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Light and electron microscopic study of the tongue in the White-eared bulbul (*Pycnonotus leucotis*)

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Summary

The aim of the present study was to investigate the light and electron microscopic structure of the tongue in White-eared bulbul (*Pycnonotus leucotis*). Six adult White-eared bulbul (3 males and 3 females) in Shahrekord district were used in the investigation. Samples of the apex, body and root of the tongue were prepared routinely for light and electron microscopic study. Results showed that the tongue is characterized by an elongated triangular format. At a point, approximately 2/3 the length of the lingual corpus there is a distinct depression, separating the caudal one-third of the lingual corpus from its rostral two-third. On the dorsal surface of the apex and body of the tongue, a median groove is found. A unique feature of the tongue in White-eared bulbul is the presence of many fine overlapping needle-shaped processes at both lateral sides of the anterior lingual apex. A single row of large conical papillae is observed symmetrically in the marginal region between the body and root of the tongue. The mucosa of the tongue is covered with a thick stratified squamous epithelium, which is cornified only on the ventral surface of the apex. The periodic acid schiff (PAS) positive lingual salivary glands can be classified as dorsolateral and mediodorsal salivary glands. The compound tubular dorsolateral glands extend from the apex of the tongue to both sides of the laryngeal cleft. The compound tubulo-alveolar mediodorsal glands are found in the lingual root. At electron microscopic level, many fine openings of these glands are scattered throughout the dorsal surface of the lingual root. Neither the morphology nor the dimensions of the tongue show sex-specific differences.

Key words: Tongue, White-eared bulbul, Salivary glands, Scanning electron microscopy

Introduction

Birds have adapted to their environments with respect to food sources. Reflecting their specific lifestyles, birds have various feeding habits, with corresponding versatility in the structures of their tongues. A considerable number of papers have been published on the lingual structure in domestic mammals (Steflik *et al*., 1983; Kumar *et al*., 1998; Tadjalli, 2004). The studies on the structure of the tongue in birds, however, have been conducted on a small number of avian species, i.e. domestic chickens (Homberger and Meyers, 1989), little tern (Iwasaki, 1992), goose (Iwasaki *et al*., 1997), penguin (Kobayashi *et al*., 1998), White tailed eagle (Jackowiak and Godynicki, 2005), cormorant (Jackowiak *et al*., 2006), owl (Emura and Chen, 2008), falcon and kestrel (Emura *et al*., 2008), ostrich (Jackowiak and Ludwig, 2008) and woodpecker (Emura *et al*., 2009). 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. 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 (Whittow, 2000). However, in the available literature, there is a lack of morphological data characterizing the

structure of the tongue in the White-eared bulbul. The present study was performed to characterize morphologically the tongue of this species using light and scanning electron microscopy, in order to compare the results with those previous reports in other birds.

Materials and Methods

Six adult White-eared bulbul (Aves: Passeriformes: Pycnonotidae: Pycnonotus), 3 males and 3 females, were used in the investigation. Samples of the apex, body and root of the tongue were fixed in the 10% buffered paraformaldehyde at room temperature for 48 h 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 7 µm of thickness were obtained and stained routinely with haematoxylin and eosin (H&E) 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 observation under the scanning electron microscope (SEM) the tongues were rinsed with 0.1 M phosphate buffer at pH = 7.4. Postfixation was made in 1% sodium tetroxide solution for 2 h at 4°C. After dehydration through a graded ethanol series and infiltration by hexamethyl disilazin, the dried specimens were mounted on aluminium stubs and coated about 20 s gold-palladinum.

