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Phylogenetic Relationships and Genetic Diversity of Some Sparid Species from The Egyptian Mediterranean Sea Using *16S rRNA* Gene

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ABSTRACT

A study of the molecular diversity and phylogenetic analysis of some Sparidae species, which are ecologically and economically important, was carried out using *16S rRNA* gene. This study focused on the phylogeny relationships amongst seven sparid species: *Boops boops*, *Crenidens crenidens*, *Diplodus annularis*, *Diplodus sargus*, *Pagellus acarne*, *Pagellus erythrinus*, and *Sparus aurata*. The sequences of *16S rRNA* were displayed in GenBank/NCBI databases to gain the accession numbers (OQ858300.1- OQ858306.1). The average frequencies of adenine (A), thymine (T), cytosine (C), and guanine (G) were 28.75, 22.42, 25.02, and 23.81%, respectively. Our findings proved the effectiveness of *16S rRNA* in phylogenetic evaluation of the sparid species and validated the earlier findings of other authors that certain Sparidea family genera are not monophyletic.

INTRODUCTION

Marine fish of the family Sparidae are found in tropical and temperate latitudes of the Atlantic, Indian, and Pacific oceans; they are seldom found in freshwater or brackish environments. It's interesting to note that many endemic species—more than one-third of all species live along South Africa's coast **(Parenti, 2019)**. The species belonging to the family Sparidae are very valuable economically, particularly in the Mediterranean area where catch fisheries capture the majority of the species for commercial uses and just a limited number are commercially cultivated **(Basurco *et al.*, 2011)**. It consists of 115 species belonging to 33 genera **(Orrell *et al.*, 2002)**. Along the shores of Egypt, thirty-three species of the family Sparidae have been identified **(FAO, 2013)**. 21 species of the family Sparidae have been found in the Egyptian waters in the Mediterranean Sea. Due to their rich meat and excellent flavor, the majority of these species are utilized as table food and are significant to the economy **(Ibrahim & Soliman, 1996; Ibrahim *et al.*, 2020)**.

Various evolutionary investigations, such as determining character homology and understanding character loss and convergent evolution, are based on the mapping of heritable character states (phenotypic or genotypic) onto a tree. Since homologous traits of two taxa are inferred from their common ancestor by definition, we can determine the traits of these ancestors. More broadly, character mapping enables us to recreate the historical path of evolution by tracking the changing character states throughout a tree. Trees and molecular data also underpin methods for fitting a timescale to the evolutionary process. They form the basis of the comparative method used to establish trends in evolutionary processes **(Telford & Budd, 2003; Kapli *et al.*, 2020)**.

Although evolutionary studies have been essential to our understanding of life, they have only recently become somewhat involved in conservation attempts **(Forest *et al.*, 2015)**. For many biological investigations, understanding the evolutionary relationships between species is essential. The precise phylogenetic tree supports our understanding of the major transitions in evolution, such as the emergence of new body plans or metabolism, and is key to inferring the origin of new genes, detecting molecular adaptation, understanding morphological character evolution, and reconstructing the demographic changes in the recently diverged species. Howeve, there are still numerous obstacles to trustworthy tree construction even with the availability of powerful analysis tools and an abundance of data **(Kapli *et al.*, 2020)**.

In vertebrates, the mitochondrial genome has proven to be a useful tool for population genetic and phylogenetic analysis. But little is known about the composition and diversity of the vertebrate mt genome **(Satoh *et al.*, 2016)**. As complete mitochondrial genome sequences from many vertebrates have been accumulated, various differences have been revealed in the properties of these genomes, including tRNA gene structure and the start/stop codons of protein-coding genes **(Bibb *et al*., 1981; Fearnley & Walker, 1987; Gadaleta *et al*., 1989; Seutin *et al*., 1994; Xiufeng & Árnason, 1994; Macey *et al*., 1997)**. Furthermore, numerous instances of gene order variation have also been discovered in the vertebrate mt genome **(Paul & Rejean, 1990; Pääbo *et al*., 1991; Kumazawa & Nishida, 1995; Macey *et al*., 1997; Boore, 1999; Inoue *et al*., 2001; Satoh *et al*., 2006)**.

One key feature that supports the utilization of the *16S rRNA* gene in particular is the existence of several conserved/hypervariable sites, which provide a variety of alternatives for designing PCR primers **(Van de Peer, 1996)**. For a variety of reasons, 16S rRNA gene sequences have been the most often utilized housekeeping genetic marker in the study of phylogeny and taxonomy. These include the following: (i) the 16S rRNA gene is found in nearly all species; (ii) the gene's function has not changed over time, indicating that random sequence changes are a more accurate indicator of time (evolution); and (iii) the gene's (1,500 bp) size is suitable for informatics applications **(Patel, 2001)**.

Due to these significant reasons, we used the *16S rRNA* gene in this work to explore the genetic diversity and phylogenetic relationships of several sparid species from the Egyptian Mediterranean Sea.

MATERIALS AND METHODS

Ethics statement

All studies were approved by the research animal care ethical committee of the Faculty of Science, Suez Canal University under protocol REC244/2023.

Samples collection and species identification

The Sparid fish were collected from the Egyptian Mediterranean Sea, specifically in Port-Said area, where seven species of family Sparidae (*Boops boops*, *Crenidens crenidens*, *Diplodus annularis*, *Diplodus sargus*, *Pagellus acarne*, *Pagellus erythrinus* and *Sparus aurata*) were grouped and identified **(Froese & Pauly, 2024)**. The muscle tissues of the samples were obtained from the dorsal muscles of the fish and stored at -20°C. The muscle tissue was utilized for DNA extraction.

