



Journal of  
**Fisheries and  
Aquatic Science**

ISSN 1816-4927



Academic  
Journals Inc.

[www.academicjournals.com](http://www.academicjournals.com)



## Research Article

# Phylogeny and DNA Barcoding of the Family Sparidae Inferred from Mitochondrial DNA of the Egyptian Waters

<sup>1</sup>Eman M. Abbas, <sup>1</sup>Taha Soliman, <sup>2</sup>Mohammed A. El-Magd, <sup>3</sup>Mahmoud M.S. Farrag, <sup>4</sup>Rania F. Ismail and <sup>5</sup>Mikio Kato

<sup>1</sup>Laboratory of Genetics, Division of Aquaculture, National Institute of Oceanography and Fisheries (NIOF), Kayed Bey, Anfushi, Alexandria, Egypt

<sup>2</sup>Department of Anatomy, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt

<sup>3</sup>Marine Science and Fishes Branch, Department of Zoology, Faculty of Science, Al-Azhar University, Assiut, Egypt

<sup>4</sup>Laboratory of Fish Reproduction and Spawning, Division of Aquaculture, National Institute of Oceanography and Fisheries (NIOF), Kayed Bey, Anfushi, Alexandria, Egypt

<sup>5</sup>Laboratory of Biology, Faculty of Liberal Arts and Sciences, Osaka Prefecture 14 University, 1-1 Gakuencho, Naka-ku, 599-8531 Sakai, Japan

## Abstract

**Background:** The family Sparidae includes about 115 species divided into 33 genera that inhabits tropical and temperate coastal waters of the Pacific, Indian and Atlantic oceans. Herein, fish of family Sparidae were molecularly barcoded using the mitochondrial gene, cytochrome oxidase subunit I (*COI*). **Materials and Methods:** Fish samples from 22 fish species of the family Sparidae were collected from Abo Qir Bay, West of the Mediterranean sea and the Gulf of Suez, North of the Red Sea. Genomic DNA was extracted from fish muscles of these species and partial coding region of the *COI* gene was amplified then sequenced and their sequences were deposited into the GenBank database. **Results:** The results of the phylogenetic tree showed that monophyly of Sparidae species. The tree was divided into two distinct clades and some sub-clades. The two major clades are including all the species under the study except *Crenidens crenidens* in a separate branch. **Conclusion:** Our results confirm the phylogenetic relationship of these Sparidae species in Egyptian Mediterranean and Red Sea and support the previous finding which concluded that the Sparidae is composed by two major lineages.

**Key words:** Sparidae, DNA barcoding, phylogeny, *COI* gene

**Received:** November 02, 2016

**Accepted:** January 13, 2017

**Published:** February 15, 2017

**Citation:** Eman M. Abbas, Taha Soliman, Mohammed A. El-Magd, Mahmoud M.S. Farrag, Rania F. Ismail and Mikio Kato, 2017. Phylogeny and DNA barcoding of the family Sparidae inferred from mitochondrial DNA of the Egyptian waters. J. Fish. Aquat. Sci., 12: 73-81.

**Corresponding Author:** Eman M. Abbas, Laboratory of Genetics, Division of Aquaculture, National Institute of Oceanography and Fisheries (NIOF), Kayed Bey, Anfushi, Alexandria, Egypt Tel: +201124852279

**Copyright:** © 2017 Eman M. Abbas *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The family Sparidae has historically been classified into six subfamilies, primarily on the basis of dentition<sup>1,2</sup>. This family includes about 115 species divided into 33 genera<sup>3</sup>. Fishes from this family are carnivorous, feeding on benthic invertebrates and inhabit tropical and temperate coastal waters of the Pacific, Indian and Atlantic oceans<sup>2,4</sup>. They swim near the shore in shallow inlet and bays at moderate depth<sup>5</sup> and can be used in mariculture and cultivated in cages<sup>6</sup>. In the Mediterranean countries *Sparus aurata* is so far, the most extensively cultured species<sup>7</sup>. Cultivation of other species such as *Pagrus pagrus*, *Diplodus sargus* and *Diplodus puntazzo* are envisaged<sup>8</sup>.

Thirty three Sparidae species were recorded in the Egyptian coasts<sup>9</sup>, most of them are economically important and commonly used as a food due to their good taste and rich flesh. Twenty one out of these 33 species found in the Egyptian Mediterranean water<sup>10</sup>. Twelve out of these 21 species are most frequent in the landed catch from Alexandria water on the Northern coast of Egypt. Seabreams represent about 15% of these landed catch fishes<sup>11</sup>. On the other hand, only 14 species of this family exist in the Red Sea<sup>12</sup>, but they are less economically importance than those of the Mediterranean Sea.

The determination and identification of the different species are considered as the initial basic steps for biodiversity monitoring and conservation<sup>13</sup>. Fish identification is traditionally based on morphological features. However, due to high diversity and morphological plasticity, in many cases, fish and their diverse developmental stages are difficult to identify by using morphological characteristics alone<sup>14</sup>. The DNA-based identification techniques have been developed and proven to be analytically powerful<sup>15-17</sup>. For ensuring rapid and accurate identification of a broad range of biological specimens<sup>18</sup> proposed "DNA barcoding" technique, using the *COI* gene, because its mutation rate is often fast enough to distinguish closely related species and also its sequence is conserved among conspecifics. For many animal taxa, nucleotides sequence divergences within the *COI* gene are generally much greater between species than within species. However, changes in its amino acid sequence occur more slowly than those in any other mitochondrial gene<sup>19</sup>. Therefore, this gene is conserved and less subjected to external forces. Many studies have shown that intraspecific variation of *COI* barcodes is generally pretty small and clearly discriminable from interspecific variation<sup>20-22</sup>. Consequently, many researchers have investigated the use of DNA barcoding to enforce traceability regulations and to fight illegal fishing and frauds<sup>21,23,24</sup>.

