

Ultrastructural Studies on the Tongue of Some Egyptian Lizards 1-Scincine Lizards *Chalcides Ocellatus* and *Chalcides Sepsoides* (Lacertilia, Scincidae)

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Abstract

Tongues of Egyptian lizards *Chalcides ocellatus* and *Chalcides sepsoides* are investigated under scanning and transmission electron microscope. Their tongues are divided into tip, anterior, middle and posterior regions.

In *C. ocellatus*, the tip region is bifurcated and protected with strong, non-papillosed, keratinized epithelium. The lingual papillae are separating with wide trenches except in the middle region. Papillae of the anterior and posterior regions have serrated and imbricated ends oriented in the posterior direction. The surface epithelia is studded by tiny or reticular microvilli. Ultra-sections show that the imbricated portions have goblet cells rich in numerous secretory granules. The lamina propria of each papilla is provided with striated muscle fibers. The distribution and orientation of the lingual muscle fibers prove that the tongue is adapted for snipping, masticate and swallowing their prey.

In *C. sepsoides* tongue, the tip region is slightly bifurcated. All lingual papillae have different shapes with narrow moats separating between them. Those of the anterior and posterior regions have free ends overlapped towards the anterior or posterior directions, respectively, while that of the middle region is not imbricated. The surface epithelia of the tongue has dispersed or aggregated microvilli.

In the tongue of the *C. sepsoides*, new structures are described for the first time. At its ventral aspect, there is an accessory lingual muscle formed of numerous muscle fibers orient to the vertical, transverse or longitudinal directions. These orientations prove that the tongue is able to mash and suck the prey's sherbets. At the end of the posterior region, there is a ciliated concave depression hosting a laryngotracheal organ, which has T-shaped crevice preceded by large fleshy highly ciliated lip supported by inflexible folds.

Keywords: Tongue; Ultrastructure; Egyptian lizards; Scincidae

Nomenclatural: Am: Accessory lingual muscle; An: Anterior region of tongue; B: Basement membrane; Bi: Biforked tip of the tongue; Bv: Blood vessel(s); C: Cilia; Cj: Cell junction(s); Cl: Ciliated lingual layer; Co: Collagen fibres; Cy: Cytoplasm; De: Dorsal epithelium; Dp: Dorsal papilla(e); E: Endomysium; Ec: Epithelial cells; El: Epithelial layer; Em: Enlarged microvilli; Ep: Elongated papilla(e); F: Fibrocyte(s); Fi: Fibroblast; G: Golgi apparatus; Gc: Goblet cell(s); Ge: Glandular epithelium; If: Inflexible fold(s) of laryngotracheal organ; Im: Imbricated papilla(e); Jm: Jagged microvilli; L: Lamina propria; Le: Lingual epithelium of laryngotracheal depression; Lf: Lingual fold(s); Lg: Large papilla(e); Lo: Laryngotracheal organ; Lon: longitudinal; Lp: Lingual papilla(e); Lt: Laryngotracheal ciliated lip; Ltd: Laryngotracheal depression; Ma: Mast cell(s); Mf: Muscle fibers; Mo: Mosaic microvilli; Mr: Microridges; Mt: Mitochondria; Mu: Mucoid secretion; Mv: Microvilli; My: Myofibril(s); N: Nucleus; Nt: Narrow trench(s); P: Plasma cell(s); Po: Posterior region of the tongue; RER: Rough Endoplasmic Reticulum; S: Sarcoplasm; Sa: Sarcolemma; Se: Stratified squamous epithelium; Sf: Serration furrow(s); Sg: Secretory granules; Sm: Small papilla(e); So: Secretory orifice; Sp: Secretory pore; Spd: Secretory pouch(es); Sr: Serrated papilla(e); St: Striated muscle fibers; Su: Surface epithelium; Sv: Secretory vesicle; T: T-shaped orifice; Tb: Taste bud orifice; Tc: Tracheal canal; Ti: Tiny microvilli; Tm: Tongue muscles; Tr: Transverse; Vl: Ventral lingual epithelium; Vm: Virtuous microvilli; V: Vertical; Wt: Wide trench(s)

Introduction

The Eyed Skink, *C. ocellatus*, is a species that can be found in a wide variety of environments closer to vegetation, such as farmland and gravel deserts around the Mediterranean coast including Greece, southern Italy, Malta, Egypt and parts of northern Africa [1]. Adults have long cylindrical bodies (6-12 inches) with small head, and short

limbs that are adapted for fossorial movement [2]. Their coloration patterns are variable with noticeable ocelli.

The Wedge-snouted Skink, *C. sepsoides* is found in Jordan, Egypt, Libya and the Palestinian territories. In Egypt it is a sand fossorial species, found in a wide variety of habitats ranging from sandy depressions of the Western Desert, to sandy spots in rocky valleys of the Eastern Desert and Sinai [3]. It swims suddenly beneath the sand surface, forming a characteristic winding track. It feeds entirely on fossorial insects and other invertebrates such as pseudoscorpions and appears to be nocturnal. They have narrow cylindrical snake-like bodies with short tail and their limbs are thin and very short. The dorsal coloration plane is sandy, often with dark faint stripes.

Numerous investigators concluded that tongue of most tetrapods are provided with voluntary and movable muscles [4-11]. Reptilian tongues are characterized by morphological and functional variations among species [12-14]. In lizards it plays important roles during olfaction, gustatory and feeding, where they can pick up and transfer

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chemicals to the vomeronasal organ [15], test palatability and capture of praise, mastication process, secretion of mucus as lubricant and pushing food to the pharynx [14,16,17]. In some lizards, differences are reported among regions of the tongue in terms of dorsum epithelium, keratinisation, lingual papillae and the presence of taste buds [18]. Ultrastructural studies on the tongue of lizards showed marked variance in the dorsal topography and cytoarchitecture of the tongue tissues [19-23].

