The peculiar syrinx of *Rhea americana* (Greater Rhea, Palaeognathae)

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**Abstract**

This work studied the skeletal and muscular syringeal anatomy of Greater Rhea (*Rhea americana*) throughout postnatal ontogeny, by using muscle staining and differential coloring of cartilage and bone techniques. Anatomical syrinx dissections on four adults (one female and three males) and eight unsexed chicks, were made. The type of the syrinx was tracheobronchial and it was entirely cartilaginous in chicks and in the adult female but showed a partially cartilaginous and osseous pessulus in male adults. A pair of intrinsic muscles were found and the extrinsic muscles were represented by the muscles sternotrachealis and tracheolateralis, and a broad dorsal medial muscular band. The syrinx of Greater Rhea was notable for having a more complex morphology than other Paleognathae birds. Future studies on how *Rhea* produces vocalizations will allow the comparison with other birds, and contribute to the understanding of the evolution of sound-production mechanisms in birds.

**Resumen**

Se estudió la anatomía esqueletaria y muscular de la siringe del Ñandú Grande (*Rhea americana*) durante su ontogenia postnatal usando técnicas de tinción diferencial de tejido óseo y cartilaginoso. Se diseccionaron cuatro ejemplares adultos (una hembra y tres machos) y ocho juveniles sin sexar. La siringe fue del tipo tracheobronchial y fue completamente cartilaginosa en juveniles y en la hembra adulta mientras en los machos adultos presentó el pessulus parcialmente cartilaginoso. Un par de músculos intrínsecos fueron encontrados y la musculatura extrínseca estuvo representada por los músculos sternotrachealis y tracheolateralis y una ancha banda muscular de posición dorsomedial. La siringe de *Rhea americana* presentó una morfología más compleja que la descrita para otras aves Paleognatas. La realización de estudios focalizados en conocer cómo el Ñandú Grande produce sus vocalizaciones permitirá realizar comparaciones con otras aves y contribuirá al conocimiento de la evolución de la producción de sonidos en las aves.

**Key words**

Rheidae, tracheobronchial syrinx, syringeal muscles, pessulus.

**Introduction**

The Greater Rhea (*Rhea americana*) is the largest of South American birds, reaching 1.5 m in height and weighing 25 kg (Folch, 1992). *Rhea* is grouped with *Apteryx* (kiwi), *Casuarius* (cassowary), *Dromaius* (emu) and *Struthio* (ostrich) in the Ratitae clade. This group together with Tinamidae (Tinamous) comprise the Palaeognathae. These birds are monophyletic and a basal group with respect to Neognathae (e.g. Livezey & Zusi, 2007;
The syrinx is the organ responsible for producing vocalizations in birds and knowledge of its anatomy in Paleognathous birds is scarce and often outdated. Since the early 19th century, studies on the anatomy of the syrinx have focused mainly on Neognathae birds (e.g. MÜLLER, 1847; HUXLEY, 1872; CHAMBERLAIN et al., 1968; AMES, 1971; WARNER, 1971, 1972; GARAN-LIMA & HÖFLING, 2006; KABAK et al., 2007; MILLER et al., 2008). On the other hand, FORBES (1881) was the first to study the macroscopic anatomy of this organ on Ratites. Subsequently, WUNDERLICH (1884), BIEDDARD (1898) and PYCRAFT (1900) corroborated these anatomical descriptions and pointed some minor anatomical variations (e.g. conformation and numbers of tracheal rings) but did not provide further details. The anatomy of the Palaeognathae syrinx would not be studied again until BEEBES (1925) works with the Variegated Tinamous (Crypturellus), and later on, a few others in the ostrich (Struthio; YILDIZ et al., 2003) and in Darwin’s Nothura (Nothura; GABAN-LIMA & HUVIG, 2006; KAKBACH & HOFLING, 2007) and in the Greater Rhea (Béver, 1884; AMES, 1971) was the first to mention the cartilaginous nature of the pessulus. However, due to methodological limitations at the time, these studies did not provide detailed anatomical descriptions, nor age or sex of the individuals studied. Also, the illustrations only show two macroscopical syrinx drawings in dorsal and ventral aspects.

