Examining evolutionary relationships and shifts in depth preferences in batfishes (Lophiiformes: Ogcocephalidae)

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ABSTRACT

Batfishes (Ogcocephalidae) are an understudied, group of marine anglerfishes that are dorsoventrally flattened and have an illicium and esca (terminal lure) used to attract prey. The family contains 10 genera and 75 recognized species from nearly all tropical and subtropical seas. Relationships among these taxa, as well as the position of Ogcocephalidae within Lophiiformes, remain poorly understood with previous studies showing conflicting, and poorly resolved results. The timing of divergence and depth of origination in the water column have also not been explored in any detail. In this study a concatenated nuclear (three genes) and mitochondrial (two genes) dataset was constructed across several anglerfish families to elucidate phylogenetic relationships among all ten batfish genera, to clarify the placement of Ogcocephaloidei within Lophiiformes, and to estimate divergence times using fossil calibrations. An ancestral state reconstruction was also conducted to examine the history of shifts in preferred habitat depths within batfishes. Phylogenetic analyses supported monophyly of each sub-order within Lophiiformes and placed Ogcocephaloidei as the sister group to Antennarioidei. Batfish genera were divided into an Eastern Pacific/Western Atlantic clade and an Indo-Pacific clade; *Halieutaea* was recovered as the sister group to all other batfishes. Based on divergence time estimations and ancestral state reconstructions of preferred depth, Ogcocephalidae is Eocene in age and originated on the lower continental shelf/upper continental slope (disphotic zone).

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1. Introduction

Anglerfishes (Order Lophiiformes) include the dorsoventrally flattened batfishes (in the suborder Ogcocephaloidei, all in the family Ogcocephalidae), and 17 other families distributed among four other suborders (Pietsch, 1984): Antennarioidei (frogfishes and handfishes – four families), Ceratioidei (deep sea anglerfishes – 11 families), Chaunacoidei (sea toads and coffinfishes – one family), and Lophioidei (monkfishes and goosefishes – one family). Within the order there are currently 360 described species (Eschmeyer and Fong, 2014) unified by a modified first dorsal fin (illicium) that is used as a luring apparatus in feeding (Pietsch, 2005). Most phylogenetic studies incorporating members of Lophiiformes have focused on the group as a whole or the deep-sea Ceratioidei (Pietsch, 1981; Pietsch and Grobecker, 1987; Pietsch and Orr, 2007; Shedlock et al., 2004; Miya et al., 2010), but few have focused specifically on the evolutionary relationships within batfishes.

Batfishes occur in almost all tropical and subtropical marine waters, and inhabit shallow to deep-water benthic habitats. Most species have dorsoventrally flattened, discoid bodies and use their pelvic and pectoral fins to "stand" on the bottom of the sea floor (Bradbury, 2003). In contrast to many anglerfishes, which are globular, loose-skinned deep-sea forms using bioluminescence, batfishes are hard bodied, benthic forms mostly found in shallow and moderately deep waters (Ho et al., 2010). Batfishes are small to medium sized and characterized by a short illicium containing only two bony elements – first pterygiophore supporting first dorsal spine (as opposed to three bony elements in other lophiiform families – first pterygiophore supporting two spines), a small pore on the dorsal surface of the esca, well-developed scales (tubercles and bucklers), and horizontal pectoral fins (Bradbury, 1967). Most batfishes retract their illicium into an illicial cavity, where the illicium and esca are housed entirely. The esca (fleshy organ at the end of the illicium) is believed to be glandular, and there is some evidence of batfishes using chemicals to attract their prey

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(Nagareda and Shenker, 2009) as opposed to the bioluminescent lure used by deep-sea anglerfishes (Pietsch, 2005), or the "worm/larvae" mimic escas of frogfishes (Pietsch and Grobecker, 1987).

There are currently 75 valid species within Ogcocephalidae organized into 10 genera, viz., *Coelophrys* (7 spp.), *Dibranchus* (13 spp.), *Halicmetus* (3 spp.), *Halieutaea* (9 spp.), *Halieutichthys* (4 spp.), *Halieutopsis* (9 spp.), *Malthopsis* (13 spp.), *Ogcocephalus* (12 spp.), *Solocisquama* (3 spp.), and *Zalieutes* (2 spp.), making it the second most speciose sub-order within Lophiiformes (Eschmeyer and Fong, 2014). Illicial skeleton and escal morphology have been useful in delineating these genera (Bradbury, 1967); however, relationships among the genera are poorly studied and have only been investigated using morphological data (Endo and Shinohara, 1999; Ho, 2010).

