ANATOMICAL ANALYSIS OF SYRINX IN CHICKENS, DUCKS AND QUAILS

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CERTIFICATION

This is to certify that we have read this research paper entitled '**Anatomical Analysis of syrinx in different birds**' by Yamunasri Kuthiah, and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course DVT 55204-Research project.

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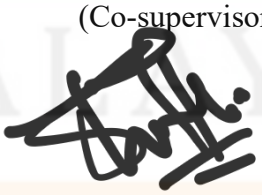

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Family

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Thank You

DEDICATIONS

I would like to dedicate this dissertation to my family, who have been a rock of stability. I am especially appreciative of my dear parents Kuthiah and Maheswari for always having constant confidence in me and my siblings Thiyagu for always having faith in me.

Additionally, I want to thank Assoc. Prof. Dr. Shanthi Ganabadi for always being supportive of me and teaching me lessons that have improved me as a person.

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ABSTRACT

An abstract of the research paper was presented to the Faculty of Veterinary Medicine, University Malaysia Kelantan, in partial requirement for the course DVT 55204- Research project.

Birds are an excellent model for researching the phonation process. Syrinx is the vocal organ of the birds. In Malaysia different bird breeds including *Gallus gallus domesticus*, *Cairina moschata* and *Cortunix japonica* have limited documentation on syrinx anatomical characteristics. There is a lack of insight and knowledge about the topographical position and anatomical features of the syrinx in these avian species The main goal of this study is to investigate the topographical and morphological characteristics of the syrinx in different avian species. In this study three adult broiler chickens, three broiler ducks and three broiler quails of mixed sexes were examined. The study revealed that the syrinx is tracheobronchial type, composed of tympanum, tracheosyringeal and bronchosyringeal groups. Additionally, there is lateral and medial tympaniform membranes, pessulus and interbronchial ligament (brachidesm) at the tracheal bifurcation. The Syrinx also consists of extrinsic muscle such as tracheolateralis and sternotrachealis muscle. Data was subjected to Kruskal-Wallis Test followed by Mann-Whitney. The result inferred that there is significant difference in the measurement of the tracheolateralis and sternotrachealis muscle length in different species of birds, where ducks and chickens have longer muscles compared to quails. The current study presented detailed gross morphological characteristics of syrinx of chickens, ducks and quails. The findings of this study will aid in knowledge on the evolution of syrinx of different birds which will add input in the field of veterinary anatomy.

Keywords: Syrinx, tracheobronchial, tympanum, phonation.

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 55204 – Projek Penyelidikan.

Burung merupakan model yang baik untuk menyelidikan proses pembunyian. Sirinks adalah organ pembunyian dalam burung. Di Malaysia kajian mengenai anatomi sirinks yang telah dilakukan dan ciri-ciri anatomi terhad .Justeru itu, terdapat kekurangan pengetahuan mengenai subjek kajian ini. Matlamat utama penyelidikan ini adalah untuk menyiasat ciri-ciri topografi dan morfologi syrinx dalam spesis burung yang berbeza. Dalam kajian ini, tiga ekor ayam pedaging, tiga ekor itik pedaging dan tiga ekor burung puyuh pedaging yang matang yang campur jantina telah diperiksa. Kajian ini mendedahkan bahawa sirinx burung yang biasa adalah jenis trakeobronkial dan terdiri daripada timpanum, trakeosyringeal dan bronkosyringeal. Selain itu, terdapat membran timpaniform sisi dan tengah, pessulus dan interbronkial ligamen (brachidesm) di bawah cabang trakea. Ia juga terdiri daripada otot trakeolateralis dan otot sternotrakealis. Ujian Kruskal-Wallis dengan Mann-Whitney telah digunakan untuk menganalisis data. Kesimpulan hasil kajian menunjukkan bahawa terdapat perbezaan yang signifikan dalam pengukuran otot trakeolateralis dan otot sternotrakealis dalam spesies burung yang berbeza dimana itik dan ayam mempunyai otot yang lebih panjang berbanding puyuh. Kajian semasa memperlihatkan secara terperinci ciri-ciri morfologi kasar sirinks ayam, itik dan puyuh. Dapatan kajian ini akan menambahkan pengetahuan tentang evolusi sirinks burung yang berbeza yang akan manambah input mengenai anatomi veterinar.

Kata kunci: Sirinks, trakeobronkial, timpanum, fonasi .

1. INTRODUCTION

Syrinx is a unique bird bone structure derived from the word sɪrɪŋks New Latin and Greek. It is also referred to as a bird's vocal organ or panpipes. Cartilage and vibrating soft components make up the syrinx. Throughout the year, some birds employ syrinx for vocalisation, whereas others only do so during courting and migration. Syrinxes are similar to mammalian larynxes, with the exception that they lack the vocal cords that vibrate in response to air currents and are rudimentary in some species, such as vultures and ostriches, according to Reem and Hamdy *et al.,* (2016). Depending on how far the cartilage of the syrinx deviates from the trachea or bronchi, there are three different varieties of syrinx: tracheal, tracheobronchial, and bronchial. The majority of birds, have a tracheobronchial syrinx that is situated at the trachea's bifurcation. When a bird inhales, air enters the nasal cavity, travels via the choana to the larynx, and then travels through the trachea to the syrinx and bronchi (Reem and Hamdy,2016).

In recent years, it has been reported that syringeal muscle variation and the pressure in the clavicular air sacs affect the production of melodic sounds, particularly in singing birds. Numerous bird species' syrinx morphologies have been investigated. According to a study by Khaksar *et al.,* (2012), the turkey tympanum is made up of two tracheal cartilaginous rings. However, according to research by Yildiz *et al.,* (2005), turkeys from the New World have tympanums with four complete tracheal rings. According to a study done on a chicken by Marwa *et al.,* (2019), the tympanum has three to five tracheal rings. In accordance with a study done on a chicken by Marwa *et al.,* (2019), the tympanum has three to five tracheal rings. Kabak *et al.,* (2007) reported that the tympanum in quail is made up of two tracheal rings. This demonstrates the variability among the various breeds of avian animals.

In Malaysia, no anatomical information regarding the syrinx, which is particularly significant for birds, was found for broiler chicken, duck, or quail. The primary goals of this study are to thoroughly evaluate the variations in the anatomic structures of the syrinx in various breeds of avian species, including chicken, duck, and quail, and to add to the body of knowledge on veterinary anatomy.

1.1 RESEARCH PROBLEM

In Malaysia, different breeds of bird species, such as chickens, ducks, and quails, have unknown syrinx anatomical structures. In these avian species, there is a paucity of understanding and knowledge regarding the topographical location and anatomical characteristics of the syrinx. Furthermore, there are no studies or data on the variations in syrinx in these breeds of bird species in Malaysia. The majority of the studies and references made mention of research carried out in other nations. In light of this, the purpose of this study is to provide additional details on the variance in morphological features of syrinx in various breeds of avian species. Future research will depend subtly on this study which could contribute toward further understanding of syrinx structures involvement in the phonation of the birds for further studies.

1.2. RESEARCH QUESTIONS

1.2.1 What are the types of syrinxes present in chickens, ducks, and quails?

1.2.2 What are the gross morphological differences in the syringeal structure between chicken, ducks and quails?

1.2.3 Is there significance in the statistical variation in the syringeal structures among chicken, ducks and quails?

1.3. RESEARCH HYPOTHESIS

1.3.1 Tracheobronchial syrinx is the most common type found in chicken, ducks and quails.

1.3.2 Gross morphological differences in the syringeal structures between chicken, ducks and quails are the numbers of cartilaginous rings of syrinx.

