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## Scanning electron-microscopic studies on the anterior larynx of adult broiler chickens

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### Abstract

The present study was conducted on scanning electron microscopy of anterior larynx of four adult broiler chickens. The larynx exhibited an oval-shaped laryngeal cleft, centrally positioned and flanked on both sides by the laryngeal mound. The laryngeal mound differentiated into medial and lateral parts, and extended caudally as the laryngeal fissure. Additionally, the laryngeal surface had several conical papillae organized in two configurations adjacent to the fissure, i.e. the median papillary rows containing four rows of papillae placed medially, and the slightly laterally positioned transverse rows of caudally directed pharyngeal papillae, forming two rows. These papillae exhibited a rough surface due to desquamated epithelial cells. The dorsal surface of the conical papillae presented squamous epithelial cells with diverse patterns and arrangements of microplicae.

**Keywords:** Larynx, laryngeal mound, median papillae, microplicae, pharyngeal papillae, Scanning Electron Microscopy (SEM)

### Introduction

The larynx retains its respiratory valve function while its caudal part has undergone modifications to form the syrinx, the sound-producing organ in case of birds. The anatomical studies have been conducted on describing the importance and versatility of the tongue in the oro-pharyngeal cavity, the avian tongue plays crucial functional roles in capturing, filtering, sucking, and manipulating food. Additionally, variations in the lingual papillary system significantly impact the feeding habits of birds (Erdogan and Iwasaki, 2014) [6]. There is a scarcity of literature on the morphology and ultrastructure of the laryngeal region in birds (Erdogan and Alan, 2012; Erdogan *et al.*, 2012; Abumandour, 2014; Erdogan and Perez, 2015) [5, 8, 1, 7]. In light of this knowledge gap, the present study was aimed to elucidate the ultrastructural characteristics of the anterior larynx in broiler chickens. Specifically, the study focuses on the conical pharyngeal papillary rows with caudally directed papillae, which have been observed in various other bird species previously (Erdogan and Alan, 2012; Erdogan *et al.*, 2012; Erdogan and Perez, 2015) [5, 8, 7].

### Materials and Methods

The present study was conducted on four healthy adult broiler chickens, each 6 weeks of age. Fresh tissues were collected from the anterior, middle, and posterior portions of the anterior larynx, along with the pharyngeal papillae. To prepare the tissues for scanning electron microscopy (SEM), the collected tissues were immediately washed with 0.1M chilled phosphate buffer (pH 7.4) after removal. Subsequently, they were fixed in a 2% glutaraldehyde solution for approximately 6–8 hours. After fixation, the tissues underwent two additional washes with chilled 0.1M phosphate buffer. The fixed tissues were then transferred to the EM Lab at AIRF, JNU, New Delhi, India, where further processing was carried out. This processing involved dehydration in increasing grades of ethanol, critical point drying, and sputter-coating with gold ions. The tissues were examined using a scanning electron microscope (SEM) to record the observations and photographs.

### Results and Discussion

The anterior larynx, protruding above the pharynx floor, displayed a medially placed laryngeal cleft with an oval outline as observed in the long-legged buzzard (Erdogan *et al.*, 2012), southern

lapwing (Erdogan and Perez, 2015), and broiler chicks (Bansal and Kumar, 2020) [8, 7, 4]. However, Abumandour and El-Bakary (2017a) reported an elongated triangular laryngeal cleft in the Eurasian coot [2]. The anterior border of the laryngeal cleft appeared straight, while the posterior border exhibited a U-shaped outline. This cleft was bounded on both sides by well-developed, elevated mucosal fold-like structures, known as the laryngeal mound, as also observed in broiler chicks by Bansal and Kumar (2020) and in southern lapwings by Erdogan and Perez (2015) [4, 7].

The laryngeal mound could be distinguished into two portions, the lateral and medial parts. The medial portion was smaller, while the lateral portion was larger and flat as reported by Bansal and Kumar (2020) in broiler chicks [4]. An absence of papillae on the laryngeal mound during the present study aligned with the reports of Abumandour (2014) in Eurasian hobby, Abumandour and El-Bakary (2017a) in Eurasian coot, Abumandour and El-Bakary (2017b) in the European kestrel, and Pasand *et al.* (2010) in ostrich [1, 2, 3, 15]. In contrast, the presence of papillae surrounding the glottis have been observed in turkey, goose, and duck (Nickel *et al.*, 1977), raven and magpie (Erdogan and Alan, 2012), long-legged buzzard (Kabak *et al.*, 2007), and goose (Onuk *et al.*, 2010) [13, 5, 10, 14]. Additionally, Jackowiak *et al.* (2010) and Abumandour and El-Bakary (2017b) reported the existence of large conical papillae on the dorsal surface of the laryngeal mound in nutcrackers and Hume's tawny owls [9, 3].

