



Gross anatomical study on the syrinx of the ostrich (*Struthio camelus*) with special reference to its microscopic structure

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Abstract

Syrinxes of different sex and age ostrich were examined; six specimens applied for the anatomical demonstration of the syrinx where it was tracheobronchial type and formed by three groups of cartilages; tympanum, syringeotracheal and syringeobronchial which was denied to the pessulus and had no extending laterally for constructing neither osseous nor membranous syringeal bulla. It includes two types of folds; paired medial and lateral tympaniform membranes. It supported dorsally by areas of ossified cartilages of the tympanum and ventrally by bi-lobed - like syringeal fat. The other four samples used for histological examination where existed that their mucosa lined by ciliated pseudo stratified columnar epithelium with variable densities of goblet cells and intraepithelial mucous glands. Densely backed fibroelastic connective tissue rich in blood vessels was demonstrated in propria submucosa. Ventral tympanum tissue sections revealed submucosal hyaline cartilaginous plates that showed central zone of branched osseous tissue plates housing bone marrow spaces in between. The elastic fibers were demonstrated in between submucosal collagen fibers. There were focal areas of lymphoid aggregations of mucosal associated lymphatic nodules where evident underlying the lining epithelium at syringeobronchial level. Higher mucosal folding where demonstrated in the medial tympanic membrane than the lateral one.

Keywords: Ostrich. Syrinx. Anatomy. Microscopic. Structure

Introduction

The syrinx is the bird voice box and similar to the mammalian larynx but it lacks the vocal cords that vibrate with air currents (King 1989). Regarding to *Baumel et al. (1993)* there were three types of syrinxes; tracheal, tracheobronchial and bronchial, depending on the deviation of the cartilages of the larynx from either the trachea or the bronchi. The tracheal elements laid cranial to the bifurcation of the airway, while the bronchial elements laid at the most

cranial part of the right and left primary bronchi. The tracheobronchial syrinx has both tracheal and bronchial elements.

The great majority of birds were described as having a tracheobronchial syrinx, located at the tracheal bifurcation and consisted from three parts; tympanum, syringeotracheal and syringeobronchial cartilages as that stated by *Ragab et al. (2016)* in turkey, *Mariana*

et al. (2013) in rhea, *Khaksar et al. (2012)* in turkey, *Onuk et al. (2010)* in goose.

The syrinx mucosa formed from bistratified squamous as mentioned by (*Bacha and Linda 1990*) or columnar epithelium (*Campbell, 1995 and Benks, 1993*). Mucosal glands are present in lamina propria mucosae (*Hodges, 1974*). **Aim of our study;** highlight anatomical observations between parts of the ostrich syrinx and their histological features of different tissue sections that are considered as basic information for the sound production phenomena in ostrich and anatomical investigation for applying of different surgical approaches of corresponding organ.

Materials and Methods

Specimens

Ten specimens from ostrich of different weight and sex were examined to be free from any morphological distortion. Syrinx samples were collected from cadavers of slaughter houses of farm ostrich on the Alex desert road ostrich's farm.

Gross anatomical examination of the syrinx

Six of specimens were collected fresh and then fixed in 10% formalin. The syringes were dissected of different sections for anatomical variations then photographed. *Baumel et al. (1993)* was used for nomenclature.

Sample processing for histological observation

Another four samples used for histological examination where tissue samples were flushed and fixed in 10% neutral buffered formalin for 72 hrs. The dorsal aspect of the tympanum was immersed in 10% EDTA as a decalcifying agent for 2 weeks with daily change of the solution. Samples were trimmed, processed and dehydrated by serial grades of ethanol alcohol, cleared in Xylene, synthetic wax infiltration and embedded into Paraplast tissue embedding media. 5 μ sections were cut by rotatory microtome. The sections were stained with Harris Hematoxylin and Eosin as a general staining method for tissue examination and Masson's trichrome stain for demonstration of collagen fibers as outlined by (*Bancroft and Stevens 2010*). In addition to orcein stain for demonstration of elastic fibers according to *Luna (1968)*.

Results

The syrinx of ostrich (figs.1 and 3/8) was a large caudal larynx compared to other species of birds, it was considered as a tracheobronchial type where it oriented between the last tracheal ring and bifurcated two main principal bronchi (figs.1,2,3,4,5,6 and 7/7), dorsal to the base of the heart at the level of 2-3 thoracic vertebrae where it started cranially by the caudal border of the last tracheal ring and terminated caudally via the cranial border of the first bronchial cartilages. It consisted of three groups of cartilages; tympanum, syringeotracheal and syringeobronchial which was denied to the pessulus and had no extending laterally for constructing neither osseous nor membranous syringeal bulla. It included two types of voice producing folds; paired medial and lateral tympaniform membranes. It supported dorsally by areas of ossified cartilages of the tympanum and ventrally by bi lobed - like syringeal fat (figs.3 and 4/11).

The tympanum (cranial group of syringeal cartilages) (figs.2,3,and 5/3) were three large complete cartilaginous rings where they described as the first one was large thick overlapped dorsally and curved ventrally to meet at a midway of two free ends. The second tympanic cartilage was a complete cartilage which continued concave dorsally and run obliquely craniolaterally to meet two free ends ventrally. The third one was incomplete largest, thickest cartilage which met dorsally forming bony prominent ridge of free ends and passed craniolateral oblique to terminate ventrally at the cranial aspect of the ventral syringeal fat (figs.3 and 4/11) by slightly overlapped ends.

The syringeotracheal group (intermediate group of syringeal cartilages) (figs. 2, 5 and 6/4) were paired two C- shaped incomplete cartilages that oriented at the tracheal bifurcation point. The first one was the larger and starts from the dorsal border of the medial tympaniform membrane and converged laterally to attache ventromedially at the ventral aspect of the commencement of the corresponding membrane and connected cranially to the caudal border of the third cartilage of the tympanum by the thin syringeotracheal membrane. The second cartilage was the smaller and attaches with the first syringeotracheal cartilage cranially and the cranial border of the first bronchial cartilage caudally by syringeotracheal membrane. The two cartilages connected medially by the medial tympaniform membrane.

