
RESULTS

The data obtained in the present study were tabulated, and figures were photographed, drawn and texted to easily investigate the identification and characterization of the studied species.

I. Scale characteristics:

The scales maintain the same morphological proportions located on the different parts of the body. The scales present below the dorsal fin, above the lateral line, are the largest. As the scale from this region depicts all the features, this scale has been designated as the "key scale" and obtained in this study.

The normal scale of each studied *Tilapia* species can be divided into anterior or rostral field (Af), posterior or caudal field (Pf), lateral fields (Lf) and the focus (F). There is no cteni at the posterior part of the scales; hence, they are cycloid scales (Figure, 6). The anterior field is embedded in the skin and overlapped by the posterior side of the proceeding scale. The ventral surface of the scales is shiny and smooth while the dorsal is rough, convex and has distinct structures, consisting of ridges and radii (R), forming nearly circular rings around the central focus and granules (tubercles).

Atomic force microscopy (AFM) studies:

Rostral field:

a) The inter-radial tongues and 1st circulus:

In the inter-radial space, the rostral rim of the scale form tongue-like projections that are free of circuli near the rim. The tongues are

convex and the 1st inter-radial circulus is straight. This form was recorded in *O. niloticus*, *S. galilaeus* and *T. zillii* (Figures, 7, 9 & 10). Part from slightly curvature on the left side of *O. aureus* scale's rim, it is rather straight. There is no tongue and the first circulus found directly below the rim (Figure, 8).

b) Radial grooves:

In the anterior and lateral parts of the scale, the circuli are partitioned by deep and narrow grooves that run radially towards the focus, they are called radii. The radial grooves of *O. niloticus* scales are deep with wide and regular split (Figure, 11), but the scales of *O. aureus* have shallow radial-grooves with narrow regular split (Figure, 12). The radial grooves of *S. galilaeus* scales are deep with wide irregular split (Figure, 13) and the radial grooves of *T. zillii* scales are deep with more wide and irregular split (Figure, 14).

Focus region:

Each scale has a focus which is a part of the scale that developed first during ontogenesis. The central part of the focus in each scale of studied *Tilapia* species has no circuli. However, the scales of *O. niloticus* have tubercular ridges around the centre of their focus (Figure, 15). But, the focus region in *O. aureus* scale appear circular with rounded to oval granules arranged in circular rings around the focus (Figure, 16). Also, *S. galilaeus* scale has focus region similar to that of *O. aureus* one. But the granules of *S. galilaeus* scale are small and few (Figure, 17). Part from few granules, the focus region of *T. zillii* scale appear nearly clear. Small tubercular ridges were observed at the margin of the focus (Figure, 18).

Caudal field:

In the external posterior part of the scale, the circuli lose their characteristic features. In this part, the scale is covered with epidermis and has several rows of rough pigmented granules (tubercles). The shape of rough tubercles varies from round to oval, semi-oval and even an oblong structure. The tubercles impart specific colour of fish as they contain chromatophores in the outer surface.

The caudal region of *O. niloticus* scale has large, rounded and randomly arranged tubercles (Figure, 19). The tubercles are close to each other with no observed space between them. The tubercles of *O. aureus* and *S. galilaeus* scales vary in shape from round to oval and oblong (Figures, 20-21). They are median size and arranged randomly with wide spaces between each other. For *T. zillii* caudal region, the tubercles are mostly oblong and arranged in definite semi-circular rows (Figure, 22).

AFM images of circuli:

From the focus, lines of growth (ridges) start appearing. These are named circuli, which overcrowded in the anterior part but not found on the posterior part of the scale. The circuli formation is due to the excess calcium salts secreted by the skin and their subsequent deposition on the scale. Each circuli is wedge shape, having a broad base and a pointed upper part. The circuli have calcium projections or small denticles. These small denticles or teeth-like structures, that can be seen only under high magnification and are called lepidonts. For studied *Tilapia* species, AFM images of these lepidonts were obtained by scanning 35 μm square of one circulus from each tilapia

scale. Then, these images were applied to Scanning Probe Image Processor (SPIP) program to obtain the 3D images and roughness analysis.

For *O. niloticus*, the image indicates that, lepidonts are close to each other and vary in size from small to large. Each denticle has a broad shape with curved end (Figure, 23A). According to the 3D image, the end of each denticle has a central curved process or projection. Also, the denticles heights are not equal to each other (Figure, 23B).

The image of *O. aureus* indicates that, the circuli bear conical denticles with pointed ends. These denticles have variable size from small to large. Also, some of them appear fused at the base (Figure, 24A). The 3D image indicates that, the denticles ends are curved and smooth with no projections (Figure, 24B).

The circuli of *S. galilaeus* bear heterogenous denticles appear as very small to very large cons with curved and pointed ends (Figure, 25A). According to the 3D image, the denticles appear long, close to each other and have teeth-like ends with numerous small pointed or curved projections (Figure, 25B).

According to the image obtained for *T. zillii*, the circuli bear broad denticles with long pointed ends. Also, denticles vary from small to large size and some of them are fused at the base (Figure, 26A). The denticles appear too long with flat smooth surface in the 3D image (Figure, 26B).

AFM image analysis:

The obtained 3D image of four tilapia scales were applied to SPIP program (version 5.0.7) to obtain different roughness values and charts (Table, 2 & figure, 27). According to the data presented in this table, the roughness average (Sa) ranges from 1.35 μm in *O. niloticus* image to 2.20 μm in *T. zillii* image. So, the scales of *O. niloticus* are considered the smoothest scales but, those of *T. zillii* are considered the roughest ones.

By comparing root mean square (Sq), this refers to the mean width of the denticle base, *O. niloticus* denticles have the less mean width value (1.62) and *T. zillii* denticles have the largest mean value (2.63).

Concerning the peak-peak (Sy) values, which refer to the total length of the denticle from the deepest point in the inter-circular space to the most high point of the denticle. Sy value of *S. galilaeus* scale records the maximum denticle height (9.30). However, *O. niloticus* scale Sy value records the minimum denticle height (6.67). Also, large denticle height was recorded for *O. aureus* (8.31) and *T. zillii* (9.11) scales (Figure, 27).

According to the obtained data (Table, 2), the top 5% height (Sdc 0-5) of the image show the maximum height (2.26) in *S. galilaeus* scale and minimum height (0.97) in *T. zillii* scale. The 5-10% height (Sdc 5-10) of the image records maximum value (0.65) in *O. aureus* scale. But, minimum value (0.17) was recoded in *T. zillii* scale. For 10-50% height (Sdc 10-50) of the image, the maximum height (4.60) was recorded in *T. zillii*. But, the minimum

height (2.17) was recorded in *O. niloticus* scale. Almost the remain height of the image - 50-95% height (Sdc 50-95) – records the maximum height (2.92) in *T. zillii* scale and the minimum height (1.68) in *O. aureus* scale.

When comparing max valley depth (Sv), which refers to the deepest point in the inter-circular space, the maximum depth (4.27) was recorded in *T. zillii* scale. And the minimum depth (2.34) was recorded in *O. niloticus* scale.

Regarding max peak height (Sp), the data presented in the table shows the maximum denticle height (5.75) on the circuli of *O. aureus* scale and the minimum denticle height (4.33) on the circuli of *O. niloticus* scale.

In conclusion, most 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.

Lateral line scale:

A lateral line scale is also divided into anterior (rostral) and posterior (caudal) parts. The focus is absent in the lateral line scale. This scale has a canal which, characteristically, lies along the anterior-posterior axis, slightly towards the posterior part with two openings. The posterior opening lies towards the posterior margin and the anterior opening towards the anterior part of the scale.

The lateral line canal of scales of *Tilapia* species is considered as a simple tube embedded within the matrix of the scale (Figure, 28).

For scales of *O. niloticus* and *T. zillii*, such canal may slightly deviate from the anterior-posterior axis of the scale. But, the canal may be straight along the axis of *O. aureus* and *S. galilaeus* scales.

The four tilapia scales have long and wide canals. The canal of *T. zillii* appeared more wide specially at the middle part. But *S. galilaeus* canal appeared more narrow and regular.

The anterior opening of the lateral line canal vary in shape from rounded (*O. niloticus* and *O. aureus*), curved (*S. galilaeus*) and pointed (*T. zillii*). The posterior opening is wider than the anterior one and opens slightly above the posterior rim of the scale causing its deviation (*O. niloticus* and *O. aureus*). However, this opening extends to the posterior rim and opens there (*S. galilaeus* and *T. zillii*).

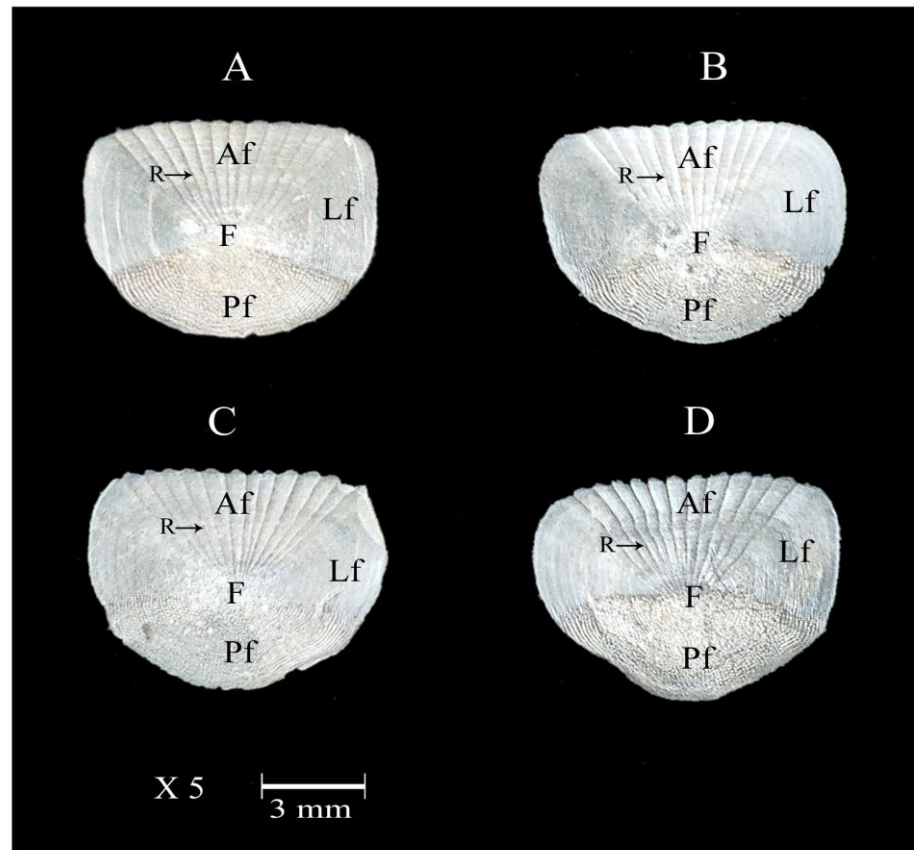
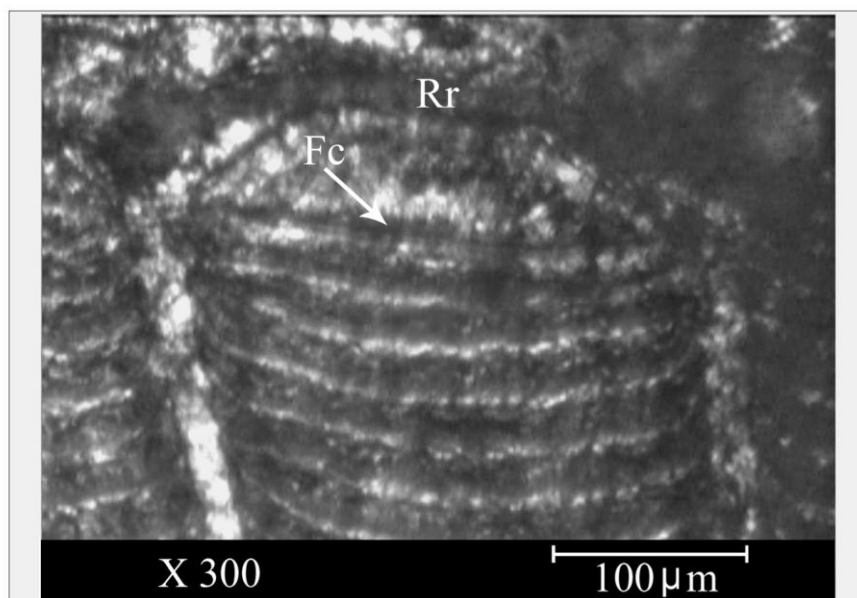
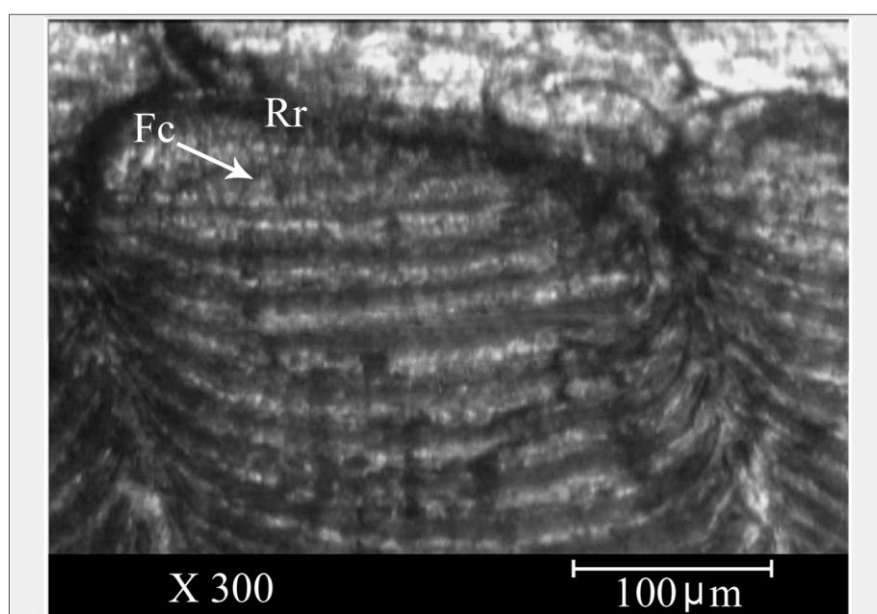


Figure (6): The normal scale of the four *Tilapia* species. (A): *O. niloticus*, (B) *O. aureus*, (C) *S. galilaeus* and (D) *T. zillii*. Each scale is divided into: Anterior (rostral) field (Af) with radii (R), Posterior (caudal) field (Pf), Lateral field (Lf) and Focus region (F).



**Figure (7): The inter-radial rostral rim of the scale of *O. niloticus*.
(Rr): Rostral rim (tongue) and (Fc): First circulus.**



**Figure (8): The inter-radial rostral rim of the scale of *O. aureus*.
(Rr): Rostral rim and (Fc): First circulus.**

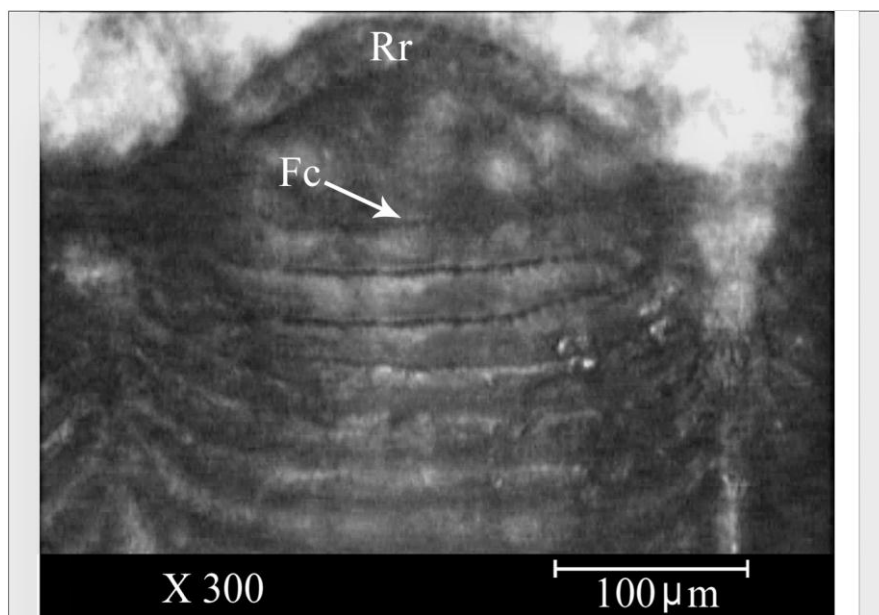


Figure (9): The inter-radial rostral rim of the scale of *S. galilaeus*.
(Rr): Rostral rim (tongue) and (Fc): First circulus.

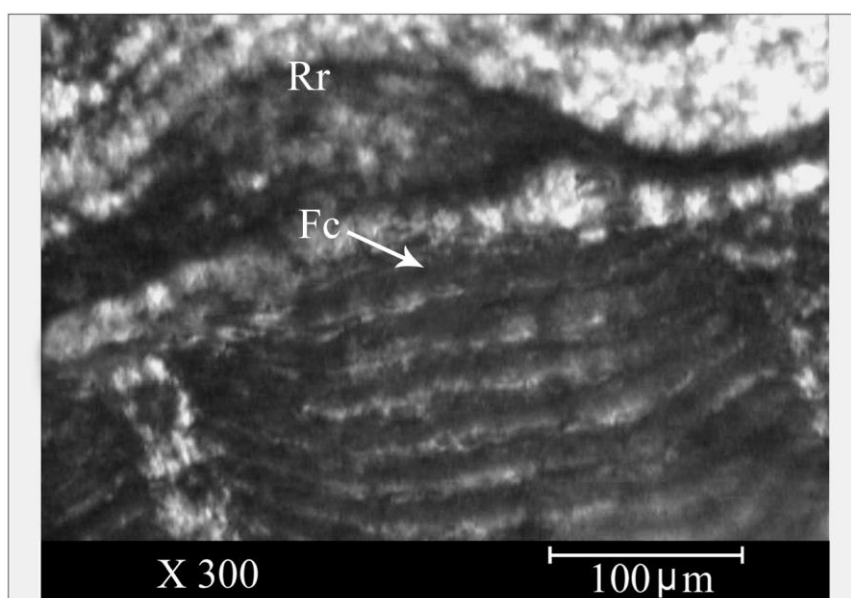


Figure (10): The inter-radial rostral rim of the scale of *T. zillii*.
(Rr): Rostral rim (tongue) and (Fc): First circulus.

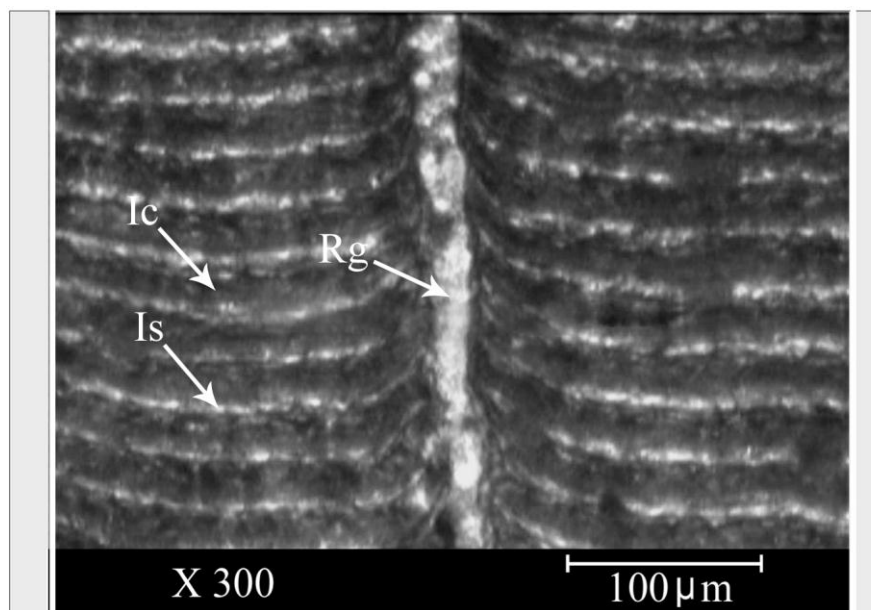


Figure (11): The radial groove of the scale of *O. niloticus*.

(Rg): Radial groove, (Ic): Inter-radial circulus and
(Is): Inter-circular space.

