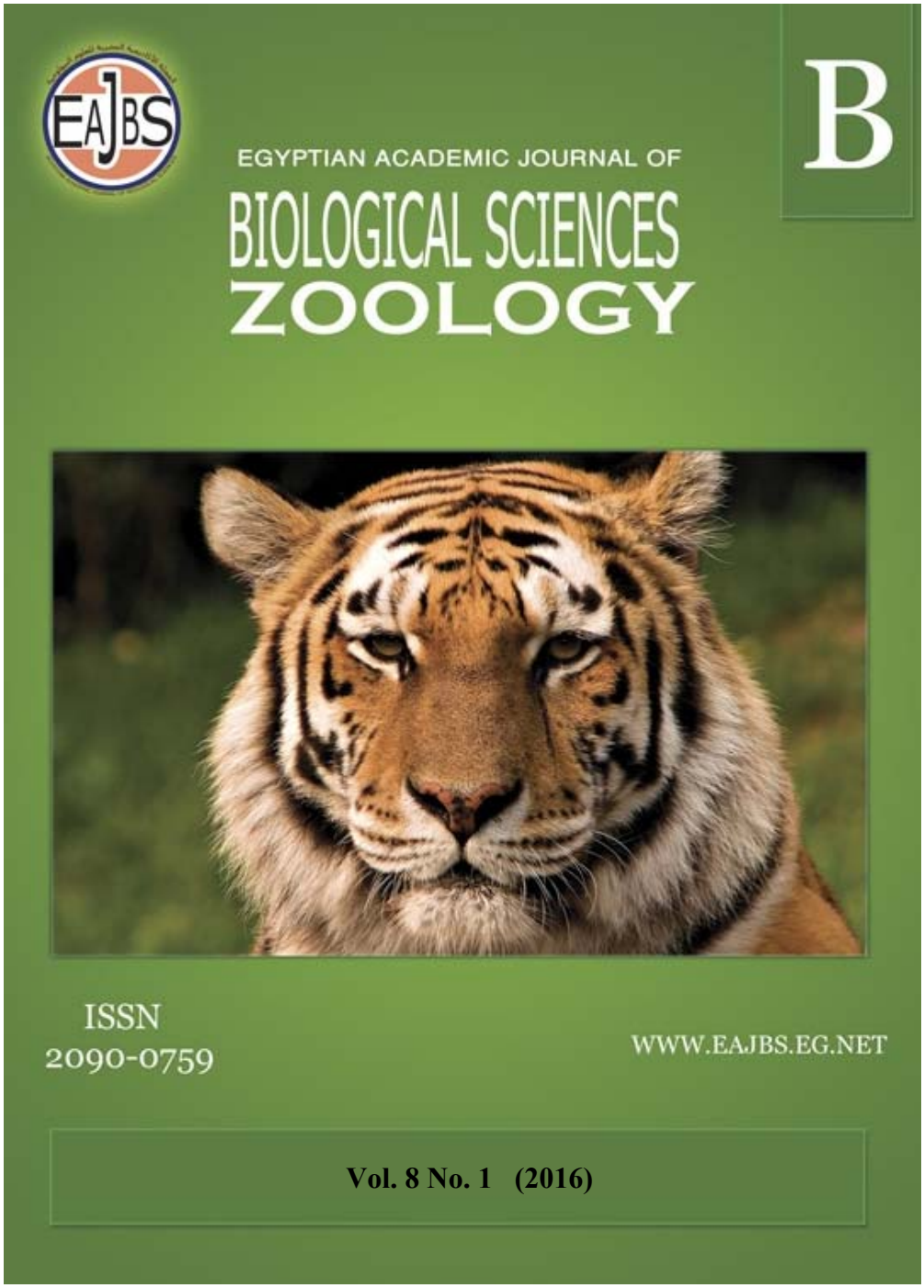

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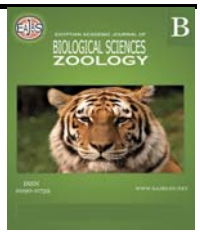

Comparative Morphological, Ultrastructural and Histological Studies on the Tongue of Eurasian Wigeon *(Anas penelope)* and Northern Shoveler *(Anas clypeata)*

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ABSTRACT

The aim of this work is to examine the morphological features of the tongue of two adult species of the Family Anatidae, Eurasian Wigeon *(Anas penelope)* and Northern Shoveler *(Anas clypeata)*, using light and scanning electron microscopy. The tongue is divided into apex, body and root. Here, our study shows that, the small hair-like filiform lingual papillae are found in the lateral sides of the apex region of the tongue of Eurasian Wigeon. The body region of the tongue of the Eurasian Wigeon has several numbers of mechanical conical papillae on its lateral sides, while the northern shoveler has two rows of small conical papillae, each single row consists of several pairs of the conical papillae on the dorsal surface. In the body and root regions of both Eurasian Wigeon and Northern Shoveler, there are large units of glands located in the lamina proparia. The anatomical characteristics of the tongue of Eurasian Wigeon and Northern Shoveler adapt to their life in the aquatic environment.

