

First distribution record of *Schistura scaturigina* McClelland, 1839 in river Tons, a tributary of river Yamuna in Uttarakhand, in morphological and molecular approach

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Abstract

Background

The Tons River, one of the significant potential tributaries of the Yamuna, flows through the Uttarakhand region of Garhwal. Fish that prefer cold water habitats often predominate in this river. The natural distribution of *Schistura scaturigina* McClelland, 1839 (Family: Nemacheilidae) was described (n = 15) from river Tons during the study period of 2019–2021 along with other fish species. For the first time, this species' geographic distribution has now been extended, spreading from hill streams in the Northeast and other regions to new habitats in the Garhwal Himalaya. According to morphometric studies and molecular examination of the mtDNA COI and 16S rRNA, this is the first instance of *S. scaturigina* being discovered in the Tons River.

Methods

The Specie (*S. scaturigina*) was confirmed through a morphological and molecular platform.

Results

Morphometric measurements and molecular analysis of mtDNA COI and 16S rRNA revealed this to be the first occurrence of *S. scaturigina* in the Tons River.

Conclusion

According to the aforementioned study, this is the first extended distribution report for the species *S. scaturigina* from a new location in the Tons River of Naitwar-Mori, Uttarakhand.

Introduction

Fish have been a vital component of human communities since the dawn of civilization. Several physical, biological, and ecological factors might influence how fish species are distributed [1]. Fish habitat preferences for lentic or lotic rivers, river morphology, food availability, and competition with other fishes are largely influenced by their morphological traits [2]. So far, in shallow water hill streams within the adjacent geographic range, habitat heterogeneity is crucial for fish distribution and fish assemblage. Yet, at a more precise spatial scale, it has been revealed that the distributions of fish abundance are correlated with habitat type, water depth, salinity, substrate, and resource availability at high altitudes [3–6].

There are 720 species of the fish known collectively as loaches, of which 241 are designated species belonging to the genus *Schistura* and have the morphological trait of a cylindrical body and a rounded or horizontal belly [7]. According to Mirza et al. [8], the genus name *Schistura* was first adopted in modern times to refer to this enormous and diverse group of Asian fishes. Making accurate diagnoses of species groupings as genera has proven challenging due to the paucity of data for the majority of *Schistura*

species and their presumed relations as well as the little genetic information that is currently accessible. Although exceedingly difficult to discern, the species in this genus are very similar in terms of overall shape [9]. In the northeastern state, Manipur has two major drainages and eight species of the genus *Schistura* have been described [10]. Its monophyletic relationship was, nevertheless, confirmed by numerous molecular phylogenetic analyses [11, 12]. Only a small number of *Schistura* species have been studied genetically to date, with the majority of studies focusing on relationships within the subfamily Cobitoidea (Tang et al., [13] – 4 species; Slechtová et al., [14] – 5 species; Liu et al., [15]– 3 species, etc.) or the connections between the family Nemacheilidae of genera *Schistura* (Min et al.,- 8 species [16]; Jamshidi et al.,- 5 species [17]; Sember et al., -7 species [18]; Deng et al., – 13 species [19]; Sayyadzadeh et al.,-13 species [20]; Siva et al.,-12 species [21]). In the most comprehensive molecular study, the species was described as *Nemacheilus schebbearei* and *Nemacheilus mugah* by Day [22]. McClelland [23] described this species as *Cobitis scaturigina*. *S. scaturigina* McClelland [23], commonly known as stone loach, belongs to the family Nemacheilidae under the order Cypriniformes and is an important ornamental fish found in high-altitude streams with gravelly bottoms. According to IUCN Red List Criteria, the species' conservation status is Least Concern (LC). It is a freshwater benthopelagic species found in tropical regions. In the Asia subcontinent, the fish species are distributed in Darjeeling and Assam in Eastern Himalayas, India, Nepal, and Bhutan [24]. Khyntiam and Sen [25] reported the distribution of *S. scaturigina* from the Northeastern region. It is also reported in Bangladesh [26]. Although the species has no food value, considered one of the potential ornamental fishes. It attains a maximum length (SL) of 5 cm. [27].

Discovering alternative habitats as a consequence of the broadening of a fish species' distribution ranges not only expands the research area but also helps to be aware of the new habitats so that conservation efforts may be made at the same time [28–30]. Together with observations on its morphology, ecology, and molecular identification, the present report intends to reveal the new record of the described *S. scaturigina* expanding its known range.

Material and methods

Study area and sampling

From May 2019 to April 2021, the current study was carried out to mount a survey to collect fish and other hydrological data from different selected sites in River Tons, Uttarakhand. Four sampling sites were selected covering a 10 km stretch of river Tons. The positional coordinates ranged from N 31° 04' 04.7", E 078° 05' 59.7" to N 31° 03' 00.9", E 078° 04' 09.9" and elevation from 1154 to 1287 m (Fig. 1). The habitat profile of the study area was recorded. The river substrate at sampling sites consists of boulders (45%), cobbles (20%), gravels (20%), and others (15%). Average water velocity ranged between 90–110 cm/sec. Good diverse bank vegetation was observed in the sampling sites which as a whole act as a filter for runoff from the surrounding land partly purifying the water before entering the stream. The studied area of the stream is surrounded by hills with rocky and gravelly banks giving the stream unique habitat.

The fish sample was collected with a conventional cast net (5–10 mm mesh size) and an indigenous trap made up of bamboo sticks from both banks. Traps were set up parallel to the shore during the night along the deep shoreline (> 1.5 m). The average width of the (wetted channel) river at the sampling site was 90–110 m in the lean period while it was in the range of 150–300 m in monsoon season. The fish sample was collected (n = 15) along with other fish species from site III in the pre-monsoon season which is downstream of the proposed barrage site.