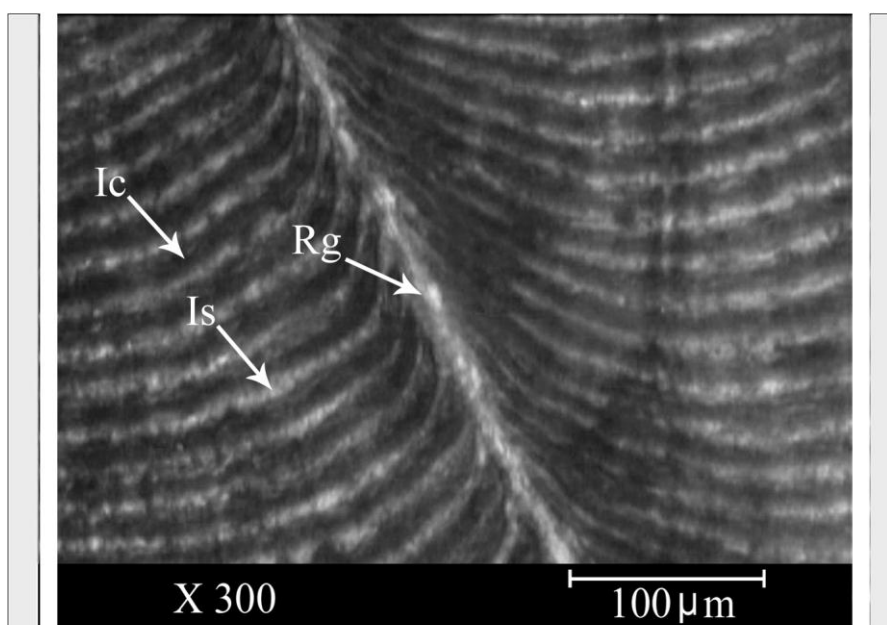


Figure (12): The radial groove of the scale of *O. aureus*.

(Rg): Radial groove, (Ic): Inter-radial circulus and
(Is): Inter-circular space.

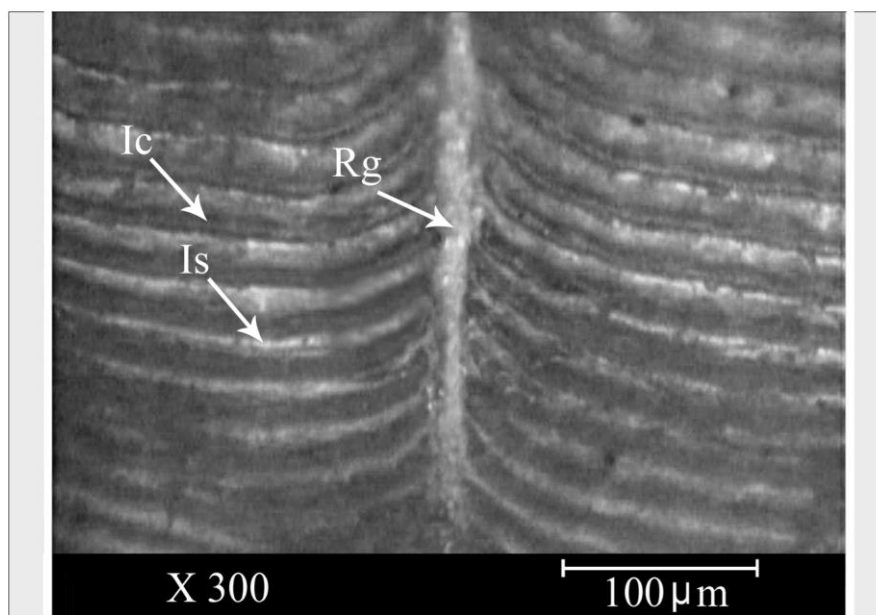


Figure (13): The radial groove of the scale of *S. galilaeus*.

**(Rg): Radial groove, (Ic): Inter-radial circulus and
(Is): Inter-circular space.**

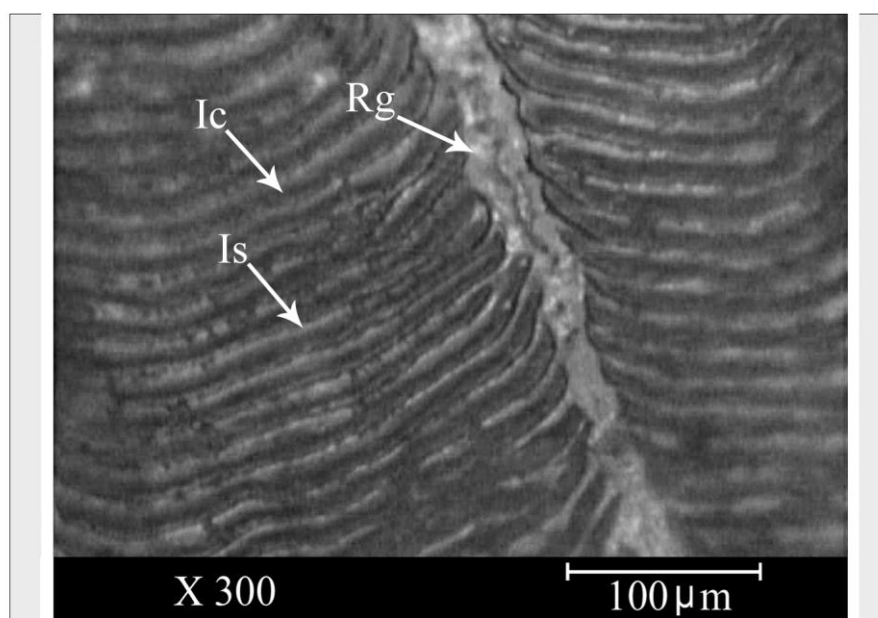


Figure (14): The radial groove of the scale of *T. zillii*.

**(Rg): Radial groove, (Ic): Inter-radial circulus and
(Is): Inter-circular space.**

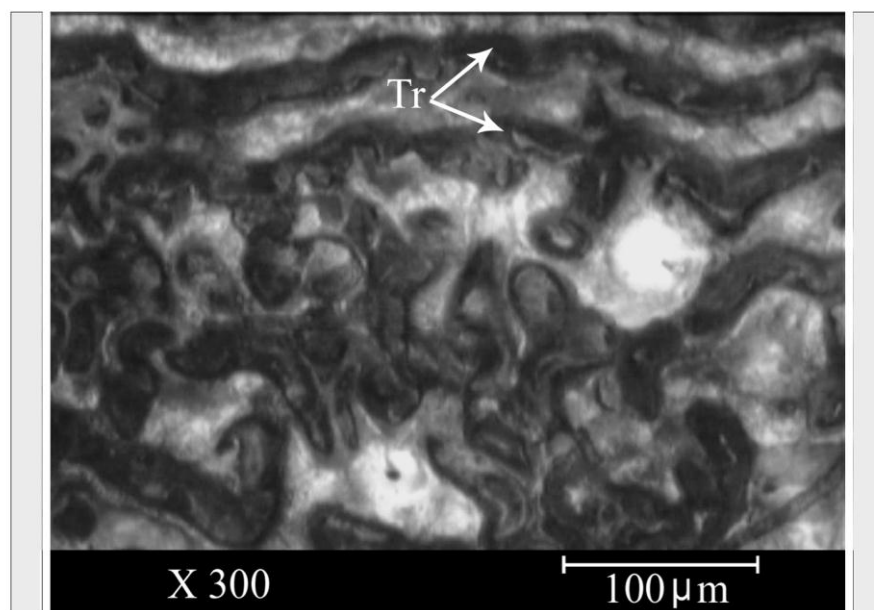


Figure (15): The focus region of the scale of *O. niloticus*.

(Tr): Tubercular ridges.

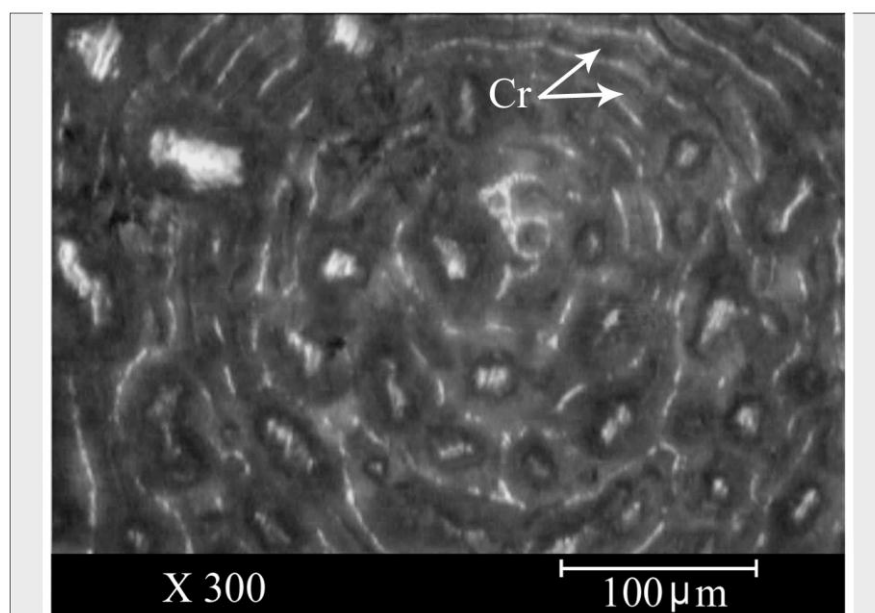


Figure (16): The focus region of the scale of *O. aureus*.

(Cr): Circular ridges.

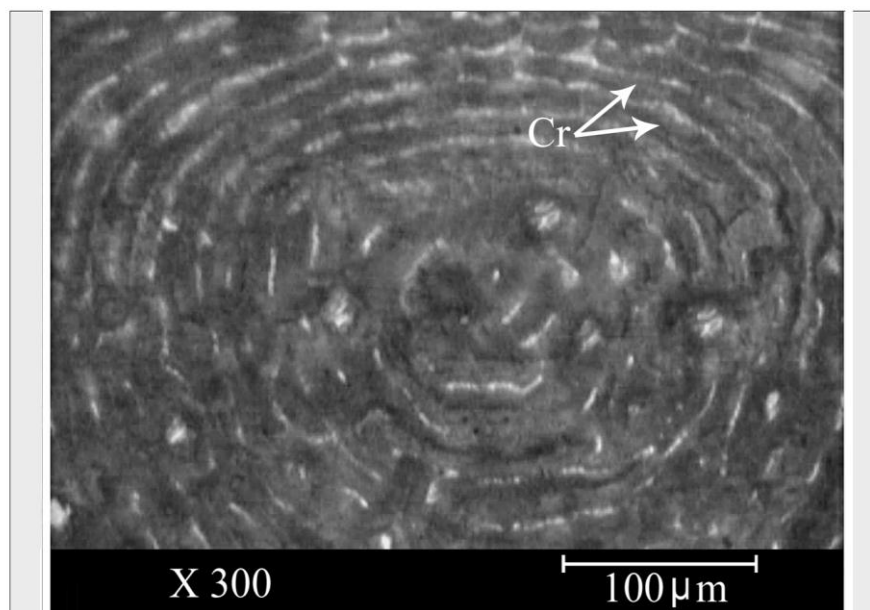


Figure (17): The focus region of the scale of *S. galilaeus*.
(Cr): Circular ridges.

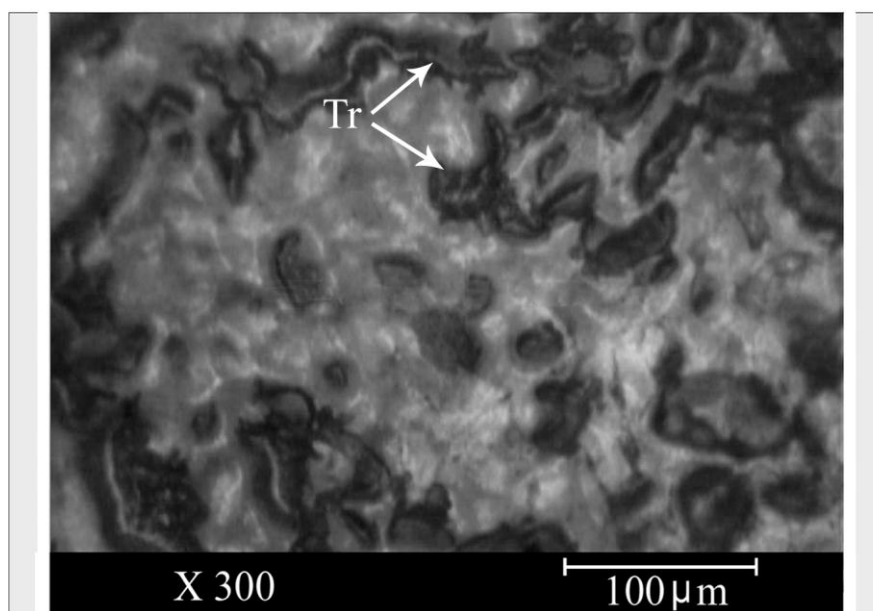
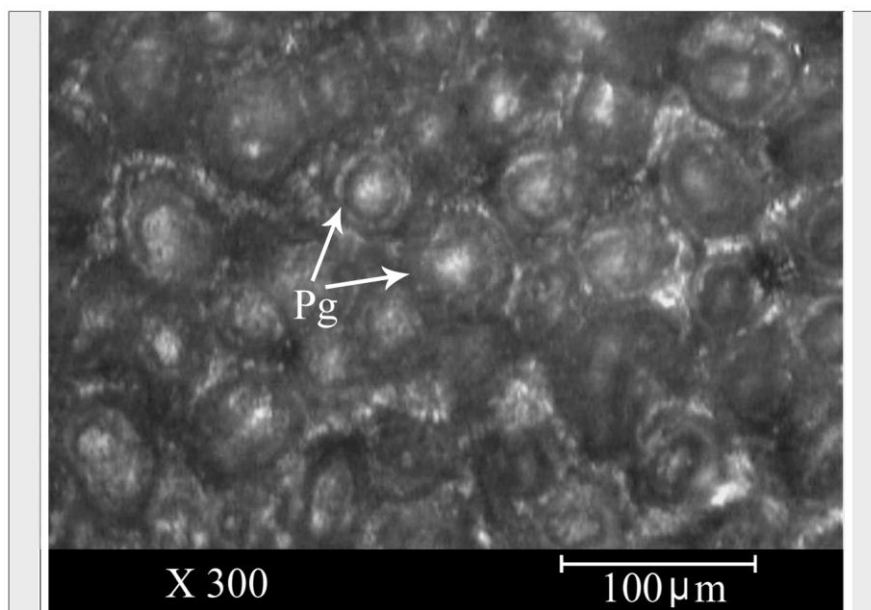
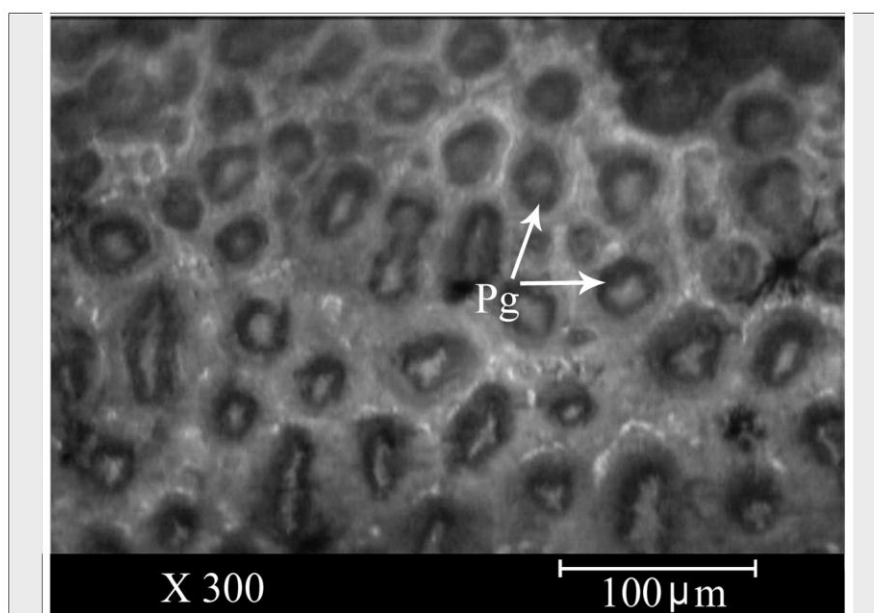


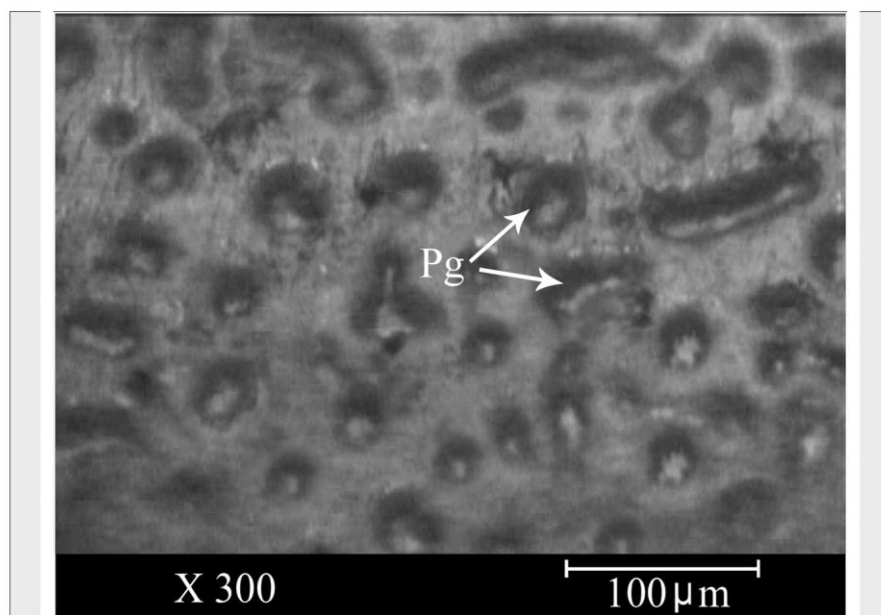
Figure (18): The focus region of the scale of *T. zillii*.
(Tr): Tubercular ridges.



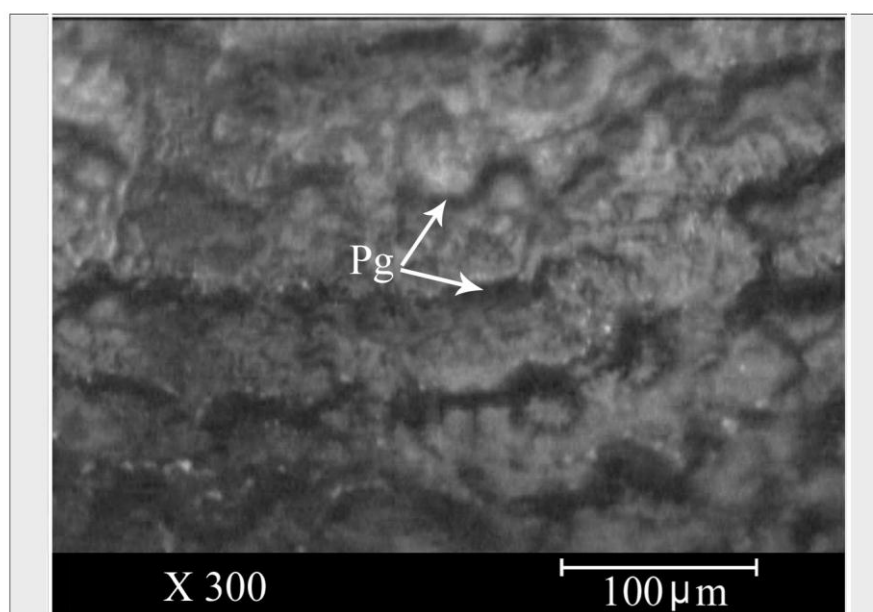
**Figure (19): The granulation area of the scale of *O. niloticus*.
(Pg): Pigmented granule.**



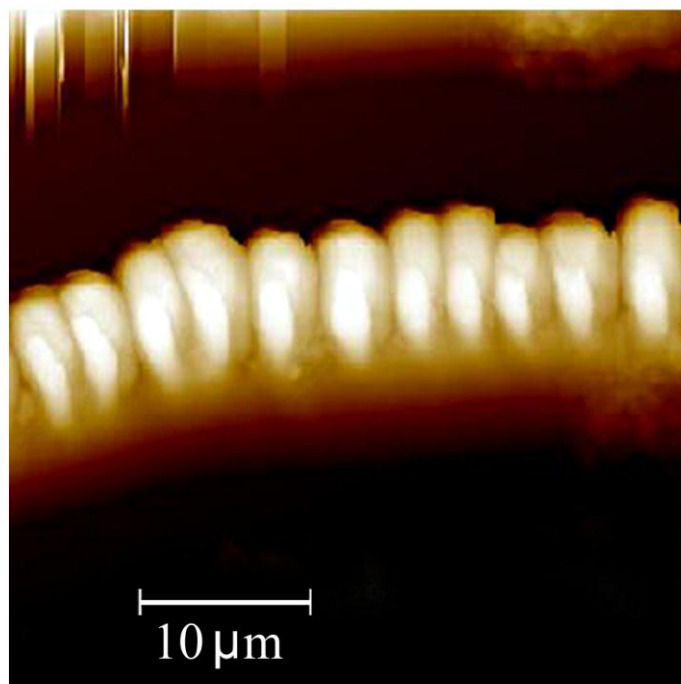
**Figure (20): The granulation area of the scale of *O. aureus*.
(Pg): Pigmented granule.**



