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Comparative Macro- and Micro-morphological Study on the Syrinx of Three Avian Species

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ABSTRACT

This study aimed to explain and compare the anatomical, histological, histochemical & histomorphometric analyses of the syrinx in different species of birds. This study includes 21 healthy birds from geese (Anser Anser Domesticus), cattle egrets (Bublucus ibis) & house sparrows (Passer domesticus), seven from each species. Results: Anatomically, the syrinx of the three studied species was tracheobronchial type composed of 2 different cartilaginous groups arranged in three parts: Tracheo-Syringeal cartilage in (cranial, intermediate part (tympanum)) and broncho-syringeal cartilage; as well as the trachea's muscles were once thought to be the syrinx's extrinsic muscles. Histologically, the lamina epithelialis of the Tracheo-Syringeal part was represented by pseudostratified ciliated columnar epithelium in the three investigated species with goblet cells in geese, house sparrows and lack goblet cells in cattle egrets. The pessulus in all three investigated species was triangular and positioned at the tip of the tracheal bifurcation. The bulla of house sparrow was considered the intrinsic syringeal muscles and these muscles in sparrows are not only present in geese or cattle egrets.

1. INTRODUCTION

Birds are classified as passerine and non-passerine (Fitch, 1999). The syrinx is a vocal organ, located at the base of the trachea in songbirds (Yıldız et al., 2003). The syrinx in birds is not responsible for only sound production but also for mating behaviors, in sex determination, sorting of bird species, and determination of their phylogenetic positions (Gaban-Lima and Heofling 2006). * Anatomically, the features of syrinx either morphological or structural are widely inconstant between different species of birds (Ibrahim et al., 2020). Generally, the syrinx is observed between the trachea and primary bronchi (Yıldız et al., 2003) at the level of the second and third thoracic vertebrae (Dursun, 2002). The syrinx comprises specialized membranes, cartilaginous structures, connective tissue masses, and several muscles (Larsen and Goller, 2002). Generally, the syrinx consists of tympanum or tracheosyringeal cartilage in the shape of a "C" and bronchosyringeal ones in the shape of half-rings. The syrinx consists of three parts of hyaline cartilage: tracheal, bronchial, and tracheobronchial according to their origin (Frank et al., 2007). In most avian species, sound production is only controlled by the extrinsic muscles of the syrinx, M.Sternotrachialis , M. Trachiolateralis, which constitute the syrinx and trachea properly for the phonation process (Brackenbury, 1989). In some bird groups such as Passeriformes and Psittaciformes, there are self-regulating sets of muscles limited to the syrinx known as syringeal muscles or intrinsic muscles of the syrinx (King, 1989). *Histologically, the cranial (tracheosyringeal), intermediate (tympanic), caudal (bronchosyringeal) parts of the syrinxes in avian species are formed of mucosa, syringeal cartilages, and syringeal muscles (Ibrahim et al., 2020).In avian species, there are differences in histology of the syrinx mucosa's epithelium since this epithelium consists of double-layer squamous or columnar or ciliated pseudostratified columnar epithelium according to its structure in syrinx either tracheosyringeal or bronchosyringeal part (Campbell, 1995). In most bird species, the lamina propria and the submucosa connected to form

Propria-Submucosa that consists of loose connective tissue with compact elastic fibers as in ostriches (Yildiz et al., 2003), and Japanese quail (Cevik-Demirkan et al., 2007).

2- MATERIALS AND METHODS

2.1- Animals and Tissue samples; In this study, 21 healthy birds (seven per species). geese (Anser Anser Domesticus) weights ranging from 2500 to 3000 g were purchased from a commercial poultry in Zagazig city, Sharkia province. Cattle egrets (Bubulcus ibis) and house sparrows (Passer domesticus), were caught from the local surroundings. Immediately after bringing the birds, they were trapped in well-aerated cages and moved to the Department of Histology and Cytology, Faculty of Veterinary Medicine, Zagazig University. Then, under observation for 2 days, the birds were kept before the beginning of tissue harvesting to enable them to adapt to their environment.

2.2- Tissue Preparation (Ethical consideration):- All birds were euthanatized, after which they were retained on their backbone, an opening made from the posterior part (vent) to the superior one (shoulder joint) laterally, then the related bones and muscles imitated, giving access to the viscera such that the whole respiratory system could be pictured, and its relationship with adjacent organs observed after retracting the trachea and getting rid of any tissues as adipose tissue to record structural measuring. This study protocol was evaluated and accepted by the Institutional Animal Care and Use Committee (IACUC) of Zagazig University, Egypt. (ZU-IACUC/ 2/F/67/2021).

2.3- Morphometrical analysis: Show the site of the syrinx in three studied species, examine the differences, and count the number of the cartilaginous syringeal rings in the three parts (tracheal, intermediate, and bronchial cartilage).

2.4- Histological study: The trachea was fixed in Bouin's solution for 6-10 hours to protect the mucous secretory cells from damage, after which the samples were collected immediately and placed in 10% neutral buffer formalin for one week. The samples were decalcified by (EDTA), dehydrated in a series of ascending Grade of ethanol, cleared with xylene (three changes), infiltrated in soft melted paraffin in a hot air oven, and embedded in hard paraffin wax to form paraffin blocks. The specimens were processed to obtain a desirable paraffin section of 5- 7 µm thickness by using a microtome. Some achieved sections were stained with usual staining; 1) As a general stain the (H & E) Harris's hematoxylin and eosin. 2) Crossmon's trichrome stain for detection of Collagen fibers. 3) (AB) Alcian Blue stain to detect acidic mucopolysaccharides. 4) The Periodic Acid Schiff (PAS) technique is used to detect neutral mucopolysaccharides and some acidic ones. 5) orcein stain for elastic fibers. The handling with tissue and staining methods were conducted consistent with Suvarna et al., (2019).

2.5- Statistical analysis: The Anderson–Darling test was used to confirm that all numerical data was evaluated for normality. The SPSS software (v.16) was used for statistical assessment. The information was stated as mean ± standard error (SEM). The Mann–Whitney U test was used to determine the significance of the disparities between AB and PAS optical densities among the three investigated species. To expose significances between the three species in the epithelial height and the area percentage of the collagen fibers, the Kruskal–Wallis H test was done.

3- RESULTS

3.1- Macroscopically:

The syrinx of the studied birds was found in the thoracic cavity, which is situated between the trachea last portion and the first part left and right primary bronchi (Fig.1). However; the anatomical positions were different. Since the syrinx of geese hidden by the heart, the heart must be detached to observe the syrinx (Fig.1 a, b). Also, the syrinx in cattle egrets (Fig.1 c) and house sparrows (Fig.1 d) was observed dorsal to the base of the heart. The syrinx type was tracheobronchial composed of 2 different cartilaginous groups arranged in three parts: Tracheo-Syringeal cartilage in (cranial, intermediate part (tympanum)) and broncho-syringeal cartilage. In the three studied species, the syrinx has two membranes the medial tympaniform membrane and the lateral tympaniform membrane (Fig. 2,3 & 4). In geese, the tracheosyringeal cartilage was composed of six rings. The first two rings had clear borders, but the rest were completely fused and ossified to form the tympanum. The last four tracheosyringeal cartilaginous rings constituted the intermediate syringeal part (tympanum) which appeared narrow (Fig. 2). The broncho-syringeal cartilage was made up of six pairs of complete rings, with the first two were 'C' shaped (Fig. 2a). The medial tympaniform membrane connected from the pessulus to the second broncho-syringeal cartilages while the lateral tympaniform membrane attached to the caudal process of dorsal and ventral tympanum (Fig. 2b). A foramen called (inter-bronchial foramen) exists between two bronchi in geese which is confined rostrally by the pessulus and caudally by inter-bronchial ligament (Fig.2). In cattle egret, the cranial part of tracheosyringeal cartilages was composed of four rings and three rings

forming the intermediate part (tympanum), the first of which is a complete ring, and the other two being c- shaped cartilage (Fig.3). The broncho-syringeal cartilages were made up of four pairs of half rings ('C' shape) (Fig.3a). The medial tympaniform membrane lasted from the pessulus to the fourth broncho-syringeal cartilage and there was no lateral tympaniform membrane or ill-developed (Fig.3b). The tracheo-syringeal cartilages of house sparrow (songbird) were comprised of five rings that are separated by fibrous connective tissue. The number of rings that form the intermediate part or tympanum could not be determined certainly in sparrows. Due to the presence of extensively modified bilaterally symmetrical dilation called the syrinx bulla (Fig. 4). Six pairs of complete rings formed up the broncho-syringeal cartilages. Because of the syrinx bulla, the medial and lateral tympaniform membranes did not appear macroscopically.

