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Short Communication

Molecular phylogenetics of New World searobins (Triglidae; Prionotinae)

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ABSTRACT

Phylogenetic relationships among members of the New World searobin genera *Bellator* and *Prionotus* (Family Triglidae, Subfamily Prionotinae) and among other searobins in the families Triglidae and Peristediidae were investigated using both mitochondrial and nuclear DNA sequences. Phylogenetic hypotheses derived from maximum likelihood and Bayesian methodologies supported a monophyletic Prionotinae that included four well resolved clades of uncertain relationship; three contained species in the genus *Prionotus* and one contained species in the genus *Bellator*. *Bellator* was always recovered within the genus *Prionotus*, a result supported by *post hoc* model testing. Two nominal species of *Prionotus* (*P. alatus* and *P. paralatus*) were not recovered as exclusive lineages, suggesting the two may comprise a single species. Phylogenetic hypotheses also supported a monophyletic Triglidae but only if armored searobins (Family Peristediidae) were included. A robust morphological assessment is needed to further characterize relationships and suggest classification of clades within Prionotinae; for the time being we recommend that *Bellator* be considered a synonym of *Prionotus*. Relationships between armored searobins (Family Peristediidae) and searobins (Family Triglidae) and relationships within Triglidae also warrant further study.

1. Introduction

Members of the family Triglidae, commonly known as searobins or gurnards, are benthic scorpeaniform fishes (*sensu* Nelson et al., 2016) inhabiting shelf habitat globally in tropical and temperate seas (Richards and Miller, 2002). In addition to producing sound, using sonic musculature associated with the swim bladder (Connaughton, 2004), searobins are characterized by greatly expanded, wing-like pectoral fins in which the three lower rays are shortened and thickened in comparison to the upper rays and free, i.e., not connected by a fleshy membrane (Gosline, 1994). These highly modified rays can move independently of each other, are sensitive to both chemical and mechanical stimuli, and are involved in the location of prey items, discovered by 'walking' the free rays across the substrate (Silver and Finger, 1984).

The Triglidae currently comprise ~125 described species distributed across nine genera and three subfamilies (Triglinae, Pterygotriglinae, and Prionotinae) identified by a number of morphological characters (Richards and Jones, 2002; Nelson et al., 2016). The Triglinae contain ~65 species in 5 genera (*Chelidonichthys*, *Eutrigla*, *Lepidotrigla*, *Trigla* and *Trigloporus*) and are distributed throughout the eastern Atlantic, Indian, and Pacific oceans (Richards and Jones, 2002; Nelson et al., 2016). The Pterygotriglinae contain 29 species in two genera (*Bovitrigla* and *Pterygotrigla*) and are distributed throughout the Indian and Pacific oceans (Nelson et al., 2016). The Prionotinae (New World searobins) comprise 31 species in two genera (*Bellator* and *Prionotus*) restricted to the western Atlantic, including the Caribbean Sea, and eastern Pacific (Richards and Jones, 2002).

The nomenclatural history of Prionotinae is complex, involving twelve nominal genera or subgenera (Miller and Richards, 1991). Only two genera, *Prionotus* and *Bellator*, are recognized currently as valid, with 23 species placed in *Prionotus* and eight placed in *Bellator* (Miller and Richards, 1991; Richards and McCosker, 1998). *Bellator* previously was placed as a subgenus of *Prionotus* (e.g., Teague, 1951) but currently is recognized as a distinct genus based on the most recent taxonomic revision of the group, including a diagnosis of the two genera based largely on external morphology (Miller and Richards, 1991). However, a number of characters used

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to distinguish *Bellator* from *Prionotus* exhibit some degree of overlap (e.g., level of squamation of the opercular membrane and number of dorsal-fin spines; Miller and Richards, 1991), and as such are not unique to *Bellator* within the Prionotinae. Imamura (1996) highlighted additional differences between *Prionotus* and *Bellator* and placed both in a sister group relationship in a monophyletic Prionotinae, based on a phylogenetic analysis of 142 morphological characters derived mostly from the musculoskeletal system. In the most recent phylogenetic study of searobins, based on morphology (Richards and Jones, 2002), *Bellator* and *Prionotus* were not placed as a clade (monophyletic group), but instead were represented as a paraphyletic group leading to a clade comprised of members of the Triglininae and Pterygotriglinae.

