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A STUDY ON MOLECULAR BIOLOGY OF *TILAPIA* SPECIES AND THEIR HYBRIDS IN THE RIVER NILE, EGYPT.

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ABSTRACT

Morphometric, meristic and DNA ripoprinting analyses of *Tilapia* species and their hybrids inhabiting the River Nile were examined. The obtained morphometric data evoke striking similarities and overlapping among tilapia species. Accordingly it could not able to differentiate tilapia species. The obtained data of meristic characters reveal that tilapia species could be differentiated into four species (*Oreochromis niloticus, O. auraeus, Sarotherodon galilaeus* and *Tilapia zillii).* The lateral line scales differed significantly among the four-studied tilapia species. While the number of fin rays in the dorsal and anal fins differentiate significantly three *Tilapia* species (Confusion between *O. niloticus* and *O. auraeus*). Furthermore, the study used the technique of restriction fragment length polymorphism (RFLP) of nuclear small sub-unit ribosomal RNA (18SsrRNA) gene. The PCR-RFLP data provide a unique pattern for each examined species with a specific restriction enzyme. So, it could be possible to detect two hybrids of tilapia fish which are given H1 and H2 symbols. The endonucleases *Sac*II and *Apa*I differentiate H1 and H2, respectively. Furthermore, the analyses of PCR-RFLP data indicate that the H1 are closer to *O. niloticus* and *S. galilaeus*, whereas the H2 is phylogenetically closer to *O. auraeus* and *T. zillii*. The data of this research evoke a monophylogenetic relationship of all the studied *Tilapia* species.

INTRODUCTION

Tilapia fish species represent the most important group of family Cichlidae. They constitute a major part of the fish fauna in the River Nile and its tributaries (Rajavarthini *et al.*,2000 ; Morals *et al.*,2001 ; Sharaf Eldeen and Abdel-Hamide,2002).So, they represent a valuable part of national income as they are characterized by delicious taste and cheap price. Furthermore, many researchers use it as a fish model to investigate different items (Abdel-Hamide, 1998;Yapi-Gnaore', 2001; Sharaf- Eldeen & Abdel-Hamide, 2002; El-Serafy *et al.*, 2003). Also, it is a successful model for aquaculture (Pullin, 1996). So, the need to characterize and to name tilapia is extremely needed (Pullin, 1996).Perdices *et al.*(2005) considered that the application of molecular techniques would permit enhanced detection of evolutionary structure and taxonomy across the widespread species. They used the mitochondrial DNA to get evolutionary history of synbrachid eels. Burridge and Smolenski (2004) also used the sequencing of mitochondrial DNA to discriminate species of families Cheilodactlidae and Latridae and to show the biogeographical effect.

In the River Nile the reproduction between different tilapia species and the production of hybrids could be fulfilled . The differentiation of the hybrids could not be possible by using the morphological and meristic characters (Rajavarthini *et al.*, 2000). Therefore, the present undertaken throw light on different classical fish identification and the molecular (RFLP) method as well. This may help to identify the different hybrids of tilapia species.

MATERIALS AND METHODS

I. Studied fishes

Live tilapia fish species were collected from El-Riah El-Tawfiqui (See El–Serafy *et al.*, 2003).

II. Morphometric characteristics

For every fish the following measurements were done: total length (TL),standard length (SL),body depth (BD), peduncle length (PedL) ,predorsal fin length (PrDFL), prepectoral fin length (PrPectFL) ,prepelvic fin length(PrPelvFL), preanal fin length (PrAnFL),Peduncle depth (Ped D),Head length (HL),head depth (HD),preorbital length (PrOL),eye diameter(ED), length of dorsal fin (LDF) ,length of pectoral fin (LpectF), length of pelvic fin (LpelvF) and length of anal fin (LanF) based on the method of Lagler *et al.*

(1977).These measurements were calculated according to the following formula:

Morphometric index = Morphometric character/TL or HL x 100

III. Meristic characteristics

The number of fin rays were counted in the dorsal fin (DFrs) ,in the anal fin (AnFrs) and in the caudal fin (CaudFrs).Also ,the number of lateral line scales (Lat.Lin.Scales) were counted from the end of the operculum to the end of the caudal peduncle. Fluctuating asymmetry (FA) of the pectoral fin rays ,pelvic fin rays and gill rackers were done by counting the rays or the gill rackers of the right and left sides .Then FA was calculated by subtracting the right value from the left one (Sanchez-Galan *et al.*, 1997).

IV. DNA extraction

Genomic DNA of the studied tilapia species was extracted from the liver tissue following the method recommended by Hugo *et al.* (1992) and El-Serafy *et al.* (2003).

V. Determination and amplification of rDNA by polymerase chain reaction (PCR)

The standard PCR mixture was used according to Kessing *et al.*(1989).The entire nuclear srDNA was amplified using the primers SSU1[5`-CGACTGGTTGATCCTGCCAGTAG-3`] and SSU2 [3` TCCTGATCCTTCTAGGTTCAC-5`] (Amresco) anchored respectively in the conserved extremities of the 18SsrRNA gene (Stohard and Rollinson,1997).The detail of the standard PCR program for amplification of nuclear SsrRNA was recorded in El-Serafy *et al.*(2003).Nine restriction enzymes were used to differentiate tilapia species. These enzymes are *Bg*11and *Eco*RI (Amersham, Life Science),*Sac*II, *Ap*uI and *Ava*I (Boehringer Mannheim) and *Sma*I, *A1wN*I,*Xma*I and *Sst*II { Sigma co. ,USA) The detail of the gel preparation ,running, sample loading and imaging was previously recorded in El-Serafy *et al.*(2003).

