

## *Indoreonectes mahadeoensis*, a new species of river loach from headwaters of the Narmada River, India (Teleostei: Nemacheilidae)

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### Abstract

A new species of nemacheilid loach, *Indoreonectes mahadeoensis* is described from the Narmada River basin, Central India. *Indoreonectes mahadeoensis* is distinguished from its congeners by the presence of 8 branched rays in the dorsal fin, 7 branched rays in the anal fin, and by the unique combination of the following characters: nasal barbels reaching anterior border of eye, dorsal-fin origin positioned posterior to vertical line drawn from pelvic-fin origin, and broken bars along lateral surface of body. Further, *I. mahadeoensis* differs from all congeners, except *I. evezardi*, in having divided vertical bars on caudal peduncle (vs. undivided bars), but can be distinguished from the latter in having larger eyes (16.5–21.8 vs. 9.8–15.9% HL) and wider gape (40.4–59.1 vs. 24.3–37.5% HL). Analysis of the mitochondrial cytochrome *b* gene sequences revealed high genetic divergence (uncorrected P-distance ranging from 11.2 to 18.6%) between *I. mahadeoensis* and congeners.

**Key words:** Central India, Madhya Pradesh, river loach, taxonomy, freshwater fish

### Introduction

The small benthic freshwater fishes belonging to the sub-order Cobitoidei (commonly known as loaches), are characterized by a flattened conical head, and prolonged cylindrical body with lateral compression on the dorsal part (Prokofiev 2010). They primarily inhabit riffles, runs and small pools within streams and rivers, preferring gravel-and pebble-filled substrates, where they seek refuge beneath submerged rocks, leaf patches and stones (Raj 1916; Rainboth 1996; Kottelat 1990; Prokofiev 2010; Kesar *et al.* 2015; Kottelat 2021).

*Indoreonectes* Rita, Bănărescu and Nalbant 1978 (family Nemacheilidae), is an endemic loach genus of peninsular India, characterized by the presence of rounded caudal fin, short lateral line, long nasal barbels, nostrils placed close to each other, and several vertical bands often split into stripes and blotches on both sides of the body (Menon 1987). The genus currently comprises eight species occurring in the Western and Eastern Ghats Mountain ranges of peninsular India. These include the type species, *Indoreonectes keralensis* (Rita *et al.* 1978) from the southern Western Ghats in Kerala, *I. evezardi* (Day 1872) from northern Western Ghats in Maharashtra and Madhya Pradesh in Central India, *I. telanganaensis* from the lower reaches of the Godavari River in Telangana (Prasad *et al.* 2020), *I. neeshi* from the Godavari River, and *I. rajeevi* from the Krishna River, both in the northern Western Ghats of Maharashtra, (Kumkar *et al.* 2021), *I. amrabad* from Telangana, and *I. kalsubai* and *I. radhanagari* from northern Western Ghats in Maharashtra (Jadhav *et al.* 2024).

During ichthyofaunal surveys in the Mahadeo stream near Pachmarhi, located in the south-eastern part of the Satpura Tiger Reserve in Madhya Pradesh, India, specimens of a nemacheilid loach of the genus *Indoreonectes* were collected. Detailed examination of these specimens revealed it to be an undescribed species, which is described herein as *Indoreonectes mahadeoensis*.

## Methods

### *Study site and sampling*

Specimens were collected from the Mahadeo stream flowing along the Gupt Mahadev Shiva Temple (22.4164° N, 78.4252° E, 1129 m above mean sea level) near the town of Pachmarhi, along the south-western part of Satpura Tiger Reserve, Madhya Pradesh State, India. The Mahadeo stream meets the Denwa River at Banganga (22.5605° N, 78.2537° E; 490 m above mean sea level/asl) approximately six kms away from the sampling site. Denwa is one of the major rivers draining the Satpura Tiger Reserve, which ultimately empties into Tawa near Dhai (22.4585° N, 78.1843° E; 410 m asl), 35 kms from Gupt Mahadeo. The GPS coordinates were recorded by GARMIN *etrex* 30, and water quality parameters using a HANNA multi-parameter HI991300 probe. Fish specimens were photographed by a Cannon 80D camera with macro 100mm lens, after which they were fixed in 10% formalin and preserved in 70% ethanol, for determining morphometric and meristic data. Fin clip was preserved in 95% ethanol.

### *Morphometric and meristic data*

Digital vernier calipers (accuracy 0.1 mm) was used to measure morphometric characters as described in Keskar *et al.* (2015). Morphometric characters associated with body were converted into percentage of standard length (SL), and those associated with head were converted into percentage of head length (HL). For meristic counts, a stereo microscope (Nikon SMZ745T) was used. The frequency of the counts are provided in parenthesis, and the values of holotype indicated by an asterisk. Body markings and bar patterns were described based on Kumkar *et al.* (2021). The type specimens are deposited in the National Wildlife Repository of the National Biodiversity Authority (NBA) at the Wildlife Institute of India (WII), Dehradun, India.

### *DNA extraction, sequencing and phylogeny*

DNA was extracted from the fin clip using PureLink™ Genomic DNA Mini Kit (Invitrogen) following the manufacturer's protocol. Subsequently, the target mitochondrial cytochrome *b* (cyt *b*) gene was amplified by polymerase chain reaction. The partial cyt *b* gene was amplified using primers L14724 (5'-G AC TTG AAA AAC CAC CGT TG-3') and H15915 (5'-CTC CGA TCT CCG GAT TAC AAG AC-3') (Irwin *et al.* 1991) with hot start at 95°C for 2 minutes, followed by denaturation at 95°C for 30 seconds; annealing at 52°C for 10 seconds and extension at 60°C for 4 minutes, followed by final hold at 4°C. The denaturation, annealing and extension processes were set for 25 cycles. The PCR products were treated with Exonuclease I (EXO I), and shrimp alkaline phosphatase (SAP) for 15 minutes each at 37°C and 80°C to remove residual primers, followed by sequencing of both direction using the BigDye® Termination Kit and (v.3.1) and an ABI 3500XL Applied Biosystems Genetic Analyzer. The sequences were edited using Sequencer version 4.9 (Gene Codes Corporation, Ann Arbor, MI) and deposited in NCBI GenBank (PP911355).

