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Title: **Fish diversity at Head Panjnad and Genetic Identification by DNA Barcoding**

Author (s): Saima Naz[1], Ahmad Manan Mustafa Chatha[2], Durali Danabas[3], Saba Iqbal[1]

Affiliation (s):
[1]Woman University Bahawalpur, Pakistan
[2]The Islamia University of Bahawalpur, Pakistan
[3]Munzur University, Fisheries Faculty, Tunceli, Turkey

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Fish Diversity at Head Panjnad and its Genetic Identification by DNA Barcoding Technology

Saima Naz[1*], Ahmad Manan Mustafa Chatha[2], Durali Danabas[3], Saba Iqbal[1]

[1]Department of Zoology, The Government Sadiq College Woman University Bahawalpur, Pakistan
[2]Department of Entomology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan.
[3] Munzur University, Fisheries Faculty, Turkey.

*Corresponding Author: saima.naz@gscwu.edu.pk

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Abstract

This study was conducted at Head Panjnad, Pakistan to collect information about the available fish diversity. The evident fish orders found were siluriformes, anabantiformes, cypriniformes, cichliformes, osteoglossiformes, and synbranchiformes. The highest number of *Hypophthalmicthys molitrix* and the lowest number of *Wallago attu* were determined from this diversity. Simpson diversity index (D) and Shannon-Weiner index (H) were measured as 0.94938868 and 3.00940719, respectively. After the keen observation of various diversities at Head Panjnad, these fish species were selected for their COI gene and phylogenetic analysis. COI is the Cytochrome C Oxidase subunit 1 of mitrochondria (a gene sequence used in molecular investigations as a DNA barcode). The K2P (Kimura two-parameter) distances measured within species, genus, family, and order were 0.57%, 0.63%, 0.68%, and 0.77%, respectively. The K2P neighbor-joining tree was built on a commonly clustered species sequence in agreement with its taxonomic classification. The purpose was to create QR codes based on DNA sequences for accurate fish species identification. The current work concludes that COI sequencing might be used for fish species identification.

1. Introduction

Panjnad is situated in a region where two rivers namely, Sutlej and Chenab merge in Punjab Province, at the confluence of five rivers. The head Panjnad is further expanded into a network of canals that are mostly used for irrigation purposes. Overfishing, habitat damage, and changes in climate have had substantial effects on the richness and organization of point community. The impact of such changes is quite visible inside the Head Panjnad as human activities have increased.

Human beings consume fish as a source of protein which is commonly used in Complementary and Alternative Medicine (CAM) also known as traditional medicine. Many pollutants found in water tend to affect the growth of fish. Fish accumulate heavy metals from water through their gills and these heavy metals cause acute toxicity in fish [1].The delimitation and

confirmation of the species of fish is crucial not only for logical categorization and systematics, but also for fisheries administration, food verification, and differentiating evidence of CAM (complementary and alternative medicine) components [2-4].

Fish health and fish abundance describe the health of whole aquatic life [5]. Population growth and economic aspects are also infected by a decline in population of fishes and fisheries. Pakistan has presented a number of foreign fish species listed as Common Carp (*Cyprinus carpio*), Silver Carp (*Hypophthalmichthys molitrix*), Grass Carp (*Ctenopharyngodon idella*), Gold Fish (*Carassius auratus*), and Bighead Carp (*Hypophthalmichthys nobilis*). Moreover, 3 species of tilapia as *Oreochromis aureus, Oreochromis niloticus,* and *Oreochromis mossambicus* in warm waters, along with 2 other trout species known as rainbow trout (*Onchorynchus mykiss*) and brown trout (*Salmotrutta fario*) are found in comparatively cooler areas. These specific species are used for particular objectives, such as yield improvement, sport fishing, biological control of mosquitoes, and aquatic weeds [6].

Due to the complexity and restrictions of morphological criteria used in traditional scientific classification, the DNA barcoding technique has gained prominence. The backbone could be universal bio-identification mechanism for living organisms' DNA barcodes based on the nucleotide sequence of the mitochondrial gene cytochrome c oxidase I (COI) [7].

DNA barcodes were developed in fisheries as a quick and precise method of identifying species using a universal primer [8]. By utilizing a verified and standardized DNA-based method, DNA barcoding elevates the chances of species-level identification [9]. FISH-BOL (www.fishbol.org) barcoding project for all fishes is aided by the use of mitochondrial cytochrome c oxidase I (COI) gene sequence for fish species identification [10]. By using molecular tags based on short and authorized mitochondrial genes, DNA barcoding provides a precise and mechanized species identification proof framework [11]. DNA barcoding can evidently assign dark instances to recognized species, while also identifying possible enigma species and hereditarily evicted populations [12]. The assuring consequences sparked international hard work to systematize species screening and speed up the identification of cryptic species.

To ensure long-term survival, fisheries management and organization must be prioritized. One of the earliest and most important challenges in fisheries management is the recognition and verification of point species. The COI diversity was measured, both, inside and across 22 fish species, the majority of which were commercially important species, intending to determine DNA barcoding's effectiveness as a tool for identifying fish species. The DNA standardized tag records produced in the current study would be utilized by the investigators to screen and protect the fish diversity in this region under research.

