A100100010000?0??10?010?000????????????1201
Arctoscopus japonicus               00?00?0?0111?1010?00?1?1?00003111100?1?100?1-??0??????00????????????10?0
Trichodon trichodon                 00?00?0?0111?1010?00?1?1?00003111100?1?101?1-??0??????00????????????1100
Cyclopterus lumpus                  00?11?0?0?11?1011?01?1-1?0000A?11101?1?101?0??0?????0?00000000000000002211
Aptocyclus ventricosus              ???1??????111??1?1001?11?????????1010111????????1??11??00???????????220?
Eumicrotremus orbis                 10?11?0?021?1101110011-1?0000A011101011101?0??1??11?00????????????2211
Liparis mucosus                     10?11?0?011?111011111-111?0000311110110111?0??1??11?000000000000000002?00
Careproctus melanurus               10?11?-??111?1011111-1-1?0000311111-111211?0??1??????10000000000000001201
Paraliparis devriesi                10?11?-??????1??1111-1????????????1??1?121?????????????1??????????????010?
Rhinoliparis barbulifer             10?11?-????-?1??1111-1????????????1??1?121??????????????11??????????????010?
Rhamphocottus richardsonii          10010110001110101001110110001010111121110201100110100000000000002211
Marukawichthys ambulator            000101010111100101010-121011001011101111200000110011010000????????2011
Hemilepidotus jordani               10010011011110011101-1210010001001011110000001101110100110000000002211
Hemilepidotus zapus                 10010011011110011101-1210010001001011110000001101110100110000000002211
Nautichthys pribilovius             10010011111100111101-121001001101011110101010111010000110000001122211
Blepsias cirrhosus                  1001000211111001111-1210010011011111111000010011011000110000000112211
Hemitripterus americanus            0001001211111001111-A2100110B110101111111001A0A1011101000011000001112211
Hemitripterus bolini                00010012101110011111-1210010011010101111110010010111010000110000001122211
Hypsagonus quadricornis             1101111210111A011111-1210111011101111211003211111101000????????????1211
Aspidophoroides monopterygius       10?1??111?11?0??1111-120101113?1111-?1?21??03????????00????????????011?
Podothecus accipenserinus           1101111101111011111-1211011131111-111211003211111101000????????????0111
Sarritor frenatus                   10?1??111?11?1??11101121101113?1111-?1?21??03????????00????????????011?
Stellerina xyosterna                10?1??111?11?0??1111-121101113?1111-????1?03????????0000110000001111?
Agonopsis vulsa                     10?1??111?11?0??1111-121101113?1111-??21?03????????00????????????121?
Xeneretmus latifrons                10?10?111111?0011111-1211011131111-?1?211?03??0??????00000100000011211
Bathyagonus nigripinnis             10?10?110111?0011111-120101113111-?1?210?03??0??????00001100000110211
Bathyagonus pentacanthus            10?10?110111?0011111-120101113111-?1?210?03??0??????00001100000111211
Scorpaenichthys marmoratus          00010011011110111101-121000011001011110001-01101101001100000000002201
Jordania zonope                     00010011011110110100111000000100101111000001101110100????????????1
Leptocottus armatus                 00010011011110111101-121000011101011120111-0110111010000110000010001001
Cephalocottus amblystomopsis        0001001101111A111101-121000011101011120111-0110111100001000001001?1
"Cottus" hangiongensis              0001001101111A111101-121000011101011120111-0110111100001000001001?1
"Cottus" poecilopus                 0001001101111A111101-121000011101011120111-011011110000100000100??1
Cottus gobio                        0001001101111A111101-121000011101011120111-011011110000110000010012?1
Uranidea bairdi                     0001001101111A111101-121000011101011120111-011011110000110000010001201
Uranidea carolinae                  0001001101111A111101-121000011101011120111-011011110000110000100???1
Batrachocottus baicalensis          11?10?1?1111?1111?01?1?1?0100A111101?1?201?0???0??????00????????????12?1
Comephorus baikalensis              ?????????????????????????????????????????????????????????????????????000?
Cottocomephorus inermis             ?????????????????????????????????????????????????????????????????????000?
Icelinus filamentosus               0001011101111011111-1210011111110111201000011011010000110000000011001
Stlengis misakia                    0001011101111011111-121001101111011112010000110111010100????????????1
Furcina ishikawae                   0001011101111011111-121001111111011120101-011011010100????????????1
Artedius fenestralis                0001011101111011101-121001100111011120100000110111010000110111000020011
Orthonopias triacis                 0001011101111011101-121001100111011120100021101110100????????????12?1
Clinocottus analis                  0001011101111011101-121001100111011120101-11101110100001101110000120011
Oligocottus snyderi                 0001011101111011101-121001100111011120101-011011010000110111000020011
Radulinus asprellus                 1001011101111011101-121001100111011112010000110111010000110000000011211
Dasycottus setiger                  10110102021111011111-021011001110111201010-011011000000110000100021011
Malacocottus zonurus                10111102021111011111-021011001110111201110-01101100000011000010002201
Cottunculus thomsonii               1111010202111101111-021011001110111201110-011011100000????????????1
Psychrolutes phrictus               10111112021111011111-021011001110111120111-001011110000110000010000002211
Triglops scepticus                  10010112011111111101-121001100111011112010001101111100????????????2001
Enophrys bison                      10010111011111111111-121001100111011120111-01101110000110000000021211
Taurulus bubalis                    10010111011111111111-121001100111011120111-0110111010000????????????20?1
Myoxocephalus octodecemspinosus     10010111011111111111-121001100111011120101-01101110000110000000011?1
Microcottus sellaris                10010111011111111111-121001100111011120101-01101110100????????????1
Myoxocephalus polyacanthocephalus   10010111011111111111-121001100111011120101-01101110000110000000012201
Porocottus camtschaticus            10010111011111111111-121001100111011120101-01101110100????????????21?1
Gymnocanthus galeatus               0001011101111011101-121001100111011120101-01101101000011100000001101
Chitonotus pugetensis               0001011101111011111-121001100111011112010000110111010000110000000011211
Icelus spiniger                     0001011101111011111-121001100111011112010000110111010100001110000001001
```

Table 3
Primers, PCR conditions, and substitution models for each amplicon analyzed in the current study.

Primer name (Source)	Primer sequence	Primary annealing temperature (°C)
12S (Tang, 2001) – whole amplicon: GTR + I + G		
Phe2-L	5′-AAAGCATAACACTGAAGATGTTAAGATG-3′	47
12Sb-H	5′-AGGAGGGTGACGGGCGGTGTGT-3′	47
tRNA-Val-16S (Titus, 1992; Feller and Hedges, 1998) – whole amplicon: GTR + I + G		
12SL13-L	5′-TTAGAAGAGGCAAGTCGTAACATGGTA-3′	48
TitusI-H	5′-GGTGGCTGCTTTTAGGCC-3′	48
16Sar-br (Kocher et al., 1989; Palumbi, 1996) – whole amplicon: GTR + I + G		
16S ar-L	5′-CGCCTGTTTATCAAAAACAT-3′	48
16S br-H	5′-CCGGTCTGAACTCAGATCACGT-3′	48
Cytochrome *b* (Schmidt and Gold, 1993) – 1st Pos.: HKY + I + G; 2nd Pos.: HKY + I + G; 3rd Pos.: GTR + I + G		
L14724	5′-GTGACTTGAAAAACCACCGTTG-3′	48
H15915	5′-CAACGATCTCCGGTTTACAAGAC-3′	48
Histone H3 (Colgan et al., 1998) – 1st Pos.: GTR + G; 2nd Pos.: JC + G; 3rd Pos.: HKY + I + G		
H3a-L	5′-ATGGCTCGTACCAAGCAGACVGC-3′	48
H3b-H	5′-ATATCCTTRGGCATRATRGTGAC-3′	48
TMO-4c4 (Streelman and Karl, 1997) – 1st Pos.: GTR + I + G; 2nd Pos.: GTR + I; 3rd Pos.: GTR + G		
TMO-f1	5′-CCTCCGGCCTTCCTAAAACCTCTC-3′	51
TMO-r1	5′-CATCGTGCTCCTGGGTGACAAAGT-3′	51
28S (Hillis and Dixon, 1991) – whole amplicon: GTR + I + G		
28SV	5′-AAGGTAGCCAAATGCCTCGTCATC-3′	48
28SJJ	5′-AGGTTAGTTTTACCCTACT-3′	48

edited in Sequencher and collated into fasta text files. The novel sequences were submitted to GenBank and assigned accession numbers KM057847–KM058054.

2.4. Phylogenetic analyses

Both partitioned likelihood and parsimony analyses were used to analyze the morphological and molecular data using equal weights. For these analyses, each of the seven amplicons was aligned individually in MAFFT (Katoh et al., 2002) using default values. The maximum-likelihood dataset was broken into 12 partitions. Two partitions were designated for the mitochondrial (12S, tRNA-Val-16S, and 16Sar-br) and nuclear (28S) ribosomal fragments. Nine partitions covered the three codon positions in each of the three protein coding genes: mitochondrial (cytochrome *b*) and nuclear (histone H3, and TMO-4c4). The 12th partition was the morphological dataset (Table 2). The optimal nucleotide substitution model for each molecular partition was determined empirically (Table 3) by comparing different models under an Akaike information criterion as executed in jModelTest (Posada, 2008). The datasets were coded, concatenated, examined, and analyzed (ancestral state reconstructions) in Mesquite v2.75 (Maddison and Maddison, 2011). The maximum likelihood analysis was conducted in GARLI v2.0 (Zwickl, 2006), and the tree with the best likelihood score from 100 independent analyses was selected as the preferred hypothesis. A nonparametric maximum-likelihood bootstrap analysis was conducted for 200 random pseudoreplicates to assess nodal support. The parsimony analysis was conducted in NONA (Goloboff, 1998) using gaps as a fifth state, and the topology with the fewest steps was used to evaluate evolutionary relationships and anatomical features. The parsimony analysis used 1000 replications with different random addition sequences of taxa. Each replication began with an initial Wagner tree followed by TBR (tree bisection and reconnection) branch swapping, keeping up to ten trees per replication. All saved trees were then submitted to a final round of TBR branch swapping. Numerous subsequent analyses using a total of 5000 rounds of ratcheting (Nixon, 1999) did not find shorter or alternative equally parsimonious trees. To assess nodal confidence, a nonparametric parsimony bootstrap analysis was conducted for 500 random

pseudoreplicates. We recognize two levels of nodal support: ⩾70% bootstrap support represents a moderately supported node or clade; ⩾95% bootstrap support represents a well-supported node or clade.

