RAPD –PCR analysis of four coral reef fish species, genus cephalopholis (Family: serranidae) in the Red sea.

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ABSTRACT

The genetic relationships between four species of groupers: *Cephalopholis oligosticta, C. sexmaculata, C. hemistiktos* and *C. argus*, common coral reef fish in the Red Sea of family Serranidae were studied by using Random Amplified Polymorphic DNA (RAPD) technique. Twenty random primers were used. Each primer was individually tested with the four fish species. Amplification products were resolved on agarose gel electrophoresis and visualized under UV light, then photographed. The number and size of amplified DNA fragments showed a wide range of variation. Tabulation and analysis of the data using the genetic similarity coefficient were carried out. The highest genetic similarity observed was between *C. hemistiktos* and *C argus* and the lowest was between *C. oligosticta* and *C. sexmaculata*. Dendrogram analysis revealed three clusters: first, comprises *C. hemistiktos* and *C. argus*; second, contains *C. oligosticta* and (*C. hemistiktos* and *C. argus*); the third incompasses *C. sexmaculata* and (*C. oligosticta*, *C. hemistiktos* and *C. argus*).

Keywords: Cephalopholis, Serranidae, RAPD –PCR, Genetic Variation, Red sea

INTRODUCTION

Random amplified polymorphic DNA (RAPD) is a PCR- based molecular marker developed by Williams *et al.* (1990) which have been demonstrated to be useful not only for the study of population genetic variation but also for taxonomic identities, systematic relationships, parent- age identifications, identification of interspecific hybridization and introgressive hybridization (Imtiaz *et al.,* 2011).

RAPD technique has several advantages and has been quite widely employed in fisheries studies. The method is simple, rapid and cheap, it has high polymorphism. Only a small amount of DNA is required, no need for molecular hybridization and most importantly no prior knowledge of the genetic make-up of the organism in question is required. The technique is based on PCR amplification of discrete regions of genome with short oligonucleotide primers of arbitrary sequence Okumus and Çiftci (2003).

Among the several available molecular markers, RAPD (randomly amplified polymorphic DNA) stands for a simple and cost-efficient assay to evaluate genetic variability, regardless any previous genomic information about the selected organism (Dinesh *et al.,* 1993; Wasko *et al.,* 2004b; Affonso and Galetti, 2007). RAPD is a highly useful technique for phylogenetic analysis among related individuals, the RAPD-PCR analysis has proven to be helpful in taxonomic studies (Sleem and Ali, 2008).

Several authors (Welsh and McClelland, 1990; Baradakci and Skibinski, 1994; Naish *et al.,* 1995) have demonstrated that the RAPD PCR method is a powerful tool

for the assessment of genetic markers that are capable of discriminating between species or subspecies in a wide range of organisms, including fishes.

Several nuclear DNA techniques have been available in accessing genetic variability in fish species and have been employed in aquaculture studies. On the other hand, the use of RAPD assay for genetic analysis of fishes has been fairly limited in comparison to published reports on microorganism, plant and insect species (Park and Moran, 1994; Mjolnerod *et al.,* 1997; Norris *et al.,* 1999 and El-Alfy *et al.,* 2009).

Cephalopholis is the most common genus of Serranidae family found in the aquarium trade. Some species of the genus *Cephalopholis* feature have colorful bodies. Most *Cephalopholis* are Indo-Pacific (12 species), some Red Sea, western and eastern Atlantic, Oceania (mid- tropical Pacific) and one from the eastern Pacific. Mostly are found in shallows to a few hundred feets in areas with lots of hiding opportunities. The fishes in this genus are robust, somewhat elongate marine predators that specialize in ambush tactics when hunting. The genus comprises 22 species (Heemstra and Randall, 1993; Fenner, 1996 and FAO, 2001).

In the present study, we applied the RAPD technique in revealing the genetic variation in four species of the genus Cephalopholis.

MATERIAL AND METHODS

Samples of *Cephalopholis oligosticta, C. sexmaculata, C. hemistiktos and C. argus* were collected from Hurghada by fishermen of the National Institute of Oceanography and Fisheries (Red Sea Branch). Fishes were dissected immediately after capture; liver, kidney, gills and gonads were isolated, bulked in 95% ethanol and stored in a freezer until processed for RAPD technique.

Extraction and Purification of Genomic DNA:

DNA extraction using the Qiagen DNeasy (Qiagen Santa Clara, CA), that was performed following the manufacturer's instructions as follows:

1- A volume of 400µl of buffer AP1 and 4µl of RNase A stock solution (100mg / ml) were added to a maximum of 100 mg of ground animal tissue and vortexed vigorously.
2- The mixture was incubated for 10 min at 65ºC, and mixed about 2-3 times during incubation by inverting tube.
3- Buffer AP2 130µl was added to the lysate, mixed and incubated for 5 min on ice.
4- The lysate was applied to the QIAshredder mini spin column, placed in a 2ml collection tube and centrifuged for 2 min at 14000 rpm.
5- Flow-through fraction from step 4 was transferred to a new tube without disturbing the cell-debris pellet.
6- 1.5 volumes of Buffer AP3/E was added to the cleared lysate and mixed by pipetting.
7- A volume of 650µl of the mixture from step 6, including any precipitate which may have formed, were applied to the DNeasy mini spin column sitting in a 2 ml collection tube. Centrifuged for 1 min at 8000 rpm and flow-through was discard.
8- Step 7 was repeated with remaining sample, the flow-through and collection tube were discard.
9- DNeasy mini spin column was placed in a new 2 ml collection tube, 500µl buffer AW was added to the DNeasy mini spin column and centrifuged for 1 min at >8000 rpm the flow-through was discard and the collection tube was reused in step 10.

10- Buffer AW 500µl was added to the DNeasy mini spin column and centrifuged for 2 min at 14000 rpm to dry the membrane.
11- The DNeasy mini spin column was transferred to a 1.5 ml microcentrifuge tube and 100µl of buffer AE was pipeted directly onto the DNeasy membrane. The microcentrifuge was incubated for 5 min at room temperature (15-25 ºC) and then centrifuged for 1 min at >8000 rpm to elute.
12- Step 11was repeated once.

Estimation of DNA Concentration:

DNA concentration was determined by diluting the DNA 1:5 in dist. H_2O. 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.

