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# Histological study of the syrinx in swan goose (*Anser cygnoides*)

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## Abstract

The current exploration was done to study the histological characteristic features of the syrinx in the swan geese (*Anser cygnoides*). For that purpose, 10 (5 males and 5 females) birds were euthanized and specimen was collected for studying the location, length, and relationship were reported. Moreover, the syrinx was histological-studied by hematoxylin and eosin (H&E), Periodic acid–Schiff stain (PAS), combined Alcian blue (AB) and PAS, and Masson's trichrome and Verhoeff stain. The results unveiled that the voice box (syrinx) of the swan goose revealed that the syringeal wall thickness was (1903.78±2.957 and 1902.5±1.476µm), for males and females, respectively. The part of the tracheosyringeal rings were ciliate-lined of pseudostratified columnar epithelium with different-sized acini in high numbers (tubular mucous glands), and epithelia-lined-goblet cells. The mean thickness of the epithelium was (204.56±2.231 and 198.92±1.513µm) in males and females, respectively. The average thickness of the cartilage was (207.78±1.601 and 199.12±1.661µm) in males and females respectively. Simple branched mucous glands showed a positive reaction to the PAS stain. Also, positive reaction for alcian blue and combined alcian blue pas stains. The diameter of the syringeal mucous alveoli was 304.02±3.205 and 298.02±4.072µm in males and females respectively. This study reveals the characteristic pictures of the syrinx of the swan geese that could be useful for future studies that deal with different sciences, such as public health and industries of this bird.

**Keywords: *Anser cygnoides*, syrinx anatomy, Swan goose.**

## Introduction

The syrinx, the phonatory organ that birds have developed through the course of evolution, varies greatly in its form depending on the species. During the process of shutting and opening the passage airways, the muscles that govern the syrinx, which, like the human larynx, control airflow and action and modify acoustic characteristics, are responsible for these functions. The categorization of birds was determined by whether or not they had a syrinx as part of their basic anatomy or whether or not they had musculature. There are birds that are silent for the whole of the year and others that only make sounds during the breeding season (1).Additionally, this is followed by a change in the functional morphology of certain organs that are responsible for the emission of sound. In spite of this, the extent to which gender dimorphism influences the control vocal center is still not entirely clear. The tympanum of different birds has a different number of rings; for example, Japanese quails have two rings, whereas pigeons have five rings (2).There has been no investigation of the histological or morphological variation of the syrinxes in the songbird. On the other hand, these modifications have been shown to occur in mammalian species, where they result in a distinct gender difference in characteristics. The labia are a very important component in the sound producers of songbirds. Labia, in particular those located laterally, contribute

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significantly to vibration. The wedge-shaped pessulus was responsible for separating the syrinx's airways (3).The black francolin males, in contrast to the black francolin females, have a characteristically high and loud voice, particularly during the mating season. This is in contrast to the black francolin females, who maintain an inaudible sound. There is a paucity of knowledge on the anatomy and histology of the syrinx in this bird, as well as the question of whether or not there is a discernible difference between the sexes, particularly in light of the fact that males have the recognized high voice (4). In birds, the syrinx is considered to be the primary vocal organ because of its function. It may be found directly inside the thorax, just next to the point where the trachea branches off into the left and right major bronchi. It is associated with the clavicular air sac, which may be found in close proximity to the heart on the dorsal side of the glandular stomach and ventrally of the esophagus. The Syrinx is particularly prevalent in Chickens and Ostriches (5). The left major and right minor chambers of the male Stock duck's pear-shaped syringeal bulla are separated by a double-walled (6).For example, Swiftlets and Oscines both have syrinxes that originate in the tracheobronchial system, much as Brown Thrashers. Although the trachea makes a little contribution to the syrinx in the duck, the bronchi are almost entirely responsible for its development. A tracheal syrinx is present in budgerigars and other members of the subfamily Furnarioidea in the order Suboscine (7).The current exploration was done to study the anatomical characteristic features of the syrinx in the swan geese (*Anser cygnoides*).

