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COI-BASED DNA BARCODING FOR IDENTIFICATION OF MARINE FISH (FAMILY: SCOMBRIDAE) FROM THE COASTAL SITE OF LASBELA, BALOCHISTAN, PAKISTAN

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ABSTRACT
The study represents the first COI barcode record of marine fish species in Pakistan, utilizing DNA barcoding to identify from Baluchistan coast, Lasbela region. COI based molecular identification method had not been extensively employed for marine species. Present study provides a foundational genetic based identification reference for future study and contribute to improving the accuracy in identification process of marine fish identification. Additionally, study also highlights the significance of various integrating molecular identification techniques management and conservational strategies in fisheries to ensure viable use of marine fisheries. By using cytochrome c oxidase subunit, I (COI) gene as a marker for identification, *Scomberomorus commerson* and *Scomberomorus guttatus* were identified accurately. Specimens of Family: Scombridae as *Scomberomorus commerson* and *Scomberomorus guttatus* were used collected, analyzed, and identified accurately resulting in the successful submission of sequences to GenBank, NCBI. BLAST analysis confirmed accuracy of identification as *Scomberomorus commerson* and *Scomberomorus guttatus* specie with exact match, with 668 and 408 base pair respectively as inferred from neighbor-joining tree analysis, further corroborated these findings. Study demonstrates the efficacy of DNA extraction method and barcoding as a reliable and efficient approach for species identification and phylogenetic lineage in marine fishes from coastal region of Pakistan.

KEYWORDS: DNA barcoding, COI, Marine Fish, Identification.

INTRODUCTION
Molecular identification by DNA barcoding base identification was considered to be an effective tool for the identification of species [1]. The identification of fish species plays a crucial role in fishery management and conservation. Another type of DNA referred to as the mitochondrial DNA is used globally in the molecular identification of fishes [2]. DNA extraction considered closely related to the high quality, and concentration as its depends on a few factors: the sample size, DNA extraction method, and the storage of a sample for sequencing [3]. The sequencing-enhanced rapid, standardized mitochondrial COI identification for molecular identification [4]. For DNA barcoding, the initial easy and inseparable process involves excising the DNA then attempting towards Polymerase Chain Reaction (PCR) amplification, which largely depends [5]. For more specific distinctions between fish species, the mitochondrial COI-gene is ideal because of close relation among the group of fish species [6]. Through the DNA markers, the identification of fish is studied on molecular basis. With the exception of billfishes, which are currently classified in the suborder Xiphioidei, the Scombridae is the most gigantic and most evolved of the four families of scombroid fishes. There are 51 species and 15 genera in the Scombridae family [7].
This study applied DNA barcoding to properly identify *Scomberomorus commerson* and *Scomberomorus guttatus* fishes collected from coastal area, Lasbela, Balochistan, Pakistan to examine the fish supply and establish benchmarks for fishermen to catch these species.

MATERIAL AND METHODS
Collection of specimens and morphological identification
A total of 10 fish specimens were collected from the coastal site, Lasbela with the help of local fishermen. The specimens, along with habitat information and were frozen on-site to preserve tissue integrity and subsequently transported to the Fisheries Research Lab, University of Balochistan, Quetta, Pakistan. Upon arrival, all samples were stored at 4°C until further processing and molecular analysis.

DNA Extraction, PCR Amplification

50mg of muscle tissue was excised from each fish specimen using a sterile scalpel. Genomic DNA was subsequently extracted using the standard phenol-chloroform extraction method [8]. 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 successfully performed using universal fish primers [9] (Table 1).

Table 1: Detail of Primers used for PCR amplification

Target Region/Gene	Primer ID	Prime Sequence 5′-3′	Temp °C	GC %	Primer Size
Mit. COI	Forward F1 COI	TCAACCAACCACAAAGA CATTGGCAC	61	46.15	26
Mit. COI	Reverse R1 COI	TAGACTTCTGGGTGGCCA AAGAATCA	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 [10].

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 [11], Phylogenetic relationships were further evaluated by constructing a maximum-likelihood tree using MEGA X software, with 1,000 bootstrap replicates to assess node support [12].