A BLASTn (Altschul *et al.* 1990) search was performed for the newly generated sequence on NCBI BLAST (<http://blast.ncbi.nlm.nih.gov>) to confirm genus-level identity against published GenBank sequences. The newly generated sequence was aligned with those downloaded from GenBank (Benson *et al.* 2017) (Appendix 1) using ClustalW (Thompson *et al.*, 1994) with default prior settings implemented in MEGA 7.1 (Kumar *et al.* 2016). We used *Balitora chipkali* and *B. mysorensis* as outgroup taxa. We checked for unexpected stop codons by translating the sequence to amino acids in MEGA 7.1 (Kumar *et al.* 2016).

We performed Maximum likelihood (ML) analysis for the dataset (1067 base pairs) using the GUI version of IQTREE (Nguyen *et al.* 2015) implemented within the PhyloSuite (Zhang *et al.* 2020). The dataset partitioned by codon positions using ModelFinder (Kalyaanamoorthy *et al.* 2017) to find the best fit partitions and models of sequence evolution for each partition suggested three different partitions, Partition 1: TNe+G4, Partition 2: HKY+F+I, Partition 3: TIM2+F+G4. Bayesian inference (BI) analysis was carried out using the program MrBayes 3.2.7a (Ronquist *et al.* 2012) implemented within PhyloSuite (Zhang *et al.* 2020), with default prior settings. The same dataset used for the ML analysis was partitioned by codon positions using PartitionFinder 2.1.1 (Lanfear *et al.*

2017) with default settings to find the best-fit model of sequence evolution. PartitionFinder suggested three different partitions, Partition 1: SYM+G, Partition 2: HKY+I, Partition 3: GTR+G. Two separate runs were set up with four Markov chains each initiated from random trees and allowed to run for 10 million generations, sampling every 1000 generations. Analyses were terminated when the standard deviation of split frequencies was less than 0.001, the first 25% of trees were discarded as burn-in, and trees were constructed under 50% majority consensus rule. We obtained the Effective Sample Size values using TRACER 1.6 (Rambaut *et al.* 2018), and confirmed values above 200 for the priors. Support for the internal branches for the ML and BI was quantified using 1000 pseudoreplicates (ultrafast bootstrap UFB) and posterior probabilities (PP), respectively. The resulting tree was visualised and edited in FigTree (<http://tree.bio.ed.ac.uk/software/figtree>). The uncorrected pairwise distances (P-distance) were calculated in MEGA 7.1, with pairwise deletion of missing data and gaps.

## Results

### *Indoreonectes mahadeoensis* sp. nov. (Figs 1 & 2, Table 1)

Holotype: WII/NWR/Type\_F 04, 53.0 mm, SL, Mahadeo stream, near Pachmarhi, Satpura Tiger Reserve, Narmadapuram District, Madhya Pradesh, India (22.4164° N, 78.4252° E, 1129 m above mean sea level); M. Ghosh, 4 June 2022.

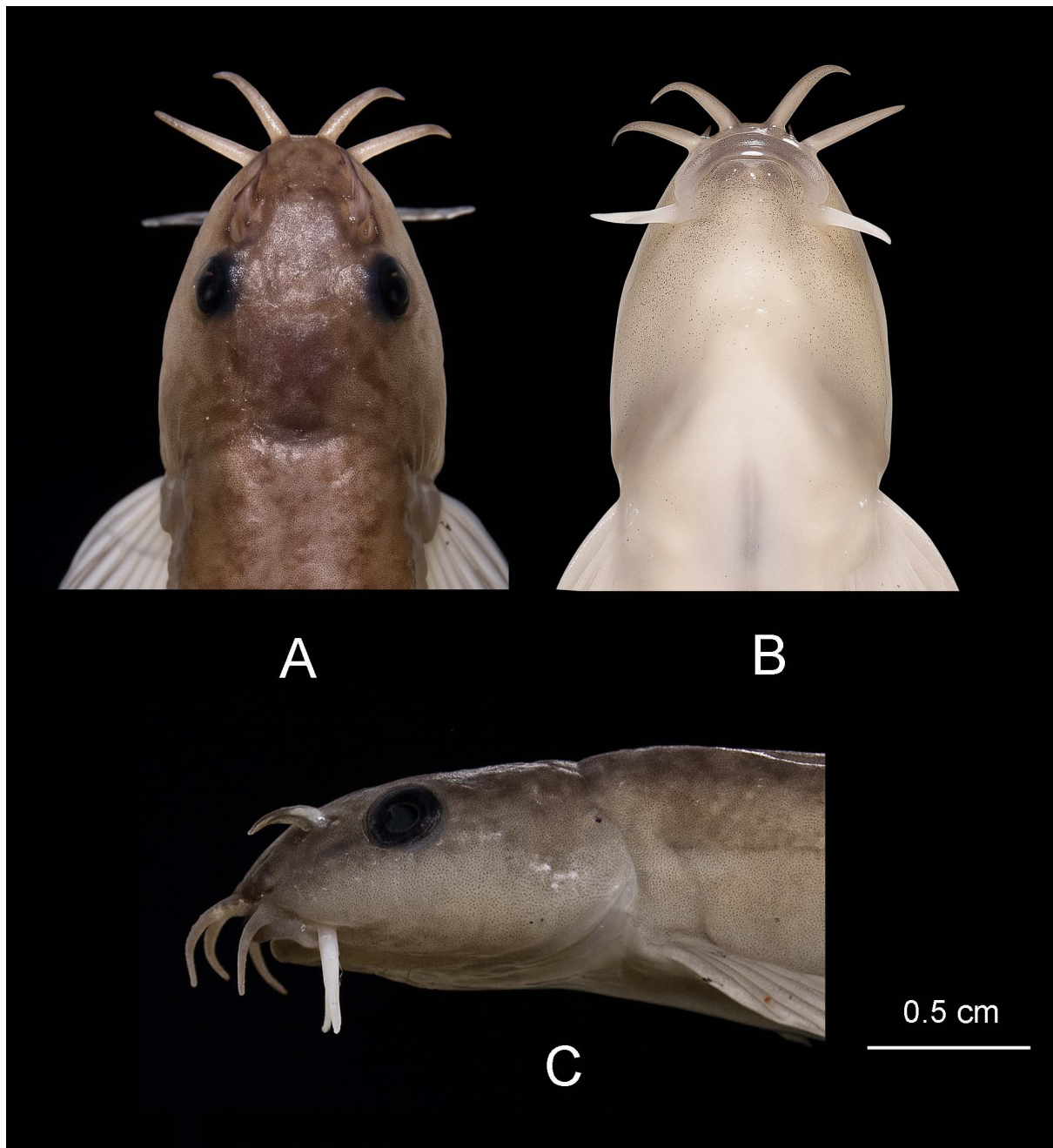
Paratypes: WII/NWR/Type\_F 05, 9 ex. 31.0–45.0 mm SL, same data as holotype.



