Microscopy Research and Technique

WILEY

MICROSCOPY RESEARCH & TECHNIQUE

RESEARCH ARTICLE

New Findings in Developmental Studies of Laryngeal Mound With a Reference to Its Attached Structures in Japanese Quail (*Coturnix coturnix Japonica*)

Mahmoud Osman Khalifa[1,2] | Wafaa Gaber[3] | Abdelmohaimen Mostafa Saleh[3]

[1]Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Aswan University, Aswan, Egypt | [2]Department of Molecular Bone Biology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan | [3]Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt

Correspondence: Mahmoud Osman Khalifa (mahmoud.othman@aswu.edu.eg)

Received: 11 February 2024 | **Revised:** 13 June 2024 | **Accepted:** 31 October 2024

Review Editor: Paul Verkade

Funding: The authors received no specific funding for this work.

Keywords: cartilages | glands | glottis | laryngeal | morphogenesis | papillae

ABSTRACT

The laryngeal mound (LM) formed the caudal part of the pharyngeal floor, which varied in position, shape, and length at different ages. This work aimed to study the morphogenesis of the LM in the embryonic and post hatching periods grossly, histologically, and by scanning electron microscopy using *forty-eight* Japanese *quails*. The LM primordia appeared on the 8th day of incubation as a raised elevation carried on a deep median symmetrical sulcus (glottis primordium). As a result of rapid differential LM parts growth, LM took different shapes with advanced ages, finally ending in a heart shape. Internally, LM was supported by hyaline laryngeal cartilages; a C-shaped cricoid cartilage that had two wings, paired fork-like two arytenoids, and a comma-shaped procricoid that had four articulations. The glottis appeared as a central longitudinal opening on the 13th day of incubation. With age advancing, it was characterized as a wide rostral commissure and a caudal narrow one that was supported on either side by arytenoid cartilages. Additionally, on the 13th day, a bilateral sagittal row to the glottis developed, consisting of 8–9 small caudally directed papillae. At that time, rostral and caudal transverse laryngeal papillary rows appeared. LM had compound tubuloalveolar submucosal laryngeal glands that were situated between *M. dilator glottidis* and *cricohyoideus* and opened on the dorsal surface of LM. Histochemically, the early post-hatching stages of the glandular secretion were PAS-positive while late post-hatching ages had alcinophilic reactions. In conclusion, the LM had rapid morphological developmental events in the early ages other than the adult ages.

1 | Introduction

The Japanese quail (*Coturnix japonica*) was classified under the order Galliformes, family Phasianidae, genus Coturnix, and species japonica which differed from the common quail or European quail (Coturnix coturnix). The first record of wild Japanese quail appeared in the eighth century in Japan. It migrates throughout Europe, Asia, Africa, and India (Huss, Poynter, and Lansford 2008). The high rate of returns and low cost of investment and rearing as well as the fact that the birds grow and reach maturity stage faster and lay eggs within 2 months compared to 6 months maturity period of chicken in

Abbreviations: AB, alcian blue stain; BH, basihyale; CB, ceratobranchiale; Cr, cricoid; CTLR, caudal transverse laryngeal papillary row; KOH, potassium hydroxide; LM, laryngeal mound; M, muscle; PAS, periodic acid-schiff stain; Post-hatch, post-hatching; prehatch, prehatching; RH, relative humidity; RTLR, rostral transverse laryngeal papillary row; SEM, scanning electron microscopy; T, tongue; Tr, trachea; UH, urohyale.

Summary

- Primordia of the laryngeal mound developed on the 8th day of incubation.
- Primordia of the aditus larynges appeared on the 10th day of incubation.
- Laryngeal cartilage ossified except for caudal processes of arytenoids.
- The transverse laryngeal papillae appeared on the 13th day of incubation.
- The compound tubuloalveolar intraepithelial mucous glands as well as the laryngeal salivary glands had alcinophilic activity in elder ages.

addition to the birds high nutritional and market value were some of the reasons that many poultry farmers were fast resorting to quail farming (Bakoji et al. 2013).

The laryngeal mound (Mons larynges) was a raised structure attaching to the root of the tongue rostrally and the tracheal rings caudally and carried different backward orientated laryngeal papillae. Laryngeal mound (LM) carries the laryngeal inlet (aditus larynges) that was represented the beginning of the upper respiratory tract in the caudal part of the pharyngeal floor (Kabak, Orhan, and Haziroglu 2007). The outline of the glottis could take different shapes such as the "v" or "u" and elliptical shape with age advancing (Mohamed 2004). The laryngeal mound was supported by laryngeal cytoskeleton that is formed of cricoid, paired arytenoid, and procricoid cartilages. These cartilages connected together by dilators and constrictor muscles, adventitia, and laryngeal salivary glands (Kabak, Orhan, and Haziroglu 2007).

Knowledge of the developmental stages of the LM was important to identify the structural features that might influence the function, as well as to provide a foundation for the recognition of pathology. In the available literature only few researches had been carried out on the development of the LM in a precise manner of post-hatching ages within various avian species (Igwebuike and Eze 2010; Erdogan and Alan 2012; Moussa and Hassan 2013; Erdogan and Perez 2015; Abumandour and El-Bakary 2017; Abumandour 2018; Mahdy 2020; Abumandour et al. 2021a; Madkour and Abdelsabour-Khalaf 2022). Very few discussed the embryonic period. We found that this field needs more investigation and many points should be focused on. Therefore, our study aims to provide more serial detailed description of the morphogenesis of the laryngeal mound of the Japanese quail in a particular manner of the embryonic and post-hatching periods grossly, histologically, and by scanning electron microscopy, with special emphasis was placed upon the development of the attached structures.

2 | Materials and Methods

The current study has been approved by The Ethical Committee of The Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt, according to The OIE standards for use of animals in research Under the No. 06/2023/0103.

2.1 | Samples Collection and Preparation

The healthy quail embryos and chicks were collected from the poultry farm, Faculty of Agriculture, Assiut University. Assiut, Egypt. The embryos were collected from a forced-draft incubator (37.5° ± 0.3°C and 60% RH). The fertilized eggs collected within 1 week of laying and preserved in a refrigerator at 4°C for ensuring the symmetrical aging of the specimens before placing into the incubator. *Forty-eight healthy Japanese quails (Coturnix coturnix japonica) were used during the incubation and post-hatch period. During incubation, embryos (n = 23) were collected at 6, 8, 10, and 13th days of incubation and at hatching. After hatching, quails (n = 25) were utilized at 7, 14, 30, and 60-day old. Embryos and quail chicks were euthanized by cervical dislocation;* the specimens have been sacrificed and all heads were cut off for ensuring complete bleeding, then the beak's angles were incised. The floor was dissected and then rinsed in running tap water to remove blood traces *and exposed accordingly to (*Khalifa et al. 2022*). Samples were fixed in different fixatives solutions according to the methods to be used. All methods were performed in accordance with the relevant guidelines and regulations with arrive guidelines instructions* https://arriveguidelines.org. *The institutional review board of the Ethics Committee of the faculty of Veterinary Medicine, Assiut University, Egypt approved this study (License No. 06/2023/0103). The oropharyngeal floor samples were incised and exposed accordingly to* (Khalifa et al. 2022).

2.2 | Alizarin Red and Alcian Blue Double Staining

Three specimens from each age 7, 14, 30, and 60-day-old quail chicks were fixed in 10% neutral buffered formalin and stained after maceration process by alizarin red and alcian blue stains (KOH 1%) (Khalifa et al. 2022).

2.3 | Histological and Histochemical Analysis

For histological investigations three specimens of each age were used after proper fixation, the samples were kept in 10% formic acid/formol saline for the process of decalcification and then histological processing. The specimens were embedded in paraplast (Sigma Aldrich). Serial 5–6 μm cross, longitudinal, and frontal sections from the oropharyngeal floor were cut by a LEICA 2155rm automatic microtome. Some of these sections were routinely stained corresponding to (Bancroft and Gamble 2002; Khalifa et al. 2022). The histological sections were photographed by the OLYMPUS BX51 microscope, and the photos were taken by the OLYMPUS DP72 camera adapted into the microscope.

2.4 | Scanning Electron Microscope (SEM)

For SEM investigations, we used three samples of the 8th, 10th, and 13th days embryonic period & 60th days old quail. Samples were prepared accordingly to (Khalifa et al. 2022). Briefly, the floor of the oropharynx was washed several times in 0.1 M phosphate buffer at pH (7.2 ± 0.1). Post-hatching samples were rinsed with acetic acid 2%, then fixed in 4% glutaraldehyde solution for 24 h. Post-fixation was made in 1% sodium tetroxide solution for

2 h at 4°C. After that, the fixed samples were washed in 0.1 M phosphate buffer at pH = (7.2 ± 0.1), then dehydrated in ascending grades of ethanol followed by critical point-dried in liquid carbon dioxide. All specimens were mounted on aluminum stubs covered with carbon tabs and sputtered with gold. The prepared specimens were examined and photographed using JEOL scanning electron microscopy (JSM-5400) at an accelerating voltage of 15 kV in the electron microscope unit of Assiut University (Abou-Elhamd, Abd-Elkareem, and El-Zuhry 2018).

2.5 | Morphometric Analysis

The samples were prepared freshly after proper washing from traces of blood and debris. After that we followed the previous procedures of sampling preparation. The measurement procedures were done using the digital caliper. Then, the photos were photographed by using the stereomicroscope (LEICAS6D). Measurement values were confirmed by Image J software (https://fiji.sc/). *Particularly, the nomenclature that we used in this study follows (Nomina Anatomica Avium). Besides that, we synonymized and homologized many names here according to former and past studies of the chicken and other avian species described in different literatures.*

3 | Results

In this study, we highlight both the LM gross and morphometric analysis prehatching and post-hatching ages. The laryngeal mound is a firm projection surrounding the pharyngeal floor which was defined rostrally by the lingual root and caudally by a group of caudal-directed laryngeal papillae at the pharyngeo-esophageal junction caudally.

3.1 | Gross, SEM, and Morphometric Studies

The hallmarks of LM primordia appearance could be found at the 8th day of incubation, had a similar rostral, middle, and caudal parts width (Table 1, Figure 1b). LM carried a shallow median groove represents the glottis primordium (Figure 2A). Compatible with SEM results revealed that two raised structures emerged from the pharyngeal floor with several small openings on its surface (Figure 3a,b). Of note, in SEM data, the LM primordia appeared like a heart-shaped well-projected structure with two AR swelling, but the median groove became deeper at the 10th day of incubation than before (Figure 3c,d). Different developmental dimensions of the laryngeal mound (LM) are given in (Tables 1–5).

TABLE 1 | The length of the laryngeal mound and width of its parts (mm) in Japanese quail.

Age	LM length ± SE	Pharyngeal floor length ± SE (P.F)	LM to pharyngeal floor ratio
6-days of incubation	0.57 ± 0.007	—	—
8-days of incubation	1.11 ± 0.009	1.85 ± 0.006	60%
10-days of incubation	1.71 ± 0.009	3.55 ± 0.003	48.2%
13-days of incubation	2.73 ± 0.015	4.39 ± 0.003	62.2%
Hatching day	3.01 ± 0.007	4.8 ± 0.003	62.7%
7-days old	4.16 ± 0.006	6.82 ± 0.009	61%
14-days old	5.1 ± 0.009	7.52 ± 0.003	67.8
30-days old	6.1 ± 0.003	9.6 ± 0.003	63.5%
60-days old	6.53 ± 0.017	10.7 ± 0.032	61%

Age	LM Width rostral part	LM Width middle part	LM Width caudal part
6-days of incubation	—	—	—
8 days of incubation	0.63 ± 0.012	0.736 ± 0.007	0.733 ± 0.009
10 days of incubation	1.81 ± 0.006	2.32 ± 0.003	2.32 ± 0.003
13 days of incubation	2.3 ± 0.006	2.77 ± 0.003	2.77 ± 0.007
Hatching	2.88 ± 0.003	3.08 ± 0.006	3.08 ± 0.006
7-days old	4.4 ± 0.003	4.82 ± 0.009	4.63 ± 0.018
14-days old	4.54 ± 0.022	5.11 ± 0.006	5.05 ± 0.006
30-days old	5.55 ± 0.003	6.3 ± 0.003	5.82 ± 0.017
60-days old	5.95 ± 0.29	6.98 ± 0.009	6.61 ± 0.01

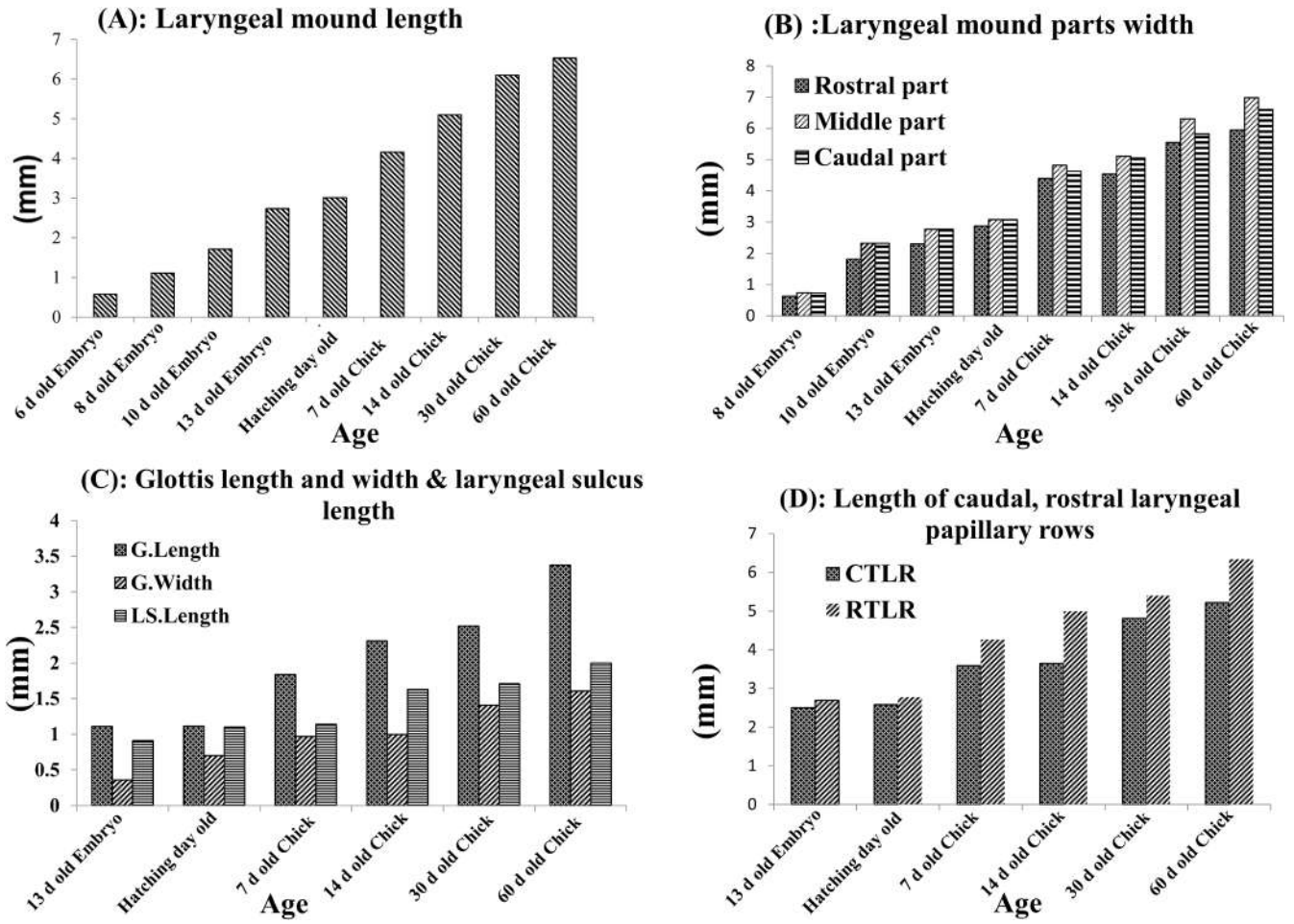

FIGURE 1 | Representative micrographs showing laryngeal mound length (a), laryngeal mound parts width (b), glottis length and width, and laryngeal sulcus length (c), caudal and rostral transverse papillary rows length (d).

