E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2018; 6(1): 01-08

www.fisheriesjournal.com
Received: 01-11-2017
Accepted: 02-12-2017

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DNA barcoding and molecular population structure of two species from genus *Diplodus* based on COI gene in the Egyptian Mediterranean Sea

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Abstract
Genetic population structure and DNA barcoding of two species *Diplodus*, (*Diplodus sargus* and *Diplodus vulgaris*) belonging to family Sparidae, were determined by using 652 bp of the mitochondrial DNA sequences of the COI gene for 114 individuals collected from six locations in the Egyptian Mediterranean Sea. Eleven haplotypes were detected for *D. sargus* including 10 polymorphic sites and 10 haplotypes for *D. vulgaris* containing 16 polymorphic sites without insertion and deletion. Two dominant haplotypes of *D. sargus* were shared among the six locations, while only one haplotype of *D. vulgaris* was common in the same mentioned locations. The haplotype and nucleotide diversities of *D. sargus* individuals were 0.652 and 0.00134 respectively, while those of *D. vulgaris* were 0.541 and 0.00188, respectively. There is no significant population subdivision between all six locations based on the *Fst* values (genetic diversity) of the population for the two species from genus *Diplodus*. Therefore, our data reflects the high connectivity between all populations indicating that these populations share the same genetic material.

Keywords: Genus *Diplodus*, population structure, COI gene, Phylogeny, Mediterranean Sea

1. Introduction
Diplodus is considered as the major genus known in family Sparidae, including 13 species and 11 subspecies [1-3]. Family Sparidae, commonly called breams or porgies, belongs to the order Perciformes. The Perciformes (perch-likes fishes) is the largest order of fishes [4]. Sparidae (porgies) are a diverse group of a large number of species with high economic value and palatable for the human. This family has about 115 species categorized in 33 genera [5]. Individuals of Sparidae were found to live in coastal waters world-wide and stay in important commercial fisheries. The *Diplodus sargus* (White Sea bream) and *Diplodus vulgaris* (the common two-banded seabream) are omnivorous species. Commercially, these fish species are considered to be important. Both fish are distributed in the eastern Atlantic ocean and along the Mediterranean Sea [2, 6]. The classification of sparid fishes is based on morphological issues such as dentition, spinous, soft fin ray counts, scalation and color of the body [1, 2].
Biological research based on the diagnosis of species and taxonomy is collapsing. In previous studies, a variety of techniques are used to identify fish species of family Sparidae. Among them, allozymes which are powerful markers for determining genetic variation and differentiation of Sparidae specs. The researchers used seventeen enzymes, revealing 24 loci and containing four resolute loci that discriminated 24 out of 28 species pairs in a dual species comparison matrix [7]. Fernández *et al.* [8] used amylase activity present in the gut of five different species of sparid fishes in the coastal water of the Mediterranean Sea for their differentiation. DNA sequence analysis, PCR-SSCP and isoelectric focusing (IEF) of water-soluble proteins of sarcoplasmic proteins were also used to differentiate between two taxonomically identified species from the family Sciaenidae and one from Sphyraenidae [9]. DNA Inter-Simple Sequence Repeat (ISSR) markers were also used to identify *Diplodus* spp. and *Dentex dentex* where eight ISSR primers produced 95 polymorphic loci with 63 of species private bands [10]. By using the random amplified polymorphic DNA (RAPD) technique, results of Ali *et al.* [11] showed a variety in the RAPD fragment patterns and the genetic variation within the family.

The application of the restriction fragment length polymorphism was used to differentiate between *Sparus aurata* and *Dentex dentex*, as well as other species [12]. Nine microsatellite markers were used for *Diplodus vulgaris*, these loci were polymorphic with a variance of allele mean of 13 showing heterozygosity [13].

Another genetic marker relating to the ecology and evolution of natural systems is DNA barcodes, which are short gene sequences taken from a standardized portion of the genome and used to identify species [14]. Among those barcodes, a mitochondrial gene known as cytochrome c oxidase I (COI), which can act as the main gene of bio-identification system for animals. Sequence divergences at COI enable the discrimination of closely allied species in all animal phyla which reflects high rates of sequence change at COI in most animal groups [15]. The genetic relationships of 24 sparidae species were analyzed by 486-bp segment of the mitochondrial 16S rDNA [16]. DNA barcoding was applied to 22 species of the family Sparidae using the barcode gene COI and the results revealed the phylogenetic relationship among the family [17].

