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Papillary Architecture of the Lingual Surface in Greater Flamingo (*Phoenicopterus roseus*, Pallas 1811)

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Abstract: The tongues of birds, which are generally adapted to their feeding habits, have a wide variety of anatomical structures. The tongue structures of flamingos are equipped with the ability to make 3 different types of feedings. The aim of this study was to examine the *lingual* structures of flamingos. *Cuticula cornificata linguae* (nail tongue) structure was not observed in flamingos, which were found to have significant size and numerous *papillae lingua rostralis*. *Micro papilla* structure was observed on *papilla lingua rostralis* in SEM images. In the Greater flamingo, the orifices of salivary glands were found to be located *latera*l to the *corpus* of the tongue. The *rugae linguae* structure was recorded for the first time in Greater Flamingos.
Keywords: Anatomy, flamingo, lingual architecture, SEM, tongue

Greater Flamingoda (*Phoenicopterus roseus*, Pallas 1811) Dil Yüzeyinin Papiller Mimarisi

Öz: Genellikle kuşların beslenme şekillerine uyum sağlayan dilleri, çok çeşitli anatomik yapılara sahiptir. Flamingoların dil yapıları 3 farklı beslenme tipine uygun biçiminde donatılmıştır. Bu çalışmanın amacı flamingoların dil yapılarını incelemektir. Önemli büyüklükte ve çok sayıda *papilla lingua rostralis*'e sahip olduğu tespit edilen flamingolarda *cuticula cornificata linguae* (dil tırnağı) yapısına rastlanmadı. SEM görüntülerinde *papilla lingua rostralis* üzerinde *micro papilla* yapısı görüldü. Greater flamingolarda tükürük bezlerinin deliklerinin *corpus lingua*'nın yan tarafında yer aldığı tespit edildi. *Rugae linguae* yapısı ilk kez Greater flamingolarında kaydedildi.
Anahtar kelimeler: Anatomi, dil yapısı, flamingo, lingua, SEM

Introduction

Birds should also be considered unique in the animal world because of the wide variety of shapes and structures of their tongues. A bird's tongue can be very long, very short, hairy at the tips, have spines of various sizes, and in some species show specific adaptations for feeding (Johnston, 2014; Ahmed et al., 2018). Birds with different lifestyles and dietary habits adapt to these situations thanks to changes in their tongue and beak structures in order to reach different food sources (Emura and Chen, 2008).

There have been some studies on tongue structures and especially papillary architecture in birds. For example, Finch (Heidweiller and Zweers, 1990), Caribbean and Greater flamingos (Zweers et al., 1995), penguins (Kobayashi et al., 1998), Andean-James' and Chilean flamingos (Mascitti and Kravetz, 2002), Western Sandpiper and Dunlin (Elner et al., 2005), White Tailed eagle (Jackowiak and Godynicki, 2005), cormorants (Jackowiak et al., 2006), Peregrine Falcon and Common Kestrel (Emura et al., 2008), ostrich (Jackowiak and Ludwig, 2008), Oriental Scops owl (Emura et al., 2009), Blue-and-White Flycatcher, Hawfinch and Japanese White-eye (Emura et al., 2010), long-legged buzzards (Erdoğan et al., 2012), storks (Tutuncu et al., 2012), quail (Pourlis, 2014), common buzzards and seagulls (Onuk et al., 2015), domestic duck (Skieresz-Szewczyk and Jackowiak, 2016) and Egyptian little owl (Ahmed et al., 2018), Guinea Fowl (Ilgun et al., 2020), barn owls (Ozkadif et al., 2023).