DNA extraction and PCR amplification

QIAamp DNA Mini kit (Qiagen, Germany) was used to extract the total genomic DNA from the separated muscles' tissues. To amplify mitochondrial ribosomal *16S rRNA* gene in the seven sparid fish species we used primers according to **Simon *et al*. (1991)**. The PCR reactions consisted of 25µL PCR master mix, 1µL of genomic DNA, and 1 µL each of forward and reverse primers, in a final reaction volume of 50µL. The PCR cycling conditions were conducted with an initial denaturation for 5 minutes at 94ºC, followed by 30 cycles of denaturation for 60s at 94ºC, annealing for 60s at 49°C and an extension at 72°C for 60sec with post cycling extension at 72ºC for 5min. 1.5% agarose gel stained with ethidium bromide was used to separate the PCR products.

The sequencing of PCR products and phylogenetic tree construction

All DNA sequencing was carried out in Macrogen (Seoul, South Korea) using conventional Sanger sequencing method. The sequences of *16S rRNA* genes were submitted to the National Center for Biotechnology Information (GenBank/NCBI) for obtaining accession numbers. CLUSTAL W **(Clustal *et al*., 1994)**, with the default settings, was used to align the sequences. For phylogenetic reconstructions, two methodologies were followed, including neighbor joining and minimum evolution by using MEGA software version 7.0 18 **(Kumar *et al*., 2016)**. To finalize the sequence divergences, we used Kimura two-parameter distances **(Kimura, 1980)**, with 1000 bootstrap iterations **(Felsenstein, 1985)**.

RESULTS

The sequences length of *16S rRNA* gene in seven Sparid fish expanded from 524bp to 561bp. The nucleotide sequences were inserted into the GenBank/NCBI with accession numbers (OQ858300.1- OQ858306.1). Our results showed that the longest nucleotide sequence (561bp) was found in *Diplodus sargus* and also in *Pagellus acarne*, while *Diplodus annularis* showed the shortest sequence (524bp). The average frequencies of the nucleotides were 28.75, 22.42, 25.02 and 23.81% for adenine (A), thymine (T), cytosine (C) and guanine (G), respectively. The *16S rRNA* gene displayed an A+T ratio bigger than the C+G ratio in all the species (Table 1).

Table 1. Accession number, nucleotide frequencies, A+T contents and their averages of 16S rRNA gene sequences in seven sparid fish

No.	Species	Accession number	Base pair length	Nucleotide number %				A+T Content (%)
				A%	T%	C %	G%	
1	*Boops boops*	OQ858300.1	559	27.91	23.43	24.87	23.79	51.34
2	*Crenidens crenidens*	OQ858301.1	560	28.93	22.32	24.46	24.29	51.25
3	*Diplodus annularis*	OQ858302.1	524	28.82	21.37	26.15	23.66	50.19
4	*Diplodus sargus*	OQ858303.1	561	29.06	22.99	24.60	23.35	52.05
5	*Pagellus acarne*	OQ858304.1	561	29.06	22.64	24.60	23.71	51.7
6	*Pagellus erythrinus*	OQ858305.1	560	29.29	22.14	24.82	23.75	51.43
7	*Sparus aurata*	OQ858306.1	560	28.21	21.96	25.71	24.11	50.17
	Average	-------	555	28.75	22.42	25.02	23.81	51.17

The sequences obtained from the seven sparid species from the Mediterranean Sea in Egypt revealed that, the final alignments included 585bp; the conserved, parsimony informative, and variable sites were 451, 45 and 113, respectively (Fig. 1).

P-distances for all the sparid species ranged from 0.0000 to 0.0209%. Overall, the distance value among all the species was 0.08%. The highest value (0.0209) was observed between *Diplodus puntazzo* (AJ247291.1) and both *Boops boops* (OQ858300.1*)* and *Boops environmental* (KU510485.1) and between *Diplodus bellottii* (AJ247289.1) and *Sarpa salpa* (AF247402.1). While, 0.000 value was observed between *Diplodus sargus* (OQ858303.1) and *Diplodus sargus* (AJ247293.1). The P-distances among the understudied sparid fish expanded from 0.0113 to 0.0187%. The highest value (0.0187) was found between *Boops boops* (OQ858300.1) and *Pagellus acarne* (OQ858304.1). While, the lowest P-distance (0.0113) was found between *Crenidens crenidens* (OQ858301.1) and *Sparus aurata* (OQ858306.1) (Table 2).

