ORIGINAL PAPER

Roars, groans and moans: Anatomical correlates of vocal diversity in polygynous deer

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Abstract

Eurasian deer are characterized by the extraordinary diversity of their vocal repertoires. Male sexual calls range from roars with relatively low fundamental frequency (hereafter f_o) in red deer *Cervus elaphus*, to moans with extremely high f_o in sika deer *Cervus nippon*, and almost infrasonic groans with exceptionally low f_o in fallow deer *Dama dama*. Moreover, while both red and fallow males are capable of lowering their formant frequencies during their calls, sika males appear to lack this ability. Female contact calls are also characterized by relatively less pronounced, yet strong interspecific differences. The aim of this study is to examine the anatomical bases of these inter-specific and inter-sexual differences by identifying if the acoustic variation is reflected in corresponding anatomical variation. To do this, we investigated the vocal anatomy of male and female specimens of each of these three species. Across species and sexes, we find that the observed acoustic variability is indeed related to expected corresponding anatomical differences, based on the source-filter theory of vocal production. At the source level, low f_o is associated with larger vocal folds, whereas high f_o is associated with smaller vocal folds: sika deer have the smallest vocal folds and male fallow deer the largest. Red and sika deer vocal folds do not appear to be sexually dimorphic, while fallow deer exhibit strong sexual dimorphism (after correcting for body size differences). At the filter level, the variability in formants is related to the configuration of the vocal tract: in fallow and red deer, both sexes have evolved a permanently descended larynx (with a resting position of the larynx much lower in males than in females). Both sexes also have the potential for momentary, call-synchronous vocal tract elongation, again more pronounced in males than in females. In contrast, the resting position of the larynx is high in both sexes of sika deer and the potential for further active vocal tract elongation is virtually absent in both sexes. Anatomical evidence suggests an evolutionary reversal in larynx position within sika deer, that is, a secondary larynx ascent. Together, our observations confirm that the observed diversity of vocal behaviour in polygynous deer is supported by strong anatomical differences, highlighting the importance of anatomical specializations in shaping

mammalian vocal repertoires. Sexual selection is discussed as a potential evolutionary driver of the observed vocal diversity and sexual dimorphisms.

KEYWORDS
acoustic variation, descended larynx, fallow deer, female contact calls, male sexual calls, polygynous deer, red deer, sexual dimorphism, sexual selection, sika deer, source-filter theory, vocal anatomy, vocal production, vocal repertoire

1 | INTRODUCTION

Recent research in vocal communication has highlighted the diversity of mammal vocalizations and led to considerable advances in our understanding of the information content and function of the key acoustic components of vocal signals. Because natural and sexual selection affect the organs of vocal production and their control, investigating the underlying anatomical bases of this diversity is a crucial step for advancing our understanding of the evolution of vocal signalling (Taylor & Reby, 2010).

While the interspecific and intersexual diversity of vocal signals has already been documented in several species of vertebrates (Cap et al., 2008; Espmark, 1964; Fischer & Price, 2017; Frey et al., 2012; Garcia, Kopuchian, et al., 2017; Kidjo et al., 2008; Ladich & Winkler, 2017; Matsunaga & Okanoya, 2009; Minami, 1997; Odom et al., 2014; Reby & McComb, 2003a; Riebel et al., 2019; Suthers et al., 2016; Suthers & Goller, 1997; Vannoni & McElligott, 2007; Volodin et al., 2019; Wich & Nunn, 2002), the extent to which this acoustic diversity does reflect an underlying anatomical diversity remains to be investigated. Indeed, it is not clear whether the bases of this vocal diversity are mainly behavioural (innate or culturally acquired), anatomical (affecting the laryngeal or supralaryngeal structures) or both. The aim of this study is thus to investigate the anatomical and physiological correlates of vocal diversity, both across species and between sexes of the same species, and to determine if anatomical specializations are likely to support the observed acoustic diversity. To do this, we focus on three species of polygynous deer characterized by acoustically very different male sexual calls: long series of stereotypical groans with low fundamental frequency in European fallow deer, *Dama dama* (Vannoni & McElligott, 2007) short bouts of non-stereotypical roars in Western European red deer, *Cervus e. elaphus* (Reby & McComb, 2003a) to single moans with extremely high fundamental frequency in Japanese sika deer, *Cervus n. nippon* (Minami & Kawamichi, 1992; Figure 1; Table 1).

A useful paradigm for describing the acoustic structure of mammalian vocal signals and predicting the information that their different components encode is the "Source-Filter" theory of vocal production. According to this theory the acoustic components of vocalizations result from a two stages process, involving an oscillator as the sound source (typically the vocal folds in the larynx) and a resonator as the sound filter (in mammals the supra-laryngeal vocal tract; Fant, 1960; Fitch, 1997; Taylor & Reby, 2010; Titze, 2000, 2006). The fundamental frequency (f_o) is determined by the rate of vibration of the vocal folds caused by the passage of air through the glottis into the larynx (the

source) and the formants are resonance frequencies produced as the glottal wave travels through the supra-laryngeal vocal tract (the filter). While f_o determines the perceived pitch of the sound, formants contribute to the timbre of the vocalization (Fant, 1960). Because of its dependence on the length of the supra-laryngeal vocal tract (Owren & Bernacki, 1998; Titze, 2000), the frequency spacing of formants (ΔF) typically provides a reliable indication of body size (rhesus macaque, *Macaca mulatta* (Fitch, 1997); domestic dog, *Canis lupus* f. familiaris (Riede & Fitch, 1999); red deer, *Cervus elaphus* (Reby & McComb, 2003a,b); fallow deer, *Dama dama* (Vannoni & McElligott, 2008); bison, *Bison bison* (Wyman et al., 2012)).

Many species of mammals retain an ancestral vocal organ configuration, that is, they possess a larynx that is on average allometrically scaled to body size and located in a high and relatively fixed position close to the root of the tongue (e.g., domestic mammals cat, dog, sheep, goat, pig, horse Nickel et al., 2004; capuchin monkeys *Cebus* sp., Cebidae Negus, 1949; Guinea baboon *Papio papio* Cercopithecidae Berthommier et al., 2017, Boë et al., 2017; American moose *Alces alces* Alceini Márquez et al., 2019). Typically, these species produce calls with a f_o and ΔF relatively close to the f_o/body mass and ΔF/body mass regression lines for mammals (cf., e.g., Charlton & Reby, 2016; Fletcher, 2004; Garcia, Herbst, et al., 2017; Riede & Brown, 2013; Tembrock, 1996). In contrast, some species have evolved an enlarged, descended and mobile larynx (e.g., goitred gazelle *Gazella subgutturosa* (Frey et al., 2011; Volodin et al., 2017); Mongolian gazelle *Procapra gutturosa* (Frey et al., 2008); impala *Aepyceros melampus* (Frey et al., 2020) and fallow deer *Dama dama* (McElligott et al., 2006; this work), see Charlton and Reby (2016) for review. In these species the enlarged larynx can accommodate larger vocal folds and a descended and mobile larynx can lengthen the vocal tract, thereby allowing the production of a low f_o, and low ΔF dramatically deviating from the f_o/- and ΔF/body mass regression line for mammals (Charlton & Reby, 2016). Surprisingly, the anatomical correlates underlying this vocal biodiversity have not been systematically investigated, despite their strong evolutionary and taxonomic relevance.

Polygynous deer species of the subfamily Cervinae, characterized by the exceptional acoustic diversity of their vocal signals (Cap et al., 2008), constitute an ideal group for conducting investigations of the anatomical bases of inter- and intra-specific vocal variation. Within Cervinae, both sexes produce loud-call vocalizations in multiple contexts, including seasonal male mating calls, mother-offspring "contact" calls and anti-predator calls (Bowyer & Kitchen, 1987; Ericson, 1972; Espmark, 1971; Lingle & Riede, 2014; Reby & McComb, 2003b; Teichroeb et al., 2013; Torriani et al., 2006; Vaňková et al., 1997; cf. Kondo & Watanabe 2009). While anti-predator and mother-offspring
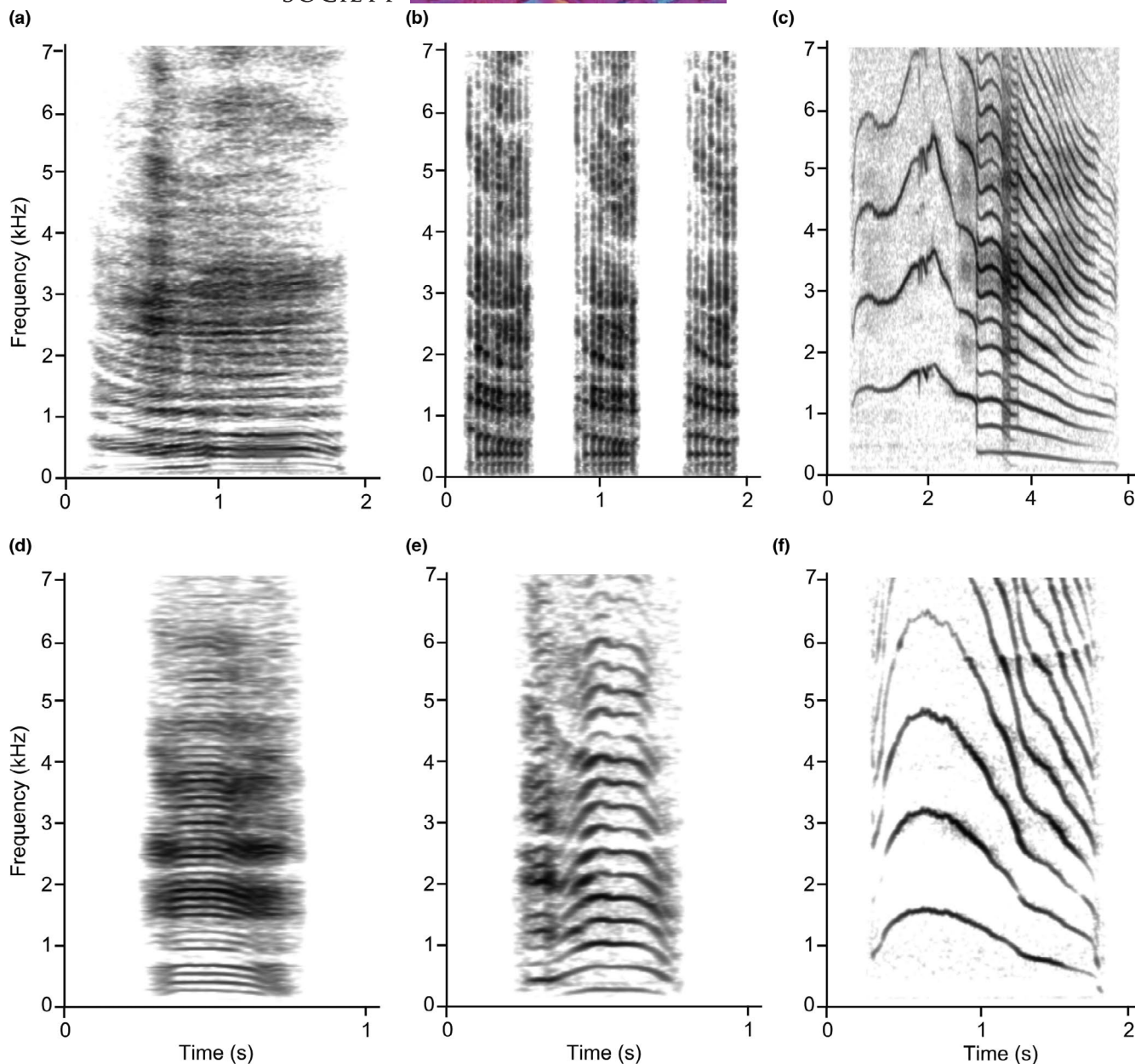
FIGURE 1 Spectrograms of male mating calls and female contact calls. Calls include examples of the following: male red deer roar (a), male fallow deer groan (b), male sika moan (c), female red deer (d), female fallow deer (e) and female sika deer (f). Female fallow deer call was provided by A. McElligott, all other calls were provided by authors (see Audio S1–S6). Spectrograms were produced in Praat v. 6.0.24 (Boersma & Weenink, 2021), with 0.05 s window length, 16-bit amplitude resolution and 44.1 kHz frequency sampling rate

"contact" calls are assumed to arise from natural and non-sexual social selection pressures (adult and offspring survival) and likely to support individual recognition (Torriani et al., 2006; Vaňková et al., 1997) and enhance offspring survival (e.g., avoiding detection; cf. Clutton-Brock et al., 1982; Torriani et al., 2006; Vaňková et al., 1997), the highly conspicuous, exaggerated and context-specific male sexual calls advertise male quality (Reby & McComb, 2003b; Vannoni & McElligott, 2008) and are assumed to primarily result from the strong sexual social selection pressures (male competition and female choice) that characterize these group-living, highly polygynous species (Clutton-Brock 2017; Hughes-Games et al., 2015; Kruuk et al., 1999; McComb, 1991; McElligott & Hayden, 2000; Pitcher et al., 2015; Reby et al.,

2005; cf. Janicke et al., 2016; Lyon & Montgomerie, 2012; West-Eberhard, 1983, 2014). These asymmetries likely contribute to the emergence of sexual dimorphism in the vocal repertoires: while male mating calls emerge after physical sexual maturity (often correlated with testosterone-dependent pronounced changes of larynx and vocal tract morphologies) and typically express "exaggerated" traits, female contact calls are typically more conservative and more acoustically similar to juvenile contact calls (Minami & Kawamichi, 1992; Torriani et al., 2006; Vaňková & Málek, 1997; Vannoni et al., 2005).

The male sexual calls and female contact calls from three species of Cervinae, namely Western European red deer (*C. e. elaphus*), European fallow deer (*D. dama*) and Japanese sika deer (*C. n. nippon*) exemplify

TABLE 1 Comparison of acoustic properties between male and female red deer, fallow deer and sika deer. Calls include the predominant sexual calls from males produced during the rut (red deer roars, fallow deer groans and sika deer moans) and contact calls from females. Mean values with standard error, along with minimum and maximum values, are reported as available from published literature

Acoustic variables[a]	Red deer		Fallow deer		Sika deer	
	Male	Female	Male	Female	Male	Female
Number of calls	475	59	153	487	144	44
Number of individuals	47	8	16	14	–	–
$\min f_o$ (Hz)	61.7 ± 0.69 min: 36.3	min: 70	22.3 ± 2.5 min: 16	min: 152.7	196 ± 18	394 ± 25
$\max f_o$ (Hz)	136.8 ± 1.60 max: 213.9	–	34.7 ± 6.2 max: 55	max: 579.1	1187 ± 81	968 ± 83
$\text{mean} f_o$ (Hz)	106.9 ± 1.19	108.35 ± 116.8	28.2 ± 3.7	365 ± 3.88	–	–
Duration (s)	3.7 ± 0.06	0.27 ± 1.7	0.38 ± 0.07	0.35 ± 0.004	4.36 ± 2.8	2.33 ± 0.39
Minimum formant dispersion (Hz)	243.5	–	298.5 ± 3.0	–	–	–
eVTL max (cm)	71.9 ± 0.01	–	58.2 ± 1.8	–	–	–
Source	Reby and McComb (2003b)	Vaňková and Málek (1997)	Vannoni and McElligott (2007)	Torriani et al. (2006)	Minami and Kawamichi (1992)	Minami and Kawamichi (1992)

[a]Variable abbreviations: $\min f_o$ = minimum fundamental frequency, $\max f_o$ = maximum fundamental frequency, $\text{mean} f_o$ = mean fundamental frequency, eVTL max = estimations of maximum vocal tract length (derived from the formant frequencies of male red deer and fallow deer).

the wide diversity of vocal behaviour observed in polygynous species of the Cervinae (Table 1; Figure 1; Audio S1–S6). Within the male sexual calls of these three species, male fallow deer groans are the shortest and lowest in f_o (duration: 0.4 s, mean f_o: 28.2 Hz, Vannoni & McElligott, 2007, rut call rate: mean up to 23.5–74.2 groans/min depending on context, McElligott & Hayden, 1999) and male sika deer moans are the longest and highest in f_o (duration: 4.4 s, f_o range: 196–1187 Hz, Minami & Kawamichi, 1992, rut call rate: up to 24 moans/h, Miura, 1984), with male red deer roars intermediate (duration: 3.7 s, mean f_o: 106.9 Hz, Reby & McComb, 2003b, rut call rate: mean of over 2.5 roars/min in harem holding males, Clutton-Brock & Albon, 1979). Formant frequencies are prominent in the male mating calls of both red deer and fallow deer but are much less conspicuous in sika deer moans (Figure 1). Red deer and fallow deer males have a descended and highly mobile larynx (Fitch & Reby, 2001; McElligott et al., 2006; Vannoni & McElligott, 2008, 2009), which they are able to retract further towards the sternum while they vocalize. The low resting position of the larynx in the mid-neck region is clearly visible externally, depending on mane length. Roaring in male red deer involves considerable extension of the head-and-neck region with roar-synchronous down-and-up movements of the larynx externally visible. The larynx starts to descend slightly before or at the onset of roaring, remains in the down position during the roar and ascends again to its resting position at the end of a roar. The production of groans in male fallow deer (typically produced in long stereotypical series, Vannoni & McElligott, 2007) involves less extension of the head-and-neck region than in red deer but is accompanied by serial descents and ascents of the larynx. The laryngeal descent allows red deer and fallow deer to increase their supra-laryngeal vocal tract length (Fitch & Reby, 2001), thereby decreasing the formant frequencies in their calls. These descending

formants are clearly visible in the spectrograms of these vocalizations (Figure 1). While minimum formant dispersion (the minimum spacing of formants during roaring), achieved when the vocal tract is fully extended, is considerably lower than expected from body size (Charlton & Reby, 2016; Reby & McComb, 2003a), it still provides an honest indication of body size to receivers, that is, larger males have smaller minimum formant dispersion (Reby & McComb, 2003b). Anatomically and physiologically, this is a consequence of two reasons (i) larger males have longer necks than smaller males of the same species or subspecies and (ii) the larynx is not retracted beyond the caudal end of the neck. In other words: within the same species or subspecies, neck length correlates with body size and as long as larynx retraction is constrained by the length of the neck, a maximally extended vocal tract still correlates with body size. In comparison, the larynx of the male sika deer is not visible externally and thus, call-synchronous movements of the larynx are not observed. Furthermore, descending formants are not visible in the spectrogram (Figure 1).

