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ORIGINAL ARTICLE

Morphology of the syrinx of three species of birds from Brazilian cerrado (*Psittacara leucophthalmus*, *Rhynchotus rufescens* and *Cariama cristata*): Gross anatomy and light microscopy study

Elton Luis Ritir Oliveira[1] | **Fernanda Zuliani**[2] | **Gabriel Corrêa de Camargo**[2] | **Salvatore Desantis**[3] | **Bruno Cesar Schimming**[1,4]

[1]Graduate Program in Wild Animals, School of Veterinary Medicine and Animal Science, São Paulo State University (UNESP), Botucatu, Brazil

[2]Center for Medicine and Research in Wild Animals, School of Veterinary Medicine and Animal Science, São Paulo State University (UNESP), Botucatu, Brazil

[3]Veterinary Clinics and Animal Production Unit, Department of Precision and Regenerative Medicine and Ionian Area, University of Bari Aldo Moro, Valenzano (Ba), Italy

[4]Laboratory of Wildlife Anatomy, São Paulo State University (UNESP), Botucatu, Brazil

Correspondence
Bruno Cesar Schimming, Laboratory of Wildlife Anatomy, São Paulo State University (UNESP), Botucatu, SP, Brazil.
Email: bruno.schimming@unesp.br

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Abstract

The aim of this study was to describe the morphology of the trachea and syrinx at macroscopic and light microscopy levels of three species of birds from different orders that inhabit the Brazilian cerrado. For that, five adult specimens (three males and two females of each species) of white-eyed parakeet (*Psittacara leucophthalmus*), red-winged tinamou (*Rhynchotus rufescens*) and red-legged seriema (*Cariama cristata*) were used. The trachea and syrinx of the birds were collected and destined for anatomical and histological studies. The trachea of the studied birds presented an elongated path and originated in the larynx and extended caudally to the syrinx. No sexual dimorphism was observed in the syrinx of the studied species, probably because it is associated with their song, which is very similar between males and females of these species. The findings of this study allowed us to classify the syrinx as tracheal in the white-eyed parakeet and tracheobronchial in the red-winged tinamou and red-legged seriema. In general, the morphological features of the trachea and syrinx were similar to those described for other species of birds, such as the presence of intrinsic and extrinsic syringeal muscles, and the lateral and medial tympaniform membranes, which would represent important anatomical structures in sound production through vibration during expiration and eventual inspiration. The morphological structure of the syrinx in the three avian species of the Brazilian cerrado is consistent with the ability of these avian species to perform a potential vocalization, especially the red-legged seriema that emits characteristic sounds very loud and can carry several kilometres.

KEYWORDS
birds, histology, red-legged seriema, red-winged tinamou, syrinx, white-eyed parakeet

1 | INTRODUCTION

The red-winged tinamou (*Rhynchotus rufescens*), white-eyed parakeet (*Psittacara leucophthalmus*) and red-legged seriema (*Cariama cristata*) are bird species that inhabit the Brazilian cerrado biome (Ciambelli, 2008; Silva et al., 2016). The red-winged tinamou belongs to the Order Tinamiformes, white-eyed parakeet to the Order Psittaciformes and red-legged seriema to the Order Cariamiformes.

Regarding the production of sounds, the red-winged tinamou emits sounds almost exclusively during the breeding season, the female is restricted to fine and spaced chirps and the male has a more elaborate song (Marques, 2014), whereas the white-eyed parakeet is capable of vocalizing in a characteristic way (Grespan & Raso, 2014), while the song of the red-legged seriema is a remarkable song, which can be heard over 1 km away (Fontenelle & Barros, 2014).

The syrinx is the organ that produces sounds in birds (Casteleyn et al., 2018; Dewi et al., 2023) and is located close to the tracheobronchial junction (Goller, 2022). It is of great importance for territorial marking, recognition of family members, socialization of the offspring in female birds and the luring of a mating partner in male birds (Casteleyn et al., 2018; Eens & Gorissen, 2005; Marler & Hamilton, 1966).

According to its anatomy and position relative to the tracheal bifurcation, the syrinx can be classified as tracheal, bronchial or tracheobronchial (Evans, 2016; Ibrahim et al., 2020; King, 1986). The tracheal syrinx is the one that is located only in the trachea, whereas the bronchial one appears only in the main bronchi, and the tracheobronchial one is found in the distal part of the trachea and in the proximal part of the main bronchi (Evans, 2016; Ibrahim et al., 2020). The tracheobronchial type of syrinx is the most common type in birds (Casteleyn et al., 2018; Ibrahim et al., 2020).

