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Polyphyly of the mail-cheeked fishes (Teleostei: Scorpaeniformes): evidence from mitochondrial and nuclear sequence data

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Abstract

Mitochondrial and nuclear DNA sequence data for 105 acanthomorph taxa are analyzed to address questions of scorpaeniform monophyly and relationships. The combination of 3425 aligned base pairs from the mitochondrial small subunit rDNA (12S), large subunit rDNA (16S), and tRNA-Val and the nuclear large subunit rDNA (28S), histone H3, and TMO-4c4 loci are analyzed. Representatives of all scorpaeniform suborders and 32 of 36 scorpaeniform families are included with most suborders represented by multiple species. In addition to 69 scorpaeniform taxa, 36 outgroup taxa, including representatives of most families previously conjectured to be related to the Scorpaeniformes, are analyzed due to serious concerns of scorpaeniform monophyly. The traditionally recognized scorpaeniform fishes are recovered as polyphyletic. The 13 representatives of the Atheriniformes, Blennioidei, Gasterosteoidei, Grammatidae, Notothenioidei, Percidae, Trichodontidae, and Zoarcoidei included in the analysis are all nested within the least inclusive clade that includes all traditionally recognized scorpaeniforms. The scorpaenoid lineage is widely polyphyletic, and its intrarelationships differed significantly from previous hypotheses. The cottoid lineage is paraphyletic with only the presence of the Trichodontidae, as the sister-taxon of the Cottoidei, disrupting the traditional subordinal hypothesis of relationships.
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1. Introduction

1.1. Background

The scorpaeniform, or mail-cheeked, fishes are one of the largest and most morphologically diverse teleostean orders with more than 1400 species classified in 24–36 families, depending on the taxonomy (e.g., Eschmeyer, 1998; Nelson, 1994; Washington et al., 1984). Representatives are found circumglobally in marine environments at depths ranging from surface water to oceanic trenches >7000 m (Andriashev, 1955). Although most species inhabit marine waters, many sculpins (e.g., *Cottus* and Lake Baikal cottoids) in the northern hemisphere and the bullrout (*Notesthes robusta*) in Australia have colonized freshwater environments (Allen et al., 2002; Berg, 1948).

Scorpaeniform fishes are best known for their venomous spines (e.g., *Pterois*, *Scorpaena*) and commercial importance (e.g., *Sebastes*, *Ophiodon*). However, most scorpaeniforms are not venomous with the presence of venom being restricted to the majority of the species in the suborder Scorpaenoidei (Halstead, 1988). This group does, however, include the world's most venomous fishes, synanceiid stonefishes, whose stings have caused countless human injuries and numerous fatalities (Burnett, 1998; Halstead, 1988; Kizer et al., 1985). Despite the danger that venomous scorpaenoids pose to divers and aquarists, humans present a much more serious threat to their survival. Scorpaeniforms are among

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the most heavily fished groups, representing 8% of the annual catch in the United States (O'Bannon, 2001). This commercial harvesting has led to drastic overfishing (Love et al., 2001), resulting in four scorpionfishes being placed on the IUCN red list and 14 additional North American species being designated as vulnerable by Musick et al. (2000). In addition to their medical and commercial importance, many researchers have studied other aspects of scorpaeniform ecology and behavior. These studies range from the examination of their antifreeze proteins (e.g., Davies et al., 1993; Slaughter et al., 1981) to studies of their bewildering array of reproductive modes (e.g., Breder and Rosen, 1966; Moser, 1967; Moyer and Zaiser, 1981; Munehara et al., 1997). Unfortunately, studies looking broadly at the evolution of these and other ecological or behavioral characteristics cannot be put into a proper phylogenetic context because the intrarelationships of scorpaeniform fishes remain problematic. Imamura and Shinohara (1998) reviewed our incomplete, and often contradictory, understanding of scorpaeniform relationships, highlighting areas that need additional study. Because a comprehensive morphological analysis examining the limits and relationships of the Scorpaeniformes is lacking, this study was undertaken to look at the higher-level relationships of the Scorpaeniformes, on a worldwide basis, using mitochondrial and nuclear DNA sequence data.

1.2. Scorpaeniform monophyly

Cuvier (1829) first united the Scorpaeniformes, his Acauthoptérigiens celle des joues cuirassées, because these taxa share a posterior extension of the third circumorbital that reaches back across the preopercle. This character, the suborbital stay, has single-handedly held this large group together for nearly 200 years. Recently, two additional scorpaeniform synapomorphies have been proposed. First, Johnson (1993) noted the presence of a lateral-line canal in the base of the parietal spine in most larval scorpaeniforms. Imamura and Yabe (2002) briefly discussed the development of this parietal lateral-line canal in a few scorpaeniform taxa and suggested that the conditions in the cottoid and scorpaenoid lineages are not homologous. Despite their criticisms, further work is still needed on the development and homology of this larval specialization. The second hypothesized synapomorphy, the presence of a swimbladder muscle (Shinohara, 1994), was refuted by Imamura (1996) because the muscles have different origins and orientations in the two main scorpaeniform lineages. Thus, corroborative evidence, beyond the suborbital stay, remains to be convincingly demonstrated and scorpaeniform monophyly continues to be questioned (Imamura and Yabe, 2002).

Despite concerns about the monophyly of the scorpaeniforms (e.g., Freihofer, 1963; Johnson, 1993; Quast, 1965), modern taxonomies continue to treat the bulk of Cuvier's (1829) mail-cheeked assemblage as a monophyletic percomorph order (e.g., Eschmeyer, 1998; Greenwood et al., 1966; Nelson, 1994) or a perciform suborder (Johnson and Patterson, 1993; Mooi and Gill, 1995). As originally conceived, Cuvier (1829) united taxa now recognized in the following families: Agonidae, Apistidae, Bembridae, Congiopodidae, Cottidae, Hemitripteridae, Hoplichthyidae, Peristediidae, Platycephalidae, Scorpaenidae, Sebastidae, Synanceiidae, Tetrarogidae, Triglidae, Dactylopteridae, Gasterosteidae, Monocentridae, and Oreosomatidae [following the taxonomy of Eschmeyer (1998), which will be followed, unless otherwise noted, throughout this paper]. For most of the last 150 years, the last three families have been excluded from the Scorpaeniformes, although dissenting views have been expressed for the exclusion of the Gasterosteidae (e.g., Jungersen, 1910; Miya et al., 2003; Regan, 1913). Finally, the dactylopterids have variously been treated as their own percomorph order, a perciform suborder, or a scorpaeniform family (reviewed in Imamura, 2000; Johnson and Patterson, 1993).

Recently, Imamura and Yabe (2002) provided morphological evidence linking the Serranidae with the scorpaenoid lineage (sensu Imamura and Shinohara, 1998) using the presence of a backwardly directed opercular spine and an *adductor dorsalis*. Other than these few minor changes to Cuvier's (1829) mail-cheeked fishes, the composition of this large assemblage has remained remarkably stable (Imamura and Shinohara, 1997, 1998; Washington et al., 1984), and its composition has only been augmented with new species and the occasional new family (as reviewed in Gill, 1888; Imamura and Shinohara, 1998; Matsubara, 1943; Washington et al., 1984).

1.3. Scorpaeniform interrelationships

Despite stability in the composition of the Scorpaeniformes, its interrelationships remain problematic (Imamura and Shinohara, 1998). Johnson and Patterson (1993) and Mooi and Gill (1995) recently reclassified this assemblage as the perciform suborder Scorpaenoidei because they found no evidence to corroborate its traditional pre-perciform placement, and they believed that this new classification would stimulate additional work on its interrelationships. Recent large-scale molecular analyses (e.g., Chen et al., 2003; Miya et al., 2001, 2003) have suggested that the Scorpaeniformes are not monophyletic, and they have hypothesized a number of novel interrelationships including a close relationship for cottoids, sticklebacks (Gasterosteoidei), and eelpouts (Zoarcoidei). Following the results of these molecular analyses, a morphological analysis (Imamura and Yabe, 2002) provided numerous morphological synapomorphies uniting the cottoid

lineage (sensu Imamura and Shinohara, 1998) and the perciform suborder Zoarcoidei. Most of these synapomorphies were originally identified as cottoid lineage synapomorphies by Quast (1965) and Shinohara (1994). These studies illustrate the utility of molecular analyses to suggest novel percomorph relationships that can be further tested and corroborated with morphological data.

1.4. Scorpaeniform intrarelationships

Given the inability to find corroborative evidence for a monophyletic Scorpaeniformes (Johnson, 1993; Imamura and Yabe, 2002), it is not surprising that its intrarelationships remain contentious. Greenwood et al. (1966) described scorpaeniforms as a "typical example of the work that needs still to be done." Gosline (1971) believed, "the classification of the group appears to be in an advanced state of confusion." Finally, Washington et al. (1984) argued, "the limits of the order, suborders, families, and distribution of families in the suborders are the subject of considerable disagreement... These problems will not be resolved without a worldwide revision of the order." As an example of the severity of the problem, Washington et al. (1984) described how anywhere between one and 17 different families have been recognized by different researchers for Imamura and Shinohara's (1998) cottoid lineage. However, even with these radically different taxonomies, the most recent reviews of the Scorpaeniformes (Imamura and Shinohara, 1997, 1998; Washington et al., 1984) recognized two major scorpaeniform lineages, the cottoid and scorpaenoid lineages of Imamura and Shinohara (1998).

