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Molecular Identification of Commercially Important Marine Fishes (Family Lutjanidae) from Southwestern Coast of Pakistan

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ABSTRACT

The management of marine fish is extremely important for commercial purposes and has a large impact on the food sector. In addition to several directed studies on fish and fisheries, taxonomy has drawn significant interest from researchers. In recent years, DNA barcoding has gained popularity for use in identification and phylogenetic relationship studies. When compared to the conventional morphological-based identifications. DNA bar-coding is seen to be a more trustworthy method of identification. The cytochrome oxidase sub-unit 1 (COI) was given a lot of attention for identifications among mitochondrial DNA (mtDNA) markers. The *COI* genetic marker was employed in the current study to identify species belonging to family Lutjanidae. Following species of this family were re-confirmed bases on strong similarity scores in NCBI nucleotide blast and bar-code of life data system (BOLD) findings such as, *Lutjanus johni*, *Lutjanus lutjanus, Lutjanus rivulatus*, *Lutjanus fulvus, Lutjanus ehrenbergi*, and *Lutjanus erythropterus*. These sequences were submitted to NCBI under accession numbers ON705767, ON706980, ON705756, ON706977, ON705716, ON705711, ON706273, ON706982, ON706952, ON706976, ON706979, ON706983, and ON705701, respectively. The phylogenetic tree described three major clades with bootstrap support values of 100%, 79%, and 100%, respectively. The results of this investigation will be useful for taxonomists and biodiversity monitors.

INTRODUCTON

The coastal areas of Pakistan, as a part of the northern Arabian Sea, is highly productive in terms of fish diversity, abundance, and endemism as it receives nutrient-rich waters from up-sloping in the Gulf of Oman and surrounding areas. These areas are bestowed with a variety of marine habitats including large Indus Delta, numerous major and minor creeks, mudflats, and mangrove forests (Psomadakis, 2015). The marine fisheries sector plays vital role in generating the economy of Pakistan and significant employment opportunities for foreign exports and representing a great source of protein for local rural population, so is the importance of teleost fishes among fishery resources. The Lutjanidae family has profound importance with 21 genera and 135 species, and commonly called as snappers mostly dwells in marine waters, nevertheless, few are reported in freshwater and estuarine ecosystem as well. The distribution of this import food source family is worldwide (Betancure *et al*., 2017).

The classification of fish species is based on morphological character like body color, scale, size, fin ray, fin spines and measurement of the body parts (Stuarts and Bond, 1990). However, this approach of morphological identification required ichthyologic expertise but it becomes complicated when morphological character change according to the life history. Fish has extremely diverse aquatic organism due to morphology character as they change through ontogenetic metamorphism and thus metamorphic character change according to ontogenetic

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developmental stage of life (Zhang and Hanner, 2011). For centuries the classification of fishes based on traditionally conducted through visible character which cause widely mislabeled, fraud and substitution that prevent the export and expansion of fishes. In modern taxonomy physical pattern, genes behavior and geographic character are included for appropriate identification (Costa and Carvalho, 2007).

The use of molecular approach for the identification of fish species is helpful for mitigating the limitation of morphological based identification of taxonomy and the lack of local fish identification expertise (Zhang and Hanner, 2011). Taxonomist used the DNA barcoding mitochondrial gene as golden tool to complement the morphological data with particular molecular technique known as barcoding (Ward *et al*., 2005). DNA barcoding has been proved as a molecular authenticity of recognition and identification of fish species due to its rapid and cost effectiveness which is robust output based on cytochrome oxidase subunit I (*COI*) gene (Hebert *et al*., 2003). identified marine fishes using the molecular identification from the different areas of Pakistan coast and including some species present in this study Amir *et al*. (2022).

This investigation of commercially important Lutjanidae fishes from southwest coast of Pakistan would be important contribution of the DNA barcoding and facilitate in management of marine fishes.