RESULTS

Taxonomic description of Scomberomorus commerson

Small and bifid interpelvic process and lack of swim bladder. The lateral line suddenly curved downward beneath the second dorsal fin's end. Intestine with three branches and two folds. Less than 20 spots are present in juveniles, while 40–50 spots are present in adults when the vertical bars on the trunk split lateral. Juveniles with huge oval dark patches on body; fins are black save for the central portion of the first dorsal fin (Figure 1).

Figure 1: ***Scomberomorus commerson***

Taxonomic description of Scomberomorus guttatus

Intestine with three branches and two fold sides that are silvery white and have multiple rows of circular, dark brownish dots that are arranged erratically in three rows along the lateral line. Whole body coated in tiny scales. Lateral line with several auxiliary branches that curve down toward the posterior peduncle and spread dorsally and ventrally in the anterior third. Black membrane on dorsal fin first. Narrow and disabilities interpelvic process. Swim bladder lacking (Figure 2).

Figure 2: ***Scomberomorus guttatus***

Molecular Identification

Isolation and quantification of genomic DNA

Extracted DNA from 10 specie tissue samples by phenol-chloroform procedure of [13] 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 3-4.

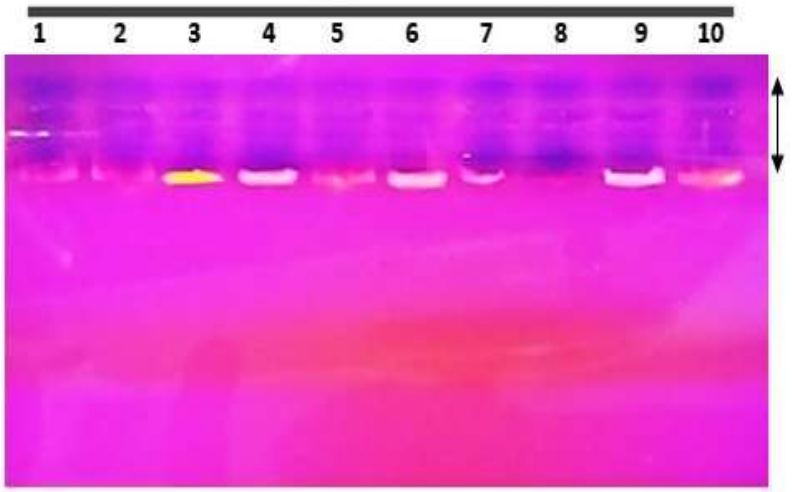

Figure 3: Bands of extracted DNA of ***Scomberomorus commerson***

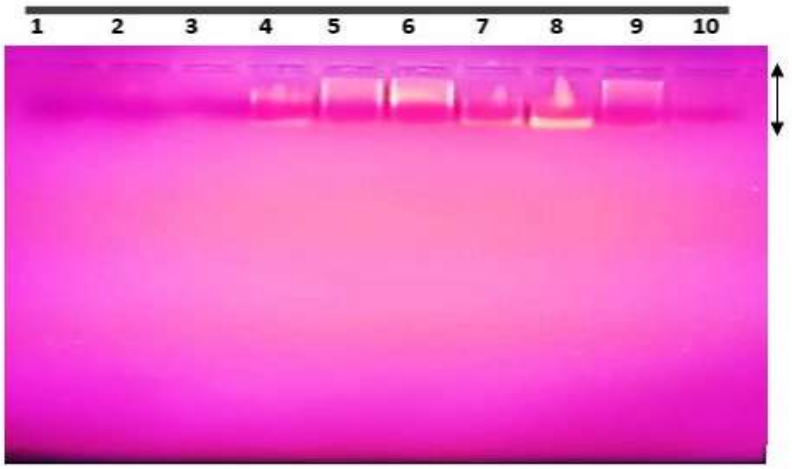

Figure 4: Bands of extracted DNA of ***Scomberomorus guttatus***

GenBank BLAST search and sequences submission:

Highlighted sample forwarded for sequencing, data obtained sequences 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 against NCBI and sequences representing COI gene were submitted to GenBank with accession numbers as *Scomberomorus commerson* noted as 668 bp, while 408 for *Scomberomorus guttatus* as sequence size as given in Table 3.