Females of all three species are known to produce contact calls in a mother–offspring context (Figure 1; Minami & Kawamichi, 1992; Torriani et al., 2006; Vaňková et al., 1997; cf. Volodin et al., 2011). These calls also differ inter-specifically but less pronouncedly than the corresponding male sexual calls. Within females, red deer produce contact calls with the lowest f_o (mean f_o: 108.6 Hz, Vaňková & Málek, 1997) with higher f_o calls produced by fallow deer (mean f_o: 365 Hz) and sika deer (f_o range: 394–968 Hz, Minami & Kawamichi, 1992). The duration of female contact calls is short in all three species (Table 1). The larynx is externally visible in female red and fallow deer. During the production of the contact and alarm calls, female red deer and fallow deer also show a down-and-up movement of the larynx (D. Reby, R. Frey, personal observations), although to a much lesser

degree than the males. The larynx of female sika deer is not visible externally. Strong intersexual differences in the f_o of male mating calls and female contact calls are observed in fallow deer (with males producing calls of lower f_o) but not in red deer or sika deer.

Hypothesis *Our general hypothesis is that the acoustic diversity of vocalizations observed in polygynous cervids is supported by specializations of the vocal anatomy, both between species and between sexes. To investigate this, we compare the vocal anatomy and behaviour of males and females in three species in which males produce high rates of vocalization during the reproductive season: Western European red deer, European fallow deer and Japanese sika deer (Figure 2).*

Based on published acoustic and mostly behavioural data, for example, Fitch and Reby (2001), Reby and McComb (2003a,b),

McElligott et al. (2006), and Vannoni and McElligott (2008), as well as our own visual observations, we expect the following:

At the level of the vocal tract filter, red and fallow deer have a low larynx resting position and an extendable vocal tract involving a mobile larynx. Both features are very pronounced in the males but, although to a much lesser extent, also occur in the females. In contrast, both sexes of sika deer have a high larynx resting position and a short vocal tract lacking adaptations for strong extension. At the level of the source, fallow deer males have a relatively large larynx with long and flaccid vocal folds specialized for the production of a very low f_o, while sika deer males and females have a relatively small larynx with short and rigid vocal folds specialized for the production of a high f_o. Red deer larynges and vocal folds are intermediate in size and structure in both the sexes. Sexual dimorphism of larynx size and macroscopic vocal fold structure is low in red deer and sika deer but strongly pronounced in fallow
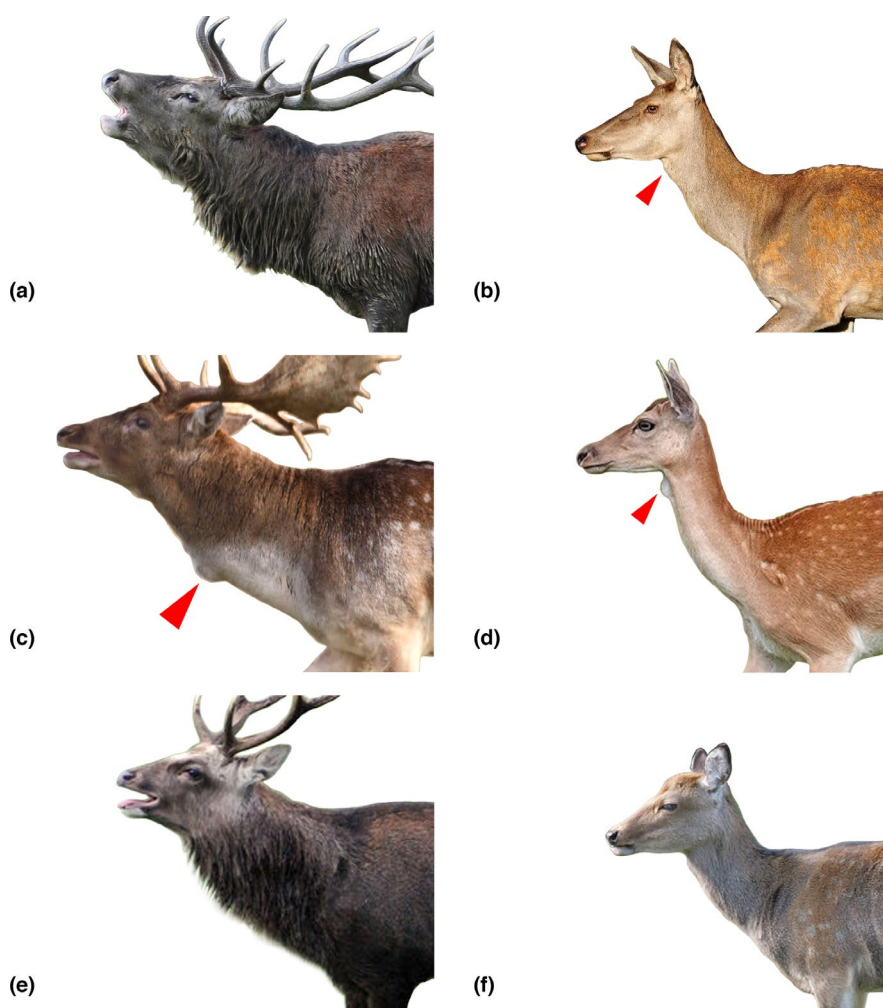

FIGURE 2 Images of Western European red deer (*Cervus e. elaphus*), male roaring (a), female (b); European fallow deer (*Dama dama*), male groaning (c), female (d); Japanese sika deer (*Cervus n. nippon*), male moaning (e), female (f). Not to scale, all individuals were brought to the same size approximately. In male and female fallow deer and in female red deer the larynx position is visible externally (red arrowheads). Sources: (a, b) © Megan Wyman; (c) © David Reby; (d) © Richard Steel 2011; (e) © Julian Dowse 2007 under a creative commons license at www.geograph.org.uk; (f) © Scandinavisk Dyrepark (Frank Vigh Larsen, director) 2014

deer. Accordingly, the relative laryngeal and vocal fold dimensions of female fallow deer are closer to red deer and sika deer of both sexes than to male fallow deer.

In order to investigate these expectations, we combine non-invasive cross-sectional imaging and dissections of the larynx and supralaryngeal vocal tract in both males and females, with examination of histological slices of one vocal fold in one male of each species. We then discuss the proximate links between vocal anatomy, production and acoustics and suggest ultimate explanations for the evolution of the diverse vocal signals observed in these polygynous deer species as well as the interspecific sexual differences in size and structure of the vocal organs.

2 | MATERIALS AND METHODS

2.1 | Specimens

Anatomical data were collected from six head and neck specimens of adult deer using both macroscopic dissections and high resolution cross-sectional imaging (computer tomography [CT] and magnetic resonance imaging [MRI]). These six primary specimens included one adult of each sex for each species (Table 2). Additionally, five secondary specimens provided limited data from partial dissections and/or CT and MRI scans for comparison with data from the primary specimens.

Specimens were collected during regularly scheduled culling events in the fall and winter of 2010 and 2011, conducted by licensed wildlife officers and rangers in parks and wildlands across southern England and western Scotland. Specifically, specimens were collected from Bushy Royal Park (London), Home Park (London), Richmond Royal Park (London), Forestry Commission England (East Sussex), Arne Royal Society for the Preservation of Birds reserve (Dorset), Lulworth Ministry of Defense (Dorset) and Forestry Commission Scotland (Argyll). Age was estimated by the wildlife officers and rangers based on tooth wear and antler size and configuration. Specimens were transported to the University of Sussex and when necessary, frozen for storage in −20°C freezers. Fresh or freshly thawed specimens were scanned with computer tomography and magnetic resonance imaging before subsequent macroscopic anatomical dissection and skeletonization.

2.2 | Nomenclature

The common and scientific species names for our three study species are taken from Burgin et al. (2020). For red deer, we additionally considered Ludt et al. (2004) and Zachos and Hartl (2011), in which a British subspecies of red deer (*Cervus elaphus scoticus*) could not be verified. In the Abstract, Sections 3 and 4 we use simplified common and scientific species names for easier reading: red deer (*Cervus elaphus*), fallow deer (*Dama dama*), sika deer (*Cervus nippon*).

2.3 | Imaging

CT and MRI scans were performed to demonstrate the mineralized and soft-tissue structures of the vocal apparatus and surrounding anatomy. Scanning was conducted at the Clinical Imaging Sciences Centre at the University of Sussex. Thin slice CT scans allow high spatial resolution reconstructions (Figure 3) which can be manipulated using post processing software to allow accurate measurement of the anatomical structures of the larynx. CT is able to clearly delineate the laryngeal cartilages from the vocal folds and surrounding soft tissues (aided by early ossification of the larynx in some deer species). For demonstration of the vocal folds, the soft palate (velum) and the strap muscles of the ventral neck region, MRI gave excellent soft tissue contrast (Figure 4).

Prior to scanning, the nasal tract, vocal tract and oesophagus of each specimen were thoroughly flushed with water to remove any debris. The whole cadaver deer heads were positioned on the CT scanner table (contained within plastic wrapping) with the nose facing upwards, the head secured at a natural resting angle, and the trachea orientated in the line of the scan plane. Scout images, that is, large field of view survey scans, were used to ensure correct alignment and alterations made prior to the full scan acquisition. This limited the need for post processing adjustments in scan orientation to ensure the most accurate measurements.

CT scans were performed on a SIEMENS Biograph 64 slice scanner with a 0.6 mm slice thickness on a pitch of 0.9 mm. Contiguous 0.6 mm slices were displayed with a 512 × 512 pixel matrix, with reconstructions performed with a 'B20s smooth'

TABLE 2 Age and size of primary and secondary specimens. Size was measured as maximal mandibula and metatarsal length. Information for secondary specimens is included in parentheses

Specimen and anatomical variables	Red deer		Fallow deer		Sika deer	
	Male	Female	Male	Female	Male	Female
Specimen code	U4 (E2[a])	BB2 (BB1[b])	U5 (T2[a])	Y1 (NA)	O8 (P1[a], ZC7[b])	W2 (W1[b])
Age (approx. years)	5 (7)	3–4 (5–6)	10–12 (5–6)	4–5 (NA)	7 (5.5, 6)	4–5 (5–6)
Maximal mandibula length (mm)	291 (302)	286 (279)	210 (203)	188 (NA)	205 (203, 195)	184 (182)
Metatarsal length (mm)	407 (399)	397 (388)	325 (322)	NA (NA)	306 (285, 288)	270 (273)

[a]Data available from partial dissection and CT and MRI scans.
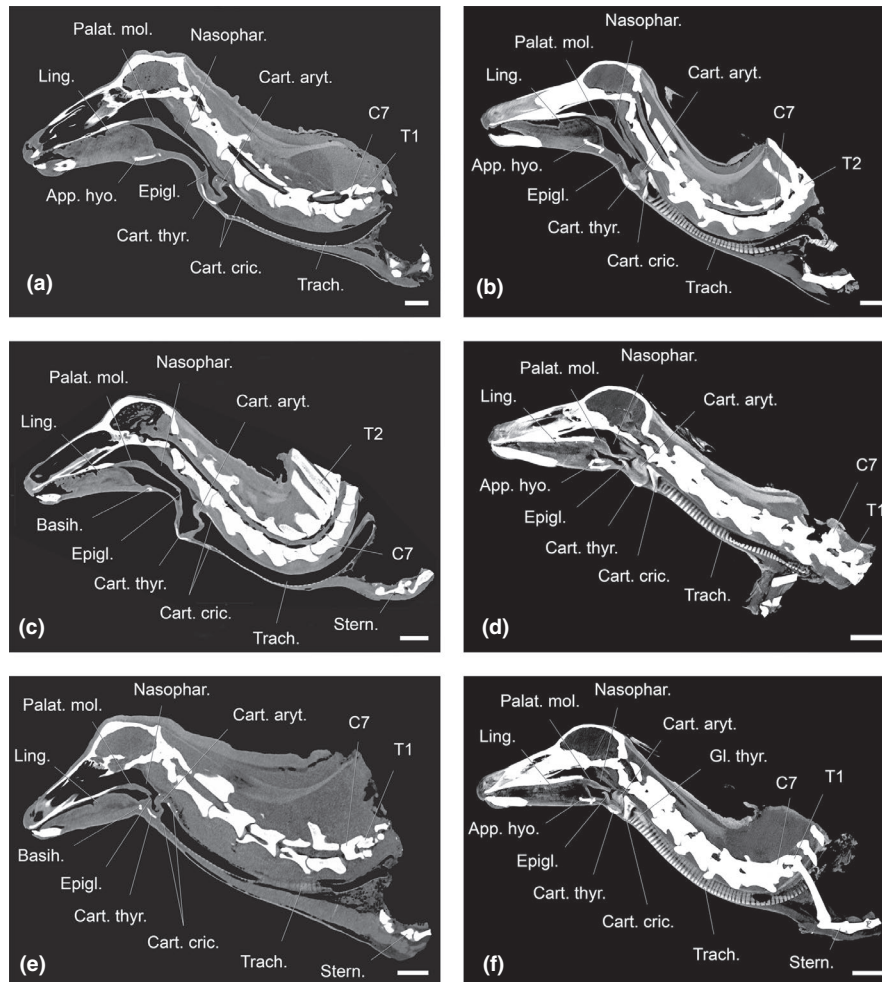
[b]Data available from CT and MRI scans only.

FIGURE 3 CT images of a male (a) and female (b) red deer, a male (c) and female (d) fallow deer, and a male (e) and female (f) sika deer. Not to scale, all specimens brought to the same size approximately. Note the pronouncedly low larynx resting position in male red and fallow deer, associated with an elongated pharynx and soft palate and a caudally shifted intrapharyngeal ostium. Scale bars 50 mm

filter. All CT scans were displayed on a 512 × 512 pixel matrix but the 'Field of View' was varied according to the size of the deer head.

MRI scans were performed on a SIEMENS Avanto 1.5T scanner. A number of scan sequences were used and the advantages and limitations of each will be discussed. The full scan parameters are displayed in the table below. The most useful scan sequences for demonstrating soft tissue anatomy were sagittal T2 sequences (T2 TSE Cor and T2 de3d we cor) with isotropic 0.8 mm voxels on a 250 FoV. These T2 sequences produced the best soft tissue contrast which is limited in post mortem specimens. T1W MPRAGE sequences were also performed although these demonstrated less soft tissue contrast and were of limited anatomical use.

Anatomical dimensions were measured with the Synedra View Personal 3.1.0.6 DICOM viewer program. CT images were loaded into the program and viewed using the multi-planar reconstruction function which constructs a 3D image of the specimen using image slices taken in three planes.

2.4 | Dissection

Macroscopic dissections were conducted on fresh or freshly thawed head-and-neck specimens. Most dissections occurred in air with only the final stages and the excised larynges dissected in water. Consecutive dissection steps were photographically documented by means of a Nikon D70S digital camera (Nikon Corp.) on a Compact Flash card. The images were downloaded to a PC and graphically processed (Adobe Photoshop 5.5 and CS4; Adobe Systems Inc.) for identifying the individual components of the vocal organs and for clarifying mutual anatomical relationships (Figure 5).

