Monitoring of genetic diversity in some parrotfish species based on inter simple sequence repeats polymorphism

Y. M. Saad[1,2;]AbuZinadah, O. A. H.[1]; and El-Domyati, F. M.[1,3]

1Dept. of Biol. Science, Fac. of Sciences, King Abdulaziz Univ., KSA.
2 Genetic Lab., National Institute of Oceanography and Fisheries (NIOF), Egypt.
3 Dept. of Genetics, Faculty of Agric., Ain Shams Univ., ShobraElndKheima, Egypt.
yasser_saad19@yahoo.com

Abstract: ISSR technique was used for monitoring of genetic diversity within and among eight parrotfish species [*Scarus niger*, *Scarus* (*chlorurus*) *sordidus*, *Scarus frenatus*, *Scarus ghobban*, *Scarus ferrugineus*, *Scarus fuscopurpureus*, *Cetoscarus ocellatus* and *Cetoscarus bicolor*]. A total of 132 ISSR bands were detected. Most of estimated bands were polymorphic. The highest number of polymorphic bands (PB=28), percentage of polymorphic loci (21.21%), actual number of alleles (na=1.21), effective number of alleles (ne=1.12), *Nei's* gene diversity (h=0.07) and Shannon's information index (I=0.11) values were calculated in *Scarus frenatus*. On the other hand, the lowest values of these parameters were calculated in *Cetoscarus ocellatus*. A total of 21 ISSR specific bands were detected and analyzed in the applied fish species. The developed DNA markers were powerful tools to estimate the genetic diversity and detecting genetic polymorphism in the applied fish species. The genetic distance values among the applied fish species were calculated. The analyses of detected ISSR loci produced well reconstruction of phylogenetic trees for the studied parrotfish species. This knowledge is fundamental for the conservation of these fish species and fish evolution studies.

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1. Introduction

The parrotfishes (family scaridae) are a distinctive group of labroid fishes. The scaridae has 80 species in ten genera.These fish are closely associated with coral reefs and form a dominant and conspicuous part of the herbivorous fish community. The development and identification of parrotfish are complex and accompanied by a series of changes in color. Almost all species are sequential hermaphrodites, starting as females (known as the initial phase) and then changing to males (Bellwood 1994).

Principal threats to marine fishes are exploitation, habitat loss, pollution, invasive species (Reynolds *et al*., 2005) and bad management.

Parrotfish (as animal protein sources) are popular in local seafood restaurants. These aquatic genetic resources may be at risk of extinction due to over exploitation and bad management.

Fishing with no good management constraints, a substantial number of aggregations, and hence species populations, have been severely depleted or become extinct locally with consequences felt regionally (Sadovy *et al*., 2004).

Genetic diversity plays a huge role in survival and adaptability of a species. During environment changes, slight gene variations are necessary for species to adapt and survive. A species that has a large degree of genetic diversity will have more variations from which to choose the fit alleles. The species that have very little genetic variation are at a great risk (Frankham 1996 and Frankham*et al.,* 2002).

Some efforts were done to study the effect of parrotfish on coral reefs (Andrew *et al.,* 2012) and biodiversity by ontogenetic changes in shape, size, coloration that correspond to discrete sexual stages and genetics (Streelman *et al*., 2002). The genetics studies on parrotfish species identification and evolution are not fully maximized. So, it is important to monitor of genetic diversity in parrotfish species as economic genetic resources using an efficient, highly polymorphic and easy molecular technique such as Inter Simple Sequence Repeats. In addition, monitoring of genetic diversity based on molecular markers such as ISSR markers (Saad *et al.,* 2012 and saad *et al*., 2013) should be conducted to provide the information needed for a sound management of parrotfish wild stocks. This way will be useful especially in conservation (Saad *et al*., 2011) of aquatic genetic resources. In addition, the capacity to conserve and to use natural resources wisely requires identifying taxa and variation at both the individual, population and/or species levels.

The application of DNA markers has allowed rapid progress in aquaculture investigations

of genetic variability, inbreeding (Rashed *et al*., 2009), parentage assignments, species & strain identification and the reconstruction of phylogenetic relations among aquatic organisms especially fish.

