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Original Scientific Article

LIGHT AND SCANNING ELECTRON MICROSCOPIC STUDY OF THE TONGUE IN THE ZEBRA FINCH *CARDUELIS CARDUELIS* (AVES: PASSERIFORMES: FRINGILLIDAE)

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Summary: The tongue of adult zebra finch *(Carduelis carduelis)* was examined by light and electron microscopy. The tongue resembles a thick rod with a pointed tip. The length of the tongue is about 8 mm. Three parts are distinguished in the dorsal surface of the tongue: the apex, the body and the root of the tongue in each bird. A unique feature of the organ is the presence of many fine densely populated needle–like processes in both lateral sides of the anterior lingual apex. The caudal processes are of equal lengths and are tangent to the tongue. Afterwards, the length of the processes increases progressively towards the free point of the organ. The median sulcus is absent on the tongue. Large conical papillae with a W-shaped arrangement are present between the body and the root of the tongue, the apices of which are pointed toward the posterior part of the organ. According to their positions, the PAS-positive compound tubuloalveolar salivary glands can be classified as lingual and laryngeal salivary glands. The lingual salivary glands extend from the lingual apex to the lingual root, whereas the laryngeal salivary glands are situated in both sides of the laryngeal cleft. The ventral side of the tongue is devoid of any glandular structure.

Key words: tongue; papillae; finch

Introduction

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 (1). 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 (2, 3). The studies on the structure of the tongue have been conducted on a small number of avian species such as woodpecker (4); cormorants (5); ostrich (6); falcon and kestrel (7); owl (8); white tailed eagle (9); penguin (10) and little tern (11). The results obtained from these studies show a close relationship of the shape of the tongue with the method of food intake and the type of food and habitat.

However, in available literature, there is a lack of morphological data characterizing the structure of the tongue in the zebra finch. The purpose of this study was to describe the morphology of the tongue in this species and to characterize the microscopic structure of the lingual mucosa using light and scanning electron microscopy in order to compare the results with those previous reports in other birds.

Materials and methods

Tongues of 5 adult female zebra finches were used in the investigations. For the observations in the light microscope (LM) the samples of the apex, body and root of the tongue were fixed in the 10% buffered paraformaldehyde (Merck, pH: 7.3) at room temperature for 48 hours and later submitted to the dehydration process in a series of ethanol at increasing concentrations (70-96%) and embedded

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in paraplast. Histological serial sections of 7μm of 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 using Balzers SCD-040.

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 zebra finch is about 8 mm long. Overall shape of the tongue resembles a thick rod with a pointed tip. Three parts are distinguished in the dorsal surface of the tongue: the apex, the body and the root of the tongue in each bird. The median sulcus is absent on the tongue of zebra finches (Fig. 1). A unique feature of the organ is the presence of many fine densely populated needle–like processes in both lateral sides of the anterior lingual apex. The caudal processes are of equal lengths and are tangent to the tongue. Afterwards, the length of the processes increases progressively towards the free point of the organ (Figs. 1, 2, 3). At light microscopic level, the muscle bundles of the tongue in the corresponding apical region of the tongue have a V shaped histological arrangement in cross section (Fig. 8).

Large conical papillae are present between the body and the root of the tongue, the apices of which are pointed toward the posterior part of the organ. These mechanical papillae are arranged like the letter W at the edges of two huge caudally directed elevations of the lingual corpus. The axial papillae are noticeably smaller and thinner than the abaxial ones (Figs 1, 6).

The mucosa of the whole dorsal and ventral surface of the apex, body and root of the tongue is covered with flat stratified non-keratinised epithelium (Figs. 8, 9). At electron microscopic level, the mucosal surface of the tongue in the apex, body and root of the tongue is flat with no papillae (Figs. 1, 2, 4, 5, 6, 7). The special delicate pattern of microridges can be seen at electron microscopic level on the surface of the tongue particularly in the body and root regions (Figs. 4, 7). Gustatory papillae are not found in the epithelium covering the tongue in the finch.