From the available literature, it was found that some studies have been published on the phylogeny of Sparid fishes using mitochondrial genes<sup>2,25,26</sup>. In addition, genetic variations between five Sparid species from the Northern coastal waters of Egypt were analyzed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of sarcoplasmic proteins and Random Amplified Polymorphic DNA (RAPD) analyses<sup>27</sup>. In the present study, DNA barcoding technique based on mitochondrial *COI* gene was adopted to identify 22 fish species of family Sparidae inhabiting the Egyptian coasts of Mediterranean and Red Seas.

## MATERIALS AND METHODS

**Sample collection:** Fish samples were collected from two locations from Abo Qir Bay, West of the Mediterranean Sea and from the Gulf of Suez, North of the Red Sea in Egypt (Fig. 1). Samples were transferred to National Institute of Oceanography and Fisheries (NIOF) Alexandria Branch, in ice box. Twenty two fish species from family Sparidae were analyzed (Table 1). Fish samples were photographed and identified *in situ* by visual inspection and taxonomically classified employing standard taxonomic guides following the FAO fish identification sheets<sup>9</sup>. For DNA samples, pieces of flesh muscle were sliced and preserved in 99% ethanol.

**DNA samples and analysis:** Total genomic DNA was extracted from fish muscles using conventional phenol-chloroform procedures described by Ward *et al.*<sup>28</sup>. In brief, tissues were first homogenized in the DNA isolation buffer TES [10 mM tris-HCl (Wako, Japan), 140 mM NaCl (Wako, Japan), 25 mM EDTA (Bio-Rad), pH 7.8] containing 1% SDS-Wako, Japan and 0.5 mg mL<sup>-1</sup> proteinase K (Biolabs, New England) and the reaction mixtures were incubated for 1 h at 50°C.

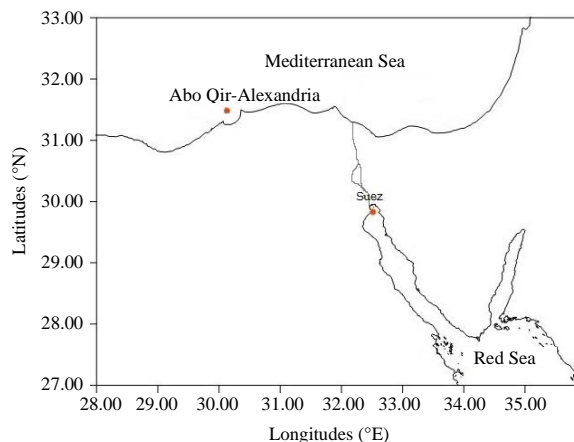


Fig. 1: Location map showing the sampling areas

Table 1: Collected samples used in the present study and the retrieved sequences of the relatives species from the GenBank database