Concerning gross and SEM data, we found that LM took a clear heart-shaped outline with age advancing per se on the 13th day of incubation. Additionally, two laryngeal transverse rows of caudally directed papillae appeared. Whereas, the rostral row showed two median giant papillae within. Furthermore, some papillae of the rostral transverse row were noticed biforked (Figure 3e,f). Surprisingly, at the first glance, we found the caudal and rostral transverse laryngeal papillary rows consisted of 18 and 13 papillae, with 2.69 ± 0.003 and 2.5 ± 0.003 mm lengths respectively. Also, we found some papillae were biforked with the same age (Figure 3e,f). The two giant papillae carry the laryngeal sulcus measuring 0.91 ± 0.007 mm. Hence, the glottis appeared clearly as a slit-like opening that measured about 1.11 ± 0.007 mm long and 0.36 ± 0.01 mm wide (Table 2). The glottal rims were guarded on each side by a sagittal row of eight small caudally directed papillae (Figure 2b). At this age, the laryngeal mound increased significantly in its length and width. While we can see the width in the rostral, middle, and caudal three transverse levels also had a great change (Table 1, Figure 1a,b).

Interestingly, the gross examinations of chick at hatching time indicated that two transverse papillary rows were slightly curved. Of note, the rostral row showed two median giant papillae followed caudally by two pairs of papillae that were connected between two transverse rows in the middle of the LM. Here, we noticed the papillae were pointed other than blunt previously. However, the papillary length had no change. Thus, the CTR had a clear demarcation between the pharyngeal and esophageal junction. The glottis became "v-shaped" with a wide rostral end and a caudal narrow one (Figure 2c). LM measurements were about 3.01 mm in length and the width of the rostral, middle, and caudal points levels were 2.88, 3.08, and 3.08 mm, respectively (Table 2, Figure 1a,b).

With age advancing, at the 7-day old, we noticed that most developmental events happened within morphometric structures. To find, the glottis became a "U-shape" in outline with a slightly wide rostral commissure that was situated in the rostral half of the LM and continued caudoventrally to the trachea. Furthermore, the caudal commissure of the glottis was continued caudally with a short narrow laryngeal sulcus which was extended beyond to the base of the two giant papillae (Figure 2d). Here, we found that the glottis measures were 1.84 mm long and 0.97 wide (Table 3, Figure 1c). While rostral and caudal transverse laryngeal papillary rows (RTLR and CTLR) were 4.26 and 3.59 mm in length and had 20 and 14 papillae, respectively (Figure 2d).

Particularly, in chicks at 14-day old, our SEM and morphological data indicated that the rostral row of the laryngeal papillae became strongly curved backward laterally to connect with the caudal row laterally because of the high growth rate of LM (Figure 2e). Besides that, (2–3) pairs of the laryngeal papillae

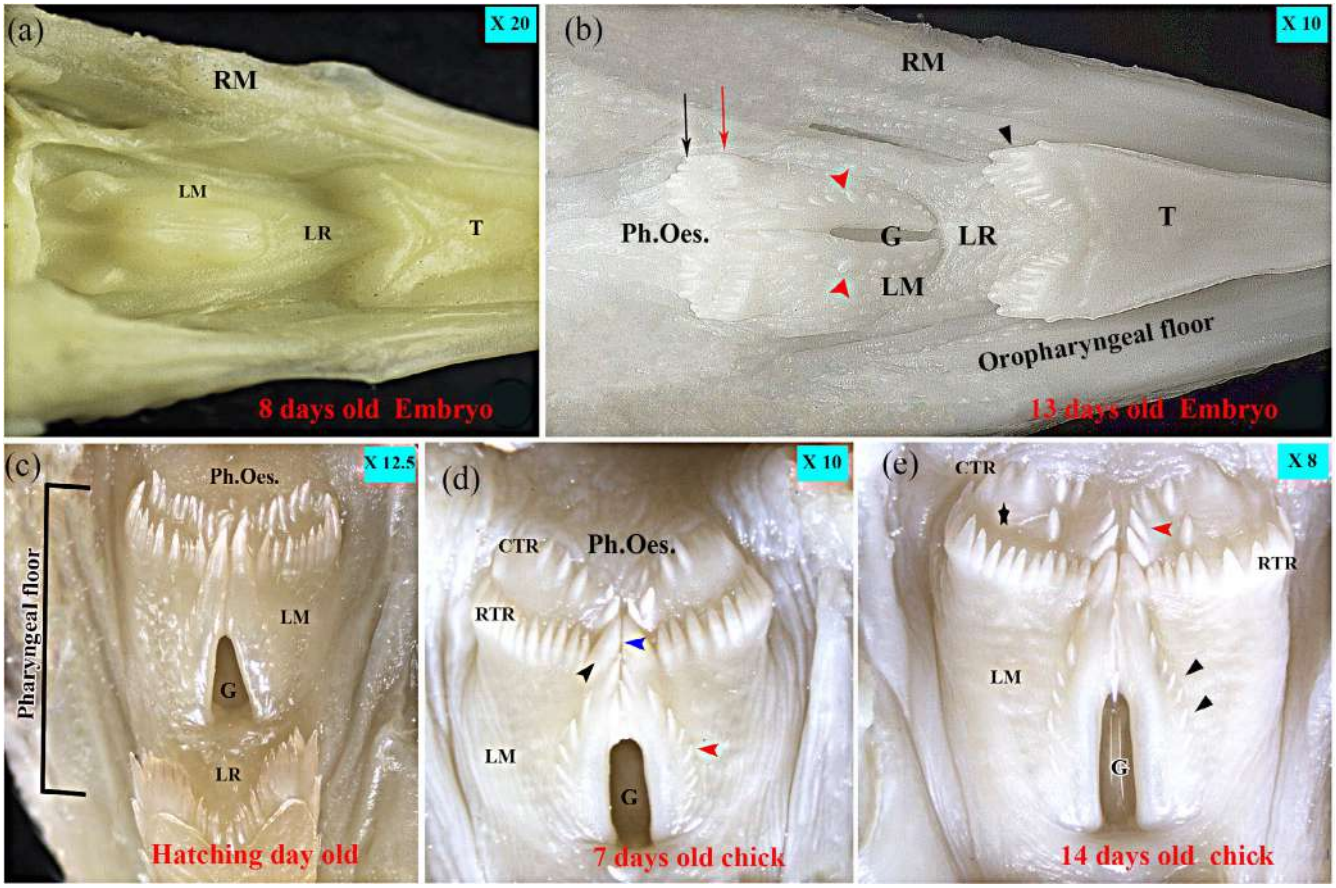

FIGURE 2 | Photographs of dorsal views of the oropharyngeal floor. (a) The laryngeal mound primordium (LM) with median shallow groove of the 8 days old embryo. Tongue (T), lingual root (LR), Ramus mandibularis (RM), (X 20). (b) A 13-day old embryo showing the laryngeal mound (LM) with rostral (red arrow) and caudal (black arrow) transverse rows of papillae. Notice, the glottis (G) is guarded by sagittal rows of papillae (red arrowheads). Tongue (T) and lingual root (LR), Ramus mandibularis (RM), pharyngeal-esophageal junction (Ph.Oes.), lingual papillary crest (black arrowhead), (X 10). (c) Hatching day-old chick showing the V-shaped glottis (G). Notice, the laryngeal papillae have pointed tips. Laryngeal mound (LM), lingual root (LR) and pharyngeal-esophageal junction (Ph.Oes.), (X 12.5). (d) Pharyngeal floor of 7 days old chick showing the U-shaped glottis (G). Notice, the median laryngeal sulcus (blue arrowhead) extends to the level of the base of giant papillae (black arrowhead). Laryngeal rostral transverse row (RTR) and caudal transverse row (CTR) taking (wide v-shape) intersected together and sagittal row (red arrowhead), Laryngeal mound (LM), (X 10). (e) Dorsal view of the pharyngeal floor of 14 days old chick showing the rostral (RTR) and caudal (CTR) of laryngeal papillae were arranged in two adjacent crowns (black star). Laryngeal mound (LM), two connecting median papillae rows (red arrowhead), and glottis (G), bilateral papillae row sided the glottis (black arrowheads), (X 8).

were present in the midline between the two rows caudal to the giant papillae of the rostral row made the two rows formed two adjacent crowns with a central papilla within each one (Figures 2e and 4a,b). Clearly, we noticed the caudal commissure of the glottis was narrower than the rostral one. Some papillae were bi-forked, and others were tri-forked (Figure 4a,b). The laryngeal sulcus extended from the caudal commissure of the glottis to the base of the two median giant laryngeal papillae and was guarded by 2–3 papillae on each side (Figure 4c). Some minuscule papillae were shown on the surface of the giant papillae (Figure 4d,e). The surface of the laryngeal papillae was covered by many scales, which show micro-plicae on their surface (Figure 4f).

At 30 days old quail chick, the glottis takes "U-shape" with much wider rostral end, that width increased and reached 1.41 mm (Figure 5a). The paired arytenoids were still cartilaginous which caudal processes support the base of the giant papillae (Figure 5b). Also, the cricoid cartilage was partially ossified with its caudal part was still cartilaginous. It was supported mid-ventrally by the urohyale (Figure 5c).

By the 60th day old quail, the cricoid cartilage had shown complete ossification, while the arytenoid was still cartilaginous (Figure 5d,e). The laryngeal mound increased in size reaching about 6.53 mm long and its width measured about 5.95, 6.98, and 6.61 mm rostral, middle, and caudal parts respectively. The glottis length reached about 3.37 mm with a width of 1.61 mm, while the laryngeal sulcus measured (2 mm) long. The sagittal rows consisted of 9 papillae for each. The rostral and caudal transverse laryngeal rows had (21 and 14) papillae with length of 6.34 and 5.22 mm respectively (Tables 4 and 5, Figure 1d).

3.2 | Histological Studies

In our histological investigation of the 6th days old quail embryo, both primordia of *sternohyoideus* and

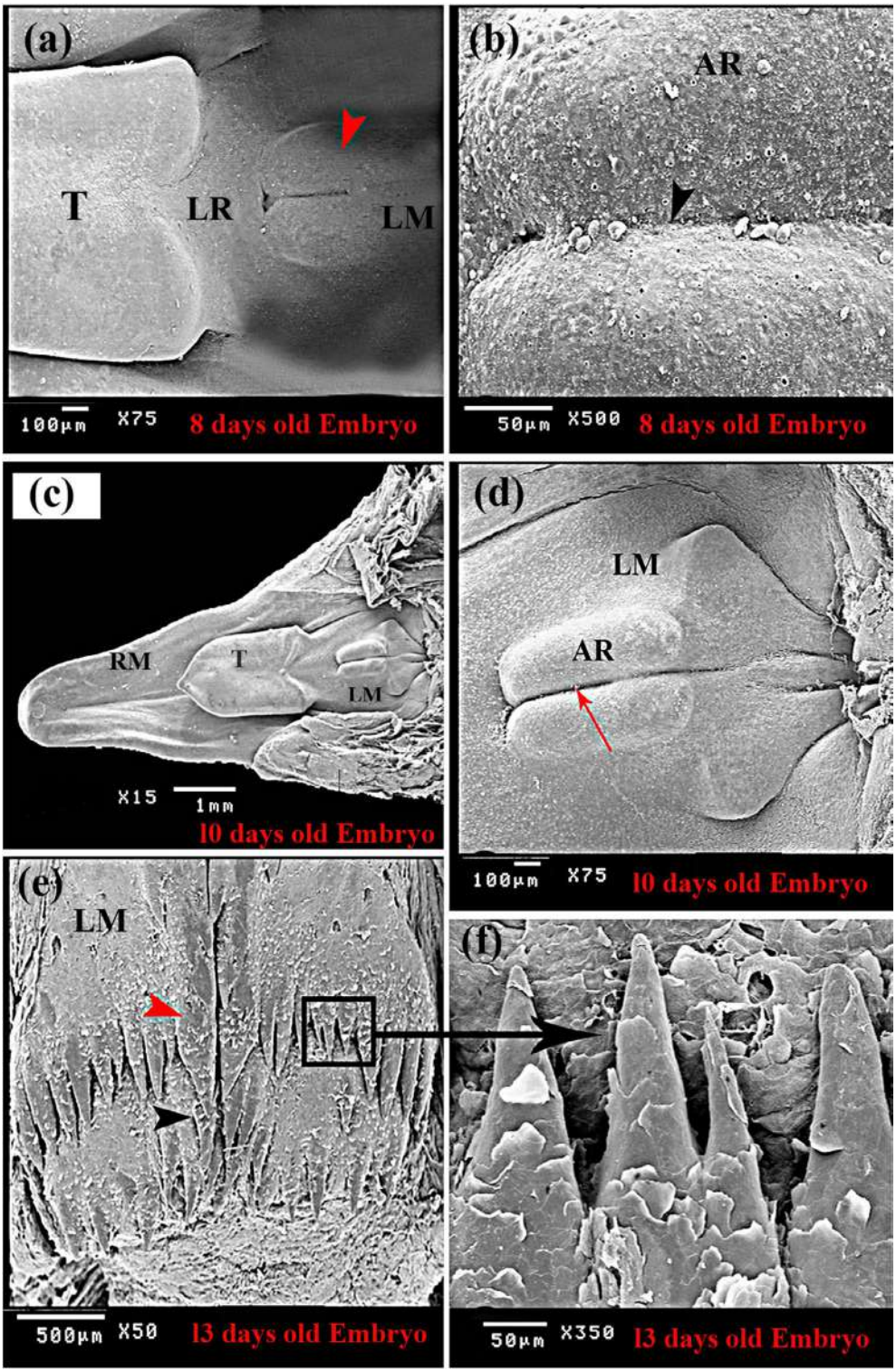

FIGURE 3 | Scanning electron micrographs of the oropharyngeal floor. (a, b) An 8-day-old embryo showing the laryngeal mound primordium (red arrowhead) with a median shallow groove (black arrowhead; B) and small openings on its surface, (X 75 and X 500). (c, d) A 10-day old embryo showing the two arytenoid swellings (AR) with deep median groove (red arrow). Laryngeal mound (LM), tongue (T), Ramus mandibularis (RM), (X15 and X 75). (e, f): Laryngeal mound of the 13 days old embryonic period showing the biforked papillae (black rectangular shape) and giant papillae (red arrowhead). Notice, two pairs of papillae (black arrowhead) caudal to giant papillae, laryngeal mound (LM), pharyngeo-esophageal junction (Ph.Oes.), (X 50 and X 350).