The syringeobronchial cartilages (caudal group of syringeal cartilages) (figs. 2, 4, 5 and 6/5) were small paired three half rings. According to their diameter; they were larger than the incomplete bronchial rings and the second syringeotracheal cartilage. They attached with each other on each side of the syrinx laterally by the lateral tympaniform membrane and supported medially by the medial tympaniform fold.

The lateral tympaniform membranes (figs. 4 and 5/12) were paired; right and left. They were considered as thin transparent folds insinuated between the last syringeotracheal, the syringeobronchial and the first

bronchial cartilages. They oriented by the concurrent cartilages and forming the lateral wall of the syrinx.

The medial tympaniform membranes (figs. 2, 4, 6 and 7/6) were double paired; right and left and they represented as a whole medial wall of the syrinx where they attached with the free ends of the last tympanic, syringeotracheal, syringeotracheal cartilages. They came in contact cranially with the caudal border of the tympanum and terminated caudally at the junction between the interbronchial and medial bronchial ligaments.

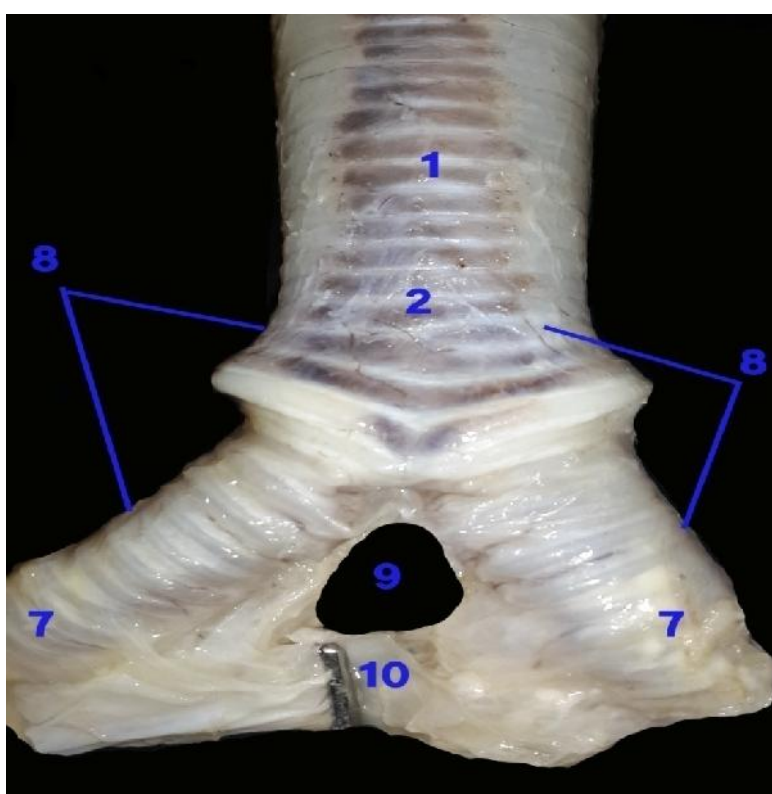


Fig. 1 Photograph showing dorsal view of the ostrich syrinx.

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|---|---------------------|----|--------------------------|
| 1 | Trachea. | 8 | Syrinx. |
| 2 | Last tracheal ring. | 9 | Foramen interbronchiale. |
| 7 | Bronchus primarius. | 10 | Lig. Interbronchiale. |

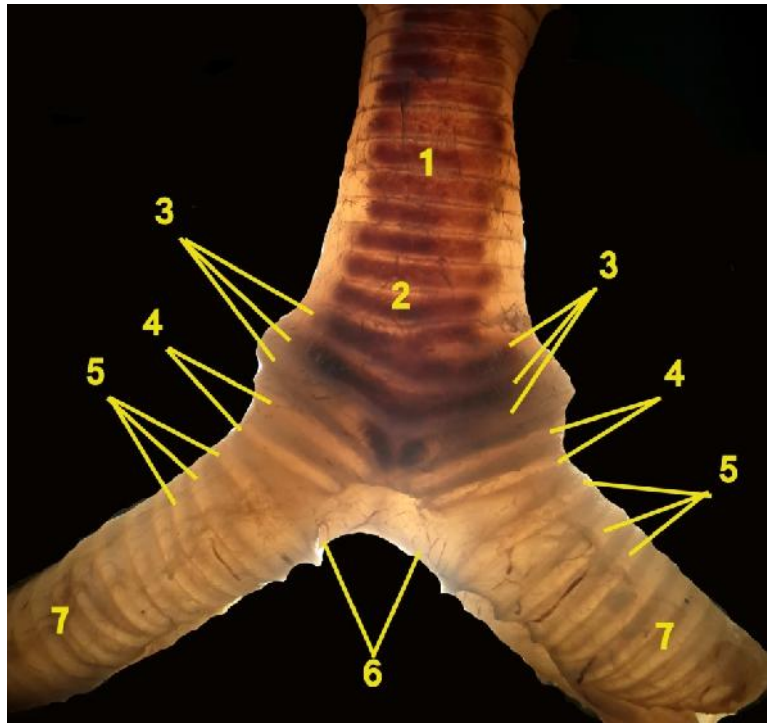


Fig. 2 Photograph showing negative x- ray of the dorsal view of the ostrich syrinx.

