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An Extensive Taxonomic Analysis of Catfish Species (Family: Bagridae) Using Mitochondrial DNA (mtDNA) from the Cauvery River Basin in Tamil Nadu, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study investigates the morphological, molecular, osteological, and evolutionary aspects of freshwater Bagrid catfish in the Western Ghats of India, focusing on the Cauvery River basin. Samples from three genera of the Bagridae family, namely *Hemibagrus, Sperata,* and *Mystus,* were collected from the Cauvery and Bhavani rivers. The study aims to assess the diversity and threatened status of Bagridae catfishes in the region, identify morphological discrimination using mitochondrial DNA (mtDNA), analyze phylogenetic relationships, and understand their evolutionary process from common ancestries. Morphological and meristic characterization was performed, and genetic analysis was conducted using the cytochrome oxidase subunit 1 (COX1) gene. DNA was extracted from tissue samples, amplified using specific primers, and sequenced. The study highlights the need for taxonomic revision of Bagridae catfishes in the Western Ghats, as previous classifications were primarily based on morphometric analysis, leading to confusion and invalid species claims. The findings contribute to a better understanding of the diversity, taxonomy, and evolutionary history of Bagrid catfishes in the Western Ghats, which is crucial for their conservation, as more than 50% of the species are threatened with extinction due to various factors such as pollution, biological resource use, and habitat modification.

Keywords: Catfish; bagridae family; river basin; diversity; morphometric analysis; phylogenetic relationships.

1. INTRODUCTION

Catfishes, classified under the order Siluriformes, represent a significantly diverse group of ray-finned fish renowned for their distinctive barbels, akin to a cat's whiskers. These species are geographically widespread, inhabiting every continent except Antarctica, and thriving in a wide array of aquatic environments including freshwater rivers, lakes, and marine ecosystems (Sharma et al., 2023) This extensive diversity is underscored by the identification of over 3,000 species, positioning catfishes as one of the most varied and widespread groups of freshwater fish globally (Felix Ouma & Barasa, 2022). The order Siluriformes, colloquially referred to as 'catfishes' (Tamil- ‘keluthi or keliru’), constitutes an integral component of wetland ichthyofauna, with many species holding significant economic value due to their high nutritional content. This order is well-defined, encompassing approximately 35 families, 437 genera, and around 2,734 species worldwide, with the Indian subcontinent hosting 158 inland species distributed across 51 genera and 13 families (Jayaram, 2010). Notable families in India include Bagridae, Siluridae, Schilbeidae, Pangasiidae, Amblycipitidae, Sisoridae, Clariidae, Heteropneustidae, Chacidae, Olyridae, Akysidae, Ariidae, and Plotosidae (Talwar & Jhingran, 1991).

Among these, the genus *Mystus* within the family Bagridae features prominently, characterized by small to medium-sized species prevalent in freshwater habitats across West, South, and Southeast Asia (Arunkumar & Arunachalam, 2018). Although global studies on Bagrid catfishes are extensive, in India, the research remains comparatively limited. While some taxonomic literature is available (Jayaram, 2009), the areas of osteology and molecular phylogeny are underexplored, leading to classifications predominantly based on morphometric analysis. Traditional taxonomy faces challenges such as synonymy and misidentification, necessitating a taxonomical revision, especially within the Western Ghats region (Dahanukar et al., 2011). Recent advancements include the identification and re-evaluation of freshwater catfishes through DNA barcoding in Northeast India (Bhattacharjee

et al., 2012). However, the diversity within the Western Ghats remains inadequately understood. This region's complex taxonomic landscape has rendered some biological studies inconclusive. An extensive systematic revision of Indian Bagridae requires comprehensive material examination, as evidenced by critical taxonomical studies in River Cauvery's tributary, River Bhavani. Morphological, molecular, and osteological variations have been observed across the genera *Mystus, Hemibagrus*, and *Sperata* underscoring the need for detailed phenotypical analyses.

The family Bagridae facing significant threats from pollution, habitat modification, and overexploitation, with more than 50% of species at risk of extinction (Dahanukar et al., 2011). Understanding their taxonomy, diversity, and evolutionary history is vital for conservation efforts. This study aims to bridge existing knowledge gaps by examining the morphological, molecular, osteological, and evolutionary attributes of freshwater Bagrid catfishes in the Western Ghats, with a focus on the Cauvery River basin.

