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A Taxonomic Study of Family Hemiramphidae from Indian Waters

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Abstract

The order Beloniformes consists of minor pelagic fish species that are considered valuable resources and can be found in marine, brackish, and freshwater habitats. In India, although they do not contribute significantly to commercial fish landings, they play a remarkable role in the pelagic fishery of Tamil Nadu state, accounting for approximately 42.6% of the total beloniform catch in the country. During this study, taxonomic analysis was conducted based on morpho-meristic characteristics, otolith morphology, and DNA techniques. The results confirmed the existence of six distinct species including *Hemiramphus far, Hemiramphus lutkei, Hemiramphus archipelagicus, Hyporhamphus limbatus, Rhynchorhamhus naga and Rhynchorhamphus georgii* belonging to family Hemiramphidae, in Indian waters. In addition, DNA barcodes were generated for all the aforementioned species. These genetic sequences have been deposited in the Gene Bank, specifically in the BOLD Systems database. Notably, the study has generated barcodes by using COI gene for *Hemiramphus archipelagicus* and *Hemiramphus lutkei* for the first time.

Keywords:
Hemiramphidae, Beloniformes, *Rhynchorhamphus naga, Hemiramphus archipelagicus*

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Introduction

The species of family Hemiramphidae (Beloniformes), commonly known as halfbeaks, represents a significant pelagic fishery of India. These fish species widely distributed across the Atlantic, Indian, and Pacific Oceans, can be found in marine, brackish water, and freshwater environments worldwide (Froese and Pauly, 2023). Historically, the family Hemiramphidae was divided into two subfamilies: Zenarcopterinae and Hemiramphinae, as recognized by Collette (2004). However, recent taxonomic revisions have resulted in the elevation of Zenarcopterinae to the status of a separate family known as elevation of subfamily Zenarcopteridae, as documented by other authors (Lovejoy *et al*., 2004 and Nelson *et al*., 2016). Currently, the family Hemiramphidae, comprising of halfbeaks, encompass a total of 62 species distributed among 8 genera, *Arrhamphus*, *Chirodorus*, *Euleptoramphus*, *Melapedalion, Hemiramphus*, *Hyporamphus*, *Oxyporamphus*, and *Rhynchoramphus*, as reported in a recent study (Fricke *et al*., 2023). Out of them *Arrhamphus, Chriodorus, Melapedalion*, and *Oxyporhampus* are monotypic. Genus *Hemiramphus* (with ten species) includes a worldwide occurring marine species and *Rhynchorhamphus* (with four species), identified by fimbriate nasal papillae, are confined to Indo-West Pacific marine waters. Gopi and Mishra (2015) gave the numbers 5 genera and 16 species from India, while listed five genera and 15 species. Further, two more species were reported from Indian waters *Rhynchorhamphus naga* (Behera *et al.*,

2022) and *Hemiramphus archipelagicus* (Behera *et al.,* 2019). The species within the family Hemiramphidae share some distinctive morphological characteristics like shorter upper jaw compared to the lower jaw, which tends to be elongated in most juveniles and adults. An exception to this pattern is found in the genus *Oxyporhamphus*, previously classified as a flying fish in the Exocoetidae family but now placed within Hemiramphidae after recent revision (Collette, 2004). In the species of *Oxyporhamphus,* the upper and lower jaws are almost equal in length. In these fishes, the premaxilla is pointed anteriorly, forming a triangular upper jaw. Additionally, many species in this family exhibit red coloration at the tip of the lower jaw, indicating the presence of pigments such as astaxanthin, zeaxanthin, and beta-doradexanthin. The species within the Hemiramphidae hold consumer preference due to their food value. For example, *Hemiramphus far* is commercially exploited in various parts of the world, including the Arabian Sea coast of Pakistan (Fishstat, 2013). In some regions, certain species from this group are also used as bait fishes, such as in Australia (Collette, 1974). Moreover, species like *Hyporamphus limbatus* have an ornamental value, contributing to the economic well-being of fisher communities. Further, correct species identification is fundamental for biodiversity studies, conservation efforts, and effective fisheries resource management. It also plays a crucial role in further research related to growth, mortality, fecundity, trophic relationships, and paleontological events. In many cases, classical taxonomy alone may not be sufficient to distinguish cryptic species. Therefore, molecular approaches, including DNA barcoding, can be valuable tools for species identification. Additionally, hard structures like otoliths are commonly used for species identification, age determination, and stock identification in fisheries. However, identifying species within these particular groups can be challenging because of overlapping morphological characteristics among species. Hence, the present study aims to provide a useful information for distinguishing the species of family Hemiramphidae examined in this research and resolving the identification challenges.