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|---|---------------------|---|-----------------------------|
| 1 | Trachea. | 5 | Cartt. Tracheosyringales. |
| 2 | Last tracheal ring. | 6 | Mem. tympaniformis medialis |
| 3 | Bronchus primarius. | 7 | Bronchus primarius. |
| 4 | Tympanum. | | |

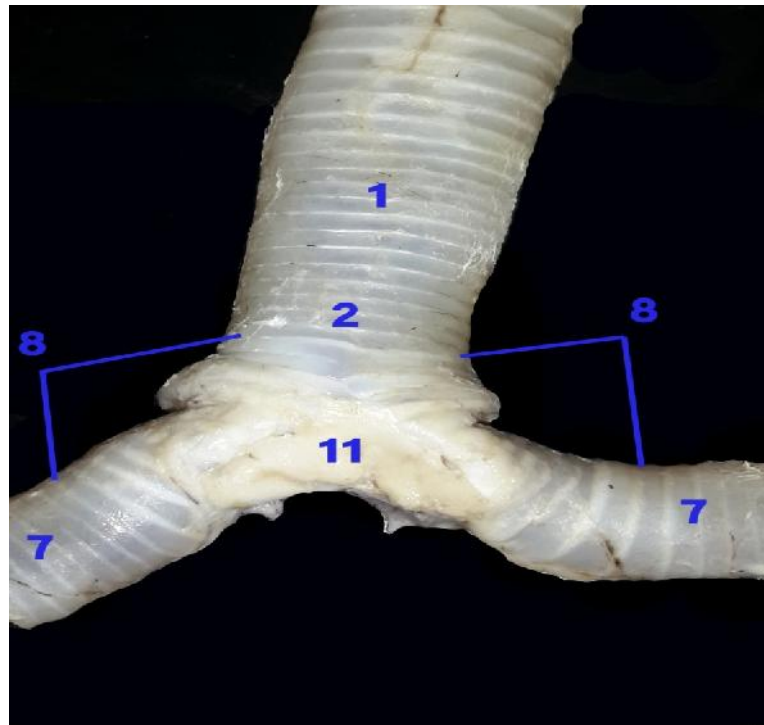


Fig. 3 Photograph showing ventral view of the ostrich syrinx.

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|---|---------------------|----|--------------------------------|
| 1 | Trachea. | 8 | Syrinx. |
| 2 | Last tracheal ring. | 11 | Adipose syringealis ventralis. |
| 7 | Bronchus primarius. | | |

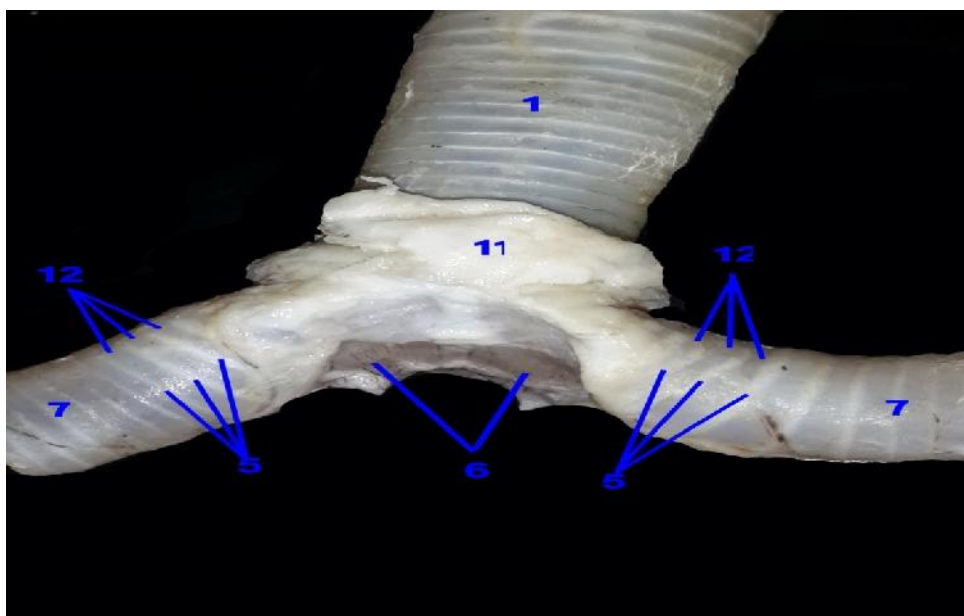


Fig. 4 Photograph showing ventral view of the ostrich syrinx after reflex of the ventral syringeal fat.

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|---|-----------------------------|----|--------------------------------|
| 1 | Trachea. | 7 | Bronchus primarius. |
| 5 | Cartt.Tracheosyringaeales. | 11 | Adipose syringealis ventralis. |
| 6 | Mem. tympaniformis medialis | 12 | Mem. tympaniformis lateralis. |

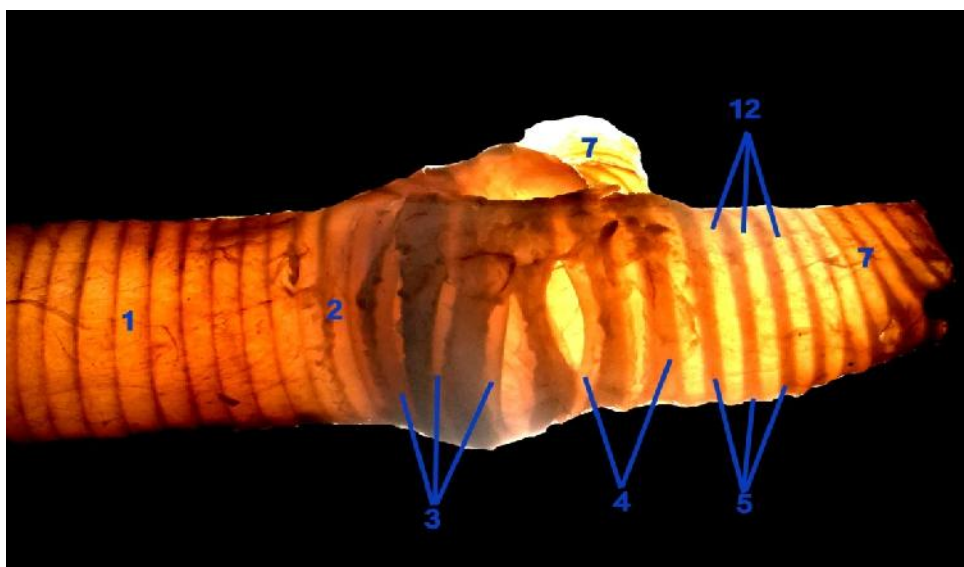


Fig. 5 Photograph showing negative x-ray of the left lateral view of the ostrich syrinx.

