Evolutionary Relationships of the Enigmatic Anglerfishes (Teleostei: Lophiiformes): Can Nuclear DNA Provide Resolution for Conflicting Morphological and Mitochondrial Phylogenies?

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A dissertation
submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

University of Washington
2014

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Program Authorized to Offer Degree:
School of Aquatic and Fishery Sciences

University of Washington

Abstract

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Anglerfishes, order Lophiiformes, are among the most spectacular fishes living today and are widely known for their modified first dorsal-fin spine that serves as a luring apparatus. They comprise 351 living species placed in 73 genera, 18 families, and five suborders found world-wide from tropical near-shore habitats to the deep ocean. Previous studies using mitogenomes and morphological characters conflict, especially within the deep-sea suborder Ceratioidei, requiring that an independent line of evidence be examined to estimate the species tree. To this end, fragments from the protein coding nuclear loci ENC1, Glyt, myh6, and zic1 were analyzed from 68 species of lophiiforms representing all five suborders and 17 of the 18 families. Partitioned Bayesian and maximum likelihood analyses recovered monophyly for the order and all suborders. The concatenated nuclear genes strongly support the most basal position of the Lophiidae, supporting previous morphological and mitogenome studies. In addition, strong support for a clade comprising Chaunacoidei + Ceratioidei was also recovered, lending support to the previous mitogenome study. An unexpected relationship was recovered with regard to the Ogcocephaloidei, being recovered for the first time as basal to a clade comprising Antennarioidei + (Chaunacoidei + Ceratioidei). In addition, novel relationships were recovered within the Antennarioidei, rendering the family Antennariidae paraphyletic. While these relationships have not been recovered in previous morphological studies, some life history characters appear to lend support to these relationships. Relationships recovered within the Ceratioidei in this study conflict with both morphological and mitogenome studies and highlight the need for faster evolving nuclear loci to be analyzed to better understand these relationships. Additionally, evidence is presented to support at least three species within the *Antennarias striatus* complex. The results of this study are presented in a revised classification of the Lophiiformes.

ACKNOWLEDGMENTS

This work was supported by the Dorothy T. Gilbert Ichthyology Research Fund through the School of Aquatic and Fishery Sciences. I am extremely grateful to all the individuals who provided tissue and loans of whole specimens, and/or helped with the procurement of specimens for DNA analyses, including Greg Arnold, Janine Baker, Andy Bentley, Diane Bray, Jason Coombs, Roxanne Fea, Ralph Foster, Brian Gim, Martin Gomon, Dave Harasti, Rob Harcourt, Amanda Hay, Barry Hutchinson, Jeff Johnson, Helen Larson, Mark McGrouther, Sue Morrison, Katherine Maslenikov, Phil Mercurio, Audrey Pang, Sally Reader, and Bill Watson. For guidance during data acquisition and analysis, I acknowledge the help provided by Adam Leaché, Ted Pietsch, and Duane Stevenson. I also thank Adam Leaché, Kerry Naish, Jay Orr, Ted Pietsch, and Duane Stevenson for providing helpful critiques of the manuscript.

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CHAPTER I – INTRODUCTION

From the walking, jet-propelling, shallow-water frogfishes to the bizarre reproductive modes of the deep-sea ceratioids, the anglerfishes, order Lophiiformes, are among the most spectacular fishes living today, captivating the attention of the general public, aquarists, recreational SCUBA divers, and professional scientists. They are a morphologically diverse group of teleost fishes found world-wide from tropical near-shore habitats to the deep ocean and are widely known for their modified first dorsal-fin spine that serves as a luring apparatus. The 18 families, 73 genera, and approximately 351 living species (Table 1) are distributed among five suborders (Pietsch, 1984a; Figure 1): Lophioidei (Caruso and Bullis, 1976; Caruso and Suttkus, 1979; Caruso, 1981, 1983, 1985, 1986) containing a single family of relatively shallow-water, dorso-ventrally flattened forms; Antennarioidei (Last et al., 1983; Pietsch and Grobecker, 1987; Last et al., 2007; Last and Gledhill, 2009), four families, shallow to moderately deep-water, benthic forms, of which nearly all are laterally compressed; Chaunacoidei (Caruso, 1989a, b), one family, deep-water benthic forms that are more or less globose; Ogcocephaloidei (Ochiai and Mitani, 1956; Bradbury, 1967, 1980, 1988, 1999; Endo and Shinohara, 1999), one family, shallow to deep-water, all dorso-ventrally flattened; and Ceratioidei (Pietsch, 1986; Pietsch and Orr, 2007), consisting of 11 families, all more or less globose, and occurring only in deep water (Figures 2 and 3).

Members of the Lophioidei, commonly known as the monkfishes or goosefishes, are the only members of the Lophiiformes that are commercially harvested for human consumption. While their flesh catches a hefty price, the liver of a monkfish, or “ankimo,” as it is known in Japan, is considered the “foie gras of the sea.” Once considered overfished, the two stocks of *Lophius* *americanus* in the United States are currently managed jointly by the New England Fishery Management Council and the Mid-Atlantic Fishery Management Council, and are considered a "good alternative" and not a "best choice" by the Monterey Bay Aquarium Seafood Watch because bycatch of other species is still a concern (e.g., the Atlantic Sturgeon, marine mammals, and sea turtles; http://www.seafoodwatch.org, retrieved 22 May 2014). Greenpeace USA, however, still includes *Lophius americanus* on their "Red List fish," citing destructive fishing methods (bottom trawling and dredging) and bycatch problems (http://www.greenpeace.org/usa/en/campaigns/oceans/seafood/red-fish/, retrieved 22 May 2014). *Lophius piscatorius* and *L. budegassa* are caught in European waters, and the TAC (total allowable catch) is currently set by ICES (International Council for the Exploration of the Sea). The discovery of the most common type of reproductive mode for lophiiform fishes also began with members of the Lophiidae. Most anglerfishes produce a pelagic, egg-filled raft, first described and illustrated by Alexandar Agassiz (1882) for a species of *Lophius*. However, even the ancient Greeks may have known the manner of oviposition, as Aristotle wrote of *Lophius* that "the female lays her spawn all in a lump close in to shore" (translation of Thompson, 1910, book 6(17), lines 30ff; see Pietsch and Grobecker, 1987, p. 352).

Members of the Antennarioidei are morphologically diverse. Distributed among four families, they are both globose and somewhat depressed (as for the sole member of the Lophichthyidae, *Lophichthys boschmai*, Boeseman, 1964), and occur in tropical, subtropical, and temperate waters around the world. However, nearly all genera (except *Antennarius*, *Antennatus*, *Fowlerichthys*, and *Histrio* in the family Antennariidae) are confined to the Indo-Australian Archipelago, a region that extends from Taiwan to Tasmania, including all of the inland seas and islands of the Philippines, Indonesia, New Guinea, the Solomons, and the continent of Australia

(Pietsch and Grobecker, 1987). Some antennarioids are well known among recreational divers for their cryptic but photogenic nature, and in the aquarium hobbyist community for their voracious appetites. Tetrabrachiidae, with only two species, and Lophichthyidae, with only one species, are relatively unknown to the general public and are somewhat rare in natural history collections as well. Members of the Brachionichthyidae, commonly known as the handfishes, are only found along the southern coast of Australia and the island of Tasmania. Some members of the Brachionichthyidae are listed as critically endangered on the IUCN Red List and/or listed as vulnerable to extinction by the Australian government (Last et al., 1983). Antennarioids have two distinct reproductive modes and life histories: the release of a gelatinous pelagic egg raft with thousands of tiny eggs that hatch into pelagic larvae before settlement (as for *Antennarius*, *Antennatus*, *Fowlerichthys*, and *Histrio* within the Antennariidae; Mosher, 1954; Rasquin 1958), or a small cluster of relatively large eggs that undergo direct development and hatch into demersal juveniles and have very limited dispersal potential (as for the remaining genera within the Antennariidae, except for *Nudiantennarius* for which reproductive habits are completely unknown, and all members of the Brachionichthyidae, Lophichthyidae, and Tetrabrachiidae; Pietsch and Grobecker, 1987; Bruce et al., 1997, 1998, 1999; DEH, 2005a, b; Pietsch et al., 2009a, b; Arnold and Pietsch 2012).

Members of the Ogcocephaloidei, commonly known as the batfishes, are probably the most peculiar in shape amongst the Lophiiformes; most are extremely dorso-ventrally flattened, having a somewhat round, pancake-like appearance. Batfishes have a relatively small lure and some or all may have evolved a novel luring mechanism: rather than using the fleshy tip of the first dorsal-fin spine (the esca) to entice would-be predators, at least a few species of batfishes apparently release a chemical attractant to lure small gastropods (Nagareda and Shenker, 2009).

Although not as popular as antennarioids, some species of ogcocephalids are sought after by SCUBA divers and a few species are collected for the aquarium trade.

There are only 16 species and two genera in the Chaunacoidei, commonly known as the coffinfishes, and they are relatively deep-dwellers, occupying depths from 90 to more than 2000 m. Much about the biology of the coffinfishes is unknown, however, recent in situ observations of seven *Chaunacops coloratus* at seamounts in the eastern North Pacific Ocean show that they may undergo an ontogenetic color change from blue in small individuals to red in large individuals (Lundsten et al., 2012). While the ability to change color is relatively well known among some species of antennariids, the color change in those cases is presumably to aid in camouflage, both to avoid predation and to conceal the fish from potential prey (known as aggressive mimicry; Pietsch and Grobecker, 1978), rather than ontogenetic.

With approximately 165 species distributed among 11 families, the Ceratioidei contains the most species diversity and is the most phylogenetically derived of these suborders (Pietsch and Orr, 2007; Pietsch, 2009). Commonly known as the deep-sea anglerfishes because they occur below 300 m, ceratioids are the only members of the Lophiiformes that possess a lure containing bioluminescent bacteria. In addition, all members of the Linophrynidae have an elaborate bioluminescent hyoid barbel that originates from a complex array of intrinsic, intracellular, paracrystalline photogenic granules (Hansen and Herring, 1977). Male members of the Ceratioidei are dwarfed; the size of the females is sometimes more than 60 times the length and approximately half a million times the weight of the males (Bertelsen, 1951; Pietsch 1976, 1986). The males attach themselves either temporarily or permanently to the bodies of the females using pincer-like denticles, becoming parasitic on the female for nourishment.

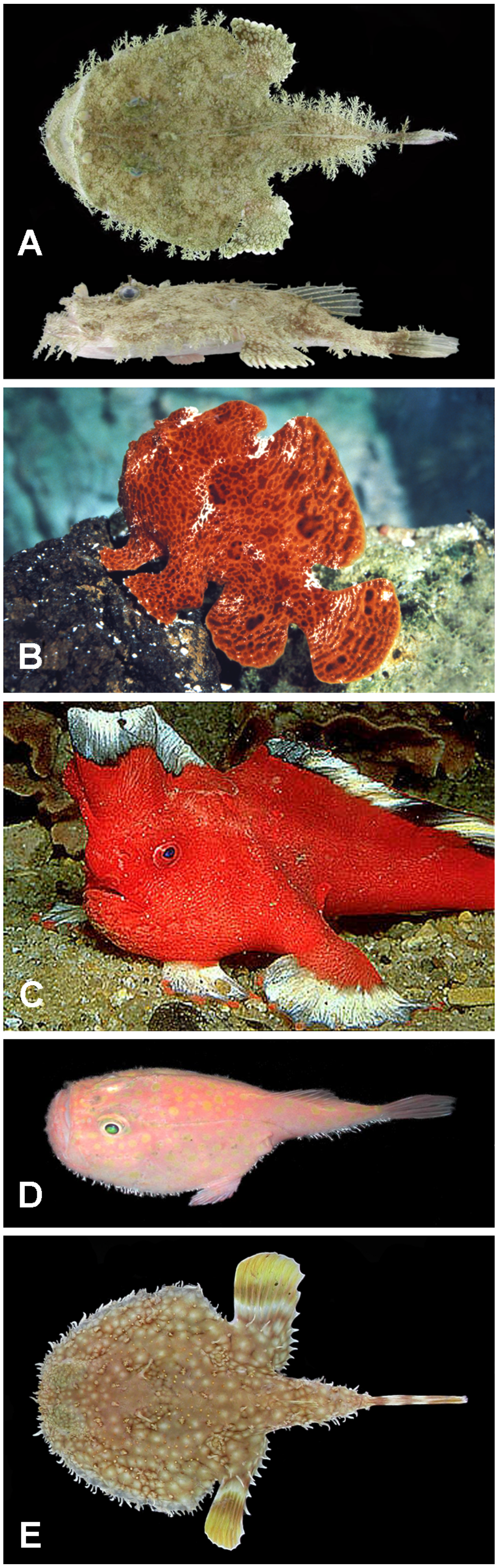
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Figure 1. Representatives of lophiiform suborders included in this study: A. *Lophiodes reticulatus* Caruso and Suttkus, 157 mm SL, UF 158902, dorsal and lateral views (photo by J. H. Caruso); B. *Antennarius commerson* (Latreille), 111 mm SL, UW 20983 (photo by D. B. Grobecker); C. *Brachionichthys politus* (Richardson), specimen not retained (photo by R. Kuiter); D. *Chaunax suttkusi* Caruso, 107 mm SL, TU 198058 (photo by J. H. Caruso); D. *Halieutichthys aculeatus* (Mitchill), 80 mm SL, specimen not retained, dorsal view (photo by J. H. Caruso); after Miya et al., 2010.

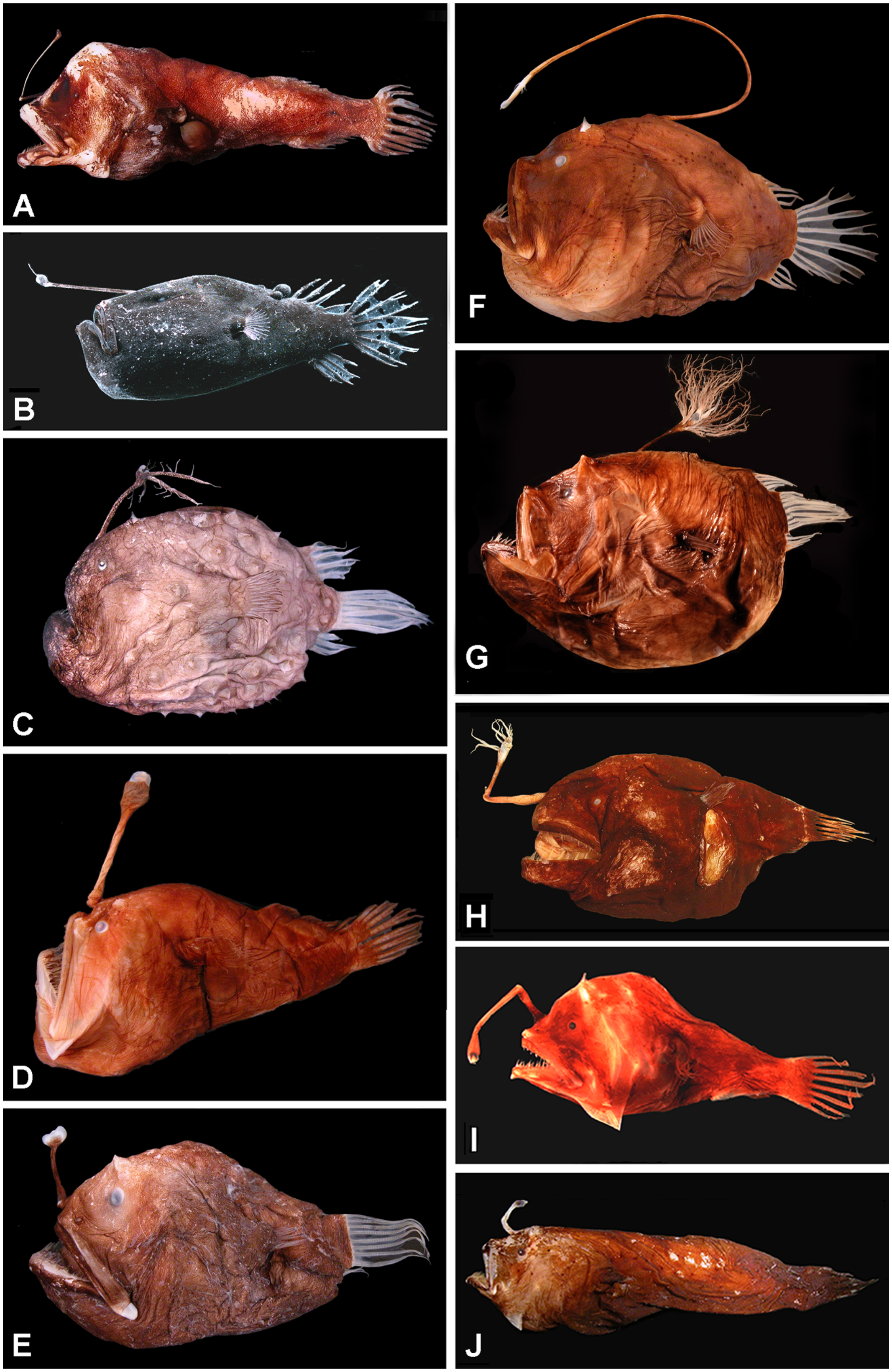
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Figure 2. Females of ceratioid taxa used in the present study: A. *Centrophryne spinulosa* Regan and Trewavas, 136 mm SL, LACM 30379-1 (photo by T. Pietsch); B. *Cryptopsaras couesii* Gill, 40 mm SL, specimen not retained (photo by E. A. Widder); C. *Himantolophus appelii* (Clarke), 124 mm SL, CSIRO H.5652-01 (photo by T. Pietsch); D. *Melanocetus eustales* Pietsch and Van Duzer, 93 mm SL, SIO 55-229 (photo by T. Pietsch); E. *Diceratias trilobus* Balushkin and Fedorov, 86 mm SL, AMS I.31144-004 (photo by T. Pietsch); F. *Bufoceratias wedli* (Pietschmann), 96 mm SL, CSIRO H.2285-02 (photo by T. Pietsch); G. *Bufoceratias shaoi* Pietsch, Ho, and Chen, 101 mm SL, ASIZP 61796 (photo by H.-c. Ho); H. *Chaenophryne quasiramifera* Pietsch, 157 mm SL, SIO 72-180 (photo by T. Pietsch); I. Oneirodidae: *Oneirodes* sp., 31 mm SL, MCZ 57783 (photo by C. P. Kenaley); J. Oneirodidae: *Spiniphryne duhameli* Pietsch and Baldwin, 117 mm SL, SIO 60-239 (photo by T. Pietsch).