The morphology presented by the trachea and syrinx has already been described in several birds species, such as ostriches (Yildiz et al., 2003), Mallard duck (Frank et al., 2007), passerines and columbidae (Casteleyn et al., 2018; Ibrahim et al., 2020; Warner, 1972a, 1972b), long-legged buzzard (Kabak et al., 2007), Japanese quail (Çevik-Demirkan et al., 2007), greater rhea (Picasso & Carril, 2013), southern cassowary (Mclerney et al., 2019), domestic fowl (Casteleyn et al., 2018; Ibrahim et al., 2020; King, 1986) and in lovebirds (Dewi et al., 2023). However, to the best of our knowledge, there have been no previous studies describing the morphology of the trachea and syrinx in the red-winged tinamou, white-eyed parakeet and red-legged seriema. Thus, the aim of this study was to provide a morphological description of the syrinx of three avian species from different orders: red-winged tinamou (*Rhynchotus rufescens*), white-eyed parakeet (*Psittacara leucophthalmus*) and red-legged seriema (*Cariama cristata*), that inhabit the Brazilian cerrado.

2 | MATERIALS AND METHODS

2.1 | Animals

Five specimens of red-winged tinamou (*Rhynchotus rufescens*), white-eyed parakeet (*Psittacara leucophthalmus*) and red-legged seriema (*Cariama cristata*) were used in this study. They were adults of both sexes (3 males and 2 females of each species), donated by the Center for Medicine and Research in Wild Animals (CEMPAS), School of Veterinary Medicine and Animal Science, UNESP, Botucatu. The birds were donated after death, for reasons beyond the scope of this study. The specimens received were destined for anatomical ($n = 2$) and light microscopy studies ($n = 3$). This study was authorized by the Committee on the Use of Animals of School of Veterinary Medicine and Animals Science, UNESP (CEUA 1218/2019).

2.2 | Anatomical and histological studies

The birds ($n = 2$) were fixed in a 10% formaldehyde solution for the anatomical studies and, subsequently, the trachea and syrinx of each individual were removed for identification and comparison of structures. The tracheae and syringes were photographed in ventral and dorsal views using a digital camera Canon EOS R5® (Canon Inc.). The anatomical structures were named according to the Nomina Anatomica Avium (Baumel et al., 1993).

Three specimens of each bird species were dissected and the trachea with syrinx was removed and fixed in 4% paraformaldehyde for 24 h and submitted to the histological routine with inclusion in Paraplast™ (Sigma). The material was sectioned using a microtome to obtain slices of 5 µm thickness. Longitudinal histological sections of tracheas and syrinx were stained with haematoxylin and eosin and Masson's Trichrome staining. The sections were analysed and photographed using an Olympus BX-41 microscope, equipped with a DP-12 digital camera (Olympus Inc.), provided by the Department of Structural and Functional Biology, Institute of Biosciences of Botucatu, UNESP.

3 | RESULTS

3.1 | Gross anatomy

The trachea of the studied birds (white-eyed parakeet, red-winged tinamou and red-legged seriema) extended caudally to the syrinx (Figures 1 and 2) and was formed by complete cartilaginous rings. The syrinx was located between the last tracheal ring and the first left and right bronchial ring, dorsally to the heart and ventrally to the oesophagus. The right and left primary bronchi had incomplete cartilaginous rings (Figures 1a and 2a,b,d,e) and were joined by the interbronchial ligament, which presented an interbronchial foramen in the birds of this study (Figures 1a,b and 2a,b,d,e).

The syrinx was formed by cartilaginous components, which together can be called the tympanum (Figures 1, 2a,b,d,e, and 3), by lateral and medial tympaniform membranes (Figures 1b, 2f, 3) and by syringeal muscles (Figure 3a). In the white-eyed parakeet, the lateral tympaniform membrane was found, laterally to the tracheosyringeal cartilages and medially to the syringeal muscles, extending to the first bronchial ring (Figures 1 and 3a). In red-winged tinamou and red-legged seriema, the lateral tympaniform membrane was observed medial to the lateral tracheosyringeal cartilages. In addition, the medial tympaniform membrane, medially at the bronchial bifurcation was also found in red-winged tinamou and red-legged seriema (Figures 2c,f and 3b,c).

The syringeal muscles could be divided into extrinsic and intrinsic syringeal muscles. The extrinsic muscles comprised the

tracheolateral and sternotracheal muscles (Figures 1–3). The tracheolateral muscle was observed laterally on both sides of the trachea of the three investigated species and extended from the cranial part of the trachea to the lateral surface of the tympanum (Figures 1a,b, and 2a,b,d,e). The sternotracheal muscle was found only in the red-legged seriema and, extending from the sternum to the lateral face of the trachea, cranially to the syrinx (Figure 2d,e). Intrinsic syringeal muscles were observed only in the white-eyed parakeet located laterally to the syrinx and medially to the tracheolateral muscles (Figure 1). They were paired and comprised the superficial and deep syringeal muscles, originating from the last tracheal rings and inserting into the tympanum (Figures 1 and 3a).

