



**Lingual Epithelium of the Rock Dove, *Patagioenas livia* (Columbidae).
A Light and Scanning Electron Microscopic Studies**

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Abstract

The aim of the present study was to investigate the light and electron microscopic structure of the tongue in lingual epithelium of the rock dove, *Patagioenas livia*. Ten adult Rock dove, (5 males and 5 females) were used in this study. Samples of the different regions of the tongue were routinely prepared for light and electron microscopic study. Results showed that the dorsal surface of the tongue is covered by a stratified squamous epithelium that is thicker in the dorsal surface than that of the ventral surface in the free portion of the tongue and is characterized by the presence of downward folds. The ventral epithelium that covers the free portion of the tongue is highly keratinized. The posterior of the free portion of the tongue ended by paired giant lingual wing. The dorsal surface of the lingual wing is smooth and carries one row of lingual papillae. The outer layers of the preglottal area are formed of squamous cells which is furnished by non-keratinized epithelium. The preglottal epithelium is characterized by the appearance of the dermal papillae.

Keywords: Tongue, Rock dove, Lingual epithelium, Scanning electron microscopy.

Introduction

All birds are adapted to their different environments with respect to food sources. Reflecting their different lifestyles, birds have different feeding habits, with corresponding differences in the structures of their tongues (Parchami et al., 2010). In the anatomy of the tongue, three parts may be distinguished: the apex, the body and the root. The body and the root of the organ are demarcated externally by a single or double crest composed of mechanical conical papillae (Vollme-rhaus & Sinowatz, 1992). The histological structure of the birds tongue has been examined in many different birds, e.g., in parrot, penguin, little tern, owl, white-tailed eagle, hen, quail, and bean goose (Homberger & Brush, 1986; Iwasaki, 1992a; Iwasaki et al., 1997; Jackowiak & Godynicki, 2005; Emura & Chen, 2008). The dorsal and ventral surfaces of the birds tongue are covered by a rather keratinized or non-keratinized thick layer of stratified squamous epithelium. (Erdoğan et al., 2012) stated that keratinized layer of the lateral and lower surfaces of the tongue in domestic birds are reduced in its thickness. Santos et al., (2011) found that stratified squamous epithelium is not keratinized in

the tongue of ratites. (Jackowiak and Ludwig, 2008) stated that the tongue of the herbivorous and granivorous birds is covered with thick keratinized mucosa. Many authors stated that the dorsal epithelium is thicker than the ventral epithelium (Jackowiak and Ludwig, 2008; Crole and Soley, 2009). However, the keratinization is lesser in the tongue of the water habitat birds (Jackowiak et al., 2006).

The present study was aimed to describe the lingual epithelium of the rock dove, *Patagioenas livia* using light and scanning electron microscopy, in order to compare the results with those previous reports in other birds.

Materials and Methods

Experimental Animals

The experimental animals of the present work included adult and healthy birds. Ten adult Rock dove were used in the present study. Birds brought alive to

the laboratory, and then were killed by chloroform inhalation.

Preparation of specimens for Light Microscopic investigation

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.

Preparation of specimens for Scanning Electron Microscopic investigations

The tongue was cut to small pieces, and directly fixed in 5% glutaraldehyde 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 time, 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 AJeol scanning electron microscope (JSM-6390-LA).

Results

The lingual epithelium

The lingual epithelium of the Rock dove, *Patagionas livia*, as well as, its floor of the mouth are covered by a stratified squamous epithelium which is composed of the main following layers; 1- the stratum basale in which the basal cell layer being columnar or cubical; 2- the stratum intermedium that forms a thick layer of polyhedral cells outer to the basal layer; 3- the stratum transitivum that lies outer to the stratum intermedium and is formed of flattened cells with flattened nuclei; 4- and the most outer layer; the stratum corneum which is formed of wavy keratin and lacking nuclei. These layers except the stratum corneum are present

as the constituent of the dorsal epithelium of the free portion of the tongue (Fig.1A).

The epithelium of the free portion of the tongue

Concerning the dorsal epithelium of the free portion of the tongue of the Rock dove, it is thicker than that of the ventral epithelium. The anterior-third of the free portion is furnished dorsally by non true-keratin, with the appearance of the deciduous epithelium outside to the keratinized layer. The ventral epithelium that covers the free portion of the tongue is highly keratinized stratified epithelium forming the differentiated lingual nail and is characterized by the disappearance of the dermal papillae (Fig. 1B).

