International Journal of Zoology Studies
ISSN: 2455-7269
Impact Factor: RJIF 5.14
www.zoologyjournals.com
Volume 3; Issue 2; March 2018; Page No. 101-111

Morphological, histological and ultrastructural (sem) characterization of the egyptian tortoise's tongue

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Abstract
The present histological and SEM ultrastructural studies designate that, the tongue of Egyptian tortoise (*Testudo kleinmanni*) is divided into hind, mid and blunted non-bifurcated fore tongue. It has a heavily papillose dorsal topographic surface due to copious lingual filiform papillae distributed all over the three tongue sectors. Flattened and/or dome-shape cuboidal papillae are widely distributed on the dorsal surface of the fore- and hind-tongue, they appeared fused with narrow inter-papillary spaces. However, long columnar papillae cover the mid-tongue and pre-glottal are in addition to seldom circumvallate and fungiform ones. Microridges, microvilli and many glandular pores are distributed on the dorsal surface of the tongue including papillae. It seems that they work on the retention and spreading of mucous, secreted by a well-developed glandular system of compound alveolar units laying underneath the different papillae and open in-between. All of the above tongue's organization in addition to intrinsic tongue musculature and relatively small cartilaginous hypoglossum are typical morphological tongue's features of tortoises that feed exclusively on dry plant food on land as the Egyptian tortoise.

Keywords: egyptian tortoise, papilla, microridges, microvilli, SEM

Introduction

The Egyptian tortoise (*Testudo kleinmanni* Lortet, 1883) is the smallest desert-living Testudines occurs in the regions around Mediterranean and Middle East (Perälä, 2001) [55] [. Its diminutive size and brightly coloured carapace provide good camouflage (Van Der Kuyl *et al*., 2002) [66]. Since its dorsal carapace is recognized by smooth high-domed of bright golden straw to dark brown reflective colour (Siroky and Fritz, 2007; Delfino *et al*., 2009) [64, 18].

It inhabits mainly arid to semi-arid areas of coastal sand dunes and shallow sandy/rocky valleys along coastal strip of Mediterranean up to about 90-120 km of Egypt from western Libya to the eastern-north Sinai Peninsula (Baha El Din *et al*., 2003; Perälä, 2005) [7, 55] in addition to some isolated patches in the Negev desert (Baha El Din, 2006) [5]. However, Attum *et al*. (2007) [3] presumed that, in the last few decades, Egyptian tortoise is under sever threat and vanished in all Egyptian territories even in Sinai Peninsula, in which it had a significant dispersion, except its present occurrence in Zaranik and El Omayed protected areas of North Sinai (Baha El Din and Attum, 2000; Baha El Din *et al*. 2003) [6, 7]. It has been eradicated primarily due to agricultural and industrial actions as well as intensification of human population and shore's events (Siren, 2007) [63]. All of the sea activities reduced dramatically the native vegetation for tortoise's food and cover therefore, diminish its habitat (Fritz and Havas, 2007) [64]. Since vegetation not only serves as food for this Testudines but also as hiding place (Schneiderand Schneider, 2008) [59]. Hamer and McDonnell (2009) [30], Dolan *et al*. (2011) [21] and Brown *et al*. (2012) [17] reported that, urbanization habitat changes is negatively affect animal's biodiversity, accordingly *Testudo kleinmanni* is globally endangered (Attum*et al*., 2013).In addition to these reasons, its collection for the illegal pet trade was also played significant role in virtually extinction of this species(IUCN, 2014; Zwartepoorte, 2015) [33, 73].

The term tortoise usually refers to any land-dwelling, non-swimming, chelonian reptile belonging to family: Testudinidae of order: Testudines (Fergus, 2007) [27]. They represents the most ancient and diverse long lived reptiles (Fritz and Havas, 2007; Thomson and Shaffer, 2010) [64, 65], although some are highly threatened since they are ectotherms, their internal body temperature varies according to the ambient environment (Vitt and Caldwell, 2013) [68]. The most obvious characteristic of *Testudo kleinmanni*, is its diminutive size and body shape, the male's average length is of only 95mm but the largest female recorded only 127mm length (Macale *et al*., 2011) [47]. It uses desert larger shrubs of *Artemisia monosperma* as an effective thermal shelter that stabilized micro-climatic variation for protection in the aestivation sites (Attum *et al*., 2013) [4]. They are diurnal, active mostly during warm periods and least active during very hot or very cold months, spending the day hiding under the cover of a bush or in rodent's burrows (Zwartepoorte, 2015) [73].The age of Egyptian tortoises, as all Testudinids, can be determined by counting the growth rings since each ring is deposited yearly during activity period (Rodriguez-Caro *et al*., 2015) [57].

Reptilian tongues, morphologically and histologically, are varied often as a result of their adaptation to environmental conditions and type of food (Mao *et al*., 1991; Iwasaki and Kumakura, 1994; Delheusy *et al*., 1994; Delheusy and Bels, 1999; Al-Zahaby *et al*., 2017) [49, 35, 15, 19, 1]. Consequently, as reported by many authors, Testudines species exhibit significant variations overall the shape of their tongue and its dorsal morphology including the form and distribution of lingual papillae in accordance with the animal's feeding nature and habitat either terrestrial, aquatic or semi aquatic. Among of them, some deal with those live on the land, tortoises(Beisser *et al*., 1995 & 2004; Bels *et al*., 1997;Sabry *et al*., 2015) [12, 11, 15, 18] others with those inhabit purely aquatic environments, turtles (Weisgram, 1985; Iwasaki *et al*., 1996a,d;Beisser and Weisgram, 2001) [69, 38, 10] and the third with for most semi-aquatic species (Iwasaki, 1992; Iwasaki *et al*., 1992; Iwasaki *et al*., 1996b,c; Beisser *et al*., 1997,1998&2001;Beisser and Weisgram, 2001) [36, 38, 9, 13, 10] to clarify the interrelationship between the morphology and fine structure of lingual epithelium and animal's feeding habit.

