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Phylogeny and Taxonomy of Flatheads, Scorpionfishes, Sea Robins, and Stonefishes (Percomorpha: Scorpaeniformes) and the Evolution of the Lachrymal Saber

W. Leo Smith[1,2], Elizabeth Everman[2,3], and Clara Richardson[2]

We report on the discovery of a remarkable defensive specialization in stonefishes that was identified during a phylogenetic study of scorpionfishes and their relatives. This newly described innovation, the lachrymal saber, involves modifications to the circumorbitals, maxilla, *adductor mandibulae*, and associated tendons. At its core, the lachrymal saber is an elongation of an anterior spine (or spines) on the ventral surface of the lachrymal that stonefishes are capable of rotating from the standard ventral position to a locked lateral position. The locking mechanism minimally includes a bony spur on the inner surface of the lachrymal and a ridged bony protuberance on the anterolateral end of the maxilla. A modified and highly subdivided *adductor mandibulae* appears to control the movement of the lachrymal saber by rotating the maxilla while it is engaged with the spur on the medial side of the lachrymal. This maxillary rotation results in a subsequent rotation of the lachrymal that we hypothesize reduces predation on stonefishes. This specialization was included in our phylogenetic analysis of scorpaenoid fishes. This study expands upon the previous higher-level taxonomic sampling reported in earlier evolutionary studies of scorpaenoid fishes and, unlike previous analyses, explicitly combines molecular and morphological data with an expanded taxonomic sampling to mitigate the conflict between these competing datasets. The resulting phylogeny based on a combination of 113 morphological and 5,280 molecular characters for 63 species is used to produce a revised taxonomy of flatheads, scorpionfishes, sea robins, and stonefishes. Our results do not support the monophyly of the traditional Scorpaeniformes, Scorpaenoidei, Scorpaenoidea, Platycephaloidea, Bembridae, Scorpaenidae, Sebastidae, Serranidae, Tetrarogidae, or Triglidae. Our monophyletic taxonomy recognizes nine monophyletic families: Bembridae, Congiopodidae, Hoplichthyidae, Neosebastidae, Platycephalidae, Plectrogeniidae, Scorpaenidae, Synanceiidae, and Triglidae. The taxonomic composition of the Congiopodidae, Hoplichthyidae, Neosebastidae, and Platycephalidae are unchanged. The Bembridae is expanded to include the recently described Parabembridae, while *Bembradium* is moved to the Plectrogeniidae. The Scorpaenidae is expanded to include the traditional Sebastidae and Setarchidae. The Triglidae is expanded to include the Peristediidae. Finally, a revised Synanceiidae, diagnosed by the lachrymal saber, is expanded to include the Apistidae, Aploactinidae, Eschmeyeridae, Gnathanacanthidae, Pataecidae, Perryenidae, and Tetrarogidae. Based on these results, we recommend treating all of these traditional scorpaenoid clades as families in an expanded Scorpaeniformes that includes a restricted Scorpaenoidei that includes all traditional scorpaenoid families except the Congiopodidae. The resulting phylogeny is then used to explore aspects of scorpaenoid evolution.

DEFENSIVE specializations have altered the evolution of major radiations of organisms because of their role in competition and species survival (Vamosi, 2005; Bosher et al., 2006). In fishes, these innovations range from the evolution of poisons or venoms (Randall et al., 1971; Smith et al., 2016) and related Batesian mimicry (Casewell et al., 2017) to the repeated evolution of armor (Yang et al., 2013) and spines (Moser, 1981; Price et al., 2015). Anti-predator defenses obviously benefit the species that possess them, and these innovations have been implicated as causative agents in the diversification of some groups (Vamosi, 2005; Nagy et al., 2012). Herein, we report on a novel specialization in stonefishes that has a presumed defensive role. This feature, which we call the lachrymal saber, involves modifications to the circumorbitals, maxilla, *adductor mandibulae*, and associated tendons. This complex defensive morphological innovation was discovered during a study exploring the phylogeny and morphology of flatheads, scorpionfishes, sea robins, and stonefishes (Scorpaenoidea *sensu* Imamura, 2004) and has implications for the evolution of the Scorpaenoidei and the monophyly of the Synanceiidae.

The Scorpaenoidea represents a tremendous assemblage of mail-cheeked fishes with approximately 818 species (Eschmeyer et al., 2017) that has been classified in 6–20 families (e.g., Matsubara, 1943; Washington et al., 1984; Imamura and Shinohara, 1998; Mandrytza, 2001; Shinohara and Imamura, 2005; Eschmeyer et al., 2017). Over the last 30 years, there have been multiple attempts to resolve the phylogenetic relationships of the scorpaenoids and the placement of its included fishes among the mail-cheeked fishes using explicit morphological data (e.g., Washington et al., 1984; Ishida, 1994; Imamura, 1996, 2004; Mandrytza, 2001; Richards and Jones, 2002; Shinohara and Imamura, 2005; Ishii and Imamura, 2008; Kawai, 2008; Honma et al., 2013; Fig. 1). These morphological studies have provided phylogenies that are both iterative improvements building upon previous work as well as evolutionary hypotheses that are incongruent with preceding studies. In addition to these morphological phylogenies, there have been DNA-sequence-based explorations of the Scorpaenoidei or major groups within it (e.g., Smith and Wheeler, 2004, 2006; Hyde and Vetter, 2007; Smith and Craig, 2007; Lautredou et al., 2013; Portnoy et al., 2017; Fig. 2). These studies have not focused on the limits and relationships of Scorpaenoidei as a whole. However, they have supported some previous morphological hypotheses (e.g., placement of *Caracanthus* among scorpaenids; Shinohara and Imamura, 2005; Smith and Craig, 2007; Lautredou et al., 2013) and refuted others (e.g., placement of

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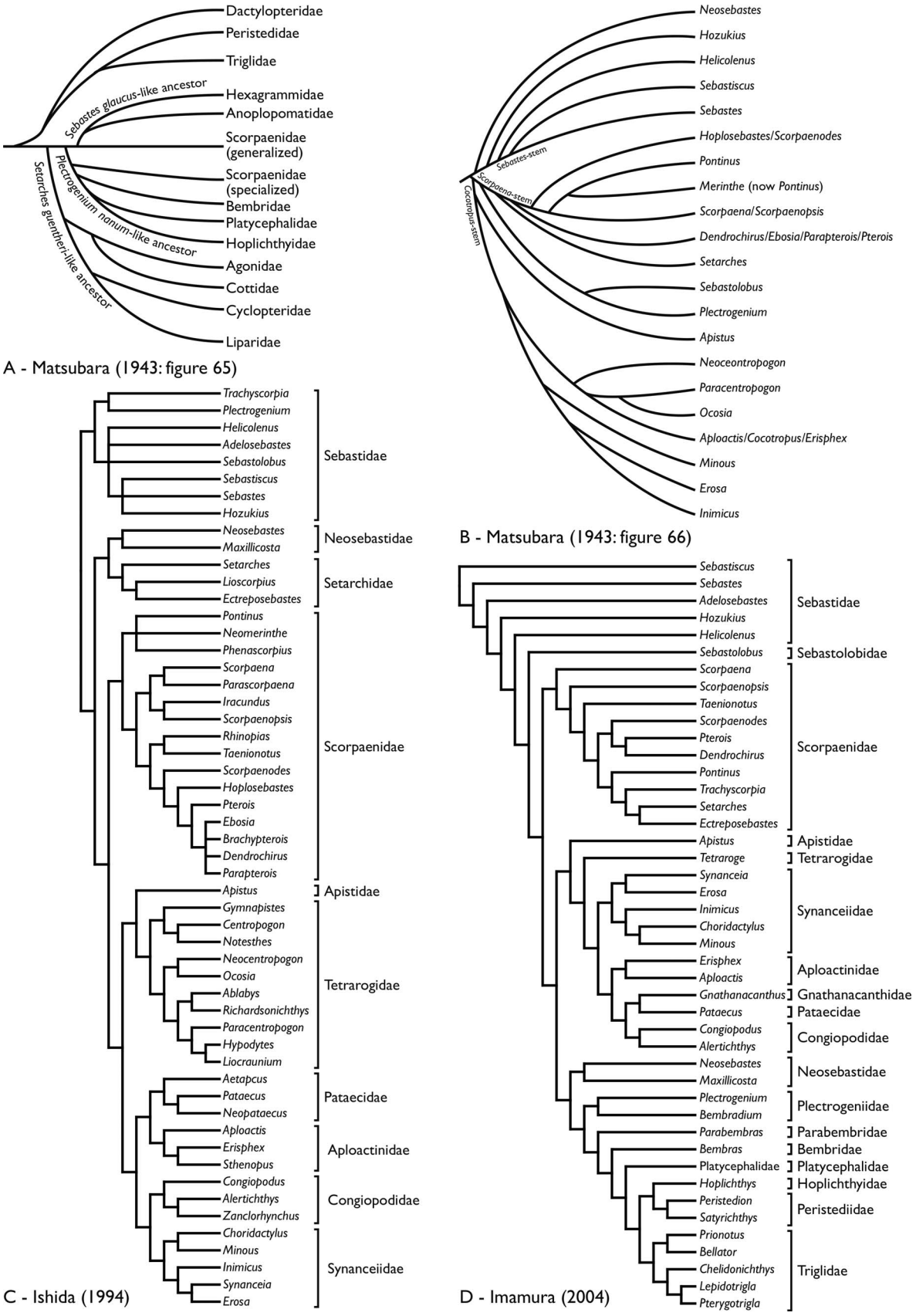

Fig. 1. Morphological hypotheses of inter- and intrafamilial relationships of the scorpaenoid lineage and allies: (A) traditional scorpaeniforms (Matsubara, 1943); (B) scorpionfishes and allies (Matsubara, 1943); (C) Scorpaenoidea (Imamura, 2004); (D) Scorpaenoidei (Ishida, 1994).

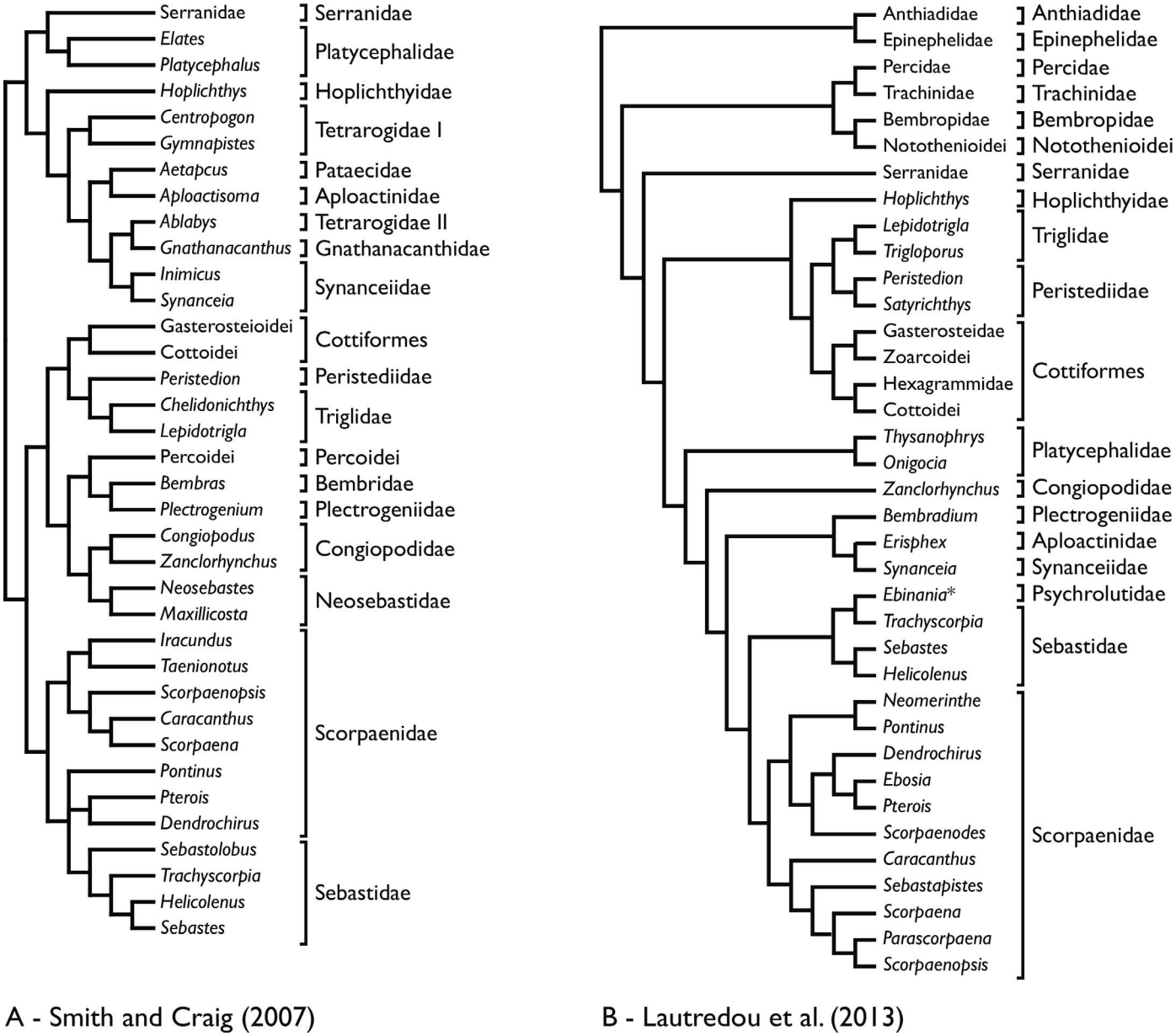

Fig. 2. Molecular hypotheses of inter- and intrafamilial relationships of the scorpaenoid lineage and allies: (A) Scorpaenoidei and allies (Smith and Craig, 2007); (B) traditional Scorpaeniformes and allies (Lautredou et al., 2013; * indicates probable problematic placement—see Smith and Busby [2014] for discussion).

Congiopodidae among the stonefishes [Ishida, 1994; Imamura, 2004] vs. outside of the stonefishes [Smith and Craig, 2007; Lautredou et al., 2013]). Differences between the results of morphological and molecular phylogenetic analyses are common (Smith, 2010; Wiley et al., 2011; Grande et al., 2013), but comparing differences among studies or datasets can often enable researchers to recognize or hypothesize previously undetected patterns of convergence and character loss (e.g., Wainwright et al., 2012; Stewart et al., 2014; Davis et al., 2016). These convergences are best explored in combined analyses, so we followed the lead of the morphological studies (Matsubara, 1943; Ishida, 1994; Imamura, 1996, 2004; Shinohara and Imamura, 2005; Honma et al., 2013; Fig. 1) and iteratively improved upon prior work by building on existing morphological data and adding species and new character data. We combined this expanded morphological dataset with a largely complementary molecular dataset to resolve conflict among the varied molecular and morphological phylogenies and generated a comprehensive family-level hypothesis of scorpaenoid relationships and corresponding monophyletic taxonomy.

MATERIALS AND METHODS

Taxon sampling.—Sixty-three taxa were analyzed in the current phylogenetic study. To provide a test of scorpaenoid monophyly and relationships, 12 outgroups were included: Anthiadidae, Bathymasteridae, Bembropidae, Bovichtidae, Cirrhitidae, Epinephelidae, Niphonidae, Percidae, Psychrolutidae, Serranidae, and Trachinidae. The topology was rooted with *Cirrhitus* (Centrarchiformes), which has long been closely allied with, but excluded from, the mail-cheeked fishes (Gill, 1888; Smith and Wheeler, 2004; Near et al., 2013). The 51 traditional scorpaenoid terminals analyzed in this study included all 20 scorpaenoid families (clade *sensu* Imamura, 2004; classification *sensu* Eschmeyer et al., 2017).

Table 1. Primers, PCR conditions, and substitution models for each amplicon analyzed in the current study.

Primer name (source)	Primer sequence	Primary annealing temperature (°C)
tRNA-Val-16S (Titus, 1992; Feller and Hedges, 1998)—whole amplicon with 16Sar-br: 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 with tRNA-Val-16S: GTR+I+G		
16S ar-L	5′–CGCCTGTTTATCAAAAACAT–3′	48
16S br-H	5′–CCGGTCTGAACTCAGATCACGT–3′	48
COI (Folmer et al., 1994)—1st Pos.: GTR+I+G; 2nd Pos.: TVM+I; 3rd Pos.: GTR+I+G		
LCO1490	5′–GGTCAACAAATCATAAAGATATTGG–3′	46
HCO2198	5′–TAAACTTCAGGGTGACCAAAAAATCA–3′	46
28S (Hillis and Dixon, 1991)—whole amplicon: GTR+I+G		
28SV	5′–AAGGTAGCCAAATGCCTCGTCATC–3′	48
28SJJ	5′–AGGTTAGTTTTACCCTACT–3′	48
Histone H3 (Colgan et al., 1998)—1st Pos.: K3Puf+I; 2nd Pos.: JC+I; 3rd Pos.: HKY+G		
H3a-L	5′– ATGGCTCGTACCAAGCAGACVGC–3′	48
H3b-H	5′– ATATCCTTRGGCATRATRGTGAC–3′	48
TMO-4c4 (Streelman and Karl, 1997)—1st Pos.: TRN+I+G; 2nd Pos.: TVM+I+G; 3rd Pos.: TVM+G		
TMO-f1	5′–CCTCCGGCCTTCCTAAAACCTCTC–3′	51
TMO-r1	5′–CATCGTGCTCCTGGGTGACAAAGT–3′	51
Glyt (Li et al., 2007)—1st Pos.: K3Puf+I+G; 2nd Pos.: K3Puf+I+G; 3rd Pos.: TVM+I+G		
Glyt_F559	5′–GGACTGTCMAAGATGACCACMT–3′	57/55
Glyt_R1562	5′–CCCAAGAGGTTCTTGTTRAAGAT–3′	57/55
zic1 (Li et al., 2007)—1st Pos.: TVM+G; 2nd Pos.: K3Puf+I; 3rd Pos.: TIM+G		
zic1_F9	5′–GGACGCAGGACCGCARTAYC–3′	58/57
zic1_R967	5′–CTGTGTGTGTCCTTTTGTGRATYTT–3′	58/57

Character sampling.—A total of 5,393 morphological and molecular characters were scored. These data included 5,280 aligned nucleotides from five nuclear and three mitochondrial loci. Previous studies have shown the effectiveness of the included molecular loci for resolving relationships among percomorphs generally or mail-cheeked fishes specifically (e.g., Chakrabarty et al., 2011a, 2011b; Li et al., 2011; Near et al., 2012a, 2012b; Girard and Smith, 2016). The molecular terminals analyzed in the present study and GenBank accession numbers corresponding to the gene fragments sequenced are listed in Supplemental Table 1 (see Data Accessibility). For these analyses, the 134 novel DNA sequences were combined with previously published DNA sequences from the following sources: Smith and Wheeler (2004, 2006); Smith and Craig (2007); Smith et al. (2009, 2016); Near et al. (2012a, 2013, 2015); Wainwright et al. (2012). The molecular matrix was 90% complete at the amplicon level and 82% complete at the cell or individual-base-pair level (Supplemental Table 1; see Data Accessibility). These molecular data were simultaneously analyzed with a morphological dataset (Supplemental Table 2; see Data Accessibility; Appendix 1) composed of 113 characters that was built from multiple sources, but focused on the work of Imamura (2004). Details about additional sources of morphological data are listed along with all character descriptions in Appendix 1. The morphological matrix was 92% complete at the cell or individual character level (Supplemental Table 2; see Data Accessibility).

