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# Evolutionary Relationships and Evolution of Body Shape of the Deep-Sea Hatchetfishes (Stomiiformes: Sternoptychidae).

Zachary A. May

# Evolutionary Relationships and Evolution of Body Shape of the Deep-Sea Hatchetfishes (Stomiiformes: Sternoptychidae).

by

Zachary A. May

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Matthew P. Davis, Chairperson
Matthew A. Tornow
Jennifer Y. Lamb

## Abstract

Within the Actinopterygii (ray-finned fishes), the Stomiiformes (dragonfishes and their allies) include 444 species of fishes found in pelagic deep-sea habitats world-wide. Within Stomiiformes, the family Sternoptychidae (deep-sea hatchetfishes) includes 78 species within ten genera. The deep-sea hatchetfishes all possess ventral bioluminescent photophores, which are hypothesized to be used for camouflage in the deep sea. The sternoptychids are commonly known for having deep bodies that resemble a hatchet, although seven genera in this family exhibit a more slender-body. Previous phylogenetic studies have examined the evolutionary relationships of this family of fishes predominately based on morphological data. Few molecular-based studies have examined the evolutionary relationships of the sternoptychids. This study investigates the evolutionary relationships of Sternoptychidae using a genome-scale dataset with ultraconserved elements (UCEs) and protein-coding gene fragments. This dataset is then combined with previously published morphological data to infer a total evidence hypothesis for the evolutionary relationships among deep-sea hatchetfishes. The phylogenetic analyses infer the Sternoptychidae to be a monophyletic family, although these results differ from previous phylogenetic studies regarding the monophyly of the subfamilies 'Maurolicinae' and Sternoptychinae. The 'Maurolicinae' consists of seven slender-bodied genera (*Araiophos*, *Argyripnus*, *Danaphos*, *Maurolicus*, *Sonoda*, *Thorophos*, *Valenciennellus*) and Sternoptychinae includes three deep-bodied genera (*Argyropelecus*, *Polyipnus*, *Sternoptyx*). The hypothesis of relationships presented herein for the family infers a polyphyletic 'Maurolicinae' with *Maurolicus* inferred to be nested within the subfamily Sternoptychinae. To investigate the evolution of body shape in the deep-sea hatchetfishes, patterns of body-shape were quantified in 684 digitized specimens using a geometric morphometric approach with seven homologous landmarks and 106 semi-landmarks. The relative warp analysis clustered species within genera together in relationship to their deep or slender body-shape. The geometric morphometric analysis provides continuous characters of body shape that allowed for a species-level ancestral character state reconstruction in combination with the total evidence phylogeny. The results presented herein infer a slender-bodied common ancestor for the family Sternoptychidae with three independent transitions to deeper bodies.

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## Introduction

### Sternoptychidae: The Deep-Sea Hatchetfishes and Their Habitat

The oceans include some of the most diverse and understudied environments on the planet and cover over 71 percent of its surface (Robison, 2004; Neill, 2019). There are over 35,000 described species of fishes, with more than half of these residing in marine habitats (Helfman, Collette, Facey, Bowen, 2009; Nelson, Grande, Wilson, 2016; Fricke, Eschmeyer, van der Laan, 2019a; Fricke, Eschmeyer, Fong, 2019b). The order Stomiiformes (dragonfishes and their allies) are a marine lineage of ray-finned fishes that contain 444 species distributed globally in pelagic deep-sea habitats. These habitats include the epipelagic (0–200 meters), mesopelagic (200–1000 meters) and bathypelagic zones (1000–4000 meters) (Weitzman, 1974; Fink & Weitzman, 1982; Fink, 1985; Harold & Weitzman, 1996; Helfman et al., 2009; Haddock, Moline, Case, 2010; Webb, Vanden Berghe & O'Dor, 2010; Davis, Holcroft, Wiley, Sparks, Smith, 2014; Nelson et al., 2016; Davis, Sparks, Smith, 2016; Hellinger, Huhn, Herlitze, 2017; Fricke et al., 2019a,b). These descending zones of the ocean create a complex pelagic environment resulting in little-to-no natural solar light, an increase in pressure, and temperature gradients resulting in a thermocline (Helfman et al., 2009; Haddock et al., 2010).

There are four families of stomiiform fishes including the Gonostomatidae (bristlemouths), Phosichthyidae (lightfishes), Stomiidae (dragonfishes), and Sternoptychidae (hatchetfishes). All stomiiforms have a variety of evolutionary adaptations for thriving in their deep-sea habitat, such as bioluminescence, which aid in communication and predator-prey interactions. (Harvey, 1952; Herring, 1978, 1982, 1987, 2000, 2007; Haddock et al., 2010; Widder, 2010; Davis et al., 2014; Davis et al., 2016). Stomiiforms emit light endogenously

through a variety of bioluminescent organs including photophores (Davis et al., 2016). The photophores of Stomiiformes are predominantly located on the ventral surface of the body and are hypothesized to be used for counter illumination to camouflage their silhouette against down-welling light (Baird, 1971; Baird & Eckardt, 1972; Weitzman, 1974; Mensinger & Case, 1990; Randall & Farrell, 1997; Priede, 2017). A few species also have light-emitting organs below the eye (orbital photophores), photophores on the lateral surface of the body (deep-sea hatchetfishes), and bioluminescent chin barbels (dragonfishes) (Fink & Weitzman, 1982; Fink, 1985; Davis et al., 2014; DeArmon, 2019). Bioluminescence in stomiiforms is hypothesized to serve a variety of functions including inter and intraspecies communication, camouflage, prey attraction, and mate recognition (Harvey, 1952; Weitzman, 1974; Herring, 1978, 1982, 1987 2000, 2007; Fink & Weitzman, 1982; Fink, 1985; Haddock et al., 2010; Widder, 2010; Davis et al., 2014; Davis et al., 2016; Marranzino & Webb, 2018).

The family Sternoptychidae (deep-sea hatchetfishes) includes 78 species across ten genera exhibit a variety of body-shape morphologies (Figure 1.1). The deep-sea hatchetfishes are known for their deep, hatchet-shaped body, although there are slender-bodied species within this group as well. All deep-sea hatchetfishes possess ventral bioluminescent photophores that aid in counter illumination (Baird, 1971; Baird & Eckardt, 1972; Weitzman, 1974; Mensinger & Case, 1990; Randall & Farrell, 1997; Priede, 2017). These deep-dwelling fishes predominately feed on planktonic crustaceans found during the hatchetfishes vertical (diurnal) migrations into the upper mesopelagic (~ 200 m) and lower epipelagic (< 200 m) zones of the ocean (Baird, 1971; Hopkins & Baird, 1973, 1985; Kinzer & Schulz, 1988).

**Taxonomy and Evolutionary Relationships of the Hatchetfishes**

The first deep-sea hatchetfish was diagnosed and described by Hermann (1781) for the deep-bodied *Sternoptyx* (Figure 1.1, I). Our knowledge of the biodiversity of these fascinating deep-sea fishes has increased over time and ten genera (Figure 1.1) are now known from this lineage of fishes (Fricke et al., 2019a,b) including *Sternoptyx* (Hermann, 1781), *Argyropelecus* (Cocco, 1829), *Maurolicus* (Gmelin, 1838), *Polyipnus* (Günther, 1887), *Valenciennellus* (Jordan & Evermann, 1896), *Argyripnus* (Gilbert & Cramer, 1897), *Thorophos* (Bruun, 1931), *Danaphos* (Bruun, 1931), *Sonoda* (Grey, 1959), and *Araiophos* (Grey, 1961). The oldest fossil evidence of this family dates back to the Eocene and Miocene with fossils attributed to the genera *Argyropelecus*, *Polyipnus*, and *Sternoptyx* (Baird, 1971; Carnevale, 2007; Afsari Yazdi, Bahrami & Carnevale, 2014).

The family Sternoptychidae has not always been hypothesized to be monophyletic with its currently recognized taxonomic composition. Prior studies hypothesized that genera within the currently recognized Sternoptychidae were more closely related to taxa within the families Gonostomatidae and Phosichthyidae (Gmelin, 1789; Gunther, 1864, 1878; Brauer, 1906, 1908; Regan, 1923; Norman, 1930; Schultz, 1938; Grey, 1959, 1960; Schultz, 1961, 1964; Weitzman, 1967; Baird, 1971; Baird & Eckardt, 1972). Baird (1971) and Baird and Eckardt (1972) focused on the systematics and zoogeography of three genera of deep-sea hatchetfishes (*Argyropelecus*, *Polyipnus*, *Sternoptyx*) inferred from 41 anatomical characters. These studies identified *Polyipnus* as the sister group to a clade including *Argyropelecus* and *Sternoptyx*. Baird and Eckardt (1972) hypothesized that *Polyipnus* was the more “primitive” of the three genera and that *Sternoptyx* was the most “advanced.”

The currently recognized taxonomic composition of the Sternoptychidae was first hypothesized by Weitzman (1974) based on morphological data (Figure 1.2, A). Weitzman (1974) inferred the family to be a monophyletic group and identified that the subfamily 'Maurolicinae' (Gill, 1885) was polyphyletic (Figure 1.2, A) (*Araiophos*, *Argyripnus*, *Danaphos*, *Maurolicus*, *Sonoda*, *Thorophos*, *Valenciennellus*) and that the subfamily 'Sternoptychinae' (Dumeril, 1805) was monophyletic (*Argyropelecus*, *Polyipnus*, *Sternoptyx*). Weitzman (1974) did not include an optimization-based phylogenetic approach, but he did identify synapomorphies that he inferred as 'primitive' for the stem lineages of the polyphyletic 'Maurolicinae', followed by 'advanced' synapomorphies in the deeper-bodied 'Sternoptychinae' (Figure 1.2, A). Subsequent studies investigating the evolutionary relationships of deep-sea hatchetfishes have consistently recovered a monophyletic Sternoptychidae with some studies including all ten genera and additional characters (Harold & Weitzman, 1996), while others were focused on a subset of taxa (Baird, 1971; Baird & Eckardt, 1972; Harold, 1993; 1994; Miya & Nishida, 1998). Ahlstrom (1974) discussed the evolution of stomiiform fishes with a focus on taxa in the Gonostomatidae and Sternoptychidae based on larval and adult anatomical characters. Ahlstrom (1974) identified differences in the formation and types of photophores found in these fishes as well as characters associated with their metamorphosis from larval to adult forms. He identified two distinct groups within Sternoptychidae based on specializations of photophore development including a group consisting of *Araiophos*, *Argyripnus*, *Danaphos*, *Maurolicus*, *Sonoda*, *Thorophos,* and *Valenciennellus* and a second group including *Argyropelecus*, *Polyipnus*, and *Sternoptyx*. These groupings were consistent with the Sternoptychidae subfamily classifications of Weitzman (1974).


*Figure 1.1*. Sternoptychidae Diversity: A) *Thorophos nexilis* USNM 92326, B) *Araiophos eastropas* USNM 203240, C) *Argyripnus brocki* USNM 207657, D) *Sonoda pauculampa* USNM 196967, E) *Valenciannellus tripunctulatus* USNM 201141, F) *Danaphos oculatus* USNM 201100, G) *Polyipnus spinifer* USNM 135528, H) *Maurolicus imperatorius* USNM 321407, I) *Sternoptyx diaphana* USNM 378001, J) *Arryropelecus aculeatus* USNM 196699.

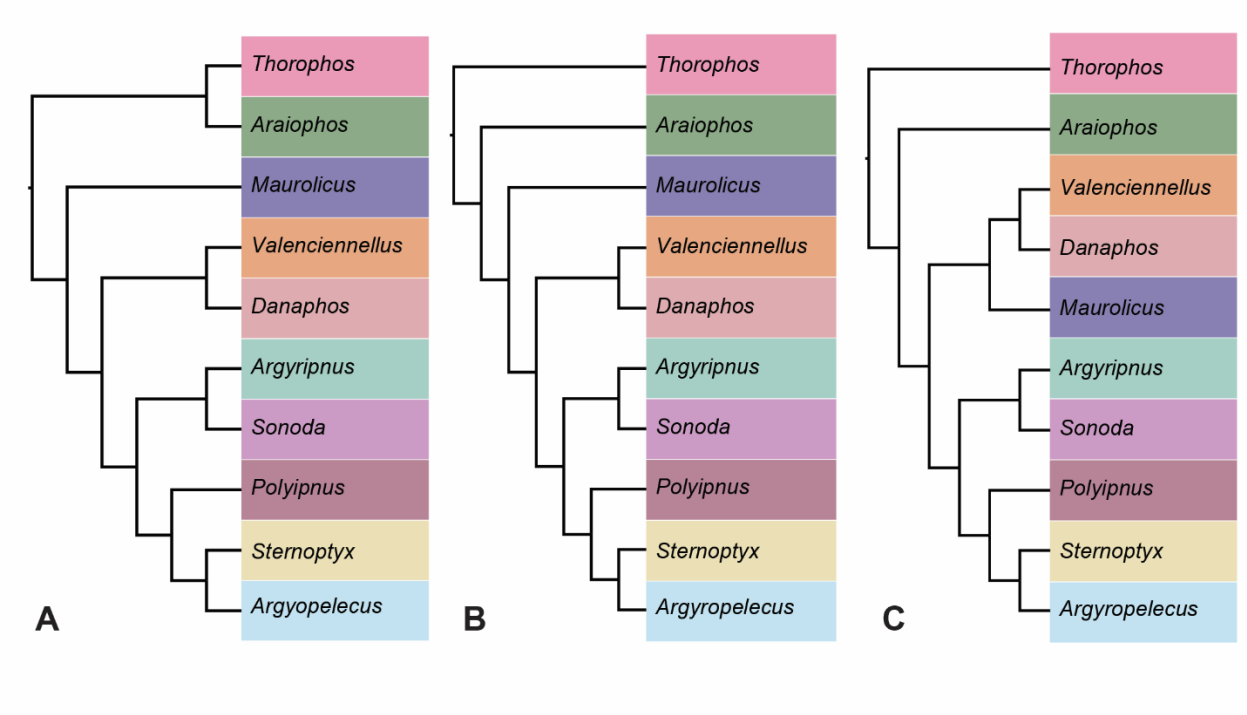


*Figure 1.2*. Previous Sternoptychidae Evolutionary Relationships: Hypotheses of evolutionary relationships among hatchetfishes based on morphological data from A) Weitzman (1974), B) Harold and Weitzman (1996) most parsimonious reconstruction, and C) Harold and Weitzman (1996) parsimony hypothesis that is one step longer than the most parsimonious hypothesis.

