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Schistura obliquofascia, a new loach from Uttarakhand, India (Cypriniformes: Nemacheilidae)

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Abstract

A new nemacheilid loach, *Schistura obliquofascia*, is described from Kalsa, a stream near Chanfi, tributary of Gola River, Ganga basin in Uttarakhand, India. The new species is readily distinguished from congeners by the following combination of characters: 12–14 oblique olivaceous dark bars on the body and three rows of black spots on the dorsal fin; lateral line complete with 104–112 pores, dorsal fin with 3–4 simple and 8½ branched rays; 8+8 branched rays in caudal fin; dorsal adipose crest prominent; males lacking suborbital flap; and 10–11 pores in preoperculo-mandibular canal. The sequence of the cytochrome *b* gene of length 307 base pairs was used for molecular characterization of the species.

Key words: Ganga basin, cytochrome *b*, phylogeny, New nemacheilid

Introduction

Fishes of the genus *Schistura* McClelland (1838) in the family Nemacheilidae are predominantly small sized with attractive coloration; medially interrupted lower lip without forming two lateral triangular pads; moderately arched mouth, 2.0–3.5 times wider than long; usually a black bar (sometimes dissociated) on caudal-fin base; dorsal fin with one or two black marks along its base (Kottelat 1990; Vishwanath & Laishram 2001). They inhabit running fresh waters of hill streams of most parts of continental Asia and adjacent islands (including Greater Sunda Islands), of Europe and northeast Africa (Ethiopia). Eschmeyer (2012) treated 182 species of *Schistura* as valid of which 12, viz., *Schistura beavani* (Günther), *S. chindwinica* (Tilak & Husain), *S. fasciata* Lokeshwor & Vishwanath, *S. minutus* Vishwanath & Shantakumar, *S. multifasciata* (Day), *S. papulifera* Kottelat, Harries & Proudlove, *S. reticulofasciata* (Singh & Banarescu), *S. savona* (Hamilton), *S. sijuensis* (Menon), *S. tigrinum* Vishwanath & Nebeshwar, *S. tirapensis* Kottelat, and *S. zonata* McClelland are distributed in the Ganga-Brahmaputra basin.

A collection of fishes from a stream near Chanfi, tributary of the Gola River, Ganga basin in Uttarakhand, India, included an unnamed species of *Schistura* which is herein described as *Schistura obliquofascia*. The phylogenetic position of the new species is also studied by comparing its nucleotide sequences of cytochrome *b* (*cyt b*) gene with that of *S. kloetzliae* Kottelat, *S. thai* (Fowler), *S. fasciolata* (Nichols and Pope), *S. beavani*, *S. longa* (Zhu) and *S. corica* (Hamilton).

Materials and methods

Live specimens were collected and tips of the caudal fins were dissected from the anesthetized specimens and kept in 95% alcohol and stored at -20°C until use. After removing the fin, specimens were preserved in 10% formalin along with two intact specimens for morphological studies. The type specimens are deposited in the Manipur

University Museum of Fishes (MUMF). Measurements and counts follow Kottelat (1990). Measurements were made point to point with dial calipers on the left side of the specimens and data recorded to nearest 0.1 mm. SL = standard length; HL = head length. Specimens in the Manipur University Museum of Fishes, Manipur (MUMF); Zoological Survey of India, Kolkata (ZSI); and Zoological Survey of India, Eastern Research Station, Shillong (ZSI/ERS) were used for comparison.

