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SYMPOSIUM ARTICLE

Evolution of Venomous Cartilaginous and Ray-Finned Fishes

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Synopsis Venom and its associated delivery systems have evolved in numerous animal groups ranging from jellyfishes to spiders, lizards, shrews, and the male platypus. Building off new data and previously published anatomical and molecular studies, we explore the evolution of and variation within venomous fishes. We show the results of the first multi-locus, ordinal-level phylogenetic analysis of cartilaginous (Chondrichthyes) and ray-finned (Actinopterygii) fishes that hypothesizes 18 independent evolutions of this specialization. Ancestral-states reconstruction indicates that among the 2386–2962 extant venomous fishes, envenomed structures have evolved four times in cartilaginous fishes, once in eels (Anguilliformes), once in catfishes (Siluriformes), and 12 times in spiny-rayed fishes (Acanthomorpha). From our anatomical studies and phylogenetic reconstruction, we show that dorsal spines are the most common envenomed structures (~95% of venomous fish species and 15 independent evolutions). In addition to envenomed spines, fishes have also evolved venomous fangs (2% of venomous fish species, two independent evolutions), cleithral spines (2% of venomous fish species, one independent evolution), and opercular or subopercular spines (1% of venomous fish species, three independent evolutions).

Introduction

Many animals use venoms—toxins injected using specialized delivery structures—for interactions with predators, prey, and conspecifics. Venomous organisms are captivating because of the potential for their toxins to kill or debilitate people and other animals. Worldwide, venomous creatures inflict countless stings, bites, and barbs. Venomous vertebrates alone cause an estimated 2.7 million venom-related injuries per year with symptoms ranging from blisters to intense pain, fever, and death (Chippaux 1998; Halstead 1988; Vetrano et al. 2002; Haddad et al. 2003). However, the chemical properties that make venoms so dangerous and fascinating, if harnessed and studied, have the potential to serve as natural products for the development of beneficial pharmaceuticals and physiological tools (Tan et al. 2003; Ault 2004; Fox and Serrano 2007; Trim and Trim 2013). Despite the potential benefits of studying venoms for both human-health and pharmaceutical advances, many venomous groups remain understudied. Studying these tens of thousands of neglected venomous animals and their phylogenetic history is critical for understanding the diversity and evolution of all venomous systems without the bias inherent in focusing on charismatic venomous groups (von Reumont et al. 2014).

Beyond its toxicity, venom is fascinating from an evolutionary perspective because of the remarkable diversity of organisms that have independently evolved the ability to inject these potent chemicals. This specialization is found in more than 100,000 species spread across more than 20 major groups of animals (Calvete et al. 2009; Casewell et al. 2013; von Reumont et al. 2014). Among vertebrates, cartilaginous fishes (Chondrichthyes); tree frogs (Hylinae); squamates (Toxicofera); the platypus (Monotremata); primates, shrews, and solenodons (Boreoeutheria); and eels, catfishes, and spiny-rayed fishes (Teleostei) have all evolved venoms, often multiple times within each clade (Fry et al. 2006; Smith and Wheeler 2006; Casewell et al. 2013;

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Jared et al. 2015). Our understanding of venomous systems and the venoms themselves in terrestrial vertebrates, particularly snakes, is substantive (reviewed in Fry et al. 2012), whereas, our understanding of piscine venom systems remains limited (Church and Hodgson 2002; Sivan 2009; Wright 2015; Ziegman and Alewood 2015). This limited understanding is partially due to the recent recognition that there are considerably more venomous fishes than previously noted (Smith and Wheeler 2006; Wright 2009). Historically, the number of venomous fishes has been reported as approximately 200 species (Halstead 1970, 1988; Church and Hodgson 2002; Haddad et al. 2003), but recent work (Smith and Wheeler 2006; Wright 2009) has expanded that number to at least 2000 venomous cartilaginous and ray-finned fishes (hereafter fishes). This increase in known venomous fish diversity has resulted in a roughly equivalent distribution of venomous vertebrate species between terrestrial and aquatic environments (Fry et al. 2006; Smith and Wheeler 2006; Wright 2009; Casewell et al. 2013). With our current taxonomy (Nelson 2006), the estimated 2000 species of venomous fishes are classified in nine orders (Smith and Wheeler 2006; Wright 2009). However, recent fish phylogenies (e.g., Vélez-Zuazo and Agnarsson 2011; Near et al. 2012, 2013; Betancur-R et al. 2013; Tang and Fielitz 2013) have dramatically altered our understanding of venomous fish relationships and provided the comparative data necessary to explore broad-scale evolutionary questions across fishes (e.g., Price et al. 2014; Sparks et al. 2014). These shifts in our understanding of fish relationships and the corresponding departure from the traditional classification (Nelson 2006) highlight our need to reassess this phenomenon and explore its evolution in a single phylogenetic framework across fishes rather than a series of isolated studies of venomous orders and clades.

Given progress on the identity of venomous fishes (Smith and Wheeler 2006; Wright 2009), advancements in our studies of the anatomy of envenomed or potentially envenomed structures (Egge and Simons 2011; Wright 2015), the discovery of new venomous groups (Conway et al. 2014), and recent advances in our understanding of fish relationships (e.g., Vélez-Zuazo and Agnarsson 2011; Near et al. 2012), the time is right for a phylogenetic investigation of the evolution and distribution of venom across both cartilaginous and ray-finned fishes. The resulting phylogenetic framework will not only be critical for exploring the evolution of this feature across fishes, but it will provide a comprehensive and updated framework for exploring venoms for future anatomical, venomic, and pharmaceutical investigations (Smith and Wheeler 2006; Wright 2009; Vetter et al. 2011). To resolve relationships among venomous fishes, we analyzed a dataset with 7067 aligned base pairs for 388 species in a phylogenetic analysis across vertebrates that included representatives of all fish orders and all venomous fish groups. Using the resulting hypothesis, the objectives of this study are to: (1) produce the first multi-locus phylogeny of all orders of cartilaginous and ray-finned fishes, (2) identify the number of evolutionary origins of venom in fishes by delimiting all venomous clades, (3) determine the number and identity of venomous fish species, and (4) explore the anatomical and behavioral similarities and variation across these disparate venomous fish groups.

Materials and methods

Taxon sampling

The 388 species analyzed in this study included representatives of 290 families (Nelson 2006) from across Craniata. The analysis includes every order and all but 10 suborders of fishes. Representatives of the Chlorophthalmoidei, Denticipitoidei, Elassomatoidei, Giganturoidei, Hexagrammoidei, Muraenoidei, Normanichthyoidei, Pholidichthyoidei, Phosichthyoidei, and Scombrolabracoidei were not included. Analyses were rooted with a hagfish (*Eptatretus burgeri*). Representatives of all nine venomous orders of cartilaginous and ray-finned fishes were included in the analyses: chimaeriform ratfishes; heterodontiform sharks; squaliform dogfishes; myliobatiform stingrays; monognathid eels; siluriform catfishes; thalassophrynine toadfishes; scorpaeniform stonefishes and scorpionfishes; and perciform blennies, clingfishes, jacks, stargazers, and surgeonfishes. Species considered venomous in the included molecular analyses have had the presence of venom or venom glands confirmed, or it has been confirmed in one of their congeners or close allies (Halstead 1970, 1988; Bertelsen and Nielsen 1987; Smith-Vaniz et al. 2001; Church and Hodgson 2002; Sosa-Rosales et al. 2005; Smith and Wheeler 2006; Wright 2009; Conway et al. 2014; current study). Institutional abbreviations for museum and collection acronyms used for anatomical and tissue vouchers follow Sabaj Pérez (2014).