TABLE 2 | The morphometric data of the glottis length and width and laryngeal sulcus (LS) length (mm) in Japanese quail from embryonic period to 60 days old.

Age	Glottis length ± SE	Glottis width ± SE	(LS) length ± SE
10-days old	0.76 ± 0.012	0.12 ± 0.007	—
13-days old	1.11 ± 0.007	0.36 ± 0.01	0.91 ± 0.007
Hatching	1.113 ± 0.009	0.7 ± 0.003	1.1 ± 0.003
7-days old	1.84 ± 0.019	0.97 ± 0.003	1.14 ± 0.003
14-days old	2.31 ± 0.006	0.996 ± 0.003	1.63 ± 0.003
30-days old	2.52 ± 0.009	1.41 ± 0.007	1.71 ± 0.01
60-days old	3.37 ± 0.009	1.61 ± 0.012	2 ± 0.12

TABLE 3 | Length of the laryngeal papillae of the rostral transverse row (RTLr) and casual transverse row (CTLr) and sagittal row in Japanese quail.

Age	(RTLr) length ± SE	(CTLr) length ± SE
13-days old	2.69 ± 0.003	2.5 ± 0.003
Hatching day	2.77 ± 0.003	2.58 ± 0.01
7-days old	4.26 ± 0.007	3.59 ± 0.006
14-days old	4.99 ± 0.006	3.65 ± 0.009
30-days old	5.4 ± 0.003	4.81 ± 0.01
60-days old	6.34 ± 0.018	5.22 ± 0.002

TABLE 4 | Number of the laryngeal papillae of the rostral transverse row (RTLr) and casual transverse row (CTLr) and sagittal row in Japanese quail.

Age	Number of papillae (RTLr)	Number of papillae (CTLr)	Number of sagittal papillae
13-days old	18	13	8
Hatching	18	13	8
7-days old	20	14	8
14-days old	20	14	8
30-days old	20	14	8
60-days old	21	14	9

tracheolateralis muscles appeared, representing infrahyoid (external laryngeal muscles) (Figure 6a,b). Also, cricoid, and arytenoid cartilages primordia could be noticed obviously as a condensation of mesenchymal cells, and *cricohyoideus* muscle primordia could be noticed at the 8-days old embryo (Figure 6c–e).

The *procricoid* cartilage primordium was found lying at the caudal portion of the larynx articulating with the wings of the cricoid cartilage on the 11th day of incubation. Notably, the *procricoid* consisted of a body and tail (Figures 6f, 7a).

By the 13th day of incubation, laryngeal cavity had a heart-shaped with its base lay caudally. The procricoid cartilage protruded rostrally into the laryngeal cavity. Consequently, we checked the laryngeal mucosa to find intraepithelial mucous glands were within caudal part of the laryngeal mucosa (Figure 7b). Additionally, at the same age, the laryngeal papillae were supported with connective tissue cores that arose from lamina propria. However, the epithelial cap consists of two types of cells; inner rounded cells with vesicular nuclei and outer stellate cells. Interestingly, we observed the desquamation phenomenon of the pharyngeal floor (Figures 7c–f, 8).

Of note, the rate of the developmental changes of LM was slowed down to be restricted to the intraepithelial mucous glands. The glands were shown a moderate positive reaction result to AB stain (blue color) and combined AB/PAS stains (purple color) and a strong positive reaction to PAS stain (magenta color) at 7 days old chick. However, we could see obviously the median giant papillae were supported by the caudal process of the arytenoid cartilage and cricoid cartilage encircle the laryngeal cavity ventrally. Also, muscle *cricohyoideus* could be seen inserted within the cricoid cartilage (Figure 9).

Surprisingly, we noticed few epithelial changes at 14 days old chick. Whereas the laryngeal papillae were shown a para-keratinization process nucleated *stratum cornium* (Figure 10a–d). Furthermore, our SEM and histological data found minuscule papillae, which were present on the surface of the giant papillae only that had the same histological structure as the parental papilla (Figure 10e).

At 30 days old quail, the surface of the laryngeal papillae was shown complete keratinization (Figure 10f). The surfaces of the laryngeal mound as well as the laryngeal inlet were covered by various epithelial types depending on the location. The rostral part of the laryngeal cavity was lined by a stratified squamous layer wholly except the floor was lined by respiratory epithelium with intraepithelial mucous glands.

TABLE 5 | Showing the histochemical properties of the laryngeal salivary gland (Gl) in Japanese quail chicks.

	Laryngeal salivary gland				
	Hatching	7-days	14-days	30-days	60-days
AB	+/−	++	++	+++	++++
PAS (magenta)	++	+++	++++	−	−
AB/PAS purple	++	+++	++++	++++	++++ Alcinophilic (greenish-purple)

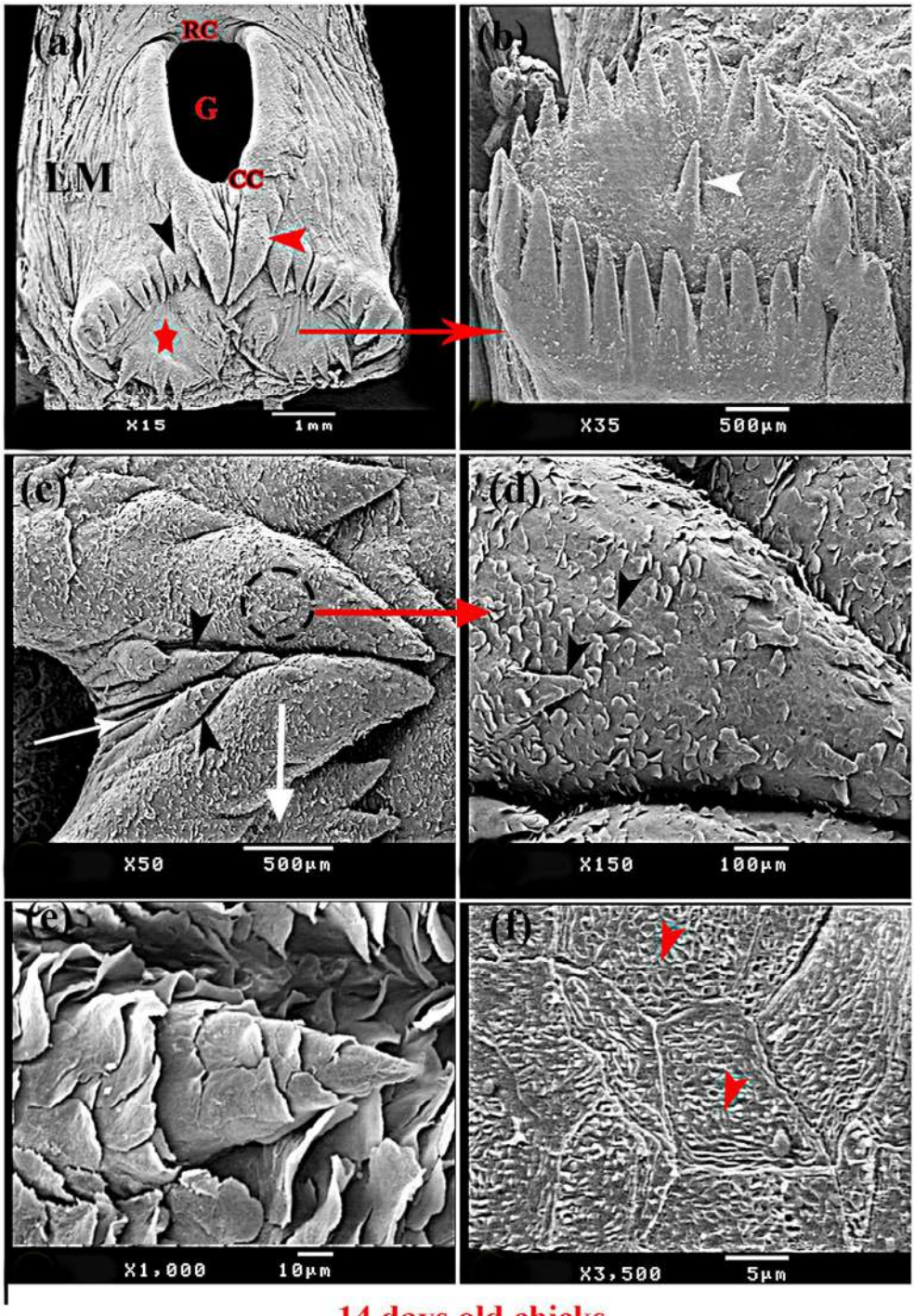

FIGURE 4 | Scanning electron micrographs of the oropharyngeal floor of a 14-day-old chick. (a, b) The rostral wide commissure (RC) and caudal narrow commissure (CC) of the glottis (G), tri-forked papillae with single stem (black arrowhead), and the giant papillae (red arrowhead). Notice, the transverse laryngeal papillary rows were arranged as two adjacent crowns (red star) with a central papilla within (white arrowhead), (X 15 and X 35). (c) The laryngeal sulcus (white arrow) is guarded by small papillae (black arrowheads), (X 50). (d) Giant papillae with minuscule papillae (black arrowheads) covering the papillary surface, (X 150). (e) Higher magnification of the minuscule papillae in Figure (d), (X 1000). (f) Microplicae (red arrowheads) on laryngeal mound surface scales. (X 3500).

While the caudal part of the laryngeal cavity is lined wholly by respiratory epithelium with intraepithelial mucous glands (Figure 11a,b).

From previous data, we focus more on the epithelial character changes. To find two lateral recesses rostro-ventrally within the laryngeal cavity (Figure 11c). Also, lymphatic aggregation was embedded between the stratified squamous epithelium and the respiratory epithelium lining (Figure 11d). The intraepithelial mucous glands were compound tubuloalveolar glands, showing a strong positive reaction to AB stain and moderate reaction to both PAS and combined AB/PAS stains (Figure 11e,f) and (Figure 12a,b).

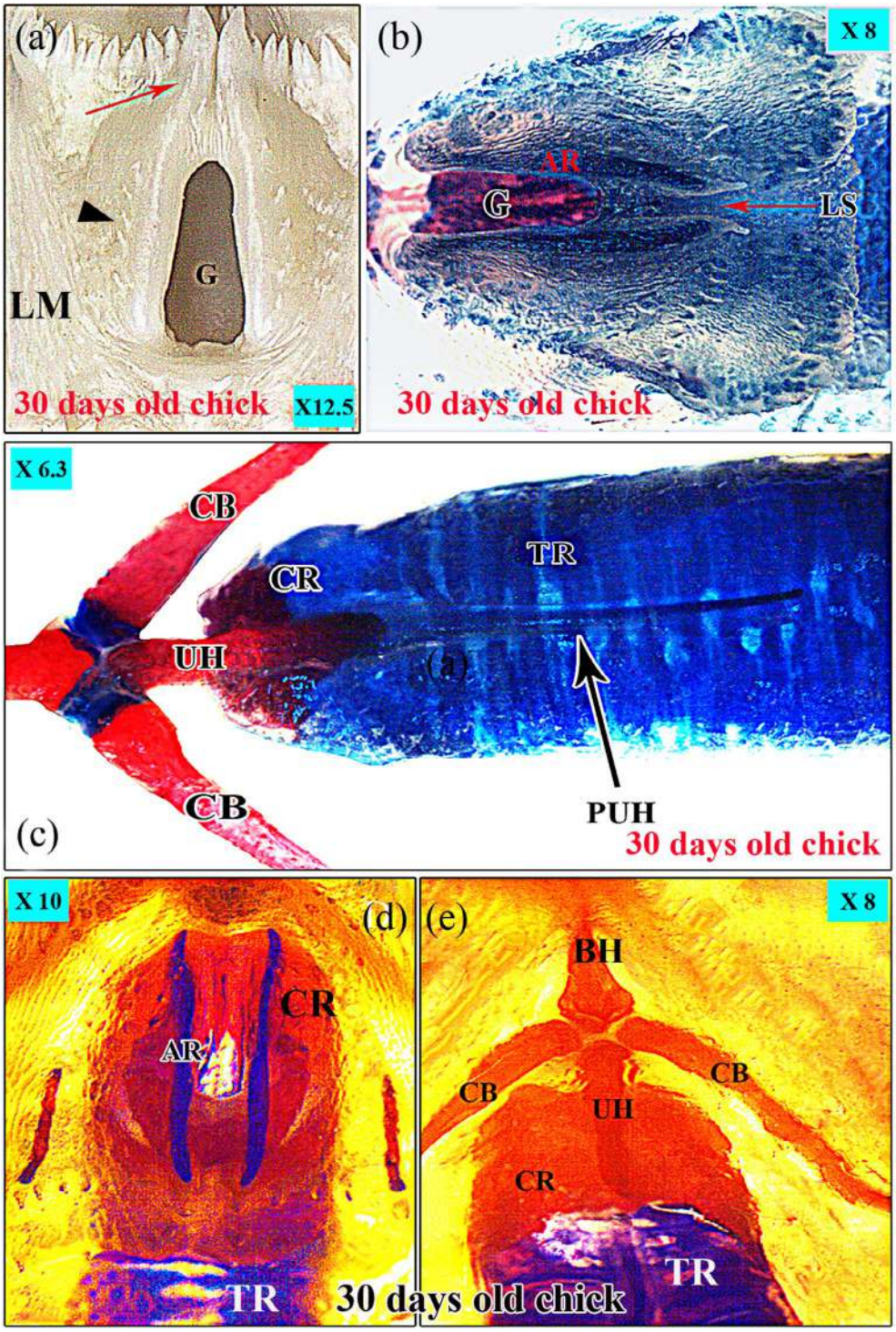

FIGURE 5 | Photographs of dorsal views of the pharyngeal floor of 30-day-old chick. (a) The glottis (G) became U-shaped with the widest rostral end. Notice, the giant papilla (red arrow), the bilateral papillae row (black arrowhead), laryngeal mound (LM), (X 12.5). (b, c) A 30-day old chick showing ventral view of laryngeal skeleton with partial ossification of the cricoid cartilage rostrally (CR). Notice: (b) arytenoid cartilages (AR) support two giant papillae; Laryngeal sulcus (LS), glottis (G). Notice: (c) urohyale (UH), process urohyale (PUH), and trachea (TR), (Alizarin red and alcian blue stains), (X 8 and X 10). (d, e) Dorsal and ventral views of 60-day old chick showing complete ossification of cricoid cartilage (CR). Cartilaginous arytenoid (AR), basihyale (BH), ceratobranchiale (CB), urohyale (UH), Basihyale (BH) and trachea (TR). (Alizarin red and alcian blue stains), (X 10 and X 8).

