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Mitochondrial-Based Phylogenetic Inference of Worldwide Species of Genus *Siganus*

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ABSTRACT

Genus *Siganus* encompasses a diverse group of fishes with a broad geographical distribution. *Siganus rivulatus* and *Siganus luridus* are the most common species of such group in Egypt. The current study introduced an inclusive framework on the molecular characterization and phylogenetic relationship of Siganus species worldwide. Partial sequence of Cytochrome oxidase 1 gene (COI) and D-loop control region were used to barcode *S. rivulatus* and *S. luridus* which have been collected from the Mediterranean Sea and the Red Sea in Egypt. Further, the newly assembled sequences were combined with 56 COI sequences representing another 17 Siganus species and 35 D-loop sequences for eight Siganus species, available at the international databases, to reconstruct the phylogenetic relationship among this group of fishes. The analyses performed in the current study included calculation of GC%, calculation of the genetic distance and reconstruction of the phylogenetic relationship among different siganus species. Based on COI sequences analysis, the average GC% in the studied Siganus species was 46.6%، the genetic distance among Siganus species ranged between 0.004 to 0.166. Based on the D-loop control region analysis, the average GC% was 30.4%, and the genetic distance ranged between 0.048 to 0.346. The COI-based phylogenetic tree clustered the studied species into two major clades. In the first clade, fusiform species inhabiting schools on the inshore reef flats were included. The second clade included deep-bodied species with brightly colored bodies that live on the reef front and those inhabiting the small schools in mangroves, estuaries. In the second clade, *S. argentanus*, the only species of family Siganidae is known to have a pelagic pre-juvenile stage, was separated into a non-clade group. Whereas, D-loop-based tree grouped *S. argentanus* in a separate sub-clade with fusiform species. The proper molecular characterization for *S. rivulatus*, *S. luridus* and the updated phylogenetic relationship for worldwide Siganus species, provided in the current study, was considered as a primary key for fisheries and aquaculture management for such species.

INTRODUCTION

In many taxonomic groups that are dispersed worldwide, it is difficult to correctly identify fishes and deduce the phylogenetic relationships among species based on their

morphology. That is due to the morphological characteristics similarity among species which are derived from convergent evolution and speciation pattern is quite complicated **(Rice and Westneat 2005; Duftner *et al.* 2007)**.

For instance, family Siganidae includes approximately 33 genera that are widely distributed in temperate and tropical water **(Shakman *et al.* 2009)**. Rabbitfishes, belonging to family Siganidae, includes 29 species in one genus, Siganus **(Froese and Pauly 2019)**. They are distributed across the Indo-Pacific Ocean, from the Red Sea and the Gulf of Suez to the Mediterranean region, and from Japan to southern Australia **(Mirbach and Brandl 2016)**. The similarity of rabbitfishes' noses to that of their terrestrial namesake and the grazing ability that compete for mammalian bunnies are the reasons for their nomenclature **(Froese and Pauly 2019)**.

Among the 29 species of genus Siganus, only four species (*Siganus rivulatus, S. stellatus, S. argenteus, S. luridus*) were recorded in Egypt **(Akel and Karachle 2017)**. However, *Siganus luridus* and *Siganus rivulatus* represent the relatively settled populations in different water bodies in Egypt. Nevertheless, *Siganus rivulatus* is the most common and abundant one **(Mehanna *et al.* 2018)**.

In 1869, Since the establishment of the Suez Canal, an unlimited number of fish species translocated from the Red Sea to invade the Mediterranean Sea. These species were listed as Lessepsian migratory species **(Por 1978)**. The two siganids, *S. rivulatus, and S. luridus* have crossed the Suez Canal **(Bariche 2005)**. *S. rivulatus,* in particular, was one of the first recorded Lessepsian migrants **(Steinitz 1929; Golani 1990)**. Populations of the two species are currently inhabiting both Seas but are most common and diverse in the Red Sea **(El-Far 2008; Gabr and Mal 2016; Akel and Karachle 2017)**.

Both species represent essential elements in shallow coral reef fish communities **(Tharwat and Al-Owafeir 2003; Gabr and Mal 2016)**. They recently received great attention as they can be successfully cultivated with mullets and milkfish **(Mehanna *et al.* 2018)**, and they can be considered as important species for aquaculture in the Middle East **(Fahmy 2019)**. Based on morphology and ecology, rabbitfishes have been divided into two groups: those that are drab- colored, fusiform and school in macro-algal habitats and those that are brightly-colored, reef- associated and pairing **(FAO 2019)**.