Materials and methods

In this present study, specimens were collected from various coasts of Indian waters, including Maharashtra, Tamil Nadu, Kerala, and Odisha during July 2018 to March 2019. Immediately after collection, the specimens were carefully preserved in iceboxes and subsequently transported to the laboratory for further examination and identification. The identification of these specimens was performed through a combination of methods. Firstly, they were categorized into different group of species based on their morphometric measurements and meristic counts. Additionally, DNA Barcoding by using two sets of universal primer (Ward et al., 2005; Hajibabaei et al., 2005) was also employed for species confirmation. The identification keys provided by Collette (1984, 1999) were used for identification. To ensure accuracy, precise morphometric measurements and meristic counts recorded by using following methodology outlined by Hubbs and Lagler (2004). These measurements were standardized with a high level of precision, accurate to 0.1 mm, using a digital Vernier calliper. Selected morphometric characters were then expressed as percentages of the standard length (SL) and head length (HL), as detailed in Tables 1 and 2. The tissue (muscle) samples were extracted from the specimens for DNA Barcoding purposes and were carefully preserved in absolute alcohol. Additionally, one specimen from each species was fixed in 10% formalin solution and stored at the Fisheries Resource Management Laboratory (Specimen ID CHB), ICAR-Central Institute Fisheries Education (CIFE) in Mumbai, India, for future reference. Descriptive statistics was applied to analyse the morphological characteristics of different species, using R (version 3.3.3). DNA Barcoding was conducted by amplifying the Mitochondrial DNA cytochrome c oxidase-1 (COI) gene, which spans 650 base pairs, using universal primers. The resulting DNA sequences for all six studied species have been submitted to the BOLD Systems. The molecular analysis was carried out using MEGA-X, as described by Kumar *et al*. (2018). The otolith, also known as the "ear stone," is a rigid calcified structure located behind the brain in bony fishes. Fish possess three distinct types of otoliths: Lapillus, Sagitta, and Astericus. Among these, Sagitta stands out as the largest in most fishes and holds significance in fish taxonomy. Further, otolith was extracted from 5 species out of the 6 identified species. To extract the otoliths, the operculum was carefully removed to reveal the gills and lower jaw, reaching down to the skull's base and the otic capsule on both the left and right sides is delicately fractured, and the otoliths are then extracted using a scalpel, taking utmost care to avoid breakage. The extracted otoliths were cleaned and scrubbed with either alcohol or distilled water to eliminate any adhering organ tissue (Secor, 1992). Subsequently, the right and left otoliths were preserved individually in small plastic vials, setting the stage for further examination. The otolith images were captured using an Olympus SZX16 microscope, equipped with a zoom capability ranging from 0.7X to 2.5X. Identification and description of the various components of the otoliths was facilitated with the aid of an otolith atlas described by Tuset *et al*. (2008).

Results and discussion

A total 6 species were confirmed out of 16 species, recorded so far from India (Mohapatra *et al.*, 2020, Behera *et al.,* 2022). Specimens examined were *Hemiramphus far*, *Hemiramphus archipelagicus*, *Hemiramphus lutkei*, *Hyporhamphus limbatus*, *Rhynchorhamhus naga* and *Rhynchorhamphus georgii*. Altogether, 23 morphometric traits and seven meristic characters were measured. Morphometric characters were expressed in terms of their % of HL and % of SL (Table 1). The morphometric characters that were