The first of these two scorpaeniform groups, the cottoid lineage, was diagnosed by Shinohara (1994) who proposed seven synapomorphies [many discussed by Quast (1965) (see Fig. 1A)]. These synapomorphies include a parasphenoid–pterosphenoid junction, six branchiostegal rays, absence of a third epibranchial tooth plate, dorsal pterygiophores arranged individually in each interneural space, lack of an accessory spine on the head of the cleithrum, and the absence of anal spines with robust pterygiophores. Yabe and Uyeno (1996) and Imamura and Yabe (2002) showed that many of these characters are also found among the Zoarcoidei. Additionally, Shinohara (1994) and Yabe (1985) (see Fig. 1B) provided evidence for the monophyly of the cottoid lineage's suborders and superfamilies: Anoplopomatoidei, Zaniolepidoidei, Hexagrammoidei, Cottoidei, Cyclopteroidea, and Cottoidea. Shinohara (1994) diagnosed the Anoplopomatoidei (=Anoplopomatidae) by the presence of *ramus lateralis accessorius* nerve pattern seven (Freihofer, 1963) and a robust hypurapophysis. Shinohara (1994) diagnosed the Zaniolepidoidei by the loss of the ascending process of the anguloarticular and the Hexagrammoidei by the presence of primary tubules on the first infraorbital, a reduced supraoccipital crest, and the operculo-mandibular canal region of preopercle being covered by the *adductor mandibulae*. Finally, Yabe (1985) and Shinohara (1994) diagnosed the Cottoidei by a number of features including the loss of both the basihyal and the third *levator externus*. In addition to these higher-level studies

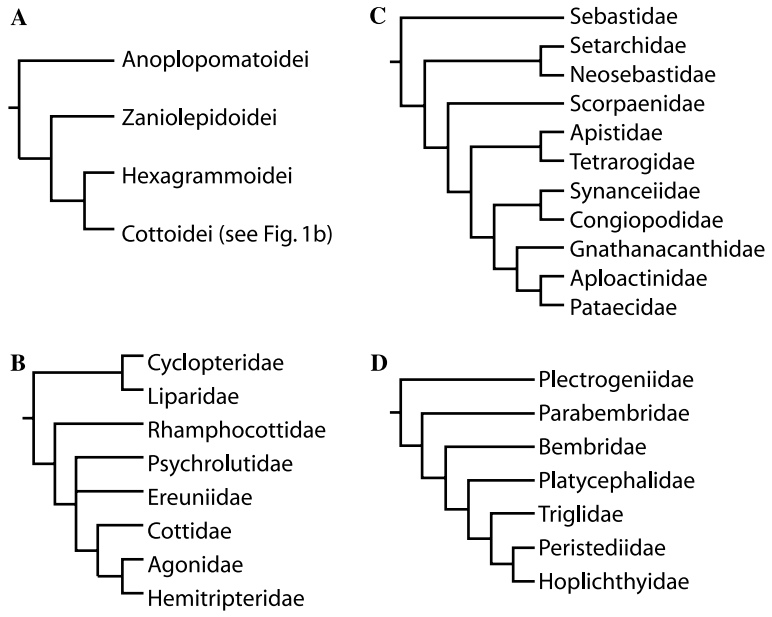

Fig. 1. Hypotheses of subordinal and familial relationships of the Scorpaeniformes (A) cottoid lineage (Shinohara, 1994), (B) Cottoidea (Yabe, 1985), (C) Scorpaenoidei (Ishida, 1994), and (D) Platycephaloidei (Imamura, 1996).

of the intrarelationships of the cottoid lineage, there have been a number of detailed studies looking at the phylogeny and relationships within the Cottoidei (e.g., Bolin, 1947; Busby, 1998; Jackson and Nelson, 1998; Kanayama, 1991; Kendall and Vinter, 1984; Kido, 1988; Leipertz, 1988; Quast, 1965; Taranets, 1941; Yabe, 1981) and additional work on their taxonomy (reviewed in Imamura and Shinohara, 1998).

The second major scorpaeniform group, the scorpaenoid lineage, was diagnosed by the presence of a backwardly directed opercular spine that extends beyond the subopercle and an extrinsic gas bladder muscle derived from the *obliquus superioris* (Imamura, 1996). In addition, Imamura used the presence of a posterior pelvic fossa to diagnose a radically redefined Platycephaloidei, which includes the former scorpaenoid groups: Triglidae, Hoplichthyidae, Peristediidae, and *Plectrogenium.* Either including or excluding the families that Imamura (1996) moved to the Platycephaloidei, the Scorpaenoidei has eluded diagnosis. Ishida (1994) (see Fig. 1C) and Imamura (1996) (see Fig. 1D) were unable to find any synapomorphies to unite this suborder; however, Mandrytza (2001) diagnosed a redefined Scorpaenoidei by the presence of three neuromasts in the lachrymal and a particular configuration of the dorsal pterygiophores and neural spines. Mandrytza's (2001) Scorpaenoidei excluded the Congiopodidae, placing them in the new scorpaeniform suborder Congiopodoidei (also see Greenwood et al., 1966), and removed the Pataecidae from the Scorpaeniformes because they lack a suborbital stay. In addition to these higher-level studies of the scorpaenoid lineage, there have been a number of studies looking at various scorpaenoid groups (e.g., Johns and Avise, 1998; Kai et al., 2003; Keenan, 1991; Kochzius et al., 2003; Matsubara, 1943; Matsubara and Ochiai, 1955; Richards and Jones, 2002; Rocha-Olivares et al., 1999a,b) and their taxonomy (reviewed in Imamura and Shinohara, 1998).

Despite concerns about the limits and the relationships of the Scorpaeniformes and its suborders, no one has undertaken a comprehensive phylogenetic analysis including representatives of all recognized suborders and percomorph groups previously conjectured to be related to the Scorpaeniformes to rigorously test scorpaeniform monophyly and relationships. Here we present the results of a molecular analysis designed specifically to address these questions. Our analysis includes representatives from all recognized scorpaeniform suborders and numerous percomorph families that have been previously allied with the mail-cheeked fishes. The resulting hypothesis of relationships is based upon the simultaneous analysis of nucleotide characters from three mitochondrial loci: the small ribosomal subunit (12S), the complete tRNA-Val, and the large ribosomal subunit (16S) and three nuclear loci: the large ribosomal subunit (28S), histone H3, and TMO-4c4. The objectives of this study are to use these nucleotide characters to: (1) test the monophyly of the Scorpaeniformes; (2) test the monophyly of the cottoid and scorpaenoid lineages; and (3) test the monophyly of the Scorpaenoidei, Platycephaloidei, Hexagrammoidei, and Cottoidei.

2. Materials and methods

2.1. Taxon sampling

The resulting hypotheses were rooted using the basal acanthomorph *Polymixia* following the work of Stiassny (1986) and Johnson and Patterson (1993). The 105 taxa sequenced in this study (Table 1) were purposefully selected to address the questions outlined above and included representatives of all previously hypothesized scorpaeniform suborders, 32 of 36 scorpaeniform families and 69 scorpaeniform species. Only the Apistidae (3 spp.), Bathylutichthyidae (1 sp.), Gnathanacanthidae (1 sp.), and Parabembridae (2 spp.) were unable to be collected for our analysis. We included 36 outgroup taxa [i.e., taxa that are not classified in the Scorpaeniformes in Eschmeyer (1998)], many of which were chosen because they have been previously allied with the Scorpaeniformes (e.g., Serranidae, Cirrhitidae, Centrogeniidae, Dactylopteridae, Champsodontidae, Gasterosteoidei, and Zoarcoidei). Additional outgroup taxa were included because they have a "type 1" epaxial morphology and/or a parietal lateral-line canal, which Johnson (1993), Mooi and Gill (1995), and Mooi and Johnson (1997) suggested might help resolve the limits of the Scorpaeniformes.

2.2. Acquisition of nucleotide sequences

Fish tissues were preserved in 70–95% ethanol or stored frozen at −70 °C prior to extraction of DNA. DNA was extracted from muscle or fin clips using a Qiagen DNeasy Tissue Extraction Kit following the manufacturers protocol. PCR was used to amplify five segments, representing six loci, from the mitochondrial and nuclear genomes. Double-stranded amplifications were performed in a 25 μl volume containing one Ready-To-Go PCR bead (Amersham Biosciences), 1.25 μl of each primer and 2–5 μl of DNA. To amplify and sequence the 12S, tRNA-Val, 16S fragment, the primers 12SL13-L 5′-TTAGAAGAGGCAAGTCGTAACATGGTA-3′ and TitusI-H 5′-GGTGGCTGCTTTTAGGCC-3′ (Feller and Hedges, 1998; Titus, 1992) were used. To amplify and sequence the remaining 16S fragment, the primers 16S ar-L 5′-CGCCTGTTTATCAAAAACAT-3′ and 16S br-H 5′-CCGGTCTGAACTCAGATCACGT-3′ (Kocher et al., 1989; Palumbi, 1996) were used. To amplify and sequence the 28S fragment, the primers 28SV 5′-AAGGTAGCCAAATGCCTCGTCATC-3′

Table 1
Classification of species following Eschmeyer (1998), except as noted in the text, with GenBank accession numbers