Notably, the muscle *cricohyoideus* was paired muscle, which originates from the dorsal surface of the *basihyale* and inserts into the ventrolateral surface of the cricoid cartilage (Figure 12c). The myenteric plexus could be seen embedded between laryngeal muscle fibers (Figure 12d) and (Figure 13a). At this age, the cricoid and the procricoid were partially ossified, while the arytenoid was still cartilaginous in agreement with the gross data (Figure 13b).

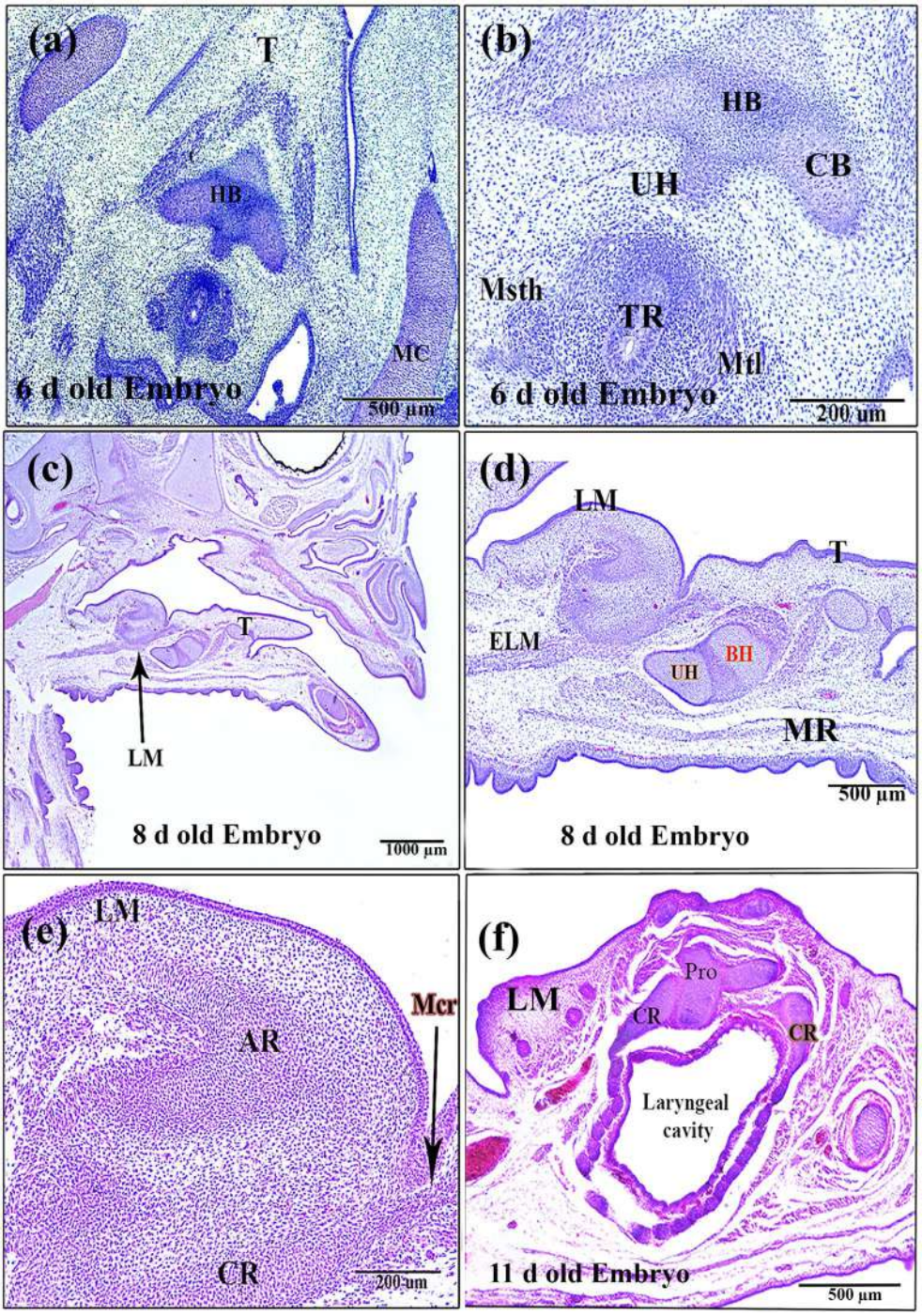

FIGURE 6 | Photomicrographs of the oropharyngeal floor. (a, b) Frontal sections in the oropharyngeal floor primordium of 6 days old embryo showing the appearance of external laryngeal muscles sternohyoideus (Msth) and tracheolateralis (Mtl). Trachea (TR), Meckel's cartilage (MC), Ramus mandibularis primordium (RM), Tongue primordium (T) and hyobranchial apparatus (HB), (H and E, scale bars 500 and 200). (c, d) Sagittal sections of embryo head and the oropharyngeal floor of 8 days old embryo showing the laryngeal mound primordium (LM), muscle cricohyoideus (Mcr), cricoid (CR), arytenoid (AR) cartilages, external laryngeal muscles (ELM), basihyale (BH), urohyale (UH), and tongue (T), (H and E, scale bars 1000 and 500 µm). (e) High magnification to Figure (d) (H and E, scale bars 200 µm). (f) Cross section in the laryngeal mound of 11 days old embryo showing the caudal of the procricoid cartilage (Pro, comma shape) articulates with cricoid cartilage (CR), laryngeal mound primordium (LM), (H and E, scale bars 500 µm).

Interestingly, at 60 days old quail, the cricoid cartilage became completely ossified and took a "C-shaped" appearance with high lateral walls caudally representing its wings. The wings decreased gradually in height rostrally. While the arytenoids appeared as forked left and right hyaline cartilages that were composed of body, rostral and caudal processes. The body articulated with the procricoid head caudally; the rostral process guarded the glottis, and the caudal process supported the giant papilla. All parts were ossified except the caudal process (Figure 13c,d).

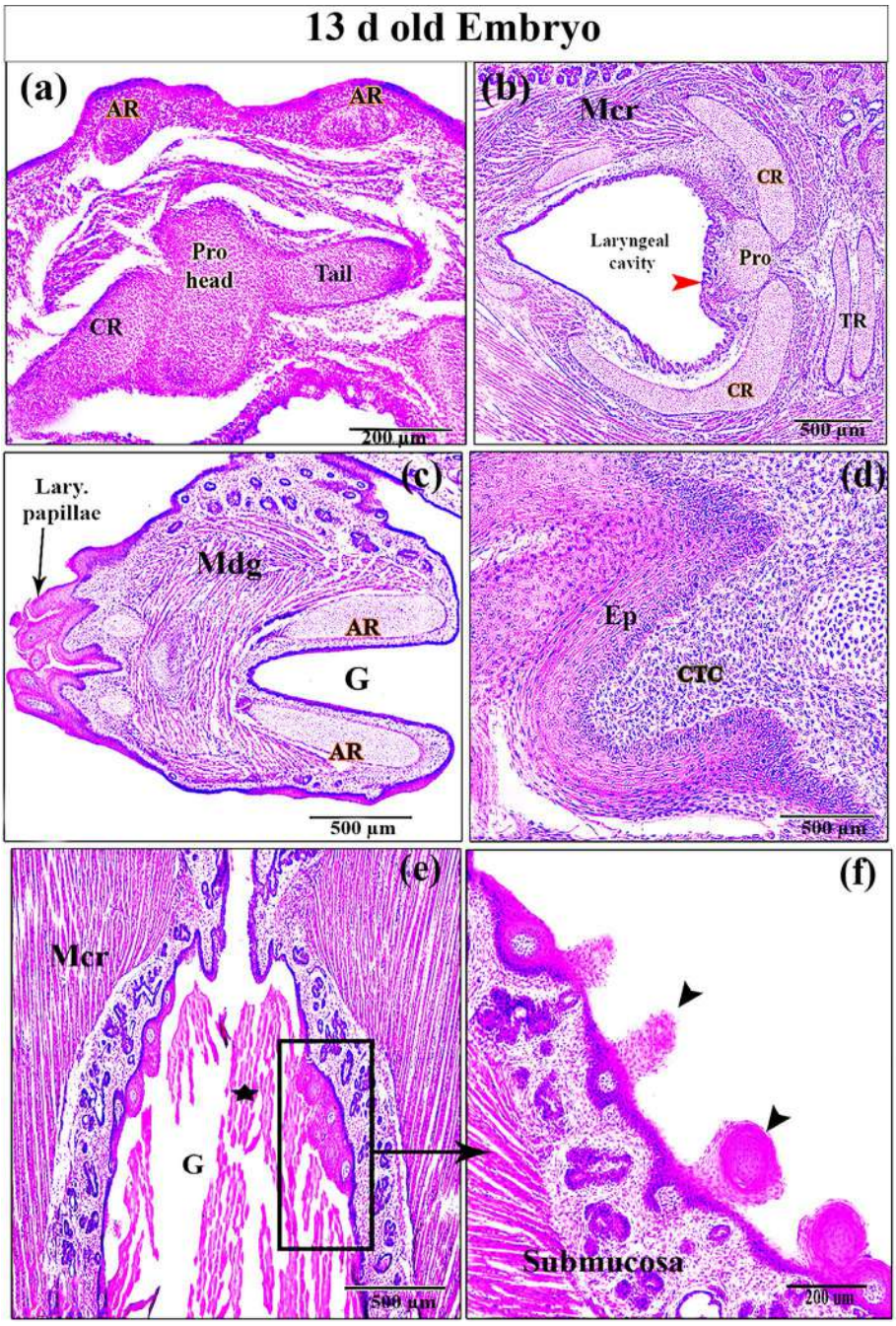

FIGURE 7 | Photomicrographs of the pharyngeal floor of 13-day old embryo. (a) Higher magnification to Figure 5f, showing procricoid cartilage (pro) parts consists of head and tail articulating with cricoid cartilage (CR). Arytenoid (AR), (H and E, scale bar 200 µm). (b) Heart shaped laryngeal cavity with presence of intraepithelial mucous gland at the caudal wide part (red arrowhead). Procricoid head (Pro), cricoid cartilage (CR) and trachea (TR), Muscle cricohyoideus (Mcr), (H and E, scale bar 500 µm). (c, d) Frontal sections showing the appearance of the transverse laryngeal papillae (Lary.papillae) consisting of epithelial cap (Ep) and connective tissue core (CTC). Arytenoid (AR), Muscle dilator glottidis (Mdg), (H and E, scale bars 500 and 100). (e, f) Frontal sections showing the desquamation phenomenon (black star) and appearance of sagittal rows papillae (rectangular area) was guarding the glottis (G). Papillae (black arrow heads), Muscle cricohyoideus (Mcr), (H and E, scale bars 500 and 200 µm).

The *procricoid* lay at the caudal portion of the larynx that had 4 articular surfaces on its head; two caudolateral surfaces for articulation with the paired cricoid wing and two rostrolateral surfaces for articulation with paired arytenoid bodies (Figure 13c,d). The intraepithelial mucous glands were shown strong positive alcianophilic activity to AB stain and combined AB/PAS stains (not shown). However, the glands were shown negative results to PAS stain (Figure 13e,f).

The laryngeal salivary glands were compound tubuloalveolar paired gland situated in the submucosa of the laryngeal mound, which were extending longitudinally between *dilator glottidis* muscle medially and *cricohyoideus* muscle laterally till beyond the level of the laryngeal papillae, which were represent the caudal continuation of the middle preglottal salivary glands. Secretomotor plexus was situated adjacent the glandular lobules (Figure 14a–c). The secretory lobules drained their secretion

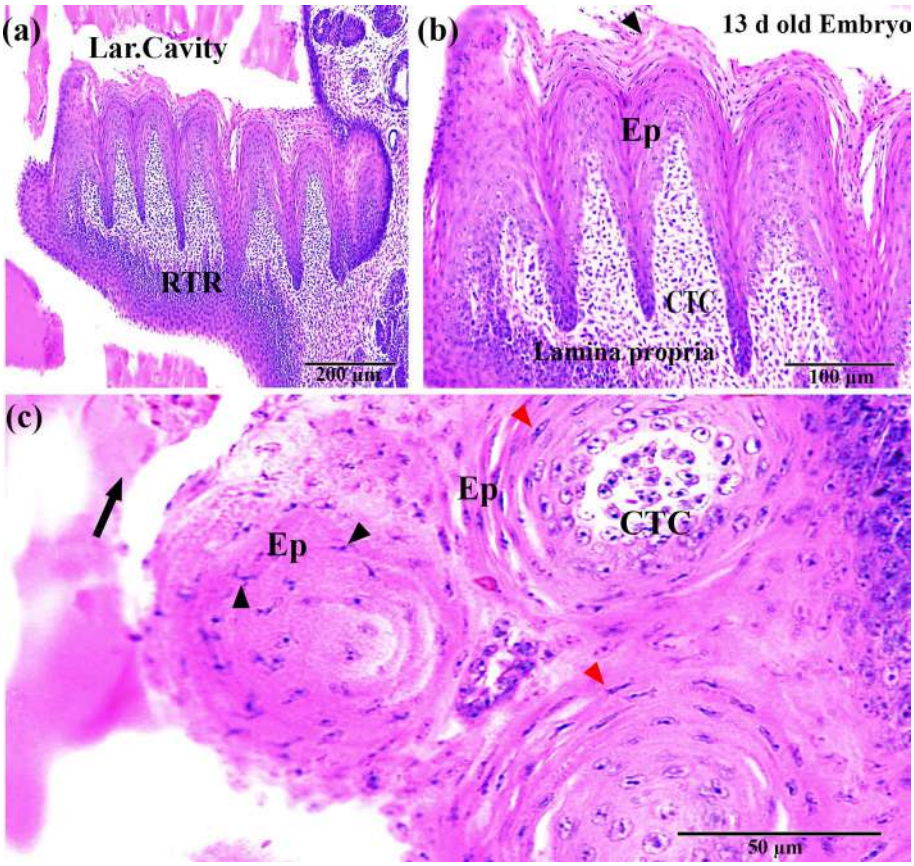

FIGURE 8 | Photomicrographs of frontal sections in laryngeal transverse papillary row of 13-day old embryo. (a–c) Show the rostral laryngeal transverse papillary row (RTR) consists of number of papillae that have connective tissue core (CTC) arose from lamina propria and covered with epithelial cap (Ep) with flatten cells (red arrowheads). Notice, the mesenchymal like desquamated cells (stellate shaped) (black arrow). Also, the basal layers of epithelium were rounded cells. (H and E, scale bars 200, 100 and 50 μm).

within intralobular ducts then to interlobar ducts leading to common secretory duct that opened dorsally to the laryngeal mound (Figure 14d,e).

