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Molecular characterization of growth hormone genes in Egyptian Tilapias

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ABSTRACT

This study presented growth hormone (GH) gene of three fish species in Egypt namely; *Oreochromis niloticus* (KT387598 accession number), *Sarotherodon galilaeus* (KT387599 accession number) and *Tilapia zillii* (KT387600 accession number) which belong to cichlids. The tilapiine species growth hormone (GH) genes were isolated and sequenced following amplification from genomic DNA by the polymerase chain reaction (PCR) technique. The sequences of GH gene were analyzed by GENEIOUS program (V8.1) software. The gene lengths were variable among the three studied species which ranged from 1572bp to 1601bp and consisted of six exons and five introns. Was also described a novel polymorphism of the six exons and the five introns fragments. Exons showed 26 nucleotide polymorphisms (NPs) while the total number of NPs in introns was 37. A comparison between the three studied species indicated that *T. zillii* species was totally different from the others. It had three insertions and nine substitutions which were not existed in the other species. The sequencing predicted a polypeptide of 204 amino acids. The GH amino acid sequence of tilapiine species exhibited the homology in all amino acids expect only four amino acids which were different [Isoleucine (I)> Threonine (T), Glycine (G)> Arginine (R), Leucine (L)> Lysine (K) and Leucine (L)> Valine (V)] in 78, 97, 132 and 133 sites, respectively; which all differences were only found in *T. zillii* species. In the phylogenetic tree the single outlier was *T. zillii* species.

Keywords: Characterization, Growth Hormone, Molecular, Egyptian Tilapias.

INTRODUCTION

Tilapia is a generic term used to designate a group of commercially important food fish belonging to the Cichlidae family; Cichlids are classified in the large order Perciformes, which consists of three aqua-cultural important genera; *Oreochromis niloticus*, *Sarotherodon* and *Tilapia*, which they inhabit in the fresh and brackish waters.

Tilapias have been introduced in many countries around the world. It has originated since more than 4000 years ago in Egypt. The first culture of tilapia was conducted in Kenya in at 1924. Of the 70 species of tilapias, only nine are used in farming and of these, only Nile tilapia (*Oreochromis niloticus*) is the main cultured species. These species is being farmed in about 85 countries worldwide (FAO, 2002). It grows to a maximum length of 62 cm (average size is 20 cm), and weigh is 3.65 kg (at an estimated 9 years of age; Bwanika *et al.,* 2004). *Sarotherodon galilaeus* growth range was about 16-34 cm in length and a maximum published weight was 1.6 kg (Stiassny *et al*., 2007). Meanwhile, *Tilapia zillii* grows up to 30 cm as a common length (max length is 40 cm) (Van Oijen, 1995) and maximum published weight was 300 g (Kapetsky & Petr, 1984).

Growth hormone (GH) has many important physiological roles in the control of growth, metabolism and reproduction (Glass, 2003, 2005; Ma *et al*., 2007; Velloso, 2008; Wood *et al.,* 2005). It also called somatotropin (ST), which is synthesized and secreted by the somatotrope cells of the anterior pituitary gland (Agellon *et al.,* 1988; Reinecke *et al*., 2005; Yowe & Epping, 1995). In most species, GH consists of 191 amino acids and contains two disulphide bonds that maintain its three dimensional structure. The amino acid sequence of GH is similar to prolactin (PRL) and placental lactogen (PL). PRL, PL and GH have overlapping biological actions (Dänicke, 2006).The GH gene in various fish species has been extensively studied for potential applications with the target to enhance the growth rate of these species in aquaculture (Pendón *et al*., 1994).

The current study aimed to investigate the genetic aspects that contribute in controlling the growth trait through the sequencing of the GH gene among three Egyptian tilapias.

MATERIALS AND METHODS

Materials

Based on the most abundant tilapias in Borolus Lake, at Kafr El-Sheikh Governorate (Egypt), three tilapia species namely *O. niloticus*, *S. galilaeus* and *T. zillii* were collected in the current study.

Methods

Growth hormone gene sequencing

DNA was extracted from ten samples for eact per species according to Lopera-Barrero *et al.* (2008). Standard PCR reaction was performed using newly designed primers based on *O. niloticus* GH gene (GenBank accession M84774.1)**.** Primers were designed to amplify the growth hormone gene in three sets of primer combinations to cover the CDS region including introns (Table 1).

Table 1: Primer combination sets, names, sequences (5' → 3'), expected (bp) and melting temperature (Tm) of the newly designed primers used in GH gene sequencing.

