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Morphological Analysis of Air Sacs in the Red-Winged Tinamou (*Rhynchotus rufescens* Temminck, 1815)

Running Title: Air Sacs in Red-Winged Tinamou

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ABSTRACT

The red-winged tinamou (*Rhynchotus rusfencens*) is a bird belonging to the order Tinaniforme, family Tinamidae, present in South America, and due to its population decline is classified as LC (Least Concern) on the BirdLife International red list. This study aimed to evaluate the air sacs of this species, as anatomical studies of partridges are scarce. Ten cadaveric specimens were collected, and latex perfusion was used to solidify the material. The cervical air sac in the red-winged tinamou is smaller and has a more irregular conformation than other air sacs. The thoracic air sacs are symmetrical, and the cranial thoracic air sacs are smaller than the caudal ones. The abdominal air sacs are asymmetrical, and the largest, extending to the cloaca. Only one clavicular air sac was found, with three subdivisions: right, left, and medial. Additionally, right, and left extra-coelomatic portions were found, passing under the clavicle. In one of the animals, the latex-filled humeri were found, and in three other ribs, vertebral diverticula were present. There is no clear relationship between taxonomy and biology versus the quantity and conformation of air sacs, as different animals with taxonomic proximity present differences. This study enhances species-specific anatomical knowledge of the red-winged tinamou.

Keywords: animal anatomy; birds; respiratory system; wild animals.

INTRODUCTION

The red-winged tinamou (*Rhynchotus rusfencens* Temminck, 1815) belongs to the order Tinaniforme, family Tinamidae (Cubas et al., 2014). It is present in South America, and despite its declining population (BirdLife International, 2022; Cubas et al., 2014), the red-winged tinamou is classified as LC (*Least concern*) on the BirdLife International red list (BirdLife International, 2022).

Given the scarcity of anatomical studies in partridges, the present study aimed to evaluate the anatomy of the air sacs in this species.

The air sacs are thin-walled structures that are found throughout the bird's body and play a crucial role in the respiratory process. They function to maintain a unidirectional flow of air through the lungs, which allows for efficient gas exchange. The air sacs are also involved in thermoregulation, sound production, and buoyancy control in some aquatic species of birds (Casteleyn et al., 2018; O'Malley, 2005).

The anatomical structure of the air sacs in birds is unique and has evolved to meet the demands of flight. Birds have a total of nine air sacs, which are connected to the lungs and trachea. The air sacs are divided into four groups: cervical, cranial thoracic, caudal thoracic, and abdominal. Each group of air sacs has a specific function in the respiratory process, and they work together to allow birds to extract oxygen efficiently from the air (Casteleyn et al., 2018).

Birds do not possess a diaphragm, so they do not present any differentiation between the thoracic and abdominal cavities. The respiratory system of birds is divided into lower and upper respiratory tract. The upper respiratory system is formed by the nasal cavity, larynx and trachea, whereas the lower respiratory tract is formed by the lungs and air sacs (8 or 9 depending on the species) (O'Malley, 2005). The physical separation (decoupling) of the lung, which serves as the gas exchanger, from the air sacs, where mechanical ventilation occurs, enables a continuous, one-way airflow within the lung. These characteristics, among others, contribute to the remarkable efficiency of the gas exchange process, supporting the highly aerobic lifestyles and substantial metabolic capacities commonly found in birds (Maina, 2006, 2007).

Air sacs have already been described in the following birds: the domestic fowl (*Gallus gallus*) (Akester, 1960; Goodchild, 1970), domestic goose (*Anser anser domesticus*) (Onuk et al., 2009), duck (*Anas spp.*) (Araújo et al. 2014), turkey (*Meleagris sp.*) (King, Atherton 1970; Ragab, Reem 2016; Rigdon et al. 1958), ostrich (*Struthio camelus*) (Deeming, 1999), quail (*coturnix coturnix*) (Bianchi et al. 2016), Japanese quail (*coturnix coturnix japonica*) (Çevik-Demirkan et al. 2006), European red-winged tinamou, of the order Galliformes, (*Alectoris graeca*) (Kürtül et al. 2004), long-legged buzzard (*Buteo rufinus*) (Orhan et al. 2009), European pidgeon (*Columbia livia*) (Akester, 1960; Gilbert, 1939; Müller, 1908), loon (*Gavia immer*) (Gier, 1952), White Cheeked Bulbul (*Pycnonotus leucogenys*) (Jaifar & Sawad, 2016) and hooded crow (*Corvus cornix*) (El-Sayed & Hassan, 2020). The clinical care of birds in wild animal medicine is considered common (Capaverde-Jr et al. 2018; Lima, Silva 2014), but it must be remembered that such birds are an extremely diversified class, comprising 32 orders and more than 10,000 distinct species (Cubas et al. 2014; Tully et al. 2010).

