Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 6(2025)

Fish Biodiversity of the Barandu River in Buner District: Morphological and Molecular Analysis

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Article Details

Keywords: Fish Fauna, Morphological Identification, Molecular Characterisation, COI Gene, River Barandu.

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ABSTRACT

Background: Aquatic fauna makes a prominent contribution to the ecosystem, as it is friendly towards the terrestrial mode of life in multiple ways. Out of the huge aquatic fauna, fish are immense and peculiar, as they have both nutritional and economic value for terrestrial inhabitants. Methods: The current study focuses on morphological identification and molecular analyses. During the study period, ranging from January to December 2023, a total of 193 species were collected from the River Barandu. Results: The current study identified 12 species belonging to 4 orders, 5 families, and 12 genera in the river Barandu. The family Cyprinidae, being the most common, shows 9 species. Family Nemacheilidae, Mastacembelidae, Cichlidae, and Channidae were represented by a single species each. Three species, i.e. Salmostoma baccila, Paraschistura abdoii, and Tariqilabeo latius, were further identified via genetic profiling. With the effective use of the COI gene as a genetic marker and phylogenetic analysis, the monophyly of most taxa and distinct clades was confirmed. Conclusion: Our current study emphasised the importance of conservation and measures to preserve the fauna of the research site, which is occasionally threatened by several factors, excessive fishing, habitat destruction and climatic uplift. The study boosts our understanding and equips us with crucial knowledge for upcoming breakthroughs on the ecological significance and evolutionary history of fish fauna in the River Barandu.

INTRODUCTION

Fish are cold-blooded aquatic vertebrate animals without limbs. They have fins for swimming and gills for breathing for adaptation to aquatic life. Humans have been interested in breeding and researching fish since ancient times, tracing back to the civilisations of ancient China, Egypt, and Greece (Betancur et al., 2013). Ichthyo-diversity describes the variety of fish in a particular environment. Ichthyologists categorise fish into three main groups such as Chondrichthyes, Agnatha, and Osteichthyes. Most existing fish species belong to the bony fish, while jawed and jawless fish are regarded as more ancient and primitive (Jelks et al., 2011).

Fish comprise around half of the overall 54,711 living vertebrates. Of these, the total fish population of 27,000 (95%) is bony fish, 970 (4%) are cartilaginous fish, and 107 (1%) are agnathans (Nair and Gopalakrishnan 2014). The freshwater bodies of Pakistan comprise 193 fish species, which belong to the class Actinopterygii, specifically subclass Teleostei. They are divided into 3 cohorts, 6 super-orders, 13 orders, and 30 families (Rafique and Khan, 2012). Butt reports 94 fish species from Khyber Pakhtunkhwa (Butt, 1986).

Several researchers have studied the variety of fish fauna in the district of Buner. (Saeed et al., 2013), Examined the fish fauna of the river Barandu and identified 11 fish species, belonging to 3 orders and 4 families. (Akhtar et al., 2014), Report 10 species, and a subsequent study was conducted by Akhtar et al. (2015), reporting the same number of species (Akhtar et al., 2015),

In Ichthyo-diversity research, accurate identification of fish is essential, as it underpins effective assessment of species diversity (Okumuş and Çiftci, 2003). The identification of the specific feature is crucial for the development of feasible management and conservation strategies for the species (Dudgeon et al., 2006). Outline the morphological traits that have assisted as a forerunner for the taxonomical and evolutionary process. Two criteria, such as morphometric and meristic measures, can be used to identify fish species (Kim et al., 2010). Species identification and classification also rely on a wide range of morphological, physiological, behavioural, and biochemical parameters. According to Strauss and Bond (1900), morphological criteria included body form, colouration, fin count and location, scale size and quantity, as well as several relative features of the body (Strauss and Bond, 1900)

Identification based solely on traditional morphological features and the use of identification keys can sometimes lead to errors, as the methods may produce ambiguous results, especially at the species level. It is relatively rare, but some species may exhibit a mix of characteristic states, further complicating the accurate identification (Teletchea et al., 2009).

To overcome the mentioned impediment, genetic markers have been introduced to assess the process of identification and to generate the phylogenetic tree depicting the relationship among the species. In contrast, DNA-based identification has a significant influence on identification as compared to the traditional method (Wong, 2011). Mitochondrial DNA (mtDNA), restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), single- nucleotide polymorphism (SNP), and microsatellite are the prominent genetic markers used in the aqua-culture community (Hemmer-Hansen et al., 2007).