Most of studies, on species of genus Siganus, dealt with growth, spawning, mortality and yield per recruit or management and stock assessment of Siganus species **(Stergiou 1988; Lundberg *et al.* 2004; Shakman *et al.* 2008; Shakman *et al.* 2009; Gabr and Ahmed 2018; Mehanna *et al.* 2018; Fahmy 2019; Saber and Gewida 2020)**. However, the molecular studies dealing with the taxonomy and phylogenetic relationships in rabbitfishes are still rare.

Bonhomme *et al.* (2003) discussed the influence of Lessepsian migration of rabbitfish *S. rivulatus* on the genetic diversity of populations inhabiting the Red Sea and Mediterranean Seas based on cytochrome b, and recorded the lack of genetic

differentiation between both populations. **Azzurro *et al.* (2006)** reported important information on the taxonomy and phylogenetic relationship of the Siganidae family, especially *S. luridus* and *S. rivulatus* as the Lessepsian species, based on mitochondrial control region marker. **(Borsa *et al.* 2007)** established the first phylogenetic relationship among species of genus Siganus based on *Cytb* and 16S markers.

However, the two Siganus species, *S. rivulatus and S. Luridus,* have an economic importance and considered as attractive models for molecular studies. But, they have never been characterized at the molecular level in Egypt **(Abbas *et al.* 2021)**. Also, there is a significant gap between the current study and previous studies of, such as **Azzurro *et al.* (2006) and Borsa *et al.* (2007**), that addressed siganids' phylogenetic relationships.

Consequently, The current study's objectives were: first, providing multiple efficient barcodes for both species, *S. Luridus* and *S. rivulatus* that were collected from the Mediterranean Sea and the Red Sea in Egypt. Second, relate the *S. rivulatus* and *S. luridus* (from Egypt) to other Siganus species distributed worldwide. Third, reconstructe the phylogenetic relationship among Siganus species based on two applied molecular markers, Cytochrome oxidase subunit I (*COI*) and D-loop control region.

The partial sequence of cytochrome oxidase subunit I (*COI*) gene that has proposed by **Hebert *et al.* (2003)** have been used in many studies for barcoding different fishes **(Hajibabaei *et al.* 2005; Steinke *et al.* 2005; Ward *et al.* 2005; Hubert *et al.* 2008)**, discriminating between morphologically similar species **(Abbas *et al.* 2017; Ali *et al.* 2019a)**, studying population structures **(Wang *et al.* 2017; Ali and Mamoon 2019)**. The D-loop (noncoding) control region was also used for the barcoding and the study of populations' structure **(Azzurro *et al.* 2006; Soliman *et al.* 2017)**.

MATERIALS AND METHODS

Sampling collection, sample preservation and DNA Isolation

Samples of Siganus species (*S. rivulatus* and *S. luridus*) were collected freshly from the commercial catch of Alexandria city. Frozen fish samples were transferred to the Genetics lab at The National Institute of Oceanography and Fisheries (NIOF), Egypt. DNA was isolated from caudal fins as described in **Ali and Mamoon (2019).** DNA concentration was measured by Nanodrop (IMPLEN, Nanophotometer, NP80, Germany) and stored for further analysis at -20°C.

Amplification and sequencing

A partial fragment of COI gene was amplified for the barcoding of *S. rivulatus* and *S. luridus* using the modified **Ward *et al.* (2005)** primer pairs **(Kochzius *et al.* 2010)**: COI Fish-F (5′-TTCTCA ACTAACCAYAAAGAYATY GG-3′) and COI-Fish-R (5′-TAGACT TCT GGG TGG CCR AAR AAY CA-3′). An additional barcode region, D-loop control region (noncoding region) was further amplified using the following primer pairs: CR-A,5′-TTCCACCTCTAACTCCCAAAGCTAG-3′and CR-E,5′-