3. Results

The likelihood analysis resulted in a single optimal tree (Fig. 3). Most of the 67 nodes recovered in the likelihood analysis were moderately to well supported with 47 (70%) nodes being supported by a bootstrap value ⩾70 and 28 (52%) nodes being supported by a bootstrap value ⩾95. The parsimony analysis resulted in ten most parsimonious trees that each had a length of 9259 steps. The strict-consensus-parsimony tree is presented in Fig. 4. Most of the 62 nodes remaining in the strict consensus were moderately to well supported with 36 nodes (58%) being supported by a bootstrap value ⩾70 and 25 nodes (40%) being supported by a bootstrap value ⩾95. In total, 36 of the 62 (58%) recovered parsimony nodes received unambiguous morphological support. Many taxa lacked all (three species) or most (nine additional species) of the morphological data (Table 2), so this limited the number of nodes that could have been supported by unambiguous morphological transformations. The results of the likelihood and parsimony analyses were largely congruent, and the likelihood analysis generally had higher levels of support. In total, 49 of 62 nodes (79%) from the less-resolved parsimony hypothesis were also recovered in the likelihood hypothesis. All moderately and well supported nodes in the parsimony analysis were recovered in the likelihood analysis. In contrast, two nodes with moderate support and two nodes with high support in the likelihood analysis were not recovered in the parsimony analysis. In the likelihood hypothesis, Trichodontidae was resolved as the sister group of Cyclopteroidea + Cottoidea instead of being in a polytomy with these two suborders as is seen in the parsimony hypothesis. In the likelihood hypothesis, *Cottus gobio* was recovered as the sister group of the Lake Baikal cottids, whereas the parsimony analysis recovered *Cottus* sister to *Uranidea* + Lake Baikal cottids. Finally, the likelihood hypothesis recovered *Gymnocanthus* sister to *Porocottus* and *Rastrinus* sister to *Icelus,* whereas the parsimony hypothesis recovered *Icelus*, *Gymnocanthus*, and *Porocottus* as

subsequent sister groups to *Chitonotus + Rastrinus*. Most of the remaining differences between the topologies were minor rearrangements among poorly supported clades. However, there was one major topological change regarding the placement of *Jordania*

as sister to all other cottoids in the likelihood analysis or sister to *Scorpaenichthys* in the parsimony analysis.

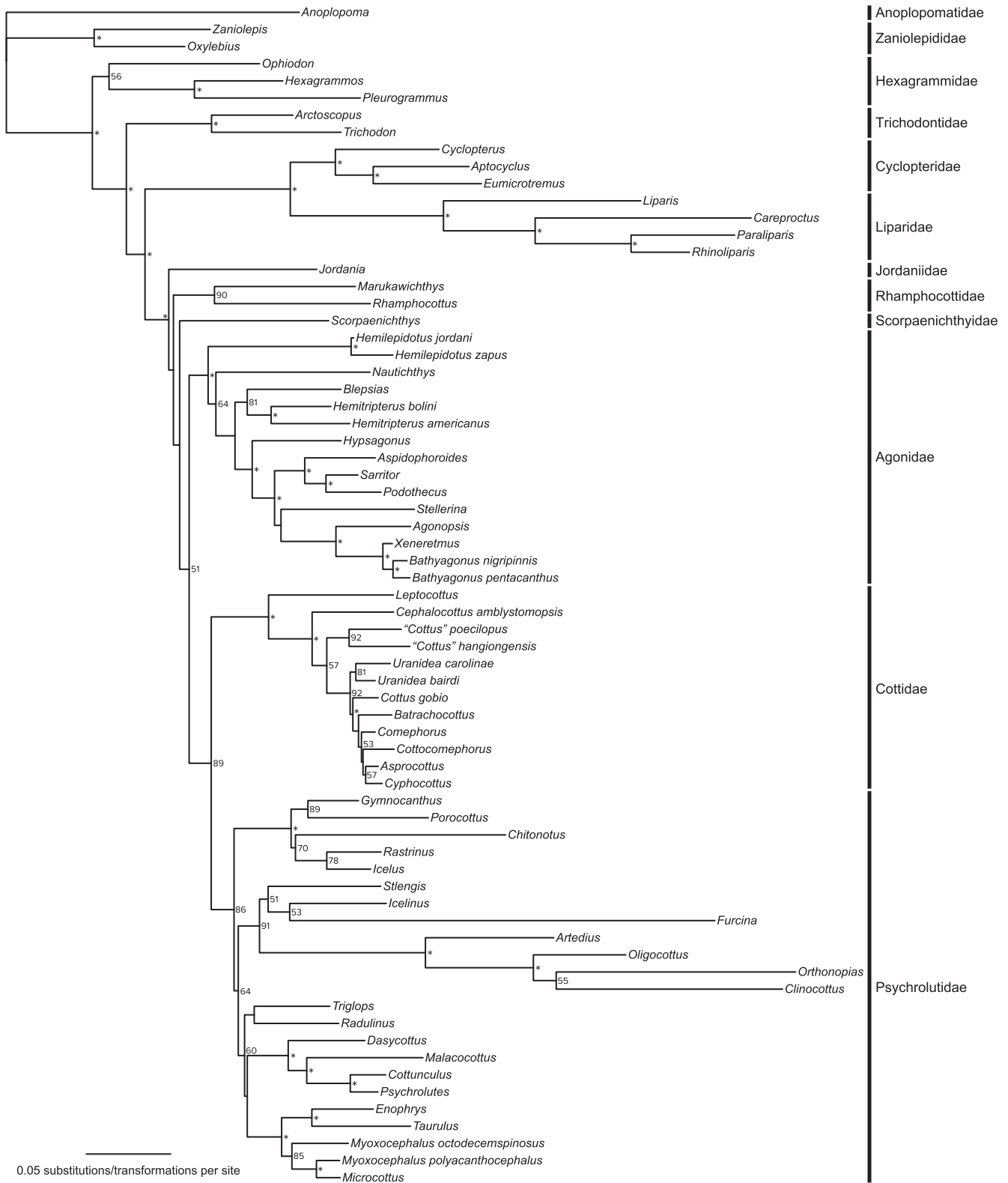In both analyses, the suborders Zaniolepidoidei, Hexagrammoidei, and Cottoidei and superfamilies Cottoidea, Cyclopteroidea, and

Fig. 3. Optimal cladogram resulting from the partitioned likelihood analysis of the data set composed of 72 phenotypic and 4518 nucleotide characters. Clades with ⩾50% clade-confidence intervals are retained and identified with their support. Nodes with clade-confidence intervals ⩾95% were marked with an "*". Family-level classification is designated on the right.
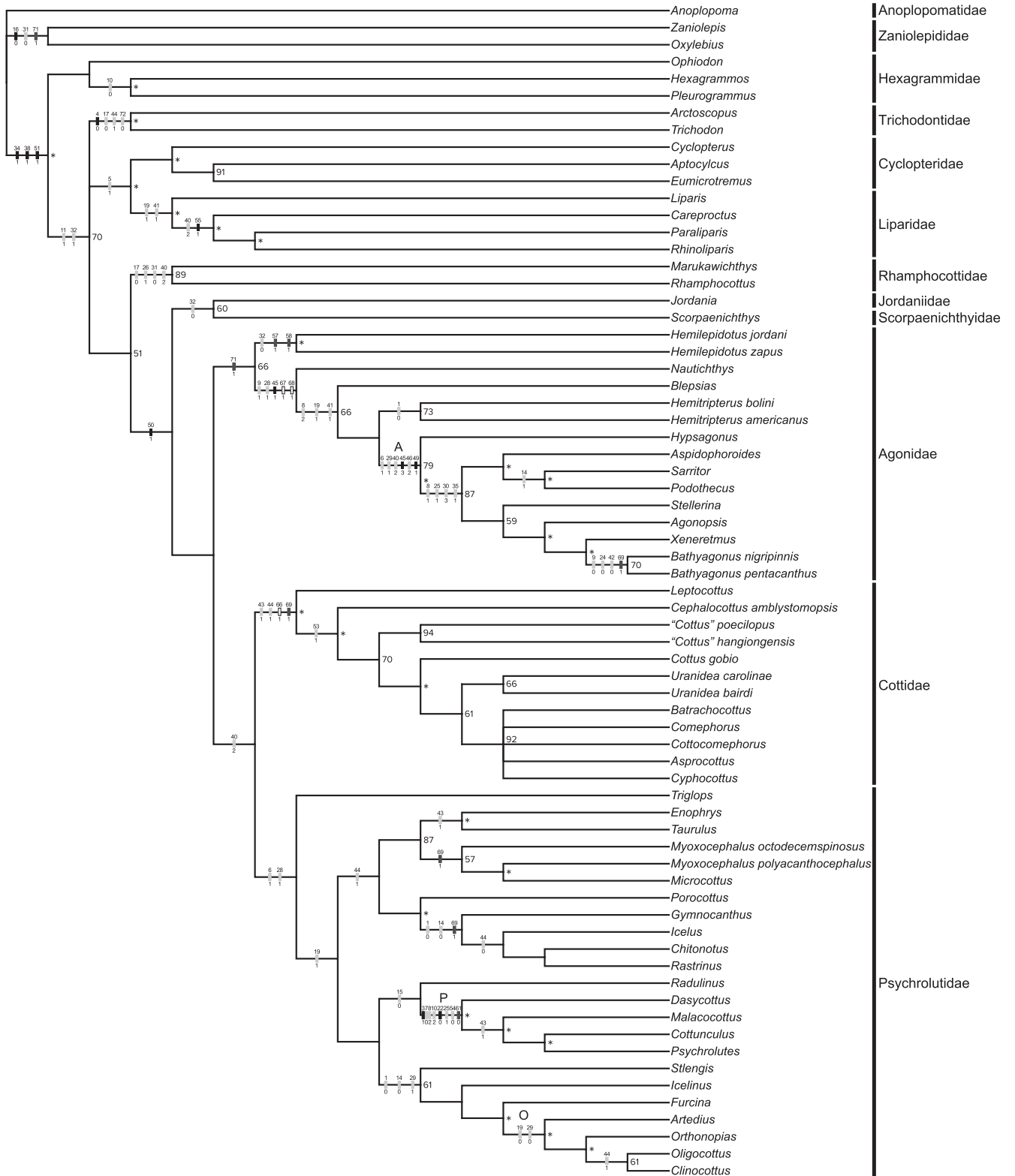
Fig. 4. Strict consensus of ten equally most parsimonious trees recovered (tree length = 9259 steps, CI 0.30, RI 0.46 when uninformative characters were retained) from the analysis of the data set composed of 72 phenotypic and 4518 nucleotide characters. Numbers above branches represent bootstrap resampling percentages (>50%). Nodes with resampling percentages ⩾95% were marked with an "*". Unambiguous morphological features that diagnose each clade are listed above hashmarks on branches with the diagnostic state listed below the hashmark. Dark-gray hashmarks represent early-life-history characters that are homoplastic synapomorphies, light-gray hashmarks represent adult characters that are homoplastic synapomorphies, white hashmarks represent early-life-history characters that are unique and unreversed synapomorphies, black hashmarks represent adult characters that are unique and unreversed synapomorphies. Family-level classification is designated on the right. Subfamilial designations are listed on the nodes: A–Agoninae; O–Oligocottinae; P–Psychrolutinae.

