Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(3): 3131 - 3152 (2025)
www.ejabf.journals.ekb.eg

DNA Barcoding as a Tool for Evaluating Genetic Diversity and Morphological Characteristics of *Sparus aurata* in Egypt

Engy S. Nour[1]*, Eman M. Abbas[2], Engy T. Megahed[2], Reda M. Fahim[1], Hatem H. Mahmoud[1], Mikio Kato[3]

[1]College of Fisheries and Aquaculture Technology - Arab Academy for Science, Technology and Maritime Transport (AASTMT), Alexandria 1029, Egypt
[2]National Institute of Oceanography and Fisheries (NIOF), Cairo 11516, Egypt
[3]Biology Laboratory, Faculty of Liberal Arts and Sciences, Osaka Prefecture University, 1-1 Gakuencho, Naka-ku, Sakai, 599-8531, Japan

*Corresponding Author: engynour2024@adj.aast.edu

ARTICLE INFO

Article History:
Received: April 12, 2025
Accepted: June 15, 2025
Online:

Keywords:
DNA Barcoding,
COI gene,
D-Loop region,
Morphometric,
Mediterranean Sea,
Sparus aurata

ABSTRACT

The Sparidae family, which includes commercially important breams and porgies within the Perciformes order, comprises various edible species of significant economic value. *Sparus aurata*, native to the Mediterranean Sea, constitutes a major portion of fishery production, particularly in the Alexandria and El-Max regions. This study combined traditional morphology-based species identification with DNA barcoding as a key complementary technique. This integrated approach also supports the evaluation of genetic diversity among different populations by analyzing the mitochondrial COI gene and D-loop region, alongside morphological characteristics. The research underscores the importance of investigating the population structure and genetic biodiversity of *S. aurata*across four distinct sites along the Egyptian Mediterranean coast. In total, 69 out of 151 samples were analyzed using the mitochondrial COI gene, while 82 samples were analyzed using the D-loop region. Additionally, morphometric and meristic traits were recorded for 101 specimens collected from Al-Mahdiyyah. Phylogenetic trees based on genetic distance calculations were constructed for each molecular marker. The COI-based tree revealed two main genetic lineages (clades) comprising seven unique genetic forms (haplotypes). In contrast, the D-loop-based tree identified three clades encompassing twelve haplotypes. Recognizing their synergistic advantages, morphological analysis and DNA barcoding are employed together as an effective taxonomic approach for fish species identification. The data generated in this study will deepen our understanding of population connectivity and gene flow dynamics, and inform conservation strategies and fisheries management. Future research should explore the relationships between genetic diversity and life-history traits to further enhance conservation efforts.

INTRODUCTION

The family Sparidae, also known as breams or porgies, is a part of the Perciformes order. The family Sparidae is made up of a wide variety of species, many species in the family Sparidae are edible and have significant economic value **(Jobling, 2011)**. In the Mediterranean Sea, numerous species are exploited by capture fisheries, and certain species are commercially farmed. Among these, the species of

family Sparidae hold significant economic importance, being highly valued as a food source due to their flavorful and soft flesh **(Basurco *et al.*, 2011; Ibrahim *et al.*, 2020)**. Based on dentition, the family Sparidae has historically been divided into six subfamilies, 33 genera, and roughly 115 species are included in this family **(Orrell *et al.*, 2002; Abbas *et al.*, 2017)**.

In the Egyptian coasts, 33 species of the family Sparidae have been identified **(FAO, 2013)**. However, the Egyptian Mediterranean Sea is home to twenty-one species belonging to the family Sparidae **(Ibrahim & Soliman, 1996; Ibrahim *et al.*, 2020)**. Twelve of these prevalent species are found in Alexandria's marine catch on Egypt's northern shore, as stated by **GAFRD (2018)**. The gilthead seabream (*Sparus aurata*) is caught using trawl fishing, locally called “denis” **(Moretti *et al.*, 1999; Aly *et al.*, 2019)**. Bottom set longlines and handlines are also frequently employed **(Sola *et al.*, 2007)**.

Sparus aurata is native to the Mediterranean Sea, but it is also found, albeit in smaller numbers, in the Black Sea and along the Eastern Atlantic coast from Great Britain to Senegal. It is common in brackish water and marine habitats, including coastal lagoons and estuaries **(Moretti *et al.*, 1999)**.

S. aurata represents about 33% of the production of Alexandria and El-Max according to **GAFRD (2020)**. The gilthead seabream inhabits zones of seagrass, and sand floors in addition to the surf zone, at depths of up to 30 metres. *S. aurata* is a protandrous hermaphrodite; for an initial two generations, it functions as a male, but at around 30cm in length, it becomes female **(Sola *et al.*, 2007)**. For males and females, the spawning season occurs at 4 years and 6 years, respectively **(Crosetti *et al.*, 2014)**.

The success of sustainable fisheries management and biodiversity conservation hinges on the ability to accurately identify commercial fish species. However, while traditional morphological taxonomy is useful, it frequently cannot distinguish closely related species, especially juvenile fish or processed specimens **(El-Tabakh *et al.*, 2024)**. Variations in fish morphometric and meristic traits across different stocks are primarily driven by a combination of genetic, environmental, and developmental factors **(Farrag & AbdAllah, 2023)**.

DNA barcoding is a molecular taxonomy technique that utilizes brief genetic markers within an organism's genome to identify species. DNA barcoding is an innovative technique for quickly and accurately identifying species. It also enables the calculation of the genetic variability between unique taxa **(Megahed *et al.*, 2020)**. Twenty-two species of the family Sparidae were determined using a DNA barcoding method based on the mitochondrial *COI* gene **(Abbas *et al.*, 2017)**. There is widespread acceptance of the control region (D-Loop) and mitochondrial DNA (mtDNA) as markers **(Ahmet Doğdu & Turan, 2021)**. Using both the markers facilitates better estimation of the genetic diversity of *S. aurata* **(Chavanne *et al.*, 2014)**.

Previous genetic studies on the family Sparidae used only one molecular marker to identify species or examine genetic variation within this family's species **(El-Deeb *et al.*, 2014; Abbas *et al.*, 2018; Ibrahim *et al.*, 2020)**. The aim of this

study was to emphasize the importance of studying the population structure and genetic biodiversity of *S. aurata* in four different locations in the Egyptian Mediterranean Sea. The findings will aid in understanding population connectivity, gene flow patterns, and conservation & fisheries management implications.

MATERIAL AND METHODS

1. Fish sampling

A total of 467 *Sparus aurata* specimens were collected from various locations in the Egyptian Mediterranean Sea, including Rashid, Damietta, Bahari (Alexandria), Burullus, and Maadia (Idku), as exhibited in Fig. (1), with 252 samples successfully retained. 101 samples were collected from Maadia for the measurement of morphometric and meristic characteristics. The samples were transported on flake ice to the fisheries lab of the Arab Academy for Science, Technology and Maritime Transport (AASTMT), Abu Qir, Alexandria. However, from 151 of these, 69 samples were identified by mitochondrial Cytochrome c oxidase subunit I (*COI*) gene, and 82 were identified using a displacement loop (D-Loop) region. The samples were transported on flake ice to the genetics lab of the National Institute of Oceanography and Fisheries (NIOF) in Alexandria. Table (1) lists the number of fish samples and their geographic locations. The sample sizes varied among locations, which may affect the morphometric characteristics and genetic diversity estimates. To account for this, statistical corrections were applied where necessary.

Table 1. Type of gene, sample locations, sizes, sample codes, and GenBank accession numbers for *Sparus aurata*

Type of identification	Location (Egypt)	Coordinates	Sample size	Sample code	GenBank accession no.
***COI* gene**	Burullus	(31.62° N, 30.85° E)	24	*S. aurata* B1- B25	OQ826468-OQ826491
	Damietta	(31.57° N, 31.79° E)	15	*S. aurata* D1-D20	OQ826121-OQ826135
	Bahari (Alexandria)	(31.22° N, 29.88° E)	14	*S. aurata* M1-M29	OQ832530-OQ832543
	Rashid	(31.51° N, 30.34° E)	16	*S. aurata* R1-R16	OQ826530-OQ826545
D-Loop region	Burullus	(31.62° N, 30.85° E)	25	*S. aurata* B1- B25	OQ835654-OQ835678
	Damietta	(31.57° N, 31.79° E)	15	*S. aurata* D1-D16	OR004163-OR004177
	Bahari (Alexandria)	(31.22° N, 29.88° E)	26	*S. aurata* M1-M29	OR023548-OR023573
	Rashid	(31.51° N, 30.34° E)	16	*S. aurata* R1-R16	OQ835638-OQ835653
Morphometric and Meristic characteristics	Maadia (Idku)	(31.30° N, 30.16° E)	101	----------	

Fig. 1. Sampling sites: Bahari, Rashid, Burullus, Damietta and Maadia highlighted (Mediterranean Sea, Egypt)

2. Morphometric and meristic characteristics

Sparus aurata's external morphology was defined by its color, length, and fin/spine counts **(Nelson *et al.*, 2016; Panprommin *et al.*, 2023)**. Using a partition and measuring board, 11 morphometric and 9 meristic measurements were collected of the far-left side for every fish, to the closest millimeter. These measures are listed below and are exhibited in Fig. (2). Each value is identified with the number that corresponds to the measure in the list. The morphometric parameters comprised (**Quvatov *et al.*, 2023)**:

1. Total length (TL).
2. Forked length (FL).
3. Standard length (SL).
4. Head length (HL).
5. Dorsal fin base length (DL).
6. Preocular distance (POL).
7. Postocular distance (PSL).
8. Eye diameter (ED).
9. Pelvic fin length (PelvicL).
10. Pectoral fin length (PectoralL).
11. Anal fin length (AnalL).

