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MOLECULAR IDENTIFICATION OF MARINE FISH (FAMILY: SPHYRAENIDAE) FROM THE COASTAL AREA OF LASBELA, BALOCHISTAN, PAKISTAN

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ABSTRACT
Molecular identification aims to identify species using mitochondrial genetic markers. DNA barcoding is a very effective method for identifying the labeling of fish products to ensure consumer protection and quality. As barcoding is a reliable technique for identifying taxa from biological material, such as microscopic tissue, body parts, or fins. The study represents of marine fish species in Pakistan, utilizing DNA barcoding to identify from coastal region of Lasbela, Balochistan. So, molecular marker like cytochrome c oxidase subunit-I (COI) based identification method had been extensively employed for identification of marine species. By using cytochrome c oxidase subunit, I (COI) gene as a marker for identification of specie *Sphyraena jello* (Cuvier, 1829), Family: Sphyraenidae were used collected, analyzed, and identified accurately resulting in successful submission to NCBI, GenBank analysis confirmed accuracy of identification as *Sphyraena jello* specie with exact match, as with sequence size of 665 and 442 base pair respectively as inferred from neighbor-joining tree analysis, further corroborated these findings. Study demonstrates the usefulness of DNA extraction method and barcoding as a reliable and efficient approach for species identification and phylogenetic lineage in marine fishes and supporting sustainable management of coastal fish bioresources.

KEYWORDS: DNA barcoding, Molecular identification, Marine Fish, Coast, *Sphyraena jello,*

INTRODUCTION

In a dynamic coastal environment, fish biodiversity supports ecosystem conservation and sustainability (da Silva et al. 2021), food security, economic viability, and socioeconomic upliftment for coastal communities. Natural fish resources in freshwater and marine habitats are being adversely affected by an increasing number of human and natural disturbances, such as habitat degradation, overexploitation, and climate change, which hasten the loss of fish variety in coastal regions. Fish biodiversity loss has been a major worry lately, especially in coastal habitats (Nagelkerken et al. 2023). In order to profile and comprehend fish biodiversity in coastal regions which is essential for managing and protecting coastal fisheries and ecosystems it is necessary to accurately identify and record fish species and create an authentic molecular-level database. Fish species are traditionally identified and categorized using morphometric and meristic traits such body form, the number of fin rays or lateral line scales, allometric features, and coloration pattern. However, these physical characteristics may change throughout the course of a species' existence and are frequently insufficient to accurately evaluate uncommon and cryptic species or incomplete samples (Sheraliev and Peng, 2021). Therefore, taxonomy that relies only on physical traits has drawbacks (Frutos et al. 2022).

Fish identification methods now integrate morphological and molecular (morpho-molecular) approaches to reduce ambiguity (Hou et al. 2020). DNA barcoding is therefore one of the key molecular techniques used in species identification, aside from morphological identification. The mitochondrial COI gene is used in DNA barcoding, a molecular method of identifying an organism. Optimal DNA barcoding allows for the investigation of more interspecies than intraspecies variety, making species identification quick, accurate, economical, and automated (Hebert et al. 2003).

DNA barcoding with COI gene sequences is an effective approach for recognizing known and unknown species by analyzing sequence variation (Li et al., 2025). The sequencing-enhanced rapid, standardized mitochondrial COI identification for molecular identification. For DNA barcoding, the initial easy and inseparable process involves excising the DNA then attempting towards Polymerase Chain Reaction (PCR) amplification, which largely

depends. For more specific distinctions between fish species, the mitochondrial COI-gene is ideal because of close relation among the group of fish species (Panprommin et al., 2023). The COI gene is a typical marker in animal metabarcoding version 30 because of its extensive taxonomic coverage in National Center for Biotechnology Information (NCBI) GenBank and BOLD. DNA-based techniques have demonstrated seafood mislabeling and confirmed species authenticity to prevent supply chain fraud globally (Pardo et al., 2020).
One of the largest groups of piscivorous teleost fishes found in tropical and subtropical marine environments is represented by the 27 species of the family Sphyraenidae that are now living.
The species occurs in tropical and warm temperate waters of the Indo-West Pacific (Hoese et al., 2006), Pakistan (Hosseini et al., 2009), and Philippines (Kulbicki et al., 1993). Hence, the aims of the present study were to ascertain information on the biology of *S. jello* for identification on molecular basis that are essential in managing fishery. This study applied DNA barcoding to properly identify *Sphyraena jello* (Cuvier, 1829) fishes collected from coastal area, Lasbela, Balochistan, Pakistan for management and conservation of marine species.