MATERIALS AND METHODS

Specimen collection

Specimen for the experiments were collected from the southwest coast of Pakistan (Fig. 1). Specimen were transferred to the center of excellence in marine biology, University of Karachi, Pakistan, identified based on morphological character (Fig. 2) as per national and international available keys (Talwar and Kacker, 1984; Talwar and Jhingran, 1991).

DNA extraction

DNA was extracted from 30 mg of fish muscles by using lysis buffer and followed by standard proteinase K-phenol-chloroform, isoamyl alcohol method (Sambrook, 1989). *CO1* gene was amplified by universal *CO1* primer (Ward *et al*., 2005) as given below.

FishF1-5′TCAACCAACCACAAAGACATTGGCAC3.

FishR1-5′TAGACTTCTGGGTGGCCAAAGAATCA3.

The polymerase chain reaction (PCR) was carried out in 25 µl reaction. The mixture was included 1.25 µL of DNA template, 1.25 µL of each primer, 12.5 µl of Es Taq, and 8.75 µl of double distilled water. The PCR conditions were set as denaturation at 94 °C for 5 min; 35 cycles of 94 °C for 30 sec, annealing at 50 °C for 30 sec, and extension at 72 °C for 30 sec; and a final extension at 72 °C for 7 min. Gel electrophoresis (1.2%) agarose gel with ethidium bromide was used to confirm successful amplification).

Fig. 1. Sampling locations at the southwestern coast of Pakistan.

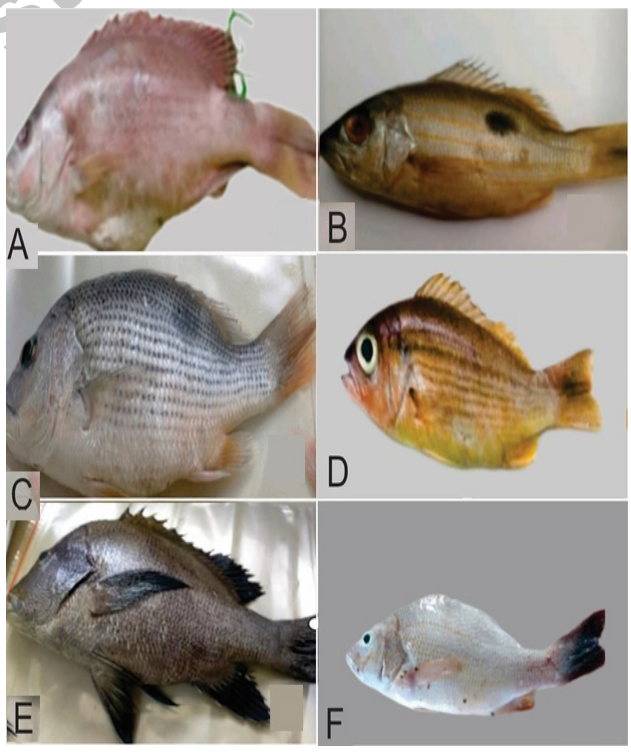

Fig. 2. The figure showing the morphology of six *Lutjanidae* species from Southwestern Coast of Pakistan. A, *Lutjanus erythropterus;* B, *L. ehrenbergi;* C, *L. johni;* D, *L. lutjanus;* E, *L. rivulatus;* F, *L. fulvus*.

