



Light and scanning electron microscopic observations on the tongue of Nile monitor, *Varanus niloticus niloticus*

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Abstract

Gross morphology of the Nile monitor's tongue revealed that it is bluish to black in colour with smooth texture. It is differentiated into an anterior free portion and other posterior laryngeal part. The free portion can be distinguished into fore- (apex), mid- (body) and hind-tongue (root). The fore-tongue is much long and deeply bifurcated. By light and SEM examination it was found that, the non-keratinized dorsal and ventral tongue's surface devoid any of lingual papillae, taste buds and salivary glands but its epithelial is encountered with glandular epithelial (goblet) cells. The lamina propria, underneath the lingual mucosa, is of loose connective tissue described by the presence of massive striated longitudinal and circular muscle bundles responsible for the free movement and protrusion of the tongue. This affirmed that the Nile monitor's tongue is adapted to chemoreception and scrabbling about its food or victims by smell sense, in cooperation with the remarkable vomeronasal organ, rather than transportation of food into the mouth and through oropharyngeal cavity.

Keywords: *Varanus niloticus*, Reptilia, Tongue, lingual papillae, SEM, gross morphology.

Introduction

The Nile monitor, *Varanus niloticus niloticus* (Linnaeus, 1766), is belonged to the largest and widely distributed African varanid lizard; *Varanus* (Polydaedalus) *niloticus* species group (Böhme and Ziegler, 1997). This varanus species group is rightly placed in subfamily Varaninae of family Varanidae (Zug et al., 2001). Based on the morphology and habitat the last authors categorized this *Varanus* (Polydaedalus) *niloticus* species group into two distinct subspecies; the Nile monitor, *Varanus niloticus niloticus* and ornate monitor, *Varanus niloticus ornatus*. The first one, of the present study, inhabits a broad diversity of vegetation zones across Africa, from tropical forests to semi-arid areas, northward along the Nile Valley throughout the central and southern Africa. It become native in Egypt,

especially in southern and central provinces (Bayless, 1997). So, it is characterized by a prolonged aestivation phase during the dry season (Angelici and Luiselli, 1999). The Nile monitor is not found in the entire desert regions but it is flourished around the river and rivulets (Spawls et al., 2002). It is semi-aquatic adaptable lizard, since it can be found in almost any habitat where permanent water bodies exist (Lenz, 2004). This includes grassland, scrub, forests, mangroves, swamps, lakes, rivers and rivulets (Pianka and King, 2004). The same authors showed also that, it is an excellent swimmer and can apparently spend up to one hour submerged in water, since its nostrils are placed high on snouts (Dowell et al., 2016). It is also a tremendous climber and quick runner on land and relatively unaffected by climate change (Dowell and Hekkala, 2016).

The Nile monitor is not only the Africa's largest lizard but also one of the continent's most voracious predators (Capula, 1990). This hard-hitting reptile have powerful robust body with elongated neck, elongated snake-like head, sharp claws and a muscular very long compressed tail lacking caudal autonomy (Zug et al., 2001). However, it is more helpless on the land and if threatened it boldly defends itself or mostly avoids hurt and escapes to the safety of deep water (Pianka and King, 2004). The Nile monitor is an active diurnal predator catching live preys with the aid of its strong jaws and sharp, conically curved teeth (Lenz, 2004). It feeds on anything it can override or find like as insects, snails, fish, frogs, crocodile eggs as well as young snakes, birds and mammals, and is also scavenger feeds on carrion (Pianka and King, 2004).

The tongue is often considered a key of advances in the evolution of terrestrial existence of vertebrates as it allows animals to transport food through the oral cavity (Herrel et al., 2005). It has been also designated for prey capture and swallowing, drinking, breathing and even defensive and conducting social behaviours (Bels et al., 1994; Darwish, 2012). The later author clarified that, there are fairly strong correlations between tongue anatomy and it's up mentioned functional role. Among vertebrates, the reptilian tongues are characterized by vast morphological and functional variations. Apparently, these variations indifferent reptiles may depended on dissimilarities in food items, feeding habits and handling of food in the mouth (Lemell et al., 2000). So, the tongue's morphology, gross anatomy and fine architecture are more interesting especially in the amazing lizard species as their tongues are specialized for other functions in different clades (Herrel et al., 2005). In most lizards, tongue is used for prey prehension, transport and swallowing in addition to defensive display and/or chemical sampling from the external environment by means of tongue flicks (Cooper, 2003; Herrel et al., 2005).

