Light and scanning electron microscopic study of the lingual structure in the budgerigar (*Melopsittacus undulatus*)

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Abstract:

BACKGROUND: The tongue, which plays a very important role in food intake by vertebrates, exhibits significant morphological variations that appear to represent adaptation to the current environmental conditions of each respective habitat. **OBJECTIVES:** The aim of the present investigation was to investigate lingual structure in adult budgerigar. **METHODS:** Tongues of 12 adult budgerigars were used in this investigation. Samples of the apex, body and root of the tongues were studied using light and electron microscopy. **RESULTS:** The tongue in budgerigar is about 5 mm in length. The deep concave rostral portion of the lingual apex is devoid of any glandular structure and is continuous with a semicircular caudal portion. The caudal portion of the lingual apex is divided into two symmetrical halves by a median longitudinal fissure. The rostral part of the lingual corpus is distinctly divided by fissures of varying depth into many irregular raised areas with different sizes. Several large caudally directed conical papillae are situated on the posterior end of the lingual corpus and along the thick border region between the lingual body and root. There are also some giant conical papillae on the laryngeal mound. According to their positions, the PAS-positive compound tubuloalveolar salivary glands can be classified as dorsal and dorsolateral salivary glands. The dorsal lingual salivary glands are situated beneath the dorsal lingual epithelium. They extended from the caudal end of the fissure on the caudal lingual apex to the front of the laryngeal cleft. The dorsolateral salivary glands on each side extend from the beginning of the body of the tongue to the level of the laryngeal cleft. The ventral side of the tongue is devoid of any glandular structure. Neither the morphology nor the dimensions of the tongue show sex-specific differences. **CONCLUSIONS:** Lingual structure shows considerable differences in budgerigars in comparison to other birds studied so far.

Introduction

Studies of the comparative morphology of the tongues of living vertebrates have revealed how variations in the morphology and function of the organ might be related to

evolutional events. The tongue, which plays a very important role in food intake by vertebrates, exhibits significant morphological variations that appear to represent adaptation to the current environmental conditions of each respective habitat (Campbell and Lack, 1985). 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 (Emura and Chen, 2008; Emura et al., 2009).

The studies on the structure of the tongue have been conducted on a small number of avian species such as woodpecker (Emura wt al., 2009); cormorants (Jackowiak et al., 2006); ostrich (Jackowiak and Ludwig; 2008), falcon and kestrel (Emura et al.; 2008), owl (Emura and Chen, 2008), white tailed eagle (Jackowiak and Godynicki, 2005), penguin (Kobayashi et al.; 1998) and little tern (Homberger and Meyers, 1989). The results of morphological studies conducted so far indicate a close correlation of the shape of the tongue with the method of food intake and the type of food, and habitat. The purpose of this study is to describe the morphological structure of the tongue in the budgerigar through light and scanning electron microscopy in order to compare the results with those of previous reports in other birds.

Materials and Methods

Tongues of 12 adult budgerigars of both sexes (6 males and 6 females) were used in this investigation. No animal was scarified in this study. The materials used in this investigation were obtained from animals that died due to different causes, but their tongues were not infected. Lengths of the tongue were measured and the samples of the apex, body and root of the tongue were fixed in 10% buffered paraformaldehyde at room temperature for 24 hours and later submitted to the dehydration process in a series of ethanol at increasing concentrations (70-96%) and embedded in paraplast. Histologic serial sections of 7µm thickness were obtained and stained routinely with haematoxylin-eosin (HE) and periodic acid Schiff (PAS) reaction. The morphometric data were obtained using a KS 400 computer morphometry system (ZEISS). The figures were documented under an Axioscope 2 plus light microscope (ZEISS).

For observations under the scanning electron microscope (SEM) the tongues were rinsed with 0.1M phosphate buffer at pH 7.3. Postfixation was made in 1% osmium tetroxide solution for two hours at 4°C. After dehydration through a graded ethanol series and infiltration by hexamethyl disilazin, the dried specimen were mounted on aluminum stubs and coated with about 20 s gold-palladinum.

