

Morphological and molecular characterization of *Cirrhinus mrigala*, Mori (Hamilton, 1822) (Cypriniformes: Cyprinidae) from Indus River district Swabi Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Indus River is the largest river of Pakistan that originated from Tibetan Plateau of Gilgit-Baltistan and flow through the entire Pakistan before merging with Arabian Sea in District Sindh. This river is a hot spot of biodiversity having more than 180 fresh water fish species. In this study a total of 10 fish samples were collected from the selected regions i.e., Ghazi, Hund, Beka, and Kund Park from Indus River district Swabi, Khyber Pakhtunkhwa, Pakistan from 24 May to 28 August 2024. The collected fish samples were identified morphologically as *Cirrhinus mrigala* through standard fish keys and preserved in 100% ethanol for the purpose of DNA extraction. The DNA were extracted from fish muscle and liver using Phenol-Chloroform protocol and characterized by using universal genetic marker Cytochrome Oxidase sub unit I (COI) gene. The new available sequence was submitted to GenBank for accession number PV562800. The phylogenetic analysis of the new reported sequence of *C. mrigala* showed 100% similarity with other reported similar species from India, China, France and Bangladesh. Fish fauna of the Indus River decline day by day due to overfishing, pollution and habitat loss. This study provides a baseline for the management of fish fauna inhibiting in River Indus.

Keywords: Tibetan plateau; Fish keys; Phenol-Chloroform; Pollution; Habitat loss; GenBank

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Introduction

Fish is a group cold blooded aquatic vertebrates that breathe on gills, use fins to swim and primarily depends on water as medium to live [1]. Fish fauna is most diverse and abundant group of chordates that present in every niche of the hydrosphere [2]. Globally, a total of 32000 fish species are reported that belonging to 515 taxonomic families, in which 77 % families belongs to fresh water fishes. Of the number total fish species, 60% are marine and 40% are fresh water fish species. There are 15000 freshwater fishes, in which 305 species are added in each year [3]. Fish fauna face many environmental threats including over fishing, water pollution, habitat loss, and climatic changes [4]. A total of 28,000 fish species were extinct up to date, of these 108 were lampreys and hag fish, 27000 were bony fishes and 992 were cartilage fishes (Skate, Rays Sharks and chimera fish) [5]. In Pakistan the total number fish species are 53, out these 189 are fresh water and 233 were marine fishes [6].

Fish is most diverse and abundant group of vertebrates that are usually identified on the base on morphology including body shape and color, number of lateral line scales, number of gills rakers and number fin rays [7]. Sometime morphological characters are insufficient to differentiate dead and decomposed species, larval stages and closely related species i.e., species of genus *Schizothorax*, *Barus* and *Microhyala* are impossible to differentiate on the base of morphology [8]. In this scenario we used molecular characterization to identified organism up to species. The most authentic technique of species based identification is the analysis of the Mitochondrial DNA (mDNA) of an organism that consist of specific genetic marker i.e., 12SrRNA, 16SrRNA, 18SrRNA, Cytochrome b and Cytochrome oxidase sub unit I (COI) [9] Among these the most suitable marker is COI gene which is stable and universal identifier gene that is responsible for intraspecific and interspecific variation [10]. Cytochrome oxidase sub unit-I (CO) gene is 650-760 bps mitochondrial gene that is accepted widely as genetic marker for species level identification for many animal taxa that provide better phylogenetic display and signals [11].

Indus River is locally known as "Darya -e- Sindh" is 21th Asian longest river and a largest river of Pakistan that of total length is 2880 km, drainage area is 11, 65,500 km and annual flow of water is 207km [12]. This river originated from Tibetan plateau, Ladakh region of Kashmir and flow the entire length of Pakistan before merging in the Arabian Sea in district Sindh. Darya-e- Sindh is hotspot and a hub of fish diversity due to ideal water temperature and food availability, this river is home of 180 fresh water fish species in which 22 fish species are exclusively found in this river [13]. Fish fauna of Indus River decline day by day due to over fishing, and water pollution this study provide a baseline for the conservation and management of fish fauna inhibiting in this river.