Trichodontoidea were recovered as monophyletic. Many of the traditionally recognized family-level clades were recovered as monophyletic: Agonidae, Cyclopteridae, Hexagrammidae, Liparidae, Psychrolutidae, Trichodontidae, and Zaniolepididae. As has been seen in previous analyses, the results of both analyses refute the monophyly of the Cottidae, Cottocomephoridae (*sensu* Eschmeyer, 2014), and Hemitripteridae. As has been previously suggested (Washington et al., 1984; Yabe, 1985) and amended by Smith and Wheeler (2004), the Agonidae + Hemitripteridae + *Hemilepidotus* was recovered as a clade (less *Nautichthys* in Smith and Wheeler (2004)). However, our results recover Hemitripteridae as a paraphyletic grade leading toward a monophyletic Agonidae. As previously argued (Smith and Wheeler, 2004; Kinziger et al., 2005), the Lake Baikal cottoids (Abyssocottidae, Comephoridae, and Cottocomephoridae *sensu* Eschmeyer, 2014) were nested within the genus *Cottus*. Representatives of the traditional Cottidae were recovered in six major clades in both the parsimony and the likelihood analyses, which necessitated the familial taxonomic revisions presented herein (Figs. 3 and 4, Appendix B). All of the remaining results and discussion below will utilize the revised classification unless noted otherwise. All of the proposed taxonomic changes and the revised classification are consistent with both the parsimony and likelihood analyses.

4. Discussion

This study was designed specifically to look at the limits and relationships of the family-level clades within the sculpins and allies (Cottoidea). In particular, we focused on genera that had been previously hypothesized to be sister groups of all other members of their respective family-level clades or stem-branching lineages (*Cottus, Dasycottus, Hemilepidotus, Hypsagonus, Jordania, Leptocottus, Nautichthys, Rhamphocottus,* and *Scorpaenichthys*) because their placement varied most dramatically across previous studies. Beyond Cottoidea, our results provided an opportunity to re-examine the higher-level relationships within Cottoidei. Our subordinal intrarelationships within Cottoidei corroborate (likelihood) or do not refute (parsimony) the hypothesis of Imamura et al. (2005), which largely followed Shinohara (1994). The Cottoidei herein was diagnosed by the loss of the basisphenoid and the reduction to a single fin ray associated with the last proximal dorsal pterygiophore (Fig. 4).

4.1. Trichodontioidea and Cyclopteroidea

Our analyses corroborate the findings of Smith and Wheeler (2004), Smith (2005) and Imamura et al. (2005), which classified Trichodontidae as a member of the Cottoidei. This relationship was first hinted at by Starks (1930) who suggested a possible link between these groups based on similarities in the pectoral skeleton. Later, Mooi and Johnson (1997) suggested a possible relationship between the Trichodontidae and the mail-cheeked fish (their scorpaenoid) radiation based on several features, most notably the presence of a parietal with an enclosed sensory canal. The distribution of this character state was corroborated by Smith (2005) who noted that this character state is better treated as a syndesmosis between the medial extrascapular and the parietal. The monophyly of Trichodontidae in this study was supported by the loss of the suborbital stay, lachyrymal-palatine articulation, and scales as well as the presence of a pharyngobranchial one (Fig. 4). Our likelihood results corroborate the phylogenetic hypothesis and classification of Imamura et al. (2005) who placed Trichodontidae sister to Cyclopteroidea + Cottoidea. The parsimony analysis recovered these three cottoid clades as a polytomy. Our results do not

support the hypotheses of Jackson (2003) or Smith and Wheeler (2004) whose results placed Cyclopteroidea within Cottoidea.

The monophyly of the Cyclopteroidea is supported by the loss of vomerine teeth (Fig. 4). Within Cyclopteroidea, our results corroborate the traditional separation of and sister-group relationship between Cyclopteridae and Liparidae. Despite limited sampling, our placement of *Aptocyclus* within Cyclopterinae suggests that the lumpsucker subfamilies Aptocyclinae and Cyclopterinae may need further evaluation. Within Liparidae, our limited results corroborate the findings of Kido (1988) and Knudsen et al. (2007) who, among examined taxa, recovered *Liparis* sister to the remainder of the liparids and a close relationship between *Rhinoliparis* and *Paraliparis* (Kido, 1988 even treating the genera as synonyms). The monophyly of the Liparidae was supported by the loss of pharyngobranchial two and the lack of branching in the caudal-fin rays (Fig. 4). In general, relationships within Cyclopteroidea require additional work. This work is particularly needed within the species-rich Liparidae.

4.2. Rhamphocottidae, Jordaniidae, and Scorpaenichthyidae

Our results agree with previous studies that recovered species classified in the Rhamphocottidae, Jordaniidae, and Scorpaenichthyidae among the earliest splitting branches within the Cottoidea (Fig. 1). Corroborating recent studies (Jackson, 2003; Smith and Wheeler, 2004; Smith, 2005), our results include the traditional Agonidae, Hemitripteridae, and Psychrolutidae within the crown "cottid" radiation.

Our analyses support previous hypotheses that placed the traditional Rhamphocottidae near the base of Cottoidea (Washington et al., 1984; Yabe, 1985; Smith and Wheeler, 2004). Given our results that place *Rhamphocottus* sister to the Ereuniidae (also recovered in Smith and Wheeler, 2004; Smith, 2005), we recommend placing the ereuniids in the synonymy of Rhamphocottidae. This expanded Rhamphocottidae is diagnosed by the presence of pharyngobranchial one, the presence of several lower pectoral-fin rays separated from the upper pectoral-fin lobe and free of the pectoral-fin membrane, the presence of dorsal- and anal-fin stays, and a complex caudal fin where all remaining hypural and parahypural elements are fused into a single complex element (Fig. 4; Appendix B).

Our two analyses (Figs. 3 and 4) differ most dramatically in the placement of Jordaniidae. Our parsimony analysis grouped *Jordania* with *Scorpaenichthys* as the sister group to the Agonidae, Cottidae, and Psychrolutidae, whereas our likelihood analysis resulted in a sister-group relationship between Jordaniidae and all other members of the Cottoidea. Despite some support, it is clear that neither relationship for Jordaniidae is demonstrably preferable. These alternative placements are consistent with prior morphological and molecular hypotheses. For example, Bolin (1947) placed jordaniids at the base of his sculpin radiation, whereas Yabe (1985) and Knope (2013) recovered *Jordania* in a modestly more apical position as the sister group to the core cottoid radiation (Agonidae, Cottidae, and Psychrolutidae; Figs. 1 and 2). In light of previous work and our hypotheses, we recommend resurrecting the family Jordaniidae because this classification is consistent with previous and current phylogenetic hypotheses and the family's aberrant anatomy; further, it ensures taxonomic stability. We are hopeful that the eventual addition of the rare and enigmatic jordaniid, *Paricelinus,* will help resolve this phylogenetic conflict. Our hypothesis and previous morphological and molecular hypotheses have consistently resulted in a placement of *Scorpaenichthys* as the earliest or one of the earliest diverging Cottoid Lineages; thus, we also recommend its classification as a family to recognize its distinctiveness and to be consistent with the current and most recent phylogenetic analyses. The familial recognition and wide

separation of *Scorpaenichthys* from other cottoids on morphological grounds has been shown herein and elsewhere (Fig. 1) with Taranetz (1941) even moving this enigmatic taxon from the Cottoidei to the Notothenioidei because of its aberrant pectoral-skeleton morphology.

4.3. Agonidae

Our analyses agree with the majority of previous explicit morphological studies in placing the species traditionally classified in the Hemitripteridae and Agonidae together. Further, our results support the findings of Smith and Wheeler (2004) and Smith (2005), which suggested that *Hemilepidotus* represents a member of this clade. In contrast, Knope (2013) recovered a widely polyphyletic *Nautichthys* and did not recover the traditional Agonidae, Hemitripteridae, and *Hemilepidotus* as a clade. Given the atypical placement (near *Icelus*) of *Nautichthys pribilovius* by Knope (2013) and the difference in placement relative to the more traditional placement in this study, it is worth further investigation to ensure that the sequences used in Knope (2013) represent *N. pribilovius*. The identification of the voucher used by Knope was corroborated by Katherine Pearson (pers. com.) and via digital image by both the authors of the current study.

The expanded Agonidae presented herein is diagnosed by the presence of scales that are modified into dermal spines in larvae or pelagic juveniles (Figs. 4 and 5; Appendix B). This familial expansion is further supported by the recognition that the traditional hypsagonine (often treated as percidines) agonids show intermediate morphology between the revised Agonidae and the traditional agonids or Agoninae. Finally, it is interesting to note that *Hemilepidotus, Hemitripterus*, and *Blepsias,* presumably not uniquely among cottoids, share a reproductive behavior where semen is released at a short distance during internal insemination rather than through copulation or close contact (Muñoz, 2010).

