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# Gross anatomy and mineralization of larynx, trachea and syrinx in gray heron (Ardea cinerea)

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Abstract: This study aims to determine the normal anatomical features of the larynx, trachea, and syrinx of the heron birds and to identify the cartilage and mineralized areas with red Alcian blue staining. Three adult heron birds' larynx, trachea, and syrinx were used as materials in the study. Sex of the birds was neglected. With the staining of alizarin red Alcian blue, cartilage tissues were blue, and mineralized areas with calcium accumulation were red. In the macroscopic examination, mons laryngealis was seen on the caudal side of the tongue on the larynx under a stereomicroscope. It was observed that there was a thin and long-looking glottis on mons laryngealis. Larynx cranialis was observed to consist of two cartilago arytenoidea and one cartilago cricoidea in alizarin red staining. Trachea was found to consist of fully mineralized rings, except for the first and incomplete mineralized cartilago trachealis. Trachea rings were found to form a complete ring except for the first cartilago trachealis. Diameters of the rings differed regionally throughout for the trachea. It was determined that the widths of the trachea rings were approximately the same size except the first one. Syrinx was found to be the tracheobronchial type, which is the typical simple type syrinx. The four rings that make up the tracheobronchial syrinx were seen to merge. This is the first study showed the detailed anatomical description of larynx trachea and syrinx belonging to heron birds with alizarin red Alcian blue staining.

Key words: Anatomy, heron, larynx, mineralization, trachea, syrinx

#### 1. Introduction

The gray heron (Ardea cinerea) is a species of bird belonging to the heron (Ardeidae) family. They are big birds, which are 90-100 cm long, have 175-195 cm wingspan, and weigh 1-2 kg. The plumage is largely gray above and dirty white below. There is a thick wedge-shaped beak. They have long necks. Adults are pale gray, white, and gray-black without spots, with a white head cut off black, and small ornamental feathers on both sides of the head. Its beak is yellow that turns green and turns pink or orange in spring. It flies very slowly, and, as it flies, it bends its wings down, pulls its head back, and stretches its legs back. They feed in shallow water, catch fish or frogs with their long beaks. Herons can also eat small mammals [1,2]. The main functions of the bird's respiratory system are to contribute to oxygen and carbon dioxide exchange, body temperature balance, and sound production. To reveal the structure and function of the respiratory system of poultry [3], parrot [4], ostrich [5], owl [6], and ducks [7], many morphometric and histological studies have been performed. Respiratory system in birds is different from mammals. These differences are generally due to the flying abilities of birds, sound production, and thermoregulation [8,9]. These differences caused birds to have the most efficient respiratory system among vertebrates [10]. During breathing, air passes through the nasal cavity, throat and continues with the trachae, then goes to the syrinx and bronchi [11,12,13,14]. In birds, laryinx consists of four partially ossified cartilage: cartilago cricoidea, cartilago procricoidea, and two cartilago arytenoidea [12,15,16]. The number of cartilage rings in the trachea varies depending on the length of the neck. Trachea usually consists of 108-126 cartilage rings [12,14,17,18]. Syrinx is the organ that produces sounds in birds [12,14,19]. Some birds make sounds all year round, while others only make sounds during the mating season or migration. Syrinx is shaped by the last part of the trachea or the beginning of the bronchi, sometimes both [20,21,22]. Therefore, syrinx is classified into 3 types: tracheobronchial, bronchial, and tracheal [14,15,23]. Syrinx has two membranes. These are membrane tympaniformis lateralis and membrane tympaniformis medialis. Sound is produced by these membranes [14,24,25].

There is no study in the literature on the anatomy of larynx, trachea, and syrinx in heron with alizarin

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red Alcian blue staining. This study aims to identify the cartilage and mineralized areas of the larynx, trachea, and syrinx in the heron with double staining to contribute to the literature and to provide basic data for the diagnosis of diseases.

## 2. Material and methods

Three adult heron birds larynx, trachea, and syrinx were used in this study. Sex of the birds was neglected. After the normal posture and position of the larynx, trachea, and syrinx in the body were examined, they were dissected using scissors and a scalpel. After the materials stored in 10 percent (10%) formaldehyde solution were washed with distilled water, they were placed in containers containing 95% ethanol. The materials waiting for 10 days were kept ready for painting after being kept in pure acetone for 24 h. A solution is created by mixing 300 mg of Alcian blue, 100 mL of 70% ethanol, 100 mg of alizarin red, and 100 mg of 95% ethanol. Then, 100 mL of glycial acetic acid and 1700 ml of 70% ethanol were added to the solution. The materials were kept in the incubator in the solution at the appropriate temperature for 4 days and then washed in running water for 2 h. After washing, the materials were stored in containers containing 2% KOH for three days, and finally kept in 100% glycerin solution [26,27]. Some of the macroscopic findings and images were taken under a stereomicroscope (Nikon SMZ 745T) (Nikon Corp., Tokyo, Japan). Other digital pictures were taken with the Fujifilm FinePix S602 (Fujifilm Holdings Corp., Tokyo, Japan).

