Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 24(3): 333 – 349 (2020)
www.ejabf.journals.ekb.eg

DNA barcoding and comparative genetic diversification among species of family Sparidae in the coastal waters of Egypt

Nermin Ibrahim[1], Eman M. Abbas[1]*, Ayman El-Seedy[2], Taha Soliman[1], Fawzia S. Ali[1]

[1]Laboratory of Genetics, Aquaculture Division, National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt 13621.

[2] Laboratory of cellular and Molecular Genetics, Department of Genetics, Faculty of Agriculture, Alexandria University, Alexandria, Egypt

***Correspondence author:** emanabbas03@yahoo.com

ARTICLE INFO

Article History:
Received: April 13, 2020
Accepted: April 29, 2020
Online: May 2020

Keywords:
Sparidae,
Genetic diversity,
RAPD,
ISSR,
Mitochondrial Cyt-*b*,
Red Sea,
Mediterranean Sea.

ABSTRACT

Species of family Sparidae, commonly called, sea breams, are widely distributed from temperate to tropical waters and are of great economic interest. However, in Egypt, limited data is available on genetic variation and evolutionary relationships of family Sparidae. Therefore, the study of the genetic diversity among sparid species is crucial for proper management and convenient strategies. The aim of this study was to evaluate the genetic diversification among 22 species belonging to family Sparidae from the Mediterranean Sea and the Red Sea in Egypt using three different molecular markers. DNA barcoding, using *Cyt-b* mitochondrial gene, was applied as an initial step for species identification and diversification. In addition, Randomly Amplified Polymorphic DNA (RAPD), and Inter Simple Sequence Repeat (ISSR) markers were employed in estimating the genetic diversity among the studied species. Based on each molecular marker, a phylogenetic tree was constructed for the studied sparid species according to the calculated genetic distance/ similarity. DNA barcoding using mitochondrial *Cyt-b* gene provided efficient DNA barcodes for most of the studied species. RAPD assays (using 20 RAPD primers) produced a total of 308 bands, of which 91.8% were polymorphic. Eight ISSR primers amplified a total of 197 bands, 97.9% of which were polymorphic. RAPD and ISSR profiles differed substantially among the 22 sparid species, enabling easy discrimination. However, ISSR had a higher power of discrimination compared to RAPD markers. The *constructed* phylogenetic trees based on the employed molecular markers provided the update for the barcoded Sparidae species evolution. The employed molecular markers in this study were efficient in species genetic diversification. *Cyt-b* was successfully utilized in species barcoding, whereas, the combination of RAPD and ISSR-PCR profiles provided substantially differentiated profiles for each species, which makes them suitable for measuring genetic divergence among species.

INTRODUCTION

Family Sparidae, commonly called, Sea breams, includes demersal fishes that spread all over the world from temperate to tropical waters; they mainly inhabit South African waters. Family Sparidae are mainly marine and frequently inshore species, but only few species occasionally enter estuaries (**Hanel and Tsigenopoulos, 2011)**. Species of family Sparidae have high economic importance, principally around the Mediterranean area, where many species are targeted by capture fisheries and few species are commercially cultured (**Basurco *et al*., 2011)**. Traditionally, this family had been categorized into several subfamilies by its dentition (**Akazaki, 1962)**; it consists of 115 species belonging to 33 genera (**Orrell *et al*., 2002)**. Thirty-three species of family Sparidae have been recorded along the Egyptian coasts (**FAO, 2013)**. Most of these species are economically important and are used as table food, owing to their good taste and rich flesh. In the Mediterranean Sea, 21 species within the family Sparidae have been recorded in the Egyptian waters **(Ibrahim and Soliman, 1996)**. Of these, twelve are common species of the landed catch of Alexandria on the northern coast of Egypt. According to **GAFRD (2018)**, family Sparidae represents about 15% of these landed caught fishes in Egypt. However, in the Red Sea, another 14 species are existing **(Golani and Bogorodsky, 2010)**. These 14 species are of less economic importance than those recorded in the Mediterranean Sea.

Species of family Sparidae have highly similar morphological features. Therefore, the morphological identification can only be achieved by skilled taxonomists. The specialized dentition is the most distinguishing characteristic used for the identification of family Sparidae **(Smith and Smith, 1986)**, and it is the basis for the taxonomy of the six subfamilies of this family. However, even when whole samples are available, the high similarity between different species of family Sparidae is very confusing, which makes it nearly impossible to discriminate the prepared or processed products during examinations.

The use of DNA markers is a powerful tool to solve the problem of misleading morphological identification **(Armani *et al*., 2012; Abou-Gabal *et al*., 2018; Ali and Mamoon, 2019)**. DNA markers, including mitochondrial DNA (mtDNA) markers, are extensively used to determine systematic relationships among species (**FAO, 2013)**. Cytochrome *b* (Cyt-*b*) is one of the effective genes for phylogenetic studies and is one the best-known mitochondrial gene with respect to the structure and function of its protein product **(Esposti *et al*., 1993; Ali *et al*., 2019)**. Additionally, various molecular markers, such as random amplified polymorphic DNA (RAPD) **(Qiubai *et al*., 2013; Hassanien and Al-Rashada, 2019)**, inter simple sequence repeat (ISSR) **(Casu *et al*., 2009; Hassanien and Al-Rashada, 2019)**, AFLP **(Simmones *et al*., 2006; Magdy *et al*., 2016)**, and microsatellite DNA **(Wachirachaikam and Na-Nakorn, 2007, Megahed *et al*., 2020)** markers, were used to analyze genetic variation and taxonomic relationships among different fish species.

Despite the economic importance of family Sparidae, few studies have evaluated the genetic variation and evolutionary relationships within family Sparidae in the Mediterranean Sea, and the Red Sea in Egypt **(El-Deeb *et al*., 2014; Abbas *et al*., 2017; Guerriero *et al*., 2017)**. The previous genetic studies on family Sparidae relied only on one molecular marker for species

identification or studying the genetic variation among the species of this family **(El-Deeb *et al*., 2014; Abbas *et al*., 2017)**. Therefore, the current study aimed to integrate three different DNA-based molecular markers, in order to: (1) Provide DNA barcodes for 22 species of family Sparidae using mitochondrial *Cyt-b*. (2) Investigate the genetic diversity among Sparidae species using RAPD and ISSR markers. (3) Reconstruct the phylogenetic relationships among the studied species of family Sparidae based on the three utilized molecular markers. The employed molecular markers, in the current study, have been extensively used to achieve the same objectives in various studies of fish species characterization and fish populations' studies **(Saad *et al*., 2009; Soliman *et al*., 2017; Hassanien and Al-Rashada, 2019)**.

