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Analysis of 16S mitochondrial ribosomal DNA sequence variations and phylogenetic relations among some Serranidae fishes

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Abstract

Reconstruction of phylogenetic relations among certain Serranidae fish could be applied to detect the biodiversity that is needed to conserve these biological resources. This study was designed to provide information about the molecular variations within and among numbers of economically important fishes (42 fish species that belong to three genera), using a simple and effective barcoding system. A comparative genetic analysis was carried out among some *16S r-RNA* sequences in various Serranidae fish genera (*Plectropomus, Cephalopholis* and *Epinephelus*). Nucleotide composition, sequence conservation, single nucleotide polymorphism (SNP), haplotype diversity and nucleotide diversity were calculated in all these fish species. All of these fish samples were differentiated by the barcoding system. Average genetic distance values within *Plectropomus*, *Epinephelus* and *Cephalopholis* were 0.03, 0.039 and 0.048, respectively. *Plectropomus* species were found to be distantly related to both *Epinephelus* and *Cephalopholis.* The DNA barcoding system should be used in fisheries and aquaculture to conserve aquatic genetic resources.

Keywords: *Epinephelus*, *Plectropomus*, *Cephalopholis*, comparative, genetics
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Introduction

In marine life there are more than 15000 aquatic species (Zemlak *et al*., 2009), including fish. Grouper fish (Noikotr *et al*., 2013) in Serranidae (the family that includes *Plectropomus, Cephalopholis*, and *Epinephelus* fish genera) inhabit tropical and subtropical waters around the world (Craig & Hastings, 2007). This family includes about 300 species, which constitute an important part of the marine environment (Smith, 1971). To date, the true phylogenetic relationships and evolution within the Serranidae family are unclear. Knowledge about characterization and evolution is needed to conserve this fish family.

Sometimes morphological characterization leads to incorrect identification (Hubert *et al*., 2008), such as in the Serranidae family, especially in sister taxa. Fish characterization based on molecular markers has been applied successfully to study fish evolution, and biodiversity, and enhance fisheries conservation (Craig & Hastings, 2007; Zhu & Yue, 2008; Zhang & Hanner, 2012).

Acceleration of speciation is affected by evolutionary forces (such as natural and sexual selection), which can contribute to the origination of new species (Civetta & Singh, 1999; Schluter, 2001). Analysis of evolutionary variations (Saad *et al*., 2012) among fish, including the Serranidae family, based on DNA sequence polymorphism, would assist in the definition of appropriate units for fish conservation. The capacity to conserve Serranidae fish resources requires identifying taxa and variations at molecular level (Rashed *et al*., 2008; Saad *et al.,* 2011).

Mitochondrial DNA markers (Ward *et al*., 2008; Zhang & Hanner, 2012) offer accurate identification systems of species, so they should be used in the fisheries and aquaculture sectors for conservation (Saad *et al*., 2011) of aquatic genetic resources. The 16S mitochondrial ribosomal DNA system is preferred owing to the sensitivity of its application in exploring the evolutionary variations in aquatic organisms (Craig *et al*., 2001; Pondella *et al*., 2003).

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The accuracy of fish genetic identification is affected by the choice of identification system, of DNA regions for barcoding and of markers. Developing clear DNA markers is widely used to explore biodiversity among and within aquatic biological taxa (Saad *et al*., 2011; Quraishia *et al*., 2015; Saad & El-Sebaie 2017). In addition, individually isolated DNA from fish eggs, larvae, fillets and fins can be characterized through DNA identification systems.

Recently, Renxie *et al*. (2018) used *COI* and *16S r-RNA* genes to develop DNA barcoding and species identification in some sparid species from the coastal waters of China. They confirmed that fish species were effectively characterized and delimited by *COI*, as well as *16S r-RNA.* The 16S mitochondrial ribosomal DNA (Lianming *et al*., 2014) system provides simple and effective approaches to exploring the real number of species (Craig *et al*., 2001; Pondella *et al*., 2003) that belong to certain genera.

The aim of the study was to produce baseline information about molecular variations within and among numbers of Serranidae fish that belong to three genera, namely *Plectropomus, Cephalopholis* and *Epinephelus*.

Materials and Methods

Samples of *Cephalopholis oligosticta and Plectropomus areolatus* were collected from Yanbu (Red Sea port in western Saudi Arabia), while samples of *Cephalopholis sonnerati* were obtained from the Biological Aquatic Resources Research Group, King Abdulaziz University, Saudi Arabia.

