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

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ABSTRACT

This study deals with the electrophoretic characterization of Tilapia species using plasma and muscle proteins, as a taxonomically tool to differentiate Tilapia species that live in Egyptian habitat of the River Nile. These species are *Oreochromis niloticus (O. niloticus)* , *O. aureus* ,*Sarotherodon galilaeus (S. galilaeus)* and *Tilapia zillii (T. zillii)*. The data of electrophoretic protein separation indicated that each species has a characteristic specific pattern with more common bands for all species as well as species specific bands characteristic for each one. In this study, the protein of plasma and muscle were examined either treated with sample buffer or not treated. The results indicated that the protein pattern among the closer species exhibits common characters. In this regard, the obtained protein pattern of *O. niloticus* looks like that of *O. aureus* indicating a monophylogenetic origin of these species. Whereas, less degree of similarity was recorded between *T. zillii* and other species, indicating a genetic distance between this species and the rest of Tilapia species.

INTRODUCTION

Fishes constitute a principal component of aquatic habitat. It is a source of protein, calcium and other elements necessary for vital activities of human body. It also contributes about 6% of the total world protein supply and about 24% of animal protein (FAO, 1984). So that, a great attention has been given to study the Egyptian fish species. Fishes of family *Cichlidae* 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 the River Nile (Sharaf Eldeen and Abdel-Hamide, 2002). 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 reproduce 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 the 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 susbstrate 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 skeletal muscle proteins (Myogens) of fish by using polyacrylamide electrophoretic techniques as one of the biochemical methods used to differentiate animal species (El-Serafy, 1994; Mamuris *et al.*, 1999; Sharaf El-Deen and Abdel-Hamide, 2002 ; 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; Shain, 1999 ; El-Serafy *et al.*, 2003.). Also, methods based on DNA analysis have also been used (El-Serafy *et al.*, 2003; Perdices *et al.*, 2005). For these reasons the present study aims to use the protein electrophoresis as a tool to differentiate Tilap*ia* species, and to define the phylogenetic relationship among the studied species.

MATERIALS AND METHODS

The fishes used in the present study were collected from El-Riyah E-Tawfequi [A branch of the River Nile] at Benha City. After fish killing by medullar transaction, the muscle samples were isolated from the dorsal epiaxial muscle.

I. Blood 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. Then it was stored in deep freezer (-20°C) until analysis.

II. Electrophoretic technique: -

Fractionation of protein in plasma and muscle were done using sodium dodecyl sulphate polyacrylamide gell electrophoresis (SDS-PAGE). Sample treatment and gell 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 destaining 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.

III. Statistical analysis:

The data obtained in this study were presented as mean ± SE (Standard error) Student *t*-test was estimated between 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 (Table 1 and Figs 1;3;5;7). Fraction number 1 presented with a percentage 100% in all Tilapia species. In *O. niloticus*, fractions number 6 and 8 appeared with the same percentage. Whereas the 2nd and 7th fractions showed a low percentage of appearance, so these bands are species specific.

Most protein fractions of *O. aureus* appeared with high percentages. The fractions number 1,3 and 7 appeared in all the tested fishes. Low percentages of appearance were recorded for fractions number 4, 9 and 11 (Table 1)

Five fractions of plasma protein of *S.galilaeus* appeared with a percentage of 85.71% (Fraction number 2,3,6,8 and 10). Only one fraction (number 5) appears with low percentage (42.86%).

In *T. zillii*, the first and 7th fractions appeared in all examined fishes. The last fractions (numbers 10 and 11) showed a low percentage of 28.57% and 42.86%, respectively. Therefore, by viewing the plasma proteinogram of Tilapia species it could be possible to differentiate Tilapia species by protein bands number 2,4,5,7, 9, 10 and 11 which appeared in the proteinogram by different percentages among the studied species (Table 1). Protein polymorphic bands are presented among Tilapia species (Numbers 1, 3 and 7).

Table (2) shows the percentage of occurrence of plasma protein fractions of Nile Tilapia species (treated electrophoretic samples), the plasma proteinogram are 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 numbers 1,2,6,7,9 and 10. The plasma protein fractions of *S. galilaeus* have a percentage appearance of 100%;these fractions are the first fraction and the last six ones (numbers 6, 7,8,9,10 and 11). So, these fractions are polymorphic.

T. zillii shows 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 be better than using the treated one.

I.2 Relative mobility of 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 ,4th , 7th , 8th and 9th fractions changed with significant differences.

Only the difference in the 1st and 8th fraction mobilities were found statistically significant in comparing *O. niloticus* and *S. galilaeus*. Concerning the comparison between *O. niloticus* and *T. zillii*, four fraction differed significantly [fractions numbers 4,5,7 and 8]. This indicates that they are polyphyltic species.

All fraction mobilities of *O. aureus* and *S. galilaeus* did not differ significantly. The fractions numbers 1,4,5,10 and 11 differed significantly 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 are statistically differed when comparing *S. galilaeus* and *T. zillii* ; this also indicates polyphylogny.

Similarity coefficient (SC) of the relative mobility of 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 show high SC which may indicate a monophylogeny of all Tilapia species except *T. zillii* which may be originated separately.