The objectives of this work are monitoring of some parrotfish genetic diversity, reconstruction the phylogenetic relationships (among some parrotfish species) and increasing the scientific information about the parrotfish biodiversity in Saudi Arabia using ISSR polymorphism.

2.Material and Methods:

Fish [*Scarus niger*, *Scarus* (*chlorurus*) *sordidus*, *Scarus frenatus*, *Scarus ghobban*, *Scarus ferrugineus*, *Scarus fuscopurpureus*, *Cetoscarus ocellatus* and *Cetoscarus bicolor*] samples were obtained from project (Monitoring of biodiversity in *Cetoscarus* sp. genetic resources in the Red sea) funded from DSR, King Abdulaziz Univ., KSA during year of (2013). Samples were coded as:

A= *S. niger,* B= *Scarus (chlorurus) sordidus,* C= *S.frenatus,* D= *S.ghobban,* E= *S.ferrugineus,* F= *S.fuscopurpureus,* I= *Cetoscarus ocellatus and* K= *C.bicolor.*

DNA samples were extracted from 80 fish individuals (10 samples were estimated from each applied fish species). From each specimen, approximately 0.2g of fish fin tissue was excised, placed in a 70 % isopropanol and held at 4°C for subsequent DNA extraction. DNA of the 10 individuals from each estimated parrotfish species were extracted as described by (Hills *et al.,* 1996).

ISSR analysis:

11 ISSR primers (Table1) were originally selected (Biotechnology Laboratory, University of British Columbia) to measure the genetic variability among the applied fish samples.

Table (1): ISSR primer names and sequences.

Code	Sequence	Code	Sequence
844	5' [CT]8RC 3'	MAO	5' [CTC]5RC 3'
7	5' [CT]8RG 3'	814	5' [CT]8TC 3'
17899B	5' [CA]6GG 3'	843	(CT)8ndRA
HB13	5'[GAG]3GC3'	John	5' [AG]7YC 3'
MANNY	(CAC)4ndRC	HB8	[GA]6GG
17898B	5' [CA]6GT 3'		

PCR reaction was prepared in a 10 µl contained a 1µl of DNA (50 ng), a 0.3 µM of primer, a 0.2mM of dNTPs, a 25 mM of $MgCl_2$, a 1 unit of Taq DNA polymerase and a 1 X buffer.

PCR program was consisted of one cycle for 2 min. at 94^{o}C, 35 cycles for (30 sec. at 94^{o}C, 45 sec. at 44^{o}C & 1.5 min. at 72^{o}C) and one cycle for 10 min. at 72^{o}C.

The amplification products were separated by standard horizontal electrophoresis in 1.5% agarose (Sigma) gels were stained with ethidium bromide (0.3ug/ml), then visually examined with UV trans illuminator and photographed using a CCD camera (UVP, UK).

Data score and analyzing:

Gel images were analyzed using GelAnalyzer3 software to determine molecular sizes, presence (1) or absence (0). In addition, frequencies, polymorphism type of the amplified fragments, the mean of band frequency and the polymorphism percentage for each primer were calculated.

Data were analyzed as described by Saad *et al.,* (2012) with some modifications. Some parameters [number of polymorphic loci, percentage of polymorphic loci, actual number of alleles (na), effective number of alleles (ne), *Nei's* gene diversity (h) and Shannon's information index (i)] were estimated to monitor the genetic diversity in applied fish species. These parameters were calculated and estimated using POPGENE (version 1.32), which is a Microsoft Windowsndbased freeware program for population genetic analysis (Yeh *et al*., 1997). Dendrogram was constructed based on *Nei's* genetic distances using UPGMA.

3.Results

A total of eleven ISSR primers (Table 1) were used for monitoring of genetic diversity within and among applied fish species. These primers generated 132 ISSR bands. Generally, most of estimated bands (loci) were polymorphic.

Genetic diversity within each applied fish species:

Some parameters were used to assess the genetic variation within applied fish species and they were: average of band frequencies (Table2), number of polymorphic loci, percentage of polymorphic loci, actual number of alleles (na), effective number of alleles (ne), *Nei's* gene diversity (h) and Shannon's information index (i).The values of these parameters were presented in Tables (2 and 3).

The averages of band frequencies were ranged from 0 to 1 within each estimated fish samples.