The phylogenetic position of Ogcocephaloidei within Lophiiformes is unresolved with incongruent hypotheses suggested by molecular (Miya et al., 2010; Shedlock et al., 2004) and morphological data (Pietsch and Grobecker, 1987; Pietsch and Orr, 2007). Both molecular studies have found a sister relationship between Chaunacoidei and Ceratioidei; however, Miya et al. (2010) recovers Ogcocephaloidei (as represented by four taxa) as the sister group to an Antennarioidei + (Chaunacoidei + Ceratioidei) clade and Shedlock et al. (2004) recovers Ogcocephaloidei (as represented by two taxa) as the sister group to a Lophioidei + (Chaunacoidei + Ceratioidei) clade. Notably, both of these studies use only mitochondrial data. The morphological studies of Pietsch and Grobecker (1987) and Pietsch and Orr (2007) both recover Ogcocephaloidei as the sister lineage of Ceratioidei.

The first attempt at determining relationships among batfishes by Endo and Shinohara (1999) used morphological characters and included nine of the ten batfish genera. The tree was poorly resolved and recovered a polytomy between *Halieutaea*, *Halieutichthys* and the remaining batfish genera (that they sampled) in a single clade. The relationships within the clade of remaining genera included *Halieutopsis* and *Coelophrys* as sister taxa (Fig. 1a). The only other study that has focused on relationships among batfish genera, by Ho (2010), used morphological and biogeographical evidence to hypothesize relationships among all ten genera (Fig 1b). Ho (2010) recovered a poorly resolved comb-like phylogeny that was very different from Endo and Shinohara (1999) and recovered *Coelophrys* as the sister group to all other batfishes, followed by *Halieutopsis*, and with *Halieutichthys* in a derived position. The conflicting results of the aforementioned studies highlight the need for further analyses of this group.

Furthermore, the age of Ogcocephalids, and where they may have originated (deep water vs. shallow water), is also unclear. As a family, they have one of the greatest vertical spans of all of the anglerfishes (Fishnet2, 2014; Ho, 2010). Estimating divergence times and examining their depth history will be important in understanding the evolutionary significance of habitat shifts between these fishes.

Here we elucidate generic relationships among all ten batfish genera using mitochondrial and nuclear markers. Our taxonomic sampling also allows us to examine the placement of Ogcocephaloidei within Lophiiformes, and includes fossil calibrations to determine the timing of evolutionary events in this group. Using these data we then examine the evolutionary history of shifts in habitat depth exhibited by batfishes. By comparing our results with previous phylogenetic hypotheses for batfishes, we are able to gain a more complete view of evolution in these enigmatic taxa.

2. Methods

2.1. Taxon sampling and DNA extraction

All ten Ogcocephalid genera are represented in this study (~13/75 species) as well as all five suborders within Lophiiformes (6/18 families) for a total of 22 ingroup taxa, 24 Lophiiform outgroups, and six additional outgroups. The additional outgroups were selected based on results from Yamanoue et al. (2007) and Near et al. (2013) and include members of Tetraodontiformes and Perciformes. Tissues were obtained from tissue loans or collected by the authors. Samples were extracted using fin clips and tissue samples preserved in ethanol following the protocol outlined in the D'Neasy Qiagen extraction kit (Hilden, Germany). GenBank accession numbers for all sequences used in this study can be found in Table 1.

2.2. Genes, alignment, and sequencing

Two mitochondrial genes, 16S (ribosomal RNA) and CO1 (Cytochrome oxidase c sub-unit 1), and three protein coding nuclear genes, GLYT (Glycosyltransferase), PTR (hypothetical protein LOC564097), and SH3PX3 (SH3 and PX domain containing 3 genes) were selected for consistency with other studies and because of their usefulness in phylogenetic inference in fishes (Li et al., 2007). Primer pairs used in the reactions are as follows: 16S – 16Sar-L, 16Sbr-H (Palumbi, 1996); CO1 – BOL-F1 5′, BOL-R1 5′ (Ward et al., 2005); GLYT – Glyt_F559, Glyt_R1562; PTR – Ptr_F458, Ptr_R1248; SH3PX3 – SH3PX3_F461, SH3PX3_R1303 (Li et al., 2007).

