COMPARATIVE STUDY ON ELECTROPHORETIC PROTEIN PATTERN OF *TILAPIA* SPECIES IN THE RIVER NILE, EGYPT.

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ABSTRACT

Tilapia fish species were gathered from the wild population inhabiting the river Nile at Benha City during the period from May to August 2000. The plasma and muscle proteinograms of *tilpaia* species were described electrophoretically to differentiate *tilpaia* species inhabiting the river Nile; where eleven and ten fractions were separated, respectively. The plasma protein fractions obtained in the present study show common polymorphic fractions for all the studied species. It is also indicated that, each species has a characteristic specific pattern with more common bands for all studied species. The results indicated that closer species have similar protein pattern. The obtained data revealed close similarity between *O. niloticus* and *O. aureus* indicating a monophylogenetic origin of these two species. Whereas, less degree of similarity was recorded between *T. zillii* and the other species, indicating a genetic distance between this species and the others.

Key words: *Tilapia*, species identification, protein electrophoresis, plasma, and muscle, Monophylogenetic.

INTRODUCTION

Fishes of family *Cilchlidae* are randomly distributed throughout Africa, Central America, northern half of South America and parts of India (Fryer and Iles, 1972). *Tilapia* species present in different parts of the world, but the origin of *O. niloticus* is the river Nile.

Tilapia species constitute the most important group of family *Cichlidae* that inhabiting river Nile. These fishes are important for the nutritional and socio -economic development of tropical and subtropical regions (Oberst *et al*., 1993;

Rajavarthini *et al*., 2000; Morals *et al*., 2001). Special attention has been given to *tilapia* species for aquaculture as it is characterized by rapid growth rate, reasonable reproductive strategy and reproduces during the first year of their age (Abdel-Hamide, 1998; Haroun, 1999). There is a wide array of aquatic species to be accurately classified and their populations to be categorized for evaluation of aquaculture potential. In river Nile, the original habitat of *tilapia,* there is a need to characterize and to name the species that is useful for researchers, farmers and consumers (Pullin, 1996). Lagler *et al*. (1977) noted that *tilapia* fishes exhibit a high degree of parental care and they are divided into mouth brooders and substrate brooder. Trewavas (1984) distinguished three genera of *tilapia* which are mouth brooders i.e., *Sarotherodon* and *Oreochromis,* and substrate brooder, i.e., *Tilapia*. Therefore, hybridization has been done between the genera of similar reproductive pattern of *tilapia* fish population that live in the river Nile (El-Serafy *et al.,* 2003). Many researchers studied fish proteins by using polyacrylamide electrophoretic techniques as one of the biochemical methods that used to differentiate animal species [El-Serafy *et al*., 1993;El-Serafy, 1994; Mamuris *et al*., 1999; Sharaf El-Deen &Abdel-Hamide, 2002 and Berrini *et al*., 2006]. They reported the efficiency of electrophoretic methods for species identification. They also added that these methods gave useful data in strain and phylogenetic identification (El-Serafy , 1994 and Shain, 1999). Also, methods based on DNA analysis have also been used (El-Serafy *et al*., 2003; Perdices *et al*., 2005). The present study embodies a comparative electrophoretic survey of four *tilapia* species to define the phylogenetic relationship among these species.

MATERIALS AND METHODS

The fishes used in the present study were collected using fish trap from El-Riyah El-Tawfequi [A branch of river Nile] at Benha City, during the period from May to August 2000.Then it was transported to the wet laboratory at faculty of Science Benha university and let for acclimatization for some hours in aerated aquaria. Thereafter, fishes measuring 14-16 cm in total length were segregated into four groups based on their recognizable characteristics. Each group consists of seven fishes. Morphologically apparent healthy fishes were used only for this study.

I. Blood and muscle sampling.

The fishes were wiped carefully especially in the region between the operculum and the gills, in order to avoid the haemolysis. To avoid the possible effect of anesthesia on blood parameters and its constituents, the fishes were not

anaesthetized before blood sampling [Abdel-Hamide, 1994]. The blood samples were collected by heart puncture in a lithium-heparinized tube to avoid blood coagulation. The blood samples were centrifuged two times at 1500g (about 4000 r.p.m.) for 10 minutes. Thereafter, the blood plasma was separated carefully from the blood cells using micropipette. The muscle samples were isolated from the dorsal epiaxial muscle .It were homogenized in 1x electrode buffer (1:1 ratio) using electric tissue homogenizer. The muscle homogenates were centrifuged at 1500g (about 4000 r.p.m.).Clear sarcoplasmic protein was separated from the muscle fibers using micropipette. The plasma and the clear sarcoplasmic protein were stored in deep freezer (-20^{o}C) until analysis.