3.3 | Histochemical Studies of the Laryngeal Salivary Glands

The laryngeal salivary glands were shown at the hatching day old quail chick, a weak positive reaction to alcian blue stain, which involved the secretion within the lumen (Figure 15a), while positive reaction to combined AB/PAS stains (dark purple coloration) (Figure 15b), and moderate positive reaction to PAS stains (Figure 15c). While at 7 days old chick, the glands were shown a moderate positive reaction to alcian blue. However, strong positive reaction to PAS stain and moderate reaction to combined AB/PAS stains (Figure 15d–f). Of note, our results at 30 days old of laryngeal glands were like preglottal salivary glands results (Khalifa et al. 2022) (Data not shown).

Hence, we found the glands progressed to react strongly to AB stain with high alcianophilic basal portion of cells at 60 days old chicks. Also, strong alcinophilic reactivity result could be shown only to combined AB/PAS stains (purple coloration). Interestingly, negative results to PAS stain at both 30 days (Data not shown) and 60 days old were found (Figure 16).

4 | Discussion

The laryngeal mound was a firm circumscribed prominence projects from the surrounding pharyngeal floor. It occupies the caudal part of the pharyngeal floor where it is sharply defined rostrally from the root of the tongue by a row of caudally directed papillary crest and separated caudally from the pharyngeo-esophageal junction by a group of caudally directed laryngeal papillae. Morphologically, LM occupies the pharyngeal floor by 67.87% and the oral cavity by 20.55% of the total length (Madkour and Abdelsabour-Khalaf 2022). However, we found that the LM to pharyngeal floor ratio was around 60%.

The current study revealed that the width of the middle and caudal parts was nearly equal at the 8th day of incubation, while the rostral part was the smallest till hatching. Later, the middle part widens, resulting in a heart-shaped laryngeal mound. These findings were consistent with those of McLelland (1990) and Kabak, Orhan, and Haziroglu (2007) in the chicken and long-legged buzzard, but contradict Lbe et al. (2008), who defined it was as nearly triangular in the West African guinea fowl. In the same concept, Bassuoni et al. (2022) investigated the tongue of the Eurasian common moorhen and reported a heart-shaped glottal enterance beside two dorsal and ventral giant papillae in the papillary crest.

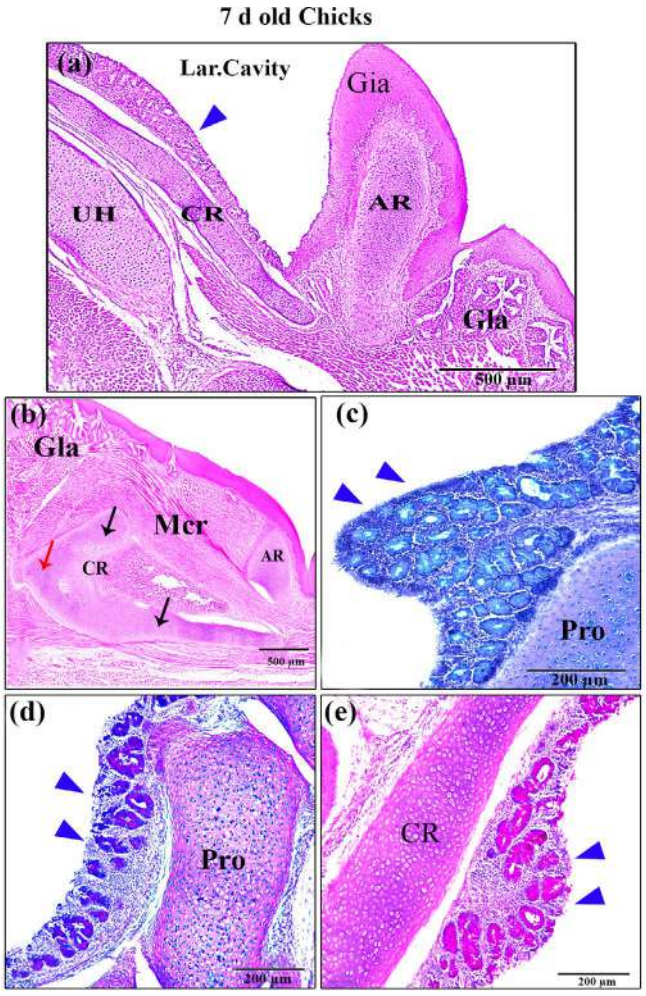

FIGURE 9 | Photomicrographs of the oropharyngeal floor of 7-day old chick. (a) Sagittal section showing the giant laryngeal papilla (Gia) was supported by caudal process of arytenoid cartilage (AR). Cricoid cartilage (CR) and urohyale (UH), Laryngeal cavity (Lar.Cavity) lined with respiratory epithelium (blue arrowhead), gland laryngealis (Gla), (H and E, scale bar 500 µm). (b) Sagittal section showing cricoid cartilage (CR) consists of caudal plate (red arrow) and rostral two wings (black arrows). Arytenoid cartilage (AR) and muscle cricohyoideus (Mcr), gland laryngealis (Gla), (H and E, scale bar 500 µm). (c–e) Frontal sections showing intraepithelial mucus glands (blue arrowheads) with moderate reaction with both AB stain (greenish blue color) and AB/PAS stains (purple color) but strong reaction with PAS stain (magenta color). Notice, Procricoid head (Pro), Cricoid (CR). (C–D), (AB and AB/PAS &PAS) stains, scale bars, 200 µm).

The glottis appeared at the 10th day of incubation with a high growth rate. However, post-hatching, the glottis took the following shapes: slit, triangular, pear-like and long finger like on the 13th day of incubation, zero-, 7-, and 14-days old respectively. The lining epithelium began as stratified squamous as an extension to the pharyngeal wall mucosa, then transitions to respiratory with intraepithelial mucous glands. Similar findings were reported by Gewaily and Abumandour (2020) as well as Madkour and Abdelsabour-Khalaf (2022).

According to our data, the rostral commissure of the laryngeal inlet was slightly wider than the caudal one. The rim of the aditus larynges was marked by a sagittal row of 8–9 small papillae that appears at the 13th day of incubation. Crole and Soley (2010) have confirmed these findings that the glottis was wide rostrally and narrow caudally (V shaped). Similarly, Saleh (2013) has pointed out that the inlet of the turkey has rostral wide and caudal narrow commissures but, he added that the rim of the laryngeal inlet appears smooth. Demirkan, Haziroğlu, and Kürtül (2007) estimated the rostral and caudal row numbers in adult Japanese quail to be (23–26) and (21–25), respectively. This attributed the lateral divergence of the arytenoid cartilages as they were preceded rostrally. The glottis in ostrich was wide therefore the general appearance of the larynx in ostrich is different from other domestic birds as it was a wide triangular slit directed caudally (Tadjalli, Mansouri, and Poostpasand 2008).

All papillae were supported with connective tissue core from the lamina propria and were covered with an embryonic epithelial cap of two cell layers; inner layer of rounded cells with vesicular nuclei and outer layer of flat cells with flattened nuclei but the desquamated cells were stellate like referring to the desquamation phenomenon at the 13 day of incubation (Khalifa et al. 2022). Parakeratinization had occurred on the papillary surface, beginning at the rostral border of the papillae in 14-day-old chicks and completing the keratinization process in 30-day-old chicks. According to Mohamed (2010), this could be because surfaces have come into contact with food.

Using alcian blue and alizarin red stains, our results revealed that the cricoid body was partially ossified in the 30 days old chicks but most laryngeal cartilages were completely ossified except the caudal processes of the arytenoid in the 60 days old chicks. In the same respect, Hogg (1982) has reported that the laryngeal cartilages of chickens were mineralized consistently in the body and wings of the cricoid cartilage, the procricoid cartilage, and the bodies of the arytenoid cartilages in the 105 days old chicks.

In the current investigation, the external laryngeal muscles (*M. tracheolateralis* and *M. sternohyoideus*) appeared at the 6th day of incubation. They originated from the trachea and the sternum respectively and insert to the dorsal surface of the basihyale lateral to the *cricohyoideus* origin. While, both the cricoid cartilage and the *cricohyoideus* muscle that originated from it emerged at the 8th day of incubation. We suggest that the reason for the appearance of both *tracheolateralis* and *sternohyoideus* muscles before the *cricohyoideus* muscle was that the trachea is earlier in development than the larynx. Myentric plexus could be observed between the muscle fibers similar to that mentioned by Homberger and Meyers (1989a) and Demirkan, Haziroğlu, and Kürtül (2007).

In contrast to birds, mammals' laryngeal skeleton consists of a pair of the arytenoid, cricoid, thyroid cartilage, and epiglottis (Lungova and Thibeault 2020). There were no vocal cords was liked those found in mammals. However, the organ of vocalization was named differently, becoming well known as the syrinx. The syrinx was made up of either tracheal or bronchial cartilaginous rings (Demirkan, Haziroğlu, and Kürtül 2007). However, our investigation discovered that the larynx was made up of three bones; the cricoid, the small dorsal procricoid, and the paired arytenoids. The cricoid possessed a pair

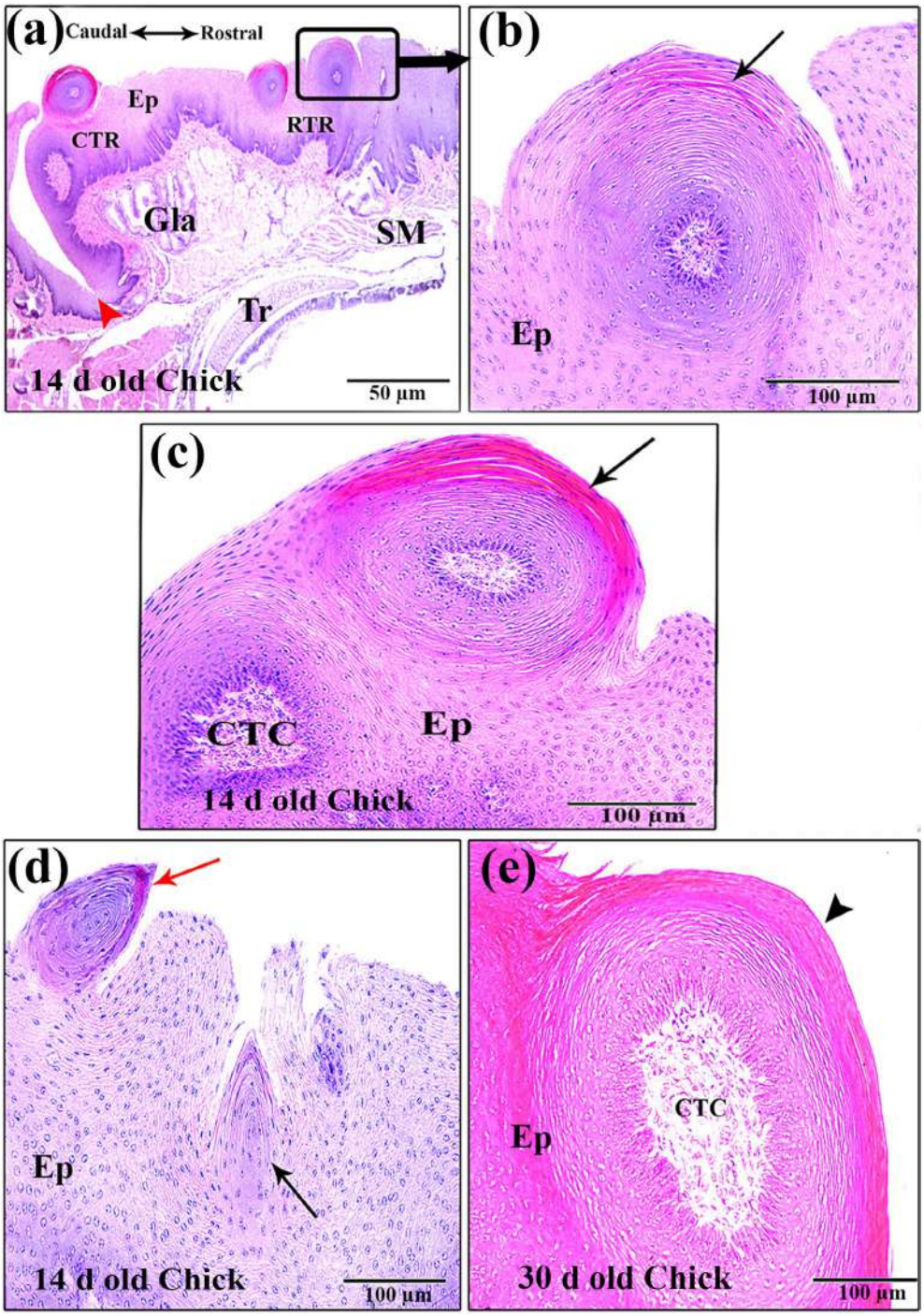

FIGURE 10 | Photomicrographs of the oropharyngeal floor showing developmental histological changes of epithelial cap of laryngeal papillae. (a–c) Sagittal sections in the 14-day old chick showing para-keratinization (black arrows in b, c) of the laryngeal papillae. Rostral (RTR) and caudal (CTR) transverse laryngeal papillary rows and pharyngeo-esophageal junction (red arrowhead), Tracheal cartilage ring (Tr), Gland laryngealis (Gla), Epithelium (Ep), Laryngeal submucosa (SM). (H and E, scale bars 500 and 100 and 50 µm). (d) Sagittal section in the giant laryngeal papilla of 14 days old chick showing minuscule papillae; old one (red arrow) and newly formed one (black arrow) and both have the same parental papilla histological structure (Ep). (H and E, scale bar 100 µm). (e) Sagittal section in the laryngeal papillae of 30 days old chick showing highly keratinized papillary surface (black arrowhead). Connective tissue core (CTC), Epithelium (Ep). (H and E, scale bar 100 µm).

of dorsal wings, while each arytenoid had a long caudodorsal process. The procricoid cartilage was an unpaired cartilage, comma-shaped and was directed dorsomedially. It lay at the caudal portion of the larynx and consisted of body and tail. It had 4 articular surfaces; two articular surfaces at its caudolateral corners for the paired dorsal cricoid wings and the other pair surfaces at its rostro-lateral corners for the paired arytenoids.

