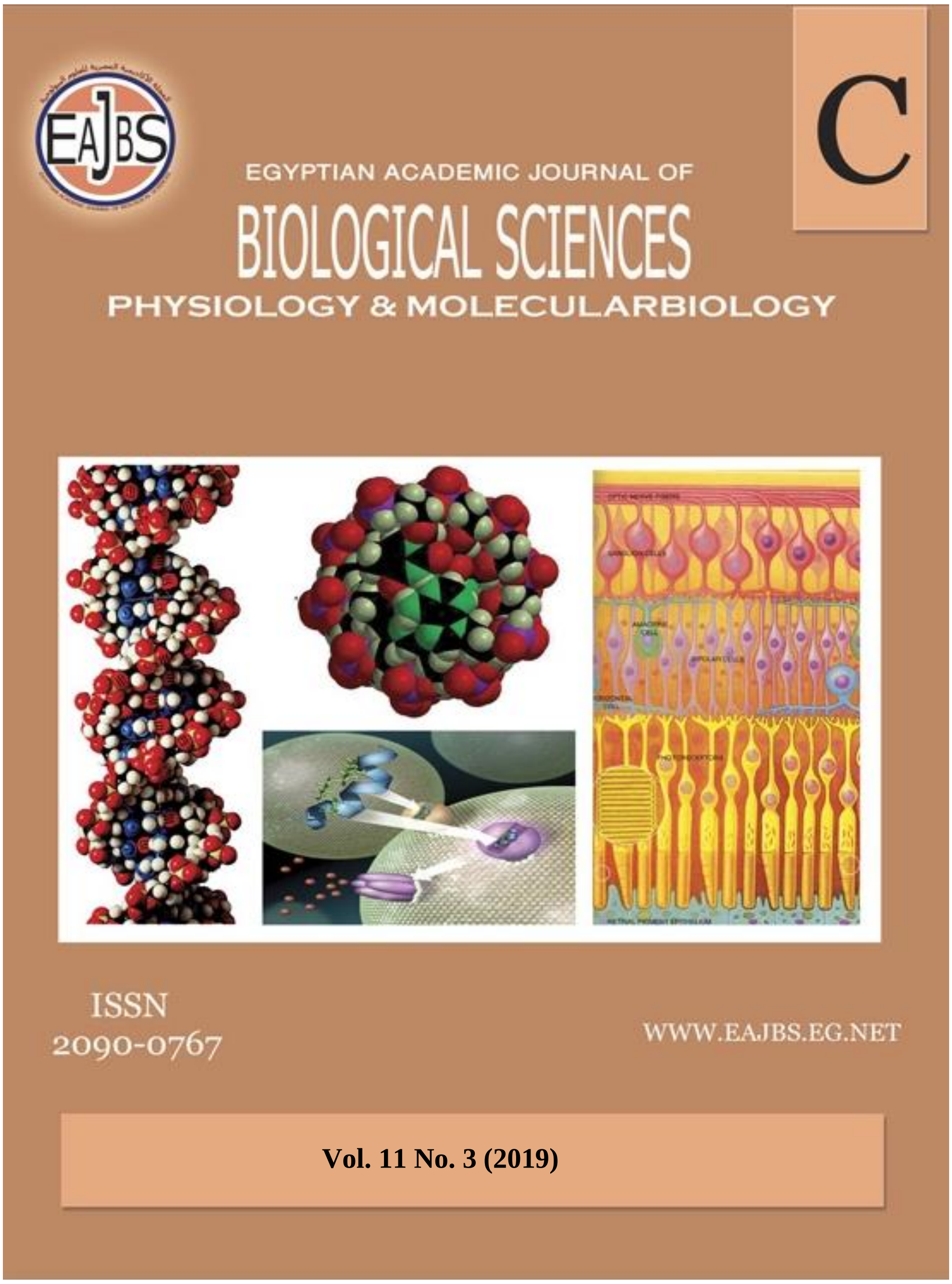

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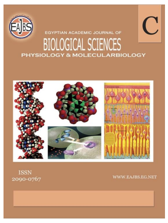

Sequence Variations and Molecular Phylogeny of some Red Sea Parrotfishes (Scaridae) Using Mitochondrial Gene Sequences

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ABSTRACT

Targeted DNA fragments were isolated from four scarids species; *Scarus collana* Rüppell 1835; *Scarus frenatus* Lacepède 1802; *Scarus (Chlorurus) sordidus* Forsskål 1775 and *Scarus niger* Forsskål 1775 using mitochondrial 16S rRNA gene-specific primers.