MATERIALS AND METHODS

Ten birds were used in this study and were obtained from the Department of Animal Improvement and Nutrition in the Animal Science course at UNESP – Botucatu Campus. The cadaveric material was collected after the slaughter of animals from projects in the production area, which were injected with latex and dissected for macroscopic evaluation. The process involved opening the skin in the cervical region, exposing the trachea, and making a vertical cut of approximately 1 cm to introduce a cannula for latex perfusion. The material was solidified by freezing it for 15 days, and then a midline incision was made from the trachea to the cloaca to expose the pectoral musculature. The pectoral muscles and the sternum were removed by separating the costochondral joints and the shoulder girdle. The viscera were removed, and the latex mold was removed from the carcass.

The methodology adopted in this study was approved by Ethics Committee Approval Statement (Committee on Ethics in the Use of Animals – CEUA) of the School of Veterinary Medicine and Animal Science at the São Paulo State University (UNESP) filed under the number 0030/2021. The project also obtained authorization from the Biodiversity Authorization and Information System– SISBIO, a division of the Brazilian Institute for the Environment and Renewable Natural Resources – IBAMA, for the temporary maintenance of wild vertebrates in captivity, under number 77786-1.

RESULTS

The partridge's air sacs are in close relationship with the viscera - liver, proventriculus and intestine - in the coelomic cavity. After removing the viscera, it is possible to visualize an anatomically organized set of abdominal, clavicular, cranial thoracic and caudal thoracic air sacs (Figure 1).