Species	Sampling location	GenBank accession No.	Species	Sampling location	GenBank accession No.
<i>Diplodus sargus</i>	Turkey	KC500582	<i>Oblada melanura</i>	Abu Qir-Alexandria, Mediterranean Sea	LC163889*
<i>Diplodus sargus</i>	Central Mediterranean Sea from the European marine	KJ709518	<i>Oblada melanura</i>	Turkey	KC501013
<i>Diplodus sargus</i>	Italy	KJ012352	<i>Oblada melanura</i>	Turkey	KC501014
<i>Diplodus sargus</i>	Abu Qir-Alexandria, Mediterranean Sea	KP308274*	<i>Pagellus acame</i>	Italy	KJ012382
<i>Diplodus sargus</i>	Turkey	KC500578	<i>Pagellus acame</i>	Portugal	JQ775090
<i>Diplodus noct</i>	Suez, Red Sea	KP308273*	<i>Pagellus acame</i>	Abu Qir-Alexandria, Mediterranean Sea	KU757073*
<i>Diplodus argenteus</i>	Brazil	JQ365343	<i>Diplodus annularis</i>	Portugal	JQ775092
<i>Diplodus bermudensis</i>	USA	KT883620	<i>Diplodus annularis</i>	Turkey	KC500526
<i>Diplodus holbrookii</i>	USA	JQ842442	<i>Diplodus annularis</i>	Turkey	KC500531
<i>Diplodus puntazzo</i>	Turkey	KC500553	<i>Diplodus annularis</i>	USA	KT883619
<i>Diplodus cervinus</i>	Canada	HQ945933	<i>Diplodus annularis</i>	Turkey	KC500521
<i>Diplodus cervinus</i>	USA	KF929819	<i>Diplodus annularis</i>	Italy	KJ012339
<i>Diplodus cervinus</i>	Turkey	KC500545	<i>Diplodus annularis</i>	Israel	KM538326
<i>Diplodus cervinus</i>	Abu Qir-Alexandria, Mediterranean Sea	KU757074*	<i>Diplodus annularis</i>	Italy	KJ012337
<i>Diplodus vulgaris</i>	Italy	KJ012359	<i>Diplodus annularis</i>	Abu Qir-Alexandria, Mediterranean Sea	LC1522205*
<i>Diplodus vulgaris</i>	Italy	KJ012358	<i>Acanthopagrus latus</i>	South China sea	EU871695
<i>Diplodus vulgaris</i>	Turkey	JQ623934	<i>Acanthopagrus bifasciatus</i>	Suez, Red Sea	LC150892*
<i>Diplodus vulgaris</i>	Abu Qir-Alexandria, Mediterranean Sea	KP308275*	<i>Acanthopagrus bifasciatus</i>	Canada	GU805011
<i>Oblada melanura</i>	Italy	KJ012379	<i>Acanthopagrus catenula</i>	USA	KT883596
<i>Rhabdosargus sarba</i>	Australia	DQ107830	<i>Boops boops</i>	Turkey	KC500342
<i>Rhabdosargus hafara</i>	Italy	KJ012422	<i>Boops boops</i>	Italy	KJ012296
<i>Rhabdosargus hafara</i>	Suez, Red Sea	KP308279*	<i>Sarpa salpa</i>	Canada	JF494420
<i>Lithognathus mormyrus</i>	Israel	KM538409	<i>Sarpa salpa</i>	Turkey	KC501256
<i>Lithognathus mormyrus</i>	Abu Qir-Alexandria, Mediterranean Sea	KP308276*	<i>Sarpa salpa</i>	Abu Qir-Alexandria, Mediterranean Sea	KU757071*
<i>Lithognathus mormyrus</i>	Israel	KM538408	<i>Sarpa salpa</i>	Turkey	KC501255
<i>Sparus aurata</i>	Turkey	KC501554	<i>Sarpa salpa</i>	Italy	KJ012425
<i>Sparus aurata</i>	USA	KT883618	<i>Sarpa salpa</i>	Italy	KJ012429
<i>Sparus aurata</i>	Turkey	KC501557	<i>Argyrops spinifer</i>	Iran	HQ149794
<i>Sparus aurata</i>	Central Mediterranean Sea from the European marine	KJ709635	<i>Argyrops spinifer</i>	Suez, Red Sea	LC151617*
<i>Sparus aurata</i>	Abu Qir-Alexandria, Mediterranean Sea	KP308280*	<i>Argyrops spinifer</i>	Australian and South African waters	DQ884968
<i>Pagellus bogaraveo</i>	Italy	KJ012387	<i>Argyrops spinifer</i>	Australia	DQ107836
<i>Spondyliosoma emarginatum</i>	Canada	JF494575	<i>Argyrops spinifer</i>	Australia	DQ107839
<i>Spondyliosoma cantharus</i>	Turkey	KC501615	<i>Argyrops filamentosus</i>	Italy	KJ012287
<i>Spondyliosoma cantharus</i>	Abu Qir-Alexandria, Mediterranean Sea	KP308281*	<i>Pagrus pagrus</i>	Alabama Deep Sea in USA	KF461214
<i>Spondyliosoma cantharus</i>	Turkey	KC501622	<i>Pagrus pagrus</i>	USA	KF930219
<i>Spondyliosoma cantharus</i>	Turkey	KC501618	<i>Pagrus pagrus</i>	Italy	KJ012417
<i>Spondyliosoma cantharus</i>	Turkey	JQ624002	<i>Pagrus pagrus</i>	Abu Qir-Alexandria, Mediterranean Sea	LC177762*
<i>Spondyliosoma cantharus</i>	Turkey	KC501613	<i>Pagrus major</i>	Turkey	KC501089
<i>Boops boops</i>	Israel	KM538238	<i>Pagrus major</i>	South China sea	EU871693
<i>Boops boops</i>	Abu Qir-Alexandria, Mediterranean Sea	LC151618*	<i>Pagellus erythrinus</i>	Europe sea	JQ178368
<i>Pagellus erythrinus</i>	Abu Qir-Alexandria, Mediterranean Sea	KP308278*	<i>Pagrus caeruleostictus</i>	China sea	KF857268
<i>Dentex dentex</i>	Turkey	KC500455	<i>Pagrus caeruleostictus</i>	Israel	KM538478

Table 1: Continue

Species	Sampling location	GenBank accession No.	Species	Sampling location	GenBank accession No.
<i>Dentex dentex</i>	Turkey	KC500460	<i>Pagrus caeruleostictus</i>	China sea	KF857267
<i>Dentex dentex</i>	Abu Qir-Alexandria, Mediterranean Sea	KP308272*	<i>Pagrus caeruleostictus</i>	Italy	KJ012409
<i>Dentex dentex</i>	Turkey	KC500467	<i>Pagrus caeruleostictus</i>	Israel	KM538481
<i>Pagrus auriga</i>	Turkey	KC501053	<i>Pagrus caeruleostictus</i>	Abu Qir-Alexandria, Mediterranean Sea	KU757072*
<i>Pagrus auriga</i>	Turkey	KC501054	<i>Crenidens crenidens</i>	Canada	JF493279
<i>Pagrus auriga</i>	Italy	KJ012405	<i>Crenidens crenidens</i>	Suez, Red sea	LC155797*
<i>Pagrus auriga</i>	Abu Qir-Alexandria, Mediterranean Sea	KP308277*	<i>Crenidens crenidens</i>	Italy	KJ767818
<i>Dentex gibbosus</i>	Abu Qir-Alexandria, Mediterranean Sea	LC152206*	<i>Crenidens crenidens</i>	Mediterranean Sea off the coast of Lebanon	KR861522
<i>Pagrus caeruleostictus</i>	Italy	KJ012407	<i>Marone chrysops</i>	Mediterranean BH	KJ553987

\*Fish under the study

Following application of phenol and chloroform, genomic DNA was then recovered by standard precipitation with ethanol and the resulting DNA was dissolved in TE buffer (100 mM tris-HCl, 10 mM EDTA, pH 8). The concentration of the extracted DNA was spectrophotometrically assessed (Eppendorf, Hamburg, Germany) and DNA stored at 4°C till use.