Isolation of genomic DNA was carried out from alcohol preserved fin tissues following the conventional protocols of Sambrook *et al.* (1989). A fragment of approximately 307 bp (base pairs) that corresponds to the *cyt b* region of the mtDNA was amplified by Eppendorf Mastercycler gradient PCR using Universal Primers “L14841-AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA “H15149-AAACTGCAGCCC CTCAGAATGATATT TGTCTCA (Kocher *et al.* 1989). DNA amplifications were performed in 50 µl volumes, containing 5µl 10X PCR-buffer (100mM Tris, pH 9.0, 500mM KCl, 15 mM MgCl₂, 0.1% Gelatin) (Bangalore, Genei, India), 200 µM of each dNTPs (dATPs, dCTPs, dGTP, dTTPs) (Bangalore, Genei, India), 25 pmol of each primer, 2 Unit of Taq DNA polymerase (Bangalore, Genei, India) and 100 ng of total genomic DNA. PCR amplification conditions were as follows: one preliminary denaturation step at 94°C for 4 min followed by 35 cycles at 94°C for 45 s, annealing at 54°C for 30 s and at 72°C for 1 min. and one step of final extension at 72°C for 10 min. PCR products were checked on 1.2% agarose gel and purified using Qiaquick columns (Qiagen, USA) following manufacturer’s instructions. After purification, sequencing was performed in both strands by cycling sequencing with the primers used for PCR amplification. Sequencing was performed in an ABI Prism 3100 automated sequencer (Applied Biosystems, USA) using Bigdye Terminator. Nucleic acid sequences were subjected to BLASTn (Altschul *et al.* 1990) searches at the National Centre for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/blast>) and they were aligned using ClustalW software (Thompson *et al.* 1994) (<http://www.ebi.ac.uk/clustalw/>).

The *cyt b* sequences of the five specimens of the new species were deposited in Genbank (Table 1). Sequences of the mitochondrial *cyt b* genes of the other two species of *Schistura* and *Schizothorax richardsonii* were downloaded from Genbank (Table 1). In the analysis, the 3' and 5' ends of the larger sequences were deleted due to differences in length. A Neighbor-joining tree for the three species of *Schistura* was constructed. Neighbor-joining tree analysis was conducted using MEGA 4 (Tamura *et al.* 2007).

TABLE 1. Genbank accession numbers for cytochrome *b* sequences of five paratypes of *Schistura obliquofascia*.

Species	GenBank Accession Nos.	MUMF Nos.
<i>Schistura obliquofascia</i> 1	JN127382	11055
<i>Schistura obliquofascia</i> 2	JN127383	11056
<i>Schistura obliquofascia</i> 3	JN127384	11057
<i>Schistura obliquofascia</i> 4	JN127385	11060
<i>Schistura obliquofascia</i> 5	JN127386	11061
<i>Schistura beavani</i>	GQ478448	-
<i>Schistura corica</i>	GQ478442	-
<i>Schizothorax richardsonii</i>	JN600503	-

***Schistura obliquofascia* sp. nov.**

(Fig. 1)

Holotype. MUMF 11058, 73.3 mm SL, female; India: Uttarakhand State, Kalsa River at Chanfi, Ganga basin, 29°22'06" N, 79°34'44" E; A. Barat and Party, 25 March 2009.

Paratypes. MUMF 11055, 11059 & 11061, 3, 80.0–98.4 mm SL, males; MUMF 11056, 11057 & 11060, 3, 67.0–67.5 mm SL, females; same data as holotype; two specimens (MUMF 11060 & 11061) were dissected for confirmation of sexes, air bladder and intestinal coil).

Diagnosis. *Schistura obliquofascia* can be distinguished from congeners in having a unique combination of the following characters: 12–14 obliquely arranged olivaceous dark bars on body; lateral line complete with 104–112 pores; dorsal fin with 3–4 simple and 8½ branched rays, marked with irregularly arranged black spots; caudal fin

with 8+8 branched rays, slightly emarginated, lobes equal in length; dorsal adipose crest prominent; 10–11 pores in preoperculo-mandibular canal; no suborbital flap.

Description. Biometric data are given in Table 2. Body elongate, thick, stout, subcylindrical in cross section anteriorly and compressed posteriorly. Dorsal profile of body almost straight, but slightly elevated at dorsal-fin origin. Head depressed, short and triangular. Snout rounded and short.

Dorsal fin with 3–4 simple and 8½ branched rays, origin midway between tip of snout and caudal-fin base, slightly in advance of pelvic-fin origin. Anterior margin of dorsal fin convex, posterior margin slightly concave. Anal fin with three simple and 5½ branched rays, reaching near base of caudal fin, leaving a gap equal to eye diameter. Pectoral fin with 11 rays, oval in shape, reaching about one-third distance to pelvic-fin origin (38.3–39.9% prepelvic length). Pelvic fin with eight rays, origin under 1st branched dorsal-fin ray, not touching the vent when adpressed, leaving a gap equal to diameter of eye. Axillary pelvic-fin lobe well developed. Caudal fin with 8+8 branched rays, slightly emarginate, lobes of equal length. Caudal peduncle 1.0–1.3 times longer than deep, with high dorsal adipose crest on entire length and low ventral adipose crest on posterior half of peduncle. Largest recorded size 98.4 mm SL male (MUMF 11059).

