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Histomorphological Study of Tongue in Moorhen (*Gallinulla chloropus)*

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Abstract

Aims: The present investigation was carried out to offer fundamental information on the morphological and histological structure of the tongue in moorhens (*Gallinula chloropus).* For the current investigation to be carried out، seven moorhens were purchased from the Baghdad, Iraq, commercial market. The samples(tongue) were then carefully fixed in 10% neutral buffered formalin. The specimens were washed with tab water then dehydrated with serial dilution of alcohol and cleared with xylene and then embedded in paraffin wax. Following a five micrometer-thick sectioning, the slides were stained with hematoxylin and eosin. according to the gross findings our results showed that the tongue was situated on the floor of the oropharyngeal cavity. A row of caudally pointed papillae arranged transversely known as lingual papillae separated the tongue's rostral and caudal regions. There was a laryngeal cleft beyond it, with one pharyngeal papillae row positioned caudally in a heart shape. There were a distinct keratinized bands on the dorsal surface of the tongue apex, the lingual mucosa's histology revealed stratified squamous epithelium with varying degrees of keratinization. Many mucosal glands were seen in the caudal part of the tongue; the secretions emptied into the ducts, this led to the tongue's dorsal surface opening. Whereas the papillae might act primarily as physical barriers to the reflexive backward movement of food that has passed over them, the glandular secretions may facilitate swallowing by lubricating the caudal region of the oropharyngeal cavity in addition to the proximal part of the esophagus. The study concluded that the morphological features of the moorhen's tongue are likely adaptations adequate to its unique feeding habits and methods of gathering food. These adaptations make the moorhen an excellent forager in ponds, marshes, and other wetland environments, that has evolved a special beak and tongue to survive in its aquatic environments. Small aquatic invertebrates and plant matter can be filtered and retained from the water by its tongue by means of papillae, which are hair-like projections pointing backward. Because of these adaptations, moorhens are great foragers in ponds, marshes, and other types of wetland habitats.

Keywords: Histological, morphological, Tongue, Moorhen, Photomicrograph, tongue papillae

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Introduction

Among all vertebrates, avians possess the second-largest number of species, adapted to their various environments, these feathered creatures have evolved distinct feeding patterns to suit their needs. In addition, their digestive systems vary greatly depending on their unique feeding behaviors (Süzer et al. 2018; AL-TAAI SA, KHALAF AS,2022; Farhan and Hussein 2024). The moorhen (Gallinula chloropus) is a versatile omnivorous bird known for its diverse diet, which includes plant seeds, flower heads, and insects. Occasionally, this adaptable bird also feeds on small fish and crustaceans (Hamdi et al. 2013; YOUSUF and KHALAF 2023). Due to its varied eating habits, the birds' tongue exhibits different shapes and structures, optimized for its well-adapted diet (Mohammed 2017 and Alshammary 2023). This adaptability plays a significant role in its survival and success across different habitats.

The morphological and histological structure of bird tongues fascinates researchers because of the diverse variety of the tongue's mucosa. This diversity is influenced by the bird's diet as well as the processes for collection and transporting food within the beak cavity. The tongue, located at the entrance of the gastrointestinal system, is crucial for food intake. The tongue in birds serves various purposes, like gathering, swallowing and manipulating food. This versatility allows birds to efficiently process their meals, as noted in studies by (Harrison 1964; King and McLelland 1984; O'Malley 2005). The number and location of salivary glands in birds vary depending on their feeding habits. In avian species that primarily eat insects or seeds, The glands that produce saliva are fully grown, this includes the sphenopterygoid, palatine, and maxillary salivary glands on the oropharyngeal roof, as well as the cricoarytenoid, mandibular, and lingual salivary glands found at the base of the oropharynx. In contrast, birds that feed on fish have less developed salivary glands. Remarkably, these gland openings can be seen without the aid of magnification (King and McLelland 1984). The morphology of bird tongues was extensively investigated in various avian species. Notable research includes studies on the

