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## NOTE

# FIRST RECORD OF HAGFISH (CYCLOSTOMATA: MYXINIDAE) IN INDIAN WATERS

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# FIRST RECORD OF HAGFISH (CYCLOSTOMATA: MYXINIDAE) IN INDIAN WATERS

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Hagfishes are exclusively marine bottom dwelling species with eel-like body shape, large eggs and direct development. Distribution of hagfish was long described with large gaps in tropical waters (Hardisty 1979). Later it became clear that hagfish occurred also in these areas but at great depth where the water was cold (Fernholm 1998). Improvement in collection technique with manned submersibles have found hagfish in new bottom environments such as volcanic hydrothermal vents and coral reefs (Møller & Jones 2007; Fernholm & Quattrini 2008).

An early morning of 13 March 2015 a shrimp trawler trawling at 500–600 m depth in the Arabian Sea off Kerala coast of India brought a hagfish as bycatch. The fish was collected from the trawl bycatch landed at Sakthikulangara fishing harbour in Kerala, India by the second author in an ice-box and brought back to the laboratory of Department of Aquatic Biology and Fisheries, University of Kerala, India. A DNA sample was taken and sequenced for mitochondrial gene cytochrome oxidase C (CO1). The CO1 sequence of the mitochondrial DNA (mtDNA) was amplified using Fish F1 and Fish R1 primers (Ward et al. 2005). PCR products were visualized on 1% agarose gels and purified using Exo Sap IT (USB). Bidirectional sequencing was performed using the PCR primers and products were labelled with BigDye Terminator V.3.1 Cycle sequencing Kit (Applied Biosystems, Inc.) and sequenced in an ABI 3730 capillary sequencer following manufacturer's instructions at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India. The CO1 sequence was run together with the other sequences used in a recent study of hagfish phylogeny (Fernholm et al. 2013).

The support for the Indian hagfish as a new separate species is strong. It is also clearly nested within the genus *Eptatretus* Cloquet, 1819 (Fig. 1).

The voucher specimen is deposited in the museum collections of the Department of Aquatic Biology and Fisheries, University of Kerala, India (DABFUK-FI-230).

**Class: Myxini**
**Order: Myxiniformes**
**Family: Myxinidae**
**Subfamily: Eptatretinae**
**Genus *Eptatretus* Cloquet, 1819**

Since the specimen has been in the trawl, frozen and preserved in ethanol (dehydrated) it is not in good shape for detailed morphological studies. It is a typical eel-shaped elongated hagfish 350mm long (Image 1) with a single nostril surrounded by four tentacles (Image 2) and a ventrally facing mouth with laterally biting keratinous tooth plates. Pattern of fused cusps of teeth is 3/2 and total cusps 44. There are eight gill openings on each side. We did not open and count the gill pouches

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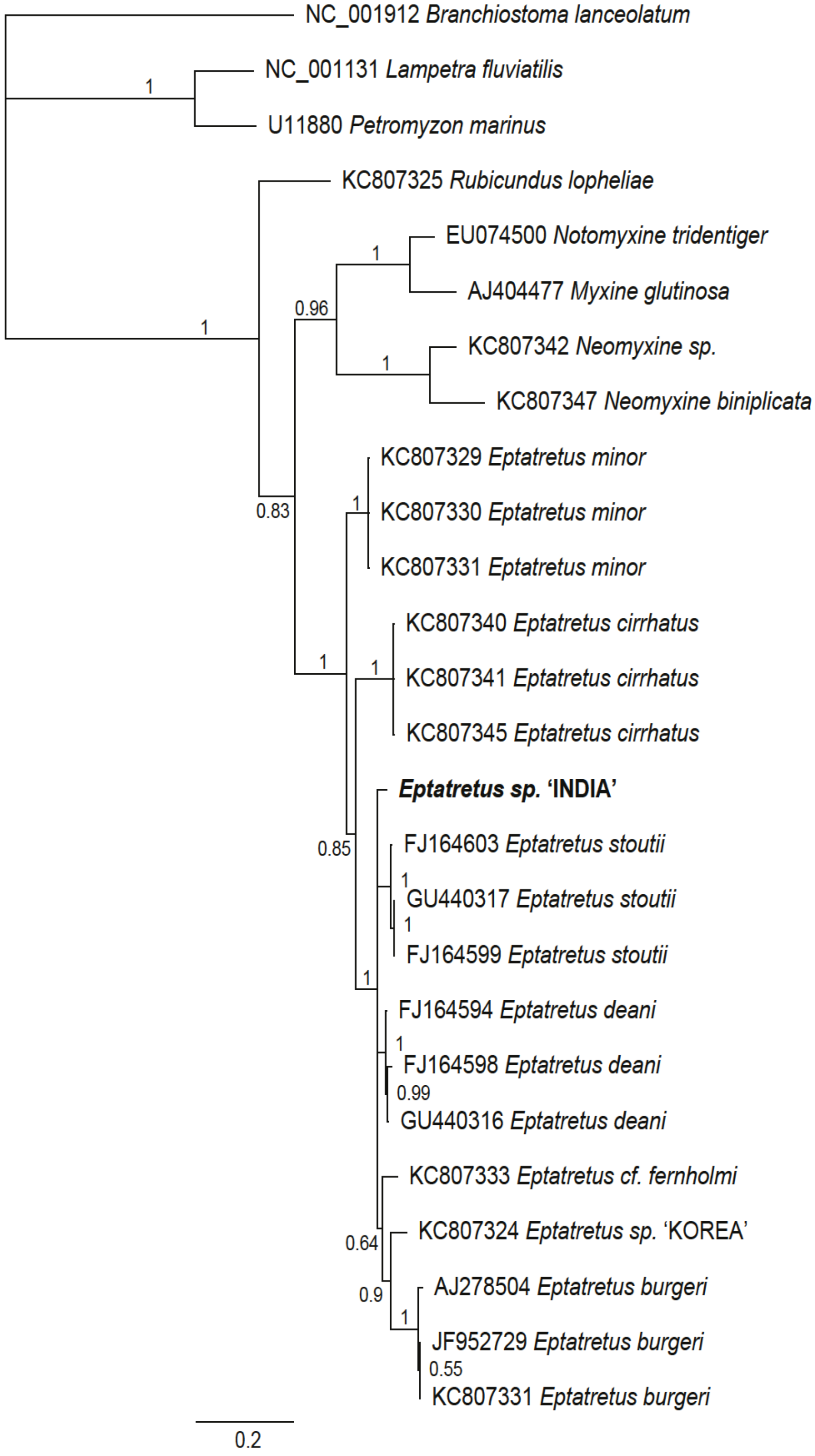