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

Results

The tongue of the adult White-eared bulbul is characterized by an elongated triangular format for both sexes (Fig. 1), conforming to the shape of the lower beak within which it lies. From the morphometric point of view, the tongue has a total length of 28 mm and a width at the root and apex of 8 and 2 mm, respectively. Its root has a length of 7.5 mm. Neither the morphology nor the dimensions of the tongue show sex-specific differences. Three parts are distinguished in the dorsal surface of the tongue: the apex, the body and the root of the tongue. At a point approximately 2/3 of the length of the lingual corpus there is a distinct depression, separating the caudal one-third of the lingual corpus from its rostral two-third (Fig. 1). On the dorsal surface on the apex and body of the tongue a median groove is found. This groove is apparently wider on the middle part of the lingual corpus in comparison to the other parts. The groove divides the apex and body of the organ into two symmetrical halves. A unique feature of the tongue in White-eared bulbul is the presence of many fine overlapping needle-shaped processes at both lateral sides of the anterior lingual apex, the apices of which are directed rostrally (Fig. 2). A single row of large conical papillae are observed symmetrically in the marginal region between the body and root of the tongue, the apices of which are pointed towards the posterior part of the tongue. The sizes of these mechanical papillae varied according to their location within the tongue. The lateral papillae are noticeably larger and thicker than the medial ones (Fig. 3). The papillae show a flat surface. At higher magnification, on the dorsal surface of the epithelium of the lingual apex and body, desquamate cells of the non-keratinized epithelium are present (Fig. 4). SEM images show that superficial epithelial cells exfoliate as isolated squamae, on the surface of which at higher magnifications, a gentle micro-ridge pattern can be seen. In SEM images, the dorsal surface of the root of the tongue presents smooth aspect with no densely distributed desquamated cells (Fig. 3).

The mucosa of the tongue is covered with a thick stratified squamous epithelium, which is cornified only on the ventral surface of the apex of the organ (Figs. 5, 6). The mean thickness of the epithelium of the tongue is 300 ± 35, 375 ± 75 and 80 ± 15 µm on the dorsal surface of the apex, body and root of the organ, respectively. Gustatory papillae are not found in the epithelium covering the tongue in the White-eared bulbul. The tongue is supported by

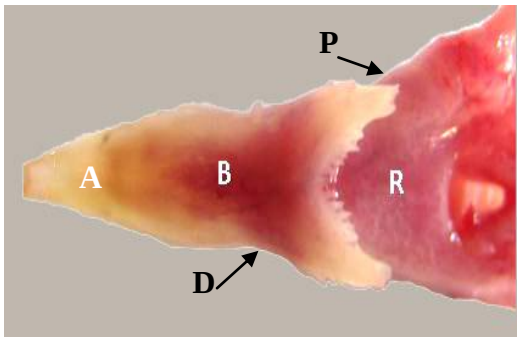

Fig. 1: Macroscopic structure of the dorsal surface of the tongue in White-eared bulbul. Apex (A), body (B), root (R), large conical papillae (P). Note the presence of distinct depression (D), separating the caudal two-third of the lingual corpus from its rostral two-third

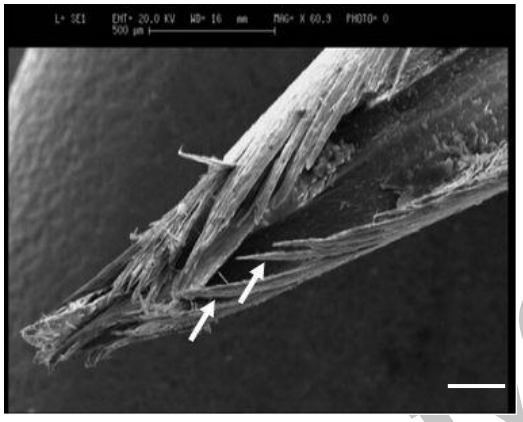

Fig. 2: Scanning electron micrograph of the dorsal surface of the lingual apex. Note the presence of many fine needle-shaped overlapping processes (arrows) at both lateral parts of the lingual apex, (scale bar = 250 µm)

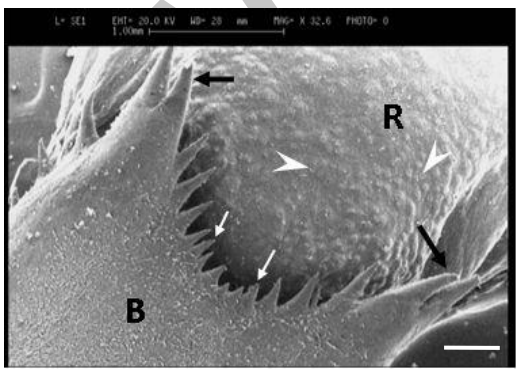

Fig. 3: Scanning electron micrograph of the large conical papillae between the lingual body (B) and root (R). Note that lateral papillae (black arrows) are noticeably larger than the medial ones (white arrows). Fine openings of mediodorsal lingual salivary glands (arrowheads) are scattered throughout the lingual root, (scale bar = 500 µm)