MATERIALS AND METHODS

Fish Sampling

Fish samples were collected from two different locations: Abo Qir Bay, west of the Mediterranean Sea, and the Gulf of Suez, north of the Red Sea, Egypt. A total of 175 samples were transferred on ice to the National Institute of Oceanography and Fisheries, Alexandria Branch. Fish Samples, belonging to family Sparidae, were sorted according to their external features into 22 fish species. The morphological characterization of each species followed FishBase **(Froese and Pauly, 2017)**.

DNA extraction

The conventional phenol-chloroform extraction was used to isolate fish DNA from muscle tissue, as described by Sambrook et al., 1989. Briefly, Tissues were homogenized in 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. Muscle tissues were lysed at 50°C for 60 min. DNA was isolated by standard ethanol precipitation. The eluted DNA in TE buffer (100 mM Tris-HCl, 10 mM EDTA, pH 8) was stored at 4°C for further analysis. The purity and concentration of DNA was assessed by a BioDrop spectrophotometer (Cambridge, UK).

DNA barcoding using *Cytochrome b* gene

A partial sequence of the mitochondrial (mt) *Cyt-b* gene was amplified using the universal primers: *Cyt-b*28-F, 5′-CGAACGTTGATATGAAAAACCATCGTTG-3′ and *Cyt-b*34-R, 5′-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3′ **(Gilles *et al*., 2000)**. PCR was performed using a Veriti Thermal cycler (Applied Biosystems, Thermo Fisher Scientific, USA), in a reaction volume of 25 µL containing 12.5 µL of MyTaq^{TM} HS Red Mix (Bioline, Meridian Life Scienc, UK), 2.0 µL of DNA template (approximately 20 ng/µL), 1.0 µL of 10 µmol/L forward primer, 1.0 µL of 10 µmol/L reverse primer. The cycling conditions were: 94°C for 5 min, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, 2 min at 72°C, and final elongation at 72°C for 7 min. Products quality were then tested on 2.5% 2.5% agarose gel, then purified using the Isolate II PCR and Gel Extraction Kit (Bioline, Meridian Life Scienc, UK). The purified

PCR products were sequenced using ABI's Big Dye Terminator kit (**Abbas *et al*., 2011)**. DNA sequences of *Cyt-b* were analysed using Chromas Lite version 2.1.

Table 1. RAPD and ISSR primers sequence used in Sparid species genotyping

	Primer code	***Nucleotide sequence (5'–3')***
RAPD	**R1**	AATCGGGCTG
	R2	GAAACGGGTG
	R3	CAATGCCCGT
	R4	GTATTGCCCT
	R5	TCCCTCGTGC
	R6	GCGCCTGGAG
	R7	AACGGGCAGG
	R8	GGCTGC GGTA
	R9	GCGGAGGTCC
	R10	CGACGCCCTG
	R11	GTGCGCAATG
	R12	GTCATGCGAC
	R13	GACAGGTTGG
	R14	CCGACTCTGG
	R15	CCTGGCACAG
	R16	CAAGCCGTCA
	R17	GTCGTAGCGG
	R18	CCGATATCCC
	R19	AGGTGACCGT
	R20	TTCCGAACCC
ISSR	**ISSR 1**	CACACACACACACACAGT
	ISSR 2	CACACACACACACACAAC
	ISSR 3	CACACACACACACACAAG
	ISSR 4	GTG GTG GTG GTG GC
	ISSR 5	GAG GAG GAG GAG GC
	ISSR 6	AGA GAG AGA GAG AGA GG
	ISSR7	GAG AGA GAG AGA GAG AC
	ISSR8	ACA CAC ACA CAC ACA CG

RAPD- and ISSR-PCR analyses

Primer selection

For RAPD-PCR, 20 random decamer primers (Operon Technologies, USA) **(Mishra *et al*., 2008; Rajakumaran *et al*., 2014)** were used to discriminate between the studied sparid species. Twenty primers were used in PCR and the sequences of RAPD primers are listed in Table 1. In addition, eight ISSR primers were used for the same purpose (**Gilles *et al*., 2000)** (Table 1).

PCR conditions and electrophoresis

PCR amplifications was performed in 25 µL reaction volumes containing 12.5 µL of PCR master mix (MyTaq^{TM} HS Red Mix; Bioline, London, UK), 2 µL of primer (10 pmol mL^{-1}), 2.5 µL of genomic DNA (approximately 20 ng/µL), and 8 µL of sterile distilled water. PCR amplification (Applied Biosystems, USA) were applied using the following thermal profile: 94°C for 5 min, 40 cycles of 94°C for 25 s, 37°C for 35 s, and 72°C for 1 min, with a final extension at 72°C for 5 min. For ISSR, the amplification conditions were as follows: 2 min at 94°C, 40 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 2 min, with a final extension for 5 min at 72°C. PCR amplicons were visualized on 1.6 % agarose gels run at 100 V for 45 min.

Statistical analysis

Cyt b sequence analysis

The obtained *Cyt b* sequences were edited using MEGA6 (**Tamura *et al*., 2013)**. Following sequence editing, sequences were compared to the archived sequences on GenBank using BLAST algorithm (https: //www.ncbi.nlm.nih.gov/). After sequences alignment, *Cyt b* sequences of different sparid species were deposited into GenBank/EMBL/DDBJ databases with accession numbers LC203045 to LC203061. The edited *Cyt b* sequences were translated into amino acids using MEGA6 software package to confirm the absence of stop codons. In addition, no insertions or deletions were detected throughout the amplified fragments. A phylogenetic tree based on the *Cyt-b* gene was constructed by MEGA6 using the UPGMA method based on Tamura-Nei model.
RAPD-PCR and ISSR profiles were analyzed using PAST (ver. 3.14, 2016). All markers profiles were scored as binary data (0, 1) based on the presence or absence of specific band (allele). The similarity indices were estimated and used for dendrogram construction by the unweighted pair group method (UPGMA) as described previously **(Nei, 1972; Jaccard, 1980)**.

RESULTS

1. Morphological Identification

The morphological examination classified the collected samples into 22 species belonging to 14 genera in family Sparidae. These species included: *Acanthopagrus bifasciatus* (Abi), *Argyrops spinifer* (Asp), *Boops boops* (Bbo), *Diplodus annularis* (Dan), *Diplodus cervinus* (Dce), *Diplodus noct* (Dno), *Diplodus sargus* (Dsa), *Diplodus vulgaris* (Dvu), *Dentex gibbosus* (Dgi), , *Dentex dentex* (Dde), *Lithognathus mormyrus* (Lmo), *Pagellus acarne* (Pac), *Pagellus erythrinus* (Per), *Pagrus auriga* (Pau), *Pagrus caeruleostictus* (Pca), *Pagrus pagrus* (Ppa), *Rhabdosargus*

haffara (Rha), *Sparus aurata* (Sau), *Spondyliosoma cantharus* (Sca), *Sarpa salpa* (Ssa), *Crenidens crenidens* (Ccr), and *Oblada melanora* (Ome).

