

The Indian handfish, *Halieutaea indica* Annandale & Jenkins, 1910 (Lophiiformes: Ogocephalidae), an additional fish species for the Gulf of Oman

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Abstract

Batfishes of the family Ogocephalidae are a group of Lophiiformes found in tropical and subtropical marine waters with specific morphological adaptations for a deep-water benthic lifestyle. This study, morphologically and genetically documents the range extension of Indo-Pacific batfish, *Halieutaea indica* into the northern marginal waters of the Indian Ocean (Gulf of Oman), and confirms the presence of more shared fish elements of the Gulf of Oman with the Indian Ocean. DNA barcoding based on the mitochondrial COI gene sequence revealed that the collected batfish specimens from the Gulf of Oman are conspecific with *H. indica*.

Keywords: Anglerfish, Batfish, Distribution, Phylogeny, Morphology, DNA barcoding.

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Introduction

The family Ogocephalidae (batfishes) currently comprises 10 genera i.e. *Coelophrys*, *Halieutopsis*, *Dibranchus*, *Halieutaea*, *Halicometus*, *Malthopsis*, *Halieutichthys*, *Ogocephalus*, *Solocisquama* and *Zalieutes* and 78 valid species, making it the most speciose family within Lophiiformes (Bradbury 1999, 2003; Fricke et al. 2019; Ho et al. 2013a, b). The ogocephalid anglerfishes live in benthic habitats of shallow to deep marine waters in nearly entire tropical and subtropical regions of the world, known mostly from continental slopes and outer continental shelves to 1,500-3,000 m and rarely from 4,000 m; some species occur inshore and rarely known upstream in rivers (Derouen et al. 2015; Nelson et al. 2016). Analyses of a concatenated nuclear and mitochondrial dataset by Derouen et al. (2015) has shown that Ogocephalidae originated in the disphotic zone (200-1,000 m) in Eocene, approximately 54 million years ago, with subsequent invasion into the aphotic zone (below 1,000 m) by the genus *Halieutopsis* and an invasion into the euphotic zone (0-200 m) by the Eastern Pacific/West Atlantic batfish genera.

The genus *Halieutaea* Valenciennes, 1837 comprises nine valid species distributed over the Indo-west and eastern central Pacific (Fricke et al. 2019; Froese and Pauly 2019). Herein, we provide morphological and molecular evidence for occurrence of *Halieutaea indica* Annandale & Jenkins, 1910 in the Gulf of Oman ecoregion for the first time and discuss its phylogenetic relationship to other ogocephalids.

Material and Methods

Taxon sampling: Between 25 and 26 October 2017, five batfish specimens were collected using bottom trawl on Ferdows-I investigation ship in the Gulf of Oman, off province of Sistan and Baluchestan, southeast of Iran, between 25°9'0.6618"N, 60°54'0.6948"E and 25°8'0.0132"N, 60°57'0.543"E at 41-57 m depth. Also, during the second fish survey on October 2018, another nine specimens were collected using the same fishing equipment in the Gulf of Oman between 24°30'0"N-25°6'0"N and 61°0'0"E-61°15'0"E, at 20-70 m depth (Fig. 1). All