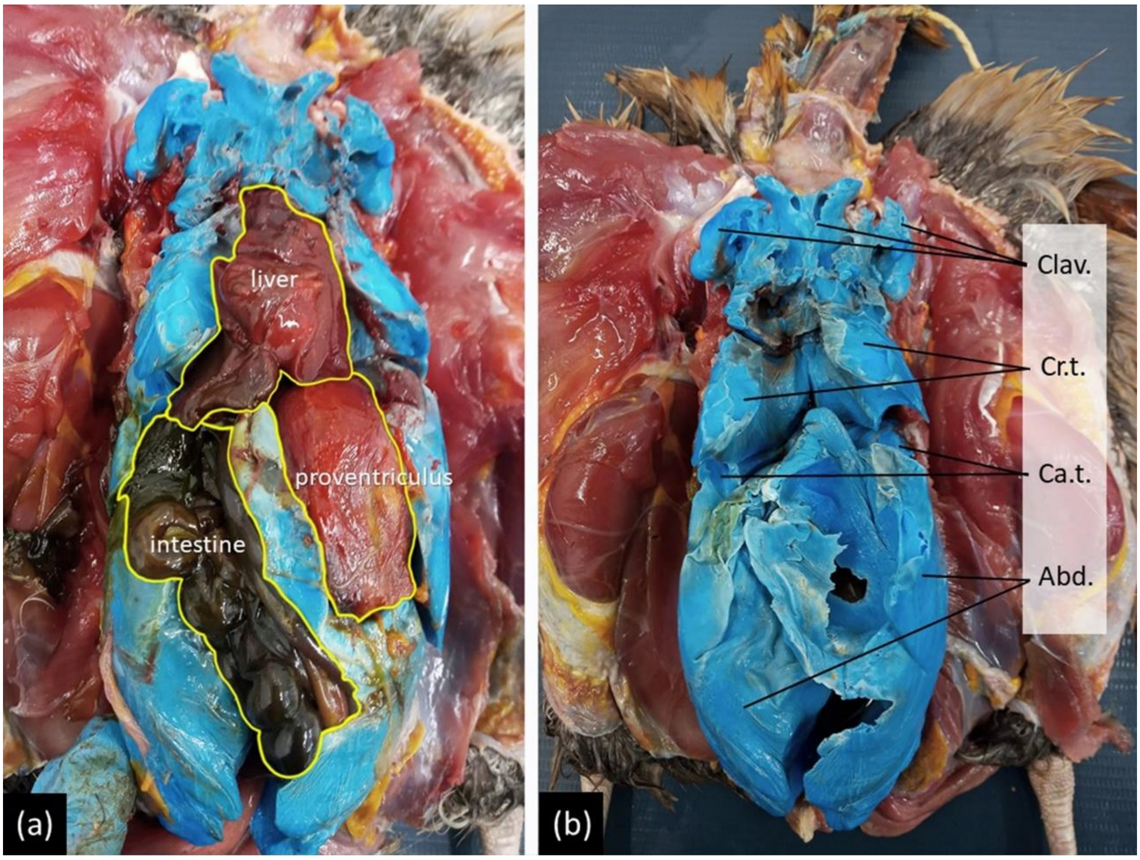

Figure 1 – (a) Relationship of air sacs and viscera in the coelomic cavity of the Partridge, where you can visualize the Liver (red), Proventriculus (yellow) and Gut (green). **(b)** Visualization of air sacs in the coelomic cavity partridge after removal of viscera. Ab.: Abdominal air sac; Clav.: Clavicular air sac; Cr.T: Cranial thoracic air sac; Ca.T: Caudal thoracic air sac.

The partridges, commonly, do not present cervical air sacs. But in the red-winged tinamou were identified a total of seven air sacs, including one cervical air sac smaller and more irregularly shaped cervical air sac compared to other birds. Furthermore, one pair of cranial thoracic air sacs, one pair of caudal thoracic air sacs, and one pair of abdominal air sacs (Figure 2). Additionally, a clavicular air sac with three subdivisions was observed: right, left and medial; there are right and left extracoelomic portions found under the clavicle (Figure 2).