The placement of *Hemilepidotus* has traditionally been a major stumbling block for cottoid systematists. Despite variation in its placement, *Hemilepidotus* has been uniformly placed near the stem of the cottoid radiation. Taranetz (1941) treated *Hemilepidotus* as one of 13 cottid subfamilies. Bolin (1947) treated *Hemilepidotus* as the first split off his core sculpin assemblage (Fig. 1B). Washington et al. (1984) placed *Hemilepidotus* in a clade with *Scorpaenichthys* as the base of the non-rhamphocottid cottoids (Fig. 1C). Washington et al. (1984) described three characters to unite this large clade, although their cladogram indicated five features. These characters included the presence of heavy, pitted, dermal bones in the cranium of larvae (our character 57); broad supraocular bony shelf, which projects laterally over the orbit in larvae (our character 58); and the reduction of the dorsalmost pectoral radial (not subsequently found in *Hemilepidotus* (Yabe, 1985; Smith, 2005)). Yabe (1985; Fig. 1D) recovered *Hemilepidotus* at the base of his Cottidae (less *Jordania* and *Scorpaenichthys;* his clade C), but this relationship was not recovered in explicit analyses of his data matrix (Jackson, 2003; Smith and Wheeler, 2004). Similar to Yabe's (1985) presented topology, Jackson (2003) recovered a cottoid placement for *Hemilepidotus* with just *Jordania* and *Scorpaenichthys* more distantly related to the core cottid radiation than *Hemilepidotus.* However, Jackson's (2003) study differed from Yabe (1985) by placing the Agoninae, Cyclopteridae, traditional Hemitripteridae, Liparidae, Psychrolutinae, and Rhamphocottidae in a more derived position than *Hemilepidotus* within the "cottid" clade. Previous studies including molecular data (Smith and Wheeler, 2004; Smith, 2005) were similar to the current study in placing *Hemilepidotus* within or sister to an expanded Agonidae. Despite variation, all of these studies consistently recover the separation of *Hemilepidotus* as one of the earliest splitting events in the cottoid radiation. This genus simply lacks the derived adult
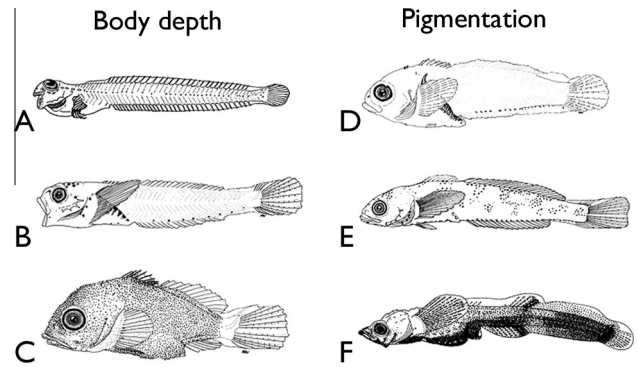

Fig. 5. Representatives of each of the various early-life-history specializations described as new in this study (characters 69–71). The larvae in the left column represent relative body depth from shallowest (A) to deepest (C) and the larvae in the right column represent pigmentation from least pigmented to (D) to heavily pigmented (F). Figures are taken from the following sources: (A) *Comephorus baicalensis* (Taliev, 1955); (B) *Leptocottus armatus* (Richardson and Washington, 1980); (C) *Rhamphocottus richardsoni* (Richardson and Washington, 1980); (D) *Artedius fenestralis* (Richardson and Washington, 1980); (E) "*Cottus*" *hangionensis* (Kojima, 1986); and (F) *Bathyagonus nigripinnis* (Busby, 1998).

features that characterize the other cottoid clades. However, as first noted by Washington et al. (1984), there are clues in larval squamation that indicate an "agonid-like" ancestry for *Hemilepidotus*. The one morphological synapomorphy supporting *Hemilepidotus* as a member of the expanded Agonidae is the presence of robust or plate-like scalation in the form of dermal spines in the larval or juvenile stages of these groups (character 71). Bolin (1947: 159) described the scales of *Hemilepidotus* as, "flat, deeply embedded plate[s] whose external surface bears a posteriorly inclined, strongly ctenoid, plate-like ridge." Similarly derived scalation, particularly in larvae, is found among the traditional hemitripterids and agonids.

A close relationship between the traditional agonids and hemitripterids has been one of the most consistently recovered results among all explicit cottoid studies, beginning with Washington et al. (1984). Yabe (1985), Jackson (2003), Smith (2005), and the current study all continued to recover this group as a clade. Washington et al. (1984) united this clade by the presence of prickled scales in larvae, a knobby fronto-parietal ridge in larvae, and broad, plate-like epurals. Yabe (1985) united this clade by the insertion of Baudelot's ligament on the first vertebra (shared with *Pseudoblennius* and *Vellitor*). The current study diagnosed this non-*Hemilepidotus* agonid clade, not surprisingly, by several of the same characters, including features from the axial skeleton, pelvic fins, scalation, the caudal skeleton, and the cranial lateral-line system (Fig. 4). Unlike the exclusively morphological studies (Bolin, 1947; Washington et al., 1984; Yabe, 1985), this study recovered *Blepsias, Hemitripterus*, and *Nautichthys* as a grade rather than an assemblage evolving from a single common ancestor. Washington et al. (1984) noted five features to unite their Hemitripteridae, but they did not enumerate them. Yabe (1985) diagnosed his hemitripterids solely by their modified scalation, which was accounted for in our analysis. Our results provide limited support for a sister-group relationship between *Blepsias* and *Hemitripterus*, which is different from Yabe's (1985) topology. However, his character optimization clearly indicated substantial homoplasy among his hemitripterids with only a reduction in the number of infraorbitals uniting *Blepsias* and *Nautichthys*. The reanalysis of Yabe's (1985) data matrix supported a sister-group relationship between *Blepsias* and *Hemitripterus* and did not support for the monophyly of his Hemitripteridae (Smith and Wheeler, 2004, but see Jackson, 2003). Additional work may clarify the inter- and intrarelationships of *Blepsias, Hemitripterus,* and

Nautichthys, but all evidence points to these genera being in the expanded Agonidae.

The monophyly of the Agoninae has been supported in all previous studies except Smith and Wheeler (2004) who recovered a paraphyletic Agoninae with the Cyclopteroidea nested within it. The placement of Cyclopteroidea within Agoninae was not supported herein or in subsequent molecular or morphological studies. Further, the results of the current study corroborate Kanayama's (1991) sister-group pairing of his Percidinae relative to all other agonines. The current study did not test the monophyly of Kanayama's (1991) Percidinae or Brachyopsinae, but the remainder of his subfamilial designations (his Agoninae and Anoplagoninae) were not recovered as monophyletic. As the current study was focused on the Cottoidea as a whole, it is premature to refute Kanayama's (1991) subfamilial designations given their strong morphological support. However, these agonid clades should now be treated at the tribal level within Agoninae. Subsequent work on the relationships of this expanded Agonidae should combine Kanayama's morphological data with explicitly coded phenotypic data from *Blepsias, Hemilepidotus, Hemitripterus,* and *Nautichthys*.

4.4. Cottidae

The large-scale restriction of the Cottidae to *Cottus, Leptocottus, Mesocottus, Trachidermus,* and Lake Baikal cottoids is well supported on evidential grounds and highlights the clade's predominantly freshwater nature when contrasted with essentially all other cottoid families (see Goto et al., 2014 for additional discussion). Richardson (1981) recognized this clade as one of her larval groupings, her clade six. Sideleva (1994), Kontula et al. (2003), Smith and Wheeler (2004), Kinziger et al. (2005) and Goto et al. (2014) all recovered these Lake Baikal cottoid families nested within *Cottus,* and most studies that have analyzed freshwater and marine cottoids have found this clade as distinct and separate from the majority of the traditional marine cottids (e.g., Clade D$_1$ of Yabe, 1985). Unfortunately, one troubling aspect of fully embracing this result, while retaining all current species of *Cottus* in *Cottus,* is that it would require the inclusion of all Lake Baikal species in this large and already widespread genus. This change has understandably concerned some ichthyologists. For example, Nelson (2006) stated, that there is "strong support for the monophyly of the whole endemic Baikalian cottoid diversity, and [it has been] found... to be nested within the Holarctic freshwater genus *Cottus*. The implications of this for cladistic classification are straightforward. Yet, I am reluctant to make the obvious step to show relationships." We are sympathetic to this position, even though it is clear that some taxonomic changes are necessary. We believe that Nelson's taxonomic reluctance stems from the undesirability of reclassifying the 90 + species in 13 currently recognized genera and multiple families to just members of the genus *Cottus.* We feel that the best solution to this real problem is to recognize all of the Lake Baikalian genera and several subdivisions within *Cottus* (originally described as cottid genera). This was recently proposed by Kinziger (2003). Kinziger et al. (2005) subsequently treated these genera as clades or subgenera within *Cottus* (Kinziger et al., 2005) and Goto et al. (2014) treated these clades as a mix of named and unnamed assemblages. As noted by Kinziger (2003), this scenario would recognize *Cephalocottus, Cottopsis, Cottus,* and *Uranidea.* Further, it would require the treatment of *"Cottus" beldingi, "C." confusus, "C." czerskii, "C." greenei, "C." hangiongensis, "C." leiopomus, "C." poecilopus, "C." pollux,* and *"C." reinii* as *Cottus* (*incertae sedis*) until their placement is further resolved. Despite these few *incertae sedis* species that require additional data before being reclassified, the recognition of four genera for the primarily riverine *Cottus* (*sensu lato*) provides the most conservative, most

information rich, and most stable classification across the 18 estuarine, riverine, and Lake Baikal cottid genera. This classification also better reflects our current understanding of the phylogeny, taxonomy, evolution, and diversity within this intriguing radiation of cottoids. Interestingly, Kinziger (2003) noted that these four core freshwater cottid genera traditionally classified in *Cottus* largely follow geographic patterns with *Cephalocottus* being restricted to eastern Eurasia and some surrounding Pacific islands, *Cottopsis* being restricted to the western United States and Canada, with *Uranidea* being restricted to North America (with one species, *U. cognata,* found in both North America and eastern Eurasia), and *Cottus* (*sensu stricto*) being restricted to western Eurasia (except *C. ricei,* which is found in northern North America). For these reasons, we believe that the taxonomic proposal put forward by Kinziger (2003) should be followed.

