FishTaxa (2022) 25: 1-8
Journal homepage: www.fishtaxa.com
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Genetic investigation of freshwater fishes from Indo-Nepal transboundary Gandak River

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Abstract

The diversity of freshwater fish in the Indo-Nepal transboundary river is minimally known to the common people. The present study adopted an integrated approach to determine the genetic diversity of fish fauna from the river Gandak. We generated 59 DNA barcodes of 25 fish species, identified through meristic and morphometric characters. The studied species revealed monophyletic clustering in the BA topology with high posterior probability supports and sufficient genetic distances. The study hinted more rigorous sampling and development of DNA data are required to develop a robust barcode library for indigenous fishes of Gandak River. A similar attempt will be useful to detect the extant fish diversity of other transboundary riverine systems in India and abroad and assist in reassessing the checklist and encourage sustainable conservation direction to protect the faunal composition in a riverine ecosystem.

Keywords: Fish fauna, Protected area, DNA barcoding, IUCN, Conservation.

Citation: Kundu S., Rath S., Shangningam B., Tyagi K., Laishram K., Kumar V., Banerjee D. 2022. Genetic investigation of freshwater fishes from Indo-Nepal transboundary Gandak River. FishTaxa 25: 1-8.

Introduction

The Indian subcontinent has different landscapes and biogeographic realms divided by international borders which affect the floral and faunal diversity in a drastic manner (Myers et al. 2000). Most freshwater fishes have an innate tendency to move across long riverine distances without the restriction of any political boundaries (Comte and Olden 2017). However, the equitable sharing of biological resources, especially for ichthyofauna, is often affected in transboundary riverine systems (Zawahri 2009; Arjoon et al. 2016). Hence, the estimation of spatial diversity and conservation of biodiversity elements in transboundary regions are a major cause of concern for protecting the biodiversity wealth of any country (Vasilijević et al. 2015).

Gandak is a major riverine system in India and Nepal originating from Dhaulagiri Mountain in Tibet. After passing the deep gorges in Nepal, the river passes through the Terai-Duar savanna and grasslands and joins with the north bank of the River Ganges in the Bihar state of India. More than 100 freshwater fish species have been reported from the river Gandak (David 1963; Srivastava 2013). These biodiversity resources are directly connected with humans as food resources and livelihood through economic importance. Thus, the modicum information on ichthyofaunal diversity in the Gandak River is urgently required through the contemporary DNA barcoding approach (Hebert et al. 2003; Hajibabaei et al. 2007).

Several small to large-scale attempts have been intended from different riverine systems and bio-geographical regions to generate the nucleotide sequence data (NSD) of freshwater fishes in India (Barman et al. 2018; Laskar et al. 2018; Kundu et al. 2019a, b). These NSDs are often utilized in rapid and reliable species identification, systematics studies, and border biosecurity (Collins et al. 2012). Subsequently, the growth of Digital Sequence Information (DSI) on Indian NSD has enormously risen in the last decades. A single DNA barcode-based attempt has been made to identify the freshwater fish diversity from the transboundary river of Indo-Bhutan (Kundu et al. 2019c). However, the exploration of freshwater fishes from many transboundary riverine systems is yet to be aimed to fill the gap of DSI from India. Thus, the present study was intended to examine the fish samples from the Gandak River and identify the specimens through an integrated approach. This DNA-based assessment allows to build of a local barcode library of selected freshwater fishes and would