Fish samples were placed on ice in the field for short-time preservation before photographing. For long-time preservation, small pieces of caudal fins were preserved in 95% ethanol (Georgescu *et al*., 2011). DNA was extracted from caudal fin tissues (with a total sample size of 30 specimens) as described by Hillis *et al*. (1996). Mitochondrial *16S r-RNA* gene fragments were amplified using polymerase chain reaction (PCR). A total of 100 microliter (µL) amplification reactions (Pondella *et al*., 2003) were prepared with 100 ng DNA, 1.5 mM magnesium chloride ($MgCl_2$), 2.5 U Taq polymerase, 200 µM of deoxynucleotide triphosphates (dNTPs), and 0.1 µM for the primer pairs 16sarL 5-CGCCTGTTTATCAAAAACAT-3 and 16sbrH 5-CCGGTCTGAACTCAGATCACGT-3 (Palumbi, 1996).

PCR amplification was performed with denaturation for 3 min at 95 °C, 30 cycles at 95 °C for 60 seconds, 45 °C for 120 seconds, 72 °C for 90 seconds, and an extension at 72 °C for 15 min. The PCR products of the gene fragments were visualized on 1.3 agarose gel and purified with a QIAGEN PCR purification kit. The most intense fragment samples were introduced as three subsamples for sequencing (Macrogen Inc., Republic of Korea) using forward primer. These fragment sequences were selected, analysed and submitted to the National Centre for Biotechnology Information (NCBI). For comparison, other *16S r-RNA* gene fragments from *Plectropomus, Cephalopholis* and *Epinephelus* genera were obtained from NCBI. Accession numbers and codes of all the DNA sequences are summarized in Tables 1a and 1b.

Some mitochondrial *16S r-RNA* gene fragments (obtained from NCBI) were analysed comparatively with sequenced samples *Cephalopholis sonnerati* (KX298698 and KX298699), *Cephalopholis oligosticta* (KX298691, KX298692, KX298695, KX298696) and *Plectropomus areolatus* (KX298700, KX298701 and KX298702). Sequences were aligned and the phylogenetic tree was re-constructed among the fish using MEGA V6 (Tamura *et al*., 2013). The evolutionary history was inferred by using the maximum likelihood method.

Analysis of the number of haplotypes, single nucleotide polymorphisms, and estimates of haplotype diversity, nucleotide diversity, theta from polymorphic sites, the average number of nucleotide differences and sequence conservation were calculated using DnaSp (version 5.10.01). A total of 131 mitochondrial *16S r-RNA* gene fragments were estimated. Only 556 bp sequences were aligned and analysed (after trimming) to detect nucleotide variations and phylogenetic reconstruction in Serranidae fishes (42 fish species belonging to the three genera). A comparative analysis was carried out among the *16S r-RNA* gene sequences and other *16S r-RNA* gene sequences obtained from NCBI in various Serranidae species. The nucleotide compositions (A, T, G and C) for each of the *16S r-RNA* gene fragments were calculated.

Results

The DNA polymorphism was detected among these three fish species, namely *C. oligosticta, C. sonnerati* and *P. areolatus*. PCR products (approximately 600 bp fragments of the mitochondrial *16S r-RNA* gene) were visualized on an agarose gel (Figure 1). The most intense fragment samples were introduced for sequencing. These fragment sequences were selected, analysed and submitted to the NCBI. For comparison, some other *16S r-RNA* gene fragments from *Plectropomus, Cephalopholis* and *Epinephelus* genera were obtained from NCBI.

Table 1a The evaluated fish species, codes and National Centre for Biotechnology information accessions

