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Structural and functional adaptation of the lingual papillae of the Egyptian fruit bat (*Rousettus aegyptiacus*): specific adaptive feeding strategies

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ABSTRACT

The current investigation was directed to clarify the correlations between the feeding strategy and lingual structure of the Egyptian fruit bat captured from the Egyptian east desert. The current work depends on twelve adult Egyptian fruit bats that observed grossly and with the help of the stereo, light, and scanning electron microscope. There were three types of the lingual papillae; one mechanical filiform and two gustatory (fungiform and circumvallate). There were seven subtypes of filiform papillae were

recognized on the seven lingual regions. There were scanty numbers of fungiform papillae distributed among the filiform papillae on the lingual tip and two lateral parts of apex and body while fungiform papillae completely absent in the median part. There were three circumvallate papillae. The central bulb of circumvallate papillae surrounded by one layer of two segmented circular annular bad. The lingual tip had cornflower-like and diamond-shaped filiform papillae. Histochemical results revealed that the lingual glands were a stronger AB-positive reaction and gave dark blue color, while the reaction for the PAS-stain was negative**.** Also, the glands exhibited a blue color as an indication of positive AB reactivity with combined AB-PAS staining.

Key words: Egyptian fruit bat, lingual papillae, scanning electron microscope (SEM), histology, histochemical examination

INTRODUCTION

The bats were classified as the second-largest mammalian order (After rodent), and organized into two suborders; Mega-chiropters and Micro-chiropters (1). The bats had an arboreal character (2) and the only mammalian-species naturally capable of flight (3). Egyptian fruit bat belonged to *Chiroptera* order, *Mega-chiroptera* suborder, *Pteropodidae* family, *Rousettus genus Rousettus aegyptiacus* species (2, 4). The *Rousettus aegyptiacus* species had six subspecies, the only subspecies that life in Egypt was *Rousettus aegyptiacus aegyptiacus*. The feeding habit of the *Pteropodidae* family depends on fruit, flowers, nectar, and pollen so classified as frugivorous species.

The effect of the feeding system on increasing viability and achievement vertebrate modification to its overall state (5, 6). Generally, there are different feeding styles reported in the different bat-species; the blood suckling bats, insectivorous bats, and frugivorous bats (7). Physiologically, to know the preservation tools of any vertebrate species, you must know its lingual structure (5, 8). The tongue was the most important structure that was modified with the distinctive nutritional ability, behavior, and different types of available food particles (9). Moreover, the lingual structure is modified to play different functions (5, 10). furthermore, the lingual papillae are the most structure on the tongue adapted according to the feeding mechanism (11).

The current investigation was prepared to give a complete morphological description of the lingual papillary system of the Egyptian fruit bat (*Rousettus aegyptiacus*) grossly and by the aid of the stereo, light, and scanning electron microscope. Then, the results were compared with the previous reports in other bat-species.

MATERIALS AND METHODS

Collection of bat samples

The present investigation was carried out on twelve tongues from adult Egyptian fruit bats (*Rousettus aegyptiacus*). The Egyptian fruit bats (*Rousettus aegyptiacus*) were collected from the fruit farms in the Siwa Oasis, Egypt. The current work was done according to the permission of Handling and care of the animal's Ethics Committee on Animal Experimentation of the Faculty of Veterinary Science, Alexandria University. The animals were euthanized by deep halothane inhalation, and the tongues were quickly transferred for stereo and Electron microscope lab. The anatomical terms followed the (12).

Morphological examination grossly and under stereomicroscope

Four tongues from adult Egyptian fruit bats (*Rousettus aegyptiacus*) were used to describe the lingual papillary system. After euthanasia, tongues were dissected, separated, examined, and photographed using a camera (Canon IXY 325, Japan) grossly, and under a stereoscopic Zeiss Stemi 2000-C microscope (Carl Zeiss, Jena, Germany).

Scanning electron microscopic examination

Four tongues from adult Egyptian fruit bats (*Rousettus aegyptiacus*) were fixed in (2% formaldehyde, 1.25% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH~7.2) at 4°C. After fixation, samples were washed in 0.1 M sodium cacodylate containing 5% sucrose, processed through tannic acid, and finally dehydrated in ascending grades of ethanol (5). The samples were then critical point dried in Polaron apparatus (E3000 CPD), attached to stubs with colloidal carbon, and coated with gold-palladium in a sputtering device (*Pelco model 3 sputter coater 91000*). Specimens were examined and

photographed using a JEOL scanning electron microscope (*JSM-6510LV, Japan*) operating at 15 Kv, at the faculty of science, Alexandria University.

Histological and histochemical investigations

Four specimens of the Egyptian fruit bat (*Rousettus aegyptiacus*) tongue were dissected out and fixed in 10% formaldehyde for 48 hours at (pH 7.4), dehydrated in graded series of ethanol, cleared with xylene, and immersed in melted paraffin wax. 5 µm cutting sections were prepared. Then, samples were sectioned in five microns using Leica rotatory microtome (RM 20352035; Leica Microsystems, Wetzlar, Germany) and stained by Haematoxylin and Eosin according to (13) to demonstrate the general histological structure. Extra sections were by Masson's trichrome (14, 15) for staining the collagen and muscle fibers.

For histochemical studies, Some sections were taken and stained by Alcian Blue (16) for acidic mucin; Periodic Acid-Schiff (17) for neutral mucin, and double stain of Alcian blue (pH = 2.5), and periodic Acid-Schiff (AB-PAS) technique for acidic and neutral mucin (18). These sections were examined and photographed under a bright field light microscope (Olympus BX 50 compound microscope).