The main aim of the present studies is to show the tongue's organization and lingual epithelial features of the Egyptian tortoise (*Testudo kleinmanni*) on the basis of gross anatomy, light and Scanning Electron Microscopy to elucidate whether this species is fits into a specific group regarding feeding preferences, as for instance diet or environment. Since, there are no any Electron-microscopic clarifications of the tongue's epithelium of this Testudines species have been published up to now.

Materials and Methods

Four adult Egyptian tortoise, *Testudo kleinmanni*, of about 85-95 mm carapace length, were brought from the North Sinai Peninsula and transferred alive to the laboratory of Experimental Zoology, Zagazig Univ., Egypt. After decapitation of the an anesthetized animals with sodium pentobarbital, following the rules approved by the Animal Ethics Committee at Zagazig University according to the Laboratory Animal Welfare guidelines, the heads of sacrificed tortoises were detached. Their tongues remove out after examination and photo-registration of buccal cavity by means of Samsung 12 mega pixel digital camera. The tongues were rinsed in 8 N hydrochloric acid at 60°C for 30 min in order to get rid of the excessive adhering mucus.

For light microscopic examination, tongues were fixed in 10% buffered paraformaldehyde (pH: 7.3) at room temperature for about 48 hours then dehydrated and paraffin-embedded. Microtomic sections of 5 micron thickness were stained with haematoxylin-eosin as recommended by Drury and Walington (1980) [23], then examined and photographed. However, for SEM, the whole tortoise's tongues were immediately fixed overnight with modified Karnovsky solution (2% paraformaldehyde and 2.5% glutaraldehyde containing 0.1 M cacodylate-buffer, pH 7.4) according to Karnovsky (1965) [41].The fixed specimens then were washed in 0.1 M cacodylate buffer, post-fixed in cacodylate-buffered solution of 1% osmium tetroxide at 37°C for 2 hours, dehydrated and dried in Hitachi Critical Point Drier (HCPD). The dried specimens were then sputtered with gold in Joel fine coat Ion Sputter (SPI-Module).Specimens were then 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.

Observations

Egyptian tortoise of the present study is a small land dwelling has a unique bony box giving the animal acute protection. It consists of above carapace and below plastron, linked by vertical bridges between the front and hind limbs. The smooth strongly arched carapace is of high-dome of bright golden straw to dark brown colour. However its plastron is paler, yellowish with 2 diagnostic, black triangular marks on the abdominal scutes. It have moderate head with smooth edged hooked beak.

Gross Morphology

The fairly triangular short tongue of the Egyptian tortoise (*Testudo kleinmanni*) is firmly sited on the floor of buccal cavity and attached to it by an arrow not extended fold of skin. It is confined by the free teeth lower jaw in front of the pre-glottal region which embracing a slit-like glottis interposed the laryngeal mound (Fig. 1). Otherwise, the roof of buccal cavity is bounded by the upper jaw with its main components; pre-maxilla and maxilla bearing a single row of pointed cone-shaped teeth. Two wide, opened and discontinuous fenestra exochoanalis are intervened palatine and are separated by an intermediate palatine ridge and posterior portion of the vomer. The two exochoanalis, within which the internal nares situated, are limited posteriorly by palatine and laterally by the upper jaw bone, definitely maxilla beyond the choanal groove as illustrated also in figure (1). Also in the same figure, more anteriorly in the roof of the tortoise's buccal cavity and just behind the premaxilla a medial nasal gland, with a direct buccal connection, is hidden. The overgrown premaxilla and maxilla as a smooth edged hooked beak may lead to a strong bite, but do not chew food items. The mouth and tongue of the active tortoise have usually a pink coloration.

Macroscopically, the fleshy tortoise's tongue can be differentiated dorsally into fore-tongue (apex), mid-tongue (body) and hind-tongue (radix) with profound median sulcus in front of the laryngeal mound. It is rather triangular, with blunted apex and broad base when observed dorsally in consistency with the outlines of lower jaw. However, it appears flattened when showed laterally, with about 10-12 mm total length and maximum width at its base of 8-10 mm (Fig. 1). It has rough dorsal surface with clearly observed, superabundant lingual papillae spreading out the whole tongue's dorsal surface. They are filiform of variable shape and size between the three tongue's sectors with interpapillary spaces in-between (Fig. 2).

Fig 1: A photograph of buccal cavity (roof and floor) of the Egyptian tortoise showing: the triangular heavily papillated tongue (T), Laryngeal mound (LM), pre-glottal region (PG) and glottis (GL), toothed upper jaw of maxilla (M) and pre-maxilla (Pm), Fenestra exochoanalis (FE), palatine (Pa), vomer (V), choanal groove (ChG), median palatine ridge (MPR), medial nasal gland (MNG). Scale bar signifies 6.3 mm.

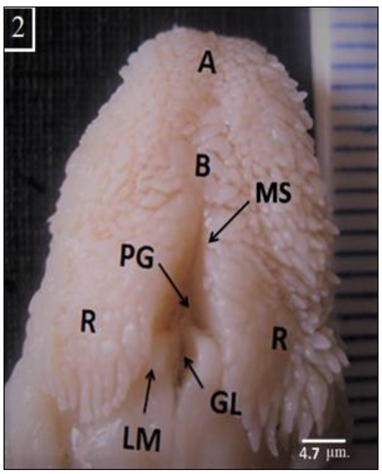

Fig 2: A photograph of dorsal surface of the Egyptian tortoise's tongue showing: The blunt apex (A), Body (B) and Radix (R). Pre-glottal region (PG), laryngeal mound (LM), slit-like glottis (GL), median sulcus (MS). Scale bar signifies 4.7 mm.