RESULTS

I. Morphometric characteristics:

According to the data in Table (1) the Standard Length / Total Length (SL/TL) ratio showed a significant differences when comparison made between *Oreochromis aureus* (*O. auraeus*) and *Sarotherodon galilaeus* (*S. galilaeus*) and *Oreochromis niloticus* (*O. niloticus*) and (*S. galilaeus*). While the differences between *S. galilaeus* and *Tilapia zillii*

(*T. zillii*) are considered statistically more highly significant. But the difference is not significant between *O. auraeus* and *O. niloticus* , *O. auraeus* and *T. zillii and O. niloticus* and *T. zillii.*

Concerning the head length (HL/TL), its values change with highly significant differences between *O. auraeus* and *S.galilaeus, S. galiaeus* and *T. zillii* , *O. niloticus* and *T. zillii.* The difference between *O. auraeus* and *T. zillii* is considered more highly significant. Whereas, there is no significant changes between *O. auraeus* and *O. niloticus and O. niloticus* and *S. galilaeus.*

Regarding the body depth (BD/TL), the differences in its values are significant between *O.niloticus* and *S.galilaeus* and highly significant between *O. auraeus* and *T. zillii.* Whereas, the changes in BD/TL between *O. niloticus* and *T. zillii* , *O. auraeus* and *S.galilaeus* and *S. galilaeus* and *T. zillii* are statistically more highly significant. The differences between *O.niloticus* and *O.auraeus* are not significant .So, *O. niloticus* and *O.auraeus* are monophylogenetic species.

The values of predorsal fin indices (PrDFL /TL) when comparing between *S.galilaeus* and *T. zillii* are differed with a statistically more highly significant value. When comparing *O. aureus* and *O. niloticus, O. aureus* and *S.galilaeus, O. aureus* and *T. zilli, O. niloticus* and *S.galilaeus* and *O. niloticus* and *T. zillii,* the differences are considered not significant .So, *tilapia* species can be divided into three groups.

When comparing prepectoral fin length (PrPectFL /TL) of the examined tilapia species, the differences were found significant between *O. aureus* and *O. niloticus* and *O. niloticus* and *S. galilaeus,* and highly significant between *O. niloticus* and *T. zillii.* Whereas, the differences are more highly significant between *O. aureus* and *S.galilaeus* and *O. aureus* and *T. zillii* . But there is no significant difference between *S.galilaeus* and *T. zillii.*

Concerning prepelvic fin length as ratio of TL (PrPelvFL/TL), it changed with significant differences between *O. niloticus* and *T. zillii,* and with highly significant differences between *O. aureus* and *T. zillii* and *O. niloticus* and *S.galilaeus.* There is more highly significant difference in the value of PrPelvFL /TL between *O. aureus* and *S.galilaeus.* But the changes between *O. aureus* and *O. niloticus* and *S.galilaeus* and *T. zillii* are statistically non-significant. So, these species are phylogenetically related ones.

Regarding the preanal fin length (PrAnFL /TL), there are highly significant differences in its values when comparing between *O. aureus*

and *O. niloticus* and more highly significant differences were found between *O. niloticus* and *S.galilaeus* and *O. niloticus* and *T. zillii.* Whereas, the differences are not significant between *O. aureus* and *S.galilaeus, O. aureus* and *T. zillii* and *S.galilaeus* and *T. zillii.*

Concerning the length of the dorsal fin as a part of TL (LDF/TL), significant differences were found between *O. auraeus* and *O. niloticus* and *O. niloticus* and *S.galilaeus.* Whereas, the differences between *O. niloticus* and *T. zillii, O. auraeus* and *S.galilaeus, O. auraeus* and *T. zillii* and *S.galilaeus* and *T. zillii* were found to be not significant.

When comparing the ratio of the length of the pectoral fin related to TL (LpectF / TL), the differences between *O. auraeus* and *T. zilli, O. niloticus* and *T. zillii* and *S.galilaeus* and *T. zillii* were considered statistically more highly significant, while between *O. auraeus* and *d O. niloticus, O. auraeus* and *S.galilaeus* and *O. niloticus* and *S.galilaeus* were not significant.

Regarding the length of the pelvic fin as a ratio of TL (LpelvF /TL), the difference is significant between *O. auraeus* and *S.galilaeus*, while differences between *O. auraeus* and *T. zillii* is more highly significant . The differences in the LpelvF /TL values are not significant between *O. auraeus* and *O. niloticus, O. niloticus* and *S.galilaeus, O. niloticus* and *T. zillii* and *S.galilaeus* and *T. zillii.*

The length of anal fin as a ratio of TL (LanF/TL), changed with significant differences between *O. auraeus* and *O. niloticus* and *O. auraeus* and *S.galilaeus*, and with highly significant differences between *O. auraeus* and *T. zillii.* But there are no significant changes between *O. niloticus* and *S.galilaeus, O. niloticus* and *T. zilli* and *S.galilaeus* and *T. zillii.*

Concerning the peduncle length divided by TL (PedL/TL), the differences in its values between each of *O. auraeus* and *T. zillii, O. niloticus* and *T. zillii* and *S.galilaeus* and *T. zillii* are considered statistically more highly significant. But there are no significant changes between *O. auraeus* and *O. niloticus, O. auraeus* and *S.galilaeus* and *O. niloticus* and *S.galilaeus.*

Peduncle depth (PedD /TL) of tilapia species varies with more highly significant differences between *O. auraeus* and *S.galilaeus, O. niloticus* and *S.galilaeus* and *S.galilaeus* and *T. zillii.* Whereas, the difference between *O. auraeus* and *O. niloticus, O. auraeus* and *T. zillii* and *O. niloticus* and *T. zillii* are statistically not significant.

