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Morphometric and Molecular Assessment of *Tor putitora* and *Neolissochilus hexagonolepis* Population in Jia Bhoroli river Ecotone Zone, Assam

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ABSTRACT

Background: Ecotones are areas of overlap between different ecosystems, referred to as 'junction zones or tension belts', that harbor a distinct collection of organisms. *Tor putitora* and *Neolissochilus hexagonolepis* are two threatened Mahseer species which requires urgent conservation attention. Bhalukpung area in the River Jia Bhoroli is an ideal example of an ecotone, as its ecosystem transforms from resembling a foothill river ecosystem to a river floodplain ecosystem. However, studies on threatened fish populations in ecotone zones, which could be used as conservation sites, are lacking. Therefore, the present study aims to generate firsthand information on the relationship between the populations of these threatened fish and their foothill-floodplain river interface ecotone in the Jia Bhoroli River.

Methods: The data on water quality, topography was collected for a period of one year from January to December, 2021 along with fish samples. The mahseer species were identified and catch per unit effort (CPUE) was also calculated.

Result: Identification of the collected mahseer fish as *T. putitora* and *N. hexagonolepis* were confirmed using both morphometric as well as molecular tools. CPUE was found to be 4.3-12.6 for *N. hexagonolepis* and 4.1-10.3 for *T. putitora*. The ecotone zone was found to have a thriving mahseer population and ecotones are believed to play a crucial role as a diverse food source for the fish. Conserving Bhalukpong as a designated ecotone site in the Jia Bhoroli River as a fish sanctuary can help to protect their populations. Further research that encompasses all aspects of ecology is recommended for the future.

Key words: Conservation, Ecotone, Jia bhoroli, Mahseer, Population.

INTRODUCTION

The concept of an ecotone was first introduced by Clements in 1905 as a way to describe the boundary between two different biological communities. An ecotone is defined as "a zone of transition between adjacent ecological systems, with a set of characteristics determined by the strength of interaction between the adjacent ecological systems" (Holland, 1988). It represents a region characterized by a relatively swift transition, giving rise to a narrow ecological region that separates two distinct and relatively uniform community types (van der Maarel, 1990). The River Jia Bhoroli originates in the upper Himalayan range of Arunachal Pradesh at an elevation of 5400 m msl where it is known as Kameng, It then flows through the Sonitpur district of Assam before joining the mighty River Brahmaputra at Tezpur (elevation 69 m msl). The middle reaches of the Jia Bharali, from Bhalukpung to Balipara in Sonitpur district, provide excellent opportunities for sport fishing and rafting. Bhalukpung area in the River Jia Bhoroli is an ideal example of an ecotone, as it shifts from resembling a foothill river ecosystem to a river floodplain ecosystem. This transition results in a high level of fish diversity, due to the unique combination of the two contrasting ecosystems (Naiman *et al*., 2005).

Mahseer, a globally renowned sport and table fish, is considered as the National Heritage of India (Nautiyal, 2014). They are flow-loving cyprinids (Order: Cypriniformes, Family: Cyprinidae) characterized by large scales and can grow to be large-bodied carps with a maximum recorded weight of 54 kg. They are taxonomically partitioned into the genera *Naziritor*, *Neolissochilus* and *Tor* (Kottelat, 2013; Froese and Pauly, 2018; Eschmeyer *et al*., 2017). Only species of the genus *Tor* are considered as true mahseer (Desai, 2003) and this genus includes 16 valid species. These species

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are considered highly potamodromous, with upstream spawning migrations often over considerable distances being necessary for successful reproduction (Nautiyal *et al*., 2001 and 2008).

Golden Mahseer, *Tor putitora*, is a species of fish that is widely distributed in the Himalayan region and across South and Southeast Asia, from Afghanistan, Pakistan, India, Nepal, Bangladesh, Bhutan, Myanmar, Sri Lanka, Western Iran to Thailand. This fish is restricted to montane and sub-montane streams and rivers in the Indus, Ganga and Brahmaputra river basins and is found at an elevation of 70-1891 m above sea level and latitude of 8°N to 36°N. The species has been declared Endangered (EN) by the International Union for Conservation of Nature (IUCN) Red List due to severe threat from overfishing, loss of habitat, decline in quality of habitat resulting in loss of breeding grounds and from other anthropogenic effects such as construction of dams leading to blocking their migrations and affecting their breeding (Jha *et al*., 2018).