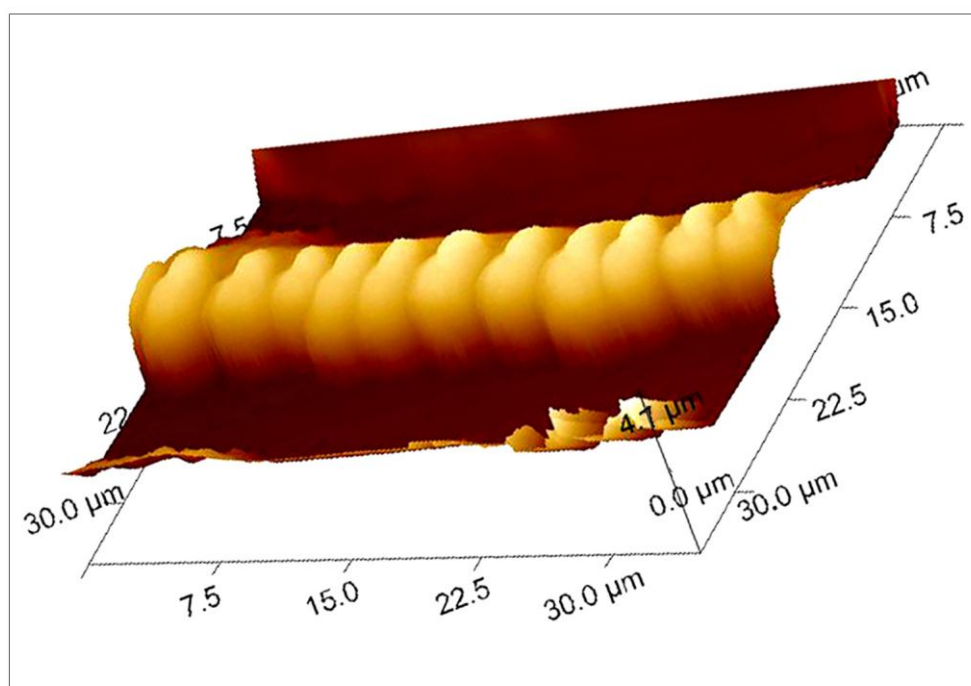
**Figure (21): The granulation area of the scale of *S. galilaeus*.
(Pg): Pigmented granule.**



**Figure (22): The granulation area of the scale of *T. zillii*.
(Pg): Pigmented granule.**

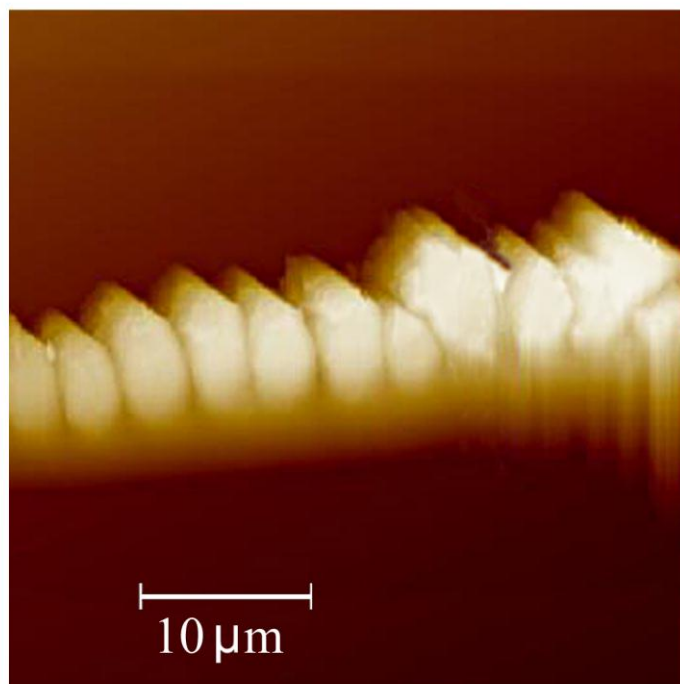


(A)

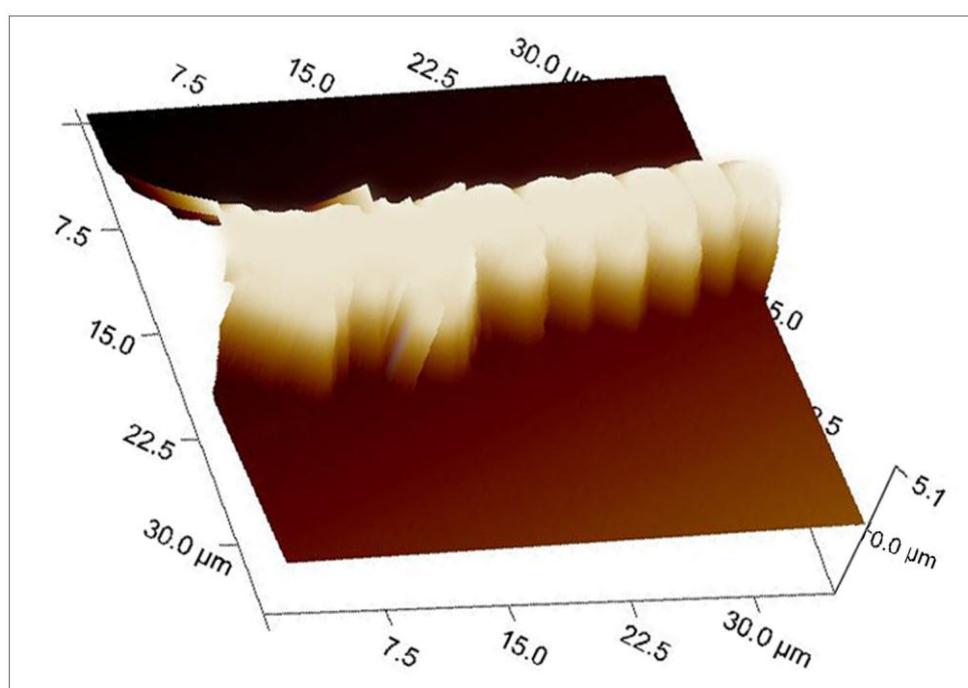


(B)

Figure (23): AFM images for 35μm square of the inter-radial circulus surface and denticles of *O. niloticus* scale. (A) AFM 2D image. (B) AFM 3D image.

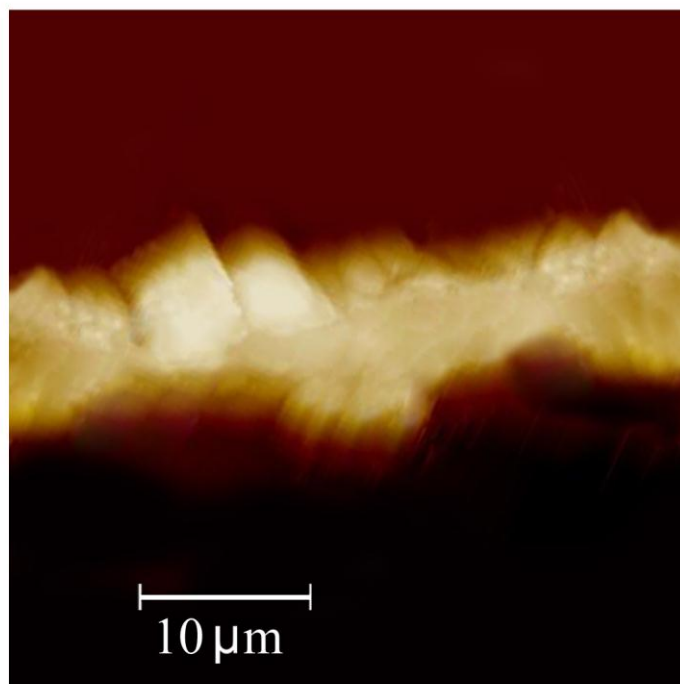


(A)

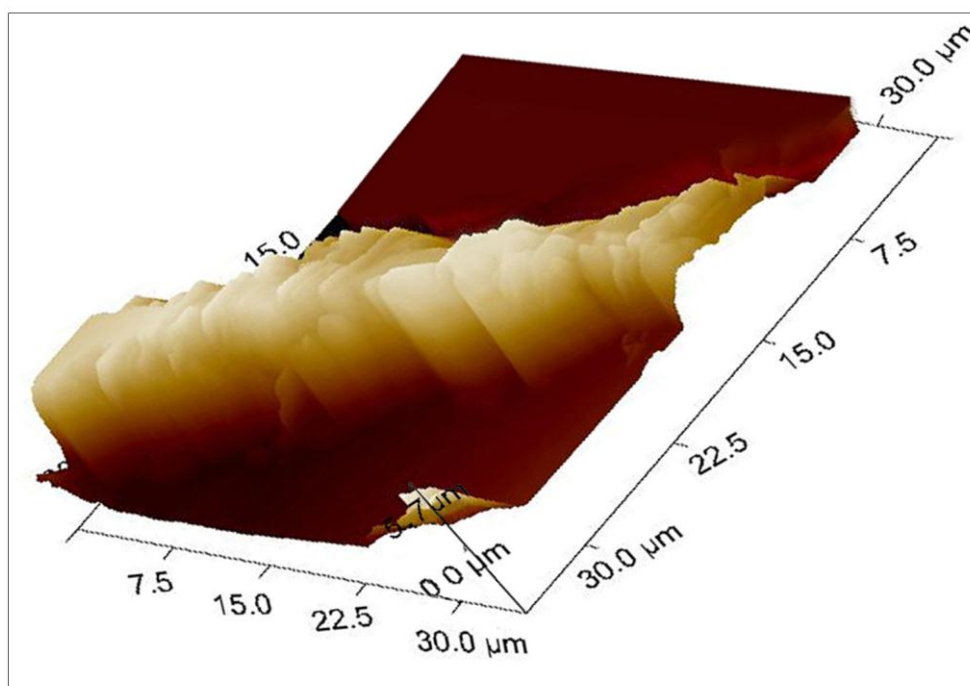


(B)

Figure (24): AFM images for 35μm square of the inter-radial circulus surface and denticles of *O. aureus* scale. (A) AFM 2D image. (B) AFM 3D image.

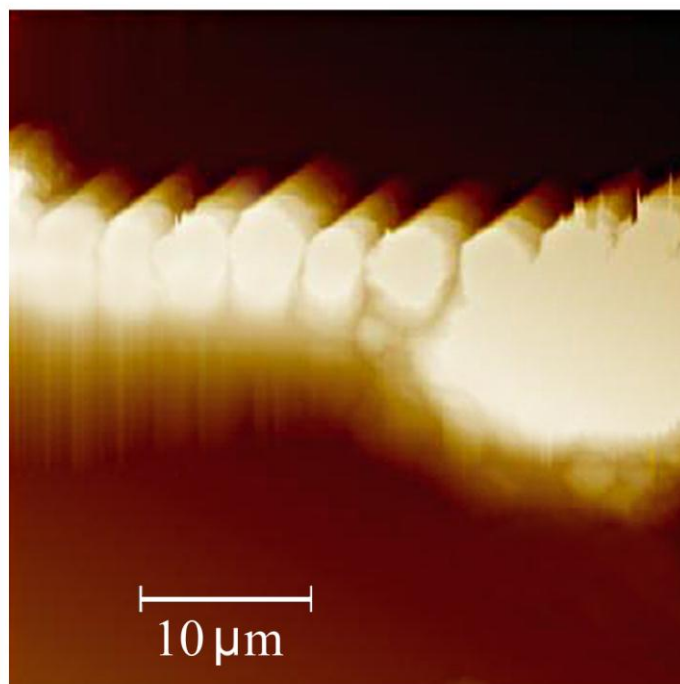


(A)

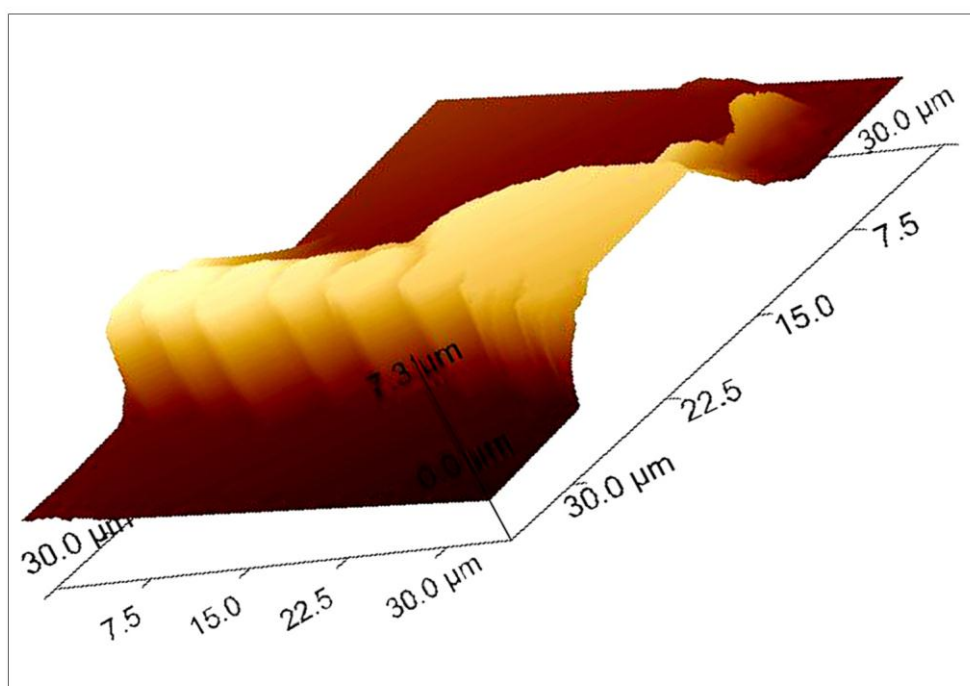


(B)

Figure (25): AFM images for 35μm square of the inter-radial circulus surface and denticles of *S. galilaeus* scale. (A) AFM 2D image. (B) AFM 3D image.



(A)



(B)

Figure (26): AFM images for 35μm square of the inter-radial circulus surface and denticles of *T.zillii* scale. (A) AFM 2D image. (B) AFM 3D image.

Table (2): The roughness values of AFM images of the four studied *Tilapia* species.

Species Parameters	<i>O. niloticus</i>	<i>O. aureus</i>	<i>S. galilaeus</i>	<i>T. zillii</i>
Sa	1.35	1.44	1.82	2.2
Sq	1.62	1.86	2.05	2.63
Sy	6.67	8.31	9.3	9.11
Sdc 0-5	2.04	1.93	2.26	0.97
Sdc 5-10	0.21	0.65	0.39	0.17
Sdc 10-50	2.17	3.64	3.34	4.6
Sdc 50-95	1.92	1.68	2.85	2.92
Sv	2.34	3.56	3.8	4.27
Sp	4.33	5.75	5.5	4.84

Sa: Roughness average.

Sq: Root mean square.

Sy: Peak-peak.

Sdc 0-5: Top 5% height.

Sdc 5-10: 5-10% height.

Sdc 10-50: 10-50% height.

Sdc 50-95: 50-95% height.

Sv: Max valley depth.

Sp: Max peak height.

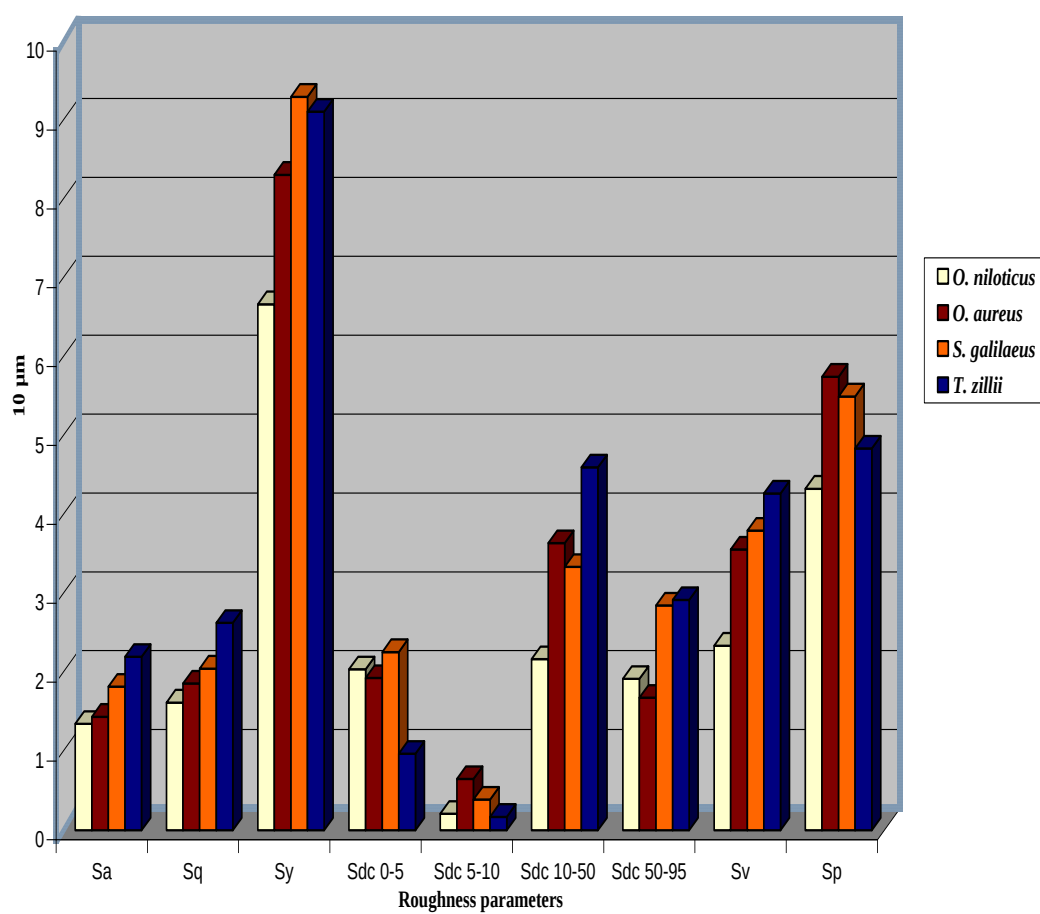


Figure (27): Histogram shows the roughness charts of AFM images of the four *Tilapia* species.

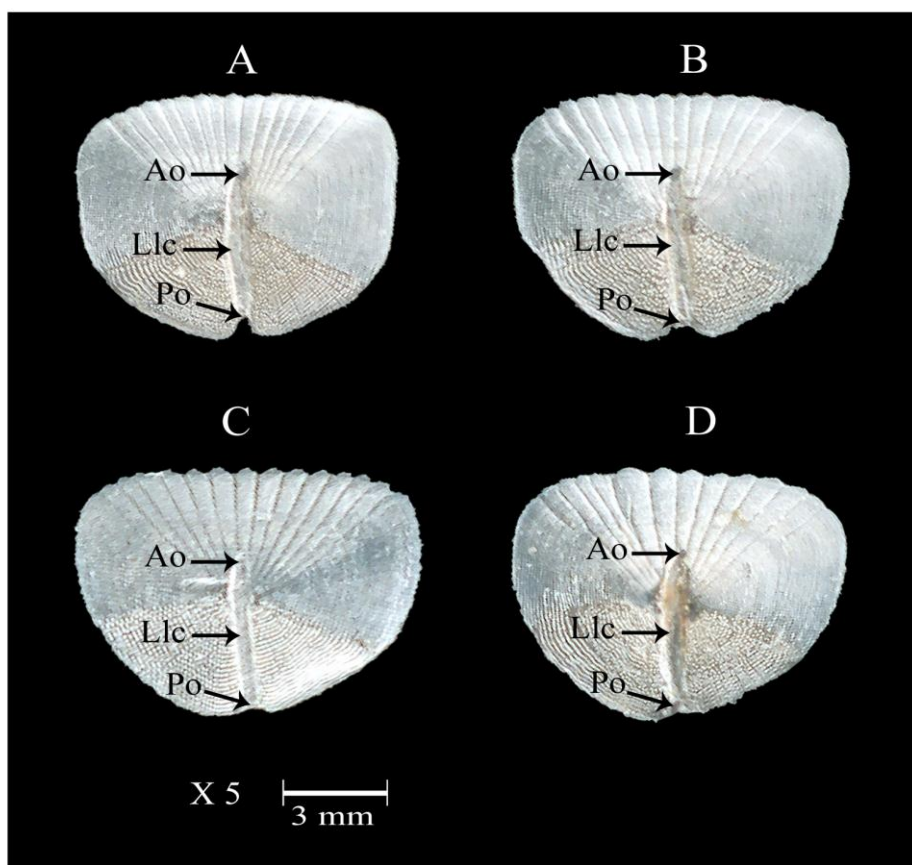


Figure (28): The lateral line scales with lateral line canal of the four *Tilapia* species. (A): *O. niloticus*, (B) *O. aureus*, (C) *S. galilaeus* and (D) *T. zillii*. Each scale has lateral line canal (Llc) with anterior opening (Ao) and posterior opening (Po).

II. Osteometric characteristics:

(A) X-ray skeleton:

The general inter-specific osteometric differences between the four *Tilapia* species were reflected qualitatively by X-ray photographs (Figures, 9-12). Such photographs reflect the differences in skull, body cavity shape, vertebral column, pelvic girdle and pectoral girdle characteristics as well as the interrelationship between the positions of different fins relative to the axial skeleton.

The axial firm skeleton of a fish is composed of the skull, the vertebral column, the ribs and the inter-muscular bones. The skull in each species has a rather triangular shape. The dorsal bone (frontal) of the skull is more curved in *S. galilaeus*, but, more straight in *T. zillii*. A less curvature is observed in the frontal bone of *O. niloticus* and *O. aureus* skulls. According to the photographs, the position of the orbit (eye region) characterize *T. zillii* in which, it is located directly below the flat dorsal bone. Also, the size of the orbit is differed from small (*O. niloticus* and *O. aureus*) to large (*S. galilaeus* and *T. zillii*). Regarding the jaws length, the jaws of *O. aureus* and *T. zillii* are more elongated than that of the other species.