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). The polymerase chain reaction (PCR) 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), 1.25 μL of each primer (10 pmol), and 2–5 μL of undiluted DNA extract. Primers and primer sources are listed in Table 1. Amplifications for all novel fragments except Glyt and zic1 were carried out using the following temperature profile: initial denaturation for 360 sec at 94°C; 36 cycles of denaturation for 60 sec at 94°C, annealing for 60 sec at 46–51°C (see Table 1 for primary annealing temperature for each locus), and extension for 75 sec at 72°C; with a final terminal extension for 360 sec at 72°C. For Glyt and zic1, the following temperature profile was used: initial denaturation for 180 sec at 94°C; ten cycles of denaturation for 45 sec at 94°C, annealing for 45 sec at 57–58°C (see Table 1 for core annealing temperature for each locus), and extension for 75 sec at 72°C; 30 cycles of denaturation for 45 sec at 94°C, annealing for 30 sec at 55–57°C (see Table 1 for secondary annealing temperature for each locus), and extension for 75 sec at 72°C, with a final terminal extension for 360 sec at 72°C. The double-stranded amplification products were desalted and concentrated using AMPure (Beckman Coulter). 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 v1.1 (Applied Biosystems) with minor modifications to the manufacturer's protocols. The sequencing reactions were cleaned and desalted using cleanSEQ (Beckman Coulter). The nucleotides were sequenced and the base pairs were called on a 3730 automated DNA sequencer (Applied Biosystems) or by Beckman Coulter Genomics (Danvers, MA). Contigs were built in Geneious v8.1.8 (Kearse et al., 2012) using DNA sequences from the complementary heavy and light strands. Sequences were edited in Geneious and collated into fasta text files. The novel sequences were submitted to GenBank and assigned accession numbers MF966393–MF966401 and MF991301–MF991425.

Morphological investigation.—Ethanol preserved, cleared and stained, and dried skeletal material was examined using several stereomicroscopes with varying magnification and lighting regimes. Photographs documenting the anatomy of specimens were taken under normal visible lighting with either a Nikon D800 with an AF-S VR Micro-NIKKOR 105mm f/2.8G IF-ED lens or a Lumenera INFINITY2-5 digital CCD camera attached to a Nikon SMZ-18 stereomicroscope. Fluorescent images were taken either using the D800 with lighting from two NightSea BlueStar flashlights (Lexington, MA) and filtered through the Nikon light-shading plate from the Nikon SMZ-18 microscope or using the Nikon SMZ-18 stereomicroscope with either a Nikon P2-EFL GFP-B or P2-ELF RFP filter cube. Drawings of specimens were aided with a camera lucida arm attachment.

Phylogenetic analyses.—A likelihood analysis was used to analyze the molecular and morphological data. For this analysis, each of the eight amplicons was aligned individually in MAFFT v7.017 (Katoh et al., 2002) using default values. The maximum-likelihood dataset was broken into 18 partitions. Two partitions were designated for the mitochondrial (12S, tRNA-Val-16S, and 16Sar-br) and nuclear (28S) ribosomal fragments. Fifteen partitions covered the three codon positions in each of the five protein-coding genes: mitochondrial (cytochrome oxidase I) and nuclear (Glyt, histone H3, TMO-4c4, and zic1). The 18th partition was the morphological dataset (Supplemental Table 2; see Data Accessibility). The optimal nucleotide substitution model for each molecular partition was determined empirically (Table 1) by comparing different models under an Akaike information criterion as executed in jModelTest v0.1 (Posada, 2008), and the morphological model was set to Mkv (Lewis, 2001). The datasets were coded, concatenated, examined, and analyzed (ancestral-state reconstructions) in Mesquite v3.04 (Maddison and Maddison, 2017). Maximum-likelihood analyses were conducted in GARLI v2.01 (Zwickl, 2006). The tree with the best likelihood score from 40 independent analyses was selected as the preferred hypothesis. A non-parametric maximum-likelihood bootstrap analysis was conducted for 200 random pseudoreplicates to assess nodal support. For the support analysis, terminals represented solely by morphological data were excluded (*Eschmeyer* and *Perryena*) because they have excessive missing data in the combined analysis; the optimal tree with these terminals excluded was otherwise identical to the complete phylogeny (results not shown). We recognize two levels of nodal support: $\geq$70% bootstrap support represents a moderately supported node or clade and $\geq$95% bootstrap support represents a well-supported node or clade.

RESULTS

Phylogenetic analyses.—The likelihood analysis resulted in a single optimal tree (Fig. 3). Most of the 59 nodes recovered in the bootstrap analysis (analysis excluding *Eschmeyer* and *Perryena*) were moderately to well supported with 36 (61%) nodes being moderately supported by a bootstrap value $\geq$70 and 24 (41%) nodes being well supported by a bootstrap value $\geq$95. In this analysis, the traditional cottoid and zoarcoid representatives were nested within the Scorpaenoidea (*sensu* Imamura, 2004), sister to the Congiopodidae. This congiopodid + zoarcoid + cottoid clade was recovered as the sister group to all non-congiopodid scorpaenoid fishes. Many traditionally recognized family-level clades (*sensu* Eschmeyer et al., 2017) that were represented by more than a single terminal were recovered as monophyletic: Congiopodidae, Neosebastidae, Pataecidae, Peristediidae, Platycephalidae, Setarchidae, and Synanceiidae. In contrast, the Bembridae, Scorpaenidae, Sebastidae, Tetrarogidae, and Triglidae were recovered as para- or polyphyletic. As has been seen in previous morphological and molecular analyses, the core scorpionfishes included *Caracanthus* (Smith and Wheeler, 2004, 2006; Shinohara and Imamura, 2005; Smith and Craig, 2007; Lautredou et al., 2013) and the traditional Setarchidae (Imamura, 2004; Smith and Wheeler, 2004, 2006; Shinohara and Imamura, 2005). Similarly, hoplichthyids were recovered as more closely related to the Triglidae + Peristediidae than to platycephalids (see also Imamura, 1996, 2004; Lautredou et al., 2013). In contrast to morphological phylogenies (Ishida, 1994; Imamura, 2004) and some molecular phylogenies (Lautredou et al., 2013), but in agreement with other molecular phylogenies (e.g., Smith and Wheeler, 2004, 2006), the live-bearing rockfishes (e.g., *Helicolenus*, *Sebastes*) were nested deeply in the scorpionfishes as opposed to a stem grade. Despite these similarities to other studies, the combined analysis of morphological and molecular data is frequently in conflict with the traditional taxonomy and prior phylogenetic work. The traditional Peristediidae was nested within the Triglidae (see also Portnoy et al., 2017), *Bembradium* was allied with *Plectrogenium* (see also Imamura, 1996, 2004), Tetrarogidae was widely polyphyletic within a "stonefish" clade (see also Mandrytza, 2001; Smith and Wheeler, 2004; Honma et al., 2013), and representatives of the Scorpaenidae and Sebastidae were variously distributed within a "scorpionfish" clade. If we compare our results to a recent classification of fishes (Betancur-R. et al., 2017), we note that their Percoidei, Platycephaloidei, Serranoidei, Scorpaenidae, Serranidae, Tetrarogidae, and Triglidae were recovered as polyphyletic. Their Scorpaenoidei was the only relevant higher-level grouping with multiple families that was recovered as monophyletic in our analysis. The differences between our results and previous hypotheses necessitate the familial taxonomic revisions presented herein (Fig. 3; Appendix 2). All of the remaining results and discussion below will use the revised classification unless noted otherwise.

Description of the lachrymal saber.—The lachrymal saber is a complex feature that involves a series of modifications to the circumorbitals, maxilla, *adductor mandibulae*, and associated tendons. Among mail-cheeked fishes, there are some modifications to these lachrymal-saber elements (e.g., *adductor mandibulae* variation as illustrated by Yabe [1985] and Mandrytza [1990]), but we treat this element as a single feature in this study. Future studies looking broadly at the phylogeny of mail-cheeked fishes should consider the historical independence of several aspects of the lachrymal saber to assess their individual phylogenetic significance. For example, many triglids have additional tendinous connections between the *adductor mandibulae* and maxilla that appear anatomically similar and potentially convergent with some synanceiids while lacking the remainder of the lachrymal-saber modifications (Yabe, 1985; Mandrytza, 1990; pers. obs.).

As expected, the circumorbitals of synanceiids lie lateral to the suspensorium (Figs. 4, 5). As with most mail-cheeked fishes, the third circumorbital is firmly attached to the preopercle posteriorly forming the characteristic suborbital stay. The circumorbitals are attached anteroventrally to the

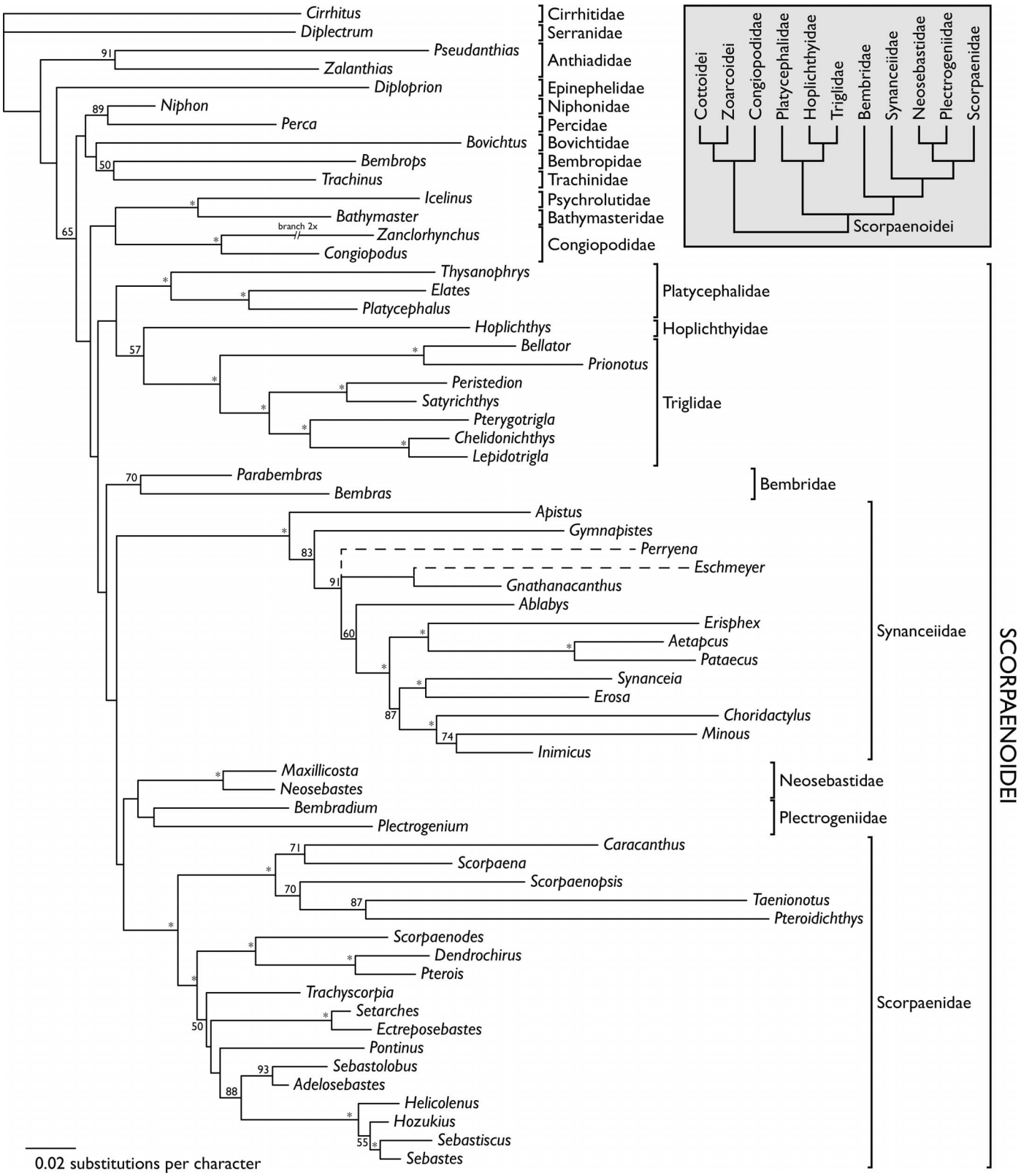

Fig. 3. Optimal cladogram resulting from the partitioned likelihood analysis of the dataset composed of 113 phenotypic and 5,280 nucleotide characters. Clades with 50% bootstrap support are retained and identified with their support. Nodes with bootstrap support of 95% were marked with an "*". Family-level classification is designated on the right. Dashed branches indicate terminals that were represented only by morphological data that were excluded from the bootstrap analyses. Family-level-phylogeny of scorpaenoids and their sister group in gray box in upper-right corner.

maxilla and anterodorsally to the lateral ethmoid via the lachrymal. Both anterior lachrymal attachments have substantive soft-connective tissue, which enables the necessary rotation of the lachrymal saber while also holding the bones in close association. The circumorbitals of mail-cheeked fishes frequently have elongations of their lateral-line canal pores that create the group's characteristic bony spines and knobs (Smith, 2005). In all species with a lachrymal saber,

the ventral spines associated with the second pore on the first circumorbital (lachrymal) are enlarged (Figs. 4–7). As noted in revisions and artificial keys separating various mail-cheeked fishes (e.g., Poss, 1999), the lachrymal is highly mobile and hinged to the lateral ethmoid dorsally in synanceiids. As the element is relatively free to rotate, it abuts, but is not as firmly bound to, the second circumorbital as is otherwise common among scorpaenoid fishes. This freedom allows for the rotation of this element such that the typical ventral lachrymal spines, including the larger spine, can be rotated laterally and projected outward making the "saber" (Fig. 5).

The lachrymal saber can be held in an outwardly projected or lateral position by a locking mechanism that functions somewhat like a ratchet and pawl that relies on the friction between highly textured (often ridged) modifications on both the medial surface of the lachrymal and the lateral surface of the maxilla. These modifications include a bony spur that ranges from a peg to a cup on the inner surface of the lachrymal (Fig. 6A) and a ridged bony protuberance on the anterolateral end of the maxilla (Fig. 6B). The lachrymal's medial projection acts as the pawl when the saber is extended laterally. This lachrymal pawl, when in a locked position, is tightly connected to a highly sculptured protuberance on the anterior end of the maxilla that acts somewhat like a ratchet allowing the lachrymal to be held firmly in place (Fig. 7). While the lachrymal is free to rotate, its motion is highly constrained by a notable amount of soft connective tissue.

Manipulations of ethanol preserved and cleared and stained specimens indicate that the lachrymal saber is likely rotated and controlled by movements of the maxilla. The maxilla can be rotated by contractions of the various subunits of the *adductor mandibulae* A_1 division that have diversified among synanceiids. These separate muscle subdivisions can facilitate the engagement of the locking mechanism as well as outward rotation of the lachrymal (Figs. 4, 5) and, to a lesser degree, the second circumorbital (Fig. 4B). The number, size, and placement of *adductor mandibulae* A_1 division subunits and their associated tendinous connections vary tremendously among synanceiids (Fig. 8; Mandrytza, 1990). The traditional *adductor mandibulae* A_1 division in synanceiids has between two and five discernible subunits that variously originate caudally from the preopercle, the suborbital stay of the third circumorbital, the ventral surface of the third circumorbital, and/or the ventral surface of the second circumorbital. These muscles attach anteriorly to the maxilla via a diversity of tendons (Figs. 7, 8; Yabe, 1985; Mandrytza, 1990; Ishida, 1994). As these tendons can be homologous with ligaments in other fish groups (Johnson and Patterson, 2001; Datovo and Vari, 2013), individual representatives of these elements are often referred to as ligaments. As emphasized in Yabe (1985) and Mandrytza (1990), the proliferation of *adductor mandibulae* A_1 division subunits and their associated tendons/ligaments in synanceiids requires a more taxonomically comprehensive study across percomorphs generally and scorpaenoids specifically before the specific homology of the elements can be determined.

Many aspects of the lachrymal saber vary: the number of *adductor mandibulae* A_1 division subunits, the caudal origination locations of the *adductor mandibulae* A_1 division subunits, the number and placement of tendons inserting on the maxilla, the length of the lachrymal spines, and the shape and size of the lachrymal and maxillary components of the locking mechanisms. Despite this substantial variation, all of the synanceiids have the core modifications to the circumorbitals, maxilla, *adductor mandibulae*, and associated tendons that constitute this novel defensive mechanism.