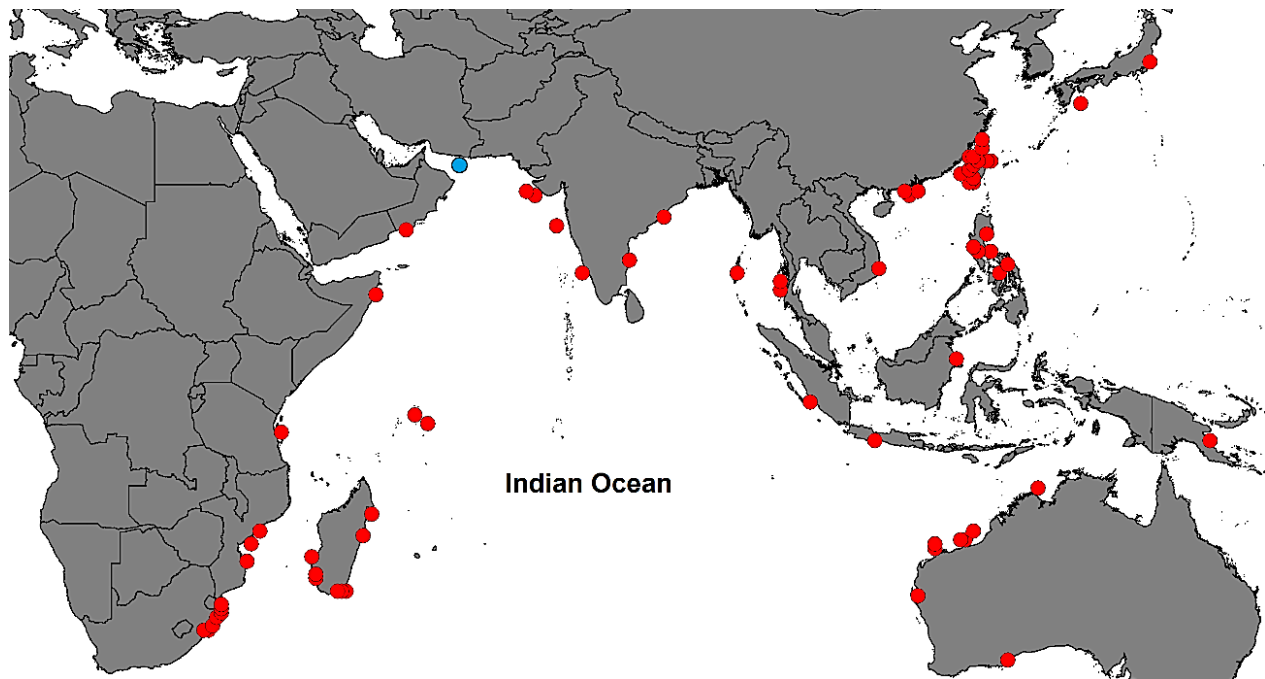


Figure 1. Point distribution map of *Halieutaea indica* (after Froese and Pauly 2020), and our sampling site (blue circle) in the Gulf of Oman.

specimens were preserved in formaldehyde (10%) and subsequently transferred to 70% ethanol for long-term storage. Voucher specimens are deposited in the Zoological Museum of Shiraz University, Collection of Biology Department, ZM-CBSU. In addition, samples of pectoral fin tissue were taken from five specimens and stored in 96% ethanol for genetic analysis.

Morphological method and definitions: The specimens were identified following Annandale and Jenkins (1910) and Ho and Shao (2008). Morphometric measurements, expressed as percentages of standard length (SL) to the nearest 0.1 mm, were made using a digital caliper. For morphological method and definitions of the characters, we followed Bradbury (1988, 1998). A stereomicroscope (Zeiss Stemi SV6) was used to count the meristic characters: D: dorsal fin rays; V: pelvic fin rays; A: anal fin rays; P: pectoral fin rays; C: caudal fin rays. Morphometric values were expressed as percentages of SL.

DNA extraction, PCR amplification and sequencing: Total DNA was extracted from two fin clips (taken from ZM-CBSU S084-5 and ZM-CBSU S084-7) using the salt method protocol described in Bruford et al. (1992). The mitochondrial cytochrome C oxidase subunit I (COI) gene sequence was amplified using a primer pair, Fish-BCL (5'-TCAACYAATCAYAAAGATATYGGCAC-3') and Fish-BCH (5'-ACTTCYGGGTGCCRAARA ATCA-3') (Baldwin et al. 2009). Amplifications were performed using a PCR Thermal Cycler with thermal profile for amplification of COI consisting of an initial step of denaturation at 95°C for 5 min followed by 35 cycles of denaturation (1 min at 94°C), annealing (45 secs at 58.4°C), and extension (1 min at 72°C) with the final extension at 72°C for 7 min. The quality of the PCR products were assayed using 1% agarose gel in TBE buffer stained with Green fluorescent dye. After purification, the high quality PCR products were sequenced by the Persian Bayan Gene Lab., Shiraz, Iran.