CCTGAAGTAGGAACCAGATG-3 **(Lee *et al.* 1995)**. For amplification of the target COI and D-loop fragments, a volume of 30 µL of PCR mixture (2X My-Taq Red Mix, BIOLINE) following manufacturer's instructions. A volume of 15 µL of the master mix, 0.7 µL of each primer (final concentration 0.25 µM), DNA template of final concentration 20 ng were included in the PCR mixture. T100 96-well Thermal Cycler, BIO-RAD, USA, was used for target region amplification. The applied thermal profile was: initial denaturation at 95°C for 5 min, 35 cycles of a denaturation step at 94°C for 30 s, annealing temperature at 57°C for 30 s (for COI region), and at 52 for 45 sec. (for D-loop region), then extension for 30 s at 72°C, with a final extension of 7 min at 72°C. For checking the quality of the PCR product, 3 µL of the PCR product was loaded onto 2% agarose gel which contains 100 mg/ml of ethidium bromide, and the amplicon was electrophoresed. The PCR products with sizes (700-800 bp) were purified by PCR/Agarose DNA purification kit (Intronbio-Korea). The purified products were then sequenced using the Applied Biosystems ABI3730 (California, USA). Sequences of COI and D-Loop of *S. rivulatus* and *S. luridus* were deposited with the accession numbers LC541544- LC541567 in the GenBank/EMBL/DDBJ genetic databases.

Sequence analysis

The available COI and D-Loop sequences for Siganus species from different countries were retrieved from the international GenBank database and then aligned with the newly collected sequences of *S. rivulatus* and *S. luridus* from Egypt using a MEGA6 software package **(Tamura *et al.* 2013)**. For all the studied species of genus Siganus, the percentage of GC content (%GC) was calculated using the GENEIOUS software program (V8.1) **(Kearse *et al.* 2012)**. For both molecular markers (D-loop and COI), inter-spacific genetic distances for genus Siganus were estimated using Group Mean Distance as implemented in MEGA X, based on Kimura two-parameter distance model (K2P) **(Kumar *et al.* 2018)**.

Bayesian phylogenetic analysis

We used BEAST v.2 to conclude the phylogenetic relationship between the two Egyptian Siganus species and other available species on the public databases **(Bouckaert *et al.* 2019)**. As compared to other phylogenetic programs, BEAST exclusively uses molecular clock models so that trees have a timescale and its reliance on the Bayesian framework. The analysis was performed for COI and D-loop species sequences separately. Initially, XML files (BEAST control files) for each marker were generated using the BEAUti application implemented in BEAST, which will be subsequently used in the phylogenetic analysis. Also, we used the simplest default model of nucleotide evolution **(Hasegawa *et al.* 1985)**. The assumption of the molecular clock was set as default (strict clock), which assumes that all branches on the tree have the same rate of evolution. Since our focus is on inferring species level relationship, we used the speciation model as the tree prior using the Yule process model **(Yule 1925)** which is the

simplest model of speciation at which each lineage was assumed to has specific fixed rate. The program has been appointed to run different runs based on Markov chain Monte Carlo simulations (MCMC). We set the number of runs as a default value of 10,000,000 with sampling every 10,000 runs. To be sure of that, the chain length was enough the resulting logfile was analyzed using Tracer v 1.7 **(Rambaut *et al.* 2018)**. After the completion of MCMC runs, single maximum clade credibility (MCC) tree was generated using TreeAnnotator v1.8.2 **(Rambaut and Drummond 2015)**. This tree represented the summary information of posterior probabilities of the nodes. The constructed MCC tree loaded in Fig Tree **(Rambaut 2011)**, which allows visualization of the tree and accompanying preliminary information produced by TreeAnnotator.

RESULTS

DNA fragments containing the target regions of COI gene and D-Loop control region were successfully amplified for the collected samples of *S. rivlatus* and *S. luridus* from Egypt. Approximately, 650 bp fragment was amplified for COI partial sequence and 390 bp fragment was amplified for D-Loop sequence without insertions or deletions. No stop codons were found after a translation of the nucleotide sequences. BLAST searches revealed a high concordance between the obtained sequences of COI and D-Loop of both species (*S. rivlatus* and *S. luridus*)compared to the reference sequences available at GenBank database (≤ 99%). For COI sequences of the studied Siganus species, the GC% ranged from 47.9% for *S. puellus* to 45.1% for *S. vulpinus* and *S. sutor as well.* The GC% were 46.6% and 47.4% for *S. rivlatus* and *S. luridus*, respectively (Table 1). A lower GC% was observed for D-Loop sequences which ranged from 28% for *S. argenteus* and 33% for *S. fuscescens.* A GC% of 30.2%, and 30.8% were observed for *S. rivlatus* and *S. luridus* D-loop sequences respectively (Table 1).

