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Ultrastructural features on the oral cavity floor (tongue, sublingual caruncle) of the Egyptian water buffalo (*Bubalus bubalis*): gross, histology and scanning electron microscope

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Abstract

The present work was focused on the morphological characters of the lingual caruncles, and tongue with its papillae of Egyptian water buffalo *(Bubalus bubalis)* using the gross, light, and scanning electron microscope. The ventral surface of the sublingual caruncle carried a small opening of the duct of both monostoamtic and mandibular salivary gland. The lingual mucosa of

dorsal, lateral border, some extent to ventral surface of apex had lingual papillae (filiform, fungiform), while the lingual mucosa of body especially at torus linguae had conical papillae, but circumvallate papillae observed at the caudal part of body and root. The dorsal surface of apex and body carried numerous long thread-like with blunt apex caudally directed filiform papillae that covered with keratinized scales without secondary papillae. The degree of keratinization classified filiform papillae into rostral part of high keratinization and caudal less keratinization. Conical papillary surface carried exfoliated epithelium with longitudinal groove on its rostral surface and had CT core does not reach the level surface of epithelium and carried secondary papillae. Fungiform papillae scattered among filiform papillae on the dorsal and ventral surface of apex and its convex surface had exfoliated keratinized epithelium. Circumvallate papillae surrounded by circular deep groove that bordered by vallum that carried small secondary papillae that ended into the primary groove. Taste buds of circumvallate papillae opened in the lateral lining epithelium facing the groove. Von Ebner's glands were observed in CT under papillae especially toward the groove and their ducts open into the base of the groove.
Key words: lingual caruncles, tongue, lingual papillae, Egyptian water buffalo, histology, scanning electron microscope (SEM)

INTRODUCTION

Egyptian water buffalo *Bubalus bubalis bubalis* (Linnaeus, 1758) subspecies was classified under *Bubalus bubalis* species, *Bubalus* genus, *Bovidae* family, *Artiodactyla* suborder, *Ruminantia* order. The Egyptian water buffalo is the most important domestic animal and their number reached 2.5 million (1) in Egypt. The Egyptian water buffalo feed mainly on the green grasses, plants, and other roughage materials with the help of large lips, and elongated protrusible tongue to collect the food materials (2, 3).

The tongue was the most important organ that was modified with characteristic feeding tendencies, behavior, and various types of food particles that could be accessed, moreover, the lingual structure was modified to play different abilities, for example, feeding input, control, and ingestion of nutrition molecules (4). The morphological appearance, prevalence, orientation, and structure of lingual papillae were modified in accordance with the nutritional requirements, the types of nutritional particles accessible, and the various environmental conditions (4). Most previously published anatomical articles concerned on the tongue and its papillae of the domestic

animal species (5-13) but there were rare data on the Egyptian water buffalo (*Bubalus bubalis bubalis*) from Egypt.

The available data about the sublingual caruncle and sublingual floor of the domestic animals including the Egyptian water buffalo (*Bubalus bubalis bubalis*) were scanty. The current work was conducted to give a full morphological characterization of the tongue with its papillae, sublingual caruncle, and the sublingual floor of the Egyptian water buffalo (*Bubalus bubalis bubalis*) using the gross, SEM, and histological techniques to describe the relation of our findings with the feeding mechanism of Egyptian water buffalo (*Bubalus bubalis bubalis*). Then, the obtained findings were compared with those reported in the formerly published articles on the ruminant and other domesticated animal species.

MATERIALS AND METHODS

Sample's collection

Twelve heads from the normal adult Egyptian water buffalo (*Bubalus bubalis bubalis*) of both sexes were collected from the local slaughterhouse in Kafrelsheikh Governate, Egypt. The samples were collected directly after slaughtering. The present investigation was prepared according to the guidelines for the using and caring of the laboratory animals and follow the animal Ethics and welfare in the Faculty of Veterinary Medicine, Alexandria University and according to the Egyptian laws.

Gross morphology observations

The tongue, sublingual caruncle, and sublingual floor were collected from four Egyptian water buffalo heads of both sexes, were prepared to demonstrate the gross morphology of the tongue, sublingual caruncle, and sublingual floor. Then, the collected samples were fixed in 10% formalin. The gross morphological images were examined and photographed by a digital camera (Cannon IXY 325, Japan). The anatomical terms followed the (14).

For histological and histochemistry studies

The tongue, sublingual caruncle, and sublingual floor from four heads of Egyptian water buffalo (*Bubalus bubalis bubalis*), were used in the histological techniques in the fresh state according to (15) to examine under a light microscope. The collected samples (tongue, sublingual caruncle, and sublingual floor) were fixed in 10% normal buffer formalin. After 24h, the samples were gently transferred to 70% alcohol. Then, they were dehydrated in ascending graded series of ethanol. Then, the samples were cleared in xylene and impregnated and embedded in paraffin wax. Sections of 5-7 µm were cut using Leica rotatory microtome (*RM 2035*) and mounted on glass slides. Paraffin sections were used for conventional staining (H&E) for general histological examinations (16).

For histochemistry processing, the sections were stained with Periodic Acid-Schiff technique (PAS) (ab150680) to demonstrate the neutral mucin (17), Alcian Blue (AB) at pH of 2.5 (ab150662) for acidic mucin (18, 19), Van Gieson (mixture of picric acid and acid fuchsin) for elastic fibers in connective tissue, trichrome for collage fibers in the connective tissue (20). The histological techniques and stains were adopted according to (15).

For scanning electron microscopy

Four Egyptian water buffalo (*Bubalus bubalis*) heads were used to collect the samples (tongue, sublingual caruncle, and sublingual floor) used in the SEM technique (21, 22). The collected samples (tongue, sublingual caruncle, and sublingual floor) were fixed at 4°C in a solution of fixation formed from; 2% formaldehyde, 1.25% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2. Once fixation occurs, the collected samples were washed in 0.1 M sodium cacodylate containing 5% sucrose, processed through tannic acid. Finally, the lingual samples were dehydrated by using an increasing concentration of ethanol (15 min each in 50, 70, 80, 90, 95, and 100% ethanol). The collected samples were then dried in carbon dioxide and attached to stubs with colloidal carbon and coated with gold palladium in a sputtering device. In the end, the collected samples were examined and photographed with a JEOL scanning electron microscope operating at 15 kV, at the faculty of science, Alexandria University.