Cast nets and traps were employed to collect fish samples from all of the sampling sites. For morphometric analysis, representative *S. scaturigina* specimens (Fig. 2) were preserved in 5% formalin. To preserve the materials for molecular analysis, 95% molecular-grade ethanol was used.

Morphometric and meristic data

The identification of the fish specimen was conducted using the keys as per Day [22], Talwar and Jhingran [31], Jayaram [32–34], and Khyntiam and Sen [25]. Measurements were done point to point with slide calipers to the nearest 0.1 mm. 19 taxonomic parameters were taken into consideration for the identification of the species, whereas fin formulae and barbells were the meristic characters that are applied here.

DNA isolation, PCR, and sequencing

Following DNA quality and quantity check using a nanodrop, total DNA was extracted from tissue samples stored in 95% ethanol using the standard method of proteinase k digestion, phenol/chloroform isolation, and ethanol precipitation [35]. DNA fragments were amplified with mitochondrial gene cytochrome oxidase subunit I (COI) and 16S fragments were amplified using universal primer, Fish1, and Fish2, respectively. In a 25µl reaction volume containing 50ng template DNA, 10pmol of each specific primer, 200µM of each dNTP, 0.75 units of *Taq* DNA polymerase, and 1x *Taq* buffer containing 1.5mM MgCl₂, and primers COI 658bp and 16S 583bp. The universal primer FishF1 5'-TCAACCAACCACAAAGACATTGGCAC-3' and FishR2 5'- ACTTCAGGGTGACCGAAGAATCAGAA-3' [36]. Similarly, 16S were amplified using the universal primer pair 16S F 5'-ACGCCTGTTTATCAAAAACAT-3' and 16S R primer 5'-CCGGTCTGAACTCAGATCACGT-3' [37]. Thermal cycling conditions included an initial step of 95°C for 4 min of denaturation, followed by 35 cycles of 94°C for 1 min of denaturation, 52°C for 1 min of annealing for COI, and 44°C for 16S and 72°C for 10 min of extension. The product was examined on 1.5% agarose gel and Agrigenome commercially sequenced both the sense and antisense strands using the corresponding PCR primers.

Results and discussion

Taxonomic description of the identified species:

Phylum : Chordata

Class : Actinopterygii

Order : Cypriniformes

Family : Nemacheilidae

Genus : *Schistura*

Species : *S. scaturigina*

Morphometric analysis

A species of *S. scaturigina* that stand out for having 10–12 dark brown transverse bars present across lateral line interspaced narrowing down at sides, not extending to ventral sides. Scales that do not overlap cover the body. Complete lateral line; 9 branching rays on the dorsal fin; moderately caudal extension of the dorsal adipose crest. Up to half of the space between the pectoral and pelvic fin is covered by the pectoral fin. caudal fin slightly emarginate. Nostril is situated nearer to the eyes' frontal edges. Mouth slightly arched; upper and lower lips both straight and median dip in the lower lip (Fig. 3). Three pairs of barbells were present among these 2 pairs of barbells. The anterior edge of the orbit is reached by the maxillary barb. Observed morphological features of *S. scaturigina* are provided in Table 1.

Table 1

Morphometric characteristic of *Schistura scaturigina* collected from river Tons, a tributary of river Yamuna in Uttarakhand

Parameters	Range (mm)	Value (mean \pm SD, n = 15)
TW (g)	1.92–4.13	3.14 \pm 0.77
TL (cm)	55.00–78.00	65.50 + 7.20
SL (cm)	46.10–68.00	55.10 + 6.50
FL (cm)	53.50–770	65.90 + 7.50
HL (mm)	10.13–12.54	11.59 + 0.85
Sn. L (mm)	4.19–5.62	4.91 + 0.42
IOD (mm)	3.27–5.34	4.14 + 0.61
ED (mm)	1.84–2.70	2.17 + 0.29
PDL (mm)	22.98–30.81	27.19 + 3.34
DFBL(mm)	5.95–9.91	7.81 + 1.33
CPBL(mm)	4.01–6.25	5.08 + 0.69
AFBL(mm)	2.84–4.95	3.96 + 0.72
GM(mm)	3.27–5.58	4.44 + 0.84
BD(mm)	6.37–9.67	8.02 + 1.12
BGL(cm)	22.50–46.00	32.00 + 7.20
CPH(mm)	4.33–6.81	5.56 + 0.77
DFH (mm)	8.60-13.62	10.97 + 1.75
CFH(mm)	9.14–12.41	10.69 + 0.98
AFH (mm)	6.82–8.93	7.73 + 0.70

TW = Total Weight; TL = Total Length; SL = Standard Length; FL = Fork Length; HL = Head Length; Sn. L = Snout Length; IOD = Inter Orbital Depth; ED = Eye Diameter; PDL = Pre-Dorsal Length; DFBL = Dorsal Fin Base Length; CPBL = Caudal Peduncle Base Length, AFBL = Anal Fin Base Length; GM = Gape of Mouth; BD = Body Depth; BGL = Body Girth Length; CPH = Caudal Peduncle Height; DFH = Dorsal Fin Height; CFH = Caudal Fin Height; AFH = Anal Fin Height.

Colour

Olive yellow or matt golden body colour on the background with 10–12 dark brown vertical bands (Fig. 4). The pre-dorsal has 3–4 bands, the dorsal has 3, and the post-dorsal has 5–6 bands. Pre-dorsal

bands are wider than post-dorsal bands. The dorsal fin and caudal fin both have noticeable black patches.