**FIGURE 1.** *Indoreonectes mahadeoensis*, Holotype, WII/NWR/Type\_F 04, 53.0 mm SL; India: Madhya Pradesh: Satpura Tiger Reserve: Pachmarhi: Mahadeo stream, lateral view.

**Diagnosis.** *Indoreonectes mahadeoensis* is distinguished from all its congeners by the presence of 8 branched rays in the dorsal fin, 7 branched rays in the anal fin, and by a unique combination of the following characters: nasal barbels reaching anterior border of eye, dorsal-fin origin positioned posterior to vertical line drawn from pelvic-fin origin, and broken bars along lateral surface of body. In addition, *Indoreonectes mahadeoensis* can be separated from its congeners by a divergence of 11.2 to 18.6% (uncorrected P-distance) in the mitochondrial *cyt b* gene sequences.

**Description.** Body almost cylindrical at anterior, sub-cylindrical towards middle and, elongated and laterally compressed towards posterior end. At dorsal-fin origin, body width 7.4 to 11.8 times in standard length and depth 5.2 to 8.0 times in standard length. Head conical (Fig. 2A), its length 3.6 to 4.6 times in standard length. Snout round and smooth, its length 2.3 to 2.6 times in head length. Eye dorso-laterally positioned, closer to tip of snout than to posterior margin of operculum. Eye diameter 4.5 to 7.3 times in head length, and inter-orbital width 2.2 to 2.6 times in head length. Pre-dorsal outline convex gradually rising up to dorsal-fin origin. A distinct hump behind nape, and post-dorsal outline rising up to base of caudal fin. Caudal peduncle short, its length as equal to caudal peduncle depth. Scales minute, embedded in skin.



**FIGURE 2.** Dorsal (A), ventral (B) and lateral (C) head view of *Indoreonectes mahadeoensis*, Holotype, WII/NWR/Type\_F 04, 53.0 mm SL.

Mouth ventral, semi-circular, with thick, fleshy lips. Upper lip continuous and lower lip interrupted medially by groove (Fig. 2B). Barbels 4 pairs. Nasal barbel well-developed (Fig. 2C), 2 pairs of rostral barbels (1 outer rostral barbel and 1 inner rostral barbel), maxillary barbel and outer rostral barbel almost equal in length. Nasal barbel long, reaching in front of eye. Inner rostral barbel reaching posteriorly to anterior margin of eye. Maxillary barbel not reaching posterior border of operculum.

Dorsal-fin origin slightly posterior to vertical from pelvic-fin origin, its insertion closer to caudal-fin base than tip of snout. Dorsal fin with 3\* (10) simple and 8\* (10) branched rays, its origin positioned posterior to vertical line drawn from pelvic-fin origin. Pectoral fin with 1\* (10) simple and 8 (7), 9\* (3), branched rays, its length equal to head length. Pelvic fin with 1\* (10) simple and 6 (1), 7\* (9) branched rays, its length almost equal to head length. Anal fin with 3\* (10) simple and 7\* (10) branched rays, its length 1.2 to 1.5 times head length. Caudal fin rounded, with 9+9\* (10) branched rays.



**Coloration in life.** Body pale yellow, darker on dorsal, than lateral profile. Brownish-black irregular broken bars on lateral and dorsal side of body. Lateral and dorsal side bars separate from each other. Lateral bars narrower than inter-bar spaces. Complete bars present along caudal peduncle. A conspicuous spot on dorsal side of head, in addition to several head spots (Fig. 2A). Lower and upper lips grey in colour due to concentrated pigments (Fig. 2B), no spots on cheeks (Fig. 2C). Dorsal-fin anterior spot well developed. Dorsal-fin membrane hyaline with 3 rows of black spots on rays (1 complete row and 2 incomplete rows). Pelvic, pectoral and anal fins hyaline. Caudal fin also hyaline, with 4 rows of dark brown spots on rays; last row towards tip comparatively darker than remaining rows (Fig. 1).

**TABLE 1.** Morphometric data of the holotype (WII/NWR/Type\_F 04) and nine paratypes (WII/NWR/Type\_F 05) of *Indoreonectes mahadeoensis* sp. nov. Data of holotype included in the calculation of mean; SD, standard deviation; and range.