Character sampling

A total of 7067 aligned nucleotides were analyzed from three mitochondrial and five nuclear loci that have been previously shown to be effective at resolving relationships among diverse fishes (Grande et al.

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2013; Davis et al. 2014; Smith and Busby 2014). The molecular terminals analyzed in the present study and GenBank accession numbers corresponding to the included fragments are listed in the Supplementary Information. A total of 242 novel DNA sequences (GenBank numbers and voucher information for all new sequences can be found in Supplementary Information) were combined with previously published DNA sequences for this analysis with most of the existing data coming from Near et al. (2012, 2013) and Wainwright et al. (2012). The final molecular matrix was 77% complete at the amplicon level and 74% complete at the cell or individual-base-pair level (Supplementary Information).

Acquisition of nucleotide sequences

Fish tissues were preserved in 95% ethanol or frozen prior to extraction of DNA. Genomic DNA was extracted from muscle or fin clips using a DNeasy Tissue Extraction Kit (Qiagen, Valencia, CA). 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, Piscataway, NJ), 1.25 μL of each primer (10 pmol), and 2–5 μL of undiluted DNA extract. To amplify and sequence these gene fragments, the following primers were used: 16S (5′-CGCCTGTTTATCAAAAACAT-3′, 5′-CCGGTCTGAACTCAGATCACGT-3′; Kocher et al. 1989; Palumbi 1996); COI (5′-GGTCAACAAATCATAAAGATATTG-3′, 5′-TAAACTTCAGGGTGACCA-3′; Folmer et al. 1994); RAG1 (5′-CTGAGCTGCAGTCAGTACCATAAGATGT-3′, 5′-CTGAGTCTTGGAGCTTCCATRAAYTT-3′; López et al. 2004); ENC1 (5′-GACATGCTGGAGTTTCAGGA-3′, 5′-ACTTGTTRGCMACTGGGTCAAA-3′; Li et al. 2007); Glyt (5′-GGACTGTCMAAGATGACCACMT-3′, 5′-CCCAAGAGGTTCTTGTTRAAGAT-3′; Li et al. 2007); zic1 (5′-GGACGCAGGACCGCARTAYC-3′, 5′-CTGTGTGTGTCCTTTTGTGRATYTT-3′; Li et al. 2007); and plagl2 (5′-CCACACACTCYCCACAGAA-3′, 5′-TTCTCAAGCAGGTATGAGGTAGA-3′; Li et al. 2007). Amplifications for mitochondrial 16S and COI were carried out in 36 cycles using the following temperature profile: initial denaturation for 6 min at 94°C; 36 cycles of denaturation for 60 s at 94°C, annealing for 60 s at 46–48°C, and extension for 75 s at 72°C; and a final terminal extension at 72°C for 6 min. All of the NADH dehydrogenase 2 (ND2) sequences were taken from GenBank (Supplementary Information), so we did not amplify any ND2 sequences. For the nuclear genes, the following temperature profile was used: initial denaturation for 3 min at 94°C; 10 cycles of denaturation for 45 s at 94°C, annealing for 45 s at 57–58°C, and extension for 75 s at 72°C; 30 cycles of denaturation for 45 s at 94°C, annealing for 30 s at 55–57°C, and extension for 75 s at 72°C; and a final terminal extension at 72°C for 6 min. The double-stranded amplification products were desalted and concentrated using AMPure (Agencourt Biosciences, Beverly, MA). The purified PCR products were used as templates and amplified for sequencing using the PCR amplification primers and a Prism Dye Terminator Reaction Kit Version 1.1 (Applied Biosystems, Foster City, CA). The sequencing reactions were cleaned and desalted using cleanSEQ (Beckman Coulter, Beverly, MA). 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 Version 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 KX230144–KX230385.

Phylogenetic analysis

Partitioned likelihood analyses were used to analyze the molecular data. The dataset was concatenated and examined in Mesquite v3.04 (Maddison and Maddison 2015). For this analysis, each of the eight amplicons was aligned individually in MAFFT (Katoh et al. 2002) using default values. The dataset was broken into 22 partitions. One partition was designated for the mitochondrial 16S fragment and 21 partitions represented the three codon positions in each of the seven protein-coding genes: mitochondrial COI and ND2 genes and nuclear RAG1, ENC1, Glyt, zic1, and plagl2 genes. All partitions were assigned a GTR + G substitution model following recommendations of Darriba and Posada (in review). The maximum-likelihood analysis was conducted in GARLI v2.01 (Zwickl 2006), and the tree with the best likelihood score from 35 independent analyses was selected as the preferred hypothesis. The monophyly of the traditionally recognized Osteichthyes, Actinopterygii, and Teleostei was constrained to expedite searches and to reduce complexities associated with comparatively limited overlap in the sampled genes among Chondrichthyes, Sarcopterygii, and Actinopterygii (Supplementary Information). A non-parametric maximum-likelihood bootstrap analysis was conducted for 200 random pseudoreplicates to assess nodal support. We recognize two levels of

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nodal support: 70% bootstrap support represents a moderately supported node or clade and 95% bootstrap support represents a well-supported node or clade. Likelihood ancestral-character-state reconstructions for the evolution of venom (0: absent; 1: present; built from known occurrences in fishes from sources above) were performed in Mesquite v3.04 (Maddison and Maddison 2015).

Morphological examination

We examined preserved museum specimens for the presence of both a venom delivery structure (e.g., spine, teeth) and a discrete venom gland. Previous studies have shown that both the gland and the delivery system are visible by dissection in the majority of fish groups, but the venom glands of most catfishes were typically obscured or too small to be examined under a dissecting microscope (Halstead 1988; Wright 2009). The presence or absence of a venom apparatus and its associated gland(s) was examined in 90 museum specimens spread across 58 families that built upon previous studies (Halstead 1988; Smith and Wheeler 2006; Wright 2009). Our sampling focused on species that were predicted to be venomous, species listed as possibly venomous by Halstead (1970), or species that were closely allied to the venomous clades recovered in our phylogeny. Using the optimization of venom-presence from our likelihood analysis and the current number of described species in each clade (Eschmeyer et al. 2016), we estimated the number of venomous fishes in each group. If the distribution of venom within a small clade that lacks diagnosed subgroups (e.g., surgeonfishes in the genus *Acanthurus*) was unclear because both venomous and non-venomous forms have been noted, a range is given (Supplementary Information).