Combination	Name	Sequence (5' → 3')	Tm	Expected (bp)
Set 1	GHTILAPIA-F0	CAGAACCACCGACTCACATCATA	60	764
	GHTILAPIA-R1	TTGTTGAGCTGACGTTGCTCCT	62	
Set 2	GHTILAPIA-F1	AGGAGCAACGTCAGCTCAACAA	62	460
	GHTILAPIA-R2	TTCTCTGCTTCATCCTGATTGGC	60	
Set 3	GHTILAPIA-F2	GCCAATCAGGATGAAGCAGAGAA	60	498
	GHTILAPIA-R0	AGACTCCACACATCAATGCAACAC	61	

PCR reaction for each amplified region was prepared in a 50 µl of 2x MyTaq Master Mix (Bioline, UK). PCR program was initiated with adenaturation step at 95 °C for 5 min, followed by 35 cycles including 94˚C for 45 sec, 60˚C for 1 min and 72˚C for 1 min, with a final extension at 72˚C for 5 min.To test the PCR success, its product were loaded on a 1.5 % agarose gel containing a 1 µl of ethidium bromide (EthBr) using tris boric EDTA buffer (TBE) as a running buffer (Peacock, & Dingman 1968). Successful PCR products (three fragments /species) were cleaned and concentrated using Gene JET PCR Purification Kit as #K0701. Then cleaned fragments were sequenced by sequencing service (Macrogen, Netherlands). The obtained sequences of GH gene of the three tilapia species

were deposited in the GenBank with the accession numbers; KT387598 for *O. niloticus*, KT387599 for *S. galilaeus* and KT387600 for *T. zillii*. http://www.ncbi.nlm.nih.gov/genbank/

Data analysis

Trace files of three tilapias species were obtained from the fragment analysis service and sorted according to sample order. All trace files were imported on inserted into the GENEIOUS program (V8.1) to automatically analyze the generated peaks and generate in the following sequence. Firstly, the three primers were designed for the GH gene, each one of them consist of forward and reverse sequences, which were collected pairwise alignment and the three fragments were collected from each primer. Secondly, a multiple alignment among them and BLAST search were done. After that, the sequence of the *O. niloticus* GH gene (M84774.1) from the Gene Bank was added as a reference for the three studied species. Then, the three studied species sequences were aligned with the reference sequence. Tow phylogenetic trees were made based on GH and their amino acids sequence. By using GENEIOUS program (V8.1) software. Introns, exons, annotations and nucleotide polymorphisms were specified. Comparison between the studied species with the similar species in the reference of GenBank by using MEGA6 program the phylogenetic trees were constructed, and generated using neighbor joining (NJ) method and confirmed by both maximum likelihood (ML) and minimum evolution (ME) methods, while the trees were tested using boot strap test of phylogeny method (many times).

RESULTS AND DISCUSSION

Growth hormone (GH) gene has a vital role in controlling the growth performance in all organisms (Gross & Nilsson, 1995; Gross *et al*., 1996). The evidence suggests that GH is the ancestral hormone in the molecular evolution of the GH/PRL/SL family and that the endocrine mechanism for growth stimulation was established at an early stage in the evolution of vertebrates (Moriyama *et al*., 2006). Therefore, a comparative analysis study of the GH gene among the studied tilapias species (*Oreochromis niloticus*, *Sarotherodon galilaeus* and *Tilapia zillii*) would help to find the explanation for the differences in the adult fish size and weight of *T. zillii* compared to its relative's *O. niloticus* and *S. galilaeus* (Van Oijen, 1995). For that reason, in the current study the growth hormone gene was isolated and analyzed (structure, features, transcription regulation…etc.) from the three tilapias species abundant in the Egyptian environment.

GH gene sequences

Gene length polymorphism

The structure of GH gene in the three tilapias species was studied. The gene lengths were variable among the three studied species which ranged from 1,572 bp to 1,601bp. It was found that the GH gene is composed of six exons and five introns (Ber & Daniel, 1992). All these exons were in the same length among the studied species. A comparison among the species intron lengths indicated that the first intron is the shortest one (84 – 96 bp), while the second intron is the longest one (403 – 436 bp). It was observed that all introns started with a GT dinucleotide and ended with an AG dinucleotide in accordance with the universal role (Wu, & Krainer, 1999). As a result of the differences in intron lengths, the lengths of GH gene were differed among the studied species. Alignment of the three sequenced gene species showed a total length of 1,654 bp in which 1,572 were identical

(88.1%), while the pairwise identity was 93.5%. Nile tilapia (*O. niloticus*) showed an exact match to the reference sequence (Ber & Daniel 1992) and showed the longest sequence which was 1,601 bp, followed by *T. zillii* and *S. galilaeus* which were 1,592 and 1,572 bp, respectively (Table 2).

Table 2: The start and end regions (bp) of each exon and intron among the three studied tilapias.

