FishTaxa (2019) 4(3): 140-144
Journal homepage: www.fishtaxa.com

Further specimens and phylogenetic position of the recently described catfish species *Mystus prabini* (Siluriformes: Bagridae)

Shantanu KUNDU, Vikas KUMAR, Kaomud TYAGI, Kailash CHANDRA
Centre for DNA Taxonomy, Molecular Systematics Division, Zoological Survey of India, M Block, New Alipore, Kolkata-700053, India.
*Corresponding author: *E-mail: vikaszsi77@gmail.com*

Abstract
The study generated six DNA barcode data of the recently described catfish, *Mystus prabini* from different localities in northeast India. Further, we assess the phylogenetic position of *M. prabini* using partial sequence data of the mitochondrial cytochrome c oxidase subunit I (cox1) gene to confirm its genetic distinctiveness. Mitochondrial data strongly suggest a sister relationship of *M. prabini* with *M. bleekeri*, *M. ngasep*, and *M. cineraceus*. According to the genetic intimacy of *M. pabini* with *M. bleekeri*, we presumed the possible gene-flow within Ganges and Brahmaputra River basins.
Keywords: Freshwater Fish, *Mystus*, Ganges, Brahmaputra, Molecular Phylogeny.
Zoobank: urn:lsid:zoobank.org:pub:610B63ED-1FCF-4594-8840-5950EA83F107

Introduction

Catfishes of the genus *Mystus*, belonging to the family Bagridae, occur in fast flowing freshwaters in Asia and Southeast Asia (Ng 2004). This small to medium-size fishes is comprised 46 valid species, among them 21 species are found in India (Eschmeyer 2019). The members of *Mystus* are mainly differentiated from other Bagrids by the osteological features of the metapterygoid and lachrymal (Hollister 1934; Mo 1991). The *Mystus* species possess ornamental value; hence, these fishes are highly exploited from different riverine systems in their range distribution (Dhar and Ghosh 2015). Most of the *Mystus* species are categorized as least concern, whereas two species of *M. bocourti* and *M. malabaricus* are vulnerable and near threatened, respectively (IUCN 2019). Several taxonomic studies have been aimed to determine the species diversity of *Mystus* from India and other countries (Ng and Pethiyagoda 2013; Plamoottil and Abraham 2014). In recent past, a new species namely *M. prabini* has been discovered from Brahmaputra river basin in Arunachal Pradesh, Northeast India (Darshan et al. 2019).

Both morphological and molecular approaches have been aimed to identify and discriminate the species from few congeners. However, the actual diversity and genetic distinctiveness of *M. prabini* is still anonymous as comparison with other species. The partial segment of cytochrome c oxidase subunit 1 (cox1) gene in mitochondrial region has been largely used in the taxonomic assessment of fishes (Hebert et al. 2003; Laskar et al. 2013, 2018a, b). Further, the DNA barcode data of *Mystus* species has been largely generated from different geographical regions, including India. Till date more than 321 DNA sequences of partial cox1 gene are available in GenBank, among them 306 are labeled up to species level and remaining 15 are unclassified. Further, many barcode data are unreliable due to the taxonomic mistake and incorrect sequence handling. In the present study, we aimed to generate the DNA barcode data of the taxonomically identified recently described species, *M. prabini* from different localities in the northeast India. We also acquired the verified barcode data of all available *Mystus* congeners from GenBank and analyzed them through genetic distances and cluster analysis to reevaluate the monophyletic criteria for species-level identification. In addition, the present effort will facilitate to screen the actual diversity of *M. prabini* in the northeast of India.

Accepted: 7 November 2019; Published: 3 December 2019

Table 1. Collection details of the generated and GenBank sequences of *Mystus prabini* in the present study.