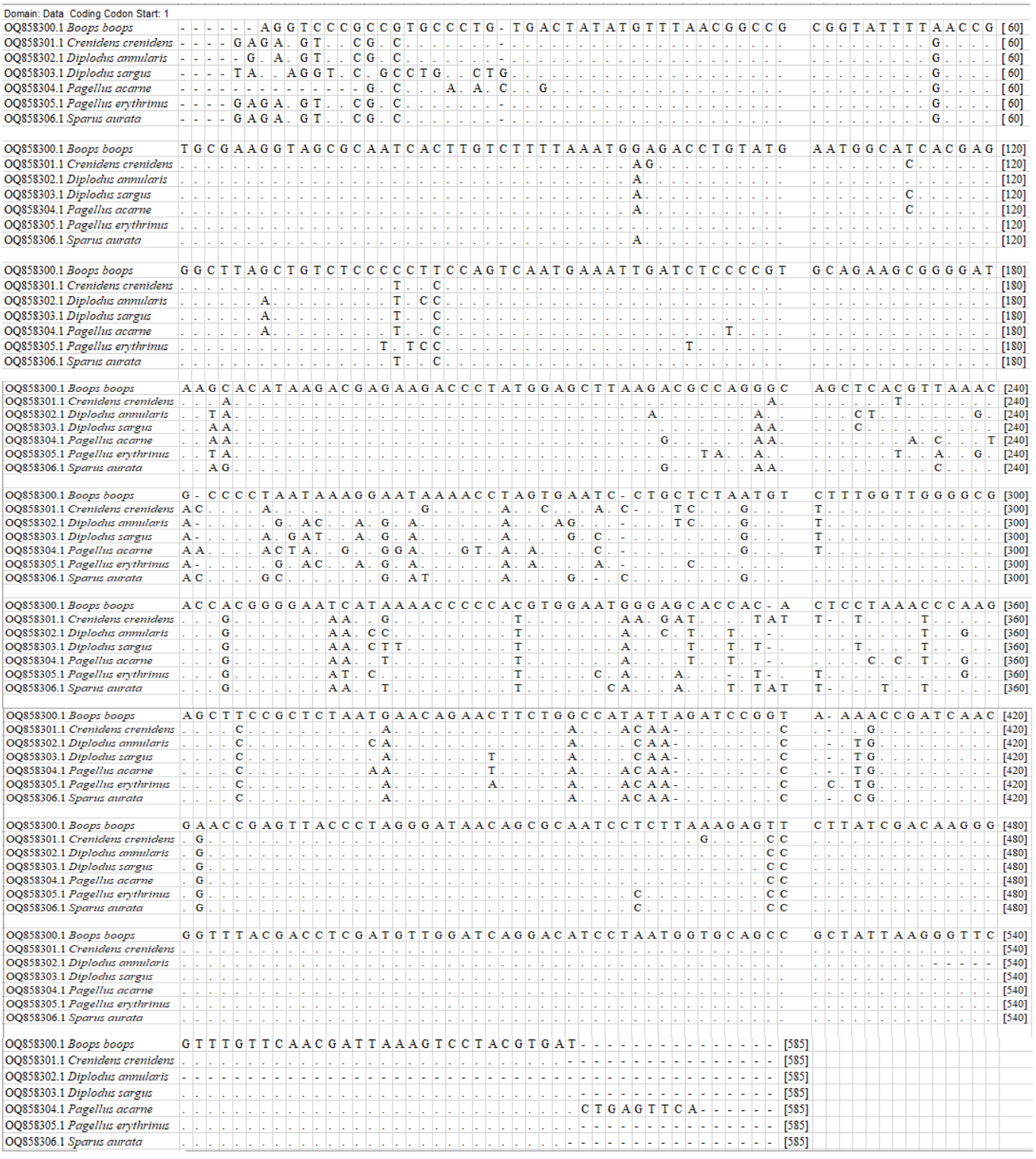

Fig. 1. Alignment of (*16S rRNA*) sequences in the seven sparid fish. Dots show similar nucleotides, while A, C, G, and T display the variance nucleotides

Table 2: Pairwise distances by the mean of *16S rRNA* gene amongst seven species of Sparidea family with their linkage species from the GenBank/NCBI