2. Materials and Method

2.1 Sample Collection

Sample collection was carried out at Panjnad Head Works as shown in Figure 1. Fish (100g) samples were collected randomly during daytime (October-February) using different types of nets available. These nets included common fish

trap nets, drag nets, cast nets, bait hooks, and hand nets. All fish samples were labeled and kept at -20°C. Initial identification and common name of fish species was confirmed with the assistance of common fish catchers and fish sellers. Later on, all species were identified taxonomically [13] and misidentifications were removed by an expert taxonomist using systematic keys.

2.2 Samples of Transportation

Fish samples were carefully transferred to the Zoological laboratory of Government Sadiq Women College and University, Bahawalpur, for further investigation. For further study fish specimens were dissected individually at working Zoology laboratory. By following optimized traditional method, genomic DNA extraction from fish muscles was carried out by applying standard Phenol-Chloroform Isoamyl alcohol (PCI) [14]. DNA confirmation was done by 1% agarose gel electrophoresis. Polymerase Chain Reactions were performed using the universal specie-specific primer pair, that is, FISH F1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ and FISH R1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′. The COI gene was approximately 658 base pairs in length, located in the mitochondrial genome which was amplified using one set of primer [15]. PCR results were checked on 2% Agarose gel electrophoresis. The obtained results were used for making barcodes after the sequencing process. Barcodes were generated by online barcode generator tool.

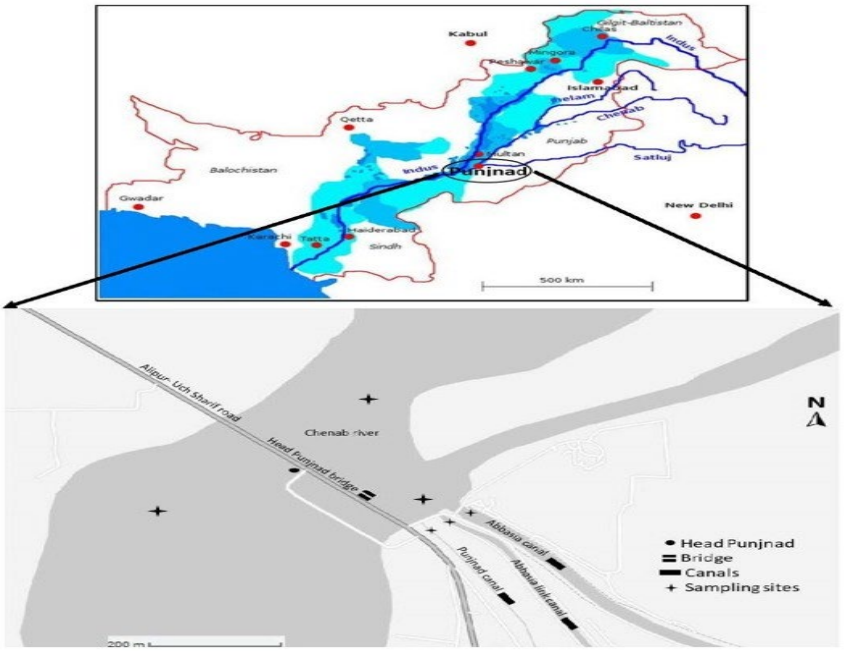

Figure 1. Map of Study Area and Location of Sampling Sites at Head Panjnad, Punjab, Pakistan

Table 1. Fish's Species, Relative Abundance (RA) and GenBank Accession Numbers

Order	Family	Genus/Species	Local name	N	RA(%)	FO(%)	GenBank Accession Numbers
Siluriformes	Schilbeidae	*Eutropiichthys vacha*	jhali	19	1.54	41.6	MK5721193.1
	Siluridae	*Wallago attu*	mulli	6	0.48	16.6	MZ461934.1
	Bagridae	*Rita rita*	Desi khagga	46	3.74	66.6	KF670723.1
		Sperata seenghala	singhari	39	3.17	50	MN171374.1
	Sisoridae	*Bagarius bagarius*	mujahid	55	4.47	75	KT983411.1
Anabantiformes	Chinnidae	*Channa marulius*	soul	53	4.31	75	HQ680827.1
		Channa punctata	Daula/ gudo	44	3.58	58.3	AB196280.1
Cypriniformes	Cyprinidae	*Catla catla*	Thaila	69	5.61	91.6	JQ87872.1
		Cirrhinus mrigala	Mori	58	4.72	83.3	JQ231112.1
		Cyprinus carpio	Gulfaam	46	3.74	75	KM492734.1
		Labeo rohita	Rohu	47	3.82	50	MN533986.1
		Labeo calbasu	Kalbans	55	4.47	75	MZ504855.1
		Labeo goninus	Sereeha	51	4.15	66.6	KT001152.1
		Labeo boga	Bhangan	54	4.39	75	AP013338.1
		Ctenopharyngodon Idella	Grass carp	60	4.88	83.3	MG570437.1
		Hypopthalmicthys molitrix	Silver carp	74	6.02	91.6	KP013119.1
		Hypopthalmicthys nobilis		26	2.11	66,6	KJ756343.1
Cichliformes	Cichlidae	*Oreochromis niloticus*	Nile tilapia	73	5.94	75	GU477627.1
		Oreochromis mossambicus	chira	67	5.45	75	AF317234.1
Osteoglossiformes	Notopteridae	*Notopterus notopterus*	But pari	66	5.37	75	AP008925.1
		Chitala chitala	Cheetal pari	60	4.88	66.6	AP008922.1
Synbranchiformes	Mastacembelidae	*Mastacembelus armatus*	Baam	17	1.38	25	KJ184553.1

