Genetic relationship between five fish species of genus scarus using rapd assay

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ABSTRACT

Scaridae fish species including *Scarus psittacus, S. frenatus, S. genazonatus, S. fuscopurpureus and S. niger,* were collected from Red Sea in Egypt. The DNA fingerprints of these species were identified using RAPD-PCR technique with eight decamer primers. A total of 93 bands were scored in all species using the selected primers, with the size range varying from 190 to 2500 bp. On average, each primer amplified 11.63 bands per species. Each species had both monomorphic and polymorphic DNA bands. The polymorphic bands represent 34.41% in all fish species. The highest similarity index (70%) was found between *Scarus psittacus* and *S. frenatus*, while the lowest one (51%) was between *Scarus psittacus* and *S. niger, S. genazonatus* and *S. fuscopurpureus* and *S. genazonatus* and *S. niger.* Therefore the phylogenetic relationships among the five studied scaridae species were clearly resolved in dendrogram, they were clustered into four clusters groups: the first one comprised, *S. fuscopurpureus and S. niger,* the second included *S. psittacus, S. frenatus* and *S. genazonatus,* the third included *S. genazonatus* and the fourth branch contained *S. psittacus* and *S. frenatus*. In conclusion, the obtained results exhibited that the five *Scarus* species are distinctive ones. In addation, the RAPD patterns are suitable tools to differentiate between fish species.

Keywords: Genetics, RAPD, Fingerprints, Taxonomy, Dendrogram, Fishes, *Scarus*.

INTRODUCTION

Parrot fishes are named for their dentition, which also is distinct from that of other labrids. Their numerous teeth are arranged in a tightly packed mosaic on the external surface of the jaw bones, forming a parrot-like beak with which they rasp algae from coral and other rocky substrates. Maximum sizes vary within the family, with the majority of species reaching 30-50 centimeters in length; however, a few species reach lengths in excess of one m and the green hump head parrot fish can reach up to 1.3 meters. Some authors (Choat and Bellwood, 1998; Westneat and Alfaro, 2005; Randall, 2007; Froese *et al*., 2009) recorded that parrot fishes are group of fishes that traditionary had been considered a family (Scaridae), but now these fishes are considered a subfamily (Scarinae) of the wrasses. Some authorities have preferred to maintain the parrot fishes as a family level taxon.

Recently, a wide range of new molecular techniques have been explored and reported for fishes and shellfishes (Lehmann *et al*., 2000 and Jayasankar, 2004). Several DNA techniques for evaluating genetic variability in fish species are available and are widely used (Harris *et al*., 1991; Mjolnerod *et al*., 1997; Coughlan *et al*., 1998; Wasko and Galetti, 2002; Barman *et al.*, 2003; Jayasankar, 2004; Matoso *et al.,* 2004). One such technique is the Random amplified polymorphic DNA (RAPD)

which was first introduced by Williams *et al.,* (1990). It is a technique based on the PCR amplification of discrete regions of genome with short oligonucleotide primers of arbitrary sequence (Welsh and McClelland, 1990; Williams *et al.,* (1990). It utilizes single, arbitrary, decamer DNA oligonucleotide primers to amplify regions of genome based on the polymerase chain reaction (Hadrys *et al*. 1992; Williams *et al*., 1993). The characters assessed through RAPD are useful for genetic studies because they provide various types of data-taxonomic population, inheritance pattern of various organisms including fishes (Brown and Epifanio, 2003; Degani, 2004; David and Pandian, 2006).

RAPD has been widely used in the last decade in species identification program, and in assessing genetic variations among different taxa at DNA level because of its cost effectiveness and simple operation without requiring prior knowledge of species DNA sequences (Schnell *et al*., 1995; Frankel *et al*., 1997). The RAPD method has been initially used to detect polymorphism in genetic mapping, taxonomy and phylogenetic studies and later in genotoxicity and carcinogenesis studies (Atienzar and Jha 2006; Fouz *et al*., 2007). In addition, the fact that the RAPD assay allows the visualization of a wide range of PCR products may explain why this assay is a preferred choice. Also the random amplified polymorphic DNA (RAPD) is a useful assay for the detection of genotoxin-induced damage and mutations. The main advantages in the use of the RAPD method lie in its rapidity, applicability to any organism without prior information on the nucleotide sequence, cell cycle, karyotype and potential detection of a variety of DNA damage and mutations at the whole genome level. Random amplified polymorphic DNA (RAPD) is one of the molecular techniques that has benefited from the advent of the PCR. This molecular method has found applications in various types of organisms, e.g. bacteria. (Atienzar *et al*., 2002; Brahmane *et al*., 2006 and 2008).