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hoopoe, emu, Egyptian goose, African pied crow, white-tailed eagle, black kite, long-legged buzzard, and common quail. Understanding the tongue morphology in these birds provides valuable insights into their feeding behaviors and ecological adaptations. Crucial research has been conducted by (El-Bakary 2011 and Parchami et al. 2010). According to (King and McLelland 1984), domestic fowl have large conical pharyngeal papillae. These are organized into two transverse rows located between the tongue's anterior and posterior regions. In herbivorous and granivorous birds, the tongue's mucous membrane is highly keratinized, according to studies by (Iwasaki, 1992; Jackowiak and Ludwing, 2008). Conversely, birds inhabiting water environments display less keratinization on their tongues (Iwasaki, 2002; Jackowiak et al., 2006). The para keratinized stratified epithelium is present on the tongue's root and dorsum, as confirmed by studies from (Iwasaki et al., 1997; Jackowiak and Godynicki, 2005).

Materials and methods:

In the current study, seven adult moorhens were sourced from a specialized bird market. These moorhens, the focus of the research, had an average weight of 500 grams. The birds were humanely euthanized through an injection of sodium pentobarbitone given intravenously at a dose of 80 mg/kg, administered via the cutaneous ulnar vein. This method follows the protocol established by (Mitchell and Smith, 1991), After the birds' death, their heads were carefully dissected and detailed photographs were taken using a Canon digital camera with 5 megapixels. To prepare tongue tissue specimens for microscopic analysis, the samples were initially cut and washed with a saline solution. They were then preserved by immersing them in a 10% formalin solution for 48 hours. The specimens then went through a dehydration procedure utilizing a sequence of graded ethanol concentrations for two hours each, progressing through 70%, 80%, 90%, and finally 100% ethanol. After dehydration, the samples were cleared with xylene for 1 hours. Finally, the specimens were embedded in paraffin wax, allowing for the preparation of blocks from which serial sections, 5 micrometers thick, were cut using a microtome. The ribbon Sections were

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stained after being placed on glass slides using hematoxylin and eosin (H&E) for enhanced visibility under light microscopy. These prepared slides were then inspected and captured on camera with a microscope, equipped with a digital camera, ensuring high-quality imaging. (Bancroft, 2008).

Ethical approval:

All techniques involved in the current study were assessed and permitted by the local Co mmittee for Animal Care and Use at the College of Veterinary Medicine, University of B aghdad, Baghdad, Iraq.

The results:

Macroscopic findings: The gross findings indicate that the moorhen's tongue is located in the beak's lower region., filling the entire cavity. It features a long, triangular shape with a narrow front edge and divided into the root, body, and apex. The free portion of the tongue has a slim ventrolateral and dorsal surface. Notably, there is a central groove in the front portion of the dorsal surface Figure (1). There is a transverse row of backward-directed lingual conical papillae between the tongue's body and root. Just behind these papillae, there is a short, flat plate-like fold. The papillae itself come in different sizes; the smallest ones are found nearer the tongue's midline. Extending caudally from each end of this transverse row, three to four large conical papillae are set up in a short row. Notably, the transverse row exhibits a distinct concavity along the tongue's middle line Figure (2). The laryngeal cleft, which is situated behind the lingual root, has conical papillae that face backward and form a heart shape. The base of this shape points forward, with a large conical papilla in the center, while the apex is situated towards the back Figures (3, 4).

Histological results:

There are layers of squamous epithelium covering the tongue. The tongue's dorsal surface has thicker mucosa than its ventrolateral surface. Notably, this layer creates a strong keratinized band in the first third of the tongue on the dorsolateral surface. Figure (5). The tongue has several conical papillae on its dorsal surface Figure (6). The connective tissues of the lamina propria are

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abundant in mucous glands and blood vessels. These glands have ducts that extend into the epithelial surface. Additionally, this area includes skeletal muscle elements and hyaline cartilage, surrounded by a distinct perichondrium, which extends from the body to the root. Notably, the lingual apex lacks cartilage Figure (7). The salivary glands (lingual) are situated within the lamina propria of the tongue's free section's second half, known as the anterior lingual glands arranged as medial and lateral group Figure (8), Additionally, there are the posterior lingual glands, which are located in the dorsal region at the base of the tongue. The posterior glands are situated between the extrinsic muscle bundles and the dorsal epithelium. Figure (9). Notably, there are no glandular structures in the tongue's anterior region. Figure (10). The cellular characteristics have been crucial in distinguishing the posterior and anterior (medial and lateral) of the lingual glands. These glands feature secretory units composed of tall, simple columnar mucous cells with base nuclei positioned Figures (11, 12).