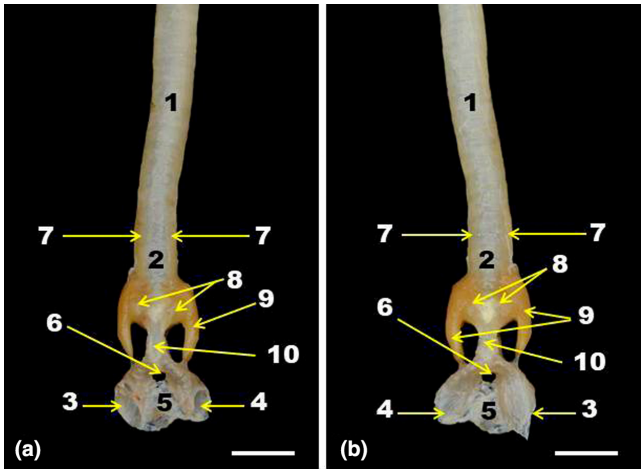

FIGURE 1 Ventral (a) and dorsal (b) views of trachea and syrinx in the white-eyed parakeet. 1. Trachea, 2. tympanum, 3. right primary bronchi, 4. left primary bronchi, 5. interbronchial ligament, 6. interbronchial foramen, 7. tracheolateral muscle, 8. deep syringeal muscle, 9. superficial syringeal muscle, 10. lateral tympaniform membrane. Bar = 0.5 cm.

3.2 | Light microscopy

The distal part of the trachea was lined by a ciliated pseudostratified columnar epithelium in white-eyed parakeets, red-winged tinamou and red-legged seriemas (Figure 4a–c). Cartilaginous rings were located deep in the mucosal epithelium and were lined by the perichondrium. Some tracheal rings showed signs of ossification in the red-legged seriema trachea (Figure 4c). The muscular layer of the trachea was composed of a longitudinal muscular layer in white-eyed parakeets, red-winged tinamous and red-legged seriemas (Figure 4a–d). The tunica adventitia covered the outer surface of the tunica muscularis and consisted of loose connective tissue (Figure 4a–c).

The histological characteristics observed in the longitudinal sections of the studied syringes allowed us to classify them as tracheal type in the White-eyed parakeet (Figure 3a) and tracheobronchial type in the red-winged tinamou and red-legged seriema (Figure 3b,c).

The histological stratigraphy of the syringes was very similar to the trachea one. The mucosa of the syringes was also lined by a ciliated pseudostratified columnar epithelium with numerous mucous goblet cells (Figure 5d,e). Cartilaginous rings formed of hyaline cartilage were found under the epithelium. These cartilaginous structures were covered by the perichondrium (Figure 5a–c). The muscular and adventitia tunics were also observed in the avian syringes of this study (Figure 5b,c).

Inside the red-winged tinamou and red-legged seriema syringes, it was possible to observe the pessulus, which is a median bridge fused with the tympanum. The pessulus was located at the tracheal bifurcation point and its concavity faced the cranial direction (Figure 6a–d). The white-eyed parakeet did not present the pessulus. The pessulus of both red-winged tinamou and red-legged seriema was lined by a pseudostratified columnar epithelium with several intraepithelial glands (Figure 6e,f). Goblet cells were also observed

