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Tor sattalensis: A new Cyprinid species of the genus *Tor* (Cypriniformes: Cyprinidae) described in the Western Himalayan aquatic eco-system of Uttarakhand, India

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Abstract

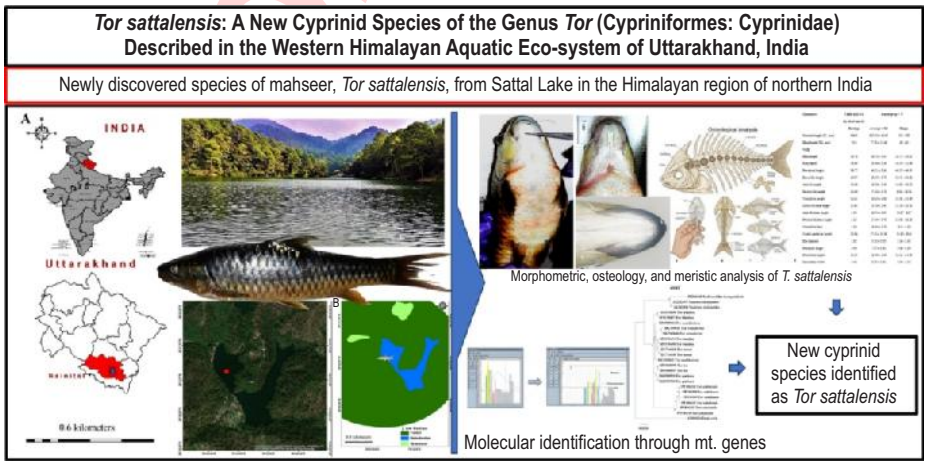
Aim: The study describes *Tor sattalensis*, a new cyprinid species under genus *Tor.* from the Western Himalayan region of India.

Methodology: Mahseer specimens with standard lengths measuring 253.0 to 382.0 mm were collected from the Sattal Lake, Uttarakhand, India. To identify the specimens, comprehensive morphometric and meristic analyses were conducted, along with osteological examinations. Molecular characterization was also performed using three mitochondrial markers: cytochrome c oxidase subunit I (cox1), cytochrome b (cytb), and ATPase subunits 6 and 8.

Results: These specimens exhibited a dark blue-black sheen, lacked a median lobe on the mandibular symphysis, possessed two pairs of reduced barbels, a 'U' shaped, partially grey lower lip, non-hypertrophic lips, 16 circumpeduncular scales, 30 caudal fin rays, 17 post-dorsal scales, anal fin 7+I, 3½ scales above and below the lateral line. The results confirmed a morphometric and genetically distinct, monophyletic lineage, supporting the recognition of a new species, *Tor sattalensis,* within the genus *Tor* (Cyprinidae), from the Sattal Lake in Uttarakhand, India.

Interpretation: *T. sattalensis* represents a novel addition to *Tor* diversity. Its discovery enhances ichthyological taxonomy, reveals hidden Himalayan biodiversity, informs cyprinid evolution and biogeography, and underscores the need for freshwater conservation.

Key words: Himalaya, India, Molecular taxonomy, New species, Osteology, *Tor*



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Introduction

The Indian Himalayas are recognized as a biodiversity hotspot, harbouring 269 freshwater fish species, including 33 endemic species. Most Western Himalayan species belong to the Ganges system, originating at the Gangotri glacier (7,000 MSL, near the Tibet border). Formed 50 million years ago by the collision of the Indian and Eurasian plates, the Himalayas support high speciation driven by diverse altitudes, climates, and aquatic habitats. Despite this richness, ichthyofaunal diversity in the Northern Himalayas remains poorly explored due to inaccessibility and sampling challenges. Species of the genus *Tor* are widely distributed in lotic and lentic systems across South-east Asian countries including Nepal, India, Pakistan, Thailand, Malaysia, Indonesia, and Bhutan (Zhou and Cui, 1996; Roberts, 1999; Nguyen *et al.,* 2008; Sarkar *et al.,* 2015; Shahi *et al.,* 2017; Pinder *et al.,* 2019). Globally, 16 valid Tor species are recognized—*T. putitora, T. tor, T. khudree, T. ater, T. barakae, T. kulkarnii, T. mosal, T. tambra, T. remadevii, T. yingjiangensis, T. polylepis, T. sinensis, T. malabaricus, T. dongnaensis, T. tambroides* and *T. laterivittatus* (Pinder *et al.,* 2019). Of these, about eight remain data deficient and lack Red List threat status. In India, five species (*T. putitora, T. tor, T. khudree, T. mosal* and *T. remadevii*) are currently accepted as distinct valid mahseer species (Khare *et al.,* 2014).

Previous studies have identified *T. macrolepis* and *T. mosal* mahanadicus as synonyms of *T. putitora* and *T. mosal,* respectively (Khare *et al.,* 2014). Non-valid taxa such as *T. macrolepis* and *T. mosal mahanadicus* show morphological plasticity in traits like mouth pattern and coloration, influenced by habitat and developmental stages, often leading to taxonomic ambiguity (Mohindra *et al.,* 2007). Mahseer comprises three genera; *Tor, Naziritor,* and *Neolissochilus* belonged to family cyprinidae. Of these, *Tor* is regarded as the true mahseer, characterized by a fleshy median lobe at the mandibular symphysis, large body scales, a lateral line with 22–28 scales, and two pairs of long barbels (Sen and Jayaram, 1982; Jaafar *et al.,* 2021). In the Northern, Central and Western Himalayas, three *Tor* species are documented: *T. putitora, T. tor* and *T. mosal* (Talwar and Jhingran, 1991; Jaafar *et al.,* 2021). While *T. tor* and *T. mosal* are native to the Himalayan plateau, *T. putitora* is endemic to Myanmar river systems (Jaafar *et al.,* 2021).

Other regional records include *T. barakae* from the Barak River system, Manipur (Arunkumar and Basudha, 2003; Chen and Yang, 2004), and the recently described cave-dwelling *Neolissochilus pnar* from Meghalaya, India (Dahanukar *et al.,* 2023). Recent studies have revised the taxonomy of genus *Tor.* The Mahseer Trust synthesis (Pinder *et al.,* 2019) and Eschmeyer's Catalogue (2021) has reduced the number of valid species from 24 (Ingram *et al.,* 2005; Nguyen *et al.,* 2008; Kottelat, 2016) to 16–17, with corresponding IUCN updates (Fricke *et al.,* 2021; Jaafar *et al.,* 2021). *Tor mahanadicus*, initially described as *Tor mosal mahanadicus* without adequate details or a type specimen, has been validated as a distinct species through morphological and mitochondrial evidence (Johnson *et al.,* 2023). Phylogenetic analyses of *Tor* and *Neolissochilus* (COI, Cyt b, 16S, ND4) resolved ambiguities, synonymizing *T. laterivittatus* with *T. sinensis* and *T. dongnaiensis* with *T. tambra* (Wu *et al.,* 2024). These analyses also confirmed the monophyly of *Tor* and revealed cryptic diversity, suggesting some South-east Asian taxa may be under- or over-described (Jaafar *et al.,* 2021).

During an ichthyological survey of Sattal Lake, Uttarakhand, India, medium-sized mahseer specimens (253.0–382.0 mm SL) exhibiting a dark blue-black shine, absence of the mandibular symphysis median lobe, and two pairs of reduced barbels were collected. Owing to the lake's geographic isolation, ecological heterogeneity, and historical stocking, its *Tor* populations provide a model for sympatric–allopatric studies. Morphological examination of four specimens confirmed their placement within *Tor* but revealed distinct differences from *T. putitora* and *T. tor,* the species typically reported from this lake. Considering the hypotheses of regional endemism and the presence of unique diagnostic characters, the detailed investigations were undertaken using an integrative taxonomic framework. Morphometric and meristic analyses, osteological studies (gill rakers, pharyngeal and vertebral bones), and molecular characterization employing three mitochondrial markers; cytochrome c oxidase subunit I (*coxI*), cytochrome b (*cytb*), and ATPase subunits 6 and 8 (ATPase 6 & 8) were conducted. The results confirmed a genetically distinct monophyletic lineage and supported the description of a new species, *Tor sattalensis,* within the genus *Tor* (Cyprinidae) from the landlocked Sattal Lake of Uttarakhand, India.

Materials and Methods

Ethics statement: The fish were captured by local fishermen, and all the specimens were dead at the time of sampling for morphometric and meristic data, and collection of fin samples for molecular analyses. The Institute Animal Care and Use Committee (IACUC), ICAR-Central Institute of Coldwater Fisheries Research (ICAR-CICFR), Bhimtal, India, approved the experimental techniques employed and the inclusion of fish specimens in this study.