Based on the Kimura two-parameter distance model (K2P), the analysis of COI partial sequence for all Siganus species revealed that, the highest genetic distance (0.166) was recoded between *S. Punctatissimus and S. spinus.* Whereas, the lowest genetic distance (0.004) was recoded between *S. lineatus* and *S. guttatus*. (Table 2). For D-Loop sequence analysis, the highest genetic distance (0.346) was recorded between *S. vermiculatus* and *S. spinus*. Whereas, the lowest genetic distance (0.048) was recorded between *S. canaliculatus* and *S. rivulatus* (Table 3).

Table 1: GC content percentage for different Siganus species.

	Species	GC Ratio
COI	***Siganus luridus***	47.4%
	Siganus rivulatus	46.6%
	Siganus argenteus	45.7%
	Siganus corallinus	46.5%
	Siganus doliatus	46.6%
	Siganus fuscescens	45.9%
	Siganus guttatus	45.9%
	Siganus javus	46.5%
	Siganus punctatus	48.3%
	Siganus spinus	47.6%
	Siganus stellatus	47.8%
	Siganus_sutor	45.1%
	Siganus vermiculatus	46.1%
	Siganus vulpinus	45.1%
	Siganus canaliculatus	46.1%
	Siganus virgatus	46.8%
	Siganus lineatus	46.1%
	Siganus_puellus	47.9%
	Siganus punctatissimus	47.5%
D-Loop	***Siganus luridus***	30.2%
	Siganus rivulatus	30.8%
	Siganus argenteus	28.0%
	Siganus canaliculatus	30.8%
	Siganus fuscescens	33.0%
	Siganus guttatus	29.3%
	Siganus spinus	32.8%
	Siganus vermiculatus	30.8%
	Siganus virgatus	30.0%
	Siganus vulpinus	28.6%

Table 2: K2P pairwise genetic distance among 19 Siganus species including the Egyptian *Siganus rivulatus* and *Siganus luridus* based on COI barcode region.

	S. Canaliculatus	***S. Argenteus***	***S. Vermiculatus***	***S. Javus***	***S. Luridus***	***S. Sutor***	***S. Rivulatus***	***S. Punctatissimus***	***S. Guttatus***	***S. Lineatus***	***S. Corallinus***	***S. Vulpinus***	***S._Doliatus***	***S. Punctatus***	***S. Spinus***	***S. Fuscescens***	***S. Stellatus***	***S. Virgatus***	***S. Puellus***
S. canaliculatus																			
S. Argenteus	0.118																		
S. Vermiculatus	0.117	0.116																	
S. Javus	0.127	0.115	0.081																
S. Luridus	0.084	0.127	0.134	0.124															
S. Sutor	0.053	0.118	0.119	0.122	0.087														
S. Rivulatus	0.062	0.122	0.123	0.124	0.082	0.042													
S.Punctatissimus	0.141	0.126	0.079	0.076	0.151	0.135	0.134												
S. Guttatus	0.123	0.114	0.034	0.083	0.120	0.126	0.120	0.069											
S. Lineatus	0.122	0.113	0.034	0.087	0.125	0.128	0.124	0.070	**0.004***										
S. Corallinus	0.114	0.093	0.029	0.084	0.127	0.115	0.121	0.073	0.032	0.031									
S. Vulpinus	0.129	0.116	0.074	0.080	0.135	0.132	0.126	0.071	0.073	0.077	0.073								
S. Doliatus	0.124	0.098	0.035	0.083	0.132	0.116	0.130	0.076	0.041	0.040	0.018	0.080							
S. Punctatus	0.123	0.098	0.075	0.068	0.123	0.124	0.125	0.065	0.071	0.072	0.071	0.051	0.078						
S. Spinus	0.094	0.137	0.134	0.144	0.102	0.079	0.091	**0.166**	0.131	0.133	0.125	0.158	0.123	0.136					
S. Fuscescens	0.006	0.117	0.114	0.126	0.088	0.054	0.063	0.139	0.121	0.119	0.112	0.127	0.122	0.122	0.096				
S. Stellatus	0.126	0.100	0.077	0.072	0.126	0.126	0.127	0.062	0.073	0.074	0.073	0.049	0.080	0.006	0.138	0.124			
S. Virgatus	0.120	0.098	0.029	0.081	0.132	0.119	0.128	0.069	0.036	0.033	0.012	0.073	0.006	0.071	0.128	0.117	0.073		
S. Puellus	0.144	0.119	0.083	0.070	0.120	0.124	0.125	0.060	0.074	0.077	0.084	0.066	0.087	0.055	0.137	0.141	0.058	0.084	

*The highest and lowest genetic distance are highlighted in bold.