DISCUSSION:

The study reveals that the tongue has a triangular shape with a narrow anterior edge. Its overall structure is divided into three parts: the root, body, and free apex. This observation aligns with previous findings from ((Parchami A, 2010) regarding the avian quail, as well as similar descriptions of the domestic chicken by (Homberger DG ,1989). The tongue's surface features numerous conical papillae situated between its body and root, a characteristic also observed in several bird species, including the Egyptian goose, ostrich, peacock, and starling. Studies by (Hasan et.al 2010, pasand et.al 2010; AL-TAAI SA, KHALAF AS, 2022; Mohamed.H.H, et.al,2023) have documented these similarities. The lingual papillae arrangement is consistent with previous observations in chickens (Hill, 1971; Iwasaki and Kobayashi, 1986). Nevertheless, in geese, the tongue features just extremely conical papillae located along the midline between the tongue's body and root (Iwasaki et al., 1997; Hassan et al., 2010). While in the common kestrel, multiple conical papillae are found positioned between the body and the

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base of the tongue (Emura et al., 2008). The lingual conical papillae in eagles are located on a single ridge at the back of the tongue, stretching from the lingual body to the root of the tongue (Jackowiak and Godynicki, 2005). According to (Hodges ,1974) highlighted the crucial role of conical papillae in aiding the movement of food toward the esophagus and preventing regurgitation. This study underscores the significance of conical papillae in digestive health and function. An ostrich's tongue has a characteristic mucosal fold which continues to face backward., situated between the body and the base of the tongue (Jackowiak and Ludwig, 2008). In the present research, we observed A brief, plate-shaped fold extending caudally towards the lingual papillae's base., a finding not stated in the research by (Hodges, (1974) on fowl. Our findings align with those of (Nickel et al. ,1977) on the common kestrel and McLelland ,1975 on the red jungle fowl. In both studies, the lingual papillae were observed to be arranged in a concave transverse row. Our findings differ from those reported by Iwasaki (1992) regarding he lingual papillae's arrangement in the little tern, which were described as a straight transverse line. Additionally, (Samara et.al, 2002) noted that the pharyngeal papillae of domestic fowl include two rows of large conical papillae. However, our research results presented variations from observations. Our findings revealed that the tongue's surface has a thick layer of stratified squamous epithelium covering it. This layer has a lot of cornification on the lingual papillae and dorsolateral surface, and it is highly keratinized at the tongue apex. These observations contrast with those of (Iwasaki and Kobayashi, 1986) in domestic fowls. Notably, the thickness of the epithelium's keratinized layer often varies based on diet. Herbivorous and granivorous birds typically exhibit a thicker keratinized epithelium (Jackowiak and Ludwing, 2008; Iwasaki, 1992). Birds that inhabit aquatic environments exhibit a lower degree of keratinization in their epithelial tissue, as noted in studies by Iwasaki (Iwasaki, 2002; Jackowiak et al., 2006). In line with our findings studies by (Iwasaki et al. 1997), Jackowiak and Godynicki (2005)) also revealed that the dorsum and the base of the tongue are covered with Para keratinized epithelium. Our findings are consistent

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with the studies by (Hill, 1971, McLelland (1975)), They demonstrate that the tongue's anterior third lacks salivary glands and musculature, but the muscles outside the tongue extend into the other sections of the tongue. The cellular characteristics play a vital role in differentiating the anterior and posterior lingual glands' medial and lateral groups. These glands consist of secretory units made up of the tall simple columnar mucosal cells with the nuclei at the base. However, these findings do not align with the results from (Kadhim et.al, 2011) used histological staining to report differences between the anterior and posterior lingual glands in chickens.

The lingual salivary glands are responsible for producing mucinous saliva, which aids in swallowing by lubricating and managing ingested food. Furthermore, the secretions from these mucous lingual glands might provide a protective layer for the mucous membranes of the upper digestive tract, a function closer to what (Samara et.al,2002) suggested.