INTRODUCTION

Anseriformes is a large and complex group including approximately 150 species and showing very high worldwide variety as reported by Gonzalez *et al.* (2009). The waterfowl (Anseriformes) are amongst the best known of all avian groups (Olson and Feduccia, 1980). Comparative studies that have been made on the tongue of different vertebrate species suggest morphological adaptations throughout the process of evolution. These evolutionary changes are considered the principles for the progress in food intake and occupation of different habitats (Iwasaki, 2002 and Guimarães *et al*., 2014).

The results of morphological studies proceed so far suggest a close connection of the tongue shape, the structure of the epithelium of the mucosa of the tongue with the mechanism of food intake and the type of food, and the habitat (Homberger and Meyers, 1989; Iwasaki *et al.,* 1997 and Jackowiak and Godynicki, 2005). The different studies on the avian tongues is attributed the structural/anatomical variations

of those tongues to the feeding habits as birds are adapted to live the different environmental conditions (Iwasaki, 2002). There are many examples for that such as white tailed eagle (Jackowiak and Godynicki, 2005), cormorant (Jackowiak *et al.,* 2006), peregrine falcon and common kestrel (Emura *et al.,* 2008), Japanese pygmy woodpecker (Emura *et al.,* 2009), blue-and-white flycatcher, hawfinch and Japanese white-eye (Emura *et al.,* 2010), common quail (Parchami *et al.,* 2010), rainbow lorikeet (Emura *et al.,* 2011), scarlet macaw (Emura *et al.,* 2012), white-breasted kingfisher (El-Bakary, 2012), muscovy duck (Igwebuike and Anagor, 2013), white throated kingfisher and common buzzard (El-Beltagy, 2013), domestic goose (*Anser anser f. domestica*), the domestic duck (*Anas platyrhynchos f. domestica*) (Skiereszand and Jackowiak, 2014) and Northern Pintali (El-Bakary, 2015).

Both species under study show a preference for the same habitat as they live in shallow permanent waters, slow-flowing large rivers, small freshwater lakes and shallow marshes with abundant emergent vegetation are preferred (Kear, 2005). Also, their feeding materials are very similar, they could feed on molluscs, worms, insects and crustaceans. During winter the species mainly feeds on the seeds of grasses, aquatic plants, leaves, stems and root bulbs of pond weeds and algae (Del Hoyo *et al.*, 1992 and Kear, 2005).

The aim of the present work was to make morphological, histological and ultra-structural comparison between the tongue in *Anas penelope* (Eurasian Wigeon) and *Anas clypeata* (Northern Shoveler) and comparing our results with the previous examined list of avian species.

MATERIALS AND METHODS

All the investigated animals were mature and in good health. The two species (*Anas penelope, Anas clypeata*) were obtained from the local markets in Port Said and Damietta. They were slaughtered and the head region of each bird was dissected, then gross photographs of the tongue were captured by a digital camera to show the morphological differences between the two investigated species.

Scanning electron microscope

The tongues were fixed in 10% formalin, post fixed with 1% osmium tetroxide for 1h at pH 7.2. Then, the specimens were dehydrated in a graded series of ethanol and critical point dried. Some of the samples were washed in distilled water after fixation. Then fixed at 1% buffered osmium tetroxide for 1h. After washing three times, the specimens were dehydrated in a series of ethanol and critical point dried (Jackowiak and Godynicki, 2005). All specimens were mounted on aluminum stubs covered with carbon tabs, sputtered with gold and observed under JEOL scanning electron microscopy (JSM-5300) at an accelerating voltage of 15kv in electron microscopy unit at the Faculty of Science, Alexandria University.

Light microscope study:

The extracted tongues of the two species were cut into the three parts representing the three main regions of the tongue; apex, body and root regions. These parts were preserved in 10% formalin fixative, then dehydrated in an ascending series of alcohol, cleared in xylene and finally embedded in paraffin wax. Histological sections of different parts were prepared and stained with haemtoxyline and eosin in a histology laboratory in the Faculty of Science.

RESULTS

First we compared each part of the tongue from each species morphologically.

Anas penelope

The total length of the tongue is 5 cm and its width is 1 cm, whereby the apex measures 0.5 cm, the body with the lingual prominence measures 3.5 cm and the root measures 1 cm. The apex of the tongue of the *Anas crecca* (common teal) is roughly rounded with no lingual papillae within its smooth edge. On the flat dorsal surface of the tongue body there is a median groove divides the lingual mucosa into two equal parts (Fig. 1).