The morphological and histological characteristics of the tongue have been studied, by using light and/or electron microscopy, in many squamate species by many authors such as; Wassif (2002), Herrel et al. (2005), Jamniczky et al. (2009), El-Sayyad et al. (2011), Darwish (2012), Taha (2013), Al-Zahaby et al. (2017) and others. Although many studies had carried on the varanids concerning their biology (Green and King, 1993), ecology and evolution (Pianka, 1995), yet the works on the anatomy and ultra structure of its lingual apparatus are scarce. Accordingly, the aim of

the present investigations have hypothesized the tight correlation of gross anatomy and fine architecture of the Nile monitor's tongue, with its feeding habit and habitation.

Materials and Methods

Three adult Nile monitor, *Varanus niloticus niloticus*, of about 80 cm were collected from Abou-Rawash area, Giza, at South-west of Cairo. The animals were transferred alive to the laboratory in Zoology Department, Faculty of Science, Zagazig University, Egypt, where they left for 24 hours prior to the different processes of investigations. Animals were sacrificed with sharp razor blades approved by the Animal Ethics Committee at Faculty of Science, Zagazig University according to the Laboratory Animal Welfare guidelines. The heads of sacrificed animals were detached to remove out tongues from their root after keeping upper and lower jaws away from each other. But, before slicing out the tongues, the oropharyngeal roof and floor as well as tongue were examined and photographed by means of Samsung 12 mega pixel digital camera, in their normal position to be studied in term of their shape and gross morphology and measured under stereomicroscopic image analysis (SMZ 1500 digital camera, Nikon, Japan).

For light microscopic investigations, small pieces from the apex, body and root of the tongue were immediately fixed in 10% buffered paraformaldehyde (Merck, pH: 7.3) at room temperature for 48 hours then dehydrated in ascending series of alcohols, cleared in xylen and processed for paraffin blocks. Histological longitudinal and transverse paraffin sections of 5 μ m thickness of the three parts of the tongue were done and stained routinely with Haematoxylin and Eosin stain (HE) according to Drury and Walington (1980) after then examined and photographed.

For SEM studies, the fixed tongues in 0.1 M phosphate buffer at pH 7.3), were washed twice in the same phosphate buffer to remove the excess of fixative. Post-fixation take place in 1% osmium tetroxide in phosphate buffer (PH 7.8) for two hours at 4°C, then rinsed twice in the same phosphate buffer for about 4 hours. Specimens were then infiltrated with isoamyl acetate for two days, and dried by the critical point drier using liquid carbon dioxide in Hitachi Critical Point Drier (HCPD). The dried specimens were then mounted on aluminum stubs, sputtered with gold in Joel fine coat Ion Sputter (SPI-Module).

Specimens were examined and photographed under JEOL SEM (JSM-5300 LV) at an accelerating voltage of 15kv, in the Regional Centre of Mycology, Al-Azhar Univ., Egypt.

Results

Gross Morphology.— The upper jaw of the Nile monitor is slightly longer than the lower one, the premaxilla and maxilla of the upper jaw as well as the outer margin of lower jaw bear one rows of pointed sharp cone-shaped teeth. The roof of the oropharyngeal cavity of the studied varanus showed two wide orifices (Jacobson's apertures) bounded by the maxilla laterally and the vomer medially. These vomeronasal apertures are somewhat anterior to the internal nares (choanae), which opens at the posterior end of the choanal groove bounded by the palatine bone. Otherwise, on the bottom of the oropharyngeal cavity, there is a median groove directly in front of the tongue's base to be settle in the resting phase. A distinctive median longitudinal glottis, opens into

the larynx and is places on the top of the laryngeal mound at the most back border of the tongue (Fig. 1).

The tongue of *Varanus niloticus* of the present studies is bifurcated with about 5-8 cm total length and 3-5 ml maximum width. It is socketed in a tongue's sheath, found at tongue's base and extends back- and forward .The tongue of this varanus is bluish to black in colour with somewhat smooth texture. It is differentiated into an anterior free portion and posterior laryngeal part. The free portion can be divided into fore-tongue (apex), mid-tongue (body) and hind-tongue (root).The apex is long and deeply bifurcated, this anterior bifurcations presents two cornu of thick proximal portion which are tapered towards the tip. These cornu protrudes out of the mouth during tongue flicking, but the posterior portion of the tongue remains almost entirely within the mouth even during protrusion. The dorsal surface of tongue's body showed a median deep depression (sulcus) which is an extension of the bifurcated tongue tip (Figs. 1&2)



Figure 1. A photograph of buccal cavity (roof and floor) of Nile monitor, showing the long bifurcated tongue (T) with its groove (G) in the lower jaw , tongue sheath (SH), upper and lower jaws with their related entity, Jacobson's aperture (JA), internal naris (IN), choanal groove (CG), cone-shaped teeth (Te), mid-dorsal sulcus (DS), premaxilla (Pm), maxilla (M), palatine (Pa), vomer (V), laryngeal mound (LM), and elongated laryngeal cleft (LC).