Sampling site, fish sample collection and voucher specimen preservation: Sattal Lake (29°21'08" N, 79°31'05" E; 1,282 m above MSL), Uttarakhand, India, is a landlocked "V"-shaped freshwater lake (Fig. 1A) surrounded by oak–pine forest, with a muddy–silty bottom and benthic weeds. It is linked to seven freshwater lakes, with a surface area of 40,000 m^2 and maximum depth of 20 m; the 2 km surrounding area consists mainly of forest and grassland (Fig. 1B). Fish (380.65 ± 104.06 g; n=13) were caught by local fishermen using nylon gill nets between October 2020 and February 2022. Each specimen was assigned a unique ID and photographed (dorsal, ventral, lateral, and head views) using a Canon EOS 760D with EFS 18–135 mm lens. Six voucher specimens, after ID assignment, were fixed in 10% formaldehyde for 3–4 days, then transferred to 4% formaldehyde for long-term

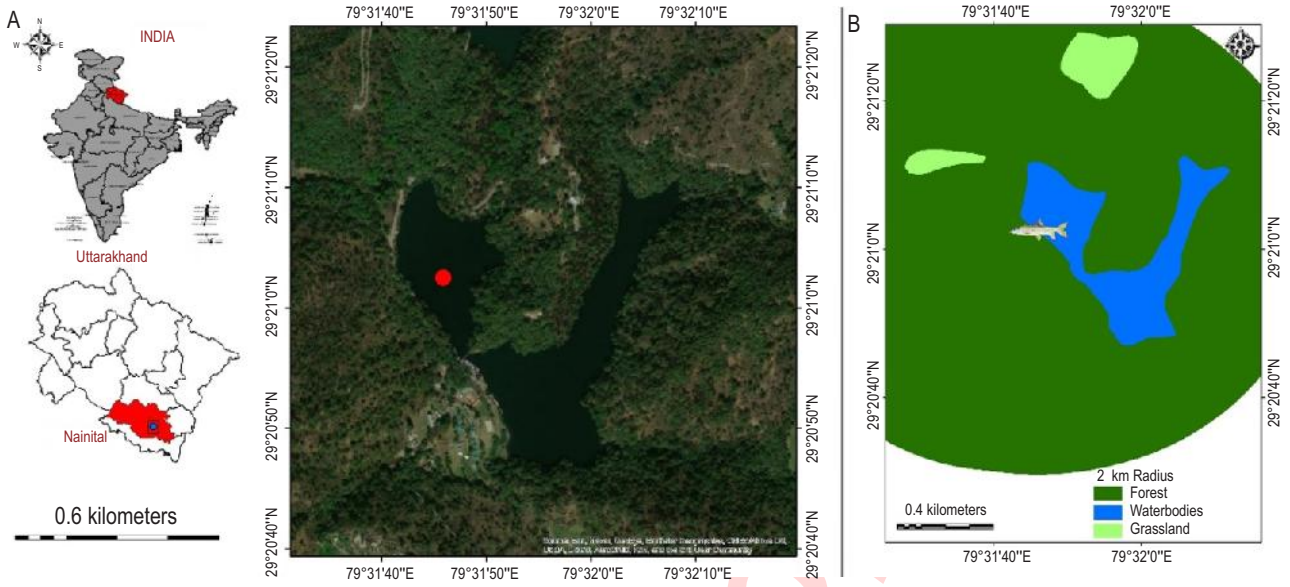

Fig. 1(A-B): Sampling site of *T. sattalensis* at Uttarakhand, India. (A) Circle in red shows the sampled site at Sattal Lake. (B) Area mapping of Sattal Lake in a 2 km radius including forest and grassland around the lake.The scale in km is shown at the bottom of the image. The map was prepared using ArcGIS (ESRI) version 10.8.1.

preservation. Prior to fixation, pectoral fin clippings were stored in 95% ethanol for DNA isolation. Four vouchers specimens were deposited at ICAR-CICFR, Bhimtal, and two at the Zoological Survey of India, Hyderabad, Telangana, for future reference.

Comparative materials examined for morphometric parameters: The comparative *Tor* speciemens examined for morphometric parameters and the places, where specimens presently held were stated as follows: *Tor tor* (Hamilton, 1822): FBRC_ZSI_F_2746, MH986131, Indravati River, Chattisgarh, 18.612N 81.876E; FBRC_ZSI_DNA640_F3329, MT118114, Andhra Pradesh, 17.7203N 81.5701E; FBRC_ZSI_DNA644_F3332, MT118117, Andhra Pradesh, 17.7021N 81.7136E; FBRC_ZSI_DNA736_F3442, MW002461, a stream near Mallavaram camp, East Godavari, Andhra Pradesh, 17.838N 81.666E. *Tor khudree* (Sykes, 1839): FBRC_ZSI_DNA374_F2833, MK560998, Talokona falls, Andhra Pradesh, 13.812N, 79.216E; FBRC_ZSI_DNA526_F3157, MN2555390, Cauveri Basins, Tamil Nadu, 12.116N 77.778. These above specimens were held at Freshwater Biology Regional Center, Zoological Survey of India, Hyderabad, Telangana.Tor putitora (Hamilton, 1822): HARC/ZSI/Solan/F-1041, Giripul, Sirmour, Himachal Pradesh and above mentioned specimen was from High Altitude Regional Centre, ZSI, Saproon, Solan, HP.

Morphometric and meristic data: Morphometric measurements and meristic counts of fish specimens followed established methods (Britz *et al.,* 2019). Morphometric measurements (Table 1) were taken with electronic digital calipers (Fisher Scientific, Pittsburgh, USA) to 0.1 mm accuracy, and meristic counts were obtained under transmitted light using a stereozoom microscope (Nikon SMZ18, Tokyo, Japan). Measurements were expressed as proportions of standard length (SL) and head length (HL). Data were recorded for all 13 specimens, with measurements taken independently at least three times. Principal Component Analysis (PCA) of size-corrected morphometric and meristic data was conducted using PAST version 4.04 (Hammer *et al.,* 2001) to test whether the new species clustered separately from other *Tor* species. Additional traits such as body and fin color, body markings, mouth and body shape, presence or absence of tubercles, mandibular median lobe, fleshy lip flap, and eye size/position were also recorded.

DNA isolation, PCR amplification, and bioinformatic analyses: From 13 fish specimens, 100–300 mg pelvic fin clippings were collected and preserved in 95% ethanol at 4 °C. Genomic DNA was extracted from 30–40 mg tissue using the Wizard Genomic DNA Purification Kit (Promega, Madison, USA), and DNA quality and quantity were assessed with a Qubit 2.0 Fluorometer (Invitrogen, MA, USA). The *coxI* gene was amplified using FishF1–FishR1 primers (Ward *et al.,* 2005), *cytb* with L14724–H15915 primers (Xiao *et al.,* 2001), and *ATPase* 6 & 8 with ATP8.2 L8331–COIII.2H9236 primers (Sivasundar *et al.,* 2001). PCR cycling profiles are provided in Table 2.

PCR amplification of mitochondrial genes was performed in a 96-well Veriti thermal cycler (Applied Biosystems Inc., Waltham, MA, USA) using 50 µl reactions containing 1× Taq buffer with KCl (without $MgCl_2$), 2.5 mM $MgCl_2$, 40 pmol of each primer, 0.25 mM dNTPs, 0.25 U recombinant Taq DNA

Table 1: Morphometric measurements (in cm) of *Tor sattalensis* (n=13). Abbreviations. TL is total length, FL is fork length, SL is standard length, HL is head length, IW is interorbital width, PoL is preorbital length, ED is eye diameter, PtoL is post orbital length, AL is anal fin length, BD is body depth, DFL is dorsal fin length, DFB is dorsal fin base length, PFL is pectoral fin length, PFB is pectoral fin base length, AFL is anal fin length, AFB is anal fin base length, VFL is ventral fin length, VFB is ventral fin base length and HCP is height of caudal peduncle