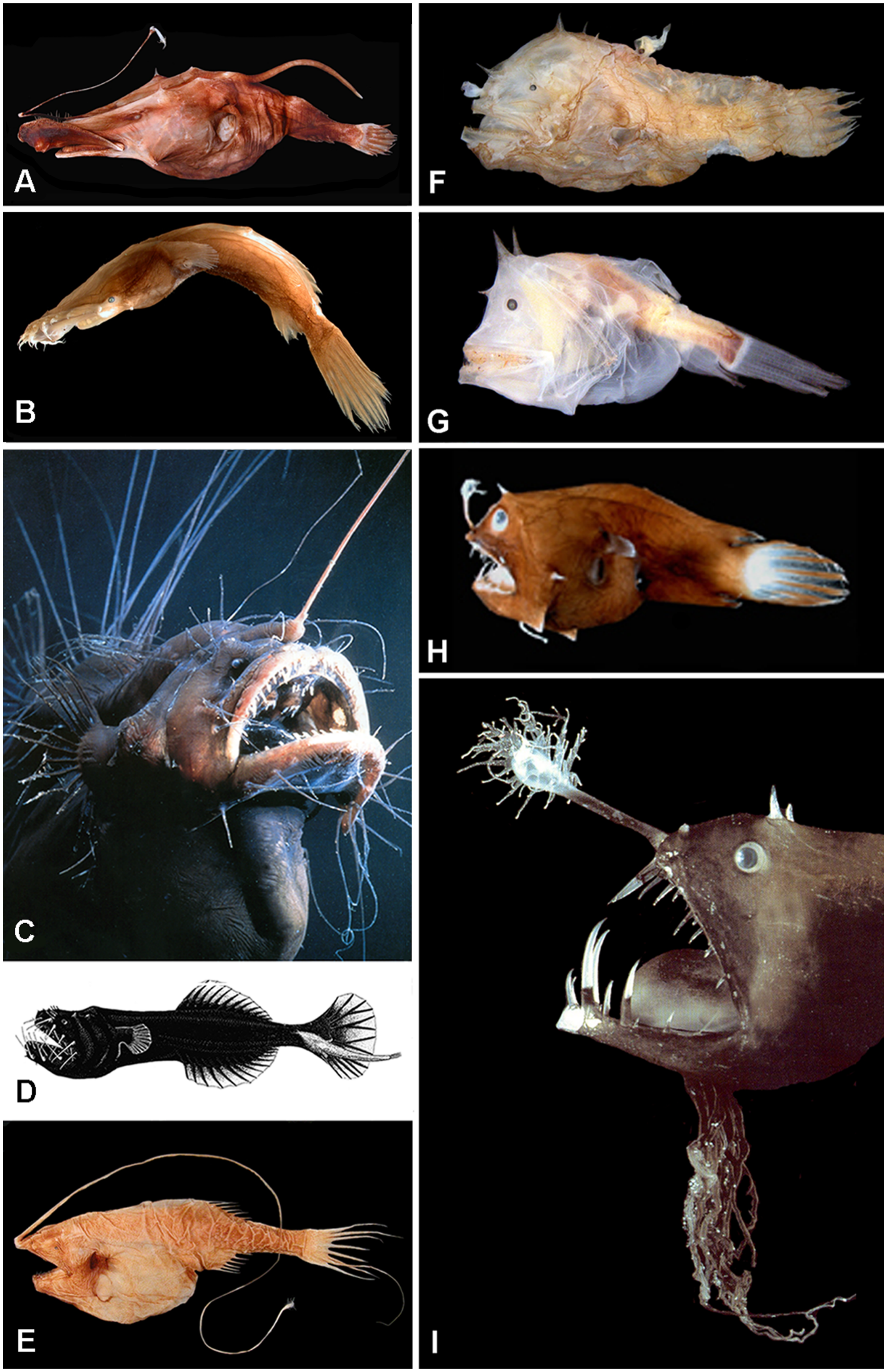
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Figure 3. Females of ceratioid taxa used in the present study: A. *Lasiognathus amphirhamphus* Pietsch, 157 mm SL, BMNH 2003.11.16.12 (photo by T. Pietsch); B. *Thaumatichthys binghami* Parr, 83 mm SL, UW 47537 (photo by C. Kenaley); C. *Caulophryne pelagica* (Brauer), 183 mm SL, BMNH 2000.1.14.106 (photo by D. Shale); D. *Neoceratias spinifer* Pappenheim, 52 mm SL, with 15.5-mm SL parasitic male, ZMUC P921726 (after Bertelsen, 1951); E. *Gigantactis gargantua* Bertelsen, Pietsch, and Lavenberg, 166 mm SL, LACM 9748-028 (photo by T. Pietsch); F. *Photocorynus spiniceps* Regan, 46-mm SL, with 6.2-mm SL parasitic male, SIO 70-326 (photo by T. Pietsch); G. *Haplophryne mollis* (Brauer), 36 mm SL, MNHN 2004-0811 (photo by T. Pietsch); H. *Linophryne macrodon* Regan, 28 mm SL, UW 47538 (photo by C. P. Kenaley); I. *Linophryne polypogon* Regan, 33 mm SL, BMNH 2004.9.12.167 (photo by P. David).

Table 1. Diversity of the Lophiiformes. Percentages indicate the coverage of each group included in the present study.

	Included in Study	**Percentage**
Lophiidae		
4 genera	4	100.0%
28 species	4	14.3%
Ogcocephalidae		
10 genera	6	60.0%
73 species	6	8.2%
Antennariidae		
14 genera	12	85.7%
49 species	27	55.1%
Brachionichthyidae		
5 genera	1	20.0%
14 species	1	7.1%
Tetrabrachiidae		
2 genera	1	50.0%
2 species	1	50.0%
Lophichthyidae		
1 genus	0	0.0%
1 species	0	0.0%
Chaunacidae		

2 genera	2	100.0%
16 species	3	18.8%
Ceratioidei		
11 families	11	100.0%
35 genera	21	60.0%
165 species	24	14.3%
18 Families	**17**	**94.4%**
73 Genera	**47**	**64.4%**
351 Species	**66**	**18.8%**

Placement of the Lophiiformes among teleosts. Anglerfishes have traditionally been allied with the toadfishes of the teleost order Batrachoidiformes, dating back to Georges Cuvier's second edition of *Le Règne Animal* (1829) in a group called the *acanthoptérygiens à pectorales pédiculées* in reference to the foot-like structure of the pectoral fins. Many authors since that time have followed this phylogenetic arrangement, or a variation of it, placing anglerfishes and toadfishes side by side (Valenciennes, 1837; Cope, 1872; Jordan and Evermann, 1898; Jordan and Sindo, 1902; Boulenger, 1904; Jordan, 1905; Regan, 1912; Eaton et al., 1954). Regan (1912) included anglerfishes and toadfishes as suborders of the Pediculati, but later (1926) changed his mind in his review of *The Pediculate Fishes of the Suborder Ceratioidea*, separating the Lophiiformes from the Batrachoidiformes, but keeping them side by side, thereby implying a sister-group relationship (Pietsch, 2009). However, recent molecular studies from whole mitogenomes and various nuclear genes have repeatedly recovered lophiiforms within higher teleosts, deeply nested within one of the larger percomorph clades and generally associated with the Tetraodontiformes (Chen et al., 2003; Miya et al., 2003; Dettai et al., 2004; Holcroft, 2004; Dettai and Lecointre, 2005; Holcroft, 2005; Miya et al., 2005; Miya et al., 2007; Yamanoue et al., 2007; Li et al., 2008; Li et al., 2009; Betancur-R. et al., 2013). The Tetraodontiformes and closely allied taxa, the Caproidei, Acanthuroidei, Chaetodontidae, Pomacanthidae, Ephippidae, and Drepanidae, were not thought to be closely associated with the Lophiiformes before the advent of molecular phylogenetics (Miya et al., 2010).

Subordinal relationships. Regan (1912) recognized two suborders within the Pediculati, the Batrachoidea and Lophioidea. Within the Lophioidea he recognized three "divisions" of equal rank: the Lophiiformes, Antennariiformes, and Ceratiiformes. Within the Antennariiformes he recognized only four families (Tetrabrachiidae and Lophichthyidae had not yet been discovered at the time); and within the Ceratiiformes he recognized only six families, one of which, the Aceratiidae, was based only on males. Pietsch (1981) recognized that the monophyly of Regan's (1912) three major lophiiform taxa had never been established, and, in an attempt to test the validity of the concept of three major lophiiform taxa using cladistic analysis, proposed a sister relationship between the Ogcocephalidae and Chaunacidae based on four synapomorphies: posteriormost branchiostegal ray exceptionally long; gill teeth tiny, arranged in a tight cluster at apex of pedicellike tooth plates; gill filaments of gill arch I absent; and illicial bone, when retracted, lying within an illicial cavity. However, Pietsch (1981) was unable to identify the sister group of the Chaunacidae and Ogcocephalidae nor any osteological characters for or against their placement within the Antennariiformes. And, despite being unable to establish monophyly for the four families of Regan's (1912) Antennariiformes, he tentatively chose to retain both Chaunacidae and Ogcocephalidae within the Antennarioidei (Figure 4).

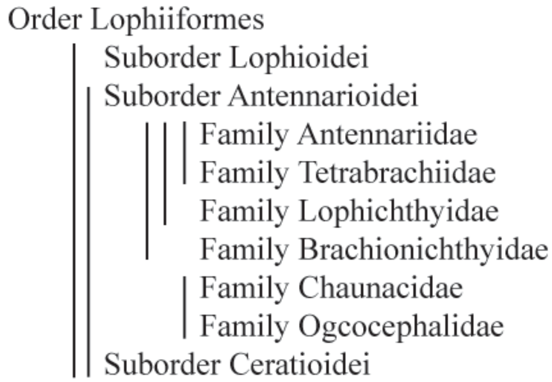

Figure 4. Classification of the Lophiiformes based on the cladistic analysis proposed by Pietsch (1981). Internested sets of vertical lines were used to indicate sister-group relationships.

On further study, however, Pietsch (1984a) and Pietsch and Grobecker (1987) erected the Ogcocephaloidei and the Chaunacoidei for the families Ogcocephalidae and Chaunacidae, respectively, after discovering support for a monophyletic assemblage containing them and the Ceratioidei based on two synapomorphies: second dorsal spine reduced and embedded beneath skin of head (Pietsch, 1981); and gill filaments of gill arch I absent (present on proximal end of ceratobranchial I of some ceratioids (Bradbury, 1967; Pietsch, 1981). Pietsch (1984a) and Pietsch and Grobecker (1987) also proposed three synapomorphies to support a sister relationship between the Ogcocephaloidei and Ceratioidei: second dorsal-fin spine reduced to a small remnant (Bertelsen, 1951; Bradbury, 1967; Pietsch, 1981) third dorsal-fin spine and pterygiophore absent (Bertelsen, 1951; Bradbury, 1967; Pietsch, 1981); and epibranchial I simple, without ligamentous connection to epibranchial II (Pietsch, 1981). The removal of the Ogcocephalidae and Chaunacidae restricted the Antennarioidei to four families: Antennariidae, Tetrabrachiidae, Lophichthyidae, and Brachionichthyidae (Pietsch, 1984a; Pietsch and Grobecker, 1987). That the Antennarioidei formed a monophyletic assemblage with the Ogcocephaloidei, Chaunacoidei, and Ceratioidei was supported by four synapomorphies: eggs and larvae small; head of larvae proportionately large relative to body; reduction in the number of dorsal fin spines from a primitive number of six in lophioids to three or less (Pietsch, 1981); and loss of pharyngobranchial IV (Pietsch, 1981). This new hypothesis of relationships required that three derived character states previously used by Pietsch (1981) were the result of evolutionary convergence or reversal: posteriormost branchiostegal ray exceptionally large; gill teeth tiny, arranged in a tight cluster at apex of pedicel-like tooth plates; and illicial bone, when retracted, lying within an illicial cavity. Lophioidei was retained as the basal member of the Lophiiformes (Figure 5A).

Using short fragments of the mitochondrial 16S rRNA and cytochrome *b* genes from 18 lophiiforms, Shedlock et al. (2004) analyzed 513 and 318 nucleotide sites, respectively, using the maximum likelihood (ML) method, with two batrachoidiform taxa as outgroups. The resulting tree (Figure 5B) differed significantly from both morphological analyses (Pietsch, 1981, 1984a; Pietsch and Grobecker, 1987; Figure 5A) and more recent molecular analyses (Miya et al., 2010; Arnold and Pietsch, 2012; Figures 5C and 6D). *Tetrabrachium*, a member of the family Tetrabrachiidae within the suborder Antennarioidei, was recovered as the basal member of all the Lophiiformes, while *Lophius*, a member of the family Lophiidae, suborder Lophioidei, was recovered deeply nested within the Lophiiformes. *Tathicarpus*, a member of the Antennariidae, was not recovered within the clade containing *Antennarius* and *Histrio*, the only other two members of the Antennariidae included in the study. The use of two short mitochondrial gene fragments and poor taxon sampling may have led to conclusions that are incongruent with morphological analyses; choice of outgroup and poor taxon sampling may have also led to conclusions incongruent with the more recent molecular analyses that used more derived perciform taxa for outgroups (Miya et al., 2010; Nabhan and Sarkar, 2012). In fact, Miya et al. (2010) discovered that the use of two batrachoidiforms as the only outgroups to root their lophiiform phylogenies disrupted the Antennarioidei at the most basal position, similar to the results obtained by Shedlock et al. (2004).

An analysis using mitogenomes by Miya et al. (2010) corroborated the monophyly of each of the five suborders hypothesized by Pietsch (1981) and Pietsch and Grobecker (1987); however, in a significant departure from the sister relationship of the Ogcocephaloidei plus Ceratioidei, the authors proposed the Chaunacoidei as sister of the Ceratioidei (Figure 5C). Miya et al. (2010) stated that the three unambiguous synapomorphies proposed by Pietsch (1984a; the first epibranchial is simple and without ligamentous connection to the second epibranchial; the third cephalic dorsal-fin spine and pterygiophore are absent; and the posttemporal is fused to the cranium) represented simplified or reductive trends and may be the result of convergent evolution, which would undermine the robustness of the phylogenetic hypotheses based on morphology. Placement of the Antennarioidei and Ogcocephaloidei was ambiguous with the three alternative hypotheses of relationships among the lineages (Ogcocephaloidei, Antennarioidei, and Chaunacoidei plus Ceratioidei) and equally likely in a statistical sense (Miya et al., 2010).