This work is the first research in a series studying the ultrastructure of tongues in the Egyptian lizards. It examines two species of scincine family and both were belong to genus *Chalcides*. The tongue of these two species has been insufficiently investigated or discussed in the literature, especially the skink *C. sepsoides*, which has not been described before. The objective of this study is to investigate the ultrastructure of the dorsum epithelium of *C. ocellatus* and *C. sepsoides* tongues, understanding the relations between its structure and function, examining those data in light of the current literatures, and comparing them with those found in other animals.

Material and Methods

Experimental animals

Thirty adults of two species of scincine lizards *C. ocellatus* and *C. sepsoides* (Family Scincidae) were purchased from Abo-Rawash region 30 km North-West of Cairo.

Experimental design

Tongues of *C. ocellatus* and *C. sepsoides* were analyzed under scanning and transmission electron microscope. Twenty healthy animals of both species were randomly selected, sacrificed and their tongues were removed, then immediately fixed in 5% cold buffered glutaraldehyde. After 24 hours, tongues are distributed into 4 separate groups; the first two groups include 10 complete tongues of both species *C. ocellatus* and *C. sepsoides* were processed for Scanning Electron Microscopy (SEM). Tongues of group 3 and 4 include 5 tongues from each two species that will be processed for Transmission Electron Microscopy (TEM). These tongues were cut into 4 slices include the tip, anterior, middle and posterior regions. All specimens were left in fresh 5% cold buffered glutaraldehyde for a further 24 hours to continue fixation. The specimens were washed 3 times in cacodylate buffer at pH 7.2 for 30 minutes.

For SEM, five tongues from each two species were postfixed in 1% osmium tetroxide solution for two hours at -4°C. The specimens were washed 3 times in cacodylate buffer, dehydrated and immersed in amyl-acetate for 48 hrs [24]. Samples were dried in critical point dryer by using carbon dioxide, stuck on metal holders and coated with gold by using the gold sputter coating apparatus in a thickness of 15 nm then examined in scanning electron microscope JSM-5400 LV, at 15Kv.

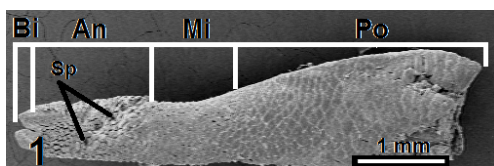


Figure 1: Electron micrograph shows the tongue of *C. ocellatus*. Note that the tip is biforked (Bi), the anterior region (An) contains numerous secretory pores (Sp), the middle region (Mi) has compacted papillae, the posterior region (Po) is formed of large elongated papillae. $\times 15$.

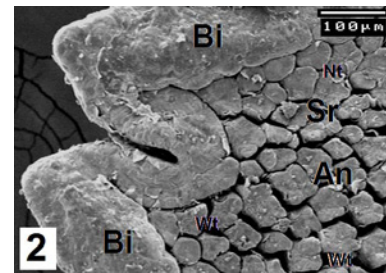


Figure 2: Electron micrograph shows biforked tip (Bi) of *C. ocellatus* tongue. Note that it is formed of strong, thick, non-papillose and keratinized lingual epithelium. The anterior region (An) is characterized by specialized multiangular lingual papillae with wide (Wt) or narrow trenches (Nt) between them. Note that some papillae are serrated (Sr) at their posterior ends. $\times 200$.

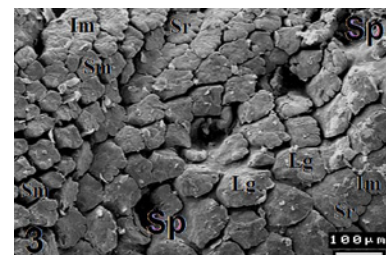


Figure 3: Magnified micrograph from figure 1 shows large secretory pores (Sp) lingual papillae of the anterior region of the tongue, which contains small (Sm) and large papillae (Lg). Note that these papillae are serrated (Sr) and imbricated (Im) toward the posterior direction. $\times 150$.

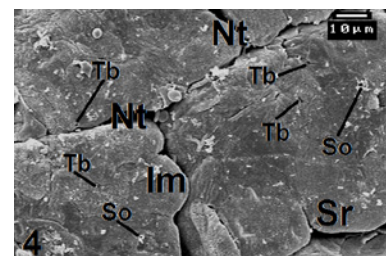


Figure 4: Electron micrograph shows large lingual papillae of the anterior region in *C. ocellatus* tongue. Some papillae are imbricated (Im) and serrated (Sr) at their posterior ends. Note the narrow trenches (Nt) between them. So: secretory orifice; Tb: Taste bud orifice. $\times 1000$.

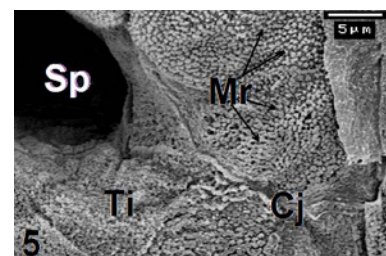


Figure 5: Electron micrograph of the anterior region of *C. ocellatus* tongue shows large secretory pore (Sp) and the cell junctions (Cj) between the epithelial cells that are studded by tiny microvilli (Ti). Note that these microvilli have spherical apices interconnected by microridges (Mr). $\times 3500$. (See Figure 7).

For TEM, the slices were cut into small blocks, 0.5-1 mm and rinsed with fresh 0.1 M sodium cacodylate buffer at pH 7.2 to remove cell depress. Postfixation was done in 1% osmium tetroxide solution for

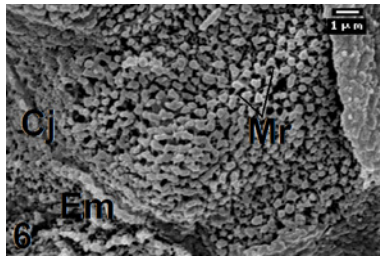


Figure 6: Magnified micrograph from Figure 5 shows tiny microvilli, with enlarged apices (Em). Some are fused together and interconnected by microridges (Mr). Cj: Cell junction. ×7500.