Specimen Number	37	38	39	40	41	42	180	192	182	187	189	200	202	Mean ± SD
TL	46.80	39.30	40.00	47.40	33.50	31.50	30.70	36.30	36.00	35.40	34.10	32.30	31.50	36.52 ± 5.496
FL	41.50	36.70	35.90	33.80	29.00	28.60	27.70	32.50	32.00	31.50	30.90	28.70	27.80	32.05 ± 4.083
SL	38.20	32.30	32.80	31.50	27.20	25.50	25.30	29.90	29.20	28.50	28.50	26.50	25.60	29.31 ± 3.689
HL	9.00	8.50	8.80	7.30	6.60	6.20	6.40	8.00	7.00	7.00	7.20	6.20	5.90	7.24 ± 1.036
IW	3.50	2.60	2.80	2.80	2.70	2.20	2.30	2.20	2.70	2.30	2.30	2.00	2.00	2.49 ± 0.415
PoL	3.00	2.80	2.90	2.10	2.00	1.70	2.00	2.50	2.30	2.30	2.20	2.00	1.80	2.28 ± 0.415
ED	1.24	1.14	1.29	1.14	0.90	0.90	0.80	1.10	0.70	1.00	11.00	1.00	0.80	1.77 ± 2.779
PtoL	5.50	4.80	4.80	4.10	3.30	3.30	3.50	4.30	3.50	3.60	3.70	3.50	3.20	3.93 ± 0.718
AL	18.00	16.40	16.90	14.80	20.00	18.90	18.20	22.50	22.00	21.70	21.70	19.70	19.20	19.23 ± 2.365
BD	8.00	6.70	6.70	6.40	5.90	5.50	3.50	4.30	3.50	3.60	3.70	3.50	3.20	4.96 ± 1.631
DFL	7.30	6.30	6.10	5.80	5.50	5.10	5.00	5.30	5.60	5.70	5.40	5.10	5.10	5.64 ± 0.640
DFB	7.10	3.90	4.30	4.00	3.10	3.50	3.50	3.20	4.00	3.70	3.60	3.20	3.60	3.90 ± 1.024
PFL	6.50	5.20	5.60	5.60	5.00	4.60	4.80	4.70	5.30	5.20	4.40	4.40	4.40	5.05 ± 0.610
PFB	1.60	1.30	1.80	1.20	1.30	1.50	1.00	1.20	1.20	1.10	1.10	1.00	1.00	1.25 ± 0.247
AFL	7.00	5.60	5.60	4.70	4.20	3.90	4.50	5.00	4.50	4.20	3.60	4.20	4.10	4.70 ± 0.915
AFB	2.40	2.10	2.20	2.70	2.00	1.60	2.00	2.00	1.90	2.00	2.10	1.70	2.00	2.05 ± 0.279
VFL	6.10	4.60	4.90	4.20	4.30	3.80	4.20	4.50	4.40	4.40	3.90	3.90	3.60	4.37 ± 0.630
VFB	1.50	1.30	1.10	1.60	1.40	1.40	0.80	1.00	1.00	1.10	1.00	1.00	1.00	1.17 ± 0.243
HCP	2.10	1.62	2.80	2.90	1.00	0.90	1.30	1.50	1.50	1.70	1.40	1.20	1.10	1.62 ± 0.632

SD: Standard deviation

Table 2: Thermal cycle profile of partial amplification of three mitochondrial genes, *coxI, cytb* and *ATPase* 6 & 8 of *Tor sattalensis*

	coxI	***cytb***	***ATPase* 6 and 8**
1 cycle of Initial denaturation	94°C for 2 min	94°C for 2 min	94°C for 2 min
35 cycles of Denaturation	92°C for 50 sec	92°C for 50 sec	92°C for 50 sec
Annealing	55°C for 1 min	42°C for 1 min	44°C for 1 min
Extension	72°C for 50 sec	72°C for 1.5 min	72°C for 50 sec
1 cycle of Final Extension	72°C for 7 min	72°C for 10 min	72°C for 10 min

polymerase (5 U μl^{-1}; Thermo Scientific™), and 40–50 ng genomic DNA. Amplified fragments were purified with Min Elute Gel Extraction Kit (Qiagen, Hilden, Germany) and bi-directionally Sanger sequenced at SciGenom Laboratories Pvt. Ltd., Kochi, India. Forward and reverse reads were assembled in CLC Genomic Workbench v11.01 (QIAGEN, Hilden, Germany). Sequences of *coxI, cytb,* and *ATPase* 6 & 8 were BLAST-searched at NCBI (https://blast.ncbi.nlm.nih.gov). Molecular evolutionary analyses and phylogenetic tree construction were performed in MEGAX (Kumar *et al.,* 2018) using the maximum likelihood method with Kimura-2-parameter model and 500 bootstrap replications. Analyses were conducted separately for each dataset (*coxI, cytb, ATPase* 6 & 8).

Genetic distance between *N. chelynoides, Tor* spp., and other taxa was assessed using *coxI, cytb* and *ATPase* 6 & 8 sequences analyzed in MEGAX (Kumar *et al.,* 2018). Distance was computed with the Kimura-2-parameter model. A minimum spanning haplotype network was built from 18 sequences of *T. sattalensis* (6 *coxI,* 6 *cytb,* 6 *ATPase* 6 & 8) and 38 sequences of other *Tor* spp., *N. chelynoides* and *Neolissochilus* spp. Genetic diversity was assessed from mitochondrial genes (*coxI, cytb, ATPase* 6 & 8). Sequence alignment was performed using Muscle in MEGAX (Kumar *et al.,* 2018), with gaps as missing data and partial sequences trimmed. Population genetic parameters (polymorphic sites, nucleotide diversity, haplotypes, neutrality statistics) were estimated with DnaSP v6.12.03 (Rozas *et al.,* 2017). Haplotype relationships were visualized with minimum spanning networks in PopART (Bandelt *et al.,* 1999; PopART: http://popart.otago.ac.nz/index.shtml, Massey University, Palmerston north, New Zealand).

Examination of gill arch, pharyngeal bones and vertebrae count: After removing the operculum of *T. sattalensis* specimens (n=6), the first gill arch from the left side was dissected, washed with distilled water, and preserved in 70% ethanol. Gill arch and

raker length, shape, number, and other characteristics were examined under a stereozoom microscope (Nikon SMZ18, Tokyo, Japan), and photographed using a mounted digital camera. Pharyngeal bones were dissected from the same specimens, cleaned, preserved in 70% ethanol, and examined for number, size, and morphology of teeth under the stereozoom microscope (Nikon SMZ18, Tokyo, Japan), with photographs taken using the mounted camera. The vertebral column and cranium of *T. sattalensis* (n = 4) were obtained by boiling specimens for 10 min, carefully dissected, cleaned, preserved in 70% ethanol, and examined for bony characteristics including vertebral centrum, hemal spine, neural spine, ribs, and skeleton. Bones were photographed using a Canon EOS 760D camera (Ota, Tokyo, Japan) with an EFS 18–135 mm lens.

Age determination of holotype: The age of the holotype was estimated from annuli counts on scales collected above the lateral line and below the dorsal fin. Cleaned scales were examined under a stereozoom microscope at 20X magnification, with true annuli distinguished as well spaced concentric rings, separate from circuli or stress marks. Three scales were analysed, and counts were independently verified by two readers.

Statistical analysis: Principal Component Analysis (PCA), a multivariate statistical test, was used to quantify variation and distinguish meristic parameters between the new species and its congeners. PAleontological Statistics (PAST) version 5.2.1 was applied for the PCA.

Results and Discussion

Morphological traits: Morphological traits such as body shape, size, fin ray counts, and median lengths have traditionally been used to differentiate *Tor* species. However, this approach has often led to confusion and debate among taxonomists. The morphological characteristics of *Tor* exhibit considerable intra- and interspecific variation, influenced by environmental conditions and geographic locality. Consequently, validating *Tor* species in South-east Asian countries and establishing reliable reference databases has been challenging (Walton *et al.,* 2017; Jaafar *et al.,* 2021). In the present study, the details of information about holotype, paratype and non-types of *Torsattalensis,* sp. nov.were stated as follows (Fig. 2 (A-J) and Table 3): Holotype: FBRC/ZSI/VS 14, 354.5 mm SL, adult, age 3 to 4 years, Sattal Lake, District-Nainital, Uttarakhand, India, 29°21'08" N and 79°32'05" E; 1,237 m above the sea level (Collected by N. Shahi, D. Sarma, B. Singh, and R. S. Haldar, 07th October 2020).