Just caudal to the laryngeal cleft, four rows of transversely aligned median papillae were observed, and laterally pharyngeal papillae were present. However, Erdogan *et al.* (2012) reported the presence of 16-18 papillae medially, continuing from the caudal border of the glottic fissure backward in long-legged buzzards [8]. In Japanese quails, medially caudally directed conical papillae were observed surrounding the rim of the glottis (Madkour, 2018) [11]. The pharyngeal papillae were arranged in two transversely oriented rows (Figs. 2, 3) which corroborates with the findings of Erdogan and Alan (2012) in ravens and magpies, Erdogan and Perez (2015) in Southern lapwings, Abumandour and El-Bakary (2017b) in common kestrels, and Bansal and Kumar (2020) in broiler chicks [5, 7, 3, 4]. In Eurasian coots, Abumandour and El-Bakary (2017a) reported the presence of large, conical, caudally directed pharyngeal papillae that exhibited a unique heart-shaped appearance at the caudal border of the laryngeal mound [2].

The surface of the laryngeal mound appeared irregular and rough due to the presence of scale-like structures. The laryngeal cleft continued caudally in the form of a fissure known as the laryngeal fissure. Similarly, the glottis continued caudally as the laryngeal sulcus, as reported by Abumandour and El-Bakary (2017a) in Eurasian coots [2]. On either side of the laryngeal fissure, four rows of transversely arranged median conical papillae were observed. The sharp tips of these papillae faced backward. The first row had only two papillae, one on either side of the laryngeal cleft, while the second row exhibited four papillae, taller than those of the first row, with two on either side of the laryngeal fissure. The third row presented five papillae on either side of the fissure, with three larger papillae arranged like the petals of a lotus flower (Fig. 1). The remaining two were positioned obliquely. The fourth row comprised four papillae on each side, situated

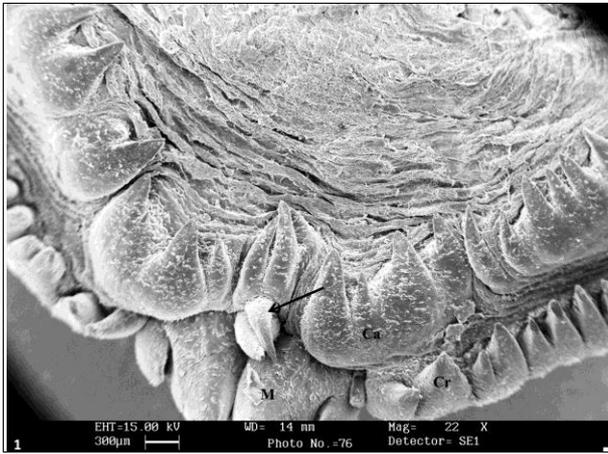
posteriorly and slightly laterally to the third row. All these papillae were covered with scale-like flat polyangular cells on their surface and displayed different patterns of microplicae, as also observed by Bansal and Kumar (2020) in broiler chicks [4].

Along with the median papillae, our studies also recorded another pattern of papillae on the surface of the anterior larynx, known as the pharyngeal papillae. These papillae can be further classified into two rows - the anterior row and the posterior row. The presence of two transverse rows of pharyngeal papillae aligns with previous works in raven (Erdogan and Alan, 2012), long-legged buzzard (Erdogan *et al.*, 2012), and Eurasian Hobby (Abumandour, 2014) [5, 8, 1]. However, Erdogan and Alan (2012) mentioned the presence of only one row of caudally directed conical pharyngeal papillae at the border of the glottis in magpies [5]. Similarly, in Hume's tawny owl, there were 20–22 large, long, mechanical, caudally directed conical papillae arranged in a single transverse row (Abumandour and El-Bakary, 2017b) [3]. Conversely, Abumandour and El-Bakary (2017a) emphasized the characteristic distribution of pharyngeal papillae in two paramedian rows: transverse and longitudinal rows, divided by a median laryngeal sulcus into two halves in Eurasian coots [2]. Madkour (2018) observed that these pharyngeal papillae were arranged in three transverse rows in Japanese quails [11].