Studies on the comparative morphology of the syrinx contribute to understand the mechanisms of sound production (e.g. GOLLER & LARSEN, 1997; LARSEN & GOLLER, 1999, 2002) and have been used to establish phylogenetic relationships in systematic studies (e.g. AMES, 1971; MOORE, 1986; PRUM, 1992; GRIFFITHS, 1994; GARAN-LIMA & HÖFLING, 2006; ZIMMER et al., 2008; MANDIWANA et al., 2011). The aim of this study was to describe the anatomy of the syrinx in the Greater Rhea americana throughout its ontogeny, by using muscle staining (BOCK & SHEAR, 1972) and differential colouring of cartilage and bone (CANNELS, 1988). This approach to the study of the anatomy of the syrinx of a Ratite bird would be useful to carry out further studies on its comparative and functional anatomy.

Results

Skeletal elements of the adult syrinx

The syrinx of Greater Rhea was of the tracheobronchial type and it was composed of cartilages, namely (cart.) tracheosyringealae (T) and cart. bronchosyringealae (B). The former were broad and conformed a well-developed tympanum (figs. 1: T1 – T4 & 2a, b) together with the first cart. bronchial (figs. 1: B1 & 2a, b). In dorsal view, cartilages T2, T3 and T4 got to fuse in the middle region of tympanum together with the pessulus and the cart. B1 (Fig. 1 & 2a), whereas cart. B1 was free in dorsal aspect (fig. 2a). Unlike other cart. tracheosyringealae, T4 was noticeably convex (fig. 1 & 2a). The cart. bronchosyringealae (figs. 1: B2 – B6 & 2a, b) were “C-shaped” and the cart. B1 showed a slightly concave shape, which added to the convexity of the T4, delimited a wide space occupied by the membrana tympaniformis lateralis (fig. 1; see below). The remaining cart. bronchosyringealae had similar form and held the ligamentum bronchiale mediale (fig. 1).

The pessulus was a thin bar, fused with the tympanum (figs. 2a,b & 3). In females, the pessulus was entirely cartilaginous (figs. 2a, b). On the other hand, in males, it was cartilaginous on its dorsal half, whereas it was osseous.

Materials and Methods

Anatomical dissections were performed on 12 specimens of Rhea americana at several ages: four adults (1 female and 3 males of two years old), four 3-month-old unsexed chicks, and four 1-month-old unsexed chicks. The birds were obtained from various commercial farms located in Buenos Aires province (Argentina) and reared in accordance with Argentinean regulations for Greater Rhea farming. Birds were sacrificed by cervical dislocation or electrical stunning, and their syringes were first observed in situ and then they were carefully removed. They were fixed in 10 % formaldehyde for 48 hours and then preserved in 70 % ethanol. An iodine solution that selectively stains muscular tissue with a reddish brown color (BOCK & SHEAR, 1972) was used to observe the muscles in preserved syringes. Lastly, the syringes were stained using a standard differential coloring technique for cartilage and bone (CANNELS, 1988), where cartilage tissues were stained blue (alcian blue) and ossified tissues were stained red (alizarin red). The anatomical nomenclature followed throughout corresponds to that proposed by BAUMEL et al. (1993). Photographs were taken with a Nikon D-40 digital camera.
on its ventral half, forming an osseous plate (figs. 2c, d). The membrana tympaniformis lateralis (fig. 1) was located between the last cart. tracheosyringeal (T4) and the first cart. bronchosyringeal (B1). It was partially covered by the pair of intrinsic muscles (fig. 1; see below). The membrana tympaniformis mediales (figs. 1 & 3a) was suspended between the free ends of the cart. bronchosyringealeals B1 and B2, and extended, making contact with the pessulus (fig. 3a). This membrane formed a pair of intrusions into the lumen of the syrinx (fig. 3b). Finally, the ligamentum interbronchiale connected the left and right bronchii (fig. 1) and between this ligament and the pessulus the foramen interbronchiale could be observed (fig. 1).