1.3.3 There is significance in statistical variation in the syringeal structures among chicken, ducks and quails.

1.4. RESEARCH OBJECTIVES

1.4.1. To determine the types of syrinx present in chickens, ducks and quails.

1.4.2. To determine the gross morphological difference in the syringeal structures between chicken, ducks and quails.

1.4.3. To study the statistical variation in the syringeal structures among chicken, ducks and quails.

2. LITERATURE REVIEW

2.1. Birds Vocalization

Birds are able to make a range of sounds, and depending on the structural differences in their vocal organs, they can be divided into passerine and non-passerine species. Pure-tone whistles, tones with discernible harmonics, broad band noises with formant-like structures, click-like sounds, and noise are examples of common bird vocalizations, according to Ismail *et al.,* (2020). Commonly occurring simultaneous, two harmonically unrelated, frequently independently modulated tones show that various membranous parts that function as acoustic sources are individually activated. Bestami *et al.,* (2012) and Ismail *et al.,* (2020) claimed that the vast differences in syringeal anatomy across different orders of birds, such as parrots and chickens, are the reason why they generate diverse sounds.

2.2. Gross morphology of Syrinx

The syrinx and larynx are both elaborated vocal tract structures that occur in other animals (Riede & Goller 2010). The location of the syrinx in the coelomic cavity is ventral to the second intercostal space and dorsal to the heart's base. The first portion of the two primary bronchi and the terminal portion of the trachea were what it was made of. Trachea, bronchosyringeal, and tracheobronchial syringeal modified cartilages can all be seen, along with the pessulus at the tracheal bifurcation, depending on where they are located according to Yildiz *et al.,* (2005). Furthermore, medially and laterally, two pairs of vibrating tympaniform membranes can be seen. The tympanum, which is made of cranial cartilage, will exhibit the cranial and median portions of the syrinx, which developed from the last tracheal rings. At the site of tracheal bifurcation, two C-shaped rings that are open dorsally create the

tracheosyringeal, an intermediate piece of cartilage. While the bronchosyringeal is a set of three half rings on either side of the syrinx, it is a caudal cartilaginous structure.

The pessulus, which is a tiny, medium-sized wedge-shaped osseous ridge positioned sagittally between the two openings leading to the primary bronchi. It had a broad base that went caudally and was linked to the medial tyampaniform membranes, with a pointed apex that projected cranially through the syrinx lumen. Next is the lateral tympaniform membrane, which is represented by thin transparent strips stretched between the first bronchial cartilaginous rings and last tracheosyringe as well as the three bronchosyringes. The medial tympaniform membrane, which was connected to the free ends of the bronchosyringeal rings, made up the most dorsomedial aspect of the divided segment of the syrinx. It continues cranially with the pessulus caudal border and caudally with the interbronchial ligament as stated by Reem and Hamzy *et al.,* (2016).

2.3. Histological morphology of syrinx

According to research on the histology of the syrinx, the trachea and bronchial epithelia are pseudostratified columnar epithelia that contain intraepithelial glands and goblet cells. The pessulus, on the other hand, had a cranial dome and pseudo-stratified columnar epithelium as its surface epithelium. According to an analysis of the membranes, the epithelium covering the first portion of the lateral membrane underwent a change in histological features from pseudo-stratified prismatic epithelium to bistratified cubic epithelium, which then underwent a pseudo-stratified prismatic epithelium transformation at the bronchi. Ibrahim *et al.,* (2020) compare it to the medial membrane.

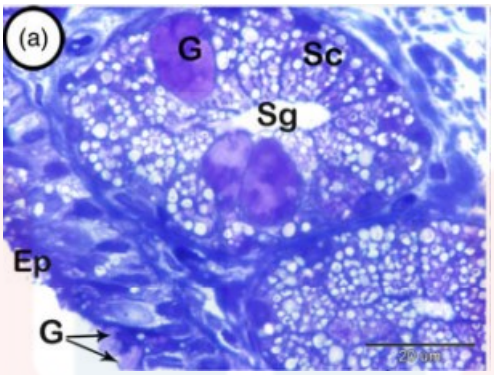

Figure 2.1: Thin section of toluidine blue stained syrinx of male domestic pigeon at the level of the tympanum part. **(a)** Lamina epithelialis is consisted of pseudostratified ciliated columnar epithelium (Ep) with goblet cells (G). The lining epithelium is interrupted by the opening of the syringeal glands (arrow). Underneath the epithelium, secretory acini of syringeal glands (Sg) are lined by pyramidal secretory cells (Sc) and goblet cells (G).

2.4. Physiology of syrinx

When air moves through the syrinx, it causes vibrations of the pessulus and some or all of the wall (membrane tympaniformis) in birds, which create sound. As a result, a self-oscillating mechanism is established, which modifies the airflow responsible for producing the sound. Changes in the tension of the membranes and bronchial apertures allow the muscles to modify the sound form. According to Reem and Hamdy (2016), these membranes are activated by intrinsic muscles, which show too much vibration in singing birds, and by the stern tracheal and tracheolateral muscles, which express only as extrinsic muscles in non-singing birds.

3. METHODOLOGY

3.1. Study Design

A descriptive cross-sectional study was conducted among different species of birds consisting of three adult broiler chickens, three ducks, and three quail. The syrinx's anatomical differences were highlighted and compared among the three bird species. The study area in this research covers the Kota Bharu state of Kelantan, where the birds were sourced for the research. The source population in this study is avian species, including chicken, quail, and duck carcasses obtained from farmed animals, wet markets, and certified breeders. The inclusion criteria of this study are any animal that fulfils the criteria of an avian species, including different species of birds such as chickens and quails, which are males and females with an average age of 28 days. Data related to Syrinx were collected, tabulated, subjected to Kruskal-Wallis test followed by comparison within the groups by pairs individually with Mann-Whitney test using -IBM® SPSS® Version 23 (IBM®, USA). Findings were later presented in tables.

3.2 Samples preparation

In this study, nine birds of mixed sexes from different species (three broiler chicken, three quail, and three duck carcasses) weighing about 100 g to 2kg were used. The birds were collected from farms, wet markets, and certified breeders. The birds were defeathered and deskinned before being placed in dorsal recumbency. The approach is made with a midsagittal section of the abdomen cut on a longitudinal plane from superior to inferior. The pectoralis major muscle and the pectoralis minor muscle were dissected. Then, a cut was made on the keel bone and furcula to expose the thoracic cavity. The syrinx is located beneath

the heart. The heart was removed to reveal the syrinx. The anatomical inspection and topographical position of the syrinx were both observed. Different measurements were taken using the RS PRO 150mm Precision Digital Vernier Calliper, (-RS Components Sdn Bhd, Malaysia)includes the paired sternotrachealis (ST) muscles, which extend from the medial aspect of the xiphisternal processes of the sternum to the lateral aspects of the caudal part of the trachea. Photographs of the syrinx were taken in situ within the intracoelomic cavity. followed by removal of the tracheal region containing the syrinx. This was done by performing dissection along the Longus coli muscle to allow tracheal manipulation until it reached the larynx. The entire larynx, trachea, and syrinx, together with the lungs, were removed from the coelomic cavity. The syringes of birds were left in a 1% Methylene Blue solution for 15 minutes, then passed through 70% alcohol for two hours for the cartilages to be more visible, and then photographed. The syringes were examined, documented, and captured using a stereomicroscope (Olympus Stereomicroscope System SZ61, (-Olympus Corporation, Japan-) fitted with a digital camera (Dino-Lite Dino-Eye C Mount Camera AM-423C,(-Anmo Electronics Corporation, Taiwan-). The same method was repeated for all the birds simultaneously by Ismail *et al.,* (2019).

3.3 Data Extraction

The measurement of syringes component was done using Vernier Calliper and tabulate in SPSS.

3.4 Data Analysis

IBM® SPSS® Version 23 (IBM®, USA) was used to analyse the data. The median for each species were calculated, and the significant difference between the different species was compared. The Kruskal-Wallis test was used to determine the difference in the

syringeal measurement between the birds, where a P-value of 0.05 was considered statistically significant. Followed by pair comparisons between the groups using the Mann-Whitney test.