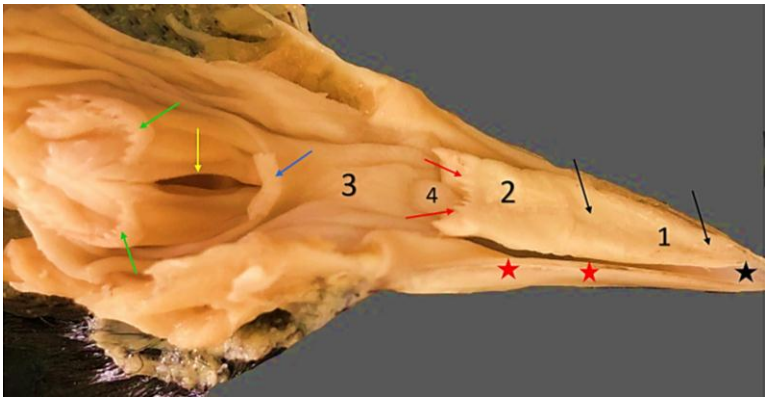

Figure 1: Photograph of moorhen tongue show: The bill (red stars), bill cavity (black star), The three parts of tongue, apex (1), body (2), Root (3), median groove (black arrows), lingual papillae (red arrows), anterior fold like plate (4), posterior fold like plate (blue arrow), pharyngeal cleft (yellow arrow), pharyngeal papillae (green arrows).

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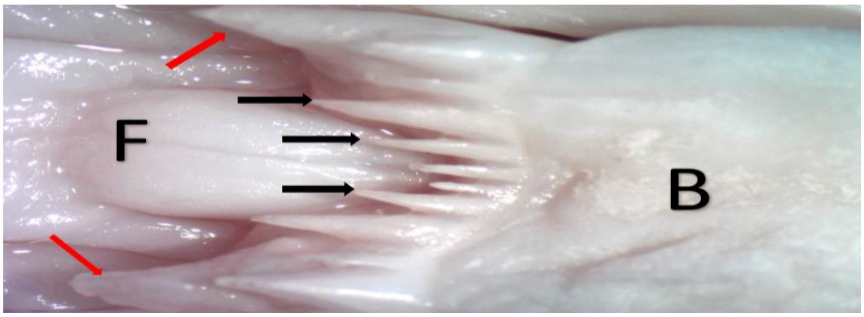

Figure 2: Photograph of moorhen tongue show: The body (B), single row of lingual papillae, small middle papillae (black arrows), large lateral conical papillae (red arrows), Tongue body(B), and fold behind lingual papillae(F).

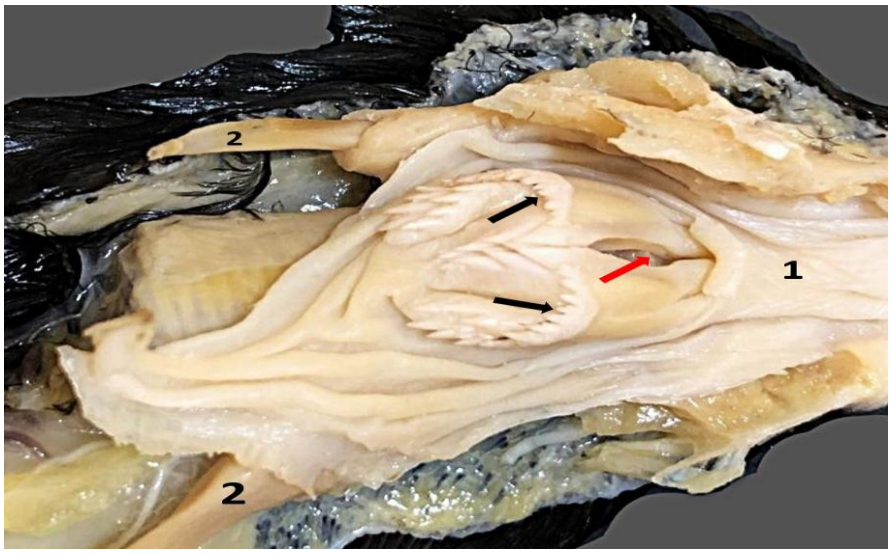

Figure 3: Photograph of moorhen tongue show: Tongue root (1), pharyngeal opening (red arrow), the pharyngeal papillae (black arrows) hyoid bone (2).