Analysis of obtained partial 16S rRNA gene nucleotide sequences showed a high level of nucleotide identity in the studied regions. This reflects a close genetic relationship and shared ancestry among studied parrotfishes. Nucleotide compositions of partial 16S rRNA gene nucleotide sequence biased towards adenine and similar preference towards thymine, cytocine, and guanine. Also, base constitutions revealed preference towards higher DNA conservations.

Phylogenetic analysis displayed patterns of assembly for studied species, and other included related taxa, which reflect their similar genetic makeup and their tendency to have similar niches. The phylogenetic trees revealed two evolutionary lineages splitting Scaridae and Wrasses which assumed that Scaridae should maintain the family status.

Obtained data could be beneficial for parootfishes classification, conservation, and their needed environments. Therefore, the acquisition of nucleotide sequences from other parrotfishes using the developed mt16S rRNA gene-specific primers utilized here would contribute in the future to the phylogenetic and evolutionary studies of parrotfish in the Red Sea territory.

INTRODUCTION

Fishes worldwide mostly represent fifty percent (50%) of all recognized vertebrates. They significantly consider major protein sources for Humankind (FAO 1997 and FAO 2000). According to Nelson (1994), more than 32,000 fish species from 482 families are known, roughly 13,000 live in the marine water surroundings. The Red sea possesses over 1000 fish species containing a diverse assemblage of Coral reef fishes that live adjacent to coral reefs (Alwany *et al.*, 2007). Coral reef fishes possess diverse colours (juveniles) or colour alterations during sexual maturity (wrasses, Labridae) (Randall, 1982).

Parrotfishes (Scaridae) are herbivorous which reside near to coral reefs and composing a clade of ninety (90) species. They profile coral reef communities using their beak-like teeth to scratch algae and detritus off coral reefs to preclude algal overgrowth of corals (Hughes, 1994; Bellwood *et al.*, 2004) and maintain a healthy resilient coral reef ecosystem (Bellwood et al., 2003; Burkepile and Hay, 2008; Cheal *et al.*, 2010). Parrotfishes were previously classified as a family (Scaridae), but have recently been considered as subfamily Scarinae (family Labridae), but others still prefer to allocate them as a family (Bellwood, 1994; Choat and Bellwood, 1998; Westneat and Alfaro, 2005; Randall, 2007).

The mitochondrial (mtDNA) genome possess much smaller gene contents, little recombination and speedy evolutionary level (Brown *et al.*, 1979; Attardi, 1985; Hayashi Jun-I *et al.*, 1985; Saccone *et al.*, 1991). mtDNA genome was significantly used for studying the evolutionary association among various species, also in estimating divergence times of some marine invertebrates species (Olivo et al., 1983; Westneat and Alfaro, 2005; Lee, 2003). The mtDNA sequences of 16S rRNA gene are amongst the most widely used genetic marker for fish species identification, description, and in fisheries management (Greig *et al.*, 2005; Kochzius *et al.*, 2010; Faddagh *et al.*, 2012; Farrag *et al.*, 2015). It is also been used for relatedness assessment and distinguishing among various taxa, and phylogeography research (Li *et al.*, 2008; Smith, *et al.*, 2008; Lakra *et al.*, 2013; Yang *et al.*, 2014).

Because of the classification and lineage history of parrotfishes is still unsettled. There is a requirement for more molecular evolutionary studies such as gene-specific markers to understand parrotfishes phylogenetic relationships, also to resolve their related taxonomic issues. The purpose of this study to analyse sequence disparities and molecular phylogenetic patterns amongst four Red Sea parrotfish species; *Scarus collana*, *Scarus frenatus*, *Scarus (Chlorurus) sordidus* and *Scarus niger* using partial mitochondrial 16S rRNA gene sequences.

MATERIALS AND METHODS

1. Fish Samples and Genomic DNA Extraction:

Fish samples used in this study were previously collected by EL-Mahdi (2018a). About ~30 mg from muscle tissue specimens were used for genomic DNA extraction (EZ10 spin column genomic DNA kit, Bio Basic Inc., Canada). The DNA purity and concentration were estimated by UV spectrophotometry.