Accepted: 07 September 2022; Published: 09 September 2022

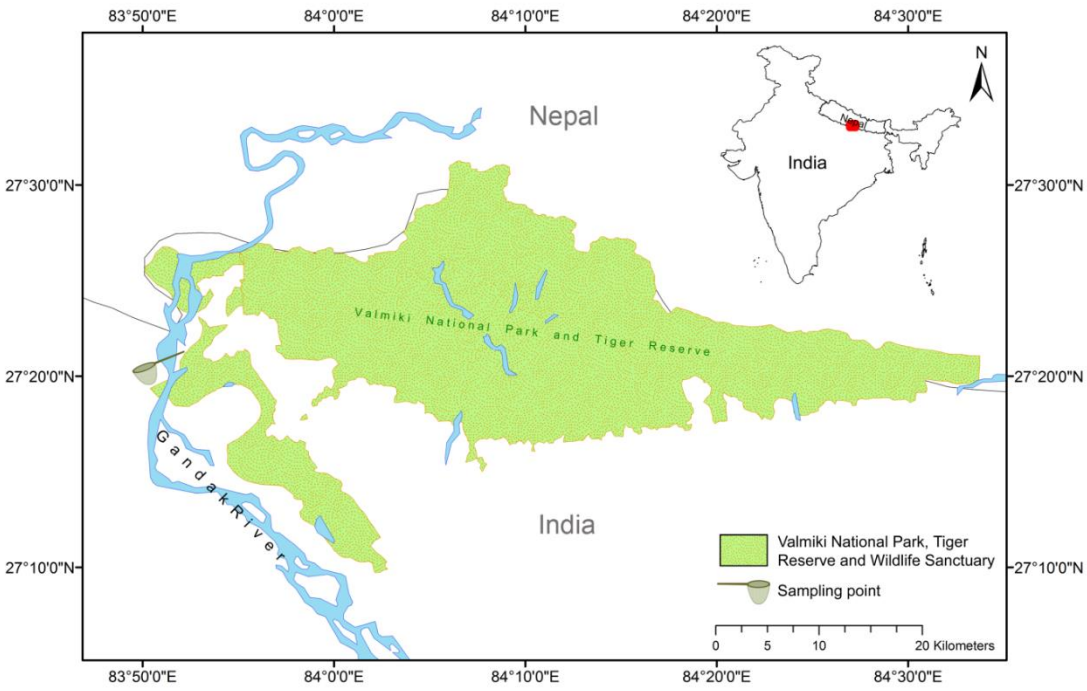

Figure 1. Map showing the topology and Gandak River in the trans-boundary of Indo-Nepal with sampling site of freshwater fishes.

assist the management schemes of Gandak River and its allied biodiversity.

Material and Methods

In the present study, we collected 59 fish samples from the Gandak River (27.33N 83.84E) in Eastern India (Fig. 1). All specimens were caught by caste net (mesh >5 cm in size) and stored in 70% ethanol for downstream genomic investigation. The collected specimens were identified using morphometric data and meristic counts (Menon 1974; Jayaram 1999; Eschmeyer 2012). The voucher specimens were preserved in the Freshwater Fish Section of the Zoological Survey of India (ZSI), Kolkata.

Genomic DNA extraction, PCR amplification with published primer pairs, and purification were executed following the standard laboratory protocols (Kundu et al. 2019c; Ward et al. 2005). The ABI-3730 DNA analyzer was used for Sanger Sequencing available in the in-house facilities of ZSI, Kolkata. The bi-directional chromatograms of the partial mitochondrial cytochrome oxidase subunit 1 (COI) gene were aligned to build the consensus sequence of each sample. The web tools, nucleotide BLAST (https://blast.ncbi.nlm.nih.gov) and ORF finder (https://www.ncbi.nlm. nih.gov/orffinder/) were employed to check the alignment, gap, and amino acid sequence array, similar to the vertebrate mitochondria. The *Apristurus ampliceps* sequence (Accession No. EU398546) of the order Carcharhiniformes was acquired from the GenBank database and incorporated as an out-group. The dataset was aligned by ClustalX (Thompson et al. 1997) and genetic distance was calculated by MEGAX (Kumar et al. 2018) with the Kimura-2 parameter. The best suitable model for the dataset was estimated by Mr. MODELTEST v2 and lowest BIC (Bayesian Information Criterion) score (Nylander 2004). The Bayesian (BA) topology was constructed by Mr. Bayes 3.1.233 with GTR + G+I model and four metropolis-coupled Markov Chain Monte Carlo (MCMC) (Ronquist and Huelsenbeck 2003). The sampling frequency for topology construction was run for 1,000,000 generations with 25% burn-in and trees saving at every 100 generations. The MCMC analysis was performed to render the convergence metrics till the standard deviation (SD) of split frequencies reached 0.01 and the potential scale reduction factor (PSRF) was reasonably close to

1.0. The generated BA topology was further illustrated by the online iTOL platform (https://itol.embl.de/) (Letunic and Bork 2007).