Phylogenetic analysis: BioEdit 7.1 (Hall 1999) was used to read and edit the DNA chromatograms, while ClustalW procedure in Mega 6.0 (Tamura et al. 2013) was used for sequence alignment. The new haplotype sequences are deposited in GenBank (Acc. no. MT093858 and MT093859). A total of 25 mtDNA COI sequences representing 25 batfish species from all 10 ogocephalid genera were obtained from GenBank. After alignment with new haplotype sequences as well as an *Fowlerichthys radiosus* COI sequence as outgroup

(family Antennariidae; GenBank Acc. no.: KP201948.1), GTR+I+G was selected as the best nucleotide substitution model based on the AICc model selection criterion in jModelTest 2.1.10 (Darriba et al. 2012). We reconstructed a Bayesian phylogeny for all species using the Bayesian inference (BI) implemented in MrBayes 3.2 (Ronquist et al. 2012) with default priors and MCMC proposal distributions, run for 20,000,000 generations and sampled every 1,000 generations. As burn-in, the first 15% of the generations were discarded. In addition, we reconstructed a Maximum Likelihood (ML) phylogenetic tree of sequences with 10,000 bootstrap replicates in RaxML 7.2.5 (Stamatakis 2014).

Results

Halieutaea indica Annandale & Jenkins, 1910

(Figs. 2, 3)

Halieutaea indica, Annandale [N.] & Jenkins [J. T.] 1910:19, Pl. 2 (fig. 4) [Mem. Indian Mus. Vol. 3 (no. 1)]; Bay of Bengal, off Orissa coast, India.

Specimens examined: ZM-CBSU S084, 9, 58.02-92.22 SL, Iran: Gulf of Oman, off Sistan and Baluchestan Province coast, between 24°30'0"N-25°6'0"N and 61°0'0"E-61°15'0"E, 20-70 m depth, F. Owfi, Oct. 2018. ZM-CBSU S085, 5, 68.57-93.20 SL, Iran: Gulf of Oman, off Sistan and Baluchestan Province coast; between 25°9'0.6618"N, 60°54'0.6948"E & 25°8'0.0132"N, 60°57'0.543"E, 41-57 m depth, R. Sadeghi, 25-26 Oct. 2017.

Morphological description: Morphometric data of nine specimens are given in Table 1. D: 4; V: 5; A: 4; P: 13; C: 9. Head relatively compressed or slightly elevated; interorbital space equals or slightly exceeds horizontal eye diameter; roof of illicial cavity (rostrum) well projects over front of disk; esca invisible from dorsal view; two axe-like patches of teeth present on tongue, each has an elongated inner prolongation; dorsal surface covered with numerous strong needle-like tubercles, many of which bifid, at the edge many trifold, and totally naked between principal tubercles; ventral surface covered with some small granules; and tail slender and tapering. In preserved specimens (Fig. 2), dorsal and ventral white; dorsal surface densely covered with minute brown dots, which grouped together in places to form thin lines and two regular H-shaped brown patterns; all fins completely pale without black margin in preservation. In live specimens, colour brown to reddish; pectoral fin reddish with broad yellowish margin and white sub-marginal band (Fig. 3).

Key to the Indian Ocean species of *Halieutaea*

- 1a. Ventral surface smooth, glandular and nearly transparent; dorsal fin with five rays.....*H. fumosa*
- 1b. Ventral surface opaque or slightly translucent, with minute stellate spines or granules.....2
- 2a. Dorsal fin with four rays3
- 2b. Dorsal fin with five rays.....4
- 3a. Rostrum extends forward as far as the disk edge; caudal and pectoral not edged*H. indica*
- 3b. Rostrum not extending to the disk edge; caudal and pectoral edged*H. stellate*
- 4a. Disk flat.....*H. nigra*
- 4b. Disk markedly convex in proximal.....*H. coccinea*

Species delimitation by DNA barcoding: In phylogenetic reconstructions (BI & ML), the two sequenced batfish specimens from the Gulf of Oman (C2743 and C2744 in Fig. 4) grouped with the only previously sequenced specimen of *H. indica* (KP201924; Derouen et al. 2015) with full statistical support (BS=100%, PP=100).