2. PCR Amplification of Mitochondrial 16S rRNA Gene:

A pair of primers were used to amplify the partial sequence of mt 16S rRNA gene. These are 16Sar-L 5’ CGC CTG TTT ACC AAA AAC ATC GCCT 3’ and 16Sbr-H 5’ CCG GTC TGA ACT CAG ATC ACG T 3’ (Palumbi 1996). The PCR reactions were performed in 25 ml final volumes containing 1.0× DreamTaq Green PCR Master Mix 2X (Thermo Scientific Inc), 10 pM of each primer and about 50ng of each DNA sample. The cycling conditions included an initial denaturation at 95°C for 2 min, 35 cycles (94°C for 1 min, 56°C for 1 min and 72°C for 2 min), and one cycle at 72°C for 9 min for final extinction. PCRs were performed in the Primus 25 advanced system (PEQLAB Biotechnologie GmbH).

3. Gel Electrophoresis and DNA Sequences:

PCR products of 10 µL were separated by 1.5% (w/v) agarose/TAE/ethidium bromide (0.5 µg/ml) at 90 V for 40 min. A 100 bp DNA ladder (0.1 µg/µl, Solis BioDyne, Estonia) was used for PCR product approximation. Gel images were taken under UV light using Elttrofor M20 SaS Photo-Gel System (Italy). PCR fragments were bidirectional sequenced (Macrogen Inc., Seoul, Republic of Korea) by the same primers used for PCR amplification.

4. DNA Sequence Analysis:

Sequence reads of both DNA strands were edited using BIOEDIT version 7.0.5.3 (Hall, 1999) and free SnapGene Viewer v3.2.1 (GSL Biotech). The obtained partial mt 16S rRNA gene sequences were compared to GenBank nucleotide sequence database for species identities. For a phylogenetic study, six selected DNA sequences were recovered from downloaded complete mtDNA genomes. These retrieved sequences correspond to DNA targeted regions. Those are flanked by 16S gene-specific primers used for PCR amplification (Table 1). Five sequences were included as in-group while, the sixth one that is related species to them, *Choerodon schoenleinii* of Wrasses was chosen as out-group.

The Muscle software (Edgar, 2004) implemented in MEGA6 version 6 (Tamura, et al., 2013) was used for sequence alignments under default options. The MEGA6 program was also used for nucleotide compositions and phylogenetic analyses. A suitable nucleotide substitution model was chosen by Maximum likelihood (ML) fits of 24 nucleotide substitution models (Nei and Kumar, 2000) and trees were constructed using ML (Tamura *et al.*, 2004) and UPMGA (Unweighted pair group method with arithmetic mean) (Sneath and Sokal, 1973) with assessment of 1000 bootstrap replicates (Felsenstein, 1985) for internal tree branches that measured in number of substitutions per site.

Table 1. DNA sequences used in this study. The out-group species is highlighted in grey.

Species Name	Family, (Subfamily), and Genus	Length in bp	Accession Number
Present study, the Red Sea species partial Sequences			
Scarus collana	Scaridae (Scarinae)	16S rRNA 574 bp	MN586283
Scarus frenatus	Scaridae (Scarinae)	16S rRNA 572 bp	MN633399
Scarus (chlourus) sordidus	Scaridae (Scarinae)	16S rRNA 572 bp	MN633397
Scarus niger	Scaridae (Scarinae)	16S rRNA 572 bp	MN633398
The Genebank/NCBI Sequences used			
Bolbometopon muricatum (Valenciennes, 1840)	Scaridae (Parrotfishes), Scarinae, Genus Bolbometopon	16, 788	NC_033901.1
Choerodon schoenleinii (Valenciennes, 1839)	Labridae (Wrasses) , (Bodianinae), Genus: Choerodon	16,504	NC_025771.1
Scarus forsteni (Bleeker, 1861)	Scaridae (Parrotfishes), (Scarinae), Genus Scarus	16,679	NC_011928.1
Scarus ghobban Forsskål, 1775	Scaridae (Parrotfishes), (Scarinae), Genus Scarus	16,676	NC_011599.1
Scarus rubroviolaceus Bleeker, 1847	Scaridae (Parrotfishes), (Scarinae), Genus Scarus	16,681	NC_011343.1
Scarus schlegeli (Bleeker 1861)	Scaridae (Parrotfishes), (Scarinae), Genus Scarus	16,701	NC_011936.1

RESULTS

1. PCR Amplification of Mitochondrial 16S rRNA Gene:

The PCR primers targeting 16S rRNA regions were reproductively amplified the DNA fragments from species under study. Expected DNA fragments of mitochondrial 16S rRNA gene generated amplicons of approximately 640 bp (Fig.1).

