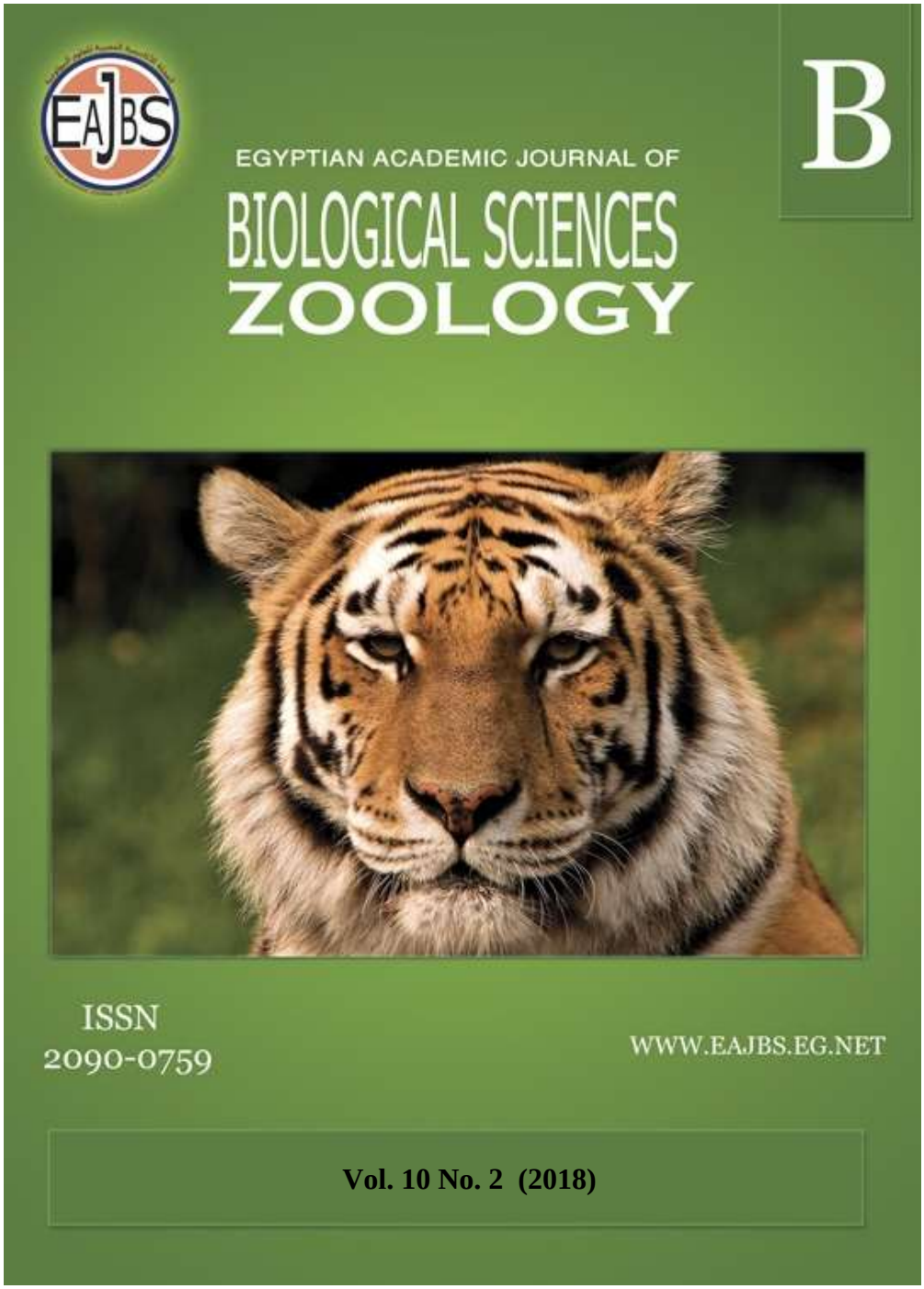

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Sequence Variations and Phylogeny Relationships Among Seven River Nile Teleosti Species from Qena, Egypt Based on Mitochondrial 16S Rrna Gene Sequences

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ABSTRACT

The amplified PCR products from mitochondrial 16S rRNA gene were sequenced for seven River Nile teleost species. The obtained sequences ranged in size from 557 (*Clarias gariepinus*) to 602 (*Bagrus bajad*) nucleotides. Nucleic acid base composition of the 16S rRNA gene sequences were found to be comparable to other vertebrates having higher AT content relative to GC content. The 16S rRNA sequences have variable regions flanked by six conserved stretches, which could be useful for phylogenetic studies. Results indicated the close genetic relations among the studied actinopterygian River Nile fish species, which are likely to share a common ancestor. These new sequence data could be beneficial for effective fish management, conservation and aquaculture purposes. The value in generating sequence data from other family representatives of River Nile telesot species using the 16S rRNA gene primers developed here could provide a useful contribution to future fish phylogenetic and evolutionary studies of fish in this region.