SEM observations

With higher magnification of SEM, These papillae are fused, flattened, ridge-like filiform on the fore-tongue (apex) surface. But, on the tongue's body surface, they are elongated of columnar appearance in addition to conical of pointed tip ones. However, on hind-tongue (radix), the filiform papillae are varied between fused flattened, ridge-like to elongated dome-shaped ones (Fig. 3).

The flattened filiform papillae cover apex's dorsum appeared as dome-shaped bulges of various forms and sizes giving it a cobblestone appearance. The apical surface of these bulges bears variously sized microridges as well as glandular orifices and interpapillary spaces in between (Fig. 4). Furthermore, on the dorsal surface of the fore-tongue (apex), taste bud is clearly evident in-between the irregular flattened filiform papillae (Fig. 5).

Otherwise, filiform papillae found on the lateral sides of the tongue's body are either flattened, cuboidal or pointed elongated raising next to each other and maintain interpapillary spaces in-between (Fig.6). They are somewhat differed on mid-tongue's middle sector where they appeared more elongated and less overlapped if compared with those cover the lateral sides (Fig.7).

It is necessary to mention also that, excessive microvilli are profusely spread on the outer surface of the filiform papillae forming a continuous covering over the papillae and embracing glandular pores in between (Fig. 8). Highly magnified SEM-Micrograph of the dorsal surface of the tortoise's mid-tongue, shows also infrequently circumvallated papillae, of broad surface and marginal depressions, appear in between the densely crowded filiform papillae with sloughing keratinized slices as illustrated in figures (9).

On the other hand, the dorsal surface of hind-tongue (radix) of the Egyptian tortoise is dominated with ridge-like flattened filiform papillae with numerous glandular pores in-between microridges on the papillae top surface (Fig. 10). Meanwhile, the dorsal surface of this hind tongue's portion around and close to the glottis is covered by blunted elongated filiform papillae or of high dome-shaped appearance (Figs. 11, 12). On the top surface of these latter filiform papillae, numerous glandular orifices in-between excessive microvilli and microridges are vastly spread also (Fig. 13).

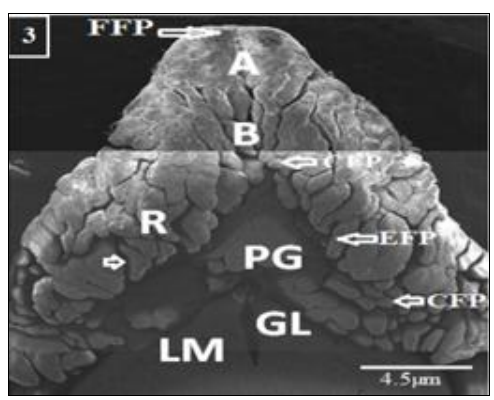

Fig 3: A panoramic SEM-Micrograph of the Egyptian tortoise's tongue showing :The three distinctive parts, Apex (A), Body (B) and Radix (R). Pre-glottal region (PG), laryngeal mound (LM), slit-like glottis (GL). Fused flattened filiform papillae (FFP) on the surface of apex and radix, elongated filiform papillae (EFP),cuboidal filiform papillae (CFP) on the mid-tongue surface. Interpapillary spaces in-between (arrow). Scale bar signifies 4.0 mm.

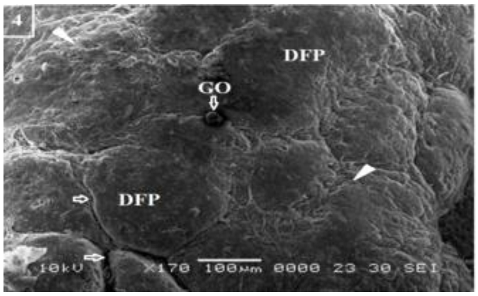

Fig 4: SEM-Micrograph of the fore-tongue (apex) dorsal surface of the Egyptian tortoise showing: Dome-shape filiform papillae (DFP) with minute microridges (Head arrow), interpapillary space (Arrow) and glandular orifice (GO).Scale bar signifies 100 µm.

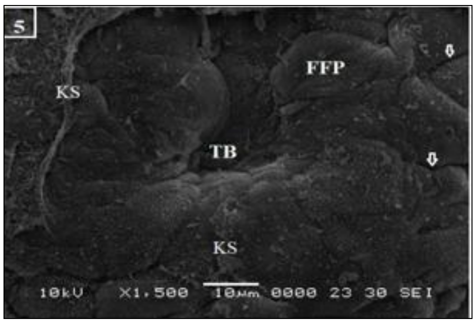

Fig 5: Higher magnified SEM-Micrograph of the dorsal surface tortoise's fore-tongue (apex) showing: Taste bud (TB) in-between flattened filiform papillae (FFP), interpapillary space (Arrow) and sloughing keratinized slice (KS). Scale bar signifies 10 µm.

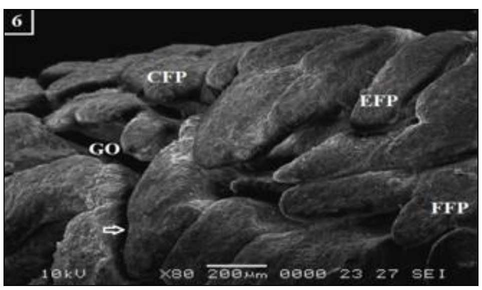

Fig 6: Dorsal surface SEM-Micrograph of the mid-tongue's lateral sides of the Egyptian tortoise showing: Cuboidal filiform papillae (CFP),elongated pointed filiform papillae (EFP),flattened filiform papillae (FFP) with inter papillary spaces (Arrow) and glandular orifice (GO) in-between. Scale bar signifies 200 µm.