The preocular length related to HL (PrOL/HL), the differences between *O. auraeus* and *T. zillii* , *O. niloticus* and *T. zillii* and *S.galilaeus* and *T.zillii* are found statistically more highly significant. But the differences between *O.auraeus* and *O.niloticus*, *O.auraeus* and *S.galilaeus* and *O.niloticus* and *S.galilaeus* are considered not significant.

When comparing eye diameter as a ratio of HL (ED/HL), the differences are highly significant between *O. auraeus* and *O. niloticus* and *O.auraeus* and *S.galilaeus*, while the more highly significant differences were found between *O.auraeus* and *T.zillii*, but there is no significant differences between *O. niloticus* and *S.galilaeus, O. niloticus* and *T.zilli* and *S.galilaeus* and *T. zillii.*

The data of head depth related to HL (HD/HL) show a significant difference between *S.galilaeus* and *T.zillii.* Whereas, when the comparison made between *O.auraeus* and *T.zillii* and *O.niloticus* and *S.galilaeus* the differences were highly significant. A more highly significant difference was found between *O. auraeus* and *S.galilaeus.* There are no significant differences between *O.auraeus* and *O.niloticus* and *O.niloticus* and *T.zillii.*

The head depth as a ratio of head length (HD/ HL)differed significantly between *S.galilaeus* and *T.zillii.* Highly significant differences were found between *O. auraeus* and *T.zillii* , *O. auraeus* and *S.galilaeus* and *O.niloticus* and *S.galilaeus.* No significant differences were recorded when the comparison was made among *O. niloticus, O. auraeus* and *T. zillii.*

II.Meristic characteristics

Seven meristic characteristics were selected in this study .The available data are tabulated in Tables (3,4 and 5). The number of dorsal fin rays (DFrs) differed significantly when comparing *S.galilaeus* and *O. auraeus* , *O. niloticus* and *S.galilaeus, O. auraeus* and *T.zillii* , *O. niloticus* and *T. zillii* and *S.galilaeus* and *T. zillii.* But no significant difference was found between *O. auraeus* and *O. niloticus.* So, DFrs differentiate *tialpia* species except *O. auraeus* and *O. niloticus.* This is an indication of the same origin for both species.

The fin rays of anal fin (AnFrs) varied with more highly significant differences when the comparison was made between all *tilapia* species except between *O. auraeus* and *O. niloticus.*

When comparing the number of caudal fin rays (CaudFrs) a significant differences were found between *O. niloticus* and *T. zillii, O. auraeus* and *O.niloticus* and *O. niloticus* and *S. galilaeus.* No significant

differences between the rest of the compared group. The number of scales in the lateral line (Lat.Lin.scales) differed significantly between all the studied tilapia species.

Fluctuating asymmetry (FA) of the pectoral, pelvic fins and the gill rackers were presented in Table (5) The obtained data show a fluctuation between the right and the left sides in the number of pectoral fin rays (PectFrs) in all tilapia species except *S.galilaeus*. The pelvic fin rays (PelvFrs) of all tilapia species are bilaterally identical (FA=0) .Whereas, the FA of the gill rackers (GRs) are highly represented in all *tilapia* species.

The data recorded in Table (2) show a high similarity coefficient (0.69) when comparing the morphometric characteristics between *O. niloticus* and *O. auraeus*, whereas the values of similarity coefficient are less than 50% when comparing the rest of tilapia species. This indicates that *O.niloticus* and *O. auraeus* are closely similar in their morphological characters. Furthermore, the degree of similarity between *O. niloticus* and *O. auraeus* reaches to 0.5 in its meristic characters in general indicating that these two species are close together (Table, 4). Zero similarity coefficient was recorded when comparing *O. niloticus* and *S.galilaeus* and *O. niloticus* and *T.zillii. Also, a* very low similarity coefficient was reported when comparing the meristic characters between *O. auraeus* and *S. galilaeus* , *O. auraeus* and *T. zillii* and *S.galilaeus* and *T. zillii* indicating that these three species display great degree of differences.

III. RFLP of 18SsrRNA gene

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used to identify the various *tilapia* species in the River Nile (El-Serafy *et al.*, 2003). Figure (1) represents the separation of tilapia DNA genome, which appear in a smear like form as it has a high molecular weight. But the 1kb DNA ladder represented in the first lane was separated into bands with different lengths. The PCR products of 18SsrRNA gene for tilapia species appeared at a length ~2000 bp (Fig. 2).

There are some restriction endonucleases (EcoRI and *Bg*1I; Figs. 3 and 4) did not differentiate between the different strains of tilapia species. *EcoR*I restriction enzyme collected the species in one cluster when fragmented their rRNA gene into two cuts (~1650 and ~350 bp; Fig. 3). The gene of all species was cut into two fragments (~1250 and ~750 bp; Fig. 4) when digested with *Bg*1I restriction endonuclease.