Chocolate mahseer, *Neolissochilus hexagonolepis*, is a freshwater fish that inhabits hill streams and rivers in India, Bangladesh, Nepal, Myanmar, Indonesia and China. In India, it is found in water bodies located at 50-2000 m above sea level in states such as Arunachal Pradesh, Assam, Bihar, Jharkhand, Meghalaya, Nagaland, Uttar Pradesh and West Bengal. It is an omnivorous, opportunistic feeder that feeds on green filamentous algae, aquatic insects, small fish, weeds and mollusks (Majhi *et al*., 2013). The species has been declared as Near Threatened (NT) as per IUCN Red List due to factors such as habitat loss, pollution and increasing water temperature in its inhabiting area (Arunachalam, 2010).

Previous researchers (Samways and Stewart, 1997; Attril and Rundle, 2002) have emphasized the function of ecotones in determining the biotic makeup of ecosystems, but there is a dearth of information regarding the role of ecotones in conserving threatened species. The lack of specific studies on Mahseer populations especially for *T. putitora* and *N. hexagonolepis* in ecotone zones makes the current study important and first of its kind. It aims to generate new information regarding the relationship between this fishes populations and their foothill-floodplain river interfaces ecotone environment in the Jia Bhoroli river. The results of this study will contribute to a better understanding of the ecology of this species and facilitate the development of informed conservation and management decisions.

MATERIALS AND METHODS

Study area and sampling

During the present study, fish samples were collected from the wild stocks in Bhalukpong area (27°0′56″N 92°38′46″E) of the Jia Bhoroli river which falls near the Nameri National Park (Fig 1). The samples were collected with the help of local fishermen using various fishing methods (gill nets, angling and cast nets) from January to December 2021, following the guidelines of the National Biodiversity Authority of India (Biological Diversity Act, 2002). The fish specimens were identified using standard taxonomic keys (Vishwanath and Nebeshwar, 2009; Kottelat, 2013). Fish that weighed less than 140 g were counted, photographed and then released back into the water, while the remaining samples were brought to the laboratory for further morphometric, meristic and molecular analysis. These samples were deposited in the Fish Museum of the Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University, Raha, Nagaon, Assam (COFAAUMU 01-55). The population abundance was calculated as:

$$\text{Total abundance or Catch Per Unit Effort (CPUE)} = \frac{\text{Total catch}}{\text{Sum of effort, Sum of the effort remains 10}}$$

(Khajiria *et al*., 2014)

Water quality parameters

Water samples were collected from Bhalukpong of River Jia Bhoroli from January to December, 2021. Some of the physical parameters like depth and surface water temperature (Mercury thermometer) were determined on the spot. Other parameters like, dissolved oxygen, pH, total alkalinity, total hardness of the water samples was analyzed in the laboratory following APHA (2017).

DNA extraction and barcoding

Further, for molecular analysis, fin and muscle tissue samples were dissected from the mahseer specimens and stored in 100% ethanol. The extraction of total DNA from the ethanol-preserved tissue was carried out according to the standard DNA barcoding methods for fish as described by (Ward *et al*., 2005). A 655 bp region from the 5′ end of the COI gene was amplified using the primers (Table 1) outlined by Ward *et al*. (2005) through a Polymerase Chain Reaction (PCR) with a total volume of 50 µl. The thermal cycling conditions consisted of an initial step of 2 minutes at 95°C followed by 35 cycles of denaturing (94°C, 30 seconds), annealing (54°C, 30 seconds) and extension (72°C, 1 minute) with a final extension of 10 minutes at 72°C. The PCR products were screened for success on a 1.0% agarose gel. The sequencing of the PCR products was outsourced to Eurofin India, Pvt. Ltd. The sequences were then manually edited, aligned and proofread using Clustal W in MEGA 10.0 software and submitted to GenBank (NCBI).

Statistical analysis

All the analysis was performed in Microsoft Excel 2016. The results are displayed either in range or Mean±SD based on applicability. Correlation analysis was done between Total Length and other external features.