Meristics:

1. Pectoral fin rays (PECFR).
2. Dorsal fin rays (DFR).
3. Dorsal fin spines (DFS).
4. Pelvic fin rays (PELFR).

5. Pelvic fin spines (PELFS).
6. Anal fin rays (AFR).
7. Anal fin spines (AFS).
8. Caudal fin rays (CFR).
9. Total number of gill rackers (TGR).

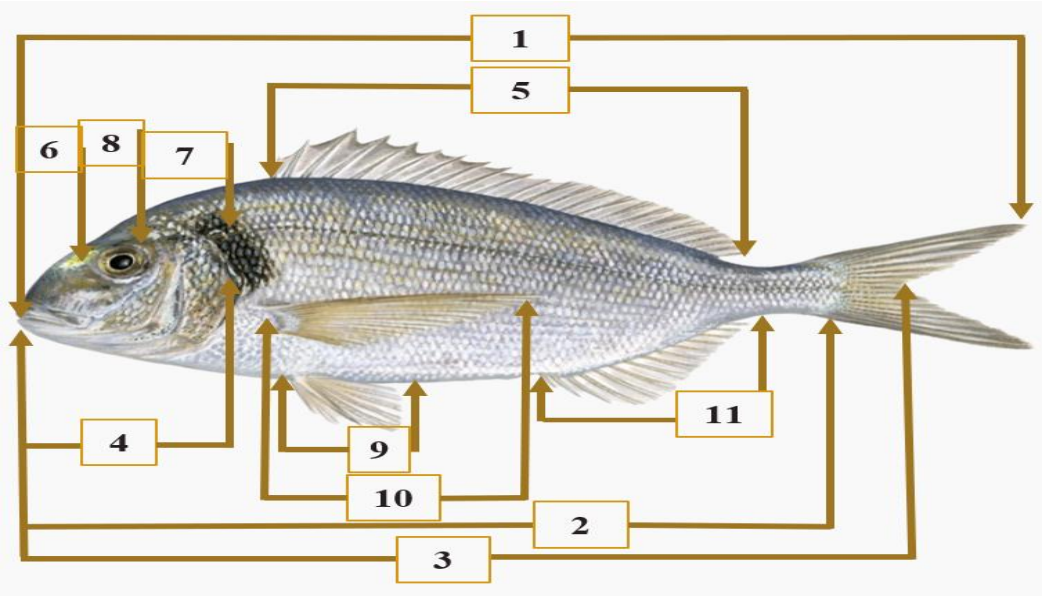

Fig. 2. Characterization determines using morphometric evaluation 1: Total Length (TL); 2: Standard length (SL); 3: Forked length (FL); 4: Head length (HL); 5: Dorsal fin base length (DL); 6: Preocular distance (POL); 7: Postocular distance (PSL); 8: Eye diameter (ED); 9: Pelvic fin length (PelvicL); 10: Pectoral fin length (PectoralL); 11:Anal fin length (AnalL) **(El-Zaeem, 2021)**

3. Statistical analysis

The mean and standard error of the morphometric measurements were calculated. To standardize morphometric measurements, each was expressed as a percentage of the standard length. The relationship between standard length and other morphometric variables was then assessed using linear regression, with the equation:

$$(\boldsymbol{Y = a + bX})$$

The linear regression model used $(Y = a + bX)$ relates the morphometric variable (Y) to the standard length (X), with the constants 'a' and 'b' (the allometric coefficient) calculated using the least squares method **(Farrag & AbdAllah, 2023)**.

The meristic data, which included counts of nine variable involved in the dorsal fin spines (DFS), the dorsal fin soft rays (DFR), the pectoral fin rays (PECFR), the pelvic fin rays (PELFR), the pelvic fin spines (PELFS), the anal fin rays (AFR), the anal fin spines (AFS), the caudal fin rays (CFR), and the total number of the gill rakers (TGR), were subjected to statistical analysis. The process included the calculation of the mean, standard deviation, and correlation coefficient, with methodologies adhering to those presented by **Snedecor and Cochran (1982)**.

4. Molecular identification

DNA extraction

Flesh tissues were dissected and preserved in 99% alcohol. To extract fish DNA from muscle tissue, the traditional phenol-chloroform extraction method was performed according to **Sambrook *et al.* (1989)**. The buffer TES [10 mM Tris-HCl, 140 mM NaCl, 25 mM EDTA, pH 7.8] containing 1% SDS and 0.5 mg mL^{-1} proteinase K was used to homogenize the specimens. For 60 minutes, muscle tissues were lysed at 56°C. By using the usual ethanol precipitation, DNA was precipitated and eluted in TE buffer (100 mM Tris-HCl, 10 mM EDTA, pH 8), then kept at 4°C for further analysis. A Biodrop (Cambridge, England) spectrophotometer was used to determine the DNA's clarity and quantity, with purity ratios (A260/A280) ranging between 1.8 and 2.0, indicating high-quality DNA.

For DNA barcoding of *S. aurata*, a portion of the *COI* gene was amplified using a modified primer set based on the study of **Ward *et al.* (2005)**. These primers were *COI* FishF1, 5′ TCAACCAACCACAAAGACATTGGCAC-3′ and FishR1, 5′-TAGACTTCT GGGTGGCCAAAGAATCA-3′. Furthermore, the D-loop control region, was amplified using the primer pair CR-A (5′-TTCCACCTCTAACTCCCAAAGCTAG-3′) and CR-E (5′-CCTGAAGTAGGAACCAGATG-3′), as described by **Lee *et al.* (1995)**.

5. Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) was performed to amplify the *COI* and D-loop fragments using a 30µL reaction mixture containing 2X My-Taq Red Mix (BIOLINE), Each reaction contained 15 µL of the mix, 0.7 µL of each primer (0.25 µM final concentration), and 20 ng of DNA template, following the manufacturer's instructions. Amplification was carried out on a BIO-RAD T100 Thermal Cycler with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation (94°C for 30 seconds), annealing (57°C for *COI*; 52°C for D-loop, each for 30 or 45 seconds, respectively and extension (72°C for 30 seconds), with a final extension at 72°C for 7 minutes. To verify the purity of the PCR products, 3µL was analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide. The PCR/Agarose DNA purification kit (Intronbio-Korea) was used to purify the PCR results, which ranged in size between 700 and 800bp. The purified molecules were then sequenced by the Applied Biosystems ABI3730 system (California, USA), adapted by **Abbas *et al.* (2011)** in the lab of Biology, Faculty of Liberal Arts and Sciences, Osaka Metropolitan University, Japan. The forward sequencing data were assembled and aligned using ClustalW within the Ugene 48.1 software **(Okonechnikov *et al.*, 2012)**. The resulting *COI* and D-Loop sequences for *S. aurata* were assigned in GenBank under the following accession numbers: *COI*: OQ826468-OQ826491, OQ826530-OQ826545, OQ832530-OQ832543, OQ826121-OQ826135; D-Loop: OQ835638-OQ835678, OR023548-OR023573, and OR004163-OR004177.

6. Data analysis

DNAsp software v.6.12 was used to analyze polymorphic sites **(Librado & Rozas, 2009)**. Haplotypes, nucleotide diversity, and the Fixation index (F_{st}) among all

populations were calculated using ARLEQUIN v3.5.2 software with a permutation value of 10,000 **(Excoffier & Lischer, 2010)**. The *COI* and D-Loop region haplotypes were utilized to build a minimum-spanning haplotype network using Pop ART software ver.1.7 **(Leigh & Bryant, 2015)**. Mega11 software was used to create a maximum likelihood phylogenetic tree for *S. aurata* **(Tamura *et al.*, 2021)**. The tree for *S. aurata* was visualized, adjusted, and produced using FigTree ver. 1.4.4 **(Rambaut, 2018)**. To ensure the effectiveness and dependability of the tree, 1,000 bootstraps were used as replicates.

RESULTS

1. Morphological and Meristic analysis

Morphometric analysis

Descriptive statistics for morphometric measurement are given in Table (1). The total length of *S. aurata* specimens investigated from Maadia ranged from 12.00 to 26.00cm TL with a mean length of 18.83 ± 3.73cm. The regression analysis between the standard length and other measurements was estimated, which confirmed that there is a strong correlation between length-length relationships. The correlation between *Sparus aurata's* morphometric measurements and standard length (SL) was determined through linear regression, with the results presented in Table (2).