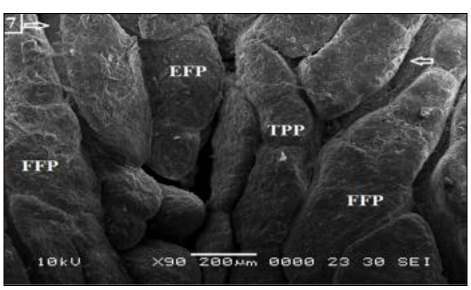

Fig 7: Dorsal surface SEM-Micrograph of the mid-tongue's midline of the Egyptian tortoise showing: Elongated filiform papillae (EFP),tall pointed filiform papillae (TPF) with interpapillary space (Arrow) in-between. Scale bar signifies 200 µm.

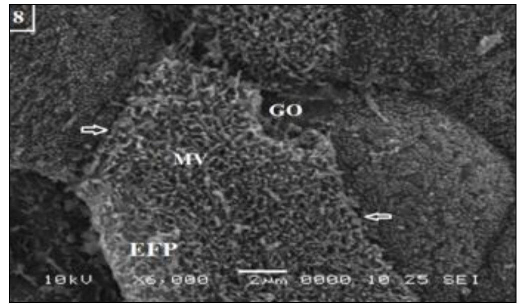

Fig 8: Higher magnified SEM-Micrograph of the dorsal surface tortoise's mid-tongue showing: Excessive microvilli (MV) widely spread on the elongated filiform papillae (EFP), glandular orifice (GO), inter papillary spaces (Arrow). Scale bar signifies 2 µm.

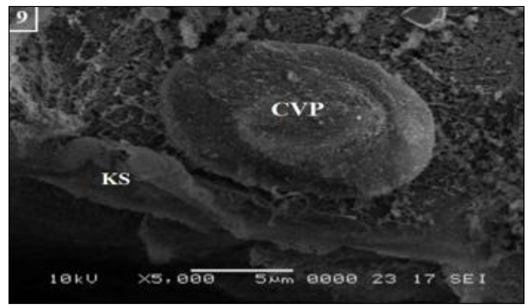

Fig 9: Higher magnified SEM-Micrograph of the dorsal surface tortoise's mid-tongue showing: Circumvallate papilla (CVP),sloughing keratinized slice (KS). Scale bar signifies 5 µm.

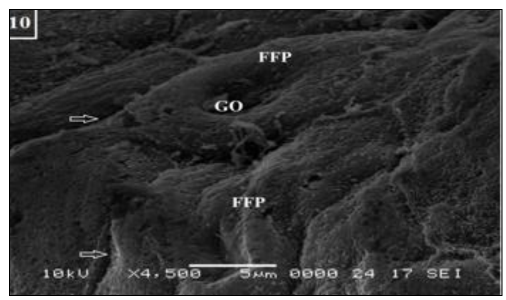

Fig 10: SEM-Micrograph of higher magnification in the dorsal surface of the tortoise' shined-tongue showing: Glandular orifice (GO) in-between different ridge-like flattened filiform papillae (FFP) and interpapillary spaces (Arrow). Scale bar signifies 5 µm.

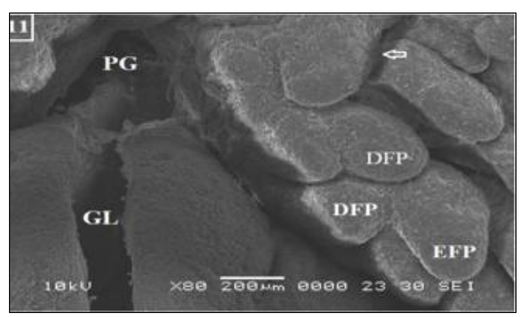

Fig 11: SEM-Micrograph of the dorsal surface of Egyptian tortoise's hind-tongue close to the glottis showing: Blunted elongated filiform papillae (EFP), dome-shaped filiform papillae (DFP), pre-glottal region (PG) and slit-like glottis (GL). Scale bar signifies 200 µm.

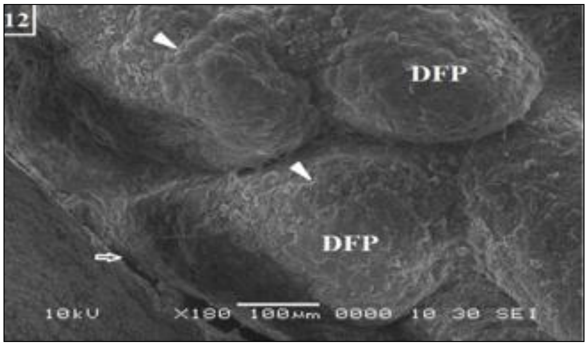

Fig 12: Magnified SEM-Micrograph of the dorsal surface of the Egyptian tortoise close to the glottis showing: Dome-shaped filiform papillae (DFP) with massive microridges (Head arrow) and inter papillary spaces in-between (Arrow). Scale bar signifies 100 µm.