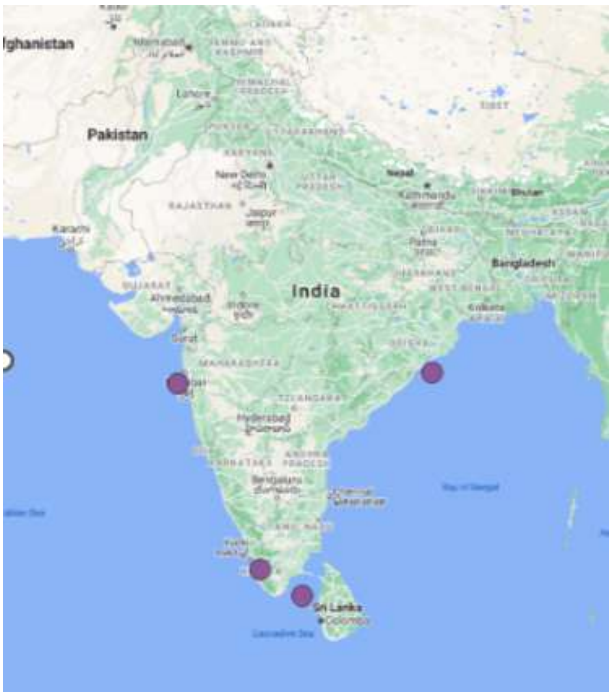

Fig.1. Map showing sampling locations of species under family Hemiramphidae from Indian waters.

found to be important in species identification are beak length, upper jaw length, upper jaw width, pre dorsal length, caudal peduncle length & width. The meristic characters included gill rakers, pre dorsal scale that varied among the different group of species. Similarly, the DNA Barcode and morphology for the otolith of identified species was also studied.

Rhynchorhamphus georgii (Valenncciennes, 1847)

Description: Body more elongated (219.33 to 278.77 mm SL) than other species. Prolonged beak,134.05 % to 181.78 % of HL, Nasal pectoral length 59.59 % to 71.83% of HL. The beak is slightly flattened with the prominent black fleshy membrane. The upper jaw length 24.97% to 32.05% of HL having prominent scales arranged over it. Pre dorsal length is 53.184 to 69.99 % of SL. A prominent line passes throughout the body from the lower part of opercular region to the origin of the caudal fin. Caudal fin slightly forked. Color: The strip passes through the lateral side of the body having black color on the upper margin and silvery on the lower side. The caudal fin is whitish with black marking on its margin. The origin of the lower jaw (beak) is glassy yellowish.

Rhynchorhamphus naga (Collette, 1976)

Description: Body slender (267.65 mm SL) with the prominent beak (171.82 % of HL) but less elongated in comparison with *R. georgii*. Nasal pectoral length is 59.34 % of HL. The upper jaw length is 25.18% of HL with prominent scales on it. Pre dorsal length is 51.62% of the standard length. A prominent silvery stripe passes throughout the body from behind the opercular region to the origin of the caudal fin. Caudal fin is slightly forked. Color: Bluish abdomen, the beak yellowish blue, a prominent lateral stripe passes throughout the body, slightly broadened towards the posterior position, lower lobe of caudal fin bluish and upper lobe yellowish blue. There is blue marking on anal fin and below the abdomen.

Hemiramphus far (Forsskål,1775)

Description: Body compressed, prolonged lower jaw (73.37-143.57 % of HL), no scales on the upper side of the upper jaw (12.76- 21.55 % of HL). Eyes are moderately large (23- 28.47 % of HL). Head length is 15.88-19.68 % of SL. Pectoral fin is short (9.41-14.37% of SL). Vomerine teeth are more than one row. Color: Body is slightly bluish in fresh condition. The upper lobe of the caudal fin and front part of dorsal fin is yellowish. Beak prominent with slightly reddish in color at its tip as well as lower side. Vertical black band is prominent in adult.

Hemiramphus lutkei (Valenciennes 1847)

Description: Body elongated with slightly triangular head, beak ranged from 29.52 mm to 68.79 mm (41.07-92.88% HL), caudal fin deeply forked compared with its closely related species *H. archipelagicus*. The white stripe on the lateral side of body is prominent and broader towards posterior. The Nasal pectoral length is longer than or nearly equal to the pectoral fin in all the specimens (65.85 - 81.46% of HL). Swimbladder with the alveolar chamber. Vomerine teeth are in a single row. Color: The fresh specimen is slightly reddish towards the posterior region. Caudal fin is deeply forked in preserved specimens. The silvery band faded in comparison with its closely related species *H. archipelagicus*.