Habitat

locally, the species is known as '*Garia*' and the common name is 'stone loach'. This species was benthos pelagic in their nature and lives in the hill streams found attached with small pebbles.

Distribution

The species is globally distributed in Bangladesh, Bhutan, India, and Nepal [38, 39]

Sequence analysis

To achieve accurate species identification, the nucleotide sequences of the COI and 16S fragments were acquired. Both the COI (493 bp) and 16S (1066 bp) gene sequences showed significant matches with the sequence of *S. scaturigina*. The COI sequences were uploaded to GenBank with the accession number ON745188 and *scaturigina* from GenBank with 95% and 98% similarity, respectively (Table 2). The sequence showed that the average nucleotide frequencies for COI were T = 27.61, C = 28.77, A = 24.47, and G = 19.14, with a GC percentage of 47.9%, while those for 16S were T = 21.76, C = 25.42, A = 30.39, and G = 22.42, with a GC content of 47.8%. the outcome of the COI and 16s gene pairwise genetic distance analysis for species identification.

Table 2
Sequence match with NCBI nucleotide database

Species Name	Accession no.	% of similarity	% of query coverage
<i>Schistura scaturigina</i>	MK993534	95.33	97
<i>Schistura rupecula</i>	MK480347	91.06	93
<i>Schistura rupecula</i>	MK480346	91.06	93
<i>Schistura rupecula</i>	MN172328	90.45	57
<i>Schistura fasciata</i>	KX951823	90.47	96
<i>Schistura khugae</i>	MG736498	90.47	57
<i>Schistura mizoramensis</i>	MF098658	90.45	95
<i>Schistura sp.</i>	KT896738	90.47	96
<i>Schistura sp.</i>	KT896735	90.47	96
<i>Schistura khugae</i>	KJ909378	90.47	96
<i>Schistura khugae</i>	KJ909377	90.47	96
<i>Schistura khugae</i>	KJ909376	90.47	96
<i>Schistura khugae</i>	KJ909375	90.47	96
<i>Schistura sp.</i>	KF598791	90.45	95
<i>Schistura cf.</i>	JN815297	90.49	57
<i>Schistura fasciata</i>	KX399157	90.24	96
<i>Schistura fasciata</i>	KY810453	90.24	95
<i>Schistura sp.</i>	KT896737	90.26	96
<i>Schistura nebeshwari</i>	KJ909450	90.6	96
<i>Schistura khugae</i>	KJ909379	90.26	96
<i>Schistura fasciata</i>	KJ936799	90.24	99
<i>Schistura cf.</i>	KF598790	90.24	95
<i>Schistura scaturigina</i>	MG238197	90.26	97
<i>Schistura cf. l</i>	KF318336	90.24	95
<i>Schistura fasciata</i>	KY810454	90.04	95
<i>Schistura nebeshwari</i>	KY810441	90.06	95
<i>Schistura nebeshwari</i>	KY810440	90.06	57

Phylogenetic analysis

The software seqscape v.2.1 (Applied Biosystems, Foster City, CA) was used to align and modify the sequence. In the National Centre for Biotechnology Information's GenBank (BLASTN) database, the resulting consensus sequences were compared with the already-existing public datasets [40]. The COI gene and 16S grouped our specimens with *S. scaturigina* sequences during phylogenetic reconstruction. Other genomes from the family *Schistura* place *scaturigina* in a distinct group (Fig. 5). The MEGA 6 software was used to calculate genetic distance among similar species. The Neighbour-joining (NJ) algorithm [41], which was created with the support analysis of bootstrap pseudo replicates, was used to create a dendrogram from the genetic distance matrix.

The Neighbor-Joining approach was used to infer the evolutionary history [41]. The ideal tree is displayed with a branch length sum of 2.71410544. The evolutionary distances, which are measured in base substitutions per site, were calculated using the Maximum Composite Likelihood technique [42]. There were 28 nucleotide sequences in the analysis. Codon positions 1st + 2nd + 3rd + Noncoding were included. Positions with holes and incomplete data were all removed. The final dataset contained 496 locations altogether. MEGA6 was used to undertake evolutionary analysis [43].

Fish morphometric characters are the evaluable characters that apply to all fishes. The individual fish shape can be examined using some markers that have been randomly chosen on a fish's body. A landmark is an object's point of correspondence that can be used to compare populations both inside and outside of it [44]. A comparative morphometric study has been carried out in a 15-nos sample of *S. scaturigina*. The measurement study revealed that no significant intra-species variations have been found. A brief description and meristic study in comparison with various workers along with the present work also support that no significant variations and changes have been found (Table 3).