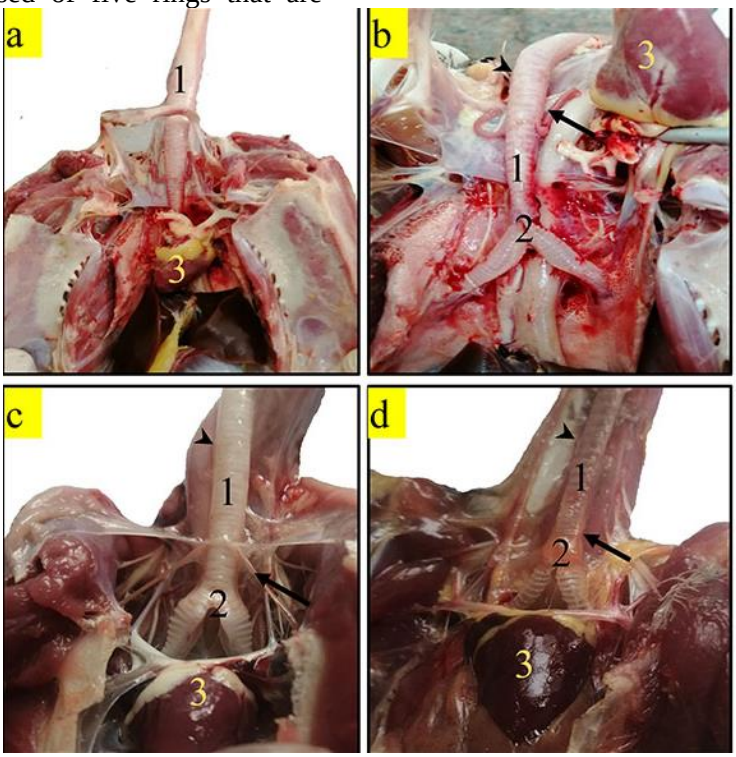

Fig. 1. In situ ventral views of the syrinx in the male domestic geese (a, b), cattle egrets (c) and house sparrows(d), showing trachea (1), syrinx (2), heart (3), Trachiolateralis muscles (arrowhead (and Sternotrachialis muscles (arrows).

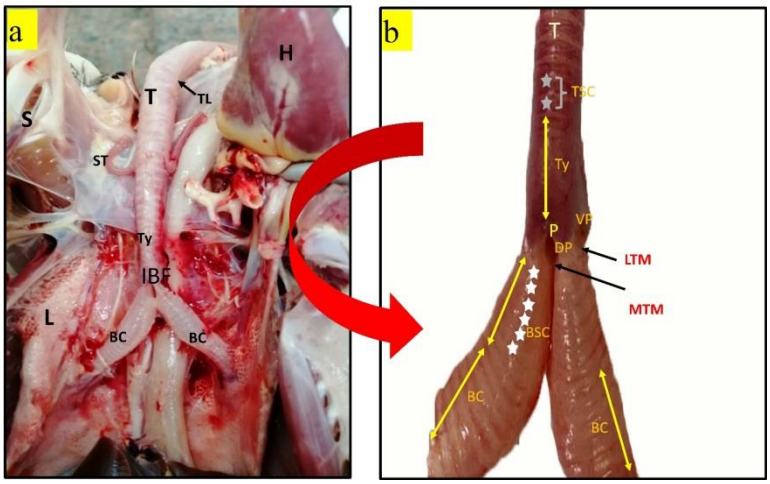

Fig. 2. Ventral view of syrinx and its surrounding of male domestic geese (a). (b) Showing the different parts of syrinx, trachea (T), tracheosyringeal cartilages (TSC), tympanum (Ty), pessulus (p), dorsal process (DP), ventral process (VP), lateral tympaniform membranes (LTM), medial tympaniform membranes (MTM), bronchosyringeal cartilages (BSC), bronchial cartilages (BC), sternum(s), lung (L), intrabronchial foramen (IBF), the heart (H), Trachiolateralis muscles (TL) and sternotrachialis muscles (ST).

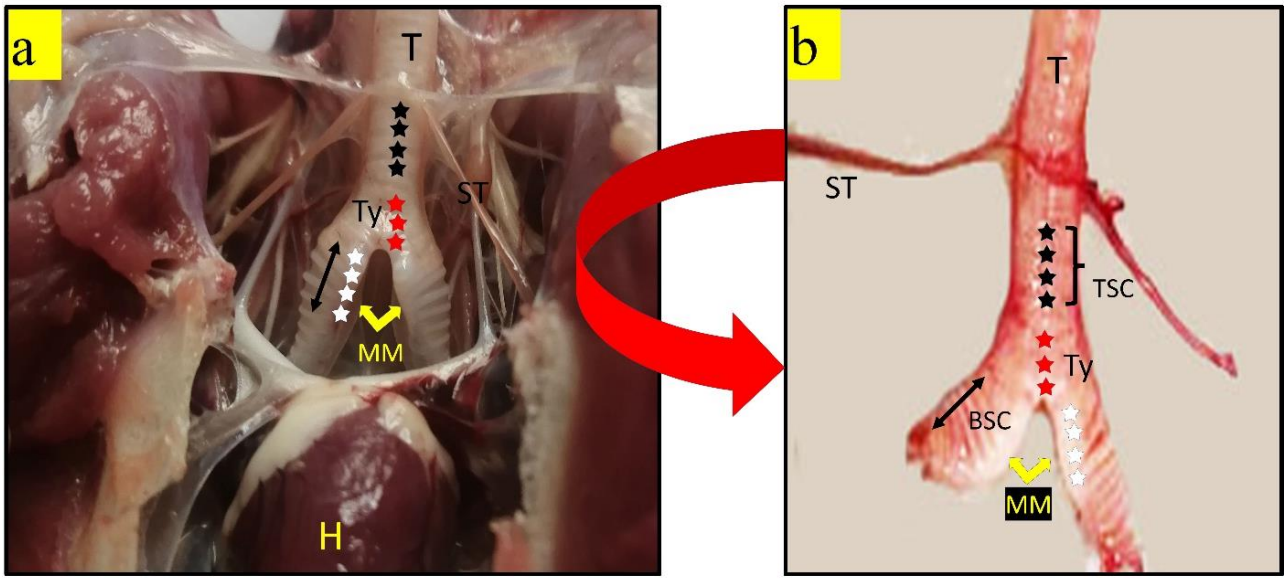

Fig. 3. Ventral view of syrinx of male cattle egrets (a, b) showing different parts of syrinx, trachea (T), tracheosyringeal cartilages (TSC), tympanum (Ty), medial tympaniform membranes (MTM), bronchosyringeal cartilages (BSC), the heart (H) and sternotrachialis muscles (ST).

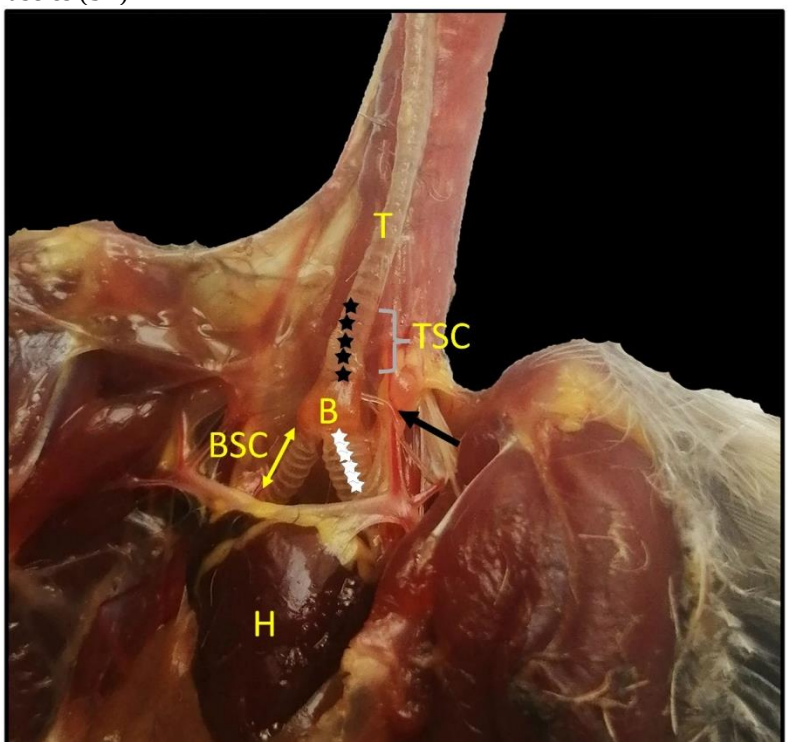

Fig. 4. A ventral view of male house sparrow's syrinx. Showing different parts of syrinx, trachea (T), tracheosyringeal cartilages (TSC), broncho syringeal cartilages (BSC), bulla tympaniformis (B)