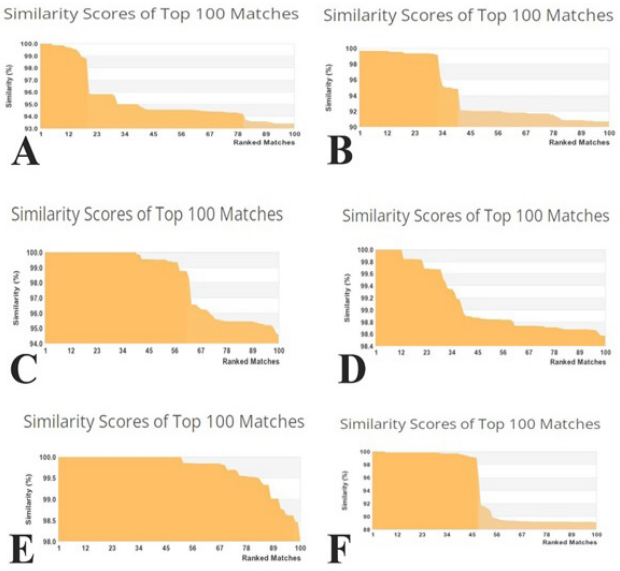

Fig. 3. Similarity score of top 100 matches gained from BOLDv4 systemes. A, *L.* erythropterus; B, *L. ehrenbergi*; C, *L. johni;* D, *L. Lutjanus;* E, *L. rivulatus;* F, *L. fulvus.*

Sequencing and construction of the phylogenetic tree

The PCR products were sequenced using the Sanger sequencing method. Software (BIOEDIT and MEGA 6) were used for the editing and alignment of sequences (Tamura *et al* 2013). Nucleotide Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastnandBLAST-SPEC=GeoBlastandPAGE_TYPE=BlastSearch) and BOLDv4 systems (https://v4.boldsystems.org/) were used to identify the possibly accurate species. Using the Kimura 2 parameter (K2P) model and MEGA 6, a neighbor-joining tree was created to compute the evolutionary history among the populations (Tamura *et al*., 2013).

RESULTS

The fishes of family Lutjanidae are commonly found in marine water of Pakistan. In present study we have identified these species with morphological and molecular based approaches, the photo morph of all these fishes are shown in (Fig. 2). The molecular based identifications were done using fragments of cytochrome oxidase sub-unit 1 (CO1). The NCBI nucleotide blast and BolDv4 systems search used. The higher similarity percentages were chosen for identification. The confirmed sequences were submitted to NCBI under accession numbers ON705767, ON706980, ON705756, ON706977, ON705716, ON705711, ON706273, ON706982, ON706952, ON706976, ON706979, ON706983, and ON705701. In the phylogenetic analysis of marine within six Lutjanus marine fish species from Gwadar showed the minimum genetic distance from 0.0-0.5% while maximum genetic divergence was observed 8.4-21.1% and mean genetic distance was observed 13.3% which declared that all species are genetically separated.

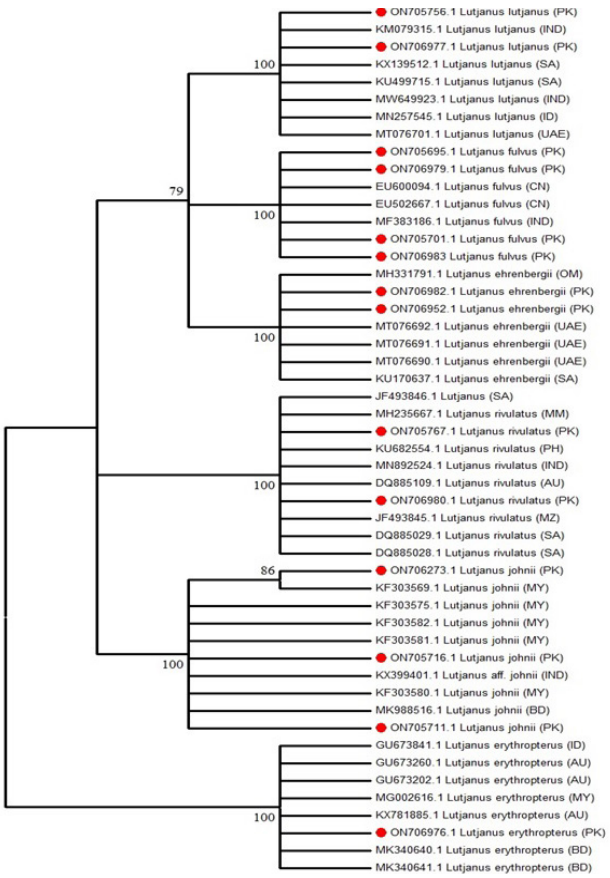

Fig. 4. Neighbour joining tree of CO1 barcode for *Lutjanus* species (maximum composite likelihood method) only bootstrap values (thousand replicates) greater than 50 % are shown with samples collected from the south coast of Baluchistan.