For example, RAPD was used to determine the genetic variation in endangered Neotropical fish species (Wasko and Galetti, 2002). Moreover, genetic monitoring of the Amazonian fish matrincha *Brycon cephalus* were studied using RAPD markers and determine the usefulness of genetic management and biodiversity conservation of this species (Wasko *et al.,* 2004). In addition genetic diversity of three cultured populations of goldfish were studied and revealed poor genetic diversity of goldfish (Xi-dong *et al.,* 2007).

RAPD analysis was applied to four fish's families in Egypt: Cichlidae (freshwater), Mugilidae (catadromus), Sparidae and Serranidae (marine). Twenty random primers were used to assay polymorphisms within and among these families. Results demonstrated that there are great differences among the first three families. Genetic differences within each family were observed. Evidence was presented showing RAPD markers useful for systematic investigation among and within different families for fish that live under different water salinities (Ali, 2003; Ali *et al*., 2004).

From the above literature, the reports on the genetic variability are very scanty in Scaridae fishes in Egypt. Hence, the present study is aimed to analyze genetic variations and determine the genetic taxonomic relationship among *Scarus psittacus, Scarus frenatus, Scarus genazonatus, Scarus fuscopurpureus and Scarus niger* species using RAPD analysis.

MATERIALS AND METHODS

Five species of marine fishes from Family Scaridae (*Scarus psittacus, S. frenatus, S. genazonatus, S. fuscopurpureus and S. niger*), were utilized. All samples were collected from Hurghada, on the Egyptian Red Sea coast by the aid of fishermen then transported to the laboratory of "National Institute of Oceanography and Fisheries (Red Sea Branch)" and kept alive until processed.

Eight primers (Alpha DNA, Montreal, Quebec, Canada) were used in RAPD-PCR analysis to study the difference between these five fish species. The code and sequences of these primers are shown in Table (1). DNA extraction was carried out using the Qiagen DNeasy (Qiagen Santa Clara, CA) from muscles of fish species. DNA concentration was determined by diluting the DNA 1:5 in bi dist. H_2O.

Table 1: Oligonucleotide sequences of primers, their annealing temperature and G+C contents.

No.	Primer code No.	Nucleotide sequence (5` to 3`)	Annealing Tm °C /Sec	GC%
1	OPA- 12	TCGGCGATAG	30.5	60
2	OPA- 14	TCTGTGCTGG	23.2	60
3	OPA- 15	TTCCGAACCC	32.6	60
4	OPA- 17	GACCGCTTGT	27.2	60
5	OPA- 18	AGGTGACCGT	25.0	60
6	OPA- 19	CAAACGTCGG	30.2	60
7	OPA- 20	GTTGCGATCC	27.8	60
8	OPO- 11	GACAGGAGGT	17.8	60

The DNA samples were electrophoresed in 1% agarose gel against 10 ng of a DNA size marker. This marker covers a range of concentration between 95 ng and 11 ng. Thus, estimation of the DNA concentration in a given sample was achieved by comparing the degree of fluorescence of the unknown DNA band with the different bands in the DNA size marker. The amplification reaction was carried out in 25 µl reaction volume containing 1X PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 1 µM primer, 1 U Taq DNA polymerase and 25 ng templates DNA. PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 1 min, an annealing step at 36ºC for 1 min, and an elongation step at 72ºC for 1.5 min. The primer extension segment was extended to 7 min at 72ºC in the final cycle. The amplification products were resolved by electrophoresis in a 1.5 % agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a Polaroid camera. Amplified products were visually examined and the presence or absence of each size class was scored as + or -, respectively.

The banding patterns generated by RAPD-PCR marker analyses were compared to determine the genetic relatedness of the studied species. Clear and distinct amplification products were scored as (+) for presence and (-) for absence of bands. Bands of the same mobility were scored as identical.

Data analysis

The genetic similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient Sneath and Sokal, (1973).

Dice formula: $GS_{ij} = 2a/(2a+b+c)$

Where GS_{ij} is the measure of genetic similarity between individuals *i* and *j*, a is the number of bands shared by *i* and *j*, b is the number of bands present in *i* and absent in *j*, and c is the number of bands present in *j* and absent in *i*. The similarity matrix

was used in the cluster analysis. The cluster analysis was employed to organize the observed data into meaningful structures to develop taxonomies. At the first step, when each accession represents its own cluster, the distances between these accessions are defined by the chosen distance measure (Dice coefficient). However, once several accessions have been linked together, the distance between two clusters is calculated as the average distance between all pairs of accessions in the two different clusters. This method is called Unweighted Pair Group Method using Arithmetic Average (UPGMA).

