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Research Article

Northern Arabian Sea: Rare Fish Diversity and Biogeographic Affinities

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Keywords
Northern Arabian sea, Genetic diversity, Phylogenetic analysis, Barcode gap, Genetic variations, Rare species

Abstract | Recent molecular approaches have revolutionized the world of species classification and identification. In this study, we delved into the fascinating domain of DNA barcoding precisely for rare marine species and delineated species population genetic variability, genetic differences, and phylogenetic relationships between families/genera. 542 COI sequences from experimental species and an online database were considered for phylogenetic and Fst analysis. Moreover, an online QR code generator was used to develop the first-ever QR codes for nucleotide information of these species. It is the first study from Pakistan to reveal the barcode gap, phylogenetic relationship, and genetic diversity of the fish species in the northern Arabian Sea. A notable genetic variation level was revealed, with the highest value of 0.75 indicating a significant differentiation between populations of Taiwan and Pakistan. In contrast, the lowest Fst value of 0.04 manifested minimal genetic differentiation between populations in the USA and Bangladesh. An average genetic distance using the Kimura 2 parameter (K2P) model using BOLD systems revealed 20.17 and 19.87 percent within genus and family respectively. Nevertheless, this study documented the COI sequence of *Caesio varilineata* and *Uranoscopus dollfusi,* for the first time. The combined use of taxonomy, DNA barcoding, and QR codes appeared to be robust approaches, and have paved the way for a better understanding of fishes rarely found in Pakistan, northern Arabian Sea.

Novelty Statement | This study demonstrates the utility of integrating DNA barcoding and QR coding for elucidating the genetic diversity and phylogenetic relationships of rare marine fish species in the northern Arabian Sea, thereby enhancing our understanding of this previously undercharacterized region.

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Introduction

Due to contemporary advancements and the involvement of molecular tools, fish taxonomy has

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grabbed the interest of fisheries biologists globally. Pakistan is rich in fish diversity due to its subtropical position on the globe. Previously, conventional taxonomy was used as a major tool to describe species (Farooq and Panhwar, 2023; Qamar *et al.*, 2016; Rauf *et al.*, 2019). Conventional taxonomy based on just morphology has many limitations, as we are unable to identify processed meat, broken samples, or individuals at their early developmental stages (Keskin and Atar, 2013; Zhang and Hanner, 2012). Cryptic species, species having incomplete morphological characters, and novel species may not be identified accurately, leading to misidentification. Moreover, there is a knowledge gap for taxonomists to properly identify some fish groups at the species level. The classification description of fish in different literature is different which can also lead to misidentification by beginners. Currently, DNA barcoding is being successively used and has been proven as an efficient tool for the rapid and accurate identification of fish (DeSalle and Goldstein, 2019). Furthermore, the identification of cryptic species, species at earlier stages of their life, and processed samples having incomplete morphology can be more reliably executed using molecular techniques as compared to identification based on morphology (Galal-Khallaf *et al.*, 2014; Raharinaivo *et al.*, 2020; Wang *et al.*, 2020). Molecular approaches together with morphological identification can serve as a potential tool in fish biology because they provide rapid, precise, and reasonable systems for identification. Moreover, the delineation of genetic distances within different species and even in the population of the same species can be measured using single gene-based studies (de Sousa *et al.*, 2022; Habib *et al.*, 2022; Khan *et al.*, 2023; Tang *et al.*, 2023). The 650 bp region of COI a mitochondrial gene is being extensively used in molecular identification (Lohman *et al.*, 2009). This gene possesses great importance as it has a slow amino acid change rate (Hebert *et al.*, 2003; Lynch and Jarrell, 1993). Practically, DNA barcoding using the COI gene is being worldwide used for the identification of freshwater and marine fish species with a success rate of up to 93% (Ward, 2012). However, there are some limitations of the *COI* gene too, such as partial lineage sorting phenomena and gene introgression, which may lead sometime towards misidentification (Eberle *et al.*, 2020; Galimberti *et al.*, 2021). So, by employing the combination of morphological and molecular techniques, many ambiguities can be removed. Moreover, many analysis tools in addition to the conventional morphological-based identification and DNA barcoding studies are applied to improve the accuracy of fish identification, which will further empower the discovery of cryptic species and will enrich the genetic diversity of fish species (Breman *et al.*, 2016; Hou *et al.*, 2018). Northern Arabian Sea possesses a complex and high ratio of biodiversity including abundantly found species as well as some unique and rarely occurring species. Normally the focus of researchers is on abundantly present fishes as they contribute a major part in daily fish landing. However, the study of rare fish holds significant importance for conservation, ecological, and scientific reasons. In this study, some rarely occurring fishes were tried to cover taxonomy at the molecular level. In total, 15 species belonging to 13 families and eight different classes were collected from daily fish landing facilities along the Karachi coast. By considering a 650 bp region of COI, a comprehensive DNA barcode database of 45 samples representing 15 marine species, 13 families, and 7 orders (*Lactarius lactarius, Rachycentron canadum, Caesio varilineata, pempheris russellii, Pomacanthus annularis, Myripristis botche, Sargocentron rubrum, Plotosus lineatus, Chanos chanos, Uranoscopus dollfusi, Terapon jarbua, Terapon puta, Drepane longimana, Scatophagus argus, Pampus argenteus)* was developed as these are rarely present in daily fish landing at Karachi coast.

This study aimed to provide robust taxonomic and molecular descriptions of rare fishes and to delineate the genetic variability, species population differentiation, and biogeographic affinities, to facilitate researchers for further findings on population structure, understanding of evolutionary processes, and conservation strategies for species inhabiting circumglobally.

Materials and Methods

Sampling and morphological identification

In total 542 sequences were used for analysis of genetic diversity in rarely occurring marine species. Detailed information on sampling and Genbank accession numbers of extracted sequences from each species is mentioned in Supplementary Table 1. Type species (*Lactarius lactarius, Rachycentron canadum, Caesio varilineata, Pempheris russellii, Pomacanthus annularis, Myripristis botche, Sargocentron rubrum, Plotosus lineatus, Chanos chanos, Uranoscopus dollfusi, Terapon jarbua, Terapon puta, Drepane longimana, Scatophagus argus, Pampus argenteus*) belonging to 13 families and 7 orders were collected from the commercial catches at the daily fish landing facility (Karachi Fisheries Harbor). The exact location of the sample collection is shown in Figure 1. Samples were transported to the fisheries laboratory, Centre of Excellence in Marine Biology (CEMB). Each species was identified based on its morphology by using taxonomic keys and an FAO field guide (Psomadakis, 2015). Photographs for each of the specimens were taken. Samples were then transported to the Department of Biotechnology, University of Sargodha for molecular analysis. Tissue excision was done under sterile conditions and properly labeled. All of the samples and excised tissues were preserved in 95% ethanol for future use. All procedures performed involving animals were approved by the ethical committee of the University of Karachi, approval no IBC KU-260/2022.

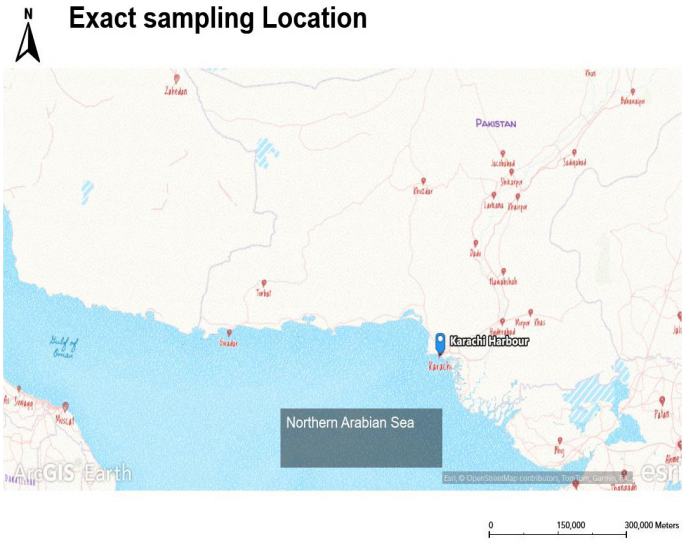

Figure 1: Exact sample collection site. The blue mark shows the exact sampling site for rare fish species.

Amplification and sequencing of DNA barcode region

The DNA extraction was accomplished using a GeneJET DNA purification kit (Catalogue no. K0721). The recommended protocol for animal tissue DNA extraction was followed for DNA extraction. The product of DNA extraction was stored at -20°C for further experimental work.

The target region of 652 bp was amplified using published primers (Fish F1 and Fish R1) for DNA barcoding of fishes (Karim *et al*., 2016). PCR amplified product was sequenced commercially. Further, the excised tissues were also sent to the Canadian Centre for DNA barcoding (CCDB), the University of Guelph for sequencing and generation of DNA barcodes. Obtained sequences are uploaded to BOLDsystems for further analysis and also submitted to NCBI Genbank, the details are mentioned in Table 1.

Barcode index number allocation

Uploaded data on the BOLD workbench is automatically referred to a unique relevant BIN (Barcode Index Number). All the samples in one bin share the similarity at the molecular level. Available sequences of type species from across the globe were also downloaded and included in the analysis to explore genetic diversity. Species identification using molecular tools was also reconfirmed by BLAST search analysis. The significant E-values that are generated pairwise also play a paramount role in the delineation of fish species. If there was any mismatch between morphological identification and molecular identification then both were revisited to reach the concrete decision after the consideration of both parameters. The genetic distances based on the COI gene were determined with the help of MEGA X by using the Kimura 2 Parameter (K2P). Additionally, other analyses including a distance summary, nucleotide diversity, and a barcode gap analysis were done using the BOLD systems workbench, while the Fst analysis used R studio.

Phylogenetic relationship of experimental species from Pakistan with specimens across the globe

A phylogenetic analysis was conducted by using sequences obtained from experimental species from Pakistan and also retrieved data of the same species from across the globe. All of the sequences were aligned using the MSA/MAFT method. The trees to the explicit phylogenetic relationship were developed using the Fast tree method. Upon formation, the GGTree method was used for the visualization of relationships (Yu, 2020). The Genbank/Accession number for each species was manifested within the center of the tree. All of the work was done via UNIX, R, and R Studio. The species belonging to the same order and families were

Table 1: Geographical coordinates, sampling location, and GenBank accession number of rare species.

Family	Specie name	Order	Location	Voucher no.	Accession no	BOLD ID
Lactariidae	*Lactarius lactarius*	Perciformes	Latitude; 24°50'53.2"N Longitude; 66°58'40.6"E Fish Harbour Rd., West Wharf Karachi,Karachi City, Sindh, Pakistan	MAK-142	OQ801201	BOLD:AAD4634
Rachycentridae	*Rachycentron canadum*	Carangiformes		MAK-73	OQ807163	BOLD:AAB2939
Caesionidae	*Caesio varilineata*	Perciformes		MAK-103	OQ825963	BOLD:ADK7119
Pempheridae	*Pempheris russellii*	Perciformes		MAK-108	OQ826120	BOLD:AAD1777
Pomacanthidae	*Pomacanthus annularis*	Perciformes		MAK-59	OQ807215	BOLD:AAF1425
Holocentridae	*Myripristis botche*	Holocentriformes		MAK-119	OQ807657	BOLD:AAX2837
Holocentridae	*Sargocentron rubrum*	Holocentriformes		MAK-120	OQ808566	BOLD:AAB9306
Plotosidae	*Plotosus lineatus*	Siluriformes		MAK-37	OQ808970	BOLD:ABY8174
Chanidae	*Chanos chanos*	Gonorynchiformes		MAK-145	OQ809068	BOLD:AAC1320
Uranoscopidae	*Uranoscopus dollfusi*	Trachiniformes		MAK-114	OQ809069	BOLD:ACX9882
Terapontidae	*Terapon jarbua*	Perciformes		MAK-05	OQ810001	BOLD:AAA9351
Terapontidae	*Terapon puta*	Perciformes		MAK-13	OQ810033	BOLD:AAB0170
Drepaneidae	*Drepane longimana*	Perciformes		MAK-44	OQ814428	BOLD:AAB0170
scatophagidae	*Scatophagus argus*	Perciformes		MAK-92	OQ814532	BOLD:AAB3530
Stromateidae	*Pampus argenteus*	Scombriformes		MAK-27	OQ815713	BOLD:AAB6557

Table 2: Values obtained from barcode gap analysis.

