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Ostrich (*Struthio camelus*) syrinx morphology and vocal repertoire across postnatal ontogeny and sex: Implications for understanding vocal evolution in birds

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Abstract

Vocal production in birds has been the target of considerable research that mostly has focused on phylogenetically well-nested songbirds. Anatomical descriptions and recordings of many non-songbirds have often only focused on a single ontogenetic stage or sex. While basic morphology of the vocal organ (syrinx) of ostrich (Palaeognathae, Struthio camelus) has been known since the 1800s, descriptions of its vocal repertoire and syrinx anatomy since then have been incomplete or inconsistent. New toolkits now enable detailed qualitative description of internal anatomy and meristic data and allow it to be compared to vocal production. Here we describe the anatomy of the syrinx in Struthio camelus for three post-hatching ontogenetic stages and both an adult male and female utilizing dissection and contrast enhanced X-ray computed tomography (diceCT). We find changes in ring geometry and spacing through ontogeny as well as lateral labia thickness. We document a small unpaired, midline, cartilaginous structure, a "pessuliform process" at the tracheobronchial juncture present throughout ontogeny and in both males and females. Investigation of the vocal repertoire of ostriches across ontogeny using a new dataset of 77 recordings led to identification of four vocalizations not previously reported in the literature, including the simultaneous production of a hiss and tonal. We find syrinx morphology largely consistent across ontogeny and in male and female adults. Both are capable of producing long duration tonal calls, but these may be more frequent in male birds. Closed-mouth boom calls remain unique to males. A detailed understanding of diversity in parts of early diverging clades is pivotal in attempting to estimate features of the ancestral syrinx in birds and how avian vocalization evolved.

KEYWORDS

diceCT, enhanced contrast CT, ostrich, Paleognathae, syrinx

1 | INTRODUCTION

Extant birds are composed of two sister clades: Paleognathae and Neognathae (e.g., Burleigh et al., 2015; Prum et al., 2015; Yonezawa et al., 2017). While extant paleognaths comprise 60 species, there are more than 10,000 species of extant neognaths (Clements et al., 2023). To better understand ancestral characteristics of all extant birds, we need to understand trait evolution within early diverging birds in Paleognathae. Paleognathae consists of the flightless ratites (a polyphyletic group that includes taxa such as the ostrich, emu,

WILEY-ANATOMICAL SOCIETY

Journal of

cassowary, rhea, and kiwi) and the flighted tinamous. For many years, phylogenetic relationships among paleognaths were controversial, but they are becoming clearer (e.g., Harshman et al., 2008; Sackton et al., 2019; Yonezawa et al., 2017). However, the timing of acquisitions of key morphologies of the vocal organ in crown birds remains ambiguous (Clarke et al., 2016). Located where the bronchi split from the base of the trachea (the tracheobronchial juncture), the syrinx is the unique vocal organ of extant birds, with most other living tetrapods utilizing a modified larynx for sound production (Ames, 1971; Elemans et al., 2015; Kingsley et al., 2018; Negus, 1954). The morphology of the syrinx varies dramatically, with avian clades developing additional muscles, modifications of tracheal and bronchial rings, and phonation of one or two pairs of lateral labia or membranes (Ames, 1971; King & McLelland, 1989). Ostriches are consistently recovered as the sister taxon to all other extant paleognaths (Harshman et al., 2008; Sackton et al., 2019; Yonezawa et al., 2017). This phylogenetic position makes them an influential taxon in estimation of the ancestral states in crown birds.

The syrinx of paleognath birds was first described in the 19th century (Forbes, 1881; Owen, 1834) when its morphology was studied primarily as a potentially taxonomically informative character rather than for its role in producing sound (Beddard, 1898; Forbes, 1881; Pycraft, 1900). Most discussions of potential homology and relationship were accompanied by illustrations showing the external structure of the tracheal and bronchial rings and muscles based on work by Forbes (1881). In the late 1800s, it was thought that ratites may be united by lacking a syrinx entirely (Forbes, 1881). Forbes (1881) refuted this by providing the most detailed ventral and dorsal illustrations of the syrinx in Struthio camelus, Apteryx mantelli,

Casuarius casuarius (previously C. galeatus), and Rhea americana of the time. He also confirmed the presence and position of a pair of lateral labia (or vocal folds) in the ostrich airway in one of the few early illustrations of this internal structure (Forbes, 1881; Figure 1, inset). Subsequently there has been comparatively little new descriptive anatomical work done. Picasso and Carril (2013) redescribed the unusual syrinx anatomy of Rhea americana through dissection and histology, confirming, but elaborating on, the observations of Forbes (1881) and Beddard (1898). Clarke et al. (2016) discuss the tracheal and bronchial ring structure in cassowary and tinamou only in the context of comparisons with neognath exemplars. McInerney et al. (2019) offered a new detailed description of the cartilaginous ring structures of Casuarius casuarius syrinx based on CT data, as well as commenting on the morphology of other paleognaths from the literature.

The literature addressing specifically the ostrich syrinx since Forbes (1881) has been inconsistent as well as lacking consideration of potential differences by sex or ontogenetic status. For instance, King and McLelland (1989) reported that they found no sign of a lateral labia (which they called a "thickened mucosa") in the ostrich syrinx. Yildiz et al. (2003) examined the morphology of the ostrich syrinx and described the labia as the lateral tympaniform membranes, despite its position and thickened morphology. These authors also did not find the m. tracheolateralis or a small cartilaginous feature near the tracheobronchial juncture which Forbes (1881) called the pessuliform process (Yildiz et al., 2003). El Karmoty and Khattab (2018) focused primarily on histology of internal soft tissue structures of the ostrich syrinx; they described the lateral labia as thin membranes (referring to it as the "lateral



FIGURE 1 Cutthroughs of ostrich syrinxes through ontogeny: a) 2-day-old ostrich syrinx (TMM M-14723). b) 2-week-old ostrich syrinx (TMM M-14777). c) adult female ostrich syrinx (TMM M-14733). d) adult male ostrich syrinx (TMM - M-15382). See Figure 2 for ring labels. (grey = tracheal and bronchial rings, pink = lateral labia, purple = medial tympaniform membrane) (scale bars = 10 mm).

Journal of Anatomy - ANATOMICAL-WILEY-

tympaniform membrane") and did not mention Forbes' pessuliform process in their discussion of rings and cartilage in the ostrich syrinx (El Karmoty & Khattab, 2018).