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1	OQ858300.1_Boops_boops		0.0150	0.0186	0.0186	0.0187	0.0162	0.0148	0.0150	0.0144	0.0019	0.0166	0.0203	0.0203	0.0203	0.0158	0.0168	0.0198	0.0195	0.0209	0.0202	0.0165	0.0171	0.0144	0.0157	0.0184	0.0153	0.0153	0.0145	0.0164	0.0174	0.0108	0.0158	0.0193	0.0190	0.0184
2	OQ858301.1_Crenidens_crenidens	0.0960		0.0157	0.0147	0.0149	0.0143	0.0113	0.0125	0.0122	0.0156	0.0157	0.0142	0.0157	0.0146	0.0127	0.0126	0.0140	0.0147	0.0143	0.0144	0.0133	0.0125	0.0127	0.0131	0.0133	0.0126	0.0132	0.0117	0.0133	0.0141	0.0157	0.0138	0.0215	0.0208	0.0202
3	OQ858302.1_Diplodus_annularis	0.1183	0.0872		0.0137	0.0151	0.0134	0.0147	0.0165	0.0140	0.0194	0.0149	0.0115	0.0089	0.0121	0.0102	0.0111	0.0123	0.0104	0.0119	0.0119	0.0100	0.0142	0.0144	0.0136	0.0116	0.0143	0.0134	0.0149	0.0151	0.0156	0.0194	0.0159	0.0207	0.0199	0.0202
4	OQ858303.1_Diplodus_sargus	0.1314	0.0871	0.0746		0.0125	0.0163	0.0133	0.0151	0.0120	0.0176	0.0153	0.0036	0.0105	0.0042	0.0089	0.0077	0.0021	0.0074	0.0073	0.0000	0.0091	0.0140	0.0133	0.0138	0.0106	0.0148	0.0122	0.0146	0.0131	0.0155	0.0194	0.0154	0.0174	0.0201	0.0198
5	OQ858304.1_Pagellus_acame	0.1338	0.0918	0.0859	0.0698		0.0158	0.0127	0.0157	0.0126	0.0189	0.0162	0.0119	0.0155	0.0118	0.0119	0.0116	0.0124	0.0139	0.0138	0.0122	0.0124	0.0150	0.0136	0.0146	0.0130	0.0151	0.0109	0.0138	0.0135	0.0141	0.0188	0.0149	0.0208	0.0216	0.0213
6	OQ858305.1_Pagellus_erythrinus	0.1065	0.0857	0.0749	0.1081	0.1033		0.0130	0.0150	0.0099	0.0170	0.0094	0.0163	0.0169	0.0156	0.0131	0.0140	0.0165	0.0156	0.0174	0.0163	0.0140	0.0133	0.0101	0.0121	0.0153	0.0135	0.0141	0.0140	0.0143	0.0157	0.0168	0.0149	0.0172	0.0169	0.0163
7	OQ858306.1_Sparus_aurata	0.0948	0.0589	0.0798	0.0780	0.0687	0.0715		0.0129	0.0109	0.0155	0.0148	0.0123	0.0143	0.0121	0.0102	0.0108	0.0127	0.0128	0.0133	0.0125	0.0106	0.0113	0.0109	0.0111	0.0133	0.0107	0.0114	0.0088	0.0093	0.0096	0.0139	0.0097	0.0198	0.0191	0.0186
8	MK919138.1_Acanthopagrus_datnia	0.0967	0.0648	0.0989	0.0935	0.0979	0.0937	0.0696		0.0127	0.0156	0.0164	0.0145	0.0164	0.0150	0.0134	0.0137	0.0152	0.0156	0.0156	0.0148	0.0144	0.0140	0.0140	0.0132	0.0145	0.0147	0.0134	0.0124	0.0125	0.0156	0.0164	0.0148	0.0204	0.0203	0.0199
9	AY857945.2_Archosargus_probatocephalus	0.0883	0.0632	0.0779	0.0688	0.0692	0.0481	0.0543	0.0702		0.0149	0.0107	0.0108	0.0135	0.0102	0.0090	0.0093	0.0112	0.0109	0.0125	0.0110	0.0092	0.0090	0.0057	0.0092	0.0115	0.0143	0.0097	0.0119	0.0117	0.0125	0.0149	0.0121	0.0165	0.0164	0.0157
10	KU510485.1_Boops_environmental	0.0018	0.1008	0.1243	0.1193	0.1362	0.1116	0.0995	0.1014	0.0919		0.0174	0.0203	0.0203	0.0203	0.0164	0.0175	0.0198	0.0195	0.0209	0.0202	0.0173	0.0180	0.0149	0.0164	0.0191	0.0158	0.0159	0.0151	0.0167	0.0182	0.0113	0.0164	0.0193	0.0198	0.0192
11	HQ592214.1_Chrysoblephus_puniceus	0.1068	0.0929	0.0872	0.0987	0.1008	0.0419	0.0853	0.1003	0.0517	0.1116		0.0158	0.0170	0.0151	0.0130	0.0143	0.0160	0.0150	0.0172	0.0158	0.0144	0.0146	0.0106	0.0135	0.0153	0.0152	0.0145	0.0155	0.0144	0.0167	0.0195	0.0169	0.0185	0.0184	0.0183
12	AF365357.1_Diplodus_argenteus_argenteus	0.1280	0.0674	0.0498	0.0063	0.0546	0.0912	0.0572	0.0748	0.0473	0.1280	0.0831		0.0104	0.0036	0.0066	0.0036	0.0042	0.0077	0.0070	0.0036	0.0060	0.0116	0.0129	0.0128	0.0088	0.0147	0.0107	0.0142	0.0134	0.0136	0.0197	0.0141	0.0197	0.0196	0.0200
