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Molecular Genetic Variations and Phylogenetic Relationship Using Random Amplified Polymorphic DNA of three species of Catfishes (Family: Schilbidae) in Upper Egypt

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Abstract: *The RAPD-PCR analysis was carried out on three species of fresh water fishes of family Schilbidae (**Schilbe mystus, Schilbe uranoscopus** and **Siluranodon auritus**) by using twenty primers. All twenty primers amplified successfully on the genomic DNA extracted from all studied fish species. The number of bands was variable in each species. **Schilbe mystus** produced number of bands 174, and **Schilbe uranoscopus** 193, while in **Siluranodon auritus** 210 bands. A total of 295 DNA bands were generated by all primers in all specimen, out of these DNA bands 97 (32.88%) were conserved among all specimens, while 198 bands were polymorphic with percentage 67.12% of all the twenty tested primers produced polymorphism in all specimens table 22. These results are discussed in relation to implications of RAPD assays in the evaluation of genetic diversity.*
Key words: *Genetics – Molecular genetics - Schilbidae, random amplified polymorphic DNA, RAPD, fingerprinting, primer, genetic diversity and Upper Egypt.*

Introduction

The history of molecular genetics goes back to early 1950 when F. Crick, J. Watson and M. Wilkins established the currently accepted model of DNA structure (the double helix). This was a Nobel Prize winning discovery in Chemistry **Hallerman *et al*. (2003**). Since then details of structure and function of DNA and genes have been clarified and started to use in determining the genetic diversity **Okumus and Ciftci, (2003**).

The development of molecular technologies has provided researchers in many different fields with powerful new tools. Fishes are the most species group of vertebrates, with more than 24, 000 species. They are characterized by great diversity in ecology, morphology, life history, behavior and physiology. Fishes range in size from the very small to the very large **Le Comber and Smith, (2004**). The PCR technique is basically a primer extension reaction for amplifying specific nucleic acids in vitro. The use of a thermostable polymerase referred to as taq allows a short stretch of DNA to be amplified to about a million fold so that one can determine its size, nucleiotide sequence, etc. **Ali, (2003**). Polymerase chain reaction (PCR) technology has become a widespread research technique and has led to the development of several novel genetic assays based on selective amplification of DNA **Erlich, (1989**).

The RAPD method is a PCR-based technique that amplifies random DNA fragments with the use of single short primers of arbitrary nucleotide sequence under low annealing conditions, this technique has been extensively used for species classification and microorganism strain determination **Bielawski** and **Pumo, (1997); Bardakci, (2001**) and **Lee *et al*. (2007**).

Bardakci, (**2001**), reported that, perhaps the main reason for the success of RAPD analysis is the gain of a large number of genetic markers that require small amounts of DNA without the requirement for cloning, sequencing or any other form of the molecular characterization of the genome of the species in question. **Weising, (1995)**, reported that, RAPD markers can be sensitive to changes in reaction conditions, resulting in low reproducibility and inconsistencies in amplification efficiencies among samples. Using RAPD-PCR is very useful in determination genetic molecular variations and relationship degree between the species which belong to same family. Randomly amplified polymorphic DNA-PCR (RAPD-PCR) is a technique widely used for studying the DNA polymorphism between closely related species without the requirement of prior knowledge of the genome (**Welsh** and **McClelland, (1990)**; **Williams *et al*. (1990**) and **Zhivotovsky, (1999))**.

Comparatively few studies related strictly to the molecular differences between some species of fresh-water fishes and especially Random amplified polymorphic DNA (RAPD) analysis on three species of fresh-water fishes in Upper Egypt namely; ***Schilbe mystus, Schilbe uranoscopus*** and ***Siluranodon auritus*** **(Family: Schilbidae- Order: Siluriformes).**

The aim of the present study was to using Randomly Amplified Polymorphic DNA-PCR (RAPD - PCR) to study the molecular genetic differences between three species of family Schilbidae, and determination the relationship degree between these species.

Materials and Methods

In the present study, three species of fresh water fishes, (***Schilbe mystus, Schilbe uranoscopus*** and ***Siluranodon auritus***) figures (1, 2 and 3) family: Schilbidae, were collected from different localities of upper – Egypt (Qena and Aswan). The Identification and classification of species were carried out using descriptions and keys provided by **Bishai and khalil, (1991)** and **Mekkawy *et al*. (2011).**

Muscles tissue from fishes immediately after capture, were put on tubes and stored in a freezer until processed for RAPD-PCR analysis. Twenty primers (Operon) were used to amplify genomic DNA. Operon 10-mer kits contained 10 base oligonucleotide primers (Table 1) used in this study.

RAPD Reaction:

DNA extraction using the Qiagen DNeasy (Qiagen Santa Clara, CA). DNA concentration was determined by diluting the DNA 1:5 in H2O. 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.

A set of twenty primers RAPD (Tables 1) was used. 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 template DNA.

Table(1): The sequence of 20 primers using for RAPD analysis of family: Shilbidae
A: Adenine, T: Thymine, G: Guanine and C: Cytosine

No.	Name of primer	Nucleotide length	Sequence (5'-3')
1	OPA - 4	10-mer	AATCGGGCTG
2	OP A -7	10-mer	GAAACGGGTG
3	OPA -9	10-mer	GGGTAACGCC
4	OPA -15	10-mer	TTCCGAACCC
5	OPA -17	10-mer	GACCGCTTGT
6	OPB -6	10-mer	TGCTCTGCCC
7	OPB -17	10-mer	AGGGAACGAG
8	OPC -1	10-mer	TTCGAGCCAG
9	OPC -11	10-mer	AAAGCTGCGG
10	OPC -18	10-mer	TGAGTGGGTG
11	OPD-5	10-mer	TGAGCGGACA
12	OPE-5	10-mer	TCAGGGAGGT
13	OPE-6	10-mer	AAGACCCCTC
14	OPE-10	10-mer	CACCAGGTGA
15	OPF-6	10-mer	GGGAATTCGG
16	OPG-2	10-mer	GGCACTGAGG
17	OPM-2	10-mer	ACAACGCCTC
18	OPM-5	10-mer	GGGAACGTGT
19	OPM-17	10-mer	TCAGTCCGGG
20	OPZ-18	10-mer	AGGGTCTGTG

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 (0.5 ug/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.