Function of the ostrich syrinx was investigated by Elemans et al. (2015), in a synoptic comparative consideration of mechanisms of avian sound production. Excised syrinxes of this taxon, a tinamou (Eudromia elegans), and neognath birds were phonated ex vivo (Elemans et al., 2015). In contrast to other works since Forbes (1881) (e.g., El Karmoty & Khattab, 2018; Yildiz et al., 2003), the authors clearly describe and figure the thickened lateral labia as the primary vibratory tissues in the ostrich and tinamou syrinx, which contrasts with hypotheses from the time of King and McLelland (1989) that this function was served by the medial tympaniform membrane in most birds. This study (Elemans et al., 2015) used two adult ostrich syrinxes of unknown sex. Similarly, all the previously mentioned studies investigating the syrinx of ostrich either do not report a known age or sex, or only examine adult males, meaning that we do not currently have any comparison between sexes or of distinct ontogenetic stages (El Karmoty & Khattab, 2018; Elemans et al., 2015; Forbes, 1881; King & McLelland, 1989; Yildiz et al., 2003).

Although ostrich behavior and physiology in farmed context has been noted (Csermely et al., 2007; Deeming, 1998), publicly available acoustic data of ostrich calls is extremely sparse. The Xeno-canto. org archive contains only 8 recordings of ostriches comprising 2 juvenile recordings, 4 adult "boom" calls from Struthio camelus (captive and wild), 1 recording of adult female egg-laying vocalizations, and 1 Struthio molybdophanes "boom" call. The Macaulay Library (Cornell Lab of Ornithology) contains only 2 recordings comprising captive prehatching and juvenile Struthio camelus vocalizations (ML794, ML793) and no adult vocalizations. Qualitative descriptions of farmed and wild ostrich vocal repertoires are limited but suggest a range of distinct calls (Duerden, 1920; Sheak, 1923). Juveniles are reported to produce whistling and peeping contact calls as early as a few days before hatching (Cramp, 1978). These peeps and whistles are said to be used to maintain contact with parents and siblings (Cramp, 1978). Some distress calls made by chicks were documented by Samson (1996) as being similar to "a pigeon's prolonged throat call" (Samson, 1996: 413).

Both male and female adult ostriches are reported to produce various open-mouthed hissing sounds during aggression, although female vocalizations are only rarely remarked (Csermely et al., 2007; Duerden, 1920; Sauer & Sauer, 1966; Sheak, 1923). One of the most widely discussed calls in both early and recent literature are the "boom" calls (sometimes referred to as "bromm" calls; Duerden, 1920). These calls are only made by sexually mature males, and they are closed-mouth vocalizations consisting of three deep "booms" accompanied by inflation of the esophagus (Duerden, 1920; Riede et al., 2016; Sheak, 1923). It is thought that these calls are a signal to potential mates and rival males (Sheak, 1923). Previous studies focused on closed-mouth vocalizations reported that these "booms" have a fundamental frequency of around 180Hz (Riede et al., 2016). However, adult vocalizations outside of these booms and hisses are rarely reported in the literature. A muted "boo" call,

ranging from hoarse to clearer and more tonal, was documented by Sauer and Sauer (1966, 1967) to be commonly made by wild adults when threatened or antagonized, but is not described elsewhere (e.g., Cramp, 1978). Sauer and Sauer (1966) also observed this call to be made during distraction displays to predators while attempting to lead them away from juveniles in the wild. However, no recordings of this call were available. In light of a lack of accessible bioacoustic data, more data are required to bolster our understanding of vocalization in the ostrich, including both sexes and through ontogeny.

Here we describe the syrinx anatomy and vocalizations of Struthio camelus at distinct ontogenetic stages and in both male and female adults. We also document ostrich vocalizations throughout ontogeny, including calls that had not been recorded before to our knowledge. We first examine the morphology of the syrinx in dissection and as visualized using iodine enhanced contrast X-ray computed tomography imaging. We assess the position and morphology of lateral labia, muscles and cartilaginous rings for ontogenetic differences and differences by sex, and compare the morphology of ostrich syrinxes to other paleognaths and neognaths in order to gain insight into the morphological evolution of the syrinx.

MATERIALS AND METHODS 2

Our anatomical dataset included syrinxes from four Struthio camelus camelus specimens of varying ages, including a 2-day-old, a 2-week-old, an adult female, and an adult male now reposited in the Texas Memorial Museum - Modern Collection, Vertebrate Paleontology Lab (TMM M). These specimens are TMM M-14723, TMM M-14777, TMM M-14733, TMM M-15382, respectively (Table 1). All were donated to the lab deceased by the Superior Ostrich Ranch, Waco, TX; syrinxes in juvenile and adult male specimens were excised per the protocol in Bilger et al. (2020) while adult female airway was donated already excised by the farm. All specimens were preserved in 70% EtOH, specimens were stained in 3.75% I2E (w/v) in 100% EtOH solution. Stained specimens were then scanned in a custom NSI helical scanner at UTCT (www.ctlab. geo.utexas.edu) in the Jackson School of Geosciences producing 16bit tiff image stacks. Scanning specifications are provided in Table 1. We then used Avizo v 2022.1 (Thermo Fischer Scientific, Waltham, MA, USA) to digitally segment and visualize the anatomy in these specimens. 3D visualizations for use in figures were also created using the volume rendering and surface view functions in Avizo. Syrinxes of several other birds including paleognaths Casuarius casuarius (TMM-12033, stored in 70% ethanol for 2 years, stained in 0.8%-1% I2/ethanol) and Nothoprocta perdicaria (UMNH 23840, stored in 70% ethanol for 2 years, stained in 1% I2/8vol ethanol), and neognaths Gallus gallus domesticus (TMM-23828, stored in 70% ethanol for 2 years, stained in 1% I2/8vol ethanol) and Cairina moschata (TMM-11681, stored in 70% ethanol for 4 days, stained in 0.25% l2/ethanol) were also examined for morphological comparison. Institutional abbreviations: TMM: Texas Memorial Museum, UMNH: Natural History Museum of Utah. Supplementary scan data

were archived on Morphosource.org at: (https://www.morphosour ce.org/projects/000490403?locale=en).

Measurements of major and minor axes of tracheal rings were taken directly from CT scan images in the program ImageJ (Schneider et al., 2012). In order to assess ontogenetic variation in the ring structure of ostrich syrinxes, we then ran Wilcoxon signedrank tests in R v 4.2.1 (R Core Team, 2020) to determine if there were significant differences in the aspect ratios (major axis length to minor axis length) of tracheal rings we measured. In our descriptions of syrinx specimens, we use tracheal diameter to mean the mediolateral diameter across the airway. "Cranial" refers to the direction towards the laryngeal opening and "lungward" to mean towards the bronchi. Ring identities (+2, -1) refer to the schema in Clarke et al. (2016), Kingsley et al. (2018) that uses the tracheobronchial airway split as a major referent.

