# Fine structure of the dorsal lingual epithelium in *Tarentola annularis* and *Crocodylus niloticus*

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The present study examined the morphological features, histological and histochemical aspect of the tongue of two reptilian species, Tarentola annularis (family: Gekkonidae) and Crocodylus niloticus (family: Crocodylidea), with different habitats, feeding patterns and behaviours, by light and scanning electron microscope. It was observed, that the bifurcation of the tongue was more visible in Tarentola annularis. Conical and filamentous papillae were observed on the lingual body of Tarentola annularis, while in Crocodylus niloticus both mechanical filiform and gustatory papillae appeared. The lingual mucosa in Tarentola annularis is covered by stratified squamous epithelium and keratinised but in Crocodylus niloticus it is highly folded and more heavily keratinised in the folded region and have a localised thickenings structure resembling taste buds. Mucous glands appeared in Tarentola annularis and compound tubular glands in Crocodylus niloticus. At scanning electron microscopy, abundant microridges and microvilli in both species were exhibited on papillae surface facilitated feeding habits. Histochemically, the tongue of two species is strongly positive for carbohydrate stain but with variable degree with others stains. In conclusion, there is a marked correlation between the structure of the tongue of the present reptilian species, habitats and feeding mechanism of the two species. (Folia Morphol 2016; 75, 2: 162–172)

Key words: tongue, reptiles, histology, histochemistry, scanning electron microscopy, *Tarentola annularis*, *Crocodylus niloticus* 

# **INTRODUCTION**

Feeding mechanism is an especially important factor that determines the success of adaptation and persistence of animals in an environment [7, 40, 49]. The tongue and hyoid apparatus in reptiles have a significant role in some physiological functions like feeding, respiration, social interaction, defence, vocalisation and the collection of particles for chemo-sensation [19, 42, 55, 56]. Moreover, there are fairly strong correlations between tongue anatomy and its functional roles and the environmental conditions in which animals use their tongues or hyobranchial system (i.e., water vs. air) [7, 12, 29, 44]. The shape and structure of the tongue differ significantly among animal species, reflecting the various functions of each respective tongue [19, 41, 49]. There have been the great number of macroscopic and light microscopic studies of the tongues of reptiles [4, 5, 19]. The results of such studies have demonstrated considerable variety in the morphology and histology of the reptilian tongue. It should be stated that, only

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a few microscopic and ultrastructural observation of the lingual epithelium of crocodile have been reported [2, 11, 26, 45]. The tongue of Crocodylia has been discussed in variety of publications, mainly in regard to the presence of lingual glands and their participation in salt secretion [47, 53]. Above mentioned papers give only a few information on the basic structure of the tongue of crocodylids, because they concerned primarily physiological studies regarding salt balance, tolerance to saline conditions and salt secretion. Reese [38] and Taguchi [48] provide any meaningful histological descriptions of the crocodilian tongue in general. The purpose of the present study aimed to illustrate a comparative morphological, histological, histochemical and functional variation of Tarentola annularis and the Nile crocodile (Crocodylus niloticus).

# **MATERIALS AND METHODS**

## Material

In the present study, 20 adult specimens (10 from each species) are used. The first species is *Tarentola annularis* (family: Gekkonidae) which are distributed at desert and inhabiting rocky wadis, ruins and old buildings and feeds on insects. The second is *Crocodylus niloticus* (family: Crocodylidea) were obtained from lake Nassir and feeds on fish, birds or mammals.

The animal was anaesthetised and decapitated according to the Local Experimental Animal Ethics Committee. After the animal was killed by decapitation, the mandible was removed and the tongues were dissected and photograph.

#### Histology and histochemistry

The dissected tongues were fixed in 4% neutral formalin, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin. Sections were then cut serially at 6  $\mu$ m thickness. Some sections were stained with haematoxylin and eosin, other section with periodic acid-Schiff (PAS), PAS-alcian and bromo-phenol blue (Table 1).