Data Analysis

The banding patterns generated by RAPD-PCR marker analyses were compared to determine the genetic relatedness of the. 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).**

Dice formula: GSij = 2a/(2a+b+c)

Where **GSij** 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

Family schilbidae characterized by the anal fin base very long, not confluent with caudal, 24–90 rays; usually four pairs of barbels. The pelvic fin is occasionally absent in species of several genera. Members of this family tend to swim in open water **Nelson, (2006**). The objectives of this experiment were focused on detection of RAPD pattern for schlibe fishes and determination of the genetic variation among these fishes **Ambak *et al*. (2006**).

In the present work three species of family Schilbidae: ***Schilbe mystus, Schilbe uranoscopus*** and ***Siluranodon auritus***, adopted in controlled environment in Upper Egypt, were molecule-genetically studied, using by using Randomly Amplified Polymorphism DNA (RAPD-PCR) technique. The short ten nucleotide primers generated discrete DNA amplified fragments of varying lengths and revealed RAPD variation among the species.

Twenty primers (table 1) were used in the present investigation to determine the genetic variations among three species of family Schilbidae (***Schilbe mystus, Schilbe uranoscopus*** and ***Siluranodon auritus***). All twenty primers amplified successfully on the genomic DNA extracted from all studied fish species. The twenty primers yielded amplification products in the three species of the family Schilbidae. The number of fragments amplified per primer varied between 7 (OPE-10) and 21 (OPG-02) (14.57 bands/ primer) and had a size range from 200 bp (OPB - 17, OPC - 1, OPE – 6, OPM – 17 - OPA – 4 and OPM - 5) to 3000 bp (OPG-02). The DNA fragments generated by the twenty primers from the genomic DNA of the three species were separated using Agarose gel electrophoresis and illustrated in figs (4, 5, 6, 7, 8, 9 and10). The banding patterns of these DNA fragments were analyzed by Gene profiler computer software program and summarized in tables (2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 and 21). The number and positions of the bands depended on species and primer as shown in these tables.

The number of bands was variable in each species. ***Schilbe mystus*** was the species that produced number of amplified bands 174, and in ***Schilbe uranoscopus*** 193, while in ***Siluranodon auritus*** 210 bands**.** A total of 295 DNA bands were generated by all primers in all specimen, out of these DNA bands 97 (32.88%) were conserved among all specimens, while 198 bands were polymorphic with percentage 67.12% of all the twenty tested primers produced polymorphism in all specimens (table22). The RAPD bands profile for these species as seen in the Figures (4, 5, 6, 7, 8, 9 and10) indicated specificity of the DNA patterns for a given species. We have found that the primers of same length but with different sequences generated different DNA patterns among fishes. The results of the (RAPD-PCR) DNA analysis were compared with those obtained from the classical methods in taxonomy using morphological and anatomical characters alone, the following are the amplification results of the three species obtained by the examined primers:

Schilbe mystus

All the primers amplified successfully on the genomic DNA from ***Schilbe mystus*** yielded distinct RAPD pattern. The twenty primers generated 174 amplified bands present in ***Schilbe mystus***. The number of fragment amplified per primer varied from 1 fragment by the primer (OPA - 4) to 14 fragments by the primers (OPE –6 and OPM - 5) and the size of these fragments arranged from 200 to 3000 bp. The primer (OPG - 2) generated the largest size of the fragments 3000 bp., while the primers (OPB - 17, OPC - 1, OPE – 6 and OPM – 17) generated the smallest size 200 bp. of fragments.

Schilbe uranoscopus

A primary evaluation of the twenty oligonucleotides, tested on the ***Schilbe uranoscopus*** species, indicated that the primers produced amplification products ranging from approximately 200 bp. by the primers OPA - 4, OPB – 17, OPE – 6 and OPM - 5 to 1500 bp. by the primer OPA - 15.

The twenty primers amplified (193) fragments presented in ***Schilbe uranoscopus*** species. The twenty primers used in the amplifications reactions produced various numbers of fragments for the species ***Schilbe uranoscopus***, the largest number of fragments was 15 fragments generated by the primers OPA – 15 and OPE – 6, while the smallest number of fragments was 3 fragments generated by the primer OPF – 6.

Siluranodon auritus

All the twenty different primers used in study, produced different band patterns. The number of amplified bands detected varied, depending on the primers and treatments. These primers amplified 210 bands presented in ***Siluranodon auritus.*** The bands ranged in size from 200 bp by primers (OPE-6 and OPM-17) to 2000 bp. by (OPE-5). The bands generated by the twenty primers, varied in number from 2 by primer OPE-10 to 17 by OPA-7.

Similarity matrix UPGMAJaccard's Coefficient:

Data of the presence / absence of DNA fragments of ***Schilbe mystus***, ***Schilbe uranoscopus*** *and* ***Siluranodon auritus***, were used to calculate the genetic similarity. Then based on the calculated genetic similarity presented in table (23) and Dendrogram as figure 11, an estimation of the relationship between the above species was concluded where the lowest genetic similarity **60.8** was observed between***, Schilbe uranoscopus*** and ***Siluranodon auritus***, while the highest value **73** was found between ***Schilbe mystus*** and ***Schilbe uranoscopus***.

The phylogenetic relationships and genome specificity among three species of Schilbe catfishes (***Schilbe mystus, Schilbe uranoscopus*** and ***Siluranodon auritus***) were investigated using RAPD markers as discriminating characters for first time in Egypt.

Discussion

Here we report a study on the genetic variation of Schilbe species throughout Upper Egypt by means of random amplified polymorphic DNA (RAPD) fingerprinting. Our aim was to investigate the genetic variation within and between the three species ***Schilbe mystus, Schilbe uranoscopus*** and ***Siluranodon auritus***). This information can improve our understanding of the relationship between the genus ***Schilbe*** and the genus ***Siluranodon***.

All the primers which amplified successfully on the genomic DNA from the samples (***Schilbe mystus, Schilbe uranoscopus*** and ***Siluranodon auritus***) yielded distinct RAPD pattern. The number of fragment amplified per primer varied between **(7- 21**) fragments and had a size arranged from (**200 – 3000** bp.). The results obtained in this study showed that RAPD could be used to generate useful fingerprints characteristic of fish species and for genotyping of individuals within the species. Thus, it provides an efficient and sensitive method which can be used to estimate genetic variability, relatedness, inbreeding levels, pedigree analyses, detection of economic traits and in other maker based studies in fishes **Shair *et al.* (2011**).

