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Swimbladder Evolution of Longfin Herrings (Pristigasteridae, Teleostei)

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Sébastien Lavoué, Sahat Ratmuangkhwang, Hsuan-Ching Ho, Wei-Jen Chen, and Mohd Nor Siti Azizah (2018) Longfin herrings form a monophyletic, circumtropically distributed family of mostly marine teleost fishes, the Pristigasteridae (Clupeoidei), that includes 38 species classified into nine genera and three main lineages (the Pelloninae, Pristigasterinae, and the genus *Ilisha*). The external morphology and osteology of the Pristigasteridae provide only a few, sometimes conflicting, informative characters which makes it difficult to reconstruct their phylogeny, but their swimbladder (a visceral gas-filled chamber that has several important functions in the Teleostei) appears to be highly specialized and variable among species. In particular, the swimbladder of most Indo-West Pacific pristigasterid species exhibits one or paired post-coelomic extensions, whereas New World species do not. The presence of these extensions conflicts with the current systematic classification, as they are only found in subsets of different taxa. To examine this conflict, the most comprehensive molecular phylogenetic tree of the Pristigasteridae to date was built using six molecular markers and 21 species. This tree deeply disagreed with the current classification in that it indicated that the subfamilies Pelloninae and Pristigasterinae are not monophyletic and neither are the genera *Ilisha*, *Pellona*, and *Opisthopterus*. Using this tree to infer the evolution of the post-coelomic extensions, it was found that their absence is the ancestral condition in the Pristigasteridae. Indo-West Pacific species with post-coelomic extensions evolved later and form a monophyletic group, inside which species with only one extension form a monophyletic group. The consequences of our findings on the evolution and classification of Pristigasteridae are discussed.

We suggest that only species of Pristigasteridae having one or paired post-coelomic extensions should be included in the genus *Ilisha*.

Key words: Evolution, Phylogeny, Morphology, Molecules, Fish.

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BACKGROUND

The Pristigasteridae, collectively called longfin herrings, are a small family of ray-finned fishes belonging to the suborder Clupeoidei (Teleostei). The Clupeoidei include four additional families, the Chirocentridae, Engraulidae, Clupeidae, and Dussumieriidae, the last two of which are likely not reciprocally monophyletic (Grande 1985; Whitehead 1985; Whitehead et al. 1988; Lavoué et al. 2014). Pristigasterids are easily recognized by their laterally compressed body, full or almost full series of well-developed ventral scutes, large eyes, reduced or absent pelvic fins, a short dorsal fin (absent in the genus *Raconda*), and a long anal fin (with more than 30 fin-rays) (Whitehead 1985). The Pristigasteridae currently comprise 38 species classified into nine genera (Eschmeyer et al. 2018) and three main lineages: the subfamilies Pristigasterinae and Pelloninae and the genus *Ilisha* (minus *I. africana*, which is placed in the Pristigasterinae) (Grande 1985) (Fig. 1). Three genera include about three-quarters of the species diversity: *Ilisha* with 16 species, and *Pellona* and *Opisthopterus* each with six species (Eschmeyer et al. 2018). Two species of the Pristigasteridae - *I. melastoma* and *I. elongata* - are commonly found in the northern South China Sea (Shao et al. 2008), of which large (> 30 cm in standard length) specimens of *I. elongata* hold some economic importance in this region.

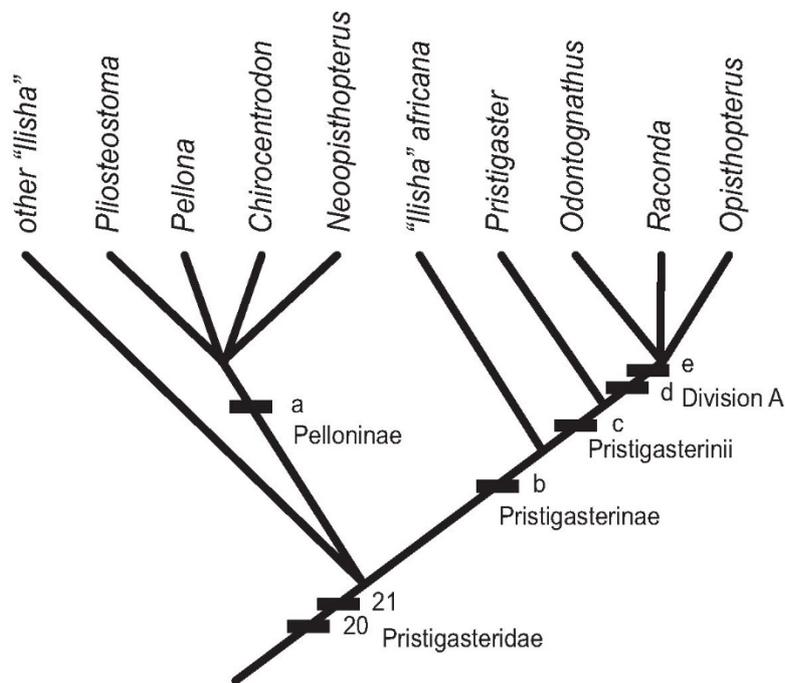


Fig. 1. Cladogram of pristigasterid fishes based on morphological characters (modified from Grande 1985). List of characters (reproduced from Grande, 1985): character 20: “*Predorsal bones oriented either vertically or inclined anterodorsally*”; character 21: “*Loss of interlobar notch in third hypural of caudal skeleton*”; character a: “*Maxillary-premaxillary gap covered by bone*”; character b: “*Presence of a bony process on the first pleural rib which articulates with the shoulder girdle*”; character c: “*Loss of pelvic fin*”; character d: “*More than 23 predorsal bones*”; character e: “*More than 57 anal pterygiophores.*”

The monophyly of the Pristigasteridae is supported by several morphological synapomorphies from the structures of the gill arches, pre-dorsal bones, and caudal fin (Nelson 1967; Grande 1985) and molecular characters (Bloom and Lovejoy 2014), but its phylogenetic position relative to the four other clupeoid families is still being debated (Grande 1985; Di Dario 2002; Li and Ortí 2007; Miyashita 2010; Lavoué et al. 2013; Bloom and Lovejoy 2014).