GenBank Accession No	Collection localities	Coordinates	References
MN520224	Dibang River, Arunachal Pradesh	28.15N 95.68E	This Study
MN520225	Siang River, Arunachal Pradesh	28.08N 95.34E	This Study
MN520226	Dibru River, Assam	27.58N 95.34E	This Study
MN520227	Dhansiri River, Assam	26.65N 93.71E	This Study
MN520228	Diphlu River, Assam	26.61N 93.43E	This Study
MN520229	Kameng River, Assam	26.72N 92.85E	This Study
KY290074	Diphlu River, Assam	-	Laskar et al. 2018a
MH175199	Sinkin River, Arunachal Pradesh	-	Darshan et al. 2019
MH175200	Sinkin River, Arunachal Pradesh	-	Darshan et al. 2019
MH175201	Sinkin River, Arunachal Pradesh	-	Darshan et al. 2019
MH175202	Sinkin River, Arunachal Pradesh	-	Darshan et al. 2019
MG582180	Sinkin River, Arunachal Pradesh	-	Darshan et al. 2019

Material and Methods

A total of six specimens of *M. prabini* were collected from its type locality and different riverine systems of the Brahmaputra basin in the northeast of India. The collection localities were mentioned in the GenBank and also pointed in the map (Fig. 1). Morphological identification of the collected specimens was confirmed by the described taxonomic characters (Darshan et al. 2019). The voucher specimens were stored in National Zoological Collections of Zoological Survey of India, Kolkata. Total genomic DNA extraction, Polymerase chain reaction (PCR), and purification of the PCR products of each sample were done as per the published laboratory protocol (Sambrook et al.1989; Ward et al. 2005). The Veriti® Thermal Cycler (Applied Bio systems, Foster City, CA) was used for PCR and ABI-3730 DNA analyzer was used for Sanger Sequencing as per standard protocol. Both forward and reverse chromatograms (>600 bp) of the partial mitochondrial cytochrome c oxidase subunit 1 (mtCOI) gene were obtained to build the consensus sequence for each studied sample. The online tool, nucleotide BLAST (https://blast.ncbi.nlm.nih.gov) and NCBI-ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/) were used to confirm the alignment and amino acid array. Total 46 mtCOI sequences of the same and congeners of *Mystus* were acquired from the public database (GenBank) to make a final dataset. The database sequence *of Apristurus ampliceps* (Carcharhiniformes) (Accession No. EU398546) was also acquired from GenBank and used as an out-group. The alignment of the studied dataset was performed by ClustalX (Thompson et al. 1997). The genetic distances and maximum-likelihood (ML) topology with 1000 bootstrap support was estimated by MEGAX (Kumar et al. 2018). The best fit model for this dataset 'HKY+G' was estimated using MEGAX with lowest BIC value=6915.456.

Results and Discussion

The generated DNA barcode sequences of *M. prabini* showed 99-100% similarity with the publicly available sequences of *Mystus* sp. in GenBank. The database sequences of these newly discovered species are nameless and needed to be updated as *M. prabini*. The overall mean K2P genetic distances of the studied dataset (22 species) was 16.2%. The intra-species genetic distance was ranging from 0% to 1.19%, however the inter-species genetic distance was ranging from 6.43% to 29.15% in the present dataset. All the studied species were clearly discriminated by sufficient K2P genetic distances and revealed monophyletic clustering in ML tree (Fig. 1). The northeast Indian species, *M. prabini* is closely clustered with *M. ngasep* and *M. cineraceus* with 11.80% genetic distances. Further, *M. prabini* also showed lowest genetic distance (9.67%) with *M. bleekeri* (Ganges River Basin, India) in comparison with other congeners. Nevertheless, *M. cineraceus* was discovered from