Figure 2. Photograph of the Nile monitor's tongue (dorsal surface), showing the free portion differentiated into; bifurcated tip(BT), lingual apex (A), cornu (Cu) mid tongue, body (B) and hind tongue, root (R), mid-dorsal sulcus (DS) and tongue sheath (SH).

Scanning electron microscopic observations.— Morphologically, the tongue's surface is smooth and missing any visible projection, but by SEM examination, slight roughness on the dorsal surface with distinct hexagonal or polygonal serrations are appeared (Fig. 3). At higher magnification, these

hexagonal or polygonal serrations appeared cells having thickened margin with fine filaments outgrowths giving the cells feathery borders, so are called plumose cells. These cells are covered also by fine protrusions, microfacets (Figs.4 & 5).

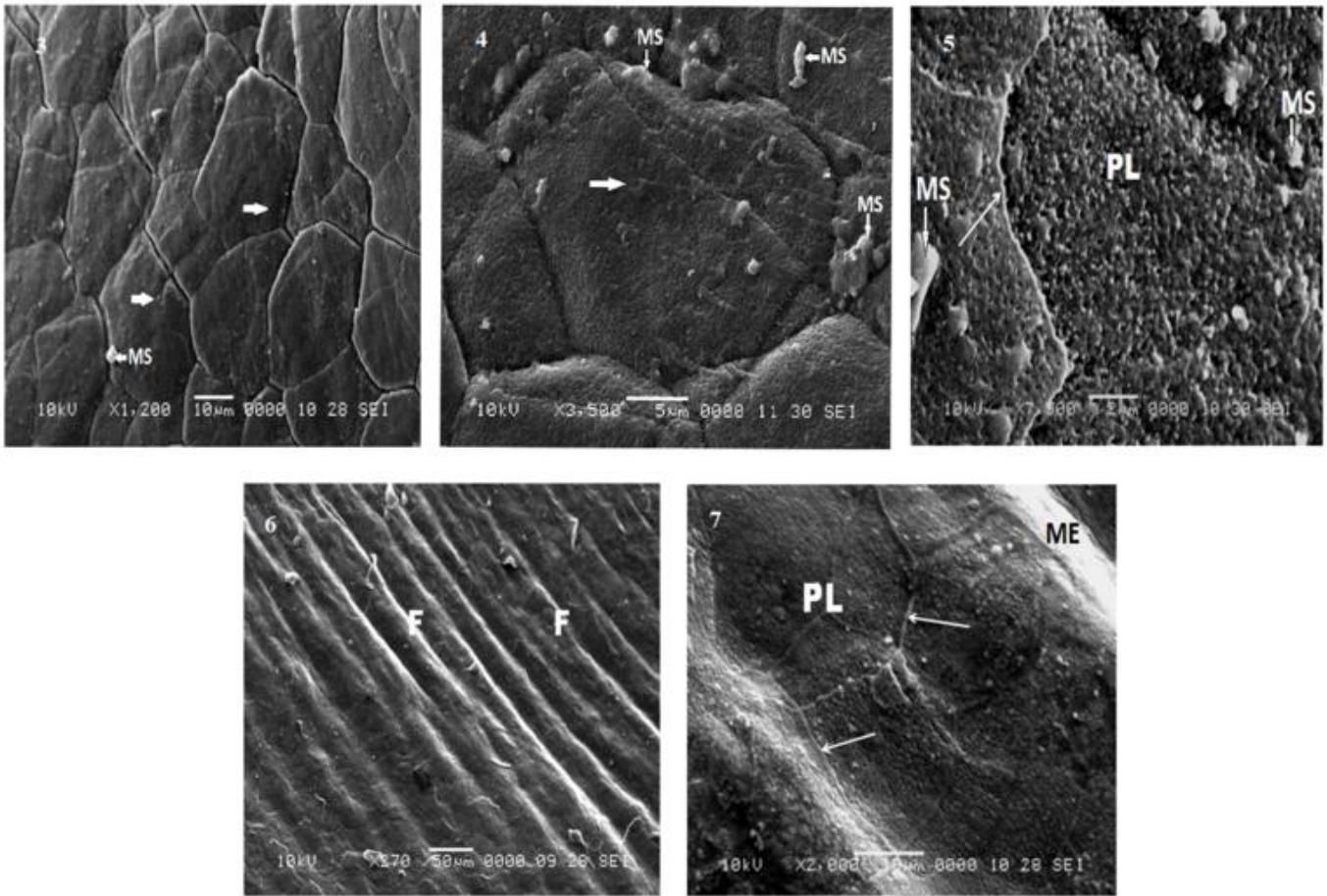


Figure 3. SEM Photomicrograph of the lingual apex of *V. niloticus niloticus*, showing, hexagonal plumose cells (arrows) on the lingual apex. Scale bar = 10 μ m.