2.1 Audio recordings

Recordings of ostrich vocalizations were also made at Superior Ostrich Ranch near Waco, Texas. We made four visits to the facility, in October and November of 2019, July of 2020, and October of 2021. Three age classes of ostriches were observed in their respective enclosures. Juveniles between less than one and around 2weeks old were recorded in indoor pens containing 10-50 individuals. Two- to 3-year-old adults (males and females) were recorded in several large open pens containing 50 or more individuals. Other mature adults were recorded in more isolated breeding pens containing one male and three to five females per pen. The recordings were made using a Wildtronics WTPMMA microphone with a parabolic reflector (Wildtronics, LLC, Newton Falls, Ohio) and a TASCAM DR-40 recorder (TASCAM, Santa Fe Springs, California). The parabolic reflector amplified the vocalizations of focal ostriches while reducing background noise in the farm. Observations of behavior were written in a journal while the ostriches were being recorded. Further documentation of behavior was made by taking videos of the ostriches using a Canon G-16 camera (Canon Inc., Tokyo, Japan) and a Nikon D5600 camera (Nikon Corporation, Tokyo, Japan). Audio was recorded at 44100 samples per second, in Waveform Audio File Format (WAV), and at 16-bit depth. Due to contamination from background noise or low signal-to-noise ratios, not all recordings made in the farm could be used for acoustic analysis. Noise was produced mostly by wind and ostriches in other pens. Using Audacity v. 2.1.2 (https://www.audacityteam.org/), 77 quality recordings with high signal-to-noise ratios were selected for additional analysis and repositioning in public archives.

To isolate discrete vocalizations, we produced spectrograms of the recordings in Audacity via a Fast Fourier Transform, using Hanning windows. The duration of the windows was manually calibrated for each vocalization, to minimize the occurrence of sidebands and to visualize the F0 trace and the harmonic traces. We reduced background noise in Audacity using the Noise Reduction function. This function subtracts the spectral envelope of background noise,

3 weeks 10 days 10 days 3 weeks 16 days Time to 9 days 9 days 6 days stain **Fime from staining** to scanning 14 months 10 days 3 weeks 10 days 3 years 2 years 2 years 4 days Aluminum Aluminum None None None Filter None None None Voxel size 102.2μm 25.4 μm 30.4 μm 54.27 μm 25.4 μm 27.2 μm 27.2 μm 89.7 μm 0.15 mA 0.21 mA 0.24 mA 0.2 mA 0.21 mA Current 0.2 mA 0.14 mA 0.2 mA Peak voltage 150kV 150kV 150kV 140kV 159 kV 150kV 160 kV 70 kV 2 weeks 2 days Adult Adult Adult Adult Adult Adult Age sex (where Unknown Unknown Jnknown Jnknown known) Female Female Female Male Specimen number UMNH-23840 UMNH-23828 UMNH-23826 FMM-14723 FMM-15832 FMM-12033 FMM-14733 TMM-14777 Nothoprocta perdicaria Casuarius casuarius Struthio camelus Struthio camelus Struthio camelus Struthio camelus Cairina moschata Gallus gallus Taxon

FABLE 1

Ostrich specimens.

which is selected by the user from sections of the recording in which only background noise is present (https://www.audacityteam.org/, Adret et al., 2018; Pantoja-Sánchez et al., 2022; Walsh et al., 2023). To maximize the dynamic range of the spectrograms, we normalized the amplitude of each recording to its maximum amplitude. After preparing the vocalizations, we classified them based on features unique to each call type such as number of notes, fundamental frequency, and presence of frequency traces that distinguished tonal sounds from hisses. Vocalizations of ostriches are produced as single notes or repetitions of the same type of note. Single notes were the most common vocalization, and they were often produced by several ostriches during any single recording. Often, the same ostrich would produce a few repetitions of the same notes consecutively. Throughout our descriptions, we use the term "note" to mean discrete units of sound that combine together to create a call (Beaver, 1978; Corfield et al., 2009; Mack & Jones, 2003). To diminish the possibility of some individuals being overrepresented in our sample, we selected 3-5 non-consecutive examples from each recording of this type of vocalization. In contrast to the single notes, we rarely saw several repetitions of series in our recordings, and thus we took all series. Recording data is archived on Xeno-canto. org (Link when available).

2.2 | Acoustic measurements

For all analyses of acoustics, we analyzed the fundamental frequency (F0) of each vocalization. The analysis of F0 in the vocalizations of Aves has been used for testing relationships between sound production and the morphology and physiology of the syrinx (Düring et al., 2017; Elemans et al., 2009, 2015; Goller & Riede, 2013; Riede et al., 2019). We took two measurements of fundamental frequency, for each call: maximum and minimum fundamental frequency. These measurements often scale to different degrees with body mass, and thus possibly age (Friis et al., 2021; Marcolin et al., 2022). The discrepancy may result from different mechanisms limiting the lowermost and uppermost frequencies that an animal produces (Elemans et al., 2009; Marcolin et al., 2022; Titze, 1994). To take our measurements, we first applied a low-pass filter to separate the fundamental frequency from the harmonics. To determine the cutoff frequency for each vocalization, we first located the trace of F0 in the spectrogram of the vocalization, which had a frequency equal to the greatest common divisor of all harmonics (Beeman, 1998). The cutoff was set at a frequency between the trace of FO and that of the first harmonic. When more than one trace of FO was found in the same vocalization (ie. gurgle, Figure 6), we isolated both of them. After filtering the recording, we generated a power spectrum, using the same bandwidth used to generate the spectrogram, and located the peak frequency. The minimum and maximum frequencies were the lower and upper frequencies, respectively, that crossed the spectral envelope at 10dB below the amplitude of the peak frequency (Brumm et al., 2017; Zollinger et al., 2012). The low-pass filter and the power spectra were generated using the library seewave in R

(R Core Team, 2020; Sueur et al., 2008). Finally, call duration corresponded to the time span during which the vocalization had an amplitude above the peak amplitude of the vocalization minus 10 dB.

2.3 | Testing for effects of age on acoustics

We tested whether maximum F0, minimum F0, and call duration vary with age in ostriches. Differences related to age can occur from scaling of F0 with body mass (Bradbury & Vehrencamp, 2011; Fletcher, 2004). Differences in call duration could be related to differences in pulmonary capacity. We used generalized linear models (GLM) in JASP (JASP Team, 2022) to test for the effect of age on each of the three measurements, while controlling for call type as a random effect. We used separate GLMs for each measurement. Given that all dependent variables were positively skewed, and thus non-normal, we used log link functions and gamma distributions to model them.

