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ECOLOGY AND MOLECULAR CHARACTERIZATION OF SOL FISH (*CHANNA MARULIUS)* IN DISTRICT SWABI, KHYBER PAKHTUNKHWA, PAKISTAN

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Abstract

Channa marulius commonly known as Sol fish is distributed across various countries of the south Asian subcontinent. The natural habitat of *Channa marulius* includes standing or slow-moving water in swamplands, water channels, lochs and deep water lagoons in rivers. These carnivorous fishes thrive in environments rich in submerged aquatic vegetation. The study was conducted to study the ecology and molecular characterization of *Channa marulius* in district Swabi, Khyber Pakhtunkhwa, Pakistan. The ecological factors such as water temperature and its quality play vital role in the distribution and population density of *Channa marulius.* Water quality parameters such as dissolved oxygen (DO), pH and availability of prey fauna are closely linked to its distribution, population density and its survival rates. A total of 200 fish specimens were collected by using hand nets, dip net and cast net from five different locations of Swabi (Garh-munara, Batakra, Zarobai, Hund and Allader). Morphometric parameters such as total length, head length and body depth were measured while meristic traits including dorsal and anal fin rays, lateral line scales and ventral fin rays were also recorded. Blood and tissues samples were collected from each fish for further genomic analysis. DNA was extracted and amplified using PCR with species-specific primers. Molecular characterization was performed by sequencing mitochondrial Cytochrome Oxidase subunit I (COI) gene. Two species of genus Channa named *Channa marulius* and *Channa punctatus* were identified. Phylogenetic analysis confirmed the presence of *Channa marulius* in district Swabi

with 97% similarity with the sequences of the *Channa marulius* from other countries validating its classification and regional presence. The results highlight the ecological significance and growing demand for *Channa marulius.* This study provides a foundational molecular database for *Channa marulius*, supporting future research on genetic aspects of this fish species, its conservation strategies and aquaculture development in the region of Khyber Pakhtunkhwa Pakistan.

Key words: Sol Fish, *Channa marulius,* Habitat, Distribution, Genomic analysis, Pakistan

1. Introduction

The aquatic fauna specially fish play a vital role in economic growth. The *Channa marulius* locally known as 'Sol Fish' is considered as the largest among the murrel species. It inhabits the larger lakes, rivers, canals, and other aquatic habitats along with other species of same genus (Dua A and Kumar K.,2006). It is widely recognized as economically important species having great potential for aquaculture and capture fisheries throughout the southern and southeastern Asia (Barman *et al*., 2023). Some species of genus Channa are highly priced and popular in the ornamental fish trade such as (*Channa barca* and *Channa stewartii*) and consumption of some snakeheads is considered therapeutic for wound healing after trauma and surgical procedure including cesarean sections (Rosmawati *et al*., 2018).

In Asia the genus Channa, currently contains 26 well known species which are having wide distribution in Iran, southern Asia and the Far East (Li *et al*.,2006) and have been reported even at an altitude of 475 m above mean sea level (Bhatti *et al*., 2014).Members of this group commonly inhabit rivers, swamps, ponds, canals, drains, reservoirs and rice fields of southern Asia, Southern China, Indo-China and Sunda Islands (Zuraini *et al*., 2006; Hossen *et al.,* 2019).

Feeding nature is carnivorous so this behavior feeding makes them an important element of fish farming which need a keen understanding of their biology and ecological requirements. The literature indicates that lots of work has been done on *Channa striata* and *C. punctatus* with respect to their breeding frequency, parental care, reproductive capability and biology. However, very little literature and knowledge is available regarding *C. marulius* (Dua and Kumar, 2006). These fish can attain maximal length of 183 cm and a weight of 30 kg approximately (Yadav *et al*., 2016).