This study calculated the mean and standard error of morphometric indices to provide basic statistical summaries of morphometric variables, as shown in Table (2).

Table 2. Correlation between measured morphometric variables and standard length of *S. aurata*, and the basic statistics (Mean Standard error and range) of morphometric indices of *S. aurata* collected from the Mediterranean Sea at Maadia, Egypt

Morphometric Index	The Equation	R^2	Mean±SE	Range
TL	TL = 2.461+ 1.133 SL	0.918	17.1±0.3*	12-21.6
FL	FL = 3.266+ 0.937 SL	0.788	15.37±0.27	10.5-20.8
HL	HL = 0.314+ 0.307 SL	0.784	4.28±0.09	3-5.9
DL	DL = 0.402+ 0.515 SL	0.843	7.06±0.14*	5.1-9.6
POL	POL = 0.107+ 0.132 SL	0.774	1.81±0.04	1-2.5
ED	ED = 0.353+ 0.07 SL	0.538	1.26±0.02*	0.9-1.9
PSL	PSL = -0.056+ 0.138 SL	0.743	1.73±0.04	1-2.5
PelvicL	PelvicL = -0.338+ 0.21 SL	0.807	2.37±0.06*	1.5-3.4
PectoralL	PectoralL = -0.723+ 0.379 SL	0.807	4.18±0.11	2.5-6.1
AnalL	AnalL = 0.406+ 0.184 SL	0.737	2.78±0.05	1.9-4.4

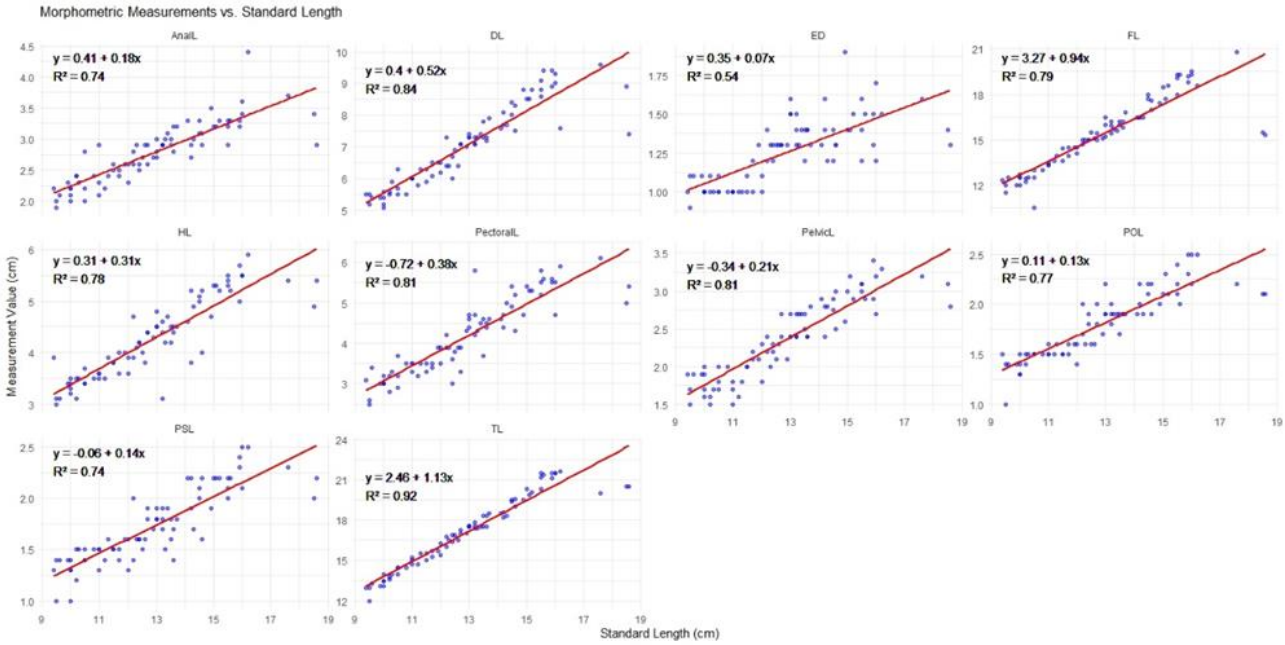

Fig. 3. The correlations between various body measurements and standard length of *S. aurata* from Maadia, Egypt. (TL: Total length, FL: Fork length, HL: Head length, DL: Dorsal fin length, POL: Preocular distance, ED: Eye diameter, PSL: Postocular distance, PelvicL: Pelvic fin length, PectoralL: Pectoral fin length, and AnalL: Anal fin length

Meristic analysis

Meristic characters are biological features defined by a numerical count, encompassing structures like fin rays, scales, and gill rakers; the observed variations are likely due to both the genes and environment. A complete understanding of the population structure of *S. aurata* requires genetic studies, alongside morphometric, meristic, and life-history data. The meristic characteristics of *S. aurata* collected from Maadia, Egypt, are shown in Table (3).

Table 3. Basic statistics (Mean ± standard error and range) for meristic indices of *S. aurata* collected from the Mediterranean Sea at Maadia, Egypt

Meristic Index	Mean±Se	Range
PECFR	13.23±0.12	10-16
DFR	12.15±0.08	10-14
DFS	10.81±0.08	7-12
AFR	10.57±0.09	9-13
AFS	2.91±0.04	2-4
TGR	11.57±0.13	10-14
CFR	15.8±0.16	12-19

2. DNA barcoding and phylogeny analysis

A sequencing 645 bp and 465 bp for a total 151 *COI* and D-Loop samples, respectively, was performed. None of the duplicated sequences contained any stop codons, insertions, or deletions in the DNA. The *COI* and D-Loop sequences from the 151 samples were compared to the information in the GenBank database using a BLAST search. Analysis showed that 96 to 100% aligned successfully with *S. aurata*. Implementing a kimura-2 parameter model (+G+I) with 1000 bootstrap repetitions, the maximum likelihood analysis was conducted. Based on the acquired sequences, all samples were used to generate maximum-likelihood phylogenetic trees (Figs. 4, 5). The phylogenetic trees revealed distinct clustering of the Egyptian *S. aurata* samples with those from other Mediterranean countries, as analyzed using the *COI* gene and D-Loop region. The phylogenetic tree based on *COI* gene was separated into two main clades. The first clade comprised *S. aurata* from the Egyptian Mediterranean such as Burullus, Rashid and Damietta (acc. no: OQ826476, OQ826474, OQ826534, OQ826129, OQ826483) with Portugal (KJ709635), Turkey (KC501572) and Greece (KC409640). The second clade included *S. aurata* from Burullus and Rashid (acc. no: OQ826468, OQ826539) with Germany (KY018850), France (KP975844), Spain (KP330354) and Italy (KJ012434). In contrast, the phylogenetic tree, based on the D-Loop region, was divided into three parts of clades. The first clade included *S. aurata* from the Egyptian Sea such as Bahari, Damietta and Burullus (acc. no: OR023564, OR023554, OR004166, OR004167, OR004165, OR004164, OQ835665, OQ835672) with Greece (KC106664). The second clade consists solely of *S. aurata* from the Egyptian Mediterranean Sea, specifically from Damietta, Rashid and Bahari (acc. no: OR004172, OQ835642, OR023571). The third clade contained only one sequence from Burullus (OQ835675).

3. Genetic variation and population structure

3.1 Genetic variation of S. aurata with COI gene

The nucleotide sequences of 69 samples were determined based on the *COI* gene (645 bp segment). The *COI* gene recorded 637 monomorphic and 8 polymorphic with 7 haplotypes. No insertions or deletions were observed. Polymorphic sites have 6 singleton variable sites (two variants) at positions 20, 36, 132, 354, 363 and 545; additionally, 2 parsimony informative sites (two variants) at positions 4 and 480. PopArt software was used to visualize the haplotypes through the most efficient network for the mitochondrial DNA *COI* gene (Fig. 6). The Hap2_*COI* haplotype showed the greatest dominance by appearing throughout all examined locations. The Hap1_*COI* haplotype exists in three sampled populations: Burullus, Bahari and Rashid. In Burullus, Hap3_*COI* 8.33%, Hap4_*COI* 4.16%, Hap1_*COI* 20.83%, and Hap2_*COI* 66.66% of the individuals at the location. In Damietta, Hap5_*COI* constituted 6.66% and Hap2_*COI* 93.33% of the individuals. In Bahari, Hap1_*COI* constituted 35.71% and Hap2_*COI* 64.28% of the individuals in the location. In Rashid, Hap1_*COI* 18.75%, Hap2_*COI* 68.75% and (Hap6_*COI* and Hap7_*COI*) each in 6.25% of the individuals. The nucleotide and haplotype diversity showed 0.00087 and 0.065, subsequently. Table (4) displays the haplotype frequencies in the

populations. It also presents the genetic diversity measures for the mitochondrial *COI* gene, including haplotypes, number of polymorphic loci, haplotype diversity, nucleotide diversity, and the average number of nucleotide differences per site. The population of Burullus has experienced the largest haplotype diversity ever documented, reaching 0.525, and the Damietta population had a haplotype diversity of 0.133, which was the lowest ever observed. Table (7) displays the F_{st} values and *P*-values between the sites. The population pairwise F_{st} values, which measure genetic diversity, were extremely low (ranging from 0.00066 to 0.00112). Furthermore, no significant population structure was detected among the four areas studied ($P > 0.05$).

