Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28 (1):1897 – 1904 (2024)
www.ejabf.journals.ekb.eg

DNA Barcoding and Phylogenetic Study as a Modern Taxonomical Tool for Identifying Commercial Catch along the Egyptian Mediterranean Coast, Alexandria

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ARTICLE INFO
Article History:
Received: Jan. 16, 2024
Accepted: Feb. 11, 2024
Online: Feb. 29, 2024

Keywords:
Fish,
COI,
DNA barcoding,
Phylogeography,
Evolutionary dynamics,
Egypt

ABSTRACT

Sustainable fisheries management and biodiversity conservation rely heavily on precisely identifying commercial fish species. Although morphological taxonomy is generally beneficial, it is frequently inadequate when clarifying uncertainties related to identifying species, particularly in specimens that are juveniles or have been treated. DNA barcoding and phylogenetic analysis were intended to be validated as modern, dependable taxonomic techniques for identifying commercial fish species. Four fish species (*Dicentrarchus labrax*, *Mullus surmuletus*, *Seriola dumerili*, and *Sparus aurata*) were obtained from frequent commercial fish landings along the Egyptian coastline of the Mediterranean Sea in Alexanderia that underwent DNA barcoding for identification. The investigated species were successfully identified using COI barcoding. Additionally, an accurate representation of established taxonomic connections was achieved through further phylogenetic analysis, which utilized the Tamura-Nei model and the neighbor-joining method to generate a tree with a strong bootstrap support. In conclusion, for the monitoring of commercial fisheries, the present research findings significantly confirmed the effeciency application of genetic approaches to species identification and highlighted their capacity to supplement or surpass conventional methods. In addition to enhancing our comprehension of species variety and evolutionary biology, the integration of DNA barcoding and phylogenetic analysis helps identify species more accurately.

INTRODUCTION

To address the ongoing global biodiversity crisis, conservation approaches must be underpinned by robust information **(Buxton *et al*., 2021)**. Animal welfare, fisheries administration, seafood traceability, and numerous other organisations rely heavily on precisely identifying fish **(Ward *et al*., 2009)**. Conventional morphological taxonomy is limited to describing cryptic species, juveniles, or processed seafood items **(Costa & Carvalho, 2007)**. Statistics on angler catch rates and species composition have been widely used worldwide to monitor changes in the abundance of commercially and recreationally important species **(Beaudreau & Levin, 2014; Florisson, 2015; Kroloff, 2016; Thurstan *et al*., 2016; Quinn, 2018; Chan *et al*., 2019; Rehage *et al*., 2019)**. DNA barcoding, using the mitochondrial cytochrome C oxidase subunit I (COI) gene, has emerged as a reliable method

for species identification and delimitation in fish **(Hubert *et al.*, 2008)**. Barcoding facilitates dependable taxonomic assignments, even among morphologically unclear or closely related species, when combined with phylogenetic reconstructions **(Costa & Carvalho, 2007)**.

DNA barcoding utilizes sequence diversity in short, standardised gene regions to identify species **(Hebert *et al.*, 2003)**. For animals, the mitochondrial cytochrome oxidase subunit I (COI) gene has been established as the standard barcode region **(Susari *et al.*, 2021)**. DNA barcoding provides a rapid, reliable tool for species identification through comparison against a reference database **(Jin *et al.*, 2020)**. It outperforms the traditional morphological taxonomy, distinguishing closely related, cryptic, juvenile, or processed species **(Ward *et al.*, 2009)**. Barcoding is well-suited for identifying fish species in fishery product authentication and wildlife forensics **(Fotedar *et al.*, 2019)**. In addition, it aids in the discovery of new species and understanding of evolutionary relationships **(Dornburg & Near, 2021)**.

DNA barcoding employing the COI gene has been extensively utilized in ichthyology to resolve taxonomic difficulties and delineate species boundaries **(Ward *et al.*, 2005)**. The Fish Barcode of Life Campaign compiled COI sequences from more than ten thousand fish species **(Ward *et al.*, 2009)**. Reference barcode libraries facilitated the identification of unidentified samples by comparing their COI sequence with that of proven and morphologically vouchered species **(DeWaard *et al.*, 2019)**. A uniform methodology is provided by barcoding, which facilitated collaborative species inventories **(Gostel & Kress, 2022)**. In general, DNA barcoding functions as a highly effective adjunct to conventional taxonomy in fish species identification.

The objective of this research endeavour was to use DNA barcoding as a means to precisely discern species of commercially significant *Dicentrargus labrax*, *Mullus surmuletus*, *Seriola dumerili*, and *Sparus aurata* that were procured from Alexandria, Egypt. The mitochondrial COI gene would serve as the barcode marker of standardisation. Additionally, this study aimed to emphasize the effectiveness of DNA barcoding as a swift and dependable method of identification, which can contribute to the sustainable management and conservation of commercial catch.