Body entirely covered with embedded scales. Cephalic lateral-line system with 8 supraorbital, 4+8 infraorbital, 10–11 preoperculo-mandibular and 3 supratemporal pores. Unculi are present on lips and barbels. Lateral line complete, with 104–112 pores.



FIGURE 1. *Schistura obliquofascia*, n. sp.: lateral aspects of (A) holotype, MUMF 11058, 73.3 mm SL, female; (B) paratype, MUMF 11055, 80.0 mm SL, male; (C) dorsal aspect of holotype.

TABLE 2. Biometric data of holotype and six paratypes of *Schistura obliquofascia*.

	Holotype	Paratypes		
	MUMF 11058	MUMF 11055–11061		
		range	mean	SD
Standard length	73.3	67.0–98.4		
% SL				
Body depth	16.8	13.0–17.2	15.7	1.4
Head depth at nape	12.3	11.3–12.4	12.0	0.4
Lateral head length	21.3	20.9–22.0	21.3	0.4
Dorsal head length	19.1	18.2–19.3	18.8	0.4
Head depth at eye	10.4	9.7–12.3	10.5	0.6
Caudal-peduncle length	12.8	10.7–14.2	12.9	1.1
Caudal-peduncle depth	10.9	10.0–12.0	11.1	0.7
Predorsal length	51.8	49.1–53.0	51.2	1.4
Prepelvic length	52.9	52.9–55.4	54.2	1.0
Preanus length	75.6	74.0–76.9	75.4	1.2
Preanal length	82.0	79.1–83.6	81.1	1.6
Dorsal-fin height	12.8	11.2–13.9	13.0	0.9
Pelvic-fin length	18.6	18.2–20.2	19.2	0.7
Anal-fin depth	15.4	15.4–16.7	16.3	0.4
Pectoral-fin length	20.5	20.5–22.1	21.3	0.6
Maximum head width at cheek	17.7	15.8–17.7	16.5	0.6
Head width at nares	12.6	11.4–12.6	12.0	0.5
Body width at anal-fin origin	7.4	4.3–7.4	6.2	1.0
Body width at dorsal-fin origin	14.5	9.9–14.5	12.3	1.7
% HL				
Snout length	50.0	50.0–59.8	53.0	3.3
Interorbital distance	47.1	40.0–48.0	43.6	2.9
Eye diameter	17.1	14.2–18.2	16.2	1.3
Mouth gape width	42.9	39.8–50.0	45.1	3.1
Head depth at eye	54.3	50.2–58.1	55.4	2.7
Head depth at nape	64.3	60.8–68.2	64.2	2.6
Body depth at dorsal-fin origin	87.9	70.3–93.5	84.1	8.9
Depth of caudal peduncle	57.1	54.1–65.9	59.4	4.3
Length of caudal peduncle	67.1	58.5–73.8	66.4	5.7
Maximum head width	92.8	85.1–92.8	88.1	2.9
Body width at dorsal-fin origin	75.7	53.4–76.6	68.5	9.3
Body width at anal-fin origin	38.6	23.0–38.6	32.2	5.8

Anterior nostril pierced in front side of low flaplike pointed tube. Mouth arched, 1.8–2.1 times wider than long. Lips fleshy and finely pleated. Lower lip with deep median interruption without forming lateral triangular pads (Fig. 2). Inner margin of lower lip slightly thicker than lateral. Upper lip with small median incision. Processus dentiformis prominent, with median notch. Lower jaw with shallow median notch. Free posterior chamber of air bladder absent. Intestine with large loop which reaches forward and touches posterior great curvature of stomach (Fig. 3).

Barbels moderately long; inner rostral barbel much shorter, and short distance from posterior margin of corner of lip; outer one much longer, reaching to vertical of anterior rim of orbit. Maxillary barbel reaches nearly to vertical from posterior rim of orbit.



FIGURE 2. Mouth of *Schistura obliquofascia*, paratype, MUMF 11055.

