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DNA Barcoding for Identification of Some Fish Species (Sparidae) in Mediterranean Sea Area

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Abstract

Background: DNA barcoding depend on a piece of the cytochrome C oxidase subunit I (COI) gene in the mitochondrial genome is broadly useful in species ID and biodiversity studies. The aim of this study is to create a complete barcoding reference database of some fishes in the Mediterranean Sea fall under the family Sparidae. . **Materials and methods**: Mitochondrial COI barcode sequences were demonstrated from 8 species of family Sparidae in the order Perciformes, the mean length of Mitochondrial COI sequences was 650 base pairs. **Results**: The results of the phylogenetic tree presented that monophyly of Sparidae species. The studied species displayed clades of conspecific sequences and showed a match between the present study and the GenBank (NCBI) database. All groups clustered with high bootstrap value, that showed next to each branch and the tree was rooted based on the out group of *Rhincodon typus*. **Conclusion**: We achieve that COI sequencing can be used to recognize different fish species, and also it is used to obtain high competence of species reorganization by DNA barcoding. We underline the power of DNA barcode and its tools to identify different species from Mediterranean Sea. Results give Species ID for each species under study by using DNA barcoding.

Keywords: DNA, Barcoding, Mediterranean, Fish, Sparidae, PCR, COI.

1. Introduction

The Roman Sea or the Shami Sea is another name of the Mediterranean or the Mediterranean Sea [1]. The joining between Mediterranean Sea and Atlantic Ocean concluded the stretch of Gibraltar underpins considerable taxonomic relationship,

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with further than 50% of the Mediterranean taxa creature of Atlantic origin together with continuing gene flow in several species. Concomitantly, examine intra-species population difference, since similar comparisons have exposed considerable lineage divergence or suggested the occurrence of cryptic species [2]. DNA barcoding is a method for taxonomy different species that usages a chosen area of a specific gene or genes to classify to the species [3]. A DNA barcode can be originated from the, chloroplast mitochondrion and nucleus. The supreme usually of nuclear DNA barcodes which used are 28S rDNA, transcribed spacer (ITS) and 18S rDNA, and internal [4]. The technique which used here uses current PCR and sequencing methodologies to detect mitochondrial DNA polymorphisms in complex matrices. mitochondrial DNA barcodes which commonly used in the animal kingdom are the genes for COI, cytochrome b gene (cytb), and control region (or displacement-loop, d-loop) [5,6]. Here, we present a wide account of DNA barcodes for Mediterranean Sea fishes undergo family Sparidae based on the mitochondrial (COI). The recognition of important divergence between populations is provides experiential scientific care for protection measures to challenge biodiversity loss. Worldwide, there are more than 200,000 marine species [7]. The first studies for barcoding marine species in Turkish waters was by [2,8]. whose DNA barcoding studied was by usage three mitochondrial genes which are cytochrome b (cyt b), 16S rRNA (16S), and COI gene for the identification of 50 marine fish species in European waters, including Turkish seas they found that cyt b and COI are good tools for instantly recognizable identification of marine fishes [9]. The Perciformes (perch-likes fishes) is the largest order of fishes [10]. It is also the most patchily sized order of vertebrates, It is also the most variably sized order of vertebrates Among the famous members of this group are mackerel and tuna in the family Scombridae, sea breams and porgies in the family Sparidae, pompanos, jack mackerels, runners in the family Carangidae, Rabbitfishes in the family Siganidae and drums or croakers in the family Sciecnidae [11,12].

2. Materials and methods

2.a. Ethics statement

All fish species were trapped in the offshore zone (not national parks, other sheltered zones, or private zones, etc.), so no specific permissions were necessary for these places/activities. This study did not need Ethical because no endangered or protected fish species were involved in study. (table 1)

Table 1. Market common name in Arabic according to vendors, English common name, scientific name, Latin family name and photo of utilized fishes.

No.	Market name	Scientific name	English Common name	Family	Photo of utilized fishes
1	دنيس	*Sparus aurata*	Gill-head bream	Sparidae	
2	شرغوش	*Dipodilus Sargus*	Sea bream	Sparidae	
3	مرجان	*Pagarus pagarus*	Red porgy	Sparidae	
4	موزة	*Boops boops*	Bug	Sparidae	
5	مرمار	*Lithognathus mormyrus*	Sand steenbras	Sparidae	
6	كحله	*Oblada melanura*	Saddled bream	Sparidae	
7	تيس	*Argyrops spinifer*	King soldier bream	Sparidae	
8	شعوم	*Spondyliosoma cantharus*	The black seabream	Sparidae	

2.b. Sample collection

Eight species which represent family Sparidae were collected from Mediterranean Sea (Alexandria-Egypt) (Fig.1) in winter from 3-5 fish samples for each species, named according to the fish market vendors as pronounced in Arabic and searching for their English Common name, scientific name and Latin family name. We stored dissected muscle tissue sample from each specimen after morphological examination and then stored in 95% ethanol at −20ºC.