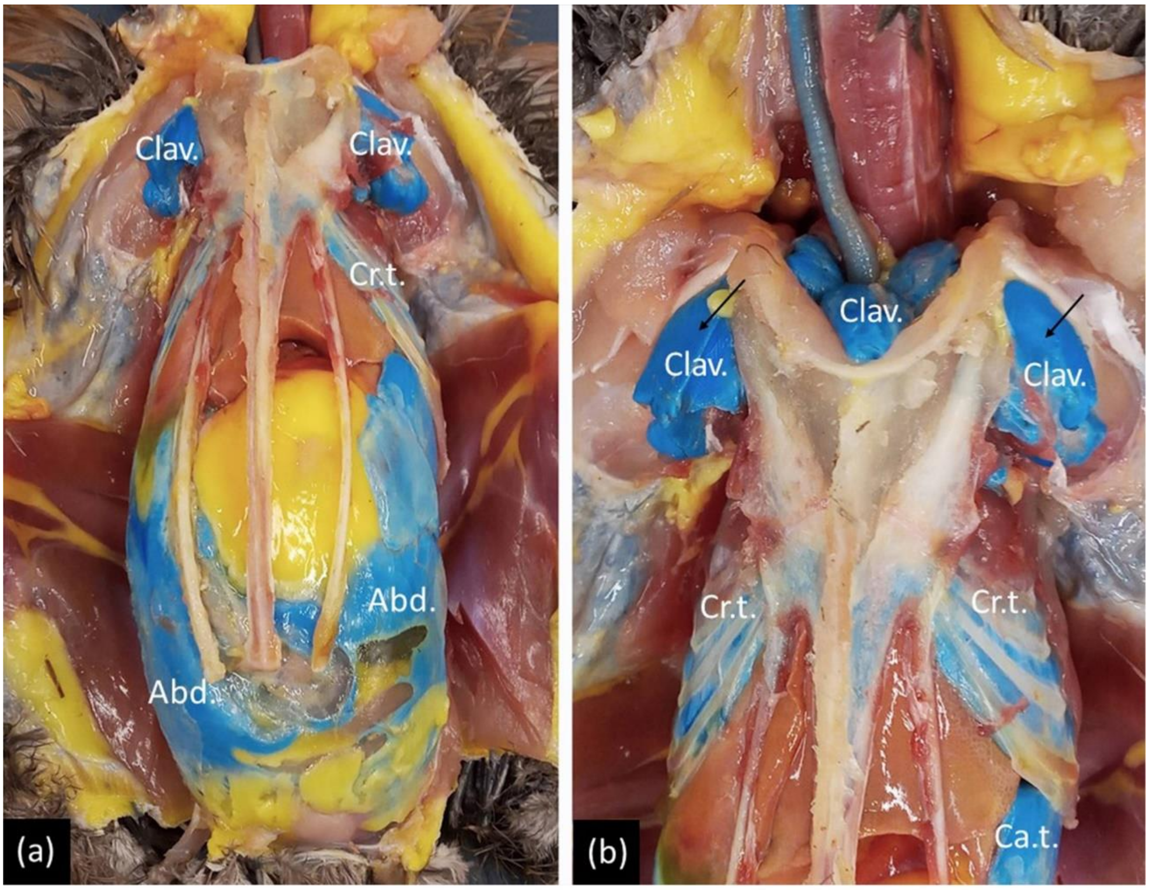

Figure 2 – (a) Visualization of partridge air sacs after skin removal and ventral musculature. **(b)** Note the extrathoracic portion of the clavicular air sac (arrows). Ab.: Abdominal air sac; Clav.: Clavicular air sac; Cr.T: Cranial thoracic air sac; Ca.T: Caudal thoracic air sac.

The thoracic air sacs are symmetrical and the cranial thoracic air sacs are smaller than the caudal ones. The cranial thoracic air sacs are in close anatomical relationship with the lungs. The abdominal air sacs are asymmetrical and larger, extending to the cloaca region (Figures 3 and 4).

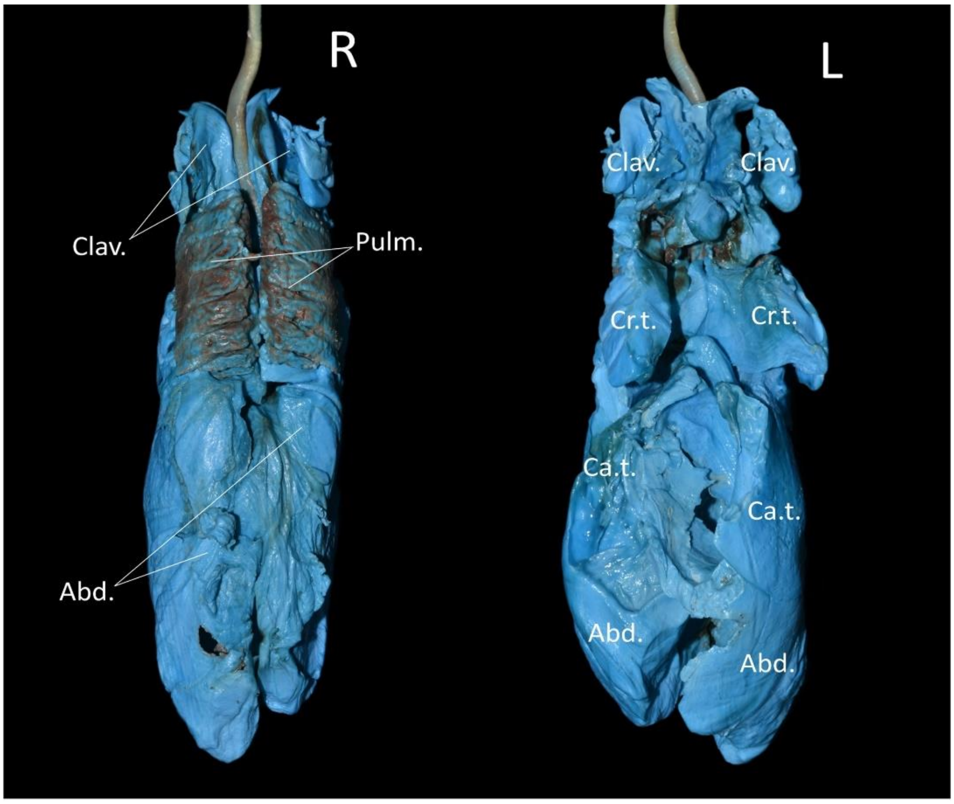

Figure 3 - Latex cast of partridge lower respiratory system, view dorsal **(a)** and ventral **(b)**. Ab.: Abdominal air sac; Clav.: Clavicular air sac; Cr.T: Cranial thoracic air sac; Ca.T: Caudal thoracic air sac; Pulm.: Lung.