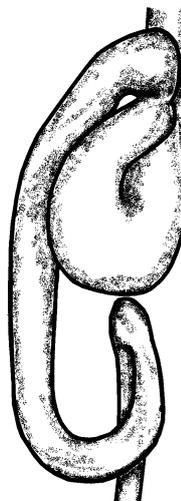


FIGURE 3. Coiling pattern of intestine of *Schistura obliquofascia*.

Sexual dimorphism. Females are smaller and body bars are well separated; width of bars almost equal to interspaces. In males, dorsal surface of body is much darker than in females, bars coalesce on back; predorsal bars are fewer and broader; pectoral fin slightly longer than in females.

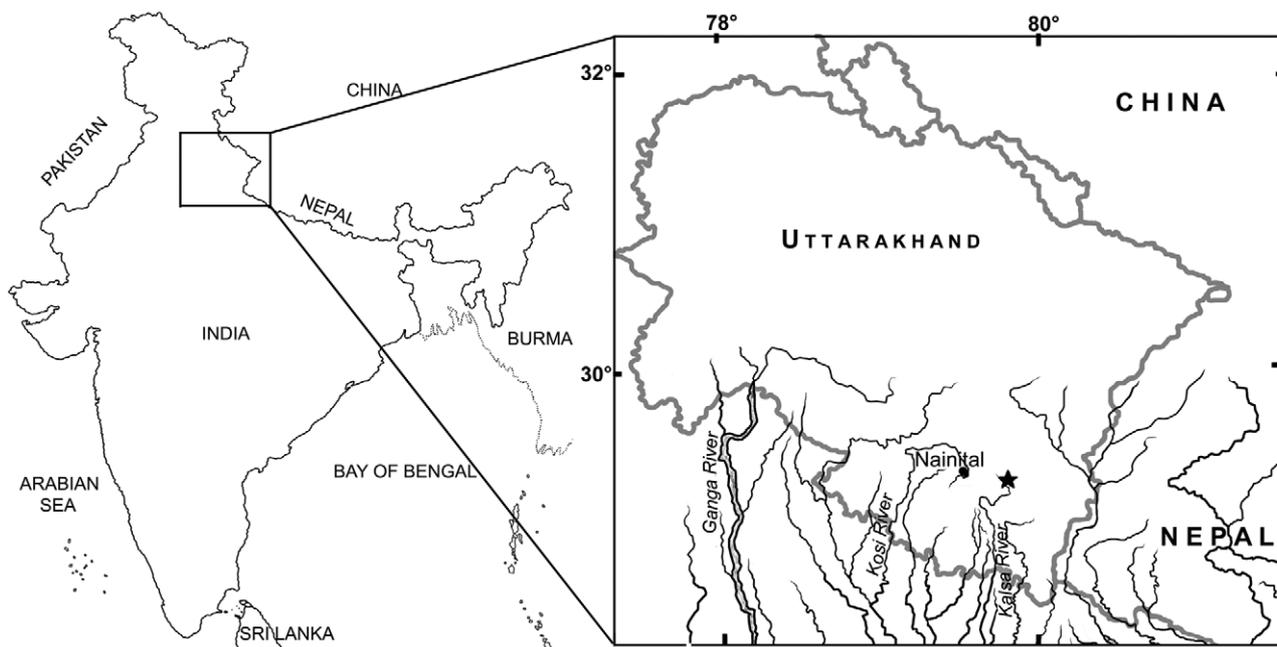


FIGURE 4. Collection site of *Schistura obliquofascia* (star).

Color. In 10% formalin: Body with 12–14 quite regularly arranged olivaceous dark bars, fading ventrolaterally, obliquely placed on creamy-white background. Four–5 bars in predorsal region, 2–3 under dorsal fin, and 6–7 between base of last dorsal-fin ray and caudal-fin base. Bars in front of anal fin, generally extend downward to horizontal level of pectoral fin while those on caudal peduncle meet on midventral line. Head dark olive above, becoming lighter ventrolaterally and much lighter on venter. Ventral side of body creamy white. Dorsal fin with irregularly arranged black spots. Base of simple and first branched rays marked with dark spot.

Anterior first to fifth pectoral-fin rays tinged with dusky coloration. Pelvic and anal fins marked with irregularly arranged dusky spots. Prominent, complete vertical black bar present at base of caudal fin. Caudal fin with irregularly arranged dark spots, sometimes arranged in three rows.