Random Amplified Bolymorphic DNA (RABD) was used to profile the genetic diversity of population **Fouz *et al.* (2007**). The RABD offered a quick and inexpensive molecular tool that assisted to distinguish, monitor and manage the genetic diversity of natural populations of fish raised in fish hatcheries fish **Brahmane *et al.* (2006**). RAPD analysis is useful since different number and size of fragments in different fish species can be obtained. Different fish species were given different number and size of bands (**Bielawski** and **Pumo, (1997); Elo *et al.* (1997); Smith *et al.* (1997); Asma, (1999**) and **Liu Z.J. *et al.* (1999**)).

Using RAPD fingerprinting on fish has been limited so in the current study, this technique was applied to analyze the genetic relationships among schlibe species. Using a RAPD analysis, the intrapopulation variation was detected with different primers in tilapia **Bardakci** and **Skibinski, (1994**). This technique was more sensitive than the mtDNA analysis which failed to reveal the variation within the tilapia populations (**Capili, (1990)** and **Seyoum** and **Kornfield, (1992**)). RAPD has been used to detect genotoxic-induced DNA damage and mutations in different organisms, including fish **Atienzar** and **Jha (2006**).

Although several amplified fragments were shared by all three Species, clearly distinguishable bands were observed only in a determined species (***Schilbe mystus, Schilbe uranoscopus*** and ***Siluranodon auritus***) was evident from high number of polymorphic marker. Present study indicated that least genetic distance was observed in ***Schilbe mystus*** and ***Schilbe uranoscopus*** these two species fell into a distinct cluster from other species ***Siluranodon auritus***. This points out that these two species are genetically closer and show divergence as compared to other species of Siluranodon auritus. These two species are considered morphologically closer in contrast to the other species of Schilbe.

RAPD is one of the simplest molecular tools for genetic and systematic analyses of various organisms and has provided important applications in catfish **Bartish *et al.* (2000).** Random amplified polymorphic DNA (RAPD) analysis has been widely used in genetic diversity studies, identification of fish species, genetic differentiation in intra- or interpopulation breeding, DNA detection and so on, as it is a quick, sensitive and easy technique (**Bardakci and Skibinski, (1994)**; **Ding *et al*. (1998); Liu, Z.H *et al*. (1999); Liu, Z.J. *et al*. (1999)** and **Yoon and Kim, (2001).**

It is further suggested that the use of more numbers of random primers from different operon series in more numbers of samples might be helpful to achieve more reliable results in the genetic studies **Chandra *et al.***

(2010). The RAPD method was successfully used to detect the variation between the different species of fish **Bardakci and Skibinski, (1994**).

In conclusion, the RAPD method was successfully used to detect the variation between the different species of fishes. The results obtained in this study showed that RAPD could be used to generate useful fingerprints characteristic of fish species and for genotyping of individuals within the species. The results indicated that each species has different molecular genetic characteristics. The cluster analysis clearly differentiated ***Schilbe mystus*** *and* ***Schilbe uranoscopus*** from ***Siluranodon auritus***. The molecular genetic taxonomic relationship among three species of Serranidae fishes (***Schilbe mystus, Schilbe uranoscopus*** and ***Siluranodon auritus***) were investigated using RAPD markers for first time in Egypt.

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Fig (1): The external features of *Schilbe mystus.*

Fig (2): The external features of *Schilbe uranoscopus.*

Fig (3): The external features of *Siluranodon auritus.*

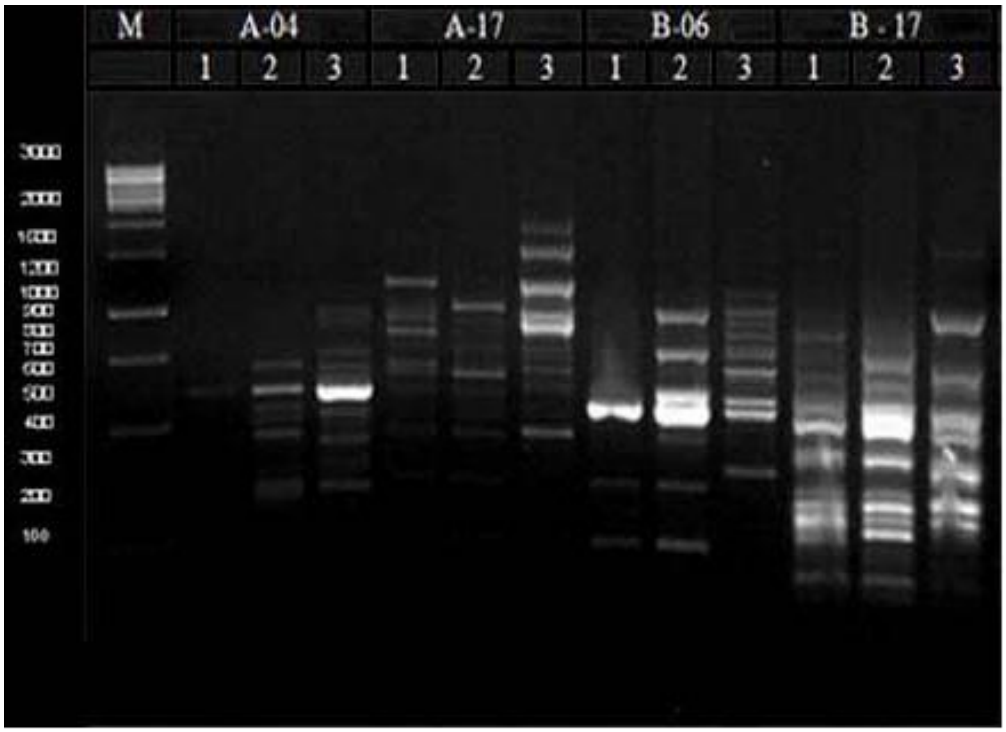

Fig (4): Agarose-gel electrophoresis products generated with primers OPA-4, OPA-17, OPB-6 and OPB-17. (1- *Schilbe mystus,* 2- *Schilbe uranoscopus* 3- *Siluranodon auritus*)

		OPA - 04		
No.	M	1	2	3
1	1000	0	1	1
2	950	0	0	1
3	820	0	0	1
4	750	0	1	1
5	680	0	0	1
6	600	1	1	1
7	570	0	1	0
8	530	0	0	1
9	500	0	1	0
10	480	0	1	1
11	370	0	0	1
12	300	0	1	1
13	220	0	1	1
14	200	0	1	0

Table (2): Survey of RAPD Markers using primer (OPA-4). (1- *Schilbe mystus,* 2- *Schilbe uranoscopus* 3- *Siluranodon auritus*), where (1) means present and (0) means absence.