Order	Family	Species	Max intra-Sp	Nearest species	Nearest neighbour	Distance to NN	Barcode gap
Carangiformes	Rachycentridae	*Rachycentron canadum*	2.74	*Drepane longimana*	SUFIS237-21	21.85	19.11
Holocentriformes	Holocentridae	*Myripristis botche*	0.74	*Terapon jarbua*	SUFIS385-21	17.17	16.43
Holocentriformes	Holocentridae	*Sargocentron rubrum*	4.23	*Myripristis botche*	SUFIS330-21	19.26	15.03
Moroniformes	Drepaneidae	*Drepane longimana*	0.31	*Terapon jarbua*	SUFIS005-20	19.26	18.95
Perciformes	Caesionidae	*Caesio varilineata*	0.96	*Myripristis botche*	SUFIS330-21	18.83	17.87
Perciformes	Pempheridae	*Pempheris russellii*	2.16	*Sargocentron rubrum*	SUFIS331-21	21.44	19.28
Perciformes	Pomacanthidae	*Pomacanthus annularis*	1.72	*Myripristis botche*	SUFIS329-21	22.02	20.3
Perciformes	Scatophagidae	*Scatophagus argus*	2.64	*Pampus argenteus*	SUFIS188-21	20.43	17.79
Perciformes	Terapontidae	*Terapon jarbua*	3.62	*Myripristis botche*	SUFIS329-21	17.17	13.55
Perciformes	Terapontidae	*Terapon puta*	1.59	*Terapon jarbua*	SUFIS386-21	19.63	18.04
Scombriformes	Stromateidae	*Pampus argenteus*	3.96	*Caesio varilineata*	SUFIS149-21	19.42	15.46
Siluriformes	Plotosidae	*Plotosus lineatus*	1.44	*Scatophagus argus*	SUFIS138-21	21.2	19.76
Trachiniformes	Uranoscopidae	*Uranoscopus dollfusi*	0.68	*Sargocentron rubrum*	SUFIS331-21	24.59	23.91

clustered under the same clade and the species belonging to different families and orders were clustered under different clades. So, a unique pattern of phylogeny was depicted based on COI nucleotide information.

Generation of DNA barcodes and QR codes

In the present era technology is very developed and Smartphones can widely be used to access 2D QR codes. At a single time, almost 4000 characters can be accessible, hence such technology can be used in fish identification coupled with genetic information. The sequence data were used to generate DNA barcodes and QR codes using online code generator tools (Ayesha *et al.*, 2019).

Results and Discussion

A series of analyses were performed on sequence data obtained from the experimental species and data retrieved from online databases (NCBI, BOLDsystems). A total of 542 sequences including sequences from experimental species were considered for Phylogenetic and Fst analysis using R studio. The metadata of all the species is given in Supplementary Table 1.

Barcode gap analysis

The barcode gap can be measured by finding the distribution of intra-species distances and the distance between their nearest neighbors. The barcode gap can be an impressive tool to delineate the species boundaries via nucleotide sequence information. All of the data from experimental species were uploaded to the online database Bold systems and were tested to reveal the barcode gap between them using the bold systems workbench. Three scatterplots are provided to confirm the existence and magnitude of the Barcode Gap. The results are listed in Table 2. The first two scatterplots show the overlap of the max and mean intra-specific distances vs the inter-specific (nearest neighbor) distances. The third scatterplot plots the number of individuals in each species against their max intra-specific distances, as a test for sampling bias (Figure 2).

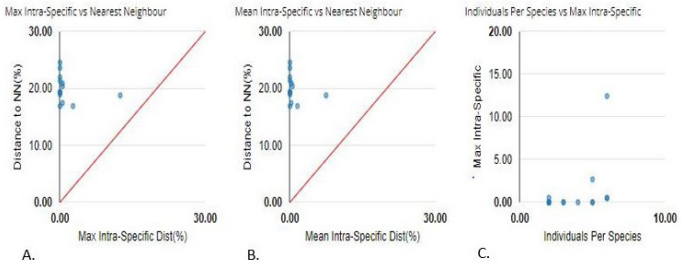

Figure 2: Barcode Gap analysis. (A) Max Intraspecific vs. Nearest Neighbors: Compares the maximum intraspecific distance of each species to the genetic distance of its nearest neighboring species. (B) Mean Intraspecific vs. Nearest Neighbors: Shows the average intraspecific genetic distance compared to the distance to the nearest neighboring species. (C) Individual per Species: Displays individual genetic distances for each species, highlighting variation within and between species.

Genetic distances and nucleotide diversity

The genetic distances based on COI nucleotide data were measured by using the K2P model and Muscel alignment on the BOLD systems workbench. Distribution of distances on different levels was manifested between all specimens and showed 20.17, and 19.87 percentages within genus and family, respectively. Table 3 shows that thymine (T) is the most common nucleotide, with a mean percentage of 29.06%, followed by adenine (A) at 23.71%. The overall GC content is 47.23%, with the first codon position being the most GC-rich at 57.60%. Interestingly, the third codon position has a lot of variability, which could affect how genes are expressed and proteins are made.

Table 3: Summary statistics for nucleotide frequency distribution are provided in the table.

Nucleotide	Min	Mean	Max	SE
G %	17.04	18.42	20.22	0.0984
C %	24.88	28.81	33.57	0.2441
A %	20.22	23.71	25.35	0.1617
T %	25.08	29.06	32.57	0.2340
GC %	42.09	47.23	51.54	0.2923
GC % Codon Pos 1	55.30	57.60	59.72	0.1217
GC % Codon Pos 2	42.11	43.03	45.50	0.0821
GC % Codon Pos 3	27.19	41.05	53.93	0.8530

F_{st} analysis

Fraction of genetic variance manifests differentiation within a population. The sequence data from experimental species and already available sequence data from NCBI were taken for each species. Overall, 542 sequences representing 12 species were used in this analysis. The metadata of each sequence is provided in Supplementary Table 1. The results were illustrated by heatmap charts and principle component analysis (Figures 3, 4, 5, 6). Fst analysis and development of heatmaps and PCA charts was done by R script. Sequence data taken from NCBI represents different geographical regions of the world.

Chanos chanos

Experimental sequences of *Chanos chanos* and sequences downloaded from NCBI were considered for Fst to determine genetic differentiation between species populations. Fst analysis revealed a maximum genetic variance of 0.5 between populations of Australia/ Iran, China/ Iran, and Bangladesh/ Iran. Moreover, a minimum of 0.04 genetic variation was revealed within the species population of the USA and Bangladesh. The values for Fst were interpreted by using heatmap charts (Figure 3A) and PCA (Figure 4A). The countries having similar

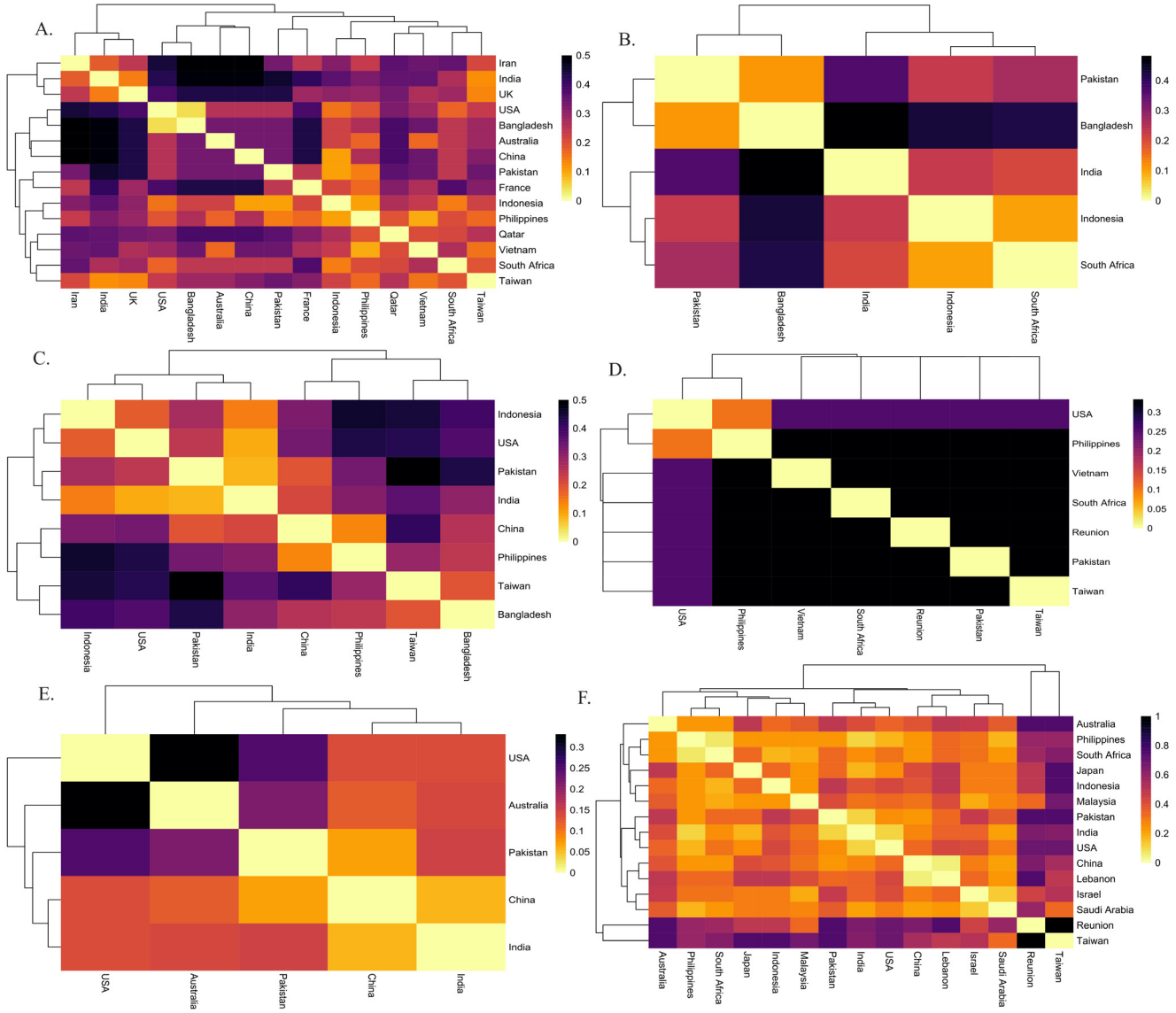

Figure 3: Heatmap illustration of Fst values (A). *Chanos chanos* (B). *Drepane longimana* (C). *Lactarius lactarius* (D). *Myripristis botche* (E). *Pampus argenteus* (F). *Plotosus lineatus* Heatmap chart is based on F_{st} analysis of sequence data of species population. The darker color shows high values and the light color shows fewer values from F_{st}.

