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Phylogenetic Diversity of Some Snappers (Lutjanidae: Perciformes) Inferred from Mitochondrial *16S rRNA* Sequences

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ABSTRACT

To conserve species and determine suitable management plans, the insidious and accurate identification of a species is fundamental. Despite the commercial importance of Lutjanidae family, the phylogeny of Lutjanidae has not been fully studied. Thus, this study focused on the phylogeny of this family using mitochondrial *16S rRNA* sequences in four species; *Lutjanus fulviflamma*, *Lutjanus monostigma*, *Lutjanus bohar* and *Lutjanus kasmira*.The generated bands of *16S rRNA* in the four species prolong from 561 to 575 bp. The sequences of *16S rRNA* were displayed in GenBank/NCBI to gain the accession numbers (OQ803478.1 - OQ803481.1). The average frequencies of adenine (A), thymine (T), cytosine (C) and guanine (G) were 28.91, 21.93, 25.51 and 23.65%, respectively.

INTRODUCTION

Several species of snappers have a commercial importance, and they play important roles in artisanal fisheries across many tropical countries. Snappers are energetic predators; they use their strength caniniform teeth to feed on large crustaceans or fishes **(Allen, 1985)**. The family Lutjanidae (Snappers) comprises medium to large- sized fishes, with five subfamilies distributed into 21 genera and about 135 species **(Johnson 1993; Miller & Cribb, 2007; Froese & Pauly, 2016; Eschmeyer *et al*., 2016)**. The subfamily Lutjaninae comprises six genera; *Pinjalo*, *Macolor*, *Hoplopagrus*, *Ocyurus*, *Rhomboplites* and *Lutjanus*. The most taxonomic diversity genus of Lutjaninae is *Lutjanus*, with about 71 species **(Iwatsuki *et al*., 2015; Froese & Pauly, 2016; Veneza *et al*., 2019)**.

The mitochondrial DNA based methods are considered more species-specific, firm and credible for species identification than the nuclear DNA-based analysis **(Branicki *et al*., 2003)**. The identification of species play an important role in the suitable management and preservation of threatened species **(Guha *et al*., 2006)**. The *16S rRNA* gene is used in wide scale to describe the phylogenetic trees of exceedingly related or symbiotic organisms **(Nakahara *et al*., 2004; Metallinou *et al*., 2012; Al-Qahtani & Amer, 2019)**. In order to restrict populations and protect marine species, genetic information is important for developing strategies **(Souza *et al*., 2019)**.

The basic goal of this work was to evaluate the phylogenetic linkages of some species of snappers belonging to family Lutjanidae by the mean of large mitochondrial rRNA (*16S rRNA*) gene.

MATERIALS AND METHODS

Samples collection and species identification

The Egyptian Red Sea is the study sampling collection location where four species of family Lutjanidae (*Lutjanus fulviflamma, Lutjanus monostigma, Lutjanus bohar* and *Lutjanus kasmira*) were grouped and identified **(Randall, 1982)**. The muscles of the samples were removed and conserved at -20ºC for DNA isolation.

DNA isolation, and PCR amplification

From the conserved muscles, total genomic DNA was extracted using the manufacturer's instructions of DNA Mini kit (Qiagen, Hidden, Germany). PCR was used to amplify partial sequence of mitochondrial *16S rRNA* with previous described primers **(Simon *et al*., 1991)**. The PCR was completed in 46µL with 23µL of 2X master mix, 1µL of genomic DNA, 1µL of each primer, and 20µL of nuclease-free water. The amplification conditions were denaturated at 95°C for 5min, followed by 30 cycles of denaturation, annealing, and extension at 94, 48 and 72°C, respectively, for 60s, with an extension at 72°C for 7min as a last step. PCR products were electrophoresed on a 1.5% agarose gel comprising ethidium bromide with 100bp DNA Ladder.

Sequences and phylogenetic analysis

The sequences were completed by Macrogen (Seoul, South Korea). To gain the accession numbers, the sequences of *16S rRNA* were displayed in GenBank/NCBI. CLUSTAL W **(Thompson *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

By dint of large subunit ribosomal RNA (*16S rRNA*) sequences, this work predestined the phylogenetic lineages of four species of family Lutjanidae, viz. *Lutjanus fulviflamma, Lutjanus monostigma, Lutjanus bohar* and *Lutjanus kasmira*.

The generated bands of *16S rRNA* in the four species prolong from 561 to 575 bp. The sequences of *16S rRNA* were displayed in GenBank/NCBI to gain the accession numbers (OQ803478.1 - OQ803481.1). The results illustrate that *Lutjanus fulviflamma* has the longest (575 bp.) sequence, while *Lutjanus monostigma* has the shortest sequence (561 bp.). The average frequencies of adenine (A), thymine (T), cytosine (C) and guanine (G) were 28.91, 21.93, 25.51 and 23.65%, respectively. The average A+T attribution was higher than the C+G attribution (Table 1). The final alignments consisted of 575 bp. The conserved, Parsimony informative, and variable sites were 535, 4 and 32, respectively.

Amidst the whole fishes, the P-distances varied from 0.0035 to 3.235%. Overall, the distance value was 0.25%. Amongst the *Lutjanus* species, the P-distances varied from 0.0035 to 0.0116%. The biggest value (0.0116) was present between *Lutjanus erythropterus* and *Lutjanus johnii*, while (0.0035) value was present between *Lutjanus griseus* and *Lutjanus jocu*. Amidst the *Lutjanus* species under study, the P-distances varied from 0.0072 to 0.0093%. The biggest proportion (0.0093) was present between *Lutjanus monostigma* and *Lutjanus bohar*. While, the lowest P-distance (0.0072) was present between *Lutjanus fulviflamma* and *Lutjanus monostigma* (Table 2).

