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Morphological, histological and histochemical studies of the lingual salivary glands of the rock dove, *Patagioenas livia* (Columbidae)

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A B S T R A C T

Morphological, histological and histochemical study of the Salivary Glands of the Rock Dove, *Patagioenas livia* was studied using macroscopic microscopic and scanning electron microscope. The glands are classified according to their location into: Glandula Lingualis, Glandula Preglottalis, Glandula Laryngeal and Glandula Sublingualis. The glandula lingualis is a paired extends along the posterior half of the dorso-lateral surface of the free part of the tongue and on the lingual wings. It composed of compound branched tubule-alveolar type. The secretory cells of the lingual salivary gland show strongly positive reaction to Periodic Acid Schiff's reaction. The preglottal gland is unpaired one which occupies the preglottal region and it consists of a compound tubulo-alvealor with many secretory lobules. The glandula laryngeal is paired gland, each of which is ovoid-shaped and is compound tubulo-alveolar type which consists of many secretory lobules. The glandula sublingualis is a paired gland that is embedded in the skin of the anterior portion of the floor of the mouth and composed of compound tubulo-alveolar type which consists of many secretory lobule. Histochemical results indicated that all glands give a positive Periodic Acid Schiff's reaction.

Introduction

The Rock dove (*Patagioenas livia*) or Rock Pigeon, is a member of the bird family Columbidae (doves and pigeons). Birds in general possess a series of paired salivary glands, which are usually described as mucous in nature (McLelland, 1979). Some birds, however, are said to possess seromucous cells that may secrete amylase (Jerrett and Goodge, 1973; Nagato and Tandler, 1986).

The salivary glands associated with the lingual apparatus of birds form glandular bodies that are invested by a connective

tissue capsule, even if they consist of several glandular tubules (Hodges, 1974; Homberger, 1986). They function as hydrostatic skeletal elements, to which muscles and fasciae may attach. The morphology of salivary gland in different types of birds has been extensively studied (Toryu *et al.,* 1960; Rossi *et al.,* 2005; Al-Mansour and Jarrar, 2007).

Recently, in various avian species, salivary glands histochemical studies of secretory granules have investigated in addition to the locations and structures of the glands (Santos *et al.,* 2011; Kadhim *et al.,* 2011; Erdoğan *et al.,* 2012; Sağsöz *et al.,* 2013). The results of these studies showed that these glands are efficient and quite functional in birds which feed mainly on small insects or seeds, less developed in those eating soft diet, and absent in some birds such as pelicans (King and McLelland, 1984; Blanks, 1993). Saliva in some birds is used mainly to lubricate and glue the insects or seeds into sticky ball to ease swallowing, while in others it is used to glue together the ingredients required for building nests (Marcone, 2005). Most electron microscopy studies of salivary glands refer to mammals and reptiles but some descriptions of the structure and ultrastructure of the secretory granules in birds have been reported (Erdoğan *et al.,* 2012).

The aim of the present study to elucidate the morphological, histological and histochemical characterizations of the salivary glands of the Rock dove.

Materials and Methods

Thirty adult specimens of the Rock dove were used in the present study. Birds brought alive to the laboratory, and then were killed by chloroform inhalation. The specimens were dissected under the binocular with camera Lucida attachment. The different techniques used in the present work were; gross anatomy, scanning electron microscopic investigations (S.E.M.), together with different histological structure.

Preparation of specimens for gross anatomy

The head and neck were dissected from the rest of the body, and then fixed in 10% formalin for 48 HRS. Then specimens were stored in 3% phenoxy-ethanol for long term preservation.

The skin that covers the head and the neck was carefully removed under the binocular. An iodine solution was used for staining the anatomical material to improve the color contrast between muscles and connective tissues. The figures were prepared by outlining the anatomical preparations with the help of the camera Lucida.

Preparation of specimens for Light Microscopic investigation

The following histological procedures were used according to Humason (1972). The tongue and larynx were fixed in Bouin's solution for about 12–24 hrs, and washed in 70% ethyl alcohol. Dehydration was done through ascending series of 70, 95 % ethyl alcohol (1- 2 hrs), followed by two changes in absolute ethyl alcohol for 2 hrs.