MATERIALS AND METHODS

Samples collection

We procured our fish species from commercial fish landings along the Egyptian coastline of the Mediterranean Sea, specifically in Alexandria, Egypt. The species obtained were *Dicentrarchus labrax*, *Mullus surmuletus*, *Seriola dumerili*, and *Sparus aurata*. The exact length in millimetres has been meticulously measured and recorded for newly acquired specimens. For future laboratory analysis, specimens of muscle tissue were preserved in ethyl alcohol at -20º C.

DNA extraction and analyses

Muscle tissue subsamples were obtained from the collected specimens and then preserved in ethyl alcohol at a concentration of 100% and frozen at -20º C. By the manufacturer's guidelines, DNA was extracted from the tissue using the DNeasy Tissue Kit (QIAGEN). 679 base pairs were amplified from the 50 sections of the COI gene, which was extracted from the mitochondrial DNA using the following newly designed primers, according to **Ward *et al.* (2005)**:

FishF1-50TCAACCAACCACAAAGACATTGGCAC30,

FishR1-50TAGACTTCTGGGTGGCCAAAGAATCA30

Each of the 25 PCR reaction mixes comprised 12.5mL of green master mix, 5.5µL of nuclease-free water, 1µL of forward primer, 1µL of reverse primer, and 5µL of isolated genomic DNA. For the amplification, a master cycler Eppendorf gradient thermal cycler was utilized. Following a 2-minute step at 95°C, the thermal regime consisted of 35 cycles of 0.5 minutes at 94°C, 0.5 minutes at 54°C, and 1 minute at 72°C, followed by 10 minutes at 72°C. It concluded at 4°C. Two-directional sequencing of the PCR product was performed at an Egyptian genetic center (Colour Laboratory Co.). To determine the yield and quality of the PCR product, the amplified DNA was seen using a transilluminator on a 1% agarose gel that was previously stained with ethidium bromide (U.V. transilluminator, Spectro-line, Westbury, USA).

Bioinformatic analysis

The assembled sequences were processed utilizing the Chromas Pro 1.5 beta software (Technelysium Pty., Tewantin, QLD, Australia). The accession numbers PP196679, PP196677, PP196678, and PP196694 for the recently obtained COI sequences of *Dicentrargus labrax*, *Mullus surmuletus*, *Seriola dumerili*, and *Sparus aurata*, respectively, were utilized to compare them with sequences already available in GenBank via the basic local alignment search tool (BLAST), which can be accessed at http://blast.ncbi.nlm.nih.gov/Blast.cgi.

For a sequence alignment, muscle alignment, which was developed in the MEGA 11.0 program, was applied. Sequence divergences were computed utilizing the Tamura model with three parameters **(Tamura, 1992)**. To represent species divergence trends visually, neighbor-joining trees were constructed using the Tamura 3-parameter approach **(Tamura, 1992)**. The bootstrapping procedure was executed using 1000 replications in MEGA 11.0. The visualization of the resultant data was further improved by utilizing the ITOl programme **(Letunic & Bork, 2021)**.

RESULTS

The examined specimens were amplified by PCR using the fish F1 and R1 primers. The amplification results were then resolved on a 1% agarose gel. The electrophoresis gel obtained displayed distinct bands corresponding to the anticipated fragment sizes of each species. This confirmed that the primer set for the target fish DNA was specific and efficient.

Employing the neighbor-joining method, the evolutionary lineage of the sampled taxa was speculated **(Saitou & Nei, 1987)**. The ideal phylogenetic tree, representing the one with the maximum log-likelihood is illustrated in Fig. (1). The bootstrap consensus tree, constructed using 1000 repetitions, is considered to be an accurate representation of the taxonomic history under investigation (Felsenstein, 1985). Collapsed branches correspond to partitions reproduced in fewer than 50 per cent of the bootstrap replicates. Branch lengths are the base substitutions per site on the to-scale diagram. In the analysis, 38 nucleotide sequences were examined. With the elimination of all ambiguous positions for each sequence pair, the final dataset contained 557 positions in total. The Tamura-Nei method **(Tamura & Nei, 1993)** was employed to calculate evolutionary distances expressed in base substitutions per site unit.

The obtained phylogenetic tree (Fig. 1) represented discrete clades corresponding to the examined fish species, namely *Dicentrarchus labrax, Mullus surmuletus, Seriola dumerili* and *Sparus aurata*. Bootstrap values strongly supported most nodes, resulting in a well-resolved tree topology. For example, the bootstrap value of 97.1 indicates that *Sparus aurata* and *Seriola dumerili* are highly connected taxa, suggesting their cluster is quite credible. An outgroup comprising *Squalus megalops* is incorporated into the tree. This outgroup is phylogenetically unique from the ingroup taxa and is a reference point for the tree's root.