The specimens were observed at various angles under a scanning electron microscope (Stereoscan 360, Leica Cambridge Ltd., England). The measurement was provided automatically by the SEM unit.

Results

The tongue of the adult budgerigar which is located in the floor of the oral cavity is about 5 mm long. Three parts are distinguished in the dorsal surface of the tongue: the apex, the body and the root of the tongue in each bird (Fig. 1). The deep concave rostral portion of the lingual apex which resembles a spatula with dorsally elevat-

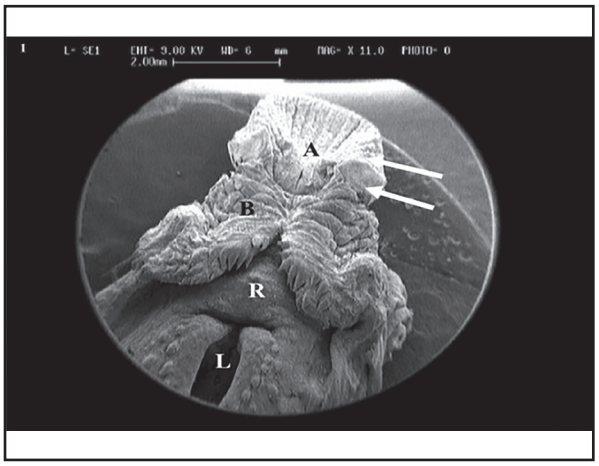

Figure 1. Scanning electron micrograph of the dorsal surface of the budgerigar tongue. Three parts are distinguished in the tongue: lingual apex (A), lingual body (B) and lingual root (R). Note the shape of the lingual apex showing spatula-shaped structure with thick lateral margins (arrows). Laryngeal cleft (L).

Figure 2. Scanning electron micrograph of the dorsal surface of the concave rostral portion of the lingual apex. Note that parallel longitudinal grooves (arrows), divide the surface into parallel slightly bulged columns of surface epithelium (two-headed arrows).

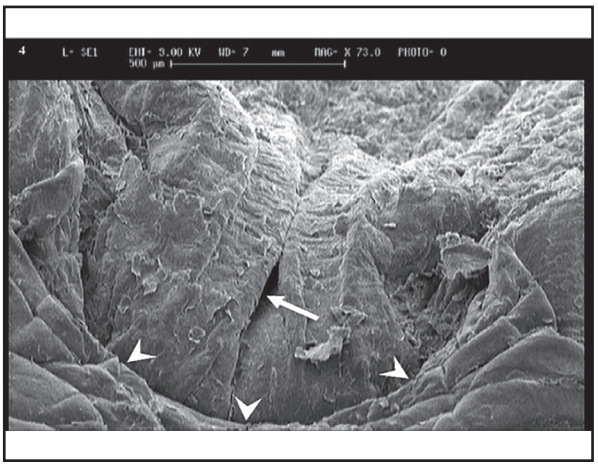

Figure 3. Scanning electron micrograph of the dorsal surface of the caudal portion of the lingual apex. Note the presence of the longitudinal fissure (arrow) and borderline separating the lingual apex and body (Arrowheads).