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|---|---------------------|----|-------------------------------|
| 1 | Trachea. | 5 | Cartt.Tracheosyringaeales. |
| 2 | Last tracheal ring. | 7 | Bronchus primarius. |
| 3 | Bronchus primarius. | 12 | Mem. tympaniformis lateralis. |
| 4 | Tympanum. | | |

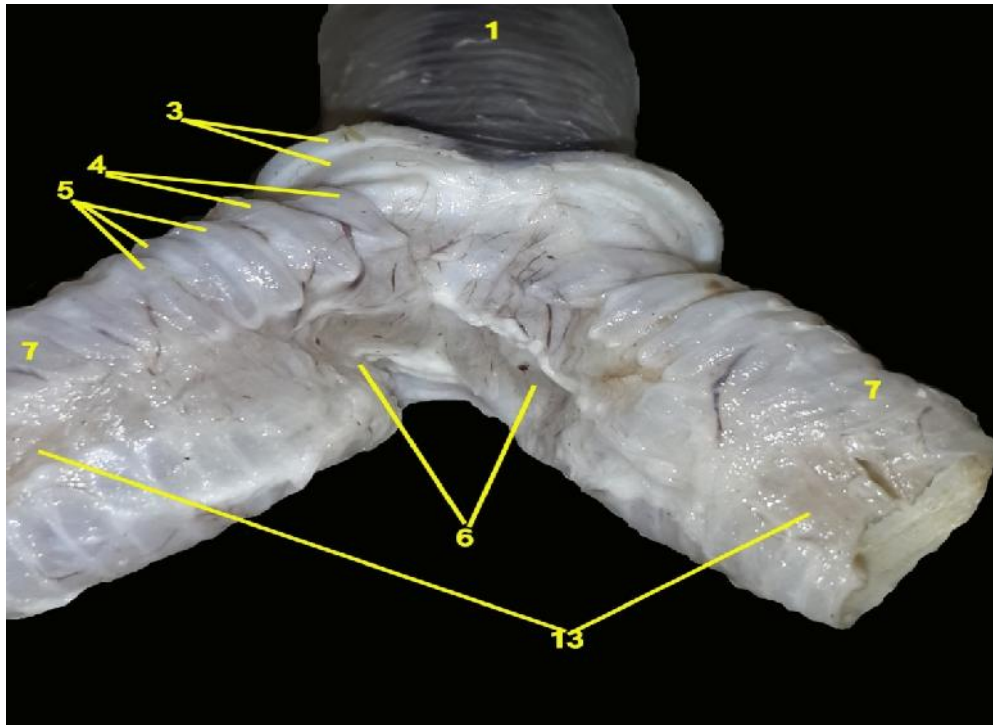


Fig. 6 Photograph showing caudal view of the ostrich syrinx.

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|---|--------------------------|----|-----------------------------|
| 1 | Trachea. | 6 | Mem. tympaniformis medialis |
| 3 | Bronchus primarius. | 7 | Bronchus primarius. |
| 4 | Tympanum. | 13 | lig. bronchiale Mediale. |
| 5 | Cartt.Tracheosyringales. | | |

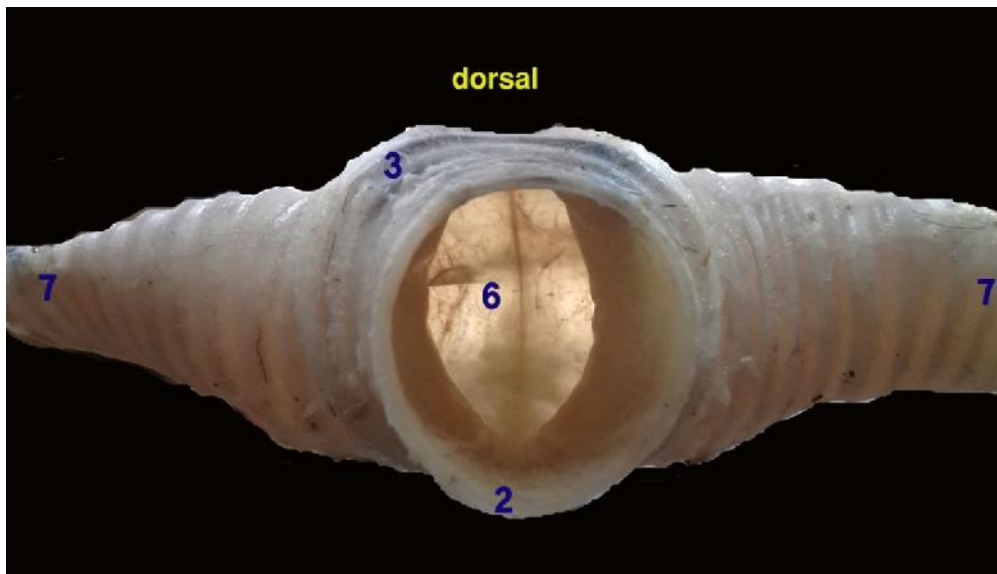


Fig. 7 Photograph showing the internal view of the ostrich syrinx.

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|---|---------------------|---|-----------------------------|
| 2 | Last tracheal ring. | 6 | Mem. tympaniformis medialis |
| 3 | Bronchus primarius. | 7 | Bronchus primarius. |

Microscopic examination of the syrinx at different levels revealed that the mucosa lined by ciliated pseudo stratified columnar epithelium with variable densities of goblet cells and intraepithelial mucous glands (plates 1, 2, 3 and 4). Densely backed fibroelastic connective tissue rich in blood vessels (plates 1, 2 and 4) was demonstrated in propria submucosa and covered externally by loose connective tissue of tunica adventitia. Dorsal and ventral tympanum tissue sections revealed submucosal hyaline cartilaginous plates (plate2), where the dorsal one showed central zone of branched osseous tissue plates housing bone marrow spaces in between (plate1). The elastic fibers were demonstrated in between submucosal collagen fibers. However; higher density of elastic fibers was oriented in deeper part of

the submucosa, intercartilagenous spaces (plate 1, 2 and 4) as well as perichondrial connective tissue. Syringeotracheal and syringeobronchial cartilages showed general histological structures of syrinx in addition to focal areas of lymphoid aggregations of mucosal associated lymphtic nodules (plate 3) where evident underlying the lining epithelium at syringeobronchial level. The lateral and medial tympanic membranes consisted of fibroelastic connective tissue containing many blood vessels and covered by pseudo stratified columnar ciliated epithelium with many goblet cells and intraepithelial mucous glands. Higher mucosal folding (plate 4) where demonstrated in the medial tympanic membrane than the lateral tympanic one.