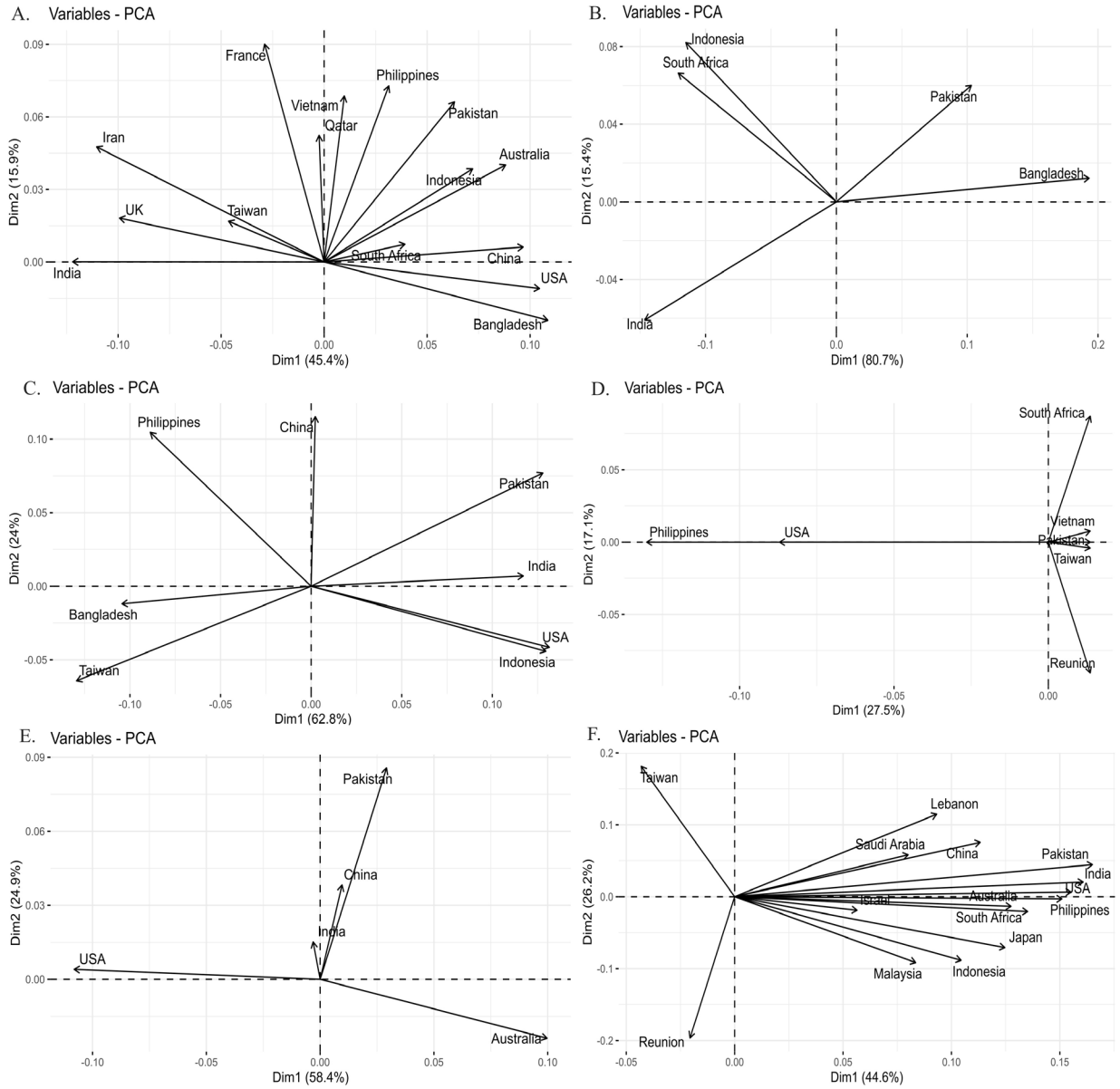

Figure 4: Principle component analysis of Fst values. (A). *Chanos chanos* (B). *Drepane longimana* (C). *Lactarius lactarius* (D). *Myripristis botche* (E). *Pampus argenteus* (F). *Plotosus lineatus* PCA is based on F_{st} analysis of sequence data of species population. Countries falling in one dimension have individuals more close to each other as compared to those falling in different dimensions.

divergence were interpreted under the same cluster. According to PCA interpretation of Fst, the maximum divergence was shown in the population of Bangladesh followed by France, the Philippines, and Pakistan.

Drepane longimana

The maximum genetic variance (0.48) was manifested by the study population of India and Bangladesh, while the minimum genetic variance of (0.09) was revealed between the species populations of Indonesia and South Africa (Figure 3B). The countries having related genetic divergence were clustered under the same cluster as compared to ones that have distant genetic variance. PCA from Fst values representing *Drepane longimana* manifested maximum genetic variation between the species population of Indonesia followed by South Africa and Pakistan (Figure 4B).

Lactarius lactarius

The COI-based Fst analysis of sequences of *Lactarius lactarius* shows a maximum value of 0.5 genetic variations from the study population of Taiwan and Pakistan. Moreover, the species population representing India and Pakistan showed the lowest genetic variance value which was 0.08. The results obtained from Fst were interpreted with heatmap charts (Figure 3C) and PCA (Figure 4C). The countries having less distant genetic variance within their population were seen to gather under a single cluster. Principle component analysis of the values obtained from Fst shows that the *L. lactarius* population of the Philippines shows significantly higher divergence followed by the population of China, Pakistan, and India.

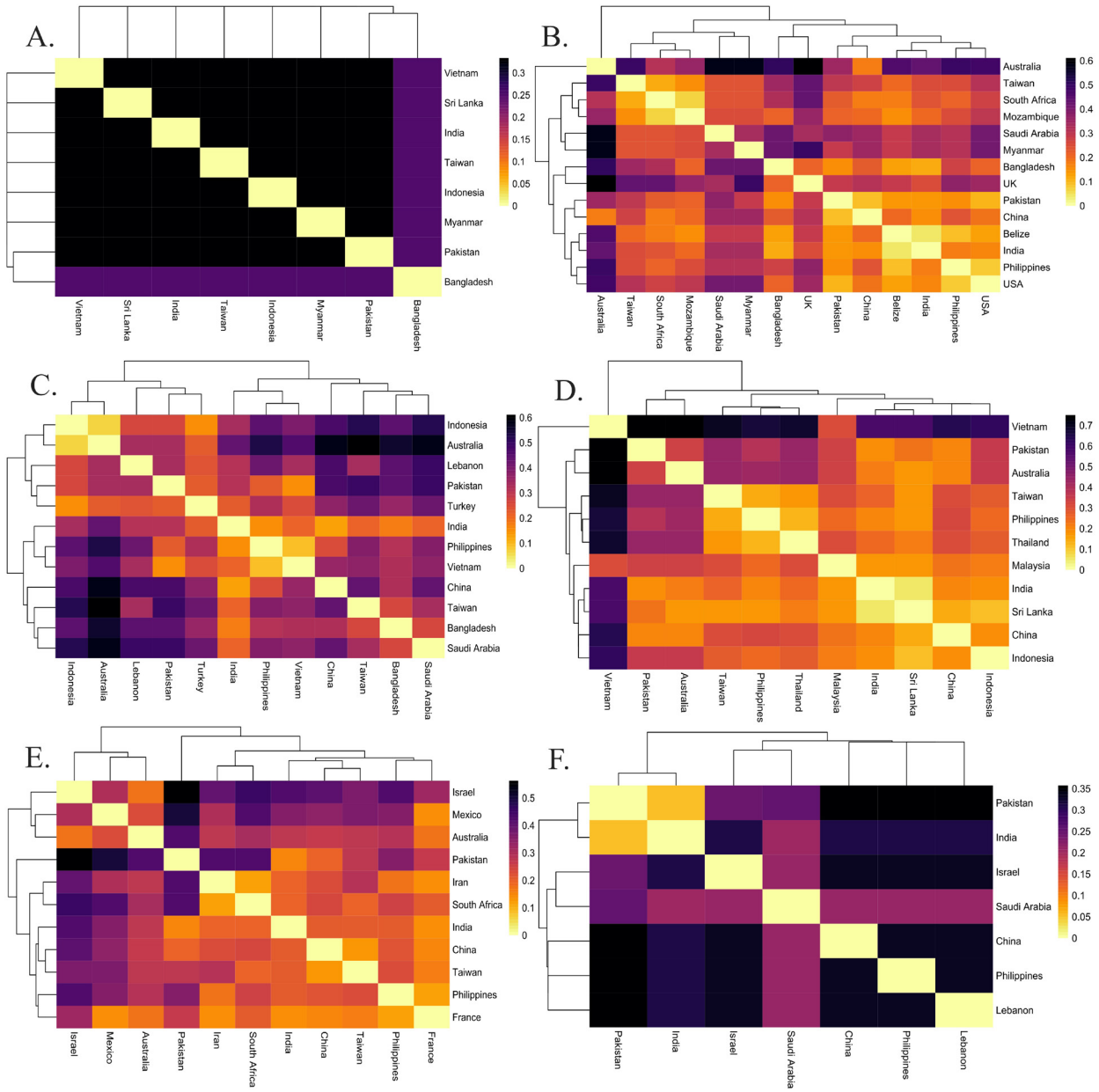

Figure 5: Heatmap Illustration of Fst values. (A). ***Pomacanthus annularis*** **(B).** ***Rachycentron canadum*** **(C).** ***Sargocentron rubrum*** **(D).** ***Scatophagus Argus*** **(E).** ***Terapon jarbua*** **(F).** ***Terapon puta*** **Heatmap chart is based on F_{st} analysis of sequence data of species population. The darker color shows high values and light color shows fewer values from F_{st}.**

Myripristis botche

From all sequences under study populations from multiple countries show the maximum genetic variance of 0.33. The minimum genetic variance was 0.1 as shown between populations of the Philippines and USA. The values of Fst were interpreted by a heatmap chart (Figure 3D) darker colors show greater values and lighter colors show lower values. Multiple countries illustrate the maximum genetic variance hence falling in darker regions. Moreover, species populations from Taiwan, Reunion, Pakistan, and South Africa made one cluster, and species populations from the USA and Philippines formed another cluster. PCA interpretation of Fst values showed a similar pattern (Figure 4D). The species population from South Africa showed maximum genetic variance followed by the population of Taiwan and Pakistan.

Pampus argenteus

Fst analysis showed maximum genetic variance (0.33) between study populations of Australia and the USA. Meanwhile, the study population from India and China showed a minimum Fst value of 0.05. PCA (Figure 4E) and heatmap charts (Figure 3E) were plotted by using values obtained from Fst. The darker colors on the heatmap represent high genetic variation and lighter colors on the heatmap chart display lower values. In heatmap charts countries that form a single cluster show relatively similar genetic variations among their populations like India, China, and Pakistan. PCA values indicate that Pakistan showed significantly higher divergence followed by Australia, China, and India.

Plotosus lineatus

The study populations of *Plotosus lineatus* representing different regions of the world were considered for Fst. Maximum genetic variance (0.75) was found between countries viz Taiwan/Pakistan, Taiwan/Australia, Reunion/Pakistan, Reunion/Australia, Japan/Taiwan, Indonesia/Taiwan, Lebanon/Reunion, and Taiwan/Australia. Similarly, minimum genetic divergence (0.05) was revealed between the species population of Lebanon and China based on COI as a potential marker. Heatmap (Figure 3F) and PCA (Figure 4D) were used for the illustration of values obtained from Fst data. Moreover, species populations from countries like Taiwan and Reunion form one cluster, meanwhile all other countries' species population was clustered under separate clusters according to Fst values. PCA based on values obtained from Fst was also done and depicts that the *Plotosus lineatus* population of Pakistan is significantly more divergent followed by the population of the USA and Philippines.

Pomacanthus annularis

The species population of *Pomacanthus annularis* depicts a range of Fst values between 0.33-0.25 taken from different geographical regions of the world. Heatmap (Figure 5A) illustrated that the species population from Pakistan and Bangladesh formed one cluster meanwhile species populations representing different regions of the world were clustered under separate clusters concerning their Fst values. Principle component analysis (Figure 6A) depicts that the most significant divergence was produced by the population of Pakistan followed by Vietnam and India.