Genetic markers have revolutionised the field of genetics, particularly animal genetics. The use of DNA-based markers has pointedly impacted the identification process in several ways, including aiding in species identification and constructing subsequent phylogenetic trees. The genetic markers are a useful tool for tracing the distinctive features of a species (Divu et al., 2008). Mitochondrial genes have unique features that make them a cautious marker for identifying aquatic fauna compared to nuclear genes (Kochzius et al., 2010).

The mtDNA is the most predominant molecular marker, affected by point mutation substitution. It is relatively small (16500 bp in fish), has the highest nucleotide exchange rate at synonymous sites, and no recombination (Parhi et al., 2019). The successful implementation of using the CO1 gene to identify fish species was primarily responsible for the establishment of FISHBOL (International Fish Barcoding Program) (Ward et al., 2009). The Barcode of Life Data Systems (BOLD) currently comprises the barcodes of 23,000 fish species, representing a substantial portion of the 30,000 fish species worldwide. It also offers comprehensive information on CO1 sequenced species, encompassing the provenance and present whereabouts of voucher specimens.

The dwindling number of taxonomists and the challenges associated with morphological identification underscore the need for more rapid taxon delimitation. Molecular approaches are generally less time-consuming, require short DNA segments as a data source, in contrast to reliance on detailed morphological or ecological data, and provide a quick and standard way to identify a wide range of taxa from diverse habitats (Wong, 2011). In terms of both time and information, the molecular identification surpasses the traditional identification (DeSalle and Amato, 2009). Still, to achieve greater resolution in distinguishing between species across various animal phyla, the integration of molecular and traditional phenotypic approaches is essential. In addition to molecular analysis, it is essential to include other sources of information, like physical, behavioural, ecological, and geographical variation (Ward et al., 2009).

MATERIALS AND METHODS

STUDY AREA

The current study assesses the morphological and molecular characterisation of the fish fauna of the river Barandu, located in the Buner district. The river Barandu is the main River in the area and merges with the river Indus in Kala Dhaka. Buner lies between 34-09 and 34-43°N latitude and 72-10 and 72-47°E longitude.

District Buner is surrounded by Swat district to the north, Malakand Agency to the south, Shangla district to the east, and Hazara division to the southeast, along with the Indus River forming its southeastern boundary.

COLLECTION SITE

The collection site is divided into Daggar, Sunigram, Takhtaband, Matwani, and Budal. From such points, as shown in Figure 1, fish were captured once a month throughout the study period from January 2023 to December 2023. The cast nets, drag nets, and hand-held seine or dip nets were used for capturing the fish. After prosper labelling, the fish were treated with 70% alcohol. For morphological and molecular identification, the fish were stored in 99.9% ethanol.