3.2 Genetic variation of S. aurata with D-Loop region

The nucleotide sequences of 82 samples were determined based on the D-Loop region (465 bp segment). The D-Loop region recorded 455 monomorphic and 10 polymorphic with 12 haplotypes. No insertions or deletions were observed. Polymorphic sites have 2 singleton variable sites (two variants) at position 241 and 336, adding to 8 parsimony informative sites (two variants) at position 162, 218, 236, 252, 261, 264, 344 and 411. PopArt software was used to visualize the haplotypes through the most efficient network for the D-Loop region (Fig. 7). The Hap1_D-Loop haplotype displayed the highest dominance by being present in every surveyed population. In Burullus, Hap1_D-Loop showed 92% and Hap2_D-Loop 4% of the individuals in the location. In Damietta, Hap1_D-Loop showed 60% and (Hap2_D-Loop and Hap7_D-Loop) 6.66% of the individuals in the location. In Bahari, Hap1_D-Loop is 46.15%, (Hap3_D-Loop, Hap8_D-Loop, Hap10_D-Loop, Hap11_D-Loop) 3.84%, (Hap4_D-Loop and Hap9_D-Loop) 7.69% and Hap6_D-Loop 15.38% of the individuals in the location. In Rashid, (Hap1_D-Loop, Hap4_D-Loop and Hap6_D-Loop) showed 25% and (Hap3_D-Loop, Hap5_D-Loop, Hap5_D-Loop and Hap12_D-Loop) each in 6.25% of the individuals. The nucleotide and haplotype diversity showed 0.00183 and 0.635, respectively. Table (5) displays the haplotype frequencies in the populations. Table (5) presents the haplotypes, number of polymorphic loci, haplotype diversity, nucleotide diversity, and average number of nucleotide differences of every site for the D-Loop region, referred to as genetic diversity. The population of Rashid has experienced the largest haplotype diversity ever documented, reaching 0.850, and the Burullus population had a haplotype diversity of 0.157, which was the lowest ever observed. Table (7) displays the F_{st} values and *P*-values between the sites. The population pairwise F_{st} values, which measure genetic diversity, were extremely low (range from 0.00117 to 0.00264). All four areas studied showed no discernible population division ($P > 0.05$).

Table 4. Percentages of different haplotypes observed in *S. aurata* samples from four separate locations by *COI* gene

	Haplotype Code	Burullus	Damietta	Bahari	Rashid	Total number
***COI* gene**	Hap1_*COI*	**5** (20.83%)	0	**5** (35.71%)	**3** (18.75%)	**13** (18.84%)
	Hap2_*COI*	**16** (66.66%)	**14** (93.33%)	**9** (64.28%)	**11** (68.75%)	**50** (72.46%)
	Hap3_*COI*	**2** (8.30%)	0	0	0	**2** (2.89%)
	Hap4_*COI*	**1** (4.16%)	0	0	0	**1** (1.44%)
	Hap5_*COI*	0	**1** (6.66%)	0	0	**1** (1.44%)
	Hap6_*COI*	0	0	0	**1** (6.25%)	**1** (1.44%)
	Hap7_*COI*	0	0	0	**1** (6.25%)	**1** (1.44%)

Table 5. Percentages of different haplotypes observed in *S. aurata* samples from four separate locations by D-Loop region

	Haplotype Code	Burullus	Damietta	Bahari	Rashid	Total number
D-Loop region	Hap1_D-Loop	**23** (92%)	**9** (60.04%)	**12** (46.15%)	**4** (25%)	**48** (58.53%)
	Hap2_D-Loop	**1** (4%)	**1** (6.66%)	0	0	**2** (2.43%)
	Hap3_D-Loop	**1** (4%)	0	**1** (3.84%)	**1** (6.25%)	**3** (3.65%)
	Hap4_D-Loop	0	**1** (6.66%)	0	**1** (6.25%)	**2** (2.43%)
	Hap5_D-Loop	0	**1** (6.66%)	**6** (23.08%)	**4** (25%)	**11** (13.41%)
	Hap6_D-Loop	0	**1** (6.66%)	0	0	**1** (1.21%)
	Hap7_D-Loop	0	**1** (6.66%)	**1** (3.84%)	**1** (6.25%)	**3** (3.65%)
	Hap8_D-Loop	0	**1** (6.66%)	**2** (7.69%)	**4** (25%)	**7** (8.53%)
	Hap9_D-Loop	0	0	**1** (3.84%)	0	**1** (1.21%)
	Hap10_D-Loop	0	0	**2** (7.69%)	0	**2** (2.43%)
	Hap11_D-Loop	0	0	**1** (3.84%)	0	**1** (1.21%)
	Hap12_D-Loop	0	0	0	**1** (6.25%)	**1** (1.21%)

Table 6. Evaluation of genetic diversity within the mitochondrial *COI* gene and D-Loop region of *S. aurata* populations

	Statistics	Burullus	Damietta	Bahari	Rashid
***COI* gene**	No. of samples	24	15	14	16
	No. of Haplotypes	4	2	2	4
	No. of Polymorphic loci	3	1	1	5
	Haplotype (gene) diversity (Hd)	0.525	0.133	0.495	0.517
	Nucleotide diversity (Pi)	0.00098	0.00021	0.00077	0.00128
	Average no. of nucleotide differences (k)	0.63406	0.13333	0.495	0.825
D-Loop region	No. of samples	25	15	26	16
	No. of Haplotypes	3	7	8	7
	No. of Polymorphic loci	2	6	7	6
	Haplotype (gene) diversity (Hd)	0.157	0.657	0.745	0.850
	Nucleotide diversity (Pi)	0.00034	0.00197	0.00218	0.00305
	Average no. of nucleotide differences (k)	0.160	0.914	1.012	1.417

Table 7. Evaluation of pairwise genetic differentiation *(Fst)* and statistical significance (p-values) among *S. aurata* populations

***COI* Gene**	**Burullus**	**Damietta**	**Bahari**	**Rashid**
Burullus				
Damietta	0.00069			
Bahari	0.00086	0.00066		
Rashid	0.00112	0.00078	0.00103	
D-Loop region	**Burullus**	**Damietta**	**Bahari**	**Rashid**
Burullus				
Damietta	0.00117			
Bahari	0.00141	0.00206		
Rashid	0.00205	0.00259	0.00264	

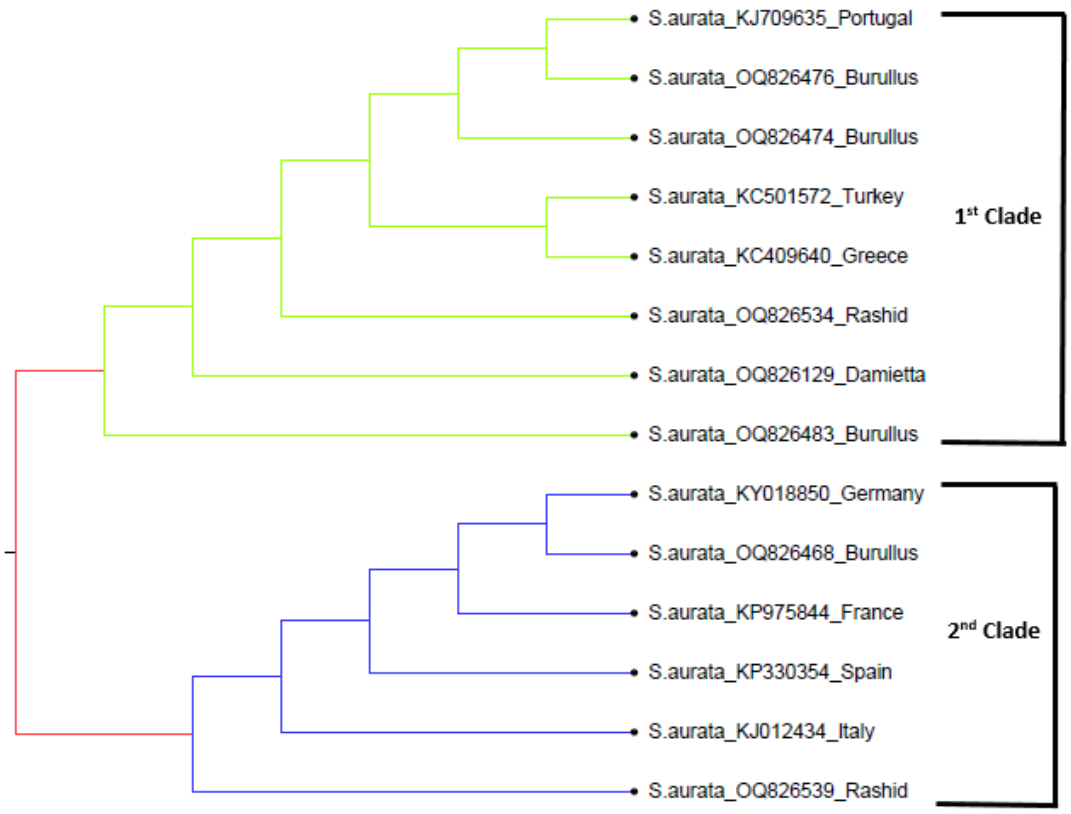

Fig. 4. Comparison of *S. aurata* from various locations in the Mediterranean Sea using 7 haplotype DNA samples isolate by *COI* gene

