

## Morphological comparison of the chukar partridge (*Alectoris chukar*) and Japanese quail (*Coturnix coturnix japonica*) syrinx

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### Article Info

#### Article history:

Received: 07 March 2022  
Accepted: 09 November 2022  
Available online: 15 June 2023

#### Keywords:

Chukar partridge  
Japanese quail  
Morphology  
Syrinx

### Abstract

Syrinx is a voice device and shows structural and functional differences between bird species. This study aimed to investigate morphological and histological structures of the syrinx in chukar partridge (*Alectoris chukar*) and Japanese quail (*Coturnix coturnix japonica*). In the present study, 12 male chukar partridges and 12 male Japanese quail were used. The syrinx tissues were photographed by digital camera and fixed in formaldehyde solution. Five syrinxes were stained with methylene blue to make the syrinx rings distinct. After anatomical examination, tissues were passed through alcohol series, cleaned in xylene, and embedded in paraffin blocks. The blocks were cut and obtained sections were stained with Crossman modified triple staining and examined under camera attached light microscope. The syrinx of chukar partridges and Japanese quail consisted of cartilaginous tracheosyngeales and bronchosyngeales in the region of *bifurcatio trachea* and at the level of *basis cordis*. The tracheal rings constituting syrinx were counted three in chukar partridge and four in Japanese quail. The bronchial rings comprising syrinx counted nine in chukar partridge and eight in Japanese quail. In the histological examination, the *pesullus* structure was hyaline cartilage and calcified with increasing ages being covered by pseudostratified columnar epithelium. The results of the study suggested that chukar partridge and Japanese quail syrinxes have some morphological differences compared to the other bird species; but, anatomically and histologically similarities to many bird species.

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### Introduction

Syrinx is the vocal organ of birds showing histological and anatomical differences according to the bird species. The sounds made by syrinxes have an important role in distinguishing the species. Birds are classified as passerine and non-passerine birds according to the structural differences of their vocal organ (syrinx).<sup>1</sup>

The syrinx is located at the level of 2<sup>nd</sup> and 3<sup>rd</sup> thoracic vertebrae, just before the *bifurcatio trachea*, and between the trachea and *bronchus primarius*.<sup>2</sup> The combination of cartilages known as syringeales forms cartilagines. If these cartilages originate from the trachea, they are called *cartilagine tracheales syringes*, and if they originate from the *tympanum*, they are called *cartilagine bronchiales syringes*. Due to the combination of *cartilagine tracheales syringes*, the cylindrical formation called *tympanum* is formed. The areas originating from the *tympanum* are called *cartilagine bronchiales syringes*, and the free ends of

these cartilages, similar to a C-shaped half ring, are supported by membranous structures called *membrana tympaniformis medialis* and *membrana tympaniformis lateralis* from the outside.<sup>2</sup> These two thin membranes play an active role in forming sound.<sup>1,3,4</sup> Syrinx is examined in three groups according to the type of cartilages making it up including tracheal, bronchial, and tracheobronchial syrinxes.<sup>2,4</sup> Most birds have a tracheobronchial syrinx.<sup>5</sup>

The cartilage called *pesullus* being located in the median part just below the *tympanum* in the syrinx directs the air from the trachea to the *bronchus primaries*.<sup>6</sup> In some species,<sup>7</sup> the *pesullus* takes part in forming sound, and the elastic fibers in its structure play a role in frequency control. In contrast, it has been suggested that *pesullus* does not have a vibration feature due to its structure; therefore, the sound formation does not occur here.<sup>8</sup> A common belief is that the sound source is the vibration of the *membrana tympaniformis lateralis et medialis*, and it is accepted that it occurs only during expiration.<sup>2</sup>

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There are five pairs of syrinx muscles in songbirds including *musculus tracheobronchialis*, *m. tracheobronchialis brevis*, *m. tracheobronchialis ventralis*, *m. syringalis dorsalis*, and *m. syringeales ventralis*.<sup>2</sup> Although the mucosal epithelium of syrinx differs among bird species, it consists of bilayer columnar, pseudostratified prismatic, and squamous epithelia.<sup>9</sup> The structure of *pessulus* in *cavum syringis* is in the form of a double-layered mucosal membrane extending dorsoventrally towards the primary bronchus.<sup>5</sup>

The purpose of this study was to investigate morphological and histological structures of the syrinx in chukar partridge (*Alectoris chukar*) and Japanese quail (*Coturnix coturnix japonica*).

