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Range extension and molecular confirmation of the critically endangered and endemic catfish *Hemibagrus punctatus* (Jerdon, 1849) (Siluriformes: Bagridae) from the southern Western Ghats

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Abstract

Hemibagrus punctatus (Jerdon, 1849) is endemic to the Western Ghats and native to the Cauvery River system. The species faces distributional uncertainty due to misidentifications and the lack of voucher specimens. This study confirms the range extension of *H. punctatus* from the Cauvery River tributary, Bhavani River to the Bharathappuzha River in Kerala. Samples were collected from both rivers, with larger number of samples collected from the Bhavani River (n=230). The analysis revealed subtle morphometric variations between the two populations in head length (21.2-27.3% *vs* 24.7-26.4% in SL), dorsal to adipose distance (16.5-21.2% *vs* 13.7-16.5% in SL) and eye diameter (12.6-17.8% *vs* 10.3-16.9% in HL). Species confirmation was done using the mitochondrial DNA (mtDNA) Cytochrome Oxidase I (COI) gene sequencing, and taxonomic position was compared with known *Hemibagrus* species. Genetic analysis revealed that *H. punctatus* differs from its sister taxon *H. menoda* with a divergence of 4.4% and other *Hemibagrus* species with 19.8% in partial mitochondrial COI gene sequences.

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Introduction

Hemibagrus are large bagrid catfishes that may attain a standard length of 800 mm and reside in major rivers of the Indian Subcontinent, South-east and East Asia (Ng *et al*., 2001). They inhabit diverse environments, ranging from fast-moving headstreams at high elevations to areas closer to estuaries (Ng and Kottelat, 2013). *Hemibagrus* species are considered as valuable food fishes throughout their geographic range, especially in South-east Asia (Dodson and Lecomte, 2015). *Hemibagrus* consists of 41 documented species. Several colourful species, notably *H. wyckii* and *H. wyckioides*, are exported as ornamental fish (Ng and Kottelat, 2013). Among the native Indian fish species, *Hemibagrus* comprises three species namely, *Hemibagrus maydelli*, *Hemibagrus menoda* and *Hemibagrus punctatus*. *H. maydelli* is so far confined to the Krishna River system of southern India (Dahanukar *et al*., 2011). *H. menoda* is known to be recorded in the Godavari, Mahanadi, Brahmaputra and Ganges Rivers in northern India and Bangladesh (Ng and Kottelat, 2013). *H. punctatus* is endemic to the Western Ghats and commonly referred to as Nilgiri mystus (Dahanukar *et al*., 2004).

H. punctatus, previously known as *Bagrus punctatus*, was reported by Jerdon (1849) from the River Cauvery in southern India. However, the exact type locality was not specified. *H. punctatus* was later documented by Day (1867, 1878) from the Bhavani River of the Nilgiri foothills. *H. punctatus* is generally regarded to be a rare species as initial records were limited to a single specimen (Rajan, 1955). The IUCN Red List (2010) categorised this species as 'Critically Endangered' (CR) due to its high risk of extinction (Dahanukar

et al., 2011). The last documented sighting was in 1998 and no detailed studies have since been conducted in the Bhavani River of the Cauvery drainage in the Western Ghats (Ali *et al*., 2013). This species was considered extinct from its native range, due to severe population declines caused by habitat degradation, pollution, siltation, overfishing and barrage construction by local communities for subsistence (Dahanukar *et al*., 2011; Ali *et al*., 2013). Subsequent works by Ali *et al*. (2013), Ng and Kottelat, (2013) and Praveenraj *et al*. (2019) confirmed the presence of the species, but its populations continue to decline in the wild (Ali *et al.*, 2013). Sustainable, community-based conservation measures are urgently needed and accurate species identification is critical for effective management and utilisation.