Paratypes: (n = 7; 253.1 to 382.6 mm) DCFR ST 37, DCFR ST 38, DCFR ST 39, DCFR ST 40, DCFR ST 41, DCFR ST 180 and DCFR ST 192. Collected from Sattal Lake, District-Nainital, Uttarakhand, India, 29°20'94" N and 79°30'91" E;1,237 m above mean sea level (Collected by N. Shahi, D. Sarma, B. Singh, R. S. Haldar and S. K. Mallik, 19th March 2022).Non-types: (n = 5): ICAR-DCFR museum identification number; DCFRMU 0127, 272.7 mm SL, Sattal Lake, District-Nainital, Uttarakhand, India, 29°20'94" N and 79°30'91" E;1,237 m above mean sea level, collected by N. Shahi, D. Sarma, B. Singh, R. S. Haldar and S. K. Mallik, 10th October 2020, DCFRMU 0146, 0148, 0149 and 0150, 265.3-292.4 mm SL, Sattal Lake, district Nainital, Uttarakhand, India; 29°21'08" N and 79°31'05" E;1, 282 m above sea level, collected by N. Shahi, D. Sarma, B. Singh, R. S. Haldar, and S. K. Mallik, 19th March 2022.

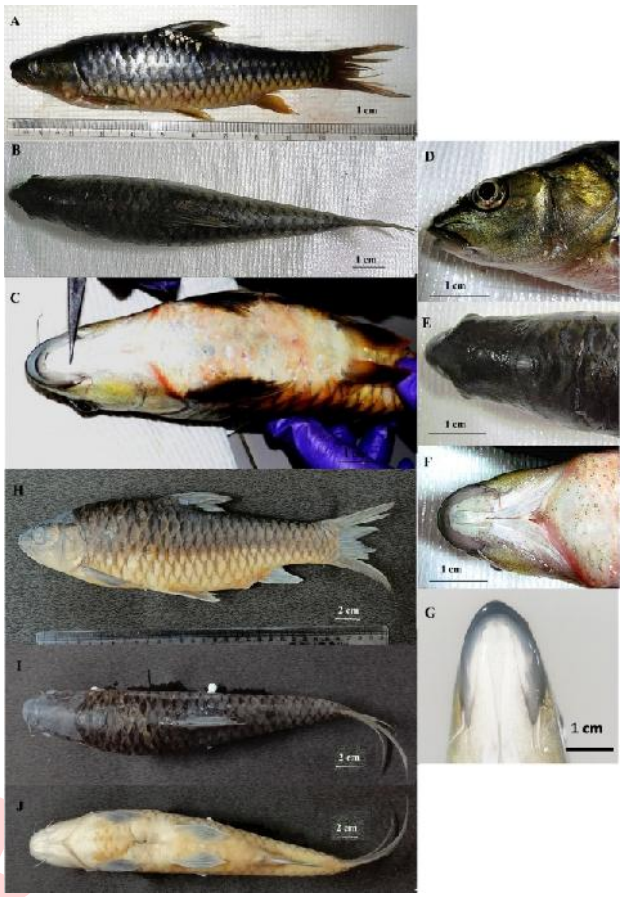

Fig. 2 (A-J): Picture of *T. sattalensis*; voucher specimen DCFRMU 0127, paratype, 272.5 mm SL collected from the Sattal Lake, Uttarakhand, India. (A) Lateral, (B) dorsal, and (C) ventral picture of fresh fish without formaldehyde fixation. (D) Lateral, (E) dorsal, and (F) & (G) ventral view of the head of fish without formaldehyde fixation. (H) Lateral, (I) dorsal, and (J) ventral view of formalin-fixed specimen of *T. sattalensis* collected from the Sattal Lake. The scale bar (in cm) is shown at the bottom left side of the images.

Type locality: *T. sattalensis* was collected along with *T. putitora* and *T. tor* from Sattal Lake, where *T. putitora* was the most common mahseer. The species name *sattalensis* was taken from its type locality Sattal Lake, a noun in opposition. Sattal in hindi means "Seven lakes". This single lake has formed due to merging of the area of seven lakes. The common name was given 'Sattal mahseer'. Similarly, *T. putitora* occurs both in the Ganga and

Table 3: Morphometric measurements of the holotype (FBRC/ZSI/VS 14, 354.5 SL, adult, age 3 to 4 years) and paratypes (n = 12) of *T. sattalensis*, collected from Sattal Lake of Uttarakhand, India

Characters	FBRC/ZSI/VS 14, 354.5 mm SL	Paratype (n = 7)	
	Holotype	Average ± SD	Range
Standard length (SL, mm)	354.5	312.50 ± 42.92	255 - 382
Head length (HL, mm)	70.0	77.33 ± 11.40	62 - 90
% SL			
Head length	19.74	24.73 ± 1.41	23.17 - 26.82
Body depth	18.89	20.94 ± 0.54	20.31 - 22.94
Pre-dorsal length	39.77	46.21 ± 2.86	42.27 - 49.53
Dorsal fin length	16.07	19.30 ± 0.70	18.41 - 20.22
Anal fin length	11.84	16.39 ± 1.30	14.92 - 18.32
Pectoral fin length	14.46	17.39 ± 0.79	6.09 - 18.38
Ventral fin length	12.41	14.85 ± 0.93	13.33 - 15.96
Dorsal-fin base length	10.43	13.59 ± 2.45	11.39 - 18.58
Anal–fin base length	5.64	16.94 ± 0.84	16.27 - 8.57
Pectoral-fin base length	3.10	17.39 ± 0.79	16.09 - 18.38
Ventral fin base	3.38	14.65 ± 0.79	13.8 - 5.88
Caudal-peduncle length	22.84	27.21 ± 10.88	21.95 - 50.4
Eye diameter	2.82	3.52 ± 0.23	3.24 - 3.92
Preorbital length	6.48	7.67 ± 0.90	6.66 - 8.84
Postorbital length	10.15	13.66 ± 1.06	12.13 - 14.86
Interorbital width	6.48	8.85 ± 0.61	8.04 - 9.92
Least height of caudal peduncle	4.79	5.45 ±1.67	4.99-7.02
Length of caudal peduncle	27.64	19.53 ± 5.87	15.76-24.80
Caudal fin height	22.84	17.23 ± 3.89	14.25-21.12
Depth at anus	14.10	10.47 ± 0.96	9.04-11.32
Depth at pectoral fin	12.41	8.67 ± 0.99	7.89-9.92
Depth at dorsal fin	18.89	16.56 ± 1.76	15.50-18.02
% of head length			
Eye diameter	14.28	14.29 ± 0.86	13.63 - 15.84
Preorbital length	32.85	30.94 ± 2.38	27.41 - 33.33
Postorbital length	51.42	55.25 ± 3.53	50 - 61.11
Interorbital width	32.85	36.65 ± 3.93	30.58 - 40.9
Head depth	71.90	68.89 ± 6.73	63.40 – 75.01
Head width	48.78	43.89 ± 4.89	41.67 -49.45
Ratios			
SL/HL	5.06	4.05 ± 0.2270	3.72 - 4.24
SL/body depth	5.29	4.77 ± 0.1239	4.61 - 4.92

SD: Standard deviation

Brahmaputra basins along the Himalayan foothills, whereas *T. tor* is distributed in the Narmada basin of Central India. The type locality of *T. mosal* is the Kosi River, a tributary of the Ganga basin in the Indian Himalayan foothills. (Hora and Mukerji, 1936; Desai, 2003; Kar and Sen, 2007; Laskar *et al.,* 2018).

Diagnostic traits: The lateral, dorsal, and ventral views of fresh *T. sattalensis* are shown in Fig. 2 (A-C). The species was distinguished from all its congeners by the difference in mandibular symphysis, meristic counts, morphometric measurements, and mitochondrial gene sequences. Diagnostic traits included complete absence of a fleshy median lobe below the mandibular symphysis (vs. present in congeners), shiny dark blue–black body pigmentation (vs. olive green to light yellow), non-hypertrophic thin lips (vs. thick protractile), and highly reduced thin maxillary and mandibular barbels (vs. thick and elongate), with the mandibular barbel not reaching the opercular margin. Within Sattal Lake, *T. sattalensis* differed from sympatric *T. tor* and *T. putitora*. Compared to *T. tor*, it had shallower body depth (20.31–22.94 vs. 24.56–25.18 %SL), longer caudal peduncle (18.46–21.91 vs. 16.47–17.31 %SL), fewer pre-dorsal scales (6–7 vs. 9–10), and more anal fin rays (8–9 vs. 5–6).

