



Exploring *Barilius bendelisis*: Unveiling as Potential Member of SIS Family

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Abstract: Aquaculture and Fisheries encompass sectors that not only fulfill basic sustenance needs but also contribute significantly to enhancing the overall quality of life for humanity and generating foreign exchange. In the Eastern Himalayan region of Cooch Behar in West Bengal, India, renowned for its rich freshwater fish diversity, the *Barilius bendelisis* (Hamilton 1807) species holds high value both as an ornamental fish, also source of food and considered as SIS (small indigenous species). The classification of *Barilius bendelisis* (Hamilton 1807) as endangered and vulnerable in the Cooch Behar region is predominantly attributed to deteriorating water quality resulting from human activities, leading to a substantial decline in biodiversity. The physicochemical properties of the water significantly affect the biology and physiology of these fish. *Barilius bendelisis*, a small native fish species, is a popular source of sustenance in the Terai region of the Eastern Himalayas. The proximate analysis of *Barilius bendelisis* also established that it is a good source of protein needs. The Barcode ID number assigned to the studied *Barilius bendelisis* species is BOLD: AAF3096. The study offers foundational information about the biodiversity in the transboundary rivers Torsa, Raidak-1, and Mansai (Jaldhaka), which could prove invaluable for the conservation and management of *Barilius bendelisis*.

Keywords: Aquaculture, Terai region of Eastern Himalaya, *Barilius bendelisis*, Proximate analysis, DNA barcoding, SIS (Small Indigenous Species)

India boasts a rich diversity of freshwater fish, encompassing 868 distinct species. Among these, 192 are considered endemic, while 327 species face threats according to the IUCN. The Genus *Barilius*, often referred to as Bariline fishes, typically resides in swiftly flowing mountain streams across Asia (De et al 2021). *Barilius* species are recognized by their flattened bodies, along with blue-black markings on their bodies and dorsal fins placed behind the midsection of their bodies (Hamilton 1822). *Barilius bendelisis* can be grouped as 'SIS' (small indigenous species). SIS can develop most extreme to a length of 25-30 cm in the development or grown-up phase of their lifecycle (De et al 2021a). Globally, there have been reports of 36 fish species of *Barilius* genus, with 24 of them found in India (Qin et al 2019). According to the Conservation Assessment and Management Plan report for India's freshwater fishes, the conservation status of *Barilius* species is labeled as 'lower risk near threatened' (LRnt) (Molur and Walker 1988).

One such species, *Barilius bendelisis*, locally holds significance both economically and ecologically due to its ornamental value and potential as a food fish (Mishra et al 2012). However, despite its importance, *Barilius bendelisis* is experiencing a rapid decline in regions like the transboundary rivers Torsa, Raidak-1, and Mansai (Jaldhaka) in West Bengal, India, primarily due to overfishing, habitat degradation, alterations in hydrology and

water pollution (Saha et al 2022a). In recent years, *Barilius bendelisis* has become increasingly scarce in these sub-Himalayan transboundary rivers (Saha et al 2022). Furthermore, the identification of *Barilius* species, including *Barilius bendelisis*, has been a significant challenge due to the species' high phenotypic variability, sexual dimorphism, and a lack of adequate identification keys for their immature stages (Mishra et al 2012). The transboundary rivers Torsa, Raidak-1, and Mansai (Jaldhaka) originate from the Sikkim, Darjeeling, Bhutan, and Tibetan Himalayas, ultimately entering Bangladesh through West Bengal, India (Fig. 1).

Over the past fifteen years, molecular tools, particularly DNA barcoding, have proven highly effective in cataloging fish diversity worldwide, including within India (Saha et al 2021). Beyond species identification, DNA barcoding has emerged as a valuable tool for resolving complex taxonomic issues in fish, such as species groupings and hidden diversities (Laskar et al 2013, Laskar et al 2018). However, the majority of integrated studies in this realm have been limited to characterizing the ichthyofaunal diversity in various riverine ecosystems in India. The current study seeks to bridge this research gap, particularly concerning *Barilius* species, with a special focus on *Barilius bendelisis*. This research aims to shed light on the phylogenetic relationships and genetic differences within these species, utilizing both newly generated and existing COI sequences from India.