Table 3: K2P pairwise genetic distance among 10 Siganus species including the Egyptian *Siganus rivulatus* and *Siganus luridus* based on D-Loop control region.

	S. Canaliculatus	***S. Vermiculatus***	***S. Guttatus***	***S. Virgatus***	***S. Fuscescens***	***S. Argenteus***	***S. Rivulatus***	***S. Spinus***	***S. Vulpinus***	***S. Luridus***
S. Canaliculatus										
S. Vermiculatus	0.293									
S. Guttatus	0.272	0.202								
S. Virgatus	0.270	0.173	0.128							
S. Fuscescens	0.068	0.287	0.269	0.271						
S. Argenteus	0.254	0.302	0.269	0.272	0.263					
S. Rivulatus	**0.048**[*]	0.286	0.285	0.275	0.062	0.251				
S. Spinus	0.105	**0.346**	0.310	0.326	0.113	0.229	0.113			
S. Vulpinus	0.239	0.194	0.191	0.196	0.224	0.232	0.237	0.260		
S. Luridus	0.075	0.324	0.263	0.281	0.083	0.218	0.085	0.107	0.225	

[*]The highest and lowest genetic distance values are highlighted in bold.

Bayesian phylogenetic analysis

A single tree was generated for each of the used markers; (Fig. 1) for COI and Fig 2 for D-loop. The generated trees showed a clear clustering pattern of Siganus species with proper placement of Egyptian Siganus together with the closest relatives with high posterior probability (darker branches colure). The constructed COI-based tree clustered the studied species into two major clades. The first clade included fusiform species that inhabit schools on the inshore reef flats (*S. fuscescens, S. canaliculatus, S. rivulatus, S. luridus, S. sutor* and *S. spinus*). The second clade included deep-bodied species with brightly colored bodies that live on the reef front and those in small schools in mangroves, estuaries (*S. corallinus, S. doliatus, S. puellus, S. punctatus, S. unimaculatus, S. virgatus, S. vulpinus, S. guttatus, S. javus, S. lineatus, S. vermiculatus, S. stellatus, S. punctatissimus*). *S. argentanus*, which is the single species of the family Siganidae is known to possess a pelagic, pre-juvenile stage, was separated in a non-clade group.

