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DNA Barcoding of Sparidae Family in Bardawil Lagoon, North Sinai, Egypt

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ABSTRACT

Bardawil Lagoon is one of Egypt's most important fishing areas, being the second-largest and least polluted lagoon in the country. A significant portion of its catch is exported, highlighting its economic value. Among the most prominent fish groups in the lagoon are species from the family Sparidae, commonly known as porgies or sea breams. A taxonomic study of Sparidae species in Bardawil Lagoon was conducted using specimens collected directly from the lagoon. Classification was based on molecular data, specifically through analysis of the mitochondrial cytochrome oxidase subunit 1 (*COI*) gene. Studying molecular barcoding and genetic diversity among Sparidae species is crucial for effective management and conservation strategies. Following DNA analysis, a modified identification key was developed to distinguish Sparidae species present in Bardawil Lagoon. The study confirmed the presence of three species: *Sparus aurata*, *Diplodus sargus*, and *Boops boops*.

INTRODUCTION

The Sparidae family consists of fish species with significant economic value, commonly found in coastal waters around the world. These species support both commercial and recreational fisheries **(Abecasis *et al.*, 2008)** and are particularly important to the fisheries and aquaculture industries in the Mediterranean Sea. Members of the Sparidae family are carnivorous, primarily feeding on benthic invertebrates **(Carpenter, 2001)**.

Accurate identification and classification of species are essential first steps in biodiversity monitoring and conservation **(Dayrat, 2005)**. Without a solid taxonomic foundation, it is impossible to confirm species identity or determine if organisms belong to the same or different taxa. Traditionally, fish identification has relied on morphological characteristics. However, due to the high diversity and morphological variability within

fish species, especially during different developmental stages, this approach can be challenging and often insufficient **(Rasmussen *et al.*, 2009)**.

In response to these limitations, **Tautz *et al.* (2002, 2003)** advocated for a DNA-based taxonomic system. While DNA sequence analysis has been used for decades to aid species identification, methods and markers have varied across taxonomic groups and laboratories. DNA barcoding offers a standardized molecular approach, using reference databases to provide precise taxonomic identification. This method not only supports biodiversity conservation but also contributes to applications in biotechnology, forensics, and combating illegal wildlife trade **(Krishnamurthy & Francis, 2022)**.

Hebert *et al.* (2003) proposed that a single gene sequence could reliably differentiate most animal species. They recommended the mitochondrial cytochrome oxidase subunit I (*COI*) gene as a universal animal barcode. Its mitochondrial origin offers advantages such as a higher mutation rate than nuclear DNA, making it especially effective for distinguishing closely related species **(Church *et al.*, 2020)**. The widespread adoption of *COI* as a standard DNA barcode has led to the development of extensive reference libraries covering a wide range of taxonomic groups. This facilitates efficient comparison of new sequences and promotes consistency and collaboration in global biodiversity research. Its application in initiatives such as BOLD (Barcode of Life Data System) underscores its importance as a universal identifier **(Geiger *et al.*, 2021)**.

The DNA barcoding process begins with sample collection, which may involve individual species (e.g., raw tissue or processed products) or multispecies samples (e.g., mixtures or preparations). Genomic DNA is then extracted from these samples, ensuring adequate quality and quantity for analysis. A specific barcode region—typically a mitochondrial gene such as *COI*—is selected for amplification **(Ankola *et al.*, 2021)**. This target region is then amplified using polymerase chain reaction (PCR) with either universal or species-specific primers. For single-species samples, Sanger sequencing is commonly used to determine the nucleotide sequence of the amplified region **(Farrance, 2019)**. The resulting sequences are submitted to reference libraries such as BOLD, GenBank, or the Molecular Marine Biodiversity Database (MMDBD). Species identification is then performed by comparing these sequences with reference data. Phylogenetic analysis and statistical techniques further support species validation, making DNA barcoding a reliable and standardized tool for taxonomic research **(Meiklejohn *et al.*, 2019; Nakazato & Jinbo, 2022)**.

The aim of this study was to describe a new species from the Sparidae family and provide a redescription of previously recorded species from Bardawil Lagoon. Additionally, the study evaluates genetic diversification among Sparidae species in the lagoon using the *COI* gene.

MATERIALS AND METHODS

Study area

This study was conducted in Bardawil Lagoon, located in El-Arish, North Sinai, Egypt (Fig. 1). It is a shallow, highly saline lagoon covering approximately 595km², with a maximum length of 95km and a width of up to 22km. Water depth ranges from 0.5 to 3m **(GAFRD, 2019)**. Situated in the northern part of the Sinai Peninsula, the lagoon is separated from the Mediterranean Sea by a sandbar, which varies in width from 100 meters to 1 kilometer.

Fig. 1. Satellite map of Bardawil lagoon, Egypt

Tissue sample collection and DNA extraction

The fish were anesthetized using tricaine methanesulfonate (MS-222) at a concentration of 50 mg/L. Muscle samples were collected from the anterior one-third of the dorsal musculature (three fish per species) and preserved at –20 °C until further analysis.