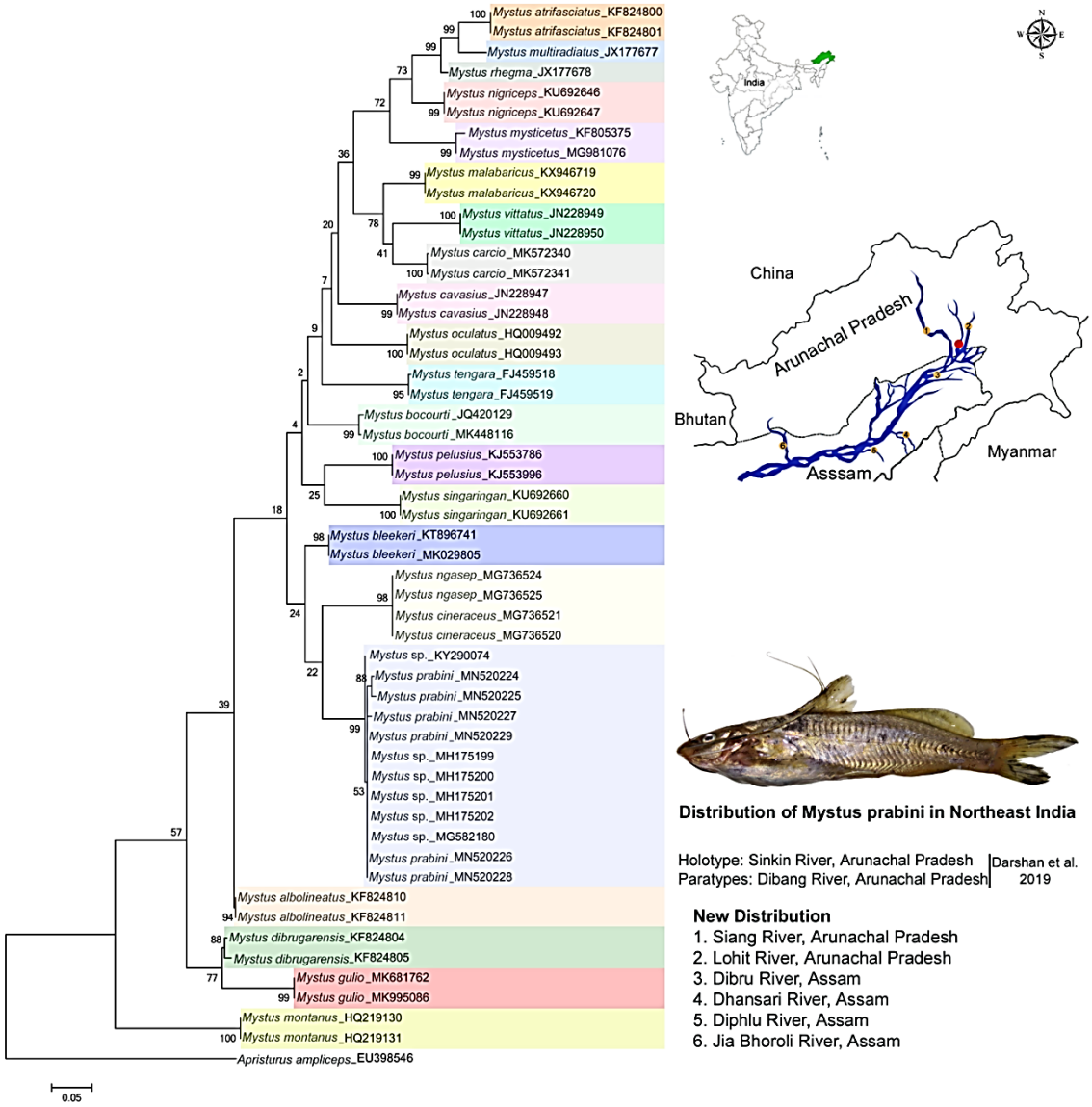

Figure 1. Maximum-Likelihood (ML) topology for *Mystus* species of 642 bp of the mtCOI gene. Numbers preceding species names are GenBank accession numbers The GenBank sequence of *Apristurus ampliceps* (order Carcharhiniformes) was used as an out-group. A lateral image of *M. prabinbi* was merged beside the ML tree. Map with red dot indicate the distribution of holotype as mentioned by Darshan et al. 2019, and orange dots indicate the new range distribution of *M. prabini* in northeast India.

Irrawaddy River drainage of Myanmar (Ng and Kottelat 2009), and *M. ngasep* was discovered from Chindwin drainage from the Manipur state in northeast India (Darshan et al. 2011). Both the valid species showed negligible genetic distances (0%) with their named DNA data available in the GenBank, which need further investigation through molecular approaches with their type or actual topotype specimens.