To complete the phylogenetic tree investigation by the dint of *16S rRNA* sequence, the sequences acquired from four fish of family Lutjanidae, as well as 29 linked sequences and the out-group species from GenBank, were exercised in this work for widely combination phylogenetic investigation (Table 3). For widely illustrative phylogenetic investigation by using *16S rRNA* gene, more than one phylogenetic method was used: Neighbor Joining and Minimum Evolution. With some variation in the support rate, the approaches produced findings that were basically similar and illustrate three basic lineaments: (1) species of the outgroup forming a separate cluster. (2) species of genus *Lutjanus* were non monophyletic. (3) species of genus *Pterocaesio* were non monophyletic (Figs. 1, 2).

Table 1. Accession number, nucleotide frequencies, A+T contents and their averages of (*16S rRNA*) sequences in four species of genus *Lutjanus*

No.	Species	Accession number	Base pair length	Nucleotide Number %				A+T Content (%)
				A%	T%	C %	G%	
1	*Lutjanus fulviflamma*	OQ803478.1	575	29.22	22.26	25.22	23.30	51.48
2	*Lutjanus monostigma*	OQ803479.1	561	28.17	21.75	26.02	24.06	49.92
3	*Lutjanus bohar*	OQ803480.1	562	29.18	22.06	25.27	23.49	51.24
4	*Lutjanus kasmira*	OQ803481.1	564	29.08	21.63	25.53	23.76	50.71
	Average %	-	565.5	28.91	21.93	25.51	23.65	50.84