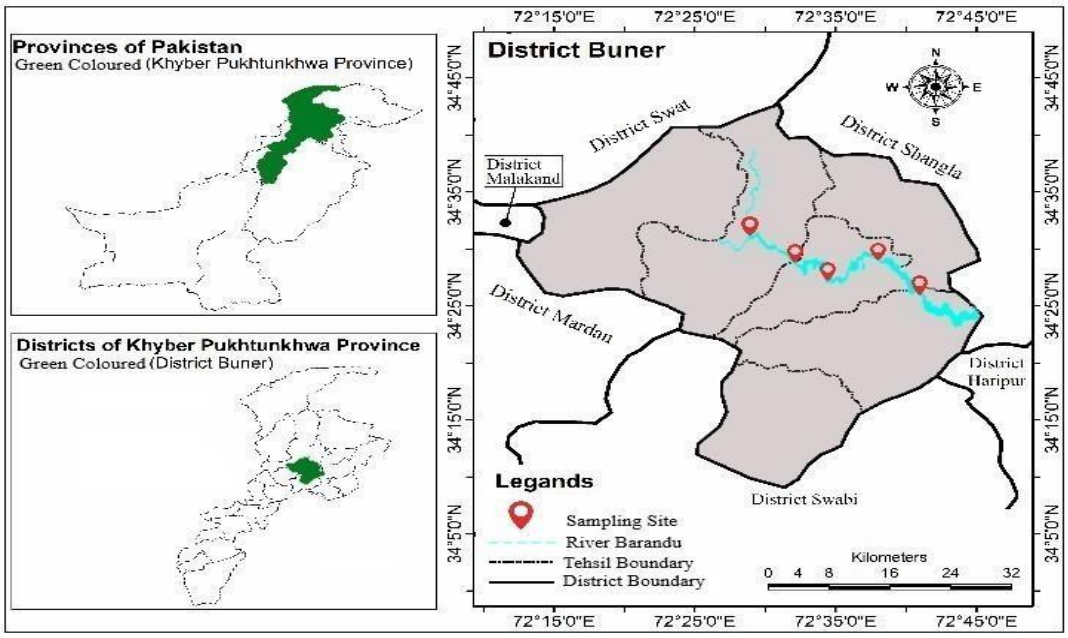


FIGURE 1. SAMPLING SITE OF THE STUDY AREA RIVER BARANDU OF THE BUNER DISTRICT, KHYBER PAKHTUNKHWA

FISH IDENTIFICATION

The fish were identified up to the species level with the help of standard identification keys like Fishes of Punjab, Pakistan (Mirza and Sandhu 2007), and Inland Fishes of India and Adjacent countries (Talwar and Jhingran, 1991).

MOLECULAR CHARACTERISATION OF FISHES

For molecular characterisation, a single specimen from each morphologically identified species was used. A moderate-sized muscle sample has been cut and collected for DNA extraction. Gently homogenised with 200 µl of 20% Chelex solution and 20 µl of proteinase K and incubated at 37°C for 40-60 minutes. The digested samples were centrifuged at 15,000 rpm for 15 minutes, and the supernatant containing DNA was collected, diluted with TE buffer, and stored at -20 °C.

ELECTROPHORESIS FOR DNA VISUALISATION

To visualise the extracted DNA, 0.3 g of agarose was mixed with 30 ml of 1X TBE buffer, maintaining a pH of 7.2. After cooling, 3 µl of ethidium bromide was added and gently mixed. The gel was prepared and exposed to electrophoresis at 125 volts to visualise using a gel documentation system.

PCR AMPLIFICATION OF THE CO1 GENE

The mitochondrial CO1 gene was targeted for amplification using universal primers CO1 F1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and CO1 R1 (5'-AGACTTCTGGGTGGCCAAAGAATCA-3') (Ward *et al.*, 2005). The 20 µl PCR mixture consists of 10 µl of DreamTaq Green Master Mix (Thermo Scientific), 1 µl of each primer, 1 µl of DNA template, and 7 µl of PCR-grade water. The initial denaturation at 95 °C for 3 minutes, followed by 35 cycles at 94 °C for 50 seconds, annealing at 58.6°C for 50 seconds, extension at 72 °C for 50 seconds, and 72 °C for 5 minutes for final extension.

VISUALISATION OF PCR PRODUCTS

PCR products were pictured on a 2% agarose gel along with 2 µl of DNA marker (Thermo Scientific Gene Ruler). Electrophoresis was performed at 125 volts for 40 minutes, and finally, bands were visualised with a gel documentation system.

DNA SEQUENCING AND PHYLOGENETIC ANALYSIS

For bidirectional sequencing, the final PCR products were purified and sent to Macrogen Inc. (Seoul, South Korea). The generated sequence was subject to trimming and eliminating contaminating nucleotides and primer sequences using BioEdit version 7.2. The trimmed sequence was analysed using BLAST against the NCBI GenBank database (Sayers *et al.*, 2012) for

species-level identification. Phylogenetic trees were constructed in MEGA X using the Maximum likelihood method with 1000 bootstrap replicates, and inter- and intra-species distances were calculated using the Kimura 2-parameter model (Kumar *et al*., 2018).

RESULTS

A total of 12 species were identified, which belong to 4 orders, 5 families, and 12 genera, as shown in Table 1.

TABLE 1: A COMPLETE LIST OF THE SCIENTIFIC NAMES OF ICHTHYO-DIVERSITY OF THE RIVER BARANDU IN THE DISTRICT OF BUNER, KHYBER PAKHTUNKHWA

S/No	Class	Order	Family	Genus and Species
1	Actinopterygii	Cypriniformes	Cyprinidae	*Barillius pakistanicus*
2				*Garra Gutyla*
3				*Puntius sophore*
4				*Tariqilabeo latius*
5				*Shoziouthorax plagiostomus*
6				*Salmostoma Bacaila*
7				*To puititora*
8				*Cyprinus carpio*
9			Nemacheilidae	*Paraschistura abdolii*
10		Synbranchiformes	Mastacembelidae	*Mastacembelus armatus*
11		Perciformes	Cichlidae	*Oreochromis niloticus*
12		Anabantiformes	Channidae	*Channa gachua*

Table 2 shows the morphometric measurements of various parts of the identified fish, including total length, forked length, standard length, and head length. The largest fish collected is *Mastacembelus armatus*, whose length is 42 cm, while the smallest fish is *Puntius sophore*, whose length is 8 cm.