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

#### 3. Results

Larynx, trachea, and syrinx were examined in detail and macro anatomic structures specific to the heron were determined. In the macroscopic examination, findings were obtained under a stereomicroscope. With the painting of alizarin red Alcian blue, cartilage tissues were blue and mineralized areas with calcium accumulation were seen as red and photographed.

## 3.1. Larynx

Bump-shaped mons laryngealis was seen on the caudal side of the tongue. It was found that this structure was in the shape of an isosceles triangle with a pointed end at the front and the base above. It was observed that there was a thin and long-looking glottis on mons laryngealis. The length of glottis was measured 13.26 mm on average. In the middle of the caudal part of glottis, it was observed that there were 2 papillae-shaped quite long protrusions on the right and left. In this section, sulcus laryngeus was not seen. It was determined that papilla exceeded the level of mons laryngeus by 3.73 mm and was directed in the dorso caudal direction. The distance between the front end of the glottis and the papilla was measured as an average of 19.45 mm. A transverse-progressive papilla sequence was found in the aboral of mons laryngealis. There were 6 to 8 papillae with free ends retrospectively in each half in the aboral of mons laryngealis (Figure 1). It was found that this papilla line extended towards the ventral after wrapping the dorsolateral part of the trachea. It was determined that the sizes of the papillae were different from each other and settled irregularly (Figure 1).

Larynx cranialis was formed by three cartilage seen in red staining in alizarin red staining (Figure 2). The double of these three cartilage was seen as cartilago arytenoidea and the single one was cartilago cricoidea. It was found that cartilago cricoidea, Larynx's largest cartilage consists of three parts: processus rostralis, corpus, and ala. It was seen that this section forms the rostral, ventral, and caudodorsal sections of the larynx and supports the ala of cart. cricoidea. Cartilago cricoidea was found to be mineralized as the ventral part was completely red. An unsharp crista ventralis structure along the median line was observed in the ventral part of the corpus of cartilago cricoidea. It was seen that the part of this part extending towards the dorsal was shaped like a saw blade and mineralized. It was seen that the part of crista ventralis extending towards the dorsal was shaped like a saw blade and mineralized. It was observed that the bilateral parts of this cartilage were blue but not mineralized yet (Figure 2). Therefore, it was observed that the corpus and ala portion of cartilago cricoidea did not combine as mineralized. Between the two sections, a 1.95 cm blue cartilage area was observed (Figure 2). This region was not fully mineralized. On the lateral upper part of the cartilago cricoidea, ala portion was detected. Ala was observed to be mineralized. From the dorsal view, trapezoid-shaped reddish mineralized areas were observed bilaterally extending towards the ventral. These were determined to be the ala of cartilago cricoidea. It was determined that there is a gap between the right and left ala of cartilago cricoidea, and there was no mineralized area in this gap. Cartilago procricoidea was not seen.

Cartilago arytenoidea, which is found as a pair of larynx cartilage, was seen to consist of corpus, processus caudalis, and processus rostralis. This cartilage was the dorsal part of the larynx. It was observed that a large part of the cartilage was painted in red, that is, it was mineralized. It was determined that approximately 2 mm of the anterior end of the rostral section was cartilage (Figure 2).

#### 3.2. Trachea

The average length of trachea was determined to be 30.8 cm. It was determined that it was floating under the esophagus on the ventral face of the neck. The median line crossed again at the junction of the neck and chest. Then,



**Figure 1.** Dorsal view of larynx. 1: Irregular papillas; 2: Papillae-shaped quite long protrusions; 3: Glottis.

it was found that it joined syrinx in the furcula around the basis of the heart. Trachea was found to consist of 195-201-203 fully mineralized rings, except how he first and incomplete mineralized cartilago trachealis. Diameters of the rings differed regionally throughout the trachea. It was determined that the rings in the starting part of trachea were of a large diameter equal to the larynx. In this section, the average diameter of the trachea rings was determined to be 6.65 mm. The average diameter of the rings, which shrank towards the middle of the trachea, was measured as 6.58 mm. In the last part of trachea, the rings had a smaller diameter (average 5.75 mm).