Table 2: Pairwise distances by the mean of *16S rRNA* gene amongst four species of genus *Lutjanus* with their linkage species and the out-group 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	36
1	*Lutjanus_fulviflamma*		0.0072	0.0085	0.0076	0.0075	0.0060	0.0097	0.0067	0.0073	0.0055	0.0071	0.0092	0.0084	0.0072	0.0066	0.0078	0.0091	0.0067	2.7784	0.0083	0.0065	0.0072	0.0066	0.0080	0.0086	0.0100	0.0094	0.0070	0.0089	0.0087	0.0090	0.0092	0.0091	0.0191	0.0190	0.0168
2	*Lutjanus_monostigma*	0.0241		0.0093	0.0079	0.0077	0.0064	0.0095	0.0081	0.0079	0.0070	0.0067	0.0094	0.0090	0.0072	0.0067	0.0077	0.0103	0.0059	2.4946	0.0078	0.0073	0.0082	0.0074	0.0086	0.0093	0.0100	0.0104	0.0078	0.0088	0.0088	0.0092	0.0097	0.0097	0.0195	0.0192	0.0178
3	*Lutjanus_bohar*	0.0357	0.0419		0.0091	0.0092	0.0076	0.0095	0.0083	0.0081	0.0080	0.0090	0.0076	0.0097	0.0081	0.0089	0.0083	0.0109	0.0084	4.2377	0.0094	0.0082	0.0075	0.0083	0.0094	0.0092	0.0095	0.0094	0.0085	0.0090	0.0090	0.0092	0.0089	0.0082	0.0192	0.0191	0.0178
4	*Lutjanus_kasmira*	0.0297	0.0320	0.0382		0.0072	0.0058	0.0088	0.0072	0.0085	0.0076	0.0083	0.0093	0.0054	0.0062	0.0069	0.0063	0.0092	0.0070	2.5321	0.0032	0.0081	0.0056	0.0069	0.0084	0.0090	0.0106	0.0097	0.0069	0.0088	0.0088	0.0090	0.0087	0.0101	0.0199	0.0191	0.0178
5	*Lutjanus_analis*	0.0292	0.0299	0.0400	0.0260		0.0068	0.0092	0.0064	0.0065	0.0077	0.0080	0.0087	0.0090	0.0070	0.0031	0.0065	0.0094	0.0074	2.2863	0.0068	0.0083	0.0076	0.0040	0.0068	0.0091	0.0100	0.0090	0.0043	0.0087	0.0087	0.0091	0.0093	0.0081	0.0188	0.0182	0.0168
6	*Lutjanus_apodus*	0.0187	0.0206	0.0286	0.0169	0.0227		0.0072	0.0073	0.0075	0.0064	0.0064	0.0082	0.0076	0.0045	0.0064	0.0045	0.0087	0.0060	2.5187	0.0064	0.0066	0.0066	0.0051	0.0076	0.0089	0.0093	0.0092	0.0072	0.0079	0.0079	0.0082	0.0086	0.0089	0.0186	0.0178	0.0169
7	*Lutjanus_argentimaculatus*	0.0448	0.0418	0.0439	0.0378	0.0410	0.0246		0.0083	0.0095	0.0087	0.0092	0.0090	0.0093	0.0069	0.0089	0.0075	0.0109	0.0091	1.4215	0.0091	0.0086	0.0091	0.0086	0.0090	0.0100	0.0092	0.0099	0.0089	0.0097	0.0098	0.0100	0.0092	0.0096	0.0190	0.0195	0.0172
8	*Lutjanus_buccanella*	0.0242	0.0326	0.0345	0.0263	0.0205	0.0251	0.0341		0.0046	0.0058	0.0081	0.0085	0.0081	0.0069	0.0062	0.0072	0.0104	0.0070	2.7237	0.0076	0.0069	0.0075	0.0055	0.0060	0.0086	0.0096	0.0091	0.0051	0.0085	0.0085	0.0088	0.0082	0.0066	0.0199	0.0190	0.0174
9	*Lutjanus_campechanus*	0.0281	0.0305	0.0324	0.0344	0.0205	0.0272	0.0422	0.0110		0.0074	0.0075	0.0089	0.0088	0.0081	0.0062	0.0077	0.0106	0.0077	1.6027	0.0082	0.0080	0.0077	0.0060	0.0043	0.0092	0.0096	0.0092	0.0052	0.0086	0.0086	0.0090	0.0088	0.0061	0.0196	0.0190	0.0179
10	*Lutjanus_carponotatus*	0.0162	0.0241	0.0317	0.0297	0.0294	0.0206	0.0371	0.0184	0.0281		0.0071	0.0087	0.0089	0.0065	0.0073	0.0067	0.0103	0.0055	2.8268	0.0078	0.0041	0.0075	0.0068	0.0083	0.0091	0.0097	0.0093	0.0072	0.0087	0.0087	0.0090	0.0088	0.0083	0.0190	0.0185	0.0166
11	*Lutjanus_decussatus*	0.0261	0.0225	0.0363	0.0340	0.0301	0.0210	0.0400	0.0321	0.0281	0.0261		0.0094	0.0091	0.0077	0.0075	0.0072	0.0102	0.0052	2.2070	0.0080	0.0067	0.0075	0.0077	0.0079	0.0087	0.0101	0.0097	0.0077	0.0076	0.0076	0.0080	0.0084	0.0099	0.0189	0.0189	0.0172
12	*Lutjanus_erythropterus*	0.0406	0.0438	0.0299	0.0417	0.0388	0.0325	0.0408	0.0339	0.0359	0.0370	0.0439		0.0093	0.0088	0.0091	0.0083	0.0116	0.0089	1.6525	0.0098	0.0095	0.0077	0.0081	0.0092	0.0085	0.0088	0.0091	0.0085	0.0096	0.0097	0.0099	0.0080	0.0092	0.0211	0.0201	0.0186
13	*Lutjanus_fulvus*	0.0358	0.0398	0.0443	0.0147	0.0400	0.0285	0.0421	0.0345	0.0386	0.0398	0.0404	0.0423		0.0071	0.0082	0.0077	0.0107	0.0079	2.1221	0.0057	0.0095	0.0075	0.0085	0.0084	0.0093	0.0107	0.0101	0.0075	0.0098	0.0098	0.0101	0.0090	0.0099	0.0215	0.0199	0.0191
14	*Lutjanus_griseus*	0.0274	0.0261	0.0341	0.0203	0.0256	0.0111	0.0236	0.0244	0.0324	0.0237	0.0302	0.0390	0.0262		0.0063	0.0035	0.0088	0.0073	1.8961	0.0065	0.0070	0.0077	0.0061	0.0079	0.0086	0.0099	0.0095	0.0071	0.0086	0.0086	0.0089	0.0088	0.0094	0.0195	0.0191	0.0177
15	*Lutjanus_guttatus*	0.0235	0.0240	0.0379	0.0241	0.0053	0.0207	0.0390	0.0186	0.0186	0.0275	0.0281	0.0408	0.0340	0.0199		0.0068	0.0088	0.0069	2.3149	0.0065	0.0079	0.0073	0.0045	0.0066	0.0089	0.0098	0.0089	0.0048	0.0083	0.0083	0.0087	0.0091	0.0079	0.0193	0.0184	0.0171
16	*Lutjanus_jocu*	0.0312	0.0299	0.0340	0.0203	0.0217	0.0111	0.0273	0.0244	0.0283	0.0237	0.0262	0.0349	0.0300	0.0071	0.0236		0.0098	0.0073	1.6196	0.0065	0.0070	0.0077	0.0055	0.0076	0.0085	0.0099	0.0094	0.0071	0.0086	0.0086	0.0089	0.0087	0.0096	0.0196	0.0189	0.0179
17	*Lutjanus_johnii*	0.0421	0.0504	0.0572	0.0405	0.0445	0.0369	0.0549	0.0474	0.0496	0.0527	0.0515	0.0614	0.0525	0.0364	0.0382	0.0446		0.0101	3.2356	0.0097	0.0105	0.0089	0.0093	0.0113	0.0113	0.0125	0.0118	0.0091	0.0116	0.0116	0.0118	0.0116	0.0113	0.0194	0.0194	0.0178
18	*Lutjanus_ophuysenii*	0.0235	0.0183	0.0337	0.0257	0.0272	0.0186	0.0407	0.0261	0.0300	0.0161	0.0146	0.0406	0.0318	0.0273	0.0253	0.0273	0.0504		2.4129	0.0067	0.0061	0.0069	0.0070	0.0080	0.0087	0.0101	0.0095	0.0068	0.0077	0.0078	0.0080	0.0084	0.0092	0.0193	0.0188	0.0166
