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Molecular Identification and Characterization of Parrotfish species from the Farasan Islands, Red Sea-Saudi Arabia

Mohamed M. Hassan [1,2], Ayman Sabry [1,3] and Mohamed Ismail [2,*]

[1] Department of Biology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; [2] Genetics Department, Faculty of Agriculture, Menoufia University, Shibin El-Kom, 32516, Egypt; [3] Cell Biology Department, National Research Center, 12622, Dokki, Giza, Egypt

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Abstract

Cheilinus trilobatus, *Cheilinus quinquecinctus*, and *Chlorurus sordidus* specimens from Saudi Arabia's Farasan Islands were collected and genotyped using inter simple sequence repeats (ISSRs) and start codon targeted (SCoT) primers. Mitochondrial cytochrome C oxidase subunit I (COI) gene fragments were used for DNA barcoding. ISSRs and SCoT primers showed moderate polymorphisms: expected heterozygosity (H_{exp}) of 0.470 and 0.435 and average polymorphism information contents (*PIC*s) of 0.359 and 0.339 for ISSRs and SCoT markers were observed, respectively. *Cheilinus quinquecinctus* had the highest genetic diversity from ISSRs (70%) and SCoT (73%). *Chlorurus sordidus* and *C. trilobatus* showed similar genetic diversities of 29% and 39% based on ISSRs, respectively, and 60.32% for both species based on SCoT. *Cheilinus quinquecinctus* had the lowest nucleotide diversity (π) of 0.003, while *C. sordidus* and *C. trilobatus* had π values of 0.065 and 0.103, respectively. Analysis of molecular variance (AMOVA) revealed greater genetic variation among species rather than within them using ISSRs (65% and 67%, respectively) and SCoT (35% and 33%, respectively). COI-based AMOVA showed similar genetic variation among (51.11%) and within (48.89%) species. The current study highlighted outperformance of COI compared to ISSRs and SCoT markers in differentiating among parrotfish species. Also, ISSR outperformed SCoT since it was able to clearly show three distinct groups in principal component analysis. This study also confirmed the presence of three distinct parrotfish species, which will provide an insight into parrotfish diversity. Moreover, the results will contribute to monitoring parrotfish migration between Farasan Islands and different geographic locations which significantly affect species conservation.

Keywords ISSR; SCoT; COI; Cheilinus trilobatus, Cheilinus quinquecinctus, Chlorurus sordidus, Farasan Islands.

1. Introduction

Parrotfish (family Scaridae) are a distinctive group of labroid fish, comprising 10 genera that contain 90 species, including *Scarus niger*, *S.* (*Chlorurus*) *sordidus*, *S. frenatus*, and *S. ghobban* (Saad *et al.*, 2013). Meanwhile, the genus *Cheilinus* belongs to the family Labridae and contains wrasses (e.g. *Cheilinus trilobatus* and *Cheilinus quinquecinctus*) that are native to the Indian and Pacific Oceans and the Red Sea (Bogorodsky *et al.*, 2016).

Parrotfish (Scaridae) and wrasse (Labridae) are diverse fish families with an ecologically important role. Feeding activities for both families involve browsing of macroalgae, hence contributing to the maintenance of coral reefs (Bonaldo *et al.*, 2014). Several parrotfish species move across large areas; their movement plays a significant role in connecting different ecosystems (Fox and Bellwood, 2007; Nystrom and Folke, 2001).

Parrotfish include two major clades (*Chlorurus* and *Cheilinus*) that diversified during the Miocene, followed by speciation during the Pliocene (Choat *et al.*, 2012). Both form a noticeable part of the herbivorous fish community. The phenotypic characterization of parrotfish is challenging due to the presence of a series of coloration changes. For instance, *Sparisoma* species color pattern varies by sex and developmental stage and is only clearly visible in fresh specimens (Bernardi *et al.*, 2000).