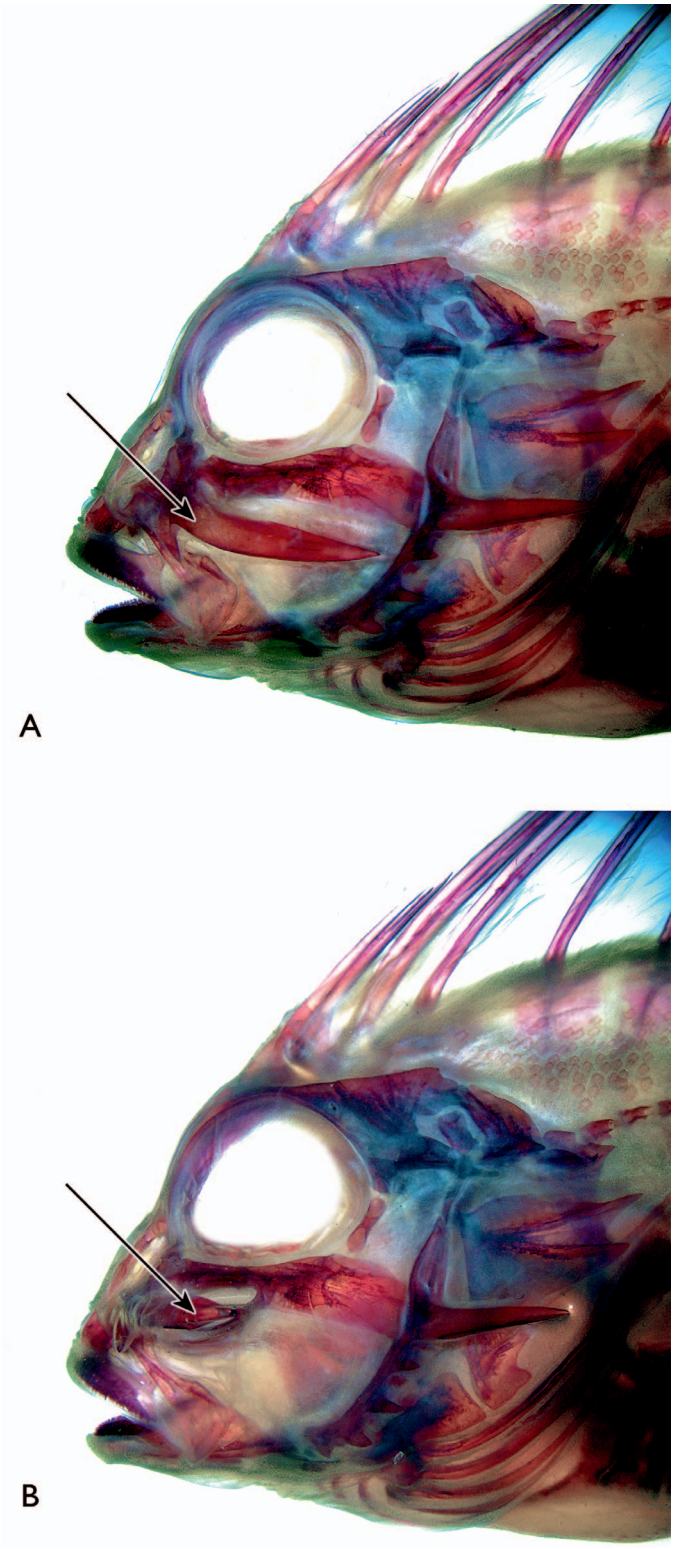

Fig. 4. Lateral view of a cleared-and-stained specimen of the synanceiid *Paracentropogon,* CAS_SU 68769. Images highlight the (A) resting position of the lachrymal saber (arrow) along the side of the waspfish's cheek and the (B) locked-out position where the lachrymal saber extends laterally from the specimen. The rotation of both the first and second circumorbital are visible in the lower image.

Fig. 5. Lateral (A), rostral (B), and dorsal (C) views of the lachrymal saber in the Soldierfish (*Gymnapistes marmoratus,* AMNH 31009). Arrow highlights both the resting and locked lachrymal saber in the various angles.

In addition to anatomical investigations using typical LED "daylight" lighting, one specimen was examined under fluorescence (Synanceiidae: *Centropogon australis*; Fig. 9). The lachrymal saber in this species biofluoresced in the green spectrum (Fig. 9B), whereas other regions of the head fluoresced red (Fig. 9C). Compared to visible light (Fig. 9A), stereomicroscopic images using fluorescence (Fig. 9B) highlight regions associated with the lachrymal saber that fluoresce in the green spectrum. Additional inspection (Fig. 9C) illuminated with two NightSea BlueStar flashlights and filtered through the Nikon light-shading plate highlights both the green lachrymal saber element and the red fluorescence associated with eye rings in this species of stonefish. Considerably more species need to be examined to assess the diversity of species that have biofluorescent lachrymal sabers or even whether additional species have this visual modification.

DISCUSSION

This study was designed to look at the limits and relationships of the Scorpaenoidea (*sensu* Imamura, 2004). In particular, we focused on the interrelationships of genera that were recovered in different phylogenetic positions in earlier studies (i.e., *Bembradium*, *Congiopodus, Ectreposebastes, Helicolenus, Hoplichthys, Hozukius, Plectrogenium, Sebastes, Sebastiscus, Sebastolobus, Setarches, Trachyscorpia,* and *Zanclorhynchus*; Figs. 1, 2). Beyond scorpaenoid generic relationships, our results provide an opportunity to re-examine the limits of the scorpionfishes generally. Imamura and Yabe (2002) grouped their Scorpaenoidea with the Serranidae (*sensu* Nelson, 2006) independent of other traditional perciform or scorpaeniform fishes. Our results (Fig. 3) did not recover this hypothesized clade or a monophyletic Serranidae or Scorpaenoidea. We recovered a polyphyletic Serranidae (*sensu* Johnson, 1983), which corroborates the findings of all recent studies that have sampled multiple subfamilies of traditional serranids and/or *Acanthistius* and *Niphon* (e.g., Smith and Craig, 2007; Lautredou et al., 2013; Near et al., 2013, 2015). Imamura and Yabe (2002) and Imamura (2004) expanded our understanding of the Scorpaenoidea over previous studies (e.g., Matsubara, 1943; Washington et al., 1984; Ishida, 1994) and diagnosed their Scorpaenoidea by a single postocular spine in larvae, an extrinsic gas bladder muscle derived from the *obliquus superioris*, a parietal lateral-line canal with spine, and a suborbital stay. Imamura (2004) and the current study did not broadly examine the larval spine feature across all scorpaenoid fishes. The postocular spine was not included in the current analysis because its distribution is insufficiently known across scorpaenoids. In

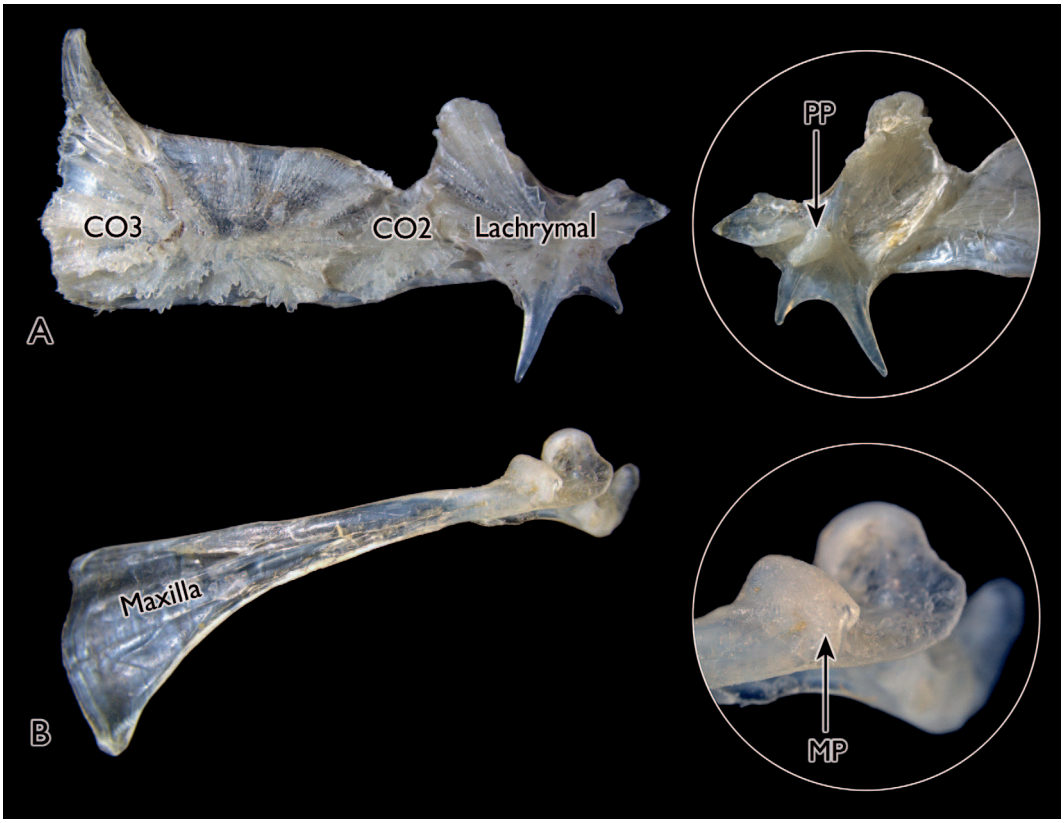

Fig. 6. Skeletal images of the key lachrymal saber components. (A) Lateral image of the lachrymal, second circumorbital (CO2), and third circumorbital (CO3) in *Minous quincarinatus,* KUI 41397. Circular inset of upper image shows medial view with medial protuberance (PP). (B) Lateral image of the maxilla in *Apistus carinatus,* KUI 41400. Circular inset of lower image shows closeup of the maxillary protuberance (MP). These two protuberances interact to lock the lachrymal saber.

our analysis, the most recent common ancestor (MRCA) of all traditional scorpaenoids included the representative cottoid and zoarcoid taxa. This larger MRCA is diagnosed by four unambiguous synapomorphies that include two of the three previously identified scorpaenoid characters: a suborbital stay with a broad distal end that is strongly connected to the preoperculum (character 8, state 3) and an extrinsic muscle that was connected to the neurocranium anteriorly and vertebrate posteriorly (character 101, state 2) and two additional characters: the reduction of supraneurals to zero (character 65, state 3) and the fusion of hypurals one and two (character 68, state 1). A more restricted scorpaenoid MRCA that excludes the congiopodids, cottoids, and zoarcoids is herein recognized as a revised Scorpaenoidei that is diagnosed by the presence of a parietal lateral-line canal with a spine (character 24, state 1).

Congiopodidae.—The Congiopodidae is a small Southern Hemisphere endemic family that has been recovered in a diversity of phylogenetic placements (Ishii and Imamura, 2008). Traditional higher-level phylogenetic studies (e.g., Regan, 1913; Greenwood et al., 1966) placed the congiopodids among the mail-cheeked fishes in their own order or suborder. Using morphological data and explicit phylogenetic analyses (Fig. 1), Ishida (1994) placed congiopodids sister to Synanceiidae (*sensu* Imamura, 2004) using five shared myological features and Imamura (2004) placed congiopodids sister to a clade composed of Gnathanacanthidae and Pataecidae (both *sensu* Imamura, 2004) using preopercular spination, the loss of the uroneural, and the separation of the *transversus ventralis* posterior. In contrast, Mandrytza (2001) recognized a congiopodoid clade (composed of a separate Congiopodidae and Zanclorhynchidae equivalent to our Congiopodidae) independent of his Scorpaenoidea. Mandrytza (2001) presented a variety of results, but his various explicit morphological analyses either allied his Congiopodoidei with a restricted Cottoidei (not including or associated with the Anoplopomatoidei, Hexagrammoidei, and Zoarcoidei) or in a polytomy with Anoplopomatoidei, Cottoidei + Hexagrammoidei, Hoplichthyoidei, Normanichthyoidei, and Scorpaenoidei (all *sensu* Mandrytza, 2001). Mandrytza (2001) also provided evidence that *Perryena* was not a congiopodid; instead, he suggested that this genus was a member of the Tetrarogidae (*sensu* Mandrytza, 2001). This finding was largely corroborated in a morphological analysis of a subset of scorpaenoids by Honma et al. (2013) who created a new family, Perryenidae, for this genus because of the non-monophyly of their two putative tetrarogids (*Perryena* and *Tetraroge*) included in their analysis. Earlier analyses of DNA-sequence data have resulted in even more hypotheses than the diversity of morphological studies. Smith and Wheeler (2004) recovered a separate *Congiopodus* (sister to Neosebastidae) and *Zanclorhynchus* (sister to the Notothenioidei). Li et al. (2009) recovered *Zanclorhynchus* sister to all other scorpaeniform fishes. Smith and Wheeler (2006) recovered *Congiopodus* sister to Bembridae. Smith and Craig (2007; Fig. 2A) recovered a clade composed of *Congiopodus* + *Zanclorhynchus* sister to Neosebastidae. Lautredou et al. (2013; Fig. 2B) recovered *Zanclorhynchus* sister to a clade composed of Plectrogeniidae + Scorpaenidae + Synanceiidae. Near et al. (2015) recovered a clade composed of *Congiopodus* + *Zanclorhynchus* sister to a clade composed of Bembridae,

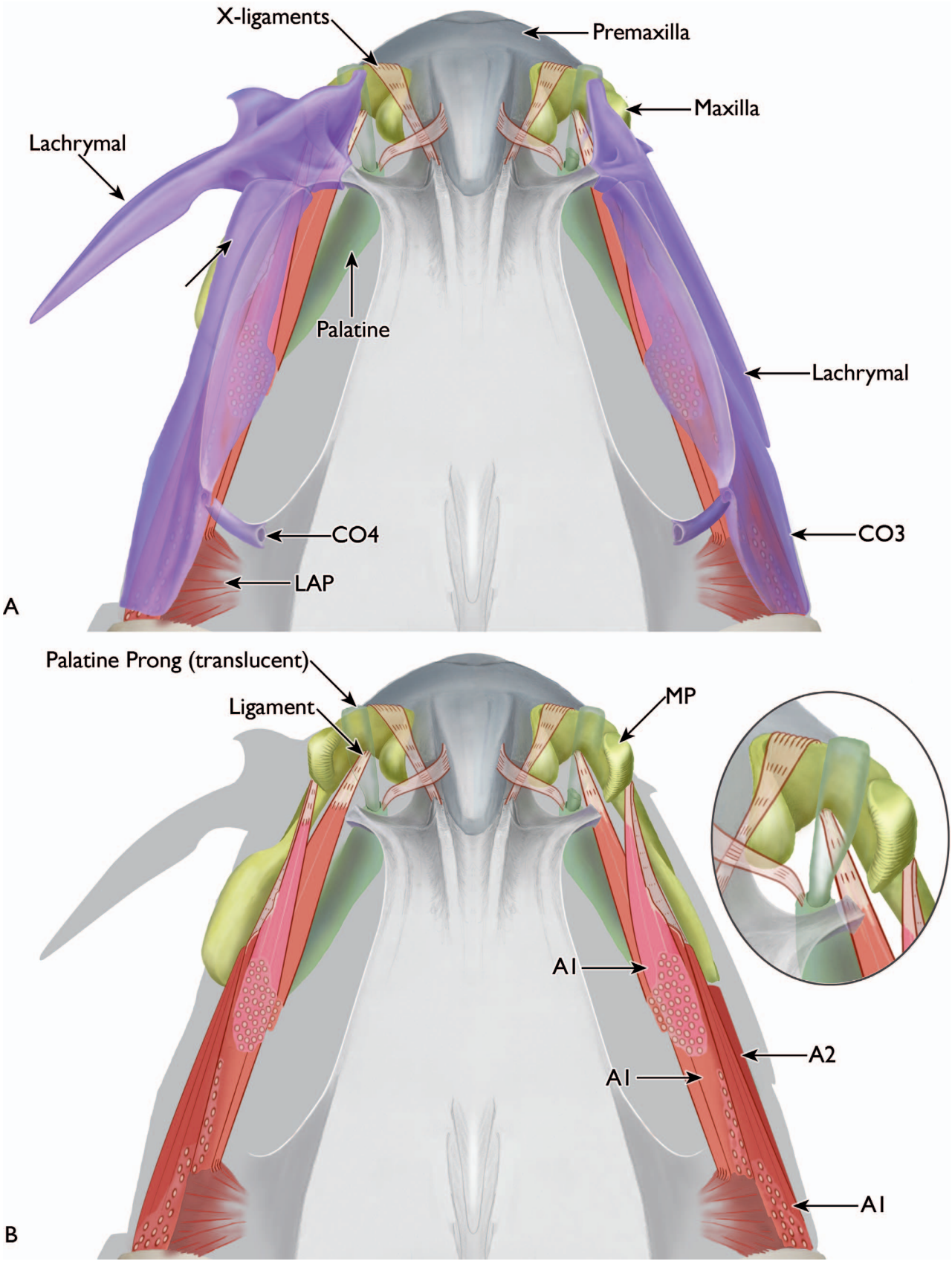

Fig. 7. Composite dorsal images of various specimens of *Paracentropogon* highlighting the morphology of the components of the lachrymal saber with the locked position on the left side and the resting position on the right side. Note that the anteriormost component of the palatine has been made largely transparent in the images to allow for a better examination of the maxilla. The upper image (A) represents the anatomy with all elements marked, and the lower image (B) represents the image with the circumorbitals shadowed to visualize underlying muscles. Muscle attachments on removed bones are denoted with pale circles at the interface. Abbreviations: A1 or A2 represent the major subdivisions of the *adductor mandibulae*; CO3–circumorbital 3; CO4–circumorbital 4; LAP–*levator arcus palatini*; MP–maxillary protuberance. Illustration by Clara Richardson.

