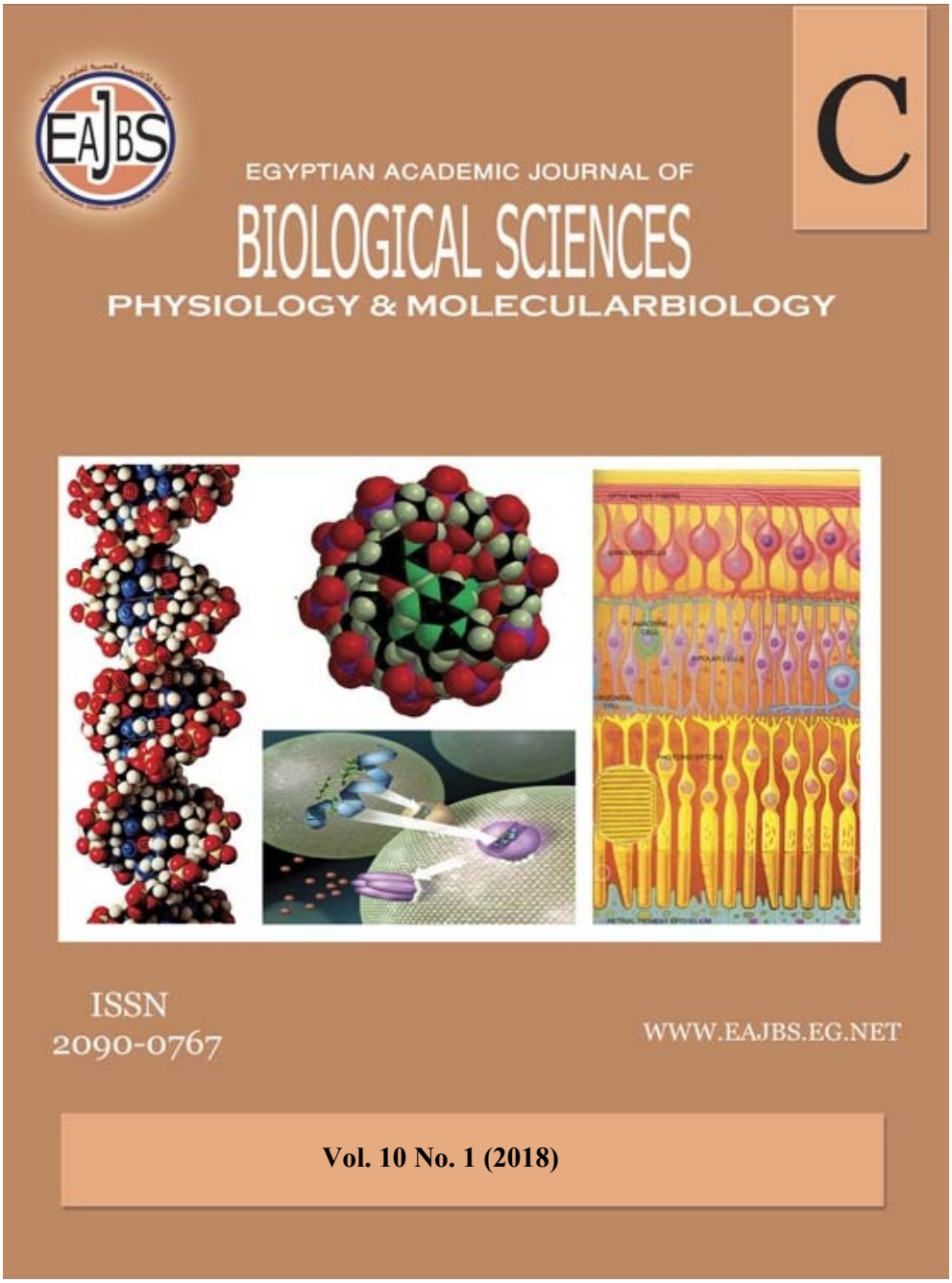

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RAPD-evaluated Genetic Polymorphisms and Relations among three River Nile Catfish (Siluriformes) Species from Qena, Egypt

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ABSTRACT

Genetic discrepancies among three River Nile catfish; *Schilbe mystus* Linnaeus, 1758 (Schilbeidae), *Bagrus bajad* Forsskål, 1775 (Bagaridae) and *Clarias gariepinus* Burchell, 1822 (Claridae) was evaluated by RAPD-PCR technique using 8 deca oligonucleotides (A-03, A-04, A-05, A-06, A-09, A-10, A-11, and A-12). A total of 52 amplified bands were produced, from them 5 bands were common with level of monomorphism of % 9.62, and 47 bands were polymorphic with level of polymorphism of 90.38 %. An instructive RAPD fingerprint profile was generated with various band size lengths ranging from 200 to 2900 base pair.