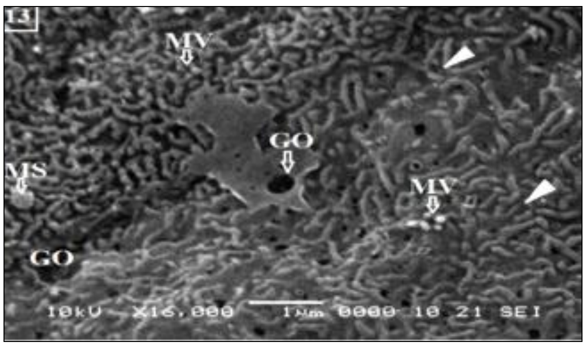

Fig 13: Higher magnified SEM-micrograph of the previous figure showing: Glandular orifices (GO) in-between excessive microvilli (MV) and microridges (Head arrow) spread on the top the dome-shaped filiform papillae (DFP), mucous secretion (MS) and interpapillary space (Arrow). Scale bar signifies 1 µm

Light Microscopic Observations

1. As visible, the light microscopic observations disclose that, the tongue dorsal surface of the Egyptian tortoise is covered with widely distributed and reasonably sized filiform papillae. These papillae are of different form and appearance within the various tongue's three segments. They are flattened cuboidal dome-shaped filiform on the surface of tongue's apex and radix, meanwhile they are elongated or tall of pointed top filiform on the tongue's body surface with small narrow epithelium depression in-between, interpapillary spaces (Figs. 14-22). Circumvallated papillae of broad surface and marginal depressions without any lingual glands (Fig. 19) as well as fungiform papillae of mushroom-shaped appearance with flattened top and narrow base (Fig. 22) are scarcely observed among the other filiform papillae on the tortoise's mid-tongue and radix dorsal surfaces, respectively. The dorsal surface of tongue including the different emitting papillae is made up of a fibrous connective tissue (corium) shielded with the universal covering of squamous stratified epithelium composed of the three fundamental cell layers, basal, intermediate and superficial squamous cell layers. The proximal basal cell layer is of short columnar cells, but the upper intermediate mucous cell layers are of cuboid to trodden spherical having visible smaller nuclei than the basal cells. However, the superficial layer is keratinized and so more intensely stained, its cells are significantly flattened losing their nuclei (Fig. 14). This surface stratified epithelium rises up the fibrous connective tissue corium embracing blood venules and mucus-secreting compound alveolar lingual glands. Some of these glands are formed of many acini around a wide lumen opens with wide orifice in the interpapillary space. Another smaller alveolar gland opens directly on the top of papilla's dorsal surface by independent glandular orifice (Fig. 15).Both types of glands are bounded by lingual intrinsic musculature (Fig. 15). It is noted also that, the epithelial cell layers enclose distinctive oval taste buds, of elongated sensory cell clusters, extended across the thickness of the epithelium with a taste pore setting on the tongue's surface (Fig. 16).

It is evident also that, the foundation of the stratified epithelium covering the papillary lateral side of the various elongated filiform papillae, on the mid-tongue surface, is obviously differed from the papillary apical epithelium, covering the apical region. On the lateral sides of the interpapillary area, the mucus lingual glands exceed in number and volume as showed in figure(15) of tongue's apex, figures (17, 18) of the tongue's body and figures (20, 21, 22) of the tongue's radix. In all, the irregularly short columnar cells of the basal layer are almost of the same features as in the apical papillary epithelium, but cells of the intermediate layer become thinner than the corresponding epithelial cells and may be disappear at the ventral base of the papillae where the glandular ducts open without any sign of keratinization.

The underlying fibrous connective tissue core (corium) invaginates and interdigitates in-between lingual glands and provides deep expansions to the center of each papilla as a dermal papillae (Figs. 14 – 22). Lingual intrinsic muscles underneath the lingual papillae, in the most superficial layer of the corium, encircles the lingual glands as well as the cartilaginous lingual support, hypoglossum which is distinctively showed in the tongue's body (Fig. 18) and tongue's radix (Fig. 20).

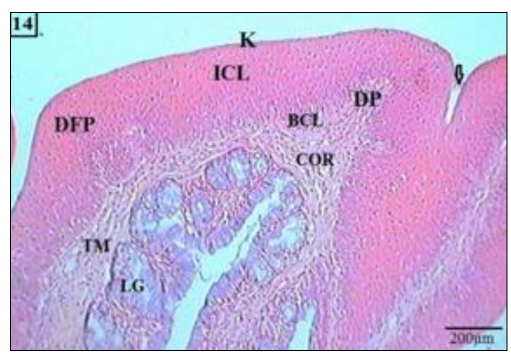

Fig 14: Light photograph of a V.S. through the dorsal surface of the tortoise's tongue apex showing: Dome-shape filiform papilla (DFP), keratinized superficial cell layer (K), Intermediate mucous cell layer (ICL),basal cell layer (BCL),connective tissue corium (COR),dermal papilla (DP), compound lingual gland, (LG), lingual intrinsic musculature(LIM),interpapillary space (Arrow). (H&E), Scale bar signifies 200 µm.

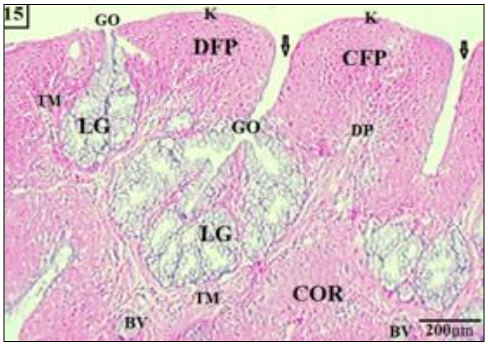

Fig 15: Light photograph of V.S. through the dorsal surface of the tortoise's tongue apex showing: Dome-shape filiform papillae (DFP),cuboidal filiform papillae (CFP), keratinized superficial layer (K), interpapillary space (Arrow).Large compound alveolar gland (LG),Glandular orifice (GO), smaller gland opens on the papilla's surface by glandular orifice (GO),both glands are bounded by lingual musculature (LIM). Connective tissue corium (COR) with dermal papilla (DP) and blood venules (BV). (H&E), Scale bar signifies 200 µm.