From *T. putitora*, it differed by shorter head length (15.76–17.91 vs. 21.47–29.93 %SL), greater body depth (20.31–22.94 vs. 15.77–18.53 %SL), and higher pre-dorsal scale counts (16–17 vs. 9–11). Variation in median lobe length was also noted across congeners; short in *T. ater, T. tambra,* and *T. yingjiangensis*; medium in *T. douronensis* and *T. mekongensis*; long in *T. dongnaiensis, T. laterivittatus, T. sinensis* and *T.*

Table 4: Meristic counts of holotype (FBRC/ZSI/VS 14, 354.5 SL, adult, age 3 to 4 years) and paratypes (n = 12) of *T. sattalensis* collected from Sattal Lake of Uttarakhand, India

Characters	n = 13
Pectoral-fin rays	16+I
Dorsal-fin rays	9+iii
Pelvic-fin rays	9+I
Anal-fin rays	7+I
Caudal fin rays	17-18
Circumpeduncular scales	16
Lateral line scales	22 ½
Pre-dorsal scales	6
Post-dorsal scales	17
Pre-pelvic scales	9
Post-pelvic scales	8
Pre-anal scales	16
Scale above the lateral line	3 ½
Scales below the lateral line	3 ½

Fig. 3: Distribution of fine black color spots on the scales of live specimens adult *T. sattalensis* collected from Sattal Lake, Uttarakhand, India

tambroides; and medium in *T. putitora* and *T. tor,* with morphotype-dependent variation across India, Pakistan, Nepal and Bangladesh (Menon, 1992; Desai, 2003; Ng, 2004; ZiMing and JunXing, 2004; Haryono and Tjakrawidjaja, 2006; Kottelat, 2013; Hoang *et al.,* 2015; Pinder *et al.,* 2019).

Description: The snout and lateral head regions, above and below the eyes, bore numerous fine black dots (0.2–0.4 mm; Fig. 2D). Eyes were positioned slightly anterior, partially visible dorsally (Fig. 2E), but not ventrally, where additional fine black dots were present (Fig. 2F–G). The lower lip was partially grey. Dorsal and caudal fins were jet black, whereas pelvic, pectoral, and anal fins were pale reddish-black at the base, fading to pale orange posteriorly. Diagnostic features separating *T. sattalensis* from *T. tor* included head length greater than body depth (vs. shorter), equally convex dorsal and ventral profiles (vs. more arched dorsal), thin non-hypertrophic lips (vs. thick hypertrophic), absence of the median lobe in the lower lip (vs. presence), and a dark blue-black shiny dorsum (vs. yellow-orange). *T. sattalensis* differed from its sister genus *Neolissochilus* by the absence of cheek tubercles (vs. present), pointed snout (vs. blunt), complete post-labial groove (vs. interrupted), dorsal fin with 9–10 rays (vs. 13), pelvic fin with 9–10 rays (vs. 8), and lateral line with 22–23 scales (vs. 20–29). *T. sattalensis* differed from its sister genus *Naziritor* (Sarkar *et al.,* 2015) by having relatively large, heavy scales (vs. small), 22–23 lateral line scales (vs. 33–37), dark blue-black shiny dorsum (vs. dark brown), black fins (vs. reddish), and larger adult size up to 350 mm SL (vs. 180 mm SL).

The body showed equally and smoothly convex dorsal and ventral profiles (Tables 3–4). Head length exceeded body depth; the body was elongated, oval anteriorly, and laterally compressed at the caudal peduncle. Standard length was 5.2–5.7 times body depth and 4.3–4.5 times head length. The head was medium-sized, pointed laterally, with the eye slightly anterior to mid-length and positioned dorsally near the snout tip. The rostral barbel reached mid-eye, and the maxillary barbel extended beyond the eye; both were thin (~1.5 mm diameter) and short. In fresh specimens, the lower lip was off-white to light pink, medium fleshy, smooth, continuous at the mouth angle, and fleshier than the upper lip. The mouth was subterminal, non-protractile, and "U"-shaped; the upper jaw overhung the lower. No rostral fold was present, and the post-labial groove was continuous. Fin ray counts were: pectoral 16+i, dorsal 9+iii, anal 7+i, pelvic 9+i. The dorsal fin was moderately concave, originating one scale before the pelvic origin, above the 7th lateral-line scale. The anal fin was convex distally, originating immediately after the anus, above the 17th lateral-line scale. The pelvic fin, short and moderately concave, did not reach the anus and originated over the 9th lateral-line scale. The pectoral fin was concave and did not reach the pelvic fin. The gill opening was moderately large; the operculum smooth-edged and crescent-shaped; interorbital space smooth and flat. The caudal fin was deeply forked, the fork comprising two-thirds of total caudal length, with both lobes pointed. The lateral line was complete with 22–28 scales, beginning at the operculum and ending at the caudal peduncle, slightly curved ventrally before running horizontally. Scales above the lateral line numbered 3–4, and below 3; pre-dorsal scales 7, post-dorsal 18–19. Scales were large, shiny, hexagonal, absent on head and belly. No lateral line band was present; fine black dots (0.2–0.4 mm) extended onto the head. Two pairs of short, thin barbels were present: rostral (shorter, dark grey) and maxillary (longer, pale cream). Tubercules were absent and operculum was smooth.

Coloration: In fresh specimens, its body coloration differed; scales above the lateral line were dark blue-black and shiny, those below were pale yellow-cream, and lateral scales bore dark grey-black spots along the posterior margins. The rostral barbel was dark grey and the maxillary barbel pale cream. Numerous fine black dots were present on the dorsal and dorsolateral body, which appeared dark brown to black with bluish–black shades

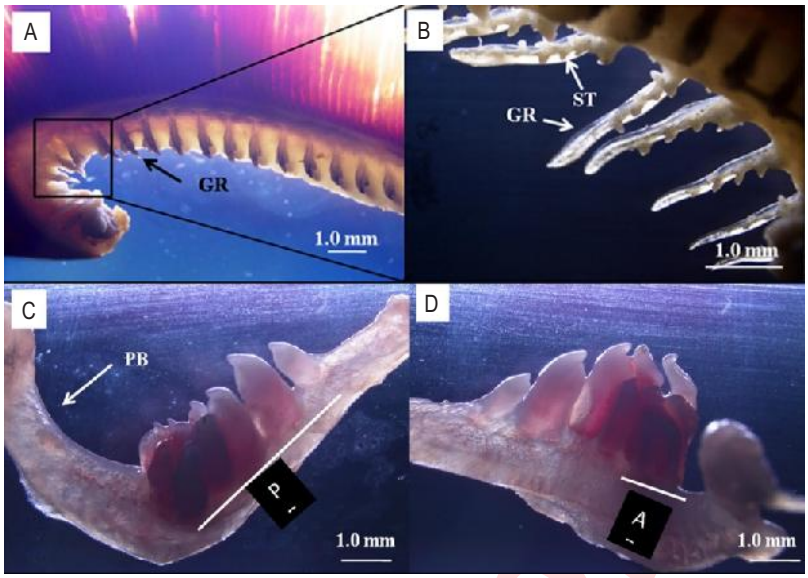

Fig. 4 (A-D): Photomicrograph of the gill rakers and the pharyngeal teeth of *T. sattalensis* discoveredfrom Sattal Lake, Uttarakhand, India. (A) Anterior row of gill rakers in upper and lower limb of first-gill arch. (B) Denticles on ventral region of the gill rakers of first gill arch. (C) Anterior view of the pharyngeal bone. (D) Second dental teeth on the main row of pharyngeal bone. The bones were fixed in 70% ethanol and pictures were taken under the bright field of the stereozoom microscope. Abbrevations- PB is pharyngeal bone; A is anterior row, P is posterior row; GR is gill raker; and ST is denticles. The scale bar is shown at the bottom right side of each image.

above the lateral line. The dorsal and caudal fins were dark black, while the pelvic, pectoral, and anal fins were pale reddish–black with light orange anteriorly. The dorsal and lateral head region, including the snout and area above the eye, was dark black, whereas the ventral head and suborbital region were off-white. In case of *T. putitora*, the body colour was reddish sap green, light orange fading to silvery white. The colour of the pelvic, anal and caudal fins was reddish gold (Menon, 1992), while grayish green body colour, reddish fin, silvery abdomen, and dark gray dorsal side were recorded in *T. tor* (Desai, 2003).

In formalin-fixed specimens, the dorsal body region was dark grey, with the sides above the lateral line grey and scales darker at the base, each posteriorly edged with fine dark grey spots (0.2–0.4 mm; Fig. 2H, I). The region below the lateral line and ventral body were pale yellow–cream, while all fins appeared light blue (Fig. 2J). The dorsal head was dark grey, extending to the snout whereas the ventral head was light grey, the lateral sides grey, and the opercula region light grey. In fresh specimens, the body above the lateral line (22½ LL scales) bore numerous fine black dots (0.2–0.4 mm) extending to the cheek region bilaterally (Fig. 3).