The salivary glands are located in the lamina propria beneath the dorsal lingual epithelium. According to their positions, these glands can be classified as lingual and laryngeal salivary glands. The lingual salivary glands extend from the lingual apex to the lingual root, and are interspersed between the stratified squamous lining the dorsal surface and the lingual muscle bundles of the tongue (Figs. 8, 9). Dorsal lingual epithelium overlying the lingual salivary glands is considerably thicker than that the other parts (Fig. 8). Lingual salivary glands are divided into two portions (laryngeal salivary glands) by the laryngeal cleft (Fig. 10). Both lingual and laryngeal salivary glands are of compound tubuloalveolar type consist of secretory endpieces composed of tall columnar cells with flattened nuclei at their basis. The glandular cells rest at a delicate basement membrane, having extensively vesicular cytoplasm, and thus stained lighter with haematoxylin and eosin stain. Secretory units forming the glands are separated by narrow connective tissue septa containing capillary vessels (Fig. 9). The ducts of the lingual glands opened onto the dorsal surface of the tongue. The secretory cells of the both lingual and laryngeal salivary glands reacted positively to PAS reaction (Fig. 9). The ventral side of the tongue is devoid of any glandular structure (Fig. 8).

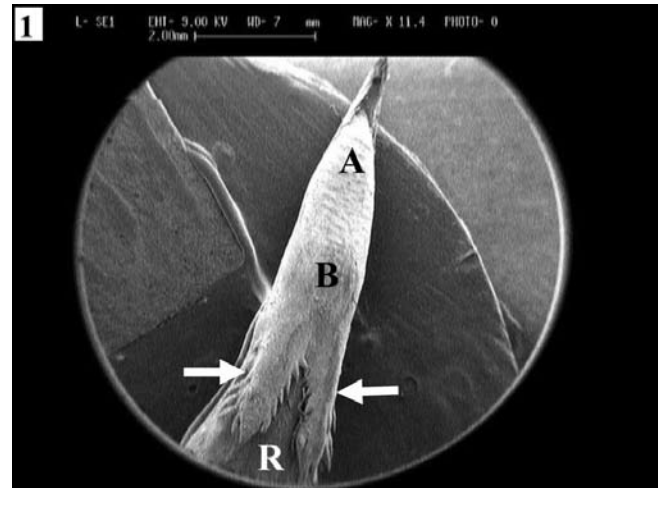

Figure 1: Scanning electron micrograph of the dorsal surface of the zebra finch tongue. Three parts are distinguished in the tongue: lingual apex (A), lingual body (B) and lingual root (R). Note the W-shaped arrangement of the conical papilla (arrows) between the lingual apex and body

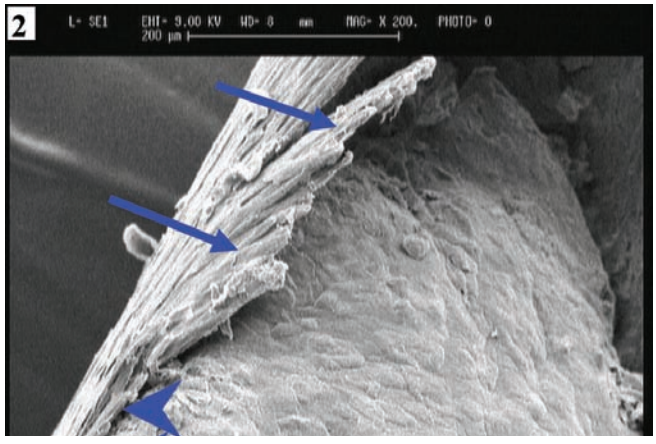

Figure 2: Scanning electron micrograph of the surface of the lingual apex, showing many needle-like processes in the lateral side of lingual apex (arrows). Note that the caudal processes (arrowheads) are completely tangent to the organ. The lingual surface is flat with no lingual papilla

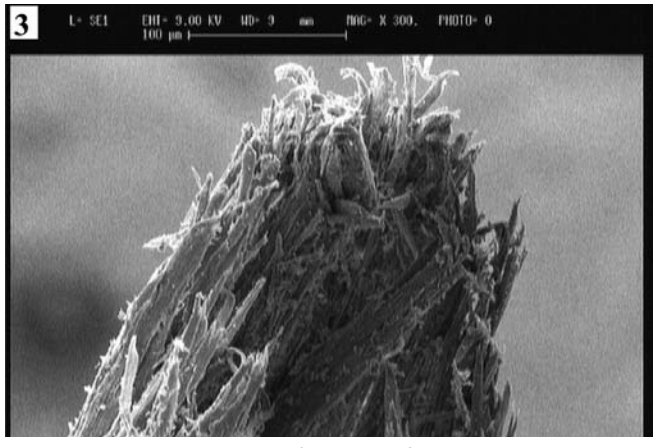

Figure 3: A higher magnification of the needle-like processes of the apex of the tongue