Characters	<i>Indoreonectes mahadeoensis</i> sp. nov. (n=10)			
	Holotype	Mean	SD	Range
Total length (mm)	64.1	48.6	7.4	38.3–64.1
Standard length (SL, mm)	53.0	40.4	6.5	30.7–53.0
Head length (HL, mm)	11.6	9.7	1.4	8.1–11.6
% in SL				
Head length	32.1	25.1	2.9	21.4–32.1
Predorsal length	60.2	58.8	4.6	55.4–70.9
Dorsal-fin origin to caudal-fin base	49.4	47.0	3.0	43.4–54.3
Prepectoral length	21.9	23.9	2.0	21.1–27.4
Prepelvic length	50.7	51.3	4.7	40.8–59.2
Preanus length	72.9	72.6	1.9	69.4–75.7
Preanal-fin length	79.9	79.5	4.2	72.5–88.1
Body depth (at dorsal-fin origin)	15.2	15.3	2.0	12.6–17.2
Body depth (at anus)	14.9	14.3	1.3	12.6–17.1
Body width (at dorsal-fin origin)	13.4	10.7	1.8	8.4–13.6
Body width (at anus)	8.1	6.7	0.9	5.6–8.1
Height of dorsal fin	19.2	19.4	1.5	17.7–22.8
Length of dorsal-fin base	12.1	11.3	1.8	9.3–4.5
Length of pectoral fin	22.4	21.1	1.7	18.8–23.1
Length of pelvic fin	19.1	19.8	2.4	16.6–26.0
Length of anal fin	17.1	18.3	2.4	15.9–23.4
Length of anal-fin base	8.9	9.0	1.3	7.3–11.8
Length of caudal fin	21.1	23.7	2.2	21.1–29.3
Depth of caudal peduncle	13.4	13.6	1.5	12.0–17.2
Length of caudal peduncle	13.7	13.2	0.7	11.7–14.0
% in HL				
Head depth	59.5	48.4	6.8	41.2–59.5
Head width	73.3	57.2	8.2	45.8–73.3
Snout length	45.7	42.1	5.8	37.3–56.8
Eye diameter	20.3	18.3	1.9	16.5–21.8
Interorbital width	45.7	38.3	4.0	31.4–45.7
Width of mouth	54.3	51.6	6.8	43.2–59.1



**Habitat.** *Indoreonectes mahadeoensis* was collected from a deep gorge-like area mainly composed of undercut bedrock and large boulders (Fig. 3). The substratum was filled with sand and gravel. The habitat was characterized by the presence of riffles, that were either flowing to meet their downstream tributaries, or ending up in small pools. The water quality parameters recorded during specimen collection (June 2022) were: temperature 23.5°C, pH 7.76, conductivity 51  $\mu$ S and dissolved oxygen 5.3 mg/l. The riparian vegetation along the entire stretch of the riverscape comprised primarily *Terminalia arjuna* (Arjuna) and *Syzygium cumini* (Jamun). *Indoreonectes mahadeoensis* co-occurs with *Devario aequipinnatus* and *Garra mullya*.

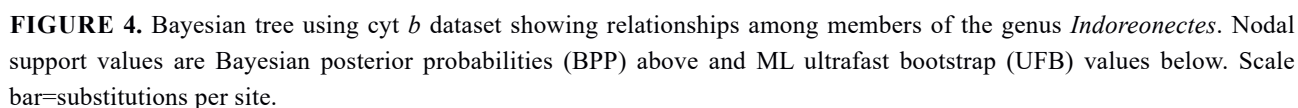


**FIGURE 3.** Mahadeo stream near Pachmarhi, Satpura Tiger Reserve, Madhya Pradesh, the type locality of *Indoreonectes mahadeoensis* (Photograph data: stream located adjacent to the Mahadeo Temple, located about 10 km from Pachmarhi Cantonment; 4 June 2022; wide camera 26 mm, f 1.8 12 MP; photo credit: Meghma Ghosh).



The species is named after its type locality—Mahadeo stream, flowing across the Gupt Mahadeo Temple near Pachmarhi hills, within the Satpura Tiger Reserve in Madhya Pradesh, Central India.