Higher classification	Species	12S-tRNA-Val-16S		16S	Histone H3	28S	TMO-4c4
Polymixiiformes							
Polymixiidae	*Polymixia lowei*	AY538862	AY539479	AY538966	AY539175	AY539071	AY539382
Beryciformes							
Trachichthyidae	*Hoplostethus mediterraneus*	AY538864	AY539481	AY538968	AY539177	AY539073	AY539384
Ophidiiformes							
Ophidiidae	*Chilara taylori*	AY538863	AY539480	AY538967	AY539176	AY539072	AY539383
Atheriniformes							
Atherinidae	*Menidia menidia*	AY538865	AY539482	AY538969	AY539178	AY539074	AY539385
Gasterosteiformes							
Aulorhynchidae	*Aulorhynchus flavidus*	AY538866	AY539483	AY538970	AY539179	AY539075	AY539386*
Aulostomidae	*Aulostomus maculatus*	AY538869	AY539486	AY538973	AY539182	AY539078	AY539389*
Gasterosteidae	*Apeltes quadracus*	AY538867	AY539484	AY538971	AY539180	AY539076	AY539387*
Pegasidae	*Pegasus volitans*	AY538868	AY539485	AY538972	AY539181	AY539077	AY539388*
Dactylopteriformes							
Dactylopteridae	*Dactylopterus volitans*	AY538870	AY539487	AY538974	AY539183	AY539079	AY539390*
Perciformes							
Blennioidei							
Blenniidae	*Salarias fasciatus*	AY538965	AY539582	AY539070	AY539279	AY539174	AY539478
Labrisomidae	*Labrisomus multiporosus*	AY538964	AY539581	AY539069	AY539278	AY539173	AY539477*
Notothenioidei							
Bathydraconidae	*Gymnodraco acuticeps*	AY538959	AY539576	AY539064	AY539273	AY539168	AY539472
Harpagiferidae	*Harpagifer kerguelensis*	AY538958	AY539575	AY539063	AY539272	AY539167	AY539471*
Percoidei							
Centrogeniidae	*Centrogenys vaigiensis*	AY538942	AY539559	AY539047	AY539256	AY539151	AY539455
Cheilodactylidae	*Cheilodactylus variegatus*	AY538955	AY539572	AY539060	AY539269	AY539164	AY539468
Cirrhitidae	*Cirrhitus rivulatus*	AY538954	AY539571	AY539059	AY539268	AY539163	AY539467
Grammatidae	*Gramma loreto*	AY538948	AY539565	AY539053	AY539262	AY539157	AY539461
Haemulidae	*Haemulon plumierii*	AY538952	AY539569	AY539057	AY539266	AY539161	AY539465
Kuhliidae	*Kuhlia rupestris*	AY538953	AY539570	AY539058	AY539267	AY539162	AY539466
Malacanthidae	*Hoplolatilus purpureus*	AY538951	AY539568	AY539056	AY539265	AY539160	AY539464
Moronidae	*Morone saxatilis*	AY538941	AY539558	AY539046	AY539255	AY539150	AY539454
Percidae	*Etheostoma blennioides*	AY538949	AY539566	AY539054	AY539263	AY539158	AY539462*
	Perca flavescens	AY538950	AY539567	AY539055	AY539264	AY539159	AY539463
Serranidae	*Diplectrum formosum*	AY538943	AY539560	AY539048	AY539257	AY539152	AY539456
	Epinephelus adscensionis	AY538944	AY539561	AY539049	AY539258	AY539153	AY539457
	Grammistes sexlineatus	AY538945	AY539562	AY539050	AY539259	AY539154	AY539458
	Hemanthias leptus	AY538946	AY539563	AY539051	AY539260	AY539155	AY539459
	Plectranthias kelloggi	AY538947	AY539564	AY539052	AY539261	AY539156	AY539460
	Pseudogramma thaumasium	AY538897	AY539514	AY539002	AY539211	AY539107	AY539415
Trachinoidei							
Champsodontidae	*Champsodon c.f. atridorsalis*	AY538960	AY539577	AY539065	AY539274	AY539169	AY539473*
Pinguipedidae	*Parapercis ramsayi*	AY538962	AY539579	AY539067	AY539276	AY539171	AY539475
Trachinidae	*Trachinus draco*	AY538963	AY539580	AY539068	AY539277	AY539172	AY539476
Zoarcoidei							
Bathymasteridae	*Bathymaster signatus*	AY538956	AY539573	AY539061	AY539270	AY539165	AY539469
Zoarcidae	*Lycodes diapterus*	AY538957	AY539574	AY539062	AY539271	AY539166	AY539470
Scorpaeniformes							
Normanichthyoidei							
Normanichthyidae	*Normanichthys crockeri*	AY538909	AY539526	AY539014	AY539223	AY539119	AY539426
Cottoid Lineage							
Anoplopomatoidei							
Anoplopomatidae	*Anoplopoma fimbria*	AY538905	AY539522	AY539010	AY539219	AY539115	AY539422
Cottoidei							
Agonidae	*Hypsagonus quadricornis*	AY538931	AY539548	AY539036	AY539245	AY539140	AY539445*
	Leptagonus frenatus	AY538932	AY539549	AY539037	AY539246	AY539141	AY539446
	Xeneretmus latifrons	AY538933	AY539550	AY539038	AY539247	AY539142	AY539447
Cottidae	*Artedius fenestralis*	AY538912	AY539529	AY539017	AY539226	AY539121	AY539428
	Asprocottus pulcher	AY538928	AY539545	AY539033	AY539242	AY539137	AY539442
	Batrachocottus baicalensis	AY538926	AY539543	AY539031	AY539240	AY539135	AY539440
	Comephorus baikalensis	AY538927	AY539544	AY539032	AY539241	AY539136	AY539441

Table 1 (*continued*)

Higher classification	Species	12S-tRNA-Val-16S		16S	Histone H3	28S	TMO-4c4
	Cottus bairdi	AY538913	AY539530	AY539018	AY539227	AY539122	AY539429
	Cottus carolinae	AY538914	AY539531	AY539019	AY539228	AY539123	AY539430
	Cottus poecilopus	AY538915	AY539532	AY539020	AY539229	AY539124	AY539431
	Hemilepidotus jordani	AY538916	AY539533	AY539021	AY539230	AY539125	AY539432
	Hemilepidotus zapus	AY538917	AY539534	AY539022	AY539231	AY539126	
	Icelinus filamentosus	AY538918	AY539535	AY539023	AY539232	AY539127	AY539433
	Jordania zonope	AY538919	AY539536	AY539024	AY539233	AY539128	AY539434
	Leptocottus armatus	AY538920	AY539537	AY539025	AY539234	AY539129	AY539435
	Microcottus sellaris	AY538921	AY539538	AY539026	AY539235	AY539130	
	Myoxocephalus polyacanthocephala	AY538922	AY539539	AY539027	AY539236	AY539131	AY539436
	Radulinus asprellus	AY538923	AY539540	AY539028	AY539237	AY539132	AY539437
	Taurulus bubalis	AY538924	AY539541	AY539029	AY539238	AY539133	AY539438
	Triglops scepticus	AY538925	AY539542	AY539030	AY539239	AY539134	AY539439
Cyclopteridae	*Aptocyclus ventricosus*	AY538937	AY539554	AY539042	AY539251	AY539146	AY539450
	Cyclopterus lumpus	AY538938	AY539555	AY539043	AY539252	AY539147	AY539451
Ereuniidae	*Marukawichthys ambulator*	AY538911	AY539528	AY539016	AY539225		
Hemitripteridae	*Hemitripterus americanus*	AY538929	AY539546	AY539034	AY539243	AY539138	AY539443
	Nautichthys pribilovius	AY538930	AY539547	AY539035	AY539244	AY539139	AY539444
Liparidae	*Careproctus melanurus*	AY538939	AY539556	AY539044	AY539253	AY539148	AY539452
	Liparis mucosus	AY538940	AY539557	AY539045	AY539254	AY539149	AY539453
Psychrolutidae	*Cottunculus thomsonii*	AY538934	AY539551	AY539039	AY539248	AY539143	AY539448
	Dasycottus setiger	AY538935	AY539552	AY539040	AY539249	AY539144	AY539449
	Malacocottus zonurus	AY538936	AY539553	AY539041	AY539250	AY539145	
Rhamphocottidae	*Rhamphocottus richardsonii*	AY538910	AY539527	AY539015	AY539224	AY539120	AY539427
Trichodontidae	*Trichodon trichodon*	AY538961	AY539578	AY539066	AY539275	AY539170	AY539474
Hexagrammoidei							
Hexagrammidae	*Hexagrammos decagrammus*	AY538906	AY539523	AY539011	AY539220	AY539116	AY539423
	Pleurogrammus azonus	AY538907	AY539524	AY539012	AY539221	AY539117	AY539424
Zaniolepidoidei							
Zaniolepididae	*Zaniolepis frenatus*	AY538908	AY539525	AY539013	AY539222	AY539118	AY539425*
Scorpaenoid Lineage							
Platycephaloidei							
Bembridae	*Bembras japonica*	AY538901	AY539518	AY539006	AY539215	AY539111	AY539419
Hoplichthyiidae	*Hoplichthys citrinus*	AY538904	AY539521	AY539009	AY539218	AY539114	
Platycephalidae	*Elates ransonnetii*	AY538902	AY539519	AY539007	AY539216	AY539112	AY539420
	Platycephalus bassensis	AY538903	AY539520	AY539008	AY539217	AY539113	AY539421
Peristediidae	*Peristedion gracile*	AY538898	AY539515	AY539003	AY539212	AY539108	AY539416
	Peristedion miniatum	AY538899	AY539516	AY539004	AY539213	AY539109	AY539417
Plectrogeniidae	*Plectrogenium nanum*	AY538900	AY539517	AY539005	AY539214	AY539110	AY539418
Triglidae	*Lepidotrigla spinosa*	AY538896	AY539513	AY539001	AY539210	AY539106	
Scorpaenoidei							
Aploactinidae	*Aploactisoma milesii*	AY538891	AY539508	AY538996	AY539205	AY539101	AY539411
Caracanthidae	*Caracanthus maculatus*	AY538895	AY539512	AY539000	AY539209	AY539105	AY539414
Congiopodidae	*Congiopodus peruvianus*	AY538893	AY539510	AY538998	AY539207	AY539103	
	Zanclorhynchus spinifer	AY538894	AY539511	AY538999	AY539208	AY539104	AY539413
Neosebastidae	*Maxillicosta whitleyi*	AY538874	AY539491	AY538978	AY539187	AY539083	AY539394
Pataecidae	*Aetapcus maculatus*	AY538892	AY539509	AY538997	AY539206	AY539102	AY539412
Scorpaenidae	*Dendrochirus brachypterus*	AY538886	AY539503	AY538990	AY539199	AY539095	AY539405
	Iracundus signifer	AY538877	AY539494	AY538981	AY539190	AY539086	AY539397
	Pontinus longispinis	AY538878	AY539495	AY538982	AY539191	AY539087	AY539398
	Pterois volitans	AY538887	AY539504	AY538991	AY539200	AY539096	AY539406
	Scorpaena brasiliensis	AY538879	AY539496	AY538983	AY539192	AY539088	AY539399
	Scorpaena guttata	AY538880	AY539497	AY538984	AY539193	AY539089	AY539400
	Scorpaena plumieri	AY538881	AY539498	AY538985	AY539194	AY539090	AY539401
	Scorpaenodes scaber	AY538882	AY539499	AY538986	AY539195	AY539091	
	Scorpaenopsis macrochir	AY538883	AY539500	AY538987	AY539196	AY539092	AY539402
	Taenionotus triacanthus	AY538884	AY539501	AY538988	AY539197	AY539093	AY539403
	Thysanichthys sp.	AY538885	AY539502	AY538989	AY539198	AY539094	AY539404
Sebastidae	*Helicolenus dactylopterus*	AY538871	AY539488	AY538975	AY539184	AY539080	AY539391
	Sebastes elongatus	AY538872	AY539489	AY538976	AY539185	AY539081	AY539392

Table 1 (*continued*)

Higher classification	Species	12S-tRNA-Val-16S		16S	Histone H3	28S	TMO-4c4
	Sebastes matsubarae	AY538873	AY539490	AY538977	AY539186	AY539082	AY539393
	Sebastolobus macrochir	AY538875	AY539492	AY538979	AY539188	AY539084	AY539395
	Trachyscorpia cristulata	AY538876	AY539493	AY538980	AY539189	AY539085	AY539396
Setarchidae	*Setarches guentheri*	AY538888	AY539505	AY538992	AY539201	AY539097	AY539407
Synanceiidae	*Synanceia verrucosa*	AY538890	AY539507	AY538995	AY539204	AY539100	AY539410
Tetrarogidae	*Ablabys taenianotus*	AY538889	AY539506	AY538993	AY539202	AY539098	AY539408
	Gymnapistes marmoratus			AY538994	AY539203	AY539099	AY539409