## Materials and Methods

### Birds and collection of syrinxes

The methods included the use of 10 birds (5 males and 5 females). The birds were euthanized and the syrinx was collected, and features, such as location, relationship, length, weight, and volume were reported.

### Histological study

The specimens were collected from syrinx from (5 males and 5 females) studied birds. Some specimens were fixed in 10% neutral buffered formalin and the other was fixed in Bouin's solution where they were $H_2O$-removed by a graded alcohol (70%, 80%, 90%, 95%, and 100%) for two hours for each concentration. The specimens were cleared in xylene for one hour in two changes and infiltrated in paraffin wax (molten). Based of sections of 5µm thickness were prepared from the blocked specimens using the rotary microtome (Series MRS3500, Histo-Line Laboratories Ltd, Italy). Mounting of sections were placed on slides and stained using H&E, PAS, AB and PAS, and Masson's trichrome and Verhoeff stain to demonstrate the general histological components of the tissues.

### Ethical approval

The study protocol was approved by the College of Veterinary Medicine, University of Al-Qadisiyah, Iraq.

## Results

In this study, histological analyses of the voice box (syrinx) of the swan goose revealed that the syringeal wall thickness was (1903.78±2.957 and 1902.5±1.476µm), for males and females, respectively. The part of the tracheosyringeal rings were ciliate-lined of pseudostratified columnar epithelium with different-sized acini in high numbers (tubular mucous glands), and epithelia-lined-goblet cells. The mean thickness of the epithelium was (204.56±2.231 and 198.92±1.513µm) in male birds and female birds, respectively (Table 1 and Fig. 1). The

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mean thickness of the epithelium was (204.56±2.231 and 198.92±1.513µm) in male birds and female birds, respectively. The average thickness of the cartilage was (207.78±1.601 and 199.12±1.661µm) in male and female respectively. Simple branched mucous glands showed a positive reaction to the PAS stain (Fig. 2). Also positive reaction for alcian blue (Fig. 3) and combined alcian blue pas stains (Fig. 4). The diameter of the syringeal mucous alveoli was 304.02±3.205 and 298.02±4.072µm in males and females respectively.

Table 1: Syringeal histological measurement in swan geese

| **Histological measurement** | **Syrinx** | | **T test** | **Calculated P value** |
|---|---|---|---|---|
| | **Male** | **Female** | | |
| **Syringeal wall thickness (µm)** | 1903.78±2.957 | 1902.5±1.476 | 0.774 | 0.461(NS) |
| **Syringeal epithelium thickness (µm)** | 204.56±2.231 | 198.92±1.513 | 4.184 | 0.003(S) |
| **Syringeal cartilage thickness (µm)** | 207.78±1.601 | 199.12±1.661 | 7.504 | 0(S) |
| **Diameter syringeal mucous alveoli (µm)** | 304.02±3.205 | 298.02±4.072 | 2.315 | 0.049(S) |