Genomic DNA was extracted from 20 mg of muscle tissue using the GeneJET™ Genomic DNA Purification Kit (Thermo Scientific, MA, USA), following the manufacturer's instructions. Samples were digested with Proteinase K, and RNA was removed by treatment with RNase A. Genomic DNA was then eluted using the provided Elution Buffer. The integrity and size of the extracted genomic DNA (gDNA) were assessed via 1% agarose gel electrophoresis. DNA concentration was measured by spectrophotometry based on absorbance at 260 nm (A260).

DNA barcoding using the *COI* gene

The *cytochrome oxidase subunit I* (*COI*) gene was used as a molecular marker to evaluate genetic similarity and diversity among three Sparidae species. Amplification of the *COI* gene was carried out using a primer pair: forward primer “FF2d-1” and reverse

primer "FR1d-1," as described by **Ivanova *et al.* (2007)**. The primer sequences, melting temperatures, and expected amplicon lengths are summarized in Table (1).

Table 1. The primer sequence, melting temperature, and the amplicon length

Gene	Primer	Oligonucleotide sequence 5́-3́	Tm	Product length
COI (NC_024236)	FF2d-1	TTCTCCACCAACCACAARGAYATYGG	64.7	650 bp
	FR1d-1	CACCTCAGGGTGTCCGAARAAYCARAA	66.8	

Genomic DNA (gDNA) was amplified in a 50µL PCR reaction mixture containing 21µL sterile nuclease-free water, 25 µL DreamTaq Green Master Mix (Thermo Scientific, MA, USA), 1.0µL of each primer (5 pmol/µL), and 2.0µL of DNA template (50 ng/µL). The PCR cycling conditions were as follows: initial denaturation at 94°C for 1 minute; followed by 35 cycles of denaturation at 94°C, annealing at 50°C, and extension at 72°C; with a final extension step at 72°C for 10 minutes **(Ahmed *et al.*, 2021)**. A 1 kb DNA molecular ladder (PeqGold 1 Kb, Peqlab, GMH) was used to estimate the size of the amplified product.

Specific gene detection

To confirm the amplification of the target *COI* gene fragment, PCR products were separated by electrophoresis using the multiSUB Mini system (Cleaver Scientific, UK) on a high-resolution agarose gel (Cleaver Scientific, UK). Band visualization was performed using a gel documentation system (Geldoc-It, UVP, England), and data were analyzed using TotalLab software (Version 1.0.1, www.totallab.com).

Amplicon purification and sequencing

Nine PCR products (triplicates for each of the three Sparidae species) were sequenced. Amplicons were purified using the GeneJET PCR Purification Kit (Thermo Scientific, MA, USA) and sequenced in both forward and reverse directions using the same primers employed in PCR. Sequencing was performed via dye-terminator cycle sequencing on an ABI PRISM® 3100 Genetic Analyzer at Macrogen Inc. (Seoul, South Korea).

Data analysis

The nucleotide sequences obtained from the *COI* gene in the various Sparidae species were subjected to BLAST searches against the NCBI nucleotide database using Web BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm species identity. Each sequence was aligned and compared with related sequences deposited in GenBank using NCBI BLAST tools (http://www.ncbi.nlm.nih.gov).

Genetic distances and multiple sequence alignments were calculated using the Pairwise Distance method in ClustalW (https://www.genome.jp/tools-bin/clustalw). Additionally, *COI* gene sequences were aligned using MEGA11 software, and phylogenetic trees were constructed using the Maximum Likelihood method under the Tamura-Nei model **(Tamura *et al.*, 1993)**.

RESULTS

Molecular analysis

I. Checking the integrity of genomic DNA

The quality of the extracted genomic DNA from different species of the family Sparidae found in the Bardawil lagoon is shown in Fig. (2). The gDNA was intact and did not show any sign of degradation.

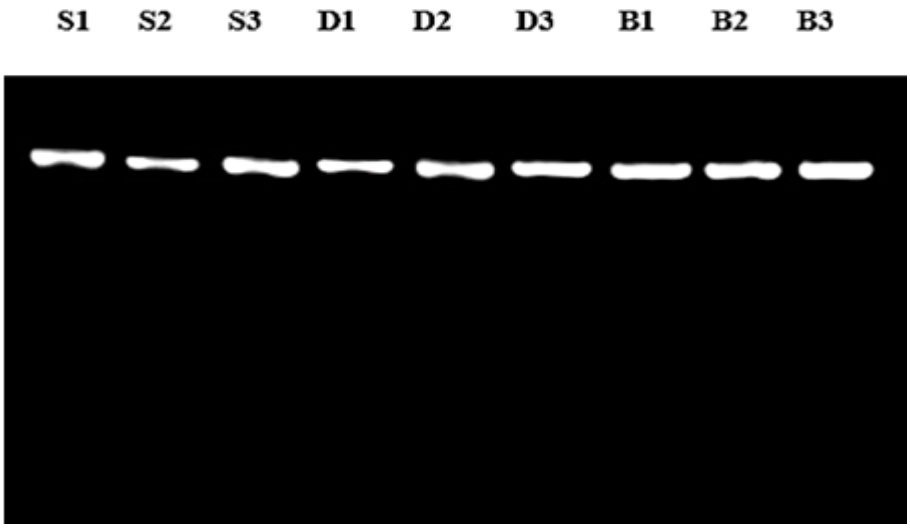

Fig. 2. The purified genomic DNA from different species of the family Sparidae: *Sparus aurata* (S1 - S3), *Diplodus sargus* (D1 - D3), and *Boops boops* (B1- B3)