II. Electrophoretic technique.

Fractionation of protein in plasma and muscle was done using sodium dodecyl sulphate polyacrylamide gel electrophoresis [SDS- PAGE]. Sample treatment and gel preparation were done according to the method recommended by Laemmli (1970). The plasma and muscle protein samples were loaded without any treatment (untreated sample) or by incubation with an equal volume of sample buffer at 95 ˚C for ten minutes (treated sample). Each sample was loaded in a separate well. Protein samples were separated using a vertical slab electrophoresis unit at a current 30 mA for each gel. Protein was stained in a gel by comassie brilliant blue (Falk *et al*., 1996). Excess stain was removed in destining solution until the bands become clearly seen and the background became colourless, then the gel was stored in 7% acetic acid. Protein bands were detected by densitometer using Hoefer GS 365 software. Band reading was done as a transmission mode. Scanning figures were selected to represent the proteinogram for each specimen.

III. Statistical analysis:

The data obtained in this study were presented as mean ± SE (Standard error) Student *t*-test was carried out between the data of every two species to show the significant differences (Pipkin, 1984). Similarity coefficient (SC) between fish species was estimated following the formula of Ferguson (1980)

$$SC = \frac{\text{Number of fractions of common mobility}}{\text{Maximum number of fractions in an individual}}$$

RESULTS

I. Plasma Protein.

I.1 Fractions Appearance.

In each studied species, untreated electrophoretic plasma protein sample showed eleven fractions. As shown in table 1; and figures 1,3,5and 7 it would clearly appear the following: (a) Fraction number 1cannot be used to differentiate between species, being it had 100% appearance in all studied species. (b) Fractions number 2 and 7 differentiate *O. niloticus* from the rest of *tilapia* species being appear in a low percentage of appearance, so these bands are species specific.(c)Fractions number 4, 9 and 11 are species characteristic which is useful for discrimination of *O. aureus*.(d) Fraction number 5 characterizes *S.galilaeus* as it appears in the proteinogram of this fish species with low percentage (42.86%).

In *T. zillii*, the last two fractions showed a low percentage of appearance (28.57% and 42.86%) compared with high appearance for other *tilapia* species, so it could be possible to differentiate this species using these two bands. Protein polymorphic bands are presented among *tilapia* species (Number 1, 3 and 7).

Table (2) showed the percentage of occurrence of plasma protein fractions of Nile *tilapia* species (treated electrophoretic samples) and the plasma proteinogram were presented in figures (2, 4,6 and 8). Similarly, eleven fractions were recorded in each *tilapia* species. Also, in *O. niloticus*, most fractions appeared with a high percentage. Regarding *O. aureus*, six fractions appeared with percentage of 100%; these are number 1,2,6,7,9 and 10. The plasma protein fractions of *S. galilaeus* have a percentage appearance of 100%; for the first fraction and the last six ones (number 6, 7,8,9,10 and 11); indicating that these fractions are polymorphic.

T. zillii showed a special protein pattern in which three fractions disappeared (number 4,5 and 11). So, this protein pattern characterizes *T. zillii* from the other *tilapia* species. From the obtained data, it could be possible to differentiate *tilapia* species by using untreated plasma protein, which is better than using the treated one.

I.2 Relative mobilities of plasma Protein Fractions.

The significant (*t*-test) among different relative mobilities of plasma protein fractions (untreated electrophoretic samples) were presented in table (4). When comparing *O. niloticus* and *O. aureus*, the relative mobilities of 3rd, 4 th , 7th , 8th and 9th fractions changed with significant differences.

Only the differences in the 1st and 8th fraction mobilities were found statistically significant between *O. niloticus* and *S. galilaeus*. Concerning the comparison between *O. niloticus* and *T. zillii*, four fractions differed significantly, namely 4,5,7 and 8; indicating that they are polyphyletic species.

All fraction mobilities of *O. aureus* and *S. galilaeus* did not differ significantly indicating certain degree of genetic relationship. On contrary, five protein fractions namely 1,4,5,10 and 11 showed significant differences in its mobilities between *O. aureus* and *T. zillii*. This means that these are dissimilar species and they are genetically differed.

Only the average value of the relative mobilities of the 4th and 11th fractions were statistically differed when comparing *S.galilaeus* and *T. zillii* ; this also indicated polyphylogeny.

