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Cover: Mixed media with fine liners, colour pencils, and watercolour background of an Indian funnel web spider. © Elakshi Mahika Molur.

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COMMUNICATION

DNA barcoding and distribution of *Osteobrama peninsularis* (Teleostei: Cyprinidae) in India

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Abstract: *Osteobrama peninsularis* has been reported in southern Indian drainages, particularly in the Krishna River drainage. This study provides new findings of *O. peninsularis* in two distant locations: the Kangsabati River (Suvarnarekha River drainage) in West Bengal and Wyra Lake (Godavari River drainage) in Telangana. This marks the first record of *O. peninsularis* in eastern India, specifically in West Bengal. The species can be distinguished from other *Osteobrama* members by its 28–31 branched anal-fin rays and 55–60 lateral line scales, along with other unique morphological features. Mitochondrial cytochrome oxidase C subunit I gene sequences from specimens collected in Suvarnarekha and Godavari River drainages cluster together in the phylogenetic analysis, indicating that the clade of *O. peninsularis* is distinct and maintains significant genetic distance from its congeners.

Keywords: Carp, genetic analysis, minnow, new record, peninsular India, range extension, ray-finned fish, taxonomy, West Bengal.

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Author contributions: BAL & AKT did field surveys and collected the specimens. BAL, AKT & SR studied the morphology and meristics of the specimens for taxonomic identification. BAL generated the DNA data, and did the molecular analysis. BAL, AKT, SR & LK wrote the article. BAL & LK reviewed the article.

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INTRODUCTION

The genus *Osteobrama* includes several key food fish species in India, thriving in both lentic and lotic systems. The species in the genus *Osteobrama* (type species *Cyprinus cotio* Hamilton, 1822) are characterized by their laterally compressed bodies, elevated dorsum, absence of procumbent predorsal spines, rounded abdomens in front of the pelvic fins, keeled abdominal edges from the pelvic-fin origin to the vent, and long anal fins with more than 10 branched rays (Talwar & Jhingran 1991). Hamilton (1822) described the type species as having a row of "5–6 black spots below the fore part of the lateral line, around which is a bluish shining depression" (plate 207).

Sykes described two species, *Rohtee vigorsii* and *R. ogilbii*, from the Deccan region of India (Sykes 1838), categorizing them under the genus *Rohtee*, which he characterized by long dorsal and anal fins, a posteriorly serrated last undivided dorsal-fin ray, and minute scales. Bleeker (1863) designated *R. ogilbii* as the type species of the genus, a classification upheld in later taxonomic work (Tilak & Husain 1989). The placement of species within the genera *Osteobrama* and *Rohtee* has been addressed by Jordan (1919), Hora (1921), and Mukerji (1934). Hora (1937) distinguished *R. ogilbii* from species now classified as *Osteobrama* by its unique procumbent predorsal spine and the long anal fin with 13 to 14 branched rays, Consequently, *Rohtee* is now recognized as valid with *R. ogilbii* as its sole species, while *Osteobrama* currently includes 10 valid species (Laskar et al. 2024).

Among the 10 valid species, the type species *O. cotio* is widespread in the Ganga basin of India and Bangladesh (Rahman et al. 2018). Three congeners, *O. feae*, *O. cunma*, and *O. belangeri* are distributed in Myanmar and the Irrawaddy drainage in India and China (Rahman et al. 2018). Doi (1997) reported *O. alfredianus*, *O. belangeri*, and *O. feae* from the Salween basin, but Laskar et al. (2024) questioned the validity of *O. alfredianus*. Silas (1952) described *O. peninsularis* from peninsular India. Two more species, *O. neilli* and *O. bakeri* are found in extreme southern peninsular India (Talwar & Jhingran 1991; Jadhav et al. 2011; Rahman et al. 2018). Shangningam et al. (2020) described *O. tikarpadaensis* from the Mahanadi River in Odisha and recognized *O. dayi* as a valid species. Laskar et al. (2024) states that *O. vigorsii* is limited to the Krishna River system in southern India, while the distribution of *O. tikarpadaensis* extends to the Godavari River drainages of South India. Morphologically, *O. peninsularis* closely resembles *O. cotio*, leading to frequent misidentifications due to their narrow range of morphological variations. The species is currently listed as Data Deficient on the IUCN Red List, raising questions about its record from Kerala (Dahanukar 2011).