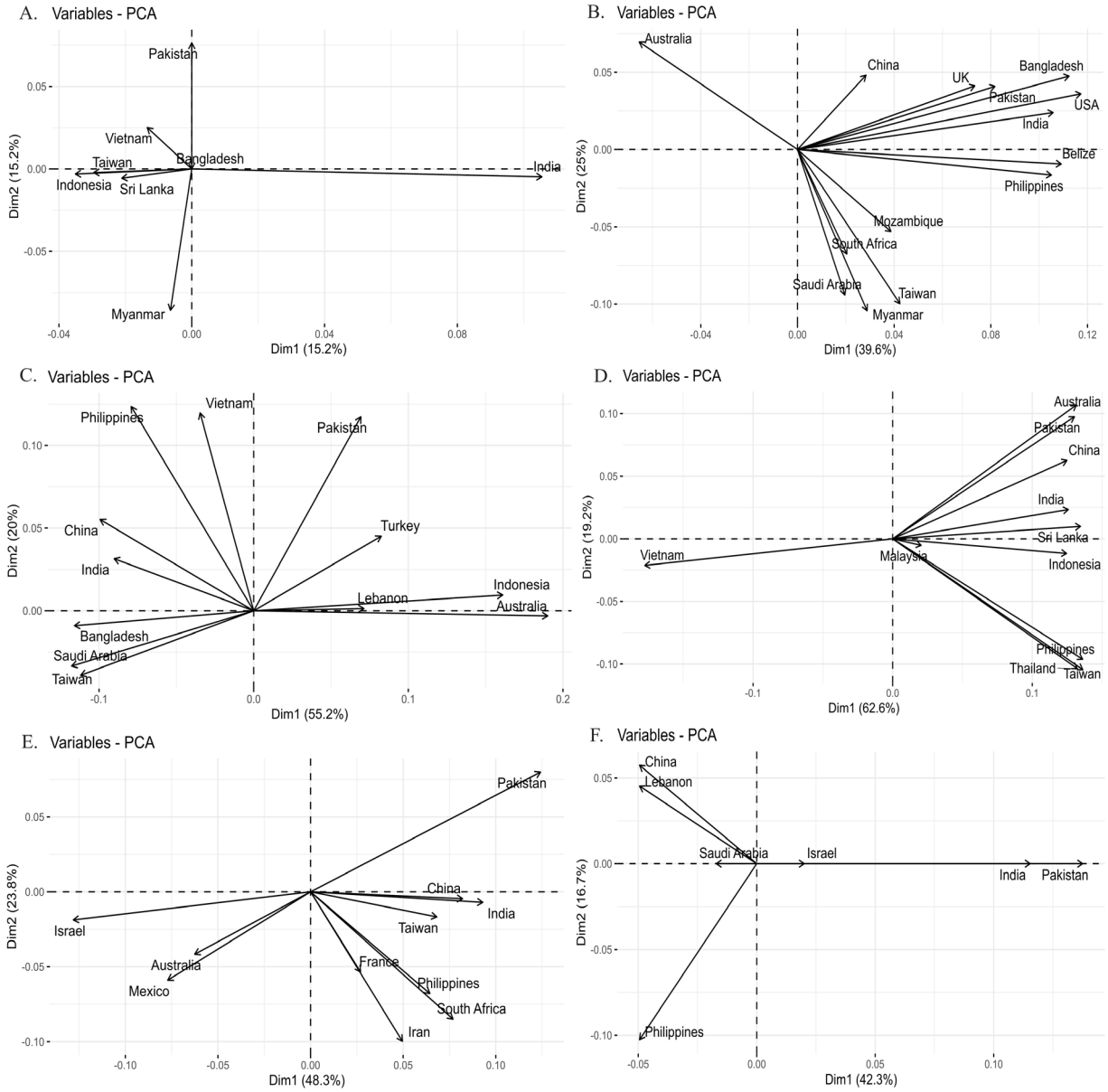

Figure 6: Principle component analysis of Fst values. (A). *Pomacanthus annularis* (B). *Rachycentron canadum* (C). *Sargocentron rubrum* (D). *Scatophagus Argus* (E). *Terapon jarbua* (F). *Terapon puta* PCA is based on F_{st} analysis of sequence data of species population. Countries falling in one dimension have individuals more close to each other as compared to those falling in different dimensions.

Rachycentron canadum

A maximum of 0.61 genetic divergence was revealed between Australia and the UK, meanwhile, a minimum of 0.06 genetic divergence was illustrated by species populations belonging to Mozambique and South Africa. So, the population species of Mozambique and South Africa are more related as compared to others. The heatmap chart depicts that species populations of countries having somehow similar genetic divergence were clustered under a single cluster, while species populations having different genetic variances were clustered under different clusters (Figure 5B). PCA analysis of data obtained from Fst showed that Bangladesh produced more significant divergence followed by Bangladesh, Pakistan, and the UK (Figure 6B).

Sargocentron rubrum

Study populations of this species belonging to different regions of the world were tested for genetic variance between them using Fst. It produces a maximum value of divergence of about 0.57 between the study population of Australia and Saudia Arabia, while minimum genetic variation (0.06) was revealed in the species populations of Australia and Indonesia. Countries that share somehow similar values were combined to form one cluster and countries that depict different colors were considered to have distant genetic divergence (Figure 5C). PCA analysis depicts that species population from Pakistan and the Philippines shows significantly higher differentiation followed by other countries (Figure 6C).

Scatophagus argus

Fst analysis revealed a maximum genetic variance (0.75) between the study population of Australia/Vietnam, and Pakistan/Vietnam, while a minimum genetic variance of 0.04 was found to be present in populations of India and Sri Lanka. The presence of low genetic variance between two populations indicates that both of them are related to each other. The study populations of countries that show similar genetic differentiation (Indonesia, China, Sri Lanka, India, and Malaysia) were clustered under the same cluster, on the other hand, species populations of (Vietnam) were clustered under separate clusters (Figure 5D). PCA analysis of values obtained from Fst illustrated that the study population of Australia showed a significantly high level of differentiation followed by that of Pakistan and China (Figure 6D).

Terapon jarbua

Terapon jarbua manifested a maximum of 0.56 genetic variance between the species population of Pakistan and Israel. While a minimum of 0.11 genetic variance was displayed by study populations of France and the Philippines. Figure 5E depicted heatmap chart of values obtained from the Fst Species population of (France/ Philippines, Taiwan/ China/ India) having similar genetic differentiation formed a single cluster, and the population of (Australia, Mexico, and Israel) having different genetic variance formed different clusters. Principle component analysis (Figure 6E) showed significantly higher differentiation for the species populations from Pakistan followed by other countries.

Terapon puta

A maximum of 0.35 genetic variance was displayed between the species populations of Philippines/Pakistan, and Lebanon/Pakistan, while a minimum of 0.05 genetic variance was observed between populations of Pakistan and India hence they are more closely related to each other. A heatmap chart (Figure 5F) was used to manifest data obtained from Fst Species populations from India and Pakistan were clustered in a single cluster as they show similar genetic divergence and all other countries' populations were gathered to form a separate cluster as they show different divergence levels. Fst values-based PCA (Figure 6F) was also conducted and showed that China shows a significantly higher level of differentiation followed by Lebanon and other countries.

Phylogenetic analysis among marine species occurring worldwide

Figure 7A illustrates a phylogenetic tree for species of *Chanos chanos* occurring worldwide to delineate evolutionary relationships and genetic similarities between them. Every country was highlighted with a unique pattern of color. The orange color represents sequences grabbed from NCBI and the blue color shows sequenced species from Pakistan. All species were clustered under clades according to evolutionary relationships. Sequences from Pakistan formed a clade surrounded by India and the Philippines. Figure 7B depicts the phylogenetic relationship of *Drepane longimana* each of the sequences representing different countries was clustered according to their evolutionary relationship. Type specimens from Pakistan formed a clade with species from Bangladesh and showed more similarity to sequences from Bangladesh and India. Figure 7C shows the evolutionary relationship of *Lactarius lactarius* occurring across the globe, the studied species from Pakistan were clustered under a clade with China, India, and USA. Figure 7D illustrates the phylogenetic tree of *Myripristis botche*. The experimental species were clustered under the clade representing species from Pakistan along with species from the island of Reunion. Figure 7E shows the phylogenetic relationship of *Pampus argenteus* samples occurring across the globe. The experimental samples from Pakistan were clustered together with sequences from India. Both regions of Pakistan share a common Arabian Sea so they also share common ancestors. Figure 7F illustrates the evolutionary relationship of *Plotosus lineatus* occurring across the globe. The experimental species formed a clade with the species from Saudi Arabia, India, and the Philippines. So, the individuals from these regions are more related to each other and are considered to have evolved from the same ancestors.

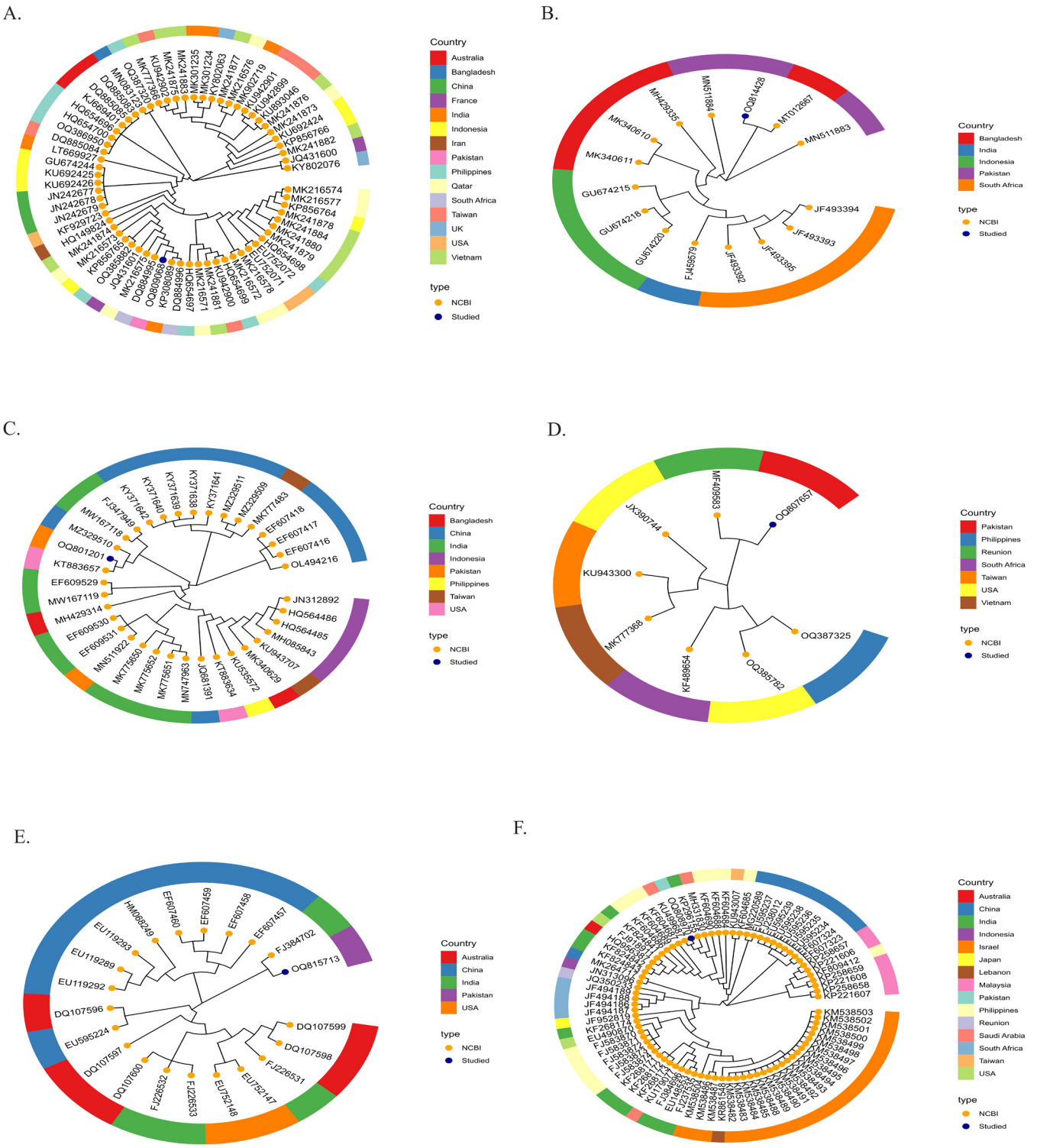

Figure 7: Species level phylogenetic relationship illustration of members occurring across the globe. (A). *Chanos chanos* (B). *Drepane longimana* (C). *Lactarius lactarius* (D). *Myripristis botche* (E). *Pampus argenteus* (F). *Plotosus lineatus*

Figure 8A illustrates a phylogenetic relationship of *Pomacanthus annularis* members occurring in different regions of the world. The experimental species from Pakistan were clustered under a clade shared by species members from Myanmar and Sri Lanka. Figure 8B depicts the evolutionary relationship of worldwide occurring members belonging to the *Rachycentron canadum* species. Our experimental species were clustered under the clade in which the members from Bangladesh and India were clustered. So, the experimental species was evolutionary more similar to members from Bangladesh and India. Figure 8C illustrates the phylogenetic relationship between members of *Sargocentron rubrum* representing different regions of the world. The studied samples were clustered in a clade with members from India and Australia. Figure 8D represents the phylogenetic relationship of *Scatophagus*

argus members from different regions of the world. All of the members were clustered under different clades according to their evolutionary relationship. Experimental species were clustered under a clade with Australia, Sri Lanka, and India. Figure 8E manifested phylogenetic relationship members of *Terapon jarbua* from available COI sequences on NCBI and sequences obtained from experimental samples. The blue color on the tips represents the type specimen and the orange color on the tips shows sequences obtained from NCBI. The studied species were clustered under the clade with the members of species from France and India. Figure 8F manifested the phylogenetic relationship of *Terapon puta* occurring across the globe. Each of the countries was labeled with a unique pattern of colors. The studied samples from Pakistan were clustered under a clade with members from Lebanon, Israel, and Saudi Arabia.

