



Two new species of the hillstream loach genus *Indoreonectes* from the northern Western Ghats of India (Teleostei: Nemacheilidae)

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<http://zoobank.org/D5FDD6A7-7D8E-471F-B1D2-425C56DF597B>

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Academic editor Ralf Britz

Received 5 January 2021

Accepted 22 July 2021

Published 16 August 2021

Citation: Kumkar P, Pise M, Gorule PA, Verma CR, Kalous L (2021) Two new species of the hillstream loach genus *Indoreonectes* from the northern Western Ghats of India (Teleostei: Nemacheilidae). *Vertebrate Zoology* 71 517–533. <https://doi.org/10.3897/vz.71.e62814>

Abstract

The hill stream loach genus *Indoreonectes* is endemic to peninsular India south of the Satpura hill ranges and is represented by three species *I. evezardi*, *I. keralensis* and *I. telanganaensis*. *Indoreonectes evezardi* has been suggested as a species complex based on recent genetic studies; however, due to lack of type material the species delimitation has been difficult. Here we redescribe *I. evezardi* collected from its type locality and describe two new species from the northern Western Ghats of India. *Indoreonectes neeleshi*, described from Mula River tributary of Godavari river system, can be diagnosed from all its congeners based on a combination of characters: inner rostral barbel reaching middle of nostril; maxillary barbel reaching midway between eye and posterior border of operculum; dorsal hump behind nape; bars on lateral side of the body wider than inter-bar space; total vertebrae 35 and dorsal fin insertion between 13th and 14th abdominal vertebrae. *Indoreonectes rajeevi*, described from Hiranyakeshi River of the Krishna river system, differs from all its congeners based on a combination of characters: inner rostral barbel reaching anterior margin of eye; maxillary barbel reaching posterior border of operculum; conspicuous black markings on lower lip, dorsal hump absent; total vertebrae 36 and dorsal fin insertion between 12th and 13th abdominal vertebrae. Further, *I. neeleshi* differs from its congeners by the raw genetic distance of 6.8–14.4% for the *cox1* gene and 5.7–16.2% for the *cytb* gene, while *I. rajeevi* differs from its congeners by the raw genetic distance of 10.9–14.0% for the *cox1* gene and 11.8–15.8% for the *cytb* gene.

Keywords

Biodiversity hotspot, Godavari river system, Krishna river system, Maharashtra

Introduction

The genus *Indoreonectes* Rita, Bănărescu and Nalbant of hill stream loaches (Cypriniformes: Nemacheilidae) was initially proposed as a subgenus of *Oreonectes* Günther, while describing *O. (Indoreonectes) keralensis* Rita

and Nalbant (Rita et al. 1978). Kottelat (1990a, 1990b, 2012) and Prokofiev (2010) considered *Indoreonectes* as a valid genus endemic to Peninsular India. The genus is diagnosed based on following combination of characters

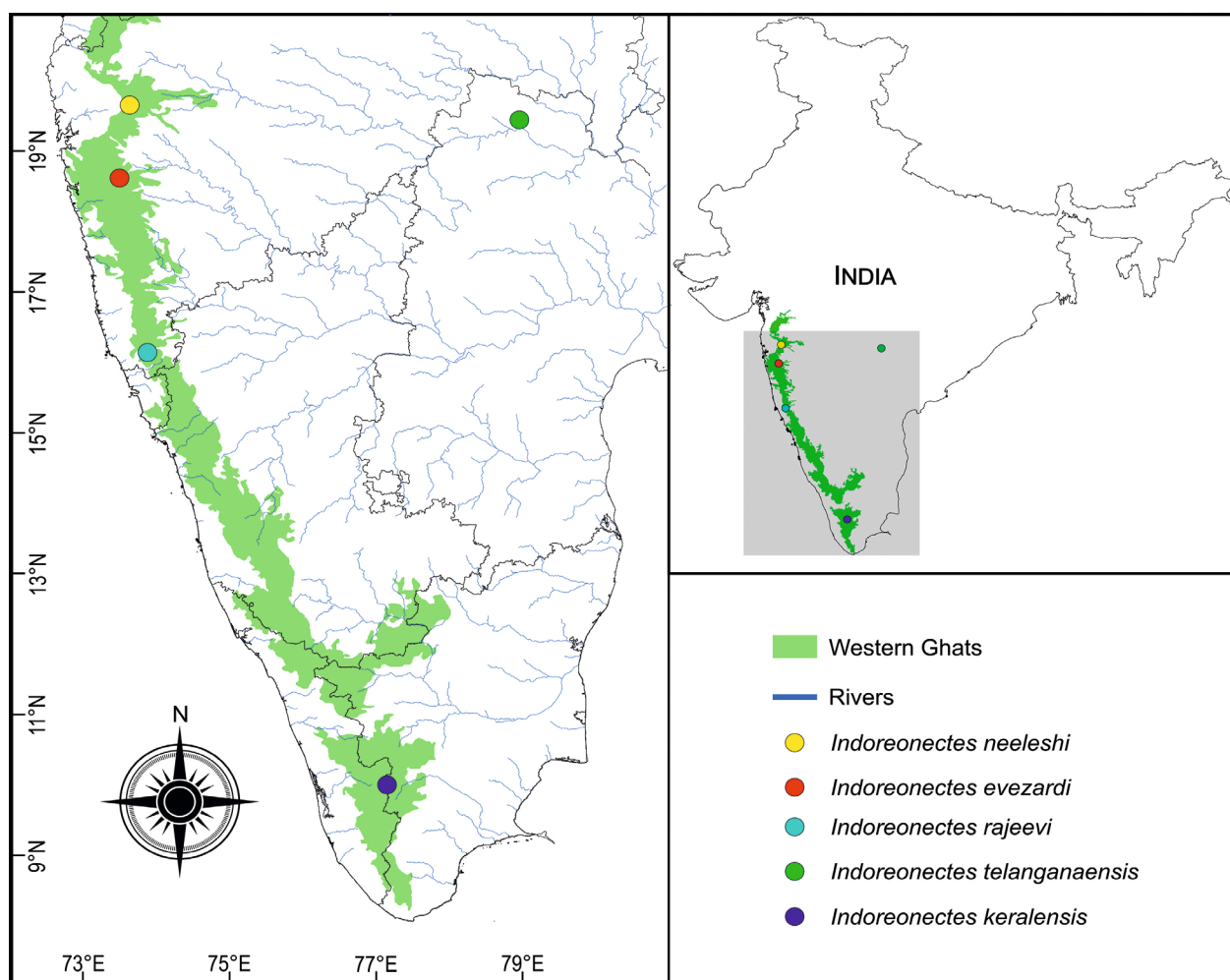


Figure 1. Type localities of species of the genus *Indoreonectes* in southern Peninsular India.

following Rita et al. (1978) and, Bănărescu and Nalbant (1995): nostrils close to each other, posterior one distant from eye; lateral line present, short; length of nasal barbels variable; free posterior part of air bladder rudimentary; bars on side of body. Currently, the genus is represented by three species, *I. evezardi* (Day, 1872), *I. keralensis* (Rita and Nalbant, 1978) and *I. telanganaensis* (Prasad, Srinivasulu, Srinivasulu, Anoop and Dahanukar, 2020).

Indoreonectes evezardi has been considered to be a widely distributed species in peninsular India north of the Palghat Gap, occurring in the east-flowing Krishna, Godavari and Cauvery river systems in addition to several west-flowing rivers in Goa, Maharashtra, and Gujarat states, while *I. keralensis* is restricted to river systems south of the Palghat gap, particularly the Periyar, Pampa, Muvattupuzha, and Meenachil rivers of Western Ghats of Kerala (Rita et al. 1978; Menon 1987; Kottelat 1990b; Rema Devi et al. 2002, 2013; Dahanukar et al. 2004; Kazi et al. 2018; Raghavan and Ali 2011). The recently described species *I. telanganaensis* is currently known only from its type locality, a seasonal stream of the Godavari River within the Kawal Tiger Reserve, Mancheriyal District, Telangana State (Prasad et al. 2020).

Based on extensive genetic sampling from the northern Western Ghats, Keskar et al. (2018) suggested that the wide-ranging taxon *Indoreonectes evezardi* is a spe-

cies complex with several undescribed species. However, lack of type material for *I. evezardi* (see for detailed discussion, Prasad et al. 2020: 344) has made it difficult to diagnose species in the *I. evezardi* complex. Although Prasad et al. (2020) recognized this issue and provided photographs of *I. evezardi* from its type locality, they did not redescribe the species in detail.

In the current study we redescribe *Indoreonectes evezardi* from its type locality using fresh collections and specimens studied by Prasad et al. (2020). Further, we describe two new species from the *I. evezardi* complex from the Mula River, a tributary of the Godavari river system and from the Hiranyakeshi River, part of the Krishna river system.

Materials and methods

Fish collection

Individuals of the new species of *Indoreonectes* were collected from two different localities, one in the upper reaches of Godavari river system in Maharashtra at Harishchandragad (19°23.64'N; 73°46.74'E, ca 1180 m a.s.l.)

