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A new species of eight-gilled hagfish (Myxinidae: *Eptatretus*) off Kerala, Western Indian Ocean

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ABSTRACT

This paper describes *Eptatretus sunilii* Ida & Bineesh, a new hagfish species from the west coast of India. The specimens were caught as bycatch by a deep-sea shrimp trawler operated at a depth of 400-600 m and were landed at Sakthikulangara Fisheries Harbour, Kollam, Kerala. The fish differs from most other *Eptatretus* Cloquet 1819 by having eight pairs of gill pouches and eight numbers of gill apertures, 3/2 multi-cusp teeth, and 68 -78 total slime pores. Though appears to be similar to *Eptatretus indrambaryai* Wongratana, (1983), *Eptatretus octatrema* Barnard (1923), *Eptatretus okinoseanus* Dean, (1904), and *Eptatretus gomoni* Mincarone and Fernholm, (2010) in having the same number of gill apertures and 3/2 multi-cusp teeth, it differs from these species by having fewer slime pores (68-78), a total cusp count of 42, and longer branchial length (10.31-12.22 % TL), and formed a distinct clade in the ML phylogenetic tree.

Keywords: Hagfish, Western Indian Ocean, Molecular Taxonomy, Myxinidae, *Eptatretus sunilii*.

1. INTRODUCTION

Hagfish have been in existence since 300 Mya (Bardack, 1991). While they are given importance mainly as primitive organisms, they play an ecological role as scavengers and burrowers. They are commercially important for their "eel-like" skin as a source of leather. Additionally, they are fished extensively as a source of food (McMillan and Wisner, 2004; Gorbman et al., 1990). They release a copious amount of slime when provoked or under stress. Hagfish slime, a deterrent, is also known to exhibit specific properties and is increasingly used in biomedical and biomimetic research. They are also used to produce strong fabrics and petroleum-free plastics (Negishi et al., 2012; Fernholm and Hubbs, 1981; Fudge et al., 2005).

Hagfishes from Indian waters are poorly known, with only a limited number of specimens collected and recorded. So far, eight species of *Eptatretus* Cloquet 1819 have been recorded from the Indian Ocean. Recently, a species has been described from Kollam: *Eptatretus wadgensis* (Augustina et al., 2022). The species described in this paper is the second one from the same locality. The morphological characters of this species are compared with previously known 8-gilled species, *Eptatretus okinoseanus* Dean, (1904), *Eptatretus octatrema* Barnard, (1923), *Eptatretus indrambaryai* Wongratana, (1983), and *Eptatretus gomoni* Mincarone and Fernholm, 2010 (Dean, 1904; Barnard, 1923; Wongratana, 1983; Mincarone and Fernholm, 2010).

2. MATERIALS AND METHODS

On April 26th, 2022, a deep-sea shrimp trawler caught 31 hagfish specimens as bycatch at a depth of 400- 600 meters from the west coast of India. The specimens were landed at Sakthikulangara Fisheries Harbour, Kollam, Kerala. A morphological analysis was conducted in this study to understand the species. Terminologies, counts, and measurements follow those described by (Fernholm and Hubbs, 1981; McMillan and Wisner, 1984). The general anatomy of the body and dissection follow those demonstrated by (Mok and McMillan, 2004). The Cusps (anterior, posterior, multicusps, and unicusps) were counted and photographed under a stereo microscope. This study used digital vernier calipers to measure each morphometric character to the nearest 0.1 mm. All measurements are recorded as a percentage of total length (TL).

The specimens are deposited in the National Zoological Collections of the Marine Biology Regional Centre, Zoological Survey of India, Chennai. All counts and the number of gill apertures were taken from the left side of the body. In the case of damaged specimens, counts from the right side of the body have been considered. Counts and measurements considered are counts of anterior multicusps, posterior multicusps, anterior unicusps, posterior unicusps, the total number of teeth, count of gill apertures, counts for prebranchial, branchial, trunk, tail, and total slime pores; as a percentage of total length (TL): Preocular length, prebranchial length, branchial length, trunk length, tail length, depth at cloaca, branchial depth, body depth (including and excluding ventral fin fold (VFF)), tail depth, body width at pharyngocutaneous duct (PCD).