The DNA based investigation is the most flourishing research in systematics and biodiversity studies (Hajibabaei et al. 2007). However, before aiming the DNA based investigation for addressing any kind of biological questions; the species identification should be reliable (Collins and Cruickshank 2013). The generated DNA sequences with other collateral data in the global database (GenBank and BOLD) provides a trustworthy platform for speedy and authentic species identification (Shen et al. 2013). Further, the taxon sampling from wide geographical regions and their DNA barcodes provides better scenario on their diversity (Bergsten et al. 2012). So far, many small to large scale effort has been made to explore the ichthyofaunal diversity in India including northeastern region (Khedkar et al. 2014; Lakra et al. 2016; Barman et al. 2018; Kundu et al. 2019a,

b, c). This present effort aids species-specific survey of other important fish species to substantiate their actual range distribution and status. In the original description of *M. prabini*, the authors were compared with eight species through molecular approaches (Darshan et al. 2019) and evidenced to be close with *M. bleekeri* (8.6%) and *M. albolineatus* (13.9%). However, the present analysis with 22 species illuminated more clear understanding of this species. Our new record from different riverine systems in Assam state corroborates the further range extension of this species and provides scope to revise the check-list of ichthyofauna locally. Although, the newly discovered species is spotted from the Brahmaputra River Basin, but the genetic intimacy with *M. bleekeri*, distributed in Ganges River Basin, India presumed gene flow within these two river basins.

Acknowledgments

We thank to the Director, Zoological Survey of India, Ministry of Environment, Forest and Climate Change (MoEF & CC) for providing necessary working facilities. 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.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study is funded by the core funding of Zoological Survey of India, Kolkata, Ministry of Environment, Forest and Climate Change (MoEF&CC), New Delhi. The funders had no role in study design, data collection and analysis or preparation of the manuscript.

Literature cited

Barman A.S., Singh M., Singh S.K., Saha H., Singh Y.J., Laishram M., Pandey P.K. 2018. DNA Barcoding of Freshwater Fishes of Indo-Myanmar Biodiversity Hotspot. Scientific Reports 8: 8579.

Bergsten J., Bilton D.T., Fujisawa T., Elliott M., Monaghan M.T., Balke M., Hendrich L., Geijer J., Herrmann J., Foster G.N., Ribera I., Nilsson A.N., Barraclough T.G., Vogler A.P. 2012. The effect of geographical scale of sampling on DNA barcoding. Systematics Biology 61: 851-869.

Collins R.A., Cruickshank R.H. 2013. The seven deadly sins of DNA barcoding. Molecular Ecology Resources 13: 969-975.

Darshan A., Abujam S., Kumar R., Parhi J., Singh Y.S., Vishwanath W., Das D.N., Pandey P.K. 2019. *Mystus prabini*, a new species of catfish (Siluriformes: Bagridae) from Arunachal Pradesh, north-eastern, India. Zootaxa 4648: 511-522.

Darshan A., Vishwanath W., Mahanta P.C., Barat A. 2011. *Mystus ngasep*, a new catfish species (Teleostei: Bagridae) from the headwaters of Chindwin drainage in Manupur, India. Journal of Threatened Taxa 3: 2177-2183.

Dhar B., Ghosh S.K. 2015. Genetic assessment of ornamental fish species from North East India. Gene 555: 382-392.

Eschmeyer W.N., Frickle, R. 2019. Catalog of fishes. California Academy of Sciences. Available from: http://research. calacademy. org/research/ichthyology/catalog/fishcatmain.asp. Accessed 15 October 2019.

Hajibabaei M., Singer G.A., Hebert P.D., Hickey D.A. 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. Trends in Genetics 23: 167-172.

Hebert P.D.N., Cywinska A., Ball S.L., deWaard J.R. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences 270: 313-321.

Hollister G. 1934. Clearing and dying fishes for bone study. Zoologica 12: 89-101.