Genes were amplified by Polymerase Chain Reactions (PCR) using a standard protocol of 94 °C for 3:00 (Initial denaturation);
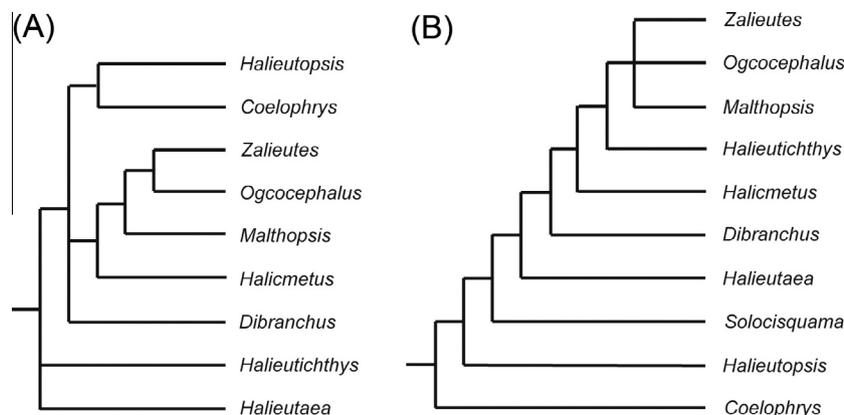

Fig. 1. Phylogenetic hypotheses for Ogcocephalidae interrelationships based on previous morphological studies. (A) Relationships hypothesized by Endo and Shinohara (1999) using morphology and nine of ten batfish genera. (B) Relationships hypothesized by Ho (2010) using morphology and biogeographic characters and all batfish genera.

Table 1
List of individuals used with museum vouchers and GenBank accession numbers.

Classification	Species	Museum voucher	16S	CO1	GLYT	PTR	SH3PX3
Order Lophiiformes							
Sub-Order Antennarioidei							
Family Antennariidae	*Antennarius sp.*	NMMB-P uncat.	KP201876	KP201925	N/A	KP202056	KP201966
	Antennarius striatus	ASIZP 62545	KP201877	KP201947	N/A	KP202057	KP201967
	Fowlerichthys radiosus	LSUMZ 13631	KP201874	KP201948	N/A	KP202062	KP201965
	Fowlerichthys radiosus	LSUMZ 14257	KP201875	KP201932	N/A	KP202045	KP201987
Sub-Order Chaunacoidei							
Family Chaunacidae	*Chaunax abei*	NMMB-P uncat.	KP201894	KP201933	KP202004	KP202039	KP201988
	Chaunax abei	NMMB-P uncat.	KP201892	KP201917	KP202011	KP202040	KP201998
	Chaunax abei	NMMB-P uncat.	KP201893	KP201918	KP202012	KP202041	KP201997
	Chaunax cf. abei	NMMB-P uncat.	KP201891	KP201957	KP202013	KP202042	KP201991
	Chaunax fimbriatus	NMMB-P uncat.	KP201890	KP201919	KP202005	KP202043	KP201992
	Chaunax fimbriatus	NMMB-P uncat.	KP201889	KP201950	KP202023	KP202044	KP201990
	Chaunax fimbriatus	NMMB-P uncat.	KP201887	KP201951	KP202014	KP202055	KP201994
	Chaunax penicillatus	NMMB-P uncat.	KP201888	KP201949	KP202028	KP202048	KP201993
	Chaunax sp.	NMMB-P uncat.	KP201886	KP201955	KP202027	KP202065	KP201995
	Chaunax sp.	NMMB-P uncat.	KP201895	KP201954	KP202018	KP202038	KP201989
	Chaunax sp.	NMMB-P uncat.	KP201885	KP201956	KP202019	KP202054	KP201996
Sub-Order Lophioidei							
Family Lophiidae	*Lophiodes mutilus*	NMMB-P uncat.	KP201884	KP201952	KP202032	KP202049	KP201970
	Lophiodes naresi	ASIZP 62537	KP201883	KP201928	N/A	KP202050	KP201972
	Lophiomus setigerus	ASIZP 62045	KP201882	KP201931	KP202008	KP202063	KP201969
	Lophiomus setigerus	ASIZP 62544	KP201881	KP201929	N/A	KP202053	KP201971
	Lophiomus setigerus	ASIZP 62543	KP201880	KP201930	KP202007	KP202059	N/A
	Sladenia gardineri	No Voucher	KP201879	KP201953	KP202031	KP202064	KP201968
Sub-Order Ceratioidei							
Family Himantolophidae	*Himantolophus sagamius*	N/A	AY920478	GU440342	JX190369	JX190776	JX191033