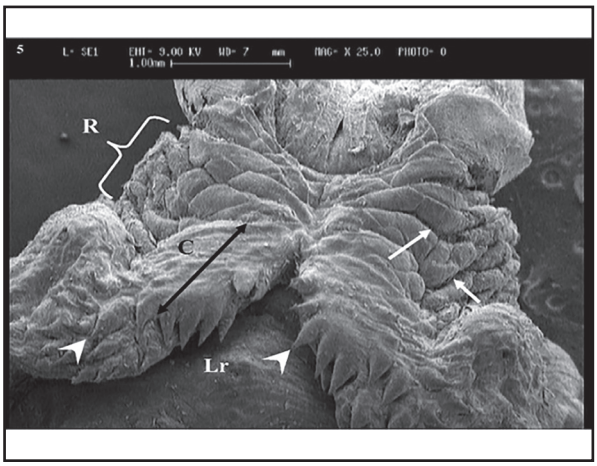

Figure 4. Scanning electron micrograph of the dorsal surface of the tongue. The rostral part of the lingual corpus (R) is distinctly divided by fissures of varying depth (Arrows) into many irregular raised areas. The deep fissures are not present in the caudal part of the body (C) of the organ. Note the presence of large conical papillae (Arrowheads) between the body and root of the tongue. Lingual root (Lr).

ed thick lateral margins is devoid of any glandular structure and is continuous with a semicircular caudal portion (Fig. 2). The caudal portion of the lingual apex is divided into two symmetrical halves by a median longitudinal fissure (Figs. 1, 3, 4). On the concavity of the rostral portion, parallel longitudinal grooves divide the surface into parallel slightly bulged columns of surface epithelium (Fig. 2). At higher magnification, the epithelial surface revealed the presence of horny scales. The curved caudal margin of the caudal portion shows the clear-cut borderline separating the lingual apex and body (Figs. 3, 4). At light microscopic level, the highly keratinized stratified squamous epithelium of the rostral concave portion of the lingual apex has a peculiar plicate arrangement causing the parallel longitudinal

Figure 5. Scanning electron micrograph of the dorsal surface of the laryngeal mound. Note the presence of large conical papillae (P). Laryngeal cleft (L).

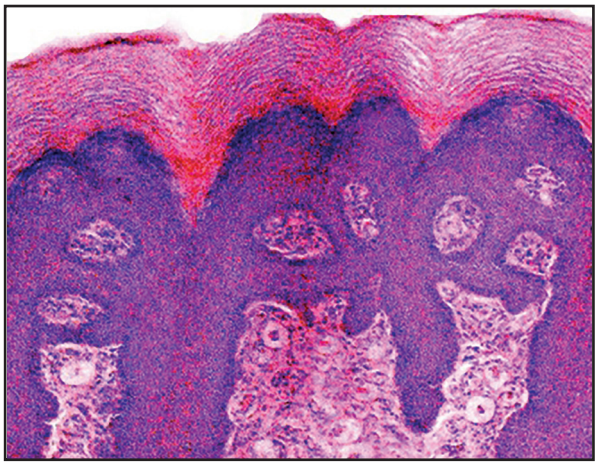

Figure 6. Cross section of the rostral portion of the lingual apex; light photomicrograph, hematoxylin and eosin staining. Ed, dorsal epithelium; K, thick keratinized layer of stratified epithelium. Note the peculiar plicated pattern of the surface epithelium (Arrowheads).Lamina properia (Lp) Scale bar, 50 µm.

grooves seen on the surface of the concavity in SEM images. The keratinized layer produced in this region reaches up to 100 µm in thickness (Fig. 6). Cross sectional images also revealed that the longitudinal fissure seen on the caudal apical part of the tongue is merely a simple invagination of surface epithelium with about 500 µm depth (Fig. 7).

The rostral part of the lingual corpus is distinctly divided by fissures of varying depth into many irregular raised areas with different sizes. The quantity of fissures varied in the same tongue when comparing the right and left sides. The deep fissures however are not present in the caudal part of the body of the organ (Fig 4).

Several large caudally directed conical papillae are situated on the posterior end of the lingual corpus and along the thick border region between the lingual body and root. The papillae close to the middle part of the tongue are noticeably thicker and larger than the laterally situated ones (Fig. 4). There are also some giant conical papillae on the laryngeal mound. Their sizes were considerably larger than the papillae between the body and root of the tongue and were easily observable in the low magnification (Figs. 1, 5).