3.2- Microscopically:

Tracheosyringeal cartilages: The cranial and intermediate parts appeared as one part as they have the same structure of mucosa and cartilage in geese (Fig. 5a) and cattle egrets (Fig. 5b). While the two parts of house sparrow were different from which cartilage but similar in mucosa. In the cranial part, the rings were hyaline cartilage or tended to ossification (Fig. 5c), and ossified cartilage in tympanum (Fig. 5d). The cranial and intermediate (tympanum) parts were formed of mucosa, syringeal cartilages, and syringeal muscles in sparrows only (Fig. 5c, d). The lamina epithelialis was represented by pseudostratified ciliated columnar epithelium with goblet cells in geese (Figs. 6A), in house sparrows (Figs. 6C) and lack goblet cells in cattle egrets (Figs. 6B). Statistically, the epithelium of the geese was the highest (Fig. 6D), (Table). The lamina propria of male geese was formed of collagen and elastic fibers as well as numerous syringeal glands (Figs. 6A). The

acini of glands appeared round and lined by secretory cells with basal flattened nuclei (Fig. 6a). The lining secretory cells were positively reacted to AB (Fig. 7c, d) and PAS (Fig. 7e, f) in both geese and house sparrows however the reaction in geese was stronger and higher than in the house sparrows. The submucosa of geese' tracheosyringeal cartilages was also made up of dense connective tissue which consisted of a network of fibro elastic C.T. but no glands (Figs.8a). The lamina propria and tunica submucosa in cattle egrets and house sparrows blend forming Propria submucosa. Lamina propria-submucosa was formed of loose connective tissue in cattle egrets (Figs.8b) and house sparrows (Figs.8c) and consisted of a network of fibro elastic C.T. In geese, cattle egrets, and house sparrows, the network of collagen fibers reacts positively to Crossmon stain (Fig. 9a, b, c). Statistically, the percentage of collagen fibers in geese was the highest (Fig. 9d and Table). The house sparrow's tympanum has a unique structure. Since it is hidden by bulla Tympaniformis in gross morphology (Figs. 10a) but in light microscope, Appeared obviously (Figs. 10b). Four completely ossified tracheosyringeal rings with the third tracheosyringeal ring being the largest in size (Figs. 10) form the tympanum part. There were three pairs of syringeal muscles (Figs. 10b) arranged in 1) Dorsomedial syringeal (DMS) muscles that extend medially from the cranial part of the tympanum to the second ring of the tympanum, 2) Dorsolateral syringeal (DLS) muscles that extend laterally from the cranial part of the tympanum to the fourth rings of tympanum and 3) Ventrolateral syringeal (VLS) muscles which extend laterally from caudal part of tympanum to the first broncho-syringeal cartilages. These muscles have been considered the intrinsic muscles of sparrows' syrinx. The pessulus was triangular, positioned at the tip of the tracheal bifurcation and formed from the mucosa, Propria-submucosa, and cartilaginous plate. From the tip to the base of the pessulus the mucosa of geese exhibited no alteration (fig. 11a). In contrast to cattle egrets, the pessulus was covered by thin mucosa (simple squamous epithelium) at the tip and turned to stratified squamous epithelium toward the base of pessulus (fig. 11b). In house sparrow changed from respiratory epithelium at the tip of pessulus to a thin mucosa of flattened cells at the base of pessulus (fig. 11c). The lining epithelium was typical respiratory epithelium (pseudostratified columnar ciliated epithelium with goblet cell) in geese (fig. 12a) and house sparrows (fig. 12c). Likewise, the pessulus of cattle egrets is lined by stratified squamous epithelium with solitary goblet cells (fig. 12b). The epithelium of the cattle egrets had the highest statistical value (Fig. 12d), (Table). The goblet cells appeared in large amounts in geese (Fig. 13a) than in-house sparrows (Fig. 13g) and in scanty amounts in cattle egrets (Fig. 13d). These secretory cells were positively reacted to AB (Fig. 13b, e, h) and PAS (Fig. 13c, f, i) in the three species while the reactivity and density in geese was the highest. The Propria submucosa of geese consisted of dense connective made up of a network of collagen and elastic fiber (fig.11a). While in cattle egrets (fig. 11b) and house sparrows (fig. 11c) Formed of loose connective tissue. The pessulus of geese (fig. 11a) and cattle egrets (fig. 11b) consisted of one cartilaginous plate that showed intra-cartilaginous ossification. While in the house sparrows displayed a unique structure known as tympanum. Since it consisted of five cartilaginous plates; three plates at the tip of pessulus and two ones at the base of it (fig. 11c). The medial and lateral membranes: In geese, the medial tympani-form membrane connected from the pessulus to the second broncho-syringeal cartilages (fig. 14a) while the lateral tympani-form membrane attached to the caudal process of dorsal and ventral tympanum (fig. 14b). In cattle egrets, the medial tympani-form membrane lasted from the pessulus to the fourth broncho-syringeal cartilage (fig. 14c, d) with no lateral tympani-form membrane or ill-developed. As well, in house sparrows the medial tympani-form membrane extended from the pessulus to the caudal part of the first broncho-syringeal cartilages, whereas the lateral tympani-form membrane ran from the fourth cartilaginous ring of tympanum to the cranial part of first broncho-syringeal cartilage (fig. 14e). In geese and house sparrows, the lateral tympaniform membrane is thicker than the medial one and the lateral tympaniform membrane of sparrow thicker than that of geese (fig. 14f) and (Table). Broncho-syringeal cartilages: The broncho-syringeal cartilages were formed from six rings in geese (Fig. 15a), four rings in cattle egrets (Fig. 15b) and six or seven rings in house sparrows (Fig. 15c). The broncho-syringeal cartilages consisted of mucosa, submucosa, fibrocartilage, and serosa (Fig. 15). The lining epithelium of broncho-syringeal cartilages was pseudostratified columnar epithelium with few or without goblet cells in geese (Fig. 15d) and cattle egrets (Fig. 15 e). As well as house sparrows were covered by simple squamous epithelium (Fig. 15 f).

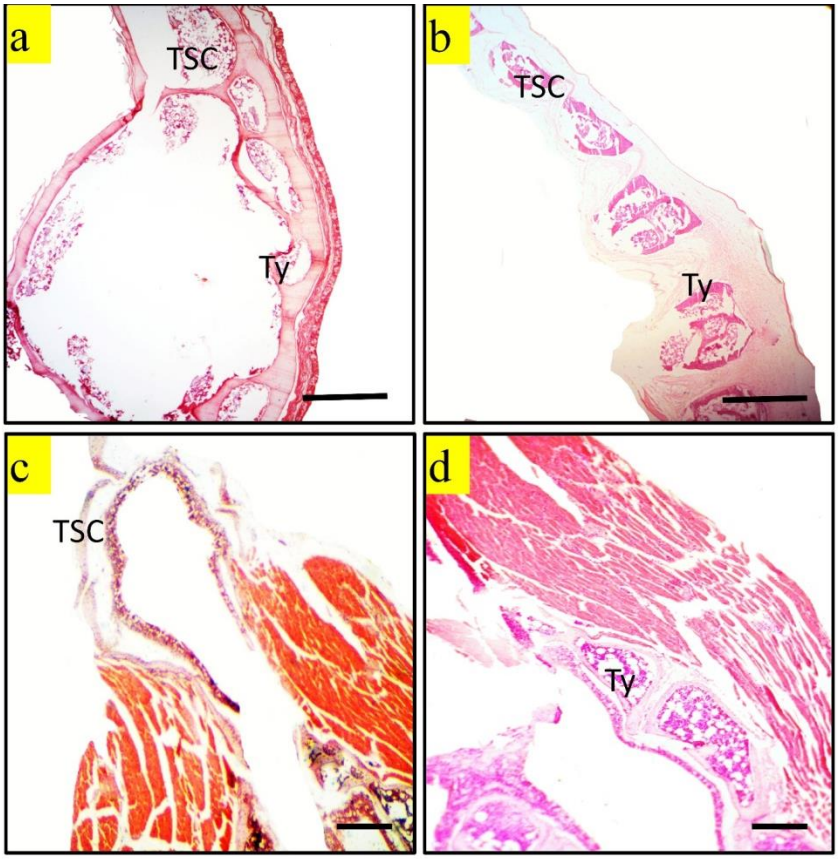

Fig. 5. Photomicrograph of tracheosyringeal parts of three studied species. geese (a), cattle egrets (b) and house sparrows (c, d). Showing the tracheosyringeal cartilages (TSC) and tympanum (Ty). Scale bars; a, b= 200 µm and c, d=50 µm (H&E stain).