2. Molecular analysis

2.1. *Cyt-b* mitochondrial gene analysis:

The applied *Cyt-b* primer pairs, *Cyt-b*28-F and *Cyt-b*34-R, showed successful PCR amplifications with most of the collected samples. The *Cyt-b* barcode sequence length was nearly 440 bp for the barcoded species. However, after many trials using different conditions, the utilized primer pairs failed to amplify the target regions of five morphologically identifiable species which are: *Diplodus noct*, *Spondyliosoma cantharus*, *Boops boops*, *Pagrus pagrus*, and *Dentex dentex*. Based on sequence similarity (≥97% cutoff), efficient barcodes were established for the other 17 species that showed successful PCR amplifications. BLAST comparisons showed complete matching with the morphological identification of those species.

Based on C*yt-b* sequence analysis, the estimated pairwise genetic distances among the 17 species of family Sparidae showed that *Diplodus cervinus* (Dce) and *Diplodus sargus* (Dsa) are the closest species with the lowest genetic distance (0.070), whereas, the highest genetic distance (0.212) was recorded between *Rhabdosargus haffara* (Rha), and *Sarpa salpa* (Ssa) (Table 2).

The molecular phylogeny that was constructed based on the mtDNA *Cyt-b* gene sequences clustered the 17 barcoded Sparid species into two major clades (Fig. 1). These two major clades included all species except *Crenidens crenidens and Sarpa salpa*, which formed non-clade groups, separately. Of the two major clades, the first clade that which was divided into two sub-clades, the first one included species of genus Diplodus that were clustered together, and *Oblada melanura*, *Pagellus acarne* and *Sparus aurata.* However, *Pagellus erythrinus*, *Lithognathus mormyrus* and *Argyrops spinifer* were clustered together in the second sub-Clade, while *Rhabdosargus haffara* was branched separately. The second major clade was divided into two branches; the first included *Acanthopagrus bifasciatu* only, while the other branch was divided into two sub-clades, one for *Pagrus caeruleostictus*, and the other included *Pagrus auriga* and *Dentex gibbosus.*

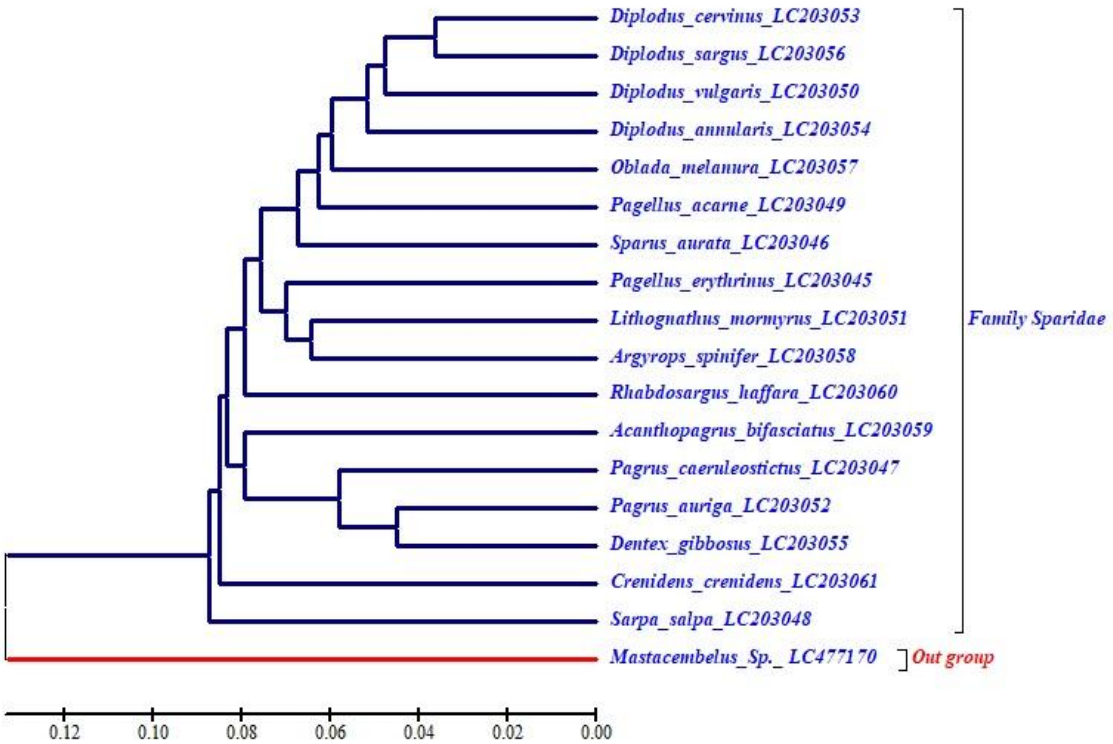

Fig. 1. *Cyt b*-based UPGMA phylogenetic tree for Sparid species in Egypt.

2.2. RAPD- and ISSR-PCR profiles

The RAPD analysis generated a total of 308 distinct bands, of which 283 (91.8%) were polymorphic and 25 (8.2%) were monomorphic. RAPD profiles differed substantially among the 22 sparid species due to the high percentage of polymorphic bands, enabling easy discrimination among different species. Based on the values of similarity indices revealed from band sharing of the RAPD markers, *Pagrus auriga* (Pau) and *Dentex dentex* (Dde) are the closest species with similarity index 0.722, whereas *Pagellus erythrinus* (Per) and *Oblada melanora* (Ome) are the farthest species with similarity index 0.455 (Table 3).

The utilized ISSR primers amplified a total of 197 bands, of which 193 bands were polymorphic (97.9%) and ranged from 100 to 2000 bp. The similarity indices among all species revealed from ISSR primers, indicates that *Pagrus caeruleostictus* (Pca) and *Lithognathus mormyrus* (Lmo) were the closest species with similarity index (0.735), whereas, *Pagellus erythrinus* "Per" and *Oblada melanora* "Ome" are the most distant species with similarity index 0.401 (Table 4).

RAPD and ISSR-based UPGMA clustering separated *Sparus aurata* "Sau" in a non-clade group. Whereas, the other 21 species were divided into two clusters; a major one including all species except *Pagellus erythrinus* "Per" (based on RAPD markers), and *Pagellus erythrinus* "Per" and *Boops boops* "Bbo" (based on ISSR markers) Fig. 2 and Fig. 3, respectively.