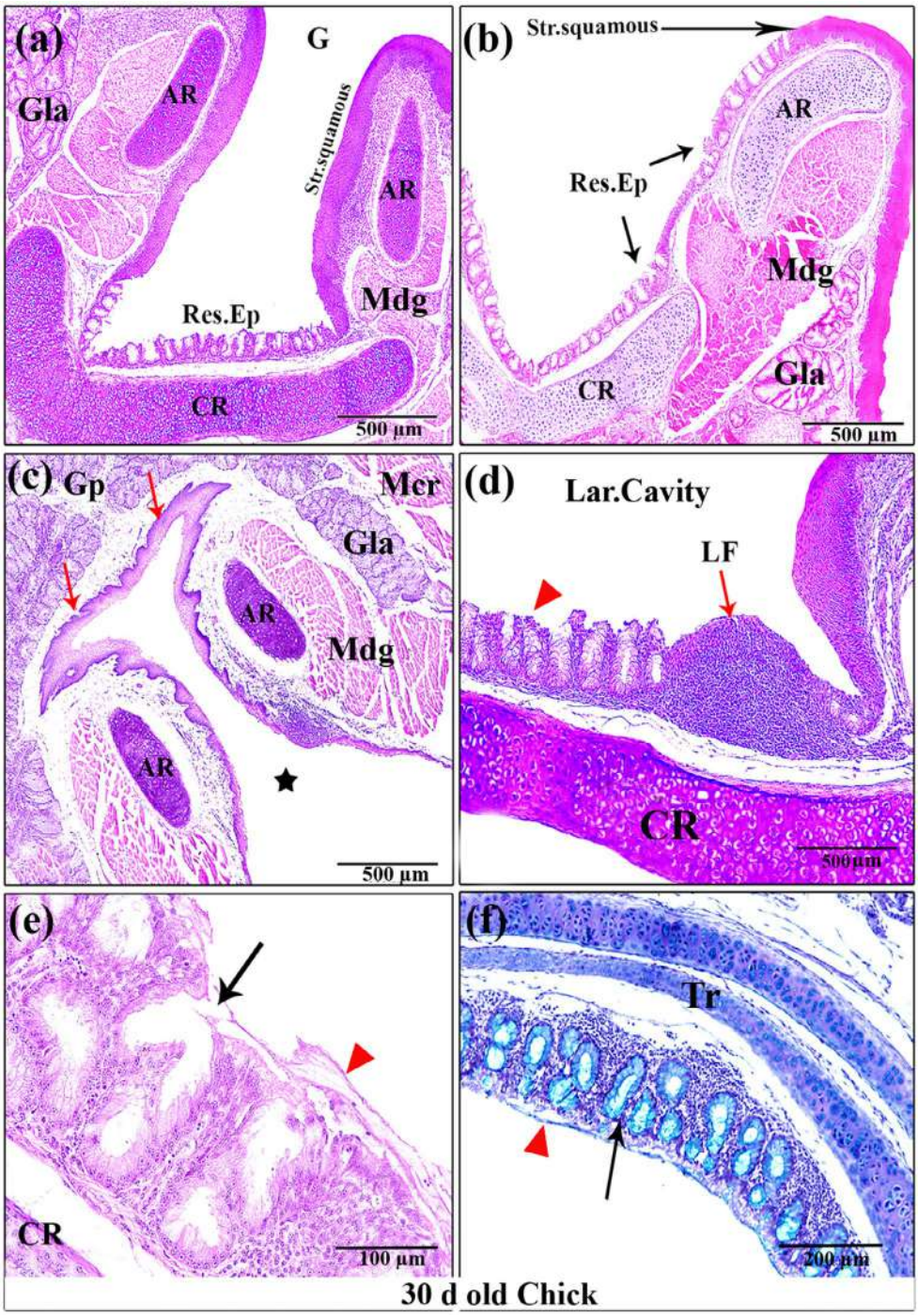

FIGURE 11 | Photomicrographs of the oropharyngeal floor showing laryngeal cavity mucosa. (a) Cross section in the rostroventral part of the laryngeal mound of 30 days old chick showing its lining epithelium. Stratified squamous epithelium (Str. Squamous) upper part and respiratory epithelium (Res.Ep) ventral part of the laryngeal cavity lining epithelium. Cricoid cartilage (CR), arytenoid (AR) and glottis (G). (H and E, scale bar 500). (b) Cross section in the caudal part of the laryngeal mound of 30 days old showing the lining epithelium. Stratified squamous epithelium (Str. Squamous) and respiratory epithelium (Res.Ep). Cricoid cartilage (CR) and arytenoid (AR), Muscle dilator glottidis (Mdg), Gland laryngealis (Gla), (H and E, scale bar 500 µm). (c) Frontal section in the ventral part of the laryngeal mound of 30 days old chick showing the laryngeal cavity (black star) with two lateral recesses (red arrows) rostrally. Arytenoid (AR) and glottis (G), Muscle dilator glottidis (Mdg), Gland laryngealis (Gla), Muscle cricohyoideus (Mcr), Gland preglottalis (Gp), (H and E, scale bar 500 µm). (d) Cross section of 30 days old chick showing lymphatic follicle (LF) within the respiratory epithelium. Cricoid cartilage (CR), intraepithelial mucous glands (Red arrowhead), (H and E, scale bar 500 µm). (e) Cross section in the respiratory epithelium of 30 days old chick showing compound intraepithelial mucous glands (black arrow), Mucous thin layer (red arrowhead), Cricoid (CR). (H and E, scale bar 100). (f) Cross section in the laryngeal mound of 30 days old chick showing strong alicinophilic reaction of intraepithelial mucous glands (black arrow), Mucous thin layer (red arrowhead), Trachea (Tr). (AB stain, scale bar 200 µm).

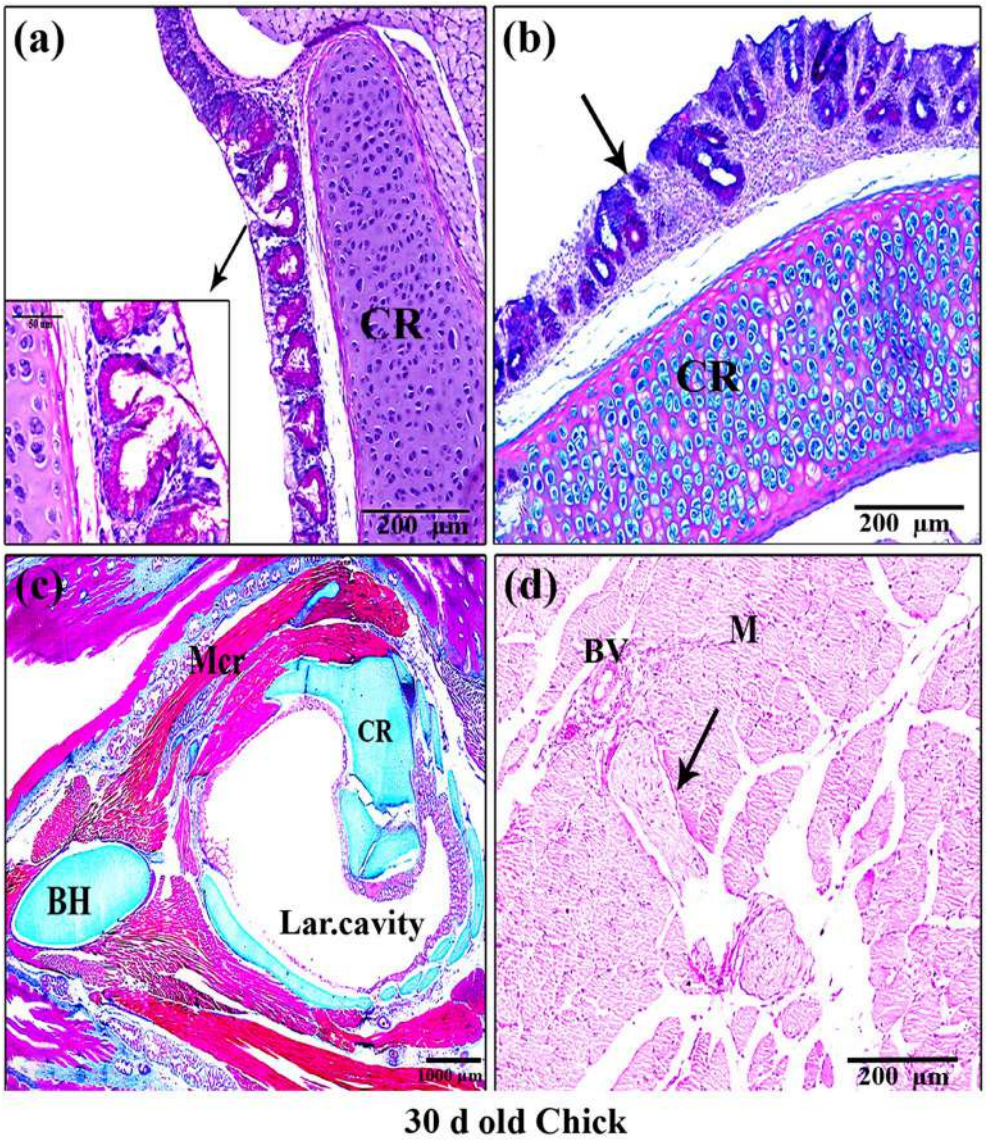

FIGURE 12 | Photomicrographs of the laryngeal mound showing laryngeal cavity mucosa of the 30 days old chick. (a) Cross section in the laryngeal mound showing the moderate reaction to PAS stain of intraepithelial mucous glands. Notice, compound tubuleoalveolar glands (inset), cricoid cartilage (CR), (PAS stain, scale bar 200 µm). (b) Cross section showing the moderate reaction to combined AB/PAS stains of intraepithelial mucous glands (black arrow), cricoid cartilage (CR), (AB/PAS stains, scale bar 200). (c) Frontal section showing muscle cricohyoideus (Mcr) originaed from basihyale (BH) dorsally and inserts into cricoid cartilage (CR) laterally, (Crossmon's trichrome stain, scale bar 1000). (d) Cross section showing myentric plexus (black arrow) is embedded between muscle fibers (M), blood vessels (BV), (H and E, scale bars 200 and 100).

According to our investigation, the cricoid cartilage appeared at 8 days pre-hatching and supported the larynx ventrolaterally. The ossification process started at 30 days and is completed at 60-days post-hatching. The procricoid ossified at 60-days post-hatching. The arytenoid had a caudal process that supports the two big laryngeal papillae and the glottis. These results were in agreement with Bock (1978), King and Mclelland (1984), Mclelland (1990), Demirkan, Haziroğlu, and Kürtül (2007) as well as Crole and Soley (2012). Worth mentioning, Fitzgerald (1970) found in quails that only cricoid and arytenoid cartilages were present. Notably, cricoid cartilage was a C-shaped in our study, but Demirkan, Haziroğlu, and Kürtül (2007) found that it was triangular. Moreover, it was scoop-like in ducks (McLelland 1990). Of note, our data revealed that the ventral surface of the cricoid articulates with the urohyale body, which was ossified rostrally, but its process was still being cartilaginous caudally.

Our results indicated that the laryngeal cavity was heart-shaped with its apex rostrally at 13 days pre-hatching age due to procricoid body protrusion within the cavity but at 60 days old the laryngeal cavity made two recesses rostrally. The lining epithelium of the laryngeal cavity was varied according to the position; in the rostral part, the epithelium was still non-keratinized stratified squamous till its transformation into respiratory epithelium with intraepithelial mucous glands ventrally. In the caudal part, the epithelium had transformed into intraepithelial mucous glands then became respiratory

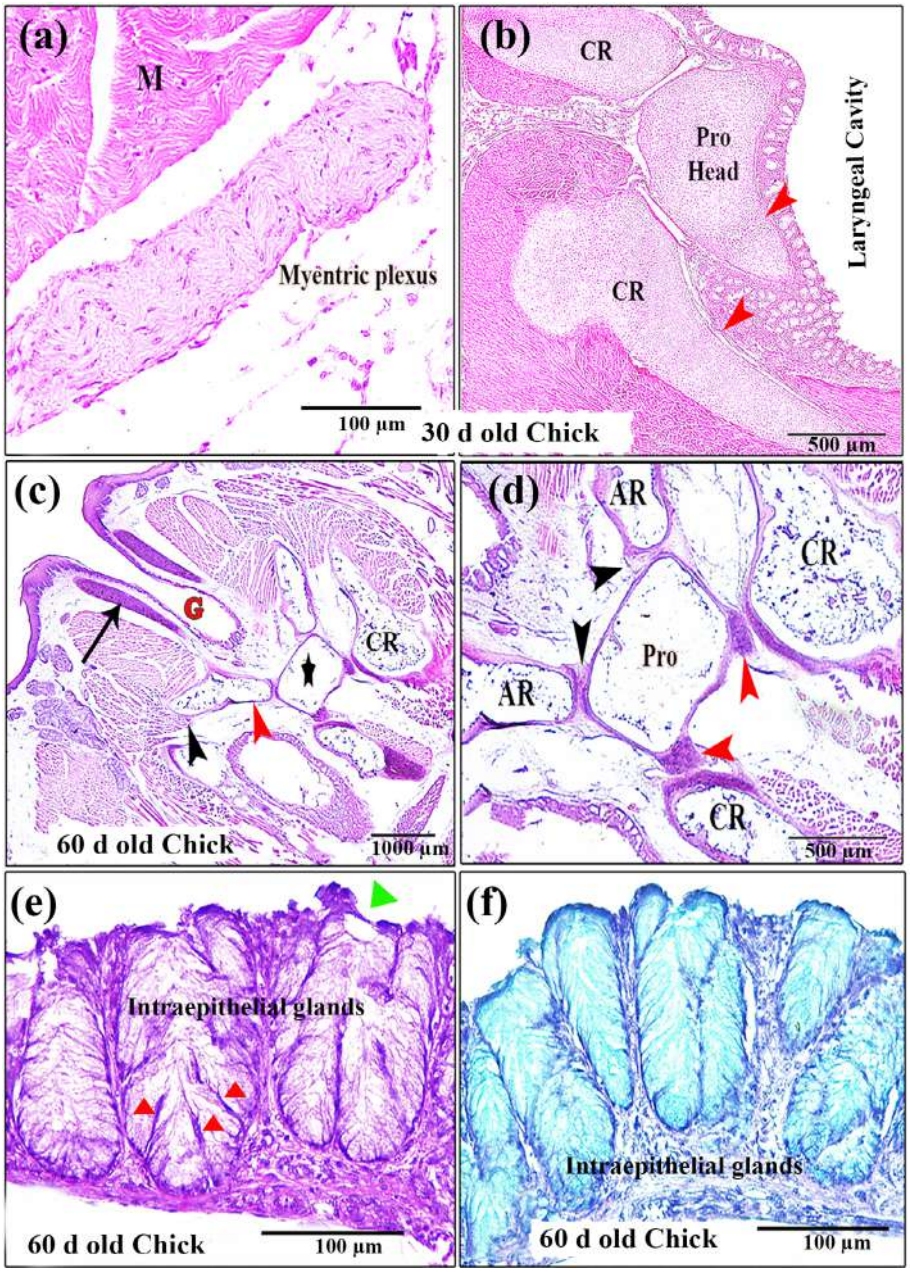

FIGURE 13 | Photomicrographs of the laryngeal mound. (a) High magnification to another myentric plexus between muscle fibers (M), (H and E, scale bars 200 and 100 µm). (b) Frontal section in the laryngeal mound of 30 days old chick showing cricoid cartilages (CR) articulate with procricoid head (Pro Head) caudally. Notice, their partial ossification (red arrow heads), Laryngeal cavity (Lar.Cavity). (H and E, scale bar 500 µm). (c) Frontal section in the 60 days old chick showing complete ossification of cricoid cartilage (CR), arytenoid rostral process (black arrowhead), arytenoid body (red arrowhead) and procricoid head (black star). Notice, caudal process of arytenoid (black arrow) was still cartilaginous. Glottis (G), (H and E, scale bar 1000 µm). (d) High magnification to (c) showing 4 articular surfaces on procricoid head (Pro); two caudolateral (red arrow heads) and two rostrolateral (black arrow heads). Cricoid cartilage (CR) and arytenoid (AR) and glottis (G). (H and E, scale bar 500 µm). (e) Cross section in the laryngeal mound of 60 days old showing negative result to PAS stain of intraepithelial mucous glands (compound tubuloalveolar, red arrowheads). Note, gland opening (green arrowhead), (PAS stain, scale bar 100). (f) Cross sections in the laryngeal mound of 60 days old chick showing strong alicinophilic reaction of intraepithelial mucous glands to both AB and combined AB/PAS stains. (AB and AB/PAS stains, scale bars 100 µm).

epithelium with intraepithelial mucous glands in agreement to King and Mclelland (1984), Mohamed (2010) and Mohamed, Abdelsabour-Khalaf, and Abdelhakeem (2018) who have assumed that the lining epithelium is transformed from stratified squamous to respiratory epithelium with intraepithelial mucous glands. However, Demirkan, Haziroğlu, and Kürtül (2007) added that olfactory epithelium might be present.