RESULTS

Using RAPD- PCR technique, the molecular genetic analysis and taxonomic phylogenetic relationship of five marine water species of fishes *Scarus psittacus, S. frenatus, S.genazonatus, S. fuscopurpureus and S. niger* (Figs. 1-5), (Family: Scaridae - Order: Perciformes) were investigated. Eight random primers were used in this study. The DNA fragments generated by the primers from the genomic DNA of the five species were separated using agarose gel electrophoresis and illustrated in Figs. (6 to 13).

Fig. (6): Agarose gel electrophoresis of RAPD products generated with OPA-12. Where, 1-*Scarus psittacus*, 2-*Scarus frenatus*, 3-*Scarus genazonatus*, 4-*Scarus fuscopurpureus and* 5-*Scarus niger.*

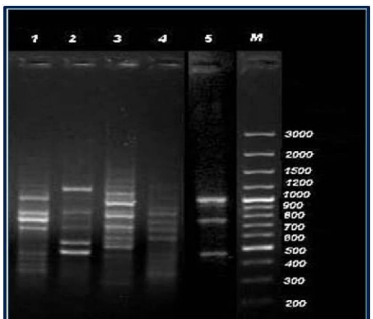

Fig.(7): Agarose gel electrophoresis of RAPD products generated with OPA-14.

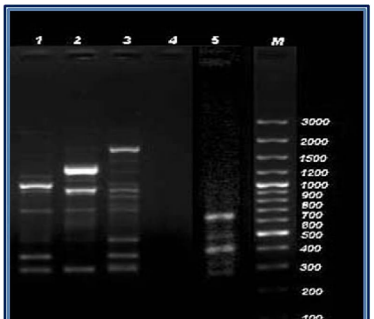

Fig.(8): Agarose gel electrophoresis of RAPD products generated with OPA-15.

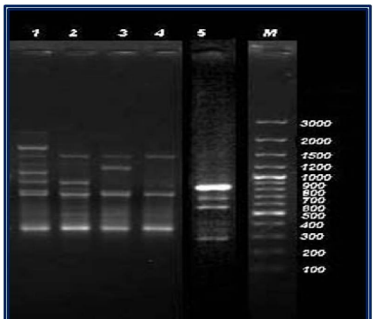

Fig.(9): Agarose gel electrophoresis of RAPD products generated with OPA-17.

Fig.(10): Agarose gel electrophoresis of RAPD products generated with OPA-18.

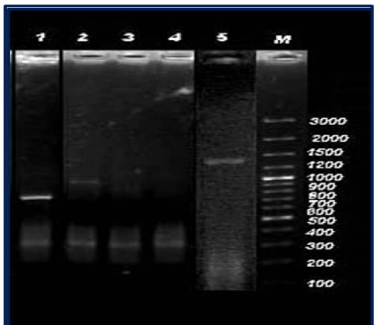

Fig.(11): Agarose gel electrophoresis of RAPD products generated with OPA-19.

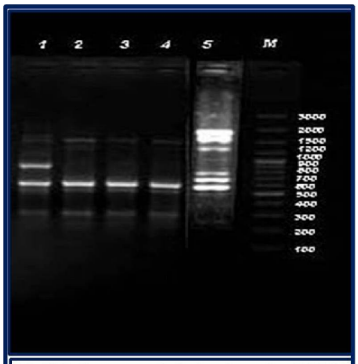

Fig.(12): Agarose gel electrophoresis of RAPD products generated with OPA-20

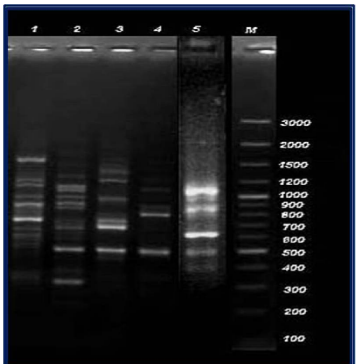

Fig.(13): Agarose gel electrophoresis of RAPD products generated with OPO-11.

The banding patterns of these DNA fragments were analyzed by Gene profiler computer software program and summarized in Table (2). RAPD analysis revealed a good polymorphism among *Scarus* species.

Table 2: Survey of RAPD Markers using eight primers. (1-*Scarus psittacus,* 2-*Scarus frenatus,* 3-*Scarus genazonatus,* 4-*Scarus fuscopurpureus and* 5-*Scarus niger*), where (+) means present and (-) means absent.