The partial coding regions of the *COI* gene was then amplified by PCR using the following primers: FishF1-5'TCAACCAACCACAAAGACATTGGCAC3' and FishR1-5'TAGACTTCTGGGTGGCCAAAGAATCA3'<sup>29</sup>. The PCR was carried out in a GeneAmp PCR system 9700 (Applied Biosystems, California, USA) with a reaction volume of 25 µL, containing 2.0 µL DNA template (approximately 20 ng), 1.0 µL 10 µmol L<sup>-1</sup> forward primer, 1.0 µL 10 µmol L<sup>-1</sup> reverse primer and 21 µL 1 × RBC SensiZyme® HotstartTaq Premix (RBC Bioscience, Taipei, Taiwan). The following thermal cycling conditions were used: Initial denaturation at 94°C for 5 min, 35 cycles of amplification (94°C for 30 sec for DNA denaturation, 55°C annealing temperatures for 30 sec, extension at 72°C for 2 min) and final extension at 72°C for 7 min. The products were then purified using Hi-Yield Gel/PCR DNA fragments extraction kit (RBC Bioscience, Taipei, Taiwan). The purified DNA fragments were sequenced using the BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, California, USA) and ABI3730 sequencer (Applied Biosystems, California, USA). The sequencing PCR reaction was performed at 96°C for 2 min, followed by 25 cycles of 10 sec at 96°C, 5 sec at 50°C and 4 min at 60°C<sup>30</sup>.

The raw sequence data of *COI* was edited by a free software Chromas Lite version 2.1 (Technelysium Pty Ltd., available from the URL <http://technelysium.com.au/>). The partial coding sequences of *COI* for 22 fish species of family Sparidae were deposited in GenBank/EMBL/DBJ International databases with accession numbers are shown with asterisks in Table 1. Eighty six *COI* sequences belonging to the same family retrieved from the databases are given the similarities with the Egyptian Sparidae species (Table 1). Best fitting model were applied for the *COI* datasets of nucleotide composition and divergence values depending on Kimura 2-parameter model<sup>31</sup> with gamma distribution among invariant sites (G+I) with a tool from MEGA7 software<sup>32</sup>. Phylogenetic tree was constructed by MEGA7 using Maximum Likelihood (ML) method with 1000 replicates of bootstrapping.

## RESULTS AND DISCUSSION

The DNA barcoding considered to be a tool to identify, invent and study specimens in order to understand the diversity of species within an ecosystem and also to evaluate

the genetic variability within species<sup>33</sup>. In the present study, the universal DNA primers, FishF1 and FishR1, amplified about a 650 bp region of the *COI* gene from 22 fish species of the family Sparidae without stop codons, insertions or deletions. Multiple alignments resulted in a consensus length of 612 positions (base-pair and gaps). While performing BLAST, the sequences of *Dentex dentex*, *Dentex gibbosus*, *Diplodus annularis*, *Lithognathus mormyrus*, *Oblada melanura*, *Pagellus acarne*, *Pagrus pagrus*, *Sarpa salpa*, *SpondylIOSoma cantharus*, *Argyrops spinifer* and *Diplodus noct* showed 100% similarity with the existing data in the GenBank databases. However, *Boops boops*, *Diplodus cervinus*, *Diplodus sargus*, *Diplodus vulgaris*, *Pagellus erythrinus*, *Pagrus Auriga*, *Pagrus caeruleostictus*, *Sparus aurata*, *Crenidens crenidens* and *Rhabdosargus haffara* resulted in 99% identity with their counterparts in the GenBank databases. *Acanthopagrus bifasciatus*, however, showed 97% sequence similarity. Accession numbers for *Dentex gibbosus* (LC152206) and *Acanthopagrus bifasciatus* (LC150892) in the current study considered to be the second one for the same species in the GenBank. The GenBank accession numbers of all Sparidae species under the study and other related species retrieved are presented in Table 1. These accession numbers were retrieved depend on the similarity and haplotype sequences. The maximum likelihood analysis of the concatenated sequence of the 22 species resulted in the phylogenetic tree shown in Fig. 2. The ML tree showed monophyly of Sparidae species. This matches with a previous report of molecular analysis for some Sparidae species<sup>34</sup>. The tree was divided into two distinct clades and some sub-clades. The two major clades, that include all the species under the study except *Crenidens crenidens* in a separate branch were supported by 53 and 98 bootstraps. The clade one was divided into some subclades, first one that has genus *Diplodus* was clustered with the two different species *Oblada melanura* and *Acanthopagrus bifasciatus*, the second one for *Lithognathus mormyrus* and *Sparus aurata* was clustered in another subclade and the third one for *SpondylIOSoma cantharus*, *Sarpa salpa* and *Boops boops* were clustered together. Both *Rhabdosargus haffara* and *Pagellus acarne* were paraphyletic and placed in the first major clade. The clade two was divided into two clades, first one that was for *Argyrops spinifer* as a paraphyletic species, while the second was for three species from genus *Pagrus*, two species from genus *Dentex* and *Pagellus erythrinus*. *Pagrus pagrus* and *Pagellus erythrinus* were clustered in one sub-clade and the other two species from genus *Pagrus* and the two species of genus *Dentex* were clustered together in the second sub-clade. These results are in agreement with the results of De la Herran *et al.*<sup>34</sup> that their phylogenetic analysis suggested that the Sparidae can be