Osteology: Morphological variation in gill raker number, shape, and size is a key taxonomic trait for fish identification (Khalaf-Allah *et al.,* 2016; Azab *et al.,* 2019). In *T. sattalensis*, the anterior row of the first gill arch bore 7–8 long pointed gill rakers (2066.67 ± 219.67 µm) on the upper limb and 12–13 shorter ones (1224.22 ± 167.53 µm) on the lower limb (Fig. 4A). These bore sporadic melanin pigments and ventral teeth-like projections (218.20 ± 49.67 µm; Fig. 4B), which were sparse in the lower limb. Posterior row rakers were shorter (1,047.53 ± 239.45 µm), round-tipped, and densely pigmented with spinules having flat or tube-like tops. Raker bases were broad and triangular. Inter-raker spacing was 531.05 ± 179.23 µm (anterior) and 723.76 ± 297.32 µm (posterior). Mid-raker widths were 169.30 ± 45.32 µm (anterior) and 238.32 ± 52.10 µm (posterior). In contrast, N. chelynoides had fewer anterior rakers (3–4 upper, 7–8 lower) with distinctly curved tips (Shahi *et al.,* 2023), and *Neolissochilus* spp. were even having fewer anterior rakers (2–6 upper, 7–12 lower).

Such differences confirmed gill raker morphology as a reliable trait distinguishing *T. sattalensis* from congeners and related genera (Claytor and MacCrimmon, 1988). The fifth branchial arch formed a three-dimensional pharyngeal bone (Fig. 4C). Pharyngeal teeth were stout with broad bases, concave depressions near the apex, and pointed, slightly curved tips (Fig. 4D). Teeth were arranged in three rows on each side: the lower row with 5 large teeth, the middle with 3 large teeth, and the upper with 2 small teeth. All teeth were continuous with the pharyngeal bone and coated with enamel to their bases. These enamel-coated extensions of the pharyngeal bones were comparable in shape and number to those reported in other Tor, Naziritor, and Neolissochilus species (Talwar and Jhingran, 1991). *T. sattalensis* possessed 33–36 vertebrae with an equal number of neural spines (33–36) and 15–16 hemal spines. The 15th neural spine was characteristically bifurcated from mid-length. The 3rd and 4th anterior ribs were bifurcated at the base. Neural and hemal spines were equal in length and curved posteriorly towards the

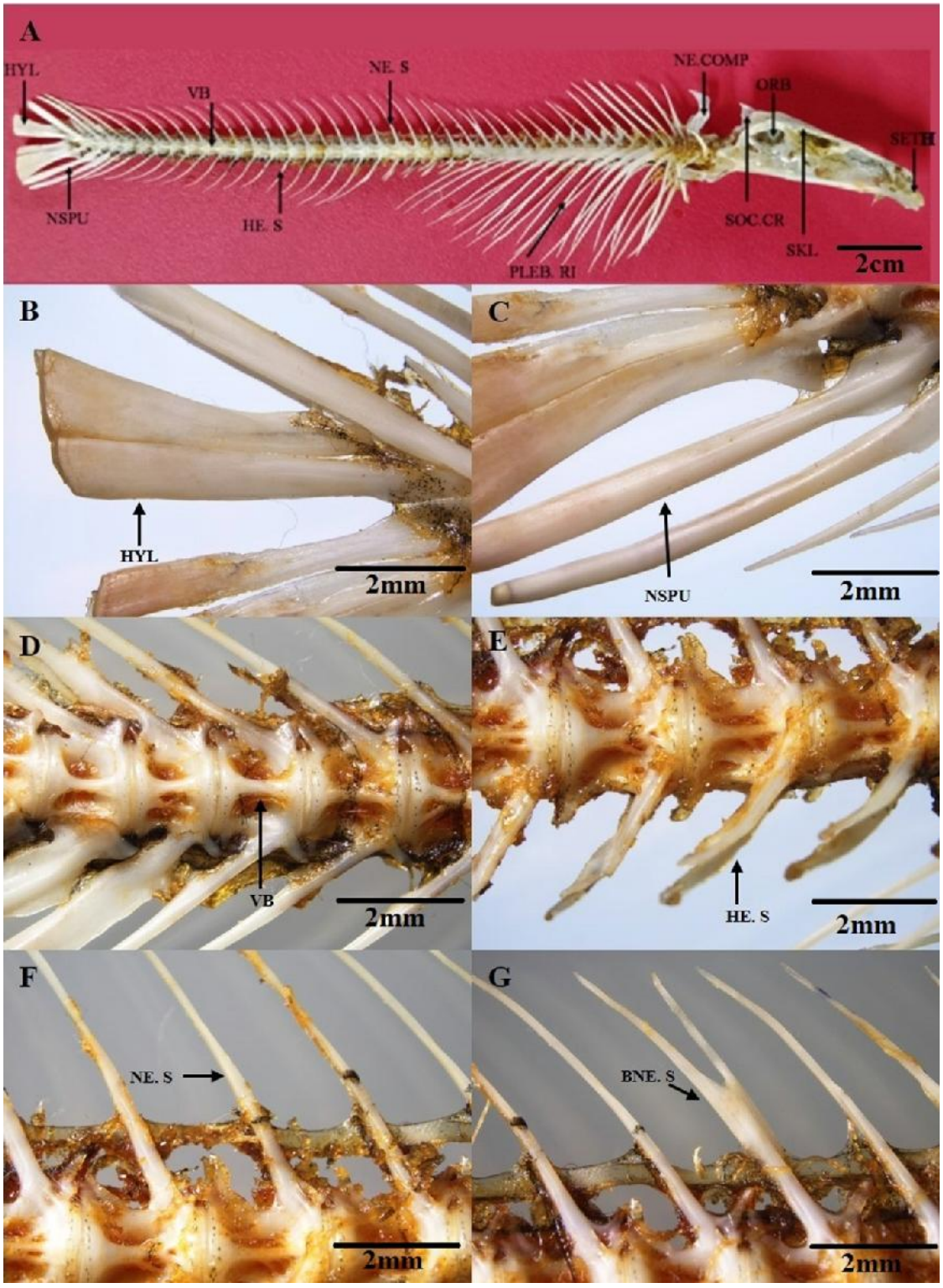

Fig. 5 (A-G): Picture of lateral view of the cleared and unstained skeleton of *T. sattalensis* (Paratype DCFRMU 149, 256.5 mm SL). (A) Skeleton of the whole fish. (B) Hypural bone of the fish. (C) Neural spine of preural centrum. (D) Vertebrae. (E) Hemal spine. (F) Neural spine. (G) Bifurcated neural spine at fifteenth position. Abbrevations: VB-Vertebrae; SKL-Skull; ORB-Orbit; SETH-Supraethmoid; SOC.CR-Supraoccipital crest; NE. COMP-Neural complex; PLEB.RI-Pleural rib; NE. S -Neural spine; He. S-Hemal spine; VB-Vertebrae; HYL-Hypural; BNE. S- bifurcated neural spine, and NSPU-Neural spine of preural centrum.

Table 5: Uncorrected p genetic distance between *T. sattalensis* and other fish taxa based on nucleotide sequences of three mitochondrial genes: *coxI*, *cytb* and *ATPase* 6 & 8 gene. The number of base substitutions per site from between sequences is shown. Analyses were conducted using the Maximum Composite Likelihood model. Codon positions included were 1st +2nd + 3rd + Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were conducted in the MEGA-X tool