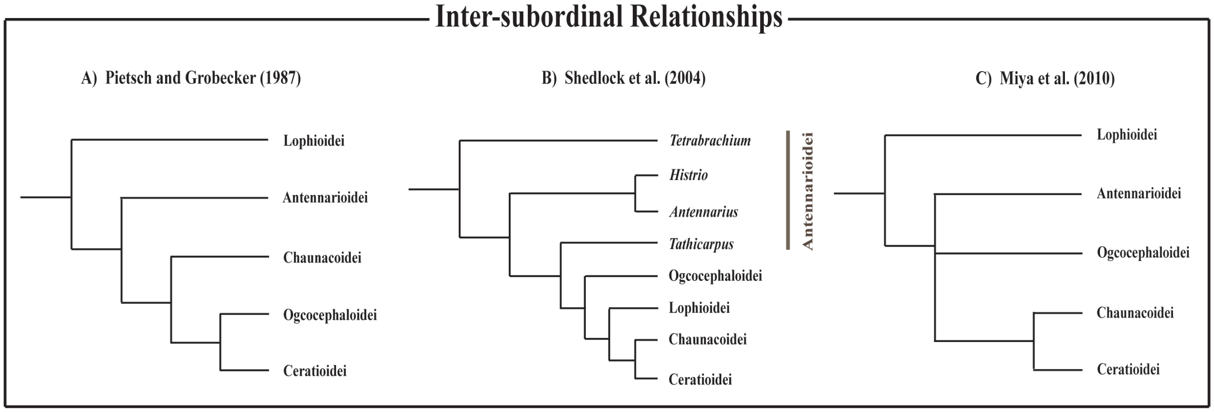

Figure 5. Inter-subordinal relationships of the Lophiiformes based on morphology (A) Pietsch, 1984a, and Pietsch and Grobecker, 1987; and mitochondrial DNA (B) Shedlock et al., 2004, and (C) Miya et al., 2010.

Familial and intergeneric relationships. Within the Lophioidei, consisting of the single family Lophiidae, Caruso (1985) proposed a phylogenetic hypothesis based on 12 shared derived states of the following morphological characters: 1) body shape; 2) anterior articular spines; 3) quadrate spines; 4) humeral spines; 5) subopercular spines; 6) interopercular spines; 7) gill opening; 8) ural centrum and transverse process; 9) frontal ridge; 10) sphenotic spines; 11) epiotic spines; and 12) pectoral fin shape and number of pectoral-fin rays. Monophyly of the Lophioidei was assumed and batrachoidiforms, paracanthopterygians and euteleosteans were used as nested taxonomic outgroups (Caruso, 1985). *Sladenia* was recovered as the basal member of the Lophiidae, as *Lophiodes*, *Lophiomus*, and *Lophius* shared derived states of characters 1–6; *Lophiodes* was recovered as sister of *Lophiomus* and *Lophius* as the latter two shared derived states of character 7–12; and *Lophius* was proposed to be the most derived of the four genera as it is autoapomorphic with regard to characters 13–15 (high number of vertebrae, high number of median fin rays, and an additional upper quadrate spine; Figure 6A; Caruso, 1985).

To place the extinct genera †*Caruso*, †*Eosladenia*, and †*Sharfia*, Carnevale and Pietsch (2012) proposed a phylogenetic hypothesis based on 38 newly examined morphological characters (Figure 6B) using two outgroup antennarioid genera, *Antennarius* and *Brachionichthys*. They proposed that †*Sharfia* is the sister group of all other known lophiid taxa, that †*Caruso* and *Sladenia* are sister groups, that these two genera together form the sister group of all remaining members of the family, that *Lophiodes* is sister to †*Eosladenia* + *Lophiomus* + *Lophius*, and that *Lophiomus* and *Lophius* are sister groups. These results are in concordance with Caruso's (1985) previous hypothesis of relationships of the extant lophiid genera.

Within the Antennarioidei, Pietsch (1981) and Pietsch and Grobecker (1987) recovered the Antennariidae as sister to the Tetrabrachiidae based on three synapomorphies: 1) posteromedial process of vomer emerging from ventral surface as a laterally compressed, keel-like structure, its ventral margin strongly convex; 2) postmaxillary process of maxilla spatulate; and 3) opercle similarly reduced in size. That these two families together form the sister group of the Lophichthyidae is supported by one synapomorphy: ectopterygoid triradiate, a dorsal process overlapping the medial surface of the metapterygoid; and that these three families and the Brachionichthyidae form a monophyletic assemblage is supported by two synapomorphies: 1) interhyal with a medial, posterolaterally directed process that comes into contact with the respective preopercle; and 2) illicial pterygiophore and pterygiophore of the third dorsal-fin spine with highly compressed, blade-like dorsal expansions (Figure 6C; Pietsch, 1981; Pietsch and Grobecker, 1987).

A detailed review by Pietsch (1984b) provided evidence for the recognition of approximately 40 more or less well-defined species of the Antennariidae—approximately 25% of the total number of described forms at that time—and provided a diagnosis and allocations for 13 genera to stabilize the nomenclature at the generic level [Pietsch and Grobecker (1987) later synonymized *Trichophryne* with *Echinophryne*]. However, Pietsch (1984b) and Pietsch and Grobecker (1987) were unable to resolve the intergeneric relationships of the Antennariidae, particularly the monophyly of the most primitive genus, *Antennarius*, because a single clearly derived feature could not be found among all taxa. The authors did recognize that two characters (loss of the mesopterygoid and loss of the epural) corroborated each other in defining the same set of nine genera (*Kuiterichthys*, *Allenichthys*, *Lophiocharon*, *Echinophryne*, *Trichophryne*, *Phyllophryne*, *Rhycherus*, *Histiophryne*, and *Tathicarpus*), but were unable to comment on the extent to which those states may have been independently acquired, and suggested that new, most likely non-osteological characters, would need to be identified and analyzed before the phylogenetic history of the Antennariidae could be reconstructed. This study came in 2012 when Arnold and Pietsch analyzed two mitochondrial genes and one nuclear gene and recovered two major lineages within the Antennariidae: the Antennariinae, comprised of *Antennarius*, *Antennatus*, *Fowlerichthys* [erected to contain the *A. ocellatus* group], *Histrio*, and *Nudiantennarius*; and the Histiophryninae, comprised of *Allenichthys, Echinophryne*, *Histiophryne*, *Kuiterichthys*, *Lophiocharon*, *Phyllophryne*, *Rhycherus*, *Tathicarpus*, and an undescribed genus and species (Figure 6D), the same subset of genera that Pietsch (1981) and Pietsch and Grobecker (1987) identified as having lost the mesopterygoid and epural. Pietsch (1984a) and Pietsch and Grobecker (1987) also identified morphological characters that correspond to the lineages Arnold and Pietsch (2012) hypothesized made up the Antennariinae: *Fowlerichthys*, the least derived genus of the group, has a unique combination of 20 vertebrae and five bifurcate pelvic-fin rays, while the rest of the members have 19 vertebrae and one bifurcate pelvic-fin ray. In addition, a sister relationship between *Lophiocharon* and *Histiophryne* was recovered by Arnold and Pietsch (2012), while Pietsch (1984b) and Pietsch and Grobecker (1987) identified these two genera as having lost pharyngobranchial I (but this was noted to also be absent in brachionichthyids and at least some ogcocephalids).

The Tetrabrachiidae contains only two monotypic genera, and the Lophichthyidae is a monotypic family. To place the extinct genera †*Histionotophorus* and †*Orrichthys*, Carnevale and Pietsch (2010b) provided a phylogenetic analysis of the Brachionichthyidae using a data matrix of seven antennarioid taxa (*Antennarius*, *Brachionichthys*,†*Histionotophorus*, *Lophichthys*,†*Orrichthys*, *Sympterichthys*, and *Tetrabrachium*) plus the basal lophiid genus

Sladenia and 36 morphological characters. They recovered *Brachionichthys* and *Sympterichthys* as sister taxa to the two extinct genera, †*Histionotophorus* and †*Orrichthys*, and recovered the Antennariidae as sister to the Tetrabrachiidae, these two forming the sister of the Lophichthyidae, and this assemblage forming the sister of the Brachionichthyidae. However, their manuscript had gone to press before Last and Gledhill (2009) described three new genera and nine new species, and so no hypothesis of the intergeneric relationships of the Brachionichthyidae exists for all currently recognized taxa.

Within the Ogcocephaloidei, consisting only of the family Ogcocephalidae, Endo and Shinohara (1999) reanalyzed Bradbury's (1967) nine transformation series using cladistics to determine the interrelationships of ogcocephalid genera (Figure 6E). They recovered a sister group relationship between *Ogcocephalus* and *Zalieutes*, this group forming the sister group to *Malthopsis*, and these groups forming the sister group to *Halicmetus*; this clade was recovered in a polytomy with the sister groups *Coelophrys* and *Halieutopsis* and the genus *Dibranchus*. This polytomy was in turn recovered in another polytomy that included *Halieutea* and *Halieutichthys*. The genus *Solocisquama* Bradbury, 1999, was not included in the analysis because it had not yet been described.

Within the Ceratioidei, consisting of 11 families, 35 genera, and 165 species, classification has been problematic. The genera are defined and distinguished primarily by characters present only in females, although some of the distinguishing meristic and osteological characters, such as fin-ray counts, are shared with the males (Pietsch and Orr, 2007). In most cases, it has not been possible to separate free-living males into taxa below the generic level, and even examining males attached to females has not revealed characters that will allow specific identification (Bertelsen, 1984). In addition, deep-sea ceratioids are rare in collections; nevertheless, Bertelsen

(1984; Figure 2F), Pietsch and Orr (2007), and Pietsch (2009), using morphological characters (Figure 2G), and Miya et al. (2010; Figure 2H), using mitogenomes, have proposed interfamilial relationships of the Ceratioidei. Miya et al. (2010) recovered monophyly for all higher taxa and the order itself, with the exception of the Thaumatichthyidae (*Lasiognathus* was recovered deeply nested within the Oneirodidae). While their results were congruent with or statistically indistinguishable from alternative morphology-based hypotheses within the relatively shallow, benthic dwellers (Lophioidei, Antennarioidei, Ogcocephaloidei, Chaunacoidei), relationships within the Ceratioidei were incongruent and could not be reconciled with the morphological phylogeny of Pietsch and Orr (2007). In addition, Miya et al. (2010) recovered the Chaunacoidei plus Ceratioidei in all datasets with high bootstrap (BS) values, departing significantly from the hypothesis of (Pietsch 1984a) and Pietsch and Grobecker (1987) of a sister relationship of the Ogcocephaloidei plus Ceratioidei (see subordinal relationships, above).

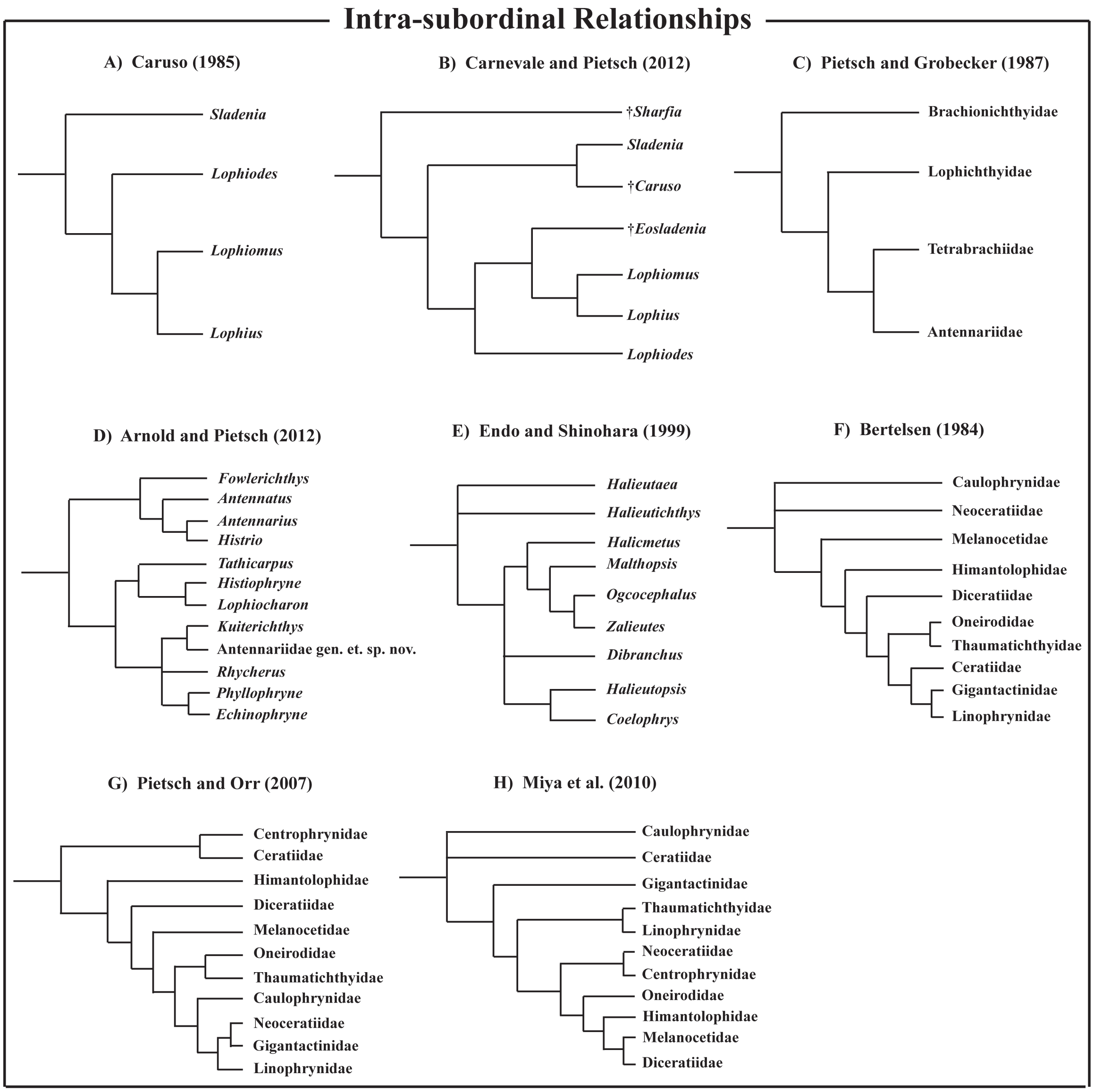

Figure 6. Intra-subordinal relationships of the (A, B) Lophioidei (Caruso, 1985; Carnevale and Pietsch, 2012); (C, D) Antennarioidei (Pietsch and Grobecker, 1987; Arnold and Pietsch, 2012); (E) Ogcocephaloidei (Endo and Shinohara, 1999); and (F, G, H) Ceratioidei (Bertelsen, 1984; Pietsch and Orr, 2007; Miya et al., 2010).

Objectives. Phylogenies derived from mitogenomes represent only the mtDNA genealogy and may not match the evolution of the species under analysis, and phylogenies based solely on mtDNA may be incongruent at both higher and lower phylogenetic levels (Springer et al., 2001; Ballard et al., 2002; Cameron et al., 2004; Leaché and McGuire, 2006). In addition, mitochondrial genes are inherited as a single linkage group and provide only one independent estimate of the species tree, while a set of nuclear loci can be selected such that each gene tree provides an independent estimate of the species tree (Moore, 1995). Because the phylogenetic relationships recovered by Miya et al. (2010) represent only the mtDNA genealogy, the primary goal of this study was to concatenate multiple nuclear loci (each providing an independent estimate of the species tree) to estimate the intra-relationships of the Lophiiformes and to compare the resulting phylogeny to the relationships proposed by previous authors using other datasets (Regan, 1912; Pietsch, 1984b; Pietsch and Grobecker, 1987; Shedlock et al., 2004; Miya et al., 2010). To this end, fragments from the protein coding nuclear loci ENC1, Glyt, myh6, and zic1 were used for this study because they are putatively single-copy gene fragments with long, uninterrupted exons, are relatively well conserved and suitable for a relatively high taxonomic level (substitution rates of 1.13, 1.18, 1.35, and 0.64, respectively), and have previously been shown to provide useful phylogenetic signal within the ray-finned fishes (Li et al., 2007). While several other genes are available for phylogenetic study, these four nuclear genes amplified well for this taxonomic group.

CHAPTER II – METHODS

Taxon sampling. This study was conducted under the assumption that the Lophiiformes are higher-level percomorphs (in close association with the Caproidei and Tetraodontiformes) based on recent molecular analyses (Betancur-R et al., 2013; Near et al., 2012, 2013; Santini et al., 2009) and morphology (Chanet et al., 2012). The outgroup sampling by Miya et al. (2010) was also based on results from recent mitochondrial phylogenomic studies (Miya et al., 2003, 2005; Yamanoue et al., 2007), and whose authors proposed that the order was closely related to members of previously unallied groups such as the Caproidei and Tetraodontiformes. To replicate as much as possible the study by Miya et al. (2010) using nuclear genes instead of mitogenomes, the same individual percomorphs were obtained for outgroup sampling in this study. In addition, as many species of lophiiform fishes are still rare in collections, new species were included in this study whenever possible. In the present study, nine outgroup taxa, 37 of the 39 species of lophiiforms used by Miya et al. (2010), plus 29 additional lophiiform taxa and two undescribed species, for a total of 68 species, were incorporated, which represent all families except the rare monotypic antennarioid family Lophichthyidae, for which tissues are unavailable (Table 1). Samples represented all five lophiiform suborders, 17 of the 18 families (94.4%), 47 of the 73 genera (64.4%), and 66 of the 351 described species (18.8%). While the study by Miya et al. (2010) included only two of the 14 genera (14.3%) and three of the 49 described species (6.1%) of the Antennariidae, this study includes 12 genera (85.7%) and 27 described species (55.1%) of antennariids.