Table 2. C*yt b*-based pairwise genetic distance among 17 species of family Sparidae.

	Per	Ccr	Sau	Pca	Ssa	Pac	Dvu	Lmo	Pau	Dce	Dan	Dgi	Dsa	Ome	Asp	Abi	Rha
Per																	
Ccr	0.167																
Sau	0.192	0.195															
Pca	0.149	0.187	0.183														
Ssa	0.149	0.187	0.183	0.000													
Pac	0.204	0.166	0.126	0.172	0.172												
Dvu	0.167	0.169	0.132	0.161	0.161	0.120											
Lmo	0.149	0.182	0.143	0.193	0.193	0.124	0.133										
Pau	0.157	0.184	0.202	0.108	0.108	0.189	0.172	0.187									
Dce	0.164	0.170	0.152	0.162	0.162	0.119	0.094	0.141	0.179								
Dan	0.137	0.158	0.133	0.168	0.168	0.116	0.105	0.129	0.183	0.102							
Dgi	0.110	0.185	0.198	0.116	0.116	0.179	0.187	0.180	0.088	0.151	0.179						
Dsa	0.180	0.161	0.127	0.172	0.172	0.105	0.095	0.118	0.182	0.070	0.088	0.183					
Ome	0.169	0.144	0.129	0.158	0.158	0.124	0.118	0.126	0.108	0.118	0.110	0.142	0.105				
Asp	0.127	0.160	0.150	0.129	0.129	0.146	0.137	0.130	0.134	0.139	0.150	0.124	0.142	0.150			
Abi	0.188	0.169	0.172	0.171	0.171	0.140	0.135	0.184	0.155	0.149	0.144	0.163	0.159	0.134	0.163		
Rha	0.149	0.185	0.154	0.202	0.212	0.161	0.157	0.169	0.194	0.162	0.149	0.170	0.147	0.151	0.180	0.178	

Table 3. Average pairwise genetic similarity based on RAPD markers among 22 Sparidae species

	Per	***Sau***	***Pca***	***Ssa***	***Pac***	***Dva***	***Lmo***	***Pau***	***Dce***	***Dde***	***Dan***	***Dgi***	***Dsi***	***Bbo***	***Ppa***	***Ome***	***Sca***	***Asc***	***Abi***	***Rha***	***Dno***	***Ccr***
Per																						
Sau	0.507																					
Pca	0.612	0.577																				
Ssa	0.540	0.560	0.533																			
Pac	0.548	0.567	0.658	0.551																		
Dva	0.511	0.535	0.606	0.540	0.628																	
Lmo	0.586	0.571	0.696	0.569	0.646	0.645																
Pau	0.644	0.558	0.703	0.577	0.653	0.616	0.715															
Dce	0.539	0.558	0.670	0.592	0.648	0.648	0.696	0.711														
Dde	0.573	0.564	0.671	0.592	0.642	0.595	0.644	0.722	0.654													
Dan	0.575	0.509	0.587	0.603	0.630	0.613	0.617	0.632	0.619	0.643												
Dgi	0.580	0.529	0.632	0.555	0.596	0.580	0.620	0.664	0.608	0.692	0.633											
Dsi	0.571	0.527	0.646	0.590	0.581	0.594	0.612	0.620	0.600	0.653	0.610	0.650										
Bbo	0.498	0.500	0.559	0.557	0.550	0.511	0.568	0.612	0.569	0.614	0.571	0.597	0.525									
Ppa	0.573	0.532	0.557	0.636	0.584	0.551	0.610	0.610	0.589	0.604	0.648	0.558	0.557	0.668								
Ome	0.455	0.485	0.514	0.570	0.531	0.551	0.543	0.545	0.544	0.535	0.569	0.500	0.512	0.602	0.636							
Sca	0.535	0.517	0.591	0.552	0.605	0.550	0.615	0.623	0.610	0.549	0.613	0.557	0.577	0.590	0.647	0.598						
Asc	0.507	0.532	0.542	0.537	0.560	0.542	0.578	0.586	0.565	0.587	0.552	0.579	0.548	0.507	0.556	0.515	0.661					
Abi	0.531	0.563	0.618	0.569	0.611	0.551	0.606	0.593	0.601	0.524	0.568	0.538	0.550	0.546	0.580	0.527	0.593	0.578				
Rha	0.522	0.533	0.562	0.560	0.553	0.529	0.591	0.551	0.593	0.543	0.566	0.578	0.584	0.537	0.594	0.556	0.569	0.533	0.660			
Dno	0.532	0.507	0.580	0.557	0.624	0.584	0.582	0.605	0.591	0.590	0.593	0.575	0.604	0.602	0.578	0.544	0.606	0.529	0.597	0.581		
Ccr	0.544	0.507	0.570	0.532	0.592	0.538	0.621	0.614	0.587	0.558	0.533	0.568	0.571	0.585	0.595	0.534	0.578	0.551	0.607	0.606	0.628	

Table 4. Average pairwise genetic similarity based on ISSR markers among 22 Sparidae species

	Per	***Sau***	***Pca***	***Ssa***	***Pac***	***Dva***	***Lmo***	***Pau***	***Dce***	***Dde***	***Dan***	***Dgi***	***Dsi***	***Bbo***	***Ppa***	***Ome***	***Sca***	***Asc***	***Abi***	***Rha***	***Dno***	***Ccr***
Per																						
Sau	0.511																					
Pca	0.652	0.577																				
Ssa	0.560	0.560	0.533																			
Pac	0.558	0.567	0.658	0.551																		
Dva	0.515	0.535	0.606	0.540	0.628																	
Lmo	0.566	0.571	0.735	0.569	0.646	0.645																
Pau	0.634	0.558	0.703	0.511	0.653	0.616	0.715															
Dce	0.532	0.558	0.670	0.592	0.648	0.648	0.696	0.711														
Dde	0.573	0.564	0.671	0.592	0.642	0.595	0.644	0.722	0.654													
Dan	0.572	0.509	0.587	0.693	0.630	0.613	0.617	0.632	0.619	0.643												
Dgi	0.585	0.529	0.632	0.555	0.596	0.580	0.620	0.664	0.608	0.692	0.633											
Dsi	0.571	0.527	0.646	0.520	0.581	0.594	0.612	0.620	0.600	0.653	0.610	0.650										
Bbo	0.498	0.500	0.559	0.557	0.550	0.511	0.568	0.612	0.569	0.614	0.571	0.597	0.525									
Ppa	0.573	0.532	0.557	0.636	0.584	0.551	0.610	0.610	0.589	0.604	0.648	0.558	0.557	0.668								
Ome	0.401	0.485	0.514	0.570	0.531	0.551	0.543	0.545	0.544	0.535	0.569	0.500	0.512	0.602	0.636							
Sca	0.535	0.517	0.591	0.552	0.605	0.550	0.615	0.623	0.610	0.549	0.613	0.557	0.577	0.590	0.647	0.598						
Asc	0.507	0.532	0.542	0.537	0.560	0.542	0.578	0.586	0.565	0.587	0.552	0.579	0.548	0.507	0.556	0.515	0.661					
Abi	0.531	0.563	0.618	0.569	0.611	0.551	0.606	0.593	0.601	0.524	0.568	0.538	0.550	0.546	0.580	0.527	0.593	0.578				
Rha	0.522	0.533	0.562	0.560	0.553	0.529	0.591	0.551	0.593	0.543	0.566	0.578	0.584	0.537	0.594	0.556	0.569	0.533	0.660			
Dno	0.532	0.507	0.580	0.557	0.624	0.584	0.582	0.605	0.591	0.590	0.593	0.575	0.604	0.602	0.578	0.544	0.606	0.529	0.597	0.581		
Ccr	0.544	0.507	0.570	0.532	0.592	0.538	0.621	0.614	0.587	0.558	0.533	0.568	0.571	0.585	0.595	0.534	0.578	0.551	0.607	0.606	0.628	