**S**: Significant difference at $p<0.05$, NS: No significant difference at $p<0.05$

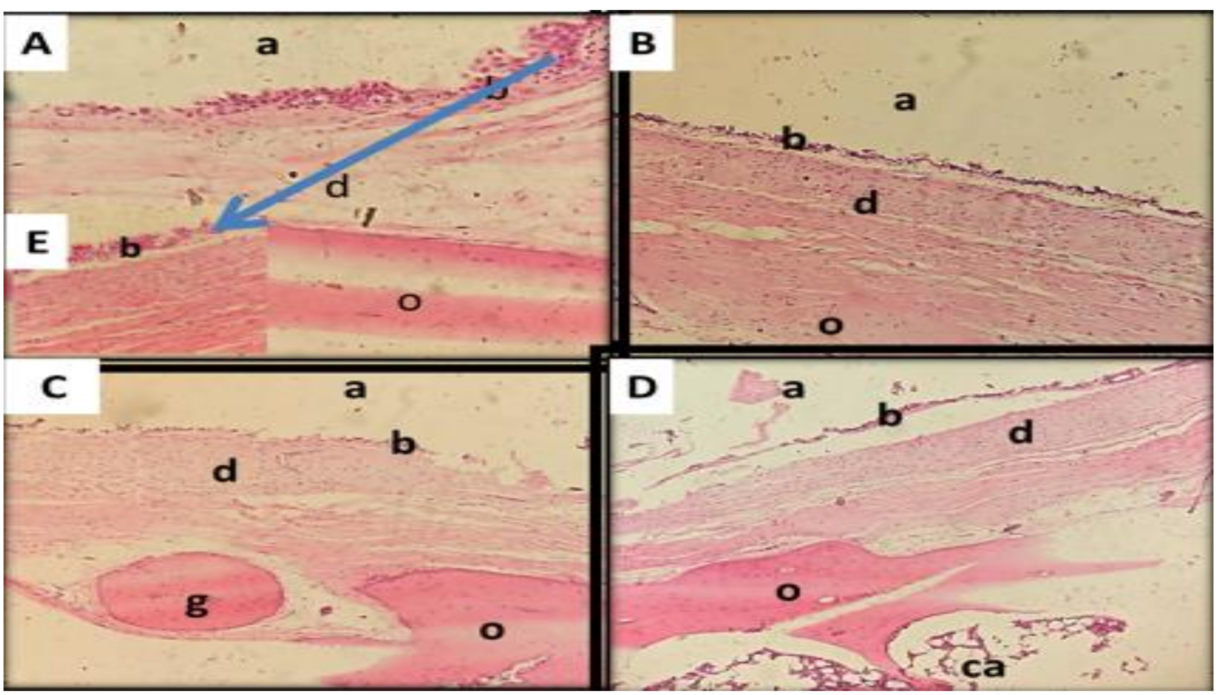


Fig. 1: cross section of A: trachiosyringeal ring: B: vibrating membrane : C: Bronchiosyringeal cartilage : D: pessulus, of swan goose shows : lumen of syrinx a), lining epithelium pesudostraified columnar (b), loose connective tissue of lamina propria (d), hyaline cartilage (g) , ossified tissue (o) , ossification of hyaline cartilage (ca) .H&E stains. A,B,C,D:X200. E :X 400

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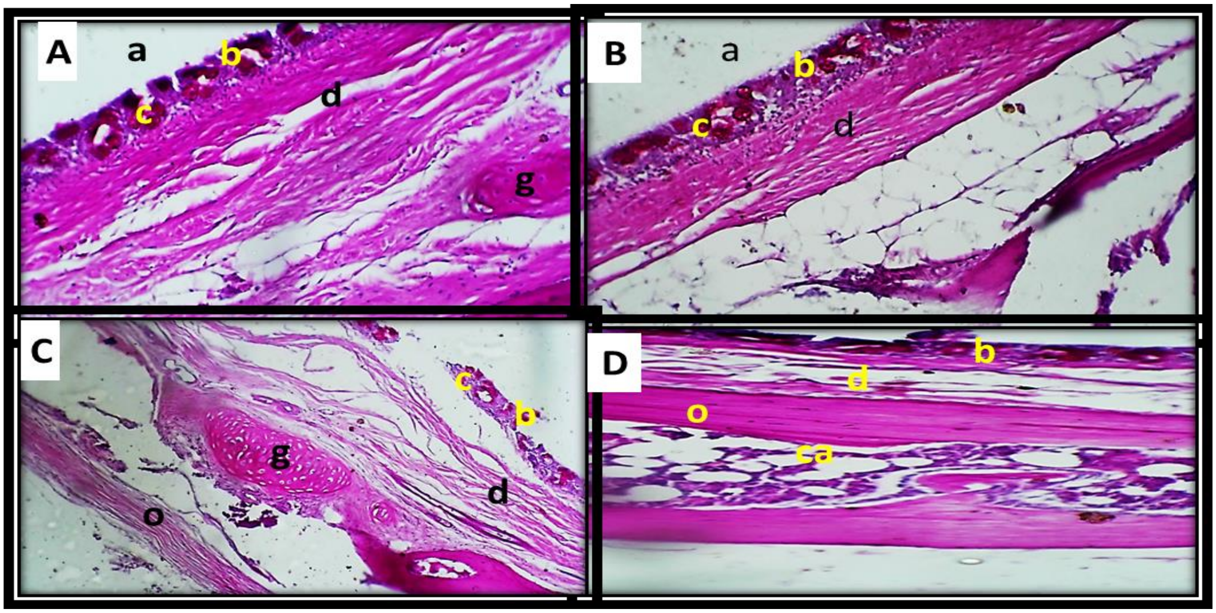