Filter feeders filter food particles and other suspended matter from water and sludge. Some flamingos, many ducks and geese, prions and avocets are among notable filter feeders. There are several different methods of how filter feeders filter food; first method is to shake the beak and use the beak to strain out food. The second method is to press the tongue and use it like a pump. Water/mud fills the *oral* cavity and then tongue is pressed against the palate and water/mud is thrown sideways through the *papillae* that capture solid food particles. Flamingos are the best known filter feeders that use both filter feeding methods. In shallow water, their large tongue acts like a piston, sucking the water in front of the beak and then pushing it out of the sides. Fringed

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plates on the tongue trap algae and crustaceans. However, they do this with their beaks upside down (upper jaw below). For larger foodstuffs, they are fed using the 'catch and throw' mechanism where the role of tongue is unknown (Zweers et al., 1995; Holliday et al., 2006; Erdoğan and Iwasaki, 2014; Johnston, 2014).

Considering that, in birds, the tongue has an important role in taking food, so its shape and morphology are affected by the nutrition method and the environment of the animal (Erdoğan and Pérez, 2015; Skieresz-Szewczyk and Jackowiak, 2016; Tabasi and Mohammadpour, 2019).

In addition, other SEM conducted on pheasant tongue, red-necked phalarope, rainbow lorikeet, scarlet macaw, grey crowned crane (*Balearica regulorum*), American flamingo (*Phoenicopterus ruber*), great egret (Ardea alba), mallard (*Anas platyrhynchos*), black-necked stilt (*Himantopus mexicanus*), Himalayan monal (*Lophophorus impejanus*) and green macaw (*Ara militaris*) show the connection between the feeding habits of birds and the shape of their tongue (Emura, 2016; Tabasi and Mohammadpour, 2019).

In flamingos, there are 20 bilaterally located *papillae lingua rostralis* on the d*orsolateral* surface of the tongue, decreasing in height from *rostral* to *caudal*. In flamingos, there are 20 *papillae lingua caudalis* located transversely in 2 series parallel to each other on the *dorsal* surface of the *caudal* end of the tongue (Zweers et al., 1995; Mascitti and Kravetz, 2002).

This study on the lingual papillae of the greater flamingo (*Phoenicopterus roseus*) species provides new findings in addition to previous studies in the literature, and these findings are thought to contribute significantly to the species' morphology. In this study, it is aimed to reveal the papillary architecture by examining the macro anatomical and electron microscopic structure of the tongue in Greater Flamingos, and to compare it with other bird species and especially other flamingo species.

Material and Methods

In this study, 2 females and 3 males Greater flamingo tongues were used. Two of them died during the operation at the Erciyes University Veterinary Faculty, where they were brought as a result of trauma, while the other three were found dead by a bird watcher around Sultan Reedy National Park and brought to the faculty.

Macro measurements were made immediately after the tongues were dissected together with the laryngeal region. Afterwards, for SEM application, the tongues were cut into pieces of appropriate sizes from different parts, washed with 0.1 M phosphate buffer solution, and then fixed with 2.5% glutaraldehyde for 6 hours. Afterwards, it was washed again with 0.1 M phosphate buffer solution and then fixed with 1% osmium tetroxide. Subsequently, dehydration with acetone, critical-point drying and coating with gold ions were performed according to the SEM procedures by Alan et al., (2020) and Onuk et al., (2013). Samples were examined and photographed with a scanning electron microscope (Leica, model Leo S440, Leica Cambridge, Cambridge, UK). Nomina Anatomica Avium (Baumel et al., 1993) is used for terminology.

Results

It was observed that the length of the examined flamingo tongues was 12.5±1 cm on average from the *apex* to the *glottis* cleft, and the average width was 2.3±0.1 cm at the widest part. It was determined that the *rostral* 1/3 of the tongue was raised at an angle of 30^{o} from the apex to the *dorsal* in harmony with the beak, and the *apex lingua*, which was flattened in the *dorsoventral* direction, expanded on both sides and formed a full *margo linguae*. *Cuticula cornificata linguae* (tongue nail) structure seen in some birds was not observed in the samples examined (Figure 1A). The presence of *sulcus linguae* continuing backwards from the *apex* was detected on the *dorsal* surface of the tongue. In the ascending part from the *apex* to the *dorsum lingua, caudomedial* directional fan-shaped, faint *rugae linguae* structures were determined (Figure 4A).