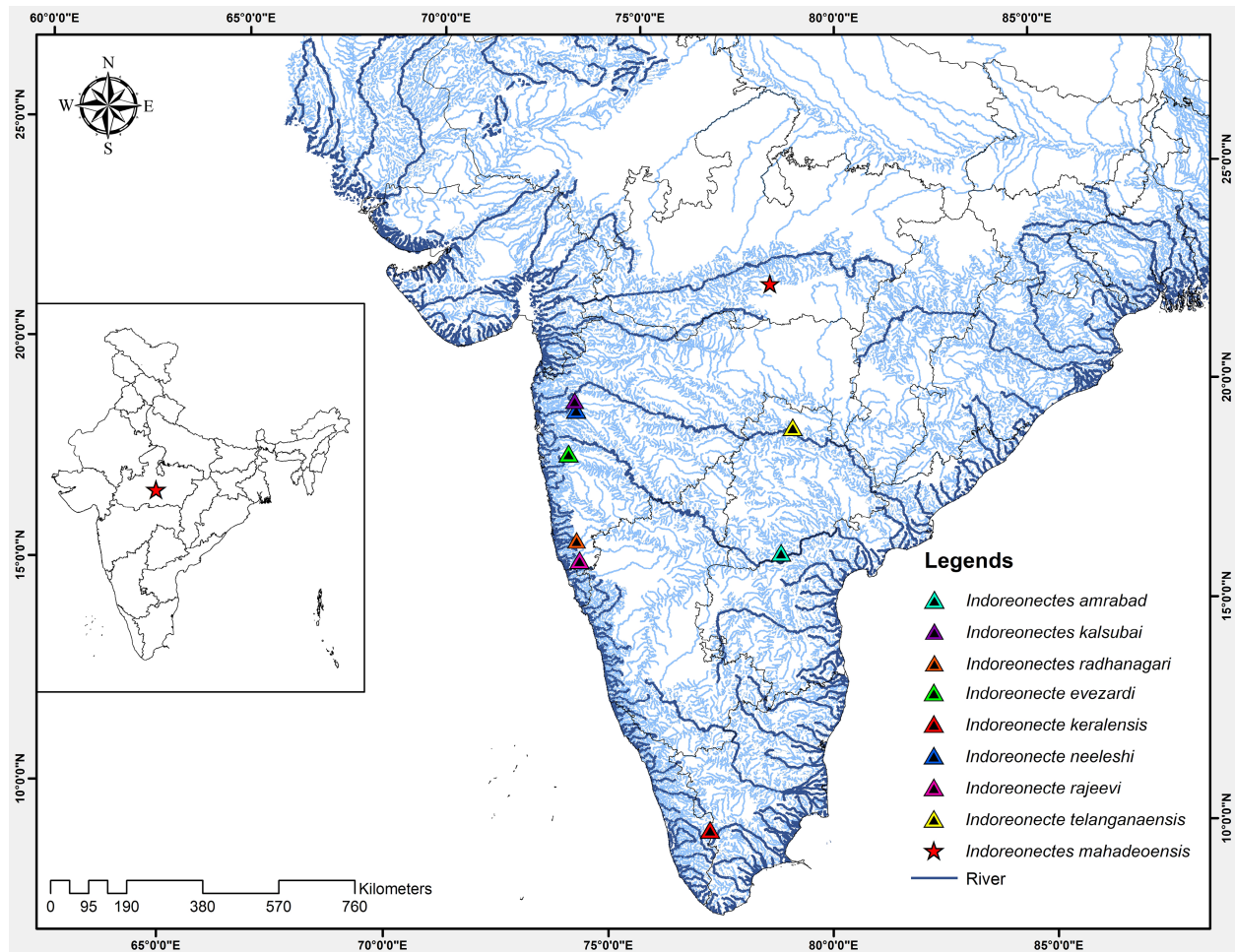
Our *cyt b* gene dataset comprised 1067 base pairs. Both ML and BI analyses resulted in the same tree topology (Fig. 4) and was largely congruent with Jadhav *et al.* (2024). *Indoreonectes mahadeoensis* was recovered within *Indoreonectes*, and showed a sister group relationship with *I. amrabad*, *I. evezardi*, *I. kalsubai*, *I. neelesh* and *I. telanganaensis*, but with low bootstrap value (UFB 58), and strong posterior probability value (PP 1) (Fig. 4). The uncorrected P-distance in the *cyt b* gene sequences between *Indoreonectes mahadeoensis* and its congeners ranges between 11.2–18.6% (Table 2).



The genus *Indoreonectes* is endemic to Central and Southern peninsular India where it has a restricted distribution in the Western and Eastern Ghats and adjoining landscapes. Until the year 2020, only two species, *Indoreonectes evezardi* and *I. keralensis* were known from Western Ghats. Subsequently, Keskar *et al.* (2018) studied the molecular phylogeny of *Indoreonectes*, where they indicated the presence of potential cryptic species and the need for a comprehensive taxonomic revision. This resulted in the description of *I. telanganaensis* from the Godavari River system by Prasad *et al.* (2020), and *I. neelesi* and *I. rajeevi* from the Krishna and Godavari River Systems in the northern Western Ghats by Kumkar *et al.* (2021). Recently three more species, *I. kalsubai*, *I. amrabad* (from the Godavari River system) and *I. radhanagari* (from the Western Ghats rivers) were described (Jadhav *et al.* 2024).

Till date, all species of *Indoreonectes* have been recorded from south of the Narmada River, and *Indoreonectes mahadeoensis* is the first member of the genus to be described from the Narmada River (Fig. 5), where it is currently known only from streams in the Mahadeo hills, near Pachmarhi in the Satpura Tiger Reserve. The Pachmarhi plateau is famously known as the Queen of the Satpura Hills, and due to its unique geographical location, harbours biodiversity with characters of the Himalayas, as well as the Western and Eastern Ghats mountains. The Mahadeo

hill is characterized by undulating terrain and deep gorges with bedrock undercut making it extremely favourable for loach species including *Indoreonectes mahadeoensis*.