Table 3

Meristic characteristics of *Schistura scaturigina* collected from river Tons and compared with other published literature

Meristic characters	Present study	Day, 1839	Talwar and Jhingran, 1991a	Rahman, 2005	Sethy et al., 2009
Dorsal fin rays	iii 9	iii 9	iii 8	9 (1/8)	iii 8
Anal fin rays	i 5	7–8 (2/5–6)	ii 5	6 (1/5)	i 5
Pelvic fin rays	i 7	8	i 7	7 (1/6)	i 7
Pectoral fin rays	i 9	11	i 9	11–12 (1/10–11)	i 9
Caudal fin rays	18	17	-	-	18
Barbels	3 pairs, well developed	Present	2–4 pairs	-	-
Nos of bands	10–12 prominent brown vertical bands with border dorsally	About 15 brown bands	9–12 dark vertical bands broader dorsally	-	10–12 prominent brown vertical bands

The body of the species was found elongated and its depth was observed as 5.45–7.95 times in standard length (SL), against the value of 4.9–7.7 times as reported by Talwar and Jhingran [27], and 6.5 by Sethy et al. [45]. While the head length was observed as 3.92–5.48 times SL, against 4.0 times as reported by Sethy et al. [45] In our study, we could find the eye diameter 5.5–5.81 times of head length, while Day [22] reported it as one-fifth of the length of the head. The maximum length of the species was reported as 100 mm TL by Shrestha [46] from Nepal, 5 cm SL by Talwar and Jhingran [27] from Indian waters, and 5.9 cm TL by Rahman (2005) from Bangladesh waters. In our observation, we could find the maximum length of the species as 78 mm.

In the current investigation, we found a link based on the COI gene that is comparable to the molecular analysis of Ng and Jiang's [47] work. On the other hand, the morphological analysis revealed a link between the COI gene and the study by Ng [48] in (*S. scaturigina*). The complexity of the evolutionary reconstruction of the *Schistura* genus is well known. In this work, the molecular analysis successfully confirmed the identification of the species as *S. scaturigina* and supported the findings provided by Kovacic et al. [49]. *S. scaturigina*.

There have been no comprehensive reports on fish diversity and distribution records in river Tons. Whatever study conducted in the river Tons has been restricted to limited selected stretches of the river with variable altitudes. Negi and Mamgain [50] reported 19 fish species from the lower stretches of river

Tons. Nautiyal [51] described only 3 species from Naitwar-Mori. In the present study, we could report a total of 10 species from river Tons during 2019–2021 with the new report of *S. scaturigina*. So far the species is reported in the Indian states of Assam, Bihar, Jharkhand, Mizoram, Odisha, Sikkim, Uttar Pradesh, and West Bengal [45, 52], but not from river Yamuna in Uttarakhand. The present finding confirms its extension of distribution ranges in river Yumana of the state Uttarakhand and kept wide open assemblages of such extension of biodiversity distribution in the studied environment.

Comparative study of identifying keys of closely related species of *Schistura scaturigina*

Key to species of the genera Schistura

1. Complete lateral line.....2

Incomplete lateral line.....2

2. 11-13 dark brown transverse bars on the body, frequently united on middorsal.....3

body with 10-12 dark brown transverse stripes and small, slender bands coming from above.....3

Body with 15-16 irregular, dark bands that are barely discernible. ***Schistura khugae***

3. The dorsal fin has eight and a half branching rays and the base has three distinct black spots4

8-9 branching rays on the dorsal fin have noticeable dark patches at the base.....4

10-11 soft rays on the dorsal fin..... ***Schistura mizoramensis***

4. On the ventral and dorsal sides of the caudal peduncle, there is a moderately high adipose crest
..... ***Schistura fasciata***

On the ventral and dorsal sides of the caudal peduncle, there is a somewhat adipose crest ***Schistura scaturigina***

Conclusion

The above study revealed that this is the first extended distribution report of the species *S. scaturigina* from a new location in the cold water zone of river Tons at Naitwar – Mori, Uttarakhand.

Declarations

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Ethics Statement

The ICAR-Central Inland Fisheries Research Institute (ICAR-CIFRI), Barrackpore, Monirampore, Kolkata, West Bengal 700120, Animal Ethics Committee approved this work. (Approval Code: CIFRI/EC/2019/62 and approval date: 10/09/2019) The fish used in the experiment were handled according to the Institute's established procedures.

Data of Availability

No data associated with the article

Conflict of Interest

The authors report they have no conflicts of interest. The article's writing and content are solely the writers' responsibility.

References

1. D.A. Jackson, P.R. Peres-Neto, J.D. Olden, What controls who is where in freshwater fish communities the roles of biotic, abiotic, and spatial factors, *Canadian Journal of Fisheries and Aquatic Sciences*. 58 (2001) 157–170.
2. D. Das, T.C. Nag, Organs of adhesion in some mountain-stream teleosts of India: structure-function relationship, in: *Functional Surfaces in Biology*, Springer, 2009: pp. 105–122.
3. D. V Fairclough, K.R. Clarke, F.J. Valesini, I.C. Potter, Habitat partitioning by five congeneric and abundant *Choerodon* species (Labridae) in a large subtropical marine embayment, *Estuarine, Coastal and Shelf Science*. 77 (2008) 446–456.
4. E.S. Harvey, M. Cappo, G.A. Kendrick, D.L. McLean, Coastal fish assemblages reflect geological and oceanographic gradients within an Australian zootone, *PloS One*. 8 (2013) e80955.
5. M.J. Travers, I.C. Potter, K.R. Clarke, S.J. Newman, J.B. Hutchins, The inshore fish faunas over soft substrates and reefs on the tropical west coast of Australia differ and change with latitude and bioregion, *Journal of Biogeography*. 37 (2010) 148–169.
6. D.L. Watson, E.S. Harvey, Influence of the Leeuwin Current on the distribution of fishes and the composition of fish assemblages, *Journal of the Royal Society of Western Australia*. 92 (2009) 147.
7. R. Fricke, W.N. Eschmeyer, R. Van der Laan, *Catalog of fishes: genera, species, references*, California Academy of Sciences, San Francisco, CA, USA [Http://Researcharchive.Calacademy.Org/Research/Ichthyology/Catalog/Fishcatmain.Asp](http://Researcharchive.Calacademy.Org/Research/Ichthyology/Catalog/Fishcatmain.Asp). (2018).
8. M.R. Mirza, T.T. Nalbant, P.M. Banarescu, A review of the genus *Schistura* in Pakistan with description of new species and subspecies (Pisces, Cobitidae, Noemacheilinae), *Bijdragen Tot de Dierkunde*. 51