Since the species characterization of parrotfish in the Red Sea is poorly investigated due to the difficulties in morphological identification of parrotfish, the use of molecular barcoding is important. Such genetic markers represent effective and reliable alternative in parrotfish identification. Accordingly, COI gene has been established as an efficient species-level DNA barcoding marker for marine fish (Veneza *et al.*, 2013; Ali *et al.*, 2019; Ali *et al.*, 2020). The reliability and accessibility of the COI barcoding enabled them to be extensively used for both fish identification and monitoring of biodiversity (Shen *et al.*, 2017).

In addition to the use of COI as the most common barcode for fish identification, inter simple sequence repeats (ISSRs) and start codon targeted (SCoT) markers have also been applied in the assessment of parrotfish diversity (Sabry *et al.*, 2015). ISSRs markers are useful in genetic divergence estimates among closely related species (Sabry *et al.*, 2015), and many studies have demonstrated the utility of ISSRs markers for monitoring fish genetic

* Corresponding author e-mail: mohamed.ismail@agr.menofia.edu.eg.

diversity (Paul *et al.*, 2018; Labastida-Estrada *et al.*, 2019). Recently, many alternative markers have been developed. SCoT markers, for instance, are reproducible markers that were developed using short, conserved regions in plant genomes that surround translation initiation codons (Amirmoradi *et al.*, 2012). Subsequently, these markers have been used to evaluate genetic diversity and population structure, and to identify cultivars (Hamidi *et al.*, 2014). However, the use of SCoT markers in animal studies remains relatively limited as compared to its use in plant studies.

The Farasan Islands are considered a high priority for conservation and a destination of annual aggregation of parrotfish to spawn (Gladstone, 2002). Therefore, from a conservation perspective, the assessment of parrotfish species identity and genetic diversity is a priority. In addition, the molecular structure of fish species is crucial for stock identification, improvement, and genetic resources preservation (Bingpeng *et al.*, 2018). However, such studies have yet to be extended to marine populations of the Farasan Islands, as one of the richest biodiversity hotspots in the Red Sea (Pearman *et al.*, 2014). Farasan Island of Saudi Arabia is underrepresented in marine species diversity assessment. In the current study, three different markers ISSRs, SCoT, and COI will be used to determine the genetic diversity and barcode three parrotfish species (*Cheilinus trilobatus*, *C. quinquecinctus*, and *Chlorurus sordidus*) from the Farasan Islands.

2. Materials and Methods

2.1. Fish Sampling and Genomic DNA Extraction

Parrotfish specimens were collected from the commercial fisheries at Farasan Islands (16°40′N, 42°00′E), on the Jeddah coast in Saudi Arabia. Samples were transferred frozen to the laboratory for further analyses. According to the morphological examination of the collected samples, most of the samples belonged to family Scaridae. The collected samples were identified by their morphological characteristics as described in FishBase (Froese and Pauly, 2019). Following morphological identification, three different species were detected: *Chlorurus sordidus*, *Cheilinus trilobatus*, and *Cheilinus quinquecinctus* (Figure 1), 10 specimens were selected from each species for DNA analyses. Approximately, 1 gm of fish muscle tissue was cut and ground in liquid nitrogen. The powdered fish muscle was used for genomic DNA extraction using the DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions.

2.2. ISSRs Genotyping

A total of nine ISSR primer pairs were used in PCR (Table 1). DNA amplification was performed in a final volume of 25 µL using a C1000^{TM} Thermo Cycler (Bio-Rad, Germany), Master mix Promega (Promega, Co., Wisconsin, USA) and 1 µL of 10 ng DNA. The PCR reaction was performed according to Hassan *et al.* (2015) using the following protocol: 94°C for 10 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at a primer-specific annealing temperature ranging from 48-54°C for 1 min and an extension at 72°C for 2 min, and a final extension at 72°C for 10 min. All PCR products were electrophoresed on 2% agarose gel electrophoresis in 0.5X TAE (20 mM Tris-HCl, 10 mM Acetic acid, 0.5 mM EDTA, pH 8.0); the gel was stained with ethidium bromide and visualized with UV light.