Concerning the comparison of treated samples between *O. niloticus* and *O. aureus*, the 2nd ,8th , 10th, 10th and 11th fraction mobilities changed significantly. Fractions number 1,2,3,9 and 11 show 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*, whereas, the 4th , 5th and 11th fractions disappeared in the prescribed species. Similarly, a special protein pattern was noticed again between *S. galilaeus* and *T. zillii*, in which, the 4th , 5th fractions were not found. 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 (0.64%) *O. niloticus* and *O. aureus*. 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 (Monophylogentic). Whereas, *T. zillii* displays another origin. So all *tilapia* species are polyphylogenetic, i.e., they are derived from separate origins.

I.3 Percentage (%) Area of Protein Fractions:

The data of % area of each protein band (untreated sample) are represented in tables 7 and 8. By comparing *O. niloticus* and *O. aureus*, only the 3rd and 11th fractions percentage area differed with significant differences. Similarly, the percentage areas of the 3rd and 5th fractions were significantly differed *between O.niloticus* and *S. galilaeus*. Regarding *O.niloticus* and *T. zillii* only the 5th fraction percentage area showed significant difference. The comparison between *O. aureus* and *S. galilaeus* showed a significant differences in fraction number 1,3 and 5. While the 3rd and 5th fractions differed significantly between *O. aureus* and *T. zillii*. But all compared fractions between *S.galilaeus* and *T. zillii* showed no significant differences, so they are closely related.

The comparison of percentage area of plasma protein fractions of treated electrophoretic samples as average t-values between different species were presented in Table (10) When comparing *O. niloticus* and *O. aureus*, the percentage area of the 1st fraction showed a significant difference, but no differences in the other fractions, so they are monophyltic species Between *O. niloticus* and *S. galilaeus* , there are significant changes of percentage area of the 8th and 11th fractions only .

Concerning *O. niloticus* and *T. zillii*, significant differences were observed when comparing the 1st , 2nd and 6th fractions. So they are genetically distant species. Comparing *O. aureus* with *S. galilaeus* only the 1st fraction differed significantly. The difference between *O. aureus* and *T. zillii* showed significant differences of percentage area of the 10th, and 6th fractions.

Regarding the differentiation between *O. aureus* and *S. galilaeus*, only the % area of the first fraction was differed significantly. The comparison between *O. aureus* and *T. zillii* showed highly significant changes of

percentage area of the 6th and 10th fractions. When comparing *S. galilaeus* and *T. zillii*, the 1st, 3rd ,6 th ,and 9th and 10th fractions showed significant differences.

II. Muscle proteins:

II.I. Fractions Appearance:

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

The eighth fraction 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 are fractions number 1,2,4,5,6 and 7. But, the 8th and 10th fractions were not found in this fish species. (Table 11).

Regarding *S.galilaeus*, four fractions appeared in all the examined fishes (100%). Whereas, one fraction, the last one was disappeared (10th). No specific band could be detected.

Fractions numbers 3 and 8 distinguish *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* can be identified using this band .The last fraction (10th) 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 12 and the muscle proteinograms were depicted in Figs 10,12.14 and 16. Fractions from 1 to 5 of muscle protein of *O. niloticus* have the percentage of appearance 100%. Only. the last fraction (10th) was not observed in the muscle proteinogram , so it discriminates *O. niloticus* from the other fishes. While in *O. aureus*, the fractions from 4 to 7 existed in all tested individuals.

A part from 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 are 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 distinguishes *T. zillii* from the other tilapias, as it appeared with 100% appearance in all species, except *T. zillii*, only 42.86% of the individuals have this fraction.

II.2. Relative Mobility of Protein Fractions:

The relative mobilities of muscle protein fractions (untreated electrophoretic samples) of *tilapia* species were presented in tables 13 and 14. 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 changed with more highly significant differences between *O. aureus* and *T. zillii* when. comparing the bands noumber 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*, 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 low value; this indicates a low similarity.

The significant (*t*-test) among different relative mobilities of muscle protein fraction (treated electrophoretic samples) of Tilapia species is presented in table 16.All protein fractions statistically have 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. But, the comparison of the other fractions is not significant.

The relative mobility of the 8th and 9th fractions was changed with significant differences between *O. niloticus* and *T. zillii*. But the comparison of other fractions between the same two species was not significant. Concerning *O. aureus* and *S. galilaeus*, the differences of fractions mobility were changed significantly when comparing the 2nd and 4th fractions .

The mobilities of fractions numbers 7,8 and 9 were differed with significant difference between *O. aureus* and *T. zillii* . The rest of fractions show negligible differences.

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

According to the data presented in table 16, the SC values of relative mobility of protein fractions are 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.*

II.3. Percentage (%) area of protein fractions:

The percentage area of muscle protein fractions (untreated electrophoretic samples) of Tilapia species were presented in tables 17 and 18. The percentage area of the 1st and 7th fractions differed significantly when comparing *O. niloticus* with *O. aureus*, whereas, the differences of other fractions did not significantly changed.