Distribution. Presently known from Kalsa River at Chanfi, a tributary of the Gola River, Ganga basin, Uttarakhand, India (Fig. 4).

Etymology. The specific epithet is derived from oblique: prefix meaning slanting or inclining in direction, and *fascia* (Latin), meaning band, referring to the obliquely arranged bars on the body.

Discussion

The genus *Schistura* includes a large number of species distributed over a vast geographical area. As a rheophilic species is restricted to a particular region and not likely to occur outside of it (Ng & Rachmatika 2005), the new species has been compared with those occurring in the Ganga-Brahmaputra basin. All of the species of the basin except *S. papulifera* have dark bars on the body. Besides the presence of the bars, *S. obliquofascia* can be distinguished from *S. papulifera* in absence (vs. presence) of skin projection on the lower half of the head and in having three (vs. five) pores in the supratemporal canal of the cephalic lateral-line system, 8+8 (vs. 9+8) branched rays in the caudal fin, and 11 (vs. 12–13) rays in the pectoral fin. The new species can be easily differentiated from the other 11 congeners in having oblique (vs. vertical) bars on the body and 16 (vs. 17–18) branched rays in the caudal fin.

Schistura obliquofascia is further distinguished from *S. beavani* in presence (vs. absence) of nasal barbel, and lower body depth (14.3% standard length vs. 20); from *S. chindwinica* in having 8+8 branched caudal rays (vs. 9+8), prominent adipose crest (vs. absence), longer snout (50.0–59.8% HL vs. 39.0–45.0) and wider interorbital distance (40.0–48.0% HL vs. 30.0–37.0). It also differs from *S. fasciata* in having fewer branched caudal-fin rays (8+8 vs. 9+8), longer pelvic-fin rays (18.2–29.2% SL vs. 13.6–17.7), and wider interorbital distance (40.0–48.0% HL vs. 25.1–34.8).

Day (1878) described *Schistura multifasciata* from Darjeeling and Assam. The holotype of this species at ZSI is in a poor state of preservation and is not suitable for comparison. Thus the data of Day (1878) and Menon (1987) were used for comparison. The new species differs from *S. multifasciata* in having 3–4 (vs. 2) simple dorsal-fin rays, 8½ (vs. 7½) branched dorsal-fin rays, 8 (vs. 9) pelvic-fin rays and 12–14 (vs. 18–30) bars on body. The new species also differs from *S. minutus* in having strongly developed (vs. weakly formed) processus dentiformes, 8 (vs. 7) pelvic-fin rays and larger (vs. smaller) size at maturity, i.e., SL = 67.0–98.5 mm vs. 26.2–38.9. It can also be distinguished easily from *S. reticulofasciata* in the presence of complete (vs. incomplete) lateral line, longer snout (50.0–59.8% HL vs. 45.9) and narrower eye (diameter 14.2–18.2% HL vs. 27.4).

Schistura obliquofascia is further distinguished from *S. savona* in having more dorsal-fin rays (3–4/8½ vs. 2/7½), pectoral-fin rays (11 vs. 10), and 12–14 broad olivaceous dark bars (vs. 9–10 narrow yellowish bars). A specimen labeled as *S. reticulofasciata* in ZSI, Shillong (ZSI VF/ERS 2629) has been examined in detail and identified as *S. sijuensis* as it has a complete lateral line with 93 pores, an important diagnostic feature of the species. The new species is distinguished from *S. sijuensis* in having shorter head (18.2–19.3% SL vs. 21.4–25.8), longer snout (50.0–59.8% HL vs. 32.4–44.4), and no suborbital flap (vs. presence). It is further distinguished from *S. tigrinum* in having more (8 vs. 7) pelvic-fin rays; complete (vs. incomplete) lateral line, wider interorbital distance (40.0–48.0% HL vs. 27.1–30.3) and wider head at check (85.1–92.8% HL vs. 74.6–81.8).

The new species can be easily differentiated from *S. tirapensis* in having complete lateral line (vs. incomplete), shorter head (18.2–19.3% SL vs. 25.5–28.6), and longer snout (50.0–59.8% HL vs. 43.5–46.2). It also differs from *S. zonata* in having spotted (vs. nonspotted) dorsal and caudal fins; 12–14 (vs. 11) bars on side of body and blunt (vs. pointed) snout.