Figure 1 Map showing the location of Alexandria in relation to the Mediterranean Sea and the location and importance of the Mediterranean for the world.

From small piece from tissue which preserved in ethanol_[13] by optical density values at 254 nm each sample of DNA was examined. The gel was illuminated with short wavelength UV light (254 nm), and visualized under UV-light and documented using a gel documentation system (SYNGENE) were used in PCR. An approximately 650 bp fragment of COI mitochondrial gene was the polymerase chain reaction -amplified using the mitochondrial COI gene. The PCR was made in a final volume of 25 µl volume using EasyTaq® DNA Polymerase (Trans, China) as follows: denaturation for 1 min at 94^{o} c, then of strand denaturation at 94^{o}c (35 cycles), followed by annealing at 50^{o}c and extension at 72^{o}c, a final at 72^{o}c for 10 min, Amplicons were tested on 1.5% w/v agrose gel electrophoresis supplement by 5 µl per each sample were loaded along with 3 µl DNA ladder "OneMARK100, GeneDireX". The gel was visualized under UV- light and documented using (InGenius^{3}, SYNGENE, UK).

All samples were subject to PCR amplification using sing the mitochondrial COI gene.

Primer	**Name**	**Sequence 5' 3'** →	Tm	**Reference**
COI	FF2d-1	TTCTCCACCAACCACAARGAYATYGG	64.7	**Ivanova *et al.* (2007)**
	FR1d-1	CACCTCAGGGTGTCCGAARAAYCARAA	66.8	

The amplification reaction is an important necessary step before automated DNA-sequencing in which we prepare the cleanup step for all PCR sample reaction when successful, to remove dimers, RNA, PCR residues then using EasyPure PCR Purification Kit (TransGen Biotech, Beijing, China). The results of purification were tested by using agarose gel electrophoresis 1.5%, then visualized under UV-light and documented using (InGenius^3, SYNGENE, UK).

After successful purification, the purified DNA was prepared to be sent to the sequence service (Macrogene, Netherlands) to proceed with the sequencing process. Samples were prepared by adding 20 µl from purified DNA together with 2 µl of primers pair (forward and reverse, separately. The Geneious software R10^9 was used to analyze the sequences. The blast tool was used for similarity search in order to confirm the studied species. Sequences of COI gene was aligned using MAFFT aligner [10]. Phylogenetic trees were constructed using the maximum likelihood method.

3. Results

3.a. DNA extraction:

The collected fish samples were subjected to DNA extraction and isolation. All samples were successfully extracted with high concentration using 1% agarose gel electrophoresis (Fig.2).

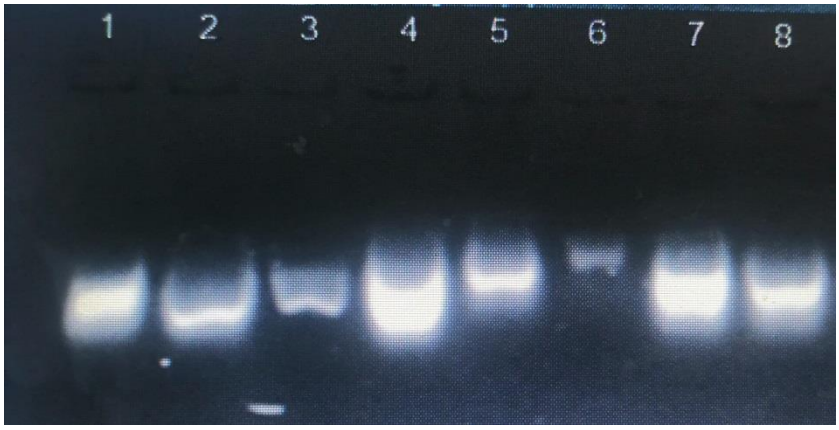

Figure 2 Agarose gel electrophoresis of total DNA isolated from 8 collected samples from Mediterranean Sea.

3.b. PCR amplification:

The region of COI gene was amplified successfully, after trimming, COI sequence length varied according to the species type. The PCR products were confirmed using 1.5% agarose gel electrophoresis (Fig.3). Total samples were purified and sequenced for both directions.