Fractions numbers 1, 6 and 8 have percentage area which are found significantly differed between *O. niloticus* and *S. galilaeus.*

Concerning *O. niloticus* and *T. zillii*, only, the percentage area of the 6th fraction showed a significant difference; this is due to fractions missing in these species.

By comparing the significant differences of percentage area of each protein fraction (Table 18) ,it was found that *O. aureus* and *S. galilaeus* have a five significantly differed bands which may reflect genetic distance. The comparison of *O. niloticus* and *S. galilaeus* yield four bands that significantly differed in the percentage area, also reflecting dissimilar genetic origin. Whereas, *S. galilaeus* when compared with *T. zillii* show three bands which were significantly differed in its % area.

The data presented in Table (20) show the *t*-values of percentage area of sarcoplasmic protein fraction (treated electrophoretic samples) of *tilapia* species. The comparison of percentage area of the 1st, 3rd, 4th, 5th and 6th protein fractions between *O. niloticus* and *O. aureus* represented significant differences which my reflect variations in the protein content in these bands.

Concerning the comparison between *O. niloticus* and *S. galilaeus*, the differences were statistically significant in five bands (1, 3, 5, 6 and

8). When comparing *O. niloticus* with *T. zillii* significant differences were recorded for the % area of bands numbers 1,3,7and 8.

When comparing the percentage area of fractions noumber 4,5 and 6 between *O. aureus* and *S. galilaeus*, it was differed significantly. Regarding the last two species (*S. galilaeus* and *T. zillii*) the 5th , 6th and 8th fractions percentage area showed significant changes,

DICUSSION

Tilapia (family: Cichlidae) is common fishes native to the fresh waters of Africa. They include the mouth brooding genera (*Orechrmois* and *Sarotherodon*) and substrate spawning genus Tilapia (Trewavas, 1991; Stiassny, 1991). Its population constitutes a bulk of fauna in the River Nile. Their species distributed all over the river Nile habitat with some degree of dominance.

The monophyly of all Tilapia species (family Cichlidae) has been proved by Oberst *et al.* (1993 and 1996).

Lovshin (1982) recorded that the systematic distance between species is the reason for a reproductive behaviour barrier. In the present study, the two genera. *Sarotherodon* were found to be more closely related to each other. The evidence of this degree of similarity may be due to that these two obvious genera related to the same group with mouth brooding reproductive behaviour. Whereas, 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 ; Berrini *et al.*, 2006)

Hanfling and Brandl (2000) proved the monophyltic relationship between subfamilies of family *Cyprinidae*, which do not seem to be monophyltic, using allozyme electrophoretic technique. The results reported in the present work indicate species-specific patterns with common bands for all the studied species as well as specific bands characterizing each species. The monophyltic relationship of *tilapia* fish has been confirmed by Oberst *et al.* (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.

Percentage appearance of plasma protein fractions indicated that the number of fractions are common for all the studied species without missing any fractions, this is due to all species are not exposed to any pollution. Sharaf-Eldeen and Abdel-Hamide (2002) investigated the exposure of *O. niloticus* to some pollutants and found that six protein fractions were missing due to exposure to high level of copper.

The plasma protein fractions obtained in the present study show common polymorphic fractions for all the studied species. These fractions are noumber 1,3,6 and 8; this proves the monophylogenetic relationship of *tilapia* species. The same observations were reported by Haroun (1999), White (2000) and El-Serafy *et al.* (2003).

Species-specific fractions for *O. niloticus* were obtained; these fractions are noumber 4,5,9,10 and 11, which show polymorphism. Fractions noumber 2.5,7 and 10 were polymorphic and characteristic for *O. aureus*. Whereas fractions noumber 2.4,7,9, 10 and 11 were specific for *S. galilaeus*, while fractions noumber 2.4.5,7 and 9 characterized *T. zillii* from the other *tilapia* species. These results are in agreement with those obtained by Haroun (1999) who used a biochemical technique for identification of three *Oreochromis* species and reported that each *Oreochromis* species has a characteristic species – specific isoelectric focusing pattern.

The comparison of serum proteinogram between the four species in the term of relative mobility indicated that there were similarity between *O. niloticus* and *S. galilaeus* (SC = 0.82) also between *O. niloticus* and *T. zillii* (SC = 0.64) and between *T. zillii* and *S. galilaeus* (SC = 0.82). Also, high similarity was recorded between *O.aureus* and *S. galilaeus*. These results are in agreement with those obtained by Zowail and Baker (1998). The authors used the sera proteinograms to identify five species of fresh water fish (*Sarotherodon galilaeus, Tilapia zillii, Oreochromis niloticus, Clarias lazera* and *Barbus bynni*). The similarity coefficients were studied for the different species. The previous authors reported that the comparison of serum proteinogram between the five species in the term of relative mobility indicated that there were 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). On the other hand similarity was lower between the other species.

The structure of blood serum proteins, muscle proteins haemoglobins as well as enzymes in blood and some organs appears to be variable (Kirpichinkove, 1981). These results are in agreement with the obtained results in which the muscle proteins differed from plasma proteins in two items: First, the muscle proteins were separated into ten fractions and to eleven fractions in plasma proteinogram, secondly, the disappearance of some fractions in the proteinogram of each species.