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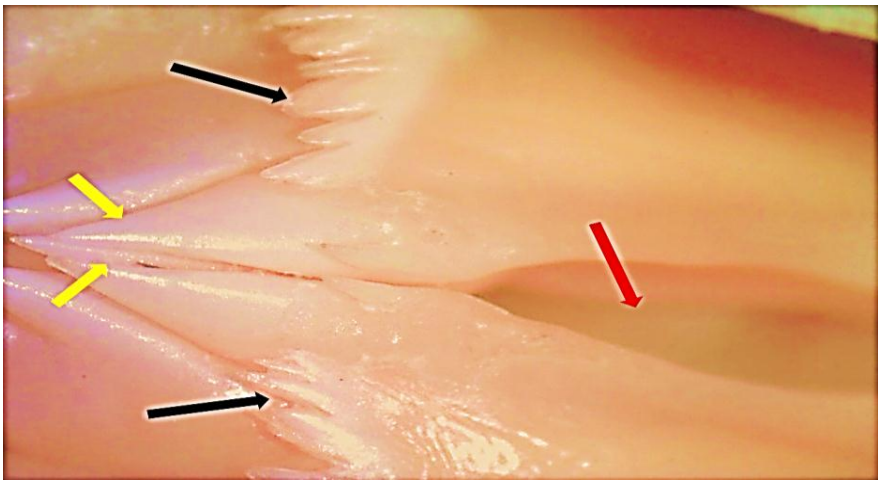

Figure 4: Photograph of moorhen tongue show: The single row of pharyngeal papillae, Small lateral pharyngeal papillae (Black arrows), large middle pharyngeal papillae (Yellow arrows) and laryngeal cleft (Red arrow).

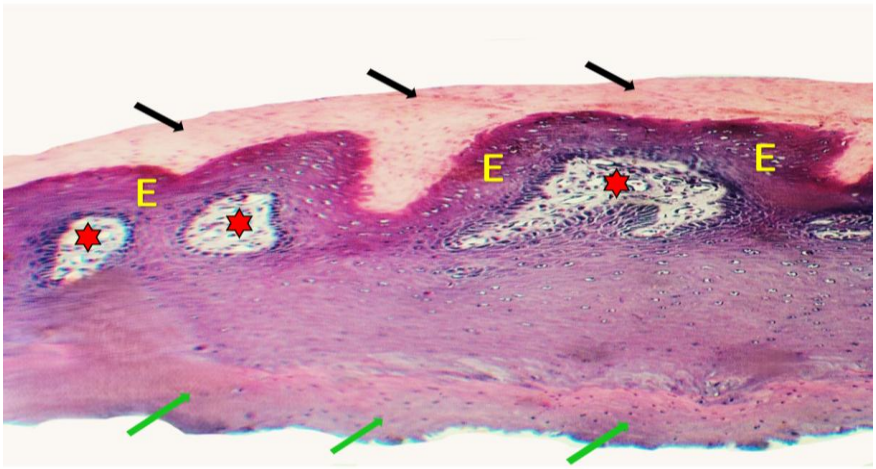

Figure 5: Photomicrograph of moorhen tongue rostral part (cross section) show: The dorsal surface Epithelium (E), cornified squamous cells epithelium (black arrows). Non keratinized ventral epithelium (Green arrows), Lamina propria (Red stars). ×10 Hematoxylin and eosin stain.