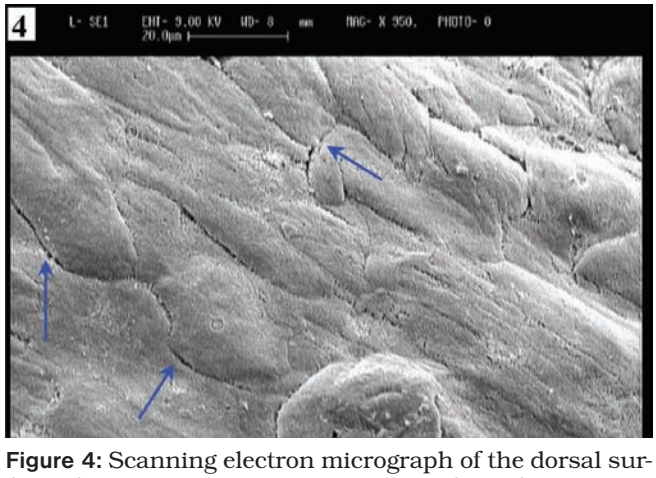

Figure 4: Scanning electron micrograph of the dorsal surface of the lingual body. The dorsal surface of the lingual body presents smooth aspect with no papilla. Note the borders between surface squamous cells (arrows) and microridges on the surface epithelium

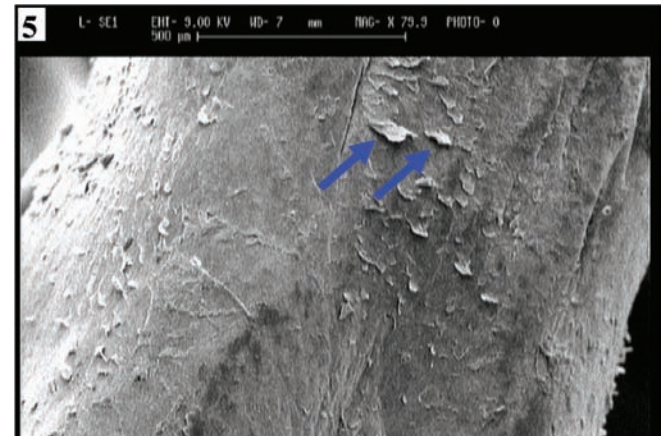

Figure 5: Scanning electron micrograph of the dorsal surface of the body of the tongue. The arrows show the desquamate cells of the non-keratinized epithelium

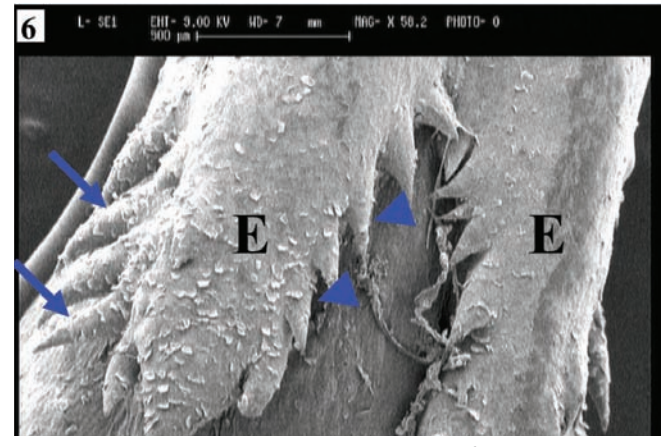

Figure 6: Scanning electron micrograph of the large conical papilla between the body and the root of the dorsal surface of the tongue. Note that the abaxial papillae (arrows) are considerably larger than the axial ones (arrowheads). Desquamated cells are clearly visible on the surface of two huge caudally directed extensions (E) from the lingual body

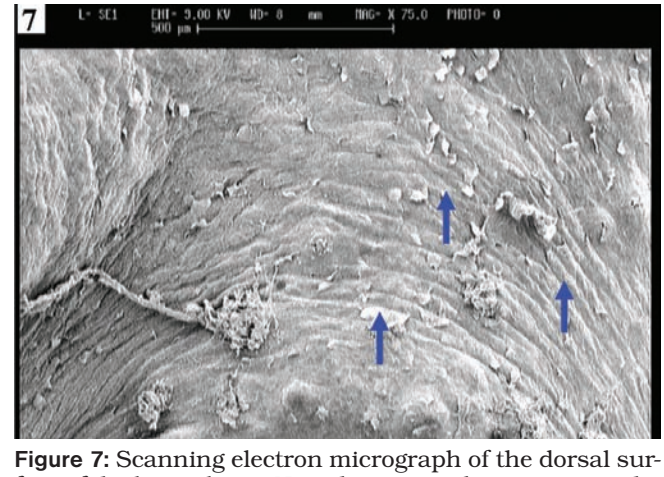

Figure 7: Scanning electron micrograph of the dorsal surface of the lingual root. Note the microridge pattern on the surface epithelium (arrows)