El-Gharabawy (1991) used electrophoresis technique to study the soluble proteins of muscles and skin of five sole species. The author found that some bands were obtained for each individual species and were considered as markers of these species. Also, several common protein bands were observed in all tested species. Also, White (2000) used protein electrophoretic technique to use skeletal muscle protein for investigating genetic variations of white bass *Morone chrysops*. The author found that there were relatively low levels of electrophoretic variations which characterize the species and low levels of allozyme variations appeared in all species in the same genus. Recenty. Berrini *et al.* (2006) used iso- electric focusing (IEF) and two dimensional electrophoresis (2-DE) of muscle protein to distinguish foure freshwater species.

In case of *tilapia* research, Oberst *et al.* (1996) studied the electropherograms of muscle protein of three species of genus *tilapia*: O. *niloticus*, *S. galilaeus* and *S. melanotherom*. They recorded species-specific protein profiles with common characteristic bands and according to the relation of these patterns. They placed the six investigated species into three groups with distinct band patterns *O. niloticus* with *S. galilaeus*, *S. melanotheron* with *T. zillii* and *T. guineensis* with *T. dageti*. Also, they found a closer relationship between the two species of genus *tilapia*. There was close relationship between genus *Orechromis* (*O. niloticus*) and genus *Sarotherodon* (*S. galilaeus*) and the genetic distance of genus *Tilapia*.

The obtained results of soluble muscle protein in the present study indicate the monophylogenetic relationship of all species in which, they all have 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 numbers 2 and 3 characterize *O. aureus* from the rest of the species. Also, fractions

numbers 2,3 and 8 appeared equally in *S. galilaeus* and *T. zillii*. Whereas, protein band number 9 distinguishes *S. galilaeus* from *T. zillii*.

Also, the obtained results of similarity coefficient indicate the polyphylogenetic relationship of different species. As well as there is 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.

Falk *et al.* (1996) studied the proteinogram of skeletal muscle proteins, especially low molecular weight parvalbumins for different tilapis species. They indicated that, the 22kDa-parvalbumin component was common to all species studied and probably characteristic for tilapia. Also, species of genus tilapia were characterized by the 24kDa component which was absent in the two other tilapia genera. They also grouped the studied tilapia species into three groups. The first group included *O. aureus, O. niloticus* and *S. galilaeus* characterized by the expression of only one major parvalbumin component (22kDa). The second group includes *T. zillii and T. busumana* characterized by 13.5 kDa component. Whereas, 18kDa component occurs only in *T. guineensis* and *T. dageti* which have been placed as the third group.

Smith and McVeagh (2000) used allozyme electrophoretic technique to differentiate tooth fish species. They proved that the allozyme data show little genetic differentiation among species. In contrast, the microsatellite DNA data indicate significant genetic heterogeneity and demonstrate significant genetic differentiation among species. The same results were recorded for tilapia by Yapi – Gnaore (2001). The author used morphometric and Meristic characteristics, as well as, electrophoresis characterization and recent genetic techniques such as microsatellite and restriction fragments length polymorphism of mitochondrial DNA, to evaluate and describe fish characterization of three tilapia species (*S. melanotheron, O. niloticus and O. aureus*). The same author also found that the molecular techniques provide god markers and significant genetic characterization for the studied species.

Species identification based on morphological criteria and protein analysis is the most reliable and widely used method. Species-specific banding patterns are typically generated by isoelectric focusing. This technique has proven to be reliable (Rehbein *et al.*, 1995). Protein-based identification techniques become less reliable with fish. However, in

some cases it is still possible to generate a banding pattern which enables identification (Berrini *et al.*, 2006).

As an alternative to protein analysis, DNA-based identification techniques have been proposed and investigated. The molecular techniques based on PCR-RFLP analysis of the DNA have been extensively used for many analyses of fish (Fernandez, 2001 ;Perdices *et al* ., 2005).

Farias *et al.* (1999) and El-Serafy *et al.* (2003) used restriction fragment length polymorphisms of nuclear and mitochondrial DNA- PCR products (RELPs / PCR) as a basis for examining relationships among *tilapia* species. They find out that *tilapia* species are polyphylogenetic species and some are monophylogenetic.

Conclusion:

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

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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 = 9.41 (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 (5)	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) of different *tilapia* species.

Compared Species	Fraction number											Similarity coefficient
	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) of different *tilapia* species.

Species	Fraction number											Similarity coefficient
	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