II. PCR amplification of *COI* gene

The 650- bp PCR amplicon of the *COI* gene was amplified successfully using FF2d1 and FR1d1 primer pair in the three species of the family Sparidae: *Sparus aurata* (Linnaeus, 1758)*, Diplodus sargus* (Linnaeus, 1758)*,* and *Boops boops* (Linnaeus, 1758), (Fig. 3).

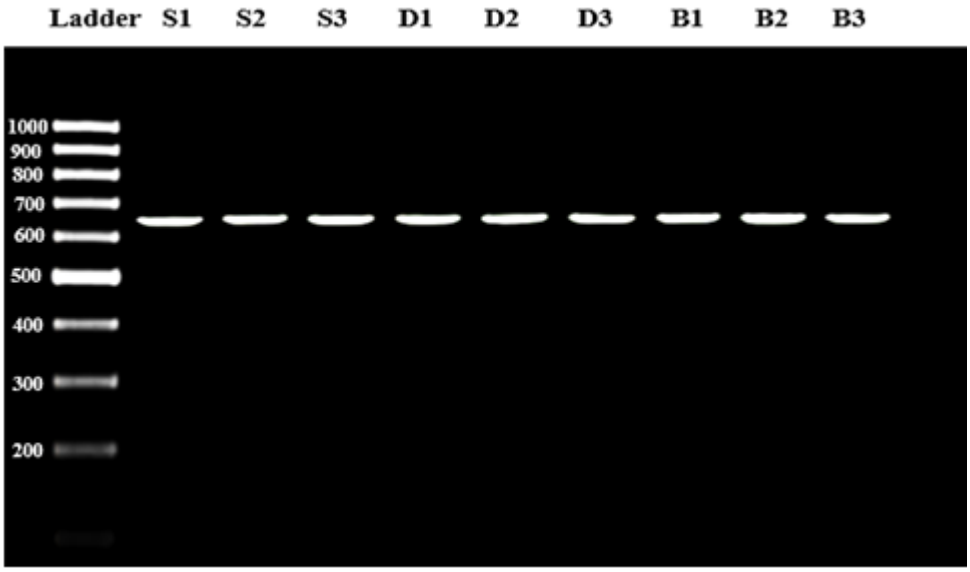

Fig. 3. 650-bp amplicon of *COI* gene in the members of the family Sparidae: *Sparus aurata* (lanes S1-S3), *Diplodus sargus* (lanes D1-D3), and *Boops boops* (lanes B1-B3)

III. Sequence analysis of *COI* gene in the family Sparidae

The nucleotide sequence of the 650-bp PCR amplicon of the *COI* gene for the different species of the family Sparidae was obtained by DNA sequencing. According to the morphological characteristics and features of the collected specimens, three species of the family Sparidae were found in the Bardawil lagoon in North Sinai. *Sparus aurata*, *Diplodus sargus*, and *Boops boops* were the species identified. To investigate the genetic relationship between the samples within each species, the obtained sequences were aligned using the Crustal W (https://www.genome.jp/tools-bin/clustalw), Figs. (4-7). Based on the alignment result, the phylogenetic tree of the samples for each species was constructed and the percent identity matrices were calculated.