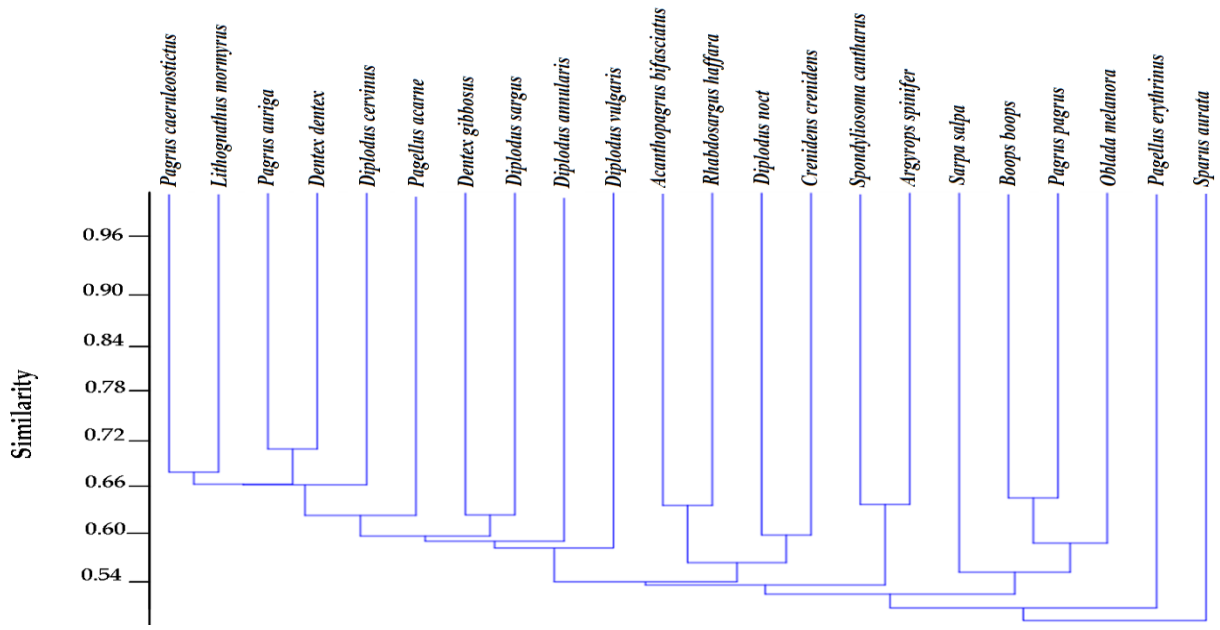

Fig. 2. Dendrogram based on RAPD markers representing the relationships among the 22 Sparidae species.

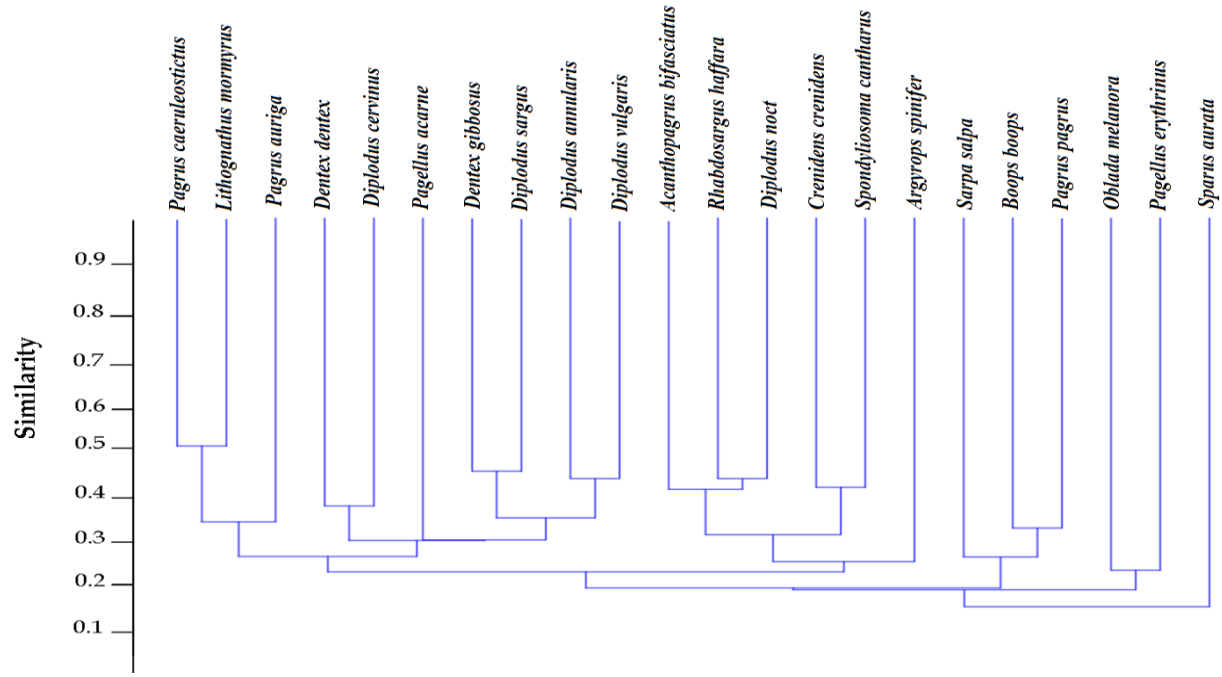

Fig 3. Dendrogram based on ISSR markers representing relationships among 22 Sparidae species.