Table (7): Mean ± SE of percentage area 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	10.77	4.65	4.90	7.23	11.42	20.34	13.4	17.27	8.42	11.73	17.2
	± SE	±1.38	±0.95	±0.89	±0.39	±0.88	±2.71	±2.94	±2.94	±2.41	±2.13	±1.6
	(n)	(7)	(3)	(6)	(4)	(5)	(7)	(7)	(7)	(6)	(6)	(4)
O.aureus	Mean	11.03	5.02	14.9	6.3	13.78	17.8	20.23	15.32	8.65	9.83	7.8
	± SE	±0.90	±0.99	±1.77	±0.0	±1.47	±4.71	±3.64	±1.28	±3.36	±1.74	±0.21
	(n)	(7)	(5)	(7)	(1)	(6)	(5)	(7)	(6)	(2)	(4)	(2)
S. galilaeus	Mean	7.34	6.25	8.9	8.64	5.83	28.9	19.98	13.2	5.4	10.63	13.45
	± SE	±1.11	±1.09	±1.43	±1.73	±1.2	±5.14	±4.27	±1.14	±0.98	±1.55	±2.46
	(n)	(7)	(6)	(6)	(5)	(3)	(6)	(5)	(6)	(5)	(6)	(4)
T. zillii	Mean	10.07	4.44	6.4	7.77	6.85	21.04	27.64	10.40	16.92	4.25	16.2
	± SE	±1.34	±0.66	±1.28	±1.34	±1.41	±3.08	±4.04	±2.16	±4.6	±0.74	±2.29
	(n)	(7)	(5)	(4)	(6)	(4)	(5)	(7)	(5)	(6)	(2)	(3)

N= Number of observations

Table (8): The significance (*t*-test) among percentage area of different plasma fractions (untreated electrophoretic samples) of different *tilapia* species.

Compared Species	Fraction number											Similarity coefficient
	1	2	3	4	5	6	7	8	9	10	11	
O.n. × O.au.	0.1580	0.2483	4.7822*	1.1883	1.3088	0.5005	1.2014	0.5717	0.0491	0.6351	3.9109*	0.18
O.n. × S.g.	1.9395	0.9370	2.3683*	0.7081	3.8323**	1.5376	1.0802	1.2086	1.0752	0.4182	1.2779	0.18
O.n. × T.z	0.3648	0.1869	0.9967	0.3184	2.8780*	0.1695	2.1481	1.7355	1.6361	1.9184	0.3716	0.09
O.au. × S.g.	2.5767*	0.8223	2.5746*	0.5520	3.4813*	1.5634	0.0462	1.2363	1.3472	0.3374	1.5302	0.27
O.au. × T.z.	0.5947	0.4876	3.3117*	0.4162	3.2315*	0.5757	1.4004	2.0439	0.9675	2.1121	2.8418	0.18
S.g. × T.z.	1.5696	1.3497	1.2164	0.4048	0.5244	1.2416	1.2764	1.2081	2.2316	2.2413	0.7895	0.0

* Significant at $P < 0.05$

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

Table (9): Mean ± SE of percentage area 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)	4.31 ±0.27 (7)	3.03 ±0.35 (4)	6.73 ±1.41 (4)	5.9 ±0.93 (3)	21.88 ±3.27 (7)	23.9 ±3.22 (7)	11.9 ±2.43 (3)	11.07 ±0.54 (6)	10.28 ±1.84 (6)	16.48 ±4.46 (6)	18.45 ±1.21 (4)
O.aureus	Mean ± SE (n)	7.76 ±6.63 (7)	3.81 ±0.28 (7)	6.43 ±1.1 (4)	6.87 ±0.19 (3)	15.0 ±2.14 (6)	22.79 ±3.05 (7)	17.43 ±2.36 (7)	11.55 ±1.96 (4	6.86 ±1.23 (7)	10.87 ±1.66 (7)	15.5 ±3.32 (2)
S. galilaeus	Mean ± SE (n)	5.77 ±0.66 (7)	3.66 ±0.30 (5)	4.28 ±0.88 (5)	5.4 ±0.58 (4)	18.78 ±1.85 (5)	18.97 ±2.98 (7)	11.69 ±2.17 (7)	8.2 ±0.57 (7)	8.81 ±0.61 (7)	12.3 ±2.13 (7)	11.66 ±1.95 (7)
T. zillii	Mean ± SE (n)	10.04 ±1.19 (7)	4.95 ±0.25 (2)	9.35 ±0.74 (2)	- - -	- - -	38.23 ±4.77 (4)	24.25 ±8.69 (7)	19.49 ±8.03 (7)	4.85 ±1.17 (2)	29.33 ±3.95 (7)	- - -

N= Number of observations

Table (10): The significance (t-test) among percentage areas of plasma protein fractions (treated electrophoretic samples) of different *tilapia* species.

Compared Species	Fraction number										
	1	2	3	4	5	6	7	8	9	10	11
O.n. × O.au.	5.0446*	1.7034	0.1681	1.0223	1.8178	0.2504	1.3803	0.2841	1.5868	1.2535	1.0818
O.n. × S.g.	2.0513	1.3724	1.5431	0.4821	0.8243	1.1239	0.0564	3.6156*	0.8086	0.8886	2.4406*
O.n. × T.z	4.6776*	3.5606	1.2152	-	-	2.5767*	1.1790	0.9632	1.5941	2.1644	-
O.au. × S.g.	2.1894*	0.3544	1.5471	2.0865	1.3053	0.8966	1.7896	2.0738	1.4207	0.5299	0.9397
O.au. × T.z.	1.6900	2.0115	1.7061	-	-	2.8705*	0.9670	0.7243	0.8158	4.3055*	-
S.g. × T.z.	3.1317*	2.4551	3.3231*	-	-	3.6281*	1.8102	1.4028	3.0407*	3.7930*	-

*Significant at $P < 0.05$

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

Table (11) 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%	10%	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 (12) 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 (13): 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)	41.95 ±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 (14): The significance (*t*-test) among different relative mobilities of muscle protein fractions (untreated electrophoretic samples) of different *tilapia* species.