Molecular phylogeny becomes an efficient tool for systematic ichthyologist for species identification or taxonomic ambiguities. A fragment of 655bp of the mitochondrial COI gene has been proposed as a universal barcode for eukaryotes including fishes (Hebert *et al*., 2003). Recent evidence from better studied taxa suggests that in most cases, DNA Barcoding permits rapid and accurate identification (Zhu *et al*., 2013)

The present study was conducted in district Swabi, Khyber pakhtunkhwa Pakistan characterised by diverse ecological and hydrological features. Geographically, swabi lies between the two major rivers, Kabul and Indus, positioned between 34°-23′ & 33°- 55′ North latitudes and 72°-49′ and 72°-13′ East longitudes. Climatically swabi experience peak rain fall in March (up to 160 mm) and during the monsoon season from July to August.It is bounded in the North by Buner, on the East by Haripur in the South by Attock and on the West by two districts named as Mardan and Nowshera. The total area of the district is 1543 km2 with an Altitude 316 m ASL. The famous Tarbela Dam and electricity power house is located here. (Fawad *et al*., 2013)

The present study investigated the ecological status, molecular characterization and phylogenetic analysis of *Channa marulius* species. Recognised for its high growth potential and commercial value, *Channa marullius* is considered an important candidate for aquaculture and biodiversity research. To assess its population genetics and confirmation of its species identity, partial sequencing of the mitochondrial cytochrome c Oxidase subunit I (COI) gene was performed. The study focused on specimens collected from district Swabi, Khyber Pakhtunkhwa (KP) Pakistan.

2. Materials and Methods

2.1. Study area

The fish were collected from 5 different localities of river Indus i.e; Garh munara (34°01'44"N, 72°30'49"E), Zarobai (34°01'38"N, 72°33'43"E), Batakra (34°02'02"N, 72°36'01"E), Hund (34°00'57"N, 72°25'58"E) and Allader (33°57'06"N, 72°16'12"E) district Swabi, Khyber Pakhtunkhwa, Pakistan. Geographical Information System (QGIS 3.28.13 'Firenze version) was used for mapping **(Figure 1)**.

2.2. Study duration

The study was conducted 1st may 2022 to 30 august 2022.

2.3. Sampling procedure

A total of 200 fish were collected from targeted localities using hand net, meshed cast nets (Kumar and Dua, 2006). Nets were set up at random spots at the study areas. The collected fish specimens were transported in 50 liters plastic drums to Fish Pathology Research Laboratory (FPRL), College of Veterinary Sciences and Animal Husbandry (CVS & AH), Abdul Wali Khan University, Mardan, Khyber Pakhtunkhwa, Pakistan. The samples were reared in aquarium (width=2feet, height=3 and length= 4feet) for further analysis.

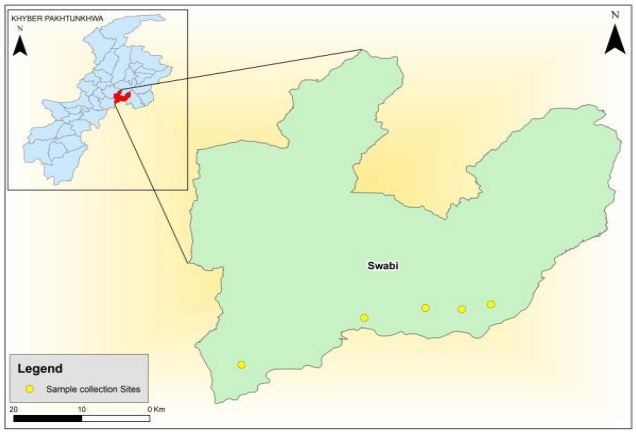


Figure 1: Geographical map of study area, district Swabi, Khyber Pakhtunkhwa, Pakistan.