Results

Phylum CHORDATA
Class ACTINOPTERYGII
Division TELEOSTEI
Order CYPRINIFORMES
Family Cyprinidae
Neolissochilus hexagonolepis (McClelland, 1839)
ZSI FF 9410, 1, 65.66 mm SL., Gandak River, Bihar, 01.03.2018, S. Rath & Party. (Fig. 2)
Cabdio morar (Hamilton, 1822)
ZSI FF 9409, 2, 49.5-50.1 mm SL., Gandak River, Bihar, 01.03.2018, S. Rath & Party. (Fig. 2)
Opsarius bendelisis (Hamilton 1807)
ZSI FF 7643, 5, 45.0-65.0 mm SL., Gandak River, Bihar, 04.03.2018, S. Rath & Party. (Fig. 2)
Puntius sophore (Hamilton 1822)
ZSI FF 5859, 3, 36.9-40.4 mm SL, Gandak River, Bihar, 03.03.2018, S. Rath & Party. (Fig. 2)
Puntius chola (Hamilton 1822)
ZSI FF 7638, 3, 32.6-60.9 mm SL, Gandak River, Bihar, 03.03.2018, S. Rath & Party. (Fig. 2)
Labeo dyochilus (McClelland 1839)
ZSI FF 9411, 1, 59.3 mm SL., Gandak River, Bihar, 01.03.2018, S. Rath & Party. (Fig. 2)
Labeo boggut (Skyes, 1839)
ZSI FF 9412, 2, 68-70 mm SL., Gandak River, Bihar, 01.03.2018, S. Rath & Party. (Fig. 2)
Labeo bata (Hamilton 1822)
ZSI FF 9413, 1, 65.2 mm SL., Gandak River, Bihar, 01.03.2018, S. Rath & Party. (Fig. 2)
Tariquilabeo latius (Hamilton 1822)
ZSI FF 7997, 7, 42.9-65.7 mm SL., Gandak River, Bihar, 01.03.2018, S. Rath & Party. (Fig. 2)

Family Botiidae
Botia dario (Hamilton 1822)
ZSI FF 7961, 1, 48.43 mm SL., Gandak river, Bihar, 01.03.2018, S. Rath & Party. (Fig. 2)
Botia lohachata Chaudhuri 1912
ZSI FF 7921, 2, 48.7-49.8 mm SL., Gandak river, Bihar, 03.03.2018, S. Rath & Party. (Fig. 2)

Family Cobitidae
Lepidocephalichthys guntea (Hamilton 1822)
ZSI FF 5858, 2, 36.9-41.3 mm SL., Gandak river, Bihar, 05.03.2018, S. Rath & Party. (Fig. 2)

Family Nemacheilidae
Paracanthocobitis botia (Hamilton 1822)
ZSI FF 7629, 4, 34.2-52.5 mm SL., Gandak river, Bihar, 05.03.2018, S. Rath & Party. (Fig. 2)

Order SILURIFORMES
Family Amblycipitidae

Amblyceps mangois (Hamilton, 1822)
ZSI FF 9408, 1, 48.2 mm SL., Gandak River, Bihar, 01.03.2018, S. Rath & Party. (Fig. 2)

Family Sisoridae
Gagata sexualis Tilak 1970
ZSI FF 7994, 4, 26.8-46.4 mm SL., Gandak River, Bihar, 01.03.2018, S. Rath & Party. (Fig. 2)

Family Bagridae
Mystus bleekeri (Hamilton 1822)