A second dataset consisting of a subset of taxa included in the study by Miya et al. (2010) was generated by concatenating the four nuclear genes from the first dataset with 14 mitochondrial genes (12S, 16S, ATPase 6, ATPase 8, COI, COII, COIII, cyt b, ND1, ND2, ND3, ND4, ND5, and ND6) from the same individuals downloaded from GenBank, with the following exception: mitochondrial genes from *Brachionichthys hirsutus* were concatenated with nuclear genes from *Brachionichthys australis*. This dataset included 48 species (nine outgroup taxa) for a total of 3,300 base pairs (bps) from nuclear genes and 14,250 bps from mitochondrial genes.

Table 2. Taxon sampling and associated Genbank numbers for lophiiforms and outgroup taxa included in this study.

Family	Species	Cat. #	ENC1	Glyt	myh6	zic1	Mito-genome
Antennariidae	*Porophryne erythrodactylus*	AMS I.43749.001	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Antennatus coccineus*	NSMT-P 68051	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282830
Antennariidae	*Antennarius commerson*	UW 117686	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Antennarius hispidus*	UW 117828	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Antennarius indicus*	UW 118818	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Antennarius maculatus*	UW 117687	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Antennarius multiocellatus*	UW 117826	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Antennatus nummifer*	T5049 / RUSI 65251	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Antennarius pictus*	UW 118986	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Antennatus rosaceus*	QS I.38177	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Antennatus sanguineus*	UW 118813	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Fowlerichthys scriptissimus*	UW 112642	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Antennarius striatus*	CBM-ZF-10514	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282828
Antennariidae	*Antennarius striatus 2*	UW 112081	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Antennarius striatus 3*	WAM 32905.001	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	

Antennariidae	*Antennarius striatus 4*	UW 118815	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Antennatus tuberosus*	UW 115750	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Echinophryne crassispina*	*	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Histiophryne cryptacanthus*	UW 117821	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Histiophryne cryptacanthus*	UW 118816	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Histiophryne maggiewalker*	QS I.38176	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Histiophryne* sp.	SAM F11719	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Histrio histrio*	Unknown	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282829
Antennariidae	*Histrio histrio*	T3016 / KU 29308	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Histrio histrio*	MNHN 2006-1712	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Kuiterichthys furcipilis*	SAM F10476	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Lophiocharon lithinostomus*	UW 115749	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Lophiocharon trisignatus*	UW 115748	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Phyllophryne scortea*	NMV A29226.005	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Phyllophryne scortea*	SAM F17721	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Rhycherus filamentosus*	NMV A29238.11	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Rhycherus filamentosus*	*	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	

Antennariidae	*Tathicarpus butleri*	WAM 32903.001	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Tetrabrachiidae	*Tetrabrachium ocellatum*	UW 049710-1	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Fowlerichthys ocellatus*	UW 150909	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Fowlerichthys ocellatus*	UW 150910	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Antennatus* sp. 2	*	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Histiophryne cryptacanthus*	UW 117816	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Phyllophryne scortea*	SAM 11720	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Histiophryne bougainvilli*	UW 118990	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Antennatus tuberosus*	UW 118814	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Antennatus tuberosus*	UW 117644	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Antennarius striatus* 7	UW 118819 (Indonesia)	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Tetrabrachiidae	*Tetrabrachium ocellatum*	UW 049710	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282831
Antennariidae	*Antennarius striatus 5*	CAS 234886 / TI 2010-132	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Antennariidae	*Antennarius pardalis*	CAS 235484/ TI 2010-109	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Brachionichthyidae	*Brachionichthys australis*	CSIRO H 4460-02	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Brachionichthyidae	*Brachionichthys australis*	CSIRO H 4466-01	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Chaunacidae	*Chaunax abei*	CBM-ZF-10508	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AP004415

Chaunacidae	*Chaunax pictus*	Unknown	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282833
Chaunacidae	*Chaunax* sp.	UW 025870	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Chaunacidae	*Chaunacops coloratus*	CAS 232088	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Caulophrynidae	*Caulophryne jordani*	Unknown	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AP004417
Caulophrynidae	*Caulophryne pelagica*	NSMT-P 93887(1)	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282836
Centrophrynidae	*Centrophryne spinulosa*	UW 047213	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282849
Ceratiidae	*Ceratias uranoscopus*	NSMT-P 99996(1)	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282851
Ceratiidae	*Cryptopsaras couesii*	Unknown	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282850
Diceratiidae	*Bufoceratias thele*	Unknown	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282841
Diceratiidae	*Diceratias pileatus*	Unknown	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282842
Gigantactinidae	*Gigantactis vanhoeffeni*	MCZ 101608	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282852
Gigantactinidae	*Rhynchactis macrothrix*	ASIZ-P 0062880	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282853
Himantolophidae	*Himantolophus albinares*	MCZ 138064	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282839
Himantolophidae	*Himantolophus groenlandicus*	MNHN-2003_1066	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282840
Linophrynidae	*Acentrophryne dolichonema*	HUMZ-189134	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282855
Linophrynidae	*Haplophryne mollis*	MNHN-2004_0811	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282856
Linophrynidae	*Linophryne bicornis*	MCZ 138063	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282854

Melanocetidae	*Melanocetus johnsonii*	HUMZ-185908	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282838
Melanocetidae	*Melanocetus murrayi*	Unknown	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AP004418
Neoceratiidae	*Neoceratias spinifer*	*	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282837
Oneirodidae	*Bertella idiomorpha*	UW 042301	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282846
Oneirodidae	*Chaenophryne melanorhabdus*	UW 049299	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282845
Oneirodidae	*Oneirodes thompsoni*	UW 048054	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282843
Oneirodidae	*Puck pinnata*	SIO-04-35	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282844
Thaumatichthyidae	*Lasiognathus sp.*	Unknown	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282848
Thaumatichthyidae	*Thaumatichthys pagidostomus*	Unknown	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282847
Thaumatichthyidae	*Thaumatichthys* sp.	*	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Linophrynidae	*Linophryne* sp.	*	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Diceratiidae	*Diceratias* sp.	*	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Gigantactinidae	*Gigantactis* sp.	*	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Gigantactinidae	*Gigantactis* sp.	*	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Gigantactinidae	*Gigantactis* sp.	*	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Himantolophidae	*Himantolophus* sp.	*	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Oneirodidae	*Dolopichthys* sp.	*	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	

Oneirodidae	*Chaenophryne* sp.	*	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Lophiidae	*Lophiodes caulinaris*	HUMZ-189431	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282826
Lophiidae	*Lophiomus setigerus*	Unknown	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AP004413
Lophiidae	*Lophius americanus*	Unknown	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AP004414
Lophiidae	*Sladenia* sp.	Unknown	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282827
Ogcocephalidae	*Coelophrys brevicaudata*	ASIZ-P 0062883	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282834
Ogcocephalidae	*Dibranchus atlanticus*	UW 025869	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Ogcocephalidae	*Halieutaea stellata*	CBM-ZF-10510	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AP005977
Ogcocephalidae	*Malthopsis jordani*	BSKU 94678	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AP005978
Ogcocephalidae	*Ogcocephalus radiatus*	UW 118987	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Ogcocephalidae	*Zalieutes elater*	HUMZ-189343	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AB282835
Outgroup	*Antigonia capros*	Unknown	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
Outgroup	*Antigonia capros*	CBM-ZF-11799	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AP002943
Outgroup	*Capros aper*	*	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AP009159
Outgroup	*Chaetodon auripes*	ORIUT198	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AP006004
Outgroup	*Lutjanus rivulatus*	ORIUT193	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AP006000
Outgroup	*Monodactylus argenteus*	*	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AP009169

Outgroup	*Pagrus major*	CBM-ZF-10896	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AP002949
Outgroup	*Ranzania laevis*	Unknown	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AP006047
Outgroup	*Triacanthodes anomalus*	*	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AP009172
Outgroup	*Triodon macropterus*	*	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	AP009170

**** denotes a specimen that is not cataloged.***

DNA extraction, PCR, sequencing, and alignment. Original DNA extractions from Miya et al. (2010) and Arnold and Pietsch (2012) were obtained and used for further sequencing. For all newly added taxa, total genomic DNA was extracted from a small piece of epaxial musculature or fin clip from fresh or ethanol-fixed specimens using a DNeasy tissue extraction kit (Qiagen), following the manufacturer's protocol. PCR amplification of four nuclear genes, ENC1, Glyt, myh6, and zic1, was carried out in a Peltier Thermo Cycler (PT-225) using previously published primers (Li et al., 2007) with the following concentrations of reagents: 1–2 µl of genomic DNA template, 0.5 µL of each 10 µM primer, 2.5 µL of 10X buffer, 2.0 µL of 25 mM $MgCl_2$, 2.0 µL of 8 mM premixed deoxynucleotide triphosphates, 0.1 µL of DNA polymerase (TaKaRa Ex Taq), and nuclease-free water to a total volume of 25 µL. PCR amplification was carried out in a Peltier Thermo Cycler (PT-225) under the following conditions: initial denaturation at 95˚C for 4 min followed by 35 cycles of denaturation at 95˚C for 40 s, annealing between 48–62˚C for 40 s, and extension at 72˚C for 90 s. After a final extension step at 72˚C for 7 min, all samples were held at 4˚C. Sequences were cleaned and generated at the High-Throughput Genomics Unit (HTGU), Department of Genome Sciences, University of Washington, Seattle. Forward and reverse chromatographs were contiged in GENEIOUS (Drummond and Rambaut, 2007) and checked against each other for base calls; instances of heterozygosity (overlapping peaks of equal strength) were coded according to the IUPAC ambiguity code. Alignment was completed using MAFFT v7.017 (Katoh and Toh, 2008) as implemented in GENEIOUS (Drummond and Rambaut, 2007). Sequences were aligned against previously published sequences (JN235977, JX188678, JX189629, EU001884) to confirm the reading frame as well as to determine the presence of insertions and deletions. Sequences from 14 mitochondrial genes downloaded from GenBank were also aligned individually using MAFFT v7.017 (Katoh and Toh, 2008) as implemented in GENEIOUS (Drummond and Rambaut, 2007). All datasets were concatenated in GENEIOUS (Drummond and Rambaut, 2007), and all new sequences were deposited in GenBank (Table 2).

Phylogenetic analysis. Partitioning strategies were assessed in PartitionFinder v.1.1.1 (Lanfear et al., 2012) using Python v.2.7 with the following parameters for both the nuclear dataset and the combined nuclear and mitochondrial dataset: branches were set to unlinked, models were limited to those used in the program MrBayes (Ronquist et al., 2012), model selection was set to AIC, and the search scheme was set to greedy. For the nuclear dataset, all codon positions and genes were assessed separately. The same parameters that were implemented for the nuclear dataset were implemented for the combined dataset, except that the codon positions were not assessed separately for each mitochondrial gene. MrBayes v.3.2 (Ronquist et al., 2012) was used to estimate phylogenetic relationships of the concatenated datasets using the following parameters: partitions and models were set as selected by PartitionFinder v.1.1.1 (Lanfear et al., 2012) and all parameters were set to unlinked. RaxML v.8.0.0 (Randomized Axelerated Maximum Likelihood; Stamatakis, 2014) was also used to estimate the phylogenetic relationships of the both datasets using the following parameters: partitions were set as selected by PartitionFinder v.1.1.1 (Lanfear et al., 2012), but all partitions were analyzed under the GTR+G model using the default settings because RaxML only implements variations of the GTR model; this appears unproblematic as Dornburg et al. (2008) found that overparameterization of the substitution model did not significantly affect the phylogeny. The topology with the best likelihood score was retained. Bootstrap support values were also calculated from 1000 replicates during the search for the best scoring maximum likelihood (ML) tree for both datasets. All analyses were completed using the CIPRES portal v.3.3 (Miller et al., 2010).

The following partitions and models were selected in PartitionFinder v.1.1.1 (Lanfear et al., 2012) for the first dataset that contained the four nuclear genes: GTR+G for the third codon positions of myh6 and ENC1; GTR+G for the third codon position of zic1; SYM+I+G for the third codon position of Glyt; and GTR+I+G for all first and second codon positions of all genes. For the dataset that included the four nuclear genes plus 14 mitochondrial genes, the following partitions and models were selected: GTR+I+G for the first codon positions of the ENC1 and zic1 genes; GTR+I for the second codon positions of the ENC1, myh6, and zic1 genes; GTR+G for the third codon positions of the ENC1 and myh6 genes; HKY+I+G for the first codon positions of the Glyt and myh6 genes, and the second codon position of the Glyt gene; K80+G for the third codon position of the Glyt gene; GTR+G for the third codon position of the zic1 gene; and separate partitions with the GTR+I+G model for each of the 14 mitochondrial genes.

CHAPTER III – RESULTS

Bayesian analysis of nuclear genes. The phylogenetic relationships recovered using Bayesian analysis of the four nuclear genes are presented in Figure 7. Monophyly was indicated for the order and for each of the five suborders of the Lophiiformes with high posterior probabilities (PP 0.84 for the suborder Ceratioidei and 1.00 PPs for all other suborders). The Ceratioidei and Chaunacoidei were recovered as sister groups (PP 1.00), these two suborders formed the sister group to the Antennarioidei (PP 0.99), this assemblage in turn formed the sister group to the Ogcocephaloidei (PP 1.00), and the suborder Lophioidei was recovered as the basal lineage of the Lophiiformes (PP 1.00).

Within the Lophioidei, for which the one family and all four genera were included, *Lophius* and *Lophiomus* were recovered as sisters (PP 1.00), these two genera made up the sister group to *Lophiodes* (PP 1.00), and *Sladenia* was recovered as the basal lineage (PP 1.00). Within the suborder Ogcocephaloidei, for which the one family and six of ten genera were included, the following relationships were recovered: *Coelophrys* and *Malthopsis* formed a sister group relationship (PP 0.55), with *Dibranchus* recovered as sister to them (PP 0.93); this assemblage made up the sister group to the sister genera *Ogcocephalus* and *Zalieutes* (PP 1.00) with a PP of 0.98, and *Halieutaea* was recovered as the basal lineage (PP 1.00).

Within the suborder Antennarioidei, the family Antennariidae was rendered paraphyletic by the addition of the families Brachionichthyidae and Tetrabrachiidae. However, the subfamily Antennariinae (Arnold and Pietsch, 2012) is recovered as monophyletic, with the genus *Fowlerichthys* recovered as basal to the remaining genera (PP 1.00). Within the Antennariinae, the *A. pictus* and *A. striatus* groups (*sensu* Arnold and Pietsch, 2012) were recovered as sisters (PP 1.00), three individuals of *Antennatus tuberosus* were recovered as sister to an individual of *Histrio histrio* from the Gulf of Mexico (0.95 PP), with a second individual of *Histrio histrio* from French Polynesia recovered as sister to them (0.85 PP). Two sister pairs, the antennariids *Antennatus sanguineus* and *Antennatus coccineus* (PP 1.00), and *Antennatus rosaceus* and an undescribed species of *Antennatus* (PP 1.00), were recovered in a large polytomy (PP 1.00) that also comprised *A. nummifer*, the *A. pictus* and *A. striatus* species groups, and the group comprised of the two individuals of *H. histrio* and *A. tuberosus*. Within the *A. striatus* group, *A. striatus* was rendered paraphyletic by *A. hispidus*. *Antennarius hispidus* was sister to individuals of *A. striatus* from off the coasts of Sao Tome and Principe, Indonesia, and Western Australia (PP 0.97). This clade was sister to *A. striatus* from Japan (PP 1.00), and this assemblage formed the sister group to *A. striatus* from the Gulf of Mexico (PP 1.00), with *A. indicus* recovered as the basal member of this clade (PP 1.00). Within the clade containing the Brachionichthyidae, the Tetrabrachiidae, and the remaining members of the family Antennariidae (subfamily Histiophryninae), two major lineages were recovered with a PP of 1.00. In the first lineage, the genus *Phyllophryne* was recovered as sister to *Echinophryne* (PP 0.99), *Rhycherus* formed a sister relationship with them (PP 1.00), and this assemblage formed a sister group to *Porophryne* (PP 0.99), while *Kuiterichthys* was recovered as the basal antennariid of this assemblage (PP 0.99); the two brachionichthyids, *Brachionichthys australis*, were recovered as the basal sister group of this clade (PP 1.00). Within the second major lineage, *Histiophryne* was recovered as sister to *Lophiocharon* (PP 1.00), the tetrabrachiid *Tetrabrachium ocellatum* was recovered as the sister group of this clade (PP 0.74), and the antennariid *Tathicarpus* was recovered as the basal lineage of this clade (PP 0.56).