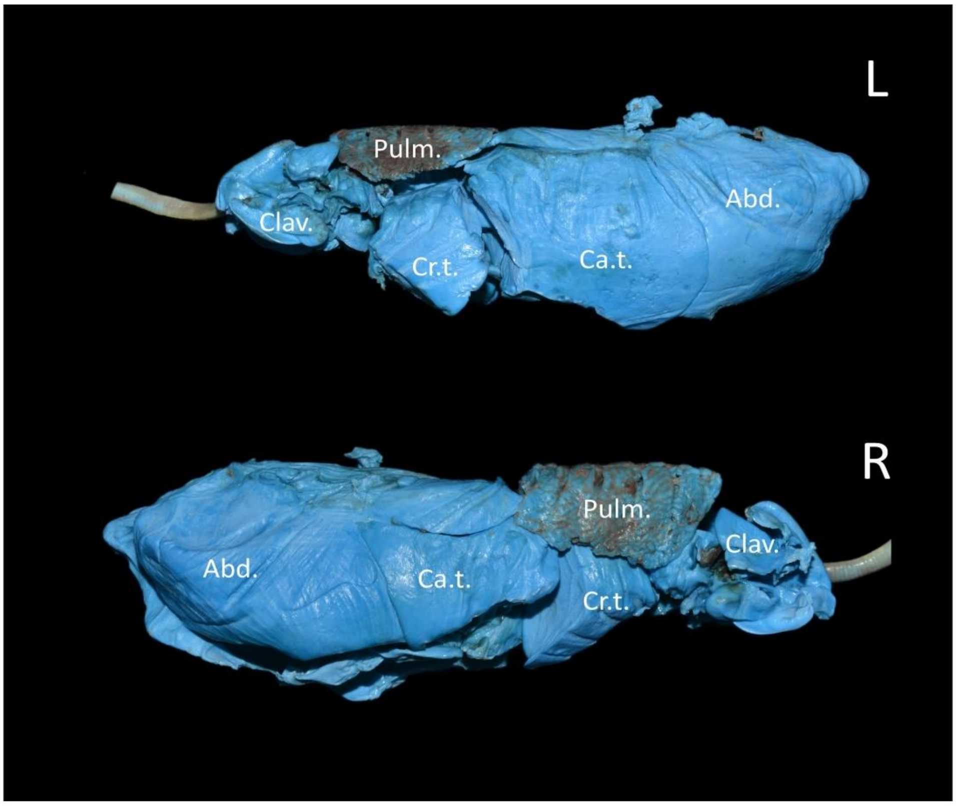

Figure 4 - Latex cast of partridge lower respiratory system, view right side (above) and left side (below). Ab.: Abdominal air sac; Clav.: Clavicular air sac; Cr.T: Cranial thoracic air sac; Ca.T: Caudal thoracic air sac; Pulm.: Lung.; R=right; L=Left.

Furthermore, in one of the animals, both humeri and vertebral diverticula were found to be full of latex (Figures 5 and 6). The filling of the intramedullary space of the partridge's humerus highlights the pneumatization of this bone.