Figure 1. Parrotfish species collected from the Farasan Islands, Saudi Arabia; (a) *Chlorurus sordidus*, (b) *Cheilinus trilobatus*, (c) *Cheilinus quinquecinctus*.

2.3. ISSRs Genotyping

A total of nine ISSR primer pairs were used in PCR (Table 1). DNA amplification was performed in a final volume of 25 µL using a C1000^{TM} Thermo Cycler (Bio-Rad, Germany), Master mix Promega (Promega, Co., Wisconsin, USA) and 1 µL of 10 ng DNA. The PCR reaction was performed according to Hassan *et al.* (2015) using the following protocol: 94°C for 10 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at a primer-specific annealing temperature ranging from 48-54°C for 1 min and an extension at 72°C for 2 min, and a final extension at 72°C for 10 min. All PCR products were electrophoresed on 2% agarose gel electrophoresis in 0.5X TAE (20 mM Tris-HCl, 10 mM Acetic acid, 0.5 mM EDTA, pH 8.0); the gel was stained with ethidium bromide and visualized with UV light.

2.4. SCoT Genotyping

A total of 10 SCoT markers were selected for specimen genotyping (Table 1). PCR reactions were performed according to Etminan *et al.*, (2016). Amplification consisted of 94°C for 5 min, followed by 45 cycles of denaturation at 94°C for 45 s, primer annealing at 45°C for 45 s and primer elongation at 72°C for 90 s. The final extension was 10 min at 72°C. All PCR products were analyzed with 1.5% agarose gel electrophoresis in 0.5X TAE (20 mM Tris-HCl, 10 mM Acetic acid, 0.5 mM EDTA, pH 8.0), and the gel was stained with ethidium bromide and visualized with UV light

Table 1. Inter simple sequence repeat **(**ISSR) and Start codon targeted (SCoT) markers used in parrotfish species genotyping.

Marker	Primer sequence (5′-3′)	Marker	Primer sequence (5′-3′)
ISSR-2	$(GA)_8A$	SCoT-1	ACGACATGGCGACCACGC
ISSR-3	$(AG)_8TG$	SCoT-2	ACCATGGCTACCACCGGC
ISSR-4	$(GA)_8TT$	SCoT-5	CAATGGCTACCACTAGCG
ISSR-8	$(TG)_8AA$	SCoT-6	CAATGGCTACCACTACAG
ISSR-9	$TAG(CA)_7$	SCoT-7	ACAATGGCTACCACTGAC
ISSR-13	$(GA)_8C$	SCoT-8	ACAATGGCTACCACTGCC
ISSR-18	$(AC)_7CG$	SCoT-10	ACAATGGCTACCACTACC
ISSR-19	$(AG)_8TT$	SCoT-11	AAGCAATGGCTACCACCA
ISSR-28	$(GTG)_6$	SCoT-12	ACGACATGGCGACCAACG
		SCoT-14	ACGACATGGCGACCACGC

2.5. DNA Sequencing of COI gene

The COI standard barcoding region (655 bp) was amplified for each of the 30 fish specimens using a pair of forward FishF1 (5′-TCAACCAACCACAAAGACATTG-GCAC-3 ′), and reverse FishR1 (5′-TAGACTTCTGGGT-GGCCAAAGAATCA-3′) universal primers, as previously described in Ivanova *et al.*, (2007). Each amplification reaction contained 2 µL of DNA, 10.5 µL of deionized water, 12.5 µL of Master Mix (Promega, Co., Wisconsin, USA), 0.5 µL of forward primer (10 µM), and 0.5 µL of reverse primer (10 µM) at a total volume of 25 µL. The PCR reactions were performed according to the following profile: initial denaturation at 95°C for 5 min, followed by a total of 35 cycles of 94°C for 45 s, 54°C for 1 min, and 72°C for 45 s, and a final extension at 72°C for 7 min. Successful amplifications were confirmed by 5% agarose gel electrophoresis in 0.5X TAE (20 mM Tris-HCl, 10 mM Acetic acid, 0.5 mM EDTA, pH 8.0), and the gel was stained with ethidium bromide and visualized with UV light. PCR amplicons of the COI gene were purified from gels using an QIAquick PCR Purification Kit (QIAGEN, Valencia, CA, USA) and sequenced on the Applied Biosystems 3500 Genetic Analyzer Sequencer (Hitachi, Japan).