Fig. 2: cross section of A: trachiosyringeal ring : B: vibrating membrane : C: Bronchiosyringeal cartilage : D: pessulus, of swan goose shows : lumen of syrinx a), lining epithelium pesudostraified columnar (b), Iintraepithelial glands (c) positive reaction for pas stain , loose connective tissue of lamina propria (d), hyaline cartilage (g) , ossified tissue (o) , ossification of hylaine cartilage (ca) . PAS stains. A,B,C,D:X200.

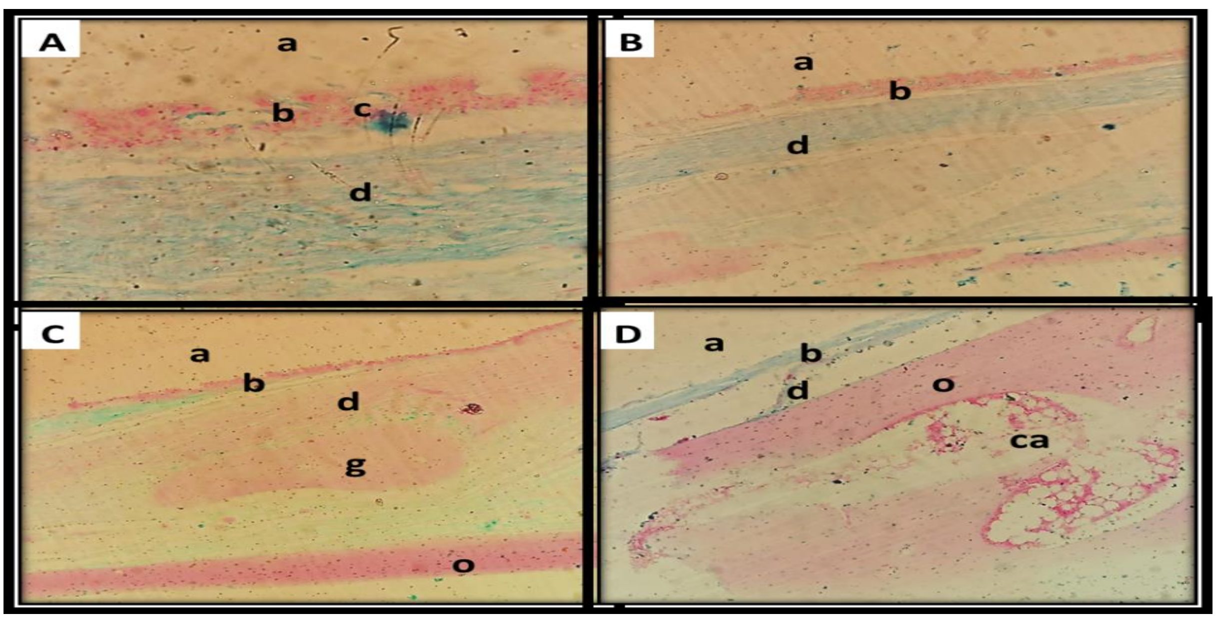


Fig. 3: cross section of A: trachiosyringeal ring : B: vibrating membrane : C: Bronchiosyringeal cartilage : D: pessulus, of swan goose shows : lumen of syrinx a), lining epithelium pesudostraified columnar (b), intraepithelial glands (c) positive reaction for alcian blue stain , loose connective tissue of lamina propria (d), hyaline cartilage (g) , ossified tissue (o) , ossification of hylaine cartilage (ca) . Alcian blue stains. A,B,C,D:X200.