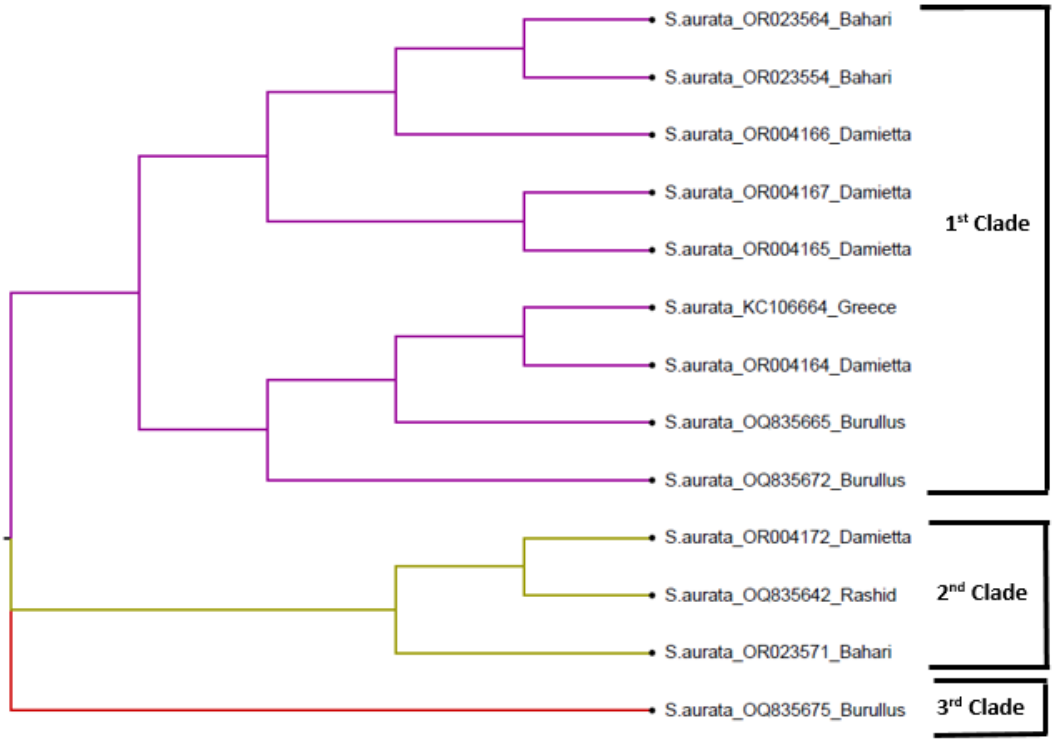

Fig. 5. Comparison of *S. aurata* from various locations in Mediterranean Sea using 12 haplotype DNA samples isolated by D-Loop region

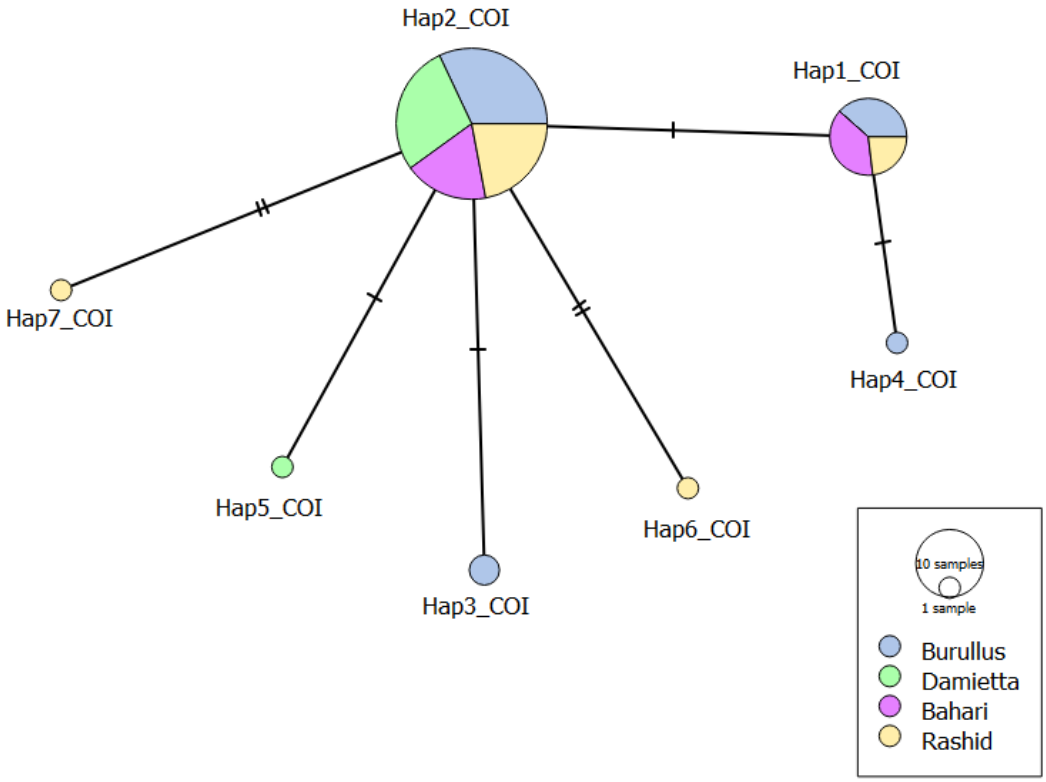

Fig. 6. The sparsest parsimonious network for mitochondrial DNA *COI* gene haplotypes of *S. aurata* species, showing 7 haplotypes. Note: the detection of the prevalent haplotype (Hap2_*COI*) at every sample site

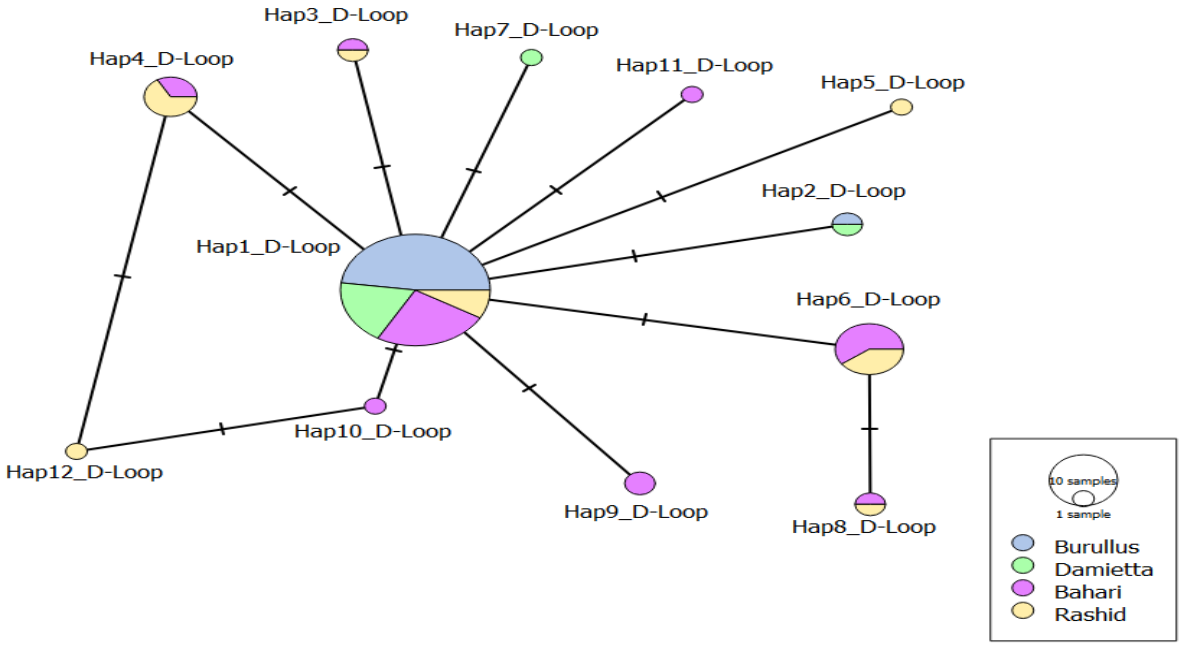

Fig. 7. The sparsest parsimonious network for D-Loop region haplotypes of *S. aurata* species, showing 12 haplotypes. Note: the detection of the prevalent haplotype (Hap1_D-Loop and Hap4_D-loop) at every sample site