TMO-4c4 sequences that are missing more than 25% of their data are followed by a "*."

and 28SJJ 5′-AGGTTAGTTTTACCCTACT-3′ (Hillis and Dixon, 1991) were used. To amplify and sequence the histone H3 fragment, the primers H3a-L 5′-ATGG CTCGTACCAAGCAGACVGC-3′ and H3b-H 5′-AT ATCCTTRGGCATRATRGTGAC-3′ (Colgan et al., 1998) were used. To amplify and sequence the TMO-4c4 fragment, the primers TMO-f1 5′-CCTCCGGCCT TCCTAAAACCTCTC -3′, TMO-f2 5′-ATCTGTGAG GCTGTGAACTA-3′, TMO-f3 5′-ATCCCCTCAGGA GATTCTGC-3′, TMO-r1 5′-CATCGTGCTCCTGGG TGACAAAGT-3′, and TMO-r2 5′- TCCACGTCAAA CTCCATCAC-3′ (Lovejoy, 2000; Streelman and Karl, 1997) were used. Amplifications for all fragments were carried out in 30–40 cycles following the following temperature profile: initial denaturation for 6 min at 94 °C, denaturation for 45–60 s at 94 °C, annealing for 45–60 s at 45–55 °C, and extension for 1–2 min at 72 °C, with an additional terminal extension at 72 °C for 6 min. The double-stranded amplification products were desalted and concentrated using an ArrayIt PCR Product Purification Kit (TeleChem International) on a Beckman BIOMEK 2000 laboratory automated pipetting workstation with minor modifications to the manufacturer's protocol. Both strands of the purified PCR fragments were used as templates and directly cycle-sequenced using the original amplification primers and an ABI Prism Dye Terminator Reaction Kit. The nucleotides were sequenced on an ABI 3700 automated DNA sequencer. Contigs were built in Sequencher (Gene Codes) using DNA sequences from the complementary heavy and light strands. Sequences were edited in Sequencher and Bioedit (Hall, 1999). All sequences were submitted to GenBank and assigned Accession Nos. AY538862–AY539582.

2.3. Phylogenetic analyses

For the phylogenetic analysis, 3425 aligned base pairs from the six loci were simultaneously analyzed under the optimality criterion of parsimony with equal weights (i.e., gaps, transitions, and transversions all given a weight of 1). Ten fragments (1.9%) could not be successfully amplified and/or sequenced and were coded as missing data in the analysis (Table 1). An additional 11 TMO-4c4 sequences (Table 1) had a significant portion (>25%) of their data missing because of problems encountered with either one of the external primers, so the absent data were coded as missing. Therefore, internal primers were used to collect as much of the data as possible (usually ~62%). The parsimony analysis was conducted using direct optimization (Wheeler, 1996) and iterative pass (Wheeler, 2003a) as implemented in the program POY (Wheeler et al., 2002) and run on the American Museum of Natural History Parallel Computing Cluster. Unlike traditional multiple sequence alignment, which is divorced from the search for most optimal tree topologies, direct optimization combines alignment and tree-search into a single procedure to produce globally most parsimonious trees. This is achieved by including insertions and deletions, in addition to transitions and transversions, as forms of character transformation during optimization.

For this analysis, ribosomal DNA sequences were divided into smaller regions according to features of ribosomal secondary structure to save computation time and to constrain the homology statements to putatively homologous stem and loop regions (e.g., Giribet and Ribera, 2000). This method is preferable to a manual rDNA sequence alignment, which is informed solely by the visual comparison to a modeled rDNA secondary structure because it is repeatable, unbiased, logically consistent, and, most fundamentally, because there is no necessary connection between functional considerations (i.e., secondary structure) and the homology of individual nucleotides within a stem or loop region. Following the analysis, the least-costly cladograms resulting from the partitioned data sets were diagnosed using the unpartitioned (raw) data set to validate the results and implied homology statements (i.e., check if both analyses result in the same tree length and relationships). The results of both analyses were identical, so constraining the homology to presumed homologous stem and loop regions did not affect homology statements or our most parsimonious phylogenetic hypothesis.

The analysis began by generating five random addition sequences (RAS) per random replicate for 50 replicates. These 250 RAS were improved with TBR branch swapping during the searches, an additional round of TBR branch swapping of all trees within 0.5% of the shortest tree(s) found per replicate, and 500 parsimony

ratchet replicates (Nixon, 1999; 10 rounds in each of the 50 replicates with ratchetpercent 20 ratchetseverity 2 or 4). In addition to TBR branch swapping and ratcheting within each replicate, all resulting trees within 1.0% of the shortest trees were examined in an additional round of TBR branch swapping. The random replicates from these initial searches resulted in six equally most parsimonious trees with a length of 11,272 steps. These six trees were submitted to POY for further tree searching using the commands iterative pass (Wheeler, 2003a) and exact (Wheeler et al., 2002). This second step of the analysis began by tree fusing (Goloboff, 1999) the six submitted topologies and one additional RAS, and it was followed by 200 rounds of parsimony ratcheting (ratchetpercent 20 ratchetseverity 2 or 5), which was followed by a final round of tree fusing and TBR branch swapping.

The length of the resulting implied alignment (Wheeler, 2003b) was verified in NONA (Goloboff, 1998) and WinClada (Nixon, 2000). To estimate the "robustness" of the clades recovered in the phylogenetic hypotheses, Bremer supports (Bremer, 1988, 1995) and jackknife percentages (1000 replications, 10 random addition sequences per replicate) were calculated in NONA based on the resulting implied alignment. Character evolution on the recovered topologies was examined using NONA and WinClada.

3. Results

The combined analysis of the six gene fragments resulted in four equally most parsimonious trees with length of 11,198 steps. The implied alignment of 3425 base pairs had a consistency index (CI, Kluge and Farris, 1969) of 0.29, and a retention index (RI, Farris, 1989) of 0.48, when uninformative characters are retained. A strict consensus of these four trees is presented in Fig. 2. The traditionally recognized scorpaeniform fishes were recovered as polyphyletic. The atheriniform, blennioid, gasterosteioid, grammatid, notothenioid, percid, trichodontid, and zoarcoid representatives included in the analysis were all nested within the traditional Scorpaeniformes (Clade S in Figs. 2, 3). The scorpaenoid lineage was widely polyphyletic, and its intrarelationships differed significantly from previous hypotheses (e.g., Imamura, 1996; Ishida, 1994; Mandrytza, 2001). The cottoid lineage was paraphyletic with only the presence of the trichodontid (*Trichodon trichodon*) as the sister-taxon of the Cottoidei (sensu Yabe, 1985; Clade 51 [C51] in Fig. 3) disrupting the monophyly and intrarelationships of Shinohara's (1994) (see Fig. 1A) subordinal hypothesis.

The Hexagrammoidei (C49) and Cottoidei (C51) were the only scorpaeniform suborders represented by multiple species that were resolved as monophyletic. Representatives of the Scorpaenoidei were found in four distinct clades (clades 8, 10, 37, and *Zanclorhynchus*). The inter- and intrarelationships of the scorpaenoid families are also different from the hypotheses suggested by Ishida (1994), Imamura (1996), and Mandrytza (2001). Representatives of Imamura's (1996) Platycephaloidei were found in four distinct clades (clades 1, 9, 29, and *Hoplichthys*), which did not match his hypothesized relationships.

Within the scorpaenoid lineage, only the Platycephalidae (C1) and Peristediidae (C31) were recovered as monophyletic. Our results do not corroborate the monophyly of the Tetrarogidae, Congiopodidae, Scorpaenidae, or Sebastidae. Within the cottoid lineage, the Hexagrammidae (C49), Liparidae (C70), Cyclopteridae (C71), and Psychrolutidae (C73) were recovered as monophyletic. Our results do not corroborate the monophyly of the Cottidae, Hemitripteridae, or Agonidae.

Most of the 101 nodes resulting from the analysis were well supported, with 70 nodes having a Bremer support $\geqslant 5$ and 47 nodes having a bremer support $\geqslant 10$. Additionally, 77 nodes were supported by a jackknife value $\geqslant 70$ and 64 nodes had a jackknife value $\geqslant 90$. In general, relationships within the eight scorpaenoid-lineage clades are better supported than relationships within the cottoid lineage, particularly for the Cottoidei, which had many nodes with jackknife values <50 and bremer supports of one.

4. Discussion

4.1. Outgroups

The interrelationships outside of clade S in Fig. 2 are not the focus of the study, but the placement of taxa historically linked to the Scorpaeniformes will be discussed. The dactylopterid (*Dactylopterus*) was resolved as the sister-group of *Aulostomus*, the only sygnathoid included in this analysis. This sister-group relationship between *Aulostomus* and *Dactylopterus* (also recovered in Chen et al., 2003) agrees, in part, with the morphological hypothesis suggested by Pietsch (1978) based on similarities in jaw morphology and the fusion of the first three vertebrae. Johnson and Patterson (1993) argued against a close relationship between these groups based primarily on dactylopterids lacking their hypothesized gasterosteiform synapomorphies and concerns about the homology of the vertebral fusion in both groups. However, our analysis and other large-scale acanthomorph molecular analyses (e.g., Chen et al., 2003; Miya et al., 2003) have not recovered a monophyletic Gasterosteiformes, so a dactylopterid–sygnathoid relationship should be explored further in light of the results of recent