Hemibagrus taxonomy remains ambiguous, with the validity of many nominal species in question. A comprehensive analysis of the type specimens is lacking and species identification and phylogenetic reconstruction are challenging due to the region's complex biogeographical history, a lack of distinct morphological characters, and high phenotypic variability compared to other catfish groups. DNA barcoding helps overcome challenges of morphological identification and misidentification in both larval and adult stages (Tapilatu *et al.,* 2021). To resolve the taxonomy of *H. punctatus* and related species, comprehensive studies are needed. This study combines morphological and DNA barcode data to address the gaps in the geographic distribution and taxonomic understanding of *H. punctatus* within the Nilgiri Biosphere Reserve of the southern Western Ghats.

Materials and methods

Fresh samples of *H. punctatus* were collected from Bhavani (11°28'37.092"N; 77°17'57.264"E), Cauvery (11°23' 1.716"N; 77°42'45.216"E) and Bharathappuzha (10°55'13.296"N; 76°17'42.972"E) rivers of southern Western Ghats, India. The fish samples were collected with multi-meshed monofilament gill nets of mesh sizes 18, 30, 45, 60, 90, 110, 120 and 150 mm operated by local fishermen. Weight measurements were taken using a digital balance with an accuracy of 0.01 g. All the specimens were identified using morphometric characters and colour patterns. Morphometric measurements were obtained with digital calipers, recorded point to point with a precision of 0.1 mm. All counts and measurements were performed on the left side wherever feasible as per Ng and Kottelat (2013). All fresh specimens were photographed after collection and the tissues were stored in absolute ethanol for molecular analysis. Vertebral counts were taken from radiographs of the representative samples *i.e.,* the first vertebra bearing fully developed ribs was counted as the sixth one as per Roberts (1994). The type specimens were deposited in the museum of the Kongunadu Arts and Science College (KASC/BHA/110-111 and KASC/BHPU/112-113 specimen collection numbers) Coimbatore, India.

DNA extraction, PCR amplification and gel electrophoresis

The phenol/chloroform method was used to extract genomic DNA from the samples (Sambrook *et al*., 2001). Purity of the DNA was checked and stored at -80°C until further use. A partial sequence of the cytochrome c oxidase subunit I (COI) gene was amplified using the primers: Fish F1 5'-TCAACCAACCACAAAGACATTGGCAC-3' and Fish R1 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3' (Ward *et al.*, 2005). PCR amplification was performed in a 25 µl reaction mixture containing 1.5 units of Taq DNA polymerase, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 5 pmol of each primer and 2 µl (25-50 ng) of genomic DNA. Reactions were run in an Eppendorf thermal cycler under the following conditions: 35 cycles with initial denaturation at 98°C for 2 min, denaturation at 95°C for 30 s, annealing at 55°C for 45 s, extension at 72°C for 45 s and a final extension at 72°C for 5 min. Amplified products were visualised on a 1% agarose gel prior to sequencing.

PCR product cleanup and DNA sequencing

The amplified PCR products were treated with exonuclease I and shrimp alkaline phosphatase (USB, Cleveland, OH) for 15 min each at 37 and 80ºC, respectively, to eliminate any residual primer. The PCR products were directly sequenced using the BigDye® Terminator kit (v3.1) and analysed on an Applied Biosystems Genetic Analyser ABI 3130. All products were sequenced in both directions. The sequences were aligned and edited using Sequencer 4.7 (Gene Code Corporation). All the raw sequences were aligned through the visual method using BioEdit version 7.1.3.0.

Data analysis

The BioEdit sequence alignment editor (version 7.0.5.2) was used for the alignment of raw DNA sequences (Hall, 1999). Multiple sequence alignments were done using CLUSTAL X (version 2.0) and incorporated in BioEdit. MEGA II software was used for phylogenetic and molecular evolutionary analysis (Tamura *et al.,* 2021). According to the Kimura two-parameter model, the standard error of pairwise sequence divergence among populations was calculated in MEGA 11 (Tamura *et al.,* 2021). A graphical representation of divergence with 1000 replications was generated using Neighbor-joining (NJ) trees of K2P distance. COI mitochondrial gene sequences were sourced from NCBI for comparison and analysis.

Results

Systematic position

Order: Siluriformes

Family: Bagridae

Genus: *Hemibagrus* (Bleeker, 1862)

Species: *Hemibagrus punctatus* (Jerdon, 1849)

Synonyms

Mystus maydelli (Rossel, 1964)

Mystus punctatus (Jerdon, 1849)

Macrones punctatus (Jerdon, 1849)

Bagrus punctatus Jerdon, 1849: (Type locality: Cauvery River and its tributaries, India).