4. RESULTS

The syrinx of different birds, including chicken, duck, and quail, was observed within the coelomic cavity (Figures 4.2, 4.7, and 4.13). It appeared ventral to the oesophagus and dorsal to the heart's base. It forms the terminal aspect of the trachea and the cranial part of the two primary bronchi. Based on its location at the tracheal bifurcation, it is a tracheobronchial type in all the different species of birds. The syrinx is divided into three sections: the tympanum, which contains the cranial cartilaginous group, the intermediate group, and the bronchosyringeal, which contains the caudal cartilaginous group. Additionally, there are pessulus at the tracheal bifurcation, lateral and medial vibrating membranes, as well as the interbronchial ligament (brachidesm).

The tympanum (Figures 4.4, 4.9, and 4.15) outlines the first part of the syrinx with C-shaped cartilage rings. The present result reveals a tympanum formed of four cartilage rings in chickens and ducks and two cartilages in quails. They are firmly attached together with dense fibrous tissue medially and laterally. The cartilage rings are different in sizes and shapes, which increase dorsoventrally to the tracheal rings above it (Figures 4.6, 4.12, and 4.17). It was determined that the syrinx in male ducks is asymmetrical with special anatomical morphologies, which are the bullae tympaniformis, which appear as a large extension on the left side (Figure 4.11). This was not reported in other birds.

The intermediate rings located caudal to the tympanum at the point of tracheal bifurcation are known as the tracheosyringeal cartilage. It was composed of four incomplete

C-shaped tracheal rings in chickens, ducks, and quails, respectively. They are compressed laterally and fused ventrally with the pessulus, with their free ends directed dorsally. The preceding tracheosyringeal rings are smaller in size than the caudal rings (Figures 4.6, 4.12, and 4.17). The bronchosyringeal rings formed the first three pairs of bronchial half-rings in chicken, on both the right and left side of the syrinx, and were directed as incomplete rings medially. In ducks, the bronchial cartilage forms asymmetrically on both sides, with the left having greater numbers of cartilage than the right, which appears more ossified. While in quail, it formed the first two pairs on each side of the syrinx. In all the birds, the first bronchosyringeal half-rings are modified and larger in size than the other caudal rings. The ends of the rings are attached to the pessulum. The second bronchosyringeal half-rings are attached to the first bronchosyringeal rings ventrally while the third bronchosyringeal half-rings attached caudally.(Figures 4.6, 4.12, and 4.17).

The ossified pessulus is a triangular or wedge shape, formed at the fusion of the right and left bronchus at the level of tracheal bifurcation. In the present findings, its apex pointed cranially through the syringeal lumen, with its broad base directed caudally in all the birds (Figures 4.6, 4.12, and 4.17).

All birds were found to have two pairs of lateral and medial tympaniform membranes.The lateral tympaniform membranes (Figures 4.6, 4.12, and 4.17) are projected inward through the syringeal lumen from the last tracheosyringeal rings to the first bronchosyringeal half-rings. The lateral tympaniform membranes in ducks have less depression of the syringeal lumen compared to chickens and quails. The medial tympaniform membranes expanded between the free ends of the first bronchosyringeal half-rings and

stretched to the caudal aspect of the pessulus to the level of the third bronchosyringeal half-ring.

The intrinsic syringeal muscles in all the birds are absent, but the extrinsic syringeal muscles are evident in all the birds. Paired tracheolateralis muscles formed laterally on both sides of the trachea and extended from the larynx to the cranial aspect of the tympanum (Figures 4.2, 4.7, and 4.13). The length of the tracheolateralis muscle in chickens is 120-125 mm, 70-75 mm in quails, and 166-186 mm in ducks. As a result of this study, the duck shows the longest tracheolateralis (TL) muscle among the birds. The paired sternotracheolateralis (ST) muscles are observed to be extended from the xiphisternal process of the sternum, which covers lateral aspects of the caudal part of the trachea (Figures 4.2, 4.7, and 4.13). Both the TL and ST muscles are linked and overlie both the dorsal and lateral aspects of the trachea. Both the left and right TL and ST muscles are well developed in all birds. The length of the right ST muscle in chickens is 13.6–18.1 mm, 7.6–9.6 mm in quail, and 29.8–31.7 mm in ducks. The length of the left ST muscle in chickens is 15.1–18.2 mm, 8.9–10.3 mm in quails, and 26.9–42.3 mm in ducks.

The interbronchial ligament in all three species of this study (Figures 4.3, 4.9, and 4.15) observed to connects the right and left primary bronchi at the distal point of the medial tympaniform membranes. It extends from the third bronchosyringeal half-rings to the fifth bronchial rings in both the chickens and ducks, from the second bronchosyringeal half-rings to the first bronchial rings in quails. The interbronchial foramen in all three species (Figure 4.6) is observed between the pessulus dorsally, the interbronchial ligament ventrally, and the median tympaniform membrane laterally.

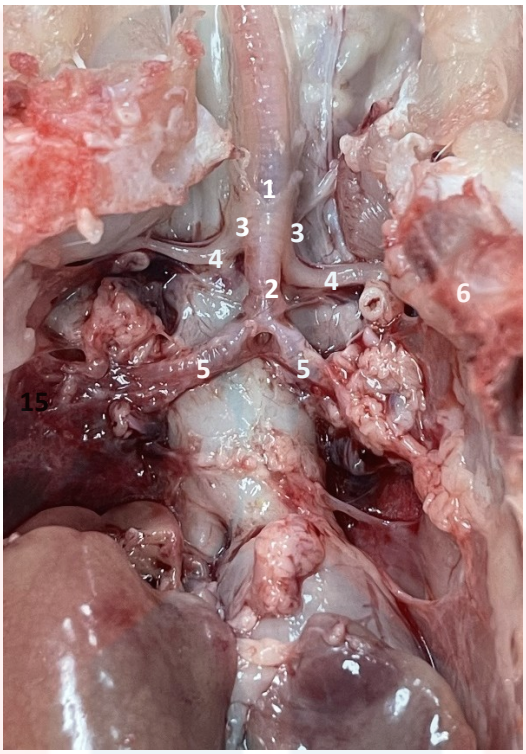

Figure 4.2. In situ ventral view of the syrinx in the chicken. 1. Trachea; 2. Tympanum; 3. Tracheolateralis muscle; 4. Sternotrachealis muscle; 5. Primary Bronchi; 6. Sternum; 15. Lung

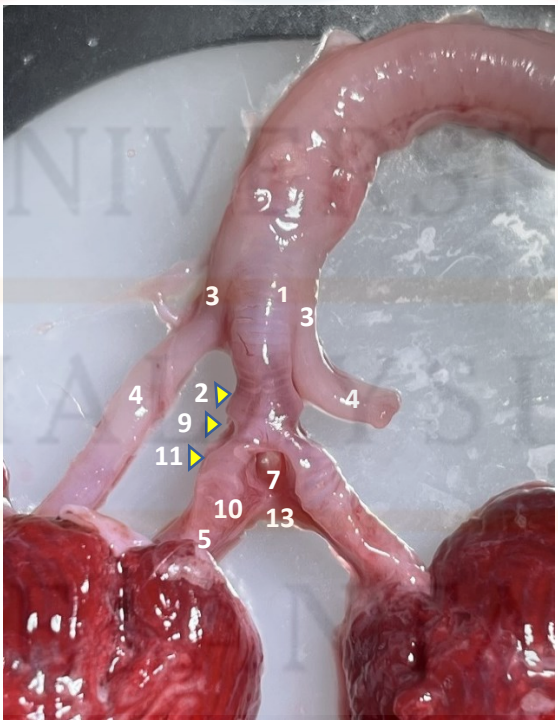

Figure 4.3. Dorsal view of the syrinx in the chicken, in fresh state. 1. Trachea; 2. Tympanum (Cranial cartilaginous group); 3. Tracheolateralis muscle; 4. Sternotrachealis muscle; 5. Primary bronchi; 7. Subpessular space; 9. Tracheosyringeal rings (Intermediate group); 10. Bronchosyringeal half-rings (caudal cartilaginous group); 11. Lateral tympaniform membrane; 13. Interbronchial liagament.