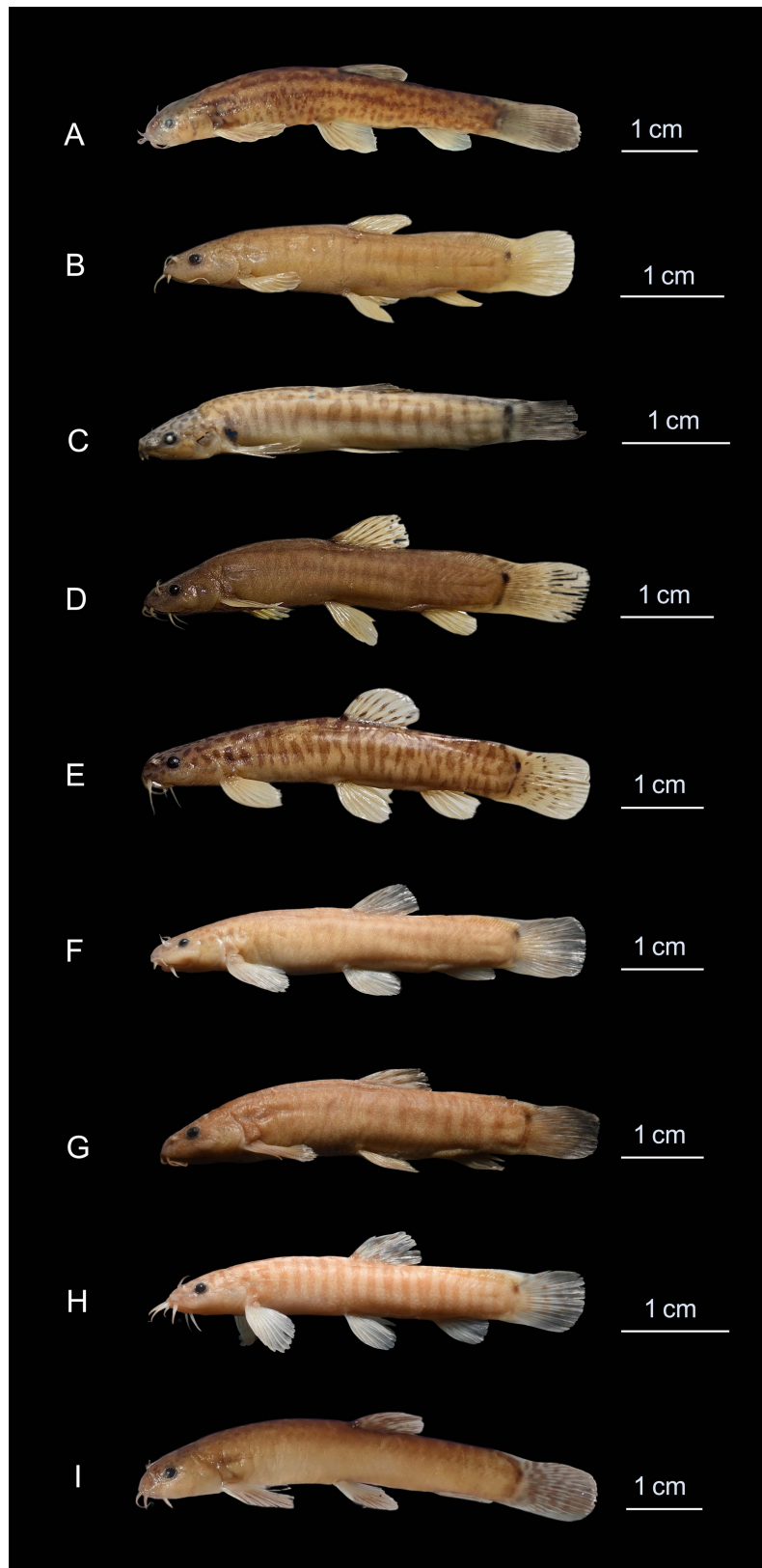
**FIGURE 5.** Map showing the type locality of the species of *Indoreonectes* in southern and central India.

*Indoreonectes mahadeoensis* can easily be distinguished from all its congeneric species (Fig. 6) by the presence of 8 branched rays in dorsal fin, 7 branched rays anal-fin. *Indoreonectes mahadeoensis* is morphologically similar to *I. evezardi* in having its caudal peduncle broader than long, and presence of broken bars on lateral side of body and caudal peduncle. However, *I. mahadeoensis* can be distinguished from *I. evezardi* by having divided vertical bars on caudal peduncle (vs. undivided), and anal fin with 7 branched rays (vs. 5); from *I. neeleshi* in having a deeper caudal peduncle (vs. caudal peduncle as deep as long), body bars narrower than interspace (vs. wider than interspace), 3 simple and 8 branched rays in dorsal fin (vs. 4 simple and 7 branched rays); from *I. rajeevi* in having a deeper caudal peduncle (vs. slightly longer than deep), conspicuous black marking on lower lip absent (vs. present), and dorsal fin insertion posterior than pelvic-fin origin (vs. vertical to pelvic-fin origin); from *I. radhanagari* by deep caudal peduncle (vs. caudal peduncle as deep as long), lateral body bars narrower than inter-bar space (vs. lateral bar and inter-bar space equal), pectoral fin with 1 simple ray (vs. 2), and anal fin with 7 branched rays (vs. 5); from *I. keralensis* by deeper caudal peduncle (vs. caudal peduncle longer than deep), presence of a dorsal hump (vs. absent), and spots on dorsal fin base and dorsal side of head (vs. absent); from *I. amrabad* by presence of divided bars along lateral side (vs. undivided bars), and anal fin with 7 branched rays (vs. 5); from *I. kalsubai* in having a deeper caudal peduncle (vs. as deep as long), dorsal fin insertion posterior to pelvic-fin origin (vs. vertical to pelvic-fin origin), and anal fin with 7 branched rays (vs. 5); from *I. telanganaensis* in having a deeper caudal peduncle (vs. as deep as long), absence of spots on cheek (vs. present), pectoral fin with 1 simple and 9 branched rays (vs. 2 simple and 10 branched rays), pelvic fin with 1 simple ray (vs. 3), and anal fin with 7 branched rays (vs. 5).