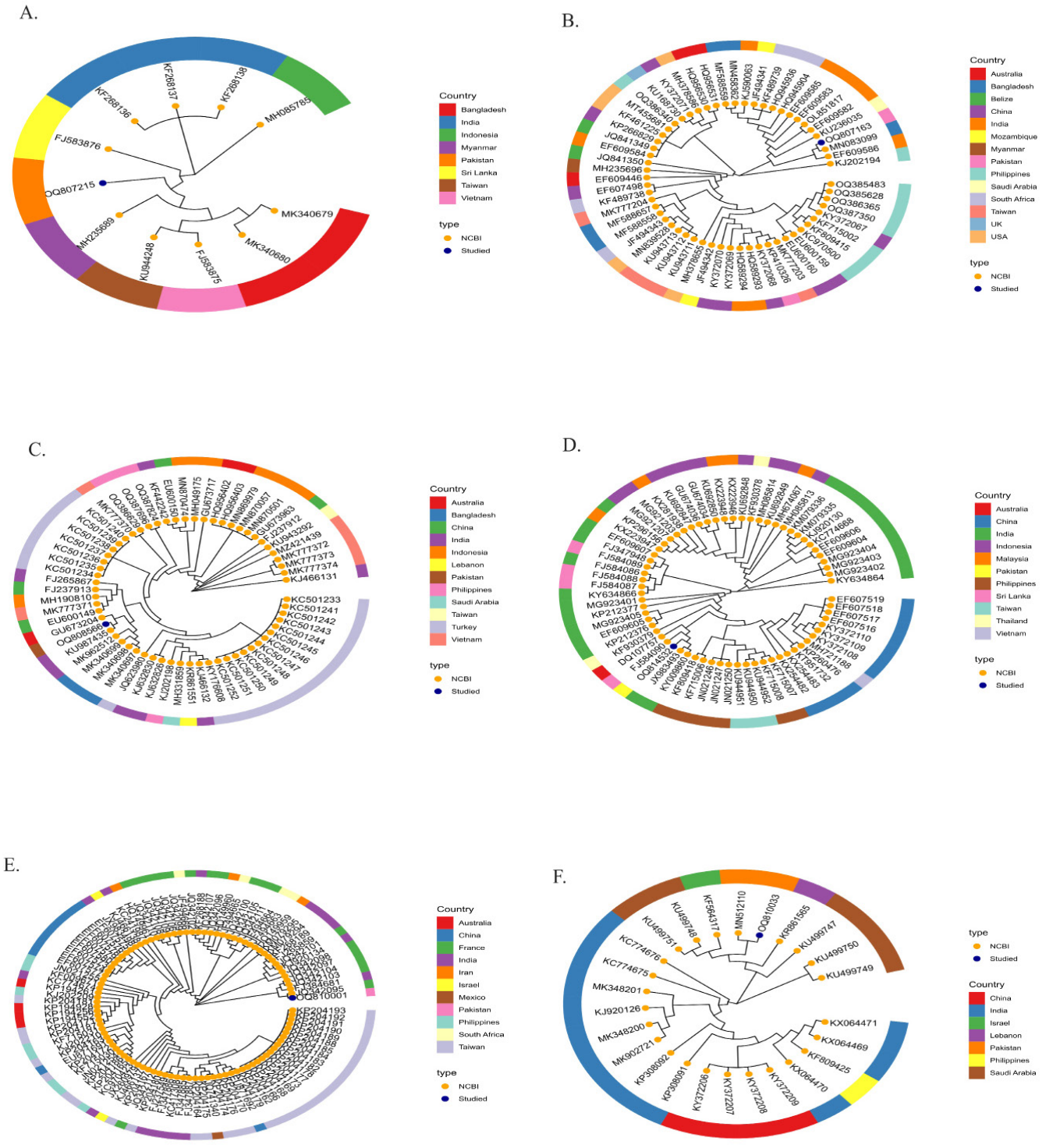

Figure 8: Species-level phylogenetic relationship illustration of members occurring across the globe (A). *Pomacanthus annularis* (B). *Rachycentron canadum* (C). *Sargocentron rubrum* (D). *Scatophagus argus* (E). *Terapon jarbua* (F). *Terapon puta*.

Phylogenetic relationship at the family level

Based on results obtained from phylogenetic analysis at the family level it was depicted that species belonging to the same family were clustered under the same clades whereas species belonging to different families were clustered under different clades (Figure 9). Moreover, the species members of *Chanos chanos* from Pakistan were more similar to South Africa, Qatar, and India. Species members of *Myripristis botche* formed a distinct clade and type member from Pakistan was surrounded by USA and Reunion depicting a close genetic resemblance. *Plotosus lineatus* from Pakistan showed close genetic resemblance with Saudia Arabia and Malaysia. *Pampus argenteus* members from Pakistan showed close resemblance with China and India. Species members of *Scatophagus argus* representing Pakistan were more closely related to India and Sri Lanka. Likewise, each of type species showed their resemblance with the members from countries that showed more closed evolutionary relationships or the members that were inferred from common ancestors.

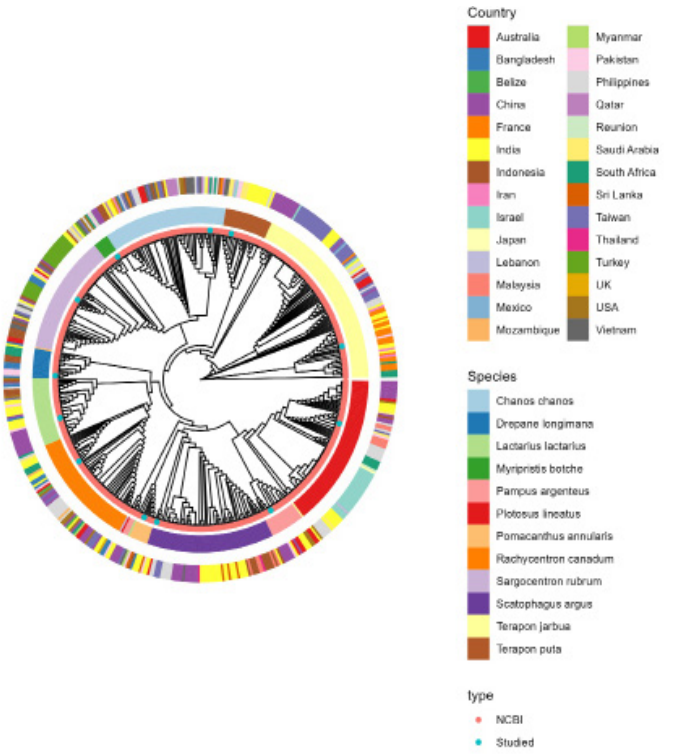

Figure 9: Phylogenetic relationship of all samples included in the study.

The results based on mitochondrial gene COI show significant differentiation between the species population of (*Lactarius lactarius, Rachycentron canadum, Caesio varilineata, Pempheris russellii, Pomacanthus annularis, Myripristis botche, Sargocentron rubrum, Plotosus lineatus, Chanos chanos, Uranoscopus dollfusi, Terapon jarbua, Terapon puta, Drepane longimana, Scatophagus argus, Pampus argenteus*) belonging to 13 families and 7 orders, representing different regions of the world. To our knowledge, our study provides the first-ever COI nucleotide sequence addition of *Caesio Varilineata,* and *Uranscopus dollfusi* to Genbank.

This study revealed genetic differentiation between various fish populations via Fst analysis. The results showed a substantial genetic differentiation (Fst = 0.75) in populations of *Plotosus lineatus* and *Scatophagus argus,* illustrating a significant divergence between them. Other species displayed varying levels of genetic differentiation (Fst = 0.51, 0.57, 0.48, and 0.3), implying some degree of population divergence. Possible factors contributing to these differences can be due to geographical barriers, limited gene flow, and local adaptation. The findings have important implications for conservation efforts, as high levels of genetic differentiation may require tailored conservation strategies. Additionally, the study contributes to our understanding of the genetic structure of the studied fish species and underscores the importance of maintaining genetically diverse populations for species resilience and adaptability.

Within the suborder Stromateoidei the family Pempheridae is the most speciose family, this family illustrated the poorly resolved systematics owing to its highly conserved anatomical features and wide distribution (Haedrich, 1967; Liu *et al*., 2013). Previously work done on *Pampus argenteus* lacked comprehensive information regarding the phylogenetics and population divergence. They showed within species genetic distance of about 0.000 to 0.005, However, Fst analysis revealed 0.05-0.33 genetic differentiation within the population of the same species this variation can be due to the large sample size (Cui *et al*., 2010). Moreover, our study has illustrated the detailed phylogenetics and population differentiation.

Nasihin-Seth *et al.* (2019) provide the DNA barcodes of *Plotosus lineatus* based on COI and the limited phylogenetic information from Malaysia. However, the members of this species particularly from Pakistan were under-explored on a molecular basis. Our study manifested the detailed phylogenetic relation as well as the species' genetic diversity and population divergence within different regions of the world. The divergence between the members of species belonging to different regions of the world was measured using Fst analysis and showed 0.05-0.75 genetic divergence. The species population of *Plotosus lineatus* from the Arabian Sea was more similar to the population of India as they share less genetic differentiation between them. This similarity is obvious, as Pakistan and India share a common border. Over time, species and their relatives may travel within these places and share common ancestors as well.

Barton *et al.* (2018) documented the detailed information of *Rachycentron canadum* on the taxonomic characters as well as based on COI sequencing of the members of species from the Australian Coast. However,

the species data from Pakistan still need to be explored. Our study provides detailed information on basic morphology, DNA barcodes, population genetic divergence, and phylogenetic relationship between the complex diversity of the Arabian Sea and all of the COI sequence data available on the online databases. The population divergence was found using Fst analysis and shows (0.06-0.61) values. The type species population showed more resemblance to Chinese and Indian populations. The low population differentiation can be due to different factors, including geographical proximity, historical gene flow, shared environmental conditions, and high dispersal ability.

Washim *et al*. (2022) illustrated the information based on morphometric measurements of *Scatophagus argus,* particularly from Bangladesh. However, in addition to morphometric measurements molecular-based exploration is required for more authentic species delimitation, particularly from Pakistan. Our study offers comprehensive insights into DNA barcodes, population divergence, genetic distances, and phylogenetic relationships between the experimental species and other species found globally. The online QR code for the nucleotide information of sequences was also generated. The population divergence was revealed using Fst analysis and illustrated (0.04-0.75) values. The phylogenetic tree shows a grouping of the taxas based on nucleotide sequence similarity. The population differentiation of type species showed maximum resemblance with populations of India and China. The possible reason can be the geographical proximity as they share common borders and shorter distances for potential migration. There can be other reasons such as high dispersal ability, historical gene flow, and shared environmental conditions.

The Fst analysis based on a single gene, COI, unravels significant genetic differentiation among species populations of type species. The maximum value of 0.75 manifested considerable genetic divergence between different populations indicating limited gene flow and potential population isolation. In contrast minimal value of 0.04 implies a lower level of genetic divergence between other populations, depicting higher gene flow and potential connectivity. These findings depict that genetic structure within type species varies across regions and populations. Higher differentiation values proposed the presence of subpopulations or local adaptations, that are typically influenced by factors such as environmental conditions, geographical barriers, and historical events. The higher degree of gene flow is illustrated by the lower values, potentially facilitated by factors like geographical proximity, shared environmental conditions, or the active dispersal mechanism. The differentiation values obtained from a single gene, such as COI give an insight about genetic divergence at that particular locus. To get a detailed insight into genetic structure it is recommended to consider additional genetic markers and broad sampling across different genomic regions. The above findings highlighted the importance of maintaining connectivity between populations having low differentiation to preserve overall genetic diversity and to prevent the loss of locally adapted potential traits. In addition, populations that manifested higher differentiation may warrant specific conservation measures to protect their unique genetic resources and ensure their long-term viability. The present study could serve as an integrative genetic analysis that unravels differentiation and its implications for the conservation and management of type species populations.

Phylogenetic trees were constructed using R script delineating the evolutionary relationship between experimental species and species occurring across the globe. The species were clustered under the clades according to their orders, families, genera, and species. The pattern of clustering obtained from phylogenetic relationships unraveled an interesting genetic relationship among species occurring across the globe. The species members of *Myripristis botche* showed close resemblance with species members of Reunion. The island of Reunion is very far from the northern Arabian Sea but due to many natural changes and adaptations some species might have traveled and may share common ancestors at the time of course. Likewise, the other species revealed their evolutionary history and genetic similarity. These findings may manifest historical migration events or they may share common ancestors across the covered regions. Further studies may be required to reveal the true phylogeny of life for these groups of fishes and could serve as a valuable insight into evolutionary relationships, and practical implications for conservation, genetics, and ecology.

