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RESEARCH PAPER

Genetic Characterization of The Freshwater Gar Fish, *Xenentodon cancila*, (Hamilton, 1822) (Beloniformes: Belonidae) from The Indus River, Khyber Pakhtunkhwa Province, Pakistan

Javed KHAN[1*]

[1] *University of Peshawar, Fisheries and Aquaculture Lab, Department of Zoology, Peshawar, Pakistan.*

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*** Corresponding Author**
E-mail: javedkhanbio71@gmail.com
https://orcid.org/0009-0008-8765-9487

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Abstract

Xenentodon cancila, commonly known as the Gar fish, Needle fish, or Needle nose fish, is an Asian fresh water fish that is widely distributed across South and South East Asia, i.e., Pakistan, India, Bangladesh, Nepal, Sri Lanka, Myanmar, and Malaysia. It is adapted for diverse aquatic habitat including fresh water, marine, and brackish water, with a preference for rivers but also inhabiting in ponds, lakes, canals, and other water bodies. In this study, a field survey was conducted from March to August 2021 along the Indus River in Swabi District, Khyber Pakhtunkhwa, Pakistan. A total of 10 samples were collected from four different locations i.e., Tarbela, Hund, Nabi and Kund Park along the Indus River. We measured 30 morphometric characters and performed statistical analysis including mean, standard deviation, range difference, correlation, and regression. We sequenced and submitted two cytochrome oxidase subunit I (COI) genes to GenBank with accession numbers PP231016 and PP230982 from Pakistan. Phylogenetic analysis of the COI gene revealed high similarity of almost 100% with other related species. We constructed Median joining haplotype network and find out the haplotype and nucleotide diversity. The haplotype diversity (HD) and nucleotide diversity (π) of the COI gene were calculated as 0.9338 and 0.0113679, respectively.

INTRODUCTION

The Indus River (Darya-e-Sindh) is one of the Asia's longest rivers that originates in the Tibetan plateau, flows through Kashmir, Gilgit-Baltistan to the Hindu Kush ranges, and passing through all of Pakistan before emptying into the Arabian Sea (Ahmad & Lodrick, 2019). The Indus River supports the diverse aquatic fish fauna and is home to over 180 fresh water species, with 22 fish species found exclusively in this river (Rafiq & Khan, 2012). The upper part of this river has fewer genera and species i.e., Diptychs, *Schizopyge, Schizothorax, Xenentodon*, Snow trout, *Triplophysa*, loaches, and cat fish. Fish diversity increases significantly after the Kabul–Indus junction at the Attock region, with species like *Cyprinadae, Rohu, Catla*, and Mahasher (Mirza & Mirza, 2014).

Xenentodon cancila (Hamilton, 1822), commonly known as the freshwater Garfish, is a species of order Beloniformes in the Belonidae family is a native Asian species that has been introduced in Hawaii (Froese & Pauly, 2012). This fish has many local names in different countries i.e., 'Phtong' in Cambodia, Sydasiatisk halvgedde in Denmark, Kokila in India, Pla katung heow mueng in Thailand, Intiannokkakala in Finland, Silver needle fish and Stick fish in Hawaii, Freshwater jar fish in the United Kingdom, Cánhói in Vietnam (Nath & Dey, 1989; Chuenpagdee, 2002; Carl, 2003; Varjo et al., 2004; Nghia, 2005; FAO-FIES, 2010) and in Bangladesh it is known as Kaikka, Kakila, and Kankely (Rahman, 1989; Al-Mamun, 2003; Nath & Dey 1989). *X. cancila* is present in diverse aquatic fresh water habitats i.e., rivers, ponds, canals, beels, and inundated fields (Rahman, 1989; Pethiyagoda, 1991; Talwar & Jhingran, 1991). It is mostly prefers slow-flowing pools with a rock or sandy substrates (Pethiyagoda, 1991). It was also

observed in clear, gravelly, perennial streams and ponds in Terai and Duars, North Bengal, India, and is fairly common in the Ganges-Brahmaputra system (Talwar & Jhingran, 1991). This solitary fish swims in mid water against the current and bursts of speed, when pursuing prey. *X. cancila* primarily feeds on crustaceans, small fish, and insects in the wild. In an aquarium, it only consumes live fish (Pethiyagoda, 1991; Rainboth, 1996). Morphologically, *X. cancila* has an elongated body with beak like jaws filled with sharp teeth, a silvery green upper body, and a lighter lower body with dark bands, maximum body length reaches to 40 cm, it has a complete lateral line, and the dorsal fin is opposite to the anal fin and very close to the caudal fin (Talwar & Jhingran, 1991). The number of rays in its fins varies: 16 to 19 in the dorsal fin, 11 to 12 pectoral fin, 6 in the pelvic fin, 16 to 19 in the anal fin, and 15 in the caudal fin (Bhuiyan, 1964). *X. cancila* is an oviparous fish that exhibits slight sexual dimorphism with male fish having anal and dorsal fins with black edge. Their eggs may be found attached to objects in the water by tendrils on the egg's surface (Breder & Rosen, 1966).