Harold and Weitzman (1996) investigated the interrelationships of the Stomiiformes and inferred a hypothesis of evolutionary relationships for Sternoptychidae using a maximum parsimony approach based on 150 anatomical characters. They inferred the family Sternoptychidae to be monophyletic within Stomiiformes (Figure 1.2, B) with the same taxonomic composition as Weitzman (1974) (Figure 1.2, A). The most parsimonious phylogenetic hypotheses were similar to the results from Weitzman (1974), with the seven slender-bodied 'Maurolicinae' genera recovered as a grade leading to a clade of the three hatchet-shaped genera in the subfamily 'Sternoptychinae' (Figure 1.2, B). Their hypothesis of relationships differs from Weitzman (1974) in that *Thorophos* is hypothesized as the sister group to the remaining genera of the Sternoptychidae (Figure 1.2, B) in their most parsimonious

reconstruction (Harold & Weitzman, 1996). Harold and Weitzman (1996) also highlighted that the phylogenetic placement of *Maurolicus* was tenuous, with the most parsimonious hypothesis inferring *Maurolicus* as the sister-group to a clade of *Danaphos* and *Valenciennellus* (Figure 1.2, B) while one step longer resulted in the placement of *Maurolicus* as the sister group to a clade that included *Danaphos, Valenciennellus, Argryipnus, Sonoda, Polyipnus, Argyropelecus,* and *Sternoptyx* (Figure 1.2, C).

Other studies have focused on species-level relationships within specific clades of hatchetfishes (Harold, 1993, 1994; Miya & Nishida, 1998). Harold (1994) investigated the evolutionary relationships within the genus *Polyipnus* using a combination of 182 photophore, morphological, and osteological characters from previous studies (e.g., Harold, 1993). Harold's (1994) taxonomic revision resulted in the recognition of 30 species of *Polyipnus*, the most species-rich genus of deep-sea hatchetfishes. Few studies have used genetic information to infer relationships among deep-sea hatchetfishes. Miya and Nishida (1998) used mitochondrial DNA (12S and 16S rRNA) gene-fragment data from four species of *Sternoptyx* (1998) to investigate phylogenetic relationships and biogeographic patterns in *Sternoptyx*.

Studies focused broadly on the evolutionary relationships of ray-finned fishes have included a limited number of Sternoptychidae genera (e.g., Near, Eytan, Dornburg, Kuhn, Moore, Davis, Wainwright, Friedman, Smith, 2012; Davis et al., 2014; Davis et al., 2016; Smith, Stern, Girard, Davis, 2016; Betancur, Wiley, Arratia, Acero, Bailly, Miya, Lecointre, Ortí, 2017; Smith, Everman, Richardson, 2018) and the family is consistently inferred to be monophyletic within the Stomiiformes although some genera such as *Sonoda* and *Araiophos* are rarely included due to scarcity of available specimens for DNA-based work. No study has investigated the evolutionary relationships of deep-sea hatchetfishes with a genome-scale molecular dataset using

ultraconserved elements (UCE) or in a total-evidence framework that incorporates previously published morphological data (Harold & Weitzman, 1996) with all ten genera of Sternoptychidae.

The objectives of this study are to investigate the evolutionary relationships among the deep-sea hatchetfishes using newly collected genome-scale data (UCEs) and with a total evidence approach incorporating molecular and morphological data. I use a combination of UCEs, nuclear and mitochondrial protein-coding gene fragments, and previously published anatomical characters (Harold & Weitzman, 1996) to conduct a total evidence approach to investigate the evolutionary relationships of deep-sea hatchetfishes. I address the following questions: 1) Is the family Sternoptychidae monophyletic? 2) Are the subfamilies of deep-sea hatchetfishes monophyletic? and 3) What are the patterns of relationships among the deep-sea hatchetfishes?

## Materials and Methods

### Taxonomic Sampling

Ultraconserved elements (UCEs) from twelve species of deep-sea hatchetfishes were sequenced including seven of ten sternoptychid genera. Additional molecular data were included from previously published studies including eight nuclear (e.g., Enc1, myh6, plagl2, ptr, RAG1, SH3PX3, tb1, zic1) and one mitochondrial gene fragment (e.g., COI). The additional nuclear and mitochondrial gene fragments included previously published data from GenBank (Table 1.1) as well as newly acquired sequences for 27 sternoptychid species representing the same seven of ten sternoptychid genera as the UCE dataset. The total evidence dataset (UCEs, nuclear and mitochondrial gene fragments, and morphological data) included 32 of 78 taxa of Sternoptychidae representing all ten genera. Outgroups included a representative of

Osmeriformes (smelts) as the rooted outgroup as Osmeriformes are inferred to be the sister group to Stomiiformes in previous studies (Near et al., 2012; Smith et al., 2016; Davis et al., 2016). Additional outgroups included eight taxa representing the other three families of Stomiiformes, with three species of Gonostomatidae, two species of Phosichthyidae, and three species of Stomiidae.

**DNA Extractions**

DNA extractions were completed using muscular tissue samples or fin clips from 21 species including twelve sternoptychids and the nine closely related outgroup using a DNeasy Tissue Extraction Kit (Qiagen) (nuclear and mitochondrial gene fragments) and a Maxwell RSC Instrument (UCE sequencing) with a Promega Blood Kit (AS1400) following manufacturer protocols. The quantified samples were sent to Arbor Biosciences (Ann Arbor, MI) for library preparation, amplification, and sequencing.

**UCE Amplification, Sequencing and Assembly**

Extractions sent to Arbor Biosciences underwent DNA shearing, size selection, cleanup, target capture using the 500 UCE Actinopterygian loci probe set (Faircloth, Sorenson, Santini, Alfaro, 2013) and enrichment. Sequencing of these UCEs were conducted on an Illumina HiSeq 2500, including the demultiplexing of samples. The UCE dataset was received as a Fastq data file which was then refined and cleaned for assembly using various software within PHYLUCE v1.5.0. This data file containing the cleaned genomic sequences were assembled into contigs using ABySS v1.3.7 (Simpson et al., 2009). This new assemblage (kmer value of 60) was then separated out using the UCE Actinopterygian loci probe set (Faircloth et al., 2013) in LASTZ v1.02.00 (Harris, 2007) by an 80% minimum coverage and identity respectively for detecting UCEs. PHYLUCE v1.5.0 (Faircloth, McCormack, Crawford, Harvey, Brumfield, Glenn, 2012)

collectively gathered and organized a database of the UCE loci by taxon, creating a FASTA file. The extracted UCE data was aligned using MAFFT to create a data matrix (Katoh & Standley, 2013) that included contigs of a 65% match or higher of the included taxa. The extracted UCE data include 419 UCEs concatenated for a total length of 265,371 base pairs.

**Gene Fragment Sequences**

The UCE dataset was concatenated with newly acquired and previously published nuclear and mitochondrial gene fragments from GenBank (Table 1.1). These included eight nuclear Glyt, Myh6, plagl2, RAG1, SH3PX3, tbr1, zic1, Ptr1, and ENC1 and one mitochondrial (COI) gene fragments (Table 1.1). These gene fragments were aligned using MAFFT (Katoh & Standley, 2013) and concatenated to the UCE dataset for a comprehensive molecular dataset.

**Morphological Data**

Morphological data from Harold & Weitzman (1996) includes 150 morphological characters (Appendix 1.1) and this character matrix (Appendix 1.2) was concatenated to the molecular dataset to make a total evidence dataset that includes all ten genera within the family Sternoptychidae. Outgroup taxa that were not coded in Harold and Weitzman (1996) were coded as unknown in this analysis (Appendix 1.2).

**Phylogenetic Analysis**

The UCE data were partitioned to assess the appropriate model of evolution using the Sliding-Window Site Characteristics – Entropy Method, SWSC-EN (Tagliacollo & Lanfear, 2018). These individual, species-specific UCE loci are fragmented into three regions; a left flanking region, a right flanking region and an ultra-conserved core through a rate of evolution. PartitionFinder v2.1.1 (Lanfear, Calcott, Kainer, Mayer, Stamatakis, 2014; Stamatakis, 2014; Lanfear, Frandsen, Wright, Senfeld, Calcott, 2017) was used rendering the proper nucleotide

substitution model for each region found. PartitionFinder v2.1.1 was also used to infer the best fitting model of molecular evolution for each codon position of the nine additional gene fragments. For the total evidence dataset, the morphological characters from Harold and Weitzman (1996) were assigned the Lewis MK model for morphological data (Lewis, 2001). Polymorphic characters were coded as unknown for the subsequent maximum likelihood analyses.

The phylogenetic analyses of the molecular dataset (concatenated ultraconserved element dataset and mitochondrial and nuclear gene fragments) and the total evidence dataset (concatenated ultraconserved elements, mitochondrial and nuclear gene fragments, and morphological characters) was conducted with a Maximum Likelihood approach using IQ-TREE v1.6.8 (Nguyen, Schmidt, von Haeseler, Minh, 2015). For each dataset, 25 independent analyses were conducted and the tree with the highest likelihood score is presented herein (Figures 1.3 & 1.4). Additionally, 100 nonparametric bootstrap replicates were also conducted (Felsenstein, 1973).

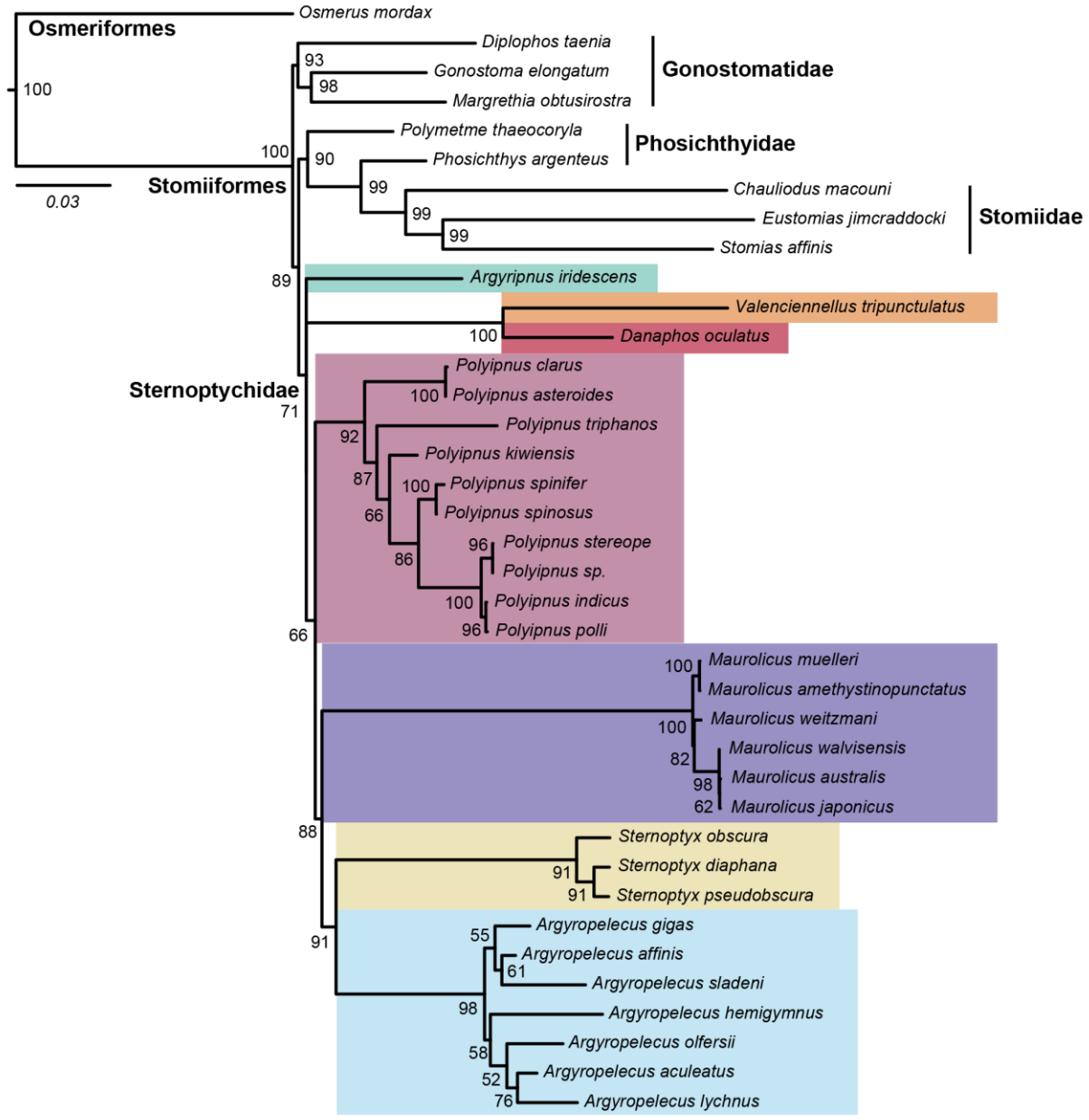


*Figure 1.3*. Hypothesized Molecular Phylogenomic Relationship of Sternoptychidae: Ultraconserved Elements (UCEs) and Protein-Coding Gene Fragments: Maximum-likelihood phylogeny of deep-sea hatchetfishes inferred from concatenated ultraconserved elements and gene-fragment data. Numbers at nodes indicate bootstrap support values.