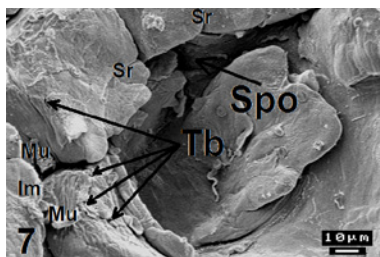


Figure 7: Magnified micrographs from Figures 3 and 5 show that the secretory pores are lined with cork-screw shaped channel system. Figure 7 shows that the secretory pore is surrounded by serrated papillae and some of them are imbricated (Im) and have taste bud orifices (Tb). Note the presence of an orifice of a secretory pouch (Spo) inside the channel system and the mucoid secretion (Mu) that emitted from deep trenches between the lingual papillae. ×750.

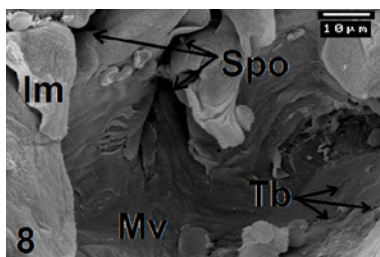


Figure 8: Magnified micrographs from Figures 3 and 5 show that the secretory pores are lined with cork-screw shaped channel system. Figure 8 shows small imbricated papilla (Im) and the epithelial cells that line channel system have very smooth microvilli (Mv). Note also the presence of secretory pouches (Spo), which can be seen along the channel system. ×1500.

two hours at -4°C. After dehydration, the specimens were embedded in Epon 812 [24]. Semithin sections (0.5-1μm thick) were done by using LKB ultramicrotome. Ultrathin (700-800 Å thick) sections were made, stained with uranyl-acetate and lead citrate then examined by JEM 100 CXII at 80 kv. Successive electron photographs were captured by CCD Camera Model XR-41. All samples were examined at EM unit, Faculty of Veterinary, Assiut University.

Results

By using SEM, at low magnification, the tongue of *C. ocellatus* appeared elongated with cone or triangular shape and biforked free tip. Dorsally, it is divided into four different parts: the tip, anterior, middle and posterior regions (Figure 1). The biforked tip of the tongue is formed of strong, non-papillose and keratinized lingual epithelium (Figure 2).

The lingual papillae of the anterior aspect have flattened gravel shape with multiangular edges. These papillae have small and large size and separated by deep trenches (Figure 2). The smaller type was, not serrated, and mostly situated near the tip region. The larger type was serrated, and occupied the rest of the anterior lingual region.

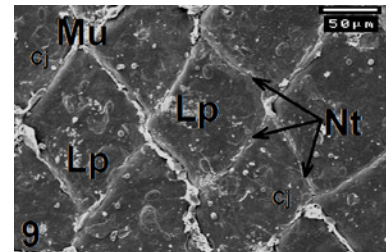


Figure 9: Electron micrograph shows lingual papillae (Lp) in the middle region of *C. ocellatus* tongue. Note their rhombus or quadric shapes, the Mucoid secretion (Mu) that emitted from the narrow trenches (Nt) between them. Note the cell junctions between the epithelial cells. ×350.

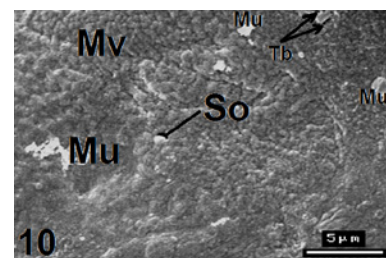


Figure 10: Magnified electron micrograph from Figure 9 shows tiny microvilli (Mv). Note the presence of few secretory orifice (So) of a goblet cell and taste bud orifices (Tb). Mu: mucoid secretory droplets. ×5000.

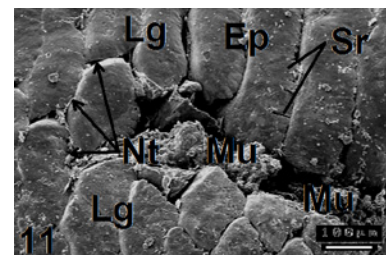


Figure 11: Electron micrograph shows large elongated (EP), rectangular papillae of the posterior part of *C. ocellatus* tongue. Most of these papillae are serrated (Sr) at their posterior ends and separated by narrow trenches (Nt). Note the mucoid secretion that emitted from a large deep trench. ×150.

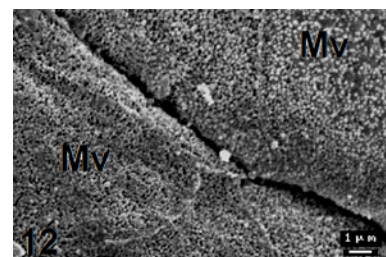


Figure 12: Magnified electron micrograph from figure 11 at the serrated portion (Sr). It shows tiny microvilli (Mv) that cover the epithelial cells. Note that these microvilli have spherical apices and inter connected by microridges. ×7500.

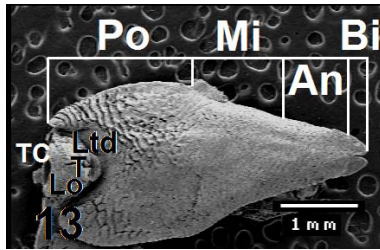


Figure 13: Electron micrograph shows the dorsal view of *C. sepsoides* tongue. It divides into bi-forked tip (Bi), anterior (An); middle (Mi) and posterior regions (Po) of the tongue. There is a laryngotracheal organ (Lo), which situated in a depression (Ltd) in the most posterior extremity of the tongue. Note also, the orifice of the tracheal canal (Tc) and T-shaped orifice (T) of the laryngotracheal organ. $\times 15$.