Materials and methods

Study area

The study area for fish samples collection was Indus river district Swabi Khyber Pakhtunkhwa, Pakistan. Geographically, Islamabad the capital territory located at the East of Swabi, district Buner is on Northern side , southern side is Province Punjab and western side is district Mardan with geographic coordination is 34.1270° N and 72.4741°E.

Sample Collection

Fish samples were collected from Ghazi, Hund, Beka and Kund region from Indus River district Swabi Khyber Pakhtunkhwa, Pakistan from 14 May to 28 August 2024. A total of 10 fish samples were collected, 4 from Ghazi, 2 from Hund, 3 from Beka and 1 from Kund Park by using hand net, Cast net and trammel net. The collected samples preserved in 100% ethanol and brought to Fisheries and Aquaculture lab department of Zoology University of Peshawar for morphological and genetic analysis.

Taxonomic identification

The collected samples were identified as *Cirrhinus mrigala* through available Standard taxonomic keys such as "Fishes of the world" [1], "The fresh water fishes of India and sub content" [14], and "Fish base" (Online data base).

DNA extraction and Phylogenetic analysis

Mitochondrial DNA (mDNA) were extracted from the muscle and liver of the collected fish samples using Phenol- chloroform protocol [15]. For the amplification of Cytochrome oxidase subunit, I gene (COI), we used us universal primer both forward and reverse primer i.e., LCO1490 FGG TCA ACA AAT CAT AAA GAT ATT GG, HCO2198R TAA ACT TCA GGG TGA CCA AA AAA TCA [16]. The PCR product was sequenced in next generation of Microgen sequencer in Korea. The available sequence was read, edited and termed using Finch TV version 1.01. The newly obtained sequence was blasted and submitted to GenBank for accession number PV562800. For Phylogenetic analysis the similar related sequences were downloaded from NCBI and aligned to construct Phylogenetic tree using MEGA version 11.

Results

Morphology

Fish body was elongated, stream lined, bilateral symmetrical and laterally compressed. Maximum total length (TL) was ranged from 16-90cm, mouth was sub terminal, broad and transverse, lower lip was indistinct with small tubercle, upper lip continuous, entire and completed with one pair of short rostral barbels, three rows of pharyngeal teeth, with formula 5, 4, 2 and 2, 4, 5. Golden eyes with black iris, body was covered by cycloid scale except head, lateral line scales was 42-44 and caudal fin was extremely forked. Body color was dark-grey and silver some time black coppery. Dorsal fin greyish, anal fin, pelvic fine and pectoral lighter blackish. Fin formula; D. 15-16, P1. 15-16, P2. 8-9, A. 6-8, C. 17-20 (Figure 1).



Fig. 1: Lateral view of *Cirrhinus mrigala*

Molecular and Phylogenetic analysis

We extracted mDNA from the collected fish sample from Indus River Khyber Pakhtunkhwa, Pakistan. The new available Cytochrome oxidase (COI) sequences were submitted into GenBank for accession number. The GenBank accession numbers for the newly generated sequences were PV562800 (432bp; Kund Park) and blast through NCBI. The Cytochrome oxidase sub unit I *COI* genes of *C. mrigala* from Pakistan shows 100 % similarity with *C. mrigala* reported from India, Bangladesh, China and France (Table 1). A total 13 sequences were downloaded from NCBI and aligned in MEGA11 to construct Phylogenetic tree. The phylogenetic tree showed two groups i.e., the out-group consist of frog species, *M. taraiensis* and the in-group was consist of fish species including *C. mrigala*, *L. rohita*, *Cirrhinus* and *C. cirrhosus* (Figure 2).