Accession no	Code	Name	Accession no	Code	Name
KC593378.1	CSo	*C. sonnerati*	KM658604.1	PA	*P. areolatus*
DQ088037.1	CSo	*C. sonnerati*	KM658603.1	PA	*P. areolatus*
KX298699.1	CSo	*C. sonnerati*	KM658602.1	PA	*P. areolatus*
KX298698.1	CSo	*C. sonnerati*	KJ101556.1	PL	*P. leopardus*
HQ592260.1	CSo	*C. sonnerati*	KJ101555.1	PL	*P. leopardus*
KX298696.1	Col	*C. oligosticta*	JF750754.1	PL	*P. leopardus*
KX298695.1	Col	*C. oligosticta*	DQ101270.1	PL	*P. leopardus*
KX298692.1	Col	*C. oligosticta*	DQ067321.1	PL	*P. leopardus*
KX298691.1	Col	*C. oligosticta*	AF297298.1	PL	*P. leopardus*
AF297323.1	CCu	*C. cruentata*	KM658637.1	PLe	*P. leopardus*
AF297292.1	CFu	*C. fulva*	KM658635.1	PLe	*P. leopardus*
KC537759.1	CBo	*C. boenak*	KM658636.1	PLe	*P. leopardus*
KJ469385.1	CSe	*C. sexmaculata*	KM658643.1	PMa	*P. maculatus*
KU891818.2	CUr	*C. urodeta*	KM658646.1	PMa	*P. maculatus*
AY947627.1	CPo	*C. polleni*	KM658645.1	PMa	*P. maculatus*
AY947599.1	CIg	*C. igarashiensis*	KM658644.1	PMa	*P. maculatus*
JX094007.1	CSo	*C. sonnerati*	KM658642.1	PMa	*P. maculatus*
KM077968.1	CSp	*C. spiloparaea*	JF750755.1	PMa	*P. maculatus*
KM077965.1	CBo	*C. boenak*	KM658622.1	PLa	*P. laevis*
JX094019.1	CSe	*C. sexmaculata*	KM658621.1	PLa	*P. laevis*
KM077969.1	CUr	*C. urodeta*	KM658620.1	PLa	*P. laevis*
EF213705.1	CUr	*C. urodeta*	KM658616.1	PLa	*P. laevis*
KM656819.1	CSe	*C. sexmaculata*	KM658615.1	PLa	*P. laevis*
AF297325.1	CUr	*C. urodeta*	KM658613.1	PLa	*P. laevis*
KM656818.1	COl	*C. oligosticta*	DQ067320.1	PLa	*P. laevis*
KM656816.1	CHe	*C. hemistiktos*	KM658624.1	PLa	*P. laevis*
KM077967.1	CMi	*C. miniata*	KM658623.1	PLa	*P. laevis*
EF213713.1	CMi	*C. miniata*	KM658626.1	PLa	*P. laevis*
AY947603.1	CFo	*C. formosa*	KM658667.1	PPe	*P. pessuliferus*
KX298702.1	PA	*P. areolatus*	KM658666.1	PPe	*P. pessuliferus*
KX298701.1	PA	*P. areolatus*	KM658665.1	PPe	*P. pessuliferus*
KX298700.1	PA	*P. areolatus*	KM658668.1	PPe	*P. pessuliferus*
KM658607.1	PA	*P. areolatus*	KM658659.1	POl	*P. oligacanthus*
KM658612.1	PA	*P. areolatus*	KM658656.1	POl	*P. oligacanthus*
KM658611.1	PA	*P. areolatus*	KM658655.1	POl	*P. oligacanthus*
KM658609.1	PA	*P. areolatus*	KM658664.1	POl	*P. oligacanthus*
KM658608.1	PA	*P. areolatus*	KM658662.1	POl	*P. oligacanthus*
KM658601.1	PA	*P. areolatus*	KM658663.1	POl	*P. oligacanthus*
KM658605.1	PA	*P. areolatus*	AY947615.1	POl	*P. oligacanthus*
HQ592227.1	EPm	*E. marginatus*	LC127005.1	EPoe	*E. poecilonotus*
AY947595.1	EPm	*E. marginatus*	KM656829.1	EPoe	*E. poecilonotus*
KT835677.1	ERa	*E. radiatus*	EF503628.1	EPol	*E. polyphekadion*
DQ067304.1	ERa	*E. radiatus*	AY947569.1	EPol	*E. polyphekadion*
KP013758.1	EFu	*E. fuscoguttatus*	JX094010.1	EMo	*E. moara*
KJ607972.1	EFu	*E. fuscoguttatus*	JF750750.1	EMo	*E. moara*

Table 1b The evaluated fish species, codes and National Centre for Biotechnology information accessions