Skeletonization was executed in water at 38°C in heated water tanks. Skeletal parts were then cleaned, dried, re-assembled, photographed, and together with photos of live animals, served as a basis for the virtual 2D reconstructions.

Anatomical terms are in accordance with the latest edition of the Nomina Anatomica Veterinaria (NAV) www.wava-amav.org/Downloads/nav_2005.pdf (see also Constantinescu & Schaller, 2012). Main
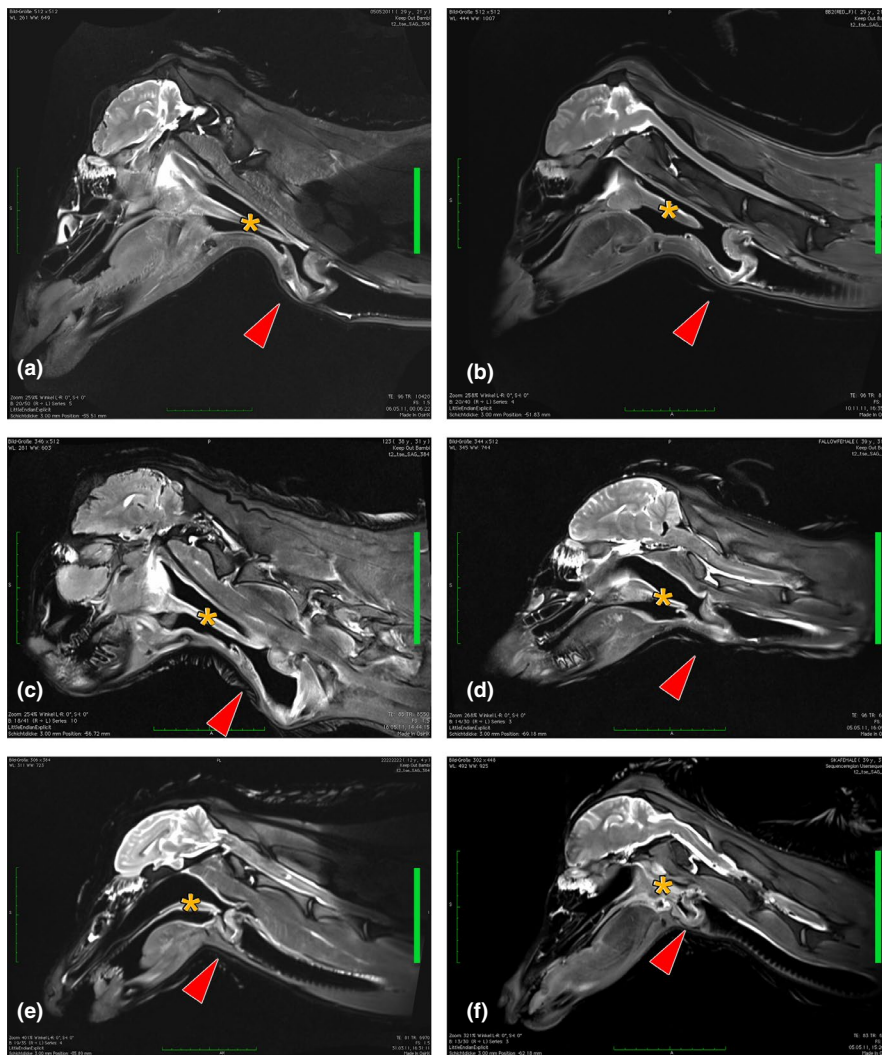
FIGURE 4 MRI scans of a male (a) and female (b) red deer, a male (c) and female (d) fallow deer, and a male (e) and female (f) sika deer. Not to scale, all specimens were brought to the same size, approximately. Note the pronouncedly low larynx resting position in male red and fallow deer, less pronouncedly in female red and fallow deer. Larynx position is high and close to the root of the tongue in male and female sika deer. Red arrowheads point to the larynx, orange asterisks mark the soft palate. Green scale bars 100 mm

dissections were conducted at the University of Sussex, UK with excised larynx dissections and skeletonization performed at the Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin. Only measures functionally relevant to the hypotheses tested are reported in the main text (see Supplemental Information S1 for additional associated measurements).

The hyoid apparatus is an important key element of the vocal tract extension mechanism as it connects the larynx to the pharynx, the tongue and cranial base. Increases in resting and maximally extended lengths of the thyrohyoid ligament between species and sexes can reflect evolutionary modification of the vocal apparatus and adjacent structures during the acquisition of laryngeal mobility. The thyrohyoid ligament, connecting the thyrohyoid of the hyoid apparatus to the rostral horn of the thyroid cartilage, was measured during both the resting state and maximum extension (achieved by manually retracting the larynx towards the sternum).

Our investigations of the muscular system are focused on the muscles most relevant for the stabilization of the larynx, laryngeal movements and throat extension. The 'strap muscles' are narrow, ribbon-like muscles that typically connect the larynx to the hyoid apparatus rostrally (thyrohyoid muscle) and the hyoid apparatus and the larynx to the sternum caudally (sternohyoid and sternothyroid muscles, respectively). In conjunction with the strap muscles, the stylopharyngeal muscle (which in ruminants terminates on the thyroid cartilage) can contribute to stabilizing the resting position of the larynx as well as moving the larynx down and up the ventral neck contour. Finally, the stylohyoid muscle, which connects the stylohyoid angle with the basihyoid can contribute to dorsoventral throat expansion. Muscle lengths were ascertained by using a string measure.

We examined the key laryngeal dimensions, including (a) the overall ventral length of the larynx from the thyroid notch to the
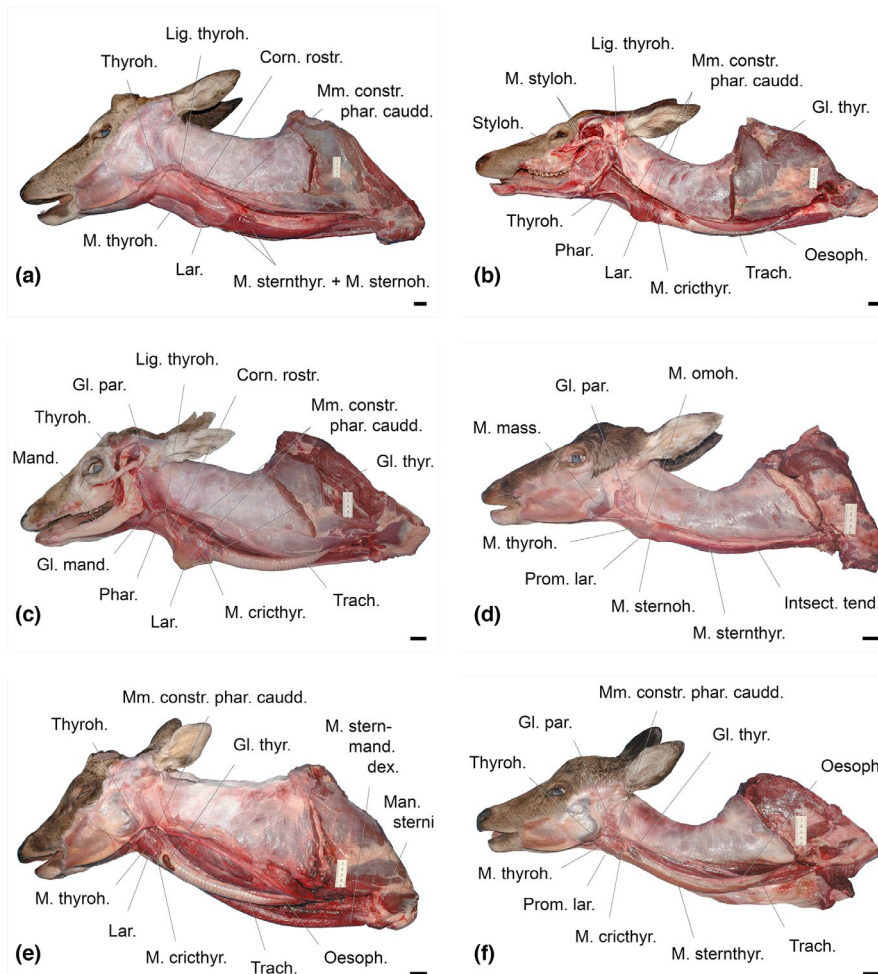
FIGURE 5 Dissection of male (a) and female (b) red deer, male (c) and female (d) fallow deer, and male (e) and female (f) sika deer, left lateral view. Not to scale, all specimens were brought to the same size, approximately. Scale bars 30 mm, respectively

caudal edge of the cricoid arch, (b) the overall lateral length of the larynx from the most laterorostral portion (tip of rostral horn) to the caudal end of the cricoid plate, (c) the maximal height of the larynx from the thyroid prominence to the most dorsal portion (dorsal tip of corniculate processes), and (d) the internal distance between the thyroid prominence and the rostral edge of the cricoid cartilage.

Measures of the nasal and oral vocal tract length were taken while the larynx was in the resting state and at the maximal extension of the larynx towards the sternum. Vocal tract extension, achieved by manually pulling the larynx/trachea in caudal direction along the ventral neck contour, was conducted to simulate the momentary retraction of the larynx via muscular contractions during vocalization, observed as down-and-up movements of the laryngeal prominence in live animals. Resting VTL was measured after removal of the skin but before removing the surrounding muscles in order to get the position of the resting larynx as accurately as possible (i.e., before the in-situ larynx position is disturbed by dissecting off surrounding structures). As any pronounced manual retraction of the larynx in a dead individual/head-and-neck

specimen is difficult to achieve with all structures in place, particularly muscles, we had to wait for later stages of the dissection (after most muscles had been removed and the pharynx opened) to simulate and measure maximal VT extension based on the resilience of the involved structures. Although the artificially induced VT lengths during rest and maximal extension were measured at different stages of the dissection process, this method produced the most realistic estimates of minimal and maximal laryngeal positions for vocalizing animals. The latter were either observed in live animals or in photographs of them.

2.5 | Histology

Larynges from three additional male specimens, an adult male fallow deer (7 years old), red deer (5 years old) and sika deer (4.5 years old), were used for histological sampling. Larynges were excised within 1–2 h of death and were subsequently fixed in 10% buffered formalin for 4 weeks. After fixation, larynges were cut open along the midsagittal plane to expose the vocal

folds. Perpendicular to the dorsoventral length of the vocal fold, transverse samples were taken at the dorsoventral midpoint of the vocal fold between the thyroid cartilage ventrally and the vocal process of the arytenoid cartilage dorsally. Samples were pinned out flat onto sylgard blocks before emersion in an ethanol gradient for dehydration (30 min each at 70%, 95%, 100%, with two changes at 100%). Samples were then placed in a clearing agent for 2 h, drained, then placed in fresh clearing agent for 18–24 h (histoclear for red and fallow samples, xylene for sika samples). Molten wax (60°C) was added to the clearing agent to give a 1:1 ratio of wax:clearing agent and samples were placed in a wax oven at 60°C for 1 h. Samples were placed into fresh wax within the oven every hour for 3 h before being placed into plastic moulds with wax and allowed to set. Samples were sliced into 7 μm sections using a microtome. Sections were stained with Haematoxylin-Eosin, Masson Trichrome, and Elastica Van Gieson and photographed and measured using a Keyence VHX 5000 digital microscope with an RZ00 0–50× zoom lens (for overviews) and a VH-Z100R 100–1000× zoom lens (for collagen and elastic fibres).

Haematoxylin-Eosin staining was conducted according to the manufacturer's instructions. Elastica Van Gieson and Masson Trichrome staining was carried out using kits HT25 and HT15, respectively, from Sigma Aldrich (L'Isle D'Abeau Chesnes F-38297 St. Quentin Fallavier) with few modifications to manufacturer recommendations. Elastica Van Gieson stain was performed after deparaffinization using 10 min exposure to Elastic stain solution. Slides were rinsed in deionized water then exposed to Ferric Chloride Solution. After being differentiated slides were rinsed in deionized water then in 95% alcohol to remove iodine. Slides were rinsed in deionized water then stained in Van Gieson Solution for 2 min, dehydrated in 95% alcohol and mounted. For Trichrome stain, sections were deparaffinized, stained in Haematoxylin for 5 min and washed in deionized water. A second stain was performed in Fuchsin for a duration of 5 min, washed in water then in solution of phosphotungstic-phosphomolybdic acids for 5 min. Final staining was performed in Anilin blue solution for a duration of 5 min, followed by washing for 2 min in acetic acid (1%) then in water before mounting.

First, an overview of the vocal folds was examined using slides from all three stains (Figure 13), with measures of the vocal ligament and the thyroarytenoid muscle derived from the slides stained with Haematoxylin-Eosin. Second, quantitative estimates of the elastic fibre and collagen fibre contents of the vocal ligament was performed in three selected histology slides stained with Elastica Van Gieson. We first intended to measure collagen fibres in the Masson Trichrome slides and elastic fibres in the Elastica Van Gieson slides. It turned out, however, that measuring both fibre types in the Elastica Van Gieson slides yielded more reliable results because in these slides elastic fibres and collagen fibres are differently coloured (black vs. red, respectively) and, hence, can be observed simultaneously. To this end, representative image sections of the mid-part of one vocal fold per

male, including the transversely sectioned vocal ligament, were imported to Photoshop (Adobe Photoshop, CC 2018; Adobe Systems Inc.) on an imac Pro 27″ retina 5k 20 and graphically analysed. The area occupied by the black coloured elastic fibres was estimated in 10 squares of 50 μm edge length, positioned at regions of high fibre concentration, by manually whitening all non-black area and then reading the pixel count in the histogram panel of Photoshop. The average of the 10 squares was used as an estimate of the elastic fibre contents of the vocal ligaments in the three adult males. The same procedure, applied to the reddish or pink coloured collagen fibres in the same three slides and in the same squares, yielded an estimate of the collagen fibre contents of the vocal ligaments in the three adult males. Also, both values provided a ratio of elastic versus collagen fibres in the vocal ligaments of the three adult males.

2.6 | Anatomical comparisons

In order to assess anatomical dimensions between sexes and species which have different relative body sizes, individual measures were normalized by dividing the original measured values by the length of the lower jaw, defined as the distance between the bottom edge of the rostral incisors and the angle of the mandibula in lateral view (Table 2). Lower jaw length is correlated with overall body size (weight or body length) in cervids (Azorit et al., 2003; Blant & Gaillard, 2004; Górecki et al., 2014; Hanzal et al., 2017; Pérez-Barbería et al., 2014; Saether, 1983) and commonly used as a proxy to body size. In our specimens, lower jaw length was strongly correlated with metatarsal length, another traditional proxy of size (Spearman rank correlation: $r = 0.964$, $p < 0.001$).

Anatomical comparisons between the sexes and species will be discussed for both the original measured values (termed 'absolute values') and normalized values relative to lower jaw length (termed 'relative values', denoted by italicized font). We acknowledge that these comparisons are based on the very small sample size of one specimen per sex and species category, with partial data from one additional specimen per category used for confirmatory purposes (Table 2). Nonetheless, the measured anatomical differences represent qualitative differences between species and sexes. To our knowledge, this is the first attempt to simultaneously investigate the vocal anatomy of three species, including both sexes, in such detail.