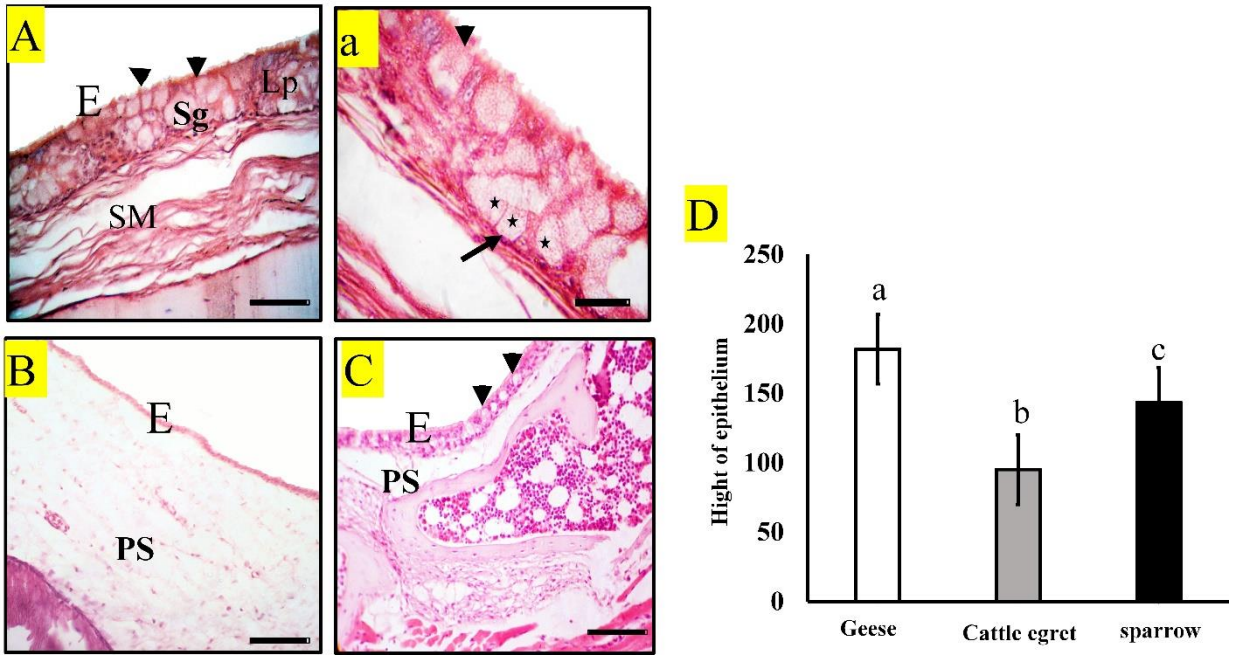

Fig. 6. Photomicrographs of the tracheosyringeal mucosa of male geese (A), cattle egrets (B) and house sparrows (C) showing the pseudostratified columnar ciliated epithelium (E) with goblet cells (arrowhead) forming syringeal gland (SG). In geese, lamina propria (LP) separated from submucosa (SM). (a) showing secretory cells of basal flattened nuclei (arrow) in mucous acini of gland and pale vacuolated cytoplasm (ostricles). In cattle egrets (B) and house sparrows (C) the propria blend with submucosa forming propria-submucosa (PS). Scale bars; A, B, and C = 50 µm a =30 µm (H&E stain). (D): Bar chart showing the epithelial height of the tracheosyringeal part of the syrinx of three investigated species. Data are expressed as means ± SEM (n = 6), (a–c) refer to statistically significant differences ($p \leq 0.05$).

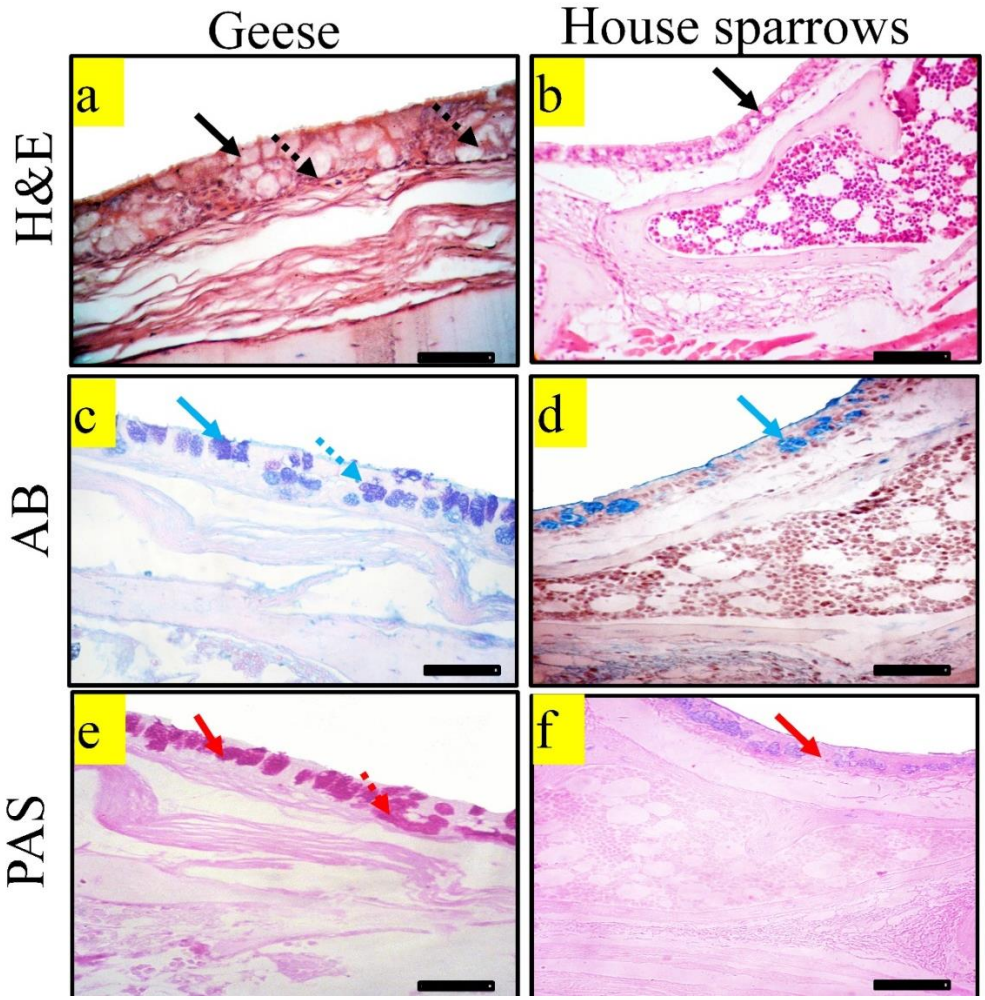

Fig.7. Photomicrographs of the tracheosyringeal mucosa from H&E (a, b), AB (c, d) and PA S (e, f) cross stained sections of male geese (a, c, e) and house sparrows (b, d, f) showing surface(arrows) and glandular (dashed arrow) goblet cells are positively reacted with AB (blue arrows) and PAS (red arrows). Notice the weak PAS reaction in house sparrows. Scale bars a, b, c, d, e, f = 50µm