		Primer OPA-12							**Primer OPA-14**				
N	**MW**	**1**	**2**	**3**	**4**	**5**	**N**	**MW**	**1**	**2**	**3**	**4**	**5**
1	**1200**	+	-	-	-	+	**1**	**1350**	-	-	+	-	-
2	**1050**	+	-	-	-	-	**2**	**1150**	+	+	+	-	-
3	**600**	-	-	-	+	+	**3**	**1050**	+	-	+	-	-
4	**530**	-	-	-	+	-	**4**	**970**	-	-	+	-	+
5	**460**	-	-	+	-	-	**5**	**860**	+	+	+	+	-
6	**400**	+	+	+	+	+	**6**	**780**	+	+	-	+	+
7	**350**	+	-	-	+	+	**7**	**700**	+	+	+	+	-
8	**310**	+	+	+	-	-	**8**	**600**	-	-	+	+	-
		Primer OPA-15					**9**	**500**	+	+	+	-	+
N	**MW**	**1**	**2**	**3**	**4**	**5**	**10**	**430**	+	-	-	+	-
1	**1700**	-	-	+	-	-	**11**	**360**	+	-	-	+	-
2	**1300**	-	+	-	-	-	**12**	**300**	-	-	-	+	-
3	**1000**	+	+	+	-	-	**13**	**250**	-	-	+	-	-
4	**900**	+	+	+	-	-			**Primer OPA-17**				
5	**700**	-	-	-	-	+	**N**	**MW**	**1**	**2**	**3**	**4**	**5**
6	**500**	+	+	+	-	+	**1**	**2500**	+	-	-	-	-
7	**400**	-	-	-	-	+	**2**	**2100**	+	-	-	-	-
8	**350**	+	-	+	-	-	**3**	**1900**	+	-	-	-	-
9	**280**	+	+	+	-	+	**4**	**1500**	+	+	+	+	-
		Primer OPA-18					**5**	**1200**	+	-	+	-	+
N	**MW**	**1**	**2**	**3**	**4**	**5**	**6**	**1000**	+	-	-	-	-
1	**2000**	+	+	-	-	-	**7**	**850**	+	+	-	-	+
2	**1500**	+	-	-	-	-	**8**	**760**	+	+	-	-	-
3	**1400**	-	+	-	-	-	**9**	**700**	+	+	+	+	+
4	**1250**	+	-	+	-	-	**10**	**550**	-	-	-	-	+
5	**1150**	-	-	+	+	+	**11**	**350**	+	+	+	+	+
6	**1050**	+	+	+	+	+	**12**	**300**	-	-	-	-	+
7	**950**	-	-	+	-	+			**Primer OPA-19**				
8	**870**	+	-	+	-	+	**N**	**MW**	**1**	**2**	**3**	**4**	**5**
9	**800**	+	-	+	+	+	**1**	**1400**	-	-	-	-	+
10	**760**	-	-	+	-	-	**2**	**900**	-	+	-	-	-
11	**700**	+	+	-	+	-	**3**	**700**	+	-	-	-	-
12	**620**	+	+	-	+	+	**4**	**300**	+	+	+	+	+
13	**550**	-	+	-	+	+			**Primer OPA-20**				
14	**500**	+	+	+	+	-	**N**	**MW**	**1**	**2**	**3**	**4**	**5**
15	**450**	+	-	+	-	-	**1**	**2000**	-	-	-	-	+
16	**370**	+	-	-	-	-	**2**	**1600**	+	-	-	-	-
17	**320**	-	+	-	-	+	**3**	**1500**	+	+	+	+	+
18	**250**	+	+	+	+	+	**4**	**1300**	-	+	-	-	+
19	**190**	-	-	-	+	+	**5**	**1100**	-	-	-	-	+
		Primer OPO-11					**6**	**900**	+	-	-	-	-
N	**MW**	**1**	**2**	**3**	**4**	**5**	**7**	**800**	-	-	-	-	+
1	**1500**	+	-	-	-	-	**8**	**600**	-	-	-	-	+
2	**1300**	+	+	+	-	-	**9**	**550**	+	+	+	+	+
3	**1200**	+	-	+	+	-	**10**	**500**	+	+	+	+	+
4	**1100**	+	+	-	+	+	**11**	**300**	+	+	+	+	+
5	**1000**	+	+	+	-	+							
6	**930**	+	+	-	+	+							
7	**870**	-	+	-	+	+							
8	**800**	+	-	+	+	+							
9	**750**	+	+	+	-	-							
10	**620**	+	+	+	-	-							
11	**550**	+	+	-	-	-							
12	**500**	+	+	+	+	+							
13	**460**	-	-	+	-	-							
14	**420**	-	+	-	-	+							
15	**370**	+	+	-	-	-							
16	**350**	+	+	-	-	-							
17	**250**	-	+	-	-	-							

An average of 11.63 bands per primer was observed in a total of 93 bands. Size of bands ranged from 190 bp (OPA-18) to 2500 bp (OPA-17). From RAPD data

65.59% of common bands and 34.41% of polymorphic bands were observed among *Scarus* species (Table3). The primer OPO-18 gave rise to maximum bands (19) and OPA-19 showed the least number of bands (4). Cluster analysis was carried out depending on the results of RAPD analysis using Gene profiler computer software program to find the diversity among the given species of *Scarus* as shown in the dendrogram Fig. (14). The following are the amplification results of the five species obtained by the examined primers:

Table 3: Number of amplified and polymorphic DNA-fragments in the five Scaridae species.