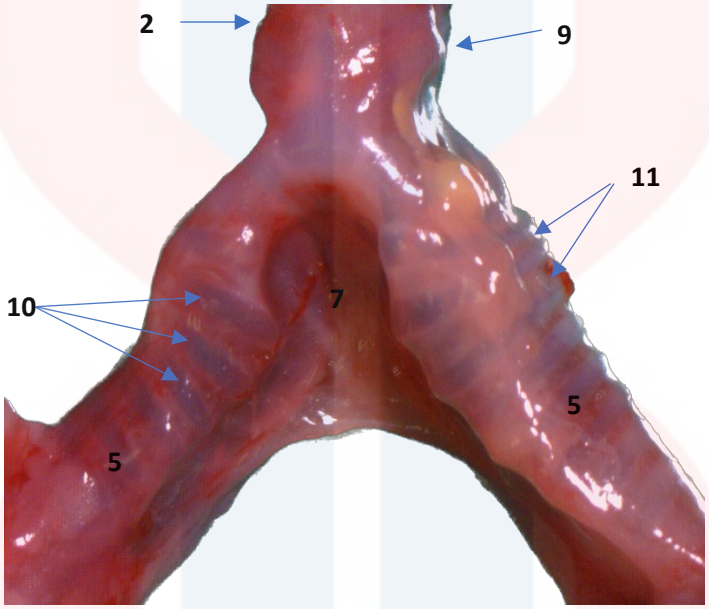

Figure 4.4. Magnified dorsal view of chick syrinx in fresh state. 2. Tympanum (Cranial cartilaginous group); 9. Tracheosyringeal rings (Intermediate group) 10. Bronchosyringeal half-rings (Caudal cartilaginous group); 5. Primary bronchi; 11. Lateral (external) tympaniform membrane; 7. Subpessular space

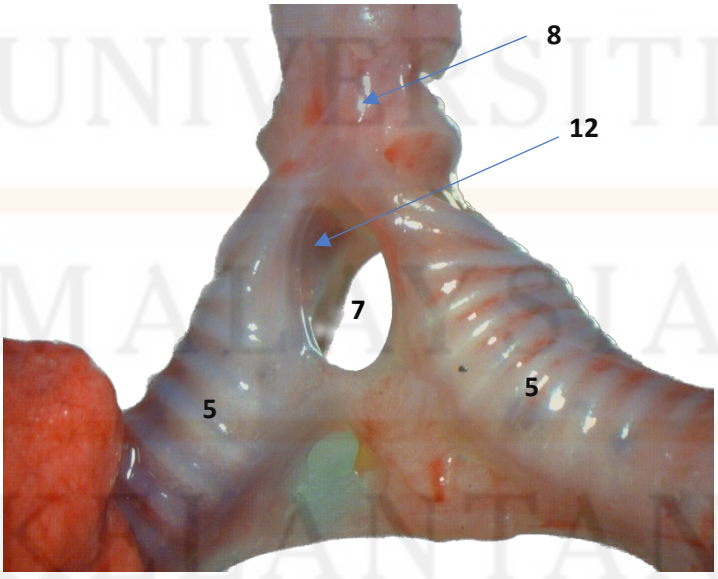

Figure 4.5. Magnified ventral view of chicken syrinx in fresh state. 5. Primary bronchi; 7. Subpessular space; 8. Pessulus; 12. Medial (Internal) tympaniform membrane

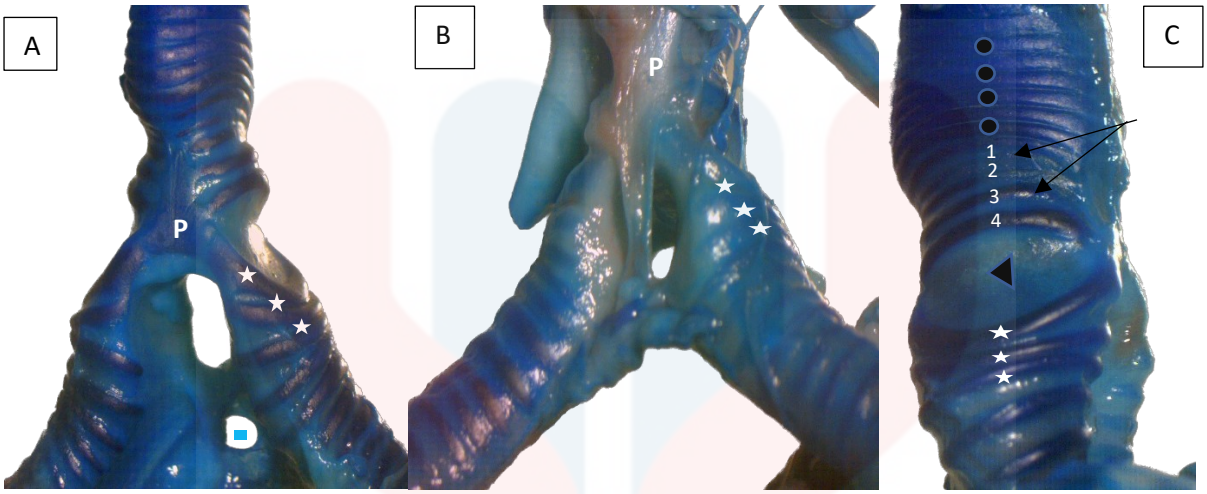

Figure 4.6. Gross anatomic appearance of the syrinx of chicken stained with methylene blue 1%; dorsal(A), ventral (B) and lateral (C) views. (A-C): Tympanum rings (black rings), Tracheosyringeal rings (arrows), Bronchosyringeal half-rings (White stars), Lateral tympaniform membrane (arrow head), Pessulus (P), Interbronchial foramen (Blue square).

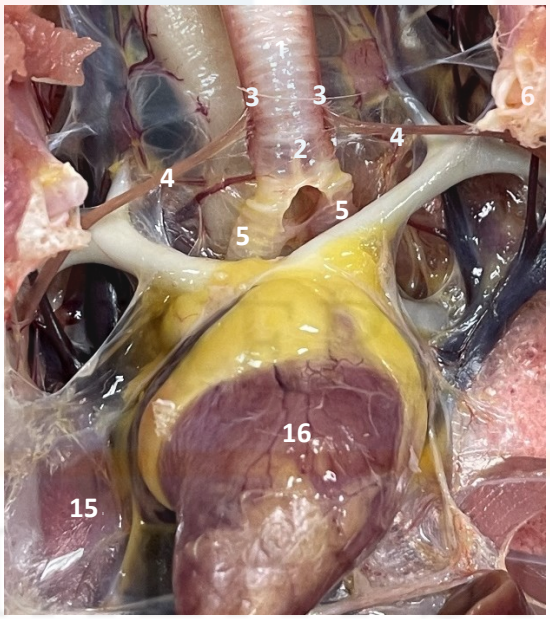

Figure 4.7. In situ ventral view of syrinx in the duck. 1. Trachea; 2. Tympanum; 3. Tracheolateralis muscle; 4. Sternotrachealis muscle; 5. Primary Bronchi; 6. Sternum; 16. Heart; 15. Lung.