Fresh specimen colouration

Light yellowish brown on the dorsal side and dirty white on the ventral side in freshly collected samples. About 9-10 black spots on

a horizontal row along the lateral line, along the black spots, vertical rows of black lines below and above lateral line are present (Fig. 1).

Description

Morphometric and meristics data are given in Tables 1 and 2 respectively. The body is moderately compressed, depth 11.1-17.7% in standard length (SL) and width 13.9-19.2% in SL. The dorsal profile rises evenly from the tip of the snout to the dorsal fin origin, then slopes downwards gently to the end of the caudal peduncle. The ventral profile extends horizontally to the anal fin origin, then inclines towards the end of the caudal peduncle. Head broad, width 15.7-21.1% in SL and depressed. Predorsal profile is 33.5-40.4% in SL. Eye is 12.6-17.8% in HL and interorbital distance is 28.1-36.1% in head length (HL). Snout rounded, length is 34.4-39.8% in HL, upper jaw longer than the lower jaw. Occipital process not connected with a basal bone of the dorsal fin. Adipose dorsal fin base short, length 12.0-18.5% in SL. Adipose fin origin is separated from the end of dorsal fin of 16.5-21.2% in SL.

Teeth villiform: The upper jaw has two rows, a slightly curved uninterrupted outer row and crescent-shaped inner row with an

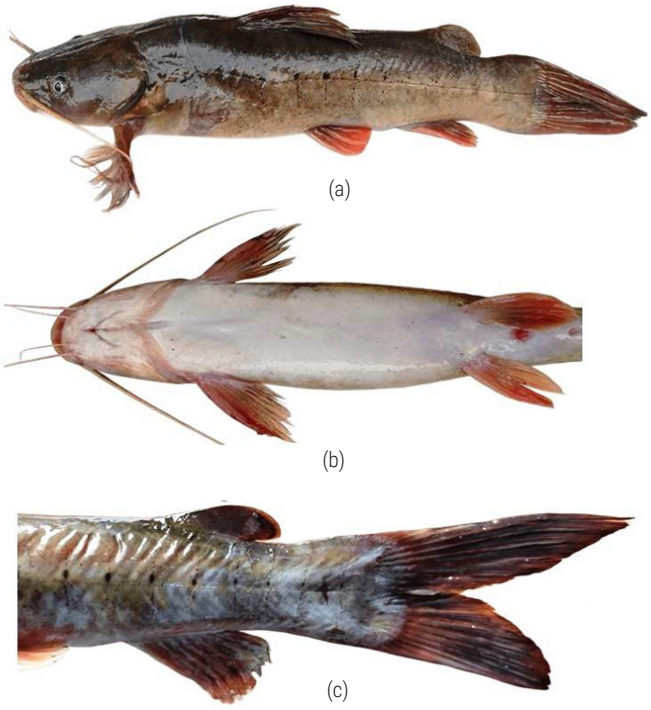


Fig. 1. *H. punctatus* from Bhavani River of southern Western Ghats, India. (a) Dorsal view; (b) Ventral view and (c) Caudal fin and adipose fin view