Despite recent advances in classification of Triglidae, intrarelationships within Prionotinae remain to be investigated using a molecular approach. Here we present the first molecular assessment of phylogenetic relationships among New World searobins, using a combination of nuclear and mitochondrial sequences. Our primary aim was to (i) assess monophyly of Prionotinae and included genera (*Bellator* and *Prionotus*), and (ii) relationships among species in the subfamily.

2. Materials and methods

2.1. Tissues, PCR amplification, and sequencing

Tissues were collected from 16 of 23 currently recognized species of *Prionotus* and from three of eight currently recognized species of *Bellator*. Additional tissues were collected from three species of non-prionotine triglids (*Pterygotrigla andertoni*, *Lepidotrigla argus* and *Chelidonichthys lucerna*) and one peristediid (*Peristedion gracile*). Details of vouchers associated with tissue samples (when available) are listed in Table 1. Tissues were stored in DMSO buffer (Seutin et al., 1991) or 95% non-denatured ETOH. DNA was extracted using a modified Chelex extraction (Estoup et al., 1996) or a standard phenol-chloroform protocol.

A portion of the mitochondrial cytochrome oxidase I (COI) and cytochrome *b* (cyt*b*) genes were sequenced from all species; a short segment of the nuclear recombination activating gene I (RAG1) was sequenced from a subset of species (Table 1). COI was amplified using combinations of previously published universal COI primer pairs (Ward et al., 2005): FishF1 (5′-TCAACCAAC CACAAAGAGATTGGCAC-3′, FishF2 (5′-TCGACT AATCATAAAGATATCGGCAC-3′) and FishR1 (5′-TAGACTTCTGGGTG GCCAAAGAATCA-3′), FishR2 (5′-ACTTCAGGGTGACCGAAGAATCA GAA-3′). Cyt*b* was amplified using custom designed primers: *Cytb_Prion*F (5′-CAG GCC TTTTCYTWGCAATAC-3′) and *Cytb_Prion*R (5′-GGNCGGAAGGTNAGRCTTCG-3′). RAG1 also was amplified using custom designed primers: RAG1_*Prion*F (5′-ACT GTC AAA GCC ACG ACT GG-3′) and RAG1_*Prion*R (5′-TGK AGY ACC ATG TTT TGA GA-3′). Twenty-five microliter PCR reactions for all genes contained 1x reaction buffer (pH 8.5), 1.5 mM $MgCl_2$, 0.25 mM of each dNTP, 25 pmol of each primer, 0.05 U/μL *Taq* polymerase, and 2 μL of template. Reaction conditions consisted of an initial denaturation at 95 °C for 4 min followed by 35 cycles of 95 °C for 1 min, 54–64 °C for 45 s, and 72 °C for 1 min, followed by a final extension of 72 °C for 10 min. Amplified products were column cleaned using Qiaquick® PCR purification kits (Qiagen) and sequenced in both forward and reverse directions at the University of Florida's Interdisciplinary Center for Biotechnology Research (http://www.biotech.ufl.edu/) or Beckman Coulter Genomics (http://www.beckmangenomics.com/genomic_services/sanger_dna_sequencing.html). Chromatograms were edited in Sequencher 4.8 (Gene Codes Corporation 2016) or Geneious v.7.1 (Kearse et al., 2012.), resulting in a 638 bp fragment for COI, a 754 bp fragment for cyt*b*, and a 694–700 bp fragment for RAGI. Additional mtDNA sequences were obtained from Genbank for the triglid *Trigla lyra* (Accession #: COI, JQ775153.1; cyt*b*, EU036516.1), and the peristediid *Peristedion cataphractus* (Accession #: COI, JQ774693.1; cyt*b*, HM049961.1). Sequences for all three genes also were obtained for the platycephalid *Platycephalus indicus* (Accession #: mtDNA, NC_022481.1; RAG1 KF141318.1). Triglidae, Peristediidae, and Platycephalidae are placed as separate families within the scorpeaniform suborder Platycephaloidei (Nelson et al., 2016). Final data sets were composed of 58 operational taxa (49 in Prionotininae) for mtDNA (COI + cyt*b*) and 44 operational taxa for RAG1 (41 in Prionotininae). All sequences were translated and aligned using Geneious v.7.1. GenBank accession numbers for all sequences are provided in Table 1.