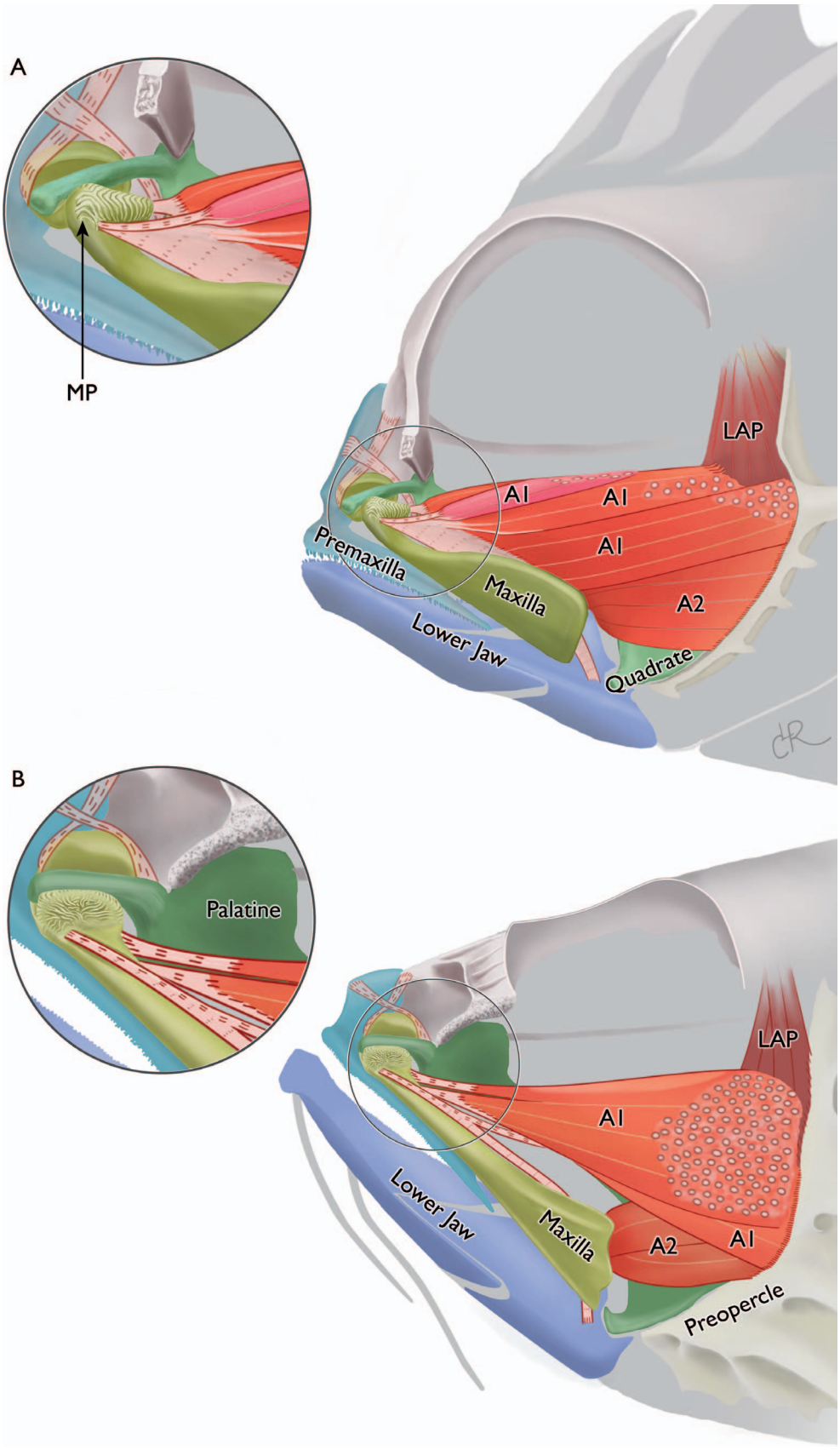

Fig. 8. Lateral view of the non-circumorbital components of the lachrymal saber in composite images of the various specimens of *Paracentropogon* above (A) and the various specimens of *Apistus* below (B). The comparisons are shown to illustrate variation in this system between species (e.g., subdivisions and attachment points of the *adductor mandibulae*). Muscle attachments on removed bones are denoted with pale circles at the interface. Abbreviations: A1 or A2 represent the major subdivisions of the *adductor mandibulae*; LAP–*levator arcus palatini;* MP–maxillary protuberance. Illustration by Clara Richardson.

Bembropidae, and Scorpaenidae. Finally, Smith et al. (2016) recovered a clade composed of *Congiopodus* + *Zanclorhynchus* sister to a clade composed of the Plectrogeniidae + Scorpaenidae + Synanceiidae. We recover (Fig. 3) the Congiopodidae sister to a clade composed of the cottoids + zoarcoids, which is most similar to the findings of Mandrytza (2001). Our results corroborate the findings of Mandrytza (2001) and Honma et al. (2013) that separate *Perryena* from other traditional tetrarogids. Our sister-group relationship between congiopodids and cottoids + zoarcoids is supported by the

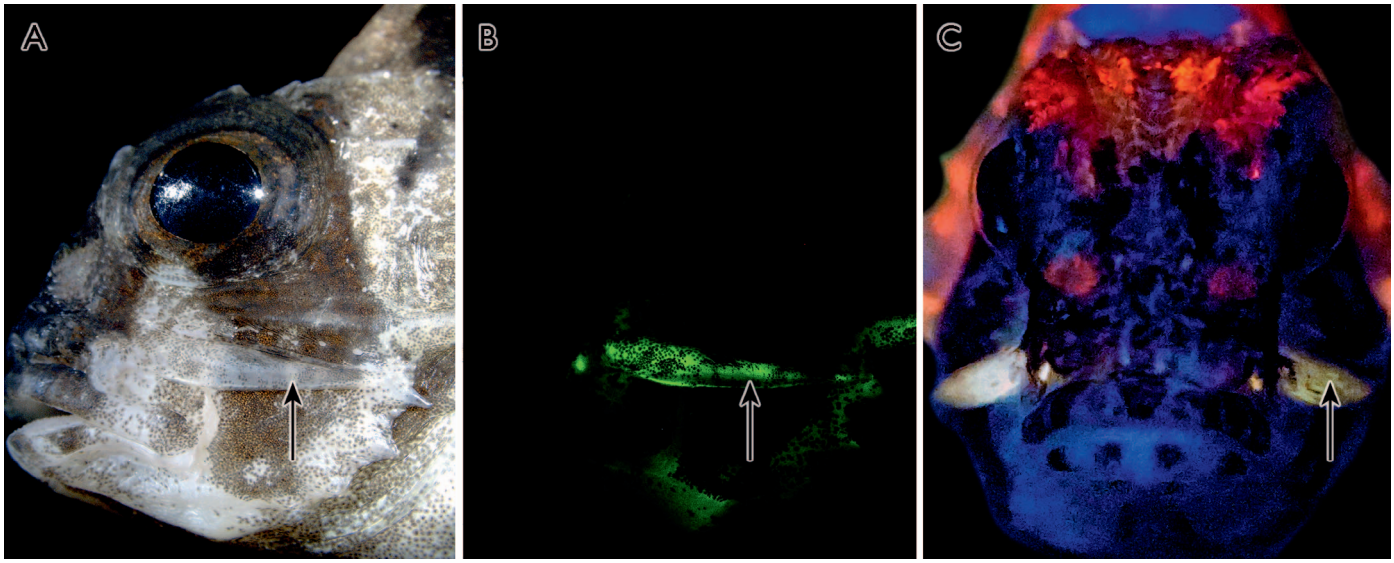

Fig. 9. Lateral (A and B) and rostral (C) images of *Centropogon australis,* KUI 41409. (A) Image represents a visible light image of *Centropogon* with the lachrymal saber in the resting position. (B) Image represents the identical placement of the image in panel A under fluorescent light with GFP filter under the Nikon SMZ-18 microscope. This image shows the bright green biofluorescence visible on the lachrymal saber. (C) Image shows a rostral view of the same individual under NightSea BlueStar flashlight illumination and the light-shading plate from the Nikon SMZ-18, which is not as restricted as the microscope filter. In this image, the green (lachrymal saber) and orange (dorsal surface of head) fluorescent emissions are visible in the waspfish.

loss of the metapterygoid lamina (character 32, state 1), the loss of one branchiostegal ray (character 37, state 1), the loss of a tooth plate on the third epibranchial (character 40, state 1), the separation of the lateral extrascapular into two elements (character 45, state 1), the fusion of the lower hypural plate and the parhypural (character 71, state 1), the origin of the *levator operculi* on the pterotic and posttemporal (character 83, state 1), the presence of *adductores* I–III (character 91, state 1), and the *obliquus superioris* not extending to the neurocranium (character 103, state 1). The overwhelming majority of explicit analyses and our results recover a monophyletic Congiopodidae that includes *Zanclorhynchus*; we recover 12 characters supporting the monophyly of this family (Appendix 2). Further, most recent studies separate the Congiopodidae from the Synanceiidae. The placement of this clade is inconsistently recovered across studies, and it remains difficult to know whether the family is more allied with the cottoid or scorpaenoid components of the scorpaeniform tree. We recommend treating this clade as a separate family within its own suborder Congiopodoidei.

Bembridae, Hoplichthyidae, Platycephalidae, and Triglidae.— Traditionally, the Bembridae, Hoplichthyidae, and Platycephalidae have been treated as a single evolutionary unit (Matsubara, 1943; Washington et al., 1984; Fig. 1). As first noted by Imamura (1996), explicit analyses of "platycephaloid" relationships do not recover bembrids in a clade with the hoplichthyids and platycephalids. Instead, Imamura's (1996) study demonstrated that Platycephalidae was sister to a clade of Hoplichthyidae + Triglidae. These three families were then hypothesized to be sister to *Bembras*, and that clade was subsequently hypothesized to be sister to *Parabembras* (Imamura, 1996; Fig. 1). Our results support Imamura's (1996) hypothesis that Platycephalidae is sister to a clade composed of Hoplichthyidae + Triglidae. However, our results place this Hoplichthyidae + Platycephalidae + Triglidae clade sister to all other scorpaenoids. This relationship supports the view that the less flattened bembrids are more closely related to scorpionfishes and stonefishes than to hoplichthyids, platycephalids, and triglids.

The Bembridae is a small, deep-water Indo-Pacific marine family whose limits have varied across studies. Jordan and Hubbs (1925) first separated the Parabembridae from the Bembridae, but this separation was not followed in most subsequent studies. For example, Washington et al. (1984) and Nelson (2006) included *Bambradon, Bembradium, Bembras, Brachybembras* (not discussed in Washington et al. [1984]), and *Parabembras* in their Bembridae. Imamura (1996) not only recognized a separate Bembridae and Parabembridae, but he also classified one traditional bembrid, *Bembradium,* as a member of the Plectrogeniidae. Molecular studies have not sufficiently sampled the Bembridae, and they have recovered their included bembrids sister to Congiopodidae, Hoplichthyidae, Platycephalidae, Plectrogeniidae, Synanceiidae, or a clade composed of the Bembropidae + Scorpaenidae (Smith and Wheeler, 2004, 2006; Smith and Craig, 2007; Lautredou et al., 2013; Near et al., 2013; Smith et al., 2016; Betancur-R. et al., 2017). Molecular studies that have included *Bembras* and *Parabembras* have consistently resulted in these genera forming a clade (Near et al., 2013, 2015; Betancur-R. et al., 2017), thus not requiring the recognition of a separate family-level status for Parabembridae. The current study is the first study with molecular data that included *Bembradium, Bembras,* and *Parabembras*. Given the alignment of *Bembradium* with *Plectrogenium* and the separate grouping of *Bembras* and *Parabembras,* we recommend classifying *Bambradon, Bembras, Brachybembras,* and *Parabembras* as a revised Bembridae (Appendix 2). Further, and as discussed below, our findings support the results of Imamura (1996) who placed *Bembradium* and *Plectrogenium* in the Plectrogeniidae. We recovered the Bembridae sister to a clade composed of Neosebastidae + Plectrogeniidae + Scorpaenidae + Synanceiidae (Fig. 3). This sister group relationship is supported by the loss of the lateral-line canal on the pterotic (character 22, state 0), two spines on the first dorsal-fin pterygiophore (character 56, state 0), and the presence of an *adductor dorsalis* (character 100, state 0).

The Hoplichthyidae is an Indo-Pacific marine family of 17 species that has been variously classified among mail-cheeked fishes but has been allied generally with the platycephalids. Traditional higher-level phylogenetic studies (e.g., Gill, 1888; Regan, 1913; Matsubara, 1943; Quast, 1965; Washington et al., 1984) placed the hoplichthyids as a separate family, closely related to the platycephalids. Greenwood et al. (1966) had a similar classification, but they treated the hoplichthyids as their own suborder, Hoplichthyoidei. Winterbottom (1993) suggested that the hoplichthyids might even be close relatives to the gobioids, so the family's placement has been historically varied. As noted above, Imamura (1996) first suggested that hoplichthyids are more closely related to the triglids than the platycephalids, and his results suggested that *Hoplichthys* was sister to a clade composed of *Peristedion* + *Satyrichthys*. Molecular data, beginning with Smith and Wheeler (2004), have suggested alternative placements for the hoplichthyids ranging from a close relationship with Ovalentaria to sister to the Bembridae, Normanichthyidae, Synanceiidae, a clade composed of Bembridae + Platycephalidae, a clade composed of the "cottoids and allies" (Anoplopomatoidei, Cottoidei, Gasterosteioidei, Hexagrammoidei, and Zaniolepidoidei) + Triglidae, a clade composed of Cottoidei + Gasterosteioidei + Hexagrammoidei + Triglidae + Zoarcoidei, and a clade composed of the "cottoids and allies" + Scorpaenidae + Triglidae (Smith, 2005; Smith and Wheeler, 2006; Smith and Craig, 2007; Lautredou et al., 2013; Near et al., 2013, 2015; Smith et al., 2016; Betancur-R. et al., 2017; Fig. 2). Our results (Fig. 3) place the Hoplichthyidae sister to the Triglidae, similar to the findings of Imamura (1996, 2004; Fig. 1). This sister group relationship is supported by the presence of tubercles on the neurocranium (character 13, state 1), the loss of one postcleithrum (character 48, state 1), the increase in the number of free pectoral-fin rays to three or more (character 49, state 3), the fusion of the cartilaginous caps on the anterior portion of the pelvis (character 51, state 1), the presence of a *hyohyoides inferioris* (character 84, state 1), the attachment of dorsal elements of pelvic-fin muscles to the pectoral girdle (character 97, state 1), and the *obliquus superioris* bypassing and lying ventrally to Baudelot's ligament (character 104, state 1). As with the Congiopodidae, the placement of the hoplichthyids is inconsistent across studies. Most studies ally the hoplichthyids more with the Triglidae (often combined with the included "cottoids and allies"). Given the ambiguity, we recommend treating the Hoplichthyidae, diagnosed by 20 morphological synapomorphies (Appendix 2), as a separate scorpaenoid family.

The Platycephalidae is a modestly large family of 84 species that is found in brackish and marine environments in the Indo-Pacific region (Nelson, 2006). As has been found in previous studies, the Hoplichthyidae and Platycephalidae were recovered as independent monophyletic groups with the traditional limits (Keenan, 1991; Imamura, 1996; Appendix 2; Fig. 3). The interrelationships of the family have been discussed above, and our results support the findings of Imamura (1996) that the Triglidae + Hoplichthyidae is recovered as the sister group to the platycephalids. This sister group relationship is supported by the presence of a tooth plate on the second epibranchial (character 39, state 1). Additionally, our limited sampling of platycephalids corroborates the phylogeny and classification presented in Imamura (1996).

The Triglidae is a large family of 171 species that are found in tropical, temperate, and deep-water habitats across all oceans (Nelson, 2006). Traditional classifications (e.g., Gill, 1888; Matsubara, 1943; Washington et al., 1984; Imamura, 1996; Eschmeyer et al., 2017) often treat the Peristediidae and Triglidae as independent, closely related families. Our study corroborates the findings of Smith (2005) and Portnoy et al. (2017) that place the traditional peristediids (our peristediines) within the Triglidae. Other than the placement of peristediines inside the triglids, our hypothesized relationships support the phylogenies of Imamura (1996) and Richards and Jones (2002). Similarly, our phylogeny supports the phylogeny of Portnoy et al. (2017), including the placement of peristediines inside the Triglidae. This placement and resulting expansion (and monophyly) of the Triglidae is supported by both morphological and molecular data, and our study recovers seven characters supporting the monophyly of this expanded family (Appendix 2).

Neosebastidae and Plectrogeniidae.—The placement of Neosebastidae and Plectrogeniidae has been historically problematic; they are often represented as early diverging scorpaenoid lineages or "ancestral" forms (Matsubara, 1943; Imamura, 1996). Although the current study is the first study to unite these two families (Fig. 3), Imamura (2004), Smith and Wheeler (2004, 2006), Smith and Craig (2007), and Smith et al. (2016) have often found them relatively closely related. In this study, this sister-group relationship was supported by the separation of the first and second hypurals (character 68, state 0).