Results obtained from the present study also revealed that the median sulcus is absent on the tongue of budgerigars. The root of the tongue is flat with no fissures or papillae (Figs. 1, 4). The special delicate pattern of microridges can be seen at electron microscopic level on the surface of the lingual root.

The dorsal and ventral surfaces of the budgerigar tongue are covered by keratinized stratified epithelium. The epithelium on the surface of the body of the tongue is 100-110 µm thick, whereas on the root of the tongue it is 80-90 µm thick. Gustatory papillae were not found in the epithelium covering the tongue in the budgerigar.

According to their locations, the lingual salivary glands of the budgerigar can be classified as dorsal and dorsolateral lingual salivary glands, all of which are compound tubuloalveolar type (Figs. 7, 8). The dorsal lingual salivary glands are situated just beneath the dorsal lingual epithelium. They extended from the caudal end of the fissure on the caudal lingual apex to the front of

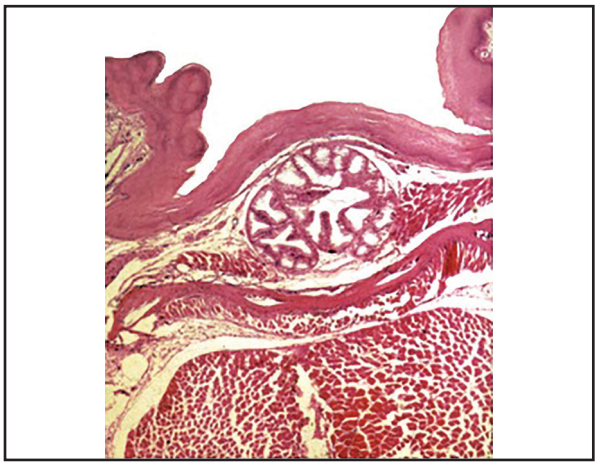

Figure 7. Cross section of the caudal portion of the lingual apex; light photomicrograph, hematoxylin and eosin staining. Ed, dorsal epithelium. Note that the longitudinal fissure (F) seen in this region is merely a simple invagination of surface epithelium. Dorsal lingual salivary glands (Dg.) Lingual muscles (M). Scale bar, 100 µm.

Figure 8. Cross section of the lingual corpus; light photomicrograph, hematoxylin and eosin staining. Ed, dorsal epithelium. El. Lateral epithelium. Dorsolateral lingual salivary glands (DLg.) Lingual muscles (M). Scale bar, 100 µm.

the laryngeal cleft. The dorsolateral salivary glands on each side extend from the beginning of the body of the tongue to the level of the laryngeal cleft. The glands are interspersed between the stratified squamous and the lingual muscle bundles of the organ. The ventral portion of the tongue is devoid of any glandular structure. The secretory acini of the lingual glands consist of tall columnar mucoserous cells with flattened nuclei at their bases. The glandular cells rest at a delicate basement membrane, having extensively vesicular cytoplasm, and thus stained lighter with haematoxylin and eosin stain. The glandular ducts of the dorsal and dorsolateral salivary glands open on the corresponding faces of the surface epithelium. Our study on histochemistry of the lingual salivary glands of the budgerigar revealed it to be PAS positive.

Neither the morphology nor the dimensions of the tongue showed sex-specific differences.

Discussion

General morphological features of the tongue in the budgerigar show considerable structural differences in comparison with the tongues of species of the birds investigated previously.