DNA Extraction, PCR Amplification, and Sequencing

DNA was extracted from the muscle tissue of the specimens using the Qiagen DNeasy Tissue Kit. FishF1 (5'TCAACCAACCACAAAGACATTGGAC-3') and FishR1 (5'TAGACTTCTGGGTGGCCAAAGAATCA-3') were the primer pair used for the PCR amplification of COI fragment (Ward et al., 2005). The extracted DNA was amplified in a 25 µl reaction volume, which contained 1.5 units of Taq DNA polymerase, 1X PCR buffer, 1.5mM MgCl2, 0.2mM of each dNTP, five pmol of each primer, and two µl l (25– 50ng) of genomic DNA. The PCR amplification was performed in a PCR thermal cycler (Eppendorf).

Amplification reactions were performed with the initial denaturation at 94°C for 5 min followed by 35 cycles at 94°C for 30 s, annealing at 50°C for 30 s followed by 72°C for 1 min and a final extension of 72°C for 10 min. PCR products were checked using 1% agarose gel electrophoresis and purified with a Qiagen PCR purification kit. The sequencing reactions were performed on an ABI 377 Genetic Analyzer. The partial COI mtDNA gene sequences obtained were deposited in GenBank with the accession numbers PQ095618–PQ095619.

Data analysis

The sequences obtained were aligned using the BioEdit software and inspected visually (Hall, 1999). They were authenticated using the NCBI BLASTn (Basic Local Alignment Search Tool) (https://blast.ncbi.nlm.nih.gov). The sequences were then compared with other closely related species, downloaded from the NCBI GenBank. Phylogenetic analysis was performed using the Maximum Likelihood method (ML) and Kimura 2-parameter model (K2P) using the MEGA 11 (Tamura et al., 2021). The bootstrap consensus tree inferred from 1000 replicates taken to represent the evolutionary history of the taxa analyzed. A discrete Gamma distribution was applied to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.5343)). The phylogenetic tree obtained was edited in the FigTree v1.4.4 (Rambaut, 2009).

Taxonomy

Eptatretus sunilii sp. nov. Ida & Bineesh.
Common name: Kerala Hagfish
(Figure 1-4; Table 1)
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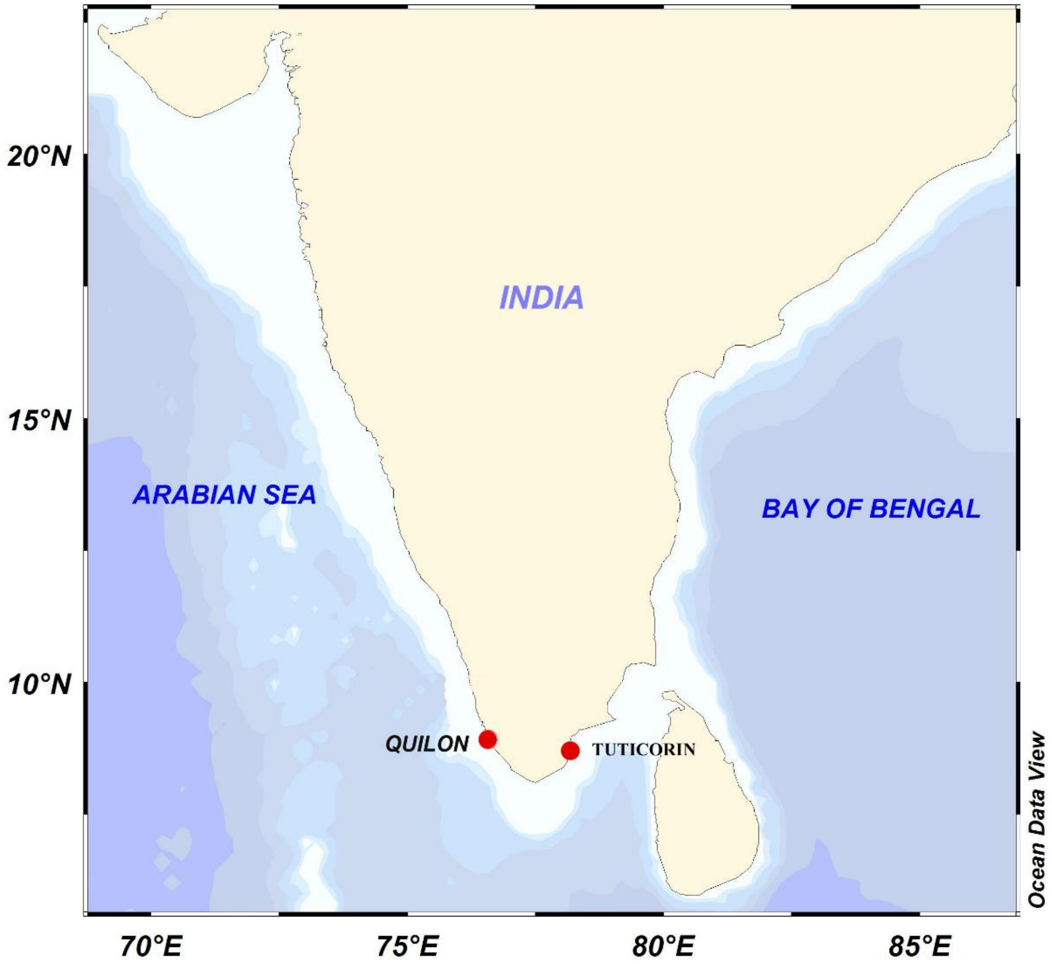