Fish identification is typically based on morphological characteristics like body shape, scale patterns, color and measurements (Strauss & Bond, 1990). However, morphometric characters may not always be sufficient for species differentiation especially for closely related species like *Barbus* (*B. ablabes, B. aboinensis, B. acuticeps, B. afrohamiltoni, B. afrovernayi, A. barbell, B. aliciae* and *B. alluaudi*) (Callejas & Ochando, 2001). In this scenario, molecular identification is valuable tool for identifying species using specific genes (Hebert et al., 2003). Mitochondrial DNA analysis, particularly the cytochrome oxidase subunit I gene, is commonly used for this purpose (Avise et al., 2003; Hebert, 2004a). In this study, we sequenced X. cancila for the first time in Pakistan, shedding light on its systematic status and haplotype analysis. This study clarifies the taxonomic status of *Xenentodon* species in Pakistan through morphological and genetic analysis.

MATERIALS AND METHODS

Study Area

The study area for collecting fish samples was the Indus River in Swabi district, Khyber Pakhtunkhwa, Pakistan. A total of 10 fish specimens were collected from four different locations: Tarbela, Hund, Nabi, and Kund Park along the Indus River using cast nets and trammel net.

Fish Identification

The fish samples collected were morphologically identified using standard taxonomic keys such as "Fishes of Punjab" (Mirza, 2003), "Fresh water Fishes of the Indian Region" (Jayaram, 1999), and "Inland Fishes of India and Adjacent Countries" (Talwar & Jhingran, 1991). A total of 30 morphometric measurements were taken from the collected samples and analyzed statistically, including mean, standard deviation, range difference, correlation coefficients and regression analysis. The morphometric characters were taken by using Caliper and digital balance which were, total length (TL), standard length (SL), forked length (FL), eye diameter (ED), inter nasal distance (IND), pre dorsal length (PDL), post dorsal length (PODL), pre pectoral length (PPL), post pectoral length (POPL), pre pelvic length (PrPL), post pelvic length (PrOPL), pre anal length (PAL), post anal length (POAL), snout length (SnL), distance b/w pelvic and pectoral fin (DPPF), distance b/w pelvic and anal fin (DPAF), distance b/w anal and pectoral fin (DAPF), Length of dorsal fin (LDF), height of dorsal fin (HDF), length of pectoral fin (LPF), height of pectoral fin (HPF), pelvic fin length (PFL), pelvic fin height (PFH), length of anal fin (LAF), height of anal fin (HAF), length of caudal fin (LCF), height of caudal fin (HCF), weight (W) and inter orbital length (IOL) (Mojekwu & Anumudu, 2015). The voucher specimens were deposited in the Fisheries and Aquaculture Laboratory at the Department of Zoology, University of Peshawar, Pakistan.

Molecular and Phylogenetic Analysis

Two fish samples were collected from Tarbela and Kund Park for molecular and phylogenetic analysis. Mitochondrial DNA was extracted from muscle tissue using the ammonium acetate method (Brufordet al., 1998). PCR amplicons were sequenced by Rehman Medical Institute and Hospital, Peshawar, Pakistan. The *COI* sequence reading and editing Finch TV (1.4.0) and Bio Edit (7.2.5), were used and then compared with reference sequences in NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences were deposited in Gen Bank with

accession number PP231016 (Tarbela) and PP230982 (Kund Park). Taxonomic status was assessed using MEGA11 (Hall, 1999), and a phylogenetic tree was constructed. Nucleotide diversity and Haplotype were calculated using DnaSPv. 5.10.1, and a median join network was constructed in PopART v.1.7. (Librado & Rozas, 2009; Leigh & Bryant, 2015).