RESULTS

Grossly, the short tongue from the adult Egyptian fruit bat (*Rousettus aegyptiacus*) was divided into three parts; lingual apex (anterior free part), lingual body (middle part), and lingual root (posterior part). The dorsal lingual surface had three lingual papillary types; one mechanical that was described as filiform, and two gustatory that described as a fungiform, and circumvallate papillae. By SEM perceptions, the shape, size, number, dispersion, direction and, terminology of lingual papillae was species-specific. Their position, shape, size, number, and direction of the papillae and their very own procedures were locale explicit as per the feeding propensities, mechanism of mastication, and sorts of sustenance particles. There were three main directions of all lingual papillae; the posterior or posterior-median or median directions. The ventral lingual surface is connected to the sublingual floor by the lingual frenulum leaving a long free end of the tongue to encourage the opportunity for lingual movement.

The dorsal surface of the lingual root was characterized by the presence of three circumvallate papillae.

Generally, the lingual papillae were located on the lateral region taken the posteriorly or medioposteriorly or medially directions, while that located on the median region and the lingual tip were taken the posterior orientation toward the pharynx and the lingual root. The papillae on the lateral region took the different orientations to help in the collected food particles in the median region of the tongue.

Gross anatomical observations

Macroscopically, the protrusible prolonged tongue of the Egyptian fruit bats (*R. aegyptiacus*) was ended with a round lingual apex and had two round lateral borders (Fig.1A, 2A and 3A). In addition, every lingual district (apex, body, and root) was subdivided into a median and two lateral parts (Fig.1A, 2A and 3A).

The tongue was narrow at the anterior rostral tip, while it was broad at the lingual root. The tongue length was 1.1±0.2cm yet reach 0.44±0.2cm wide at its center part. There were seven subtypes of papillae that were disseminated on seven papillary regions (Fig. 1A, 2A and 3A\Cfr, Dr, Gr, Cr, Lr, Rr, LPr).

Scanning electron microscopy

The anterior lingual region was subdivided into four regions in form of a U-shape: lingual tip, two lateral regions, and median region **(Fig. 1A, B and F\1, 2, 3 and Lt)**. There were two types of lingual papillae: mechanical and gustatory papillae.

Mechanical filiform lingual papillae

There were seven subtypes of filiform papillae that were recognized on the tongue of Egyptian fruit bats and described as the following;

Cornflower-like filiform papillae (Fig. 1B, 1C and 1D\Cfp). This type of filiform papillae took a posterior direction and located on the dorsal surface of the rostral part of the lingual tip and the rostral and two lateral borders of the tongue. These papillae had apex caudally with an elongated posteriorly directed process, and wide base rostrally and elongated body with two elevated lateral borders that bent on the base, in addition to each

one of the lateral border carrying 8-12 posteriorly directed small, pointed processes (Fig. 1D\white and red arrowheads).

Diamond-shaped conical filiform papillae (Fig. 1B and 1E\Dcp). These diamond-shaped conical filiform papillae were posteriorly directed and located on the dorsal surface of the median part of the lingual apex (just rostral to the region of trifid filiform papillae). These papillae had elongated apex caudally with an elongated posterior process, wide base rostrally and diamond-shape body with non-elevated serrated borders that curved rostrally on the base, in addition to non-elevated serrated borders had 30 – 32 posteriorly directed small-pointed processes.

Trifid (Giant) filiform papillae **(**Fig. 1B, 1E, 1F, 1G and 1H\Gfp). The trifid filiform papillae were located on the dorsal surface of the median region of the lingual apex and the anterior part of the lingual body. The organization of papillae was observed as they overlapped on each other and each papilla had a wide rectangular smooth body that carrying 18-22 small pointed posteriorly directed anterior processes (Fig. 1E and 1H\blue arrowheads). While the body was ended posteriorly by a posteriorly directed large three finger-like posterior processes (Fig. 1E, 1G and H\white*).

Round or rectangular conical filiform papillae (Fig. 1G and 1H\Rcp). These papillae were located on the dorsal surface of two lateral regions of the lingual apex (the area around the region of the trifid papillae) and the anterior part of the lingual body. Each papilla carried 26-28 posteriorly directed processes that arisen from all borders of the papillae (Fig. 1H\white arrowheads).

Leaf-like filiform papillae (Fig. 2\Lfp). These papillae had a different orientation; the laterally situated papillae took the median direction, while that located near the median region took the posteromedian direction, but the median situated papillae took the posterior direction (Fig. 2B and 2C\ Lfp1 and Lfp2). These leaf-like filiform papillae were located on all dorsal surface of the posterior part of the lingual body; each papilla had an ovoid-shaped body that carried 14 - 16 posteriorly directed processes that originated from all borders (Fig. 2C, 2D, 2E and 2F\white arrowheads).

Rosette-like filiform papillae (Fig. 3\Rfp). These papillae were located on the dorsal surface of the median part and part of the two lateral parts near the median part of the lingual root till the beginning of the triangular region of the circumvallate papillae. Each

papilla had a round base and body with an apex that terminated by posteriorly directed numerous small processes forming a basket-like shape (Fig. 3C\Rfp).