## Materials and Methods

**Anatomical analysis.** In the present study, 12 male chukar partridges (*A. chukar*; 2-3 years old) were bought from Chukar Partridge Production Farm' from the region of Merzifon in Amasya, Türkiye, and 12 male Japanese quail (*C. coturnix japonica*; 4 - 6-month-old) were provided from Ataturk University Livestock Research and Application Farm, Erzurum, Türkiye. All animals were sacrificed under diethyl ether anesthesia (~ 4.00 - 5.00 mL via inhalation route) and syrinx tissues were obtained from the *bifurcatio trachea* region. The study was approved by the Local Ethics Committee of the Ataturk University, Erzurum, Türkiye (Approval Number: 2015-8/150). Then, the tissues were photographed by digital camera (Axio Scope A1; Zeiss; Jena, Germany) and after that, fixed in 10.00% formaldehyde solution for morphological and histological analyses. Five syringes were stained with Methylene blue to make the syrinx rings distinct. Morphologically, bronchial and tracheal rings were also counted.

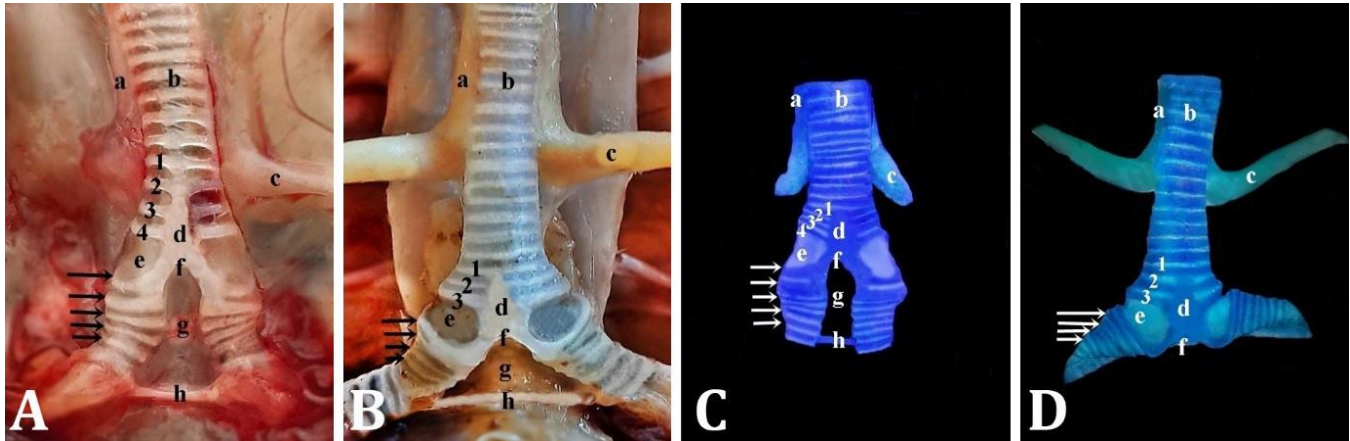
**Histological analysis.** After anatomical examination, tissues were passed through alcohol series, cleaned in xylene and embedded in paraffin blocks. The blocks were cut and obtained sections (5.00 - 7.00 µm thickness) were stained with Crossman modified triple staining for histological analysis and examined under light microscope (Eclipse 50i; Nikon, Tokyo, Japan).