2.7 | Abbreviations

Ang. styloh.	Angulus stylohyoideus	Stylohyoid angle
App. hyo.	Apparatus hyoideus	Hyoid apparatus
Basih.	Basihyoideum	Basihyoid
Cart. aryt.	Cartilago arytenoidea	Arytenoid cartilage
Cart. cric.	Cartilago cricoidea	Cricoid cartilage

Cart. thyr.	Cartilago thyroidea	Thyroid cartilage
Cart. thyroh.	Cartilago thyrohyoidea	Thyrohyoid cartilage
Corn. rostr.	Cornu rostrale	rostral horn
Ceratoh.	Ceratohyoideum	Ceratohyoid
C1	Vertebra cervicalis 1	1st cervical vertebra
C7	Vertebra cervicalis 7	7th cervical vertebra
Epigl.	Epiglottis	Epiglottis
Epih.	Epihyoideum	Epihyoid
Gl. mand.	Glandula mandibularis	Mandibular gland
Gl. par.	Glandula parotis	Parotid gland
Gl. thyr.	Glandula thyroidea	Thyroid gland
Intsect. tend.	Intersectio tendineae	Tendinous intersection
Lar.	Larynx	Larynx
Lig. thyroh.	Ligamentum thyrohyoideum	Thyrohyoid ligament
Ling.	Lingua	Tongue
Man. sterni	Manubrium sterni	Sternal manubrium
Mand.	Mandibula	Lower jaw
M. cricthyr.	Musculus cricothyroideus	Cricothyroid muscle
M. mass.	Musculus masseter	Masseter muscle
M. omoh.	Musculus omohyoideus	Omohyoid muscle
M. sternmand.	Musculus sternomandibularis	Right sternomandibular
dex.	dexter	muscle
M. sternoh.	Musculus sternohyoideus	Sternohyoid muscle
M. sternthyr.	Musculus sternothyroideus	Sternothyroid muscle
M. styloh.	Musculus stylohyoideus	Stylohyoid muscle
M. thyroh.	Musculus thyrohyoideus	Thyrohyoid muscle
Mm. constr.	Musculi constrictores	Caudal pharyngeal
phar. caudd.	pharyngis caudales	constrictor muscles
Nasophar.	Nasopharynx	Nasal part of pharynx
Nars.	Naris	Nostril
Oesoph.	Oesophagus	Oesophagus
Orophar.	Oropharynx	Oral part of pharynx
Ost. intrphar.	Ostium intrapharyngeum	Intrapharyngeal ostium
Palat. mol.	Palatum molle	Soft palate (Velum)
Palat. dur.	Palatum durum	Hard palate
Phar.	Pharynx	Pharynx
Plic. voc.	Plica vocalis	Vocal fold
Proc. ling.	Processus lingualis	Lingual process
Prom. lar.	Prominentia laryngea	Laryngeal prominence
Stern.	Sternum	Sternum
Styloh.	Stylohyoideum	Stylohyoid
Thyroh.	Thyrohyoideum	Thyrohyoid
Trach.	Trachea	Trachea
Tympoh.	Tympanohyoideum	Tympanohyoid
T1	Vertebra thoracica 1	1st thoracic vertebra
T2	Vertebra thoracica 2	2nd thoracic vertebra

3 | RESULTS

3.1 | Features of the filter

3.1.1 | Laryngeal position

The resting position of the larynx was assessed from CT (Figure 3), MRI (Figure 4) and dissection (Figure 5). The extent of permanent laryngeal descent in the resting position was estimated using dissections as the ratio of the rostral distance from the angle of the mandibula to the laryngeal prominence over the entire distance from the mandibular angle to the sternum (higher number indicates a lower resting position; Figure 6; Table 3).

This ratio was *0.27* (male) and *0.21* (female) in red deer, *0.31* (male) and *0.19* (female) in fallow deer and *0.08* (male) and *0.09* (female) in sika deer. CT and MRI scans show that, accordingly, the resting position of the larynx was at the level of neck vertebrae 3/4 (male) and 2/3 (female) in red deer, 2/3 (male) and 1/2 (female) in fallow deer, and at the level of neck vertebrae 1/2 for male and female sika deer (Figures 3 and 4). It should be noted here that CT, MRI and dissection data tend to underestimate the larynx resting position compared to that in live animals because, after separating the neck from the body, sectioned structures passively contract.

FIGURE 6 Estimation of permanent laryngeal descent as the ratio of the distance from the angle of the mandibula to the laryngeal prominence (red line) over the distance from the angle of the mandibula to the sternal manubrium (yellow line) exemplified in a male Iberian red deer (*C. elaphus hispanicus*) lacking a neck mane (modified from Frey et al., 2012)

TABLE 3 Dimensions of filter-related vocal apparatus anatomy. Measurement values indicate the original measure, taken in millimetres, paired with the normalized value relative to body size (italicized, in parentheses). Relative values were calculated by dividing the original measure by the maximal mandibula length for each specimen. DV = dorsoventral, RC = rostrocaudal, and TV = transverse; L = Left, R = Right

Anatomical variables	Red deer		Fallow deer		Sika deer	
	Male	Female	Male	Female	Male	Female
Maximal mandibula length	291	286	210	188	205	184
Laryngeal position						
Laryngeal prominence to sternum	480 (*1.65*)	480 (*1.68*)	370 (*1.76*)	320 (*1.70*)	445 (*2.17*)	395 (*2.15*)
Laryngeal prominence to angle of mandibula	180 (*0. 62*)	130 (*0.45*)	165 (*0.79*)	75 (*0.40*)	40 (*0.20*)	40 (*0.22*)
Vocal tract length						
Oral VTL (resting position)	583 (*2.00*)	435 (*1.52*)	405 (*1.93*)	305 (*1.62*)	278 (*1.36*)	275 (*1.49*)
Nasal VTL (resting position)	653 (*2.25*)	498 (*1.74*)	453 (*2.16*)	338 (*1.80*)	331 (*1.62*)	293 (*1.59*)
Oral VTL (maximal extension)	680 (*2.34*)	487 (*1.70*)	510 (*2.43*)	345 (*1.84*)	313 (*1.53*)	285 (*1.55*)
Nasal VTL (maximal extension)	NA	563 (*1.97*)	583 (*2.78*)	400 (*2.13*)	NA	NA
Ratio of the relative maximal extension/resting oral VTL	1.17	1.12	1.26	1.14	1.13	1.04
Hyoid apparatus and thyrohyoid ligament						
Tympanohyoid, DV length	10 (*0.03*)	6 (*0.02*)	4 (*0.02*)	5 (*0.03*)	2 (*0.01*)	2 (*0.01*)
Stylohyoid, DV length	132 (*0.45*)	115 (*0.40*)	88 (*0.42*)	73 (*0.39*)	80 (*0.39*)	67 (*0.36*)
Stylohyoid angle, RC length	38 (*0.13*)	37 (*0.13*)	22 (*0.11*)	19 (*0.10*)	23 (*0.11*)	20 (*0.11*)
Epihyoid, DV length	40 (*0.14*)	30 (*0.11*)	17 (*0.08*)	12 (*0.06*)	13 (*0.06*)	12 (*0.07*)
Ceratohyoid, DV length	52 (*0.18*)	45 (*0.16*)	25 (*0.12*)	17 (*0.09*)	23 (*0.11*)	18 (*0.10*)
Basihyoid, maximum TV width	40 (*0.14*)	33 (*0.12*)	23 (*0.11*)	18 (*0.10*)	20 (*0.10*)	15 (*0.08*)
Basihyoid, maximum RC length	14 (*0.05*)	11 (*0.04*)	8 (*0.04*)	5 (*0.03*)	7 (*0.03*)	7 (*0.04*)
Thyrohyoid, total RC length	65 (*0.22*)	60 (*0.21*)	47 (*0.22*)	35 (*0.19*)	32 (*0.16*)	30 (*0.16*)
Thyrohyoid cartilage	28 (*0.10*)	14 (*0.05*)	8 (*0.04*)	5 (*0.03*)	7 (*0.03*)	7 (*0.04*)
Thyrohyoid ligament length (resting position)	80 (*0.27*)	20 (*0.07*)	70 (*0.33*)	10 (*0.05*)	2 (*0.01*)	2 (*0.01*)
Thyrohyoid ligament length (maximal extension)	200 (*0.69*)	50 (*0.17*)	100 (*0.48*)	30 (*0.16*)	4.5 (*0.02*)	4.5 (*0.02*)
Resilience (ratio of the relative maximal extension/resting thyrohyoid ligament length)	2.56	2.43	1.45	3.20	2.00	2.00
Hyoid muscles						
Thyrohyoid muscle length	200 (*0.69*)	145 (*0.51*)	145 (*0.69*)	75 (*0.40*)	45 (*0.22*)	50 (*0.27*)
Sternohyoid muscle length	460 (*1.58*)	450 (*1.57*)	360 (*1.71*)	L: 425 (*2.26*) R: 330 (*1.76*)	420 (*2.05*)	365 (*1.98*)
Sternothyroid muscle length	430 (*1.48*)	440 (*1.54*)	340 (*1.62*)	315 (*1.68*)	420 (*2.05*)	365 (*1.98*)
Stylohyoid muscle length	155 (*0.53*)	120 (*0.42*)	100 (*0.48*)	90 (*0.48*)	85 (*0.41*)	65 (*0.35*)
Stylothyroid muscle length	240 (*0.82*)	185 (*0.65*)	180 (*0.86*)	90 (*0.48*)	84 (*0.41*)	70 (*0.38*)
Hyoepiglottic muscle length resting	185 (*0.64*)	95 (*0.33*)	75 (*0.36*)	50 (*0.27*)	40 (*0.20*)	35 (*0.19*)

As a result of the lacking caudal tension the larynx slightly ascends and, thus, rests a bit higher than in the live animal. Dissections revealed a clear difference in larynx resting position between red and fallow deer on the one hand and sika deer on the other. Among males, there was little apparent interspecific difference in the relative resting laryngeal position between red and fallow deer. In contrast, the larynx of the male sika deer exhibits a low, if any, degree of descent in comparison to the other species. This pattern is repeated in the female specimens, but with a smaller degree of difference in laryngeal descent between the female sika deer and both the female red and fallow deer. Larynx resting position was considerably lower in males than in females in red and fallow deer, but not in sika deer who showed almost no difference in resting position. The intersexual difference of larynx resting position was largest in fallow deer.

3.1.2 | Vocal tract length

Averaged across all specimens, the absolute nasal vocal tract length (VTL) was 1.13 ± 0.04 (Mean ± SD) times longer than the absolute oral VTL (Table 3).

Amongst males, the relative resting oral and nasal VTL was longest in red deer (oral, nasal: *2.00, 2.25*) and fallow deer (*1.93, 2.16*) and smallest in the sika deer (*1.36, 1.62*; Table 3). Amongst females, there were smaller differences of relative resting oral and nasal VTL: ranging from *1.62* and *1.80* in fallow deer, via 1.52 and 1.74 in red deer, to *1.49* and *1.59* in sika deer (Table 3). Large sexual dimorphisms of relative resting oral and nasal VTL were present in red and fallow deer, with males having considerably longer resting oral and nasal VTLs than females, while only minor differences were noted between the sexes in sika deer (Table 3).

Interspecific and intersexual differences of the relative maximally extended oral VTL (i.e., after the larynx was maximally descended in the caudal direction) resembled the pattern of the resting oral and nasal VT. Amongst males, the relative maximally extended oral VT was longest in fallow deer (*2.43*) and red deer (*2.34*) and shortest in sika deer (*1.53*) while differences between females were less pronounced: from *1.84* in fallow deer, via 1.70 in red deer, to *1.55* in sika deer (Table 3). Strong sexual dimorphisms of the maximally extended normalized oral VT were present in red and fallow deer but not in sika deer. Maximal extension measures for nasal VTL were not available for all specimens (see Table 3).

The greatest increase in oral VTL during maximal extension [absolute and relative (in italics)] occurred in male red deer, 97 mm (*0.33*), and male fallow deer, 105 mm (*0.50*), representing a 1.17 fold and a 1.26 fold increase (ratio of relative maximal extension length/resting length) in oral VTL, respectively (Table 3). Female red and fallow deer showed smaller increases in oral VTL during extension: 52 mm (*0.18*) and 40 mm (*0.21*), respectively, representing 1.12 and 1.14 fold increases in oral VTL. Sika deer showed the smallest absolute and relative increases in oral VTL during extension, with the male increasing 35 mm (*0.17*) and the female increasing 10 mm (*0.05*). Similar to the pattern observed in red and fallow deer, the male sika deer exhibited a larger increase in oral VTL during maximal extension than the female sika deer (male, female: 1.13, 1.04 fold increases, Table 3).

3.1.3 | Larynx descent and mobility in females

Larynx descent and mobility is not restricted to the males of red and fallow deer. Though to a much lesser extent than in the males, the resting position of the larynx, often visible in the live animals, is also lowered in female red and fallow deer. Furthermore, when producing alarm and contact calls, female red and fallow deer, though less pronounced than the males, also visibly descend the larynx (D. Reby, R. Frey, personal observations), thereby extending the vocal tract. This restricted laryngeal descent and mobility in the females can be expected to affect call acoustics in a way similar to males.

3.1.4 | Hyoid apparatus and thyrohyoid ligament

The hyoid apparatus in all six specimens (Figure 7) corresponded to the basic hyoid structure in ruminants. Measurements of the individual parts of the hyoid apparatus are provided in Table 3. Overall there is a close correspondence of the hyoid apparatuses between the species and the sexes, qualitatively (Figure 7) and regarding the normalized values of the measurements (Table 3). However, one major difference exists between the two species with pronounced larynx
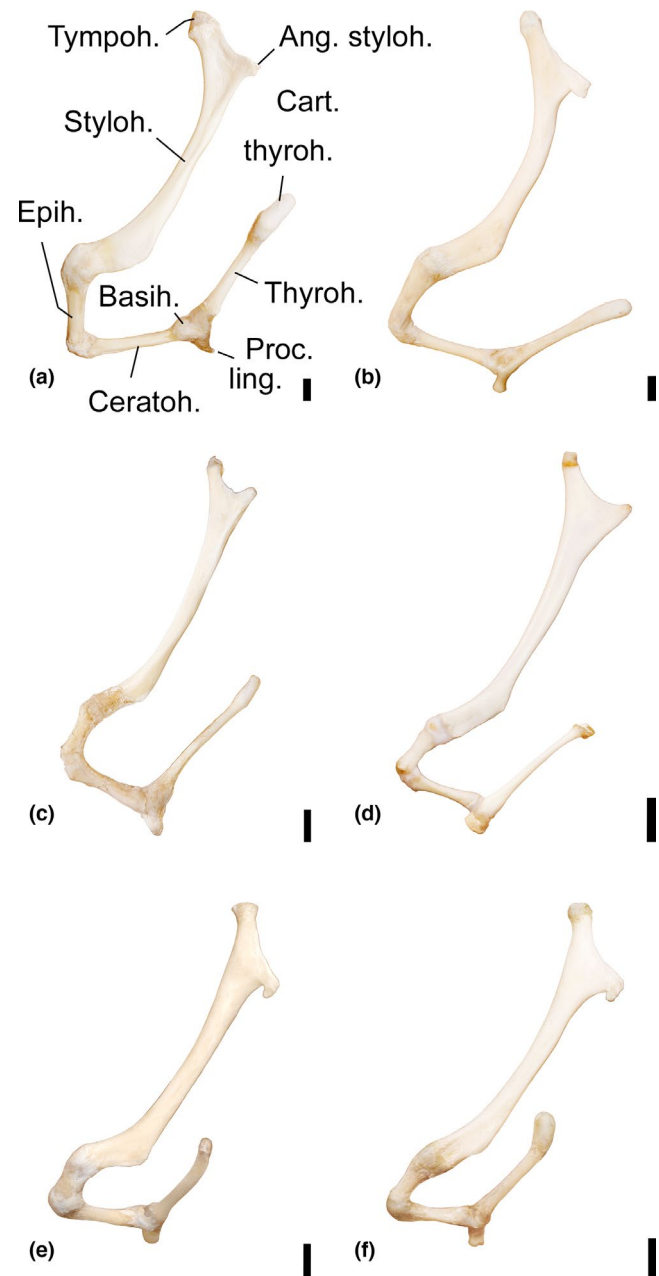

FIGURE 7 Hyoid apparatuses of male (a) and female (b) red deer, male (c) and female (d) fallow deer, and male (e) and female (f) sika deer, left lateral view. Not to scale, all specimens brought to the same size, approximately. Scale bars 10 mm, respectively

retraction and the one without. The total rostrocaudal length of the thyrohyoid is relatively longer in the males of red and fallow deer than in the sika deer male (*0.22* and *0.22* vs. *0.16*, respectively, Table 3). The normalized values of the thyrohyoid length of the females show a similar trend. A minor qualitative difference regards the shape of the stylohyoid angle in the three species. In both sexes of red and sika deer the stylohyoid angle is broad, rectangular and directed caudally whereas in both sexes of fallow deer it is rather pointed and directed dorsally towards the occipitohyoid muscle. In both sexes of sika deer there is a small osseous knob of the stylohyoid angle directed ventrally at the origin of the stylohyoid muscle (Figure 7).

The resting length and maximally extended length of the thyrohyoid ligament are in accordance with respective resting and maximally retracted positions of the larynx (see below), at both intra- and inter-specific levels. In the red and fallow deer specimens, the absolute and relative length (in italics) of the thyrohyoid ligament was considerably larger in males than in females for both the resting state (male vs. female: 80 mm (*0.27*) vs. 20 mm (*0.07*) in red deer; 70 mm (*0.33*) vs. 10 mm (*0.05*) in fallow deer) and the extended state (male vs. female: 200 mm (*0.69*) vs. 50 mm (*0.17*) in red deer; 100 mm (*0.48*) vs. 30 mm (*0.16*) in fallow deer; Table 3). The red deer male has a substantially longer relative thyrohyoid ligament length in the extended state than the fallow deer male while in red and fallow females the relative thyrohyoid ligament length in the resting and extended states are similar.