		OPA - 17		
No.	M	1	2	3
1	1800	0	0	1
2	1500	0	0	1
3	1300	1	0	1
4	1250	0	1	1
5	1000	1	1	1
6	920	1	1	1
7	850	0	1	0
8	750	1	0	1
9	700	1	1	0
10	650	1	1	1

11	580	0	1	0
12	500	1	1	1
13	480	0	0	1
14	400	1	1	0
15	270	0	1	1

Table (3): Survey of RAPD Markers using primer (OPA-17).

		OPB – 06		
No.	M	1	2	3
1	1100	0	0	1
2	1000	0	1	1
3	900	0	0	1
4	830	0	0	1
5	780	0	0	1
6	750	0	1	1
7	700	0	0	1
8	650	1	1	1
9	600	1	1	1
10	500	0	1	0
11	400	0	0	1
12	380	1	1	0
13	260	1	1	0

Table (4): Survey of RAPD Markers using primer (OPB-6).

		OP: B17		
No.	M	1	2	3
1	1500	0	0	1
2	1000	1	1	1
3	850	1	1	0
4	750	0	1	0
5	680	1	1	1
6	600	1	1	1
7	550	1	1	1
8	450	1	1	1
9	420	1	0	1
10	400	0	0	1
11	350	1	1	0
12	300	1	1	1
13	270	1	1	1
14	230	1	1	1
15	200	1	1	0

Table (5): Survey of RAPD Markers using primer (OPB-17).

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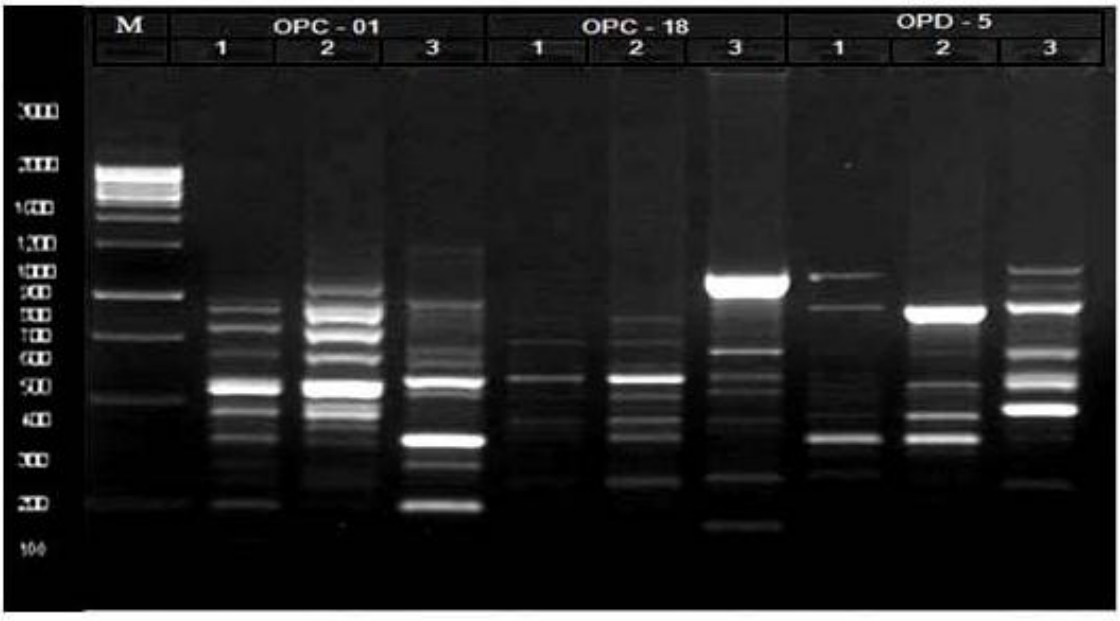

Fig (5): Agarose-gel electrophoresis products generated with primers OPC-1, OPC-18 and OPD-5.

		OPC - 01		
No.	M	1	2	3
1	1500	0	0	1
2	1200	0	1	0
3	1000	1	1	1
4	900	1	1	0
5	800	1	1	0
6	750	0	0	1
7	700	1	1	1
8	600	1	1	1
9	550	1	1	1
10	500	0	1	0
11	480	1	1	0
12	450	1	1	0
13	400	1	1	1
14	350	1	0	1
15	300	1	1	0
16	250	1	1	1
17	200	1	0	0

Table (6): Survey of RAPD Markers using primer (OPC-1).

		OP: C18		
No.	M	1	2	3
1	1050	0	0	1
2	900	1	1	1
3	750	1	1	0
4	700	0	0	1
5	650	0	1	0
6	600	1	1	1
7	500	1	1	0
8	450	1	1	1
9	400	1	1	0
10	300	1	1	1
11	220	0	0	1

Table (7): Survey of RAPD Markers using primer (OPC-18).

		OPD - 05		
No.	M	1	2	3
1	1200	1	0	1
2	1100	0	0	1
3	950	1	1	1
4	700	1	1	1
5	600	1	0	1
6	550	1	1	1
7	500	1	1	0
8	420	1	1	1
9	380	1	1	1
10	350	1	1	0
11	300	1	0	1

Table (8): Survey of RAPD Markers using primer (OPD-5).