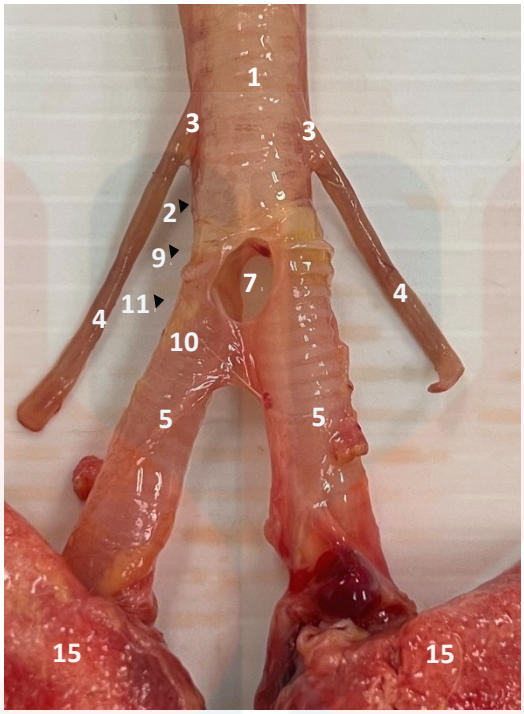

Figure 4.8. Dorsal view of the syrinx in the duck, in fresh state. 1. Trachea; 2. Typanum; 3. Tracheolateralis muscle; 4. Sternotrachealis muscle; 5. Primary Bronchi; 15. Lung; 9. Tracheosyringeal rings; 10. Bronchosyringeal half-rings; 11. Lateral tympaniform membrane; 7. Subpessular space.

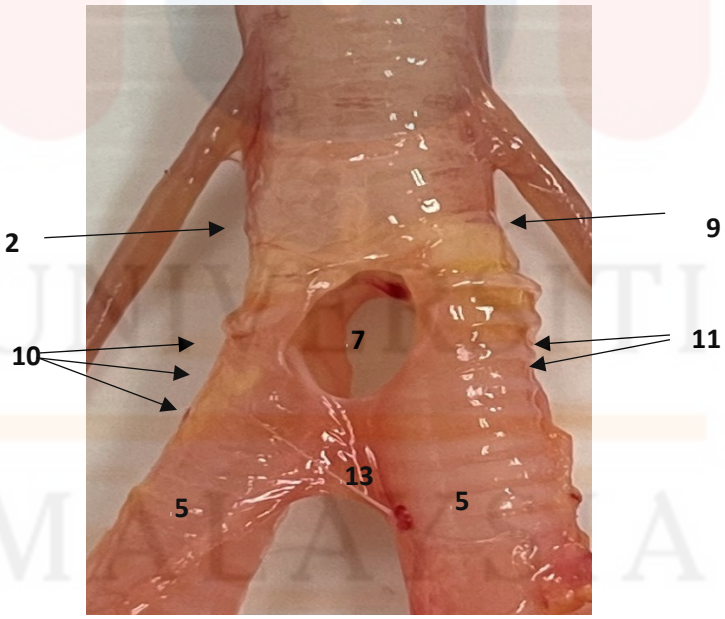

Figure 4.9. Magnified dorsal view of duck syrinx in fresh state. 2. Tympanum (Cranial cartilaginous group); 9. Tracheosyringeal rings Intermediate group; 10. Bronchosyringeal half-rings (Caudal cartilaginous group); 5. Primary bronchi; 11. Lateral (external) tympaniform membrane; 7. Subpessular space; 13. Interbronchial ligament.

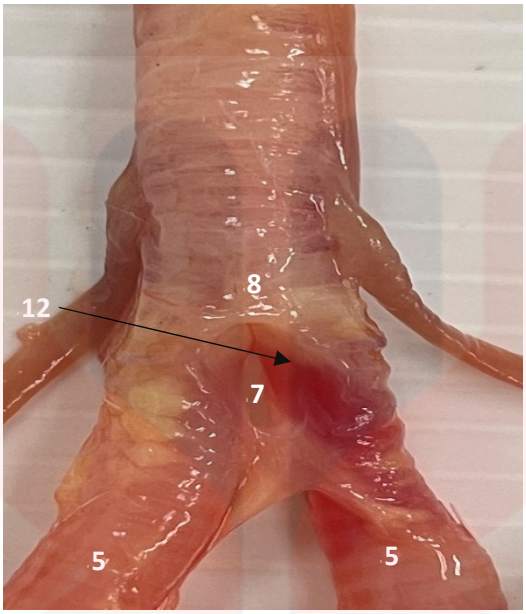

Figure 4.10. Magnified ventral view of duck syrinx in fresh state. 5. Primary bronchi; 7. Subpessular space; 8. Pessulus; 12. Medial (Internal) tympaniform membrane

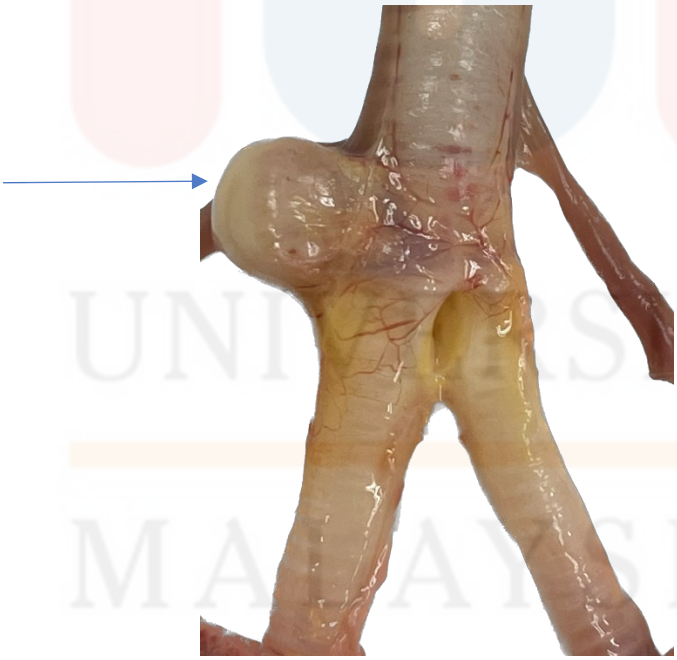

Figure 4.11. Syringeal bullae in duck (blue arrow)

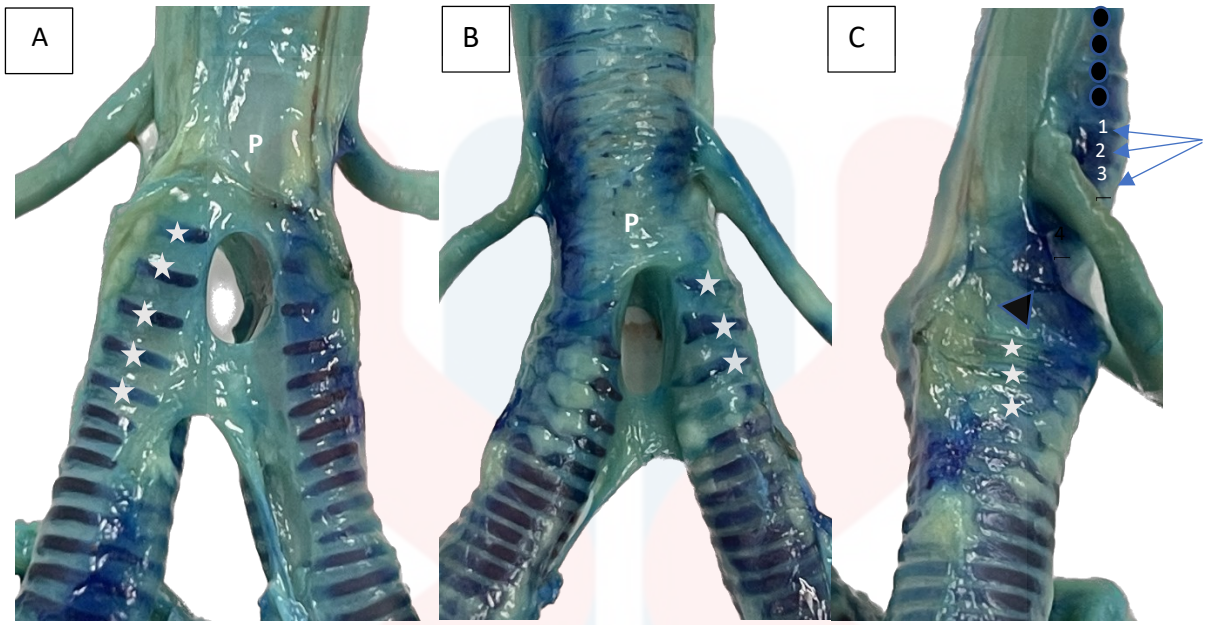

Figure 4.12. Gross anatomic appearance of the syrinx of duck stained with methylene blue 1%; dorsal(A), ventral (B) and lateral (C) views. (A-C): Tympanum rings (black rings), Tracheosyringeal rings (arrows), Bronchosyringeal half-rings (White stars), Lateral tympaniform membrane (arrow head), Pessulus (P).