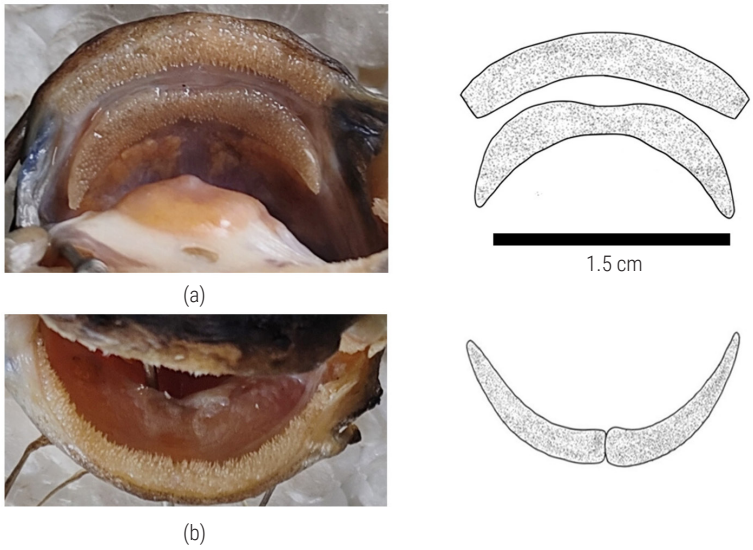


Fig. 2. Teeth pattern of *H. punctatus* from Bhavani River of southern Western Ghats, India (a) Upper jaw and (b) Lower jaw)

Table 1. Morphometric measurements of *H. punctatus* collected from various rivers in the present study, in comparison with that of Ng and Kottelat (2013) (*n* = Number of individuals)

Morphometric measurements	Bhavani River (n=30)	Cauvery River (n=5)	Bharathappuzha River (n=10)	Ng and Kottelat (2013) (n=4)
In SL%				
Head length	21.2-27.3	26.5-27.1	24.7-26.4	28.1–29.6
Head width	15.7-21.1	17.1-17.5	14.3-18.5	16.4–20.5
Head depth	8.3-14.5	12.9-13.6	11.0-12.6	11.9–14.3
Pre-dorsal distance	33.5-40.4	38.2-40.3	36.4-40.4	39.8–42.0
Pre-anal length	69.0-73.1	75.4-75.4	68.2-74.6	71.0–74.2
Pre-pelvic length	48.8-53.4	56.2-56.5	49.3-56.4	53.9–55.7
Pre-pectoral length	19.5-24.3	23.9-24.4	20.1-23.4	26.2–26.6
Body depth at anus	12.0-14.7	10.2-14.9	11.8-14.2	11.9–14.3
Length of caudal peduncle	14.3-18.5	16.4-18.1	13.8-18.9	16.1–18.6
Pectoral-spine length	13.4-20.2	16.3-17.2	13.8-17.7	15.6–18.1
Pectoral-fin length	16.8-22.2	17.4-17.7	15.0-18.9	18.7–21.1
Length of dorsal fin	20.1-26.0	18.5-19.5	15.8-19.7	24.7–27.6
Length of dorsal-fin base	14.5-16.8	16.7-17.5	14.4-17.1	14.7–17.4
Dorsal-spine length	12.3-17.9	13.4-13.4	10.8-13.6	13.9–15.9
Pelvic-fin length	11.0-17.2	11.2-12.8	12.3-14.7	14.9–17.2
Length of anal-fin base	10.7-13.5	12.5-13.2	11.6-12.9	11.8–14.3
Length of adipose-fin base	12.0-18.5	11.6-15.0	10.3-15.9	10.1–13.2
Maximum height of adipose fin	3.3-5.5	4.0-4.3	3.4-4.6	3.9–5.4
Dorsal to adipose distance	16.5-21.2	21.9-22.3	18.7-22.7	16.3–19.4
Post-adipose distance	12.9-17.0	14.3-16.0	13.7-16.5	14.8–16.2
In %HL				
Snout length	34.4-39.8	10.4-10.4	32.8-38.3	35.7–38.9
Interorbital distance	28.1-36.1	9.5-10.2	29.2-35.4	31.3–32.5
Eye diameter	12.6-17.8	3.0-3.8	10.3-16.9	13.8–15.7
Nasal barbel length	31.5-57.2	10.7-11.4	27.6-48.0	27.5–40.3
Maxillary barbel length	191.2-290.7	53.5-57.2	148.6-236.2	163.2–203.4
Inner mandibular barbel length	30.6-69.2	12.1-13.3	38.1-55.9	31.7–45.6
Outer mandibular barbel length	76.8-121.6	20.8-22.1	65.2-95.9	68.8–80.2

Table 2. Meristic counts of *H. punctatus* collected in the present study in comparison with that of Ng and Kottelat (2013) (*n* = No. of individuals)