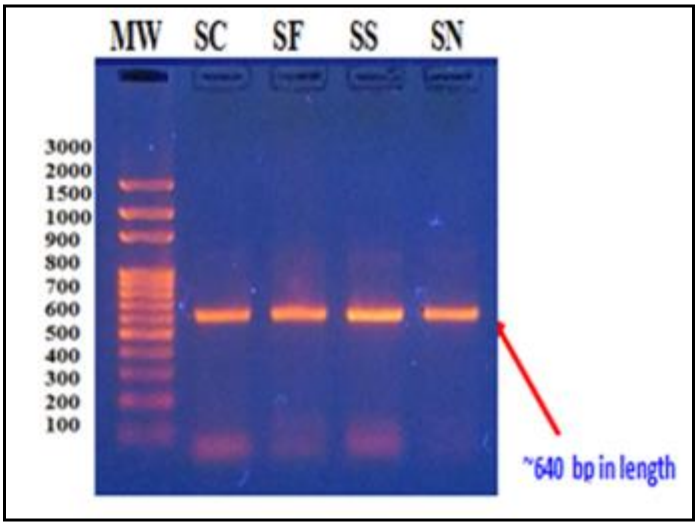

Fig. 1: Electrophoretic separation of PCR products of mt 16S rRNA gene amplified from four studied parrotfish species SC: *Scarus collana*; SF: *Scarus frenatus*; SC: *Scarus (Chlorurus) sordidus*, and SN: *Scarus niger*; MW: DNA ladder (100-3000 bp).

2. Sequence Analysis and Nucleotide Composition of mt 16S rRNA Gene:

BLAST search of partial 16S rRNA gene nucleotide sequences against nr database/parrofises (taxid: 8247) verified the identity of studied species. After excluding primers bases, sequence alignment produced an average nucleotide length of 572.5 base pairs and a consensus length of 574 sites (Fig. 2) which included base pairs, gaps, and 2 indel (insertion/deletion) sites. In average, nucleotides composition (Table 2) was T(U) =23.0, C=25.1, A=29.0 and G= 23.0. Overall, the G+C=45.40% and A+T=54.60% exhibited nucleotides favor towards AT contents.

Analysis of 574 sites of the 16S rRNA gene revealed 539 (93.90%) conserved nucleotides and 33 (5.75%) variable nucleotides. From the variable nucleotides, 12 (2.09%) were parsimony informative, and 21 (3.66%) were singletons. A majority of the 16S rRNA gene was conserved (93.90%) however, a less sequence divergence of 5.75% was observed (Table 2). Also, nucleotide analysis showed part of 16S rRNA gene analysed here contains 2 indels in three species (red dashed, Fig. 2).

```
Scarus_collana   CTTGCAAAATCAACAAATAAGAGGTCCCGCCTGCCCTGTGACTATATGTTTAACGGCCGC [ 60]
Scarus_frenatus  ....................AGA.GT.....G............................ [ 60]
Scarus_sordidus  ............................................................ [ 60]
Scarus_niger     ............................................................ [ 60]

Scarus_collana   GGTATTTTGACCGTGCGAAGGTAGCGCAATCACTTGTCTTTTAAATGGAGACCTGTATGA [120]
Scarus_frenatus  ............................................................ [120]
Scarus_sordidus  ............................................................ [120]
Scarus_niger     ............................................................ [120]

Scarus_collana   ATGGCACGACGAGGGCTTAACTGTCTCCTTTCCTAAGTCAATGAAATTGATCTCCCCGTG [180]
Scarus_frenatus  ......................................................T..... [180]
Scarus_sordidus  ............................................................ [180]
Scarus_niger     ......................................................T..... [180]

Scarus_collana   CAGAAGCGGGGATAAACCCATAAGACGAGAAGACCCTATGGAGCTTTAGACACTAAAACA [240]
Scarus_frenatus  ..............G...............................C............. [240]
Scarus_sordidus  ..............CG.T.......................................... [240]
Scarus_niger     ..............G...............................C............. [240]

Scarus_collana   GCCCATGTTAAGAACCCTCACACAAGAGACCAAACTAGATGGCCCCTGTCCTAATGTCTT [300]
Scarus_frenatus  ......................A...................T................. [300]
Scarus_sordidus  ..T.....C..A.C.....C........G............A...........T...... [300]
Scarus_niger     ..T........A..........A.....G............A.................. [300]

Scarus_collana   TGGTTGGGGCGACCACGGGGTAACAAAAAACCCCCGCGCGGAACAGAAACATTGTTTCCA [360]
Scarus_frenatus  ...................................................C........ [360]
Scarus_sordidus  ............................................................ [360]
Scarus_niger     ...................................................C........ [360]

Scarus_collana   AAACCAAGAGTACCCACTCTAAGTAACAAGACCTACTTGACCTTTGAGATCCGGCACTTA [420]
Scarus_frenatus  G.........C....G.................C.......................--. [420]
Scarus_sordidus  G..........TT...........................................T--. [420]
Scarus_niger     G.........C....G............G....C.......................--T [420]

Scarus_collana   AGCCGATCAACGGACCGAGTTACCCTAGGGATAACAGCGCAATCTTCTTCGAGAGTTCAT [480]
Scarus_frenatus  ............................................................ [480]
Scarus_sordidus  ........................................................C... [480]
Scarus_niger     ............................................................ [480]

Scarus_collana   ATCGACGAGAGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCAGCCGCT [540]
Scarus_frenatus  ..........A................................................. [540]
Scarus_sordidus  ..........A................................................. [540]
Scarus_niger     ............................................................ [540]

Scarus_collana   ATTAAGGGTTCGTTTGTTCAACGATTAAAGTCCT [574]
Scarus_frenatus  .................................. [574]
Scarus_sordidus  .................................. [574]
Scarus_niger     .................................. [574]
```