Table 1: Total number of fish collected from district Swabi, Khyber Pakhtunkhwa, Pakistan

S.No	**Locations**	**Altitude**	**Number of fish collected**
1	Garh munara	34°01'44"N, 72°30'49"E	62
2	Batakra	34°02'02"N, 72°36'01"E	21
3	Zarobai	34°01'38"N, 72°33'43"E	37
4	Hund	34°00'57"N, 72°25'58"E	29
5	Allader	33°57'06"N, 72°16'12"E	51
Total samples			**200**

2.4. Morphometry and Morphological identification

The morphological identification and classification were executed through standard taxonomic keys followed (Ahmed *et al*., 2018) **(Figure 2).**

2.5. Fish morphometric measurements

In the laboratory, a total of 10 morphometric measurements were recorded each by using ordinary scale. The morphometric measurements included total Length, Standard Length, Pre-orbital length, Head Length, Eye diameter, Body depth, Pre-anal length, pre dorsal length, pre-pelvic length, pre-pectoral length (cm) and body weight (gm), (Yousafzai *et al*., 2013) and (Rosmawati *et al*., 2018).

2.6. ***Blood and tissue sampling***

Blood and tissues samples were collected from *Channa marulius* specimens for molecular analysis. Blood was drawn from its peritoneal region using 5cc sterilized syringe and immediately transferred to the EDTA tubes to prevent coagulation. These samples were then stored at -20 ºC to preserve DNA integrity. Tissue samples were carefully dissected, chopped, homogenized and preserved in TNES-urea (100 mM Tris-HCl pH 7.5, 125 mM NaCl, 10 mM EDTA pH 7.5, 1% SDS, 3 M urea), following a modified protocol based on (Yousafzai *et al*., 2013). For long term preservation, tissue samples were also stored in 95% ethanol prior to DNA extraction.

2.7. **Molecular analysis and DNA Extraction**

2.7.1. **DNA Extraction**

DNA was extracted using organic phenol chloroform method as described by (Rakhi *et al*., 2023). The concentration and purity of isolated DNA were assessed using Spectrophotometry and 1% agarose gel electrophoresis. The extracted DNA samples were stored at -20°C for further analysis (Barman *et al*., 2023).



Figure 2: Morphology of Sol fish (*C. marulius)* lateral side(A) and dorsal side(B)

2.7.2. Primers used

The Following primers including and Forward primer FishF1 (5'-TGATCCGGCTTAGTCGGAACTGC-3') and Reverse primer FishR1 (5'GATGTGTGTTGAATTACGGTCGG-3') were used which are considered as universal primers (Ward *et al.*, 2005).

2.7.3. **Polymerase Chain Reactions (PCR)**

Polymerase chain reaction (PCR) was carried in a 50µL reaction mixture comprising 22.5µL Prime STAR Max DNA polymerase, 22.5µL double distilled water, 2µL DNA template and 1.5µL each of forward and reverse primers. The thermal cycling conditions included an initial denaturation temperature of 95°C for 2 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 30 seconds. Final extinction was carried out at 72°C for 5 minutes. PCR products were then analyzed on 1% agarose gel electrophoresis stained with 0.5 µg/ml ethidium bromides for 85-95 min at 80v in 1X TBE buffer (90mM Tris-HCL, 90 mM Boric Acid, 2 mM EDTA) and DNA bands were visualized under ultraviolet (UV) light using as transilluminator, following the protocol of (Ghatee *et al*., 2013)**.**

DNA amplification, Sequencing and Phylogenetic analysis

The amplified PCR products were purified using the Gene Clean II Kit according to the manufacturer instructions. The purified PCR products were sent to First BASE Laboratories SdnBhd, Malaysia for sequencing. The sequencing data from the chromatograms were curated and converted into FASTA format. A BLAST search of the COI sequences was performed using nucleotide database of National Center for Biotechnology Information (NCBI) to determine the closest homologus matches. The barcodes determined in this study were submitted to the GenBank/EMBL database under the Accession No. PP266703. Evolutionary analyses of the aligned sequences were conducted in the program MEGA X (Kumar *et al*. 2018). Based on the Kimura 2-parameter (K2P) model, Neighbour joining (NJ) and Maximum parsimony (MP) trees were constructed using MEGA 5.05 (Kumar *et al*., 2018) with *Channa punctatus* from NCBI (GenBank Accession No. HQ611124) as an out group. Node support for the two approaches (NJ and maximum likelihood [ML]) was inferred with bootstrap analysis (1000 replicates). (Zhu *et al*., 2013)

2.8. **Statistical Analysis**

For the statistical analysis of data, SPSS software version 16.0 (SPSS) was used for the calculation of different relations. Different statistical assessment including one-way ANOVA and standard deviation, were found to find the relations among different variables and there effect on each other (Song *et al*., 2013).