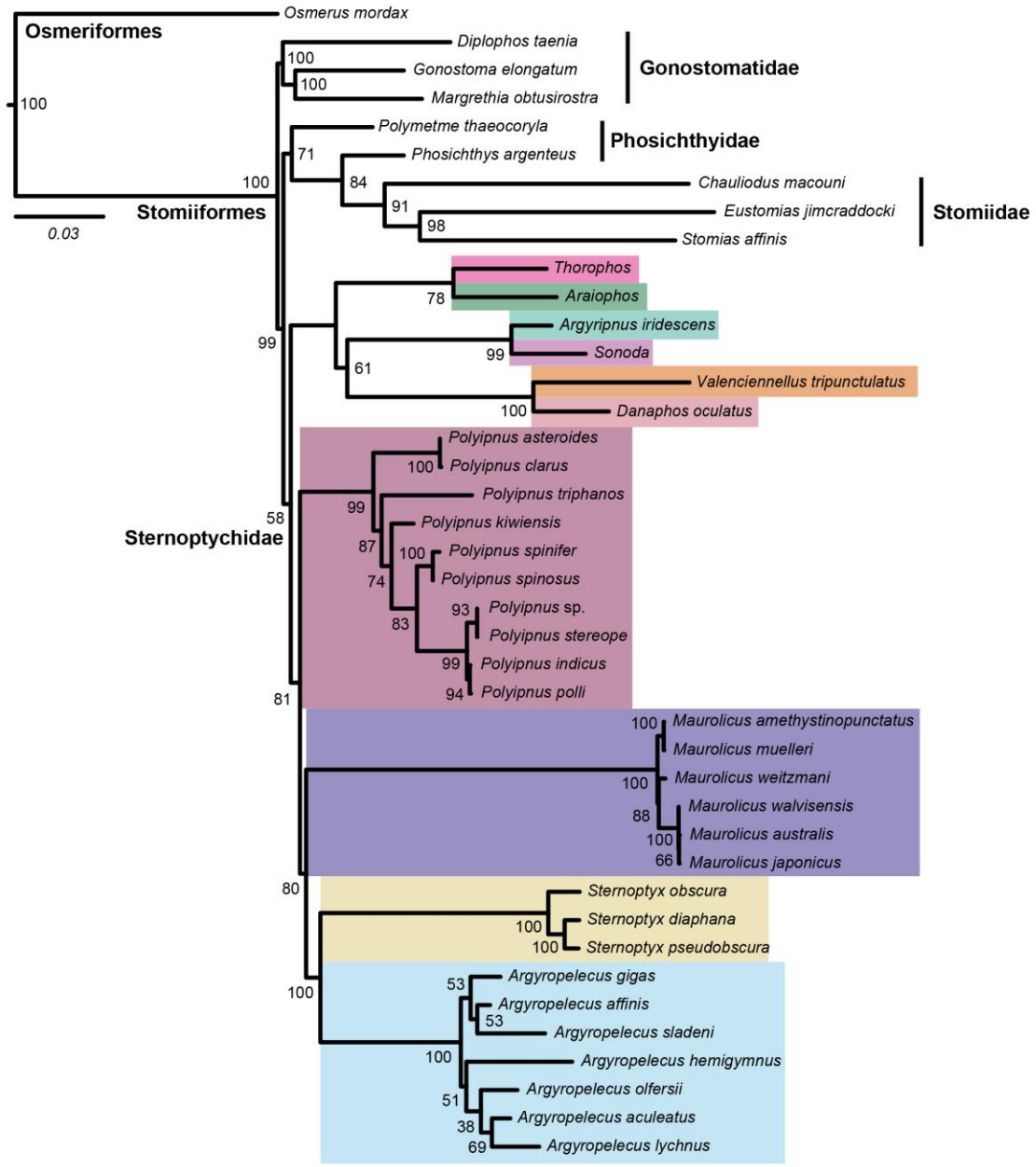


*Figure 1.4*. Hypothesized Total Evidence Phylogenomic Relationship of Sternoptychidae: Ultraconserved Elements (UCEs), Protein-Coding Gene Fragments and Morphological Characters: Maximum-likelihood phylogeny of the deep-sea hatchetfishes inferred from concatenated ultraconserved elements, gene fragments, and anatomical character states. Numbers at nodes indicate bootstrap support values.

Table1.1

*Genbank Accession Numbers*

| Specimen | CO1 | Glyt | Myh6 | plagl2 |
|---|---|---|---|---|
| Outgroups | | | | |
| *Chauliodus macouni* | JQ354039.1 | | KF139964.1 | |
| *Diplophos taenia* | KJ190028.1 | KX227820.1 | FJ918866.1 | EU366676.1 |
| *Eustomias jimcraddocki* | | | GQ860491.1 | |
| *Gonostoma elongatum* | KX228026.1 | KF139754.1 | KX227886.1 | KX227908.1 |
| *Margrethia obtusirostra* | KJ190029.1 | | KX227868.1 | |
| *Osmerus mordax* | FJ205606.1 | JN132509.1 | JN132546.1 | JN132584.1 |
| *Phosichthys argenteus* | GQ860371.1 | | GQ860477.1 | |
| *Polymetme thaeocoryla* | KJ190039.1 | | KX227878.1 | |
| *Stomias affinis* | GQ860375.1 | | GQ860482.1 | |
| Sternoptychidae | | | | |
| *Argyropelecus aculeatus* | KX098553.1 | | GQ860473.1 | |
| *Argyropelecus affinis* | KJ190035.1 | | KF139927.1 | KF140677.1 |
| *Argyropelecus gigas* | MG856721.1 | | GQ860474.1 | JX190574.1 |
| *Argyropelecus hemigymnus* | MF041549.1 | | | |
| *Argyropelecus lychnus* | HQ010059.1 | | | |
| *Argyropelecus olfersii* | EU148090.1 | | | |
| *Argyropelecus sladeni* | MG856544.1 | | GQ860475.1 | |
| *Danaphos oculatus* | HQ010057.1 | | | |
| *Maurolicus amethystinopunctatus* | KU958037.1 | | | |
| *Maurolicus australis* | KU958032.1 | | GQ860470.1 | |
| *Maurolicus japonicus* | KU199192.1 | | | |
| *Maurolicus muelleri* | KU958039.1 | | | |
| *Maurolicus walvisensis* | KU958031.1 | | | |
| *Maurolicus weitzmani* | KU958025.1 | | GQ860471.1 | |
| *Polyipnus asteroides* | KF930288.1 | | | |
| *Polyipnus clarus* | FJ918932.1 | | FJ918870.1 | |
| *Polyipnus indicus* | JF494209.1 | | | |
| *Polyipnus kiwiensis* | GQ860367.1 | | | |
| *Polyipnus polli* | JF494212.1 | | | |
| *Polyipnus spinifer* | KU893054.1 | | KF140086.1 | |
| *Polyipnus spinosus* | KP267658.1 | | | |
| *Polyipnus stereope* | KC442066.1 | | | |
| *Polyipnus triphanos* | KU943035.1 | | | |
| *Sternoptyx diaphana* | MF957051.1 | | | |
| *Sternoptyx obscura* | GQ860368.1 | | | |
| *Sternoptyx pseudobscura* | GU440537.1 | | KX227887.1 | KX227909.1 |
| *Valenciennellus tripunctulatus* | GQ860363.1 | | GQ860472.1 | |

Table1.1

*Genbank Accession Numbers*

| Specimen | RAG1 | SH3PX3 | tbr1 | zic1 |
|---|---|---|---|---|
| Outgroups | | | | |
| *Chauliodus macouni* | | | KF140327.1 | KF140513.1 |
| *Diplophos taenia* | EU366724.1 | KX227956.1 | KC830313.1 | EU366768.1 |
| *Eustomias jimcraddocki* | GQ860333.1 | | | |
| *Gonostoma elongatum* | KJ190081.1 | KF141476.1 | KF140358.1 | KJ190116.1 |
| *Margrethia obtusirostra* | KJ190080.1 | KX227963.1 | KX227999.1 | KJ190115.1 |
| *Osmerus mordax* | EU621579.1 | JN132720.1 | JX255615.1 | JN132785.1 |
| *Phosichthys argenteus* | GQ860318.1 | | | |
| *Polymetme thaeocoryla* | KX228059.1 | KX227970.1 | KX228006.1 | KJ190124.1 |
| *Stomias affinis* | GQ860324.1 | | | |
| Sternoptychidae | | | | |
| *Argyropelecus aculeatus* | GQ860311.1 | | | |
| *Argyropelecus affinis* | KX228066.1 | KF141405.1 | KX227990.1 | KJ190121.1 |
| *Argyropelecus gigas* | GQ860312.1 | | JX191177.1 | JX191296.1 |
| *Argyropelecus hemigymnus* | | | | |
| *Argyropelecus lychnus* | | | | KJ190120.1 |
| *Argyropelecus olfersii* | | | | |
| *Argyropelecus sladeni* | GQ860313.1 | | | |
| *Danaphos oculatus* | | | | |
| *Maurolicus amethystinopunctatus* | | | | |
| *Maurolicus australis* | | | | |
| *Maurolicus japonicus* | | | | |
| *Maurolicus muelleri* | | | | |
| *Maurolicus walvisensis* | | | | |
| *Maurolicus weitzmani* | GQ860309.1 | | | |
| *Polyipnus asteroides* | | | | |
| *Polyipnus clarus* | FJ896445.1 | | | FJ906666.1 |
| *Polyipnus indicus* | | | | |
| *Polyipnus kiwiensis* | GQ860314.1 | | | |
| *Polyipnus polli* | | | | |
| *Polyipnus spinifer* | KF141323.1 | KF141572.1 | | |
| *Polyipnus spinosus* | | | | |
| *Polyipnus stereope* | JN230897.1 | | | |
| *Polyipnus triphanos* | | | | |
| *Sternoptyx diaphana* | KJ190083.1 | | | KJ190122.1 |
| *Sternoptyx obscura* | GQ860315.1 | | | |
| *Sternoptyx pseudobscura* | KX228054.1 | KX227975.1 | KX228011.1 | KX228033.1 |
| *Valenciennellus tripunctulatus* | GQ860310.1 | | | |

Table1.1

*Genbank Accession Numbers*

| Specimen | Ptr1 | ENC1 |
|---|---|---|
| Outgroups | | |
| *Chauliodus macouni* | | |
| *Diplophos taenia* | KX227920.1 | EU366630.1 |
| *Eustomias jimcraddocki* | | GQ860447.1 |
| *Gonostoma elongatum* | | KX227809.1 |
| *Margrethia obtusirostra* | KX227930.1 | KX227801.1 |
| *Osmerus mordax* | JN132618.1 | KF139359.1 |
| *Phosichthys argenteus* | | GQ860427.1 |
| *Polymetme thaeocoryla* | KX227938.1 | GQ860429.1 |
| *Stomias affinis* | | |
| Sternoptychidae | | |
| *Argyropelecus aculeatus* | | GQ860420.1 |
| *Argyropelecus affinis* | KX227913.1 | KX227794.1 |
| *Argyropelecus gigas* | JX190708.1 | GQ860421.1 |
| *Argyropelecus hemigymnus* | | |
| *Argyropelecus lychnus* | | KX230203.1 |
| *Argyropelecus olfersii* | | |
| *Argyropelecus sladeni* | | GQ860422.1 |
| *Danaphos oculatus* | | |
| *Maurolicus amethystinopunctatus* | | |
| *Maurolicus australis* | | GQ860417.1 |
| *Maurolicus japonicus* | | |
| *Maurolicus muelleri* | | JX121736.1 |
| *Maurolicus walvisensis* | | |
| *Maurolicus weitzmani* | | GQ860418.1 |
| *Polyipnus asteroides* | | |
| *Polyipnus clarus* | | GQ860423.1 |
| *Polyipnus indicus* | | |
| *Polyipnus kiwiensis* | | GQ860424.1 |
| *Polyipnus polli* | | |
| *Polyipnus spinifer* | KF141090.1 | KF139368.1 |
| *Polyipnus spinosus* | | |
| *Polyipnus stereope* | | |
| *Polyipnus triphanos* | | |
| *Sternoptyx diaphana* | | |
| *Sternoptyx obscura* | | GQ860419.1 |
| *Sternoptyx pseudobscura* | | GQ860425.1 |
| *Valenciennellus tripunctulatus* | | GQ860426.1 |

## Results

### Monophyly of Sternoptychidae and Relationships to other Stomiiformes

The results of the maximum likelihood analyses for both the molecular and total evidence datasets support the monophyly of the order Stomiiformes (Figures 1.3 & 1.4). Within Stomiiformes, the families Gonostomatidae and Stomiidae were inferred to be monophyletic. The included Phosicthyidae taxa (*Polymetme thaeocoryla*, and *Phosichthys argenteus*) were not recovered as monophyletic. The results (molecular and total evidence) indicate that the family Sternoptychidae is monophyletic (Figures 1.3 & 1.4) and includes the same taxonomic composition as proposed by Weitzman (1974). The family Sternoptychidae was recovered as the sister group to a clade including "Phosichthyidae" and Stomiidae in both the molecular and total evidence analyses (Figures 1.3 & 1.4).

### Molecular Phylogeny of Sternoptychidae inferred from Ultraconserved Elements and Protein-Coding Gene Fragments

The molecular dataset included 29 species of sternoptychids representing seven of ten genera and all genera with more than one taxonomic representative were inferred to be monophyletic with high bootstrap support values (Figure 1.3). *Argyripnus* was recovered as the sister group to all other sternoptychids (Figure 1.3). A clade including *Valenciennellus* and *Danaphos* is the sister group to a clade including *Polyipnus, Maurolicus, Argyropelecus,* and *Sternoptyx* (Figure 1.3). *Polyipnus* was recovered as the sister group to a *Maurolicus, Sternoptyx,* and *Argryropelcus* clade (Figure 1.3). *Maurolicus* was inferred to be the sister group to a *Sternoptyx* and *Agryropelecus* clade (Figure 1.3).

### Total Evidence Phylogeny of Sternoptychidae Inferred from Ultraconserved Elements, Protein-Coding Gene Fragments, and Morphological Characters

The total evidence dataset included 32 species of sternoptychids including representatives of all ten genera. All genera with more than one taxonomic representative were inferred to be monophyletic with high bootstrap support values (Figure 1.4). In the total evidence analysis, there are two main clades of deep-sea hatchetfishes that are sister to each other. The first clade is predominantly comprised of slender-bodied taxa and includes *Thorophos, Araiophos, Argyripnus, Sonoda, Valenciennellus,* and *Danaphos* (Figure 1.4). Within this clade, *Thorophos* is the sister group to *Araiophos,* with this clade sister to the remaining four genera in this monophyletic group (Figure 1.4). Within the clade of the remaining four genera, *Argyripnus* is the sister group to *Sonoda* and this clade is the sister group to a clade including *Valenciennellus* and *Danaphos* (Figure 1.4).

