JJBS

Volume 12, Number 3,August 2019
ISSN 1995-6673
Pages 379 - 383

Jordan Journal of Biological Sciences

Molecular Analysis and Phylogenetic Assessment of the Red Sea Fish of *Plectropomus pessuliferus*

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Received September 20, 2018; Revised November 3, 2018; Accepted November 20, 2018

Abstract

A fish sample related to *Plectropomus pessuliferus* was obtained from Mastoora on the Red Sea in Saudi Arabia, and was identified morphologically by Polymerase Chain Reaction (PCR). The *Plectropomus pessuliferus* sample, locally known as Najil, was 53.8 cm in length and 2110 gm in weight. The sequence alignment of the TMO-4C4 gene (466 bp in length) obtained by PCR revealed a 84 % identity with eleven accessions in GenBank from five different *Plectropomus* species. Analysis of the TMO-4C4 gene sequence alignment showed positional differences of sixty-nine nucleotides with base-pair substitutions and forty-four transversions interchanges, thirteen transitions from A to G, and seven from T to C. Blast protein alignment revealed a 66 % identity between the amino acids' sequence of the TMO-4C4 gene of *Plectropomus pessuliferus* and other five *Plectropomus* species. A nine-amino-acid domain only has appeared in the sample sequence of the current study compared to other *Plectropomus* sequences which may be referred to as a unique sample with new traits. These results should be focused and completed in order to have a good understanding of the genetic information for this fish species.

Keywords*: Plectropomus pessuliferus*, TMO-4C4 gene, base-pair substitutions, Mastoora**,** Red Sea, Saudi Arabia

1. Introduction

Plectropomus pessuliferus (Coral Reef Guide), locally known as Najil, is one of important fish species of the Red Sea which can be found in some countries such as Egypt, Saudi Arabia, Jordan and Sudan; rare species can be found in the Indo-Pacific regions (Ashworth *et al*. 2006). *Plectropomus pessuliferus* fishes have been found to live in coral reef and seaward reefs at a depth range of 25 - 147 m. The *Plectropomus pessuliferus* fish can reach up to a maximum length of 120 cm in the Red Sea and to a minimum length of 63 cm in the Indo-Pacific (Heemstra *et al*. 1993; Morris *et al*. 2000). These large fishes have very variable colors ranging from white or beige to red and the body is covered with blue dots (Durville *et al*. 2003; Randall *et al*. 2003; Sattar *et al*. 2005). This species is rather similar to and is often misidentified as *Plectropomus maculatus*.

The intense fishing pressure on coral-reef fish resources throughout the Red Sea and Indo-Pacific region especially *Plectropomus pessuliferus* (Sluka 2002) creates an urgent need for protection actions in some countries. In the Ras Mohammed National Park (Egypt) *Plectropomus pessuliferus* is protected, as well as in Dungonab and Sanganeb Marine Parks (Sudan). Also, the Ministry of Agriculture of Saudi Arabia has taken measures to protect *Plectropomus pessuliferus* and *Plectropomus areolatus* along the coast, such as prohibiting fishing during the seasons in 1994, 1995, 1999, 2001. Therefore, the landed catch was markedly decreased during these periods (Fallatah 2005).

Fish identification is based not only on morphological features but also on DNA techniques. Santos *et al.* (2013) used mitochondrial and nuclear markers to identify and differentiate between two species of a grouper (*Plectropomus maculatus* and *Plectropomus leopardus*. Harrison *et al*. 2014 used microsatellites to discriminate between two closely-related species of coral reef fish, *Plectropomus leopardus* and *P. maculatus* (Serranidae). The TMO-4C4 gene was previously analyzed in a new *Plectropomus areolatus* fish sample obtained from Yanbu coast on the Red Sea in Saudi Arabia (Gharbawi. 2015).

The aim of this work is to determine and analyze the TMO-4C4 gene sequence in a new *Plectropomus pessuliferus* fish sample obtained from Mastoora on the Red Sea in Saudi Arabia.

2. Materials and Methods

Plectropomus pessuliferus (Red Sea reef fishes) fish sample was obtained from Mastoora on the Red Sea in Saudi Arabia (natural habitat).

2.1. DNA Extraction

A tissue sample of about 2-4 mm^3 was used to extract DNA according to the procedure described in a Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN) (Streelman and Karl 1997).