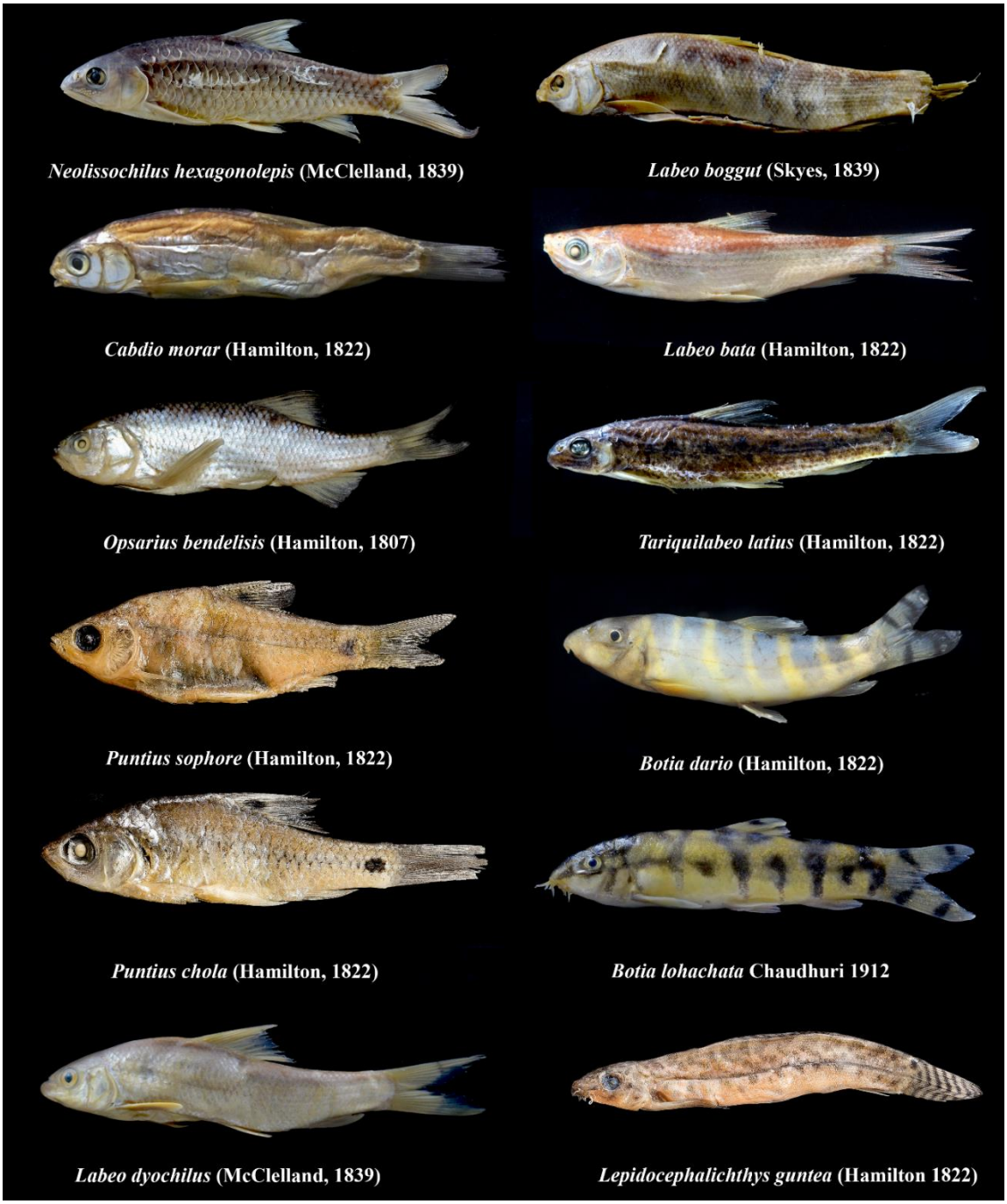

Figure 2. The selected fish species from the Gandak River.