Base compositional bias:

In *Scomberomorus guttatus* the base composition of COI sequences ranged as 30.3-31.4% (T), 27.5-28.8% (C), 23.8-24% (A) and 17-17.4% and for *Scomberomorus commerson* 29.9% (T), 27.7% (C), 22.5% (A) and 19.9% (G). 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 5.

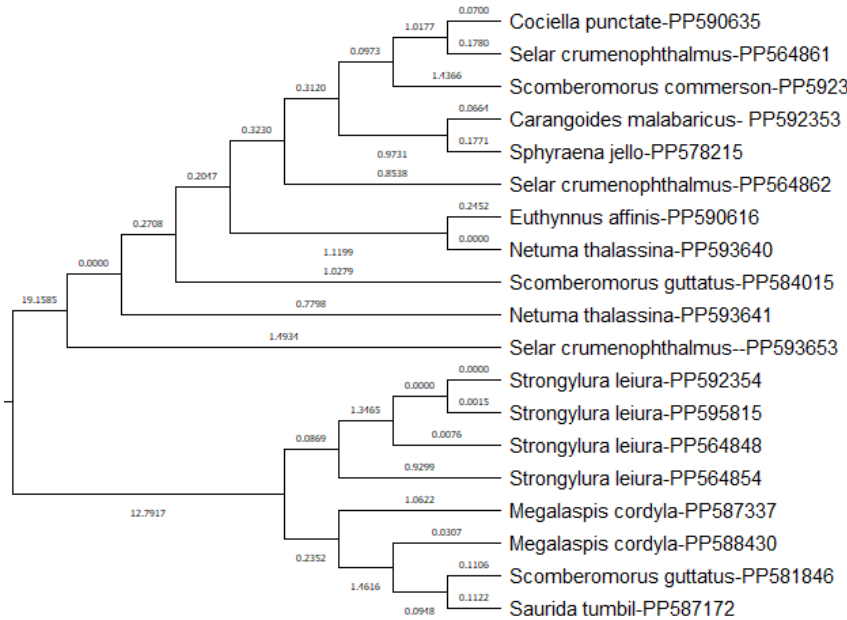

Figure 5: NJ tree showing the relationship between species and outgroup in *Scomberomorus commerson* and *Scomberomorus guttatus.*

DISCUSSION

Study is confined to 2 species belonging to family Scombridae were collected and studied for morphometric measurements for their characterization collected from coastal site of Balochistan, Pakistan for generation of DNA barcodes. A reliable and affordable method must be chosen for the isolation of genomic DNA since it is an essential step in the processes of PCR amplification, DNA barcoding, and genetic diversity analysis of species [14]. For most genetic analyses, high-quality DNA extraction is necessary. Fish DNA is mostly retrieved from their muscle, liver, and blood, all of which require the sacrifice of an animal [15]. Only a limited amount of DNA retrieved because of these tissues' hardness and minute size, but in the current study, we have effectively isolated high-quality and concentrated DNA from muscle tissues in fish.

Present study revealed that in consistent with the study of [16] in N. notopterus [13, 17-18], the Phenol Chloroform approach was shown to have an adequate DNA concentration when compared to other 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 may contain contaminants such as proteins and other elements The samples that are not within the purity range may contain contaminants such as proteins and other elements, even if they are the purest [8] (Chowdhury et al., 2016). In Phenol Chloroform method almost all the samples were found within purity range for successful amplification of PCR as [19] statement fish for the PCR amplification. Utilizing the mitochondrial COI genetic marker, DNA appropriateness for PCR amplification was evaluated in order amplify the 668 and 408 base pairs.

In present study, fish species identification was confirmed by barcoding the specimens using varied base pair sequences as [20] employed 650 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 [21]. BLAST analysis of barcode sequences in current study provided identity with reference sequence for identification of desired reference analysis of identified species identity match, as [2] used BLAST analysis figures for molecular

identification of *Scomberomorus commerson,* and *Scomberomorus gutatus.*

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 *Scomberomorus commerson*, and *Scomberomorus gutatus* 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.

Ethical of approval: Not applicable

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