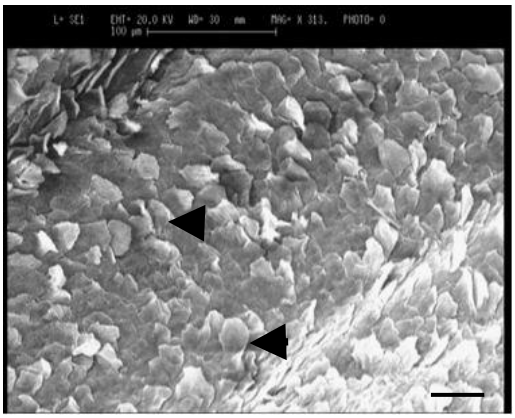

Fig. 4: A higher magnification of the dorsal surface of the body of the tongue. Note the presence of desquamate cells (arrows), (scale bar = 50 µm)

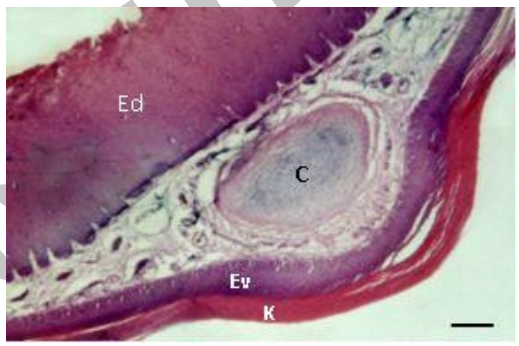

Fig. 5: Cross section of the apex of the tongue, light photomicrograph. Haematoxylin and eosin staining. Dorsal epithelium (Ed), ventral epithelium (Ev), keratinized layer (K), hyoid cartilage (C), (scale bar = 100 µm)

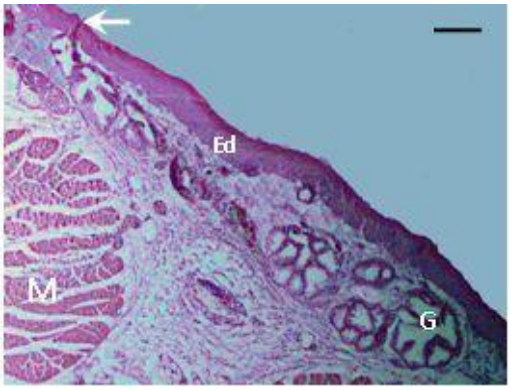

Fig. 6: Cross section of the root of the tongue, light photomicrograph. Periodic acid schiff (PAS) staining. Dorsal epithelium (Ed), lingual skeletal muscle (M), dorsomedial tubuloalveolar lingual salivary glands (G). Note the opening of a secretory unit on the dorsal lingual epithelium, (white arrow), (scale bar = 65 µm)

cartilaginous hyoid apparatus extending from the lingual root to lingual apex and enclosed by intrinsic lingual muscle bundles (Fig. 5).

The lingual salivary glands of the White-eared bulbul can be classified as dorsolateral and dorsomedial salivary glands according to their positions. The dorsolateral glands are located in the lamina propria of the dorsal lingual plate and extend from the apex of the tongue to both sides of the laryngeal cleft. The glands are of compound tubular type and consist of secretory endpieces composed of tall columnar cells with extensive vesicular cytoplasm resting at a delicate basement membrane. Secretory units forming the glands are separated by narrow connective tissue septa containing capillary vessels. The compound tubulo-alveolar mediodorsal glands were found in the lingual root. The ducts of these glands open onto the dorsal surface of the tongue (Fig. 6). At electron microscopic level, many fine openings of these glands are scattered throughout the dorsal surface of the lingual root (Fig. 3). The ventral surface of the tongue was devoid of any glandular structure. All the lingual salivary glands in White-eared bulbul show strongly PAS positive reaction.