Compared Species	Fraction number										Similarity Coefficient
	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 (15): 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 (16): The significance (*t*-test) among different relative mobilities of muscle protein fractions (treated electrophoretic samples) of different *tilapia* species.

Compared Species	Fraction number										Similarity Coefficient
	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

Table (17): Mean ± SE of percentage area 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)	14.59 ±1.50 (7)	7.3 ±1.06 (3)	- - -	8.94 ±1.75 (5)	7.61 ±1.41 (7)	25.51 ±2.16 (7)	9.28 ±1.60 (6)	32.2 =2.91 (7)	- - -	16.4 ±0.0 (1)
O.aureus	Mean ± SE (n)	18.6 ±0.99 (7)	10.07 ±1.52 (7)	10.05 ±0.49 (6)	7.86 ±0.44 (7)	7.47 ±0.64 (7)	21.6 ±1.74 (7)	19.64 ±3.07 (7)	- - -	14.43 ±2.99 (3)	- - -
S. galilaeus	Mean ± SE (n)	33.39 ±5.45 (7)	4.93 ±1.26 (4)	4.82 ±0.59 (6)	8.65 ±1.62 (6)	6.5 ±2.0 (6)	4.2 ±0.79 (6)	5.3 ±0.54 (7)	12.73 ±1.67 (7)	23.93 ±3.87 (7)	- - -
T. zillii	Mean ± SE (n)	15.87 ±1.58 (7)	9.63 ±0.99 (7)	7.74 ±1.3 (7)	11.01 ±0.90 (7)	6.9 ±0.93 (7)	5.57 ±0.73 (7)	12.90 ±1.80 (6)	32.26 =3.60 (7)	- - -	- - -

N= Number of observations .

Table (18): The significance (*t*-test) among percentage area of muscle protein fractions (untreated electrophoretic samples) of different *tilapia* species.

Compared Species	Fraction number									
	1	2	3	4	5	6	7	8	9	10
O.n. × O.au.	2.2305*	1.1122	-	0.7003	0.0903	1.4121	2.8385*	-	-	-
O.n. × S.g.	3.3235*	1.3965	-	0.1216	0.4639	8.7578*	2.5111*	5.7931*	-	
O.n. × T.z	0.5882	1.3745	-	1.1461	0.4191	8.7482*	1.5028	0.0130	-	-
O.au. × S.g.	2.6681*	2.806*	6.7834*	0.506	0.495	8.7293*	4.5952*	-	1.4886	-
O.au. × T.z.	1.4666	0.2419	1.556	3.1425*	0.5048	8.511*	1.8081	-	-	-
S.g. × T.z.	3.086*	1.2317	1.928	1.3271	0.1908	1.3359	4.3261*	4.9167*	-	-

* Significant at $P < 0.05$

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

Table (19): Mean ± SE of percentage area 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)	21.34 ±1.9 (7)	8.83 ±1.02 (7)	12.33 ±1.05 (7)	8.94 ±1.56 (7)	3.54 ±0.55 (7)	10.57 ±1.79 (6)	26.17 ±5.27 (6)	23.07 ±1.99 (3)	12.8 ±6.08 (2)	- - -
O.aureus	Mean ± SE (n)	8.97 ±1.15 (7)	7.73 ±0.81 (7)	8.53 ±0.82 (7)	4.87 ±0.32 (7)	5.37 ±0.45 (7)	28.27 ±1.06 (7)	15.96 ±2.35 (7)	9.9 ±4.17 (4)	3.1 ±0.0 (1)	19.4 ±2.57 (5)
S. galilaeus	Mean ± SE (n)	8.24 ±1.39 (7)	5.4 ±1.21 (7)	8.37 ±1.05 (7)	8.42 ±1.21 (6)	7.03 ±0.57 (7)	17.65 ±1.84 (6)	13.37 ±3.62 (6)	6.06 ±0.06 (7)	24.32 ±4.50 (6)	23.97 ±4.79 (3)
T. zillii	Mean ± SE (n)	12.5 ±2.19 (7)	7.64 ±0.92 (7)	7.07 ±0.53 (3)	11.66 ±1.61 (7)	5.18 ±53 (6)	6.41 ±1.04 (7)	9.06 ±1.75 (7)	11.74 ±1.13 (7)	16.63 ±5.48 (6)	22.53 ±5.06 (6)

N= Number of observations.

Table (20): The significance (*t*-test) among percentage area of muscle protein fractions (treated electrophoretic samples) of different *tilapia* species.