***coxI* /Species**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**		
1. *Tor sattalensis*										
2. *Tor tor*	0.730									
3. *Tor putitora*	0.713	0.000								
4. *Tor barakae*	0.690	0.021	0.021							
5. *Tor khudree*	0.729	0.024	0.024	0.027						
6. *Tor malabaricus*	0.701	0.023	0.023	0.018	0.024					
7. *Tor mosal*	0.701	0.028	0.030	0.007	0.029	0.021				
8. *Tor remadeviae*	0.693	0.033	0.035	0.023	0.031	0.028	0.028			
9. *N. chelynoides*	0.936	0.771	0.766	0.782	0.778	0.774	0.788	0.801		
***cytb* /Species**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1. *Tor sattalensis*										
2. *N. hexagonolepis*	0.058									
3. *Tor tambroides*	0.056	0.062								
4. *Tor barakae*	0.045	0.074	0.037							
5. *Tor malabaricus*	0.072	0.083	0.055	0.051						
6. *Tor khudree*	0.047	0.068	0.043	0.029	0.061					
7. *Tor douronensis*	0.100	0.044	0.088	0.058	0.067	0.064				
8. *Tor sinensis*	0.060	0.049	0.055	0.058	0.067	0.056	0.047			
9. *Tor putitora*	0.005	0.058	0.051	0.045	0.072	0.048	0.080	0.057		
10. *Tor tor*	0.023	0.058	0.052	0.042	0.063	0.046	0.077	0.055	0.019	
11. *N. chelynoides*	0.128	0.130	0.126	0.137	0.137	0.132	0.121	0.130	0.125	0.128
***ATPase* 6 & 8 /Species**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**		
1. *Tor chelynoides*										
2. *N. chelynoides*	0.134									
3. *N. hexagonolepis*	0.074	0.115								
4. *Tor tor*	0.019	0.119	0.080							
5. *Tor khudree*	0.050	0.119	0.070	0.053						
6. *Tor douronensis*	0.066	0.134	0.088	0.063	0.072					
7. *Tor putitora*	0.027	0.152	0.084	0.028	0.056	0.073				
8. *P. titteya*	0.264	0.256	0.262	0.262	0.266	0.296	0.274			
9. *P. chalakkudiensis*	0.184	0.196	0.189	0.182	0.197	0.215	0.194	0.190		

caudal peduncle. The neural spine of the preural centrum was broad throughout its length (Fig. 5A–G).

Evolutionary relationship: Analyses of nucleotide sequences of *coxI* (680 bp), *cytb* (1121 bp) and *ATPase* 6 & 8 (842 bp) of *T. sattalensis* with congeners and related genera (*Tor, Naziritor, Neolissochilus*) resolved it as a distinct genetic cluster, separate from all known *Tor* species (Fig. 6) (Nguyen *et al.,* 2008). Genetic distance analyses (Kimura 2-parameter model) revealed raw distances of 0.690–0.730 (*coxI*), 0.005–0.100 (*cytb*), and 0.019–0.066 (*ATPase* 6 & 8) (Table 5). For *coxI,* the maximum and minimum distances were 0.729 (T. khudree) and 0.690 (*T. barakae*); for *cytb,* 0.100 (*T. douronensis*) and 0.005 (*T. putitora*); and for *ATPase* 6 & 8, 0.100 (*T. douronensis*) and 0.005 (*T. putitora*). Nucleotide composition of all three genes is presented in Table 5.

Haplotype network: Haplotype network analysis of *coxI, cytb,* and *ATPase* 6 and 8 revealed significant genetic divergence of *T. sattalensis* from congeners, particularly *T. putitora* and *T. tor,* as shown by distinct SNP patterns. SNP differences between *T. sattalensis* and *T. putitora* were 18 (*coxI*), 6 (*cytb*) and 7 (*ATPase* 6 & 8), and with *T. tor* were 20, 7 and 8, respectively. These substantial mutational steps, especially the higher divergence in *coxI*, indicated unique haplotypes not shared with other mahseer species, supporting *T. sattalensis* as a distinct evolutionary lineage shaped by geographic isolation or historical divergence (Fig. 7). It displayed stronger affinity to the genus *Tor* than to *Neolissochilus* or *Naziritor*. The molecular confirmation using the three mitochondrial genes was further reinforced by PCA of 13 individuals based on morphometric and meristic traits, which formed a distinct, non-overlapping cluster along PC1 and PC2, separated from other Tor species (Fig. 8). High loading values for head depth, body depth, gill raker counts, and fin lengths highlighted its morphological distinctiveness, confirming *T.*

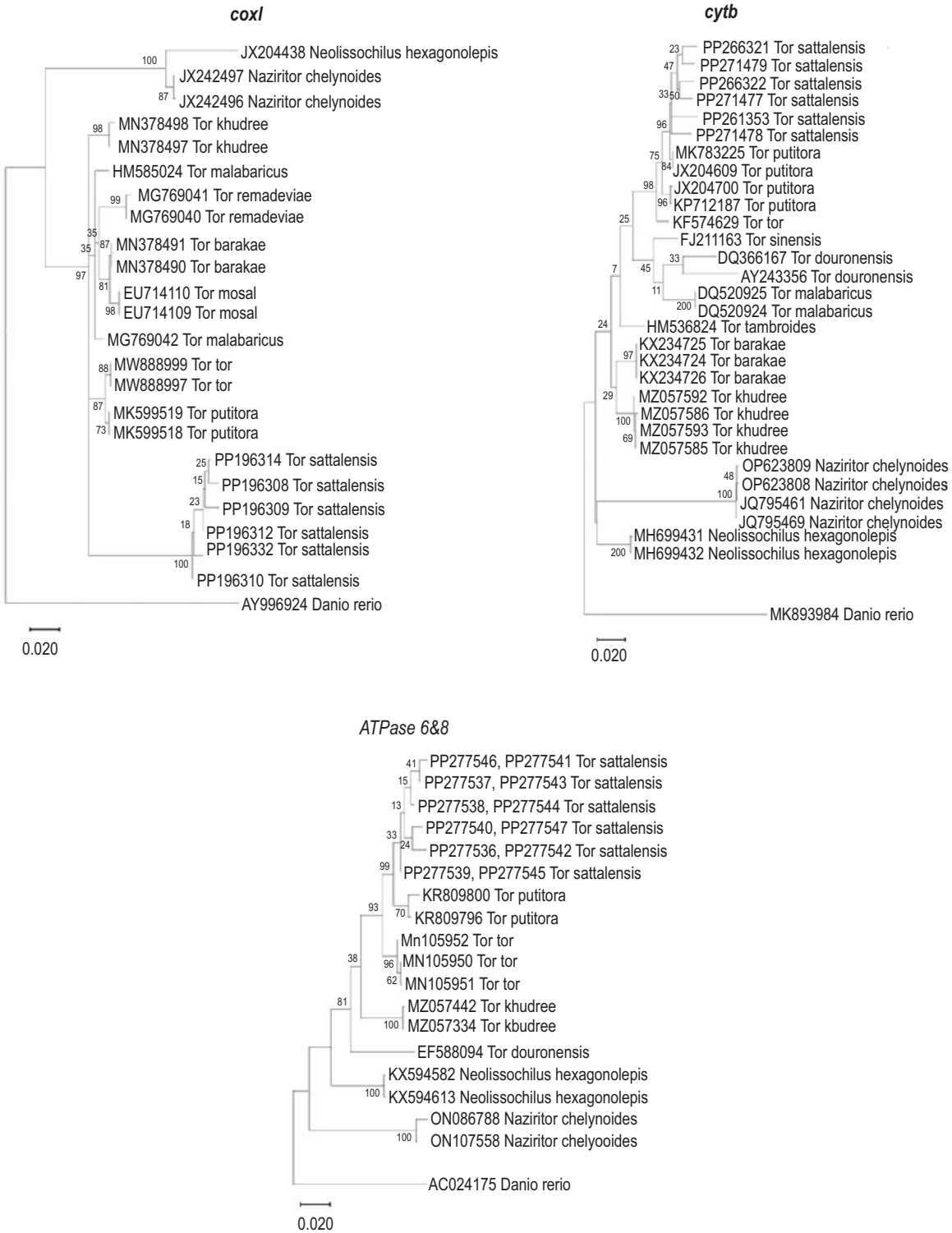

Fig. 6: Molecular phylogenetic relationships of various mahseer species. Phylogenetic analyses for the three genes; *coxI, cytb* and *ATPase* 6 & 8 were carried out by Maximum Likelihood Method, and the Kimura-2-parameter with 500 bootstrap replications using MEGAX. The analyses were made individually for each independent dataset of the genes. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site, and the *T. sattalensis* is shown in bold letter in the tree. The NCBI GenBank accession number of each nucleotide sequences is given in front of scientific names of the species.