TABLE 2. MORPHOMETRIC MEASUREMENT (CM) OF THE FISH FAUNA OF THE RIVER BARANDU AT THE DISTRICT OF BUNER, KHYBER PAKHTUNKHWA

S/No	Fish Species	Total Length	Forked Length	Standard Length	Head Length
1	*Barillius*	13	11	12	2

	pakistanicus				
2	*Garra Gutyla*	11.7	10	10.5	2.3
3	*Puntius sophore*	8	6.5	7.5	1.3
4	*Tariqilabeo latius*	11	9.5	10.5	2
5	*Shoziouthorax plagiostomus*	18.7	16	17.3	3
6	*Salmostoma Bacaila*	10.5	9	9.6	2.2
7	*To putitora*	12.9	10.5	11.8	2.9
8	*Cyprinus carpio*	28	24	25	5
9	*Paraschistura abdolii*	11.3	9.5	10.5	1.5
10	*Mastacembelus armatus*	42	39.5	41	3.6
11	*Oreochromis niloticus*	10.5	9	9.6	3
12	*Channa gachua*	17	14.5	16	3

In the present study, DNA was extracted from the muscle tissue of the selected fish. Muscle samples were used to extract DNA, and the samples were successfully visualised on agarose gel electrophoresis, as shown in Figure 2. Clear, distinct bands were observed under UV light, indicating good quality and integrity of the DNA.

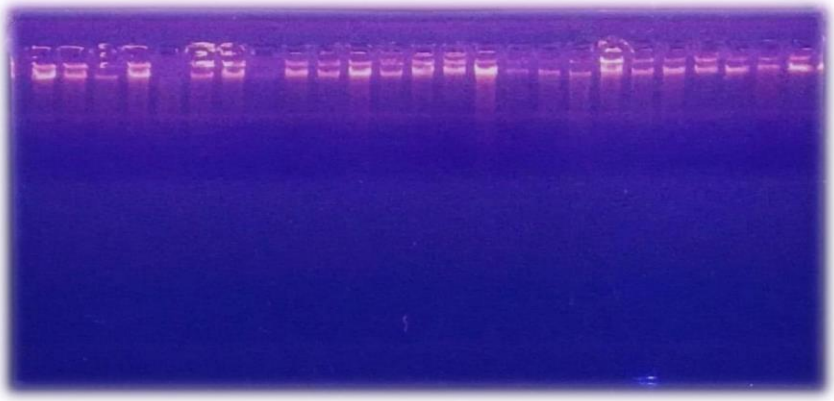


FIGURE 2. EXTRACTED DNA GEL PICTURE (IN 0.1% AGAROSE)

Using a universal primer, the cytochrome c oxidase 1 gene was effectively amplified for selected fish species. Afterwards, the amplified products were stained with ethidium bromide and separated on a 1% agarose gel, as shown in Figure 3(A), 3(B), and 3(C).