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2.2. Primer Design and PCR Amplification of the TMO-4C4 Gene

Two specific primers forward (5′ CGCTAATGTTTTACGCTGAG 3′) and reverse (5′ GATGTGTTACCGAGTATTTA 3′) were designed for the TMO-4C4 gene obtained from accession EF517751 of NCBI GenBank. The PCR-amplification reaction was used in a final volume of 25 µL containing 12.5 µL of My *Taq* Red Mix Master mix (BIOLINE), 1.5 µL of 20 mM of each forward and reverse primers, 50 ng of template DNA. PCR conditions started with the initial denaturation step at 94°C for two minutes, followed by forty cycles of thirty seconds at 94°C, the annealing step at 49°C for one minutes, and the elongation step at 72°C for one minute with a final extension of five minutes at 72°C. The PCR amplified product was analyzed using 1.2 % agarose gel electrophoresis in a 1X TBE buffer by staining using 0.8 µg/µL of ethidium bromide, and was visualized under UV light. A TMO-4C4 fragment size of 466 bp was estimated based on a 50 bp DNA ladder (Bioron, Germany).

2.3. Purification and Sequence Alignment of the TMO-4C4 Gene

A 466 bp of the PCR product was purified with the Zymoclean^{Tm} Gel DNA Recovery Kit (Epigenetics Company) according to the manufacturer's instructions. The TMO-4C4 fragment was sent for sequencing, and sequence was compared with the sequences of the most closely-related *Plectropomus* fish samples deposited in GenBank and the sequencing-genome databases using BLAST search (http://www.ncbi.nlm.nih.gov/blast). Highly-conserved residues have a black background, whereas partially conserved residues are shown with a grey shaded background. Numbering at the end of each line refers to the position in the alignment.

2.4. Phylogenetic Analysis

The Kimura's two-parameter model was used to obtain genetic distances (Kimura 1980). The construction of the phylogenetic tree and the dendrogram were obtained using multiple alignment of the TMO-4C4 sequence from *Plectropomus* by the neighbor-joining method (Saitou and Nei 1987) with the Geneious Pro 4.5.4 program.

3. Results

3.1. Morphological Features of the Plectropomus pessuliferus Fish Sample

The *Plectropomus pessuliferus* sample, locally known as Najil, was collected from Mastoora. It is related to the family Serranidae, and was 53.8 cm in length and 2110 gm in weight. This fish sample was orange, and the whole body is covered with blue dots as shown in Figure 1.

Figure 1. *Plectropomus pessuliferus* fish sample obtained from Mastoora on the Red Sea, Saudi Arabia.

3.2. PCR Amplification and Sequence Analysis of the Plectropomus pessuliferus' TMO-4C4 Gene

A fragment corresponding to the partial sequence of the TMO-4C4 gene of an expected size of 466 bp was amplified by PCR from *Plectropomus pessuliferus*. The gene sequence was aligned and compared to GenBank databases using BLAST website. Blast alignment data revealed a 84 % identity between the TMO-4C4 gene sequence of *Plectropomus pessuliferus* and eleven accessions from five different *Plectropomus* species, namely *laevis*, *oligacanthus*, *leopardus*, *maculates* and *areolatus* (Table 1).

Sixty nine base-pair substitutions in nucleotide sequences have been revealed from the nucleotide sequence analysis of the TMO-4C4 gene alignment data compared with other *Plectropomus* species. Forty-four transversions interchanges from purine to pyrimidine and from pyrimidine to purine were detected, while thirteen transitions from purine to purine (A) ⇆ (G), and seven from pyrimidin to pyrimidine (T) ⇆ (C). These results and base-pair substitutions are shown in green color as presented in Table 2 and Figure 2.

Table 1. Blast alignment results for the TMO-4C4 gene sequence of *Plectropomus pessuliferus*

No.	Accession	Description	Identity %
1	KM078024	*Plectropomus leopardus* TMO-4C4 protein (TMO-4C4) gene	84
2	AY949211	*Plectropomus leopardus* TMO4C4 (TMO4C4) gene	84
3	EF517751	*Plectropomus maculatus* TMO-4C4 gene	84
4	EF517747	*Plectropomus leopardus* TMO-4C4 gene	84
5	EF517750	*Plectropomus areolatus* TMO-4C4 gene	84
6	AY949211	*Plectropomus leopardus* TMO4C4 (TMO4C4) gene	84
7	KM078023	*Plectropomus laevis* TMO-4C4 protein (TMO-4C4) gene	84
8	AY949320	*Plectropomus laevis* TMO4C4-like (TMO4C4) gene	84
9	AY949300	*Plectropomus oligocanthus* TMO4C4-like (TMO4C4) gene	84
10	AY949267	*Plectropomus areolatus* TMO4C4 (TMO4C4) gene	84
11	EF517748	*Plectropomus oligacanthus* TMO-4C4 gene	84