The neighbor joining tree clearly distinguishing these species and Lutjanus Species west coast of Pakistan are clustered with other similar species of different locations of world (Fig. 4) such as Australia (AU), Bangladesh (BD), China (CN), India (IND), Indonesia (ID), Malaysia (MY), Mozambique (MZ), Myanmar (MM), Oman (OM), Philippines (PH), Saudi Arabia (SA) and United Arab Emirates (UAE).

DISCUSSION

The west coast of Pakistan is rich in bio-diversity so is the diverse species of fishes, Astoal Island, mangrove ecosystem and typical geological process make the area

favorable habitat for many species. Presently, scientists are using molecular approaches for accurate taxonomy. Therefore, CO1 mitochondrial gene is being used in large scale.

In present study fishes of family Lutjanidae were investigated using *CO1* gene. The six species are listed as native and having high commercial values (*Lutjanus johni, L. fulvus*, *L. lutjanus*, *L. erythroptreus*, *L. ehranbergi*, *L. rivulatus*). The composition of species greatly varied in catches from different areas of Gwadar. *L.johni* and *L. rivulatus* were highly present. *L. erythropterus* was found limited in Jiwani and Pasni and their difference in catches reflect the different habitats. Local fisher man could not differentiate in different species of *Lutjanus*. The group of *snapper* fish species were largely sharing color pattern from yellow to dark brown (Iwatsuki *et al*., 2015).

DNA barcoding analyses the close boundary of identical species, which is helpful in the divergence of close resembling neighbors within the same species. DNA barcoding of our *Lutjanus* species from Gwadar was similar to Lutjanus barcode of Malaysian fishes while it has very close recognized resemblance with Indian and Pacific Ocean, many process are involved in temperature and sedimentation and direction of Ocean connectivity during the time of high Sea level.

Genetic distance and phylogenetic analysis

Present study of phylogenetic distance is also sustained by Bakar *et al*. (2018) worked on Malaysian commercial snappers of Lutjanidae species. DNA barcoding is helpful for identification of Lutjanus family and provides potential pattern in identification of *Lutjanus* (Hajibabaei *et al*., 2007; Chue *et al*., 2013) found the phylogenetic distance of *Lutjanus* species taxonomy. DNA barcoding with mitochondrial *CO1* gene sequence provided attractive and authentic fish identification (Heberet *et al*., 2004). *Lutjanus* taxonomy is carried out by several workers (Lakra *et al*., 2011; Wang *et al*., 2010; Allen *et al*., 2013; Iwastuki *et al*., 2013) who declared that DNA barcoding is helpful in identification of fishes. DNA barcoding analyses the close boundary of identical species, which is helpful in the divergence of close resembling neighbors within the same species. Generally, intraspecific divergence is equal to 2% mostly are less than 1%, but there is no universal level of intraspecific divergence. In the present study minimum genetic distance was 0.0% to 0.5% which was observed in *L. johni* and *L. erythropterus*, respectively. Observe maximum genetic distance was 8.4% to 21.1% in *L. fulvus* and *L. erythropterus*. The mean genetic distance was 13.3%. DNA barcoding of our *Lutjanus* species from Gawadar were similar to *Lutjanus* barcode of Malaysian fishes while it is very close recognized resemblance with Indian and Pacific Ocean. The geographically distribution of these species has very close resemblance in monophyletic and genetic observance of the result that is due to the same process of biogeography of *Lutjanus* fishes, many process are involved in temperature and sedimentation and direction of Ocean connectivity during the time of high Sea level. This result of genetic distance is supported by many researchers (Haleem *et al*., 2022) worked on Malaysian *Lutjanus* species through *CO1* gene. They observed genetic distance in Lutjanus species from 0.0 - 0.5 % and 8.4 -21.1 %. Present study of phylogenetic distance is also sustained by Bakar *et al*. (2018) worked on Malaysian commercial snappers of *Lutjanidae* species. They determined the minimum genetic distance 0% to 0.4% while intraspecific genetic distance ranged from 4.5% to 21.3% Templonuevo *et al*. (2018) also observed genetic distance in *Lutjanus* species from 0.0% to 15.90% between species. DNA barcoding is helpful in identification of *Lutjanus* family and provides potential pattern in identification of *Lutjanus* (Hajibabaei *et al*., 2007; Chue *et al*., 2013). Heberet *et al*. (2004) found the phylogenetic distance of *Lutjanus* species DNA taxonomy and DNA barcoding with mitochondrial *CO1* gene sequence provided attractive and authentic fish identification. *Lutjanus* taxonomy is carried out by many scientist (Lakra *et al*., 2011; Wang *et al*., 2010; Allen *et al*., 2013; Iwastuki *et al*., 2013).