The purpose of this study was to examine the genetic population structure and DNA barcoding of *Diplodus sargus* and *Diplodus vulgaris* of family Sparidae collected from different locations in Egypt to examine the phylogenetic relationship between both fish species and other species belonging to the same family using mitochondrial DNA for the COI region.

2. Materials and Methods

2.1 Sample collection and Identification

Diplodus sargus and *Diplodus vulgaris* samples were collected from six different locations in Egypt. These locations are Abo Qir, Bahari (Alexandria), Lake Burullus, Damietta, Rashid and Marsa-Matrouh. The map of the studying area was performed by surfer ® v12 (Fig. 1) [18]. Total samples of both species were 114. The number of *Diplodus sargus* specimens were 56, while *Diplodus vulgaris* were 58. The number of fish and their geographical location are listed in Table 1. Morphologically, *Diplodus sargus* has a flat body, its normal length ranged from 15 to 30 cm, it may reach 45 cm. It has a light gray coloration with silver reflections and eight or nine transversal dark gray strips alternating with lighter ones that disappear with age. The caudal fin is black-rimmed and the caudal peduncle is dark-spotted [4]. However, *Diplodus vulgaris* can reach 45 centimeters in length, its body is gray-silver, with two well-defined vertical black bands, one near the gills and the other before the base of the caudal fin. Another black stripe, less marked, is present close to the eyes. There are golden lines along the body, typically on the back [3].

2.2 PCR and DNA sequencing

A small part of flesh tissue was dissected and preserved in 95% alcohol [19], extraction of total genomic DNA was performed by Phenol-Chloroform technique [20, 21].

The COI mitochondrial gene was amplified using the following primer pairs [22]

FishF1-5'TCAACCAACCACAAAGACATTGGCAC3'

FishR1- 5'TAGACTTCTGGGTGGCCAAAGAATCA3'

The PCR was carried out in Applied Biosystems verity 96 well thermal cycler. The reaction volume was 25µl containing 2µl DNA template (about 20 ng), 12.5 Bioline MyTaq™ Red master mix, 1.0 µl (10 µmole) forward primer, 1.0 µL (10 µmole) reverse primer and complete the reaction with dH_2o water to 25 µl.

Reactions of PCR were performed with 94 °C initial denaturation temperature for 5 min, 35 cycles of amplification (94 °C for 30 Sec for denaturation of DNA, annealing temperature 52 °C for 30 Sec and 72 °C extension for 2 min) and final extension at 72 °C for 3 min. The PCR product was purified using Bioline ISOLATE II PCR and Gel Kit. The purified PCR product was sent for sequencing to Sangon Company China. The gained forward and reverse sequences were assembled and aligned using ClustalW with the Ugene software version 1.26 [23]. The COI sequences for all samples were uploaded to GenBank/EMBL/DDBJ databases. Sample sizes, codes and accession numbers were shown in Table 1.

2.3 Data Analysis

Analysis of polymorphic sites and DNA Polymorphism was performed using DNAsp software [24]. ARLEQUIN v3.5 software [25] was used to calculate haplotypes and nucleotides diversity and to calculate the Fixation index (*Fst)* among all populations, using permutation value of 10,000. The Popart software version 1.7. (http://popart.otago.ac.nz/index.shtml) was used to construct a minimum-spanning haplotype network for COI gene haplotypes [26]. A Maximum likelihood phylogenetic tree was constructed among *D. sargus*, *D. vulgaris* and other allied species whose sequences were retrieved from the GenBank database, using Mega7 software [27]. 1,000 bootstraps were applied as replicates for assuring the tree's efficiency and reliability.