Table 2. Nucleotide sequence analysis of the TMO-4C4 gene of *Plectropomus pessuliferus* compared to other *Plectropomus* species.

present in sample as: present in NCBI as:	Nucleotide sequence range	A T	A G	A C	A ---	T A	T C	T G	T ---	C A	C T	C G	G A	G T	G C	G ---
Nucleotide positions change	T=69	29 33 51 53 54 86 100	30 172 249 290 352 263 79	44 74 102 280 396	78 90	19 22 185 201 243 294 337 390	47 81 114 326 372	298 383	165	135 162 191 280 351 357 384	92 186	76 307 334 338	43 138 230 250 283 388	18 48 161 199 228 366 371	225 246 319 380	85 164
		7	7	5	2	8	5	2	1	7	2	4	6	7	4	2

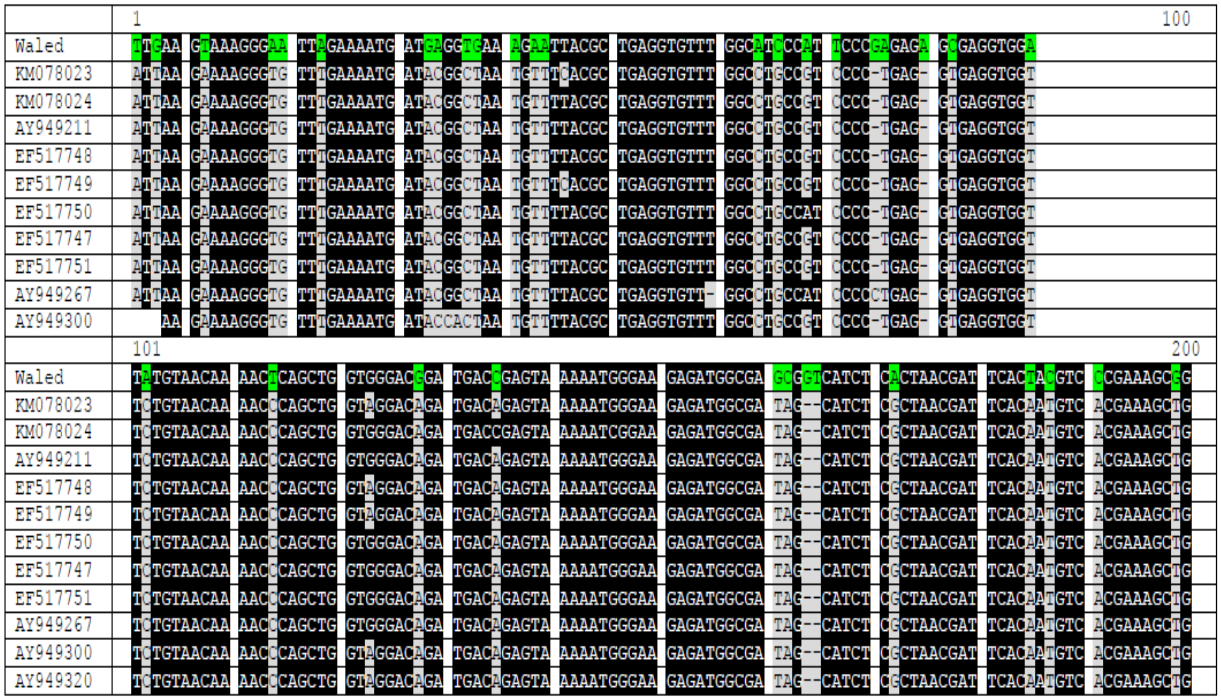

Figure 2. TMO-4C4 gene nucleotides sequence alignment of *Plectropomus pessuliferus* sample of Mastoora compared to other *Plectropomus* species by Blast. Conserved nucleotides are appeared in black, Putative conserved between the different isolates with no identity with isolates are boxed in grey, nucleotides appeared only in our isolate are boxed in green.