Importantly, this change to the Cottidae represents a substantive break from its relative stability over the last 25 + years. Historically, the traditional Cottidae was spread among as many as 17 different families. Our results, with just a handful of exceptions, illustrate just how much support there is for Yabe's (1985) phylogeny. However, our altered classification of the former Cottidae and Psychrolutidae, combined with revisions to a few enigmatic genera, are the only major changes to Yabe's (1985) hypothesis. In that study, Yabe's (1985) single unique and unreversed diagnostic feature of his Cottidae was the presence of a lateral process of the hyomandibular. Subsequent examination of several cottoids (e.g., Smith, 2005; Smith, pers. obs.) indicate that this feature was not as uniformly seen across cottids (*sensu* Yabe, 1985). For example, the process was not found in several genera (e.g., *Hemilepidotus, Radulinus* (Smith, 2005)). This suggests that there is more intrageneric or intraspecific variation in this feature then Yabe (1985) witnessed. Because of the variation seen in the lateral process of the hyomandibular and the lack of additional support for the previously recognized Cottidae, this revised phylogeny and taxonomy proposed herein should be followed. This revised Cottidae is diagnosed by the branchiostegal membranes being fused to the isthmus, the presence of scales, first proximal dorsal pterygiophores not distinctly slender in larvae, and larvae of moderate depth (Fig. 4; Appendix B).

Finally, the relationships that we recovered among the Lake Baikal cottoids differ from Hunt et al. (1997), Kontula et al. (2003) and Kinziger et al. (2005), particularly the placement of *Batrachocottus.* Despite these differences, the remainder of our revised cottid relationships correlates well with those presented in the mitochondrial study of Kinziger et al. (2005). Future work on the Cottidae needs to resolve the classification of the nine species left as *incertae sedis* as well as examine the relationships of *Trachidermus* and the bulk of the cottids using morphological and nuclear DNA sequence data, building upon Kinziger et al. (2005).

4.5. Psychrolutidae

The taxonomic changes to the Psychrolutidae recommended herein treat all of the former marine cottids (except *Hemilepidotus, Jordania, Leptocottus, Melletes, Paricelinus, Scorpaenichthys,* and *Trachidermus*) as psychrolutids. This change represents a substantive expansion of the family, but one necessary for a phylogenetic classification. Like the almost exclusively freshwater Cottidae, this revised, overwhelmingly marine, Psychrolutidae exemplifies the simplification, cohesiveness, and power that our improved phylogenetic hypothesis has on our evolutionary understanding of this clade and the predictive potential (e.g., habitat) of the resulting classification. This almost exclusively marine radiation is also noteworthy for its predominantly Northern Hemisphere distribution. Only 25 of the 214 psychrolutid species are found south of the equator, and all of these species (except those in *Antipidocottus*)

are traditional psychrolutids (species in *Ambophthalmos, Cottunculus, Ebinania, Neophrynichthys,* and *Psychrolutes*).

The recognition of this clade is also surprisingly consistent with Clade E in Yabe (1985; Fig. 1D) except that it includes the former psychrolutids, herein treated as the Psychrolutinae. This expansion of the Psychrolutidae also continues the consistent growth of the family since Taranetz (1941) and Nelson (1977) began uniting the former Cottunculidae, Dasycottidae, Gilbertididae, and Neophrynichthyidae into the ever-expanding marine Psychrolutidae recognized herein. More recent studies of cottoids (Jackson, 2003; Smith and Wheeler, 2004; Smith, 2005; current study) have furthered this trend of expansion, resulting in a revised Psychrolutidae now comprising some 64 genera and 225 species (Appendix B). If we compare our revised classification to Jordan's (1923) classification, we see that he classified this assemblage across six families. Similarly, Taranetz (1941) and Watanabe (1960) recognized 12 and eight families/subfamilies for this expanded monophyletic Psychrolutidae, respectively. In short, a series of incremental refinements over the last 50 years have resulted in a clearer understanding of this assemblage as the dominant clade of marine sculpins. Psychrolutidae can now be diagnosed by the loss of the palato-cranial articulation and the reduction of pelvic-fin rays to three or fewer (Fig. 4; Appendix B).

With this expansion of the Psychrolutidae, a natural question is: where did previous studies place the psychrolutines? Early studies (e.g., Jordan, 1923; Berg, 1940; Taranetz, 1941) had the various psychrolutine genera distributed across many families and subfamilies with most of the taxa falling out across six to 13 cottid subfamilies/families. Bolin (1947) did not examine psychrolutines. Richardson (1981) used larval features to break up the cottoids, and she recovered a clade composed of the Psychrolutinae but did not hypothesize its interrelationships. Three years later, Richardson and colleagues (Washington et al., 1984) neither recovered nor rejected a monophyletic traditional Psychrolutidae and placed their *Psychrolutes* group and *Malacocottus* group in a polytomy with their Agonidae and *Myoxocephalus, Artedius* Part A, *Cottus,* and *Hemitripterus* groups (Fig. 1C). Yabe (1985) recognized the Psychrolutinae (his Psychrolutidae), but felt that the family was more distantly related to his cottids than his agonids or hemitripterids (Fig. 1D). However, this separation was not supported in computer-aided analyses of Yabe's (1985) matrix (Jackson, 2003; Smith and Wheeler, 2004). In a molecular study, Smith and Wheeler (2004) recovered the Psychrolutinae within a paraphyletic cottid radiation in, more or less, the general placement recovered in this study. Jackson's (2003) cottoid study, while much broader than the Psychrolutinae, appears to have focused, in part, on the origin and placement of this clade. Through detailed morphological study, Jackson interestingly recovered psychrolutines sister to *Artediellus* based on primarily on fin-ray branching; this result was supported in Knope's (2013) molecular study. Jackson's (2003) traditional Psychrolutidae + *Artediellus* was recovered in a large polytomy made up of the Agonidae, Cottidae, Cyclopteridae, Liparidae, Psychrolutidae, and Rhamphocottidae (as classified in the current study). Smith (2005) recovered the historic Bathylutichthyidae deeply nested within the Psychrolutinae (as previously suggested by Nelson (1994)) based on a number of features ranging from the anteriormost dorsal pterygiophores lacking dorsal spines (i.e., not supraneurals) to the loss of the vomerine teeth, branched caudal-fin rays, and scales. In short, we have come a long way toward resolving the limits and relationships of the psychrolutines and how they fit into this marine cottoid radiation.

Six clades were consistently recovered among the revised psychrolutids between the likelihood and parsimony analyses: (1) *Dasycottus, Malacocottus, Cottunculus,* and *Psychrolutes*; (2) *Enophrys, Microcottus, Myoxocephalus,* and *Taurulus*; (3) *Radulinus*; (4) *Triglops*; (5) *Artedius, Clinocottus, Furcina, Icelinus, Oligocottus,*

Orthonopias, and *Stlengis;* (6) *Chitonotus, Gymnocanthus, Icelus, Porocottus,* and *Rastrinus*. We will briefly discuss the support for these groupings from previous analyses and their interrelationships.

The first group, *Dasycottus, Malacocottus, Cottunculus,* and *Psychrolutes,* represents the traditional Psychrolutidae or Psychrolutinae as classified in this study. The relationships among these taxa were similar to previous morphological studies except that *Psychrolutes* was resolved as the sister group of *Cottunculus* in contrast to *Malacocottus* (Yabe, 1985; Jackson and Nelson, 1998; Jackson, 2003). Presumably, the remainder of the traditional psychrolutids (*sensu* Jackson and Nelson, 1998) and *Bathylutichthys* (Smith, 2005) would group with these taxa. Lautredou et al. (2013), however, recovered the psychrolutine *Ebinania* among the scorpaenoids in a molecular study. While the current study did not include *Ebinania,* morphological data (Jackson and Nelson, 1998; Jackson, 2003) and unpublished mitochondrial sequence data from *Ebinania costaecanariae* (FMNH 118064) places *Ebinania* in its traditional placement among the psychrolutines and not among the scorpaenoids.

Group two (*Enophrys, Microcottus, Myoxocephalus,* and *Taurulus*) was largely recovered in Yabe (1985) and Knope (2013), except that among examined taxa, they recovered *Porocottus* and some species of *Triglops* within this assemblage (Figs. 1 and 2). Neither this clade, nor this assemblage with *Porocottus* was recovered in the computer-aided analyses of Yabe's (1985) data (Jackson, 2003; Smith and Wheeler, 2004) unless Yabe's characters were treated with irreversible character evolution (Jackson, 2003: his Figs. 2 and 3). Similarly, Taranetz (1941) recognized this assemblage as his Myoxocephalini and Enophryini with the same inclusion of *Porocottus*. In the likelihood analysis (Fig. 3), this clade was recovered sister to Psychrolutinae. These results provide some corroborative evidence of Jackson's (2003) hypothesis linking *Artediellus* and the Psychrolutinae given the relatively close relationship between *Artediellus* (and six additional genera) and *Enophrys, Taurulus, Microcottus,* and *Myoxocephalus* in Yabe's (1985) study. Our results suggest that *Myoxocephalus* is paraphyletic relative to *Microcottus.* A previous study of *Myoxocephalus* (Kontula and Väinölä, 2003) recovered one deep branch within the genus that would separate *M. octodecemspinosus* and *M. polyacanthocephalus,* suggesting that *Myoxocephalus* may need minor revision. In general, the relationships within this group are similar to those proposed explicitly by Yabe (1985) or Knope (2013). In addition to this second group, our third and fourth groups (*Radulinus* and *Triglops,* respectively) were recovered as close allies to the above clades in the likelihood analysis (Fig. 3). In contrast, the parsimony analysis (Fig. 4) recovered *Radulinus* as the sister group of the Psychrolutinae, and *Triglops* at the stem of the Psychrolutidae. These two placements are somewhat consistent with previous studies where *Radulinus* and *Triglops* were recovered as stem members of the revised Psychrolutidae by Knope (2013; Fig. 2) or near *Artediellus* in the reanalysis of Yabe's (1985) morphological matrix (Smith and Wheeler, 2004).

The fifth clade, *Artedius, Clinocottus, Furcina, Icelinus, Oligocottus, Orthonopias,* and *Stlengis,* includes one of the most consistently recovered groups, the Eastern Pacific endemic Oligocottinae (represented by *Artedius, Clinocottus, Oligocottus,* and *Orthonopias;* Bolin, 1947; Richardson, 1981; Washington et al., 1984; Ramon and Knope, 2008; Knope, 2013). The relationships among these taxa are identical to those recovered by Knope (2013) and similar to those hypothesized by Bolin (1947) and Ramon and Knope (2008). This Oligocottinae was recovered as the sister group to the Eastern and Western Pacific grouping of *Furcina, Icelinus,* and *Stlengis* in the likelihood analysis (Fig. 3). *Stlengis* and *Icelinus* have often been allied together (Bolin, 1936; Yabe, 1985), but a close relationship among these genera and *Furcina* was first suggested in a reanalysis of Yabe's (1985) morphological matrix (Smith and

Wheeler, 2004) and then corroborated in the molecular study of Knope (2013). This clade of seven genera was diagnosed in this study by the presence of six circumorbitals, palatine teeth, and the loss of one pelvic-fin ray to a total of two pelvic-fin rays (Fig. 4; Appendix B).