Accession no	Code	Name	Accession no	Code	Name
JX119192.1	EFu	*E. fuscoguttatus*	KX147236.1	EMo	*E. moara*
HQ840452.1	EHe	*E. hexagonatus*	KP009977.1	EMo	*E. moara*
HQ840446.1	EHe	*E. hexagonatus*	JQ518290.1	EMo	*E. moara*
DQ067312.1	EFu	*E. fuscoguttatus*	JX094008.1	ECo	*E. corallicola*
AF297295.1	ECi	*E. cifuentesi*	JN637834.1	ECo	*E. corallicola*
AY947585.1	ECa	*E. caninus*	LC127002.1	EAr	*E. areolatus*
KC593373.1	EEp	*E. epistictus*	KM077974.1	ECo	*E.coeruleopunctatus*
DQ067306.1	EAm	*E. amblycephalus*	AY947568.1	ECo	*E. corallicola*
KM656827.1	ELa	*E. latifasciatus*	DQ067303.2	EMo	*E. moara*
KC480177.1	ELa	*E. latifasciatus*	AF297314.1	EAd	*E. adscensionis*
HQ840449.1	EHe	*E. hexagonatus*	LC126985.1	EBr	*E. bruneus*
DQ088044.1	ELa	*E. latifasciatus*	LC126984.1	EBr	*E. bruneus*
HQ840444.1	EHe	*E. hexagonatus*	LC126983.1	EBr	*E. bruneus*
HQ840447.1	EHe	*E. hexagonatus*	KF556648.1	EBl	*E. bleekeri*
KM656834.1	ESu	*E. summana*	LC126988.1	ECh	*E. chlorostigma*
KT835676.1	ELa	*E. latifasciatus*	KM077972.1	EAr	*E. areolatus*
DQ154106.1	EHa	*E. hexagonatus*	KC593374.1	EAr	*E. areolatus*
KM656824.1	ECh	*E. chabaudi*	KC466080.1	EAr	*E. areolatus*
EF503629.1	ECo	*E.coeruleopunctatus*	DQ088038.1	EAr	*E. areolatus*
KT835678.1	EUn	*E. undulosus*	LC127001.1	EAr	*E. areolatus*
LC127006.1	EPo	*E. poecilonotus*			

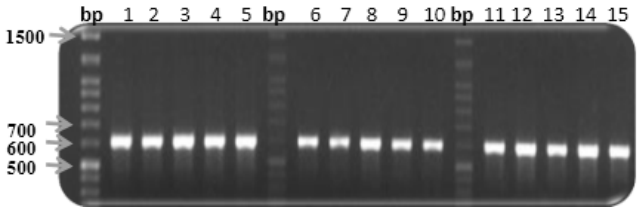

Figure 1 Polymerase chain reactions of *16S r-RNA* gene fragments generated by specific primer pairs (16sarL and 16sbrH) from *Cephalopholis oligosticta* (samples from 1 to 5)*, Cephalopholis sonnerati* (samples from 6 to 10) and *Plectropomus areolatus* (samples from 11 to 15)

The averages of nucleotide composition values are summarized in Table (2). Minor differences in nucleotide compositions were observed among the genera. The number of haplotypes (h = 66), single nucleotide polymorphisms (SNPs = 149), estimates of haplotype diversity (hd = 0.983), nucleotide diversity (Pi = 0.086), theta from polymorphic sites (Θ = 0.062), average number of nucleotide differences (k = 44.85), and sequence conservation value (Sc = 0.707) were calculated for overall sites.

A total of seven *Plectropomus* species were evaluated. The number of haplotypes (h = 13), single nucleotide polymorphism (SNPs = 39), estimates of haplotype diversity (hd = 0.922), nucleotide diversity (Pi = 0.029), theta from polymorphic sites (Θ = 0.016), average number of nucleotide differences (k = 16.1), and sequence conservation value (Sc = 0.928) were calculated in genus *Plectropomus 16S r-RNA* gene fragments. A total of 13 *Cephalopholis* species were evaluated. The values of (h), (SNPs), (hd), (Pi), (Θ), (k) and (Sc) within *Cephalopholis* were 23, 112, 0.985, 0.046, 0.056, 24.69 and 0.795 respectively. A total of 22 *Epinephelus* species were evaluated. The values of (h), (SNPs), (hd), (Pi), (Θ), (k) and (Sc) within *Epinephelus* were 30, 87, 0.963, 0.037, 0.03, 20.36 and 0.837 respectively (Table 2).