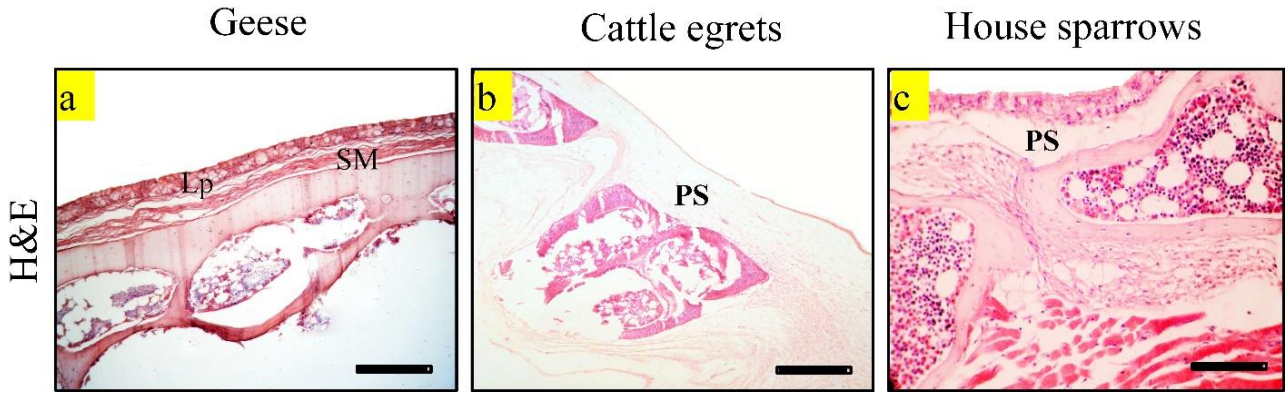

Fig. 8. Photomicrographs of the tracheosyringeal part from H&E-stained sections showing the lamina propria and tunica submucosa. The lamina propria (LP) and tunica submucosa (SM) in geese (a) detached from each other but in cattle egrets (b) and house sparrows (c) blend forming propria submucosa (PS). Scale bars a, b = 200 µm, c = 50 µm.

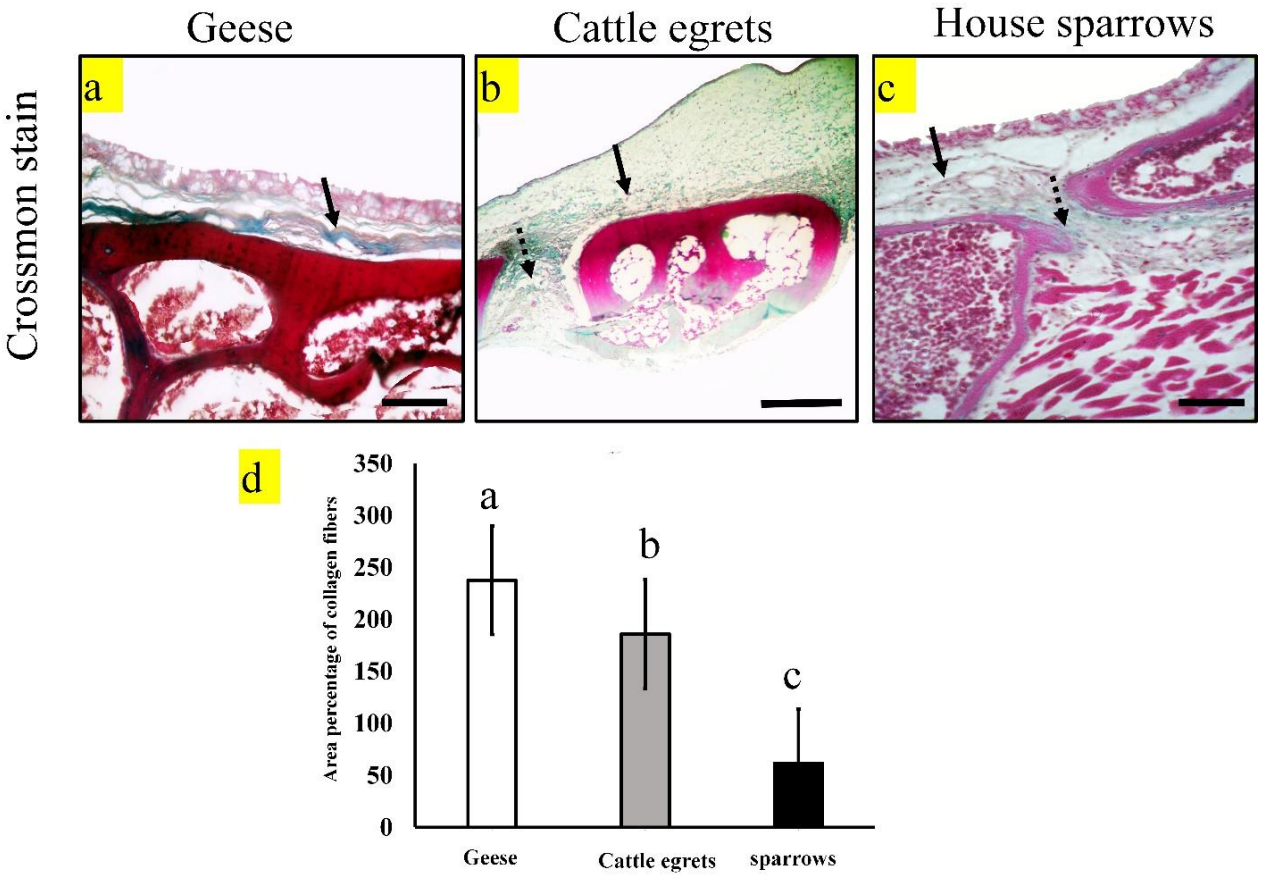

Fig. 9. Photomicrographs of the tracheosyringeal part from male geese (a) cattle egrets (b) and sparrows (c) from Crossman's trichrome stained sections, showing the collagen fibers are prominent in propria submucosa (arrows) and between the cartilaginous plates (dashed arrows), Scale bars a, c = 50 µm, b = 200 µm. (d): Bar chart showing the area % of the collagen fibers from all studied species. Data are expressed as means ± SEM (n = 6), (a, b, c) refer to statistically significant differences ($p \leq 0.05$).

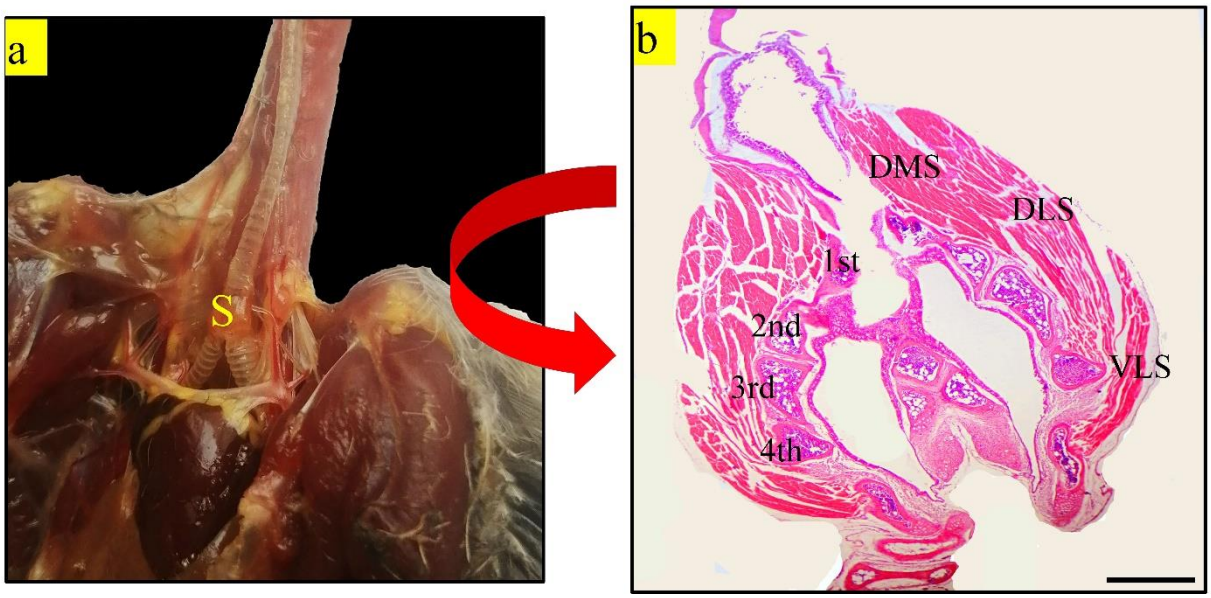

Fig. 10. In situ ventral view of syrinx (S) of male house sparrows (a) Showing bulla tympaniformis. (b) Longitudinal section of the syringeal tympanum and muscles of male house sparrows. All cartilages of tympanum (1st to 4th) with the third one is the largest in size. Syringeal muscles; Dorsomedial syringeal (DMS), Dorsolateral syringeal (DLS) and Ventrolateral syringeal (VLS) muscles. scale bar = 400um (H&E stain).