Within the monotypic suborder Chaunacoidei, for which both genera were included in this study, three species of *Chaunax* were recovered as sister to the genus *Chaunacops* (PP 1.00). Within the suborder Ceratioidei, the Linophrynidae was recovered as the basal member (PP 0.84). The Centrophrynidae and Caulophrynidae were recovered as sister families (PP 0.62) and were the next to diverge in this suborder. Two minor lineages were then recovered with a PP of 0.66. The first minor lineage was composed of a sister group relationship between the sister families Ceratiidae and Gigantactinidae (PP 0.57) and the sister families Neoceratiidae and Thaumatichthyidae (PP 0.86) with a PP of 0.82. The second minor lineage was composed of the sister families Himantolophidae and Melanocetidae (PP 0.51), with the Diceratiidae recovered as sister to them (PP 1.00), and the Oneirodidae (including the genus *Lasiognathus*, see below) recovered as the basal member of this clade (PP 1.00). Within the Linophrynidae, *Linophryne bicornis* was recovered as sister to *Haplophryne mollis* and an unidentified species of *Linophryne* (PP 1.00), with *Acentrophryne* recovered as the basal species of this family (PP 1.00). Within the family Oneirodidae, the genera *Bertella* and *Dolopichthys* were recovered as sister genera (PP 1.00), and the genus *Chaenophryne* was recovered as sister to them (PP 1.00). *Puck* and *Lasiognathus* were recovered as sister genera (PP 1.00) and were recovered as the sister group to the assemblage that included *Bertella*, *Dolopichthys*, and *Chaenophryne* (PP 1.00), with *Oneirodes* recovered as the basal member of this family (PP 1.00).

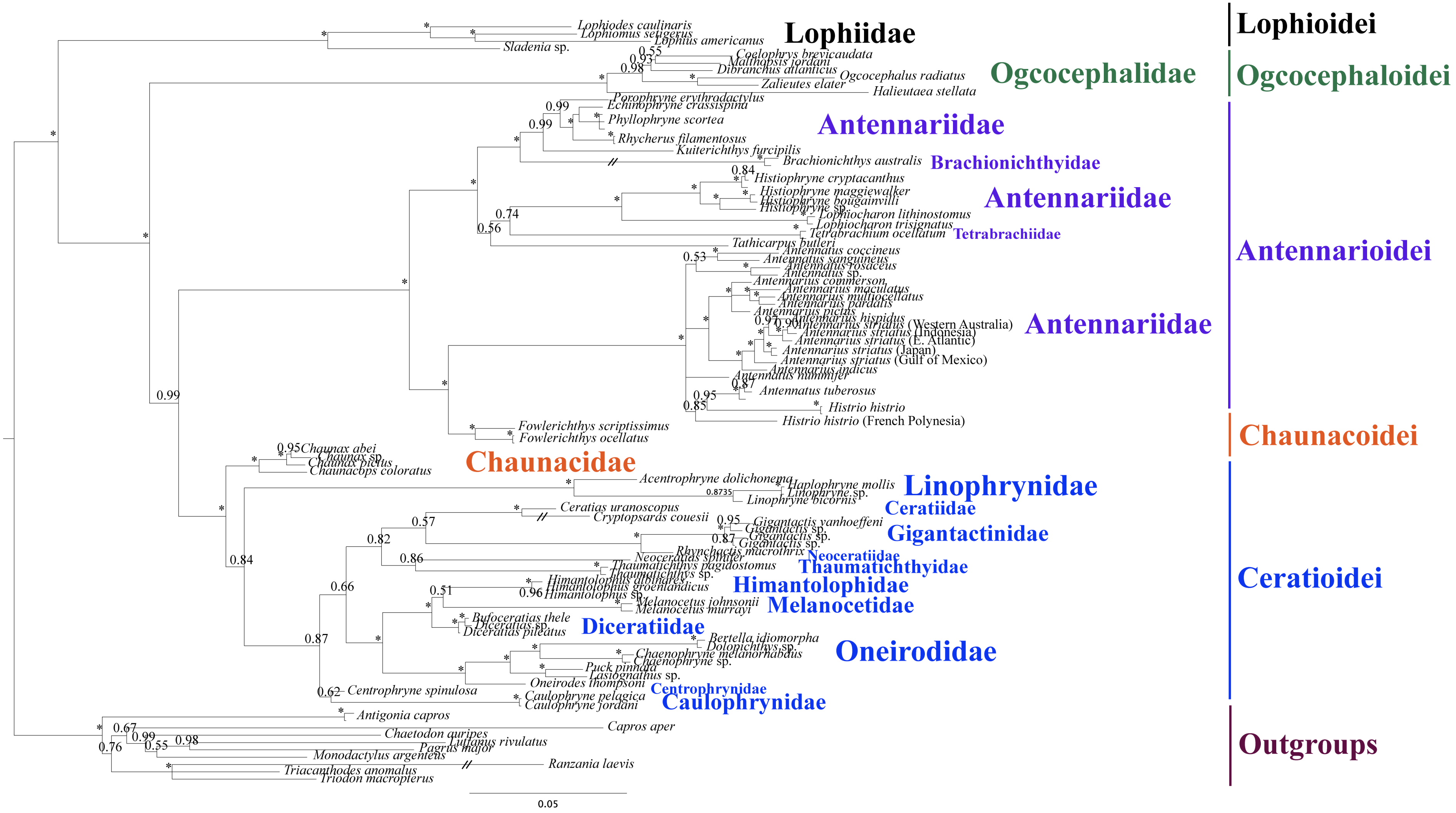

Figure 7. Bayesian analysis of four concatenated nuclear genes (ENC1, Glyt, myh6, and zic1) for the Lophiiformes and nine outgroup taxa. An asterisk (*) represents a posterior probability (PP) of 1.00.

Bayesian analysis of nuclear and mitochondrial genes. The phylogenetic relationships recovered using the concatenated dataset of nuclear and mitochondrial genes subjected to Bayesian analysis are presented in Figure 8. All five suborders of the Lophiiformes were recovered as monophyletic with PPs of 1.00. The Lophioidei was recovered as the basal suborder (PP 1.00), followed by a sister relationship (PP 1.00) between the sister suborders Ogcocephaloidei and Antennarioidei (PP 1.00) and the sister suborders Chaunacoidei and Ceratioidei (PP 1.00).

Within the Lophioidei, which included the single family Lophiidae and all four genera, *Lophiomus* was recovered as sister to *Lophius* (PP 1.00), this assemblage was recovered as sister to *Lophioides* (PP 1.0), and *Sladenia* was recovered as the basal lineage of this clade (PP 1.00). Within the Ogcocephaloidei, which included the single family Ogcocephalidae and four of ten genera, *Halieutaea* was recovered as the basal member of this group (PP 1.00), *Malthopsis* diverged next (PP 0.50), and *Coelophrys* and *Zalieutes* formed a sister relationship at the crown of the clade (PP 0.50).

Within the Antennarioidei, which included three of the four families, the Antennariidae was recovered as sister to the Tetrabrachiidae (PP 1.00), and the Brachionichthyidae was recovered as the basal member (PP 1.00). Within the Antennariidae, only three of 14 genera were included. *Antennatus* formed a sister relationship with *Histrio* (PP 1.00), and these two species formed the sister to *Antennarius* (PP 1.00).

Within the Ceratioidei, the Linophrynidae was the first family to diverge from all other members (PP 1.00). A sister group relationship between the Caulophrynidae and Ceratiidae diverged next (PP 1.00), then the Gigantactinidae (PP 1.00), the Thaumatichthyidae (PP 1.00), and a sister relationship between the Centrophrynidae and Neoceratiidae (0.72) diverged from the lineage in a step-wise fashion (PP 0.72); this left a crown group with PP of 1.00 comprised of the sister families Diceratiidae and Melanocetidae (PP 1.00), this assemblage made up the sister group to the Himantolophidae (PP 1.00), while the Oneirodidae was recovered as the basal member of this lineage (PP 1.00). Within the Linophrynidae, *Haplophryne* was recovered as the basal member (PP 1.00) to an assemblage that included *Acentrophryne* and *Linophryne* as sister families (PP 1.00). Within the Oneirodidae, *Puck* and *Lasiognathus* were recovered in a sister relationship (PP 1.00), this assemblage formed the sister to *Bertella* (PP 1.00), and this assemblage formed the sister to *Chaenophryne* (PP 1.00), with *Oneirodes* recovered as the basal lineage of this family (PP 1.00)

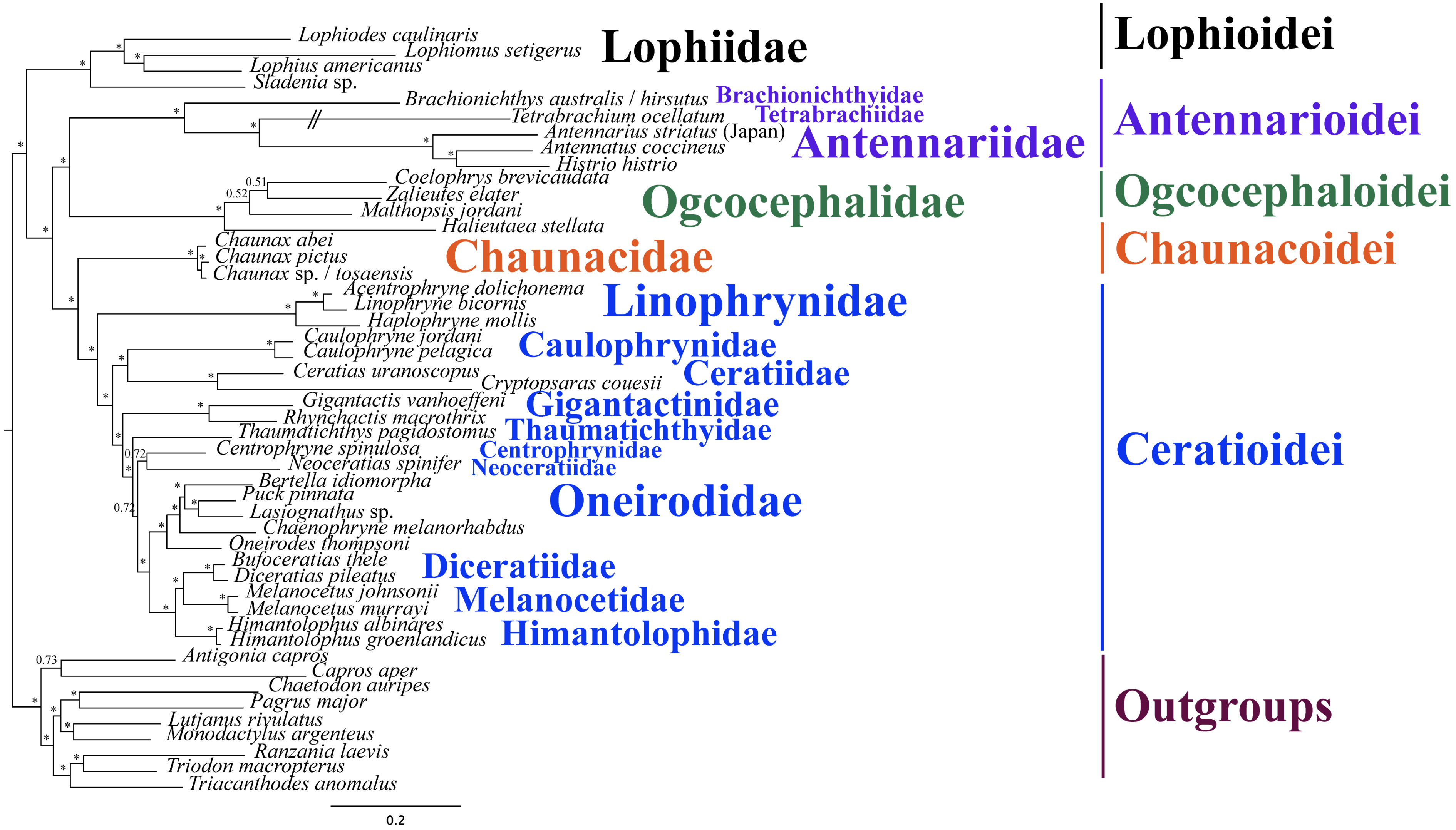

Figure 8. Bayesian analysis of a concatenated set of four nuclear genes (ENC1, Glyt, myh6, and zic1) and 14 mitochondrial genes (12S, 16S, ATPase 6, ATPase 8, COI, COII, COIII, cyt b, ND1, ND2, ND3, ND4, ND5, and ND6) for the Lophiiformes and nine outgroup

Maximum likelihood analysis of nuclear genes. The phylogenetic relationships recovered using the first dataset of nuclear genes subjected to ML analysis are presented in Figure 9. In this analysis, the Lophiiformes was recovered as a monophyletic assemblage with a bootstrap (BS) value of 100. All five suborders were also recovered as monophyletic, with Lophioidei recovered as the basal group (BS 100), the Ogcocephaloidei diverged next (BS 100) in a step-wise fashion, this assemblage sister to the Antennarioidei, Chaunacoidei, and Ceratioidei (BS 61), with the latter two suborders recovered in a sister relationship (BS 73).

Within the Lophioidei, a sister relationship was recovered between *Lophiomus* and *Lophius* (BS 100), this assemblage formed the sister to *Lophiodes* (BS 100), and *Sladenia* was recovered as the basal member of the family Lophiidae (BS 100). Within the Ogcocephaloidei, *Coelophrys* and *Malthopsis* were recovered as sisters (BS 39), *Dibranchus* was recovered as sister to them (BS 65), and this assemblage was sister to a clade (BS 34) containing the sister genera *Ogcocephalus* and *Zalieutes* (BS 100), while *Halieutaea* was the basal member of the family Ogcocephalidae.

Within the Antennarioidei, the Antennariinae formed a sister relationship to the clade containing the Histiophryninae, Brachionichthyidae, and Tetrabrachiidae (BS 100). Within the latter clade, two minor lineages were recovered. The first minor lineage recovered a member of the Brachionichthyidae, *Brachionichthys australis*, as the basal member (BS 89), then the antennariids *Kuiterichthys* (BS 72), *Porophryne* (BS 92), and *Rhycherus* (BS 88) diverged in a step-wise fashion, which left *Echinophryne* and *Phyllophryne* as sister genera at the crown of this lineage (BS 89). Within the second minor lineage, the antennariids *Lophiocharon* and *Histiophryne* formed a sister relationship (BS 100), and this assemblage was recovered as sister

(BS 34) to an assemblage that included the antennariid *Tathicarpus* and the tetrabrachiid *Tetrabrachium ocellatum* as sister groups (BS 48).

Within the Chaunacoidei, which included both genera of the family Chaunacidae, *Chaunacops* was recovered as sister to a group of species within the genus *Chaunax* (BS 79). Within the Ceratioidei, members of the Linophrynidae made up the basal clade of the suborder (BS 54), while the Caulophrynidae diverged next in a step-wise fashion (BS 55). The crown group was made up of two smaller clades, the first containing the sister families Ceratiidae and Gigantactinidae (BS 61) as sister (BS 42) to the sister families Thaumatichthyidae and Neoceratiidae (BS 44). The second clade recovered Centrophrynidae as the basal member (BS 33); the Oneirodidae (BS 90) and then the Himantolophidae (BS 100) diverged next in a step-wise fashion, and the sister groups Diceratiidae and Melanocetidae diverged next (BS 56). Within the Linophrynidae, *Haplophryne* and an unidentified species of *Linophryne* formed a sister relationship (BS 100), this assemblage was recovered as sister to *Linophryne* (*Linophryne bicornis*; BS 76), and this larger assemblage was recovered as sister to the basal member *Acentrophryne* (BS 100). Within the Oneirodidae, *Bertella* and *Dolopichthys* formed a close sister relationship (BS 100), these two genera made up the sister group to *Chaenophryne* (BS 93), and this assemblage made up the sister group (BS 100) to a group that included a sister relationship between *Lasiognathus* and *Puck* (BS 100); this larger assemblage formed the sister group to the basal *Oneirodes* (BS 100).