In strong contrast with these two species, there was only a very limited elasticity and no sexual dimorphism of the thyrohyoid ligament in the sika deer specimens in either the resting or extended state: thyrohyoid ligament length was 2 mm for both sexes in the resting state and 4.5 mm for both sexes in the maximally extended state. In contrast with the red and fallow deer, there was a slight overlap between the rostral horn of the laryngeal thyroid cartilage and the caudal tip of the thyrohyoid, that is, no rostrocaudal distance between these two structures, during the resting state in male and female sika deer.

The relative resting length of the thyrohyoid ligament is greatest in male fallow deer (*0.33*) but coincides with the lowest resilience (ratio of relative maximal extension/resting length: 1.45, Table 3). The relative resting length of the thyrohyoid ligament in female fallow deer is sixfold shorter than that of the male (male, female: *0.33*, *0.05*) but has the highest resilience of any specimen (3.00). Despite a comparable difference in the relative thyrohyoid ligament resting lengths in the male and female red deer (male, female: *0.27*, *0.07*), both sexes of this species showed about the same level of resilience (male, female: 2.56, 2.43). Both sexes of sika deer had the same relative thyrohyoid ligament resting length (male, female: *0.01*, *0.01*) and resilience (male, female: 2.00, 2.00, Table 3).

3.1.5 | Hyoid muscles

In general, the resting length of the infrahyoid strap muscles (Figure 8), were consistent with species and sex differences in the resting position of the larynx. See Table S1 for weights of external laryngeal muscles.

The absolute and relative length (in italics) of the thyrohyoid muscle, connecting the lamina of the thyroid cartilage to the thyrohyoid of the hyoid apparatus, was substantially longer in the male than in the female red deer (male vs. female: 200 mm (*0.69*) vs. 145 mm (*0.51*)) and fallow deer (male vs. female: 145 mm (*0.69*) vs. 75 mm (*0.40*)) but slightly shorter in the male than the female sika deer (male vs. female: 45 mm (*0.22*) vs. 50 mm (*0.27*); Table 3). Interspecifically, the relative thyrohyoid muscle lengths were similar in male red and fallow deer, but considerably shorter in male sika deer. Between females, the relative thyrohyoid muscle length was longest in red deer and smallest in sika deer.

In contrast with most mammals (Table S2), the termination site of the sternohyoid muscle, ancestrally connecting the sternal manubrium to the basihyoid, is shifted caudally from the basihyoid to the thyroid cartilage in all three investigated species. In other words, the sternohyoid muscle has lost its connection to the basihyoid. Topographically, it is therefore no longer a 'hyoid' muscle. Functionally, this transforms the sternohyoid muscle into an "additional" sternothyroid muscle, and, thereby, increases the muscle power available for retracting the larynx away from the hyoid apparatus.

The changed termination of the sternohyoid muscle was at the ventrocaudal edge of the thyroid cartilage covering the origin of the thyropharyngeal muscle, one of the caudal pharyngeal constrictors. Remarkably, the female fallow deer exhibited a left/right asymmetry of the termination of the sternohyoid muscle: the right muscle, as in the other specimens, inserted onto the ventrocaudal edge of the thyroid cartilage covering the origin of the thyropharyngeal muscle. The left muscle, however, proceeded rostrally up to the basihyoid as a very narrow and thin muscular band (Figure 8d).

In red and sika deer the rostral portion of the sternohyoid muscle fuses with the sternothyroid muscle and terminates on the thyroid cartilage in both sexes. If the short rostral lobe in red deer that projects rostrally from the fused portion (Figure 8a,b) represents the rostral end of the sternohyoid muscle, the length of the sternohyoid muscle would slightly exceed that of the sternothyroid muscle in red deer whereas the length of both muscles are identical in sika deer (Figure 8e,f; Table 3). In fallow deer, the rostral portion of the sternohyoid muscle is separate from and slightly longer than the rostral portion of the sternothyroid muscle in both sexes, except the left sternohyoid muscle in the female, which reaches the basihyoid and, thus, is pronouncedly longer than the rostral portion of the sternothyroid muscle. The caudal portion of the sternohyoid muscle is fused to the caudal portion of the sternothyroid muscle in all three species and sexes.

In all species and sexes, the sternothyroid muscle connected the sternum to the lamina of the thyroid cartilage, caudally adjacent to the origin of the thyrohyoid muscle. The caudal portions of the sternohyoid and sternothyroid muscles are fused (except for a cleft from 100 to 175 mm rostral to the sternum in the female sika deer) and separated from the rostral portions by a transverse tendinous
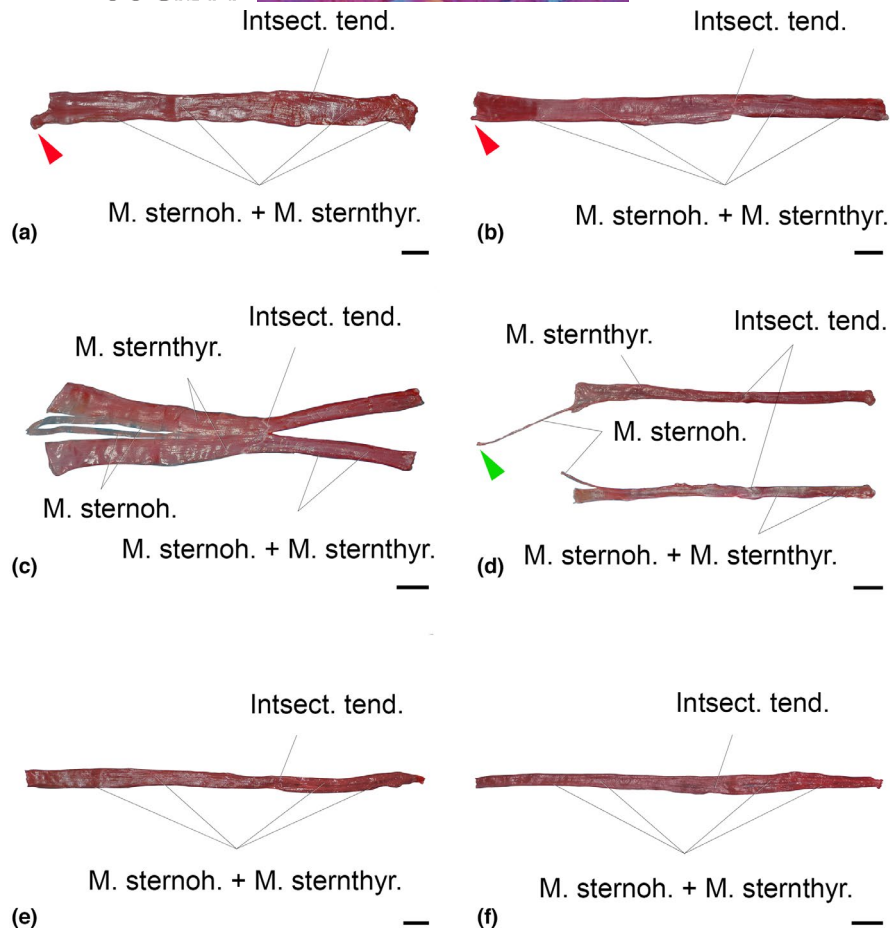
FIGURE 8 Left infrahyoid strap muscles (a, b, d, e, f) in left lateral view and left and right strap muscles (c) in ventral view of male (a) and female (b) red deer, male (c) and female (d) fallow deer, and male (e) and female (f) sika deer. In the male fallow deer the muscles of both sides were removed simultaneously and demonstrate the full set of the main retractors of the larynx. In all species and sexes the caudal portions of the sternohyoid and sternothyroid muscles are fused and separated from the rostral portions by a transverse tendinous intersection. The rostral portions of the sternohyoid and sternothyroid muscles are mostly separate in fallow deer but inseparably fused in red and sika deer. Remarkably, the left sternohyoid muscle of the female fallow deer retained its ancestral termination on the basihyoid (green arrowhead). In male and female red deer a slender ventral bundle possibly represents the termination of the sternohyoid muscle (red arrowheads). Scale bars 30 mm, respectively

intersection. As mentioned, the rostral portions of the sternohyoid and sternothyroid muscles are mostly separate in fallow deer but inseparably fused in red and sika deer. In male and female red deer, a slender ventral bundle reaches a bit more rostrally than the remaining muscle, possibly representing the termination of the sternohyoid muscle (Figure 8a,b). In sika deer, there was no structural distinction between the sternothyroid and the sternohyoid muscles and, consequently, no measurable difference between sternohyoid and sternothyroid muscle length (Figure 8e,f; Table 3).

The relative length of the sternothyroid muscle in the resting position was slightly longer in the female than in the male red deer (male, female: *1.48*, *1.54*) and fallow deer (male, female: *1.62*, *1.68*) but slightly longer in the male than in the female sika deer (male, female: *2.05*, *1.98*; Table 3).

In red and fallow deer, the relative <u>sternohyoid muscle</u> length was almost identical in red deer (male, female: *1.58*, *1.57*) but longer in the female than in the male fallow deer (male, female: *1.71*, *1.76* on right side and *2.26* on left side). The much larger value on the left side is a consequence of the ancestral termination of the left sternohyoid muscle in female fallow deer (see above). In both sexes of red and fallow deer, sternohyoid muscle length was a bit larger than sternothyroid muscle length because the rostral portion of the sternohyoid muscle exceeded that of the sternothyroid muscle (provided the rostroventral lobe in red deer is correctly taken as the rostral end of the sternohyoid muscle – Figure 8). In sika deer, the lengths of the sternohyoid and sternothyroid muscle are identical because a structural distinction between the two muscles was not possible (male, female: *2.05*, *1.98* in relative length, Table 3).

The longer relative lengths of the sternohyoid and sternothyroid muscles in the sika specimens compared to the fallow and red deer specimens reflects the higher resting position of the larynx in male and female sika deer compared to the more descended larynx resting position in the two other species. Between sexes, the slightly

longer relative sternothyroid muscle lengths in female red and fallow deer arises from the more pronounced larynx descent in the males entailing a shortening of the relative sternothyroid muscle length. In sika deer, relative sternothyroid muscle length is slightly longer in the male than in the female, indicating a slightly lower relative larynx position in the female.

In contrast with most other mammals, the caudal stylopharyngeal muscle in ruminants has shifted its termination from the wall of the pharynx to the dorsal edge of the thyroid cartilage lamina (Constantinescu & Schaller, 2012, p. 152). Therefore, it might be better called a "stylothyroid muscle" in ruminants. Owing to the establishment of a direct muscular connection between the stylohyoid and the larynx, this muscle can stabilize the resting position of the larynx and assist in protraction of the larynx. At larynx retraction, distension of this muscle will exert a caudally directed pulling force on the stylohyoid and, thereby, assist in extension of the hyoid apparatus. The stylohyoid muscle connects the angle of the stylohyoid to the basihyoid and, thus, can be expected to be involved in the regulation of the expanse of the pharynx. The stylohyoid and stylothyroid (caudal stylopharyngeal) muscles, connecting the stylohyoid to, respectively, the basihyoid and thyroid cartilage, were measured in the resting position of larynx and hyoid apparatus.

The longer relative length of the stylothyroid (caudal stylopharyngeal) muscle in the red deer (male, female: 0.82, 0.65) and fallow deer (0.86, 0.48) compared to sika deer (male, female: 0.41, 0.38) is in accordance with the lower resting position of the larynx in the former and the high resting position of the larynx (at the level of C1) in sika deer (Table 3). Between sexes, the difference in the relative length of the stylothyroid muscle in red and fallow deer reflects the pronounced descent of the larynx in the males (longer stylothyroid muscle) and the less pronounced larynx descent in the females (shorter stylothyroid muscle). In sika deer, the relative values of male and female are close and small because larynx length and position are almost identical and there is no larynx descent in either sex (short stylothyroid muscle).

The length of the stylohyoid muscle might be indicative of the potential for dorsoventral expansion of the pharynx. Relative values of stylohyoid muscle length were higher in red deer (male, female: 0.53, 0.42) and fallow deer (male, female: 0.48, 0.48) than in sika deer (male, female: 0.41, 0.35). Relative stylohyoid muscle lengths differed considerably between male and female red deer but did not differ between male and female fallow deer. As in red deer, though less pronounced, relative stylohyoid muscle length in sika deer is larger for the male than for the female. It appears, therefore, that the production of roars in male red deer would require the highest expansion of the pharynx, while the groaning of male fallow deer involves less pharynx expansion. Least expansion seems to occur in the moaning of male sika deer. The larger relative stylohyoid muscle lengths of red and sika deer males suggests higher potential for pharynx expansion than in the females, whereas this does not hold for fallow deer. See Table S3 for measurements of the pharynx in resting and maximal extension positions.

Connecting the hyoid apparatus to the epiglottis, the relative length of the hyoepiglottic muscle was markedly longer in the male than in the female red deer (0.64 vs. 0.33) and fallow deer (0.36 vs. 0.27) but not in sika deer (0.20 vs. 0.19). Interspecific comparisons were similar within each sex: The relative hyoepiglottic muscle length was longest in red deer, shortest in sika deer and intermediate in fallow deer. Considering the similarly expressed laryngeal descent in male red and male fallow deer, the much shorter length of the hyoepiglottic muscle in male fallow deer is surprising. This might arise from a different position of the epiglottis, a large laryngeal vestibulum and a high resilience of the epiglottis/thyroid cartilage connection (see Section 4). In the male and female sika deer the relative length of the hyoepiglottic muscle was particularly short.

3.1.6 | Head dimensions and proportions

When taking the length of the lower jaw as a proxy of body size and the condylobasal length of the skull as a proxy for head size (Figure 9) for evaluating relative head size, no pronounced clear-cut differences between the species emerged (Table 4, Line 12). Relative head size of the males was almost identical. Relative head size of the sika and fallow female were close while the relative head size of the female red deer was moderately larger (longer). This is supported by the ratio of male to female lower jaw length: red deer 1.02, fallow deer 1.12, sika deer 1.11 (Table 3).

Within species, relative head size (length) of the females was throughout a bit larger than that of the males (Table 4, Line 12).
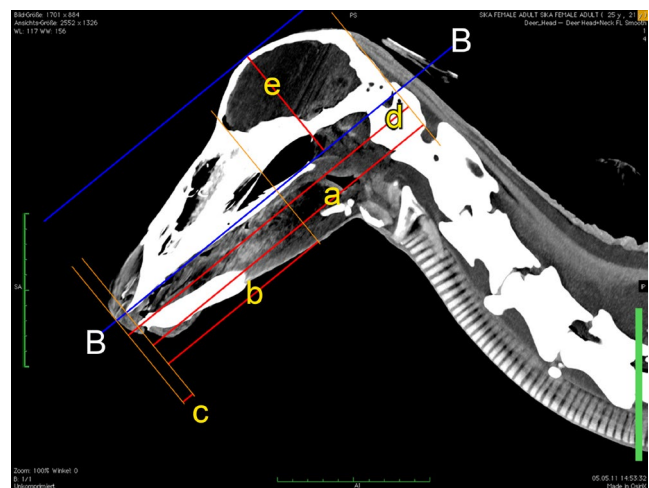

FIGURE 9 Head dimensions and proportions. This figure illustrates the distances taken for the comparison between the sexes and the species (see Table 4). B–B: baseline; a: condylobasal length; b: osseous snout length; c: length of nasal soft tissue rostral to incisive bone; d: condylobasal length plus rostral soft tissue length; e: maximal height of the skull; background: CT scan of sika female W2. Scale bars 100 mm

TABLE 4 Head dimensions and proportions. Skull and nose distances (mm): Lines 1–5; relative skull proportions (%): Lines 6–9; and head size relative to body size: Lines 10–12. See Figure 9 for visualization of distance measures

Line	Measure	From	To	Red deer Male U4	Red deer Female BB2	Fallow deer Male U5	Fallow deer Female Y1	Sika deer Male O8	Sika deer Female W2
1	Distance a	Rostral tip incisive bone	Caudal edge occipital condyle	373.4	340.5	278.1	241.4	262.4	228.1
2	Distance b	Rostral tip incisive bone	Rostral edge cranial cavity	237.8	213.1	165.1	145.3	148.4	126.7
3	Distance c	Tip of nose (soft tissue)	Rostral tip incisive bone	14.2	17.3	12.6	5.6[a]	7.3	8.3
4	Distance d	Tip of nose (soft tissue)	Caudal edge occipital condyle	387.8	358	291	247.1	269.7	236.6
5	Distance e	Base line skull	Most dorsal point skull	123.9	101	95	89.7	96.3	79.1
6	Ratio b:a			63.7	62.6	59.4	60.2	56.5	55.5
7	Ratio c:a			3.8	5.1	4.5	2.3	2.8	3.6
8	Ratio e:a			33.2	29.7	34.2	37.2	36.7	34.7
9	Ratio (b+c):d			65	64.4	61.1	61.1	57.7	57.1
10	Length of mandibula M (proxy of body size)	Bottom edge rostral incisors	Angle of mandibula	291	286	210	188	205	184
11	Distance a (proxy of head size)	As above	As above	373.4	340.5	278.1	241.4	262.4	228.1
12	Ratio M:a			0.78	0.84	0.76	0.78	0.78	0.81

[a]This value appears to be unrealistically low because of a compression of the nostril region in this specimen.