RAPD-PCR protocol:

A set of twenty random 10-mer primers as shown in Table (1) were used in the detection of polymorphism. 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 1 or 0, respectively.

Table 1: Primers and primer sequences used for amplification and sequencing, where A: Adenine, T: Thymine, G: Guanine and C: Cytosine

No.	Primer code	Nucleotide sequence (5` to 3`)	Nucleotide length	GC%
1	OPA-04	AATCGGGCTG	10-mer	60
2	OPA-06	GGTCCCTGAC	10-mer	70
3	OPA-11	CAATCGCCGT	10-mer	60
4	OPA-17	GACCGCTTGT	10-mer	60
5	OPB-14	TCCGCTCTGG	10-mer	70
6	OPD-01	ACCGCGAAGG	10-mer	70
7	OPD-14	CTTCCCCAAG	10-mer	60
8	OPE-05	TCAGGGAGGT	10-mer	60
9	OPG-03	GAGCCCTCCA	10-mer	70
10	OPG-07	GAACCTGCGG	10-mer	70
11	OPG-13	CTCTCCGCCA	10-mer	70
12	OPG-19	GTCAGGGCAA	10-mer	60
13	OPH-13	GACGCCACAC	10-mer	70
14	OPM-11	GTCCACTGTG	10-mer	60
15	OPM-12	GGGACGTTGG	10-mer	70
16	OPO-09	TCCCACGCAA	10-mer	60
17	OPZ-01	TCTGTGCCAC	10-mer	60
18	OPZ-04	AGGCTGTGCT	10-mer	60
19	OPZ-13	GACTAAGCCC	10-mer	60
20	OPZ-19	GTGCGAGCAA	10-mer	60

Data Analysis:

The banding patterns generated by RAPD-PCR marker analyses were compared to determine the genetic relatedness of the four sample fish accessions. Clear and distinct amplification products were scored as '1' for presence and '0' for absence of bands. Bands of the same mobility were scored as identical.

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

$$\mathbf{GS_{ij} = 2a/(2a+b+c)}$$

Where $\mathbf{GS_{ij}}$ 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) (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

Twenty single 10-mer primers as shown in Table (1) were used in the present study to determine the genetic differences among four species of genus Cephalopholis (*C. oligosticta, C. sexmaculata, C. hemistiktos and C. argus*). The G+C contents of the twenty primers were between 60% &70%. All primers were successfully amplified on the genomic DNA extracted from all studied fish species.

The results of RAPD analysis obtained by primer OPA-04 reacted with genomic DNA of the samples (*Cephalopholis oligosticta, C. sexmaculata, C. hemistiktos* and *C. argus*) and gave 12 bands (fragments) ranged in size between 160 to 680 as shown in Table (2) and Fig. (1) while it (OPA-04) generated 16 fragments in *Oreochromis niloticus*, *O. aureus* and *Tilapia zillii* from family *Cichilidae* (El-Alfy *et al.*, 2009), primer OPA-06 generated 10 DNA bands from 110 to 520 bp (Table 3 & Fig 2), OPA-11 12 fragments between 120 and 720 bp. (Table 4 & Fig 3), OPA-17 14 DNA bands from 150 to 500 bp (Table 5 & Fig. 4) and primer OPB-14 generated 9 fragments ranged in size between 200 and 750 bp (Table 6 & Fig. 5).

Table 2: Survey of RAPD markers using Primer (OPA-04) in bulked samples of, *Cephalopholis speciemens*. Fig (30): Agarose-gel electrophoresis of RAPD products generated with Primer OPO-09 with the four species

Band No.	RAPD Marker bp	1	2	3	4
1	160	1	0	1	1
2	180	0	1	1	1
3	210	1	1	1	0
4	220	0	0	1	1
5	230	0	0	0	1
6	250	1	0	1	1
7	280	1	1	1	1
8	300	0	0	1	1
9	350	0	1	0	0
10	510	0	1	1	1
11	620	0	0	1	1
12	680	0	0	1	0

Table 3: Survey of RAPD markers using Primer (OPA-06) in bulked samples of, *Cephalopholis speciemens*.

Band No.	RAPD Marker bp	1	2	3	4
1	110	1	0	1	1
2	130	0	1	0	0
3	150	1	1	1	0
4	170	1	0	1	1
5	190	1	0	1	1
6	210	1	1	1	1
7	230	1	0	1	1
8	270	1	0	0	0
9	310	1	0	1	0
10	520	0	1	0	0

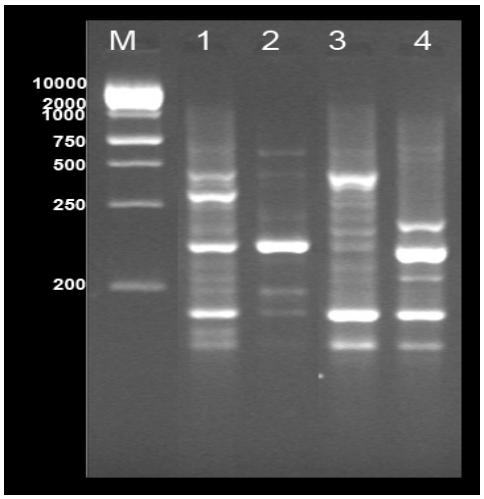

Fig. 1: Agarose-gel electrophoresis of RAPD products generated with Primer OPA-04 with the four species.
Where 1-*Cephalopholis oligosticta*, 2-*Cephalopholis sexmaculata*, 3-*Cephalopholis hemistiktos* and 4-*Cephalopholis argus*.

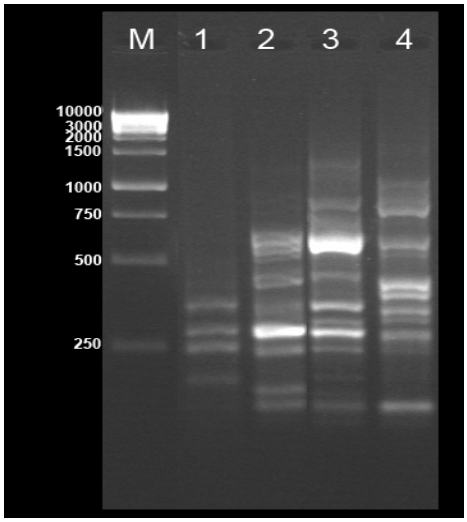

Fig. 2: Agarose-gel electrophoresis of RAPD products generated with Primer OPA-06 with the four species.