2.2. Phylogenetic analysis

Maximum-likelihood analysis was run using PhyML v3.0 (Guindon et al., 2010) as implemented in Geneious v.7.1, with the TrN + I + G model of nucleotide evolution (Tamura and Nei, 1993) for concatenated COI + cyt*b* and the K80 + G model of nucleotide evolution (Kimura, 1980) for RAG1. Models of nucleotide evolution were chosen by evaluating the AICc, BIC, and DT outputs in jModelTest 2 (Darriba et al., 2012). Initial trees were determined using neighbor joining; branch swapping employed subtree pruning and regrafting and nearest neighbor interchange. Branch support was assessed using 10,000 bootstrap replicates.

Bayesian analyses were run using Beast v.2.4 (Drummond et al., 2012); data were partitioned by the 1st, 2nd, and 3rd codon position, with mutation rates independent across partitions. Models of nucleotide evolution for each codon position were chosen using jModelTest 2, as above, and two independent runs for each gene tree (concatenated mtDNA or RAG1) were used to assess whether a strict or relaxed (log normal) molecular clock was more appropriate for the data. For mtDNA sequences, runs consisted of 100 million steps after a burn-in of 50 million steps; while for RAG1 sequences, runs consisted of 20 million steps after a burn-in of five million steps. For both mitochondrial and nuclear sequences, the sampling interval was 1000 trees. To ensure adequate run length, effective sample size was assessed in Tracer v.1.6 (Rambaut et al., 2014) and clock models were compared using Akaike Information Criterion based on Monte Carlo sampling (AICM; Baele et al., 2012), using 100 bootstrap replicates. For each of the gene trees, runs also were carried out after imposing one of two topology constraints. The first constraint excluded species of *Bellator* and forced all nominal species of *Prionotus* to be monophyletic; the second forced all three gene sequences (COI, cyt*b*, and RAG1) of *P. alatus* and *P. paralatus* to be reciprocally monophyletic. For each of the constrained and unconstrained models, AICM values were calculated in Tracer as above. Species trees (*Beast) were constructed for the entire data set (RAG1 and mtDNA sequences) for all available taxa (allowing for missing data), and for a smaller data set that included only those taxa for which sequences from all three genes were available. Data partitioning and models of nucleotide evolution were as above, a relaxed molecular clock was used for each of the seven partitions (2×3 codon positions of the mtDNA genes, plus RAG1), and the appropriate ploidy was specified for the nuclear and mitochondrial genes (the mtDNA genes shared a single gene tree). Runs consisted of 400 million generations, sampling every 5000 steps, and discarding 50% of samples as burn-in. An additional run included a topology constraint forcing *Prionotus* to be monophyletic; the AICM for unconstrained and constrained models were calculated as above. For each analysis, TreeAnnotator v.2.4 (part of the Beast suite) was used to estimate a maximum clade credibility tree and clade posterior probabilities.

Table 1
Genbank accession numbers and available museum voucher numbers for samples used in the study. Museum collection abbreviations: AMS, Australian Museum, Sydney; KU, University of Kansas Biodiversity Institute and Natural History Collection, Lawrence, KS; SIO, Scripps Institution of Oceanography, La Jolla, CA; and TCWC, Biodiversity Research and Teaching Collections, Texas A&M University, College Station, TX.