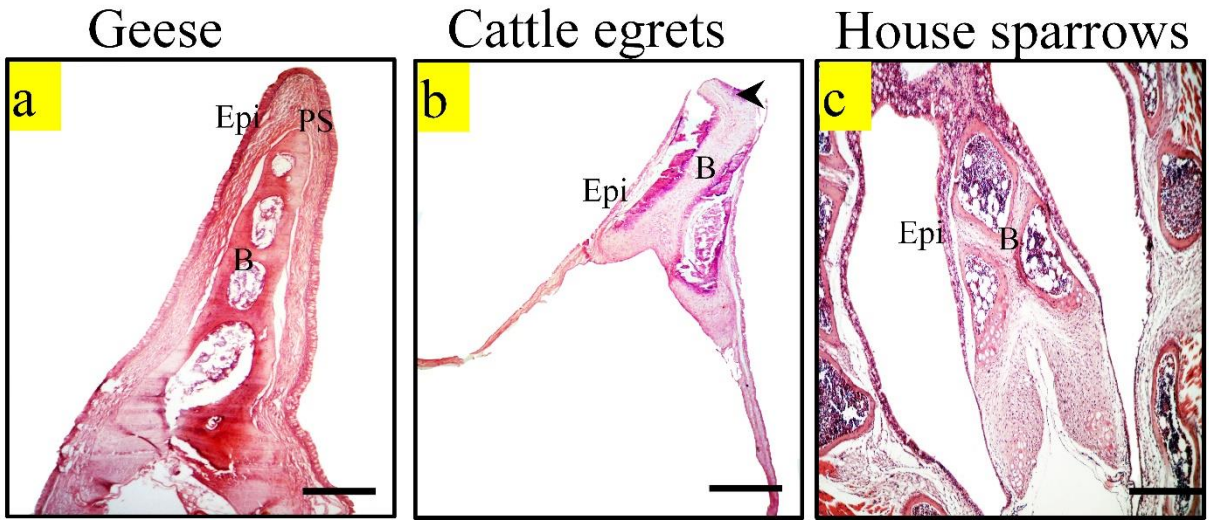

Fig. 11. Photomicrographs of the syrinx at the pessulus levels from H&E stained cross sections of male geese (a), cattle egrets (b) and house sparrows. The pessulus composed of mucosa with lining epithelium (Epi.) that turned to thin mucosa (arrow) at tip of pessulus in cattle egrets and at the base of pessulus in house sparrows, propria submucosa (PS) and bony plate(B). scale bars; a, b= 600um and c=200 um.

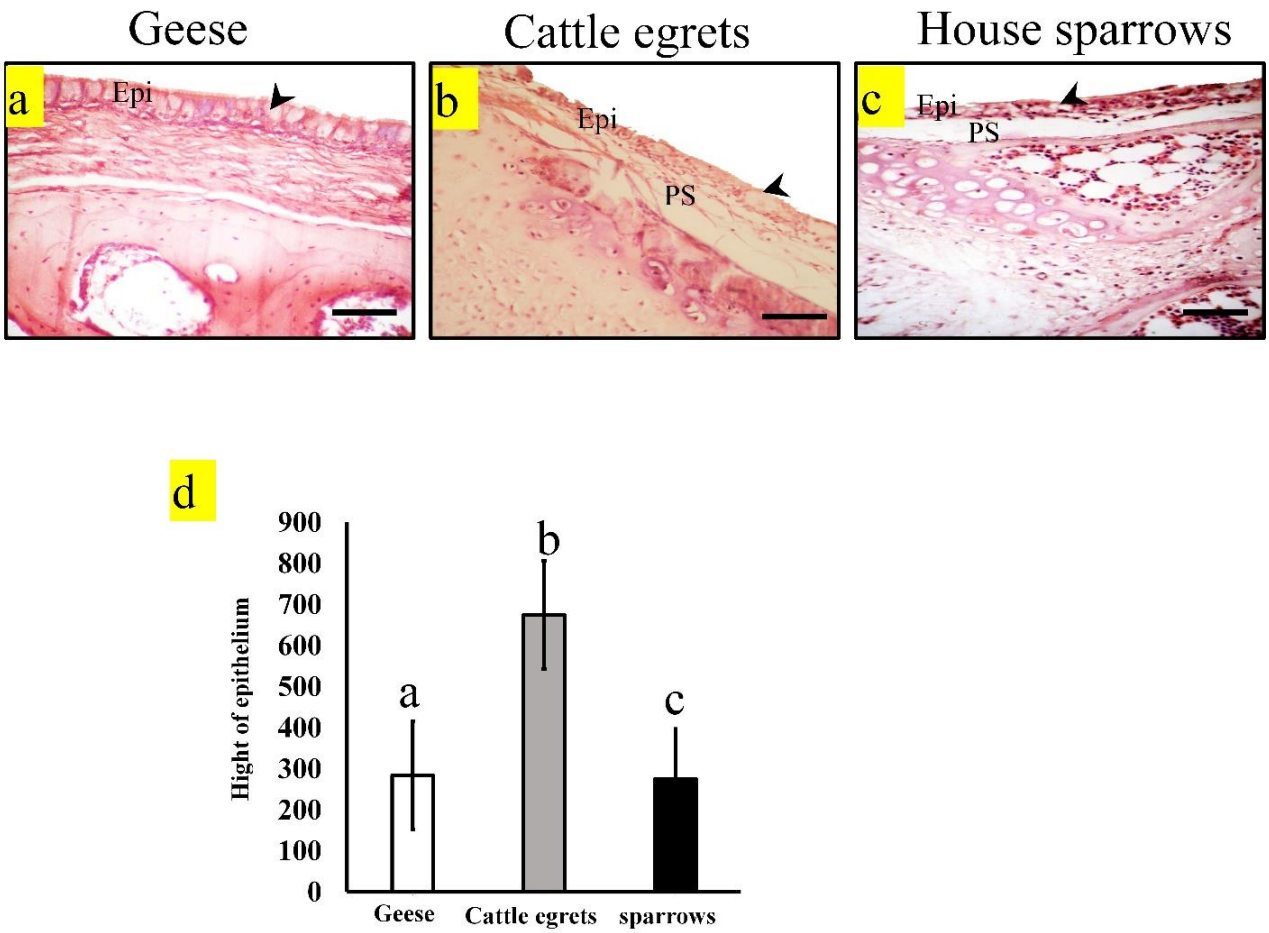

Fig. 12: Photomicrographs showing the lining epithelium (Epi.) of pessulus; pseudostratified columnar ciliated epithelium with goblet cells (arrowhead) in male geese (a), male house sparrows (c) and stratified squamous epithelium with solitary goblet cells (arrowhead) in male cattle egrets (b). Scale bars; all = 50 µm. (d): Bar chart showing the epithelial height of the pessulus of three investigated species. Data are expressed as means ± SEM (n = 6), (a, b, c) refer to statistically significant differences ($p \leq 0.05$) (H&E stain).