INTRODUCTION

Archaeological evidence of preserved fossils, engravings, hieroglyphic inscriptions of Egyptian tombs and temples indicate various fish species such as Nile tilapia, catfish eels, Nile perch, carps, mullets, tiger fishes, elephant-snout fishes, and moonfish's from Egypt. Some species still form an important component of the Egyptian fishery catches from the River Nile (Duell 1938; Brewer 1989; Danelius *et al.*, 1967). An estimated 128 fish species belonging to different families populate the River Nile (Ali 1984; Witte *et al.*, 2009; van Zwieten *et al.*, 2011; El-Sayed 2016). Fish families of Schilbeidae, Cyprinidae, Cichlidae, Bagaridae, Mormyridae, Latidae and Clariidae within the River Nile possess a number of species with considerable economic and important aquaculture purposes (Abd el Rahman and El Moghraby, 1984; Nwadukwe, 1995; Ayoade, *et al*., 2008; Mohammed and Ali 2008; Alhassan, *et al*., 2011; Mekkawy and Hassan, 2012; El-Saidy, 2015).

The mitochondrial genome (mtDNA) are a relatively small circular double stranded DNA (approximately 16,500 base pairs in fishes), maternally inherited

(Kondo *et al.,* 1990; Gyllestein *et al.,* 1991) and undergo little recombination (Clayton, 1982; Hayashi *et al.,* 1985). Genes of mtDNA are packed together, consisting of short intergenic sequence and characterised by some nucleotide functional overlap, a polycistronic-based transcription, high mutation rate and nucleotide compositions divergence (Cantatore and Saccone, 1987; Song *et al.,* 2005).

Molecular genetic techniques are reliable tools to evaluate DNA sequence variation and sequence divergence among genomes of different taxa (Wolf *et al.,* 1999). The conserved mtDNA 16S rRNA gene has been widely used as a universal molecular marker for phylogenetic reconstruction and taxonomy due to genic hypervariable regions (species-specific signature sequences) for species identification across the animal kingdom (Mitani *et al.,* 2009; Pereira *et al.,* 2010; Kolbert and Persing, 1999; Yang *et al.,* 2014).

In fishes, numerous studies have used 16S DNA sequences to resolve evolutionary relationships, genomic variations, and gene conservation patterns at various taxonomic levels (Hillis and Dixon, 1991; Palumbi and Benzie, 1991; Cunningham *et al.,* 1992; Stackebrandt and Rainey, 1995; Wiley *et al.,* 1998).

Analysis of mtDNA genome sequences offers an important genetic resource, for which the information can be useful for understanding genetic variation and evolutionary studies based on comparing the nucleotide DNA sequence among species. The aim of this work was evaluation of partial 16S rRNA gene sequence regions for molecular sequence variation and phylogenetic relationship among seven River Nile teleost species. These species are *Schilbe mystus* (Linnaeus, 1758), *Barbus bynni* (Forskål, 1775), *Oreochromis niloticus* (Linnaeus, 1758) *Bagrus bajad* (Forsskål, 1775) *Mormyrus kannume* (Forsskål, 1775), *Lates niloticus* (Linnaeus, 1758) and *Clarias gariepinus* (Burchell, 1822).

.

MATERIALS AND METHODS

Fish Sampling and DNA Extraction:

Fish samples (Figure 1) were obtained from Elsihreg fish market, Qena, Egypt in August 2017 (freshwater captured economic fishes) and were stored at -20°C. On return to the laboratory, fish were morphologically identified to species level (Bailey 1994; Bishai and Khalil, 1997; Froese and Pauly, 2017). Tissue samples were removed and preserved in ethanol at -20°C prior to DNA extraction. Total genomic DNA of approximately 30mg was extracted using EZ-10 spin column genomic DNA extraction kit for animal tissue (Bio Basic Inc., Canada) according to the manufacturer's instructions. DNA concentration and purity was assessed using spectrophotometric UV absorption.

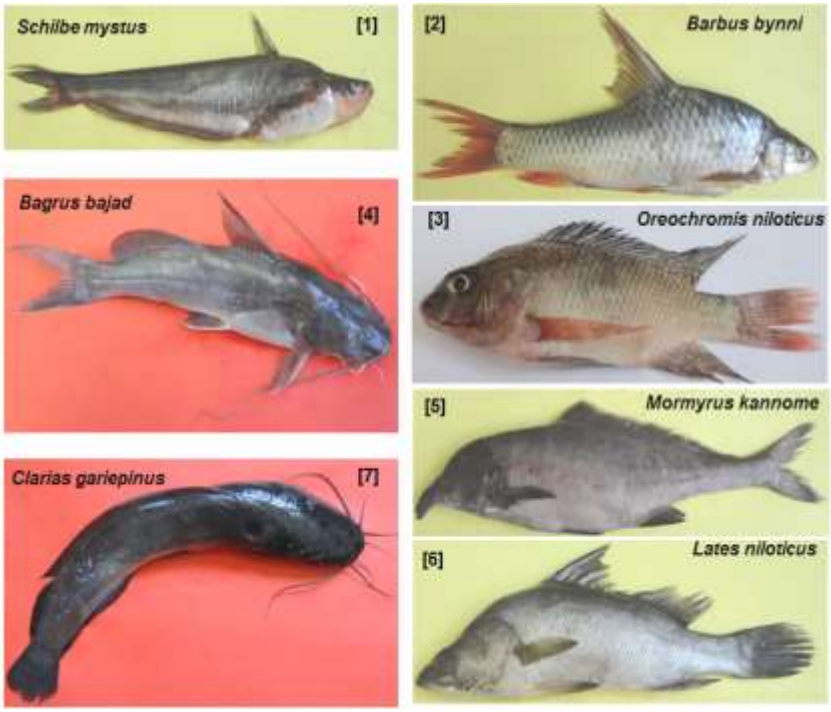

Fig.1 Images for the seven River Nile teleost species investigated in this study. Images 1, 4 and 7 after EL-Mahdi (2018).