DISCUSSION

The current study discusses an interesting research point since few studies have focused on the genetic variation and evolutionary relationships in family Sparidae in Egypt (**Abbas *et al*., 2017)**. Fish species of family Sparidae are economically important fishes, therefore, the study of the genetic diversity among species is crucial for proper management and conversation strategies. In this study, three different molecular techniques were integrated, mitochondrial *Cyt-b*, RAPD, and ISSR markers, to better evaluate the genetic diversity among different species belonging to this family which is more effective compared to other studies which relied only on a single molecular marker (**Abbas *et al*., 2017)**.

The applied molecular markers, in the current study, were relatively efficient in estimating the genetic diversity among the studied species. Regarding the use of mitochondrial *Cyt-b* in DNA barcoding of sparid species, *Cyt-b*, in the current study, has established efficient DNA barcodes for 77% of the studied species, which seems to be less efficient than *Cytochrome Oxidase subunit l* (*COI)* that was used by **Abbas *et al*. (2017)**. In that study **Abbas *et al*. (2017)**, the utilized *COI* primer pairs described by **Ward *et al*. (2005)** were able to provide DNA barcode for the same 22 species of family Saparidae in Egypt. Therefore, it is recommended in future studies to modify the sequences of the utilized *Cyt-b* primers to increase its efficiency in barcoding the following species; *Diplodus noct, Spondyliosoma cantharus, Boops boops, Pagrus pagrus*, and *Dentex dentex*.

The *Cyt-b* pairwise genetic distance, in the current study, revealed that *Diplodus cervinus* (Dce) and *Diplodus sargus* (Dsa) are the closest species with the lowest genetic distance (0.070). Whereas, in **Abbas *et al*. (2017)** that studied the same sparid species in Egypt based on COI, *Diplodus cervinus* and *Diplodus noct* were the closest species with a genetic distance (0.01). On the other hand, the highest genetic distance (0.212) based on *Cyt-b* was recorded between *Rhabdosargus haffara* (Rha), and *Sarpa salpa* (Ssa) which fully disagreed with **Abbas *et al*. (2017)** that recorded the highest genetic distance (0.210) between *Diplodus cervinus* and *Argyrops spinifer*.

The constructed *Cytb*-based UPGMA tree is in accordance with a previous molecular identification of various Sparidae species by **Chiba *et al*. (2009)** for family Sparidae using the *Cyt-b* gene. The study reported the non-monophyly of genera; Diplodus, Dentex, Pagrus, and Pelagius which agreed with the Cyt-b based clustering pattern in the current study. These results are also consistent with those of **Abbas *et al*. (2017)** using *Cytochrome Oxidase Subunit I* (*COI*) gene which demonstrated similar phylogenetic clustering of family Sparidae with two major lineages. However, based on *Cyt-b* in the current study, two different species formed a non-clade group, *Crendinus crendinus* and *Sarpa salpa,* while, in **Abbas *et al*. (2017)** based on COI, only one non-clade group was formed by *Crendinus crendinus.* The recorded differences between the COI and *Cyt-b*-based genetic distances and the phylogenetic tree clustering is apparently due to the nature and function of the two mitochondrial regions.

On the other side, RAPD and ISSR markers were integrated in the current study to assess the genetic diversity among the studied sparid species and both markers contributed to the discrimination among the studied species. However, by comparing the number of alleles and the percentage of polymorphic ones generated by 20 RAPD markers and that generated by eight ISSR markers, it was obvious that ISSR generated a higher percentage of polymorphic bands (97%), which makes ISSR markers are more powerful. This power of discrimination of ISSR markers can be due to the fact that the ISSR primers often target coding regions of the genome, which tend to be highly polymorphic (**Costa *et al*., 2016)**, whereas, The RAPD markers amplifies both coding and non-coding regions of the genome (**Costa *et al*., 2016)**.

However, the clustering pattern of the phylogenetic tree based on RAPD and ISSR markers are relatively similar, where, based on both markers, *Sparus aurata* "Sau" formed a non-clade group and the other species were clustered in another clade. The similar clustering pattern based on RAPD and ISSR markers may be attributed to being nuclear markers. The use of RAPD and ISSR for the initial assessment of genetic variation among fish species or populations was reported by many studies **(Barman *et al*., 2003; Rashed *et al*., 2008; Saad *et al*., 2009; Pereira *et al*., 2010; Abdul-Muneer *et al*., 2011, Hassanien and Al-Rashada, 2019)**. Particularly, with the simplicity and the low cost of both nuclear markers, RAPD and ISSR, compared to other molecular markers (**Costa *et al*., 2016)**.

By comparing the clustering pattern based on *Cyt-b* as a mitochondrial barcode marker and that based on (RAPD and ISSR) as nuclear markers, it was obvious that the clustering pattern based on *Cyt-b* is more reliable than that based on RAPD and ISSR marker. In *Cyt-b* based phylogenetic tree, species belonging to the same genus were clustered into the same clade, for example, species of genus Diplodus, species of genus Pagrus and that of genus Pagellus. On the other hand, the RAPD/ISSR- based phylogenetic tree showed a degree of mixing among all species.

CONCLUSION

In conclusion, the applied molecular markers, in the current study, were relatively efficient in estimating the genetic diversity among the studied species. DNA barcoding using mitochondrial *Cyt-b* provided efficient barcodes for most of sparid species. RAPD and ISSR markers were combined in the current study to assess the genetic diversity among the studied sparid species. Both markers apparently contributed to the discrimination among the studied species. However, the discrimination power of ISSR was higher. The clustering pattern based on Cyt-b is more reliable than that based on RAPD and ISSR marker.

ACKNOWLEDGMENT

This framework was supported by STDF funding from the Ministry of Scientific Research, Egypt.

REFERENCES

Abbas, E. M.; Soliman, T.; El-Magd, M. A.; Farrag, M. M., Ismail, R. F. and Kato M. (2017). Phylogeny and DNA barcoding of the family Sparidae inferred from mitochondrial DNA of the Egyptian waters. J. Fish. Aqua. Sci. 12(2):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. Mol. Res. 10(4): 3306-3315.

Abdul-Muneer, P. M.; Gopalakrishnan, A.; Shivandem, P.; Basheer, V. S. and Ponniah, A. G. (2011). Genetic variation and phylogenetic relationship between two species of yellow catfish, Horabagrus brachysoma and H. nigricollaris (Teleostei: Horabagridae) based on RAPD and microsatellite markers. Mol. Bio. Rep. 38: 2225-2232.