3. Results

A total of 200 Fish samples of *Channa marulius* were collected from five different locations named Garh munara, Zarobai, Batakra, Hund and Allader. The locations targeted for the collection of fish speciemens were natural flowing fresh water bodies. It included medium shallow and some deep water bodies with sandy, rocky and muddy bottoms along with rocky sandy banks. This specific species inhabit the muddy water lochs and drains located under the flowing Indus River. All collected specimens were belonging to the family Channidae. Among these five points the population of *Channa marullius* was 60% in Garh munara, 55% in Zarobai, 45% in Batakra, 50% in Hund and55% in Allader. In Garh munara the high population density is due to the presence of large number of deep loches and these fishes prefer these areas to live in **(Figure 3)** (Rakhi *et al*., 2023).

As *Channa marulius* tends to underground in winter, the samples were collected in mid-summer season when the aquatic conditions were favourable. At Allader, water temperature ranged from 25-30°C with pH 7.2-8, Total Suspended Solids (TSS) 85 mg/L, Water Conductivity 169.25 µS/cm, TDS 125 mg/L, Dissolved Oxygen (D.O) 6.5 ppm. In Hund temperature was 23-25°C, pH 7.1-8, (TSS) 79 mg/L, conductivity 165.65 µS/cm, TDS 120 mg/L, D.O 6.4 ppm. Garh munara recorded 24-27°C, pH 7-8, TSS 80 mg/L, conductivity 163.35 µS/cm, TDS 126 mg/L and D.O 6.1 ppm. Zarobai showed 25-30°C, pH 7.4-8.2, TSS 87 mg/L, conductivity 167.35 µS/cm, TDS 125 mg/L and D.O 6.4 ppm. Batakra had 26-30°C, pH 7.2-8.1, TSS 87 mg/L, conductivity 169.75 µS/cm, TDS 121mg/L and D.O 6.7 ppm. These water quality parameters affected the availability and distribution of *Channa marulius* across the sampling sites.

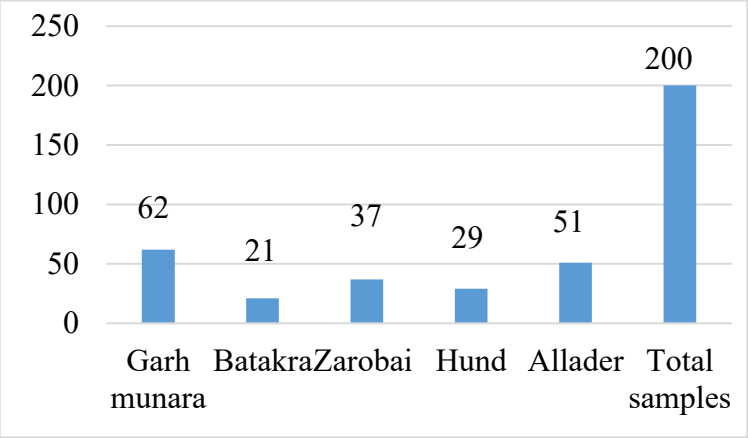


Figure 3: Number of Fish collected from study area, district Swabi Khyber Pakhtunkhwa, Pakistan

The elements of the Habitat in these five different collection sites differ greatly along with the pollution level caused by sewerage system and industrial waste accumulation, as a result of which the quality and number of fish were affected greatly. The availability of flora and fauna along with water quality parameters affect the weight and length of fish greatly. But overall conditions of the water quality were favourable for culture of fish species. Water quality parameters measured during study period **(Table 2).**