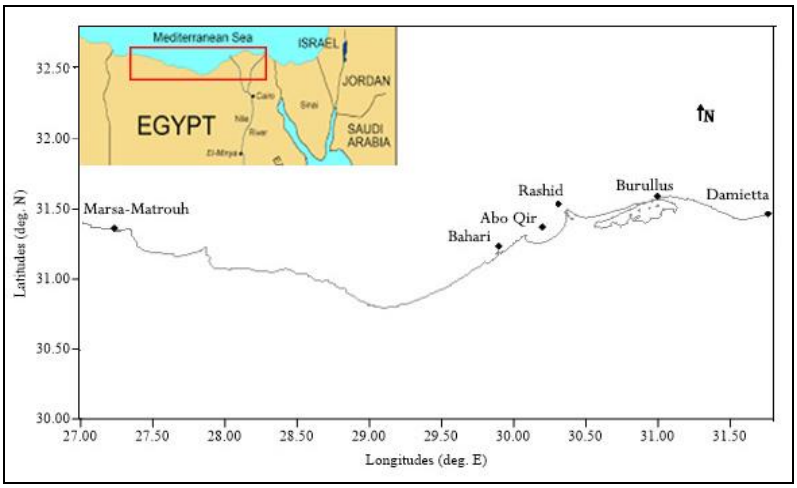

Fig 1: Map of the studying area showing sampling location sites (performed by surfer ® v12).

Table 1: Locations of the samples, their coordinates, sample sizes, sample codes and GenBank accession no. for *Diplodus sargus* and *D. vulgaris*

	Locality (Egypt)	Their coordinates	Sample size	Sample code	GenBank accession no.
Diplodus sargus	Rashid	(31.51° N, 30.34° E)	12	Dsar1-Dsar12	LC203112-LC203123
	Marsa-Matrouh	(31.40° N, 27.26° E)	9	Dsamm1-Dsamm9	LC203124-LC203132
	Damietta	(31.57° N, 31.79° E)	4	Dsadm1-Dsadm4	LC203081-LC203084
	Bahari	(31.22° N, 29.88° E)	9	Dsabh1-Dsabh2	LC203097-LC203101
	(Alexandria)				KU379675-KU379678
	Lake Burullus	(31.62° N, 30.85° E)	12	Dsabb1-Dsabb12	LC203085-LC203096
	Abo Qir	(31.31° N, 30.09° E)	10	Dsaabk1-Dsaabk10	LC203102-LC203111
Diplodus vulgaris	Rashid	(31.51° N, 30.34° E)	13	Dvur1-Dvur13	LC203490-LC203502
	Marsa-Matrouh	(31.40° N, 27.26° E)	6	Dvumm1-Dvumm6	LC203508-LC203513
	Damietta	(31.57° N, 31.79° E)	5	Dvudm1-Dvumm5	LC203503-LC203507
	Bahari	(31.22° N, 29.88° E)	10	Dvubh1-Dvubh10	KU379679-KU379688
	(Alexandria)				
	Lake Burullus	(31.62° N, 30.85° E)	10	Dvubb1-Dvubb10	LC195189 -LC195197
					LC203489
	Abo Qir	(31.31° N, 30.09° E)	14	Dvuabk1-Dvuabk14	LC203514-LC203527

3 Results

3.1 DNA barcoding and phylogeny

For all 114 samples of both *Diplodus sargus* and *Diplodus* vulgaris, 652 bp were sequenced for COI. The sequences gained from these samples were compared against the BOLD (Barcode of Life Data) and GenBank databases. The results revealed successful matches ranged from 98%-100%. The Maximum Likelihood analysis, based on kimura-2 parameter model (+G+I) with 1000 bootstraps replicates, of 11 COI gene haplotype from genus *Diplodus* for our present study (*D. sargus* and *D. vulgaris*) and some Egyptian sequences for *Diplodus* resulted in the phylogenetic tree shown in Fig. 2. The tree displayed monophyly of genus *Diplodus*. The ML tree was divided into two distinct clades including genus *Diplodus* under the study and the sequences were retrieved from the database except the *Diplodus annularis* in a separate branch. The two major clades were supported by 93 and 98 bootstraps.

The clade one was divided into two subclades, the first one included *Diplodus sargus* allocated from the different locations in Egypt under the study (acc. no. LC203107, LC203102, LC203085, LC203130 and LC203100) with *D. sargus* collected from Eastern Atlantic and Tukey (acc. no. JX192125 and KC500582), and Egyptian *Diplodus noct* with *D. sargus* collected from South Africa (acc. no. KP308273 and JX192292). On the other hand, *Diplodus cervinus* was located in a separate subclade.