It was determined that trachea started from the end of cartilago cricoidea and extended to syrinx. It was determined that all the rings were red and mineralized, forming a complete ring except howhe first cartilago trachea ring. It was found that mineralization was completed in the rings and the back sections were more mineralized from the front side because they were painted in darker color. It was observed that the first trachea ring was narrower (cranio caudal length) (average 0.87 mm). It was determined that the ends of this ring did not join in the dorsal part, the ends were overlapped. The width of the first 7 tracheal rings had a larger diameter to fit the width of the larynx. It was observed that the diameter of the tracheal rings close to syrinx decreased. A notch was observed in the ventral of each cartilago trachealis, both in front and behind. It was observed that not all these



**Figure 2.** Lateral view of larynx with Alizarin red Alcian blue staining. 1: Papillae-shaped quite long protrusions; 2: Cart. arythenoidea; 3: Proc. rostralis of cart. Arythenoidea; 4: Cart. cricoidea, asterisk: not mineralized arae.

notches were properly located in the middle. Notch was observed on both sides of the dorsal part of each tracheal ring (Figures 3 and 4). Thanks to these notches, it was determined that the tracheal rings overlap each other, increasing (Figure 3) or reducing (Figure 4) the length of the trachea. Therefore, it was found that the rings how a telescope-like arrangement and they were interlocked with each other thank to these notches. In this process, one side of the tracheal ring was found on top of its neighbor, while the other side was found on the bottom. It was observed that when the trachea rings diverged, the total length increased. Besides that the cranio-caudal length of the tracheal rings was approximately the same size, excluding the first.

# 3.3. Syrinx

Syrinx was found to be the tracheobronchial type (Figure 5), which is the typical simple type syrinx. The four rings that make up the tracheabrochial syrinx were seen to merge. It was determined that these rings did not merge fully mineralized, capillaries remained between them and formed the tympanum. The joining parts were found to have much less width (craniocaudal length) than previous tracheal rings. It was found that syrinx formed a hard and strong structure together with the mineralization of the last four rings, rings in the broncho syringeal part and pessulus. Cartilago bronchosyringealis were seen when looking at syrinx laterally. In total, 26-31 cartilago bronchosyringealis were counted. When viewed from the lateral, 5-7 fully mineralized cartilago bronchosyringealis were detected on one side. The middle parts of the sixth (in two materials) and the seventh (in one material) cartilago bronchosyringealis were partially mineralized (Figure 2). The dorsoventral length at the widest point of cartilago



**Figure 3.** Dorsal view of long trachea. 1:Tracheal notch, asterisk: trachea ring.

bronchosyringealis was measured as 13.18 mm. It was observed that the rings that formed the tympanum and partially joined together were fully joined at the dorsal, extending towards the median or even the ventral, joining the dorsal part of the pessulus. The dorsal ends of the first three cartilago bronchosyringealis curved towards the medial. It was determined that the gap between the free ends of cartilago bronchosyringealis was closed by the membrane tympaniformis medialis. Ligamentum interbronchiale was found to bind right and left bronchus primarius. A foramen interbronchiale was observed between the pessulus and the ligamentum interbronchiale.

Pessulus (Figure 5) was found to be one of the major parts of syrinx. It was located in the dorso-ventral position, and its dorsal-ventral ends and body were seen. From the first cartilago tracheasyringealis, it was determined that it consisted of cartilage mineralized in the form of 2 parallel rods towards the ventral. It was found that it combined with the first brochosyringealis in the ventral and formed the pessulus. It was observed that the front edge was pointed and concave, while the back edge extended flat from top to bottom.

#### 4. Discussion

In our study, it was determined that the red colored areas painted with alizarin red were mineralized and showed calcium accumulation. Hogg [3] ve Atalgin et al. [5] declared that red staining of alizarin can only provide an



**Figure 4.** Dorsal view of short (overlapped ring) trachea. 1: Tracheal notch, asterisk: trachea ring.



**Figure 5.** Dorsal view of syrinx. 1: Pessulus, form of two parallel rods; 2: Cartt. Bronchosyringeales.

idea for the detection of ossification and that the exact ossification diagnosis will be made with histological sections. Since the hardness of the red-painted areas seen in the presented study is important, it was not necessary to take histological sections as the boundaries of the mineralized areas, and histological section could not be determined exactly.

In a developmental study [3] with Alizarin red Alcian blue staining, it was reported that the mineralization of trachae was on the 98th day, and in another study, mineralization continued from caudal to cranial [28]. Since our materials in the study belong to mature individuals, mineralization is mostly completed except laryinx, and it is compatible with the studies reported since it is determined that the posterior parts are more mineralized. In addition, the declaration of Myers [29] that the most mineralized regions are cartilago bronchosyringealis is similar to our study findings. It was observed that 5-7 of cartilago bronchosyringealis, which were 26-31 in total, were mineralized. It was observed that the middle parts of the sixth cartilago bronchosyringealis were mineralized in two material and the middle parts of the seventh cartilago bronchosyringealis in one materials. Therefore, there were 18-24 unmineralized cartilago bronchosyringealis from 26-31 half-ring structure cartilago bronchosyringealis.