The current phylogenetic hypothesis and classification of the family Pristigasteridae proposed by Grande (1985) (Fig. 1) need to be evaluated for two reasons. First, while the diagnoses for the six monospecific or species-poor (≤ 3 species) genera are straightforward (Norman 1923; Berry 1964; Whitehead 1985), the largest genus, *Ilisha*, which includes almost half of all pristigasterid species, is poorly diagnosed because a synapomorphy is absent. Some authors, including Grande (1985), cast doubt about the monophyly of *Ilisha*, arguing that it merely represents a grade (Whitehead 1985; de Pinna and Di Dario 2003). However, no recent taxonomic revision of this genus or new hypothesis on the Pristigasteridae phylogeny has been published. Second, some of the characters used by Grande (1985) to support clades above the level of genus either conflict with

others or are absent (*i.e.*, the absence of the pelvic fin conflicts with the presence of post-coelomic extensions) (Fig. 1).

Whereas the external morphology and osteology of species of *Ilisha* and several other pristigasterids offer limited information regarding their systematics, the morphology of their swimbladder is highly specialized and variable among species (Talwar and Whitehead 1971; Seshagiri Rao 1975, 1976; Whitehead and Blaxter 1989). The swimbladders of teleosts serve several important functions, such as regulating buoyancy or maintaining the hydrostatic position in the water column (Whitehead and Blaxter 1989; Pelster 2011). Specialization of Pristigasteridae swimbladders may have additional purposes such as sound production, reception, or amplification (Allen et al. 1976; Wahlberg and Westerberg 2003; Wilson et al. 2004; Yan 2004; Wang et al. 2017), although there is no direct evidence for such functions (Whitehead and Blaxter 1989).

The most striking specialization in the swimbladders of most Indo-West Pacific (IWP) pristigasterid species is the one or two post-coelomic extensions (Valenciennes 1847; de Beaufort 1909), which is seen as a rare condition throughout the Teleostei (Poll 1969; Tominaga et al. 1996). In six IWP species of *Ilisha* (*I. kampeni*, *I. lunula*, *I. compressa*, *I. melastoma*, *I. obfusca*, and *I. striatula*), IWP species of *Opisthopterus* and *Raconda*, and the West African *I. africana*, the posterior end of the swimbladder divides into two extensions (= diverticula) that pass backward on either side of the haemal spine and anal pterygiophores (Fig. 2A) (Kailola 1986; Whitehead and Blaxter 1989; Randall 1994). The diverticula lie just above the distal tips of the anal pterygiophores and extend to the anal fin. A second condition is found only in five IWP species of *Ilisha* (*I. elongata*, *I. filigera*, *I. megaloptera*, *I. macrogaster*, and *I. pristigastroides*). In these species, there is only one diverticulum, always on the right side of the body (Fig. 2B). In the third and last condition, which is the most common, the swimbladder has no post-coelomic diverticulum. This condition is found in *Pellona*, five endemic New World genera (*Pliosteostoma*, *Chirocentrodon*, *Neopisthopterus*, *Odontognathus*, and *Pristigaster*), all New World representatives of *Ilisha* and *Opisthopterus*, as well as in two IWP species of *Ilisha* (*I. sirishae* and *I. novacula*).