Features	*O. niloticus* (KT387598)	*S. galilaeus* (KT387599)	*T. zillii* (KT387600)
5' Partial Exon1	1-10 (10)	1-10 (10)	1-10 (10)
Intron1	11-106 (96)	11-94 (84)	11-102 (92)
Exon2	107-240 (134)	95-228 (134)	103-236 (134)
Intron2	241-660 (420)	229-631 (403)	237-672 (436)
Exon3	661-777 (117)	632-748 (117)	673-789 (117)
Intron3	778-900 (123)	749-871 (123)	790-891 (102)
Exon4	901-1,044 (144)	872-1,015 (144)	892-1,035 (144)
Intron4	1,045-1,116 (72)	1,016-1,087 (72)	1,036-1,107 (72)
Exon5	1,117-1,263 (147)	1,088-1,234 (147)	1,108-1,254 (147)
Intron5	1,264-1,342 (79)	1,235-1,313 (79)	1,255-1,333 (79)
Exon6	1,343-1,601 (259)	1,314-1,572 (259)	1,334-1,592 (259)

Insertions and Deletions (Indels)

Concerning the insertions and the deletions (Indels) recording, 56 indel code (CAAAGTAAACCG = 12 bp), 269 indel code (TCAGACAGACAAGAGCTGACCAACAC-CTTCA = 31 bp) and 609 indel code (CTAACACTGG = 10 bp) were found to be unique in *T. zillii*. While 101 indel code (TGTC = 4 bp), 618 indel code (TGTTACCA = 8 bp) and 887 indel code (GTACCAGAGGTACTCTGCCCA = 21 bp) were found in both of *O. niloticus* and *S. galilaeus* but were deleted from *T. zillii.* 105 Indel code (TGTCTGTCTGTC = 12 bp) and 689 indel code (TTCTTTAATTCTACACA = 17 bp) were only found in *O. niloticus* and absent from the other two species (Table 3).

Table 3: Codes, start nucleotide number (min), end nucleotide number (max), length (bp), sequence and presence among the studied species for the GH gene.

Indel Code	Min	Max	Bp	*O. niloticus*: M84774.1	*O. niloticus*	*S. galilaeus*	*T. zillii*
56	56	67	12	-	-	-	CAAAGTAAACCG
101	101	104	4	TGTC			-
105	105	116	12	TGTCTGTCTGTC		-	- -
269	269	299	31	-	-	-	TCAGACAGACAAGAGCTGACCAACACCTTCA
609	609	619	10	-	-	-	CTAACACTGG
618	618	688	8	TGTTACCA			-
689	689	705	17	TTCTTTAATTCTACACA		-	- -
887	887	907	21	GTACCAGAGGTACTCTGCCCA			-

Substitutions

It was observed that, *O. niloticus* reference (M84774.1), *O. niloticus* and *S. galilaeus* sequences were identical, with one exception to *S. galilaeus* which was a single difference at 383 site where the reference sequence was AC which substituted with (>) AT sequence. While, *T. zillii* was totally different from the other species in nine substitutions (383AC> GT, 490AGA> GAG, 802TC> CT, 912AT> CA, 1086CT> AA, 1133TG> CT, 1162CT> GA, 1165TA> GG and 1168AG> GT) (Table 4).

Table 4: Alignment codes, frequencies and ungapped base positions of these substitutions in the three different tilapias species.

Alignment Code	*O. niloticus*: M84774.1	*O. niloticus*	Ungapped bases	*S. galilaeus*	Ungapped bases	*T. zillii*	Ungapped bases
383AC>GT	AC	AC	340	AT	328	GT	367
490AGA>GAG	AGA	AGA	447	AGA	435	GAG	474
802TC>CT	TC	TC	749	TC	720	CT	761
912AT>CA	AT	AT	859	AT	830	CA	850
1086CT>AA	CT	CT	1033	CT	1004	AA	1024
1133TG>CT	TG	TG	1079	TG	1050	CT	1070
1162CT>GA	CT	CT	1109	CT	1080	GA	1100
1165TA>GG	TA	TA	1112	TA	1083	GG	1103
1168AG>GT	AG	AG	1115	AG	1086	GT	1106

Nucleotide polymorphisms

Exon sequences showed 26 nucleotide polymorphisms (NPs). The first partial exon sequence was totally conserved among the compared species. The sixth exon sequence had the highest number (12) of nucleotide polymorphism (NPs). Nucleotide substitutions were seen in the form of 20 transitions (A-G; T-C) and six trans-versions (G-C; G-T) in the exon sequence of the GH gene.

The total number of NPs in the intron sequences were 37. In comparison, the second intron sequence had the highest number (22) of NPs. While only one of NP was in the third intron sequence. Most of these NPs were found in *T. zillii* species. Nucleotide substitutions were seen in the form of 20 transitions (A-G; T-C) and 17 trans-versions (T-A; G-C; G-T) in the intron sequences. The sequences, nucleotides polymorphism (NPs), substitutions, insertions and deletions (Indels) are shown in (Figs. 1, 2 and 3).