13	AJ247289.1_Diplodus_bellottii	0.1280	0.0777	0.0333	0.0448	0.0832	0.0947	0.0725	0.0903	0.0677	0.1280	0.0915	0.0425		0.0103	0.0094	0.0100	0.0110	0.0089	0.0104	0.0105	0.0088	0.0133	0.0147	0.0124	0.0126	0.0148	0.0127	0.0161	0.0167	0.0171	0.0209	0.0162	0.0206	0.0206	0.0209
14	AF365359.1_Diplodus_bermudensis	0.1280	0.0701	0.0548	0.0085	0.0546	0.0856	0.0547	0.0775	0.0424	0.1280	0.0777	0.0064	0.0425		0.0066	0.0042	0.0048	0.0077	0.0074	0.0042	0.0060	0.0117	0.0122	0.0128	0.0091	0.0146	0.0107	0.0142	0.0135	0.0137	0.0203	0.0142	0.0198	0.0197	0.0201
15	KU510487.1_Diplodus_environmental	0.0973	0.0652	0.0431	0.0416	0.0639	0.0762	0.0502	0.0733	0.0399	0.1021	0.0717	0.0216	0.0377	0.0216		0.0057	0.0067	0.0044	0.0075	0.0063	0.0026	0.0102	0.0110	0.0103	0.0089	0.0111	0.0097	0.0123	0.0120	0.0127	0.0159	0.0127	0.0180	0.0174	0.0171
16	AF247421.1_Diplodus_holbrooki	0.1086	0.0620	0.0497	0.0302	0.0584	0.0822	0.0532	0.0765	0.0423	0.1139	0.0813	0.0064	0.0402	0.0085	0.0186		0.0043	0.0077	0.0074	0.0036	0.0055	0.0109	0.0109	0.0109	0.0077	0.0133	0.0091	0.0121	0.0118	0.0122	0.0169	0.0122	0.0179	0.0169	0.0171
17	AF365360.1_Diplodus_noct	0.1222	0.0672	0.0549	0.0021	0.0595	0.0966	0.0622	0.0800	0.0521	0.1222	0.0884	0.0085	0.0473	0.0106	0.0216	0.0085		0.0079	0.0078	0.0021	0.0061	0.0121	0.0128	0.0131	0.0095	0.0143	0.0111	0.0146	0.0140	0.0141	0.0198	0.0140	0.0199	0.0198	0.0202
18	AF365353.1_Diplodus_prayensis	0.1168	0.0699	0.0404	0.0239	0.0726	0.0859	0.0622	0.0823	0.0474	0.1168	0.0779	0.0262	0.0331	0.0262	0.0085	0.0262	0.0262		0.0077	0.0074	0.0044	0.0113	0.0128	0.0120	0.0107	0.0137	0.0112	0.0148	0.0147	0.0150	0.0192	0.0153	0.0201	0.0201	0.0204
19	AJ247291.1_Diplodus_puntazzo	0.1280	0.0648	0.0548	0.0240	0.0728	0.0969	0.0650	0.0775	0.0573	0.1280	0.0915	0.0218	0.0425	0.0241	0.0261	0.0242	0.0263	0.0262		0.0073	0.0069	0.0121	0.0151	0.0131	0.0067	0.0148	0.0126	0.0131	0.0144	0.0147	0.0208	0.0139	0.0209	0.0205	0.0213
20	AJ247293.1_Diplodus_sargus	0.1252	0.0699	0.0524	0.0000	0.0570	0.0938	0.0596	0.0773	0.0496	0.1252	0.0856	0.0063	0.0448	0.0085	0.0194	0.0063	0.0021	0.0239	0.0240		0.0056	0.0117	0.0125	0.0127	0.0090	0.0147	0.0108	0.0143	0.0136	0.0138	0.0201	0.0142	0.0196	0.0194	0.0199
21	GQ485269.1_Diplodus_vulgaris	0.0994	0.0650	0.0410	0.0401	0.0637	0.0794	0.0491	0.0800	0.0380	0.1046	0.0784	0.0172	0.0331	0.0172	0.0038	0.0154	0.0172	0.0085	0.0216	0.0150		0.0097	0.0113	0.0103	0.0089	0.0127	0.0098	0.0128	0.0125	0.0122	0.0166	0.0127	0.0192	0.0178	0.0182
22	JX282345.1_Gymnocrotaphus_curvidens	0.1046	0.0597	0.0773	0.0807	0.0829	0.0715	0.0548	0.0751	0.0348	0.1101	0.0784	0.0525	0.0654	0.0525	0.0461	0.0505	0.0574	0.0502	0.0525	0.0549	0.0415		0.0102	0.0095	0.0122	0.0134	0.0108	0.0126	0.0134	0.0132	0.0163	0.0125	0.0210	0.0185	0.0192
23	MG459222.1_Lagodon_rhomboides	0.0886	0.0678	0.0827	0.0777	0.0759	0.0501	0.0542	0.0834	0.0180	0.0921	0.0537	0.0623	0.0782	0.0571	0.0567	0.0549	0.0621	0.0625	0.0782	0.0595	0.0556	0.0481		0.0098	0.0131	0.0150	0.0096	0.0117	0.0122	0.0128	0.0146	0.0118	0.0161	0.0161	0.0153