2. MATERIALS AND METHODS

2.1 Sample Collection

The samples were collected from the rivers Cauvery and Bhavani, targeting three genera within the family Bagridae: *Hemibagrus, Sperata*, and *Mystus* (Map. 1). The species identified in this study included *Hemibagrus punctatus*, *Sperata aordies*, *Mystus cavasius* and *Mystus bleekeri*. Sample collection involved direct collection from the rivers using nets, as well as procurement from local fish markets. The collected samples were preserved in 4% formalin, while tissue samples were transferred to 98% ethanol for subsequent DNA extraction. Collection efforts took place every Sunday, coordinating with local fishermen to gather the necessary specimens.

2.2 Morphological and Meristic Characterization

Total 30 morphological characters such as Standard length, Pre dorsal length, Preanal length, Pre pelvic length, Pre pectoral length, Dorsal-spine length, Dorsal-fin length, Length of dorsal-fin base, Length of anal-fin base, Pelvic-fin length, Pectoral-fin length, Pectoral-spine length, Caudal-fin length, Length of adipose-fin base, Maximum height of the adipose fin, Dorsal to adipose distance, post-adipose distance, Length of caudal peduncle, Depth of caudal peduncle, Body depth at anus, Head length, Head width, Head depth, Snout length, Interorbital distance, Eye diameter Nasal barbel length, Mandibular barbel length, Inner mandibular barbel length and Maxillary barbel length were taken for the morphological analysis. The meristic characters such as Dorsal fin, Pectoral fin, Pelvic fin, Anal fin And Caudal fin were measured according to Ng et al., (2013).

2.3 Genetic Analysis

In the current study, a total of 70 samples were collected from the rivers Cauvery and Bhavani. These included samples from three genera within the family Bagridae: *Hemibagrus, Sperata*, and *Mystus*. Specifically, the species *Hemibagrus punctatus, Sperata aordies, Mystus cavasius* and *Mystus bleekeri* were included. The samples were collected directly from the rivers using nets, as well as from local fish markets. Upon collection, the samples were stored in 4% formalin for preservation, while tissue samples were transferred to 98% ethanol for DNA extraction. For DNA extraction, two samples from each species were used. The DNA was extracted from tissue and gill samples kept in 100% ethanol, following protocols outlined by Ali et al., (2013) and Dahanukar et al., (2011). Tissue samples were digested at 60°C for 2 hours using STE buffer (0.1M NaCl, 0.05 M Tris-HCl, 0.01M EDTA, 1% SDS) with 15 µl Proteinase K (20 mg/ml) per 500 µl of STE buffer. The conventional phenol-chloroform method was employed to extract DNA, which was then re-suspended in nuclease-free water. The quality of extracted DNA was verified using 1% Agarose gel electrophoresis and Nanodrop analysis, and the DNA was stored at -20°C.