Table 2 Number of fragments, number of species, single nucleotide polymorphisms, average T, C, A, G contents, average nucleotide composition, nucleotide diversity, theta from site, nucleotide differences, haplotype diversity, and sequence conservation in each Serranidae fish

Genera parameters	***Plectropomus***	***Cephalopholis***	***Epinephelus***	**All genera**
Number of fragments	*49*	*29*	*53*	*131*
Number of species	*7*	*13*	*22*	*42*
SNP	39	112	87	*149*
T	0.244±0.3	0.244±0.3	0.235±0.4	0.241±0.6
C	0.235±0.5	0.236±0.5	0.241±0.5	0.238±0.6
A	0.295±0.5	0.287±0.4	0.290±0.4	0.292±0.6
G	0.224±0.4	0.231±0.4	0.232±0.3	0.229±0.6
Pi	0.029	0.046	0.037	0.086
Ɵ	0.0168	0.056	0.039	0.062
K	16.1	24.69	20.36	44.85
h	13	23	30	66
hd	0.922	0.985	0.963	0.983
SC	0.928	0.795	0.837	0.707

Single nucleotide polymorphism= (SNP), the averages of T content = (T), the averages of C content = (C), the averages of A content = (A), the averages of G content = (G), nucleotide diversity= (Pi), theta from site = (Ɵ), number of haplotypes= (h), average number of nucleotide differences =(K), haplotype diversity= (hd) and sequence conservation = (SC)

The phylogenetic relations among the Serranidae fish showed that they were clustered in two main groups. The first group included only *Plectropomus* species. *Epinephelus* and *Cephalopholis* (distance = 0.08) were subgroups that were included in the second group.

The percentage of genetic distance values between the congeneric fish species reached 5.8%, 8%, and 10.9% in *Plectropomus* species*, Epinephelus* species, and *Cephalopholis* species*,* respectively. *Plectropomus* species samples were distantly related to both *Epinephelus* and *Cephalopholis.* The overall distance value (0.09) was obtained among all these fish species (Table 3; Figure 2). Averages of genetic distance values within the genera *Plectropomus*, *Epinephelus* and *Cephalopholis* were 0.03, 0.039, and 0.048, respectively. The genetic distance values within samples of genus *Plectropomus* ranged from 0% to 5.6%. Within this genus*, P. oligacanthus* and *P. areolatus* are the most distantly related species relatively. Low genetic distance value (0.006) was obtained between sequenced *16S r-RNA* gene fragments of *P. areolatus* and those from NCBI. The genetic distance values within samples of genus *Cephalopholis* ranged from 0% to 10.5%. Within this genus*, C. sonerati* and *C. polleni* were the most distantly related species relatively. The distance value between sequenced *C. oligosticta* samples and the samples obtained from NCBI was very low (D = 0.01). The genetic distance values within samples of the genus *Epinephelus* ranged from 0% to 7.7%.

Table 3 Estimated genetic distance values among the Serranidae genera based on consensus sequence variations

	Plectropomus	***Cephalopholis***	***Epinephelus***
Plectropomus	0.03		
Cephalopholis	0.132	0.048	
Epinephelus	0.134	0.08	0.039

Within this genus*, E. cifuentesi* and *E. bleekeri* were the most distantly related species relatively

The phylogenetic relations among the fish species revealed from the analysis of consensus sequences are presented in Figure 3. Many sister groups were formed based on sequence variations within each genus. No molecular variation was calculated between EMo and EBr, and EEp and EAm. With reference to genus *Cephalopholis,* the Col is a sister to CSo.

All fish species belonging to genus *Plectropomus* formed a monophyletic construction. In addition, PA constituted a paraphyletic relation with PMa, PPe, PL and PLe groups.