RESULTS

Morphological identification

X. cancila was morphologically identified as extremely elongate, needle-like body, slightly compressed or sub-cylindrical, tapering at both ends, with a total length (TL) ranging 16.9 to 30cm. The head is small and pointed with small yellow colored superior and moderate eyes, a pointed snout with a triangular cavity near the eyes, the mouth with elongated beak like jaws containing unequal sharp teeth. The lower jaw is slightly longer than the upper jaw, and the body lacks a keel. The lateral line is complete and present near the lateral profile, with more than 200 pre-dorsal scales. The fins do not have spines, with the dorsal and anal fins opposite each other and very close to the caudal fin. The caudal fin is round, truncated or emarginated with 15 rays while the pectoral fins are short and high up on the body sides. The skin scales are small, cycloid, and deciduous. The fins formula was D. 16-19; A. 16-19; P1. 10-12; P2. 6, C. 15; LI.250 (Figure 1).

Body color: The body is silvery green, darker above and lighter below with a dark band running to the flank. Male fish has anal and dorsal fins with black edges.

Sexual dimorphism: *X. cancila* exhibits sexual dimorphism, with males having a hump near the head region below the dorsal fin and black dots on their dorsal and anal fins. Females lack the hump and black dots on their fins.

Habitat and niche: Found in freshwater bodies, some common habitats are ponds, ditches, inundated fields, canals, beels, floodplains, haors, baors and mostly found in rivers. It also inhabits slow running pools, with rocks and sandy substrates.

Morphometric Measurements

Morphometric measurements of 10 samples were taken by using digital caliper, standard ruler, and digital balance. A total of 30 morphometric characters were documented and analyzed statistically, including mean, standard deviation, range, correlation coefficients and regression analysis. All measurements were recorded in centimeters (cm) (Table 1 & 2).

Among the 30 morphometric characters of *X. cancila*, the eight characters were vast in range (environmentally controlled), thirteen characters were intermediate and nine characters were narrow ranged (genetically controlled) (Table 3). Out of 29 characters, the 22 characters showed high value of correlation which were statistically significant and 7 characters showed low correlation values, which were not statistically significant (Table 4).

Figure 1. Lateral view of *X. cancila*, needle like beak with sharp teeth's, eye with golden iris and black pupil, golden-olive color transparent body, caudal fin with black dot.

Table 1. Morphometric measurements of 10 samples of *X. cancila* from River Indus.

S:NO	Acronym	1	2	3	4	5	6	7	8	9	10
1	TL	30	22.9	22.7	20.5	20.6	21	20.2	20.7	21.1	16.9
2	SL	27.7	21.5	21.2	19.3	19.3	19	18.9	19.3	18.9	15.9
3	HL	9.1	8	7.8	7	7.1	7.4	7.2	7.1	7.3	5.9
4	FL	28.6	22.3	22	19.9	20	20.4	19.6	19.6	20.2	16
5	ED	0.8	0.5	0.6	0.5	0.6	0.6	0.6	0.6	0.6	0.5
6	IND	0.8	0.5	0.5	0.5	0.6	0.5	0.5	0.5	0.6	0.5
7	PDL	21.9	16.9	16.7	15.3	15	15.3	15	15.2	15.3	12.4
8	PODL	1	1	0.9	0.7	0.9	0.9	0.9	1	1	0.9
9	PPL	9.9	8.4	8.4	7.5	7.8	7.5	7.6	7.6	7.5	6.5
10	POPL	17.4	12.9	15.5	11	11.2	12	11.5	11.6	12	9.2
11	PrPL	17.9	13.6	13.6	15	15.6	12.9	12	12	13	9.9
12	PrOPL	10	7.8	7	7.3	7	7.6	7.2	7.2	7.7	6
13	PAL	22.5	16.9	16.5	15.3	15.3	15.4	15	15.1	15.3	12.5
14	POAL	1.5	1	1	0.5	1	1	1	1.2	1	1
15	SnL	5	4.8	5	4.4	4.4	4.5	4.5	4.4	4.5	3.8
16	DPPF	7.6	5.4	5.3	5	5	5	4.5	4.7	5	4
17	DPAF	3.6	2.9	3.4	2.9	3	2.5	3	2.7	2.5	2.5
18	DAPF	4.5	3.5	3.5	3.4	3.3	3.3	3.3	3.2	3.1	3
19	LDF	4.4	3	3	2.9	2.7	3	3	2.7	2.9	2.9
20	HDF	2.5	2	2.1	2	2	2	2	2	2	1.8
21	LPF	0.4	0.4	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3
22	HPF	2	1.6	1.4	1.5	1.3	1.2	1.3	1.4	1.3	1
23	PFL	0.4	0.3	0.4	0.3	0.3	0.3	0.2	0.3	0.3	0.2
24	PFH	1.2	1	1	0.8	0.7	0.7	0.7	0.8	0.7	0.6
25	LAF	3.6	2.8	3	2.6	2.4	2.7	2.9	2.7	2.7	2.2
26	HAF	2.8	2.2	2	2	2	2.1	2	2.2	2	2
27	LCF	2.9	2	2.3	2	1.9	2	2	2	2	1.9
28	HCF	2.1	1.5	1.9	1.7	1.2	1.7	1.7	1.7	1.6	1.1
29	W	57	24	22	18	16	17	15	19	16	10
30	IOL	1	0.9	0.8	0.7	0.6	0.5	0.6	0.7	0.5	0.5