- (1981) 105–130.
9. P.K. Talwar, A.G. Jhingran, Inland fisheries of India and adjacent countries, Vol. I & II. (1991) 1–1158.
 10. W. Vishwanath, K. Nebeshwar, *Schistura reticulata*, a new species of balitorid loach from Manipur, India, with redescription of *S. chindwinica*, *Ichthyological Exploration of Freshwaters*. 15 (2004) 323–329.
 11. X. Guo, S. He, Y. Zhang, Phylogenetic relationships of the Chinese sisorid catfishes: a nuclear intron versus mitochondrial gene approach, *Hydrobiologia*. 579 (2007) 55–68.
 12. M. Yu, S. He, Phylogenetic relationships and estimation of divergence times among Sisoridae catfishes, *Science China Life Sciences*. 55 (2012) 312–320.
 13. Q. Tang, H. Liu, R. Mayden, B. Xiong, Comparison of evolutionary rates in the mitochondrial DNA cytochrome b gene and control region and their implications for phylogeny of the Cobitoidea (Teleostei: Cypriniformes), *Molecular Phylogenetics and Evolution*. 39 (2006) 347–357.
 14. V. Šlechtová, J. Bohlen, H.H. Tan, Families of Cobitoidea (Teleostei; Cypriniformes) as revealed from nuclear genetic data and the position of the mysterious genera *Barbucca*, *Psilorhynchus*, *Serpenticobitis* and *Vaillantella*, *Molecular Phylogenetics and Evolution*. 44 (2007) 1358–1365.
 15. S. Liu, R.L. Mayden, J. Zhang, D. Yu, Q. Tang, X. Deng, H. Liu, Phylogenetic relationships of the Cobitoidea (Teleostei: Cypriniformes) inferred from mitochondrial and nuclear genes with analyses of gene evolution, *Gene*. 508 (2012) 60–72.
 16. R.U.I. Min, X.-Y. Chen, J.-X. Yang, R. Winterbottom, R.L. Mayden, Phylogenetic relationships of the genus *Homatula* (Cypriniformes: Nemacheilidae), with special reference to the biogeographic history around the Yunnan-Guizhou Plateau, *Zootaxa*. 3586 (2012) 78–94.
 17. S. Jamshidi, A. Abdoli, M. Sherafatian, K. Golzarianpour, Analysis of mitochondrial DNA sequences of *Turcinoemacheilus* genus (Nemacheilidae Cypriniformes) in Iran, *Iranian Journal of Fisheries Sciences*. 12 (2013) 592–604.
 18. A. Sember, J. Bohlen, V. Šlechtová, M. Altmanova, R. Symonova, P. Rab, Karyotype differentiation in 19 species of river loach fishes (Nemacheilidae, Teleostei): extensive variability associated with rDNA and heterochromatin distribution and its phylogenetic and ecological interpretation, *BMC Evolutionary Biology*. 15 (2015) 1–22.
 19. H. Deng, H. Wen, N. Xiao, J. Zhou, A new blind species of the cave genus *Oreonectes* from Guizhou, China (Nemacheilinae), *ZooKeys*. (2016) 47.
 20. G. Sayyadzadeh, S. Eagderi, H.R. Esmaeili, A new loach of the genus *Oxyoemacheilus* from the Tigris River drainage and its phylogenetic relationships among the nemacheilid fishes (Teleostei: Nemacheilidae) in the Middle East based on mtDNA COI sequences, *Iranian Journal of Ichthyology*. 3 (2016) 236–250.
 21. C. Siva, R. Kumar, L. Sharma, M.A. Laskar, S. Sumer, A. Barat, P.K. Sahoo, The complete mitochondrial genome of a stream loach (*Schistura reticulofasciata*) and its phylogeny, *Conservation Genetics Resources*. 10 (2018) 829–832.