Specimens were cleared in ethyl alcohol and xylene (v/v; 1:1) for 1–2 hrs and cleared by absolute xylene (2–3 hrs), and finally embedded in paraffin wax .Serial sections of 6µm thickness were prepared according to normal histological techniques for staining with Haematoxylin-Eosin method .Sections were dehydrated through 90, 95 and two changes in 100% ethyl alcohol, then cleared in xylene and mounted in Canada Balsam

Periodic acid Schiff's technique (P.A.S.)

The histological procedures were used according to (Gurr, 1962).

Preparation of specimens for Scanning Electron Microscope

The tongue was cut to small pieces, and directly fixed in 5% glutraldehyde in a cacodylate buffer for at least 48 HRS at pH=7.2 and at 4 C , then these pieces were washed three times in 0.1% cacodylate buffer, then the specimens were post-fixed in a cacodylate buffered solution of 1% Osmium tetroxide for 2 HRS at 37 C.

The specimens were washed in the same buffer three times, dehydrated and then infiltrated with amyl acetate for two days. The drying of specimens is accomplished by the critical point drying using liquid carbon dioxide, mounted and sputter-coated with gold. The specimens were examined under A Jeol scanning electron microscope (JSM-6390-LA).

Results and Discussion

The salivary glands

The salivary glands of the Rock dove, *Patagioneas livia*, were studied as derivatives of the lingual epithelium. Consequently, they interact with the mechanical performance of the lingual system.

The glands are classified according to their location as follow:

1- Glandula Lingualis (GL.L.).
2- Glandula Preglottalis (GL.PR.).
3- Glandula Laryngeal (GL.LAR.).
4- Glandula Sublingualis (GL.SL.)

Glandula Lingualis (GL.L.)

The glandula lingualis is a paired one which extends along the posterior half of the dorso-lateral surface of the of the free part of the tongue, Glandula Lingualis anterior (G.L.A) and Glandula Lingualis posterior (G.L.P.) (Fig. 1A). The glandula lingualis is a compound branched tubule-alveolar type, and consist of secretory end-pieces composed of tall columnar cells with extensive vesicular cytoplasm resting at a delicate basement membrane (Figs. 2A,B). The secretory cells of the lingual salivary gland show strongly positive reaction to Periodic Acid Schiff's reaction (P.A.S.) (Figs. 3A,B). The gland delivers its mucoid secretion via many openings on the dorso-lateral surface of the free portion of the tongue, and the dorsal surface of the lingual wings. Also, each gland is encased inside a connective tissue sheath which is flexible but not elastic. The glandula lingualis performs many functions; it lubricates the food items by its mucoid secretion. Mean-while, the gland acts as a hydroskeleton due to its relationship with the Os paraglossale and the lingual papillae.

Glandula Preglottalis (GL.PR.)

The preglottal gland is unpaired one which occupies the preglottal region (Fig. 1). The preglottal gland is a compound tubulo-alvealor which consists of many secretory lobules (Fig. 2C). The gland is enveloped by a connective tissue capsule which is connected to the epimysium of the dorsal surface of the muscle cricohyoideus dorsalis, posteriorly, the capsule spreads over the dorsal surface of the cricoids cartilage and attached on the anterior edge of the arytenoids cartilage. The secretory cells of the preglottal salivary gland show strongly positive reaction to Periodic Acid Schiff's reaction (Fig. 3C). The glandula preglottalis

opens at the epithelial surface by multiple openings which deliver their mucoid secretion for lubricating the food items and facilitate their passage. Meanwhile, the glandula preglottalis acts as a hydroskeletion due to its relation-ships with the lingual papillae, the glottis and the muscle cricohyoideus dorsalis. Moreover, the gland which acts as a hydraulic structure contributes in move the free portion of the tongue.

Glandula Laryngeal (GL.LAR.)