Figure 4. Higher magnified micrograph of figure(3) showing, hexagonal plumose cells (arrow) with fine protrusions (microfacets) and mucous granules (MS). Scale bar = 5 μ m.

Figure 5. More magnified micrograph of figure (3) showing, plumose cells (PL) with its fine protrusions (microfacets), regularly spaced pores and serrated thickened borders (arrow) and covering copious of mucous secretions (MS). Scale bar = 2 μ m.

Figure 6. SEM Photomicrograph of the dorsal surface of the posterior lingual apex of *Varanus niloticus* showing many successive meanders or folds (F). Scale bar = 50 μ m.

Figure 7. Higher magnified micrograph of figure (6) showing, plumose cells(PL)with its thickening borders (arrow) in between the successive meanders. Scale bar = 15 μ m.

Many meanders or folds are recognized at the dorsal surface of the posterior extremity of the fore-tongue (Fig.6) facilitating retraction and flocking of tongue. At higher magnification, plumose cells are clearly noticed with their thickening borders (Fig.7). These cells are noticed in-between the successive meanders or folds and sometimes they appear as rows of cells separated by their serrated thickening margin (Fig. 8).

From the SEM examination it was found also that, the mid-tongue's surface of the studied lizard showed

outstanding irregular fine protrusions, microfacets (Figs. 9 & 10). However, dorsal surface of the hind-tongue is described wavy folds like as those of the fore-tongue's surface (Fig. 11) where plumose cells with their fine protrusions, microfacets (FP) and copious of mucous secretions (ME) are distributed in between these successive folds (Fig. 12). It is exceedingly important to point out that, the whole tongue of *Varanus niloticus* lacking lingual papillae and/or taste buds.