3 | RESULTS

3.1 | Anatomy

The ostrich lateral labia in all age classes and both sexes lie at the tracheobronchial juncture (Figure 1) (Supplementary Data S1). The cartilaginous tracheal and bronchial rings themselves are not well mineralized and show no sign of fusion into a tympanum. There is no sign of asymmetry in the overall geometry of the syrinx. Approaching the tracheobronchial juncture (rings +2, +1, and +0). the ventral side of the tracheal rings develop a chevron shape on their midline which points caudally. The last two to three tracheal rings (rings +2, +1, and +0) are dorsally incomplete. Across these last three tracheal rings, there is a notable increase in the diameter of the trachea, reaching a maximum width at ring +0 (Figure 1) (Table 2). The diameter of the airway decreases slightly above and below the tracheobronchial juncture, occurring in the first two bronchial half rings (rings -1 and -2). Bronchial rings remain medially incomplete throughout the length of the bronchi (to the lungs). The adult male specimen (TMM M-15382) exhibited several ventrally forked bronchial half rings (Figure 2). No other specimens in our dataset

TABLE 2 Tracheal diameters.

Specimen number	Sex (where known)	Average tracheal diameter rings 3–5 (mm)	Tracheal diameter ring 0 (mm)
TMM-14733	Unknown	4.226	5.472
TMM-14777	Unkown	5.5	7.501
TMM-14723	Female	20	29.86
TMM-15832	Male	22.096	35.763



FIGURE 2 External view of adult male ostrich syrinx (TMM M-15382). a) Dorsal view. b) Ventral view showing forked rings. (grey=tracheal and bronchial rings, pink=lateral labia, purple=medial tympaniform membrane, blue=pessuliform process) (scale bars=10 mm).



FIGURE 3 Semi-transparent cutthroughs of ostrich syrinxes highlight the tab-like, unpaired pessuliform process on the dorsal side at the tracheobronchial juncture: a) 2-week-old ostrich syrinx (TMM M-14777) and b) Adult male ostrich syrinx (TMM M-15382). (transparent grey = tracheal and bronchial rings, transparent pink = lateral labia, transparent purple = medial tympaniform membrane, solid blue = pessuliform process) (scale bars = 10 mm).

or those figured or described in prior literature showed this forked morphology.

The ostrich syrinx lacks a complete cartilaginous or mineralized bar supporting the tracheobronchial split called a pessulus in birds or a carina in other amniotes (Kingsley et al., 2018). However, we confirm a small, roughly rectangular midline unpaired cartilaginous element located on the dorsal side of the syrinx, just cranial to the tracheobronchial juncture (Figure 3), which was originally noted by Forbes (1881). It sits in a gap created by the last two to three tracheal rings (rings +2 to +0) above the tracheobronchial juncture, which is incomplete dorsally (Figure 3). This structure, which Forbes (1881) called a "pessuliform process," has not been discussed since it was initially described, although Forbes' (1881) observation was repeated in Beddard (1898). It is typically a blunted rectangle or oval shape, though its dimensions vary slightly from individual to individual. It is approximately as well mineralized as the tracheal and bronchial rings around it. This feature is present in all four specimens we observed, ranging from 2 days old to fully mature, as well as in both males and females.

Although the overall structure of the syrinx is similar throughout ontogeny in the ostrich specimens we examined, we find potential ontogenetic shifts in cross-sectional cartilaginous ring geometries and the spacing of rings. The 2-day-old and 2-week-old juvenile specimens exhibit relatively round tracheal rings with crosssectional aspect ratios ranging from 0.60 to 1.01 and 0.71 to 1.15 respectively. The adult male specimen also starts with relatively round rings in cross-section with aspect ratios between 1.30 and 1.60 near the tracheobronchial juncture that then shifts towards flatter rings with higher aspect ratios (between 1.95 and 3.17) moving cranially. There is specifically a divergence around ring +7 where aspect ratio begins to increase in the adult male, while the juvenile aspect ratios stay relatively similar (Figure 4).

Journal of

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All specimens had both primary extrinsic muscles present in most other birds: that is, m. tracheolateralis and m. sternotrachealis (Figure 5). The m. tracheolateralis is fairly thin, but is well developed enough to be clearly visible on the trachea during dissection and in CT scans. It originates on the upper trachea and follows the lateral sides of the trachea tapering before terminating cranial to the syrinx. The m. sternotrachealis inserts near the terminal end of the m. tracheolateralis expanding as the m. tracheolateralis tapers. In the juveniles, the m. tracheolateralis follows the lateral sides of the trachea before terminating at rings +31 to +28; the m. sternotrachealis inserts on rings +35 to +31 along the edge of the m. tracheolateralis. In the adult male, the *m. tracheolateralis* also follows the lateral side of the trachea and the *m. sternotrachealis* inserts on rings +20 to +25. The m. sternotrachealis originates in its typical location on the dorsal surface of the craniolateral processes of the sternum, although no previous authors had commented on the *m*. sternotrachealis origin. We find no sign of intrinsic muscles in any ostrich specimen.

All specimens show the lateral labia centered around the tracheobronchial juncture. They show thickened lateral labia (Figure 1) that extend between mediolaterally broader rings +6 to +4 cranially

and rings -2 to -4 in the bronchi of both juveniles and adults. In juveniles, the thickest portion of the labia sits just slightly cranial to the tracheobronchial juncture. Their location is similar in both male and female adults, with the thickest portion of the labia possibly very slightly higher (cranial) within the trachea than in juveniles. In the adult male, the labia in cross-section show a shelf-like appearance (Figure 1) which is less pronounced in the adult female. The thickest portions of the labia are thicker relative to the width of the lower tracheal airway in the adult male than in the juveniles and adult female. However, the labia in the female specimen are distorted and may be likely closer to the shape and size of the male's in life than they now appear (Figure 1). The extent and shape of the labia in the ostrich are similar in some ways to that of the Chilean Tinamou (Nothoprocta perdicaria), with both having a shelf-like shape primarily sitting within an expansion in tracheal diameter just cranial to the tracheobronchial juncture (Figure 8). The location of the labia and even the presence of intrinsic and extrinsic muscles, such as the lack of an *m*. sternotrachealis in Nothura darwinii, are highly variable among tinamous and not necessarily similar to the morphology of ostriches (Beebe, 1925; Garitano-Zavala, 2009). Labia are also present in the cassowary (Casuarius casuarius), but they extend more significantly into the bronchi and have several thickened "lobes" not

ANATOMICAL SOCIETY-WILEY-



FIGURE 4 Differences in tracheal ring geometries between ostrich syrinx specimens. a) CT slice showing syrinx and lower trachea of the 2-week-old specimen (TMM M-14777) (Ring shape highlighted in orange). b) CT slice showing syrinx and lower trachea of the adult male specimen (TMM M-15382) (Ring shape highlighted in orange). c) Ring aspect ratios of the two juvenile specimens and the adult male specimens show the divergence in aspect ratio at ring 7.