Discussion

The syrinx of the Greater Rhea corresponds to the tracheobronchial type, as in the remaining Palaeognathae birds (FORBES, 1881; YILIDZ et al., 2003; GARITANO-ZAVALA, 2009) and most Neognathae birds (BEDDARD, 1898; KING, 1989; BAUMEL et al., 1993). But, the Greater Rhea syrinx was notable for having a well-developed tympanum, and for the presence of a pair of intrinsic muscles. In the rest of the Palaeognathae birds, there are no intrinsic muscles and the tympanum has often been described as simple due to the presence of a lower degree of fusion between the components and the presence of a pessulus of connective tissue (FORBES, 1881; WUNDERLICH, 1884; YILIDZ et al., 2003; GARITANO-ZAVALA, 2009). FORBES (1881) described the presence of a “vocal cord” inside the syrinx of *Rhea americana* (p. 240). It is possible that this author called “vocal cord” the two intrusions of the membranae tympaniformes mediales into the lumen of the syrinx (see fig. 3a). These intrusions are noticeable when the syrinx is fixed and preserved, whereas in, unpreserved specimens this is not as evident. In regard to the extrinsic muscles, the results of this study showed some differences with respect to information given by previous authors (i.e.: FORBES, 1881, WUNDERLICH, 1884; BEDDARD, 1898; Pycraft, 1900). Initially, these authors described the presence of a single pair of extrinsic muscles which were identified as the “lateral tracheal muscle” (FORBES, 1881 p. 240), without giving further details on their origin and extension. In our work, we identified the two typical pairs of extrinsic muscles of the syrinx (mm. sternotracheales and tracheolaterales). When comparing it with other Palaeognathae birds, FORBES (1881) found that the ostrich syrinx had no intrinsic or extrinsic muscles, but WUNDERLICH (1884), Pycraft (1900) and YILIDZ et al. (2003) described the presence of the m. sternotrachealis. Regarding the remain genera (*Casuarius, Dromiaus* and *Apteryx*), these authors described the presence of the two typical pairs of extrinsieal muscles. These disparities indicate that variations in the musculature (e.g. presence or absence of a muscle) is a common trait in birds (BERGER, 1956; BERMAN et al., 1990).

Lastly, concerning the medium-dorsal muscular band present in the Greater Rhea, FORBES (1881) called it “fibrous band” and mentioned its presence also in the Cassowary. After a review of the available bibliography, we could not find a similar muscle in other birds.

The presence of intrinsic muscles in the syrinx of the Greater Rhea is interesting. They are a distinctive feature of Passerine birds (especially the Oscines songbirds), that have at least four pairs that contribute to the control of a wide variety of vocalizations (SUTHERS, 2001; LARSEN & GOLLER, 2002). Some groups of non-Passeriformes birds also have intrinsic muscles, although in lesser numbers, namely hummingbirds (Trochiliformes, MOLLER, 1847; GAUNT, 1983), parrots (Psittaciformes, GAUNT & GAUNT, 1985; GABAN-LIMA & HOFLING, 2006) and the oilbird...
Fig. 1. *In situ* ventral view of the syrinx of Greater Rhea, B1–B4: cartilages bronchosyringealis, T1–T4: cartilages tracheosyringeales, bp: bronchus primarius, fi: *foramen* interbronchiale, lbm: *ligamentum bronchiale mediale*, li: *ligamentum interbronchiale*, mtl: *membrana tympaniformis lateralis*; ty: tympanum. Dotted line indicate the pair of intrinsic muscles.

