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

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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
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 Kawai 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 species 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.)
(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. neeleshi* – 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 pterygiophore 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. neeleshi* (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 neeleshi* – 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

Figure 2. Diagram of colour markings in *Indoreonectes* and their terminology.
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 WFW 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 WFW 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. rajiervi*); 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. rajiervi*). 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. neeleshi* by having lateral bars narrower than inter-bar spaces (vs. wider in *I. neeleshi*). *Indoreonectes evezardi* can be distinguished from *I. rajiervi* 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*).

**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; postdorsal 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).
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 vertebral. 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 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. 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 mooreeh*, *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-
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 3. *Indoreonectes evezardi* (BNHS FWF 1070, 33.5 mm SL), topotype, collected from Kuran, Mutha River, Pune, in life (A) and preserved (B–D).
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, 38.5 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 congener 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-
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*Indoreonectes neeleshi* 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 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 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. neeleshi* (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. neeleshi* is malformed in caudal region.
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 procurent rays 10(8) or 11(2) and ventral procurent 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 Dahanukar 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.
Habitat and Distribution. *Indoreonectes neeleshii* 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, *Indoreonectes neeleshii* 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 WFW 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 WFW 1082–1092, 11 ex., 26.4–36.3 mm SL, same data as holotype. Paratypes BNHS WFW 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. neeleshi 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 and anal fin rays 12(8) or 14(4) and ventral fin 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.
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-
formally 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 neeleshi* 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. neeleshi* differs from *I. evezardi* in caudal peduncle bar not divided into two spots (vs. divided into two spots). *Indoreonectes neeleshi* further differs from *I. evezardi* and *I. rajeevi* in dorsal fin insertion between 13th and 14th abdominal vertebrae (vs. between 12th and 13th). *Indoreonectes neeleshi* also differs from *I. telanganaensis* and *I. keralensis* in the absence of spots on cheek below eye (vs. presence).

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 ..............................................

   - 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 Nasal barbel longer, reaching posterior border of eye; maxillary barbels 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 ..............................................................................................................

   - 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 Lateral bars wider than inter-bar spaces ........................................................................................................................................

   - Lateral bars narrower than inter-bar spaces ........................................................................................................................................

4 Caudal peduncle as long as deep; caudal-peduncle bar not divided into two spots; spots on cheeks below eye

   - Caudal peduncle deeper than long; caudal-peduncle bar expressed as two separate spots; no spots on cheeks below eye ........................................................................................................................................

**Molecular analysis**

The best partitioning scheme and nucleotide substitution model for the partition scheme for concatenated sequences was identified as TN+F+I for combined partition of first two codon positions of *cox1* and *cytb* genes (BIC = 10659.524), HKY+F for third codon position of *cox1* (BIC = 2158.262) and TN+F+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. neeleshi* showed a sister group relationship with *I. telanganaensis*, while *I. rajeevi* was recovered as the sister group to the clade containing *I. evezardi*, *I. neeleshi* and *I. telanganaensis*. *Indoreonectes neeleshi* differs from all its congeners, for which genetic data are available, by a raw genetic distance of 6.8% 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. neeleshi* 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.
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Table 2. Percentage raw genetic distances in cytochrome oxidase subunit 1 gene.

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

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

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

Figure 9. Maximum likelihood phylogram based on *cox1* 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 *cox1* 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 (Perdices 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-
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, Idukki (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).

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**Figure 10.** *Indoreonectes keralensis* preserved (KUFOS.2017.11.217, 76.11 mm SL) in lateral (A) dorsal (B) and ventral (C) views.
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## Appendix I

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

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