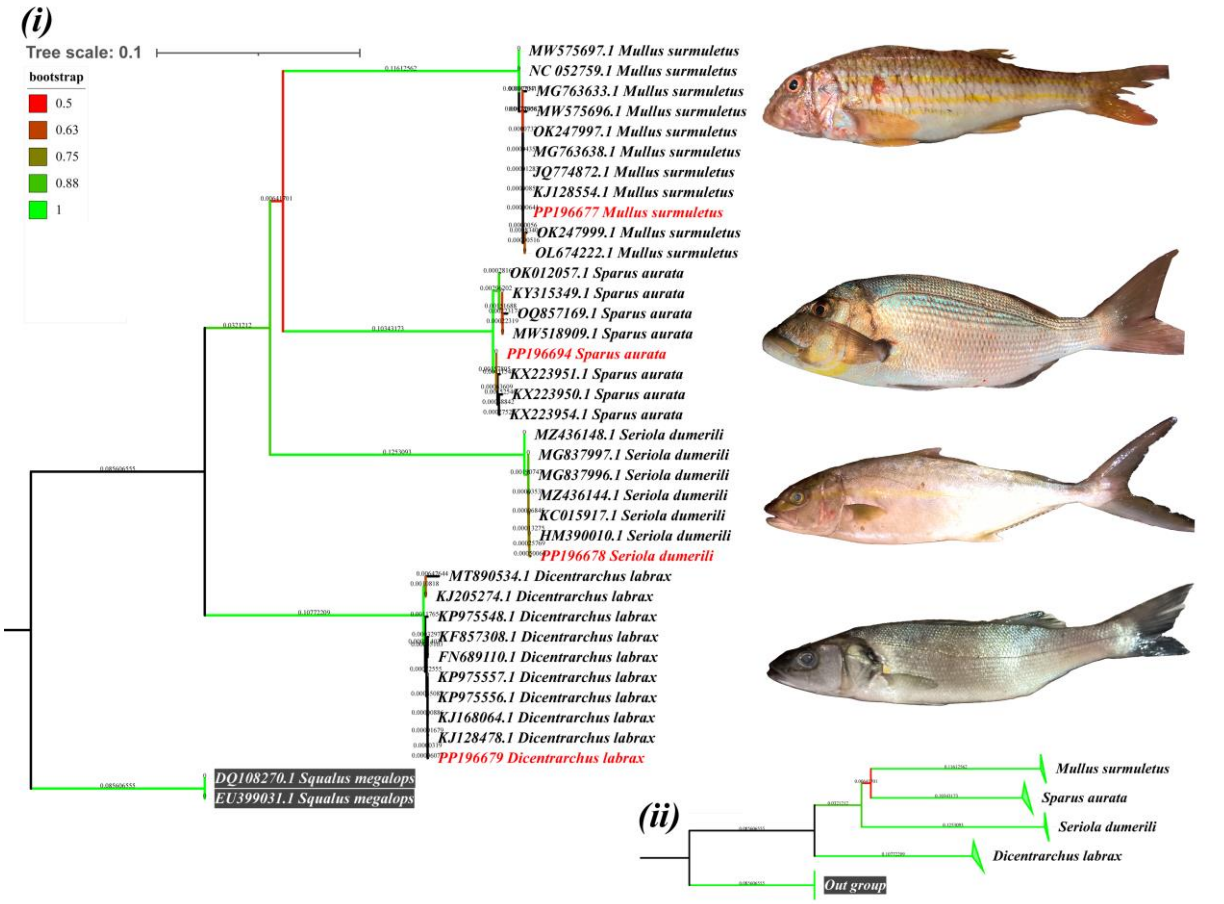

Fig. 1. Evolutionary phylogenetic analysis using neighbor-joining method

DISCUSSION

In numerous spheres of marine resource management and consumer consciousness, the precise identification of commercial fish is of the utmost importance. Underline that, initially, it improves traceability and labelling, thereby empowering consumers to make well-informed decisions that advocate for sustainable fishing techniques **(Cusa *et al.* 2021)**. The promotion of sustainable aquaculture and fishing is aided by such well-informed judgments. Furthermore, in order to successfully monitor fishing activities, establish sustainable catch limits, and execute conservation measures, fishery management is profoundly dependent on the accurate identification of species. Furthermore, in order to evaluate the condition of populations and safeguard endangered species, an accurate identification of fish species is critical to species conservation initiatives **(Hughes *et al.*, 2023)**.

Furthermore, the gathering of fishery-dependent data, including information acquired from commercial catches, is essential for measuring the number, distribution, and health of fish populations. This dataset serves as a cornerstone of efficient fishery management **(Howard *et al*., 2023)**. Ensuring the integrity of fishing records also necessitates the use of rigorous identification when verifying claims, including those submitted to the Irish Specimen Fish Committee. In summary, the precise fish identification is critical for scientific investigation and conservation efforts since it empowers scholars to analyze population dynamics, migration patterns, and ecological functions. This knowledge ultimately contributes to the development of effective approaches to safeguarding marine ecosystems.