Results

The likelihood analysis resulted in an optimal topology (lnL = –567054.73; Fig. 1) with 62% of nodes being moderately supported and 46% of nodes being well supported (Supplementary Information). Based on the ancestral-states reconstruction on the optimal maximum-likelihood phylogeny, venom apparatuses have evolved 18 independent times across fishes (Fig. 1; Supplementary Information): chimaeras (Chimaeriformes), stingrays (Myliobatoidei), hornsharks (some heterodontiforms), dogfishes and allies (some squaliforms), one-jawed eels (Monognathidae), catfishes (Callichthyidae + some siluroids), toadfishes (some porichthyines + Thalassophryninae), leatherjacket jacks (Scomberoidini), fang-tooth blennies (*Meiacanthus*), clingfishes (some gobiesocines), stargazers (Uranoscopidae), surgeonfishes (some acanthurids), scats (Scatophagidae), rabbitfishes (Siganidae), weeverfishes (Trachinidae), gurnard perches (Neosebastidae *sensu* Eschmeyer et al. [2016]), stonefishes and wasp fishes (Apistidae, some aploactinids, Eschmeyeridae, Gnathanacanthidae, Synanceiidae, and Tetrarogidae), and scorpionfishes (Scorpaenidae, Sebastidae, and Setarchidae *sensu* Eschmeyer et al. [2016]). In light of these findings, we identify a minimum of 50 (50–55) families (*sensu* Nelson 2006) or 58 (58–63) families (*sensu* Eschmeyer et al. 2016) of fishes that have venomous representatives (Supplementary Information). In addition to these independent evolutions, we see nine losses of a venom apparatus, mostly in siluroid catfishes (whalelike catfishes and allies [Cetopsidae]; banjo catfishes [Aspredinidae]; loach catfishes [Amphiliidae]; electric catfishes [Malapteruridae]; driftwood catfishes [Auchenipteridae]; heptapterids [Heptapteridae]; sisorid and erethistid catfishes [Sisoridae and Erethistidae]; prowfishes [Pataecidae]; manta rays [Myliobatidae]; and velvetfishes [Aploactinidae]; Supplementary Information). The combination of this phylogenetic hypothesis and the current diversity of fishes (Eschmeyer et al. 2016) suggest an increase in known venomous fish diversity to 2386–2962 venomous species (Supplementary Information).

Our morphological examination of 90 specimens from 58 families demonstrates that our molecular phylogeny is highly effective at predicting the presence or absence of venom glands in fishes. Of the 58 families examined in the morphological study, we were unable to find a conspicuous venom gland, grooved spines, or any indications of a venom apparatus in at least one representative of 42 families (Supplementary Information). Because of the diverse phylogenetic distribution of venomous fin spines, it is not surprising that there is variation in the morphology of these structures in venomous fishes (Fig. 2). The majority of venomous fishes have a fin spine with one to three longitudinal grooves with associated glandular tissue resting within the groove (Fig. 2). Venomous spines are found in the dorsal fins of the majority of venomous fishes, but envenomed spines can also be found in the pectoral, pelvic, and anal fins (Fig. 2A–G). This largely convergent anatomy of grooved spines can be seen across extant groups ranging from lanternsharks (Etmopteridae; Fig. 2A) to leatherjacket jacks (Carangidae: Scomberoidini; Fig. 2B). In addition to these more typical envenomed structures, we find the same previously described major modifications to the dorsal-spine venom apparatus in

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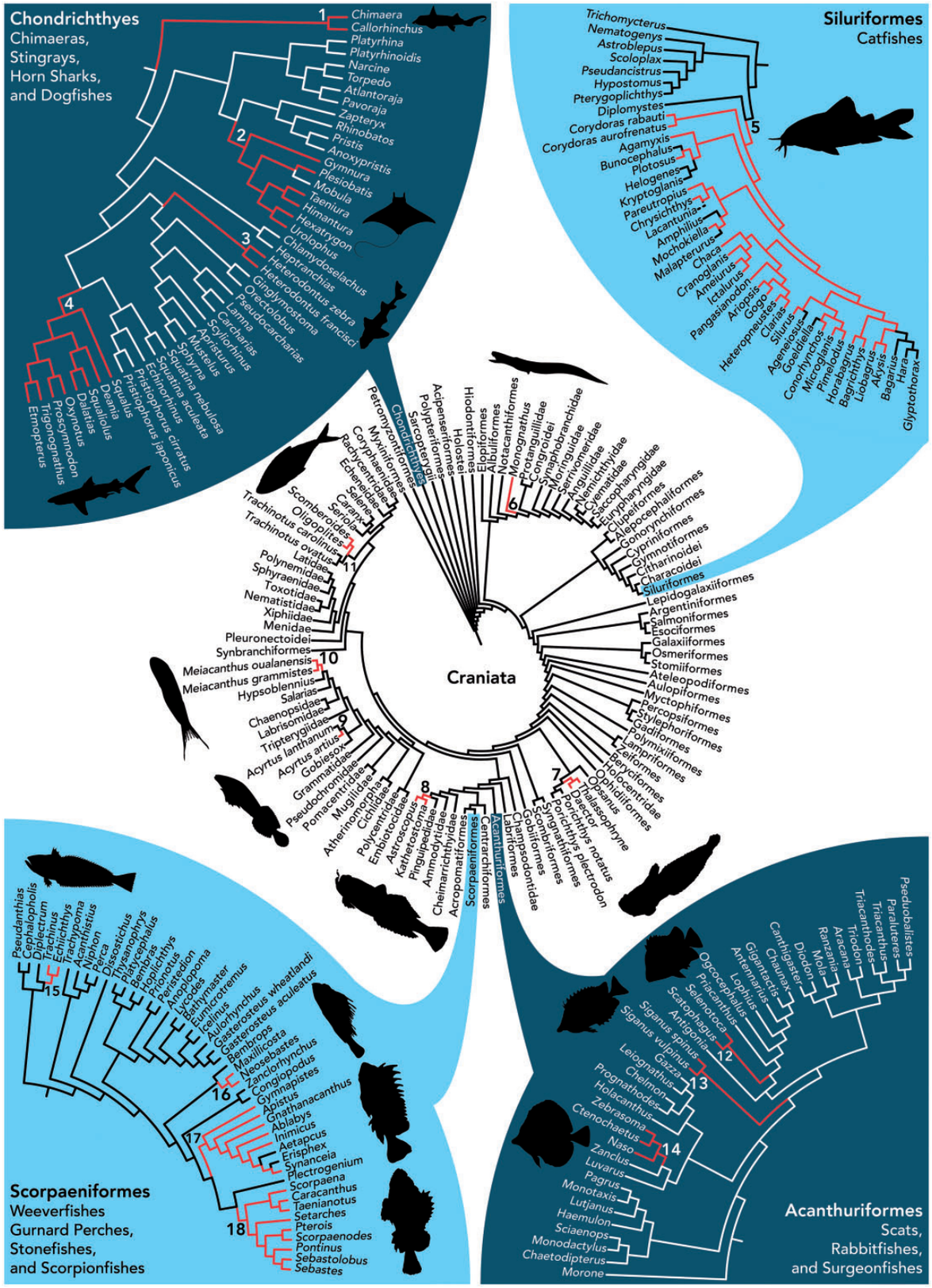