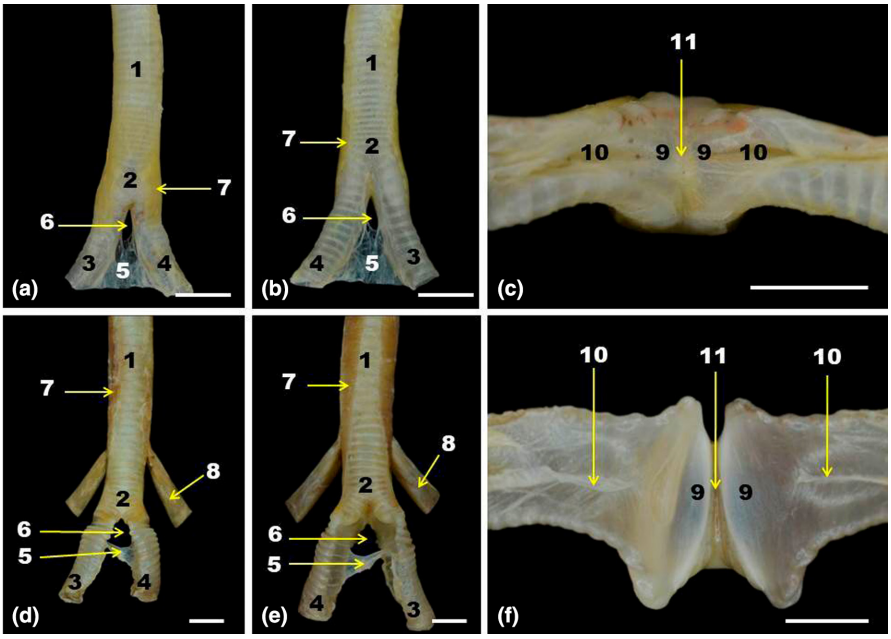

FIGURE 2 Ventral (a and d), dorsal (b and e), and caudal (c and f) views of trachea and syrinx in the red-winged tinamou (a–c) and red-legged seriema (d–f). 1. Trachea, 2. tympanum, 3. right primary bronchus, 4. left primary bronchus, 5. interbronchial ligament, 6. interbronchial foramen, 7. tracheolateral muscle, 8. sternotracheal muscle, 9. medial tympaniform membrane, 10. medial bronchial ligament, 11. pessulus. Bar = 0.5 cm.

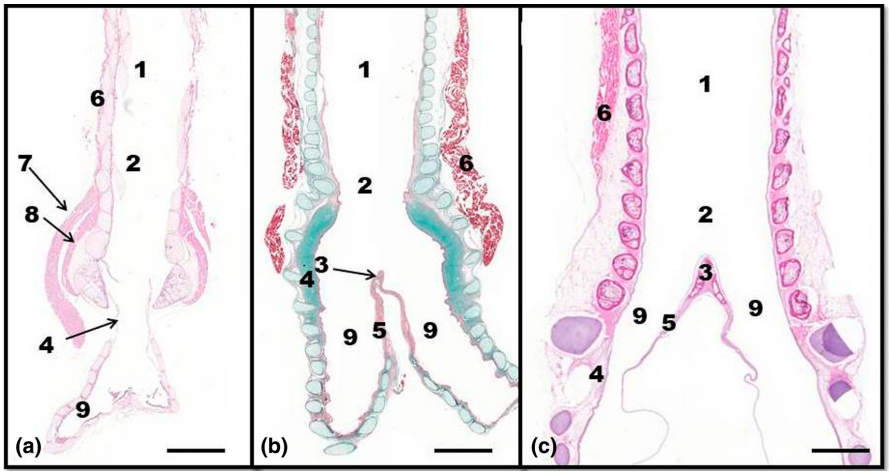

FIGURE 3 Longitudinal histological sections of the syrinx in the white-eyed parakeet (a), red-winged tinamou (b), and red-legged seriema (c). 1. Trachea, 2. tympanum, 3. pessulus, 4. lateral tympaniform membrane, 5. medial tympaniform membrane, 6. tracheolateral muscle, 7. superficial syringeal muscle, 8. deep syringeal muscle, 9. primary bronchi. H&E staining (a and c), Masson's Trichrome staining (b). Bar = 2000 μm.