Scanning electron microscopy (S.E.M.) reveals the presence of deciduous epithelium that is directed medially towards the median sulcus (Fig.1C). However, the keratinization decreases posteriorly and then the posterior surface becomes smooth, as well as, it is associated with the appearance of the pores of the salivary glands (Fig. 1D).

Histological investigation of the free portion of the tongue revealed that the mucosa of the dorsal epithelium is characterized by the presence of downward folds (rete ridges) which interdigitate with the upper projections of the submucosa, dermal papillae resulting in the formation of the mucoso-submucosal junctions and secondary connective tissue papillae (Fig.2A)

Considering the most posterior of the free portion of the tongue, the paired giant lingual wing (Fig.2B) that is enveloped by a well non-keratinized squamous epithelium with short mucoso-submucosal junctions, dermal papillae. Meanwhile, the epithelium that covering the dorsal surface of the lingual wing is smooth and carries one row of lingual papillae (Fig. 2C), each lingual papilla is composed of the mucosa and submucosa . That envelope of the lingual papillae lacks the dermal papillae, like the epithelium covering the lingual wing.

The surface of the lingual nail which is smooth with detached keratin means that its keratin is continuously regenerated (Fig. 2D). Therefore, the covering epithelium of the free portion of the tongue, just posterior to the lingual nail exhibits a gradual decreasing of keratinization until it becomes non-keratinized epithelium with the appearance of mucoso-submucosal junctions, dermal papillae.

The epithelium of the preglottal area

The preglottal area of the Rock dove is furnished by non-keratinized epithelium which is characterized by the absence of true keratin. The outer layers of the preglottal area are formed of squamous cells with their flattened nuclei. The preglottal epithelium is characterized by the appearance of the dermal papillae

which are shorter and fewer than ones associated with the dorsal parakeratinized epithelium of the free portion of the tongue (Fig. 2E). Scanning electron microscopy investigation shows the presence of intercellular borders of the outer cellular layer and the pores of the preglottal salivary gland which cannot be seen by the light microscope (Fig. 2F).