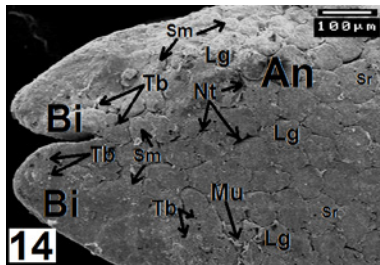


Figure 14: Electron micrograph shows bi-forked, non-papillosed, keratinized tip (Bi) and large (Lg), multiangular papillae of anterior region of *C. sepsoides* tongue. Note the serration in the posterior ends of these papillae, the narrow trenches and mucoid secretion that emitted between them. $\times 150$.

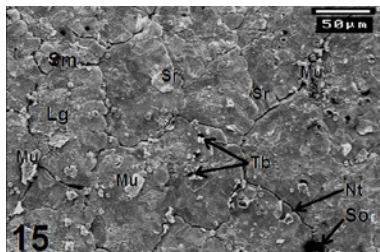


Figure 15: Electron micrograph shows the dorsal surface of lingual papillae in the middle region of *C. sepsoides* tongue. Most of the lingual papillae are large (Lg) with few smaller ones (Sm). Note the serrated ends (Sr), secretory orifice (So) and taste bud (Tb) orifices. $\times 350$.

The serrated parts are flattened, overlapped and their posterior ends oriented towards the posterior direction. The anterior dorsal aspect of *C. ocellatus* tongue contains numerous secretory pores (Figures 1,3 and 4). By using high magnification, these pores appeared lined with cork-screw shaped channel system (Figures 5,7 and 8). The surface epithelial cells of this region were studded by tiny microvilli (Figure 5), which may be fused together or provided with small blebs at their terminal ends. Most of these microvilli are interconnected by microridges (Figure 6). Some taste buds orifices were observed in the anterior portion of the tongue especially around the secretory pores (Figure 7).

The papillae of the middle region of the tongue appeared compacted, more strong, and characterized by their rhombus shape. They were not imbricated like that papillae in the anterior and posterior regions. These papillae were separated by narrow trenches that emitted secretion effusiveness (Figure 9). The epithelial cells are covered by a network of

tiny microvilli, the cell borders are elevated and some orifices of goblet cells can be seen between them (Figure 10).

The posterior region of the tongue has large rectangular papillae that are arranged perpendicular to their long axis. All of them are serrated at their posterior end (Figure 11). The epithelial cells of the serrated portion are provided with tiny microvilli (Figure 12).

In *C. sepsoides*, low magnification by SEM, showed that the tongue is more or less flattened with biforked tip and the dorsum epithelia is divided also into 4 regions tip, anterior, middle and posterior parts that terminated at the laryngotracheal depression (Figure 13). The lingual papillae of the anterior and middle regions were slightly different from each other.

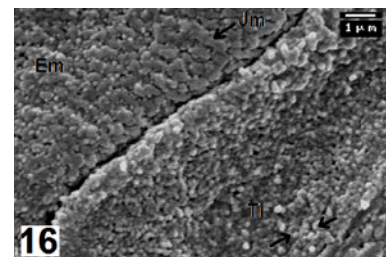


Figure 16: Magnified micrograph from figure 15 shows the microvilli that cover epithelial cells in the serrated area of a lingual papilla. Upper left part provided with thick, enlarged microvilli (Em), which may fused together to form jagged microvilli (Jm) that are connected with each other by microridges (Mr). Lower right shows tiny microvilli (Ti), some of them have spherical terminus (arrows). $\times 10,000$.

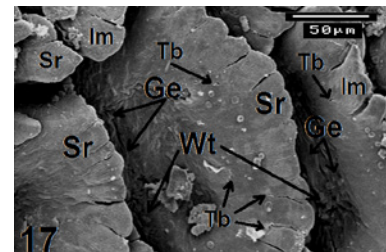


Figure 17: Electron micrograph shows the lingual papillae of the posterior region of *C. sepsoides* tongue, which imbricated (Im) toward the posterior direction. Most of them are large, elongated, arranged in a transverse pattern and deeply serrated (Sr) at their posterior ends (arrows). Note the presence of taste bud orifices (Tb) and wide trenches (Wt) separated between these papillae. The serrated parts at the end of each papilla cover and protect the glandular epithelium (Ge) of the anterior part in the next papilla beneath it. $\times 500$.

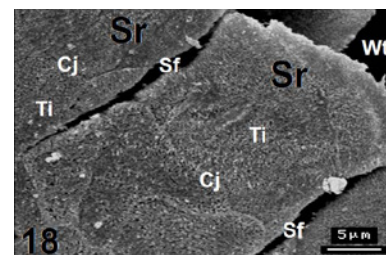


Figure 18: Magnified micrograph from figure 17 shows the serrated (Sr) part of a lingual papilla in the posterior region of the tongue. Two serration furrows (Sf) are dividing the terminal end of the present lingual papilla. Note the cell junctions (Cj) between them and their tiny microvilli (Ti). Wt: wide trench. $\times 3500$.

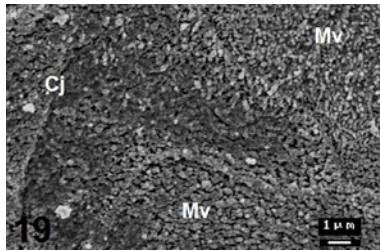


Figure 19: Magnified micrographs from figure 17 shows different shapes of microvilli (Mv) in the posterior region of *C. sepsoides* tongue. Figure 19 shows loosely arranged (upper right) or aggregated (lower middle region) microvilli. Note the cell junctions (Cj) between the epithelial cells. ×7500.

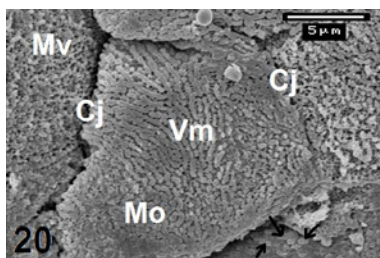


Figure 20: Magnified micrographs from figure 17 shows different shapes of microvilli (Mv) in the posterior region of *C. sepsoides* tongue. Figure 20 displays other different patterns of microvilli in the serrated portion of lingual papilla. The central epithelial cell is studded by a condensed architectural pattern of mosaic (Mo) and virtuous microvilli (Vm) with narrow spaces separating between them. Left epithelial cell shows that the microvilli (Mv) have fine cylindrical shapes with spherical terminus, while that in the right are provided microvilli with large spherical terminus (arrow). Cj: Cell junction. ×5000.