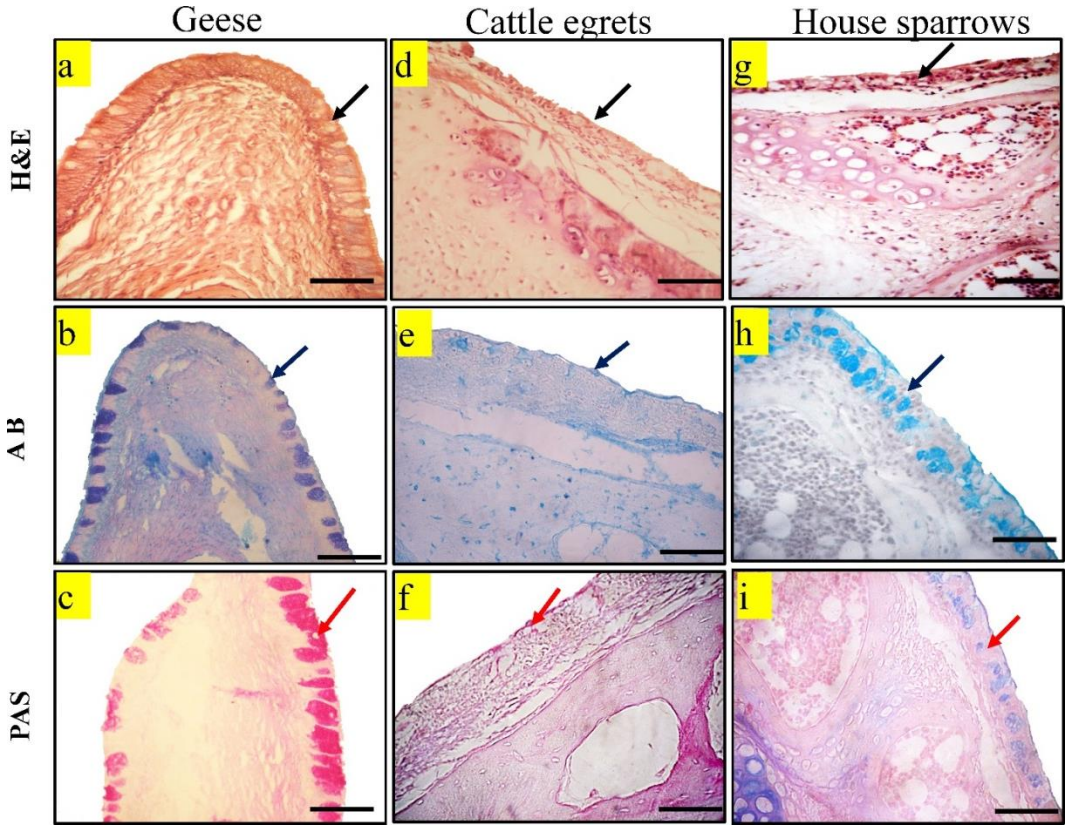

Fig. 13. Photomicrographs of the pessulus mucosa from H&E (a, d, g), AB (b, e, h) and PAS (c, f, i) of male geese (a, b, c), cattle egrets (d, e, f) and house sparrows (g, h, i). The H&E (black arrows), AB (blue arrows) and PAS (red arrows) in geese and house sparrows positively stained goblet cells. Notice the PAS show weak reaction in cattle egrets (f) and sparrows (i). Scale bars a, b, c, d, e, f = 50µm.

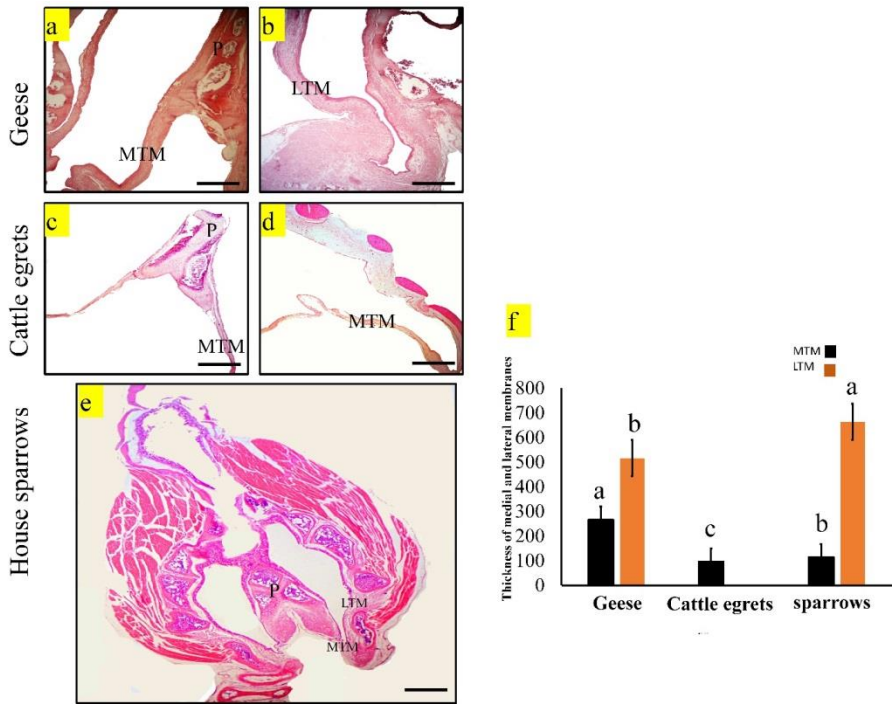

Fig. 14. Longitudinal sections in the syrinx of male domestic geese (a, b), male cattle egrets (c, d) and male house sparrows (e) showing the medial and lateral tympaniform membrane. Pessulus (P), medial tympaniform membrane (MTM) and lateral tympaniform membrane (LTM). Scale bar; all=500 µm. (f): Bar chart showing the thickness of medial and lateral tympaniform membrane from all studied species. Data are expressed as means ± SEM (n = 6), (a, b, c) refer to statistically significant differences ($p \leq .05$) (H&E stain).

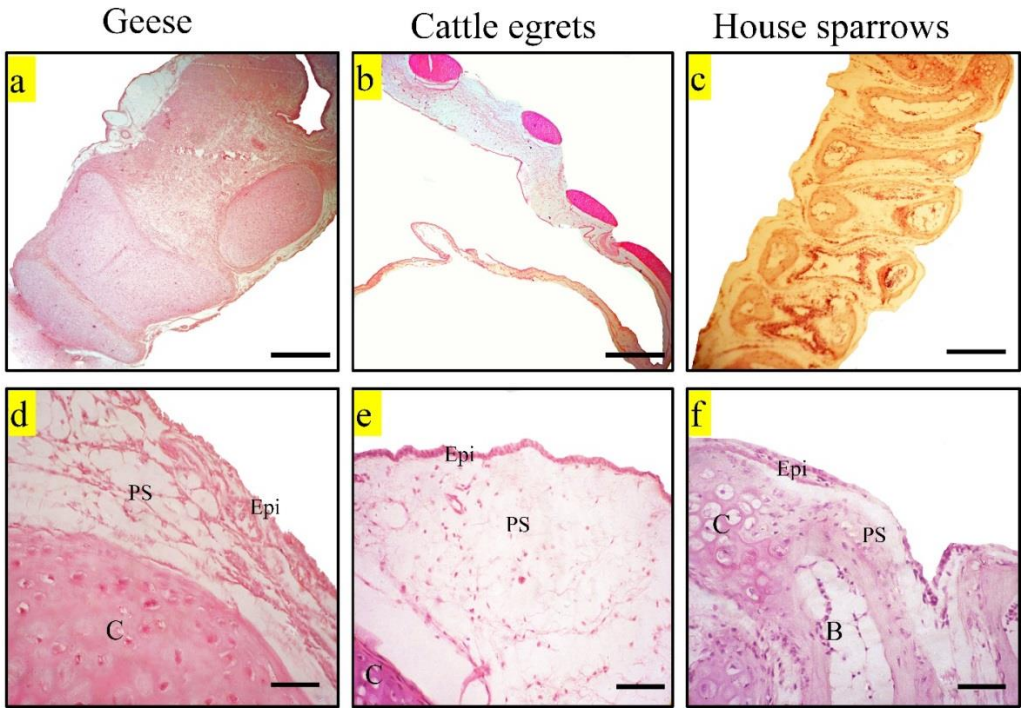

Fig.15. Photomicrograph in the syrinx of male domestic geese (a, d), male cattle egrets (b, e) and male house sparrows (c, f) showing the broncho-syringeal cartilaginous rings. (Epi) is the epithelium of these cartilages, it is pseudostratified columnar epithelium in geese (d), cattle egrets (e) and simple squamous epithelium in house sparrows (f). (PS) showing the propria submucosa that consisted of loose connective tissue. C = The cartilaginous rings. B= bone marrow. Scale bars; a, b, c = 600 µm and d, e, f = 50 µm (H&E stain).

4- DISCUSSION

The main way of communication is the voice production process. Birds can create sounds and sing with their vocal organ. Birds have a characteristic vocal organ (syrinx), have significantly different in position, structure, and musculature between species (Edwards et al., 2005). Many earlier research have looked at the morphologic structure of the syrinx (Yildiz et al., 2005, Cevik-Demirkan et al., 2007, Tsukahara et al., 2008, Riede and Goller 2010, Yilmaz et al., 2012, Erdogan et al., 2015, Al-Aameli and Kadhim 2017, and Abdel-Maksoud et al., 2020), but either anatomically or histologically are the first study to look at the syrinx of cattle egrets and house sparrows and histologically in geese. The obtained results of the syrinx location were parallel to those described by Arican et al., (2007) in white turkey, Yilmaz et al., (2012) in mallard, AL-badri (2014) in Bulbul, Mohamed (2017b) in white Pekin ducks, Ibrahim et al., (2020) in fowl and pigeon who showed that the syrinx was located inside the thoracic cavity lies ventral to the esophagus and at the base of the heart. Also, the position of the syrinx in geese matches with AL-badri (2014) in indigenous Pigeon who determined that the syrinx is located beneath the heart.