Figure 1. Bayesian majority-rule tree from analysis of mitochondrial COI DNA data. Values at nodes indicate Bayesian posterior probability estimates. Branch lengths are proportional to number of expected substitutions per site.

Image 1. Whole body photograph of *Eptatretus* sp. collected from the Indian coast

Image 2. Head of *Eptatretus* sp. collected from the Indian coast

but it is very likely an eight-gilled *Eptatretus* species. Slime glands cannot be accurately counted in the trunk because the animal has been severely damaged ventrally. Prebranchial, branchial and caudal slime pore counts are 11, 7 and 10 respectively.

Based on these data the specimen seems morphologically related to *Eptatretus octatrema* from South Africa, *E. indrambaryai* from Andaman Sea and *E. gonomi* from northwestern Australia since they are all eight-gilled, with tooth pattern 3/2. There are no DNA data published for any of these Indian Ocean species.

The 687 base-pair long 5' fragment of the mtCOI gene, the "barcode", from the hagfish from Kerala (GenBank accession no. KU320188) was aligned to a subset of the mtCOI dataset used in Fernholm et al. (2013), for a total of 28 sequences representing 15 species. A Bayesian analysis was performed as in Fernholm et al. (2013). The software Geneious (Kearse et al. 2012) with the plug-in Species Delimitation (Masters et al. 2010) was used to calculate P ID (Liberal), the probability of reciprocal monophyly under a model of random coalescence.

Genetic distance has been used as an aid in delimiting putative species in numerous studies, and empirically the mtCOI barcodes of two members of the same species typically differ by <1% (uncorrected pairwise *p*-distance), whereas ≥2% distance suggests that they belong to different species (for e.g., see Hebert et al. 2003; Ward 2009). A search of the GenBank non-redundant (*nr*) database and of the Barcode of Life database (BOLD) found no exact matches, and the closest match was *E. deani* (3.4%).

The phylogenetic analysis, summarized in Fig. 1, shows that *Eptatretus* sp. India is distinct from all other included species, and that the closest relative included in the analysis is *E. deani* from the west coast of USA. P ID (Liberal) for *Eptatretus* sp. INDIA is 0.96, indicating a high probability that an unknown member of the putative species would be correctly identified as a member. All genetic analyses support viewing *Eptatretus* sp. INDIA as separate and distinct from all other species in the analyses.

The most recent phylogenetic study of hagfish (Fernholm et al. 2013) divided the hagfishes into three subfamilies and described a new genus. Based on the results from that study and genetic, spatial and morphological information from our specimen we conclude that we have the found the first species of *Eptatretus* in Indian waters. Many hagfish species are known from only one specimen (Fernholm 1991). However, to formally name this particular specimen as a new species we believe it is advisable to wait for more specimens in order to provide for a more complete morphological analysis and preferably DNA data also for the other eight-gilled Indian Ocean species (*E. octatrema, E. indrambaryai* and *E. gonomi*) that seem to be morphologically most closely related.

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