Fig. 1 Results of the maximum-likelihood phylogeny of venomous craniates with the ancestral-states reconstruction of venomous fishes highlighted in red (online) and in gray (in print), unknown states dashed, and non-venomous species in black (or white on dark background). All 18 venomous clades are accompanied by a silhouette and are numbered: 1–chimaeras; 2–stingrays; 3–horn sharks; 4–dogfishes; 5–catfishes; 6–one-jawed eels; 7– toadfishes; 8–stargazers; 9–clingfishes; 10–fang-tooth blennies; 11–leatherjacket jacks;

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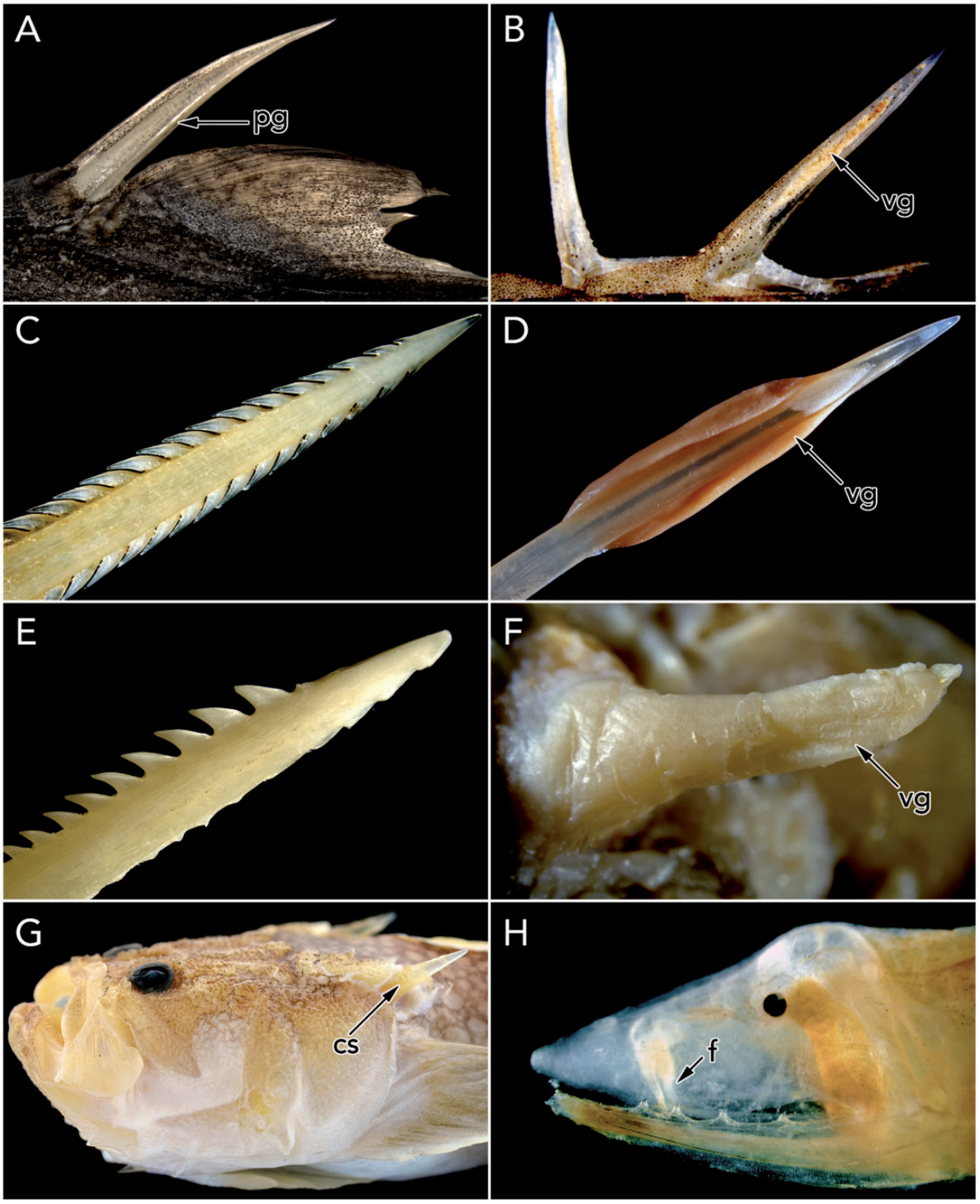

Fig. 2. Venom apparatus morphology. (**A**) Venomous dorsal spines from the lantern shark, *Etmopterus splendidus*, FMNH 120756. (**B**) Venomous dorsal spine from the jack, *Oligoplites saurus*, KU 17205. (**C**) Barbed dorsal spine of the stingray, *Taeniura lymma*, KU 29279. (**D**) Venomous dorsal spine with enlarged venom glands in the stonefish, *Synanceia verrucosa*, FMNH 75888. (**E**) Barbed pectoral spine of the sea catfish, *Sciades seemanni*, KU 20093. (**F**) Venomous opercular spine of the toadfish, *Daector schmitti*, KU 18413. (**G**) Cleithral spine of the stargazer, *Kathetostoma albigutta*, KU 27026. (**H**) Venomous fang of the one-jawed eel, *Monognathus rosenblatti*, SIO 87-29. Abbreviations: cs, cleithral spine; f, fang; pg, posterior groove; vg, venom gland.

Fig. 1 Continued

12–scats; 13–rabbitfishes; 14–surgeonfishes; 15–weeverfishes; 16–gurnard perches; 17–stonefishes; and 18–scorpionfishes. See Supplementary Information for support values, branch lengths, and specific species included in the analysis with associated higher-level classification.

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stingrays (barbed spines in Myliobatoidei; Fig. 2C), toadfishes (distinct venom gland surrounding dorsal spines in Batrachoididae: Thalassophyrinae), and stonefishes (distinct venom glands and ducts in Synanceiidae: Synanceiinae; Fig. 2D). Further, catfishes were often found to have barbs and other modifications to their envenomed pectoral-fin spines (barbed pectoral fin in Ariidae; Fig. 2E). In addition to fin spines, venom glands associated with opercular spines were examined in weeverfishes (Trachinidae) and toadfishes (Batrachoididae: Thalassophyrinae; Fig. 2F). Similarly, stargazers (Uranoscopidae) had venomous glands associated with a cleithral spine (Fig. 2G). Finally, two groups of fishes had venomous teeth: fang-tooth blennies (venomous fangs in lower jaws of *Meiacanthus*) and one-jawed eels (venomous rostral fang in Monognathidae; Fig. 2H).

Discussion

This is the first study to analyze all cartilaginous and bony fish orders together in a single, explicit, and multi-locus molecular phylogenetic study. The results of this analysis show that venom is distributed widely across fishes with 18 independent evolutions of envenomed structures, 58 or more (58–63) venomous families (*sensu* Nelson 2006), and 7–9% of all fish species expected to be venomous. Using our phylogenetic hypothesis, we were able to refine our anatomical understanding of envenomed structures and explore the evolution of this system and morphological variation within it. Because our phylogenetic results and other recent studies (e.g., Smith and Craig 2007; Betancur-R et al. 2013; Near et al. 2013; Davis et al. 2016) have resulted in relationships that are inconsistent with the traditional fish classification, we will frequently use updated ordinal names for clarity (Fig. 1; Supplementary Information).