According to Nei-72 distance matrix of genetic and the unweighted pair group method average (UPGMA), the three studied catfish species are related to each other and likely have the most sharing common ancestor. However, *Schilbe mystus* (Schilbeidae) and *Bagrus bajad* (bagaridae) are genetically more close to each other than to *Clarias gariepinus*. The subset of *Clarias gariepinus as sister clade to Schilbe mystus and Bagrus bajad suggested* monophyly of the family Clarridae.

The study suggested occurrence of genetic variations among the investigated River Nile catfish, with high level of genetic convergence/relation between *Schilbe mystus* and *Bagrus bajad.* This probably be useful for enhancing their potential use in aquaculture breeding programs, as well as providing insights on their taxonomic status within the Siluriformes.

INTRODUCTION

Genetic variation (polymorphisms) caused by mutational alterations are necessitated for species survival and adaptations in diverse environmental conditions (Fisher, 1930).The patterns of genetic discrepancy to species level can be exposed using tools of molecular genetic markers (Linda and Paul, 1995). Such information of molecular markers-based on decipherable polymorphism at DNA and protein levels are source of knowledge for several research applications including molecular

phylogeny, conservation, animal breeding, management of fish populations, and monitoring of farmed species stocks (Perkins and Krueger, 1993; Ferguson *et al.*, 1995; Liu and Cordes, 2004).

The catfish (whisker-like barbells) are a diverse group of fishes containing ~4100 species, and represent ~12% of teleosts counting for ~6.3% of known vertebrates species. The catfish (Siluriformes) have a tremendous economic value for being an important dietary proteins source (*Ferraris,* 1999; Eschmeyer and Fong, 2014; Wilson and Reeder, 2005) and are significantly important species for aquaculture purpose worldwide (Kim, 1997; USDA, 2002; Buton, 1979; Aremu and Ekunode, 2008).

Clarias gariepinus Burchell, 1822 (Claridae) is omnivorous predatory fish widely distributed throughout African aquatic systems and possess diverse habitat preferences, rapid growth as well as withstanding to severe water conditions (Welcome, 1979; Bruton, 1986; Nwadukwe, 1995). *Schilbe mystus* Linnaeus, 1758 (Schilbeidae) is omnivorous surface feeders native to African fresh water lakes possessing rapid growth rate and becoming commercially important fish species (Omondi, and Ogari 1994; Adedolapo 2007; Amer *et al.,* 2008; Ayoade *et al.,* 2008; Kareem *et al.,* 2015). *Bagrus bajad* Forsskål, *1775* (Bagaridae) is carnivorous fish widely distributed in African freshwater systems (Risch, 1986) being commercially importance and potential aquaculture candidate (El-Drawany and Elnagar, 2015; Alhassan and AnsuDarko, 2011).

RAPD is a PCR-based assay utilizing a single short deca-oligonucleotide sequence to amplify a complementary arbitrary fractions (markers) of a particular genome (Williams *et al.,* 1990; Welsh and McClelland, 1990). RAPD markers have been improved significantly to observe genetic variations and been widely used in fish population genetic studies, wild and cultured fish stocks and inter/intraspecific fish genetic variation (You *et al,* 2007; Mamuris *et al.*, 1999a; Islam *et al.,* 2007; Abdul Muneer *et al*., 2009; Rahman *et al*., 2009; Danish *et al*., 2012; Popoola *et al*., 2015; Verma and Serajuddin, 2017).