The relative height of the skull was 30%–37% of the condylobasal length for all specimens without a pronounced clear-cut trend between species or between the sexes (Table 4, Line 8). The relative length of the soft tissue between the rostral tip of the incisive bone and the tip of the nose ("soft snout") was ≈3%–5% when excluding the female fallow deer because of a compression of its nostril region.

In contrast with these estimates, a clear-cut trend in skull proportions, that is, the length of the snout region compared to the length of the entire skull or head, emerged. Both the ratio of the osseous snout length (distance b) to the skull length (distance a) and the ratio of the overall snout length (distances b + c) to the head length (distance d) produced the same result (Figure 9). The length of the snout region is similar in male and female conspecifics but differs between species (Table 4, Lines 6 and 9). Sika deer male and female have the shortest snout region, red deer male and female the longest while fallow deer male and female are intermediate.

3.2 | Features of the source

3.2.1 | Laryngeal morphology

Figures 10 and 11 present rescaled photographs of the excised, sagittally sectioned larynx and of the laryngeal cartilages that enable general interspecific and intersexual comparisons of larynx morphology. The overall shape of the male red deer larynx is horizontal-rectangular or landscape-like whereas the overall shape of the male fallow deer larynx is short, deep, upright-rectangular or portrait-like. The overall shape of the male sika deer larynx is compressed horizontal-rectangular and, hence, more or less quadratic (Figure 10). The overall shape of the female larynges is more uniform than that of the males and can be described as being horizontal-rectangular or landscape-like (Figure 10).

The difference in the overall shape of the male larynges is reflected in the shape of the thyroid cartilage (Figure 11) The rostral horns of red and fallow deer are long and thin while they are short
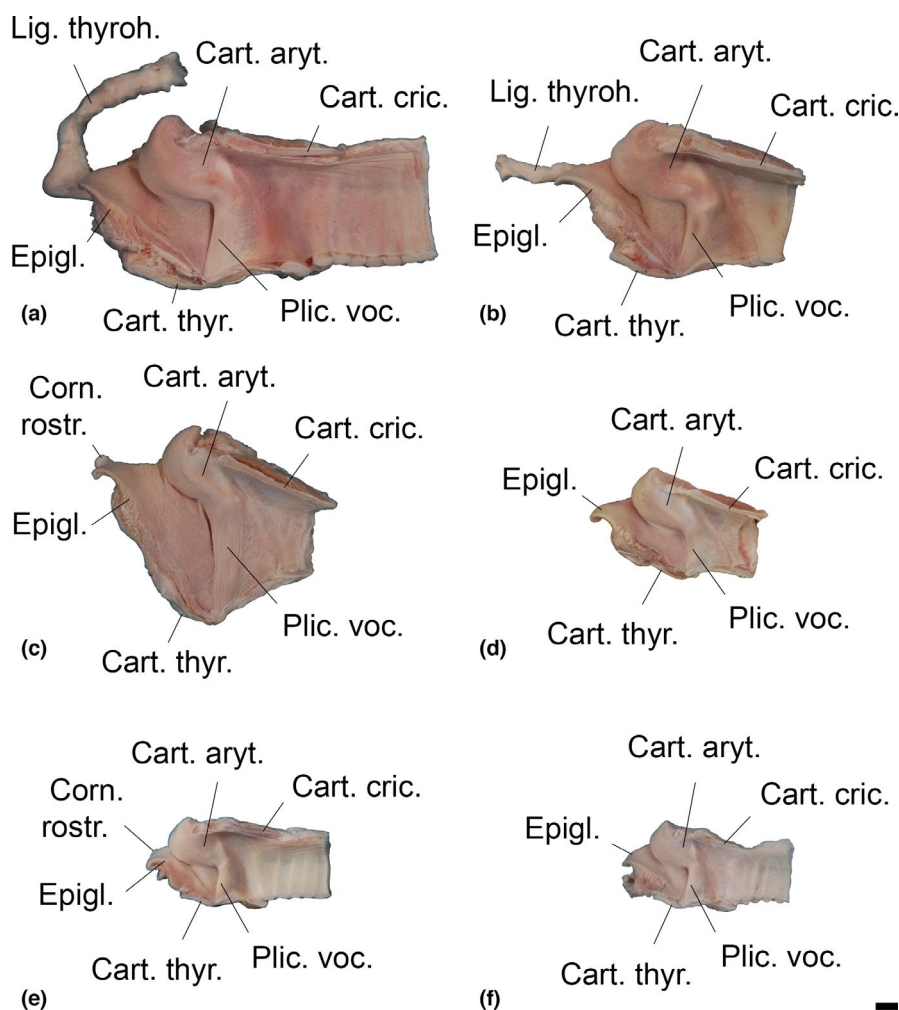

FIGURE 10 Right halves of the excised larynges, medial view, of male (a) and female (b) red deer, male (c) and female (d) fallow deer, and male (e) and female (f) sika deer. Subfigures to scale, scale bar 10 mm. Slight sexual dimorphism of larynx size in red deer, no sexual dimorphism in sika deer and pronounced sexual dimorphism in fallow deer by evolutionary enlargement of the male larynx
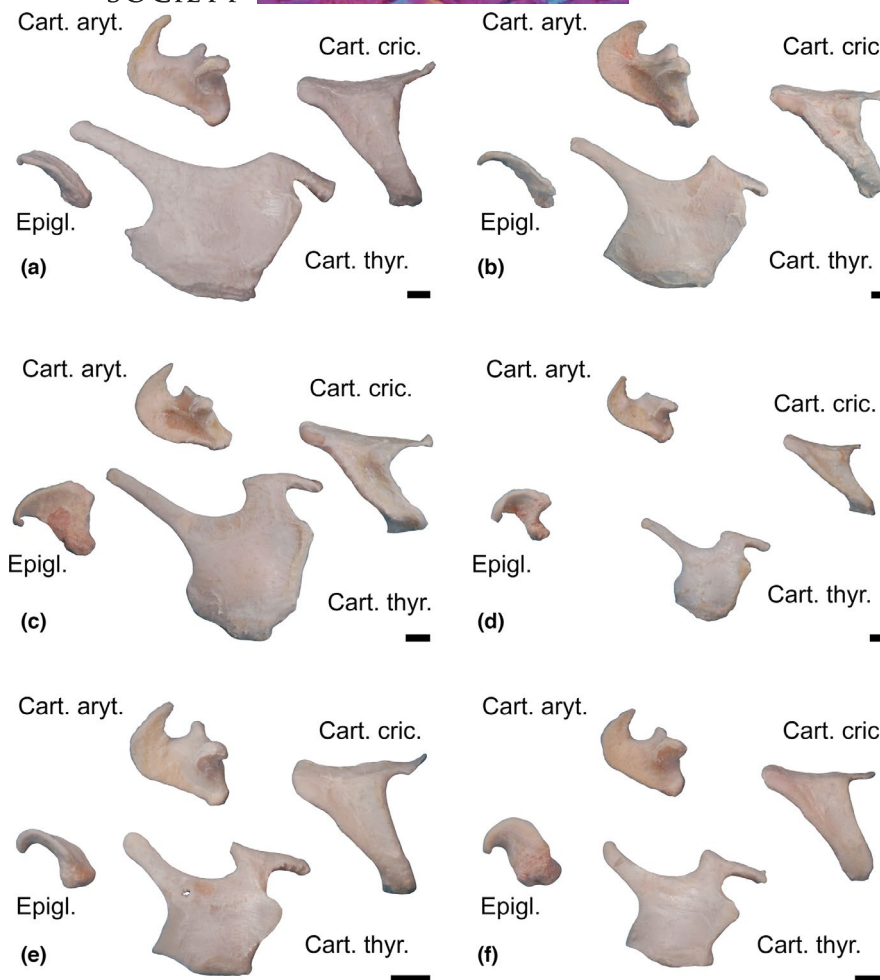
FIGURE 11 The laryngeal cartilages of male (a) and female (b) red deer, male (c) and female (d) fallow deer, and male (e) and female (f) sika deer. The cartilages are not to scale between species but they are to scale between the sexes allowing the depiction of sex-related size differences. Sexual dimorphism increases from sika deer, via red deer to fallow deer. All scale bars 10 mm

and stout in sika deer (Figure 11). Qualitatively, sexual dimorphism in larynx shape appears minimal in sika deer and small in red deer, but extreme in fallow deer as the male fallow deer exhibits a strongly enlarged larynx (Figures 10 and 11; Table 5).

The angle between the laminae of the thyroid cartilage was measured along the outside edges of the thyroid laminae in a virtual transverse section based on the CT-scan of each specimen. The viewing slice for the measure was aligned through the rostral portion of the vocal folds. The angle between the thyroid laminae was similar in the male and female red deer and the female fallow deer (respectively, 49.4°, 42.2° and 45.1°). In comparison, the angle was larger in the male and female sika deer (respectively, 66.2° and 59.7°) but much smaller in the male fallow deer (21.9°). There was little sexual dimorphism in the thyroid laminae angle size in red deer and sika deer, although males did exhibit slightly larger angles than the females. In contrast, fallow deer showed a large sexual dimorphism in thyroid laminae angle, with the angle in the male specimen less than half that of the female.

In the course of the excision, handling and dissection of the larynges, we gained the impression that the ossification state of the larynges differed between species and between male and female fallow deer. The larynx of the fallow deer male was highly flexible/pliable, whereas the larynges of the sika deer male and female were highly rigid. The larynges of the fallow deer female and of male and female red deer were of intermediate flexibility. These assumed differences in the ossification of the larynges could not be quantified here but might be quantifiable by applying other methods, for example, calculation of attenuation numbers (Hounsfield units) in CT datasets (cf. Schreiber et al., 2011). Probably, the different laryngeal flexibilities have functional relevance in terms of respective vocal production modes (see Section 4).

3.2.2 | Laryngeal dimensions

Relative larynx size differed dramatically between species. Relative to body size male fallow deer have the largest larynx by far in all specimens examined. Among males, relative larynx size is largest in fallow deer, intermediate in red deer and smallest in sika deer (fallow deer vs. red deer vs. sika deer: relative ventral length = 0.38 vs. 0.29

TABLE 5 Dimensions of source-related vocal apparatus anatomy. Measurement values indicate the original measure, taken in millimetres, paired with the normalized value relative to body size (italicized, in parentheses). Relative values were calculated by dividing the original measure by the maximal mandibula length for each specimen. DV = dorsoventral, RC = rostrocaudal, and TV = transverse; L = Left, R = Right, T = Total

Anatomical variables	Red deer		Fallow deer		Sika deer	
	Male	Female	Male	Female	Male	Female
Maximal mandibula length	291	286	210	188	205	184
Laryngeal dimensions (excised)						
Overall ventral length of larynx (thyroid incisura to caudal end of cricoid)	85 (0.29)	80 (0.28)	80 (0.38)	48 (0.26)	48 (0.23)	40 (0.22)
Overall lateral length of larynx (most laterorostral to caudal end of cricoid)	130 (0.45)	115 (0.40)	115 (0.55)	70 (0.37)	70 (0.34)	50 (0.27)
Maximal height of larynx (laryngeal prominence to most dorsal part)	80 (0.27)	70 (0.24)	85 (0.40)	45 (0.24)	35 (0.17)	40 (0.22)
Distance between thyroid prominence and rostral edge of cricoid cartilage (internally)	35 (0.12)	34.5 (0.12)	40 (0.19)	21 (0.11)	12 (0.06)	8 (0.04)
Glottis, total DV length[a]	60.5 (0.21)	54.8 (0.19)	69.3 (0.33)	35.6 (0.19)	32.7 (0.16)	30.8 (0.17)
Glottis, intermembraneous DV length[a]	34.8 (0.12)	31.7 (0.11)	52.5 (0.25)	18.5 (0.10)	16.3 (0.08)	17.5 (0.10)
Glottis, intercartilaginous DV length[a]	25.7 (0.08)	23.1 (0.08)	16.8 (0.08)	17.1 (0.09)	16.4 (0.08)	13.3 (0.07)
Ratio of intermembraneous/ intercartilaginous DV length[a]	1.35	1.37	3.13	1.08	0.99	1.32
Laryngeal cartilages						
Epiglottis, max RC length	43 (0.15)	35 (0.12)	45 (0.21)	35 (0.19)	30 (0.15)	25 (0.14)
Epiglottis, max TV width	L: 28 (0.10) T: 56 (0.19)	R: 21 (0.07) T: 42 (0.15)	R: 21 (0.10) T: 42 (0.20)	L: 18 (0.10) T: 36 (0.19)	R: 13 (0.06) T: 26 (0.13)	L: 13 (0.07) T: 26 (0.14)
Cart. thyr., max RC length	120 (0.41)	88 (0.31)	90 (0.43)	55 (0.29)	49 (0.24)	44 (0.24)
Cart. thyr., max DV height	70 (0.24)	60 (0.21)	72 (0.34)	33 (0.18)	38 (0.19)	32 (0.17)
Cart. thyr. max. ventral length lamina (straight)	56 (0.19)	46 (0.16)	35 (0.17)	21 (0.11)	26 (0.13)	23 (0.13)
Cart. thyr. max length rostral horn	55 (0.19)	44 (0.15)	52 (0.25)	28 (0.15)	23 (0.11)	19 (0.10)
Cart. cric. max DV length	77 (0.26)	67 (0.23)	67 (0.32)	47 (0.25)	45 (0.22)	39 (0.21)
Cart. cric., max RC length	60 (0.21)	55 (0.19)	56 (0.27)	31 (0.16)	35 (0.17)	30 (0.16)
Cart. aryt., max RC length	51 (0.18)	53 (0.19)	42 (0.20)	31 (0.16)	30 (0.15)	25 (0.14)
Cart. aryt. max DV height	30 (0.10)	30 (0.10)	25 (0.12)	18 (0.10)	18 (0.09)	15 (0.08)
Intrinsic laryngeal muscles						
M. arytenoideus transversus, max TV width	22 (0.08)	26 (0.09)	13 (0.06)	10 (0.05)	14 (0.07)	14 (0.08)
M. arytenoideus transversus, max RC length	25 (0.09)	15 (0.05)	13 (0.06)	15 (0.08)	18 (0.09)	8 (0.04)
M. cricothyroideus, max DV length	60 (0.21)	47 (0.16)	43 (0.20)	32 (0.17)	35 (0.17)	30 (0.16)
M. cricothyroideus, max RC length	45 (0.15)	31 (0.11)	26 (0.12)	20 (0.11)	23 (0.11)	20 (0.11)
M. cricoarytenoideus dorsalis, max TV width	26 (0.09)	25 (0.09)	15 (0.07)	11 (0.06)	14 (0.07)	14 (0.08)
M. cricoarytenoideus dorsalis, max RC length	60 (0.21)	53 (0.19)	58 (0.28)	33 (0.18)	32 (0.16)	30 (0.16)
M. cricoarytenoideus lateralis, max DV length	45 (0.15)	35 (0.12)	40 (0.19)	25 (0.13)	31 (0.15)	26 (0.14)
M. cricoarytenoideus lateralis, max RC length	22 (0.08)	19 (0.07)	16 (0.08)	13 (0.07)	12 (0.06)	11 (0.06)

(Continues)

TABLE 5 (Continued)

Anatomical variables	Red deer		Fallow deer		Sika deer	
	Male	Female	Male	Female	Male	Female
M. thyroarytenoideus, max DV length	53 (0.18)	45 (0.16)	79 (0.33)	30 (0.16)	26 (0.13)	27 (0.15)
M. thyroarytenoideus, max RC length	55 (0.19)	45 (0.16)	70 (0.33)	47 (0.25)	30 (0.15)	27 (0.15)
Fat pad covering M. thyroarytenoideus, max DV length	55 (0.19)	44 (0.15)	50 (0.24)	30 (0.16)	30 (0.15)	25 (0.14)
Fat pad covering M. thyroarytenoideus, max RC length	55 (0.19)	48 (0.17)	70 (0.33)	27 (0.14)	30 (0.15)	25 (0.14)
Vocal folds						
Vocal folds, DV length	35 (0.12)	31 (0.11)	47 (0.22)	17 (0.09)	14 (0.07)	15 (0.08)
Vocal folds, RC length	12 (0.04)	11.5 (0.04)	15 (0.07)	6 (0.03)	5.5 (0.03)	5 (0.03)

[a]Glottis measures were taken from dissection photos. The intermembraneous DV length is the average value of 10 measurements and the intercartilaginous DV length is calculated as the total glottis length minus the average intermembraneous length. Primary specimens were used for all glottis measurements except for the male sika deer, which used secondary specimen P1 and the associated mandibular length of 203 mm for calculations of relative glottis length.

vs. *0.23*; lateral length = 0.*55* vs. *0.45* vs. *0.34*; max. height = 0.*40* vs. *0.27* vs. *0.17*; Table 5; Figure 10). The upright-rectangular overall shape of the male fallow deer larynx is not well reflected here by the lateral length of the larynx, probably because of its long rostral horns (Figure 11). Among females, relative larynx size is largest in red deer, smaller in fallow deer and smallest in sika deer (red deer vs. fallow deer vs. sika deer: relative ventral length = 0.*28* vs. *0.26* vs. *0.22*; lateral length = 0.*40* vs. *0.37* vs. *0.27*; max. height = 0.*24* vs. *0.24* vs. *0.22*; Table 5; Figure 10).