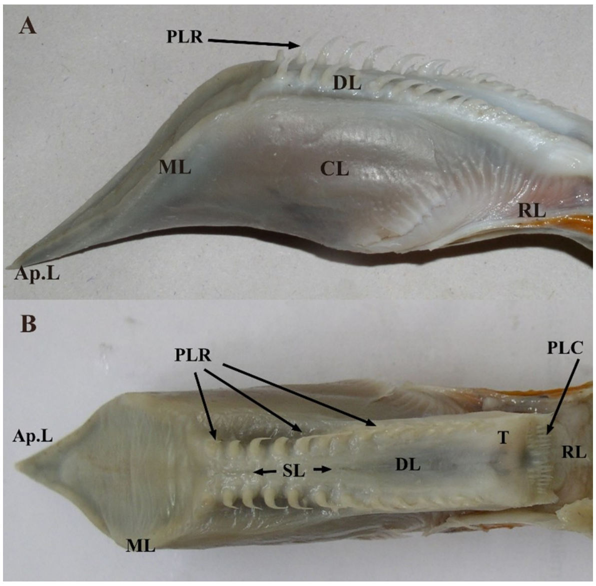

Figure 1. A. Dorsolateral; **B.** Dorsal view of tongue. **Ap.L:** Apex lingua; **CL:** Corpus lingua; **DL:** Dorsum lingua; **ML:** Margo lingualis; **PLC:** Papilla lingua caudalis; **PLR:** Papilla lingua rostralis; **RL:** Radix lingua; **SL:** Sulcus lingua; **T:** Torus lingua

In the *dorsum lingua*, it was determined that there were 15 bilateral single rows of backward-curving spines (*papilla lingua rostralis*) on the right and left of the *sulcus lingua* (Figure 1, 2, 5). One or two *irregular*, eccentric and small *intermediate papillae* were found in each flamingo tongue studied. It was observed that the size of the *papilla lingua rostralis* decreased from 4.5 mm to 2 mm caudally. The presence of a second set of *papillae* parallel to the *caudal* 10 *papillae* was noted (Figure 2). The placement of the *papillae* forming this *lateral* row coincided with the intervals of the inner row of *papillae linguae rostralis*, like the opposite teeth of the zipper.

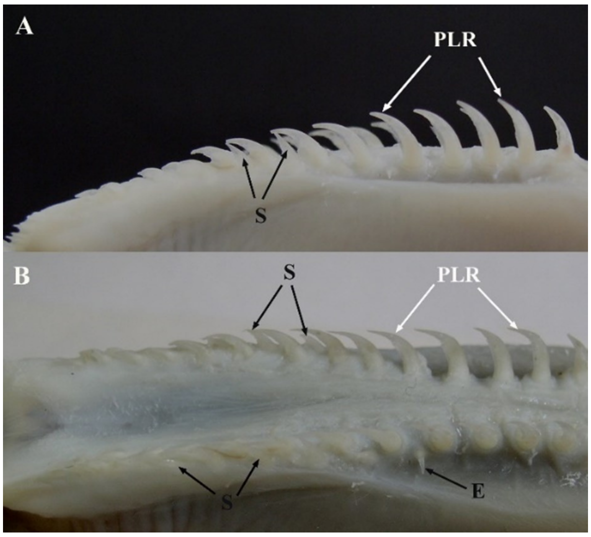

Figure 2. **A.** Lateral **B.** Dorso-lateral view of dorsum linguae. **PLR:** Papilla linguae rostrales; **E:** Exantric papilla; **S:** Second set of papillae