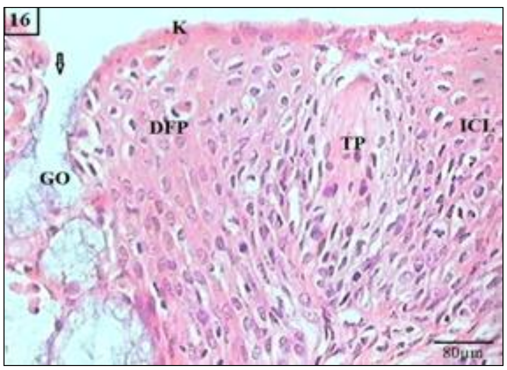

Fig 16: Light photograph of V.S. through the dorsal surface of the tortoise's tongue apex showing: Dome-shape filiform papillae (DFP), taste bud of elongated sensory cells (TB),intermediate epithelial layer (ICL),keratinized superficial layer (K), compound alveolar gland (LG), glandular orifice (GO) opens in the interpapillary space (Arrow). (H&E) Scale bar signifies 80 µm.

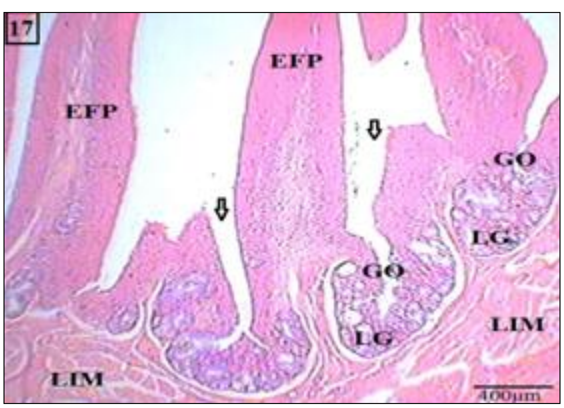

Fig 17: Light photograph of V.S. through the dorsal surface of the tortoise's tongue body showing: Elongated filiform papillae (EFP),acinar lingual glands (LG) open with distinctive glandular orifices (GO),lingual intrinsic musculature (LIM) encircles lingual glands. (H&E), Scale bar signifies 400 µm.

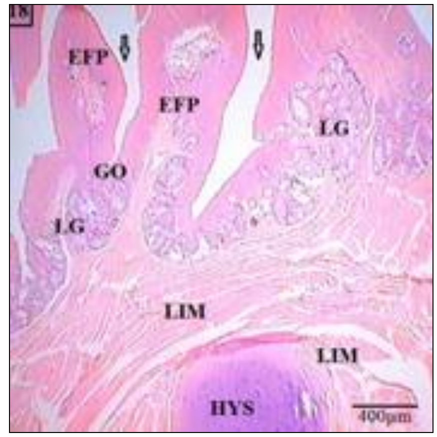

Fig 18: Light photograph of V.S. through the dorsal surface of the tortoise's tongue body showing: Elongated filiform papillae (EFP), acinar lingual gland (LG) opens by wide glandular orifices (GO), hyolingual skeleton(HYS), lingual intrinsic muscles (LIM) encircles both glands and hypoglossum. (H&E), Scale bar signifies 400 µm.

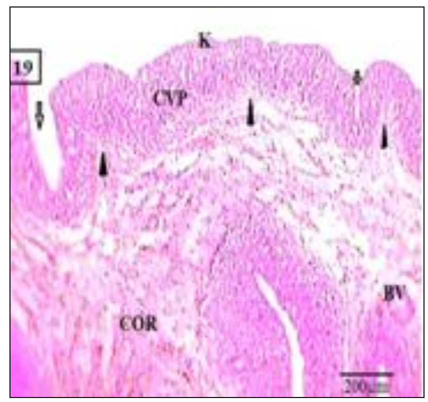

Fig 19: Light photograph of V.S. through the dorsal surface of the tortoise's tongue body showing: Circumvallate papilla (CVP),blood venules (BV), corium (COR) is extended up as dermal papilla (DP), interpapillary space (Arrow). (H&E),Scale bar signifies 300 µm.

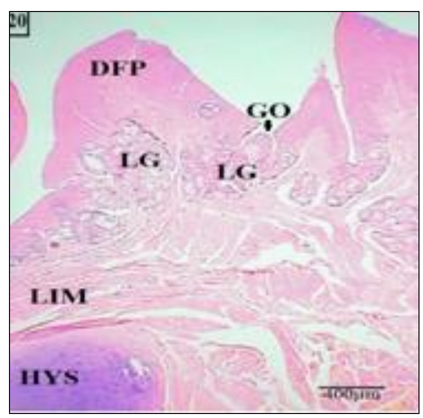

Fig 20: Light photograph of V.S. through the dorsal surface of the tortoise's tongue radix showing: Dome-shaped filiform papillae (DFP), acinar alveolar glands (LG), glandular orifices (GO), hyolingual skeleton(HYS), lingual intrinsic muscles (LIM) en circles both the lingual glands and hyolingual skeleton. (H&E), Scale bar signifies 400 µm.

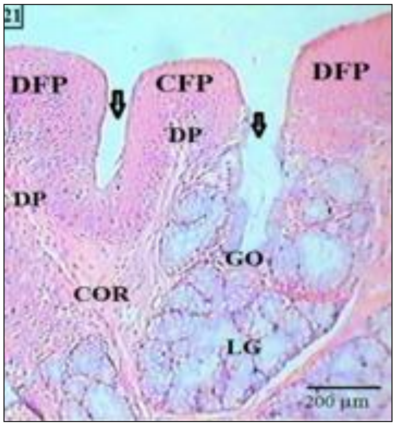

Fig 21: Light photograph of V.S. through the dorsal surface of the tortoise's tongue radix showing: Multi acinar large lingual glands (LG), cuboidal filiform papillae (CFP), dome-shaped filiform papillae (DFP), glandular orifice (GO). Corium (COR) is extended up as dermal papillae (DP). (H&E),Scale bar signifies 200 µm.