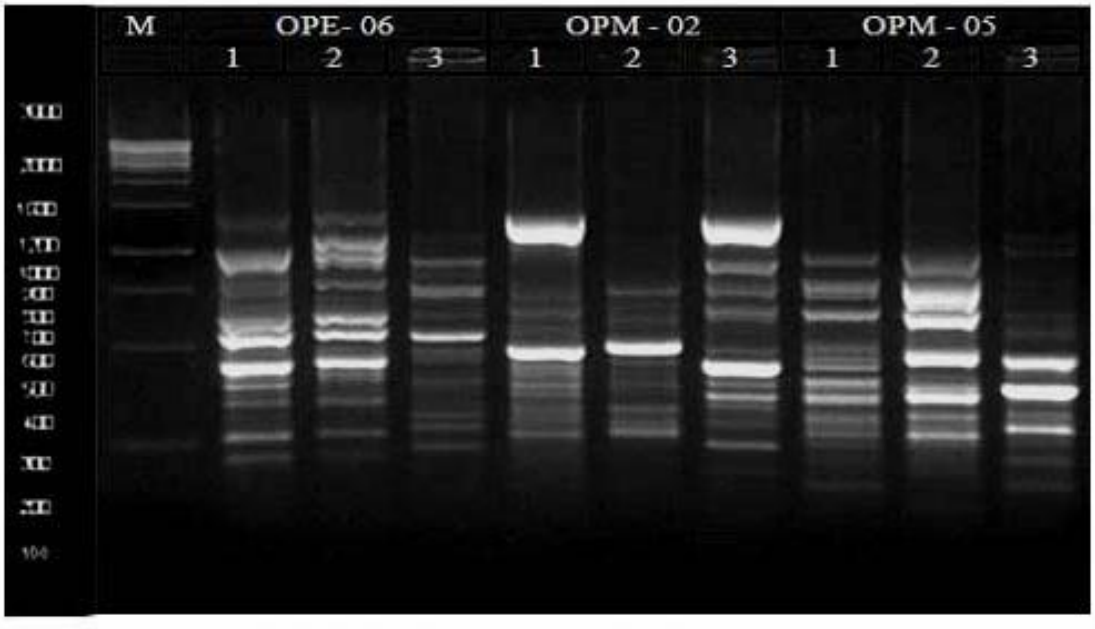

Fig (6): Agarose-gel electrophoresis products generated with primers OPE-6, OPM-2 and OPM-5.

		OP:E06		
No.	M	1	2	3
1	1300	1	1	0
2	1100	0	0	1
3	1000	0	1	0
4	900	1	1	1
5	850	1	1	0
6	750	1	1	1
7	700	1	0	1
8	650	1	1	0
9	580	1	1	1
10	500	1	1	1
11	470	1	1	1
12	450	1	1	0
13	400	0	1	0
14	380	1	1	1
15	350	1	1	1
16	300	0	0	1
17	250	1	1	1
18	200	1	1	1

Table (9): Survey of RAPD Markers using primer (OPE-6).

		OPM - 02		
No.	M	1	2	3
1	1200	1	0	1
2	900	0	0	1
3	800	1	1	1
4	750	0	1	0
5	700	1	0	1
6	600	0	1	0
7	580	1	0	1
8	550	1	1	1
9	500	0	0	1
10	450	1	1	1
11	400	1	0	1
12	370	0	0	1
13	300	1	1	1
14	270	0	1	1
15	250	1	1	0
16	230	0	0	1

Table (10): Survey of RAPD Markers using primer (OPM-2).

No.		OPM - 05		
	M	1	2	3
1	1150	0	0	1
2	1000	0	0	1
3	900	1	1	0
4	780	1	0	0
5	750	1	1	0
6	720	0	1	0
7	700	1	1	1
8	600	0	0	1
9	550	1	0	0
10	500	1	1	1
11	470	1	0	0
12	450	0	1	0
13	430	1	0	0
14	420	1	1	1
15	400	1	1	0
16	300	1	1	1
17	260	1	1	1
18	240	1	1	1
19	220	1	0	1
20	200	0	1	0

Table (11): Survey of RAPD Markers using primer (OPM-5).

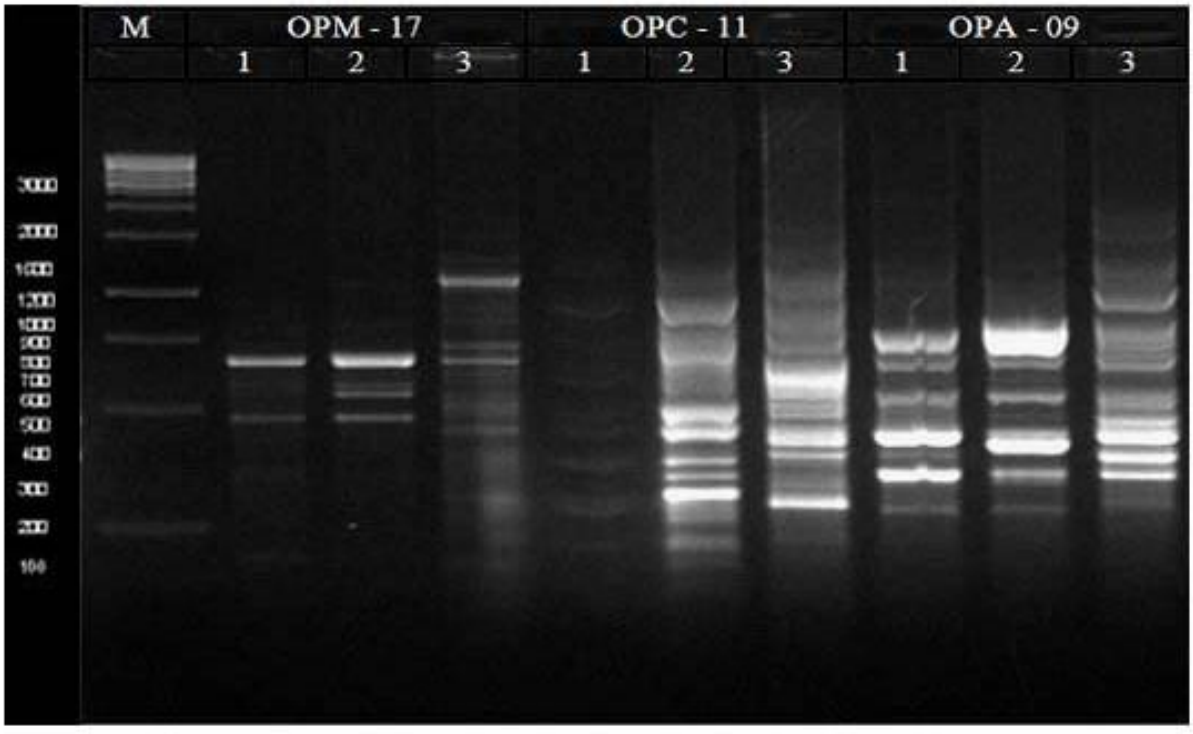

Fig (7): Agarose-gel electrophoresis products generated with primers OPM-17, OPC-11 and OPA-9.