Fig. 2: Aligned partial sequences of mt 16S rRNA gene among investigated four Red Sea parrotfishes. Sequences from sense strand and nucleotide identities are designated by dots. The indels are in the red dash.

Table 2: Nucleotide constitutions of mt 16S rRNA gene sequences analyzed for 4 scarid species. C= conserved; V= variable; PI= parsimony informative; S= singleton sites.

<table>
<tr><th>Species/Nucleotide Constitution</th><th>T(U)</th><th>C</th><th>A</th><th>G</th><th>Total</th><th>G+C%</th><th>A+T%</th><th>C</th><th>V</th><th>PI</th><th>S</th></tr>
<tr><td>Scarus collana</td><td>23.2</td><td>24.9</td><td>29.3</td><td>22.6</td><td>574.0</td><td>47.50</td><td>52.50</td><td rowspan="5">539
93.90%</td><td rowspan="5">33
5.75%</td><td rowspan="5">12
2.09%</td><td rowspan="5">21
3.66%)</td></tr>
<tr><td>Scarus frenatus</td><td>22.4</td><td>25.0</td><td>29.4</td><td>23.3</td><td>572.0</td><td>48.30</td><td>51.80</td></tr>
<tr><td>Scarus sordidus</td><td>23.6</td><td>25.2</td><td>28.5</td><td>22.7</td><td>572.0</td><td>47.90</td><td>52.10</td></tr>
<tr><td>Scarus niger</td><td>22.7</td><td>25.2</td><td>28.8</td><td>23.3</td><td>572.0</td><td>48.50</td><td>51.50</td></tr>
<tr><td>Average</td><td>23.0</td><td>25.1</td><td>29.0</td><td>23.0</td><td>572.5</td><td>48.05</td><td>51.98</td></tr>
</table>

3 Molecular Phylogentic Analysis of mt 16S rRNA Gene:

Analysis of ten sequences (4 from current study, and 6 retrieved from Genbank/NCBI) produced ML and UMPGA trees rotted with *Choerodon schoenleinii* of Wrasses (Figs 3, 4). The suitable nucleotide substitution was found to be the K2 (Kimura 2-parameter) +G model (BIC=3213.230; AIC=3087.115; lnL= -1524.490; transition/transversion bias (R) = 3.35; (+G) = 0.13; Nucleotide frequencies: f (A), f (T), f(C), and f (G), were 0.250, 0.250, 0.250, and 0.250 receptively).

The pairwise genetic distances among the 10 sequences of labriformes species computed by the K2+G model (rate variation among sites modeled with a gamma distribution, shape parameter = 0.13) is shown in Table 3. The distance values among species ranged from 0.00 (*Scarus ghobban* with *Scarus rubroviolaceus*) to 0.437 (*Bolbometopon muricatum* with *Choerodon schoenleinii*). Among the four Red Sea parrotfishes, the highest genetic distance was between *Scarus frenatus* and *Bolbometopon muricatum* (0.136), whilst the lowest was between *Scarus frenatus* and *Scarus niger* (0.030).