#### Scanning electron microscopy

The dissected tongue were cut into small pieces and fixed in glutaraldehyde in 0.1 M cacodylate buffer. The specimen were post-fixed in a solution of 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 1 h, and washed several times in the 0.1 M sodium cacodylate buffer. The tissues were dehydrated through either acetone or alcohol series, critical point drying and platinum-palladium ion-sputtering were applied. The specimens were then investigated by scanning electron microscopy (SEM) JSM-5400LV; JEOL.

### RESULTS

#### Macroscopic observation

*T. annularis* tongue appeared flattened with broad and conical free border (Fig. 1A). While in *C. niloticus*, it is firmly fixed on the floor of the oral cavity. It appeared roughly triangular in shape, being much broader caudally than its free border. The surface of the tongue was generally creamy-yellow in colour and had a slightly spongy texture even in the fixed specimens (Fig. 1B).

Light microscopy. In *T. annularis*, the lingual mucosa is covered by stratified squamous epithelium and keratinised. On the dorsal surface of lingual body occur two types of lingual papillae; conical and filamentous papillae (Fig. 2A, B). The tips of the flattened papillae curved towards the tongue root. Both papillae have dense connective tissue core. The lateral walls of the filamentous papillae exhibit mucous glands (Fig. 2B).

The epithelium in the dorsal surface of the tongue in the present species *C. niloticus* possessed a few shallow folds. This folds supported by the underlying connective tissue and clearly demarcated a diposed tissue core (Fig. 3A) and they were rapidly increased in number and complexity towards the lateral borders of the tongue. The epithelium in the highly folded zone appeared more heavily keratinised (Fig. 3B).

The epithelial lining of the tongue demonstrated localised thickenings structures resembling taste buds (Fig. 3C). The lingual body of the tongue of

#### Table 1. Histochemical staining and specyficity

Technique	Chemical group identified
Periodic acid-Schiff (PAS)	Glycoconjugates with vicinal diol groups (glycoproteins and glycogen) [34]
PAS-alcian	Acid and neutral mucopolysaccharides [32]
Mercuric bromophenol blue	Total proteins [28]



**Figure 1.** Photomacrograph of the tongue (T) of *T. annularis* (**A**); *C. niloticus* (**B**), showing the fibrous membrane attaching the tongue to the mandible (white arrow), the glottis (GT) and the laryngeal mound (LM).

the present species *C. niloticus* has numerous form of filiform papillae. Some of them takes small conical structure (Fig. 4A), flattened ones with pointed edge facing the tongue root (Fig. 4B) and others situated around the gustatory circumvallate papilla (Fig. 4C). The gustatory papillae were observed and included two forms; fungiform and circumvallate papillae. The gustatory papillae were enclosed by dense connective tissue core and have apical taste buds (Fig. 4C, D).



**Figure 2.** Photomicrograph of transverse section of the tongue of *T. annularis* showing: **A.** Conical filiform papillae (CP), CT — connective tissue; haematoxylin and eosin (H&E) stain;  $\times 100$ ; **B.** Flattened shaped filiform papillae (FP) with mucous gland (GT); H&E stain;  $\times 100$ .

The caudal two third of the tongue was dominated by the presence of large collections of compound tubular glands. Glandular tissue appeared to be composed of lobes and lobules separated by tracts of connective tissue (Fig. 4E).

**Histochemistry.** The lingual mucosa of the tongue of two studied species are stained strongly with PAS and PAS-alcian reaction (Fig. 5A, B). The bromophenol stain showed significant variations in the degree of reactivity in the present species. The lingual mucosa of *C. niloticus* exhibited strong positive reaction with bromophenol than in *T. annularis* (Fig. 5C).