This study suggests the extension of the distribution of *O. peninsularis*, which was previously documented only in southern India, by identifying the species from a new location in eastern India: Kangsabati River in the state West Bengal. DNA barcoding of specimens from southern India and eastern India further confirms the identification of *O. peninsularis*. These findings align with the original description of the species, with minor variations, and is a first report of *O. peninsularis* in eastern Indian drainage.

MATERIALS AND METHODS

Morphometric and meristic data were recorded following Jayaram (1999). Measurements were taken on the left side of the specimens using digital calipers to the nearest 0.1 mm. Fin rays and scale counts were performed under transmitted light with a stereomicroscope. All pored scales were counted for reporting the lateral line scale. The count of transverse scale rows, between the lateral line and the origin of the dorsal-fin, include the lateral line scale. Body subunits are expressed as a percentage of standard length (SL), while head subunits are presented as a percentage of head length (HL). The specimens have been deposited at the Zoological Survey of India (ZSI), Kolkata, and at the Freshwater Biology Regional Centre of the Zoological Survey of India, Hyderabad.

Genetic analysis. Tissue samples were obtained from freshly collected specimens of *O. peninsularis* and preserved in 90% ethanol. The genomic DNA was extracted through QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. The published primer pair (Ward et al. 2005): FishF1-5'TCAACCAACCACAAAGACATTGGCAC3' and FishR1-5'TAGACTTCTGGGTGGCCAAAGAATCA3' was used to amplify the partial segment of mitochondrial cytochrome oxidase C subunit I gene (COI). The 30 µl PCR mixture contains 10 pmol of each primer, 100 ng of DNA template, 1 × PCR buffer, 1.0–1.5 mM of MgCl2, 0.25 mM of each dNTPs, and 1U of Taq polymerase (Takara BIO Inc., Japan). The thermal profile was set to initial 2 min at 95 °C followed by 35 cycles of 0.5 min at 94 °C, 0.5 min at 54 °C, and 1 min at 72 °C, followed in turn by 10 min at 72 °C and subsequent hold at 4°C. The PCR products were further purified using QIAquickR Gel extraction Kit

(Qiagen, Valencia, CA).

The cycle sequencing and Sanger sequencing was executed commercially. Both forward and reverse chromatograms were checked through SeqScanner V1.0 (Applied Biosystems Inc., CA, USA), nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/), and ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/) to trim the low-quality reads and gaps. The DNA sequences generated as part of the current study have been deposited in GenBank with accession No. MT896379 & PQ333057 for *O. peninsularis*; MZ854239 & MZ854240 for *O. cotio*. We retrieved all the available COI sequences of *Osteobrama* species from GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/, assessed on 28 August 2023) and ran a test of neighbor-joining phylogeny. Based on the cohesive clustering, a maximum of five representative database sequences from each conspecific clade were used in the refined analysis. Following Rahman et al. (2018), uncertain sequences of *O. cotio* from Narmada River basin as well as from Karnafuli and Sangu Rivers were not included in the dataset. Further, a maximum of five representative sequences of three congeners used in Rahman et al. (2018), *O. belangeri*, *O. cunma*, and *O. feae* were used in the dataset. The dataset was aligned using ClustalX (Thompson et al. 1997) and the Kimura 2 parameter (K2P) genetic distances were estimated by using MEGAX (Kumar et al. 2018).

RESULTS

Material examined

Osteobrama peninsularis Silas 1952 (Image 1, and Table 1 and 2): ZSI FF 9901, 1, 69.4 mm SL, Kangshabati (or Kansai) River, Paschim Medinipur District, West Bengal, India (22.406^{0}N & 87.307^{0}E), collected by S. Rath, 14 October 2022. Genbank accession for mtCOI sequence: PQ333057; FBRC/ZSI/F3549, 1, 68.0 mm SL, Wyra lake, Godavari River drainage, Khammam District, Telangana, India, collected by Sudipta Mandal, 20 July 2020. Genbank accession for mtCOI sequence: MT896379.