19	*Stereolepis_gigas*	3.7492	3.6232	4.5537	3.4969	3.6259	3.6080	3.2079	3.6259	3.2001	3.8806	3.6153	3.3053	3.5670	3.3982	3.7492	3.3522	4.1786	3.8100		2.0374	2.9739	3.2420	2.2530	1.3767	2.2512	1.5096	1.5742	1.7234	1.8935	1.8935	1.8181	1.6347	2.4656	4.7714	2.7882	2.4057
20	*Lutjanus_quinquelineatus*	0.0352	0.0318	0.0401	0.0054	0.0238	0.0207	0.0393	0.0283	0.0323	0.0313	0.0320	0.0452	0.0166	0.0220	0.0219	0.0219	0.0446	0.0236	3.3982		0.0084	0.0063	0.0071	0.0080	0.0092	0.0107	0.0092	0.0065	0.0084	0.0084	0.0086	0.0089	0.0097	0.0196	0.0192	0.0172
21	*Lutjanus_russelli*	0.0219	0.0261	0.0338	0.0337	0.0335	0.0226	0.0353	0.0242	0.0321	0.0089	0.0242	0.0432	0.0439	0.0258	0.0315	0.0258	0.0550	0.0200	3.9458	0.0354		0.0076	0.0074	0.0090	0.0093	0.0095	0.0101	0.0078	0.0088	0.0088	0.0091	0.0089	0.0089	0.0195	0.0193	0.0175
22	*Lutjanus_stellatus*	0.0261	0.0339	0.0282	0.0167	0.0282	0.0207	0.0384	0.0267	0.0287	0.0280	0.0285	0.0301	0.0281	0.0284	0.0263	0.0283	0.0403	0.0241	3.9344	0.0205	0.0300		0.0070	0.0084	0.0072	0.0089	0.0081	0.0068	0.0083	0.0083	0.0086	0.0069	0.0093	0.0195	0.0189	0.0177
23	*Lutjanus_synagris*	0.0235	0.0279	0.0339	0.0241	0.0089	0.0130	0.0370	0.0148	0.0186	0.0236	0.0281	0.0329	0.0360	0.0199	0.0107	0.0161	0.0424	0.0253	3.5099	0.0257	0.0276	0.0243		0.0065	0.0083	0.0100	0.0089	0.0054	0.0084	0.0085	0.0088	0.0086	0.0076	0.0193	0.0179	0.0173
24	*Lutjanus_vivanus*	0.0320	0.0346	0.0407	0.0323	0.0225	0.0272	0.0381	0.0167	0.0091	0.0341	0.0301	0.0379	0.0365	0.0304	0.0205	0.0263	0.0540	0.0320	3.1079	0.0303	0.0382	0.0328	0.0205		0.0097	0.0107	0.0101	0.0055	0.0090	0.0090	0.0093	0.0090	0.0072	0.0209	0.0198	0.0191
25	*Caesio_caerulaurea*	0.0392	0.0438	0.0399	0.0397	0.0413	0.0365	0.0493	0.0360	0.0401	0.0412	0.0379	0.0352	0.0420	0.0355	0.0393	0.0354	0.0569	0.0391	3.5787	0.0414	0.0433	0.0281	0.0353	0.0421		0.0075	0.0075	0.0087	0.0072	0.0072	0.0075	0.0032	0.0103	0.0206	0.0197	0.0185
26	*Caesio_cuning*	0.0492	0.0500	0.0440	0.0501	0.0494	0.0409	0.0432	0.0442	0.0442	0.0471	0.0503	0.0390	0.0547	0.0455	0.0473	0.0454	0.0703	0.0511	3.2520	0.0516	0.0453	0.0401	0.0473	0.0506	0.0277		0.0066	0.0091	0.0092	0.0092	0.0095	0.0072	0.0104	0.0215	0.0217	0.0197
27	*Macolor_niger*	0.0450	0.0521	0.0440	0.0440	0.0411	0.0407	0.0511	0.0401	0.0401	0.0430	0.0461	0.0409	0.0485	0.0433	0.0391	0.0432	0.0635	0.0469	3.1652	0.0415	0.0495	0.0342	0.0391	0.0463	0.0295	0.0236		0.0085	0.0087	0.0087	0.0090	0.0072	0.0102	0.0208	0.0199	0.0189
28	*Ocyurus_chrysurus*	0.0273	0.0319	0.0359	0.0241	0.0107	0.0266	0.0390	0.0129	0.0129	0.0275	0.0301	0.0349	0.0300	0.0256	0.0125	0.0256	0.0403	0.0253	3.2976	0.0219	0.0315	0.0243	0.0162	0.0148	0.0373	0.0412	0.0371		0.0081	0.0081	0.0084	0.0083	0.0070	0.0197	0.0191	0.0177
29	*Pterocaesio_digramma*	0.0425	0.0415	0.0397	0.0394	0.0387	0.0304	0.0467	0.0379	0.0379	0.0408	0.0317	0.0466	0.0479	0.0369	0.0367	0.0368	0.0588	0.0346	3.4583	0.0370	0.0430	0.0358	0.0367	0.0399	0.0255	0.0393	0.0370	0.0348		0.0000	0.0018	0.0069	0.0099	0.0194	0.0196	0.0180
30	*Pterocaesio_marri*	0.0408	0.0415	0.0397	0.0394	0.0390	0.0304	0.0471	0.0379	0.0379	0.0408	0.0317	0.0469	0.0479	0.0372	0.0370	0.0371	0.0588	0.0349	3.4583	0.0370	0.0430	0.0358	0.0370	0.0399	0.0255	0.0393	0.0371	0.0351	0.0000		0.0018	0.0070	0.0100	0.0194	0.0196	0.0180
31	*Pterocaesio_pisang*	0.0427	0.0435	0.0416	0.0413	0.0409	0.0324	0.0490	0.0398	0.0398	0.0427	0.0337	0.0489	0.0499	0.0391	0.0389	0.0390	0.0608	0.0368	3.4084	0.0389	0.0450	0.0378	0.0389	0.0419	0.0274	0.0412	0.0390	0.0370	0.0018	0.0018		0.0073	0.0102	0.0194	0.0196	0.0180
32	*Pterocaesio_tile*	0.0426	0.0457	0.0377	0.0376	0.0428	0.0344	0.0428	0.0339	0.0379	0.0389	0.0358	0.0329	0.0400	0.0370	0.0408	0.0369	0.0589	0.0366	3.3134	0.0392	0.0412	0.0261	0.0368	0.0379	0.0053	0.0256	0.0274	0.0349	0.0234	0.0235	0.0254		0.0095	0.0202	0.0196	0.0181
33	*Rhomboplites_aurorubens*	0.0409	0.0460	0.0338	0.0461	0.0314	0.0388	0.0470	0.0224	0.0185	0.0352	0.0442	0.0407	0.0483	0.0433	0.0294	0.0433	0.0572	0.0408	3.6904	0.0435	0.0394	0.0403	0.0294	0.0244	0.0495	0.0514	0.0471	0.0236	0.0487	0.0490	0.0510	0.0447		0.0194	0.0193	0.0175
34	*Trachinotus_blochii*	0.1236	0.1290	0.1313	0.1349	0.1236	0.1236	0.1264	0.1336	0.1306	0.1258	0.1241	0.1467	0.1510	0.1323	0.1264	0.1349	0.1287	0.1284	5.1814	0.1319	0.1320	0.1293	0.1294	0.1396	0.1406	0.1529	0.1432	0.1294	0.1310	0.1310	0.1310	0.1372	0.1320		0.0102	0.0069
35	*Trachinotus_baillonii*	0.1244	0.1304	0.1247	0.1258	0.1165	0.1140	0.1308	0.1224	0.1224	0.1193	0.1218	0.1330	0.1330	0.1278	0.1193	0.1247	0.1278	0.1241	3.9915	0.1275	0.1278	0.1227	0.1137	0.1283	0.1333	0.1510	0.1356	0.1221	0.1324	0.1324	0.1324	0.1327	0.1275	0.0484		0.0086
36	*Trachinotus_rhodopus*	0.0999	0.1111	0.1111	0.1093	0.0999	0.1031	0.1054	0.1056	0.1085	0.0994	0.1022	0.1163	0.1248	0.1111	0.1026	0.1137	0.1085	0.0992	3.7043	0.1052	0.1085	0.1090	0.1054	0.1173	0.1198	0.1311	0.1218	0.1054	0.1157	0.1157	0.1157	0.1160	0.1080	0.0214	0.0361	