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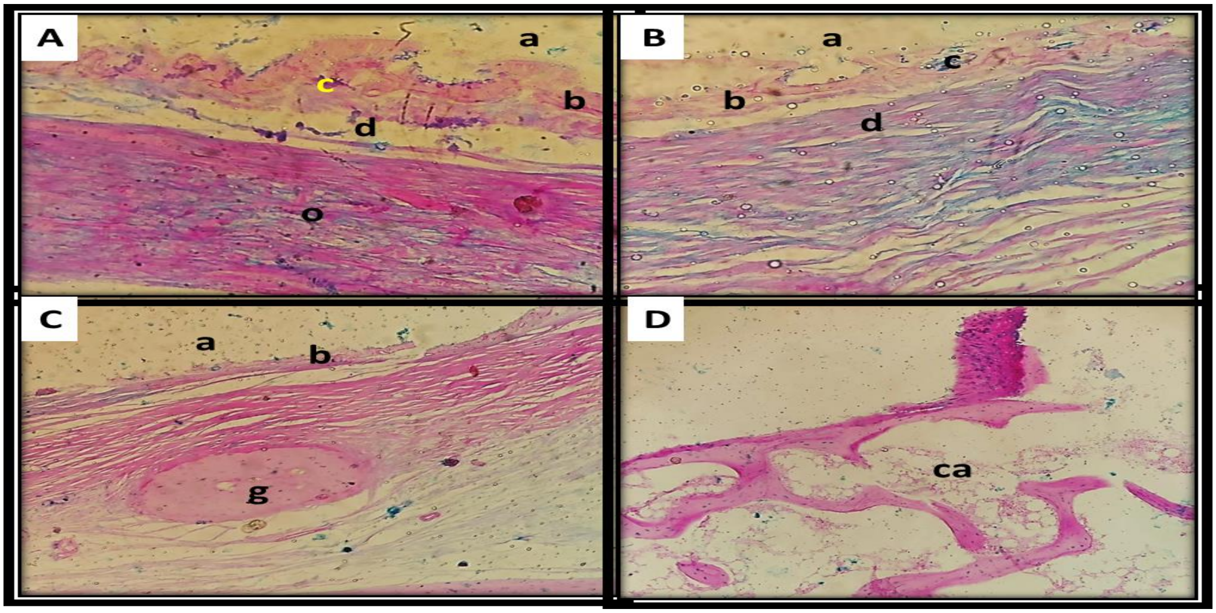


Fig. 4: cross section of A: trachiosyringeal ring : B: vibrating membrane : C: Bronchiosyringeal cartilage : D: pessulus, of swan goose shows : lumen of syrinx a), lining epithelium pesudostraified columnar (b), Iintraepithelial glands (c) positive reaction for combined alcian blue –pas stains , loose connective tissue of lamina propria (d), hyaline cartilage (g) , ossified tissue (o) , ossification of hylaine cartilage (ca) . combined alcian blue –pas stains. A,B,C,D:X200.

There was loose connective tissue in the lamina propria and submucosa. the tracheosyringeal hyaline cartilages were contained by the perichondrium, which was continued. Large collagen bundles and elastic based fibers were present in them, and the adventitial connective tissue that surrounds all syringeal structures continues from the perichondrium connective tissue (Fig. 5, 6). Intermediate Syringeal Cartilages, their structure was comparable to that of the tracheosyringeal cartilages, but there were less epithelial glands and more goblet cell groups to be found. While the vibrating membranes, same structures were present in both the medial and lateral vibrating membranes. The hyaline cartilages and the thin layer of circular smooth muscle fibers compensated for the goblet cell groups, which were more complex than simple branches, tubular mucous glands, and branches (Fig. 2).