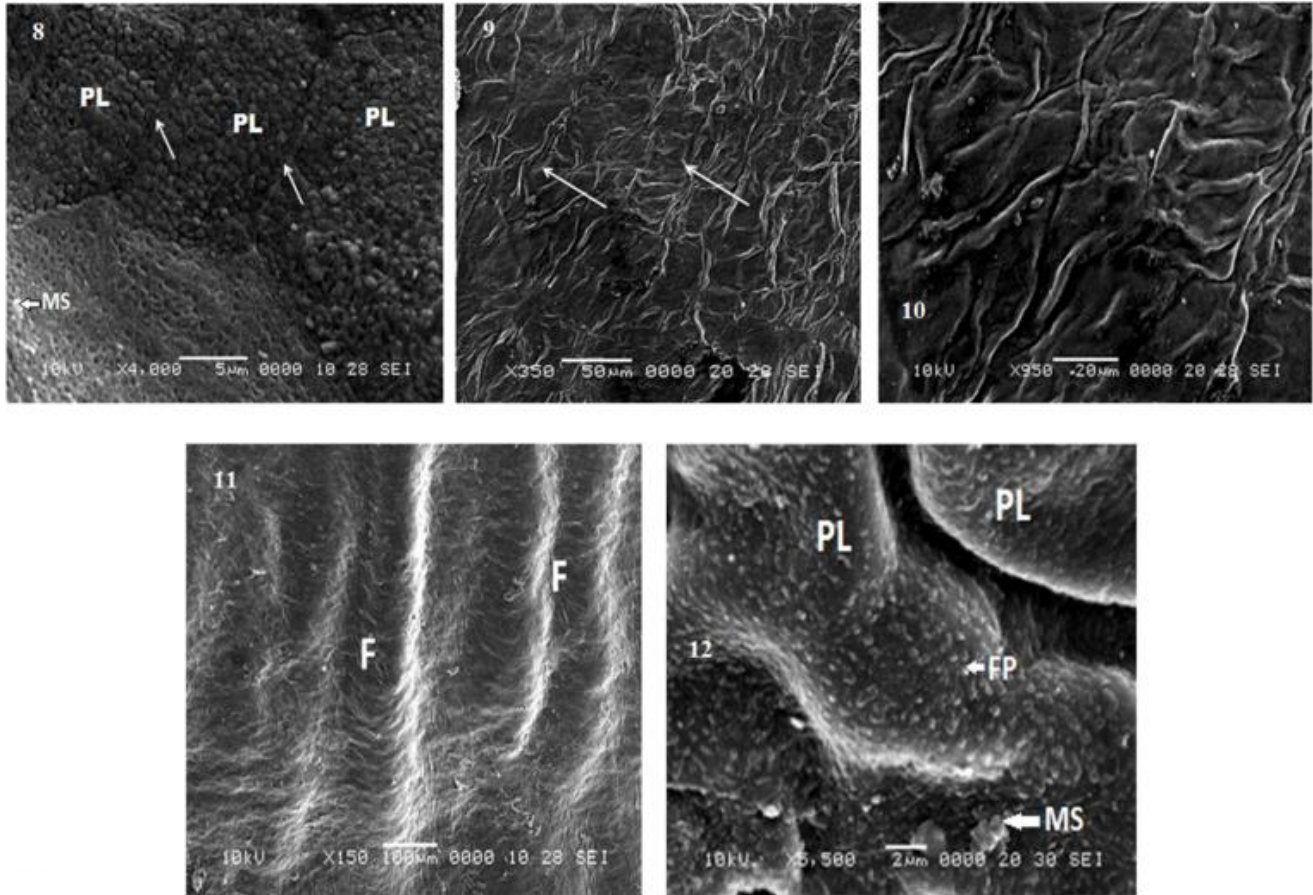


Figure 8. More magnified Photomicrograph of the dorsal surface of the posterior lingual tongue's apex of Nile monitor showing, row of plumose cells (PL) separated by their serrated thickened cell margins, (arrow) Regularly spaced pores, fine protrusions (microfacets) (FP) and copious of mucous secretions (MS) are also seen. Scale bar = 5 μ m.

Figure 9. SEM Photomicrograph of the dorsal surface of the mid-tongue (body) of Nile monitor showing irregular folds or projections (arrow). Scale bar = 50 μ m

Figure 10. Higher magnified micrograph of figure (9) showing irregular folds or projections in the dorsal surface of the mid-tongue (Arrow). Scale bar = 20 μ m

Figure 11. SEM Photomicrograph of the dorsal surface of the hind-tongue of *Varanus niloticus* showing many successive wavy folds (F). Scale bar = 100 μ m

Figure 12. Higher magnified micrograph of figure (11) showing, plumose cells (PL) with their fine protrusions (microfacets) (FP), regularly spaced pores and mucous secretions (MS) distributed in-between the successive wavy folds. Scale bar = 2 μ m