Table 2. Mean, standard deviation, range, range difference, correlation coefficient and regression equation of morphometric characters.

S:NO	Acronym	Mean	SD	Range	Range difference	Correlation coefficient	Regression equation
1	TL	21.66	3.35	30-16.9	13.1		
2	SL	20.1	3.07	27.7-15.9	11.8	0.994	Y=1.087+-0.191X
3	HL	7.39	0.82	9.1-5.9	3.2	0.966	Y=3.950+-7.533X
4	FL	20.86	3.2	28.6-16	12.6	0.997	Y=1.044+0.107X
5	ED	0.59	0.09	0.8-0.5	0.3	0.809	Y=30.956+3.3956X
6	IND	0.55	0.1	0.8-0.5	0.3	0.798	Y=27.523+6.518X
7	PDL	15.9	2.43	21.9-12.4	9.5	0.998	Y=1.377+-0.2489X
8	PODL	0.92	0.09	1-0.7	0.3	0.374	Y=13.65+9.094X
9	PPL	7.87	0.89	9.9-6.5	3.4	0.979	Y=3.688+-7.370X
10	POPL	12.43	2.36	17.4-9.2	8.2	0.929	Y=1.317+5.277X
11	PrPL	13.55	2.21	17.9-9.9	8	0.815	Y=1.233+4.939X
12	PrOPL	7.48	1.02	10-6.0	4	0.948	Y=3.115+-1.646X
13	PAL	15.98	2.56	22.5-12.5	10	0.998	Y=1.303+0.827X
14	POAL	1.02	0.24	1.5-0.5	1	0.619	Y=8.503+12.986X
15	SnL	4.53	0.35	5-3.8	1.2	0.826	Y=7.912+-14.185X
16	DPPF	5.15	0.95	7.6-4	3.6	0.986	Y=3.479+3.741X
17	DPAF	2.9	0.38	3.6-2.5	1.1	0.764	Y=6.789+1.971X
18	DAPF	3.41	0.41	4.5-3	1.5	0.958	Y=7.736+-4.721X
19	LDF	3.05	0.49	4.4-2.7	1.7	0.878	Y=6.027+3.274X
20	HDF	2.04	0.18	2.5-1.8	0.7	0.975	Y=18.401+15.878X
21	LPF	0.33	0.05	0.4-0.3	0.1	0.578	Y=40.095+8.428X
22	HPF	1.4	0.27	2.0-1.0	1	0.93	Y=11.687+5.297X
23	PFL	0.3	0.07	0.4-0.2	0.2	0.776	Y=39+9.96X
24	PFH	0.79	0.18	1.2-0.6	0.6	0.889	Y=16.629+8.522X
25	LAF	2.76	0.37	3.6-2.2	1.4	0.926	Y=8.278+-1.188X
26	HAF	2.13	0.25	2.8-2	0.8	0.885	Y=11.875+-3.634X
27	LCF	2.1	0.3	2.9-1.9	1	0.923	Y=10.243+0.147X
28	HCF	1.62	0.3	2.1-1.1	1	0.751	Y=8.465+7.946X
29	W	21.4	13.1	57-10	47	0.969	Y=0.248+16.349X
30	IOL	0.68	0.18	1-0.5	0.5	0.814	Y=15.586+11.060X

Table 3. Vast range, intermediate range and narrow range of morphometric characters.

S:No	Range difference	Morphometric characters
1	Vast range	TL, SL, FL, PDL, POPL, PrPL, PAL, W
2	Intermediate Range	HL, PPL, PrOPL, PAL, SnL, DPAF, DPAF, LDF, HPF, LAF, LCF, HCF, POAL
3	Narrow range	ED, IND, PODL, IOL, HAF, PFH, LPF, PFL, HDF,

Table 4. High and low value of correlation coefficient of morphometric characters.