Similarity coefficient (SC) of the relative mobility of plasma protein fractions was calculated between the examined species. A high SC value was found between *O. niloticus* and *S. galilaeus* (0.82); *O. aureus* and *S.galilaeus* (1.0); and S. *galileaus* and *T. zillii* (0.82). Whereas, a low value of SC was recorded between *O. aureus* and *T. zillii* (table 4). So, *T. zillii* is only closer to *S. galilaeus*. Whereas, the other *tilapia* species showed high SC which might indicate a monophylogenric of all *tilapia* species except *T. zillii* which might be originated separately.

Concerning the comparison of treated samples between *O. niloticus* and *O. aureus* (table 6), the 2nd, 8 th, 10th, 10 th and 11th fraction mobilities changed significantly. Fractions number 1,2,3,9 and 11 th showed significant differences in its mobilities between *O. niloticus* and *S. galilaeus*. Only the relative mobilities of the 6 th and 9th fractions changed significantly between *O. niloticus* and *T. zillii*; so, they are dissimilar species. Among *O. aureus* and *S. galilaeus*, the differences of the 8 th,9 th and 10th fractions were statistically significant.

Significant differences were noticed only when the 9th and 10th fractions were compared between *O. aureus* and *T. zillii*. Similarly, a special protein pattern was noticed between *S. galilaeus* and *T. zillii*. Only the mobility of the 6th fraction

differed between *S. galilaeus* and *T. zillii*. This difference was statistically significant. The rest of the fractions in *S. galilaeus* and *T. zillii* showed no significant differences.

Similarity coefficient (SC) of relative mobility (treated samples) was found high (0.73) when comparing *O. aureus* and *S. galilaeus ;* and *O. niloticus* and *O. aureus(0.64%).* Whereas low values of SC were recorded among *O. niloticus* and *T. zillii ;* and *O.aureus* and *T. zillii* (table, 6). This means that the species *O. niloticus, O. aureus* and *S. galilaeus* are derived from one origin (monophylogeneric). Whereas, *T. zillii* displays another origin. So all *tilapia* species are polyphyletic, i.e., they are derived from separate origins**.**

Table (1) Percentage appearance of plasma protein fractions (untreated electrophoretic samples) of different *tilapia* species.

Species	**Fraction number**										
	1	2	3	4	5	6	7	8	9	10	11
O.niloticus	100 %	42.86%	85.71%	57.14%	71.43%	100%	42.86%	100%	85.71%	85.71%	57.14%
O.aureus	100 %	71.43%	100%	14.29%	85.71%	71.43%	100%	85.71%	28.57%	57.14%	28.57%
S. galilaeus	100 %	58.71%	85.71%	71.43%	42.86%	85.71%	71.43%	85.71%	71.43%	85.71%	57.14%
T. zillii	100 %	71.43	57.43%	85.71%	57.14	71.34%	100%	71.43%	85.71%	28.57%	42.86

Table (2) Percentage appearance of plasma protein fractions (treated electrophoretic samples) of different *tilapia* species.

Species	**Fraction number**										
	1	2	3	4	5	6	7	8	9	10	11
O. niloticus	100 %	57.14%	57.14%	42.86%	71.43	100%	42.86%	85.71%	85.71%	85.71%	57.14%
O. aureus	100 %	100%	57.14%	42.86%	85.71%	100%	100%	57.14%	100%	100%	28.57%
S. galilaeus	100 %	71.43%	71.43%	57.14%	71.43	100%	100%	100%	100%	100%	100%
T. zillii	100 %	28.75%	28.57%	-	-	57.14%	57.14%	100%	28.57%	100%	-

Table (3): Mean ± SE of relative mobility of plasma protein fractions (untreated electrophoretic samples) of different *tilapia* species.

Species		Fraction number										
		1	2	3	4	5	6	7	8	9	10	11
O. niloticus	Mean ± SE (n)	6.99 ±0.57 (7)	15.97 ±1.33 (3)	24.08 ±0.67 (6)	35.35 ± 0.41 (4)	44.88 ± 1.09 (5)	54.51 ±1.17 (7)	57.33 ± 0.52 (3)	63.49 ±0.87 (7)	75.28 ± 0.77 (6)	85.58 ± 1.42 (6)	91.73 ±1.38 (8)
O. aureus	Mean ± SE (n)	7.66 ± 0.47 (7)	16.1 ±0.51 (5)	27.06 ±0.51 (7)	38.8 ±0.0 (1)	46.05 ± 1.29 (6)	54.22 ± 1.19 (5)	62.56 ± 1.0 (7)	67.08 ± 0.61 (6)	79.4 ± 0.21 (2)	82.6 ±1.27 (4)	91.5 ±0.35 (2)
S. galilaeus	Mean ± SE (n)	7.21 ±0.73 (7)	13.92 ±1.27 (6)	25.87 ±0.65 (6)	35.70 ±0.83 (5)	4393 ±2.02 (5)	54.22 ±0.8 (6)	60.22 ± 1.12 (5)	67.45 ± 0.5 (6)	76.74 ± 1.49 (5)	85.18 ±1.04 (6)	91.78 ±0.23 (4)
T. zillii	Mean ± SE (n)	5.43 ±0.71 (7)	15.86 ±0.69 (5)	25.15 ±0.75 (4)	32.93 ± 0.75 (6)	41.43 ±0.67 (4)	55.18 ±0.79 (5)	61.27 ± 0.46 (7)	96.98 ± 1.48 (5)	76.70 ±0.88 (6)	88.1 ±0.35 (2)	94.73 ±0.73 (3)