Abou-Gabal, A. A. A.; Abbas, E. M.; Mohamed, H. M.; El-Baramawi, N.; Khaled, A. A. and El Deeb, S. I. (2018). Molecular Identification of Grey Mullet species in the Mediterranean Sea of Egypt. Egy. J. Aqua. Biol. Fish. 22 (4): 125-139.

Akazaki, M. (1962). Studies on the spariform fishes. Anatomy, phylogeny, ecology, and taxonomy. Misaki Marine Biology Institute, Kyoto University, Spec. Rep. 1. Osaka: Kosugi Ltd.

Ali, F. S. and Mamoon, A. (2019). Population genetic studies of genus Dicentrarchus reveal loss of genetic diversity in Egyptian waters. Reg. Stud. Mar. Sci., 31.

Ali, F. S.; Ismail, M. and Mamoon, A. (2019). Comparative molecular identification of genus Dicentrarchus using mitochondrial genes and internal transcribed spacer region. Egy. J. Aqua. Bio. Fish. 23 (3): 371-384.

Armani, A.; Castigliego, L. and Guidi, A. (2012). Fish fraud: the DNA challenge. CAB Anim. Sci. Rev. 7: 227e239.

Barman, H. K.; Barat, A.; Yadav; B. M.; Banerjee, S.; Meher, P. K. and Jana, R. K. (2003). Genetic variation between four species of Indian major carps as revealed by random amplified polymorphic DNA assay. Aquaculture, 217: 115-123.

Basurco, B.; Lovatelli, A. and Garcia, B. (2011). Current status of Sparidae aquaculture, pp. 1–50 in Sparidae: Biology and Aquaculture of Gilthead Sea Bream and Other Species, edited by Pavlidis, M., and C.C. Mylonas. Wiley-Blackwell, Oxford.

Bhati, A. A.; Haniffa, M. A.; Divya, P. R.; Gopalakirshnan, A.; Milton, M. J.; Kumer, R. and Paray, B. A. (2012). Molecular characterization of eight Indian snakehead species (Pisces: Perciformes Channidae) using RAPD markers. Mol. Bio. Rep. 39: 4267-4273.

Casu, M.; Tiziana, L.; Curini-Galletti, M.; Alberto, R. and Antonio, P. (2009). Identification of Mediterranean *Diplodus spp*. and *Dentex dentex* (Sparidae) by means of DNA Inter-Simple Sequence Repeat (ISSR) markers. J. Experim. Marine Bio. Ecol. 368: 147–152.

Chiba, S. N.; Iwatsuki, Y.; Yoshino, T. and Hanzawa N. (2009). Comprehensive phylogeny of the family Sparidae (Perciformes: Teleostei) inferred from mitochondrial gene analysis. Genes & Genetic Systems, 84: 153-170.

Costa, R.; Pereira, G.; Garrido, I.; Tavares-de-Sousa, M. M. and Espinosa, F. (2016). Comparison of RAPD, ISSR, and AFLP molecular markers to reveal and classify Orchardgrass (*Dactylis glomerata L.*) germplasm variations. PLoS ONE, 11(4): e0152972.

De La Herrán, R.; Ruiz-Rejón, C.; Ruiz-Rejón, M. and Garrido-Rames, M. A. (2001). The molecular phylogeny of the Sparidae (Pisces, Perciformes) based on two satellite DNA families. Heredity. 87:691-697.

El-Deeb, S. I.; El-Seedy, A. S.; Abo Gabal, A. A.; El-Wakeel, H. E. and Ibrahim, N. A. (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 (11): 779-789.

Esposti, D. M.; De Vries, S.; Crimi, M.; Ghelli, A.; Patarnello, T. and Meyer, A. (1993). Mitochondrial cytochrome b: evolution and structure of the protein. Biochm. et Biophys. Acta, 1143: 243–271.

FAO. (2013). The State of World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations, Rome.

Froese, R. and Pauly, D. (2017). FishBase 2017, version (March, 2017). World Wide Web electronic publication Home page at: http://www.fishbaseorg.

GAFRD. (2018). The general authority for fishery resources development: summary production statistics. Cairo, Egypt.

Gilles, A.; Miquelis, A.; Quignard, J. P. and Faure, E. (2000). Molecular phylogeography of western Mediterranean dusky grouper *Epinephlus marginatus*. Comptes Rendus de l'Academie des Sciences Series III Science de la Vie, 300:195-205.

Golani, D. and Bogorodsky, S. V. (2010). The fishes of the Red Sea - Reappraisal and updated checklist. Zootaxa, 2463: 1-135.

Guerriero, G.; Rabbito, D.; Alwany, M. A.; Madonna, A.; Temraz, T. A.; Sulaiman, O. O.; Bassem, S. M.; Trocchia, S.; Abdel-Gawad, F. K. and Ciarcia, G. (2017). Fisheries and biodiversity along Mediterranean Sea: Italian and Egyptian coast overview. Euro-Mediterranean J. Environm. Integr. 2:16.

Hadreys, H.; Balick, M. and Schierwater, B. (1992). Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol. Ecol. 1: 55-63.

Hanel, R. and Tsigenopoulos, C. (2011). Phylogeny, evolution, and taxonomy of Sparids with some notes on their ecology and biology. Pavlidis M.A, Mylonas CC, (Eds.), Sparidae Biology and Aquaculture of Gilthead Sea Bream and Other Species, Wiley-Blackwell, 51-73.

Hassanien, H. A. and Al-Rashada, Y. (2019). Estimation of genetic variation in green tiger prawn, *Penaeus semisulcatus* by using random amplified polymorphic DNA, inter simple sequence repeat and simple sequence repeat markers. Aqua. Res. 50 (6): 1626-1633.

Ibrahim, M. A. and Soliman, I. A. (1996). Check list of the bony fish species in the Mediterranean waters of Egypt. Bull. Inst. Oceanogr. Fish. 22: 43 - 57.

Jaccard, P. (1908). Nouvelles recherches sur la distribution florale. Bulletin de la Société vaudoise des sciences naturelles, 44:223-270.

Magdy, M.; Eshak, M. G. and Rashed, M. A. (2016), Genetic structure of *Mugil cephalus L*. populations from the northern coast of Egypt. Vet. Wor. 9 (1): 53-59.

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. Egy. J. Aqua. Biol. Fish. 24 (1).

Mishra, P. S.; Lakra, W. S. and Chaudhari, A. (2008). Genetic analysis of East and West Coast populations of *Penaeus Monodon* from India based on random amplified polymorphic DNA. J. Indian Fish. Associ. 35: 1-8.

Nei, M. (1972). Genetic distance between populations. The American Naturalist, 106:283-292. Orrell, T. M. and Carpenter, K. E. (2004). A phylogeny of the fish family Sparidae (porgies) inferred from mitochondrial sequence data. Mol. Phyl. Evol. 32: 425-434.