## Results

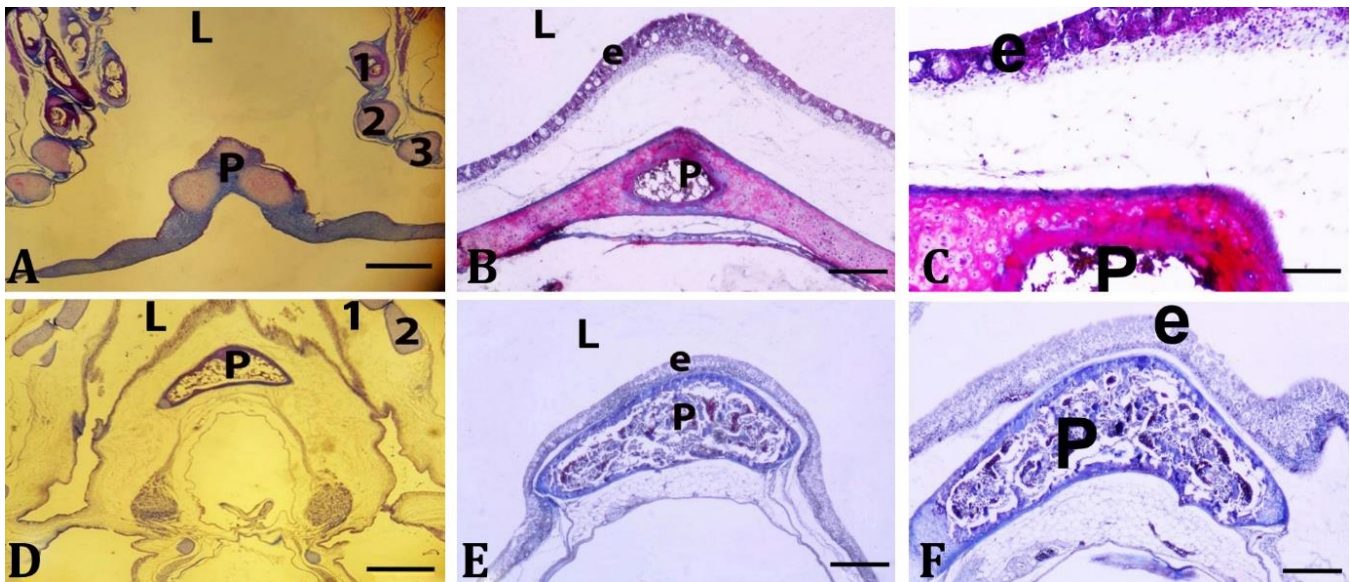
**Macroscopical findings.** It was determined that in chukar partridge (*A. chukar*) and Japanese quail (*C. coturnix japonica*), the syrinx is located at the craniodorsal part of the sternum, in the *bifurcatio trachea* region, and at the level of the *basis cordis*. It is composed of *cartilagine tracheales syringes* and *cartilagine bronchiales syringes*, and it was found to be a tracheobronchial type. The *cartilagine tracheales syringes* and *cartilagine bronchiales syringes* were flattened in the dorsoventral direction. It

was determined that *cartilagine tracheales syringes* took their origin from the trachea and participated in syrinx formation. There were three in chukar partridge and four in Japanese quail, and they came together to form the *tympanum*. It was determined that these cartilages merged among themselves in the median part. It was observed that *cartilagine bronchiales syringes* were C-shaped and had a wider lumen than bronchial cartilages. The *cartilagine bronchiales syringes* making up the syrinx were nine in chukar partridge and eight in Japanese quail (Fig. 1). In both species, it was determined that the C-shaped *cartilagine bronchiales syringes* of the *membrana tympaniformis medialis*, producing sound in birds due to its vibration, tightly closed their free ends. The *membrana tympaniformis lateralis* was localized between the last (2<sup>nd</sup>) ring of the *tympanum* and the first ring of *cartilagine bronchiales syringes*. It was observed that the ossified *pessulus*, dividing the airflow into two sending it to the *bronchus primarius*, was located in the *bifurcatio trachea* region. It was determined that the ligament connecting the right and left bronchial rings was *ligamentum interbronchialis*, and the *foramen interbronchialis* was formed between *pessulus* and this ligament (Fig. 1). In chukar partridge and Japanese quail, it was observed that *m. tracheolateralis* was the muscle bilaterally surrounding the trachea and *m. sternotrachealis syringis* from the outside. The *m. tracheolateralis* was started from larynx and extended bilaterally in trachea to the first *cartilago bronchialis syringis*, and the *m. sternotrachealis* was located bilaterally in the distal 1/3 of the trachea (Fig. 1).