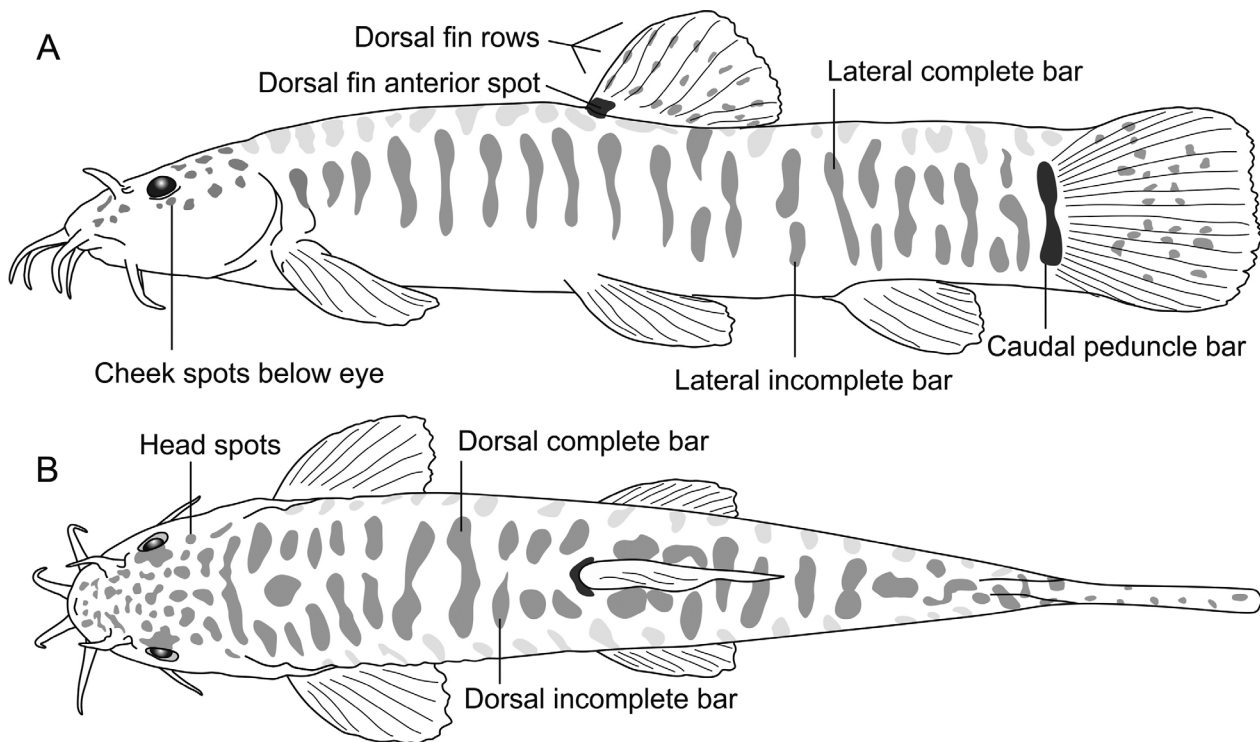


Figure 2. Diagram of colour markings in *Indoreonectes* and their terminology.

(ten individuals) and another from Krishna river system in Maharashtra at Amboli (15°58.02'N; 74°0.66'E, ca 692 m a.s.l.) (twelve individuals) (Fig. 1). Three topotypes of *Indoreonectes evezardi* were collected from Mutha River at Kuran (18°23.28'N; 73°38.52'E, ca 580 m a.s.l.) Pune, Maharashtra, and one topotype of *Indoreonectes keralensis* was collected from Santhampara, Idukki, (9°58.08'N; 77°12.72'E, ca 1050 m a.s.l.), Kerala. After anesthetization one individual each of the new species was stored in absolute alcohol and the remaining in 10% formalin. Collected specimens are housed in the museum of the Bombay Natural History Society (BNHS), Mumbai, Maharashtra, India (*I. evezardi* – BNHS FWF 1068–1070, *I. neeleshii* – BNHS FWF 1071–1080, *I. rajeevi* – BNHS FWF 1081–1092) and in the collection of the Kerala University of Fisheries and Ocean Studies (KUFOS), Kochi, India (*I. keralensis* – KUFOS.2017.11.217). Additional specimens were studied from the museum collection of BNHS and the Wildlife Information Liaison Development Society (WILD), Coimbatore, India.

Morphology, morphometry and osteology

Measurements were taken for each specimen to the nearest 0.1 mm using digital callipers (Mitutoyo, Japan). Morphometric methods follow Keskar et al. (2015). Subunits of the body are presented as a percent of standard length (SL), and subunits of the head as a percent of head length (HL). Fin rays were counted using a stereomicroscope (Magnus, India). Frequency of the count is provided in parentheses after the count. The last two fin rays of the dorsal and anal fins, which articulate with a single pte-

rygiophore and often referred to as “last ray split to the base” were counted as one ray. Terminology used for describing the pattern of bars and other markings is defined in Figure 2. Reach of barbels is based on the condition when folded back artificially.

Two paratypes of *I. neeleshii* (BNHS FWF 1074–75), three paratypes of *I. rajeevi* (BNHS FWF 1090–92), two specimens of topotypic *I. evezardi* (BNHS FWF 1068–1069) and one specimen of *I. keralensis* (KUFOS.2017.11.217) were cleared and stained to study skeletal morphology following the procedure described by Potthoff (1984) and used to obtain vertebral counts, including Weberian apparatus (counted as four vertebrae) and compound centrum.

Molecular analysis

Gill tissues were extracted from a paratype of each species preserved in absolute alcohol (*Indoreonectes neeleshii* – BNHS FWF 1080 and *I. rajeevi* – BNHS FWF 1089). DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the protocol given by the manufacturer. Mitochondrial cytochrome oxidase subunit I (*cox1*) gene was amplified using Polymerase Chain Reaction with primers FishF1 and FishR1 (Ward et al. 2005). Gene amplification, purification and sequencing protocols follow Ali et al. (2013). Sequencing was performed by Apical Scientific SDN, BHD., Malaysia. The raw chromatograms were manually assembled and verified for potential mistakes using free software FINCHTV 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; <http://www.geospiza.com>). The BLAST tool (Altschul et al. 1990) was used to see the similarity to the listed sequences in

the database of NCBI (<http://www.ncbi.nlm.nih.gov/>). Gene sequences are deposited in GenBank with the accession number MW136273 and MW136274. Additional sequences, for *cox1* and cytochrome b (*cytb*) genes from our earlier studies Keskar et al. (2018) and Kumkar et al. (2016) and *cytb* gene from Prasad et al. (2020), were retrieved from NCBI GenBank database (Appendix I).

Gene sequences were aligned with MUSCLE (Edgar 2004) for individual genes and the aligned sequences were concatenated to get the combined data matrix of *cox1* and *cytb*. The combined matrix had 1747 base pairs. *Balitora chipkali* (Cypriniformes: Balitoridae) was used as an outgroup. Data were partitioned in two genes and their respective codon positions to create a full partition. The best partitioning scheme and the nucleotide substitution model for the partition scheme were estimated using partition analysis (Chernomor et al. 2016) and MODELFINDER (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE 1.6.12 (Nguyen et al. 2015). Maximum likelihood (ML) analysis was performed in IQ-TREE 1.6.12 (Nguyen et al. 2015) with the best partition scheme and ultrafast bootstrap support for 1000 iterations (Hoang et al. 2018). The resulting maximum likelihood phylogram was edited in FIGTREE v1.4.4 (Rambaut 2018). Uncorrected raw (p) distances between pairs of sequences were calculated in MEGA X 10.1 (Kumar et al. 2018).

Results

Taxonomy

Indoreonectes evezardi (Day, 1872)

Figs 3–6

Nemacheilus evezardi Day, 1872: p. 182

Oreonectes (*Indoreonectes*) *evezardi* (Day, 1872): Rita, Bănărescu and Nalbant (1978, 186)

Material examined. BNHS FWF 1068–1070, 3 ex., 33.1–43.81 mm SL, India: Maharashtra, Mutha River, Krishna river system, Pune (18°23.28'N; 73°38.52'E, ca 580 m a.s.l.), coll. M. Pise, P. Gorule and P. Kumkar 8 Oct. 2017. BNHS FWF 299–300, 2 ex., 40.5–44.6 mm SL, Mutha River at Warje (18°28.32'N; 73°48.48'E), Pune, Maharashtra, India, coll. N. Dahanukar and M. Paingankar, 10 Jul. 2008; WILD-17-PIS-350–358, 9 ex., 35.0–43.0 mm SL, Mutha River at Warje (18°28.32'N; 73°48.48'E), Pune, Maharashtra, India, coll. N. Dahanukar and M. Paingankar, 10 Jul. 2008; WILD-17-PIS-359, 1 ex., 33.5 mm SL, Mutha River at Panshet (18°26.16'N; 73°38.1'E), Pune, Maharashtra, India, coll. P. Kumkar, 8 Oct. 2017.

Diagnosis. *Indoreonectes evezardi* can be distinguished from all other congeners by having caudal peduncle bar divided as two spots (vs. not divided in *I. keralensis*, *I. telanganaensis*, *I. neeleshi* and *I. rajeevi*); caudal peduncle deeper than long (vs. longer than deep in *I. keralensis*,

as deep as long in *I. telanganaensis* and *I. neeleshi*, slightly longer than deep in *I. rajeevi*). Further, *Indoreonectes evezardi* can be distinguished from *I. keralensis* by having long nasal barbel reaching mid of eye (vs. short nasal barbel barely reaching anterior border of eye); inner rostral barbel reaching middle of nostril (vs. reaching anterior margin of eye); presence of dorsal hump behind nape (vs. absent); dorsal-fin insertion between neural spines of 12th and 13th abdominal vertebrae (vs. between 13th and 14th); presence of a dark brown to black spot at base of first dorsal-fin ray and distinct spots on dorsal side of head (vs. absent); spots on cheek below eye absent (vs. present). *Indoreonectes evezardi* can be distinguished from *I. telanganaensis* by having inner rostral barbel reaching middle of nostril (vs. reaching further posteriorly to anterior margin of eye); spots on cheek below eye absent (vs. present). *Indoreonectes evezardi* can be distinguished from *I. neeleshi* by having lateral bars narrower than inter-bar spaces (vs. wider in *I. neeleshi*). *Indoreonectes evezardi* can be distinguished from *I. rajeevi* by having inner rostral barbel reaching middle of nostril (vs. reaching further posteriorly to anterior margin of eye); maxillary barbel not reaching posterior border of operculum (vs. reaching); absence of conspicuous black marking on lower lip (vs. presence); presence of dorsal hump behind nape (vs. absence); total vertebrae 35 (vs. 36).