Table 4: Survey of RAPD markers using Primer (OPA-11) in bulked samples of, *Cephalopholis speciemens*.

Band No.	RAPD Marker bp	1	2	3	4
1	**120**	0	1	1	1
2	**150**	1	1	1	1
3	**170**	1	1	1	1
4	**180**	0	0	1	1
5	**200**	1	1	1	1
6	**210**	1	1	1	1
7	**220**	0	0	0	1
8	**240**	0	1	1	1
9	**270**	0	1	0	1
10	**310**	0	1	1	1
11	**520**	0	0	1	1
12	**720**	0	0	1	0

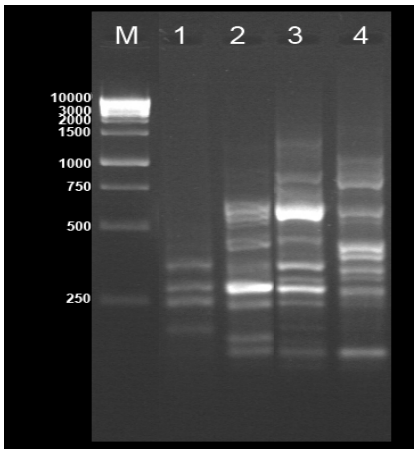

Fig. 3: Agarose-gel electrophoresis of RAPD products generated with Primer OPA-11 with the four species.

Table 5: Survey of RAPD markers using Primer (OPA-17) in bulked samples of the four species.

Band No.	RAPD Marker bp	1	2	3	4
1	**150**	1	0	0	0
2	**160**	1	1	1	1
3	**180**	1	1	1	1
4	**190**	1	0	1	1
5	**200**	1	1	1	1
6	**210**	1	1	1	1
7	**220**	1	1	0	0
8	**230**	0	1	1	1
9	**240**	0	0	1	1
10	**250**	1	0	0	0
11	**270**	1	1	1	1
12	**300**	1	0	0	1
13	**420**	1	1	0	1
14	**500**	1	1	1	1

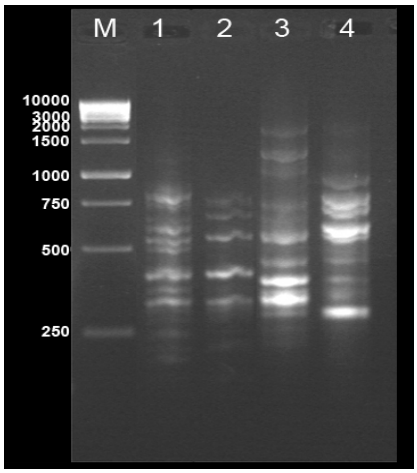

Fig. 4: Agarose-gel electrophoresis of RAPD products generated with Primer OPA-17 with the four species.

Table 6: Survey of RAPD markers using Primer (OPB-14) in bulked samples of the four species.

Band No.	RAPD Marker bp	1	2	3	4
1	**200**	0	1	1	1
2	**250**	0	1	1	1
3	**260**	0	0	0	1
4	**280**	0	1	0	1
5	**350**	0	1	0	1
6	**450**	0	1	0	0
7	**580**	1	1	1	1
8	**650**	1	0	0	1
9	**750**	0	1	0	0

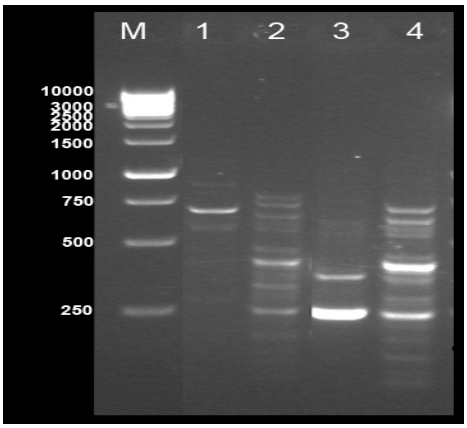

Fig. 5: Agarose-gel electrophoresis of RAPD products generated with Primer OPB-14 with the four species.

The primer OPD-01 reacted with genomic DNA of the four species generated 10 fragments ranged in size between 230 and 800 bp (Table 7 & Fig. 6), while it generated 17 bands all polymorphic bands and fragments size range 1356-135bp in *Plectropomus maculates, Plectropomus leopardus* and *Plectropomus areolatus* from family *Serranidae* (Saad *et al.,* 2012) , primer OPD-14 generated 9 DNA bands between 270 and 900 bp (Table 8 & Fig. 7) (89 %) of these fragments (8 fragments) were polymorphic between the four species, primer OPE-05 gave 6 bands between 300 to 1000 bp (Table 9 & Fig. 8), OPG-03 generated 8 DNA bands from 150 to 750 bp. (Table 10 & Fig. 9) and primer OPG-07 generated 14 DNA bands ranged in size from 150 to 1200 bp (Table 11 & Fig. 10).

Table 7: Survey of RAPD markers using Primer (OPD- 01) in bulked samples of the four species.

Band No.	RAPD Marker bp	1	2	3	4
1	**230**	0	0	1	1
2	**270**	1	0	0	1
3	**320**	1	0	0	1
4	**370**	1	0	0	0
5	**460**	1	0	0	0
6	**530**	0	0	0	1
7	**550**	1	1	0	1
8	**680**	1	0	0	1
9	**700**	1	0	0	0
10	**800**	1	0	0	0

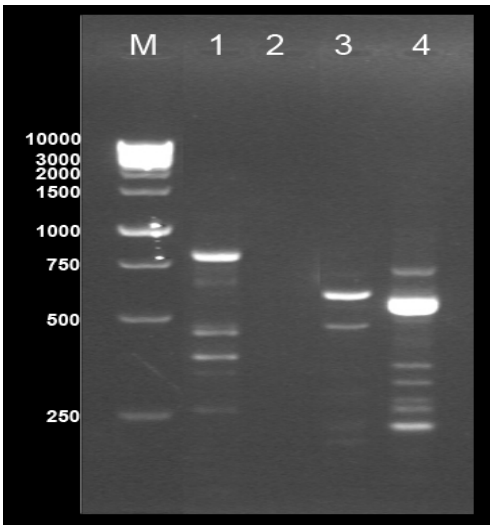

Fig. 6: Agarose-gel electrophoresis of RAPD products generated with Primer OPD-01 with the four species.