FIGURE 5 Position of extrinsic tracheal muscles in adult male ostrich (TMM M-15382). a) Ventral aspect of lower trachea and syrinx. The dotted line shows the region where muscle was cut. b) Left lateral aspect of the trachea. Anatomical abbreviations: (TL) *m. tracheolateralis*, (ST) *m. sternotrachealis*, (MTM) medial tympaniform membrane, (IBL) interbronchial ligament (Scale Bars = 10 mm).

seen in ostrich (Figure 8). In *Rhea*, the primary vibratory structures appear to be the medial tympaniform membrane and a thin lateral tympaniform membrane present in the lower trachea rather than lateral labia as in other paleognaths discussed here (Beaver, 1978; Picasso & Carril, 2013) but more similar to Domestic Chicken (*Gallus gallus domesticus*) (Figure 8). The Muscovy Duck (*Cairina moschata*) has lateral labia with a slightly shelf-like appearance, but the labia are located more closely adjacent to the medial tympaniform membrane than in ostrich (Figure 8).

The medial tympaniform membrane is extensive and uniformly thin in all specimens (Figure 1), showing no visible signs of a thickened semilunar membrane consistent with King and McLelland (1989). The membrane covers the entire medial surface of the tracheobronchial juncture (Figure 1). In addition, because all rings in the bronchi are half rings, the medial tympaniform membrane also forms the internal wall of the bronchi all the way to the lungs as noted by Forbes (1881). The interbronchial ligament was not well preserved in most scanned specimens, but in the adult male specimen we dissected, we found the ligament just below the tracheobronchial juncture, connecting the two inner walls of the bronchi (Figure 5).

3.2 | Vocal repertoire

Juvenile ostriches are very vocal, producing a series of complex whistles and peeps that start even before hatching. We did not observe adult ostriches vocalizing as frequently. Adults tended to vocalize primarily when threatened, during aggression, and when displaying to potential mates or rivals. However, we find the diversity of adult

ostrich vocalizations to be greater than previously reported, and we documented three tonal call types and a new variant of hiss that have not been recorded to our knowledge. We measured the effect of age on fundamental frequency by fitting generalized linear models and then modeling contrasts between juvenile and adult calls. We found a significant effect of age on maximum fundamental frequency (2=6.07, d.f. = 1, p=0.014) and minimum fundamental frequency (2=8.76, d.f. = 1, p=0.003). Adult calls were all recorded to be lower in fundamental frequency (both minimum and maximum) than juvenile calls, as would be expected with the very large increase in body size through ontogeny, as shown among other avian and tetrapod clades (e.g., Bradbury & Vehrencamp, 2011; Fletcher, 2004; Friis et al., 2021; Marcolin et al., 2022). This is not necessarily indicative of a significant relationship between syrinx size and body size in ostriches specifically, as this remains to be investigated with a larger sample size. For juveniles, the minimum and maximum fundamental frequency were 1031 ± 153 Hz (median \pm s.d.; n = 15) and 1400±156Hz, respectively. For adults, these values were 151 ± 128 Hz and 188 ± 211 Hz, respectively. The timing of the transition between the very vocal, higher frequency juvenile stage and the less vocal, lower frequency adult stage is not well studied with reference to sound production and will require further investigation. In addition to frequency, we tested for an effect of ontogenetic status on call duration, which could be related to differences in pulmonary capacity between juveniles and adults. However, we did not find such an effect (2 = 3.41, d.f. = 1, p = 0.065).

Juvenile ostriches produced several different call types which we split into two main categories: those that are composed of a series of separate repeated notes and those that are longer with



FIGURE 6 Recorded vocalizations of Struthio camelus displayed as spectrograms. (Juvenile Silhouette = Juvenile, M Silhouette = Adult Male, F Silhouette = Adult Female? Adult Silhouette = Adult of Unknown Sex). Spectrogram settings: Hanning windows; window lengths: 2048 (Boom window length: 8192); 50% overlap between window; sampling frequency 44.1 kHz. The amplitude of power spectra was normalized to the peak amplitude. Note that time and frequency axis scales differ between calls due to differences in call duration. For the scream, boo, and coo calls in which notes were not repeated, we show the complete vocalization (n.b. a much shorter duration than other call types).

oscillations in fundamental frequency. It is worth noting that juvenile ostriches can rapidly switch between a series of separate notes and long calls. In the former category, juvenile ostriches produced repeated staccato notes, which we call peeps, before hatching and continued doing so for several weeks after hatching. Peeps resemble inverted U's when viewed in a spectrogram (Figure 6). Juveniles also produced separate notes in very rapid succession, which we call chirps. When viewed as spectrograms, the beginnings and ends of the chirp calls sometimes feature notes linked into similar inverted U shapes to peeps (Figure 6). In the latter category, we also recorded longer juvenile calls that oscillated between two frequencies, which we call vibrato calls (Figure 6). Recorded instances of these calls lasted between 0.7 and 2.3 s. A variation of this call, which we call a "gurgle," additionally features structures resembling biphonation in

spectrogram view, with two overlapping bands of sound (Figure 6). They range between 0.8 and 3.5 s in duration.

The best-documented vocalization of adult ostriches is the male "boom" call. As has been previously noted, this call is made only by mature male ostriches. It is a closed-mouth, low-frequency vocalization with inflation of the gular region (Duerden, 1920; Riede et al., 2016; Sheak, 1923) and consists of two shorter notes followed by a longer one (Figure 6).

We also documented non-hissing tonal vocalizations primarily made by aggressive or defensive adult ostriches. One of these is the "boo" call. We use this name, which was used by Sauer and Sauer (1966) for aggressive, tonal calls they observed in wild settings.

The "boo" call is a tonal, open-mouthed vocalization so far recorded only in males associated during threat displays. The call is



FIGURE 7 Recorded hissing vocalizations of Ostrich (*Struthio camelus*), Domestic Goose (*Anser* sp.), and American Alligator (*Alligator mississippiensis*) displayed as spectrograms. Stacked structures in the Alligator hiss spectrogram are from background noise. (M Silhouette = Adult Male, F Silhouette = Adult Female? Adult Silhouette = Adult of Unknown Sex). Spectrogram settings: Hanning windows; window lengths: 2048; 50% overlap between window; sampling frequency 44.1 kHz. Amplitude of power spectra was normalized to the peak amplitude. Note that time axis scales differ between calls due to differences in call duration.

short, consisting of one note lasting between 0.215 and 0.5 seconds (Figure 6). There is a noticeable variation in the clarity of the tone of the "boo," with some utterances being clearer and more tonal and others being noisier in spectrogram view. Most recordings of this call were made by males and two of them by individuals that were not seen when they were recorded, and thus of unknown sex. A similar call, which we tentatively term the "scream" has a spectrogram, like the "boo" call, that resembles an inverted U but shows a greater maximum frequency and lacks the clear stacking of harmonics of the "boo" call.