The final clade, *Chitonotus, Gymnocanthus, Icelus, Porocottus,* and *Rastrinus,* represents a geographically widespread and enigmatic clade of sculpins. Many of these genera have been recovered in varied places across previous molecular and morphological studies (Figs. 1 and 2). Several of these genera (e.g., *Chitonotus, Gymnocanthus*) have been recovered in more stem positions in previous explicit analyses, and the relationships of these groups to the diversity of psychrolutids remain problematic.

4.6. Evolution of Cottoidei

As noted above, cottoids are perhaps best known for their diverse reproductive modes, and tracing the evolution of these features on an ever more refined phylogeny could help us understand the macroevolution of this diverse clade. Unfortunately, most of this reproductive mode diversity needs additional observational data to be thoroughly explored at the higher levels emphasized in the current study because the presence or absence of particular modes are not broadly known. For example, copulation and egg depositing into invertebrates (egg hiding) is known from six species in the revised Psychrolutidae and Agonidae (Abe and Munehara, 2009), but its confirmed presence or absence is largely unknown in marine forms outside of these six species. As such, egg hiding cannot be used to diagnose any particular clades of cottoids. In contrast, the one feature that is somewhat broadly characterized across cottoids is copulatory vs. non-copulatory mating. This reproductive mode was previously explored using Yabe's (1985) phylogenetic hypothesis by Abe and Munehara (2009) with limited explanatory success. An ancestral states reconstruction on the revised phylogenies indicates minor improvements in the understanding of copulatory behavior across Cottoidei compared to Abe and Munehara (2009). The reconstruction of this feature set (Supplemental Fig. 1) on the revised phylogeny does suggest that copulating optimizes on the node representing the most recent common ancestor of the traditional Hemitripteridae + Agoninae. The revised placement of *Hemilepidotus* as the sister group to this copulating clade supports this view, but the optimization at the base of the Agonidae is equivocal because the missing data renders optimizations at broad levels unknown. Similarly, the revised, principally freshwater, Cottidae appears to be primitively and overwhelmingly non-copulatory. In contrast, the distribution and optimization within and across the marine psychrolutids is not clarified in the current study. The reproductive mode with psychrolutids is overwhelmingly equivocal or unknown because of the substantive missing reproductive data and comparatively higher species richness (>55% of species in Cottoidea are psychrolutids). As researchers continue to revise the limits and relationships of cottoids and collect additional observational reproductive data, we will be able to explore and predict these features with improved accuracy and possibly explore their role in cottoid diversification as has been done in other groups (e.g., McMahan et al., 2013; Davis et al., 2014).

The revised phylogeny and classification (Appendix B) highlights the importance of early-life-history specializations in diagnosing the major groups of cottoid fishes. In the analysis, just 13 of the 72 morphological features were larval features, but half of the examined families (Agonidae, Cottidae, Trichodontidae, and Zaniolepididae) that had their monophyly tested and supported with morphological data were diagnosed by these early-life-history characters. In particular, the only diagnostic feature for the revised Agonidae was a larval scalation feature. This study

corroborates earlier work (e.g., Washington et al., 1984; Tyler et al., 1989) that has demonstrated that larval features can provide critical features for resolving higher-level relationships, particularly for stem taxa in groups with tremendous morphological and phylogenetic diversity like the cottoids. These diagnostic morphological features for major groups are critical for placing taxa, particularly when DNA sequence data are not available. Further, the simultaneous analysis of morphological and molecular data results in cladograms that take all available phylogenetic data into account and reduce the conflicting signal common to independent analyses. This allows researchers to optimize any of the included features or other behavioral, functional, or anatomical characters onto a phylogeny using morphological and/or molecular data or place fossil terminals into phylogenetic analyses for an explicit temporal context. These studies are critical for future macroevolutionary studies of this interesting and diverse group of fishes.

Acknowledgments

Appendix A

Characters examined in the phylogenetic analyses. Data for characters 1-54 and 72 taken from Yabe (1985) and augmented with data from the following sources (when available) for the following genera: *Anoplopoma, Arctoscopus, Bathyagonus, Batrachocottus, Careproctus, Cyclopterus, Eumicrotremus, Hexagrammos, Liparis, Ophiodon, Oxylebius, Pleurogrammus, Trichodon, Xeneretmus,* and *Zaniolepis* (Smith, 2005); *Agonopsis, Aspidophrooides, Leptagonus,* and *Stellerina* (Kanayama, 1991); *Paraliparis* and *Rhinoliparis* (Kido, 1988). Data (when available) for characters 55–56 was taken from Kido (1988), Smith (2005) and Yabe (1985). Data for characters 57–68 were taken from Washington et al. (1984). Data for characters 69–71 were taken from Dunbar (1947), Taliev (1955), Gorbunova (1962), Kobayashi (1962), Khan (1972), Ahlstrom and Stevens (1976), Russell (1976), Chernyaev (1979, 1985), Richardson and Washington (1980), Marliave (1981), Richardson (1981), Kendall and Vinter (1984), Matarese and Vinter (1985), Kido and Kitagawa (1986), Washington (1986), Feeney (1987), Kojima (1988), Okiyama (1988), Shiogaki (1988), Matarese et al. (1989), Goto (1990), Ambrose (1996), Busby (1998), Pinder (2001), Busby and Cartwright (2006), Fahay (2007), Cartwright (2009) and Blood and Matarese (2010). In a few cases, the sources used for character coding deviate from the general description above; these are noted below. All characters are preferentially coded at the species level, but, when necessary, they were coded in congeners. Characters associated with early life history are marked with an[*].

1. *Loss of circumorbital six* (based in part on Yabe (1985) character 1); data for *Hemilepidotus* from Smith (2005):
   (1_0) = six circumorbitals
   (1_1) = five or fewer circumorbitals
2. *Loss of circumorbital five* (based in part on Yabe (1985) character 1):
   (2_0) = five or more circumorbitals
   (2_1) = four circumorbitals
3. *Infraorbital sensory canal connection to operculomandibular canal* (Yabe (1985) character 2):
   (3_0) = infraorbital sensory canal not connected with the operculomandibular canal
   (3_1) = infraorbital sensory canal connected with the operculomandibular canal
4. *Suborbital stay* (Yabe (1985) character 3; data for *Aptocylus* from Kido (1988):
   (4_0) = suborbital stay absent
   (4_1) = suborbital stay present
5. *Vomerine teeth* (Yabe (1985) character 5):
   (5_0) = vomerine teeth present
   (5_1) = vomerine teeth absent
6. *Palatocranial articulation* (Yabe (1985) character 6; data for *Hemitripterus* and *Psychrolutes* from Smith (2005):
   (6_0) = articular head of prefrontal joined to small face on palatine
   (6_1) = palatocranial articulation absent
7. *Pterosphenoid-parasphenoid junction* (Yabe (1985) character 7):
   (7_0) = pterosphenoid separated from parasphenoid by prootic
   (7_1) = pterosphenoid joined to parasphenoid due to posterior displacement of prootic
8. *Trigeminofascialis chamber* (Yabe (1985) character 8, unordered); data for *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
   (8_0) = broad vertical bridge crosses over the trigeminofascialis chamber
   (8_1) = narrow vertical bridge crosses over the trigeminofascialis chamber
   (8_2) = vertical bridge absent
9. *Baudelot's ligament* (Yabe (1985) character 10):
   (9_0) = ligament inserts on basioccipital
   (9_1) = ligament inserts on first vertebra
10. *Lateral extrascapular morphology* (Yabe (1985) character 11, unordered):
    (10_0) = lateral extrascapular forming a single t-shaped bone with two horizontal and one vertical opening
    (10_1) = lateral extrascapular is composed of two tubular bones (one vertical and one horizontal)
    (10_2) = lateral extrascapular composed of just the horizontal element
11. *Basisphenoid* (Yabe (1985) character 12):
    (11_0) = basisphenoid present
    (11_1) = basisphenoid absent
12. *Intercalar* (Yabe (1985) character 13):
    (12_0) = large intercalar, extends to prootic
    (12_1) = small intercalar, does not extend to prootic
13. *Posttemporal fossa* (Yabe (1985) character 14):
    (13_0) = posttemporal fossa deep and inserted by the lateral head of the epaxialis musculature
    (13_1) = posttemporal fossa shallow and not inserted by numerous muscular fibers
14. *Palatine teeth* (Yabe (1985) character 16); data for *Hypsagonus* from Kanayama (1991) and Smith (2005):

(14_0) = palatine teeth present
(14_1) = palatine teeth absent
15. *Lateral process of hyomandibular* (Yabe (1985) character 17); data for *Hemilepidotus* and *Radulinus* from Smith (2005):
    (15_0) = lateral process absent
    (15_1) = lateral process present
16. *Metapterygoid lamina* (Yabe (1985) character 18):
    (16_0) = metapterygoid lamina present
    (16_1) = metapterygoid lamina absent
17. *Pharyngobranchial one* (Yabe (1985) character 19):
    (17_0) = pharyngobranchial one present
    (17_1) = pharyngobranchial one absent
18. P*haryngobranchial four* (based in part on Yabe (1985) character 20); data for *Careproctus* and *Liparis* from Kido (1988), *Bathyagonus* and *Xeneretmus* from Kanayama (1991), and *Ophiodon* from Shinohara (1994):
    (18_0) = pharyngobranchial four present
    (18_1) = pharyngobranchial four absent
19. *Pharyngobranchial two* (based in part on Yabe (1985) character 20); data for *Careproctus* and *Liparis* from Kido (1988) and *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
    (19_0) = pharyngobranchial two present
    (19_1) = pharyngobranchial two absent
20. *Basihyal* (based in part on Yabe (1985) character 22); data for *Careproctus* and *Liparis* from Kido (1988) and *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
    (20_0) = basihyal present
    (20_1) = basihyal absent
21. *Basihyal size* (based in part on Yabe (1985) character 22); data for *Careproctus* and *Liparis* from Kido (1988) and *Ophiodon* from Shinohara (1994):
    (21_0) = basihyal large
    (21_1) = basihyal small
22. *Branchiostegal rays* (Yabe (1985) character 23); data for *Careproctus* and *Liparis* from Kido (1988) and *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
    (22_0) = seven branchiostegal rays
    (22_1) = six branchiostegal ray, loss of anteriormost ray
23. *Scapula foramen* (Yabe (1985) character 24, unordered); data for *Bathyagonus* and *Xeneretmus* from Kanayama (1991), *Ophiodon* from Shinohara (1994), and *Careproctus, Cyclopterus,* and *Eumicrotremus* from Smith (2005):
    (23_0) = scapula foramen closed
    (23_1) = scapula foramen closed, but crack present
    (23_2) = scapula foramen present
24. *Scapula-coracoid connection* (Yabe (1985) character 25); data for *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
    (24_0) = scapula attached to the coracoid
    (24_1) = scapula separated from the coracoid