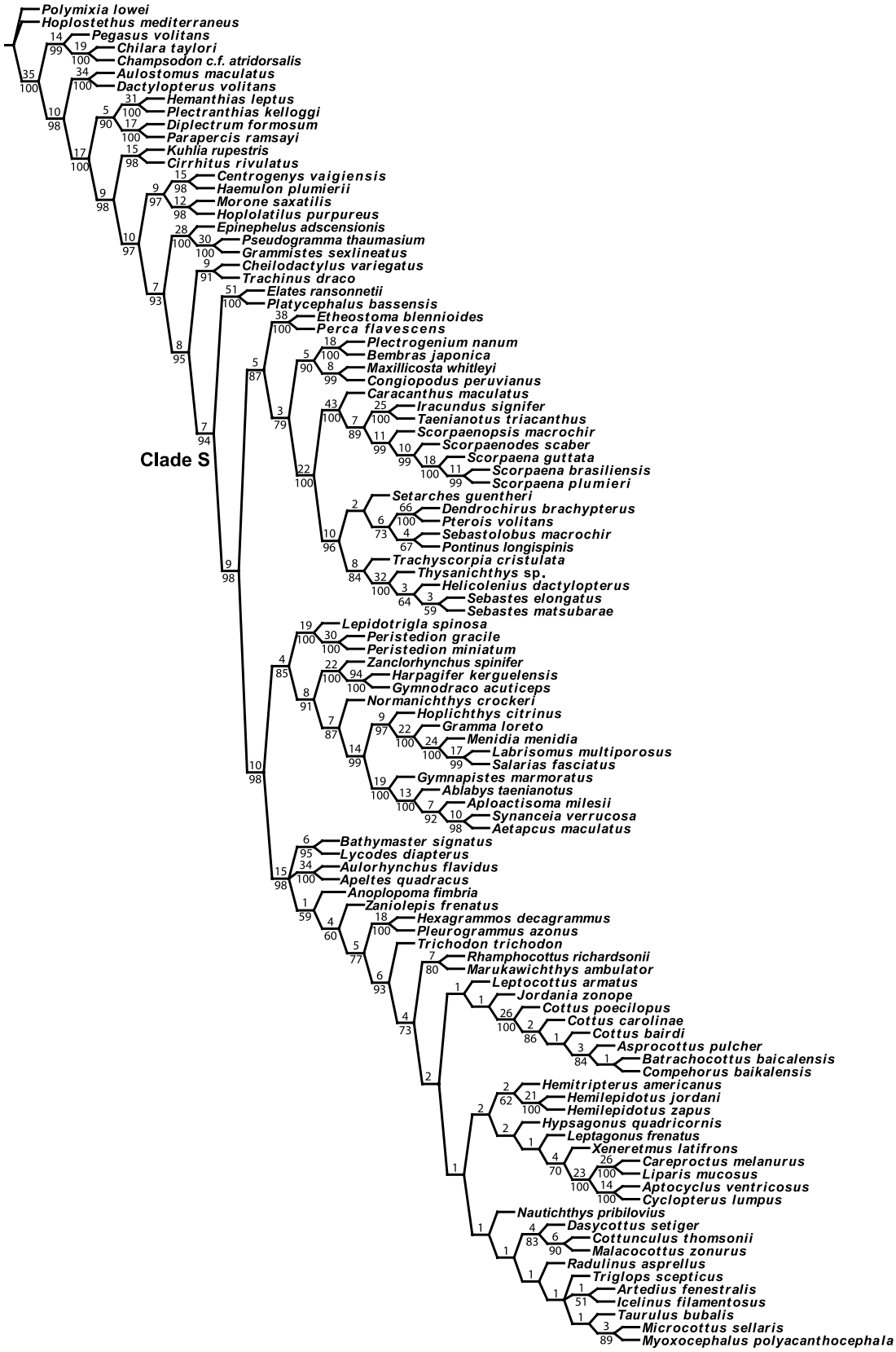

Fig. 2. Strict consensus of four equally most parsimonious trees recovered (tree length = 11,198 steps) by direct optimization of data set composed of 12S, 16S, tRNA-Val, 28S, histone H3, and TMO-4c4 nucleotide characters. Numbers on branches represent Bremer support/jackknife resampling percentages (>50%) for each recovered node. The least inclusive clade that contained all scorpaeniform taxa is labeled clade S.

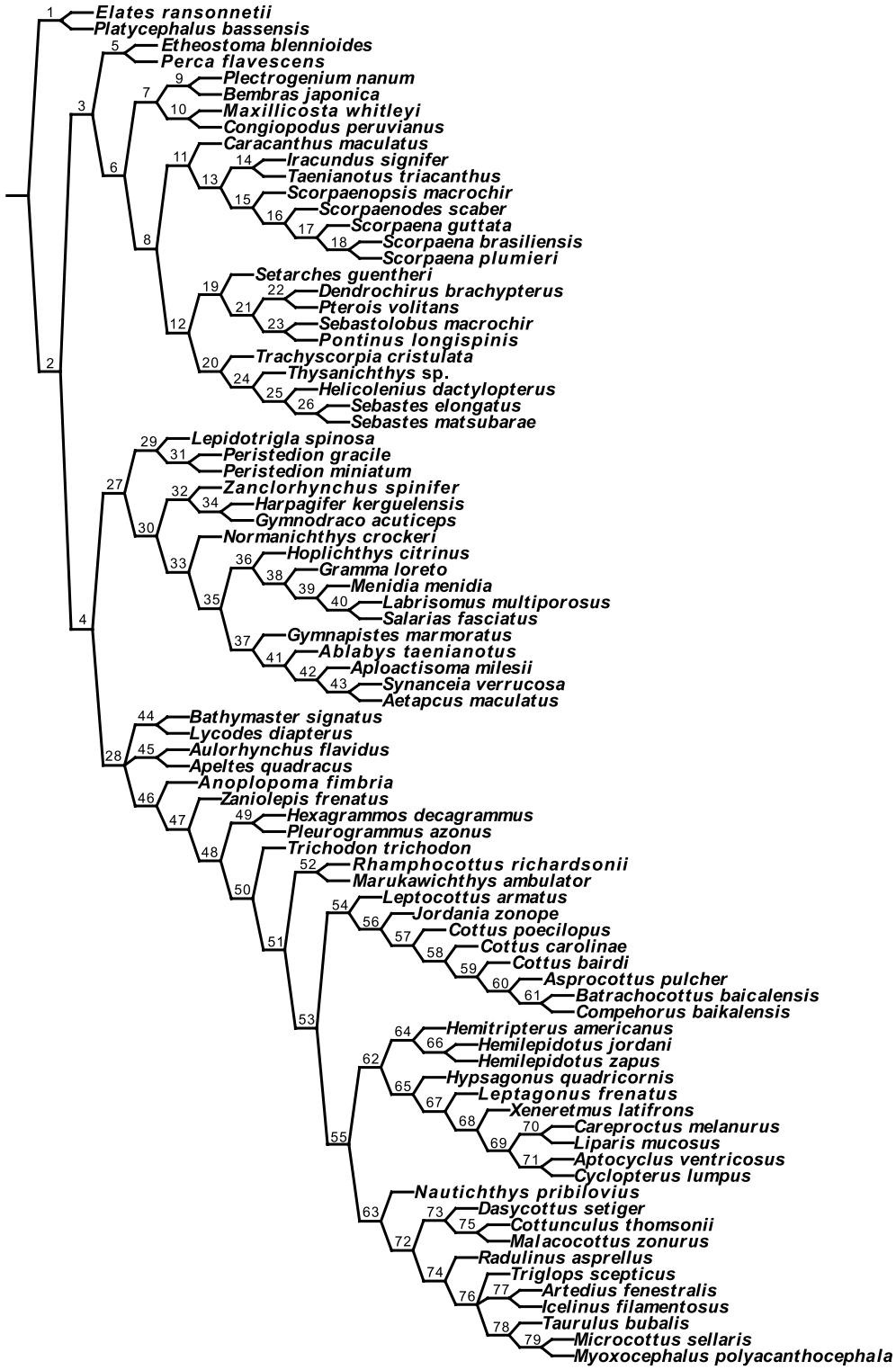

Fig. 3. Intrarelationships of clade S from Fig. 2. Numbers on nodes are for reference in the text, and they do not represent support measures.

molecular analyses. Additionally, Imamura's (2000) hypothesis suggesting a sister-group relationship between malacanthids (e.g., *Hoplolatilus*) and dactylopterids was not supported in our analysis.

Mooi and Johnson's (1997) hypothesis linking the Champsodontidae (*Champsodon*) and the Scorpaeniformes was not supported in our analysis (also see Imamura and Yabe, 2002). Instead, we recovered a close relationship between *Champsodon* and the ophidiiform *Chilara.* This ophidiiform–champsodontid relationship was not examined by Mooi and Johnson (1997), although they explored the possibility of a

paracanthopterygian–champsodontid relationship. In their discussion, Mooi and Johnson (1997) gave the distribution of the 19 characters that Johnson and Patterson (1993, J&P characters 15–33) used to define the Acanthopterygii and its various sub-groups. For comparison, we will use the characters coded by Wiley et al. (2000) for the ophidiid, *Petrotyx sanguineus*, in their total-evidence analysis of the Acanthomorpha. *Petrotyx* and champsodontids lack free pelvic radials (J&P 16 and 31), a chain-link articulation of the dorsal fin spines (J&P 22), distally ossified supraneural(s) (J&P 23), and transforming ctenoid scales (J&P 30). Additionally, both groups have a pelvic spine (J&P 15), Baudelot's ligament originating on the occiput (J&P 18), a dorsal fin originating anterior to the fourth neural spine (J&P 19), anterior epineurals on vertebrae 3–10 originating on the ribs (J&P 20), ventral procurrent rays that are not proximally shortened (J&P 24), an interarcual cartilage and uncinate process on the first epibranchial (J&P 26), second ural centrum fused with first preural centrum + ural centrum (J&P 27), five or fewer hypurals (J&P 28), pelvic fins with fewer than six soft rays (J&P 29), point of origin of all but the first two epineurals displaced ventrally into horizontal septum (J&P 32), and a caudal fin with 17 or fewer principal caudal-fin rays (J&P 33). Petrotyx and champsodontids differ in the presence or absence of only three of Johnson and Patterson's (1993) characters. These characters are associated with the pelvic girdle morphology, which is highly modified and anteriorly displaced (when present in adults) in the Ophidiiformes. These three characters are present in champsodontids and absent in *Petrotyx*: an anteromedial process of the pelvic fin (J&P 17), a complex pelvic spine (J&P 21), and a myoseptal ligament from postcleithrum to posterolateral corner of pelvic girdle (J&P 25). Because both groups share an uncommon and similar distribution of Johnson and Patterson's (1993) 19 acanthopterygian characters, particularly when the pelvic characters are excluded, this relationship should be explored further; this is particularly important in light of concerns about the monophyly of the Paracanthopterygii, Percomorpha, and the Ophidiiformes (e.g., Gill, 1996; Gosline, 1968; Rosen, 1985).

The Pegasidae (*Pegasus*), which has often been allied with the Scorpaeniformes, was not resolved as a member or ally of any scorpaeniform lineage. This supports the views of many authors (e.g., Johnson and Patterson, 1993; Pietsch, 1978) who have argued for pegasids being more closely related to other percomorph groups such as the gasterosteiforms. The interrelationships of the Gasterosteiformes remain problematic (Chen et al., 2003; Fig. 2), and the pegasids were not allied with any gasterosteioid or sygnathoid taxa in our analysis, so additional work is needed on the interrelationships of this enigmatic family.