2.3 Diversity and Relative Abundance

The relative abundance of all fish species was calculated by applying the specific formula given as: R.A (%) = ni/N×100

Here, 'ni' denotes the number of individuals of a particular fish species in a sample, whereas 'N' represents the total number of individuals across all fish species (calculated relative abundance shown in Table 1). Further diversity indices, that is, Shannon-Weiner index (H) and Simpson diversity index (D) were also calculated using the formula mentioned below:

$$H' = -\sum_{i=1}^{s} \frac{ni}{N} ln \frac{ni}{N} \quad ; \quad D = 1 - \frac{\sum_{i=1}^{s} ni(ni-1)}{N(N-1)}$$

Here 'ni' signifies individual's number and 'N' indicates total species number.

2.4 Data Analysis

Nucleotide sequences were aligned by BLAST, (Basic Local Alignment Search Tool), whereas sequencing was performed using MEGA X (version 10.1). The degree of sequence variations across the species was assessed by the average pair-wise comparison of sequence variation throughout all the individuals. The COI gene sequences of the selected 22 individuals were used to align a total of 642 base pairs. Using the software application, that is, MEGA X (Molecular Evolutionary Genetics Analysis) [16], pair-wise evolutionary distance between haplotype was calculated by K2P [17] method. The determined parameters were mentioned as the nucleotide diversity (pi), number of transitions, and transversion between species, Evolutionary Divergence between the sequence's nucleotide composition, and Maximum likelihood estimation of substitution Matrix. MEGA X was used to create a Neighbor Joining (NJ) and Maximum Parsimony (MP) tree. Bootstrap analysis with 1,000 pseudo replicates was used to test the resilience of the interior nodes of NJ and MP trees [18].

2.5 Evolutionary Pathway and Phylogeny Determination

The neighbor-joining method was used to create a phylogenetic tree after eliminating non-coding areas and modifying sequences. The greatest composite likelihood technique was also used to determine the rate of nucleotide substitution in the bases, that is, pyrimidine and purine. The process of resampling and tree reconstruction was replicated 500 times for the NJ method to determine the bootstrap probability of each tree. The occurrence frequency was also used to determine the bootstrap probability of each tree.

In order to conduct the current study, a total number of 22 species belonging to Phylum Chordata, Class Actinopterygii 6 Orders Siluriformes, Anabantiformes, Cypriniformes, Cichliformes, Osteoglossiformes, and Synbranchiformes were detected. Moreover, 9 families namely Schilbeidae, Siluridae, Bagridae, Sisoridae, Channidae, Cyprinidae, Cichlidae, Notopteridae, and Mastacembelidae were also detected.

3. Results

The current study was conducted in two steps. In the first step, diversity of different fisheries was determined at Head Punjnad district Bahawalpur Punjab, Pakistan. During the investigation 22 species were detected belonging to Phylum Chordata, Class Actinopterygii 6 Orders Siluriformes, Anabantiformes, Cypriniformes, Cichliformes, Osteoglossiformes, and Synbranchiformes. Moreover, 9 families including Schilbeidae, Siluridae, Bagridae,

Sisoridae, Channidae, Cyprinidae, Cichlidae, Notopteridae, and Mastacembelidae were detected as well. However, the maximum specimens observed were of the family Cyprinidae, that is, 540 specimens and minimum specimens observed were of the family Mastacembelidae, that is, 17 specimens. The Shannon-Wiener index (H) and Simpson's diversity index (D) for Head Panjnad canal were 3.00940719 and 0.94938868 respectively. Total number of 1085 species was observed at Head Panjnad. The relative abundance RA% of these fisheries was calculated to be 88.22%. In the second step of the current study, phylogenetic analysis was carried out. 22 diverse species were selected for phylogenetic analysis and identification was performed by COI gene. Total number of 22 Mitochondrial Cytochrome C Oxidase (COI) barcodes sequences, which belonged to 6 orders, 9 families, and 16 genera were downloaded from NCBI. The accession numbers of downloaded species are shown in Table 1. Sequences' consensus length was measured to be 630 after editing and in any sequence no insertion, deletion, and stop codons were observed.