There are various fish species to be considered for evaluation as aquaculture potentials. Nevertheless, the successful conservation and management of species will be based on comprehensive knowledge of their genetic structure, relatedness and pattern of genetic variation (Allendorf and Phelps, 1981).Thus, using diverse molecular genetic tools will be important for understanding and management of a particular fish species for farming and culturing purpose. The current work aimed to investigate the genetic polymorphisms and relations among three River Nile catfish (Siluriformes) species; *Schilbe mystus* Linnaeus 1758**,** *Bagrus bajad* Forsskål 1775, and *Clarias gariepinus* Burchell 1822 using RAPD-PCR fingerprint analysis.

MATERIALS AND METHODS

Fish Sampling :

The three River Nile catfish (Siluriformes) species (Fig. 1) used in this study were collected by the author from fish market (Elsehreg), Qena, Egypt during August/October 2017, brought to the laboratory and were placed down to species level (Bailey, 1994; Bishai and Khalil, 1997; FishBase 2017). Tissue samples (muscle, fins and scales) were individually preserved at -20°C for genomic DNA extraction and future analyses.

Fish Genomic DNA Extraction:

Genomic DNA from each fish sample (~30mg of muscle tissues) was

extracted using EZ-10 spin column genomic DNA extraction kit for animal tissue (Bio Basic Inc., Canada) according to the manufacturer's guidelines. The concentrations and clarity of DNA were approximated under spectrophotometric UV absorption at A260 and A280 and 1% agarose gel. DNA samples were stored at −20 °C.

RAPD-PCR Assay:

RAPD assay was performed based on Williams *et al.,* (1990). Each RAPD-PCR reaction was accomplished in a final volume of 25 µl of 1.0× pre-mixed OnePCR^{TM} 2X (GeneDireX Inc, USA), ~50 ng genomic DNA of each sample, and 10 pM of each deca-nucleotide primer alone. Eight (8) deca-nucleotide primers were used for the DNA amplification [A-03, A-04, A- 05, A-06, A-09, A-10, A-11, and A-12] (Bio Basic Inc, Canda).

PCR amplifications were performed in a thermocycler (Primus 25 advanced, PEQLAB Biotechnologie GmbH) under cycling sittings fitted an initial denaturation at 95°C for 2 min, 45 cycles (94°C for 1 min, 36°C for 1 min and 72°C for 2 min), then a 10 minutes cycle of final extension at 72°C. Amplification products (15 µl) were electrophoresed using 1.5% (w/v) agarose gels in TAE buffer (0.40 mM Tris, 0.20 mM acetate, 2 mM EDTA pH 8), stained with ethidium bromide (0.5 µg/ml) and imaged under UV light (Elttrofor M20 SaS Photo-Gel System, Italy) with Nikon Coolpix LB40 digital camera. Products size was compared with 100 bp DNA ladder (0.1 µg/µl, Solis BioDyne, Estonia).

Data Analysis:

For RAPD valuation, RAPD images were analysed for polymorphic DNA bands using the PyElph gel image analysis program (Pavel and Vasile, 2012). DNA fingerprints were scored for the presence/absence (1/0) of RAPD bands of identical molecular sizes in a binary matrix. The POPGENE version 1.32 (Yeh *et al.,* 1999) was used to analyse the binary matrix data to compute the Nei's original measures of genetic identity and genetic distance (Nei, 1972). A dendrogram was constructed based on Nei's original measure of genetic distance using the unweighted pair group method average 'UPGMA' clustering method (Sneath and Sokal, 1973), and displayed with the Molecular evolutionary genetics analysis Version 6.0 (MEGA6) software (Tamura *et al*., 2013). For each primer used, the total number of scored bands, number of polymorphic bands, and number of monomorphic bands were recorded.

Fig. 1: Photographs of the three River Nile catfish (siluriformes) included in this study. These are *Schilbe mystus* Linnaeus 1758 (family: Schilbeidae)**,** *Bagrus bajad* Forsskål, 1775 (family: Bagaridae) and *Clarias gariepinus* Burchell, 1822 (family: Claridae)

RESULTS

RAPD evaluated genetic polymorphism :

A set of 8 deca-nucleotide primers were monitored for the capability to generate fingerprint banding pattern and to assess polymorphism among three catfish species (Table 1). All primers produced a total of 52 amplified bands with an average of 6.5 bands per primer from which 5 bands were common exhibiting low level of monomorphism of % 9.62, and 47 bands were polymorphic displaying high level of polymorphism of 90.38 % with an average number of polymorphic fragments/primer of 5.9. An instructive RAPD fingerprint profile was generated by the 8 primers with various band size lengths ranging from 200 to 2900 base pair comparing to a 100 bp DNA Ladder (Solis Bio Dyne).