The shape of the tongue in birds is a species specific trait and closely fits the shape of the lower part of the bill (Iwasaki, 2002). Tongues used to manipulate food, such as in piscivorous species, are nonprotruding and covered with stiff, sharp, caudally directed papillae. In birds of prey, the tongue is a rasp-like structure with the rostral portion, frequently being very hard and rough. On the tongue of birds that typically strain food particles (e.g. ducks), the rostral portion forms a scoop-like structure with the lateral borders having a double row of overlapping bristles. The bristles work in conjunction with the lamellae of the bill to filter particles (Whittow, 2000). In both lateral sides of anterior lingual apex in the Japanese pygmi woodpecker, some conical processes are observed and in the posterior part of the lingual apex, there are many needle processes, the apices of which are pointed towards

the posterior part of the tongue (Emura et al.; 2009) .Results obtained from the present study showed that a unique feature of the budgerigar's tongue is the presence of a deep concave spatula-shaped portion of the lingual apex. Budgerigars are primarily seed-eating birds, as their beaks are adapted for dehusking small seeds. Using the tongue as an effective manipulating device, they rotate seeds and dehusk them with their peculiar curved beaks. Therefore, it seems that the bird uses the deep concave portion of the tongue as a ventral support for manipulation and rotation of the seeds during their dehusking process.

The peculiar plicate arrangement of the lingual epithelium in conjunction with the thick cornified layer produced by it, converts the apical lingual concavity to parallel slightly bulged columns of surface epithelium, making it a rough and frictional surface against the smoothness of globular seeds. There have been no reports regarding apical concavity of the lingual apex of the birds studied so far.

An unexpected finding of the present study was the presence of a median longitudinal fissure on the caudal portion of the lingual apex. Cross sectional LM images showed that the fissure is merely an invagination of surface epithelium and the dorsal lingual salivary glands begin from the its caudal part. Therefore the fissure may be considered as a reservoir for the mucus produced by the dorsal lingual salivary glands. Keeping in mind that the apical lingual fissure is located just behind the lingual apical concavity which is devoid of any glandular structure (probably because of the fact that mucus imbibition interfere negatively with seed rotation on its rough surface), it seems that the husked seed must be weltered in an adequate amount of mucus reserved in the fissure, preventing its regurgitation from the oropharyngeal cavity. So far the presence of a lingual fissure has not been reported in birds.

Data obtained in the present study demonstrate that the rostral part of the lingual corpus is distinctly divided, with fissures of varying depth into many irregular raised areas of different sizes. These fissures may be considered homologous to microridges of the lingual surface epithelium. Such microridges have generated discussion in the literature regarding their functionality. Some authors believe that the microridges could be linked to mucous retention (Iwasaki et al.; 1988). They, on the other hand, may enhance the transportation of seed through the lingual surface.

Our results also showed that median sulcus is absent on the tongue of budgerigars. The median groove is a characteristic feature found on the tongue of white tailed eagle, ducks and geese, whereas it is absent on the tongue of chickens and penguins (Jackowiak and Godynicki, 2005; Whittow, 2000; Homberger and Meyers, 1989; Iwasaki and Kobayashi, 1986; Kobayashi et al.; 1998). On the dorsal surface of the short tongue of the Cormorant, in the midline a crest is found, resembling a ridge, reaching both ends of the organ (Kobayasi et al.; 2006).

In most of the species of birds examined, the tongue, except for its apical part, is covered by a flat epithelium (Whittow, 2000). Emura et al. stated that in pygmi woodpecker, the dorsal surface of the lingual body presents a smooth aspect. In penguins, the whole dorsal lingual surface is covered by long conical papillae that help to hold ingested food (Kobayashi et al.; 1998). Many processes were observed densely distribut-

ed over the entire anterior 2/3 of the lingual dorsal surface in the chicken tongue (Iwasaki and Kobayashi, 1986), over the entire lingual apex of the dorsal surface, except in the tip of the apex in the owl (Emura and Chen, 2008) and over the entire lingual apex in the peregrine falcon and common kestrel (Emura et al., 2008). The results obtained from the present study also showed that the whole dorsal surface of the tongue of the budgerigar is flat and completely devoid of lingual papillae.

As shown by the light microscopic studies on the tongue in the budgerigar, the mucosa of the whole dorsal and ventral surface of the body and root of the tongue is covered with flat stratified keratinized epithelium. In most of the other species of birds examined, the whole dorsal surface of the tongue up to conical papillae is covered by horny epithelium, whereas the stratified epithelium without the horny layer usually covers a part of the root of the tongue (Whittow, 2000).