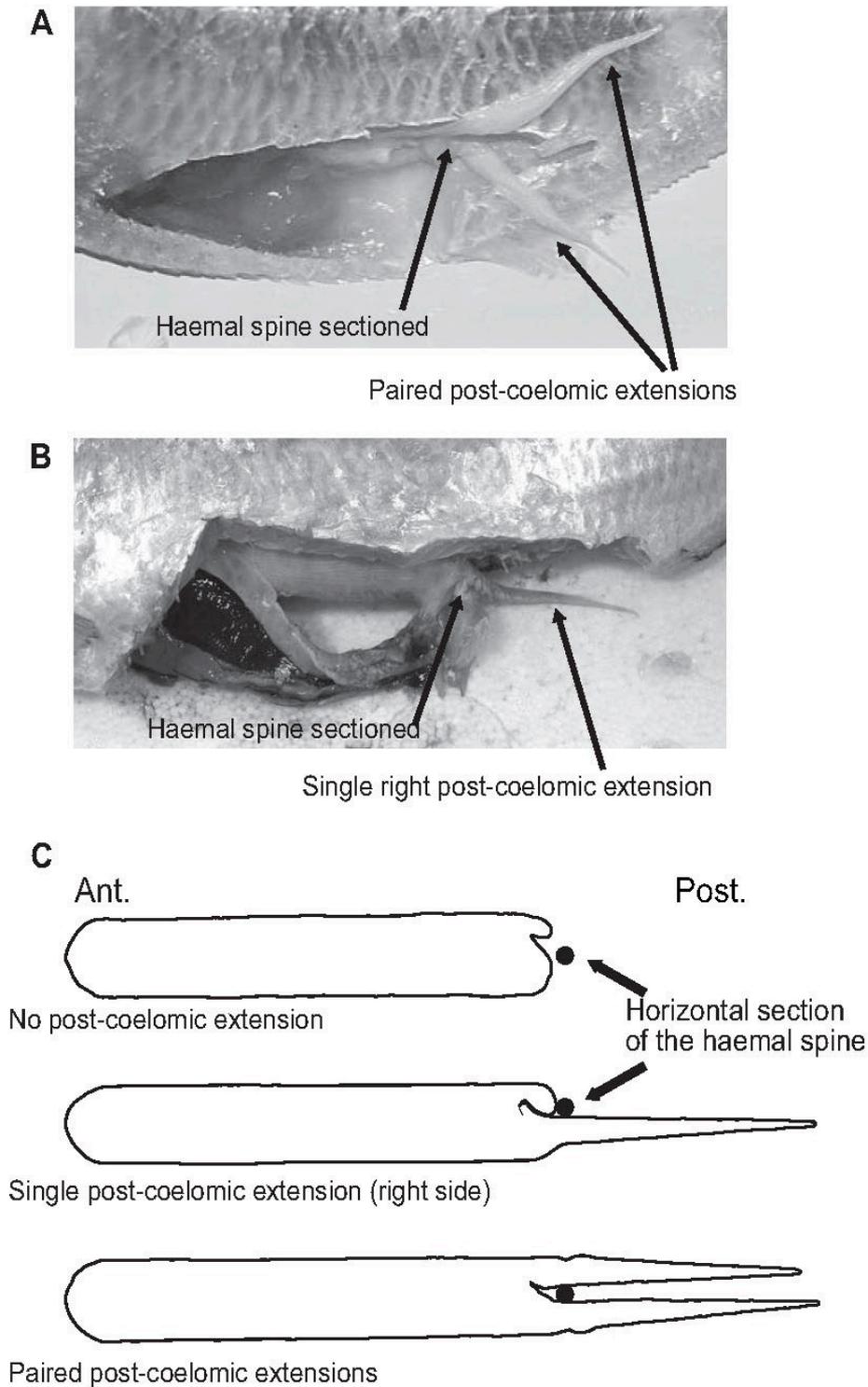


Fig. 2. Posterior end of swimbladder showing post-coelomic diverticula. (A) *Ilisha melastoma*, dissected on the left side (12 cm standard length) showing the presence of two post-coelomic diverticula; (B) *Ilisha elongata*, dissected on the left side (42 cm standard length) showing the presence of one post-coelomic diverticulum; (C) Three diagrams (ventral view) summarizing the three conditions regarding to the presence (versus absence) and number (one or two) of post-coelomic extensions in Pristigasteridae.

The presence or absence of these post-coelomic diverticula contradict the current classification because species with diverticula are found both within and outside of the genera *Ilisha* and *Opisthopterus*. Whitehead and Blaxter (1989) noted this disagreement and admitted that these two genera could be split based on features of their swimbladders, regardless of their overall external similarity. Those authors, however, did not modify the classification. Whitehead and Blaxter (1989) observed that all New World species have swimbladders that lack post-coelomic diverticula, while species with one or paired post-coelomic diverticula are only found in the IWP region along with the West African species *I. africana* (Table 1).

In this study, we examined the evolution of the swimbladder structure in the Pristigasteridae to determine whether the presence of post-coelomic diverticula is derived. For that, we reconstructed the most comprehensive, molecular-based phylogenetic tree of the Pristigasteridae to date, paying special attention to IWP species. We then used this tree to reconstruct and discuss the origin and evolution of post-coelomic extensions and, finally, we comment on the classification of the Pristigasteridae.

MATERIALS AND METHODS

Taxonomic and character samplings

For this study, we obtained novel nucleotide sequences from 33 specimens of longfin herrings (family Pristigasteridae) of 11 IWP species which were collected from local fish markets in Taiwan, Malaysia, Thailand, and Sri Lanka (Table 1). We dissected most specimens to examine the morphology of the swimbladder and report on the presence or absence of post-coelomic diverticula as described by Whitehead and Blaxter (1989). For each specimen, a muscle sample was taken and preserved in 95% ethanol.

To build our character sampling, we selected the following four mitochondrial genes as markers to reconstruct the phylogeny of the Pristigasteridae: the partial 12S ribosomal (r)RNA (about 600 base pairs [bp]) and 16S rRNA (about 600 bp), the partial cytochrome oxidase I (COI; 650 bp), and the complete cytochrome *b* (1140 bp). Our character sampling was completed with the addition of the partial nuclear genes, Rag1 (about 1200 bp) and Rag2 (about 1100 bp).

Table 1. List of species of Pristigasteridae (21), specimens (45) and molecular markers (with GenBank accession numbers) examined in this study (abbreviations: GB, GenBank; COI, cytochrome oxidase I; cyt**b**, cytochrome *b*; rag1, recombination activating gene 1; rag2 recombination activating gene 2). GenBank accession numbers of newly determined sequences in this study are in bold. “/” indicates that the respective sequence is not determined. Generic classification of Pristigasteridae follows Whitehead (1985)