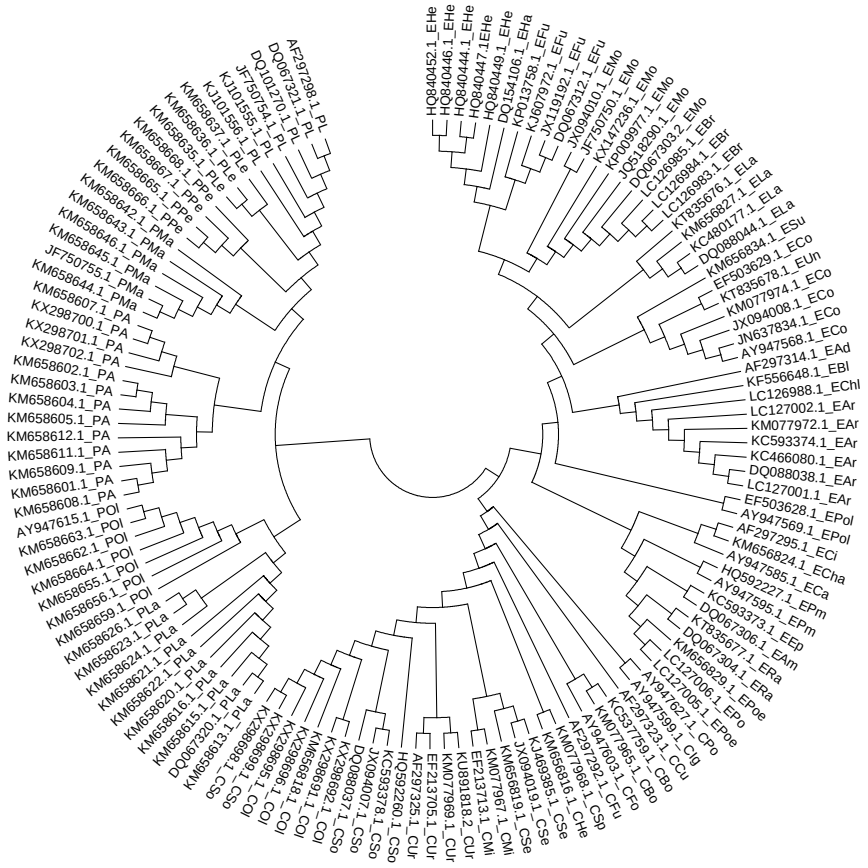

Figure 2 Phylogenetic relations among certain Serranidae fishes based on some 16S ribosomal RNA gene nucleotide variations

Discussion

The accurate relationships within the Serranidae fishes (especially in the Red Sea) are not clear. In addition, the evolution of Serranidae fish has long been poorly understood (Craig & Hastings, 2007; Saad *et al*., 2102). This may be because of abundant taxa, which should be evaluated, and the large distribution of these aquatic biological resources. Therefore, interrelationships within this family should be demonstrated based on molecular markers.

DNA barcoding systems as techniques for characterizing species by using short DNA sequences were recommended for facilitating biodiversity investigations. Many DNA barcoding systems have been applied to identifying aquatic organisms, including fish (Quraishia *et al*., 2015). Lianming *et al.* (2014) reported that *16S r-RNA* is a better choice than COI (universal barcoding system) for DNA barcoding hydrozoans.

DNA barcoding methods (Ward *et al*., 2008; Lianming *et al*., 2014; Quraishia *et al*., 2015) provide an opportunity to detect species efficiently. Conducting an accurate DNA barcoding to investigate fish biodiversity is essential for ecological assessments (Ulises *et al*., 2018). Detecting biodiversity of Serranidae fish through accurate molecular techniques would provide information that is needed to conserve these biological resources (especially endangered species). Analysis of a standardized region of DNA barcoding (Ward *et al*., 2005; Zemlak *et al*., 2009; Jefri *et al*., 2015) is widely used to detect genetic variations among fish genera, species and populations.