**Histological findings.** It was observed that the structure of the *pessulus* was hyaline cartilage in chukar partridge; ossification begins with adolescence and increases with age. It was covered by pseudostratified columnar epithelium, and the loose connective tissue was located under the epithelial tissue. It was determined that there were goblet cells from place to place in the epithelial tissue. It was found that the cartilaginous rings were covered by a muscular layer (Figs. 2A - 2C). It was determined that this muscular layer was formed by the longitudinal and transversal skeletal muscles. While it was observed that the cartilage structure of the *pessulus* continued to the sides towards the *membrana tympaniformis medialis* in the chukar partridge, it was determined that the cartilage structure of the *pessulus* did not extend to the sides in the Japanese quail (Figs. 2A and 2B). Also, it was observed that the fibro-elastic structure of the *pessulus* of the Japanese quail syrinx was more than that of the chukar partridge. In comparison, the cartilage structure of *pessulus* was more in the chukar partridge than Japanese quail (Figs. 2A and 2B). In both species, the external tympanic membrane epithelium was pseudostratified columnar and it was simple squamous epithelium in the internal one (Fig. 2), and tracheosyringeal or bronchosyringeal cartilages had hyaline structures.



**Fig. 1.** Macro-anatomical view of syrinx in Japanese quail (*Coturnix Coturnix Japonica*) and chukar partridge (*Alectoris chukar*). **A)** Syrinx in Japanese quail. a) *Musculus tracheolateralis*; b) Trachea; c) *Musculus sternotrachealis*; d) Tympanum; e) *Membrana tympaniformis lateralis*; f) *Pessulus*; g) *Foramen interbronchialis*; h) *Ligamentum interbronchialis*; 1, 2, 3 and 4) *Cartilagine tracheales sryingis*; Arrows: *Cartilagine bronchiales sryingis*. **B)** Syrinx in chukar partridge. a) *Musculus tracheolateralis*; b) Trachea; c) *Musculus sternotrachealis*; d) Tympanum; e) *Membrana tympaniformis lateralis*; f) *Pessulus*; g) *Foramen interbronchialis*; h) *Ligamentum interbronchialis*; 1, 2, 3 and 4) *Cartilagine tracheales sryingis*; Arrows: *Cartilagine bronchiales sryingis*. **C)** Syrinx with Methylene blue staining in Japanese quail. a) *Musculus tracheolateralis*; b) Trachea; c) *Musculus sternotrachealis*; d) Tympanum; e) *Membrana tympaniformis lateralis*; f) *Pessulus*; g) *Foramen interbronchialis*; h) *Ligamentum interbronchialis*; 1, 2, 3 and 4) *Cartilagine tracheales sryingis*; Arrows: *Cartilagine bronchiales sryingis*. **D)** Syrinx with Methylene blue staining in chukar partridge. a) *Musculus tracheolateralis*; b) Trachea; c) *Musculus sternotrachealis*; d) Tympanum; e) *Membrana tympaniformis lateralis*; f) *Pessulus*; g) *Foramen interbronchialis*; h) *Ligamentum interbronchialis*; 1, 2, 3 and 4) *Cartilagine tracheales sryingis*; Arrows: *Cartilagine bronchiales sryingis*.



**Fig. 2.** Histological view of syrinx in Japanese quail (*Coturnix Coturnix Japonica*) and chukar partridge (*Alectoris chukar*). **A, B and C)** Syrinx with Crossman modified triple staining in chukar partridge. 1, 2 and 3) *Cartilagine tracheales sryingis*, e) Pseudostratified columnar epithelium; L) Lumen, P) *Pessulus*; (bars = 200, 60.00, and 20.00  $\mu$ m). **D, E and F)** Syrinx with Crossman modified triple staining in Japanese quail. 1 and 2) *Cartilagine tracheales sryingis*, e) Pseudostratified columnar epithelium; L) Lumen, P) *Pessulus*, (bars = 200, 60.00, and 20.00  $\mu$ m).