The glandula laryngeal is paired gland, each of which is ovoid-shaped that lies between the tunica mucosa of the laryngeal mound and the dorsal surface of muscle dilator glottidis and the muscle cricohyoideus dorsalis (Fig. 1). The glandula laryngeal is compound tubulo-alveolar type which consists of many secretory lobules that open into the dorsal surface of the laryngeal mound. The gland is enveloped by glandular capsule which adheres with the muscle cricohyoideus dorsalis. Consequently, the gland allows site of insertion for the muscle cricohyoideus dorsalis. The laryngeal gland gives a positive Periodic Acid Schiff's reaction (Fig. 3D). That mucous secretion lubricates the food items during its passage in the buccal cavity. Meanwhile, the glandula laryngeal acts as a hydroskeleton due to its relationships with the laryngeal papillae.

Glandula Sublingualis (GL.SL.)

The glandula sublingualis is a paired gland that is embedded in the skin of the anterior portion of the floor of the mouth and lateral to the muscle genioglossus and muscle branchiomandibularis anterio. Also, the lateral sides of the fascia sublingualis are connected with the median surfaces of the lower jaw (Fig. 1). The glandula sublingualis is a compound tubulo-alveolar type which consists of many secretory lobules that open into the floor of the mouth via many orifices (Fig. 2D). The gland is enveloped by glandular capsule which adheres with the muscle branchiomandibularis anterior and the muscle cleidotrachealis. Consequently, the gland allows site of insertion for the muscle cleidotra-chealis. That mucous secretion lubricates the food items during its passage in the buccal cavity in addition, the gland acts as a hydroskeletal structure due to its connection with the muscle cleidotracheali.

A considerable number of papers been published on histochemistry of the lingual salivary glands secretions of vertebrates, mainly mammals (Quintarelli *et al.,* 1961). Development of the salivary glands in avian species has been classified according to the type of food and feeding habits. Granivorous birds fed on dry food had well developed salivary glands than those of carnivorous species (Farner and Ziswiller, 1972). Nevertheless, little is known about the nature of salivary secretion in non-mammalian vertebrates, especially birds (Fujii and Tamura, 1966; Jerret and Goodge, 1973; Samar *et al.,* 1995; Taib and Jarrar, 1998, 2001; Al-Mansour and Jarrar, 2004). In birds with soft diet, definitive compound salivary glands are absent but there may be intramural branched or unbranched simple tubular glands in the lamina propria of the buccal or lingual muscoa (King and McLelland, 1984; Blanks. 1993).

The lingual salivary glands studied extensively in different types of birds (Duke, 1986; Rossi *et al.,* 2005; Al-Mansour and Jarrar, 2007). The secretory cells of the chicken salivary gland contain both neutral and sulphate mucin (Suprasert *et al.,* 1986; Suprasert and Fujioka, 1987; Gargiulo *et al.,* 1991). The lingual salivary glands were located in the lamina propria of the second half of the free part of the tongue (anterior

lingual glands) and in the dorsal part of its base (posterior lingual glands). The anterior part of the tongue was devoid of any glandular structure. After staining, the cellular features enabled to identification of lateral and medial groups of the anterior lingual glands. The secretory cells were P.A.S. positive. An intense reaction at the medial groups of the anterior and posterior lingual glands was greater than in the lateral group of the anterior lingual glands. There were no changes in the intensity of the P.A.S. reaction after digestion with amylase. The rostral part of the dorsal surface had a median groove. Between the body and the root of the tongue lies a transverse row of backward directed lingual conical papillae. In addition, a short flat plate-like fold, extending for a short distance over the base of these papillae. The papillae vary in size, the smallest being closer the midline. A short row with three to four large conical papillae extended caudally from each end of the transverse row.