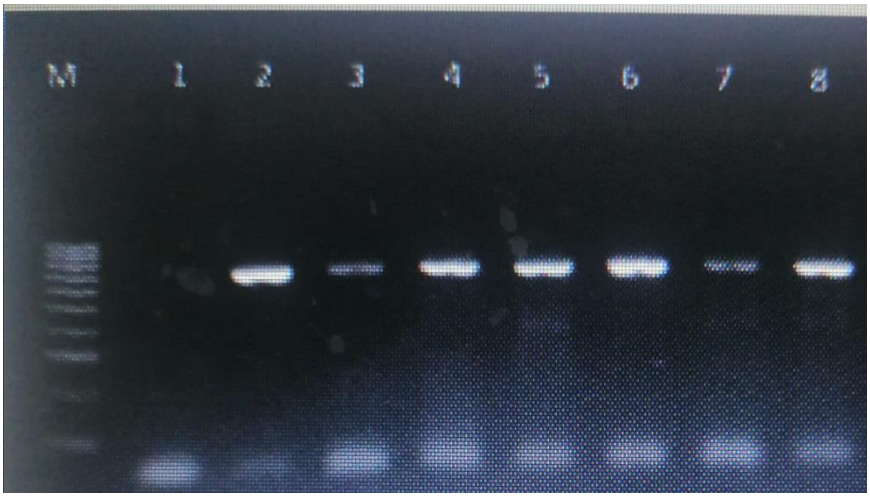

Figure 3 Gel electrophoresis of successfully amplified COI gene of 8 samples and all bands are presenting in ~650 bp length.

3.c. Blast result

Each consensus sequence was subject to the BLAST search tool in the NCBI database, for comparison with previously submitted sequences in the GenBank. Based on the BLAST results, the best matched sequences were retrieved from the database and aligned with all other results for all samples using MAFFT aligner [8]. The blast

Table 2. Blast results for COI gene; for 8 samples**.** Including sample code, percentage of pairwise %, GC content, organism name, and the accession number.

Sample code	Pairwise	GC	Organism	Accession
S1	100%	45.1%	*Sparus aurata*	MF438138
	100%	44.1%	*Sparus aurata*	KC217652
	99.9%	44.9%	*Sparus aurata*	OD909577
	99.9%	44.9%	*Sparus aurata*	LK022698
S2	100%	46.2%	*Diplodus sargus*	LC203132
	100%	46.2%	*Diplodus sargus*	LC203114
	100%	46.2%	*Diplodus sargus*	LC203112
	100%	46.2%	*Diplodus sargus*	LC203107
S3	100%	45.4%	*Pagrus pagrus*	MN756424
	100%	45.4%	*Pagrus pagrus*	KF461214
	99.8%	45.6%	*Actinopterygii*	MH566283
	99.8%	45.6%	*Pagrus pagrus*	MG837962
S4	100%	46.9%	*Boops boops*	MZ127226
	100%	46.9%	*Boops boops*	MZ127221
	100%	46.9%	*Boops boops*	MT666082
	100%	46.9%	*Boops boops*	KM538238

result including species number, pairwise identity %, GC content % and the accession number listed in table (2).

	100%	**47.7%**	***Lithognathus mormyrus***	**KY176516**
S5	100%	47.7%	*Lithognathus mormyrus*	KM538412
	100%	47.7%	*Lithognathus mormyrus*	KM538409
	100%	47.7%	*Lithognathus mormyrus*	KJ012376
S6	100%	45.6%	*Oblada melanura*	HM590730
	99.9%	45.8%	*Oblada melanura*	MT410923
	99.9%	45.5%	*Boops boops*	HM590725
S7	100%	45.7%	*Argyrops spinifer*	MW009820
	100%	45.7%	*Argyrops spinifer*	MW009819
	100%	45.7%	*Argyrops spinifer*	LC543879
	100%	45.7%	*Argyrops spinifer*	LC543878
S8	100%	47.1%	*Spondyliosoma cantharus*	KP861919
	100%	47.1%	*Spondyliosoma cantharus*	KJ012436
	100%	47.1%	*Spondyliosoma cantharus*	KJ012435
	99.8%	47.3	*Spondyliosoma cantharus*	KJ012438

3.d. Compartive moleculer phylogenetic analysis

All samples were aligned and utilized to construct a maximum-likelihood phylogenetic tree based on the obtained sequences along with the top four results in order to infer the fish species delimitation. The studied species displayed clades of conspecific sequences and showed a match between the present study and the GenBank (NCBI) database. All groups clustered with high bootstrap value, that showed next to each branch and the tree was rooted based on the outgroup of *Rhincodon typus* (Fig. 4).