Some average of band frequency values were equal 1 while the other values were ranged from 0 to 0.98 over all bands generated by the eleven ISSR primers.

Band frequency values were averaged (Table 2) across all used ISSR primers for each fish species. It were ranged from 0.68 (F samples) to 0.93 (A samples).

The highest number of polymorphic loci (PB=28), percentage of polymorphic loci (21.21%), actual number of alleles (na=1.21), effective number of alleles (ne=1.12), *Nei's* gene diversity (h=0.07) and Shannon's information index (i=0.11) values were detected in (C) species. On the other hand, the

lowest values of these parameters were detected in (i) species (Table 3).

Table (2): Average frequency of bands obtained using the ISSR primers with the applied fish species.

species	A	B	C	D	E	F	I	K
Primer code								
844	0.93	0.85	1	1	0.98	1	1	0.81
7	1	0.9	0.83	1	0.98	1	0.97	0.72
17899B	0.88	1	1	0.97	1	0.87	0.35	0.71
HB13	0.86	0.81	1	0.98	0.85	1	0.8	1
MANNY	0.77	0.53	1	1	0.70	0.83	0.76	1
17898B	0.92	1	0.80	1	0.91	0.91	0.98	0.97
MAO	1	0	0	0.32	0.92	0	0	1
814	1	0.81	0.51	0	1	0.5	0.81	0.78
843	0.86	0.73	0.64	0.88	0.81	0.83	1	0.76
John	1	1	0.57	1	0.79	0	1	0.95
HB8	1	0.76	0.65	0.98	0.81	0.58	0	0
Mean ± SD	0.93±0.07	0.76±0.2	0.73±0.3	0.91±0.2	0.89±0.1	0.68±0.3	0.69±0.3	0.79±0.2

A=*Scarus niger*, B=*Scarus* (*chlorurus*) *sordidus*, C= *Scarus frenatus*, D=*Scarus ghobban*, E=*Scarusf errugineus*, F=*Scarus fuscopurpureus*, I=*Cetoscarus ocellatus* and K=*Cetoscarus bicolor*.

Table (3): Mean ± SD (Standard deviation) of Actual number of alleles (na), Effective number of alleles (ne), *Nei's* gene diversity (h), Shannon's information index (i), polymorphic bands and percentage of polymorphic loci (%PL) for each studied fish species.

Parameter	na	ne	h	i	PB	%PL
species						
S. niger	1.083±0.27	1.06±0.22	0.03±0.12	0.05±0.17	11	8.33
S.(chlorurus) sordidus	1.12±0.33	1.052±0.18	0.032±0.1	0.05±0.15	17	12.88
S. frenatus	1.21±0.41	1.12±0.28	0.07±0.15	0.11±0.22	28	21.21
S.ghobban	1.08±0.27	1.054±0.19	0.03±0.1	0.046±0.15	11	8.33
S. ferrugineus	1.12±0.33	1.08±0.24	0.047±0.13	0.069±0.19	17	12.88
S.fuscopurpureus	1.07±0.26	1.03±0.15	0.024±0.09	0.037±0.13	10	7.58
C.ocellatus	1.05±0.22	1.029±0.13	0.018±0.08	0.027±0.12	7	5.30
C.bicolor	1.17±0.38	1.11±0.28	0.067±0.15	0.098±0.22	23	17.42

Genetic diversity among applied fish species:

1- ISSR markers for each applied fish species:

A total of 21 ISSR specific bands were detected and analyzed in the eight applied fish species using the eleven tested ISSR primers. These bands number was divided into 1 (for A), 2 (for B), 1 (for C), 6 (for D), 2 (for E), 1 (for F), 1 (for I) and 7 (for K). The Molecular sizes (bp) of the specific ISSR markers for the eight applied fish species were presented in Table (4).