22. F. Day, The fauna of British India, including Ceylon and Burma: Fishes, I and II, 548 and 509, Search In. (1889).
23. J. McClelland, Indian Cyprinidae, Asiatic Researches, 19, 2, 217-471, Search In. (1839).
24. A.G.K. Menon, Check list–fresh water fishes of India, Survey, 1999.
25. D. Khyriam, N. Sen, Taxonomic study on Nemacheiline Loaches of North East India, Rec. Zool. Surv. India, Occ. Paper No. 358 (2014) 1–37.
26. A.K.A. Rahman, Freshwater fishes of Bangladesh, Zoological Society of Bangladesh, 1989.
27. P.K. Talwar, A.G. Jhingran, Inland fishes of India and adjacent countries. Vol-1 and Vol-2, IBH Pub. Co. Pvt. Ltd. Delhi. (1991).
28. D. Bhakta, W.A. Meetei, S.P. Kamble, G. Vaishak, J.K. Solanki, T.N. Chanu, S.K. Koushlesh, P. Gogoi, S.K. Das, S. Samanta, Extension of Distributional Range with a New Record of *Garra annandalei* Hora, 1921, from River Tapti: Drainage System of the West coast of India, National Academy Science Letters. 45 (2022) 139–143.
29. D. Bhakta, B.K. Das, S.P. Kamble, S. Solanki, D. Maji, A.K. Sahoo, S. Samanta, *Systemus spilurus* (Günther, 1868) Cyprinidae, A New Distribution Record from Narmada Riverine Biodiversity, Gujarat, India, Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. 92 (2022) 193–197.
30. B.K. Das, D. Bhakta, W.A. Meetei, J.K. Solanki, A.K. Sahoo, L. Kumar, S. Samanta, First report of near threatened Arabian carpetshark, *Chiloscyllium arabicum* Gubanov, 1980, from Narmada Estuary, India, National Academy Science Letters. 45 (2022) 155–159.
31. V.G. Jhingran, Fish and Fisheries of India, Hindustan Publ, Co., New Delhi. 727 (1991).
32. K.C. Jayaram, The freshwater fishes of the Indian region, (1999).
33. K.C. Jayaram, The Freshwater Fishes of The Indian Region. Revised 2nd Edifion, (2010).
34. K.C. Jayaram, Catfishes of India, Narendera Publishing House, 2006.
35. J. Sambrook, Molecular cloning: a laboratory manual, Synthetic Oligonucleotides. (1989).
36. R.D. Ward, T.S. Zemlak, B.H. Innes, P.R. Last, P.D.N. Hebert, DNA barcoding Australia's fish species, Philosophical Transactions of the Royal Society B: Biological Sciences. 360 (2005) 1847–1857.
37. S.R. Palumbi, Nucleic acids II: the polymerase chain reaction, Molecular Systematics. (1996) 205–247.
38. C. Barooah, L. Sarma, Vertebrates of Assam: A Checklist with IUCN status, Assam Science Technology and Environment Council, 2016.
39. R. Froese, D.P. Froese, R. and D. Pauly. Editors. 2016. FishBase, World Wide Web Electronic Publication. Www. Fishbase. Org, Version (06/2016). (2016).
40. C. Camacho, G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos, K. Bealer, T.L. Madden, BLAST+: architecture and applications, BMC Bioinformatics. 10 (2009) 1–9.
41. N. Saitou, M. Nei, The neighbor-joining method: a new method for reconstructing phylogenetic trees., Molecular Biology and Evolution. 4 (1987) 406–425.

42. K. Tamura, M. Nei, S. Kumar, Prospects for inferring very large phylogenies by using the neighbor-joining method, *Proceedings of the National Academy of Sciences*. 101 (2004) 11030–11035.
43. K. Tamura, G. Stecher, D. Peterson, A. Filipski, S. Kumar, MEGA6: molecular evolutionary genetics analysis version 6.0, *Molecular Biology and Evolution*. 30 (2013) 2725–2729.
44. T.O. Mojekwu, C.I. Anumudu, Advanced techniques for morphometric analysis in fish, *Journal of Aquaculture Research & Development*. 6 (2015) 354.
45. P.G.S. Sethy, P. Sahu, S.S. Mishra, On the Occurrence of Two Balitorid Fishes of the Genus *Schistura* McClelland from Similipal Biosphere Reserve, Orissa, *Records of the Zoological Survey of India*. 108 (2008) 37–40.
46. T.K. Shrestha, *Ichthyology of Nepal: a study of fishes of the Himalayan waters*, (2008).
47. H.H. Ng, W.S. Jiang, Intrafamilial relationships of the Asian hillstream catfish family Sisoridae (Teleostei: Siluriformes) inferred from nuclear and mitochondrial DNA sequences, *Ichthyological Exploration of Freshwaters*. 26 (2015) 229–240.
48. H.H. Ng, Phylogenetic systematics of the Asian catfish family Sisoridae (Actinopterygii: Siluriformes), *Ichthyological Exploration of Freshwaters*. 26 (2015) 97–157.
49. M. Kovačić, R. Šanda, K. Čekovská, T. Soukupová, J. Vukić, *Zebrus pallaoroi* sp. nov.: a new species of goby (Actinopterygii: Gobiidae) from the Mediterranean Sea with a dna-based phylogenetic analysis of the Gobiidae-lineage, *Contributions to Zoology*. 90 (2021) 285–317.
50. R.K. Negi, S. Mamgain, Species diversity, abundance and distribution of fish community and conservation status of Tons river of Uttarakhand State, India, *Journal of Fisheries and Aquatic Science*. 8 (2013) 617–626.
51. P. Nautiyal, A.S. Mishra, K.R. Singh, U. Singh, Longitudinal distribution of the fish fauna in the river Ganga from Gangotri to Kanpur, *Journal of Applied and Natural Science*. 5 (2013) 63–68.
52. D. Kar, C.B. Kumar, S.L.K. Bohra, *Fishes of Barak drainage, mizoram and Tripura*, Environment, Pollution and Management, APH Publishing Corporation, New Delhi. 604 (2003) 203–211.