Measurements of water quality parameters

Water temperature was checked by Ordinary Centrifuge Thermometer. PH was calculated by pH meter whose model is (Mettler Delta 320. Halstead, C09 2dx, England. Model 320. Made in UK). Dissolve Oxygen (D.O) was measured by D.O meter (WPA, ox20, UK). Water Conductivity and total dissolved solid (TDS) was measured by conductivity meter and total dissolved solid meter (Model 4520, Jenway, UK). For Water conductivity, the water temperature was fixed at 25°C. Total suspended solid (TSS) was measured by evaporating 100ml sample of water on water bath, were dried in oven (Mammert 854, West Germany), and weighted through digital balance (Sartorious Germany, ME 414s, 0.0001g). Total Solids (TS) was calculated by using formula (Total Solid = Total Dissolved Solid + Total Suspended solid) (Khan *et al*., 2014).

Morphometric measurements of specimens

Measurement of morphometric characters were conducted using Vernier capilers and fish board measurement (Rosmawati *et al*., 2018).

Table 2: Effect of water quality parameters on growth of Sol fish (*Channa marulius)* in district Swabi, Khyber Pakhtunkhwa, Pakistan

S.No	Water quality parameters	Allader	Hund	Garh Munara	Zarobai	Batakra
1	Temperature (°C)	26.5±72	25.3±25	25.46±62	27.2±51	27.3±39
2	PH	7.2±0.02	7.6±0.03	7.8±0.02	7.8±0.02	7.2±0.0.1
3	Total Suspended Solids (TSS) (mg/L)	85±29	79±34	80 ± 56	87±40	86 ±41
4	Water Conductivity (µS/cm)	169.2±56	165.6±76	163.3±62	167.3±48	169.7±41
5	TDS (mg/L)	125±7.1	120±7.8	126±5.8	125±6.9	121±3.5
6	Dissolved Oxygen(DO) (ppm)	6.5±0.50	6.4±0.41	6.1±0.61	6.4±0.72	6.7±0.24

Values are mean ±S.D

Table 3: Morphometric parameters of fish collected from five different regions of district Swabi, Khyber Pakthunkhwa, Pakistan

S.No	Morphometric parameters(cm,Average)	Sample Collection sites				
		Garh munara	Batakra	Zarobai	Hund	Allader
1	Total Length (cm)	32.56	22.45	29.56	27.65	24.34
2	Standard Length	28.22	19.64	25.6	23.40	21.10
3	Pre-orbital length	1.61	1.11	1.47	1.36	1.20
4	Head Length	7.94	5.47	7.21	6.74	5.93
5	Body depth	4.1	2.85	3.75	3.51	3.09
6	Eye Diameter	0.97	0.67	0.88	0.82	0.72
7	Pre-anal length	14.6	10.1	13.3	12.4	10.8
8	Pre-dorsal length	9.21	6.35	8.36	7.82	6.88
9	Pre-pelvic length	8.88	6.12	8.06	7.54	6.64
10	Pre-pectoral length	8.14	5.61	7.39	6.91	6.08
11	Body weight (gm)	229.56	103.65	204.74	183.87	133.5

The mentioned values are mean of the all the values calculated from the total sample collected from five different localities.

Morphological identification and Meristic counts of *Channa marulius*

The coloration of *Channa marulius* observed was grayish green with slight light coloration at the ventral side, adults showing 6-7 cloudy bands below the lateral lines along with large black eyespot (ocellus) with white edges at upper part of base of caudal fin (Plamoottil, 2017). Meristic counts of *Channa marulius* shows: D- 46-49; P- 18-19; V-6-8; A- 29-37; C- 14-16, LLS- 62-69 (Mirza and Sandhu, 2007) (Plamoottil, 2017).