3.3. Phylogenetic Tree Based on TMO-4C4 gene Sequence of Plectropomus pessuliferus

The *Plectropomus pessuliferus* TMO-4C4 gene sequence alignment with other *Plectropomus* species obtained from GenBank was used to build a phylogenetic tree in which *Plectropomus pessuliferus* appeared as the main root origin of all other *Plectropomus* species' clusters as shown in Figure 3.

3.4. Analysis of the TMO-4C4 gene Amino Acid Sequence

The TMO-4C4 gene sequence was translated and aligned on GenBank with Blast databases. Six accessions for three *Plectropomus* species, namely *leopardus*, *laevis*, and *areolatus* all revealed a 66 % identity with amino acid sequences of the gene (TMO-4C4) of *Plectropomus pessuliferus* under investigation (Table 3). Amino acid sequences of the TMO-4C4 gene of *Plectropomus pessuliferus* have shown a gap in the first twenty-five amino acids compared to other amino acid sequences of the TMO-4C4 gene of other *Plectropomus* accessions as shown in Figure 4, whereas a nine-amino-acid domain <<< LQKLTPRVf >>> appeared only in the sample sequence of this study compared to the other *Plectropomus* accessions.

Table 3. Identity % of TMO-4C4 translated amino acids sequence from *Plectropomus pessuliferus* compared with other *Plectropomus* accessions.

Accession	Putative protein TMO4C4	Identity %
AAY68548	*Plectropomus areolatus*	66
ABS72105	*Plectropomus leopardus*	66
ABS72107	*Plectropomus laevis*	66
AIJ03033	*Plectropomus leopardus*	66
AAY68519	*Plectropomus leopardus*	66
ABS72105	*Plectropomus leopardus*	66

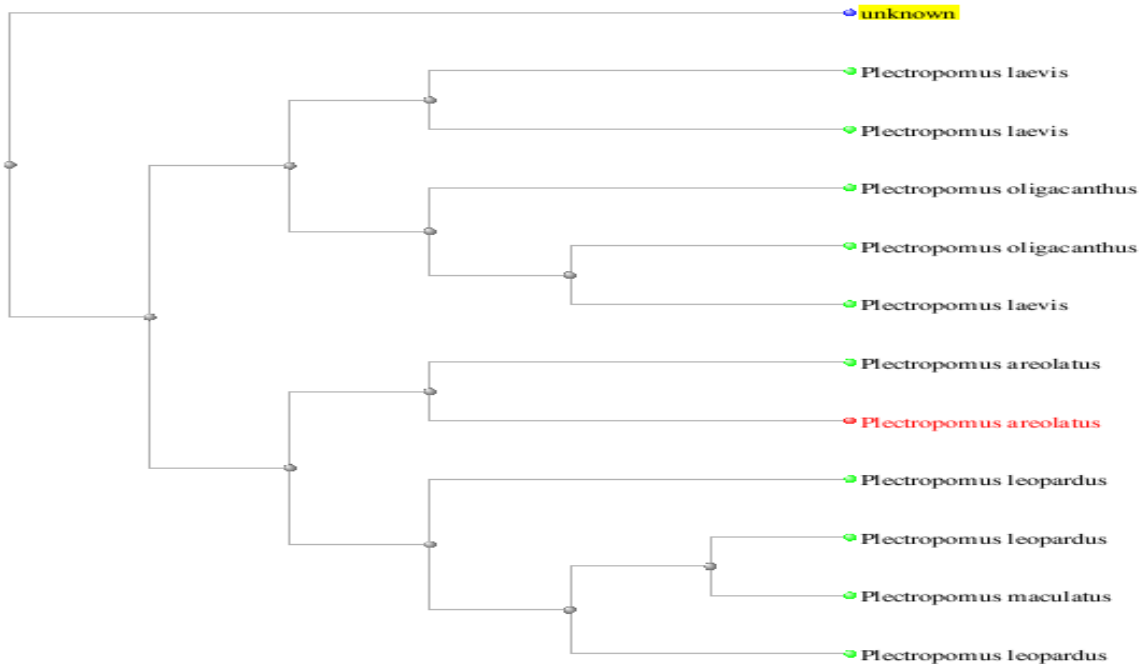

Figure 3. Phylogenetic tree based on alignment of TMO-4C4 gene sequence of *Plectropomus pessuliferus* with other *Plectropomus* accessions.