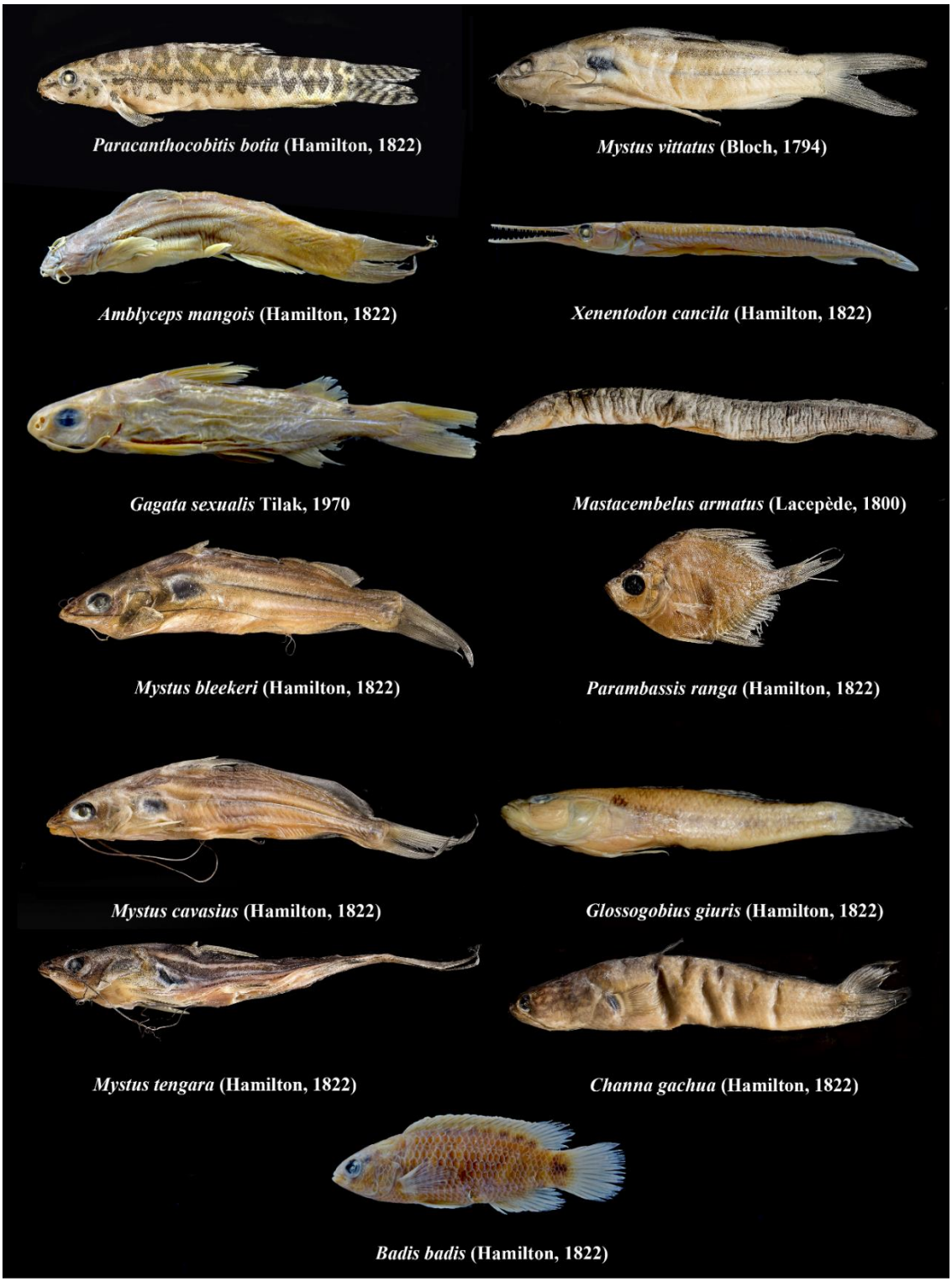

Figure 2. Continued.