24	AF247410.1_Lithognathus_mormyrus	0.1008	0.0705	0.0707	0.0788	0.0816	0.0639	0.0529	0.0755	0.0401	0.1057	0.0765	0.0597	0.0575	0.0597	0.0466	0.0528	0.0621	0.0548	0.0597	0.0595	0.0444	0.0414	0.0463		0.0110	0.0135	0.0105	0.0120	0.0125	0.0125	0.0164	0.0128	0.0172	0.0166	0.0160
25	AF247399.1_Oblada_melanura	0.1185	0.0662	0.0586	0.0549	0.0718	0.0890	0.0709	0.0808	0.0570	0.1241	0.0860	0.0310	0.0573	0.0333	0.0384	0.0284	0.0356	0.0450	0.0195	0.0332	0.0359	0.0573	0.0702	0.0504		0.0155	0.0110	0.0123	0.0134	0.0139	0.0184	0.0131	0.0183	0.0178	0.0174
26	GU946590.1_Pachymetopon_blochii	0.0951	0.0677	0.0799	0.0917	0.0927	0.0782	0.0546	0.0867	0.0816	0.0988	0.0872	0.0752	0.0779	0.0752	0.0585	0.0767	0.0750	0.0701	0.0752	0.0777	0.0689	0.0689	0.0907	0.0788	0.0881		0.0137	0.0130	0.0144	0.0159	0.0170	0.0148	0.0188	0.0189	0.0187
27	AF247411.1_Pagellus_acarne	0.0958	0.0681	0.0707	0.0677	0.0561	0.0820	0.0596	0.0759	0.0464	0.1006	0.0811	0.0450	0.0575	0.0450	0.0448	0.0403	0.0498	0.0476	0.0549	0.0473	0.0425	0.0481	0.0463	0.0505	0.0485	0.0746		0.0117	0.0126	0.0130	0.0150	0.0127	0.0163	0.0151	0.0156
28	GU946632.1_Rhabdosargus_globiceps	0.0944	0.0627	0.0846	0.0895	0.0796	0.0848	0.0400	0.0669	0.0670	0.0991	0.0947	0.0727	0.0858	0.0727	0.0671	0.0663	0.0778	0.0778	0.0648	0.0751	0.0672	0.0664	0.0647	0.0614	0.0660	0.0722	0.0593		0.0087	0.0101	0.0148	0.0077	0.0193	0.0183	0.0180
29	OM831065.1_Rhabdosargus_haffara	0.1093	0.0769	0.0877	0.0765	0.0808	0.0835	0.0407	0.0704	0.0631	0.1123	0.0835	0.0690	0.0957	0.0690	0.0634	0.0624	0.0741	0.0794	0.0769	0.0715	0.0632	0.0721	0.0674	0.0665	0.0759	0.0862	0.0712	0.0365		0.0048	0.0152	0.0096	0.0200	0.0194	0.0190
30	KY855474.1_Rhabdosargus_sarba	0.1139	0.0809	0.0916	0.0951	0.0824	0.0930	0.0425	0.0952	0.0661	0.1196	0.0996	0.0695	0.0964	0.0695	0.0668	0.0643	0.0746	0.0799	0.0774	0.0719	0.0618	0.0706	0.0707	0.0639	0.0783	0.0977	0.0735	0.0469	0.0118		0.0162	0.0100	0.0217	0.0194	0.0198
31	AF247402.1_Sarpa_salpa	0.0552	0.0986	0.1206	0.1377	0.1331	0.1100	0.0814	0.1157	0.0907	0.0587	0.1296	0.1218	0.1311	0.1280	0.0981	0.1095	0.1222	0.1168	0.1280	0.1252	0.1005	0.0986	0.0861	0.1043	0.1194	0.1125	0.0949	0.0930	0.0987	0.1047		0.0153	0.0202	0.0193	0.0190
32	AF247430.1_Sparodon_durbanensis	0.0981	0.0794	0.0948	0.1001	0.0902	0.0884	0.0446	0.0877	0.0652	0.1030	0.1045	0.0753	0.0886	0.0753	0.0703	0.0701	0.0751	0.0833	0.0726	0.0778	0.0668	0.0663	0.0629	0.0675	0.0745	0.0882	0.0700	0.0304	0.0433	0.0444	0.0946		0.0195	0.0186	0.0183
33	EU099495.1_Hemilepidotus_hemilepidotus	0.1314	0.1501	0.1372	0.1181	0.1470	0.1099	0.1326	0.1451	0.1069	0.1327	0.1210	0.1270	0.1332	0.1270	0.1211	0.1205	0.1329	0.1304	0.1364	0.1298	0.1280	0.1350	0.1019	0.1120	0.1205	0.1224	0.0996	0.1292	0.1362	0.1452	0.1425	0.1311		0.0040	0.0020
34	AY539021.2_Hemilepidotus_jordani	0.1305	0.1472	0.1354	0.1506	0.1582	0.1092	0.1304	0.1479	0.1070	0.1365	0.1249	0.1268	0.1329	0.1268	0.1196	0.1169	0.1327	0.1301	0.1329	0.1296	0.1208	0.1213	0.1046	0.1090	0.1222	0.1271	0.0946	0.1246	0.1323	0.1310	0.1378	0.1273	0.0080		0.0042
35	EF458449.1_Hemilepidotus_spinosus	0.1291	0.1426	0.1386	0.1487	0.1561	0.1056	0.1264	0.1449	0.1032	0.1350	0.1277	0.1301	0.1364	0.1301	0.1186	0.1188	0.1361	0.1335	0.1395	0.1329	0.1257	0.1241	0.0985	0.1060	0.1188	0.1301	0.0993	0.1234	0.1309	0.1359	0.1365	0.1261	0.0019	0.0094	