Table 3. The understudied four species of genus *Lutjanus* and their related species in addition to the out-group species from the GenBank/NCBI by the mean of large subunit ribosomal RNA sequences

No.	Species	Accession number	No.	Species	Accession number
1	*Lutjanus fulviflamma*	OQ803478.1	19	*Stereolepis_gigas*	AY072683.1
2	*Lutjanus_monostigma*	OQ803479.1	20	*Lutjanus_quinquelineatus*	DQ784736.1
3	*Lutjanus_bohar*	OQ803480.1	21	*Lutjanus_russelli*	DQ784737.1
4	*Lutjanus_kasmira*	OQ803481.1	22	*Lutjanus_stellatus*	DQ444483.1
5	*Lutjanus_analis*	AY857938.2	23	*Lutjanus_synagris*	AY857939.2
6	*Lutjanus_apodus*	JQ741057.1	24	*Lutjanus_vivanus*	KX354248.1
7	*Lutjanus_argentimaculatus*	NC_016661.1	25	*Caesio_caerulaurea*	DQ784724.1
8	*Lutjanus_buccanella*	KX354282.1	26	*Caesio_cuning*	DQ784725.1
9	*Lutjanus_campechanus*	KX354240.1	27	*Macolor_niger*	DQ784740.1
10	*Lutjanus_carponotatus*	DQ784730.1	28	*Ocyurus_chrysurus*	AY857942.2
11	*Lutjanus_decussatus*	AF247445.1	29	*Pterocaesio_digramma*	LC549803.1
12	*Lutjanus_erythropterus*	NC_031331.1	30	*Pterocaesio_marri*	DQ784742.1
13	*Lutjanus_fulvus*	MK335865.1	31	*Pterocaesio_pisang*	DQ784743.1
14	*Lutjanus_griseus*	AY857944.2	32	*Pterocaesio_tile*	NC_004408.1
15	*Lutjanus_guttatus*	KT724723.1	33	*Rhomboplites_aurorubens*	AY857941.2
16	*Lutjanus_jocu*	AY857943.2	Out-group	*Trachinotus_blochii*	MT102364.1
17	*Lutjanus_johnii*	MW888468.1		*Trachinotus_baillonii*	LC646890.1
18	*Lutjanus_ophuysenii*	NC_056806.1		*Trachinotus_rhodopus*	HM778174.1

DISCUSSION

Phylogenetic parentage amongst Western Atlantic lutjanines are not well confirmed due to the similarities of morphological and behavioral features within the group, as well as the occurrence of both interspecific and intergeneric hybrids **(Domeier & Clarke, 1992; Sarver *et al*., 1996; Gold *et al*., 2011)**.

For samples' identification, the molecular ways should be credible, affordable, and attainable methods to differentiate amongst distinct genera and the species composing those genera. These advantages are preserved by the identifying mechanism (*16S rRNA*). Therefore, it is recommended for the reconstruction of beneficial phylogenetic linkages and suitable identification methods to investigate the evolution of fish **(Saad, 2019)**.