RESULTS AND DISCUSSION

Odum and Barrett, (1971) defined an ecotone as an area where two or more different ecosystems overlap and interact. They described it as a transition zone between communities, such as between a forest and grassland or between a soft bottom and hard bottom marine community. Odum also referred to the ecotone as a "junction zone or tension belt" that contains a unique set of organisms from the overlapping communities, as well as organisms that are specific to and often restricted to the ecotone itself. Mahseer, a globally renowned sport and table fish, is considered the national heritage of India (Nautiyal, 2014). *T. putitora* and *N. hexagonolepis* are two threatened Mahseer fishes which need to be conserved (Jha *et al*., 2018; Arunachalam, 2010). Even though there are multiple reports of assessment of these species in different rivers and other ecosystems. But no specific study has been conducted in an ecotone zone of a river. Thus, the current study is the first of its kind to study of two important threatened in a foothill-floodplain river interfaces ecotone zone.

Water quality parameters of River Jia Bhorali at Bhalukpong during the sampling period are given in Table 2. The study site is a flowing stream characterized by a moderate current and an average width of 60 m and depth of 1.5 m. The riverbed is composed of boulders (5%), cobbles (35%), coarse gravels (40%) and fine gravels (20%) according to the Indian Standard Soil Classification System (Ranjan and Rao, 2000). The riparian zone is made up of shrubs, bushes and small trees like *Ziziphus mauritiana* in the terrestrial part and pools and riffles in the aquatic part. The banks of the river are covered by the dense forest of Nameri National Park.

Water quality in the study area was found to be intermediate between that of foothill river water and floodplain river water when compared to previous reports of this river (Khound *et al*., 2012; Singh *et al*., 2020). The continuous input of organic materials, such as plant litter, into the river may be a primary factor in this classification and the river continuum concept provides a suitable framework for its description. However, the riparian flora and fauna require further study.

The current study thoroughly examined and evaluated each collected specimen. The general body shape of identified *T. putitora* and *N. hexagonolepis* can be seen in Fig 2 (a and b). The body colour of fresh specimens *T. putitora* were greenish and silvery on the side of the body, but turns reddish yellow or golden on the anal and pectoral fins. Mouth with lower jaw slightly shorter than the upper jaw, caudal fin deeply forked. The dorsal fin ray of the specimens found to be 11 (2/9); pectoral fin ray was 14-15, anal fin ray 7, pelvic fin ray count 8-9 while caudal fin ray count was 20. Lateral line scale found to vary between 25-27; scales above lateral line 4.5 and below lateral line 2.5 (Table 3 and 4). Based on

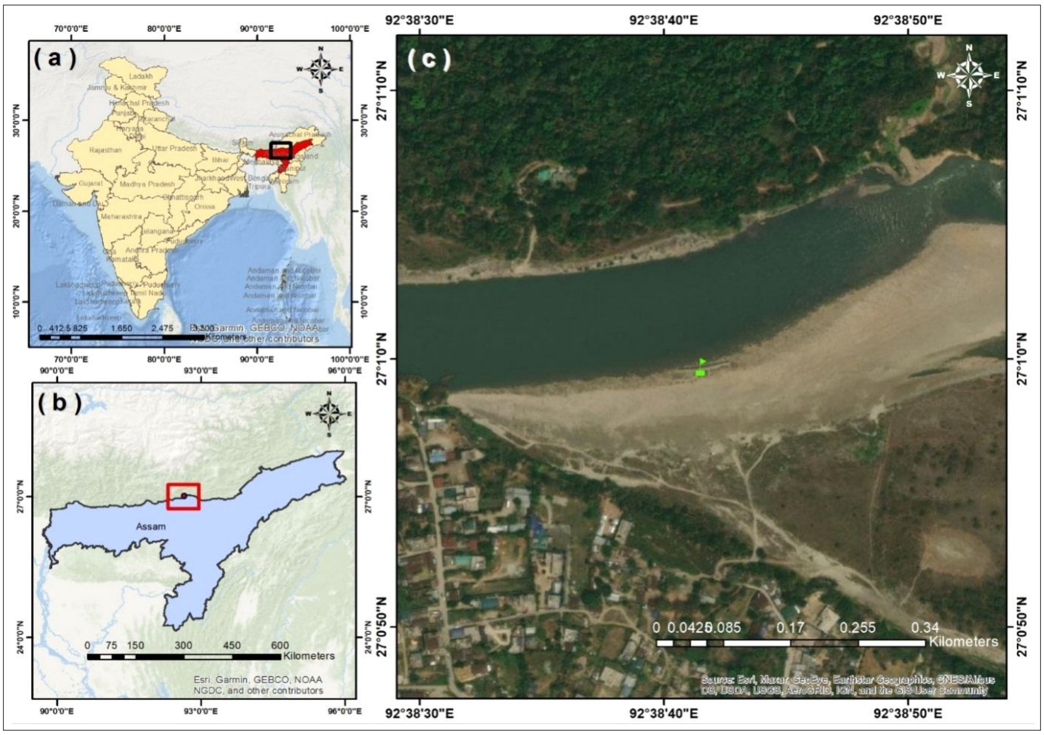

Fig 1: Map showing sample collection area in Bhalukpong ecotone zone of River Jia Bhoroli.