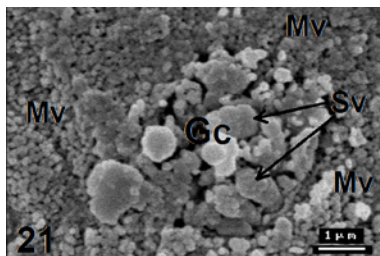


Figure 21: Electron micrograph shows orifice of a goblet cell (Gc) in a lingual papilla in the posterior region of *C. sepsoides* tongue. Note the secretory vesicles (Sv) that are protruded and the free surface of neighbor cells bear thick and compact microvilli (Mv). ×7500.

The biforked tip was formed of weak bifurcation, non-papillosed, keratinized region. In comparison with that observed in *C. ocellatus*, which had deepest bifurcation furrow and the two branches of the tongue tip was stronger than in the present species.

The anterior region was covered with compact, multiangular papillae that are separated by narrow moats (Figure 14). These papillae were flattened and slightly serrated. The anterior extremities of these papillae were imbricated towards the anterior direction as shown in semithin section (Figures 27 and 28).

In the middle region, the lingual papillae became more compacted with their narrow trenches. The posterior extremities of these papillae had irregular and serrated ends also some mucous glands and apical pores of taste buds appeared (Figure 15). Using higher magnification,

the surface epithelia was covered with tiny microvilli and some of them were fused to form bulging ends (Figure 16).

Posteriorly, the lingual papillae appeared larger, more elongated and arranged in transverse pattern with highly serrated ends, which imbricated towards the posterior direction (Figure 17). The epithelial cells of this serrated parts were provided with microvilli that appeared dispersed or aggregated (Figures 18 and 19). Figure 20 shows three epithelial cells bear different forms of microvilli in the posterior serrated portion of lingual papilla. The central cell bears condensed architectural pattern of longitudinal compact microvilli appeared separated by narrow dykes. Other cells bear microvilli with fine cylindrical shape, while the rest of them were provided with globoid ends. Numerous secretory granules exposed from an active goblet cell that found in this region as shown in Figure 21.

Figure 22 shows a large laryngotracheal depression lies in the posterior aspect of the tongue. This region contains laryngotracheal organ that provided with T-shape crevice. The horizontal slot was preceded by large fleshy lip, which was lined with cilia that arranged in

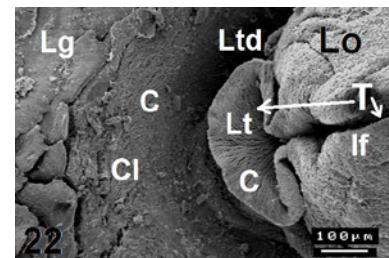


Figure 22: Electron micrograph shows the cilia (C), the ciliated lingual layer (Cl) in the laryngotracheal depression (Ltd), which is nestling the laryngotracheal organ in *C. sepsoides* tongue. Note the lingual papillae (Lg) of the posterior region (left side), ciliated lingual tracheal lip (Lt), inflexible folds (If) and T-shaped orifice of the laryngotracheal organ (Lo). ×150.

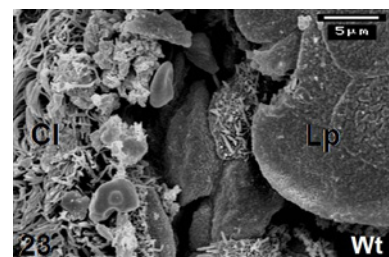


Figure 23: Magnified micrographs from figure 22 show lingual papillae (left) and ciliated lingual layer (Cl) of laryngotracheal depression (right). Wt: Wide trench. X 3500 and 7500 respectively.

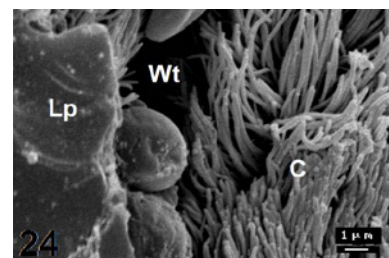


Figure 24: Magnified micrographs from figure 22 show lingual papillae (left) and ciliated lingual layer (Cl) of laryngotracheal depression (right). Wt: Wide trench. X 3500 and 7500 respectively.

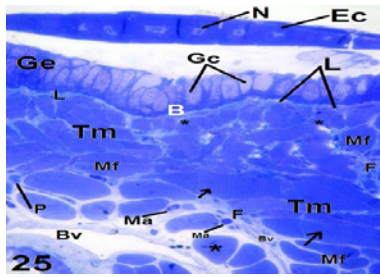


Figure 25: Light micrograph shows transverse semithin section in the anterior region of *C. ocellatus* tongue. The upper side shows a single layer of epithelial cells (Ec) with their central nuclei (N) at the posterior extremity of a lingual papilla. The lower side shows underneath papilla formed of glandular epithelium (Ge), thick basement membrane (B), lamina propria (L), tongue muscles (Tm). The muscle fibers (Mf) are cut in a transverse (stars) and longitudinal plan (arrows). Note the presence of some blood vessels (Bv), numerous fibrocytes (F), few mast cells (Ma) and plasma cells (P). $\times 160$.

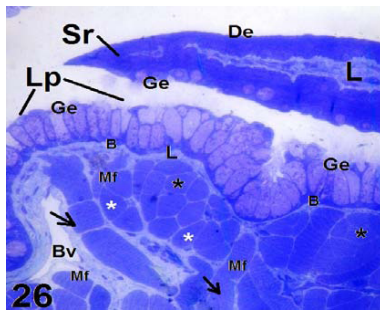


Figure 26: Light micrograph shows longitudinal semithin section in the posterior region of *C. ocellatus* tongue. The upper side shows posterior serrated (Sr) part of a lingual papilla. It is formed of dorsal epithelium (De), lamina propria (L) and ventral glandular epithelium (Ge). The lower side shows the anterior part of another lingual papilla follow the previous one. Note the basement membrane (B), Blood vessels (Bv). The muscle fibers (Mf) are cut in a transverse (stars) and vertical (arrows) plan. $\times 160$.