2.6. ISSRs and SCoT Data Analysis

For ISSRs and SCoT analysis, all genotypes were screened based on the presence/absence of a specific band (allele). Following genotype screening, GenAlex v6.5 (Peakall and Smouse, 2006) was used to estimate the percentage of polymorphisms in each species. Additionally, the iMEC program (Amiryousefi *et al.*, 2018) was used to calculate the expected heterozygosity (H_{exp}), polymorphism information content (*PIC*), discriminating power (*D*), and resolving power (*R*). NTSYS-pc v2.01 (Rohlf, 2000) was also used to determine the phylogenetic relationship among the three species. Phylogenetic trees were produced according to the unweighted pair group method with arithmetic mean (UPGMA). Principal component analysis (PCA) was also performed using GenAlex. To determine the level of genetic variation of each species, the analysis of molecular variance (AMOVA) was also obtained using GenAlex with 1000 permutations.

2.7. COI Sequence Analysis

All sequences were aligned and edited using BioEdit, version 7.0.9 (Hall, 1999). Following sequence alignment and editing, genetic polymorphisms of each species were estimated as nucleotide diversity (π) using DnaSP v6 (Rozas *et al.*, 2017). Genetic distance between species was estimated based on Kimura's two-parameter distance model (K2P) (Kimura, 1980), as implemented in MEGA-X (Kumar *et al.*, 2018). To determine genetic differentiation among species, AMOVA was conducted using Arlequin v3.5 (Excoffier and Lischer, 2010) with 1000 permutations. Using the 30 sequences from parrotfish species along with a single COI sequence belonging to Tuna (*Thunnus tonggol*, gene bank accession number JN635369.1) as an outgroup, the phylogenetic tree was constructed according to Neighbor joining (NJ) method based on p-distance (Nei and Kumar 2000). The relative robustness of individual tree branches was estimated by bootstrapping using 1000 pseudoreplicate datasets, with 80% cut-off. PCA was also performed on the K2P genetic distance matrix using GenAlex to determine species grouping. A total of 30 sequences of the COI region were deposited in GeneBank with accession numbers MN692884-MN692913.

3. Results

3.1. Parrotfish Genetic Diversity

The number of alleles at ISSRs ranged between 5 and 17, with an average of 10.77 alleles per locus (Table 2), while the number of alleles at SCoT markers ranged between 9 and 27, with an average of 12.5 alleles per locus. The expected heterozygosity (H_{exp}) was similar for both markers, with an average of 0.470 and 0.435 for ISSRs and SCoT markers, respectively. In contrast, both markers showed a comparable moderate level of *PIC*, with average of 0.359 and 0.339 for ISSRs and SCoT markers,

Table 2. Polymorphism of ISSR and SCoT markers in parrotfish species.

Marker	No. of alleles	H_{exp}	*PIC*	*D*	*R*
ISSR-2	10	0.499	0.374	0.777	4.933
ISSR-3	13	0.467	0.358	0.862	7.133
ISSR-4	17	0.386	0.311	0.932	8.067
ISSR-8	11	0.5	0.375	0.754	6.933
ISSR-9	12	0.5	0.375	0.762	7.733
ISSR-13	12	0.498	0.374	0.783	9.067
ISSR-18	9	0.42	0.332	0.511	4.467
ISSR-19	5	0.477	0.363	0.847	3.933
ISSR-28	8	0.486	0.368	0.661	3.2
Mean	10.778	0.47	0.359	0.765	6.163

D = discriminating power; *H* = expected heterozygosity; *PIC* = polymorphism information content; *R* = resolving power.