		OP:M17		
No.	M	1	2	3
1	1200	0	0	1
2	1000	0	1	1
3	800	1	1	1
4	750	0	0	1
5	700	1	1	1
6	650	0	0	1
7	620	1	1	1
8	580	0	1	1
9	520	0	0	1
10	500	1	1	0
11	450	0	0	1
12	350	1	0	1
13	300	0	0	1
14	200	1	0	1

Table (12): Survey of RAPD Markers using primer (OPM-17).

		OPC - 11		
No.	M	1	2	3
1	1100	1	1	1
2	1000	0	0	1
3	900	1	1	1
4	800	1	1	1
5	750	0	1	0
6	700	1	0	1
7	600	1	1	1
8	500	0	0	1
9	470	1	1	1
10	400	1	1	1
11	370	0	0	1
12	350	1	1	1
13	300	0	1	0
14	270	1	1	1
15	250	1	1	0
16	220	1	1	0

Table (13): Survey of RAPD Markers using primer (OPC-11).

		OP:A09		
No.	M	1	2	3
1	1500	0	0	1
2	1300	0	0	1
3	1000	1	1	1
4	900	0	0	1
5	800	0	1	1
6	750	1	1	1
7	700	0	0	1
8	680	1	1	1
9	550	1	1	1
10	500	0	0	1
11	430	0	0	1
12	380	1	1	1
13	370	0	1	0
14	350	1	0	1
15	320	1	1	1
16	300	0	1	0
17	250	1	1	1

Table (14): Survey of RAPD Markers using primer (OPA-9).

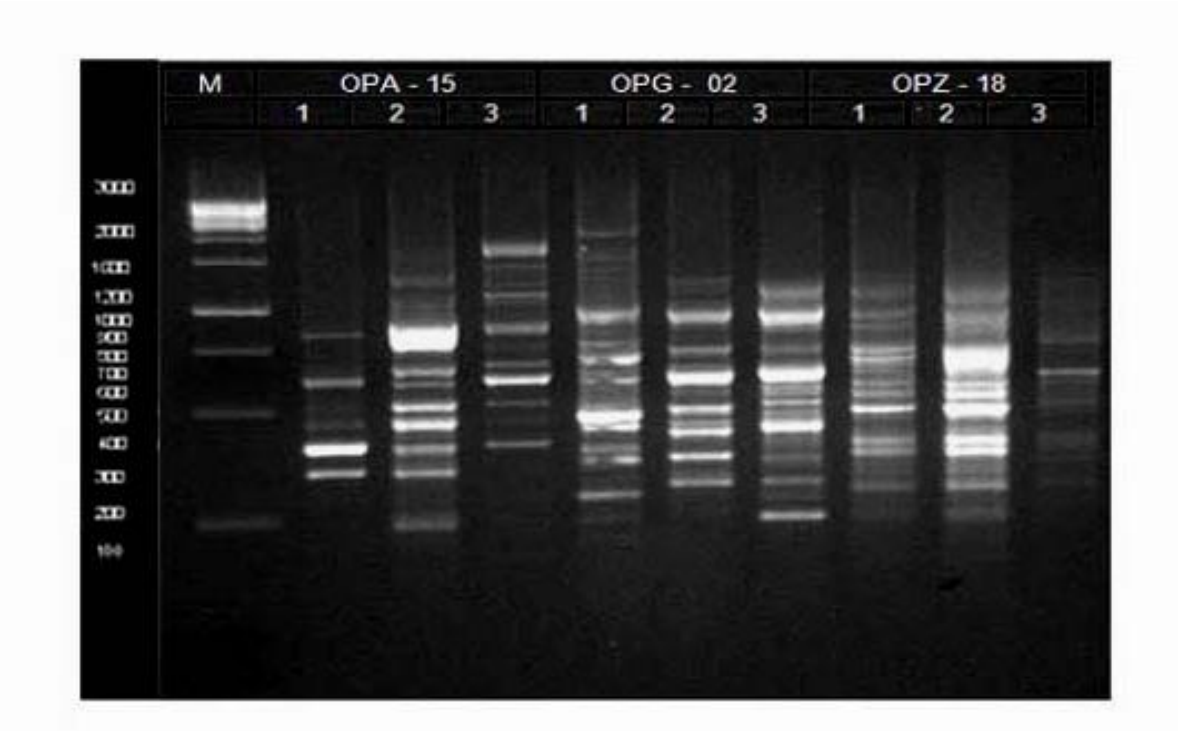

Fig (8): Agarose-gel electrophoresis products generated with primers OPA-15, OPG-02 and OPZ-18.

		OP:A15		
No.	**M**	**1**	**2**	**3**
1	1500	0	1	1
2	1400	0	0	1
3	1300	0	1	0
4	1200	0	0	1
5	1100	0	0	1
6	1000	0	1	1
7	830	1	1	1
8	800	0	1	1
9	750	0	1	0
10	700	0	1	0
11	660	1	0	1
12	640	0	1	0
13	610	1	1	1
14	500	1	1	1
15	450	1	1	1
16	400	1	1	1
17	300	1	1	0
18	250	0	1	0
19	230	0	1	0

Table (15): Survey of RAPD Markers using primer (OPA-15).

		OP:G02		
No.	M	1	2	3
1	3000	1	0	0
2	2500	1	0	0
3	1300	0	1	0
4	1200	0	1	1
5	1150	0	0	1
6	1100	0	1	0
7	1000	1	1	1
8	950	1	0	1
9	850	1	0	0
10	750	1	1	1
11	650	1	1	1
12	550	1	1	1
13	500	0	0	1
14	480	0	1	1
15	450	1	1	0
16	430	0	1	1
17	400	1	0	0
18	350	1	1	1
19	300	0	1	1
20	280	1	0	1
21	250	1	0	1

Table (16): Survey of RAPD Markers using primer (OPG-02).