DISCUSSION

The accurate identification of commercial fish is critical in marine resource management and awareness among consumers. Improved identification and labelling of species allow consumers to make informed choices that support sustainable fishing practices **(Cusa *et al.*, 2021)**. Therefore, informed decisions help advance sustainable fishing and aquaculture. Additionally, fisheries management relies heavily on recognizing species to effectively monitor fishing activities, set sustainable limits, and implement conservation measures. Accurate species identification is necessary to protect biological diversity by determining their health status and saving endangered species from extinction **(Hughes *et al.*, 2023; El-Tabakh *et al.*, 2024)**. Genetics, ecology, and environment all play a role in determining an organism's body shape **(Sarà *et al.*, 1999; Mohadasi *et al.*, 2014)**. Morphological phenotypes, including morphometric and meristic characters, are modulated by a confluence of variables, such as habitat, collection period, selective breeding, genotype, and environmental factors, and are subsequently subject to selective pressures. **(Dobzhansky, 1970; Karaiskou *et al.*, 2009; Rogdakis *et al.*, 2011; Ahmet Doğdu & Turan, 2021)**. DNA barcoding is a genetic technique that uses small, standardized gene segments as molecular identification to immediately, accurately and effectively identify species. Traditional morphology and biometrics (meristic and morphometric) measures were accurately and quickly confirmed by molecular approaches **(El-Tabakh *et al.*, 2024)**. This research combines morphological analysis with DNA sequencing techniques to determine the *S. aurata* species as demonstrated in earlier studies that used morphometric data and meristic traits to verify fish types **(Safi *et al.*, 2014; Jawad, 2015; Mahmoud *et al.*, 2016; Mustafa *et al.*, 2021)**. Population genetics theory predicts that mitochondrial DNA will undergo evolutionary changes at a rate four times faster than the typical nuclear gene. Therefore, mitochondrial DNA (mtDNA) can be utilized to track the process of differentiation in closely related taxa and even within a single species. One clear benefit of mtDNA is its capacity to determine the geographic ancestry of the species under their study **(Lencová *et al.*, 2019)**. The research we conducted employed standard identification techniques for the precise identification of the collected fish species. To address the difficulties in identifying species with insufficient morphological data, both morphological and molecular analyses were employed. Precise species identification can be challenging in the absence of comprehensive morphological characteristics **(Ng *et al.*, 2017)**. Consequently, it is advisable to employ a combination of identification approaches to guarantee correctness, as dependence on a singular method may result in incorrect findings **(Ali *et al.*, 2020; Abbas *et al.*, 2021)**. The identification of genetic diversity in populations with various geographical locations is made easier by molecular technologies (**Abbas *et al.*, 2017; Ali *et al.*, 2019)**. This work is the initial study to use the morphometric characteristics, *COI* gene, and D-Loop region to define the species various genetic genealogies and biogeographies of the marine fish species *S. aurata* in the Mediterranean Sea and along the Egyptian coast. As a useful tool for genetic tagging, molecular genetic markers *S. aurata* **(De Innocentiis *et al.*, 2005;**

Miggiano *et al.*, 2005). *S. aurata* is highly valued economically and is edible to human. 151 samples were collected from four distinct places in the Egyptian Mediterranean Sea waters: Burullus, Damietta, Bahari and Rashid. The obtained samples' *COI* and D-Loop sequences were compared to the information present in the GenBank database using a BLAST search, showing a 96 to 100% alignment success rate. To elucidate DNA variation between the distinct haplotypes identified in this research and the haplotypes of a species (*Sparus* Linnaeus, 1758) from Egypt retrieved from the GenBank database, the phylogenetic analysis was carried out. The present study also emphasizes the usage of DNA barcoding of species based on the mitochondrial *COI* gene and D-Loop region. The *COI* gene has 7 haplotypes while the D-Loop has 12 haplotypes. The study presents a comparative phylogenetic analysis of *COI* gene samples from Egypt and other Mediterranean countries. The first, clade of the phylogeny tree comprised Burullus (accession no. OQ826476, OQ826474 and OQ826483), Damietta (accession no. OQ826129), Rashid (accession no. OQ826534), Portugal (accession no. KJ709635), Turkey (accession no. KC501572) and Greece (accession no. KC409640). The second clade encompassed Burullus (accession no. OQ826468), Rashid (accession no. OQ826539), Germany (accession no. KY018850), France (accession no. KP975844), Spain (accession no. KP330354) and Italy (accession no. KJ012434). A comparison of the D-Loop region was performed using this phylogenetic tree divided into three clades. The presence of genetic mutations in Egypt is similar to Turkey, Portugal, Greece, France, Italy, Germany and Spain. The analysis ultimately demonstrated a clear similarity among the sequences of Mediterranean countries. The *COI* gene and D-Loop region analysis of all species revealed low genetic diversity and nucleotide diversities. This indicates that all populations were highly connected and that they exchanged genetic material through mating or, at the very least, in the near vicinity. This hypothesis aligns with the populations' parsimony haplotype network for the haplotypes of the *COI* gene, which represents 10.14% (Fig. 6) and haplotypes of the D-Loop region also which represents 14.63% (Fig. 7). The F_{st} pairwise values within individuals were very low and approached almost zero, indicating no discernible individual groups in the four locations (Table 6). The analysis reveals a lack of genetic variation, likely due to extensive marine connections among *S. aurata* groups. One of the main goals of DNA sequence evaluation is phylogeny restoration to comprehend the origins and evolution of species. The *COI* barcodes generated in the current study will support species identification, aligning with the findings of this research **(Sajjad *et al.*, 2023)**. The study of **Becker *et al.* (2015)** demonstrated a minimal genetic divergence (0%-0.2%) and a significant physical similarity between *Caranx sexfasciatus* and *Caranx tille*. The data analysis of *S. aurata* from our sites indicates little genetic diversity alongside uniform physical traits. **Aquilino *et al.* (2011)** employed *COI* gene DNA barcoding to investigate the ichthyofauna of Taal Lake, Philippines, identifying 23 species. The finding that *COI* sequences distinctly differentiated all 23 species at the genus level corroborates the success of our investigation in classifying the Family Sparidae. While **Vergara-Chen *et al.* (2009)** showed the efficacy of PCR-RFLP targeting the mitochondrial Cyt b gene for identifying *Cynoscion* species, especially larvae, in the

Bay of Panama, our research presents a more thorough methodology. Through the application of the *COI* gene and D-loop area, along with morphological and meristic traits, we assert that our results offer enhanced accuracy and authenticity relative to their approach.

Similarly, **Abbas *et al.* (2021)** employed the *COI* gene to successfully identify various commercial marine fish species in the Suez Gulf, Egypt. Their findings revealed strong phylogenetic connections among the 32 species, which aligns with the results of the present study. The principle that combining multiple markers and considering genetic or morphological characteristics improves accuracy, especially when *COI* has limited discriminatory ability, supports our study's methodology, which includes the *COI* gene and morphological data for identifying *S. aurata*. This is consistent with **Al-Amry *et al.* (2024)** findings suggesting that while the 16S rRNA gene is a superior marker to *COI* for Sparidae species identification, the value of a multi-marker approach remains crucial, particularly when one marker's resolution is limited. *COI* gene analysis from **Abbas *et al.* (2018)** created 21 DNA patterns in *Diplodus sargus* and *Diplodus vulgaris* that prove its usefulness for Sparidae classification along with patterns that match closely with our phylogenetic branches. Phylogenetic analysis based on the *COI* gene demonstrated monophyly within the Sparidae family. The resulting phylogenetic tree exhibited two distinct clades and several sub-clades. Notably, *Crenidens crenidens* formed a separate branch, while all other studied species grouped together within the two major clades, findings that agree with those reported by **Abbas *et al.* (2017)**. Initial analysis of the D-loop sequences revealed no significant intraspecific genetic differentiation among populations inhabiting the lake and surrounding rivers, which aligns with the findings of this current study **(O'Bryan *et al.*, 2010)**. On the other hand, our study showed different results, showing the swordfish D-loop region contained 841 base pairs and studies of 175 sequences discovered 133 genetic differences and 142 specific haplotypes, which represented greater genetic diversity than in *S. aurata*. According to **Cecconi *et al.* (1995)**, the European seabass (*Dicentrarchus labrax*) has a longer D-loop region than teleosts and chondrosteans, which shows that *S. aurata* has a shorter D-loop region and little length variation. The analysis indicates that *S. aurata* possesses a shorter D-loop area with minimal length variation.