As we move up the tree towards the scorpaeniform clade S, a number of percoid groups form a roughly pectinate grade leading to this scorpaeniform clade. Of note, the Serranidae was not recovered as a monophyletic group, although the serranid subfamilies sensu Johnson (1983) were monophyletic. The multiple placements of the Serranidae outside the Scorpaeniformes contradict the hypothesis of Imamura and Yabe (2002) that argued for a sister-group relationship between the Serranidae and the scorpaenoid lineage. Similarly, the false scorpionfish (*Centrogenys*) and hawkfish (*Cirrhitus*), which have been allied frequently with the Scorpaeniformes (e.g., Gill, 1888; Leis and Trnski, 1999), were found outside of this clade S. Instead, our analysis recovered a trachinid (*Trachinus*) + cheilodactylid (*Cheilodactylus*) clade as the sister-group of the "scorpaeniform" clade S.

4.2. Congruence of our scorpaeniform results with other percomorph molecular analyses

As described in Section 3, the Scorpaeniformes was recovered as a polyphyletic assemblage. Because there are a large number of non-scorpaeniform taxa resolved within clade S, the detailed interrelationships of most of these non-traditional scorpaeniforms will be discussed in the context of their allied scorpaeniform groups. However, we will comment briefly on the placement of the eight non-scorpaeniform clades (13 taxa) found within our clade S in other large-scale molecular phylogenies.

Of these eight percomorph clades nested within our clade S, seven have been included in previous molecular analyses looking broadly at the Acanthomorpha: Atheriniformes, Blennioidei, Gasterosteoidei, Notothenioidei, Percidae, Trichodontidae, and Zoarcoidei. The Grammatidae have not been previously examined using molecular data, so a comparison of our results with other molecular data cannot be discussed further. Large-scale molecular studies examining the interrelationships of the Percomorpha include Le et al. (1993), Wiley et al. (2000), Miya et al. (2001, 2003), Elmerot et al. (2002), and Chen et al. (2003). For additional discussion of these studies, in the context of previous morphological hypotheses, see Stiassny et al. (in press).

The study of Wiley et al. (2000) did not include any scorpaeniforms and had limited taxonomic overlap with our study (i.e., Atheriniformes, *Morone*, Dactylopteridae, Ophidiiformes, and *Polymixia*), so a comparison of our results with theirs would be of limited utility. Among the remaining studies, only Chen et al. (2003) and Miya et al. (2003) examined the interrelationships of the Atheriniformes and the Blennioidei. In both of these studies, the authors report a clade composed of the Atherinomorpha and Blennioidei (in addition to Mugilidae and Gobieosocoidei [dissenting

view were published on the inclusion, or not, of Synbranchiformes], which were not included in our analysis). Our study corroborates their findings by recovering a close relationship between the Blennioidei and the Atheriniformes. These two studies (Chen et al., 2003; Miya et al., 2003) also recovered a monophyletic clade composed of the Cottoidei, Zoarcoidei, and Gasterosteoidei. Neither of these studies included a trichodontid, so their results, for the relevant taxa, corroborate our findings. Furthermore, Elmerot et al. (2002), which did not include any zoarcoid or cottoid taxa, found a sister-group relationship between the Trichodontidae and the Gasterosteoidei, which further corroborates the findings of our study. Finally, we have the placement of the Percidae and Notothenioidei. The only large-scale molecular analyses to include either of these clades are Le et al. (1993) and Chen et al. (2003). Le et al. (1993), with very limited taxonomic sampling, found a sister-group relationship between the scorpaeniform *Trigla* and their percid *Perca*. In a more comprehensive analysis, Chen et al. (2003) found a sister-group relationship between notothenioids and percids. This clade was nested within a larger assemblage composed of these two groups, trachinids, scorpaenoids, and serranids. This group largely corresponds to our clade S and its two subsequent outgroups. Chen et al. (2003) did not include multiple scorpaenoid taxa in their analysis, which accounts for the different relationships that we recovered. For example, their analysis did not include congiopodids, which we found sister to the notothenioids instead of the percids. Regardless of these minor differences, the congruence of our results and these other large-scale molecular studies looking broadly at the Percomorpha is striking for the placement of these 13 non-scorpaeniform taxa that were resolved within our clade S. The topological similarity among these various studies, despite the use of different taxa, analytical methods, alignment methods, and gene fragments, provides corroborative evidence for our unconventional placements of these taxa within our clade S.

4.3. Scorpaeniformes

It is interesting to note that most of the groups in our clade S (e.g., scorpaeniforms, zoarcoids, notothenioids, blennioids, and trichodontids) have a sensory canal associated with the parietal as described by Eakin (1981), Johnson (1993), Mooi and Gill (1995), Mooi and Johnson (1997), and Imamura and Yabe (2002). This suggests that this character, originally cited as a possible scorpaeniform synapomorphy (Johnson, 1993), may support the monophyly of this larger clade. A detailed study of this character complex should provide insight into the relationships of this large clade.

The first major scorpaeniform clade (C1) is composed of the two platycephalids included in the analysis. The next major ingroup clade (C3) includes many of the groups included in Imamura and Shinohara's (1998) scorpaenoid lineage. These include the Bembridae, Neosebastidae, Congiopodidae (in part), Caracanthidae, Scorpaenidae, Sebastidae, Setarchidae, Plectrogeniidae, and the non-scorpaeniform Percidae. The next major ingroup clade (C27) includes the remainder of the scorpaenoid lineage, the enigmatic *Normanichthys*, and four non-scorpaeniform groups (Atheriniformes, Blennioidei, Grammatidae, and Notothenioidei). The scorpaenoid lineage families included in this clade are the Triglidae, Peristediidae, Congiopodidae (in part), Hoplichthyidae, Pataecidae, Aploactinidae, Tetrarogidae, and Synanceiidae. The relationships of the final major clade (C28) resulting from the analysis are composed of the cottoid lineage, Gasterosteoidei, Zoarcoidei, and the Trichodontidae.

4.4. Scorpaenoid lineage (clades 1, 7, 8, 29, 37, Hoplichthys, and Zanclorhynchus)

The scorpaenoid lineage was not recovered as monophyletic in our analysis. Neither the composition nor the intrarelationships of the scorpaenoid lineage match previous morphological hypotheses (e.g., Imamura, 1996; Imamura and Shinohara, 1998; Imamura and Yabe, 2002). The only authors to provide synapomorphies to unite the scorpaenoid lineage were Imamura (1996) and Imamura and Yabe (2002). Imamura (1996) united the Scorpaenoid lineage by the presence of an extrinsic swim bladder muscle derived from the *obliquus superioris* and a backwardly directed opercular spine. Later, Imamura and Yabe (2002) expanded the former scorpaenoid lineage (their Scorpaenoidea) to include the Serranidae. This revised Scorpaenoidei was diagnosed by the opercular spine discussed above and the presence of an *adductor dorsalis*. These authors also used the presence of a parietal sensory canal with spines and the extrinsic swim bladder muscle to diagnose the Scorpaenoidei + Platycephaloidei. Therefore, the distribution of these four characters in the other members of the clade S and its sister-group need to be examined further.

First, many pteroine scorpaenoids, triglids, peristediids, and apistids have intrinsic (vs. extrinsic) swim bladder muscles (Hallacher, 1974; Ishida, 1994; pers. obs.), and many scorpaenoids (e.g., *Sebastolobus*, *Maxillicosta*, *Aploactis*, and *Congiopodus*), cottoids, zoarcoids, and non-scorpaeniforms nested within clade S or its sister-group (e.g., *Cheilodactylus*, notothenioids, and *Trichodon*) lack swim bladders. Therefore, the utility of this swim bladder muscle as a synapomorphy is doubtful, particularly in light of its optimization on our phylogeny (not shown), which shows numerous losses.

The second character used by Imamura (1996) to diagnose the scorpaenoid lineage was the presence of a backwardly directed opercular spine reaching across the subopercle. This character is found in most scorpaenoid lineage taxa, some percoids (e.g., Serranidae, Epigonidae; Johnson, 1983) and trachinids (Johnson, 1983). If the distribution of this character is optimized onto our cladogram, it optimizes outside of clade S because serranids and trachinids have this character. Perhaps it is a synapomorphy of a larger clade, but as with the swim bladder muscle above, the homology of these spines requires further attention (G.D. Johnson, pers. com.; pers. obs.).

The third character, the presence of an *adductor dorsalis*, is also questionable as a synapomorphy of the Scorpaenoidei without a better understanding of percomorph intrarelationships because of its wide distribution (e.g., Acanthuridae, Apogonidae, Callanthiidae, Centrarchidae, Kuhliidae, Lutjanidae, Nandidae, Nototheniidae, Pinguipedidae, Serranidae, Sparoidea, and Tetraodontiformes) and its absence in the scorpaenoid lineage families: Synanceiidae, Aploactinidae, Triglidae, Peristediidae, Hoplichthyidae, and Platycephalidae (Winterbottom, 1974; Imamura and Yabe, 2002; pers. obs.). Based on the optimization of this character on our cladogram (not shown), it appears that the loss of the *adductor dorsalis* unites members of clade 4 rather than uniting the Serranidae + scorpaenoid lineage [as described by Imamura and Yabe (2002)].

Finally, the fourth character, the parietal sensory canal with spines needs to be examined further. As was discussed above, most of the groups in clade S have a sensory canal associated with the parietal, which is unusual among percomorphs (Mooi and Gill, 1995; Mooi and Johnson, 1997). Imamura and Yabe (2002) discussed the development of this sensory canal in a handful of scorpaeniform taxa and hypothesized that the development was different between the cottoid and scorpaenoid lineages. They suggested that the development was different because the canal in scorpaenoid lineage taxa develops as projections of the parietal and nuchal spines that connect forming this parietal canal. Although we agree with the authors that most scorpaenoid species have spines associated with this parietal canal, the distribution of this character in the cottoid lineage is much wider than their study suggests. Furthermore, this character is found in other percomorph groups (e.g., Champsodontidae; Mooi and Johnson, 1997). Optimization of this character on our hypothesis suggests that it might diagnose the larger clade S, not just the Scorpaenoidei.