Concerning the vertebral column and ribs, the backbone of a fish is composed of a series of segments (vertebrae). The curvature of the vertebral column is differed from more curved in the trunk region of *S. galilaeus* to more straight in that of *O. niloticus*. Moderate curvature is observed in trunk regions of *O. aureus* and *T. zillii*. The vertebrae in each species are modified generally according to body region. Throughout the length of the trunk, the bodies of the

vertebrae (centra) often have lateral processes that bear ribs, which enclose a characteristic shaped body cavity and air bladder.

The dorsal fin of *S. galilaeus* is more curved, resulted in increasing in the body depth. A less curvature is observed in dorsal fin of *O. aureus*. The dorsal fins of *O. niloticus* and *T. zillii* are straighter so, less body depth is observed. According to the photographs, there is a general close relationship between *O. niloticus* and *O. aureus* which are in a distance with the tow different other species.

(B) Teeth feature:

During the present work, attention has been directed towards the types and arrangements of teeth in the four *Tilapia* species (Figures, 13-16). These figures show that, in the four *Tilapia* species the lower jaw teeth are arranged in two rows and they are bicuspid in *O. niloticus* and *O. aureus*. While, the teeth in *S. galilaeus* appeared rather numerous and thin when compared to the other species also, they are bicuspid ones. The lower jaw teeth in *T. zillii* were considered fewer in number and they are of a bicuspid type.

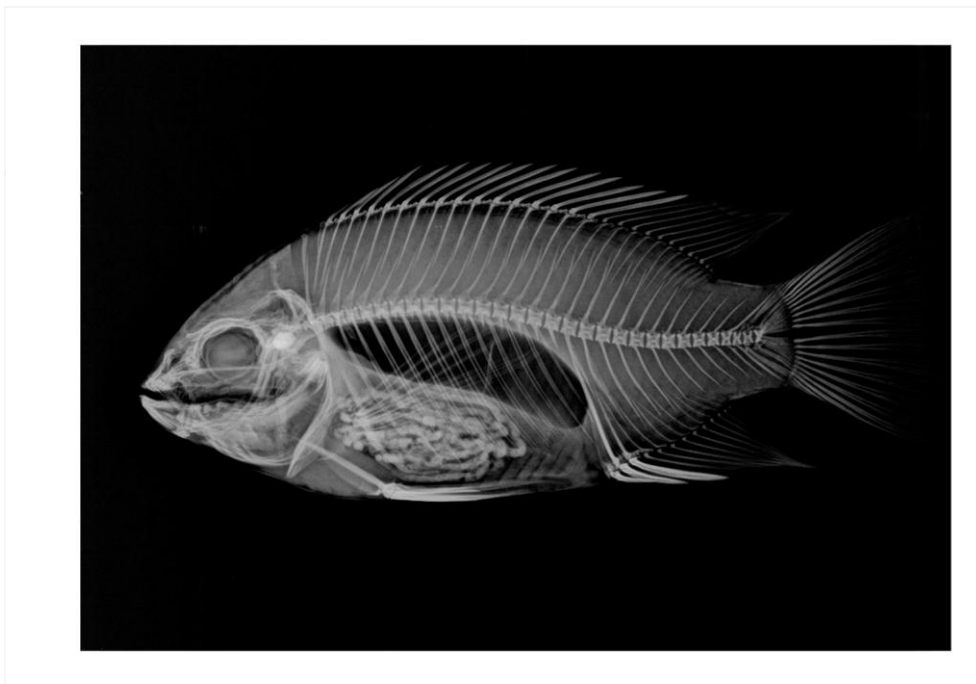
On the floor of the pharynx, two lower pharyngeal bones were existed. Each lower pharyngeal bone in the four *Tilapia* species has several teeth rows (Figures, 17-20). These figures showed that, the lower pharyngeal teeth of both *O. niloticus* and *O. aureus* are bicuspid, arranged in several rows and rather crowded. By comparing *S. galilaeus* lower pharyngeal teeth they arranged in many several rows and they appeared very thin, very crowded and unicuspid. Regarding the four *Tilapia* species, only *T. zillii* lower pharyngeal teeth are the biggest, not crowded and of tricuspid type.



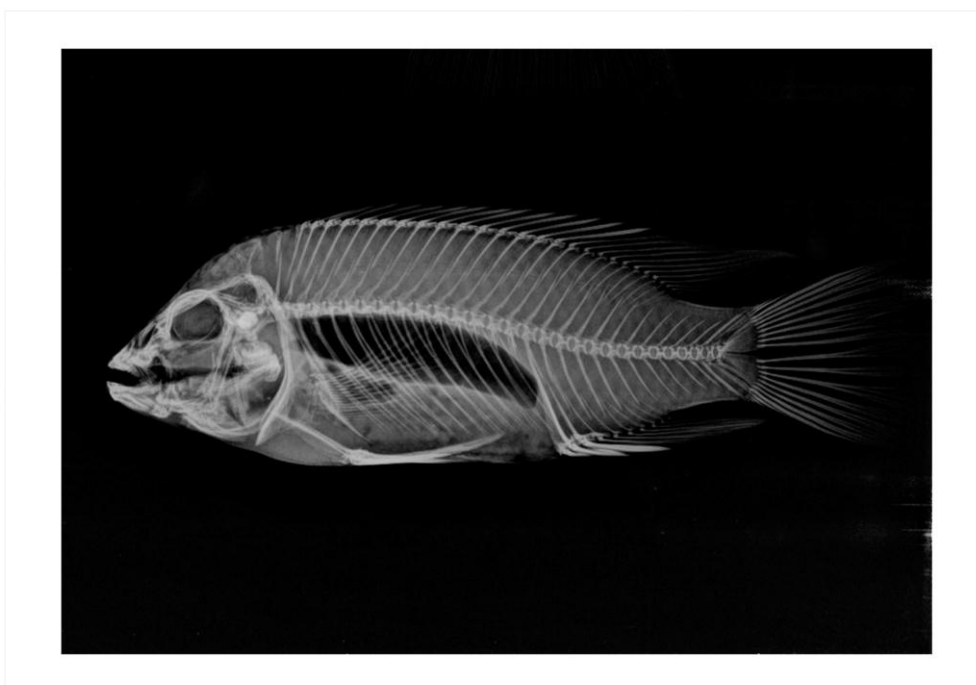
Figure (29): X-ray photograph illustrates the skeleton feature of *O. niloticus*.



Figure (30): X-ray photograph illustrates the skeleton feature of *O. aureus*.



**Figure (31): X-ray photograph illustrates the skeleton
feature of *S. galilaeus***



**Figure (32): X-ray photograph illustrates the skeleton
feature of *T. zillii*.**

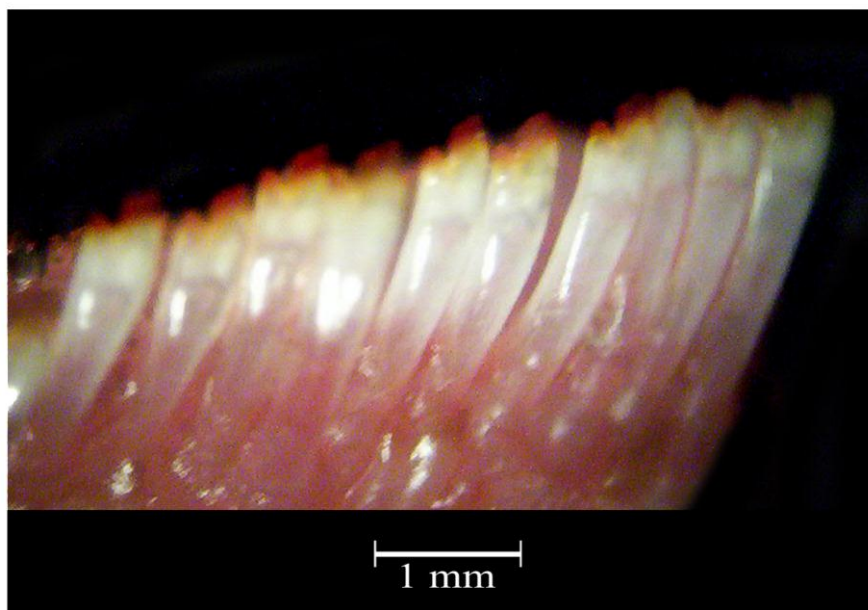


Figure (33): Photograph illustrates the teeth feature in the lower jaw of *O. niloticus*.

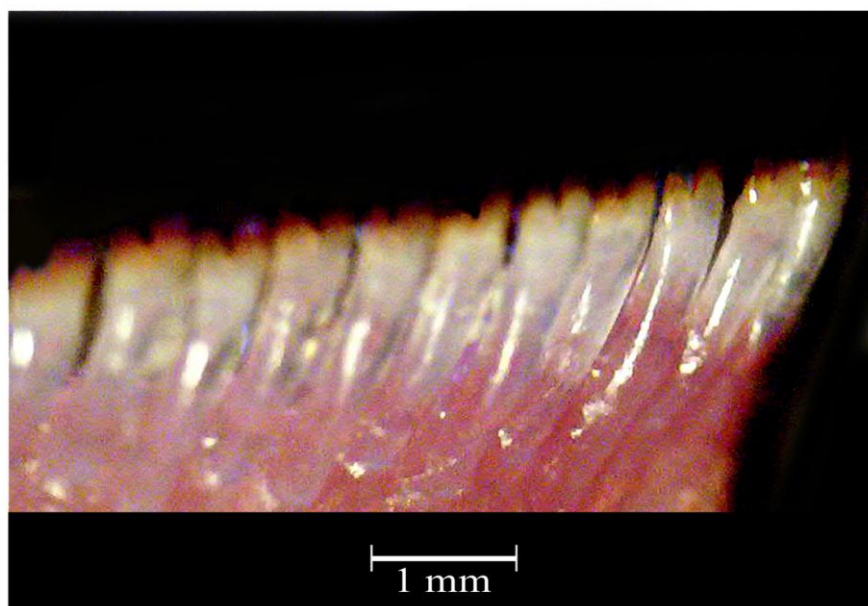


Figure (34): Photograph illustrates the teeth feature in the lower jaw of *O. aureus*.

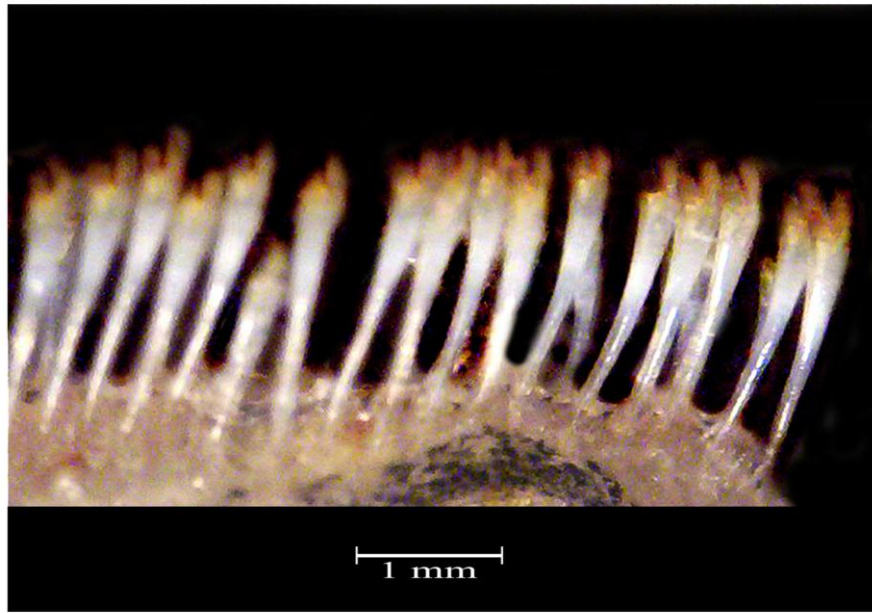


Figure (35): Photograph illustrates the teeth feature in the lower jaw of *S. galilaeus*.

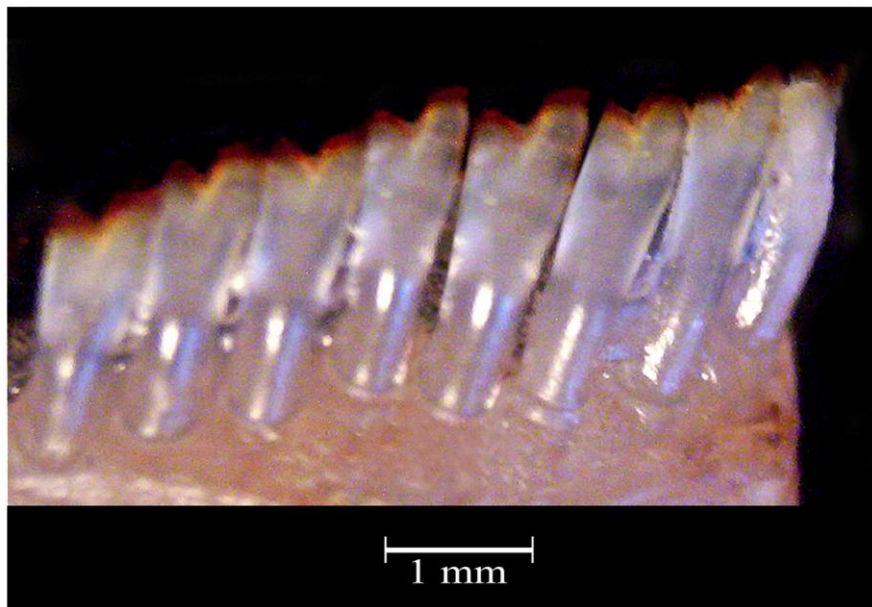


Figure (36): Photograph illustrates the teeth feature in the lower jaw of *T. zillii*.

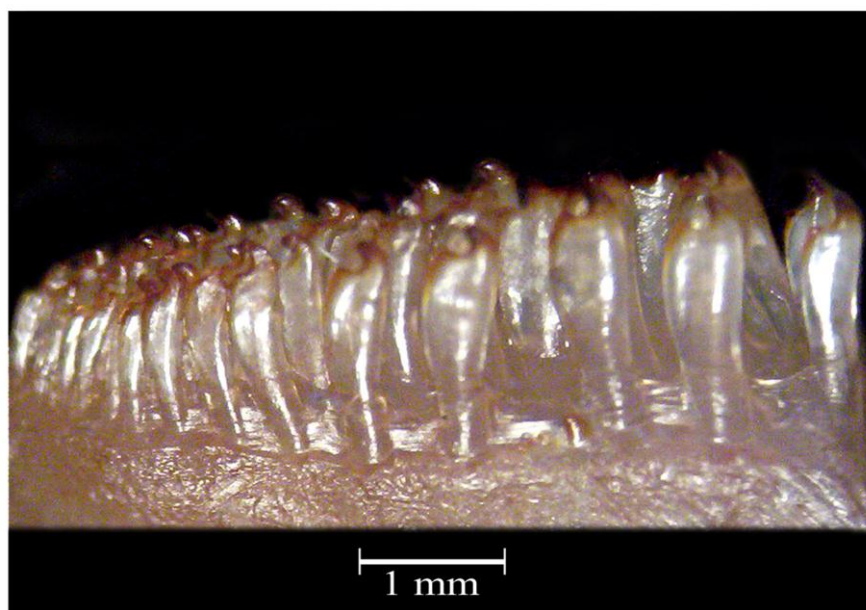


Figure (37): Photograph illustrates the lower-pharyngeal teeth feature in the lower jaw of *O. niloticus*.

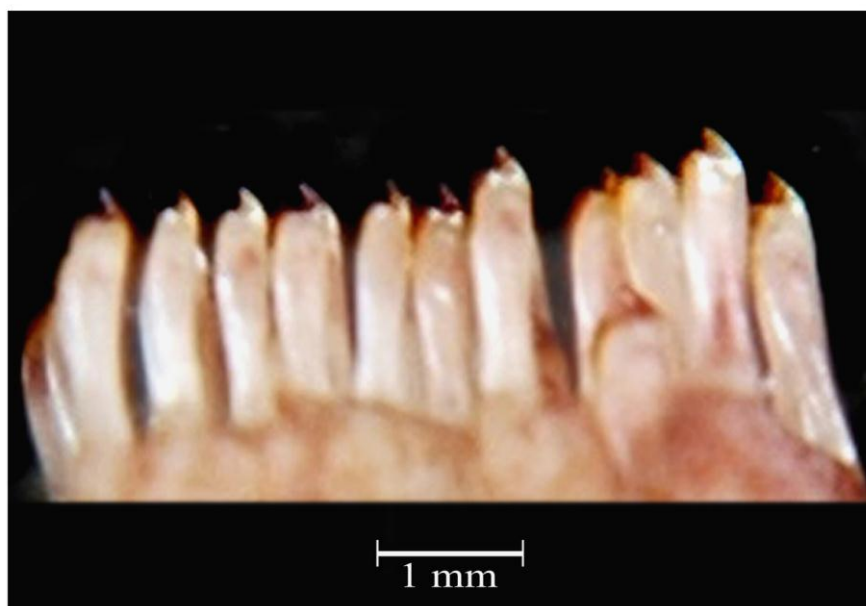


Figure (38): Photograph illustrates the lower-pharyngeal teeth feature in the lower jaw of *O. aureus*.

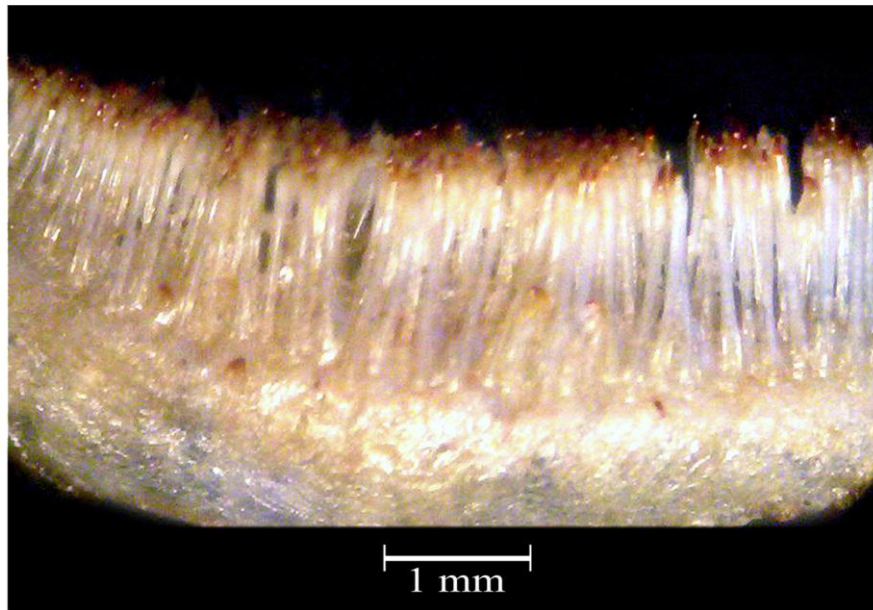


Figure (39): Photograph illustrates the lower-pharyngeal teeth feature in the lower jaw of *S. galilaeus*.

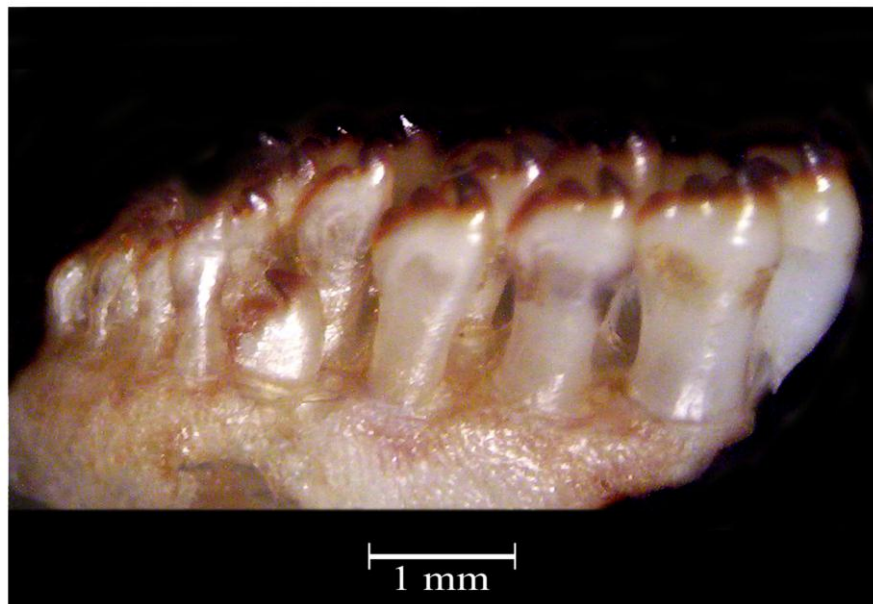


Figure (40): Photograph illustrates the lower-pharyngeal teeth feature in the lower jaw of *T. zillii*.

III. Molecular techniques:

Polymerase chain reaction (PCR) and sequencing techniques were used to differentiate the various *Tilapia* species in Egypt.