S:No	Correlation	Morphometric characters
1	High value	SL, HL, FL, ED, PDL, PPL,POPL, PrPL, PrOPL, PAL, SnL, DPPF, DAPE, LDF, HDF, HPF, PFH, LAF, HAF, LCF, W, IOL
2	Lower value	IND, PODL, POAL, DPAF, LPF, PFL, HCF

Molecular and Phylogenetic Analysis

We extracted DNA from two fish samples, collected from two different geographic localities along the Indus River, specifically Tarbela and Kund Park in the Swabi District of Khyber Pakhtunkhwa, Pakistan. We sequenced two universal identifier mitochondrial cytochrome oxidase subunit-I gene (*COI*/mDNA/650-710bp) for taxonomic identification of the species and submitted to GenBank for accession. The GenBank accession numbers for the newly generated sequences are PP231016 (480bp; Kund Park) and PP230982 (672bp; Tarbela) for phylogenetic analysis. The phylogenetic analysis of COI genes of *X. cancila* from Pakistan shows 100 % similarity with *X. cancila* reported from India (Table 5 & Figure 2).

Table 5. Species name, location, accession number, accession length and similarity index of *X. cancila*.

Species name	Location	Accession number	Accession length	Similarity index%
Xenentodon cancila	India	JX983513	652	100
Xenentodon cancila	Pakistan	PP231016	480	100
Xenentodon cancila	Pakistan	PP230982	672	100
Xenentodon cancila	India	OR148240	652	99.79
Xenentodon cancila	India	JX983511	652	99.58
Xenentodon cancila	Bangladesh	MK572631	652	99.58
Xenentodon cancila	Bangladesh	MK572629	634	98.96
Xenentodon cancila	India	KF742433	652	98.96
Xenentodon cancila	Bangladesh	MK572630	652	98.96
Xenentodon cancila	India	JX260995	612	98.96
Xenentodon cancila	India	OR148241	657	99.79
Xenentodon cancila	India	OR148238	671	99.57
Xenentodon cancila	India	KX399216	655	98.75
Xenentodon cancila	India	OR148239	665	98.75
Xenentodon cancila	India	KU685524	655	98.12
Xenentodon cancila	India	KU685520	655	98.12
Xenentodon cancila	India	KU685521	655	98.12
Xenentodon cancila	India	KU685523	655	98.12
Xenentodon cancila	India	KU685522	655	98.12