Hemiramphus archipelagicus (Collette & Parin, 1978)

Description: Body slightly cylindrical and robust. The anterior part of the dorsal surface is concave. Compared to dorsal profile, the ventral profile is slightly arched from the tip of the snout to the base of the caudal fin. Tip of the upper jaw is flat than the *Hemiramphus lutkei*. Upper jaw is 12.83-20.13 % of HL. Nasal pectoral length is 6.73-8.33% of HL, prominent lower jaw is 74.86%-132.43% of HL. Color: The beak is prominent, reddish. The margin of both lobes of the caudal fin is blackish. Upper portion of the dorsal fin is blackish colour. Caudal fin forked but not prominently deep when compared with closely resembling species *H. lutkei*.

Hyporhamphus limbatus (Valenciennes,184)

Description: In the present study, the specimens examined were of medium size (136.31 to 170.05mm SL) with slightly elongated body, body depth is 10.05% to 12.22% of the standard length. An elongated beak is 65.25 - 128.57% of SL. The Nasal pectoral length is 65.89-88.42% of the SL. The pectoral fin length is 9.49% to 13.85% of the SL, Pre dorsal length is 45.97% to 70.19% of the SL. Prominent silvery white lateral line stripe. Caudal fin slightly forked and lower lobe larger than an upper. Swim bladder without alveolar

Rhynchorhamphus georgii

Rhynchorhamphus naga

Hemiramphus far

Hemiramphus archipelagicus

Hemiramphus lutkei (with alveolar chamber swim bladder)

Hyporhamphus limbatus

Fig: 2. 6. Species identified under family Hemiramphidae during the study

Table 1. Descriptive statistics (Mean) of morphometric ratios in 6 different species under family *Hemiramphidae*

Species	*R. georgii*	*R. naga*	*H. limbatus*	*H. far*	*H. lutkei*	*H. archipelagcus*
Locality	Mumbai	Kerala	Mumbai, Odisha	Kerala, Tamilnadu, Odisha	Tamil Nadu	Odisha
No. of specimens (mean SL)	15 (259.11 mm)	1 (267.65 mm)	14 (159.248 mm)	27 (201.396 mm)	26 (296.553 mm)	16 (236.028 mm)
Morphometric characters – ratio with HL						
ED	0.220	0.20	0.273	0.259	0.228	0.279
SNL	0.461	0.44	0.360	0.338	0.350	0.301
UJL	0.295	1.72	0.184	0.158	0.142	0.152
BL	1.603	0.252	1.045	1.205	1.028	1.213
IOD	0.283	0.237	0.265	0.275	0.232	0.242
IND	0.151	0.124	0.139	0.181	0.166	0.160
NPL	0.665	0.593	0.768	0.784	0.733	0.761
HL	0.166	0.167	0.171	0.180	0.187	0.187
PDL	0.572	0.551	0.628	0.594	0.624	0.618
PAL	0.600	0.592	0.648	0.645	0.672	0.656
PPvL	0.503	0.104	0.461	0.519	0.551	0.547
PPeL	0.186	0.184	0.193	0.201	0.205	0.204
DFBL	0.128	0.110	0.126	0.109	0.118	0.127
DFL	0.072	0.063	0.091	0.090	0.078	0.088
AFL	0.056	0.054	0.083	0.055	0.050	0.065
ABL	0.085	0.084	0.112	0.062	0.062	0.082
PeFL	0.094	0.081	0.114	0.121	0.135	0.128
BD	0.087	0.071	0.108	0.110	0.116	0.126
BW	0.058	0.056	0.076	0.067	0.065	0.073
DAD	0.085	0.070	0.099	0.101	0.101	0.105
CPL	0.037	0.055	0.054	0.062	0.053	0.058
CPW	0.042	0.033	0.045	0.049	0045	0.049

Note: Standard length or SL; Snout length SNL(1); Eye Diameter ED(2); Upper jaw length UJL(3); Beak length BL(4); Inter orbital Depth IOD(5); Inter Nasal Depth IND(6); Nasal Pectoral length NPL(7); Head length HL(8); Pre dorsal length PDL(9); Pre Anal length PAL(10); Pre pelvic length PPvL (11); Pre pectoral length PPeL (12); Dorsal fin length DFL(13); Dorsal fin Base length DBL(14); Anal fin Length ABL(15); Pectoral fin Length PeFL (16); Body Depth BD(17); Body Width BW(18); Dorsal Anal Distance DAD(19); Caudal peduncle Length CPL(20); Caudal peduncle Width CPW (21)