**TABLE 2.** The uncorrected P-distance between various *Indoreonectes* species based on the cyt *b* gene sequences.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1. <i>Indoreonectes mahadeoensis</i> sp. nov. PP911355													
2. <i>Indoreonectes neelshi</i> KY497101	11.9												
3. <i>Indoreonectes rajeevi</i> KY497093	14.9	13.5											
4. <i>Indoreonectes keralensis</i> MG018981	18.6	16.4	16.2										
5. <i>Indoreonectes evezardi</i> KY497107	11.9	5.6	12.0	16.4									
6. <i>Indoreonectes evezardi</i> KY497109	11.8	5.7	11.9	16.3	0.7								
7. <i>Indoreonectes telanganaensis</i> MT569389	11.5	6.5	13.3	16.0	6.6	6.5							
8. <i>Indoreonectes telanganaensis</i> MT569390	11.6	6.6	13.4	16.0	6.7	6.6	0.1						
9. <i>Indoreonectes kalsubai</i> MW752810	11.9	6.4	13.8	15.5	7.3	7.2	5.1	5.2					
10. <i>Indoreonectes kalsubai</i> MW752811	11.9	6.4	13.6	15.5	7.3	7.2	5.1	5.2	0.2				
11. <i>Indoreonectes amrabad</i> MW752826	11.2	5.9	12.8	15.5	6.5	6.2	6.2	6.3	6.3	6.4			
12. <i>Indoreonectes amrabad</i> MW752827	11.2	5.7	12.8	15.5	6.5	6.2	6.2	6.3	6.3	6.3	0.2		
13. <i>Indoreonectes radhanagari</i> MW752829	15.2	14.0	5.1	16.3	12.8	12.9	13.6	13.7	14.0	13.8	12.8	12.7	
14. <i>Indoreonectes radhanagari</i> MW752830	14.8	14.0	5.2	16.2	12.8	12.9	13.4	13.5	14.2	14.0	12.8	12.8	0.4



**FIGURE 6.** Lateral view of nine species of *Indoreonectes* [A—*Indoreonectes keralensis*, WII/JAJ-F 313, 50 mm SL; B—*Indoreonectes evezardi*, BNHS/FWF1070, 33.5 mm SL; C—*Indoreonectes telanganaensis*, FBRC/ZSI/VS 01.ii, 32 mm SL; D—*Indoreonectes neelshi*, BNHS FWF 1071, 38.5 mm SL; E—*Indoreonectes rajeevi*, BNHS FWF 1081, 45.7 mm SL; F—*Indoreonectes amrabad*, ZSI/FBRC/F/4036, 49.3 mm SL; G—*Indoreonectes kalsubai*, ZSI/FBRC/F/4039, 44.3 mm SL; H—*Indoreonectes radhanagari*, ZSI/FBRC/F/4041, 32.1 mm SL; I—*Indoreonectes mahadeoensis* sp. nov., WII/NWR/Type\_F 04, 53.0 mm SL].

In addition to the various morphological differences, *I. mahadeoensis* can also be separated from its congeners in the genetic divergence of the cyt *b* gene sequences which ranges from 11.2 to 18.6% (Table 2). Previous studies on loaches suggested that the interspecific variation of the cyt *b* gene ranges from 2.0% in Balitoridae (Tang *et al.* 2006), 3.0% in Cobitidae (Perdices *et al.* 2018) and 5.4% in Nemacheilidae (Bohlen *et al.* 2020). However, recent studies on *Indoreonectes* loaches demonstrated a variation of > 11.0% in the cyt *b* sequences (Prasad *et al.* 2020; Kumkar *et al.* 2021; Jadhav *et al.* 2024), which has also been substantiated in the present study. Previous research has shown a strong correlation between genetic distance and geographic distance (see Jadhav *et al.* 2024), such that pairs of species that are located farther apart are genetically more different from those that are located close to each other (Muehlenbein 2015).

### Key to the species of *Indoreonectes*

1. Nasal barbels short, not reaching anterior border of eye; dorsal-fin origin posterior to pelvic-fin base; anterior half devoid of lateral bars, but with a dark horizontal mid-lateral bar ..... *Indoreonectes keralensis*
- Nasal barbels reaching anterior border of, middle, or beyond eye; dorsal-fin origin opposite to, or slightly behind pelvic-fin origin; body with bars or blotches throughout lateral surface ..... 2
2. Nasal barbels reaching anterior border of eye; dorsal-fin origin slightly behind vertical from pelvic-fin origin; body with broken bars throughout lateral surface ..... *Indoreonectes mahadeoensis*
- Nasal barbels reaching middle of eye, or beyond. .... 3
3. Nasal barbel long, reaching posterior border of eye; maxillary barbel long, reaching posterior border of operculum; inner rostral barbel long, reaching anterior margin of eye; conspicuous black marking on lower lip; no hump behind nape. .... *Indoreonectes rajeevi*
- Nasal barbel reaching middle of eye; maxillary barbel short, reaching midway between eye and posterior border of operculum; inner rostral barbel short, reaching middle of nostril; conspicuous black marking on lower lip absent; distinct dorsal hump behind nape. .... 4
4. No distinct spots on dorsal side of head ..... *Indoreonectes kalsubai*
- Distinct spots on dorsal side of head ..... 5
5. Lateral bars wider or narrower than inter-space. .... 6
- Lateral bars and inter-bar spaces equal, blotches on lateral body ..... 8
6. Lateral bars wider than inter-bar spaces ..... *Indoreonectes neeshi*
- Lateral bars narrower than inter-bar spaces ..... 7
7. Caudal peduncle as long as deep; caudal-peduncle bars not divided into two spots; spots on cheeks below eye. .... *Indoreonectes telanganaensis*
- Caudal peduncle deeper than long; caudal-peduncle bars expressed as two separate spots; no spots on cheeks below eye. .... *Indoreonectes evezardi*
8. Lateral bars and inter-bar spaces equal; caudal-peduncle as long as deep; no spots on cheeks below eye ..... *Indoreonectes radhanagari*
- Black blotches on lateral body; Caudal peduncle deeper than long; spots on cheeks below eye ..... *Indoreonectes amrabad*

### Comparative material

*Indoreonectes evezardi*: Topotype: BNHS/FWF1070, 1 ex., 33.5 mm SL., India, Mutha River, Krishna River system, Pune. Coll. M. Pise, P. Gorule and P. Kumkar 8 Oct 2017.