Table 1: Species name, Accession, Query cover, Accession length, Similarity index and Country name of *Cirrhinus mrigala*

Species name	Accession	Query cover %	A. length	S. index %	Country name
<i>C. mrigala</i>	PV562800	100	432	100	Pakistan
<i>C. mrigala</i>	KX946610	100	621	100	India
<i>C. mrigala</i>	GU195070	100	638	100	India
<i>C. mrigala</i>	OP050514	100	647	100	China
<i>L. rohita</i>	JN412817	100	16611	100	India
<i>C. mrigala</i>	JX983257	100	652	100	India
<i>C. mrigala</i>	MK820367	100	656	100	Pakistan
<i>C. mrigala</i>	MK572126	100	655	100	Bangladesh
<i>C. mrigala</i>	KC631190	100	643	100	France
<i>C. mrigala</i>	GU195083	100	665	100	India
<i>C. mrigala</i>	PP430705	100	631	100	India
<i>C. mrigala</i>	MK581194	100	619	100	India
<i>C. mrigala</i>	KJ936736	100	655	100	India

Discussion

Cirrhinus mrigala commonly known is Mori, Mirgal and Morakhi is fresh water fish that native to Pakistan, Nepal, Thailand, India, china, Russia and Sri Lanka [17]. This fish is bottom feeder and mostly found in fresh water rivers, pond and lakes[18].The present study was conducted to investigate the population *C. mrigala* on the base of morphology and molecular characterization collected from Indus River district Swabi Khyber Pakhtunkhwa, Pakistan from 24 may to 28 August 2024. Our morphological finding i.e., Fish body was elongated, stream lined, bilateral symmetrical and laterally compressed. Maximum total length (TL) was ranged from 16-90cm, mouth was sub terminal, broad and transverse, lower lip was indistinct with small tubercle, upper lip continuous, complete and entire with one pair of short rostral barbels, three rows of pharyngeal teeth, with formula 5, 4, 2 and 2, 4, 5. Golden eyes with black iris, body was covered by cycloid scale accept head, literal line scales was 42-44 and caudal fin was extremely forked. Body color was dark-grey and silver some time black coppery. Dorsal fin greyish, anal fin, pelvic fine and pectoral lighter blackish were found similar to the morphological study of Ghayyur et al.,

(2021) [19], Muhammad et al., (2018) [20], kaur et al., (2013) [21] and Li et al., (2023) [22]. we sequenced Cytochrome oxidase sub unit I (COI) gene and compared with other sequence of *C. mrigala* reported from India, Bangladesh, Pakistan and China showed almost 100% similarity. Additionally, we also constructed a Maximum-likelihood Phylogenetic tree for further species based conformation. In this we compared the morphological and genetic record of *C. mrigala* form the first time from Pakistan that provide a baseline for further research on this species in Pakistan.

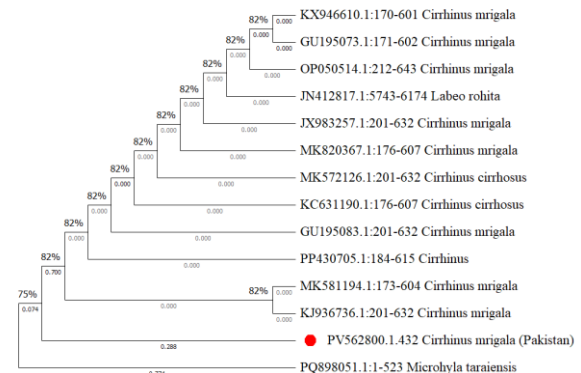


Fig. 2: Maximum-likelihood phylogenetic tree of *C. mrigala* using the *COI* gene. *Microhyla taraiensis* was used as the out group, while *Cirrhinus mrigala*, *Cirrhinus cirrhosus*, and *Labeo rohita* were included in the group.

Conclusion

This study was aimed to document the distribution of *C. mrigala* based on morphology and molecular characterization in Indus River of district Swabi. Our results based on maximum-likelihood inference assess the taxonomic status of the sample obtained from Pakistan. We provided the genetic record of *C. mrigala* from Kund Park, Indus River district Swabi using Cytochrome oxidase Subunit I (COI) gene, which provides baseline data for *Cirrhinus* genus and its distribution in the rest of Pakistan.