The second clade includes Egyptian *Diplodus vulgaris* collected from the different locations and the obtained haplotypes sequences from GenBank database. Worth to mention that these sequences were separated into three groups. Group one included *D. vulgaris* collected from Rashid, Damietta and Greece (acc. no. LC203502, LC203503 and KC409523). The second group represented the sequences of *D. vulgaris* collected from Burullus, Bahari and Eastern Atlantic (acc. no. LC195195, KU379681 and JX192137) and they were separated in one subclade. *D. vulgaris* collected from Abo Qir, Marsa-Matrouh, Western Mediterranean and Portugal were grouped together (LC203516, LC203509, KJ012355 and KJ709520). The Third clade was for Egyptian *Diplodus annularis* (acc. no. LC152205) located in a single branch.

3.2 Genetic variation and Population structure

3.2.1 *Diplodus sargus*

Nucleotide sequence (652bp-segment) of the COI gene was determined for 56 individuals of *D. sargus*. Eleven haplotypes were detected among *D. sargus* and represented by 19.64% containing 10 polymorphic sites without insertions or deletions. These polymorphic sites contained 6 singleton variable sites with two variants at these positions 136, 370, 406, 493, 501 and 565 and contained 4 parsimony informative sites with two variants at these positions 75, 262, 358 and 413. The most parsimonious network for the mitochondrial DNA COI gene was done by PopART software [26] for identifying the haplotypes and their foundation in different locations (Fig. 3). There were two dominant haplotypes (SH2 and SH5) that were shared among all samples of the selected locations. There were four haplotypes characteristic for Rashid location (SH1, SH3, SH4, and SH6), each represents 8.33% of the population site there. In addition, two haplotypes specific for Lake Burullus location (SH10 and SH11), representing (16.66% and 8.33%) respectively, and only one haplotype (SH9) for Bahari, representing 11.11% of the population site there. The haplotype (SH8) shared between Bahari and Marsa-Matrouh. The haplotype (SH7) shared between Marsa-Matrouh and Lake Burullus. The haplotype and nucleotide diversities of *D. sargus* individuals were 0.652 and 0.00134, respectively. The haplotype frequencies within the populations are shown in Table 2. The genetic diversity of the mitochondrial COI gene is shown in Table 3 including the haplotypes, number of polymorphic loci, haplotype diversity, nucleotide diversity and the average number of nucleotide differences of each location. The highest haplotype diversity recorded in the population of Burullus Lake since it reached 0.788. While, the lowest haplotype diversity recorded in Abo Qir population was 0.356. It was found that the nucleotide diversity was low in all populations. The F_{st} values and P values between the locations are shown in Table 4. The F_{st} values (genetic diversity) of the population pairwise were very low (ranging between 0 and 0.05518). There was no significant population subdivision among all the six locations tested (P>0.05).

3.2.2 *Diplodus vulgaris*

Nucleotide sequence (652bp-segment) of the COI gene was determined for 58 individuals of *D. vulgaris*. Ten haplotypes were detected among *D. vulgaris*, represented by 17.24% containing 16 polymorphic sites without insertions or deletions. These polymorphic sites contained 8 singleton variable sites with two variants at these positions 85, 377, 526, 541, 625, 626, 628 and 629 and 8 parsimony informative sites with two variants at these positions 73, 211, 250, 265, 271, 340, 619 and 646. The most parsimonious network for the mitochondrial DNA COI gene was accomplished by PopART software [26] identifying the haplotypes and their foundation in different locations (Fig. 4). There was only one haplotype that was shared among all locations (VH1). The haplotype VH2 was shared between Abo Qir and Rashid. VH4 was common between Abo Qir, Damietta and Burullus. VH5 was mutual between Abo Qir, Marsa-Matrouh and Rashid. VH6 was shared between Bahari, Rashid and Lake Burullus. VH9 was common between Rashid and Lake Burullus. VH3 was found to be characteristic for Abo Qir, representing 7.14% of the population site there. VH7 was specific for Damietta, representing 14.28% of the population site there. VH8 was found to be specific for Rashid, represented by 7.69%. The Haplotype and nucleotide diversities of *D. vulgaris* were 0.541 and 0.00188, respectively.