Description. General morphology is shown in Figure 3; morphometric data are provided in Table 1.

Body sub-cylindrical, elongate; head and anterior part of body almost cylindrical; body laterally compressed posteriorly; predorsal outline convex, gradually rising up to dorsal-fin origin, a distinct hump behind nape; post-dorsal outline straight up to base of caudal fin; ventral profile almost straight. Caudal peduncle deeper than long. Lateral line present (14) or absent (1), when present then incomplete, short, ending above middle of adpressed pectoral fin. Scales minute.

Head small, slightly longer than a quarter of SL. Snout round, its length more than one-third of head length. Eye dorsolaterally positioned, closer to tip of snout than to posterior margin of opercle, its diameter 10–16% HL. Mouth semi-circular, with thick fleshy lips, lower lip interrupted medially by a deep groove (Fig. 4A). Barbels four pairs. Two pairs of rostral barbels, inner rostral barbel extending to middle of nostril, outer rostral barbel reaching anterior margin of eye; Maxillary barbel longest, originating at vertical from nostril, reaching midway between eye and posterior border of operculum. Nasal barbel well developed, reaching middle of eye.

Dorsal-fin origin slightly posterior to pelvic-fin origin; slightly closer to caudal-fin base than tip of snout, its posterior edge rounded, with 4 (15) simple and 7 (15) branched rays. Pectoral fin slightly shorter than head length; with 1 (15) simple and 9 (3) or 10 (12) branched rays. Pelvic fin with 1 (15) simple and 7 (15) branched rays. Anal fin with 3 (15) simple and 5 (15) branched rays. Caudal fin rounded, with 9+9 (15) principal caudal-fin rays. Dorsal procurent rays 11(8) or 13(7) and ventral procurent rays 6(10) or 7(5).

Table 1. Morphometric data of *Indoreonectes evezardi* topotypes (BNHS FWF 299–300, 1068–1070, BNHS FWF, WILD-17-PIS-350–359), *I. neeleshii* holotype (BNHS FWF 1071), paratypes (BNHS FWF 1072–1080) and *I. rajeevi* holotype (BNHS FWF 1081) and paratypes (BNHS FWF 1082–1092). Mean, standard deviation (sd) and range of new species include holotype.

Characters	<i>I. evezardi</i> (n = 15)		<i>I. neeleshii</i> (n = 10)			<i>I. rajeevi</i> (n = 12)		
	Mean (sd)	Range	Holotype	Mean (sd)	Range	Holotype	Mean (sd)	Range
Total length (mm)	45.2 (4.4)	40.5–53.3	47.9	39.6 (4.1)	34–47.9	55.9	41.2 (5.7)	33.2–55.9
Standard length (SL, mm)	37.7 (3.8)	33.5–44.6	38.5	32.1 (3.2)	28.2–38.5	45.8	33.9 (4.8)	26.4–45.8
Head length (HL, mm)	8.9 (0.8)	8.0–10.8	8.3	7.1 (0.8)	5.9–8.3	8.8	6.8 (1.0)	5.3–8.8
%SL								
Head length	23.7 (0.8)	22.3–25.2	21.4	22 (0.9)	20.8–24.1	19.3	20 (1.1)	17.7–21.4
Predorsal length	55.4 (1.5)	52.0–57.2	55.3	56.5 (1.2)	54.6–58.7	52.5	53.9 (1.2)	52.2–56.1
Dorsal-fin origin to caudal-fin base	46.4 (1.4)	43.8–48.8	48.6	46.3 (1.3)	45.2–48.7	48.7	47.8 (1.0)	45.8–49.0
Prepectoral length	24.1 (1.1)	21.9–25.3	23.8	22.9 (0.9)	21.3–23.9	20.3	21.7 (0.9)	20.3–22.8
Prepelvic length	54.1 (1.7)	50.8–56.0	50.7	52.6 (1.7)	50.7–55.7	50.9	49.9 (2.4)	42.7–51.4
Preamble length	75.2 (1.2)	72.3–77.1	72.0	72.6 (1.9)	70.1–76.3	71.4	70.1 (2.1)	66.9–73.3
Preamble-fin length	80.9 (1.4)	78.1–82.8	78.1	79.1 (1.6)	76.3–81.4	77.3	76.5 (1.7)	73.6–78.9
Body depth (at dorsal-fin origin)	17.8 (1.7)	15.4–21.7	14.9	14.4 (1.8)	11.2–16.1	14.7	14.8 (1.2)	12.3–16.1
Body depth (at anus)	15.9 (1.3)	14.5–19.0	14.2	13.7 (1.5)	10.3–15.2	13.7	12.9 (0.9)	10.9–13.8
Body width (at dorsal-fin origin)	12.6 (1.5)	10.3–14.9	13.9	12.1 (1.6)	8.6–13.9	12.7	11.2 (1.3)	8.5–13.5
Body width (at anus)	9.6 (1.4)	7.1–11.6	10.4	9.3 (1.4)	6.4–10.6	10.3	8.9 (1.3)	6.0–10.9
Height of dorsal fin	17.7 (1.2)	16.1–19.9	18.9	18.5 (1.3)	15.8–20.4	17.1	18.4 (1.3)	16.6–20.7
Length of dorsal-fin base	10.7 (0.8)	9.5–12.2	12.7	10.6 (1.3)	8.1–12.7	11.8	11.6 (1.5)	9.0–13.7
Length of pectoral fin	18.2 (1.9)	12.1–20.0	20.2	19.7 (0.6)	18.6–20.4	17.5	17.8 (0.9)	16.9–19.9
Length of pelvic fin	16.5 (1.2)	14.4–18.1	17.7	17.4 (1.0)	15.8–19.0	16.5	16.8 (0.9)	15.5–18.2
Length of anal fin	14.8 (0.7)	13.7–15.8	15.7	14.7 (1.1)	13.0–15.9	15.6	15.6 (1.3)	13.0–17.6
Length of anal-fin base	6.9 (0.6)	5.9–7.9	6.9	6.4 (0.6)	5.1–7.0	7.8	7.4 (0.8)	5.8–8.3
Length of caudal fin	20.4 (1.1)	18.5–22.4	23.1	22.5 (0.9)	20.9–23.3	21.4	20.6 (0.9)	18.7–21.8
Depth of caudal peduncle	14.8 (1.5)	12.5–16.9	14.7	13.9 (2.2)	9.7–16.4	13.1	13.6 (0.8)	12.1–14.6
Length of caudal peduncle	11.6 (1.4)	9.8–15.5	14.6	14.1 (1.7)	12.0–17.7	13.8	15.3 (1.6)	12.7–17.5
% HL								
Head depth	55.1 (4.7)	45.4–60.1	64.0	60.6 (2.9)	55.6–64.0	62.4	59.6 (5.6)	47.0–66.6
Head width	69.1 (4.3)	59.3–74.5	78.9	74.5 (4.3)	66.5–78.9	75.9	72.8 (6)	60.5–79.1
Snout length	41.3 (2.5)	36.1–45.1	46.2	43.1 (3.4)	36.5–46.3	46.8	45 (4.2)	38.5–51.9
Eye diameter	12.2 (1.7)	9.8–15.9	15.7	15.6 (1.0)	14.2–17.9	16.7	17.5 (1.9)	14.1–20.7
Interorbital width	37.3 (2.5)	32.9–41.4	45.3	40.1 (3.8)	33.3–45.3	39.6	38.2 (5.2)	28.1–45.3
Width of mouth	29.6 (4.4)	24.3–37.5	37.4	32.9 (3.1)	29.5–37.8	42.6	34.9 (4)	28.0–42.6

Total vertebrae 35 (Fig. 5A) with 17 (2) abdominal and 18 (2) caudal vertebrae. Dorsal-fin insertion in both cleared and stained specimens is between neural spines of 12th and 13th abdominal vertebrae. Fifth ceratobranchial (Fig. 6A) with single row of 15 to 16 small curved teeth with pointed tips; anterior teeth longer than posterior ones.

Colouration. In life (Fig. 3A), background colour pale yellow, slightly darker on dorsal profile than lateral profile; with brownish-black irregular bars on lateral and dorsal side of body, lateral and dorsal bars separated from each other; lateral bars narrower than inter-bar spaces; lateral complete bars 13 (3), 14 (6), 15 (4) or 16 (2); lateral incomplete bars 4 (6), 5 (6), 6 (2) or 7 (1). Head dorsally covered with dark brown spots; cheek spots below eye absent. Caudal peduncle bar split into two conspicuous spots. Dorsal fin anterior spot dark brown to black in colour; dorsal-fin membrane hyaline with three rows of black spots on rays. Pectoral, pelvic and anal fins hy-

aline, lacking spots; caudal-fin with membrane hyaline, and three to four rows of dark-brown spots on rays. Ventral surface without any markings. In preservative (Fig. 3B–D), colouration similar to that in life, but less conspicuous.

Habitat and distribution. *Indoreonectes evezardi* was collected from fast-flowing clear streams with substrate consisting of rock, pebbles and sand. Co-occurring fish species include *Paracanthocobitis mooreh*, *Schistura denisoni*, *Rasbora dandia* and *Devario malabaricus*. *Indoreonectes evezardi* sensu stricto is currently known only from its type locality in Pune (see Keskar et al. 2018) from Mutha River a tributary of the east flowing Krishna river system, Maharashtra, India (Fig. 1).