As a whole, the COI barcode data obtained from members of these 13 families have delineated species explicitly. However, only two species (*Pempheris russellii* and *Caesio varilineata*) were not able to be discriminated against and shared barcode index numbers with *Pempheris nesogallica*, *Pempheris mangula*, and *Pterocaesio chrysozona* respectively, keeping in mind their morphological characters were considered again and were matched with all members representing that bin, so a strong decision was made after considering morphological characters as we cannot rely solely on one method for identification. Moreover, there is a need for more molecular markers for the exact identification of these species.

Just like supermarkets that contain a specific QR code for each product, QR codes for species nucleotide sequences can easily be developed and accessed via mobile applications QR code scanners, etc. Before this study QR codes for fish species were developed (Ghouri *et al*., 2020)

but codes for our experimental species were developed for the first time. Bio-Rad DNA barcodes are also being generated using online tools (Yang *et al.*, 2019) for species identification on a molecular basis. Our study developed both DNA barcodes on BOLD systems and the QR codes containing the sequence information are given as a Supplementary Figure 1. As a whole DNA barcoding together with taxonomy can be an effective approach to developing strategies for the management, conservation, and monitoring of the fisheries sector. The present study targets some rare 15 species belonging to 13 families and 7 orders to explore them on morphology and molecular basis. DNA barcoding is not commonly practiced in Pakistan so identification on a molecular basis and generation of DNA-based QR codes can be validated as a basic approach for this purpose.

Conclusions and Recommendations

The present data could serve as a baseline for the identification of new species, environmental studies, and biogeographic patterns. It documented the COI-based DNA database of 15 rarely occurring marine species and two species (*Caesio varilineata, Uranoscopus dollfusi*) for the first time in Genbank from a coastal area inhabiting Pakistan. Moreover, the COI sequence of five species (*Chanos chanos, Sargocentron rubrum, Plotosus lineatus, Myripristis botche,* and *Pomacanthus annularis*) was first time documented from Pakistan to BOLD systems and NCBI repository. The QR codes and DNA barcodes for robust nucleotide information availability were introduced. Moreover, this study serves as a crucial tool for developing management and conservation strategies for marine fish diversity.

Declarations

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IRB approval

This study was approved by the Institutional Review Board of University of Karachi approval no IBC KU-260/2022.

Ethical statement

This study utilized fish samples collected from local markets in Karachi, Pakistan, ensuring minimal harm and no direct impact on fish populations. No harmful or destructive sampling methods were employed.

Supplementary material

The supplementary material associated with this article is given after the references.

Conflict of interest

The authors have declared no conflict of interest.

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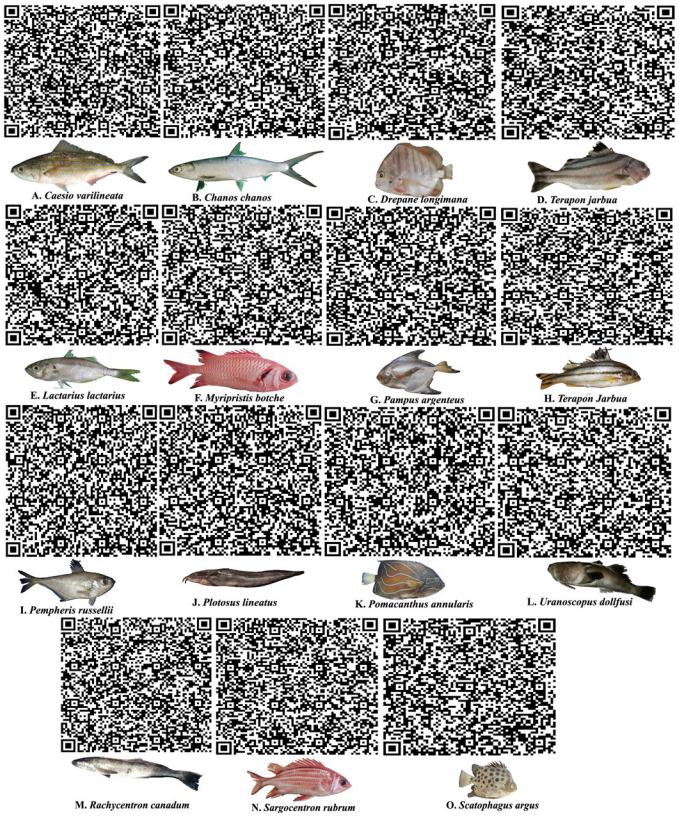
Supplementary Material



Supplementary Figure 1: Specimen pictures together with QR codes that contain nucleotide information for each species.

Supplementary Table 1: Metadata for each species included in the study.

Specie name	Accession no	Country	Type
Rachycentron canadum	OQ807163	Pakistan	Studied
Rachycentron canadum	OQ387350	Philippines	NCBI
Rachycentron canadum	OQ386365	Philippines	NCBI
Rachycentron canadum	OQ386340	Philippines	NCBI
Rachycentron canadum	OQ385628	Philippines	NCBI
Rachycentron canadum	OQ385483	Philippines	NCBI
Rachycentron canadum	KF809415	Philippines	NCBI
Rachycentron canadum	KJ202194	Philippines	NCBI
Rachycentron canadum	KF715002	Philippines	NCBI
Rachycentron canadum	KC970500	Philippines	NCBI
Rachycentron canadum	MT455681	USA	NCBI
Rachycentron canadum	KF461225	USA	NCBI
Rachycentron canadum	MN839528	USA	NCBI
Rachycentron canadum	MH378655	USA	NCBI
Rachycentron canadum	MH378586	USA	NCBI
Rachycentron canadum	HQ956531	Australia	NCBI
Rachycentron canadum	HQ956530	Australia	NCBI
Rachycentron canadum	EF609446	Australia	NCBI
Rachycentron canadum	KY372071	China	NCBI
Rachycentron canadum	KY372070	China	NCBI
Rachycentron canadum	KY372069	China	NCBI
Rachycentron canadum	KY372068	China	NCBI
Rachycentron canadum	KY372067	China	NCBI
Rachycentron canadum	EU600160	China	NCBI
Rachycentron canadum	EU600159	China	NCBI
Rachycentron canadum	EF607498	China	NCBI
Rachycentron canadum	KP266829	China	NCBI
Rachycentron canadum	JF494343	South Africa	NCBI
Rachycentron canadum	HQ945936	South Africa	NCBI
Rachycentron canadum	HQ945904	South Africa	NCBI
Rachycentron canadum	KF489739	South Africa	NCBI
Rachycentron canadum	KF489738	South Africa	NCBI
Rachycentron canadum	JF494342	Mozambique	NCBI
Rachycentron canadum	JF494341	Mozambique	NCBI
Rachycentron canadum	JQ841350	Belize	NCBI
Rachycentron canadum	JQ841349	Belize	NCBI
Rachycentron canadum	KU236035	Saudi Arabia	NCBI
Rachycentron canadum	MH235696	Myanmar	NCBI
Rachycentron canadum	MK777204	Taiwan	NCBI
Rachycentron canadum	MK777203	Taiwan	NCBI
Rachycentron canadum	KU943713	Taiwan	NCBI
Rachycentron canadum	KU943712	Taiwan	NCBI
Rachycentron canadum	KU943711	Taiwan	NCBI
Rachycentron canadum	MN458362	Bangladesh	NCBI
Rachycentron canadum	MN083099	Bangladesh	NCBI
Rachycentron canadum	MF588559	Bangladesh	NCBI
Rachycentron canadum	MF588558	Bangladesh	NCBI
Rachycentron canadum	MF588657	Bangladesh	NCBI
Rachycentron canadum	KU168730	UK	NCBI
Rachycentron canadum	KP410326	Pakistan	NCBI
Rachycentron canadum	KJ590063	India	NCBI
Rachycentron canadum	EF609586	India	NCBI
Rachycentron canadum	EF609585	India	NCBI
Rachycentron canadum	EF609584	India	NCBI

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Specie name	Accession no	Country	Type
Rachycentron canadum	EF609583	India	NCBI
Rachycentron canadum	EF609582	India	NCBI
Rachycentron canadum	HQ589294	India	NCBI
Rachycentron canadum	HQ589293	India	NCBI
Rachycentron canadum	OL851817	India	NCBI
Lactarius lactarius	OQ801201	Pakistan	Studied
Lactarius lactarius	EF607416	China	NCBI
Lactarius lactarius	EF607417	China	NCBI
Lactarius lactarius	EF607418	China	NCBI
Lactarius lactarius	JQ681391	China	NCBI
Lactarius lactarius	MZ329509	China	NCBI
Lactarius lactarius	MZ329510	China	NCBI
Lactarius lactarius	MZ329511	China	NCBI
Lactarius lactarius	OL494216	China	NCBI
Lactarius lactarius	KY371638	China	NCBI
Lactarius lactarius	KY371639	China	NCBI
Lactarius lactarius	KY371640	China	NCBI
Lactarius lactarius	KY371641	China	NCBI
Lactarius lactarius	KY371642	China	NCBI
Lactarius lactarius	EF609529	India	NCBI
Lactarius lactarius	EF609530	India	NCBI
Lactarius lactarius	EF609531	India	NCBI
Lactarius lactarius	FJ347949	India	NCBI
Lactarius lactarius	MK775650	India	NCBI
Lactarius lactarius	MK775651	India	NCBI
Lactarius lactarius	MK775652	India	NCBI
Lactarius lactarius	MN747963	India	NCBI
Lactarius lactarius	MW167118	India	NCBI
Lactarius lactarius	MW167119	India	NCBI
Lactarius lactarius	HQ564485	Indonesia	NCBI
Lactarius lactarius	HQ564486	Indonesia	NCBI
Lactarius lactarius	JN312892	Indonesia	NCBI
Lactarius lactarius	MH085843	Indonesia	NCBI
Lactarius lactarius	KT883634	USA	NCBI
Lactarius lactarius	KT883657	USA	NCBI
Lactarius lactarius	KU535572	Philippines	NCBI
Lactarius lactarius	KU943707	Taiwan	NCBI
Lactarius lactarius	MK777483	Taiwan	NCBI
Lactarius lactarius	MH429314	Bangladesh	NCBI
Lactarius lactarius	MK340629	Bangladesh	NCBI
Lactarius lactarius	MN511922	Pakistan	NCBI
Pomacanthus annularis	OQ807215	Pakistan	Studied
Pomacanthus annularis	MH235689	Myanmar	NCBI
Pomacanthus annularis	MK340680	Bangladesh	NCBI
Pomacanthus annularis	MK340679	Bangladesh	NCBI
Pomacanthus annularis	MH085785	Indonesia	NCBI

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Specie name	Accession no	Country	Type
Pomacanthus annularis	KU944248	Taiwan	NCBI
Pomacanthus annularis	KF268138	India	NCBI
Pomacanthus annularis	KF268137	India	NCBI
Pomacanthus annularis	KF268136	India	NCBI
Pomacanthus annularis	FJ583876	Sri Lanka	NCBI
Pomacanthus annularis	FJ583875	Vietnam	NCBI
Myripristis botche	OQ807657	Pakistan	Studied
Myripristis botche	KU943300	Taiwan	NCBI
Myripristis botche	JX390744	USA	NCBI
Myripristis botche	OQ385782	USA	NCBI
Myripristis botche	OQ387325	Philippines	NCBI
Myripristis botche	MF409583	Reunion	NCBI
Myripristis botche	KF489654	South Africa	NCBI
Myripristis botche	MK777368	Vietnam	NCBI
Sargocentron rubrum	OQ808566	Pakistan	Studied
Sargocentron rubrum	OQ387824	Philippines	NCBI
Sargocentron rubrum	OQ387696	Philippines	NCBI
Sargocentron rubrum	OQ386629	Philippines	NCBI
Sargocentron rubrum	KJ202198	Philippines	NCBI
Sargocentron rubrum	MZ421439	Vietnam	NCBI
Sargocentron rubrum	MK777374	Vietnam	NCBI
Sargocentron rubrum	MK777373	Vietnam	NCBI
Sargocentron rubrum	MK777372	Vietnam	NCBI
Sargocentron rubrum	MK777371	Vietnam	NCBI
Sargocentron rubrum	MK777370	Vietnam	NCBI
Sargocentron rubrum	GU673963	Indonesia	NCBI
Sargocentron rubrum	GU673717	Indonesia	NCBI
Sargocentron rubrum	MN870501	Indonesia	NCBI
Sargocentron rubrum	MN870474	Indonesia	NCBI
Sargocentron rubrum	MN870057	Indonesia	NCBI
Sargocentron rubrum	MN869979	Indonesia	NCBI
Sargocentron rubrum	MH049175	Indonesia	NCBI
Sargocentron rubrum	MH190810	Indonesia	NCBI
Sargocentron rubrum	GU673204	Australia	NCBI
Sargocentron rubrum	HQ956403	Australia	NCBI
Sargocentron rubrum	HQ956402	Australia	NCBI
Sargocentron rubrum	KR861551	Lebanon	NCBI
Sargocentron rubrum	KC501252	Turkey	NCBI
Sargocentron rubrum	KC501251	Turkey	NCBI
Sargocentron rubrum	KC501250	Turkey	NCBI
Sargocentron rubrum	KC501249	Turkey	NCBI
Sargocentron rubrum	KC501248	Turkey	NCBI
Sargocentron rubrum	KC501247	Turkey	NCBI
Sargocentron rubrum	KC501246	Turkey	NCBI
Sargocentron rubrum	KC501245	Turkey	NCBI
Sargocentron rubrum	KC501244	Turkey	NCBI