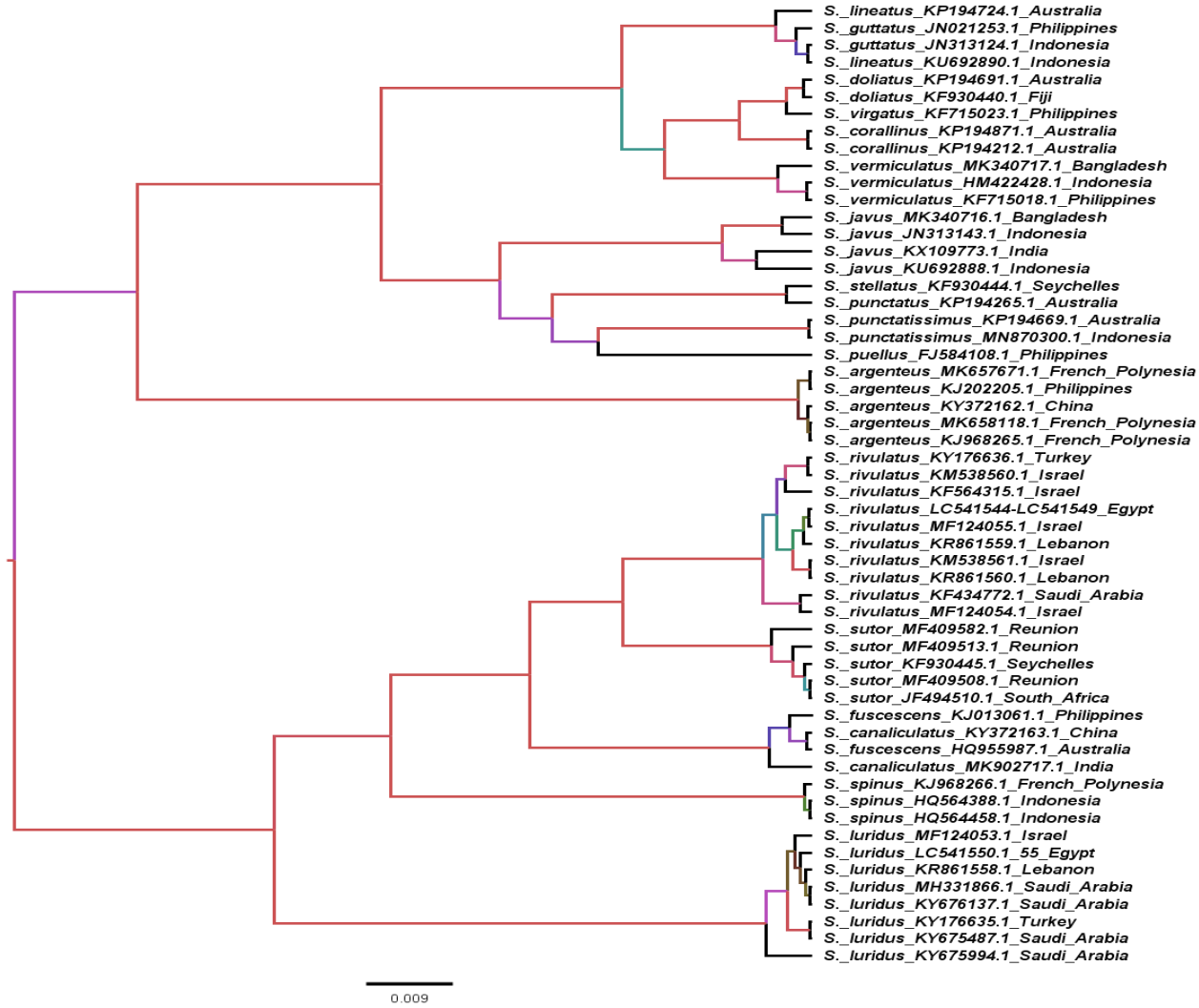

Fig. 1. COI- based phylogenetic relationship of worldwide Siganus species.

The D-loop- based tree was constructed for ten Siganus species (including the Egyptian species, *S. rivulatus* and *S. luridus*) and another eight species retrieved from the GenBank database). Such species were clustered into two main clades. The first clade has grouped the *S. argentenus*, in a separate sub-clade, with fusiform species *(S. canaliculatus, S. fuscescens* and *S. spinus, S. rivulatus and S. luridus*) in another separate sub-clade. The second clade was branched into two sub-clades included *S. vulpinus* in a separate branch while *S. guttatus, S. virgatus* and *S. vermiculatus* were separated in another subclade (Fig. 2).