Table 2. Continued.

Marker	No. of alleles	H_{exp}	*PIC*	*D*	*R*
SCoT-1	27	0.352	0.290	0.948	12.33
SCoT-2	15	0.422	0.333	0.909	7.467
SCoT-5	9	0.48	0.365	0.841	5.467
SCoT-6	10	0.349	0.288	0.95	5.867
SCoT-7	12	0.388	0.313	0.931	5.267
SCoT-8	12	0.492	0.371	0.81	7.667
SCoT-10	11	0.442	0.345	0.892	6.2
SCoT-11	9	0.458	0.353	0.874	5.2
SCoT-12	10	0.499	0.375	0.73	3.333
SCoT-14	10	0.468	0.358	0.861	3.6
Mean	12.5	0.435	0.339	0.875	6.24

D = discriminating power; *H* = expected heterozygosity; *PIC* = polymorphism information content; *R* = resolving power.

respectively. SCoT outperformed ISSR in discriminating among the three species, with average discriminating powers (*D*) of 0.765 and 0.875 for ISSR and SCoT markers, respectively. Both ISSR and SCoT markers showed similar magnitudes of resolving power (*R*), with average *R* values of 6.163 and 6.240, respectively.

Using ISSR and SCoT markers, *Cheilinus quinquecinctus* showed the highest genetic diversity, with 70% and 73% for ISSR and SCoT markers, respectively (Table 3). Both *Chlorurus sordidus* and *Cheilinus trilobatus* showed comparable magnitudes of genetic diversity using ISSR and SCoT markers. Using ISSRs, *C. sordidus* and *C. trilobatus* showed a genetic diversity of 29% and 39%, respectively, while both species had a genetic diversity of 60.32% using SCoT markers.

COI showed different patterns of genetic diversity. *Cheilinus quinquecinctus* had the lowest nucleotide diversity of 0.003, while the nucleotide diversity of *Cheilinus trilobatus* was 0.103, 1.5-fold higher than that of *Chlorurus sordidus*. The COI sequence length varied among the species, with averages of 625, 695, and 684 bp for *Chlorurus sordidus*, *Cheilinus trilobatus*, and *Cheilinus quinquecinctus*, respectively.

Table 3. Genetic diversity of parrotfish species based on inter simple sequence repeats (ISSRs), start codon targeted markers (SCoT), and mitochondrial cytochrome C oxidase subunit I (COI).

	Species	% Polymorphism
ISSR	*Chlorurus sordidus*	29.90
	Cheilinus trilobatus	39.18
	Cheilinus quinquecinctus	70.10
	Mean	46.39
SCoT	*Chlorurus sordidus*	60.32
	Cheilinus trilobatus	60.32
	Cheilinus quinquecinctus	73.02
	Mean	64.55
COI*	*Chlorurus sordidus*	0.065
	Cheilinus trilobatus	0.103
	Cheilinus quinquecinctus	0.003
	Mean	0.057

*Genetic diversity at COI estimated as nucleotide diversity (π).

3.2. Analysis of Molecular Variance (AMOVA)

The AMOVA of the three species based on ISSRs showed 65% of the molecular variance occurred between species, while 35% of the molecular variance occurred within species (Table 4). Similarly, using SCoT markers, the amount of molecular variance among species was 67%. However, using COI, the amount of molecular variance showed a similar magnitude among and within species, at 51.11% and 48.89%, respectively.

Table 4. AMOVA of parrotfish species based on inter simple sequence repeats (ISSR), start codon targeted markers (SCoT) and mitochondrial cytochrome C oxidase subunit I (COI).

		df	SS	Est. var.	%V
ISSR	Among species	2	321.40	15.25	65.00*
	Within species	27	222.60	8.24	35.00
	Total	29	544.00	23.49	
SCoT	Among species	2	192.07	8.006	67.00*
	Within species	27	431.20	15.97	33.00
	Total	29	623.27	23.977	
COI	Among species	2	5.40	0.24	48.89*
	Within species	27	6.90	0.26	51.11
	Total	29	12.30	0.50	

df; degree of freedom, SS; sum of squares, Est. var.; estimated variance; %V; percentage of total variation; *significant at $P < 0.05$ based on 1000 permutations.