N= Number of fish samples

RA (%) = Relative Abundances

FO (%) =Frequency of occurrences

Cumulatively, 22 gene sequences were produced from freshwater fish species. For entire dataset, the analysis of Nucleotide pair frequency revealed that 39 of 957 (4.07%) sites were conserved, 756 of 957 (78.99%) sites were variable, 689 of 957 (71.99%) sites were parsimony informative, and 65 of 957 (6.79%) singleton were present. The average number of identical pairs (ii) was 190, with a si/sv (R) ratio of 0.5 for the given data. The transversional pairs (sv=240) were observed to be more reoccurring than the transitional pairs (si=127). The analysis of Nucleotide base frequencies (Table 2) was also revealed and the overall observed mean for these sequences was (T =22.9%), (C=24.7%), (A=33.0%), and (G=19.5%). This analysis of base composition for COI sequences manifested that the A content average was high, while the average G content was low, and the AT content 36% was greater than the GC content (22%). The use of T (17 %) was lowest at the location of first codon, and the usage of other nucleotide bases was A (24.40%), G (25.20%), and C (26.00%). At the location of second codon, A usage was highest, that is, (24.20%), while the other base usage was T (20.42%), G (19.30%), and C (15.00%). Lastly, at 3rd codon position the content of C (31.10%) was highest, while the use of other bases was given as: G (27.00%), A (16.00%), and T (9.50%).

Table 2. Analysis of Nucleotide Composition

Species	T	C	A	G	Total
Eutropicthys vacha	21.3	24.3	32.7	21.7	630
Wallago attu	21.0	23.0	38.9	17.1	630
Rita rita	20.5	22.5	39.5	17.5	630
Sperata seenghala	22.1	25.7	32.1	20.2	630
Bagarius bagarius	22.9	24.4	33.5	19.2	630
Channa marulius	19.7	25.2	33.0	22.1	630

Species	T	C	A	G	Total
Channa punctata	19.4	26.0	32.5	22.1	630
Catla catla	30.8	23.5	30.2	15.6	630
Cirrhinus mrigala	32.5	21.9	31.7	13.8	630
Cyprinus carpio	30.5	22.9	31.3	15.4	630
Labeo rohita	32.4	19.0	35.7	12.9	630
Labeo calbasu	20.5	25.4	32.4	21.7	630
Labeo goninus	30.8	22.9	31.0	15.4	630
Labeo bata	19.7	26.0	33.0	21.3	630
Ctenopharynodon Idella	19.0	26.5	32.1	22.4	630
Hypopthalymicthys molitrix	19.5	25.7	31.7	23.0	630
Hypopthalymicthys nobilis	19.5	26.2	32.4	21.9	630
Oreochromis niloticus	18.3	27.3	34.9	19.5	630
Oreochromis mossambicus	19.8	27.1	30.8	22.2	630
Notopterus notopterus	19.5	26.3	32.5	21.6	630
Chitala chitala	21.1	24.8	32.9	21.3	630
Mastacembelus armatus	22.4	25.6	31.3	20.8	630
Average	22.9	24.7	33.0	19.5	630

3.1 Genetic Divergence among Species

To calculate the genetic distance Consortium for the Barcode of Life, (CBOL) recommended Kimura -2 – parameter model to be used. In the current study, to calculate the genetic distance as shown in (Table 3) within and between species, the Kimura -2 parameter model was used. The average genetic distance within species, genus, family, and order was 0.57%, 0.63%, 0.68%, and 0.77% respectively. This data showed that genetic distance (K2P) was higher for upper taxonomic levels and above the species level. However, the increase in genetic distance (K2P) was comparatively smaller and less apparent at higher taxonomic levels.

Table 3. Genetic Divergence (Percentage, K2P distance) within Various Taxonomic Levels

Comparison within	Mean (%)	S.E
Species	0.57	0.01
Genus	0.63	0.02
Family	0.68	0.05
Order	0.77	0.07

3.2 Lineage and Tree Construction

The same species was clustered together in the phylogenetic tree as shown in (Figure 2), indicating an earlier taxonomic classification based on fish morphology. There was no taxonomic variation found at the species level, suggesting that most of the species inspected could be validated

using barcodes. *Hypopthalymicthys molitrix, Ctenophargodon Idella*, and *Hypopthalymicthys nobilis* all belong to the order Cypriniformes and family Cyprinidae, that are narrowly related to the evolutionary tree. *Sperata seenghala* and *Eutropicthys vacha* are sister species belonging to order Silurifomes, family bagridae, and schilbeidae respectively. Similarly, *Chitala chitala* is closely related to *Oreochromis mossambicus* and *Channa punctata* which are sister species to each other. *Oreochromis mossambicus* fits in to order Cichliformes and family Cichlidae. However, Channa *punctata* belongs to the order Anabantiformes and family Chinnidae. *Notopterus notopterus* and *Labeo bata* are also sister species. *Notopterus notopterus* goes to order Osteoglossiformes, whereas family Notopteridae and *Labeo boga* goes to order Cypriniformes and family Cyprinidae. *Labeo rohita* and *Cyprinus carpio* are also sister species belonging to order cypriniformes and cyprinidae. *Labeo calbasu* and *Cirrhinus mrigala* are sister species and narrowly linked to *Catla catla* belonging to order Cypriniformes and family Cyprinidae. *Bagarius bagarius* and *Oreochromis niloticus* are closely related and also relate with two sister species *Wallago attu* and *Rita rita.*