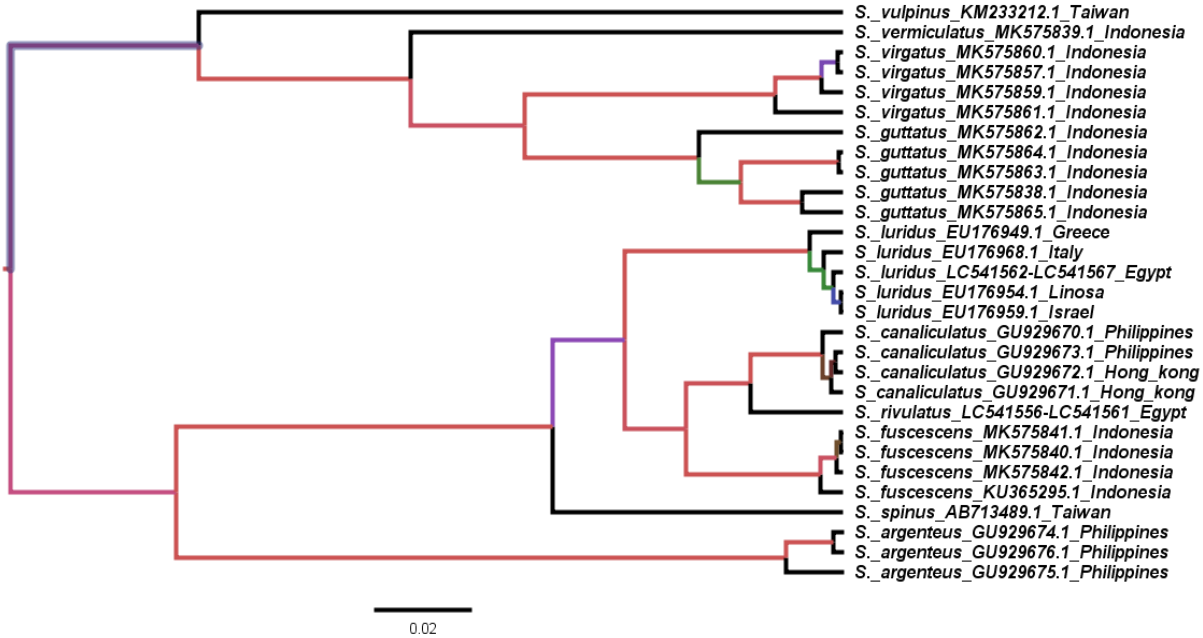

Fig. 2. D-Loop- based phylogenetic relationship of worldwide Siganus species.

DISCUSSION

Rabbitfishes are belonging to family Siganidae, and are widely distributed across the Indo-Pacific Ocean from the Red Sea to the Mediterranean region via Suez Canal **(Mirbach and Brandl 2016)**. However, only *S. rivulatus* and *S. luridus* are the most common Siganus populations in Egypt. They have been recorded as primary Lessepsian migrants, thus their molecular investigation and phylogenetic relationship with other Siganids are of interest; especially, with the scarcity of molecular studies that have been carried out on genus Siganus **(Bonhomme *et al.* 2003; Hassan *et al.* 2003; Azzurro *et al.* 2006; Borsa *et al.* 2007)**, The current study employed the technology of DNA barcoding in achieving our goals. DNA barcoding is a powerful tool for species identification **(Hajibabaei *et al.* 2007b)**, as the sequencing of a target region of the genome provides comprehensive information about the species of living creatures **(Ali *et al.* 2019b)**. DNA barcoding has been widely used for delineating species boundaries, discriminating closely related species of fish **(Hajibabaei *et al.* 2007a)**. Similarity or

percentage identity ranged from 99–100% with the identified species in the GenBank databases that was commonly recorded among the fish species and demonstrated the potency of such genomic technique in confirming individual fish similarity **(Debenedetti *et al.* 2014; Bellagamba *et al.* 2015; Abbas *et al.* 2017; Ali *et al.* 2019a; Ali *et al.* 2020; Ibrahim *et al.* 2020)**. On the nucleotide level, the sequence alignment detected considerable polymorphism at different consensus positions in 28 fish species, the differences had been widely verified **(Persis *et al.* 2009)**. In the present study, two mitochondrial regions, COI gene and D-Loop, were successfully amplified for two commercial fish species of genus Siganus in Egypt, *S. rivlatus* and *S. luridus.*

The lack of stop codons in the obtained sequences supported the fact that an entire coding region was amplified. The concordance between the obtained COI sequences and the reference sequences in the GenBank and BOLD databases was used to confirm that our data set was free from nuclear mitochondrial DNA (numts), support the evidence of their existence in Actinopterygii **(Bensasson *et al.* 2001)**. The average percentage of the GC content of the amplified 650 bp mitochondrial COI region in the studied Siganus species was (46.5%). This percentage was comparable to that reported in other fish species **(Ward *et al.* 2005; Wang *et al.* 2017; Ali *et al.* 2020)**. While, a lower GC content was observed for the D-Loop sequences with an average of 30.6% that was nearly agreed with the result of **Martins *et al.* (2003)**. This lowered GC content could be due to the shortened length of the D-Loop sequence. The observed variation of GC content at different codon positions among Siganus species may reflect a sign of adaptation. Since a region with high GC is likely to be less affected by selective forces as compared to a region with lower GC; leading to the higher retention potential for ancestral polymorphisms **(Romiguier and Roux 2017)**.

In this study, the average Kimura two-parameter (K2P) for COI gene varied between (0.004) and (0.166) among Siganus species. Such variation in the genetic distance between species is consistent with the fish barcoding studies that support the genetic divergence at the species level **(Hubert *et al.* 2008; Lakra *et al.* 2011; Keskin and Atar 2012)**. The constructed phylogenetic tree clustered the closely related species together in distinct clades. By examining the COI-based tree, the phylogenetic relationship at the species level could be informative that showed by clear clustering pattern. **Ward *et al.* (2005)** proposed that the gathering information from the approximately 655-bp fragment of the mitochondrial gene could be used as a plan for the phylogenetic study. The clustering pattern inferred from COI analyses closely matches that inferred from the analysis of *Cytb* and 16s rRNA gene fragments **(Borsa *et al.* 2007)**. This may be attributed to the similar nature of the applied mitochondrial molecular markers. The D-Loop-based (K2P) genetic distance among species (0.346) was relatively higher than that obtained in the COI gene. This higher genetic distance might be related to the fewer number of D-Loop sequences that are available on the GenBank database for