The second main clade is comprised of four genera including the deep-bodied *Polyipnus, Sternoptyx,* and *Argyropelecus*, and the slender-bodied *Maurolicus* (Figure 1.4). The species-rich *Polyipnus* is the sister-group to a clade including *Maurolicus, Sternoptyx,* and *Argyropelecus* (Figure 1.4). *Maurolicus* was inferred to be the sister group to a *Sternoptyx* and *Agryropelecus* clade (Figure 1.4).

## Discussion

The goal of this study was to investigate the evolutionary relationships of deep-sea hatchetfishes and their placement within the order Stomiiformes using a novel genome-scale dataset combined with morphological characters resulting in a total evidence approach. My results from the molecular and total evidence analyses indicate that the family Sternoptychidae as defined by Weitzman (1974) is monophyletic. In both of the molecular dataset (UCEs and

additional gene fragments) and total evidence dataset (UCEs, additional gene fragments and morphological data) the family Sternoptychidae was inferred to be the sister group to a clade including taxa from the polyphyletic “Phosichthyidae” (lightfishes) and Stomiidae (dragonfishes) as seen in Figures 1.3 & 1.4. Previous studies (Weitzman, 1974; Harold & Weitzman 1996), that investigated the evolutionary relationships among stomiiforms based primarily on morphological characters hypothesized that the Sternoptychidae were more closely related to taxa currently recognized in the Gonostomatidae (bristlemouths). Other molecular based studies that included gene fragment data inferred Sternoptychidae as monophyletic but either closely related to gonostomatid taxa (Near et al., 2012; Davis et al., 2014) or closely related to “Phosichthyidae” and Stomiidae (Davis et al., 2016) as recovered herein.

The results of my total evidence analyses that included all ten genera of deep-sea hatchetfishes inferred hypotheses of relationships that are both consistent with the findings of previous studies and differ in some substantial ways. The currently recognized subfamily ‘Maurolicinae’ (Fricke et al., 2019a,b) that includes the genera *Araiophos*, *Argyripnus*, *Danaphos*, *Maurolicus*, *Sonoda*, *Thorophos*, and *Valenciennellus* is not monophyletic in my analyses (Figures 1.3 & 1.4). This subfamily was also not monophyletic in the previous phylogenetic studies of Weitzman (1974) and Harold and Weitzman (1996) based on anatomical data alone (Figure 1.2), and I recommend that future studies refrain from using this subfamily name as it does not reflect a monophyletic group in any study that has included all of the genera associated with it.

Our total evidence analyses inferred a clade including six of the slender-bodied sternoptychid genera typically associated with the subfamily ‘Maurolicinae’, although this clade did not include *Maurolicus* (Figure 1.4). While this clade had low bootstrap support, it is

supported by an unambiguous anatomical synapomorphy from the study of Harold and Weitzman (1996), including the presence of a fenestra in the Mesopterygoid (40-1, Appendix 1.1 & 1.2). While the relationship among lineages in this clade of six genera differs from previous studies (Weitzman, 1974; Harold & Weitzman, 1996) there is also considerable overlap in sister group relationships between genera and these sister group relationships are supported with high bootstrap values (Figures 1.3 & 1.4) and unambiguous synapomorphies.

Within the clade of predominantly slender-bodied sternoptychids, the genera *Thorophos* and *Araiophos* were inferred in this study to be a monophyletic group, which was previously hypothesized by Weitzman (1974). Weitzman (1974) identified numerous similarities between these two genera, particularly in relation to the morphology of their skulls and also upper jaw. Further support for this clade includes the premaxillary-proethmoid crossed ligament being present (63-1, also present in *Sternoptyx*) from Harold and Weitzman (1996). *Thorophos* and *Araiophos* are the sister group to a clade including *Argyripnus*, *Sonoda*, *Danaphos*, and *Valenciennellus*. This clade is novel to this study and is supported by one unambiguous morphological synapomorphy associated with a very short otolith sagitta rostrum (114-1, Harold & Weitzman, 1996; Appendix 1.1 & 1.2).

The genera *Argyripnus* and *Sonoda* were recovered in this study as sister groups, and this relationship was also hypothesized by Weitzman (1974) and Harold and Weitzman (1996). Synapomorphies that support the sister group relationship from Harold and Weitzman (1996) between *Argyripnus* and *Sonoda* include a small posterior myodome (18-1, also present in *Valenciennellus*), a palatine shape that is a cartilaginous bar with tooth plate ventrally (37-2), interopercle shape which is an elongate dorsal process (57-1), the presence of the ethmoid prenasal process (95-1), the flat lateral surface of the otolith sagitta (115-1, also present in

*Argyropelecus*), the ligamentous attachment of pterygiophores immediately anterior and posterior to anal-fin hiatus (125-1), an absent anterior portion of pelvic girdle ischial process (126-1), and photophores posterior inferior OP size being greatly enlarged (131-1) (Harold & Weitzman, 1996; Appendix 1.1 & 1.2). Finally, within this assemblage, the genera *Danaphos* and *Valencienellus* were inferred to be sister groups, which is consistent with the studies of Weitzman (1974) and Harold and Weitzman (1996). This relationship is supported by numerous unreversed anatomical synapomorphies from Harold and Weitzman (1996) including an anteriorly fused interfrontal joint (1-1), an anteriorly joined longitudinal frontal fossa (3-1), the anteriorly absent frontal crest (5-1), a narrow interorbital space (7-1), the posttemporal fossa is bounded by frontal (21-1), the overall symplectic shape being very elongated (42-1), mandibulohyoid ligament system that is separate to interopercle and epihyal to interopercle (48-1), subopercle ossification that is incomplete (58-1), a dorsal supraethmoid relative position to frontals (82-1), and absent lateral vomerine teeth (90-1) (Harold & Weitzman, 1996; Appendix 1.1 & 1.2). Weitzman (1974) identified a number of similarities shared between these two genera including specializations for enlarged eyes that included a suite of modifications to the interorbital-ethmoid region of the skull.

A unique finding of this study is the phylogenetic placement of *Maurolicus* within a clade that includes the deep-bodied taxa *Polyipnus* as the sister group to a clade including *Maurolicus* sister to a clade including the remaining deep-bodied taxa *Argyropelecus* and *Sternoptyx*. This work also supports the findings from Harold (1994) that the species-rich genus *Polyipnus* is monophyletic. Many previous studies have hypothesized a close evolutionary relationship among the deeper-bodied hatchetfish taxa that are currently classified in the subfamily ‘Sternoptychinae’ (e.g., Baird, 1971; Baird & Eckardt, 1972; Ahlstrom, 1974; Weitzman, 1974;

Harold, 1993, 1994; Harold & Weitzman, 1996; Miya & Nishida, 1998; Davis et al., 2014; Davis et al., 2016). While no previous studies have hypothesized that *Maurolicus* is the sister group to a clade including *Sternoptyx* and *Argyropelecus* as inferred from my molecular (Figure 1.3) and total evidence (Figure 1.4) analyses with high bootstrap support values, there is significant evidence to support these findings in addition to the results from my genome-scale analyses.

Weitzman (1974) noted a number of anatomical similarities shared among *Maurolicus* and the deeper-bodied hatchefishes, including a deep mandible that is shared with *Polyipnus* and the lack of a postcaudal trough on the rostrum of the sagitta of the otolith that is also absent in *Argyropelecus*, *Sternoptyx*, and *Polyipnus*. This anatomical character was used in Harold and Weitzman (1996) and is an unreversed synapomorphy that supports the clade of *Polyipnus, Maurolicus, Argyropelecus*, and *Sternoptyx* sagita: postcaudal trough presence (111-1, Appendix 1.1 & 1.2). An additional synapomorphy that supports this clade is the shape of the palatine bone which lacks a posterior head in these four genera (37-1, Appendix 1.1 & 1.2). Bassot (1966) noted that the enclosed space of the ventral photophores in stomiiform fishes have a number of differing anatomical properties related to glandular luminous cells distributed there called A-photocytes. Of particular interest is that he indicated that the photophores of *Maurolicus, Argyropelecus*, and *Sternoptyx* possessed comparatively small, spherical or polyhedral A-photocytes that were arranged side by side in the space of the photophore. He referred to this as a type Alpha photophore, whereas taxa within the gonostomatids (*Bonapartia, Cyclothone, Diplophos, Gonostoma, Manducus*) exhibited type Beta photophores with A-photocytes grouped in tubes, or Type Gamma found in “phosichthyid” and stomiid taxa where A-photocytes were arranged radially in a limited number (Bassot, 1966). While Bassot (1966) did not examine all of the different genera currently recognized within Sternoptychidae, he did note that the

arrangement of glandular cells and the anatomical structure of the photophores of *Maurolicus* most closely resembled those found in *Argyropelecus*.

While there has been some debate regarding which of the deep-bodied hatchetfishes was the sister group to the comparatively extreme anteriorly-posteriorly compressed *Sternoptyx* (e.g., Baird, 1971; Baird & Eckardt, 1972; Weitzman, 1974; Ahlstrom, 1974; Harold & Weitzman, 1996) the results of phylogenetic hypotheses from both morphological (Weitzman, 1974; Harold & Weitzman, 1996) and molecular data (e.g., Near et al., 2012; Davis et al., 2014; Davis et al., 2016) have consistently recovered a sister group relationship between *Sternoptyx* and *Argyropelecus*. This relationship is strongly supported in this study with high bootstrap values and is also supported by numerous unreversed anatomical synapomorphies from Harold and Weitzman (1996) including a strongly convex parasphenoid (14-1), a not visible otic bullae (29-1), basioccipital that is not as deep as exoccipital (31-1), a slender, elongate ectopterygoid shape (41-1), the absent of the antorbital (49-1), the shape of the interopercle being a highly elongate, narrow dorsal process (57-2), relative size of preopercular limbs that is much longer than ventral (61-1), a greatly constricted anterior ceratohyal shape (77-1), the presence of specialized supraneurals (a dorsal blade) (103-1), otolith sagitta rostrum being absent or low eminence (114-2), otolith sagitta length relative to cranial length (117-1), the palatopremaxillary ligament originating on palatine and subdivided into branches to premaxilla, maxilla, and supraethmoid (137-1), a ventral anterior portion highly angular cleithrum shape (140-1), a pectoral radial articulation that has radial II articulating only with scapula (141-1), the number of hypobranchial 1 gill rakers is three or fewer (146-1), the presence of the hypobranchial 1 middorsal tabular process (147-1), pubic process relationship to posterior pleural rib in which the shaft of pubic

process tightly bound and parallel to distal end of last pleural rib (148-1), and curved dorsally in an arc hypobranchial 1 shape (149-1) (Appendix 1.1 & 1.2).

## Conclusion

Our study is the first analysis of the evolutionary relationships among the deep-sea hatchetfishes to include genome-scale data (UCEs) and is also the first study to incorporate a total evidence approach to investigate their evolutionary history. While many of the sister group pairings are consistent among my hypotheses with those of previously published studies, there are substantial differences in the overall evolutionary relationships of lineages at deeper nodes. My results indicate that neither subfamily ‘Maurolicinae’ nor ‘Sternoptychinae’ are monophyletic as currently defined. I recommend that future studies refrain from recognizing either subfamily name and simply refer to the ten genera as belonging to the family Sternoptychidae to better reflect our knowledge on the evolutionary history of this group of fishes. My total evidence analyses inferred two distinct lineages of deep-sea hatchetfishes, including one that includes six of the slender-body shaped genera (*Araiophos, Argyripnus, Danaphos, Sonoda, Thorophos, Valenciennellus*) and one that includes the deeper-bodied genera (*Argyropelecus, Polyipnus, Sternoptyx*) in addition to *Maurolicus*. These findings provide a novel roadmap for future studies investigating the evolution and diversification of this ecological important and fascinating lineage of pelagic deep-sea fishes.

# Chapter II: The Evolution of Body Shape in Sternoptychidae (Hatchetfishes)

## Introduction

Earth's oceans comprise 71 percent of the surface and 97 percent of water on the planet (Robison, 2004; Neill, 2019). The open ocean consists of three major zones: the epipelagic (0–200 meters), the mesopelagic (200–1000 meters), and the bathypelagic (1000–4000 meters) zones (Randall & Farrell, 1997; Helfman et al., 2009; Nelson et al., 2016; Priede, 2017). Fishes that live in the open ocean have evolved various adaptions (e.g., decreased bone density, bioluminescence, tubular eyes) for conditions that change in these oceanic zones as depth increases, such as pressure, temperature, and solar light penetration (Baird, 1971; Baird & Eckardt, 1972; Weitzman, 1974; Herring, 1978, 1982, 1987 2000, 2007; Fink & Weitzman, 1982; Fink, 1985; Haddock et al., 2010; Widder, 2010; Davis et al., 2014; Davis et al., 2016; Marranzino & Webb, 2018). Fishes that have evolved in pelagic habitats exhibit a diversity of body sizes and shapes, including fusiform (Clupeidae, herrings), laterally compressed (Carangidae, jacks and pompanos), deep/spade shaped (Lampridae, opahs), elongated (Regalecidae, oarfishes), and round (Lophioidei, anglerfishes) (Randall & Farrell, 1997; Helfman et al., 2009; Nelson et al., 2016; Priede, 2017). Functional adaptations for these body shapes include locomotion for capturing prey and escaping predation, and camouflage by limiting the silhouette of the body.

The Stomiiformes (dragonfishes and their allies) are a diverse radiation of fishes (444 species) that live exclusively in pelagic marine habitats. Stomiiform fishes possess a wide variety of body shapes ranging from fusiform to hatchet-shaped. All stomiiform fishes exhibit intrinsic bioluminescence through ventral photophores (Davis et al., 2014). The bioluminescence associated with stomiiform ventral photophores is hypothesized to be used for predator

avoidance through counter illumination (Haddock et al., 2010; Davis et al., 2014; Davis et al., 2016). The stomiiform family Sternoptychidae, also known as the deep-sea hatchetfishes, have the broadest diversity of body shape within the Stomiiformes (Figure 1.1), ranging from slender and fusiform species to those with deep hatchet-shaped bodies. The diversity of body shapes among the deep-sea hatchetfishes establish them as a prime case study for exploring the evolution of body shape in deep-sea pelagic habitats.

