SUMMARY

In Egypt, there are mainly four *Tilapia* species naturally distributed in the River Nile and its branches. These species include the mouth brooding genera; *Oreochromis* (*O. niloticus* & *O. aureus*) and *Sarotherodon* (*S. galilaeus*) and substrate spawning genus; Tilapia (*T. zillii*). Striking similarities and overlapping of morphological characteristics have been recoded among these *Tilapia* species. So, classical methods as well as molecular biological techniques have been applied to differentiate between these species.

As the normal cycloid scales have general morphological characteristics, they can be helpful in the identification of fishes. For that reason, the Atomic Force Microscopy (AFM) was used to scan the anterior regions with their rostral rims, radial grooves and inter-radial circuli of the normal scales of the studied species. Also, the focus regions as well as the posterior regions with pigments were scanned by the same technique. The images of 35 µm square of the inter-radial circuli were obtained and analyzed using SPIP program to obtain the 3D images and roughness charts for the scale of each studied species. Furthermore, the lateral line scales were scanned and the lateral line canals with their anterior and posterior openings were studied. The analyzed AFM images indicated that, most of the roughness parameters recorded the maximum values in *T. zillii* scale and minimum values in *O. niloticus* scale. These results indicate that, *T. zillii* scale is the roughest scale but, *O. niloticus* scale is the smoothest one.

The general inter-specific osteometric differences between the four *Tilapia* species were reflected qualitatively by X-ray photographs. The photographs verify the more closely relationship between *O. niloticus* and *O. aureus*, furthermore, the less degree of similarity was reflected between *S. galilaeus*, *T. zillii* and the previous species of genus *Oreochromis*.

During the present work, attention has been directed toward the types and arrangement of lower jaw and lower pharyngeal teeth in the four *Tilapia* species. The results obtained for lower jaw teeth support the monophylogenetic relationship in which, all studied species have a bicuspid teeth. But the results of lower pharyngeal teeth confirm the pholyphylogenetic relationship between the studied three genera, *sarotherodon* (unicuspid teeth). *Tilapia* (Tricuspid teeth) and *Oreochromis* which contains two monophylogenetic species, *O. niloticus* and *O. aureus*, each of them has bicuspid lower pharyngeal teeth.

In brief, the classical methods such as, osteometric analysis can successfully differentiate genera. But at the species level, there is great perplexity in identification studies.

Thus, advances in molecular biology techniques have enabled specific identification and genetic differentiation between fish species. The polymerase chain reaction (PCR) has been useful in resolving the complex taxonomy and identification process of the species. Nearly complete sequences (1916-1986bp) of nuclear small subunit ribosomal RNA (n18S rRNA) genes from different studied *Tilapia* species were obtained. Then, the analysis of DNA nucleotide composition using Bio-edit program indicated the variation between species, as well as, the % C + G content for each *Tilapia* species.

The alignment of 18S rRNA genes sequences for different *Tilapia* species obtained by gene bee service appeared with homology percent 67.7%, indicating the monophylogenetic relationship of all studied species. The data indicated the presence of 10 variable regions most of them clustered *O. niloticus* & *O. aureus* as one group and *S. galilaeus* & *T. zillii* as the other one. Also, depending on the percent sequence differences (2.19%-40.19%) and number base differences (43-770) between species, the four *Tilapia* species were differentiated into the same two polyphylogenetic groups.

Several different phylogenetic analyses were used for the sequence data of nuclear 18S rDNA by using Gene Bee service. Three methods were used for nucleotide sequence analysis. The data presented from parsimony tree and seqboot values for 100 bootstrap comparisons supported the clustering of *O. niloticus* & *O. aureus* as one monophylogenetic group and *S. galilaeus* & *T. zillii* as the second (monophylogenetic) group. As well as support the polyphylogenetic relationship between these two groups. The 100 X bootstraps of bootstrapping tree gave a tree with strong bootstrap values for the two main groups. The value for each group (100) suggested that the nodes indicated were significant.

The distance tree constructed for nuclear 18S rRNA genes for the studied species identified the same two clusters with distance 0.829, in which *O. aureus* branched with *O. niloticus* with distance

0.076 and *S. galilaeus* & *T. zillii* grouped together in the same group with distance 0.042.

Small subunit ribosomal RNA which transcribed from 18S rDNA is a single strand consists of several complementary sequences (paired) stems and single stranded (unpaired) loops. These stems and loops formed a distinct structure known as RNA secondary structure. This structure obtained by RNA secondary structure prediction. The obtained results indicated a unique RNA secondary structure for each species with definite number of stems, not only stems differed in number but also, they differed in sequences and positions.

In conclusion, the data obtained from scale characteristics, osteometric characteristics and distinctive teeth as well as from molecular analysis can differentiate the four studied *Tilapia* species as a polyphylogenetic species. However, the species related to the same genus are closely related to each other.