Taxa	Specimen code	COI	12S	16S	cytb	rag1	rag2
Raconda							
<i>Raconda russeliana</i>	KU02b	MH325626	MH325563	MH325533	MH325593	MH325506	MH325475
	KU03b	MH325627	MH325564	MH325534	MH325594	/	MH325476
Ilisha							
<i>Ilisha elongata</i>	WJC2067	MH325628	MH325565	MH325535	MH325595	MH325507	MH325477
	I LEL (GB)	AP009141	AP009141	AP009141	AP009141	JX255541	DQ912160
	KU23b	MH325629	MH325566	MH325536	MH325596	MH325508	MH325478
<i>Ilisha melastoma</i>	HH51	MH325630	MH325568	MH325537	MH325597	MH325509	MH325479
	HH70	MH325631	MH325569	MH325538	MH325598	MH325510	MH325480
	MAY22	MH325632	MH325570	MH325539	MH325599	MH325511	MH325481
	MAY29	MH325633	MH325571	MH325540	MH325600	MH325512	MH325482
	MAY219	MH325634	MH325572	MH325541	MH325601	MH325513	MH325483
	SRI79	/	MH325573	MH325542	MH325602	MH325514	MH325484
	SRI80	/	MH325574	MH325543	MH325603	MH325515	MH325485
<i>Ilisha cf. megaloptera</i>	DDB3240 (GB)	/	/	KJ158118	KJ158079	/	KJ158099
<i>Ilisha filigera</i>	KU15	MH325635	MH325575	MH325544	MH325604	MH325516	MH325486
	KU17	MH325636	MH325576	MH325545	MH325605	/	MH325487
	KU30b	MH325637	MH325567	MH325546	MH325606	MH325517	MH325488
	MAY362	/	MH325577	MH325547	MH325607	MH325518	MH325489
<i>Ilisha pristigastroides</i>	MAY543	MH325638	MH325578	MH325548	MH325608	MH325519	MH325490
	MAY544	MH325639	MH325579	MH325549	MH325609	MH325520	MH325491
	KU15b	MH325640	MH325580	MH325550	MH325610	MH325521	MH325492
	KU16b	MH325641	MH325581	MH325551	MH325611	MH325522	MH325493
<i>Ilisha macrogaster</i>	KU14	/	MH325582	MH325552	MH325612	MH325523	MH325494
	SI05	MH325642	MH325583	MH325553	MH325613	MH325524	MH325495
<i>Ilisha striatula</i>	SRI03	/	MH325584	MH325554	MH325614	MH325525	MH325496
	SRI04	/	MH325585	MH325555	MH325615	/	/
	Ra37	MH325643	/	/	MH325616	MH325526	MH325497
<i>Ilisha kampeni</i>	CL04 (GB)	AP011609	AP011609	AP011609	AP011609	/	/
	Bk03	MH325644	/	/	MH325617	MH325527	MH325498
	Ra42	MH325645	/	/	MH325618	MH325528	MH325499
<i>Ilisha sirishae</i>	SRI77	MH325646	MH325586	MH325556	MH325619	/	/
	SRI78	MH325647	MH325587	MH325557	MH325620	/	MH325500
<i>Ilisha africana</i>	ILAF (GB)	AP009140	AP009140	AP009140	AP009140	/	/
<i>Ilisha amazonica</i>	DDB717 (GB)	/	/	KJ158134	KJ158097	KJ158151	KJ158115
Opisthopterus							
<i>Opisthopterus tardoore</i>	MAY20	MH325648	MH325588	MH325558	MH325621	/	MH325501
	MAY118	MH325649	MH325589	MH325559	MH325622	MH325529	MH325502
	SRI01	/	MH325590	MH325560	MH325623	MH325530	MH325503
	SRI02	/	MH325591	MH325561	MH325624	MH325531	MH325504
<i>Opisthopterus valenciennesi</i>	KU21b	/	MH325592	MH325562	MH325625	MH325532	MH325505
Pellona							
<i>Pellona ditchela</i>	/(GB)	JF494121	/	/	/	/	/
<i>Pellona flavipinnis</i>	/(GB)	AP009619	AP009619	AP009619	AP009619	AY430206	DQ912134
<i>Pellona harroweri</i>	DDB4309 (GB)	JX124858	KJ158143	KJ158126	KJ158088	KJ158143	KJ158107
Pristigaster							
<i>Pristigaster cayana</i>	DDB657 (GB)	/	/	KJ158133	KJ158096	/	KJ158114
<i>Pristigaster whiteheadi</i>	DDB4397 (GB)	/	/	KJ158127	KJ158089	KJ158144	/
Odontognathus							
<i>Odontognathus mucronatus</i>	DDB3595 (GB)	JX124826	/	KJ158122	KJ158082	/	/
Chirocentrodon							
<i>Chirocentrodon bleekermanus</i>	DDB3452 (GB)	/	/	KJ158121	/	/	/
Ougroup (family Engraulidae):							
<i>Coilia nasus</i>	/(GB)	AP009135	AP009135	AP009135	AP009135	DQ912123	DQ912157

We combined our data with previously published data from 12 specimens of ten species, mostly from the New World (Lavoué et al. 2007; Lavoué et al. 2013; Bloom and Lovejoy 2014). In total, our dataset included 5296 characters and 45 specimens from 21 species belonging to seven genera (only the New World genera *Pliosteostoma* and *Neoopisthopterus* are missing). *Coilia nasus*, an anchovy, was used to root the phylogenetic trees.

DNA extraction, amplification, and sequencing

Total genomic DNA of each tissue sample was extracted using the LabTurbo DNA Mini Kit LGD480-220 (TAIGEN Bioscience, Taipei, Taiwan), a semi-automatic method, following the manufacturer's protocol. We then used the polymerase chain reaction (PCR) technique to amplify the targeted nucleotide markers from the extracted DNA. For each marker, the following PCR primer pairs were used (the names and sequences of the reverse primers are indicated in italics): 12S_229F (5'-GYCGGTAAAAYTCGTGCCAG-3') and *12S_954R* (5'-YCCAAGYGCACCTTCCGGTA-3') (Li and Ortí 2007); 16S_135F (5'-GCAATAGAVAWAGTACCGCAAGG-3') and *16S_1072R* (5'-CCTTYGCACGGTYARAATAC-3') (Li and Ortí 2007); Cytb_AnchF (5'-TGACTTGAAAAACCACCGTTGTTATTCAAC-3') and *Cytb_AnchR* (5'-CTAGCTTTGGGAGYTAGDGGTGGRAGTT-3') (Bloom and Lovejoy 2012); COI_FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and *COI_FishR2* (5'-ACTTCAGGGTGACCGAAGAATCAGAA-3') (Ward et al 2005); newly designed primer pair RAG1_Prif (5'-CAGATCTTCCAGCCCCTGCACACC-3') and *RAG1_Prir* (5'-AGTTGGTGATCTTGCCGTCGTAGCGG-3'); and newly designed primer pair RAG2_Prif (5'-TCAAGCTGCGCCCCATCTCCTTCTCCA-3') and *RAG2_Prir* (5'-CTTCATGCACTGGGCGTGGACCCA-3').

PCR products were purified using the AMPure magnetic bead cleanup protocol (Agencourt Bioscience, Taipei, Taiwan) before being sent for sequencing in both directions (forward and reverse) at the Center of Biotechnology (National Taiwan University), using the Sanger sequencing method (Sanger et al. 1977).

To check the quality of the chromatograms, we used the software 4Peaks vers. 1.7 (available at <http://nucleobytes.com/4peaks/>). The consensus sequence of each gene and each specimen was built from the forward and reverse sequences using the software CodonAligner 3.7.2 (Codoncode, Dedham, MA, USA).

For each marker (*i.e.*, 12S, 16S, COI, cytochrome *b*, Rag1 and Rag2), we built a matrix “specimens*characters” that was used for subsequent phylogenetic analyses. Alignments of the protein-coding genes, cytochrome *b*, COI, rag1 and rag2, were straightforward. The 12S and 16S

rRNA genes were separately aligned with the software Proalign vers. 0.5 (Löytynoja and Milinkovitch 2003) using default parameter settings. Regions with posterior probabilities of $\leq 90\%$ were excluded from the alignments.