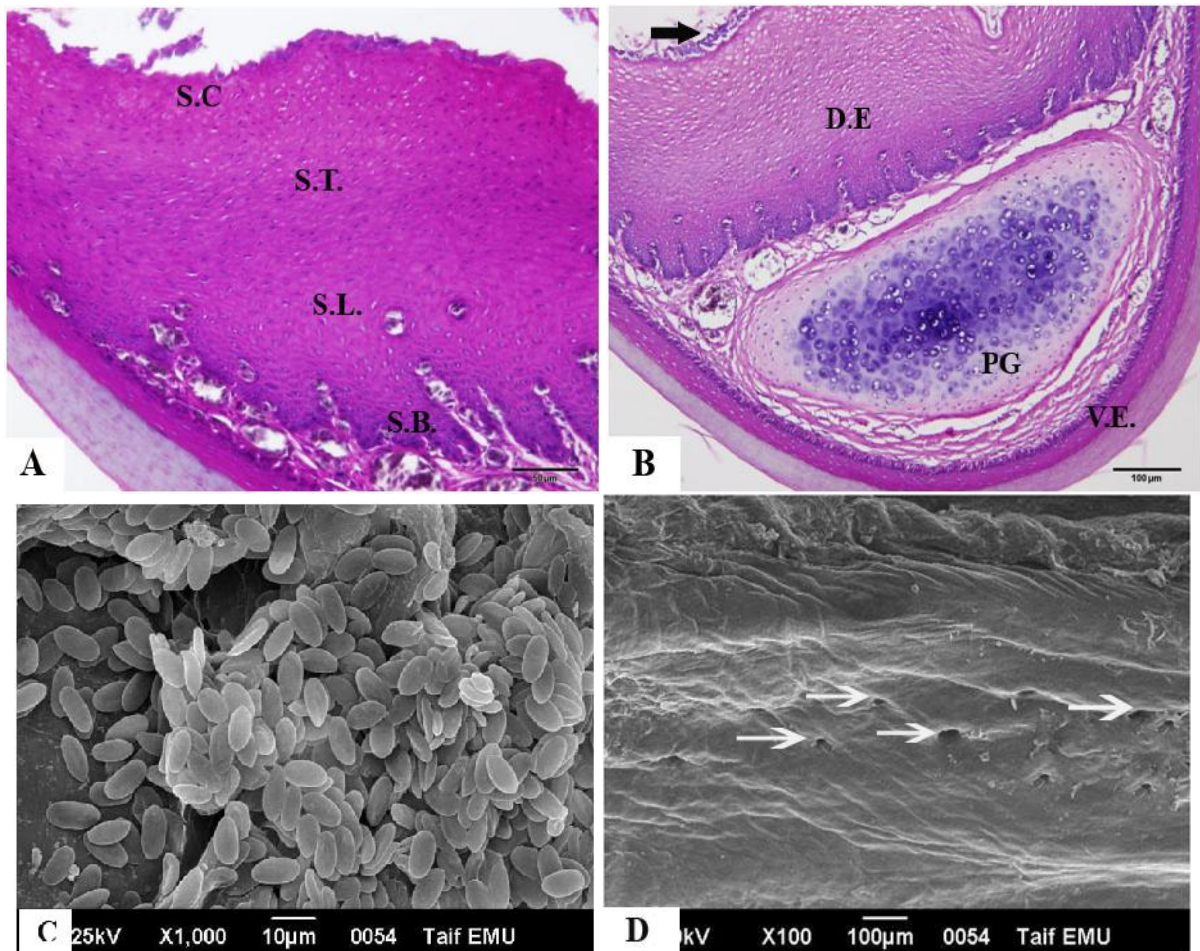


Fig.1. A: Photomicrograph of a transverse section of the dorsal lingual epithelium of the anterior of the free portion of the tongue of *Patagioenas livia*, showing the main cellular layers consisting the keratinized stratified squamous epithelium; stratum basale (S.B.), stratum intermedium (S.I.), stratum transitivum (S.T.) and stratum corneum (S.C.). (H.&E., 400x).

B: Photomicrograph of a transverse section through the anterior-third of the free portion of the tongue showing the dorsal epithelium (D.E.) is furnished by non-true keratin "parakeratosis" with the some projections on the surface (arrow). In addition, the dorsal epithelium (D.E) is thicker than the ventral epithelium (V.E.). (H.&E., 200x).

C: Scanning electromicrograph of the dorsal surface of the anterior of the free portion of the tongue showing the numerous crowded deciduous epithelia (1000x).

D: Scanning electromicrograph of the ventral surface of the free portion of the tongue, showing that the posterior surface becomes smooth, as well as, it is associated with the appearance of the pores of the salivary glands (arrows) (100x).