For *Sparus aurata* samples, the nucleotide sequences were as follows:

```
S2
ATGCGTGCTTTGGTGCTTGGGCCGGAATAGTAGGAACTGCCCTAAGCCTGCTCATTCGAG
      60
S1
ATGCGTGCTTTGGTGCTTGGGCCGGAATAGTAGGAACTGCCCTAAGCCTGCTCATTCGAG
      60
S3
ATGCGTGCTTTGGTGCTTGGGCCGGAATAGTAGGAACTGCCCTAAGCCTGCTCATTCGAG
      60

************************************************************

S2
CTGAGCTTAGCCAGCCTGGCGCTCTCCTTGGAGACGACCAGATTTATAATGTAATTGTTA
      120
S1
CTGAGCTTAGCCAGCCTGGCGCTCTCCTTGGAGACGACCAGATTTATAATGTAATTGTTA
      120
S3
CTGAGCTTAGCCAGCCTGGCGCTCTCCTTGGAGACGACCAGATTTATAATGTAATTGTTA
      120

************************************************************

S2
CAGCACATGCGTTTGTAATAATTTTCTTTATAGTTATACCAATTATGATCGGTGGCTTTG
      180
S1
CAGCACATGCGTTTGTAATAATTTTCTTTATAGTTATACCAATTATGATCGGTGGCTTTG
      180
S3
CAGCACATGCGTTTGTAATAATTTTCTTTATAGTTATACCAATTATGATCGGTGGCTTTG
      180

************************************************************

S2
GGAACTGATTAATTCCACTTATGATCGGTGCCCCTGACATAGCATTCCCCCGAATAAATA
      240
S1
GGAACTGATTAATTCCACTTATGATCGGTGCCCCTGACATAGCATTCCCCCGAATAAATA
      240
S3
GGAACTGATTAATTCCACTTATGATCGGTGCCCCTGACATAGCATTCCCCCGAATAAATA
      240
```

```
************************************************************

S2
ACATGAGCTTCTGACTTCTTCCTCCCTCGTTCCTCCTTCTATTGGCCTCTTCTGGAGTTG
      300
S1
ACATGAGCTTCTGACTTCTTCCTCCCTCGTTCCTCCTTCTATTGGCCTCTTCTGGAGTTG
      300
S3
ACATGAGCTTCTGACTTCTTCCTCCCTCGTTCCTCCTTCTATTGGCCTCTTCTGGAGTTG
      300

************************************************************

S2
AAGCCGGAGCCGGCACCGGATGAACAGTTTACCCCCCGCTGGCAGGAAACCTTGCCCACG
      360
S1
AAGCCGGAGCCGGCACCGGATGAACAGTTTACCCCCCGCTGGCAGGAAACCTTGCCCACG
      360
S3
AAGCCGGAGCCGGCACCGGATGAACAGTTTACCCCCCGCTGGCAGGAAACCTTGCCCACG
      360

************************************************************

S2
CAGGTGCATCAGTTGATTTAACAATCTTTTCCCTTCATTTAGCTGGAATTTCATCTATTC
      420
S1
CAGGTGCATCAGTTGATTTAACAATCTTTTCCCTTCATTTAGCTGGAATTTCATCTATTC
      420
S3      CAGGTGCATCAGTTGATTTAACAATCTTTTCCCTTCATTTAGCTGGAT----
--------     408

**********************************************

S2
TTGGCGCTATTAATTTTATCACTACCATTATTAACATAAAACCCCCAGCCATTTCACAAT
      480
S1
TTGGCGCTATTAATTTTATCACTACCATTATTAACATAAAACCCCCAGCCATTTCACAAT
      480
S3      -
TGGCGCTATTAATTTTATCACTACCATTATTAACATAAAACCCCCAGCCATTTCACAAT
      467
```

```
**************************************************************

S2
ACCAAACACCACTATTCGTTTGAGCCGTTTTAATTACTGCTGTCCTACTTCTTCTGTCCC
      540
S1
ACCAAACACCACTATTCGTTTGAGCCGTTTTAATTACTGCTGTCCTACTTCTTCTGTCCC
      540
S3
ACCAAACACCACTATTCGTTTGAGCCGTTTTAATTACTGCTGTCCTACTTCTTCTGTCCC
      527

**************************************************************

S2
TTCCTGTTCTTGCCGCCGGGATTACAATGCTCCTTACAGACCGAAACCTAAACAACTTAG
      600
S1
TTCCTGTTCTTGCCGCCGGGATTACAATGCTCCTTACAGACCGAAACCTAAACACTACCT
      600
S3
TTCCTGTTCTTGCCGCCGGGATTACAATGCTCCTTACAGACCGAAACCTAAACACTACCT
      587

**************************************************

S2      CTT---------CAGGGATTGCATCTAGCTTTCAGGCAGTTACCCAAA-
      639
S1      TCTTTGACCCAGCAGGGATTGCATCTAGCTTTCAGGCAGTTTGCGCAAA
      649
S3      TCTTTACCAATTCAGCGATTGCATCTAGCTTTCAGGCAGTTACGGCAA-
      635
          *         *** ************************     **
```