No.	Primer code	No. of amplified bands					Total amplified bands	No. of polymorphic bands (Unique bands)	Polymorphism %
		(1) *Scarus psittacus*	(2) *Scarus frenatus*	(3) *Scarus genazonatus*	(4) *Scarus fuscopurpureus*	(5) *Scarus niger*			
1	OPA-12	5	2	3	4	4	8	3	37.50
2	OPA-14	8	5	9	7	3	13	3	23.08
3	OPA-15	5	5	6	0	4	9	4	44.44
4	OPA-17	10	5	4	3	6	12	6	50.00
5	OPA-18	12	9	10	9	10	19	4	21.05
6	OPA-19	2	2	1	1	2	4	3	75.00
7	OPA-20	6	5	4	4	9	11	6	54.54
8	OPO-11	13	13	8	6	7	17	3	17.65
total		61	46	45	34	45	93	32	34.41

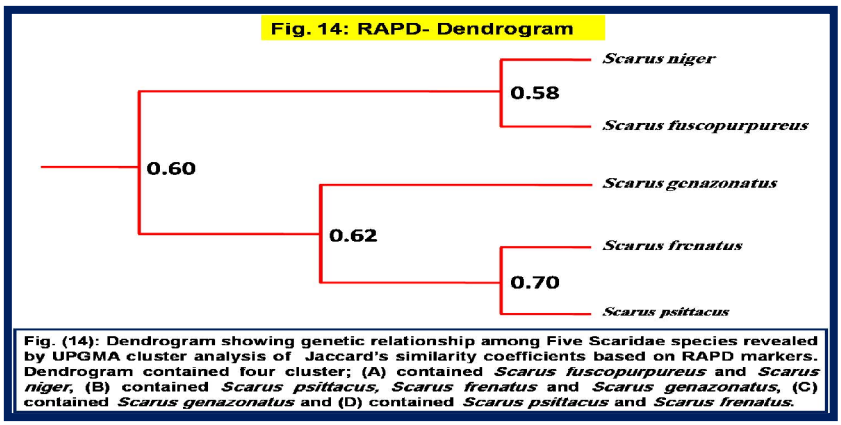

Fig. (14): Dendrogram showing genetic relationship among Five Scaridae species revealed by UPGMA cluster analysis of Jaccard's similarity coefficients based on RAPD markers. Dendrogram contained four cluster; (A) contained *Scarus fuscopurpureus* and *Scarus niger*, (B) contained *Scarus psittacus*, *Scarus frenatus* and *Scarus genazonatus*, (C) contained *Scarus genazonatus* and (D) contained *Scarus psittacus* and *Scarus frenatus*.

***Scarus psittacus*:** The RAPD-DNA analysis of S. *psittacus* produced 61 fragments with all eight primers, varied in number from 2 by the primer OPA-19 to 13 by the primer OPO-11, the size of these fragments ranged from 250 bp by the primer OPA-18 to 2500 bp by the primer OPA-17.

***Scarus frenatus*:** This fish produced different RAPD band patterns of number of 46 bands ranged from 250 bp by the primer OPO-11, OPA-18 to 2000 bp by the primer OPA-18. The generated bands ranged in number from 2 by the primers OPA-12 and OPA-19 to 13 by the primer OPO-11.

***Scarus genazonatus*:** The eight primers produced 45 bands in *S. genazonatus*. The number of bands amplified per primer varied from 1 band by the primer OPA-19 to 10 bands by the primer OPA-18 and had a size ranged from 250 bp by the primers OPA-14 and OPA-18 to 1700 bp by the primer OPA-15.

***Scarus fuscopurpureus*:** All the primers amplified successfully yielded distinct RAPD patterns with *S. fuscopurpureus* except OPA-15, the seven primers generated 34 fragments, the number of fragments varied from 1 by the primer OPA-19 to 9 by the primer OPA-18. The size of fragments ranged from 190 bp by the primer OPA-18 to 1500 bp by the primer OPA-17 and OPA-20.

***Scarus niger*:** All primers amplified yielded distinct RAPD patterns of 45 bands. The number of fragments varied from 2 by primer OPA-19 to 10 by the primer OPA-18 and the size of these fragments ranged from 190 bp by the primer OPA-18 to 2000 bp by the primer OPA-20.