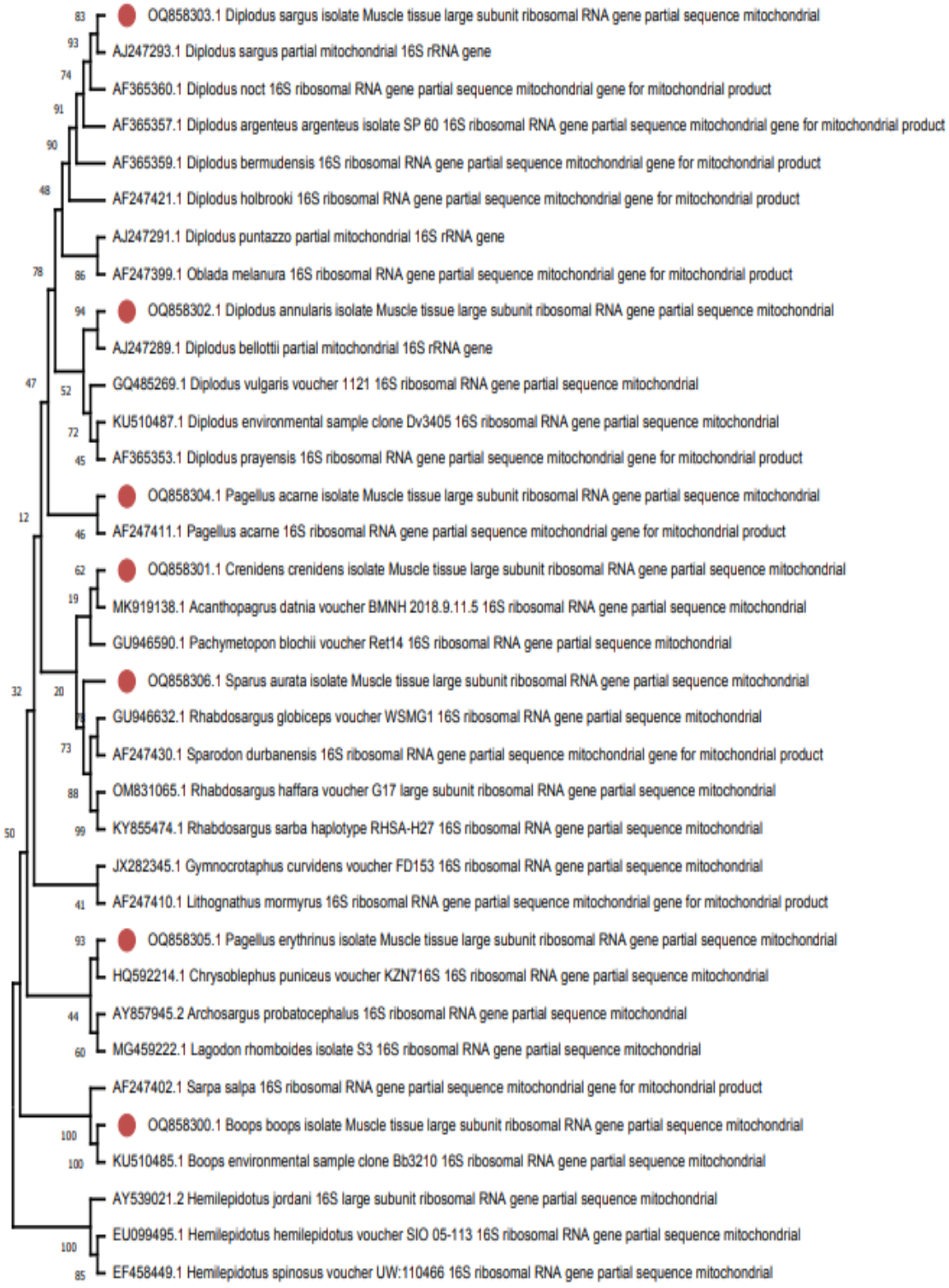

Fig. 2. Neighbor joining phylogenetic tree in the seven sparid fishes and their linked sparid species with the outgroup by employing the *16S rRNA* gene

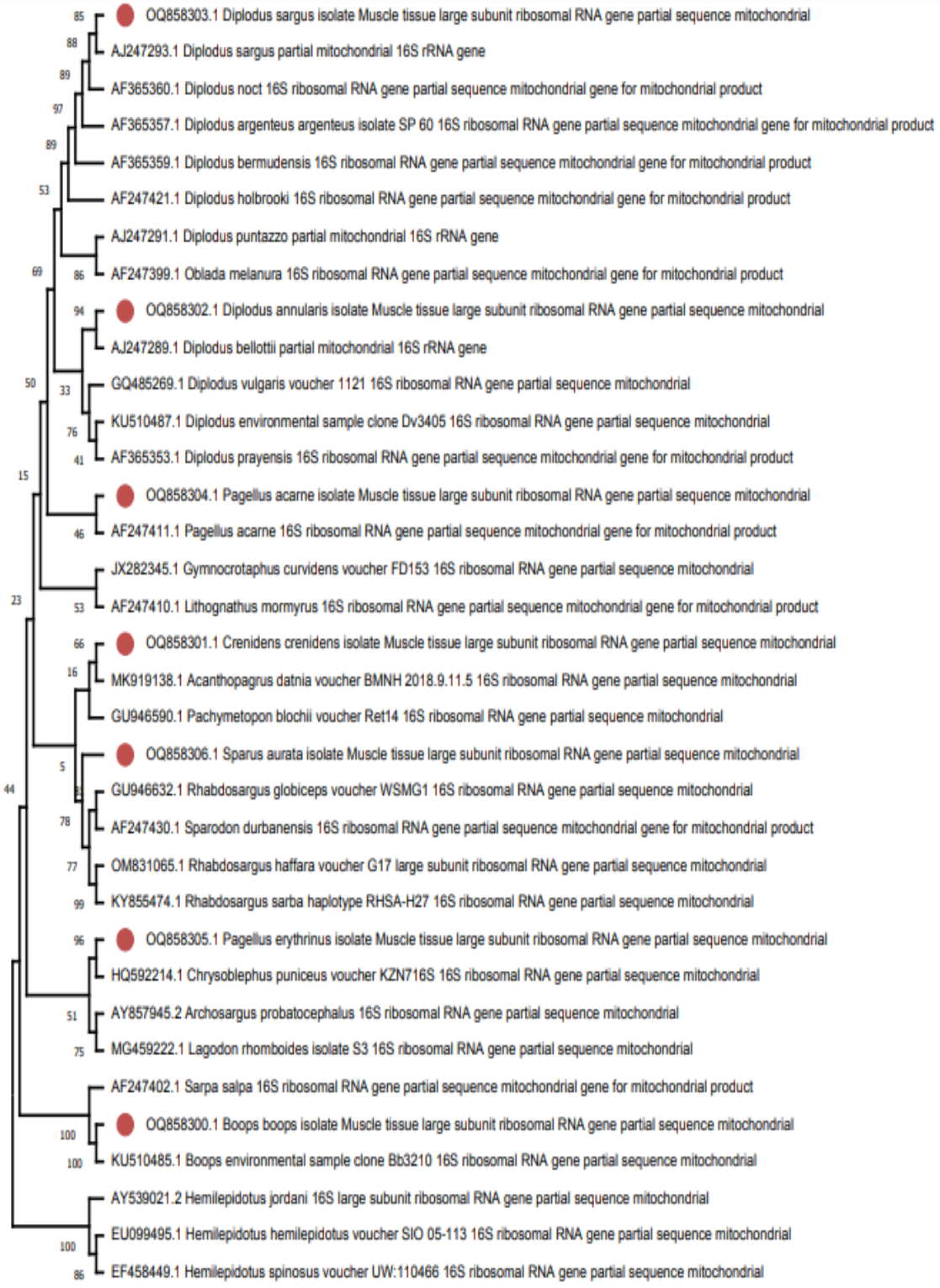

Fig. 3. Minimum evolution phylogenetic tree in seven sparid fish and their linked sparid species with the outgroup by employing the *16S rRNA* gene