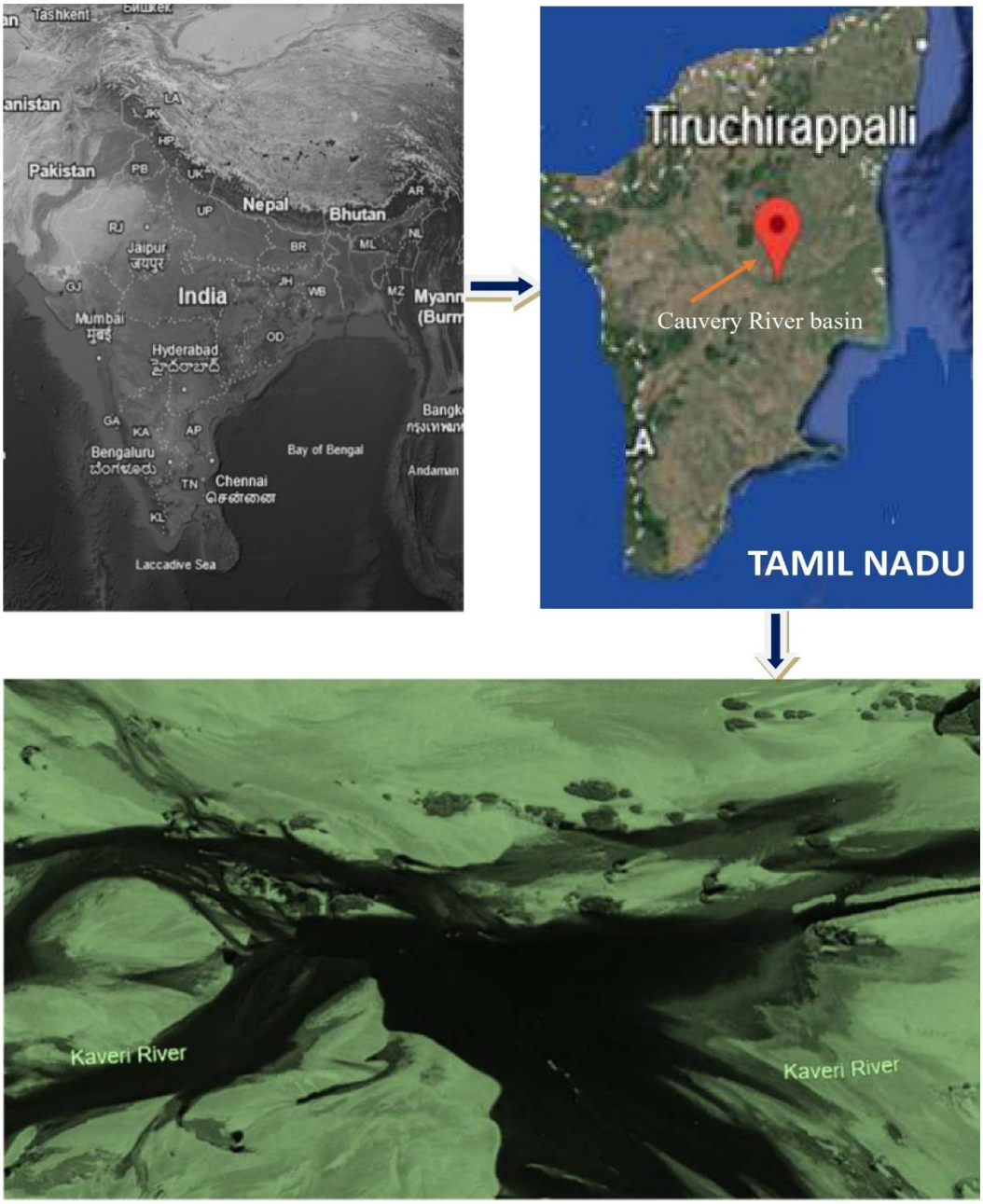
2.4 PCR Analysis

Total genomic DNA was extracted from alcohol-preserved tissue using a DNA assay Blood and Tissue Kit (Qiagen, UK). The primers Fish F1 (5' - TCA ACC AAC CAC AAA GAC ATT GGC AC - 3') and Fish R1 (5' - TAG ACT TCT GGG TGG CCA AAG AAT CA - 3') were employed to amplify a partial sequence of the mitochondrial COI gene, following the protocol described by Divya et al. (2017). PCR was performed for the Cytochrome Oxidase subunit 1 gene, and the PCR product was assessed for purity and length using 1% Agarose Gel, observed under a UV Transilluminator EP-04. Raw DNA sequences were edited and aligned using BioEdit version

7.0.5.2 (Hall, 1999), and uncorrected pairwise distances were calculated in MEGA XI (Kumar et al., 2018).

2.5 Bioinformatics Analysis

Maximum likelihood analysis was conducted using Mega X, employing BLAST (Altschul et al., 1990) and DAMBE (Xia, 2013). Phylogenetic trees were generated using MEGA 6 (Tamura et al., 2013). For comparative analysis, 70 sequences from the Bagridae family were downloaded from NCBI GenBank. A Maximum Likelihood (ML) tree for the COX1 gene was constructed with the reliability of clustering assessed through 1000 bootstrap iterations. In this comparative study, Channa striata was utilized as an outgroup for both genes.



Map 1. Aerial perspective of the study site concerning catfish species within the Cauvery River basin in Tamil Nadu, India

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown below the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. This analysis involved 17 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 583 positions in the final dataset. Evolutionary analyses were conducted in MEGA11.

2.6 Osteological Analysis

The osteological analysis was done by using an x-ray. The fish x-ray was taken and the skull structure and vertebral column numbers were studied (Rahul et al., 2017).

3. RESULTS

A total of four species were collected during the study from the Bhavani River and Cauvery River. These included one species from the *Hemibagrus* genus, one from the *Sperata* genus, and three from the *Mystus* genus. Morphometric and meristic characters, along with molecular analysis, were used to confirm the species as *Hemibagrus punctatus, Sperata aorides, Mystus cavasius* and *Mystus bleekeri*. The taxonomic analysis effectively revealed the identity of each of these species, contributing to the understanding of their diversity and classification within the Bagridae family.

3.1 *Hemibagrus punctatus*

Hemibagrus punctatus, described by Jerdon, (1849) from the Cauvery River and its tributaries in southern India, has a light brown body that fades to an unclean white ventrally. Black spots arranged in horizontal rows are present on the lateral surface, with dull greyish-brown fins that have scattered melanophores. the morphometrics were shown in Table 1. The fins have a lighter hue along the posterior edge, and the fin formula was Dorsal fin 1+8, Pectoral fin 1+7, Pelvic fin 1+6, Anal fin 1+8, and Caudal fin U=8; L=7 (Table 2). The species' color pattern includes large spots aligned with the sense organ, similar to *Hemibagrus menoda* and *Hemibagrus peguensis*. However, *H. punctatus* is distinguished by the consistently larger spots in the middle of the columns along the lateral line organ (Fig. 1A).