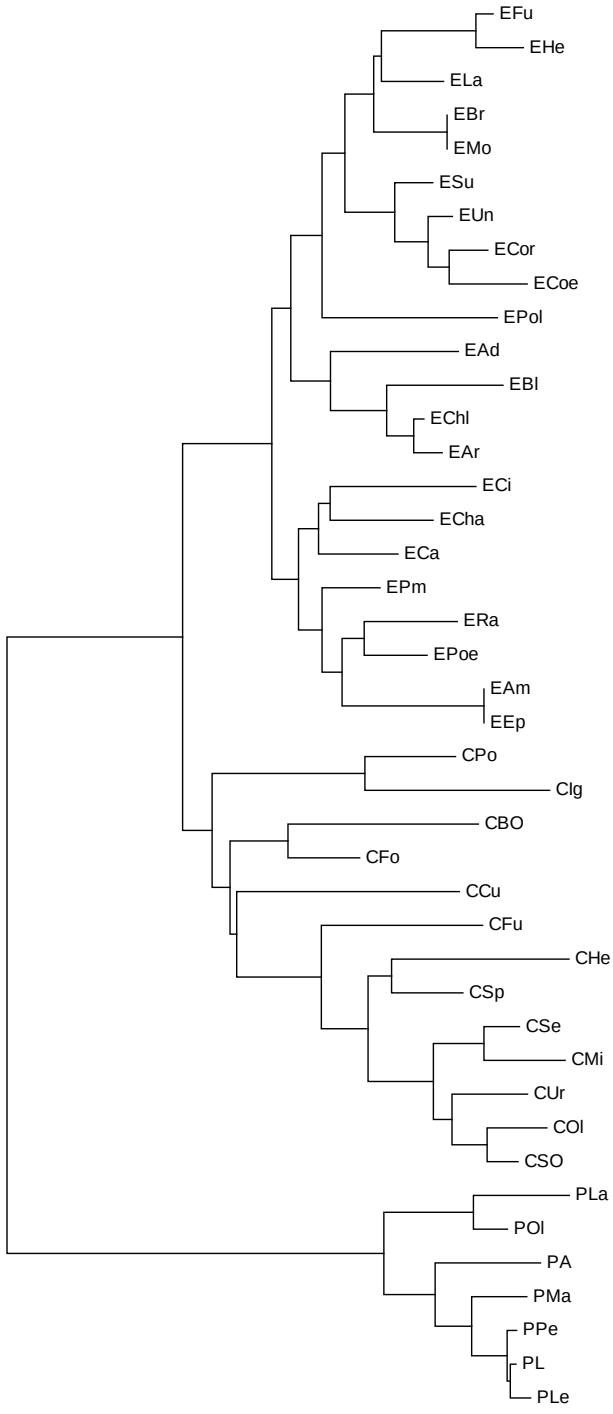

Figure 3 Phylogenetic relations among certain *Serranidae* fish species based on partial 16S ribosomal RNA gene consensus sequence variations

The 16S barcoding system was selected owing to its successful application in arriving at inference and calculating the genetic distance among other related marine fishes (Craig *et al*., 2001; Pondella *et al*., 2003). One of the benefits of using the 16S identification system for barcoding the fishes is that the sequences are easier to amplify and sequence. In addition, it has been the system of choice for fish identification in many studies (Miglietta *et al*., 2009; Moura *et al*., 2011; Ulises *et al*., 2018).

The results indicated that the *16S r-RNA* system was efficient (Quraishia *et al*., 2015) in detecting genetic variations among these fish species. The lowest values of the parameters (except Sc value) were detected within the *Plectropomus* species. The highest variability was detected in genus *Cephalopholis*. The results of these parameters were affected by the numbers of SNPs. Calculations of such parameters were recommended (Saad & El-Sebaie, 2017) for exploring the molecular genetic variations among some animal taxa, including aquatic organisms.

Calculation of SNPs revealed from molecular marker analysis would allow for accurate identification of biological taxa, including plants (Gao *et al*., 2016), and aquatic animal species (Saad & El-Sebaie 2017; Wenne, 2018). These DNA markers were developed to investigate the evolutionary variations within and among fish taxa (Wenne, 2018). The author calculated and explored the genetic distance values within and among the fish genera. For 16S, interspecific variations were higher than the intraspecific variations. *E. bruneus* is usually characterized as *E. moara* (Heemstra & Randall, 1993). Therefore, no molecular variation was calculated between EMo and EBr. The 16S sequence identity that was detected between EEp and EAm may be due to problems in morphological characterization.

When genetic distances between species and variations within species overlap, the utilization of DNA barcoding is less effective (Ulises *et al*., 2018). The percentage of genetic distance values between the congeneric fish species reached 5.8%, 8%, and 10.9% in *Plectropomus, Epinephelus*, and *Cephalopholis,* respectively. The average genetic distance values were 0.03, 0.039, and 0.048, respectively.

These levels of divergences were higher than those calculated among other fish taxa such as mullet (*Liza* sp*, Crenimugil* sp*, Chelon* sp, *Mugil* sp and *Oedalechilus* sp*)* using two barcoding systems (*16S r-RNA* and cytochrome b). The highest levels of differences between these mullet fishes (Aurelle *et al*., 2008) using cytb and *16S r-RNA* systems were 4.2% and 3.8%, respectively. The calculated divergence levels indicated that the 16s barcoding system is a beneficial marker to identify species boundaries in the fish.