Family	Subfamily	Species	Voucher	ID	COI	CytB	RAG1
Platycephalidae	–	*Platycephalus indicus*	NA	NA	NC_022481.1	NC_022481.1	KF141318.1
Peristediidae	–	*Peristedion gracile*	TCWC	14555.01	KX811042	KX810987	NA
Peristediidae	–	*Peristedion gracile*	TCWC	14556.01	KX811043	KX810988	NA
Peristediidae	–	*Peristedion cataphractus*	NA	NA	JQ774693	HM049961.1	NA
Triglidae	Prionotinae	*Bellator gymnostethus*	SIO	09-293	KX810993	KX810938	NA
Triglidae	Prionotinae	*Bellator loxius*	SIO	11-374	KX810994	KX810939	NA
Triglidae	Prionotinae	*Bellator loxius*	SIO	11-374	KX810995	KX810940	NA
Triglidae	Prionotinae	*Bellator militaris*	TCWC	14553.01	KX810996	KX810941	NA
Triglidae	Prionotinae	*Bellator militaris*	TCWC	14558.01	KX810997	KX810942	KX811050
Triglidae	Prionotinae	*Bellator militaris*	TCWC	14531.01	KX810998	KX810943	KX811051
Triglidae	Prionotinae	*Prionotus alatus*	TCWC	14367.01	KX810999	KX810944	KX811052
Triglidae	Prionotinae	*Prionotus alatus*	TCWC	14373.01	KX811000	KX810945	KX811053
Triglidae	Prionotinae	*Prionotus alatus*	TCWC	14375.01	KX811001	KX810946	KX811054
Triglidae	Prionotinae	*Prionotus alatus*	TCWC	14368.01	KX811002	KX810947	KX811055
Triglidae	Prionotinae	*Prionotus alatus*	TCWC	14376.01	KX811003	KX810948	KX811056
Triglidae	Prionotinae	*Prionotus albirostris*	SIO	10-122	KX811004	KX810949	KX811057
Triglidae	Prionotinae	*Prionotus carolinus*	KU	T1086	KX811005	KX810950	KX811058
Triglidae	Prionotinae	*Prionotus carolinus*	KU	T1183	KX811006	KX810951	KX811059
Triglidae	Prionotinae	*Prionotus evolans*	KU	T8274	KX811007	KX810952	NA
Triglidae	Prionotinae	*Prionotus evolans*	KU	T1465	KX811008	KX810953	NA
Triglidae	Prionotinae	*Prionotus evolans*	KU	T8273	KX811009	KX810954	NA
Triglidae	Prionotinae	*Prionotus longispinosus*	TCWC	14551.02	KX811010	KX810955	KX811060
Triglidae	Prionotinae	*Prionotus longispinosus*	TCWC	14551.03	KX811011	KX810956	KX811061
Triglidae	Prionotinae	*Prionotus longispinosus*	TCWC	14522.03	KX811012	KX810957	KX811062
Triglidae	Prionotinae	*Prionotus martis*	TCWC	14401.01	KX811013	KX810958	KX811066
Triglidae	Prionotinae	*Prionotus martis*	TCWC	14359.01	KX811014	KX810959	KX811067
Triglidae	Prionotinae	*Prionotus martis*	TCWC	14377.01	KX811015	KX810960	KX811068
Triglidae	Prionotinae	*Prionotus ophryas*	TCWC	14557.01	KX811016	KX810961	KX811063
Triglidae	Prionotinae	*Prionotus ophryas*	TCWC	14404.01	KX811017	KX810962	KX811064
Triglidae	Prionotinae	*Prionotus paralatus*	TCWC	14551.04	KX811018	KX810963	KX811069
Triglidae	Prionotinae	*Prionotus paralatus*	TCWC	14551.05	KX811019	KX810964	KX811070
Triglidae	Prionotinae	*Prionotus paralatus*	TCWC	14551.06	KX811020	KX810965	KX811071
Triglidae	Prionotinae	*Prionotus paralatus*	TCWC	14552.01	KX811021	KX810966	KX811072
Triglidae	Prionotinae	*Prionotus paralatus*	TCWC	14552.03	KX811022	KX810967	KX811073
Triglidae	Prionotinae	*Prionotus punctatus*	TCWC	15653.03	KX811023	KX810968	KX811075
Triglidae	Prionotinae	*Prionotus roseus*	TCWC	14556.02	KX811024	KX810969	KX811076
Triglidae	Prionotinae	*Prionotus roseus*	TCWC	14528.01	KX811025	KX810970	KX811077
Triglidae	Prionotinae	*Prionotus roseus*	TCWC	14528.02	KX811026	KX810971	KX811078
Triglidae	Prionotinae	*Prionotus rubio*	TCWC	14363.01	KX811027	KX810972	KX811079
Triglidae	Prionotinae	*Prionotus rubio*	TCWC	14365.01	KX811028	KX810973	KX811080
Triglidae	Prionotinae	*Prionotus rubio*	TCWC	14366.01	KX811029	KX810974	KX811081
Triglidae	Prionotinae	*Prionotus ruscarius*	SIO	08-146	KX811030	KX810975	NA
Triglidae	Prionotinae	*Prionotus scitulus*	TCWC	14396.01	KX811031	KX810976	KX811082
Triglidae	Prionotinae	*Prionotus scitulus*	TCWC	15648.01	KX811032	KX810977	KX811083
Triglidae	Prionotinae	*Prionotus scitulus*	TCWC	15648.01	KX811033	KX810978	NA
Triglidae	Prionotinae	*Prionotus stearnsi*	TCWC	14554.01	KX811034	KX810979	KX811086
Triglidae	Prionotinae	*Prionotus stearnsi*	TCWC	14361.01	KX811035	KX810980	KX811085
Triglidae	Prionotinae	*Prionotus stearnsi*	TCWC	14371.01	KX811036	KX810981	KX811084
Triglidae	Prionotinae	*Prionotus stephanophrys*	SIO	04-79	KX811037	KX810982	KX811087
Triglidae	Prionotinae	*Prionotus stephanophrys*	KU	T462	KX811038	KX810983	NA
Triglidae	Prionotinae	*Prionotus tribulus*	KU	T5101	KX811039	KX810984	KX811089
Triglidae	Prionotinae	*Prionotus tribulus*	TCWC	15348.01	KX811040	KX810985	KX811088
Triglidae	Prionotinae	*Prionotus tribulus*	TCWC	15348.02	KX811041	KX810986	KX811090
Triglidae	Pterygotriglinae	*Pterygotrigla andertoni*	AMS	I.44761-035	KX811047	KX810992	KX811049
Triglidae	Triglinae	*Lepidotrigla argus*	NA	NA	KX811045	KX810990	NA
Triglidae	Triglinae	*Lepidotrigla argus*	NA	NA	KX811046	KX810991	KX811048
Triglidae	Triglinae	*Trigla lyra*	NA	NA	JQ775153.1	EU036516.1	NA
Triglidae	Triglinae	*Chelidonichthys lucernus*	NA	NA	KX811044	KX810989	NA