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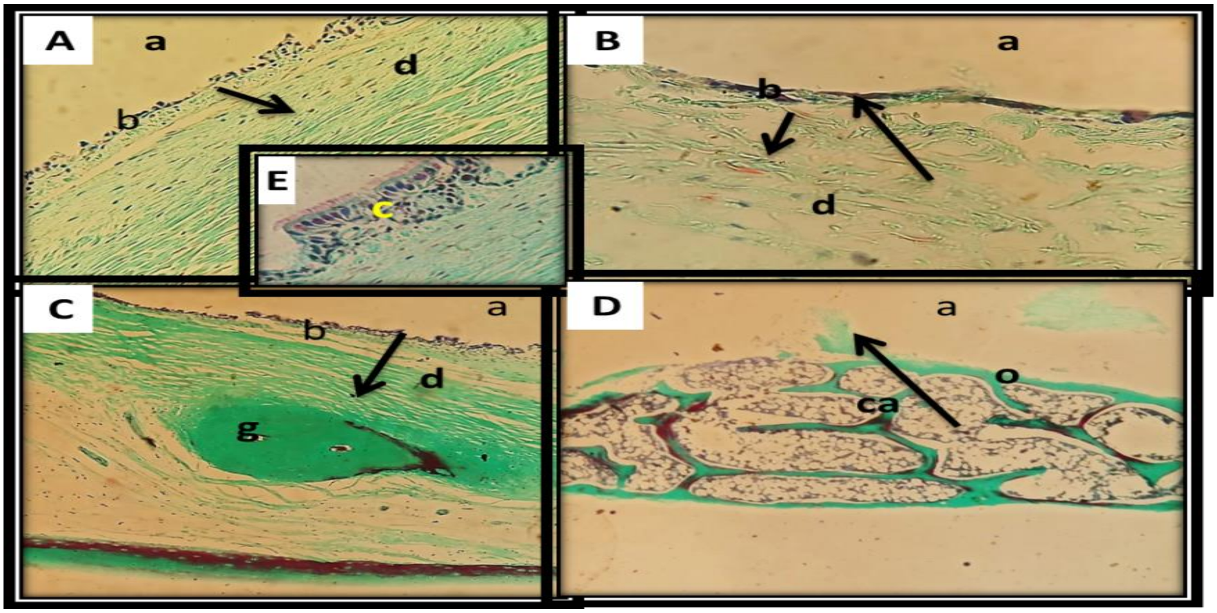


Fig. 5: cross section of A: trachiosyringeal ring: B: vibrating membrane: C: Bronchiosyringeal cartilage : D: pessulus, of swan goose shows : lumen of syrinx (a), lining epithelium pesudostraified columnar (b), intraepithelial glands (c), collagen fiber(black arrows) in loose connective tissue of lamina propria (d), hyaline cartilage (g) , ossified tissue (o) , ossification of hylaine cartilage (ca) . Masson trichrom stains. A,B,C,D:X200. E:X400.