The body region of the tongue has several numbers of mechanical conical papillae on its lateral sides. There is a triangular lingual prominence above the root region with 6 pairs of large and small conical papillae. Those are arranged in a single row at its end. The root region of the tongue is a relatively short region and communicated with the laryngeal prominence. The conical lingual papillae protruding from it and caudally directed. The glottis, a large oval opening, is apparent caudal to the root region (Fig. 1).

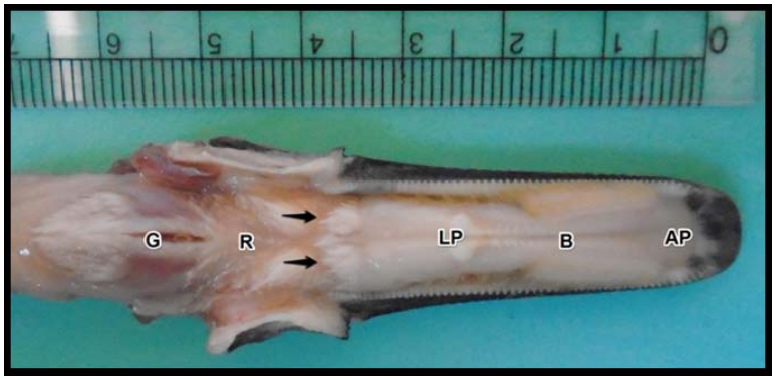

Fig. 1: Dorsal view of *Anas penelope* tongue, showing its parts; apex (AP), body (B), root (R), lingual prominence (LP), Glottis fissure (G) and caudally directed lingual papillae (Black arrows).

Anas clypeata

The tongue of the *Anas clypeata* (northern shoveler*)* is relatively large in size and elongated, the body region is broader than the other two parts, apex and root. The tongue had a total length of 7 cm and its width is 1.7 cm, whereby the apex measured about 0.5 cm, the body with the lingual prominence 6 cm and the root 0.5 cm. The apex of the tongue is roughly rounded, on the flat dorsal surface of the tongue body there is a median groove dividing the lingual mucosa into two equal parts (Fig. 2).

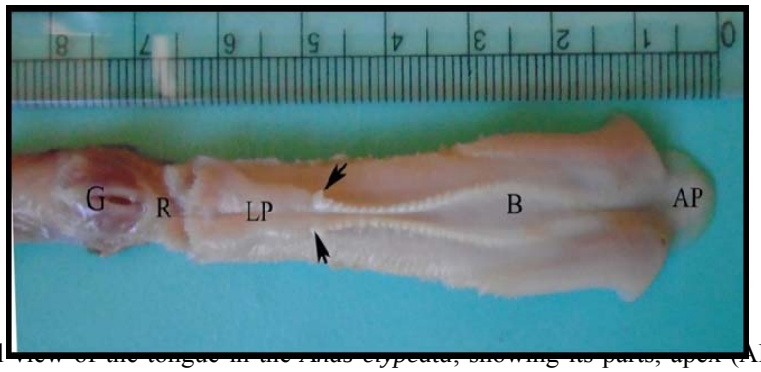

Fig. 2: Dorsal view of the tongue in the *Anas clypeata*, showing its parts; apex (AP), body (B), root (R), lingual prominence (LP) - Glottis fissure (G) and lingual papillae (black arrows).

On the dorsal surface of the body region, there are two rows of lingual conical papillae each single row consists of several numbers of small conical papillae. The lingual prominence which can be seen above the body region of the tongue is triangular in shape, its basal part lies towards the root region and lined with a row of lingual papillae. The root region of the tongue is communicated with the laryngeal prominence. There is a large and oval glottis (Fig 2).

***Scanning electron microscopy* (SEM)**

In this part of study we address the ultra-comparison between the tongues from the two species investigated in the study using the scanning electron microscopy

Anas crecca

Our results show that the lingual apex region is short and most of its dorsal surface was relatively smooth, without any lingual papillae (Fig. 3 a). With higher magnification of scanning electron microscope, there are several superficial leaf-like cells in the dorsal side of the apex region (Fig. 3 b). There are several folds in the dorsal surface of the apex (Fig. 3 c). The apex region is distinguished by the presence of the lingual glands openings in the apex region (Fig. 3 d).