3.2 *Sperata aorides*

Sperata aorides is endemic to the Cauvery River and its principal tributaries in southern India. The body of the fish is silvery at the edges, white on the underside, and bluish-grey on the back. Paired fins are tinged with red at the bases. Dorsal and anal fins are dark with chromatophores, while the outer margins are notably dark. The anal fin's leading edge and the lowest rays of the tail fin are almost white, with the rest being grey. A dark spot near the adipose fin's posterior margin stands out. The body slopes evenly from the snout to the dorsal fin and then to the caudal peduncle. The mouth is sub-terminal with an exposed pre-maxillary tooth band. The maxillary barbel is long, reaching past the adipose fin to the caudal peduncle, while the nasal barbel is short, and the inner and outer mandibular barbels reach the orbit and fin base, respectively. The cranium bones are visible under a thin layer of skin (Fig. 2A). The morphometrics and meristic characters were shown in Tables 3 and 4.

3.3 *Mystus cavasius*

Mystus cavasius described from the Atrai River (Hamilton, 1822), is now recognized as occurring in the northern part of the Indian subcontinent, while populations from the south and Myanmar are identified as *M. seengtee* and *M. falcarius*, respectively (Chakrabarty & Ng, 2005). The body is elongated and moderately compressed, with a dorsal profile that rises gently from the snout to the fin origin and slopes down to the caudal peduncle. The head is conical with a narrow occipital process. The mouth is terminal with a slightly longer maxilla. The species has four pairs of barbels: maxillary, nasal, and two mandibular pairs. The pectoral spine is pointed and smooth externally. The body is greyish, turning yellowish along the abdomen, with dusky maxillary barbels and dull white paired fins (Fig. 3A). The morphometrics and meristic characters were shown in Tables 5 and 6.

3.4 *Mystus bleekeri*

Mystus bleekeri, described from the Ganges River drainage and Myanmar by Day, (1878), is now restricted to the Ganges River drainage (Khan et al., 2014). It is distributed across Asia in Pakistan, India, Bangladesh, Nepal, Myanmar, and Indonesia. This small-sized fish has a moderately elongated and compressed body with a rounded abdomen. The head is also moderate in size and compressed, with eyes that are moderately large and positioned supra-laterally. The mouth is terminal, transverse, and nearly half the length of the head. The head and opercles are granulated, with a shallow median groove reaching the base of the occipital process. *Mystus bleekeri* features four pairs of barbels. Maxillary barbels extend to or beyond the anal fin, nasal barbels reach the hind edge of the eye, and mandibular barbels (both inner and outer) extend near the base of the pectoral fin. The pectoral spine is stronger than the dorsal spine, which is smooth and rarely serrated. The adipose dorsal fin is large, starting just behind the rayed dorsal fin, and the caudal fin is forked with the upper lobe longer than the lower (Fig. 4A). The morphometrics and meristic characters were shown in Tables 7 and 8.

3.5 Gill Rackers

The morphology of gill rakers is so diverse that they are often used as a taxonomic tool to identify and classify fish species (e.g., gill raker counts can differentiate species on a dichotomous key). The role of the gill raker apparatus is related to prey retention efficiency, where the gill rakers function as a cross-flow filter (Sanderson et al., 2001; Smith & Sanderson, 2013). An increasing number of gill rakers enhance crossflow filtering and the closely spaced gill rakers also limit the escape possibilities of small prey. However, a dense gill raker apparatus is more likely to be clogged by sediments than more sparse gill rakers, and foraging in the muddy bottom of the profundal most likely requires other gill raker adaptations. Accordingly, a high number of long gill rakers is common in planktivorous fish species and morphs, whereas benthic species and morphs usually display a lower number of shorter gill rakers (Janssen, 1978; Schluter & McPhail, 1992; Robinson & Parsons, 2002). Gill rakers on the first-gill arch have been considered important taxonomical characters for *Mystus Species.* The gill raker counts for the species studied show distinct variations. *Hemibagrus punctatus* has a total of 16 gill rakers, with 5 developed and 2 rudimentary on the upper part, and 9 developed on the lower part. *Sperata aorides* possesses 20 gill rakers, with 4 developed on the upper and 14 developed plus 2 rudimentary on the lower part. *Mystus cavasius* has 18 gill rakers, with 3 (1 rudimentary) on the upper and 11 developed plus 4 rudimentary on the lower part. *Mystus bleekeri* has the fewest, with a total of 8 gill rakers, 2 on the upper and 6 on the lower part. This data highlights the morphological differences in gill raker counts used for taxonomic classification.