Figure 27: Light micrograph shows longitudinal semithin section of *C. sepsoides* tongue. The dorsal papillae (Dp) is a large, flattened and are separated by narrow trenches (Nt). Most of them are imbricated (Im) at their anterior ends. The ventral lingual epithelium (Vl) contains numerous lingual folds (Lf). The lingual connective tissue rich with lingual muscle fibers (Mf) more than that, described in *C. ocellatus* tongue. Note that the tongue is supported by an accessory lingual muscle (Am), which may increase potency of tongue movements. This muscle is supported by muscle fibers (Mf) that was cut in longitudinal (Lon), Transverse (Tr) and vertical plans (V). $\times 25$.

a radial pattern. The slot was conquering into a longitudinal cleft, which was supported by two inflexible folds (Figures 22-24). The lingual layer, which surrounds the laryngotracheal structure, was lined with ciliated epithelium. Figures 23 and 24 illustrate the posterior lingual papillae and the ciliated portion.

Semithin sections of *C. Ocellatus* tongue shows the covering epithelium, glandular epithelium, lamina propria and tongue muscle fibers. Figure 25 shows a transverse semithin section through lingual papillae in the anterior region of the tongue. The upper part shows a single epithelial layer of the posterior imbricated portion of pre-lingual papillae, while the lower part represents the anterior portion of post-lingual papilla, which lies underneath the former structure. It is formed of glandular epithelium, basement membrane, thin lamina propria and numerous muscle fibers that were cut in transverse and longitudinal planes. The intermuscular connective tissue shows numerous fibrocytes and some mast cells. In the posterior region of the tongue, Figure 26

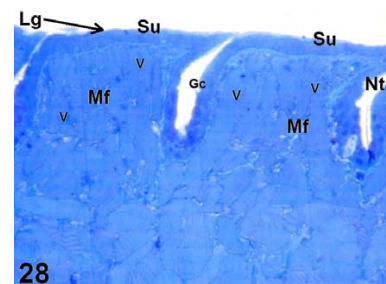


Figure 28: Light micrograph magnified from 27 shows large lingual papillae (Lg) of the anterior lingual region, surface epithelium (Su), narrow trenches (Nt) and few goblet cells (Gc). Note that each papilla is supplied by large number of muscle fibers (Mf) that are cut in a vertical plan to facilitate expansion of lingual papillae and protrusion of the tongue. $\times 100$.

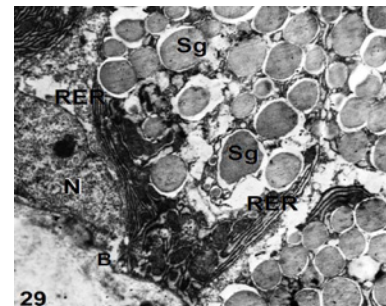


Figure 29: Transmission electron micrograph shows magnified active goblet cell, rested on basement membrane (B), in the middle region of *C. ocellatus* tongue. Note the nucleus (N) is basely situated, rough endoplasmic reticulum (RER) and numerous secretory granules (Sg). $\times 12000$.

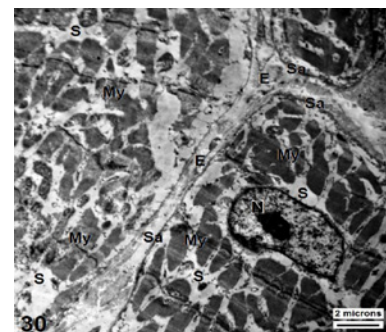


Figure 30: Transmission electron micrograph shows the muscle fibers of *C. ocellatus* tongue. It is enclosed in a sarcolemma (Sa) and their sarcoplasm (S) rich in numerous myofibrils (My). Note the presence of a peripheral nucleus, nucleolus and a thin outer connective tissue coat, endomysium (E). $\times 9940$.

shows longitudinal semithin section of similar structures explained before in Figure 25. The upper part shows a longitudinal section of a posterior extension of one lingual papilla. It is formed of dorsal and

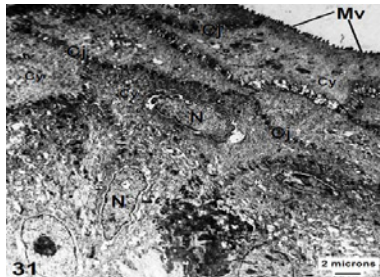
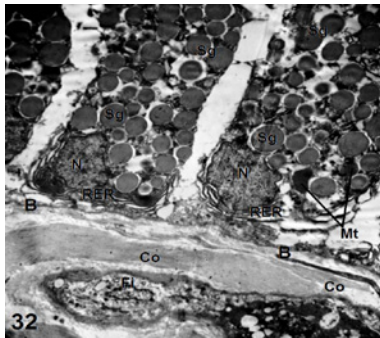
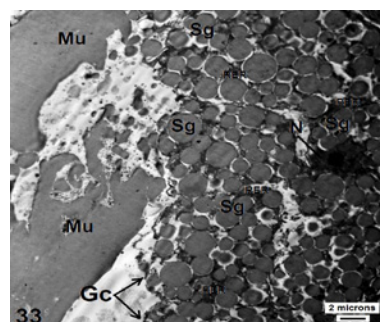


Figure 31: Transmission electron micrograph shows stratified squamous keratinized epithelium of the tip region of *C. sepsoides* tongue. Note the cell junctions (CJ), microvilli (Mv), their electron dense cytoplasm (Cy) and elongated nuclei (N). $\times 6000$.