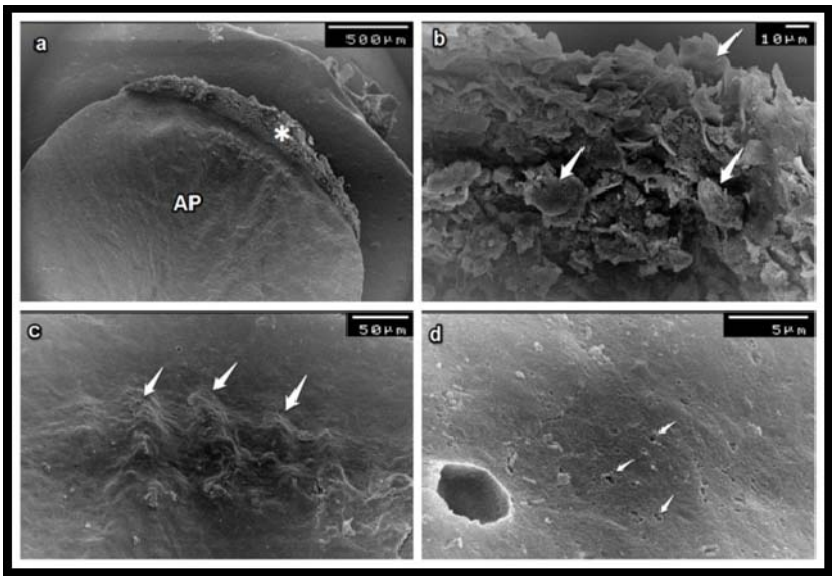

Fig. 3: Scanning electron micrograph of dorsal lingual surface of the apex region of the tongue of *Anas Penelope*, showing a horny tip (star) of the apex (Ap). Scale bar: 500 µm (a), higher magnification of the horny tip on the dorsal surface of the apex region, leaf-like processes (white arrows). Scale bar: 10 µm (b), higher magnification of the epithelium on the dorsal surface of the apex region, white arrow shows small folds. Scale bar: 50 µm (c), the openings of the lingual glands (white arrows). Scale bar: 5 µm (d).

Large conical papillae and foliate papillae were located in many transverse rows in the body region. The outer surface of the large conical papillae is tended to be smooth, with relatively large numbers of desquamating cells were seen on its surface (Fig. 4 a,b). The desquamating cells could be easily seen on the wrinkled surface of the epithelium in the body region (Fig. 4 c). There are well-developed, significant microridges on the surface of all the outer epithelial cells in the lingual body region which is a distinguishing feature of *Anas penelope* (Fig. 4 d).

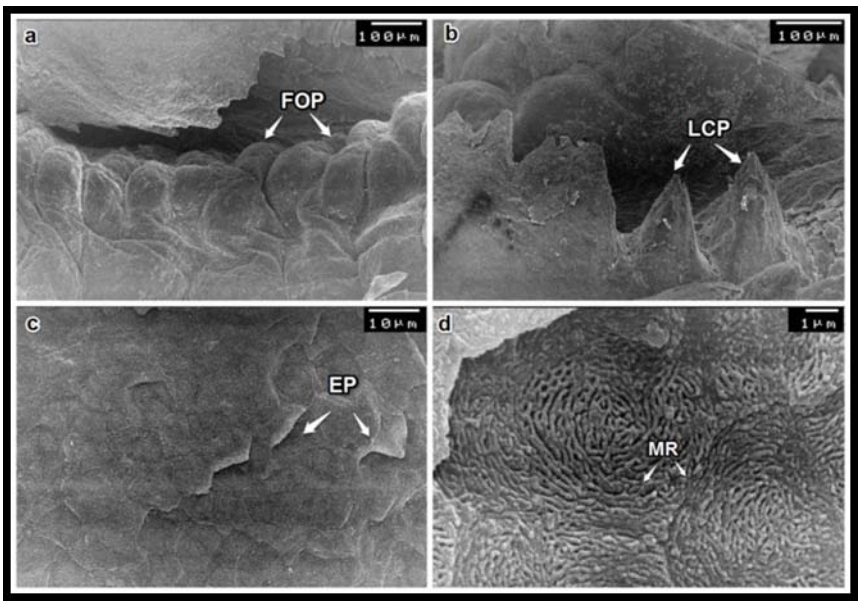

Fig. 4: Scanning electron micrograph of dorsal lingual surface of the body region of the tongue in *Anas Penelope*, showing the foliate papillae (FOP). Scale bar: 100 µm (a), the large conical papillae (LCP) in the body region (B). Scale bar: 100 µm (b), higher magnification of the mucosal epithelium (EP) on the dorsal surface of the body region, white arrow shows superficial cell. Scale bar: 10 µm (c), microridges (MR) on the body surface. Scale bar: 1 µm (d).

In the root region of the tongue, many rows of small conical papillae and large conical papillae could be seen, with pointed processes directed sideways and slightly backwards (Fig. 5 a). Large numbers of lingual projections appear in the region between the conical papillae (Fig. 5 b).