Fig.1A Ventral view of the tongue after isolation from the mandible and head, showing anterior free portion of the tongue, glandula lingualis anterior (GL.L.A.) and glandula lingualis posterior GL.L.P.), In addition, the Glandula Preglottalis (GL.PR.), Glandula Laryngeal (GL.LAR.). **B**: Ventral view of the head and gular region of *Patagioenas livia*, showing glandula sublin-gualis (GL.SL.) position

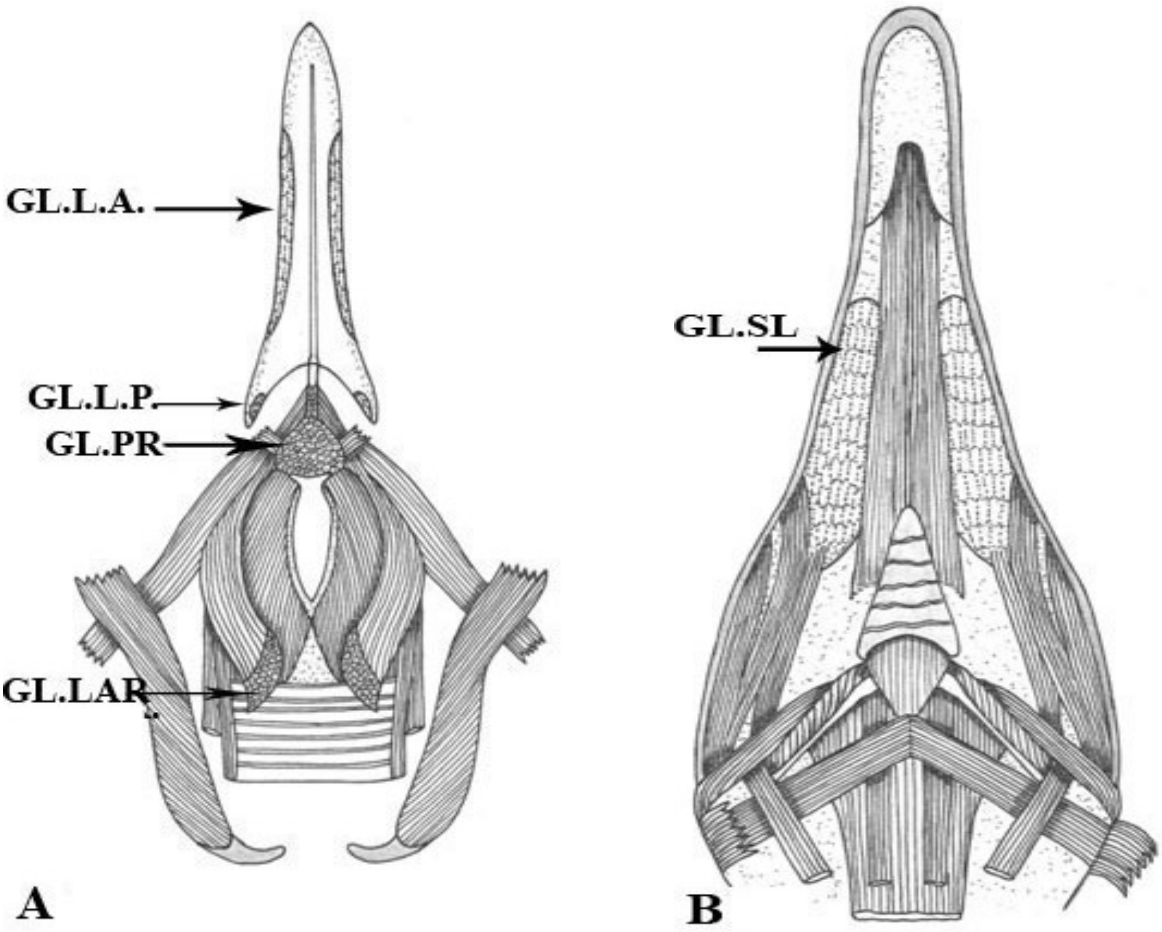


Fig.2A Photomicrograph of a transverse section through the middle part of the free portion of the tongue of *Patagioenas livia*, showing the dorsal epithelium (D.E.), as well as, it is associated with the lingual salivary gland anterior (GL.L.A.) and ventrally, the lingual anterior is attached to the dorsal surface of the paraglossale (PG.) (H.andE., 100x). **B**: the lingual papillae (L.P.), Glandula lingualis posterior (GL.L.P.) (H.andE., 100x). **C:** the preglottal epithelium (E.PG.) and Glandula Preglottalis (GL.PR.) (H.andE., 100x). **D:** Photomicrograph of a transverse section through the floor of mouth of *Patagioenas livia*, showing the Glandula Sublingualis (GL.SL.) is a compound tubule alveolar type which consists of many mucoid secretory lobules, the epithelium of the floor of the mouth (E.F.M.) (HandE, 200x)