Family Melanocetidae	*Melanocetus johnsonii*	N/A	AY292587	GU440403	N/A	HM050180	N/A
	Melanocetus murrayi	LSUMZ 13532	KP201878	KP201923	N/A	N/A	N/A
Sub-Order Ogcocephaloidei							
Family Ogcocephalidae	*Coelophrys brevicaudata*	ASIZP tissue	KP201906	KP201922	KP202030	KP202035	KP201984
	Dibranchus atlanticus	UW 025869	AY292594	KC015319	KP202002	KP202034	KP201977
	Halicmetus reticulatus	NMMB-P uncat.	KP201916	KP201934	N/A	KP202051	KP201983
	Halieutaea fitzsimonsi	NMMB-P uncat.	KP201898	KP201938	KP202009	N/A	KP201962
	Halieutaea fumosa	ASIZP 63240	KP201897	KP201941	KP202010	N/A	KP201960
	Halieutaea indica	ASIZP 625P4	KP201896	KP201924	KP202017	N/A	KP201959
	Halieutaea sp.	ASIZP 63238	KP201901	KP201942	KP202006	N/A	KP201961
	Halieutaea stellata	NMMB-P uncat.	KP201900	KP201939	KP202003	N/A	KP201963
	Halieutaea stellata	NMMB-P uncat.	KP201899	KP201940	KP202015	N/A	KP201964
	Halieutichthys intermedius	LSUMZ 14223	KP201902	KP201935	KP202022	N/A	KP201973
	Halieutichthys intermedius	LSUMZ 14234	KP201903	KP201948	KP202001	N/A	KP201974
	Halieutichthys intermedius	LSUMZ 13629	KP201905	KP201944	KP201999	KP202052	KP201975
	Halieutichthys intermedius	LSUMZ 13632	KP201904	KP201945	KP202000	N/A	KP201976
	Halieutopsis ingerorum	ASIZP 0911273	KP201911	KP201937	KP202024	KP202061	KP201986
	Halieutopsis ingerorum	ASIZP 0911274	KP201912	KP201958	N/A	N/A	N/A
	Malthopsis annulifera	NMMB-P uncat.	KP201913	N/A	KP202016	KP202060	KP201985
	Malthopsis annulifera	NMMB-P uncat.	KP201914	KP201926	N/A	KP202058	KP201978
	Ogcocephalus declivirostris	LSUMZ 14272	KP201907	KP201936	KP202029	KP202046	N/A
	Ogcocephalus declivirostris	LSUMZ 13593	KP201908	KP201943	KP202025	KP202047	KP201982
	Solocisquama sp.	NMMB-P uncat.	KP201915	KP201927	KP202026	KP202037	KP201981
	Zalieutes elater	LSUMZ 14789C	KP201909	KP201920	KP202020	KP202033	KP201979
	Zalieutes elater	LSUMZ 14789D	KP201910	KP201921	KP202021	KP202036	KP201980
Order Tetraodontiformes							
Family Diodontidae	*Diodon holocanthus*	N/A	AY679639	JQ842441	JX188833	JX190239	JX189526
Family Tetraodontidae	*Tetraodon miurus*	N/A	AB741971	N/A	JX190378	JX190784	JX191040
Family Triacanthidae	*Triacanthus biaculeatus*	N/A	N/A	JF952881	JX190379	JX190785	JX191041
Family Molidae	*Mola mola*	N/A	DQ532911	JQ775088	JX190375	JX190781	JX191038
Order Perciformes							
Sub-Order Caproidei							
Family Caproidae	*Antigonia capros*	N/A	AY679618	N/A	KF027657	KF027899	KF028051
Sub-Order Acanthuroidei							
Family Scatophagidae	*Scatophagus argus*	N/A	DQ532954	FJ584089	JX188820	JX190227	JX189515