Fig. 4. Multiple sequence alignment of *Sparus aurata COI* gene sequences

For *Diplodus sargus* samples, the nucleotide sequences were as follows:

```
D2
AACTTAGTCCTCTTGTATTTGGTGCTTGGGCCGGAATAGTAGGAACTGCCTTAAGCCTGC
      60
D1
AACTTAGTCCTCTTGTATTTGGTGCTTGGGCCGGAATAGTAGGAACTGCCTTAAGCCTGC
      60
```

```
D3      -
ACTTAGTCCTCTTGTATTTGGTGCTTGGGCCGGAATAGTAGGAACTGCCTTAAGCCTGC
       59

************************************************************

D2
TCATTCGAGCCGAACATAAGCCAGCCTGGCGCTCTCCTGGGAGACGACCAGATTTATAAT
       120
D1
TCATTCGAGCCGAACATAAGCCAGCCTGGCGCTCTCCTGGGAGACGACCAGATTTATAAT
       120
D3
TCATTCGAGCCGAACATAAGCCAGCCTGGCGCTCTCCTGGGAGACGACCAGATTTATAAT
       119

************************************************************

D2
GTAATTGTTACAGCACATGCGTTTGTAATAATTTTCTTTATAGTAATACCAATCATGATT
       180
D1
GTAATTGTTACAGCACATGCGTTTGTAATAATTTTCTTTATAGTAATACCAATCATGATT
       180
D3
GTAATTGTTACAGCACATGCGTTTGTAATAATTTTCTTTATAGTAATACCAATCATGATT
       179

************************************************************

D2
GGAGGCTTTGGAAACTGACTAATCCCACTTATGATCGGTGCCCCTGACATAGCATTCCCC
       240
D1
GGAGGCTTTGGAAACTGACTAATCCCACTTATGATCGGTGCCCCTGACATAGCATTCCCC
       240
D3
GGAGGCTTTGGAAACTGACTAATCCCACTTATGATCGGTGCCCCTGACATAGCATTCCCC
       239

************************************************************

D2
CGAATAAATAACATGAGCTTCTGACTTCTACCCCCGTCATTCCTCCTCCTGCTAGCCTCG
       300
```

```
D1
CGAATAAATAACATGAGCTTCTGACTTCTACCCCCGTCATTCCTCCTCCTGCTAGCCTCG
      300
D3
CGAATAAATAACATGAGCTTCTGACTTCTACCCCCGTCATTCCTCCTCCTGCTAGCCTCG
      299

************************************************************

D2
TCCGGAGTTGAAGCTGGGGCCGGTACTGGGTGAACTGTTTACCCGCCCCTGGCAGGTAAC
      360
D1
TCCGGAGTTGAAGCTGGGGCCGGTACTGGGTGAACTGTTTACCCGCCCCTGGCAGGTAAC
      360
D3
TCCGGAGTTGAAGCTGGGGCCGGTACTGGGTGAACTGTTTACCCGCCCCTGGCAGGTAAC
      359

************************************************************

D2
CTCGCTCACGCAGGTGCATCAGACGGATTATGGCTCCCTTGACTTAACTATCTTTTCTCT
      420
D1      CTCGCTCACGCAGGTGCATCAG----------------
TTGACTTAACTATCTTTTCTCT    404
D3      CTCGCTCACGCAGGTGCATCAG----------------
TTGACTTAACTATCTTTTCTCT    403
        **********************
**********************

D2
CCACCTAGCCGGAATTTCATCTATTCTTGGTGCCATTAATTTCATTACCACAATTATTAA
      480
D1
CCACCTAGCCGGAATTTCATCTATTCTTGGTGCCATTAATTTCATTACCACAATTATTAA
      464
D3
CCACCTAGCCGGAATTTCATCTATTCTTGGTGCCATTAATTTCATTACCACAATTATTAA
      463

************************************************************

D2      CATGAAACCTCCAGCA---
CCACAATATCAGACGCCATTATTTGTATGAGCCGTCTTAAT    537
```

```
D1
CATGAAACCTCCAGCTATCTCACAATATCAGACGCCATTATTTGTATGAGCCGTCTTAAT
       524
D3
CATGAAACCTCCAGCTATCTCACAATATCAGACGCCATTATTTGTATGAGCCGTCTTAAT
       523
         ***************
****************************************

D2
TACCGCCGTACTTCTTCTTCTATCTCTCCCAGTTCTTGCTGCCGGAATTACACTTAGTTC
       597
D1
TACCGCCGTACTTCTTCTTCTATCTCTCCCAGTTCTTGCTGCCGGAATTACAATGCTCCT
       584
D3       TACCGCCGTACTTCTTCTTCACCAGTTCTTGCTACCTGCAA-----------
-TGCTCCT     571
         ********************      **     * * ***
*

D2       CAGAC-----------CCGAGGCATTGACGTAGGACTTCTTAAAC
       631
D1       AACAGAGGGAGGAGACCCGAGGCATTGACGTAGGACTTCTTAAAC
       629
D3       AACAGAGGGAGGAGAACTTAGCCCGTAGGACTT------------
       604
           * *              *   * *   *
```