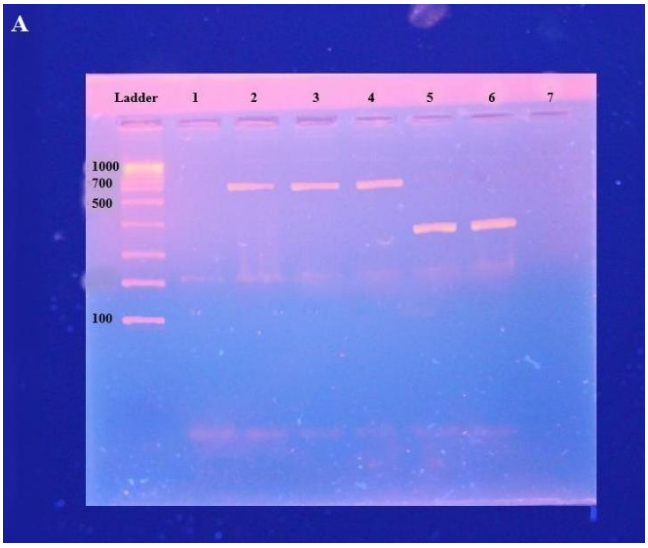


FIGURE 3 (A). BANDS IN THE 2ND, 3RD, AND 4TH (656BP) WELL REPRESENT THE *PARASILURUS ASOTUS*

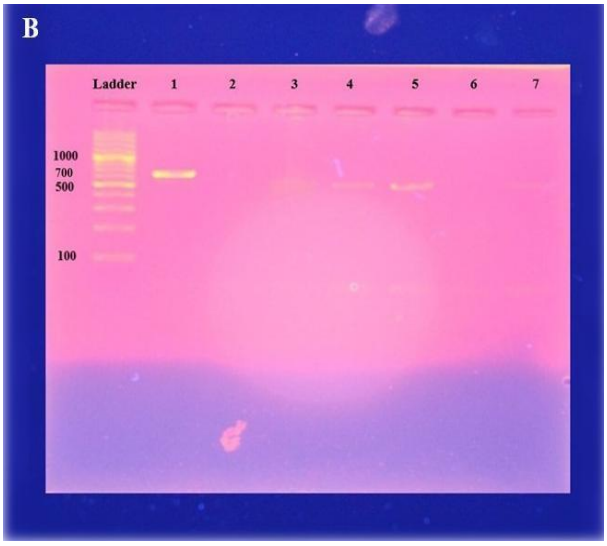


FIGURE 3 (B). BANDS IN WELL 1 REPRESENT THE COI GENE (641 BP) OF *SALMONETLA BACILLUS*

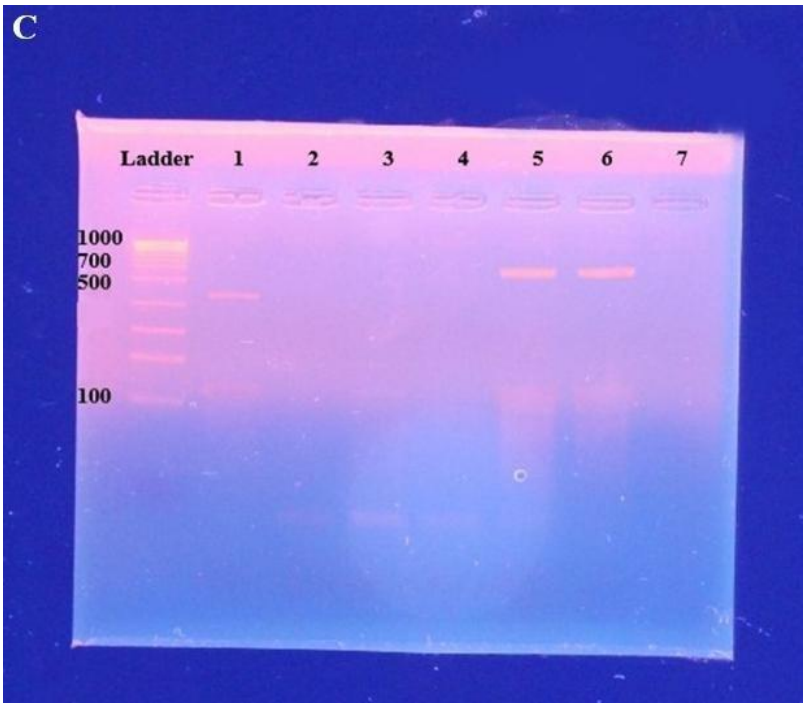


FIGURE 3 (C). BANDS THE 5TH AND 6TH (667 BP) REPRESENT THE *TARIQILABEO LATIUS*

Subsequently, the amplified products were purified according to the manufacturer's instructions for sequencing. To ensure sequencing accuracy, both forward and reverse sequencing were carried out. The sequencing was carried out by a commercial provider, Macrogen, Inc. (based in Seoul, South Korea), to ensure accurate identification.

Fish species selected for sequencing were *Salmostoma baccila*, *Paraschistura abdoii*, and *Tariqilabeo latius*. A primary consideration was the frequent morphological misidentification associated with these taxa, which led to taxonomic ambiguities in our research. Additionally, some of these fish species were recorded for the first time in the study area, and no prior documentation regarding their morphological characteristics and molecular profiles was available. These three species were chosen to contribute to a more accurate taxonomic assessment and establish a baseline morphological and macular data for future research. All selected samples were sequenced. The quality of each sequence was assessed using FinchTV version 4.0, and consensus sequences for each sample were generated using BioEdit version 7.2.