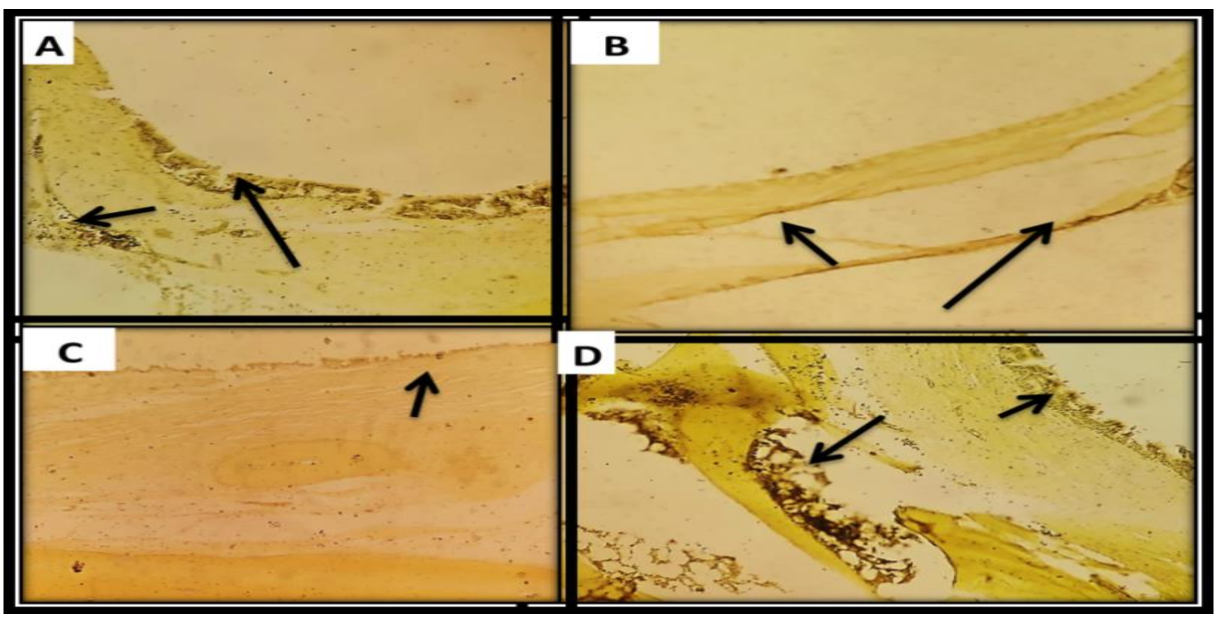


Fig. 6: cross section of A: trachiosyringeal ring : B: vibrating membrane : C: Bronchiosyringeal cartilage : D: pessulus, of swan goose shows : elastic fiber(black arrows) . Verhoffe van-gasin stains . A,B,C,D:X200.

However, there were large goblet cells arranged in groups in large numbers, which caused compression and bronchiosyringeal based cartilage dislodged with epithelial cells. Additionally, there were a small number of tubular mucous gland branches of the epithelia, that a positive reaction to the PAS stain (Fig. 2). Also positive reaction for alcian blue (Fig. 3) and combined alcian blue pas stains (Fig. 4). Pessulus, However, the hyaline cartilage was

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more ossified than the other syringeal cartilages, and a small bundle of collagen and elastic fibers was present in the submucosa (Fig. 5, 6). It had similar structures to those in the bronchiosyringeal cartilages.

## Discussion

Although histological descriptions of syringeal cartilage, the most recent histological studies on the syrinx in swan geese revealed ciliated, pseudostratified columnar epithelium with a considerable number of mucous glands and goblet cell groups of various sizes. In this study, histological analyses of the voice box (syrinx) of the swan goose revealed that the syringeal wall thickness was (1903.78±2.957 and 1902.5±1.476μm), for males and females, respectively. These findings are compatible with (8) in Chukar Partridge. They also differ with Hüseyin Yildiz et al (9) in the bursa roller pigeon. The submucosa and lamina propria were both made up of loose connective tissue. These perichondrium included the hyaline cartilages of the syrinx, and the findings partially correspond with those found by Hüseyin Yildiz et al (9) in bursa roller pigeons. In the measurements of the histological features of the syrinx components, the mean thickness of the epithelium was (204.56±2.231 and 198.92±1.513μm) in male and female birds, respectively. While the mean thickness of the cartilage was (207.78±1.601 and 199.12±1.661μm) in male and female birds, respectively. Moreover, the diameter of the syringeal mucous alveoli was 304.02±3.205 and 298.02±4.072μm in males and females respectively. The thickness of the hyaline cartilage in the bronchiosyringeal rings was referred to more than the thickness of the hyaline cartilage in the tracheosyringeal rings; however, the pessulus contains more thickness and fully ossified hyaline cartilages. These characteristics can be traced back to the pessulus, which serves as a supporting and connecting component of this organ. As one moves closer to the bronchiasyringesl cartilages, the number of mucus alveoli gradually decreases, but the number of mucus cells group increases in the same direction. This indicates that there is a significant quantity of mucins, which plays an important role in the body's defense mechanisms (10).

**Conclusion**

This study reveals the characteristic pictures of the syrinx of the swan geese, such as the distinct wall and epithelial layer thickness that are differ from those in different birds, that could be useful for future studies that deal with different sciences, such as public health and industries of this bird.

**Conflict of interest**

The study has no conflict of interest.

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