3. Results

Final mtDNA alignment, composed of concatenated cyt*b* and COI sequences, consisted of 1392 bp, with no gaps. There were 588 variables sites and 556 parsimony-informative substitutions among the 58 taxa in the analysis. Mean nucleotide composition was similar between the two mtDNA genes: T = 29.5%, C = 32.2%, A = 22.2%, G = 16.1% for COI, and T = 29.2%, C = 29.6%, A = 22.0%, G = 19.1% for cyt*b*. The final RAGI alignment consisted of 700 bp with a two amino-acid (6 bp) insertion in the sequence for *Lepidotrigla argus* and a single amino-acid (3 bp) insertion in the sequence for *Pterygotrigla andertoni*. There were 178 variables sites and 130 parsimony-informative sites among the 44 taxa in the analysis; mean nucleotide composition was T = 24.5%, C = 24.4%, A = 22.0%, G = 29.2%. Phylogenetic hypotheses inferred from these data have been submitted to Treebase (Submission # 20204).

Phylogenetic hypotheses generated were congruent between methodologies (Bayesian and maximum likelihood) but differed slightly between sequence type (mtDNA vs nuclear), with mtDNA sequences providing greater resolution of relationships toward the tips of trees (Fig. 1, Supplemental Figs. 1–5). Nodes generally had higher support with Bayesian analysis relative to ML bootstrap