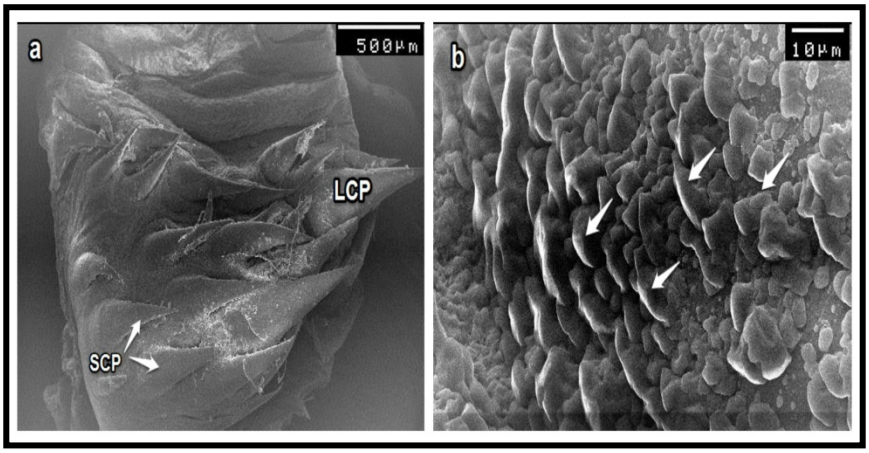

Fig. 5: Scanning electron micrograph of dorsal lingual surface of root region of the tongue in *Anas penelope*, showing the large conical papillae (LCP) and small conical papillae (SCP). Scale bar: 500 µm (a), the region between the conical papilla with several projections (white arrows). Scale bar: 10 µm (b).

Anas clypeata

The lingual apex region is short and most of its dorsal surface was relatively smooth and has no lingual papillae on its tip though horny folds were found (Fig. 6 a). Using magnification of scanning electron microscope, superficial cells are present in the dorsal side of the apex region as epithelial surface of the lamina propria of the lingual mucosa (Fig. 6 b). The desquamating cells could be easily seen on the wrinkled surface of the epithelium in the apex region (Fig. 6 c).

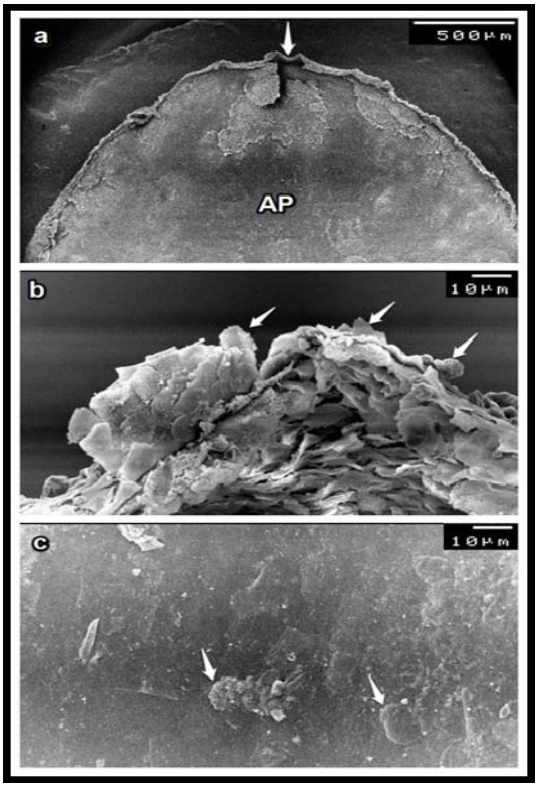

Fig. 6: Scanning electron micrograph of dorsal lingual surface of the apex region of the tongue in (*Anas clypeata*), showing the horny tip (white arrow) of the tongue apex (Ap). Scale bar: 500 µm **(a),** the dorsal epithelial surface of the lamina proparia of the lingual mucosa of the apex (white arrows). Scale bar: 10 µm **(b),** higher magnification of the mucosal epithelium on the dorsal surface of the apex region. Scale bar: 10 µm **(c).**

The lingual body region has small conical papilla have the shape of flattened plates whose ends are sharpened with blunt processes pointing to the back of the tongue, also many rows of relatively small and very densely arranged filiform papillae were conspicuous as hair like papillae arranged laterally in the body region of the tongue (Fig. 7 a,b).