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Specie name	Accession no	Country	Type
Sargocentron rubrum	KC501243	Turkey	NCBI
Sargocentron rubrum	KC501242	Turkey	NCBI
Sargocentron rubrum	KC501241	Turkey	NCBI
Sargocentron rubrum	KC501240	Turkey	NCBI
Sargocentron rubrum	KC501239	Turkey	NCBI
Sargocentron rubrum	KC501238	Turkey	NCBI
Sargocentron rubrum	KC501237	Turkey	NCBI
Sargocentron rubrum	KC501236	Turkey	NCBI
Sargocentron rubrum	KC501235	Turkey	NCBI
Sargocentron rubrum	KC501234	Turkey	NCBI
Sargocentron rubrum	KC501233	Turkey	NCBI
Sargocentron rubrum	KY176608	Turkey	NCBI
Sargocentron rubrum	JQ623980	Turkey	NCBI
Sargocentron rubrum	EU600150	China	NCBI
Sargocentron rubrum	EU600149	China	NCBI
Sargocentron rubrum	FJ237913	China	NCBI
Sargocentron rubrum	FJ237912	China	NCBI
Sargocentron rubrum	FJ265867	India	NCBI
Sargocentron rubrum	MK962512	India	NCBI
Sargocentron rubrum	KU987435	India	NCBI
Sargocentron rubrum	KJ632830	India	NCBI
Sargocentron rubrum	KJ632826	India	NCBI
Sargocentron rubrum	KJ466132	India	NCBI
Sargocentron rubrum	KJ466131	India	NCBI
Sargocentron rubrum	KF442242	India	NCBI
Sargocentron rubrum	MK340699	Bangladesh	NCBI
Sargocentron rubrum	MK340698	Bangladesh	NCBI
Sargocentron rubrum	MK340697	Bangladesh	NCBI
Sargocentron rubrum	MH331859	Saudi Arabia	NCBI
Sargocentron rubrum	KU943292	Taiwan	NCBI
Plotosus lineatus	OQ808970	Pakistan	Studied
Plotosus lineatus	EF607323	China	NCBI
Plotosus lineatus	EF607324	China	NCBI
Plotosus lineatus	EU595234	China	NCBI
Plotosus lineatus	EU595235	China	NCBI
Plotosus lineatus	EU595236	China	NCBI
Plotosus lineatus	EU595237	China	NCBI
Plotosus lineatus	EU595238	China	NCBI
Plotosus lineatus	EU595239	China	NCBI
Plotosus lineatus	FJ238012	China	NCBI
Plotosus lineatus	MG220589	China	NCBI
Plotosus lineatus	MK264717	China	NCBI
Plotosus lineatus	EU148553	India	NCBI
Plotosus lineatus	FJ237535	India	NCBI
Plotosus lineatus	FJ384696	India	NCBI
Plotosus lineatus	KF268171	India	NCBI

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Specie name	Accession no	Country	Type
Plotosus lineatus	KF268172	India	NCBI
Plotosus lineatus	KF268173	India	NCBI
Plotosus lineatus	KF268174	India	NCBI
Plotosus lineatus	KF824841	India	NCBI
Plotosus lineatus	KF824842	India	NCBI
Plotosus lineatus	KF824843	India	NCBI
Plotosus lineatus	KP296155	India	NCBI
Plotosus lineatus	EU490870	USA	NCBI
Plotosus lineatus	FJ918911	USA	NCBI
Plotosus lineatus	FJ583870	Philippines	NCBI
Plotosus lineatus	FJ583871	Philippines	NCBI
Plotosus lineatus	FJ583872	Philippines	NCBI
Plotosus lineatus	FJ583873	Philippines	NCBI
Plotosus lineatus	FJ583874	Philippines	NCBI
Plotosus lineatus	KF604684	Philippines	NCBI
Plotosus lineatus	KF604685	Philippines	NCBI
Plotosus lineatus	KF604686	Philippines	NCBI
Plotosus lineatus	KF604687	Philippines	NCBI
Plotosus lineatus	KF604688	Philippines	NCBI
Plotosus lineatus	KF604689	Philippines	NCBI
Plotosus lineatus	KF604690	Philippines	NCBI
Plotosus lineatus	KF809412	Philippines	NCBI
Plotosus lineatus	HQ956387	Australia	NCBI
Plotosus lineatus	JF494186	South Africa	NCBI
Plotosus lineatus	JF494187	South Africa	NCBI
Plotosus lineatus	JF494188	South Africa	NCBI
Plotosus lineatus	JF494189	South Africa	NCBI
Plotosus lineatus	JF952819	Japan	NCBI
Plotosus lineatus	JN313096	Indonesia	NCBI
Plotosus lineatus	JQ350233	Reunion	NCBI
Plotosus lineatus	KM538482	Israel	NCBI
Plotosus lineatus	KM538483	Israel	NCBI
Plotosus lineatus	KM538484	Israel	NCBI
Plotosus lineatus	KM538485	Israel	NCBI
Plotosus lineatus	KM538486	Israel	NCBI
Plotosus lineatus	KM538487	Israel	NCBI
Plotosus lineatus	KM538488	Israel	NCBI
Plotosus lineatus	KM538489	Israel	NCBI
Plotosus lineatus	KM538490	Israel	NCBI
Plotosus lineatus	KM538491	Israel	NCBI
Plotosus lineatus	KM538492	Israel	NCBI
Plotosus lineatus	KM538493	Israel	NCBI
Plotosus lineatus	KM538494	Israel	NCBI
Plotosus lineatus	KM538495	Israel	NCBI
Plotosus lineatus	KM538496	Israel	NCBI
Plotosus lineatus	KM538497	Israel	NCBI

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Specie name	Accession no	Country	Type
Plotosus lineatus	KM538498	Israel	NCBI
Plotosus lineatus	KM538499	Israel	NCBI
Plotosus lineatus	KM538500	Israel	NCBI
Plotosus lineatus	KM538501	Israel	NCBI
Plotosus lineatus	KM538502	Israel	NCBI
Plotosus lineatus	KM538503	Israel	NCBI
Plotosus lineatus	KM538504	Israel	NCBI
Plotosus lineatus	KP221606	Malaysia	NCBI
Plotosus lineatus	KP221607	Malaysia	NCBI
Plotosus lineatus	KP221608	Malaysia	NCBI
Plotosus lineatus	KP258657	Malaysia	NCBI
Plotosus lineatus	KP258658	Malaysia	NCBI
Plotosus lineatus	KP258659	Malaysia	NCBI
Plotosus lineatus	KR861548	Lebanon	NCBI
Plotosus lineatus	KU179077	Saudi Arabia	NCBI
Plotosus lineatus	KU499687	Saudi Arabia	NCBI
Plotosus lineatus	MH331830	Saudi Arabia	NCBI
Plotosus lineatus	KU943007	Taiwan	NCBI
Chanos chanos	OQ809068	Pakistan	Studied
Chanos chanos	DQ884995	South Africa	NCBI
Chanos chanos	DQ884996	South Africa	NCBI
Chanos chanos	DQ885083	Australia	NCBI
Chanos chanos	DQ885085	Australia	NCBI
Chanos chanos	KJ669401	Australia	NCBI
Chanos chanos	EU752071	USA	NCBI
Chanos chanos	EU752072	USA	NCBI
Chanos chanos	KF929723	USA	NCBI
Chanos chanos	GU674244	Indonesia	NCBI
Chanos chanos	KP856764	Indonesia	NCBI
Chanos chanos	KP856765	Indonesia	NCBI
Chanos chanos	KP856766	Indonesia	NCBI
Chanos chanos	KU692424	Indonesia	NCBI
Chanos chanos	KU692425	Indonesia	NCBI
Chanos chanos	KU692426	Indonesia	NCBI
Chanos chanos	HQ149824	Iran	NCBI
Chanos chanos	HQ654696	Philippines	NCBI
Chanos chanos	HQ654697	Philippines	NCBI
Chanos chanos	HQ654698	Philippines	NCBI
Chanos chanos	HQ654699	Philippines	NCBI
Chanos chanos	HQ654700	Philippines	NCBI
Chanos chanos	OQ385882	Philippines	NCBI
Chanos chanos	OQ386950	Philippines	NCBI
Chanos chanos	OQ387320	Philippines	NCBI
Chanos chanos	JN242677	China	NCBI
Chanos chanos	JN242678	China	NCBI
Chanos chanos	JN242679	China	NCBI

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Specie name	Accession no	Country	Type
Chanos chanos	JQ431600	France	NCBI
Chanos chanos	JQ431601	France	NCBI
Chanos chanos	KP308089	India	NCBI
Chanos chanos	LT669927	India	NCBI
Chanos chanos	MK301234	India	NCBI
Chanos chanos	MK301235	India	NCBI
Chanos chanos	MK902719	India	NCBI
Chanos chanos	DQ885084	Taiwan	NCBI
Chanos chanos	KU893046	Taiwan	NCBI
Chanos chanos	KU942899	Taiwan	NCBI
Chanos chanos	KU942900	Taiwan	NCBI
Chanos chanos	KU942901	Taiwan	NCBI
Chanos chanos	KU942902	Taiwan	NCBI
Chanos chanos	KY802063	UK	NCBI
Chanos chanos	KY802076	UK	NCBI
Chanos chanos	MK216571	Qatar	NCBI
Chanos chanos	MK216572	Qatar	NCBI
Chanos chanos	MK216573	Qatar	NCBI
Chanos chanos	MK216574	Qatar	NCBI
Chanos chanos	MK216575	Qatar	NCBI
Chanos chanos	MK216576	Qatar	NCBI
Chanos chanos	MK216577	Qatar	NCBI
Chanos chanos	MK216578	Qatar	NCBI
Chanos chanos	MK241873	Qatar	NCBI
Chanos chanos	MK241874	Vietnam	NCBI
Chanos chanos	MK241875	Vietnam	NCBI
Chanos chanos	MK241876	Vietnam	NCBI
Chanos chanos	MK241877	Vietnam	NCBI
Chanos chanos	MK241878	Vietnam	NCBI
Chanos chanos	MK241879	Vietnam	NCBI
Chanos chanos	MK241880	Vietnam	NCBI
Chanos chanos	MK241881	Vietnam	NCBI
Chanos chanos	MK241882	Vietnam	NCBI
Chanos chanos	MK241883	Vietnam	NCBI
Chanos chanos	MK241884	Vietnam	NCBI
Chanos chanos	MK777366	Vietnam	NCBI
Chanos chanos	MN083123	Bangladesh	NCBI
Terapon jarbua	OQ810001	Pakistan	Studied
Terapon jarbua	EF607573	China	NCBI
Terapon jarbua	EF607574	China	NCBI
Terapon jarbua	EF607575	China	NCBI
Terapon jarbua	EF607576	China	NCBI
Terapon jarbua	EF607577	China	NCBI
Terapon jarbua	EF607578	China	NCBI
Terapon jarbua	EF607579	China	NCBI
Terapon jarbua	EF607580	China	NCBI