3.6 Verterbral Coloum

The vertebral column analysis of the species studied revealed specific characteristics. Hemibagrus punctatus has 25 pre-caudal vertebrae and 21 caudal vertebrae, resulting in a total of 46 vertebral columns, with 4 being fused (Fig. 1B). Sperata aorides shows 26 pre-caudal vertebrae and 23 caudal vertebrae, totaling 48 vertebral columns with 5 fused (Fig. 2B). *Mystus cavasius* has 21 pre-caudal vertebrae and 17 caudal vertebrae, making a total of 38 vertebral columns, with 2 fused (Fig. 3B). Mystus bleekeri possesses 21 pre-caudal vertebrae and 18 caudal vertebrae, resulting in a total of 39 vertebral columns (Fig. 4B). This data is crucial for understanding the anatomical and taxonomic distinctions among these species.

3.7 Molecular Analysis

The phylogenetic tree analysis of cytochrome oxidase 1 (cox1) was sequenced in submitted to NCBI with the accession number ON076064.1- *Hemibagrus punctatus*, ON076068.1- *Sperata aorides*, ON076065.1- *Mystus cavasius*, OP661359.1- *Mystus bleekeri*. The phylogenetic tree depicted in the image is constructed to show the evolutionary relationships among various species of fish (Fig. 5). Each branch of the tree represents a different species or a unique sequence associated with that species. The species included in this tree are *Mystus cavasius, Mystus bleekeri, Hemibagrus punctatus, Sperata aorides*, and *Channa striata* was taken as the out group. The branching points, or nodes, on the tree indicate common ancestors shared by the species branching out from those points. Bootstrap values are displayed at these nodes, with a value of 100% indicating very high confidence in the branching pattern at that point. Red dots are used to highlight specific sequences or nodes within the

tree. The phylogenetic tree also includes a scale bar at the bottom, representing a genetic distance of 0.050. This scale helps in understanding the degree of evolutionary divergence between the sequences. The shorter the branches, the closer the genetic relationship; conversely, longer branches indicate more significant evolutionary differences. Overall, the tree visually represents how closely or distantly related these fish species are, providing insights into their evolutionary history and genetic diversity.

Table 1. Morphometric measurement of *Hemibagrus punctatus*

S.No.	Character	% in SL	SL
1	Standard length	12.5-13.7	12.5-13.7
2	Pre dorsal length	25.54-35.2	2.84-3.91
3	Preanal length	51.09-57.6	1.73-1.95
4	Pre pelvic length	33.6-37.22	2.68-2.97
5	Pre pectoral length	15.32-16.8	5.95-6.52
6	Dorsal-spine length	5.839-12.8	7.81-17.1
7	Dorsal-fin length	12.4-16	6.25-8.05
8	Length of dorsal-fin base	9.48-12.8	7.81-10.5
9	Length of anal-fi n base	6.56-7.2	13.8-15.2
10	Pelvic-fin length	10.21-12.8	7.81-9.78
11	Pectoral-fin length	12.4-13.6	7.35-8.05
12	Pectoral-spine length	10.21-11.2	8.92-9.78
13	Caudal-fin length	23.35-16.8	4.28-5.95
14	Length of adipose-fin base	31.38-36	2.77-3.18
15	Maximum height of adipose fin	4.37-6.4	15.6-22.8
16	Dorsal to adipose distance	0.729-0.8	125-137
17	post-adipose distance	8.759-8	11.4-12.5
18	Length of caudal peduncle	13.86-19.2	5.2-7.21
19	Depth of caudal peduncle	8.029-8.8	11.3-12.4
20	Body depth at anus	18.24-19.2	5.2-5.48
21	Head length	16.05-16	6.22-6.25
22	Head width	13.13-12	7.61-8.33
23	Head depth	13.13-10.4	7.61-9.61
24	Snout length	5.10-7.2	13.8-19.5
25	Interorbital distance	5.83-7.2	13.8-17.1
26	Eye diameter	2.18-3.2	31.2-45.6
27	Nasal barbel length	7.29-8	12.5-13.7
28	outer Mandibular barbel length	12.4-13.6	7.35-8.05
29	Inner mandibular barbel length	14.59-16	6.25-6.85
30	Maxillary barbel length	51.09-56	1.78-1.95

Table 2. Meristic count in *Hemibagrus punctatus*

S.No.	Meristic characters	Fin counts
1	Dorsal fin	I+8
2	Pectoral fin	I+7
3	Pelvic fin	I+6
4	Anal fin	I+8
5	Caudal fin	U=8; L=7