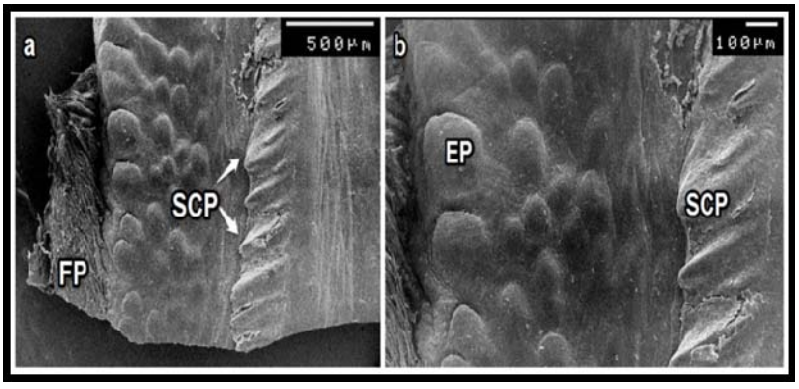

Fig. 7: Scanning electron micrograph of dorsal lingual surface of body region of the tongue in *Anas clypeata*, showing the filiform papillae (FP) on lateral side and small conical papillae (SCP). Scale bar: 500 µm (a), higher magnification of the mucosal epithelium (Ep) with several cells with definite border on the dorsal surface of the body region and small conical papillae (SCP). Scale bar: 100 µm (b).

In the root region, the large and small conical papillae were distributed. Conical papillae of this row were found to be located closer to each other and each papilla was longer than the other (Fig. 8).

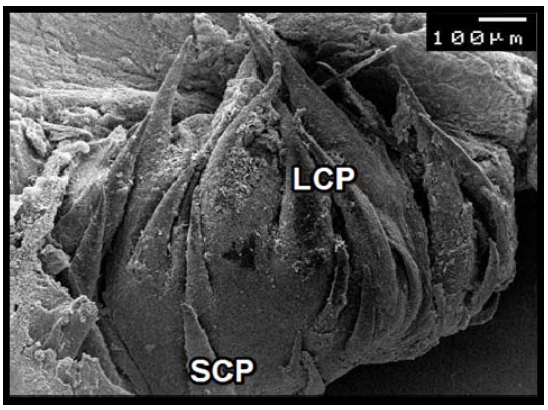

Fig. 8: Scanning electron micrograph of dorsal lingual surface of root region of the tongue in *Anas clypeata*, showing the small conical papillae (SCP) and large conical papillae (LCP). Scale bar: 100 µm.

Histology

1. *Anas penelope*

In the apex region of *Anas penelope,* the dorsal and ventral surfaces of the tongue are lined by non-keratinized stratified squamous epithelium. The ventral epithelium is thicker than the dorsal epithelium. The lamina proparia contains many units of lingual glands (Fig. 9 a). The lingual papillae are covered by keratinized stratified squamous epithelium. The hyaline cartilage surrounded by striated muscles is found in the center of the tongue (Fig. 9 b).

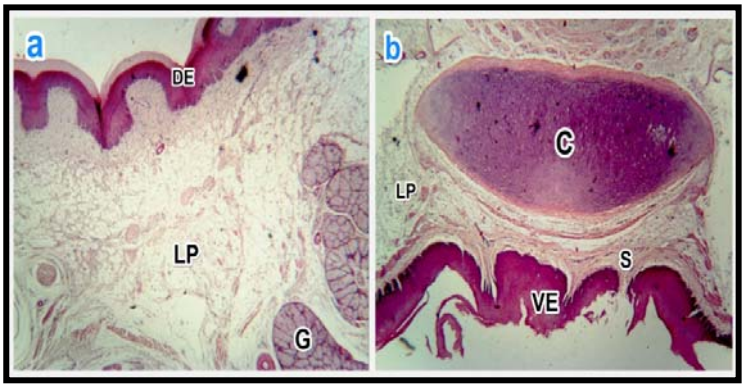

Fig. 9: Light photomicrograph of transverse section of the apex region of the tongue of *Anas penelope* (Eurasian Wigeon), showing the dorsal epithelium (DE), lamina proparia (LP) filled with glandular units (G) Haematoxyline and eosin staining (40x) **(a),** apex region showing venteral lingual epithelium (VE), lamina proparia (LP), skeletal muscles (S) and a centrally located hyaline cartilage (C), Haematoxyline and eosin staining (40x) **(b).**

Large numbers of simple branched tubular glands are present in the lamina proparia of both body and root regions (Fig. 10 a, b). The glandular units are surrounded by a richly vascularized, condensed layer of connective tissue and containing round and oval glandular aciai. Each acinus is composed of typical mucus-secreting cells, with basally displaced dark nuclei and lightly stained cytoplasm (Fig. 10 a, b).