MATERIAL AND METHODS

Specimens Collection and morphological identification

Present study was conducted between 2024 and 2025 in the coastal regions of Lasbela, Balochistan, Pakistan (Figure 1) and were immediately brought to the Fisheries Research Laboratory, University of Karachi Centre of Excellence in Marine Biology, Karachi, Pakistan. Tissues of fish were excise and preserved in graded ethanol, then stored and processed for further analysis. Initially species are identified on the basis of morphometric characters by descriptions and voucher specimens are labelled.

Morphometric data and analyses

Morphometric data of sampled specimens by using standard methods including fin and other morphometric parameters.

DNA Extraction, PCR Amplification

50-mg of tissue from muscles were excised from each sample specimen for genomic DNA extraction. Genomic DNA was subsequently extracted using the standard phenol-chloroform extraction method. The extracted DNA was stored at –20°C until further analysis. DNA purity and concentration were assessed using a NanoPhotometer (IMPLEN) by measuring absorbance at A260/280. Polymerase chain reaction (PCR) amplification of the mitochondrial COI gene was performed using primers (Table 1).

Table 1: Detail of Primers used for amplification by PCR

Target Region/Gene	Primer ID	Prime Sequence 5′-3′	Temp ℃	GC %	Primer Size
Mit. COI	Forward F1 COI	TCAACCAACCACAAAGACATTGGCAC	61	46.15	26
Mit. COI	Reverse R1 COI	TAGACTTCTGGGTGGCCAAAGAATCA	61	46.15	26

The total PCR reaction volume was 25 µL, comprising 12.5 µL of TaqNova Red PCR Master Mix (BLIRT S.A.), 1.5 µL of DNA template, 0.1 µL of each primer, and 10.8 µL of sterile nuclease-free water. PCR amplification was performed under the following thermal cycling conditions: an initial denaturation step at 95°C for 2 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 54°C for 40 seconds, and extension at 72°C for 1 minute. A final extension step was conducted at 72°C for 7 minutes. The amplified products were subsequently visualized by electrophoresis on a 2% (w/v) agarose gel.

Molecular Analysis

The purified PCR products were submitted for DNA barcode sequencing at First BASE Laboratories Sdn Bhd, Malaysia. MEGA X software for subsequent analysis, Phylogenetic relationships were further evaluated by constructing a maximum-likelihood tree using MEGA X software.