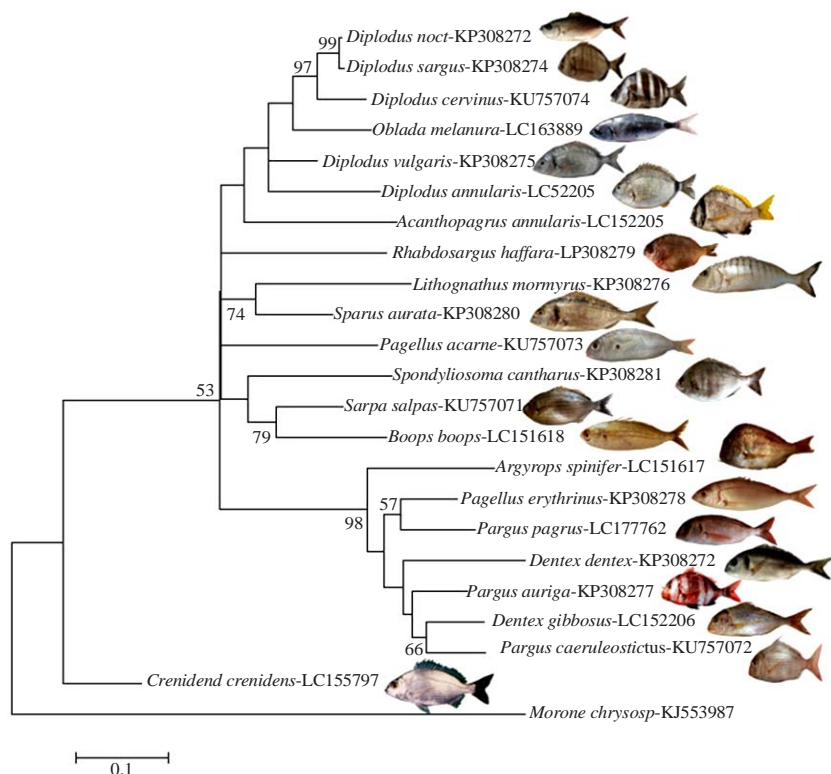


Fig. 2: Maximum Likelihood (ML) phylogenetic tree for the *COI* gene sequences for the common Egyptian Sparidae fish species

assigned into two major lineages: Lineage one, composed of the species of the genera *Sparus*, *Diplodus*, *Lithognathus*, *SpondylIOSoma*, *Boops* and *Sarpa* and lineage two composed of the genera *Pagrus* and *Dentex*, plus *Pagellus erythrinus*. The phylogenetic relationship of the family Sparidae is also supported by previous data obtained from the analysis of using satellite DNA found within the genome of some Sparidae species<sup>35,36</sup>. Their study with the different phylogenetic inferences methods provisions the existence of two monophyletic groups within the family Sparidae. On the other hand, our data contradict the results that have considered the family Sparidae as non-monophyletic on the basis of mitochondrial cytochrome b (*Cyt-b*) gene and 16S ribosomal RNA data<sup>2,25,37</sup>. The species of *Diplodus* appeared as a monophyletic group as also presented by De la Herran *et al.*<sup>34</sup>. The more closely related species within this genus were *D. noct* that was collected from the Red Sea and *D. sargus* from the Mediterranean Sea. Special consideration should be given to the species *Oblada melanura*, which appeared in our results clustered with the genus *Diplodus* in a sub-clade and this results are in the agreement with Orrell *et al.*<sup>2</sup> and Chiba *et al.*<sup>37</sup>. Our results showed that the genus *Pagrus*, *Dentex* are closely related with *Pagellus erythrinus*<sup>34</sup> while *Pagellus acarne* separated in the other group and associates with species of *Boops*, *Sarpa* and

*SpondylIOSoma*. This means that the two species from genus *Pagellus* are monophyletic and appeared in our study as highly divergent species. *SpondylIOSoma cantharus*, *Sarpa salpa* and *Boops boops* separated in a sub-clade. Another phylogenetic analysis based on mitochondrial DNA<sup>38</sup> suggested that the existence of a third lineage within the Sparidae composed of last three species. As considered traditionally, *S. salpa* and *B. boops* are closely related species (the common synonym of *S. salpa* was *Boops salpa*<sup>39</sup> and the genetic distance between these two species is 0.11. *Acanthopagrus bifasciatus* is clustered with genus *Diplodus* in one sub-clade and the genetic distance between this species and the species from genus *Diplodus* is ranged from 0.119-0.139. Therefore, our molecular results suggested that *A. bifasciatus* is closely related *Diplodus* spp. *Lithognathus mormyrus* and *Sparus aurata* are clustered in another sub-clade and the genetic distance between them is 0.125. Our results also showed genetic relationship between *Rhabdosargus haffara* and the latter two species, but no support for this finding.