We also documented two other adult calls for which we were unable to determine a clear behavioral context. The first of these is a "rattle" call, which consists of a rapid series of repeated notes (approximately 0.03 seconds from the beginning of one note to the start of the next) (Figure 6). At least two of the three instances of this vocalization that we recorded were made by a male, but the exact behavioral context is uncertain outside of the fact that male ostriches were being aggressive towards each other in the vicinity. Another of these calls is the "coo" call, which was recorded once in an ostrich of unknown sex. Its spectrogram forms a similar U shape as the boo and scream call, but it has a shorter duration (0.2 s) and is overall less intense and less chaotic than the spectrogram of the scream call (Figure 6). The behavioral context of this call is entirely unknown, as we did not see the individual that made it.

The most common vocalizations made by adult ostriches were hisses, which were produced by male and female ostriches when engaged in aggressive or defensive behavior, both towards other ostriches and humans. Most hisses are straightforward turbulent expulsions of air with a spectrogram resembling white noise from only a few 100 to over 8000 Hz, similar to hisses produced by other

birds such as geese and other reptiles such as crocodilians (Figure 7). However, we documented some hisses that had noticeable tonal components, as demonstrated by clearly stacked harmonics visible in spectrograms. We distinguish between tonal and atonal hisses by the presence of harmonics in spectrogram view (Figure 7). These harmonics are much clearer in some spectrograms than others, potentially due to background noise interference or microphone distance from individuals vocalizing. There are also several instances in which harmonics were seen, but a fundamental frequency is not clearly visible where it would be expected (Figure 7). The reason for this is unclear, but it is possible the fundamental was filtered out through resonance in the vocal tract. Tonal hisses were only definitively recorded in males, although one of the female hisses' spectrograms had subtle structures similar to those in tonal hisses recorded in males (Figure 7). We cannot rule out that females are able to produce this call and we simply did not record it clearly. In both atonal and tonal hisses, we observed ostriches moving their tongues forward or towards the roof of the mouth to varying degrees while vocalizing and during open-mouthed threat displays. However, this movement cannot be clearly linked to control of airflow during hisses and could simply be a part of a visual threat display not associated with vocalization.

4 | DISCUSSION

We examined the morphology of the ostrich syrinx for evidence of anatomical correlates to sound source number and changes in morphology through ontogeny and by sex. All ostrich syrinxes consistently lacked ring fusion, a pessulus, or intrinsic muscles. Early authors Journal of Anatomy - ANATOMICAL-WILEY

Specimen	R labium thickness between ring 0 and – 1 (mm)	L labium thickness between rings 0 and – 1 (mm)	Total tracheal width (mm)	Airway width (mm)	Labia thickness to airway width
TMM-14723	0.909090909	N/A (Distorted)	5.636363636	N/A (Distorted)	N/A (Distorted)
TMM-14777	1.086956522	1.086956522	7.608695652	5.434782609	0.4
TMM-14733	2.777777778	5	29.4444444	21.66666667	0.358974359
TMM-15382	10	7.692307692	34.61538462	16.92307692	1.045454545

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pessulus origin in neognaths, or the structure in ostrich may represent a vestigial pessulus homologous with the structure observed in galloanserines and other Neognathae, suggesting that at least a cartilaginous pessulus may be ancestral to Aves. Cartilaginous support of the tracheobronchial junction like a pessulus is known to arise multiple times across amniotes and can comprise the edge of a single ring or multiples (Kingsley et al., 2018). More work on the development of the pessuliform process in ostrich, the structure in rhea, and neognaths may help shed light on whether the pessuliform process may be homologous to pessulus of neognaths. Cross-sectional ring geometry across the tracheobronchial juncture in all ostriches studied shows a simple transition from more rounded at the juncture to more elongated away from the juncture. Emu (Dromaius novaehollandiae) and cassowary (Casuarius casuarius) show more variation in cross-sectional ring shape cranial to the syrinx, through the tracheobronchial juncture, and into the bronchi (Figure 8). Casuarius and Dromaius syrinxes also differ from that of the ostrich in that in Casuarius and Dromaius there is a much more gradual transition from tracheal ring morphology to bronchial ring structures overall (McInerney et al., 2019; Pycraft, 1900). The ring geometries in ostrich are more similar to the Chilean Tinamou (Nothoprocta perdicaria) (Figure 8). Nothoprocta perdicaria also shares a similar single expansion in tracheal diameter above the tracheobronchial juncture. This single expansion in tracheal diameter is also similar to illustrations of the kiwi (Apteryx mantelli) syrinx (Forbes, 1881). This is unlike Casuarius, which expands in tracheal diameter moving lungward adjacent to the labia, contracts at the level of the tracheobronchial juncture, and then expands again moving into the bronchi (Figure 8). Nothoprocta, Apteryx, Casuarius, and Dromaius also feature the downward deflecting chevron shape of the ventral midline and the incomplete dorsal side of the lower few tracheal rings that we see in ostrich (Forbes, 1881; Pycraft, 1900).

We consistently identify prominent lateral labia consistent with Forbes (1881) and extensive medial tympaniform membrane bridging the tracheobronchial juncture and extending to the lungs in the ostrich. We did not find anything that we would term a lateral tympaniform membrane (contra El Karmoty & Khattab, 2018; Yildiz et al., 2003). Both the *m. sternotrachialis* and *m. tracheolateralis* are present, and the *m. tracheolateralis* does not extend to the tracheobronchial juncture. King and McLelland (1989) consider lateral labia to be fairly uncommon in birds as a primary vocal fold but this deserves reassessment along with a reconsideration of the number of sound sources ancestral to Aves. With two pairs in rhea (Picasso & Carril, 2013), a single pair was found experimentally in ostrich

based on the absence of ring fusion. However, Forbes (1881) largely settled this debate with his descriptions and illustrations of paleognath syrinxes focusing instead on potential lateral labia and membranes. Forbes (1881) documented the ostrich lateral labia as being located just above the tracheobronchial juncture, thickest around the last few tracheal rings and extending minorly to the upper bronchi (Elemans et al., 2015; Forbes, 1881). We confirm this morphology in all specimens we observed, including throughout ontogeny and in both sexes (Figure 1). One of the differences we observed between the well-preserved adult syrinx specimen and the juvenile specimens is that its lateral labia are thicker relative to the width of the airway; as the female airway was distorted this was primarily remarked in the male (Figure 1) (Table 3). Cross-sectional ring geometry changes significantly across postnatal ontogeny from observed juveniles to adults, but rings at the tracheobronchial juncture change less and may be more constrained. We show for the first time that female ostriches have a syrinx similar in form to males despite long-noted differences in vocal behavior and a lack of prior data on female vocal anatomy. Specifically, the production of the closed-mouth boom call is known to be restricted to males, but there has been limited data on female calls. Given the small sample size and a lack of body mass data for specimens, we were not able to use geometric morphometric approaches to inform potential changes in scaling in the syrinx of ostrich across ontogeny. However, further studies with larger sample sizes may benefit from these quantitative approaches.