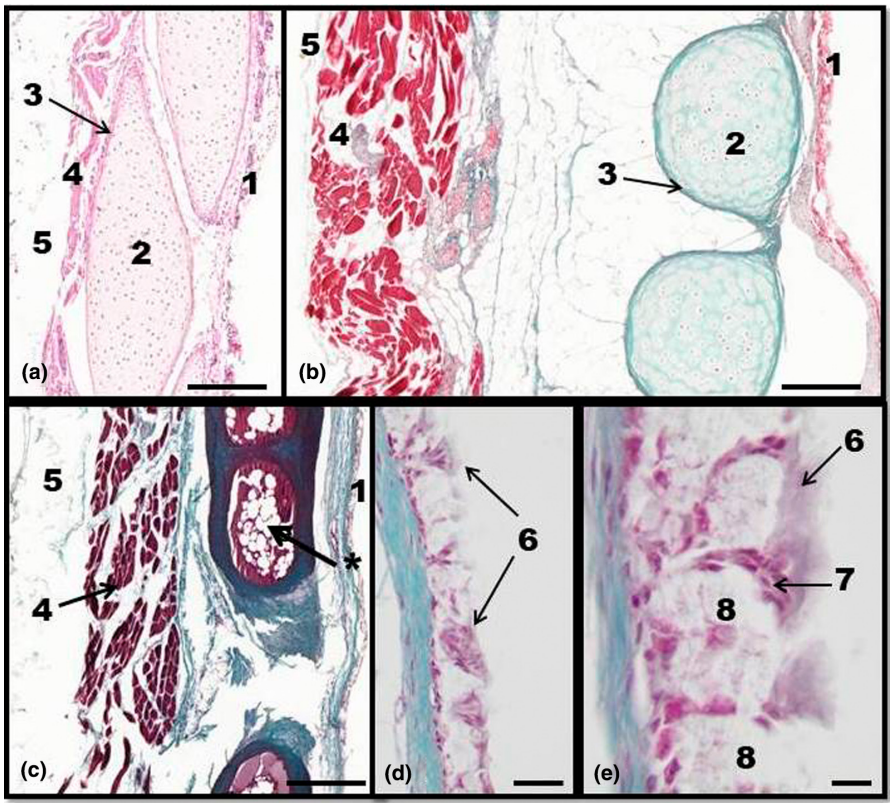

FIGURE 4 Histological sections of the trachea in the white-eyed parakeet (a), red-winged tinamou (b), and red-legged seriema (c). High magnification of the epithelial lining of the seriema trachea (d and e). 1. Trachea epithelial lining, 2. cartilaginous layer, 3. perichondrium, 4. muscular coat, 5. adventitia, 6. cilia, 7. goblet cells nuclei, 8. intraepithelial glands. Note also ossification rings in the seriema trachea (asterisk). H&E staining (a), Masson's Trichrome staining (b, c, d, e). Bar = 200 μm (a–c), 20 μm (d) and 10 μm (e).

in the epithelium lining of pessulus (Figure 6e,f). The wall of the red-winged tinamou pessulus was essentially formed by connective tissue with the lamina propia underlying submucosa constituted of dense connective tissue (Figure 6a). The submucosa of the red-legged seriema pessulus exhibited hyaline cartilaginous tissue that was not demarcated by perichondrium (Figure 6b–d).

The cartilaginous rings were absent in the medial region of the primary bronchi in the red-winged tinamou and red-legged seriema, being replaced by dense non-modelled and poorly vascularized connective tissue, constituting the medial tympaniform membrane (Figures 3b,c and 7a,b). The same characteristic occurred in the lateral region of the primary bronchi, originating the lateral tympaniform membrane (Figures 3b,c, 5a–c, and 7c). The pessulus lining epithelium was continuous with the medial tympaniform membrane epithelium, which was constituted of a squamous epithelium (Figure 7d–f). In the seriema, it was possible to observe the transition between these two types of epithelium lining (Figure 7d,e).

4 | DISCUSSION

This study demonstrated the morphological features of the syrinx in three avian species that inhabit the Brazilian cerrado biome (white-eyed parakeet, red-winged tinamou and red-legged seriema). The syrinx is a vocal organ unique to birds (Goller, 2022). The topographic relationships of the syrinx of the three studied avian species were similar, being located dorsally to the sternum and heart and, ventrally to the oesophagus.

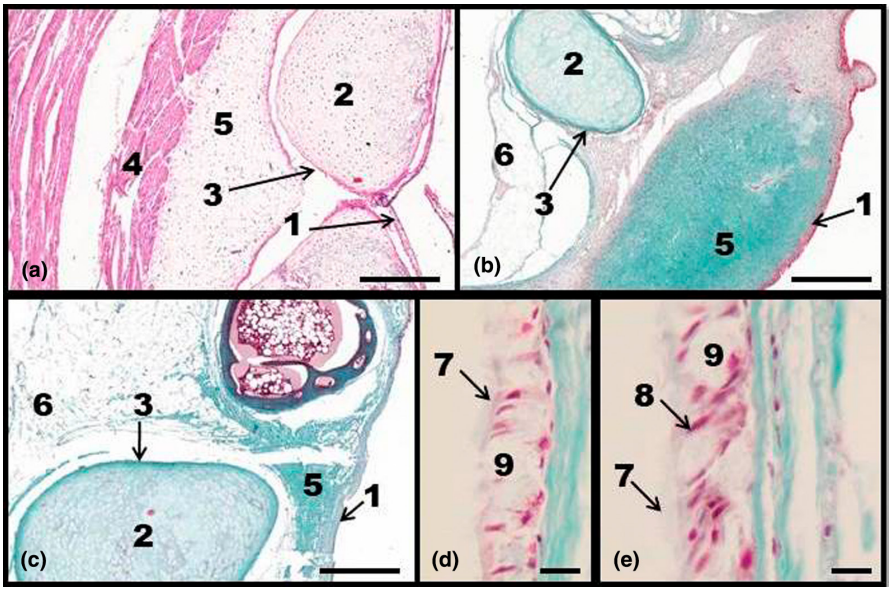

FIGURE 5 Histological section of the White-eyed parakeet (a), red-winged tinamou (b) and red-legged seriema (c) syringes. High magnification of the syringeal epithelium in the red-legged seriema (d and e). 1. Epithelial lining, 2. cartilaginous ring, 3. perichondrium, 4. muscular coat, 5. lateral tympaniform membrane, 6. adventitia, 7. cilia, 8. goblet cells nuclei, 9. intraepithelial glands. H&E staining (a), Masson's Trichrome staining (b–e). Bar = 200 μm (a–c) and 10 μm (d and e).