For *COI* gene-based pairwise distances among all 22 Sparidae species showed the highest genetic distance (0.210) between *Diplodus cervinus* and *Argyrops spinifer* and the lowest genetic distance (0.010) between *Diplodus noct* and *Diplodus sargus* (Table 2). Taking into consideration that

Table 2: Pairwise genetic distances among family Sparidae inferred from COI gene of the Egyptian waters

Parameters	<i>Morone chrysops</i> -KJ553987	<i>Spondylisoma cantharus</i> -KP308281*	<i>Dentex glibbosus</i> -LC152206*	<i>Diplodus annularis</i> -LC152205*	<i>Diplodus cervinus</i> -KU75704*	<i>Diplodus noct</i> -KP308273*	<i>Diplodus sargus</i> -KP308274*	<i>Diplodus vulgaris</i> -KP308275*	<i>Lithognathus mormyrus</i> -KP308276*	<i>Obiada melanura</i> -LC163889*	<i>Pagellus acarne</i> -KU75703*	<i>Pagellus erythrinus</i> -KP308278*	<i>Pagrus auriga</i> -KP308277*	<i>Pagrus caeruleostictus</i> -KU75702*	<i>Pagrus pagrus</i> -LC17762*	<i>Rhabdosargus hafra</i> -KP308279*	<i>Sarpa salpa</i> -KU75701*	<i>Sparus aurata</i> -KP308280*	<i>Acanthopagrus bifasciatus</i> -LC150892*	<i>Argyrops spinifer</i> -LC151617*	<i>Boops boops</i> -LC151618*	<i>Crenidens crenidens</i> -LC155797*	<i>Dentex dentex</i> -KP308272*	
<i>Morone chrysops</i> -KJ553987	0.223																							
<i>Spondylisoma cantharus</i> -KP308281*	0.254	0.187																						
<i>Dentex glibbosus</i> -LC152206*	0.211	0.162	0.189																					
<i>Diplodus annularis</i> -LC152205*	0.229	0.157	0.203	0.122																				
<i>Diplodus cervinus</i> -KU75704*	0.230	0.145	0.189	0.108	0.058																			
<i>Diplodus noct</i> -KP308273*	0.230	0.141	0.180	0.105	0.064	0.010																		
<i>Diplodus sargus</i> -KP308274*	0.230	0.134	0.162	0.110	0.093	0.084	0.080																	
<i>Diplodus vulgaris</i> -KP308275*	0.229	0.188	0.194	0.158	0.175	0.156	0.153	0.135																
<i>Lithognathus mormyrus</i> -KP308276*	0.229	0.153	0.176	0.122	0.101	0.092	0.094	0.095	0.170															
<i>Obiada melanura</i> -LC163889*	0.225	0.147	0.169	0.144	0.169	0.149	0.153	0.138	0.164	0.141														
<i>Pagellus acarne</i> -KU75703*	0.239	0.183	0.106	0.171	0.177	0.153	0.157	0.153	0.176	0.165	0.151													
<i>Pagellus erythrinus</i> -KP308278*	0.233	0.176	0.097	0.172	0.180	0.163	0.153	0.136	0.178	0.158	0.159	0.112												
<i>Pagrus auriga</i> -KP308277*	0.262	0.197	0.089	0.182	0.181	0.184	0.177	0.151	0.192	0.184	0.185	0.112	0.095											
<i>Pagrus caeruleostictus</i> -KU75702*	0.241	0.194	0.120	0.179	0.196	0.184	0.178	0.153	0.178	0.160	0.176	0.096	0.124	0.107										
<i>Pagrus pagrus</i> -LC17762*	0.222	0.169	0.190	0.146	0.135	0.143	0.141	0.144	0.173	0.151	0.161	0.165	0.165	0.183	0.188									
<i>Rhabdosargus hafra</i> -KP308279*	0.227	0.132	0.176	0.137	0.148	0.126	0.126	0.144	0.154	0.152	0.144	0.142	0.150	0.187	0.177	0.147								
<i>Sarpa salpa</i> -KU75701*	0.242	0.155	0.166	0.134	0.158	0.138	0.129	0.116	0.125	0.137	0.149	0.155	0.157	0.161	0.140	0.126	0.144							
<i>Sparus aurata</i> -KP308280*	0.254	0.167	0.202	0.139	0.119	0.119	0.119	0.130	0.180	0.149	0.181	0.179	0.213	0.200	0.200	0.148	0.161	0.157						
<i>Acanthopagrus bifasciatus</i> -LC150892*	0.232	0.183	0.143	0.178	0.210	0.199	0.201	0.177	0.175	0.172	0.183	0.132	0.119	0.141	0.129	0.186	0.178	0.172	0.202					
<i>Argyrops spinifer</i> -LC151617*	0.216	0.146	0.195	0.170	0.157	0.141	0.135	0.149	0.179	0.147	0.156	0.152	0.169	0.178	0.175	0.146	0.110	0.151	0.161	0.177				
<i>Boops boops</i> -LC151618*	0.217	0.170	0.200	0.151	0.163	0.163	0.163	0.164	0.199	0.187	0.187	0.178	0.173	0.203	0.188	0.158	0.158	0.179	0.176	0.182	0.138			
<i>Crenidens crenidens</i> -LC155797*	0.244	0.206	0.105	0.176	0.194	0.199	0.199	0.172	0.207	0.172	0.176	0.130	0.117	0.138	0.139	0.168	0.177	0.189	0.201	0.182	0.199	0.183		
<i>Dentex dentex</i> -KP308272*																								



Orrell *et al.*<sup>2</sup> and Hanel *et al.*<sup>38</sup> found that subfamilies of Sparidae are defined mostly by trophic levels. The interpretation of the agreement between our results and De la Harran *et al.*<sup>34</sup> that might be due to they investigated the family under study in the same water body, Mediterranean Sea with quite similar surrounding ecological factors including the available food items.