debated whether ostrich or other "ratites" even had a syrinx largely

All juvenile and adult ostriches show a small, single, unpaired, cartilaginous element at the tracheobronchial juncture (Figure 3) which Forbes (1881) called the "pessuliform process." The pessuliform process in ostrich does not seem to be an extension or fusion of any paired tracheal or bronchial rings, being solely a midline element. While all other paleognaths lack any sign of a pessulus, Rhea americana shows a thin cartilaginous carina formed of bronchial rings fused to tracheal rings they identify as a pessulus (Picasso & Carril, 2013), while in studied neognaths a pessulus is not known to arise through ring fusion (Kingsley et al., 2018). The pessulus of neognathae, as an ossified carina present at the tracheobronchial split, is present in nearly all extant neognaths including Domestic Chicken (Gallus gallus domesticus) and Muscovy Duck (Cairina moschata) (Figure 8); it varies in degree of mineralization across taxa with a well-ossified pessulus hypothesized to be at least ancestral to neognaths (Clarke et al., 2016). The midline structures in ostrich and Rhea may represent multiple independent developments of a cartilage support at the tracheobronchial split in birds independent from



FIGURE 8 Comparison of labia and membrane position in the a) adult male Ostrich (*Struthio camelus*), b) Cassowary (*Casuarius casuarius*) syrinx, c) Chilean Tinamou (*Nothoprocta perdicaria*), d) Domestic Chicken (*Gallus gallus domesticus*), e) Muscovy Duck (*Cairina moschata*) syrinx. (Grey=Tracheal and Bronchial Rings, Pink=Lateral Labia, Purple=Medial Tympaniform Membrane) (Scale Bars=10mm).

(consistent with what we observed in our specimens) and elegantcrested tinamou (Eudromia elegans) (Elemans et al., 2015), and two pairs ambiguously optimized as present in basal Neognathae (Clarke et al., 2016), further data on the ancestral traits for Aves require further developmental and functional data. The position of the labia just above the tracheobronchial juncture in our adult ostrich specimens appears consistent with the single sound source found by Elemans et al. (2015). The less cranial position of the labia in juvenile ostriches, with the thickest portion sitting just slightly above the tracheobronchial juncture, as well as our new recordings of juvenile ostrich calls, which we call gurgles, containing notable structures resembling biphonation, make this more ambiguous in the juvenile specimens. However, because structures like these can be produced by weakly coupled vibration in the labia ("true" biphonation) as well as by multiple sound sources, physiological studies will be required to determine which of these two phenomena cause these structures in spectrogram view in juvenile ostriches (Digby et al., 2014).

Comparing the vocal repertoire and habits of ostriches, we observed juvenile ostriches vocalizing more frequently than adults, which were generally quiet and primarily vocalized during display or aggression. However, adult ostriches have a wider vocal repertoire than is often discussed, with our study identifying at least 5 distinct adult tonal call types (not including hisses) (Figure 6). The fundamental frequencies of adults were significantly lower than juveniles, as would be expected in a bird that goes through an extreme change in body size through ontogeny even among other paleognaths, reaching an average adult body mass of 111kg (Bradbury &

Vehrencamp, 2011; Dunning, 2008; Fletcher, 2004; Friis et al., 2021; Marcolin et al., 2022). One hypothesis that has been lightly discussed in ostriches and other paleognaths is that a biomechanical or energetic constraint on the ostrich syrinx such as higher driving pressures required to vibrate increasingly large labia prevents adults from vocalizing as frequently or for as long as juveniles. It has been noted by some authors in the past that the adult male booming display calls seem to "cost [them] considerable exertion" (Sheak, 1923, p. 638), and a similar idea was suggested in Rhea by Beaver (Beaver, 1978), who noted that the medial tympaniform membranes intrude further into the bronchial airway in juveniles than in adults and that adult medial tympaniform membranes "do not seem capable of closing the air passages to the extent necessary to produce the resonance of the chick call-notes" (Beaver, 1978). A higher cost to produce vocalizations in adults may also explain why we did not find significant differences in call duration between juveniles and adults, despite the latter having higher pulmonary capacity. However, Picasso and Carril (2013) did not substantiate the claim that the medial tympaniform membranes intrude further into the airway in adults in their description of the rhea vocal organ through ontogeny, suggesting that this idea was based on an artifact of specimen preservation and that the change in frequency of vocalization in rhea may need to be examined from other perspectives (Picasso & Carril, 2013). The communicative function of the vocalizations reported here needs to be further elucidated in studies in which ostriches are recorded in settings where the distance to the sound source and the background noise can be controlled for. These studies will allow the ability to estimate the sound pressure

Journal of Anatomy - ANATOMICAL-WILEY-

level at the source, which paired with playback experiments, will allow researchers to shed light on the distance range at which the vocalizations are used, the identity of the intended receivers (e.g., relatives, mates), and the behavioral context in which they are produced.

The morphology of ostrich syrinxes does not appear to be clearly sexually dimorphic in adults, while more tonal calls appear more common in males and the boom call remains unique to males. The males in which we recorded most tonal sounds being produced did so in aggressive contexts with other males, a context we observed less frequently among females. If the females are engaging in direct confrontational behavior less often than males, there is less reason for them to produce aggressive tonal sounds. Thus, behavioral differences may better explain why longer tonal calls are not more common in adult female ostriches. It is also possible that a combination of behavioral and biomechanical factors could explain this, with drawnout, potentially energetically taxing tonal calls being unnecessary for most behavioral situations resulting in these longer calls being relatively uncommon. Vocalizations of ostrich and other birds of both sexes during breeding seasons, egg-laying, and other contexts are also important to getting a full picture of avian vocalizations. One recording uploaded to Xeno-Canto from Morioka Zoological Park includes a female ostrich in the process of egg-laying repeatedly producing sounds with harmonics and subharmonics in the lower frequency range (Min-Max fundamental frequency of 150-250 Hz) that are much longer than any call type we recorded in adults in an outdoor farm setting (duration of between 4 and 7 seconds) (Morioka Zoological Park ZOOMO, XC675445. Accessible at www.xenocanto.org/675445). The video included with the file depicts these vocalizations as being closed-mouth or nearly so. These calls do not fit solidly into any call type category that we recorded, likely because these were vocalizations brought on by exertion during egg-laying, which we did not record and are restricted to a very specific context in female animals. This recording clearly demonstrates that adult ostriches are physically capable of producing longer vocalizations than are typically recorded, and this is not restricted to males.