Afterwards, the resulting consensus sequences were aligned and compared with the CO1 sequence available in the GenBank database using sequence similarity analysis. Species identification was performed based on the highest percentage of pairwise identity. According to

the NCBI database, the consensus sequences *for Salmostoma baccaila, Paraschistura abdoii,* and *Tarigilabeo latius* showed clear alignment within the 97–100% similarity range. Each species yielded an E-value of 0.0 in GenBank, indicating highly significant matches.

The COI gene sequence obtained from *Paraschistura abdoii* showed 97% similarity to reference sequences available in the NCBI database. Phylogenetic analysis confirmed its grouping with previously reported *Paraschistura abdoii* specimens from Pakistan and India. Similarly, the COI sequences of *Salmostoma bacaila* exhibited 100% identity with previously reported sequences from the same geographic regions and clustered with them in the phylogenetic tree. For *Tariqilabeo latius*, COI sequences demonstrated 99% identity and also clustered with conspecific sequences from Pakistan and India, as shown in Figure 4.

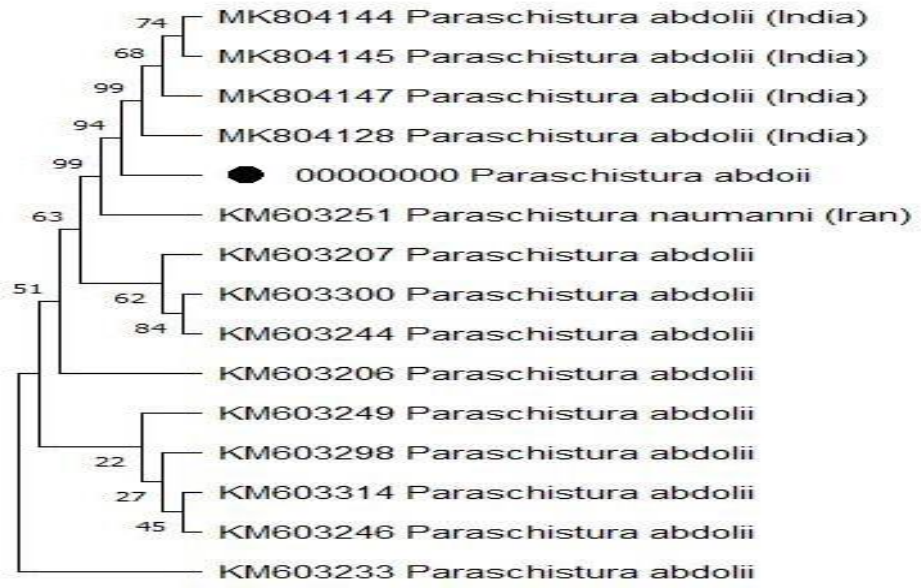


FIGURE 4 (A). The N-J phylogenetic tree. The COI sequence for *Paraschistura abdoii* spp. Each node on the tree is annotated with bootstrap values to indicate the reliability of the branching, and the sequence generated in the current study is marked with a black circle for clear identification.



FIGURE 4 (B). Neighbour-Joining tree for *Salmostoma bacaila* spp., illustrating the evolutionary relationships between the species and relevant outgroups. Bootstrap values are indicated at each node to reflect the robustness of the groupings. The sequence obtained in the present study is marked with a black circle for identification.



FIGURE 4 (C). N-J phylogenetic tree (COI gene sequences for *Tariqilabeo latius* spp, showing the connection among species and outgroups. The bootstrap values are represented by nodes, and the solid circle signifies the sequence from the present study.

DISCUSSION

The current study was conducted in the river Barandu and identified 12 species belonging to 4 orders, 5 families, and 12 genera. The family Cyprinidae shows 9 species, i.e., *Garra Gutyla*,

Puntius sophore, Tariqilabeo latius, Shoziouthorax plagiostomus, Salmostoma Bacaila, To putitora, and *Cyprinus carpio*. Family Nemacheilidae, Mastacembelidae, Cichlidae, and Channidae were represented by a single species, i.e., *Paraschistura abdolii, Mastacembelus armatus, Oreochromis niloticus, and Channa gachua*, respectively.