The Neosebastidae is a predominantly anti-tropical Indo-Pacific marine family of 18 species that has been often separated from the core scorpionfishes in a separate family or subfamily in modern phylogenies and classifications (Imamura, 2004; Motomura, 2004; Nelson, 2006). Beginning with Matsubara (1943) and supported by Washington et al. (1984) and Nelson (2006), the Neosebastidae has been treated as a separate subfamily (Neosebastinae) of the Scorpaenidae. Matsubara (1943) hypothesized that the neosebastids were allied with the sebastines, and Ishida (1994; Fig. 1) allied the neosebastids with the setarchines and recognized them as a separate family. Imamura (2004; Fig. 1) also recognized the clade as a separate family and suggested a non-scorpaenid relationship for the neosebastids; he resolved them with the more flattened scorpaenoids in the families Bembridae, Hoplichthyidae, Platycephalidae, Plectrogeniidae, and Triglidae. Molecular studies have grouped the neosebastids with several non-scorpaenoid groups (e.g., *Acanthistius* or bembropids; Smith and Wheeler, 2006; Smith et al., 2016) or with congiopodids (Smith and Wheeler, 2004; Smith and Craig, 2007). Our finding of a Neosebastidae + Plectrogeniidae clade adds additional complications to the placement of the Neosebastidae, but this result is closer to the findings of Imamura (2004) and several molecular studies (e.g., Smith and Wheeler, 2004, 2006) that have plectrogeniids among the closest relatives of the neosebastids.

Species in the Plectrogeniidae are relatively widespread with collections ranging from the western Indian Ocean to Hawaii despite the family including just four species (Imamura, 1996; Nelson, 2006; Eschmeyer et al., 2017). Fowler (1938) first emphasized the distinctiveness of *Plectrogenium*. Matsubara (1943) supported Fowler's (1938) assertion, suggesting that the genus may represent the ancestral condition of some of the deeper water, flattened scorpaenoids. He noted that *Plectrogenium* shared the loss of the gas bladder and the presence of several rows of prominent head spines and notched pectoral fins with some scorpaenids (e.g., *Sebastolobus*) while also showing characteristics in common with the bembrids. Washington et al.

(1984) further corroborated this hypothesis by pointing to similarities in the scales and caudal fin between *Parabembras* and *Plectrogenium*. Subsequently, Imamura (1996, 2004) supported a placement of an expanded Plectrogeniidae (including *Bembradium*) sister to the clade composed of Bembridae + Hoplichthyidae + Platycephalidae + Triglidae that was united by the presence of a posterior pelvic fossa. Molecular studies have not fully supported or refuted these morphological hypotheses. Smith and Wheeler (2004) and Smith and Craig (2007) recovered *Plectrogenium* sister to Bembridae, Lautredou et al. (2013) recovered *Bembradium* sister to Synanceiidae (their analysis did not include any bembrids), and Smith et al. (2016) recovered *Plectrogenium* sister to the Scorpaenidae. This is the first molecular study to include both *Bembradium* and *Plectrogenium,* and we found a unique relationship for plectrogeniids sister to Neosebastidae. The monophyly of the Plectrogeniidae (Appendix 2) is supported by five characters and corroborates Imamura's (1996) hypothesis and the recognition of this distinct family.

Scorpaenidae and Synanceiidae.—The scorpaenids and synanceiids are the most species-rich clades of scorpaenoids, and the species in these families have often been classified together in whole or in part. Gill (1888) grouped these clades together to the exclusion of all other mail-cheeked fishes. Regan (1913) modified Gill's (1888) arrangement and united scorpaenids, synanceiids, and triglids in his Scorpaeniformes (their studies did not include any representatives of the Neosebastidae or Plectrogeniidae). Matsubara (1943; Fig. 1B) distributed the core scorpionfishes across his "*Cocotropus*-stem," "*Scorpaena*-stem," and "*Sebastes*-stem" based primarily on circumorbital differences. His "*Cocotropus*-stem" was composed of the Japanese synanceiids. His "*Scorpaena*-stem" was composed of *Plectrogenium* and all non-sebastine scorpaenids. Finally, his "*Sebastes*-stem" was composed of the Neosebastidae and Sebastinae. The classification of Washington et al. (1984) largely followed Matsubara (1943) except that they combined his "*Scorpaena*-stem," "*Sebastes*-stem," Synanceiinae, *Apistus,* and *Cheroscorpaena* into their more inclusive Scorpaenidae. They distributed the remaining synanceiids across four additional families (Aploactinidae, Gnathanacanthidae, Pataecidae, and Tetrarogidae) and recognized *Caracanthus* as a distinct family from the Scorpaenidae. Ishida's (1994) phylogenetic hypothesis (Fig. 1C) and classification recognized 12 families. As noted above, this phylogeny included Congiopodidae, Neosebastidae, and *Plectrogenium* nested among the included representatives of the Scorpaenidae and Synanceiidae. Ishida's (1994) major groupings largely followed Washington et al. (1984) except that Ishida often recognized clades at higher taxonomic levels. Ishida (1994) elevated Washington et al.'s (1984) Apistinae, Neosebastinae, and Setarchinae to the family level. Ishida's (1994) Sebastidae included Washington et al.'s (1984) Plectrogeniinae, Sebastolobinae, and Sebastinae. Ishida's (1994) classification included a Synanceiidae that incorporated Washington et al.'s (1984) Choridactylinae, Minoinae, and Synanceiinae. Finally, his Scorpaenidae was restricted to Washington et al.'s (1984) Pteroinae and Scorpaeninae. As noted by Smith and Wheeler (2004), a computer-aided re-analysis of Ishida's (1994) matrix recovered many equally optimal trees that were shorter than the tree presented by Ishida (1994). This large assortment of most parsimonious trees resulted in a poorly resolved phylogeny with just three of his families represented by more than one species being recovered as monophyletic: Aploactinidae, Congiopodidae, and Pataecidae. Relative to Ishida (1994), Imamura (2004; Fig. 1D) increased the taxon sampling among closely related groups (e.g., Platycephalidae, Triglidae) and recovered a largely complementary phylogenetic classification with a few changes. Imamura (2004) recognized a Sebastolobidae at the family level and relegated the Setarchidae of Ishida (1994) to a clade within Scorpaenidae. Subsequent work by Shinohara and Imamura (2005) also placed *Caracanthus* into the Scorpaenidae. Most recently, Honma et al. (2013) recognized a new family Perryenidae for a member of Mandrytza's (2001) Tetrarogidae. This new family and Mandrytza's (2001) earlier treatment of Eschmeyeridae as an additional monotypic family based on former waspfishes casts doubt on tetrarogid monophyly. The proliferation of new waspfish families and Smith and Wheeler's (2004) re-analysis of Ishida's (1994) dataset that recovers 5–6 distinct clades of tetrarogids (*sensu* Ishida, 1994) highlights that traditional "stonefish" taxonomy is becoming complicated with substantial evidence for tetrarogid polyphyly and a diversity of families with three or fewer species (traditional Apistidae, Eschmeyeridae, Gnathanacanthidae, Pataecidae, and Perryenidae).

As seen with the morphological studies, molecular phylogenies that have included representatives of both the Scorpaenidae and Synanceiidae have recovered varied phylogenetic results, but the clades themselves have been largely repeated. These studies have also echoed some morphological results. For example, molecular studies, like morphological studies, consistently recover a polyphyletic Tetrarogidae (*sensu* Ishida, 1994) and *Plectrogenium* separate from the sebastines (Smith and Wheeler, 2004, 2006; Smith and Craig, 2007; Smith et al., 2016). The molecular results have also largely recovered reciprocally monophyletic scorpaenid and synanceiid clades. Smith and Wheeler (2004) recovered independent clades of the Scorpaenidae and Synanceiidae with a diversity of included taxa (including non-scorpaeniforms) in the MRCA. Smith and Wheeler (2006) recovered the scorpaenids sister to epinephelids and synanceiids sister to the triglids with an MRCA that includes the "cottoids and allies" as well as scorpaenoid and serranid fishes. Smith and Craig (2007) recovered relationships similar to Smith and Wheeler (2006) except that the MRCA excluded the Epinephelidae and included the Anthiadidae, Niphonidae, Percidae, Serranidae, and Trachinidae. With more families sampled, Lautredou et al. (2013) and Smith et al. (2016) recovered a clade composed of Plectrogeniidae + Scorpaenidae + Synanceiidae. Finally, Betancur-R. et al. (2017) recovered a clade composed of Scorpaenidae + Synanceiidae, but their analysis only included two synanceiids and did not include any congiopodids, neosebastids, or plectrogeniids, so their phylogeny is of limited comparative value. Our current analysis is the first to recover a clade composed of Neosebastidae + Plectrogeniidae + Scorpaenidae + Synanceiidae, and this clade was supported by the loss of the fourth circumorbital (character 9, state 1).

Despite continued iterative improvement, the march toward a monophyletic taxonomy based on morphological and molecular data has been incompletely accepted by the major fish classifications (e.g., Nelson, 2006; Nelson et al., 2016; Eschmeyer et al., 2017). For example, Nelson (2006) largely follows the pre-phylogenetic study of Washington et al. (1984) except for the placement of Ishida's (1994) Tetrarogidae in their Scorpaenidae. Nelson et al. (2016) followed Nelson (2006) except they placed *Caracanthus* in the Scorpaenidae and recognized Eschmeyeridae as a separate family from their Tetraroginae. Curiously, they left the Tetraroginae within the Scorpaenidae and continued to recognize *Perryena* in the Congiopodidae despite the evidence for both tetrarogine changes being presented in the

same study (Mandrytza, 2001). In contrast, Eschmeyer et al. (2017) largely followed Ishida (1994) except for the placement of *Caracanthus* in the Scorpaenidae (presumably following Shinohara and Imamura, 2005) and the recognition of a separate Plectrogeniidae (presumably following Imamura, 1996), Eschmeyeridae (presumably following Mandrytza, 2001), and Perryenidae (presumably following Honma et al., 2013). Betancur-R. et al. (2017) largely followed Eschmeyer et al. (2017) including the retention of a non-monophyletic (in their study) Scorpaenidae. Presumably, their minimal changes relative to Eschmeyer et al. (2017) are due to limited sampling where only 11 of their 21 platycephaloid, scorpaenoid, or trigloid families were examined. All of these previous studies highlight the need to combine molecular and morphological data to generate a complete and holistic phylogenetic hypothesis that can become stable and more widely accepted.

The Scorpaenidae is a worldwide marine family of 370 species that have been collected in environments ranging from shallow to deep water and from the poles to the tropics (Nelson, 2006; Eschmeyer et al., 2017). This group includes the traditional Caracanthidae, Scorpaenidae, Sebastidae, and Setarchidae (*sensu* Ishida, 1994; Appendix 2) and includes animals with reproductive modes ranging from more traditional broadcast spawning to live birth (Breder and Rosen, 1966; Muñoz, 2010). Most inexplicit and explicit morphological studies and one large-scale molecular study generally have resolved the rockfishes (Sebastinae) as an ancestral or stem grade within the scorpaenoid radiation (Matsubara, 1943; Ishida, 1994; Imamura, 1996, 2004; Lautredou et al., 2013). In contrast, Smith and Wheeler (2004, 2006), Smith and Craig (2007), Smith et al. (2016), and Betancur-R. et al. (2017) have recovered the Sebastinae as a deeply nested lineage. This revised phylogenetic hypothesis implies that the scorpaenoids originated in warmer waters and transitioned to deeper (e.g., Setarchinae) and colder habitats (e.g., Sebastinae) rather than the previous hypotheses that would necessitate transitioning from cooler waters into more temperate and tropical regions. This more traditional hypothesis may have been largely driven by the evolutionary perspective that the colder, overwhelmingly North Pacific cottoids and allies were the closest allies to a sebastine-stem scorpaenoid radiation (Smith and Busby, 2014). This Sebastinae was resolved as the sister group to a clade composed of *Adelosebastes* + *Sebastolobus,* which have generally been allied with or nested among the core sebastines in previous morphological and molecular studies (Figs. 1, 2). As might be expected with the revised placement of these core rockfishes, we recover this clade nested within a larger assemblage that includes all other sampled deeper and cooler water genera (i.e., *Ectreposebastes, Pontinus, Setarches,* and *Trachyscorpia*). In this study, this colder-habitat clade of scorpaenids was recovered as the sister group to a *Scorpaenodes* + Pteroinae clade. This is in contrast to several previous studies (e.g., Imamura, 2004; Smith and Craig, 2007) that have found multiple clades of deep-water scorpaenoids sister to the pteroine lionfishes and allies (Figs. 1, 2). One of the most consistent results across scorpaenid studies is the sister-group relationship between *Scorpaenodes* and pteroine lionfishes (e.g., Ishida, 1994; Imamura, 2004; Lautredou et al., 2013; Smith et al., 2016; Betancur-R. et al., 2017). The final scorpaenid clade recovered in our analysis is a primarily tropical and subtropical clade composed of *Caracanthus, Pteroidichthys, Scorpaena, Scorpaenopsis,* and *Taenionotus*. This clade was sister to the cold-water scorpaenoids + lionfishes and allies and has been consistently recovered in molecular studies (Smith and Craig, 2007; Lautredou et al., 2013; Smith et al., 2016; Betancur-R. et al., 2017). In contrast, morphological studies have typically recovered these fishes as a grade with *Scorpaenodes* and Pteroinae (and potentially other genera) nested within the group (Ishida, 1994; Shinohara and Imamura, 2005). It is clear that the inversion of scorpaenid relationships with sebastines deeply nested with the family that is recovered in this combined study and several other molecular studies (Smith and Wheeler, 2004, 2006; Smith and Craig, 2007; Smith et al., 2016; Betancur-R. et al., 2017) has dramatically altered the polarity of morphological transformations and the impact this has on the evolutionary relationships in this commercially important clade.

The Synanceiidae, a family of 133 species, is primarily a marine clade with a few fresh- or brackish-water representatives (e.g., *Gymnapistes, Neovespicula*) that is distributed from the western Indian Ocean to the South Pacific Ocean (Eschmeyer and Rama-Rao, 1973; Nelson, 2006). The largest taxonomic change we are recommending in this study is the consolidation of the traditional Apistidae, Aploactinidae, Eschmeyeridae, Gnathanacanthidae, Pataecidae, Perryenidae, Synanceiidae, and Tetrarogidae (all *sensu* Eschmeyer et al., 2017) into a monophyletic Synanceiidae. This expanded Synanceiidae is diagnosed by the evolution of the lachrymal saber as well as five additional morphological transformations (Appendix 2). Additionally, Leis and Rennis (2000) provided evidence from larval morphology that separates these fishes from the remainder of the core scorpaenoids. We recommend this higher-level change because of the proliferation of family-level names that are already emanating from the former Tetrarogidae (i.e., Eschmeyeridae, Perryenidae) and that are likely to continue. Our results, previous molecular studies (Smith and Wheeler, 2004, 2006; Smith and Craig, 2007; Smith et al., 2016), and the computer aided re-analysis of Ishida's (1994) scorpaenoid study in Smith and Wheeler (2004) that have all sampled multiple species of tetrarogids (*sensu* Eschmeyer et al., 2017) have suggested that upwards of four or five additional small or monogeneric families will likely be needed to generate a monophyletic taxonomy. Instead of describing a diversity of new waspfish families, the alternative strategy is chosen here where the diversity of existing comparatively small families of stonefishes and waspfishes can be consolidated into a single, well supported, consistently supported, and taxonomically stable family with the retention of subfamilies as warranted and needed (e.g., Apistinae, Aploactininae, Pataecinae, Synanceiinae). Further, our classification largely returns the subfamilial taxonomy to that recommended by Matsubara (1943). Our results recover the typical placement for the Apistinae as the earliest diverging lineage in the Synanceiidae. Regan (1913) included this group among his Scorpaenidae, which was composed of our Scorpaenidae, *Apistus, Erisphex* (an aploactine), and six genera of "tetrarogids" (*sensu* Ishida, 1994). Matsubara (1943), Ishida (1994), and Imamura (2004) all treated *Apistus* as the earliest branching lineage in his clade that is largely equivalent to our Synanceiidae. The placement of Apistinae is one of the most consistently recovered relationships in scorpaenoid phylogenetics, but it is important to note that Washington et al. (1984) highlighted a number of features that potentially group the Apistinae with the Triglidae: a bilobed gas bladder with an intrinsic muscle, elongate pectoral-fin rays (also found in hoplichthyids, *Choridactylus, Inimicus*, and *Minous*), and an expansion of the circumorbitals. Thus, continued work is warranted. Our phylogeny, like previous morphological studies (Ishida, 1994; Imamura, 2004), recovers a monophy-

letic Synanceiinae deeply nested within the Synanceiidae. Our study recovers a clade composed of the Aploactininae and Pataecinae sister to the restricted Synanceiinae. Other than his inclusion of the Congiopodidae in this clade, Ishida (1994) recovered this same relationship. Finally, we have a grade composed of the various "tetrarogid" or formerly "tetrarogid" genera and *Gnathanacanthus* as a diversity of lineages more closely related to Synanceiinae than to Apistinae.

Evolution of the Scorpaenoidei.—The combined morphological and molecular phylogeny presented herein provides an opportunity to reconcile the often conflicting datasets and look at the implications for this updated hypothesis on the evolution of this species-rich clade. One of the major findings in this study was the discovery of the lachrymal saber. As noted above, this specialization is hypothesized to have a primarily defensive role. The maxillary rotation of the lachrymal saber has two major anti-predator impacts. First, it expands the width of the head by projecting the spine(s) outward (Figs. 4–7). This expansion increases the rostral width of the fish by 10–25% and would greatly increase the gape required by a would-be predator (Price et al., 2015). Second, the presence of an outwardly directed and sharp spine should reduce predation because of the potential for the saber to pierce a would-be predator. Cowan (1969) described a similar defensive role for the outward projection of the preopercular spines in the closely allied psychrolutids that have enlarged antler-like modifications (e.g., *Enophrys, Icelinus;* Yabe, 1985). In addition to its role in avoiding or reducing predation, it is possible that the lachrymal saber is used for intraspecific competition. As seen in the evolution of antlers and horns (Chapman, 1975), the lachrymal saber could play a role when synanceiids compete for mates or territories. The one synanceiid species examined for biofluorescence (*Centropogon australis*) had a green fluorescent lachrymal saber that contrasted with non-fluorescent or red-fluorescent regions on the head of these animals (Fig. 9). Recent studies (e.g., Sparks et al., 2014; Anthes et al., 2016; Gruber et al., 2016) have demonstrated that a number of fish groups have green and red fluorescence that appears to be playing an ecological and/or evolutionary role. As such, it is possible that the synanceiids could be advertising or highlighting this specialization with this fluorescence in a similar role as the bioluminescence associated with the defensive dorsal spines in etmopterid sharks (Claes et al., 2013) or that the lachrymal saber is involved in intraspecific competition and mate choice where synanceiid species are advertising their sabers to conspecifics.