Long pointed filiform papillae (Fig. 3\Lpf). These papillae were located on the lateral border (Fig. 3B and 3C\Lpf), in addition to the triangular area of circumvallate papillae (Fig. 3D and 3E\Lpf) and the small area posterior to the triangular area (Fig. 3F and 3G\Lpf). The papillae that were situated on the lateral border took the median direction while that on the lateral region took the posteriomedian direction, but those located on the triangular area of the circumvallate papillae took the posterior direction. Each papilla was overlapped the other long pointed tongue-shape without any secondary processes with a central groove (Fig. 3C\dotted line).

Gustatory lingual papillae

The gustatory papillae were recorded with characteristic position, dispersion, and number all through the entire tongue length. These papillae were the fungiform and circumvallate papillae, and described as the following;

Fungiform papillae. There were scanty numbers of the fungiform papillae that randomly distributed among the filiform papillae on the lingual tip (Fig. 1B, 1C and 1E\Fu) and the two lateral parts of the lingual apex and body (Fig.1F\Fu) while, the fungiform papillae were completely absent in the median lingual part (region of trifid filiform papillae and leaf-like filiform papillae and the median region of the lingual root). There was one shape of the fungiform papillae was the quadrilateral appearance. The dorsal surface of the fungiform papillae had micro-ridges and micro-scales in addition to the one or two small depressions for taste pores (Fig. 2F\red arrowheads).

Circumvallate papillae (Fig. 3D, 3E, 3F and 1G\V). There were three rounded circumvallate papillae distributed on the posterior part of the lingual root that took the triangular arrangement; its base directed rostrally with two laterally located papillae while its apex directed posteriorly with one median located papilla. Each papilla consisted of a round central bulb surrounded by a continuous deep papillary groove (Fig. 3D, 3E, 3F and 1G\cb and Ag). The central round bulb was surrounded by one layer of two segmented circular annular bad laterally but, from the rostral and posterior side, not surrounded by this circular bad (Fig. 3D, 3E, 3F and 1G\Ap). The dorsal surface of the

central bulb had an irregular surface with microtubercles, microfolds, microgrooves, and numerous taste buds (Fig. 3F).

Light microscopic examination

Histological observation of the tongue of the Egyptian fruit bat (*Rousettus aegyptiacus*) cleared three types of lingual papillae (filiform, circumvallate, and fungiform papillae) with different subtypes and density that distributed over the whole dorsal lingual surface of the tongue. Each papilla was covered by a keratinized multilayered stratified squamous epithelium and supported by connective tissue core, and underneath the lamina propria and muscle fiber layers. The lingual apex showed numerous filiform papillae that widely dispersed over the dorsal surface, their tips are pointed posteriorly (Fig. 4A, B). Dense connective tissue rich with collagen fibers and blood vessels penetrates deeply into the core of the papillae and continuous with the underneath connective tissue layer (Fig. 4C).

At the lingual body, the dorsal epithelium showed numerous fungiform papillae with a quadrilateral appearance. Their epithelium was covered by a thin detached keratin layer and containing few taste buds (Fig. 4D-F). The three triangular arranged circumvallate papillae were covered the dorsal surface of the lingual root. The papillae were covered with a keratinized mucosal surface (Fig. 5A&B). They were also supported by collagen connective tissue fibers and skeletal muscle bundles. Collagen fibers, lymphoid cells, and blood vessels are occupied the connective tissue layer and are supported by an underlying layer of muscle fibers are arranged in many directions. Abundant fatty cells have been seen between the bundles of muscle fibers (Fig. 5C).

Histochemical results revealed that the lingual glands displayed a stronger AB-positive reaction and gave dark blue color (Fig. 6A, B), while the reaction for the PAS-stain was negative (Fig. 6C, D). In addition, the glands exhibited a blue color as an indication of positive AB reactivity with combined AB-PAS staining (Fig. 6E, F).

DISCUSSION

The variations of the feeding mechanism with the different available feeding particles were reflected on the position, structure, number, shape, nomination and,

orientation of the lingual papillae (5, 8, 11, 19, 20). The common example for the adaptation of the different bat-species of the different feeding mechanism with the feeding on different food particles during the flying lead to the species-variations in the morphological appearance of the lingual papillae as appeared in the previously published data (5, 9, 10, 21-23) and confirmed by the current study on the two bat-species of the different feeding mechanism. The mammalian tongue exhibited numerous morphological adaptations to perform numerous functions including the food particles collection, manipulation, and direction of the food particles towards the esophagus (5, 6).

The tongue of the Egyptian fruit bat (*Rousettus aegyptiacus*) was described as subdivided into three regions; lingual apex, body, and root, similar to that reported in all vertebrate species (5, 6, 8, 21, 24). Moreover, in the Egyptian fruit bat, there was a subdivision of these three regions into; two lateral and single median parts in addition to the anterior lingual region was subdivided into four regions in form of U-shape; lingual tip, two lateral regions and median region, also these subdivision parts carrying lingual papillae of different shape, orientation, nomination, and function, similar to that observed by (5, 9, 25).

Morphologically, the most of researchers were focused on the description of the lingual papillae due to their direct relationship with the feeding style of each species, available food particles (6). Functionally, the papillary system was adapted and this appears in the structure, number, appearance, orientation, and dispersion of the lingual papillae (5). The present description confirmed that the lingual papillae classified functionally into; mechanical and gustatory papillae according to their specific function (5, 9, 19, 21, 26). Moreover, these papillae described as three types in Egyptian fruit bat (*Rousettus aegyptiacus*); one mechanical papilla that described as filiform, and two gustatory papillae that described as fungiform and circumvallate, these findings were similar to that reported in some bats species (10, 19, 27, 28). While (5, 9) observed the presence of four types of lingual papillae; two mechanical and two gustatory in Egyptian fruit bat (*Rousettus aegyptiacus*). However (28) observed the presence of two types of lingual papillae; filiform and fungiform in hematophagous bats.