Meristics	Bhavani River (n=30)	Cauvery River (n=5)	Bharathappuzha River (n=10)	Ng and Kottelat (2013) (n=4)
Unbranched dorsal fin rays	II	II	II	II
Branched dorsal fin rays	7	7	7	7
Unbranched anal fin rays	iv	iv	iv	iv
Branched anal fin rays	8	8	8-9	8 or 9
Unbranched pelvic fin rays	i	i	i	i
Branched pelvic fin rays	5	5	5	5
Unbranched pectoral fin rays	I	I	I	I
Branched pectoral fin rays	9	9	8-9	9 or 10
Caudal fin rays	i,7,8,i	i,7,8,i	i,7,8,i	i,7,8,i
Gill rakers on upper lobe	4-5	5	4-5	4 or 5
Gill rakers on lower lobe	13-15	14	13-14	8 or 13
Precaudal vertebrae	5 (fused)+20	5 (fused)+20	5 (fused)+20	25
Caudal vertebrae	20	20	20	21
Total vertebrae	45	45	45	46

uninterrupted tooth band, the lower jaw is narrow and rounded with an interrupted tooth band (Fig. 2). Lateral line complete along the midway of the body with 9-10 pores.

Pectoral fin rays have a spine and 9 branched rays. Pectoral spine stout and outer surface smooth and inner surface serrated with (22-spiny) serrations. The dorsal fin origin is nearer to the snout (Predorsal length - 33.5-40.4% in SL). A dorsal fin ray consists of a spine and 7 branched rays. Dorsal spine stout and the outer surface smooth, with 10-16 serrations posteriorly inside. Adpressed pelvic fin rays does not reach anal fin rays and have 1 simple ray

and 5 branched rays. Its pre-pelvic length is 48.8-53.4% in SL and its length is 11.0-17.2% in SL. Anal fin rays have 4 unbranched and 8 branched rays and its pre-anal distance is about 69.0-73.1% in SL. Caudal peduncle is broad (8.5-12.1% in SL). Caudal fin forked and upper lobe is longer than the lower lobe. Barbels four pairs. Maxillary barbel extends upto the pelvic fin. Nasal barbel extends to posterior border of eyes. Inner mandibular barbel extends along the midway of snout and pectoral fin. Outer mandibular barbel extends to the base of pectoral fin. Gill rakers 12-15 on lower, 4-5 on upper.

Distribution

H. punctatus is known to be distributed along the river Cauvery and its tributaries, yet type locality is not clear. The present study mainly explored Bhavani River and observed 230 samples of *H. punctatus* during the survey (Kodiveri, Nellithurai). This is comparatively the highest sample size studied from the entire Cauvery Basin (Fig. 3). In addition, the present intensive exploration revealed that *H. punctatus,* previously considered endemic to the Cauvery and its tributaries, has extended its range to the Bharathappuzha River in Kerala of southern Western Ghats, India (10 samples).

Vertebra

For the vertebral analysis, 2 samples from each site (Bharathappuzha and Bhavani rivers) were examined; no variation in counts was noticed *i.e.,* 5 (Fused) +20+20=45 (Fig. 4).

Biological observation

Mature female *H. punctatus* samples (Average weight : 270 g) were observed from Bhavani River. The paired gonads (Average weight : 60 g) appeared black in colour (Fig. 5).

Molecular analysis

The species was identified as *H. punctatus* based on 27 morphometric, 6 meristic characters and mitochondrial COI gene sequence analysis. Phylogenetic analysis confirmed *H. punctatus* as a genetically distinct species. COI analysis revealed genetic divergence within the genus ranging from 14.4 to 19.8%, with *H. punctatus* forming a sister clade to *H. menoda* with genetic distance of 4.4%, showing the greatest divergence from *H. filamentus* by 19.48% (Fig. 6).