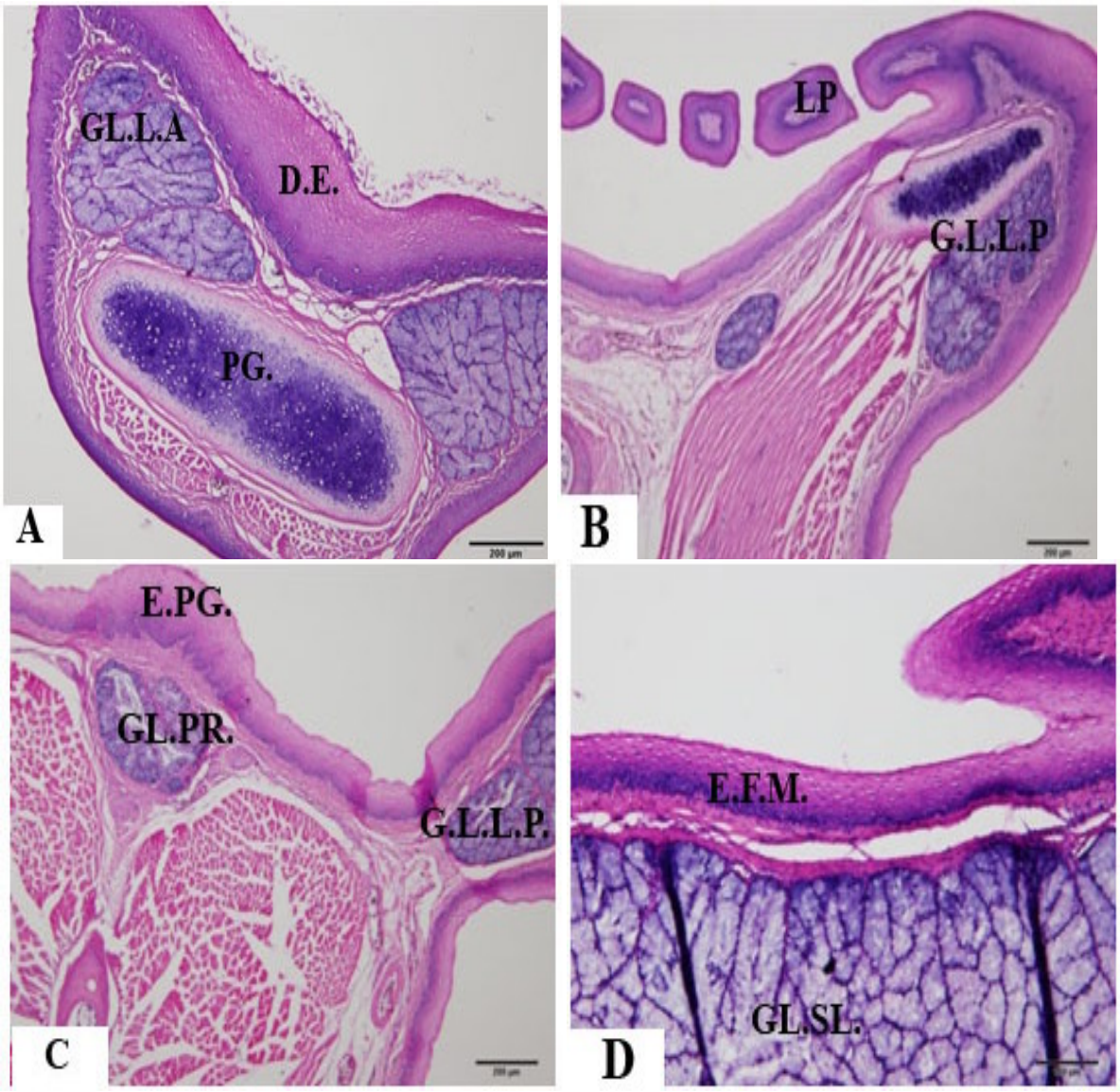


Fig.3A Photomicrograph of a transverse section of the Glandula lingualis anterior (GL.L.A.) of *Patagioenas livia*, showing the lingual salivary gland gives a strong positive Periodic Acid Schiff's reaction (P.A.S) which means it secretes mucopolysaccharides (P.A.S., 200x). **B:** Photomicrograph of a transverse section through the Glandula lingualis posterior (GL.L.P.), showing the lingualis posterior salivary gland gives a positive Periodic Acid Schiff's reaction (P.A.S., 400x). **C:** Photomicrograph of a transverse section through the Glandula preglottalis (GL.PR.) , showing the preglottalis salivary gland gives a positive Periodic Acid Schiff's reaction (P.A.S., 200x)

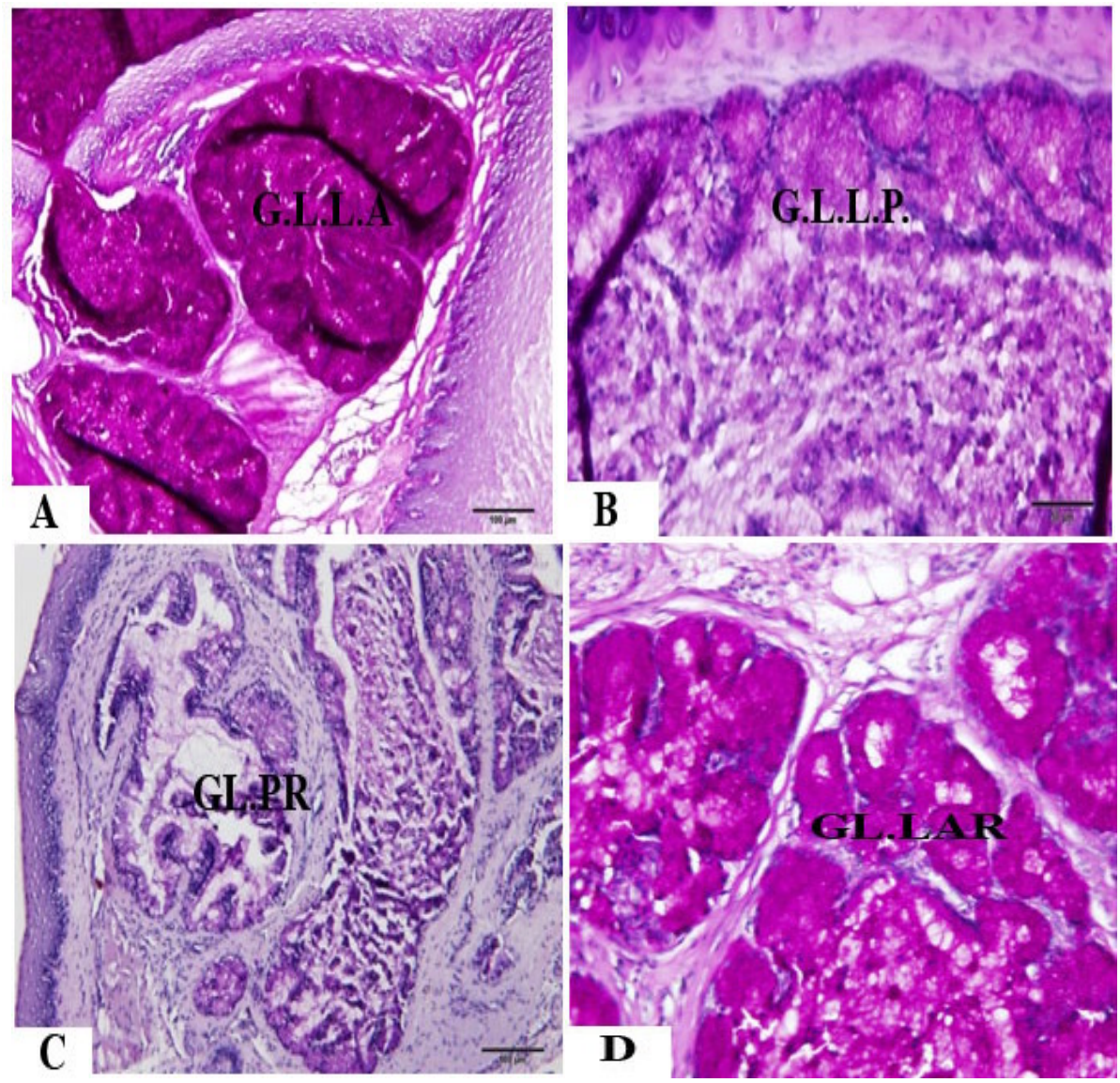
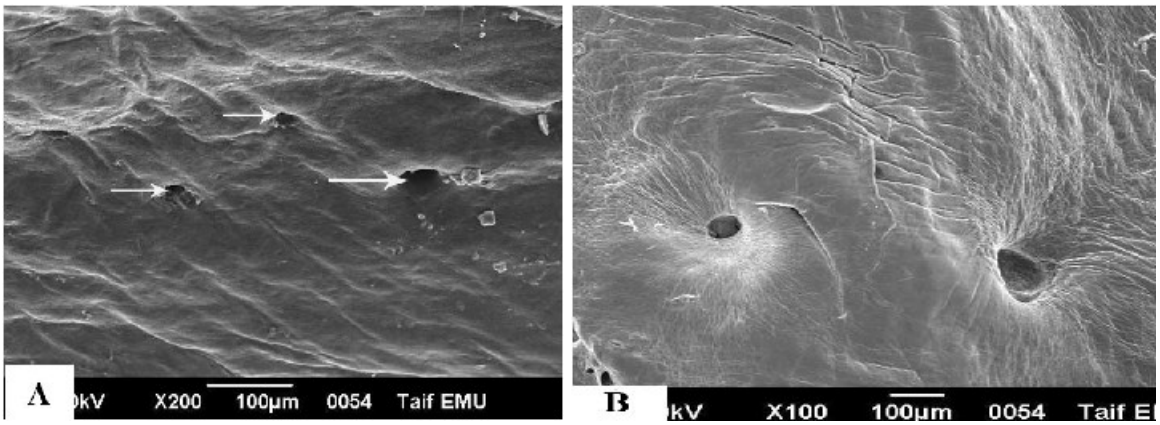