MATERIAL AND METHODS

Barilius bendelisis specimens were obtained from three different locations along the Torsa river, Raidak-1, and Mansai (Jaldhaka) in West Bengal (Fig. 1.1, 1.2, 1.3). In the GIS layouts presented in Figures 1.1, 1.2 and 1.3, ArcGIS software has been used.

These specimens were captured using a cast net and authenticated based on established taxonomic references (Jayaram 2010). Following both morphological and molecular examinations, the specimens were preserved in a solution of 10% formalin. The voucher specimens have been stored in the laboratory at NBFGR, India. Aseptically collected muscle tissue from the specimen was stored at NBFGR, India, for future reference. Genomic DNA was extracted and PCR was conducted with published primer pairs: COIF1 and COIR1 (Saha et al 2021) using the Thermal Cycler. The PCR products underwent purification and sequencing according to the protocol (Laskar et al 2018). Bi-directional chromatograms were scrutinized through MEGA6 (Tamura et al 2013) to remove any noisy segments and generate consensus sequences. Online tools, such as BLAST and ORF finder, were employed to identify insertions/deletions (indels) and stop codons. The sequences were further cross-referenced with NCBI and BOLD databases. The final dataset comprised 66 COI sequences of *Barilius*, which included the newly generated sequences by us for *Barilius bendelisis* (NCBI accession no. MN810961, MN810962, MN810963, MN810964, MN810965, MN994439, MN994440, MN994441, MN994442). *Tor* (NCBI accession no. JX983505) served as