Waleed	----------SV--FDVLQKLTPRVIEPAPGLRIEVELDENEVKEFEKQVKIITIPEY-------
AIJ03032	RSVALVVVVSQEVRFMPAPPAVTHQHVMEFDVEEDDSSRS-PSPQEILLEVELDENEVKEFEKQVKIITIPEYTADNKSM
AAY68519	RSVALVVVVSQEVRFMPAPPAVTHQHVMEFDVEEDDSSRS-PSPQEILLEVELDENEVKEFEKQVKIITIPEYTADNKSM
AAY68548	RSVALVVVVSQEVRFMPAPPAVTHQHVMEFDVEEDDSSRS-PSPQEILLEVELDENEVKEFEKQVKIITIPEYTADNKSM
AIJ03033	RSVALVVVVSQEVRFMPAPPAVTHQHVMEFDVEEDDSSRS-PSPQEILLEVELDENEVKEFEKQVKIITIPEYTADNKSM
ABS72107	RSVALVVVVSQEVRFMPAPPAVTHQHVMEFDVEEDDSSRS-PSPQEILLEVELDENEVKEFEKQVKIITIPEYTADNKSM
ABS72105	RSVALVVVVSQEVRFMPAPPAVTHQHVMEFDVEEDDSSRS-PSPQEILLEVELDENEVKEFEKQVKIITIPEYTADNKSM

Figure 4. Amino acids' sequence of TMO-4C alignment of *Plectropomus pessuliferus* compared with other *Plectropomus* accessions.

4. Discussion

The morphological features of the *Plectropomus pessuliferus* sample under study agreed with those described by Durville *et al*. (2003) who maintain that this species of fish has very variable colors ranging from white or beige to red and the body is covered with blue. This species is rather similar to and is often misidentified as *Plectropomus maculates http://www.fishbase.se/summary/ Plectropomus-pessuliferus.html*.

However, in morphological taxonomy, characters are delimited usually without any explicit criteria for character selection or coding, and the morphological data sets have the potential to be quite arbitrary. For example, morphologists do not generally report their criteria for including or excluding characters, and when criteria are given, they vary considerably among studies (Wiens 2000). While DNA barcoding such as the TMO-4C4 gene provides taxonomic identification for a specimen, the accuracy of such an assignment depends on whether species are monophyletic with respect to the sequence variations of the genes. That is, individuals of a given species are more closely related to all other conspecifics than to any member of other species. The factors responsible for deviations from taxonomic monophyly may be varied and complex (Funk and Omland 2003); one potential cause of the species-level of polyphyly is the occasional mating between distinct species, resulting in hybrid offsprings carrying a mixture of genes from both parent species. In such cases, combinations of morphological and genotypic data are needed for the species assignment of hybrids. Biological mechanisms, water dynamics, or historical events may cause deep genetic structuring of populations in marine species (Barber *et al.* 2000).

The molecular analysis of the TMO-4C4 gene of an expected size of 466 bp revealed a 84 % identity between the TMO-4C4 gene sequence of *Plectropomus pessuliferus* and eleven accessions from five different *Plectropomus* species, namley *laevis*, *oligacanthus*, *leopardus*, *maculates*, and *areolatus*.

Sixty-nine base-pair substitutions in nucleotide sequences have been revealed from the nucleotide sequence analysis of the TMO-4C4 gene alignment data compared with other *Plectropomus* species. The genetic changes observed in the TMO-4C4 gene of *Plectropomus pessuliferus* suggested that the nucleotides' variation may be attributed to a change in the genetic composition as mentioned by (Gharbawi 2015). Amino acid sequences of the TMO-4C4 gene of *Plectropomus pessuliferus* showed a nine-amino-acid domain. These results suggest a unique sample with new traits which should be fully investigated in order to have a good understanding of the genetic information of this fish species to help those interested in this field including fisheries' management and phylogeographic studies (Williams *et al.* 2003). The nature and extent of genetic changes observed in the TMO-4C4 gene of the *Plectropomus pessuliferus* sample obtained from Mastoora on the Red Sea in Saudi Arabia suggested

that fluctuations in nucleotide sequences are underlain by significant changes in the genetic composition and population integrity. The development of robust hypotheses for phylogenetic relationships within and among coral reef fish will have a major impact on the ability to analyze the evolutionary biology of these colorful, diverse, and ecologically important sea animals.

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