### CONCLUSION

So far, this is the first study to report the molecular identification and DNA barcoding of the available and commercial fish of family Sparidae in Egyptian Mediterranean and Red Sea using *COI* gene as a genetic marker. This will provide a complete vision of the phylogenetic, evolutionary relationships of the Egyptian Sparidae and will enrich all the genetic database with the sequences from the Egyptian fauna for their biodiversity and conservation.

### ACKNOWLEDGMENT

This study was funded by Science Technology Development Fund (STDF), Ministry of Scientific Research, Egypt.

### REFERENCES

1. Akazaki, M., 1962. Studies on the Spariform fishes: Anatomy, phylogeny, ecology and taxonomy. Misaki Marine Biology Institute, Kyoto University, Special Report 1, Kosugi Ltd., Osaka, Japan, pp: 1-368.
2. Orrell, T.M., K.E. Carpenter, J.A. Musick and J.E. Graves, 2002. Phylogenetic and biogeographic analysis of the Sparidae (Perciformes: Percoidei) from cytochrome *b* sequences. *Copeia*, 3: 618-631.
3. Nelson, J.S., 2006. *Fishes of the World*. 4th Edn., John Wiley and Sons, New Jersey, USA., ISBN: 0471250317, Pages: 601.
4. Carpenter, K.E., 2001. Family Sparidae. In: *The Living Marine Resources of the Western Central Pacific*, Volume 5: Bony Fishes Part 3 (Menidae to Pomacentridae), Carpenter, K.E. and V.H. Niem (Eds.). FAO, Rome, Italy, pp: 2990-3003.
5. Froese, R. and D. Pauly, 2005. FishBase. World Wide Web Electronic Publication. <http://www.fishbase.org/search.php>.
6. Skaramuca, B., V. Kozul, I. Katavic, N. Glavic, P. Tutman, L. Grubisic and B. Glamuzina, 2000. Recent advances on the diversification of marine finfish species in Croatia. *Cahiers Options Mediterranennes*, 47: 359-363.
7. Grigorakis, K., M.N. Alexis, K.D.A. Taylor and M. Hole, 2002. Comparison of wild and cultured gilthead sea bream (*Sparus aurata*); composition, appearance and seasonal variations. *Int. J. Food Sci. Technol.*, 37: 477-484.
8. Colombo, L., A. Barbaro, A. Francescon, A. Libertini and P. Benedetti *et al.*, 1996. Potential gains through genetic improvements: Chromosome set manipulation and hybridization. *Proceedings of the International Workshop on Sea Bass and Sea Bream Culture: Problems and Prospects*, October 16-18, 1996, Verona, Italy, pp: 343-362.
9. FAO, 2012. *State of the World Fisheries and Aquaculture 2012*. Food and Agriculture Organization (FAO), Rome, Italy, ISBN-13: 9789251072257, Pages: 209.
10. Ibrahim, M.A. and I.A. Soliman, 1996. Check list of the bony fish species in the Mediterranean waters of Egypt. *Bull. Natl. Inst. Oceanogr. Fish.*, 22: 43-57.
11. GAFRD., 2008. *Annual Statistics Year Book*. General Authority for Fish Resources Development Cairo, Egypt.
12. Golani, D. and S.V. Bogorodsky, 2010. The fishes of the Red Sea-reappraisal and updated checklist. *Zootaxa*, 2463: 1-135.
13. Dayrat, B., 2005. Towards integrative taxonomy. *Biol. J. Linnean Soc.*, 85: 407-415.
14. Rasmussen, R.S., M.T. Morrissey and P.D. Hebert, 2009. DNA barcoding of commercially important salmon and trout species (*Oncorhynchus* and *Salmo*) from North America. *J. Agric. Food Chem.*, 57: 8379-8385.
15. Zhang, J., L. Huang and H. Huo, 2004. Larval identification of *Lutjanus* Bloch in Nansha coral reefs by AFLP molecular method. *J. Exp. Mar. Biol. Ecol.*, 298: 3-20.
16. Comi, G., L. Iacumin, K. Rantsiou, C. Cantoni and L. Cocolin, 2005. Molecular methods for the differentiation of species used in production of cod-fish can detect commercial frauds. *Food Control*, 16: 37-42.
17. Teletchea, F., 2009. Molecular identification methods of fish species: Reassessment and possible applications. *Rev. Fish Biol. Fish.*, 19: 265-293.
18. Hebert, P.D., A. Cywinska, S.L. Ball and J.R. deWaard, 2003. Biological identifications through DNA barcodes. *Proc. Biol. Sci.*, 270: 313-321.
19. Cox, A.J. and P.D.N. Hebert, 2001. Colonization, extinction and phylogeographic patterning in a freshwater crustacean. *Mol. Ecol.*, 10: 371-386.
20. Swartz, E.R., M. Mwale and R. Hanner, 2008. A role for barcoding in the study of African fish diversity and conservation. *South Afr. J. Sci.*, 104: 293-298.
21. Ward, R.D., R.H. Hanner and P.D.N. Hebert, 2009. The campaign to DNA barcode all fishes, FISH-BOL. *J. Fish Biol.*, 74: 329-356.
22. Kochzius, M., C. Seidel, A. Antoniou, S.K. Botla and D. Campo *et al.*, 2010. Identifying fishes through DNA barcodes and microarrays. *PLoS ONE*, Vol. 5. 10.1371/journal.pone.0012620
23. Yancy, H.F., T.S. Zemlak, J.A. Mason, J.D. Washington and B.J. Tenge *et al.*, 2008. Potential use of DNA barcodes in regulatory science: Applications of the Regulatory Fish Encyclopedia. *J. Food Protect.*, 71: 210-217.