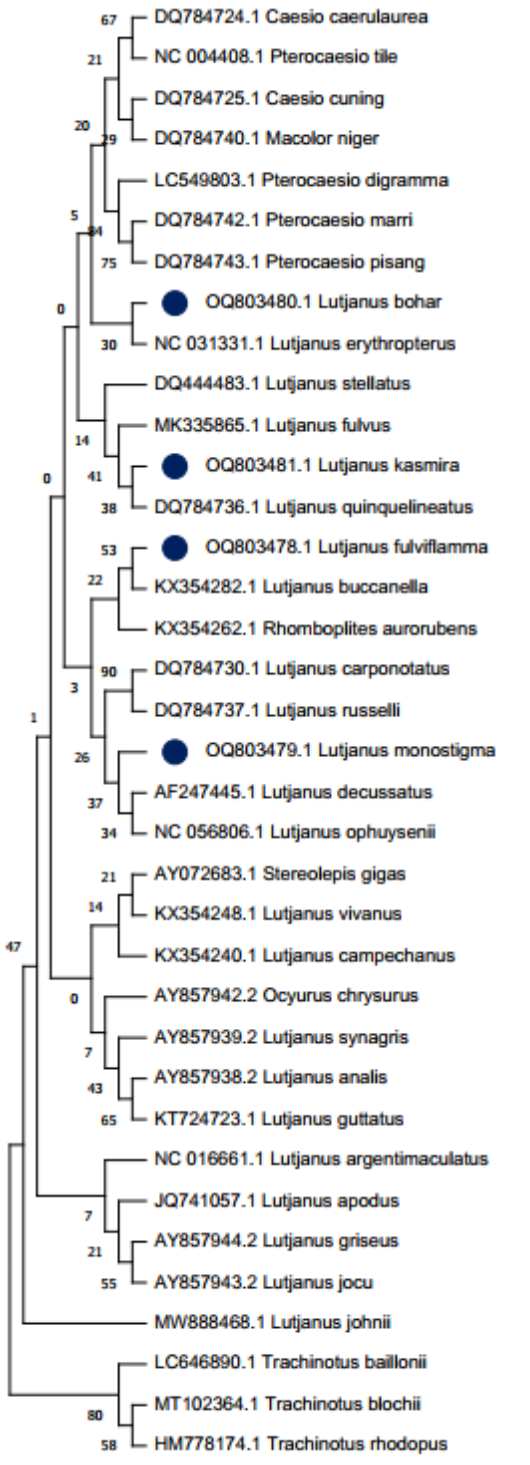

Fig.1. Neighbor Joining phylogenetic tree amongst four species of genus *Lutjanus* and their related species, with the outgroup utilizing (*16S rRNA*) gene.

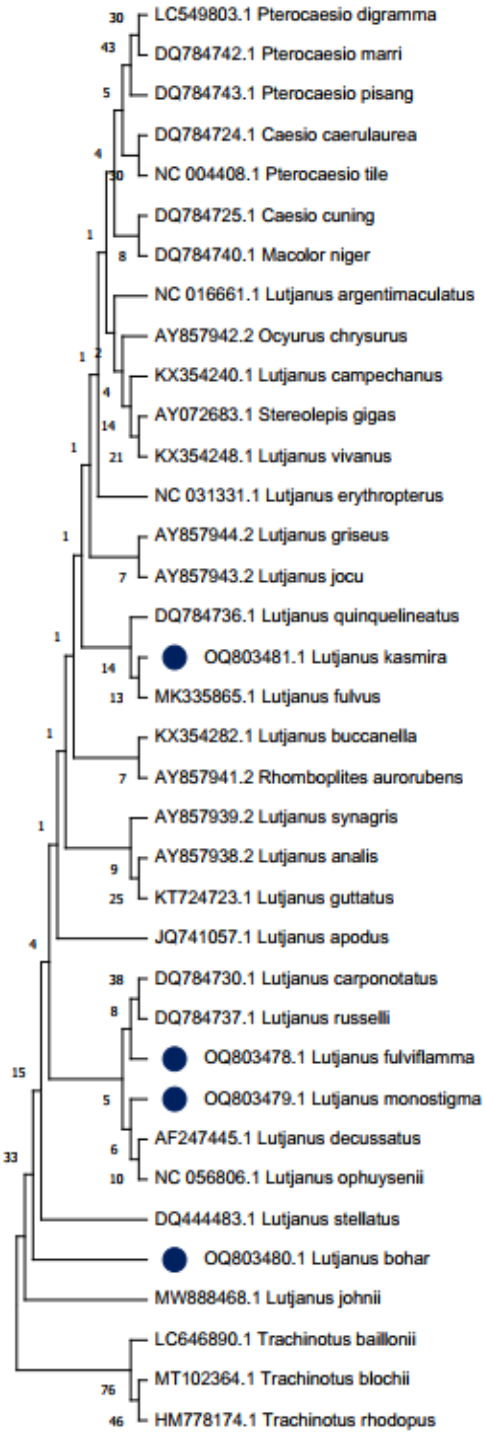

Fig.2. Minimum Evolution phylogenetic tree amongst four species of genus *Lutjanus* and their related species, with the outgroup utilizing (*16S rRNA*) gene.

This work determined that the understudied fishes have (A+T) an average bigger, compared to C+G value. This finding coincides with consistent with several previous studies. **Bo *et al.* (2013)** reported that the entire *16S rRNA* gene exhibits A+T affluence, compared to C+G. **Basheer *et al.* (2015)** observed small C+G value of *16S rRNA*, compared to A+T through the study on *Rastrelliger* species. Moreover, **Mar'ie and Allam (2019)** found in two puffer fishes a bigger A+T ratio compared to C+G. Our results of the *16S rRNA* gene displayed C+G content ranging from 48.52 to 50.08. The GC diversity among the four species of family Lutjanidae may be considered as a notation of adaptation **(Ali *et al*., 2021)**.