The restriction enzyme, *Sma*I, cut the studied gene of one species only (*T. zillii*)into two fragments (~1250 & ~ 950 bp). While the rest of tilapia species genes were not fragmented and separated parallel to 2000 bp of DNA ladder (Fig. 5). So, it differentiates *T. zillii* from the rest of tilapia species.

The 18SsrRNA gene of *O. niloticus* was digested into two distinct bands (~1750 bp & ~ 300bp) by using the enzyme *Alw*NI (Fig. 6). Whereas, the gene was not splitted in the rest of tilapia species. Only *O. auraeus* 18SsrRNA gene was digested by the enzyme *Xma*I producing two fragments at lengths ~ 1100bp and ~ 900 bp (Fig. 7). The gene pattern in the other species is identical. So, the prescribed endonucleases differentiate *O. niloticus* and *O. auraeus* from the other tilapia species.

Three restricted fragments (~1000bp,~650bp & ~350 bp) belonging to *T. zillii* , *O. niloticus*, *O. auraeus*, *S.galilaeus* and H2 produced after digestion with the enzyme *Sac*II (Fig.9).The enzyme cuts the examined gene of H1 into two fragments which appeared at ~1650bp and ~ 350 bp. For this reason the H1 could be detected by testing the PCR product of 18srRNA gene after digestion with the enzyme *Sac*II .

Enzyme *Apa*I cuts the undertaken genes of *T. zillii*, *O. niloticus*, *O. auraeus* , *S. galilaeus* and H1 into three bands with lengths ~950bp,~800bp and ~250 bp (Fig. 10). Whereas, only two bands were reported for species H2 (~1200 bp and ~800 bp).So, by using the enzyme *Apa*I ,H2 can be separated from *tilapia* species inhabiting the River Nile in the Egyptian waters.

Tilapia species and their hybrids (H1 and H2) were separated into three distinct groups according to the results of 18srRNA-gene digestion with the enzyme *Ava*I. The first group includes *T. zillii*, *O. auraeus* and *S. galilaeus* in which five distinct bands (~700 bp, ~550bp, ~300bp, ~250 bp and ~200 bp) appeared after digestion (Fig., 11). The second group is represented only by *O. niloticus* in which six restricted fragments were separated with base pairs of ~650,~500, ~350, ~250,~150 and ~100.*Tilapia* hybrids (H1 and H2) constitute the third group, where four distinct fragments were with lengths ~800 bp,~700bp,~300bp and ~200 bp. So, this enzyme is not species specific and could partially differentiate tilapia fish.

DISCUSSION

The species identification of fish species including tilapia depends on the first step for morphometric and Meristic characters of the body parts (Yapi-Gnaore, 2001). The morphological identification of tilapia species is so complicated by the extensive intraspecific variations of the morphometric measurements used for quick species identification (Albertson *et al.*, 1999). The results of the present work indicate a great morphological identity between the three tilapia genera *Oreochromis, Sarotherodon* and *Tilapia.* Regarding the data of morphometric and meristic characters, two species are very closely related ; these are *O. niloticus* and *O. auraeus.* Suggesting that they are monophyltic species (derived from the same genus). This phenomenon was previously reported by Oberst *et al.* (1996) .They morphologically differentiated three species of genus tilapia (*T. dageti, T. zillii and T. guineensis*) indicating striking similarities ,so they are monophyltic species. This agrees with the work of El-Serafy *et al.* (2003) on the same species inhabiting the River Nile.

Lovshin (1982) found that the systematic distance between the species is the main reason for reproductive behaviour barrier. In the present study the monophylogenetic relationship between the genus *Oreochromis* and the genus *Sarotherodon* are recorded. They are both mouth brooding species. For this reason natural hybridization between them is possible with a concomitant propagation of tilapia hybrids in the River Nile habitat. The analysis of morphometric and merisitic characteristics can differentiate species but not strains or hybrids (Pante *et al.*, 1988). By comparing the PCR-RFLP product of specific endonucleases activity with the gene of H1 and H2 with those of the rest of tilapia species. It was found that in case of H1 the length cut is ~350 and ~ 1650 bp. The closer species of H1 regarding the length of bp cut is *O. niloticus* and *S. galilaeus* .On the other hand ,the length of gene cut of H2 by specific enzyme is ~1200 bp and ~ 800 bp .According to the length of bp *cut ,T. zillii* and *O. auraeus* are closer with H2. Whereas, the obtained results indicate a lesser degree of similarity between genus *Tilapia* and the other two genera showing a polyphyltic species. This phenomenon was recorded previously in case of synbranchid eel genera in different habitat (Perdices *et al* ., 2005).

Furthermore, the data of FA of the gill rackers discriminate tilapia species into three groups that present confusion between *O. niloticus* and *O. auraeus* and a higher degree of similarity between *O.*

auraeus and *S.galilaeus*. The observed data differentiate *T. zillii* as a separate group with less degree of similarity. Thus, according to the data of FA tilapia species can be sorted into three groups. This result coincides with the results of Falk *et al.* (1996) and Oberst *et al.* (1996).

Rognone and Guyomard (2003) states that the morphological parameters of fishes are influenced by both genetic and environmental factors.