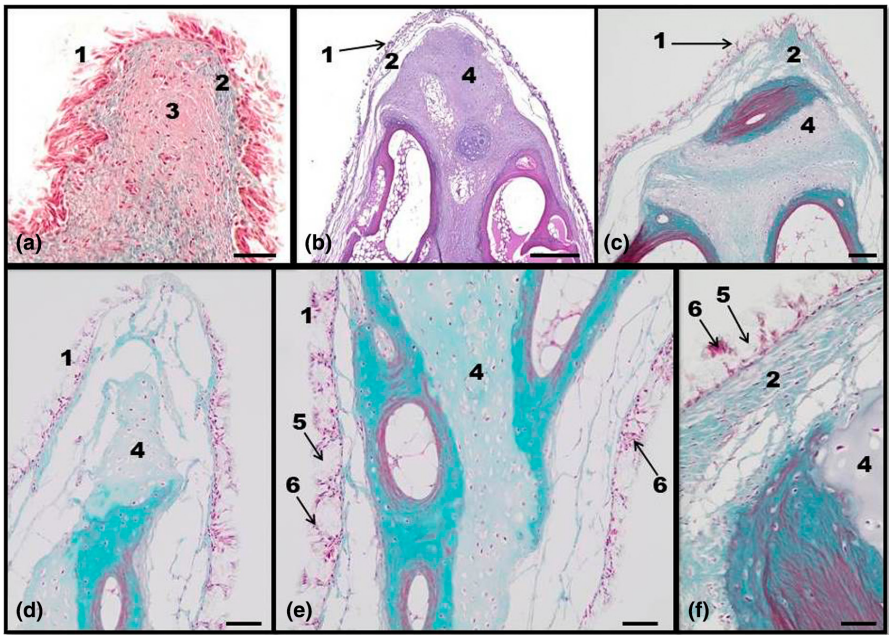

FIGURE 6 Longitudinal sections of the pessulus in the red-winged tinamou (a) and red-legged seriema (b–d). Higher magnification of the seriema pessulus (e and f). 1. epithelial lining, 2. lamina propria, 3. connective tissue, 4. hyaline cartilage plates, 5. intraepithelial glands, 6. goblet cells nuclei. Masson's Trichrome staining (a, c, d, e, f). H&E staining (b). Bar = 50 μm (a, d, e), 500 μm (b), 100 μm (c) and 20 μm (f).

Three morphological types of syrinx are described for birds according to the topographic position of the constituent elements of the syrinx, which are the tracheobronchial, tracheal and bronchial type (Çevik-Demirkan et al., 2007; King & McLelland, 1989; Mclerney et al., 2019). The tracheobronchial type is the most common among birds and, in this case, the syrinx is located near the bifurcation of the trachea and in the primary bronchi, whereas in the tracheal type, the syrinx is located only in the trachea and, in the bronchial one, only in the primary bronchi (Casteleyn et al., 2018; Çevik-Demirkan et al., 2007; Evans, 2016; Ibrahim et al., 2020; King & McLelland, 1989; Mclerney et al., 2019).

The red-winged tinamou and red-legged seriema syringes were of the tracheobronchial type, as well as in guinea fowl (Bottino et al., 2006), cinnamon-tailed buzzard (Kabak et al., 2007), goose (Onuk et al., 2010), greater rhea (Picasso & Carril, 2013), chukar partridge (Erdögan et al., 2015), turkey (Ragab et al., 2016) and Mallard duck (Mohamed, 2017). The white-eyed parakeet syrinx, in turn, was of the tracheal type, as well as that of other parrots (Gaunt & Gaunt, 1985). In this study, none of the birds studied presented a bronchial-type syrinx.

The basic structure of the syrinx consists of cartilaginous components, lateral and medial tympaniform membranes and syringeal muscles. Medial tympaniform membranes are situated on the medial aspect of the rostral region of each bronchus and the lateral tympaniform membranes extend over the lateral and dorsal area between the tracheosyringeal cartilaginous rings (Al-Badri et al., 2014). Medial and lateral tympaniform membranes were observed in the red-winged tinamou and red-legged seriema, whereas only the lateral tympaniform membrane was found in the white-eyed parakeet. Both membranes are present in passerines (Warner, 1972a),