Discussion

H. punctatus was first described by Jerdon (1849) as *Bagrus punctatus* from the Cauvery River, without mentioning the type locality of sample collections. Majority of the previous records were from the Bhavani River region (Ali *et al.,* 2013) and were based on a single specimen (Mukerji 1931; Hora 1937; Rajan 1955). The species is generally regarded as rare. Later, based on four specimens from the Cauvery tributaries in Tamil Nadu and Karnataka, this species was also thought to be extinct from its native range (Ali *et al.,* 2013). Previous records of *H. punctatus* by Singit *et al*. (1987) and Sugunan (1995) from the Tungabhadra Reservoir and Krishna River are likely misidentifications of *H. maydelli. H. punctatus* is confined to the Cauvery River, including its tributaries (Ali *et al*., 2013). On the other hand, *H. menoda* is typically found in the Brahmaputra, Ganges, Mahanadi and Godavari rivers in India, Nepal and Bangladesh, but it has also been reported from Pamba, Achankovil and Bharathappuzha rivers of Kerala and are not backed by voucher specimens which needs verification (Ali *et al.,* 2013). However, records of *H. punctatus* from Karuvannur (Thomas *et al.,* 2002) and Bharathappuzha, Chaliyar in Kerala (Biju, 2005) are taken into account as misidentifications (Raghavan and Ali, 2011), as there are no voucher specimens to support their distribution confirmation (Ali *et al*., 2013).

The findings from the 230 samples examined from the Bhavani River, suggest the type locality of the species as Bhavani River. In addition to correcting earlier misidentifications from Bharathappuzha,

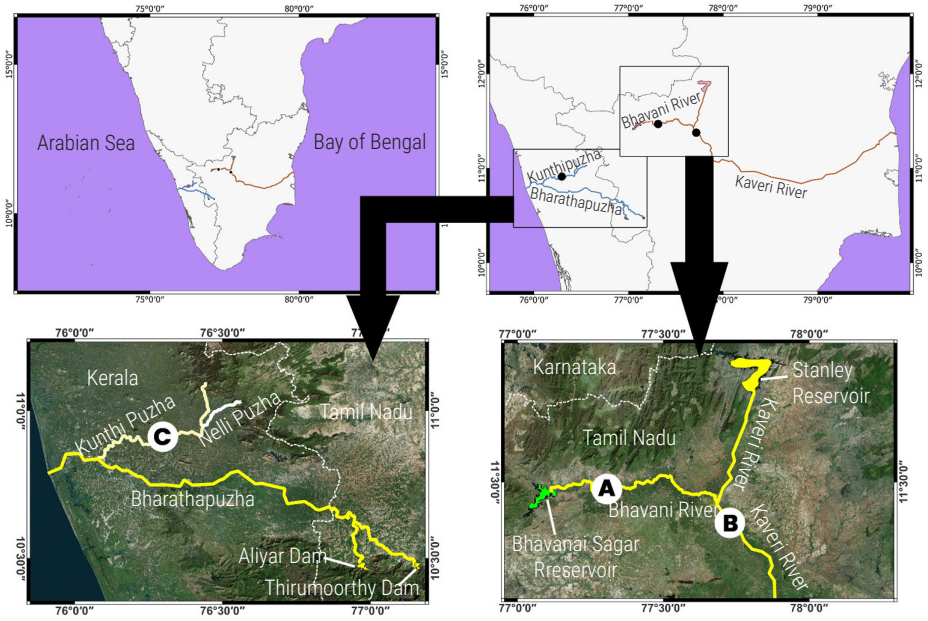
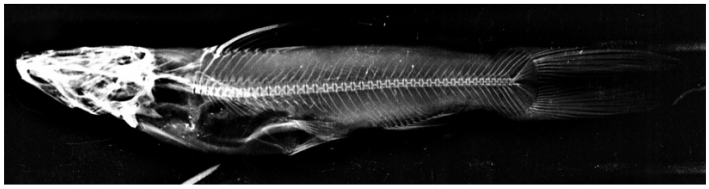
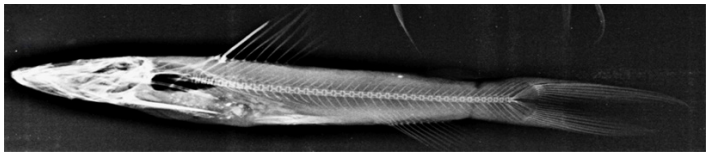