Shrestha (2008) reported *Schistura himachalensis* (Menon), *S. horai* (Menon), *S. rupecula* McClelland, and *S. sikmaiensis* (Hora) from the Ganga basin, but all might be due to misidentifications. *Schistura himachalensis*, *S. horai* and *S. rupecula* were originally described from the Indus basin, while *S. sikmaiensis* was described from the Chindwin basin. *Schistura obliquofascia*, however, is distinguished from *S. rupecula* in having 11 (vs. 10) pectoral-fin rays and 16 (vs. 18) caudal-fin branched rays; from *S. himachalensis* in having 12–14 (vs. 8–11) bars on side of body, 8½ (vs. 7) branched dorsal-fin rays, 11 (vs. 10) pectoral-fin rays, 8 (vs. 7) pelvic-fin rays, caudal fin emarginate (vs. deeply forked) with 16 (vs. 18) branched rays, lateral line complete (vs. incomplete), and

spotted (vs. unspotted) fins; and from *S. horai* in having 3 (vs. 2) simple anal-fin rays, narrower eye diameter (14.2–18.2% HL vs. 40.3), and wider interorbital distance (40.0–48.0% HL vs. 34.8). The new species is also distinguished *S. prashadi* and *S. sikmaiensis*, respectively, in having fewer branched caudal-fin rays (8+8 vs. 9+8) and shallower head depth at nape (11.3–12.4% SL vs. 13.0–14.5).

Genetic variation. A total of 5 sequences of *cyt b* (307 bp) gene of *Schistura obliquofascia* were successfully amplified and analyzed to determine genetic variation within the species. The average frequency of four nucleotides was A = 26.3%, T = 30.4%, C = 27.6% and G = 15.8% in all five sequences. Nucleotide sequences of *cyt b* were A+T rich (56.8%) with an anti-G bias (15.8%). *Cyt b* gene revealed 10 variable sites of which two were parsimony informative and 8 were singleton variable sites.

Although only three species of *Schistura* from Ganga-Brahmaputra Basin were included in this analysis, the Neighbor-joining (NJ) tree (Fig. 5) of *cyt b* gene sequences consisted of two divergent clusters. Bootstrap values higher than 66% are indicated. *Schistura obliquofascia* was closer with *S. beavani* in a single cluster, while *S. corica* was clustered in another group. Mean genetic distance (*P* distance) over all species was 0.175. Mean genetic distance between species ranged from 0.179 to 0.320 (Table 3). The lowest genetic distance was between *S. obliquofascia* and *S. beavani* (0.179). Neighbor-joining tree and genetic distance data show *S. corica* to be genetically distant from the *S. obliquofascia* and *S. beavani*.