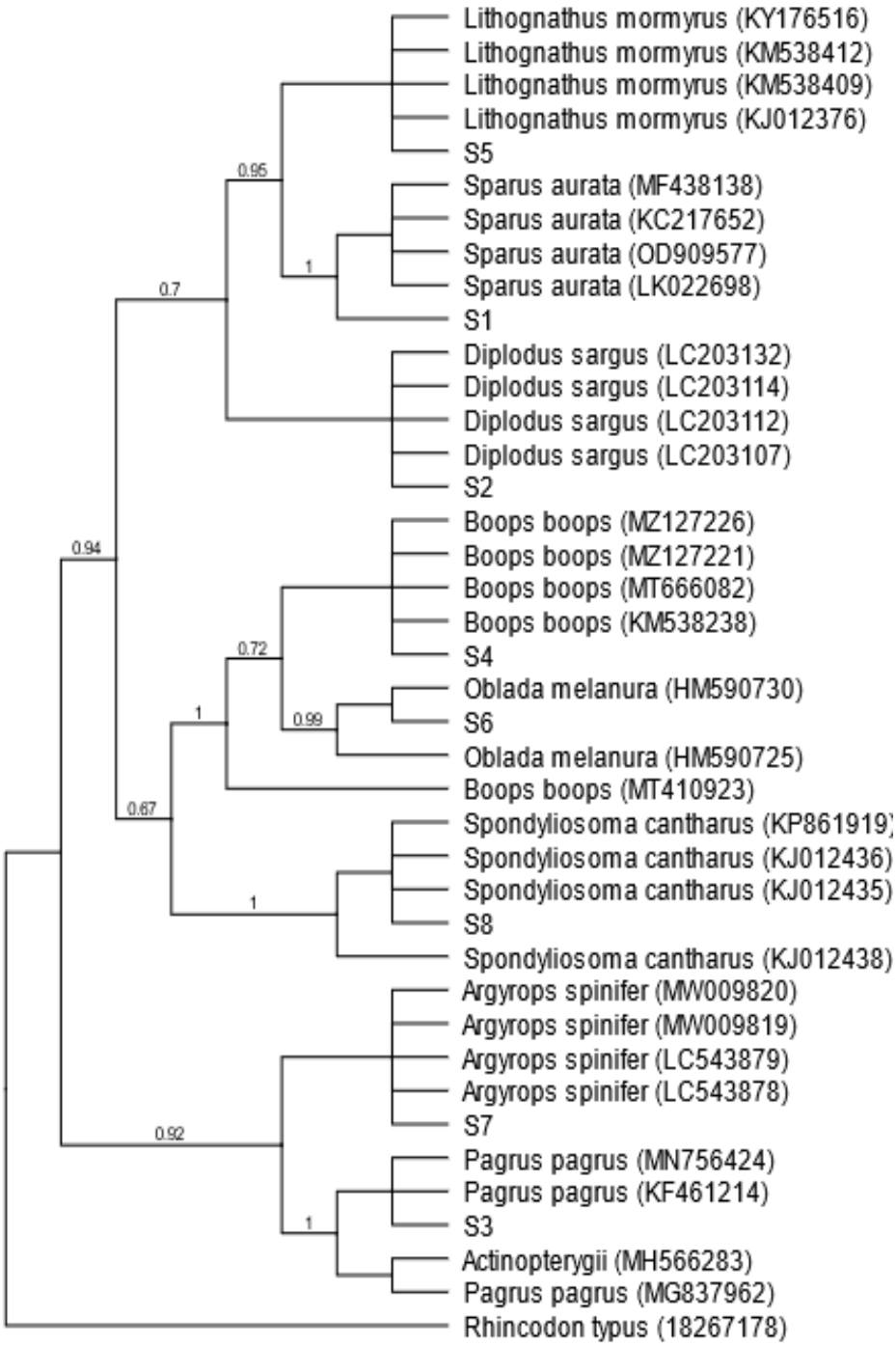

Figure 4. The phylogenetic tree between different species of the Mediterranean Sea, showing a match between the present study and sequence in the GenBank database, using the maximum-likelihood method based on the mitochondrial COI gene.

4. Discussion

barcoding is a method which has very power to recognize and identification and differentiation between different and dissimilar species and also used to detect genetic differentiation within the same species that take place through differentiation and compare between DNA sequences of different groups by using DNA barcoding which attempt to revise the morphological classical classification according to findings of mitochondrial genomic results [1,7,8,14,15].

.Family Sparidae include different species such as Diplodus sargus, Pagrus pagrus, Boops boops, Sparus aurata, Lithognathus mormyrus and Oblada melanura [16].