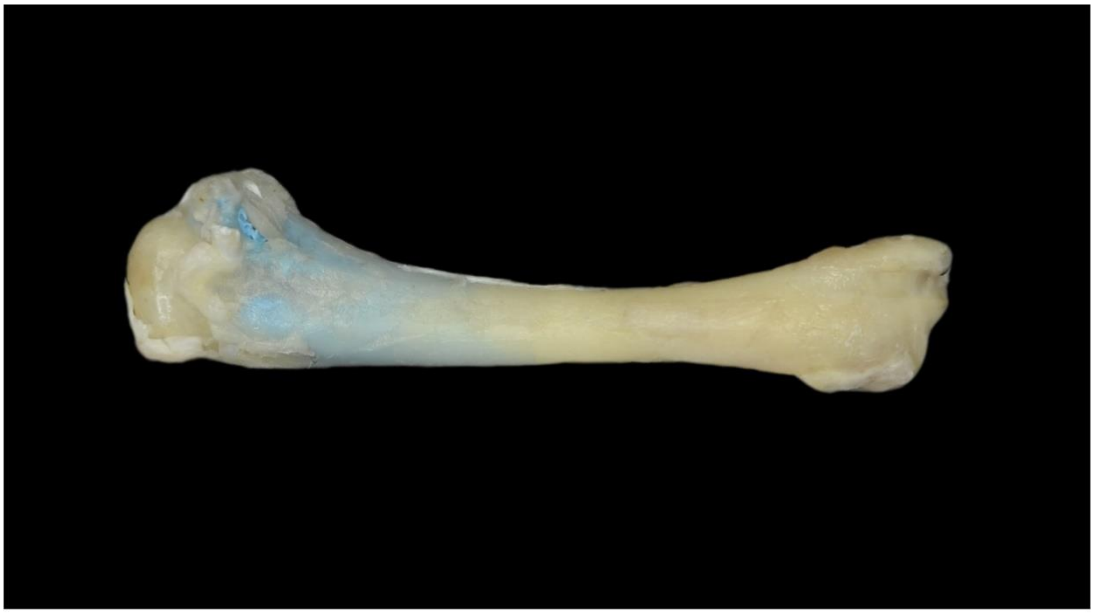

Figure 5 – Partridge humerus filled with látex. In the intramedullary, showing pneumatization of this bone.

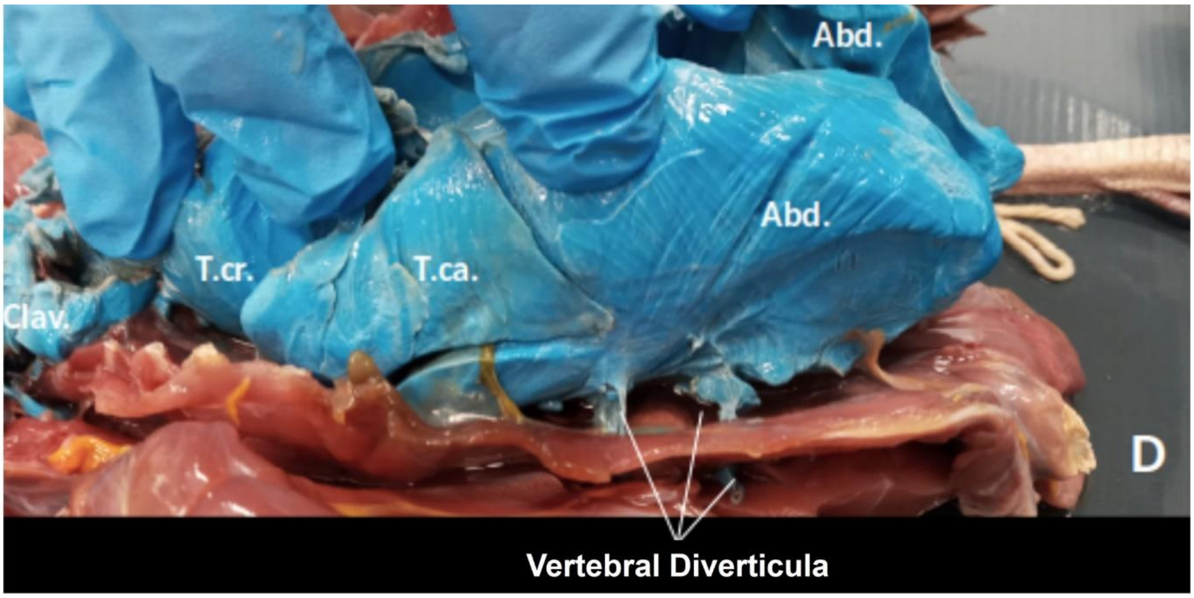

Figure 6 - Lateral view of partridge air sacs, with visualization insertion of latex into vertebral diverticula.