25. *"Pores" between each pectoral actinost* (Yabe (1985) character 27); data for *Bathyagonus, Hyspagonus, Podothecus,* and *Xeneretmus* from Kanayama (1991):
    (25_0) = one to four small "pores" present between pectoral actinosts
    (25_1) = "pores" between pectoral actinosts absent
26. *Free pectoral-fin rays* (Yabe (1985) character 28); data for *Bathyagonus* and *Xeneretmus* from Kanayama (1991) and *Rhamphocottus* from Mecklenberg (2003) and Smith (2005):
    (26_0) = all pectoral-fin rays are interconnected by pectoral-fin membranes

(26_1) = several lower pectoral-fin rays are separated from the upper lobe and free of pectoral-fin membrane

27. *Loss of one pelvic-fin ray* (based in part on Yabe (1985) character 31); data for *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
(27_0) = five pelvic-fin rays
(27_1) = four or fewer pelvic-fin rays

28. *Loss of second pelvic-fin ray* (based in part on Yabe (1985) character 31); data for *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
(28_0) = four or five pelvic-fin rays
(28_1) = three or fewer pelvic-fin rays

29. *Loss of third pelvic-fin ray* (based in part on Yabe (1985) character 31); data for *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
(29_0) = three or more pelvic-fin rays
(29_1) = two pelvic-fin rays

30. *Vertebral insertion of the anterior proximal pterygiophore(s) of the dorsal series* (Yabe (1985) character 32, unordered); data for *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
(30_0) = anteriormost proximal pterygiophore inserts into second interspace
(30_1) = anteriormost proximal pterygiophore inserts into first interspace
(30_2) = anteriormost two pterygiophores insert into first interspace
(30_3) = anteriormost proximal pterygiophore posteriorly displaced beyond third interspace

31. *Loss of dorsal- and anal-fin stays* (Yabe (1985) character 33):
(31_0) = dorsal- and anal-fin stays present
(31_1) = dorsal- and anal-fin stays absent

32. *Number of fin rays on last proximal pterygiophore of dorsal fin* (based in part on Yabe (1985) character 34); data for *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
(32_0) = two fin rays on last proximal pterygiophore of dorsal fin
(32_1) = one fin ray on last proximal pterygiophore of dorsal fin

33. *Number of fin rays on last proximal pterygiophore of anal fin* (based in part on Yabe (1985) character 34); data for *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
(33_0) = two fin rays on last proximal pterygiophore of anal fin
(33_1) = one fin ray on last proximal pterygiophore of anal fin

34. *Loss of anal-fin spines* (Yabe (1985) character 35); data for *Careproctus* and *Liparis* from Kido (1988) and *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
(34_0) = anal-fin spine present
(34_1) = anal-fin spine absent

35. *Loss of pleural ribs* (based in part on Yabe (1985) character 36); data for *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
(35_0) = pleural ribs present
(35_1) = pleural ribs absent

36. *Posterior displacement of pleural ribs* (based in part on Yabe (1985) character 37); data for *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
(36_0) = pleural ribs begin on third vertebra
(36_1) = pleural ribs displaced posteriorly such that they begin on or after the sixth vertebra

37. *Condition of neural spine on preural centrum II* (Yabe (1985) character 38); data for *Careproctus* and *Liparis* from Kido (1988):
(37_0) = neural spine of preural centrum II is suturally attached to centrum
(37_1) = neural spine of preural centrum II is fused with centrum

38. *Loss of hypurapophysis* (Yabe (1985) character 39):
(38_0) = hypurapophysis developed
(38_1) = hypurapophysis absent

39. *Condition of preural centrum I* (Yabe (1985) character 40); data for *Careproctus* and *Liparis* from Kido (1988) and *Ophiodon* from Shinohara (1994):
(39_0) = preural centrum I not fused with hypurals
(39_1) = preural centrum I fused with hypural-parhypural complex

40. *Condition of hypural-parhypural complex* (Yabe (1985) character 41, unordered); data for *Careproctus* and *Liparis* from Kido (1988) and *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
(40_0) = complex composed of three or more elements
(40_1) = complex composed of upper and lower elements
(40_2) = complex composed of a single element

41. *Loss of branched caudal-fin rays* (Yabe (1985) character 42); data for *Careproctus* and *Liparis* from Kido (1988) and *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
(41_0) = branched caudal-fin rays present
(41_1) = branched caudal-fin rays absent

42. *Neural spine of preural centrum II* (Yabe (1985) character 43):
(42_0) = neural spine of preural centrum II not elongate, so it does not support procurrent rays
(42_1) = neural spine of preural centrum II elongate, so it does support procurrent rays

43. *Condition of isthmus* (Yabe (1985) character 44):
(43_0) = branchiostegal membranes joined and free from isthmus
(43_1) = branchiostegal membranes fused to isthmus

44. *Presence of body scales or plates* (based in part on Yabe (1985) character 45); data for *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
(44_0) = body scales or plates present
(44_1) = body scales or plates absent

45. *Type of body scales* (based in part on Yabe (1985) character 45, unordered); data for *Bathyagonus* and *Xeneretmus* from Kanayama (1991):
(45_0) = ctenoid scales
(45_1) = spiny scales
(45_2) = multifid scales
(45_3) = bony plates

46. *Position of anus* (Yabe (1985) character 46, unordered):
(46_0) = anus immediately anterior to anal fin
(46_1) = anus situated midway between pelvic- and anal-fin origins
(46_2) = anus situated immediately posterior to pelvic fins

47. *Loss of hyohyoides inferioris* (Yabe (1985) character 49):
(47_0) = hyohyoides inferioris present
(47_1) = hyohyoides inferioris absent

48. *Loss of levator externus III* (Yabe (1985) character 51):
(48_0) = levator externus III present
(48_1) = levator externus III absent

49. *Loss of rectus ventralis II* (Yabe (1985) character 52):
(49_0) = rectus ventralis II present
(49_1) = rectus ventralis II absent

50. *Rectus ventralis interconnecting urohyal and third hypobranchial* (Yabe (1985) character 53):

(50_0) = connection absent
(50_1) = connection present

51. *Loss of extensor proprius* (Yabe (1985) character 54):
(51_0) = extensor proprius present
(51_1) = extensor proprius absent

52. *Extrinsic "gas bladder" muscle* (Yabe (1985) character 57):
(52_0) = muscle inserts on vertebrae
(52_1) = muscle inserts on cleithrum

53. *Connection between the adductor mandibulae section A2 and ligamentum primordium* (Yabe (1985) character 59):
(53_0) = connection absent
(53_1) = connection present

54. *Loss of coracoradialis* (Yabe (1985) character 60):
(54_0) = coracoradialis present
(54_1) = coracoradialis absent

55. *Loss of pterosphenoid* (Kido, 1988 character 5):
(55_0) = pterosphenoid present
(55_1) = pterosphenoid absent

56. *Loss of parietal* (Kido, 1988 character 4):
(56_0) = parietal present
(56_1) = parietal absent

57[*]. *Presence of heavy, pitted, dermal bones in the cranium of larvae* (Washington et al., 1984 character 13):
(57_0) = in larvae, pitted dermal bones absent
(57_1) = in larvae, pitted dermal bones present

58[*]. *In larvae, development of a broad supraocular bony shelf, which projects laterally over the orbit* (Washington et al., 1984 character 14):
(58_0) = in larvae, bony shelves absent
(58_1) = in larvae, bony shelves present

59. *Condition of neural spine of first vertebra* (Washington et al., 1984 character 19):
(59_0) = neural spine of first vertebra present
(59_1) = neural spine of first vertebra absent

60[*]. *In larvae, fusion of the first neural spine* (Washington et al., 1984 character 21):
(60_0) = in larvae, first neural spine fused
(60_1) = in larvae, first neural spine unfused, forming a broad U-shape

61[*]. *In larvae, development of a bony shelf on the anterior portion of preopercle* (Washington et al., 1984 character 14):
(61_0) = in larvae, bony shelves absent
(61_1) = in larvae, bony shelves present

62[*]. *In larvae, multiple preopercular spines* (Washington et al., 1984 character 23):
(62_0) = in larvae, preopercular spines not multifid
(62_1) = in larvae, multifid preopercular spines

63[*]. *In larvae, enlargement and expansion of the anterior neural arches* (Washington et al., 1984 character 24):
(63_0) = in larvae, anterior neural arches not expanded
(63_1) = in larvae, anterior neural arches expanded

64[*]. *In larvae, condition of first three neural arches* (Washington et al., 1984 character 25):
(64_0) = in larvae, first three neural arches fused
(64_1) = in larvae, first three neural arches unfused

65. *Development of heavy bony arches on cranium, which form late in larval development* (Washington et al., 1984 character 35):
(65_0) = absence of heavy bony arches on cranium
(65_1) = heavy bony arches on cranium

66[*]. *In larvae, first proximal pterygiophores simple and slender* (Washington et al., 1984 character 36):
(66_0) = in larvae, first proximal dorsal pterygiophores not distinctly slender

(66_1) = *in larvae, first proximal dorsal pterygiophores simple and slender*

67[*]. *Development of separate parhypural* (Washington et al., 1984 character 37):
(67_0) = parhypural forms
(67_1) = even in larvae, a distinct parhypural never forms

68[*]. *In larvae, fronto-parietal ridge forms knobby projections* (Washington et al., 1984 character 44):
(68_0) = in larvae, fronto-parietal ridge lacks knobby
(68_1) = in larvae, fronto-parietal ridge knobby

69[*]. *Post-flexion larval body depth at pectoral-fin insertion* (Fig. 5 unordered; *Nautichthys* and *Hemitripterus bolini* from Busby et al. (2012); M. Busby, pers. obs.):
(69_0) = slender larva (body depth <20% of standard length)
(69_1) = moderate larva (body depth between 20% and 25% of standard length)
(69_2) = deep larva (body depth >25% of standard length)

70[*]. *Larval pigmentation* (Fig. 4, unordered; *Nautichthys* and *Hemitripterus bolini* from Busby et al. (2012); M. Busby, pers. obs.):
(70_0) = light larval pigmentation (no or little pigmentation, restricted to a few melanophores on the head, dorsal surfaces of the gut, and a row of post-anal ventral (pvms); the lateral body is generally unpigmented)
(70_1) = moderate larval pigmentation (body with scattered melanophores or patches of pigmentation on head and lateral body, but never completely covered)
(70_2) = heavy larval pigmentation (body completely or nearly completely covered with melanophores)

71[*]. In *larvae or pelagic juveniles, scales modified as dermal spines* (based, in part, on Washington et al. (1984) character 43, *Nautichthys* and *Hemitripterus bolini* from Busby et al. (2012); M. Busby, pers. obs.):
(71_0) = no dermal spines in larvae or pelagic juvenile
(71_1) = scales modified into dermal spines in larvae or pelagic juveniles (marked with a + in Fig. 4)

72. *Lachrymal-palatine articulation* (Yabe (1985) character 4):
(72_0) = lachrymal-palatine articulation absent
(72_1) = lachrymal-palatine articulation present

Appendix B

Proposed familial classification, morphological diagnoses, support, and composition of the Cottoidea based principally on the resulting parsimony phylogeny. The proposed taxonomy is consistent with both the parsimony and likelihood analyses. There is occasional variation in the sister group between the parsimony and likelihood analyses; this is noted parenthetically and discussed in the text.