35–40 cycles of 94 °C for 0:30–0:45, 54–59 °C for 0:30–0:45, and 72 °C for 0:55–1:30; and 72 °C for 7:00 (final extension). Samples were held at 4–10 °C. Temperatures and times varied depending on the gene being amplified. Reagent concentrations for 25 μl reactions were as follows: 1 μl of each primer (10 mM), 1 μl of 10 mM deoxynucleotide solution mix, 2.5 μl of 10X standard taq buffer, 3 μl of 25 mM Magnesium Chloride, and 0.3 μl of Taq DNA Polymerase (New England Biolabs). Two μl of DNA were used as the template for all reactions.

Samples were sent to Beckman Coulter Genomics (Danvers, MA) for sequencing. The samples were sequenced using standard Sanger sequencing protocols in both forward and reverse directions
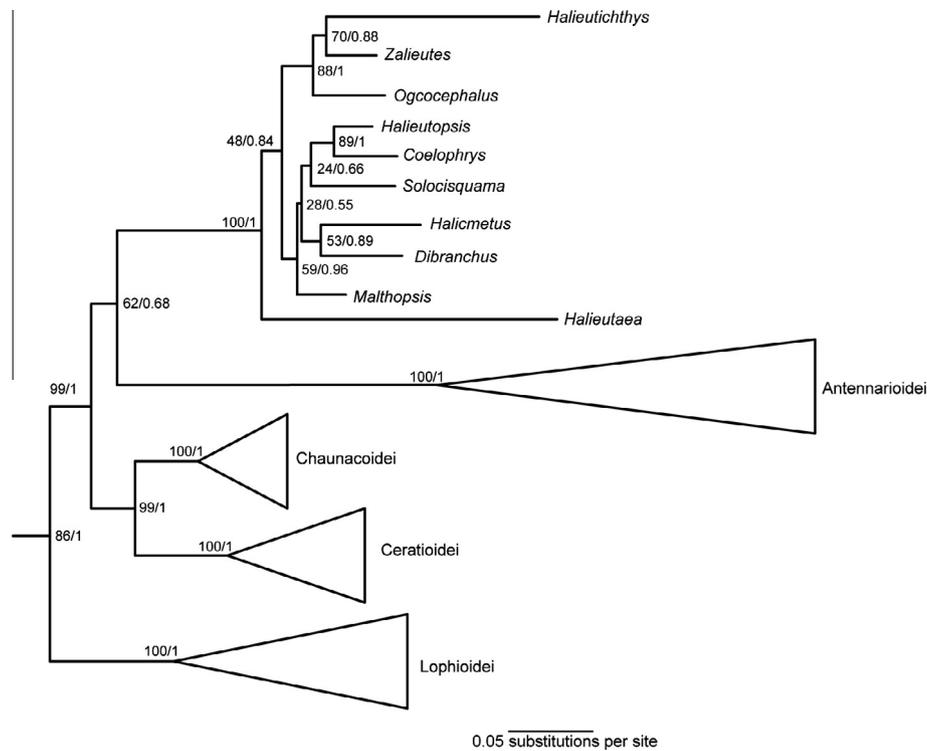
Fig. 2. Results of Garli and Mr. Bayes analyses with bootstrap proportions and posterior probabilities listed respectively. Sub-orders were collapsed except for Ogcocephalidae.

and aligned in Geneious v6.0.5 (Drummond et al., 2007). Sequences were concatenated using the program Sequence Matrix v1.7.8 (Vaidya et al., 2011) prior to phylogenetic analysis.

2.3. Phylogenetic analyses

To determine relationships among batfish genera and the placement of Ogcocephaloidei within Lophiiformes, we used both Maximum Likelihood and Bayesian approaches. Appropriate models for each gene were selected with JModeltest ver. 2.1.3 (Darriba et al., 2012) using a Fixed BIONJ-JC base tree with 11 substitution schemes. Models were differentiated under the Akaike Information Criterion with sample size correction.

The maximum likelihood analysis was carried out in Garli 2.0 (Zwickl, 2006), using a concatenated dataset that was partitioned by gene using the appropriate substitution model for each gene. Models were as follows: 16S and CO1 – TIM + I + G, GLYT – TIM + G, PTR – HKY + I + G, and SH3PX3 – TrN + I + G. Two search replicates were run with 1000 bootstrap pseudoreplicates. Results were summarized using Sumtrees in the Dendropy package of Python (Sukumaran and Holder, 2010). Trees were visualized in Figtree ver. 1.4.0 (Rambaut, 2012).

The Bayesian analysis was carried out in Mr. Bayes ver. 3.2.1 (Huelsenbeck and Ronquist, 2001) and the data were partitioned by gene using the most appropriate model for each gene. Because Mr. Bayes does not implement all of the models selected by JModeltest, the next best compatible models were used. Models were as follows: 16S, CO1, and SH3PX3 – GTR + I + G; GLYT – GTR + G; and PTR – HKY + I + G. The Markov chain Monte Carlo option was selected with all parameters unlinked. The analysis was carried out over three runs, with four chains per run for one million generations per run. Convergence was checked among trees, branches, and parameters using the average standard deviation of split frequencies in Mr. Bayes ver. 3.2.1, AWTY (Wilgenbusch et al., 2004), and Tracer (Rambaut and Drummond, 2007). Effective sample size (ESS) values were all over 200. Results were summarized into a majority rule consensus with a burn-in of 200 trees (indicated by Tracer).

2.4. Divergence time estimation

Divergence times were estimated using the Bayesian program BEAST ver. 1.7.5 (Drummond and Rambaut, 2007) using an uncorrelated lognormal relaxed clock model. The template XML file was created in BEAUTI ver. 1.7.5 with the tree prior set to a Yule process of speciation. The dataset was partitioned by gene. For efficiency, Ogcocephalidae, Lophiiformes, and Tetraodontiformes were constrained to be monophyletic in BEAST; these constraints were justified by our maximum likelihood and Bayesian phylogenetic results, as well as previous studies (Miya et al., 2010).