DISCUSSION

The size ratio of air sacs follows what has already been described in the literature for the other species: the cervical air sac has a more irregular conformation and a smaller size (Akester 1960; Çevik-Demirkan 2006; El-Bably et al. 2014). But, in the partridge, we observe that the thoracic air sacs are symmetrical and the cranial thoracic air sacs are smaller than the caudal ones. The abdominal air sacs are the largest and, despite being asymmetrical, both antimeres extend to the cloaca. A single individual presented the right antimere smaller than the left while in *Anas spp.* Ducks, the left abdominal air sac was described as smaller than the right one (Araújo et al., 2014; Çevik-Demirkan, Kürtül, et al., 2006).

The extrathoracic portion of the clavicular air sac pneumatizes the humerus and sternum and extends to the syrinx where it plays an essential role in vocalization (O'Malley, 2005). In one of the dissected animals, the latex filling evidenced the pneumatization of the humerus, and in three other ribs, revealed presence of the vertebral diverticula. Latex viscosity means that pneumatized bones are not necessarily filled, and bone pneumatization is studied via a thorough osteological examination to locate diverticula and visualization of trabeculae through sectioning of the bones. Given that the present work did not focus on the description of pneumatic bones, it was limited to reporting bones describable by latex filling, thus leaving no basis to affirm whether other bones are pneumatized in the species and whether there is intraspecific variation.

There is no clear relationship between the bird's taxonomy and biology versus the quantity and conformation of its air sacs. Animals with taxonomic proximity present differences, as exemplified by partridges, which lack a cervical sac and possess a single clavicular one, totaling seven air sacs, while ostriches (*Struthio camelus*) have two cervical sacs and one clavicular sac, totaling nine air sacs (Bezuidenhout et al., 1999).

The morphology of the respiratory system found in the red-winged tinamou (*R. rufescens*) is scarcely described in the literature, being similar only to the morphology reported in the loon (*Gavia immer*), a water bird belonging to the Gaviiformes order (BirdLife International, 2020; Gier, 1952). The other birds with seven air sacs are geese (*Anser anser domesticus*) (Cubas et al., 2014; Onuk et al., 2009) and turkeys (genus *Meleagris*) with a single cervicoclavicular air sac and the others paired (King & Atherton, 1970; Ragab & Reem, 2016).

Research on the respiratory system of birds has been ongoing for many years, with several studies published on the topic. One study by Maina et al. (Maina, 2006) examined the anatomy of the avian respiratory system and found that the air sacs play a vital role in the respiratory process. The researchers concluded that the unique respiratory system in birds allows for a more efficient extraction of oxygen from the air.

The researchers used computational modeling to simulate the air flow in bird lungs and found that the air sacs played a crucial role in the process of flight (Maina, 2022), showing that the functional efficiency of the avian respiratory system is correlated to its structural complexity.

CONCLUSION

Based on the results obtained, it was possible to conclude that the red-winged tinamou (*Rhynchotus rusfencens* Temminck, 1815) presents a total of seven air sacs, differentiated into a single clavicular air sac, a pair of cranial thoracic air sacs, a pair of caudal air sacs and a pair of abdominal air sacs.

The air sacs in birds are a unique and essential part of their respiratory system. They play a crucial role in efficient gas exchange and provide the necessary air flow for sustained flight. The ongoing research in this area is helping to increase our understanding of the respiratory system of birds and its unique adaptations.

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Ethical approval. The methodology adopted in the development of the present work was approved by the Committee on Ethics in the Use of Animals – CEUA of the School of Veterinary Medicine and Animal Science at the São Paulo State University (UNESP) filed under the number 0030/2021. The project also obtained authorization from the

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Declaration of interest. The authors declare that there is no conflict of interest in relation to the publication of this article.

Authors Contributions:

Katia Aparecida da Silva Viegas and André Luis Filadelpho idealized the study in its conceptualization, design and supervision, contributed to acquisition and curation data, formal analysis, data interpretation, original draft, review and editing of the manuscript.

Karina Padula, Eduardo Henrique Martins, Luiz Eduardo Cruz dos Santos Correia, Josineudson Augusto II de Vasconcelos Silva, contributed to the dissection of biological material, acquisition, curation and interpretation of data.

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