RESULTS

I- Sublingual floor and sublingual Caruncle

A- Gross morphological appearance of the sublingual floor and sublingual caruncles

The floor of the oral cavity had the tongue, the sublingual caruncles, and the musculocutaneous wall. The sublingual floor of the oral cavity was crescentic in shape and located under the tongue within the dental arch (Fig. 1A). It was subdivided into two parts: the prefrenular part rostrally and the two sublingual recesses caudally. The prefrenular part was located caudal to the central incisors and extended caudally to the level of frenulum linguae. This part carried two sublingual caruncles (Fig. 1A/SC). The ventral surface of the sublingual caruncle carried a small opening at which opened the duct of both monostoamtic sublingual salivary gland and the mandibular salivary gland.

The frenulum lingua was a mucosal membrane fold connecting the ventral surface of the tongue with the floor of oral cavity. The frenulum lingua was wide, extensive, and single fold, and it located caudal to the level of the central incisors. The lateral sublingual recesses (Fig. 2A/SLR) were bounded laterally by the lower cheek teeth and medially by the lateral lingual surface. It was extended from the frenulum linguae rostrally till the level of the pterygomandibular fold caudally. This part had a conical papillae of pointed apex rostrally, while its caudal part was smooth. These conical papillae arranged in two rows; the upper row had about 15 papillae and the lower one had about 10 papillae only (Fig. 1A/CP).

B- Histological observations of the sublingual floor and sublingual caruncles

The floor of oral cavity was lined by stratified squamous keratinized epithelium. Under the epithelium, there was a thick dense irregular connective tissue layer of propria-submucosa of the dermal layer. There are numerous epidermal pegs interdigitated with numerous dermal papillae (Fig. 1B). The sublingual caruncle was lined by stratified squamous keratinized epithelium. Under the epithelium, there was a thick dense irregular connective tissue layer that had two ducts, which opened to the ventral surface of the sublingual caruncle (Fig. 1C). The sublingual caruncle had two surfaces; the dorsal (like that of the floor) and the ventral surface that had aggregations of the lymphocytic nodule's (Fig. 1D). The two ducts in the submucosa of the ventral; one duct of the mandibular and other of monostoamtic sublingual salivary glands (Fig. 1D/MO, GO).

The sublingual recess consisted of stratified squamous keratinized epithelium. The submucosa was highly vascular, highly innervated dense irregular connective tissue contained

the sublingual salivary glands. These glands were mucoserous in nature that had positive reaction of PAS and AB stain (Fig. 2C).

The frenulum lingua was lined with stratified squamous keratinized epithelium, and the submucosa consisted of dense irregular connective tissue that highly vascular and innervations. The submucosal layer sends dermal connective tissue papillae to invaginate the mucous membrane and interdigitated with the epidermal pegs. These connective tissue papillae carried secondary papillae (Fig.1E and 2D).

II- Tongue

A- Gross morphological characters of tongue

The tongue occupied the oral cavity proper in the sublingual floor when the upper and lower teeth closed with each other. It reaches to 36-38 cm in length (from the lingual root to the lingual apex) and 32 cm (from the palatoglossal fold to the apex). The free lingual part measured about 9-10 cm in length that formed about 25% of the total lingual length. The lingual width was wider rostrally than caudally. The tongue divided into three parts: the apex, the body and the root. The rostral lingual part (apex) was flattened dorsoventrally with two surfaces (dorsal and ventral) and two borders. While the caudal lingual part (body and root) had only the dorsal surface and two lateral borders (Fig. 3A). The dorsal lingual surface had a large, rounded prominence (torus linguae) that located infront of its deep fossa (fossa linguae) (Fig. 3A, 4A/TL, FL).

The mechanical papillae represented by three papillary types: filiform, conical and lentiform papillae. The filiform papillae were the numerous papillae observed on the lingual mucosa that give the raspy appearance of the buffalo tongue. These papillae distributed all over the dorsal lingual surface till the beginning of the torus linguae and extended to the lateral border and to some extension on the ventral surface (Fig. 3A). Also, these papillae observed on the lateral border of the body. The caudally directed conical papillae were restricted only to the torus linguae, especially on its central part. These papillae of different size; the large ones observed at the center of torus linguae and their size decrease at the periphery of laterally directed (Fig. 3A-C/CP). The different sized lentiform papillae observed on the dorsal lingual surface at the torus linguae only (Fig. 3A-C/blue arrowheads).

The gustatory papillae represented by two papillary types; fungiform and circumvallate papillae. The fungiform papillae were widely distributed all over the dorsum surface and lateral border of the tongue. There were two subtypes of fungiform papillae; the first subtype was the pigmented papillae and concentered only on the rostral part of apex and the lateral border of the tongue (Fig. 3A-B/yellow arrowheads). The second subtype was non-pigmented and observed on the caudal part of the apex and torus linguae (Fig. 3C/FU). The circumvallate papillae were observed on the caudal lingual part infront of the palatoglossal fold (Fig. 4A-B and 7A-B). These papillae arranged in 2-3 irregular rows with different sized papillae (Fig. 4B). They were rounded and surrounded by groove which in turn surrounded by an elevated part named vallum. There are two papillae with one groove and vallum (Fig. 4B/green ovoid). Their number ranged from 18-24 in number on each side of the tongue. There is some variation from right to left ranged from 1-2 papillae. The foliate papillae were absent and instead there were low mucosal folds infront of the glossopalatine fold.

The smooth lingual root was free from any papillary type but contain the openings of the posterior lingual gland that covered the root surface with their secretions (Fig. 4A). The ventral lingual surface was attached to the sublingual floor by the lingual frenulum, its mucous membrane is somewhat loosely attached to the underlying muscles.

B- SEM characters of tongue

The dorsal lingual surface of the apex and body carried numerous long band thread-like with blunt apex caudally directed filiform papillae (Fig. 3D). The papillary surface covered with keratinized scales without secondary papillae (Fig. 3E/black arrow). The origin of the filiform papillae appeared as a depressed area (Fig. 3D/red star). The interpapillary space had some exfoliated keratinized epithelia-like filiform papillae surface (Fig. 3E-F/red arrow). The filiform papillae on the ventral surface were less numerous, shorter than that observed on the dorsal surface, in addition it carried pointed tips with longitudinal groove without secondary papillae.