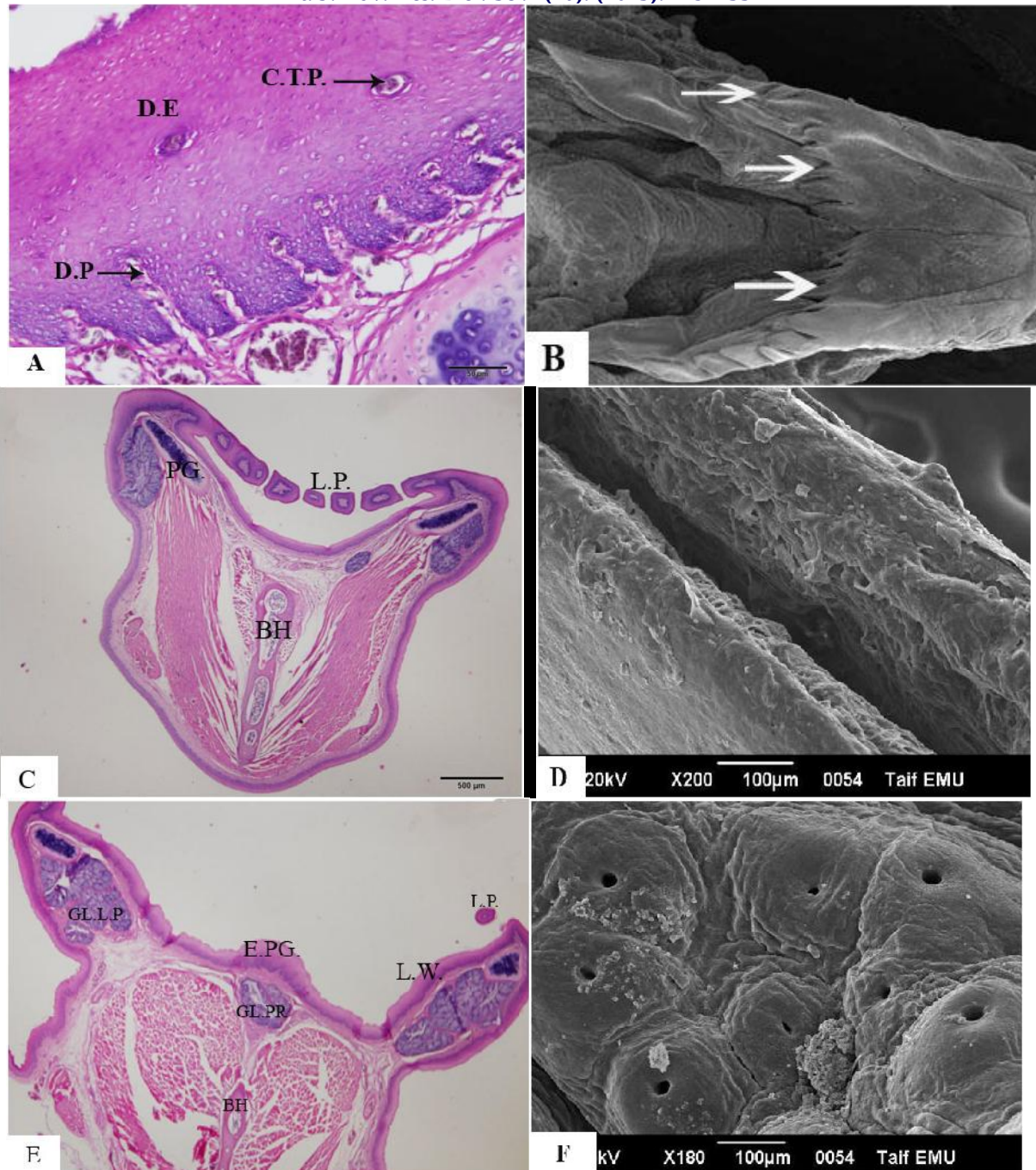


Fig.2. A: Photomicrograph of a transverse section through the anterior-third of the free portion of the tongue of *Patagioenas livia*, showing the dorsal epithelium (D.E.) of the free portion of the tongue, besides the presence of dermal papillae (D.P.) and secondary connective tissue papillae (C.T.P.) (H.&E., 400x).
B: Scanning electromicrograph of the dorsal surface of the posterior part of the free portion of the tongue, showing the short preglottal region. In addition, the row of the lingual papillae (arrows) (20x).
C: Photomicrograph of a transverse section through the posterior part of the free portion of the tongue showing the lingual papillae (L.P.), (H.&E., 40x).
D: Scanning electromicrograph of the dorsal surface of the anterior of the free portion of the tongue showing the dorso-medial groove with detached keratin (200x).
E: Photomicrograph of a transverse section through the preglottal area of the tongue showing the dorsal epithelium of the preglottal region (E.PG.) which is characterized by non-keratinized epithelium its associated with the Glandula Preglottalis (GL.PR.) (H.&E., 40x).
F: Scanning electromicrograph of the dorsal surface of the short preglottal region of the tongue showing the pores of the preglottal salivary glands (180x).

The Rock dove, *Patagioenas livia*, is domesticated birds lives in dry environment. The Rock dove depend fed on its tower beak elevation and depression also, a lingual protraction and retraction. Finally the tongue transfers the food items (grains and seeds) toward the oesophageus. The lingual apparatus of the Rock dove, like the tongue of all birds, is an integrated system in the mechanism of feeding process, which shows special adaptations to meet the requirements of its feeding type. In the Rock dove, the lining epithelium of the tongue and floor of the mouth shows changes in the type and degree of keratinization at different regions; as well as the presence of invested salivary glands which may act as hydraulic structures. Also, the relationships between the epithelium (mucosa) and the underlying submucosa have reflected the functional performance of the tongue and its adaptation during the feeding process. The lingual epithelium is the main component which is directly exposed to these forces; externally from the environment and abrasion force of the food items, and internally from the mechanical forces. The Rock dove tongue, like almost avian tongue, is a complex biomechanical organ which is connected with the floor of the mouth. Stratification and keratinization of the avian lingual epithelium is a common feature (Iwasaki, 1992a; Iwasaki et al., 1997 and Kobayashi et al., 1998). Also, non-keratinized and parakeratinized areas were found among the avian tongue. The epithelium covering the tongue and floor of the mouth of Rock dove exhibits extreme regional variations. Since, non-keratinized epithelium of transitional type lining the connection of the tongue with the floor of the mouth, these regions coincide with the main hyoid joints, which mean that it is the place from which arise the main movements of the tongue. Moreover, the transitional epithelium has a stretching ability and consequently its existence is a demand adaptive for the mechanical performance of the tongue during its protraction and retraction. Also, during the elevation and depression of the free portion of the tongue. Non-keratinized stratified epithelium is covering many regions along the tongue and the floor of the mouth; the dorsal surface of the free portion just anterior to the lingual wing and papillae, the ventral region of the free portion between the posterior end of the lingual nail and the base of the lingual wing, the preglottal area and the floor of the mouth. These regions may be less exposed to abrasive forces which may explain the presence of the multiple pores of the salivary glands.