In addition to exploring the evolution of the lachrymal saber, the revised scorpaenoid hypothesis has implications for the evolution of viviparity in this clade. The deeply nested placement of Sebastinae within the Scorpaenidae corroborates Wourms' (1991) assertion about the evolution of live birth and corresponding intermediate stages in the transition from an ovuliparous (classical oviparous) ancestor among the non-scorpaenid scorpaenoids. As noted by Smith and Wheeler (2004), there appears to be an evolutionary transition from a more common ovuliparous ancestor in the platycephalids, synanceiids, and triglids to an oviparous species that releases fertilized eggs within a gelatinous egg mass in genera such as *Dendrochirus, Pterois, Scorpaena, Scorpaenodes, Scorpaenopsis,* and *Sebastolobus* (Wourms, 1991; Koya and Muñoz, 2007). This intermediate reproductive mode is further modified in *Helicolenus* where species in the genus have internal fertilization and zygoparity where fertilized ova are held by the mother before being released into the ocean (Wourms, 1991). The reproductive mode of *Hozukius* is unknown, but it shares a II-3 ovarian type with most scorpaenids (e.g., *Caracanthus, Dendrochirus, Helicolenus, Scorpaena*; Cole, 2003; Koya and Muñoz, 2007). The more derived viviparous sebastine rockfishes (*Sebastes* and *Sebastiscus*) have a type II-1 ovarian type (Koya and Muñoz, 2007). This suggests that *Hozukius* is likely to be more similar to *Helicolenus* or *Scorpaena* and not be live bearing. The evolution of reproductive modes and live birth in scorpaenoids has been discussed in considerably more detail in other studies (Wourms, 1991; Koya and Muñoz, 2007; Muñoz, 2010; Pavlov and Emel'yanova, 2013), but their interpretations have implicitly or explicitly relied on the hypothesized placement of sebastines at the base of the scorpaenoid tree. The inversion of the phylogeny of scorpaenoids proposed in this study is more consistent with traditional views on the evolution of viviparity where there is a transition from external fertilization to internal fertilization with the retention of either the developing eggs or embryos within the mother (Wourms, 1991; Wourms and Lombardi, 1992). These are two examples demonstrating the impact of the proposed phylogeny on the evolution of the scorpaenoid fishes. We hope that this revised hypothesis for the relationships of scorpionfish and allies will allow researchers to test additional evolutionary hypotheses for this important percomorph clade.

MATERIAL EXAMINED

Ablabys taenianotus: KUI 41345

Adelosebastes latens: KUI 28431

Aetapcus maculatus: NMV A 11847

Apistus carinatus: CAS 15975, FMNH 55557, FMNH 119634, KUI 41400

Bathymaster signatus: SIO 93-174

Bellator militaris: AMNH 084578

Bembradium roseum: CAS (SU_ICH) 8653

Bembras japonicus: CAS 67504

Bembrops macronema: AMNH 49698SW

Bovichtus chilensis: AMNH 49664SW

Caracanthus unipinna: AMNH 18105

Centropogon australis: KUI 41409

Chelidonichthys kumu: AMNH 91672SD

Choridactylus multibarbus: CAS 15071

Cirrhitus rivulatus: SIO 59-225

Congiopodus leucopaecilus: AMNH 222767

Dendrochirus brachypterus: AMNH 41595SW

Diplectrum formosum: AMNH 76688

Diploprion bifasciatum: AMNH 97446SW

Ectreposebastes imus: AMNH 27991SW

Elates ransonnetii: FMNH 63930

Erisphex potti: CAS 30316

Erosa erosa: FMNH 121063

Eschmeyer nexus: USNM 233855

Gnathanacanthus goetzeei: AMNH 223040, USNM 47852

Gymnapistes maculatus: AMNH 31009

Helicolenus dactylopterus: AMNH 76550

Hoplichthys citrinus: AMNH 89898

Hozukius embremarius: SIO 75-497

Icelinus quadriseriatus: SIO 84-91

Inimicus didactylus: KUI 41391

Inimicus japonicus: AMNH 38131SW

Lepidotrigla multispinosa: SAIAB 035527

Liocranium praepositum: KUI 41399

Maxillicosta scabriceps: CAS 33304

Minous quincarinatus: FMNH 121037, KUI 41397

Minous trachycephalus: AMNH 098704SD

Neosebastes thetidis: CAS 31111

Niphon spinosus: FMNH 57109, USNM 57737

Parabembras curtus: CAS 49456

Paracentropogon sp.: CAS_SU 68769

Paracentropogon longispinis: KUI 41398

Paracentropogon rubripinnis: FMNH 89090

Pataecus fronto: CAS 67408

Perca flavescens: AMNH 43887SW

Peristedion miniatum: AMNH 75393

Platycephalus indicus: AMNH 88066

Plectrogenium nanum: CAS 70174

Pontinus longispinis: AMNH 83416

Prionotus evolans: AMNH 41773SW

Pseudanthias pleurotaenia: AMNH 38119SW

Pterodichthys amboensis: AMNH uncat.

Pterois volitans: AMNH 38132SW

Pterygotrigla hemisticta: FMNH 120607

Satyrichthys welchi: AMNH 98721

Scorpaena guttata: SIO 52-214

Scorpaenodes guamensis: AMNH 213867

Scorpaenopsis diabolus: FMNH 121052

Sebastes ruberrimus: AMNH 37946SD

Sebastolobus altivelis: KUI 28282

Sebastiscus marmoratus: AMNH 97547SW

Setarches guentheri: AMNH 64334

Synanceia horrida: AMNH 213070

Taenianotus triacanthus: FMNH 63586

Thysanophrys celebicus: CAS 80464

Trachinus draco: AMNH 36487

Trachyscorpia eschmeyeri: AMNH 098598SD

Zalanthias kelloggi azumanus: FMNH 57230

Zanclorhynchus spinifer: MNHN 2003-0266

DATA ACCESSIBILITY

Supplemental material is available at http://www.copeiajournal.org/cg-17-669.

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LITERATURE CITED

Anthes, N., J. Theobald, T. Gerlach, M. G. Meadows, and N. K. Michiels. 2016. Diversity and ecological correlates of red fluorescence in marine fishes. Frontiers in Ecology and Evolution 4:126.

Betancur-R., R., E. O. Wiley, G. Arratia, A. Acero, N. Bailly, M. Miya, G. Lecointre, and G. Ortí. 2017. Phylogenetic classification of bony fishes. BMC Evolutionary Biology 17: 162.

Bosher, B. T., S. H. Newton, and M. L. Fine. 2006. The spines of the Channel Catfish, *Ictalurus punctatus*, as an anti-predator adaptation: an experimental study. Ethology 112:188–195.

Breder, C. M., and D. E. Rosen. 1966. Modes of Reproduction in Fishes. T.F.H. Publications, Neptune City, New Jersey.

Casewell, N. R., J. C. Visser, K. Baumann, J. Dobson, H. Han, S. Kuruppu, M. Morgan, A. Romilio, V. Weisbecker, S. A. Ali, J. Debono, I. Koludarov, I. Que, G. C. Bird, G. M. Cooke, A. Nouwens, W. C. Hodgson, S. C. Wagstaff, K. L. Cheney, I. Vetter, L. van der Weerd, M. K. Richardson, and B. G. Fry. 2017. The evolution of fangs, venom, and mimicry systems in blenny fishes. Current Biology 27:1184–1191.

Chakrabarty, P., M. P. Davis, W. L. Smith, Z. H. Baldwin, and J. S. Sparks. 2011a. Is sexual selection driving diversification of the bioluminescent ponyfishes (Teleostei: Leiognathidae)? Molecular Ecology 20:2818–2834.

Chakrabarty, P., M. P. Davis, W. L. Smith, R. Berquist, K. M. Gledhill, L. R. Frank, and J. S. Sparks. 2011b. Evolution of the light organ system in ponyfishes (Teleostei: Leiognathidae). Journal of Morphology 272:704–721.

Chapman, D. I. 1975. Antlers—bones of contention. Mammal Review 5:122–172.

Claes, J. M., M. N. Dean, D.-E. Nilsson, N. S. Hart, and J. Mallefet. 2013. A deepwater fish with 'lightsabers'—dorsal spine-associated luminescence in a counterilluminating lanternshark. Nature Scientific Reports 3:1308.

Cole, K. S. 2003. Hermaphroditic characteristics of gonad morphology and inferences regarding reproductive biology in *Caracanthus* (Teleostei, Scorpaeniformes). Copeia 2003: 68–80.

Colgan, D. J., A. McLauchlan, G. D. F. Wilson, S. P. Livingston, G. D. Edgecombe, J. Macaranas, G. Cassis, and M. R. Gray. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Australian Journal of Zoology 46:419–437.

Cowan, G. I. M. 1969. The cephalic and caudal musculature of the sculpin, *Myoxocephalus polyacanthocephalus* (Pisces: Cottidae). Canadian Journal of Zoology 47:841–850.

Datavo, A., and R. P. Vari. 2013. The jaw adductor muscle complex in teleostean fishes: evolution, homologies and revised nomenclature (Osteichthyes: Actinopterygii). PLOS ONE 8:e60846.

Davis, M. P., J. S. Sparks, and W. L. Smith. 2016. Repeated and widespread evolution of bioluminescence in marine fishes. PLOS ONE 11:e0155154.

Eschmeyer, W. N., R. Fricke, and R. van der Laan (Eds.). 2017. Catalog of Fishes: Genera, species, references. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp. Electronic version accessed 4 July 2017.

Eschmeyer, W. N., and K. V. Rama-Rao. 1973. Two new stonefishes (Pisces, Scorpaenidae) from the Indo-West Pacific, with a synopsis of the subfamily Synanceiinae. Proceedings of the California Academy of Sciences (Series 4) 39:337–382.

Feller, A. E., and S. B. Hedges. 1998. Molecular evidence for the early history of living amphibians. Molecular Phylogenetics and Evolution 9:509–516.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3:294–299.

Fowler, H. W. 1938. Description of new fishes obtained by the United States Bureau of Fisheries steamer "Albatross," chiefly in Philippine seas and adjacent waters. Proceedings of the United States National Museum 85:31–135.

Gill, T. 1888. On the classification of the mail-cheeked fishes. Proceedings of the United States National Museum 11:567–592.

Girard, M. G., and W. L. Smith. 2016. The phylogeny of marine sculpins of the genus *Icelinus* with comments on the evolution and biogeography of the Pseudoblenninae. Zootaxa 4171:549–561.

Grande, T., W. C. Borden, and W. L. Smith. 2013. Limits and relationships of Paracanthopterygii: a molecular framework for evaluating past morphological hypotheses, p. 385–418. *In*: Mesozoic Fishes 5–Global Diversity and Evolution. G. Arratia, H.-P. Schultze, and M. V. H. Wilson (eds.). Verlag Dr. Friedrich Pfeil, München, Germany.

Greenwood, P. H., D. E. Rosen, S. H. Weitzman, and G. S. Myers. 1966. Phyletic studies of teleostean fishes, with a provisional classification of living forms. Bulletin of the American Museum of Natural History 131:341–455.

Gruber, D. F., E. R. Loew, D. D. Deheyn, D. Akkaynak, J. P. Gaffney, W. L. Smith, M. P. Davis, J. H. Stern, V. A. Pieribone, and J. S. Sparks. 2016. Biofluorescence in catsharks (Scyliorhinidae): fundamental description and relevance for elasmobranch visual ecology. Nature: Scientific Reports 6:24751.

Hillis, D. M., and M. T. Dixon. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Quarterly Review of Biology 66:411–453.

Honma, Y., H. Imamura, and T. Kawai. 2013. Anatomical description of the genus *Perryena*, and proposal to erect a new family for it based on its phylogenetic relationships with related taxa (Scorpaeniformes). Ichthyological Research 60:122–141.

Hyde, J. R., and R. D. Vetter. 2007. The origin, evolution, and diversification of rockfishes of the genus *Sebastes* (Cuvier). Molecular Phylogenetics and Evolution 44:790–811.

Imamura, H. 1996. Phylogeny of the family Platycephalidae and related taxa (Pisces: Scorpaeniformes). Species Diversity 1:123–233.

Imamura, H. 2004. Phylogenetic relationships and new classification of the superfamily Scorpaenoidea. Species Diversity 9:1–36.

Imamura, H., and G. Shinohara. 1998. Scorpaeniform fish phylogeny: an overview. Bulletin of the National Science Museum (Tokyo), Series A 24:185–212.

Imamura, H., and M. Yabe. 2002. Demise of the Scorpaeniformes (Actinopterygii: Percomorpha): an alternative phylogenetic hypothesis. Bulletin of the Faculty of Fisheries, Hokkaido University 53:107–128.

Ishida, M. 1994. Phylogeny of the suborder Scorpaenoidei (Pisces: Scorpaeniformes). Bulletin of Nansei National Fisheries Research Institute 27:1–112.

Ishii, N., and H. Imamura. 2008. Phylogeny of the family Congiopodidae (Perciformes: Scorpaenoidea), with a proposal of new classification. Ichthyological Research 55: 148–161.

Johnson, G. D. 1983. A primitive epinepheline serranid, with comments on the monophyly and intrarelationships of the Serranidae. Copeia 1983:777–787.

Johnson, G. D., and C. Patterson. 2001. The intermuscular system of acanthomorph fishes: a commentary. American Museum Novitates 3312:1–24.

Jordan, D. S., and C. L. Hubbs. 1925. Record of fishes obtained by David Starr Jordan in Japan. Memoirs of the Carnegie Museum 10:93–346.

Katoh, K., K. Misawa, K. Kuma, and T. Miyata. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30:3059–3066.

Kawai, T. 2008. Phylogenetic systematics of the family Peristediidae (Teleostei: Actinopterygii). Species Diversity 13:1–34.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Mentjies, and A. Drummond. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649.

Keenan, C. P. 1991. Phylogeny of Australian species of flatheads (Teleostei, Platycephalidae) as determined by allzoyme electrophoresis. Journal of Fish Biology 39:237–249.

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Pääbo, X. F. Villablanca, and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences of the United States of America 86:6196–6200.

Koya, Y., and M. Muñoz. 2007. Comparative study on ovarian structures in scorpaenids: possible evolutional process of reproductive mode. Ichthyological Research 54:221–230.

Lautredou, A. C., H. Motomura, C. Gallut, C. Ozouf-Costaz, G. Lecointre, and A. Dettaï. 2013. New nuclear markers and exploration of the relationships among Serraniformes (Acanthomorpha, Teleostei): the importance of working at multiple scales. Molecular Phylogenetics and Evolution 67:140–155.

Leis, J. M., and D. S. Rennis. 2000. Scorpaenidae (scorpionfishes, stonefishes), p. 226–235. *In:* The Larvae of Indo-Pacific Coastal Fishes: An Identification Guide to Marine Fish Larvae. J. M. Leis and J. M. Carson-Ewart (eds.). Brill, Leiden, Netherlands.

Lewis, P. O. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. Systematic Biology 50:913–925.

Li, B., A. Dettaï, C. Cruaud, A. Couloux, M. Desoutter-Meniger, and G. Lecointre. 2009. RNF213, a new nuclear marker for acanthomorph phylogeny. Molecular Phylogenetics and Evolution 50:345–363.

Li, C., R. Betancur-R., W. L. Smith, and G. Ortí. 2011. Monophyly and interrelationships of snook and barramundi (Centropomidae *sensu* Greenwood) and five new markers for fish phylogenetics. Molecular Phylogenetics and Evolution 60:463–471.

Li, C., G. Ortí, G. Zhang, and G. Lu. 2007. A practical approach to phylogenomics: the phylogeny of ray-finned fish (Actinopterygii) as a case study. BMC Evolutionary Biology 7:44.

Maddison, W. P., and D. R. Maddison. 2017. Mesquite: a modular system for evolutionary analysis. Version 3.2 http://mesquiteproject.org

Mandrytza, S. A. 1990. The condition of the muscular *adductor mandibulae* of the scorpaeniform fishes. Proceedings of the Zoological Institute of Leningrad 22:75–93. [in Russian]

Mandrytza, S. A. 2001. Seismosensory System and Classification of Scorpionfishes (Scorpaeniformes: Scorpaenoidei). Perm State University Press, Perm, Russia. [in Russian]

Matsubara, K. 1943. Studies of the scorpaenoid fishes of Japan: anatomy, phylogeny, and taxonomy (I). The Transactions of the Sigenkagku Kenyusyo 1:1–170.

Moser, H. G. 1981. Morphological and functional aspects of marine fish larvae, p. 89–131. *In*: Marine Fish Larvae: Morphology, Ecology, and Relation to Fisheries. R. Lasker (ed.). University of Washington Press, Seattle, Washington.

Motomura, H. 2004. Revision of the scorpionfish genus *Neosebastes* (Scorpaeniformes: Neosebastidae), with descriptions of five new species. Indo-Pacific Fishes 37:1–76.

Muñoz, M. 2010. Reproduction in Scorpaeniformes, p. 65–89. *In*: Reproduction and Sexuality in Marine Fishes: Patterns and Processes. K. S. Cole (ed.). University of California Press, Berkeley, California.

Nagy, L. G., J. Házi, B. Szappanos, S. Kocsubé, B. Bálint, G. Rákehly, C. Vágyölgyi, and T. Papp. 2012. The evolution of defense mechanisms correlate with the explosive diversification of autodigesting *Coprinellus* mushrooms (Agaricales, Fungi). Systematic Biology 61:595–607.