For this reason the molecular techniques data based on PCR-RFLP analysis of the 18SsrRNA gene have been extensively used as a precise tool of species identity of fishes (Fernandez, 2001; El-Serafy *et al.*, 2003 and Perdices et al., 2005). Farias *et al.* (1999) and El-Serafy *et al.* (2003) used the RFLP –PCR products of nuclear and mitochondrial DNA as a tool to identify *Tilapia* species. The results obtained indicate that the rstriction enzyme *Sma*I differentiate all species as one group except for *T. zillii*, this confirmed the monophylogenetic relationships of all species except *T. zillii* which displays a polyphylogenetic relationship.

By using the endonuclease *Sac*II the RFLP profile discriminates H1 from the rest of the examined species, so this enzyme is a specific for H1 gene and it could be possible to used for H1 identification.On the other hand ,the data obtained after using the endonuclease *Apa*I are characteristic for H2 gene.The RFLP data discriminate H2 from the rest of *tilapia* species ,so it could be a useful tool to identify H2 fish species.

All studied species may be differentiated into three groups when using the enzyme *Ava*I: group (1) include *T. zillii, O. auraeus* and *S. galialeus,* group (2) include H1, and H2 and group include *O. niloticus.* These results suggest that this endonuclease can be used to identify hybrids.. Sequencing PCR fragments has become a standard technique in laboratories applying recombinant DNA technologies. Several authors declaired that the RFLP option is simpler and faster in addition to its less cost (Ram *et al.*, 1996; Cespedes *et al.*, 1998 ; Quinteiro *et al.*, 1998).

Conclusion

The present study shows that the use of the PCR – RFLP profile is a simple and rapid method for the detection of tilapia hybrids, which may be important for fish farming, and research protocols. The degree of genetic relation was found between H1 and *O. niloticus* and *S.galilaeus,* whereas H2 is genetically related to *T. zillii* and *O. niloticus.*

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Table (1): Morphometric indices (Average ± SE) of different tilapia species.

1- In Total Length :

Morphometric ratio	*O. niloticus*		*O. oureus*		*S. galilaeus*		*T. zillii*	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
SL/TL	81.55	± 0.25	81.45	±0.26	80.32	± 0.37	82.41	± 0.41
HL/TL	25.53	± 0.32	25.80	±0.20	25.08	± 0.16	23.94	± 0.30
BD/TL	36.04	± 0.48	35.15	±0.37	37.59	±0.30	33.23	±0.40
PrDFL/TL	27.09	± 0.50	27.24	±0.48	26.53	±0.21	28.03	±0.34
PrPectFL/TL	29.26	± 0.30	30.31	±0.23	28.22	±0.37	27.89	±0.29
PrPelvFL/TL	34.11	± 0.50	34.29	±0.36	32.49	±0.30	32.33	±0.42
PrAnFL/TL	59.64	± 0.20	58.68	±0.23	57.82	±0.43	58.02	±0.36
LDF/TL	51.88	± 1.22	48.87	±0.22	48.47	±0.73	48.80	±1.30
LpectF/TL	26. 43	± 0.51	27.0	±0.32	26.78	±0.88	21.38	±0.42
LpelvF/TL	18.31	± 0.53	19.40	±0.13	18.33	±0.41	17.64	±0.38
LAnF/TL	17.76	± 1.16	14.51	±0.20	16.09	±0.58	18.74	±1.23
PedL/TL	10.52	± 0.24	10.94	±0.28	10.34	±0.25	13.88	±0.12
PedD/TL	13.02	±0.22	12.81	±0.17	14.54	±0.16	12.45	±0.35

2-In head length:

Morphometric ratio	*O. niloticus*		*O. oureus*		*S. galilaeus*		*T. zillii*	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
PrOL/HL	31.24	±0.51	30.78	±0.68	31.50	±0.52	35.24	±0.66
ED/HL	29.54	±0.44	27.58	±0.37	29.16	±0.38	30.10	±0.45
HD/HL	111.49	±2.59	110.29	±1.46	121.35	±1.30	116.16	±1.53

Table (2): The significance (t-test) among different morphometric indices of different tilapia species:

1- **In total length :**

Morphometric ratio	*O.n* × *O.au*	*O.n* × *S.g*	*O.n* × *T.z*	*O.au* × *S.y*	*O.au* × *T.z*	*S.g* × *T.z*
SL/TL	0.2794	2.7437*	1.8048	2.5019*	2.0031	3.7995***
HL/TL	0.7221	1.2736	3.6580***	2.7986**	5.1534***	3.3568**
BD/TL	1.4791	2.7531*	4.5478***	5.1147***	3.5507**	8.7671***
PrDFL/TL	0.2179	1.0361	1.5608	1.3677	1.3546	3.7719***
PrPectFl/TL	2.7290*	2.1545*	3.2752**	4.7284***	6.5299***	0.6999
PrPelvFL/TL	0.2926	2.7840**	2.7442*	3.7916***	3.5345**	0.3092
PrAnFL/TL	3.1418**	3.8622***	3.8937***	1.7767	1.5330	0.3569
LDF/TL	2.4366*	2.4057*	1.7310	0.5264	0.0532	0.2216
LPectF/TL	0.9493	0.3453	7.6263***	0.2365	10.9607***	5.5600***
LPelvF/TL	1.9802	0.0296	1.0174	2.4677*	4.3282***	1.2223
LAnF/TL	2.7580*	1.2844	0.5782	2.5665*	3.3849**	1.9411
PedL/TL	1.1526	0.5186	12.7062***	1.6015	9.8094***	12.7139***
PedD/TL	0.9966	7.4327***	1.5307	7.3434***	0.9223	5.4005***