List of Flora and Fauna collected from five different collection points in district Swabi

The fish species collected from samples collection points were categorized based on their growth position relative to water surface (habitat) as floating, emergent, free floating, immersed and swampy plants. Specimen identification was carried out using the *Flora of Pakistan*, online botanical resources and expert consultation from the Department of Botany, Abdul Wali Khan University Mardan Khyber Pakhtunkhwa Pakistan **(Table 4)**.

Sequence and phylogenetic analysis

A sequences of 20 nucleotides with 678-679bp were obtained in this research activity which showed 97% identity in nucleotide sequences. A total of 10 sequences of sole fish mitochondrial cytochrome c oxidase subunit I (COI) gene were downloaded from NCBI and were compared to the experimental nucleotide sequence with showing 97-100% similarity with (KX389280.1, MF496842.1, KJ847102.1, MF496849.1, MF496846.1, KY425544.1, MN939751.1). The sequence generated in this study was submitted to GenBank PP266703. In phylogenetic analysis the sequence was compared with *Channa punctatus*.

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model. The tree with the highest log likelihood (-1444.88) **(Figure 5)**. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. This analysis involved 11 nucleotide sequences. There were a total of 683 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

Table 4: List of Flora collected from five different regions of district Swabi, Khyber Pakthunkhwa, Pakistan

S/N	Scientific Names	English Names	Family	Habitat	Collection Points	Abundance	Plant Type
1	Alternanthere sassilis L.	Sessile joyweed	Amaranthaceae	River banks	Garhmunara,Allader, zarobai	Common	E
2	Salix tetrasperma Roxb.	Willow tree	Salicaceae	River banks	Allader,zarobai,batakra,Hund	Rare	E
3	Potamegeton crispus L.	Curly leaf pondweed	potamogetonaceae	Running water	Garh munara,allader,zarobai,Hund	Common	S
4	Eichornia crassipes (Mart.) Solms.	Water Hycinth	pontederiaceae	Running water	allader,zarobai,batakra,Hund,Garh Munara	Very common	FF
5	Marsilea quadrifoliata L.	Pepperwort water clover	Marsileaceae	Standing pond water	allader,zarobai,batakra	Very Common	AF
6	Cyperus brevifolius (Rottb.) Hassk.	globe kyllinga	Cyperaceae	River and stream banks	allader,zarobai,batakra,Hund,Garh Munara	Very Rare	E
7	Apluda mutica L.	Mauritian grass	Poaceae	Irrigation channel	allader,zarobai,batakra,Garh Munara	Rare	E
8	Lemna azolla L.	Duck weed	Lemnaceae	Stagnant pond	allader,zarobai,batakra,Garh Munara	Abundant	FF
9	Sagittaria latifolia Willd	Duck Potato	Alismataceae	Stagnant water	zarobai,batakra,Garh Munara	Rare	M

E = Emergent, S = Submerged, F = Floating, AF = Anchored Floating, M = Marshy, FF = Free Floating

At the time of fish sample collection, different fauna were also observed at the collection sites.

Table 5: List of Fauna collected from study area, district Swabi, Khyber Pakthunkhwa, Pakistan

S.No	Genus	Species	Abundance
1	Schizothorax	*Schizothorax plagiostomis*	Very Common
2	Nangra	*Schizothorax Labitous*	Common
3	Tor	*Tor putitora*	Common
4	Osteochilus	*Osteochilus lini*	Rare
5	Crossocheilus	*Crossocheilus diplocheilus*	Common
6	Agabus	*Agabus bipustulatus*	Common
7	Corixa	*Corixa affinis*	Very common
8	Allacanthos	*Allacanthos yawi*	Common
9	Schistura	*Schistura nagodiensis*	Less abundant
10	Channa	*Channa Striata*	Common
11	Corixa	*Corixa affinis*	Rare
12	Euphlyctis	*Euphlyctis cyanophlyctics*	Very common
13	Metrobates	*Metrobates trux*	Common
14	Macrognathus	*Macrognathus Lacepede*	Less common
15	Cyprinus	*Cyprinus carpio*	More common
16	Osteochilus	*Osteochilus lini*	Common
17	Carassius	*Carassius Carassius*	Very common
18	Cyprinella	*Cyprinella spilopter*	Common
19	Labeo	*Labeo Calbasu*	Very common
20	Channa	*Channa marulius*	Less common



Figure 4: Fauna collected from study area, district Swabi ,Khyber Pakhtunkhwa, Pakistan.