DNA barcoding is an evolutionary methodology that detects species automatically, precisely, and efficiently by utilizing tiny, standardised gene segments as internal identifiers. Molecular methodologies precisely and rapidly validated conventional morphological and biometric (meristic and morphometric) methods; this was achieved over the course of this work.

The effectiveness and specificity of the fish F1 and fish R1 primers for amplification of DNA fragments, as confirmed by their visualisation on a 1% agarose gel, are highly indicative of the primers' affinity for the target fish DNA. The DNA fragment sizes predicted for each species are accurately reflected by separate bands, which confirms that the PCR method utilized for the genetic analysis of the analyzed fish specimens was accurate. This observation is consistent with current molecular identification techniques that emphasize the importance of primer specificity in ensuring that the appropriate target sequences are amplified **(Meyer, 1993)**.

The application of **Saitou and Nei**'s **(1987)** neighbor-joining method for phylogenetic analysis enabled the conjectured determination of the evolutionary lineage of the taxa under consideration. The phylogenetic tree that emerges, ideally the one with the highest log probability, provides further support for this approach's efficacy in molecular evolutionary research. **Felsenstein (1985)** proposed that the bootstrap consensus tree constructed using 1000 replicates offers a dependable representation of the evolutionary lineage of the taxa being investigated. The inclusion of a considerable quantity of duplicates in the bootstrap analysis guarantees that the phylogenetic inferences are statistically well-supported.

The final dataset of 557 sites, obtained from analyzing 38 nucleotide sequences and subsequent removal of ambiguous positions, adheres to the stringent criteria for sequence alignment and data quality in phylogenetic investigations (**Thompson *et al*., 1997)**. The utilisation of the Tamura-Nei method **(Tamura *et al*., 2021)** to compute evolutionary distances presents an enhanced methodology for approximating the number of base substitutions per site, therefore facilitating a more intricate comprehension of the genetic diversity between species.

The molecular phylogenetics-determined taxonomic relationships are evident in the derived phylogenetic tree, which classifies the taxa *Dicentrarchus labrax, Mullus surmuletus, Sparus aurata,* and *Seriola dumerili* into different clades. The considerable bootstrap values attributed to the majority of the nodes serve to emphasize the tree topology's dependability. As an illustration, the bootstrap value of 97.1 assigned to the node connecting *Sparus aurata* and *Seriola dumerili* signifies a substantial degree of confidence in the clustering of these taxa. This finding may imply that these taxa share a more intimate evolutionary connection. The incorporation of *Squalus megalops* as an outgroup is crucial for establishing the foundation of the phylogenetic tree, thus furnishing a benchmark for ancestral connections **(Hillis & Bull, 1993)**. Its phylogenetic differentiation from the taxa of the ingroup strengthens the interpretive structure of the evolutionary ancestry of the fish target.

In brief, the molecular methodologies and phylogenetic examination implemented in this research have undeniably clarified the fish species' evolutionary lineage and genetic connections under investigation. These discoveries are important in biodiversity protection, taxonomy, and understanding of evolutionary dynamics in marine environments.

CONCLUSION

Our research findings offered a significant support for the efficacy of contemporary taxonomic techniques, namely DNA barcoding and phylogenetic analysis, in discerning commercial fish species from maritime harvests. The unique band patterns observed in agarose gel electrophoresis, which indicate the effective amplification and resolution of DNA fragments via species-specific primers, provide more evidence for the dependability of DNA barcoding in species identification. In addition, the high bootstrap values supporting the phylogenetic tree created with the neighbor-joining method provide a graphical and statistical validation of the evolutionary relationships among the species under study. The strong correspondence between the several divisions of clades in the tree and conventional taxonomic classification serves to bolster the validity of phylogenetic investigations since they pertain to the natural lineage and divergence of commercial fish species. In addition to validating the taxonomic classification of the examined fish species, this research established a foundation for the continuous advancement of DNA-based identification systems. The precise labelling of commercial fish catches, which is critical for ecological conservation efforts and the management of fisheries, is dependent on such systems. Furthermore, these developments carry substantial ramifications for regulatory action against illicit, unreported, and unregulated (IUU) fishing practices and the surveillance of fish populations.

In summary, the amalgamation of DNA barcoding and phylogenetic investigations signifies a formidable strategy for contemporary taxonomy, providing a rigorous, accurate, and empirically supported technique for classifying and investigating commercial fish species. By adopting this methodology, our capacity to manage marine resources sustainably and safeguard biodiversity amidst escalating anthropogenic pressures can be significantly improved.

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