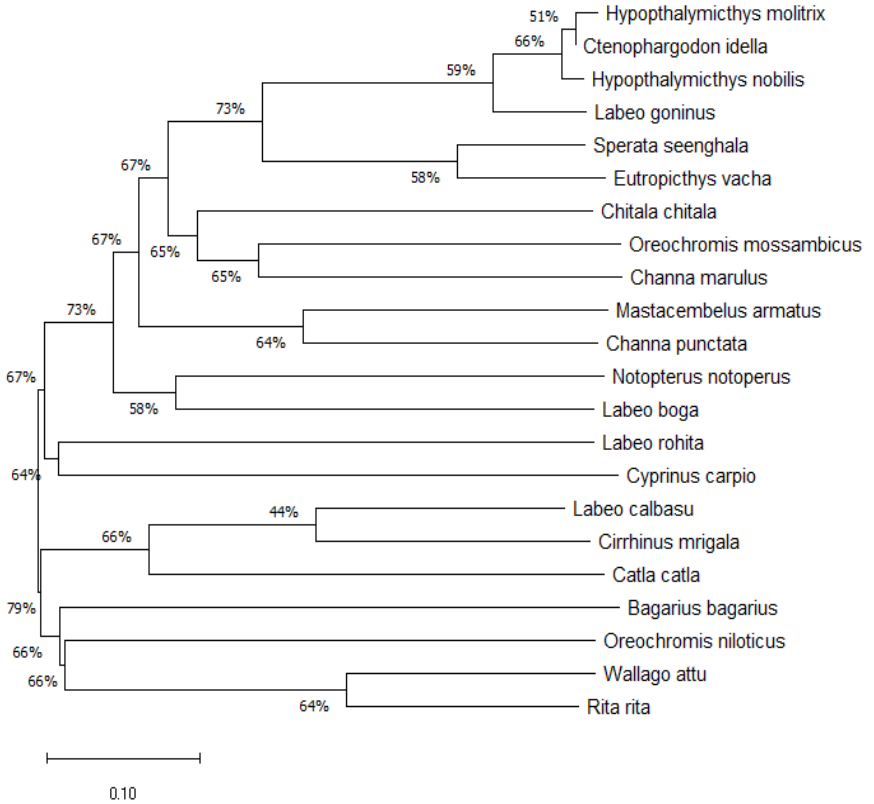

Figure 2. Phylogenetic Analysis of Fish Species Based on the COI Gene

4. Discussion

Fish diversity is distributed into two factors based on the richness, such as the number of species in a particular area and precision, for instance population size [19]. The richness of fish in the regions of tropical to subtropical rivers is associated with the river basin. Asian countries consist of huge number of basin areas for nurturing and growing fishes. These river ecosystems' vibrant characteristics are the outcome of differences in degree of water due to alteration in downpour in nesting and development places [20]. These dynamics cause variation in fish community morphology, frequently rendered by the consequence of climate related factors inside the river ecosystem, species interchanges, food availability and fish movements [21]. Primarily, the lotic water fishes' co-ordinate their nesting action with the flooding season and move to the upstream when there is rainy spell and shift back again when the weather is dry. Some of the fishes are economically crucial, because they are used as a source of food, decoration, and medical purposes as well [22]. According to [23] number of inland fish species has declined due to various factors, for instance water pollution, irreversible impact of heavy metals, construction of dams, habitat loss, wetlands drainage, eutrophication etc. Therefore, effective measures have been suggested to stabilize the major carps and other fish fauna at Downstream Indus River [24]. It is predictable that the population of species has also deteriorated as much as 50% in the past and if unnecessary conditions remain untouched, the population may be affected even up to 80% in the future [25]. Over the last three decades, the distributional ranges of some species have shrunk immensely. Habitat loss and environmental deterioration have had severe effects on various species such as *Danio rerio, Rit rita, Megarasbora elonga, Badis badis, Nandus nandus, Macrognathus aral,* and *Monopterus cuchia* [26].

The current research was conducted on 22 fish species at Head Panjnad. Various fish species were found at the study area. Among these species, *Catla catla, Cirrhinus mrigala, Cyprinus carpio, Labeo rohita, Labeo calbasu, Labeo gonius, Labeo boga, Ctenopharyngodon Idella, Hypophthalmichthys molitrix,* and *Hypophthalmichthys nobilis* had a relative abundance value of 43.91%. Catfishes (*Eutropiichthys vacha, Wallago attu, Rita rita, Sperata sarwari, and Bagarius bagarius*) were 13.40%, *Channa punctata, and Channa marulius* were 7.89%, *Oreochromis niloticus, and Oreochromis mossambicus* were 11.39%, *Notopterus notopterus and Chitala chitala* were 10.25%, *whereas, Mastacembelus armatus* were 1.38% abundant at the current study site (Table 1). A similar study conducted by [27] at river Barandu District Buner, Khyber Pakhtunkhwa, Pakistan, reported that 10 species were found belonging to 3 orders and 4 families. These species included *Tor putitora, Schizothorax plagiostomus, Channa gachua, Schistura punjabensis Barilius pakistanicus, Garagotyla, Mastacembelus armatus, Crossocheilus latius Puntius sophore,* and *Triplophysa naziri*. In another study, in the spring of 2015, 28 samples of fish were collected from the Swat River. The sampled fish species belonged to the Schizothorax (Heckle) and Schizothoraicthys (Misra). Six species, that is, *Schizothoraicthys macropthalmus, Schizothoraicthys Labiatus, Schizothorax richardsonii, Schizothoraicthys esocinus, Schizothorax sinuautus,* and *Schizothorax plagiostomus* were sampled from 12 different locations on the Swat River. The specie which was