Our results showed that several large caudally directed conical papillae are situated on the posterior end of the lingual corpus and along the thickened border region between the lingual body and root. Some giant conical papillae are observed on the laryngeal mound. It has been reported that development of lingual conical papillae of avian species is related to their feeding habits and the crest of the papillae is well developed in birds such as white tailed eagle and owl which feed on fish or small animals and is absent in birds such as woodpecker and ostrich which feed on insect or plants (Emura et al.; 2009; Jackowiak and Ludwig, 2008). The present results show that budgerigars have very well-developed conical papillae despite the fact that they feed on seeds. The discrepancy between the results might be due to the genetic variations in the different avian species. However, more work is needed for clarification.

Salivary glands also show considerable species variation in birds. While salivary glands are generally well developed in granivorous species, they are less developed in birds of prey, poorly developed in piscivores, and absent in the Anhinga and Great Cormorant (Whittow, 2000). The results of studies on the distribution of lingual glands, conducted so far on only a few bird species, make it possible to distinguish anterior and posterior lingual glands (Emura and Chen, 2008). In the Ostrich, however, the lamina propria of the lingual mucosa is filled with mucous glands whose openings are found on both the dorsal and ventral surfaces of the tongue (Jackowiack and Ludwig, 2008). The localization of the compound tubuloalveolar lingual salivary glands of budgerigar seems to be a species-specific trait since the glands exist beneath the entire surface of dorsal and dorsolateral surfaces of the lingual epithelium in the body and root of the organ. The ventral side of the tongue is devoid of any glandular structure. The secretory cells of the lingual salivary glands show strongly positive reaction to PAS reaction, indicating that the saliva of the budgerigar, similar to that of many other birds is rich in glycoproteins.

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References

Campbell, B., Lack, E. (1985) A Dictionary of Birds. (1st ed.) The British Ornithologists'

Union, T. & A. D. Poyser, Calton. Buteo Book, London, UK.

Emura, S., Chen, H. (2008) Scanning electron microscopic study of the tongue in the owl (Strix uralensis). Anat Histol Embryol. 37: 475-478.

Emura, S., Okumura, T., Chen, H. (2009) Scanning electron microscopic study of the tongue in the Japanese Pygmi Woodpecker (*Dendrocopos kizuki*). Okajimas Folia Anat Jpn. 86: 31-35.

Emura, S., Okumura, T., Chen, H. (2008) Scanning electron microscopic study of the tongue in the peregrine falcon and common kestrel. Okajimas Folia Anat Jpn. 85: 11-15.

Homberger, D.G., Meyers, R. (1989) Morphology of the lingual apparatus of the domestic chicken Gallus gallus, with special attention to the structure of the fasciae. Am J Anat. 186: 217-257.

Iwasaki, S. (2002) Evolution of the structure and function of the vertebrate tongue. J Anat. 201: 1-13.

Iwasaki, S. (1992) Fine structure of the dorsal lingual epithelium of the little tern, *Sterna albifrons* Palas (Aves, Lari). J Morphol. 212: 13-26.

Iwasaki, S., Kobayashi, K. (1986) Scanning and transmission electron microscopical studies on the lingual dorsal epithelium of chickens. Acta Anat Nippon. 61: 83-96.

Iwasaki, S., Miyata, K., Kobayashi, K. (1988) Scanning-electron microscopic study of the dorsal lingual surface of the squirrel monkey. Acta Anat. 132: 225-229.

Jackowiak, H., Andrzejewski, W., Godynicki, S. (2006) Light and scanning electron microscopic study of the tongue in the cormorant *Phalacrocorax carbo* (Phalacrocoracidae, aves). Zoolog Sci. 23: 161-167.