Genetic distance was lowest between *S. psittacus* and *S. frenatus,* while was highest between *S. psittacus* and *S. niger*. Genetic Similarity index was from 51% to 70%. The phylogenetic tree constructed from genetic distances (Table 4) showed that the dendrogram is divided into four clusters groups Figs. (14 & 15).

Table 4: Similarity Matrix UPGMA Jaccard`s Coefficient.

	(1) *Scarus psittacus*	(2) *Scarus frenatus*	(3) *Scarus genazonatus*	(4) *Scarus fuscopurpureus*	(5) *Scarus niger*
1	**100**				
2	**70**	**100**			
3	**67**	**57**	**100**		
4	**55**	**55**	**51**	**100**	
5	**51**	**57**	**51**	**58**	**100**

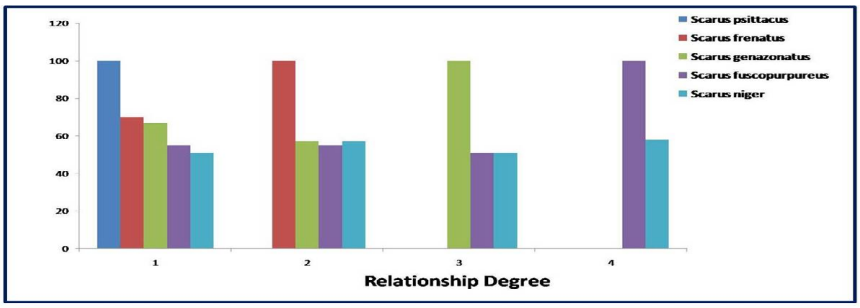

Fig. (15): Demonstration of the relationship degree among *Scarus psittacus, S. frenatus, S. genazonatus, S. fuscopurpureus and S. niger,* based on data recorded from polymorphism of RAPD markers.

DISCUSSION

Random primer seems to be a good candidate for developing markers. In this study, RAPD-PCR technique was used for identification of DNA polymorphism, genotypes and genetic diversity of DNA between studied varieties to determine DNA markers among species. The present study documents the genetic variability within and between the species of scaridae fishes. RAPD markers showed a high level of polymorphism and a high number of clearly amplified bands. The low level of genetic variation between *S. psittacus* and *S. frenatus* and the high level of genetic variation was found between *S. psittacus* and *S. niger*. The genetic distance is more between genus than between species and the hypothesis is also proved in this study by this marker. This was also proved in earlier studies in Indian major carps (Barman *et al.,* 2003). Dendrogram was constructed from similarity matrix values using UPGMA algorithm. The dendrogram obtained from the RAPD data clearly depicts the relationships among *S. psittacus, S. frenatus, S. genazonatus, S. fuscopurpureus and S. niger*. The phylogenetic relationships among the five studied Scaridae species were clearly resolved in dendrogram, they were clustered into four clusters groups: the first one comprised, *S. fuscopurpureus and S. niger,* the second included *S. psittacus, S. frenatus* and *S. genazonatus,* while the third includes *Scarus genazonatus* and the fourth branch contains *S. psittacus* and *S. frenatus*. The present study is the first report on the use of RAPD markers for studying genetic variation in Scaridae fishes in Egypt.

Although a small number of primers was enough to get molecular characteristic indicators of each of the five species, a large number of prefixes was necessary to study the relationships between different species, because it gave a powerful tool to discriminate the species based on their genetic component. This allows obtaining more accurate results about the genetic similarities and differences among the studied species. The RAPD method was employed in the past successfully for the detection of genetic relationship among individuals (Goswami and Tripathi, 2010).

Optimization of PCR conditions is necessary to get the highest specificity and product yield (Williams *et al.,* 1990; McPherson and Moller, 2001; Bakr, 2006; AlHashimi, 2008). Therefore, optimization of PCR conditions, that include reagents, temperature, number of cycles and other parameters are very necessary to get a successful RAPD-PCR reaction. Some primers can bind with DNA since they have the same complementary sequence for template DNA while others didn't bind with DNA because they generate poor amplification products and/or non-repeatable banding patterns (Abdellaoui *et al*., 2010). So we test about a hundred oligonucleotide primers, but we choose only 8, for the reasons above. The principles of RAPD-PCR depend on existence or nonexistence of bands, the number of bands and the molecular weight of resulted bands (Mayer *et al.,* 2000). The absence or presence of main band from any variety is considered a marker for that variety. The other principle of RAPD markers is the band numbers. That illustrated the number of sites which that a primer detects and bind with them. The number of these sites affected by two factors, genome size and primer sequences, (Al-Asei, 2002).