We recorded multiple types of open-mouth tonal calls and one type of closed-mouth tonal call in ostriches. Most open-mouth vocalizations we recorded had higher fundamental frequencies than the boom call (Figure 6). One of these open-mouth vocalizations, the "boo" call, had a similar fundamental frequency and inverted U shape in spectrogram view to the closed-mouth boom call (Figure 6). The most notable difference between these two calls (and the open- and closed-mouth vocalizations more generally) is a clear inflation of the esophagus, which was only observed in the closed-mouth boom calls. Inflation of the esophagus was noted as a major component of the boom call early in the study of ostrich vocalizations (Duerden, 1920). In addition, spectrograms of the two call types show that the boo call has many clear harmonics which the boom call appears to lack, especially at higher frequencies (Figure 6). This is consistent with a previous experimental study by Riede et al. (2004), which examined esophageal inflation in Ring-Necked Doves (Streptopelia risoria). Riede et al. (2004) found that an inflated esophagus served as a vocal filter for closed-mouth vocalizations in Streptopelia. Although this

has not been experimentally tested in other taxa, based on morphological observations, this phenomenon has been noted to be present in a wide variety of birds such as and has been suggested to have evolved many times within birds (Riede et al., 2004, 2014, 2016). Spectrogram data are consistent with the hypothesis that esophageal inflation in ostrich does serve to filter out certain frequencies as it does in Streptopelia (Riede et al., 2004). Most open-mouth calls in ostrich appear to be associated with intraspecific aggressive behavior, whereas the closed-mouth boom call is associated with display behaviors (Duerden, 1920; Sheak, 1923). This is a trend noted across birds, with many closed-mouth vocalizations being associated with male mating displays (Riede et al., 2014, 2016).

Our recordings also show adult ostriches producing both hisses and tonal sounds simultaneously. To our knowledge, this is the first time that this phenomenon has been documented in ostriches. While several studies have addressed the modification of sound through the use of the airway in birds (Riede et al., 2006; White, 1968), the mechanisms of avian hissing are generally understudied (Brackenbury, 1978; Dutour et al., 2020; Gridley & Gardiner, 2021). One study examined changes in air pressure and airflow rate in the trachea and air sacks of geese and found that syringeal resistance was minimal (Brackenbury, 1978). Because of this, they suggested that hisses are produced by the expulsion of air through a constricted glottis in the goose larynx (Brackenbury, 1978). This is consistent with examinations of hiss production in other tetrapods, which are better documented than in birds (Gans & Maderson, 1973; Göppert, 1937; Russell & Bauer, 2020). This would suggest that hisses in ostrich may similarly be produced in the glottis. However, the tonal portion of these calls has very similar fundamental frequencies to other tonal calls that are likely syringeal in origin, typically below 400 Hz. If correct, this would mean that the tonal hisses we recorded in ostriches are created by two separate sources, one by the vibration of the labia in the syrinx and one by the expulsion of air through the larynx and glottis. Alternatively, the hissing component might originate from air turbulence in the vocal tract, which has been well-documented for human vocalizations but not in birds (Stevens, 2005; Strevens, 1960). It is also possible that hisses are produced in the syrinx through chaotic vibration of the labia (Fletcher, 2000). At present, our data does not allow us to determine which of these mechanisms would produce the tonal hisses recorded. Experimental approaches such as those conducted by Riede et al. (2004) or Elemans et al. (2015) could provide insight into these hissing mechanisms. Either way, further study on avian hisses may shed further light on the evolution of avian vocalizations; the ability of the ostrich to simultaneously produce a larynx-based hiss and a syrinx-based tonal sound could suggest that the functional overlap of these vocal organs is indeed possible (Kingsley et al., 2018).

5 CONCLUSIONS

The morphology of the ostrich syrinx lacks ring fusion, a true pessulus, or intrinsic muscles, as has been reported by authors as early Journal of Anatomy

as Forbes (1881). However, nuance exists in the syrinx of this taxon that has not been previously well documented such as potential shifts in cross-sectional ring geometries across ontogeny and presence of an additional midline cartilaginous structure, called the pessuliform process, not examined since the late 1800s. Convergence amongst paleognaths makes it difficult to tell if certain structures, like the pessuliform process in ostrich and the pessulus in Rhea, are homologous with the pessulus of neognaths or represent convergent appearances of midline airway cartilaginous support elements in the syrinx. Despite the overall simplicity of the ostrich syrinx, adult ostriches have a wider vocal repertoire than is often discussed. Female calls can be tonal and long in duration; no major differences in morphology are present in male and female syrinx anatomy. Hisses and tonal sounds produced simultaneously may hint at functional overlap between the larynx and syrinx during aggressive adult vocalizations in this taxon. More work on the transition between juvenile and adult calls, in both males and females, as well as a larger syrinx specimen sample size with a wider range of ages would be useful in completing our understanding of ostrich vocal ontogenetic shifts. Further study into the vocalizations and syringeal morphology of paleognaths will help to shed light on the evolution of the vocalization within the group. Having a more robust understanding of the evolution of vocalization in paleognaths as a clade will also prove invaluable for unraveling the evolution of the syrinx itself as we continue the search for syrinxes preserved in the fossil record.

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AUTHOR CONTRIBUTIONS

J. A. C., M. A. C., and C. R.-S. conceived the study; C. R.-S. and M. A. C. collected anatomical specimens; Z. L. and M. A. C. processed anatomical data; M. A. C. and Z. L. visualized and performed analyses on anatomical data; C. R.-S. and M. A. C. collected bioacoustics data and performed statistical analyses; M. A. C., C. R.-S, and J.A.C wrote the paper; all authors discussed and contributed to the final draft of the manuscript.

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DATA AVAILABILITY STATEMENT

CT scans and segmentation data are reposited on morphosource.org (https://www.morphosource.org/projects/000490403?locale=en). All acoustic data and associated metadata have been uploaded to xeno-canto.org under *Struthio camelus*.

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