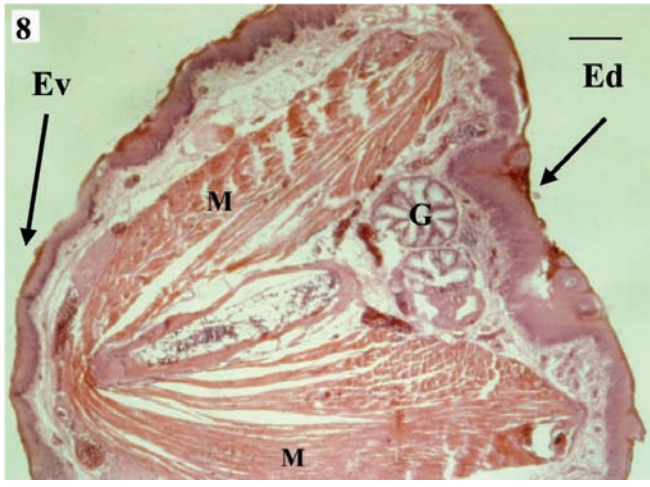

Figure 8: Cross section of the apex of the tongue; light photomicrograph, hematoxylin and eosin staining. Ed, dorsal epithelium; Ev, ventral epithelium; G, lingual salivary glands. Note the v-shaped arrangement of the skeletal muscle bundles (M). Scale bar, 100 μm

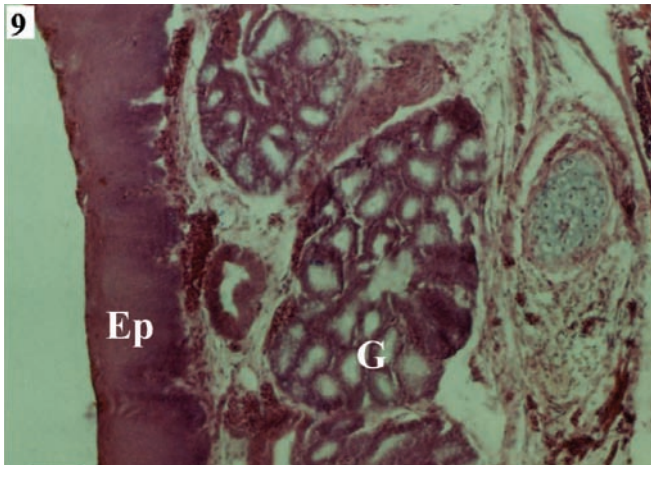

Figure 9: Cross section of the body of the tongue; light photomicrograph, PAS staining. Dark cytoplasm in the cells of the lingual salivary glands (G) presents a positive PAS reaction. Ep, dorsal lingual epithelium. Scale bar, 70 μm

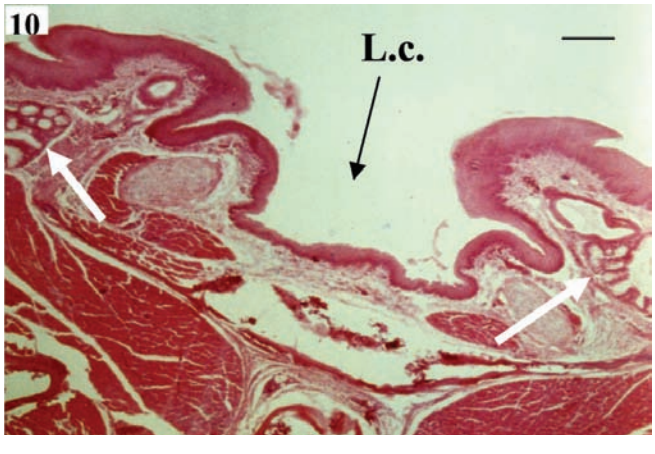

Figure 10: Dorsal surface of the tongue. The laryngeal salivary glands (arrows) at both sides of the glottis-laryngeal cleft (L.c.). hematoxylin and eosin staining. Scale bar, 40 μm

Discussion

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

The shape of the tongue in birds is a species specific trait (3, 12). 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 scooplike 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 (13). 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 (4).