Phylogenetic analyses

We inferred partitioned maximum likelihood (ML) phylogenetic trees using the software RAxML (Stamatakis 2006) from the previously built combined six-marker matrix. PartitionFinder vers. 1.1.1 (Lanfear et al. 2012) was used to calculate the best partition scheme from seven basic partitions (*i.e.*, the first, second, and third codon positions of the two combined mitochondrial coding-protein genes plus the first, second, and third positions of the two combined nuclear coding-protein genes plus the concatenated 12S/16S rRNAs). A four-partition scheme was inferred. We then performed ML heuristic phylogenetic searches under the general time-reversible model with discrete gamma-distributed rate heterogeneity [GTR + Γ] applied to each of the four partitions. We executed 100 searches and found the best ML tree by comparing the final likelihoods among the 100 inferred trees. To evaluate the robustness of the internal branches of the ML tree, 1000 bootstrap replicates were calculated for each matrix using the GTR + Γ model.

Statistical tests for alternative topologies

The likelihoods of different topologies, in which the monophyly of different taxonomic groups is separately constrained, were evaluated using the Shimodaira-Hasegawa (SH) and approximately unbiased (AU) tests as implemented in the program CONSEL vers. 0.1j (Shimodaira and Hasegawa 2001; Shimodaira 2002). First, a four-partition ML tree search of the combined dataset was performed using RAxML and the GTR + Γ model, in which we *a priori* constrained the monophyly of different groups (the constrained topology tree was first built using Mesquite vers. 3.31; Maddison and Maddison 2009). Site-wise log-likelihoods of the constrained and unconstrained topologies were calculated with RAxML (-f g option in RAxML), and *p* values were finally calculated with CONSEL.

Reconstruction of swimbladder evolution

We reconstructed the evolution of the presence and number (one or two) post-coelomic diverticula of the swimbladder onto our ML phylogenetic tree, using a symmetrical one-rate (“Mk1”) model of character evolution. This model assumes that transitions between each

morphological condition occurred at the same rate. Character state reconstructions were performed using Mesquite vers. 3.31 (Maddison and Maddison 2009). Data on the presence and number of post-coelomic diverticula of the swimbladder of the Pristigasteridae at the species level are summarized in appendix 1 and were assembled from our direct observations and from Whitehead and Blaxter (1989) (see their Table 3, page 353). We coded three conditions: the absence of a diverticulum (coded “0”), the presence of only one diverticulum that always lies on the right side (coded “1”), and the presence of two diverticula (coded “2”).

RESULTS

Phylogenetic structure

The ML phylogenetic tree is shown in figure 3. This tree was fully resolved, but several relationships - mostly at the base of the tree, among New World taxa - were not well supported by bootstrap proportions (BPs) of $> 75\%$. Each species represented by more than one specimen was monophyletic. This tree conflicted with the current classification of the Pristigasteridae in several aspects. None of the three main lineages identified by Grande (1985) (Fig. 1), *i.e.*, the Pelloninae, Pristigasterinae, and *Ilisha* (minus *I. africana*), were monophyletic. Our results showed that the genus *Ilisha* did not form a monophyletic group because 1) *I. sirishae*, *I. africana* and *I. amazonica* were only distantly related to other species of *Ilisha* and 2) *Raconda russelliana* and the two species of *Opisthopterus* were nested within the IWP species of *Ilisha* (minus *I. sirishae*). The genera *Pellona* and *Opisthopterus* were also not respectively monophyletic relative to the remaining pristigasterids or to *R. russelliana*.

In contrast to the current classification, we found support (BP = 88%) for the monophyly of a large group including all IWP pristigasterid species (except *Ilisha sirishae* and *Pellona ditchela*). This group was the sister group of a clade comprising the West African *I. africana* and New World *Chirocentrodon bleekermanus*. The phylogenetic position of *C. bleekermanus* should be considered with caution, because only 16S rRNA was sampled and its terminal branch was significantly longer than those of other species. When *C. bleekermanus* was excluded from the dataset (data not shown), *I. africana* was sister of the IWP pristigasterid species; this clade was supported by a BP of 60%.

The SH and AU tests statistically rejected the monophylies of *Ilisha*, *Opisthopterus*, *Pellona*, the Pelloninae, and Pristigasterinae, but they did not reject the hypothesis that the IWP *Ilisha* (minus *I. sirishae*), *Raconda*, and *Opisthopterus* along with *I. africana* form a monophyletic group.