DNA genome has a high molecular weight, so it remained in the wheel of the agarose gel (Figure, 41). But the 1kbDNA Ladder represented in the first lane was separated into bands with different lengths.

Sequences of complete nuclear 18S rRNA genes (18S rDNA) were obtained for all *Tilapia* species from the PCR products (Figure, 42). The size of the nuclear 18S rRNA genes ranged between 1916-1986 bp for different *Tilapia* species. The nucleotide sequence was obtained and nucleotide bases were recorded from X-ray film (Figure, 43).

The analysis of DNA nucleotide composition using Bio-edit program indicated that the percentage of every base was different for each nuclear 18S rRNA gene of each species (Table, 3 & Figures, 44-47). Also, the table shows the % C + G which averaged 51.25% in *O. niloticus*, 51.38% in *O. aureus*, 52.31% in *S. galilaeus* and 52.37% in *T. zillii*.

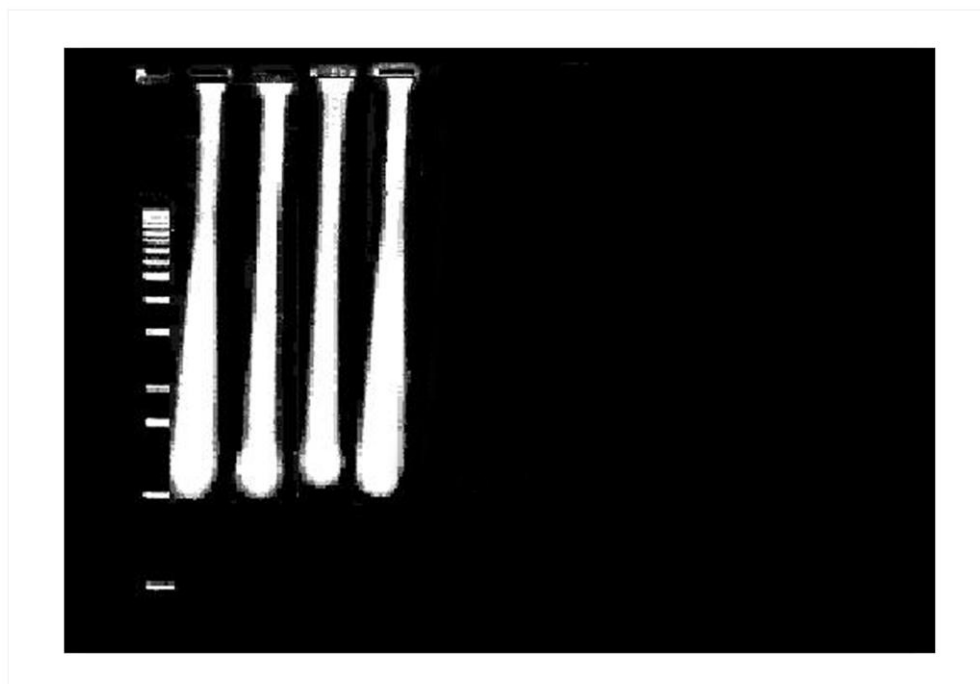
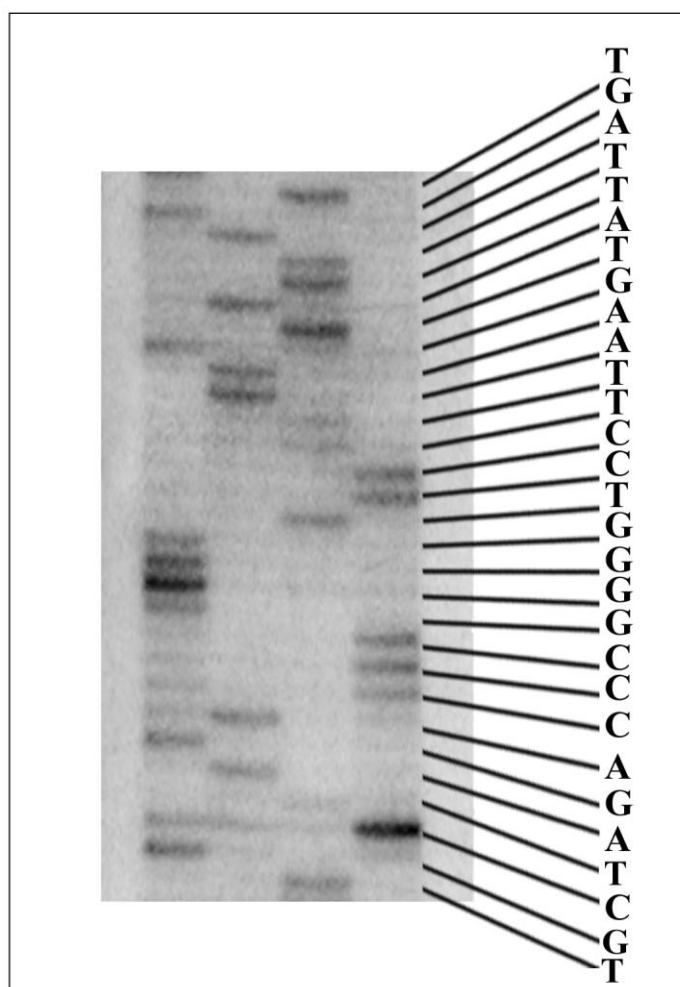


Figure (41): DNA genome of the four *Tilapia* species.



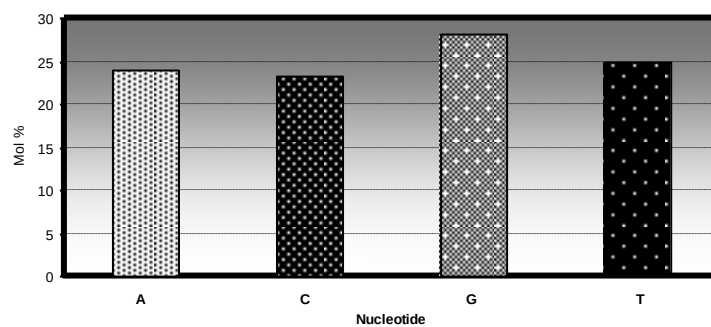
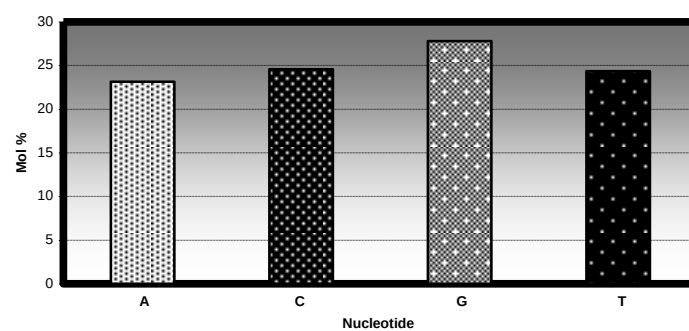
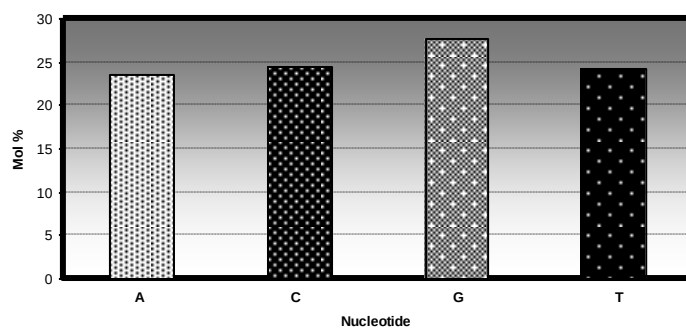
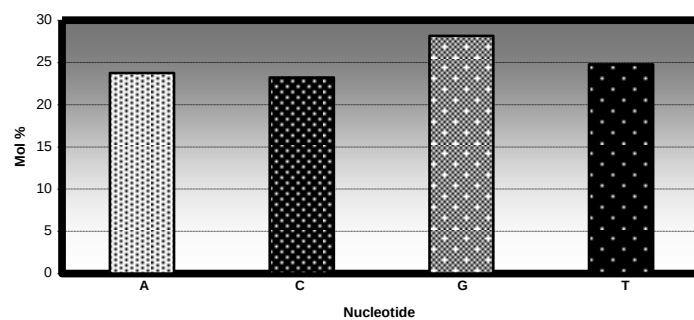
Figure (42): PCR products of 18S rRNA gene for *O. niloticus*, *O. aureus*, *S. galilaeus* and *T. zillii* (Lanes 2-5, respectively). 1kb DNA ladder represents at lane 1.



**Figure (43): Model of DNA nucleotide sequence
illustrated by X-ray film.**

Table (3): The nucleotide composition analysis of nuclear 18S rRNA gene of the four *Tilapia* species.

DNA Molecule Species	Length (bp)	Molecular weight (Daltons)		Nucleotide number				Nucleotide mol%				C+G Content	A+T Content
		Single strand	Double strand	A	C	G	T	A	C	G	T		
<i>O. niloticus</i>	1916	5805 58	1165 189	458	445	537	475	23.90	23.23	28.03	24.79	51.25 %	48.70 %
<i>O. aureus</i>	1925	5832 22	1170 711	457	447	542	477	23.74	23.22	28.16	24.78	51.38 %	48.52 %
<i>O. gallileus</i>	1967	5968 80	1196 565	461	483	546	475	23.44	24.56	27.76	24.15	52.31 %	47.59 %
<i>T. zillii</i>	1986	6025 23	1208 141	460	488	552	484	23.16	24.57	27.79	24.37	52.37 %	47.53 %



Figures (44-46):Percentage of nucleotide composition.

IV. Alignment and phylogenetic analysis of 18S rRNA gene sequences:

1. 18S rRNA gene sequences and their variable regions (alignment):

The alignment of 18S rRNA gene sequences for the four *Tilapia* species were obtained by Ali Bee-Multiple alignment (Gene bee service, <http://www.genebee.msu.ru/cgi-bin/nph-malign.pl>) with homology percent 67.7 % (Panel, I). The sequences for all species were highly conserved over most of the nuclear 18S rRNA genes except in 10 variable regions (figures, 48-57).

The first variable region sequence clustered the four *Tilapia* species into two clusters. The first cluster includes three species; *O. niloticus*, *O. aureus* and *T. zillii* with identical 18S rDNA sequences, when the other cluster contains *S. galilaeus* (Figure, 48).

The four *Tilapia* species were grouped into two groups according to the sequence of the variable regions from 2-9 of their 18S rRNA genes. The first group includes *O. niloticus* & *O. aureus* with identical sequence, and the second group contains *S. galilaeus* & *T. zillii* with identical sequence (Figures, 49-56).

The last variable region restricted the species *O. niloticus* as one group and the rest identical three species as the other dissimilar one (Figure, 57).

++++++
O. niloticus (1) GTCGACAACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGC
O. aureus (1) GTCGACAACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGC
S. galilaeus (1) GTCGACAACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGC
T. Zillii (1) GTCGACAACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGC
 ++++++
O. niloticus (61) ATGTCTAAGTATAAGCTTGTGTTTATACGGCGAGACTGCGGATGGCTCATTAAATCAGTTAT
O. aureus (61) ATGTCTAAGTATAAGCTTGTGTTTATACGGCGAGACTGCGGATGGCTCATTAAATCAGTTAT
S. galilaeus (61) ATGTCTAAGTATAAGCTTGTGTTTATACGGCGAGACTGCGGATGGCTCATTAAATCAGTTAT
T. Zillii (61) ATGTCTAAGTATAAGCTTGTGTTTATACGGCGAGACTGCGGATGGCTCATTAAATCAGTTAT
 ++++++ ++++++
O. niloticus (121) AGTTTATTGATGGTCTCTTTTGTCT-TTTTTTTACCTACTTGGATAACCGTGGTAATTCT
O. aureus (121) AGTTTATTGATGGTCTCTTTTGTCT-TTTTTTTACCTACTTGGATAACCGTGGTAATTCT
S. galilaeus (121) AGTTTATTGATGGTCTCTTTTGTCT-TTTTTTTACCTACTTGGATAACCGTGGTAATTCT
T. Zillii (121) AGTTTATTGATGGTCTCTTTTGTCT-TTTTTTTACCTACTTGGATAACCGTGGTAATTCT
 ++++++
O. niloticus (180) aaRGAGCTAATACATGCGCAAGGTCCCGAGCGCGGGGGGCGGGGCTTCACGGCCCCGTCC
O. aureus (180) --AGAGCTAATACATGCGCAAGGTCCCGAGCGCGGGGGGCGGGGCTTCACGGCCTCGTCC
S. galilaeus (181) --AGAGCTAATACATGCGCAAGGTCCCGAGCGCGGGGGGACGGGGCTTCACGGCTCTGTTC
T. Zillii (180) --AGAGCTAATACATGCGCAAGGTCCCGAGCGCGGGGGATGGGGCTTCACGGCTCTGTTC
 ++++++
O. niloticus (240) TCGCATGCGCAGAGGGATGATTTTATTAGGTTAAAAACCAGC--AGCCGGCAACGGCT--
O. aureus (238) TCGCATGCGCAGAGGGATGATTTTATTAGGTTAAAAACCAGC--AGCCGGCAACGGCT--
S. galilaeus (239) TCGCATGCGCAGAGGGATGATTTTATTAGGTTAAAAACCAGCGTAGCCAGCAATGGCTAC
T. Zillii (238) TCGCATGCGCAGAGGGATGATTTTATTAGGTTAAAAACCAGCGTAGCCAGCAATGGCTAC
 ++++++
O. niloticus (296) TCAA-CTCCTGGTGATTTCATAGTAACCTCTTCGGATCGCATTCATGTCCTCCTTGTGGGG
O. aureus (294) TCAA-CTCCTGGTGATTTCATAGTAACCTCTTCGGATCGCATTCATGTCCTCCTTGTGGGG
S. galilaeus (299) TCAATCTCCTGGTGATTTCATAGTAACCTCTTCGGATCGCATTCATGTACTCCTTGTGGGG
T. Zillii (298) TCAATCTCCTGGTGATTTCATAGTAACCTCTTCGGATCGCATTCATGTCCTCCTTGTGGGG
 ++++++ ++++++ ++++++ ++++++
O. niloticus (355) ACGGCGACGATTTCATCAAAATTTCTGCCCTATCAACTTTTCG-ATGGTAGGATAGA-GGCC
O. aureus (353) ACGGCGACGATTTCATCAAAATTTCTGCCCTATCAACTTTTCG-ATGGTAGGATAGA-GGCC
S. galilaeus (359) ACGGCGACGATTTCATCAAAATTTCTGGCCATCAACTTTTCGaaATGGTAGGATAGACGGCC
T. Zillii (358) ACGGCGACGATTTCATCAAAATTTCTGCCCTATCAACTTTTCG-ATGGTAGGATAGA-GGCC
 ++++++ ++++++
O. niloticus (413) TACCATGGTCGTAACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGA
O. aureus (411) TACCATGGTCGTAACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGA
S. galilaeus (419) TACCATGGACGAAACGGGTAAC-----GGAGAGGGAGCCTGAGA
T. Zillii (416) TACCATGGTCGTAACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGA
 ++++++ ++++++ ++++++ ++++++ ++++++ ++++++
O. niloticus (473) AATGGCTACCACCTTCTAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGG
O. aureus (471) AATGGCTACCACCTTCTAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGG
S. galilaeus (458) AATGGCTACCACCTTCTAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGG
T. Zillii (476) AATGGCTACTACTTCTAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCCGACTCGGGG
 ++++++ ++++++ ++++++ ++++++ ++++++ ++++++
O. niloticus (533) AGGTAGTGACAATAAATAACAATACAGGCGCTCGATAAGAGTCTTGTAAATGGAATGAGT
O. aureus (531) AGGTAGTGACAATAAATAACAATACAGGCGCTCGATAAGAGTCTTGTAAATGGAATGAGT
S. galilaeus (518) AGGTAGTGACGAAAAATAACAATACAGGACTCTTTCGAGGCCC-TGTAATTGAATGAGT
T. Zillii (536) AGGTAGTGACGAAAAATAACAATACAGGACTCTTTCGAGGCCC-TGTAATTGAATGAGT

79

[illegible]

```

+++++++..+.+.+++++++.....+++++++
O. niloticus (1189) AAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACG
O. aureus (1191) AAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACG
S. galilaeus (1038) AAGAACGAAAGTCGGAGGTTCTGAAGACGATCAGATACCGTCGTAGTTCGGACCATAAACG
T. Zillii (1057) AAGAACGAAAGTCGGAGGTTCTGAAGACGATCAGATACCGTCGTAGTTCGGACCATAAACG

+++++.+.+++++.+.+.++++.
O. niloticus (1249) ATGCCGACCAGCGATTAGGAGACGTTGAATACAAAACACCACCATCGGTGCGGTCTGCTCT
O. aureus (1251) ATGCCGACCAGCGATTAGGAGACGTTGAATACAAAACACCACCATCGGTGCGGTCTGCTCT
S. galilaeus (1098) ATGCCAACTAGCGATCCGGCGGCGTT-----
T. Zillii (1117) ATGCCAACTAGCGATCCGGCGGCGTT-----

..... +++
O. niloticus (1309) TGGCG--TCGGTCTTTC--GGGGCC--GGCGCGGGGACGGCTTAGCCCGGTGGCACCGGT
O. aureus (1311) TGGCGtcTCGGTCTTTCacGGGGCCgGGCGCGGGGGTGGCTTAGCCCGGTGGCACCGGT
S. galilaeus (1124) -----ATTC-----
T. Zillii (1143) -----ATTC-----

..+++++.+.+.+++++.+.+.+++++.+.+++++++.....
O. niloticus (1363) GAATGACTCCCTAGCAGCTTGTGAGAAATCATAAGTCTTTGGGTTCCGGGGGAGTATG
O. aureus (1371) GAATGACTCCCTAGCAGCTTGTGAGAAATCATAAGTCTTTGGGTTCCGGGGGAGTATG
S. galilaeus (1128) CCATGACCCGCCGGGCAGCGTCCGGGAAACCA-AAGTCTTTGGGTTCCGGGGGAGTATG
T. Zillii (1147) CCATGACCCGCCGGGCAGCGTCCGGGAAACCA-AAGTCTTTGGGTTCCGGGGGAGTATG

++.++++.+++++++.....
O. niloticus (1423) GTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGC-
O. aureus (1431) GTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCG
S. galilaeus (1187) GTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCG
T. Zillii (1206) GTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCG

+++++++..+.+.+++++.+.+++++.+.+++++++
O. niloticus (1482) GCTTAATTTGACTCAACACGGGGAAACTTACCAGGTCCGGACATAGTAAGGATTGACAGA
O. aureus (1491) GCTTAATTTGACTCAACACGGGGAAACTTACCAGGTCCGGACATAGTAAGGATTGACAGA
S. galilaeus (1247) GCTTAATTTGACTCAACACGGGGAAACTTACCAGGTCCGGACATAGTAAGGATTGACAGA
T. Zillii (1266) GCTTAATTTGACTCAACACGGGGAAACTTACCAGGTCCGGACATAGTAAGGATTGACAGA

+++++++..+++++.+++++++.....
O. niloticus (1542) TTGATAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGT
O. aureus (1551) TTGATAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGT
S. galilaeus (1307) TTGATAGCTCTTTCTCGATTCTGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGC
T. Zillii (1326) TTGATAGCTCTTTCTCGATTCTGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGC

+++++++..+++++.+++++++.....+.+.+++++.+.
O. niloticus (1602) GATTTGTCTGGTTAATTCCGTTAACGAACGAGACCTTAACCTGCTAAATA-----
O. aureus (1611) GATTTGTCTGGTTAATTCCGTTAACGAACGAGACCTTAACCTGCTAAATA-----
S. galilaeus (1367) GATTTGTCTGGTTAATTCCGATAACGAACGAGACTCCGACATGCTAACTAGTTACTCGAC
T. Zillii (1386) GATTTGTCTGGTTAATTCCGATAACGAACGAGACTCCGACATGCTAACTAGTTACTCGAC

....+++..+.+.+.++++.
O. niloticus (1652) ---TGC---CGCG-CTAAC-----CCGTCCATCAAAA-----
O. aureus (1661) ---TGC---CGCG-CTAAC-----CCGTCCATCAAAA-----
S. galilaeus (1427) CCCGTGCGGTCCGAGTCCAACCTCTTAGAGGGACAAGTGGCGTTCAGCCACACGAGATTG
T. Zillii (1446) CCCGTGCGGTCCGAGTCCAACCTCTTAGAGGGACAAGTGGCGTTCAGCCACACGAGATTG

.....
O. niloticus (1677) -----
O. aureus (1687) -----CCATGCGCGGCTCAC-----
S. galilaeus (1487) AGCAATAACAGGTCTGTGATGCCCTTAGATGTCCGGGGCTGCACGCGGCCACACTGAGT
T. Zillii (1506) AGCAATAACAGGTCTGTGATGCCCTTAGATGTCCGGGGCTGCACGCGGCCACACTGAGT

```

Continue.

The signs at the top of the alignment identify nucleotide differences; (+) indicates that all bases at that position are identical to *O. niloticus*, (.) indicates that at least one base at that position is dissimilar to *O. niloticus*. Lower case sign in the sequence (-) indicates a gap occurs in that position.