n= Number of observations

Table (4): The significance (*t*-test) among relative mobilities of different plasma protein fractions (untreated electrophoretic samples) and similarity coefficient (SC) of different *tilapia* species.

Compared Species	Fraction number											Similarity coefficient (SC)
	1	2	3	4	5	6	7	8	9	10	11	
O.n. ×O.au.	0.9296	0.0883	3.5977*	3.8096*	0.6774	0.1693	3.2525*	3.2746*	2.9173*	1.4653	0.1109	0.55
O.n. × S.g.	7.9218*	0.9983	1.9137	0.3472	0.4600	0.1979	1.8717	3.7847*	0.9170	0.2278	0.0359	0.82
O.n. × T.z	1.7429	0.0819	1.0432	2.4394*	2.5300*	0.4335	4.9718*	4.0466*	1.2151	0.9723	1.7254	0.64
O.au. × S.g.	0.5178	1.3711	1.4561	1.5215	0.9223	0.0	1.5437	0.4687	1.0664	1.5759	0.6874	1.0
O.au. × T.z.	2.6134*	0.2224	2.1739	2.9536*	2.7317*	0.6730	1.1736	1.9407	1.6816	2.8799*	3.2872*	0.55
S.g. × T.z.	1.7479	1.2624	0.7135	2.4772*	1.3438	0.8487	0.9706	1.7551	0.0241	1.5364	4.3956*	0.82

*Significant at $P < 0.05$

O.n : Oreochromis niloticus , O. au: Oreochrmis aureus , S.g: Sarotherodon galiliaeus , T.z: Tilapia zillii

Table (5): Mean ± SE of relative mobility of plasma protein fractions (treated electrophoretic samples) of different *tilapia* species.

Species		Fraction number										
		1	2	3	4	5	6	7	8	9	10	11
O.niloticus	Mean ± SE (n)	5.51 ± 0.50 (7)	11.85 ±1.24 (4)	24.4 ± 0.9 (4)	36.63 ±1.06 (3)	48.1 ±0.93 (5)	52.93 ±0.76 (7)	56.8 ±0.9 (3)	66.15 ±0.65 (6)	78.1 ± 0.23 (6)	83.97 ±0.75 (6)	96.2 ±0.56 (4)
O.aureus	Mean ± SE (n)	6.0 ±0.37 (7)	15.5 ±0.43 (7)	26.18 ± 1.15 (4)	36.23 ±0.83 (3)	46.03 ±0.87 (6)	53.49 ±1.11 (7)	61.74 ±1.47 (7)	69.68 ±1.34 (4)	80.19 ±1.70 (7)	87.83 ±1.43 (7)	90.6 ±1.98 (2)
S. galilaeus	Mean ± SE (n)	7.62 ±0.60 (7)	16.3 ±0.85 (5)	28.06 ±0.67 (5)	35.6 ±1.56 (4)	47.04 ± 0.45 (5)	52.34 ±0.51 (7)	58.91 ± 1.07 (7)	64.64 ±1.46 (7)	73.66 ±1.53 (7)	82.3 ±0.56 (7)	90.11 ±0.94 (7)
T. zillii	Mean ± SE (n)	6.31 ±0.56 (7)	16.0 ±1.06 (2)	26.9 ±0.85 (2)	- - -	- - -	56.8 ±0.88 (4)	58.85 ±0.91 (4)	65.96 ±1.31 (7)	71.1 ±0.57 (2)	83.74 ±0.77 (7)	- - -

n= Number of observations

Table (6): The significance (*t*-test) among relative mobilities of different plasma protein fractions (treated electrophoretic samples) and similarity coefficient (SC) of different *tilapia* species**.**