Table 2: Descriptive statistics (range) of meristic characters of 6 different species

Species	*R. georgii*	*R. naga*	*H. limbatus*	*H. far*	*H. lutkei*	*H.archipelagcus*
PeFR	9-11	9	9-11	9-12	9-11	10-11
DFR	14-16	14	13-14	8-13	12-15	13-14
PvFR	6	6	6	6	6	6
AFR	13-15	14	13-15	9-11	9-12	10-12
PDS	41-45	42	32-37	33-41	38-48	35-44
GR	45-58	41	24-28	25-34	31-45	23-28
BSR	7-10	10	8-10	9-16	8-16	9-11

Note: PeFR of Pectoral fin rays; DFR=Dorsal fin rays; PvFR= Pelvic fin rays; AFR=Anal fin rays; PDS=Pre-Dorsal scale; GR=Gill Raker; BSR= Branchiostegal Rays

chamber. A cartilaginous mass like projection just below the lower portions of the beak. Color: In the fresh specimen, body is greenish on the dorsal, greenish-yellow transparent with marking on the dorsal cephalic region. The silver stripe is prominent. Anal and dorsal fins are white with black color at the origin. Tip of the beak is fleshy and reddish.

In the R*hynchorhamphus* genus, which has four known species worldwide, *Rhynchorhamphus georgii* stands out due to its elongated lower jaw and longer upper jaw. *R. georgii* has rounded nasal pits, while in R. naga *it is* pointed. *R. naga* was not previously reported in Indian waters but is now documented in Kerala's Chetty Harbour. These species can be distinguished by gill raker count, jaw size, and a broad silvery stripe on the body. In Hemiramphidae, *Hemiramphus far* is the sole species with black vertical bands (Hata et al., 2018). *Hemiramphus lutkei* has thin, serration-free gill rakers, longer nasal-pectoral length, and a noticeable silvery stripe. *H. archipelagicus* differs with serrated gill rakers, shorter pectoral fins, longer nasal-pectoral length relative to head length, no silver stripe, smaller scales, elongated body, and is reddish when fresh. In this study *Hemiramphus lutkei* is having alveolar chamber on air bladder which is similar to earlier study Tibbettes (2007). *Hyporhamphus* species lack an alveolar chamber on the air bladder and have the shortest lower jaw length (BL) among Hemiramphidae genera. *Hypohramphus limbatus* is the species with cartilaginous mass like projection just below the lower portions of the extended lower jaw body greenish on the dorsal side in the fresh specimen, greenish-yellow transparent marking on the dorsal cephalic region and identified which is similar to earlier finding (Johny and Dominic, 2016). Furthermore, we have examined only two species i.e., *Rhynchorhamphus georgii* and *R.naga* out of 4 species under genus *Rhnchorhamphus*. *Rhynchorhamphus* is the only genus with a prolonged beak (Table 1) in comparison to head length (HL) and having fimbriae nasal papillae Rhynchorhamphus. *Rhunchorhamhus georgii* differs from other species, having dome shaped upper jaw and round shape nasal pit and a prominent strip passing through the lateral side of the body having black on the upper margin and silvery on the lower side. The caudal fin is whitish with black marking on its margin. The origin of the lower jaw (beak) is glassy yellowish. *Rhynchorhamphus georgii,* gillrakers (45-58), dorsal fin rays (14-16), anal fin rays (13-15) were found almost similar to the description of Collette (1976). However, a small variation (45-58 vs 47-67) in gillraker matching the range of earlier report (Varghese, 2005). A narrow range of gillrakers within the genus *Rhynchorhamphu*s may lead to misidentification of species. But as per description of Collette (1976), comparatively lower number of gillrakers, rays on dorsal + anal fin (28), and narrowest interorbital space, is sufficient to differentiate *R. naga* from the rest of the species within this genus. With the help of morpho-meristic characters, the species has been identified from Northeast coast of India (Behera *et al.*, 2019). Additionally, a new record of *Rhynchorhamphus naga* from Kerala, India, South Eastern Arabian Sea has been reported (Behera *et al.*, 2022). According to the previous checklist of halfbeaks of India, there were reports regarding the distribution of *Hemiramphus far* from east coast (Kayalvizhi *et al.*, 2018, Rameshkumar *et al.*, 2015, Gopalakrishnan *et al.*, 2010) as well as the west coast of India (Shaji et al., 2000, Bijukumar and Raghavan, 2015; Froese & Pauly (2023), *Hemiramphus archipelagicus* from both east coast (Varghese, 2005, Kayalvizhi *et al.,* 2018 and Behera *et al.,* 2019) as well as west coast (Bijukumar and Raghavan, 2015) and *H. lutkei* from east coast (Mohanty *et al.,* 2020, Kayalvizhi *et al.*, 2018) as well as west coast (Bijukumar and Raghavan, 2015) of India. Subsequently, species have