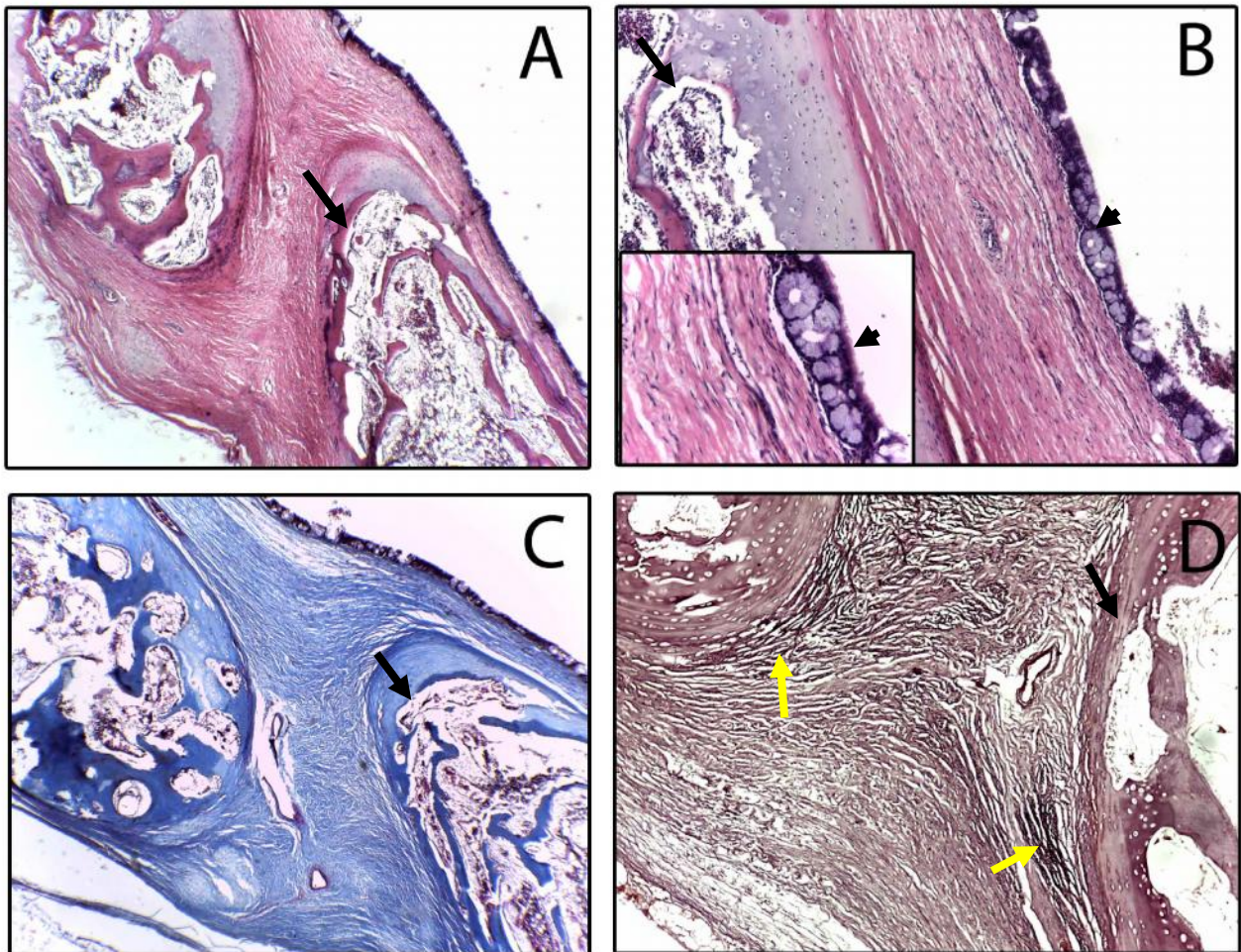


Plate 1 Showing dorsal tympanum tissue sections. A) H&E stain at 40X. B) H&E stain at 100X with inset box at 400X demonstrating intraepithelial mucous glands. C) Masson's trichrome stain at 40X. D) Orcein stain at 100X. Hyaline cartilages plates with central zone of branched osseous tissue housing bone marrow spaces (Arrows), Pseudostratified columnar ciliated epithelium rich with intraepithelial mucous glands (arrow heads). submucosal and intercartilagenous spaces rich in elastic fibers (yellow arrows)