*Indoreonectes keralensis*: Topotype: WII/JAJ-F 313, 5 ex., 46.5–50.0 mm SL., India, Vattaparai stream, Periyar River system, Kerala. Coll. J.A. Johnson, 31 Aug 2022.

*Indoreonectes telanganaensis*: Holotype: FBRC/ZSI/VS 01.i, 33.6 mm SL., India, Kawal Tiger Reserve, Maisamma Loddi, Telangana State. Coll. Krishna Prasad, K., G. Devender and G. Chethan Kumar, 3 Oct 2015. Paratypes: FBRC/ZSI/VS 01.ii, 2 ex., 28.4–32.0 mm SL., Same data as holotype.

*Indoreonectes neeshi*: Holotype: BNHS FWF 1071, 38.5 mm SL., India, Mutha River, Krishna River system, Pune. Coll. M. Pise and P. Kumkar 30 Mar. 2018. Paratypes: BNHS FWF 1073, 2 ex., 28.2–32.8 mm SL. Same data as holotype.

*Indoreonectes rajeevi*: Holotype: BNHS FWF 1081, 45.7 mm SL., India: Maharashtra, Hiranyakeshi River, Amboli. Coll. P. Gorule and A. Gorule 23 Oct 2017. Paratypes: BNHS FWF 1082 & 1085, 2ex., 27.7–36.8 mm SL., same data as holotype.

*Indoreonectes amrabad*: Holotype: ZSI/FBRC/F/4036, 49.3 mm SL, India: Eastern Ghat, Telangana State, Amrabad Tiger Reserve, Stream near Vatavarlappally, 01 Oct 2020. Coll. S. Jadhav and M. Karuthapandi. Paratypes: ZSI/ FBRC/F/4038, 2 ex., 38.0–38.4 mm, SL. Same data as holotype.

*Indoreonectes kalsubai*: Holotype: ZSI/FBRC/F/4039, 44.3 mm SL., India: Western Ghats, Maharashtra State, Ahmednagar District, Kalsubai hills, Stream near Baari Village. Coll. S. Jadhav and M. Karuthapandi, 16 Jan. 2021. Paratypes: ZSI/FBRC/F/4040 2 ex., 41.2–43.2 mm SL. Same data as holotype.

*Indoreonectes radhanagari*: Holotype: ZSI/FBRC/F/4041, 32.1 mm SL., India: Western Ghats, Maharashtra State, Kolhapur District, Radhanagari Wildlife Sanctuary, Rautwadi waterfall, 23 Nov. 2020. Coll. S. Jadhav and M. Karuthapandi. Paratypes: ZSI/FBRC/F/4041, 2 ex, 27.8–29.3 mm SL., India: same data as holotype.

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**Appendix A.** GenBank accession numbers for *cyt b* sequences used in the current study.

Species	Locality	Voucher number.	Accession number ( <i>cyt b</i> )	References
<i>Indoreonectes mahadeoensis</i> sp. nov.	Mahadeo stream, Satpura Tiger Reserve, Narmadapuram, Madhya Pradesh	WII/NWR/Type_F 04	PP911355	Present study
<i>I. telanganaensis</i>	Maisamma Loddi, Telangana	NHMOU.F.18	MT569389	Prasad <i>et al.</i> (2020)
<i>I. telanganaensis</i>	Maisamma Loddi, Telangana	NHMOU.F.19	MT569390	Prasad <i>et al.</i> (2020)
<i>I. kalsubai</i>	Baari village, Kalsubai hills, northern Western Ghats, Maharashtra	FBRC-ZSI-F-4040i	MW752810	Jadhav <i>et al.</i> (2024)
<i>I. kalsubai</i>	Baari village, Kalsubai hills, northern Western Ghats, Maharashtra	FBRC-ZSI-F-4040ii	MW752811	Jadhav <i>et al.</i> (2024)
<i>I. evezardi</i>	Pune, Maharashtra	BNHS FWF 322	KY497107	Keskar <i>et al.</i> (2018)
<i>I. evezardi</i>	Pune, Maharashtra	BNHS FWF 300	KY497109	Keskar <i>et al.</i> (2018)
<i>I. neeleshii</i>	Harishchandragad, Maharashtra	BNHS FWF 318	KY497101	Keskar <i>et al.</i> (2018)
<i>I. amrabad</i>	Mallelateertham waterfall, Amrabad Tiger Reserve, Telangana	FBRC-ZSI-F-4038iv	MW752826	Jadhav <i>et al.</i> (2024)
<i>I. amrabad</i>	Stream near Vatavarlappally, Amrabad Tiger Reserve, Telangana	FBRC-ZSI-F-4038i	MW752827	Jadhav <i>et al.</i> (2024)
<i>I. radhanagari</i>	Rautwadi waterfall, Radhanagari WLS, District Kolhapur, northern Western Ghats, Maharashtra	FBRC-ZSI-F-4042	MW752829	Jadhav <i>et al.</i> (2024)
<i>I. radhanagari</i>	Stream near Kariwade village, Radhanagari WLS, District Kohlapur, northern Western Ghats, Maharashtra	FBRC-ZSI-F-4043	MW752830	Jadhav <i>et al.</i> (2024)
<i>I. rajeevi</i>	Amboli, Maharashtra	BNHS FWF 316	KY497093	Keskar <i>et al.</i> (2018)
<i>I. keralensis</i>	Erratayar, Kerala	WILD-17-PIS-343	MG018981	Keskar <i>et al.</i> (2018)
<i>Balitora chipkali</i>	Astoli River, Kali River system, Ramnagar, Karnataka	BNHS FWF 194	KU378017	Kumkar <i>et al.</i> (2016)
<i>Balitora mysorensis</i>	Sivasamudram, Cauvery River system, Karnataka	BNHS FWF 197	KU378019	Kumkar <i>et al.</i> (2016)