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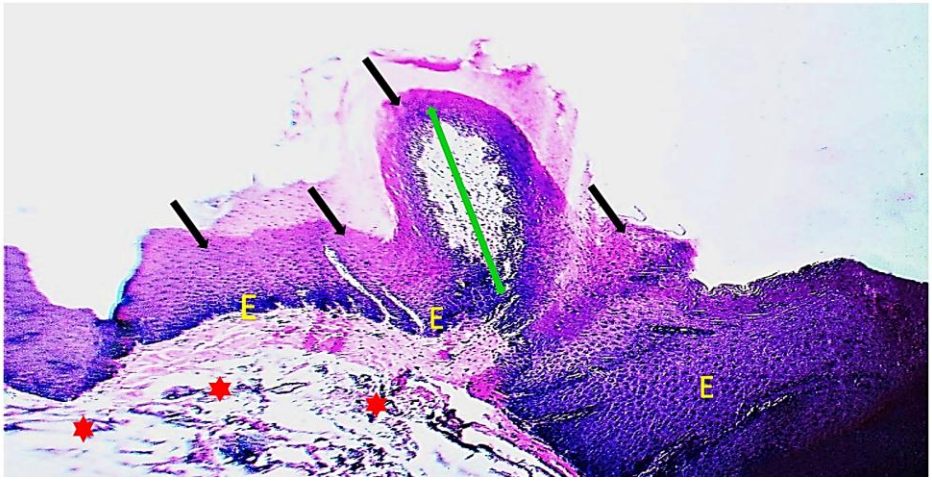

Figure 6: photomicrograph of moorhen tongue (cross section) show: Epithelium (E), conical papillae (green double head arrow), keratin layer (black arrows) lamina propria connective tissue (red stars). Hematoxylin and eosin stain. **H&E.10X**

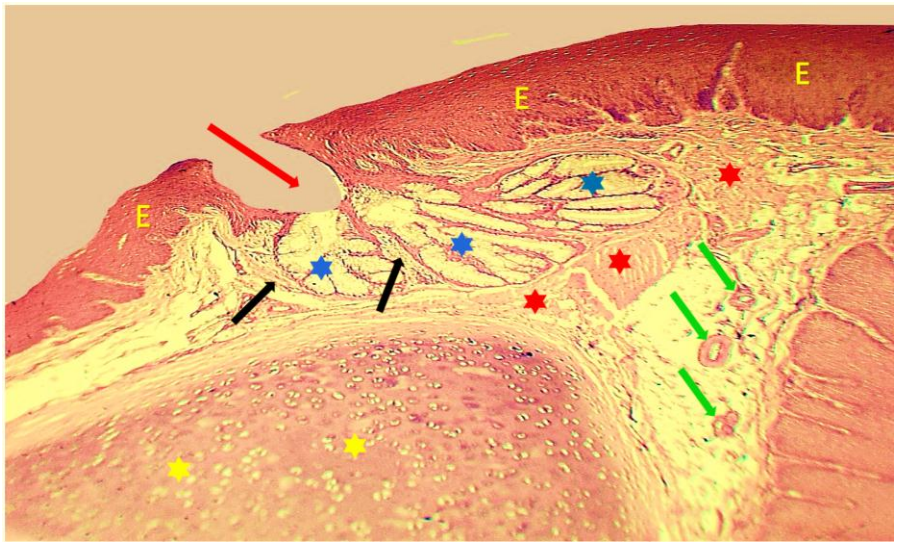

Figure 7: Photomicrograph of moorhen tongue (Longitudinal section) show: non-keratinized epithelium (E), (, tubulo-alveolar gland (Blue stars) surrounded by connective tissue capsule (black arrows), salivary gland opening (Red arrow) Skeletal lingual muscle (red stars), blood vessel (Green arrows), Hyalin cartilage (yellow stars) .10x. Hematoxylin and eosin stain.

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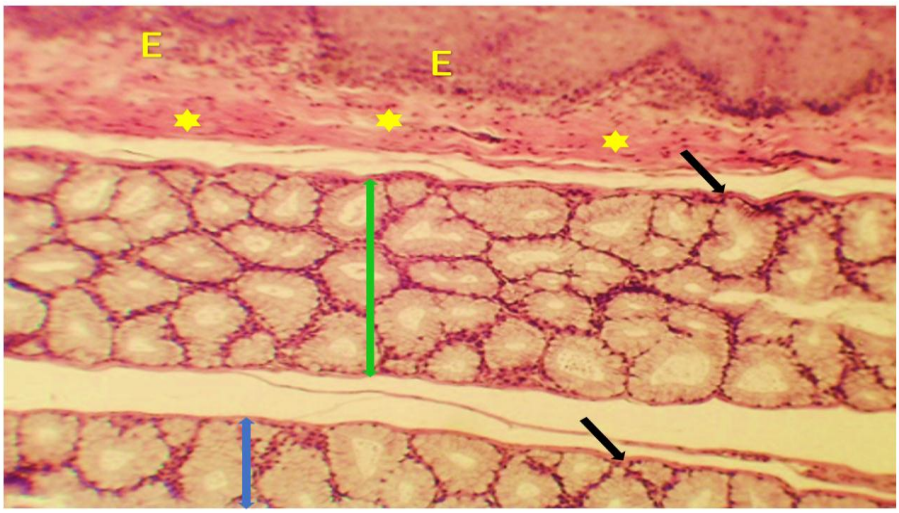