		OP: Z18		
No.	M	1	2	3
1	1300	1	0	1
2	1200	1	1	0
3	1000	1	1	0
4	850	1	1	0
5	800	1	1	1
6	750	1	1	0
7	700	0	0	1
8	650	1	1	1
9	600	1	1	0
10	550	1	1	1
11	460	1	1	1
12	430	1	1	1
13	380	0	0	1
14	350	0	1	0
15	300	1	1	1
16	250	1	1	0

Table (17): Survey of RAPD Markers using primer (OPZ-18).

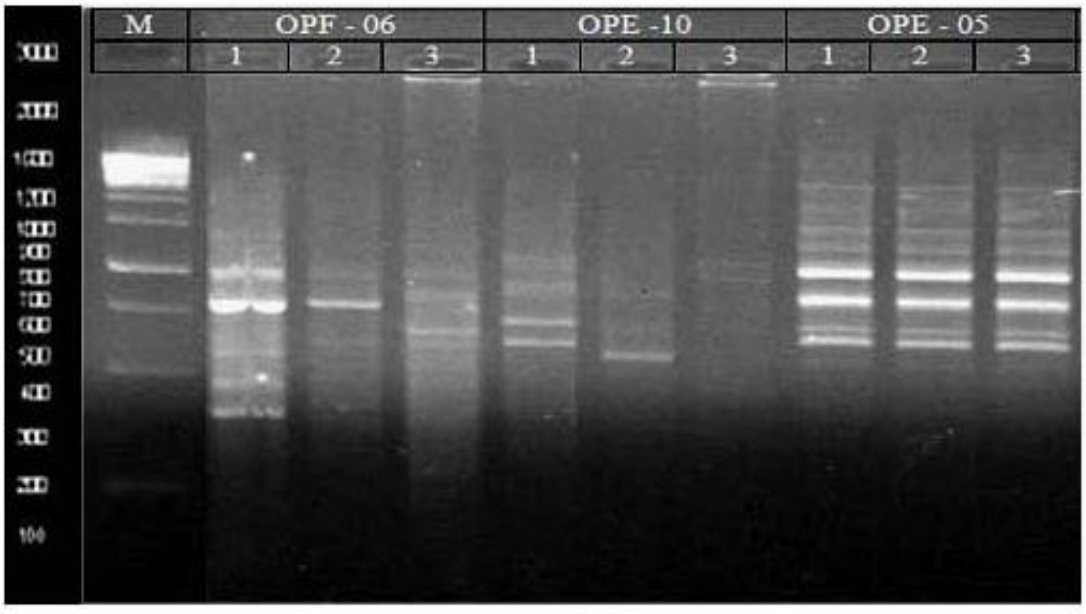

Fig (9): Agarose-gel electrophoresis products generated with primers OPF-6, OPE-10 and OPE-05.

		OP:F06		
No.	M	1	2	3
1	1200	1	0	1
2	900	1	1	1
3	750	1	1	1
4	600	0	0	1
5	550	1	1	1
6	530	1	0	0
7	450	1	0	0
8	400	1	0	0

Table (18): Survey of RAPD Markers using primer (OPF-6).

		OPE -10		
No.	M	1	2	3
1	1100	0	1	0
2	1000	1	0	0
3	800	1	1	1
4	600	0	1	0
5	580	1	0	0
6	530	1	1	0
7	520	0	0	1

Table (19): Survey of RAPD Markers using primer (OPE-10).

		OPE - 05		
No.	M	1	2	3
1	2000	1	1	1
2	1500	1	0	0
3	1300	1	1	1
4	1050	1	1	1
5	900	1	1	1
6	750	1	1	1
7	620	1	1	1
8	590	1	1	1
9	520	1	1	1

Table (20): Survey of RAPD Markers using primer (OPE-05).

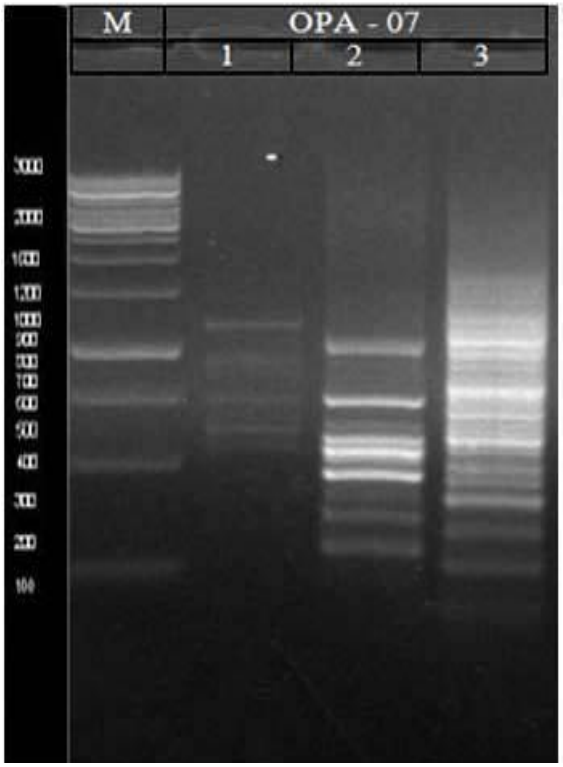

Fig (10): Agarose-gel electrophoresis products generated with primers OPA-7.

		OPA - 07		
No.	M	1	2	3
1	1500	0	0	1
2	1200	1	0	1
3	1100	0	0	1
4	1050	0	1	1
5	950	1	0	1
6	850	0	0	1
7	750	1	1	1
8	700	0	0	1
9	600	1	1	1
10	550	0	1	1
11	520	1	0	1
12	500	0	1	0
13	480	0	0	1
14	450	0	1	1
15	400	0	1	1
16	350	0	1	1
17	300	0	1	1
18	230	0	0	1

Table (21): Survey of RAPD Markers using primer (OPA-7).