Fig. 2. Stained and clarified syrinx, (a,b): adult female in ventral and dorsal view respectively; (c,d): adult males in ventral and dorsal view respectively, note the differences between the female pessulus (p) (entirely cartilaginous) and the male pessulus (partially osseous and cartilaginous) (op/cp); (e,f): chick of one month old in ventral and dorsal view respectively, B1: cart. bronchosyringeal, T1–T4: cart. tracheosyringeales, p: pessulus, ty: tympanum, arrows indicates the *cart.* B1 not fused with the *tympanum*, asterik indicate tracheal rings with partial bifurcations.

Fig. 3. Location and extension of the *membrana tympaniformis medialis* (mtm) and pessulus (p) in fixed syringes, (a) Caudal view of syrinx showing the pessulus and the *membrana tympaniformis medialis* (specimen of three months old); (b) Internal view of the syrinx showing the intrusions of the *membrana tympaniformis medialis* (adult specimen), lbm: *ligamentum bronchiale mediale*, bp: bronchus primarius.
Steatornis caripensis (Caprimulgiformes, Suthers & Hector, 1985). These birds do not have a large repertoire of vocalizations, but they can imitate sounds (e.g. parrots) and are capable of vocal learning (e.g. hummingbirds and parrots) (Gaunt, 1983; Gaunt & Gaunt, 1985; Suthers, 2001). Moreover, in the echolocating oilbird, the intrinsic muscles have evolved for the production of sonar clicks (Suthers & Hector, 1985). The role that intrinsic muscles perform in the functioning of the syrinx of the Greater Rhea is still unknown, however, it is interesting to note that adults of R. americana produce sounds like “hisses” and during breeding season, male adults make a deep-toned “grunt” (termed booming call), that can be heard at great distances (Rakow, 1969; Brunning, 1974; Beaver, 1978; Folch, 1992; Codenotti & Alvarez, 2001; Davies, 2002). In contrast, Beaver (1978) found that young birds have a wide repertoire of sounds (consisting of about five types of vocalizations), that impoverishes and disappears as the chicks grow. This author related this vocal modification with the more developed membranae tympaniformes mediales in chicks than in adults. Nonetheless, he did not perform measurements on this membrane to corroborate this statement and the published figures are unclear (see Beaver, 1978 fig. 3, p. 387), without scale and taken in different views. In our study no macroscopic differences were found in the membranae tympaniformes mediales when comparing chicks to adults. We also believe that the degree of intrusion of the membrane could be a fixation artifact. Therefore, we conclude that the impoverishment of richness of sounds found by Beaver (1978) should be studied from other perspectives.

The syrinx of the Greater Rhea was previously described as being completely cartilaginous (Forbes, 1881; Wunderlich, 1884; Pycraft, 1900), but the differential staining techniques incorporated in this study showed the presence of a pessulus in the male adult formed by osseous and cartilaginous tissues. A similar condition (yet of unknown significance) was found in the male of the Tufted Duck, Aythya fuligula (Warner, 1971). Sexual dimorphism in syrinx anatomy has been found in other birds (Miller et al., 2008), varying from the presence of larger syrinxes in males (e.g. in the collared dove, Streptopelia decaocto, Ballintijn & Cate, 1997), to the presence of specialized structures such as the syringeal bulla in males of Anatidae (e.g. Frank et al., 2007; Warner, 1971), to differences in structures like labia and cartilaginous rings (e.g. in the Zebra Finch, Taeniopygia guttata; Riede et al., 2010). Also, differences in vocalizations between male and female could be associated with sexual dimorphism in syrinx anatomy (Ballintijn & Cate, 1997; Miller et al., 2008; Riede et al., 2010; Warner, 1971), but in the Greater Rhea this topic still remains to be explored.

In conclusion, the present study points to the complexity in the morphology of the syrinx of Rhea americana and the more attention it deserves. Future studies on air flow and air sac pressure, intrinsic and extrinsic muscles electromyography and endoscopic filming of the syrinx during the generation of sounds are needed to complement these findings. Such information will allow to compare if the mechanisms of sound production of Palaeognathae differs from those known for Neognathae birds, and eventually shed new light on the evolution of this feature in birds.
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References