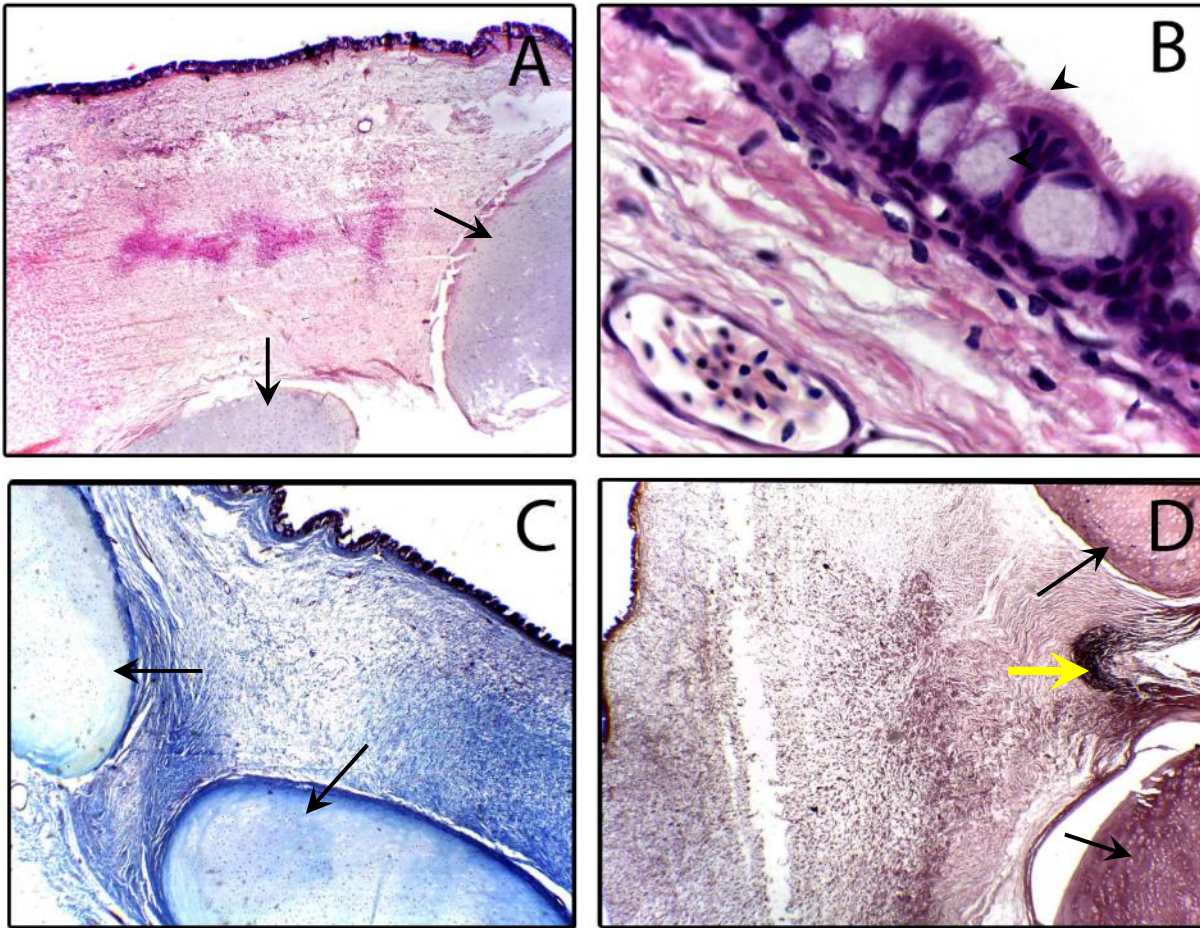


Plate 2 Showing ventral tympanum tissue sections. A) H&E stain at 40X. B) H&E stain at 400X. C) Masson's trichrome stain at 40X. D) Orcein stain at 40X. Hyaline cartilages plates (Arrows), Pseudostratified columnar ciliated epithelium rich in goblet cells (arrow head). submucosal and intercartilagenous spaces rich in elastic fibers (yellow arrows)

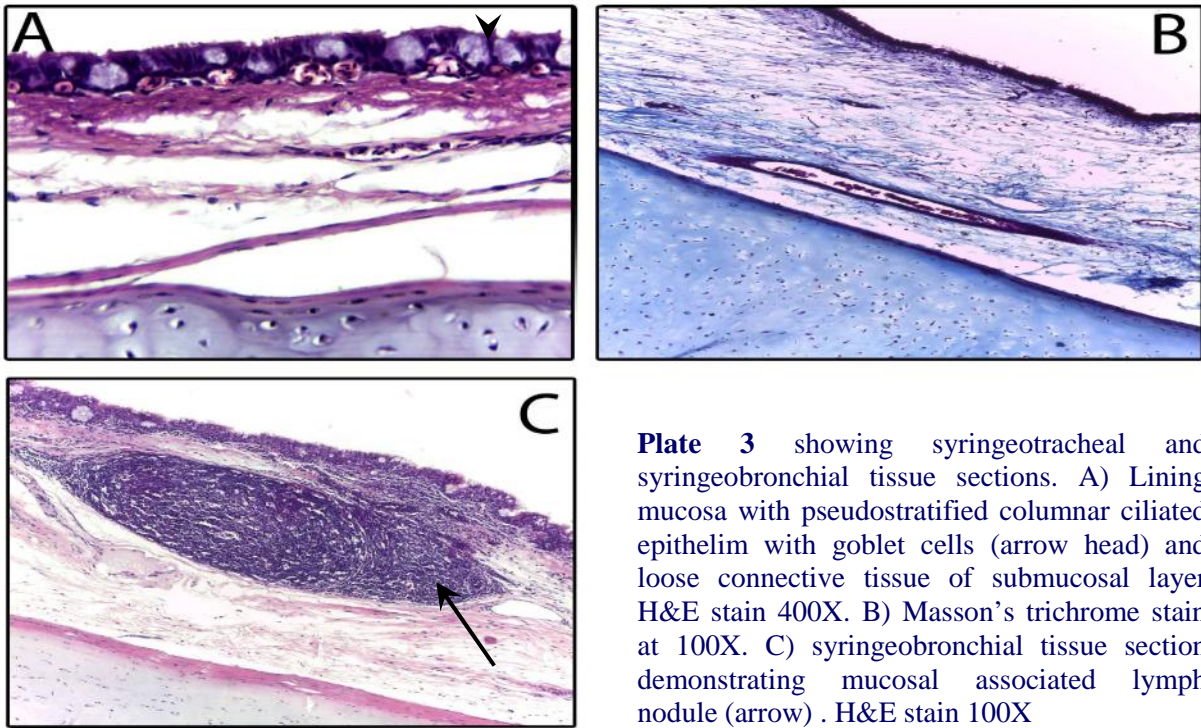


Plate 3 showing syringeotracheal and syringeobronchial tissue sections. A) Lining mucosa with pseudostratified columnar ciliated epithelium with goblet cells (arrow head) and loose connective tissue of submucosal layer H&E stain 400X. B) Masson's trichrome stain at 100X. C) syringeobronchial tissue section demonstrating mucosal associated lymph node (arrow) . H&E stain 100X