Remarks. The types of *I. evezardi* are not traceable and are suspected to be lost (see for details, Prasad et al. 2020). Specimens we examined, which include the specimens studied by Prasad et al. (2020) from the type locali-

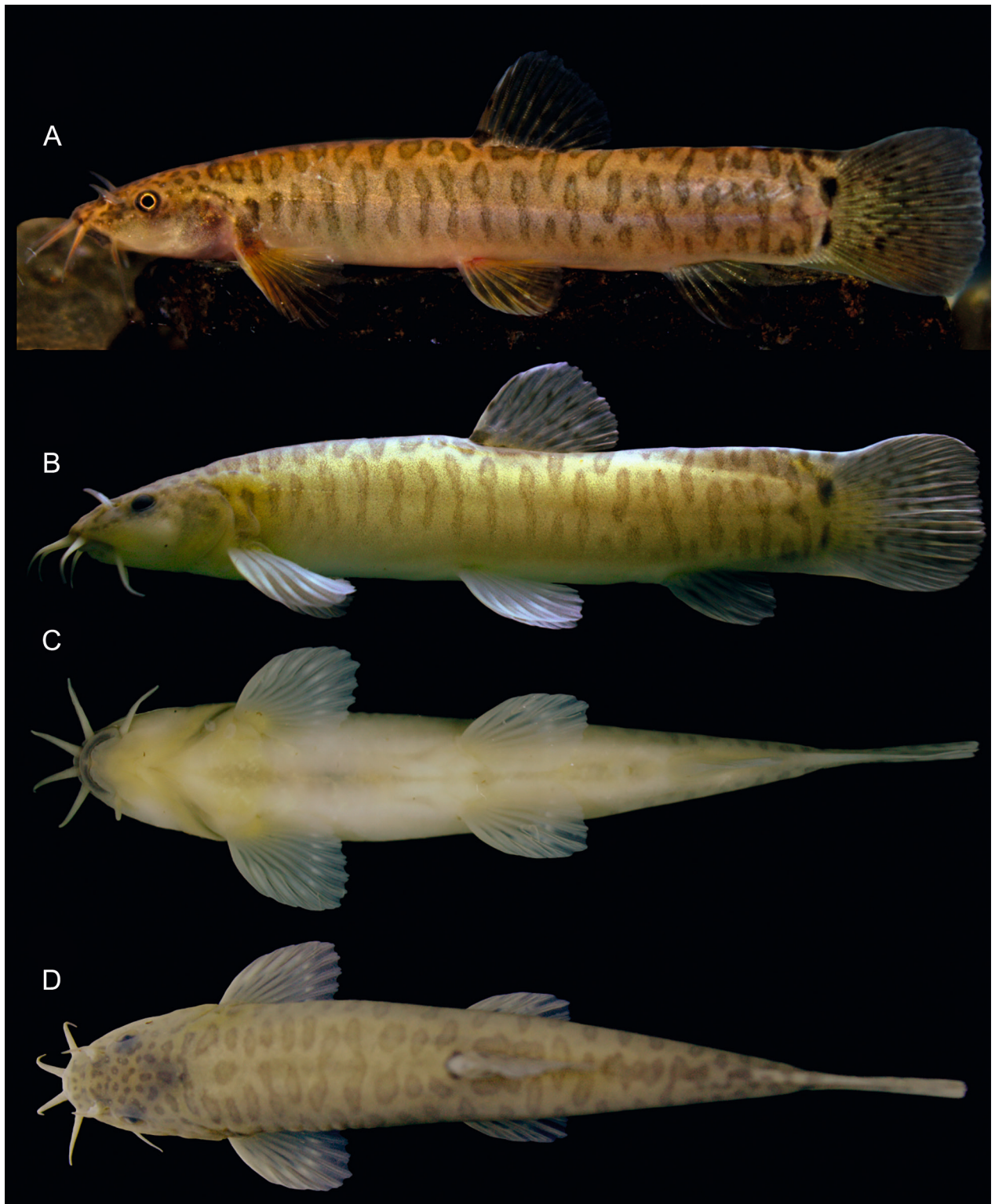


Figure 3. *Indoreonectes evezardi* (BNHS FWF 1070, 33.5 mm SL), topotype, collected from Kuran, Mutha River, Pune, in life (A) and preserved (B–D).

ty in Pune, closely resemble the specimens in the original description of the species as pointed out by Prasad et al. (2020). However, it should be mentioned that in the original description Day (1872) mentions that the lateral line is absent in *I. evezardi*. However, this is a relatively rare

character state and out of total 15 individuals we examined, the lateral line is absent in only one specimen. In all other specimens the lateral line is present, but incomplete and does not extend beyond the middle of the adpressed pectoral fin.

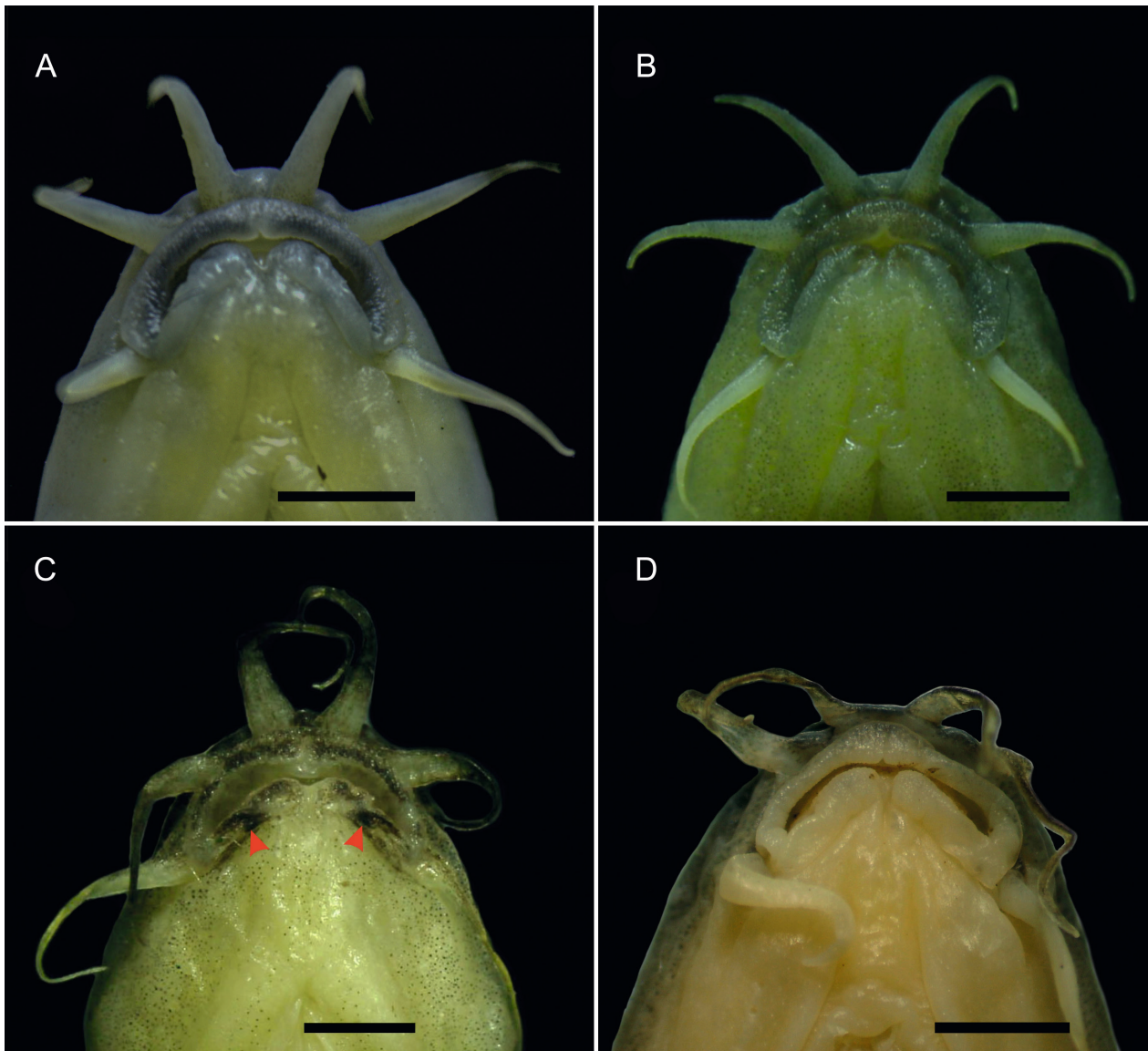


Figure 4. Mouth of *Indoreonectes* species, in ventral view. (A) *Indoreonectes evezardi* (BNHS FWF 1070, 33.5 mm SL), (B) *I. neeleshi* holotype (BNHS FWF 1071, 38.5 mm SL), (C) *I. rajeevi* holotype (BNHS FWF 1081, 45.8 mm SL) and (D) *I. keralensis* (KU-FOS.2017. 11.217, 36.1 mm SL). Scale bar 2 mm. Red arrows in C points to conspicuous black markings on lower lip in *I. rajeevi*.

Indoreonectes neeleshi sp. nov.

Common name: Neelesh's hill stream loach

Figs. 4–7

<http://zoobank.org/8C331B78-D079-43D4-8243-60CB-C2A4F314>

Holotype. BNHS FWF 1071, 38.54 mm SL, India: Maharashtra, Mula River, Godavari river system, Harishchandragad (19°23.64'N; 73°46.74'E, ca 1180 m a.s.l.), coll. M. Pise and P. Kumkar 30 Mar. 2018.

Paratypes. BNHS FWF 1072–1080, 9 ex., 28.2–36.3 mm SL, same data as holotype. Paratypes BNHS FWF 1074 (36.26 mm SL), and BNHS FWF 1075 (32.43 mm SL) used for clearing and staining.