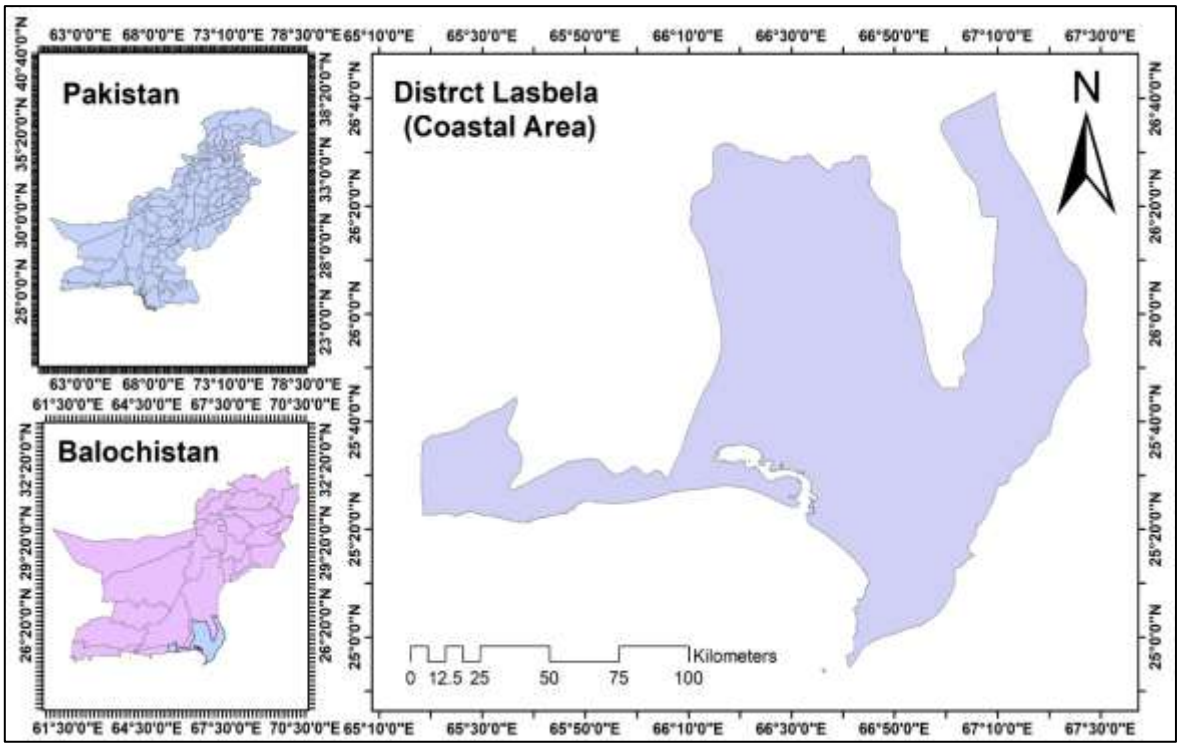

Figure 1: Map of Study Area (Coastal area of Lasbela, Balochistan

RESULTS

Morphometric based Identification

Diagnosis

Anal spines: 2; Anal soft rays: 7 – 9. Dorsal spines (total): 6; Dorsal soft rays (total): 9.

Description

The caudal fin is primarily yellowish, with the dark bars crossing the lateral line on the body obliquely on the upper half and almost horizontal on the bottom half. The Pickhandle Barracuda's coloration is typical of the species. It is silver with about twenty wavy bars along the sides of the body. There is a golden pectoral fin (Figure 2).

Figure 2: *Sphyraena jello* (Cuvier, 1829)

Molecular Identification

Isolation and quantification of genomic DNA

Extracted DNA from *Sphyraena jello* tissue samples from phenol-chloroform protocol Sambrook et al. (1989) with minor adjustment in temperature. Then, qualitative and quantitative estimation of genetic DNA assessed by gel agarose electrophoresis (0.8%) with UV spectrophotometer (at A260/280nm). The values of DNA concentration and quantities are varying as shown in Table 2; Figure 3.

Samples of quality concentration and yield used for further processing as sample ASJ_02 and 07 is used for Polymerase chain reaction.

Table 2: Concentration of the isolated DNA samples and absorbance at 260/280nm

Sphyraena jello	**Sample ID**	**260/280**	**Concentration (µg/ µl)**
	ASJ_01	2.08	312.5
	ASJ_02	1.98	1112.5

	ASJ_04	1.97	1212
	ASJ_05	1.98	1212.5
	ASJ_06	1.85	625
	ASJ_07	1.8	1237.5
	ASJ_08	1.95	487.5
	ASJ_09	1.95	487.5
	ASJ_10	2.12	425