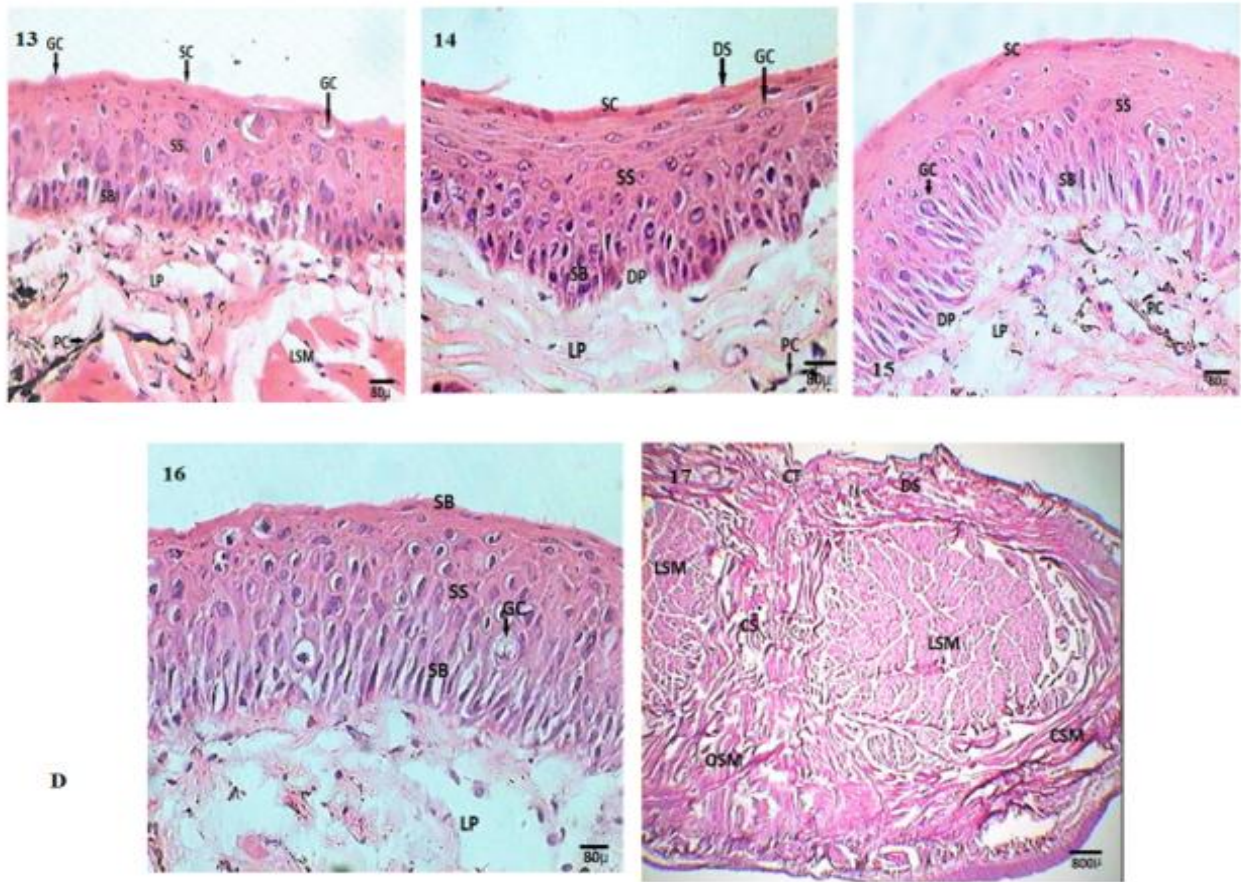


Figure 13. Photomicrograph of VS (vertical section) in the dorsal epithelium of fore-tongue of *V. niloticus* showing; non-keratinized squamous epithelium (arrow) establishing of stratum basale (SB), stratum corneum (SC) and stratum spinosum (SS) encountered with glandular epithelium cells (GC). Underneath loose connective tissue of lamina propria (LP) containing plentiful pigment cell (PC), striated muscle bundles (LMS) and dermal papilla (DP). (H&E), Scale bar = 80 μ m.

Figure 14. Photomicrograph of VS in the dorsal epithelium of mid-tongue of *V. niloticus* showing; mid-dorsal sulcus (DS), non-keratinized squamous epithelium (arrow), stratum basale (SB), stratum corneum (SC), stratum spinosum (SS), glandular epithelium cells (GC), lamina propria (LP), dermal papilla (DP) and pigment cell (PC). (H&E), Scale bar = 80 μ m.

Figure 15. Photomicrograph of VS in the dorsal epithelium of mid-tongue of *V. niloticus* showing; somewhat thicker non-keratinized squamous epithelium (arrow), stratum basale (SB), stratum corneum (SC) and stratum spinosum (SS), glandular epithelium cells(GC), lamina propria (LP),dermal papilla (DP) and pigment cell (PC),. (H&E), Scale bar = 80 μ m.

Figure 16. Photomicrograph of VS in the dorsal epithelium of hind-tongue of *V. niloticus* showing; somewhat thickest non-keratinized squamous epithelium (arrow), stratum basale (SB), stratum corneum (SC) and stratum spinosum (SS), glandular epithelium cells(GC), lamina propria (LP), dermal papilla (DP) and pigment cell (PC), (H&E), Scale bar = 80 μ m.

Figure 17. Photomicrograph of a transverse section in the mid-tongue segment of the studied varanus showing; longitudinal (LSM), circular (CSM) and Oblique (OSM) striated muscle bundles. Connective tissue septum (CS), connective tissue insertion (CT) and mid-dorsal sulcus (DS). (H&E), Scale bar = 800 μ m.

Histological Observations.— The present observations demonstrated that, the dorsal lingual epithelium and even the whole tongue's histological architecture of *Varanus niloticus* have the same pattern with some little variation in the organizing segments (fore-, mid- and hind-tongue) of the tongue's free portion. They missing any of the lingual papillae and manifests non-keratinized stratified squamous epithelium which gives the tongue smooth texture. Its stratum basale was composed of a single layer of cuboidal to short columnar cells, with vertically oriented nuclei, resting on a basement membrane. Whereas, the stratum spinosum is composed of a variable layers of polygonal cells in the different tongue's segments. Its cells, adjacent to the stratum basale, are cuboidal in shape, while the more superficial cell and are almost flattened with elliptical centrally located nuclei. This later surface cell layer of the tongue is non-keratinized and packed with frequently scattered glandular epithelial cells (goblet cells) giving heavy mucus coat. The three segments of the tongue's free portions are differed only in the thickening of the squamous epithelium. It is thicker in the hind-tongue than that of the mid-tongue and in turn that of the fore-tongue is the thinnest (Figs. 13, 15&17).

Underneath the tongue epithelium lies a layer of loose connective tissue, lamina propria, together with the epithelium and basement membrane constitute the tongue's mucosa. It provides nutrition to the above epithelium through the crowded blood capillaries infiltrated in-between the connective tissue. It holds also nerve endings and extensively scattered pigment cells (melanocytes) forming continuous row underneath the basement membrane especially in the tip and fore-tongue giving them dark-bluish colour (Figs. 13,15 & 16). The existing irregularities of lamina propria, dermal papillae of the same cellular organization, are to increase the area of contact of the lamina propria and tongue epithelium (Fig.13, 14 & 15). It has been noticed also that, in the studied species, as mention above, no lingual papillae, salivary gland and taste buds in the all tongue's segment.