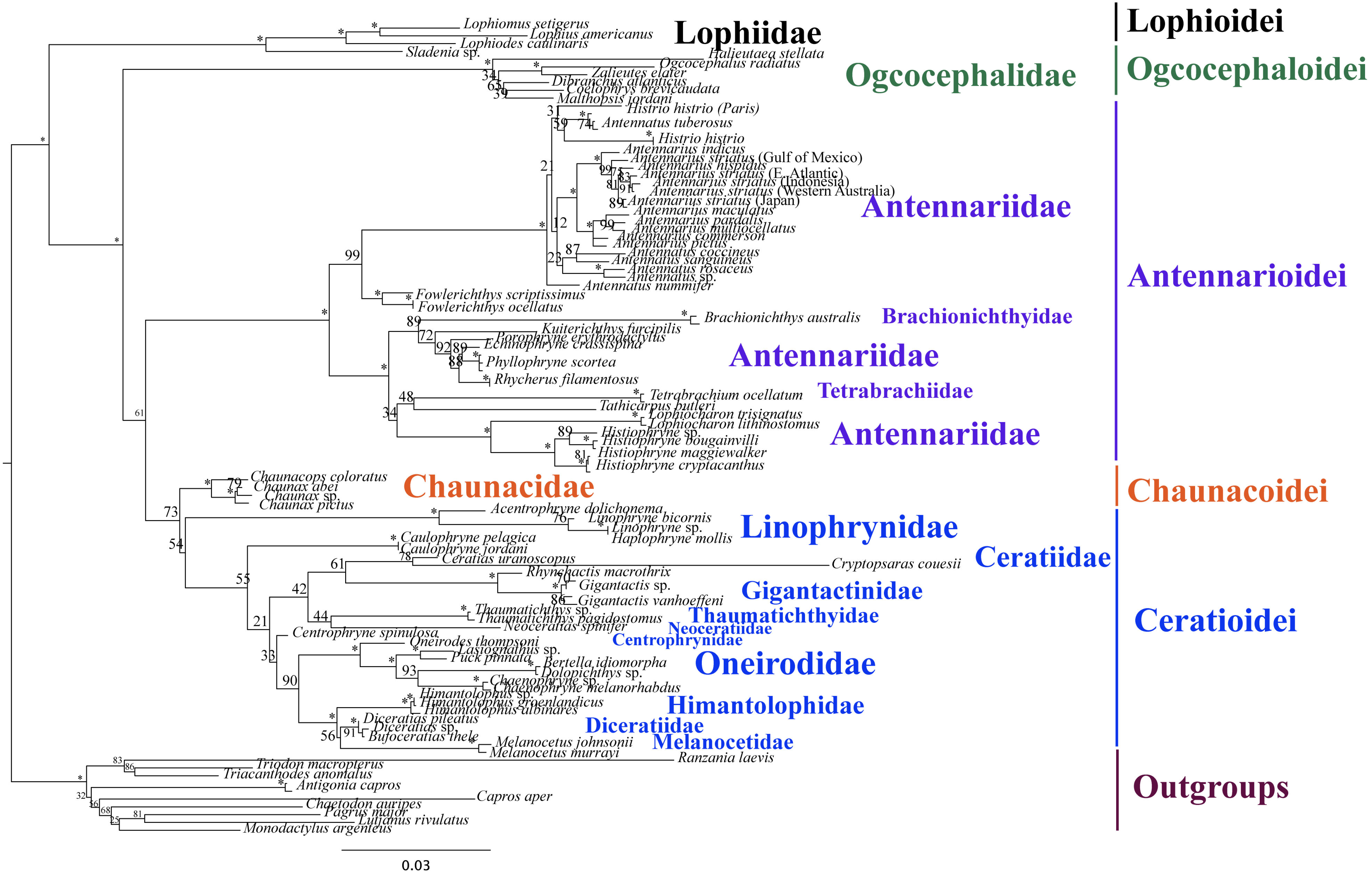

Figure 9. Maximum likelihood (ML) analysis of a concatenated set of four nuclear genes (ENC1, Glyt, myh6, and zic1) for the Lophiiformes and nine outgroup taxa. An asterisk (*) represents a bootstrap (BS) value of 100.

Maximum likelihood analysis of nuclear and mitochondrial genes. The phylogenetic relationships recovered using the second dataset of concatenated nuclear and mitochondrial genes subjected to ML analysis are presented in Figure 10. In this analysis, the Lophiiformes was recovered as monophyletic (BS 100). All five suborders were also recovered as monophyletic, with the Lophioidei diverging first (BS 100) and making up the basal member of the order, the Ogcocephaloidei and Antennarioidei (BS 54) diverged next (BS 100), leaving the Chaunacoidei as sister to the Ceratioidei (BS 100).

Within the Lophioidei, *Sladenia* was recovered as the basal member (BS 100), followed by the divergence of *Lophioides* (BS 100), and then a sister relationship between *Lophiomus* and *Lophius* (BS 100). Within the Antennarioidei, a sister relationship was recovered for the Tetrabrachiidae and Antennariidae (BS 100), and this assemblage made up the sister group to the Brachionichthyidae (BS 100). Within the Antennariidae, the genus *Antennarius* was recovered as basal (BS 100) to a sister relationship recovered between the genera *Antennatus* and *Histrio* (BS 99). Within the Ogcocephaloidei, the genus *Malthopsis* was recovered as the basal member (BS 100), followed by the divergence of *Zalieutes* (BS 23), and then a sister relationship between the genera *Coelophrys* and *Halieutaea* (BS 10).

Within the Ceratioidei, members of the Linophrynidae formed the basal members of this suborder (BS 100). The Caulophrynidae diverged next in a step-wise fashion (BS 97), followed by the divergence (BS 27) of a sister relationship between the families Ceratiidae and Gigantactinidae (BS 25). The Thaumatichthyidae diverged next in step-wise fashion (BS 94), followed by the divergence (BS 32) of a sister relationship between the families Neoceratiidae and Centrophrynidae (BS 33). The crown group was well supported and consisted of a sister relationship between the Diceratiidae and Melanocetidae (BS 100), this assemblage formed the sister group to the Himantolophidae (BS 100), and the Oneirodidae was recovered as the basal lineage (BS 100). Within the Linophrynidae, *Haplophryne* and *Acentrophryne* were recovered in a sister relationship (BS 91), and this assemblage formed the sister to *Linophryne* (BS 100). Within the Oneirodidae, *Oneirodes* was recovered as the basal member (BS 100), followed by the divergence of *Chaenophryne* (BS 100), then the divergence of *Bertella* (BS 63), leaving a sister relationship between *Lasiognathus* and *Puck* (BS 100).

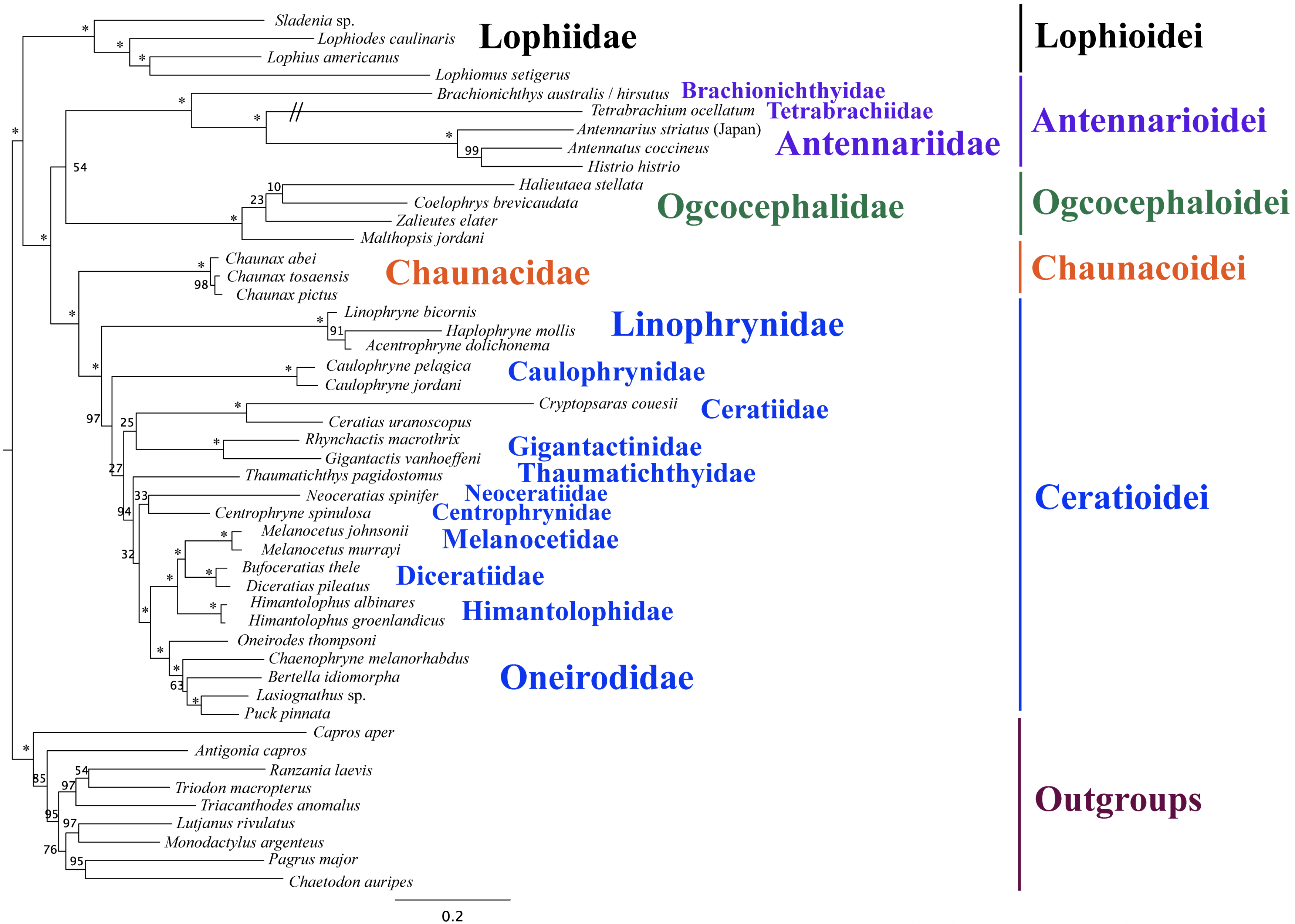

Figure 10. Maximum likelihood analysis of a concatenated set of four nuclear genes (ENC1, Glyt, myh6, and zic1) and 14 mitochondrial genes (12S, 16S, ATPase 6, ATPase 8, COI, COII, COIII, cyt b, ND1, ND2, ND3, ND4, ND5, and ND6) for the Lophiiformes and nine outgroup taxa. An asterisk (*) represents a bootstrap (BS) value of 100.

CHAPTER IV – DISCUSSION

Subordinal relationships of the Lophiiformes. All molecular analyses conducted in this study, in addition to the mitogenome study by Miya et al. (2010), recovered as monophyletic the five suborders of the Lophiiformes proposed by Pietsch (1981) and Pietsch and Grobecker (1987; Figures 3–6). Furthermore, all analyses recovered a basal Lophioidei, containing the goosefishes and monkfishes (Family Lophiidae), and provided no support for the deeply nested position of the Lophiidae proposed by Shedlock et al. (2004; see also Carnevale and Pietsch, 2012).

Results from both the Bayesian and maximum likelihood (ML) analyses, using only the nuclear dataset, recovered the Ogcocephaloidei as sister of the remaining members of the Lophiiformes (Antennarioidei, Chaunacoidei, and Ceratioidei) with strong bootstrap (BS) values and posterior probabilities (Figures 3 and 5). However, in the combined nuclear and mitochondrial DNA dataset, both Bayesian and ML analyses recovered the Ogcocephaloidei as sister to the Antennarioidei, with these two suborders making up the sister group of the Chaunacoidei plus Ceratioidei. In their study based on the mitogenome, Miya et al. (2010) found the best scoring ML tree resulted from the $12_n3_rRT_n$ dataset, which used RY-coding (Phillips and Penny, 2003) to convert the third codon positions into purine (“R”) and pyrimidines (“Y”) to remove possible noise while retaining all positions within the dataset (Miya and Nishida, 2000); the latter under a ML analysis resulted in the Ogcocephaloidei as sister of the Antennarioidei + (Chaunacoidei + Ceratioidei) with a BS value of 100. When combined with the other two datasets (one that included the third codon position and the other that excluded the third codon position), a strict consensus of the three ML trees recovered the Ogcocephaloidei within a polytomy with the Antennarioidei and (Chaunacoidei + Ceratioidei; Miya et al., 2010). Therefore, it appears that noise within the third codon position of the mitochondrial dataset may have swamped out signal from the nuclear and first and second codon positions of the mitochondrial data and erroneously supported the sister relationship, similar to what Miya et al. (2010) may have encountered in their other datasets. As there is high support for a basal Ogcocephaloidei relative to the Antennarioidei, Chaunacoidei, and Ceratioidei in both the nuclear dataset in this study, and the mitochondrial dataset with RY-coding from Miya et al. (2010), the Ogcocephaloidei is here considered to be the sister of the Antennarioidei + (Chaunacoidei + Ceratioidei).

Results from the nuclear dataset, in addition to the combined nuclear and mitochondrial DNA dataset, supported the sister group relationship between the Ceratioidei and Chaunacoidei first proposed by Shedlock et al. (2004; Figure 1B) based on two mitochondrial genes, and supported in the mitogenome study by Miya et al. (2010; Figure 1C), rather than the sister relationship between the Ceratioidei and Ogcocephaloidei proposed by Pietsch (1981) and Pietsch and Grobecker (1987; Figure 1A). In addition, this study corroborated the hypothesis that the ceratioids make up the most phylogenetically derived members of the Lophiiformes (Bertelsen, 1984; Pietsch and Orr, 2007; Pietsch, 1981, 1984a, 2009).

Intergeneric relationships within the Lophioidei. The Lophioidei comprises a single family, the Lophiidae, and 25 extant species distributed among four genera (Table 1). Caruso (1985) used 19 morphological characters to present the first cladogram of lophiid genera (Figure 2A), which recovered *Lophiomus* and *Lophius* as sister groups, this assemblage recovered as sister to *Lophiodes*, and *Sladenia* recovered as the basal member (as corroborated morphologically by Carnevale and Pietsch, 2012). All analyses conducted in this study, in addition to the mitogenome study by Miya et al. (2010), recovered the same phylogenetic relationships with BS values of 100 and PPs of 1.0.

Intergeneric relationships within the Ogcocephaloidei. The Ogcocephaloidei comprises a single family, the Ogcocephalidae, made up of 73 species distributed among 10 genera (Table 1). Endo and Shinohara (1999) analyzed nine of Bradbury's (1967) morphological characters for nine of the 10 genera (Figure 2E), and Miya et al. (2010) included four of the 10 genera in their mitogenomic study. This study included six genera (60.0%) in the nuclear dataset; both analyses recovered a sister relationship between *Coelophrys* and *Malthopsis*, these two made up the sister to *Dibranchus*, and this clade was sister to an assemblage made up of the sister genera *Ogcocephalus* and *Zalieutes*, with *Halieutaea* recovered as the basal lineage of the Ogcocephalidae (Figures 3 and 5). Miya et al. (2010) had high support for *Halieutaea* as the basal lineage, *Malthopsis*, *Coelophrys*, and *Zalieutes* diverged next (BS 89/96/71), but had very low support for a sister relationship between *Coelophrys* and *Zalieutes* (BS 31/28/61). This study also had reasonable support for a clade including *Dibranchus*, *Coelophrys*, and *Malthopsis* (BS 65; PP 0.93), but low support for a sister relationship between *Coelophrys* and *Malthopsis* (BS 39; PP 0.55). While this study and the mitogenome study provided additional evidence that corroborated the cladistic analysis for a primitive placement of *Halieutaea*, and the analyses of the nuclear dataset in this study corroborated the sister relationship between *Ogcocephalus* and *Zalieutes* recovered in the cladistic analysis, more extensive taxon sampling is needed to get a better picture of the intergeneric relationships of the Ogcocephalidae.