The surface of the large sized broad blunted short conical papillae was rough and carried exfoliated epithelium (Fig. 3I/CP). The higher magnification clarified that the presence longitudinal groove on its rostral surface (Fig. 3G/CG) and the interpapillary surface was exfoliated (Fig. 3G-H/Sc).

The circular or rounded or dome-shaped fungiform papillae were scattered among the filiform papillae on the dorsum of the tongue and the ventral surface of the apex (Figs. 3D and 4F/FU). The convex surface of the fungiform papillae had exfoliated keratinized epithelium and three circular openings of the taste pores (Fig. 3I-J/white arrows).

The rounded circumvallate papillae surrounded by circular deep groove that bordered by an elevated ridge (vallum) as appeared in (Fig. 4C/white arrow). The vallum carried small secondary papillae that ended into the primary groove (Fig. 4C/black arrowheads). The surface of the circumvallate papillae was flattened and raised above the lingual surface and carried numerous small and rounded opening of taste pores (Fig. 4D).

The lingual root had numerous folds and depression. By high magnification, the root revealed irregular shaped opening of posterior lingual glands (Fig. 4E/black arrow). The edges were elevated. The surface of the cells of root carried numerous microridges (Fig. 4E).

C- Histological characters of tongue

The lingual epithelium followed by a vascular highly innervated dense irregular connective tissue. The lingual core consisted of striated muscular fibers took different orientations (Fig. 4G, 5-7). The lingual mucosa of the dorsal, lateral border, some extent to the ventral surface of the rostral part of the lingual apex had lingual papillae (filiform, and fungiform). While the lingual mucosa of body especially at the torus linguae had conical papillae, but the circumvallate papillae observed at the caudal part of the lingual body and root.

The lingual papillae divided into mechanical and gustatory papillae according to the presence of the taste buds. The mechanical papillae included the filiform, conical, and lentiform papillae while the gustatory papillae included fungiform and circumvallate papillae (Fig. 5-7).

The filiform papillae were cornified and appeared as a thread-like with two surfaces: caudal concave and rostral convex surfaces. The keratinized layer differentiated into two parts according to the degree of keratinization: the rostral part of highly keratinization and the caudal part less keratinization papillae (Fig. 5B-D). The conical papillae consisted of highly keratinized stratified squamous epithelium and connective tissue core which does not reach the level surface of epithelium and carried secondary papillae (Fig. 6D).

The fungiform papillae surrounded by a shallow furrow (Fig. 6A-B, 6E). It covered with stratified squamous keratinized epithelium, and its basal cell layers contained pigmented

granules in some papillae. The fungiform papillae contain taste buds that opened by taste pore to the dorsal surface (Fig. 6B, 6E). The connective tissue core was highly vascular dense irregular connective from the secondary papillae originated (Fig. 6A-B).

The rounded circumvallate papillae covered by stratified squamous epithelium that keratinized at its dorsal surface and less keratinized at its lateral border. The taste buds observed as a lightly stained small areas in the lateral lining epithelium facing the groove (Fig. 7C-D/yellow arrowheads, Cg) that opened by taste pore into the groove with taste hair on the taste duct (Fig. 7D-E/TP, TH). The papillary core consisted of highly vascular and innervated dense irregular connective tissue carrying secondary papillae that penetrated the epithelium lining of the papillae. The lobules of the serous glands (von Ebner's glands) observed in the connective tissue under the papillae especially toward the groove and their ducts opened into the base of the groove (Fig. 7C/white arrowheads).

The lingual root had seromucoid and mucous glands were observed between the skeletal muscular bundles. Their ducts open in the dorsal lingual surface. The seromucoid glands were numerous than mucous ones (Fig. 4G).

DISCUSSION

The present study prepared to give a complete description of the gross, SEM, histological, and histochemistry characters of the sublingual floor with their caruncles, and the tongue with its papillary system of the Egyptian water buffalo. Similar to that previously published articles (8, 12, 23-26), the tongue of the mammalian species is subdivided into three parts; the apex, the body and the root. The current work described that the flattened dorsoventrally lingual apex had two surfaces (dorsal and ventral) and two borders, while the caudal lingual part (body and root) had only the dorsal surface and two lateral borders. Moreover, (12) classified the ventral lingual surface of the apex into the papillary and non-papillary region by the presence of the projected U-shaped line of fungiform papillae.

The current observation observed that the sublingual caruncle lined with stratified squamous keratinized epithelium and the ventral surface of the sublingual caruncle had two ducts: one duct of mandibular and the other of monostoamtic sublingual salivary glands. These observations were similar to those reported by (27).

The ruminant's species similar to the herbivorous mammals characterized by the presence of the large, rounded prominence named the torus linguae that bordered rostrally by the deep fossa named the fossa linguae on the dorsal lingual surface of the body as described in the published textbooks about the ruminant's species (27), in addition to the some published articles about the ruminant's species as in; Egyptian water buffalo (12, 13), cattle-yak *Bos taurus* (6), reeves' muntjac deer *Muntiacus reevesi* (28), Alpaca *Vicugna pacos* (29). Also, the characteristic lingual prominence was described in other herbivorous mammalian species as in; New Zealand white rabbits (26), Nile grass rat *Arvicanthis niloticus* (30). Meanwhile, this lingual prominence was absent in the tongue of the carnivorous, omnivorous monkeys and pigs (24, 31). Functionally, the presence of the lingual prominence on the dorsal surface of the body was correlated to the herbivorous habits of numerous mammalian species due to this prominence play an important role in the mastication process with the hard palate. The presence of the deep fossa lingua is a characteristic feature of the ruminant species (6, 12, 13)

The species-variations about the appearance, size, number, distribution, nomenclature, microstructure, and directions of the lingual papillae among different animal species, was related to the feeding habits and the available food particles (6, 12, 13, 24, 26, 28-32). The current work confirmed the classifications of the lingual papillae into mechanical and gustatory papillae. The current study observed the presence of five types of lingual papillae, three types of mechanical papillae (filiform, conical, and lentiform) and two types of gustatory papillae (fungiform and circumvallate), similar to that described in other ruminant's species such as in; Markhoz Goat *Iranian Angora* (33), cattle-yak *Bos taurus* (6) and chital deer *Axis axis* (34). Meanwhile, the presence of four type that described as two mechanical (filiform and conical) and two gustatory (fungiform and circumvallate) were described in other ruminant's species such as; Barking deer (35), and Bactrian Camel (36), but the four papillae described as one mechanical (filiform) and three gustatory (fungiform, foliate and circumvallate) were observed in other herbivorous species such as; rabbit (26), and rat (30, 37). Functionally, the description of the presence of the different five types of lingual papillae in the current examined Egyptian water buffalo had an important role in the identification and selection of the food particles in the Egyptian farms.