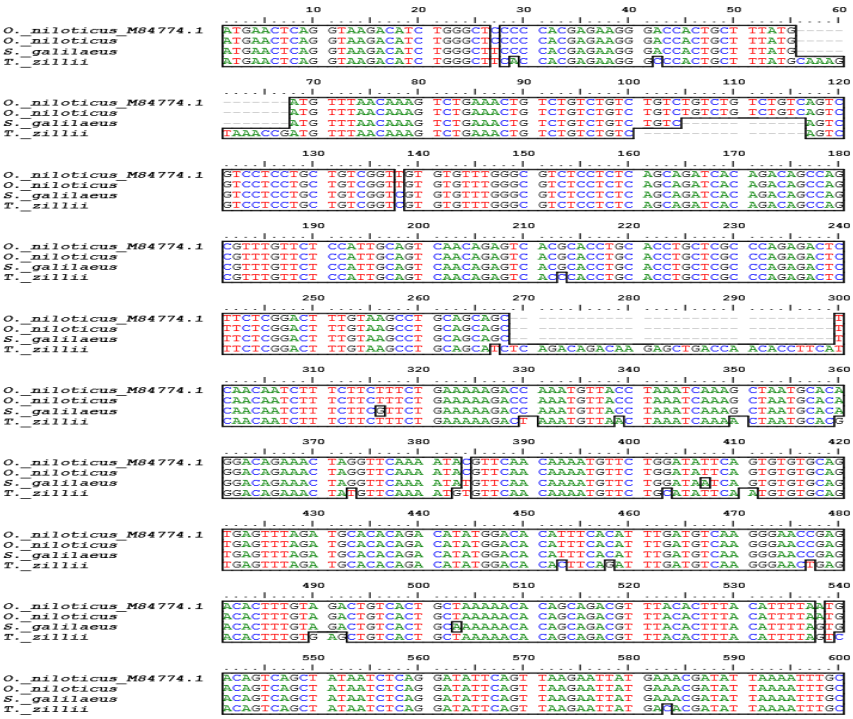

Fig. 1: Comparative of nucleotide alignment for GH gene sequence among tilapias species assembled to the reference sequence of *O. niloticus* (GenBank: M84774.1). Indels are the insertion/deletion sites (gaps) which written as (-) in the nucleotide alignment from 1 pb to 600 pb.

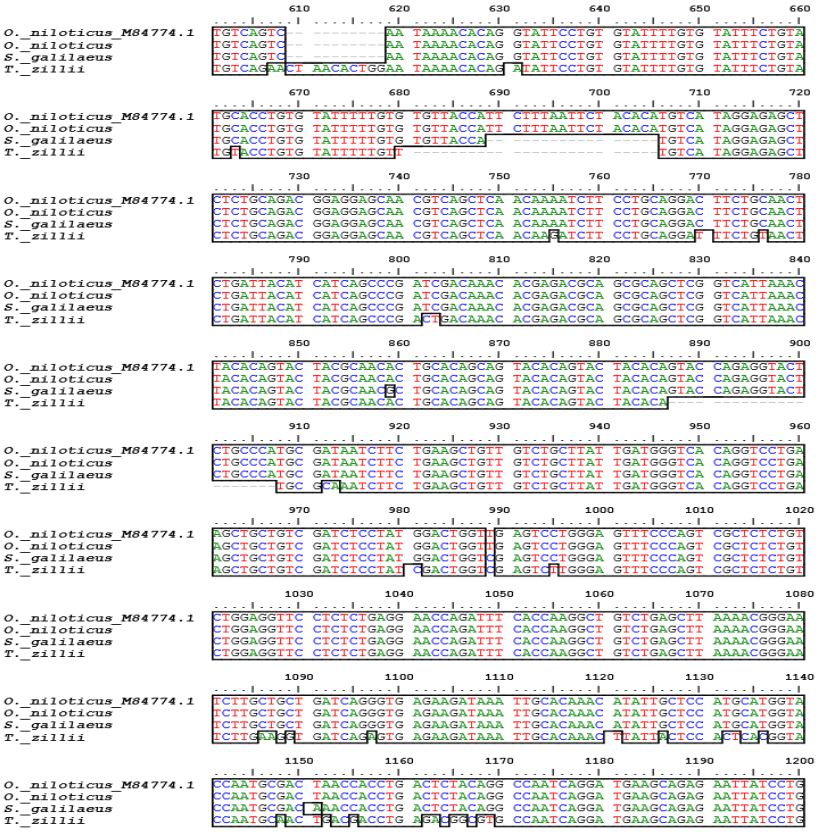

Fig. 2: Comparative of nucleotide alignment for GH gene sequence among tilapias species assembled to the reference sequence of *O. niloticus* (GenBank: M84774.1). Indels are the insertion/deletion sites (gaps) which written as (-) in the nucleotide alignment from 601 pb to 1200 pb.