Familial and intergeneric relationships of the Antennarioidei. The Antennarioidei is composed of four relatively shallow-water families, the Antennariidae, Brachionichthyidae, Lophichthyidae, and Tetrabrachiidae, of which three were included in this study. Both ML and

Bayesian analyses of the combined nuclear and mitochondrial dataset recovered the Tetrabrachiidae as sister to the Antennariidae, and the Brachionichthyidae as the basal member of the Antennarioidei, which supported both the mitogenome study (Miya et al., 2010) and morphological studies (Pietsch, 1981; Pietsch and Grobecker, 1987; Figures 4 and 6). However, the combined dataset from this study and the mitogenome study (Miya et al., 2010) only included three members of the subfamily Antennariinae (*sensu* Arnold and Pietsch, 2012), while the nuclear dataset in this study included 11 species (+1 undescribed species of *Histiophryne*) of the subfamily Histiophryninae (*sensu* Arnold et al., 2014) and 14 (+1 undescribed species of *Antennatus*) of the subfamily Antennariinae. Both analyses of the nuclear dataset recovered a paraphyletic Antennariidae, with the subfamily Antennariinae sister to a second clade that contained the Brachionichthyidae, Tetrabrachiidae, and the subfamily Histiophryninae (Figures 3 and 6). Within this second clade, the Brachionichthyidae was recovered as sister to the antennariids *Echinophryne*, *Kuiterichthys*, *Phyllophryne*, *Porophryne*, and *Rhycherus*. The genera *Echinophryne* and *Phyllophryne* were recovered as sister groups, *Rhycherus* formed a sister relationship with them, this assemblage formed the sister group to *Porophryne* (as Antennariidae gen et. sp. nov. in Arnold and Pietsch, 2012), and *Kuiterichthys* was the basal antennariid of this clade. The relationships of these five genera are similar to the relationships recovered by Arnold and Pietsch (2012; figs. 5 and 7), except that *Kuiterichthys* and *Porophryne* formed a sister relationship basal to *Echinophryne*, *Phyllophryne*, and *Rhycherus*. In the ML analysis, the Tetrabrachiidae was recovered in a sister relationship with the antennariid genus *Tathicarpus*, and this assemblage formed the sister of the antennariid genera *Histiophryne* and *Lophiocharon*. In the Bayesian analysis, the antennariid genus *Tathicarpus* was recovered as the basal lineage of this clade, with the Tetrabrachiidae recovered as sister to the antennariid genera

Histiophryne and *Lophiocharon*. The sister relationship between *Histiophryne* and *Lophiocharon* was recovered in both analyses of the nuclear dataset as corroborated by Arnold and Pietsch (2012). While the sister relationship between the brachionichthyids and the five genera of antennariids is fairly well supported (BS 89; PP 1.00), the sister relationship recovered in the ML analysis between *Tetrabrachium* and *Tathicarpus*, with those genera sister to the antennariids *Histiophryne* and *Lophiocharon*, are not (BS of 48 and 34, respectively). In addition, the relationships recovered in the Bayesian analysis between (*Histiophryne* + *Lophiocharon*) + *Tetrabrachium* (PP 0.74), with *Tathicarpus* as the basal lineage (PP 0.56), are also not well supported. In their analysis of the 16S rRNA sequences, Shedlock et al. (2004) noted that sequence divergences between *Tathicarpus* and both *Antennarius* and *Histrio* were on the same order or greater than those found between members of recognized families in different suborders. In the key of Pietsch and Grobecker (1987), *Tathicarpus* is entered as the most derived genus in the key to the known genera of the Antennariidae due to its distinct morphology relative to other frogfishes. It is evident that more data, possibly faster evolving nuclear loci, are required to more fully assess the relationships within this second largest clade that includes members of the Histiophryninae, Brachionichthyidae, and Tetrabrachiidae.

Members of the Antennariinae have both the mesopterygoid and epural, have double scroll-shaped ovaries (Pietsch and Grobecker, 1987:pl. 10, fig. 161), are broadcast spawners with a distinct larval stage, and have a broad geographic range, with all genera found circumglobally throughout the tropics and subtropics. As defined by Arnold et al. (2014), members of the Histiophryninae have lost the mesopterygoid and epural, have simple oval-shaped ovaries, undergo direct development and display various degrees of parental care, and are restricted geographically to the Indo-Australian Archipelago. Members of the Brachionichthyidae and

Tetrabrachiidae have also lost the epural and mesopterygoid (Pietsch 1981, 1984a), have simple, oval-shaped ovaries, and undergo direct development and display various degrees of parental care (Pietsch and Grobecker, 1987; Bruce et al., 1997, 1999; Pietsch et al., 2009b), and are restricted geographically to the Indo-Australian Archipelago, lending further evidence for a monophyletic assemblage that includes the Brachionichthyidae, Histiophryninae, and Tetrabrachiidae. In addition, *Lophichthys boschmai*, the only member of the Lophichthyidae, not included in this study or the study by Miya et al. (2010) due to the absence of tissues suitable for genetic analyses, has lost the mesoptergygoid and has a greatly reduced or absent epural (Pietsch, 1981, 1984a), has simple, oval-shaped ovaries, and is endemic to the seas between New Guinea and Australia (reproductive modes and behaviors are unknown), and is, therefore, a likely member of this clade as well. While the sister relationship between the antennariids *Histiophryne* + *Lophiocharon* and the tetrabrachiid *Tetrabrachium* is not highly supported (0.74 PP) in the Bayesian analysis, these three genera (and likely the tetrabrachiid *Dibrachichthys*, not included in this study and for which no reproductive behaviors are known) are the only known antennarioids to carry their eggs in some fashion (e.g., within a pocket formed by the dorsal, anal, and caudal fins in *Histiophryne* (Pietsch and Grobecker, 1987; Pietsch et al., 2009a); attached to the side of the body in *Lophiocharon* (Pietsch and Grobecker, 1980, 1987); and attached to the dorsal-fin spines in *Tetrabrachium* (Pietsch and Grobecker, 1987; Pietsch et al., 2009), and all are endemic to the tropical and subtropical waters of the Indo-Australian Archipelago, north of approximately 35˚ S latitude (an exception to this is the genus *Histiophryne*, which has members that occur in tropical, subtropical, and temperate zones within the Indo-Australian Archipelago). All members of the second major lineage comprised of the remaining members of the subfamily Histiophryninae and the family Brachionichthyidae appear to guard nests of demersal eggs and occur in temperate waters along the southern coast of Australia, including Tasmania, south of approximately 30˚ S latitude.

Intergeneric relationships within the Chaunacoidei. The Chaunacoidei is composed of only one family, the Chaunacidae, and 16 nominal species distributed between two genera (Caruso, 1989; Caruso et al., 2006). Both Bayesian and ML analyses of the nuclear dataset recovered *Chaunacops* as the primitive sister group to a monophyletic assemblage that included two species plus one unidentified specimen of *Chaunax* with high support (BS 100, PP 1.00; Figures 3 and 6). This is the first study that included *Chaunacops* in a phylogenetic hypothesis. As *Chaunacops* is distinguished from *Chaunax* by a number of morphological characters (e.g. coarsely spinulose skin, a deep notch in the postmaxillary process of the premaxilla, longer anteroventral process of the articular bone, and greater intersphenotic width; Caruso, 1989), this hypothesis may lend insight into the polarity of some of the morphological characters of this group.

Familial relationships of the Ceratioidei. The Ceratioidei, commonly known as the deep-sea anglerfishes, is composed of 11 families (Pietsch, 2009a), all of which were included in this study. The relationships recovered from all four analyses are summarized below in 50% majority rule ML and Bayesian consensus trees (Figure 11).

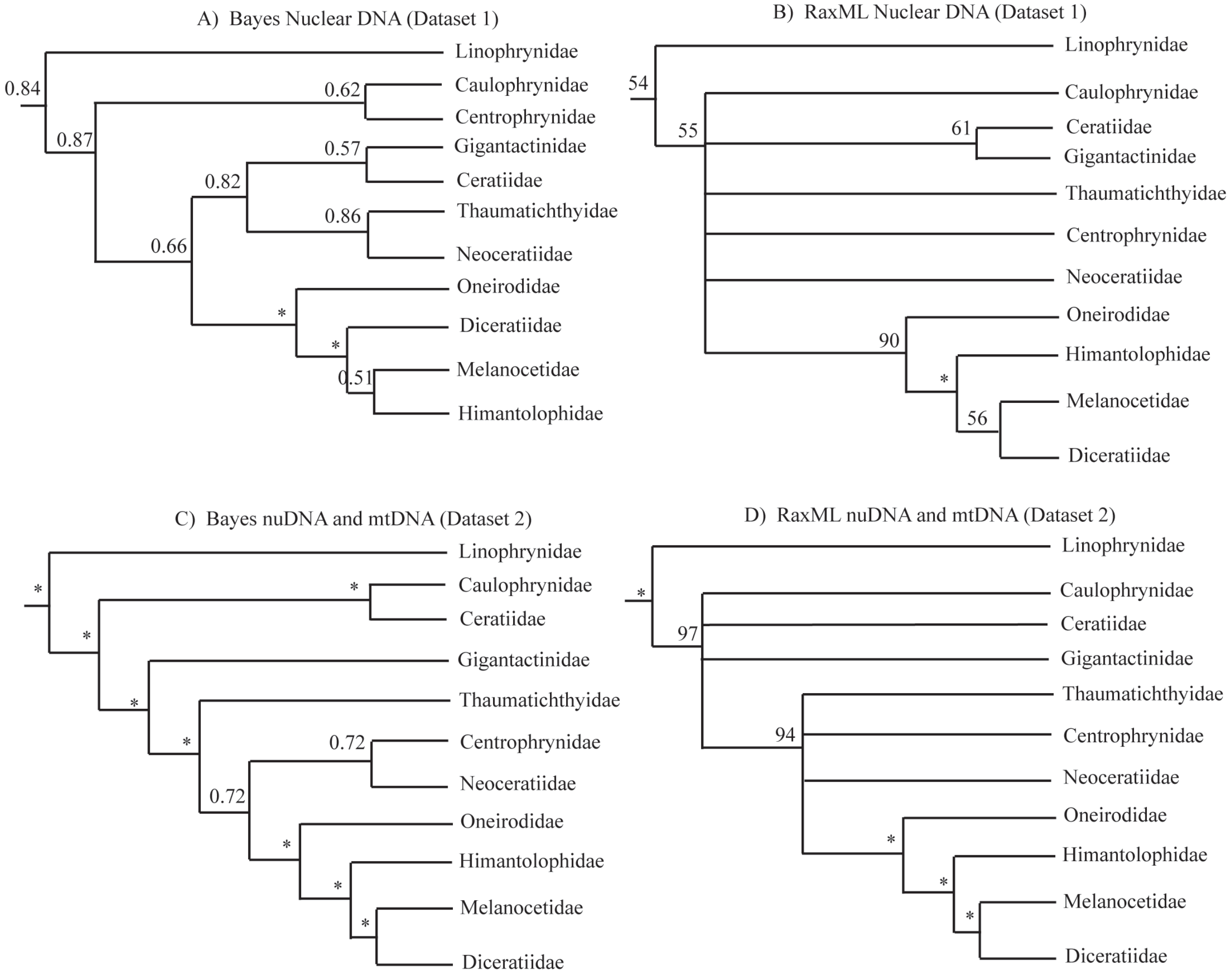

Figure 11. 50% majority rule consensus trees of the Ceratioidei from all four data analyses conducted in this study: A) relationships recovered using four nuclear genes (ENC1, Glyt, myh6, and zic1) using Bayesian analysis; B) relationships recovered using the same four nuclear genes using maximum likelihood analysis; C) relationships recovered using the same nuclear genes concatenated with 14 mitochondrial genes generated by Miya et al. (2010); and D) relationships recovered using the same concatenated set of nuclear and mitochondrial genes using maximum likelihood analysis. An asterisk (*) represents a posterior probability (PP) of 1.0 or a bootstrap (BS) value of 100.

All molecular analyses recovered a monophyletic Ceratioidei that included the Linophrynidae as the most basal lineage (Figures 7–11) in stark contrast to the morphological studies of Bertelsen (1951, 1984) and Pietsch and Orr (2007) and the mitogenome study by Miya et al. (2010; Figure 12). Despite the potential pitfall of the mitochondrial DNA swamping out signal from the nuclear DNA in the combined dataset, linophrynids were still recovered as the basal member of the Ceratioidei with a BS of 100 and PP of 1.0 (Figures 8, 10, and 11). In the strict consensus tree of the mitogenome study (Miya et al., 2010: fig. 6), the Caulophrynidae and Ceratiidae were recovered in a polytomy that included a clade composed of the remaining members of the Ceratioidei; however, this clade was poorly supported, with bootstraps of 59, 24, and 32 for each mitochondrial dataset. The next clade, which placed linophrynids as sister to Neoceratiidae, Centrophrynidae, Oneirodidae, Himantolophidae, Melanocetidae, and Diceratiidae, was also poorly supported, with bootstraps of 45, 19, and 49. Even the next clade, which recovered the sister families Neoceratiidae and Centrophrynidae (a relationship also poorly supported in two of the three datasets, with BS values of 58, 51, and 83) as sister to the Oneirodidae, Himantolophidae, Melanocetidae, and Diceratiidae, is poorly supported, with BS values of only 42, 44, and 62. Extremely low bootstrap support values may correspond to clades that are not repeatable or accurate (Felsenstein, 1985; Hillis et al., 1993), therefore the tree is redrawn here (Figure 12) to a standard 50% majority rule tree from the best scoring ML tree derived from the $12_n3_rRT_n$ dataset preferred by Miya et al. (2010).

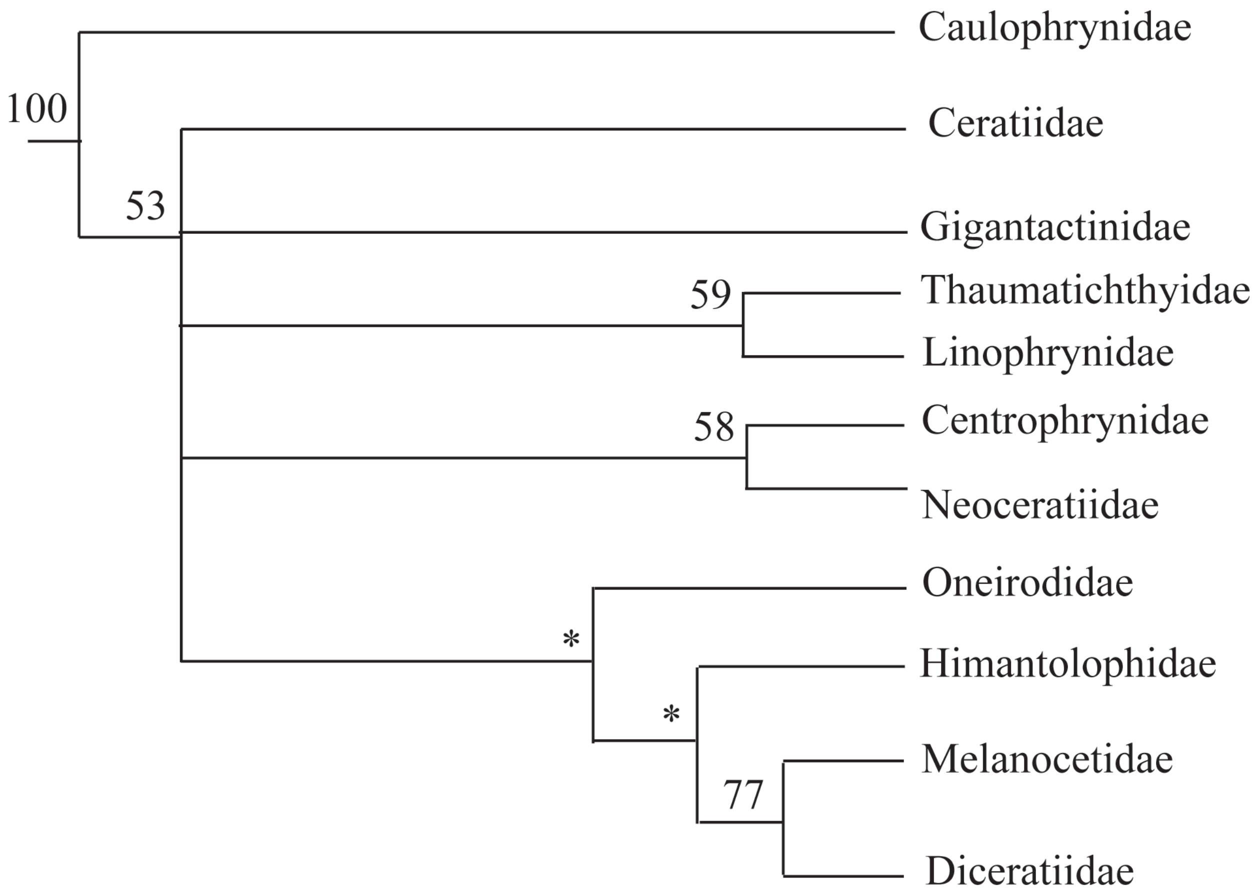

Figure 12. Redrawn 50% majority rule tree from the best-scoring maximum likelihood (ML) tree derived from the $12_n3_rRT_n$ dataset preferred by Miya et al. (2010). An asterisk (*) represents a bootstrap (BS) value of 100.