ZSI FF 5896, 2, 43.8-62.9 mm SL., Gandak River, Bihar, 03.03.2018, S. Rath & Party. (Fig. 2)

Mystus cavasius (Hamilton 1822)

ZSI FF 5897, 2, 52.6-67.0 mm SL., Gandak River, Bihar, 03.03.2018, S. Rath & Party. (Fig. 2)

Mystus tengara (Hamilton 1822)

ZSI FF 5865, 1, 41.0 mm SL., Gandak River, Bihar, 01.03.2018, S. Rath & Party. (Fig. 2)

Mystus vittatus (Bloch 1794)
ZSI FF 5882, 5, 52.1-57.3 mm SL., Gandak River, Bihar, 04.03.2018, S. Rath & Party. (Fig. 2)

Order BELONIFORMES
Family Belonidae
Xenentodon cancila (Hamilton 1822)
ZSI FF 7969, 1, 121.0 mm SL., Gandak River, Bihar, 01.03.2018, S. Rath & Party. (Fig. 2)

Order SYNBRANCHIFORMES
Family Synbranchidae
Mastacembelus armatus (Lacepède 1800)
ZSI FF 7634, 1, 204.8 mm SL., Gandak River, Bihar, 05.03.2018, S. Rath & Party. (Fig. 2)

Order PERCIFORMES
Family Ambassidae
Parambassis ranga (Hamilton 1822)
ZSI FF 5887, 3, 36.5-43.7 mm SL., Gandak River, Bihar, 04.03.2018, S. Rath & Party. (Fig. 2)

Family Gobiidae
Glossogobius giuris (Hamilton 1822)
ZSI FF 5885, 1, 68.83 mm SL., Gandak River, Bihar, 01.03.2018, S. Rath & Party. (Fig. 3)

Family Channidae
Channa gachua (Hamilton 1822)
ZSI FF 5901, 2, 92-94 mm SL., Gandak River, Bihar, 02.03.2018, S. Rath & Party. (Fig. 2)

Family Badidae
Badis badis (Hamilton 1822)
ZSI FF 5900, 2, 42.5-43.9 mm SL., Gandak River, Bihar, 05.03.2018, S. Rath & Party. (Fig. 2)

Molecular investigation: The study identified 25 selected freshwater fish species through morphological characters. The generated DNA barcodes depicted 99-100% resemblance with the available reference sequences of the representative species through the nucleotide BLAST searching engine in GenBank. The similar search results of the generated barcodes were congruent with the taxonomic identification. The overall mean K2P genetic distances of the studied dataset with the selective freshwater fishes was 22.9%. The BA phylogeny showed monophyletic clades and readily delineated all the studied fish species with high posterior probability supports (Fig. 3). The status of each studied species was further followed by the International Union for Conservation of Nature Red List of Threatened Species (IUCN) and found most of the species are Least Concern and their population trends are unknown. Thus, the generated DNA barcode data is not only enriching the global DNA barcode library for accurate species identification; but also seems to be helpful for estimating fish diversity from Gandak riverine systems in India. The barcode data and tagged collateral information will further assist the Freshwater Fish Specialist Group (FFSG) to update the status of different taxa in IUCN system.

Sharing the water system and associated bio-resources support India and neighboring countries regarding water usage and socioeconomic growth. Fish and fishery resources are the moneymaking components and are