Species	Fraction number											Similarity coefficient (SC)
	1	2	3	4	5	6	7	8	9	10	11	
O.n.* × *O.au.	0.7819	3.4285*	1.2189	0.2965	1.6217	0.4179	2.0702	2.6515*	1.1234	2.2711*	3.7966*	0.64
O.n.* × *S.g.	2.2336*	3.0672*	3.3363*	0.5041	1.0270	0.6454	1.1888	0.8918	2.6414*	1.8209	4.5710*	0.55
O.n.* × *T.z	1.0677	2.1140	1.7283	-	-	3.2103*	1.5656	0.1231	14.3136*	0.2124	-	0.55
O.au.* × *S.g.	1.7798	0.9190	1.4868	0.3203	0.9687	0.9419	1.5565	2.2902*	2.8506*	3.6075**	0.2417	0.73
O.au.* × *T.z.	0.4628	0.5241	0.3997	-	-	2.0318	1.3790	1.8385	2.7141*	2.5220*	-	0.55
S.g.* × *T.z.	1.1607	0.1793	0.9595	-	-	4.7274*	0.0377	0.6729	0.8467	1.5201	-	0.64

* Significant at P < 0.05

O.n : Oreochromis niloticus , O. au: Oreochrmis aureus , S.g: Sarotherodon galiliaeus , T.z: Tilapia zillii

II. Muscle proteins.

II.I. Fractions Appearance.

The muscle proteinograms of *tilapia* species (untreated sample) exhibited ten fractions (table 7 and figures 9,11,13 and 15). In *O. niloticus* fractions number 1,5 and 6 appeared in all examined fishes, so they were polymorphic bands. The 3rd and 9th fractions were not detected.

The fraction number eight was appeared with the same percentage in all species except in *O. aureus*, in which this fraction was not detected. In *O. aureus*, six fractions appeared with the percentage of 100%; these were fractions number 1,2,4,5,6 and 7. But, the 8th and 10th fractions were not found in this species (table, 7).

Regarding *S.galilaeus*, four fractions were of absolute appearance in all the examined fishes. Whereas the 10th fraction was disappeared. So, no specific band could be detected.

Fractions number 3 and 8 distinguished *O. niloticus* and *O. aureus* from the other *tilapia* species, in which this fraction disappeared in all tested individuals of this species. While, fraction noumber 9 was absent in *O. niloticus* and *T. zillii*. So, *T. zillii* could be identified using this band. The 10th fraction disappeared in all the tested species except *O. niloticus*, in which this fraction appeared in very low percentage.

The percentage of appearance of muscle protein fraction (treated electrophoretic samples) of *tilapia* species were presented in table 8 and the muscle proteinograms were depicted in figures 10,12,14 and 16. Fractions from 1 to 5 of muscle protein of *O. niloticus* had the percentage of appearance 100%. Only the 10th fraction was not observed in the muscle proteinogram, so it discriminated *O. niloticus* from the other fishes. While, in *O. aureus*, the fractions from 4 to 7 existed in all tested individuals.

With the exception of the 4th fraction, the fractions of *S. galilaeus* from 1 to 5 and the 8th were appeared with percentage of 100%. Six muscle protein fractions of *T. zillii* were found in all the tested individuals; these fractions were number 1,2,4,6,7 and 8.

Except fraction number 3, the fractions from 1 to 7 appeared with high percentages in all the studied species. The 3rd fraction distinguished *T. zillii* from the other tilapias, as it appeared with 100% appearance in all species, except *T. zillii*, only 42.86% of the individuals had this fraction.

II.2. Relative Mobilities of muscle Protein Fractions.

The relative mobilities of muscle protein fractions (untreated electrophoretic samples) of *tilapia* species are presented in tables 9 and 10. Comparing *O. niloticus* and *O. aureus*, only the 7th fraction showed a significant difference.

When comparing the mobility of the different fractions between *O. niloticus* and *S. galilaeus*, it was found that the differences were statistically significant if comparing the 4th, 5th, 6th, 7th and 8th fractions. So, they are widely arrayed species.

The fractions from 5th to 8th and fraction noumber 2 changed with significant differences when comparing the relative mobility between *O. niloticus* and *T. zillii*. Regarding *O. aureus* and *S. galilaeus*, the differences in the relative mobility were considered statistically significant when the 1st, 3rd, 5th, 6th, 7th and 9th fractions were compared, indicating wide genetic distance. The fraction mobility differed significantly between *O. aureus* and *T. zillii* when comparing the bands number 2,3,6, and 7.The differences of the relative mobility values changed significantly when comparing the 1st, 2nd, 3rd, and 8th fractions between *S. galilaeus* and *T. zillii*.