Base pair sequences

The sequence were analyzed and it revealed that overall content of AT% was 53.10% higher than GC% was 46.89% similar result were also observed by many other researchers like Ghouri *et al*. (2020) found in edible fishes of Pakistan with GC content in marine fishes 46.26% and AT content was 53.73%. In Bangladesh nucleotide composition of CO1 sequences was AT content 53.50% and GC content 46.50% by Ahmad *et al*. (2021). Same pattern was observed by Templonuevo *et al*. (2018) in Lutjanus fishes from Cuyo Palawan, Philippines, Osteichthyes in Australia from marine fishes and brackish fishes from Surbandar (Ward *et al*., 2005; Habib *et al*., 2021).

DNA barcoding proved as a basic tool of molecular approach for identification and biodiversity of fresh water marine fishes throughout the World. Wang *et al*. (2010) found a phylogenetic relationship of 13 snapper fishes from South China with the help of mitochondrial *CO1* gene. Velamala *et al*. (2019) found *Lutjanus* identification which is a challenging task and DNA barcoding is helpful for overlapping the morphological character of *Lutjanus* species from coastal area of India. Lakra *et al*. (2011) assessed the genetic diversity of fishes through DNA barcoding. DNA barcoding provided a vision for identification of fishes and it avoided the difficulties associated with field of morphological identification. DNA barcoding proved as a molecular tool with high quality

for identification and genetic diversity among marine Lutjanus species from Gwadar. DNA barcoding is helpful for the resolution of hybridization which occurred in close genetically resembling species and leading to species misidentification Lamendin *et al*. (2015). DNA barcoding provide worth in taxonomy and genetic identification of fishes. DNA barcoding minimize the ambiguousity and complexity in biodiversity and taxonomy of fishes.

CONCLUSION

Six species of family Lutjanidae from coastal area of Gwadar Baluchistan (*L. johni, L. rivulatus*, *L. erythropterus*, *L. lutjanus*, *L. fulvus* and *L. ehrenbergi* were successfully recognized molecular identification through mt DNA gene, namely *CO1*. The research proposed that *CO1* gene is valid and reliable genetic marker for identification of Lutjanidae distributed in coastal area of Gwadar. We hope that diversity of *Lutjanus* species will be used as a useful resource for future researchers and conserving fisheries manager for authentic information on composition of *Lutjanus* species from Baluchistan and ultimately it will be effective for conserving and protective agencies for management plan of *Lutjanus* species west coast is diverse and having abundant marine fauna, accurate species identification will be a key for up-lifting economy of marine resources. Further diversity exploration of *Lutjanus* is on a large scale can be possible by huge number of sampling and different genetic markers are needed to fill the gap of current study.

DECLARATIONS

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Ethical statement

Permission to undertake surveys in Gwadar district was granted by the Director General Fisheries, Balochistan (BFD/DG/Misc/2021-22/613). In present study we have strictly avoided for sampling of endangered species.

IRB approval

Approval from Institutional Review Board was obtained from the Advance Atudy and Research Board, University of Balochistan, Quetta (UOB/Reg/GSO/464).

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Statement of conflict of interest

The authors have declared no conflict of interest.

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