Results obtained from the present study showed that a unique feature of the tongue in zebra finch is the presence of many fine densely populated needle–like processes in both lateral sides of the anterior lingual apex. These processes may help bird in direct food caudally towards the caudal parts of the oropharyngeal cavity. Peculiar V-shaped arrangement of skeletal muscle bundles found in the apex of the tongue show that the bird can move the papillae in appropriate directions. There have been no reports regarding rostrally directed needle processes of the lingual apex in various birds.

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, pheasants and penguins (2, 3, 9, 14, 15). 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 (5). Our results also showed that the median sulcus is absent on the tongue of zebra finches.

In most of the species of birds examined, the tongue except for its apical part, is covered by a flat epithelium. Emura *et al.* (2009) stated that in pygmi woodpecker, the dorsal surface of the lingual body presents smooth aspect (4). In penguins, the whole dorsal lingual surface is covered by long conical papille that help to hold ingested food (10). Many processes were observed densely distributed over

the entire anterior 2/3 of the lingual dorsal surface in the chicken tongue (14), over the entire lingual apex of the dorsal surface, except in the tip of the apex in the owl (8) and over the entire lingual apex in the peregrine falcon and common kestrel (7). The results obtained from the present study also showed that the whole dorsal surface of the tongue of the finch is flat and completely devoid of lingual papillae.

As shown by the light microscopic studies on the tongue in the zebra finch, the mucosa of the whole dorsal and ventral surface of the apex, body and root of the tongue is covered with flat stratified non-keratinised epithelium. This finding is in accordance with that of Jackowiak and Ludwig in study of the ostrich tongue (9). 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 (14-16). Microridges, found on superficial cells over the entirety of the finch's tongue, have been described in both mammals and in birds. Microridges have been interpreted as structures that increase the adhesion of mucus to the epithelium (6).

Our results showed that large conical papillae are found in the posterior part of the lingual body, the apices of which were pointed towards the posterior part of the tongue. 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 feeds on insect or plants (6-9). Zebra Finches are primarily seed-eating birds, as their beaks are adapted for dehusking small seeds. They prefer millet, but will consume many other kinds of seeds as well. The present results show that this species of birds has very well-developed conical papillae despite the fact that it feeds 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 explanation. It also needs to be added that there have been no reports regarding conical paillae with peculiar W-shaped arrangement in tongues of species of birds investigated previously.

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 (13). The results of studies on the distribution of lingual glands, conducted so far on few bird species, make it possible to distinguish anterior and posterior lingual glands (15-17). 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 surface of the tongue (6). The localization of the compound tubuloalveolar lingual salivary glands of zebra finch seems to be a species-specific trait since the glands exist beneath the entire surface of dorsal lingual epithelium and their ducts opened onto the dorsal surface of the tongue. 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 finch similar to that of other birds is rich in glycoproteins.

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PROUČEVANJE JEZIKA LIŠČKA *CARDUELIS CARDUELIS* (AVES: PASSERIFORMES: FRINGILLIDAE) S POMOČJO SVETLOBNE IN VRSTIČNE ELEKTRONSKE MIKROSKOPIJE

R.A.F. Dehkordi, A. Parchami, S. Bahadoran

Izvleček: V članku je bil proučevan jezik odraslega liščka *(Carduelis carduelis)* s pomočjo svetlobne in vrstične elektronske mikroskopije. Jezik je podoben debeli palici z zašiljeno konico. Dolžina jezika je približno 8 mm. Pri vsaki ptici je dorzalna površina jezika ločena na tri dele: konico, telo in koren jezika. Posebna značilnost organa je prisotnost mnogih finih, na gosto razporejenih, kot igla tankih podaljškov na obeh vzdolžnih straneh jezika. Dolžina podaljškov stopnjujoče narašča proti prostemu koncu organa. Na jeziku ni sredinskega žleba. Velike stožčaste okušalne brbončice so v obliki črke W razporejene med telesom in korenom jezika, njihova konica pa je obrnjena proti zadnji strani organa. Glede na njihov položaj lahko PAS-pozitivne sestavljene cevkasto-mešičkaste slinske žleze razvrstimo v jezične in žrelne slinske žleze. Jezične slinske žleze segajo od konice do korena jezika, medtem ko so žrelne slinske žleze na obeh straneh žrelne razpoke. Na ventralni strani jezika ni žlez.

Ključne besede: jezik; brbončice; ščinkavec