As shown in Table 1, out of 8 primers used; primers A-06, A-05, and A-11 produced higher number of 9, 8 and 7 bands respectively compared to other primers. The highest number of polymorphic bands (9) was obtained with primer A-06 while the lowest ones (2) was obtained with primer A-12. The band frequency per species were 0.4615, 0.4423 and 0.5000 for *Schilbe mystus, Bagrus bajad and Clarias gariepinus* respectively while band frequency for each primer ranged from 0.0577 to 0.1731 as depicted (Fig. 2). The RAPD genotyping profile obtained for catfish (Siluriformes) under study with 8 tested primers is shown in Fig.3.

Table 1. Features of primers used to generate RAPD fingerprint profile of three River Nile catfish (Siluriformes). Number of amplified bands per species (NABands/Species), Band frequency for each primer (Band Freq/Primer), Band frequency per species (Band Freq/Species), Total number of amplified bands (TNABands), Number of polymorphic bands (NPBands), Number of monomorphic bands (NMBands), Polymorphism percentage (POL%), and Range of amplified band in base pair (RAB [bp]).

Primer Code	Sequence 5'--------3'	NABands/Species *Schilbe mystus*	*Bagrus bajad*	*Clarias gariepinus*	TNA Bands	Band Freq/ Primer	NP Bands	NM Bands	% POL	RAB [bp]
A-03	AGTCAGCCAC	3	2	2	5	0.0962	4	1	80.00	250-700
A-04	AATCGGGCTG	5	4	5	8	0.1538	6	2	75.00	200-900
A-05	AGGGGTCTTG	4	2	4	8	0.1538	8	null	100.00	250-1000
A-06	GGTCCCTGAC	2	3	4	9	0.1731	9	null	100.00	300-2900
A-09	GGGTAACGCC	4	2	3	6	0.1154	5	1	83.33	400-1300
A-10	GTGATCGCAG	1	3	3	6	0.1154	6	null	100.00	350-1250
A-11	CAATCGCCGT	3	4	4	7	0.1346	7	null	100.00	350-1200
A-12	TCGGCGATAG	2	3	1	3	0.0577	2	1	66.67	400-1400
Total		24	23	26	52		47	5	90.38	
Band Freq/ Species		0.4615	0.4423	0.5000						

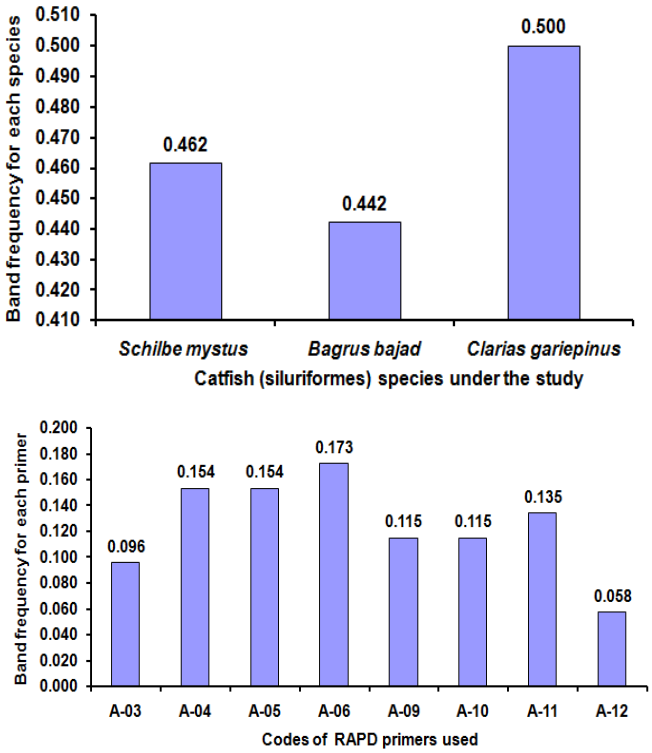

Fig. 2: Band frequencies computed for the three River Nile catfish species, *Schilbe mystus*, *Bagrus bajad and Clarias gariepinus* (**Above**) and for every used deca-nucleotide primers (**below**).