Figure 4.13. In situ ventral view of syrinx in the quail. 1. Trachea; 2. Tympanum; 3. Tracheolateralis muscle; 4. Sternotrachealis muscle; 5. Primary Bronchi; 6. Sternum; 7. Lung

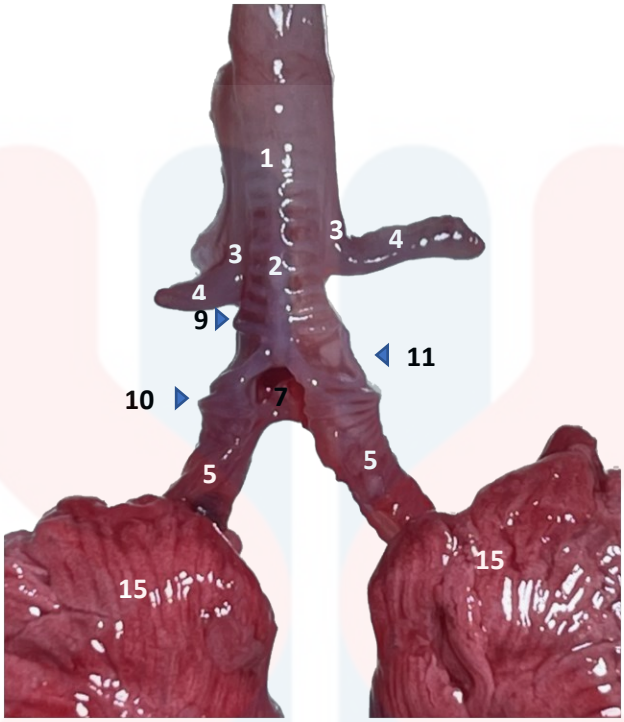

Figure 4.14. Dorsal view of the syrinx in the quail, in fresh state. 1. Trachea; 2. Typanum; 3. Tracheolateralis muscle; 4. Sternotrachealis muscle; 5. Primary Bronchi; 15. Lung; 9. Tracheosyringeal rings; 10. Bronchosyringeal half-rings; 11. Lateral tympaniform membrane; 7. Subpessular space.

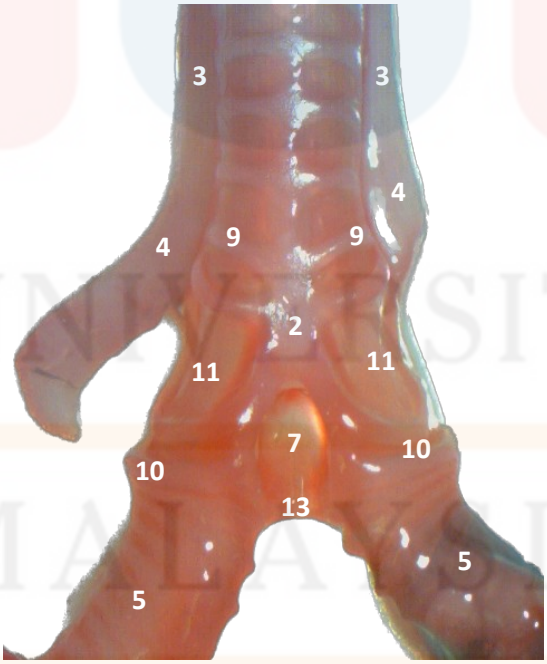

Figure 4.15. Magnified dorsal view of quail syrinx in fresh state. 2. Tympanum (Cranial cartilaginous group); 3. Tracheolateralis muscle; 4. Sternotrachealis muscle; 9. Tracheosyringeal rings Intermediate group; 10. Bronchosyringeal half-rings (Caudal cartilaginous group); 5. Primary bronchi; 11. Lateral (external) tympaniform membrane; 7. Subpessular space; 13. Interbronchial ligament.

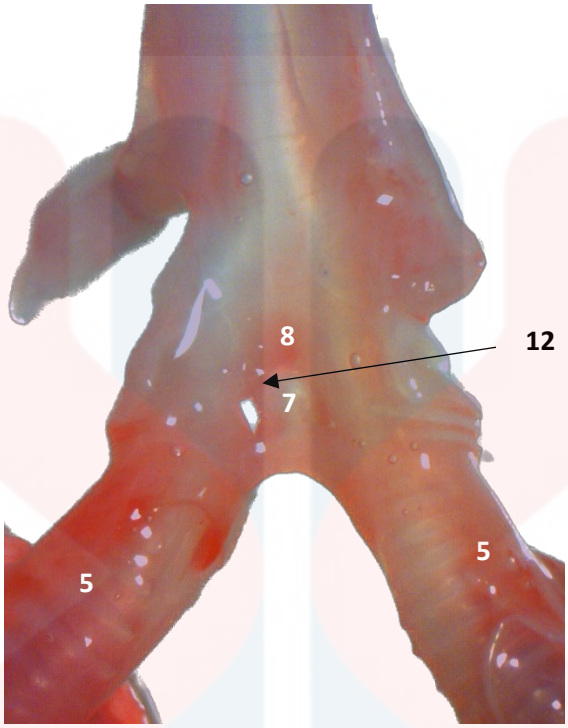

Figure 4.16. Magnified ventral view of quail syrinx in fresh state. 5. Primary bronchi; 7. Subpessular space; 8. Pessulus; 12. Medial (Internal) tympaniform membrane

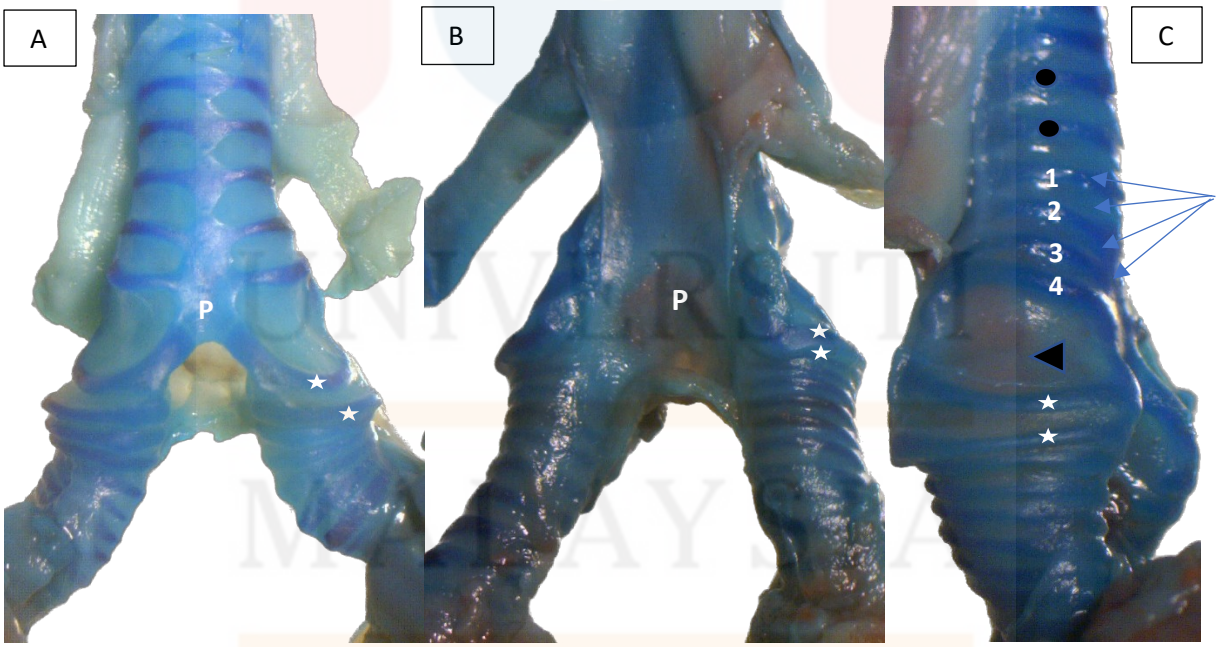

Figure 4.17. Gross anatomic appearance of the syrinx of quail stained with methylene blue 1%; dorsal(A), ventral (B) and lateral (C) views. (A-C): Tympanum rings (black rings), Tracheosyringeal rings (arrows), Bronchosyringeal half-rings (White stars), Lateral tympaniform membrane (arrow head), Pessulus (P).