Table 8: Survey of RAPD markers using Primer (OPD- 14) in bulked samples of the four species.

Band No.	RAPD Marker bp	1	2	3	4
1	**270**	1	0	0	1
2	**300**	1	0	1	1
3	**400**	1	1	1	1
4	**500**	1	0	0	1
5	**560**	1	0	1	1
6	**650**	1	0	0	1
7	**750**	0	0	1	0
8	**850**	1	0	1	1
9	**900**	1	0	0	1

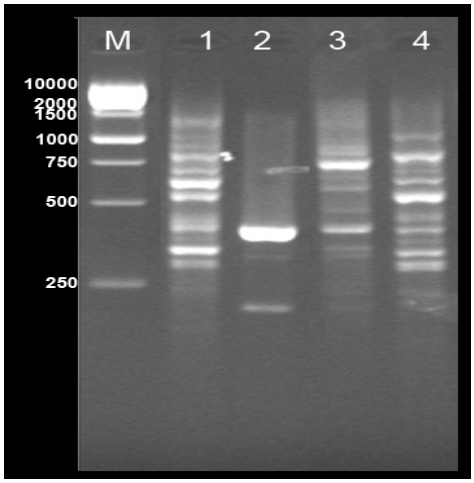

Fig. 7: Agarose-gel electrophoresis of RAPD products generated with Primer OPD-14 with the four species.

Table 9: Survey of RAPD markers using Primer (OPE-05) in bulked samples of the four species.

Band No.	RAPD Marker bp	1	2	3	4
1	300	1	0	1	0
2	450	1	1	0	1
3	500	1	1	0	0
4	600	1	1	0	1
5	800	1	1	0	1
6	1000	0	0	0	1

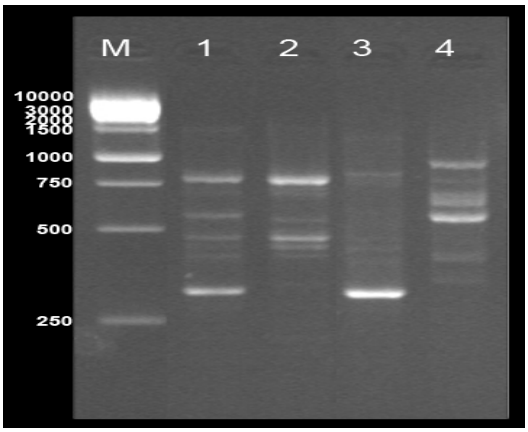

Fig. 8: Agarose-gel electrophoresis of RAPD products generated with Primer OPE-05 with the four species.

Table 10: Survey of RAPD markers using Primer (OPG- 03) in bulked samples of the four species.

Band No.	RAPD Marker bp	1	2	3	4
1	150	0	0	0	1
2	200	1	1	1	0
3	300	1	0	1	1
4	350	1	0	1	1
5	400	1	1	1	1
6	600	1	0	1	1
7	700	1	1	0	1
8	750	0	0	0	1

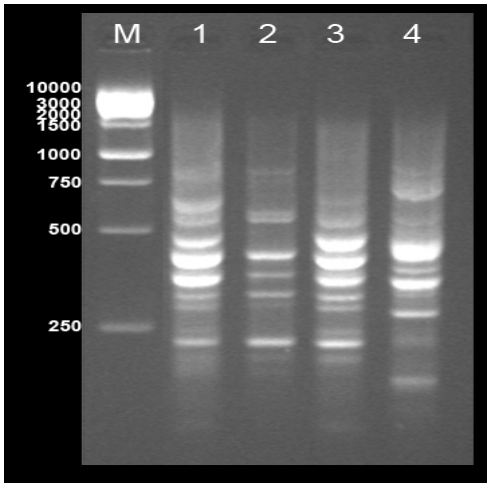

Fig. 9: Agarose-gel electrophoresis of RAPD products generated with Primer OPG-03 with the four species.

Table 11: Survey of RAPD markers using Primer (OPG- 07) in bulked samples of the four species.

Band No.	RAPD Marker bp	1	2	3	4
1	150	0	1	0	0
2	200	1	0	1	1
3	320	0	1	1	1
4	350	0	1	1	1
5	380	0	0	1	0
6	390	0	1	1	1
7	450	1	0	1	1
8	480	1	0	1	0
9	500	0	1	0	1
10	550	0	1	0	1
11	750	0	1	1	1
12	800	0	1	1	1
13	1000	1	1	1	1
14	1200	0	1	0	0

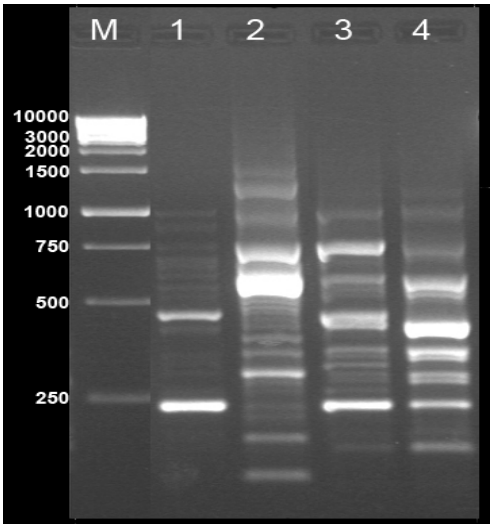

Fig. 10: Agarose-gel electrophoresis of RAPD products generated with Primer OPG-07 with the four species.

Primer OPG-13 generated 7 DNA fragments ranged in size between 200 and 650 bp (Table 12 & Fig. 11) (71 %) of these fragments (5 fragments) were polymorphic between the four species of *Cephalopholis*, primer OPG-19 generated 7 fragments between 150 and 850 bp (Table 13 & Fig. 12), OPH-13 gave 9 fragments from150 to 680 bp (Table 14 & Fig. 13), (same primer OPH-13 gave 48 bands, 9 of them polymorphic in *Tenualosa ilisha* Ham of the *Clupeidae* family (Shifat *et al.*, 2003), OPM-11 gave 13 fragments in the four species of *Cephalopholis* ranged in size between 120 and 580 bp (Table 15 & Fig. 14) and primer OPM-12 generated 10 fragments from150 to 600 bp (Table 16 & Fig. 15).