Near, T. J., A. Dornburg, R. I. Eytan, B. P. Keck, W. L. Smith, K. L. Kuhn, J. A. Moore, S. A. Price, F. T. Burbrink, M. Friedman, and P. C. Wainwright. 2013. Phylogeny and tempo of diversification in the superradiation of spiny-rayed fishes. Proceedings of the National Academy of Sciences of the United States of America 110:12738–12743.

Near, T. J., A. Dornburg, R. C. Harrington, C. Oliveira, T. W. Pietsch, C. E. Thacker, T. P. Satoh, E. Katayama, P. C. Wainwright, J. T. Eastman, and J. M. Beaulieu. 2015. Identification of the notothenioid sister lineage illuminates the biogeographic history of an Antarctic adaptive radiation. BMC Evolutionary Biology 15:109.

Near, T. J., A. Dornburg, K. L. Kuhn, J. T. Eastman, J. N. Pennington, T. Patarnello, L. Zane, D. A. Fernández, and C. D. Jones. 2012b. Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. Proceedings of the National Academy of Sciences of the United States of America 109:3434–3439.

Near, T. J., R. I. Eytan, A. Dornburg, K. L. Kuhn, J. A. Moore, M. P. Davis, P. C. Wainwright, M. Friedman, and W. L. Smith. 2012a. Resolution of ray-finned fish phylogeny and timing of diversification. Proceedings of the National Academy of Sciences of the United States of America 109:13698–13703.

Nelson, J. S. 2006. Fishes of the World. Fourth edition. John Wiley and Sons Inc., New York.

Nelson, J. S., T. C. Grande, and M. V. H. Wilson. 2016. Fishes of the World. Fifth edition. John Wiley and Sons Inc., Hoboken, New Jersey.

Palumbi, S. R. 1996. Nucleic acids II: the polymerase chain reaction, p. 205–247. *In*: Molecular Systematics. Second edition. D. M. Hillis, C. Moritz, and B. K. Mable (eds.). Sinauer, Sunderland, Massachusetts.

Pavlov, D. A., and N. G. Emel'yanova. 2013. Transition to viviparity in the order Scorpaeniformes: brief review. Journal of Ichthyology 53:52–69.

Portnoy, D. S., S. C. Willis, E. Hunt, D. G. Swift, J. R. Gold, and K. W. Conway. 2017. Molecular phylogenetics of New World searobins (Triglidae; Prionotinae). Molecular Phylogenetics and Evolution 107:382–387.

Posada, D. 2008. jModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25:1253–1256.

Poss, S. G. 1999. Scorpaenidae. Scorpionfishes (also lionfishes, rockfishes, stingfishes, stonefishes, and waspfishes), p. 2069–2790. *In*: FAO Species Identification Guide for Fishery Purposes. The living marine resources of the Western Central Pacific. Vol. 4. Bony fishes part 2 (Mugilidae to Carangidae). K. E. Carpenter and V. H. Niem (eds.). Food and Agricultural Organization, Rome.

Poss, S. G., and V. G. Springer. 1983. *Eschmeyer nexus*, a new genus and species of scorpaenid fish from Fiji. Proceedings of the Biological Society of Washington 96:309–316.

Price, S. A., S. T. Friedman, and P. C. Wainwright. 2015. How predation shaped fish: the impact of fin spines on body form evolution across teleosts. Proceedings of the Royal Society of London, B 282:20151428.

Quast, J. C. 1965. Osteological characteristics and affinities of the hexagrammid fishes, with a synopsis. Proceedings of the California Academy of Sciences (Series 4) 31:563–600.

Randall, J. E., A. Katsumi, H. Takashi, N. Mitsuura, K. Hisao, and Y. Hashimoto. 1971. Grammistin, the skin toxin of soapfishes, and its significance in the classification of the Grammistidae. Publications of the Seto Marine Biological Laboratory 19:157–190.

Regan, C. T. 1913. XIV. The osteology and classification of the teleostean fishes of the order Scleroparei. Annals and Magazine of Natural History (Series 8) 11:169–184.

Richards, W. J., and D. L. Jones. 2002. Preliminary classification of the gurnards (Triglidae: Scorpaeniformes). Marine and Freshwater Research 53:275–282.

Shinohara, G., and H. Imamura. 2005. Anatomical description and phylogenetic classification of the orbicular velvetfishes (Scorpaenoidea: *Caracanthus*). Ichthyological Research 52:64–76.

Smith, W. L. 2005. The limits and relationships of mail-cheeked fishes (Teleostei: Percomorpha) and the evolution of venom in fishes. Unpubl. Ph.D. diss., Columbia University, New York.

Smith, W. L. 2010. Promoting resolution of the percomorph bush: a reply to Mooi and Gill. Copeia 2010:520–524.

Smith, W. L., and M. S. Busby. 2014. Phylogeny and taxonomy of sculpins, sandfishes, and snailfishes (Perciformes: Cottoidei) with comments on the phylogenetic significance of their early-life-history specializations. Molecular Phylogenetics and Evolution 79:332–352.

Smith, W. L., and M. T. Craig. 2007. Casting the percomorph net widely: the importance of broad taxonomic sampling in the search for the placement of serranid and percid fishes. Copeia 2007:35–55.

Smith, W. L., K. R. Smith, and W. C. Wheeler. 2009. Novel mitochondrial intergenic spacer in fairy basslets (Serranidae: Anthiinae) and the simultaneous analysis of nucleotide and genomic data. American Museum Novitates 3652: 1–10.

Smith, W. L., J. H. Stern, M. G. Girard, and M. P. Davis. 2016. Evolution of venomous cartilaginous and ray-finned fishes. Integrative and Comparative Biology 56:950–961.

Smith, W. L., and W. C. Wheeler. 2004. Polyphyly of the mail-cheeked fishes (Teleostei: Scorpaeniformes): evidence from mitochondrial and nuclear sequence data. Molecular Phylogenetics and Evolution 32:627–646.

Smith, W. L., and W. C. Wheeler. 2006. Venom evolution widespread in fishes: a road map for the bioprospecting of piscine venoms. Journal of Heredity 97:206–217.

Sparks, J. S., R. C. Schelly, W. L. Smith, M. P. Davis, D. Tchernov, V. A. Pieribone, and D. F. Gruber. 2014. The covert world of fish biofluoresence: a phylogenetically widespread and phenotypically variable phenomenon. PLOS ONE 9:e83259.

Stewart, T. A., W. L. Smith, and M. I. Coates. 2014. The origins of adipose fins: an analysis of homoplasy and the serial homology of vertebrate appendages. Proceedings of the Royal Society of London, B 281:20133120.

Streelman, J. T., and S. A. Karl. 1997. Reconstructing labroid evolution with single-copy nuclear DNA. Proceedings of the Royal Society of London, B 264:1011–1020.

Titus, T. A. 1992. A phylogenetic analysis of the Desmognathinae (Caudata: Plethodontidae): evolutionary patterns inferred from mitochondrial DNA sequences. Unpubl. Ph.D. diss., University of Kansas, Lawrence, Kansas.

Vamosi, S. M. 2005. On the role of enemies in divergence and diversification of prey: a review and synthesis. Canadian Journal of Zoology 83:894–910.

Wainwright, P. C., W. L. Smith, S. A. Price, K. L. Tang, J. S. Sparks, L. A. Ferry, K. L. Kuhn, R. I. Eytan, and T. J. Near. 2012. The evolution of pharyngognathy: a phylogenetic and functional appraisal of the pharyngeal jaw key innovation in labroid fishes and beyond. Systematic Biology 61:1001–1027.

Washington, B. B., W. N. Eschmeyer, and K. M. Howe. 1984. Scorpaeniformes: relationships, p. 438–447. *In:* Ontogeny and Systematics of Fishes. H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall, Jr., and S. L. Richardson (eds.). American Society of Ichthyologists and Herpetologists, Special Publication No. 1, Lawrence, Kansas.

Wiley, E. O., P. Chakrabarty, M. T. Craig, M. P. Davis, N. I. Holcroft, R. L. Mayden, and W. L. Smith. 2011. Will the real phylogeneticists please stand up? Zootaxa 2946:7–16.

Winterbottom, R. 1993. Search for the gobioid sister group (Actinopterygii: Percomorpha). Bulletin of Marine Science 52:395–414.

Wourms, J. P. 1991. Reproduction and development of *Sebastes* in the context of the evolution of piscine viviparity. Environmental Biology of Fishes 30:111–126.

Wourms, J. P., and J. Lombardi. 1992. Reflections on the evolution of piscine viviparity. American Zoologist 32:276–293.

Yabe, M. 1985. Comparative osteology and myology of the superfamily Cottoidea (Pisces: Scorpaeniformes), and its phylogenetic classification. Memoirs of the Faculty of Fisheries, Hokkaido University 32:1–130.

Yang, W., I. H. Chen, B. Gludovatz, E. A. Zimmermann, R. O. Ritchie, and M. A. Meyers. 2013. Natural flexible dermal armor. Advanced Materials 25:31–48.

Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Unpubl. Ph.D. diss., The University of Texas, Austin, Texas.

APPENDIX 1

Characters examined in the phylogenetic analyses

Character descriptions for characters 1–111 from Imamura (2004), 112 from Shinohara and Imamura (2005), and character 113 is new in this study. Data for characters 1–111 for *Adelosebastes, Apistus, Bellator, Bembradium, Bembras, Chelidonichthys, Choridactylus, Congiopodus, Dendrochirus, Diploprion, Ectreposebastes, Elates, Erisphex, Erosa, Gnathanacanthus, Helicolenus, Hoplichthys, Hozukius, Inimicus, Lepidotrigla, Max-*

illicosta, Minous, Neosebastes, Niphon, Parabembras, Pataecus, Peristedion, Platycephalus, Plectrogenium, Pontinus, Prionotus, Pterois, Pterygotrigla, Satyrichthys, Scorpaena, Scorpaenodes, Scorpaenopsis, Sebastes, Sebastiscus, Sebastolobus, Setarches, Synanceia, Taenionotus, Thysanophrys, Trachyscorpia, and *Zalanthias* are from Imamura (2004). Data for character 112 for above genera and characters 1–112 for *Caracanthus* and *Pteroidichthys* are from Shinohara and Imamura (2005). Data for *Eschmeyer*, in part, are from Poss and Springer (1983). Data for *Perryena* are from Honma et al. (2013). Myological data (characters 79–106) come from many sources: *Ablabys, Aetapcus,* and *Gymnapistes* (Ishida, 1994); *Bathymaster* (Imamura and Yabe, 2002); *Icelinus* (Yabe, 1985); and *Zanclorhynchus* (Ishii and Imamura, 2008). All remaining character codings are new in this study (see Material Examined). Characters 2, 5, 18, 43, 45, and 54 were modified from Imamura (2004) because of variation in the species added to the analysis.

1. First through third circumorbitals:
   (1_0) = elements closely associated with each other
   (1_1) = elements separated from each other
2. First and third circumorbitals (modified from Imamura, 2004):
   (2_0) = separated by second circumorbital
   (2_1) = attached
   (2_2) = separated by second circumorbital and ectopterygoid
   (2_3) = loss of second circumorbital
3. Middle portion of second circumorbital sensory canal:
   (3_0) = bridge absent
   (3_1) = bridge present with no sensory openings
   (3_2) = bridge present with a single sensory opening
   (3_3) = bridge present with more than two sensory openings
4. Third circumorbital and lateral ethmoid:
   (4_0) = separated
   (4_1) = attached
5. Third and fifth circumorbitals (modified from Imamura, 2004):
   (5_0) = separated by fourth circumorbital
   (5_1) = attached
   (5_2) = loss of fourth or fifth circumorbital
6. Direction of posterior opening of third circumorbital, continuous with sensory canal of fourth to sixth circumorbitals:
   (6_0) = upward
   (6_1) = backward
7. Position of upward (or backward) opening of third circumorbital:
   (7_0) = on dorsal (or posterior) margin of element
   (7_1) = below (or anterior to) margin
8. Suborbital stay on third circumorbital (ordered via additive binary coding):
   (8_0) = absent
   (8_1) = present with distal end not strongly connected with preopercle
   (8_2) = present with distal end narrowly truncated and strongly connected with preopercle
   (8_3) = present with distal end broad and strongly connected with preopercle
9. Fourth and fifth circumorbitals (ordered via additive binary coding):
   (9_0) = both present
   (9_1) = fourth circumorbital absent
   (9_2) = fourth and fifth circumorbital absent
10. Sixth circumorbital:
   (10_0) = attached to sphenotic
   (10_1) = fused to sphenotic
   (10_2) = absent
11. Sensory canal of third circumorbital:
   (11_0) = canal continues into fourth or fifth circumorbitals
   (11_1) = canal separate/not continuous with fourth or fifth circumorbital
12. Postotic sensory canal:
   (12_0) = canal continuous with circumorbital series
   (12_1) = canal separate/not continuous with circumorbital series
13. Tubercles on neurocranium:
   (13_0) = absent
   (13_1) = present
14. Nasal and neurocranium:
   (14_0) = loosely attached
   (14_1) = sutured
15. Nasals:
   (15_0) = separated
   (15_1) = nasals sutured together medially
16. Number of vomerine tooth plates:
   (16_0) = one
   (16_1) = two
   (16_2) = none
17. Lateral ethmoids:
   (17_0) = separated
   (17_1) = meeting in midline
18. Parasphenoid and pterosphenoid:
   (18_0) = separated
   (18_1) = connected
19. Basisphenoid:
   (19_0) = present, posterior margin connected directly with neurocranium
   (19_1) = present, posterior margin free from neurocranium
   (19_2) = absent
20. Prootic and intercalar:
   (20_0) = in contact
   (20_1) = separated
21. Intercalar and posttemporal:
   (21_0) = with ligamentous articulation
   (21_1) = sutured
22. Lateral lateral-line canal pore on pterotic:
   (22_0) = absent
   (22_1) = present
23. Skinny lateral-line sensory canal between pterotic and preopercle:
   (23_0) = present
   (23_1) = absent
24. Spines associated with lateral-line sensory canal on parietal:
   (24_0) = absent
   (24_1) = present
25. Baudelot's ligament:
   (25_0) = originating from basioccipital
   (25_1) = originating from basioccipital and first vertebra
   (25_2) = originating from first vertebra
   (25_3) = absent
26. Ascending process of premaxilla:
   (26_0) = continuous with remaining part of premaxilla
   (26_1) = separated from the remaining part of premaxilla
27. Notch between ascending process and cranial condyle:
   (27_0) = present
   (27_1) = absent

28. Teeth on oral jaws:
  (28_0) = present
  (28_1) = absent
29. Palatine and ectopterygoid:
  (29_0) = connected
  (29_1) = separated
30. Teeth on palatine:
  (30_0) = present
  (30_1) = absent
31. Ectopterygoid and metapterygoid:
  (31_0) = separated
  (31_1) = meeting medially
32. Metapterygoid lamina:
  (32_0) = present
  (32_1) = absent
33. Space between metapterygoid and hyomandibula:
  (33_0) = prominent
  (33_1) = rudimentary to absent
34. Preopercular margin:
  (34_0) = with serrations
  (34_1) = with prominent spine(s)
  (34_2) = without serrations or prominent spines
35. Backwardly directed opercular spine:
  (35_0) = present
  (35_1) = absent
36. Backwardly directed opercular spine:
  (36_0) = ossified
  (36_1) = cartilaginous
  (36_2) = absent
37. Number of branchiostegal rays:
  (37_0) = seven
  (37_1) = six
  (37_2) = five
38. Interarcual cartilage:
  (38_0) = present
  (38_1) = absent
39. Tooth plate on second epibranchial:
  (39_0) = absent
  (39_1) = present
40. Tooth plate on third epibranchial:
  (40_0) = present
  (40_1) = absent
41. First pharyngobranchial:
  (41_0) = present and ossified
  (41_1) = present and cartilaginous
  (41_2) = absent
42. Tooth plate on second pharyngobranchial:
  (42_0) = present
  (42_1) = absent
43. Second through fourth pharyngobranchials (modified from Imamura, 2004):
  (43_0) = separated
  (43_1) = third and fourth continuous
  (43_2) = second to fourth continuous
  (43_3) = only third pharyngobranchial present
44. Medial extrascapular:
  (44_0) = present
  (44_1) = absent
45. Lateral extrascapular (modified from Imamura, 2004):
  (45_0) = single element with three sensory openings
  (45_1) = two elements (horizontal and longitudinal tubes) with two sensory openings
  (45_2) = single element (longitudinal tube) with two sensory openings
46. Cleithrum and coracoid (ordered via additive binary coding):
  (46_0) = without ventromedial connection
  (46_1) = with partial ventromedial connection
  (46_2) = with complete ventromedial connection
47. Dorsalmost actinost and scapula:
  (47_0) = separated
  (47_1) = fused
48. Number of postcleithra (ordered via additive binary coding):
  (48_0) = two
  (48_1) = one
  (48_2) = zero
49. Number of free lower pectoral-fin rays (ordered via additive binary coding):
  (49_0) = zero
  (49_1) = one
  (49_2) = two
  (49_3) = three or more
50. Branched pectoral-fin rays:
  (50_0) = present
  (50_1) = absent
51. Cartilaginous caps on anterior portion of pelvis:
  (51_0) = separated
  (51_1) = fused
52. Posterior pelvic fossa:
  (52_0) = absent
  (52_1) = present, opposing fossae meeting
  (52_2) = present, opposing fossae separated
53. Opposing posteromedial parts of pelvis:
  (53_0) = sutured
  (53_1) = separated
54. Number of pelvic-fin spines and rays (ordered via additive binary coding; modified from Imamura, 2004):
  (54_0) = six
  (54_1) = five
  (54_2) = four
  (54_3) = three
  (54_4) = zero
55. Branched pelvic-fin rays:
  (55_0) = present
  (55_1) = absent
56. Number of spines on first dorsal-fin proximal pterygiophore:
  (56_0) = two
  (56_1) = one
57. Dorsal spines:
  (57_0) = stout
  (57_1) = slender
58. First spine on first anal-fin proximal pterygiophore:
  (58_0) = present
  (58_1) = absent
59. Second element on first anal-fin proximal pterygiophore:
  (59_0) = spine
  (59_1) = soft ray
  (59_2) = absent
60. Element on second anal-fin proximal pterygiophore:
  (60_0) = spine
  (60_1) = soft ray
61. Branched dorsal- and anal-fin rays:
  (61_0) = present
  (61_1) = absent