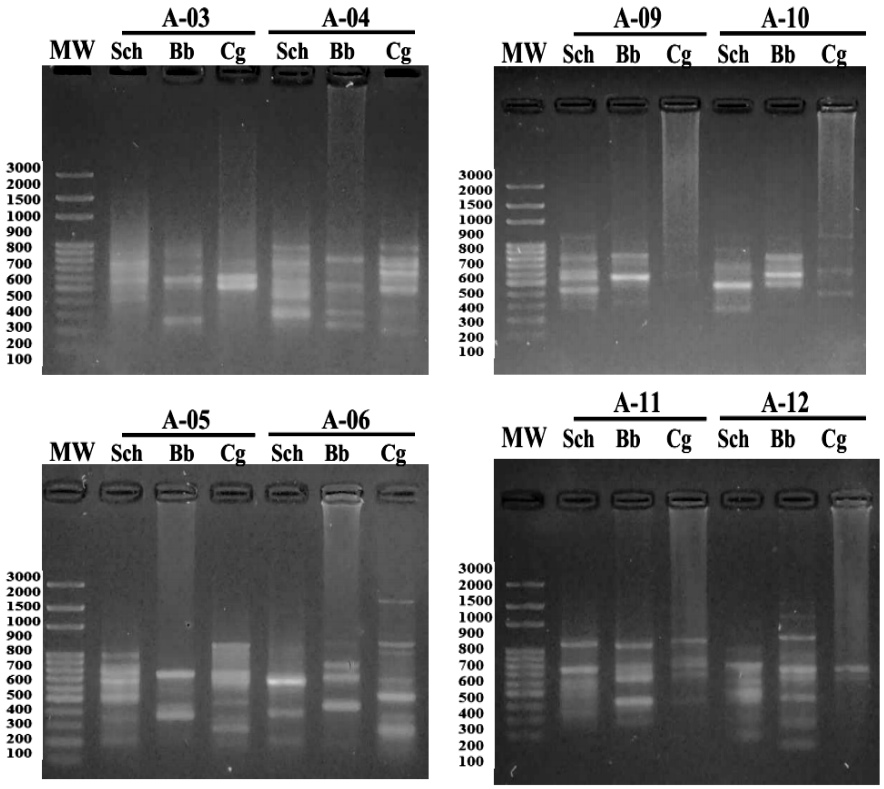

Fig. 3: RAPD fingerprint profiles for three River Nile catfish species using 8 different primers. **MW**: Molecular weight (100-3000 base pair), **Sch**: *Schilbe mystus*, ***Bb**: Bagrus bajad*, and **Cg**: *Clarias gariepinus*

Phylogentic and genetic relationship analyses:

Nei's original measures of genetic identity and genetic distance was used to construct a genetic distance and identity matrix (Table 2). As shown in the table, the lowest distance values of 0.8157 (high identity of 0.4423), between *Schilbe mystus* and *Bagrus bajad*, while the highest value of 1.0068 (low identity of 0.3654), between *Bagrus bajad* and *Clarias gariepinus*. Accordingly, *Schilbe mystus* and *Bagrus bajad* are closest to each other, while *Bagrus bajad* and *Clarias gariepinus* are distant to each other.The UPGMA phylogentic tree based on Nei's original measures of genetic distance was constructed (Fig. 4), accordingly revealed that the studied catfish (Siluriformes) segregated into two main clusters distinguishing both *Schilbe mystus* and *Bagrus bajad* in single cluster as sister group that possess a closer position and share a common node (node B). While, the *Clarias gariepinus* split up in a separate group forming sister clade to *Schilbe/Bagrus* group. The phylogenetic tree shows that *Schilbe mystus* is more closely related to *Bagrus bajad* than to *Clarias gariepinus* and all the three catfishes species share common ancestor (node A).