Figure 1 Collection localities of the new species *Eptatretus sunilii*

Figure 2 Holotype *Eptatretus sunilii*

Figure 3 Dentition of *Eptatretus sunilii* sp. nov.

Figure 4 Anatomy of *Eptatretus sunilii* sp. nov. showing gill pouches

Table 1 Morphometric and meristic data for *Eptatretus sunilii* sp. nov.

Characters	**Holotype**	**Paratypes**
Total length (TL, mm)	447	349 - 415
Sex	F	1F & 3M (2 not specific)
Gill aperture (GA)	8	8
Gill pouch (GP)	8	8
Cusps		
Multicusp pattern	3/2	3/2
Anterior unicusps	8	8
Posterior unicusps	8	8
Total cusps	42	
Slime pores		
Prebranchial	12	10-12
Branchial	7	7
Trunk	47	41-48
Tail	12	10-12
Total pores	78	68-76
Length (mm)		
Preocular	23.81	18.82-24.92
Prebranchial	98.77	68.6-87.05
Branchial	46.13	38.4-50.3
Trunk	230.43	192.45-225.69
Tail	59.75	43.65-58.27
Length in % TL		
Preocular	5.32	5.03-6.14
Prebranchial	22.10	17.27-21.03
Branchial	10.31	10.40-12.22
Trunk	51.55	51.87-59.47
Tail	13.37	11.22-14.78
Body Depth (mm)		
Branchial region	31.51	22.23 – 35.2
Body depth incl. VFF	36.95	23.6 – 36.95
Body depth excl. VFF	36.34	21.51 – 36.34
Tail depth	35.13	24.72 – 35.92
Cloaca	24.6	21.09 – 24.99
Depth in % TL		
Branchial region	7.05	5.71 – 8.48
Body depth incl. VFF	8.27	6.76 – 9.48
Body depth excl. VFF	8.13	5.53 – 9.17
Tail depth	7.86	6.35 – 8.65
Cloaca	5.9	5.42 – 7.16
Body width at PCD	30.02	23.22 – 37.8

Holotype

MBRCF 2893, Mature female, 447 mm TL, Sakthikulangara, Kollam, Kerala, south-west coast of India, 8°29′34″(N) 77°03′41″(E) 400-600 m depth, Collected on 26th April 2022 by Bineesh KK.

Paratypes

MBRCF 2894, Male, 415 mm TL; Paratype 2, Regd. No. MBRCF 2895, 415 mm TL; Paratype 3, Regd. No. 2896, Male, 389 mm TL; Paratype 4, Regd No. 2897, 7 specimens, Male & Female, 349-374 mm TL, Sakthikulangara, Kollam, Kerala, south-west coast of India, 8°29′34″(N) 77°03′41″(E) 400-600 m depth, Collected on 26th April 2022 by Bineesh KK.

Diagnosis

An eight-gilled species of *Eptatretus* with 3/2 multicusp pattern, eight anterior and posterior unicusps; 42 total cusps; total pores 68-78 (prebranchial 10-12, branchial 7, trunk 41-48 and tail 10-12). Brown-colored body; prominent eyespot; VFF inconspicuous.