Table 1: Primers used during the study.

Gene	Primer sequence (5′ to 3′)	Product size (bp)	Reference
COIF1	TCAACCAACCACAAAGACATTGGCAC	655 bp	Ward *et al*., 2005
COIR1	TAGACTTCTGGGTGGCCAAAGAATCA		

this, the derived fin formula of *T. putitora* is D11(2/9)P14-15V8-9A7(2/7)LLS25-27. The standard length (SL) of *T. putitora* ranges 234-219 mm. A positive correlation was observed between total length and all the external body parts in *T. putitora* (Table 5). In relation to TL, the highly correlated body parameters were FL (0.983), SL (0.932), VFL (0.906) and GL (0.905).

The scales of *N. hexagonolepis* were hexagonal in shape as its name derived from it, snout length was less than that of the body depth and body color was dark brown in freshly collected specimens. The specimens were found to have 26-32 lateral line scales, 3.5-4.5 scales above lateral line and 2.5 scales below lateral line. The dorsal fin ray of the specimens found to be 11 (2/9); pectoral fin ray was 14-15, anal fin ray 7, pelvic fin ray count 8-9 while caudal fin ray count was 18-21. Lateral line scale found to be 25; scales above lateral line 3.5 and below lateral line 2.5 (Table 3 and 4). Based on this, the derived fin formula of *N. hexagonolepis*is D11(2/9)P14-15V8-9A7(2/7)LLS25. The standard length (SL) of *N. hexagonolepis* ranged from 211-256 mm. Positive correlation was observed between total length and the external body parts of *N. hexagonolepis* except ED, LJL, GL, GW, DFH and DoM. The highly correlated body parameters in relation to TL were VFL (0.910), DaE (0.898), HD (0.895) and PFL (0.895).

The morphometric as well as meristic counts of *T. putitora* and *N. hexagonolepis* specimens recorded during the present study were found to be similar as described by previous

Table 2: Water quality parameters of River Jia Bhoroli at Bhalukpong.

Physico-chemical parameters	Range
Temperature	13-29°C
pH	7.2-8.2
Dissolved oxygen	4.9-7.7 mg/L
Free carbon dioxide	0-13.3 mg/L
Total alkalinity	88.5-119.6 mg/L
Total hardness	74.3-96.5 mg/L

Table 3: Morphometric count of two important Mahseer *i.e. Tor putitora and Neolissochilus hexagonolepis.*