Table (22): Number of amplified and polymorphic DNA – fragments in *Schilbe mystus* , *Schilbe uranoscopus* and *Siluranodon auritus.*

No.	Primer code	Total of amplified bands (a)	No. of amplified bands			No. of Common bands	No. of polymorphic bands (b)
			Schilbe mystus	*Schilbe uranoscopus*	*Siluranodon auritus*		
1	OPA - 4	14	1	9	11	1	13
2	OPA - 17	15	7	10	11	4	11
3	OPB - 17	15	12	12	11	8	7
4	OPB - 6	13	4	7	10	2	11
5	OPC - 1	17	13	13	9	6	11
6	OPC - 18	11	7	8	7	4	7
7	OPD - 5	11	10	7	9	5	6
8	OPE - 6	18	14	15	12	9	9
9	OPM - 2	16	9	8	13	4	12
10	OPM - 5	20	14	12	10	6	14
11	OPC - 11	16	11	12	12	8	8
12	OPM - 17	14	6	6	13	3	11
13	OPA - 9	17	8	10	15	7	10
14	OPA - 15	19	7	15	12	5	14
15	OPG - 2	21	13	12	14	5	16
16	OPZ - 18	16	13	13	9	6	10
17	OPF - 6	8	7	3	5	3	5
18	OPE - 10	7	4	4	2	1	6
19	OPE - 5	9	9	8	8	8	1
20	OPA - 7	18	5	9	17	2	16
Total		295	174	193	210	97	198

Table (23): Genetic similarity values calculated from the DNA fragments amplified from *Schilbe mystus, Schilbe uranoscopus* and *Siluranodon auritus* using twenty OPERON primers.

Siluranodon auritus	*Schilbe uranoscopus*	*Schilbe mystus*	
		100	*Schilbe mystus*
	100	73	*Schilbe uranoscopus*
100	60.8	64.1	*Siluranodon auritus*

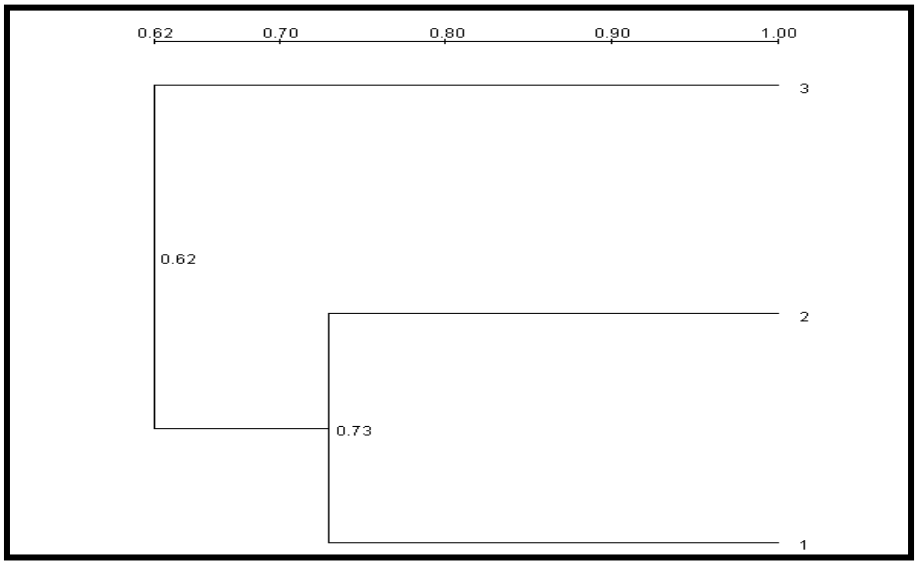

Fig. (11): Dendrogram demonstrating the relationship among *Schilbe mystus, Schilbe uranoscopus* and *Siluranodon auritus*, based on data recorded from polymorphism of RAPD markers. 1- *Schilbe mystus*, 2- *Schilbe uranoscopus* and 3-*Siluranodon auritus*. Dendrogram consisted of two clusters; (A) contained *Siluranodon auritus* and (B) contained *Schilbe mystus* and *Schilbe uranoscopus*.

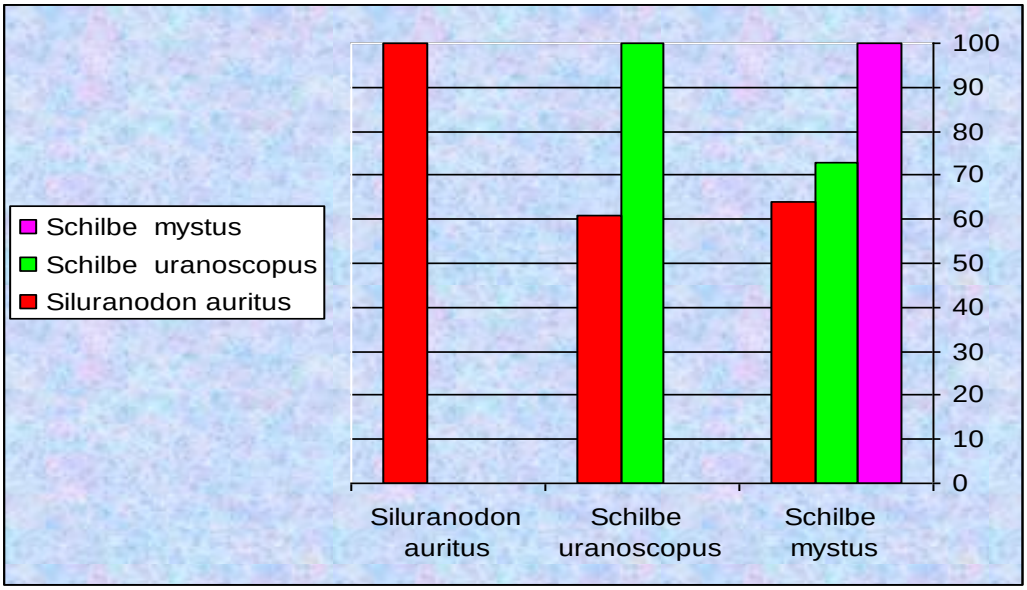

Fig. (12): Demonstrating the relationship degree among *Schilbe mystus, Schilbe uranoscopus* and *Siluranodon auritus*, based on data recorded from polymorphism of RAPD markers.