The haplotype frequencies within the populations are shown in Table 2. The genetic diversity of the mitochondrial COI gene includes the number of haplotypes, number of polymorphic loci, haplotype diversity, nucleotide diversity and the average number of nucleotide differences of each (Table 3). The highest haplotype diversity was recorded in Damietta population as it reached 0.7. While, the lowest haplotype diversity recorded in Bahari was found to be equal to 0.356. The nucleotide diversity was found to be low in all populations. The F_{st} values and P values between the locations are shown in Table 4. The *Fst* values (genetic diversity) of the population pairwise were very low (ranging between 0 and 0.11538). It was shown that there was no significant population subdivision among all the six locations tested (P>0.05).

Table 2: Haplotype frequencies of *Diplodus sargus* and *Diplodus vulgaris* samples collected from six different locations.

	Haplotype code	Rashid	Marsa-Matrouh	Damietta	Bahari	Lake Burullus	Abo Qir	Total number (56)
Diplodus sargus	SH1	1	0	0	0	0	0	1
	SH2	6	5	3	4	5	8	31
	SH3	1	0	0	0	0	0	1
	SH4	1	0	0	0	0	0	1
	SH5	2	1	1	3	3	2	12
	SH6	1	0	0	0	0	0	1
	SH7	0	2	0	0	1	0	3
	SH8	0	1	0	1	0	0	2
	SH9	0	0	0	1	0	0	1
	SH10	0	0	0	0	2	0	2
	SH11	0	0	0	0	1	0	1
Diplodus vulgaris	VH1	8	4	3	8	6	10	39
	VH2	1	0	0	0	0	1	2
	VH3	0	0	0	0	0	1	1
	VH4	0	0	1	0	1	1	3
	VH5	1	2	0	0	0	1	4
	VH6	1	0	0	2	1	0	4
	VH7	0	0	1	0	0	0	1
	VH8	1	0	0	0	0	0	1
	VH9	1	0	0	0	1	0	2
	VH10	0	0	0	0	1	0	1

Table 3: Genetic diversity of mitochondrial COI gene for *Diplodus sargus* and *Diplodus vulgaris* samples

	Statistics	Rashid	Marsa-Matrouh	Damietta	Bahari	Lake Burullus	Abo Qir
Diplodus sargus	No. of samples	12	9	4	9	12	10
	No. of Haplotypes	6	4	2	4	5	2
	No. of Polymorphic loci	5	3	1	3	4	1
	Haplotype (gene) diversity (Hd)	0.758	0.694	0.5	0.75	0.788	0.356
	Nucleotide diversity (Pi)	0.00177	0.00128	0.00077	0.00145	0.00179	0.00055
	Average no. of nucleotide differences (k)	1.15152	0.83333	0.5	0.94444	1.16667	0.35556
Diplodus vulgaris	No. of samples	13	6	5	10	10	14
	No. of Haplotypes	6	2	3	2	5	5
	No. of Polymorphic loci	8	1	4	3	9	4
	Haplotype (gene) diversity (Hd)	0.641	0.533	0.7	0.356	0.667	0.505
	Nucleotide diversity (Pi)	0.00189	0.00082	0.00245	0.00164	0.00348	0.00106
	Average no. of nucleotide differences (k)	1.23077	0.53333	1.6	1.06667	2.26667	0.69231