Table 12: Survey of RAPD markers using Primer (OPG-13) in bulked samples of the four species.

Band No.	RAPD Marker bp	1	2	3	4
1	**200**	1	1	0	1
2	**250**	1	0	1	1
3	**320**	1	1	1	1
4	**380**	1	1	1	1
5	**400**	1	1	1	0
6	**500**	1	0	0	0
7	**650**	1	1	1	0

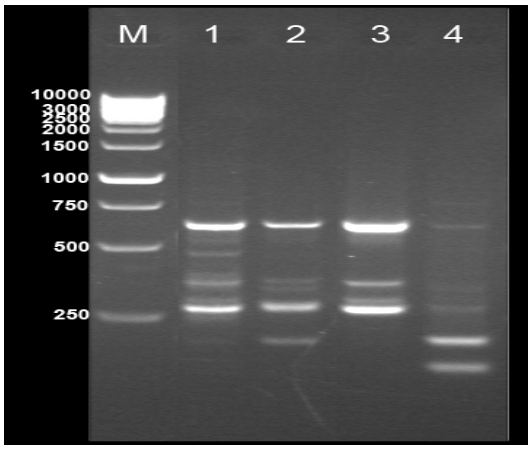

Fig. 11: Agarose-gel electrophoresis of RAPD products generated with Primer OPG-13 with the four species.

Table 13: Survey of RAPD markers using Primer (OPG- 19) in bulked samples of the four species.

Band No.	RAPD Marker bp	1	2	3	4
1	**150**	0	1	1	1
2	**250**	1	0	1	1
3	**300**	1	0	1	0
4	**350**	1	1	1	1
5	**450**	1	0	0	1
6	**500**	1	0	0	1
7	**850**	1	0	0	1

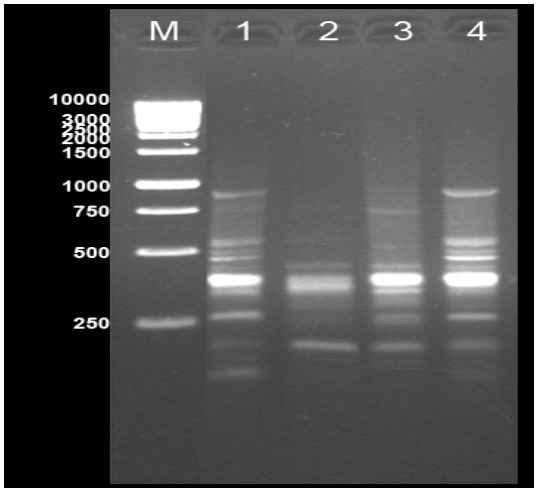

Fig. 12: Agarose-gel electrophoresis of RAPD products generated with Primer OPG-19 with the four species.

Table 14: Survey of RAPD markers using Primer (OPH- 13) in bulked samples of the four species.

Band No.	RAPD Marker bp	1	2	3	4
1	**150**	1	1	0	0
2	**200**	1	0	0	0
3	**220**	1	1	1	1
4	**250**	0	1	0	0
5	**300**	0	1	1	1
6	**400**	0	0	1	1
7	**420**	1	1	0	0
8	**500**	1	0	0	1
9	**680**	0	0	1	1

Table 13: Survey of RAPD markers using Primer (OPG- 19) in bulked samples of the four species.

Band No.	RAPD Marker bp	1	2	3	4
1	**120**	1	0	0	0
2	**150**	1	1	0	1
3	**200**	0	0	0	1
4	**220**	1	1	1	1
5	**250**	1	1	1	1
6	**300**	1	1	1	0
7	**350**	1	0	0	0
8	**370**	1	1	1	0
9	**400**	1	1	0	1
10	**450**	0	0	1	0
11	**480**	1	1	1	1
12	**500**	0	0	0	1
13	**580**	0	0	1	0

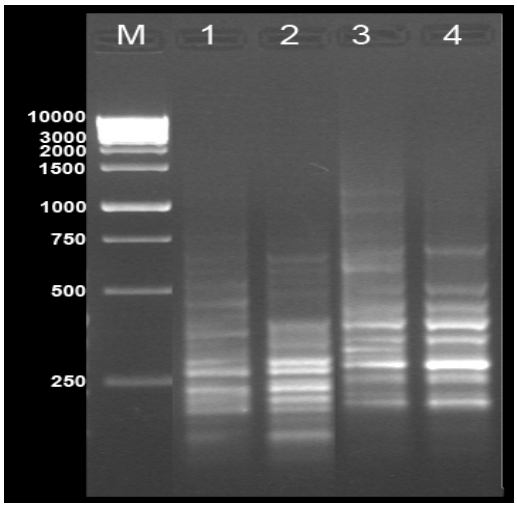

Fig. 13: Agarose-gel electrophoresis of RAPD products generated with Primer OPH-13 with the four species.

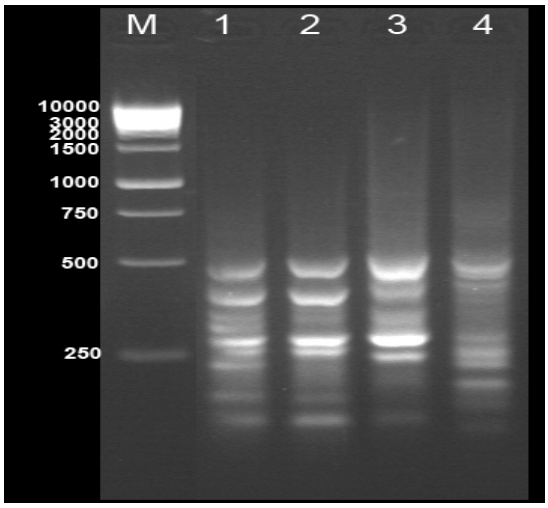

Fig. 14: Agarose-gel electrophoresis of RAPD products generated with Primer OPM-11 with the four species.

Table 16: Survey of RAPD markers using Primer (OPM-12) in bulked samples of the four species.