Morphometric	*Tor putitora*			*Neolissochilus hexagonolepis*		
count	Mean	Median	SD	Mean	Median	SD
Weight (gm)	164.636	166.5	11.713	187.455	190	20.124
Total length (TL) (mm)	268.818	270	8.168	287.182	286	14.058
Fork length (FL) (mm)	212.455	213	9.122	252.303	251	22.099
Standard length (SL) (mm)	223.682	223.500	4.357	236.182	236	15.220
Head length (HL) (mm)	52.666	52.705	1.641	53.834	53.580	2.623
Pre-dorsal length (PdL) (mm)	93.470	93.245	1.100	83.175	93.980	30.571
Snout length (SnL) (mm)	21.075	20.210	2.148	21.937	21.900	1.970
Inter-orbital width (IoW) (mm)	24.188	21.695	6.253	23.573	23.650	2.542
Post-orbital length (PoL) (mm)	27.429	26.275	3.359	28.634	27.980	4.801
Eye diameter (ED) (mm)	17.306	15.955	3.679	13.686	13.270	2.730
Upper jaw length (UJL) (mm)	15.357	13.830	4.226	14.779	14.920	1.367
Lower jaw length (LJL) (mm)	18.023	17.305	2.151	16.131	15.760	2.617
Anal length (AL) (mm)	146.177	143.170	6.681	156.505	157.870	35.476
Girth length (GL) (mm)	146.334	145.435	3.420	149.385	163.800	36.696
Body depth (BD) (mm)	50.666	49.580	3.393	63.147	65.480	5.716
Head depth (HD) (mm)	36.290	35.330	2.537	40.250	39.730	4.631
Gape width (GW) (mm)	18.948	18.210	2.090	15.265	15.110	2.942
Dorsal fin height (DFH) (mm)	58.855	58.305	2.358	60.084	58.800	6.077
Dorsal fin base (DFB) (mm)	23.155	22.785	1.521	28.053	26.540	5.796
Pectoral fin length (PFL) (mm)	38.921	37.910	2.725	43.092	43.420	5.313
Pectoral fin base (PFB) (mm)	9.625	9.130	1.481	13.286	11.920	3.608
Anal fin length (AFL) (mm)	40.200	39.215	2.604	43.656	43.480	2.910
Anal fin base (AFB) (mm)	15.483	14.905	2.520	15.774	15.120	2.522
Ventral fin length (VFL) (mm)	32.998	32.335	2.612	37.777	37.900	3.924
Ventral fin base (VFB) (mm)	15.631	14.360	2.699	12.697	12.120	1.973
Depth of mouth (DoM) (mm)	10.246	9.485	2.226	10.013	10.180	2.869
Depth of eye (DaE) (mm)	26.915	26.030	2.602	31.410	31.960	3.944
Depth at dorsal fin (DaDF) (mm)	49.265	48.845	1.726	58.739	58.300	6.474
Depth at pectoral fins (DaPF) (mm)	40.854	39.975	2.663	46.341	47.570	4.567
Depth at anus (DaA) (mm)	35.870	34.505	2.811	39.700	40.940	4.751
Caudal fin height (CFH) (mm)	28.704	27.930	2.612	43.211	33.240	16.039
Length of the caudal peduncle (LoCP) (mm)	24.255	23.680	2.324	29.979	26.710	7.593

authors (Vishwanath *et. al*., 2011; Langer *et al*., 2013 and Laskar *et al*., 2013). The positive correlation between total length and other external morphological features observed during the present study indicates isometric growth pattern of *T. putitora* in natural condition. Langer *et al*. (2013) also reported similar morphometric relationship in Golden Mahseer from Jhajjar stream, J and K, India.

Table 4: Morphometric relationship table of *Tor putitora* and *Neolissochilus hexagonolepis.*

Co-relation	*Tor putitora*	*Neolissochilus hexagonolepis*
TL/FL (mm)	0.983	0.646
TL/SL (mm)	0.932	0.867
TL/HL (mm)	0.839	0.712
TL/PdL (mm)	0.745	0.709
TL/SnL (mm)	0.886	0.691
TL/IoW (mm)	0.845	0.739
TL/PoL (mm)	0.858	0.506
TL/ ED (mm)	0.742	-0.503
TL/UJL (mm)	0.862	0.793
TL/LJL (mm)	0.844	-0.519
TL/AL (mm)	0.876	0.392
TL/GL (mm)	0.905	-0.242
TL/BD (mm)	0.856	0.747
TL/HD (mm)	0.831	0.895
TL/GW (mm)	0.850	-0.406
TL/DFH (mm)	0.855	-0.457
TL/DFB (mm)	0.879	0.873
TL/PFL (mm)	0.884	0.895
TL/PFB (mm)	0.869	0.608
TL/AFL (mm)	0.801	0.772
TL/AFB(mm)	0.871	0.593
TL/VFL (mm)	0.906	0.910
TL/VFB (mm)	0.878	0.256
TL/DoM (mm)	0.865	-0.034
TL/ DaE (mm)	0.868	0.898
TL/DaDF (mm)	0.847	0.848
TL/DaPF (mm)	0.804	0.862
TL/DaA (mm)	0.832	0.846
TL/CFH (mm)	0.818	0.885
TL/LoCP(mm)	0.846	0.810

DNA barcoding

Identification of the Mahseer species were confirmed through DNA barcoding, the generated mitogenome sequences were submitted to NCBI and accession numbers were obtained (Table 5). The construction of a phylogenetic tree using maximum likelihood and neighbor-joining algorithms showed that the COI gene sequences of the specimens were closely related to those of *N. hexagonolepis* and *T. putitora* from other parts of the world (Fig 3). The overall genetic distance calculated within the species was 0.002 and 0.01 for *T. putitora* and *N. hexagonolepis*, respectively.