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

Pereira, J. C.; Lino, P. G.; Leitão, A.; Joaquim, S.; Chaves, R.; Pousão-Ferreira, P. and Neves dos Santos, M. (2010). Genetic differences between wild and hatchery populations of Diplodus sargus and D. vulgaris inferred from RAPD markers: implications for production and restocking programs design. J. Appl. Genet. 51: 67-72.

Qiubai, Z.; Fengbo, I.; Li, Z. and Jianfang, G. (2006). RAPD markers between yellow catfish (*Pelteobagrus fulvidraco*) and long whiskers yellow catfish (*P. enpogen*). Acta Hydrobiol Sinica, 30: 482-485.

Rajakumaran, P.; Vaseeharan, B.; Jayakumar, R. and Chidambara, R. (2014). Conformation of phylogenetic relationship of Penaeidae shrimp based on morphometric and molecular investigation. Cytol. Genet. 48, 357-363.

Rashed, M. A.; Saad, Y. M.; Ibrahim, M. M. and El- Seoudy, A. A. (2008). Genetic structure of natural Egyptian *Oreochromis niloticus* evaluated using dominant DNA markers. Global Veterinaria, 2: 87- 91.

Saad, Y. M. ; Hanafi, M. S. ; Essa, M. A. ; Guerges, A. A. and Ali, S. F. (2009), Genetic signatures of some Egyptian Clarias gariepinus populations. Glob. Vet. 3 (6): 503-508

Sambrook, J.; Fritschi, E. F. and Maniatis, T. (1989). Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press, New York.

Simmones, M.; Mickett, K.; Kucuktas, H.; Li, P.; Dunham, R. and Lui, J. (2006). Comparison of domestic and wild channel catfish (*Ictalurus punctatus*) populations provides no evidence for genetic impact. Aquaculture, 252: 133-146.

Smith, L. B. and Smith, M. M. (1986). Sparidae. In M. M. Smith, & P. C. Heemstra (Eds.), Smiths' sea fishes. Berlin: Springer-Verlag.

Soliman, T.; Aly, W.; Fahim, R. M., Berumen, M. L.; Jenke-Kodama, H. and Bernardi, G. J. E. (2017). Comparative population genetic structure of redbelly tilapia (Coptodon zillii (Gervais, 1848) from three different aquatic habitats in Egypt. Ecol. Evol. 7: 11092–11099.

Stoeckle, M. (2003). Taxonomy, DNA, and the bar code of life. BioScience, 53(9): 796-797.

Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A. and Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.

Wachirachaikam, A. and Na-Nakorn, U. (2007). Application of population genetic improvement for African catfish (*Claris gariepinus*) Proceedings it. The 45th Kasetstart University Annual Conference, 82-89, Kasetstart University, Bangkok, Thailand.

Welsh, J. and McClelland, M. (1990). Fingerprinting genome using PCR with arbitrary primers. Nucl. Acids Res. 18: 7213-7218.

Williams, J. G.; Kubelik, A. R.; Livak, K. J.; Rafalski, J. A. and Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18: 6531-6535.

التعريف والتنوع الجينى المقارن لبعض اسماك العائلة المرجانية فى المياه الساحلية المصرية

نيرمين عبدالعزيزابراهيم[١]، ايمان ممدوح عباس[١]، ايمن الصعيدى[٢]، طه بشير سليمان[١] ،فوزية صلاح على[١]

[١] معمل الوراثة- شعبة تربية الأحياء المائية- المعهد القومى لعلوم البحار والمصايد- مصر.

[٢] معمل الوراثة الخلوية والجزيئية- قسم الوراثة- كلية الزراعة- جامعة الأسكندرية- مصر.

تتوزع انواع العائلة المرجانية على نطاق واسع من المياه المعتدلة إلى المياه الإستوائية وتعد ذات فوائد اقتصادية كبيرة. ومع ذلك في مصر تتوفر بيانات محدوده عن الإختلافات الوراثية والعلاقات التطورية للعائلة المرجانية. لذلك يعتبر دراسة التنوع الجيني بين انواع العائلة المرجانية أمرا بالغ الأهمية للإدارة السليمه والإستراتيجيات المناسبة. والهدف من هذه الدراسة هو التقييم الجيني بين ٢٢ نوع من العائلة المرجانية فى البحرالمتوسط والبحرالأحمر في مصر بإستخدام ثلاث معلمات جزيئية مختلفة.

تم تطبيق شفرة الحمض النووي باستخدام الجين الميتوكندري *Cyt-b* كخطوة أولية لتحديد الأنواع وتنوعها. بالإضافة إلى ذلك جرى توظيف تقنيتي الدنا متعدد الشكل المضخم عشوائياً (RAPD) DNA polymorphic amplified Random وتكرارات التسلسل البسيط البيني Sequence Simple Inter (ISSR) Repeats في تقدير التنوع الوراثي بين الأنواع المدروسة. بناء على كل علامة جزيئية، تم بناء شجرة وراثية لأنواع العائلة المرجانية المدروسة وفقا للمسافة الوراثية المحسوبة. واستخدام الجين الميتوكندري *Cyt-b* اثبت فعاليته كشفرة وراثية فعالة لمعظم الأنواع المدروسة. فحص RAPD باستخدام عشرين بادئ أنتج إجمالي ٣٠٨ حزمة منها ٩١.٨% متعددة الأشكال. بوادئ التضخيم ISSR الثمانية بإجمالي ١٩٧ حزمه ضمت ٩٧.٩% متعددة الأشكال. مظاهر RAPD و ISSR اختلفت بشكل كبير بين الأنواع ٢٢ للعائلة المرجانية مما اتاح سهولة التمييز. ومع ذلك كان لISSR قوة تمييز أعلى مقارنة بمعلمات الRAPD.

قدمت الشجرة الفيلوجينية المبنية على جين *Cyt-b* تحديثا لشفرة الحمض النووي لأنواع العائلة المرجانية. وحيث ان الشجرة المبنية على أساس تحليل بيانات كل من RAPD و ISSR قسمت الأنواع إلى قسمين رئيسيين: القسم الأول اشتمل *Sparus aurata* ، بينما انقسم القسم الثاني إلى مجموعتين إضافيتين تضم باقي انواع العائلة المرجانية. وتعتبرالمعلمات الجزيئية المستخدمة في هذه الدراسة فعالة في التنويع الجيني للانواع. تم إستخدام *Cyt-b* بنجاح في تمييزالأنواع وبعضها جينيا، في حين أن الجمع بين RAPD و ISSR يوفر ملامح متباينه إلى حد كبير لكل نوع، مما يجعلها مناسبة لقياس الإختلاف الجيني بين الأنواع.