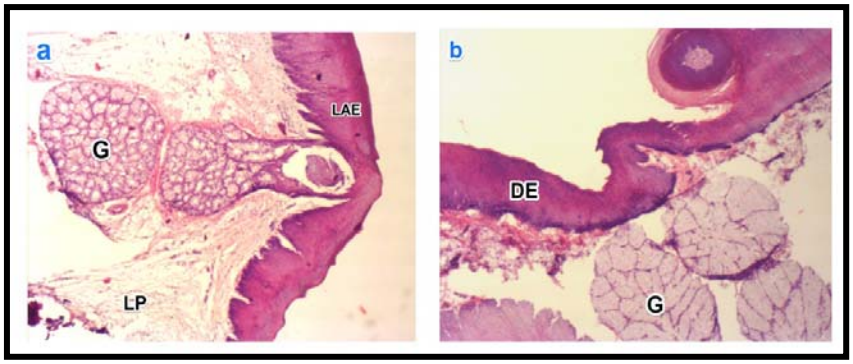

Fig. 10: Light photomicrograph of transverse section of the body and root regions of the tongue of *Anas penelope* (Eurasian Wigeon), body region showing lamina proparia (LP) filled with large glandular units (G) and the lateral epithelium (LAE). Haematoxyline and eosin staining (40x) (a), root region showing dorsal epithelium (DE) and large glandular units (G). Haematoxyline and eosin staining (40x) (b).

2. *Anas clypeata*

In the apex region of *Anas clypeata*, the dorsal epithelium is thicker than the ventral epithelium. Both ventral and dorsal epithelial linings are lined by non-keratinized stratified squamous epithelium. The hyoid cartilage is surrounded by skeletal muscles (Fig. 11 a). The lamina proparia penetrates the epithelium of the tongue to form the lingual papillae with connective tissue cores (Fig. 11 b).

The lingual submucosa of both body and root regions contains large numbers of simple branched tubular glands (Fig. 12 a, b). Using higher magnification, the glandular units are surrounded by a richly vascularized, condensed layer of connective tissue and containing round and oval glandular acinai. Each acinus is composed of typical mucus-secreting cells, with basally displaced dark nuclei and lightly stained cytoplasm (Fig. 12 a, b).

Fig. 11: Light photomicrograph of transverse section of the apex region of *Anas clypeata* (Northern Shoveler), showing dorsal epithelium (DE) and ventral epithelium (VE), lamina proparia (LP) and hyaline cartilage (C). Haematoxyline and eosin staining (40x) **(a),** apex region showing, lingual papillae (P) Haematoxyline and eosin staining (40x) **(b).**

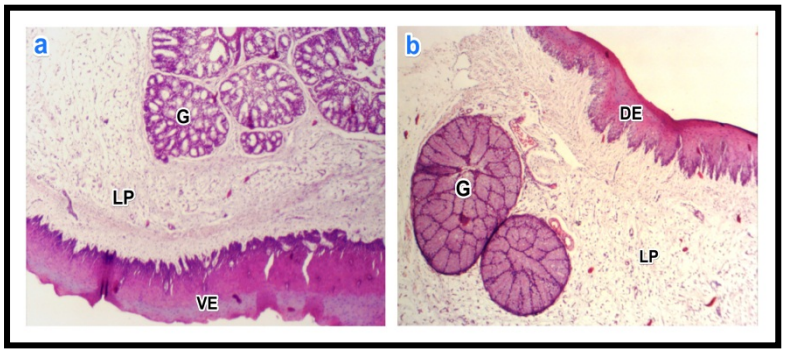

Fig. 12: Light photomicrograph of transverse section of the body and root regions of *Anas clypeata* (Northern Shoveler), body region showing connective tissue of lamina proparia (LP), large glandular units (G). Haematoxyline and eosin staining (40x) (a), root region showing dorsal epithelium (DE), lamina proparia (LP) and large glandular units (G). Haematoxyline and eosin staining (40x) (b).

DISCUSSION

All birds adapt to their habitats according to feeding mechanisms. Reflecting their different lifestyles, birds have various feeding habits, with parallel differences in the structures of their bills and tongues (Emura *et al.,* 2008). The oropharynx and tongue anatomy is studied according to many important determinants as an adaptation of a bird to its habitat, mode of food procuration and habits of feeding (Igwebuike and Eze, 2010). The morphological adaptations of birds tongues are closely related to different eating habits and lifestyle in several environments, so the diversity of feeding adaptations among avian species can be reflected in the form and function of their feeding apparatus (Emura *et al*., 2008 and Parchami *et al.,* 2010).

There is a wide variation in the diet and feeding mechanisms among different species of the Anseriformes, the Anatinae subfamily which includes ducks this study feeds on grains, plants by using a filtration tool, called lamellae. Other species in this subfamily feed on small aquatic organisms like bivalves, other mollusks and fish and in contrast to the Anserinae subfamily which includes geese and swans feeds mainly on vegetative parts of plants (Van Der Leeuw *et al.,* 2003).