Table 3: Pairwise genetic distance concerning 10 nucleotide sequences (4 Red Sea parrotfishes + 6 retrieved sequences) computed by Kimura 2-parameter +G. Sites rate variation modeled with gamma distribution (shape parameter = 0.13). The out-group species is highlighted in grey.

	Species name	1	2	3	4	5	6	7	8	9	10
1	*Scarus_collana*	--									
2	*Scarus_frenatus*	0.041	--								
3	*Scarus_sordidus*	0.034	0.075	--							
4	*Scarus_niger*	0.035	0.030	0.048	--						
5	*Bolbometopon muricatum*_NC_033901.1	0.113	0.136	0.126	0.111	--					
6	*Choerodon schoenleinii*_NC_025771.1_Wrasses	0.343	0.494	0.369	0.373	0.437	----				
7	*Scarus forsteni*_NC_011928.1	0.036	0.035	0.052	0.011	0.107	0.351	----			
8	*Scarus ghobban*_NC_011599.1	0.043	0.055	0.053	0.032	0.117	0.374	0.027	----		
9	*Scarus rubroviolaceus*_NC_011343.1	0.043	0.055	0.053	0.032	0.117	0.374	0.027	0.000	----	
10	*Scarus schlegeli*_NC_011936.1	0.013	0.052	0.048	0.041	0.127	0.412	0.047	0.054	0.054	---

The ML phylogentic tree (Fig. 3) with the highest log likelihood (-1522.6888), demonstrated three major clades (groups). In clade A, *Scarus frenatus*, *Scarus niger*, *Scarus forsteni*, *Scarus ghobban*, and *Scarus rubroviolaceus* are together assembled, however, both *Scarus ghobban* and *Scarus rubroviolaceus* are greatly closed to each other with bootstrap support of 100 (identical). Clade/group B contains *Scarus collana* and *Scarus schlegeli* (support of 88). Both *Scarus sordidus* and *Bolbometopon muricatum* are grouped together. While the *Choerodon* schoenleinii of Wrasses (outgroup) is separated from other species that pointed to its potential evolutionary divergence.

The UMPGA phylogenic tree (Fig. 4) is similar to that produced by ML method, however in the group C the *Bolbometopon muricatum* separately positioned. Furthermore, the tree displayed two evolutionary lineages, where scarid parroffihes (scarainae) are clustered based on their genetic closeness. While the out-group represented by *Choerodon schoenleinii* of wrasses may potentially represent a different evolutionary lineage.

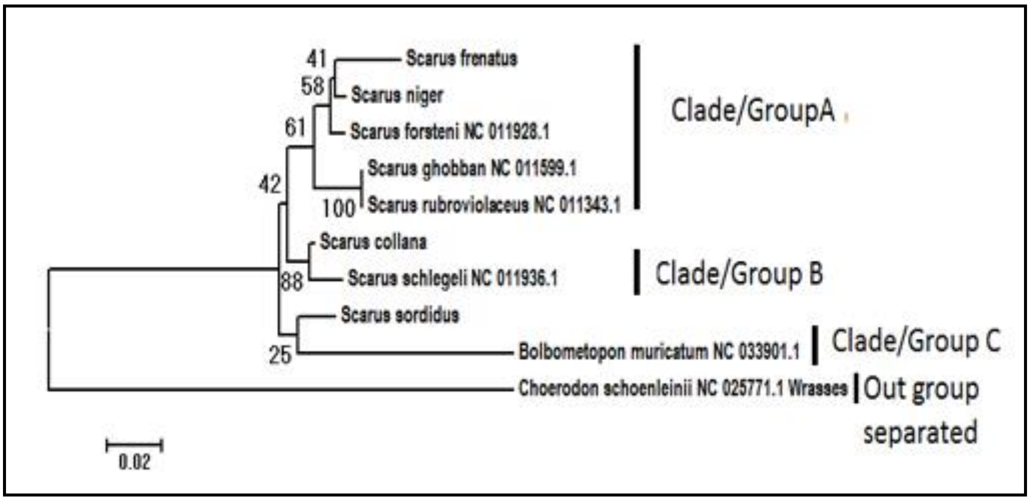

Fig. 3: Phylogenetic tree inferred by the Maximum Likelihood method based on the Kimura 2-parameter model using partial 16S gene sequences from four Red Sea Parrotfishes and other included species. Tree branch lengths measured in the number of substitutions per site, the rate variation among sites modeled with a gamma distribution (+G, shape parameter = 0.13). The bootstrap of 1000 replicates support is depicted next to the branches.