Noikotr *et al*. (2013) explored the biodiversity among some grouper fishes (*Epinephelus bleekeri* and *Epinephelus malabaricus*) using two barcoding systems (*16S r-RNA* and cytochrome c oxidase). The nucleotide variations among the *Epinephelus* species ranged from 0.037 to 0.159 in *16S r-RNA* and from 0.003 to 0.157 cytochrome c oxidase.

In Salmonidae, the *16S r-RNA* (of 864 analysed sites, only 66 were variable) barcoding system was more efficient than 12s rRNA (of 745 analysed sites, only 2 were variable) for reconstructing phylogenetic relations (Georgescu et al., 2011) among salmonid fishes (*Salmo trutta fario, S. labrax, Salvelinus fontinalis* and *Thymallus thymallus*). The efficiency of this barcoding system was confirmed in other aquatic animals such as hydrozoans. Lianming *et al*. (2014) evaluated two molecular identification systems for barcoding hydrozoans in China. They found that *16S r-RNA* is more informative than *COI* for DNA barcoding hydrozoans.

The current results suggest that Red Sea Serranidae fishes do not form a monophyletic group. These results were confirmed by the genetic distance values among these genera. Compared with other groupers, Craig & Hastings (2007) found that the American *Epinephelus* species did not constitute a monophyletic group, as had been postulated by Smith (1971).

The risk of extinction is affected by genetic variation levels in certain species (Frankham *et al*., 2002) and populations (Saad *et al*., 2011). Detection of biodiversity within species via molecular markers can be explored and be used to detect the homogeniety and heterogeneiety levels. Species with little genetic variations would be at risk. The low variability values within the three fish genera (*Plectropomus*, *Epinephelus* and *Cephalopholis*) indicated that these fish may be at risk, especially genus *Plectropomus.* This may be due to overexploitation and bad management of these fish genetic resources. So, there is an urgent need to redesign management programmes to avoid or minimize the risk of extinction (Sadovy *et al*., 2004).

Analysis of nucleotide variations (Noikotr *et al*., 2013) among fish species and populations is considered the basic principle for exploring biodiversity via molecular markers (Saad & EL Sebaie, 2017). In the present study, the sequences of *16s r-RNA* gene clustered the fish species in some unique clades, which confirmed the morphological characterization in these fishes.

The reconstructed phylogenetic relations among the Serranidae showed that these fishes were clustered in two main groups. The first group included only *Plectropomus* species. *Epinephelus* and *Cephalopholis* (distance = 0.08) were subgroups of the second group. The *Plectropomus* species was distantly related to both *Epinephelus* and *Cephalopholis.*

The results of the present study showed that the sequenced *16S r-RNA* gene from *C. oligosticta* samples differed from *C. oligosticta* samples obtained from NCBI. The distance value (D = 0.01) between samples was relatively low. Thus, both sample groups may belong to the same species, which confirms the morphological characterization of the samples. The same note was recorded in *P. areolatus* samples (D = 0.006).

Generally, interspecific hybridization and incorrect characterization of the original specimen lead to confusion in taxonomy (Verspoor & Hammar, 1991; Ward *et al*., 2005;) based on molecular variations. This problem was observed by Ward *et al*. (2005) in barcoding some Australian fish species using the *Cox1* gene system, especially in *Pristiophorus* and *Plectropomus* genera.

Conclusion

Analysis of nucleotide variations among fish species and populations is regarded as the basic principle for exploring biodiversity via molecular markers. The efficiency of *16S r-RNA* gene as barcoding for some Serranidae fishes (42 fish species belonging to the genera *Plectropomus, Cephalopholis*, *and Epinephelus*) was examined. The results suggested that the Red Sea Serranidae fishes do not form a monophyletic group. The 16S as a molecular barcoding system offered informative markers for discriminating among these fish. More molecular markers (especially nuclear DNA markers) are needed to recover the true phylogeny in the Serranidae fishes.

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Author's Contribution

Yasser Mohamed Saad conceptualized the idea and wrote the paper titled 'Analysis of 16S mitochondrial ribosomal DNA sequence variations and phylogenetic relations among some Serranidae fishes'.

Conflict of Interest Declaration

The author has no financial or personal relationship with other people or organizations that could influence or bias this paper inappropriately.

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