Table 2. Nei's (1972) original measure of genetic distance and identity from three studied catfish species computed for RAPD data. Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Species	*Schilbe mystus*	*Bagrus bajad*	*Clarias gariepinus*
Schilbe mystus	****	0.4423	0.3846
Bagrus bajad	0.8157	****	0.3654
Clarias gariepinus	0.9555	1.0068	****

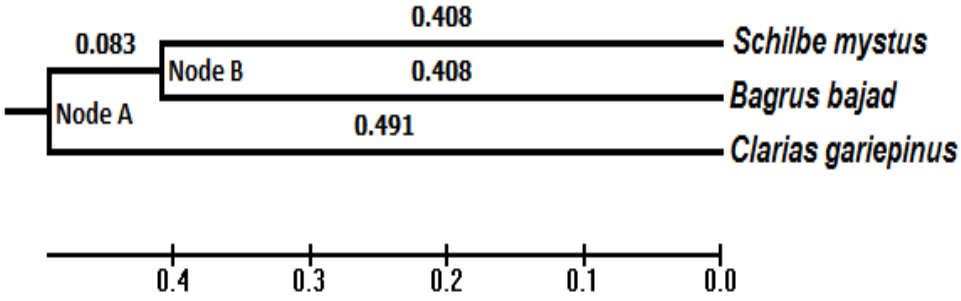

Fig. 3: Dendrogram demonstrating the genetic relationships among studied catfish (Siluriformes) based on estimation of Nei's genetic distance and identity using RAPD data (Table 2) and clustered using the UPGMA method. The branch lengths and tree nodes A, and B are shown.

DISCUSSION

Herein, RAPD-PCR assay with deca-oligonucleotide primers was applied to amplify diverse DNA fragments (RAPD markers/loci) of genomic DNA from three River Nile catfish species. These loci are inherited in Mendelian manner (Rothuizen and Van Wolferen, 1994). The presence or absence of a particular DNA fragment signify the dominant or recessive allele fashions (Williams *et al.,* 1990; Welsh and McClelland, 1990). Results obtained here demonstrated the capability of the used randomly primers to generate an instructive RAPD banding patterns and to assess polymorphism among catfish species under study. Because of PCR arbitrary primers are of valuable in spotting polymorphic genetic markers (Hunt and Page, 1992).

From a total of 52 amplified markers/bands produced, 5 bands were common exhibiting low level of monomorphism (%9.62). While 47 bands were polymorphic displaying high level of polymorphism (90.38 %) which suggest existence of interspecies high genetic variations among studied catfish species. Some studies reported that a higher polymorphism symbolize higher genetic variation, while the low polymorphism indicate the opposite (Yoon and Park, 2002; Rahman *et al.*, 2009; Yusuf *et al.,* 2017).

The highest primer band frequency of 0.1731 was recorded for primer A-06, while the lowest one of 0.0577 with primer A-12 which suggested high and low occurrence of both primers binding sites receptively within genomes of the studied catfish. Data showed detection of high molecular size fragment of ~ 2900 bp that was recorded only for *Clarias gariepinus* with primer A-06. This may consider primer-specific band for *Clarias gariepinus* indicating its internal genotype-specific variation.

The band/marker frequency per species were values of 0.4615, 0.4423 and 0.5000 for *Schilbe mystus, Bagrus bajad* and *Clarias gariepinus* respectively. Among them, *Clarias gariepinus* recorded highest band frequency of 0.5000 which suggested its higher rate of heterozygosity. This could be a reason for its adaptable phenotype, rapid growth, broad habitat diversity and globally distributed as reported (e.g Winemiller and Kelso-Winemiller, 1996; Thakur, 1998; Alves *et al.*, 1999; Yalçin *et al.,* 2001b; Senanan *et al.*, 2004; Vitule *et al.,* 2006).

The RAPD fingerprint obtained for the thee River Nile catfish (from natural populations) under study displayed various band size lengths ranging from 200 to 2900 base pair which could be reasonable with observations by other studies applied on cultured and wild fish population (Liu *et al.,*1999; Ambak *et al*., 2006; Abd El-Kader *et al*., 2013; Mostafa *et al*., 2009; Popoola *et al*., 2014).