**Scanning electron microscopy.** The tongue of gecko, *T. annularis* is thick and fleshy, an appearance principally caused by its covering of papillae. It was seen to be flattened, with bifurcated apex (Fig. 6A). The most anterior region of the tongue lacks a roughened surface. Conical flattened papillae are compactly distributed all over the tongue surface (Fig. 6B) and their surfaces possessed hexagonal structures with



**Figure 3.** Photomicrograph of transverse section of the tongue in *C. niloticus* showing: **A.** Number of folds on the surface (arrow), the thick layer of subepithelial connective tissue (CT) and the adipose tissue (A). Haematoxylin and eosin (H&E) stain;  $\times$ 400; **B.** Highly fold with heavily keratinised epithelium, groove seen surrounding the folding structures (arrows); H&E stain;  $\times$ 400; **C.** Magnification of the thickened epithelium to form an enlarged oval core of a taste bud (TB). Note the vertical orientation of the core cells (arrows); H&E stain;  $\times$ 400.

microridges and microvilli (Fig. 6C–E). The elevated intercellular borders were clearly visible (Fig. 6F). The apical surface of the conical papillae was seen to be covered with keratinised layer (Fig. 6G). Opening pores are observed at the tongue surface (Fig. 6H). Filamentous papillae appeared curved towards the tongue root (Fig. 6I).

The dorsal surface of the tongue in *C. niloticus* was composed of a number of round units (Fig. 7A). Peripheral to the units were small, spherical, semi like fungiform papillae with central nipple-like surface extensions. Some of them have taste buds (Fig. 7B, C). At the base of the tongue, there are more than one

flattened, disc-shaped structure with raised domeshaped structures semi-like circumvallate papillae on each unit (Fig. 7D). The surface of these papillae have many microridges giving the surface a sponge-like appearance (Fig. 7E, F).

The posterior two-thirds of the tongue exhibited a round shaped units with centrally positioned pore in the triangular glandular region. This pores are the opening of the main secretory duct of the underlying lingual salivary glands. Many grooves radiated outwards the pore and varied in length (Fig. 7G). The positioned flattened, disc-shaped structures appeared in the caudal region of the tongue (Fig. 7H).

## DISCUSSION

Reptilian tongues are characterised by morphological and functional variations among various species. The most interesting features of the histological structures of reptilian tongues reflect adaptations to a dry habitat or to seawater, but stratification and keratinisation of the lingual epithelium are common features [19, 20]. The alligator is an aquatic reptile and its lingual epithelium is strongly keratinised [19, 45]. In the present study, T. annularis and C. niloticus, the epithelium were typically keratinised stratified squamous in structure but in C. niloticus the keratinisation appeared thin and uniform in thickness throughout all regions of the tongue except for thickenings containing taste buds and highly folded zone. Reese [38] and Taguchi [48] found that the tongue in Florida alligator covered by a keratinised stratified squamous epithelium which thickened appreciably towards the body and base of the tongue.

Keratinisation of the dorsal lingual epithelium has been recognised in higher vertebrates. Among reptiles [14, 17] the keratinisation of the lingual epithelium occurred, in evolutionary terms, in conjunction with adaptation to dry land from a fresh-water environment

The investigated crocodile tongue, *C. niloticus* appeared triangular in structure with broad base and rounded apex and intimately fixed to the oral cavity. The tongue exhibited the presence of grooves parallel to tongue apex which facilitated flexion. The same result was observed in *Alligator sinensis*, *Crocodylus vulgaris* [48]. Fixed tongue and macroscopic structure accommodated the animal for mode of feeding [26]. On the other hand, the examined *T. annularis* tongue appeared marked short triangular structure relative to the structure of the oral cavity. *T. annularis* is entirely



**Figure 4.** Photomicrograph of transverse section of the lingual mucosa of the tongue in *C. niloticus* showing: **A.** Conical structure filiform papillae (CFP); CT — connective tissue. Haematoxylin and eosin (H&E) stain; ×100; **B.** Flattened shaped filiform papillae (FFP) with pointed edge facing the tongue root; H&E stain; ×400; **C.** Circumvallate papilla (CVP) with connective tissue core (CT) and taste buds (TB) and flattened filiform papillae (FFP) around them in the form of finger-like protrusion. H&E stain; ×100; **D.** Fungiform papillae (FU) with taste bud (TB); H&E stain; ×400; **E.** Large branched coiled compound tubular glands; H&E stain; ×400.

carnivorous and depend mainly on insect feeding and these required fleshy tongue structure [31, 49].