Table (4): Molecular sizes (bp) for detected specific markers for each applied fish species.

species	A	B	C	D	E	F	I	K
Primer code								
	-	-	-	82	-	-	-	-
844	-	-	-	-	-	-	-	-
7	-	-	-	66&65	-	-	-	68
17899B	62	-	-	-	-	-	-	102,98,96,94 &88
HB13	-	-	-	-	800	-	-	-
MANNY	-	-	-	792,642&560	-	-	-	-
17898B	-		478	-	-	-	358	-
MAO	-	778	-	-	-	742	-	-
814	-	-	-	-	82	-	-	-
843	-	64	-	-	-	-	-	-
John	-	-	-	-	-	-	-	-
HB8	-	-	-	-	-	-	-	450

A=*Scarus niger*, B=*Scarus* (*chlorurus*) *sordidus*, C= *Scarus frenatus*, D=*Scarus ghobban*, E=*Scarusf errugineus*, F=*Scarus fuscopurpureus*, I=*Cetoscarus ocellatus* and K=*Cetoscarus bicolor*.

2-Genetic distance among the applied fish species:

The genetic distance values among the applied fish species were calculated. The distance values among the applied fish species were presented in Table (5). Results showed that, the lowest distance values(less than 0.40) based on ISSR polymorphism were 0.31, 0.34, 0.33, 0.36 and 0.38between (B & C), (B & F), (C& D), (C& F) and (D & E) fish pairs

respectively. On the other hand, the highest distance value (0.62) was calculated between (D &I). The analyses of 132 ISSR loci produced well reconstruction of phylogenetic trees for the nine parrotfish species (Fig. 1). Each of the detected ISSR DNA fragments were provided useful phylogenetic signal for clear phylogenetic resolution. Figure (1) reflect the genetic distance among the applied fish species.

Table (5): Nei's genetic distance among applied fish species based on ISSR polymorphism

species	A	B	C	D	E	F	I
A							
B	0.42						
C	0.42	0.31					
D	0.54	0.42	0.33				
E	0.40	0.49	0.45	0.38			
F	0.45	0.34	0.36	0.56	0.46		
I	0.52	0.53	0.54	0.62	0.47	0.43	
K	0.52	0.52	0.42	0.59	0.54	0.50	0.48

A=*Scarusniger*, B=*Scarus* (*chlorurus*) *sordidus*, C= *Scarus frenatus*, D=*Scarus ghobban*, E=*Scarus ferrugineus*, F=*Scarus fuscopurpureus*, I=*Cetoscarus ocellatus* and K=*Cetoscarus bicolor*.

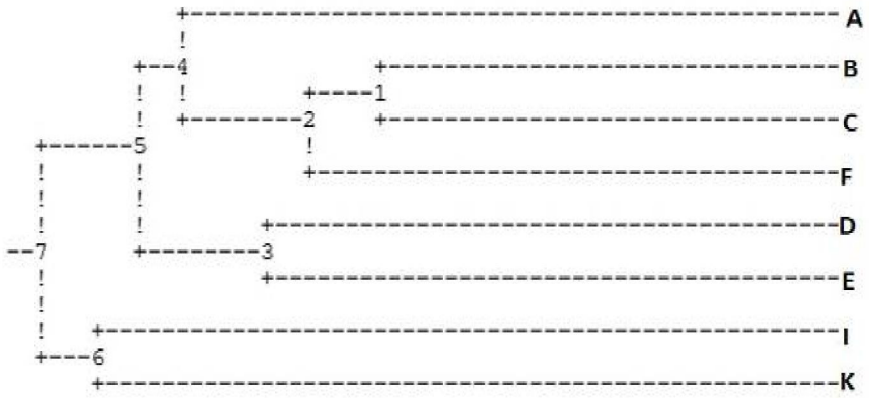

Figure (1): Reconstruction of phylogenetic relationships among the applied fish species A, B, C, D, E, F, I and K based on ISSR polymorphism. A=*Scarusniger*, B=*Scarus* (*chlorurus*) *sordidus*, C= *Scarus frenatus*, D=*Scarus ghobban*, E=*Scarusferrugineus*, F=*Scarusfuscopurpureus*, I=*Cetoscarus ocellatus* and K=*Cetoscarus bicolor*.

Table (6): The lengths among applied fish species and nodes on the phylogenetic tree.

Between	A	Length
7	5	3.00967
5	4	1.28223
4	A	21.79622
4	2	4.05862
2	1	2.23639
1	B	15.50121
1	C	15.50121
2	F	17.7376
5	3	4.05569
3	D	19.02275
3	E	19.02275
7	6	2.02512
6	I	24.063
6	K	24.063

The lengths among applied fish species and nodes on the phylogenetic tree were presented in Table (6).