Intraepithelial mucous glands were compound tubuloalveolar glands that embedded within the respiratory epithelium with tall columnar mucous cells and basal round nuclei. It was shown different AB/PAS stains reactivity from AB/PAS stains positive results to AB positive result indicated increasing in the alcianophilic reactivity. These findings were in accordance with Demirkan, Haziroğlu, and Kürtül (2007), and Madkour and Abdelsabour-Khalaf (2022).

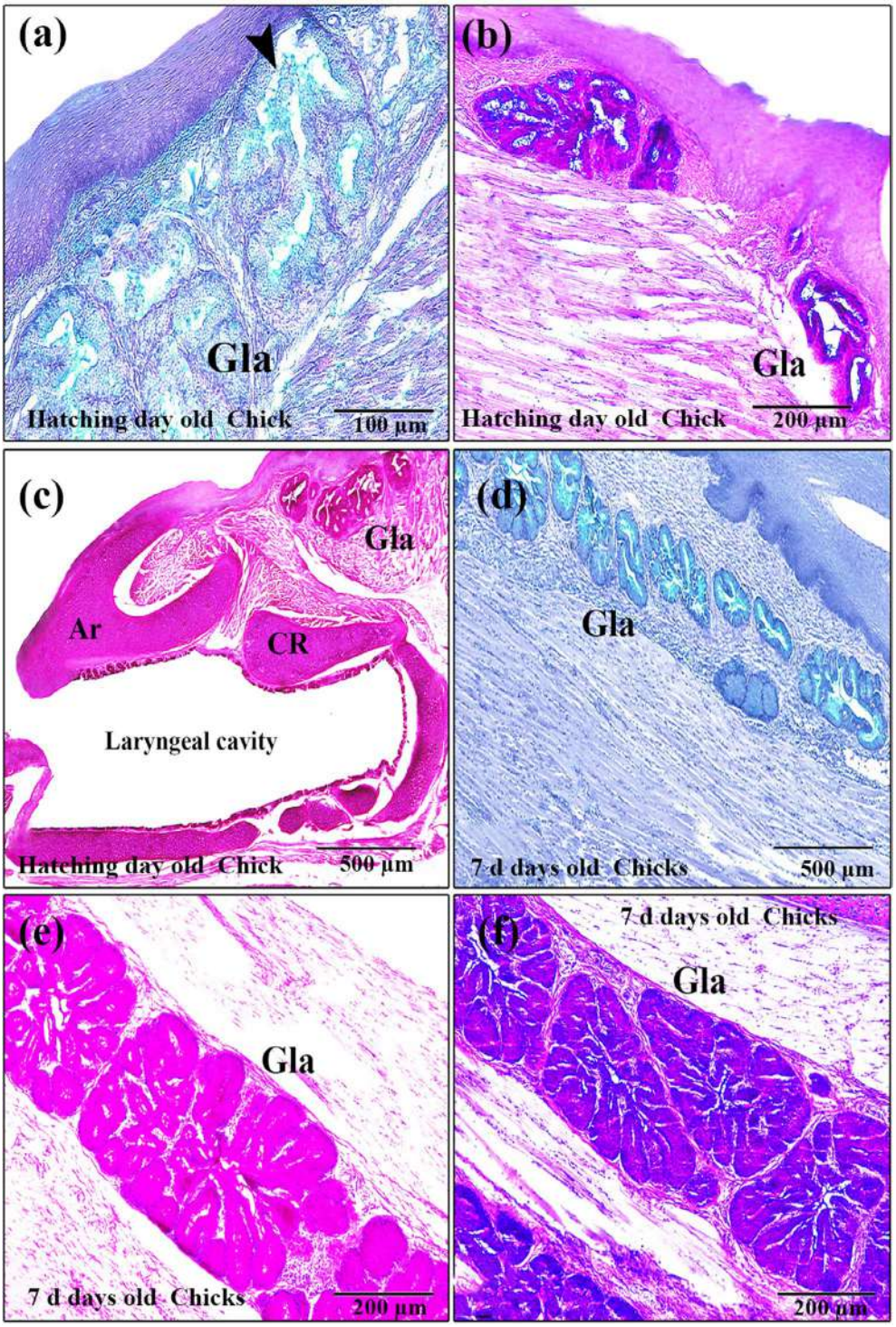

FIGURE 14 | Photomicrographs of the laryngeal mound showing laryngeal cavity submucosa. (a) Cross section in the 60 days old chick showing the gland laryngealis (Gla) lies between muscle cricohyoideus (Mcr) and muscle dilator glottidis (Mdg). Gland preglottalis lateralis (Gpl), cricoid cartilage (CR), arytenoid cartilage (Ar). (H and E, scale bar 500 µm). (b) A frontal section in the caudal part of the laryngeal mound of 60 days old chick showing the gland laryngealis (Gla) extend to the level of the laryngeal papillae (Lar. pap.). (H and E, scale bar 500). (c) High magnification to (A, green rectangle) showing the gland laryngealis (Gla) adjacent secretomotor plexus (SMP). (H and E, scale bar 50). (d, e) Frontal sections in the 60 days old chick showing the duct system of gland laryngealis and positive alcinophilic reaction. Secretory lobules (Lb), intralobular duct (red arrowhead), interlobar duct (red arrow), common secretory duct (CSD), Gland laryngealis (Gla). (AB stain, scale bars 200 and 100 µm).

The laryngeal glands opened on the dorsal surface of the LM. They were paired compound tubuloalveolar glands within the submucosa that was represent the continuation of the middle preglottal glands that lay between muscle *dilator glottidis* dorsolaterally and the muscle *cricohyoideus* medially in agreement to Mclelland (1975), Homberger and Meyers (1989b) and Liman, Bayram, and Koçak (2001). Moreover, Abumandour et al. (2021a) in rock pigeon found that the laryngeal salivary glands were

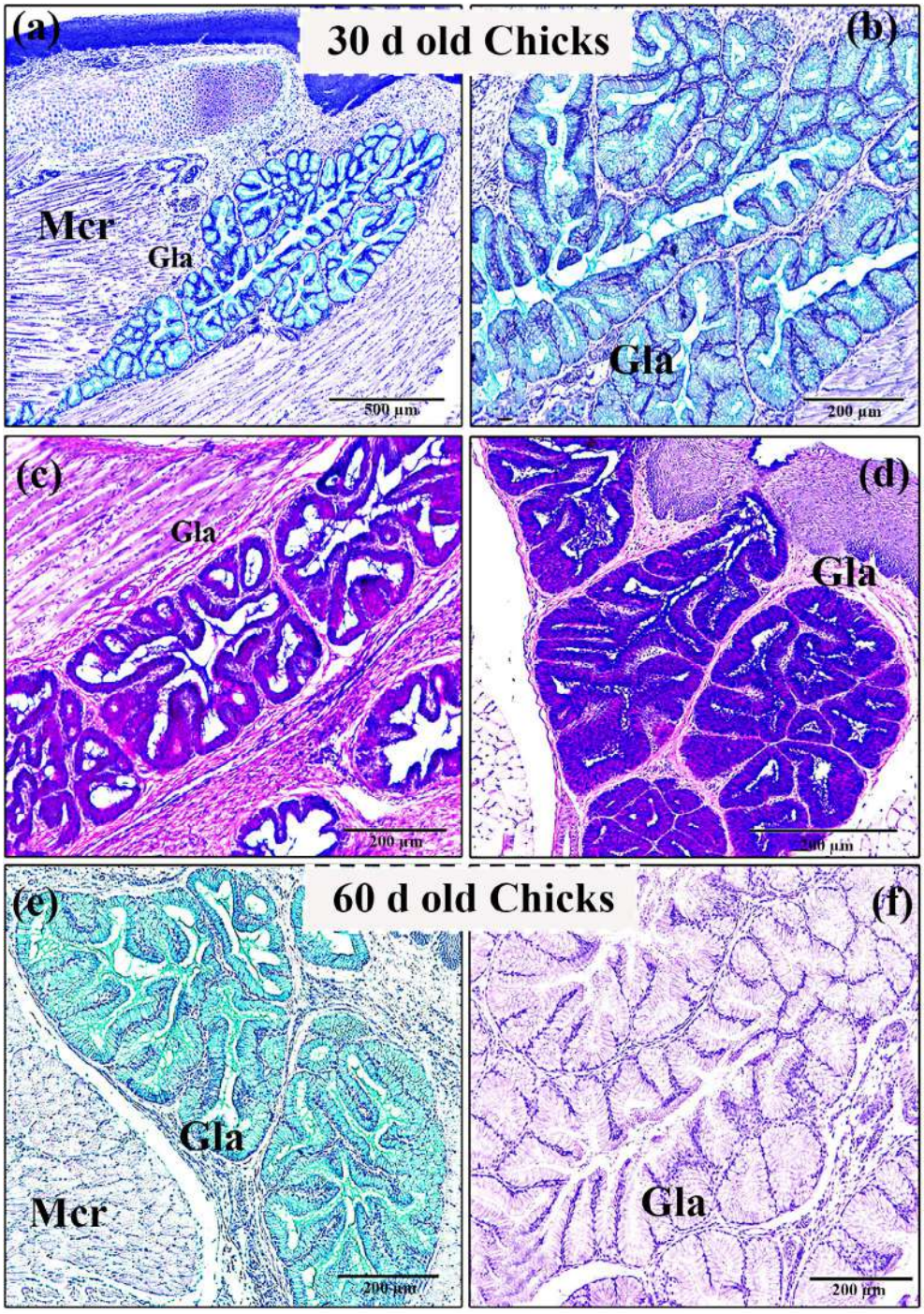

FIGURE 15 | Photomicrographs of the laryngeal mound showing laryngeal cavity submucosa. (a) Sagittal section in the hatching day old chick showing the positive reaction of the gland laryngealis (Gla) to AB stain (bluish green coloration) restricted to secreting border. (AB stain, scale bar 100). (b) Sagittal section in the hatching day-old chick showing the moderate positive reaction of the gland laryngealis (Gla) to combined AB/PAS stains (purple coloration). (AB/PAS stains, scale bar 200). (c) Sagittal section in the hatching day-old showing the moderate positive reaction of the gland laryngealis (Gla) to PAS stain (magenta coloration). Cricoid cartilage (CR), arytenoid cartilage (Ar). (PAS stain, scale bar 500). (d) Frontal section in the laryngeal mound of 7 days old chick showing the moderate positive reaction of the gland laryngealis (Gla) to AB stain (bluish green coloration). (AB stain, scale bar 500 µm). (e) Frontal section in the 7 days old chick showing the strong positive reaction of the gland laryngealis (Gla) to PAS stain (magenta coloration). (PAS stain, scale bar 200 µm). (f) Frontal section in the laryngeal mound of 7 days old chick showing the strong positive reaction of the gland laryngealis (Gla) to combined AB/PAS stains (purple coloration). (AB/PAS stains, scale bar 200 µm).

compound tubuloalveolar with muscular fibers in between and the glands were separated by lymphatic diffusion however; Abumandour et al. hadn't focused on the bony architecture of the laryngeal mound. Madkour and Abdelsabour-Khalaf (2022) reported that the laryngeal glands were tubular glands that present in the lamina propria which was controversial with our findings.