	+++++++..++..+++++++.....+++++++
<i>O. niloticus</i>	TACCATGGTCGTAACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGA
<i>O. aureus</i>	TACCATGGTCGTAACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGA
<i>S. galilaeus</i>	TACCATGGACGAAACGGGTAAC-----GGAGAGGGAGCCTGAGA
<i>T. zillii</i>	TACCATGGTCGTAACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGA

Figure (48): Alignment of the 1st variable region of 18S rDNA of the four *Tilapia* species, *O. niloticus* represents the consensus to which all other species are compared. The signs at the top of the alignment identify nucleotide differences; (+) indicates that all bases at that position are identical to *O. niloticus*, (.) indicates that at least one base at that position is dissimilar to *O. niloticus*. Lower case sign in the sequence (-) indicates a gap occurs in that position.

	+++.....++..+..+++++++.....+++++.++..+++++++..+..+
<i>O. niloticus</i>	AGGCGCTCGATAAGAGTCTTGTAATTGGAATGAGTACAATTTAAACCCCTTAACGAGTAACAA
<i>O. aureus</i>	AGGCGCTCGATAAGAGTCTTGTAATTGGAATGAGTACAATTTAAACCCCTTAACGAGTAACAA
<i>S. galilaeus</i>	AGGACTCTTTTCGAGGCCC-TGTAATTGGAATGAGTACACTTTAAATCCTTTAACGAGGATCCA
<i>T. zillii</i>	AGGACTCTTTTCGAGGCCC-TGTAATTGGAATGAGTACACTTTAAATCCTTTAACGAGGATCCA

Figure (49): Alignment of the 2nd variable region of 18S rDNA of the four *Tilapia* species, *O. niloticus* represents the consensus to which all other species are compared. The signs at the top of the alignment identify nucleotide differences; (+) indicates that all bases at that position are identical to *O. niloticus*, (.) indicates that at least one base at that position is dissimilar to *O. niloticus*. Lower case sign in the sequence (-) indicates a gap occurs in that position.

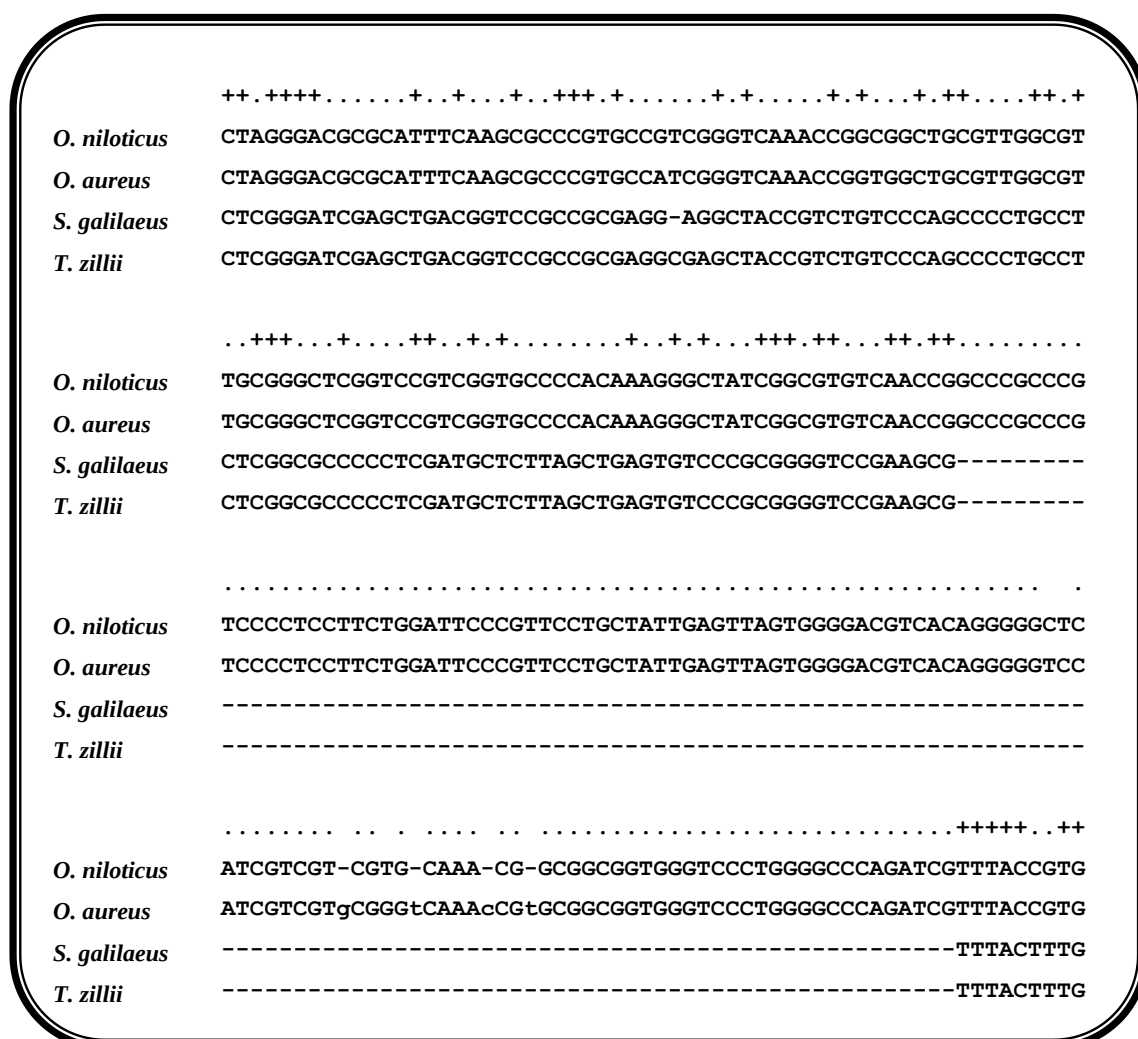


Figure (50): Alignment of the 3rd variable region of 18S rDNA of the four *Tilapia* species, *O. niloticus* represents the consensus to which all other species are compared. The signs at the top of the alignment identify nucleotide differences; (+) indicates that all bases at that position are identical to *O. niloticus*, (.) indicates that at least one base at that position is dissimilar to *O. niloticus*. Lower case sign in the sequence (-) indicates a gap occurs in that position.

++...+++.+...++.....+++++.+++++.+++.++
O. niloticus GCAGATCCAATTTTCTGCCACCGAATACATTAGCATGGGAT
O. aureus GCAGATCCAATTTTCTGCCACCGAATACATTAGCATGGGAT
S. galilaeus GC----CCGGTCGCCT-----GAATACCGCAGCTAGGAAT
T. zillii GC----CCGGTCGCCT-----GAATACCGCAGCTAGGAAT

: Alignment of the 4th variable region of 18S rDNA of the four *Tilapia* species, *O. niloticus* represents the consensus to which all other species are compared. The signs at the top of the alignment identify nucleotide differences; (+) indicates that all bases at that position are identical to *O. niloticus*, (.) indicates that at least one base at that position is dissimilar to *O. niloticus*. Lower case sign in the sequence (-) indicates a gap occurs in that position.

	+.+.+. . .+.+++++. .++.+++++. . . .+.+.+.+++ .+++ .+++++. .++++. .+. .
<i>O. niloticus</i>	CCCTGTCTCTCTATTTTCAGTTGGTTTTGGCAGCGCGAGGACTAGGGTAATGATTAATAGGGATAGTT
<i>O. aureus</i>	CCCTGTCTCTCTATTTTCAGTTGGTTTTGGCAGCGCGAGGACTAGGGTAATGATTAATAGGGATAGTT
<i>S. galilaeus</i>	CTCCG--GTTCTATTTT--GTGGGTTTT--CTCTC-TGAACGGGGCCATGATTAAGAGGGACGGCC
<i>T. zillii</i>	CTCCG--GTTCTATTTT--GTGGGTTTT--CTCTC-TGAACGGGGCCATGATTAAGAGGGACGGCC
	+++++. .++++. . . .+. .++
<i>O. niloticus</i>	GGGGGCATTAATATTTAATTGTCAG
<i>O. aureus</i>	GGGGGCATTAATATTTAATTGTCAG
<i>S. galilaeus</i>	GGGGGCATTTCGTATTGTGCCGCTAG
<i>T. zillii</i>	GGGGGCATTTCGTATTGTGCCGCTAG

: Alignment of the 5th variable region of 18S rDNA of the four *Tilapia* species, *O. niloticus* represents the consensus to which all other species are compared. The signs at the top of the alignment identify nucleotide differences; (+) indicates that all bases at that position are identical to *O. niloticus*, (.) indicates that at least one base at that position is dissimilar to *O. niloticus*. Lower case sign in the sequence (-) indicates a gap occurs in that position.

	++.....+.++++...++.....+++++++.....++++++.++
<i>O. niloticus</i>	GATTTATGAAAGATTAAC TTCTGCGAAAGCATCTGCCAAGGAT
<i>O. aureus</i>	GATTTATGAAAGATTAAC TTCTGCGAAAGCATCTGCCAAGGAT
<i>S. galilaeus</i>	GACCGGCGCAAGACGGACGAAAGCGAAAGCATT TGCCAAGAAT
<i>T. zillii</i>	GACCGGCGCAAGACGGACGAAAGCGAAAGCATT TGCCAAGAAT

Figure (53): Alignment of the 6th variable region of 18S rDNA of the four *Tilapia* species, *O. niloticus* represents the consensus to which all other species are compared. The signs at the top of the alignment identify nucleotide differences; (+) indicates that all bases at that position are identical to *O. niloticus*, (.) indicates that at least one base at that position is dissimilar to *O. niloticus*. Lower case sign in the sequence (-) indicates a gap occurs in that position.

	++ . . + . ++ . ++++++ . . . +++++
<i>O. niloticus</i>	GTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCA
<i>O. aureus</i>	GTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCA
<i>S. galilaeus</i>	GTCGGAGGTTCTGAAGACGATCAGATACCGTCGTAGTTCGGACCA
<i>T. zillii</i>	GTCGGAGGTTCTGAAGACGATCAGATACCGTCGTAGTTCGGACCA

Figure (54): Alignment of the 7th variable region of 18S rDNA of the four *Tilapia* species, *O. niloticus* represents the consensus to which all other species are compared. The signs at the top of the alignment identify nucleotide differences; (+) indicates that all bases at that position are identical to *O. niloticus*, (.) indicates that at least one base at that position is dissimilar to *O. niloticus*. Lower case sign in the sequence (-) indicates a gap occurs in that position.

	+...+.+.++++.....+++.
<i>O. niloticus</i>	TTAGGAGACGTTGAATACAAAACACCACCATCGGTGCGGTCGTCCTTGGCG--TCGGTCTTTC--G
<i>O. aureus</i>	TTAGGAGACGTTGAATACAAAACACCACCATCGGTGCGGTCGTCCTTGGCGtcTCGGTCTTCacG
<i>S. galilaeus</i>	TCCGGCGGCGTT-----ATTC--
<i>T. zillii</i>	CGATCCGGCGGCGTT-----ATTC--
	+++++.+.+.+++++.+.+.+
<i>O. niloticus</i>	GGGCC--GGCGCGGGACGGCTTAGCCCGGTGGCACCAGTGAATGACTCCCCTAGCAGCTTGTGAGA
<i>O. aureus</i>	GGGCCggGGCGCGGGGTGGCTTAGCCCGGTGGCACCAGTGAATGACTCCCCTAGCAGCTTGTGAGA
<i>S. galilaeus</i>	-----CCATGACCCGCCGGGCAGCGTCCGGGA
<i>T. zillii</i>	-----CCATGACCCGCCGGGCAGCGTCCGGGA

Figure (55): Alignment of the 8th variable region of 18S rDNA of the four *Tilapia* species, *O. niloticus* represents the consensus to which all other species are compared. The signs at the top of the alignment identify nucleotide differences; (+) indicates that all bases at that position are identical to *O. niloticus*, (.) indicates that at least one base at that position is dissimilar to *O. niloticus*. Lower case sign in the sequence (-) indicates a gap occurs in that position.

++.....++.....++.....+++.....++..+..+..+++.....+

O. niloticus ACCTTAACCTGCTAAATA-----TGC-----CGCG-CTAAC-----C

O. aureus ACCTTAACCTGCTAAATA-----TGC-----CGCG-CTAAC-----C

S. galilaeus ACTCCGACATGCTAACTAGTTACTCGACCCCGTGCGGTCCGAGTCCAACCTTCTTAGAGGGACAAGTGG

T. zillii ACTCCGACATGCTAACTAGTTACTCGACCCCGTGCGGTCCGAGTCCAACCTTCTTAGAGGGACAAGTGG

++..+..+..+.....

O. niloticus CGTCCATCAAAA-----

O. aureus CGTCCATCAAAAC-----CCATGCGC

S. galilaeus CGTTCAGCCACACGAGATTGAGCAATAACAGGTCTGTGATGCCCTTAGATGTCCGGGGCTGCACGCGC

T. zillii CGTTCAGCCACACGAGATTGAGCAATAACAGGTCTGTGATGCCCTTAGATGTCCGGGGCTGCACGCGC

.....+++..+..+..++.....+..+++.....+..+.....+..+..++.....+++..

O. niloticus -----GAT--GGTTTAGTGAGGTCTCGGATCGGCCCGCCGGGGTCGGTCACGGCCCTG

O. aureus GGCTCAC-----GGAT--GGTTTAGTGAGGTCTCGGATCGGCCCGCCGGGGTCGGTCACGGCCCTG

S. galilaeus GCCCACTGAGTGGATCAGCGTGTGTCTACCCCTTCGCCGAGAGGCGTGGGTAACCCGTTGAACCCAC

T. zillii GCCCACTGAGTGGATCAGCGTGTGTCTACCCCTTCGCCGAGAGGCGTGGGTAACCCGTTGAACCCAC

..++.....

O. niloticus GCG-----

O. aureus GCG-----

S. galilaeus TCGTGATAGGGATTGGGGATTGCAATTATTTCCCATGAACGAGGAATTCCCAGTAAGCGCGGGTCATA

T. zillii TCGTGATAGGGATTGGGGATTGCAATTATTTCCCATGAACGAGGAATTCCCAGTAAGCGCGGGTCATA

.....

O. niloticus -----

O. aureus -----

S. galilaeus AGCTCGCGTTGATTAAGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAATGGTCCG

T. zillii AGCTCGCGTTGATTAAGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAATGGTCCG

.....++

O. niloticus -----GAGCGC

O. aureus -----GAGCGC

S. galilaeus GTGAAATCCTCGGAGCCGTGGCCTCTACGCAATCCGGGCAACCGGGTTGTGAGGTCTCCCCTTTTGGC

T. zillii GTGAAATCCTCGGAGCCGTGGCCTCTACGCAATCCGGGCAACCGGGTTGTGAGGTCTCCCCTTTTGGC

..+..++++.++++..+++..+..++..++..++

O. niloticus CGAGAAGACGATCAAACCTTGACTATCTA

O. aureus CGAGAAGACGATCAAACCTTGACTATCTA

S. galilaeus GGCGAAGTCGATTGAACCTTACCATTTA

T. zillii GGCGAAGTCGATTGAACCTTACCATTTA

	++ .++++	+++
<i>O. niloticus</i>	ACACCGAgcggcccgccctgtggtGCA	
<i>O. aureus</i>	ACRCCGA-----GCA	
<i>S. galilaeus</i>	ACRCCGA-----GCA	
<i>T. zillii</i>	ACRCCGA-----GCA	

Figure (57): Alignment of the 10th variable region of 18S rDNA of the four *Tilapia* species, *O. niloticus* represents the consensus to which all other species are compared. The signs at the top of the alignment identify nucleotide differences; (+) indicates that all bases at that position are identical to *O. niloticus*, (.) indicates that at least one base at that position is dissimilar to *O. niloticus*. Lower case sign in the sequence (-) indicates a gap occurs in that position.

2. Percent and numbers of base differences among sequences:

The percent sequence differences of the nuclear 18S rDNA among all species ranged from 2.19-40.19%. The number of base differences in the sequences ranged from 43-770 (Table, 4).

Depending on the percent sequence differences and number of base differences between species for nuclear 18S rDNA, the four *Tilapia* species were differentiated into two main clusters (groups), *O. niloticus* & *O. aureus* were grouped as one group and *S. galilaeus* & *T. zillii* were grouped as the second group. The percent sequence difference of the nuclear 18S rDNA between the species of the first group was 3.50% and the number of base differences was 67 bp. The percent sequence difference between the species of the second group was 2.19% and the number of base differences was 43 bp.

Table (4): The percent of nucleotide sequence differences (above the diagonal), and the number of nucleotide differences (below the diagonal) in pair-wise comparisons of the nuclear 18S rRNA gene of the four *Tilapia* species.

	<i>O. niloticus</i>	<i>O. aureus</i>	<i>S. galilaeus</i>	<i>T. zillii</i>
<i>O. niloticus</i>	***	3.50	40.19	38.99
<i>O. aureus</i>	67	***	39.38	38.29
<i>S. galilaeus</i>	770	758	***	2.19
<i>T. zillii</i>	747	737	43	***

3. Phylogenetic analysis and identification of sequence clusters:

Several different phylogenetic analyses were used for the sequence data of nuclear 18S rDNA by using Gene Bee Service. Informative sites (ditypic changes) were used from aligned sequences for phylogenetic, parsimony and bootstrapping analysis.

For distance analysis, the complete gene sequences were used including the informative sites with the exception of all unalignable variable regions which were removed. The most parsimonious trees (drawn by Ali Bee-Multiple alignment rectangular cluster algorithm and unrooted topological algorithm) of the nuclear 18S rRNA gene sequence relationships give two main clusters; *O. niloticus* & *O. aureus* in the first group and *S. galilaeus* & *T. zillii* in the second group (Figures, 58 & 59).

The 100 X bootstraps of the maximum parsimony analysis of the four *Tilapia* species give trees with strong bootstrap values for the two main groups. The value for each group (100) suggested that the nodes are significant (Figures, 60 & 61). According to the figures, the bootstrapping trees are drawn by tree top-phylogenetic tree prediction rectangular cluster algorithm and slanted topological algorithm.

The distance matrix values are generated by the DNADIST topological algorithm (Table, 5). Distance tree constructed for the four *Tilapia* species 18S rRNA genes was drawn by tree top-phylogenetic tree prediction as phylogram topological algorithm (Figure, 62). The distance matrix and the tree identified the same two clusters as the parsimony trees 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.

Table (5): Distance matrix values generated by gene bee topological algorithm for nuclear 18S rRNA gene of the four *Tilapia* species.

	<i>O. niloticus</i>	<i>O. aureus</i>	<i>S. galilaeus</i>	<i>T. zillii</i>
<i>O. niloticus</i>	0.000			
<i>O. aureus</i>	0.076	0.000		
<i>S. galilaeus</i>	0.858	0.829	0.000	
<i>T. zillii</i>	0.829	0.800	0.042	0.000

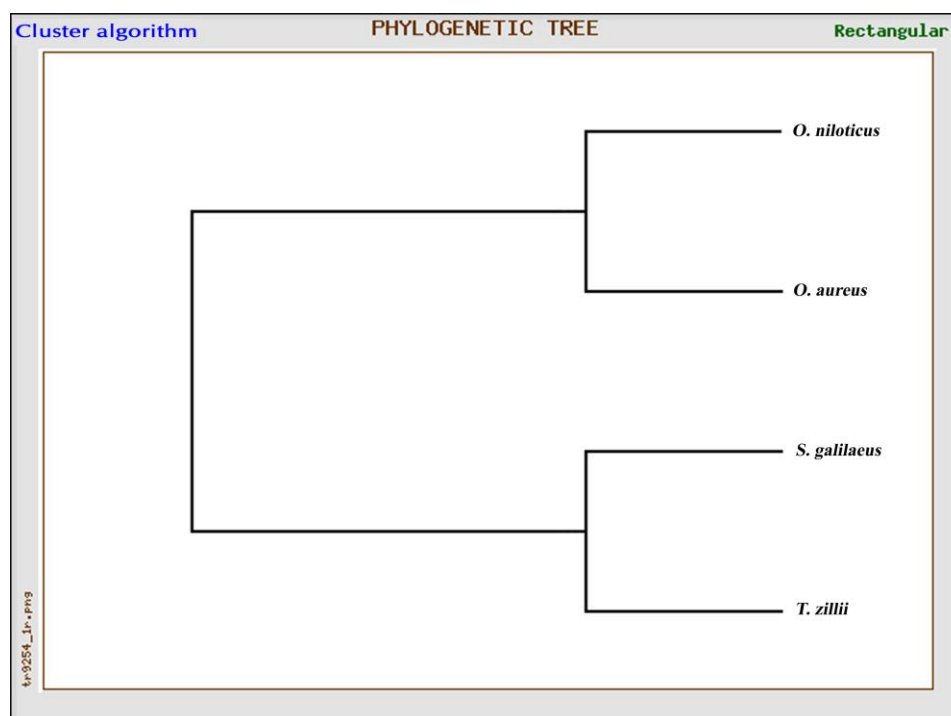


Figure (58): Parsimony tree based on alignment of nucleotide sequences of 18S rRNA gene of the four *Tilapia* species; Drawn by Ali Bee-multiple alignment (Rectangular cluster algorithm).