Phylogeny of venomous fishes

Our resulting phylogenetic hypothesis was similar to the results of recent studies of cartilaginous fishes with a few notable exceptions. Within the skates and rays (Batoidei), our ordinal relationships were similar to Aschliman et al. (2012) except that the skates (Rajiformes) were sister to electric rays (Torpediniformes) rather than sister to the electric rays and thornback rays (Platyrhiniformes). Our relationships among the venomous stingrays (Myliobatoidei) were different from Aschliman et al. (2012) in several ways, but like changes with the skates, these changes do not affect the evolution of venom within batoids (Fig. 1). Within the sharks (Selachimorpha), our order-level results were largely congruent with the results of Vélez-Zuazo and Agnarsson (2011) except that seven-gilled sharks (Hexanchiformes) were recovered sister to all other selachimorphs rather than being nested within the squalean sharks (Squalimorphii). Further, our relationships among the largely venomous dogfishes and allies (Squaliformes; Fig. 1; Supplementary Information), while different from the relationships found by Vélez-Zuazo and Agnarsson (2011), were congruent with the more densely sampled squaliform results of Straube et al. (2015) except for our placement of gulper sharks (Centrophoridae) sister to kite-fin sharks (Dalatiidae).

Among ray-finned fishes, our phylogenetic hypothesis was similar at the ordinal level (and higher) to recent large-scale phylogenies (e.g., Near et al. 2012, 2013; Betancur-R et al. 2013) with a few notable changes (e.g., ribbonfishes and allies [Lampriformes] sister to dories [Zeiformes], bony tongues and allies [Osteoglossomorpha] sister to all other teleosts). In addition to similarities at the higher levels, the relationships among the larger venomous clades were similar to other hypotheses focused on these clades (e.g., scorpionfishes [Scorpaenidae]; Smith and Wheeler 2004; Lautredou et al. 2013). As was found in Tang et al. (1999), Smith and Wheeler (2006), and Sanciangco et al. (2016), the current study recovered the venomous rabbitfishes and scats as independent clades, suggesting that these families independently evolved venom (Fig. 1). It is noteworthy that some studies (Holcroft and Wiley 2008; Betancur-R et al. 2013; Near et al. 2013) have recovered these two venomous families as a clade, so this potential relationship should continue to be examined. Finally, the relationships among the species-rich catfishes were similar to previous studies (e.g., Sullivan et al. 2006) where the larger clades (e.g., ''Big Africa,'' ''Big Asia'') were recovered as monophyletic. However, our results did have one significant change for venom evolution that has not been recovered in earlier morphological or molecular catfish phylogenies (e.g., Mo 1991; de Pinna 1993; Sullivan et al. 2006). We did not recover a monophyletic armored catfishes and allies (Loricarioidei). Instead, the one traditional loricarioid family that is venomous, the armored catfishes (Callichthyidae), was recovered as the sister group of the siluroid catfishes; this result suggests a single evolution of venom in catfishes at this callichthyid + siluroid node in our likelihood analysis (Fig. 1). Molecular studies (e.g., Sullivan et al. 2006) have typically recovered loricarioids sister to all other catfishes with Diplomystidae (velvet

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catfishes) sister to the remaining non-loricarioid catfishes. Our nuclear-gene-only analysis of the ostariophysans was included in this study to more carefully examine the placement of Callichthyidae (Supplementary Information). This analysis retains the separation of Callichthyidae from the remainder of the loricarioids as was seen in our combined analysis (Fig. 1). In the nuclear-only analysis, Callichthyidae is sister to all non-loricarioid catfishes. In contrast, morphological studies have typically recovered Diplomystidae sister to all other extant catfishes with a monophyletic Loricarioidei nested among the remaining siluriforms (e.g., Mo 1991; de Pinna 1993). While morphological data support the placement of Callichthyidae within the Loricarioidei, de Pinna (1993) did note that callichthyids share conical (vs. bifid) teeth with non-loricarioid catfishes. Additionally, callichthyids share the *retractor tentaculi* originating from the posterior portion of the suspensorium (vs. originating from the frontals or lateral ethmoids) and the absence of a discrete metapterygoid-palatine ligament (vs. presence) with all catfishes except non-nematogeniid loricarioids. With the current molecular phylogenetic results, the presence of venom in callichthyids and non-loricarioid catfishes, and limited soft-tissue and dental support, the separation of the callichthyids from the remainder of the loricarioids should be explored further. Clearly, more in-depth work on the phylogeny of catfishes is needed.

Evolution of venomous fishes

Our ancestral-states reconstruction on the optimal maximum-likelihood hypothesis suggests that venom glands have evolved 18 independent times across fishes (Fig. 1). Smith and Wheeler (2006) hypothesized 11 independent evolutions of venom across spiny-rayed fishes and suggested nine other families or orders of fishes that have evolved venoms. Wright (2009) hypothesized two to three evolutions of venom within the species-rich siluriform catfishes. No molecular studies have explicitly assessed the evolution of venom in cartilaginous fishes, one-jawed eels, or clingfishes in a broader phylogenetic context. Examining the distributions of all of these venomous groups on existing phylogenies (e.g., Smith and Wheeler 2006; Wright 2009; Vélez-Zuazo and Agnarsson 2011; Near et al. 2012) would suggest 19–20 independent evolutions of envenomed structures, so the resulting hypothesis of 18 independent evolutions refines the current understanding of venom evolution in fishes. This shift results primarily from the incorporation of all venomous fish groups in one analysis and refinements to the phylogeny of catfishes, specifically the transition from two to three independent gains in catfishes (Wright 2009) to a single evolution of venom in the ancestor of Callichthyidae + Siluroidei. The likelihood ancestral-states reconstruction of venom evolution on the nuclear-gene-only dataset in ostariophysans is somewhat ambiguous as to whether there are one or two evolutions of venom among catfishes given variation in the placement of the Diplomystidae between the more comprehensive combined analysis and the smaller nuclear-gene-only analysis (Supplementary Information). Clearly, future work on catfish phylogenetics will have implications on the evolution of venom in this species-rich clade.