Phylogenetic relationships: Within Ogocephalidae, both major monophyletic batfish clades defined by Derouen et al. (2015) including East Pacific/West Atlantic group (*Halieutichthys*, *Ogocephalus* and *Zalieutes*), and Indo-Pacific group (*Halicmetus*, *Dibranchus*, *Coelophrys*, *Malthopsis*, *Halieutopsis* and *Solocisquama*)

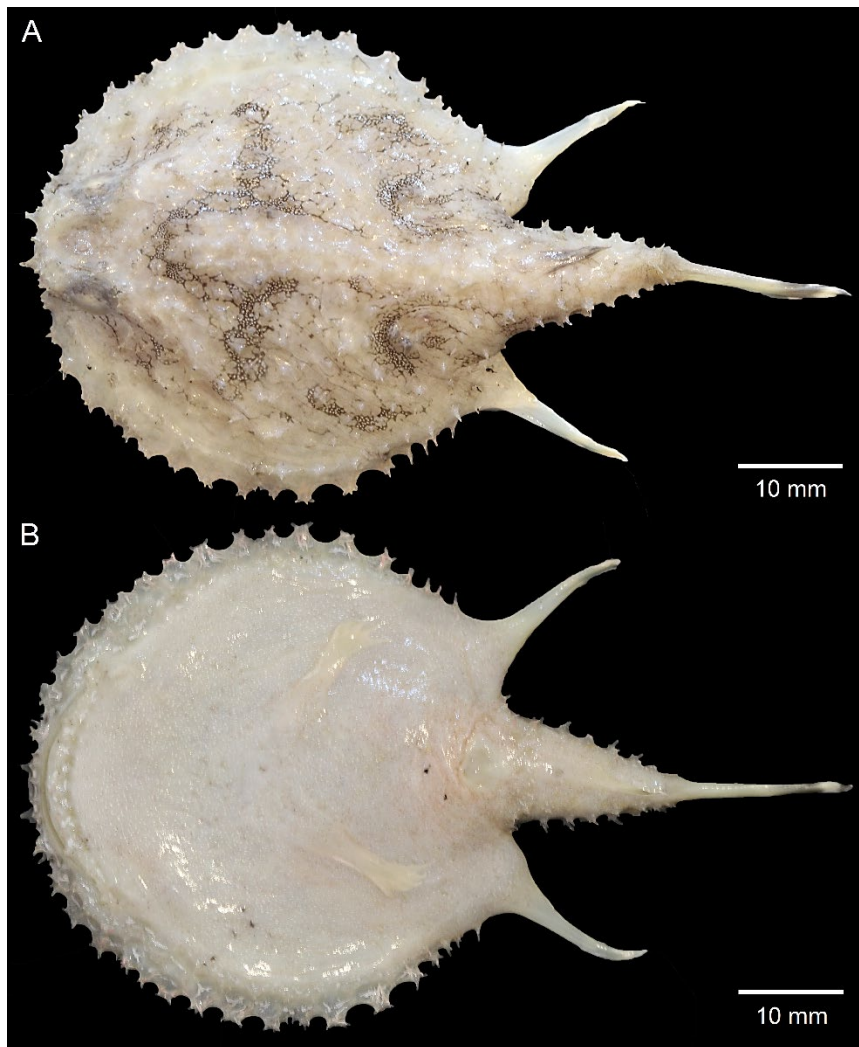


Figure 2. Preserved specimen of *Halieutaea indica* collected from the Gulf of Oman. (A) Dorsal and (B) ventral views, ZM-CBSU S084-8, 58.75 mm SL.

were recovered as paraphyletic. In addition, *Halieutaea*, as a monophyletic group, including *H. coccinea*, *F. fitzsimonsi*, *H. fumosa*, *H. indica* and *H. stellate* was proved to be the sister group of all other batfish taxa of the family Ogcocephalidae (Fig. 3).