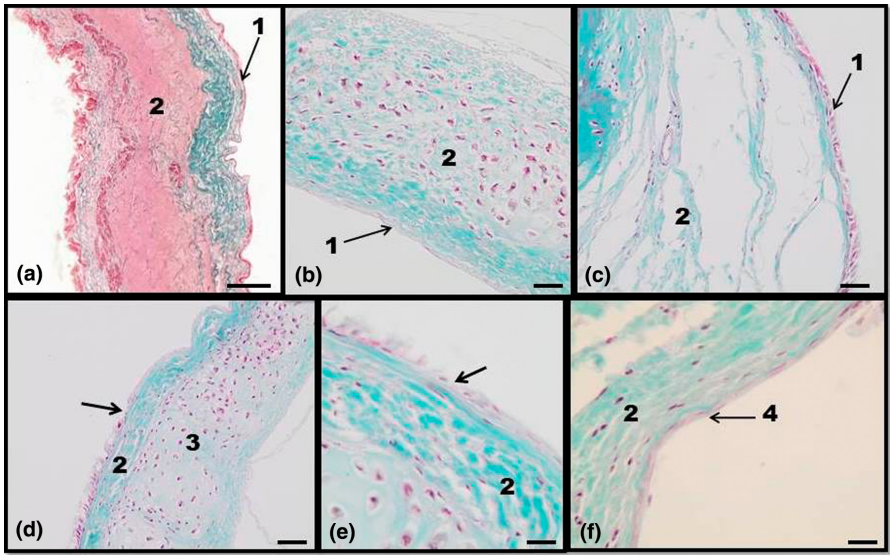

FIGURE 7 Longitudinal sections of the medial tympaniform membrane in the red-winged tinamou (a) and red-legged seriema (b, d, e, f), and lateral tympaniform membrane in the red-legged seriema (c). Histological section demonstrating the transition (arrow) of the respiratory epithelium to a squamous one at the medial tympaniform membrane in the red-legged seriema (d and e). 1. Epithelial lining of the medial tympaniform membrane, 2. connective tissue, 3. hyaline cartilage, 4. squamous epithelium of the medial tympaniform membrane. Masson's Trichrome staining. Bar = 100 μm (a), 20 μm (b–d) and 10 μm (e and f).

pigeon (Al-Badri et al., 2014), goose (Onuk et al., 2010), greater rhea (Picasso & Carril, 2013), turkey (Ragab et al., 2016) and Mallard duck (Mohamed, 2017). Regarding psitacids such as white-eyed parakeet, there is no consensus on the number of functional membranes (Gaunt & Gaunt, 1985). There are studies that state that both types are present in the psitacids (King & McLelland, 1989) and others studies that state which the only functional membranes are the lateral tympaniform membranes (Gaunt & Gaunt, 1985). Probably, the absence of the medial tympaniform membrane in parrots does not harm them, as it seems that the lateral tympaniform membrane plays an important role in the production of sounds (Elemans et al., 2008). Moreover, Dewi et al. (2023) cited only the lateral tympaniform membrane in the lovebirds that also belongs to the Psittacidae family. To these authors, the lateral tympaniform membrane produces the lovebirds's primary sound.

The interbronchial foramen was observed in the three species of birds in this study, cranially to the interbronchial ligament and medially to the right and left primary bronchi. This foramen probably helps the lateral tympaniform membrane to vibrate and produce sounds (Yildiz et al., 2003). This interbronchial foramen is described for songbirds (Warner, 1972a) and is absent in the ostrich, probably because it is not a songbird. Probably, the presence of the interbronchial foramen in the birds of this study contributes to their considerable vocalization.

Another important syringeal structure in the production of sounds are the syringeal muscles. According to Larsen and Goller (2002), the syringeal muscles are instrumental in preparing the syrinx for phonation by moving the labia or lateral tympaniform membranes into the airway and tensing the syrinx appropriately. Syringeal muscles can be divided into intrinsic and extrinsic syringeal muscles (Picasso & Carril, 2013; Yildiz et al., 2003).

The superficial and deep syringeal muscles are examples of intrinsic muscles and, in this study, they were found only in the white-eyed parakeet, similar to what was described for other parrots (Dewi et al., 2023; Gaunt & Gaunt, 1985). According to Casteleyn et al. (2018), the most domestic bird species had no intrinsic syringeal muscles. In passerines, the intrinsic muscles are powerful and run from one syringeal ring to another, regulating the tension of the medial tympanic membranes and controlling the configuration of the airway (Warner, 1972a). In the greater rhea, a single pair of intrinsic muscles was observed, which consisted of wide and thin muscle bands with an oblique course that extended from the dorsal region of the tracheal cartilages (immediately anterior to the tympanum) to the ventral surface of the first three bronchosyringeal cartilages (Picasso & Carril, 2013). Species that lack functional intrinsic syringeal muscles are supposedly only capable of making simple noises (grunts, hisses) such as storks, New World vultures, chickens, geese and ostriches (Appel, 1929; Gill, 1995; McLelland, 1990; Onuk et al., 2010; Yildiz et al., 2003). In this study, red-winged tinamou and red-legged seriema were also examples of birds without these muscles; however, unlike the red-winged tinamou, red-legged seriema vocalizations are sequences of long, intense and structurally complex tonal notes (Redford & Peters, 1986).