2- **In head length:**

Morphometric ratio	*O.n* × *O.au*	*O.n* × *S.g*	*O.n* × *S.g*	*O.au* × *T.z*	*O.au* × *T.z*	*S.g* × *T.z*
PrOL/HL	0.5375	0.8399	0.3569	4.7895***	4.6968***	4.4696***
ED/HL	3.4189**	2.9841**	0.6589	0.8915	4.3066***	1.5974
HD/HL	0.4042	5.6582***	3.4033**	1.5547	2.7829**	2.5857
Similarity coefficient	0.69	0.44	0.5	0.44	0.31	0.44

Number of tested fishes = 15
* Significant at P < 0.05
** Significant at P < 0.01
*** Significant at P < 0.001

O.n.: Orechromis niloticus
O. au.: Oreochromis aureus
S.g.: Sarotherodon galilaeus
T.z.: Tilapia zillii

Table (3) Meristic characteristics (Mean ± SE) of different tilapia species.

Meristic Count	*O. niloticus*		*O. oureus*		*S. galilaeus*		*T. zillii*	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
DFrs	29.57	±0.13	29.65	±0.12	28.95	±0.17	27.0	±0.08
AnFrs	12.07	±012	12.35	±015	14.05	±0.20	11.35	±0.15
CaudFrs	16.79	±0.11	16.25	±0.11	16.05	±0.10	16.30	±0.14
Lat.Lin. Scales	33.11	±0.15	33.65	±0.20	32.24	±0.15	31.0	±0.27

Table (4) : The significance (t-test) among different meristic characteristics of different tilapia species.

Meristic Count	*O.n* ×*O.au*	*O.n* × *S.g*	*O.n* × *T.z*	*O.au* × *S.g*	*O.au* × *T.z*	*S.g* × *T.z*
DFrs	0.4516-	2.9443**	17.1664***	3.3552**	17.9618***	10.4873***
AnFrs	1.4805	8.3885***	3.8070***	6.7586***	4.8045***	10.7343***
CaudFrs	3.5200**	5.1269***	2.7343*	1.3498	0.2743	1.4307
Lat.Lin. Scales	2.1468*	3.5769**	6.8399***	5.0123***	7.8108***	3.7218***
Similarity coefficient	0.5	0.0	0.0	0.25	0.25	0.25

Table (5) Fluctuating asymmetry (FA) of different tilapia species.

Species		Pectroal Fin Rays				Pelvic Fin Rays				Gill Rackers			
		Right	Left	FA	%FA	Right	Left	FA	%FA	Right	Left	FA	%F
O.niloticus	Mean ±SE	13.36 0.19	13.50 0.13	0.14 0.09	13.3 %	6.0 0.0	6.0 0.0	0.0 0.0	0.0%	30.50 0.45	30.21 0.34	1.29 0.32	60%
O. aureus	Mean ±SE	13.7 0.12	13.7 0.12	0.10 0.08	13.3 %	6.0 0.0	6.0 0.0	0.0 0.0	0.0%	32.75 0.46	32.05 0.53	1.20 0.19	100%
S. galilaeus	Mean ±SE	12.90 0.11	12.90 0.11	0.0 0.0	0.0%	6.0 0.0	6.0 0.0	0.0 0.0	0.0%	26.80 0.47	25.95 0.51	1.43 0.29	100%
T. zillii	Mean ±SE	13.45 0.13	13.45 0.15	0.10 0.08	6.0 0.0	0.0 0.0	0.0 0.0	0.0 %	14.95 0.35	14.95 0.35	15.0 0.30	0.40 0.17	53.3%

Number of tested fishes = 15

* Significant at P < 0.05

** Significant at P < 0.01

*** Significant at P < 0.001

O.n. : Orechromis niloticus
O. au.: Oreochromis aureus
S.g.: Sarotherodon galilaeus
T.z.: Tilapia zillii

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Fig. (1): DNA genome from tilapia species. Lane 1 represents 1 kb DNA marker. Lanes 2 –7 represent DNA of *T. zillii, O. niloticus, O. auraeus. S. galilaeus*, H1 and H2, respectively.

Fig. (2): Shows the PCR- RFLPs patterns of 18SsrRNA gene of *tilapia* species. Lane 1 represents 1 kb DNA marker. Lanes 2 –7 represent gene pattern of *T. zillii, O. niloticus, O. auraeus, S. galilaeus*, H1 and H2, respectively.

Fig. (3): Shows the PCR- RFLPs patterns of 18SsrRNA gene restricted by enzyme *EcoR*I. Lane 1 represents 1 kb DNA ladder. Lanes 2 –7 represent gene pattern of *T. zillii, O. niloticus, O. auraeus, S. galilaeus*, H1 and H2, respectively.

Fig. (4): Shows the PCR- RFLPs patterns of 18SsrRNA gene restricted by enzyme *Bg*1I. Lane 1 represents 1 kb DNA ladder. Lanes 2 –7 represent gene pattern of *T. zillii, O. niloticus, O. auraeus. S. galilaeus*, H1 and H2, respectively.

Fig. (5): Shows the PCR- RFLPs patterns of 18SsrRNA gene restricted by enzyme *Sma*I. Lane 1 represents 1 kb DNA ladder. Lanes 2 –7 represent gene pattern of *T. zillii, O. niloticus, O. auraeus, S. galilaeus*, H1 and H2, respectively.