The final alignments of incomplete *16S rRNA* sequences in the four species of family Lutjanidae illustrated highly conserved sites. **Basheer *et al.* (2015)** found 575 consistent locations of 590 bp in three *Rastrelliger* species by using *16S* rRNA aligned sequences. **Sokefun (2017)** employing the *16S* gene in cichlid phylogenetic analysis and found 337 conserved regions of 463.

The low genetic distance between *Caesio cuning* and *Macolor niger* is attributed to the close linkage between them. This result concurs with the data reported in the study of **Kaleshkumar *et al.* (2015)** who stated that, strongly related species have low genetic distance values, whereas cases with great genetic divergence are caused by the highest genetic distance.

Our finding confirms previously recorded results indicating that genus *Lutjanus* is not monophyletic **(Miller & Cribb, 2007; Gold *et al*., 2011; Frédérich & Santini, 2017)**. Additionally, **Frédérich & Santini (2017)** reported that phylogenetic findings determined many additional genera (e.g., *Pristipomoides, Pinjalo, Caesio, Pterocaesio*) that were non-monophyly and need revision.

Heino (2014) reported that, the morphological features of a living thing can differ when it lives in unique and different ecological conditions. Fish display wide diversity in physical characteristics, both within and between groups **(Brraich & Akhter, 2015)**. Morphological changes of fishes represent a type of environmental adaptation **(Hossain *et al*., 2010)**. The physical features can be affected by ecological and genetic factors **(Sala *et al*., 2022)**. These may reflect the non-monophyly of some genera in family Lutjanidae.

CONCLUSION

By dint of large subunit ribosomal RNA (*16S rRNA*) sequences, this work predestined the phylogenetic lineages of some species of family Lutjanidae. Our data support the previously results of other authors that genus *Lutjanus* is not monophyletic as well as some genera in this family were non-monophyly and need revision.

REFERENCES

Ali, F.; Mamoon, A. and Abbas, E. (2021). Mitochondrial-Based Phylogenetic Inference of Worldwide Species of Genus *Siganus*. Egyptian Journal of Aquatic Biology and Fisheries, 25: 371–388. doi:10.21608/ejabf.2021.143930.

Allen, G. R. (1985). FAO species catalogue. Snappers of the world. An annotated and illustrated catalogue of luthjanid species known to date. FAO Fish. Synopsis, 125(6): 208 pp.

Al-Qahtani, A. and Amer, S. (2019). First molecular identification of *Euphlyctis ehrenbergii* (Anura: Amphibia) inhabiting southwestern Saudi Arabia. The European Zoological Journal 86: 173–179.

Basheer, V. S.; Mohitha, C.; Vineesh, N.; Divya, P. R.; Gopalakrishnan, A. and Jena, J. K. (2015). Molecular phylogenetics of three species of the genus *Rastrelliger* using mitochondrial DNA markers. Molecular Biology Reports, 42(4): 873–879. doi:10.1007/s11033-014-3710-8.

Bo, Z.; Xu, T.; Wang, R.; Jin, X. and Sun, Y. (2013). Complete mitochondrial genome of the Bombay duck *Harpodon nehereus* (Aulopiformes, Synodontidae). Mitochondrial DNA, 24(6): 660–662. doi:10.3109/19401736.2013.772988.

Branicki, W.; Kupiec, T. and Pawlowski, R. (2003) Validation of cytochrome b sequence analysis as method of species identification. Journal of Forensic Sciences 48*:* 83–7.

Brraich, O. S. and Akhter, S. (2015). Morphometric characters and meristic Counts of a Fish, *Crossocheilus latius latius* (Hamilton-Buchanan) from Ranjit Sagar Wetland, India. International Journal of Fisheries and Aquatic Studies, 2(5): 260- 265.

Domeier, M. L. and Clarke, M. E. (1992). A laboratory produced hybrid between *Lutjanus synagris* and *Ocyurus chrysurus* and a probable hybrid between *L. griseus* and O. *chrysurus* (Perciformes, Lutjanidae. *Bulletin of Marine Science* 50: 501–507.

Eschmeyer, W. N.; Fricke, R. and Van Der Laan, R. (2016). *Catalog Of Fishes*. Available From http://www.calacademy.org/scientists/projects/catalog-of-fishes.

Felsenstein, J. (1985). Confidence Limits On Phylogenies: An Approach Using The Bootstrap. Evolution; International Journal of Organic Evolution, 39(4): 783–791. doi:10.1111/j.1558-5646.1985.tb00420.x.

Frédérich, B. and Santini, F. (2017). Macroevolutionary analysis of the tempo of diversification in snappers and fusiliers (Percomorpha: Lutjanidae). Belgian Journal of Zoology 147 (1): 17–35.

Froese, R. and Pauly D. (2016). *FishBase*. Available from www.fishbase.org.

Gold, J. R.; Voelker, G. and Renshaw, M. A. (2011). Phylogenetic relationships of tropical western Atlantic snappers in subfamily Lutjaninae (Lutjanidae: Perciformes) inferred from mitochondrial DNA sequences. Biological Journal of the Linnean Society, 2011, 102, 915–929.