3.3. Phylogenetic Relationship and Principal Component Analysis

The phylogenetic relationship between the three species was determined based on ISSRs, SCoT, and COI markers. Phylogenetic trees based on ISSR and SCoT markers are presented in supplementary Figures S1 and S2. The COI-based NJ phylogenetic tree showed three distinct groups representing the three species: *Chlorurus sordidus*, *Cheilinus trilobatus*, and *Cheilinus quinquecinctus* (Figure 2). Using PCA, the parrotfish species formed three distinct groups using ISSRs and COI markers (Figure 3). However, based on SCoT markers, the three species formed three groups, with some individuals shared among groups.

Figure 2. Neighbor-Joining (NJ) phylogenetic tree based on COI sequences for the three parrotfish species (*Chlorurus sordidus*, *Cheilinus trilobatus*, and *Cheilinus quinquecinctus*), numbers above branches are bootstrap values > 60%.

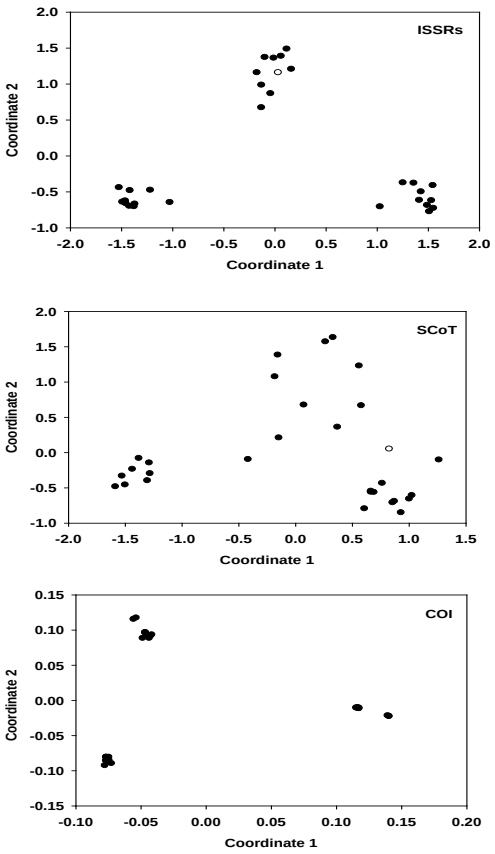

Figure 3. Principal component analysis based on inter simple sequence repeats (ISSRs), start codon targeted markers (SCoT), and cytochrome oxidase subunit I (COI) for three parrotfish species (*Chlorurus sordidus*, *Cheilinus trilobatus*, and *Cheilinus quinquecinctus*).