Jackowiak, H., Godynicki, S. (2005) Light and scanning electron microscopic study of the tongue in the white tailed eagle (*Haeliaeetus albicilla*, Accitripidae, Aves). Ann Anat. 187: 197-222.

Jackowiak, H., Ludwig, M. (2008) Light and scanning electron microscopic study of the structure of the Ostrich (*Strutio camelus*) tongue. Zoo Sci. 25: 188-194.

Kobayashi, K., Kumakura, M., Yoshimura, K. (1998) Fine structure of the tongue and lingual papillae of the penguin. Arch Histol Cytol. 61: 37-46.

Whittow, G.C. (2000) Sturkie's Avian Physiology. Academic press, NewYork, London, USA.

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بررسی ساختار زبان مرغ عشق در سطح میکروسکوپ نوری و الکترونی

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چکیده

زمینه مطالعه: زبان که نقش مهمی در دریافت غذا در مهره‌داران ایفا می‌کند تفاوت‌های ریختی چشم‌گیری نشان می‌دهد که نمایان‌گر سازگاری حیوان با شرایط محیطی موجود در زیستگاه مربوطه به‌شمار می‌رود. **هدف:** هدف از مطالعه حاضر بررسی ساختار زبان در مرغ عشق بالغ بود. **روش کار:** زبان ۱۲ مرغ عشق بالغ در این پژوهش مورد استفاده قرار گرفت. نمونه‌هایی از رأس، بدنه و ریشه زبان با استفاده از میکروسکوپ نوری و الکترونی اسکنینگ مورد مطالعه قرار گرفت. **نتایج:** زبان در مرغ عشق بالغ (*Melopsittacus undulatus*) به‌وسیله میکروسکوپ نوری و الکترونی مورد مطالعه قرار گرفت. زبان در مرغ عشق حدود ۵mm طول دارد. بخش عمیق مقعر قدامی رأس زبان فاقد هرنوع ساختار غده‌ای بوده و در امتداد بخش خلفی نیم‌دایره‌ای این اندام قرار می‌گیرد. بخش خلفی رأس زبان با یک شیار طولی میانی به دو نیمه قرینه تقسیم شده است. بخش قدامی بدنه زبان با شیارهایی با عمق گوناگون به شمار زیادی نواحی نامنظم برآمده با اندازه‌های گوناگون تقسیم شده است. چندین پرز مخروطی‌شکل بزرگ در راستای خلفی روی انتهای خلفی بدنه زبان و در راستای لبه ضخیم موجود بین بدنه و ریشه اندام جای گرفته‌اند. شماری پرز مخروطی غول‌پیکر نیز روی برآمدگی حنجره‌ای یافت می‌شوند. غدد بزاقی لوله‌ای‌آلوئولی PAS-مثبت زبان را می‌توان برمبنای جایگاه در دو گروه پشتی و پشتی‌جانبی جای داد. غدد بزاقی زبانی پشتی زیر اپیتلیوم پشتی زبان جای گرفته‌اند. این غدد از انتهای خلفی شکاف موجود روی رأس زبان تا جلو شکاف حنجره‌ای امتداد یافته‌اند. غدد بزاقی پشتی‌جانبی در هر سو از ابتدای بدنه زبان آغاز شده و تا سطح شکاف حنجره‌ای امتداد می‌یابند. سطح شکمی زبان فاقد هر نوع ساختار غده‌ای است. ریخت‌شناسی و ابعاد زبان تفاوتی در دو جنس نشان نمی‌دهند. **نتیجه‌گیری نهایی:** ساختار زبان در مرغ عشق در مقایسه با پرندگانی که تا کنون مورد مطالعه قرار گرفته‌اند تفاوت‌های چشم‌گیری نشان می‌دهد.

واژه های کلیدی: مرغ عشق، میکروسکوپ نوری، غدد بزاقی، میکروسکوپ الکترونی اسکنینگ، زبان

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