recorded abundantly in the River of Swat was *Schizothtorax plagiostomus,* (that is, 6.82%). It was followed by *Schizothoraicthys esocinus, Schizothoraicthys labiatus, Schizothorax richardsonii, Schizothorax sinuautus,* and *Schizothoraicthys macropthalmus* with abundance of (17.85 %), (14.28 %), (10.92 %), (7.14 %), and (3.57 %) respectively. The only species that was thoroughly distributed in Swat River was *Schizothtorax plagiostomus* [28].

In the current study the analysis of Nucleotide pair frequency revealed that 39 of 957 (4.07%) sites were conserved, 756 of 957 (78.99%) sites were variable, 689 of 957 (71.99%) sites were more informative, whereas 65 of 957 (6.79%) singleton was existing. The average of identical pairs (ii) was 190 and transversional pairs (sv=240) were observed as reoccurring than transitional pairs (si=127), with a si/sv (R) ratio of 0.5 for the given data. The analysis of Nucleotide base frequencies (Table 2) revealed the overall observed mean for these sequences which was (T =22.9%), (C=24.7%), (A=33.0%) and (G=19.5%). This analysis of base composition for COI sequences manifested that the A content average was high, and the G nucleotide content average was low. Whereas, the AT bases content 36% was higher than the GC bases content (22%). The results of the current study were in accordance with the previous research conducted in Taiwan Strait fishes (AT=53.20%, GC=46.80%) [29].

In the current study, for the calculation of genetic distance (Table 3) within and between species, the K2P model (Kimura - 2 parameter) was used. The average genetic distance determined within species was 0.57%, the average genetic distance within genus was 0.63%. Whereas, the average genetic distance observed within family was 0.68% and the average genetic distance between orders was 0.77%. This data showed that genetic distance (K2P) was higher at upper taxonomic levels and above the species level however, the increase in genetic distance (K2P) was comparatively smaller and less apparent at higher taxonomic levels. These results showed a high degree of consistency with the previous observations [7]. In the current study, it was found that the average distance between species within families was relatively 13.10%, while between species within order was 17.16%. There has been a steady increase in the genetic variation through an increase in classification level, which supports a clear change in the genetic deviation within the range of species.

Moreover, all of these 22 species were further used in making DNA barcodes. DNA barcoding has sparked debate in a few logical circles [30, 31]. Later findings revealed a few evident advantages of using an authorized species-specific molecular tag derived from the COI gene for species-level differentiating [7, 32, and 8]. The goal of DNA barcoding was to put forth an effective approach for species-level identification by employing a variety of species-specific molecular markers derived from COI gene sequences [25]. The partial COI gene was used as a DNA barcode in 22 freshwater fish species from six orders (Cypriniformes, Siluriformes, Anabantiformes, Cichliformes, Osteoglossiformes, Synbranchiformes, and Synbranchiformes) and nine families. The intensity of the target fish in all species was increased by universal primers, resulting in 22 630-bp COI barcodes. None of the DNA sequences had any deletions, insertions, or stop codons, indicating that all of the sequences intensified were from a functioning mitochondrial COI sequence. There was no discernible scrambling of

species in the NJ tree, which showed an unclear evolutionary connection among the species. Congeneric species and, in most cases, non-familial species were discovered and grouped together. High bootstrap values (90–100 percent) backed up all of the nodes. Despite the fact that barcode analysis was used to illustrate species boundaries, there were definitely a few phylogenetic signals in the COI sequence, as seen in prior research [33].

Based on morphological features specified in FAO recognized proof sheets, the outcomes of the current research's investigation were compatible with the taxonomic stratification of the finfish under consideration. DNA barcoding centers were used to create a tree of life and perform DNA scientific categorization. However, they may also be used to offer a widely used molecular identification key based on considerable organized information in a standardized identification reference library. The undeniable effectiveness of DNA barcoding was performed for the fact that it improves present methods in the field of molecular differentiating evidence, and standardization provides limitless applications for various users [34]. In comparison to one-of-a-kind sequences that were unique form each other, the COI gene was effective in recognizing freshwater species with assigned barcodes, as all freshwater fish species were examined. The findings of the current study conclusively confirmed the COI gene's potential value in fish barcoding.