Fig. 3. Morphological view of otolith (sagitta) of five different species under family *Hemiramphidae*.

been identified and described with the help of otolith atlas (Tusset *et al*., 2008) and DNA Barcoding.

Hemiramphus archipelagicus, previously documented in both the western and eastern Indian Ocean, has been recorded along the Northeast coast of India in this study. Notably, it displayed a lower gillraker count (23-28), setting it apart from other species in its genus. Morphometric data in this study is aligned with prior findings for dorsal fin rays (13-14), anal fin rays (10-13), and Pre dorsal scales (32-34). Three specimens with higher pre-dorsal scale counts (39, 40, 41) were exclusively collected from the Tamil Nadu coast. Furthermore, *Hyporhamphus limbatus* was confirmed to inhabit both the East Madhusudhana (2014); Suresh et al., (2018) and West coasts, contrary to earlier reports of it being limited to one coast. *H. xanthopterus* was notably absent, likely due to its preference for freshwater-influenced environments in the Western Ghats. Meristic counts for *H. limbatus* matched with previous report (dorsal fin rays: 14, anal fin rays: 14, pectoral fin rays: 10-11, pelvic fin rays: 5). Key variables, including pre dorsal length, anal fin length, inter nasal distance, and nasal pectoral length, proved crucial for species differentiation. The meristic traits are established during early embryonic development and remain consistent throughout an individual's life. In this study, otolith shape variations among six species were observed which are likely to be influenced by environmental factors during early development. Otoliths, with their significant inter-specific variability, are valuable tools for fish species identification (Battaglia *et al.,* 2010). As only one specimen of *R. naga* could be collected, its otolith was preserved for further reference. Previous research noted otolith asymmetry in adult *R. georgii* regarding otolith length and width (Al-Rasady *et al.,* 2010). The study also generated reference DNA barcodes for six species by

Table. 3. Otolith morphology of six different species under family *Hemiramphidae*

Species	***Hyporhamphus limbatus***	***Rhynchorhamphus georgii***	***Hemiramphus far***	***Hemiramphus archipelagicus***	***Hemiramphus lutkei***
Features					
Otolith shape	Elliptical, margin lobated in small otoliths	Elliptic to cuneiform truncated	Elliptic to pentagonal, posterior and dorsal margin sinuate	Elliptic to pentagonal, posterior and dorsal margin sinuate	Elliptic to pentagonal, posterior and dorsal margin sinuate
Dorsal margin	Crenate, uneven	Lobate	Serrated irregular in case of small size	Crenate	Crenate
Ventral margin	Lobate	Round	Slightly lobate	Serrated	Serrated
Posterior margin	Rounded peak, slightly pointed	Round	Angled to round irregular	Round irregular	Round irregular
Excisura	Shallow without notch	Without notch	Without notch	Without notch	Without notch
Ostium and	Undifferentiated	Undifferentiated	Undifferentiated	Undifferentiated	Undifferentiated
Rostrum	Anterior part slightly peaked, short and broad	Peakedm rostrum short, broad & round to pointed	Peaked, rostrum short, broad, sharp-ended or round	Peaked, rostrum prominent, pointed, sharp-ended or slightly round	Peaked, rostrum prominent, pointed, sharp-ended or slightly round