Band No.	RAPD Marker bp	1	2	3	4
1	150	0	1	1	0
2	170	1	0	0	1
3	200	1	1	1	1
4	250	0	0	1	0
5	300	1	1	1	1
6	350	1	0	0	1
7	400	1	0	1	1
8	500	1	0	1	1
9	550	1	0	1	1
10	600	1	0	0	1

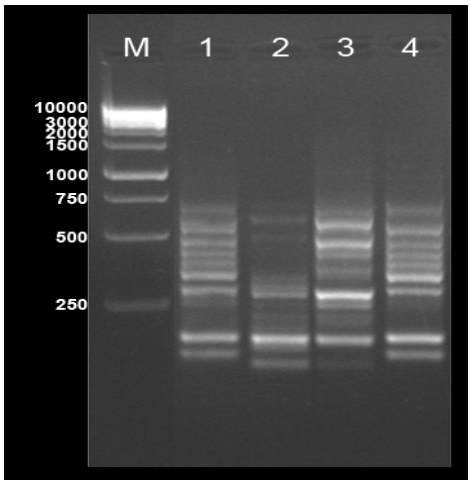

Fig. 15: Agarose-gel electrophoresis of RAPD products generated with Primer OPM-12 with the four species.

Primer OPO-09 generated 11 fragments ranged in size between 150 and 580 bp (Table 17 & Fig. 16), OPZ-01 9 bands from 150 to 500 bp (Table 18 & Fig. 17), OPZ-04 9 bands between 150 and 500 bp (Table 19 & Fig. 18), OPZ-13 12 bands from 200 to1000 bp (Table 20 & Fig. 19) and OPZ-19 gave 14 bands between 180 and 800 bp (Table 21 & Fig. 20).

Table 17: Survey of RAPD markers using Primer (OPO- 09) in bulked samples of the four species.

Band No.	RAPD Marker bp	1	2	3	4
1	150	1	0	1	0
2	170	1	0	1	1
3	240	1	1	1	1
4	250	1	1	0	1
5	300	1	1	1	1
6	350	0	1	1	1
7	370	1	0	1	0
8	420	1	1	1	1
9	440	1	1	1	1
10	480	0	0	0	1
11	580	0	1	0	0

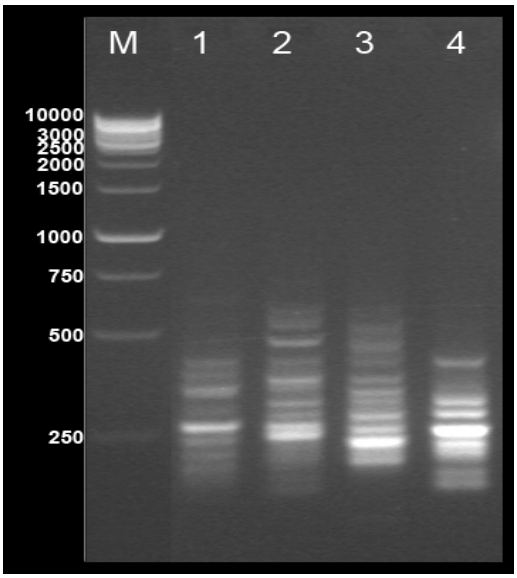

Fig. 16: Agarose-gel electrophoresis of RAPD products generated with Primer OPO-09 with the four species.

Table 18: Survey of RAPD markers using Primer (OPZ- 01) in bulked samples of the four species.

Band No.	RAPD Marker bp	1	2	3	4
1	**150**	1	0	1	0
2	**180**	1	1	1	1
3	**240**	1	1	1	1
4	**250**	0	0	0	1
5	**300**	1	1	0	1
6	**320**	1	1	1	0
7	**350**	1	1	1	1
8	**450**	1	1	1	0
9	**500**	0	0	0	1

Table 19: Survey of RAPD markers using Primer (OPZ- 04) in bulked samples of the four species.

Band No.	RAPD Marker bp	1	2	3	4
1	**150**	1	1	1	0
2	**180**	1	1	1	1
3	**200**	0	1	1	1
4	**230**	1	0	0	1
5	**250**	1	1	1	1
6	**320**	1	0	1	1
7	**350**	1	1	1	0
8	**400**	1	1	1	0
9	**500**	0	0	1	0

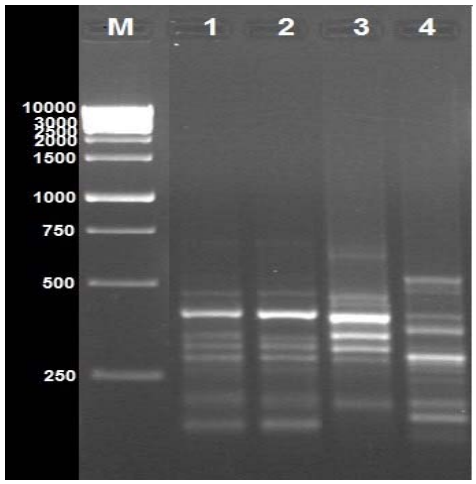

Fig. 17: Agarose-gel electrophoresis of RAPD products generated with Primer OPZ-01 with the four species.

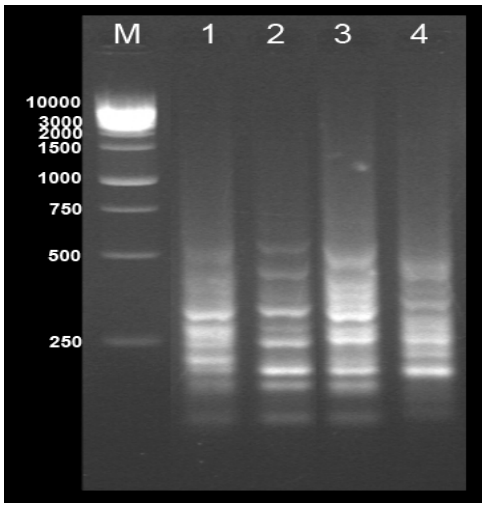

Fig. 18: Agarose-gel electrophoresis of RAPD products generated with Primer OPZ-04 with the four species.

Table 20: Survey of RAPD markers using Primer (OPZ-13) in bulked samples of the four species.