B.1. Rhamphocottidae Gill 1888

Type genus: *Rhamphocottus* Günther 1874.
Sister taxon: Agonidae + Cottidae + Jordaniidae + Psychrolutidae + Scorpaenichthyidae (Agonidae + Cottidae + Psychrolutidae + Scorpaenichthyidae in likelihood analysis)
Concept and content: Four species classified in three genera: *Ereunias*, *Marukawichthys*, and *Rhamphocottus*.
Phenotypic diagnosis: Pharyngobranchial one (suspensorial pharyngobranchial) present (Ch: 17, 1 → 0); several lower pectoral-fin rays separated from the upper lobe and free of intra-ray membrane (Ch: 26, 0 → 1); dorsal- and anal-fin stays present

(Ch: 31, 1 → 0); hypural-parhypural complex bone is composed of a single element (Ch: 40, 1 → 2).

Support statistics: The bootstrap support for this clade was 0.89 for parsimony and 0.90 for likelihood.

Systematic comment: This revised Rhamphocottidae includes the former Ereuniidae because our phylogenies and previous work (Smith and Wheeler, 2004; Smith, 2005) recovered these two small groups as sister.

B.2. Agonidae Swainson 1839

Type genus: *Agonus* Bloch and Schneider 1801.

Sister taxon: Cottidae + Psychrolutidae.

Concept and content: Approximately 60 species classified in 26 genera: Agonomalus, Agonopsis, Agonus, Anoplagonus, Aspidophoroides, Bathyagonus, Blepsias, Bothragonus, Brachyopsis, Chesnonia, Freemanichthys, Hemilepidotus, Hemitripterus, Hypsagonus, Leptagonus, Nautichthys, Occella, Odontopyxis, Pallasina, Percis, Podothecus, Sarritor, Stellerina, Tilesina, Ulcina, and Xeneretmus.

Phenotypic diagnosis: Scales modified into dermal spines in larvae or pelagic juveniles (Ch: 71, 0 → 1).

Support statistics: The bootstrap support for this clade was 0.66 for parsimony and 0.98 for likelihood.

Systematic comment: This revised Agonidae includes the former Hemitripteridae (as was done by Jackson (2003) and Smith (2005)) and *Hemilepidotus*. Taranetz (1941), Bolin (1947), Yabe (1985), Kanayama (1991), Jackson (2003) and Smith (2005) have all discussed relationships among many of these taxa. Frequently, these discussions only included either the traditional "agonids" or "hemitripterids," so comprehensive comparisons are minimal.

B.3. Scorpaenichthyidae Jordan, Evermann, and Clark 1930

Type genus: *Scorpaenichthys* Girard 1854.

Sister taxon: Jordaniidae (Agonidae + Cottidae + Psychrolutidae in likelihood analysis).

Concept and content: A single species classified in the monotypic genus *Scorpaenichthys*.

Phenotypic diagnosis: First (anteriorly) proximal pterygiophore of the dorsal fin inserts into first interneural space (Ch: 30, 0 → 1); body scales absent (Ch: 44, 0 → 1).

Support statistics: Because this entity is monotypic, the statistics are not comparable or are not calculable.

Systematic comment: The placement of *Scorpaenichthys* has been a source of much confusion for researchers studying cottoids, ranging from a fairly frequent placement of this taxon near the root of the Cottidae (Bolin, 1947; Yabe, 1985; Jackson, 2003) to a grouping with the hexagrammid *Ophiodon* (Crow et al., 2004) to the placement of the genus within the Notothenioidei (Taranetz, 1941).

B.4. Jordaniidae Jordan, Evermann, and Clark 1930

Type genus: *Jordania* Starks 1895.

Sister taxon: Scorpaenichthyidae (Agonidae + Cottidae + Psychrolutidae + Rhamphocottidae + Scorpaenichthyidae in likelihood analysis).

Concept and content: Two species classified in two monotypic genera: *Jordania* and *Paricelinus*.

Phenotypic diagnosis: Pharyngobranchial one (suspensorial pharyngobranchial) present (Ch: 17, 1 → 0).

Support statistics: Because a single terminal represented this entity, the statistics are not comparable or are not calculable.

Systematic comment: This family includes *Jordania* as well as *Paricelinus*. *Paricelinus* was included in the Jordaniidae following

the work of Taranetz (1941) and Bolin (1947). Specific characters that place it here include five pelvic rays (included in the analysis) and a diversity of similarities that had not broadly been examined across groups including a lengthening of the body and anal fin, separation of the two dorsal fins, and gill reductions (Bolin, 1947). Taranetz (1941), Bolin (1947), Yabe (1985), Jackson and Nelson (1999) and Jackson (2003) have all discussed relationships among many of these taxa.

B.5. Cottidae Bonaparte 1832

Type genus: *Cottus* Linnaeus 1758.

Sister taxon: Psychrolutidae.

Concept and content: Approximately 107 species classified in 18 genera: Abyssocottus, Asprocottus, Batrachocottus, Cephalocottus, Comephorus, Cottinella, Cottocomephorus, Cottopsis, Cottus, Cyphocottus, *Leptocottus*, Limnocottus, Mesocottus, Neocottus, Paracottus, Procottus, *Trachidermus*, and Uranidea.

Phenotypic diagnosis: Branchiostegal membranes fused to isthmus (Ch: 43, 0 → 1); body scales absent (Ch: 44, 0 → 1); first proximal dorsal pterygiophores simple and slender in larvae (Ch: 66, 0 → 1); post-flexion larval body depth at pectoral-fin insertion moderate (Ch: 69, 0 → 1).

Support statistics: The bootstrap support for this clade was 1.00 for parsimony and 1.00 for likelihood.

Systematic comment: This revised Cottidae includes the former Abyssocottidae, Comephoridae, Cottocomephoridae, *Cottus*, *Leptocottus*, and *Mesocottus*. It also excludes the overwhelming majority of former marine cottids. Taranetz (1941), Bolin (1947), Yabe (1985), Jackson (2003), Smith and Wheeler (2004), Kinziger et al. (2005), Smith (2005), and Goto et al. (2014) have all discussed relationships among many of these taxa.

B.6. Psychrolutidae Günther 1861

Type genus: *Psychrolutes* Günther 1861.

Sister taxon: Cottidae.

Concept and content: Approximately 214 species in 64 genera: Alcichthys, Ambophthalmos, Andriashevicottus, Antipodocottus, Archistes, Argyrocottus, Artediellichthys, Artediellina, Artedielloides, Artediellus, Artedius, Ascelichthys, Astrocottus, Atopocottus, Bathylutichthys, Bero, Bolinia, Chitonotus, Clinocottus, Cottiusculus, Cottunculus, Dasycottus, Daruma, Ebinania, Enophrys, Eurymen, Furcina, Gilbertidia, Gymnocanthus, Icelinus, Icelus, Leiocottus, Lepidobero, Malacocottus, Megalocottus, Micrenophrys, Microcottus, Myoxocephalus, Neophrynichthys, Ocynectes, Oligocottus, Orthonopias, Phallocottus, Phasmatocottus, Porocottus, Pseudoblennius, Psychrolutes, Radulinopsis, Radulinus, Rastrinus, Ricuzenius, Ruscarius, Sigmistes, Stelgistrum, Stlengis, Synchirus, Taurocottus, Taurulus, Thyriscus, Trichocottus, Triglops, Triglopsis, Vellitor, and Zesticelus.

Phenotypic diagnosis: Palatocranial articulation obscure (Ch: 6, 0 → 1); three (or fewer) pelvic fin rays (Ch: 28, 0 → 1).

Support statistics: The bootstrap support for this clade was <0.50 for parsimony and 0.86 for likelihood.

Systematic comment: Following Smith (2005), this revised Psychrolutidae includes the former Bathylutichthyidae. It its revised form, this family includes the Psychrolutidae and Bathylutichthyidae of Nelson (2006) and nearly all former marine cottid genera (except *Hemilepidotus, Jordania, Leptocottus, Paricelinus, Scorpaenichthys,* and *Trachidermus*). Taranetz (1941), Bolin (1947), Yabe (1985), Jackson and Nelson (1998), Jackson (2003), Smith and Wheeler (2004), Smith (2005) and Knope (2013) have all discussed relationships among these taxa.

Appendix C. Supplementary materials

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014.06.028.

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