The lingual filiform papillae had species-specific and region-specific characterization and described as the lingual papillae structure adapted with the feeding

mechanism and the available food particles. For that reason; the filiform papillae had a characteristic appearance and functional adaptation and this appears in their characteristic shape, subdivision, number, directions, and positions (11). Functionally, the subdivision of the filiform papillary system is the most important classification of due to their important role in food particles intake and transportation (5, 9, 11). The anatomical subdivision of the lingual filiform was reported previously in the published articles especially in bat (11). The filiform papillary system was subdivided into seven as reported in the current work in the Egyptian fruit bat (*Rousettus aegyptiacus*), similar to that observed by (29) in bat, however, the six subtypes observed in; Egyptian fruit bat (*Rousettus aegyptiacus*) (5) and Japanese long-fingered bats (30). The five subtypes were noted in lesser dog-faced fruit and nectarivorous bats (19, 28), but the four reported in frugivorous bats (28, 31) and Egyptian fruit bat (*Rousettus aegyptiacus*) (9). However, the three subtypes were observed by (10, 26, 27).

Another factor is affecting the anatomical subdivision of the filiform papillae is the geographical distribution of the bats. This adaptation appears in some cases; The first case in the little red flying-fox bat that feeding on the nectar-feeding so its filiform papillary system adapted by the presence of numerous long giant pointed filiform papillae on the lingual apex (32, 33), while when subjected to the migration to research on food particles other than nectar-feeding, the filiform papillary system modified to depend on the feeding on fruit instead of nectar-feeding (34). The second case in the Egyptian fruit bat that caught from the fruit farms in the Nile Delta of Egypt, the filiform papillary system divided into six subtypes (5, 11), while that catch from the fruit farms in the Siwa Oasis of Egypt in the current study was carried seven subtypes, moreover that caught from Japan had five subtypes but, that catched from Saudi Arabia carried four subtypes only (9), furthermore that caught from Poland had only three subtypes (26). The third case was observed in the filiform papillary system on the lingual tip, the current study reported that the Egyptian fruit bat carried cornflower filiform papillae, while the Egyptian fruit bat that catched from Poland, the lingual tip had numerous small filiform papillae (26), but that catched from japan, the lingual tip had numerous scales-like filiform papillae (35). The current findings agree with previously published data (9, 11, 36) that the anatomical shape, distribution, and nomination of the lingual papillae had a

characteristic features that reflected on the evolutionary taxonomic status of the bat species (9, 37).

The gustatory fungiform papillae were subjected to three main classifications; the first classification according to its function and had three types the gustatory papillae as in the present work in that shown in the current work in Egyptian fruit bat (*Rousettus aegyptiacus*), the same results described by (5, 9, 25, 38), the second one is the named mechanical papillae that not had any taste buds, this type not recorded in any bat species but noted in other species as in donkey (39), the third one is the mixed type that some papillae had taste buds and other do not have in the same animal as this recorded in Australian Megachiroptera (21). The second classification according to the distribution of these papillae as described by (40); the first one described that these papillae found on the entire lingual length (27) in bats, but the second one described that these papillae found on the lingual boundaries (41) in *S. species*, however, the third one described that these papillae found on the lingual tip and side edge areas as observed in; fruit bat (5, 19), flying squirrel (42), while the fourth one noted the presence of these papillae at the lingual center and tip as reported in bank vole and Japanese grass vole (43, 44). Moreover, the present study described that the scanty numbers of these papillae found on the lingual tip and the two lateral parts of the lingual apex and body while, these papillae completely absent in the median lingual part, similar to that described by (5). The third classification according to the shape of these papillae; from the previously published data, all species had only one shape that differs from species to species; the present study observed the quadrilateral shape, similar to that described by (9) in Egyptian fruit bat captured from Saudi Arabia while that captured from Egypt take the dome-shape fungiform as reported by (25), but the round fungiform recorded by (8) in the Egyptian Water Buffalo (Bubalus bubalis), the elliptical shape (27), the dome-shape fungiform (42) in the flying squirrel, the mushroom shape (45), the discoid fungiform observed by (9) in Egyptian tomb bat, and the fungus shape fungiform observed by (46) in the rat. While (5) in Egyptian fruit bat observed the presence of two shapes of the fungiform papillae; rectangular and round.

According to the previously published articles, there are species variations in the number, shape, distribution, position, and shape of its lingual dorsal surface with taste

buds. The most characteristic point is the number of the circumvallate papillae that differ from the completely absent to more than twenty papillae; the circumvallate papillae completely absent in the blood-drinking *Desmodus rotundus* and hematophagous bats (7, 47), but the presence of only one circumvallate papilla observed in some mammalian species (48, 49). Moreover, the most common number is the presence of two papillae as this reported in; common European bat (10); Korean greater horseshoe bat (50); lesser dog-faced fruit and Flying Fox (19, 51); free-tailed bat (20); Korean long-fingered bats (27). However, the presence of three circumvallate papillae found in the present study in Egyptian fruit bat (*Rousettus aegyptiacus*), similar to (5, 9, 25, 26) in Egyptian fruit bat, (19, 51) in frugivorous bats, (52) in *S. australis* and *Pteropus*, (31) in E. wahlbergi and the long-eared hedgehog (*H. auritus*) (49). Moreover, the four circumvallate papillae described by; (28) in long-nosed bat and frugivorous and nectarivorous bats, (52) in Australian Megachiroptera (*N. Robinsoni).* However, there were some mammalian species that carried several circumvallate lingual papillae such as; deer (53) and buffalo (8).