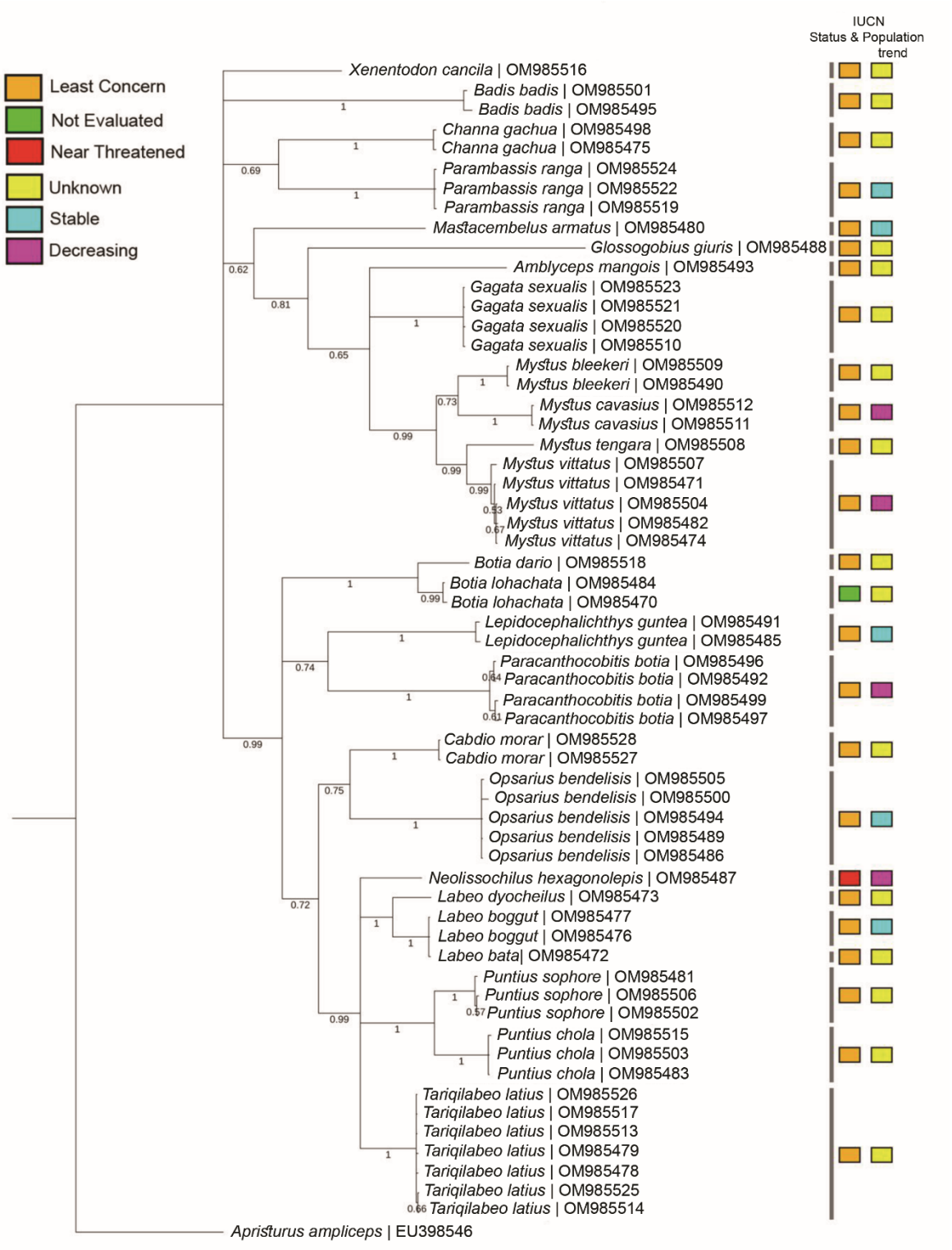

Figure 3. The BA phylogeny showing the monophyletic clades of the studied fish species from Gandak River. The IUCN status and population trends of each species were also illustrated by different color box.

straightaway connected to human and commercial products (Sarkar and Pal 2018). Nevertheless, the exotic/invasive species may enter different geographical regions through the transboundary river and possess major menaces to the indigenous species by invading their eco-regions (Collins et al. 2012). Prior to this study,

the detection of freshwater fish diversity in Gandak River had never been tried through molecular tools. Hence, we confirm the diversity of selected freshwater fishes in the India-Nepal transboundary riverine system.

Acknowledgments

We thank to the Director, Zoological Survey of India, Ministry of Environment, Forest and Climate Change (MoEF & CC) for providing necessary working facilities. We thank to Mr. Rudra Chakraborty for the vouchering and photography of the fish specimens. The first author (S.K) acknowledges the fellowship grant received from Council of Scientific and Industrial Research (CSIR) Senior Research Associateship (Scientists' Pool Scheme) Pool No. 9072-A.

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