4.5. Scorpaenoidei

Matsubara (1943), working in a pre-cladistic framework, argued that the scorpaenoids were composed of three distinct lineages (sea robins, rockfishes, and scorpionfishes), with rockfishes (Sebastidae) and the enigmatic *Plectrogenium* representing the ancestral scorpaenoids. Ishida (1994) also recovered the rockfishes (including *Plectrogenium*) as the plesiomorphic scorpaenoid family. Unfortunately, Ishida's (1994) analysis was optimized by hand, so his published cladogram is 8% longer than any of the shortest trees resulting from our reanalysis of his data matrix. Most of the nodes shown in his phylogeny (Fig. 1D) collapse in a strict consensus of the most parsimonious trees (Fig. 4A), so many of his conjectures of monophyletic families and hypotheses of relationships are simply not supported. In addition to Matsubara (1943) and Ishida (1994), there have been a few recent molecular studies that have examined the relationships of various scorpaenoid groups. None of these molecular studies have looked broadly at the inter- and intrarelationships of the suborder. Instead, they have focused on *Sebastes* (e.g., Johns and Avise, 1998; Kai et al., 2003; Rocha-Olivares et al., 1999a,b) or lionfishes (Kochzius et al., 2003). Our analysis is the first molecular study looking broadly at the intrarelationships of the Scorpaenoidei. The resulting phylogeny recovered a polyphyletic Scorpaenoidei with four independent clades.

One clade, scorpaenoid clade 8, is composed of the "core" scorpionfishes and rockfishes, which were left largely unresolved in the reanalysis of Ishida's (1994) data matrix (Fig. 4B). This group includes the Caracanthidae, Scorpaenidae, Sebastidae, and Setarchidae. Traditionally (e.g., Ishida, 1994; Matsubara, 1943), the sebastids have been treated as the basal members of the scorpaenoid radiation. However, Eschmeyer and Hureau (1971) and Hallacher (1974) suggested that sebastids were a poor choice for the plesiomorphic scorpaenoid because they have live birth and derived swim bladder muscle morphologies. The results of our analysis agree with Eschmeyer and Hureau's (1971) hypothesis that rockfishes represent a more derived lineage. Interestingly, live-bearing sebastids were found among scorpaenoids with a characteristic-spawning mode that includes the production of a gelatinous egg mass (e.g., *Sebastolobus*, *Pterois*; Koya and Matsubara, 1995). This suggests that there might be an evolutionary transition from the more typical planktonic spawning found among many scorpaenoids (e.g., *Scorpaena*; Leis and Rennis, 2000) to the production of a nutrient-rich gelatinous egg mass, and then a final transition to live birth in sebastid rockfishes.

Scorpionfish clade 10 is composed of members of the Neosebastidae and the Congiopodidae (in part). There are no unique characters to unite these two groups, and a number of morphological characters (see Ishida, 1994) to unite *Congiopodus* with the other congiopodid included in our analysis (*Zanclorhynchus*). Ishida (1994) (see Fig. 1C) suggested a close relationship between the

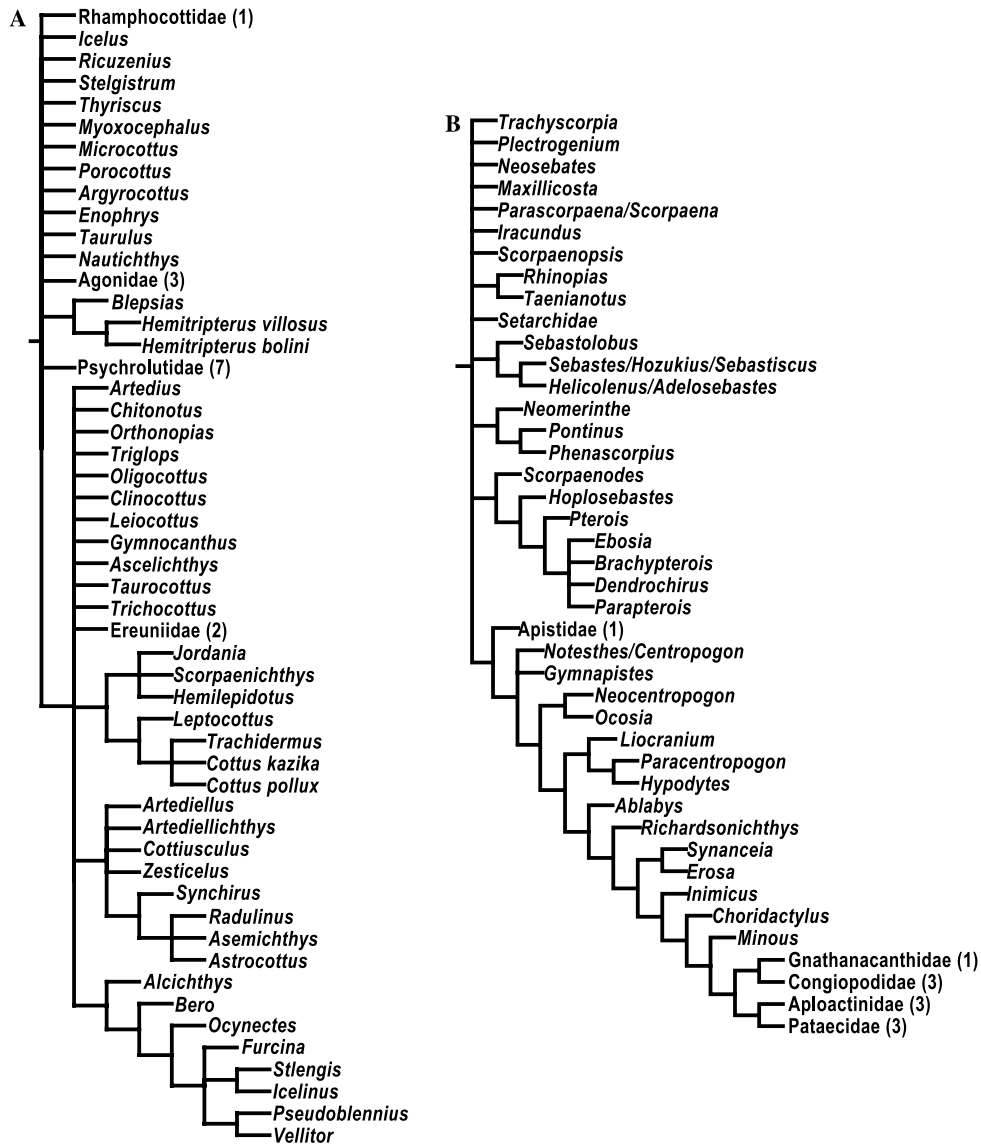

Fig. 4. Cladograms resulting from reanalyses of previous morphological studies of scorpaeniform suborders that did not find the most parsimonious trees. (A) Cottoidea (Yabe, 1985), (B) Scorpaenoidei (Ishida, 1994). Families recognized by Yabe (1985) in (A) and Ishida (1994) in (B) that were resolved as monophyletic in our reanalysis are designated by the family name (instead of generic names) and include the number of genera that these authors examined following the familial name.

Congiopodidae and the Synanceiidae and between the Neosebastidae and Setarchidae, but the reanalysis of his data (Fig. 4B) did not support these relationships. It is interesting to note that *Zanclorhynchus* was resolved as the sister-group of the Notothenioidei. Both of these groups are distributed in the southern ocean and share a number of uncommon features (e.g., lack of a basisphenoid, loss of anal spines; Balushkin, 2000; Ishida, 1994; Washington et al., 1984), so the possible relationship between notothenioids and congiopodids should be explored further in light of our results.

Scorpaenoid clade 37 is composed of the stonefishes their relatives (Synanceiidae, Tetrarogidae, Aploactinidae, and Pataecidae). Leis and Rennis (2000) provided evidence from larval morphology that separated these families from the remainder of the core Scorpaenoidei. Additionally, Ishida's (1994) analysis used the lack of a metapterygoid lamina and dermosphenotic in conjunction with a presumed fusion of the scapula and uppermost pectoral radial to unite these families. This is one of the few major groups in the Scorpaenoidei that is supported by morphological features (both adult and larval) and molecular data, which together provide strong evidence supporting its monophyly.

As is clear from this discussion, scorpaenoid intrarelationships remain among the least understood of all scorpaeniform suborders. Ishida (1994) could not provide a single synapomorphy to unite this group, but retained it stating that it is "a natural group because the other scorpaeniforms have been shown to be derived

from non-scorpaenoid ancestors." Recently, Mandrytza (2001) provided two synapomorphies for his Scorpaenoidei: presence of three neuromasts in the lachrymal and a particular configuration of the dorsal pterygiophores and neural spines. However, the condition of three lachrymal neuromasts is found in numerous percomorphs (e.g., Serranidae, Centrarchidae, and Normanichthyidae; Baldwin and Johnson, 1993, pers. obs.; Freihofer, 1978), so the distribution of this character needs further study. The second proposed scorpaenoid synapomorphy suggested by Mandrytza (2001), the condition of the first dorsal spines, has two distinct, unrelated character states, so it cannot be used as evidence for scorpaenoid monophyly. The first state, the first and second dorsal pterygiophores located between the neural spines of the second and third vertebrae, is found in numerous percoid groups (e.g., Aplodactylidae, Centropomidae, Cirrhitidae, and Haemulidae; Johnson, 1984). The second condition has the first few dorsal pterygiophores shifted forwards on to the dorsal surface of the neurocranium; this is found in all tetrarogids, other scorpaenoids, and many acanthomorph groups (e.g., Lophiiformes; Tetraodontiformes; pers. obs.). The results of our molecular analysis and the historical problems in diagnosing the Scorpaenoidei provide convincing evidence that this suborder is not monophyletic.

4.6. Platycephaloidei

Imamura (1996) provided the first explicit phylogeny for the Platycephaloidei, which included a different composition from previous hypotheses (Fig. 1D). He included a number of former scorpaenoid clades (e.g., Triglidae, *Plectrogenium*). The single diagnostic character he used for this group is the presence of a posterior pelvic fossa. The homology of the posterior pelvic fossa is somewhat questionable across all of these taxa. *Plectrogenium*, for example, has a minute opening with the anterior pelvic processes oriented rostrally and that are not visible from a ventral view; whereas, platycephalids and hoplichthyids have medially directed anterior pelvic processes and a widely separated pelvis (Imamura, 1996, Figs. 5 and 32; pers. obs.). Because there are concerns about the homology of this character across the four platycephaloid clades recovered in this analysis, the detailed anatomy and development of the pelvic girdle should be examined further. Imamura's (1996) hypothesis linking *Plectrogenium* with the bembrids and other platycephaloids was supported in our phylogeny (Fig. 3). However, the other novel interrelationships suggested by Imamura (1996) were not supported in our analysis.