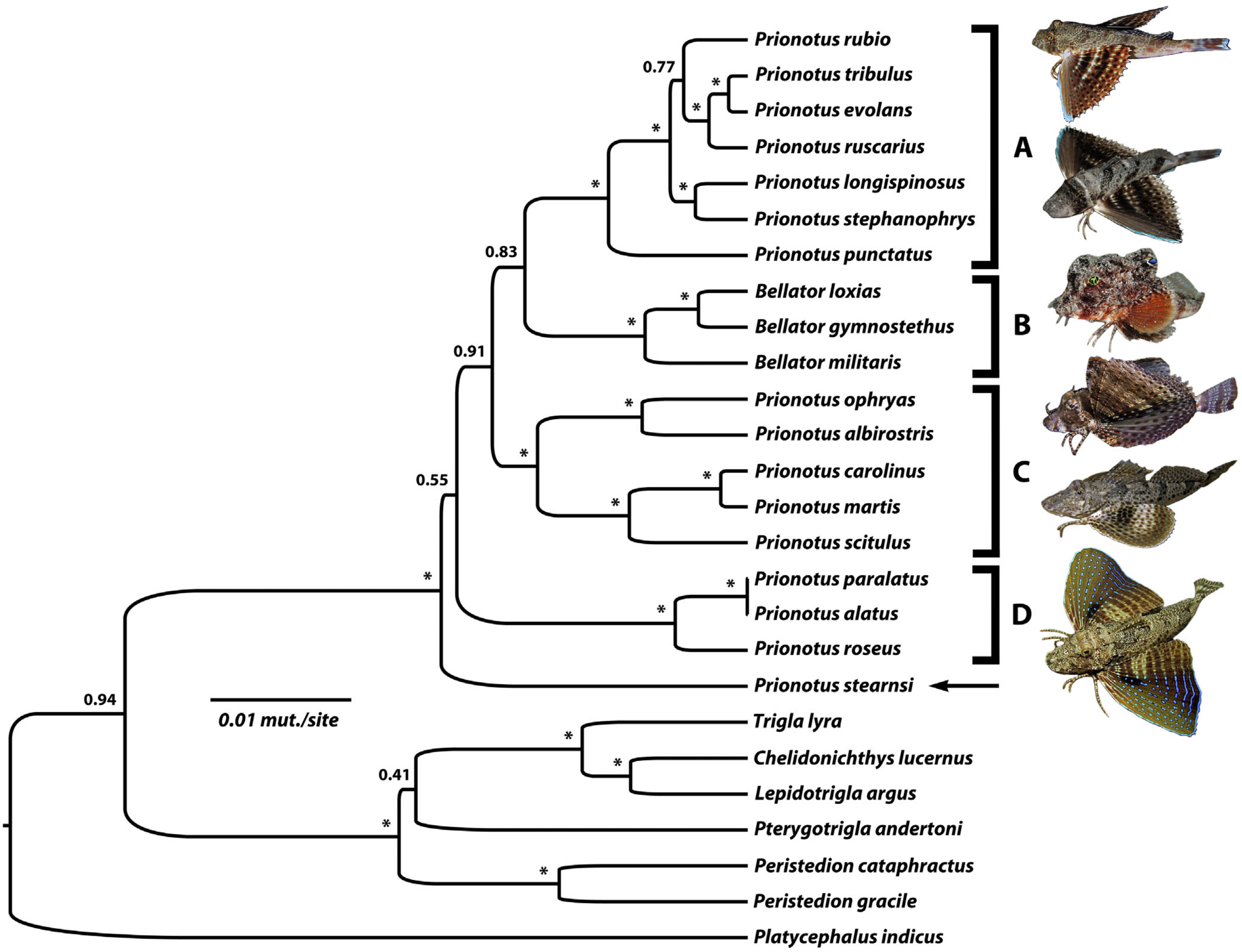

Fig. 1. Species tree of Triglidae inferred from *Beast analysis, using mitochondrial and nuclear data. Values along branches represent posterior probability support (∗ denotes 0.99–1.0). Images of searobins include (from top to bottom): *Prionotus rubio* (photo by Kevin Bryant), *P. punctatus* (Eric Riesch), *Bellator militaris* (Linda Lanniello), *P. ophryas*, *P. scitulus*, and *P. roseus* (Mike Bryant).

percentages for both mtDNA and nuclear data. Model testing showed that relaxed molecular clocks were preferred for all partitions. The species trees recovered a monophyletic Prionotinae (*Prionotus* + *Bellator*) in a sister-group relationship to a clade comprising all remaining triglid taxa plus the two peristediid taxa included in the data set (Fig. 1, Suppl. Fig. 5). This species tree was largely congruent with the individual gene trees (Suppl. Figs. 1–4). In all cases, the two peristidid species were nested inside the clade containing the triglids, rendering Triglidae paraphyletic.