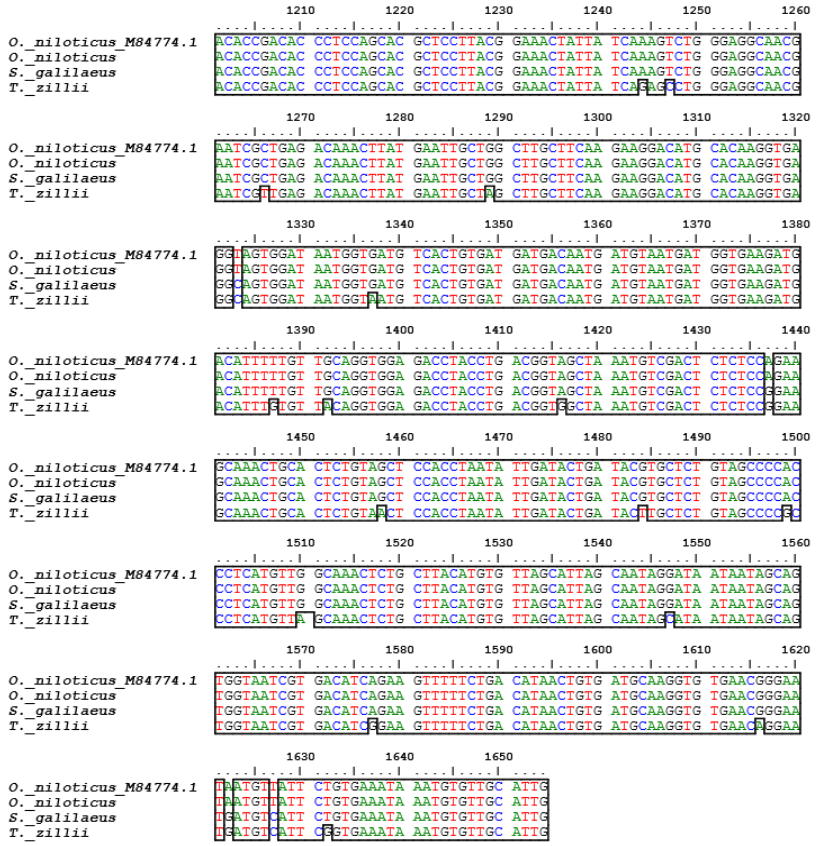

Fig. 3: Comparative of nucleotide alignment for GH gene sequence among tilapias species assembled to the reference sequence of *O. niloticus* (GenBank: M84774.1). Indels are the insertion/deletion sites (gaps) which written as (-) in the nucleotide alignment from 1201 pb to 1654 pb.

Amino acid polymorphisms

The length of individual exon was the same in all the tilapias species with an open reading frame (ORF) of 615 bp (204 amino acids codons +1 stop codon). The sequence started by the start codon of Methionine (M) according to the standard translation table and ended by the codon of Leucine (L). The most frequent amino acid was Leucine (91 time, 14.9%), followed by Serine (78 time, 12.7%), while the least frequent was Tryptophan (3 times, 0.5%) (Table 5).

The GH amino acid sequences of tilapias species exhibited homology in all amino acids expect four amino acids which were different [Isoleucine (I)> Threonine (T), Glycine (G)> Arginine (R), Leucine (L)> Lysine (K) and Leucine (L)> Valine (V)] in 78, 97, 132 and 133 sites, only of in *T. zillii* (Table 6).

Table 5: Amino acids frequency of GH gene from tilapiine species. The lowest and highest amino acids are written in bold.

Amino acid	Abbreviation	Frequency	Percentage %
Tryptophan	**Trp (W)**	**3**	**0.5**
Methionine	Met (M)	6	1.0
Cysteine	Cys (C)	15	2.5
Histidine	His (H)	15	2.5
Proline	Pro (P)	18	2.9
Phenylalanine	Phe (F)	21	3.4
Glycine	Gly (G)	23	3.8
Alanine	Ala (A)	24	3.9
Tyrosine	Tyr (Y)	24	3.5
Lysine	Lys (K)	25	4.1
Aspartic Acid	Asp (D)	27	4.4
Isoleucine	Ile (I)	29	4.7
Asparagine	Asn (N)	30	4.9
Arginine	Arg (R)	34	5.6
Threonine	Thr (T)	34	5.6
Valine	Val (V)	34	5.6
Glutamic Acid	Glu (E)	39	6.4
Glutamine	Gln (Q)	42	6.9
Serine	Ser (S)	78	12.7
Leucine	**Leu (L)**	**91**	**14.9**

Table 6: Differences in amino acids of GH gene sequences among tilapias species.

Amino Acid site	*O. niloticus*	*S. galilaeus*	*T. zillii*
aa78	Isoleucine (I)	Isoleucine (I)	Threonine (T)
aa97	Glycine (G)	Glycine (G)	Arginine (R)
aa132	Leucine (L)	Leucine (L)	Lysine (K)
aa133	Leucine (L)	Leucine (L)	Valine (V)

*aa = Amino Acid

Majority of the detected mutations were located in intron sequences, which ultimately led to divergence in the length of the GH gene. Additionally, *T. zillii* species showed high variation in four amino acid sites against none between *O. niloticus* and *S. galilaeus* species. Based on this fact, this discrepancy might be the logical explanation for the growth delay in *T. zillii* species.