The circumvallate papillae had a round central bulb encircled by a continuous deep groove in Egyptian fruit bat (*Rousettus aegyptiacus*), similar to that described by (5, 9, 54). Moreover, the present investigation described that the central round bulb encircled only by one layer of two segmented circular annular bad laterally but, from the rostral and posterior side, not surrounded by this circular bad. There was some variation in the number of pad layers surrounding the circumvallate papillae; the presence of only one layer of the annular pad was observed in mouse, rat and other rodents (55, 56) and primates (57), while the two layers of the pad were observed in some bat species (5, 41).

The dorsal surface of the circumvallate papillae had an irregular surface by the SEM observations, the same findings were described by; (5, 9) in Egyptian fruit bat and (38) in the rabbit. However, the smooth papillary surface was recorded in fox (58).

The triangular arrangement of the circumvallate papillae was a characteristic appearance that appeared in some mammalian species. The present examination on the two bat-species of various feeding habits had this triangular arrangement of the circumvallate papillae, like that revealed in; fruit bat (5, 9, 25, 26, 31, 51), Egyptian long-eared hedgehog (49), and the Persian squirrel (59).

The obtained histological findings showed that the dorsal surface of the tongue was covered with a thick keratinized multilayered stratified squamous epithelial layer that covering all the dorsal surface with its papillae, similar results recorded by (5, 9, 25) in the Egyptian fruit bat (*Rousettus aegyptiacus*).

The obtained histological findings showed that there were numerous taste buds in both fungiform and circumvallate papillae in the Egyptian fruit bat (*Rousettus aegyptiacus*) tongue. The taste buds were also recorded in most bat species; (9) in the Egyptian tomb bat (*Taphozous perforatus*) and (9, 25) in the Egyptian fruit bat (*Rousettus aegyptiacus*).

The present results corresponding with that reported by (24, 26, 60) that, the presence of the taste buds was related to the process of chemoreception of the gland secretion of the tongue. Furthermore, the description of the lingual glands in the current work matched with the obtained results of (61-64).

Among vertebrate animals, the lingual gland secretions act as a lubricant that assists in moistening the food particles, then facilitates food movements, transport, and swallowing (65-67). The current histochemical results revealed that the lingual glands showed a stronger AB-positive reaction and gave dark blue color, while the reaction for the PAS-stain was negative. This negative reaction with PAS-stain similar to that noted by (25). The current study reported that the glands give a blue color as an indication of positive AB reactivity with combined AB-PAS staining, while (25) reported the AB positive granules with blue color stain the acidic mucins.

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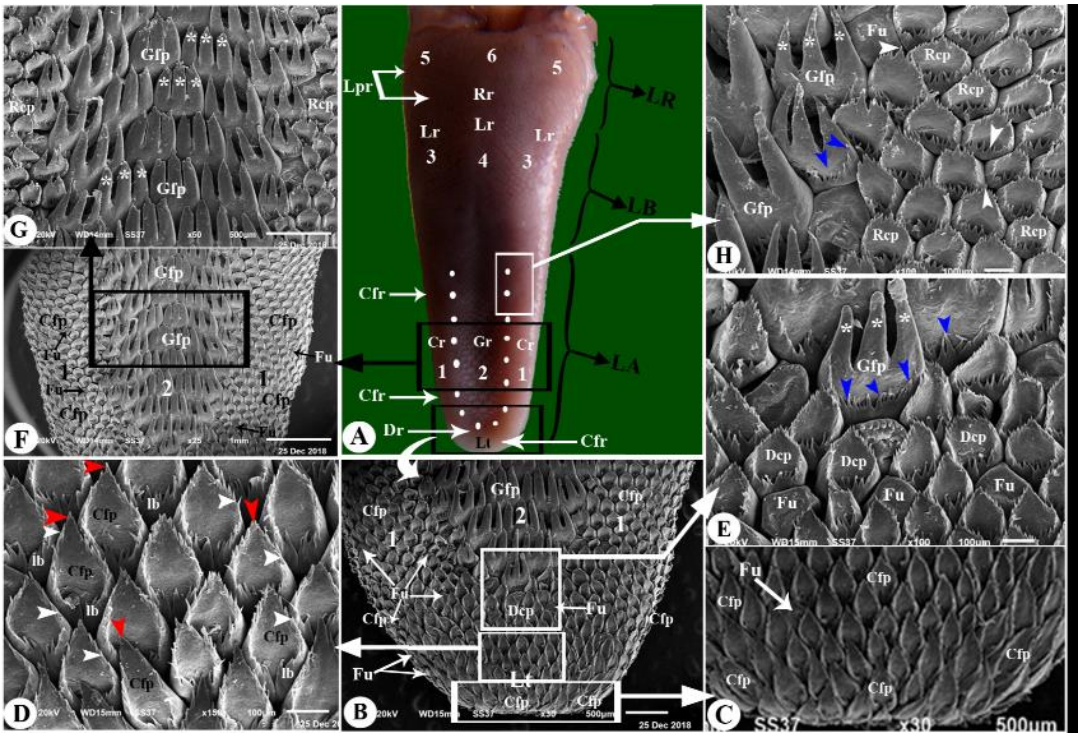