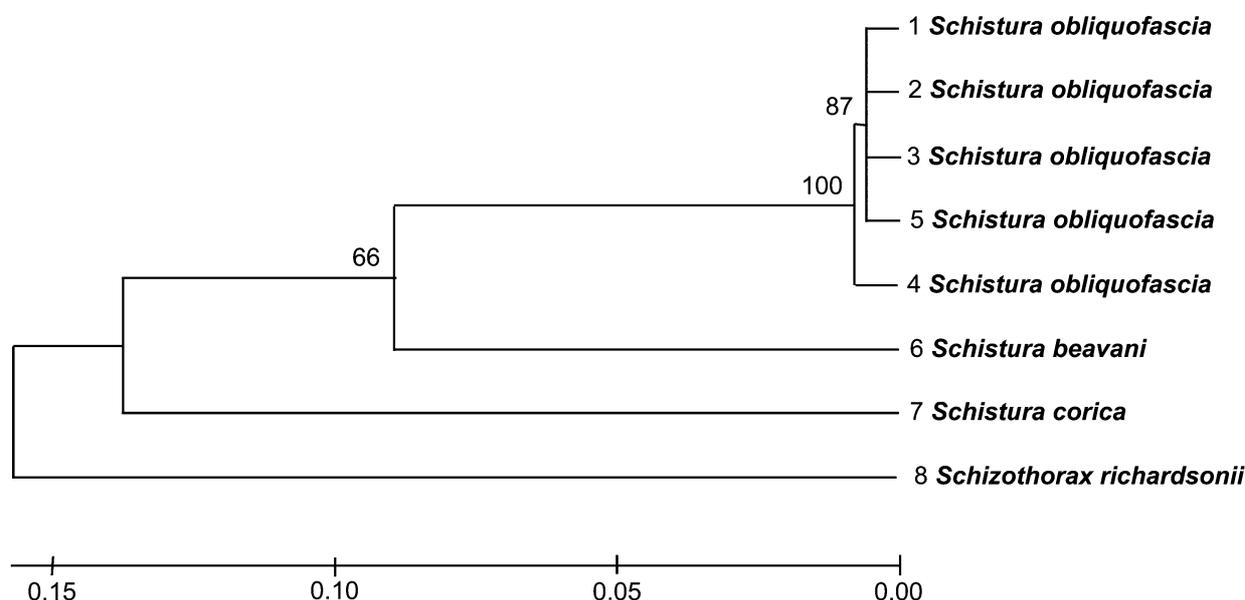


FIGURE 5. Neighbor-joining tree based on mitochondrial cytochrome *b* DNA sequences for three species of *Schistura* and *Schizothorax richardsonii* as the outgroup.

TABLE 3. Below the diagonal: *p*-genetic distances among 4 species.

	<i>S. obliquofascia</i>	<i>S. beavani</i>	<i>S. corica</i>	<i>Schizothorax richardsonii</i>
<i>S. obliquofascia</i>	-			
<i>S. beavani</i>	0.179	-		
<i>S. corica</i>	0.290	0.202	-	
<i>Schizothorax richardsonii</i>	0.320	0.282	0.317	-

Comparative materials and sources

Schistura chindwinica, MUMF 3001, 1, 54.0 mm SL; tributary of Irang River, Tamenglong district, Manipur (Brahmaputra basin), India. MUMF 4150, 1, 53.9 mm SL; Barak River at Karong, Senapati District, Manipur (Brahmaputra basin), India.

Schistura fasciata, MUMF 11010, 51.5 mm SL, holotype; Barak River at western side of Maram hill, Senapati District, Manipur (Chindwin basin), India. MUMF 11001–11021, 20 ex., 37.5–68.3 mm SL, paratypes; collection site same as holotype.

Schistura horai, ZSI F 637/2, 52.3 mm SL, holotype; Bener Khand, south of Kangra, Himachal Pradesh (Indus basin), India.

Schistura minutus, ZSI FF 3749, 35.0 mm SL, holotype; Iyei River at Noney, Tamenglong District, Manipur (Brahmaputra basin), India. MUMF 1001–1003, 3 ex., 26.2–38.3 mm SL, paratypes; collection site same as holotype.

Schistura multifasciata: ZSI F2677/1, holotype; Darjeeling, India; (poor state of preservation not fit for morphometric data).

Schistura reticulofasciata, ZSI V/ERS 3062, 40.2 mm SL, holotype; Barani Shillong, Meghalaya (Brahmaputra basin), India. ZSI VF/ERS 1929, 1 ex., 43.0 mm SL; Sumer Ri-bhoi, Meghalaya (Brahmaputra basin). MUMF 11070–11071, 2 ex., 37.0–48.3 mm SL; Barapani near ICAR complex, Meghalaya (Brahmaputra basin), India.

Schistura sijuensis, ZSI VF/ERS 2629, 1 ex., 42.8 mm SL; stream from Achingre Nokrek Biosphere Reserve (336 m), N 25°25'45.8"; E 090°10'00.5", West Garo Hills, Meghalaya (Brahmaputra basin), India; (labeled as *S. reticulofasciata* at ZSI/ERS Shillong).

Schistura sikmaiensis, MUMF 11100–03, 3 ex., 57.5–74.5 mm SL; Sanalok stream at Kultuk village, Ukhrul District, Manipur (Chindwin basin), India.

Published information used for comparison: Day (1878) and Menon (1987) for *S. multifasciata*, Günther (1868) for *S. beavani*, Hamilton (1822) for *S. savona*, Kottelat *et al.* (2007) for *S. papulifera*, Kottelat (1990) for *S. tirapensis*, McClelland (1838; 1839) for *S. rupecula* and *S. zonata*, and Menon (1987) for *S. himachalensis*.

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