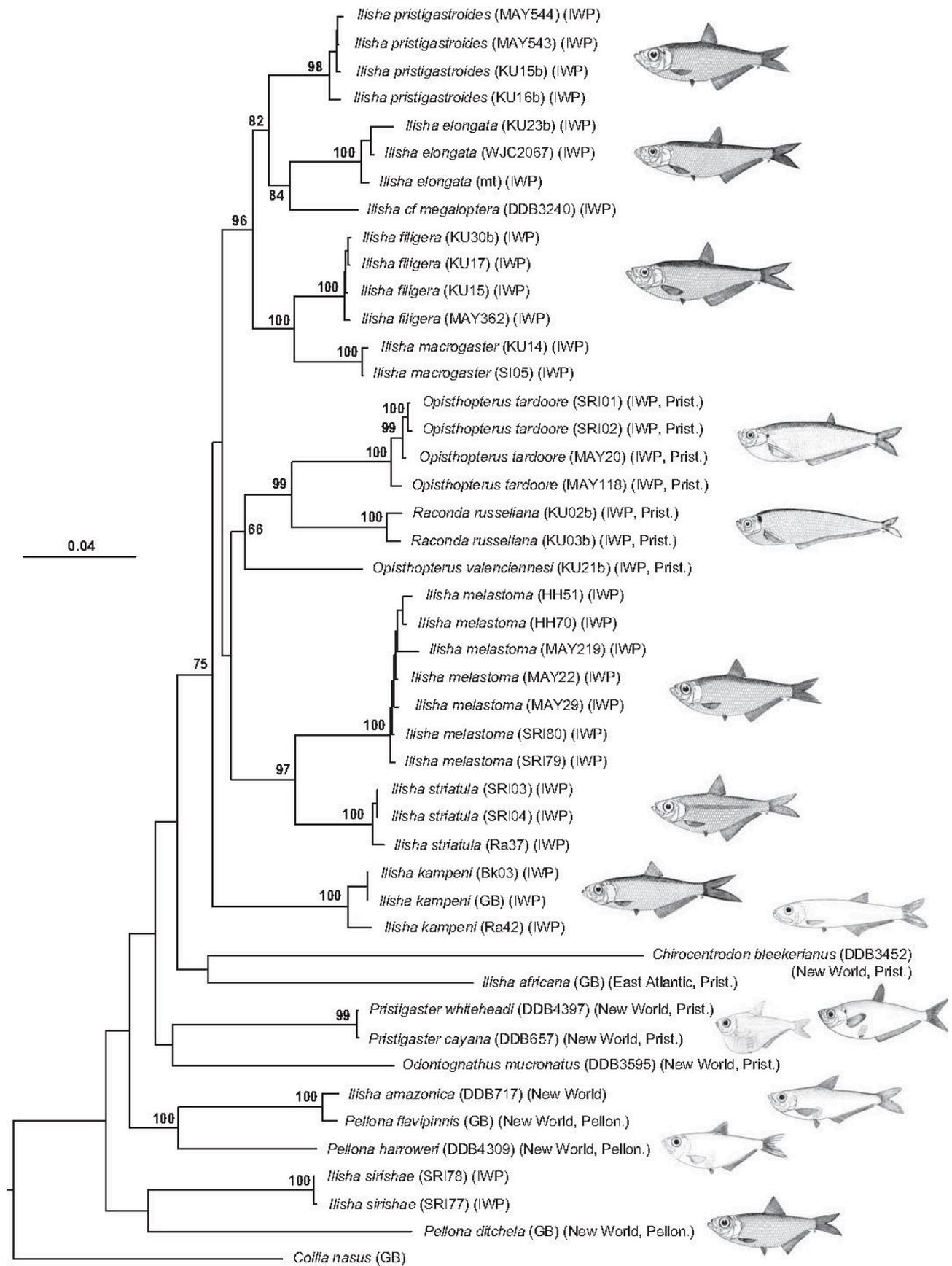


Fig. 3. Maximum likelihood phylogenetic tree of Pristigasteridae. *Coilia nasus* is used to root the tree. Bootstrap proportions are indicated at nodes when > 65%. Branch lengths are proportional to number of substitutions. Generic classification follows Whitehead (1985). The specimen code of

each specimen is indicated after its species name, followed by its geographic distribution (Indo-West Pacific [IWP] or East Atlantic or New World) and its subfamily assignment as established by Grande (1985) (Pristigasterinae [Prist.] or Pelloninae [Pellon.] or no subfamily). Fish illustrations Source: Whitehead (1985).

Reconstruction of swimbladder morphology evolution

The ancestral state reconstruction of the swimbladder morphology onto our ML tree (Fig. 4) showed that the absence of post-coelomic diverticula was the ancestral condition in the Pristigasteridae. The presence of paired post-coelomic diverticula originated later, most likely once in the ancestor of the clade ((*C. bleekermanus*, *I. africana*), IWP taxa), followed by one complete reversal in *C. bleekermanus*. When *C. bleekermanus* was excluded from the dataset (see previous section), the reconstruction strongly supported the hypothesis that the presence of paired post-coelomic diverticula evolved only once with no reversion. Finally, the presence of only one diverticulum in some species of *Ilisha* evolved once from a condition of paired post-coelomic diverticula, after the loss of the left diverticula.