Functionally, the fungiform papillae are classified into three classifications according to function, distribution, and appearance. They classified according to function into three; the gustatory fungiform type was described in the current examined Egyptian water buffalo, similar

to that reported by (12, 26, 38) in rabbit, Egyptian water buffalo, and pampas deer. Meanwhile, the mixed fungiform papillae (some papilla have taste buds and some do not have them) were described in the horse, buffalo, and cattle (7, 39). While, the mechanical fungiform papillae (no taste buds) were described in small ruminants goat (40) and the donkey (41). The other classification depending on the SEM appearance of the fungiform papillae; the current work observed the presence of the circular or rounded or dome-shaped fungiform papillae among the filiform papillae on the dorsum of the tongue and the ventral surface of the apex, while the two appearances were rectangular on the dorsal surface of the apex, and round on the dorsal surface of the body were described in Egyptian fruit bat (42) meanwhile, the only one fungiform appearance was described in most mammalian species but with different shape; dome papillae were observed in the raccoon dog and fox (31), round papillae were observed in the tiger and in the pampas deer (38, 43), Mushroom papillae were observed in the Saanen goat (40, 44), fungus papillae in mouse (45).

The current observed circular or rounded or dome-shaped fungiform papillae were scattered among the filiform papillae on the dorsum of the lingual apex and body, in addition to some extent to the lateral border and the ventral surface of the apex. Moreover, the presence of the round fungiform papillae observed among the filiform papillae on the two lateral regions of the dorsal surface of the anterior and middle lingual part, in addition to the papillary region of the ventral surface of the lingual tip and the two lateral borders of the tongue (12) in Egyptian Water Buffalo *Bubalus bubalis*. Generally, the fungiform papillae were scattered among the filiform papillae on the dorsal surface of the apex as described in some herbivorous ruminants (11, 23, 35, 36, 40), but in the deer (34, 38), and Mazama species (46), the fungiform papillae distribution were extended to the torus linguae.

The current findings described the presence of the papillary circular groove surrounded the fungiform papillae, similar to that reported in some ruminant species (11, 12, 23, 26, 35, 36, 40), meanwhile this papillary groove was completely absent as described in some ruminant species (38) in pampas deer.

The lenticular papillae were observed only on the torus linguae, the same findings were described in other ruminants (12, 36), meanwhile the completely absent of the lenticular papillae on the torus linguae was reported in reeves, barking deer, or mazama species (28, 35, 46, 47). Functionally, the presence of the mechanical conical and lenticular papillae had a great role in

the holding of the food particles during the mastication processes within the oral cavity and help in the movement of masticated food particles toward the pharyngeal cavity (12).

The SEM observations noted that the rough large-sized broad blunted short conical papillae were restricted only to the torus linguae especially on its central part and carried exfoliated epithelium with the presence of longitudinal on its rostral surface. The presence of these papillae on the torus linguae was reported in numerous ruminant species (11, 35, 38) while, (12) observed the presence of the large conical papillae on the dorsal lingual surface of root just posteriorly to the torus linguae. However, the complete disappearance of the conical papillae were described in the Bactrian camel *Camelus bactrianus* (36). Functionally, the presence of the conical papillae was joined with the fixation of the food particles during the mechanical mastication of the green grasses in all ruminant species.

The number of circumvallate papillae differ among mammalian species of the different feeding habits; the only one circumvallate papillae was described by (30) in Nile grass rat (*Arvicanthis niloticus*), while the two circumvallate papillae were observed in New Zealand white rabbit (26), and horse (8), meanwhile, the three circumvallate papillae were described by (30) in Egyptian long-eared hedgehog *Hemiechinus auritus*, (48) in koala, and (42) in Egyptian fruit bat. Moreover, the four circumvallate papillae were found in Japanese marten and tiger (43). Furthermore, the current work agree with the previous work on other ruminants species that, there were numerous circumvallate papillae such as in; Egyptian water buffalo (12), cattle (49), and reeves' muntjac deer *Muntiacus reevesi* (28), goat (10, 50), lambs (23), and deer (35, 51). The observation of the prominent papillary groove surrounded the circumvallate papillae were described in the Egyptian water buffalo (12), gazelle (52), goat (11, 40), lamb (23), Alpaca (29) and cattle (53). The current observation reported that the dorsal surface of the circumvallate papillae was rough and irregular, similar to the noticed by (12, 31) in Egyptian water buffalo, dog, and fox however, the smooth dorsal surface of the circumvallate papillae was observed in; fox (54).

The degree of keratinization of the tongue, especially its dorsal surface, is one of the most important lingual adaptations regarding the different feeding mechanisms with available food particles moreover, this keratinization extended to the lingual papillae (30). The filiform papillae had keratinized layer differentiated into two parts according to the degree of keratinization: the rostral part of high keratinization and the caudal part less keratinization

papillae, also the conical papillae had a highly keratinized stratified squamous epithelial layer. Moreover, the fungiform papillae had stratified squamous keratinized epithelium, but the circumvallate papillae had a stratified squamous keratinized layer at its dorsal surface and less keratinized at its lateral border.

The circumvallate taste bud's distribution had some variation among species. The current findings described the presence of numerous taste buds always along the entire length of the papillary wall faced the papillary groove, similar description were reported in camel (55, 56), cattle (57, 58) and deer (35).

CONCLUSIONS

The ventral surface of the sublingual caruncle carried a small opening of the duct of both monostoamtic and mandibular salivary gland. The lingual mucosa of the dorsal, lateral border, some extent to the ventral surface of apex had lingual papillae (filiform, fungiform). The dorsal surface of the apex and body carried numerous long thread-like with blunt apex caudally directed filiform papillae that covered with keratinized scales without secondary papillae. The degree of keratinization classified filiform papillae into rostral part of highly keratinization and caudal less keratinization part. Fungiform papillae scattered among filiform papillae on the dorsal and ventral surface of apex. Taste buds of circumvallate papillae opened in the lateral lining epithelium facing the groove. Von Ebner's glands were observed in CT under papillae especially toward the groove and their ducts opened into the base of the groove.