Fig 22: Light photograph of V.S. through the dorsal surface of the tortoise's tongue radix showing: Elongated filiform papillae (EFP), fungiform papilla (FUP)both are covered with keratinized epithelium (K), acinar lingual glands (LG) opens in a common glandular orifice (GO), interpapillary space (Arrow). (H&E), Scale bar signifies 300 µm

Discussion

Within Testudines, which are the oldest living reptiles, their tongue's shape and dorsal surface topography show extensive variations perhaps reflect adaptation of these animals to different feeding niches water or land (Bonin *et al*., 2006; Orenstein, 2012) [16, 54]. Although a lot of works has been done on the tongue's ultra-morphology and histology of different aquatic Testudines species, scarce of works has been done on terrestrial species (Tortoises) especially Egyptian tortoise, *Testudo kleinmanni*. This land dwelling species have fleshy triangular not bifurcate tongue firmly situated on the floor of buccal cavity in front of the glottis. The observed unique hidden medial nasal gland having direct buccal connection homologous to vomeronasal organ. Gerlach (2005) [29] declared that, this structure facilitates nasal drinking in the dipsochelys giant tortoises, *Dipsochelys dussumieri*.

In the present study it was found that, the tortoise's tongue is distinguished dorsally into a blunted fore-tongue (apex), mid-tongue (body) and hind-tongue (radix) with deep median sulcus in front of the laryngeal mound. The tongue's dorsal surface is wrapped with plentiful lingual papillae of different shape and size. They are either flattened ridge-like, elongated and/or cuboidal dome-shape filiform widely distributed on the three distinguishable tongue's sectors in addition to infrequently circumvallate and fungiform ones with interpapillary areas in between. Many studies have demonstrated that the dorsal surface of the reptilian tongue is rich with lingual papillae (Iwasaki, 1992; Elsheikh *et al*., 2013; Al-Zahaby *et al*., 2017) [36, 24, 1]. The form and pattern of distribution of these papillae exhibit significant variations even amongst different species belonging to Testudinidae (tortoises) species (Beisser *et al*., 1995 &2004; Bels *et al*., 1997; Beisser *et al*., 1998; Beisser and Weisgram, 2001; Marycz *et al*., 2009; Sabry *et al*., 2015) [12, 11, 15, 13, 10, 50, 58].Nonetheless, no lingual papillae of any form were visible on the tongue's dorsal surface of purely aquatic Testudines (Iwasaki *et al*., 1996a,d) [36].

The apical and lateral side wall's epithelium of the above mentioned lingual papillae is generally stratified epithelium consisted of the universal three epithelial cell layers; the short columnar basal cell layer, the intermediate polygonal mucous cell layers in addition to the most outer intensely stained keratinized surface cell layer. This realization, resembles that of other terrestrial Testudines (tortoises) investigated by Beisser *et al*. (1995) [12], Beisser and Weisgram (2001) [10] and Beisser*et al*. (2004) [11] as well as semi aquatic Testudines studied by Iwasaki (1992) [36], Iwasaki *et al*. (1992) [36], Iwasaki *et al*. (1996c) [38] and Beisser *et al*. (1998) [13].

The alveolar lingual glands observed in the present investigated *Testudo kleinmanni*, are positioned mostly underneath the lingual papillae and open in-between through distinctive tubular glandular ducts of wide orifices. Such glands, are established also in Testudines species famous to be adapted in terrestrial habitats (Wocheslӓnder *et al*., 1999; Beisser *et al*., 1995; Sabry *et al*., 2015) [72, 12, 58]. Beisser *et al*. (2004) [11] further, distinguished three different areas for the lamellar epithelium; a stratified apical area at the top of the papillae, a stratified lateral area as that of the apical area but involved also mucus cells of prevalent mucus granules and finally an unstratified glandular area consisting of distinct glandular ducts with mucus cells. So, as observed in the present *Testudo kleinmanni*, the intermediate cell layers of the universal stratified epithelium covering lingual papillae become thinner and even disappear toward the papillae base, where the glandular ducts of the underneath lingual glands open. To supplement this, somewhat early Beisser *et al*. (1995) [12] also showed that the intermediate epithelial cell layer are not so distinct in the lateral papillary areas where mucus production takes place, since it can deform the epithelium stratification (Beisser *et al*., 2004) [11].

This massive mucous secretion lubricates tongue's surface facilitating ingestion, transport and swallowing of dry plant food items used by Testudines living under comparable life circumstances (Winokur, 1988; Schwenk, 2000b) [71, 61]. It is necessary to mention also that, the copious of microvilli and/or microridges wrapping the different lingual papillae surfaces, may support adhering of secreted mucus on tongue surface and provide rough structures for the uptake of dry food items (Iwasaki *et al*., 1996b) [38].The microvilli copious was also detected on the tongue's papillae surface of the red-eared turtle, *Trachemys scripta elegans* by Marycz *et al*.

(2009) [50]. So, in spite of the terrestrial Testudines have prominent complexly arranged lingual gland system (Heiss *et al*., 2011) [32], the aquatic turtles showed simple mucous secreting cells open directly on the tongue's dorsal surface (Weisgram *et al*., 1989; Beisser *et al*., 2001) [10]. The departure between the lingual epithelium directly involved in food uptake (the surface of the lingual papillae) and those produce mucus (the lingual glands) as observed in the present tortoise become increasingly more distinct in land live turtles (Beisser *et al*., 2004) [11]. The authors also hypothesized that this is necessary to live in dry habitats where keratinization of the lingual epithelium is an adaptational changes during evolution on land.