Compared Species	Fraction number									
	1	2	3	4	5	6	7	8	9	10
O.n. × *O.au.*	5.5685*	0.8447	2.8395*	2.5597*	2.5792*	8.8385*	1.8653	2.5269	0.9209	-
O.n. × *S.g.*	5.5664*	2.1635	2.6554*	0.2569	4.3811*	2.7584*	2.0017	8.2914*	1.3242	-
O.n. × *T.z*	3.0464*	0.8636	3.0991*	1.2158	2.1279	2.0895	3.2877*	5.2695*	0.3681	-
O.au. × *S.g.*	0.4053	1.5979	3.1196	3.0501*	2.2824*	5.1832*	0.6177	1.1483	1.7827	0.9334
O.au. × *T.z.*	1.3252	0.0732	1.0888	4.1449*	0.2774	147368*	2.3550*	0.5429	0.9334	0.517
S.g. × *T.z.*	1.5458	1.4676	0.7659	1.5657	2.3416*	5.5167*	1.1240	3.6621**	1.0848	0.1791

* Significant at $P < 0.05$

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

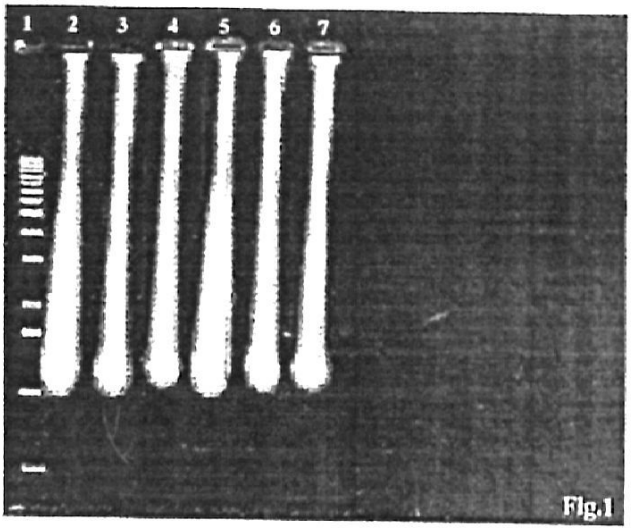

Fig.1

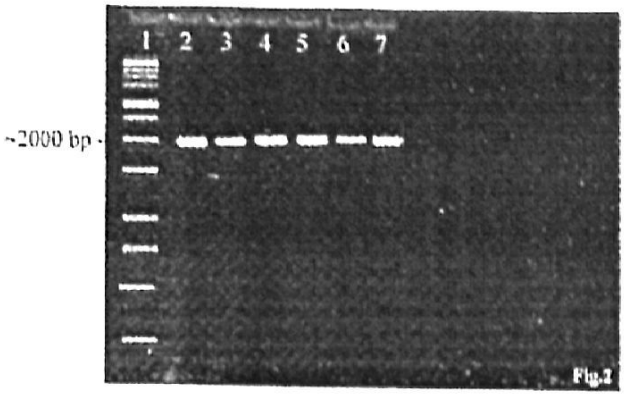

Fig.2

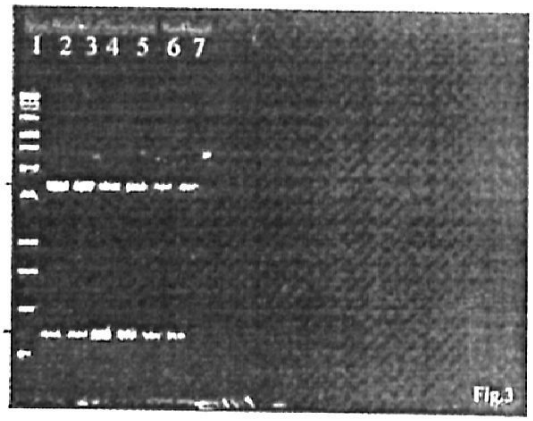

Fig.3

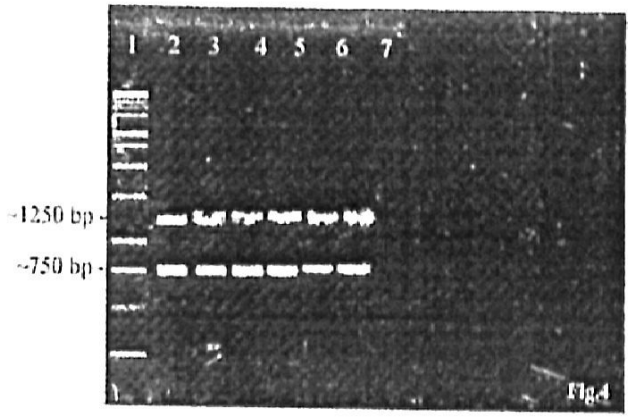

Fig.4

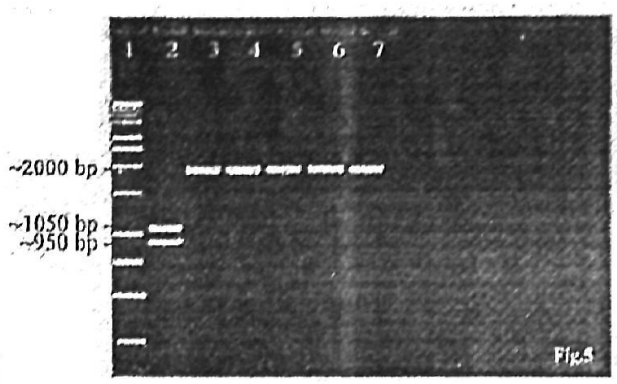

Fig.5

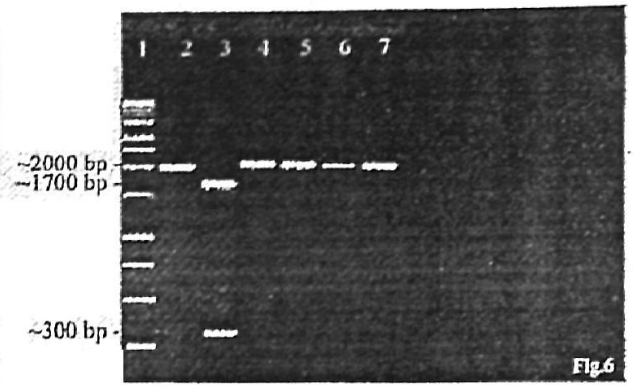

Fig.6

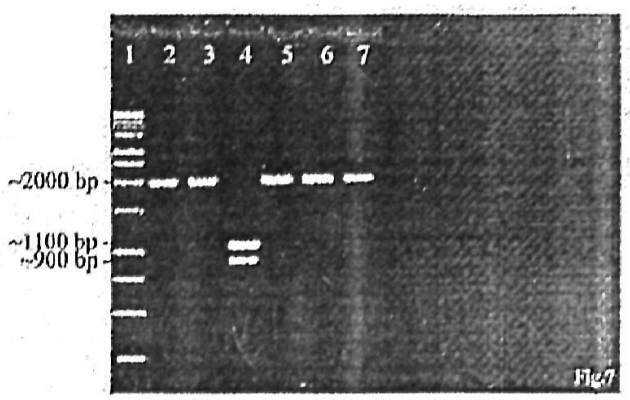

Fig.7

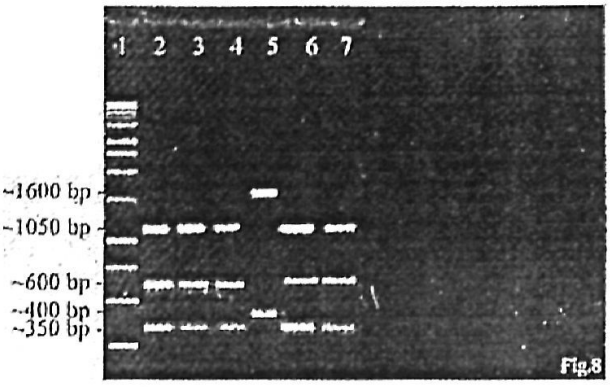

Fig.8

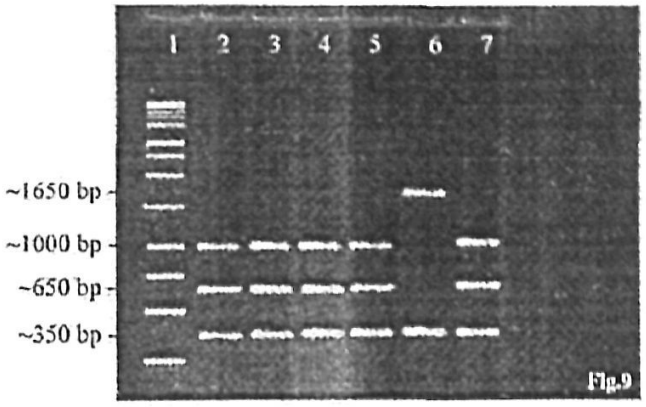

Fig.9

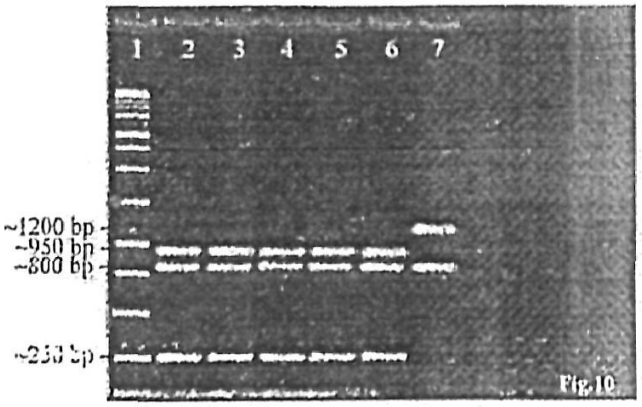

Fig.10

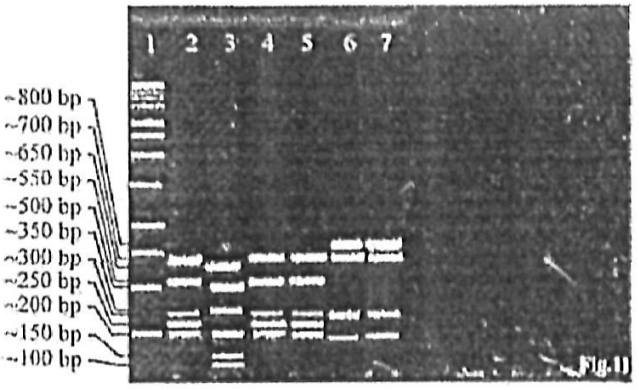

Fig.11