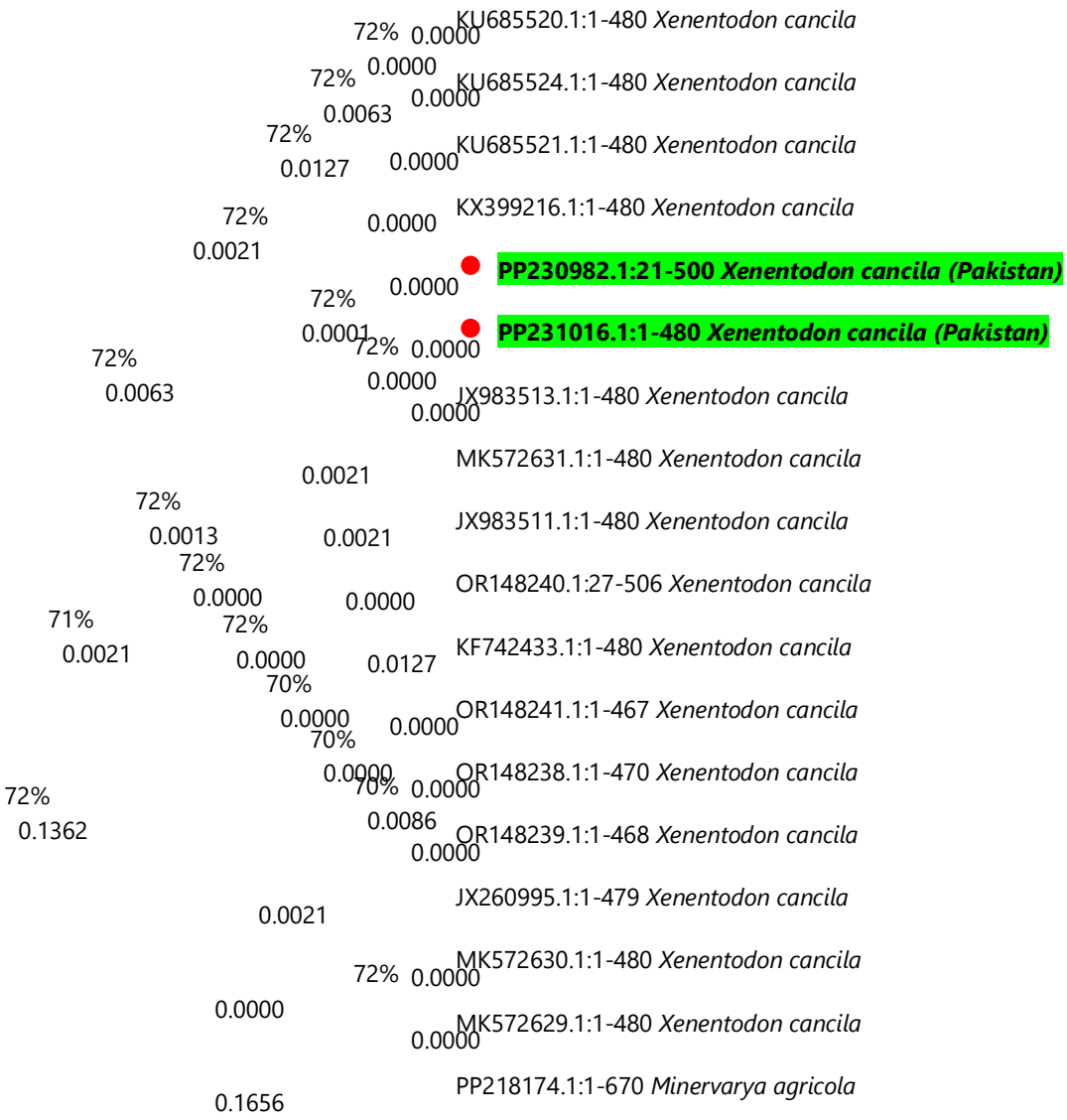

Figure 2. Maximum-likelihood phylogenetic tree of *X. cancila* using the *COI* gene. *Minervarya agricola* (Frog) was used as the out-group and shows 0.1656 nodal distance, while the *X. cancila* was included in the group. Which shows almost 0.0000 nodal distance.

Median Joining Haplotype Network and Nucleotide Diversity

For the construction of a median joining haplotype network and calculation of nucleotide diversity, DNAsp and Popart software were used. Two *COI* sequences of *X. cancila* were randomly selected for genetic analysis from the samples collected in the study area. A total of 17 *COI* sequences were downloaded from NCBI GenBank and aligned in MEGA11 to create a Nexus file. The Nexus file was then used in DnaSP to identify approximately 10 haplotypes (h) with haplotypes diversity (Hd) and nucleotide diversity (π) are 0.9338 and 0.0113679, respectively. The haplotype data file and trait file used in Pop Art to create a Median-joining haplotype network. The network shows intraspecific relationships of *X. cancila* from Pakistan with India and Bangladesh (Figure 3 & 4).

DISCUSSION

Xenentodon cancila (Hamilton) is the freshwater species that is widely distributed across almost all lentic and lotic habitats of the tropical and sub-tropical regions of South and South East Asia (Bano et al., 2012; Suba & Meheta, 2012; Hossain et al., 2013; Chakrabarti & Banerjee, 2015; Gupta & Banerjee, 2017). Previously, several studies have been conducted on *X. cancila* to investigate their morphology, physiology and anatomy. These include a SEM study on an intestinal parasite (Bucephalopsis karvei Bhalerao) (Pandey & Tewary, 1984),