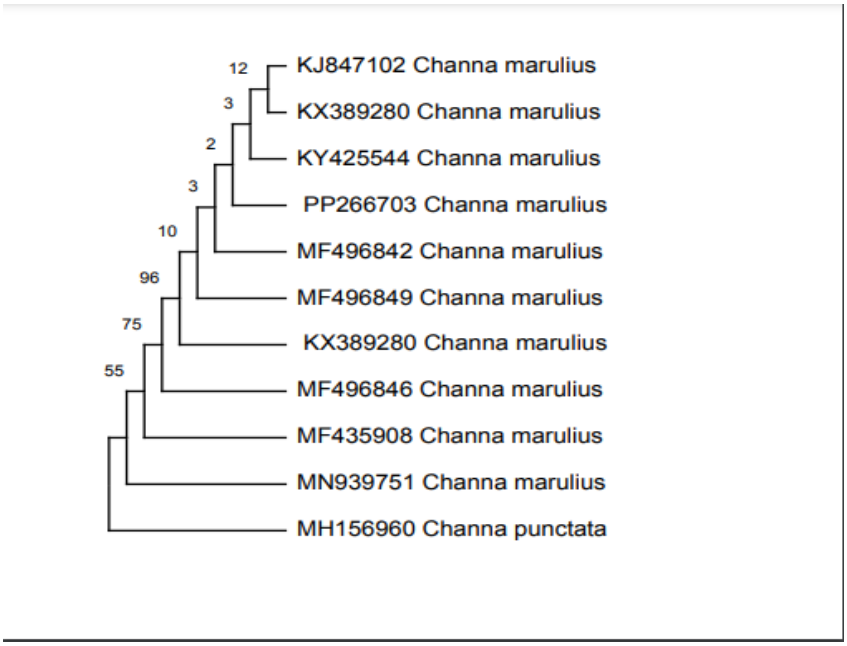


Figure 5: Evolutionary analysis by Maximum Likelihood method and Tamura-Nei model

4. Discussion

Analysis of amplified PCR products was performed by gel electrophoresis with clear bands of DNA on 1% agarose gel. Results were entered in GenBank/EMBL under Accession no. PP266703 and evolutionary analysis was conducted. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Ahmed.M *et al*., 2018). The tree with the highest log likelihood (-1444.88) revealed that next to the branches is the proportion of trees where the related taxa were clustered together. By employing the Neighbor-Join and BioNJ algorithms on a matrix of pairwise distances calculated using the Maximum Composite Likelihood (MCL) technique, the initial tree or trees for the heuristic search were automatically generated. The topology with the highest log likelihood value was then chosen. There were eleven nucleotide sequences in this investigation. The final dataset contained 683 locations in total. In MEGA X, evolutionary studies were performed (Miyan *et al*., 2014)**.**

Maximum population density (60%) of *Channa marulius* was observed in Garh munara, followed by 55% in Zarobai and Allader, 50% in Hund, and least 45% in Batakrra. Garh munara region was

preferred by the fishes to live in due to the presence of large number of deep loches. Using the conventional procedures listed in **(Table 2)**, various water quality parameters, including pH, temperature, dissolved oxygen, total suspended particles, total dissolved solids, and water conductivity, were measured. A higher impact on fish availability as a percentage was observed as a result of variations in water quality characteristics. Fish culture thrives in warmer climates, such as those experienced in the middle of summer. Pollution level along with flora and fauna at different sites vary greatly which has directly affected the quality and number of fish at each location. But overall conditions of the water quality were favourable for culture of fish species. Flora **(Table 4)** and fauna (**Table 5)** were recorded at different sites.