IUCN. 2019. The IUCN red list of threatened species, Version 2019.2. Accessed on 15 October 2019.

Khedkar G.D., Jamdade R., Naik S., David L., Haymer D. 2014. DNA barcodes for the Narmada, one of India's longest rivers. PLoS One 9: e101460.

Kumar S., Stecher G., Li M., Knyaz C., Tamura K. 2018. MEGAX: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35: 1547-1549.

Kundu S., Chandra K., Tyagi K., Pakrashi A., Kumar V. 2019a. DNA barcoding of freshwater fishes from Brahmaputra River in Eastern Himalaya biodiversity hotspot. Mitochondrial DNA Part B: Resources 4: 2411-2419.

Kundu S., Rath S., Laishram K., Pakrashi A., Das U., Tyagi K., Kumar V., Chandra K. 2019b. DNA barcoding identified selected ornamental fishes in Murti river of East India. Mitochondrial DNA Part B: Resources 4: 594-598.

Kundu S., Tyagi K., Pakrashi A., Kumar B., Laishram K., Rath S., Das U., Chandra K. 2019c. DNA barcoding of freshwater fishes from the transboundary river of Indo-Bhutan: multiple clades and cryptic diversity. Mitochondrial DNA Part B: Resources 4: 2527-2532.

Lakra W.S., Singh M., Goswami M., Gopalakrishnan A., Lal K.K., Mohindra V., Sarkar U.K., Punia P.P., Singh K.V., Bhatt J.P., Ayyappan S. 2016. DNA barcoding Indian freshwater fishes. Mitochondrial DNA. Part A, DNA Mapping, Sequencing, and Analysis 27: 4510-4517.

Laskar B.A., Bhattacharjee M.J., Dhar B., Mahadani P., Kundu S., et al. 2013. The species dilemma of Northeast Indian Mahseer (Actinopterygii: Cyprinidae): DNA barcoding in clarifying the riddle. PLoS One 8: e53704.

Laskar B.A., Kumar V., Kundu S., Tyagi K., Chandra K. 2018a. Taxonomic quest: validating two mahseer fishes (Actinopterygii: Cyprinidae) through molecular and morphological data from biodiversity hotspots in India. Hydrobiologia 815: 113-124.

Laskar B.A., Kumar V., Kundu S., Darshan A., Tyagi K., Chandra K. 2018b. DNA barcoding of fishes from River Diphlu within Kaziranga National Park in northeast India. Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis 30: 126-134.

Mo T.P. 1991. Anatomy, relationships and systematics of the Bagridae (Teleostei: Siluroidei) with a hypothesis of siluroid phylogeny. Theses Zoologicae 17: 1-216.

Ng H.H., Kottelat M. 2009. A new species of *Mystus* from Myanmar (Siluriformes: Bagridae). Copeia 2009: 245-250.

Ng H.H., Pethiyagoda, R. 2013. *Mystus zeylanicus*, a new species of bagrid catfish from Sri Lanka (Teleostei: Bagridae). Ichthyological Exploration of Freshwaters 24: 161-170.

Ng, H.H. 2004. New Estuarine Species of *Mystus* (Teleostei: Bagridae) from the Malay Peninsula. Copeia 4: 883-887.

Plamoottil M., Abraham N.P. 2014. *Mystus keralai* (Siluriformes: Bagridae), a new fish species from Kerala, India. International Journal of Pure and Applied Zoology 2: 231-240.

Sambrook J., Fritsch E.F., Maniatis, T. 1989. Molecular cloning: A Laboratory Manual. 2nd Edn., Cold Spring Harbour Laboratory Press, New York.

Shen Y.Y., Chen X., Murphy R.W. 2013. Assessing DNA barcoding as a tool for species identification and data quality control. PLoS One. 8: e57125.

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F., Higgins D.G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876-4882.

Ward R.D., Zemlak T.S., Innes B.H., Last P.R., Hebert P.D.N. 2005. DNA barcoding of Australia's fish species. Philosophical Transactions of the Royal Society B: Biological Sciences 360: 1847-1857.