Fig. 5. Multiple sequence alignment of *Diplodus sargus COI* gene sequences

For *Boops boops* samples, the nucleotide sequences were as follows:

```
B2
ATCGGCAACTTAGGCGTTGTGCTTGAGCCGGAATAGTAGGGACTGCCTTAAGTCTGCTCA
       60
B1
ATCGGCAACTTAGGCGTTGTGCTTGAGCCGGAATAGTAGGGACTGCCTTAAGTCTGCTCA
       60
B3
ATCGGCAACTTAGGCGTTGTGCTTGAGCCGGAATAGTAGGGACTGCCTTAAGTCTGCTCA
       60

************************************************************

B2
TTCGAGCGGAACTAAGCCAGCCTGGCGCCCTTTTAGGAGACGACCAAATCTATAATGTCA
       120
```

```
B1
TTCGAGCGGAACTAAGCCAGCCTGGCGCCCTTTTAGGAGACGACCAAATCTATAATGTCA
      120
B3
TTCGAGCGGAACTAAGCCAGCCTGGCGCCCTTTTAGGAGACGACCAAATCTATAATGTCA
      120

************************************************************

B2
TTGTTACAGCACACGCATTTGTAATAATTTTTTTCATAGTAATACCAATTATAATCGGAG
      180
B1
TTGTTACAGCACACGCATTTGTAATAATTTTTTTCATAGTAATACCAATTATAATCGGAG
      180
B3
TTGTTACAGCACACGCATTTGTAATAATTTTTTTCATAGTAATACCAATTATAATCGGAG
      180

************************************************************

B2
GTTTCGGAAACTGACTTATTCCACTCATGATCGGTGCCCCCGACATAGCATTCCCCCGTA
      240
B1
GTTTCGGAAACTGACTTATTCCACTCATGATCGGTGCCCCCGACATAGCATTCCCCCGTA
      240
B3
GTTTCGGAAACTGACTTATTCCACTCATGATCGGTGCCCCCGACATAGCATTCCCCCGTA
      240

************************************************************

B2
TGAATAATATGAGCTTCTGACTCCTCCCCCCTTCATTCCTTCTACTCCTTGCCTCCTCTG
      300
B1
TGAATAATATGAGCTTCTGACTCCTCCCCCCTTCATTCCTTCTACTCCTTGCCTCCTCTG
      300
B3
TGAATAATATGAGCTTCTGACTCCTCCCCCCTTCATTCCTTCTACTCCTTGCCTCCTCTG
      300

************************************************************
```

```
B2
GTGTTGAAGCCGGGGCCGGTACTGGGTGAACAGTCTACCCACCACTAGCAGGGAACCTTG
      360
B1
GTGTTGAAGCCGGGGCCGGTACTGGGTGAACAGTCTACCCACCACTAGCAGGGAACCTTG
      360
B3
GTGTTGAAGCCGGGGCCGGTACTGGGTGAACAGTCTACCCACCACTAGCAGGGAACCTTG
      360

************************************************************

B2
CCCACGCAGGTGCATCAGTTGACTTAACTATCTTTTCCCTCCACCTAGCCGGAATTTCAT
      420
B1
CCCACGCAGGTGCATCAGTTGACTTAACTATCTTTTCCCTCCACCTAGCCGGAATTTCAT
      420
B3      CCCACGCAGGTGCATCAGTTGAACTTA-------
GCCCCTCCACCTAGCCGGAATTTCAT      413
        **********************  * *
************************

B2
CCATTCTTGGGGCCATTAATTTCATTACTACCATTATTAATATGAAACCCCCAGCCATTT
      480
B1
CCATTCTTGGGGCCATTAATTTCATTACTACCATTATTAATATGAAACCCCCAGCCATTT
      480
B3
CCATTCTTGGGGCCATTAATTTCATTACTACCATTATTAATATGAAACCCCCAGCCATTT
      473

************************************************************

B2
CACAATACCAAACGCCACTATTTGTGTGAGCCGTCTTAATTACAGCTGTCCTTCTTCTTC
      540
B1
CACAATACCAAACGCCACTATTTGTGTGAGCCGTCTTAATTACAGCTGTCCTTCTTCTTC
      540
B3
CACAATACCAAACGCCACTATTTGTGTGAGCCGTCTTAATTACAGCTGTCCTTCTTCTTC
      533

************************************************************
```

```
B2
TGTCCCTCCCAGTACTTGCTGCCGGAATTACTATGACCAAACCTAAACACCCCACCCAGC
      600
B1
TGTCCCTCCCAGTACTTGCTGCCGGAATTACTATGCTCCTCACAGACCGAAACCTAAACA
      600
B3      TGTCCCTCCAAAGC--------
CGGAATTACTATGCTCCTCACAGACCGAAACCTAAACA585
        ********* *           *************  *    *  * *
*    *

B2      CACG-------------TAGTGACCCAATTCGGTAGC      624
B1      CCCCACCCAGCCGGAGGAGGGGACCCAATTCGGTAGC      637
B3      CCCCACCCAACTTAGTC--GGGACCCAATTCGGTAGC      620
        * *                * ****************
```