The result from this redrawn tree is a highly unresolved Ceratioidei; the only clade with appreciable support is the suborder itself (BS 100), and the clade that included the Oneirodidae, Himantolophidae, Melanocetidae, and Diceratiidae, and the relationships within it (BS values of 100 except for the sister relationship between the Melanocetidae and Diceratiidae, with a BS value of 77; Figure 12). The sister relationship of the Melanocetidae and Diceratiidae, with the primitive sister relationship of the Himantolophidae, and the Oneirodidae as the basal lineage of this clade, was fully congruent with the analyses in this study (the single exception to this is the nuclear dataset that was subjected to Bayesian analysis, in which the himantolophids and melanocetids formed a sister group to the diceratiids, but this is not well supported, with a PP of only 0.51). Based on osteological evidence, Regan and Trewavas (1932) also thought that the Diceratiidae, Himantolophidae, and Melanocetidae were closely related families, with the Oneirodidae being somewhat more closely related to the Diceratiidae, but that this assemblage would be placed first, and the Photocorynidae and Linophrynidae [the former since synonymized with the latter; Bertelsen, 1951] would be placed at the end of the series. In fact, both Bertelsen (1984) and Pietsch and Orr (2007) recovered the Linophrynidae at or near the crown of the Ceratioidei. Linophrynids are unique among deep-sea anglerfishes in having the largest mouths and teeth, a complex bioluminescent chin barbel, and a sinistral anus (hence the common name for the group, Leftvent Seadevils; Pietsch, 2009). Interestingly, however, Shedlock et al. (2004; fig. 4) analyzed the 16S rRNA and cytochrome b mitochondrial genes for six of the 11 families of ceratioids and also recovered linophrynids as basal. Therefore based on the molecular evidence provided by concatenated nuclear and mitochondrial genes, linophrynids would have diverged early in the evolution of the ceratioids and gained their autapomorphic characters as other lineages of ceratioids diverged.

Discrepancies in the Ceratioidei between datasets. In comparing the nuclear and combined datasets, Bayesian and ML results were fully congruent with each other for the Ceratioidei in the 50% majority rule trees (Figure 11). However, the relationships recovered for the Ceratioidei in the nuclear dataset were not fully congruent with those of the combined dataset (Figure 11). In fact, the relationships recovered within the Ceratioidei, with the exception of the linophrynids as the basal member and the monophyletic assemblage comprised of Oneirodidae + (Himantolophidae + (Diceratiidae + Melanocetidae)), bear little resemblance to one another between datasets. It is likely that the mitochondrial DNA swamped out the signal from the nuclear DNA (14,250 vs 3,300 bps).

Estimation of species trees using ML on concatenated data can be statistically inconsistent when substantial incomplete lineage sorting results from short internal branches in the species tree (such as those within the Ceratioidei), and under these conditions the use of a bootstrap to measure support for the inferred phylogeny can result in moderate to strong support for an incorrect tree (Kubatko and Degnan, 2007). Degnan and Rosenberg (2006, 2009) also found that for any species-tree topology that included five or more species, the most likely gene tree topology to evolve along the branches of a species tree would be different from the species phylogeny and, therefore, using the most frequently observed gene tree topology is guaranteed to produce an incorrect estimate. Therefore, the relationships within the Ceratioidei, save for the monophyletic assemblage of the Oneirodidae, Himantolophidae, Melanocetidae, and Diceratiidae, are here considered questionable until a coalescent approach can be taken to mitigate these problems (see *Concatenation versus the Coalescent*, below).

Phylogenetic relationships within the* Antennarius striatus *species group. Differences in color, color pattern, filamentous appendages on the body, and variation in the number of wormlike appendages of the esca have long made the taxonomic history of the *Antennarius striatus* group complex and problematic. In 1987, however, Pietsch and Grobecker synonymized 28 nominal forms with a single species, *Antennarius striatus*, in the absence of any other significant differences except for geographic variation in escal morphology. Arnold and Pietsch (2012), in their analysis of two mitochondrial genes and one nuclear gene, recovered three distinct clades for *A. striatus sensu* Pietsch and Grobecker (1987) among individuals originating from Kōchi Prefecture in Japan, Western Australia, and the Gulf of Mexico. In this study, additional individuals from Indonesia and the Eastern Atlantic grouped with the specimen originating from Western Australia, and the individuals from the Kōchi Prefecture and the Gulf of Mexico were again recovered as two distinct lineages (Figures 7 and 9). In this study and the study by Arnold and Pietsch (2012), *A. striatus* from the Gulf of Mexico was basal to *A. hispidus* and all other clades of *A. striatus*. Based on examination of 642 specimens, Pietsch and Grobecker (1987) recognized distinct variation in pectoral-fin ray counts and escal morphology within *A. striatus*: nearly all (99%) Western Atlantic individuals had 11 or 12 pectoral-fin rays and bifid escae; 95% of individuals from the coastal waters of the eastern Atlantic, Australia, and the Hawaiian Islands had 9 or 10 pectoral-fin rays and most had trifid escae; and in Japan most individuals (88%) had 11 or 12 pectoral-fin rays and all but one of the 110 specimens examined had trifid escae. The addition of genetic distinction and phylogenetic placement to the corresponding morphological differences recognized by Pietsch and Grobecker (1987) is considerable evidence for these three clades to be recognized as distinct species. Therefore, *A. scaber* (Cuvier, 1817) is hereby recognized for western Atlantic specimens with bifid escae, and *A. tridens* (Temminck and Schlegel, 1845) for those individuals originating from Japan with trifid escae. It is also possible that other cryptic species exist within this complex, such as represented by the two specimens from China (BMNH 1884.2.26.36 and SU 27997) and one from Formosa (USNM 201570) that were recognized as morphologically distinct by Pietsch and Grobecker (1987). However, further analysis of this complex is postponed until additional material and tissue is available.

In both analyses of the nuclear dataset, this study recovered a sister relationship between *A. hispidus* and the individuals of *A. striatus* from the eastern Atlantic, Indonesia, and Western Australia. However, Arnold and Pietsch (2012) recovered *A. hispidus* as sister to the individuals originating from both Kōchi Prefecture and Western Australia. As more data (both in number of genes and number of specimens) were analyzed in this study, the relationships recovered in this study are considered a better hypothesis of the relationships of the *A. striatus* group. *Antennarius indicus*, as first proposed by Arnold and Pietsch (2012), was recovered as the basal member of the *A. striatus* species group in both analyses of the nuclear dataset (BS 100, PP 1.00).

Lasiognathus *within the Oneirodidae.* Originally placed in a family of its own, the Thaumatichthyidae Smith and Radcliffe 1912, *Thaumatichthys* was included along with *Lasiognathus* within the Oneirodidae by Regan (1925, 1926), followed by Regan and Trewavas (1932), Bertelsen (1951), and Maul (1961, 1962). Pietsch (1972) resurrected the Thaumatichthyidae to include both *Thaumatichthys* and *Lasiognathus* (using the premaxillae extending anteriorly far beyond lower jaw and enlarged dermal denticles associated with the esca to diagnose the Thaumatichthyidae) and Pietsch and Orr (2007) supported the placement of *Lasiognathus* within the Thaumatichthyidae. Bertelsen and Struhsaker (1977) compared the osteology of *Lasiognathus* and considered it to be a subjective choice to include it within the Oneirodidae or the Thaumatichthyidae, or each in a family of their own, but chose to retain the Thaumaticthyidae in the enlarged sense as proposed by Pietsch (1972); they also added the following character to diagnose the Thaumatichthyidae: dorsal portion of opercle divided into 2 or more branches. Using mitogenomes, Miya et al. (2010) recovered *Lasiognathus* within the Oneirodidae. In both analyses and datasets in this study, *Lasiognathus* was recovered nested within the Oneirodidae with high support (BS 100 and PP 1.00 for both datasets), and so it is here considered a member of the Oneirodidae rather than the Thaumatichthyidae.

Concatenation versus the Coalescent. The phylogenetic tree derived from sequences from a single locus, known as the gene tree, has been studied for decades, while research on the phylogeny of species using molecular data remains in its early stages (Edwards, 2009; Liu et al., 2008, 2009). Each gene tree may conflict both in terms of branch length and topology due to different biological phenomena, including incomplete lineage sorting. Incomplete lineage sorting occurs when ancestral polymorphisms are not fully resolved into two monophyletic lineages when a second speciation event occurs, resulting in alleles becoming randomly fixed in the descendants, and can occur at both shallow and deep phylogenetic levels. In the case of short branch lengths, multiple gene lineages tend to persist into deeper portions of the species tree, allowing coalescences to occur between lineages that are not from the most closely related species; incomplete lineage sorting then becomes more probable in shorter branch lengths because lineages do not necessarily ‘sort’ by species when they are coalescing (Degnan and Rosenberg, 2009). Concatenation of the data can also result in moderate to strong support for an incorrect tree when substantial incomplete lineage sorting results from short internal branches in the species tree (Kubatko and Degnan, 2007). In the case of the Lophiiformes, short internal branches have been recovered within the Ceratioidei (Miya et al., 2010), making this group especially problematic when using both a single locus (the mitogenome) and concatenation. Therefore, an alternative approach to concatenation is using a coalescent methodology, which takes incomplete lineage sorting into account and treats the likelihood of the coalescent history on each lineage independently. However, Maddison and Knowles (2006) found that for shallower species trees, sampling more individuals had better results than sampling more loci. As ceratioids are still rare in ichthyological collections, and a single individual still only represents most groups in this study, analyzing the data using a coalescent approach is postponed until more samples can be obtained.

CHAPTER V – CONCLUSIONS

Monophyly and subordinal relationships of the Lophiiformes. The monophyly and primitive placement of the Lophiidae within the Lophiiformes seems clear; all analyses to date, whether morphological or molecular, including this study, have recovered a monophyletic Lophiiformes, with the Lophiidae recovered as the basal lineage (with the sole exception of Shedlock et al., 2004). The results from this study suggest that the Ogcocephaloidei diverged next, and this assemblage makes up the primitive sister group of the antennarioids, chaunacoids, and ceratioids. The results of this study and all molecular analyses to date have recovered the Chaunacoidei as the sister group of the Ceratioidei; therefore, adding additional molecular data is unlikely to support a sister group relationship between the Ogcocephaloidei and Ceratioidei as proposed by Pietsch (1981) and Pietsch and Grobecker (1987).

Revised classification of the Lophiiformes.

Order Lophiiformes
  Suborder Lophioidei
    Family Lophiidae Rafinesque, 1810
  Suborder Ogcocephaloidei
    Family Ogcocephalidae Gill, 1893
  Suborder Antennarioidei
    Family Antennariidae Gill, 1863

Suborder Chaunacoidei

- Family Chaunacidae Gill, 1863

Suborder Ceratioidei

- Family Linophrynidae Regan, 1925
- Family Caulophrynidae Goode and Bean, 1896
- Family Centrophrynidae Bertelsen, 1951
- Family Neoceratiidae Regan, 1926
- Family Ceratiidae Gill, 1861
- Family Gigantactinidae Boulenger, 1904a
- Family Thaumatichthyidae Smith and Radcliffe, 1912
- Family Himantolophidae Gill, 1861
- Family Diceratiidae Regan and Trewavas, 1932
- Family Melanocetidae Gill, 1879b
- Family Oneirodidae Gill, 1879a

Intrarelationships of the Lophioidei, Ogcocephaloidei, and Chaunacoidei. The results of this study corroborated previous molecular and morphological hypotheses for the evolutionary relationships of the Lophioidei. In the case of the Ogcocephaloidei, *Halieutaea* was recovered as the primitive member of the group. Morphological and molecular data supported a sister group relationship between *Zalieutes* and *Ogcocephalus*. However, more taxon sampling is needed to assess evolutionary relationships within the Ogcocephaloidei. Within the Chaunacoidei, *Chaunacops* was included for the first time in a phylogenetic hypothesis and was recovered as the primitive sister group of *Chaunax*.

Intrarelationships of the Antennarioidei. It is apparent that additional information is needed regarding the relationships within the Antennarioidei; however, it is also clear, based on the present research, that the Antennariidae is paraphyletic, and that the Brachionichthyidae and Tetrabrachiidae formed a closer relationship with members of the Histiophryninae than did members of the Antennariinae. Therefore, members of the Brachionichthyidae and Tetrabrachiidae are here included within the Antennariidae and are considered members of the Histiophryninae. In addition, it is likely that the sole species of the Lophichthyidae, *Lophichthys boschmai*, based on previously mentioned morphological characters (see Discussion), is more closely related to members of the Histiophryninae, and therefore it is tentatively included as a member of the Histiophryninae as well.

Revised classification of the Antennariidae.

- Antennariidae Gill, 1863
  - Antennariinae Arnold and Pietsch, 2012
    - *Antennatus* Schultz, 1957
    - *Antennarius* Daudin, 1816
    - *Histrio* Fischer, 1813
    - *Fowlerichthys* Barbour, 1941
    - *Nudiantennarius* Schultz, 1957
  - Histiophryninae Arnold, Harcourt, and Pietsch, 2014
    - *Echinophryne* McCulloch and Waite, 1918
    - *Phyllophryne* Pietsch, 1984b

Rhycherus Ogilby, 1907

Kuiterichthys Pietsch, 1984b

Porophryne Arnold, Harcourt, and Pietsch, 2014

Allenichthys Pietsch, 1984b

Lophiocharon Whitley, 1933

Histiophryne Gill, 1863

Tathicarpus Ogilby, 1907

Brachionichthys Bleeker, 1855

Brachiopsilus Last and Gledhill, 2009

Pezichthys Last and Gledhill, 2009

Sympterichthys Gill, 1878

Thymichthys Last and Gledhill, 2009

Dibrachichthys Pietsch, Johnson, and Arnold, 2009

Tetrabrachium Günther, 1880

Lophichthys Boeseman, 1964

Phylogenetic relationships of the Ceratioidei. The preferred tree is the topology recovered by the Bayesian and ML analyses of the nuclear dataset (Figures 7 and 9), as it contains greater taxon sampling and the signal is not swamped out by mitochondrial data. Both the nuclear and nuclear + mitochondrial datasets from this study recovered a basal Linophrynidae and a monophyletic assemblage that included the Oneirodidae, Himantolophidae, Melanocetidae, and Diceratiidae. However, placement of the other families of the Ceratioidei remains problematic, perhaps due in part to the concatenation of nuclear and mitochondrial loci; therefore, the remaining relationships are considered questionable and placement of them is postponed until further analysis using a coalescent approach can be undertaken to mitigate problems of incomplete lineage sorting.

Overall conclusions. The goal of this study was to recover the subordinal and familial relationships of the Lophiiformes as inferred from nuclear DNA and to compare the resulting phylogeny with previous hypotheses. Mitochondrial DNA is inherited as a single unit and therefore represents only one independent estimate of the species tree, but each nuclear gene tree represents an independent estimate of the tree. While the results from Miya et al. (2010) are based on a single independent estimate (the mitogenome), the results from the combined dataset in this study are based on five independent estimates.

A major goal of this study was to use nuclear DNA to test whether the Ogcocephalidae or Chaunacidae (or neither) is the sister of the Ceratioidei, which this study did with high confidence. While this goal was accomplished, some familial relationships, especially within the Antennarioidei and Ceratioidei, are still problematic and require further research. Because there are short branches within the Ceratioidei, faster evolving nuclear DNA will likely help recover these rapid speciation events. In addition, using a coalescent approach will likely help mitigate the problems encountered in this study for resolving the relationships within both the Antennarioidei and Ceratioidei. The results of this study highlight the need for independent lines of evidence to be considered when attempting to reconstruct phylogenetic relationships.

Further studies. Lophiiforms are very rare in the fossil record, with all of the recorded ages falling in the Cenozoic from 7.6 to 40 MYBP (million years before present). Only three lophiids (Family Lophiidae, Eocene), two handfishes (Family Brachionichthyidae, Eocene), one batfish (Family Ogocephalidae, Eocene), two frogfishes (Family Antennariidae, Eocene and Upper

Miocene), and six ceratioids (Family Linophrynidae and Oneirodidae, Upper Miocene) are known from the fossil record (Bannikov, 2004; Carnevale and Pietsch, 2006; Carnevale et al., 2008; Carnevale and Pietsch, 2009a, b, 2010a, b; Pietsch and Carnevale, 2011; Carnevale and Pietsch, 2012). Using molecular estimation of divergence times, Miya et al. (2010) offered a novel hypothesis of the evolutionary history of the Lophiiformes that could not be inferred from the fossil data. Assuming a sister group relationship of the Tetraodontiformes and Lophiiformes and using a minimum time constraint based on a fossil tetraodontid from the mid Cretaceous, in addition to 30 other time constraints, Miya et al. (2010) estimated the Lophiiformes to have diverged from an ancestral lineage of Tetraodontiformes 157 MYBP (145–172 MYBP; 95% credible interval). The five subordinal lineages diverged and occupied various marine habitats in a relatively short time interval of 18 MYBP, between 117 and 135 MYBP, the period roughly corresponding to the beginning of the Gondwanian fragmentation (Miya et al., 2010). However, Near et al. (2012), using nine nuclear genes, found that whole mitochondrial genomes dramatically overestimated ages for derived teleost lineages. Without using fossil calibrations for these younger lineages, their estimates for the Lophiiformes and other derived teleost lineages were much closer to the fossil age estimates, with the Lophiiformes estimated to have diverged 68.1 MYBP. A future study using the nuclear dataset from this study will be to estimate divergence times and compare the results with those recovered by Miya et al. (2010) and Near et al. (2012). Similarly, diversification rates and ancestral state reconstructions using the nuclear dataset in this study can be estimated and compared to those proposed by Miya et al. (2010) and Pietsch and Orr (2007).

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