Discussion

The Oman Sea is being considered as a zoogeographic frontier between the Indo-Polynesian and Western Indian Ocean biogeographic provinces (Briggs 1974; Béarez et al. 2008a). In comparison to the Persian Gulf (another ecoregion located in the the Somali/Arabian Sea area) (Spalding et al. 2007), the Oman Sea has more stable physical and chemical conditions with direct link to the Indian Ocean that makes it geophysically more open and deeper than the Persian Gulf (in most cases deeper than 1,000 m) (Reynolds 1993), embracing more shared fish elements with the Indian Ocean. Morphological and genetic analyses of this study documented a significant range extension into the northern part of the Oman Sea for a wide-ranging Indo-Pacific batfish, *H. indica*, and further confirms the presence of more shared fish elements of the Oman Sea with the Indian Ocean. This

Detailed morphology of *H. indica* was consistent with those previously described by Annandale and Jenkins (1910) and Ho and Shao (2008), showing almost conservative morphology. In phylogenetic analysis, the batfish samples from the Oman Sea grouped with *H. indica*, and proved to be genetically conspecific, making *Halieutaea* as a basal/sister group to all other batfish species. Based on analyses of a concatenated nuclear and mitochondrial dataset, Derouen et al. (2015) recovered three major monophyletic clades within