Table 3. Morphometric measurement of *Sperata aorides*

S.No.	Character	% in SL	SL
1	Standard length	30.0-44.50	30-44.5
2	Pre dorsal length	29.0-42.03	2.34-3.97
3	Preanal length	36.17-76	1.31-2.76
4	Pre pelvic length	38-54.27	1.88-3.17
5	Pre pectoral length	19.42-24.94	4-6.64

S.No.	Character	% in SL	SL
6	Dorsal-spine length	4.49-8.98	11.1-22.2
7	Dorsal-fin length	12.8-25.33	3.94-7.8
8	Length of dorsal-fin base	8.53-13.33	7.5-11.7
9	Length of anal-fi n base	5.84-9.333	10.7-17.1
10	Pelvic-fin length	8.98-13.9	6.66-11.1
11	Pectoral-fin length	10.5-16.8	5.26-9.46
12	Pectoral-spine length	6.29-19.33	5.17-15.8
13	Caudal-fin length	18.87-24.66	4.05-5.29
14	Length of adipose-fin base	13.03-21.66	4.61-7.67
15	Maximum height of adipose fin	2.92-6.06	16.6-34.2
16	Dorsal to adipose distance	6.292-9.333	10.7-15.8
17	post-adipose distance	8.98-14.6	6.66-11.1
18	Length of caudal peduncle	10.11-18.33	5.45-9.88
19	Depth of caudal peduncle	4.49-8.08	12.5-22.2
20	Body depth at anus	9.88-16.4	6.66-10.1
21	Head length	17.97-27.66	3.61-5.56
22	Head width	7.86-15.9	6.66-12.7
23	Head depth	6.741-9.333	10.7-14.8
24	Snout length	5.842-11.33	8.82-17.1
25	Interorbital distance	3.37-6.666	15-29.6
26	Eye diameter	1.79-1.79	33.3-55.6
27	Nasal barbel length	4.927-7.191	18.7-26.1
28	outer Mandibular barbel length	8.405-9.438	8.57-15.3
29	Inner mandibular barbel length	15.05-19.33	5.17-6.64
30	Maxillary barbel length	56-63.74	1.26-1.86

Table 4. Meristic count in *Sperata aorides*

S.No.	Meristic characters	Fin counts
1	Dorsal fin	I + 7
2	Pectoral fin	I + 10
3	Pelvic fin	I + 5
4	Anal fin	I + 11
5	Caudal fin	13 U ;14 L

Table 5. Morphometric measurement of *Mystus cavasius*

S.No.	Character	% In SL	SL
1	Standard length	16.9-14.2	14.2-16.2
2	Pre dorsal length	32.9-34.59	2.89-3.1
3	Pre anal length	71-72.32	1.38-1.4
4	Pre pelvic length	46.45-47.33	2.12-2.13
5	Pre pectoral length	19.01-21.29	4.81-5.1
6	Dorsal-spine length	11.83-14.08	7.15-7.95
7	Dorsal-fin length	30.98-27.81	3.25-3.38
8	Length of dorsal-fin base	11.88-18.7	5.48-8.41
9	Length of anal-fin base	9.09-12.25	8.36-11
10	Pelvic-fin length	16.9-19.35	5.3-5.95
11	Pectoral-fin length	14.76-16.12	6.21-6.36
12	Pectoral-spine length	15.48-18.33	5.5-6.62
13	Caudal-fin length	26.76-23.22	3.76-4.41
14	Length of adipose-fin base	40.84-46.45	2.2-2.46
15	Maximum height of adipose fin	6.338-6.451	15.8-15.9
16	Dorsal to adipose distance	1.408-1.29	71.5-79.5
17	post-adipose distance	12.9-14.08	7.15-7.95
18	Length of caudal peduncle	19.71-23.22	4.41-5.1

S.No.	Character	% In SL	SL
19	Depth of caudal peduncle	10.32-10.56	9.53-9.93
20	Body depth at anus	23.23-16.12	4.33-6.36
21	Head length	21.12-23.87	4.29-4.76
22	Head width	12.67-13.54	7.57-7.94
23	Head depth	11.97-10.32	8.41-9.93
24	Snout length	8.45-9.032	11.3-11.9
25	Interorbital distance	6.338-8.387	12.2-15.8
26	Eye diameter	2.112-3.87	26.5-47.6
27	Nasal barbel length	15.49-20.64	4.96-6.5
28	outer Mandibular barbel length	27.74-30.98	3.25-3.69
29	Inner mandibular barbel length	44.36-49.03	2.09-2.26
30	Maxillary barbel length	88.02-120	0.85-1.14