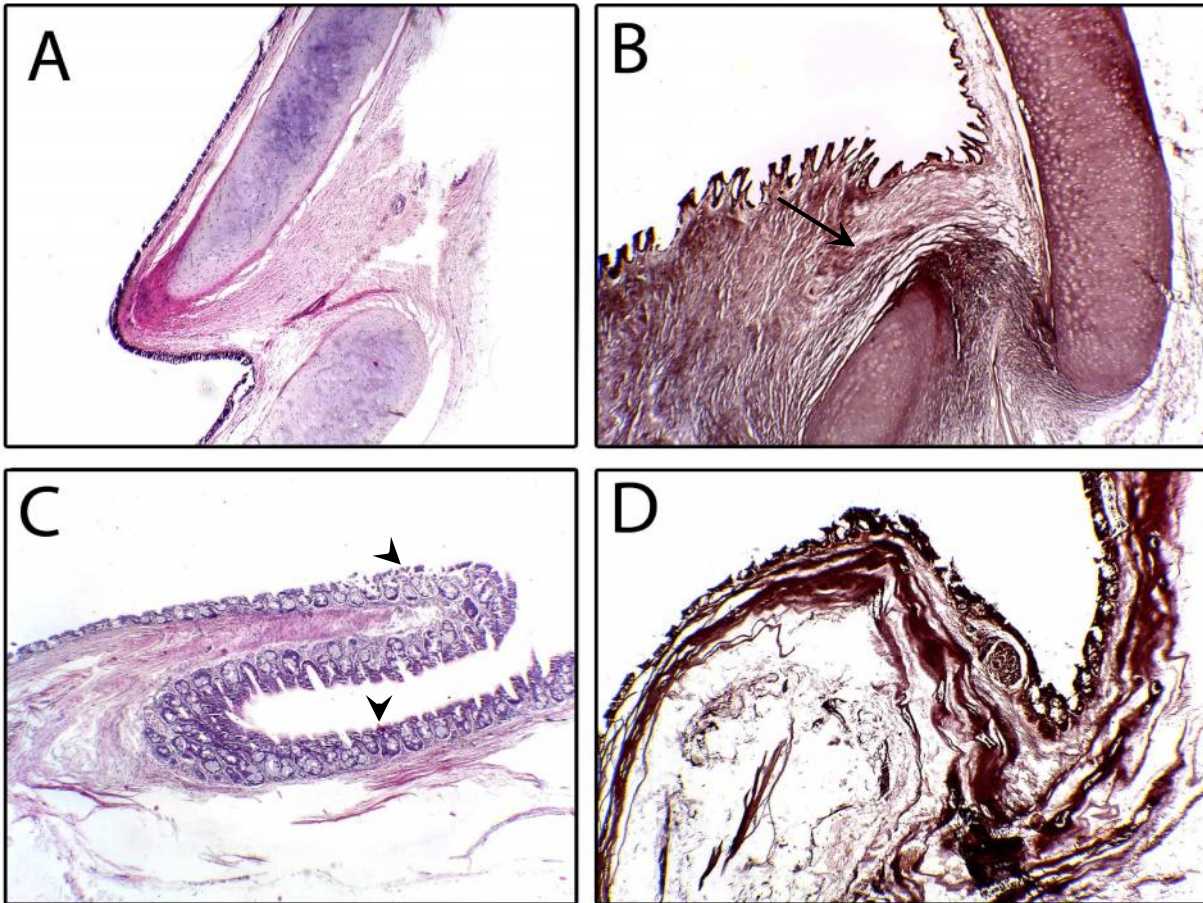


Plate 4 showing A) lateral tympanic Membrane H&E 40X. B) Lateral tympanic Membrane Rich in fibroelastic connective tissue (arrow), Orcein stain 100X. C) Medial Tympanic Membrane Lined by pseudo stratified columnar ciliated epithelium with many goblet cells and intraepithelial mucous glands With Higher mucosal folding (arrow head) H&E 40X. D) Medial tympanic Membrane with Orcein stain 100X.

Discussion

In our study the syrinx of ostrich is a large caudal larynx compared to other species of birds, was oriented between the last tracheal ring and bifurcated two main principal bronchi, dorsal to the base of the heart at the level of 2-3 thoracic vertebrae where it started cranially by the caudal border of the last tracheal ring and terminated caudally via the cranial border of the first bronchial cartilages. These results were similar to that stated by (*Ragab et al., 2016*) in turkey, (*Khaksar et al., 2012*) in turkey, (*Onuk et al., 2010*) in goose, (*Yildiz et al., 2003*) in male ostriches.

According to the composition of the ostrich syrinx; it consisted of three groups of cartilages; tympanum, syringeotracheal and syringeobronchial. These statements were simulated to that mentioned by (*Ragab et al., 2016*) in turkey, (*Mariana et al., 2013*) in rhea, (*Khaksar et al., 2012*) in turkey, (*Onuk et al., 2010*) in goose, (*Yildiz et al., 2003*) in male ostriches. On other hand (*Erdogan et al., 2014*) in chukar partridge said that the syrinx was formed by the last two tracheal and first eight bronchial cartilages.

All available literatures revealed that the syrinx of different birds had a pessulus except ostrich (*Illanes et al., 2014*) as well as (*Frank et al., 2007*) and (*King and Mclelland, 1984*) in the male duck added that the syrinx contained the osseous bulla. These statements in centrally with that found by our examined syrinx where it denied to the pessulus and had no extending laterally for constructing neither osseous nor membranous syringeal bulla in addition to it supported dorsally by areas of ossified cartilages of the tympanum and ventrally by bi lobed - like syringeal fat.