Table 1. Morphometric Measurements of *Osteobrama peninsularis* from West Bengal (ZSI FF 9901). The table presents various body dimensions, expressed in millimeters and as percentages of standard length (SL) and head length (HL), providing a detailed overview of the species' morphological characteristics.

	Parameters	value
1	Standard Length	69.4 mm
	% SL	
2	Body Depth	42.22
3	Head Length (Lateral)	23.92
4	Head depth (Occiput)	19.45
5	Snout Length	6.20
6	Eye Diameter	8.36
7	Inter orbital Width	8.79
8	Max. Head width	12.39
9	Gape Width	5.48
10	Internerial space	4.76
11	Body width at anal fin origin	9.51
12	Body width at dorsal fin origin	11.67
13	Caudal Peduncle Length	9.37
14	Caudal Peduncle Depth	12.54
15	Dorsal-fin base Length	13.40
16	Dorsal-fin Length	26.37
17	Pectoral-fin Length	17.00
18	Pelvic-fin Length	14.99
19	Anal-fin base Length	37.03
20	Anal-fin Length	40.35
21	Caudal fin length	24.93
22	Median caudal fin Length	11.53
23	Predorsal Length	51.30
24	Prepectoral Length	22.05
25	Prepelvic Length	39.48
26	Preanal Length	53.31
27	Pelvic anal distance	15.85
	% HL	
28	Snout Length	25.90
29	Eye Diameter	34.94
30	Inter Orbital Width	36.75
31	Max. Head Width	51.81
32	Gape Width	22.89
33	Internerial space	19.88

Description

Body deep, laterally compressed. Dorsal profile sloping upward linearly to nape, then in a broad curve to dorsal fin origin, forming a distinct hump, then sloping gradually downward towards caudal peduncle. Ventral profile strongly curved from tip of snout to origin of anal fin. Head compressed longer than deep. Eye large situated anteriorly on head, visible from dorsal and ventral side. Mouth terminal, obliquely directed upwards. Barbels absent.

Dorsal fin with iii unbranched and eight branched rays, last unbranched ray stiff and serrated. Pectoral fin with i unbranched and 14 branched rays. Pelvic fin i unbranched and eight branched rays. Anal fin long with iii

unbranched rays and 29 branched rays. Caudal fin deeply forked with 9+8 branched rays. Scales small in size. Pre-dorsal scale 24. Lateral line complete with 58 scales.

Coloration

In preserved specimens, dorsal and dorsolateral surfaces of head and body faint brown, lateral surface of body greyish, become lighter ventrally. Dorsal, pectoral, pelvic, anal and caudal fin is pale white. An oblique black streak immediately posterior to opercle, parallel to upper opercular margin present.

Genetic analysis

The mtCOI sequences (*denovo*) of both the specimens of *Osteobrama peninsularis* in the study, cluster together. In the phylogram (Figure 1), the *denovo* sequences of *O. peninsularis* along with a few sequences borrowed from GenBank (with taxa name *O. cotio*) form a distinct clade. The sequences in the *O. peninsularis* clade show 0.0 to 0.62 % pairwise genetic distance (intraspecies divergence) and maintain 5.28 to 5.68% genetic distance (interspecies divergence) with the sequences in the clade of *O. cotio*. The clade of *O. peninsularis* corresponds with one of the subclades of Clade A referred in Rahman et al. (2018). Notwithstanding to having a considerable range of genetic divergence among the three subclades in Clade A of *O. cotio*, Rahman et al. (2018) stated that "The haplotype group represented by the sequences from the Narmada, Karnafuli, Sangu, and Godavari drainages may represent a distinct species but not necessarily undescribed. Based on the very brief description (Silas 1952) and data on topotypes in Jadhav et al. (2011), the oldest alternative available name may be *O. peninsularis*, with type locality Pune (Maharashtra, India) in the upper Krishna River drainage". Based on the COI sequences, we consider that the three sequences (KF550101 to KF550103) with no locality information but identified as *O. cotio* in NCBI are in fact *O. peninsularis*.