Band No.	RAPD Marker bp	1	2	3	4
1	200	1	1	1	1
2	220	0	1	1	0
3	280	1	1	0	0
4	300	1	0	0	1
5	340	1	0	1	0
6	400	1	1	1	1
7	420	1	1	0	1
8	460	1	1	0	0
9	500	0	1	0	1
10	680	1	0	1	0
11	750	1	0	1	0
12	1000	1	0	1	1

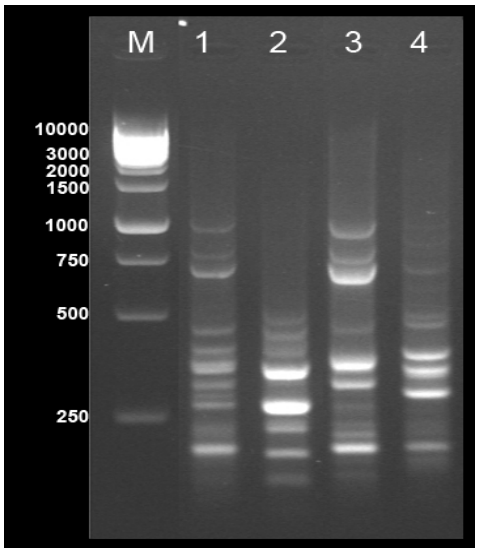

Fig. 19: Agarose-gel electrophoresis of RAPD products generated with Primer OPZ-13 with the four species.

Table 21: Survey of RAPD markers using Primer (OPZ-19) in bulked samples of the four species.

Band No.	RAPD Marker bp	1	2	3	4
1	180	0	0	0	1
2	200	1	1	1	0
3	250	1	1	0	1
4	280	1	1	0	1
5	320	1	1	0	1
6	380	1	1	1	1
7	420	1	1	1	0
8	460	1	1	0	0
9	480	1	1	1	0
10	500	1	1	1	1
11	580	1	1	1	1
12	600	0	0	1	1
13	750	0	0	1	0
14	800	0	1	0	0

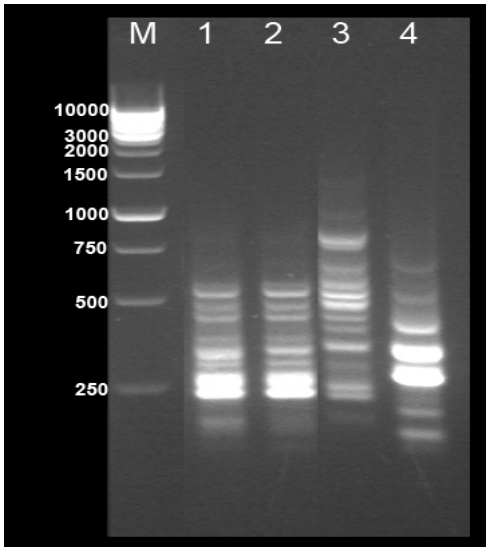

Fig. 20: Agarose-gel electrophoresis of RAPD products generated with Primer OPZ-19 with the four species.

The number of bands was variable in each species. *Cephalopholis argus* was the species that produced the greatest number of bands (141), and *Cephalopholis sexmaculata* was the lowest (111). In the other two species, *C. oligosticta* and *C. hemistiktos*, the total number of bands was 138 and 125 respectively. Generally, a total of 515 DNA bands were generated by all primers in all specimens, out of these DNA bands 39 (19.02%) were conserved among all specimens while 166 bands were polymorphic with percentage 81% of all the twenty tested primers as shown in Table (22).

Table 22: Number of amplified and polymorphic DNA – fragments in the four speciemens.

No.	Primer code	Total of amplified bands	No. of amplified bands				No. of common bands	No. of polymorphic bands
			1	**2**	**3**	**4**		
1	OPA-04	12	4	5	10	9	1	11
2	OPA-06	10	8	4	7	5	1	9
3	OPA-11	12	4	8	10	11	4	8
4	OPA-17	14	12	9	9	11	6	8
5	OPB-14	9	2	7	3	7	1	8
6	OPD-01	10	8	1	1	6	0	10
7	OPD-14	9	8	1	5	8	1	8
8	OPE-05	6	5	4	1	4	0	6
9	OPG-03	8	6	3	5	7	1	7
10	OPG-07	14	4	10	10	10	1	13
11	OPG-13	7	7	5	5	4	2	5
12	OPG-19	7	6	2	4	6	1	6
13	OPH-13	9	5	5	4	5	1	8
14	OPM-11	13	9	7	7	7	3	10
15	OPM-12	10	8	3	7	8	2	8
16	OPO-09	11	8	7	8	8	4	7
17	OPZ-01	9	7	6	6	6	3	6
18	OPZ-04	9	7	6	8	5	2	7
19	OPZ-13	12	10	7	7	6	2	10
20	OPZ-19	14	10	11	8	8	3	11
Total		205	138	111	125	141	39	166

Data of presence/absence of DNA fragments of the four species were used to calculate the genetic similarity. Based on the calculated genetic similarity presented in Table (23) and dendrogram Figure (21), an estimation of the genetic relationship between the above species was concluded where the highest genetic similarity value 67.1% was observed between *C. hemistiktos* and *C. argus*, while the lowest value 59.8% was found between *C. oligosticta* and *C. sexmaculata.* The UPGMA dendrogram shows three clusters; (A) contained *Cephalopholis hemistiktos* and *C. argus,* cluster (B) contained *C. oligosticta* and *(C. hemistiktos* and *C. argus)* and cluster (C) contained *C. sexmaculata* and *(C. oligosticta*, *C. hemistiktos* and *C. argus*).