Light microscopic examination also revealed two intrinsic longitudinal muscle bundles run and extend parallel to the tongue length in both sides of the forked apex and tongue's body. These longitudinal muscle bundles are joined and surrounded by a series of circular in addition to short oblique muscle fibers, run laterally in the perimeter of the tongue. The encircling muscles of the two sides are separated by a midline

connective tissue septum with few fibers cross this septum to inter digitate with those of the opposite side (Fig. 17).

Discussion

The tongues of reptiles are variable in their gross anatomy and microscopic structure, as well as their functions (Wassif, 2002). In the present *Varanus niloticus* as all lacertilian species tongue is differentiated into an anterior free portion and posterior laryngeal part. The free portion is divided into; fore-tongue, mid-tongue and hind-tongue. The apex of the anterior free portion is long and deeply bifurcated into two thick and terminal tapered cornu. The monitor's tongue is enveloped in a sheath extends forward and back as also illustrated by Satish et al. (2015) in Bengal monitor lizard.

The forked tongue is a phenomenon always associated with the searching mode of foraging (Schwenk, 1994). Generally, all monitor lizard has a long forked tongue, which flicks in and out the mouth (King and Green, 1999). El-Sayyad et al. (2011) illustrated that, the tongue's body and cornu of snakes protrude out of the mouth during tongue flicking, but the tongue's root remains exclusively within the mouth even during protrusion. This forked tongue may provide more surface available for chemoreception sensory function (Darwish, 2012; Al-Fartwsy et al., 2016). However, *Trachylepis vittata* lizard uses its tongue effectively for both chemosensation and prey transport (Taha, 2013).The bifurcation of tongue's apex as well as the dorsal tongue's sulcus which extends as extension of apex bifurcation were also apparent in different squamate lizards of families; Lacertidae, *Takydromus takydromoides* (Iwasaki & Miyata, 1985); Anguidae, *Anguis fragilis*, (Toubeau et al., 1994); Scincidae, *Chalcides ocellatus* (Wassif, 2002) and *Chalcides sepsoides* (Sarhan and Hussein, 2013) and Gekkonidae, *Gecko japonicus* (Iwasaki, 1990), *Tarentola annularis* (El-Bakry and Hamdi, 2016), *Stenodactylus petrii* (Darwish, 2012) and *Hemidactylus flaviviridis* (AL-Fartwsy et al., 2016) as well as Agamidae, *Uromastix aegyptia* of (Al-Zahaby et al., 2017). However, this anterior tongue bifurcation is not discerned in Black rat snake, Colubridae (Morgans and Heidt, 1978); legless blind lizard, *Anelytropsis dibamus*, Dibamidae (Greer, 1985) and *Ptyodactylus guttatus*, Phyllodactylidae (Darwish, 2012).

The tongue's dorsum epithelium of the Nile monitor displayed relatively trivial folds that increase in number and complexity towards the lateral margin of the tongue. This enable tongue's varanus to sweep up- and downwards as well as wrapping and twisting laterally around the sides of the mouth during tongue flicking (Kier and Smith, 1985). The folded surface nature of the tongue is also confirmed in different Crocodylian species (King & Burke, 1997 and El-Bakry & Hamdi, 2016), living also in the vicinity of water bodies as Nile monitor. Otherwise, the lingual epithelium of the studied varanus is of smooth texture lacking any of lingual papillae, taste buds and keratinized structures. It is composed of non-keratinized stratified epithelia, its single cell layer stratum basale resting on a basement membrane, however the stratum spinosum is of superficial flattened cell teeming with glandular epithelial cells (goblet cells), assuming its breathing function as reported also by Bels et al. (1994) and Schwenk (1994 & 1995) in other lizard and snakes having forked tongue. The keratinization which is the most interesting histological feature of the reptilian tongue surface and reflects their adaptation to the dry habitat (Iwasaki and Kumakura, 1994) not established in varanus of the present study and some other squamates like as snakes, *Psammophis sibilans* (El-Sayyad et al., 2011) and lacertid lizards, *Lacerta parva* and *Lacerta trilineata* (Herrel et al., 2005) and house gecko, *Hemidactylus flaviviridis* (AL-Fartwsy et al., 2016).

On the same way, Schwenk (1985) early had reported that taste buds are absent on the surface of the tongue of Varanidae species. Taste buds are also lacked in gekkonoid species, *Eublepharis macularius* (Jamniczky et al., 2009) and *Ptyodactylus guttatus* and *Stenodactylus petrii* (Darwish, 2012). But, considering the presence of taste buds on the animal surface tongue play an essential role in receiving chemical information of food (Nasr et al., 2012). So, the present Nile monitor used its tongue as a chemoreceptor through vomeronasal organ (smell sense) and not with taste bud (taste intelligence).