O. niloticus when compared with *O. aureus* had exhibited a high similarity coefficient, as a result of comparing relative mobility of muscle protein fraction (untreated electrophoretic sample), the recorded SC value was 0.5. Whereas, the SC between the rest of the species is of low value.

The significant (*t*-test) among different relative mobilities of muscle protein fraction (treated electrophoretic samples) of *tilapia* species is presented in table 12. All protein fractions statistically had a non-significant change when comparing *O. niloticus* and *O. aureus*. The comparison of the 2nd and 4th fractions between *O. niloticus* and *S. galilaeus* showed a significant difference in the relative mobility. Also, the relative mobility of the 8th and 9th fractions was differed significantly between *O. niloticus* and *T. zillii*.

Concerning *O. aureus* and *S. galilaeus*, the differences of fraction mobility were changed significantly when comparing the 2nd and 4th fractions.

The mobilities of fractions number 7,8 and 9 were differed significantly between *O. aureus* and *T. zillii*. The rest of fractions showed negligible differences.

By comparing the relative mobility of fractions number 4, 5,7 and 10 between *S. galilaeus* and *T. zillii*, the differences were found statistically significant. Meanwhile, the rest of fraction mobilities were slightly differed in the sarcoplasmic protein of the prescribed species.

According to the data presented in table 12, the SC values of relative mobility of protein fractions were 1.0 and 0.8 which resulted from comparing *O. niloticus* with *O. aureus* and *O. aureus* with *S. galilaeus* , respectively. However, a low SC value (0.6) was recorded when comparing *S. galilaeus* with *T. zillii.*

Table (7) Percentage appearance of muscle protein fractions (untreated electrophoretic samples) of different *tilapia* species.

Species	**Fraction number**									
	1	2	3	4	5	6	7	8	9	10
O.niloticus	100 %	42.86%	-	71.43%	100%	100%	85.71%	100%	-	14.29%
O.aureus	100 %	100%	85.71%	100%	100%	100%	100%	-	42.86%	-
S. galilaeus	100 %	57.14	85.71	85.71%	85.71	85.71%	100%	100%	100%	-
T. zillii	100 %	100%	100%	100%	100%	100%	85.71%	100%	-	-

Table (8) Percentage appearance of muscle protein fractions (treated electrophoretic samples) of different *tilapia* species.

Species	Fraction number									
	1	2	3	4	5	6	7	8	9	10
O.niloticus	100 %	100 %	100 %	100%	100%	85.71%	85.71%	42.86%	28.57%	-
O.aureus	100 %	100 %	100 %	100%	100%	100%	100%	57.14%	14.29%	71.43%
S. galilaeus	100 %	100 %	100 %	85.71%	100%	85.71	85.71%	100%	85.71%	42.86%
T. zillii	100 %	100 %	42.86%	100%	85.71%	100%	100%	100%	85.71	85.71%

Table (9): Mean ± SE of relative mobility of muscle protein fractions (untreated electrophoretic samples) of different *tilapia* species.

Species		Fraction number									
		1	2	3	4	5	6	7	8	9	10
O.niloticus	Mean ± SE (n)	6.14 ± 0.59 (7)	14.93 ±2.02 (3)	- - -	33.32 ±0.77 (5)	41.93 ±0. 71 (7)	50.83 ±0.67 (7)	54.77 ±1.12 (6)	59.89 ±1.16 (7)	- - -	87.8 ±0.0 (1)
O.aureus	Mean ± SE (n)	5.49 ±0.42 (7)	14.54 ±0.95 (7)	20.37 ±0.09 (6)	33. 64 ±1.71 (6)	41.64 ±0.64 (7)	51.49 ±1.03 (7)	59.87 ±0.79 (7)	- - -	89.3 ±1.31 (3)	- - -
S. galilaeus	Mean ± SE (n)	9.14 ±0.32 (7)	15.68 ±1.41 (4)	26.1 ±0.94 (6)	37.23 ±1.21 (6)	46.08 ±0.84 (6)	55.15 ±0.59 (6)	63.9 ±0.38 (7)	74.59 ±0.65 (7)	81.5 ±0.58 (7)	- - -
T. zillii	Mean ± SE (n)	5.69 ±0.29 (7)	10.69 ±0.56 (7)	23.13 ±0.45 (7)	34.29 ±0.74 (7)	47.67 ±1.11 (7)	56.86 ±0.69 (7)	65.4 ±1.55 (6)	69.0 ±0.80 (7)	- - -	- - -

n= Number of observations .

Table (10): The significance (t-test) among different relative mobilities of muscle protein fractions (untreated electrophoretic samples) and similarity coefficient (SC) of different *tilapia* species.