Another study was conducted by Akhtar *et al* in 2015 on the river Barandu, district of Buner, to identify the fish fauna, which reported 10 species (Akhtar *et al*., 2015). Similarly, Saeed *et al* in 2013 also identified 11 species that belong to 3 orders and 4 families. In comparison to the previous literature, *Triplophysa naziri* and *Glyptothorax punjabensis* were not found in the current investigation, and *Salmostoma bacaila* and *Oreochromis nilotius* are novel to the site and reported for the first time (Saeed *et al.*, in 2013)

Molecular taxonomy, a DNA-based identification methodology, is widely applicable and serves as a straightforward and efficient tool for species identification. This approach relies on variations in a particular region of the gene, specifically a segment of the mitochondrial DNA cytochrome c oxidase subunit I (COI) gene (Ahmed *et al.*, 2022).

To our knowledge, DNA barcoding has not been employed before in the Buner district in conjunction with the traditional morphology-based approach to identify fish at the molecular level. However, in the present study, fish species were identified via a combination of both morphological features and genomic DNA analysis.

In the present study, three distinct fish species were identified from the Barandu River in the Buner district of Khyber Pakhtunkhwa, Pakistan (*Salmostoma bacaila, Paraschistura abdoii,* and *Tariqilabeo latius*) using DNA barcoding analysis. Malkani *et al* (2022) also employed a DNA barcoding approach in their work, focusing on the CO1 gene in 6 different species (*Clupisoma naziri, Schizothorax skarduensis, Naziritor zhobensis, Nemacheilus naziri, Barilius vagra pakistanicus,* and *Nemacheilus griffithi hazarensis)*. In that study, the 655-base-pair COI barcode sequence was used to examine genetic variation among the species. Similarly, in the present study, the COI barcode sequences of the three selected species were analysed and found to align with the standard barcode length, confirming their suitability for species identification (Malkani *et al*., 2022),

This study represents the first comprehensive analysis of inter- and intra-species genetic variation among fish in the Barandu River, integrating both morphological and molecular data. These results are expected to contribute significantly to the accurate identification of local fish

species and support efforts toward their conservation and sustainable management in the region.

CONCLUSION

The research successfully integrated morphological and molecular methods to detect and describe fish species in the River Barandu. The results provide significant understanding of species diversity, abundance, and genetic relationships, delivering crucial data for upcoming conservation and ecological diversity management strategies in the area.

ETHICAL STATEMENT: This study is approved by the ASRB Committee of Abdul Wali Khan University, Mardan, Pakistan, No. Dir/A&R/AWKUM/2023/0014 dated July 2023.

REFERENCES

1. Betancur RR, RE Broughton, EO Wiley, K Carpenter, JA López, C Li, NI Holcroft, D Arcila, M Sanciangco, IJC Cureton, F Zhang, T Buser, MA Campbell, JA Ballesteros, A RoaVaron, S Willis, WC Borden, T Rowley, PC Reneau, DJ Hough, G Lu, T Grande, G Arratia and G Ortí, 2013. The tree of life and a new classification of bony fishes. PLOS currents The Tree of Life, 5: 1-45.
2. Walsh, S.J., Jelks, H.L. and Burkhead, N.M., 2011. The decline of North American freshwater fishes. Am Curr, 36(4), pp.10-17.
3. Nair, R.J. and Gopalakrishnan, A., 2014. A review on the fisheries, taxonomy, and status of the flatfishes in tropical waters. Reviews in Fisheries Science & Aquaculture, 22(3), pp.175- 183.
4. Rafique, M. and Khan, N.U.H., 2012. Distribution and status of significant freshwater fishes of Pakistan. Rec. Zool. Surv. Pakistan, 21, pp.90-95.
5. Butt, J.A. (1986). Fish and Fisheries of KPK, Pakistan. Biologia (Pak) special Supplement. 21-34.
6. Saeed, K., Khan, S. and Haq, F., 2013. Diversity and population status of fish fauna of the river Barandu district, Buner Khyber Pakhtunkhwa Province, Pakistan. Journal of Biodiversity and Environmental Sciences, 83.
7. Akhtar, N., Saeed, K., & Khan, S. (2014). Fresh water record on fish fauna of River Barandu District Buner Khyber Pakhtunkhwa, Pakistan. Journal of Zoology Studies, 1(6), 23-26.
8. Akhtar, N., Saeed, K., Khan, N. and Rafiq, N., 2015. Fish fauna of the river Barandu with a new record (Cyprinus carpio) from from district Buner, Khyber Pakhtunkhwa Pakistan. World Journal of Fish and Marine Sciences, 7(6), pp.418-420.