Diagnosis. *Indoreonectes neeleshi* can be distinguished from all congeners by having the lateral bars wider than inter-bar spaces (vs. narrower in *I. evezardi*, *I. keralensis*, *I. telanganaensis*, and *I. rajeevi*). Further, *Indoreonectes neeleshi* can be distinguished from *I. keralensis* by having long nasal barbel reaching middle of eye (vs. short nasal barbel barely reaching anterior margin of eye); inner rostral barbel reaching middle of nostril (vs. reaching further posteriorly to anterior margin of eye); presence of dorsal hump behind nape (vs. absence); presence of a dark brown to black spot on base of first dorsal-fin ray and distinct spots on the dorsal side of head (vs. absence); caudal peduncle as deep as long (vs. longer than deep); spots on cheek below eye absent (vs. present). *Indoreonectes neeleshi* can be distinguished from *I. evezardi* by having caudal peduncle as deep as long (vs. deeper than long); caudal peduncle bar not divided (vs. divided into two spots). *Indoreonectes neeleshi* can be distinguished from *I. telanga-*

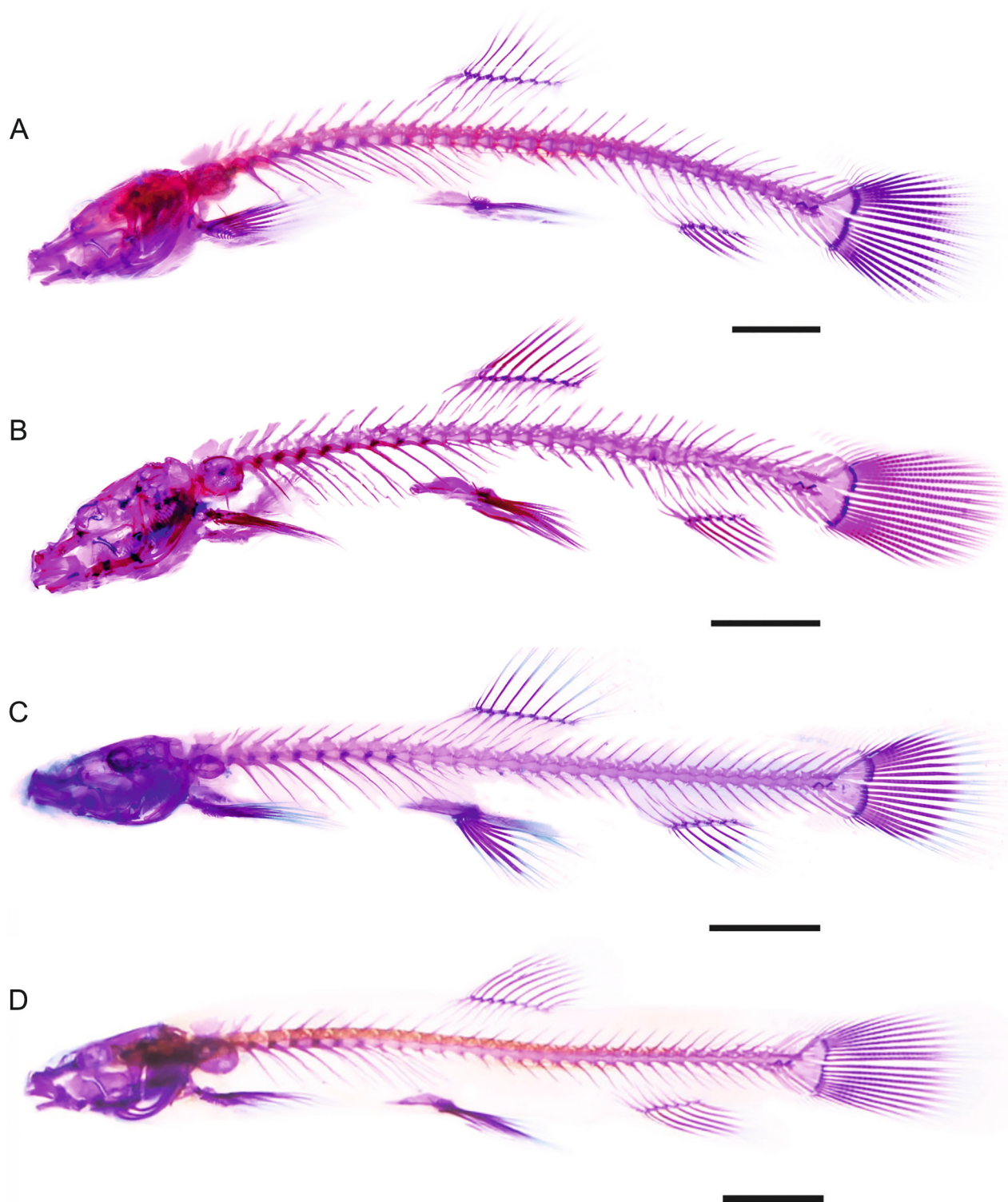


Figure 5. Cleared and stained specimens of *Indoreonectes* showing vertebral column and insertion of dorsal fin, in lateral view. (A) *Indoreonectes evezardi* (BNHS FWF 1068, 43.8 mm SL), (B) *I. neeleshii* (BNHS FWF 1074, 36.2 mm SL), (C) *I. rajeevi* (BNHS FWF 1090, 35.1 mm SL) and (D) *I. keralensis* (KUFOS.2017.11.217, 36.1 mm SL). Scale bar 5 mm. Note that vertebral column of *I. neeleshii* is malformed in caudal region.

naensis by having inner rostral barbel reaching middle of nostril (vs. reaching further posteriorly to anterior margin of eye); spots on cheek below eye absent (vs. present). *Indoreonectes neeleshii* can be distinguished from *I. rajeevi* by having inner rostral barbel reaching middle of nostril (vs. reaching further posteriorly to anterior margin of eye); maxillary barbel not reaching posterior border of opercu-

lum (vs. reaching to posterior border of operculum); absence of conspicuous black marking on lower lip (vs. presence); presence of dorsal hump behind nape (vs. absence); dorsal-fin insertion between neural spines of 13th and 14th abdominal vertebrae (vs. between 12th and 13th abdominal vertebrae); caudal peduncle as deep as long (vs. slightly longer than deep); total vertebrae 35 (vs. 36).

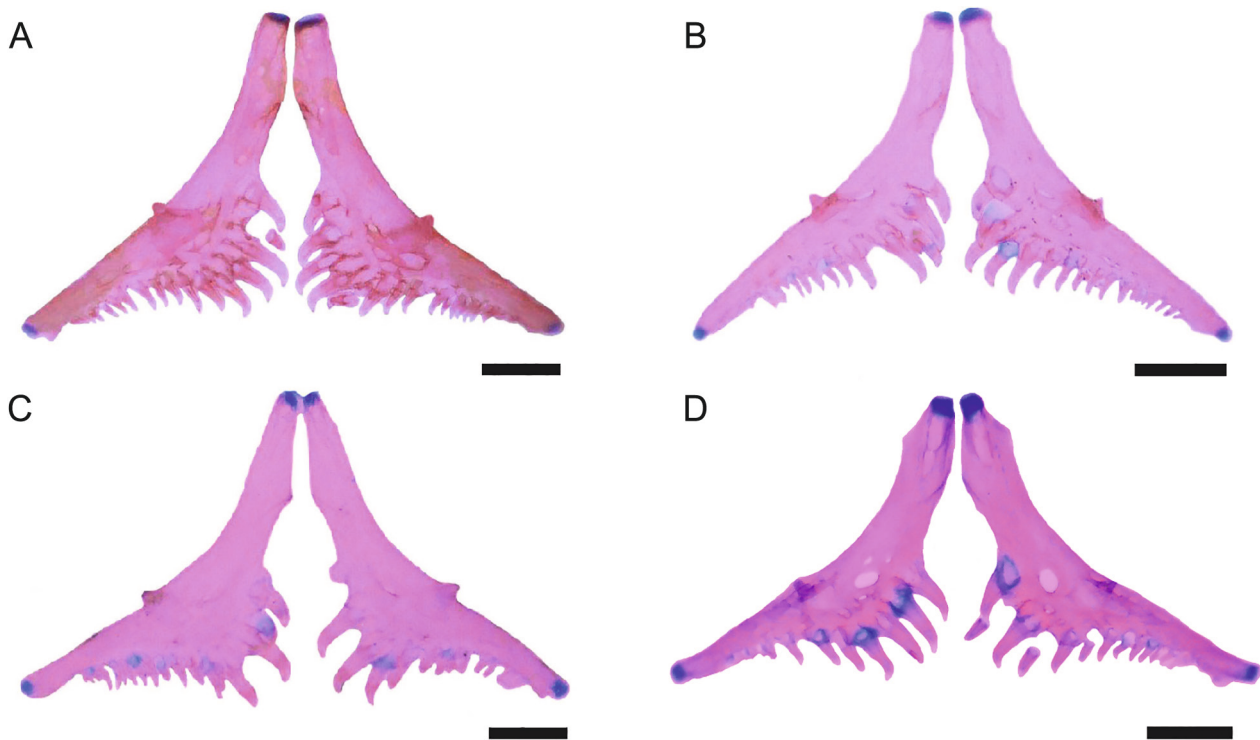


Figure 6. Cleared and stained fifth ceratobranchials showing dentition pattern, in dorsal view. (A) *Indoreonectes evezardi* (BNHS FWF 1068, 43.8 mm SL), (B) *I. neeleshii* (BNHS FWF 1074, 36.2 mm SL), (C) *I. rajeevi* (BNHS FWF 1090, 35.1 mm SL) and (D) *I. keralensis* (KUFOS.2017. 11.217, 36.1 mm SL). Scale bar 0.5 mm.