The RAPD fingerprints of studied fresh water catfish would be of help revealing their interspecies genetic variation and genetic relationship based on presence or absence of identical size RAPD markers. Result of the UPGMA tree-based Nei's genetic distance (Fig. 3) revealed two segregated main clusters where *Schilbe mystus* is genetically more close to *Bagrus bajad* forming sister group (node **B**), than to *Clarias gariepinus* that is separately positioned as sister clade sharing common ancestor to them (node **A**).This may indicate more genetic makeup resemblance between *Schilbe mystus* and *Bagrus bajad.* For that *Schilbe mystus* is more closely related to *Bagrus bajad* than to *Clarias gariepinus.* Studies reported the capability of RAPD assay to evaluate various genomic loci and facilitate analysis of genetic distance and phylogenetic relationships (Clark and

Lanigan, 1993), as well as the genetic variation and level of similarity among fish species (Barman *et al.,* 2003). The separation of *Clarias gariepinus* as sister clade to both *Schilbe mystus* and *Bagrus bajad* may point to the monophyly of family clarridae which supported by other studies (Agnese and Teugels, 2005; Sullivan *et al.,* 2006; Pouyaud *et al*., 2009; Yu and Quilang 2014).

In conclusion, results showed the consistency and informative efficiency of RAPD PCR fingerprint technique in fish phylogenetic studies down to the species level and produced characteristic DNA fingerprint profiles for the three studied River Nile catfish species. The three species are closer to each other, however *Schilbe mystus (*Schilbeidae) and *Bagrus bajad* (Bagaridae) are genetically more close. The subset of *Clarias gariepinus* as sister clade to *Schilbe mystus* and *Bagrus bajad* may be a sign to the monophyly linage of the family Clarridae. The study outcomes suggested occurrence of genetic polymorphisms (variations) among the investigated River Nile catfish, with high level of genetic convergence/relation between *Schilbe mystus* and *Bagrus bajad* which probably be useful to improve their potential use in aquaculture breeding programs, as well as providing insights on their taxonomic status within the siluriformes.

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ARABIC SUMMARY

التغايرات الوراثية والعلاقة بين ثلاثة أنواع من آسماك نهر النيل القطية من قنا، مصر باستعمال مؤشرات التضاعف العشوائي متعدد الأشكال لسلسلة الدنا (RAPD)

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أخضعت المادة الوراثية (DNA) لثلاثة أنواع من آسماك نهر النيل القطية وهى الشلباية (*Schilbe mystus* Linnaeus, 1758) البياض (*Bagrus bajad* Forsskål, 1775) و القرموط (*Clarias gariepinus* Burchell, 1822) للتنميط الوراثى بواسطة التضاعف العشوائي (RAPD) باستخدام البادئات العشوائية الآتية : **(A-03 ، A-04 ، A-05 ، A-06 ، A-09 ، A-10 ، A-11 و A-12).**

أظهرت البادئات تعددية شكلية بين انواع آسماك نهر النيل القطية قيد الدراسة و سجلت كل البادئات حزم مؤشرة فريدة مختلفة تتراوح من ٢٠٠-٢٩٠٠ زوج قاعدة.

أعطت البادئات الثمانية عدد اثنين و خمسون (٥٢) حزمه منها (٥) حزم أُحادِيُّ الشَّكْل (Monomorphic) بمعدل تباين احادى بنسبة 9.6 % و سبع واربعون (٤٧) كانت متعددة الشكل (polymorphic) مع معدل تباينى متعدد بنسبة 90.38% .كان أعلى معدل للتردد الحزمى لسمكة القرموط مقارنة بالشلباية و البياض بينما سجل البادئ A-06 اعلى معدل للتردد الحزمى بالنسبة للبادئات الآخرى المستخدمة.

وفقا لمصفوفة معامل البعد الوراثي (معامل Nei ٧٢) والتحليل العنقودي باستخدام طريقة المجموعة الزوجية غير الموزونة مع المتوسط الحسابي (UPGMA)، وضح الآرتباط الوراثى بين آسماك نهر النيل القطية المدروسة بعضها لبعض ولها سلف مشترك، لكن كل من سمكتى الشلباية و البياض اقرب وراثيا. ان انشقاق سمكة القرموط مكوناً مجموعة مستقلة و شقيقة لمجموعة سمكتى الشلباية و البياض ربما يعزى الى نمطها الأحادى فى نسبها التطورى.

اقترحت الدراسة وجود تغايرات وراثية بين آسماك نهر النيل القطية الثلاثة مع ارتفاع درجة التقارب الوراثى بين سمكتى الشلباية و البياض و الذى ربما يكون مفيداً لبرامج تربيتهم وتنمية استزراعهم السمكى وكذلك تقديم رؤى حول الوضع التصنيفى لهم ضمن الآسماك القطية.