Compared Species	Fraction number										Similarity Coefficient (SC)
	1	2	3	4	5	6	7	8	9	10	
O.n.* × *O.au.	0.8982	0.2032	-	0.1494	0.3044	0.5367	3.7998*	-	-	-	0.50
O.n.* × *S.g.	1.6495	0.3162	-	2.6002*	3.8177*	4.7805*	8.2115*	11.0796*	-	-	0.2
O.n.* × *T.z	0.6826	2.8446*	-	0.8857	4.3691*	6.2487*	5.5414*	6.4846*	-	-	0.2
O.au.* × *S.g.	6.3974*	0.6966	4.9315*	1.6618	4.2854*	2.9436*	4.5934*	-	6.4544*	-	0.2
O.au.* × *T.z.	0.3900	3.5123*	3.4367*	0.3494	4.7167*	4.3156*	3.3168*	-	-	-	0.2
S.g.* × *T.z.	7.9629*	3.9359*	2.9987*	2.1397	1.1130	1.8447	1.008	5.433*	-	-	0.4

* Significant at $P < 0.05$

O.n : Oreochromis niloticus , O. au: Oroechrmis aureus , S.g: Sarotherodon galiliaeus , T.z: Tilapia zillii

Table (11): Mean ± SE of relative mobility of muscle protein fractions (treated electrophoretic samples) of different *tilapia* species.

Species		Fraction number									
		1	2	3	4	5	6	7	8	9	10
O.niloticus	Mean ± SE (n)	7.11 ± 0.55 (7)	14.6 ± 1.07 (7)	23.1 ± 1.22 (7)	35.07 ±1.84 (7)	42.3 ±0.91 (7)	53.55 ±1.90 (7)	61.12 ±1.55 (6)	67.57 ±2.22 (3)	77.6 ±0.07 (2)	- - -
O.aureus	Mean ± SE (n)	6.51 ±0.36 (7)	14.4 ±0.71 (7)	22.21 ±0.42 (7)	33.73 ±0.72 (7)	41.45 0.45 (7)	52.77 ±0.60 (7)	60.04 ±0.68 (7)	65.3 ±2.33 (4)	87.6 ±0.0 (1)	92.64 ±1.26 (5)
S. galilaeus	Mean ± SE (n)	5.19 ±0.88 (7)	11.43 ±0.56 (7)	21.19 ±1.19 (7)	29.03 ±1.09 (6)	39.87 ±0.92 (7)	50.68 ±1.45 (6)	57.32 ±1.65 (6)	67.61 ±2.66 (7)	76.92 ±2.53 (6)	86.83 ±2.52 (3)
T. zillii	Mean ± SE (n)	6.17 ±1.0 (7)	12.43 ±1.15 (7)	23.5 ±0.43 (3)	34.37 ±0.92 (7)	43.32 ±1.0 (6)	54.07 ±0.90 (7)	64.23 ±1.12 (7)	72.47 ±0.63 (7)	79.97 ±0.40 (6)	94.9 ±0.71 (6)

n= Number of observations .

Table (12): The significance (t-test) among different relative mobilities of muscle protein fractions (treated electrophoretic samples) and similarity coefficient (SC) of different *tilapia* species.

Compared Species	Fraction number										Similarity Coefficient (SC)
	1	2	3	4	5	6	7	8	9	10	
O.n. × O.au.	0.9175	0.1551	0.6889	0.6761	1.2481	0.418	0.6734	0.6821	8.165	-	1
O.n. × S.g.	1.8546	2.6151*	1.12	2.6948*	1.8824	1.1236	1.676	0.0091	0.1470	-	0.7
O.n. × T.z	0.827	1.3825	0.2048	0.3394	0.755	0.2594	1.6622	2.9604*	2.9518*	-	0.7
O.au. × S.g.	1.3859	3.265*	0.8095	3.6866*	1.1362	1.4059	1.6116	0.5805	1.5974	2.3283	0.8
O.au. × T.z.	0.3202	1.4595	1.7976	0.5454	2.1958	1.3177	3.2817*	3.7907*	7.2082*	1.6272	0.7
S.g. × T.z.	0.7364	0.7836	1.2195	3.7683*	2.5481*	2.0452	3.5555*	1.7777	1.1921	4.1374*	0.6

* Significant at $P < 0.05$

O.n : Orechromis niloticus , O. au: Orechrmis aureus , S.g: Sarotherodon galiliaeus , T.z: Tilapia zillii

DISCUSSION

The systematic distance between species is the reason for a reproductive behaviour barrier[Lovshin, 1982]. In the present study, genera *Oreochromis* and *Sarotherodon* were found to be more closely related to each other. The evidence of this degree of similarity might be related to mouth brooding behaviour in these two above-mentioned genera. On the other hand, the results indicated a lesser degree of similarity between genus *Tilapia* and the other two genera resulted from the differed reproductive behaviour of genus *Tilapia* (substrate spawning).