Figures

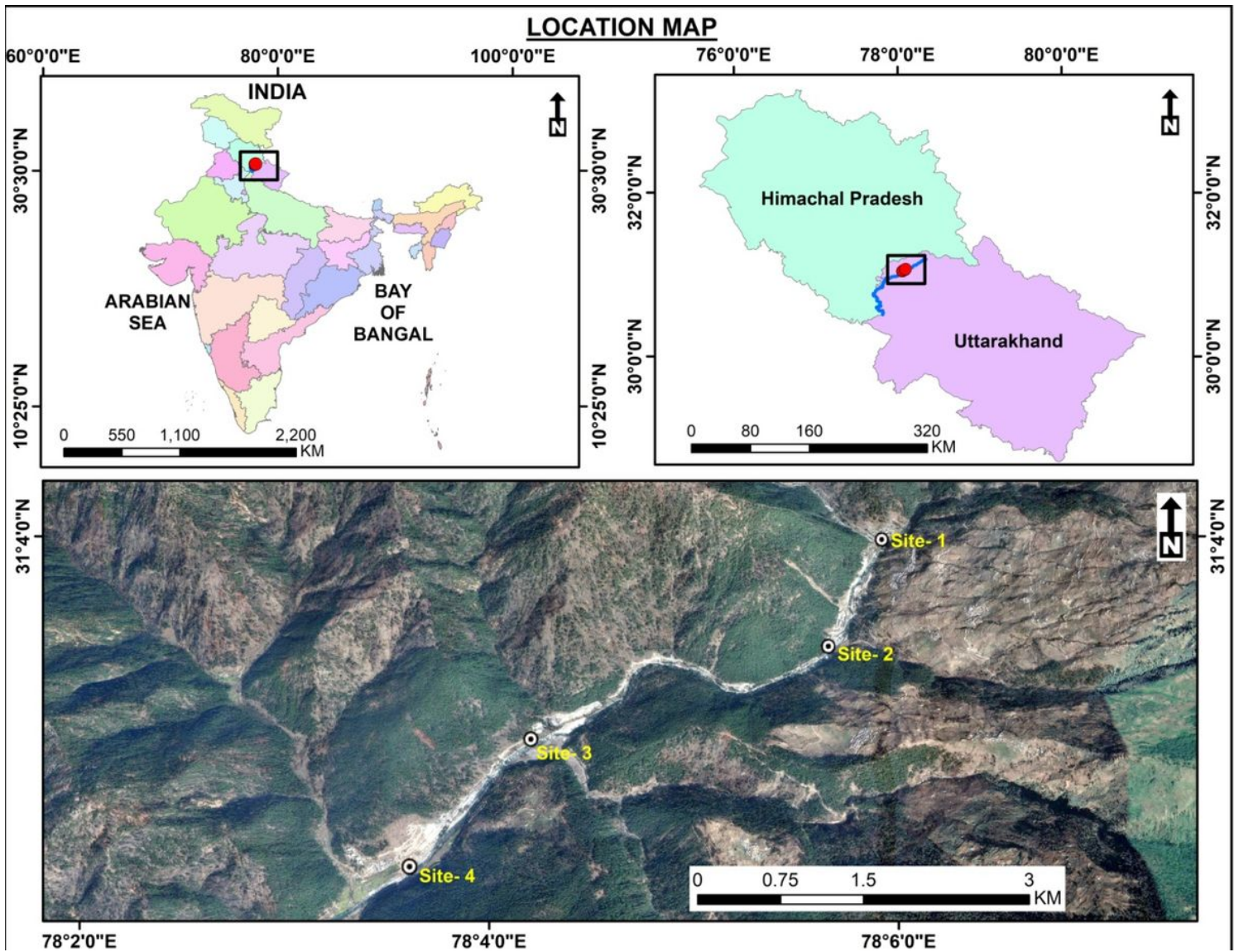


Figure 1

Map showing sampling sites of river Tons at Naitwar Mori, Uttarakhand

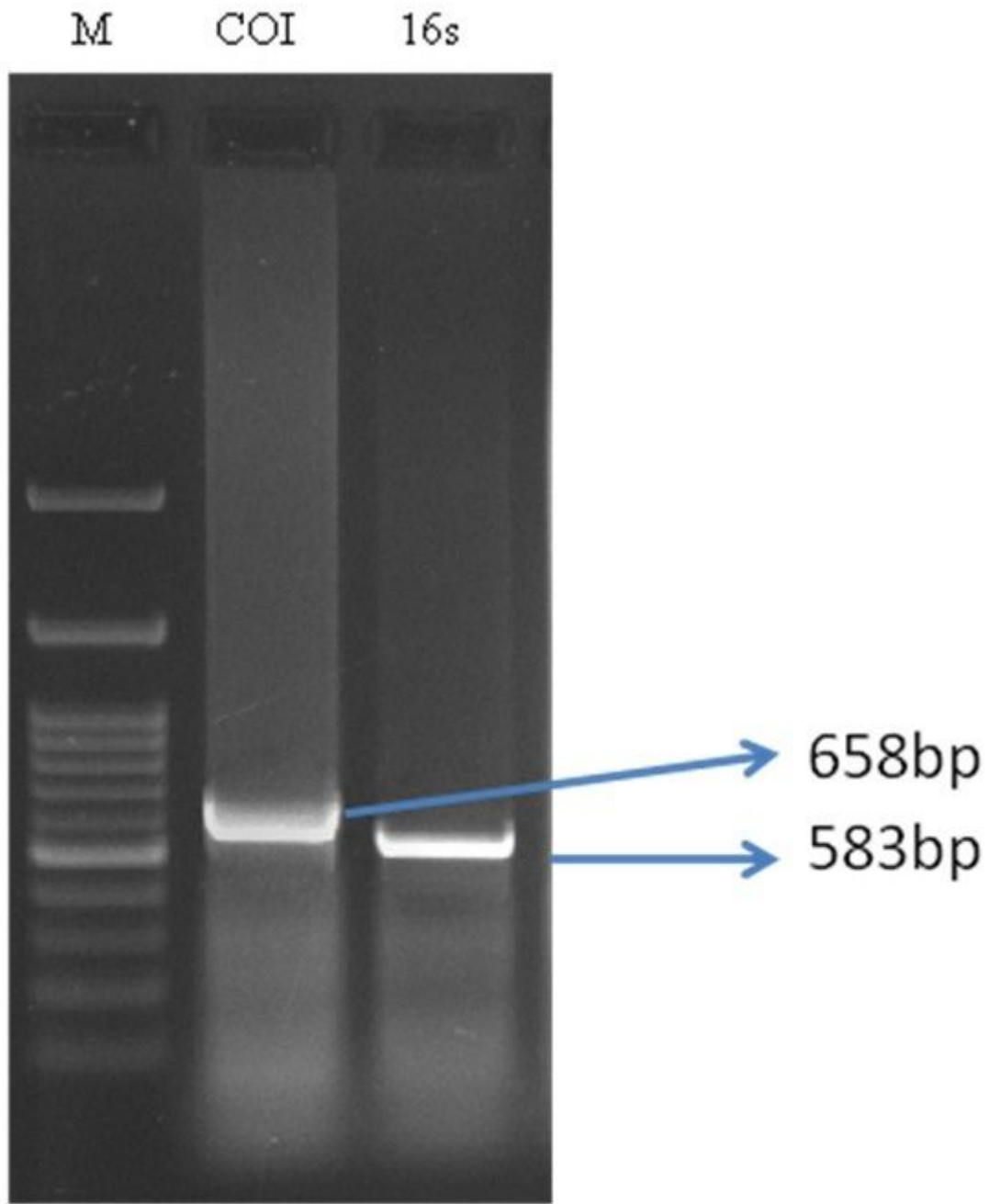


Figure 2

Expression of COI and 16s (M-100bp Marker, COI- Cytochrome