Amplification and Sequencing of mtDNA 16S rRNA Gene:

PCR reactions were carried out in a final volume of 25µl including ~50ng of each sample DNA, 10 pM of each primer, and 1 X final concentration of premixed OnePCR^{TM} 2X (GeneDireX Inc, USA). Amplifications were performed in a thermal cycler (Primus 25 advanced, PEQLAB Biotechnologie GmbH) under the following cycling conditions: initial denaturation at 95°C for 2 min, 35 cycles (94°C for 1 min, 56°C for 1 min and 72°C for 2 min), and one cycle at 72°C for 10 min as final extinction. The primers 16Sar-L 5' CGC CTG TTT ACC AAA AAC ATC GCCT 3' and 16Sbr-H 5' CCG GTC TGA ACT CAG ATC ACG T 3' (Palumbi 1996) were used for PCR amplification of the mtDNA. Electrophoresis separation for 8 µl PCR product was done on a 1.5% (w/v) agarose/TAE, and then stained with ethidium bromide (0.5 µg/ml). Molecular weight of the PCR product was estimated by comparison to 100 bp DNA ladder (0.1 µg/µl, Solis BioDyne, Estonia), and PCR products were approximately 640 bp. Gel was photographed under UV light using the Elttrofor M20 SaS Photo-Gel System (Italy). PCR products were sequenced on both strands by Macrogen Inc. (Seoul, Republic of Korea) using the same amplification primers.

Data Analysis:

Viewing and editing of the forward and reverse sequences for each individual were carried out using BIOEDIT sequence alignment version 7.0.5.3 (Hall, 1991) and SnapGene Viewer v3.2.1(Freeware License, SnapGene® software/GSL Biotech). Sequences were analysed for noise peaks and base calling errors and then aligned to complete the targeted DNA fragment. The determined nucleotide sequences were compared to fish mitogenomes, partial/complete 16S gene nucleotide sequences (Genebank/NCBI database). For phylogenetic analysis, corresponding sequences of four species from four other families were retrieved from the downloaded GenBank sequences. The retrieved sequences correspond to sequence regions flanked by

primers used in this study. Representatives from three families; *Nemachilichthys rueppelli* Sykes, 1839 (Cypriniformes; Nemacheilidae); *Xenomystus nigri* Günther, 1868 (Osteoglossiformes, Notopteridae) and *Hiodon alosoides* Rafinesque, 1819 (Hiodontiformes; Hiodontidae) were included for use as an in-group. The *Polypterus bichir bichir* Lacépède 1803 (Polypteriformes; Polypteridae) was chosen as outgroup. Multiple sequence alignments were conducted using Muscle software (Edgar, 2004) implemented in MEGA6 version 6 (Tamura *et al.*, 2013) using default options. The MEGA software was also used for nucleotide base composition and phylogenetic analyses. The best model for nucleotide substitution rate for sequences was selected by Maximum Likelihood fits of 24 different nucleotide substitution models (Nei and Kumar, 2000). The selection was based on Bayesian information criterion (BIC) and Akaike Information Criterion (AIC). Tree reconstruction was carried out using two approaches; the maximum likelihood (ML, Tamura et al., 2004) and the neighbour-joining (NJ) method (Saitou and Nei 1987). Statistical support for branches on the ML and NJ trees was determined by 1000 bootstrap replicates (Felsenstein, 1985). The branch length was used as a measure of the number of substitutions per site.

RESULTS

PCR Amplification of Partial mtDNA16S rRNA Gene :

PCR primers targeting the 16S rRNA gene successfully amplified the desired DNA fragments from the seven species. Different lengths of DNA fragments were amplified in size up to 640 base pairs (Fig. 2).

Sequence Analysis and Nucleotide Composition:

The obtained partial 16S rRNA gene sequences were verified and confirmed as being derived from species under study using a GenBank similarity search. Nucleotide sequences from this study have been deposited in GeneBank with accession numbers: MH458865, MH458866, MH458868, MH458867, MH458869, MH458870, and MH458871 (Table 1).