Figure 1: Gross morphological image of the tongue *(View A)* and SEM images *(View B-H)* of the lingual apex of the Egyptian fruit bat to show; **(View A) to show: The lingual regions:** lingual apex (LA) with its lingual tip (Lt), two lateral (1) and median parts (2) and lingual body (LB) with its two lateral (3) and median parts (4); lingual root (Lr) with its two lateral (5) and median parts (6). **The seven regions of the filiform papillae**; region of cornflower filiform papillae (CFr); region of diamond filiform papillae (Dr); region of giant filiform papillae (Gr); region of round and rectangular filiform papillae (Cr); region of leaf-like filiform papillae (Lr); region of rosette-like filiform papillae (Rr) and region of long pointed filiform papillae (Lpr). **(View B-H) show the;** cornflower filiform papillae (Cfp) with the posterior pointed process (red arrowheads) and numerous processes (white arrowheads) on two elevated lateral border that bent on the base (Ib); diamond filiform papillae (Dcp); giant filiform papillae (Gfp); round and rectangular filiform papillae (Rcf), leaf-like filiform papillae (Lfp) fungiform papillae (Fu).

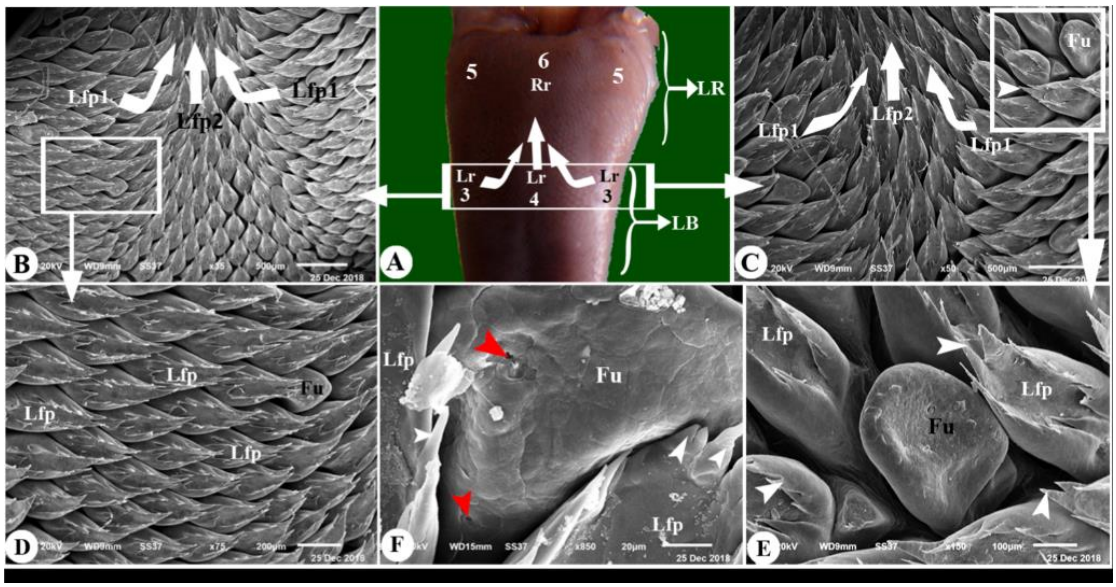

Figure 2: Gross morphological image of the lingual body and root *(View A)* and SEM images *(View B-H)* of the posterior part of the lingual body of the Egyptian fruit bat to show; **(View A) show:** lingual body (LB) and it's two lateral (3) and median parts (4) with the region of leaf-like filiform papillae (Lr). Lingual root (LR) and it's two lateral (5) and median parts (6) with region of rosette-like filiform papillae (Rr). **(View B-H) show the;** region of leaf-like filiform papillae (Lfp1) of medioposteriorly direction on the lateral region of lingual body; region of leaf-like filiform papillae (Lfp2) of posterior direction on the median region of the lingual body. Leaf-like filiform papillae (Lpf); the processes on the margin of leaf-like filiform papillae (white arrowheads) and taste pores (red arrowheads) on the fungiform papillae (Fu).