For example, Imamura (1996) united the Hoplichthyidae and the Peristediidae to the exclusion of the Triglidae. Our analysis (Fig. 3) found the traditional placement of the Triglidae as the sister-group of the Peristediidae. Our results united the Hoplichthyidae and a diverse group of non-scorpaeniform percomorphs (C36). The composite nature of clade 36, with representatives of four percomorph suborders suggests that their close relationship may be due to the limited taxon sampling of these groups and the lack of many of their traditional allies in this analysis (see above). Despite the non-traditional relationships suggested in clade 36, these basic relationships have been proposed in <u>all</u> other large-scale molecular analyses that have included blennioids and atherinomorphs (Chen et al., 2003; Miya et al., 2003). In addition to this relationship, which has been supported in other molecular analyses, there is morphological data to support these relationships. For example, the grammatids, blennioids, and atherinomorphs all have demersal, adhesive eggs with chorionic filaments, which are uncommon elsewhere among acanthomorph fishes [also found in Apogonidae, Cichlidae, Gobiesocidae, Gobioidei, Kurtidae, Opistognathidae, *Plesiops*, Pomacentridae (Breder and Rosen, 1966; Gill and Mooi, 1993; Parenti, 1993)]. Additionally, many of these groups share all or some of the following uncommon features: presence of a fourth internal levator sling, the loss of an interarcual cartilage, loss of supraneurals, and a reduced number of pharyngobranchials (Johnson, 1993; Parenti, 1993; pers. obs.; Rosen and Patterson, 1990).

4.7. Normanichthyoidei

The enigmatic mote sculpin (*Normanichthys crockeri*), which has often been included in the cottoid lineage (e.g., Norman, 1938; Washington et al., 1984), was resolved outside the cottoid lineage (C46). This placement agrees with Yabe and Uyeno (1996) who excluded *Normanichthys* from the cottoid lineage because it lacked half of the synapomorphies of the Cottoidei (following Yabe, 1985) and three of the nine cottoid lineage synapomorphies (following Shinohara, 1994). The placement of *Normanichthys* in our analysis is tentative because there are few morphological characters that support its placement with the other taxa in clade 27. Recent work by Velez et al. (2003) suggests some novel placements for *Normanichthys* based on larval morphology that should be examined further, although their suggestion that *Normanichthys* and cheilodactylids are related was not supported in our analysis.

4.8. Cottoid lineage + Gasterosteoidei + Zoarcoidei + Trichodontidae

The close relationship recovered in our analysis for the Zoarcoidei and Gasterosteoidei was also recovered in Chen et al. (2003) and Miya et al. (2003) as discussed above. Imamura and Yabe (2002) provided

morphological evidence linking the Zoarcoidei + cottoid lineage and Bowne (1994) provided some evidence linking the gasterosteioids and cottoids, although she preferred a sygnathoid sister-group for the gasterosteioids. A comprehensive morphological analysis is needed to help resolve relationships among these three percomorph assemblages.

4.9. Cottoid lineage (clade 46)

As mentioned above, the interrelationships of the cottoid lineage suborders were identical to those proposed by Shinohara (1994) (see Fig. 1A), except for the inclusion of the historically problematic Trichodontidae. For a detailed description of the interrelationships of these suborders, see Quast (1965), Yabe (1985), and Shinohara (1994). Only the placement of the Trichodontidae and the intrarelationships of the Cottoidei will be discussed because they differ from the relationships presented by previous morphology-based phylogenetic hypotheses.

Our placement of the historically enigmatic family Trichodontidae within the cottoid lineage is well supported by our molecular study (also see Elmerot et al., 2002) as well as morphological evidence (Mooi and Johnson, 1997), so we recommend classifying Trichodontidae as a cottoid family. This close relationship between these groups was first suggested by Starks (1930) based on *Trichodon* having a "shoulder girdle [that] is strikingly like that of some cottoid fishes." Additionally, Mooi and Johnson (1997) discussed the possibility of a close relationship between trichodontids and the Scorpaeniformes (their Scorpaenoidei). In addition to sharing all of the cottoid lineage synapomorphies, except the parasphenoid–pterosphenoid junction, the placement of the Trichodontidae in the Cottoidei is supported by the following synapomorphies: spawning demersal eggs, loss of basisphenoid, loss of pharyngobranchial four, presence of four large preopercular spines, an intercalar that does not reach anteriorly to the prootic, and the loss of the metapterygoid lamina (Mooi and Johnson, 1997; Nazarkin and Voskoboinikova, 2000; pers. obs.; Rosen and Patterson, 1990; Shinohara, 1994; Yabe, 1985). Furthermore, the Trichodontidae lacks a number of the features of the Cottoidea (Yabe, 1985), corroborating its placement in our analysis: presence of pharyngobranchial one (absent in Cottoidea), presence of basihyal (absent in Cottoidea), presence of ribs on vertebrae three through six (ribs begin on vertebrae six or more posteriorly in Cottoidea) (Nazarkin and Voskoboinikova, 2000; pers. obs.; Rosen and Patterson, 1990; Yabe, 1985).

Despite the overwhelming morphological evidence for a cottoid placement for the Trichodontidae, Nazarkin and Voskoboinikova (2000) rejected it because trichodontids lack a suborbital stay. Furthermore, Imamura and Yabe (2002) used the lack of four of their cottoid lineage + Zoarcoidei characters to refute a scorpaeniform placement for the Trichodontidae (lack of parasphenoid–pterosphenoid junction, lack of a circular element of *transversus dorsalis anterior*, the absence of *adductors* I–III, and the lack of an extrinsic swimbladder muscle). However, the first three of these characters are not found in any members of the Cyclopteroidea (Imamura and Yabe, 2002; pers. obs.; Kido, 1988), which Yabe (1985) found as the sister-group of the Cottoidea. Because of the weak molecular support within the Cottoidei in our analysis, a potential sister-group relationship between the Cyclopteroidea and Trichodontidae should be examined further. It is important to note that the fourth cottoid lineage + zoarcoid character lacking in the Trichodontidae, the absence of an extrinsic swimbladder muscle, needs further attention. First, it is problematic to use the term "swimbladder" muscle for this character because Imamura and Yabe (2002) used the loss of a swimbladder to unite the Zoarcoidei + cottoid lineage, so the cottoid lineage should not be diagnosed further up the tree by the evolution of a swimbladder muscle without the subsequent gain/evolution of a swimbladder. Our preliminary examination of this muscle in a variety of percomorph taxa suggests that this muscle could be a second division of the *levator pectoralis* as described in *Batrachus* (Winterbottom, 1974; his Fig. 25). A more detailed examination of this muscle and its homology is currently being undertaken.

Despite the similarity of our molecular results with the morphological hypothesis presented by Shinohara (1994), our cottoid intrarelationships are not as congruent with the hypothesis presented by Yabe (1985). Some of the discrepancies between our results and the results presented by Yabe (1985) are due to his optimization of characters. As was described above for Ishida (1994), Yabe (1985) optimized characters by hand, so he did not find the shortest tree(s) for his data set. Instead, the consensus tree presented by Yabe (1985) had a length of 177 steps, and the resulting relationships were not among the most parsimonious trees found when we reanalyzed his morphological matrix in NONA. A reanalysis of Yabe's data set finds a significantly larger number of trees >10,000 with a length of 156 steps. The strict consensus of the first 10,000 trees resulting from our reanalysis of his data matrix is presented in Fig. 4A using his most plesiomorphic taxon, *Rhamphocottus*, to root the tree. As shown in the figure, little resolution is recovered from his data set, but when his characters are examined in light of our phylogenetic hypothesis, his characters support many of our proposed relationships.

Our analysis recovered a sister-group relationship between the Rhamphocottidae and Ereuniidae (C52) as the sister-group of the remaining Cottoidei (C53). This sister-group relationship between these two families is

supported by their shared elongate pectoral rays that are thickened and free of membrane, expansion of the head, and a single postocular spine in their larvae (Matarese et al., 1989; Okiyama, 1988). The remainder of the Cottoidei (C53) is supported by three of Yabe's (1985) characters: the loss of pharyngobranchial one (although present in *Jordania*; Yabe, 1985), the loss of the last distal pterygiophore, or "stay," (sensu Weitzman, 1962) of both the anal and dorsal fins, and the presence of the *rectus ventralis* connecting the urohyal and the third hypobranchial (Yabe, 1985; pers. obs.).

Within the Cottoidei, one of the well-supported clades (C57) supports the monophyly of the freshwater cottoids. This includes the placement of the Lake Baikal cottoids (*Asprocottus*, *Batrachocottus*, and *Comephorus*) within *Cottus.* Because this analysis and recent morphological (Sideleva, 1994) and molecular (Kontula et al., 2003) analyses have found this result, we recommend synonymizing Eschmeyer's (1998) Abyssocottidae, Comephoridae, and Cottocomephoridae with the Cottidae because they are nested within the type genus of the Cottidae (*Cottus*).

Another cottoid clade (C63), which is composed of members of Yabe's (1985) Psychrolutidae (C73), the hemitripterid (*Nautichthys*), and various cottids. There are no synapomorphies that unite this clade, but there are morphological characters grouping *Nautichthys* with the other cottids that are lacking in non-*Nautichthys* hemitripterids (e.g., a narrow vertical bridge crosses over the trigeminofacialis chamber and branched caudal rays; Taranets, 1941), contradicting Yabe's (1985) placement for *Nautichthys* in the Hemitripteridae.

The intrarelationships of the Cottoidei presented in our analysis are not well supported with the exception of a few nodes (e.g., *Cottus* + Lake Baikal cottoids, Cyclopteroidea, Psychrolutidae, *Hemilepidotus* spp.). All of these strongly supported nodes have morphological support. Many of the unconventional relationships resolved in our cottoid phylogeny are not well supported. These nodes are likely to be overturned by the addition of a comprehensive morphological data set. The weak support is due, in large part, to limited variation within the Cottoidei for the loci we sequenced for this project, which was designed to look at the monophyly of the Scorpaeniformes as a whole. Future molecular work examining the intrarelationships of the Cottoidei should include additional mitochondrial sequence data or faster evolving regions of the nuclear genome. Ideally, the next step in examining the intrarelationships of the Cottoidei would be to combine the morphological data presented in Yabe (1985) and other recent studies (e.g., Busby, 1998; Imamura and Yabe, 2002; Kendall and Vinter, 1984; Richardson, 1981; Shinohara, 1994; Washington et al., 1984) with an expanded molecular data set that includes additional cottoid lineage taxa, zoarcoids, and gasterosteoids as outgroups.

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