Description. General morphology is shown in Figure 7; morphometric data are provided in Table 1.

Body sub-cylindrical, elongate; head and anterior part of body almost cylindrical; body laterally compressed posteriorly; predorsal outline convex, gradually rising up to dorsal-fin origin, a distinct hump behind nape; postdorsal outline straight up to base of caudal fin; ventral profile almost straight. Caudal peduncle as deep as long. Lateral line present, incomplete, short, ending above middle of adpressed pectoral fin. Scales minute.

Head small, less than a quarter of SL. Snout round, its length more than one-third of head length. Eye dorso-laterally positioned, closer to tip of snout than to posterior margin of operculum, its diameter about 15% HL. Mouth semi-circular, with thick fleshy lips, lower lip interrupted medially by a deep groove (Fig. 4B). Barbels four pairs. Two pairs of rostral barbels, inner rostral barbel extending to middle of nostril, outer rostral barbel reaching anterior margin of eye; Maxillary barbel longest, originating at vertical from nostril, reaching midway between eye and posterior border of operculum. Nasal barbel well developed, reaching middle of eye.

Dorsal-fin origin at vertical from ventral-fin origin; slightly closer to caudal-fin base than tip of snout, its posterior margin rounded, with 4 (10) simple and 7 (10) branched rays. Pectoral fin slightly shorter than head length; with 1 (10) simple and 9 (10) branched rays. Pelvic fin with 1 (10) simple and 7 (10) branched rays. Anal fin with 3 (10) simple and 5 (9) or 6 (1) branched rays. Caudal fin rounded, 8+9 (1) or 9+9 (9) principal caudal-fin rays. Dorsal procurrent rays 10(8) or 11(2) and ventral procurrent rays 6(6) or 7(4).

Total vertebrae 35 (Fig. 5B) with 17 (2) abdominal and 18 (2) caudal vertebrae, but vertebral column malformed in both cleared and stained specimens. Dorsal-fin insertion between 13th and 14th abdominal vertebrae. Fifth ceratobranchial (Fig. 6B) with single row of 12 to 17 small curved teeth with pointed tips; anterior teeth longer than posterior.

Colouration. In life (Fig. 7A), background colour yellow ochre slightly darker on dorsal profile of anterior side; with grey irregular bars on lateral and dorsal side of body, lateral and dorsal bars separated from each other; lateral bars wider than inter-bar spaces; lateral complete bars 12 (4), 13 (2), 14 (3) or 16 (1); lateral incomplete bars 4 (2), 5 (5) or 6 (3). Head dorsally studded with dark brown spots; cheek spots below eye absent. Caudal peduncle bar continuous but sometimes faint in the middle. Dorsal fin anterior spot dark brown to black in colour; dorsal fin membrane hyaline with three rows of black spots on rays. Pectoral, ventral and anal fins hyaline, lacking spots; caudal fin with membrane hyaline, and three to four rows of dark-brown spots on rays. Ventral surface without any markings. In preservative (Fig. 7B–D), colouration similar to that in life, but faded.

Etymology. The species name honours Neelesh Dahanurkar researcher from Indian Institute of Science Education and Research (IISER), Pune, India, for his remarkable contributions to the understanding of the systematics and evolution of Indian freshwater fishes.



Figure 7. *Indoreonectes neelesi* holotype (BNHS FWF 1071, 38.54 mm SL) (A) in life, in lateral view; preserved in lateral (B), dorsal (C) and ventral (D) views.

Habitat and Distribution. *Indoreonectes neelesi* was found in a fast-flowing clear stream with a rocky substrate. Co-occurring fish species include *Parapsilorhynchus* sp. and the exotic *Gambusia affinis*. Currently, *In-*

doreonectes neelesi is known only from its type locality in the Mula tributary of East flowing Godavari River at Harishchandragad, Maharashtra, India (Fig. 1).

***Indoreonectes rajeevi* sp. nov.**

Common name: Rajeev's hill stream loach

Figs. 4–6, 8

<http://zoobank.org/29930F6A-A87B-4C35-9ADA-9172A1739BFA>

Holotype. BNHS FWF 1081, 45.76 mm SL, India: Maharashtra, Hiranyakeshi River, Krishna river system, Amboli (15°58.02'N; 74°0.66'E, ca 692 m a.s.l.), coll. P. Gorule and A. Gorule 23 Oct. 2017.

Paratypes. BNHS FWF 1082–1092, 11 ex., 26.4–36.3 mm SL, same data as holotype. Paratypes BNHS FWF 1090 (35.1 mm SL), 1091 (33.0 mm SL) and 1092 (35.2 mm SL) used for clearing and staining.

Diagnosis. *Indoreonectes rajeevi* can be distinguished from all congeners by having maxillary barbel reaching the posterior border of operculum (vs. not reaching); presence of conspicuous black marking on the lower lip of mouth (vs. absence).

Indoreonectes rajeevi is further distinguished from *I. keralensis* by having long nasal barbel reaching middle of eye (vs. short nasal barbel barely reaching anterior margin of eye); dorsal-fin origin vertical from pelvic-fin base (vs. posterior to vertical at pelvic-fin base); dorsal-fin insertion between neural spines of 12th and 13th abdominal vertebrae (vs. between 13th and 14th); presence of a dark brown to black spot on base of first dorsal-fin ray and distinct spots on dorsal side of head (vs. absence); spots on cheek below eye absent (vs. present); total vertebrae 36 (vs. 35).

Indoreonectes rajeevi is distinguished from *I. evezardi* by having inner rostral barbel reaching anterior margin of eye (vs. reaching middle of nostril); absence of dorsal hump behind nape (vs. presence); dorsal fin origin at vertical from pelvic-fin base (vs. posterior to vertical at pelvic-fin base); caudal peduncle slightly longer than deep (vs. deeper than long); caudal peduncle bar not divided into two spots (vs. divided); total vertebrae 36 (vs. 35).

Indoreonectes rajeevi distinguished from *I. telanganaensis* by lacking spots on cheek below eye (vs. spots present); absence of dorsal hump behind nape (vs. presence); caudal peduncle slightly longer than deep (vs. as long as deep).

Indoreonectes rajeevi distinguished from *I. neeshi* by having inner rostral barbel reaching anterior margin of eye (vs. reaching middle of nostril); absence of dorsal hump behind nape (vs. presence); dorsal-fin insertion between neural spines of 12th and 13th abdominal vertebrae (vs. between 13th and 14th); caudal peduncle slightly longer than deep (vs. as long as deep); lateral bars narrower than inter-bar spaces (vs. wider); total vertebrae 36 (vs. 35).

Description. General morphology is shown in Figure 8; morphometric data are provided in Table 1.

Body sub-cylindrical, elongate; head and anterior part of body almost cylindrical; body laterally compressed posteriorly; pre-dorsal outline slightly convex, gradually rising up to dorsal-fin origin, no hump behind nape; post dorsal outline straight up to base of caudal fin; ventral profile almost straight. Caudal peduncle as deep as long or slightly longer. Lateral line present, incomplete, short, ending above middle of adpressed pectoral fin. Scales minute.

Head small, about one fifth of SL. Snout round, its length more than one-third of head length. Eye dorsolaterally positioned, closer to tip of snout than to posterior margin of operculum, its diameter about 14–20% HL. Mouth semi-circular, with thick fleshy lips, lower lip interrupted medially by a deep groove with conspicuous black markings on the either side of the groove (Fig. 4C). Barbels four pairs. Two pairs of rostral barbels, inner rostral barbel extending to anterior margin of eye, outer rostral barbel reaching posterior margin of eye; Maxillary barbel longest, originating at vertical from nostril, reaching posterior border of operculum. Nasal barbel well developed, reaching middle of eye.

Dorsal-fin origin at vertical from ventral-fin origin; slightly closer to caudal-fin base than tip of snout, its posterior margin rounded, with 3 (12) simple and 7 (12) branched rays. Pectoral fin slightly shorter than head length; with 1 (12) simple and 9 (12) branched rays. Pelvic fin with 1 (12) simple and 7 (12) branched rays. Anal fin with 3 (12) simple and 5 (12) branched rays. Caudal fin rounded, 8+9 (1) or 9+9 (11) principal rays. Dorsal procurent rays 12(8) or 14(4) and ventral procurent rays 6(10) or 7(2).

Total vertebrae 36 (Fig. 5C) with 17 (3) abdominal and 19 (3) caudal vertebrae. Dorsal fin insertion between 12th and 13th abdominal vertebrae. Fifth ceratobranchial (Fig. 6C) with single row of 14 to 18 small curved teeth with pointed tips; anterior teeth longer than posterior.

Colouration. In life (Fig. 8A), background colour golden yellow; with umber-coloured irregular vertical bars on lateral and dorsal side of body, lateral and dorsal bars usually separated from each other or rarely continuous; lateral bars narrower than inter-bar spaces; lateral complete bars 14 (4), 15 (2), 16 (3) or 17 (3); lateral incomplete bars 5 (4), 6 (3), 7 (2), 8 (2) or 11 (1). Head dorsally studied with dark brown spots; no spots on cheeks below eye. Caudal peduncle bar continuous. Dark brown to black spot on base of first dorsal-fin ray; dorsal fin membrane hyaline with three rows of black spots on rays. Pectoral, ventral and anal fins hyaline, lacking spots; caudal fin membrane hyaline, with three to four rows of dark-brown spots on rays. Ventral surface without any markings. In preservative (Fig. 8B–D), colouration similar to that in life, but faded.