Keratinization of the tongue's dorsal epithelium of the present studied *Testudo kleinmanni* is pronounced also in many terrestrial reptiles as land dwelling snake (Mao *et al*., 1991; Iwasaki and Kumakura, 1994) [49, 35] and of course land living Testudines (tortoises) as *Gopherusgopherus* (Sabry *et al*., 2015) [58]. Nonetheless, the purely aquatic Testudines either marine species (Weisgram, 1985; Iwasaki, 1996a,d) [69, 36] or fresh water (Beisser *et al*., 1997, 2001 and Lemell *et al*., 2002& 2010) [9, 10, 45, 44] has nearly reduced tongue with smooth dorsal surface lacking any morphological protrusion without any indication of keratinization or cell death. They are carnivorous, grasp and with drawn preys by enormous suction force with the water current in wards induced by fast oropharyngeal volume expansion without contact with the jaws (Anderson, 2009; Lemell *et al*., 2010; Heiss *et al*., 2010) [2, 44, 31]. Meanwhile, the tongue of Testudines species that live under amphibious style life near water bodies tend to have both keratinised and non-keratinised lingual epithelia in various parts of the tongue as illustrated by Marycz *et al*. (2009) [50], Brown *et al*. (2012) [17] and Lintner *et al*. (2012) [46].

Otherwise, the presented highly developed lingual intrinsic musculature surrounds lingual glands in the Egyptian tortoise, appears to squeeze glands to thrust their mucous secretion on the tongue's surface (Winokur, 1988) [71]. However, the aquatic freshwater turtle, *Pelusios castaneus*, lives in rivers, lakes, and shallow ponds have tongue with smooth topographic dorsal surface with irregularly ridge-like papillae and poorly developed lingual intrinsic musculature since it is an omnivore feeds mainly on fish, snails and floating water lettuce (Ernst and Barbour, 1989) [25].Otherwise, since Wocheslánder *et al*. (1999) [72] accepted that, in Testudines the more flexible tongue the more feed on land, so the organization of the hyolingual complex (hypoglossum) in tortoise in combination with the surrounded musculature in the present investigated *Testudo kleinmanni*, reflect the tongue's mobility and the dependence on it for food manipulation (Natchev *et al*., 2009; Heiss *et al*., 2011) [51, 32].

The present detectible barrel shaped taste buds with slightly long tapered cells are imbedded in the lingual stratified epithelium of *Testudo kleinmanni*, as they play a role in tasting and gustation of food items (Manteifel *et al*., 1992) [48]. The setaste organs are also detected early in many other terrestrial and semi aquatic Testudines such as *Clemmys japonica* and *Geoclemys reevesii* (Uchida, 1980) [66], *Chrysemys scripta elegans* (Korte, 1980) [42], *Platemys pallidipectoris* (Beisser*et al*., 1995) and *Trachemys scriptaelegans* (Beisser*et al*., 1998).They are often associated with the tortoise life style where enable them to differentiate between food items and allow fast decision for accepting or rejecting food (Lintner *et al*., 2012) [46].

The tongue's topography of *Testudo kleinmanni* is comparable with that of the other terrestrial herbivores such as *Testudo hermanni* (Weisgram*et al*., 1989; Wocheslánder *et al*., 1999) [72]. Their well-built papillary large tongues with complex well-developed mucous lingual gland system to lubricate sufficiently the tongue's surface during arid food manipulation through the oral cavity (Schwenk, 2000a, b) [60, 61]. Since most of the recent tortoises rely on diets mainly composed of plant material (Ernst*et al*., 2000). The Egyptian tortoise show a clear tendency towards herbivory and have lost their ancestral capability to feed under water and depend exclusively on plants in its terrestrial habitat (Bonin *et al*., 2006) [16]. It eats a wide variety of native vegetation, in arid semi-desert habitats, ranging from grasses to broadleaf plants as perennials and herbal plants which used also as hiding places (Baha El Din, 2006; Schneider and Schneider, 2008; Zwartepoorte, 2015) [6, 59, 73]. They exhibit slow feeding movements (Lemell *et al*., 2002) [45], since they are not in hurry and may prolong the duration of the food uptake (Natchev *et al*., 2015b) [53]. Nevertheless, aquatic turtles, like as *Emys orbicularis*, with their flat relatively small tongue withpoorly developed lingual papillae and glands (Beisser *et al*., 2001; Lemell *et al*., 2002; Heiss *et al*., 2010) [10, 45, 31], provides exclusively hydrodynamic feeding mechanisms under water (Drobenkov, 2014) [22].They are highly specialized predators, trust fast feeding movements to capture prey (Natchev *et al*., 2015a and Kummer *et al*., 2017) [52, 43].

The tortoise's tongue traces the food item prior to food uptake. Since it is used as a prehensile organ for food ingestion as in other tetrapod animals (Schwenk, 2000a; Schwenkand Wagner, 2001) [60, 62]. It seems that, tortoises in general developed behaviour of food uptake on land via lingual food contact prior to jaw closure (Bels *et al*., 2008) [14]. The tongue-food contact further provides tactile information on the position of the food item, since the tortoise's eyes are placed laterally on the head and the turtles are not able to permanently observe the food item (Natchev *et al*., 2015b) [53]. The same authors also approved that, the involvement of the tongue during food uptake in tortoises serves as a tactile sensory tool for the localization of the food item prior to jaw prehension and the tongue is not used as the main food collecting organ.

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