**Discussion**

As in the syrinx of long-legged buzzard,<sup>4</sup> ostrich,<sup>6</sup> Denizli rooster,<sup>10</sup> and many winged species,<sup>4,6,11,12</sup> it was found to be a tracheobronchial type in chukar partridge and Japanese quail, being flattened in the dorsoventral direction. While it has been reported that there are three

or four *cartilagine tracheales sryingis* in sparrows<sup>8</sup> and four in songbirds and domestic birds,<sup>3,10</sup> this number was three in the chukar partridge and four in Japanese quail. The study determined that the *cartilagine bronchiales sryingis*, participating in the syrinx formation, were C-shaped, as in the sparrowhawk.<sup>13</sup> While it has been reported that these rings are three in sparrows,<sup>8</sup> three in

songbirds and domestic birds,<sup>3,10</sup> and five in sparrow-hawks,<sup>13</sup> nine and eight rings were found in chukar partridge and Japanese quail, respectively.

Reportedly, *tympanum* in duck, seagull and pigeon, is shaped by the combination of five *cartilagine tracheales syringes*, and in sparrowhawk, ostrich and long-legged buzzard, is shaped by the combination of three *cartilagine tracheales syringes*.<sup>1,4,6,13-15</sup> In this study, three *cartilagine tracheales syringes* in the chukar partridge and four in Japanese quail came together and formed the *tympanum*.

Unlike our findings, the *membrana tympaniformis lateralis* is between the first *tympanum* ring and the first bronchosyringeal ring in long-legged buzzard,<sup>4</sup> male mallards<sup>1</sup> and ostrich.<sup>6</sup> In contrast, it has been reported in the 1<sup>st</sup> and 2<sup>nd</sup> bronchosyringeal sites in seagulls.<sup>16</sup> It was determined in the current study that the *membrana tympaniformis lateralis* was located between the third *cartilago tracheales syringis* in chukar partridge (the last cartilaginous ring forming the *tympanum* in chukar partridge) and the fourth *cartilago tracheales syringis* in Japanese quail (the last cartilaginous ring forming the *tympanum* in Japanese quail) and the first *cartilago tracheales syringis*. Consistent with the literature,<sup>17,18</sup> it was determined that the C-shaped *cartilagine bronchiales syringes* of the *membrana tympaniformis medialis* tightly closed their free ends.

Unlike our findings, it has been reported that *pessulus* is absent in raptors,<sup>3</sup> ostrich,<sup>6</sup> and pigeon.<sup>8,12</sup> Similar to former findings,<sup>16,19</sup> it was determined that the ligament connecting the right and left bronchial rings was *ligamentum interbronchialis*, and the *foramen interbronchialis* was formed between *pessulus* and this ligament in both species.

It has been reported that syrinx muscles in sparrowhawk consist of skeletal muscles being circumscribed externally.<sup>13</sup> Reportedly, it has been demonstrated that syrinx muscles surround the syrinx externally and internally.<sup>12</sup> In the present study, similar to the findings in sparrowhawks,<sup>13</sup> seagulls<sup>16</sup> and gray heron,<sup>19</sup> the *m. tracheolateralis* started from larynx and extended bilaterally to the first *cartilago bronchealis syringes* in the trachea, and *m. sternotrachealis* was located bilaterally in the distal third of trachea.

While *pessulus* consists of connective tissue in pigeons and ostrich, it is a hyaline cartilage in sparrowhawk and ossified cartilage in ducks and songbirds.<sup>3,6,7,13,15</sup> Herein, it was observed that *pessulus* was shaped by hyaline cartilage in both species, similar to the sparrowhawk.<sup>13</sup> It was also reported that *pessulus* is columnar in sparrowhawk.<sup>13</sup> Warner<sup>8</sup> has reported that this structure cannot contribute to the formation of sound due to the lack of vibration. This study determined that *pessulus* was covered by pseudostratified epithelium, similar to several bird species.<sup>4,13,14</sup>

As a result, as in most bird species, the syrinx was tracheobronchial type and formed by *cartilagine tracheales syringes* and *cartilagine bronchiales syringes*. It was concluded that the differences between the syrinx of chukar partridge (*A. chukar*) and Japanese quail (*C. coturnix japonica*) and from other bird species might be due to the environment and living conditions. It is believed that this research will contribute to future scientific studies regarding the subject.

## Acknowledgments

This study was financially supported by Ataturk University Scientific Research Projects, Erzurum, Türkiye (Grant No. BAP-2015-287).

## Conflict of interest

None declared.

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