CONCLUSION

This study used DNA barcoding (*COI* and D-loop) to assess *S. aurata genetic* diversity in the Egyptian Mediterranean, revealing low genetic diversity likely due to migration. This confirms the value of mitochondrial DNA, especially *COI*, for species identification and conservation. Our findings support using genetic diversity, particularly *COI*, as a marker of population health. Further research should investigate links between genetic diversity and life-history traits for improved conservation. This work contributes to the limited *S. aurata* research and emphasizes the need for further taxonomic study of this economically important fish.

REFERENCES

Abbas, E. M.; Ismail, M.; El-Ganainy, A. and Ali, F. S. (2021). First DNA barcoding-based inventory of Suez Gulf fishes in Egypt and its implication for species diversity. *Journal of Ichthyology*, *61*(3): 386-395. https://doi.org/10.1134/S0032945221030012

Abbas, E. M.; Megahed, E. T.; Hemeda, S. A. and El-nahas, A. (2018). DNA barcoding and molecular population structure of two species from genus Diplodus based on *COI* gene in the Egyptian Mediterranean Sea. *International Journal of Fisheries and Aquatic Studies*, *6*(1): 1-8. http://popart.otago.ac.nz/index.shtml

Abbas, E. M.; Soliman, T.; El-Magd, M. A.; Farrag, M. S.; Ismail, R. F. and Kato, M. (2017). Phylogeny and DNA Barcoding of the Family Sparidae Inferred from Mitochondrial DNA of the Egyptian Waters. *Journal of Fisheries and Aquatic Science*, *12*(2): 73–81. https://doi.org/10.3923/jfas.2017.73.81

Abbas, E. M.; Takayanagi, A.; Shimizu, N. and Kato, M. (2011). Methylation status and chromatin structure of the myostatin gene promoter region in the sea perch *Lateolabrax japonicus* (Perciformes). *Gene, Molecular Research*, *10*(4): 3306–3315. https://doi.org/10.4238/2011.December.8.7

Ahmet Doğdu, S. and Turan, C. (2021). Genetic and morphological impact of the cultured gilthead sea bream (*Sparus aurata* Linnaeus, 1758) populations on wild stocks. *Egyptian Journal of Aquatic Biology and Fisheries*, *25*(4): 499–511. https://doi.org/10.21608/ejabf.2021.191035

Al-Amry, W. N.; Al-Ansari, A. S. and Leduc, A. O. (2024). Identification of Sparidae species based on COI and 16S rRNA DNA barcoding along the coast of Oman. *Regional Studies in Marine Science*, *77*: 103677.

Ali, F. S.; Ismail, M. and Aly, W. (2020). DNA barcoding to characterize biodiversity of freshwater fishes of Egypt. *Molecular Biology Reports*, *47*(8): 5865–5877. https://doi.org/10.1007/s11033-020-05657-3

Ali, S. F.; Ismail, M. and Mamoon, A. (2019). Comparative molecular identification of genus *Dicentrarchus* using mitochondrial genes and internal transcribed spacer region. *Egyptian Journal of Aquatic Biology and Fisheries*, *23*(3): 371–384.

Aly, S.; Sharaf, S. and Griesh, A. (2019). Seasonal reproductive biology of gilthead seabream (*Sparus aurata*). *Suez Canal Veterinary Medical Journal (SCVMJ)*, *24*(1): 67-76. https://doi.org/10.21608/scvmj.2019.59206

Aquilino, S. V.; Tango, J. M.; Fontanilla, I. K.; Pagulayan, R. C.; Basiao, Z. U.; Ong, P. S. and Quilang, J. P. (2011). DNA barcoding of the ichthyofauna of Taal Lake, Philippines. *Molecular Ecology Resources*, *11*(4): 612–619.

Basurco, B.; Lovatelli, A. and García, B. (2011). Current status of Sparidae aquaculture. *In Sparidae: Biology and aquaculture of gilthead sea bream and other species* (pp. 1–50). Wiley-Blackwell. https://doi.org/10.1002/9781444392210.ch1

Becker, R. A.; Sales, N. G.; Santos, G. M.; Santos, G. B. and Carvalho, D. C. (2015). DNA barcoding and morphological identification of neotropical ichthyoplankton from the Upper Paraná and São Francisco. *Journal of Fish Biology*, *87*(1): 159–168.

Cecconi, F.; Giorgi, M. and Mariottini, P. (1995). Unique features in the mitochondrial D-loop region of the European seabass *Dicentrarchus labrax. Gene, 160*(2): 149–155.

Chavanne, H.; Parati, K.; Cambuli, C.; Capoferri, R.; Jiménez, C. A. and Galli, A. (2014). Microsatellites markers to depict the reproductive and genetic patterns of farmed gilthead seabream (*Sparus aurata*): illustration by a case study on mass spawning. *Aquaculture Research, 45*(4): 577–590. https://doi.org/10.1111/are.12013

Crosetti, D.; Rossi, A. R.; and De Innocentiis, S. (2014). *Gilthead sea bream (Sparus aurata) - AquaTrace Species leaflet*. D. Crosetti, A. R. Rossi, & S. de Innocentiis (Eds.). https://aquatrace.eu/documents/80305/142567/gilthead+sea+bream+leaflet.pdf

Cusa, M.; Falcão, L.; De Jesus, J.; Biolatti, C.; Blondeel, L.; Bracken, F. S. A.; Devriese, L.; Garcés-Pastor, S.; Minoudi, S.; Gubili, C.; Acutis, P. L. and Mariani, S. (2021). Fish out of water: Consumers' unfamiliarity with the appearance of commercial fish species. *Sustainability Science, 16*(4): 1313–1322. https://doi.org/10.1007/s11625-021-00932-z

De Innocentiis, S.; Miggiano, E.; Ungaro, A.; Livi, S.; Sola, L. and Crosetti, D. (2005). Geographical origin of individual breeders from gilthead seabream (*Sparus auratus*) hatchery broodstocks inferred by microsatellite profiles. *Aquaculture, 247*(1–4): 227–232. https://doi.org/10.1016/j.aquaculture.2005.02.022

Dobzhansky, T. (1970). *Genetics of the evolutionary process* (Vol. 139). Columbia University Press.

El-Deeb, S. I.; El-Seedy, A. S.; Gabal, A.; El-Wakeel, H. E. D. and Ibrahim, N. (2014). Genetic divergence and phylogenetic relationship among five sparid species from the coastal waters of Egypt based on protein profiling and RAPD molecular markers. *Life Science Journal, 11*(11): 779–789.

El-Tabakh, M. A. M.; El-Serafy, S. S.; El-Ganiny, A. A.; Hussein, M. M. and El-Gharabawy, M. M. (2024). DNA barcoding and phylogenetic study as a modern taxonomical tool for identifying commercial catch along the Egyptian Mediterranean coast, Alexandria. *Egyptian Journal of Aquatic Biology and Fisheries, 28*(1): 1897–1904. https://doi.org/10.21608/ejabf.2024.344271

El-Zaeem, S. (2021). Phylogenetic differentiation of wild and cultured sea bream (*Sparus aurata*) populations: 1. Phenotypic analysis. *Journal of Animal, Poultry & Fish Production, 10*(1): 93–99. https://doi.org/10.21608/japfp.2021.264470

Excoffier, L. and Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources, 10*(3): 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Farrag, M. and AbdAllah, E. (2023). Morphometric and meristic analysis of *Lates niloticus* (Linnaeus, 1758) (Teleostei: Latidae) from Lake Nasser and the River Nile, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries, 27*(6): 445–458. https://doi.org/10.21608/ejabf.2023.329270

FAO. (2013). The State of World Fisheries and Aquaculture. *Food and Agriculture Organization of the United Nations (FAO)*, Rome, Italy, 209 pp. ISBN: 978-92-5-107225-7.

GAFRD. (2018). Yearbook of Fisheries Statistics of Egypt. *General Authority for Fish Resources Development*, Ministry of Agriculture and Land Reclamation, Cairo, Egypt, 104 pp.

GAFRD. (2020). Yearbook of Fisheries Statistics of Egypt. *General Authority for Fish Resources Development*, Ministry of Agriculture and Land Reclamation, Cairo, Egypt, 102 pp.

Hughes, A.; Auliya, M.; Altherr, S.; Scheffers, B.; Janssen, J.; Nijman, V.; Shepherd, C. R.; D'Cruze, N.; Sy, E. and Edwards, D. P. (2023). Determining the sustainability of legal wildlife trade. *Journal of Environmental Management, 341*: 117987. https://doi.org/10.1016/j.jenvman.2023.117987

Ibrahim, M. A. and Soliman, I. A. (1996). Checklist of the bony fish species in the Mediterranean waters of Egypt. *Bulletin of the National Institute of Oceanography and Fisheries, 22*: 43–57.