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Figure legends

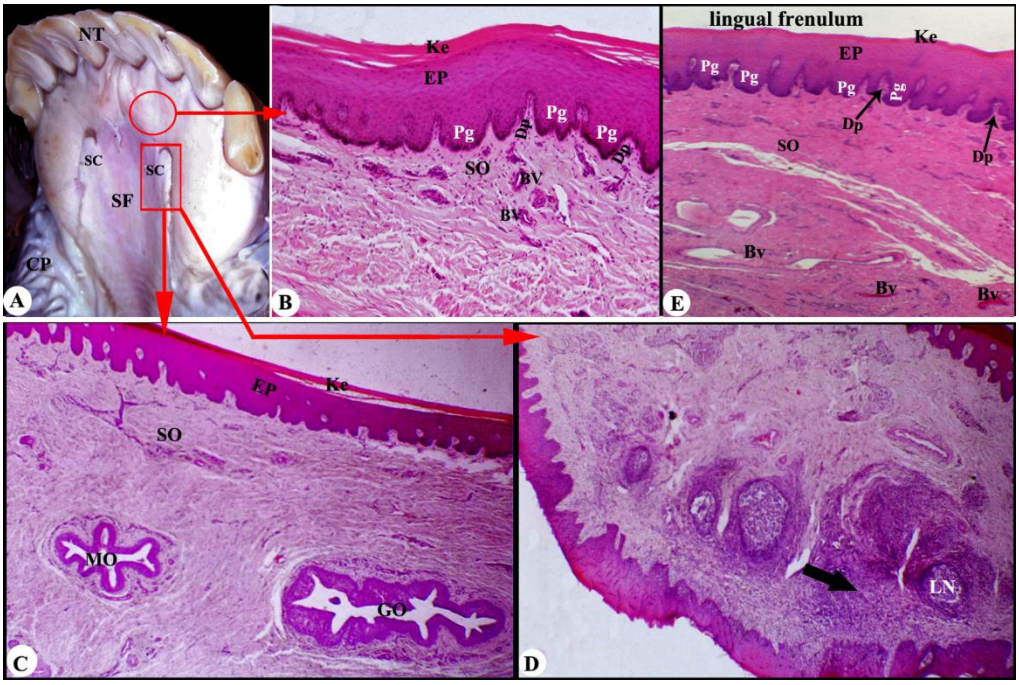

Figure 1: Macroscopic (View A) and Histological (Views B, C, D, and E) of the sublingual floor of the egyptian water buffallo. View A to show; incisive teeth (NT), sublingual floor (SF), sublingual caruncles (SC), conical papillae of check (CP). **View B-D of the sublingual caruncle** to show: sligthly keratinized (Ke), stratified squamous epithelium (EP), epidermal peg (Pg), dermal papillae (Dp), submucosa (SO), blood vessls (BV), opening of minor sublingual salivary galnd (MO), opening of minor sublingual salivary galnd (GO), diffuse lymphatic tissue (black arrow), and lymph nodule (Ln). **View E of lingual frenulum** to show: keratinized (Ke),

stratified squamous epithelium (EP), epidermal peg (Pg), dermal papillae (Dp), submucosa (SO), blood vessls (BV)

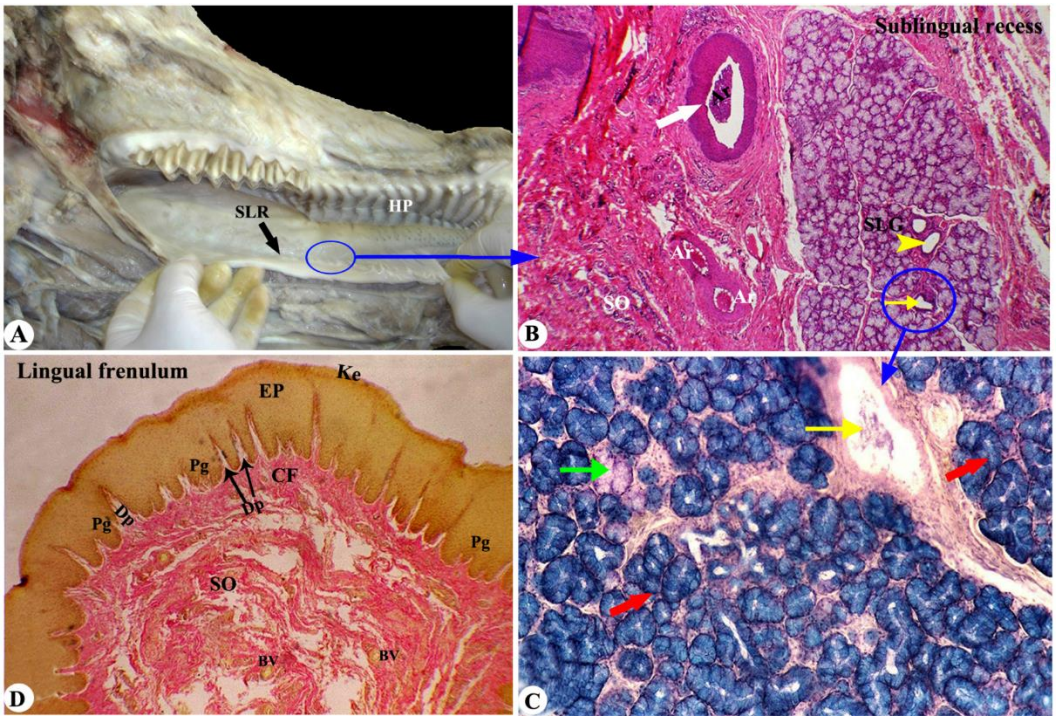

Figure 2: Macroscopic (View A) and Histological (Views B, C, and D) of the sublingual caruncles of the egyptian water buffallo. View A to show; sublingual recess (SLR), hard palate (HP). View B-C to show: Artery (Ar), sublingual gland (SLG), submucosa (SO), stained acini (red arrow), unstained acini (green arrows), interlobular duct (yellow arrow), stratiated duct (yeloow arrowheads). **View D of lingual frenulum** to show: sligthly keratinized (Ke), stratified squamous epithelium (EP), epidermal peg (Pg), dermal papillae (Dp), submucosa (SO), blood vessls (BV).