The COI gene sequence of *T. putitora* and *N. hexagonolepis* was compared to other sequences from various locations around the world available in the NCBI GenBank database. The blast alignment showed that the specimen was 98% identical to the other sequences of *T. putitora* and *N. hexagonolepis*, which, according to Ward *et al*. (2005) confirms their identity as the species with 98-99% resemblance. The mitogenome sequences of the Mahseer species generated from the River Jia Bhoroli, Brahmaputra drainage is the first such kind of information. Phylogenetic analysis supports the close evolutionary relationship between the species, with a highest bootstrap value of 100. Despite the geographical distance, the COI gene sequences of the compared species are genetically similar, suggesting conservation throughout the evolutionary process. Based on both morphological and phylogenetic analyses from COI gene sequences, it is concluded that the barb species are *T. putitora* and *N. hexagonolepis.*

Population

The survey carried out over the course of one year studied a total of 1026 nos. of *N. hexagonolepis* and 937nos. of *T. putitora* in the Bhalukpong region of Jia Bhoroli river with the density of *N. hexagonolepis* (with a CPUE of 4.3-12.6) higher in the study area compared to *T. putitora* (with a CPUE of 4.1-10.3). Both species showed the highest density during March and the lowest in January. The current study found a high

Table 5: DNA barcoding details of Mahseer species from River Jia Bhoroli generated during the study.

Species	River	Primer	Identified	Barcode submitted	Accession no.
Neolissochilus hexagonolepis	Jia Bhoroli	(COX1)	Yes	Yes	MZ540413
Neolissochilus hexagonolepis	Jia Bhoroli	(COX1)	Yes	Yes	MZ540411
Neolissochilus hexagonolepis	Jia Bhoroli	(COX1)	Yes	Yes	MZ540314
Neolissochilus hexagonolepis	Jia Bhoroli	(COX1)	Yes	Yes	MZ520586
Neolissochilus hexagonolepis	Jia Bhoroli	(COX1)	Yes	Yes	MZ664315
Neolissochilus hexagonolepis	Jia Bhoroli	(COX1)	Yes	Yes	MZ664305
Neolissochilus hexagonolepis	Jia Bhoroli	(COX1)	Yes	Yes	MZ664303
Neolissochilus hexagonolepis	Jia Bhoroli	(COX1)	Yes	Yes	OM060678
Neolissochilus hexagonolepis	Jia Bhoroli	(COX1)	Yes	Yes	OM060688
Tor putitora	Jia Bhoroli	(COX1)	Yes	Yes	OM060681
Tor putitora	Jia Bhoroli	(COX1)	Yes	Yes	OM060682
Tor putitora	Jia Bhoroli	(COX1)	Yes	Yes	OM060687

abundance of adult as well as young individuals of both fish species in this area. The riffles and pools provide excellent habitats for young and small fish (de Moraris *et al*., 1995). The reproductive success of these populations is highly sensitive to any modifications of this ecotone zone (Schiemer and Zalewski, 1992). The fish fauna in this ecotone zone was found to be rich and diverse and these ecotones likely play an important role as a diversified food source for the fish (Khajuria *et al*., 2014). The higher abundance of fish in the study area indicates favorable conditions for these fish in this ecotone.

According to the IUCN, *T. putitora* and *N. hexagonolepis* are classified as endangered and near threatened, respectively (Jha *et al*., 2018; Arunachalam, 2010). The major reasons of this classification are rise in water temperature, overfishing, loss of habitat, decline in quality of habitat resulting in loss of breeding grounds and from other anthropogenic effects (Sarma *et al*., 2022). Hence, urgent efforts for conservation of these fish species are need

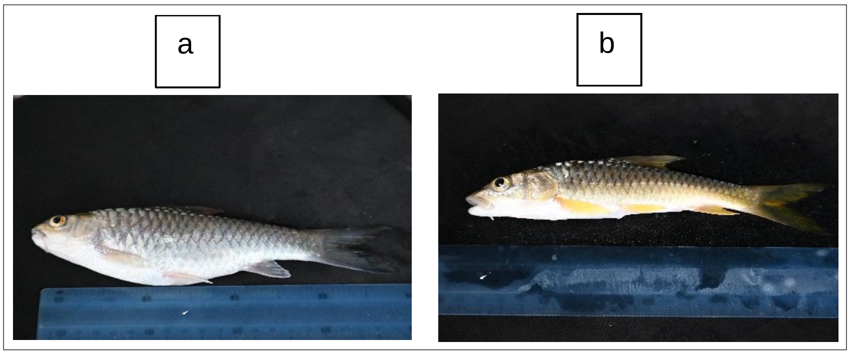

Fig 2: Preserved specimen of (a) *Neolissochilus hexagonolepis* (b) *Tor putitora.*