Four major clades (A–D) within the Prionotinae were obtained in all phylogenetic analyses; exact relationships among these clades, however, differed among analyses (Fig. 1 and Suppl. Figs. 1–5). Clade A contained seven species: five whose placement was based on mtDNA and nuclear sequences, and two whose placement was based only on mtDNA sequences. Within Clade A, *P. punctatus* was the sister taxon to the remaining six species which were divided into two smaller clades: (1) *P. rubio* (*P. ruscarius* (*P. evolans*, *P. tribulus*)), and (2) *P. longispinosus* and *P. stephanophrys*. Clade B contained the three species currently placed in the genus *Bellator*. Clade C contained five species that were consistently divided into two smaller clades: (1) *P. albirostris* and *P. ophryas*, and (2) *P. scitulus* (*P. carolinus*, *P. martis*). Clade D contained *P. alatus*, *P. paralatus* and *P. roseus*. It was not possible to distinguish between *P. alatus* and *P. paralatus* as they formed a single group with both nuclear and mtDNA markers (Fig. 2; Sup. Figs. 1–4), with sequences obtained from both species placed within a single clade. The position of *P. stearnsi* was inconsistent among analyses and poorly supported in terms of bootstrap and posterior probability values (Fig. 1, Sup Figs. 1–5). Analysis involving mtDNA either placed *P. stearnsi* as sister taxon to Clade D (maximum likelihood) or sister taxon to clade B + A + C (Bayesian), while both analysis involving RAG1 placed *P. stearnsi* as sister taxon to clade B + D.

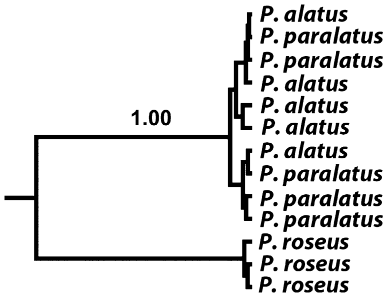

Fig. 2. Example of phylogenetic relationship recovered between individuals of *P. alatus* and *P. paralatus*. The topology is from a relaxed-clock gene tree, based on concatenated mitochondrial COI and cyt*b* sequences and inferred using *Beast. Values above branches represent posterior probability support.

Table 2

Akaike Information Criterion from Monte Carlo Markov Chain samples (AICM) for constrained and unconstrained phylogenetic analysis of Triglidae, calculated from 100 bootstrap samples of each posterior. Models with lower AICM values are preferred. For each dataset, the AICM, difference in AICM (Δ AICM) from the preferred model, and standard error (S. E.) of bootstrap samples for each model are listed. Species tree (a) includes only species with both mitochondrial and nuclear data, while species tree (b) includes all species and missing nuclear data for some taxa.

	mtDNA			RAG1		
Model	AICM	Δ AICM	S.E.	AICM	Δ AICM	S.E.
Unconstrained	4858.8	0	±0.158	26914.4	0	±0.241
Monophyletic *Prionotus*	4873.4	14.6	±0.123	26963.4	48.9	±0.488
Monophyletic *P. alatus/P. paralatus*	4874.4	15.6	±0.133	27002.4	88.0	±0.238
	Species tree (a)			Species tree (b)		
Model	AICM	Δ AICM	S.E.	AICM	Δ AICM	S.E.
Unconstrained	25657.4	0	±0.427	31771.1	0	±0.337
Monophyletic *Prionotus*	25688.8	31.4	±0.382	31785.8	14.6	±0.262
Monophyletic *P. alatus/P. paralatus*	na	na	na	na	na	na

The species tree analysis placed *P. stearnsi* as sister taxon to the rest of the Prionotini (Fig. 1).

In all hypotheses (gene trees and species trees), *Bellator* was nested within *Prionotus*. We tested the robustness of this grouping against trees where *Prionotus* was forced to be monophyletic (Table 2), and found strong support for *Bellator* nested inside of a paraphyletic *Prionotus*. Similarly, we compared a model of reciprocal monophyly of sequences (gene trees only) for *P. alatus* and *P. paralatus* to the unconstrained resolution and found strong support for interdigitating haplotypes for these species (Table 2).