Siganus species compared to COI sequences and to the fine nature of the D-Loop barcode region, which can differentiate between the morphologically close species **(Lee *et al.* 1995; Azzurro *et al.* 2006)**. It is worth noting that the two employed molecular markers have located the Egyptian Siganus species in a similar pattern in respect to each other, which can also be attributed to the similar nature of both markers as mitochondrial barcodes.

CONCLUSION

In the current study, *Siganus rivulatus* and *Siganus luridus* collected from the Mediterranean Sea and the Red Sea in Egypt were efficiently characterized based on DNA barcoding using two barcode markers, COI and D-Loop control region. The analysis of these barcode regions provided detailed data about the phylogenetic relationship among the studied species of genus Siganus. The current study represents one of the rare studies dealing with siganids. Extensive work is needed to explore precision the taxonomy of the genus Siganus, especially with the economic importance of its species.

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Arabic Summery

استنباط العلاقة التطورية القائمة على جينات الميتوكوندريا لأنواع جنس السيجان .

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يشتمل جنس السيجان على مجموعة متنوعة من الأسماك ذات توزيع جغرافى واسع. ويعتبر النوعين *Siganus luridus* و *Siganus rivulatus* هما أكثر أنواع هذه المجموعة شيوعًا في مصر. تقدم الدراسة الحالية إطارًا شاملاً للوصف الجزيئي والعلاقة التطورية لأنواع السيجان في جميع أنحاء العالم. تم استخدام التسلسل الجزيئي لجين Cytochrome oxidase 1 (COI) ومنطقة التحكم D-loop في *S. rivulatus* و *S. luridus* التي تم تجميعهما من البحر المتوسط والبحر الأحمر في مصر وذلك للتحديد الجينى. علاوة على ذلك ، تم الجمع بين التتابعات الجينية المجمعة حديثًا مع 56 تسلسلًا من COI تمثل 17 نوعًا آخر من Siganus و 35 تسلسل D-loop لثمانية أنواع Siganusمتوفرة في قواعد البيانات الدولية لإعادة بناء علاقة النشوء والتطوربين هذه المجموعة من الأسماك. تضمنت التحليلات التي تم إجراؤها في الدراسة الحالية حساب نسبة GC ٪ ، وحساب المسافة الجينية وإعادة بناء علاقة النشوء والتطور بين أنواع Siganus المختلفة. بناءًا على تحليل تسلسل COI ، كان متوسط GC ٪ في أنواع Siganusالمدروسة 46.6 ٪ ، وتراوحت المسافة الوراثية بين أنواع Siganus بين 0.004 إلى 0.166. بناءًا على تحليل منطقة التحكم في حلقة D-loop ، كان متوسط نسبة30.4 GC ٪ ، وتراوحت المسافة الجينية بين 0.048 إلى 0.346. جمعت الشجرة الوراثية المبنية القائمة على COI الأنواع المدروسة في كتلتين رئيسيتين. في الفرع الأول، تم تضمين الأنواع المغزلية التي تسكن المدارس على مسطحات الشعاب المرجانية الشاطئية. علي الجانب الاخر، تضمن الفرع الثاني من الشجره الوراثية أنواعًا عميقة الجسم بأجسام ملونة زاهية تعيش على واجهة الشعاب المرجانية وتلك التي تعيش في غابات المانغروف ومصبات الأنهار. في الفرع الثاني ، تم فصل *S. argentanus* إلى مجموعة غير فرعية و هو النوع الوحيد من عائلة Siganidae المعروف بان له طورًا سطحيًا من قبل اليفوع. في حين أن الشجرة القائمة على D-loop جمعت *S. argentanus* في فرع منفصل من الشجرة الوراثية مع أنواع مغزلية. يعتبر الوصف الجزيئي المناسب لـ *S. rivulatus* و *S. luridus* والعلاقة التطورية المحدثة لأنواع Siganus في جميع أنحاء العالم المقدمة في الدراسة الحالية، تعد مفتاحًا أساسيًا لإدارة مصايد الأسماك وتربية الأحياء المائية لهذه الأنواع.