It was observed that the *torus lingua* was formed with a ridge at the end of the *papillae linguae rostrales*. A total of 30 *papillae lingua caudalis* were seen on the *transversal axis* just behind the *torus lingua*, in two parallel rows, the anterior row being shorter. The lengths of 14 *papillae* (Figure 3) in the anterior row ranged from 1-1.5 mm. In the posterior row, there were a total of 16 *papillae* with a length of 1.5-2.5 mm (Figure 3). On both sides of the transversal rows of the *papillae lingua caudalis*, a different row of longitudinally arranged *papillae* was detected. Each of these two different sets of *papillae* on the left and right sides of the tongue consisted of 10-12 *papillae* (Figure 3). These *papillae* can also be called *lateral longitudinal papillae linguae caudalis* because of their position and arrangement.

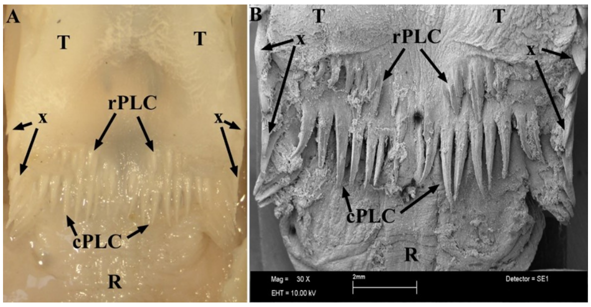

Figure 3. Dorsal view of radix lingua. **A.** Dissection microscope, **B.** Scanning Electron microscope. **T:** Torus lingua; **rPLC:** Rostral series of Papillae linguae caudales; **cPLC:** Caudal series of Papillae linguae caudales; **x:** Series of lateral longitudinal papillae; **R:** Radix lingua.

In SEM examinations, it was observed that the apex lingua had a flat surface and was covered with rough and scaly small *lamellae*, desquamating *superficial* cells (*epithelium stratificatum squamosum*) towards the *corpus* (Figure 4A2, B1, C2, Fig. 5 B1, B2). On the *lateral* surface of the *corpus lingua*, many orifices belonging to the ducts of the *glandula linguales* were observed (Figure 4C). When these *orifices* were examined more closely, taste buds were detected nearly located at the exit of the *orifices* (Figure 4C).

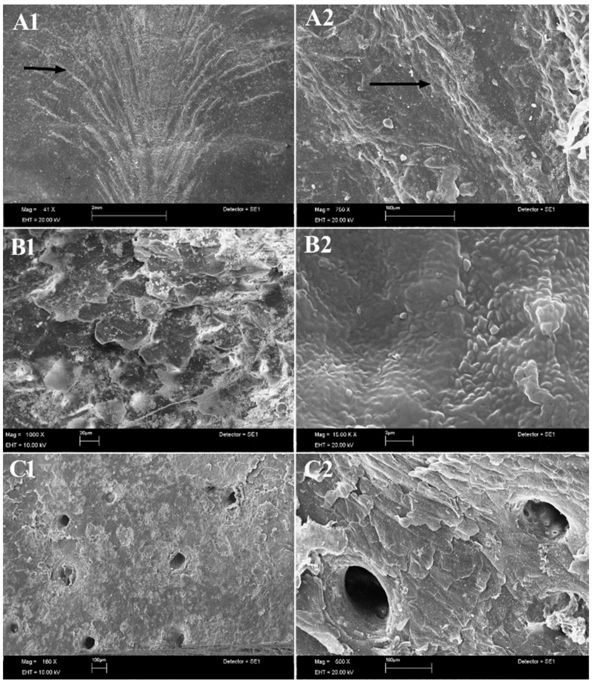

Figure 4. SEM images of surface of lingua. Surface of apex lingua. **A1.** 41X; **A2.** 750X; **arrow:** Rugae linguae; **B1.** Desquamating superficial cells on surface of apex lingua 1000X; **B2.** 15000X; Lateral surface of corpus lingua: Orificies of glandulae linguales; **C1.** 160X; **C2.** 500X. Taste buds in orifices.