Image 1. *Osteobrama peninsularis*: i—ZSI FF 9901, 69.4 mm SL, Kangsabati River, West Bengal, India (© Shibananda Rath) | ii—FBRC/ZSI/F/3549, 68.0 mm SL; Wyra lake, Godavari River drainage, Khammam District, Telangana, India (© Boni Amin Laskar).

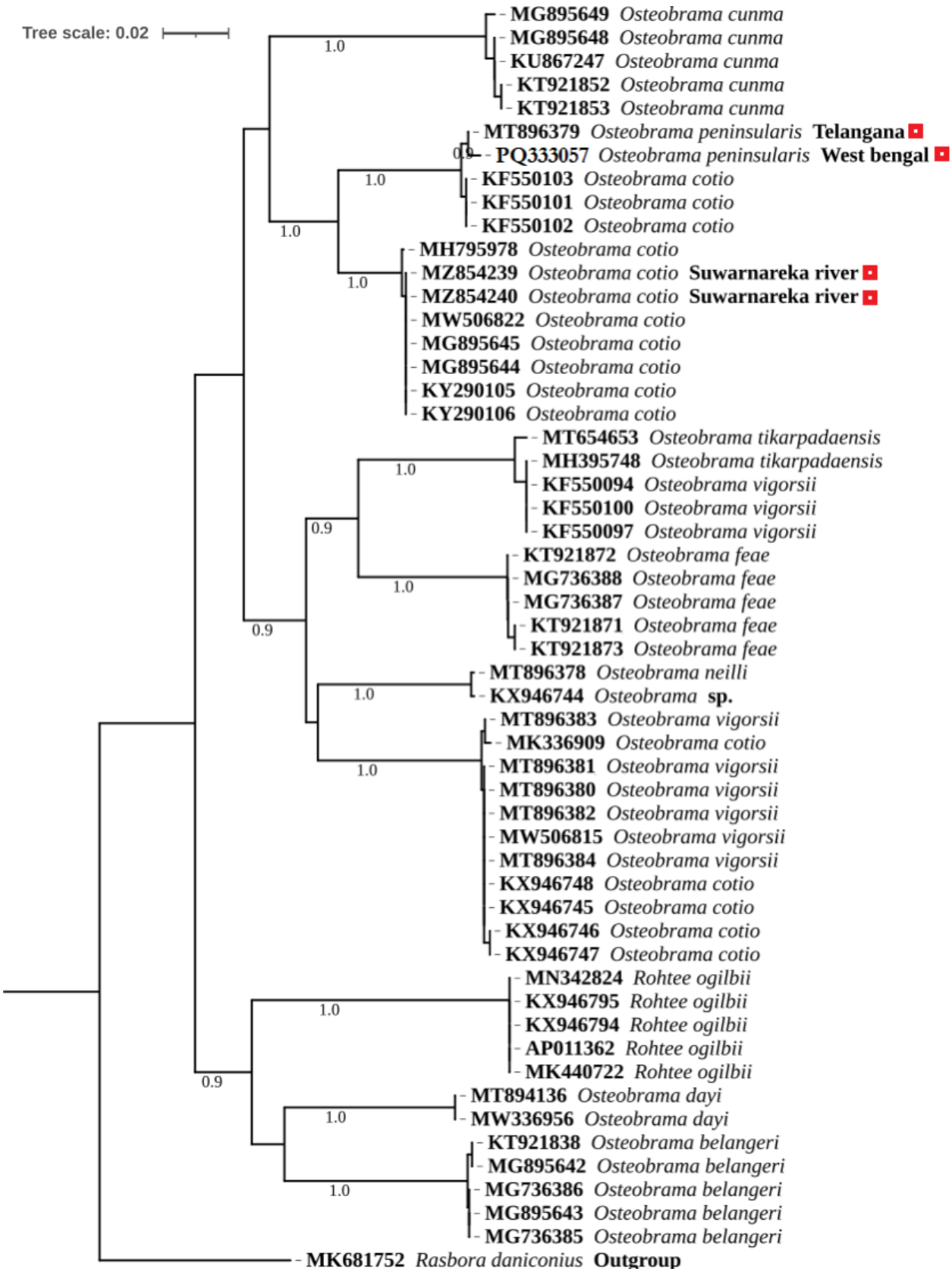
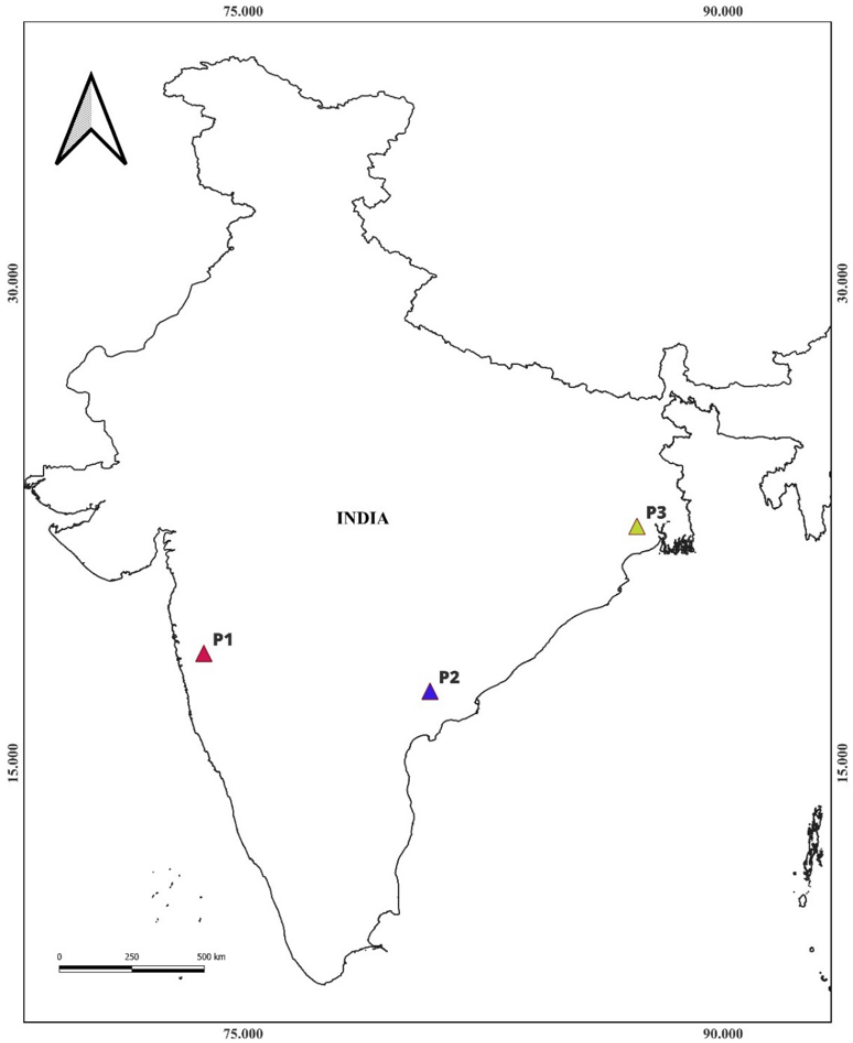