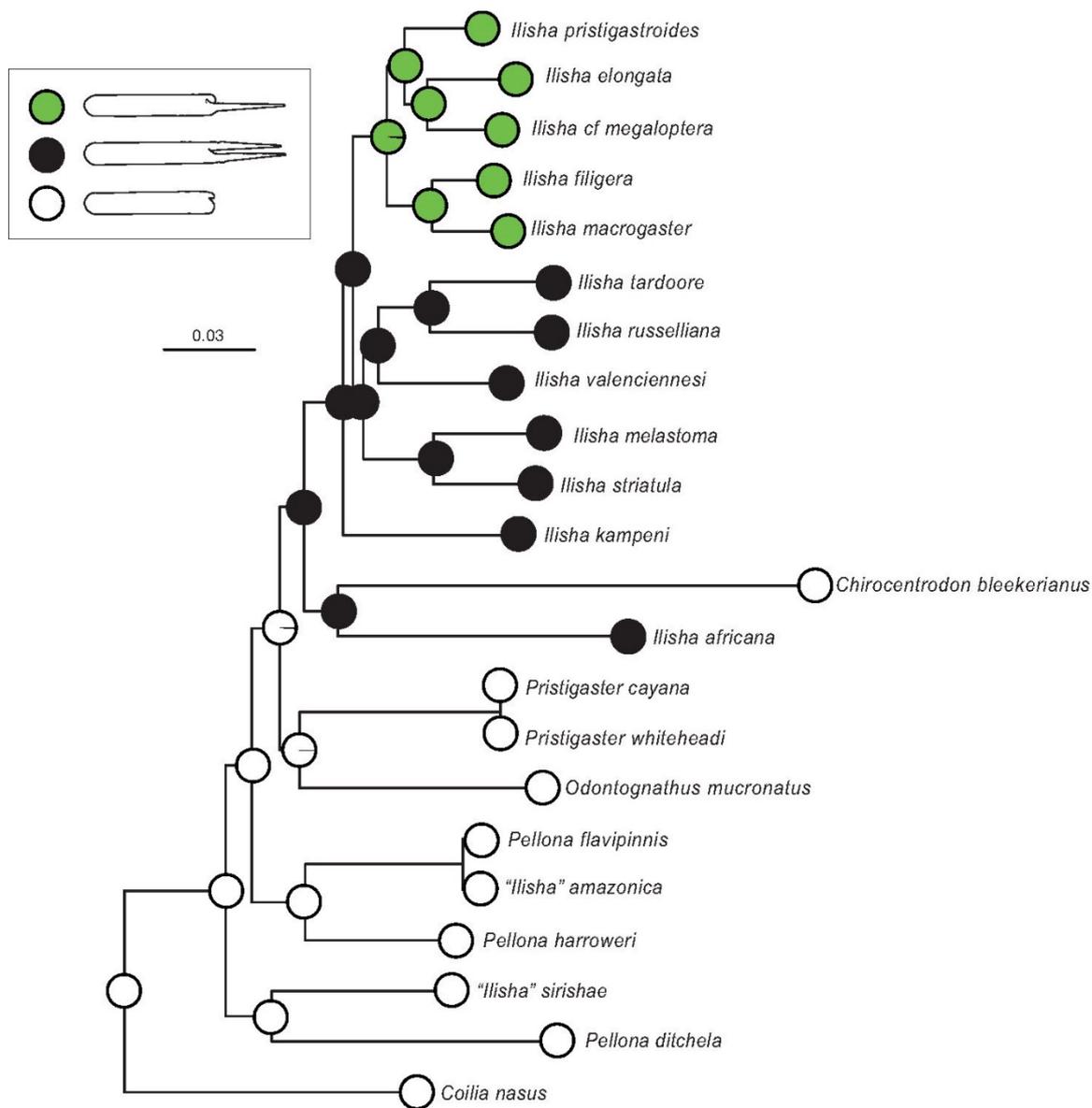


Fig. 4. Reconstruction of the evolution of post-coelomic diverticula within the Pristigasteridae. Ancestral conditions [no post coelomic extension (white), paired post-coelomic extensions (black) and single post coelomic extension (green)] at nodes reconstructed using a maximum likelihood method of ancestral character inference under the model "Mk1." Pie charts show likelihood support for ancestral habitat states (sum = 1) for the corresponding nodes. Note that the phylogenetic position of *Chirocentrodon bleekermanus* sister to *Ilisha africana* is not supported and additional data are needed to confidently resolve it. Classification is revised according to our results (see DISCUSSION).

DISCUSSION

Phylogenetic relationships

We reconstructed the most comprehensive molecular-based phylogeny of Pristigasteridae (longfin herrings) in order to examine the evolution of its swimbladder and discuss previous phylogenetic hypotheses. Longfin herrings were early recognized as a natural subgroup (tribe or subfamily) of the family Clupeidae because of their distinctive long anal fin (Günther 1868; Norman 1923; Berry 1964; Nelson 1967, 1970). Nelson (1967) noted that “*in terms of gill-arch structure, the Dussumieriinae and Pristigasterinae appear to be the most primitive*” and, consequently, Nelson (1970) removed the Pristigasterinae from the Clupeidae and elevated it to the family rank (*i.e.*, the Pristigasteridae). The phylogenetic position of the Pristigasteridae relative to other clupeoid families was left unresolved by Nelson (1970), and it is still being debated (Di Dario 2002; Li and Ortí 2007; Miyashita 2010; Lavoué et al. 2013; Bloom and Lovejoy 2014). Since the recent taxonomic reassignment of the Late Cretaceous-Paleocene[?] fossil †*Gasteroclupea branisae* from Bolivia to the extinct order †Ellimmichthyiformes (Marramà and Carnevale 2017), there are no more pristigasterid fossils and, consequently, no direct paleontological information available on the evolution of these fishes.

Prior to this work, only one phylogenetic hypothesis of the Pristigasteridae based on morphological characters was published (Grande 1985) (Fig. 1), in which the family Pristigasteridae [= Pristigasteroidea in Grande (1985)] was divided into three groups: the Pelloninae [= Pellonidae in Grande (1985)], the Pristigasterinae [= Pristigasteridae in Grande (1985), which also includes *I. africana*], and the genus *Ilisha* (minus *I. africana*). According to Grande (1985), the Pelloninae was supported by one character, the “*maxillary-premaxillary gap covered by bone*”, whereas the subfamily Pristigasterinae was supported by the presence of a “*bony process on the first pleural rib which articulates with the shoulder girdle*”. No character was given for the genus *Ilisha* (minus *I. africana*), and relationships among these three groups were left unresolved. de Pinna and di Dario (2003) briefly commented on the phylogeny of the Pristigasteridae, and they mentioned that the Pelloninae and Pristigasterinae are likely not reciprocally monophyletic. Bloom and Lovejoy (2014) examined the molecular phylogeny of 11 pristigasterid species, eight of them from the New World. They found *I. africana* to be sister to the rest of the Pristigasteridae, and the two species of IWP *Ilisha* were deeply nested within the New World lineages, making this group paraphyletic.

Although our taxonomic sampling is still incomplete, with two missing New World genera, *Pliosteostoma* and *Neopisthopterus* classified in the subfamily Pelloninae, along with some other New World species (such as those belonging to the genus *Opisthopterus*), our results (see ML tree on Fig. 3) refute the phylogenetic hypothesis of Grande (1985) and expand the results of Bloom and Lovejoy (2014) to support the non-monophyly of several taxa. We found that species of

Opisthopterus and *Raconda* are nested within IWP species of *Ilisha* and do not form a monophyletic group with *Odontognathus* and *Pristigaster*. This means that the loss of pelvic fins, which supported the monophyly of these four genera in Grande's (1985) phylogenetic hypothesis (Fig. 1), evolved independently, at least twice, in the family Pristigasteridae.

We confirm that *I. africana* forms a distinct lineage, only distantly related to other species of *Ilisha*. The other species of *Ilisha*, however, still do not form a monophyletic group (see Fig. 3) because: 1) *I. amazonica* is more closely related to some New World species of *Pellona* than to IWP species of *Ilisha*, 2) *I. sirishae* is more closely related to *Pellona ditchela* than to other IWP species of *Ilisha*, and 3) *Raconda* and *Opisthopterus* are nested within IWP species of *Ilisha* (minus *I. sirishae*), making this group paraphyletic.