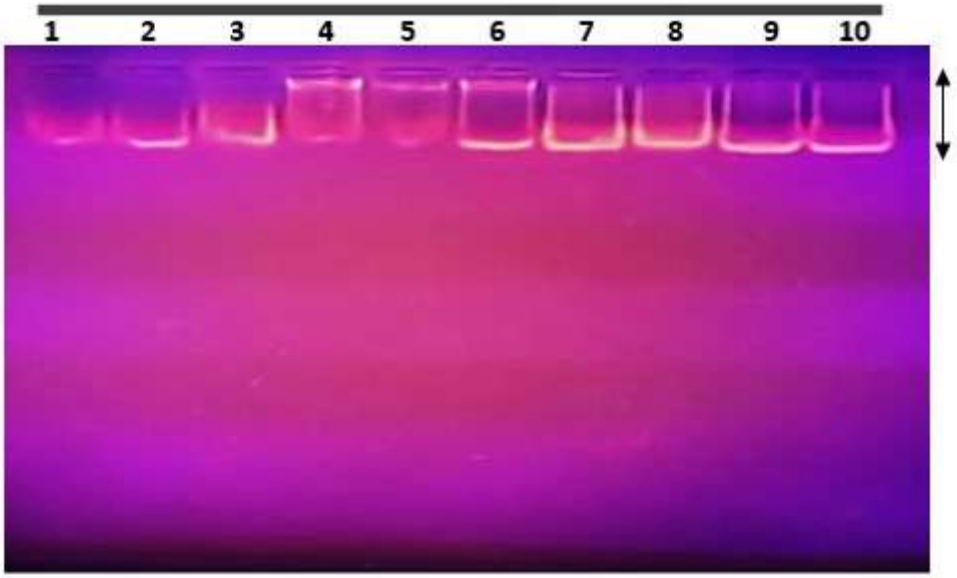

Figure 3: Bands of extracted DNA of *Sphyraena jello*

GenBank BLAST, Base composition and Phylogenetic analysis*:*
Sequencing, of samples were aligned and compared with other GenBank Cytochrome C Oxidase I sequences. The percent similarity scores of the consensus sequences from each species were compared to the maximum percent pairwise identity of the consensus sequences from each species, which were blasted on NCBI and sequences representing COI gene were submitted to GenBank with accession numbers as *Sphyraena jello* as sequence size as given in as the accession number was PP578215, and PP579407 Table 3.
In the molecular study, a consensus sequence of 665 and 442 base pairs of COI-gene was for species that were clustered under matches as shown in Table …
The base composition of COI sequences ranged as 29.9-31.3% (T), 26.5-26.8% (C), 23.5-24.1% (A) and 17.9-20.1%. The Neighbour-Joining tree (NJ) approach was utilized to infer the evolutionary by employing the Kimura 2-parameter model association between the organisms given outgroup. The phylogenetic relationships employing the NJ tree method is shown in Figure 4.

Table 3: Species, number of specimens and GenBank accession numbers ofspecimens for this study.

Species	**Number of specimen**	**GenBank accession numbers**
Sphyraena jello *Sphyraena jello* PP578215 *Sphyraena jello*-PP579407	10	PP578215, PP579407 665 base pairs 442 base pairs

Table 4: Comparison of the average GC% and AT% Nucleotide composition

	T(U)	C	A	G	Total base pairs
Sphyraena jello-PP578215	31.3	26.8	24.1	17.9	665
Sphyraena jello-PP579407	29.9	26.5	23.5	20.1	422