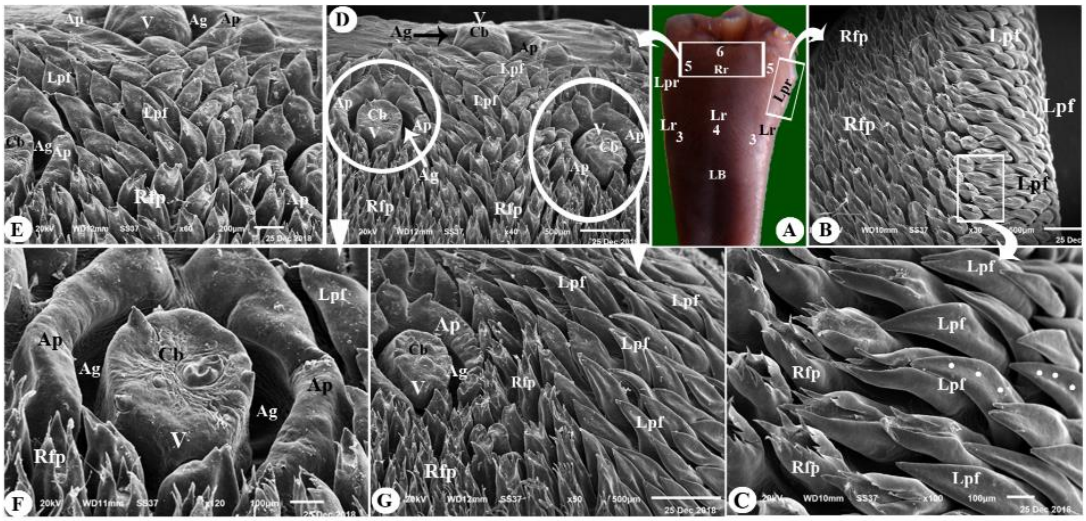

Figure 3: Gross morphological image of the lingual body and root *(View A)* and SEM images *(View B-G)* of the lingual root of the Egyptian fruit bat to show; **(View A) show:** lingual body (LB) and it's two lateral (3) and median parts (4) with the region of leaf-like filiform papillae (Lr). Lingual root (LR) and its two lateral (5) and median parts (6) with the region of rosette-like filiform papillae (Rr) and region of long pointed filiform papillae (Lpr). **(View B-G) show the;** Long pointed filiform papillae (Lpf) with the groove on it's dorsal surface (white dotted line); circumvallate papillae (V) with central bulb (cb) and surrounded by a continuous deep the groove (Ag) that surrounded by one layer of two segmented circular annular bad (Ap).

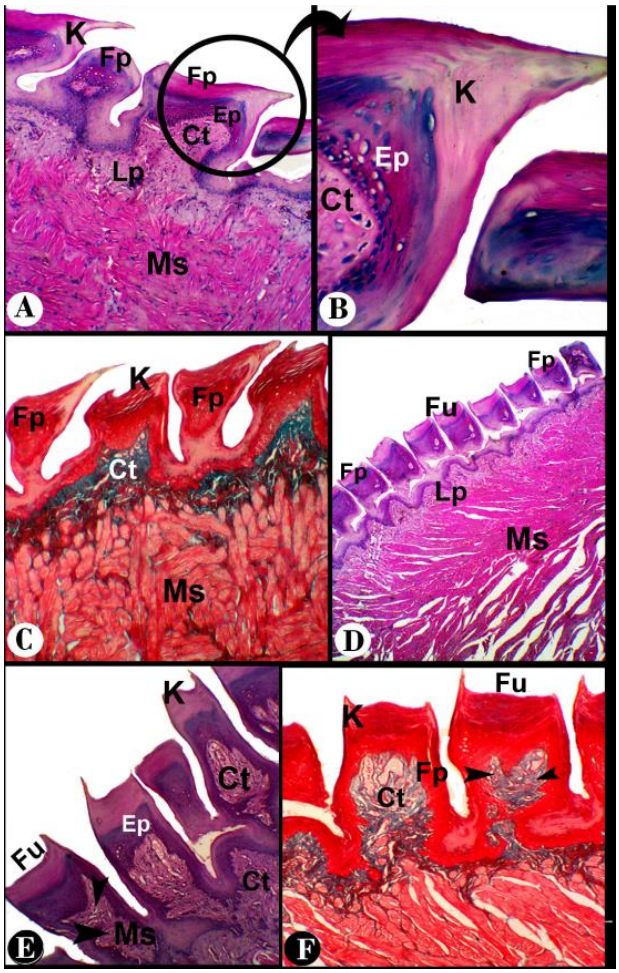

Figure 4: Histological image of the lingual apex (Views A-C) and body (Views D-F) of the Egyptian fruit bat to show; **View A**: Represent the dorsal view of the lingual apex of the Egyptian fruit bat (*Rousettus aegyptiacus*), **View B represents the** higher magnification of the filiform papillae (**Fp). H&**E. **View C represents the *MASSON'S TRICHROME stain* of** the lingual apex to clear the collagen connective tissue (**Ct)**. **View D represents the** dorsal view of the lingual body of the Egyptian fruit bat (*Rousettus aegyptiacus*), and **View E represents the** higher magnification of the dorsal view of the lingual body of the Egyptian fruit bat (*Rousettus aegyptiacus*). **H&E. View F represents the *MASSON'S TRICHROME stain* of** the lingual body of the Egyptian fruit bat (*Rousettus aegyptiacus*). ***Abbreviation:*** Filiform papillae (**Fp)** with a thick keratinized layer (**K)**, the dorsal epithelium (**Ep)**, lamina propria (Lp), connective tissue core (**Ct)**, muscles (**Ms),** fungiform papillae (**Fu),** and the **black arrowheads** refer to the taste buds in the fungiform papillae.