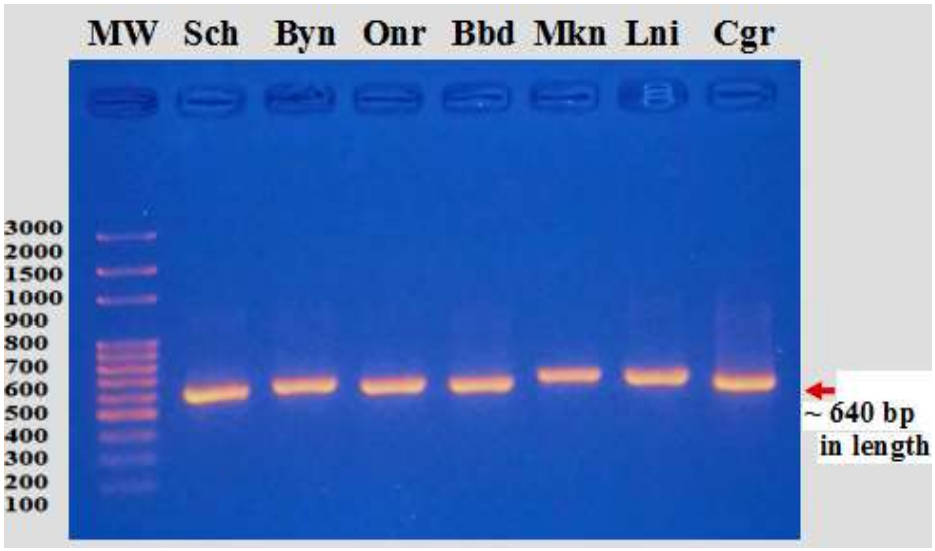

Fig. 2. PCR amplification pattern of mitochondrial 16S rRNA gene in the seven species studied. **Sch**: *Schilbe mystus*; **Byn**: *Barbus bynni*; **Orn**: *Oreochromis niloticus*; **Bbd**: *Bagrus bajad*; **Mkn**: *Mormyrus kannume*; **Lni**: *Lates niloticus*; **Cgr**: *Clarias gariepinus*; **MW**: DNA ladder (100-3000 bp).

Table 1. The GeneBank accession numbers for nucleotide sequences in this study.

Name of species	Order	Family	Accession No.
Present study, the River Nile species partial Sequences			
Schilbe mystus Linnaeus, 1758	Siluriformes	Schilbeidae	MH458865
Barbus bynni Forskål 1775	Cypriniformes	Cyprinidae	MH458866
Oreochromis niloticus Linnaeus, 1758	Perciformes	Cichlidae	MH458868
Bagrus bajad Forsskål, 1775	Siluriformes	Bagaridae	MH458867
Mormyrus kannume Forsskål, 1775	Osteoglossiformes	Mormyridae	MH458869
Lates niloticus Linnaeus, 1758	Perciformes	Latidae	MH458870
Clarias gariepinus Burchell, 1822	Siluriformes	Clariidae	MH458871
The Genebank/NCBI Sequences used			
Nemachilichthys rueppelli Sykes, 1839	Cypriniformes	Nemacheilidae	AP011305.1
Xenomystus nigri Günther, 1868	Osteoglossiformes	Notopteridae	NC_012715.1
Hiodon alosoides Rafinesque, 1819	Hiodontiformes	Hiodontidae	AP004356.2
Polypterus bichir bichir Lacépède, 1803	Polypteriformes	Polypteridae	HM143927.1

After excluding the forward and reverse primers sequence regions, partial 16S rRNA sequences of varies nucleotide lengths were obtained. The resulting fragments were 557 bp (*Clarias gariepinus*), 560 bp (*Schilbe mystus*), 573 bp (*Barbus bynni*), 573 bp (*Oreochromis niloticus*), 600 bp (*Momyrus kannume*), 601 bp (*Lates niloticus*) and 602 bp (*Bagrus bajad*). Multiple alignments of partial 16S rRNA gene sequences from the seven River Nile fish species produced an average nucleotide length of 575.1 base pairs (ranging from 557 bp in *Clarias gariepinus* to 602 bp in *Bagrus bajad)* and a consensus length of 627 sites (Table 2, Fig. 3) which included base pairs, and gaps with 39 indel (insertion/deletion) sites.

The average nucleotides composition (Table 2) was T= 22.4%, C=24.3%, A=30.8% and G= 22.5%. Overall, the A+T=53.19% and G+C= 46.81% showed a bias towards AT content. Analysis of 627 sites of the 16S rRNA gene revealed 379 (60.44%) conserved nucleotides and 209 (33.33%) variable nucleotides. From the variable nucleotides, 110 (17.54%) were parsimony informative, and 95 (15.15%) were singletons. A majority of the 16S rRNA gene was conserved (60.44%), but sequence divergence of 33.33% was detected. Nucleotide analysis also showed that part of 16S ribosomal gene analysed contains 6 conserved regions (underlined, Fig. 3) and variable sequences regions flanked by primer sequences.