Figures 32: Transmission electron micrographs from *C. sepsoides* tongue show goblet cells that are situated in the floor of deep trenches between the lingual papillae. Figure 32 shows vertical section of some goblet cells rested on a basement membrane (B). Their cytoplasm shows basal nuclei (N), few mitochondria (Mt), rough endoplasmic reticulum (RER) and numerous secretory granules (Sg). Note that the lamina propria contains active fibrocytes (Fi) and a longitudinal thick bundle of collagen fibre (Co). $\times 7450$. Figure 33 shows some goblet cells (Gc) cut in oblique plan. Note the secretory granules (Sg) are fused at the cell surfaces to form mucoid secretions (Mu). N: Nucleus, RER: Rough endoplasmic reticulum. $\times 6000$.



Figures 33: Transmission electron micrographs from *C. sepsoides* tongue show goblet cells that are situated in the floor of deep trenches between the lingual papillae. Figure 32 shows vertical section of some goblet cells rested on a basement membrane (B). Their cytoplasm shows basal nuclei (N), few mitochondria (Mt), rough endoplasmic reticulum (RER) and numerous secretory granules (Sg). Note that the lamina propria contains active fibrocytes (Fi) and a longitudinal thick bundle of collagen fibre (Co). $\times 7450$. Figure 33 shows some goblet cells (Gc) cut in oblique plan. Note the secretory granules (Sg) are fused at the cell surfaces to form mucoid secretions (Mu). N: Nucleus, RER: Rough endoplasmic reticulum. $\times 6000$.

ventral layers of lingual epithelia and a thin lamina propria between them. The posterior extremity of this structure is similar to that observed in the anterior region of the tongue (Figure 25). The dorsal layer is protective, while that in the ventral aspect is glandular epithelium. The lower part of the same figure illustrates the nature of their epithelial layer which is rich in active goblet cells. Note also, the presence of thick basement membrane, muscle fibers, which were cut in a transverse or vertical plane. A large and medium sized lymphatic ducts can be seen in figures 25 and 26, respectively.

In *C. sepsoides* tongue, figure 27 shows the anterior region was provided with large sized, flattened papillae that can be described as lingual folds, which may be facilitating the retractable function of the tongue. Note that the frontal parts of these papillae were slightly imbricated towards the anterior direction. The ventral surface epithelium of the tongue has deep lingual folds. However, there is an accessory ventral lingual muscle observed, which may increase the tongue length to become more adapted for snipe the fossorial preys before escaping (Figure 27). Using higher magnification, Figure 28 shows two papillae covered with lingual epithelium and the epithelial layer that lines the trenches between them contains few goblet cells (Figure 32). Each papilla has a narrow lamina propria and supported with numerous muscle fibers that were oriented vertically or transversely to facilitate tongue flicking. The lingual muscle fibers were cut in transverse and longitudinal plane to support food sniping and retraction.

TEM examination of *C. ocellatus* tongue shows a goblet cell and its secretory granules of different sizes in the dorsal epithelium (Figure 29). The lingual muscle fibers of the tongue contain numerous myofibrils that have a different shape and size (Figure 30). The tip region of the tongue is covered by keratinized stratified squamous epithelium that is provided with numerous microvilli (Figure 31).

TEM examination of *C. sepsoides* tongue shows the tip region of the tongue covered with keratinized stratified squamous epithelium that is provided with numerous microvilli (Figure 31). Figure 32 shows the bases of some goblet cells with numerous secretory granules and some of them are fused at the free surfaces of these goblet cells as shown in Figure 33.

Discussion

The present study revealed that the microscopic structure of the tongue of selected lizards varied widely. According to [25-29] lizards showed different morphological and histological structures of the tongue among species in different habitats. The morphology of the tongue of both *C. ocellatus* and *C. sepsoides* is characterized by smooth and bifurcated distal portion followed by different typed of lingual papillae that are distributed in the anterior, middle and posterior lingual regions. This difference is correlated with their type of food and habitat. This result is in agreement with [30] who states that the morphological studies indicated a close correlation of the shape of the tongue with the method of food intake and the type of food and their habitat.

The anterior bifurcation of the tongue of both *C. ocellatus* and *C. sepsoides* is also present in all squamates except legless lizards, Dibamidae [31]. A deep bifurcation was reported in snakes and varanids [32-35]. Similar structural pattern of forked tongue was reported by [36] in *Python molurus*. The tongue is slightly bifurcated in Gekkonidae [37] but absent in turtles [20], lizards *Sphenodon* [26], *Tarentola annularis* [35], *Phrynocephalus arabicus* [38], in snake *Psammophis sibilans* [35] and in crocodilians [35,39]. In varanids and snakes the bifurcated tongue tip is associated with the transmitting of

chemicals from the environment to the vomeronasal (Jacobson) organ [32] or prey odor discrimination by lizards [40].

The present observations revealed that the dorsal surface of the tongue tip is covered with keratinized squamous epithelium. This is similar to the results reported in *Eumeces schneideri* [16], *Gekko japonicas* [17] and *Anguis fragilis* [29]. Keratinization of the dorsal lingual epithelium has been recognized in snake *Elaph quadrivirgata* [41], and in some turtles [20, 42-44]. This keratinization is considered as an adaptation to the dry land environment and used to protect the tongue epithelia during prey sniping, mastication and swallowing.

Four areas were observed on the dorsal lingual epithelium of both *C. ocellatus* and *C. sepsoides*: tongue tip, fore, mid and hind tongue. This result was also demonstrated by [17] who worked on *C. ocellatus*, [21] who worked on the iguanid lizard *Oplurus cuvieri* and by [40] who worked on a lacertid lizard.

The present SEM examination revealed that the dorsal region of the tongue of both *C. ocellatus* and *C. sepsoides* is covered with various types of papillae that showed clear differences between the fore, mid and the most posterior part of the tongue. These differences appeared in shape, size, number and distribution. This is in agreement with [26,29,45]. The lingual papillae of the anterior region of *C. ocellatus* are of two types, the most anterior region are smaller and have deep trenches with numerous secretory pores and the rest of the lingual papillae are larger with serrated and flattened ends oriented to the posterior direction while that of *C. sepsoides* appeared more compact, multiangular separated with narrow trenches. These inter-papillary spaces filled with mucous would serve the adherence between the tongue and the food.