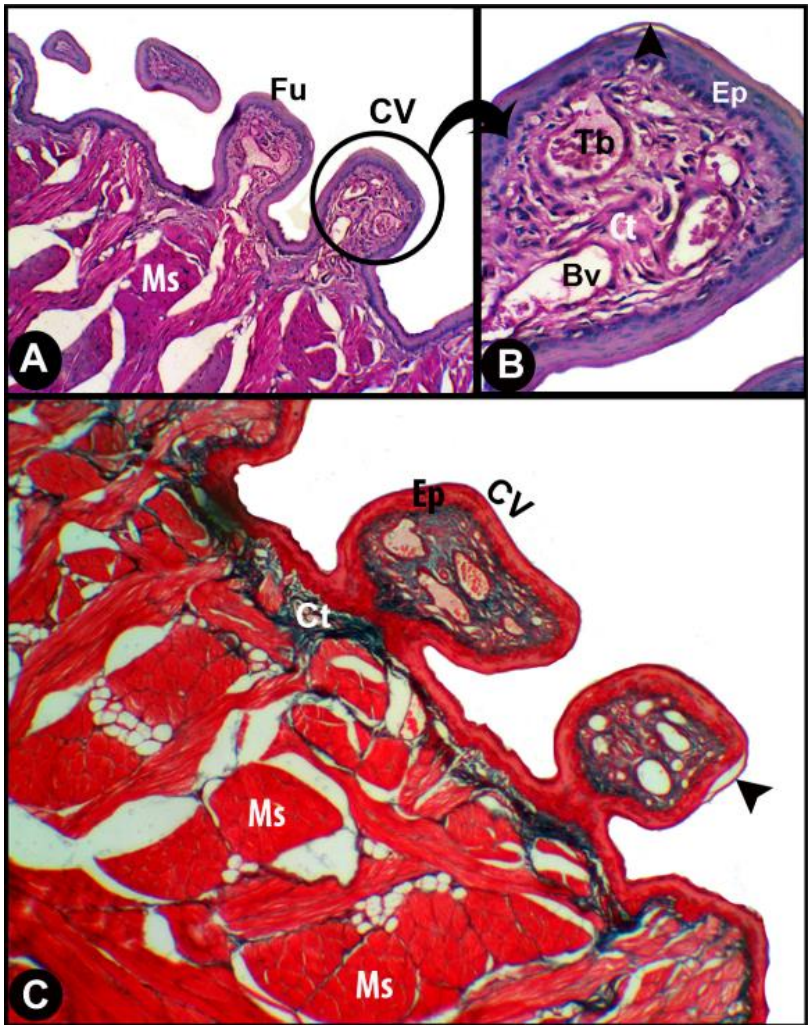

Figure 5: Histological image of the lingual root (Views A-C) of the Egyptian fruit bat; **View A**: Represent the dorsal view of the lingual root of the Egyptian fruit bat (*Rousettus aegyptiacus*), **View B represents the** higher magnification of the circumvallate papillae (**CV). H&E stain. View C represent the *MASSON'S TRICHROME* (AB) *stain* of** the lingual root to clear the collagen connective tissue (**Ct)**. ***Abbreviation:*** The dorsal epithelium (**Ep)**, connective tissue core (**Ct)**, lingual muscles (**Ms),** circumvallate papillae (**CV)** with a keratinized layer (**black arrowhead**), taste bud (**Tb)**, and blood vessel (**Bv)**.

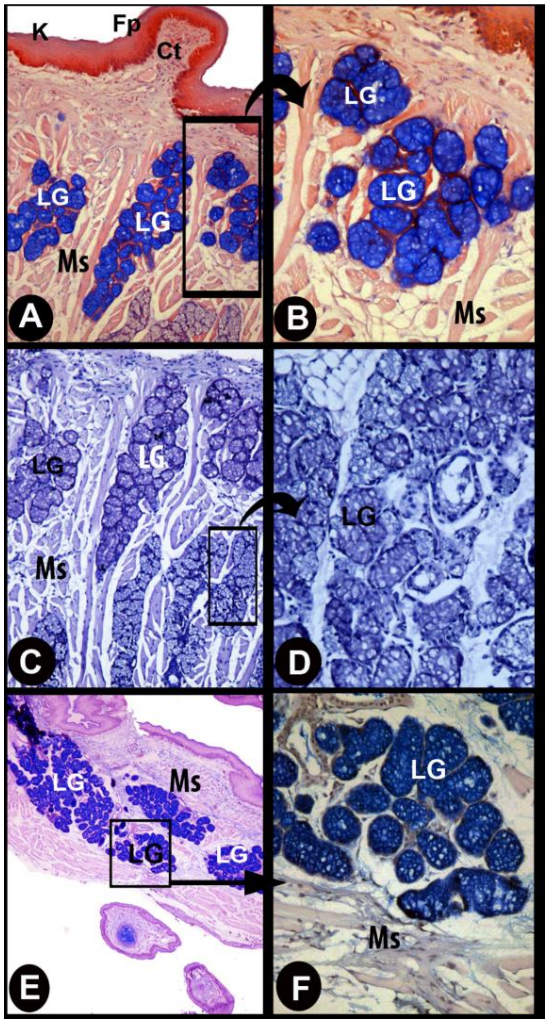

Figure 6: Transverse Histological image of the lingual root (Views A-F) of the Egyptian fruit bat; **(View A** and B) represent the transverse sections of the tongue of the Egyptian fruit bat (*Rousettus aegyptiacus*) showing a positive acidic mucin reaction of the **LG**: lingual glands. **ALCIAN BLUE stain**. **(View C** and D) represent the transverse sections of the tongue of the Egyptian fruit bat (*Rousettus aegyptiacus*) showing a negative neutral mucin reaction of the **LG**: lingual glands. **PERIODIC ACID-SCHIFF (PAS) stain**. **(View E** and F) represent the transverse sections of the tongue of the Egyptian fruit bat (*Rousettus aegyptiacus*) showing a positive AB reaction and a negative PAS reaction of the **LG**: lingual glands. **ALCIAN BLUE & PERIODIC ACID-SCHIFF (AB&PAS) stain**. ***Abbreviation:*** Circumvallate papillae (**CV),** keratinized layer (**K**), and connective tissue core (**Ct)**, lingual gland (**LG)**, and lingual muscles (**Ms).**