The Cytochrome c oxidase subunit I gene is a suitable molecular marker which very important at DNA barcoding to fishes of Mediterranean Sea. The best method to get the whole image to the patchwork that constitutes worldwide biodiversity is a multidisciplinary attitude, joining different kinds of data and changed levels of in formativeness [17]. This is equal extra significant in marine environments, where complete sampling areas may generate incomplete implication and hidden biodiversity [18].

The successful assignments typically displayed query sequences embedded within the reference haplotypes cluster, showing little or no divergence between North-East Atlantic and Mediterranean populations (66 species). These results support our initial premise on the feasibility of using an annotated reference library from the temperate North-East Atlantic for species assignments across a neighboring oceanic basin (i.e. the Mediterranean Sea), and strengthens the robustness of DNA barcode-based approaches for fish species identifications regardless of geographic distance, as observed elsewhere, However, because the overlap of the species analyzed in the two datasets (reference versus query) was only partial,several sequences could not be assigned using the reference library from North -East Atlantic. Nevertheless, through BOLD-IDS and GenBank searches it remained possible to assign species to 83% and 72%, respectively, of these sequences. For both BOLD-IDS and GenBank, however, the reliability of the assignments could not be confirmed or verified due to the presence of unpublished sequences in these databases.

According to the results here, it is suggested to make more deep studies concentrating on resolving ambiguities of morphological taxonomic and compare the result with Data base on Genebank to insure previous Identification and classification studies. Because of constant presence of environmental changes which affects living organisms to adapt to change, the definition and classification of different organisms is a wide world that needs renewal and research always to confirm the many different types of many organisms.

The results are comparing with taxonomic data which also known as GeneBank data which have acontrolled group in which based on more than one marker

5. Conclusion

So far, this study to attempt ID cards for each species under study based on DNA barcoding sequence (molecular identification) by using cytochrome c oxidase subunit I gene as genetic marker that lead to genetic data base of Egyptian fish species from Mediterranean Sea fauna.

Molecular genetic markers had successfully generated reproducible polymorphic products to study the genetic variability [19]. DNA-based molecular markers differ in their resolving power to detect genetic variations, identification, genetic structure, and type of data they generate for each species. Each technique has its own advantages and limitations [20]. The DNA-based markers seemed to be the best-suited molecular assay for fingerprinting and assessing genetic structure [21]. DNA barcoding approaches expand in African fisheries to solve the problems of fish species identifications, evolutions, population differentiations and biogeographic distributions [22]. Socio-economic and biological findings provide important contributions for evaluating the status of commercially important fish, and for sustainably conserving both the fish stocks and the ecosystem [23]. The Suez Gulf is one of the most important water bodies north of the Red Sea that contribute significantly in fish production in Egypt[24]. High genetic diversity is distinguished by high genetic diversity based on variation in morphological and anatomical features [25].

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الملخص العربي

ترميز الحمض النووي الشريطي لتحديد بعض انواع الاسماك من عائلة الاسبوريه في منطقة البحر الابيض المتوسط

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ج- قسم الانتاج السمكي – كلية الزراعة – جامعة الازهر

الخلفية والأهداف: تعد الأسماك المصدر الرئيسي للبروتين الحيواني للبشر وتلعب دوراً حيوياً في النظم الإيكولوجية المائية وشبكات الأغذية. يمكن أن يكون تحديد الأسماك أمرًا صعبًا، خاصة في المناطق الاستوائية (بسبب التنوع الكبير)، وهذا ينطبق بشكل خاص على أشكال اليرقات أو البقايا المجزأة. يعتبر تشفير الحمض النووي DNA، الذي يستخدم وحدة السيتوكروم سي أوكسيديز **c** الفرعية في الميتوكوندريا كجينة مستهدفة ، طريقة فعالة لتحديد مستوى الأنواع القياسية لتقييم التنوع البيولوجي وحفظه ، ريثما يتم إنشاء مكتبات تسلسل مرجعي.

المواد والطرق: في هذه الدراسة، تم جمع الأسماك من البحر المتوسط، تم تحديدها شكليا، وتصويرها رقميا. تم استخراج الحمض النووي، تضخيم PCR، وتم تسلسل منطقة الباركود القياسية بشكل ثنائي ل 8 أنواع من عائلة الفرخيات (Sparidae) . تم تسجيل جميع بيانات مصدر العينة ومعلومات التسلسل المرتبطة بها في الرمز الشريطي لنظم بيانات الحياة (BOLD؛www.barcodinglife.org). تم استخدام الأدوات التحليلية على BOLD لتقييم أداء التشفير الشريطي لتحديد الأنواع. وتم عمل بطاقه تعريفيه لكل نوع.