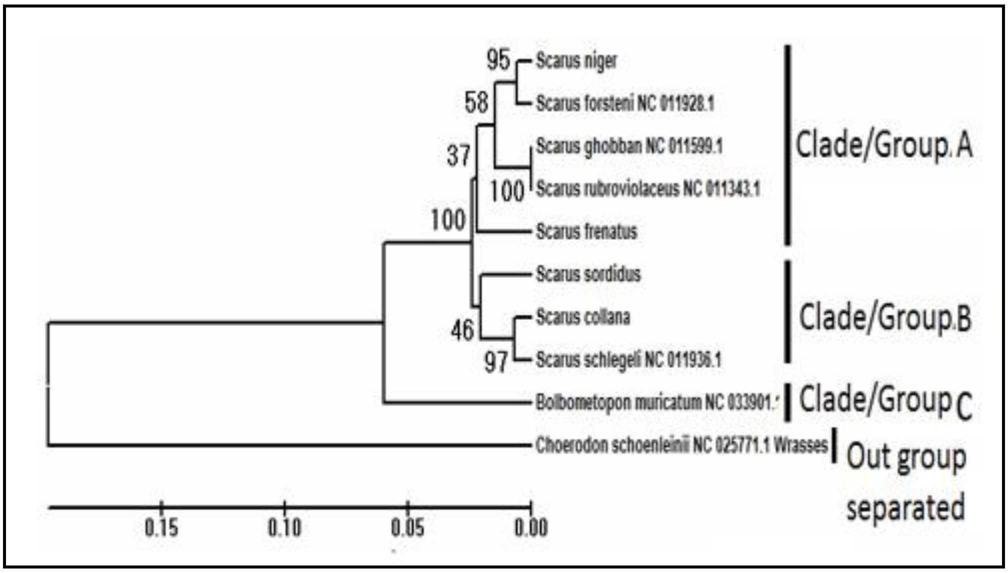

Fig. 4: The UPMGA phylogenetic tree based on the Kimura 2-parameter model using partial 16S gene sequences from four Red Sea Parrotfishes and other included species. The tree with branch lengths in the number of substitutions per site with rate variation among sites modeled with a gamma distribution (shape parameter = 0.13). The bootstrap support of 1000 replicates is shown next to the branches.

DISCUSSION

In this study, the mitochondrial 16S gene fragments were sequenced from four Red Sea parrotfish species and then were analysed for sequence variations and molecular phylogeny patterns. The 16S rRNA gene primers applied here were successfully amplified the targeted DNA fragments from each DNA sample. Similar studies used the same primers for generating 16S rRNA fragments in other fish species (Lee *et al.*, 2014; Carvalho *et al.*, 2004; EL-Mahdi, 2018b).

In general, nucleotides conservative contents can indicate conserved similar structure or function. Nucleotide sequence alignments confirmed the reservation pattern of the 16S rRNA gene (93.90%) with less sequence divergence (5.75%) that supported by other studies where, the 16S ribosomal gene is fairly conserved and therefore often used to inspect the relationships among different species and genera (Orrell *et al.,* 2004; Mitani *et al.,* 2009).

Nucleotide composition analysis of studied 16S rRNA gene confirmed a bias towards adenine and similar preference towards thymine, cytocine and guanine shifting the favoritism towards AT content. This was supported by reported fish molecular phylogeny studies (Lakra *et al.,* 2009; Mohanty *et al.,* 2013; EL-Mahdi, 2018b).

Related taxes possess similar DNA base constitutions in comparison to those are distantly related. Results here demonstrated a high level of 16S rRNA gene nucleotide base identity and similarities for species under study (see alignment, Fig. 3). This suggests their close genetic background and evolutionary relationship which may reflect sequence building-function relationships. As reported, organisms having similar/identical nucleotide base composition are closely related than those are not related (Zeigler, 2003; Gadagkar *et al.,* 2005).

Remarkably patterns from phylogenetic analysis of 16S rRNA DNA sequences demonstrated a clear grouping of analyzed parrotfishes sequences (4 present study +6 retrieved from Genbank/NCBI) into Scarid parrotfishes (*Bolbometopon muricatum, scarus (Chlorurus) sordidus, Scarus forsteni, Scarus ghobban, Scarus rubroviolaceus, and Scarus schlegeli*), while the *Choerodon schoenleinii* (outgroup) that correspond to Wrasses species is separated alone mirroring a separate evolutionary lineage. This patterns of assembling go with Bellwood's (1994) of parrotfish relationships, also with other reporters (Randall, 2007; Randall and Parenti 2014), but disagree with Schultz's (1958) and Streelman *et al.* (2002) division of the Scaridae into two subfamilies (Scarinae and Sparisomatinae). Here, the outlined phylogenetic analysis possibly assume that Scaridae is supposed to maintain the family position.