Description

Morphometric and meristic data are provided in (Table 1). The TL of the specimens studied ranged from 371-447 mm. The body is eel-like with smooth, delicate skin that effortlessly dissociates when handling. The skin lacks scales. The species has a strongly compressed tail region that ends bluntly. Two rows of teeth: outer row consists of a 3-cusp multicusp and eight unicusps; the inner row consists of a 2-cusp multicusp and eight unicusps. The slime glands are bulb-like. The slime pores can be observed from the prebranchial region to the tail region, appearing as small white spots arranged almost in a straight line. The eighth efferent branchial duct (EBD) converges with the PCD on the left side of the body. VFF is very small. The first two barbels are almost the same length while, the third barbel is longer than the first two.

Coloration

The body is uniformly brown on dorsal and ventral regions in fresh specimens.

Distribution

Known from off the south-west coast of India in the Arabian Sea, at depths of 400–600 m

Etymology

The species is named in honor of Mr. Sunil Raj, a fisherman from Kollam, Kerala, in recognition of his contributions to the deep-sea fish collection from the south-west coast of India.

3. RESULTS AND DISCUSSION

The study of the specimens collected, in comparison with the other 8-gilled species of the genus *Eptatretus* Cloquet 1819, revealed a new species of the *Eptatretus* Cloquet 1819 that is described in this paper. *Eptatretus sunilii* sp. nov. is similar to *E. indrambrayai* Wongratana, (1983), *E. okinoseanus* Dean, (1904), *E. octatrema* Barnard, (1923), and *E. gomoni* Mincarone and Fernholm, (2010) in having 8-gill apertures and a 3/2 multicusp pattern.

However, it differs from *E. indrambrayai* Wongratana, (1983) in the number of slime pores (68-78 *v.* 77-82); total cusps (42 *v.* 45-48); branchial length (10.31% - 12.22% *v.* 8.61% -10.59 %); tail length (11.22% - 14.78% *v.* 15.78% - 18.36%); greatest body depth excluding fin fold (5.42% -9.17% *v.* 8.3% -10.3%); from *E. gomoni* Mincarone and Fernholm, (2010) in the number of trunk pores (41-48 *v.* 57-58), tail pores (10-12 *v.* 14-15) and total pores (68-78 *v.* 91-93), total cusps (40 *v.* 50), prebranchial pores (10-12 *v.*12-13); from *E. octatrema* Barnard, (1923) in total cusps (40 *v.* 42-46), prebranchial pores (10-12 *v.* 22-26), trunk pores (41-48 *v.* 63-68), total pores (68-78 v. 104-117); from *E. okinoseanus* Dean, (1904) in the total number of cusps (42 *v.* 46-49), prebranchial pores (10-12 *v.* 16-17), the total number of pores (68-78 *v.* 88-95).

An NCBI BLAST search identified the new species very close to the genus *Eptatretus* Cloquet 1819 (98-90%). Twenty-two downloaded COI gene sequences representing 12 species in the genus *Eptatretus* Cloquet 1819 and the sequence generated in this study of the newly described species *Eptatretus wadgensis* Augustina et al., (2022) (PQ095620- PQ095621) from the same locality were used for further analysis. In the ML phylogenetic analysis, the COI gene sequences of the new species formed a distinct clade with high posterior probability values. The percentage of similarity showed *Eptatretus sunilii* sp. nov. species related to *Eptatretus* sp. by 89.9%, *Eptatretus okinoseanus* Dean, (1904) by 72.3%, and *Eptatretus deani* by 74.5% - all with high posterior probability values (Figure 5). The phylogenetic findings of this study largely align with morphological character-based analyses and confirm the identification of the examined species as a new species.