Regarding to the tympanum were three large complete cartilaginous rings where the first one was large thick overlapped dorsally and curved ventrally to meet at a midway of two free ends. The second tympanic cartilage was a complete cartilage which continues concave dorsally and runs obliquely cranio-laterally to meet two free ends ventrally. The third one is incomplete largest, thickest cartilage which met dorsally forming bony prominent ridge of free ends and passed cranio-lateral oblique to terminate ventrally at the cranial aspect of the ventral syringeal fat by slightly overlapped ends. These results were in a line with the description that declared by (*Ragab et al., 2016*) in turkey and (*Yildiz et al., 2003*) in male ostriches. While (*Mariana et al., 2013*) in rhea, (*Kabak et al., and Cevik-dermikan et al., 2007*) in the

chicken, (*Yildiz et al., 2005*) in the pigeon, (*Bettina and Pablo, 2001*) in new world turkeys recorded that the corresponding part of the syrinx was composed of four cartilages. On the other hand (*Khaksar et al., 2012*) in turkey and (*Onuk et al., 2010*) in goose cited that the tympanum was consisted of two rings.

Concerning to the syringeotracheal group of the studied syrinx were paired two C- shaped incomplete cartilages that oriented at the tracheal bifurcation point. The first one was the larger and started from the dorsal border of the medial tympaniform membrane and converged laterally to attach ventromedially at the ventral aspect of the commencement of the corresponding membrane and connected cranially to the caudal border of the third cartilage of the tympanum by the thin syringeotracheal membrane. The second cartilage was the smaller and adhered with the first syringeotracheal cartilage cranially and the cranial border of the first bronchial cartilage caudally by syringeotracheal membrane. The two cartilages connected medially by the medial tympaniform membrane. These observations come in contact with results that observed by (*Ragab et al., 2016*) in turkey. While the current part of the syrinx was formed from two complete rings that mentioned by (*Khaksar et al., 2012*) in turkey and (*Onuk et al., 2010*) in goose. On the other hand it was composed of four complete rings by (*Yildiz et al., 2003*) in male ostriches. However (*Kabak et al., 2007*) and (*Cevik-dermikan et al., 2007*) in chicken and (*Yildiz et al., 2005*) in pigeon, (*Yildiz et al., 2003*) in ostrich, (*Bettina and Pablo, 2001*) in world turkey observed that the corresponding part was consisted of Four C- shaped rings.

The results under discussion achieved that the syringeobronchial cartilages were small paired three half rings. These observed data were matched with that noted by (*Ragab et al., 2016*) in turkey and (*Yildiz et al., 2003*) in male ostriches. Although these statements were disagreed with the opinions of (*Mariana et al. (2013)*) in rhea and (*Khaksar et al., 2012*) in turkey where they were four C-shaped bronchosyringeal rings on each side. Further the more, (*Onuk et al., 2010*) in goose reported that the bronchosyringeal cartilages were formed from six pairs of 'C' shaped components.

In our findings reported that the lateral tympaniform membrane was considered as thin transparent fold insinuated between the last syringeotracheal, the syringeobronchial and the first bronchial cartilages where it was forming the lateral wall of the syrinx. These results were correlated with that declared by

(Ragab *et al.*, 2016) in turkey, (Yildiz *et al.*, 2003) in male ostriches, (Mariana *et al.*, 2013) in rhea and (Khaksar *et al.*, 2012) in turkey.

The results applied in our data revealed that the medial tympaniform membrane was a double and represented the whole medial wall of the syrinx where it was attached with the free ends of the last tympanic, syringeotracheal, syringeotracheal cartilages. It come in contact cranially with the caudal border of the tympanum and terminated caudally at the junction between the inter-bronchial and medial bronchial ligaments. This description was in respect to that coincided by (Mariana *et al.*, 2013) in rhea and (Yildiz *et al.*, 2003) in male ostriches. On the other hand these statements were in disagreement with that described by (Ragab *et al.*, 2016) and (Khaksar *et al.*, 2012) in turkey where the corresponding membrane was just a well developed membrane which it was stretched from the caudo-lateral wall of the pessulus and the caudal end of inter-bronchial ligament. Although (Onuk *et al.*, 2010) in goose mentioned that the current membrane was connected the pessulus and the second syringeobronchial cartilage.

Although the histological features of syrinx were studied in birds with special emphasize to chicken. However; few studies were published focused on ostrich species. The current study demonstrated the main histological features of syrinx of the ostrich at different levels. The Syringeal wall of the ostrich was formed of mucosa, submucosa and adventitia as a general structure like other birds species. The mucosa was lined by respiratory epithelium formed from pseudostratified columnar ciliated with goblet cells in agreement with (Campbell, 1995; Benks, 1993 ; Hodges, 1974) and in chicken. Vast amount of intraepithelial tubuloalveolar mucous glands were observed along syringeal different parts. These results were in agreement with (Juolio *et al.*, 2014) and (Yildiz *et al.*, 2003). These finding may be related to environmental adaptation to produce large amount of mucous as a protective physical barrier for trapping of inhaled foreign particles as well as helping in regulate humidity of inspired air and body temperature. In agreement with (Juolio *et al.*, 2014) solitary subepithelial lymph nodules were detected in syringeobronchial level which plays an important protective role as a member of mucosal associated lymphatic tissue. Submucosal connective tissue was rich in collagened and elastic fibers as mentioned by (Juolio *et al.*, 2014) and (Yildiz *et al.*, 2003). However; in the current study deeper part of the

submucosa, intercartilagenous spaces as well as perichondrial connective tissue express higher positive reactivity to orcein stain which demonstrated higher amount of elastic fibers in these zones. Presence of more elastic fibers at the previous mentioned regions impacted for flexibility role is needed for membranes and cartilage movement which is needed in sound production process.

Our examined specimen revealed obvious presence of central zone from branched ossified plates housing bone marrow spaces inside the submucosal hyaline cartilages at the level of dorsal aspect of tympanum. These results were not recorded in any of the available literatures.

In conclusion, the corresponding study explained the gross anatomical and histological observations that are considered as a basic guide lines for; sound production phenomena of ostrich, diagnosis and surgical approach treatment of any affections that introducing to ostrich syrinx.

Conflict of interest

The authors declare that there is no conflict of interest.

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