Figure 8: photomicrographs of moorhen tongue (Longitudinal section) show: Epithelium (E), sub epithelium connective tissue (Yellow stars), the lateral (Double head green arrow) and medial group (Double head blue arrow) of anterior lingual glands with Connective tissue capsule (Black arrows). 10x.Hematoxylin and eosin stain.

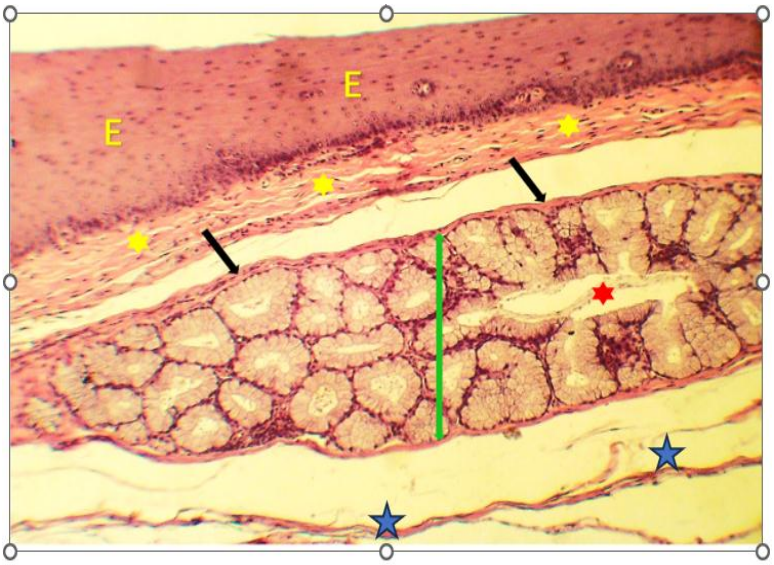

Figure 9: Photomicrographs of moorhen tongue (Longitudinal section) show: Non keratinized Epithelium(E), the secretory cells of posterior lingual glands (Double head green arrow), gland duct (Red star), connective tissue capsule (Black arrows), Sub epithelium connective tissue (Yellow stars) and muscle fibers (Blue stars). 10x.Hematoxylin and eosin stain.

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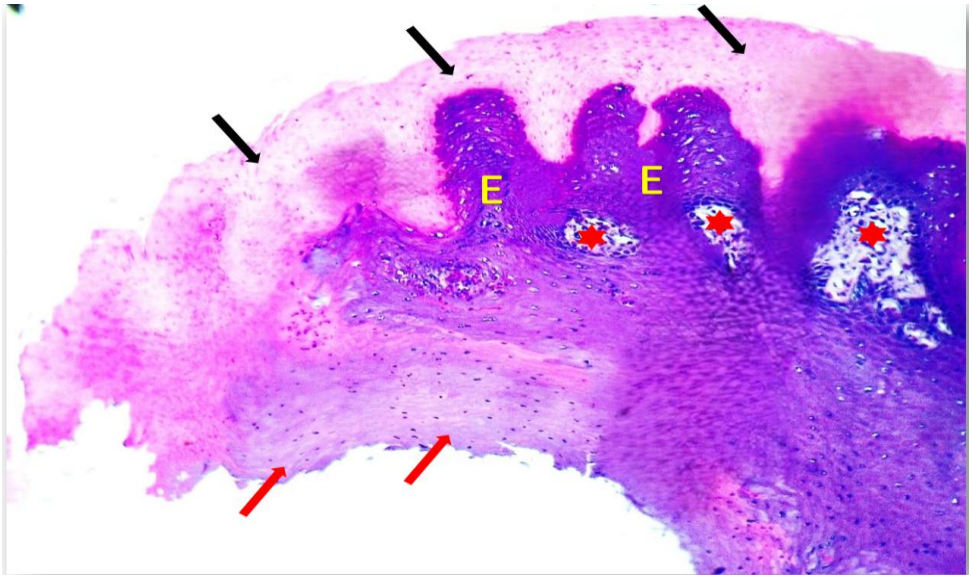