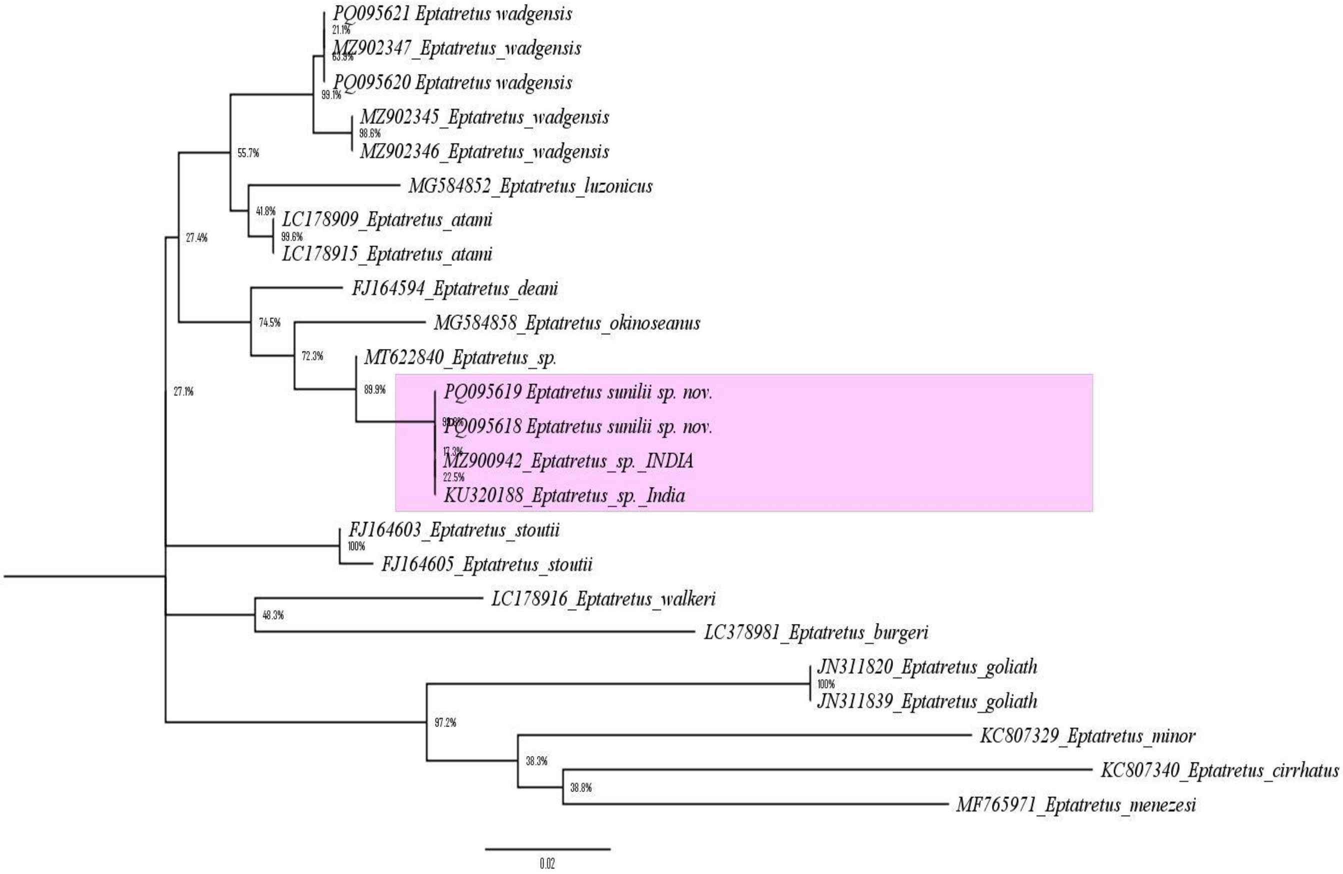

Figure 5 Maximum Likelihood method (ML) phylogenetic tree from the partial mitochondrial sequences of the species genus *Eptatretus*

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Author Contribution

IEG wrote the manuscript with support from BKK and EAS
BKK, IEG & RB prepared Figures 1-5 and Table 1.
BKK & IEG carried out morphological measurements and identification.
EAS & IEG worked out molecular identification. RB & BKK analyzed the results.
BKK, RB, HM & DB supervised and helped with conceptualization and manuscript correction and contributed to interpreting the results.

Ethical approval & declaration

In this article, as per the animal regulations followed in Zoological Survey of India, Marine Biology Regional Centre, Chennai, India, & Centre for Marine Living Resources & Ecology (CMLRE), Kerala, India & Zoological Survey of India, Kolkata, India, the authors observed a new species of eight-gilled hagfish (Myxinidae: *Eptatretus*) off Kerala, Western Indian Ocean, India. The Animal ethical guidelines are followed in the study for species observation, identification & experimentation.

Informed consent

Not applicable.

Conflicts of interests:

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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