4.1. Statistical variation in syringeal structures between different birds

A Vernier calliper was used to measure the syringeal component, which included the tracheolateralis and sternotrachealis muscles, in different birds (Table 4.1). The average length of the tracheolateralis muscle ranges between 120 and 125 mm for chickens, 70 and 75 mm for quail, and 160 and 186 mm for ducks. The measurement of the right sternotrachealis muscle revealed that chickens have average lengths of 13.1–18.1 mm, 7.9–9.6 mm in quails, and 29.8–31.7 mm in ducks. The measurement of the left sternotrachealis muscle shows an average length of 15.1–18.2 mm for chickens, 8.9–10.3 mm for quail, and 26.9–30.8 mm for ducks. The Kruskal-Wallis test results (Table 4.2) show that chickens have a median measurement of 125, quails have a median measurement of 70, and ducks have a median measurement of 186, with a P value of 0.26. The median measurement for the right sternotrachealis muscles in chicken is 15.1, in quail it is 9.6, and in duck it is 31.7, with a P value of 0.27. Whereas, for the median length of left sternotrachealis muscle, chicken shows a median of 15.8, quail 9.7, and duck 30.8. Results for pairwise comparison between groups of birds for the tracheolateralis muscle using the Mann-Whitney test (Table 4.3) reveal that groups of birds have significant p-values with chicken and quail (P = 0.43), chicken and duck (P = 0.46), and quail and duck (P = 0.46) The pairwise comparison of the right sternotrachealis between the groups of birds (Table 4.4) reveals significant results with chicken and quail (P = 0.46), chicken and duck (P = 0.50), and quail and duck (P = 0.46). For the left sternotrachealis muscle, the result for pairwise comparison between the groups of birds was (Table 4.5) shows significant results with chicken and quail (P = 0.46), chicken and duck (P = 0.50), and quail and duck (P = 0.46).

Table 4.1: Comparison of measurement of syringeal component in different birds

	Birds	Chicken			Quail			Duck		
		A	B	C	A	B	C	A	B	C
Syrinx Measurements	Length of trachea muscle Tracheolateralis (mm)	120	125	125	70	70	75	186	235	166
	Length of Sternotrachealis muscle (R) (mm)	18.1	13.6	15.1	9.6	9.6	7.9	31.7	32.6	29.8
	Length of Sternotrachealis muscle (L) (mm)	18.2	15.8	15.1	9.7	10.3	8.9	30.8	42.3	26.9

Table 4.2: Comparison of parameters between three different birds

Variables	Birds	n	Median	X^2 statistic (df)[a]	*P*- value
Length of Tracheolateralis muscle (mm)	Chicken	3	125	7.322	.026
	Quail	3	70		
	Duck	3	186		
Length of Sternotrachealis muscle (R) mm	Chicken	3	15.1	7.261	.027
	Quail	3	9.6		
	Duck	3	31.7		
Length of Sternotrachealis muscle (L) mm	Chicken	3	15.8	7.200	.027
	Quail	3	9.7		
	Duck	3	30.8		

Note. a = Kruskal-Wallis test

The medians of Tracheolateralis muscle length (mm) are significantly different (P = .026) between the different birds.

The medians of Right Sternotrachealis muscle length (mm) are significantly different (p = .027) between the different birds.

The medians of Left Sternotrachealis muscle length (mm) are significantly different (P = .027) between the different birds.

Mann -Whitney Test (Post Hoc Analysis)

Table 4.3: Comparison median length of Tracheolateralis muscle variation between birds.

Birds	n	Median	X^2 statistic (df)[a]	*p*-value
Chicken	3	125	6.000	0.43
Quail	3	70		
Chicken	3	125	6.000	0.46
Duck	3	186		
Quail	3	70	6.000	0.46
Duck	3	186		

Note: a= Kruskal-Wallis test

Post hoc multiple comparison test: chicken vs quail, p=0.43; chicken vs duck, p=0.46; quail vs duck, p=0.46

Table 4.4: Comparison median length of Right Sternotrachealis muscle variation between birds

Birds	n	Median	X^2 statistic (df)[a]	*p*-value
Chicken	3	125	6.000	0.46
Quail	3	70		
Chicken	3	125	6.000	0.50
Duck	3	186		
Quail	3	70	6.000	0.46
Duck	3	186		

Note: a= Kruskal-Wallis test

Post hoc multiple comparison test: chicken vs quail, p=0.46; chicken vs duck, p=0.50; quail vs duck, p=0.46

Table 4.5: Comparison median length of Left Sternotrachealis muscle variation between birds

Birds	n	Median	X^2 statistic (df)[a]	*p*-value
Chicken	3	125	6.000	0.46
Quail	3	70		
Chicken	3	125	6.000	0.50
Duck	3	186		
Quail	3	70	6.000	0.46
Duck	3	186		

Note: a= Kruskal-Wallis test

Post hoc multiple comparison test: chicken vs quail, p=0.46; chicken vs duck, p=0.50; quail vs duck, p=0.46

5. DISCUSSION

Birds' main method of communication is through phonation. They produce the voice through the syrinx, where they can produce extreme variations of sounds. The syrinx resembles the vocal cords in the larynx in humans, and it is the organ that allows birds to sing, as explained by Riede & Goller *et al.,* (2010). Sound is produced by vibrations in the syrinx walls together with tympaniform membranes that are generated when air passes through the syrinx.

The topographical findings of the syrinx in the present study among chicken, duck, and quail (Figures 4.2, 4.7, and 4.13) were similar to those of Denizli roosters by Marwa *et al.,* (2019). Moreover, in mallards, Frank et al., (2007), and turkeys, Marwa *et al.,* (2019), described similar findings. It can be classified as tracheobronchial in type as described in most birds, such as Denizli roosters by Marwa *et al.,* (2019), mallards by *Frank et al.,* (2007), and geese by Mohamed *et al.,* (2017).

The current study revealed that the tympanum of chickens consisted of four complete tracheal rings (Figure 4.6), similar to the Denizli roosters by Marwa *et al.,* (2019) and sparrow hawks by Ozudogru *et al.,* (2015). The findings from the present investigation also agree with Ragab *et al.,* (2016). The reasons for the similarity in the findings could be due to different breeds from different location of studies that might undergone evolution according to Freeman Dyce *et al.,* the tympanum in chickens is made up of three to five cartilaginous tracheal rings, which differs from the current findings with four tracheal rings. At the same time, present studies revealed that quail have two tracheal rings forming the tympanum (Figure 4.17). This is in agreement with Bayram and Liman *et al.,* (2000). In the current study, four

tracheal rings forming the tympanum in ducks were identified (Figure 4.12). This was in agreement with the findings by Bestami *et al.,* (2012).

The current study recorded highly modified, four incomplete C-shaped tracheosyringeal (intermediate) rings lying dorsally and attached to the pessulus ventrally in all the different species, including chickens, quails, and ducks (Figures. 4.6, 4.17, and 4.12). This was similar to the report by Ragab *et al.,* (2016) on chickens. Six C-shaped intermediate rings were reported in mallards by Bestami *et al.,* (2012), which are dissimilar from the present findings. As for the quail, there are no reported findings on the tracheosyringeal rings. It is suggested that the difference is due to the older age of birds in this study.