Figure 1. Neighbour-joining phylogram of the *Osteobrama congeners* based on mtCOI partial shows a distinct clade of the studied species, *O. peninsularis*. Numbers at branches show bootstrap. The NCBI accession numbers are given with the organism's name, the de novo sequences are marked with red square.

Table 2. Meristic counts of *Osteobrama peninsularis* from different locations. This table summarizes the meristic characteristics of *O. peninsularis* specimens from West Bengal (ZSI FF 9901) and Wyra Lake in Telangana (FBRC/ZSI/F3549), alongside counts from previous literature (Silas 1952).

Parameters	*O. peninsularis* from W.B.: ZSI FF 9901	*O. peninsularis* from, Wyra Lake, Godavari Drainage, Telangana: FBRC/ZSI/F3549	*O. peninsularis* original descriptions by Silas (1952)
Dorsal fin	iii 8	iii 8	iii 9
Pectoral fin	i 14	i 14	16
Pelvic fin	i 8	i 9	i 9
Anal fin	ii 29	ii 30	iii 28-31
Lateral line scales	58	58	55-60
Predorsal scales	24	24	21-24
Pre-anal scales	21	-	-



▲ P1: Poona Upper Krishna Drainage, Maharashtra

▲ P2: Wyra Lake, Telangana

▲ P3: River Kansai, West Bengal

Figure 2. *Osteobrama peninsularis* known distribution across southern Indian drainages, highlighting its presence in the Krishna River drainage, as well as newly recorded locations in the Kangsabati River (Suvarnarekha River drainage) in West Bengal and Wyra Lake (Godavari River drainage) in Telangana.

DISCUSSION

In this study, specimens were identified as *Osteobrama peninsularis* based on morphological characteristics, including 58 lateral line scales, the absence of barbels, and an anal fin with 29 branched rays. This species, originally described from Pune (Poona) in the upper Krishna River drainage of Maharashtra, has been frequently reported in Maharashtra (Silas 1952; Tonapi & Mulherkar 1963; Kharat et al. 2000, 2003; Arunachalam et al. 2002; Wagh & Ghate 2003; Chandanshive et al. 2007; Heda 2009; Jadhav & Yadav 2009).

Biju et al. (1999) reported *O. peninsularis* from the Periyar River in Central Kerala, indicating a range extension into Kerala. They, along with Talwar & Jhingran (1991) and Jayaram (1999), recognized the species as distributed solely in peninsular India, including Maharashtra, Odisha, Andhra Pradesh (erstwhile), and Kerala. The species has also been documented in the Tungabhadra River, Karnataka (Shahnawaz & Venkateshwarlu 2009; Shahnawaz et al. 2010). Although previous studies (Jayaram & Mazumdar 1976; Mohanty et al. 2015) noted its occurrences in Odisha, Dutta et al. (1993) did not include it in the state fauna series of Odisha.

Morphologically, *O. peninsularis* is superficially similar to *O. belangeri*, *O. cotio*, *O. cunma* because of lack of barbels. However, it is distinguished from all the three congeners in having pre-dorsal 21 to 24 scales and Lateral line scales 55 to 60. Furthermore, it is distinguished from *O. belangeri* in having more branched anal-fin rays (28–31 vs. 17–18), less pre-dorsal scales (21–24 vs. 31–34), less lateral line scales (55–60 vs. 70–78); from *O. cotio* in having less branched anal-fin rays (28–31 vs. 33–38), less lateral line scales (55–60 vs. 65); from *O. cunma* in having more branched anal-fin rays (28–31 vs. 25–29), less pre-dorsal scales (21–24 vs. 28–30) and more lateral line scales (55–60 vs. 42–53). This study largely aligns with the original description of *O. peninsularis*, noting only minor variations in body morphometry (see Table 1). Parameters of meristic counts, provide insights into the species' morphological consistency across different populations. The specimens examined were smaller than the type specimens, and minor variations may relate to their distribution. The findings indicate that the distribution of *O. peninsularis* extends through the river basins of the Godavari and Krishna in Maharashtra, Telangana, Andhra Pradesh, and into the Mahanadi basin in Odisha and the Subarnarekha river basin in West Bengal. In a recent study, amendment of description of *O. vigorsii* and the expansion of distribution of *O. tikarpadaensis* have also been reported (Laskar et al. 2024). With the addition of *O. peninsularis*, the state fauna of West Bengal now includes two species of *Osteobrama*. A distribution map of *O. peninsularis* is given in Figure 2.

The presence or absence of barbels is a crucial taxonomic feature in *Osteobrama* (Hora & Misra 1940; Shangningam et al. 2020). When present, the barbels may be either one pair of maxillary barbels or both maxillary and rostral, sometimes being minute or rudimentary. The rostral barbels can be hidden or barely visible, while in some species, they extend to the base of the maxillary barbels. *Osteobrama* species are categorized into three groups based on their barbels: (i) with four well-defined barbels, (ii) with two rudimentary maxillary barbels, and (iii) without barbels (Hora & Misra 1940). Recently, *O. vigorsii* was revised and placed in Group (i), alongside *O. bakeri*, *O. feae*, *O. neilli*, and *O. tikarpadaensis* (Laskar et al. 2024).

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