Fig. (6): Shows the PCR- RFLPs patterns of 18SsrRNA gene restricted by enzyme *AlwN*I. Lane 1 represents 1 kb DNA ladder. Lanes 2 –7 represent gene pattern of *T. zillii, O. niloticus, O. auraeus, S. galilaeus*, H1 and H2, respectively.

Fig. (7): Shows the PCR- RFLPs patterns of 18SsrRNA gene restricted by enzyme *Xma*I. Lane 1 represents 1 kb DNA ladder. Lanes 2 –7 represent gene pattern of *T. zillii ,O . niloticus ,O . auraeus , S. galilaeus* ,H1 and H2, respectively.

Fig. (8): Shows the PCR- RFLPs patterns of 18SsrRNA gene restricted by enzyme *Sst* II. Lane 1 represents 1 kb DNA ladder. Lanes 2 –7 represent gene pattern of *T. zillii, O. niloticus, O. auraeus, S. galilaeus*, H1 and H2, respectively.

Fig. (9): Shows the PCR- RFLPs patterns of 18SsrRNA gene restricted by enzyme *Sac* II. Lane 1 represents 1 kb DNA ladder. Lanes 2 –7 represent gene pattern of *T. zillii, O. niloticus, O. auraeus. S. galilaeus*, H1 and H2, respectively.

Fig. (10): Shows the PCR- RFLPs patterns of 18SsrRNA gene restricted by enzyme *Apa*I. Lane 1 represents 1 kb DNA ladder. Lanes 2 –7 represent gene pattern of *T. zillii, O. niloticus, O. auraeus, S. galilaeus*, H1 and H2, respectively.

Fig. (11): Shows the PCR- RFLPs patterns of 18SsrRNA gene restricted by enzyme *Ava*I. Lane 1 represents 1 kb DNA ladder. Lanes 2 –7 represent gene pattern of *T. zillii, O. niloticus, O. auraeus, S. galilaeus*, H1 and H2, respectively.

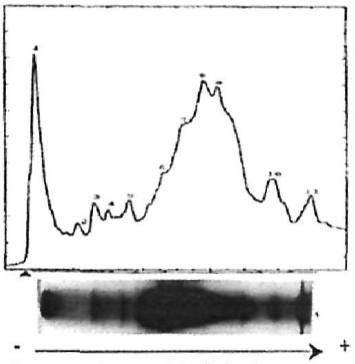

Fig. (1): Plasma proteinogram of *O. niloticus* (untreated samples).

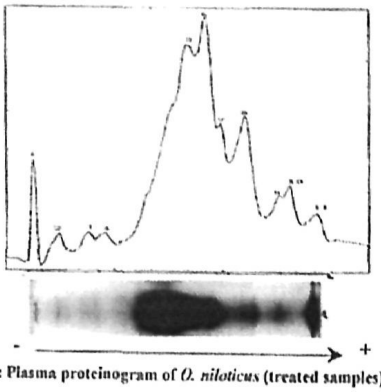

Fig. (2): Plasma proteinogram of *O. niloticus* (treated samples)

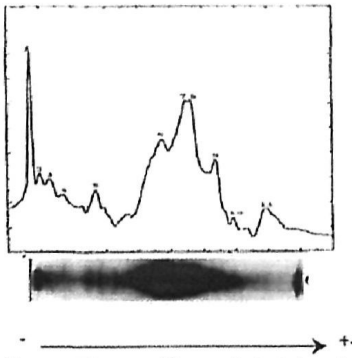

Fig. (3): Plasma proteinogram of *O. aureus* (untreated samples).

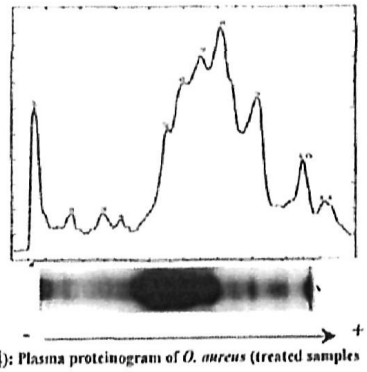

Fig. (4): Plasma proteinogram of *O. aureus* (treated samples

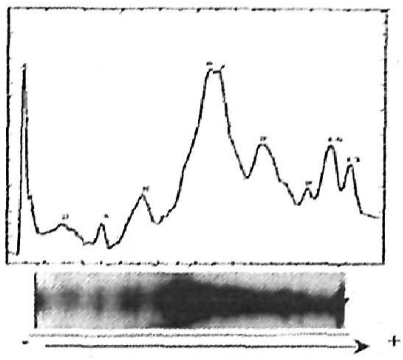

Fig. (5): Plasma proteinogram of *S. galilaeus* (untreated samples).

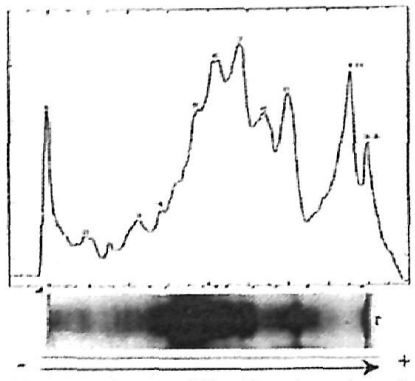

Fig. (6): Plasma proteinogram of *S. galilaeus* (treated samples).