Fig. 6. Multiple sequence alignment of *Boops boops COI* gene sequences

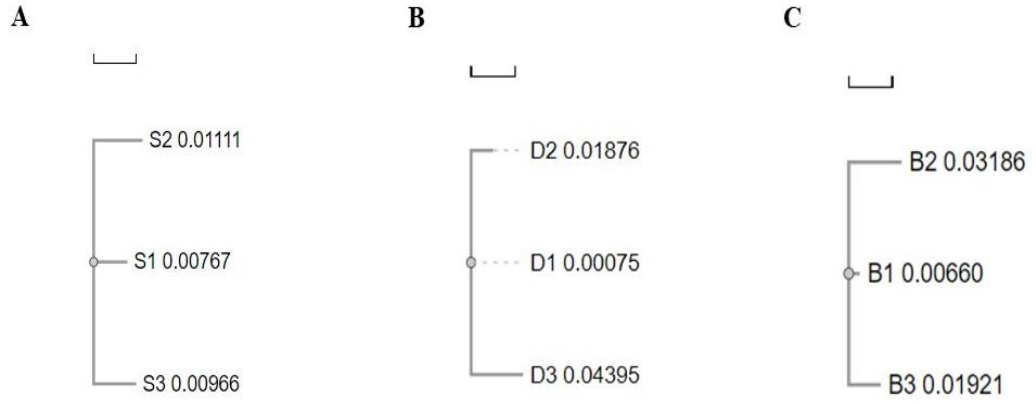

Fig. 7. Phylogenetic tree for (A) *Sparus aurata* Samples, (B) *Diplodus sargus* Samples, and (C) *Boops Boops* samples according to the *COI* gene sequences

IV. Nucleotide sequence identification and molecular characterization of the family Sparidae

The blast result contained the species number, identity %, and accession number. For *Sparus aurata* samples, the sequences were confirmed that they belong to *Sparus aurata*, bony fish with a percent identity of 100% (Table 2).

Table 2. *Sparus aurata* samples identification data based on the *COI* gene

Item	Highest homology sp.	Accession number	Identity %

S1	***Sparus aurata* *COI* mRNA, partial cds; mitochondrial**	GenBank: MF438138.1	100.00%
S2	***Sparus aurata* *COI* mRNA, partial cds; mitochondrial**	GenBank: MF438138.1	100.00%
S3	***Sparus aurata* *COI* mRNA, partial cds; mitochondrial**	GenBank: MF438138.1	97.67%

For *Diplodus sargus* samples, the sequences were confirmed that they belong to *Diplodus sargus*, bony fish with a percent identity of 99% (Table 3).

Table 3. *Diplodus sargus* samples identification data based on the *COI* gene

Item	Highest homology sp.	Accession number	Identity %
D1	***Diplodus sargus* mitochondrial *COI* gene, partial cds, isolate: Dsamm8**	GenBank: LC203131.1	99.83%
D2	***Diplodus sargus* mitochondrial *COI* gene, partial cds, isolate: Dsamm8**	GenBank: LC203131.1	96.56%
D3	***Diplodus sargus* mitochondrial *COI* gene, partial cds, isolate: Dsamm8**	GenBank: LC203131.1	99.81%

For *Boops boops* samples, the sequences were confirmed that they belong to *Boops boops*, bony fish with a percent 99% identity (Table 4).

Table 4. *Boops boops* samples identification data based on the *COI* gene

Item	Highest homology sp.	Accession number	Identity %
B1	***Boops boops voucher 821 COI* gene, partial cds; mitochondrial**	GenBank: KY176408.1	98.71%
B2	***Boops boops isolate Br1 COI* gene, partial cds; mitochondrial**	GenBank: MK317921.1	100.00%
B3	***Boops boops isolate Br1 COI* gene, partial cds; mitochondrial**	GenBank: MK317921.1	96.75%

All samples were aligned and used to construct a maximum likelihood phylogenetic tree based on the obtained *COI* gene sequences, alongside the most homologous sequences retrieved from GenBank. This analysis aimed to assess species delimitation among the studied fish.

The resulting phylogenetic tree revealed distinct clades corresponding to conspecific sequences, each of which matched previously submitted sequences in the NCBI GenBank database. All clades clustered with high bootstrap support values, which are shown next to each branch. The tree was rooted using *Boops boops* (GenBank accession: MK317921.1) as an outgroup.

Three major clades were identified:

1. A clade comprising *Sparus aurata* samples grouped with related GenBank sequences,
2. A clade containing *Diplodus sargus* samples and corresponding GenBank references,
3. A separate *Boops boops* clade, which included all sampled individuals of that species.

These results are illustrated in Fig. (8).