Table 23: Similarity matrix UPGMA Jaccard's Coefficient

	Cephalopholis oligosticta	***Cephalopholis sexmaculata***	***Cephalopholis hemistiktos***	***Cephalopholis argus***
Cephalopholis oligosticta	**100**			
Cephalopholis sexmaculata	**59.8**	**100**		
Cephalopholis hemistiktos	**65**	**62.4**	**100**	
Cephalopholis argus	**66.7**	**62.2**	**67.1**	**100**

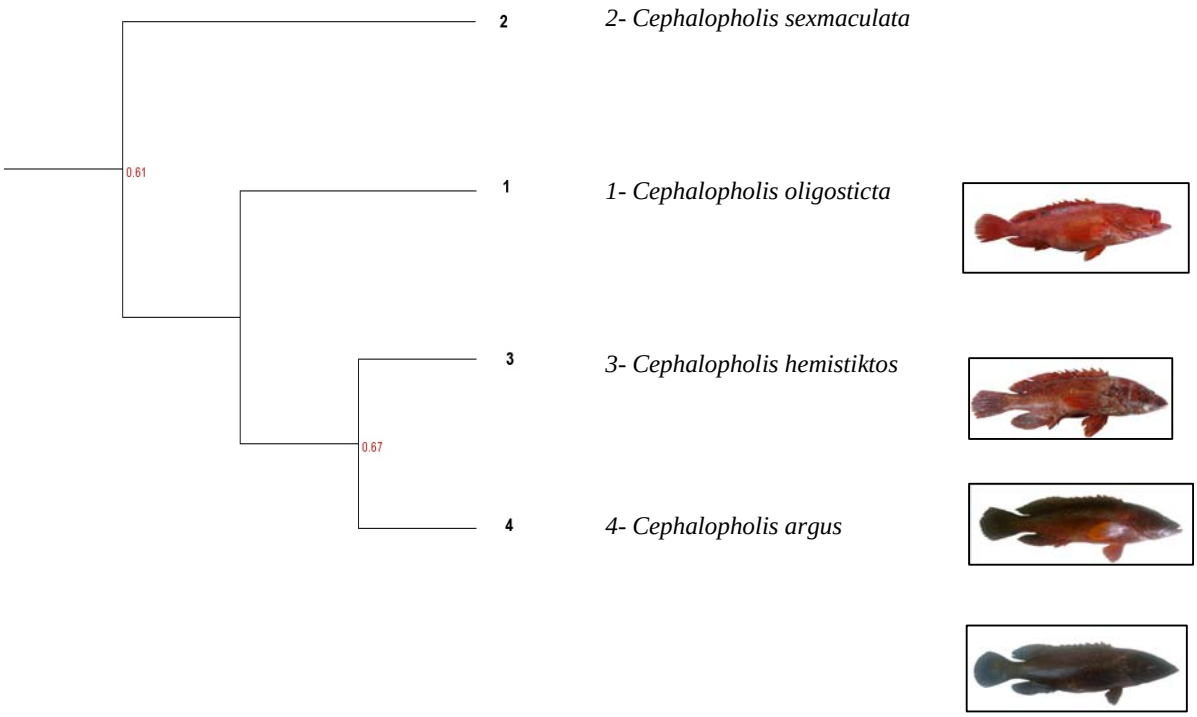

Fig. 21: Dendogram demonstrating the relationship between the four species, based on data recoreded from polymorphism of RAPD markers.

Based on the previous results, it was confirmed that RAPDs reveal similar patterns of genetic diversity when compared with other marker types and can be performed more rapidly than most other methods. Moreover, it can provide vital information for the development of genetic sampling, conservation and improvement strategies. RAPD method has been initially used to detect polymorphism in genetic mapping, taxonomy, phylogenetic studies (Chalmers *et al*., 1994; Morell *et al*., 1995) and later in genotoxicity and carcinogenesis studies (Atienzer and Jha, 2006).

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ARABIC SUMMARY

دراسات وراثية علي ٤ أنواع من اسماك الشعاب المرجانية من جنس السفالوفوليس: عائلة سيرانيدي بالبحر الأحمر

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تمت دراسة الفروق الوراثية بين ٤ انواع تنتمي الي جنس (Cephalopholis) وهم سيفالوفوليس اوليجوستيكتا *Cephalopholis oligosticta*،سيفالوفوليس سيكس ماكيولاتا *Cephalopholis sexmaculata*، سيفالوفوليس هيميستيكتوس *Cephalopholis hemistiktos* وسيفالوفوليس ارجوس *Cephalopholis argus* والتي تنتمي الي عائلة سيرانيدي Serranidae من اسماك الشعاب المرجانية بالبحر الاحمر من خلال :

أ- تحديد بعض مقاطع الحمض النووى الدى اوكسى ريبوز (د.ن.أ) باستخدام تفاعل سلسلة البلمرة العشوائى RAPD

ب- دراسة الفروق الجزيئية بين هذه الانواع من خلال استخدام عشرين بادئ بطول عشر نيكليوتيدات.

ت- توضيح العلاقة التقاربية بين هذه الانواع.

اثبتت النتائج في هذا البحث على جنس سيفالوفوليس *Cephalopholis* باستخدام تفاعل البلمرة العشوائي (RAPD) أن العلاقة التقاربية بين سمكتى كشر سيفالوفوليس هيميستيكتس ***Cephalopholis hemistiktos*** و كشر ارجوس***Cephalopholis argus*** كانت الاكبر بنسبة ٦٧،١٠% ، فى حين كانت الاصغر بين سمكتى كشر سيفالوفوليس اوليجوستيكتا ***Cephalopholis oligosticta*** وكشر سكس ماكيولاتا ***Cephalopholis sexmaculata*** بنسبة ٥٩،٨٠% ، و كانت بين كشر اوليجوستكتا ***Cephalopholis oligosticta*** ،كشر سكس ماكيولاتا ***Cephalopholis sexmaculata***، كشر هيميستكتوس ***Cephalopholis hemistiktos*** وكشر ارجوس ***Cephalopholis argus*** بنسب ٥٩،٨٠%، ٦٥% و٦٦،٧٠%على الترتيب ، و كانت بين كشر سكس ماكيولاتا***Cephalopholis sexmaculata***، كشر هيميستكتوس ***Cephalopholis hemistiktos*** وكشر ارجوس***Cephalopholis argus*** بنسب ٦٢،٤٠% و ٦٢،٢٠% ، بينما كانت بين كشر هيمي ستكتوس***Cephalopholis hemistiktos*** وكشر ارجوس ***Cephalopholis argus*** بنسبة ٦٧،١٠%.

ومن النتائج السابقة يتبين لنا أن استخدام التقنيات الحديثة كتفاعل سلسلة البلمرة العشوائى يساهم كثيراً في معرفة العلاقات الوراثية بين الكائنات الحية ومدي التقارب والتباعد فيما بينها.