Two fossil calibrations were used, one at the base of Tetraodontiformes and one at the base of Ogcocephalidae. The fossil placed at the base of Ogcocephalidae is †*Tarkus squirei* (Lophiiformes: Ogcocephalidae) from the Alveolina dainelli zone limestone (SBZ 11 Biozone) in Pesciara Cave in Monte Bolca, Italy (Carnevale and Pietsch, 2010). The fossil is approximately 50 million years old (Ypresian stage of Eocene) and represents the earliest known batfish (Carnevale and Pietsch, 2010). The fossil placed at the base of Tetraodontiformes is the jaw of †*Eomola bimaxillaria* (Tetraodontiformes: Molidae) from Kumsky Horizon on the Pshekha River of North Caucasus in Russia (Tyler and Bannikov, 1992). The fossil is dated to be 37.2 million years old (around the Bartonian stage of the Eocene). This is the same fossil Near et al. (2013) used to age the most recent common ancestor of Molidae and Triacanthus (two groups represented in this study).

Fossil calibrations were assigned a lognormal prior and given a hard minimum age (indicated by the fossil) and soft maximum age. The maximum age was set to about 95 million years old based on the most recent common ancestor of Acanthomorpha following McMahan et al., 2013. A random start tree was used with MCMC

set to run for 50,000,000 generations, four separate times. Convergences were again checked with AWTY (Wilgenbusch et al., 2004) and Tracer (Rambaut and Drummond, 2007). Using the program Log Combiner ver. 1.7.5 in the BEAST package, the four runs were combined with a burnin of 5,000,000, which was selected by Tracer. The ESS values were all over 200. The program Tree Annotator ver. 1.7.5 was used to create a maximum clade credibility tree with a burnin of 100 trees and a posterior probability limit of 60%. The final time calibrated tree was viewed in FigTree ver. 1.4.0.

2.5. Ancestral state reconstruction

To determine the depth origins of batfishes, ancestral state reconstructions were performed using the program Mesquite (Maddison and Maddison, 2011), using the results obtained from the Mr. Bayes analysis. Ogcocephaloidei was coded by genera and the rest were coded by suborder. Standard categorical character states were defined as follows: euphotic (0–200 m), disphotic (200–1000 m), and aphotic (below 1000 m). To determine the character states for each group (sub-order or genus), depth records were averaged. Averages for each genus in Ogcocephalidae came from Ho (2010). Depth data used for each suborder were obtained from museum collection data uploaded on Fishnet2, (2014). The character history was traced using the likelihood ancestral state reconstruction function.

3. Results

The concatenated alignment contained 3351 base pairs (16S – 660 bp, CO1 – 680 bp, GLYT – 535 bp, PTR – 776 bp, SH3PX3 – 700 bp). The majority of taxa were sampled for all genes, and overall there was 87.9% coverage of the data matrix. Counting each genus as a terminal, the maximum likelihood analysis resolved 44% of the nine internal nodes with bootstrap support ⩾70% (55% with bootstrap support ⩾ 50%). The Bayesian analysis resolved 44% of the nine internal nodes with posterior probabilities ⩾95% (75% with posterior probabilities ⩾75%). All analyses confirmed the monophyly of Ogcocephaloidei, and place this taxon as the sister group to Antennarioidei (Fig. 2). The maximum likelihood and Bayesian analyses obtained identical results for generic relationships.