A total of 78 species in ten genera have been described within Sternoptychidae, and previous studies have explored the evolutionary relationships among sternoptychid taxa using morphological data (e.g., Weitzman, 1974; Fink & Weitzman, 1982; Fink, 1985; Harold & Weitzman 1996). A limited number of studies have used molecular data to infer relationships within sternoptychids and most did not include representatives from all ten genera (Near et al., 2012; Davis et al., 2014; Davis et al., 2016; Smith et al., 2016; Betancur et al., 2017; Smith et al., 2018). In this thesis (Chapter I), I inferred a hypothesis of evolutionary relationships for the Sternoptychidae using a total evidence dataset that concatenated ultraconserved elements (UCEs), mitochondrial and nuclear gene fragments, and morphological characters. This hypothesis of relationships (Chapter I) included 32 sternoptychids, with at least one representative of each of the ten genera and nine closely related outgroups (Figure 1.4).

The newly inferred evolutionary relationships for Sternoptychidae are consistent in recovering the family as monophyletic within Stomiiformes (Figures 1.3 & 1.4). The total evidence analyses inferred two main clades of sternoptychids. The first clade included the slender-bodied genera *Thorophos, Araiophos*, *Argyripnus*, *Sonoda, Valenciennellus,* and *Danaphos*, and the second clade included a mix of deep-bodied and slender-bodied genera including *Polyipnus*, *Maurolicus*, *Sternoptyx,* and *Argyropelecus* (Figures 1.3 & 1.4). No study

has quantified the body shape of the sternoptychids with a geometric morphometric approach or explored the evolution of body shape in the context of their evolutionary relationships within this taxonomically diverse group. This study aims to examine the evolution of body shape with a geometric morphometric based approach while using the inferred total evidence phylogenetic analysis of the Sternoptychidae as an evolutionary framework (Figure 1.4). The objectives for this study include: 1) What is the pattern of body shape evolution within the Sternoptychidae? 2) What is the ancestral body shape of the deep-sea hatchetfishes? and 3) How many times have deep and/or slender body shapes evolved throughout this lineage of fishes?

## Materials and Methods

### Taxonomic Sampling

This study involved the digitization of Sternoptychidae specimens from museum collections from loans and institution visits (Table 2.1). Specimens were digitized using a Canon DSLR EOS Rebel T7i with a 60 mm macro lens and Canon Utilities software. Specimens were arranged to image the left side of each fish with a lateral view. Geometric morphometric analyses included 661 images of adult hatchetfish specimens that represented 52 of 78 described species and all ten genera. The nine outgroups from the hypothesis of evolutionary relationships (Figures 1.3 & 1.4) were also included, with 23 adult specimens representing the nine genera. These outgroups included eight genera from the Stomiiformes, *Chauliodus*, *Diplophos*, *Eustomias*, *Gonostoma*, *Margrethia*, *Phosichthys*, *Polymetme*, *Stomias* and the root taxa *Osmerus* (Osmeriformes, smelts).

**Relative Warp Analysis**

The TPS-Package software tpsDig 2.32 (Rohlf, 2017, 2018a,b) was used to digitally place seven homologous landmarks based on the recommendations (McMahan, Murray, Geheber, Boeckman, Piller, 2011) on each of the included specimens (Figure 2.1). The seven homologous landmarks include the: (1) anterior insertion of dorsal fin, (2) posterior insertion of dorsal fin, (3) dorsal insertion of caudal fin, (4) ventral insertion of caudal fin, (5) posterior insertion of anal fin, (6) anterior insertion of anal fin, and (7) anterior tip of snout. In addition to the seven homologous landmarks, an additional 106 semi-landmarks were placed on each image of the outmost edge of each fish's body, creating a full outline of each individual (Figure 2.1).

The 684 land-marked images were then analyzed with a relative warp analysis in the TPS-Package software tpsRelw (Rohlf, 2017, 2018a,b). This analysis is a type of principal components analysis (PCA) that quantifies differences in body-shape for each specimen with no *a priori* groupings. The analysis creates a Procrustes consensus configuration which superimposes each landmark-based shape and scales and aligns them relative to every included landmark based shape in the analysis (Rohlf, & Slice, 1990; MacLeod, 2002; Webster & Sheets, 2010). The results of the relative warp analysis were examined and color-coded by genus (Figures 2.2 & 2.3), ten within the family Sternoptychidae (*Araiophos*, *Argyripnus*, *Argyropelecus*, *Danaphos*, *Maurolicus*, *Sonoda*, *Thorophos*, *Valenciennellus*) and nine outgroups (*Chauliodus*, *Diplophos*, *Eustomias*, *Gonostoma*, *Margrethia*, *Osmerus*, *Phosichthys*, *Polymetme*, *Stomias*).

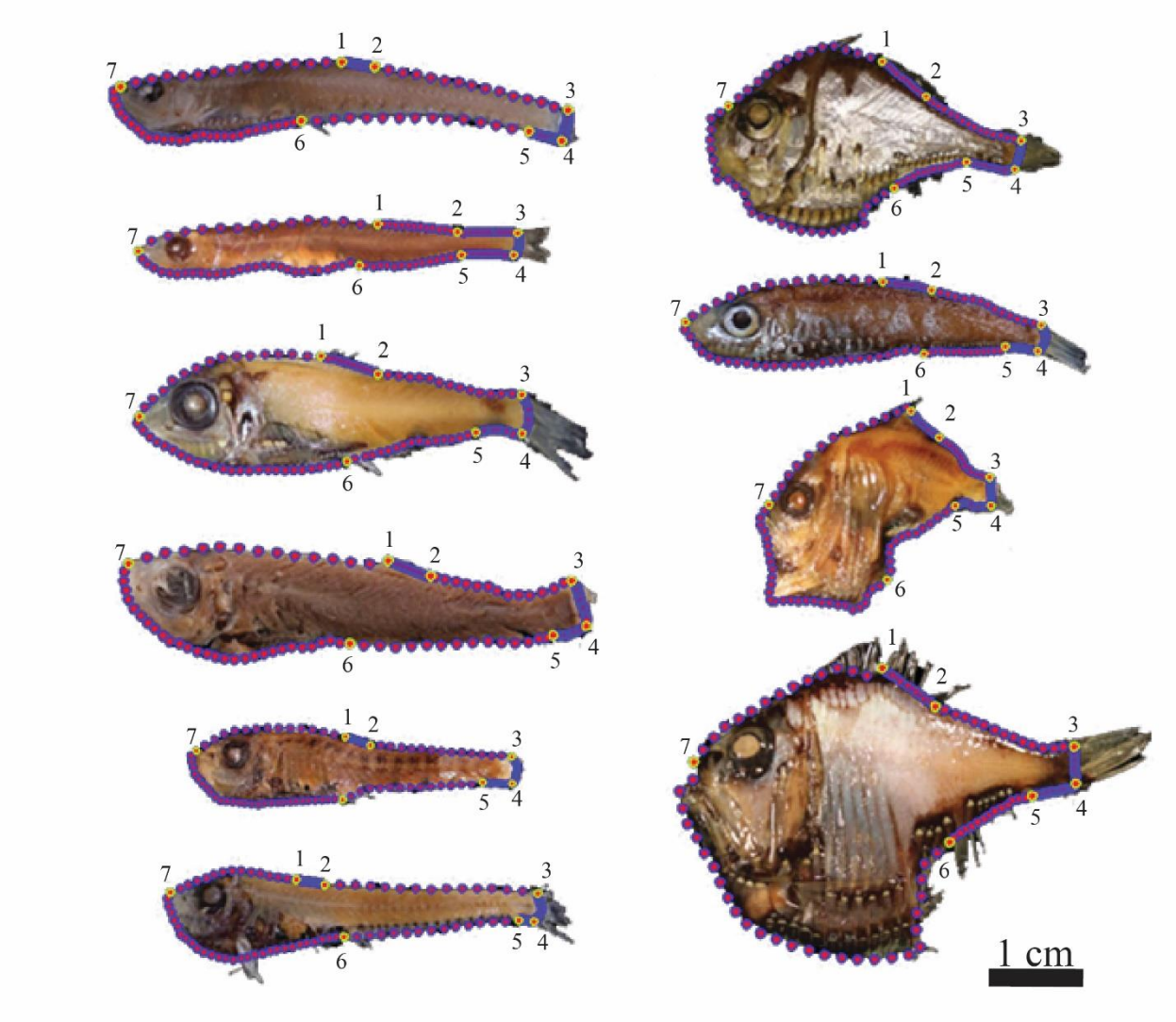


*Figure 2.1*. Locations of Landmarks: Homologous landmarks (yellow outline circles) based on the recommendations of McMahan et al. (2011) including; (1) anterior insertion of dorsal fin, (2) posterior insertion of dorsal fin, (3) dorsal insertion of caudal fin, (4) ventral insertion of caudal fin, (5) posterior insertion of anal fin, (6) anterior insertion of anal fin and (7) anterior tip of snout. An additional 106 semi-landmarks (blue outline circles) are used to outline the body shape.

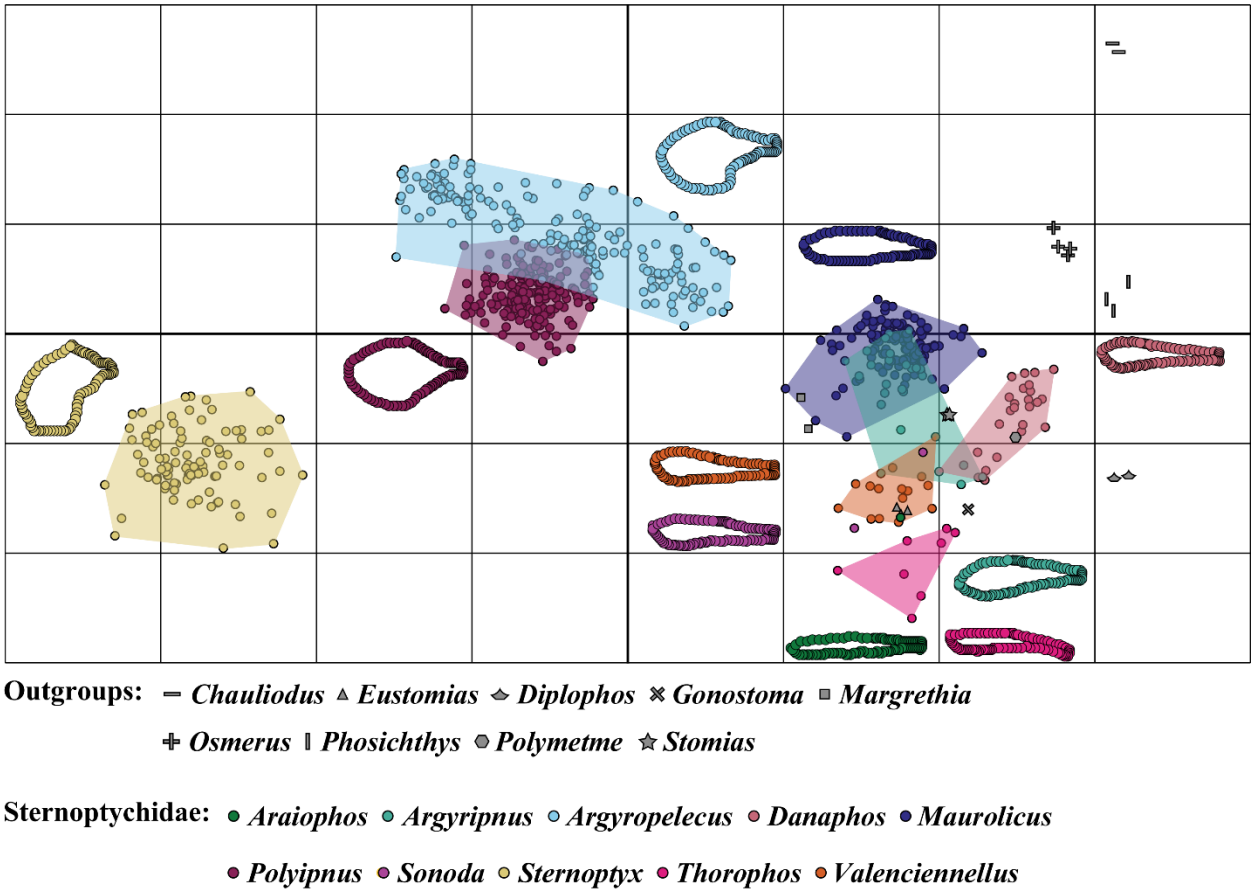


*Figure 2.2*. Relative Warp Analysis: Results of the relative warp analysis for 684 specimens of deep-sea hatchetfishes and outgroups. Colors represent different hatchetfish genera.

## Character Evolution of Body Shape

The results from the relative warp analysis were used to infer the evolution of body shape in combination with the total evidence phylogeny using the software Mesquite (Maddison & Maddison, 2018). A consensus shape for each species that was included in the geometric morphometric study which was also included in the total evidence phylogeny from Chapter 1 was created comprising the 113 landmarks and semi-landmarks. These consensus landmark continuous characters were combined with a trimmed to species-level phylogeny based on the total evidence phylogeny of sternoptychids presented in Chapter I (Figure 1.4). A continuous

character ancestral state reconstruction was conducted using a maximum parsimony reconstruction that included the 113 landmarks for each sternoptychid genus and outgroups (Figures 2.3 & 2.4).