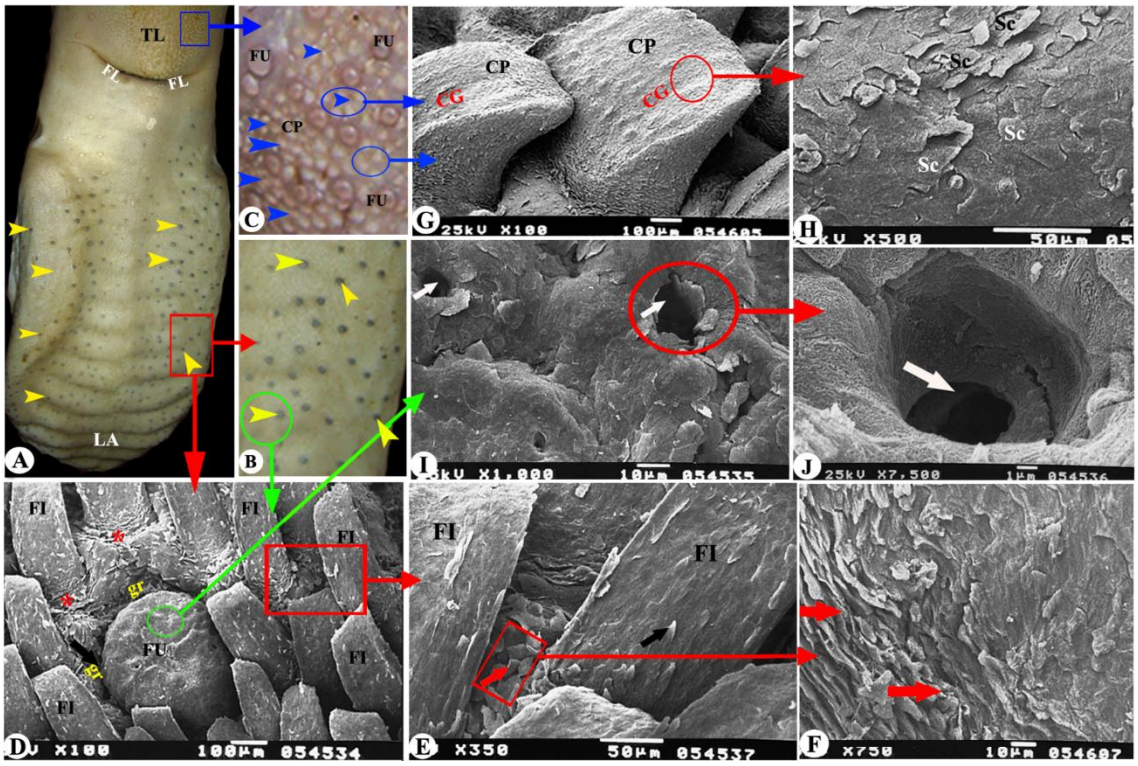

Figure 3: Macroscopic (View A-C) and SEM (Views D-J) images of the tongue of the Egyptian water buffallo. Views A-C to show; lingual apex (LA), Torus linguae (TL), fossa lingua (FL), pigmentated fungifrom papillae (yellow arrowheads), non-pigentated fungiform papillae (FU), conical papillae (CP), lentiform papillae (blue arrowheads). **View D-J to show:** filiform papillae (FI) with its depressed origin (red star) and keratinized scales (black arrow), interpapillary space had some exfoliated keratinized epithelia-like filiform papillae surface (red arrow), fungidorm papillae (FU) surrounded by circular groove (gr) and carried taste pores on its dorsal surfae (white arrow), conical papillae (CP) with its central groove (CG) and keratinized scales (Sc) on its rostral surface.

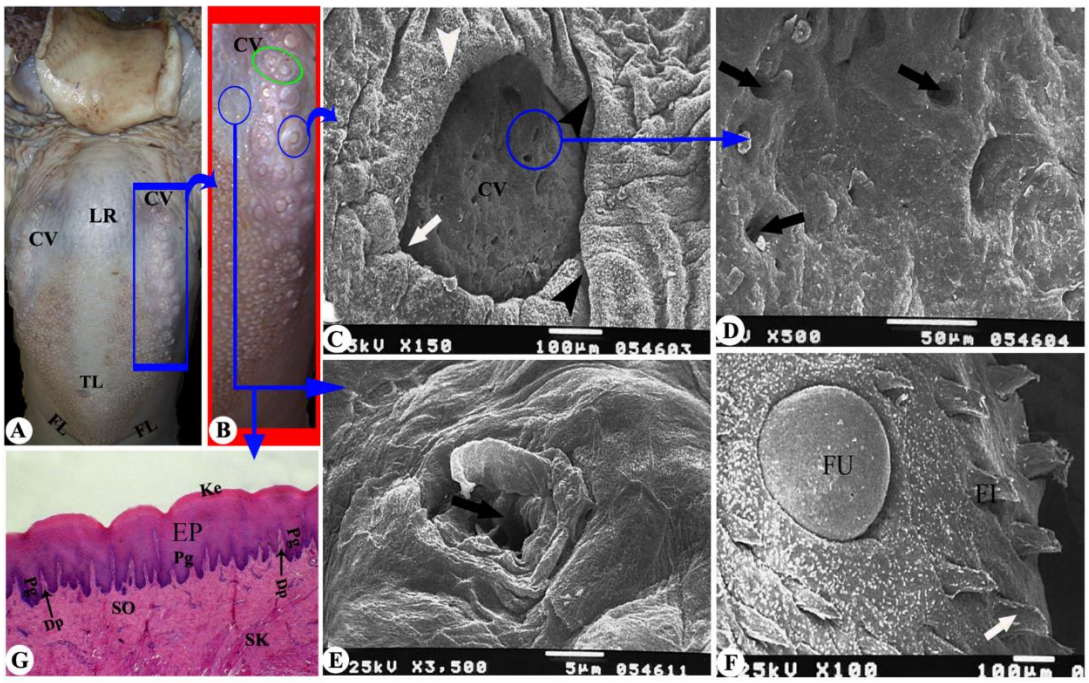

Figure 4: Macroscopic (Views A-B), SEM (Views C-F) and Histological (View G) images of the lingual root of the Egyptian water buffallo. Views A-B to show; lingual root (LR), Torus linguae (TL), fossa lingua (FL), circumvallate papillae (CV), two circumvallate papillae surrounded by one vallate (green circle). **View C-F to show:** circumvallate papillae (CV) with numerous small and rounded opening of taste pores (black arrow) and surrounded by circular deep (white arrow) groove that bordered by an elevated ridge named vallum (white arrowheads) that carried small secondary papillae that ended into the primary groove (black arrowheads). **View G to show:** kertainized (Ke) stratified squamous (EP) with numerous epidermal peg (Pg) and dermal papillae (Dp), submucosa (SO), skeletal muscles (SK).