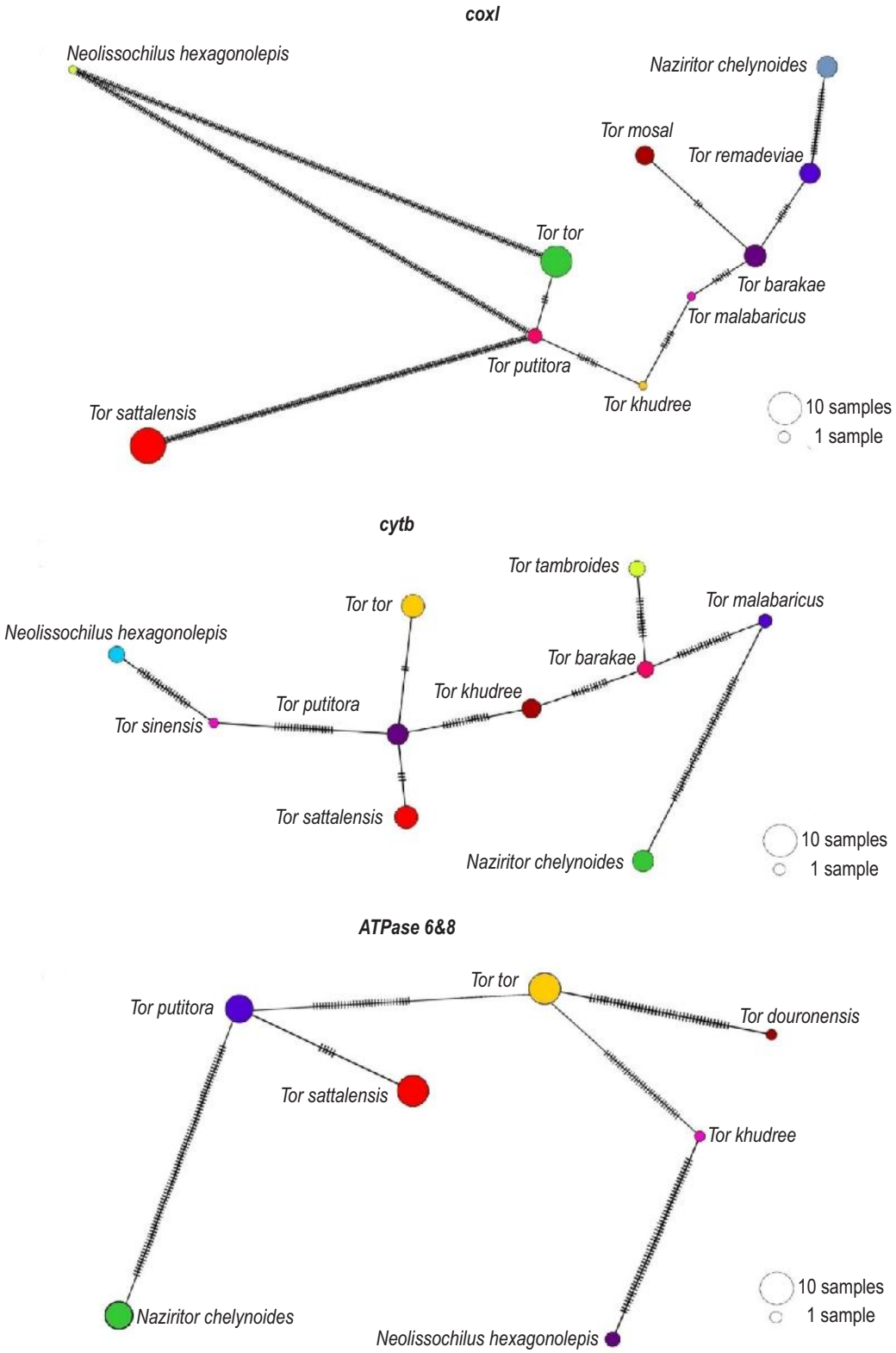

Fig. 7: The minimum spanning method (MSM) haplotype network of *T. sattalensis*, along with other mahseer species of the genus *Tor*, *Neolissochilus* and *Naziritor*, focusing on *coxI*, *cytb* and *ATPase* 6 & 8 genes. In the network representation, the diameter of each representing circle corresponds to the number of sequences, while perpendicular lines extending from the network lines indicate the number of SNPs differences among the connected species.

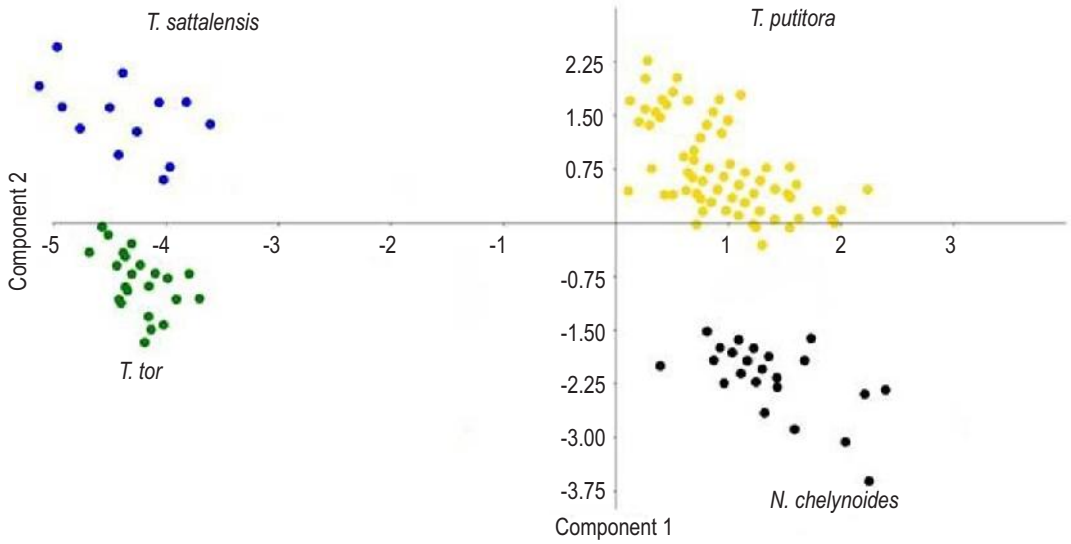

Fig. 8: Principal component analysis (PCA) of morphometric and meristic parameters of *T. sattalensis* (n = 13). Component 1 and component 2 were loaded on the axis 1 and axis 2, respectively for PCA analysis.

sattalensis as a valid species. The possibility of *T. sattalensis* being a phenotypic variant or hybrid of known *Tor* species was refuted by multiple lines of evidence. PCA showed a distinct, cohesive cluster of 13 individuals, indicating stable morphometric and meristic traits rather than plasticity or hybrid variability. Comparative analysis of mitochondrial genes (*cox1, cytb* and *ATPase* 6/8) revealed significant divergence from congeners, ruling out hybrid origin. Its occurrence in the isolated Sattal Lake (1,282 m) with unique hydrology and ecology suggests allopatric speciation, as gene flow with nearby *Tor* populations was unlikely. The absence of intermediate morphotypes or sympatric congeners further supported reproductive isolation. Thus, the combined morphological uniformity, genetic distinctiveness, ecological separation, and geographic isolation confirm *T. sattalensis* as a valid and previously unrecognized *Tor* species.

Habitat and ecology: The type locality (depth 20.318 m) had clear water with a thermal range of 8.0–27.6°C, macroalgal growth on the bottom, and was forest-surrounded. Habitat water parameters were: pH 8.6–8.7, DO 7.58–7.89 mg l^{-1}, TDS 43–56 mg l^{-1}, oxygen saturation 102–104%, and conductivity 111.7–121.5 µS cm^{-1}. Co-occurring congeners included *T. putitora* and *T. tor* (personal observation).

A novel *Tor* species, *T. sattalensis*, was identified from Sattal Lake, Uttarakhand, mid-Western Himalaya, and named after its type locality. Its distinctiveness was confirmed through morphology, osteology, and mitochondrial DNA markers (*cox1, cytb* and *ATPase* 6 and 8). Further studies on its ecology, reproduction, and genetics are vital for conservation and to explore potential speciation within the lake cluster.

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Authors' contribution: N. Shahi: Writing–original draft, Conceptualization, Methodology, Sampling, Visualization, Review and Editing; **D. Sarma:** Sampling, Funding acquisition, Supervision and Monitoring; **B. Singh:** Sampling, Methodology and Formal Analysis; **R. Haldar:** Sampling; **R. Posti:** GIS map preparation; **S.K. Mallik**: Writing-final draft, Reviewing and editing; **R. Riyaz:** Visualisation; **R. Dutta:** Visualisation.

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Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: The study complies with the current animal welfare laws in India following the guidelines of the CPCSEA [(Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment & Forests (Animal Welfare Division), Govt. of India] on care and use of animals in scientific research. The Institutional Animal Ethics Committee, corresponding author's affiliation, ICAR-DCFR ref. No. DCFR/IACUC/23/07/2021/1 approved the protocols for handling and sampling fish during experiments.

Conflict of interest: The authors declare that they have no conflict of interests that could have appeared to influence the work reported in this paper.

Data availability: The supplement data generated and analysed during the current study are available from the corresponding author on reasonable request.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

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