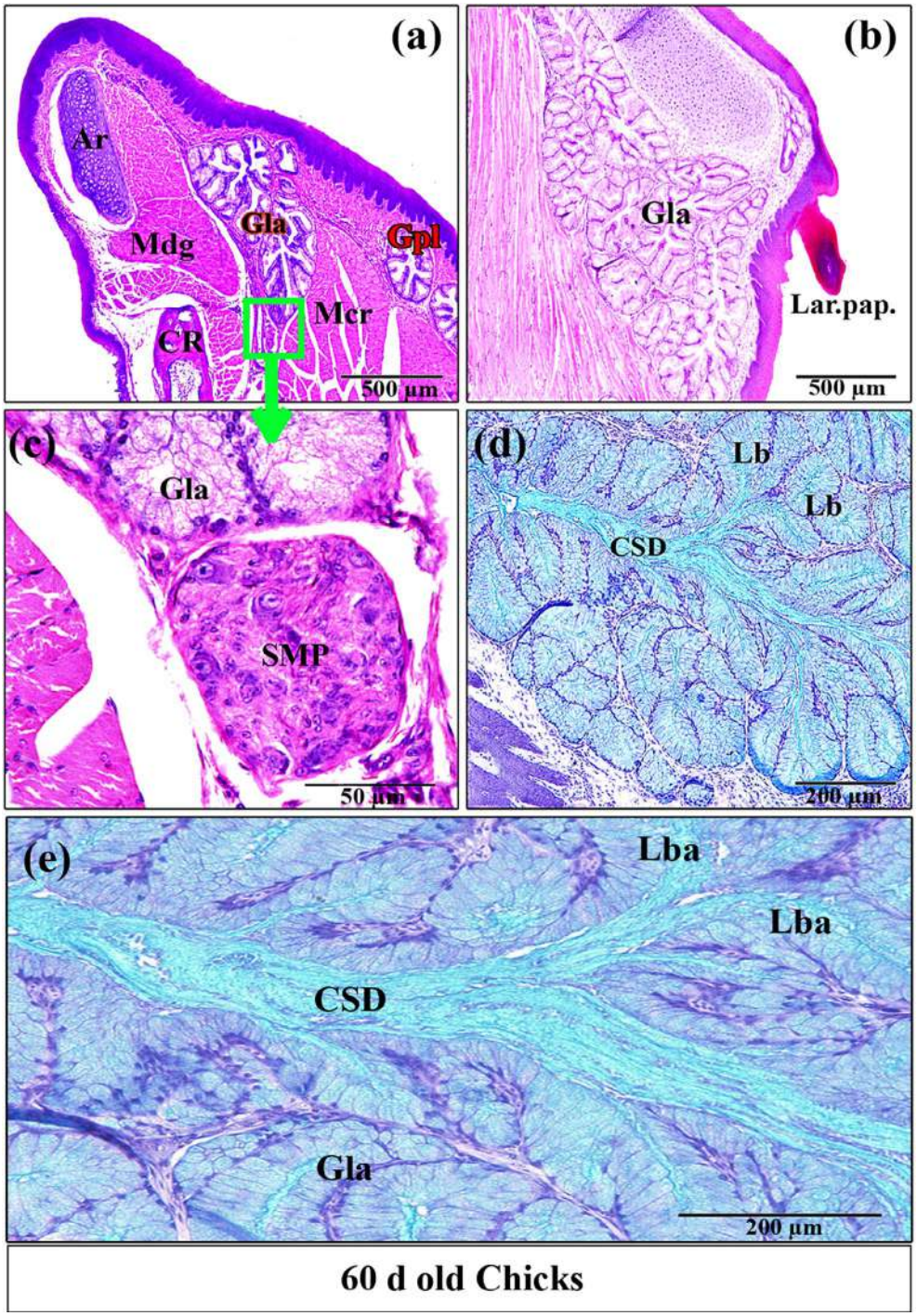

FIGURE 16 | Photomicrographs of the laryngeal mound showing the gland laryngealis histochemical reaction. (a, b) Frontal sections in the laryngeal mound of 30 days old showing the strong positive reaction of the gland laryngealis (Gla) to AB stain (bluish green coloration), Muscle cricohyoideus (Mcr). (AB stain, scale bars 500 and 200 µm). (c, d) Frontal and cross sections of 30 days old chick showing the strong positive reaction of the gland laryngealis (Gla) to combined AB/PAS stains (purple coloration). (AB/PAS stains, scale bars 200 µm). (e) Frontal section in the 60 days old chick showing the very strong alcianophilic reaction of the gland laryngealis to AB stain and combined AB/PAS (bluish green coloration), (AB and AB/PAS stains, scale bar 200 µm). (f) Frontal section of 60 days old chick showing the negative reaction of the gland laryngealis to PAS stain. (PAS stain, scale bar 200 µm).

In contrast to our results, Mclelland, Baumel, and Lucas (1979) and Hodges (1974) synonymized the laryngeal salivary gland as gland *cricoarytenoideus*, while Madkour and Abdelsabour-Khalaf (2022) represented the gland as lateral and medial gland *cricoarytenoideus* corresponding to the lateral and middle portion of the preglottal glands (Khalifa et al. 2022).

Our tentative histochemical investigation resembled our previous studies on the preglottal salivary glands that started with strong PAS positive reacted mucous secretion in early post-hatching ages and ended by alcianophilic (strong AB) reaction later to indicate more acidic mucous secretion within the glands. Hence, the laryngeal salivary glands play a role in

food prehension (Abumandour et al. 2021b; Khalifa et al. 2022; Madkour and Abdelsabour-Khalaf 2022).

5 | Conclusion

In conclusion, the laryngeal mound took different shapes and various ossification level of its cytoskeleton with age advancing. Thus, the laryngeal mound does not only play a role in the respiration and phonation but also help in bolus deglutition regarding its backwards pointed papillae and alcinophilic salivary glands.

Author Contributions

Mahmoud Osman Khalifa: conceptualization, data curation, formal analysis, methodology, validation, investigation, project administration, writing – review and editing, visualization, writing – original draft. **Wafaa Gaber:** data curation, visualization, software. **Abdelmohaimen Mostafa Saleh:** supervision, investigation, data curation, software.

Ethics Statement

The current study has been approved by The Ethical Committee of The Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt, according to The OIE standards for use of animals in research Under the No. 06/2023/0103.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

Abou-Elhamd, A. S., M. Abd-Elkareem, and Z. A. El-Zuhry. 2018. "Morphogenesis of Lingual Papillae of One-Humped Camel (*Camelus dromedarius*) During Prenatal Life: A Light and Scanning Electron Microscopic Study." *Anatomia, Histologia, Embryologia* 47, no. 1: 38–45. https://doi.org/10.1111/ahe.12321.

Abumandour, M. M., N. E. El-Bakary, E. R. Elbealy, et al. 2021b. "Ultrastructural and Histological Descriptions of the Oropharyngeal Cavity of the Rock Pigeon *Columba livia dakhlae* With Special Refer to Its Adaptive Dietary Adaptations." *Microscopy Research and Technique* 84, no. 12: 3116–3127. https://doi.org/10.1002/jemt.23870.

Abumandour, M. M. A. 2018. "Surface Ultrastructural (SEM) Characteristics of Oropharyngeal Cavity of House Sparrow (*Passer domesticus*)." *Anatomical Science International* 93, no. 3: 384–393. https://doi.org/10.1007/s12565-017-0426-6.

Abumandour, M. M. A., and N. E. R. El-Bakary. 2017. "Morphological Characteristics of the Oropharyngeal Cavity (Tongue, Palate and Laryngeal Entrance) in the Eurasian Coot (*Fulica atra*, Linnaeus, 1758)." *Anatomia, Histologia, Embryologia* 46: 347–358.

Abumandour, M. M. A., F. A. Farrag, A. El-Mansi, et al. 2021a. "Posthatching Developmental Studies on the Tongue and Laryngeal Entrance of the Common Quail (*Coturnix coturnix*, Linnaeus, 1758) in Different Five Age-Stages." *Microscopy Research and Technique* 84, no. 8: 1649–1672. https://doi.org/10.1002/jemt.23725.

Bakoji, I., M. K. Aliyu, U. Haruna, S. A. Jibril, R. M. Sani, and H. Danwanka. 2013. "Economic Analysis of Quails Bird (*Cortunix Cortunix*) Production in Bauchi Local Government Area, Bauchi. State, Nigeria." *Research Journal of Agriculture and Environmental Management* 2, no. 12: 420–425.

Bancroft, J. D., and M. Gamble. 2002. *Theory and Practice of Histological Techniques*. 5th ed, 593–620. Edinburgh: Churchill Livingstone.

Bassuoni, N. F., M. M. A. Abumandour, K. Morsy, and B. G. Hanafy. 2022. "Ultrastructural Adaptation of the Oropharyngeal Cavity of the Eurasian Common Moorhen (*Gallinula chloropus chloropus*): Specific Adaptive Dietary Implications." *Microscopy Research and Technique* 85, no. 5: 1915–1925. https://doi.org/10.1002/jemt.24053.

Bock, W. J. 1978. "Morphology of the Larynx of *Corvus Brachyrhynchos* (Passeriformes: Corvidae)." *Wilson Bull* 90, no. 4: 553–565.

Crole, M. R., and J. T. Soley. 2010. "Gross Morphology of the Intra-Oral Rhamphotheca, Oropharynx and Proximal Oesophagus of the Emu (*Dromaius novaehollandiae*)." *Anatomia, Histologia, Embryologia* 39, no. 3: 207–218. https://doi.org/10.1111/j.1439-0264.2010.00998.x.

Crole, M. R., and J. T. Soley. 2012. "What Prevents Struthio Camelus and *Dromaius novaehollandiae* (Palaeognathae) From Choking? A Novel Anatomical Mechanism in Ratites, the Linguo-Laryngeal Apparatus." *Frontiers in Zoology* 9, no. 1: 11. https://doi.org/10.1186/1742-9994-9-11.

Demirkan, C. A., R. M. Haziroğlu, and I. Kürtül. 2007. "Gross Morphological and Histological Features of Larynx, Trachea and Syrinx in Japanese Quail." *Anatomia, Histologia, Embryologia* 36, no. 3: 215–219.

Erdogan, S., and A. Alan. 2012. "Gross Anatomical and Scanning Electron Microscopic Studies of the Oropharyngeal Cavity in the European Magpie (*Pica pica*) and the Common Raven (*Corvus corax*)." *Microscopy Research and Technique* 75, no. 3: 379–387. https://doi.org/10.1002/jemt.21067.

Erdogan, S., and W. Perez. 2015. "Anatomical and Scanning Electron Microscopic Characteristics of the Oropharyngeal Cavity (Tongue, Plate and Laryngeal Entrance) in the Southern Lapwing (Charadriidae: Vaneelus Chilensis, Molina 1782)." *Acta Zoologica (Stockholm)* 96, no. 2: 127–272. https://doi.org/10.1111/azo.12075.

Fitzgerald, C. T. 1970. *The Coturnix Quail Anatomy and Histology*. Ames, IA: Iowa State University Press.

Gewaily, M. S., and M. M. Abumandour. 2020. "Gross Morphological, Histological and Scanning Electron Specifications of the Oropharyngeal Cavity of the Hooded Crow (*Corvus cornix pallescens*)." *Anatomia, Histologia, Embryologia* 50, no. 1: 72–83.

Hodges, R. D. 1974. *The Histology of the Fowl*. London; New York: Academic Press.

Hogg, D. A. 1982. "Ossification of the Laryngeal, Tracheal and Syringeal Cartilages in the Domestic Fowl." *Journal of Anatomy* 134, no. 1: 57–71.

Homberger, D. G., and R. A. Meyers. 1989a. "Morphology of the Lingual Apparatus of the Domestic Chicken *Gallus gallus*, With Special Attention to the Structure of the Fasciae." *American Journal of Anatomy* 186: 217–257. https://doi.org/10.1002/aja.1001860302.

Homberger, D. G., and R. A. Meyers. 1989b. "Morphology of the Lingual Apparatus of the Domestic Chicken *Gallus gallus*, With Special Attention to the Structure of the Fasciae." *American Journal of Anatomy* 186: 217–257.

Huss, D., G. Poynter, and R. Lansford. 2008. "Japanese Quail (*Coturnix japonica*) as a Laboratory Animal Model." *Laboratory Animals* 37, no. 11: 513–519. https://doi.org/10.1038/1108-513.

Igwebuike, U. M., and U. U. Eze. 2010. "Anatomy of the Oropharynx and Tongue of the African Pied Crow (*Corvus albus*)." *Veterinarski Arhiv* 80: 523–531.

Kabak, M., I. O. Orhan, and R. M. Haziroglu. 2007. "The Gross Anatomy of Larynx, Trachea and Syrinx in the Long-Legged Buzzard (*Buteo rufinus*)." *Anatomia, Histologia, Embryologia* 36, no. 1: 27–32. https://doi.org/10.1111/j.1439-0264.2006.00708.x.

Khalifa, M. O., M. Abd-Elkareem, W. Gaber, T.-S. Li, and A. M. Saleh. 2022. "Developmental Morphological Analyses on the Preglottal Salivary Gland in Japanese Quails (*Coturnix japonica*)." *Microscopy Research and Technique* 85, no. 1: 156–168.

King, A. S., and J. Mclelland. 1984. *Birds Their Structure and Function*. 2nd ed. London: Bailliere Tindall.

Lbe, C. S., B. I. Onyeanusi, S. O. Salami, A. D. Umosen, and S. M. Maidawa. 2008. "Studies of the Major Respiratory Pathways of the West African Guinea Fowl (*Numida meleagris* Galeata): The Morphometric and Macroscopic Aspects." *International Journal of Poultry Science* 7, no. 10: 997–1000.

Liman, N., G. Bayram, and M. Koçak. 2001. "Histological and Histochemical Studies on the Lingual, Preglottal and Laryngeal Salivary Glands of the Japanese Quail (*Coturnix coturnix* Japonica) at the Post-Hatching Period." *Anatomia, Histologia, Embryologia* 30, no. 6: 367–373. https://doi.org/10.1046/j.1439-0264.2001.00353.x.

Lungova, V., and S. L. Thibeault. 2020. "Mechanisms of Larynx and Vocal Fold Development and Pathogenesis." *Cellular and Molecular Life Sciences* 77: 3781–3795. https://doi.org/10.1007/s00018-020-03506-x.

Madkour, F. A., and M. Abdelsabour-Khalaf. 2022. "Morphological and Ultrastructural Features of the Laryngeal Mound of Egyptian Cattle Egret (*Bubulcus ibis*, Linnaeus, 1758)." *BMC Zoology* 7, no. 1: 44. https://doi.org/10.1186/s40850-022-00147-4.

Mahdy, M. A. A. 2020. "Comparative Morphological Study of the Oropharyngeal Floor of Squabs and Adult Domestic Pigeons (*Columba livia* Domestica)." *Microscopy Research and Technique* 84, no. 3: 499–511. https://doi.org/10.1002/jemt.23606.

Mclelland, J. 1975. "Aves Digestive System." In *Sission and Grossman's, the Anatomy of the Domestic Animals*, vol. 2, 5th ed. Philadelphia: Getty R. Saunders.

Mclelland, J. 1990. *A Color Atlas of Avian Anatomy*. Aylesbury, England: Wolfe Publishing Ltd. Hazell books Ltd.

Mclelland, J., A. S. K. Baumel, and A. M. Lucas. 1979. "Systema digestorium." In *Nomina Anatomica Avium*, 267–287. Nuttall Ornithological Club: Cambridge, London.

Mohamed, A. A. 2010. *Some Developmental Studies on the Floor of the Oropharynx of the Duck*. Egypt: South Valley University.

Mohamed, S. A. 2004. "Developmental Studies on the Floor of the Oropharynx of the Post-Hatching Quail." *Assiut Veterinary Medical Journal* 50: 100.

Mohamed, S. A., M. Abdelsabour-Khalaf, and F. Abdelhakeem. 2018. "Morphological Characterization of the Laryngeal Mound of the Egyptian Geese." *Assiut Veterinary Medical Journal* 64, no. 156: 26–33.

Moussa, E. A., and S. A. Hassan. 2013. "Comparative Gross and Surface Morphology of the Oropharynx of the Hooded Crow (*Corvus cornix*) and the Cattle Egret (*Bubulcus ibis*)." *Journal of Veterinary Anatomy* 6, no. 1: 1–15.

Saleh, A. 2013. "Macro-and Micromorphological Studies on the Laryngeal Mound of Turkey." *Assiut Veterinary Medical Journal* 59, no. 136: 88–94.

Tadjalli, M., S. H. Mansouri, and A. Poostpasand. 2008. "Gross Anatomy of the Oropharyngeal Cavity in the Ostrich (*Struthio camelus*)." *Iranian Journal of Veterinary Research Shiraz University* 9, no. 4: 316–322.