There is a high level of sexual dimorphism in the fallow deer with the male having a substantially larger larynx than the female in absolute and relative measures: the absolute ventral length and larynx height of the male fallow deer are 1.67 and 1.89 times those of the female. The relative male/female ratios are *1.46* and *1.67*, respectively. In red deer, there is little sexual dimorphism in absolute or relative larynx size. The relative male/female ratios of ventral length and max. height are *1.04* and *1.12*, respectively. In sika deer, the ventral length of the larynx relative to body size is similar in both sexes. The relative male/female ratio is *1.05*. However, the max. height of the larynx relative to body size is larger in the female than in the male. The relative male/female ratio is *0.77*. Hence, regarding this measure, there is potentially a reversed sexual dimorphism in sika deer. However, additional specimens should be examined to verify this statement.

Estimates of dorsoventral glottis length (total, intermembraneous and intercartilaginous) were measured using the original photographs for Figure 10. Relative intercartilaginous glottis lengths were highly similar between all specimens, ranging from *0.07* in the female sika deer to *0.09* in the male red deer and female fallow deer (Table 5). However, relative intermembraneous glottis length was by far the longest in fallow deer (*0.25*) compared to the other specimens which ranged from the smallest relative length in the male sika deer (*0.08*) to the next largest relative length in the male red deer (*0.12*; Table 5). Estimates of glottal proportions (ratio of intermembraneous/intercartilaginous length) yielded: red male 1.35; red

female 1.37; fallow male 3.13; fallow female 1.08; sika male 0.99; sika female 1.32. This would mean that the dorsoventral vocal fold length (=the intermembraneous portion of the glottis) in male fallow deer is about threefold the length of the intercartilaginous portion. In female fallow deer and male sika deer the two portions of the glottis are about the same length while in female sika deer the intermembraneous portion of the glottis exceeded the length of the intercartilaginous portion 1.3-fold. In male and female red deer the intermembraneous portion is about 1.4-fold the length of the intercartilaginous portion.

3.2.3 | Laryngeal cartilages

For an explanation of how the laryngeal cartilages were measured see Figure S1. The relative size of the epiglottis was larger in fallow deer than in red and sika deer. Both relative rostrocaudal length and transverse width of the epiglottis were larger in male and female fallow deer (male, female max. RC length and max. TV width: *0.21*, *0.19* and *0.20*, *0.19*) than in red deer (*0.15*, *0.12* and *0.19*, *0.15*) and sika deer (*0.15*, *0.14* and *0.13*, *0.14*). Regarding transverse width of the epiglottis, as in max. height of the larynx (see above), there is again a slight reversed sexual dimorphism in sika deer (Table 5; Figure 11).

The relative size of the thyroid cartilage was similar in red and fallow deer (except the male fallow deer's larger dorsoventral height) but smaller in sika deer (Table 5). Among males, the normalized dorsoventral height of the thyroid cartilage was largest in fallow deer, smaller in red deer and smallest in sika deer (*0.34* vs. *0.24* vs. *0.19*). Among females, the relative dorsoventral height of the thyroid cartilage corresponded more closely (*0.21* vs. *0.18* vs. *0.17*). A similar pattern arose for the relative length of the rostral horn. Among males, the normalized length of the rostral horn was longest in fallow deer, shorter in red and shortest in sika deer (*0.25* vs. *0.19* vs. *0.11*). Among females, the relative length of the rostral horn corresponded more closely (*0.15* vs. *0.15* vs, *0.10*).

The relative size of the cricoid cartilage was largest in the male fallow deer, probably reflecting its upright-rectangular overall shape of the larynx. Among males, the relative maximum dorsoventral length was highest in fallow deer, intermediate in red deer and smallest in sika deer (0.32 vs. 0.26 vs. 0.22). The relative rostrocaudal length followed the same pattern (0.27 vs. 0.21 vs. 0.17). Among females, the relative values of the cricoid cartilage corresponded more closely: max. dorsoventral length = 0.23 vs. 0.25 vs. 0.21; max. rostrocaudal length = 0.19 vs. 0.16 vs. 0.16.

The relative size of the arytenoid cartilage was similar in red and fallow deer but smaller in sika deer (Table 5).

Sexual dimorphism in the sizes of the laryngeal cartilages was strongest in fallow deer and moderate to low in red deer. In contrast, practically no sexual dimorphism occurred in sika deer (Table 5).

3.2.4 | Intrinsic laryngeal muscles and fat pad

The attachment sites of the intrinsic laryngeal muscles in all six specimens (Figure 12) corresponded to the basic laryngeal structure in ruminants (Nickel et al., 2004) and in cervids (Köhler, 1982). Measurements of the intrinsic laryngeal muscles are provided in Table 5 (see Table S1 for weights of intrinsic laryngeal muscles).

There was little interspecific or intersexual difference in the intrinsic laryngeal muscles relative to body size except in the male fallow deer. Male fallow deer had the highest relative values for the dorsal cricoarytenoid muscle (0.28) and for both measures of the thyroarytenoid muscle: max. dorsoventral length (0.33) and max. rostrocaudal length (0.33; Figure 12; Table 5). The corresponding
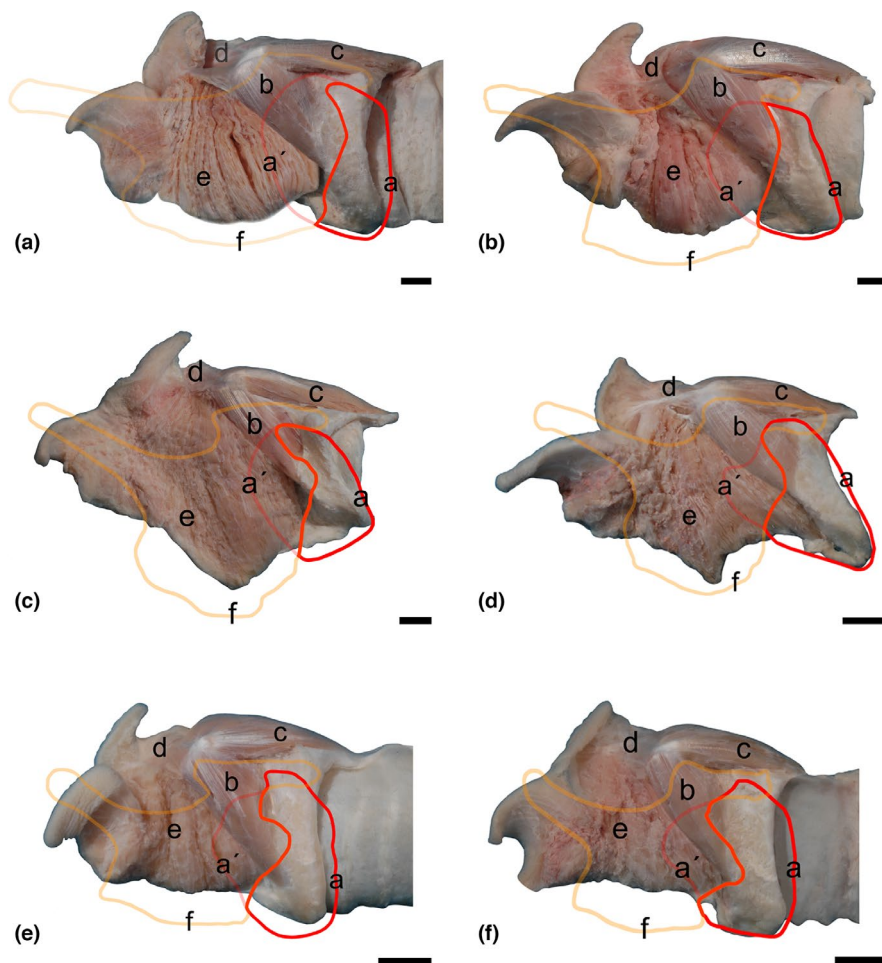
FIGURE 12 The intrinsic laryngeal muscles of male (a) and female (b) red deer, male (c) and female (d) fallow deer, and male (e) and female (f) sika deer. The six sub-illustrations are overlays reconstructed from several original dissection photos because it was not possible to show all intrinsic laryngeal muscles in one photo. The overlays are not to scale but presented at about the same size to facilitate inter-specific comparison of the intrinsic laryngeal muscles. The contour of the removed thyroid cartilage is shown in light orange; the contour of the removed cricothyroid muscle is shown in bright red (portion lateral to the thyroid cartilage) and in light red (portion medial to the thyroid cartilage). The distance between the ventral edge of the thyroarytenoid muscle and the ventral contour of the thyroid cartilage is artificial and results from separating this muscle from the thyroid cartilage when removing the latter. a = lateral portion of cricothyroid muscle; a′ = medial portion of cricothyroid muscle; b = lateral cricoarytenoid muscle; c = dorsal cricoarytenoid muscle; d = transverse arytenoid muscle; e = thyroarytenoid muscle; f = contour of thyroid cartilage. All scale bars 10 mm

measures (in the same order) for male red and sika deer were: *0.21, 0.16* and *0.18, 0.13* and *0.19, 0.15* (Table 5).

The large size of the thyroarytenoid muscle and of the dorsal cricoarytenoid muscle in the male fallow deer is most likely related to its enlarged larynx size, particularly the dorsoventrally and rostrocaudally enlarged thyroid and cricoid cartilages.

In both sexes of all three species, a large fat pad covers the entire lateral surface of the thyroarytenoid muscle. The fat pad extends rostrocaudally from the lateral edge of the epiglottis up to the rostral edge of the lateral cricoarytenoid muscle and dorsoventrally from the muscular process of the arytenoid cartilage to the ventral edge of the thyroid cartilage lamina. Caudodorsally, the portion of the cricothyroid muscle that terminates on the medial surface of the thyroid cartilage lamina is intercalated between the fat pad and the lamina of the thyroid cartilage (Figure 12; Figure S2).

Conforming to the large size of the thyroarytenoid muscle in male fallow deer, the relative size of the fat pad is largest in male fallow deer, intermediate in male red deer and smallest in male sika
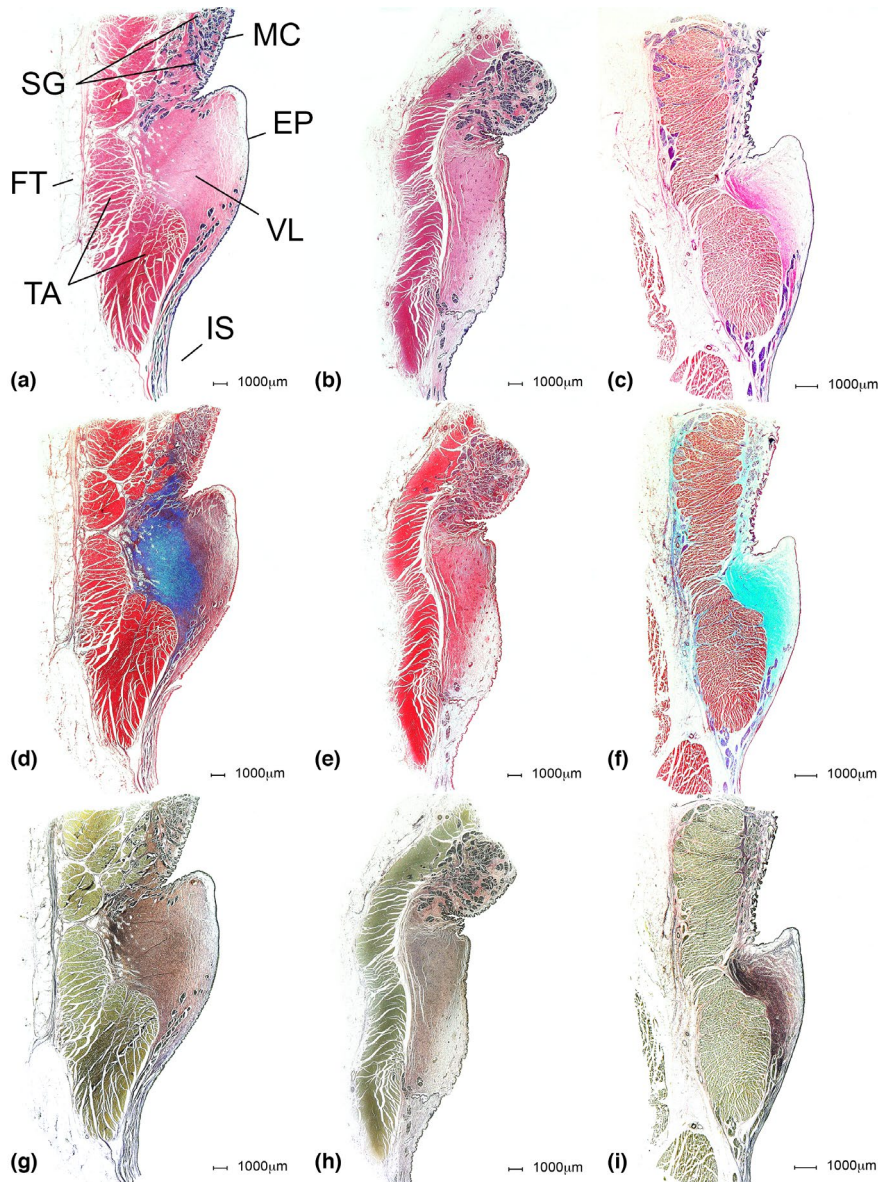
FIGURE 13 Transverse sections of the mid-region of one vocal fold of an adult male red deer (a, d, g), fallow deer (b, e, h) and sika deer (c, f, i) stained with Hematoxylin/Eosin (a, b, c), Masson Trichrome (d, e, f), and Elastica/Van Gieson (g, h, i). Samples were sliced perpendicular to the dorsoventral length of the vocal fold. This overview reveals the similarity in basic structure of the male red and sika deer vocal fold and the distinct shape of the male fallow deer vocal fold. EP = Epithelium, FT = Fat, IS = Infraglottic space, MC = Mucosa, SG = Seromucous glands, TA = Thyroarytenoid muscle, and VL = Vocal ligament

deer (max. dorsoventral length *0.24* vs. *0.19* vs. *0.15*; max. rostrocaudal length *0.33* vs. *0.19* vs. *0.15*; Table 5; Figure S2).

3.2.5 | Vocal folds: Macroscopic examination

The vocal fold dimensions of male fallow deer were remarkable and stood out from all others. Relative to body size, the male fallow deer was unique in having a dorsoventral vocal fold length almost double that of male red deer and threefold that of male sika deer: *0.22* vs. *0.12* vs. *0.07* (Table 5). Relative rostrocaudal vocal fold length was also largest in male fallow deer, intermediate in male red deer and smallest in male sika deer: *0.07* vs. *0.04* vs. *0.03* (Table 5). Among females, both measures of the vocal fold varied less than in the males: red deer *0.11*, *0.04*; fallow deer *0.09*, *0.03*; sika deer *0.08*, *0.03* (Table 5). The sexual dimorphism in dorsoventral and rostrocaudal vocal fold length was strong in fallow deer (0.22/0.09 and 0.07/0.03), whereas there was almost no sexual dimorphism in red deer (0.12/0.11 and 0.04/0.04) and sika deer (0.07/0.08 and 0.03/0.03; Table 5).

The vocal fold shape in both sexes of red and sika deer and in female fallow deer conformed more or less to a typical mammalian vocal fold, that is, they were rather taut and had a sharp, flexible rostral edge extending internally between the thyroid prominence and the vocal process. However, the vocal fold of the male fallow deer was different. In addition to its remarkable dorsoventral and dorsocaudal dimensions, it lacked a sharp, tightly stretched rostral edge and, overall, appeared to be loose and flabby. Caudally, the vocal folds blended in smoothly with the lateral walls of the infraglottic cavity in all specimens.