Morphometric measurements of fish with greatest total length, standard length, pre-orbital length, body depth, head length, eye diameter, pre-anal length, pre-dorsal length, pre-pelvic length, pre-pectoral length, and highest body length were observed in Garh munara region followed by Zarobai, Hund, Allader, and Batakra. **(Table 3)**. Observed coloration of *Channa marulius* was grayish green with slightly light coloration at the ventral side. Adult fish show 6-7 cloudy bands below the lateral lines along with large black eyespot (Ocellus) with white edges at upper part of base of caudal fin (Plamoottil, 2017). Meristic counts of *Channa marulius* shows D- 46-49; P- 18-19; V-6-8; A- 29-37; C- 14-16, LLS- 62-69 (Mirza and Sandhu, 2007) (Plamoottil, 2017).

In past, the only tools used for identification of fish were morphometric and meristic characters. But fishes, especially Channidae are difficult to differentiate due to similar external morphological features (Khan *et al*. 2013, Miyan *et al*. 2014). In the present study, initial identification of *Channa marulius* was based on morphological and meristic characters. To get rid of ambiguity in identification of *Channa marulius*, mitochondrial COI gene was used for identifying fish in the present study as it serves as a universal barcode as (Ali *et al*., 2020). Therefore, because of the intricate evolutionary changes in their morphological and physiological characteristics, the creation of phylogenetic trees based solely on morphology is contentious. In Pakistan, there is a wide range of snakeheads (Channadie), and it might be difficult to distinguish them just by looking at their morphological traits. Worldwide, there have been numerous reports of Channidae species being mislabelled (Zhu *et al*., 2013). In this experiment, results obtained from morphometric, meristic and molecular characterization were similar, thus clearing out the ambiguities.

Further research can use these sequences to discover the genetic difference of Channa species that are dispersed throughout various geographical regions. In order to help them in survival and conservation in case of threat of extinction, Pakistan must help to regulate their number in the wild. It is important to instruct locals and fishermen about the need to protect these species. Therefore, molecular identification will be useful to fish experts in understanding these fishes' adaptive radiation and in the near future in producing hybrid species. Additionally, as this fish is highly nutritious and beneficial to health, particularly for mothers struggling with postpartum depression, it's breeding and rearing in in-captive cultural settings such as ponds and bio-floc should be practiced to improve the availability of this fish to the general public.

5. Conclusion

It is concluded from the explored study that the ecology, habitat and distribution of sole fish *Channa marulius* in district Swabi Khyber Pakhtunkhwa Pakistan is confirmed along with their molecular characterization. Two species of sole fish was reported abundant named *Channa marulius* and *Channa punctate.* The molecular characterization at Cox 1 gene and phylogenetic analysis in comparison with other regions around the world shows similarity with *Channa marulius* and *Channa punctate* in Bangladesh, India and China. So as a result of this research the presence of *Channa marulius* in district Swabi is confirmed and it provides a base to explore it further at molecular level. Pakistan As it is a high profile fish with increasing demand by the community hence it provides a gap to study its abundance and presence at other different regions of the Khyber Pakhtunkhwa, Pakistan.

Author's Contribution

Maryam Begum: Investigation, Writing-original draft preparation; **Asad Ullah***: Conceptualization, Supervision, Project administration; **Yousaf Ali:** Methodology; **Raheela Taj**: Formal analysis; **Shumaila Gul**: Resources; **Sulha Syed:** Data Curation; **Imad Khan:** Visualization; Ali Gohar: Writing-review and editing, **Rainaz Begum**: Funding acquisition; **Tahira Tayyeb:** Software; **Mansoor Ahmad:** Data Curation; **Noor Us Saba Shujaat**: Validation.

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Conflicts of Interest

The authors declare no conflict of interest.

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