Diversity of venomous species

The current study provides the first explicit order-level phylogeny of fishes, which we used to delimit venomous clades. Using the predictive capabilities of phylogeny, prior knowledge of the distribution of venomous fishes, and surveys for the presence or absence of conspicuous venom glands, we have estimated the number and identity of venomous fishes (Supplementary Information). Our results expand beyond initial estimates of 200–250 venomous species by Halstead (1988), 1535–1850 venomous species by Smith and Wheeler (2006), and 2035–2475 venomous species by Wright (2009) to a revised estimate of 2386–2962 venomous fish species. This range should be considered as a minimum estimate as an ancestral-states reconstruction of envenomed structures is not necessarily the same as the number of independent evolutions of the venoms themselves among fishes. First, it is possible, or even likely, that venoms evolve prior to envenomed structures (Cameron and Endean 1973). For example, porichthyine toadfishes have opercular and dorsal spines, but there is no macroscopic evidence of venom glands in this subfamily (Smith and Wheeler 2006; current study). Interestingly, Lopes-Ferreira et al. (2014) revealed the presence of venom proteins in *Porichthys porosissimus*, which has traditionally been treated as a non-venomous toadfish. This highlights the need to expand our exploration of the diversity of venomous fishes and their close allies using transcriptomic and proteomic analyses. With this approach, we may find additional groups like porichthyine toadfishes that are venomous and allied with "known" venomous species. Second, most venomous animals have multiple venoms in their venom cocktail (e.g., Casewell et

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al. 2013; de Oliveira et al. 2015), so fine-scaled analyses of the diversity of venomous groups will continue to find additional venoms within known venomous groups and refine the identity of different piscine venoms within and between species (Chuang and Shiao 2014).

In light of our improved understanding of the diversity of venomous fishes, it is interesting to note that the distribution of venomous fishes is approximately equally divided between freshwater and marine habitats. Approximately 58% of venomous fishes are found in freshwater habitats (Supplementary Table S1; Halstead 1988; Eschmeyer et al. 2016). Despite having relatively similar species-level diversity, the diversity of independent venomous clades is not equally distributed between freshwater and marine habitats (Supplementary Table S1; Eschmeyer et al. 2016). Thirteen of the 18 venomous fish groups are found exclusively in marine or brackish environments (Chimaeriformes, Heterodontiformes, Squaliformes, Monognathidae, Scomberoidini, *Meiacanthus*, Gobiesocinae, Uranoscopidae, Acanthuridae, Siganidae, Trachinidae, Neosebastidae, and scorpionfishes [Scorpaenidae, Sebastidae, and Setarchidae]). This discrepancy is largely explained by the tremendous diversity of venomous catfishes (Callichthyidae + Siluroidei; Wright 2009, 2015). With our current estimates of fish diversity, catfishes represent more than 95% of all venomous freshwater fishes and approximately 58% of all venomous fishes.

Anatomical investigations of venomous fishes

Our results (Figs. 1 and 2; Supplementary Information) indicate that the most common venom apparatus found among the 2386–2962 venomous fishes is venom glands associated with fin spines. Venomous fin spines have convergently evolved in 15 independent clades and are found in 95% of venomous fish species including both cartilaginous and ray-finned fishes. Further, venomous opercular or subopercular spines have evolved in three clades of ray-finned fishes (1% of venomous fish species), venomous fangs have evolved in two clades of ray-finned fishes (2% of venomous fish species), and venomous cleithral spines have evolved in one clade of ray-finned fishes (2% of venomous fish species).

Venomous spines are found in the dorsal fins of the majority of venomous fishes, but they are often found in the pectoral fins of venomous catfishes (Siluriformes; Halstead 1988; Wright 2009, 2015) and the pelvic and anal fins of most venomous spiny-rayed fishes (Acanthomorpha; Halstead 1988; Smith and Wheeler 2006). Nearly all fishes with venomous spines (except the cartilaginous fishes, catfishes, toadfishes, and some stonefishes) have distinct anterolateral grooves on the lateral surfaces of the fin spines (Fig. 2) where the venom gland is situated. The anatomical convergence of venomous fin spines is remarkable among venomous spiny-rayed fishes. As described and documented by Halstead (1970, 1988), Smith and Wheeler (2006), and the current study, the venomous dorsal spines in jacks (Fig. 2B), gurnard perches, rabbitfishes (Smith and Wheeler 2006: fig. 3G), scats, most scorpionfishes, most stonefishes (Smith and Wheeler 2006: fig. 3E), tangs, and weeverfishes have converged on an anatomy where there are multiple anterolateral grooves on each fin spine that contain yellow to orange venomous tissue. The repeated evolution of these passive, grooved venomous spines in eight independent groups of spiny-rayed fishes suggests that this system is both functional and comparatively probable to evolve. The venomous dorsal-fin spines in catfishes are not visible macroscopically and require histological examination (Halstead 1988; Wright 2009; Egge and Simons 2011); however, venom glands in some catfishes have been identified macroscopically in their pectoral fins (Wright 2009: fig. 8). Interestingly, the only clades in which we predict a reversal from the presence to the absence of venomous fin-spine glands nested within anterolateral grooves (the velvetfishes and prowfishes [stonefishes]; Fig. 1) show a loss of the anterolateral grooves (Smith and Wheeler 2006: fig. 3D). Given the high fidelity of having grooved fin spines and being venomous in fishes and similar anatomical correlations in other vertebrates (e.g., Mitchell et al. 2010), it is possible, and even likely, that the varied fossil fish groups that have grooved fin spines (e.g., †hybodontiform sharks, †*Eosiganus* rabbitfishes; Tyler and Bannikov 1997; Wang et al. 2009) could also be venomous.

Defensive role of fish venoms

Venoms have evolved in more than 20 major groups of animals where they typically facilitate intraspecific behavior, defense, or, most commonly, feeding (Sunagar and Moran 2015). Among terrestrial vertebrates, most groups (except helodermatid lizards) use venoms as a foraging adaptation (Casewell et al. 2013). Interestingly, the primary use of vertebrate venoms in aquatic habitats generally, and among fishes specifically, is different from terrestrial vertebrates. Venomous cartilaginous and ray-finned fishes primarily use venom for defensive roles (Casewell et al. 2013; Sunagar and Moran 2015). Only two

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venomous fish groups use venom in a predominantly feeding role (one-jawed eel and fang-toothed blennies), and these two clades are comparatively depauperate, representing less than 2% of all venomous fishes. Given these data, previous suggestions that venoms play a primarily defensive role in fishes are corroborated. This defensive use in fishes is consistent with the earlier hypothesis of fish-venom origins (Cameron and Endean 1973). These authors suggested that venom glands in fishes were defensive and consistently developed as a thickening and aggregation of toxin-producing epidermal cells near fin spines, eventually evolving into more complex envenomed structures. The association with fin spines limits their potential role in feeding or other predatory behaviors (but see studies on lionfishes [Scorpaenidae: Pteroinae] and stingrays [Myliobatoidei]; Halstead 1970).

Future work

Ultimately, the current study expands upon the findings of Smith and Wheeler (2006) and Wright (2009) to provide the first explicit order-level phylogeny across all cartilaginous and bony fishes, which we used to delimit venomous fish clades. Our hypothesis and previous and new anatomical investigations provide a framework for studying the biological activity of fish venoms in a phylogenetic context. Researchers should undertake further phylogenetic and venomic studies within these 18 clades as our understanding of piscine venom systems is still well behind the understanding of the venom systems in terrestrial vertebrates (Sivan 2009; Fry et al. 2012; Ziegman and Alewood 2015). Although much work remains, recent investigations have begun reporting interesting findings in venomous fishes ranging from intersexual variation in toadfish toxins (Lopes-Ferreira et al. 2016) to the first fish studies exploring evidence for venom gene duplications and selection (Chuang and Shiao 2014). It is our hope that these and other novel findings combined with the phylogenetic framework provided in this study will facilitate future research on fish venoms in an explicit evolutionary context.