oxidase subunit I and 16s)



Figure 3

A view of ventral part of mouth showing barbells



Figure 4

Dorsal view of *Schistura scaturigina*

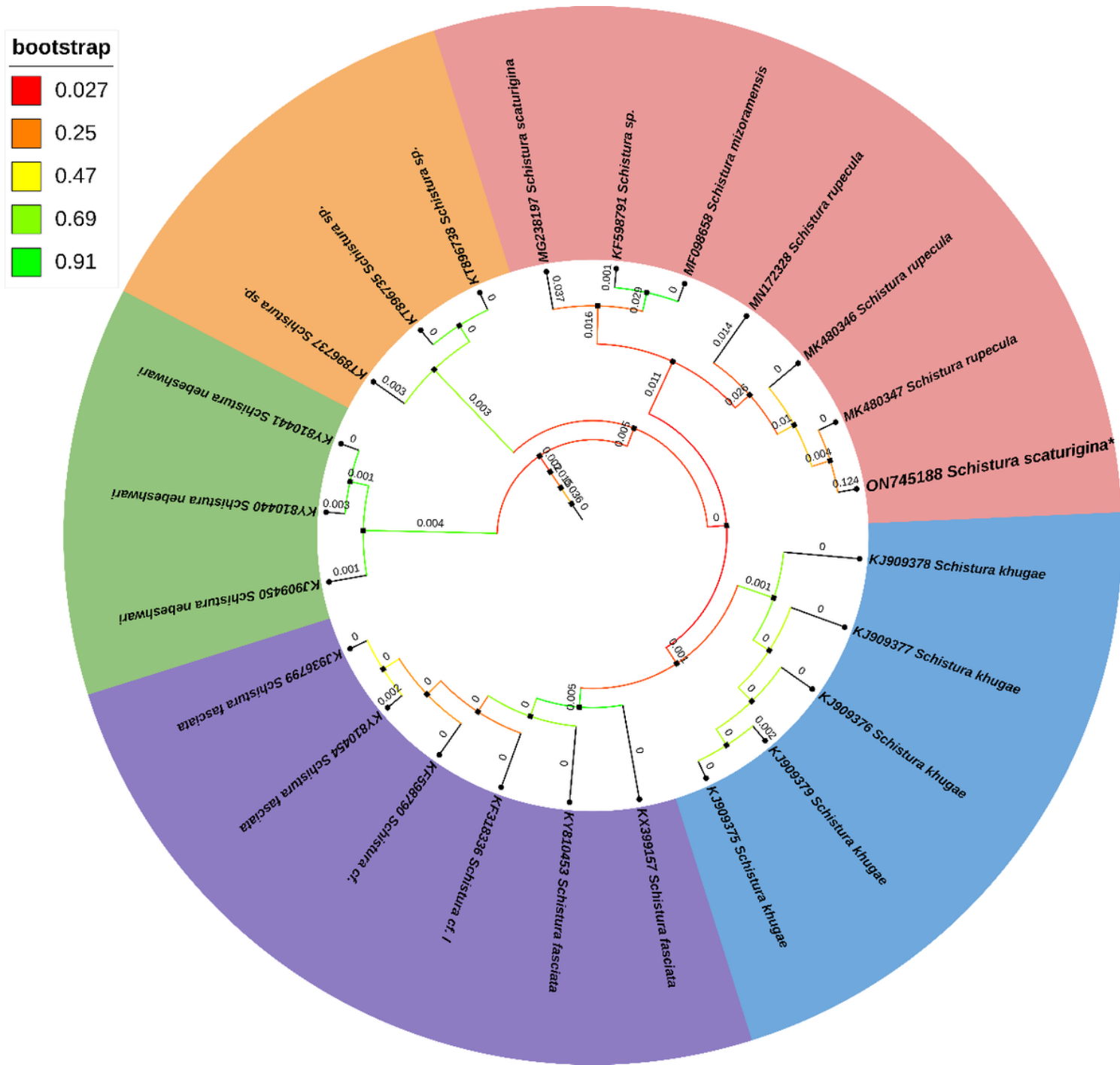


Figure 5

Evolutionary relationships of taxa