The *T. annularis* possessed bifurcation of the lingual apex which is completely missing in *C. niloticus*. It is known that forked tongues may provide more surface available for sensory function in lizards [7]. Bifurcated tongue apex was observed in different reptilian species such as *Takydromus takydromoides* [15] and *Gecko japonicas* [18]. In the bifurcated insectivorous lizards, the insect is swallowed and bifurcation may facilitate this process [7, 44]. The tongue is not bifurcated in *Sphenodon* [43], *Anolis carolinensis* [37], *Geoclemys reevesii* and *Clemys japonica* [17].

The epithelium on the dorsum of the tongue of the present Nile crocodile displayed a relative small number of folds that increase in number and complexity towards the lateral margin of the tongue. The folded nature of the tongue surface revealed in this study was also confirmed in "Florida alligator" (*A. mississippiensis*) [38], in *A. sinensis*, *C. vulgaris* [48] and in *C. niloticus* [26].

Tongue papillae are different in shape, size, number, nomenclature and distribution among different groups of vertebrates [7, 20].

The present study confirmed that in the lingual body in *T. annularis* two types of lingual papillae, conical and filamentous filiform papillae occurred, while in *C. niloticus* the numerous form of filiform and gustatory papillae (fungiform and circumvallate) were stated. Similar structural pattern of lingual papillae were reported in lizard species *Takydromus tachydromides, Gecko japonicas* and Crocodilian [4, 13, 26].

Shimada et al. [45] reported that the dorsal surface of the tongue of *A. mississippiensis* is covered with



**Figure 5.** Histochemical micrograph of transverse section of the tongue showing periodic acid-Schiff (PAS) activity in *T. annularis*;  $\times 100$  (**A**); PAS-alcian blue reaction in *C. niloticus*;  $\times 100$  (**B**); Bromophenol blue stain in *C. niloticus*  $\times 100$  (**C**).

small, evenly distributed cone-shaped filiform papillae spread across the dorsal surface of the tongue. The differences in papillae on the tongue surface depends on diet variety, feeding habits and mouth handling of the food [7, 15, 33]. The different shapes of the filiform papillae, which changed gradually from the apex to the caudal part of the tongue in *C. niloticus*, have also been observed in the mongoose [16]. Filiform papillae form the primary pathway of food transport which comes into contact with the palate during mastication and swallowing. It provides tongue with a rough surface suited for the movement and grinding of food [25, 33, 54].

In majority of mammals, keratinisation of the epithelium begins with the appearance of the non-gustatory lingual papillae, namely, the filiform papillae, just before birth [21]. By contrast, the gustatory papillae, such as the fungiform, circumvallate and foliate papillae, appear at an earlier embryonic stage without any obvious relationship to the keratinisation of the lingual epithelium [19, 30].

In the present gecko, *T. annularis*, microridges are predominant on the dorsal lingual surface. This result agree with that done in turtles, *Gecko japonicas*, *G. reevesii* and *C. japonica* [17]. The development of mentioned microridges in the present gecko is less pronounced than in *Takydromus tachdromoides* [15]. Microridges may play a role in the retention and spread of mucus on the epithelial cell surfaces so their function is as a supporting structure for food mastication and swallowing [46]. Microridges. Both microridges and microridges. Both microridges and microridges as supporting structure for food uptake, mastication, and swallowing [18].