Table 2. Nucleotide constitution for part of mitochomderial 16S gene sequences analysed for seven 7 River Nile teleosts species. C= conserved; V= variable; PI= parsimony informative; S= singleton sites

Species/Nucleotide constitution	T%	C %	A%	G%	Total	G+C %	A+T%	C	V	PI	S
Schilbe mystus	21.8	23.6	31.6	23.0	560.0	46.61	53.39	379	209	110	95
Barbus bynni	21.6	24.1	31.8	22.5	573.0	46.60	53.40				
Oreochromis niloticus	23.0	25.0	29.7	22.3	573.0	47.29	52.71				
Bagrus bajad	24.4	23.0	30.1	22.6	562.0	45.55	54.45				
Momyrus kannume	19.8	25.8	31.3	23.0	600.0	48.83	51.17				
Lates niloticus	24.5	24.1	29.8	21.6	601.0	45.76	54.24				
Clarias gariepinus	21.7	24.4	31.2	22.6	557.0	47.04	52.96				
Average.	22.4	24.3	30.8	22.5	575.1	46.81	53.19				

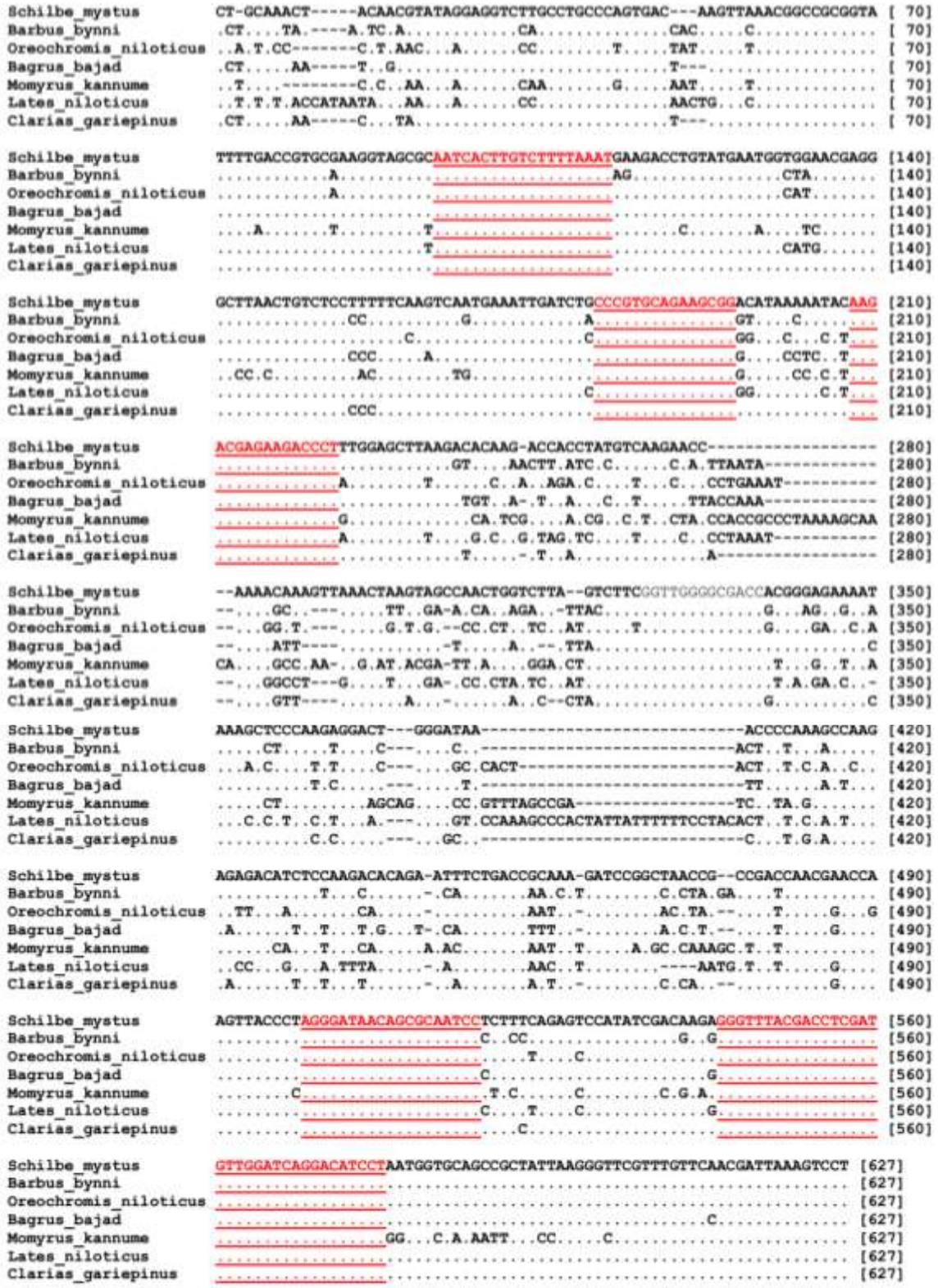

Fig. 3. Aligned partial sequences of cytochrome-*b* gene among the investigated seven River Nile teleost fishes under study. The sequences from the sense-strand and sequence identities are designated by dots. The conserved regions are underlined.

Molecular Phylogenetic Analysis:

For molecular phylogentic analysis, a total of eleven sequences (seven analysed in the present study, and 4 retrieved from Genbank) were used. All trees were rooted using Polypterus *bichir bichir* (Polypteridae) as an outgroup. The GTR+G model was selected using MEGA6 as the most appropriate nucleotide substitution model (BIC= 5497.969; AIC= 5311.844; lnL= -2627.780; transition/transversion bias (R)= 2.79; (+G) = 0.26; Nucleotide frequencies : f(A), f(T), f(C), and f(G), were 0.296, 0.225, 0.243, 0.236 receptively).