Conclusion:

In this study, we evaluated sequence variations and molecular phylogeny of scarids species; *Scarus collana* Rüppell 1835; *Scarus frenatus* Lacepède 1802; *Scarus (Chlorurus) sordidus* Forsskål 1775 and *Scarus niger* Forsskål 1775 using partial mitochondrial 16S rRNA genes nucleotide sequences.

Targeted DNA fragments were isolated and sequenced from four scarids species; *Scarus collana* Rüppell 1835; *Scarus frenatus* Lacepède 1802; *Scarus (Chlorurus) sordidus* Forsskål 1775 and *Scarus niger* Forsskål 1775 using mitochondrial 16S rRNA gene-specific primers.

Results showed a high level of nucleotide identity in the studied regions, which reflects a close genetic relationship and shared ancestry among studied parrotfishes. Nucleotide compositions of partial 16S rRNA gene DNA sequence biased towards adenine and similar preference towards thymine, cytocine, and guanine. Also, it revealed a preference for higher nucleotide conservations.

Phylogenetic analysis displayed grouping patterns of assembly for targeted species and included other related taxa, which confirmed their genetic similar constitutions and tendency to *have* similar *niche*s. The phylogenetic trees clearly revealed two evolutionary lineages separating both Scaridae and Wrasses, which outlined an assumption that Scaridae is supposed to maintain the family status.

Obtained data could be beneficial for parrotfishes classification, conservation, and their needed environments. Therefore, the acquisition of nucleotide sequences from other parrotfishes using the developed mt16S rRNA gene-specific primers utilized here would contribute in the future to the phylogenetic and evolutionary studies of parrotfishes in the Red Sea territory.

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ARABIC SUMMARY

تباينات التسلسل النيكليوتيدى والتطور الجزيئي لبعض أسماك الببغاء (Scaridae)
في البحر الأحمر باستخدام تسلسلات المورث الميتوكوندري

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فى تلك الدراسة، تم عزل شظايا الحمض النووي المستهدفه لآربعة أنواع من أسماك الببغاء (Scaridae) *Scarus collana* Rüppell 1835 *Scarus frenatus* Lacepède 1802*; Scarus (Chlorurus) sordidus* Forsskål 1775, and *Scarus niger* Forsskål 1775, بواسطة بادئات مخصصة للمورث 16S الميتوكوندرى.

أظهر تحليل تسلسل الحمض النووي ارتفاع مستوى تشابه التتابعات النيكلوتيدية في المناطق التي شملتها الدراسة، مشيرا الى وجود علاقة وراثية وثيقة وأصل مشترك بين انواع أسماك الببغاء قيد الدراسة.

أوضحت الدراسة أن التتابعات النيكلوتيدية لآجزاء المورث 16S الميتوكوندرى المدروسة تتميز بارتفاع نسبة قاعدة الأدينين بالمقارنة مع الثلاثة قواعد الآخريات (الثيمين، السيتوسين والجوانيين) اللاتى تمثلن بنسب متقاربة، كذلك بينت الدراسة الآرتفاع المحافظ لترتيب التتابعات النيكلوتيدية لشظايا الدنا(DNA) المدروسة.

أظهر التحليل العنقودى الوراثى النمط التجميعى لأنواع الاسماك قيد الدراسة، وغيرها من الأنواع المدرجة ذات الصلة، حيث دل ذلك على تركيبهم الوراثي المتماثل والتشابه البيئى المعيشى. كما اشارايضا الى احتمالية وجود مسارين للتطور يفصلا كل من Scaridae وWrasses والذى ربما يدعم الوضع التصنيفى لآسماك الببغاء (Scaridae) كعائلة مستقلة.

ان النتائج المستحصلة من هذه الدراسة ربما تكون مفيدة لتصنيف أسماك الببغاء، وكذلك سبل المحافظة عليها ومتطلباتها البيئية. لذلك فالحصول على تسلسلات نيوكليتيدية من أسماك الببغاء الأخرى بواسطة البادئات المخصصة للمورث 16S الميتوكوندرى المستخدمة فى تلك الدراسة، من شأنه أن يساهم مستقبلا في الدراسات التطورية لأسماك الببغاء في البحر الأحمر.