Phylogenetic and evolutionary relationship

By using MEGA6 program the phylogenetic trees for GH gen sequences were made and were generated using neighbor joining (NJ) and confirmed by both maximum likelihood (ML) method and minimum evolution (ME) methods for the three studied species along with approximate sequence obtained from GenBank database. Phylogenetic trees were performed based on both complete sequence (exons and introns) data and coding DNA sequence data (CDS: exons only).

Complete sequence

The evolutionary divergence among the three studied tilapias species based on the complete GH gene sequence was determined including the available GenBank accessions in which the complete GH gene is recorded. The largest genetic divergence was detected between *T. zillii* species and *O. mossambicus* Y11732 accession (0.57), followed by the divergence between *T. zillii* and *O. niloticus* species (0.51). While the smallest genetic divergence was detected between *O. niloticus* M97766 accession and

O. mossambicus Y11732 accession as well as between *O. niloticus* species and *O. mossambicus* Y11732 accession (0.08), followed by *S. galilaeus* and *O. niloticus* species (0.09) (Table 7).

Table 7: Estimates of evolutionary divergence among the three studied tilapias species based on complete GH gene sequence.

Species	*O. niloticus*	*S. galilaeus*	*T. zillii*	*O. niloticus* (M97766)	*O. mossambicus* (Y11732)
O. niloticus	0.00				
S. galilaeus	0.09	0.00			
T. zillii	0.51	0.49	0.00		
***O. niloticus* (M97766)**	0.00	0.09	0.51	0.00	
***O. mossambicus* (Y11732)**	**0.08**	0.14	**0.57**	**0.08**	0.00

The phylogenetic tree based on the complete GH gene sequence was generated as shown in (Fig. 2). The tree shared one main cluster with a single outlier. The main cluster included *O. niloticus* species and *O. niloticus* (GenBank: M97766 accession) together which the bootstrap values of NJ, ML and ME method were 89, 88 and 86, respectively. *O. mossambicus* (GenBank: Y11732 accession) was located together with *O. niloticus* species in the same cluster, which the bootstrap values of NJ and ML methods were 100 and 99 respectively, while *S. galilaeus* species was paraphyletic (sub clade). On the other hand, the only single outlier was *T. zillii* species.

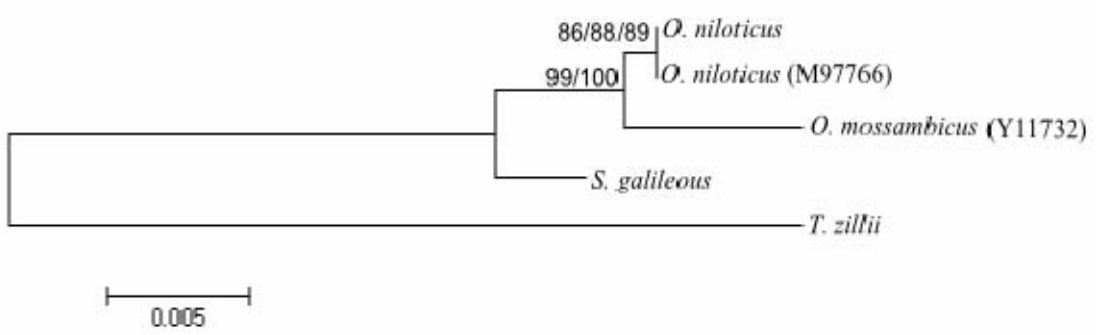

Fig. 2: Phylogenetic relationship tree of the three studied tilapias species based on the complete GH gene.

Complete CDS

The evolutionary divergence among the three studied tilapias species based on the complete CDS was determined including the available GenBank accessions in which the complete coding DNA sequence is recorded. The largest genetic divergence was detected between *T. zillii* species and *O. mossambicus* Y11732 accession (0.49), followed by the divergence between *T. zillii* species and *O. urolepis* EF371465 accession (0.44). While the smallest genetic divergence was detected between *O. niloticus* and *S. galilaeus* species as well as between *S. galilaeus* species and *O. niloticus* (M26916) accession (0.05), followed by *O. niloticus* species and *O. urolepis* EF371465 accession (0.12) (Table 8).

Table 8: Estimates of evolutionary divergence among the three studied tilapias species based on complete CDS.

Species	*O. niloticus*	*S. galilaeus*	*T. zillii*	*O. mossambicus* (AF033805)	*O. urolepis* (EF371465)	*O. niloticus* (M26916)
O. niloticus	0.00					
S. galilaeus	**0.05**	0.00				
T. zillii	0.35	0.30	0.00			
***O. mossambicus* (AF033805)**	0.13	0.18	**0.49**	0.00		
***O. urolepis* (EF371465)**	0.12	0.13	0.44	0.18	0.00	
***O. niloticus* (M26916)**	0.00	**0.05**	0.35	0.13	0.12	0.00

The phylogenetic tree based on complete CDS was generated as shown in (Fig. 3). The tree was divided into two main clusters with a single outlier. The first cluster included *O. niloticus* species and *O. niloticus* (GenBank: M97766) accession together, which the bootstrap values of NJ, ML and ME methods were 57, 56 and 55, respectively. *Oreochromis mossambicus* (GenBank: AF033805) accession and *O. urolepis* (GenBank: EF371465) accession were located together with *O. niloticus* species in the same cluster, which the bootstrap values of NJ, ML and ME methods were 80, 75 and 69, respectively. While the second cluster included *S. galilaeus* and *T. zillii* species which the bootstrap values of NJ, ML and ME methods were 97, 96 and 90, respectively. On the other hand, *T. zillii* species was the only single outlier.