24. Handy, S.M., J.R. Deeds, N.V. Ivanova, P.D. Hebert and R.H. Hanner *et al.*, 2011. A single-laboratory validated method for the generation of DNA barcodes for the identification of fish for regulatory compliance. *J. AOAC Int.*, 94: 201-210.
25. Orrell, T.M. and K.E. Carpenter, 2004. A phylogeny of the fish family Sparidae (porgies) inferred from mitochondrial sequence data. *Mol. Phylogenet. Evolut.*, 32: 425-434.
26. Armani, A., L. Guardone, L. Castigliero, P. D'Amico and A. Messina *et al.*, 2015. DNA and Mini-DNA barcoding for the identification of Porgies species (family Sparidae) of commercial interest on the international market. *Food Control*, 50: 589-596.
27. El-Deeb, S.I., A.S. El-Seedy, A.A.M.A. Gabal, H.E.D. El-Wakeel and N.A.E.A. Ibrahim, 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 Sci. J.*, 11: 779-789.
28. Sambrook, J., E.F. Fritish and T. Maniatis, 1989. *Molecular Cloning: A Laboratory Manual*. 2nd Edn., Cold Spring Harbor Laboratory Press, New York, USA., ISBN-13: 978-0879693091, Pages: 397.
29. Ward, R.D., T.S. Zemlak, B.H. Innes, P.R. Last and P.D.N. Hebert, 2005. DNA barcoding Australia's fish species. *Philos Trans. R. Soc. London B: Biol. Sci.*, 360: 1847-1857.
30. Abbas, E.M., K.M. Abdelsalam, K. Mohammed-Geba, H.O. Ahmed and M. Kato, 2016. Genetic and morphological identification of some crabs from the Gulf of Suez, Northern Red sea, Egypt. *Egypt. J. Aquat. Res.*, 42: 319-329.
31. Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.*, 16: 111-120.
32. Kumar, S., G. Stecher and K. Tamura, 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evolut.*, 33: 1870-1874.
33. Rajkumar, G., P.S. Bhavan, R. Udayasuriyan and C. Vadivalagan, 2015. Molecular identification of shrimp species, *Penaeus semisulcatus*, *Metapenaeus dobsoni*, *Metapenaeus brevicornis*, *Fenneropenaeus indicus*, *Parapenaeopsis stylifera* and *Solenocera crassicornis* inhabiting in the Coromandel coast (Tamil Nadu, India) using MT-COI gene. *Int. J. Fish. Aquat. Stud.*, 2: 96-106.
34. De la Herran, R., C.R. Rejon, M.R. Rejon and M.A. Garrido-Ramos, 2001. The molecular phylogeny of the Sparidae (Pisces, Perciformes) based on two satellite DNA families. *Heredity*, 87: 691-697.
35. Garrido-Ramos, M.A., M. Jamilena, R. Lozano, S. Cardenas, C.R. Rejon and M.R. Rejon, 1995. Phylogenetic relationships of the Sparidae family (Pisces, Perciformes) inferred from satellite-DNA. *Hereditas*, 122: 1-6.
36. Garrido-Ramos, M.A., R. de la Herran, M. Jamilena, R. Lozano, C.R. Rejon and M.R. Rejon, 1999. Evolution of centromeric satellite DNA and its use in phylogenetic studies of the Sparidae family (Pisces, Perciformes). *Mol. Phylogenet. Evolut.*, 12: 200-204.
37. Chiba, S.N., Y. Iwatsuki, T. Yoshino and N. Hanzawa, 2009. Comprehensive phylogeny of the family Sparidae (Perciformes: Teleostei) inferred from mitochondrial gene analyses. *Genes Genet. Syst.*, 84: 153-170.
38. Hanel, R. and C. Sturmbauer, 2000. Multiple recurrent evolution of trophic types in Northeastern Atlantic and Mediterranean seabreams (Sparidae, Percoidei). *J. Mol. Evol.*, 50: 276-283.
39. Hureau, J.C. and M.L. Bauchot, 1986. Sparidae. In: *Fishes of the North-Eastern Atlantic and the Mediterranean*, Whitehead, P.J.P., M.L. Bauchot, J.C. Hureau, J. Nielsen and E. Tortonose (Eds.). Vol. II, UNESCO, Paris. pp: 883-907.