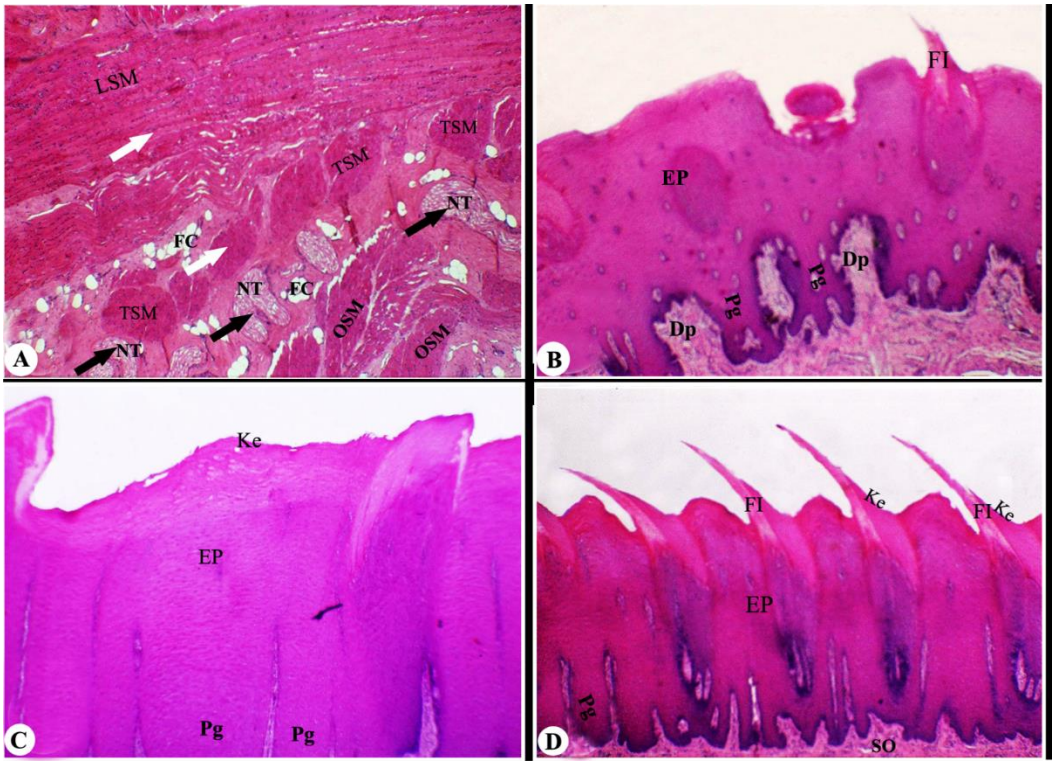

Figure 5: Histological images of the tongue of the Egyptian water buffalo to show; the longitidinal (LSM), transverse (TSM), and oblique (OSM) lingual muscles, nerve trunk (NT, black arrows), fat cells (FC), filiform papillae (FI), kertainized (Ke) stratified squamous (EP) with numerous epidermal peg (Pg) and dermal papillae (Dp), submucosa (SO).

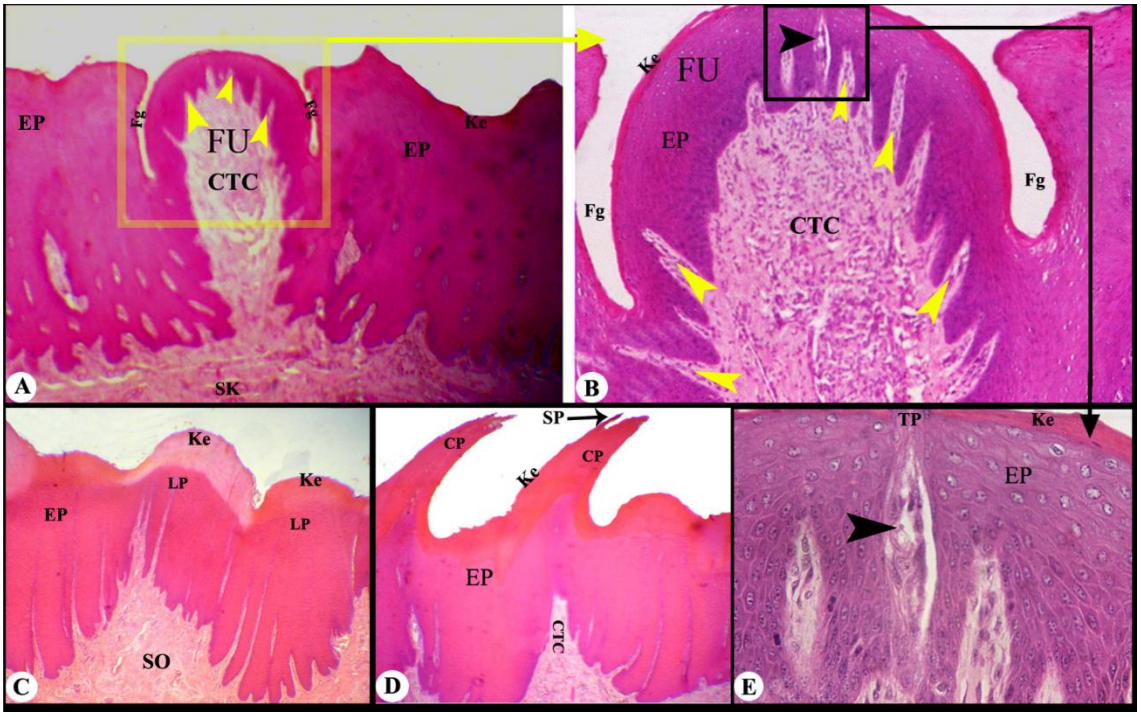

Figure 6: Histological images of the tongue of the Egyptian water buffalo to show; the fungiform papillae (FU) with taste buds (black arrowheads), taste pore (TP) and connective tisssue core (CTC) with secondary papillae (yellow arrowheads) and surrounded by circular groove (Fg), kertainized (Ke) stratified squamous epithelium (EP), skeletal muscles (SK) and dermal papillae (Dp), submucosa (SO), lentiform papillae (LP), conical papillae (CP), secondary papillae (SP).