Table 6. Meristic count in *Mystus cavasius*

S.No.	Meristic characters	Fin counts
1	Dorsal fin	I +7
2	Pectoral fin	I +8
3	Pelvic fin	I+ 5
4	Anal fin	I + 7
5	Caudal fin	7 U;9 L

Table 7. Morphometric measurement of *Mystus bleekeri*

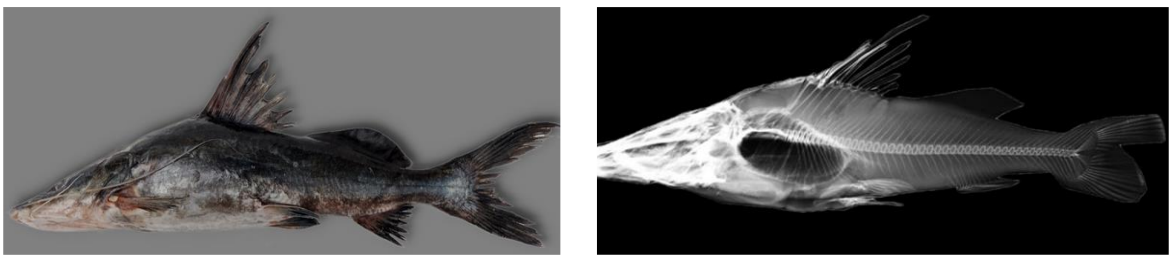
S.No.	Character	% in SL	SL
1	Standard length	12.5-13.7	12.5-13.7
2	Pre dorsal length	25.54-35.2	2.84-3.91
3	Pre anal length	51.09-57.6	1.73-1.95
4	Pre pelvic length	33.6-37.22	2.68-2.97
5	Pre pectoral length	15.32-16.8	5.95-6.52
6	Dorsal-spine length	5.839-12.8	7.81-17.1
7	Dorsal-fin length	12.4-16	6.25-8.05
8	Length of dorsal-fin base	9.489-12.8	7.81-10.5
9	Length of anal-fi n base	6.569-7.2	13.8-15.2
10	Pelvic-fin length	10.21-12.8	7.81-9.78
11	Pectoral-fin length	12.4-13.6	7.35-8.05
12	Pectoral-spine length	10.21-11.2	8.92-9.78
13	Caudal-fin length	23.35-16.8	4.28-5.95
14	Length of adipose-fin base	31.38-36	2.77-3.18
15	Maximum height of adipose fin	4.379-6.4	15.6-22.8
16	Dorsal to adipose distance	0.729-0.8	125-137
17	post-adipose distance	8.759-8	11.4-12.5
18	Length of caudal peduncle	13.86-19.2	5.2-7.21
19	Depth of caudal peduncle	8.029-8.8	11.3-12.4
20	Body depth at anus	18.24-19.2	5.2-5.48
21	Head length	16.05-16	6.22-6.25
22	Head width	13.13-12	7.61-8.33
23	Head depth	13.13-10.4	7.61-9.61
24	Snout length	5.109-7.2	13.8-19.5
25	Inter orbital distance	5.839-7.2	13.8-17.1
26	Eye diameter	2.189-3.2	31.2-45.6
27	Nasal barbel length	7.299-8	12.5-13.7
28	outer Mandibular barbel length	12.4-13.6	7.35-8.05
29	Inner mandibular barbel length	14.59-16	6.25-6.85
30	Maxillary barbel length	51.09-56	1.78-1.95

Table 8. Meristic count in *Mystus bleekeri*

S.No.	Meristic characters	Fin counts
1	Dorsal fin	I+8
2	Pectoral fin	I+7
3	Pelvic fin	I+6
4	Anal fin	I+8
5	Caudal fin	U=8; L=7