The pairwise genetic distances among the eleven sequences from actinopterygian species computed by the ML/GTR+G model with rate variation among sites modelled with a gamma distribution (shape parameter = 0.26) is shown in Table 3. The distance values among represented species ranged from 0.094 (*Bagrus bajad* with *clarias gariepinus*) to 0.668 (*Bagrus bajad* with *Polypterus bichir bichir*). Between the seven River Nile teleosts amplified in this study, the highest genetic distance was between *Bagrus bajad* and *Momyrus kannume* (0.558), whilst the lowest was between *Schilbe mystus* and *Clarias gariepinus* (0.071), and also between *Bagrus bajad* and *Clarias gariepinus* (0.094).

Table 3. Pair wise genetic distance involving 10 nucleotide sequences (7 investigated River Nile teleost species + 3 retrieved sequences) and conducted using the Maximum Composite Likelihood model. The rate variation among sites was modelled with a gamma distribution (shape parameter = 0.26).

	Species name	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	*Schilbe mystus*	---										
2	*Barbus bynni*	0.216	----									
3	*Oreochromis niloticus*	0.254	0.318	---								
4	*Bagrus bajad*	0.129	0.225	0.341	---							
5	*Momyrus kannume*	0.428	0.475	0.539	0.558	---						
6	*Lates niloticus*	0.310	0.354	0.141	0.358	0.509	---					
7	*Clarias gariepinus*	0.071	0.188	0.265	0.094	0.473	0.329	---				
8	*Polypterus bichir bichir*	0.604	0.529	0.554	0.668	0.613	0.571	0.626	---			
9	*Nemachilichthys rueppelli*	0.262	0.169	0.431	0.264	0.558	0.386	0.230	0.503	---		
10	*Xenomystus nigri*	0.480	0.467	0.493	0.577	0.179	0.445	0.468	0.596	0.546	---	
11	*Hiodon alosoides*	0.284	0.258	0.265	0.342	0.273	0.365	0.275	0.469	0.372	0.322	---

The ML/GTR+G model produced a phylogenetic tree (Fig. 4) with highest log likelihood -2627.6851, based on pairwise evolutionary distances (Table 2). In general, the bootstrap confidence levels were high for all nodes within the tree. The phylogenetic tree showed two major clades. Clade A showed the Siluriformes catfish species of families Schilbidae, Bagridae, and Claridae with a high bootstrap support value of 97, Cypriniformes species including *Barbus bynni* (cyprinidae) and *Nemachilichthys rueppelli* (nemachilichydae) clustered together with support of 78, and perciformes species, including *Oreochromis niloticus* (cichlidae) and *lates niloticus* (Lattidae) were clustered together with a high branch support value of 100. A second, Clade B, was also strongly supported and contains the Hiodontiformes; *Hiodon alosoides* (Hiodontidae) and both Osteoglossiformes *Mormyrus kannume* (Mormyridae) and *Xenomystus nigri* (Notopteridae) species. Also, the tree revealed two evolutionary lineages where the investigated River Nile species and the primitive

Hiodon alosoides clustered according to their genetic closeness as supported by evolutionary pairwise distances (Table 3).

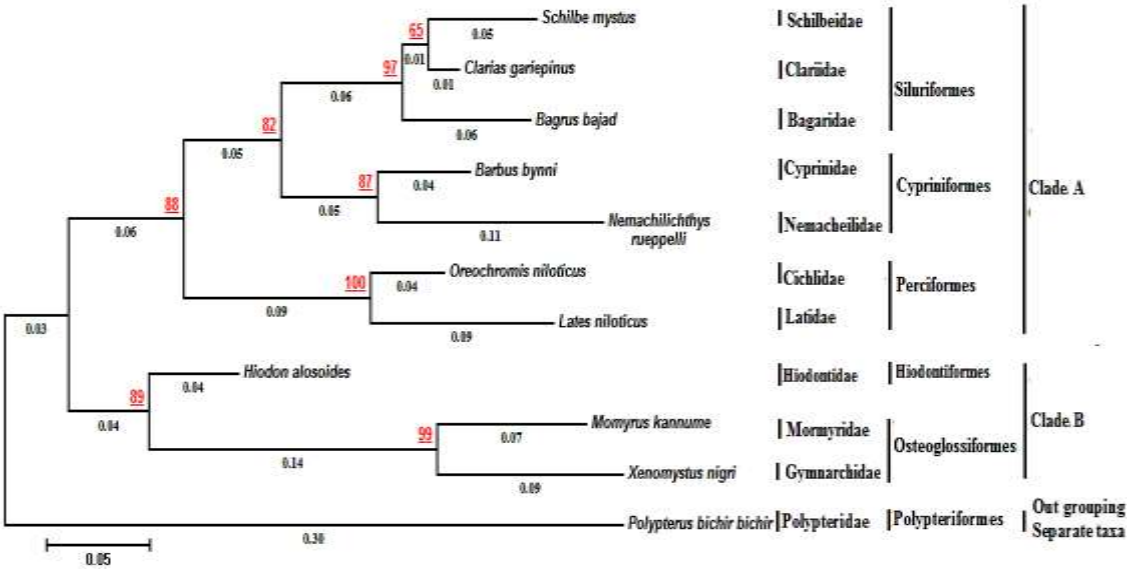

Fig. 4. Molecular phylogenetic analysis by the maximum likelihood method/GTR+G model based on partial 16S gene sequences of seven River Nile teleost species and other related actinopterygians species. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (below the branches) and a discrete Gamma distribution was used to model evolutionary rate differences among sites (+G, parameter = 0.26). the bootstrap support of 1000 replicates is shown next to the branches