Polymorphism detected in this study among Scaridae species could be used as a DNA marker to differentiate between them. In addition, some primers produced monomorphic bands, while other primers produced polymorphic ones that provided chances to find specific unique bands for species. The variation in the number of bands amplified by different primers is influenced by variable factors such as primer structure and less number of annealing sites in the genome (Kernodle *et al*., 1993). RAPDs reveal similar patterns of genetic diversity when compared with other marker types and can be performed more rapidly than most other methods (Morell *et al*., 1995) and can provide vital information for the development of genetic sampling, conservation and improvement strategies (Chalmers *et al*., 1992). Guo *et al*., (2001) tested the applicability of RAPD analysis for identification of three marine fish cell lines FG, SPH, and RSBF, and as a possible tool to detect cross-contamination. RAPD primers were useful for identification of these cell lines and for characterization of the genetic variation of these cell lines in relationship to the species of their origin.

Investigation of genetic variations for *Poecilia latipinna* and *Poecilia sphenops* from different locations of Chennai, Tamil Nadu was performed using RAPD assay. High degree of polymorphism was observed, suggesting the degree of genetic variability between *P. latipinna* and *P. sphenops*. Shanmughavalli, *et al*., (2013). RAPD technique has been useful for detection of genetic variation in various fish species. It has been used for phylogenetic studies for species and subspecies identification in fish. (Welsh and McClelland, 1990; Williams *et al*., 1990; Wilkerson *et al*., 1995; Nadig *et al*., 1998; Mollah *et al*., 2005 and 2009).

Molecular patterns showed by RAPD bands represent specific DNA fingerprints and apparently are suitable tool to distinguish among *Scarus* species. The results clearly revealed that the five *Scarus* species are distinctive species. The present investigation involves the evaluation of the genetic diversity and the molecular phylogeny within and between the five species of Scaridae, based on RAPD technique

which in turn will be useful to provide valuable information for breeding, conservation, systematic, and ecological and evolutionary studies in *Scarus* species.

CONCLUSION

RAPD analysis is a rapid and convenient technique for estimating genetic variation and taxonomic relationship between *Scarus psittacus, S. frenatus, S. genazonatus, S. fuscopurpureus and S. niger,* and to generate useful genetic markers in scaridae fishes. RAPD fragments observed in the five species, showed a reasonable degree of genetic variation within and between the species. The population specific bands could not be discerned from the fragment patterns generated. This observation clearly indicated both the population's genetic similarity index and genetic distance within the species. RAPD analysis for diversity can provide practical information for the management of genetic resources in Scaridae fishes. This research is a first study reporting the RAPD analysis of five Scaridae species in Egypt. Further studies with other molecular methodologies are essential to clarify and confirm genetic relationships among fish species depicted using RAPD.

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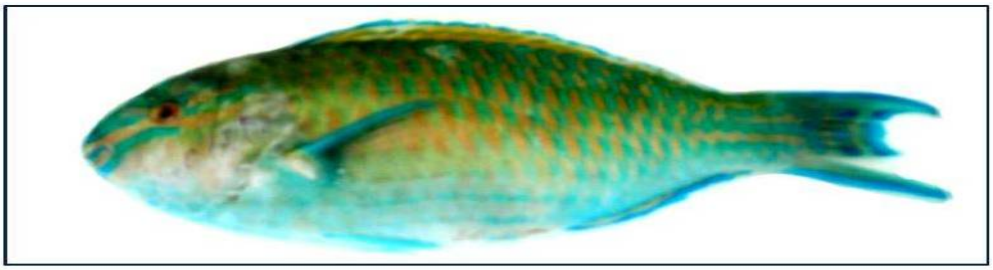