Within Ogcocephalidae, two major clades were recovered – an Eastern Pacific/Western Atlantic clade consisting of *Halieutichthys*, *Ogcocephalus*, and *Zalieutes*; and an Indo-Pacific clade consisting of *Coelophrys*, *Dibranchus*, *Halicmetus*, *Halieutopsis*, *Malthopsis*, and *Solocisquama* (Fig. 2). *Halieutaea* was recovered as the sister group to all other batfishes. BEAST analyses recovered the crown age of Ogcocephalidae to be in the Eocene at 54 million years old (50–65 mya 95% CI), and the crown age of Lophiiformes to be in the Cretaceous at 88 million years old (65–120 mya 95% CI; Fig. 3).
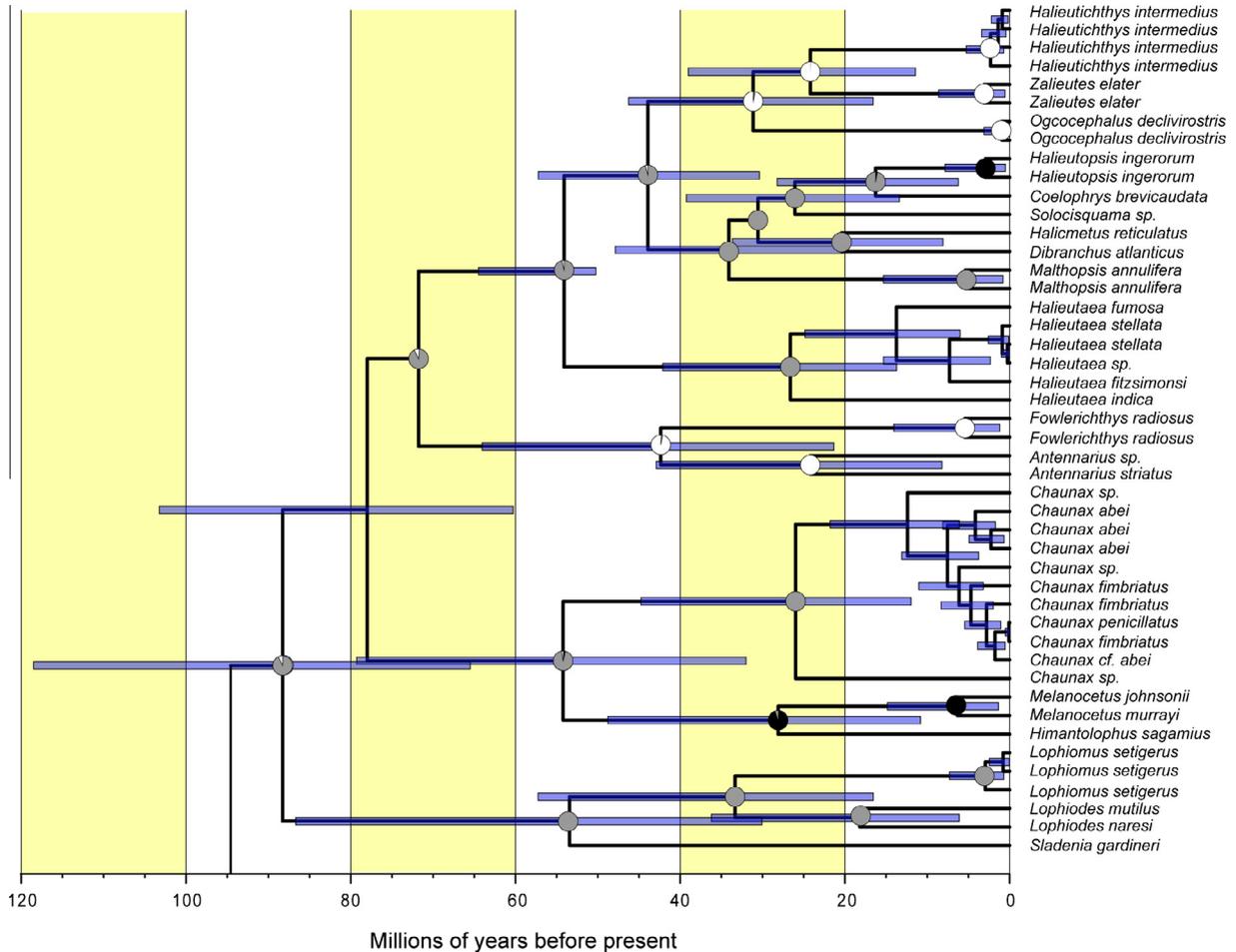

Fig. 3. Results of divergence time estimation and ancestral state reconstruction. Age estimates are in millions of years. 95% confidence intervals are shown. The character history was traced using the likelihood ancestral states. White circles represent the euphotic zone (0–200 m). Gray circles represent disphotic zone (200–1000 m). Black circles represent aphotic zone (below 1000 m).

Based on this analysis, the batfishes most likely originated in the disphotic zone (200–1000 m). The results of ancestral state reconstruction within Ogcocephalidae (Fig. 3) suggest one invasion into the aphotic zone (defined as below 1000 m) by *Halieutopsis* and an invasion into the euphotic zone (defined as 0–200 m) by the Eastern Pacific/Western Atlantic batfish genera.

4. Discussion

The goal of this study was to produce a fossil calibrated phylogeny clarifying the relationships among Ogcocephalid genera, as well as to place Ogcocephaloidei within Lophiiformes. The evolutionary history of the group was also revealed including the likely timing of divergence and original habitat depth.

4.1. Position of Ogcocephaloidei within Lophiiformes

Each sub-order was recovered as monophyletic with strong statistical support. Ogcocephaloidei was found to be the sister taxon of Antennarioidei with moderate support. This clade is the sister group to a Chaunacoidei + Ceratioidei clade. The sister relationship between Chaunacoidei and Ceratioidei is consistent with the mitochondrial studies of Miya et al. (2010) and Shedlock et al. (2004). Although this relationship has not been directly supported by morphological studies, a close relationship was suggested by Pietsch and Grobecker (1987) based on a reduced 2nd dorsal-fin spine under the skin of the head and an absence of gill filaments on the first gill arch. Lophioidei was recovered as the sister group to the rest of the anglerfish suborders. This result agrees with past molecular (Miya et al., 2010) and morphological (Pietsch and Grobecker, 1987) studies.