Fig.4A Scanning electromicrograph of the lateral surface of the middle part of the free portion of the tongue of *Patagioenas livia*, showing some pores of the anterior lingual salivary gland (arrows). **B:** Scanning electromicrograph of the dorsal surface of the floor of the mouth of the tongue of *Patagioenas livia*, illustrating pores of sublingual salivary gland (arrows)



The transverse row of the lingual papillae showed a marked concavity in the middle line of the tongue. The openings of the anterior and posterior lingual salivary glands were seen in the lateral surface and at the base of the tongue respectively. The posterior lingual glands extended to the laryngeal cleft. Posterior to the lingual root was the laryngeal cleft which is provided with backward conical papillae and illustrated a single row of pharyngeal papillae behind the laryngeal cleft (Kadhim *et al.*, 2011). *Larus argentatus* has three salivary glands. These glands within the frame of the lingual system are raised from their important role as hydrostatic elements, in addition to their secretion function (Shawki and Al-Jalaud, 1994b). The lingual salivary glands of the domestic Pigeon are of branched tubuloalveolar type and consist of secretory endpices composed of tall columnar cells with extensive vesicular cytoplasm resting at a delicate basement membrane. The ventral surface of the tongue is devoid of any glandular structure. All the lingual salivary glands in domestic pigeon show strongly P.A.S. positive reaction. In many other birds so far studied, the salivary glands have been considered as anterior and posterior lingual glands without any anatomical continuity between them. The secretory cells of the lingual salivary glands shows strongly positive to P.A.S. reaction, indicating that the saliva of the domestic pigeon similar to that of other birds is rich in glycoproteins. The saliva may lubricate ingested food for ease of swallowing, protect the mucous membrane of the upper digestive tract (Parchami and Fatahian Dehkordi, 2011).

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