Supplementary data

Supplementary data are available at *ICB* online.

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References

Aschliman NC, Nishida M, Miya M, Inoue JG, Rosana KM, Naylor GJP. 2012. Body plan convergence in the evolution of skates and rays (Chondrichthyes: Batoidea). Mol Phylogenet Evol 63:28–42.

Ault A. 2004. Ventures with venoms. The Scientist 18:43–5.

Bertelsen E, Nielsen JG. 1987. The deep sea eel family Monognathidae (Pisces, Anguilliformes). Steenstrupia 13:141–98.

Betancur-R R, Broughton RE, Wiley EO, Carpenter K, López JA, Li C, Holcroft NI, Arcila D, Sanciangco M, Cureton JC II, et al. 2013. The tree of life and a new classification of bony fishes. PLOS Curr Tree Life 5:ecurrents.tol.53ba26640df0ccaee75bb165c8c26288.

Calvete JJ, Sanz L, Angulo Y, Lomonte B, Gutiérrez JM. 2009. Venoms, venomics, antivenomics. FEBS Lett 583:1736–43.

Cameron AM, Endean R. 1973. Epidermal secretions and the evolution of venom glands in fishes. Toxicon 11:401–10.

Casewell NR, Wüster W, Vonk FJ, Harrison RA, Fry BG. 2013. Complex cocktails: The evolutionary novelty of venoms. Trends Ecol Evol 28:219–29.

Chippaux J-P. 1998. Snake-bites: Appraisal of the global situation. Bull World Health Org 76:515–24.

Chuang P-S, Shiao J-C. 2014. Toxin gene determination and evolution in scorpaenoid fish. Toxicon 88:21–33.

Church JE, Hodgson WC. 2002. The pharmacological activity of fish venoms. Toxicon 40:1083–93.

Downloaded from http://icb.oxfordjournals.org/ at University of Kansas on September 29, 2016

Conway KW, Baldwin C, White MD. 2014. Cryptic diversity and venom glands in Western Atlantic clingfishes of the genus *Acyrtus* (Teleostei: Gobiesocidae). PLOS One 9:e97664.

Darriba D, Posada D. In Review. The impact of partitioning on phylogenomic accuracy. (http://dx.doi.org/10.1101/023978).

Davis MP, Holcroft NI, Wiley EO, Sparks JS, Smith WL. 2014. Species-specific bioluminescence facilitates speciation in the deep sea. Mar Biol 161:1139–48.

Davis MP, Sparks JS, Smith WL. 2016. Repeated and widespread evolution of bioluminescence in marine fishes. PLOS One. 11:e155154.

de Oliveira U, Candido DM, Dorce VAC, Junqueira-de-Azevedo IDM. 2015. The transcriptome recipe for the venom cocktail of *Tityus bahiensis* scorpion. Toxicon 95:52–61.

de Pinna MCC. 1993. Higher-level phylogeny of Siluriformes, with a new classification of the order (Teleostei, Ostariophysi) [Unpublished Ph.D. Dissertation]. City University of New York.

Egge JJD, Simons AM. 2011. Evolution of venom delivery structures in madtom catfishes (Siluriformes: Ictaluridae). Biol J Linn Soc 102:115–29.

Eschmeyer WN, Fricke R, van der Laan R, editors. 2016. Catalog of fishes: Genera, species, references. (http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp; electronic version accessed 3 March 2016).

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–9.

Fox JW, Serrano SMT. 2007. Approaching the golden age of natural product pharmaceuticals from venom libraries: An overview of toxins and toxin-derivatives currently involved in therapeutic or diagnostic applications. Curr Pharm Des 13:2927–34.

Fry BG, Casewell NR, Wüster W, Vidal N, Young B, Jackson TNW. 2012. The structural and functional diversification of the Toxicofera reptile venom system. Toxicon 60:434–48.

Fry BG, Vidal N, Norman JA, Vonk FJ, Scheib H, Ramjan SFR, Kuruppu S, Fung K, Hedges SB, Richardson MK, et al. 2006. Early evolution of the venom systems in lizards and snakes. Nature 439:584–8.

Grande T, Borden WC, Smith WL. 2013. Limits and relationships of Paracanthopterygii: A molecular framework for evaluating past morphological hypotheses. In: Arratia G, Schultze H-P, Wilson MVH, editors. Mesozoic fishes 5—global diversity and evolution. München, Germany: Verlag Dr. Friedrich Pfeil. p. 385–418.

Haddad V Jr, Martins IA, Makyama HM. 2003. Injuries caused by scorpionfishes (*Scorpaena plumieri* Bloch, 1789 and *Scorpaena brasiliensis* Cuvier, 1829) in the southwestern Atlantic Ocean (Brazilian coast): Epidemiologic, clinic, and therapeutic aspects of 23 stings in humans. Toxicon 42:79–83.

Halstead BW. 1970. Poisonous and venomous marine animals of the world, Vol. 3. Washington, DC: US Government Printing Office.

Halstead BW. 1988. Poisonous and venomous marine animals of the world, 2nd ed. Princeton (NJ): The Darwin Press, Inc.

Holcroft NI, Wiley EO. 2008. Acanthuroid relationships revisited: A new nuclear gene-based analysis that incorporates tetraodontiform representatives. Ichthyol Res 55:274–83.

Jared C, Mailho-Fontana PL, Antoniazzi MM, Mendes VA, Barbaro KC, Rodrigues MT, Brodie ED Jr. 2015. Venomous frogs use heads as weapons. Curr Biol 25:2166–70.

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30:3059–66.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–9.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca XF, Wilson AC. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proc Natl Acad Sci USA 86:6196–200.

Lautredou AC, Motomura H, Gallut C, Ozouf-Costaz C, Lecointre G, Dettai A. 2013. New nuclear markers and exploration of the relationships among Serraniformes (Acanthomorpha, Teleostei): The importance of working at multiple scales. Mol Phylogenet Evol 67:140–55.

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

Lopes-Ferreira M, Ramos AD, Martins IA, Lima C, Conceição K, Haddad V Jr. 2014. Clinical manifestations and experimental studies on the spine extract of the toadfish *Porichthys porosissimus*. Toxicon 86:28–39.

Lopes-Ferreira M, Sosa-Rosales I, Bruni FM, Ramos AD, Portaro FCV, Conceição K, Lima C. 2016. Analysis of the intersexual variation in *Thalassophryne maculosa* fish venoms. Toxicon 115:70–80.

López JA, Chen W-J, Ortí G. 2004. Esociform phylogeny. Copeia 2004:449–64.

Maddison WP, Maddison DR. 2015. Mesquite: A modular system for evolutionary analysis. Version 3.04 (http://mesquiteproject.org).

Mitchell JS, Heckert AB, Sues H-D. 2010. Grooves to tubes: Evolution of the venom delivery system in a Late Triassic ''reptile.'' Naturwissenschaften 97:1117–21.