Fig.(1): A coloured photograph of *Scarus psittacus.*

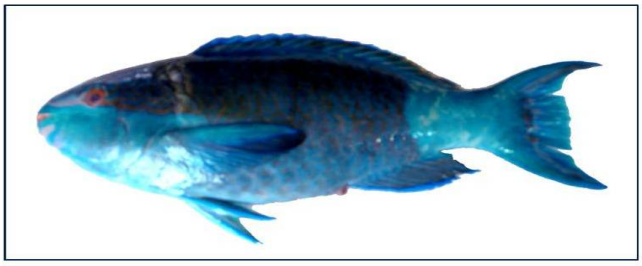

Fig.(2): A coloured photograph of *Scarus frenatus.*

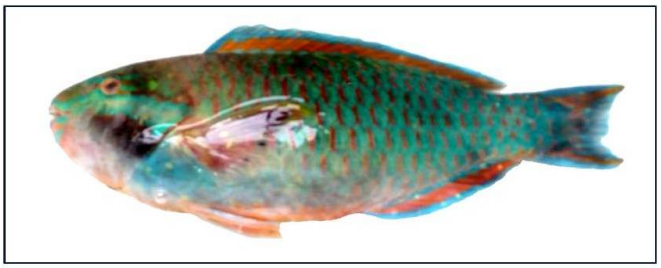

Fig.(3): A coloured photograph of *Scarus genazonatus.*

Fig.(4): A coloured photograph of *Scarus fuscopurpureus.*

Fig.(5): A coloured photograph of *Scarus niger.*

ARABIC SUMMARY

العلاقه الوراثيه بين خمسه انواع من الاسماك من جنس الحريد باستخدام تقنيه بلمره الدنا المتضخم العشوائي

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تناول البحث التحليل الوراثى باستخدام بلمره الدنا المتضخم العشوائى (RAPD) لخمس انواع من الاسماك البحريه تم تجميعها من البحر الاحمر من جنس الحريد وهي: الحريد الشائع *Scarus psittacus* ، حريد برايد ليد *Scarus frenatus* ، حريد مخطط أورجوان *Scarus genazonatus* ، حريد بنى اورجوانى *Scarus fuscopurpureus* و حريد داسكى *Scarus niger*. وقد اظهرت الدراسه النتائج التاليه:-

١- الحريد الشائع: نتج عن البادئات الثمانية المستخدمه طرز حزمية مميزه لكل بادئ حيث اظهر هذا النوع عدد ٦١ حزمة مع كل البادئات، تراوحت أعداد هذه الحزم من اثنين الى ١٣ حزمة وكانت احجامها ما بين ٢٥٠ الى٢٥٠٠ زوج من القواعد .

٢- الحريد برايد ليد: بلغ العدد الكلى للحزم المتضاعفه في هذا النوع والناتجه من استخدام البادئات الثمانية ٤٦ حزمة حيث كان أقلها حزمتين واقصاها ١٣ حزمة و تراوحت أحجام الحزم من ٢٥٠ الى٢٠٠٠ زوج من القواعد.

٣- الحريد مخطط اورجوان: تم الحصول على عدد ٤٥ حزمة باستخدام البادئات الثمانية، و تراوح عددها من حزمة واحدة الى ١٠ حزم . وكانت احجام هذه الحزم ما بين ٢٥٠ الى ١٧٠٠ زوج من القواعد.

٤- الحريد بنى اورجوانى: كما هو في الانواع الاخرى تم الحصول على طرز حزميه مميزه لكل بادئ، وبلغ العدد الكلى للحزم في هذا النوع ٣٤ حزمة مع البادئات الثمانيه. تراوحت اعداد الحزم من حزمة واحدة مع الى ٩ حزم، وبلغت احجام هذه الحزم من ١٩٠ الى١٥٠٠ زوج من القواعد.

٤- الحريد داسكى: وصل عدد الحزم المتضاعفه في هذا النوع الى ٤٥ حزمه وذلك باستخدام البادئات الثمانيه وكان عدد الحزم ما بين اثنتين الى ١٠ حزم. و تفاوتت الاحجام من ١٩٠ الى ٢٠٠٠ زوج من القواعد

بلغ العدد الكلى لحزم الدنا المتضاعفه في الانواع الخمسه من الاسماك ٩٣ حزمه وذلك باستخدام البادئات الثمانيه كان من بينهم ٣٢ حزمه متباينه بين جميع الانواع محل الدراسه وذلك بنسبه ٣٤,٤١ % من عدد الحزم الكلى. تم دراسه التباين الوراثي بين الانواع الخمسه من جنس الحريد وتوضيح درجه القرابه الوراثيه بين هذه الانواع. كما تم رسم الشجره التطوريه لهذه الانواع، وتم توزيعها في اربع مجموعات تطوريه. واسفرت التحليلات المختلفه عن وجود درجات متفاوته من القرابه والاختلاف بين تلك الانواع. وكانت اعلى درجه قرابه بين الحريد الشائع والحريد "برايد ليد" حيث وصلت قيمتها ٧٠ %. وكانت اقل درجه قرابه على سبيل المثال بين الحريد الشائع والحريد داسكى٥١ %.

التحليلات الوراثيه الى ان كل نوع من الانواع الخمسه لاسماك الحريد محل الدراسه يعتبر نوعا مميزا ومختلفا عن باقى الانواع الاخرى. ويمثل هذا البحث اهميه كبيره في استخدام التحليل الوراثي في التصنيف ودراسه العلاقه بين الانواع المختلفه لجنس معين من الكائنات الحيه ومن بينها الاسماك، هذا الى جانب الصفات المورفولوجيه والتشريحيه.