4.1 QR Codes Based on DNA Sequence

QR codes based on DNA sequences were generated (Figure 3). These codes could be scanned with smart phone apps in the same manner as the barcodes are being read in super stores. According to the current research, it the major reason for developing QR codes was the identification of molecular-based fish species that is, to make it easily accessible. Kocher et al. [35] have already developed a DNA barcode that can be used as a blueprint to accurately identify Teleost fish species. Unlike [36], a Bio-Rad DNA barcode generator was used to construct QR codes based on DNA sequences. By using species verification in conjunction with DNA, barcoding may result in an effective strategy to fisheries division inspection, management, and preservation. In Pakistan, species-level fish identification is uncommon, thus, the current study has recommended using DNA barcoding as a starting point for identifying confirmation and affirmation using QR barcodes.

Figure 3. Web generated DNA Sequence Based QR Codes

4.2 Conclusion and Recommendations

The increase in consumption of fish and its products, as well as physical similarities across the species, has resulted in accidental and deliberate false labeling of fishes available in the markets. Without taking in account the morphological or meristic features, barcoding provides a unique method for confirming fish species by sequencing the quality of their mitochondrial DNA. As a result, DNA barcoding is proved to be a dependable tool for locating fish and improving food security. The Universal Standardized Identification of Life expressed that "DNA barcoding can be utilized to distinguish the different species, as well as a basic store scanner which can use as black strip that encodes the Universal Product Code (UPC) to recognize the purchase products". An advanced standardized tag 3d image is eventually required to recognize fish species by employing a standardized identification per user. Computerized information could be utilized as well to sit well with the standardized identification arrangements for fish species of every origin. In this research, fish diversity of Panjnad canal was examined. Moreover, fish diversity of Abbasia and Abu Dhabi canals should also be examined.

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Author Contribution

Saima Naz had the initial idea about the manuscript and was involved in the experimental studies, laboratory studies, data analysis, writing, and the development of the article, as well as the final proof stage. Ahmad Manan Mustafa Chatha and Saima Naz were involved in experimental studies, laboratory studies, and writing of the article. Duarli Danabas was involved in the writing and development of the article, as well as in the final proof stage. In

addition,Saima Naz assumed the responsibility of the corresponding author. All the authors have read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Naz S, Javed M, Chatha, AMM. Investigation of acute toxicity of lead-manganese mixture to fish under laboratory conditions. *Iran J Fish Sci.* 2020;19(2):934-941. https://doi.org/10.22092/IJFS.2018.117476
2. Patwardhan B, Warude D, Pushpangadan P, Bhatt N. Ayurveda and traditional Chinese medicine: A comparative overview. *Evi Based Comple Alter Med*. 2005;2(4):465-473. https://dx.doi.org/10.1093%2Fecam%2Fneh140
3. Rasmussen RS, Morrissey MT, Hebert PD. DNA barcoding of commercially important salmon and trout species (Oncorhynchus and Salmo) from North America. *J Agri Food Chem*. 2009;57(18):8379-8385. https://pubs.acs.org/doi/10.1021/jf901618z
4. Victor BC, Hanner R, Shivji M, Hyde J, Caldow C. Identification of the larval and juvenile stages of the Cubera Snapper, Lutjanus cyanoptems, using DNA barcoding. *Zootaxa*. 2009;2215:24-36. https://doi.org/10.11646/zootaxa.2215.1.2
5. Hamzah S, Yatim NI, Alias M, Ali A, Rasit N, Abuhabib A. Extraction of hydroxyapatite from fish scales and its integration with rice husk for ammonia removal in aquaculture wastewater. *Ind J Chem*. 2019;19(4):1019-1030. https://doi.org/10.22146/ijc.40907
6. Khan AM, Ali Z, Shelly SY, Ahmad Z, Mirza MR. Aliens; A catastrophe for native freshwater fish diversity in Pakistan. *J Animal Plant Sci*. 2011;21(2):435-440.
7. Hebert PD, Cywinska A, Ball SL, DeWaard JR. Biological identifications through DNA barcodes. *Proc Royal Soc London B*. 2003;270(1512):313-321. https://doi.org/10.1098/rspb.2002.2218
8. Ward RD, Zemlak TS, Innes BH, Last PR. Hebert PDN. DNA Barcodig Australia sfish species. Philosoph. *Transac Royal Soc B-Biolo Sci*. 2005;360(1):1847-1857. https://doi.org/10.1098/rstb.2005.1716
9. Landi M, Dimech M, Arculeo M, Biondo G, Martins R, Carneiro M, Carvalho GR, Brutto SL. Costa FO. DNA barcoding for species assignment: The case of Mediterranean marine fishes. *Pub Lib Sci One*. 2014;9(9):e106135. https://doi.org/10.1371/journal.pone.0106135
10. Ward RD, Hanner R. Hebert PD. The campaign to DNA barcode all fishes, FISH-BOL. *J Fish Biol*. 2009;74(2):329-356. https://doi.org/10.1111/j.1095-8649.2008.02080.x
11. Teletchey F. After 7 years and 1000 citations: Comparative assessment of the DNA barcoding and the DNA taxonomy proposals for taxonomists and non-taxonomists. *Mitocho DNA*. 2010;21:206-26.

https://doi.org/10.3109/19401736.2010.532212

12. Radulovici AE, Archambault P, Dufresne F. DNA barcodes for marine biodiversity: Moving fast forward? *Diversity*. 2010;2:450-72. http://dx.doi.org/10.3390/d2040450

13. Rafique M, Khan NU. Distribution and status of significant freshwater fishes of Pakistan. *Zoolo Survey Pak*. 2012;21:90-95.