Guha, S.; Goyal, S. P. and Kashyap, V. K. (2006). Genomic variation in the mitochondrially encoded cytochrome b (MT-CYB) and 16S rRNA (MT-RNR2) genes: characterization of eight endangered Pecoran species. Animal Genetics, 37: 262–265.

Heino, M. (2014). Quantitative Traits. In: Cadrin SX, Karr LA, Mariani S (eds) Stock Identification Methods: Applications in Fishery Science. 2nd Edition. Academic Press – Elsevier, London, UK.

Hossain, M. A. R.; Nahiduzzaman, Md.; Saha, D.; Khanam, Mst. U. H. and Alam, Md. S. (2010). Landmark-Based morphometric and meristic variations of the endangered carp, Kalibaus *Labeo calbasu*, from stocks of two isolated Rivers, the Jamuna and Halda and a Hatchery. Zool. Stud., 49(4): 556-563.

Iwatsuki, Y.; Tanaka, F. and Allen, G. R. (2015). *Lutjanus xanthopinnis*, a new species of snapper (Pisces: Lutjanidae) from the Indo-west Pacific, with a redescription of *Lutjanus madras* (Valenciennes 1831). *Journal of the Ocean Science Foundation* 17: 22–42.

Johnson, G. D. (1993). Percomorph phylogeny: progress and problems. Bull Mar Sci., 52(1):3-28.

Kaleshkumar, K.; Rajaram, R.; Vinothkumar, S.; Ramalingam, V. and Meetei, K. B. (2015). Note DNA barcoding of selected species of pufferfishes (Order : Tetraodontiformes) of Puducherry coastal waters along south-east coast of India. Indian Journal of Fisheries, 62(2): 98–103.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16(2): 111–120. doi:10.1007/BF01731581.

Kumar, S.; Stecher, G. and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution, 33(7): 1870–1874. doi:10.1093/molbev/msw054.

Mar'ie, Z. A. and Allam, M. (2019). Molecular Phylogenetic Linkage for Nile and Marine Puffer Fishes Using Mitochondrial DNA sequences of Cytochrome b and 16S rRNA. Egyptian Journal of Aquatic Biology & Fisheries. 23(5): 67-80.

Metallinou, M.; Arnold, E. N.; Crochet, P.-A.; Geniez, P.; Brito, J. C.; Lymberakis, P.; Baha El Din, S.; Sindaco, R.; Robinson, M. and Carranza, S. (2012). Conquering the Sahara and Arabian deserts: systematics and biogeography of Stenodactylus geckos (Reptilia: Gekkonidae). BMC evolutionary biology, 12: 1-17.

Miller, T. L. and Cribb, T. H. (2007). Phylogenetic relationships of some common Indo-Pacific snappers (Perciformes: Lutjanidae) based on mitochondrial DNA sequences, with comments on the taxonomic position of the Caesioninae. *Molecular Phylogenetics and Evolution* 44: 450–460.

Nakahara, M.; Handa, S.; Watanabe, S. and Deguchil, H. (2004). Choricystis minor as a new symbiont of simultaneous two-species association with *Paramecium bursaria* and implications for its phylogeny. Symbiosis, 36(2):127-151

Randall, J. E. (1982). The diver guide to Red Sea reef fishes. Publishing limited 20 Berkeley street, Berkeley square London Wix 5AE.

Saad, Y. M. (2019). Analysis of 16S mitochondrial ribosomal DNA sequence variations and phylogenetic relations among some Serranidae fishes. South African Journal of Animal Science,49 (1): 80-89.

Sala, R.; Kusuma, A. B.; Bataradewa, S.; Pranata, B. (2022). Morphometrics Diversity and Phenotypic Relationship of the Red Snapper (*Lutjanus gibbus*) in Northern Papua Waters. Egyptian Journal of Aquatic Biology & Fisheries, 26(5): 1211-1227.

Sarver, S. K.; Freshwater, D. W. and Walsh, P. J. (1996). Phylogenetic relationships of Western Atlantic snappers (Family Lutjanidae) based on mitochondrial DNA sequences. *Copeia* 1996: 715–721.

Simon, C.; Franke A. and Martin, A. (1991). The polymerase chain reaction: DNA extraction and amplification. In Molecular Techniques in taxonomy. Eds. G. M. Hewitt, A. W. B. Johnston and J. P. W. Young. NATO AS1 Series H 57: 329 355.

Sokefun, O. B. (2017). The cichlid 16S gene as a phylogenetic marker: Limits of its resolution for analyzing global relationship. International Journal of Genetics and Molecular Biology, 9(1): 1–7. doi:10.5897/ijgmb2016.0131.

Souza, A. S.; Dias Júnior, E. A.; Perez, M. F.; Cioffi, M. B.; Bertollo, L. A. C.; Garcia-Machado, E.; Vallinoto, M. N. S.; Galetti, P. M. Jr and Molina, W. F. (2019). Phylogeography and Historical Demography of Two Sympatric Atlantic Snappers: *Lutjanus analis* and *L. jocu*. Front. Mar. Sci., 6:545. doi: 10.3389/fmars.2019.00545.

Thompson, J. D.; Higgins, D. G. and Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22(22): 4673–4680. doi:10.1093/nar/22.22.4673.

Veneza, I.; da Silva, R.; da Silva, D.; Gomes, G.; Sampaio, I. and Schneider, H. (2019). Multiloci analyses suggest synonymy among *Rhomboplites*, *Ocyurus* and *Lutjanus* and reveal the phylogenetic position of *Lutjanus alexandrei* (Lutjanidae: Perciformes). Neotropical Ichthyology, 17(1): e180109.