Table 4: F_{st} value in pairwise comparison of sample locations

Diplodus sargus	**Rashid**	**Marsa-Matrouh**	**Damietta**	**Bahari**	**Lake Burullus**	**Abo Qir**
Rashid						
Marsa-Matrouh	0.03779 (0.1687)					
Damietta	0 (0.9999)	0 (0.8418)				
Bahari	0 (0.9425)	0.0137 (0.5344)	0 (0.9999)			
Lake Burullus	0 (0.6675)	0.05518 (0.1696)	0 (0.9999)	0 (0.5998)		
Abo Qir	0 (0.647)	0.01421 (0.3124)	0 (0.9999)	0.03997 (0.3624)	0.01852 (0.2862)	
Diplodus vulgaris	Rashid	Marsa-Matrouh	Damietta	Bahari	Lake Burullus	Abo Qir
Rashid						
Marsa-Matrouh	0 (0.69736)					
Damietta	0.01792 (0.46966)	0.07901 (0.25512)				
Bahari	0 (0.55480)	0.11538 (0.19157)	0.07193 (0.15791)			
Lake Burullus	0 (0.51233)	0.04155 (0.1881)	0(0.42471)	0 (0.9999)		
Abo Qir	0 (0.85239)	0.03289 (0.32165)	0.04652 (0.34165)	0.08964 (0.08148)	0.06105 (0.11167)	

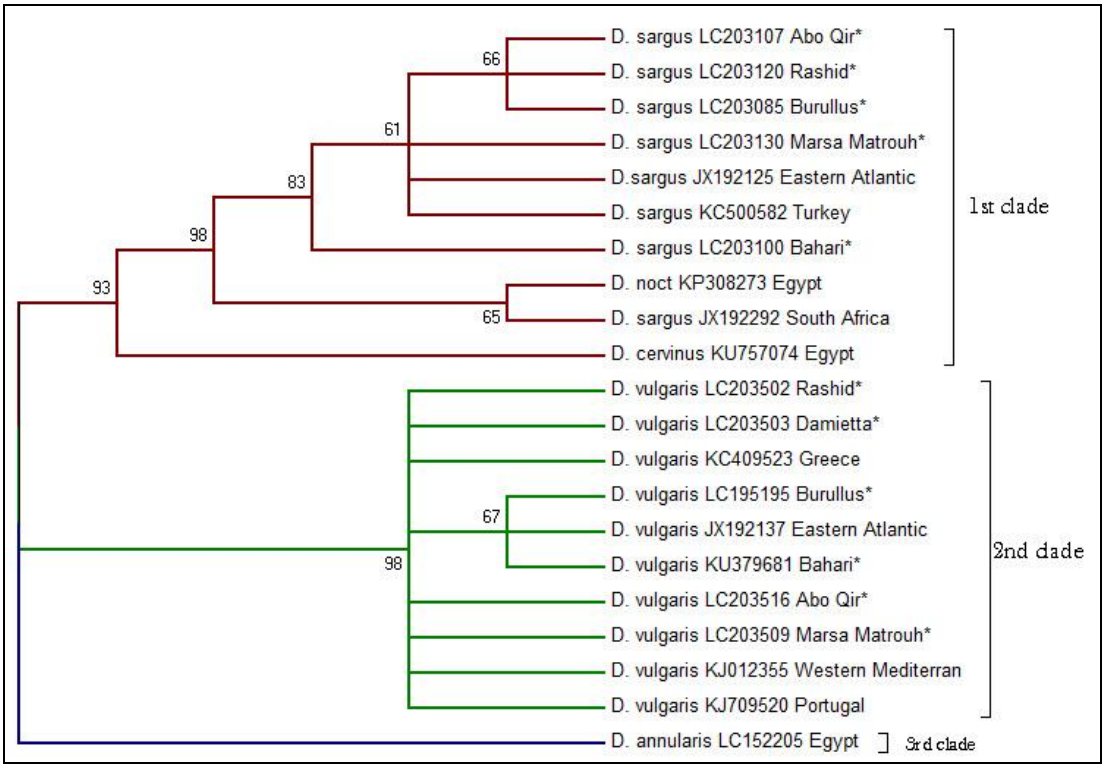

Fig 2: Maximum Likelihood tree based on kimura-2 parameter model (+G+I) of 11 haplotype samples from our study, compared with species of the same genus from different localities and the number of bootstraps replications=1000. Samples with asterisks represented that were used in the current study.