62. Number of rays supported by posteriormost proximal dorsal- and anal-fin pterygiophores:
(62_0) = two
(62_1) = one
63. Anterior dorsal-fin proximal pterygiophores:
(63_0) = separated from neurocranium
(63_1) = sutured to neurocranium
64. Dorsal-fin proximal pterygiophores:
(64_0) = not exposed
(64_1) = laterally exposed along first dorsal fin
(64_2) = laterally exposed along first and second dorsal fins
65. Number of supraneurals (ordered via additive binary coding):
(65_0) = three
(65_1) = two
(65_2) = one
(65_3) = zero
66. Dorsal-fin stay:
(66_0) = present (separate and ossified)
(66_1) = present (fused with proximal pterygiophore and ossified)
(66_2) = present (cartilage)
(66_3) = absent
67. Anal-fin stay:
(67_0) = present (separate and ossified)
(67_1) = present (fused with proximal pterygiophore and ossified)
(67_2) = present (cartilage)
(67_3) = absent
68. First and second hypurals:
(68_0) = separated
(68_1) = continuous
69. Third and fourth hypurals:
(69_0) = separated
(69_1) = continuous
70. Fifth hypural:
(70_0) = present
(70_1) = absent
71. Lower hypural plate and parhypural:
(71_0) = separated
(71_1) = fused
72. Hemal spine and third preural centrum:
(72_0) = separated
(72_1) = fused
73. Hemal spine and second preural centrum:
(73_0) = separated
(73_1) = fused
74. Urostyle and upper hypural plate:
(74_0) = separated
(74_1) = fused
75. Urostyle and lower hypural plate:
(75_0) = separated
(75_1) = fused
76. Uroneural:
(76_0) = present
(76_1) = absent
77. Number of epurals:
(77_0) = three
(77_1) = two
(77_2) = one
78. Branched caudal-fin rays:
(78_0) = present
(78_1) = absent
79. Posterior end of *adductor mandibulae* section one:
(79_0) = connected to preopercle (and hyomandibula)
(79_1) = connected only to hyomandibula
(79_2) = free from posterior bony element(s)
80. *Adductor mandibulae* section one:
(80_0) = absent
(80_1) = present
81. Origin of *adductor mandibulae* section two-three:
(81_0) = lateral to *levator arcus palatini*
(81_1) = partially medial to *levator arcus palatini*
(81_2) = completely medial to *levator arcus palatini*
(81_3) = not closely associated with *levator arcus palatini*
82. Position of *adductor arcus palatini* (ordered via additive binary coding):
(82_0) = dorsal surface of entopterygoid
(82_1) = medial margin of entopterygoid
(82_2) = ventral surface of entopterygoid
83. Origin of *levator operculi* (ordered via additive binary coding):
(83_0) = on pterotic
(83_1) = on pterotic and posttemporal
(83_2) = on posttemporal
84. *Hyohyoides inferioris*:
(84_0) = absent
(84_1) = present
85. Anterior portion of *transversus dorsalis* anterior:
(85_0) = branched
(85_1) = unbranched
86. Posterior portion of *transversus dorsalis* anterior (ordered via additive binary coding; modified from Imamura, 2004):
(86_0) = unbranched
(86_1) = branched, not continuous with anterior branch of same muscle
(86_2) = branched, continuous with anterior branch of same muscle, comprising circular muscle elements
87. *Obliquus dorsalis* II:
(87_0) = absent
(87_1) = present
88. Posterior *levator internus*:
(88_0) = lateral to *obliquus dorsalis*
(88_1) = sandwiched by *obliquus dorsalis*
89. *Levator externus* III:
(89_0) = present
(89_1) = absent
90. *Levator posterior*:
(90_0) = present
(90_1) = absent
91. *Adductores* I–III:
(91_0) = absent
(91_1) = present
92. *Transversus ventralis* anterior and posterior:
(92_0) = overlapping
(92_1) = separated
93. *Transversus ventralis posterior*:
(93_0) = without a tendon anteriorly
(93_1) = with a tendon anteriorly
94. *Protractor pectoralis*:
(94_0) = sheet-like muscle only
(94_1) = composed of sheet-like anterior and robust posterior elements
95. Division of bundles of *adductor superficialis* serving free pectoral-fin rays:
(95_0) = absent
(95_1) = present

96. Origin of *coracoradialis*:
(96_0) = posteromedial face of posterior process of coracoid
(96_1) = posteromedial face of posterior process of coracoid and posterolateral face of posteroventral process of cleithrum
97. Dorsal elements of pelvic-fin muscles:
(97_0) = not attached to pectoral girdle
(97_1) = attached to pectoral girdle
98. *Extensor proprius*:
(98_0) = present
(98_1) = absent
99. *Flexor ventralis externus*:
(99_0) = present
(99_1) = absent
100. *Adductor dorsalis*:
(100_0) = present
(100_1) = absent
101. Extrinsic muscle:
(101_0) = absent
(101_1) = present, connected to neurocranium anteriorly and gas bladder posteriorly
(101_2) = present, connected to neurocranium anteriorly and vertebrae posteriorly
(101_3) = present, free from neurocranium anteriorly and connected to vertebrae posteriorly
102. Intrinsic muscle:
(102_0) = absent
(102_1) = present
103. *Obliquus superioris*:
(103_0) = extending to neurocranium
(103_1) = not extending to neurocranium
104. *Obliquus superioris* and Baudelot's ligament:
(104_0) = *obliquus superioris* penetrated by Baudelot's ligament
(104_1) = *obliquus superioris* bypassing and lying ventrally to Baudelot's ligament
105. *Supracarinalis* anterior:
(105_0) = present
(105_1) = absent
106. *Inclinator dorsalis* associated with first dorsal spine:
(106_0) = present
(106_1) = absent
107. Gill membranes (ordered via additive binary coding; modified from Imamura, 2004):
(107_0) = free from isthmus
(107_1) = gill opening wide
(107_2) = broadly fused with isthmus, gill opening narrow
108. Sensory ducts in lateral line:
(108_0) = simple
(108_1) = with two or more branches
109. Body lateral line:
(109_0) = with scales
(109_1) = with tube-like bones
(109_2) = with bony plates
110. Spines on lateral-line scales:
(110_0) = absent
(110_1) = present
111. Body scales:
(111_0) = present
(111_1) = absent except in dorsal region
(111_2) = entirely absent
(111_3) = present as bony plates
112. Caudal peduncle:
(112_0) = straight
(112_1) = bent dorsally
113. Lachrymal saber:
(113_0) = absent
(113_1) = present

APPENDIX 2

Proposed subordinal, familial, and subfamilial classification, morphological diagnoses, support, and composition of the flatheads, scorpionfishes, sea robins, and stonefishes

CONGIOPODOIDEI

Congiopodidae Gill, 1889

Type genus: *Congiopodus* Perry, 1811

Sister taxon: Cottoidei + Zoarcoidei

Concept and content: Eight species classified in three genera: *Alertichthys, Congiopodus,* and *Zanclorhynchus*

Phenotypic diagnosis: Loss of fourth or fifth circumorbital (character 5, state 2); loss of fourth circumorbital (character 9, state 1); interacalar and posttemporal sutured together (character 21, state 1); lateral lateral-line canal pore on pterotic absent (character 22, state 0); Baudelot's ligament absent (character 25, state 3); palatine and ectopterygoid separated (character 29, state 1); teeth on palatine absent (character 30, state 1); uroneural absent (character 76, state 1); two epurals (character 77, state 1); *levator posterior* absent (character 90, state 1); gill membranes broadly fused with isthmus, gill opening narrow (character 107, state 2); body lateral line with tube-like bones (character 109, state 1).

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

Systematic comment: Familial and generic composition and species recognition follow Eschmeyer et al. (2017).

SCORPAENOIDEI

Bembridae Kaup, 1873

Type genus: *Bembras* Cuvier in Cuvier and Valenciennes, 1829

Sister taxon: Neosebastidae + Plectrogeniidae + Scorpaenidae + Synanceiidae

Concept and content: Nine species classified in four genera: *Bambradon, Bembras, Brachybembras,* and *Parabembras*

Phenotypic diagnosis: First and second hypurals are separated (character 68, state 0).

Support statistics: The bootstrap support for this clade was 0.70.

Systematic comment: This revised Bembridae includes the recently recognized Parabembridae because our phylogeny and previous work (e.g., Near et al., 2013) have resolved these two families as a single clade. We retain *Bambradon* and *Brachybembras* in Bembridae following Eschmeyer et al. (2017). Additional work on their placement is warranted. Generic composition and species recognition follow Eschmeyer et al. (2017).

Hoplichthyidae Kaup, 1873

Type genus: *Hoplichthys* Cuvier in Cuvier and Valenciennes, 1829

Sister taxon: Triglidae

Concept and content: 17 species classified in one genus: *Hoplichthys*

Phenotypic diagnosis: Middle portion of second circumorbital sensory canal bridge present with more than two

sensory openings (character 3, state 3); basisphenoid absent (character 19, state 2); prootic and intercalar in contact (character 20, state 1); lateral lateral-line canal pore on pterotic absent (character 22, state 0); skinny lateral-line sensory canal between pterotic and preopercle present (character 23, state 1); Baudelot's ligament absent (character 25, state 3); metapterygoid lamina absent (character 32, state 1); rudimentary to no space between metapteryoid and hyomandibula (character 33, state 1); first pharyngobranchial cartilaginous (character 41, state 1); tooth plate on second pharyngobranchial absent (character 42, state 1); dorsal spines slender (character 57, state 1); lower hypural plate and parhypural fused (character 71, state 1); hemal spine and third preural centrum fused (character 72, state 1); hemal spine and second preural centrum fused (character 73, state 1); *levator externus* III absent (character 89, state 1); *levator posterior* absent (character 90, state 1); gill opening wide (character 107, state 1); body lateral line with bony plates (character 109, state 2); spines on lateral-line scales present (character 110, state 1); body scales absent (character 111, state 1).

Support statistics: This family was represented by a single taxon in the analysis, so it does not have bootstrap support values.

Systematic comment: Familial and generic composition and species recognition follow Eschmeyer et al. (2017).

Neosebastidae Matsubara, 1943

Type genus: *Neosebastes* Guichenot, 1867

Sister taxon: Plectrogeniidae

Concept and content: 18 species classified in two genera: *Maxillicosta* and *Neosebastes*

Phenotypic diagnosis: No unambiguously optimized morphological synapomorphies diagnose Neosebastidae, although the family is supported by a morphological transformation through accelerated character optimization.

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

Systematic comment: Familial and generic composition and species recognition follow Eschmeyer et al. (2017).

Platycephalidae Swainson, 1839

Type genus: *Platycephalus* Bloch, 1795

Sister taxon: Hoplichthyidae + Triglidae

Concept and content: 84 species classified in two subfamilies and 17 genera: Oniigociinae: *Ambiserrula, Cociella, Cymbacephalus, Grammoplites, Inegocia, Kumococius, Onigocia, Papilloculiceps, Ratabulus, Rogadius, Solitas, Suggrundus, Sunagocia,* and *Thysanophrys*; Platycephalinae: *Elates* and *Platycephalus; incertae sedis: Leviprora*

Phenotypic diagnosis: No unambiguously optimized morphological synapomorphies diagnose Platycephalidae, although the family is supported by a morphological transformation through accelerated character optimization.

Support statistics: The bootstrap support for this clade was 0.97.

Systematic comment: Familial and subfamilial classification follows Imamura (1996). Generic composition and species recognition follow Eschmeyer et al. (2017) except for the synonymy of *Sorsogona* in *Ratabulus* following Imamura (1996).

Plectrogeniidae Fowler, 1938

Type genus: *Plectrogenium* Gilbert, 1905

Sister taxon: Neosebastidae

Concept and content: Four species classified in two genera: *Bembradium* and *Plectrogenium*

Phenotypic diagnosis: Skinny lateral-line sensory canal between pterotic and preopercle absent (character 23, state 1); posterior pelvic fossa present, fossae meeting (character 52, state 1); dorsal-fin stay cartilaginous (character 66, state 2); anal-fin stay cartilaginous (character 67, state 2); *hyohyoides inferioris* present (character 84, state 1).

Support statistics: The bootstrap support for this clade was 0.34.

Systematic comment: Familial classification follows Imamura (1996). Generic composition and species recognition follow Eschmeyer et al. (2017).

Scorpaenidae Risso, 1827

Type genus: *Scorpaena* Linnaeus, 1758

Sister taxon: Neosebastidae + Plectrogeniidae

Concept and content: 371 species classified in four subfamilies and 34 genera: Pteroinae: *Brachypterois, Dendrochirus, Ebosia, Parapterois,* and *Pterois*; Scorpaeninae: *Caracanthus, Iracundus, Parascorpaena, Pteroidichthys, Scorpaena, Scorpaenopsis, Sebastapistes,* and *Taenianotus*; Sebastinae: *Helicolenus, Hozukius, Sebastes,* and *Sebastiscus*; Setarchinae: *Ectreposebastes, Lioscorpius,* and *Setarches*; *incertae sedis: Adelosebastes, Hipposcorpaena, Hoplosebastes, Idiastion, Neomerinthe, Neoscorpaena, Phenacoscorpius, Pogonoscorpius, Pontinus, Rhinopias, Scorpaenodes, Sebastolobus, Trachyscorpia,* and *Ursinoscorpaenopsis*

Phenotypic diagnosis: Posterior opening of third circumorbital posteriorly directed (character 6, state 1); sensory canal of third circumorbital separate/not continuous with fourth or fifth circumorbital (character 11, state 1); *transversus ventralis posterior* with a tendon anteriorly (character 93, state 1).

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

Systematic comment: Familial and subfamilial classification based on the resulting phylogeny in this study. *Iracundus* included in Scorpaeninae following Smith and Craig (2007), and *Parascorpaena* and *Sebastapistes* included in Scorpaeninae following Lautredou et al. (2013). Generic composition and species recognition follow Eschmeyer et al. (2017).

Synanceiidae Swainson, 1839

Type genus: *Synanceia* Bloch and Schneider, 1801

Sister taxon: Neosebastidae + Plectrogeniidae + Scorpaenidae

Concept and content: 134 species classified in seven subfamilies and 53 genera: Apistinae: *Apistops*, *Apistus*, and *Cheroscorpaena*; Aploactininae: *Acanthosphex, Adventor, Aploactis, Aploactisoma, Bathyaploactis, Cocotropus, Erisphex, Kanekonia, Matsubarichthys, Neoaploactis, Paraploactis, Peristrominous, Prosoproctus, Pseudopataecus, Ptarmus, Sthenopus,* and *Xenaploactis*; Eschmeyerinae: *Eschmeyer*; Gnathanacanthinae: *Gnathanacanthus*; Pataecinae: *Aetapcus, Neopataecus*, and *Pataecus*; Perryeninae: *Perryena*; Synanceiinae: *Choridactylus, Dampierosa, Erosa, Inimicus, Leptosynanceia, Minous, Pseudosynanceia, Synanceia,* and *Trachicephalus; incertae sedis: Ablabys, Centropogon, Coccotropsis, Cottapistus, Glyptauchen, Gymnapistes, Liocranium, Neocentropogon, Neovespicula, Notesthes, Ocosia, Paracentropogon, Pseudovespicula, Richardsonichthys, Snyderina, Tetraroge, Trichosomus*, and *Vespicula*

Phenotypic diagnosis: Sixth circumorbital fused to sphenotic (character 10, state 1); metapterygoid lamina absent (character 32, state 1); lower hypural plate and parhypural fused (character 71, state 1); origin of *adductor*

mandibulae section two-three partially medial to *levator arcus palatini* (character 81, state 1); origin of *levator operculi* on pterotic and posttemporal (character 83, state 1); lachrymal saber present (character 113, state 1).

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

Systematic comment: Familial and subfamilial classification based on the resulting phylogeny in this study. Subfamilial composition follows familial composition of Eschmeyer et al. (2017) except for their Tetrarogidae. Generic composition and species recognition follow Eschmeyer et al. (2017).

Triglidae Rafinesque, 1815

Type genus: *Trigla* Linnaeus, 1758

Sister taxon: Hoplichthyidae

Concept and content: 173 species classified in four subfamilies and 15 genera: Peristediinae: *Gargariscus, Heminodus, Paraheminodus, Peristedion, Satyrichthys,* and *Scalicus*; Prionotinae: *Bellator* and *Prionotus*; Pterygotriglinae: *Bovitrigla* and *Pterygotrigla*; Triglinae: *Chelidonichthys, Eutrigla, Lepidotrigla, Trigla,* and *Trigloporus*

Phenotypic diagnosis: First and third circumorbitals attached (character 2, state 1); third circumorbital and lateral ethmoid attached (character 4, state 1); nasal and neurocranium sutured (character 14, state 1); notch between ascending process and cranial condyle absent (character 27, state 1); *transversus ventralis anterior* and *posterior* separated (character 92, state 1); *transversus ventralis posterior* with a tendon anteriorly (character 93, state 1); division of bundles of *adductor superficialis* serving free pectoral-fin rays present (character 95, state 1).

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

Systematic comment: Familial classification and treatment of Peristediinae as a triglid subfamily follows our phylogeny and the phylogeny from Portnoy et al. (2017). The remaining triglid subfamilies follow the tribes from Richards and Jones (2002). Generic composition and species recognition follow Kawai (2008) and Eschmeyer et al. (2017).