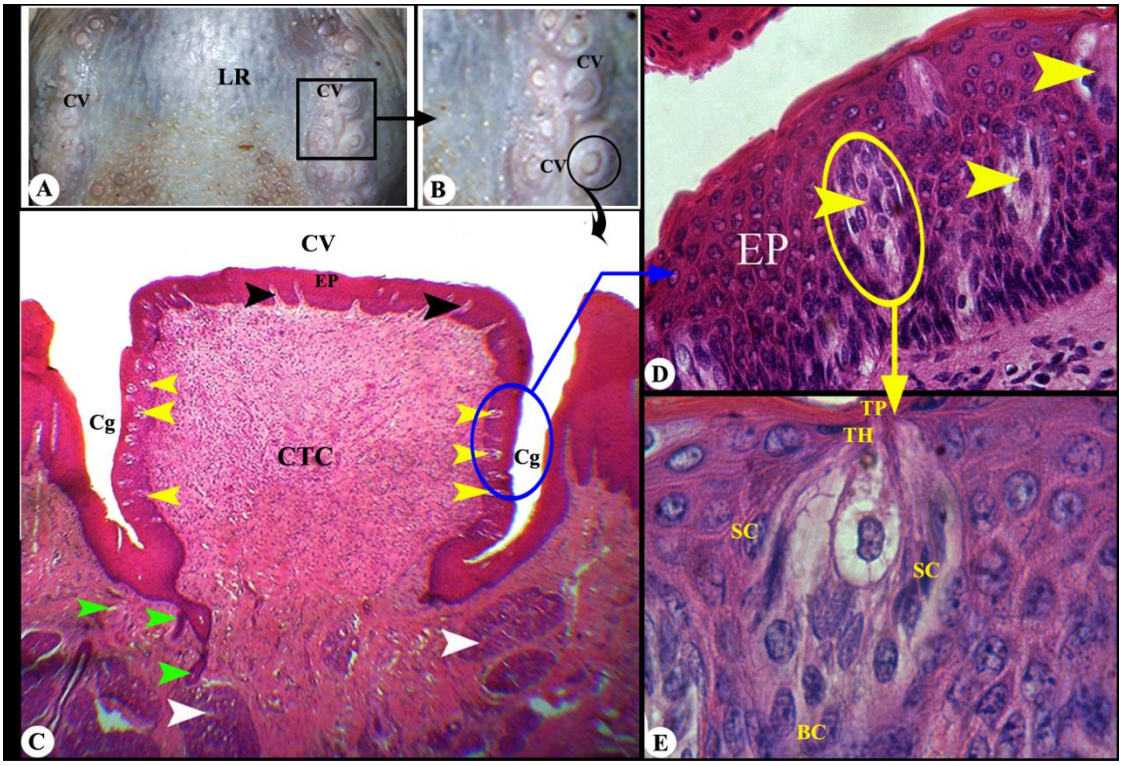

Figure 7: Gross (Views A-B) and Histological (C-E) images of the lingual root of the Egyptian water buffalo to show; the lingual root (LR), circumvallate papillae (CV) with taste buds (yellow arrowheads), taste pore (TP) facing the groove (Cg) and surrounded by sustentacular cells (SC) and basal cells (BC), taste hair (TH), and connective tisssue core (CTC) with secondary papillae (black arrowheads), lobules of von Ebner's glands (white arrowheads) with their ducts (green arrowheads).

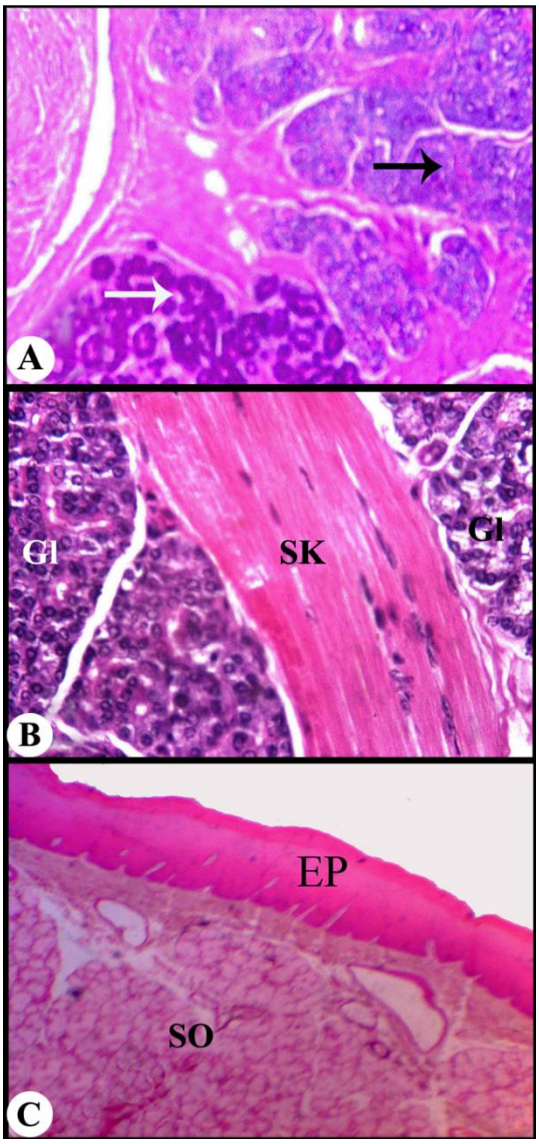

Figure 8: Histological images of the lingual root of the Egyptian water buffalo to show; sublingual glands (GI, black arrows), kertainized (Ke) stratified squamous epithelium (EP), skeletal muscles (SK), submucosa (SO).