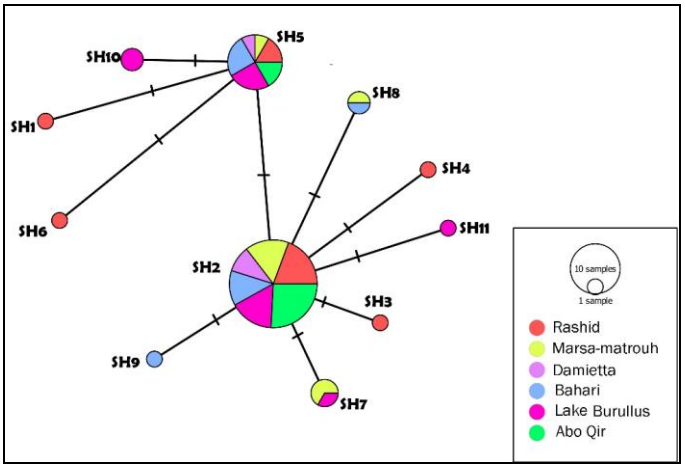

Fig 3: The most parsimonious network for haplotypes of mitochondrial DNA COI gene of *Diplodus sargus* species showing 11 haplotypes. Note also, dominant haplotype (SH2 and SH5) are detected in all sample locations.

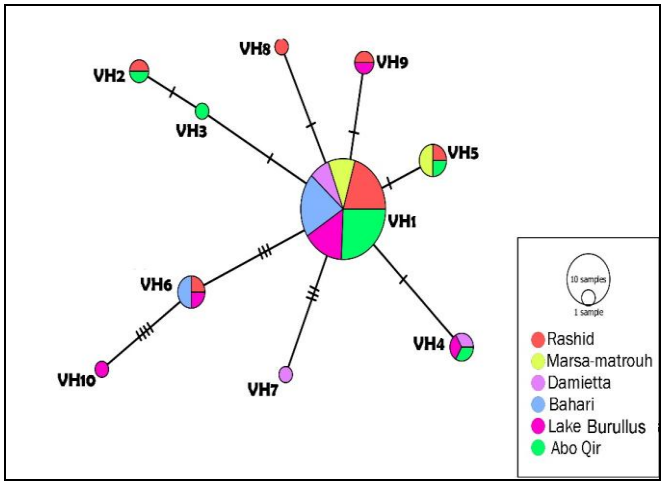

Fig 4: The most parsimonious network of haplotypes of mitochondrial DNA COI gene of *Diplodus* vulgaris species showing 10 haplotypes where dominant haplotype (VH1) is detected in all sample locations.

4 Discussion

Recently, the use of molecular tools facilitates the recognition of the genetic diversity among populations with differential geographic distribution. This study is considered to be the first collective report for population structure and DNA barcoding for two important species of genus *Diplodus* in the eastern parts of the Mediterranean Sea. It could be useful for appropriate identification and conservation of some individuals of the most economic Egyptian fish species, *D. sargus* and *D. vulgaris*.

We collected 114 samples of both *D. sargus* and *D. vulgaris* from six different locations in the Egyptian Mediterranean sea waters. 652 bp of the COI gene of all the samples were sequenced and compared against the BOLD and GenBank databases. The results revealed successful matches ranged from 98%-100% and the DNA barcoding data agree with most previous studies [17, 28-30]. However, the previously published research in Portugal contradicts our results since there are two samples published as *D. sargus* which resemble *D. vulgaris* in our study [31].

The phylogenetic analysis was performed in order to elucidate the genetic variation among the different haplotypes found in the present study and the haplotypes of genus *Diplodus* from Egypt available in the GenBank database. In addition, the current investigation highlights on the use of DNA barcoding of the species based on the mitochondrial COI gene. Concerning species haplotypes, only one sequence was found to retrieve from the GenBank database from 3 different geographical regions for *D. sargus* and 4 different geographical regions for *D. vulgaris*. The species under investigation on the Maximum Likelihood tree represented some species of genus *Diplodus*. This matches with a previous report of molecular analysis for some Sparidae species [17, 32].

The present phylogenetic analysis showed that Egyptian *D. sargus* collected from Marsa-Matrouh and Bahari (accession no. LC203130, LC203100) clustered in the same clade with the same species from Eastern Atlantic and Turkey (accession no. JX192125, KC500582). Also, all the *D. sargus* from Egypt clustered in the same main clade with Egyptian *D. noct* and *D. cervinus* (accession no. KP308273, KU757074) and this result was in accordance with that of Abbas *et al.* [17].