Ibrahim, N.; Abbas, M. E.; El-Seedy, A.; Soliman, T. and Ali, F. S. (2020). DNA barcoding and comparative genetic diversification among species of family Sparidae in the coastal waters of Egypt. *Egyptian Journal of Aquatic Biology and Fisheries, 24*(3): 333–349. https://doi.org/10.21608/ejabf.2020.91939

Jawad, L. A. (2015). Study of the vertebral column of the onion trevally, *Carangoides caeruleopinnatus* (Teleostei: Carangidae) collected from the Sea of Oman. *Italian Journal of Zoology, 82*(1): 41–47. https://doi.org/10.1080/11250003.2014.1000983

Jobling, M. (2011). Pavlidis, M. A. and Mylonas, C. C. (eds): Sparidae: biology and aquaculture of gilthead sea bream and other species. *Aquaculture International*, *19*(4): 809–810. https://doi.org/10.1007/s10499-011-9455-7

Karaiskou, N.; Triantafyllidis, A.; Katsares, V.; Abatzopoulos, T. J.; and Triantaphyllidis, C. (2009). Microsatellite variability of wild and farmed populations of *Sparus aurata*. *Journal of Fish Biology*, *74*(8): 1816–1825. https://doi.org/10.1111/j.1095-8649.2009.02186.x

Lee, W. J.; Conroy, J.; Howell, W. H.; and Kocher, T. D. (1995). Structure and evolution of teleost mitochondrial control regions. *Journal of Molecular Evolution, 41*: 54–66.

Leigh, J. W. and Bryant, D. (2015). *popart*: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution, 6*(9): 1110–1116. https://doi.org/10.1111/2041-210X.12410

Lencová, S.; Zdenkova, K.; Akhatova, D. and Demnerova, K. (2019). Current trends of fish species identification by molecular-biological methods. *Chemicke Listy, 113*(5): 292–300.

Librado, P. and Rozas, J. (2009). *DnaSP v5*: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics, 25*(11): 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Mahmoud, U. M.; El-Gamal, F.; Mehanna, S. and Mansour, E. (2016). Morphometric and meristic characters of *Mullus sermuletus* (Linnaeus, 1758) and *Mullus barbatus* (Linnaeus, 1758) from the Mediterranean Sea, Alexandria,

Egypt. *International Journal of Advanced Research, 4*(8): 48–59. https://doi.org/10.21474/IJAR01/1185

Megahed, E. T.; Abbas, E. M.; El Nahas, A. F.; and Hemeda, S. A. (2020). Genetic variation of *Diplodus sargus* and *Diplodus vulgaris* in four Mediterranean coastal regions of Egypt based on microsatellites. *Egyptian Journal of Aquatic Biology and Fisheries, 24*(1): 203–215. https://doi.org/10.21608/ejabf.2020.70235

Miggiano, E.; De Innocentiis, S.; Ungaro, A.; Sola, L. and Crosetti, D. (2005). AFLP and microsatellites as genetic tags to identify cultured gilthead seabream escapees: Data from a simulated floating cage breaking event. *Aquaculture International, 13*(1–2): 137–146. https://doi.org/10.1007/s10499-004-9024-4

Mohadasi, M.; Eagderi, S.; Shabanipour, N.; Hosseinzadeh, M. S.; AnvariFar, H. and Khaefi, R. (2014). Allometric body shape changes and morphological differentiation of Shemaya, *Alburnus chalcoides* (Guldenstadf, 1772), populations in the southern part of Caspian Sea using elliptic Fourier analysis. *International Journal of Aquatic Biology, 2*(3): 164–171.

Moretti, A.; Fernandez-Criado, M. P.; Cittolin, G.; and Guidastri, R. (1999). *Manual on hatchery production of seabass and gilthead seabream: Volume 1*. Food and Agriculture Organization of the United Nations.

Mustafa, A. A.; Mohammad, A. S.; Farrag, M. M. S.; and Osman, A. G. M. (2021). Sexual dimorphism of morphological characters in three carangid species from the Red Sea, Egypt. *Iranian Journal of Ichthyology, 8*(3): 223–235. https://doi.org/10.22034/iji.v8i3.634

Nelson, J. S.; Grande, T. C.; and Wilson, M. V. H. (2016). *Fishes of the world* (5th ed.). Wiley.

Ng, C. K.-C.; Ooi, P. A.-C.; Wong, W. L.; and Khoo, G. (2017). A review of fish taxonomy conventions and species identification techniques. *Journal of Survey in Fisheries Sciences, 4*(1). https://doi.org/10.18331/sfs2017.4.1.6

O'Bryan, D. M.; Xie, Z.; Wang, Y.; Du, J.; Brauner, C. J.; Richards, J. G. and Murray, B. W. (2010). Phylogeography and conservation genetics of Lake Qinghai scaleless carp *Gymnocypris przewalskii*. *Journal of Fish Biology, 77*(9): 2072–2092.

Okonechnikov, K.; Golosova, O.; Fursov, M.; Varlamov, A.; Vaskin, Y.; Efremov, I.; Grehov, G.; Kandrov, D.; Rasputin, K.; Syabro, M. and Tleukenov, T. (2012). Unipro UGENE: A unified bioinformatics toolkit. *Bioinformatics, 28*(8): 1166–1167. https://doi.org/10.1093/bioinformatics/bts091

Orrell, T. M.; Carpenter, K. E.; Musick, J. A. and Graves, J. E. (2002). Phylogenetic and biogeographic analysis of the Sparidae (Perciformes: Percoidei) from cytochrome b sequences. *Copeia, 2002*(3): 618–631.

Panprommin, D.; Soontornprasit, K.; Tuncharoen, S.; Pithakpol, S.; Kannika, K. and Wongta, K. (2023). DNA barcoding for fish species identification and diversity assessment in the Mae Tam reservoir, Thailand. *Fisheries and Aquatic Sciences, 26*(9): 548–557. https://doi.org/10.47853/FAS.2023.e47

Quvatov, A. Q.; Kuchboev, A. E.; Mirzayev, U. T.; Amirov, O. O.; Atamuratova, M. S. and Narboev, U. Z. (2023). Morphometric and molecular characteristics

of *Cottus jaxartensis* (Scorpaeniformes: Cottidae). *Egyptian Journal of Aquatic Biology and Fisheries*, *27*(6): 215–223. https://doi.org/10.21608/ejabf.2023.328089

Rambaut, A. (2018). *FigTree* (Version 1.4.4). Institute of Evolutionary Biology, University of Edinburgh. http://tree.bio.ed.ac.uk/software/figtree/

Rogdakis, Y. G.; Koukou, K. K.; Ramfos, A.; Dimitriou, E. and Katselis, G. N. (2011). Comparative morphology of wild, farmed and hatchery-released gilthead sea bream (*Sparus aurata*) in western Greece. *International Journal of Fisheries and Aquaculture*, *3*(1): 1–9. http://www.academicjournals.org/IJFA

Safi, A.; Khan, M. A. and Khan, M. Z. (2014). Study of some morphometric and meristic characters of striped piggy fish, *Pomadasys stridens* (Forsskål, 1775) from Karachi Coast, Pakistan. *The Journal of Zoology Studies*, *1*(4): 1–6. http://www.fishbase.org

Sajjad, A.; Jabeen, F.; Ali, M. and Zafar, S. (2023). DNA barcoding and phylogenetics of *Wallago attu* using mitochondrial *COI* gene from the River Indus. *Journal of King Saud University - Science*, *35*(6): 102725.

Sambrook, J. and Maniatis, T. (1989). *Molecular cloning: A laboratory manual* (2nd ed.). Cold Spring Harbor Laboratory Press.

Sarà, M.; Favaloro, E. and Mazzola, A. (1999). Comparative morphometrics of sharpsnout seabream (*Diplodus puntazzo* Cetti, 1777), reared in different conditions. *Aquacultural Engineering*, *19*(3): 195–209.

Snedecor, G. W. and Cochran, W. G. (1982). *Statistical methods* (7th ed.). Iowa State University Press.

Sola, L.; Moretti, A.; Crosetti, D.; Karaiskou, N.; Magoulas, A.; Rossi, A. R.; ... and Tsigenopoulos, C. S. (2007). Gilthead seabream—*Sparus aurata*. In *Genetic impact of aquaculture activities on native populations* (p. 47).

Tamura, K.; Stecher, G. and Kumar, S. (2021). *MEGA11*: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution*, *38*(7): 3022–3027. https://doi.org/10.1093/molbev/msab120

Vergara-Chen, C.; González-Wangüemert, M.; Bermingham, E. and D'Croz, L. (2009). Identification of weakfish *Cynoscion* (Gill) in the Bay of Panama with RFLP markers. *Journal of Fish Biology*, *75*(5): 1101–1107.

Ward, R. D.; Zemlak, T. S.; Innes, B. H.; Last, P. R. and Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1462): 1847–1857. https://doi.org/10.1098/rstb.2005.1716