4. Discussion

The identification of parrotfish species from the Red Sea was modified and redescribed as a distinct group from the parrotfish species mainly found in the western Indian and western Pacific oceans (Bodorodsky *et al.*, 2016). Few studies have addressed the level of genetic diversity of parrotfish species, and mainly focused on their taxonomy and evolution (Almeida *et al.*, 2017). In the present study, the molecular characterization and genetic diversity of three parrotfish species, *Chlorurus sordidus*, *Cheilinus trilobatus*, and *Cheilinus quinquecinctus*, from the Farasan Islands, Saudi Arabia, were studied. The level and pattern of genetic diversity is one of the major factors affecting the biological potential of any species. Since the species under consideration contribute significantly to the Farasan Islands ecosystem, it is crucial to understand its genetic diversity. To better assess the genetic diversity of parrotfish species, three different molecular markers were used; ISSRs, SCoT, and COI that were previously documented in assessing species genetic diversity. Different marker systems have distinctively distributed across the genomes while exhibiting different patterns of genetic diversity (Veneza *et al.*, 2013; Hamidi *et al.*, 2014). Therefore, the combination of different molecular markers in addressing genetic diversity could assist in understanding species biodiversity. The number of SCoT alleles was higher than that of ISSRs; however, both markers had comparable mean *PICs*. The studied ISSRs and SCoT markers were reasonably informative ($0.50 > PIC > 0.25$); however, the discrimination power of SCoT was higher than that of ISSRs, which was previously reported in discriminating among closely related species (Etminan *et al.*, 2016, Etminan *et al.*, 2018). Among the studied species, *Cheilinus quinquecinctus* had the highest genetic diversity based on ISSRs and SCoT markers, whereas *Chlorurus sordidus* and *Cheilinus trilobatus* showed similar magnitudes of genetic diversity. The higher genetic diversity of *Cheilinus quinquecinctus* observed at the two dominant markers might be associated with its life-history characteristics (e.g., early maturity) (Romiguier *et al.*, 2010). In contrast, COI showed a contrasting pattern where *Cheilinus quinquecinctus* had the lowest genetic diversity (i.e., low π). The observed low nucleotide diversity of *Cheilinus quinquecinctus* is a probable sign of genetic bottleneck followed by a sudden expansion (Grant and Bowen, 1998). Moreover, mitochondrial COI lacks recombination as compared to intergenic regions of nuclear DNA (Veneza *et al.*, 2013), while ISSRs and SCoT are highly polymorphic. A similar pattern of low nucleotide diversity was also observed at COI in *Konosirus punctatus*, a coastal marine fish species in China (Liu, 2020). The genetic differentiation among species was apparent, where AMOVA revealed higher genetic differentiation among species compared to that within species, based on ISSR and SCoT markers. However, based on COI, the genetic variance within species was similar to that among species. The discrepancies in genetic variance associated with nuclear genetic markers (i.e., ISSRs and SCoT) as compared to that of mitochondrial genetic markers (COI) has been reported in other fish species and mainly associated with the different recombination rates at nuclear versus mitochondrial genetic markers. For instance, *Xenophysogobio nudicorpa* populations from Yangtze River showed a six-fold increase in genetic variation among groups using nuclear genetic markers compared to that recorded using mitochondrial genetic markers (Dong *et al.*, 2019). Species grouping was examined using cluster analysis and PCA, which is considered an efficient tool in defining the relationships among individuals of the same and/or different species. The phylogenetic trees of the three species and the PCA showed a comparable grouping pattern. However, the most supported phylogenetic tree and PCA were obtained based on COI, where all the specimens from the same species grouped together with grouping pattern matched species morphological identification.

5. Conclusion

The Farasan Islands, Saudi Arabia, is considered a major aggregation spot of fish species. Hence it is crucial to identify parrotfish (family Scaridae), one of the most common fish species, with significant ecological impacts on coral reefs. The current study is the first to document the identification and assessment of the genetic diversity of three parrotfish species using three different genetic markers. Nuclear genetic markers indicated presence of higher genetic variance between, rather than within, species; however, COI highlighted an equivalent variance within and among species. COI barcoding clearly separated the studied species into three distinct groups based on phylogeny and PCA. Additionally, this study indicated that the genetic diversity of mitochondrial genetic markers is a prevailing tool in discriminating between different parrotfish species even while exhibiting less variability. On the other hand, SCoT performed poorly in differentiating among parrotfish species calling for caution in using SCoT while investigating marine fish genetic diversity.