9. Okumuş, İ. and Çiftci, Y., 2003. Fish population genetics and molecular markers: II- molecular markers and their applications in fisheries and aquaculture. Turkish Journal of Fisheries and Aquatic Sciences, 3(1).
10. Dudgeon D, Arthington AH, Gessner MO, Kawabata Z, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard AH, Soto D, Stiassny ML, Sullivan CA. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol Rev Camb Philos Soc. 2006 May;81(2):163-82.
11. Kim, H., Lee, W. and Lee, S., 2010. Morphometric relationship, phylogenetic correlation, and character evolution in the species-rich genus Aphis (Hemiptera: Aphididae). PloS One, 5(7), p.e11608.
12. Strauss, R.E. and Bond, C.E., 1990. Taxonomic methods: morphology. Methods for fish biology, pp.109-140.
13. Teletchea, F., Gardeur, J.-N., Kamler, E., & Fontaine, P. (2009). The relationship of oocyte diameter and incubation temperature to incubation time in temperate freshwater fish species. Journal of Fish Biology, 74(3), 652-668.
14. Wong, L.L., 2011. DNA Barcoding and Related Molecular Markers for Fish Species Authentication, Phylogenetic Assessment and Population Studies (Doctoral dissertation, Auburn University).
15. Hemmer-Hansen, J., Nielsen, E.E., Frydenberg, J. and Loeschcke, V., 2007. Adaptive divergence in a high gene flow environment: Hsc70 variation in the European flounder (Platichthys flesus L.). Heredity, 99(6), pp.592-600.
16. Divu, D., Khushiramani, R., Malathi, S., Karunasagar, I. and Karunasagar, I., 2008. Isolation, characterisation, and evaluation of microsatellite DNA markers in giant freshwater prawn Macrobrachium rosenbergii, from South India. Aquaculture, 284(1-4), pp.281-284.
17. Kochzius, M., Seidel, C., Antoniou, A., Botla, S.K., Campo, D., Cariani, A., Vazquez, E.G., Hauschild, J., Hervet, C., Hjörleifsdottir, S. and Hreggvidsson, G., 2010. Identifying fishes through DNA barcodes and microarrays. PloS one, 5(9), p.e12620.
18. Parhi J, Tripathy PS, Priyadarshi H, Mandal SC, Pandey PK. Diagnosis of mitogenome for robust phylogeny: A case of Cypriniformes fish group. Gene. 2019 Sep 10;713:143967.
19. Ward RD, Hanner R, Hebert PD. The campaign to DNA barcode all fishes, FISH-BOL. J Fish Biol. 2009 Feb;74(2):329-356.

20. Ratnasingham, S. and Hebert, P.D., 2007. BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). Molecular ecology notes, 7(3), pp.355-364.
21. DeSalle, R. and Amato, G., 2009. The expansion of conservation genetics. Conservation genetics in the age of genomics, pp.1-24.
22. Mirza, M. R. and Sandhu, A. A., 2007. Fishes of the Punjab, Pakistan. Polymer Publications Pvt. Ltd. Lahore.
23. Talwar, P.K. and Jhingran, A.G. 1991. Inland Fishes of India and Adjacent Countries. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi. p. 18
24. Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PD. DNA barcoding Australia's fish species. Philos Trans R Soc Lond B Biol Sci. 2005 Oct 29;360(1462):1847-57. doi: 10.1098/rstb.2005.1716. PMID: 16214743; PMCID: PMC1609232.
25. Sayers, E.W., Barrett, T., Benson, D.A., Bolton, E., Bryant, S.H., Canese, K., Chetvernin, V., Church, D.M., DiCuccio, M., Federhen, S. and Feolo, M., 2012. Database resources of the national center for biotechnology information. Nucleic acids research, 40(D1), pp. D13-D25.
26. Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution, 35(6), p.1547.
27. Ahmed, S., Ibrahim, M., Nantasenamat, C., Nisar, M.F., Malik, A.A., Waheed, R., Ahmed, M.Z., Ojha, S.C., and Alam, M.K., 2022. Pragmatic applications and universality of DNA barcoding for substantial organisms at the species level: a review to explore a way forward. BioMed Research International, 2022.
28. Malkani, N., Khalid, S., Ashraf, S., Kamran, M., Khan, R. U., Yaqub, A., & Ullah, A. (2022). Molecular identification of Museum preserved type specimens of fish species using DNA barcoding. LGU Journal of LIFE SCIENCE, 6(3), 200-215.