4. Discussion

All phylogenetic hypotheses were consistent with a monophyletic Prionotinae that included species of *Bellator* and *Prionotus*. The species of *Bellator* formed a clade that was the sister taxon to one of three clades containing species of *Prionotus*, depending on the sequence and phylogenetic methodology used. Placement of *Bellator* within *Prionotus* also was supported by *post hoc* model testing (Table 2). These findings suggest that *Prionotus*, as currently recognized, is paraphyletic and that all New World searobins belong within a single genus (*Prionotus*), an arrangement favored by Teague (1951). An alternative would be to retain *Bellator* (following Miller and Richards, 1991) and divide the clades of *Prionotus* into different genera. The work required to diagnose and properly circumscribe genera of New World searobins is beyond the scope of this study. There are multiple names available from the synonymy of *Prionotus* that could accommodate splitting the group into separate genera (e.g., Gill, 1905; Jordan and Everman, 1898). However, until a robust phylogenetic analysis, including morphological and molecular characters, of all currently recognized species of New World searobins becomes available, we suggest that *Bellator* be considered a synonym of *Prionotus*.

None of the phylogenetic hypotheses were consistent with a monophyletic Triglidae. In all hypotheses, the two peristediids (*P. gracile* and *P. cataphractus*) were placed in a clade with members of Pterygotriglinae and Triglininae. Inclusion of armored searobins (peristediids) within Triglidae as a subfamily (Peristediidinae) was accepted previously (Nelson, 1994) but fell out of favor after Imamura (1996), based on morphology, hypothesized a sister-group relationship between armored searobins and ghost flatheads (Hoplichthyidae). A sister-group relationship between Peristediidae and Triglidae also was supported in recent molecular studies (Smith and Craig, 2007; Betancur-R et al., 2013) and favored by Nelson et al. (2016). However, the molecular studies included taxa representing only one of the three subfamilies of Triglidae, hence it is not possible to compare directly results of those studies with those presented here, at least with respect to relationships between triglids and peristediids.

The species tree in this study supported a clade comprised of species of Triglidae and Peristediidae and where Prionotininae is the sister taxon to all other Triglidae and Peristediidae. However, Bayesian gene trees placed Prionotininae as the sister taxon to all other sampled taxa, including the outgroup, *Platycephalus indicus*, currently placed in the family Platycephalidae in the suborder Platycephaloiodei (Nelson et al., 2016). This apparent discrepancy may be the result of applying Yule (interspecific) models of branching in gene trees analysis (Drummond et al., 2002) as our study included several intraspecific samples. This issue likely was not encountered in species-tree analyses which also includes an intraspecific (coalescent) model of branching (Heled and Drummond, 2010). Inclusion of *Platycephalus* within Triglidae in the gene trees likely is artefactual and is inconsistent with a morphological assessment of the Platycephaloidei (Imamura, 1996). Regardless, our data support the idea of a basal split between New World searobins (Prionotini) and Old World searobins (Triglinae + Pterygotriglinae); a more detailed analysis involving additional taxon sampling and at deeper taxonomic levels including Triglidae, Peristediidae, and other platycephaloid fishes is clearly warranted.

Our study of New World searobins also identified an issue at the species level, as it was not possible to discern between the two nominal species *P. alatus* and *P paralatus* which formed a single cluster in all analyses. These two species are thought to represent sister species that meet in a suture zone in the northern Gulf of Mexico (Portnoy and Gold, 2012). Suspected hybrids between *P. alatus* and *P. paralatus* have been described from this area, based on individuals with a mixture of morphological characters attributed either to *P. alatus* or *P. paralatus* (McClure and McEachran, 1992). Results obtained from mtDNA sequences could therefore reflect introgression and the capture of the mitogenome of one species by the other (Avise, 1993). However, none of the analyses involving nuclear sequences supported exclusivity. These observations suggest that *P. alatus* and *P. paralatus* either are morphotypes of the same species, incipient species that are currently diverging, or formally isolated genetic entities in the process of re-homogenizing (Funk and Onland, 2003). Resolving which of these possibilities is correct likely will require an in depth population-level study involving high resolution genetic markers such as SNPs.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2016.11.017.

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