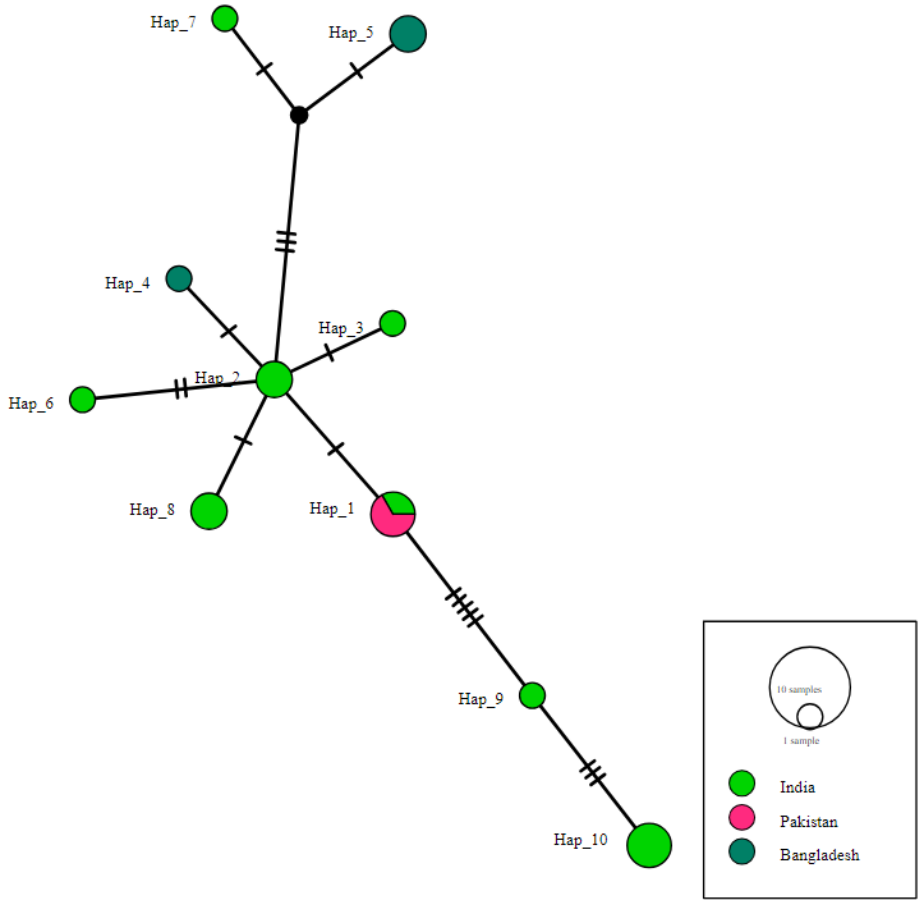

Figure 3. A median joining haplotype network of *X. cancila* from Pakistan, India and Bangladesh.

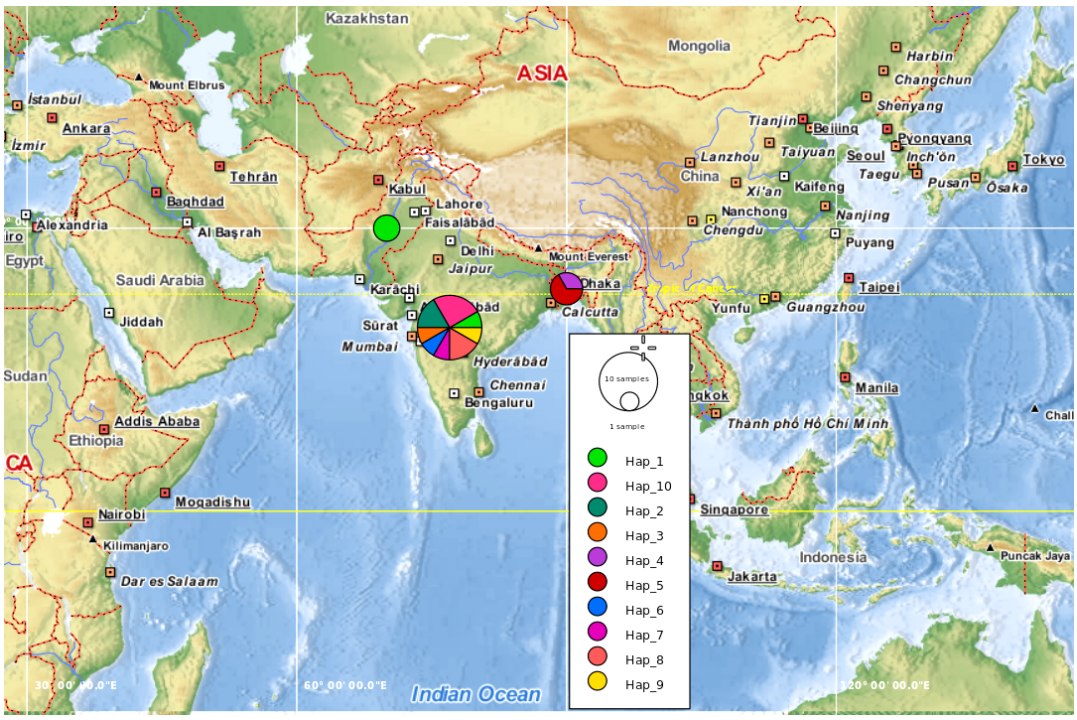

Figure 4. Map of haplotype network of *X. cancila* from India, Pakistan and Bangladesh. A total of 10 haplotypes, the green color haplotype-1 reported from Pakistan, similarly two haplotypes reported from Bangladesh and eight haplotypes reported from India.

length-weight relationships (Chandrika & Balasubramonian, 1986), reproduction biology (Yamamoto & Tagawa, 2000) and a study of the gills parasite (*Trichodina cancilae*) of a freshwater gar *X. cancila* (Asmat, 2001).