The bronchosyringeal part presented in the chicken (Figure 4.6) are the first three pairs of bronchial half-rings on both the right and left side of the syrinx and were directed as incomplete rings medially in agreement with Ragab *et al.,* (2016). Yildiz *et al.,* (2003) reported similar findings in songbirds.While in long-legged buzzards, the first four bronchial-half rings were reported by Kabak *et al.,* (2007). The bronchosyringeal part of the quail in this finding revealed two pairs on each side of the syrinx(Figure 4.17). This was supported by Bayram and Liman *et al.,* (2000). In ducks (Figure 4.12), asymmetry was seen on both sides, with the left having more cartilage than the right, which appeared more ossified. This was in agreement with Bestami *et al.,* (2012). This was dissimilar from findings reported by Mohamed *et al.,* (2017) with six bronchosyringeal half-rings in geese. Bronchosyringeal rings plays a crucial role in sound production in birds. The vibration produced by the syrinx are amplified by the resonance of the bronchosyringeal rings. This allows birds to produce a wide variety of sounds from simple chirps to complex songs, which are used for communication and territorial defence.

In the current review, the pessulum is defined as a non-ossified cartilaginous triangular or wedge shape formed at the tracheal bifurcation by the fusion of the right and left bronchus, with its apex pointed cranially through the syringeal lumen and its broad base directed caudally in chickens and quails (Figures. 4.6 and 4.17). Ragab *et al.,* (2016) discovered similar results in fowls. Ossified pessulus was recorded in ducks in the current studies. This was in agreement with similar findings by Onuk *et al.,* (2010). According to Marwa *et al.,* (2019), it was not present in penguins. The ossified pessulus leads to the medial tympaniform membranes becoming stiffer and vibrating more powerfully to make a sharper sound, which is produced by ducks (Figure 4.12). If the pessulus is made of cartilaginous structure, which causes insufficient stretching in the tympaniform membrane, a weaker sound will be produced, as exhibited by chickens and quails according to Ismail *et al.,* (2020).

The lateral and medial tympaniform membranes formed in the syrinx engage in the voice production of birds. In this present study, it was observed that two pairs of lateral and medial tympaniform membranes were present in all the birds (Figures 4.6, 4.17, and 4.12). The chicken and quail reveal thinner lateral and medial tympaniform membranes, while the duck shows thinner lateral tympaniform membranes and thicker medial tympaniform membranes. The lateral tympaniform is projected inward through the syringeal lumen from the last tracheosyringeal rings to the first bronchosyringeal half-rings. This was in agreement with findings by Ragab *et al.,* (2016) in chickens. However, different presentations have been reported in other birds, including pigeons with membrane extension from the third and fourth tracheosyringeal rings by Yildiz *et al.,* (2015). Similar findings for ducks were reported by Bestami *et al.,* (2012). While there was no report on the lateral tympaniform membrane in quails, The location of medial tympaniform membranes expanded between the free ends of the

first bronchosyringeal half-rings and stretched to the caudal aspect of the pessulus to the level of the third bronchodyringeal half-rings in the present work was in agreement with Ragab *et al.,* (2016). This finding differs in geese, where the medial tympaniform membrane is attached to the pessulus and second bronchosyringeal half-ring according to Onuk *et al.,* (2010). The medial tympaniform membrane in the ducks also presented thicker compared to the quail and chickens in this study, which causes the amplification of the sounds to be higher and increases the loudness. The sound oscillation in the chicken and quail was much reduced and produced lower attenuated sound due to the thinner membrane's sound oscillation. Ducks, in particular, often need to communicate over long distances in noisy environments, such as in a group of other ducks or in a body of water with a lot of ambient noise. This is why they need louder sounds to be heard by other ducks over these long distances and in these noisy environments. Chickens and quails, on the other hand, do not typically need to communicate over long distances, and they are not typically found in noisy environments. They may use soft calls to communicate with each other over short distances and in quieter environments

The present investigation recorded the extrinsic muscles, including tracheolateralis and sternotrachealis muscles, in all the birds (Figures 4.2, 4.7, and 4.13), which was in agreement with Ragab *et al.,* (2016). These muscles are important structures for communications as it helps sound-generating mechanism Both of our findings demonstrated the presence of paired tracheolateralis muscles on both sides of the trachea, which extended from the larynx to the cranial part of the tympanum. The paired sternotrachealis muscle extended from the medial aspect of the xiphisterna process of the sternum to the lateral aspect of the caudal part of the trachea. These two muscles aid in the adduction of the syrinx caudally, which controls airflow and sound characteristics. Similar findings were reported by Bestami *et*

al., (2012) in mallards. The intrinsic muscles were lacking in this study but recorded in songbirds and parrots by Ismail *et al.,* (2020).

A thick interbronchial ligament composed of collagen and elastic fibres was revealed in the present studies among all the birds (Figures. 4.3, 4.9, and 4.15), which agreed with Ragab *et al.,* (2016). It was also observed in geese by Onuk *et al.,* (2010). These structures help the median tympanic membrane vibrate and produce more intense sounds. Chickens, quails, and ducks need a more intense sound in order to communicate effectively with each other and to respond to potential threats in their environment. For example, chickens use vocalizations to communicate with other members of their flock, and the intensity of their calls can convey different messages, such as to warn of danger, to attract a mate, or to quails and ducks use vocalizations to communicate with each other and to establish territories. In some cases, the intensity of their calls may also be used to deter potential predators or to warn other members of their group of potential danger. In addition, these birds may need a more intense sound to compensate for the acoustic properties of their environment. For example, quails may use their calls to communicate in dense vegetation, which can absorb sound and make it more difficult to hear. By producing a more intense sound, they can more effectively communicate with each other and respond to potential threats. However, this structure is absent in pigeons according to Yildiz *et al.,* (2015).

Statistical analysis was performed using the non-parametric Kruskal-Wallis test to compare the variation between the median measurement of the syringeal component among the three different species of birds in the group. The Kruskal-Wallis test was used in this study as the data were not normally distributed (Tables 4.1 and 4.2). The medians of TL muscles are significantly different ($p = 0.26.$) between the different species of birds. The difference in

length of the tracheolateralis and sternotrachealis muscles among the birds indicates that birds can change the sound by changing the tension in the muscles around the syrinx, which changes the stiffness of the tympaniform membranes and changes the pitch or tone of the sound. The fact that the duck shows greater length of the TL and ST muscles compared to the chicken and quail indicates that these structures help in producing a louder sound. The Mann-Whitney test (Tables 4.1, 4.2, and 4.3) was used for pairwise comparisons between two independent groups. For pairwise comparison, the median measurements of the TL, right SL, and left SL are significantly different among the different species of birds. For the variation of the TL muscle, both the groups with chicken and duck have significantly longer lengths compared to the groups with quail and chicken. For both the right and left SL muscles, the chicken and duck has significantly longer length compared to the other groups. The longer length of the syringeal muscles in ducks and chickens leads to increased tension in the muscles, which causes the tympaniform membrane to vibrate intensely and produce a high-pitched sound.

6. CONCLUSION

In conclusion, for the first time in Malaysia, detailed information about the gross anatomy of the syrinx was provided, with statistical variation in different birds. There was a significant difference between the syrinx in different birds (p -value<0.05). Tracheobronchial

types were the most common types of syrinxes reported among birds. Although it was consistent with the non-singing birds, some anatomical variations were determined. Hence, this preliminary study will pave the way for further studies on this subject, which remains a crucial structure for phonation in birds.

7. RECOMMENDATION

The data presented in this current study is limited to represent the syrinx anatomy of the entire bird's species in Malaysia. Therefore, future investigations involving more replicates should be done to provide more accurate and consistent results.

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