Although it was stated in Yılmaz et al.'s [7] study that sulcus laryngeus was present in a narrow groove, sulcus laryngeus was not observed in our study. In the study presented in the aboral of mons laryngealis, in one row and succession, there are 5-6 rows of caudal-oriented papillae on each side, although in some studies [6,16,30,31,32], in the caudal part of mons laryngeus, 2 rows of rostral and caudal-oriented were seen. Onuk et al. [33] stated in their study that there were many papillae scattered and out of order. In our study, unlike the literature, a single papilla row was observed.

Although no papilla was observed along the lateral boundaries of glottis in our study, it was reported that papillae were encountered in this section in some studies [30,31].

The discovery of two papilla-shaped protrusions on the caudal border of the glottis, with a median or even rather long and exceeding the level of mons laryngea, was similar to that of Al-aameli and Kadhim's study [32]. However, it was determined that papilla-like protrusions differ due to the level of mons laryngeus.

Similar to our findings, cartilago procrocoid was not mentioned in quail [34], chicken [13], turkey [17] and penguin [30] either. In some studies, cartilago procrocid has been reported to be found [8,12,14]. McLelland [35] reported that the cartilago cricoidea in the duck was in the form of a scoop; in the current study it was determined that this cartilage structure was triangular. Although cartilago cricoidea is stated to be not mineralized in the front parts of the midline and the back midline even in adults [3], these sections were found to be mineralized in our study. In parallel with the notifications of Hogg [3], the wings of cartilago cricoidea were observed to be mineralized.

Although some studies [36] were reported to have papillae on the dorsal sides of the glottis, the papilla was not observed in the current study in accordance with the declaration of Getty [12] in turkeys.

The number of trachea rings differs in the literature. In the Denizli rooster [36] 102-130, in chickens (Gallus domesticus) 108–126, in ducks [37] 112–116, in quail [34] 83–91, in geese [33] 137-140, in scarlet hawk [16] 89-96, and also in Aseel race rooster and chickens [31] 111-118 trachea rings were reported to have. Piperno and Peirone [18] reported 90 rings in quail with ligamentum anulare. In our study, 195-201 and 203 rings were observed more than all reported. In addition, unlike the report of Piperno and Peirone [18], ligamentum anulare was not seen.

It was found that the ends of the first cartilago trachealis did not join in the dorsal part, and the ends tapered and overlapped each other. While this finding seems to be similar to McLelland's [38] statement, it is reported that the ends of the first three rings do not meet with each other and not overlapping in quails [39]. Cevik-Demirkan et al. [39] reported that the quail rings do not overlap each other. In the study, it was determined that the trachea rings overlap each other to increase and decrease the total length of the trachea. Although it was stated that some of the rings were bifurcated in the dorsal and ventral parts with other rings [33], no such association was observed in our study. Hogg [3] reported in his study that cartilago trachea has cartilage that is not exactly a ring. In our study, it was found that all the rings were completed.

In our study, 4 rings constituted tympanium. The last 5 to 7 tracheal rings in chickens [40], two rings in quail [39] and turkeys [41], 2 rings or more in domestic chickens [14], and 3 rings in Black Francolin birds [32]. It is stated that it forms a compact structure, tympanium.

It is reported that there are no pessulus in predatory birds [14,20], ostriches [42], and pigeons [36,43]. It has been stated that it is found in the form of the wedge in chickens [12,14], and it develops well in songbirds [43]. In our study, pessulus was observed. However, unlike other studies, it was found that the body of the pessulus consists of two cartilage mineralized as two parallel rods from the dorsal to the ventral (Figure 5).

Getty [12] reported that pessulus merged with 4 cartilago tracheasyringealis in the ventral, and Taşbaş et al. [36] with the last 2 rings. In our study, it was observed that pessulus was merged with a single cartilago tracheasyringealis by the reports of Kabak et al. [16].

It was determined that the gap between the free ends of the "C" shaped cartilago bronchosyringealis was closed by the membrane tympaniformis medialis that provides sound formation. This finding was consistent with the statements of Getty [12], Taşbaş et al. [36], and Yıldız et al. [42].

For the first time in the study, larynx trachea and syrinx belonging to heron birds were examined, and detailed

macroanatomical features were determined about these formations. Mineralized regions have been identified using alizarin red Alcian blue staining, which is rarely used in such studies. Since mineralized areas cannot be detected by macroanatomical and some histological methods in many studies, this staining method has provided many advantages to see mineralized and hardened areas and

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to determine their borders. In the presented study, the characteristics that have never been reported before were seen, and similarities were identified and specified.

## **Conflict of Interest**

The authors declare that they have no conflict of interest.

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