4.2. Relationships among Ogcocephalid genera

Within Ogcocephalidae, genera mainly found in the Eastern Pacific/Western Atlantic ocean, *Ogcocephalus*, *Zalieutes*, and *Halieutichthys*, were recovered as monophyletic with strong statistical support and recovered *Halieutichthys* and *Zalieutes* as sister taxa (Fig. 2). These results corroborate the morphological and biogeographical study of Ho (2010), which also suggested a close relationship between the Eastern Pacific/Western Atlantic lineages. Indo-Pacific genera excluding *Halieutaea* were also recovered as a clade and are the sister taxon to the Eastern Pacific/Western Atlantic clade. Within the Indo-Pacific clade, *Coelophrys* and *Halieutopsis* formed a sister group with high support. This relationship was also recovered in the morphological studies of Endo and Shinohara (1999) and Bradbury (1967) with synapomorphies such as the lack of a groove in the frontal bones, the spine-like shape of illicial bone, reduced pectoral fins, and absent or reduced teeth on the palate. *Halieutaea* was recovered as sister to all other batfishes, which is also similar to the results of Endo and Shinohara (1999) and was highly supported in this study.

4.3. Divergence time estimation

Based on the BEAST analyses, the age of Ogcocephaloidei is estimated to be approximately 54 million years old (50–65 mya 95% CI; stem age ~71 mya) diverging during the Eocene/Paleocene or Late Cretaceous. The age of Lophiiformes is estimated to be 88 million years old (65–120 mya 95% CI; stem age ~94 mya) diverging during the Cretaceous. Miya et al. (2010) recovered an age for Lophiiformes between 100 and 130 million years old, with batfishes diverging around 60 mya; however that study had limited taxonomic sampling of batfishes (four Ogcocephalid taxa represented) and did not include any nuclear genes. Using only nuclear data,

Near et al. (2012) aged Lophiiformes around 68 million years old and Ogcocephalidae between 20 and 35 million years old; however, that study also had very limited taxonomic sampling (seven lophiiform taxa represented; two Ogcocephalids). Also their recovered age for batfishes is dubious, as there is an Eocene (50 mya) Ogcocephalid fossil that is twice the age of their reported dates for the Ogcocephalids.

4.4. Ancestral state reconstruction

The ancestral state reconstruction was conducted to examine general patterns among batfishes. Many of the species' habitats span across the designated categories, so average depths were used as an estimate. Because of the prevalence of missing species in the dataset, a more conservative approach of coding by genus and sub-order was taken; this analysis is a first-pass attempt to examine the habitat depth history of these fishes. Focusing on batfishes, it appears that their most recent common ancestor inhabited the lower continental shelf/upper continental slope of the disphotic zone (200–1000 m), where most of the taxa currently reside. There was a deep-water invasion by *Halieutopsis*; most species in this genus are found on the continental slope (bathyal zone below 1000 m). There was a shallow water invasion by the Eastern Pacific/Western Atlantic clade, which has species that reside mainly on the continental shelf in the euphotic zone (0–200 m). Looking across anglerfishes, it appears that there were two invasions into the deep sea – the Ceratioids and members of the Ogcocephalid genus, *Halieutopsis*. There were also two invasions into shallow water – the Antennarioids and the Eastern Pacific/Western Atlantic Ogcocephalids. Within Ogcocephalidae, the shift from the Indo Pacific Ocean to the Eastern Pacific/Western Atlantic may have facilitated colonization of shallower waters on the continental shelf. Batfishes are unique among anglerfishes because they are the only suborder that spans oceanic zones from 0 to about 4000 m. Examining the evolutionary history of these depth range shifts in habitat at the species level would be worthwhile, but unfortunately beyond the scope of our deeper level study.

4.5. Conclusions

This study represents the first fossil calibrated molecular phylogeny including all ten Ogcocephalid genera and the first study to examine habitat depth shifts among anglerfishes. The phylogenetic analyses support monophyly of each suborder within Lophiiformes, and recovers a novel sister relationship between Ogcocephaloidei and Antennarioidei. Relationships among batfish genera suggest an Eastern Pacific/Western Atlantic clade and an Indo-Pacific clade, with *Halieutaea* recovered as the sister group to all other batfishes. Based on our divergence time estimations and ancestral state reconstructions, Ogcocephalidae diverged in the Eocene about 54 million years ago, most likely on the lower continental shelf/upper continental slope of the disphotic zone (200–1000 m). Additional taxonomic sampling, including more Ogcocephalid species, as well as a total evidence approach including both morphological and molecular data may help increase the support of some nodes within Lophiiformes. Because Ogcocephalidae contains shallow water as well as deep-water species, knowing when and whether or not they came from deep waters or shallow waters can help extrapolate patterns of deep-sea invasions setting a foundation for future deep-sea research.

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