The NJ-based GTR+R model (Fig. 5) with sum of branch length 1.52848800 based on pairwise distance estimated ML resulted in a tree with equivalent topology to the ML tree, where the bootstrap confidence levels were high for all nodes within the tree. This supports clear groupings between similar/related species, which would have similar evolutionary lineages.

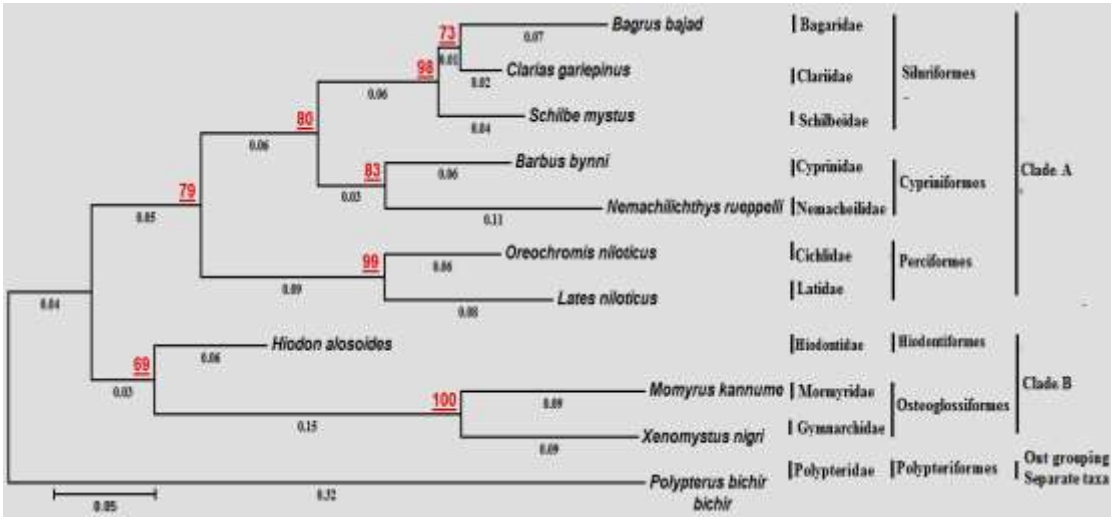

Fig. 5. Molecular phylogenetic analysis by NJ method using 16S rRNA gene partial sequences of seven fish species based on evolutionary distance computed using the maximum composite likelihood method. Branch lengths (above the branches) in the same units of the number of base substitutions per site and the bootstrap support of 1000 replicates is shown next to the branches

DISCUSSION

Amplification and DNA Sequence Analysis:

In this study, the mitochondrial 16S gene fragments were sequenced for seven River Nile teleost species. The mtDNA 16S rRNA gene primers were sensitive and successfully amplified the targeted DNA fragments for each species. Similar 16S regions generated fragments by the same primer pair in other species have been reported (Lee *et al.*, 2014; Quraishi *et al.*, 2015; Carvalho *et al.*, 2004).

Alignment analysis of a total 627 base pairs revealed indels (insertion/deletion) among the species, which was confirmed by amplification of various sequences lengths for the DNA fragment flanked by the primers sequences. Sequence length differences and variations in the region of the mitochondrial 16S gene being analysed may due to the presence of indels among partial sequences of species under study. As reported, the presence of indels are the main sources of structural variation commonly found across the genomes of diverse species (Smit *et al.*, 2007).

The studied 16 rRNA gene sequence showed both conserved and variable regions. This could be responsible for the differences in sequence length generated. In addition, this may allow selection of appropriate taxa-specific primers for phylogenetic applications. Several studies have reported the usefulness of highly conserved sequencing flanking high variable sequence regions in phylogenetic studies in diverse organisms and have recommended their selection as potential primer-binding sites (Smit *et al.*, 2007; Slatkin and Hudson,1991).

Related species have similar DNA fragments than those that are distant depending on the level of DNA base constitutions. The results here demonstrate high level of nucleotide sequence identity in the 16S gene part being analysed for the species under study (see alignment, Fig.3). This suggests their close genetic and evolutionary relationship and may reflect functional sequence and structural relationships. As reported, organisms with similar/identical sequences are closely related in comparison to those are not related (Zeigler 2003; Gadagkar et al., 2005). Nucleotide composition frequencies confirmed a nucleotide preference in bias of adenine and a nearly similar bias towards thymine, cytocine and guanine where favouritism towards adenine increased the AT content, which is supported by other fish molecular phylogenetic studies (Asakawa *et al.*, 1991; Lakra et al., 2009; Mohanty *et al.*, 2013; Lakra *et al.*, 2013; Wang *et al*., 2016). Multiple sequence alignment of a total of 627 nucleotide of the 16S gene studied region reveals a favouritism towards conservations by 60.48% for part of the 16S gene analysed with probable sequence divergence of ~ 33.44%. As reported, the 16S ribosomal gene is fairly conserved and therefore often used to inspect the relationships among different species and genera (Orrell *et al.*, 2004; Mitani *et al.*, 2009).