In this study, we present the first morphological and molecular record of *X. cancila* from the River Indus in Swabi District, Khyber Pakhtunkhwa, Pakistan. We collected a total of 10 specimens of *X. cancila* were collected from four different sites: Tarbela, Hund, Nabi and Kund Park along the River Indus. All specimens were found morphologically similar as the characteristics were described by Hamilton (1822), Mayer (1960), Talwar and Jhingran, (1991), Jayaram (1999) and Mirza (2003).

Exos cancila (*X. cancila*) was first morphologically reported by Hamilton (1822) from River Ganges. This specie was characterized as their elongated slender body, reaching up to one feet in length, beak-like jaws with sharp teeth, small yellow eyes, a greenish color on top with silver below, distinctive black spots on its tail fin and fins with varying numbers of rays. Previously this species were identified under various synonyms including *Beloni graii, Exos indica, Xenentodon canciloides, Exos hindostonicus Belone cancila,* and *Belone beloni* (Falconer, 1868; Sykes, 1839; McClelland, 1842; Bleeker, 1853; Day, 1877; Sterba, 1962). Regan (1911) reclassified later and changed the species name from *Exos cancila* to *Xententodon cancila*. Talwar and Jhingran (1991) provided a detailed description of *X. cancila* and highlighted distinctive characteristics such as an emarginated caudal fin, falcate pectoral fins, and scaled bases of the dorsal and anal fins. The fin formula reported was D, 17-21; A, 23-25; P, 10-11; V, 6. Currently, the genus *Xenentodon* is considered to containonly one freshwater species, although some researchers have suggested the possibility of additional cryptic species within the genus (Rainboth, 1996). In this study, we analyzed 30 morphometric characters and conducted statistical analyses including mean, standard deviation, range, correlation coefficients, and regression, to resolve the species ambiguity. Dhanze et al. (2018) studied sexual dimorphism in adult specimens of this species. Males can be distinguished from females of this species based on combination of 16 different morphometric proportions. Hossain (2013) calculated the sex ratio, length-frequency distributions, length-weight relationships, length-length relationships, Fulton's relative condition factors, allometric condition factors and relative weight of *X. cancila* specimens of various body sizes from the Ganges River in NW Bangladesh. Molecular and genetic study on *X. cancila* is limited, only few researchers investigate the genetic aspects of this species, such as Khedkar et al. (2014), Pandey et al. (2016) and Modeel et al. (2024) have provided the phylogenetic record of *X. cancila* from India. Rehman et al. (2019) analyzed the *COI* gene of *X. cancila* from Bangladesh. Panprommin et al. (2019) provided a Phylogenetic record from Thailand. For the first time, we sequenced two *COI* genes of *X. cancila* from two different geographic localities along the Indus River, specifically Tarbela and Kund Park in the Swabi District of Khyber Pakhtunkhwa, Pakistan. We selected two fish samples for molecular characterization and phylogenetic analysis from two regions because these two regions have different geographic and climatic conditions, such as water temperature, food availability and water vegetation. These changes have great impact on aquatic ecosystem, including fish health, distribution and behavior. We aimed to assess how changes in water quality, water temperature, and vegetation affect the fish populations. The phylogenetic analysis of *COI* genes of *X. cancila* from Pakistan showed 100% similarity with *X. cancila* population reported from India. For further confirmation, we added median joining network and haplotype diversity to investigate the intraspecific relationships of *X. cancila* from Pakistan with other neighbor countries. The present study provided a baseline to the understanding of *X. cancila* diversity and distribution in Pakistan and highlighting its significance in freshwater fish research.

CONCLUSION

This study was aimed to document the distribution of *X. cancila* based on morphological characters and *COI* identification. Our results based on maximum-likelihood inference assess the taxonomic status of the sample obtained from Pakistan. We provided first genetic record of *X. cancila* from two different locations along the Indus River: Tarbela and Kund Park in Swabi District. These findings provide baseline data for *Xenentodon* genus and its distribution in other regions of Pakistan.

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