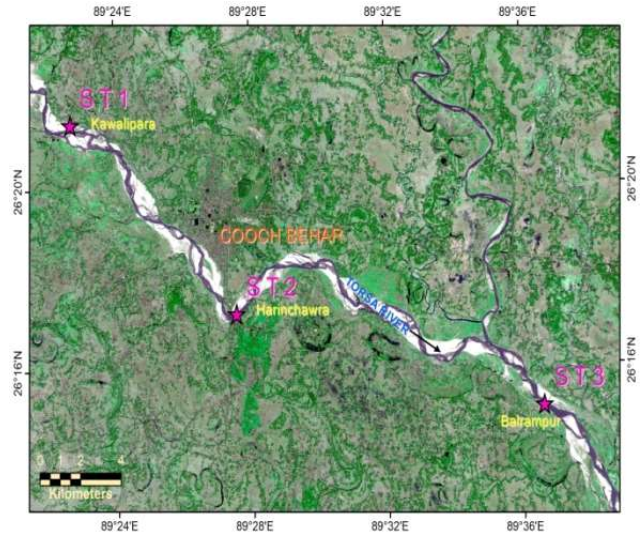


Fig. 1.1. GIS layouts of sampling spots at Torsa River



Fig. 1.2. GIS layouts of sampling spots at Raidak-1 river

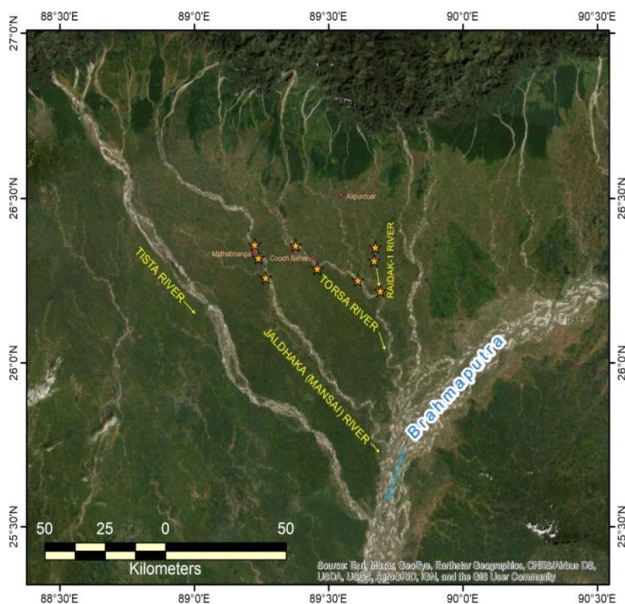


Fig. 1. Torsa, Raidak-1, and Mansai (Jaldhaka) River



Fig. 1.3. The GIS layouts of sampling spots at Mansai (Jaldhaka) river

the out-group in our study. Alignment of the dataset was accomplished using ClustalX software, and genetic divergence was calculated in MEGA6 utilizing the Kimura-2-Parameter (K2P) model. To assess species delimitation following the reciprocal monophyletic criterion, Bayesian analysis (BA) was conducted. The best-fit model, GTR+I+G, was determined using Partition Finder version 1.1.1 with the lowest Bayesian Information Criterion score. MrBayes 3.1 (Ronquist and Huelsenbeck 2003) was employed for BA, with Markov Chain Monte Carlo (MCMC) runs extending to 10,000,000 generations, with tree sampling every 100 generations (the first 1000 trees were discarded as 'burn in'). Convergence of the MCMC analysis was confirmed when the standard deviation of split frequencies fell below 0.01, and the potential scale reduction factor (PSRF) approached 1.0. The resulting BA tree was visualized using the web-based tool Interactive Tree Of Life (iTOL) (Letunic and Bork 2007).

RESULTS AND DISCUSSION

The worldwide practice of combining classical taxonomy and DNA barcoding has been widely embraced to shed light on various aspects of species diversity, including cryptic diversity, species complexes, and the pathways of invasion by invasive species (Tyagi et al 2017, Saha et al 2021). In present study, generated COI sequences of *Barilius bandelisis* collected from the Torsa, Raidak-1, and Mansai (Jaldhaka) Rivers in West Bengal, India. The final dataset consisted of approximately 648 base pairs, encompassing 67 nucleotide sequences representing 10 different *Barilius* species. *Tor* (NCBI accession no. JX983505) was used as an out group species. The Bayesian analysis (BA) phylogeny demonstrated that our generated sequences formed coherent clusters alongside the sequences from the reference database. Within the phylogenetic tree, 11 distinct lineages were discerned among the 10 morphospecies, including multiple clades within *Barilius bandelisis*. The overall mean genetic divergence in our dataset was estimated to be 6.1%, the highest mean intraspecific genetic divergence (17.1%) was observed within *Barilius bandelisis*. The two clades of *Barilius bandelisis* showed an average genetic divergence of 2.3% in our dataset. The Barcode ID for the *Barilius bandelisis* specimens was recorded as BOLD: AAF3096. This study underscored the effectiveness of DNA barcoding in distinguishing *Barilius* species at the individual level and contributed to our comprehension of *Barilius bandelisis* across various river segments in the northern region of West Bengal, particularly focusing on the Cooch Behar district of West Bengal, India (Saha et al 2021).

CONCLUSION

The identification of several distinct groups within the *Barilius bandelisis* species, each exhibiting considerable genetic divergence, suggests the presence of concealed variations and the potential emergence of separate species of *Barilius bandelisis* in different regions of eastern, north-eastern, central, and northern India. Nevertheless, to substantiate this hidden diversity, further taxonomic examinations employing multiple molecular markers are imperative. This research serves as an initial endeavor to gauge the genetic disparities within the said genus in India. The study will help to supplement the global database with barcode sequences of accurately identified *Barilius bandelisis*. Climate change, pollution, and habitat degradation have all had a significant impact on freshwater biodiversity, posing an extinction risk.

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