Electrophoretic techniques have been used to estimate genetic distances and taxonomic relationships among several groups of organisms including fish (Haroun, 1999; Hanfling and Brandl , 2000 and Berrini *et al*., 2006). Percentage appearance of plasma protein fractions indicated that the number of fractions were common for all the studied species without missing any fractions, this may be due to all species were not exposed to any pollution [Sharaf-Eldeen and Abdel-Hamide 2002] .The plasma protein fractions obtained in the present study showed common polymorphic fractions for all the studied species. The results recorded in the present study indicated species-specific patterns with common bands for all the studied species as well as specific bands characterizing each species. These results were in harmonizing with those obtained by Oberst *et al.,(*1996) ; Haroun (1999) and Berrini *et al*.,(2006). Species-specific fractions for *O. niloticus* were obtained; these fractions were number 4,5,9,10 and 11, which showed polymorphism. Fraction number 2,5,7 and 10 were polymorphic and characteristic for *O. aureus*. Whereas fractions number 2,4,7,9, 10 and 11 were specific for *S. galilaeus*, while fractions number 2,4,5,7 and 9 characterized *T. zillii* from the other *tilapia* species. Close relationship between *O. niloticus ,S. galilaeus* and a genetic distance of genus *Tilapia* was reported [Oberst *et al*.,1996].

Hanfling and Brandl (2000) proved the monophyltic relationship between subfamilies of family *Cyprinidae*, which did not seem to be monophyletic, using allozyme electrophoretic technique. The monophyltic relationship of *tilapia* fish has been confirmed by Oberst *et al*. (1993 & 1996), Zowail and Baker (1998), Yapi-Gnaore (2001) and Rognon and Guyomard (2003) by using several electrophoretic techniques including polyacrylamide gel electrophoresis, isoelectric focusing, immunoelectrophoresis and allozyme electrophoresis.

Comparison of plasma proteinogram between the four species in the term of relative mobility showed a sign of similarity between *O. niloticus* and *S. galilaeus* (SC = 0.82) and between *T. zillii* and *S. galilaeus* (SC = 0.82). Also, high similarity was recorded between *O.aureus* and *S. galilaeus* (SC= 1). Some other studies declared similarity between O. *niloticus* and *T. zillii* (SC = 0.75) also *between O. niloticus* and *S. galilaeus* (SC = 0.63) and between *T. zillii* and *S. galilaeus* (SC = 0.75)[Zowail and Baker ,1998].

The present results are pointed out that the muscle proteins differed from plasma proteins in two terms: First, the muscle proteins were separated into ten fractions and while eleven fractions were separated in case of plasma proteins; secondly, the disappearance of some fractions in the proteinogram of each. The structure of blood serum proteins, muscle proteins haemoglobins as well as enzymes in blood and some organs appeared to be variable (Kirpichnikove, 1981).

The obtained results of soluble muscle protein in the present study pointed out the monophylogenetic relationship of all species in which, they all had the same number of protein fractions. Untreated muscle samples present five common fractions (Numbers 1,4,5,6 and 7) in all the studied species. Only the protein fraction number 8 was considered as species-specific fraction for *O. niloticus*, whereas, fractions number 2 and 3 characterize *O. aureus* from the rest of the species. Also, fractions number 2,3 and 8 appeared equally in *S. galilaeus* and *T. zillii*. Whereas, protein band number 9 distinguished *S. galilaeus* from *T. zillii*.

Also, the obtained results of similarity coefficient indicated the polyphyletic relationship of different species. There was relatively high similarity (0.5) between *O. niloticus* and *O. aureus.* Meanwhile the recorded similarity coefficient between other different species was very low (0.2), indicating that these species belong to different genera, i.e., have a polyphyletic relationship [Haroun, 1999; White, 2000; El-Serafy *et al*., 2003]. This also supported the results of plasma proteinogram obtained in the present study.

Conclusion:

The present study found a species specific protein pattern of *tilapia* species, by using protein fractionation. Furthermore, the present study attained that the use of untreated sample gave data that was not completely differed in most cases from treated one. So, it is recommended to use untreated sample for electrophoretic identification of fish species. The uses of plasma and muscle proteinogram data are confirmatory for the species discrimination. It is also rescued that less

degree of similarity between *T. zillii* and the other species that point toward a genetic distance between this species and the others.

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