Geese, cattle egrets, and house sparrows have tracheo-bronchial type syrinx which has been recorded in other bird species as fayomi fowl (Abd elmohdy, 1980), ostriches (Yildiz et al., 2003), quails (Bayram and Liman, 2000 and Cevik-Demirkan et al., 2007), long-legged buzzards (Kabak et al., 2007), eagle owls (Cevik-Demirkan and Ozdemir 2011), seagulls (Ince et al., 2012), turkeys (Arican et al., 2007, Khaksar et al., 2012 and Ragab et al., 2016), mallards (Frank et al., 2007; Yilmaz et al., 2012; Mohamed 2017b), geese (Onuk et al., 2010; Mohamed, 2017a), black Francolin (Al-Aameli and Kadhim, 2017), fowls (Al-Bishtue, 2014), pigeon (Yildiz et al., 2005; Ibrahim et al., 2020) and budgerigars (Abdel-Maksoud et al., 2020). .(

The current study revealed that the syrinx was formed of two main parts including the Tracheo-Syringeal part and broncho-syringeal part, as well as the pessulus and tympani-form membranes. The obtained results of geese reported that, the Tracheo-Syringeal cartilages; the first two cartilage rings of them were separated, while the rest of these cartilages were fused and ossified to form the tympanum, this agrees with Onuk et al., (2010) and Mohamed (2017a) in geese and this ossification gives pitched sound to geese. And disagree with Khaksar et al., (2012) on turkey,and Abdel-Maksoud et al., (2020) on budgerigars who reported that, the tympanum is formed by 2, 3, 5 or 6 cartilaginous rings. Tracheo-

syringeal cartilages in cattle egrets and house sparrows were formed from four cartilaginous rings. The number of these cartilages approved Yildiz et al., (2003) in ostriches, and Ibrahim et al., (2020) in fowl and pigeon. In geese and house sparrows, the lamina epithelial was represented by pseudostratified ciliated columnar epithelium with goblet cells and this in line with the finding of (Frank et al., 2006) in male mallards, (Khaksar et al., 2012) in turkeys, and (Erdogan et al., 2015) in partridges, (Al-Aameli and Kadhim 2017) in francolin, (and Ibrahim et al., 2020) in pigeon and (Abdel-Maksoud et al., 2020) in budgerigars; and dissimilar with Ibrahim et al. (2020) in fowl who stated that the lining epithelium of Tracheo-Syringeal part was stratified cuboidal epithelium. The sound released from geese and house sparrows may also linked to the surface and Glandular goblet cells secret neutral and sulfated mucins in geese and surface goblet cells in house sparrows and this secretion may related to the protection of this tract for nice singing. The syringeal muscles are located on the opposing sides of the body, and there are two types of muscle; the extrinsic and intrinsic muscles .

In the three studied species, the extrinsic syringeal muscles are well developed. The Trachiolateralis and Sternotrachialis muscles and, by many domestic birds. The Cleidotrachealis muscle is another pair of extrinsic syringeal muscles seen in geese but not in the other two birds, and this harmonizes with Onuk et al. (2010) and Mohamed (2017a). The intrinsic syringeal muscles are very effective in voice formation and are responsible for the mechanical control of voice production (Goller and Larsen, 1997). These intrinsic muscles are seen in passerine birds such as house sparrows and the results are comparable to those described in singing birds and parrots (Abdel-Maksoud et al., 2020). The House sparrows have three pairs of intrinsic syringeal muscles; Dorsomedial syringeal (DMS) muscles, Dorsolateral syringeal (DLS) muscles, and Ventrolateral syringeal (VLS) muscles these intrinsic syringeal muscles give singing sound to house sparrow. Microscopic examination of the syrinx in the three species revealed that the pessulus was located at the tip of the tracheal bifurcation and this consistent with results from many birds as partridge (Erdogan et al., 2015), black francolin (Al-Aameli and Kadhim, 2017), fowl and pigeon (Ibrahim et al., 2020), budgerigars and canaries (Gündemir and Alpak, 2020). The pessulus plays an important role in sound production (Goller and Suthers, 1997, Larsen and Goller 2002). The present result revealed that the medial tympaniform membrane connected from the pessulus to the second broncho-syringeal cartilages in geese and this disagrees with Mclelland (1990) and Ibrahim et al., (2020) in fowl, while the lateral tympani one attached to the caudal process of dorsal and ventral tympanum. As well as, in house sparrows the medial tympaniform membrane extended from the pessulus to the caudal part of the first broncho-syringeal cartilages, whereas the lateral tympaniform membrane ran from the fourth cartilaginous ring of tympanum to the cranial part of first broncho-syringeal cartilage and this consistent with the findings of (Mclelland, 1990, Goller and Larsen 1997, Yildiz et al., 2005 as well as Ibrahim et al., 2020) in pigeons who found that the lateral tympaniform membranes filled the distance between two syringeal rings. From this result presence or absence, of the lateral tympani form membrane has a significant role in producing sound in cattle Egret its absence is related to its low sharp sound. As well as its thickness since morphometric data recorded that the thickness of lateral tympani form membranes in house sparrows was significantly higher than in geese. This may relate to the deep-pitched sound in geese than sparrows due to the higher thickness, the much lower possibility of oscillation, and the amplitude with an attenuated sound (Ibrahim et al., 2020). These explanations were like those recorded by Frank et al., (2007) in Mallards and Al-Aameli and Kadhim (2017) in black francolin who explained the different frequencies between the higher sound of males and the deeper voice of females. The broncho-syringeal cartilages were usually paired, C-shaped, and gave way to the extrapulmonary primary bronchial cartilages. Six pairs of cartilaginous rings make up the broncho-syringeal part in geese and house sparrows. These findings agree with Onuk et al. (2010) & Mohamed (2017a) in geese and dissimilar with C¸ evil-Demirkan et al. (2007) who reported that there are two bronchosyringeal in Japanese quail and Mclelland (1990) and Ibrahim et al. (2020) in fowl that, the broncho-syringeal cartilages formed from three cartilaginous rings and five broncho-syringeal cartilages in sparrowhawks (Ozudogru, 2015) and pigeon (yieldiz et al., 2005). In cattle Egret the broncho-syringeal cartilages were four in number which is in line with the results of (Kabak et al., 2007) in Long-Legged Buzzard and (Khaksar et al., 2012) in Turkey .

From these results, we can conclude that the more broncho-syringeal cartilages, the pitched and the loudest sound, as demonstrated by Erdogan et al., (2015) in Partridge who proved that the broncho-syringeal cartilages are made up of eight Cartilaginous rings and it consisted of 8-9 cartilages in canaries and 6-7 cartilages in budgerigars

(Gündemir and Alpak 2020). Also, Ince et al., (2012) in seagulls and Al- Badri (2016) in laying hens proved that, the broncho-syringeal cartilages consisted up of seven rings.

5- CONCLUSIONS

The syrinx of three species showed different anatomical and histological structures and this leads to different pitches of sound:

1- The more ossified syrinx in geese gives it the pitched sound

2- The bull tympaniformis in house sparrow gives it the singing sound

3- The absence of lateral tympaniform membrane in cattle egret gives it the less pitched sound

LIST OF ABBREVIATIONS

(H&E): Hematoxylin and eosin; (PAS): Periodic acid Schiff; (AB): Alcian Blue stain; (EDTA): Ethylene Diamine Tetra Acetic Acid.

AUTHORS' DECLARATIONS

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study protocol was evaluated and accepted by the Institutional Animal Care and Use Committee (IACUC) of Zagazig University, Egypt. (ZU-IACUC/2/f/67/2021).

CONSENT FOR PUBLICATION

EACH AUTHOR HAS DEMONSTRATED their consent for the publication of the current manuscript

AVAILABILITY OF DATA AND MATERIALS

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SAA, SH, HE & AS participated in the design of the study and collected the samples, SAA was published the article " corresponding author". And AS drafted the manuscript. ALL authors read and approved the final manuscript

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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