## Results

### Patterns of Body Shape Evolution Among Sternoptychidae

The results of the relative warp analysis (Figure 2.2) indicate that there is considerable variation in body shape among hatchetfishes, with species within a genus clustering together in various areas of morphospace compared to the consensus configuration. The deep-bodied hatchetfishes *Sternoptyx, Argyropelecus,* and *Polyipnus* are predominantly located on the left side of the relative warp analysis. *Sternoptyx,* occupying the bottom left corner of the warp analysis below the x axis and on the left side of the y axis (Figure 2.2), has a unique deep-body among hatchetfishes with significant anterior-posterior compression in body shape. The remaining two deep-bodied hatchetfishes, *Polyipnus* and *Argyropelecus* are found primarily above the x axis near the consensus configuration in morphospace. In contrast, the seven fusiform to slender-bodied Sternoptychidae genera are all located in morphospace on the right side of the y axis and predominantly below the x axis on the lower right side of warp analysis (Figure 2.2). While species within the slender-bodied genera form distinct clusters, there is more overlap in morphospace among slender-bodied species. These seven genera of hatchetfishes have similarities in their slender body shape, but there is variation regarding the overall depth of their bodies, with *Maurolicus* and *Argyripnus* possessing bodies with less dorsal-ventral compression then the other genera (Figure 2.2).

**Ancestral Character State Reconstruction of Body Shape**

The ancestral character state reconstruction for body shape evolution among the Sternoptychidae (Figures 2.3 & 2.4) indicate that the common ancestor of the sternoptychids was most likely a fusiform fish that that was similar in body-shape to that observed in *Maurolicus*. The deeper nodes in the phylogeny are fusiform in shape, with trends towards a slender-body in the genera *Thorophos* and *Araiophos* (Figures 2.3 & 2.4). The remaining slender-bodied hatchetfishes including *Argyripnus*, *Danaphos*, *Maurolicus*, *Sonoda*, *Thorophos*, and *Valenciennellus* have fusiform bodies that differ slightly from their respective inferred common ancestors (Figures 2.3 & 2.4). The three deep-bodied hatchetfishes (*Argyropelecus*, *Polyipnus*, *Sternoptyx*) are all inferred to have evolved their hatchet-shaped bodies independently from a common ancestor that was more fusiform in shape (Figures 2.3 & 2.4), indicating that there have been three separate evolutionary trends towards deeper-bodies in the hatchetfishes.

## Discussion

In this study I quantify the variation in body shape among deep-sea hatchetfishes and infer that there is considerable variation in the evolution of their body plans in deep-sea pelagic habitats. I find that that the deep-bodied taxa *Argyropelecus*, *Polyipnus*, and *Sternoptyx* occupy differing regions in morphospace from the more fusiform to slender-bodied taxa in the family. In terms of species richness, 42 percent (33 species) of Sternoptychidae reside in the genus *Polyipnus* which has the greatest variation in body shape among the deep-bodied taxa (Figure 2.2). Overall, the deep-bodied species *Argyropelecus* (seven species), *Polyipnus* (33 species), and *Sternoptyx* (four species) constitute nearly 60 percent (44 of 74) of all sternoptychid diversity. While the remaining seven genera that are fusiform to slender-bodied are all concentrated in morphospace (Figure 2.2), many of those lineages are depauperate in species

richness other than *Maurolicus* (15 species), which I infer to be more closely related to the deep-bodied taxa *Sternoptyx* and *Argyropelecus* (Figures 1.4, 2.3, 2.4).

The results of my study, which combined a newly inferred phylogeny using a total evidence approach with ultraconserved elements (UCEs), protein-coding gene fragments and morphological data (Figure 1.4) with a with landmark-based geometric morphometric analysis and ancestral character reconstruction (Figures 2.3 & 2.4) indicate that the ancestral body-shape of the Sternoptychidae was a more fusiform fish (Figure 2.4). This is consistent with the hypotheses of previous studies (e.g., Weitzman, 1974; Harold & Weitzman, 1996), which suggested that the likely body-shape of the sternoptychid hatchetfishes resembled a more typical fusiform fish. The majority of ancestral nodes in the hypothesis of evolutionary relationships for Sternoptychidae are inferred to be a more fusiform body-plan, with the six genera (*Thorophos, Araiophos, Argyripnus, Sonoda, Valenciennellus, Danaphos*) that form one of the two main clades of sternoptychids having variations in body plans, from fusiform to more slender bodies.

Previous studies (Weitzman, 1974; Harold & Weitzman, 1996) hypothesized that a deep-body evolved once within Sternoptychidae in the common ancestor of *Argyropelecus, Polyipnus,* and *Sternoptyx*. However, my results do not support the hypothesis that a deep body only evolved a single time in the Sternoptychidae. The ancestral character state reconstruction

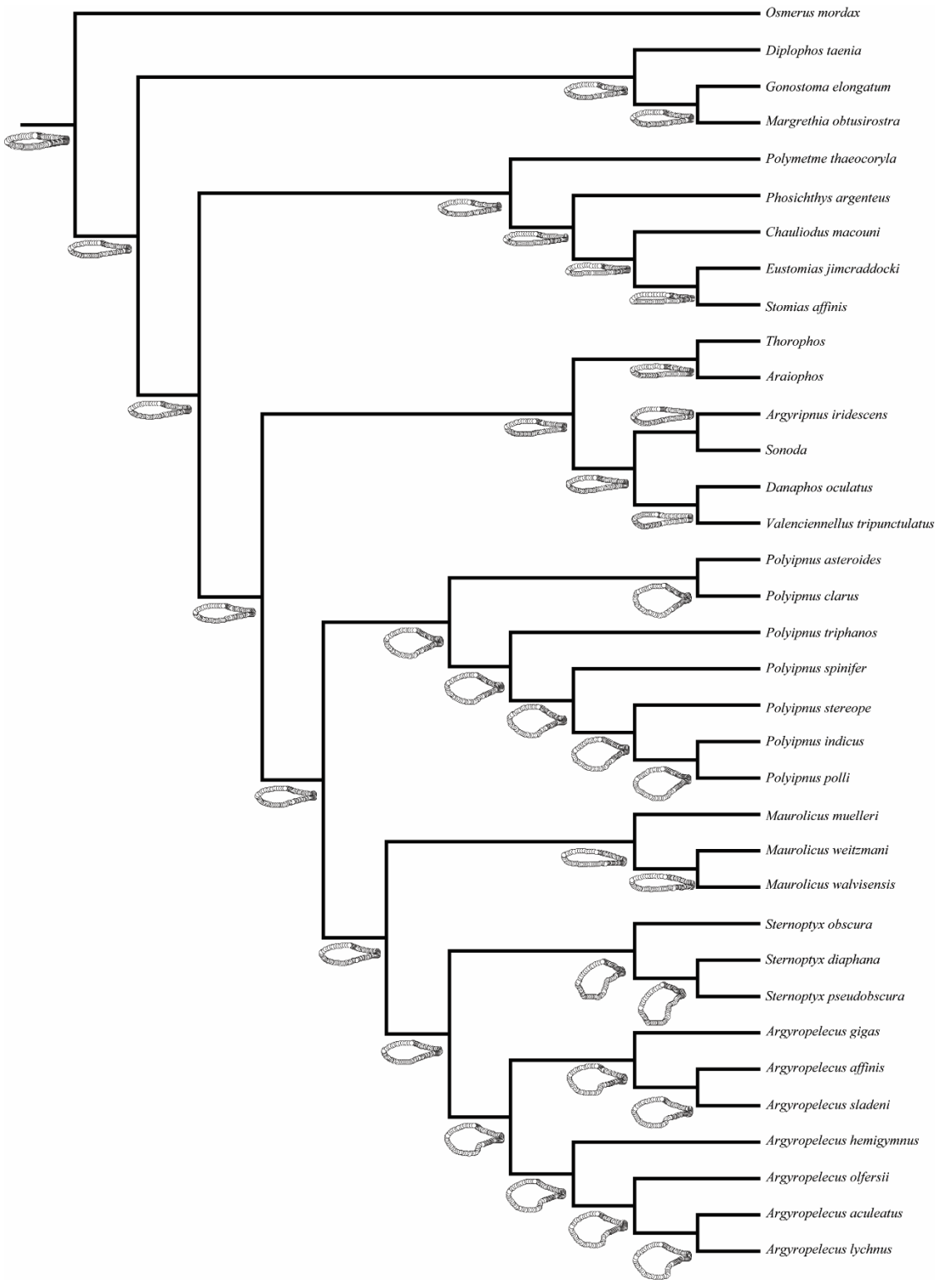


*Figure 2.3*. Total Evidence Species-Level Phylogeny Reconstruction: Ancestral character state reconstruction for body shape of deep-sea hatchetfishes with outgroups. Phylogeny based on total evidence dataset (Chapter I, Figure 1.4) representing all species used in the analysis.

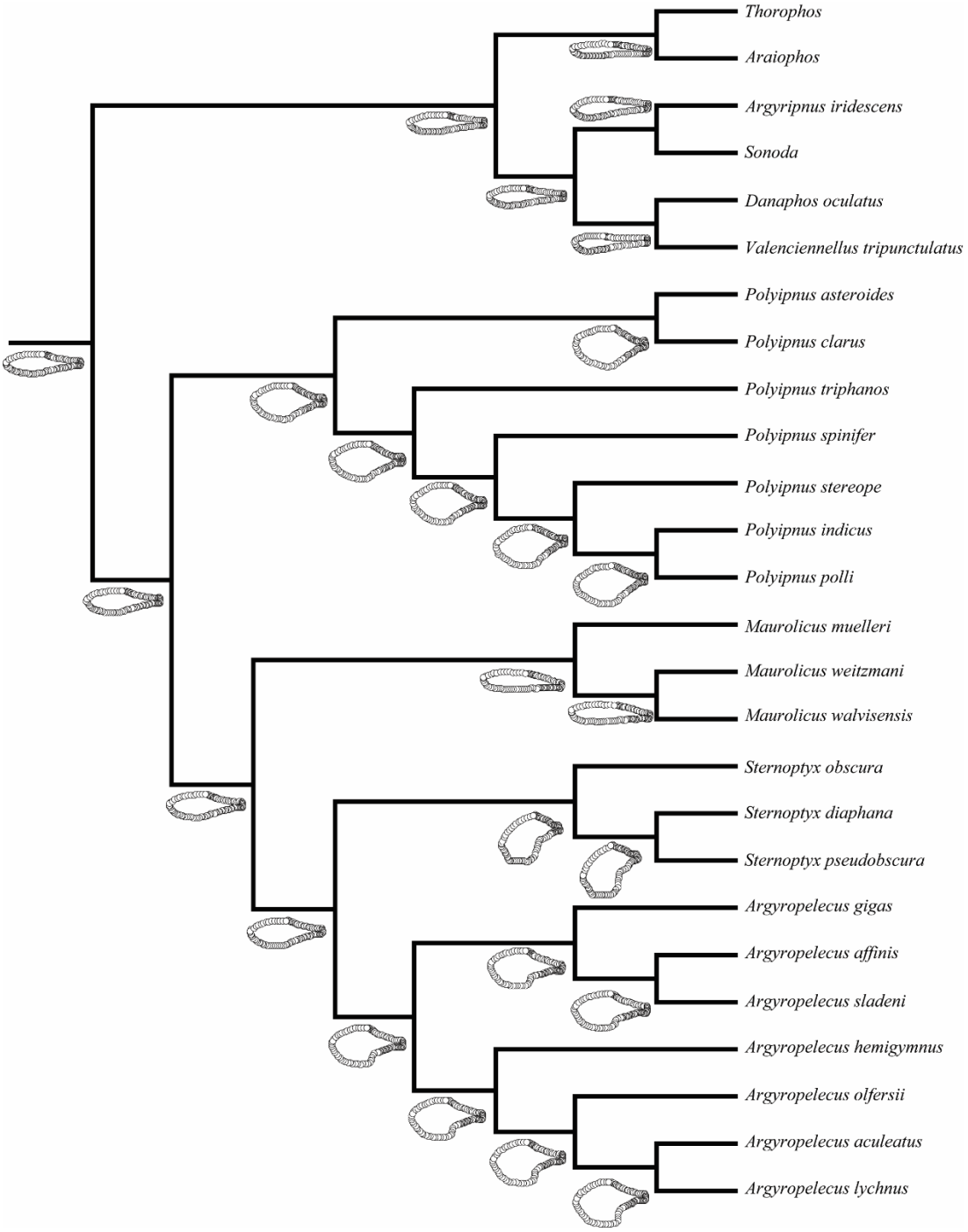


*Figure 2.4*. Sternoptychidae Species-Level Ancestral Reconstruction: Ancestral character state reconstruction for body shape of deep-sea hatchetfishes with outgroups removed from figure. Phylogeny based on total evidence dataset (Chapter I, Figure 1.4) representing all species used in the analysis.

indicates that the clade including the three deep-bodied hatchetfishes *Polyipnus*, *Argyropelecus*, *Sternoptyx* and the fusiform *Maurolicus* have undergone multiple evolutionary changes in body shape (Figures 2.3 & 2.4). There have been three independent evolutionary transitions to deep-bodies in the hatchetfishes from a fusiform ancestor, indicating that this body-shape has conferred some selective advantages in this pelagic deep-sea environment.

All sternoptychids are bioluminescent with ventral photophores, and these photophores are used for camouflage during diurnal migrations while feeding (Hopkins & Baird, 1973, 1985; Kinzer & Schulz, 1988). However, the photophores of the deep-bodied hatchetfishes extend farther up the lateral surface of the body than they do in the slender bodied taxa and are also greatly enlarged. Other lineages of pelagic deep-sea fishes with photophores on their lateral surface such as lanternfishes have been documented to have increased rates of speciation and these photophores are hypothesized to serve additional functional roles for communication rather than just with counter illumination (Davis et al., 2014; Davis et al., 2016). The photophore patterns of *Polyipnus* (33 species) are known to be species-specific (Harold, 1993) and are often used in association with their taxonomic identifications. It is possible that the deeper-bodied hatchetfishes are utilizing bioluminescence for functional reasons other than camouflage which has contributed to their present-day species richness which accounts for over sixty percent of all hatchetfish diversity. Further work is needed to explore bioluminescent patterns and structures in the deep-sea hatchetfishes.

## Conclusion

The Sternoptychidae are diverse group of deep-sea fishes that live exclusively in pelagic environments. In this study, I quantified for the first time the evolution of body shape in this unique lineage that includes taxa with both deep, fusiform, and slender body shape

morphologies. I found that the ancestor of the deep-sea hatchetfishes likely had a fusiform body plan, with a deep body evolving three independent times within the Sternoptychidae.