Etymology. The species name honours Rajeev Raghavan from Kerala University of Fisheries and Ocean Studies (KUFOS), Kochi, India, for his remarkable contributions to the understanding of the Systematics and Evolution of Indian freshwater fishes.

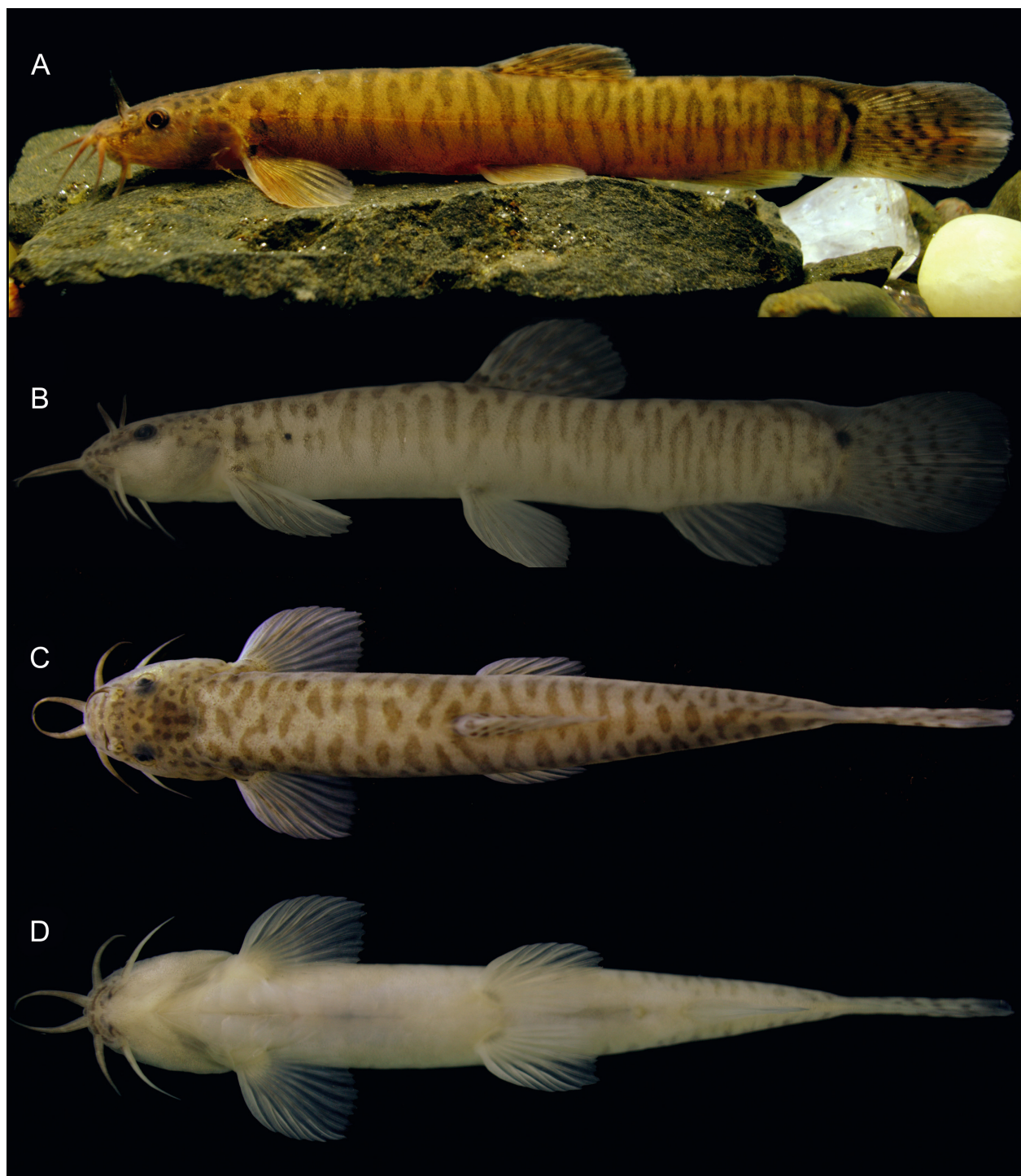


Figure 8. *Indoreonectes rajeevi* holotype (BNHS FWF 1081, 45.76 mm SL) (A) in life, in lateral view; preserved in lateral (B) dorsal (C) and ventral (D) views. Sides reversed in A and B.

Habitat and Distribution. *Indoreonectes rajeevi* was found in a slow-flowing clear stream with boulders, pebbles and mud as major substratum. Co-occurring fish species includes *Parapsilorhynchus* sp., *Schistura* sp., *Balitora laticauda*, *Rasbora dandia* and *Garra mullya*. Currently, *Indoreonectes rajeevi* is known only from its type locality in the Hiranyakeshi tributary of east flowing Krishna River at Amboli, Maharashtra, India (Fig. 1).

Species comparison and key to the species of *Indoreonectes*

The type species of the genus *I. keralensis* can be distinguished from all its congeners based on the following characters: nasal barbels short, barely reaching anterior border of eye (vs. long, reaching middle of eye or beyond); dorsal fin origin posterior to pelvic fin origin (vs. dorsal fin origin opposite to pelvic fin origin); no spot on base of first dorsal-fin ray (vs. dark brown to black spot on base of first dorsal-fin ray); dorsal surface of head uni-

formly coloured with no distinct spots (vs. distinct spots on the dorsal side of head). In addition to these characters, *I. keralensis* differs from *I. evezardi* and *I. rajeevi* in the dorsal fin insertion between 13th and 14th abdominal vertebrae (vs. dorsal fin insertion between 12th and 13th abdominal vertebrae) and from *I. rajeevi* in having 35 total vertebrae (vs. 36).

Indoreonectes neeleshii can be distinguished from all its congeners based on characters such as lateral bars wider than inter-bar spaces (vs. narrower than inter bar spaces). In addition, *I. neeleshii* differs from *I. evezardi* in caudal peduncle bar not divided into two spots (vs. divided into two spots). *Indoreonectes neeleshii* further differs from *I. evezardi* and *I. rajeevi* in dorsal fin insertion between 13th and 14th abdominal vertebrae (vs. between 12th and 13th). *Indoreonectes neeleshii* also differs from *I. telanganaensis* and *I. keralensis* in the absence of spots on cheek below eye (vs. presence).

Indoreonectes rajeevi differs from all its congeners in having very long barbels, in particular nasal barbels long reaching posterior border of eye (vs. reaching between anterior border to mid of eye); maxillary barbels long reaching posterior border of operculum (vs. maxillary barbels short not reaching beyond midway between eye and posterior border of operculum); inner rostral barbel long reaching anterior margin of eye (vs. inner rostral barbel short reaching middle of nostril); conspicuous black marking on lower lip of mouth (vs. no marking on lower lip of mouth). In addition, *I. rajeevi* differs from *I. evezardi*, *I. neeleshii* and *I. telanganaensis* in the absence of a distinct hump behind nape (vs. present). It also further differs from *I. evezardi*, *I. neeleshii* and *I. keralensis* in having 36 (3) vertebrae (vs. 35 (2), 35 (2), 35 (1) respectively).

Based on the comparison provided above, the species of *Indoreonectes* can be identified using the following key modified from Prasad et al. (2020):

- 1 Nasal barbel short, barely reaching anterior border of eye; dorsal-fin origin posterior to pelvic-fin origin; no spot on base of first dorsal-fin ray; dorsal surface of head uniformly coloured, without distinct spots *Indoreonectes keralensis*
- Nasal barbel reaching middle of eye or beyond; dorsal-fin origin opposite to pelvic-fin origin; dark brown to black spot on base of first dorsal-fin ray; distinct spots on dorsal side of head 2
- 2 Nasal barbel longer, 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 present; hump behind nape absent *Indoreonectes rajeevi*
- Nasal barbel shorter, 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 3
- 3 Lateral bars wider than inter-bar spaces *Indoreonectes neeleshii*
- Lateral bars narrower than inter-bar spaces 4
- 4 Caudal peduncle as long as deep; caudal-peduncle bar not divided into two spots; spots on cheeks below eye *Indoreonectes telanganaensis*
- Caudal peduncle deeper than long; caudal-peduncle bar expressed as two separate spots; no spots on cheeks below eye *Indoreonectes evezardi*

Molecular analysis

The best partitioning scheme and nucleotide substitution model for the partition scheme for concatenated sequences was identified as TN+I for combined partition of first two codon positions of *cox1* and *cytb* genes (BIC = 10659.524), HKY+I for third codon position of *cox1* (BIC = 2158.262) and TN+I for third codon position of *cytb* (BIC = 3651.901). In the maximum likelihood tree all the species formed reciprocally monophyletic groups (Fig. 9). Genetically, *I. neeleshii* showed a sister group relationship with *I. telanganaensis*, while *I. rajeevi* was recovered as the sister group to the clade containing *I. evezardi*, *I. neeleshii* and *I. telanganaensis*. *Indoreonectes neeleshii* differs from all its congeners, for which genetic data are available, by a raw genetic distance of 6.8% and above for *cox1* gene (Table 2) and 5.7% and above for *cytb* gene (Table 3). In particular, *I. neeleshii* differs from its sister taxon *I. telanganaensis* by a raw genetic distance of 6.5–6.7 % in the *cytb* gene (Table 3). *Indoreonectes rajeevi* differs from all its congeners, for which genetic data are available, with a raw genetic distance of 10.9% and

above for the *cox1* gene (Table 2) and 11.8% and above for the *cytb* gene (Table 3).