References

- Nelson JS. Fishes of the World, 4th Ed. John Wiley & Sons, Inc, New York; 2006:p.601
- Bone Q, Moore R. Biology of fishes. Taylor & Francis; 2008 Mar 19.
- Reid GM, Contreras MacBeath T, Csatádi K. Global challenges in freshwater-fish conservation related to public aquariums and the aquarium industry. International Zoo Yearbook. 2013 Jan;47(1):6-45.
- Aminur Rahman M, Arshad A, Nurul Amin SM. Growth and production performance of threatened snakehead fish, *Channa striatus* (Bloch), at different stocking densities in earthen ponds. Aquaculture Research. 2012 Jan;43(2):297-302.
- Vörösmarty CJ, McIntyre PB, Gessner MO, Dudgeon D, Prusevich A, Green P, Glidden S, Bunn SE, Sullivan CA, Liermann CR, Davies P. Global threats to human water security and river biodiversity. nature. 2010 Sep 30;467(7315):555-61.
- Rafique MO. Fish diversity and distribution in Indus River and its drainage system. Pakistan Journal of Zoology. 2000;32(4):321-32.
- Strauss RE, Bond CE. Taxonomic methods: morphology. Methods for fish biology. 1990:109-40.
- Callejas C, Ochando MD. Molecular identification (RAPD) of the eight species of the genus *Barbus* (Cyprinidae) in the Iberian Peninsula. Journal of Fish Biology. 2001 Dec;59(6):1589-99.
- Hebert PD, Ratnasingham S, De Waard JR. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London. Series B: Biological Sciences. 2003 Aug 7;270(suppl_1):S96-9.
- Costa FO, DeWaard JR, Boutillier J, Ratnasingham S, Dooht RT, Hajibabaei M, Hebert PD. Biological identifications through DNA barcodes: the case of the Crustacea. Canadian journal of fisheries and aquatic sciences. 2007 Feb 1;64(2):272-95.
- Dawney N, Ogden R, McEwing R, Carvalho GR, Thorpe RS. Validation of the barcoding gene COI for use in forensic genetic species identification. Forensic science international. 2007 Nov 15;173(1):1-6.
- Khan J. The Vital Role of Fish in Nutrition, Income, Employment and Ecosystem.
- MIRZA MR. Temporal variations in commercial fish community of a floodplain of the River Ravi, Pakistan. BIOLOGIA (PAKISTAN). 2014;60(1):73-80.
- Jayaram KC. The freshwater fishes of the Indian region. 1999.
- Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. 1989.
- Folmer RH, Nilges M, Folkers PJ, Konings RN, Hilbers CW. A model of the complex between single-stranded DNA and the single-stranded DNA binding protein encoded by gene V of filamentous bacteriophage M13. Journal of molecular biology. 1994 Jul 21;240(4):341-57.
- FAO Fisheries & Aquaculture National Aquaculture Sector Overview (NASO)". www.fao.org. Retrieved 2019-09-27.
- Ayyappan S, Jena JK. Sustainable freshwater aquaculture in India. Sustainable Indian fisheries. 2001:88-133.
- Ghayyur S, Khan MF, Tabassum S, Ahmad MS, Sajid M, Badshah K, Khan MA, Ghayyur S, Khan NA, Ahmad B, Qamer S. A comparative study on the effects of selected pesticides on hemato-biochemistry and tissue histology of freshwater fish *Cirrhinus mrigala* (Hamilton, 1822). Saudi Journal of Biological Sciences. 2021 Jan 1;28(1):603-11.
- Muhammad N, Umair M, Khan A, Yaqub M, Haider MS, Khan Q, Abbasi AR. Assessment of uses of Cultural Mrigal Carp in Gujran wala division Pakistan. Journal of wild life and Ecology. 2018 2(1):1-9.
- Kaur HA, Kalotra RO, Walia GK, Handa DI. Dyeing industry effluent induced behavioural and morphological changes in the fish, *Cirrhinus mrigala*. IJZR. 2013;3:13-20.
- Li W, Zhou Z, Tian X, Li H, Su J, Liu Q, Wu P, Wang S, Hu J, Shen Z, Zeng L. Gynogenetic *Cirrhinus mrigala* produced using irradiated sperm of *Cyprinus carpio* exhibit better cold tolerance. Reproduction and Breeding. 2023 Mar 1;3(1):8-16.