In the SEM images of the *papilla lingua rostralis* found in the *dorsum lingua*, the presence of grooves starting from the ends of the *papillae* and continuing along their bodies was remarkable (Figure 5A1). On the *papilla lingua rostralis* and towards the *radix*, there were flat-conical secondary micro-papillary spines (Figure 5B1, B2).

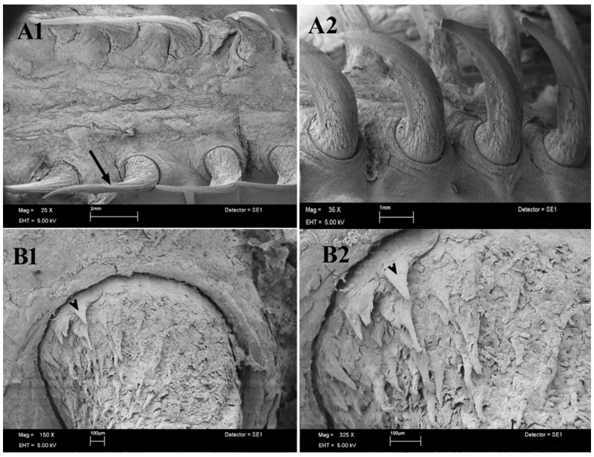

Figure 5. Papilla lingua rostralis and micropapillas. **A1.** Dorsal view 25X; **Arrow:** groove on the Papilla lingua rostralis; **A2.** Lateral view 35X; **B1.** Medial view 150X; **B2.** Medial view 325X; **Arrowhead:** micropapillar spines (desquamating cells).

Discussion and Conclusion

As reported in other studies examining the macro anatomical tongue in flamingos Zweers et al., (1995); Mascitti and Kravetz, (2002), the large and fleshly tongue in Greater flamingos rises from the *apex* to the *dorsum lingua* in line with the beak and on either side of the *apex lingua* it formed a plump *margo lingualis* structure. In the research of Emura (2016), the tongue of an American (*Chilean*) flamingo has a length of about 8.5 cm and a width of 1.9 cm. The Greater Filamingo tongues we examined in our study had an average length of 12.25±1.25 cm and an average width of 2.3±0.1 cm.

According to Zweers et al., (1995); Erdogan and Iwasaki (2014) and Onuk et al., (2015) in long-legged buzzards and seagulls, the *sulcus lingualis* structure, which is seen in the *median* line on the *dorsal* surface of the tongue in *anseriform* birds and flamingos, was also seen in the Greater flamingos examined in this study.

Rugae linguae structure has not been reported in the tongue of any poultry in the literature as reported by Zweers et al., (1995) and Mascitti and Kravetz (2002) in flamingos, Onuk et al., (2015) in long-legged buzzards and seagulls, Tutuncu et al., (2012) in storks, (other bird species can be added if you wish).The presence of rugae linguae structure, which was also reported terminologically in Nomina Anatomica Avium (Baumel et al.,1993), was recorded for the first time in Greater flamingos (Figure 4A1 and A2).

Mascitti and Kravetz (2002) report that Chilean (*Phoenicopterus chilensis*), Andean (*Phoenicoparrus andinus*) and James' (*Phoenicoparrus jamesi*) flamingos have *papillae linguae rostralis*, which consists of approximately 20 flexible spines, the height of which decreases from *rostral* to *caudale* (3.5 to 1.0 mm). Again, according to this study, there are 20 *papillae linguae rostralis* in phoenicopterus species and the *papilla* height varies between 1.0 and 1.5 mm. Zweers et al., (1995) reported that Caribbean Flamingo (*Phoenicopterus ruber ruber*) tongues have approximately 20 flexible *spinae*, *papilla lingua rostralis,* and their length is shortened from 5 mm to 3 mm from *rostral* to *caudal*. According to our study, the number of *papillae linguae rostrales* was found to be 30 in the Greater flamingo. The lengths of these *papillae* decreased from 4.5 mm to 2 mm from *rostral* to *caudal*.