Considering the keratinized epithelium of the tongue of Rock dove, it has been observed the presence of two types; a parakeratinized and highly strong keratinized epithelium. The parakeratinized epithelium spreads on the dorsal surface of the anterior-third of the free portion of the tongue. Those parakeratinized epithelium posses filiform papillae. This parakeratinized epithelium counteracts the abrasive forces acting on it, as well as helps in spreading of the mucus on its surface. This result agrees with a similar study done on the Chicken and Black kite by (Iwasaki & Kobayashi, 1986 and Shawki, 2006). The occurrence of the above mentioned type of stratified epithelium is common in birds i.e. in the Chicken and Quail tongues (Iwasaki & Kobayashi, 1986 and Parchami et al., 2010), terminal modification of the dorsal epithelium is more distinctly different in the anterior and posterior regions, so-called "filiform papillae" or distinct protrusions of the deciduous epithelial cells that are widely distributed over the anterior region of Chick tongue, whereas they are not present in the posterior region. In the little tern (Iwasaki, 1992b), the protrusion of the deciduous epithelial cells is prominent only in the anterior region of the tongue. In the white-tailed eagle (Jackowiak & Godynicki, 2005), most of the dorsal surface of the lingual corpus and root is covered with the intensively desquamate non-keratinized epithelium "parakeratinized epithelium" and were suggested that may be considered species-specific trait. The highly keratinized epithelium establishes the most prominent keratinized elements among the avian tongue; the lingual nail. The lingual nail is the highest keratinized element in the Rock dove tongue. The investigation of the lingual nail during the movement of the tongue illustrates that; it is pliable. Thus it can change its shape according to its use. Therefore, it is affected by the internal forces acting on the tip of the tongue. Although, the lingual nail is a supportive structure for the anterior half of the free portion of the tongue, it is facilitating picking up and manipulating the food items also help the bird in drinking, that has been described in the tongues of other avian species (Susi, 1969; Homberger & Brush, 1986 and Igwebuike & Eze, 2010).

The lingual and laryngeal papillae are non-keratinized pointed. They can adjust their position to perform their function in pushing the food items posteriorly during the retraction of the tongue. Iwasaki and Kobayashi (1986) concluded that the lingual papillae are common not only in mammals and amphibian, but also in birds.

In fact, from the previous anatomical studies and the present work on the Rock dove, one may suggest that both the lingual and laryngeal papillae are prominent in the avian tongue. Jackowiak and Godynicki (2005) were suggested that the parakeratinized epithelium may be considered species-specific trait. Soft keratin overspreads the dorsal surface of the laryngeal mound. That keratin disappears during routine histological preparations. Chen and Myer (1971) pointed out the presence of different types of keratins in the oral epithelium of birds. Meanwhile, Homberger and Brush (1986) concluded that in the parrot the beta keratin is expressed in the antero-ventral region of the free portion of the tongue, while alpha keratin is detected in all epithelium of the tongue. Moreover, Sawyer et al., (1986) and Landman (1986) clarified that alpha keratins are found in all vertebrate epithelia, but beta keratins are detected only in specific epithelial tissues of birds and reptiles. Carver and Sawya (1989) concluded that the beta keratins appear to be produced only in the differentiated epithelial cells on the antero-ventral region of the tongue. In fact, Shawki (2006) observed the different properties of the keratinized structural elements among the black kite tongue and pointed out about this point that these changes may be due to the difference of the chemical composition of these keratins, which will be of great interest to be studied in the future.

The transitional epithelium of the floor of the mouth and its connection with the tongue helps to withstand the expansion and mechanical performance of the tongue during swallowing process, this result agrees with a similar study done on the Black kite by (Shawki, 2006).

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