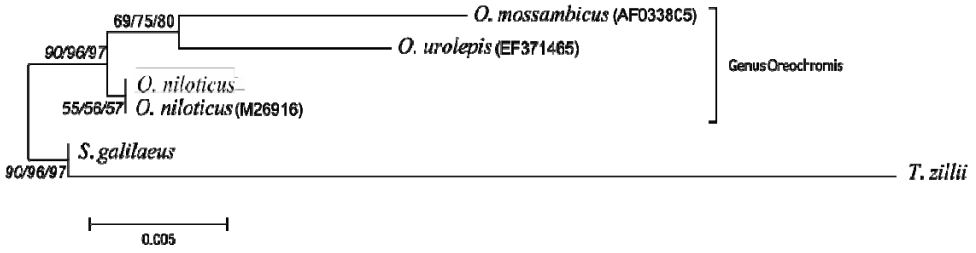

Fig. 3: Phylogenetic relationship tree of the three studied tilapias species based on the complete CDS.

Tilapia zillii taxonomically belongs to the genus Tilapia, while *O. niloticus* belong to genus *Oreochromis* and *S. galilaeus* belongs to genus *Sarotherodon*. Even though each of the three studied tilapias belongs to different genus, it appeared from our results that *T. zillii* species is far more distant to *O. niloticus* species than to *S. galilaeus* species. Schwarzer *et al*., (2009) constructed a phylogenetic tree for cichlid fishes in East African Cichlid using multi-locus DNA analysis and four mitochondrial genes. The phylogenetic tree separated the so called "Tilapia" in a paraphyletic assemblage, and established three new monophyletic groups; "*Oreochromini"*, "*Boreotilapiini*" and "*Austrotilapiini*" they supported the high genetic distance between *T. zillii* species and the other studied fish species.

In conclusion, by studying the GH gene structure, *T. zillii* species was found to be clearly different from the other studied fish species (gene length, interspecies nucleotide polymorphism and variation in amino acid composition). Such assumption,

moreover, was supported by the phylogenetic analysis based on the complete GH gene sequence, which showed a clear genetic divergence between *T. zillii* species and the other studied species.

REFERENCES

Agellon, L., Davies, S., Chen, T. and Powers, D (1988). Structure of a fish (*rainbow trout*) growth hormone gene and its evolutionary implications. Proceedings of the National Academy of Sciences, 85: 5136-40.

Ber, R. and Daniel, V (1992). Structure and sequence of the growth hormone-encoding gene from *Tilapia nilotica*. *Gene*, 113: 245-50.

Bwanika, G., Makanga, B., Kizito, Y., Chapman, L., and Balirwa, J. (2004). Observations on the biology of Nile tilapia, *Oreochromis niloticus* L., in two Ugandan crater lakes. African Journal of Ecology, 42(s1): 93-101.

Dänicke, S. (2006). Hormonal regulation of farm animal growth, K.L. Hossner (Ed.). CABI Publishing (2005). *Animal Feed Science and Technology,* 128: 173-174.

Food and Agriculture Organisation (FAO), (2002). Global Agro-ecological Assessment for Agriculture in the 21st Century: Methodology and Results, *Food and Agriculture Organisation,* Rome, Italy.

Glass, D. J. (2003). Molecular mechanisms modulating muscle mass. *Trends in molecular medicine*, 9:344-50.

Glass, D. J. (2005). Skeletal muscle hypertrophy and atrophy signaling pathways. *The international journal of biochemistry and cell biology*, 37:1974-84.

Gross, R. and Nilsson, J (1995). Application of heteroduplex analysis for detecting variation within the growth hormone 2 gene in brown trout. (*Salmo trutta*). *Heredity (Edinb), 74*(3):286-95.

Gross, R., Stein, H. and Rottmann, O (1996). Detection of allelic variation within the growth hormone gene in common bream using heteroduplex analysis. *Journal of fish biology*, 48:1283-87.

Kapetsky, J. M. and Petr, T (1984). Status of African reservoir fisheries. FAO.

Lopera-Barrero, N. M., Povh, J. A., Ribeiro, R. P., Gomes, P. C., Jacometo, C. B. and Silva Lopes, T. D (2008). Comparación de protocolos de extracción de ADN con muestras de aleta y larva de peces: extracción modificada con cloruro de sodio. Ciencia e investigación agraria, 35:77-86.