It is assumed that, during searching action, the varanus tongue protrudes, oscillates and flicks while collecting airborne chemical particles. These particles stick by the microfacets and minute pores frequently spread out on the plumose cells surface covering the tongue's apex. They are transferred and coming in the well-developed vomeronasal (Jacobson's) organ as also mentioned early in black rat snake by Morgans and

Heidt (1978). A good affirmation of this is given also by Smith (1986) who assumed that, the highly protrusible tongue is a varanids feature, since its elongation, bifurcation and narrowing nature are most likely related to chemosensation in arid environment. Similarly, Graves and Halpern (1990) demonstrated that, the flicking of lizard's tongue is attendant to external stimuli of the prey and/or predators existence. It involves also behavioral and seasonal aspects accompanied with not only of food recognition but also of sex identification and mating (Cooper, 1998). In the same way, Lambert (2005) and Pianka and Vitt (2006) also reported that, the Varanid lizards uses its long, forked and highly flexible tongue only for chemoreception and not for gathering food and water as other lizards.

Besides, the glandular cells (goblet cells) frequently scattered in the monitor's tongue epithelium, are also densely-packed in clusters particularly in the mid- and hind-tongue, producing a thick mucus coat on the tongue surface in the gekkonoid lizards, *Eublepharis macularius* (Jamniczky et al., 2009) *Tarentola annularis* (El-Sayyad et al., 2011), *Ptyodactylus guttatus* and *Stenodactylus petrii* (Darwish, 2012) as well as inbridled skink, *Trachylepis vittata* (Taha, 2013). The last author affirmed that these glandular cells are the source of dense mucous secretion coat on the tongue.

The tongue's lamina propria (corium) of the present varanus, presents loose fibrous connective tissue embracing as usual, blood capillaries and nerve endings in addition to the extensively scattered pigment cells (melanocytes) and massive bilateral voluntary longitudinal and circular as well as oblique striated muscle bundles. These striated muscle bundles of different arrangement was also recognize the tongue's lamina propria of *Psammophis sibilans* (snake) and *Tarentola annularis* (geckos) (El-Sayyad et al., 2011), *Trachylepis vittata* lizard (Taha, 2013) and *Hemidactylus flaviviridis* gecko (AL-Fartwsy et al., 2016). The simultaneous activity of these muscle bundles get done the tongue's free movement. Otherwise, the dark colour of tongue's free portion is due to the heavily condensed pigment cells established in tongue's lamina propria of Nile monitor as also demonstrated in *Trachylepis vittata* lizard (Taha, 2013).

On the other hand, it is known by Smith (1986) as well as Schwenk and Throckmorton (1989) that, the varanus are of inertial feeding, they use the jaws alone

for prey prehension, and tongue does not take any part in the food and water transport. This is different from that seen in any of the fleshy-tongued lizards, where the tongue is specialized for prey capture as in Iguanian lizards (Herrel et al., 1995), prey transport and swallowing as in *Agama stellio* (Herrel et al., 1997) and/or it has been also designated for drinking water as in desert lizards and Geckos (Sherbrooke et al., 2007 and Jamniczky et al., 2009, respectively).

The present investigations an attempt to elucidate how the feeding habit and habitat of Nile monitor mirrored the tongue's morphology and fine structure. It is dominantly inhabit the semi-arid habitat uses its jaws alone for small prey prehension, tongue does not take any part in food transport (inertial feeding). Thus, the protruding, freely movement, long and deeply bifurcated monitor's tongue showing non-keratinized smooth texture epithelium. It is lacking lingual papillae, taste buds and salivary glands but is encountered with mucous (goblet) cells. So, the present study hypothesize that Nile monitor's tongue performs chemoreception and breathing functions as well.

Acknowledgments

We obtained the approval of Ethical Committee of Faculty of Science – Zagazig University in dealing with the studied animal.

-The authors would like to express their sincere thanks to Prof. Dr. Abdel-Badie E. El-Attar, Professor of Comparative Anatomy, Department of Zool., Faculty of Science, Zagazig University for his help in preparing the material and for the linguistic revision of the manuscript.

Compliance with Ethical Standards

Disclosure of potential conflicts of interest:

None

The animals were cared for in accordance with guidelines such as the Guide to the Care and Use of Experimental Animals and that the use of animals was reviewed and approved by the animal care review committee at Zagazig University where the experiments were carried out.

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How to cite this article:

AL-Zahaby A. Sheren, Nasr E.S., Hassan S.S. (2018). Light and scanning electron microscopic observations on the tongue of Nile monitor, *Varanus niloticus niloticus*. *Int. J. Adv. Res. Biol. Sci.* 5(4): 1-11.
DOI: <http://dx.doi.org/10.22192/ijarbs.2018.05.04.001>