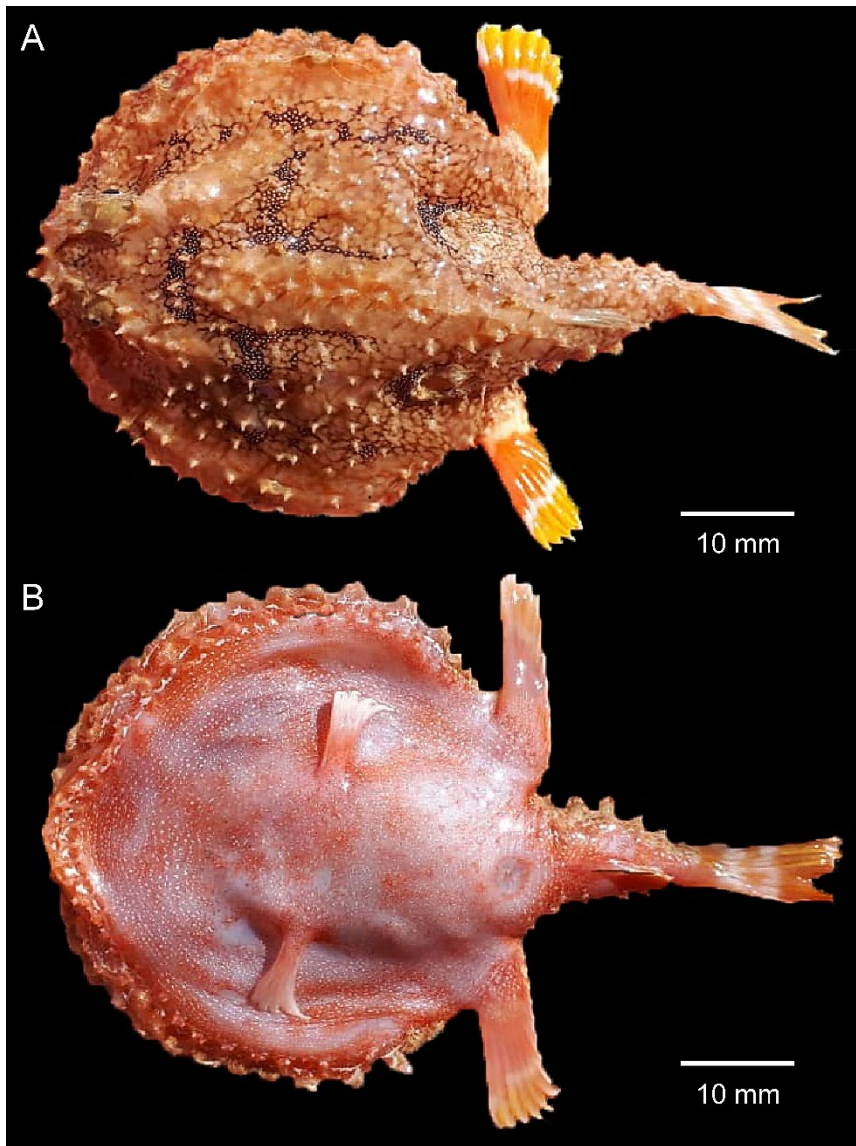


Figure 3. Freshly collected specimen of *Halieutaea indica* from the Gulf of Oman. (A) Dorsal and (B) ventral views, ZM-CBSU S084-7, 60.21 mm SL.

Ogcocephalidae: (i) an East Pacific/West Atlantic clade (*Halieutichthys*, *Ogcocephalus* and *Zalieutes*); (ii) an Indo-Pacific clade (*Halicmetus*, *Dibranchus*, *Coelophrys*, *Malthopsis*, *Halieutopsis* and *Solocisquama*); and (iii) genus *Halieutaea* as the sister taxa to all other batfish species. In our phylogenetic study, a basal/sister position for *Halieutaea* was recovered with full statistical support, which is consistent with the molecular study of Derouen et al. (2015); however, our COI phylogeny did not support the monophyly of both major clades, the East Pacific/West Atlantic clade and the Indo-Pacific clade (Fig. 4). Thus, unlike the single-gene phylogenies, the multi-gene method of phylogenetic reconstruction produces highly resolved trees, even when the combined sequences have evolved with very different substitution rates (Gadagkar et al. 2005).

Halieutaea indica is known to have a wide distribution range in the Indo-west Pacific from eastern coast of South Africa, Seychelles and Madagascar east to Philippines and New Guinea, north to southern Japan, south to northern Australia (Ho and Shao 2008; Fricke et al. 2019; Froese and Pauly 2019), and now we report it for the first time from the Oman Sea (Fig. 1). Almost all occurrence records of this species, including our record, are pertaining to deep seawaters, however there is also one report from shallow brackish water coastal ecosystem in Chilika Lagoon, India (Mahapatro et al. 2018). Recent field investigations in the Persian Gulf, Strait of Hormuz and Makran/Oman Sea have resulted in the discovery or new records of different fish groups (Randall 1995; Béarez et al. 2008b; Uiblein and Heemstra 2011; Sadeghi et al. 2017; Mehraban and Esmaeili

Table 1. Morphometric measurements of nine *Halieutaea indica* specimens collected from the Gulf of Oman. Total length (TL) and standard length (SL) in mm.

Morphometric measurements	Min.	Max.	Mean
Total length (TL)	77.25	121.80	102.01
Standard length (SL)	58.02	92.22	76.93
% of standard length			
Mouth width	28.10	31.25	29.61
Illicial cavity width	6.35	7.83	7.18
Predorsal distance	76.27	81.43	78.23
Preanal distance	81.49	87.10	83.83
Snout length	9.07	11.31	10.80
Dorsal fin length	12.71	14.38	13.28
Pectoral fin length	26.50	33.15	29.90
Pelvic fin length	17.29	19.27	18.37
Anal fin length	15.93	18.93	16.87
Caudal fin length	29.92	37.87	32.95
Interorbital space	8.56	10.21	9.47
Eye diameter	7.24	9.32	8.36
Disc length	72.15	78.42	74.15
Disc width	78.59	88.92	83.86
Tail length	60.23	66.82	63.76