Discussion

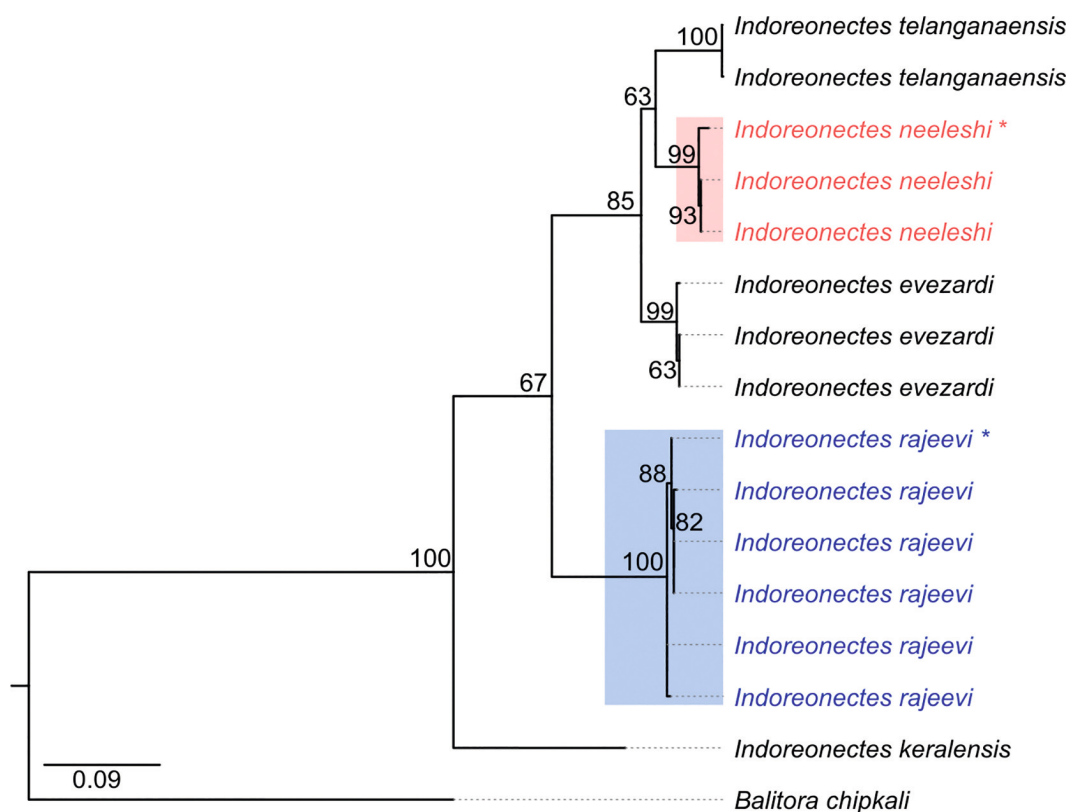
Keskar et al. (2018: fig. 3) delineated 13 clades of *Indoreonectes* from northern Western Ghats of India based on multiple molecular methods. Of these, clade 4 containing specimens from Harishchandragad and clade 13 containing specimens from Amboli are described in the current study as *I. neeleshii* and *I. rajeevi*, respectively. Both, clades 4 and 13, are distinct from clade 3, which contained topotypic *I. evezardi*, in all the molecular delimitation methods used by Keskar et al. (2018). However, it is important to note that clades 11, 12 and 13 in Keskar et al. (2018) were not distinct in one of the four genetic species delimitation methods and contained specimens from adjacent areas, namely Gaibi, Mahabaleshwar, Patan, Medha, and Kumbharli (see Keskar et al.

Table 2. Percentage raw genetic distances in cytochrome oxidase subunit 1 gene.

Species	[1]	[2]	[3]	[4]
<i>Indoreonectes evezardi</i> [1]	0.0–0.2			
<i>Indoreonectes neeleshi</i> [2]	6.8–8.1	0.0–0.8		
<i>Indoreonectes rajeevi</i> [3]	12.4–13.5	10.9–12.5	0.0–1.1	
<i>Indoreonectes keralensis</i> [4]	14.2–14.8	13.7–14.4	14.0	0.0

Table 3. Percentage raw genetic distances in cytochrome b gene.

Species	[1]	[2]	[3]	[4]	[5]
<i>Indoreonectes evezardi</i> [1]	0.0–0.6				
<i>Indoreonectes neeleshi</i> [2]	5.7–5.9	0.1			
<i>Indoreonectes rajeevi</i> [3]	11.8–11.9	13.2–13.3	0.0		
<i>Indoreonectes telanganaensis</i> [4]	6.6–6.8	6.5–6.7	13.1–13.2	0.1	
<i>Indoreonectes keralensis</i> [5]	16.0–16.1	16.1–16.2	15.8	15.7	0.0

**Figure 9.** Maximum likelihood phylogram based on *coxI* and *cytb* gene sequences illustrating the position of *Indoreonectes neeleshi* and *I. rajeevi* marked with different colours. *Balitora chipkali* (Family: Balitoridae) is used as an outgroup. Values along the nodes are bootstrap values.

2018 for locality details), suggesting that *I. rajeevi* could be distributed in areas other than its type locality. However, further taxonomic sampling is necessary to test this hypothesis.

Genetically, the new species are quite distinct from their congeners. Although the genetic data for *coxI* gene are not available for *I. telanganaensis*, both *I. neeleshi* and *I. rajeevi* are distinct from it in *cytb* gene sequence. For loaches, the interspecific genetic distances for *cytb* gene have been observed to range from 2.0% in Balitoridae (Tang et al. 2006) and 3.0% in Cobitidae (Perd-

ices et al. 2018) to 5.4% in Nemacheilidae (Bohlen et al. 2020). As a result, the pairwise genetic distance of more than 5.7% between *I. neeleshi* from all its known congeners and 11.8% between *I. rajeevi* from all its congeners is high. Interestingly, although both *I. rajeevi* and *I. evezardi* are known from Krishna river system and both *I. neeleshi* and *I. telanganaensis* are known from Godavari river system, they show large genetic divergence.

Consistent with the arguments raised by Dahanukar et al. (2011), Keskar et al. (2018) and Katwate et al. (2020) among others, our study further suggests that the earli-



Figure 10. *Indoreonectes keralensis* preserved (KUFOS.2017. 11.217, 76.11 mm SL) in lateral (A) dorsal (B) and ventral (C) views.

er notion that northern Western Ghats are species poor is the result of a lack of extensive taxonomic reviews rather than an actual species poverty. Further studies in the northern Western Ghats are likely to reveal much higher diversity among loaches in general and in the genus *Indoreonectes* in particular.

Comparative material

Indoreonectes keralensis: Paratypes, ZSI FF 1326, 3 ex., Pampadumpara, Periyar River, Kerala, India, coll. S. D. Rita, 1976 (only photographs examined); KUFOS.2017.11.217 (Fig. 10) (c&s) (Fig. 5D), 1 ex., 36.1 mm SL, India: Kerala, Iddukki (9°58.08'N; 77°12.72'E, ca 1050 m a.s.l.), coll. Anoop V. K. 2017. Additional data from Rita et al. (1978) and Prasad et al. (2020).

Indoreonectes telanganaensis: Data from Prasad et al. (2020).

Acknowledgements

The authors are grateful to Sanjay Molur for his encouragement, Ashwini Keskar for her support during the study and Anoop V. K. for providing specimens of *Indoreonectes keralensis*. Authors also thank Rahul Khot, Assistant Director, Natural History Collections department, for his help

during the registration of specimens in BNHS, Mumbai. The critical comments and suggestions of the anonymous reviewers and Ralf Britz greatly improved the manuscript.

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Appendix I

Sequences used for genetic analysis, with localities and GenBank accession numbers.

Species	Location	Latitude (N)	Longitude (E)	<i>coxI</i>	<i>cytb</i>	Reference
<i>Indoreonectes neeleshi</i>	Harishchandragad, MH	19.394	73.779	MW136273	—	Current Study
<i>Indoreonectes neeleshi</i>	Harishchandragad, MH	19.507	73.698	KX384756	KY497100	Keskar et al. (2018)
<i>Indoreonectes neeleshi</i>	Harishchandragad, MH	19.507	73.698	KX384757	KY497101	Keskar et al. (2018)
<i>Indoreonectes rajeevi</i>	Amboli, MH	15.967	74.011	MW136274	—	Current study
<i>Indoreonectes rajeevi</i>	Ajara, MH	16.14	74.206	KX946668	—	Patil et al. (2018)
<i>Indoreonectes rajeevi</i>	Ajara, MH	16.14	74.206	KX946669	—	Patil et al. (2018)
<i>Indoreonectes rajeevi</i>	Ajara, MH	16.14	74.206	KX946670	—	Patil et al. (2018)
<i>Indoreonectes rajeevi</i>	Amboli, MH	16.1	74.123	KX384748	—	Keskar et al. (2018)
<i>Indoreonectes rajeevi</i>	Amboli, MH	16.1	74.123	KX384749	KY497093	Keskar et al. (2018)
<i>Indoreonectes keralensis</i>	Erratayar, KL	9.799	77.106	MG018976	MG018981	Keskar et al. (2018)
<i>Indoreonectes evezardi</i>	Pune, MH	18.436	73.635	KX384763	KY497107	Keskar et al. (2018)
<i>Indoreonectes evezardi</i>	Pune, MH	18.472	73.808	KX384764	KY497108	Keskar et al. (2018)
<i>Indoreonectes evezardi</i>	Pune, MH	18.472	73.808	KX384765	KY497109	Keskar et al. (2018)
<i>Indoreonectes telanganaensis</i>	Maisamma Loddi, TS	19.192	78.977	—	MT569389	Prasad et al. (2020)
<i>Indoreonectes telanganaensis</i>	Maisamma Loddi, TS	19.192	78.977	—	MT569390	Prasad et al. (2020)
<i>Balitora chipkali</i>	Joida, KA	15.342	74.441	KU378004	KU378017	Kumkar et al. (2016)