Evolution of the swimbladder morphology

The swimbladder is a visceral gas-filled chamber that serves several important functions, such as buoyancy, floatability, and hydrostatic positioning. The morphology of the Clupeoidei's swimbladder is overall quite uniform with only a few specializations, except in the family Pristigasteridae where the swimbladder is both specialized and variable among species. Whitehead and Blaxter (1989) wrote that “*of the [five] clupeoid families, the pristigasterids show the most striking specializations of swimbladder form, with their post-coelomic diverticula, muscular tunica interna and pleural rib pockets*”. Among these three specializations, the presence of post-coelomic diverticula in some species of the Pristigasteridae was noted as early as the mid-19th Century (Valenciennes 1847), and this condition appears to be rare within the Teleostei (Poll 1969; Tominaga et al. 1996). The function of these diverticula is unknown. The presence of these extensions in only some species of the Pristigasteridae is especially intriguing, because the ecology and biology of all species of the Pristigasteridae seem similar (Blaber et al. 1998). So far, there is no reported ecological difference associated with the presence or absence of the diverticula, but more studies are needed.

Seshagiri Rao (1975 1976) [but see also Talwar and Whitehead (1971)] was among the first authors who considered the presence/absence and the number of post-coelomic diverticula in the Pristigasteridae as taxonomic characters. That author distinguished three groups among IWP *Ilisha* species, which are otherwise morphologically very similar. Seshagiri Rao (1975 1976), however, did not indicate whether this character, alongside its taxonomic value within *Ilisha*, could be used within the Pristigasteridae to define monophyletic groups. Later, Whitehead and Blaxter's (1989) review of the morphology of the swimbladder of the Clupeoidei explicitly stated that this character

potentially has some phylogenetic importance in the Pristigasteridae, but those authors did not reorganize the phylogeny and classification based on this.

We showed that the most ancestral condition in the Pristigasteridae is the absence of post-coelomic diverticula (Fig. 4); this is also the most common condition within the Clupeoidei and Teleostei (Poll 1969; Tominaga et al. 1996). Thus, our ancestral character state reconstruction supports the hypothesis of only one evolutionary origin for the presence of post-coelomic diverticula in the Pristigasteridae, challenging the current phylogeny and classification and confirming the hypothesis of Whitehead and Blaxter (1989). Whether the absence of diverticula in *Chirocentrodon* is a consequence of a reversal event should be further investigated for three reasons (which might not be independent of each other): 1) our data do not statistically reject the phylogenetic hypothesis that *I. africana* is sister to the IWP clade (instead of being sister to *Chirocentrodon*); 2) only the 16S rRNA gene is available for *Chirocentrodon*; and 3) the phylogenetic branch of *Chirocentrodon* is significantly longer than the others. Finally, one further specialization of the swimbladder occurred within the IWP clade with the loss of the left diverticulum in five species of *Ilisha*, making this condition the most derived one in the Pristigasteridae.

Implications for classification of the Pristigasteridae

Characters from the visceral morphology of the Teleostei are not frequently used in systematics, although they potentially contain as much phylogenetic information as other characters. This is particularly true in the Clupeoidei, where the visceral morphology has only been used to distinguish two closely related clupeid genera, *Sprattus* and *Clupea* (absence versus presence of a pterotic bulla) or in the taxonomy of the “gizzard” shads [gut anatomy; (Nelson and Rothman 1973)].

The presence of post-coelomic diverticula in the Pristigasteridae was noted a long time ago, but never considered a phylogenetic character until Whitehead and Blaxter (1989) suggested it. Considering the work of Whitehead and Blaxter (1989), our own phylogenetic results, and the necessity to establish a phylogenetic classification for the Pristigasteridae, we herein propose to diagnose the genus *Ilisha* Richardson 1846 (type species: *I. elongata*) by the presence of one or two post-coelomic diverticula in the swimbladder. Therefore, the new definition of *Ilisha* includes all IWP pristigasterid species from the genera *Raconda*, *Opisthopterus*, and *Ilisha* (except *I. sirishae* and *I. novacula*, which do not have such a post-coelomic extension), and the East Atlantic *I. africana*. *Raconda* and *Opisthopterus* are synonymized with *Ilisha* according to the new definition. The New World species of *Ilisha* (*I. amazonica* and *I. furthii*) and *Opisthopterus* (four species)

along with IWP *I. sirishae* and *I. novacula* are left *insertae sedis* within the Pristigasteridae until a more-complete systematic study of the New World species can be undertaken.

This phylogenetic-based modification classifying the Pristigasteridae has several advantages: 1) it clarifies the taxonomic situation of the genus *Ilisha*, particularly the situation of IWP species of *Ilisha*; 2) it minimizes the number of changes without the need to erect several new genera for species-poor lineages that are problematic to diagnose; and 3) it simplifies the taxonomic revision of New World pristigasterid species of “*Ilisha*” and “*Opisthopterus*.”

Finally, we underscore the need to revise the classification of the genus *Pellona*, especially that of the two Indo-West Pacific species, *P. ditchela* and *P. dayi*. However, a denser taxonomic sampling among New World taxa is necessary to achieve this goal.

CONCLUSIONS

Our phylogenetic results show that the subfamilies Pristigasterinae and Pelloninae and the genera *Ilisha*, *Pellona*, and *Opisthopterus* are not monophyletic, leading to the conclusion that the classification of the family Pristigasteridae must be revised. We found that the presence of post-coelomic diverticula in the swimbladder represents a derived condition, and it is phylogenetically conserved with a unique origin. Furthermore, species of *Ilisha* with only one diverticulum in their swimbladder secondarily evolved from a common ancestor that had paired diverticula. Given the multiple functions of the swimbladder in the Teleostei, it is likely that the presence of one or two diverticula in the Pristigasteridae is associated with some function that remains to be determined. Further studies should expand taxonomic sampling within the Pristigasteridae and investigate the function of swimbladder specializations in these fishes, which otherwise exhibit similar life traits and histories. Finally, we formalized the taxonomic implication of our phylogenetic results in re-diagnosing the genus *Ilisha* by the presence of post-coelomic diverticula in the swimbladder.

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Appendix 1. List of all species of Pristigasteridae (38 species) along with corresponding information concerning their distribution and the absence (“No”) or presence of one (“1”) or two (“2”) post-coelomic diverticula. Data from Whitehead and Blaxter (1989) and Eschmeyer et al. (2018). Classification follows Grande (1985); see figure 1. (download)