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Supplementary Figures

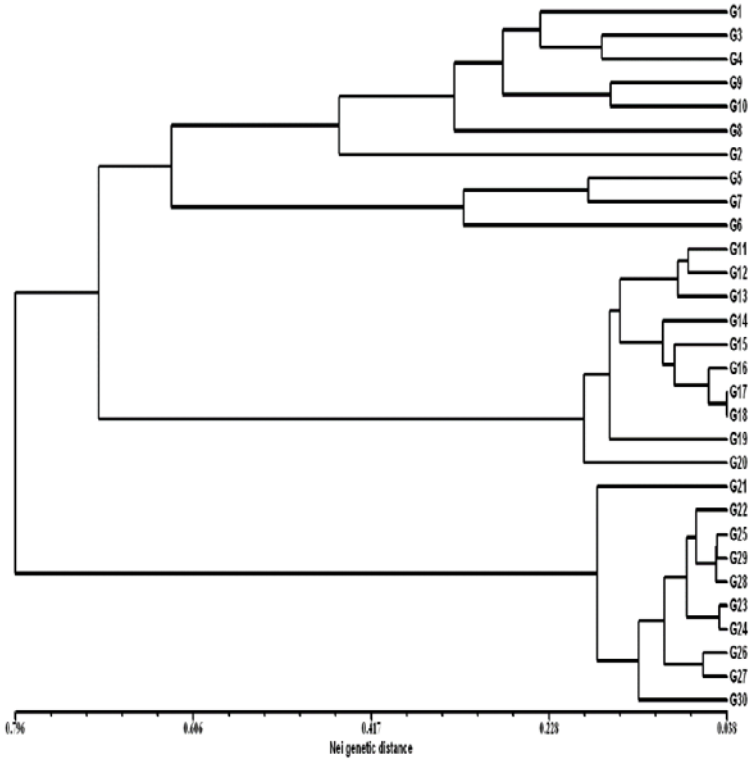

Figure S1. UPGMA phylogenetic tree based on ISSR of the parrotfish species. *Chlorurus sordidus*; G1-G10, *Cheilinus trilobatus;* G11-G20, *Cheilinus quinquecinctus* G21-G30

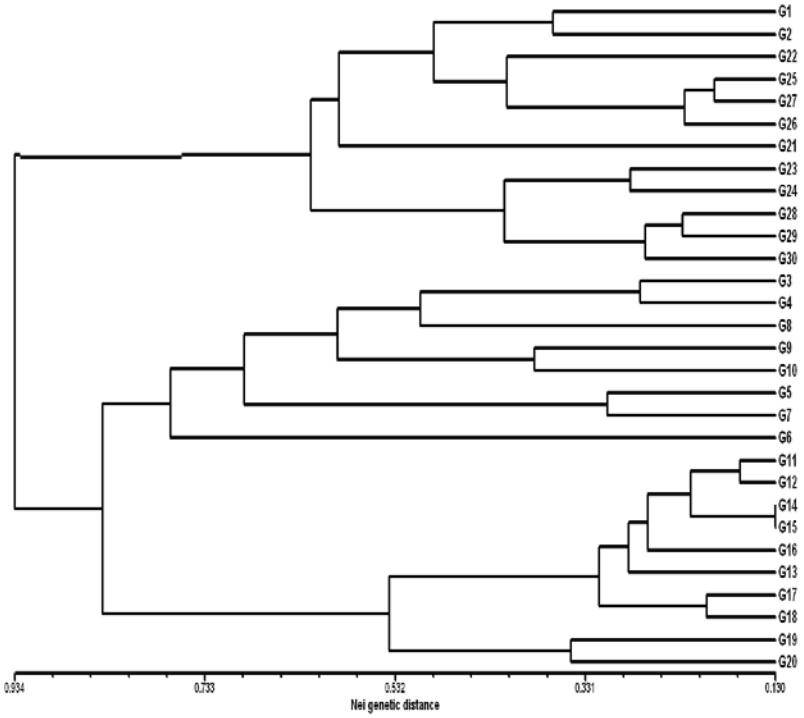

Figure S2. UPGMA phylogenetic tree based on SCoT of the parrotfish species. *Chlorurus sordidus*; G1-G10, *Cheilinus trilobatus;* G11-G20, *Cheilinus quinquecinctus* G21-G30.