The tongue of the present gecko, T. annularis and crocodylids, C. niloticus has a glandular tissue, this fact is mentioned in many earlier literature [18, 27, 38, 48, 58]. In Gecko japonicas, a majority of the epithelial cells was found to contain granules with bipartite structure as observed in the present species [18]. Taguchi [48] described the lingual glands in the three studied crocodiles as being of the branched tubular type and this appear to be typical for the glandular tissue observed during this study. The secretion of large amounts of mucus acts as a lubricant that facilitates food movements, transport and swallowing [57]. The tongue in Ptyodactylus guttatus and Stenodactylus petrii showed abundant glandular distribution associated with dense distribution of microvilli and microridges on lingual papillae, that facilitates food movements, transport and swallowing [7].

The histological study of the tongue of *A. missis-sippiensis* described that the tongue has a glandular tissue as probably mucous or slime secretion and the structure of glands as being compound tubular alveolar [38]. Many authors have noted that the tongue of *C. porosus*, marine or estuarine crocodile, contains 40 complex tubular salt glands that secrete



**Figure 6.** Scanning electron micrograph of the dorsal surface of the *T. annularis* showing: **A.** Flat tongue with bifurcated apex. Scale bar:  $500 \mu$ m; **B.** Conical flattened papillae. Scale bar:  $200 \mu$ m; **C.** Hexagonal structure on the papillae surface. Scale bar:  $20 \mu$ m; **D.** Microridges (MR) on the papillae surface. Scale bar:  $5 \mu$ m; **E.** Microvilli (MV) on the surface of papillae, Scale bar:  $5 \mu$ m; **F.** Elevated borders (arrows). Scale bar:  $2 \mu$ m; **G.** Keratinised layer (KL) on the apical surface of the conical papillae. Scale bar:  $20 \mu$ m; **H.** A central pore (arrow). Scale bar:  $100 \mu$ m; **I.** Filamentous papillae (FP). Scale bar:  $50 \mu$ m.

a concentrated solution of NaCl when the species is in a saline environment [11, 51]. According to Taplin [51], the ultrastructural features of the salt secreting glands revealed by transmission electron microscopy were the characteristically complex interdigitations of lateral cell membranes, expanded intercellular spaces,



**Figure 7.** Scanning electron micrograph of the dorsal surface of the *C. niloticus* showing: **A.** Number of clearly demarcated round units. Bar: 50  $\mu$ m; **B.** Small, spherical, semi-like fungiform papillae with central nipple-like surface (arrow). Bar: 50  $\mu$ m; **C.** The semi-like fungiform papillae (FU) with apical taste bud (TB). Bar: 50  $\mu$ m; **D.** A flat, disc-shaped structure (arrow) and raised dome-shaped structures semi-like circum-vallate papillae (circle). Bar: 200  $\mu$ m; **E.** A complex arrangement of microridges on the surface of papillae. Bar: 10  $\mu$ m; **F.** The spongy appearance of the surface of the papillae. Bar: 5  $\mu$ m; **G.** A surface unit found on the glandular area of the tongue. Note the large, centrally positioned gland opening (P), the grooves (black arrow) and the dome-shaped structures (white arrow). Bar: 100  $\mu$ m; **H.** Flat disc-shaped structures in the caudal region of the tongue (arrow). Bar: 100  $\mu$ m.

abundant mitochondria, and extensive network of blood vessels and unmyelinated nerve fibres.