It has been reported that approximately 20 *papillae lingua caudalis* in two *transversal* rows in Greater flamingos Zweers et al., (1995). Consistent with the literature, in our study, it was determined that the *papillae lingua caudalis* were in the form of two transversal rows, but the number was 30 different from the literature.

The difference in the number of *papillae* reported in the literature and the number of *papillae* reported in our study was attributed to the fact that the counting process in the literature (Zweers et al., 1995, Mascitti and Kravetz, 2002) was not performed clearly and was reported as an approximate number. In addition, although two different flamingo species were studied in the study of Zweers et al., (1995), it was not clearly stated from which flamingo the *papilla* findings were obtained. If Zweers et al., (1995) obtained their numerical findings from Caribbean flamingos, it is normal to have different findings from the Greater flamingos we studied.

Desquamating cells structure has been reported to exist on the surface of the tongue in orientel scops owl (Emura et al., 2009), on the surface of *conical papillae* in Japanese white-eye (Emura et al., 2010), on the surface of the tongue in Cormorants (Jackowiak et al., 2006), in the body of the tongue in white tailed eagle (Jackowiak and Godynicki, 2005), and in the root of the tongue in ostrich (Jackowiak and Ludwig, 2008). In this study, the structure of desquamating cells, *epithelium stratificatum squamosum*, was determined on the *apex lingua*, on the *dorsal* surface of the tongue, on the *papilla lingua rostralis* and on the *papilla lingua caudales* on the *radix lingua* in flamingos.

It has been reported that there are orifices in the ducts of the *lingual* glands in various parts of the tongue in birds. It has been reported that these orifices, through which the tongue salivary glands open, were found on the *corpus lingua* in sandbirds (Elner et al., 2005), falcons and kestrels (Emura et al., 2008); on the *radix lingua* in the striped owl (Emura et al., 2009), the blue flycatcher, the great-headed Finch, and the Japanese white-eye bird (Emura et al., 2010); the base of the tongue in the Finch (Heidweiller and Zweers, 1990), and on the surface of the tongue in the ostrich (Jackowiak and Ludwig, 2008). In our study, orifices of lingual glands were observed in accordance with the literature. In the Greater flamingo, these orifices were found to be located *lateral* to the *corpus* of the tongue.

In a study on duck tongues, it was stated that food filtration apparatus on the tongue surface includes *conical* and *filiform papillae* (Skieresz-Szewczyk and Jackowiak, 2016). In addition to the *lingua rostralis* and *caudalis*, which form large *conical papillae* in the Great flamingo, there is also a *longitudinal caudolateral* series of *papillae*. The flamingos we examined do not have *filiform papillae.*

Skieresz-Szewczyk and Jackowiak, (2016) reported that structures related to *intraoral* transport include the *sulcus medianus linguae, lingual* combs, the *rostral* border of the *lingual* process, and prominent *conical* rows of *papillae* on the *lingual eminent*. In our study, it was determined that flamingos have *conical papillae*, *sulcus lingua* and *torus lingua* structures from these structures.

The tongues of flamingos, which can catch and swallow large pieces of live food, swallow small pieces by distinguishing them from water, and feed by filtering microorganisms from the water, are designed in such a way that these 3 different types of feedings can be very successful. Flamingos can perform these 3 types of feeding thanks to the presence of pointed apex lingua and spina-shaped *papillae lingua rostralis*, which are developed to catch and swallow large pieces; *papillae lingua rostralis* and *caudalis,* which help to separate small pieces from water and swallow them; and all other papillae, which help to filter microorganisms in the water. With this study, the anatomical structure specific to its functions and the species-specific anatomy and papillary architecture of the flamingo tongue were revealed.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest related to this report.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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