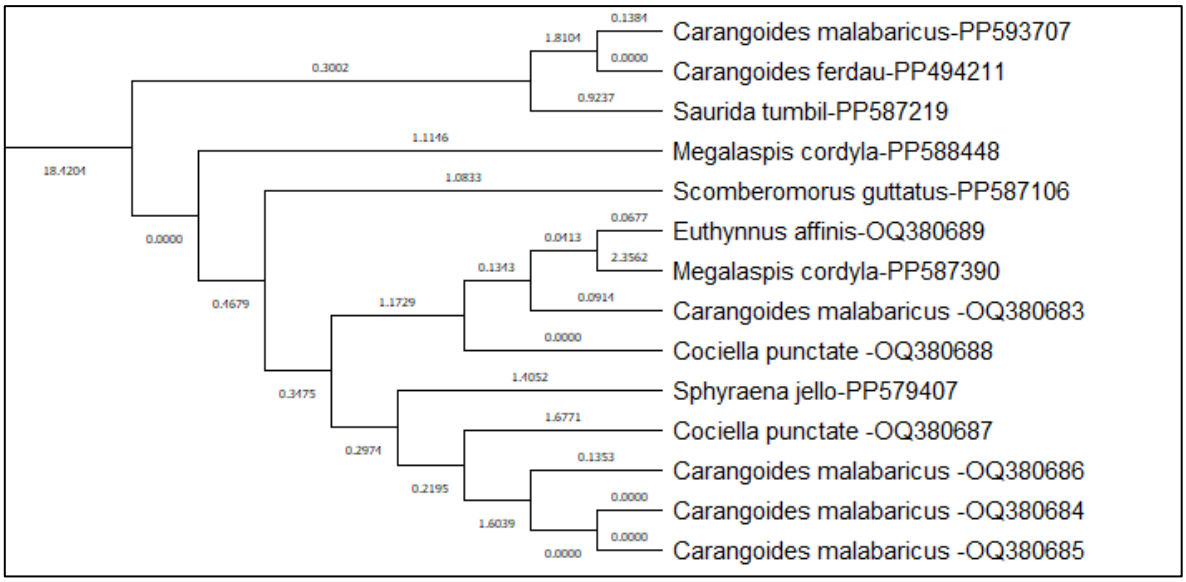

Figure 5: NJ tree showing the relationship between species and outgroup. Bootstrap values (>50%) for the analysis are above the branches.

Barcode of life data search and analysis

For species comparisons, bold data produced for the research can be viewed in the way that is indicated on the barcode of the life data system (BOLD System) with 100 percent match with data on BOLD System (Figure 5).