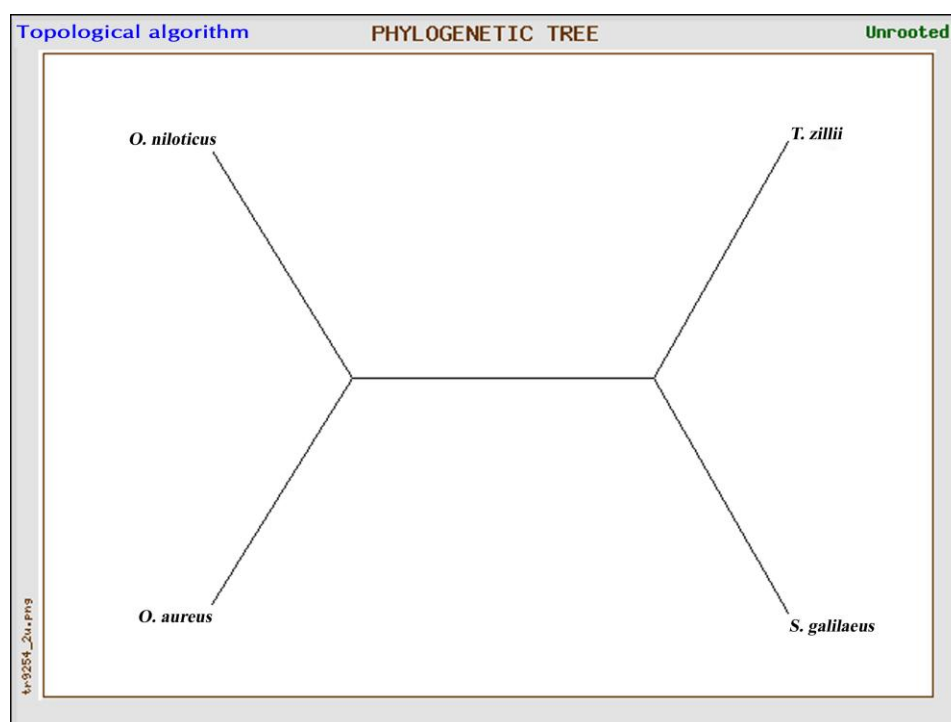


Figure (59): Parsimony tree based on alignment of nucleotide sequences of 18S rRNA gene of the four *Tilapia* species; Drawn by Ali Bee-multiple alignment (Unrooted topological algorithm)

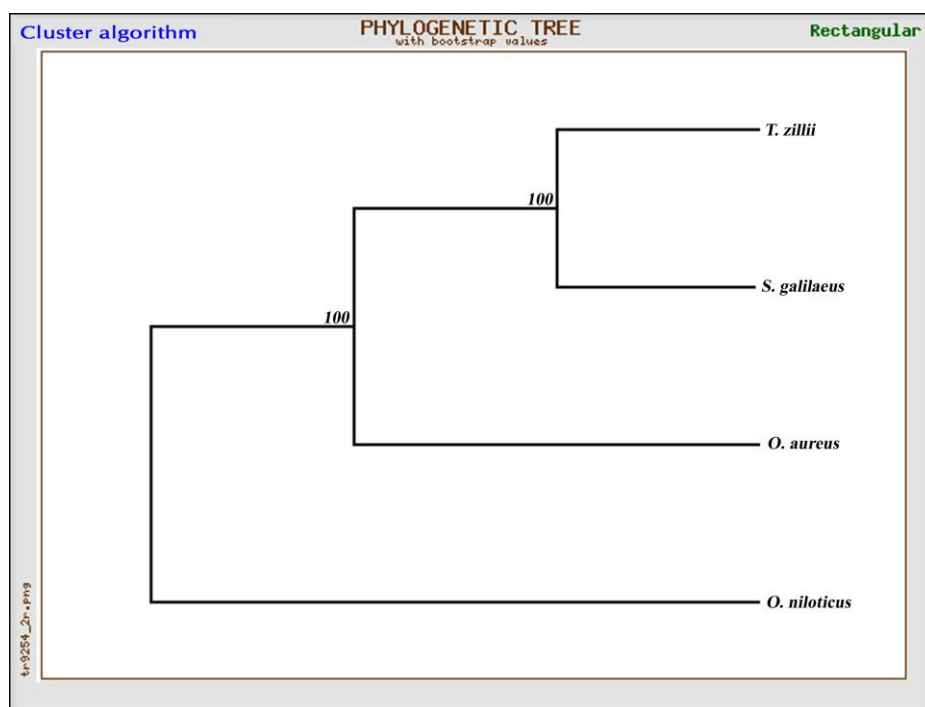


Figure (60): Bootstrapping tree with seqboot values at the nodes based on alignment of nucleotide sequences of 18S rRNA gene of the four *Tilapia* species; Drawn by tree top-phylogenetic tree prediction (Rectangular cluster algorithm).

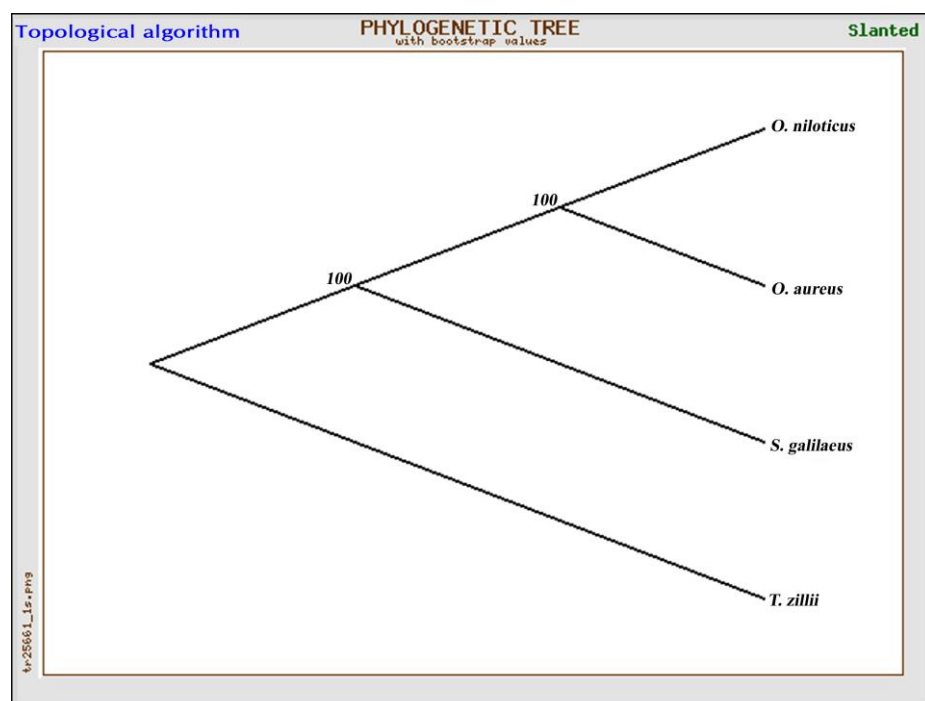


Figure (61): Bootstrapping tree with seqboot values at the nodes based on alignment of nucleotide sequences of 18S rRNA gene of the four *Tilapia* species; Drawn by tree top-phylogenetic tree prediction (Slanted topological algorithm).

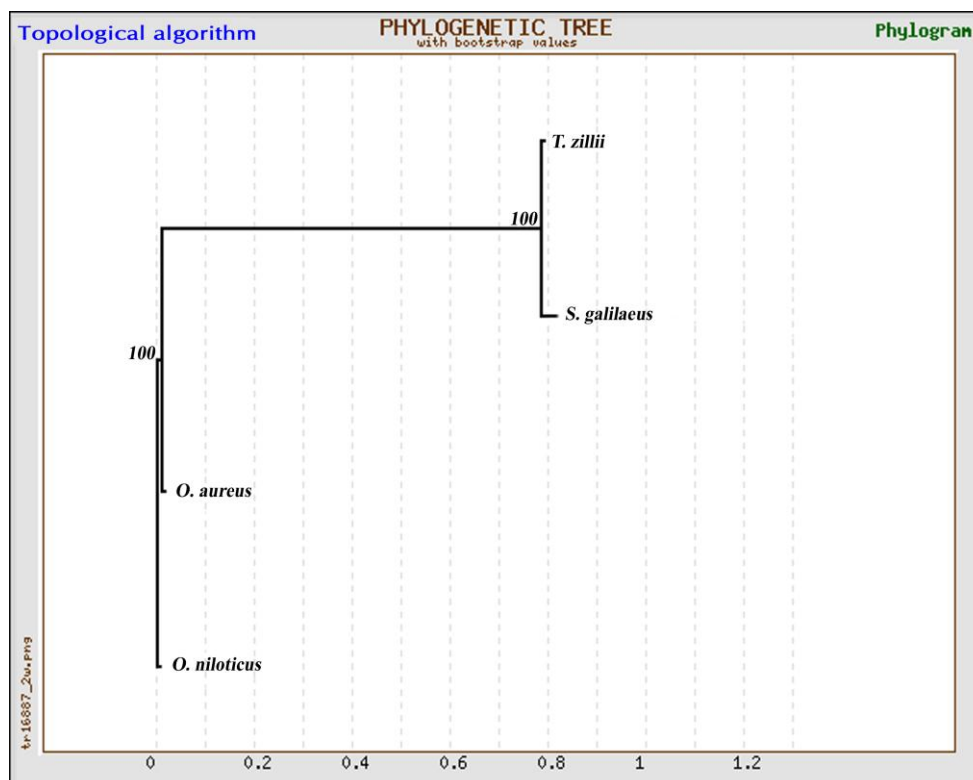


Figure (62): Distance tree with seqboot values at the nodes based on alignment of nucleotide sequences of 18S rRNA gene constructed for the four *Tilapia* species using tree top-phylogenetic tree prediction (Phylogram topological algorithm).

V. RNA secondary structure prediction:

Small subunit ribosomal RNA which transcribed from 18S rDNA is a single strand; this strand has several complementary sequences (stems) and single stranded (loops). The stems with complementary nucleotides and resulted loops formed a distinct structure known as RNA secondary structure.

The number of 18S rRNA secondary structure stems ranged from 71-86 among the four *Tilapia* species and most of them differed in their positions and nucleotide sequences (Table, 6).

For each *Tilapia* species, the 18S rRNA secondary structure stems position and sequence are recorded as well as, the distinct secondary structure is drawn using gene bee RNA secondary structure prediction (Figures, 63-66). The four RNA secondary structure indicated degrees of similarities and dissimilarities between *O. niloticus* & *O. aureus* and *S. galilaeus* & *T. zillii*. Also, according to the figures, the free energies of the 18S rRNA secondary structures are -402.9 kkal/mol (*O. niloticus*), -410.3 kkal/mol (*O. aureus*), -452.3 kkal/mol (*S. galilaeus*) and -459.7 kkal/mol (*T. zillii*).

Table (6): 18S rRNA secondary structure stems with their positions and sequences of the four *Tilapia* species.

Stem	<i>O. niloticus</i>		<i>O. aureus</i>		<i>S. galilaeus</i>		<i>T. zillii</i>	
	Position	Sequence	Position	Sequence	Position	Sequence	Position	Sequence
1	1385-1367 1910-1901	GCACCGGUGA CGUGGCCACU	218-225 1739-1732	GGCGGGGC CCGCCCCG	755-765 1581-1571	GCCUCUCGGCG CGGAGAGCCGC	774-784 1601-1591	GCCUCUCGGC G CGGAGAGCCG C
2	1462-1470 1580-1572	GCACCACCA CGUGGUGGU	1470-1478 1589-1581	GCACCACCA CGUGGUGGU	1225-1233 1344-1336	GCACCACCA CGUGGUGGU	1245-1253 1364-1365	GCACCACCA CGUGGUGGU
3	232-239 1333-1326	CGGCCCCG GCCGGGGC	822-828 1340-1334	CCGGCCC GGCCGGG	503-511 1621-1613	CCCAAUCCC GGGUUAGGG	228-235 1780-1773	CACGGCUG GUGCCGAG
4	646-653 767-760	GCAGCCGC CGUCGGCG	1665-1671 1700-1694	GCCGCGC CGGCGCG	1116-1122 1144-1138	CCGGCGG GGCCGCC	1135-1141 1163-1175	CCGGCGG GGCCGCC
5	354-361 840-833	GGGGACGG CCCCUGCC	1172-1179 1317-1310	GCCAAGGA CGGUUCCU	796-802 1432-1426	CGGGGUC GCCCCAG	815-821 1452-1446	CGGGGUC GCCCCAG
6	1170-1177 1315-1308	GCCAAGGA CGGUUCCU	637-643 1370-1364	GGUGCCA CCACGGU	248-291 1900-1893	GUAGCCAG CAUCGGUC	1826-1831 1953-1948	GGCGGC CCGCCG
7	240-246 1069-1063	UCCUCGC AGGAGCG	1379-1385 1430-1424	ACUCCCC UGAGGGG	1051-1057 1408-1402	GUCGGAG CAGCCUC	1070-1076 1428-1422	GUCGGAG CAGCCUC
8	1371-1377 1422-1416	ACUCCCC UGAGGGG	832-838 880-874	CGUCCCC GCAGGGG	406-412 1726-1720	GGUAGGA CCAUCCU	387-394 1635-1628	CCCUAUCA GGGAUAGU
9	777-782 832-827	GCGGGC CGCCCCG	644-649 765-760	GCAGCC CGUCGG	1465-1470 1541-1536	GUGGCG CACCGC	402-409 424-417	AUGGUAGG UACCAUCC
10	399-406 421-414	AUGGUAGG UACCAUCC	397-404 419-412	AUGGUAGG UACCAUCC	842-846 926-922	GGCCC CCGGG	283-289 300-294	GUAGCCA CAUCGGU
11	382-387 633-628	UGCCCU ACGGGA	888-893 1033-1028	GGGUCC CCAGG	196-201 252-247	UGCGCA ACGCGU	1485-1490 1561-1556	GUGGCG CACCGC
12	1696-1701 1886-1881	CCUCGG GGAGCC	776-781 1362-1357	CGGGCU GCCCGA	943-947 991-987	GCCGG CGGCC	502-507 694-689	GCAGCA CGUCGU
13	918-923 998-993	CGGUGG GCCACC	977-982 996-991	GGCAGA CCGUCU	789-794 1463-1458	UGUCCC ACAGGG	861-865 945-941	GGCCC CCGGG
14	1709-1713 1736-1732	CCGCC GGCGG	229-234 1597-1592	ACGGCC UGCCGG	560-564 616-612	GCCCU CGGGA	431-437 1615-1609	AACGGGU UUGCCCA
15	205-209 1343-1339	GUCCC CAGGG	380-385 631-626	UGCCCU ACGGGA	714-719 741-736	GACGGU CUGCCA	364-369 1739-1734	GCGACG CGCUGC
16	885-890 1034-1029	ACAGGG UGUCCC	1740-1744 1862-1858	GGGGU CCCCA	1910-1915 1958-1953	CCGGUG GGCCAC	962-966 1010-1006	GCCGG CGGCC
17	468-472 564-560	GCCUG CGGAC	1035-1040 1072-1067	GUCCUC CAGGAG	1104-1110 1362-1356	CCAACUA GGUUGAU	808-813 1483-1478	UGUCCC ACAGGG
18	1840-1843 1880-1877	CGGC GCCG	212-216 1905-1901	GCGGG CGCCC	385-362 1801-1797	GGGGA CCCCU	578-582 634-630	GCCCU CGGGA
19	1703-1707 1744-1740	UCGGC AGCCG	359-363 1438-1434	GCGAC CGCUG	630-634 676-672	GCAGC CGUCG	732-737 760-755	GACGGU CUGCCA
20	56-61 108-103	AGCCAU UCGGUA	466-470 562-558	GCCUG CGGAC	685-690 713-708	AGCUCG UCGAGC	1123-1129 1382-1376	CCAACUA GGUUGAU
21	1454-1459 1625-1620	ACGGAA UGCCUU	56-61 108-103	AGCCAU UCGGUA	204-208 221-217	GUCCC CAGGG	212-216 1966-1962	GCGGG CGCCC

Table (6): Continue.

22	796-799 809-806	GCCC CGGG	283-287 296-292	AGCCG UCGGC	453-457 492-488	GCCUG CGGAC	56-61 108-103	AGCCAU UCGGUA
23	1256-1260 1307-1303	CGACC GCUGG	236-240 358-354	CGUCC GCAGG	56-61 108-103	AGCCAU UCGGUA	525-529 1863-1867	CUCCC GAGGG
24	724-727 738-735	GCGC CGCG	795-798 1350-1347	CCCC GGGG	415-419 1717-1713	GACGG CUGCC	1858-1881 802-806	GGAGA CCUCU
25	481-485 541-537	CUACC GAUGG	1258-1262 1309-1305	CGACC GCUGG	1582-1586 1604-1600	GUGGG CACCC	1576-1572 1439-1497	GCUGA CGACU
26	642-645 773-770	GCCA CGGU	1645-1649 1722-1781	GACCU CUGGA	1843-1847 1865-1861	GGAGA CCUCU	1549-1545 721-724	CAGCC GUCGG
27	1634-1638 1891-1887	GAGAC CUCUG	368-373 1378-1373	CAUUCA GUAAGU	783-787 1556-1552	GCUGA CGACU	766-763 1640-1643	GGGA CCCU
28	282-286 864-860	AGCAG UCGUC	722-725 736-733	GCGC CGCG	1920-1924 1938-1934	CCCUC GGGAG	1658-1655 968-971	GGGA CCCU
29	977-980 992-989	CAGA GUCU	1758-1761 1768-1765	GCGC CCGC	363-366 1758-1755	CGGC GCCG	984-981 221-224	GGCA CCGU
30	523-526 536-533	UCCC AGGG	479-483 539-535	CUACC GAUGG	1473-1477 1529-1525	CAGCC GUCGG	1821-1818 739-742	GGGG CCCC
31	431-434 608-605	GGGU CCCA	277-281 308-304	ACCAG UGGUC	449-452 1638-1635	GGGA CCCU	750-747 833-839	CGCC GCGG
32	213-216 1338-1335	CGCG GCGC	1906-1910 1919-1915	CACCG GUGGC	518-521 747-744	GGGA CCCU	858-852 516-522	CUUUGAA GAAACUU
33	276-281 1056-1051	AAAACC UUUUGG	521-524 534-531	UCCC AGGG	949-952 965-962	GGCA CCGU	662-656 1967-1971	AAUUACC UUA AUGG
34	1791-1795 1810-1806	AGGUU UCCAA	1797-1801 1853-1849	AGGAA UCCUU	374-380 1735-1729	CAUUCAA GUAAGUU	1967-1971 1980-1976	CACCG GUGGC
35	7-11 45-41	GACAA CUGUU	429-432 606-603	GGGU CCCA	814-820 839-833	CUUUGAA GAAACUU	1287-1290 1302-1299	CGGG GCCC
36	1671-1675 1684-1680	CCAUC GGUAG	1126-1130 1332-1328	GUGAA CACUU	1663-1666 1712-1709	GCGG CGCC	1682-1685 1700-1697	CGCG GCGC
37	292-296 858-854	AACGG UUGCC	920-923 951-948	CGGU GCCA	1267-1270 1282-1879	CGGG GCCC	301-306 1752-1747	UCAAUC AGUUAG
38	1216-1219 1232-1229	GACG CUGC	245-249 336-332	AUGCG UACGC	302-307 1823-1818	UCAAUC AGUUAG	1077-1081 1417-1413	GUUCG CAAGC
39	784-787 826-823	CGGU GCCA	664-668 712-708	UCCAA AGGUU	1058-1062 1397-1393	GUUCG CAAGC	1794-1797 1805-1802	CCGG GGCC
40	1480-1483 1519-1516	CCUG GGAC	7-11 45-41	GACAA CUGUU	1774-1777 1785-1782	CCGG GGCC	1856-1860 1945-1941	AGAGG UCUCC
41	454-458 591-587	AUUCC UAAGG	205-208 1923-1920	CCCG GGGC	1152-1155 1179-1176	CCGG GGCC	470-473 485-482	AGCC UCGG
42	1433-1435 1896-1894	GGC CCG	1867-1870 1898-1895	ACCC UGGG	1304-1308 1332-1328	ACAGA UGUCU	1171-1174 1198-1195	CCGG GGCC
43	1144-1148 1325-1321	AAAGA UUUCU	1486-1489 1496-1493	AGCC UCGG	1762-1765 1794-1791	CCUC GGAG	1324-1328 1352-1348	ACAGA UGUCU

Table (6): Continue.