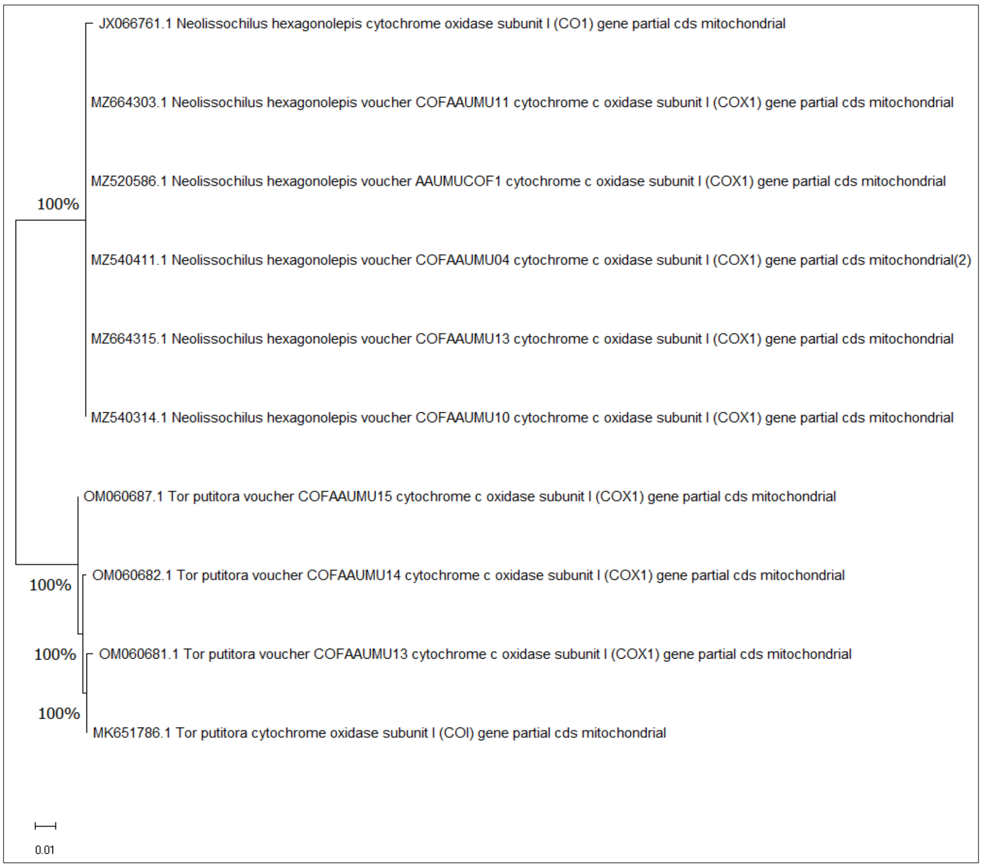

Fig 3: Neighbour-joining tree based on mtDNA COI gene sequence of *Tor putitora* and *Neolissochilus hexagonolepis* generated during the present study.

of the hour. Past studies suggested that ecotones may act as hotspots for speciation, making them valuable areas for conservation investment as they can serve as centers for biodiversity. Chapman *et al*. (1996) claimed ecotones as refugia for endangered fishes as populations in ecotones are often pre-adapted to changing environments and may be more resilient to environmental changes, such as climate change and invasions by non-native species. As ecotones are small in size yet rich in biodiversity, conserving these areas as *in-situ* conservation may be a cost-effective strategy for maintaining biodiversity (Karl, 2017).

CONCLUSION

In conclusion, the findings of this study suggest that the ecotone zones of the Jia Bhoroli river are a refuge for threatened Mahseer population as it provides food and shelter. Both *T. putitora* and *N. hexagonolepis* are in need of conservation efforts as their populations are declining due to various anthropogenic activities. Conserving Bhalukpong as designated ecotone sites in Jia Bhoroli river as fish sanctuaries can give a big boost to restoration efforts of these threatened fish species. Further research with all the other factors of ecology should be conducted for better understanding of importance of ecotones for conservation of threatened fish species.

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Conflict of interest

All the authors declare no potential conflict of interest.

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