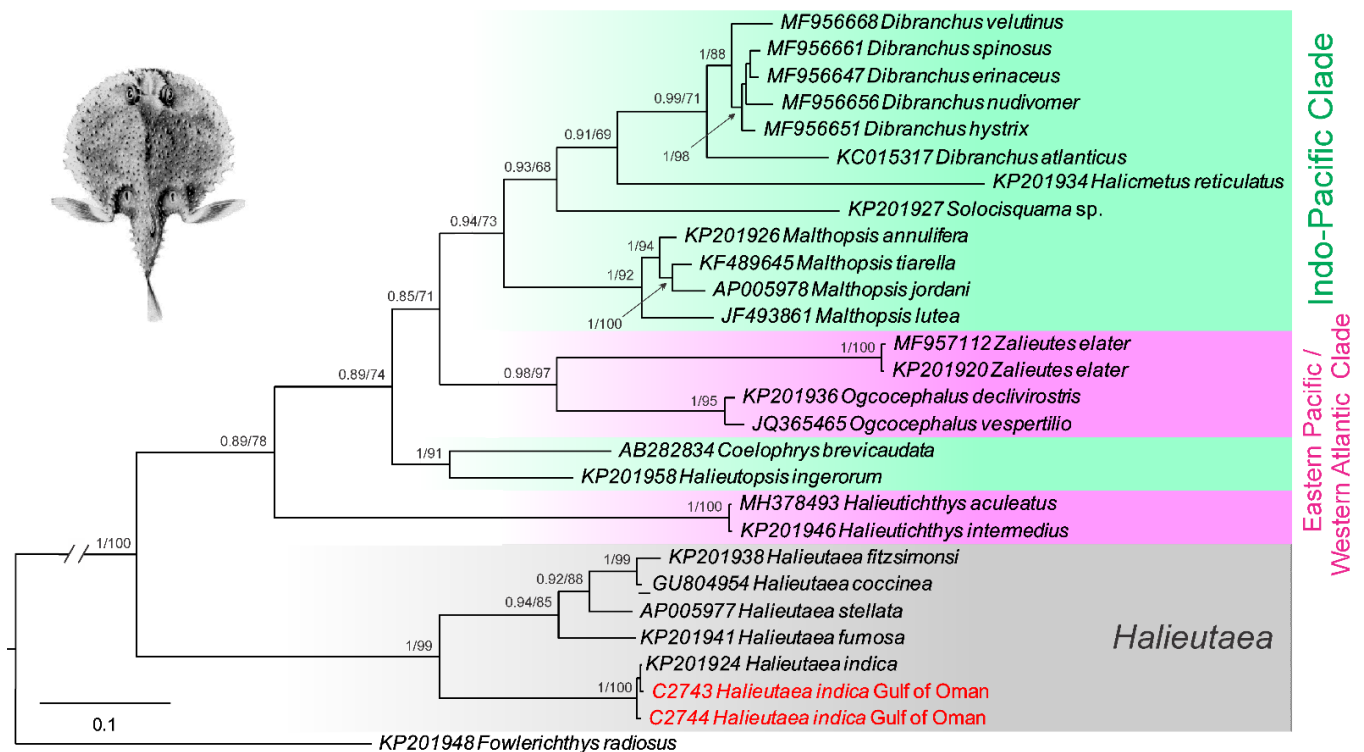


Figure 4. Bayesian and Maximum Likelihood phylogeny of Ogcocephalidae based on the COI dataset, showing the phylogenetic position of *Halieutaea indica* samples collected from the Gulf of Oman. Double bar at the root indicates that branch has been reduced in length and is not proportional to the scale. The values beside the branches before and after a slash are BI posterior probability and ML bootstrap values, respectively.

2018). Nevertheless, work still remains to be done on systematics, phylogeny, phylogeography, biology and ecology of many fish species in this ecologically and historically interesting part of the world. The discovery of *H. indica* in the Oman Sea indicates that a suitable habitat for this species occurs in this area and probably in the neighboring ecoregion, the Persian Gulf, and that additional ogcocephaliid species may be discovered in the near future.

Acknowledgments

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