In order to use the *16S rRNA* sequence phylogenetic tree analysis, seven species of the sparid fish were examined in conjunction with the 26 related species of the sparid fish and the outgroup species from the GenBank/NCBI. By utilizing the *16S rRNA* gene, two phylogenetic techniques were applied to produce more interpretive phylogenetic relationships. Neighbor joining and minimum evolution were these methods. The methods yielded results that were nearly identical and showed three main patterns with minor adjustments to the support parameters: (1) species of the outgroup were located in a different cluster; (2) *Oblada melanura* (AF247399.1) found amongst the species of the genus *Diplodus*; and (3) the non-monophyly of the genera; *Diplodus*, *Rhabdosargus*, and *Pagellus* (Figs. 2- 3).

DISCUSSION

Similar morphological characteristics among the species are derived from the convergent evolution, and speciation patterns are highly complex in most taxa of the coastal fish that are distributed worldwide, making it challenging to infer accurate phylogenetic relationships and to classify the fish appropriately **(Westneat *et al*., 2005; Duftner *et al*., 2007)**.

Species belonging to the family Sparidae have highly similar characteristics. Consequently, only proficient taxonomists are capable of achieving the morphological identification. The most distinctive feature that allows the family Sparidae to be identified is their specialized dentition. Even with complete samples available, it is practically impossible to distinguish between prepared and processed items during exams due to the great degree of resemblance across the various species in the family Sparidae **(Smith & Smith, 1986; Ibrahim *et al*., 2020)**.

One effective method for resolving the issue of misleading morphological identification is the application of DNA markers **(Armani *et al*., 2012; Abou-Gabal *et al*., 2018; Ali & Mamoon, 2019)**. Fish phylogenetic relationships at various taxonomic levels are frequently investigated using the mitochondrial (*16S rRNA*) gene **(Faddagh *et al*., 2012)**. This region's sequence segments can be effectively utilized to build phylogenies **(Singh *et al*., 2015)**.

The average length of nucleotide sequences obtained from the understudied sparid fish using *16S rRNA* was approximately 550bp. This length was within the expectations according to **Simon *et al*. (1991)**, who reported that the 16Sar and 16Sbr primers amplify a 500–650 base fragment. In addition, other investigations detected the same result **(Mar'ie & Allam, 2019; Alyamani *et al*., 2023)**. The final alignments of incomplete *16S rRNA* sequences in the seven sparid species included 585bp. The conserved, parsimony informative, and variable sites were 451, 45 and 113, respectively, which illustrated highly conserved sites. According to **Basheer *et al*. (2015)**, the *16S rRNA* aligned

sequences of the three Rastrelliger species have 575 consistent sites, totaling 590bp in length. A phylogenetic analysis of cichlids using the *16S rRNA* gene by **Sokefun (2017)** showed 463bp of alignment with 72.7% (337) conserved sites. Our results of the *16S rRNA* gene displayed a (A+T) content higher than the (C+G) content. This was supported by multiple investigations **(Singh *et al.*, 2015; Mahrous & Allam, 2022; Ramadan *et al.*, 2023)**.

Several studies utilizing various DNA markers were carried out to assess the evolutionary relationship between the family Sparidae's species and genera. **Chiba *et al.* (2009)** used cytochrome b gene data to offer molecular phylogeny of 15 sparid species in the family Sparidae. **Abbas *et al.* (2017)** created a genetic barcode on 22 fish species belonging to the family Sparidae, utilizing the mitochondrial gene cytochrome oxidase subunit I (COI). **Ibrahim *et al.* (2020)** using the Cyt-b mitochondrial gene, assessed the genetic diversity of 22 species of the family Sparidae from Egypt's Red and Mediterranean Seas.

The low P-distance of 0.0113 between *Crenidens crenidens* (OQ858301.1) and *Sparus aurata* (OQ858306.1) indicates a close relationship between them. Similarly, the low P-distance between *Oblada melanura* (AF247399.1) and *Diplodus puntazzo* (AJ247291.1) indicates a close relationship between *Oblada melanura* and species of the genus *Diplodus*. This observation aligns with **Kaleshkumar *et al.* (2015)**, who reported that significant genetic divergence results in higher genetic distance, whereas closely related species exhibit low genetic distance values.

The non-monophyly of the genera *Diplodus*, *Rhabdosargus*, and *Pagellus* is consistent with the findings of **Chiba *et al.* (2009)**. They reported the non-monophyly of the genera *Diplodus*, *Dentex*, *Pagrus*, and *Pelagius* within the family Sparidae using the Cytochrome b (Cyt-b) gene.

CONCLUSION

Through the analysis of large subunit ribosomal RNA (*16S rRNA*) sequences, this study aimed to determine the phylogenetic lineages of certain species in the Sparidae family. Our results indicated a close relationship between *Oblada melanura* and species of the genus *Diplodus*. Additionally, our data support previous findings of other authors, suggesting that some genera in the Sparidae family are not monophyletic and require further revision.

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