Table 2.1

*Materials Examined*

| **Outgroups** | **Museum Catalog Number** |
|---|---|
| *Chauliodus sloani* | KU 14368 |
| *Diplophos taenia* | KU 38771, KU 38778 |
| *Eustomias enbarbatus* | FMNH 49871 |
| *Gonostoma elongatum* | MCZ 140833 |
| *Margrethia obtusirostra* | KU 38770 |
| *Osmerus mordax* | KU 3786 |
| *Polymetme thaeocoryla* | USNM 407308 |
| *Phosichthys argenteus* | USNM 329550 |
| *Stomias boa* | KU 38764, MCZ 129197 |
| **Sternoptychidae** | **Museum Catalog Number** |
| *Araiophos eastropas* | USNM 203240 |
| *Argyripnus atlanticus* | USNM 324583; USNM 405230 |
| *Argyripnus brocki* | FMNH 88986; USNM 207654; USNM 207655; USNM 207656; USNM 207657; USNM 207659; USNM 207660; USNM 207661 |
| *Argyripnus electronus* | USNM 230723 |
| *Argyripnus sp.* | USNM 347160 |
| *Argyropelecus aculeatus* | FMNH 49829; FMNH 64352; FMNH 64363; FMNH 64364; FMNH 64365; FMNH 71632; FMNH 71633; FMNH 85171; KU 28502; MCZ 137459; USNM 196699; USNM 206624; USNM 247619; USNM 248053; USNM 395248 |
| *Argyropelecus affinis* | FMNH 120601; FMNH 122178; FMNH 122179; FMNH 122181; FMNH 122182; FMNH 122183; FMNH 122184; FMNH 122185; KU 27950; KU 28166; USNM 192819; USNM 206827; USNM 287381 |
| *Argyropelecus amabilis* | FMNH 49785; FMNH 49786; USNM 4414; USNM 10877; USNM 11560 |

| | |
|---|---|
| *Argyropelecus antrorsospinus* | USNM 33393; USNM 35561; USNM 43855; USNM 102987; USNM 102989 |
| *Argyropelecus gigas* | FMNH 45943; FMNH 71647; USNM 11491; USNM 357444 |
| *Argyropelecus hawaiensis* | USNM 265435 |
| *Argyropelecus heathi* | USNM 51632 |
| *Argyropelecus hemigymnus* | FMNH 78437; USNM 207237; USNM 219950 |
| *Argyropelecus intermedius* | USNM 201083; USNM 301852 |
| *Argyropelecus lynchus hawaiensis* | USNM 164003; USNM 177931 |
| *Argyropelecus lynchus* | USNM 57885; USNM 120239; USNM 120240; USNM 120241; USNM 170966; USNM 196705; USNM 274422 |
| *Argyropelecus olfersi* | FMNH 71639; USNM 60174; USNM 247715; USNM 301851 |
| *Argyropelecus pacificus* | FMNH 76259; USNM 87563; USNM 177930; USNM 203389 |
| *Argyropelecus sladeni* | FMNH 66634; FMNH 71641; FMNH 122194; FMNH 122195; FMNH 122198; FMNH 122200; KU 27949; KU 28158; USNM 298084; USNM 298922 |
| *Danaphos oculatus* | FMNH 71020; FMNH 71921; FMNH 71934; FMNH 122244; USNM 201100; USNM 203264; USNM 203265; USNM 203286; USNM 207110; USNM 207113; USNM 401092; USNM 438499 |
| *Maurolicus borealis* | USNM 23027 |
| *Maurolicus breviculus* | USNM 321408 |
| *Maurolicus imperatorius* | USNM 321407 |
| *Maurolicus inventionis* | USNM 321404 |
| *Maurolicus kornilovorum* | USNM 321409 |
| *Maurolicus meulleri* | FMNH 66292; FMNH 66300; FMNH 66607; FMNH 71583; FMNH 71724; USNM 40070; USNM 201138; USNM 202396; USNM 302396 |
| *Maurolicus pennanti* | FMNH 63123 |
| *Maurolicus rudjakovi* | USNM 321403 |

| | |
|---|---|
| *Maurolicus stehmanni* | USNM 321402 |
| *Maurolicus walvisensis* | USNM 321405 |
| *Maurolicus weitzmani* | KU 26994; KU 28081; USNM 317840; USNM 317841; USNM 410500; USNM 436565 |
| *Polyipnus aquavitus* | USNM 256966 |
| *Polyipnus asper* | USNM 273282 |
| *Polyipnus asteroides* | FMNH 64350; FMNH 71614; FMNH 71615; FMNH 71621; FMNH 71626; USNM 102978; USNM 102979; USNM 190472; USNM 298936 |
| *Polyipnus bruuni* | USNM 298923 |
| *Polyipnus clarus* | USNM 192847; USNM 273283; USNM 298941; USNM 306471 |
| *Polyipnus fraseri* | USNM 92324 |
| *Polyipnus indicus* | USNM 179897; USNM 307881; USNM 307918 |
| *Polyipnus laternatus* | FMNH 64355; FMNH 64359; FMNH 64360; FMNH 64362; FMNH 71627; USNM 179050; USNM 298237; USNM 298924; USNM 327096 |
| *Polyipnus matsubarai* | USNM 179793; USNM 179794 |
| *Polyipnus nuttingi* | USNM 51599; USNM 51693 |
| *Polyipnus omphus* | USNM 256967 |
| *Polyipnus polli* | USNM 179878 |
| *Polyipnus soelae* | USNM 135537 |
| *Polyipnus spinifer* | FMNH 52447; USNM 103036; USNM 135524; USNM 135528; USNM 135536 |
| *Polyipnus stereope* | USNM 51451 |
| *Polyipnus tridentifer* | USNM 179851 |
| *Polyipnus triphanos* | FMNH 120802; KU 40361; USNM 103027; USNM 103028 |
| *Polyipnus unispinus* | USNM 103153; USNM 298928 |
| *Sonoda paucilampa* | FMNH 71585; USNM 196967 |

| | |
|---|---|
| *Sternoptyx diaphana* | FMNH 49762; FMNH 49767; FMNH 64348; FMNH 66137; FMNH 78436; FMNH 121613; FMNH 121642; FMNH 121665; KU 36167; MCZ 134728; USNM 192846; USNM 192850; USNM 248230; USNM 249312; USNM 378001 |
| *Sternoptyx obscura* | USNM 57884; USNM 120294; USNM 177888; USNM 177889; USNM 177933; USNM 324258 |
| *Sternoptyx pseudobscura* | FMNH 78421; FMNH 78434; FMNH 121614; USNM 309663; USNM 316826; USNM 436509 |
| *Sternoptyx pseudodiaphana* | USNM 192845; USNM 206810; USNM 207243 |
| *Thorophos nexilis* | USNM 92326; USNM 92328; USNM 122276; USNM 151400 |
| *Valenciennellus punctulatus* | FMNH 62906-62911 |
| *Valenciennellus stellatus* | USNM 203260; USNM 203267 |
| *Valenciennellus tripunctulatus* | FMNH 71933; FMNH 78465; FMNH 78477; FMNH 112613; FMNH 121645; USNM 126770; USNM 201141; USNM 301369 |

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**Appendix 1.1: Morphological Character Matrix Used in Total Evidence Analyses**

*Morphological characters adapted from Harold & Weitzman* (1996).

| Outgroups | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Chauliodus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Diplophos* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Eustomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Gonostoma* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Margrethia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Osmerus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Phosichthys* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Polymetme* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Stomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| Sternoptychidae | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| *Araiophos* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| *Argyripnus* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| *Argyropelecus* | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 |
| *Danaphos* | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| *Maurolicus* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| *Polyipnus* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| *Sonoda* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| *Sternoptyx* | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| *Thorophos* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| *Valenciennellus* | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |

| Outgroups | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Chauliodus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Diplophos* | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| *Eustomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Gonostoma* | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| *Margrethia* | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| *Osmerus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Phosichthys* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Polymetme* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Stomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| Sternoptyhcidae | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
| *Araiophos* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 |
| *Argyripnus* | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 |
| *Argyropelecus* | 0 | 1 | 2 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 3 | 1 | 1 | 1 | 1 |
| *Danaphos* | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| *Maurolicus* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 |
| *Polyipnus* | 1 | 0 | 2 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 3 | 0 | 1 | 0 | 1 |
| *Sonoda* | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 1 | 0 | 0 |
| *Sternoptyx* | 0 | 0 | 3 | 0 | 1 | ? | 0 | ? | ? | ? | ? | ? | ? | 1 | 1 |
| *Thorophos* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Valenciennellus* | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |

| Outgroups | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Chauliodus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Diplophos* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Eustomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Gonostoma* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Margrethia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Osmerus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Phosichthys* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Polymetme* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Stomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| Sternoptyhcidae | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 |
| *Araiophos* | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 |
| *Argyripnus* | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 |
| *Argyropelecus* | 1 | 0 | ? | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 2 | 0 | 1 |
| *Danaphos* | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 |
| *Maurolicus* | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 2 | 0 | 1 | 1 | 1 |
| *Polyipnus* | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 2 | 1 | 1 |
| *Sonoda* | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 |
| *Sternoptyx* | 1 | 1 | 1 | 2 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 2 | 0 | 1 |
| *Thorophos* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| *Valenciennellus* | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 |

| Outgroups | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Chauliodus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Diplophos* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Eustomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Gonostoma* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Margrethia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Osmerus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Phosichthys* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Polymetme* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Stomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| Sternoptyhcidae | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 |
| *Araiophos* | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| *Argyripnus* | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| *Argyropelecus* | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 0 | 1 | 1 |
| *Danaphos* | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 |
| *Maurolicus* | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| *Polyipnus* | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 |
| *Sonoda* | 1 | 1 | 0 | ? | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| *Sternoptyx* | 1 | 0 | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 0 | 2 | 0 | 1 | 1 |
| *Thorophos* | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Valenciennellus* | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 |

| Outgroups | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Chauliodus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Diplophos* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Eustomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Gonostoma* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Margrethia* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Osmerus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Phosichthys* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Polymetme* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Stomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| Sternoptyhcidae | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 |
| *Araiophos* | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Argyripnus* | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| *Argyropelecus* | 1 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| *Danaphos* | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 |
| *Maurolicus* | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| *Polyipnus* | 0 | 1 | 0 | 1 | 0 | 2 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 |
| *Sonoda* | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| *Sternoptyx* | 1 | 1 | 1 | 0 | 0 | 3 | 1 | 1 | 2 | 0 | 0 | 1 | 1 | 1 | 1 |
| *Thorophos* | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Valenciennellus* | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 |

| Outgroups | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Chauliodus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Diplophos* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Eustomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Gonostoma* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Margrethia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Osmerus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Phosichthys* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Polymetme* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Stomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| Sternoptyhcidae | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 |
| *Araiophos* | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 0 |
| *Argyripnus* | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 2 | 1 | 1 | 3 | 1 | 1 | 1 | 0 |
| *Argyropelecus* | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 3 | 1 | 1 | 1 | 0 |
| *Danaphos* | 0 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| *Maurolicus* | 0 | 2 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 1 | 0 |
| *Polyipnus* | 1 | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 3 | 1 | 1 | 1 | 0 |
| *Sonoda* | 0 | 2 | 1 | 1 | 0 | 1 | ? | ? | ? | 1 | 1 | 1 | 1 | 1 | 0 |
| *Sternoptyx* | 1 | 1 | 0 | 1 | 0 | 0 | ? | ? | ? | 1 | 1 | 1 | 1 | 2 | 0 |
| *Thorophos* | 0 | 2 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 |
| *Valenciennellus* | 0 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 2 | 1 |

| Outgroups | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 | 101 | 102 | 103 | 104 | 105 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Chauliodus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Diplophos* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Eustomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Gonostoma* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Margrethia* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Osmerus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Phosichthys* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Polymetme* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Stomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| Sternoptyhcidae | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 | 101 | 102 | 103 | 104 | 105 |
| *Araiophos* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 3 | 1 |
| *Argyripnus* | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| *Argyropelecus* | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 2 | 1 |
| *Danaphos* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 2 | 0 |
| *Maurolicus* | 0 | 0 | 2 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| *Polyipnus* | 1 | 0 | 1 | 1 | 0 | 2 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 |
| *Sonoda* | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| *Sternoptyx* | 0 | 1 | 0 | 0 | 0 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | ? | 1 |
| *Thorophos* | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| *Valenciennellus* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 |

| Outgroups | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 | 120 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Chauliodus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Diplophos* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Eustomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Gonostoma* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Margrethia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Osmerus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Phosichthys* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Polymetme* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Stomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| Sternoptyhcidae | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 | 120 |
| *Araiophos* | 0 | 1 | 1 | 1 | 1 | ? | ? | ? | ? | ? | ? | 0 | 1 | ? | 0 |
| *Argyripnus* | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| *Argyropelecus* | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 |
| *Danaphos* | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 |
| *Maurolicus* | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| *Polyipnus* | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 |
| *Sonoda* | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | ? | 1 |
| *Sternoptyx* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 0 | 1 | 1 | 1 | 1 | 2 |
| *Thorophos* | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| *Valenciennellus* | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 |

| Outgroups | 121 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 130 | 131 | 132 | 133 | 134 | 135 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Chauliodus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Diplophos* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Eustomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Gonostoma* | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Margrethia* | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Osmerus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Phosichthys* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Polymetme* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Stomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| Sternoptyhcidae | 121 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 130 | 131 | 132 | 133 | 134 | 135 |
| *Araiophos* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| *Argyripnus* | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 |
| *Argyropelecus* | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 |
| *Danaphos* | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 |
| *Maurolicus* | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| *Polyipnus* | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 |
| *Sonoda* | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | ? |
| *Sternoptyx* | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 |
| *Thorophos* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 |
| *Valenciennellus* | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |

| Outgroups | 136 | 137 | 138 | 139 | 140 | 141 | 142 | 143 | 144 | 145 | 146 | 147 | 148 | 149 | 150 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Chauliodus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Diplophos* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Eustomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Gonostoma* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Margrethia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Osmerus* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Phosichthys* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Polymetme* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| *Stomias* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| Sternoptyhcidae | 136 | 137 | 138 | 139 | 140 | 141 | 142 | 143 | 144 | 145 | 146 | 147 | 148 | 149 | 150 |
| *Araiophos* | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Argyripnus* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Argyropelecus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 |
| *Danaphos* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Maurolicus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Polyipnus* | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Sonoda* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| *Sternoptyx* | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| *Thorophos* | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Valenciennellus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |

**Appendix 1.2: Abbreviated Morphological Character List from Harold & Weitzman (1996)**

1. Interfrontal joint. 0, separate; 1, fused anteriorly.

2. Dorsal frontal surface. 0, smooth; 1, pitted.

3. Longitudinal frontal fossa. 0, separate; 1, joined anteriorly.

4. Longitudinal frontal fossa. 0, shallow; 1, deep.

5. Frontal crest. 0, present; 1, absent anteriorly.

6. Fontal crest. 0, low or absent; 1, prominent.

7. Interorbital space. 0, broad; 1, narrow.

8. Parietal surface. 0, smooth; 1, pitted.

9. Parietal-sphenotic relationship. 0, no contact; 1, contact.

10. Parietal-pterotic relationship. 0, contact; 1, no contact.

11. Parietal-intercalar relationship. 0, no contact; 1, contact.

12. Parietals. 0, not separated by supraoccipital; 1, separated.

13. Parietal crest. 0, absent; 1, present.

14. Parasphenoid shape. 0, straight to slightly convex; 1, strongly convex.

15. Parasphenoid glossopharyngeal tunnel. 0, absent; 1, present.

16. Parasphenoid lateral wing. 0, moderately well developed; 1, posterior process and wings form a cap.

17. Basisphenoid. 0, present; 1, absent.

18. Posterior myodome. 0, large; 1, small; 2, moderate size; 3, dorsally elongate.

19. Posterior myodome. 0, horizontal; 1, vertical.

20. Sphenotic size. 0, small; 1, moderate to large.

21. Posttemporal fossa bounded by frontal. 0, yes; 1, no.

22. Neural arch attachment. 0, some free; 1, all fused.

23. Posttemporal fossa bounded by sphenotic. 0, none; 1, small to large amount.

24. Posttemporal fossa bounded by pterotic. 0, large amount; 1, moderate amount.

25. Posttemporal fossa bounded by epioccipital. 0, large amount; 1, moderate amount; 2, small amount.

26. Posttemporal fossa bounded by parietal. 0, large amount; 1, moderate amount; 2, small amount; 3, reduced and specialized.

27. Posttemporal fossa bounded by intercalar. 0, none; 1, small amount.

28. Posttemporal fossa bounded by exoccipital. 0, none; 1, varying amounts.

29. Otic bullae. 0, well-developed, large; 1, not visible.

30. Exoccipital pedicles. 0, moderately well-developed; 1, enlarged.

31. Basioccipital. 0, as deep or deeper than exoccipital, in posterior view; 1, not as deep as exoccipital.

32. Exoccipital plates dorsal to foramen magnum. 0, in contact; 1, no contact.

33. Centrum-like face of basioccipital. 0, moderate size; 1, shallow or small.

34. Epioccipital process. 0, small to prominent; 1, prominent posterodorsal process; 2, slender spine-like process.

35. Palatine teeth. 0, one row present; 1, absent.

36. Palatine posterior process. 0, present; 1, absent.

37. Palatine shape. 0, double-headed; 1, posterior head lost; 2, cartilaginous bar with tooth plate ventrally.

38. Mesopterygoid teeth. 0, present; 1, absent.

39. Mesopterygoid shape. 0, short; 1, long.

40. Mesopterygoid fenestra. 0, absent; 1, present.

41. Ectopterygoid shape. 0, broad, elongate, moderate size; 1, slender, elongate; 2, triangular to quadrangular, large.

42. Symplectic shape, overall. 0, elongate slender; 1, very elongate.

43. Symplectic shape, terminations. 0, dorsal end enlarged, not club-shaped; 1, both ends equal; 2, club-shaped.

44. Articulation of dorsal border of quadrate. 0, with metapterygoid; 1, ectopterygoid and/or mesopterygoid plus metapterygoid.

45. Position of lower jaw adductor pocket. 0, posterior 1/3 or 1/4 of mandible; 1, middle or anterior of mandible.

46. Mandible shape. 0, elongate, posterior portion 2- 3 times depth of anterior; 1, deep, 3- 4 times depth of anterior.

47. Mandible tooth line and coronoid platform. 0, platform absent; 1, platform present.

48. Mandibulohyoid ligament system. 0, mandible to epihyal and to interopercle; 1, separate to interopercle and epihyal to interopercle; 2, no fibers to interopercle.

49. Antorbital. 0, present; 1, absent.

50. Supraorbital. 0, present; 1, absent.

51. Infraorbital series. 0, six bones, excluding antorbital; 1, reduced to four or fewer.

52. Opercle shape, notch in dorsal border. 0, present but altered or reduced relative to outgroups; 1, modified.

53. Opercular spine. 0, present; 1, developed into a lateral ridge; 2, strong lateral spiny process.

54. Opercle shape. 0, roughly rectangular or quadrangular; 1, elongate rectangular.

55. Subopercle shape. 0, rectangular to half rectangular; 1, triangular; 2, dorsoventrally elongate.

56. Relative size of interopercle and subopercle. 0, interopercle length about equal to that of subopercle; 1, interopercle much longer than subopercle.

57. Interopercle shape. 0, short dorsal process; 1, elongate dorsal process; 2, highly elongate, narrow dorsal process.

58. Subopercle ossification. 0, complete; 1, incomplete.

59. Preopercular angle. 0, gradual, up to right angle; 1, abrupt right angle.

60. Preopercular spines. 0, absent; 1, present.

61. Relative size of preopercular limbs. 0, dorsal and ventral limbs about equal; 1, dorsal limb much longer than ventral.

62. Interpremaxillary ligament. 0, strong; 1, weak.

63. Premaxillary-proethmoid (rostrodermethmoid lateral process) crossed ligament. 0, present; 1, absent.

64. Premaxillary-proethmoid (rostrodermethmoid lateral process) uncrossed ligament. 0, present; 1, absent.

65. Palatopremaxillary ligament. 0, separate maxillary head-palatine and maxillary-premaxillary ligament; 1, continuous palatopremaxillary ligament; ?, absent.

66. Palatomaxillary ligament. 0, short; 1, long; 2, moderate length; 3, very short.

67. Maxillary-proethmoid (rostrodermethmoid lateral process) ligament. 0, present; absent.

68. Suspensory palatine ligament. 0, long; 1, short.

69. Premaxillary ascending process. 0, short to moderately long; 1, elongate; 2, almost none.

70. Maxillary angle. 0, not angulate; 1, angulate.

71. Maxillary width. 0, slender; 1, posteriorly expanded.

72. Maxillary toothed border. 0, convex; 1, concave.

73. Anterior supramaxilla. 0, present; 1, absent.

74. Hyomandibular length. 0, moderately long, about half of cranial length; 1, very long, about three-quarters of cranial length.

75. Hyomandibular spine. 0, present; 1, absent.

76. Posterior ceratohyal length. 0, short, less than length of anterior ceratohyal; 1, elongate, greater than half of anterior ceratohyal length.

77. Anterior ceratohyal shape. 0, moderately constricted in middle; 1, greatly constricted; 2, not greatly constricted.

78. Largest end of anterior ceratohyal. 0, posterior or both ends about equal; 1, anterior.

79. Total number of branchiostegal rays. 0, 12 to 22, rarely 11 in *Ichthyococcus*; 1, 10 or fewer.

80. Urohyal shape. 0, incised posterior margin; 1, not incised.

81. Basihyal. 0, present; 1, absent.

82. Supraethmoid relative position to frontals. 0, ventral; 1, dorsal; ?, supraethmoid absent.

83. Length of posterior supraethmoid process. 0, long; 1, process absent; 2, short; ?, supraethmoid absent.

84. Relative size of supraethmoid. 0, large; 1, small to moderate; ?, supraethmoid absent.

85. Proethmoids (lateral processes of rostrodermethmoid). 0, present; 1, absent.

86. Capsular ethmoids. 0, present, well-developed; 1, absent; 2, fused together; 3, fused to supraethmoid.

87. Ventral ethmoid. 0, present; 1, absent.

88. Myodome bone. 0, well-developed, separate; 1, absent.

89. Lateral ethmoid. 0, well-developed; 1, small to moderate size; 2, absent.

90. Lateral vomerine teeth. 0, present; 1, absent.

91. Median vomerine teeth. 0, absent; 1, present.

92. Vomer. 0, present; 1, absent.

93. Vomer, anterodorsal extension. 0, not reaching supraethmoid; 1, reaching supraethmoid; 2, special process dorsal to supraethmoid; 2, extends dorsally, ventral to supraethmoid.

94. Ethmoid cornu. 0, absent or weakly developed; 1, moderately to well-developed.

95. Ethmoid prenasal process. 0, absent; 1, present.

96. Ethmoid cartilage. 0, broad; 1, narrow; 2, broad, modified.

97. Rib-bearing vertebrae. 0, first rib associated with vertebra 2; 1, first rib associated with vertebra 3.

98. Enlarged ribs. 0, absent; 1, present.

99. Ribs directly supporting pelvic girdle. 0, absent; 1, present.

100. Epipleurals. 0, present; 1, absent.

101. Epineurals. 0, present; 1, absent.

102. Expanded neural and haemal spines. 0, absent; 1, present.

103. Specialized supraneurals (dorsal blade). 0, absent; 1, present.

104. Epurals. 0, three separate elements; 1, two separate elements; 2, one element; 3, epurals absent; ?, fused to uroneural

105. Caudal radials, other than epurals. 0, present; 1, absent.

106. Uroneurals. 0, two present; 1, one present (second uroneural absent).

107. Parhypural. 0, free from preural centrum 1 and hypural 1; 1, fused to preural centrum 1 and/or hypural

108. Hypurals 1 and 2. 0, autogenous; 1, fused.

109. Hypurals 3, 4, and 5. 0, autogenous; 1, fused (3-5 or 3-6 fused).

110. Ural centrum 2. 0, not fused to PUl + Ul; 1, fused to PUl + Ul.

111. Sagitta: postcaudal trough. 0, present; 1, absent.

112. Sagitta: crista superior. 0, present; 1, absent.

113. Sagitta: crista inferior. 0, present; 1, absent.

114. Sagitta: rostrum. 0, well-developed, prominent; 1, very short; 2, absent or low eminence.

115. Sagitta: lateral surface. 0, convex; 1, flat.

116. Sagitta: lateral profile. 0, longer than deep (height about 1.3 to 2.0 times in length; 1, deeper than long (height about 0.4 to 0.9 times in length).

117. Sagitta: length relative to cranial length. 0, large (4.7 to 7.0 times in length of cranium); 1, small (15 to 50 times in length of cranium).

118. Photophore development. 0, in situ formation through white phase; 1, budding (photophores in clusters).

119. Adipose fin shape. 0, short-based; 1, longbased.

120. Number of pelvic radials. 0, three; 1, six; 2, one.

121. Body shape. 0, highly elongate with shallow head; 1, deep body and head.

122. Photophores ventrally on caudal peduncle. 0, singly or in clusters of 2; 1, clusters of 4 or more.

123. Position of anal-fin origin. 0, posterior to dorsal-fin origin; 1, anterior to dorsal-fin origin. Highly variable in the Gonostomatidae, hence coded "? " for that outgroup.

124. Anal-fin hiatus. 0, absent; 1, present.

125. Attachment of pterygiophores immediately anterior and posterior to anal-fin hiatus. 0, nonligamentous; 1, ligamentous.

126. Anterior portion of pelvic girdle ischial process. 0, present; 1, absent.

127. Pelvic girdle orientation. 0, horizontal; 1, approximately vertical.

128. Abdominal keel-like structure. 0, absent; 1, present.

129. Body depth. 0, 3.7 to 7.7 percent of standard length; 1, 0.8 to 2.0 percent of standard length.

130. Iliac spines. 0, absent; 1, present.

131. Photophores: posterior inferior OP size. 0, about equal to other OP; 1, greatly enlarged.

132. Photophores: SO. 0, present; 1, absent.

133. Photophores: OA. 0, more than 1; none or 1.

134. Posterior infraorbitals. 0, posterior infraorbitals, behind eye, present; 1, posterior part of series, those posterior to eye, absent (equivalent to infraorbitals 5 and 6 and possibly 4).

135. Number of anterior infraorbitals. 0, three or four present; 1, entire series represented by two anterior elements (probably equivalent to numbers 1 and 2 in other taxa).

136. NPU2 shape. 0, narrow; 1, broad and flat.

137. Palatopremaxillary ligament. 0, single slip; 1, ligament originating on palatine and subdivided into branches to premaxilla, maxilla, and supraethmoid.

138. Posttemporal. 0, short and weak, not well-ossified; 1, elongate and strong, well-ossified.

139. Cleithrum ventral lateral wing. 0, no posterior notch; 1, posterior notch through which fin rays pass.

140. Cleithrum shape. 0, ventral anterior portion smoothly curved; 1, ventral anterior portion highly angular.

141. Pectoral radial articulation. 0, radial II articulating with scapula and coracoid; 1, radial II articulating only with scapula.

142. Photophores: L (lateral). 0, absent; 1, present.

143. Posttemporal and supracleithrum relationship. 0, free; 1, fused.

144. Distal pterygiophore perichondral ossifications. 0, present; 1, absent.

145. Urohyal size. 0, moderate to large; 1, small.

146. Number of hypobranchial 1 gill rakers. 0, more than three; 1, three or fewer.

147. Hypobranchial 1 middorsal tabular process. 0, absent; 1, present.

148. Pubic process relationship to posterior pleural rib. 0, not parallel or bound together; 1, shaft of pubic process tightly bound and parallel to distal end of last pleural rib.

149. Hypobranchial 1 shape. 0, approximately straight; 1, curved dorsally in an arc.

150. Photophores: PV number. 0, more than 10; 1, 10.