In the present study, by scanning microscope the glandular tissue in *C. niloticus*, identified as a triangular area occupying the posterior two-thirds of the tongue, was demarcated by a raised, domed unit with a centrally positioned, pigmented pore which indicated the opening of the secretory duct. This result agree with that done by Chen et al. [3] who examined the lingual glands of the Chinese alligator, Alligator sinensis, and reported that they were either simple tubular or complex acinotubular in nature and appeared in the posterior two third of the tongue and functioned as salt secreting glands as well as serving to lubricate food. In species of Alligatorinae, many more pores are present with 100 being reported in *A. sinensis* by Chen et al. [3] and 200 or more in *A. mississippiensis* [52]. The structure of the lingual glands observed in the Nile crocodile is similar to that described in *C. porosus*, despite the difference in classification of the glands as "branched coiled tubular" (*C. niloticus* in present study) or "compound tubular glands" (*C. porossus*) [50]. In the tongues of the peregrine falcon and common kestrel, the many openings of the lingual glands existed in the entire surfaces of the lingual body and lingual root [10].

In the mammals, some openings of the glandular ducts at the dorsal surfaces of the conical papillae of the lingual radix were observed in the tiger [8], fox [22] and mole [23]. However, the openings of the lingual glands in the mammals are a small number than that of the white tailed eagle, peregrine falcon and common kestrel [9].

In the present work, taste buds in Nile crocodile, C. niloticus, are associated with localised epithelial thickenings located throughout the tongue. These epithelial thickenings appear to represent the flattened, disc-shaped structures seen by SEM. The presence of short processes seen emanating from the central pore of these structure lends further support to this observation. Taste buds were also seen to be concentrated in the thickened epithelium forming the surface opening of the lingual salivary glands. The morphology of the lingual taste buds was similar to that reported for taste buds in the palate and gingivae of the Nile crocodile [35, 36] and reflects the features previously described by Bath [1] and Taguchi [48] in this region of the oral cavity. In some turtles the taste buds are missing [57] but they are scattered in the buccopharyngeal mucosa in other turtles [14].

Taste buds are the peripheral sensory organs of gestation, these structures have the task of monitoring the chemical environment of the oral cavity and particularly of sensing ingested foods are palatable, toxic, aversive, nutritive, etc. In summary, taste buds help oversee the first stage of energy balance, food intake [39]. The presence of taste buds on the tongue tip and on the fore tongue may play an important role in receiving chemical and mechanical information of food [33].

Histochemically, results of present study showed that the tongue in the two present species *T. annularis* and *C. niloticus*, is strongly positive for carbohydrates stains (PAS, PAS-alcian reaction), indicates a considerable activity of this glands in the production of the mucous secretion containing glycoproteins. It has been referred that these substances are helping the tongue adhesion and swallowing of the food easily; this has been also documented in other vertebrates [6, 24, 41, 49]. The tongue shows stronger reaction for protein stained with bromophenol technique in the lingual glands of C. niloticus compared with moderate reaction of T. annularis. This result does not agree with that obtained in the lizard Trachylepis vittata that lack aggregations of protein [49]. The histochemical properties of the present gecko and crocodiles correlate strongly with diet and methods of intraoral food movement, which suggests that they are adaptive features [19].

The mucosal folds in *C. niloticus* divide the surface of the tongue in this study into small demarcated fields or units, which was confirmed by SEM. This result agree with the study done by Taguchi [48]. The more discrete surface units occupying the caudal regions of the tongue were characterised by the presence of numerous flattened, disc-shaped structures at the centre of which there was a small pore. The small, nipple-like surface projections seen by SEM in the present species *C. niloticus* probably represent the papillae observed in the *American alligator* [38, 45].

Finally, the tongue of *T. annularis* is similar to other lizard's tongue and it is more appropriate in feeding on insect. The present crocodile tongue possessed characteristic structures mostly similar to mammalian species, especially for the most differentiation of gustatory papillae with taste buds, besides the ordinary structure of the filiform papillae that allowing the *C. niloticus* to use the tongue effectively during chemoreception, prey transport and choosing its food with a high nutritional value.

## CONCLUSIONS

We can conclude from the present study that the histological, histochemical and morphological differences in the lingual epithelium of the tongue among the two reptilian species examined exhibited important specific features reflecting the mode of life, feeding habits and to some extent to phylogenetic trend in these animals.

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