The Egyptian *D. vulgaris* collected from Rashid and Damietta (accession no. LC203502, LC202503) clustered with the same species from Greece (accession no. KC409523). While, *D. vulgaris* from Abo Qir and Marsa-Matrouh (accession no. LC203516, LC203509) clustered together with the same species from the Western Mediterranean and Portugal (accession no. KJ012355, KJ709520). However, one subclade was derived from the second main clade of *D. vulgaris* when the sequences from Burullus and Bahari (accession no. LC195195, KU379681) clustered with the sequence from the Eastern Atlantic (accession no. JX192137). Whilst, *Diplodus annularis* collected from Egypt (accession no. LC152205) was located in a separate clade and grouped with *D. vulgaris* in the phylogenetic tree. This result confirmed the work that used COI gene of phylogenetic analysis for the grouping of these two species [17]. From the results of the clusters obtained in the present study, it can be reported that there is a strong relationship between our sequences and the other sequences from Turkey, Greece, Western Mediterranean, Portugal and Eastern Atlantic, as well as those supported by the biographic analysis and explanation of structuring and sparid evolution [33].

Nucleotide and haplotype diversities can be an informative tool about the history of White Sea bream (*Diplodus sargus*) and the common two-banded seabream (*Diplodus* vulgaris) in Egypt. Low genetic variation and nucleotide diversities were observed in all samples of both species analyzed by the mtDNA marker (COI), which means that there was a high connectivity between all populations and these latter share their genetic material by crossing or, at least, close proximity. This pattern of genetic diversity was ensured by previous studies that used mitochondrial DNA marker (*cytb*) [34-37]. This explanation is also congruous with the parsimony haplotype network detected for COI in *D. sargus* and *D. vulgaris* populations (Fig. 3 and Fig. 4).

A low number of unique haplotypes was found in the populations from different locations of the Mediterranean sea (11 haplotypes among the *D. sargus* species and 10 among *D. vulgaris*). González-wangüemert *et al.* [35] disagree with the current results concerning a low number of haplotypes, as they found a greater number of haplotypes (131 haplotypes) among populations of 188 individuals in Mediterranean Sea using mtDNA (cytb), representing 69.68% among populations. In contrast, our study on *D. sargus* of 56 individuals, we found 11 haplotypes, represented by 19.64%. In addition, we recorded 10 haplotypes represented by 17.24% on *D. vulgaris* of 58 individuals. This may be due to the use of a different mitochondrial DNA marker (COI) in the present work.

Our results showed that there is no significant population subdivision between all six locations as the *Fst* pairwise values between populations were very low reached approximately Zero. Moreover, the *P* value > 0.05 was found to be equal to 0.9999 either in *D. sargus* or *D. vulgaris* populations (Table 4). Bargelloni *et al.* [38] used mtDNA in the analysis of the genetic population structure which was in accordance with our study and revealed that there was no significance between populations of *D. sargus* as the *Fst* = 0.007 and *P* = 0.2. Furthermore, this study utilized the allozymes to analyze of the genetic structure and their results revealed that the *Fst* value = 0.009 that means no difference between populations. Planes and Lenfant [39] worked on five different populations and revealed that there was no significance between populations of *D. sargus.* In contrast, González-Wangüemert *et al.* [40] found that there was a significance between populations of *D. sargus* when they used allozymes in the analysis of the genetic distance, revealing that *Fst*= 0.012 and *P* value < 0.001.

5. Conclusion

The Egyptian specimens of the two species from genus *Diplodus* (*D. sargus* and *D. vulgaris*) did not show any significant difference by using mtDNA COI for the analysis of *Fst* values of both species. Furthermore, the number of haplotypes was found to be very low along with the difference in the collection sites. Moreover, the detection of the diversity by using COI gene is low and logical in marine fishes where the variation is not very high because of the absence of natural barriers to migration in the sea (unlike rivers where waterfalls, springs, etc.). Since the movement of fish is always between multiple environments such as Sparidae, few genetic variations are expected.

6. Acknowledgement

We delighted to accomplish the current research with the help of Science Technology Development Fund (STDF; Project

ID: 5609), Ministry of High Education and Scientific research, which provided this work with the complete support and supplied it with the financial fund.

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