The lingual papillae in mid region of both *C. ocellatus* and *C. sepsoides* appeared more compact with narrow trenches and irregular serrated ends. In *C. sepsoides*, the smooth epithelium of mid tongue facilitates sucking of the prey's sherbets and food movement towards the pharynx. This finding is supported by [21,46,47]. The posterior region of the tongue of *C. ocellatus* shows large rectangular papillae. This result is in agreement with [48], while that of *C. sepsoides* appears serrated. These papillae have rough surfaces that act on the prey during the feeding phase. This result is also reported by [21,40] worked on *O. cuvieri* and lacertid lizards respectively, where they are stated that the hind tongue has a more diverse array of papillae that presumably help to generate friction between the tongue and the prey.

Imbrications of lingual papillae to the posterior or anterior direction in the lingual papillae were not discussed before. The present investigation suggests that the epithelia of the imbricated parts may used to cover and protect the underneath glandular portions. On the other hand, the orientation of the imbricated regions either to the posterior, *C. ocellatus*, or anterior directions, *C. sepsoides*, may be related to the drinking behavior of lizards. In *C. ocellatus*, the imbricated parts serve in sipping water, while the imbrications towards the anterior direction indicates that the lizard may suck food solutions rather than drinking it and used to expel the prey's remnant after sucking their sherbets. The imbricated lingual papillae need more investigations.

In the present work, the microvilli and microridges are widely distributed over the surface epithelia. These microstructures of the tongue may be helpful for collecting and receiving chemicals, respectively during tongue flicking as demonstrated by [29]. The present study reveals that the presence of microridges is commonly associated with mucous secreting epithelia. The described microridges have been interpreted as structures that increase the adhesion of mucus to the epithelium [49,50]. The microridges may protect the surface

of the tongue from an abrasive contact with the anterior teeth or the substrate during tongue flicking or substrate licking [51]. However, these microstructures serve to hold in place a residual layer of mucus, which acts as a lubricant and facilitate movement of material over a cell surface [52-54].

Ultra thin sections examined in both species showed that a collagenous connective tissue (lamina propria) is positioned just under the epithelium and penetrates into the papilla. Skeletal muscle fibers pass through the lamina propria and run into the papillae. Similar observations were noticed in the skink *Eumeces schneideri* [16], Lacertid lizards [40]. The collagenous fibers may support the attachment between the muscle fibers and the epithelia of lingual papillae [55]. The contraction of the muscle will cause either shortening or bending of the papillae. These muscles were also attached to the base of the mucous glands to squeeze their secretions [19]. The extensive development of the lingual muscles appears to reflect that they have an essential role in multiple tongue functions [40].

In both species, the present study reveals the presence of numerous goblet cells, especially in the posterior lingual region. TEM examination shows the ultrastructure of goblet cell in the dorsal epithelium of the tongue and the secretory granules of different sizes in *C. ocellatus*, and *C. sepsoides*. Similar glands are found in *E. Schneideri* [17], terrestrial tortoises [19], iguanid lizard *O. cuvieri* [21], *Sphenodon* [25] and in *Acanthodactylus boskianus* [56]. The secretion of large amounts of mucus acts as a lubricant that facilitates food movements, transport and swallowing [19,35,56,57].

Taste buds in the lingual epithelium observed in the present study are scarcely described in the tip region, while moderately distributed in the other lingual regions. Taste buds are observed also in lizards [21,25,26], turtles [19], and crocodilians [38]. These taste buds are testing the palatability during prey's capture [21]. They are stimulated by the molecules transferred during tongue flicking [29]. These previous findings support the present observations in *C. ocellatus* and *C. sepsoides*.

The ciliated lingual layer in the laryngotracheal depression was not described before. This layer situated in the most caudal regions of the tongue. It may cover and plug the laryngotracheal cervices during the swallowing process. This region needs more attention to shed light on its function.

On the other side, the accessory lingual muscle which is described in the tongue of *C. sepsoides* may support the tongue protrusion to snip their praise. This muscle was not described or discussed before.

In conclusion, the tongue of *C. ocellatus* divides into tip, anterior, middle and posterior regions. The tip region is bifurcated and characterized by the absence of lingual papillae. The anterior region has four secretory pores. The lingual papillae are posteriorly imbricated and separated by wide trenches. The epithelial cells are provided by tiny microvilli. In the middle region, the papillae are not imbricated and have strong rhombus-shaped with narrow trenches. The microvilli of the surface epithelium have reticular pattern and attached with each other by microridges. The papillae of the posterior region have a rectangular shape with deep serrated ends. The imbricated part in the anterior portion of each papilla is provided with numerous goblet cells. Also, the ventral epithelium of the posterior serrated ends has few goblet cells. The striated muscle fibers are distributed in the lamina propria of the lingual papillae as well as in the tongue connective tissue. The distribution and orientation of the muscle fibers prove that the tongue is able to catch the preys by biforked tip and facilitate the swallowing behavior.

The tongue of *C. sepsoides* shows the same four regions. Tip region shows slight bifurcation and covered with non-papillosed keratinized epithelium. Papillae of the anterior region have multiangular shaped with serrated ends, which are imbricated towards the anterior direction. The middle region shows compact papillae with goblet cell and taste bud orifices. The epithelial cells have tiny microvilli. The posterior region shows elongated papillae with highly serrated ends that are imbricated towards the posterior direction. The microvilli of the surface epithelium have a cylindrical or reticular pattern. The most posterior end of the tongue shows ciliated concavity region that hosting larengotracheal organ. The ventral aspect of the tongue has an accessory lingual muscle with myofibers oriented to the vertical, horizontal and longitudinal directions. The laryngotracheal complex and the accessory lingual muscle are described in the present work for the first time.

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