The sternotracheal and tracheolateral muscles are considered extrinsic syringeal muscles. The tracheolateral muscle was identified in the white-eyed parakeet, red-winged tinamou and red-legged seriemas in this study, similar to that described for other

bird species such as the long-legged buzzard (Kabak et al., 2007), Darwin's nothura and ornate tinamou (Garitano-Zavala, 2009) and greater rhea (Picasso & Carril, 2013). In this study, the sternotracheal muscle was observed only in the red-legged seriema. Previous studies claimed that in the most non-songbirds, which possess a tracheobronchial syrinx type, only the tracheolateral muscle is reported (Casteleyn et al., 2018). The findings of this study suggest that the songbirds species also can possess only the tracheolateral muscle such as white-eyed parakeet that are able to emit characteristic sounds when flying in flocks. Moreover, probably these disparities indicate that variations in the musculature are a common trait in avian species (Berman et al., 1990; Picasso & Carril, 2013).

The pessulus is a syringeal structure found near the bifurcation of the trachea. In this study, the pessulus was observed in red-legged seriema and red-winged tinamou. No pessulus is present in the white-eyed parakeet, similarly to reported for other Psittacidae family birds such as monk parakeet and Patagonian parrot (Dyce et al., 2010; Gaunt & Gaunt, 1985) and loverbirds (Dewi et al., 2023). The composition of pessulus, dividing the airway within the syrinx, varies significantly in the avian species (Frank et al., 2006). In passerines, the pessulus is well developed and bony, providing a rigid attachment for the membranes attached to its base, but varying greatly in shape and length (Warner, 1972a). Ossified pessulus are present in the songbirds (Warner, 1972a). In the greater rhea, it was observed that the pessulus is formed by bone and cartilaginous tissue (Picasso & Carril, 2013). Pessulus composed of connective tissue is found in the other birds such as pigeons (Warner, 1972b) and ostriches (Yildiz et al., 2003). Pessules formed by connective tissue as in the red-winged tinamou (from this study) and ostriches are due to the fact that these birds are not songbirds and, therefore, do not need a cartilaginous or osseous structure to keep the pessule tight. Based on the findings of this study and the consulted literature, the presence of a cartilaginous pessulus in the red-legged seriema is probably consistent with the fact that seriema songs are very loud and can carry several kilometres (Redford & Peters, 1986).

At the level of light microscopy, the stratigraphy of the trachea and syrinx of the studied species herein was similar to that described for other birds such as the ostrich (Yildiz et al., 2003). The tunica adventitia of the birds in this study was composed of loose connective tissue, similar to that described for the ostrich (Yildiz et al., 2003). The muscular layer of the trachea and syrinx, in the avian species of this study, was composed of a longitudinal muscular layer, presenting similarities with the Japanese quail; however, in this one, transverse muscles are also present (Çevik-Demirkan et al., 2007). The submucosal layers showed loose connective tissue in all birds in the study, as well as in ostrich (Yildiz et al., 2003) and Japanese quail (Çevik-Demirkan et al., 2007). In this study, respiratory epithelium with ciliated cells and goblet cells was found along all syringeal structures. Çevik-Demirkan et al. (2007) reported that the epithelium of the Japanese quail syrinx was ciliated pseudostratified cuboidal epithelium.

No sexual dimorphism was found in the white-eyed parakeet, red-winged tinamou and red-legged seriema syringes. Previous studies with syrinx in orange-winged parrot (Nottebohm, 1976), Japanese quail (Çevik-Demirkan et al., 2007), common murre and thick-billed murre (Miller et al., 2008), also did not observe sexual dimorphism. The opposite was observed in studies carried out with guinea fowl (Bottino et al., 2006), where males have a higher number of cranial and intermediate cartilages than females, thus favouring sound amplification. The Mallard duck syrinx is characterized by a remarkable sexual dimorphism. Male Mallard presented a large bony bulla on the left side of the syrinx (Frank et al., 2006; Mohamed, 2017). It is believed that this functions as a resonance chamber (König et al., 2016). In the birds of this study, the absence of sexual dimorphism is probably associated with the singing of these species, which is very similar between males and females.

In conclusion, this study provides a morphological description of the syrinx in the three avian species that inhabit the Brazilian cerrado. The findings of this study show that despite being of different orders, the white-eyed parakeet, red-winged tinamou and red-legged seriema have several syringeal features in common, which is consistent with the fact that vocalization is very important in these species. Moreover, this study demonstrated that the syrinx in the white-eyed parakeet, red-winged tinamou and red-legged seriema possessed some differences and some similarities as well in some aspects when compared with other avian species. The morphological structure of the syrinx in the three avian species of the Brazilian cerrado is consistent with the ability of these avian species to perform a potential vocalization, especially the red-legged seriema that emits characteristic sounds very loud and can carry several kilometres and the white-eyed parakeet that also vocalizes in a characteristic way when flying in flocks.

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CONFLICT OF INTEREST STATEMENT

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Bruno Cesar Schimming https://orcid.org/0000-0002-0837-9612

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