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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).

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);

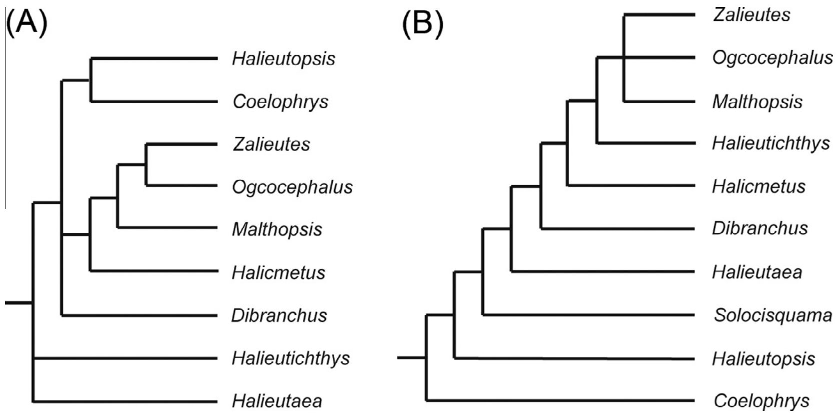

Fig. 1. Phylogenetic hypotheses for Ogcocephalidae interrelationships based on previous morphological studies. (A) Relationships hypothesized by Endo and Shinohara (1999) using morphology and nine of ten batfish genera. (B) Relationships hypothesized by Ho (2010) using morphology and biogeographic characters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