14. Yue GH, Orban L. A simple and affordable method for high-throughput DNA extraction from animal tissues for polymerase chain reaction. *Electrophoresis*. 2005;26:3081-3083. https://doi.org/10.1002/elps.200410411

15. Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PD. DNA barcoding Australia's fish species. *Philos Trans R Soc B: Biol Sci*. 2005;360:1847-1857. https://doi.org/10.1098/rstb.2005.1716

16. Kumar S, Tamura K, Jackobson IB, Nei M. MEGA 3.1: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform*. 2004;5:150-163. https://doi.org/10.1093/bib/5.2.150

17. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Molecul Evol*. 1980;16(2):111-120. https://doi.org/10.1007/BF01731581

18. Felsinstin J. PHYLIP (Phylogeny Inference Package) version 3.50 distributed by the author. Department of Genetics, University of Washington, Seattle, USA. 1993.

19. Magurran AE. *Measuring biological diversity*. John Wiley and Sons; 2003.

20. Thorp JH, Thoms MC, Delong MD. *Theriverine ecosystem synthesis*. Towards Conceptual Press; 2008.

21. Taylor BW, Flecker AS, Hall RO. Loss of a harvested fish species disrupts carbon flow in a diverse tropical river. *Science*. 2006;313(5788):833-836. https://www.science.org/doi/10.1126/science.1128223

22. Dudgeon D. Endangered ecosystems: A review of the conservation status of tropical Asian rivers. *Hydrobiologia*. 2002;248(3):167-191. https://doi.org/10.1007/BF00006146.

23. Khan A, Huq T, Salmieri S, Khan RA, Le Tien C, Riedl B. Lacroix M. Nanocrystalline cellulose (NCC) reinforced alginate based biodegradable nanocomposite film. *Carbohyd Polym*. 2012;90(4):1757-1763. https://doi.org/10.1016/j.carbpol.2012.07.065

24. Sheikh M, Laghari MY, Lashari PK, Khooharo AR, Narejo NT. Current status of three major carps (*Labeo rohita*, *Cirrhinus mrigala* and *Catla catla*) in the downstream Indus River, Sindh. *Fishe Aquaculture J*. 2017;8:222. https://doi.org/10.4172/2150-3508.1000222

25. Conand C, Polidoro B, Mercier A, Gamboa R, Hamel JF, Purcell S. The IUCN red list assessment of aspidochirotid sea cucumbers and its implications. *SPC Beche-de-mer Inform Bullet*. 2014;34(5):3-7.

26. Rafique M, Akhtar S, Niazi HK. Fish fauna of Jinnah Barrage and adjoining areas. *Pak J Zool*ogy. 2003;35:95-98.

27. Akhter MS, Sharma NK, Mir JI, Singh R, Pandey NN. Length–weight relationships for eight fish species from the Ravi River, north-western India. *J Appl Ichthyol*. 2014;31(6):1146-1147. https://doi.org/10.1111/jai.12836

28. Khan SAR, Yu Z, Belhadi A, Mardani A. Investigating the effects of renewable energy on international trade and environmental quality. *J Environ Managt*. 2020;272:e111089. https://doi.org/10.1016/j.jenvman.2020.111089

29. Bingpeng X, Heshan L, Zhilan Z, Chunguang W, Yanguo W, Jianjun W. DNA barcoding for identification of fish species in the Taiwan Strait. *Public Lib Sci.* 2018;13(6):e0198109. https://doi.org/10.1371/journal.pone.0198109

30. Hubert N, Hanner R, Holm E, Mandrak NE, Taylor E. Identifying Canadian freshwater fishes through DNA barcodes. *Public library Sci One*. 2008;3:e2490. https://doi.org/10.1371/journal.pone.0002490

31. Ebach MC, Holdredge C. DNA barcoding is no substitute for taxonomy. *Nature*. 2005;434:e697. https://doi.org/10.1038/434697b

32. Will KW, Rubinoff D. Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics*. 2004;20(1):47-55. https://doi.org/10.1111/j.1096-0031.2003.00008.x

33. Lakra WS, Verma MS, Goswami M, Lal KK, Mohindra V, Punia P, Gopalakrishnan A, Singh KV, Ward RD, Hebert P. DNA barcoding Indian marine fishes. *Molecul Ecol Resour*, 2011;11:60–71. https://doi.org/10.1111/j.1755-0998.2010.02894.x

34. Pradhan V, Kamble Y, Ladniya V, Mogul M. An overview of species identification by DNA barcoding. *Int J Curr Microbiol Appl Sci*. 2015;4:127-140.

35. Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc National Acad Sci*. 1989;86(16):6196–200. https://doi.org/10.1073/pnas.86.16.6196

36. Yang CH, Wu KC, Chuang LY, Chang HW. Decision theory-based COI-SNP tagging approach for 126 Scombriformes species tagging. *Front Genet*. 2019;10:259. https://doi.org/10.3389/fgene.2019.00259