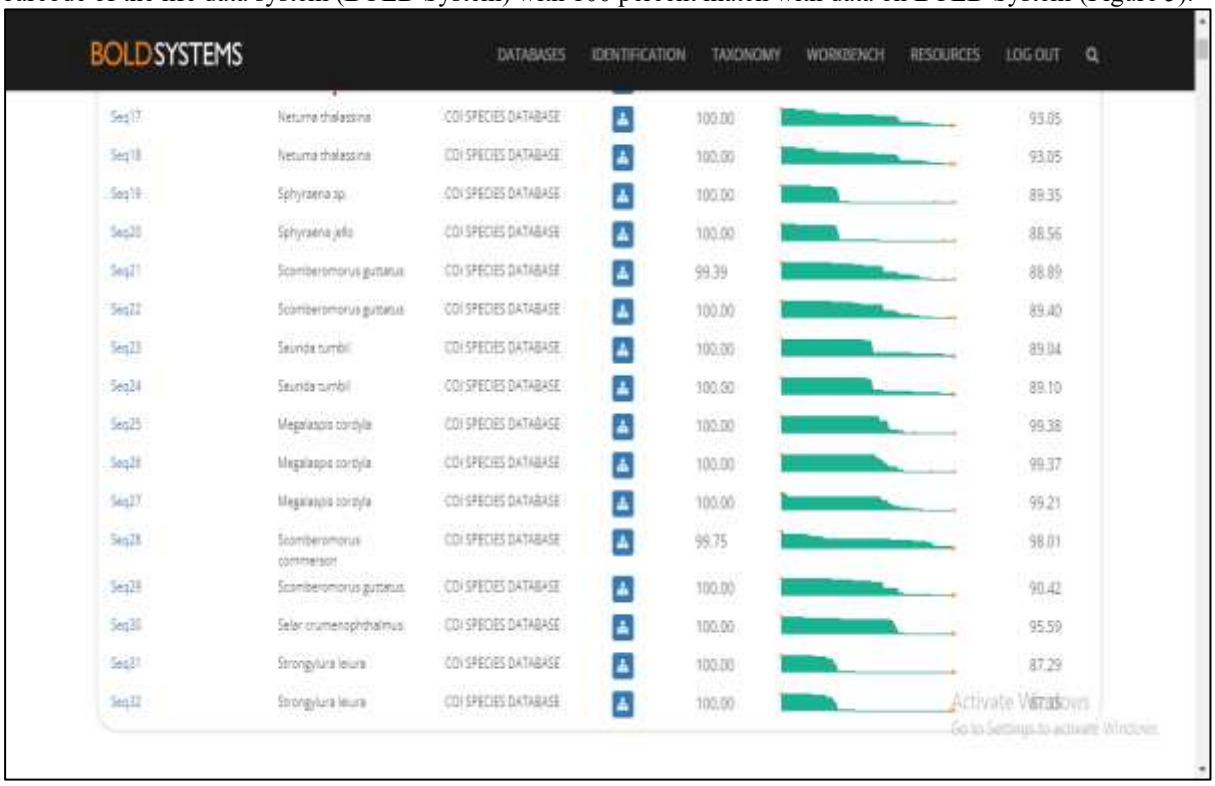

Figure 5: BOLD data analysis of different studied species

DISCUSSION

All fish samples from the coastal region of Balochistan, Pakistan, which is rich in biodiversity, were critically examined morphologically to provide a thorough understanding of their categorization and diversification. Selected fish samples were properly morphologically characterized. Thus, the results of Bhakta and Bandhyopadhyay (2008) on morphological fish identification are supported by the current study.

DNA sequences from fish samples were used for molecular identification. As a result, additional species variation analysis using K2P (Kimura, 1980) and reference sequences gathered from NCBI for estimating genetic gaps revealed clear genetic sequence divergence at the species and genus levels (Tamura et al. 2011). For each organism, at least two sequences including reference sequences from the whole data set from the current investigation and

GenBank were used to estimate the intraspecific and interspecific K2P distances within and within species, respectively.

The current work employs a combination of morpho-taxonomy and DNA barcoding as the CO1 barcode area has already been recognized as the standard technique for animal species identification (Ahmed et al., 2022). To accurately identify and categorize the specimens into species in this instance, morphological categorization should validate DNA data. The genetic divergence found in this study and the K2P distance across fishes are likely explained by random mutations and genetic drift, which may result in substantial genetic differentiation (Alam et al., 2022).

Present study revealed that in consistent with the study of DNA extraction techniques by determining the absorbance value at A260/A280, the purity and quality of extracted DNA were assessed; the purity range of most samples fell within 1.7–2.0 (Chowdhury et al., 2016). In Phenol Chloroform method almost all the samples were found within purity range for successful amplification of PCR as statement fish for the PCR amplification in order amplify the 665 and 442 base pairs.

One of the best solutions to the growing issue of mislabeling in fisheries and aquaculture is molecular species identification, particularly DNA barcoding, which can quickly and accurately provide genetic pathway to confirm the species of food products, protecting consumers from financial fraud and helping to conserve endangered species (Wong et al., 2011). Although it occasionally lacks discriminating power at the genome level, DNA barcoding is a quick, standardized approach for species identification that may be used at any stage of life due to its high degree of universality when compared to other molecular markers. We were able to successfully sequence about 450-650 bp barcodes from the Cytochrome C Oxidase I gene in our investigation.

At the species level, identities varying from 99.85 to 100% were found when the COI gene sequences were compared to those found in public databases (GenBank and BOLD). About 650 bp barcodes from the COI gene for imported frozen fish filets from Egyptian markets were successfully sequenced in the current investigation. As a result, the COI barcodes were used to accurately identify every imported fish filet. The DNA barcode (Ashour et al., 2025) is another name for fish species identification using the COI gene sequence. According to our findings, every sample of imported frozen fish filets matched the COI gene standard sequences in both GenBank and BOLD databases, with each sample under study having a distinct sequence that was distinct from that of other species.

In present study, fish species identification was confirmed by barcoding the specimens using varied base pair sequences as (Lohman et al., 2009) employed 665 and 442 base pairs to identify species, and the barcode sequences generated during this study were uploaded to the GenBank Database, which may serve as a reference for future research. Primary goal keeping records is DNA barcoding (Naeem et al., 2020a,b). BLAST analysis of barcode sequences in current study provided identity with reference sequence for identification of desired reference analysis of identified species identity match, used BLAST analysis figures for molecular identification of *Sphyraena jello*.

CONCLUSION

DNA barcode based molecular identification is not commonly used technique in Pakistan. Present research highlighted molecular identification techniques for identification of fish species. The principal goal of the current research was done to identify *Sphyraena jello* of Pakistan via DNA barcoding of its mitochondrial COI gene and submit the DNA barcode sequence in the GenBank. The present study also revealed the efficiency of DNA barcoding approach evaluate genetic divergence of fish assessed, success rate of identification for accurate and quick species identification as compared to numerous traditional morphometric and meristic identification methods.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

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