44	139-144 188-183	CUCUUU GAGAAA	651-654 757-754	CGGU GCCA	928-934 1041-1035	UGAUUAA ACUAAUU	1782-1785 1814-1811	CCUC GGAG
45	72-77 89-84	GUAUAA CAUAUU	829-831 1104-1101	GCC CGG	7-11 45-41	GACAA CUGUU	346-349 362-359	GUCC CAGG
46	1288-1291 1301-1298	CACC GUGG	1218-1221 1283-1231	GACG CUGC	1901-1904 1964-1961	CCCG GGGC	440-443 462-459	CGGA GCCU
47	154-158 178-174	UUACC AAUGG	1822-1825 1841-1838	UCCG AGGC	337-340 1813-1810	UCGC AGCG	947-953 1060-1054	UGAUUAA ACUAAUU
48	660-663 714-711	UCCA AGGU	452-456 589-585	AUUC UAAGG	589-593 604-600	AUCCU UAGGA	7-11 45-41	GACAA CUGUU
49	739-741 750-748	CCG GGC	72-77 89-84	GUAUAA CAUAUU	1241-1244 1251-1248	AGCC UCGG	205-208 1984-1918	CCCG GGGC
50	1351-1353 1913-1911	CCC GGG	1630-1633 1728-1752	UCCG AGGC	1065-1068 1081-1078	GACG CUGC	607-611 622-618	AUCCU UAGGC
51	1424-1427 1900-1897	UGGU ACCA	1480-1483 1507-1504	GAGU CUCA	1737-1740 1754-1751	UCCG AGGC	1261-1264 1271-1268	AGCC UCGG
52	1749-1752 1786-1783	ACGA UGCU	1290-1293 1303-1300	CACC GUGG	1146-1149 1200-1197	CAGC GUCG	1084-1087 1100-1097	GACG CUGC
53	424-427 616-613	UCGU AGCA	154-158 178-174	UUACC AAUGG	495-498 1627-1624	GCAA CGUU	239-242 255-252	CCUC GGAG
54	1643-1646 1695-1692	ACCU UGGA	1753-1755 1856-1854	GCC CGG	72-77 89-84	GUAUAA CAUAUU	463-466 720-717	GAGA CUCU
55	66-69 97-94	GUCU CAGA	737-739 748-746	CCG GGC	1235-1238 1262-1259	GAGU CUCA	1165-1168 1220-1217	CAGC GUCG
56	1715-1717 1730-1728	GGG CCC	930-933 940-973	CUGG GACC	155-159 179-175	UUACC AAUGG	72-77 89-84	GUAUAA CAUAUU
57	13-16 29-26	CUGG GACC	422-425 614-611	UCGU AGCA	135-138 281-278	UGGU ACCA	1903-1906 1914-1911	AGGA UCCU
58	189-192 1350-1347	CUAA GAUU	66-69 97-94	GUCU CAGA	722-724 734-732	GCC CGG	1255-1258 1282-1279	GAGU CUCA
59	1526-1530 1569-1565	CAUAG GUAUC	813-815 1345-1343	GCG CGC	1284-1286 1335-1333	CCC GGG	154-158 178-174	UUACC AAUGG
60	892-894 1009-1007	CCU CGA	13-16 29-26	CUGG GACC	548-551 621-618	GACU CUGA	769-771 1606-1604	CCC GGG
61	927-930 976-973	CCUG GGAC	1515-1517 1531-1529	GGA CCU	1217-1220 1352-1349	ACGG UGCC	1304-1306 1355-1353	CCC GGG
62	654-656 758-756	GGU CCA	1535-1539 1578-1574	CAUAG GUAUC	66-69 97-94	GUCU CAGA	566-569 639-636	GACU CUGA
63	46-50 134-130	UCAAA AGUUU	905-907 999-997	GGU CCA	1091-1094 1380-1377	ACCA UGGU	277-280 1757-1754	ACCA UGGU
64	62-64 100-98	GCA CGU	46-50 134-130	UCAAA AGUUU	13-16 29-26	CUGG GACC	486-490 712-708	ACUAC UGAUG

Table (6): Continue.

65	878-880 1039-1037	GGA CCU	62-64 100-98	GCA CGU	188-193 270-265	CUAAUA GAUUAU	1237-1240 1372-1369	ACGG UGCC
66	1476-1478 1522-1520	GGA CCU	485-488 496-493	CUUC GAAG	293-297 1834-1830	AAUGG UUACC	66-69 97-94	GUCU CAGA
67	487-490 498-495	CUUC GAAG	1615-1654 1712-1709	AACC UUGG	422-425 441-438	UACC AUGG	510-512 677-675	CGC GCG
68	1210-1213 1253-1250	AUCG UAGC	846-848 858-856	GGA CCU	869-871 889-887	GGA CCU	1110-1113 1400-1397	ACCA UGGU
69	721-723 753-751	GAC CUG	1212-1215 1255-1252	AUCG UAGC	514-517 1609-1606	CACG GUGC	13-16 29-26	CUGG GACC
70	248-250 1060-1058	UGC ACG	251-254 324-321	AGAG UCUC	648-650 658-656	GCU CGA	888-890 908-906	GGA CCU
71	1812-1814 1837-1835	CGG GCC	719-721 751-749	GAC CUG	847-849 861-859	GGU CCA	1916-1918 1927-1925	GCU CGA
72			139-142 186-183	CUCU GAGA	891-894 919-916	GUUC CAAG	866-868 880-788	GGU CCA
73			915-917 976-974	UGC ACG	46-50 134-130	UCAAA AGUUU	910-913 938-935	GUUC CAAG
74					62-64 100-98	GCA CGU	46-50 134-130	UCAAA AGUUU
75					1158-1161 1175-1172	AACC UUGG	62-64 100-98	GCA CGU
76					472-475 483-480	CUUC GAAG	333-335 1742-1740	GGA CCU
77					444-446 1654-1652	GGA CCU	371-375 1663-1659	UUCAU AAGUA
78					808-881 1047-1044	CGUU GCAA	1177-1180 1194-1191	AACC UUGG
79					139-142 187-184	CUCU GAGA	1892-1895 1938-1935	GAAC CUUG
80					1490-1492 1510-1508	GCA CGU	311-313 1746-1744	GGU CCA
81							1899-1901 1932-1930	CGG GCC
82							827-830 1066-1063	CGUU GCAA
83							139-142 186-183	CUCU GAGA
84							1667-1669 1712-1710	GGA CCU
85							1510-1512 1530-1528	GCA CGU
86							256-258 1768-1766	GAU CUA

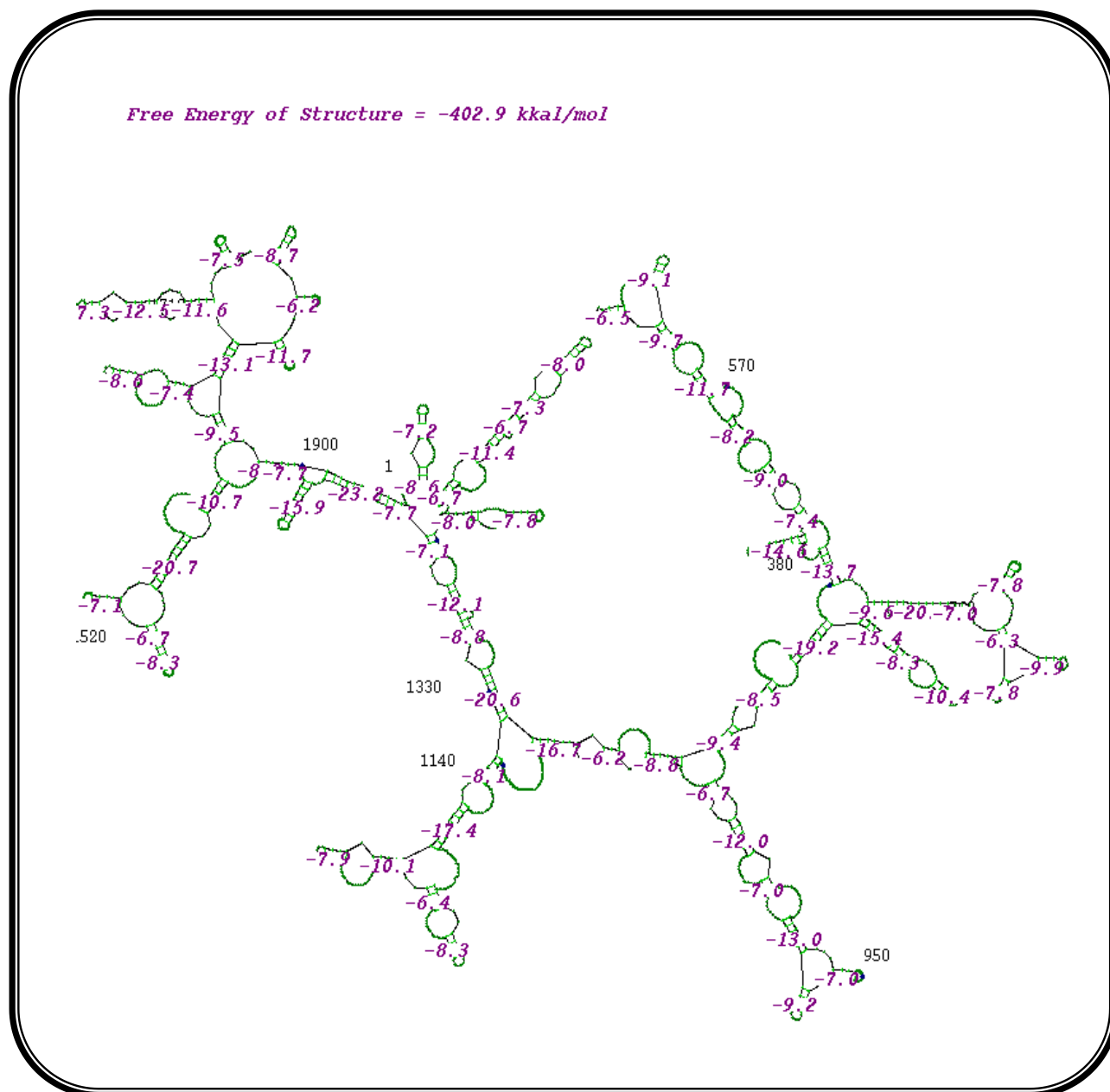


Figure (63): 18S rRNA secondary structure predicted
for *O. niloticus*.

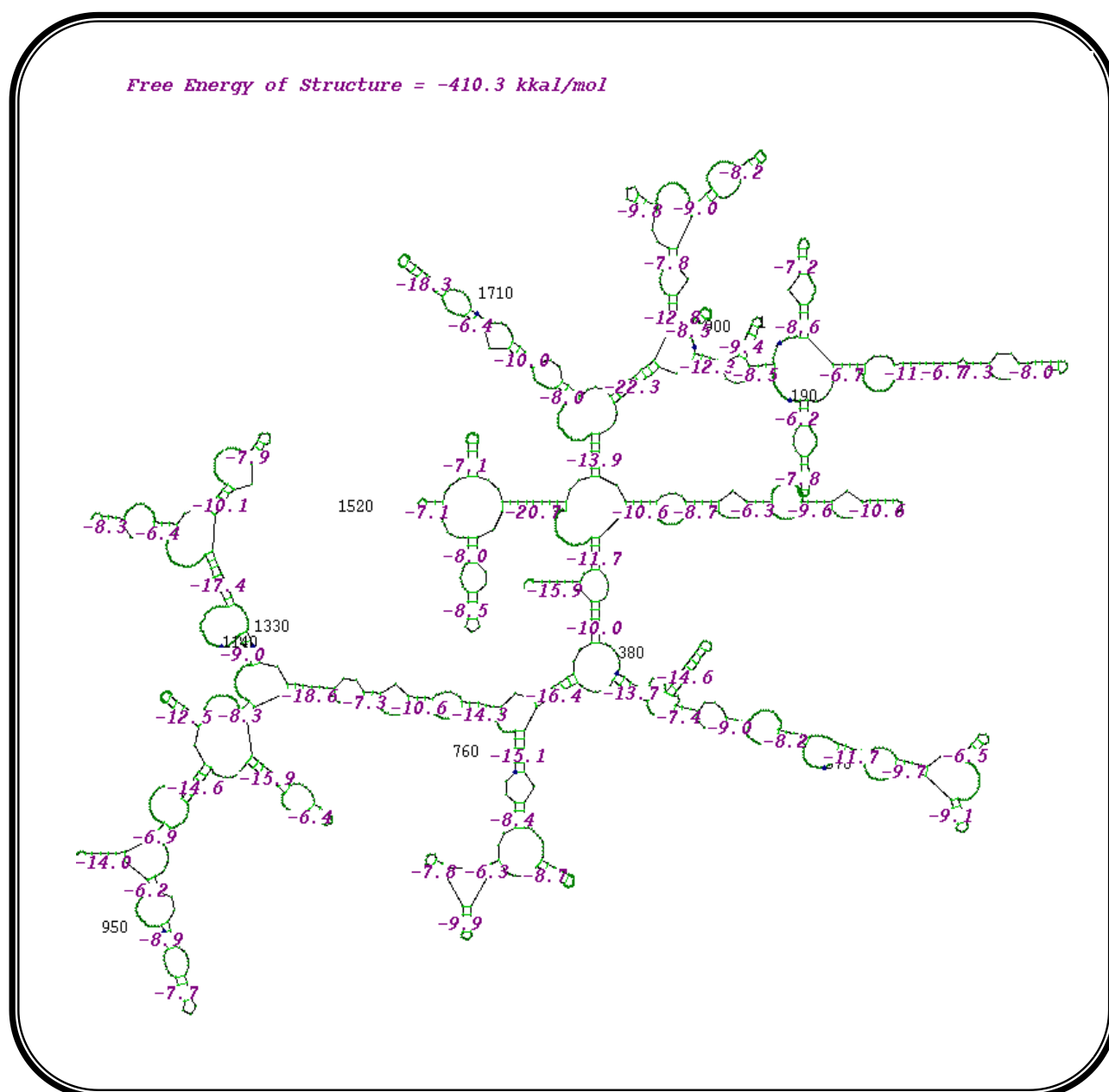
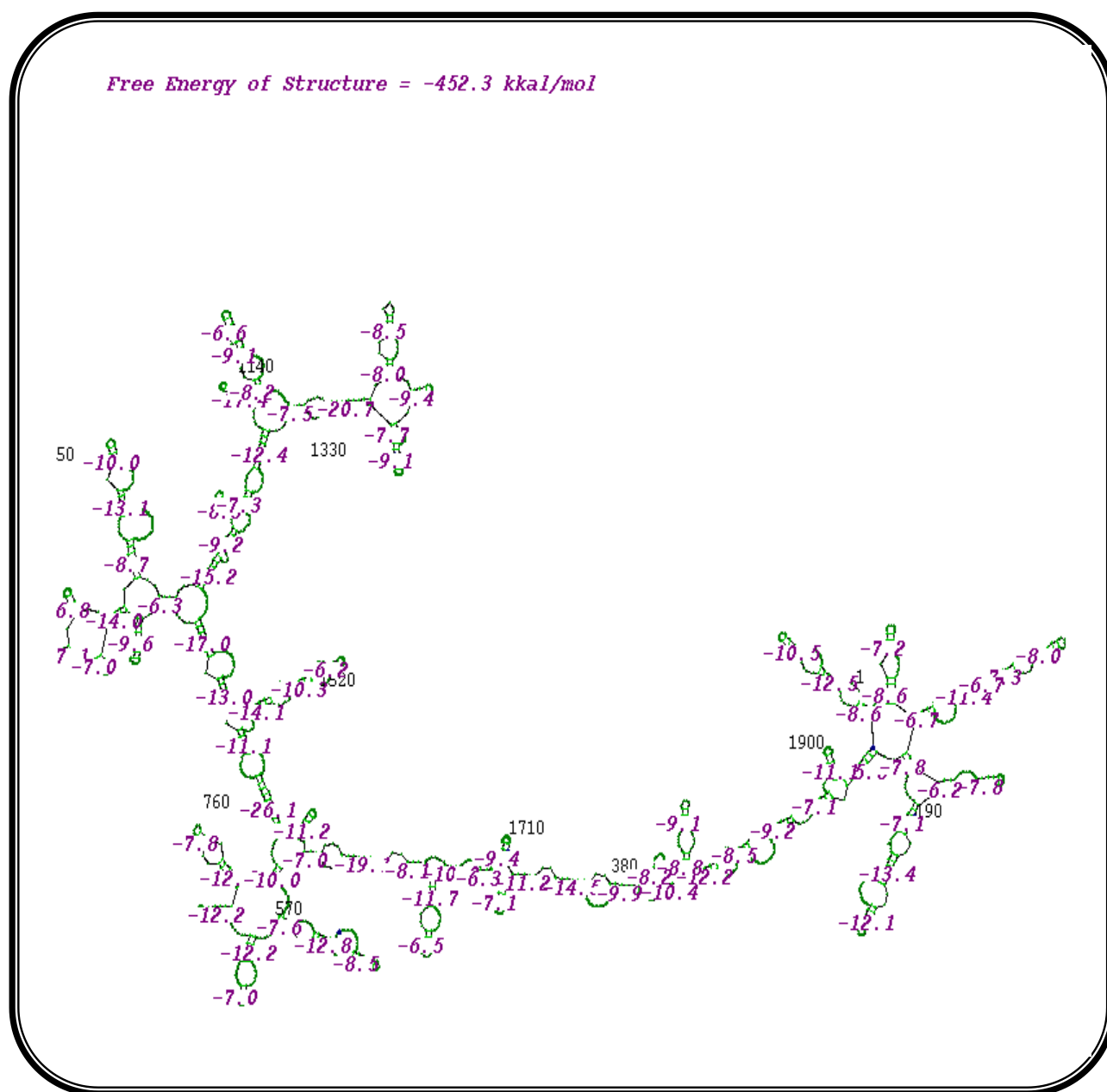


Figure (64): 18S rRNA secondary structure predicted
for *O. aureus*.



**Figure (65): 18S rRNA secondary structure predicted
for *S. galilaeus*.**

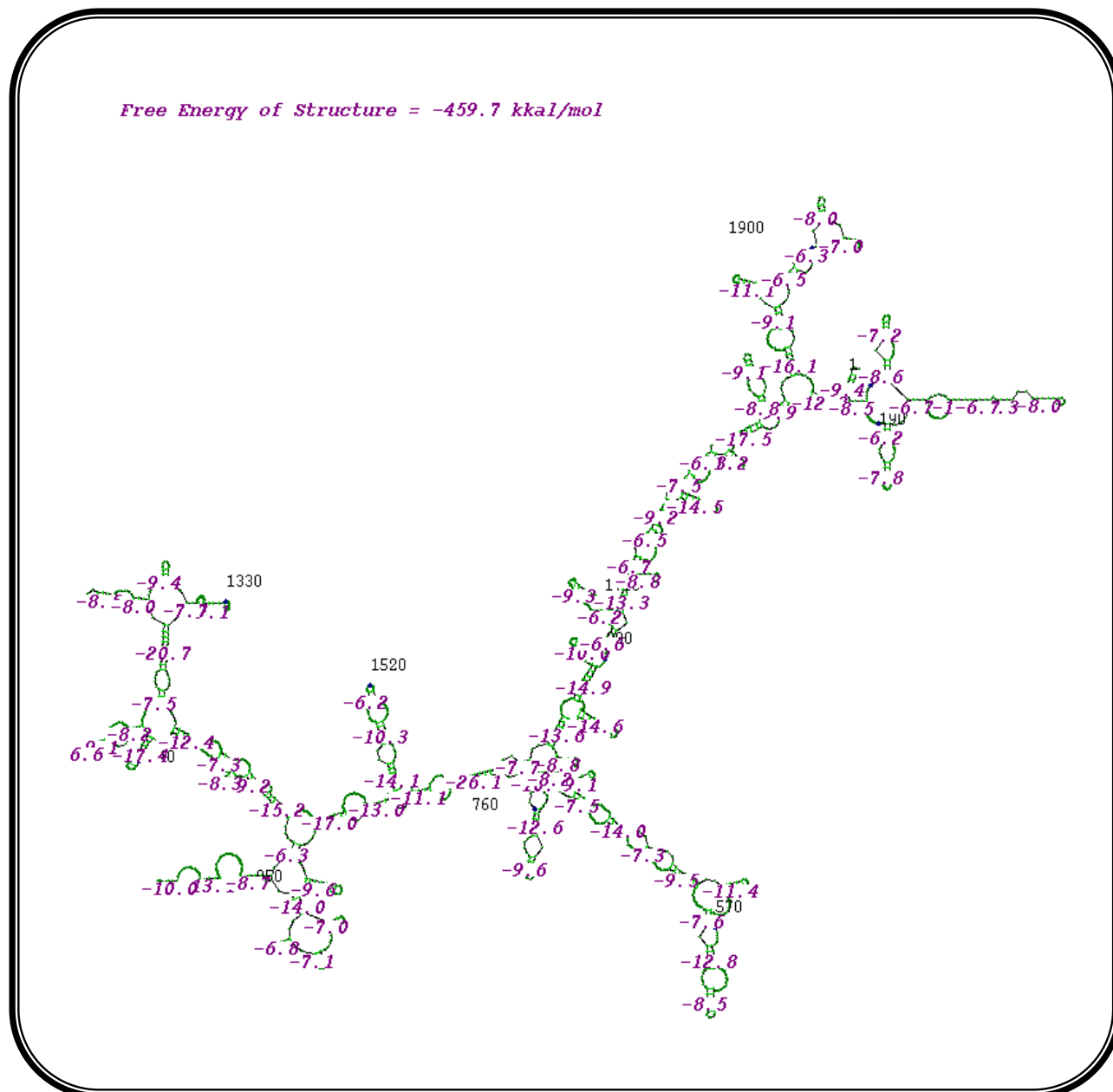


Figure (66): 18S rRNA secondary structure predicted
for *T. zillii*.