4.Discussion:

In the present study, ISSR technique was used for monitoring of genetic diversity in eight Parrotfish species because this technique overcomes most of other widely usable techniques (Gupta *et al.,* 1994; McGregor *et al*., 2000 and Saad *et al.,* 2013) limitations in this field such as low reproducibility of RAPD and high cost of AFLP.

The results (in the present study) showed that, the average of band frequency values (within each studied fish species) were ranged from 1 to 0. A total of 28 average of band frequency values were equal 1(monomorphic banding pattern). The other values were ranged from 0 (No detected bands) to 0.98 (polymorphic banding pattern) over all generated bands by the eleven ISSR primers. Band frequency values were calculated to reflect the homogeneity and heterogeneity levels (Rashed *et al*., 2008) within each estimated fish species.

In the present study, genetic diversity within applied fish species was estimated using different parameters such as *Nei's* gene diversity and Shannon's information index because these parameters has a value in detecting the genetic variations within and among biological samples such as fish samples (Saad *et al*., 2009). We found that, both *S. frenatus* and *C.bicolor* has high *Nei's* (h) gene diversity and Shannon's information index (i) values relatively. In addition, we noted that, these values (i and h) affected by the number of polymorphic bands as detected in Table (3).

Generally, genetic variation is generated by mutation and lost by genetic drift, which increases inter population differentiation (Hanfling and Brandl1998). The genetic drift increases inter-population genetic variation, (Frankham *et al.,* 2002). Moreover, natural selection can also reduce genetic variation, leading to the fixation of alleles or promoting their retention as a result of balancing or diversifying selection (Frankham 1996). The highest number of polymorphic loci (PB=28), percentage of polymorphic loci (21.21%), actual number of alleles (na=1.21) and effective number of alleles (ne=1.12) values were detected in (*Scarus frenatus*) species. On the other hand, the lowest values of these parameters were detected in *Cetoscarus ocellatus*. So, the *Scarus frenatus* will be more adapted for environmental changes than other estimated fish species especially *Cetoscarus ocellatus* or (I) species.

In the present study, a total of 21 ISSR specific bands were detected and analyzed in the applied fish species using the eleven tested ISSR primers. These markers are useful for parrotfish species identification and characterization because it were species specific DNA markers. The application of DNA markers (Saad *et al.,* 2009) has allowed rapid progress in aquaculture investigations of genetic variability, inbreeding (Rashed*et al*., 2009), parentage assignments, species & strain identification, and the construction of high-resolution genetic linkage maps for aquatic species.

In the present study, the calculated genetic distance among applied fish species reflect the phylogenetic relations among them based on molecular level. Rapid progress in the molecular phylogenetic of diverse groups of organisms has created opportunities to combine phylogenetic studies with exploration of molecular evolution (Smith *et al*., 2008). The strength of phylogenetic signal at the species level was high balanced with ISSR (Saad *et al*., 2012 and Saad *et al.,* 2013) markers.

In the present study, ISSR markers are easy, and efficient tool for applied fish species identification and characterization. The developed ISSR markers were powerful tools to estimate the genetic diversity and detecting genetic polymorphism in the applied fish species.

Maintenance of genetic diversity is a primary objective in the management of wild (Saad *et al.,* 2012) and captive fish populations and/or species (Rashed *et al*., 2009).

Our results will be used for promoting increased knowledge on the Parrotfish genetic structure and their response to environmental changes. The present study detect the genetic diversity (level of biodiversity that refers to the total number of genetic characteristics in the genetic makeup of a species) within and among the applied fish species which is needed for species adaptation and taxa speciation (Frankham *et al*., 2002 and Saad *et al.,* 2012). This knowledge is fundamental to the management of fish species and/or populations and the establishment of Evolutionary Significant Units capable of conserving genetic integrity (Piorski *et al.,* 2008).

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Corresponding author
Y.M. Saad[1&2]
[1]Dept. of Biol. Science, Fac. of Sciences, King Abdulaziz Univ., KSA.
[2] Genetic Lab., National Institute of Oceanography

and Fisheries (NIOF), Egypt.
yasser_saad19@yahoo.com

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