Mo T. 1991. Anatomy, relationships and systematics of the Bagridae (Teleostei: Siluroidei) with a hypothesis of siluroid phylogeny. Koenigstein, Germany: Koeltz Scientific Books.

Near TJ, Dornburg A, Eytan RI, Keck BP, Smith WL, Kuhn KL, Moore JA, Price SA, Burbrink FT, Friedman M, et al. 2013. Phylogeny and tempo of diversification in the superradiation of spiny-rayed fishes. Proc Natl Acad Sci USA 110:12738–43.

Near TJ, Eytan RI, Dornburg A, Kuhn KL, Moore JA, Davis MP, Wainwright PC, Friedman M, Smith WL. 2012. Resolution of ray-finned fish phylogeny and timing of diversification. Proc Natl Acad Sci USA 109:13698–703.

Nelson JS. 2006. Fishes of the world, 4th ed. Hoboken (NJ): John Wiley and Sons.

Downloaded from http://icb.oxfordjournals.org/ at University of Kansas on September 29, 2016

Palumbi SR. 1996. Nucleic acids II: The polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK, editors. Molecular systematics, 2nd ed. Sunderland (MA): Sinauer. p. 205–47.

Price SA, Schmitz L, Oufiero CE, Eytan RI, Dornburg A, Smith WL, Friedman M, Near TJ, Wainwright PC. 2014. Two waves of colonization straddling the K−Pg boundary formed the modern reef fish fauna. Proc Roy Soc B Biol Sci 281:20140321.

Sabaj Pérez, MH, editor. 2014. Standard symbolic codes for institutional resource collections in herpetology and ichthyology: An Online Reference. Version 5.0 (22 September 2014). (http://www.asih.org; electronic version accessed 3 March 2016).

Sanciangco MD, Carpenter KE, Betancur-R R. 2016. Phylogenetic placement of enigmatic percomorph families (Teleostei: Percomorphaceae). Mol Phylogenet Evol 94:565–76.

Sivan G. 2009. Fish venom: Pharmacological features and biological significance. Fish Fish 10:159–72.

Smith WL, Busby MS. 2014. Phylogeny and taxonomy of sculpins, sandfishes, and snailfishes (Perciformes: Cottoidei) with comments on the phylogenetic significance of their early-life-history specializations. Mol Phylogenet Evol 79:332–52.

Smith WL, Craig MT. 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 WL, Wheeler WC. 2004. Polyphyly of the mail-cheeked fishes (Teleostei: Scorpaeniformes): Evidence from mitochondrial and nuclear sequence data. Mol Phylogenet Evol 32:627–46.

Smith WL, Wheeler WC. 2006. Venom evolution widespread in fishes: A road map for the bioprospecting of piscine venoms. J Hered 97:206–17.

Smith-Vaniz WF, Satapoomin U, Allen GR. 2001. *Meiacanthus urostigma*, a new fangblenny from the northeastern Indian Ocean, with discussion and examples of mimicry in species of *Meiacanthus* (Teleostei: Blenniidae: Nemophini). Aqua J Ichthyol Aquatic Biol 5:25–43.

Sosa-Rosales JI, Piran-Soares AA, Farsky SH, Takehara HA, Lima C, Lopes-Ferreira M. 2005. Important biological activities induced by *Thalassophryne maculosa* fish venom. Toxicon 45:155–61.

Sparks JS, Schelly RC, Smith WL, Davis MP, Tchernov D, Pieribone VA, Gruber DF. 2014. The covert world of fish biofluorescence: A phylogenetically widespread and phenotypically variable phenomenon. PLOS One 9:e83259.

Straube N, Li C, Claes JM, Corrigan S, Naylor GJP. 2015. Molecular phylogeny of Squaliformes and first occurrence of bioluminescence in sharks. BMC Evol Biol 15:162.

Sullivan JP, Lundberg JG, Hardman M. 2006. A phylogenetic analysis of the major groups of catfishes (Teleostei: Siluriformes) using rag1 and rag2 nuclear gene sequences. Mol Phylogenet Evol 41:636–62.

Sunagar K, Moran Y. 2015. The rise and fall of an evolutionary innovation: Contrasting strategies of venom evolution in ancient and young animals. PLOS Genet 11:e1005596.

Tan PTJ, Khan AM, Brusic V. 2003. Bioinformatics for venom and toxin sciences. Brief Bioinform 4:53–62.

Tang KL, Berendzen PB, Wiley EO, Morrissey JF, Winterbottom R, Johnson GD. 1999. The phylogenetic relationships of the suborder Acanthuroidei (Teleostei: Perciformes) based on molecular and morphological evidence. Mol Phylogenet Evol 11:415–25.

Tang KL, Fielitz C. 2013. Phylogeny of moray eels (Anguilliformes: Muraenidae), with a revised classification of true eels (Teleostei: Elopomorpha: Anguilliformes). Mitochond DNA 24:55–66.

Trim SA, Trim CM. 2013. Venom: The sharp end of pain therapeutics. Br Pain Soc 7:179–88.

Tyler JC, Bannikov AF. 1997. Relationships of the fossil and recent genera of rabbitfishes (Acanthuroidei: Siganidae). Smithsonian Contrib Paleo 84:1–35.

Vélez-Zuazo X, Agnarsson I. 2011. Shark tales: A molecular species-level phylogeny of sharks (Selachimorpha, Chondrichthyes). Mol Phylogenet Evol 58:207–17.

Vetrano SJ, Lebowitz JB, Marcus S. 2002. Lionfish envenomation. J Emerg Med 23:379–82.

Vetter I, Davis JL, Rash LD, Anangi R, Mobli M, Alewood PF, Lewis RJ, King GF. 2011. Venomics: A new paradigm for natural products-based drug discovery. Amino Acids 40:15–28.

von Reumont BM, Campbell LI, Jenner RA. 2014. Quo vadis venomics? A roadmap to neglected venomous invertebrates. Toxins 6:3488–551.

Wainwright PC, Smith WL, Price SA, Tang KL, Sparks JS, Ferry LA, Kuhn KL, Eytan RI, Near TJ. 2012. The evolution of pharyngognathy: A phylogenetic and functional appraisal of the pharyngeal jaw key innovation in labroid fishes and beyond. Syst Biol 61:1001–27.

Wang N-Z, Zhang X, Zhu M, Zhao W-J. 2009. A new articulated hybodontoid from Late Permian of northwestern China. Acta Zool 90(Suppl. 1):159–70.

Wright JJ. 2009. Diversity, phylogenetic distribution, and origins of venomous catfishes. BMC Evol Biol 9:282.

Wright JJ. 2015. Evolutionary history of venom glands in the Siluriformes. In: Gopalakrishnakone P, Malhotra A, editors. Evolution of venomous animals and their toxins. Netherlands: Springer. p. 1–19.

Ziegman R, Alewood P. 2015. Bioactive components in fish venoms. Toxins 7:1497–531.

Zwickl DJ. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion [Unpublished PhD Dissertation]. [Austin]: The University of Texas.

Downloaded from http://icb.oxfordjournals.org/ at University of Kansas on September 29, 2016