Ma, X., Liu, X., Zhang, Y., Zhu, P., Ye, W. and Lin, H. (2007). Two growth hormone receptors in Nile tilapia (*Oreochromis niloticus*): Molecular characterization, tissue distribution and expression profiles in the gonad during the reproductive cycle. Comparative Biochemistry and Physiology Part B: *Biochemistry and Molecular Biology*, 147(2):325-339.

Moriyama, S., Oda, M., Takahashi, A., Sower, S. A. and Kawauchi, H. (2006). Genomic structure of the sea lamprey growth hormone-encoding gene. *General and comparative endocrinology*, 148:33-40.

Peacock, A. C. and Dingman, C. W (1968). Molecular weight estimation and separation of ribonucleic acid by electrophoresis in agarose-acrylamide composite gels. *Biochemistry*, 7:668-74.

Pendón, C., Martínez-Barberá, J. P., Pérez-Sánchez, J., Rodríguez, R. B., Grenett, H. and Valdivia, M. M (1994). Cloning of the sole (*Solea senegalensis*) growth hormone-encoding cDNA. *Gene*, 145: 237-40.

Reinecke, M., Björnsson, B. T., Dickhoff, W. W., Mccormick, S. D., Navarro, I., Power,D. M. and Gutiérrez, J (2005). Growth hormone and insulin-like growth

factors in fish: where we are and where to go. *General and comparative endocrinology,* 142:20-24.

Schwarzer, J., Misof, B., Tautz, D. and Schliewen, U. K (2009). The root of the East African cichlid radiations. *BMC Evolutionary Biology*, 9: 186.

Stiassny, M. L., Teugels, G. G. and Hopkins, C. D (2007). Fresh and Brackish Water Fishes of Lower Guinea, West-Central Africa, IRD Editions.

Van Oijen, M. (1995). Key to Lake Victoria fishes other than haplochromine cichlids. *Fish* stocks and fisheries of Lake Victoria: A handbook for field observations. Samara Publishing Limited, Dyfed, *Great Britain*, pp. 209-300.

Velloso, C. (2008). Regulation of muscle mass by growth hormone and IGF-I. *British journal of pharmacology*, 154:557-68.

Wood, A. W., Duan, C., and Bern, H. A. (2005). Insulin-like growth factor signaling in fish. *International review of cytology*, 243: 215-285.

Wu, Q., & Krainer, A. R. (1999). AT-AC pre-mRNA splicing mechanisms and conservation of minor introns in voltage-gated ion channel genes. *Molecular and cellular biology*, *19*(5):3225-3236.

Yowe, D. L. and Epping, R. J (1995). Cloning of the barramundi growth hormone- encoding gene: a comparative analysis of higher and lower vertebrate GH genes. *Gene*, 162:255-59.

ARABIC SUMMARY

التوصيف الجزيئى لجينات هرمون النمو فى البلطى المصرى

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تم دراسة جين هرمون النمو فى الأنواع الأكثر انتشاراً من البلطى فى مصر وهم (البلطى النيلى و الجاليلى والزيللى). تم استخلاص الDNA وعمل تتابع لجين هرمون النمو لأنواع البلطى تبعاً لتضخيم ال DNA بواسطة تفاعل البلمرة المتسلسل (PCR). وقراءة تتابع جين هرمون النمو عن طريق برنامج GENEIOUS. وكان طول الجين مختلف بين الأنواع المدرسة والذى يتراوح ما بين ١٥٧٢ قاعدة فى الجاليلى إلى ١٦٠١ قاعدة فى البلطى النيلى ويحتوى الجين على ٦ اكسونات و٥ انترونات كما سبق وصفة. تم وصف ايضاً تعدد فى الأشكال المظهرية فى الاكسونات والإنترونات. وأظهرت الإكسونات ٢٦ نوكليوتيدة وكان العدد الكلى في الإنترونات ٣٧. وبالمقارنة بين الأنواع الثلاثة المدروسة وجدنا أن البلطى الزيللى كان مختلفا تماما، فهو يحتوى على ثلاثة مواقع يوجد بها تتابعات من النيوكليوتيدات لم تكن موجودة فى الأنواع الاخرى، وأيضا يختلف عن الأنواع الاخرى في تسعة مواقع متباينة فى قاعدتين.

أما على مستوى البروتين فوجدنا أن جين هرمون النمو يحتوى على ٢٠٤ حمض أمينى متماثلين فى الأنواع المدروسة ماعدا ٤ من الأحماض الأمينية مختلفين فى البلطى الزيللى عن الأنواع الاخرى وهم [الايزوليوسين (I) تحول إلى ثريونين (T) فى الزيللى، جليكاين (G) إلى أرجينين (R)، ليوسين (L) إلى ليسين (K) وليوسين (L) إلى فالين (V)] في المواقع ٧٨ و ٩٧ و ١٣٢ و ١٣٣، على التوالي. وعند عمل شجرة القرابة كان البلطى الزيللى بعيداً عن الأنواع الاخرى.