Molecular Phylogeny and Evolutionary Relationship:

Phylogenetically, related species tend to be grouped together due to having shared inherited DNA sequence characters. Phylogenetic analysis based on mtDNA16S rRNA gene partial sequences using ML/GTR+G model and NJ trees (Figs 4, 5) demonstrated similar tree topologies and showed clusters of genetically similar/related species assigned closely and having common ancestor. However, the degree of the genetic closeness varies, which depends on the shared genetic constitutions as indicated by the nucleotide identities shown. In fact, the phylogenetic trees indicate that species with high sequence identity are clustered together or closely clustered. Both tree architectures support data of evolutionary pairwise

distances computed based on mitochondrial 16S ribosomal gene sequence information (Table 3). As reported, genetic divergence and phylogenetic relationships among related species/taxa can be delineated from informational DNA contents (Kocher *et.al.,* 1989; Thomas *et al*, 1989; Infante *et al.,* 2004).

Conclusion:

The mitochondrial 16S gene fragments for seven River Nile teleost species were sequenced and analysed for molecular sequence variation and phylogenetic patterns. The studied 16S rRNA gene DNA sequence showed the presence of both conserved and variable regions, which supported the gene's suitability as a DNA genetic marker in fish phylogenetic studies. The sequence data generated are reliable and convincing and reflect the evolutionary relationships and phylogenetic patterns among the fish species under study. Results of the current study indicate the close genetic relationship among the eleven species of actinopterygians (including those included in the present study), which may be beneficial for effective management, conservation resources, and aquaculture purposes. Data obtained makes a beneficial contribution to further actinoptergian molecular phylogeny studies.

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ARABIC SUMMERY

تباينات التتابع النيكليوتيدى وعلاقات التطور السلالاتى بين سبعة انواع من أسماك نهر النيل العظمية، قنا، مصر استنادا الى متواليات التتابع النيكليوتيدى للمورث الميتوكوندريى ١٦S rRNA

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تناولت الدراسة تحديد متواليات تتابع القواعد النتروجينية (التسلسل النيكليوتيدى) للمورث الميتوكوندريى 16S rRNA باستعمال تقانة تفاعل البلمرة المتسلسل (PCR) لسبعة انواع من اسماك نهر النيل العظمية وهى الشلباية (*Schilbe mystus*, Linnaeus, 1758) ، البنى (*Barbus bynni* Forskål, 1775)، البلطى النيلى (*Oreochromis niloticus* , Linnaeus, 1758) البياض النيلى (*Bagrus bajad*, Forsskål, 1775)، البويز(*Mormyrus kannume*, Forsskål, 1775) ، قشر البياض (*Lates niloticus*, Linnaeus, 1758) بالآضافة الى القرموط الافريقى (*Clarias gariepinus*, Burchell, 1822)

أعطت الدراسة متواليات مختلفة الآوزان الجزيئية، تراوحت ما بين ٥٧٧ زوج قاعدة للقرموط الآفريقى الى ٦٠٢ زوج قاعدة للبياض النيلى وأوضحت الدراسة تماثل محتوى التتابع النيوكليوتيدي للمورث الميتوكوندريى 16S rRNA للآسماك المدروسة مع الحيوانات الفقارية الآخرى حيث ارتفاع أعلى لزوج القاعدة AT مقارنا مع زوج القاعدة.CG

لقد دلت الدراسة على ان متواليات التتابع النيكليوتيدي للمورث الميتوكوندريى 16S rRNA فى الاسماك قيد الدراسة تحتوى على مناطق تتابع نيكليوتيدى متغيرة و محصورة بين ٦ مناطق ثابتة، التى ربما تعزى لكونها مفيدة لدراسة العلاقات التطورية الوراثية. أشارت النتائج ايضا إلى العلاقات الوراثية الوطيدة لشعاعيات الزعانف من اسماك نهر النيل قيد الدراسة حيث من المحتمل لها سلف مشترك.

و تجدر الآشارة الى المتواليات النيكليوتيدية المستحصله من هذه الدراسة يمكن أن تكون مفيدة فى تنمية و ادارة الثروة السمكية لتقديم رؤى وراثية مستقبليه لبرامج تربية و تحسين الآستزراع السمكى.

نوهت الدراسة الى ان الحصول على متواليات التسلسل النيكيولوتيدى لعائلات اخرى من اسماك نهر النيل العظمية و ذلك باستخدام بادئات المورث الميتوكوندريى 16S rRNA المستخدمة فى تلك الدراسة يعتبر مساهمة فعالة لدراسات مستقبلية عن العلاقات التطورية الوراثية للاسماك في هذه المنطقة.