Discussion

Owing to their different lifestyles, birds show considerable differences in the structures of their bills and tongues. The tongue in many species of birds is a triangular organ that fills the whole lower part of the bill (Campbell and Lack, 1985; Vollmerhaus and Sinowatz, 1992). It is an elongated tubular organ in woodpeckers (Emura *et al*., 2009) and elongated flat in geese and ducks (Vollmerhaus and Sinowatz, 1992; Iwasaki *et al*., 1997). The tongue of the cormorant is only a small, mushroom-shaped connective tissue structure joined with the hyoid cartilage and the lingual root is nonexistent (Jackowiak *et al*., 2006). Macroscopic features of the tongue in ostrich, such as the proportion of its size to the beak and its shape, indicate a morphological reduction of this organ (Jackowiak and Ludwig, 2008). Results obtained from the present study show that the tongue of the adult White-eared bulbul like that of many other birds is a well-developed elongated triangular organ with three distinct anatomical parts, i.e. apex, body and root.

Data obtained from the present study also showed that a distinct median groove divides the apex and body of the tongue of the White-eared bulbul into two symmetrical halves. The groove can be considered as the origin and/or insertion site of the lingual intrinsic muscles. More researches however may be needed for explanation. 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 (Homberger and Meyers, 1989; Vollmerhaus and Sinowatz, 1992; Iwasaki *et al*., 1997; Kobayashi *et al*., 1998; Jackowiak and Godynicki, 2005). 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 (Jackowiak *et al*., 2006). Iwasaki (1992) stated that, there is a median line in the anterior part of the tongue in the little tern and the apex of the tongue is slightly bifurcated. Results obtained from the present investigation also showed that at a point approximately 2/3 of the length of the lingual corpus of the White-eared bulbul there is a distinct depression, separating the caudal one-third of the lingual corpus from its rostral two-third. No one has described this peculiar morphological appearance in the tongues of species of birds that have been examined so far. These differences in the structures of the tongues may be due to the different feeding habits. However, since the White-eared bulbuls like many other birds are primarily seed-eating birds, more investigations may be needed for explanation.

Results obtained from the present study also showed that a unique feature of the tongue in White-eared bulbul 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 particles caudally towards the caudal parts of the oropharyngeal cavity. However, it seems that more investigation is needed for explanation of this finding.

The conical papillae found between the

body and root of the tongue show considerable differences in their distribution and development between the different avian species. The papillae are 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 insects or plants (Jackowiak and Godynicki, 2005; Emura and Chen, 2008; Jackowiak and Ludwig, 2008; Emura *et al*., 2009). Despite the fact that the White-eared bulbuls are primarily seed-eating birds, our results show that a single row of well developed conical papillae are observed symmetrically in the marginal region between the body and root of the tongue. The discrepancy between the results might be due to the genetic variations in the different avian species and more investigations are needed for precise explanation. The crest of these papillae aid in the transfer of the swallowed food towards the esophagus and at the same time prevent its regurgitation (Jackowiak and Godynicki, 2005).

Results obtained from the present study also showed that the mucosa of lingual apex, body and root is covered with squamous stratified nonkeratinized epithelium. This finding is similar to those documented by Jackowiak and Godynicki (2005) in the White tailed eagle and by Jackowiak and Ludwig (2008) in the ostrich. 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 (McLelland, 1975; Iwasaki and Kobayashi, 1986; Homberger and Meyers, 1989). The microridges present on the superficial exfoliated cells, have been described in both mammals and in birds. Microridges have been interpreted as structures that increase the adhesion of mucus to the epithelium (Kullaa-Mikkonen and Sorvari, 1985; Iwasaki, 1992).

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). Our results showed that the salivary glands of the White-eared bulbul form two separate morphological groups. 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. Exceptional cases include the lingual salivary glands in the ostrich in which 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 (Jackowiak and Ludwig, 2008). The secretory cells of the lingual salivary glands show strongly positive reaction to PAS reaction, indicating that the saliva of the White-eared bulbul 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.

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