Figure 10: Photomicrographs of the moorhen tongue apex (Longitudinal section) show: Highly keratinized Epithelium (Black arrows), Dorsal Epithelium (E), Lamina propria (Red stars), the apex shows devoid of lingual glands, non-keratinized ventral epithelium (Red arrows). 10x. Hematoxylin and eosin stain.

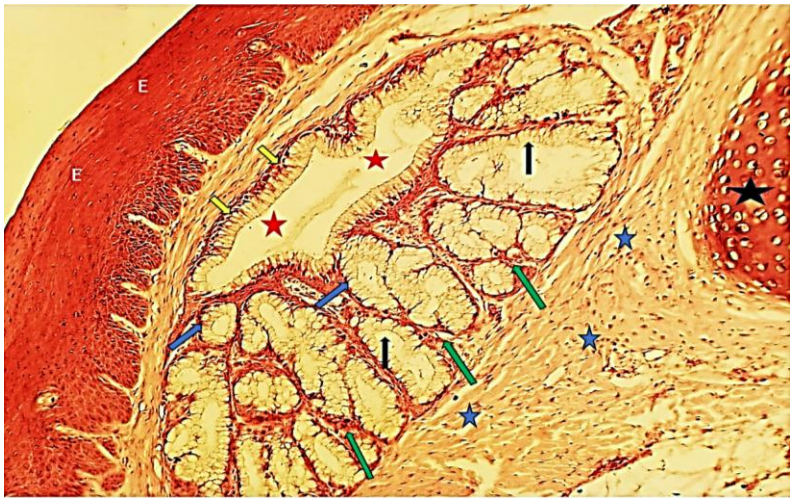

Figure 11: Photomicrographs of moorhen tongue (cross section) show: Non keratinized stratified squamous Epithelium(E), The secretory cells of lingual glands (Black arrows), gland duct (Red strs), connective tissue septa between the glandular tissue(Green arrows),skeletal muscles(Blue stars), Basal nuclei of simple columnar epithelium in duct (yellow arrows) and secretory glandular tissue(Blue arrows) ,Hyalin cartilage(Black star).10x Hematoxylin and eosin stain.

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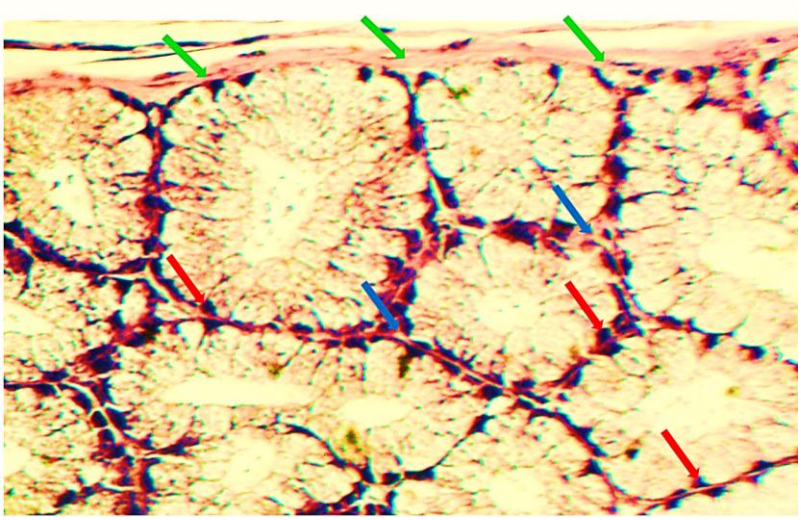

Figure 12: Magnified section of moorhen tongue (Longitudinal section) shows: simple columnar cells lining Alveoli of lingual glands with basal located nuclei (Red arrows), septa (Blue arrows) and Surrounding connective tissue (green arrows). 40x. Hematoxylin and eosin stain

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