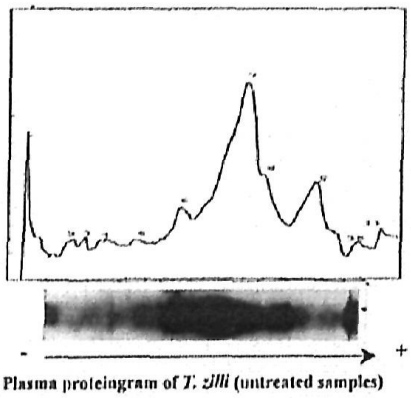

Fig. (7): Plasma proteingram of *T. zilli* (untreated samples)

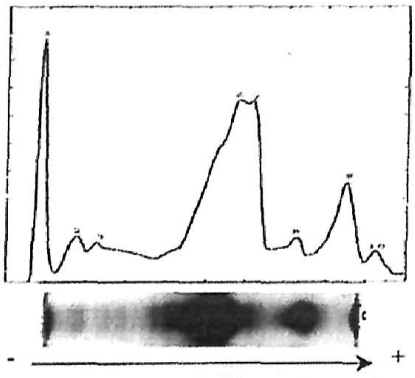

Fig. (8): Plasma proteinogram of *T. zilli* (treated samples)

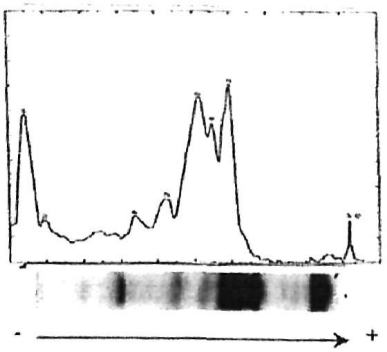

Fig. (9): Muscle proteinogram of *O. niloticus* (untreated samples)

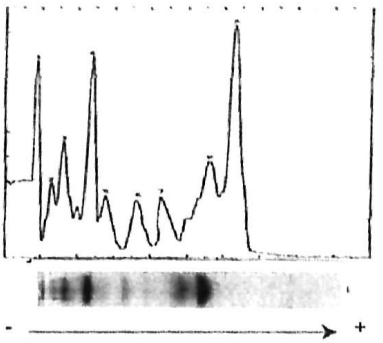

Fig. (10): Muscle proteingram of *O. niloticus* (treated samples)

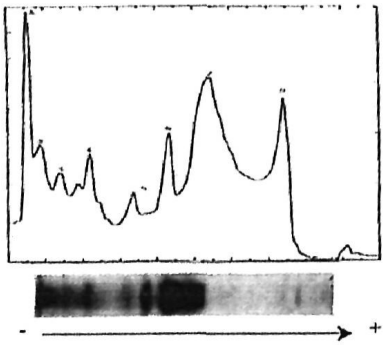

Fig. (11): Muscle proteinogram of *O. aureus* (untreated samples)

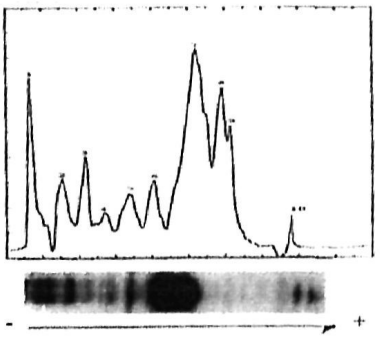

Fig. (12): Muscle proteinogram of *O. aureus* (treated samples)

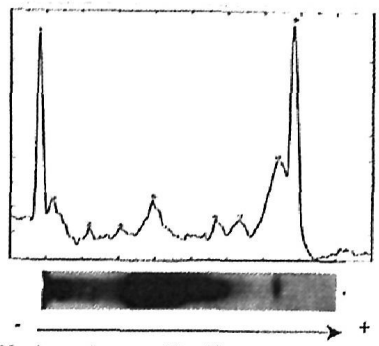

Fig. (13): Muscle proteinogram of *S. galilaeus* (untreated samples)

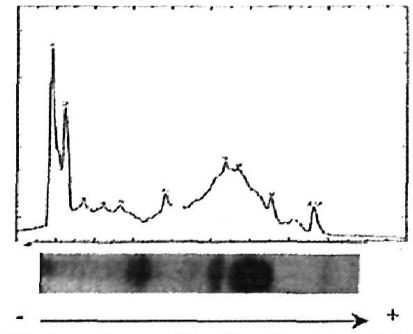

Fig. (14): Muscle proteingram of *S. galilaeus* (treated samples)

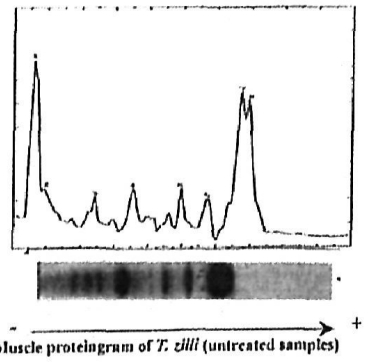

Fig. (15): Muscle proteingram of *T. zilli* (untreated samples)

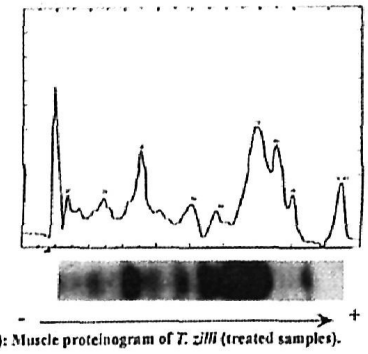

Fig. (16): Muscle proteinogram of *T. zilli* (treated samples).