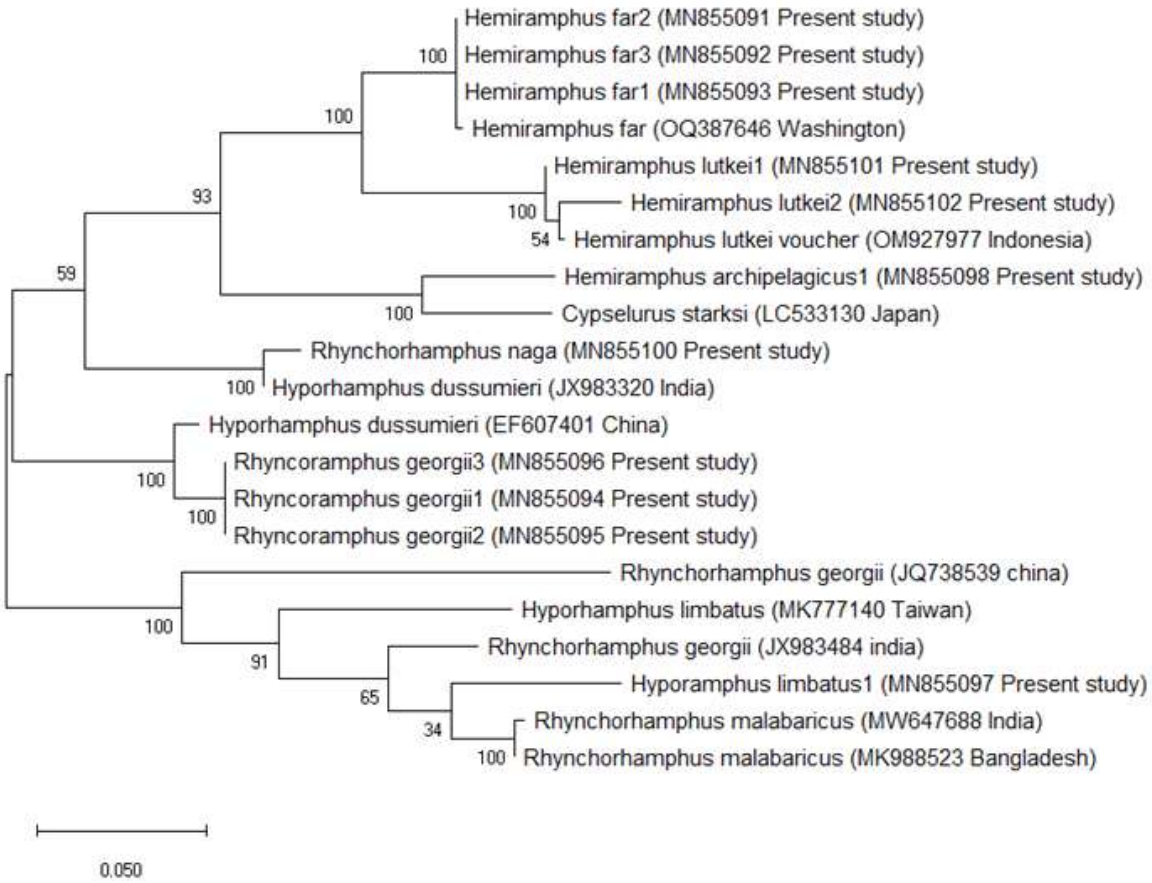

Fig. 4. Maximum likelihood tree of COI gene of 6 different species of the family *Hemiramphidae*

sequencing a segment of the COI gene, has low intraspecific divergence values (0.2%) compared to earlier reports. The congeneric divergence value (5%) was relatively low, possibly due to the small sample size. A maximum likelihood analysis was conducted to establish the phylogenetic relationship among the species, using *Cypselurus starksi* as the outgroup.

Conclusion

A study has been conducted for taxonomic assessment of species within the Hemiramphidae family, employing a range of methodologies such as the examination of morpho-meristic characters, otolith morphology, and DNA barcoding techniques. Instead of relying solely on traditional morphological traits, the study utilized DNA barcoding of the COI gene to confirm species identities, with the resulting sequences being deposited in the GenBank database. This research serves to resolve taxonomic ambiguities and contributes to the existing knowledge concerning this group of species. The present findings will be of considerable value to the scientific community, researchers, and policymakers. The implications of this study extend to various fields, including biology and stock assessment, and it may prompt further exploration to identify additional species.

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Conflict of interest

On behalf of all the authors I declare that there is no conflict of interest among the authors.

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