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Specie name	Accession no	Country	Type
Terapon jarbua	EU871691	China	NCBI
Terapon jarbua	EU871692	China	NCBI
Terapon jarbua	FJ237549	India	NCBI
Terapon jarbua	FJ265859	India	NCBI
Terapon jarbua	FJ347885	India	NCBI
Terapon jarbua	FJ347886	India	NCBI
Terapon jarbua	FJ347887	India	NCBI
Terapon jarbua	FJ384681	India	NCBI
Terapon jarbua	JX260979	India	NCBI
Terapon jarbua	JX983498	India	NCBI
Terapon jarbua	KC241987	India	NCBI
Terapon jarbua	KC417308	India	NCBI
Terapon jarbua	KC774674	India	NCBI
Terapon jarbua	KF268188	India	NCBI
Terapon jarbua	KF268189	India	NCBI
Terapon jarbua	KJ920134	India	NCBI
Terapon jarbua	KM079294	India	NCBI
Terapon jarbua	KM079295	India	NCBI
Terapon jarbua	HQ149959	Iran	NCBI
Terapon jarbua	HQ149960	Iran	NCBI
Terapon jarbua	HQ149961	Iran	NCBI
Terapon jarbua	JF494663	South Africa	NCBI
Terapon jarbua	JF494664	South Africa	NCBI
Terapon jarbua	JF494665	South Africa	NCBI
Terapon jarbua	JF494666	South Africa	NCBI
Terapon jarbua	JN021254	Philippines	NCBI
Terapon jarbua	JN021255	Philippines	NCBI
Terapon jarbua	KC970423	Philippines	NCBI
Terapon jarbua	KF009671	Philippines	NCBI
Terapon jarbua	KF715030	Philippines	NCBI
Terapon jarbua	KF715031	Philippines	NCBI
Terapon jarbua	KJ202209	Philippines	NCBI
Terapon jarbua	KJ202210	Philippines	NCBI
Terapon jarbua	JQ342095	France	NCBI
Terapon jarbua	JQ342096	France	NCBI
Terapon jarbua	JQ342097	France	NCBI
Terapon jarbua	JQ342098	France	NCBI
Terapon jarbua	JQ342099	France	NCBI
Terapon jarbua	JQ342100	France	NCBI
Terapon jarbua	JQ342101	France	NCBI
Terapon jarbua	JQ342102	France	NCBI
Terapon jarbua	JQ342103	France	NCBI
Terapon jarbua	JQ342104	France	NCBI
Terapon jarbua	JQ342105	France	NCBI
Terapon jarbua	JQ342106	France	NCBI
Terapon jarbua	JQ342107	France	NCBI

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Specie name	Accession no	Country	Type
Terapon jarbua	JQ342108	France	NCBI
Terapon jarbua	JQ342109	France	NCBI
Terapon jarbua	JQ342110	France	NCBI
Terapon jarbua	JQ342111	France	NCBI
Terapon jarbua	JQ342112	France	NCBI
Terapon jarbua	JQ741340	Mexico	NCBI
Terapon jarbua	KJ466137	Israel	NCBI
Terapon jarbua	KJ466138	Israel	NCBI
Terapon jarbua	KP194256	Australia	NCBI
Terapon jarbua	KP194261	Australia	NCBI
Terapon jarbua	KP194552	Australia	NCBI
Terapon jarbua	KP194928	Australia	NCBI
Terapon jarbua	KP204162	Taiwan	NCBI
Terapon jarbua	KP204163	Taiwan	NCBI
Terapon jarbua	KP204164	Taiwan	NCBI
Terapon jarbua	KP204165	Taiwan	NCBI
Terapon jarbua	KP204166	Taiwan	NCBI
Terapon jarbua	KP204167	Taiwan	NCBI
Terapon jarbua	KP204168	Taiwan	NCBI
Terapon jarbua	KP204169	Taiwan	NCBI
Terapon jarbua	KP204170	Taiwan	NCBI
Terapon jarbua	KP204171	Taiwan	NCBI
Terapon jarbua	KP204172	Taiwan	NCBI
Terapon jarbua	KP204173	Taiwan	NCBI
Terapon jarbua	KP204174	Taiwan	NCBI
Terapon jarbua	KP204175	Taiwan	NCBI
Terapon jarbua	KP204176	Taiwan	NCBI
Terapon jarbua	KP204177	Taiwan	NCBI
Terapon jarbua	KP204178	Taiwan	NCBI
Terapon jarbua	KP204179	Taiwan	NCBI
Terapon jarbua	KP204180	Taiwan	NCBI
Terapon jarbua	KP204181	Taiwan	NCBI
Terapon jarbua	KP204182	Taiwan	NCBI
Terapon jarbua	KP204183	Taiwan	NCBI
Terapon jarbua	KP204184	Taiwan	NCBI
Terapon jarbua	KP204185	Taiwan	NCBI
Terapon jarbua	KP204186	Taiwan	NCBI
Terapon jarbua	KP204187	Taiwan	NCBI
Terapon jarbua	KP204188	Taiwan	NCBI
Terapon jarbua	KP204189	Taiwan	NCBI
Terapon jarbua	KP204190	Taiwan	NCBI
Terapon jarbua	KP204191	Taiwan	NCBI
Terapon jarbua	KP204192	Taiwan	NCBI
Terapon jarbua	KP204193	Taiwan	NCBI
Terapon puta	OQ810033	Pakistan	Studied
Terapon puta	KC774675	India	NCBI

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Specie name	Accession no	Country	Type
Terapon puta	KC774676	India	NCBI
Terapon puta	KJ920126	India	NCBI
Terapon puta	KP308091	India	NCBI
Terapon puta	KP308092	India	NCBI
Terapon puta	KX064469	India	NCBI
Terapon puta	KX064470	India	NCBI
Terapon puta	KX064471	India	NCBI
Terapon puta	MK348200	India	NCBI
Terapon puta	MK348201	India	NCBI
Terapon puta	MK902721	India	NCBI
Terapon puta	KF564317	Israel	NCBI
Terapon puta	KF809425	Philippines	NCBI
Terapon puta	KR861565	Lebanon	NCBI
Terapon puta	KU499747	Saudi Arabia	NCBI
Terapon puta	KU499748	Saudi Arabia	NCBI
Terapon puta	KU499749	Saudi Arabia	NCBI
Terapon puta	KU499750	Saudi Arabia	NCBI
Terapon puta	KU499751	Saudi Arabia	NCBI
Terapon puta	KY372206	China	NCBI
Terapon puta	KY372207	China	NCBI
Terapon puta	KY372208	China	NCBI
Terapon puta	KY372209	China	NCBI
Terapon puta	MN512110	Pakistan	NCBI
Drepane longimana	OQ814428	Pakistan	Studied
Drepane longimana	FJ459579	India	NCBI
Drepane longimana	GU674215	Indonesia	NCBI
Drepane longimana	GU674218	Indonesia	NCBI
Drepane longimana	GU674220	Indonesia	NCBI
Drepane longimana	JF493392	South Africa	NCBI
Drepane longimana	JF493393	South Africa	NCBI
Drepane longimana	JF493394	South Africa	NCBI
Drepane longimana	JF493395	South Africa	NCBI
Drepane longimana	MH429335	Bangladesh	NCBI
Drepane longimana	MK340610	Bangladesh	NCBI
Drepane longimana	MK340611	Bangladesh	NCBI
Drepane longimana	MT012667	Bangladesh	NCBI
Drepane longimana	MN511883	Pakistan	NCBI
Drepane longimana	MN511884	Pakistan	NCBI
Scatophagus argus	OQ814532	Pakistan	Studied
Scatophagus argus	DQ107757	Australia	NCBI
Scatophagus argus	EF607516	China	NCBI
Scatophagus argus	EF607517	China	NCBI
Scatophagus argus	EF607518	China	NCBI
Scatophagus argus	EF607519	China	NCBI
Scatophagus argus	KP260476	China	NCBI
Scatophagus argus	KT951732	China	NCBI

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Specie name	Accession no	Country	Type
Scatophagus argus	KX254482	China	NCBI
Scatophagus argus	KX254483	China	NCBI
Scatophagus argus	KY372108	China	NCBI
Scatophagus argus	KY372109	China	NCBI
Scatophagus argus	KY372110	China	NCBI
Scatophagus argus	EF609604	India	NCBI
Scatophagus argus	EF609605	India	NCBI
Scatophagus argus	EF609606	India	NCBI
Scatophagus argus	EF609607	India	NCBI
Scatophagus argus	FJ347948	India	NCBI
Scatophagus argus	FJ584086	India	NCBI
Scatophagus argus	JX983493	India	NCBI
Scatophagus argus	KC774668	India	NCBI
Scatophagus argus	KJ920130	India	NCBI
Scatophagus argus	KM079335	India	NCBI
Scatophagus argus	KM079336	India	NCBI
Scatophagus argus	KP212376	India	NCBI
Scatophagus argus	KP212377	India	NCBI
Scatophagus argus	KP296156	India	NCBI
Scatophagus argus	KY009860	India	NCBI
Scatophagus argus	KY634864	India	NCBI
Scatophagus argus	KY634866	India	NCBI
Scatophagus argus	MG923401	India	NCBI
Scatophagus argus	MG923402	India	NCBI
Scatophagus argus	MG923403	India	NCBI
Scatophagus argus	MG923404	India	NCBI
Scatophagus argus	MG923405	India	NCBI
Scatophagus argus	FJ584087	Sri Lanka	NCBI
Scatophagus argus	FJ584088	Sri Lanka	NCBI
Scatophagus argus	FJ584089	Sri Lanka	NCBI
Scatophagus argus	FJ584090	Sri Lanka	NCBI
Scatophagus argus	GU674034	Indonesia	NCBI
Scatophagus argus	GU674036	Indonesia	NCBI
Scatophagus argus	KU692847	Indonesia	NCBI
Scatophagus argus	KU692848	Indonesia	NCBI
Scatophagus argus	KU692849	Indonesia	NCBI
Scatophagus argus	KU692850	Indonesia	NCBI
Scatophagus argus	MG921203	Indonesia	NCBI
Scatophagus argus	MG921207	Indonesia	NCBI
Scatophagus argus	MH085813	Indonesia	NCBI
Scatophagus argus	MH085814	Indonesia	NCBI
Scatophagus argus	JN021246	Philippines	NCBI
Scatophagus argus	JN021247	Philippines	NCBI
Scatophagus argus	JN021250	Philippines	NCBI
Scatophagus argus	KF715006	Philippines	NCBI
Scatophagus argus	KF715007	Philippines	NCBI

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Specie name	Accession no	Country	Type
Scatophagus argus	KF715008	Philippines	NCBI
Scatophagus argus	KF809418	Philippines	NCBI
Scatophagus argus	KF930378	Thailand	NCBI
Scatophagus argus	KF930379	Thailand	NCBI
Scatophagus argus	KU944950	Taiwan	NCBI
Scatophagus argus	KU944951	Taiwan	NCBI
Scatophagus argus	KU944952	Taiwan	NCBI
Scatophagus argus	KX223946	Malaysia	NCBI
Scatophagus argus	KX223947	Malaysia	NCBI
Scatophagus argus	KX223948	Malaysia	NCBI
Scatophagus argus	KX281938	Malaysia	NCBI
Scatophagus argus	MH674067	Malaysia	NCBI
Scatophagus argus	MH721188	Vietnam	NCBI
Pampus argenteus	OQ815713	Pakistan	Studied
Pampus argenteus	DQ107596	Australia	NCBI
Pampus argenteus	DQ107597	Australia	NCBI
Pampus argenteus	DQ107598	Australia	NCBI

Table continued on next column.....

Specie name	Accession no	Country	Type
Pampus argenteus	DQ107599	Australia	NCBI
Pampus argenteus	DQ107600	Australia	NCBI
Pampus argenteus	EF607457	China	NCBI
Pampus argenteus	EF607458	China	NCBI
Pampus argenteus	EF607459	China	NCBI
Pampus argenteus	EF607460	China	NCBI
Pampus argenteus	EU119289	China	NCBI
Pampus argenteus	EU119292	China	NCBI
Pampus argenteus	EU119293	China	NCBI
Pampus argenteus	EU595224	China	NCBI
Pampus argenteus	HM068249	China	NCBI
Pampus argenteus	EU752147	USA	NCBI
Pampus argenteus	EU752148	USA	NCBI
Pampus argenteus	FJ226531	India	NCBI
Pampus argenteus	FJ226532	India	NCBI
Pampus argenteus	FJ226533	India	NCBI
Pampus argenteus	FJ384702	India	NCBI