Fig. 3. Distribution map showing collection localities of *H. punctatus* from Bhavani and Bharathappuzha rivers of the southern Western Ghats, India

(a)



(b)



(c)

Fig. 4. (a) *H. punctatus* of southern Western Ghats, India. X-Ray image of *H. punctatus* from (b) Bharathappuzha River and (c) Bhavani River

Fig. 5. *H. punctatus* female with mature gonads from Bhavani River of the southern Western Ghats, India

this study provides a valid comparison to support accurate species identification and inform conservation. The present study documented 10 specimens from Bharathappuzha River, Kerala, confirmed as *H. punctatus* based on morphological and molecular analyses, making the first verified record of the species from the Bharathappuzha River. The findings highlight the need for intensive periodical survey of the Bhavani River basin and adjacent rivers to assess population status and guide conservation efforts for *H. punctatus*. The species differs from *H. caveatus* in having a pattern of dark spots arranged in vertical columns (thin vertical lines and a midlateral line) along the sides of the body. It differs from its congener *H. maydelli* by the absence of melanophores in the fins. In contrast, *H. maydelli* posses melanophores in an inter-radial manner along the pectoral, pelvic, anal and caudal fins, except the dorsal fin (where the melanophores evenly distributed). *H. punctatus* differs from its congener *H. menoda* in having shorter head length (32.7-33.5% *vs* 21.2-27.3% in SL) and in pre-dorsal distance (42.2-45.3% *vs* 33.5-40.4% in SL) and also grey fins *vs* reddish fins. Morphological difference is noted in certain characters such as head length (28.1-29.6% *vs* 21.2-27.3% in SL), pre-dorsal length (39.8-42.0% *vs* 33.5-40.4% in SL), pre-pelvic length (53.9-55.7% *vs* 48.8-53.4% in SL), length of adipose fin base (10.1-13.2% *vs* 12.0-18.5% in SL) and inter-orbital distance (31.3-32.5% *vs* 28.1-36.1% in HL) between the data recorded by Ng and Kottelat

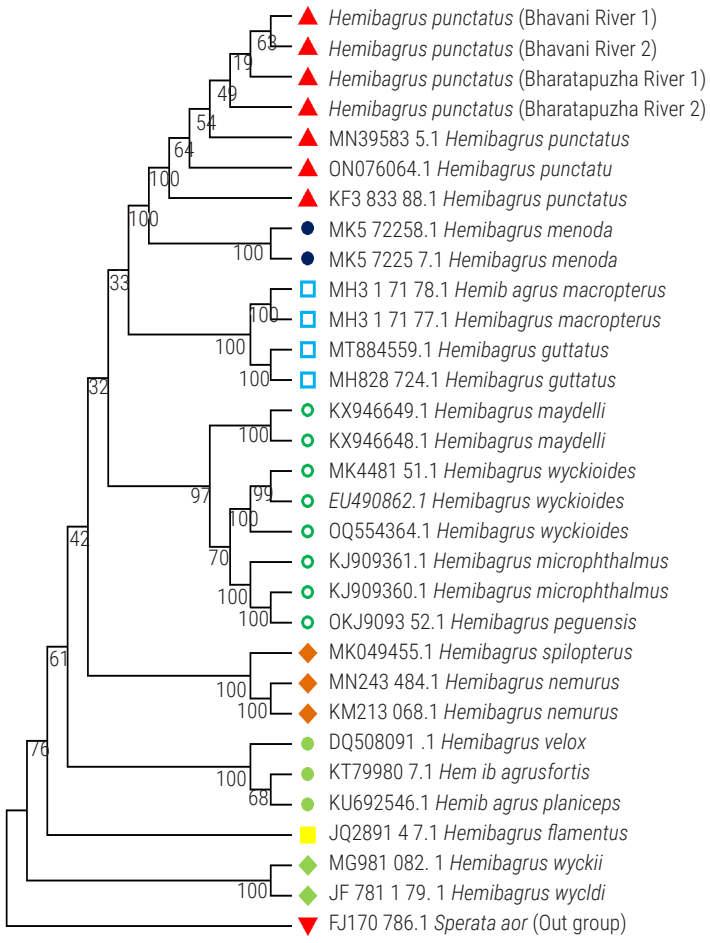


Fig. 6. Phylogenetic tree (phylogram) of *H. punctatus* with closely related *Hemibagrus* species based on Bayesian analysis of 610 bp fragments of the mitochondrial COI gene