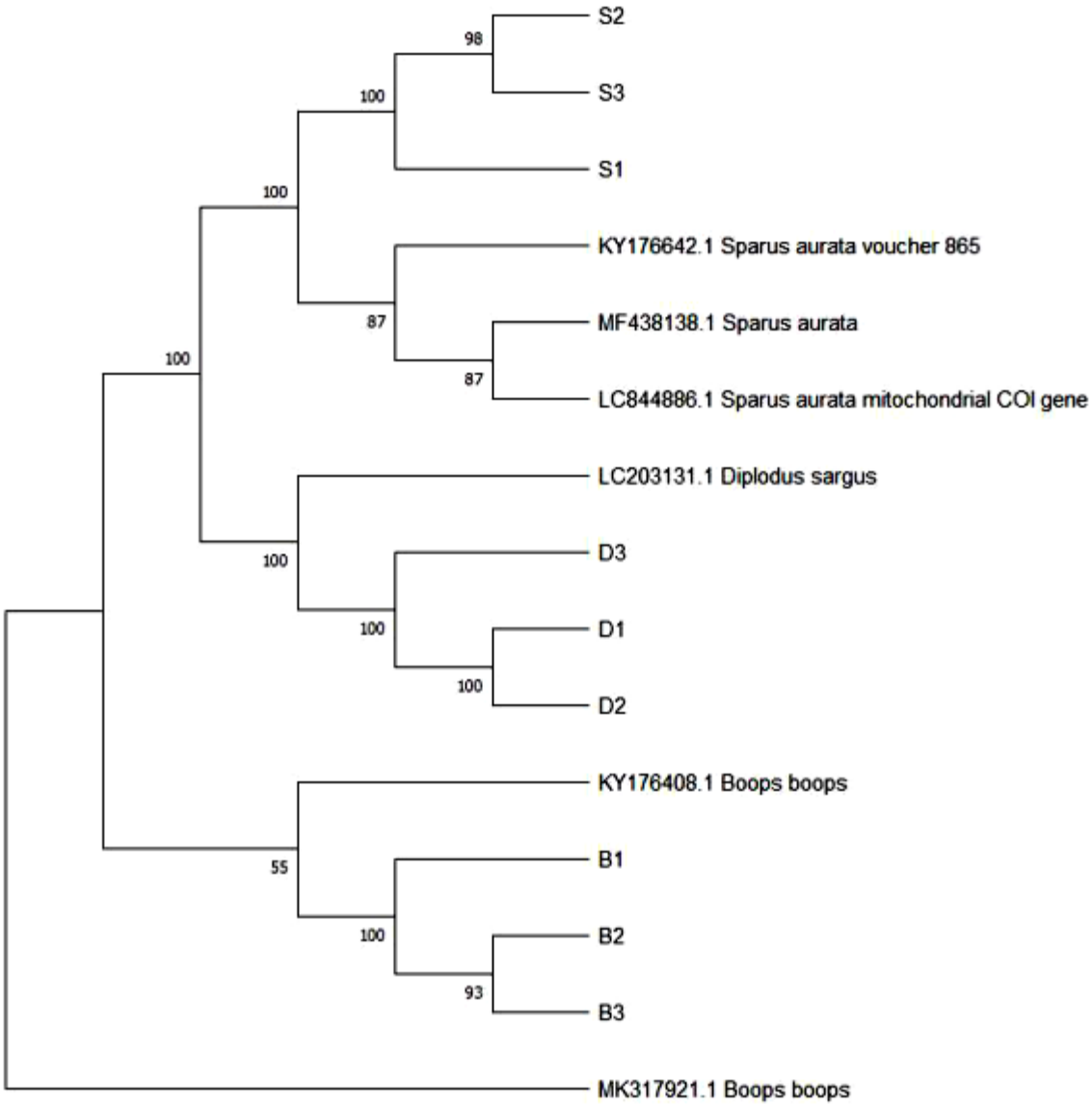

Fig. 8. Phyllogenetic tree for three samples of *Sparus aurata, Diplodus sargus,* and *Boops boops* along with the most related species from GenBank database according to the *COI* gene sequences using MEGA 11 software

DISCUSSION

The present study addresses a significant research gap, as limited investigations have explored the genetic variation and evolutionary relationships within the Sparidae family in Egypt, particularly in Bardawil Lagoon **(Abbas *et al.*, 2017)**. Our findings demonstrate that *COI* barcoding enables clear and reliable identification of most fish species and offers a more comprehensive assessment of genetic diversity within this family.

Given the substantial economic importance of Sparidae species, studying their genetic diversity is essential for informing effective fisheries management and conservation strategies. **Ward *et al.* (2005)** noted that continued application of DNA barcoding will likely uncover previously unrecognized fish species, due to significant divergence observed in *COI* sequences within currently recognized taxa. Conversely, some species thought to be distinct may share identical *COI* sequences, suggesting possible species fusion. Resolving such cases requires thorough morphological examination by expert taxonomists before definitive conclusions can be made. Therefore, a combined approach—integrating both molecular barcoding and traditional morphological analysis—is recommended for robust species identification.

In a related study, **Abbas *et al.* (2017)** successfully generated DNA barcodes for 22 Sparidae species in Egypt using *COI* primers originally designed by **Ward *et al.* (2005)**. They also tested the *cyt b* gene for species identification but found it less effective than *COI* in distinguishing species.

CONCLUSION

In the current study, specimens were randomly collected from Bardawil Lagoon and transported for taxonomic analysis and morphological measurement. The research primarily focused on the molecular characteristics of fresh samples. Upon examination, three species—*Sparus aurata*, *Diplodus sargus*, and *Boops boops*—were identified in Bardawil Lagoon, North Sinai. The morphological traits observed in these species corresponded closely with those described in earlier studies.

The overall process included DNA extraction, amplification of the *COI* barcode region, sequencing, and bioinformatics analysis. Species identification was achieved by comparing obtained sequences with reference databases, confirming the effectiveness of DNA barcoding as a standardized and reliable method for taxonomic classification and biodiversity monitoring in Egyptian coastal waters.

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