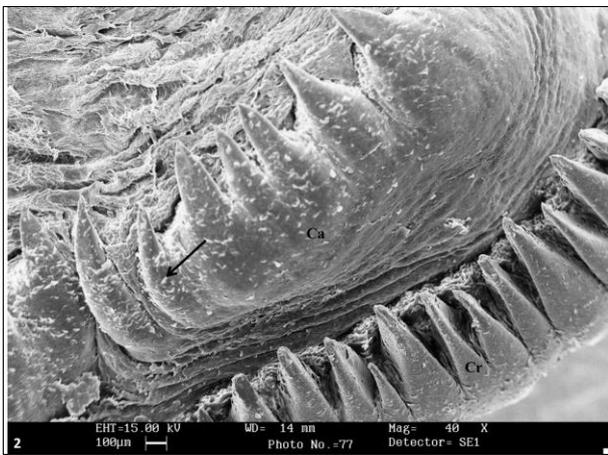
Regarding the number of papillae in each row, our study revealed approximately 18–20 transversely placed papillae on each side of the laryngeal fissure in the anterior row (Fig. 1). However, Erdogan *et al.* (2012) mentioned that the rostral row of pharyngeal papillae in long-legged buzzards was composed of 28–30 conical papillae [8]. Similarly, Abumandour and El-Bakary (2017b) reported 18–20 small conical papillae in the rostral row in common kestrels [3]. These conical papillae were positioned laterally to the third and fourth rows of median papillae, with their pointed tips directed downwards and backwards (Figs. 2, 3), while their base portions were united with adjacent ones.

Small and large papillae were uniformly distributed similar to the findings by Bansal and Kumar (2020) in broiler chicks [4]. The surfaces of these papillae were rough and irregular due to the presence of desquamated epithelial cells, and microplicae of different patterns were observed between the adjacent epithelial cells. The pharyngeal papillae of the second or caudal row were positioned posterior to the first row on each side (Figs. 2, 3) and had a globular shape towards the base with pointed tips. This row consisted of 4-5 papillae on each side. In contrast, Erdogan *et al.* (2012) stated that the caudal row of pharyngeal conical papillae in long-legged buzzards was formed by 22–25 papillae [8]. Similarly, Abumandour and El-Bakary (2017b) reported the distribution of 20–24 papillae in the caudal row in common kestrels [3]. Some of these globular-based papillae appeared bifid and exhibited shallow grooves between adjacent papillae. Additionally, these papillae displayed varying patterns of microplicae on their surface.

Apart from the median and pharyngeal papillae caudal to the laryngeal fissure, some irregularly placed papillae were also observed on the laryngeal surface. Similar freely distributed pharyngeal papillae around the laryngeal sulcus were reported by Abumandour and El-Bakary (2017a) in Eurasian coots [2].



**Fig 1:** Scanning electron-micrograph of anterior larynx of adult chicken showing cranial (Cr) and caudal (Ca) rows of pharyngeal papillae, medial papillae (M), and irregularly placed free papillae (arrow); Increase- 22X (Bar 300 µm)



**Fig 2:** Scanning electron-micrograph of anterior larynx of adult chicken showing cranial (Cr) and caudal (Ca) rows of pharyngeal papillae, and desquamated cells over surface of papillae (arrow); Increase- 40X (Bar 100 µm)



**Fig 3:** Scanning electron-micrograph of anterior larynx of adult chicken showing cranial (Cr) and caudal (Ca) rows of pharyngeal papillae, and desquamated cells over surface of papillae (arrow); Increase- 48X (Bar 100 µm)

## Conclusion

The present study revealed that the larynx exhibited a centrally placed laryngeal cleft bounded on both sides by the laryngeal mound, extending caudally as the laryngeal fissure. On either side of the fissure, papillae were organized in to the median papillae forming four rows, and the pharyngeal

papillae aligned in two transverse rows of caudally directed structures. The dorsal surface of these papillae exhibited varying patterns of microplicae on their epithelial cells. These findings highlight the specialized nature of the avian larynx, where the pharyngeal and median papillae served as essential guiding tools, directing the movement of food boluses unidirectionally towards the esophagus. This specialized arrangement helps prevent food from escaping to the glottic fissure, ensuring efficient swallowing and respiratory protection.

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