(2013) and morphometric measurements of the samples from the Bhavani River of the present study. The meristic counts of the present study agree with the meristics by Ng and Kottelat (2013) except gill rakers (13-15, 4-5 *vs* 4+8 or 5+13). However, the vertebral counts of *H. punctatus* in the present study (45) differ from those reported by Ng and Kottelat (2013), which is 46.

H. punctatus from the rivers Bhavani and Bharathappuzha showed noticeable differences in morphometrics. Samples from the Bharathappuzha River revealed longer pre-dorsal length (36.4-40.4% vs 33.5-40.4% in SL) and dorsal to adipose distance (18.7-22.7% *vs* 16.5-21.2% in SL) compared to specimens of the Bhavani River. Samples from the Bhavani River had slightly larger eyes (12.6-17.8% *vs* 10.3-16.9% in HL) and longer head length (21.2-27.3% vs 24.7-26.4% in SL) and the number of gill rakers in the first arch (13-15, 4-5 *vs* 13-14, 4-5). The morphometric analysis of *H. punctatus* specimens from the Bharathappuzha and Bhavani rivers showed noticeable variation in certain body proportions. *H. punctatus* is morphologically distinct from its congener *H. menoda* (Ng and Kottelat, 2013) by having a longer head (21.2-27.3% *vs* 32.7-33.5% in SL), shorter adipose fin base (12.0-18.5% *vs* 13.0-15.8% in SL), and a larger eye diameter (12.6-17.8% *vs* 11.9-12.3% in HL).

Additionally, differences were also observed in the number of gill rakers on the first gill arch (13-15, 4-5 *vs* 3+9 or 4+14). *H. punctatus* is morphologically distinguished from *H. maydelli* (Ng and Kottelat, 2013) by shorter head (21.2-27.3% *vs* 30.8-32.4% in SL), shorter pre-dorsal length (33.5-40.4% *vs* 42.0-46.7% in SL), and longer distance between the dorsal and adipose fins (16.5-21.2% *vs* 4.0-7.0% in SL). It also has a longer snout (34.4-39.8% *vs* 31.1-35.2% in HL), a larger eye (12.6-17.8% *vs* 11.5-12.3% in HL) and a higher number of gill rakers on the first gill arch (13-15, 4-5 *vs* 3+9).

In the molecular analysis, DNA barcoding with the mtDNA cytochrome c oxidase subunit I (COI) gene proved effective for species identification using short sequences. The obtained mitochondrial fragment was 704 bp in length. A phylogenetic tree depicts the evolutionary ancestry of a species, organism, or common ancestor (Hall, 2011). The phylogenetic tree reveals genetic relationships both within and between populations (Sowmiya *et al.,* 2025). According to Maistrenko *et al.* (2020), phylogenetics helps organise biological diversity for structural classification and offers insights into evolutionary events. A Neighbor-joining (NJ) tree was constructed using the present study samples from the Bhavani and Bharathappuzha rivers, along with reference sequences retrieved from the NCBI database. *H. punctatus* from the Bhavani River differed from that of the Bharathappuzha River by a K2P genetic

distance of 0.18% in the COI gene. BLAST analysis indicated that *H. punctatus* was most closely related to *H. menoda* showing 4.4% genetic variation, followed by *H. macropterus* (12.54%), *H. maydelli* (12.97%) and the highest divergence with *H. filamentus* (19.48%).

These findings enhance understanding of catfish biology and support the development of effective conservation and management plans. The study reveals a highly diverse lineage that genetically confirms its status as a distinct species. However, comprehensive morphological and molecular analyses from additional locations are required to identify potential multiple lineages in the southern Western Ghats. This research refines knowledge of the species' distribution and taxonomy while underscoring the urgent need for targeted conservation measures to protect this critically endangered species and its habitat.

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