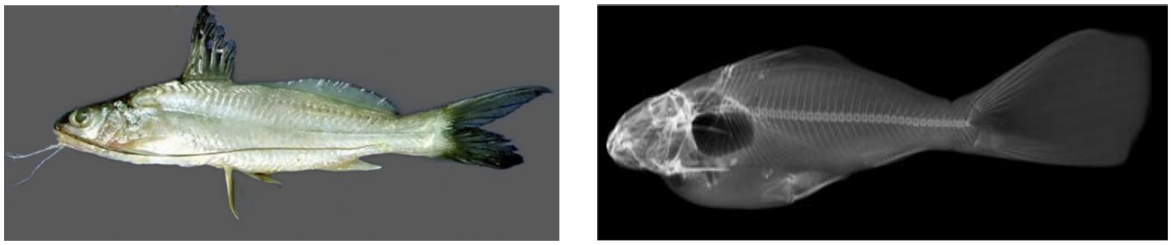
A B

Fig. 1. *Hemibagrus punctatus*



A B

Fig. 2. *Sperata aorides*



A B

Fig. 3. *Mystus cavasius*

A B

Fig. 4. *Mystus bleekeri*

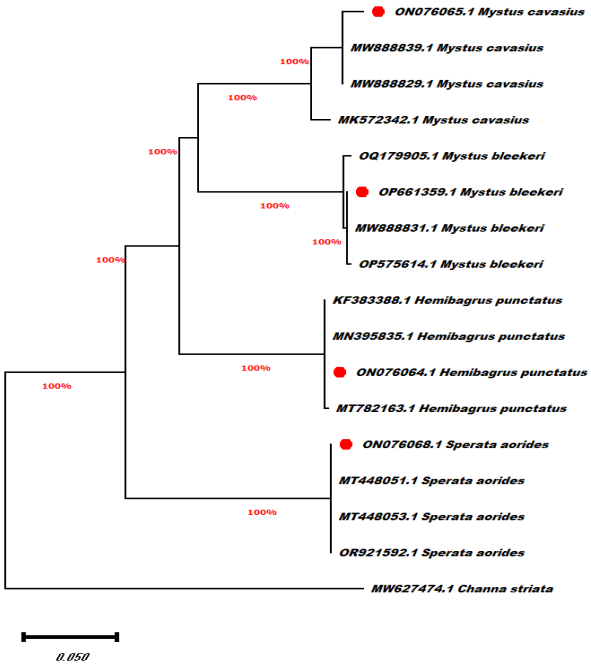


Fig. 5. Phylogenetic analysis by using neighbor-joining method

4. DISCUSSION

A detailed study on freshwater fish in India was documented by Jayaram, (2010). Further research by Arunkumar & Arunachalam, (2018) highlighted freshwater fish species in the Cauvery River. The Bagrid catfish genus, particularly *Mystus*, consists of small to medium-sized species predominantly found in freshwater habitats (Darshan et al., 2016). While catfishes of the family Bagridae are extensively studied worldwide, there are only a few notable studies in India. Despite some available taxonomic literature, osteology and molecular phylogeny are less studied, and much of the classification relies on morphometric analysis due to traditional taxonomy challenges (Patil et al., 2024). About 50 species of the order Siluriformes are found in the Western Ghats of India and associated river systems, with 21 species belonging to the family Bagridae (Kolwalkar & Deb 2023). These catfishes hold significant market value due to their high consumption. The study focuses on *Hemibagrus punctatus* and *Sperata arodies*, which are endemic to the Cauvery River and are classified as endangered (Ali et al., 2013) Catfishes of the family Bagridae face various anthropogenic threats, including pollution, biological resource use, and habitat modification, with over 50% of species threatened with extinction (Easa & Shaji, 1997). Morphometric and molecular analysis reveals certain haplotype variations in mitochondrial DNA sequences. The species of *Mystus* remain enigmatic, showing no notable variation in morphometric measurements. The molecular variation may result from habitat changes, exacerbated by pollution in the Cauvery and Bhavani rivers due to human activities (Chowdhury et al., 2024). These environmental changes may affect the fishes' habitats and gene sequences. There is an urgent need for conservation and commercialization efforts, preceded by comprehensive taxonomical and biological studies to ensure the effective conservation and sustainable use of these species.

5. CONCLUSIONS

The genus *Mystus* found in the perennial rivers of the southern Western Ghats of Tamil Nadu, India, exhibits significant hidden diversity. Several species complexes display phylogenetic and molecular variations, indicating a rich genetic diversity within the genus. *Mystus bleekeri* and Cox1 morphometric analysis suggest the presence of undescribed species of *Mystus bleekeri. Mystus cavasius* is considered a separate species, likely distributed throughout India. The catfish species *Sperata aroidies* has been recorded for the second time in the Cauvery River of Tamil Nadu.

No detailed biological studies are available on *Hemibagrus punctatus*, now categorized as an

endangered species by the IUCN. First-time detailed osteological studies in Tamil Nadu on *Sperata sp., Hemibagrus sp.,* and *Mystus sp*. reveal that the number of gills rakers, gill arches, and vertebrae help differentiate these species. For instance, gill rakers are used as a separating character based on feeding type. The study suggests that some species, like *Mystus cavasius*, have widespread distributions throughout India, with *M. seengtee* likely being synonymous with *M. cavasius*. Additionally, *M. bleekeri* appears to have undescribed species. The study provides insights into the biogeography of bagridae species in the Western Ghats, with morphometric, osteological, and molecular analyses revealing their evolutionary patterns.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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