The shape of the avian tongue is correlated to the form of the lower beak (Emura *et al.,* 2008 and Parchami *et al*., 2010). The tongue in many species of birds is a triangular organ that takes the shape of the lower part of the bill as it fills it (Mclelland, 1990). The tongues of *anas penelope* (Eurasian Wigeon) and *anas clypeata* (Northern Shoveler) have special morphological characteristics that differ from ostrich (*Strutio camelus*) (Jackowiak and Ludwig, 2008), Hoopoe (*Upupa Epops*) (El Bakary, 2011), Nutcracker (*Nucifraga caryocatactes*) (Jackowiak *et al.,* 2010) and common kingfisher (*Alcedo atthis*) (Al-Zahaby and Elsheikh, 2014). The tongue is long and broad and has a shovel shaped tip. In this study, we show that the tongue of the investigated species is elongated and oval, this is in accordance with Getty (1975), Hassan *et al.* (2010) and Iwsaki *et al.* (1997).

The tongue in avian species is divided into three parts as apex, lingual body, and root (Jackowiak and Godynicki, 2005). In the dorsal surface of the lingual body, there is a median groove that divides the lingual mucosa into two equal parts continues till the root region found in the tongue of four investigated species (*Anas penelope* and *Anas clypeata*) which also is considered as a distinguishing mark for Middendorff's bean goose (Iwasaki *et al.,* 1997). White-tailed eagle (Jackowiak and Godynicki, 2005), Egyptian goose (Hassan *et al*., 2010), and nutcracker (Jackowiak *et al*., 2010). Although median groove in raven is vague and shorter, it is not present in magpie, penguin (Kobayashi *et al*., 1998) and *Rhea americana*, a member of ratite birds (Carlesso *et al*., 2011). The main function of the median groove of the tongue is directing food transport (Jackwiak and Godynicki, 2005 and El Bakary, 2015).

This study was in agreement with the study of Skieresz-Szewczyk and Jackowiak, (2016) in the occurrence of the filtration apparatus in the domestic ducks consists of small and large conical papillae and the filiform papillae which helped in filtering the food immersed in water. The liquids containing food are collected. The tongue is then lifted and passed to the palate. Water is then removed outside oral cavity and food stays on the laminae of the beak and on the surface of filiform papillae of the tongue (Skieresz-Szewczyk and Jackowiak, 2016).

The dorsal and ventral surfaces of the tongue of the two studied species of ducks are lined with non-keratinized stratified squamous epithelium that in agreement with the study of Muscovy duck (Igwebuike and Anagor, 2013). Non-keratinized lingual epithelium is also found in the emu (Crole and Soley, 2008) and the ostrich

(Jackowiak and Ludwig, 2008). This finding is obviously different from the keratinized lingual epithelium detected in Japanese quail (Warner *et al.,* 1967) and in most birds as reported by Kobayashi *et al.* (1998). the tongue of the Magellanic penguin showed a highly keratinized epithelium both in the dorsal and ventral regions (Guimarães *et al.,* 2014) as it was observed with other penguin species (Kobayashi *et al.,* 1998) and in other birds, the cormorant (Jackowiak *et al*., 2006), the oriental scops owl, and the Japanese pigmy woodpecker (Emura *et al.,* 2009 a, b), whose feeding habits depend on a more rigid and resistant tongue structure. Our study showed that the dorsal surface of the lingual apex of *ansa clypeata* is thicker than the dorsal surface of the lingual apex of *anas Penelope*.

Mucous lingual glands can be found in the lamina proparia of the lingual submucosa in the apex, body and root regions of the tongue of *Anas Penelope*, while apex region of *Anas clypeata* lacks lingual glands. The lingual salivary glands have important functions in food lubrication and moisture, which help in rolling or sliding the food over the smooth tongue surface towards the esophagus (Jackowiak and Ludwig, 2008) Also, salivary glands play a role in the protection of the tongue surface from large material and desiccation (Tabak *et al*., 1982 and Igwebuike and Anagor, 2013).

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ARABIC SUMMERY

دراسات مورفولوجيه وتركيبيه وهستولوجيه مقارنه على لسان الصواى والكيش

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الهدف من هذا العمل هو دراسة الخصائص المورفولوجية للسان فصيلتين من عائله البط الصواى والكيش. وذلك باستخدام الميكروسكوب الضوئى و الميكروسكوب الالكترونى. ينقسم اللسان إلى ثلاث مناطق وهى قمة اللسان وجسم اللسان وجذر اللسان. هذه الدراسه تظهر وجود الحليمات الصغيرة خيطية الشكل على جانبى منطقه قمه اللسان فى الصواى. منطقه جسم اللسان تحتوى على العديد من الحلمات المخروطيه الميكانيكيه على الجانبين بينما فى الكيش تحتوى منطقه جسد اللسان على صفين من الحلمات المخروطيه الصغيره كل صف منها يحتوى على عدد من الحلمات على سطحه الظهرى. فى كل من منطقه جسم اللسان ومنطقه الجذر لكل من الصواى و الكيش يلاحظ وجود وحدات كبيره من الغدد. الصفات التشريحيه للسان الصواى و الكيش تمكنها من التأقلم مع البيئه المائيه.