3.2.6 | Vocal fold histology

General morphology and dimensions of the vocal fold
Histological transverse sections of the vocal fold, taken perpendicular to the dorsoventral length of the vocal fold at its midpoint, confirm that the vocal fold of male fallow deer is substantially different in shape and size than the vocal fold of the male red deer and male sika deer. The fallow deer vocal ligament has a larger rostrocaudal dimension than in the other two species and is of almost the same transverse diameter along this axis whereas the vocal ligaments of the red deer and sika deer are more triangular in shape. The epithelium of the male fallow deer's vocal fold differs from the smooth stratified squamous epithelium of the male red and male sika deer's vocal fold. The epithelium of the male fallow deer's vocal fold resembles the mucosa of the laryngeal vestibule and seems to be looser than the vocal fold epithelium in male red and sika deer. The cover of the male fallow deer's vocal fold appears to consist of a multi-row ciliated epithelium. The thyroarytenoid muscle indents the vocal fold cover most strongly in red deer, less in sika deer and not at all in fallow deer (Figure 13).

Relative measures for the dimensions of the vocal fold derived from histology slices were created by dividing the absolute values

by the dorsoventral vocal fold length, as measured during dissections. The maximum transverse diameter and rostrocaudal length of the vocal ligament (VL) and the transverse diameter of the thyroarytenoid (TA) muscle was largest, relative to dorsoventral vocal fold length, in the male sika deer and smallest in the male fallow deer (e.g., sika, red, and fallow transverse diameter of VL: *0.46*, *0.35*, and *0.14*; and TA, *0.45*, *0.27*, and *0.09*, Table 6). In other words, fallow deer had the longest dorsoventral vocal fold length compared to the other vocal fold dimensions measured.

Elastic and collagen fibres in the vocal ligaments
Area estimates of the elastic fibre contents of the vocal ligament in representative histology slides of one vocal fold of a male red, fallow and sika deer yielded 60.1% (±4.40 SD), 22.0% (±3.38 SD) and 35.5% (±3.67 SD), respectively (Table 7). Corresponding estimates of the collagen fibres in the same slides yielded 18.4% (±7.13 SD), 26.1% (±6.87 SD) and 10.3% (±6.36 SD), respectively (Table 7). From this, the interspecific ratio of elastic fibres in one vocal ligament of a male red, fallow and sika deer is 2.7:1:1.6. The corresponding interspecific ratio of collagen fibres is 1.8:2.5:1. The intraspecific ratios of elastic: collagen fibres in one vocal fold of a male red, fallow and sika deer are ≈3.3:1, ≈0.8:1, and ≈3.4:1 (Table 7).

4 | DISCUSSION

In accordance with our expectations based on behavioural and acoustic observations, our anatomical investigations revealed pronounced differences at several levels of the anatomy of the vocal production system, both between the species and between the sexes within species. Synthetic graphical reconstructions of the investigated macroscopic structures are presented in Figure 14. Below we discuss the implications of our investigations for our understanding of vocal diversity in polygynous deer, starting with the filter-related anatomy.
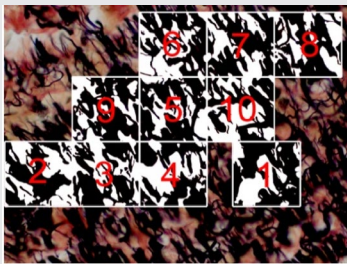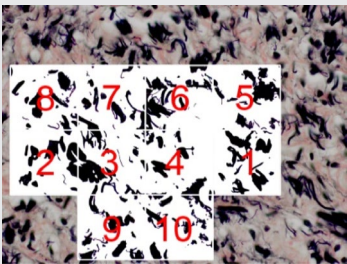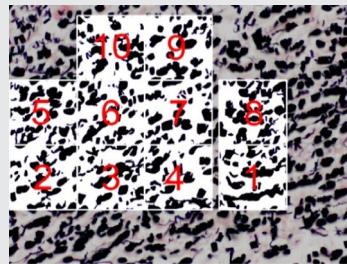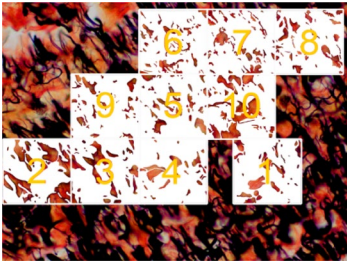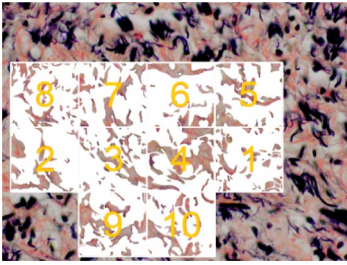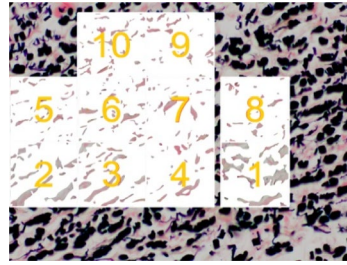
4.1 | Filter-related anatomy: Hyoid apparatus and thyrohyoid ligament, larynx position, muscles, vocal tract length

At the level of the filter, we observed strong differences in the overall dimensions of the vocal tract affecting both nasal and oral vocal tracts. In line with our expectations, fallow deer and red deer of both sexes were characterized by a low resting position of the larynx, which was more pronounced in males than females, resulting in longer nasal and oral vocal tracts in males (Table 3; Figures 3, 4 and 6). In contrast, sika deer had a high, non-sexually dimorphic resting position of the larynx, resulting in a short VT in both sexes. Supporting anatomical evidence for these differences was found in a variety of related features. Lower resting positions of the larynx were characterized by longer thyrohyoid muscles and thyrohyoid ligaments, stylothyroid (=caudal stylopharyngeal) muscles but shorter

TABLE 6 Dimensions of vocal fold anatomy in males: vocal ligament and thyroarytenoid muscle (TA). Measures were derived from histology slices sectioned from the center of the vocal fold and stained with Haematoxylin-Eosin. Measures include mean ± SD and the relative value ratio of each mean divided by the dorsoventral vocal fold length, as measured during dissections (italicized, in parentheses). TV = transverse, RC = rostrocaudal

Anatomical variables	Red deer (7 slices)	Fallow deer (9 slices)	Sika deer (9 slices)
Vocal ligament, maximum TV diameter (mm)	12.36 ± 1.32 (0.35)	6.63 ± 0.31 (0.14)	6.46 ± 0.36 (0.46)
Vocal ligament, maximum RC length (mm)	21.61 ± 1.20 (0.61)	24.64 ± 1.26 (0.52)	17.58 ± 0.57 (1.26)
Vocal ligament, cross-sectional area (mm^2)	123.37 ± 14.29	111.89 ± 11.46	53.91 ± 2.57
Thyroarytenoid muscle, maximum TV diameter (mm)	9.55 ± 0.38 (0.27)	4.38 ± 0.18 (0.09)	6.30 ± 0.33 (0.45)

TABLE 7 Area estimates and interspecific and intraspecific ratios of elastic and collagen fibre content in histology slides of the male vocal ligaments. Fibre percentage values represent the percentage of area within 50 μm squares containing elastic fibre (appearing as black in images) or collagen fibre (appearing as reddish or pink in images). Ratios are based on the mean values within 10 squares per species. Slides were stained with Elastica Van Gieson

	Red deer		Fallow deer		Sika deer	
	Elastic fibre %	Collagen fibre %	Elastic fibre %	Collagen fibre %	Elastic fibre %	Collagen fibre %
Mean ± SD (N)	60.1 ± 4.40 (10)	18.4 ± 7.13 (10)	22.0 ± 3.38 (10)	26.1 ± 6.87 (10)	35.5 ± 3.67 (10)	10.3 ± 6.36 (10)
Elastic fibre						
Collagen fibre						
Interspecific ratio of elastic fibre (E)	2.7		1.0		1.6	
Interspecific ratio of collagen fibre (C)	1.8		2.5		1.0	
Intraspecific ratio E:C	≈3.3:1		≈0.8:1		≈3.4:1	

sternothyroid and sternohyoid muscles (Table 3; Figure 8). Longer relative snout lengths in red deer and fallow deer, in comparison to sika deer (Table 4, Line 9; Figure 9), would also contribute to longer oral and nasal vocal tract lengths.

Our expectations on laryngeal mobility, and subsequent vocal tract extension, were also supported by anatomical evidence on both an interspecific and intersexual level. Red deer and fallow deer had substantially longer relative oral vocal tract lengths after maximal extension than sika deer, with sexual dimorphism in relative vocal tract extension present in red deer and fallow deer but not in sika deer (Table 3). The considerable elasticity of the thyrohyoid

ligament (which connects the larynx to the hyoid apparatus and constrains rostrocaudal movements of the larynx) in both sexes of red and fallow deer likely contributes to laryngeal mobility during phonation. The thyrohyoid ligament was sexually dimorphic in both red deer and fallow deer: In males, thyrohyoid ligaments were longer at rest and had a higher potential for elongation than in females. No such sexual dimorphism was observed in sika deer, where the thyrohyoid ligaments were very short with little potential for elongation in both sexes.

Apparently, the lowest achievable larynx position during maximal extension is, among other features, a function of both
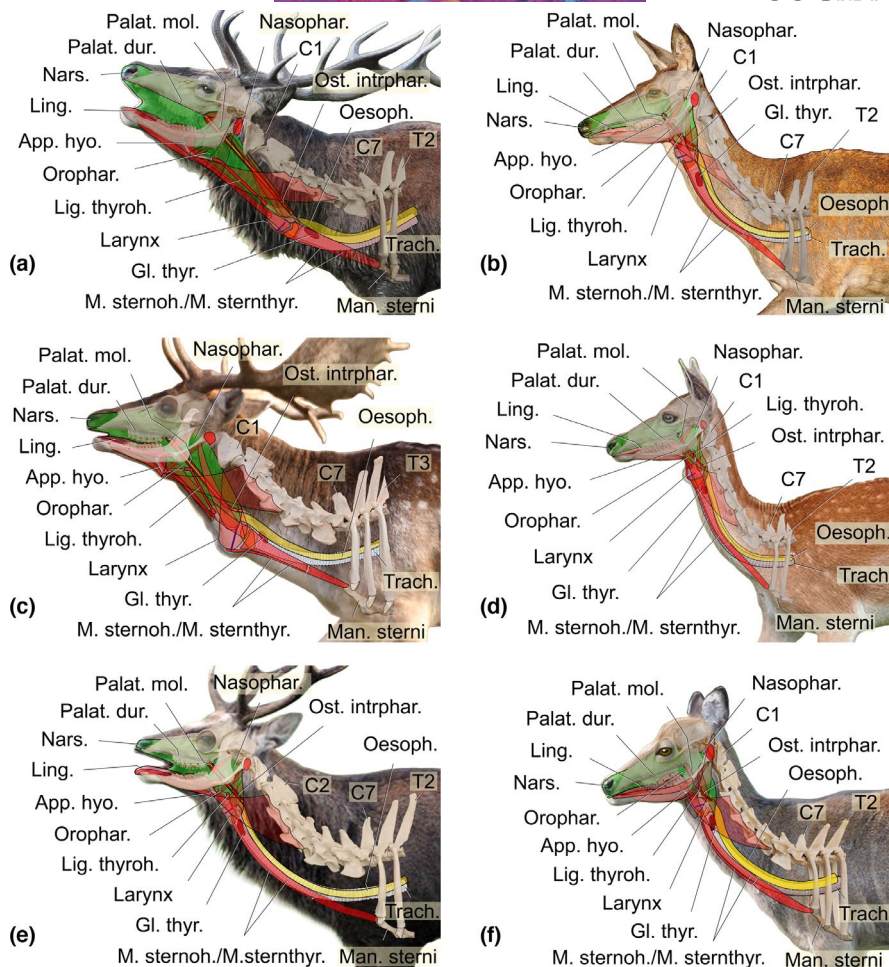
FIGURE 14 Graphical reconstructions of relevant parts of the vocal organs in male (a) and female (b) red deer, male (c) and female (d) fallow deer, and male (e) and female (f) sika deer. Not to scale, all specimens have been brought to the same size approximately. The reconstructions comprise findings on vocal gesture in males, head, neck and rostral thoracic skeleton, hyoid apparatus, larynx position, oral cavity and tongue, pharynx and vocal tract length, hyoid and extrinsic laryngeal muscles, trachea and oesophagus. (a, c, e) In addition to filter-relevant anatomical features, male sexual calls are affected by behavioural features (neck extension, head angle, lip and tongue configuration)

thyrohyoid ligament length and resilience. Between species, fallow deer males have the greatest relative resting length of the thyrohyoid ligament but the lowest resilience (Table 3). This might represent a specialization for emitting amazingly large numbers of groans separated by very short inter-call intervals during the rut (cf. McElligott et al., 1999). For this type of vocalization it might be advantageous to retain the larynx in a low position between the groans and just shortly retract it moderately more during groan production. This would allow maximal vocal tract extension in combination with an extremely high calling rate.

It should be noted that, in addition to filter-relevant anatomical features, there are behavioural features affecting call acoustics. Generally, vocal postures, that is, body posture during call emission including head and neck extension, are distinguished from vocal gestures, that is, call-synchronous lip and tongue configurations (Frey & Gebler, 2010). For example, the protraction of the mouth corners in roaring red deer stags, a gesture similar to pronouncing the long vowel [u] in the English language (i.e., "goose"), contributes to elongation of the vocal tract and, thereby to a lowering, closer spacing and high resolution of the formants. In contrast, the retraction of the mouth corners in moaning sika deer stags, a gesture similar to a human smile or pronouncing the long vowel [e] in the English language (i.e., "bee"), will entail a further shortening of the anatomically short vocal tract and, thereby decrease formant resolution in these high-frequency calls (cf. Fitch, 1997; Fitch et al., 2016). Groaning fallow deer stags are closer to sika than to red deer in terms of mouth opening and lip configuration, possibly a correlate of their extended and rapid series of short groans (up to over 3000 groans/h, McElligott & Hayden, 1999; McElligott et al., 1999) that do not leave enough time for call-synchronous pro- and retraction of the mouth corners.

Of the strap muscles both the sternohyoid and the sternothyroid muscles were shorter in red deer and fallow deer than in sika deer (Table 3). This observation is in accordance with the respective laryngeal position and mobility (or lack thereof) in these species.

The potential evolutionary shifting of the termination site of the sternohyoid muscle is instructively demonstrated by its asymmetry in the female fallow deer. Most probably, the observed asymmetry of the sternohyoid muscle represents a peculiarity of this female's vocal anatomy. One possible interpretation is that of a unilateral atavism. Under this perspective, the left sternohyoid muscle retained the ancestral character state (cf. Table S2) while the right sternohyoid muscle represents the derived character state (Figure 8; Table 3).

The length of the stylothyroid muscle (= the caudal stylopharyngeal muscle), owing to its course between the stylohyoid and the thyroid cartilage, can be taken as an indicator of larynx descent. In accordance with a pronounced larynx descent, the relative stylothyroid muscle length in male red deer and male fallow deer is dramatically greater than in male sika deer. Moderate larynx descent in female red deer and female fallow deer is reflected by a relative stylothyroid muscle length that is greater than in male and female sika deer but considerably less than in male red deer and male fallow deer. In contrast, relative stylothyroid muscle length is short and does not differ much between the sexes in sika deer, indicating their undescended, high-positioned larynx.

Together these observations follow acoustic data, as male fallow deer and male red deer produce mating calls with formants considerably lower than expected from body size, and also have the ability to further lower their formant frequencies (and overall spacing) by extending their vocal tract, an ability which has not been reported in male sika deer and which appears to be excluded by the structure of relevant elements of the vocal apparatus (e.g., a short and inextensible thyrohyoid ligament).

The stylohyoid muscle connects the angle of the stylohyoid with the basihyoid and, therefore, its length may indicate potential for dorsoventral expansion of the pharynx: longer muscle = more dorsoventral expansion. Interspecifically, the longest relative stylohyoid muscle lengths occurred in red deer and fallow deer and the shortest in sika deer, suggesting higher potential for pharyngeal expansion in red and fallow deer but lower or lacking of such potential in sika deer. Regarding the rutting calls, the potential for expansion of the throat region is highest in male red deer (roaring), intermediate in male fallow deer (groaning) and least in male sika deer (moaning). A large sexual dimorphism was found in red deer, with a longer relative stylohyoid muscle length in the male than in the female, whereas sexual dimorphism was moderate in sika deer and lacking in fallow deer. In fallow deer, this might indicate either a lower potential for pharynx expansion in the male or a higher potential for pharynx expansion in the female when compared to the other two species.

Interestingly, a comparison of the hyoid apparatus (Table 3; Figure 7) reveals that the thyrohyoids (total RC length of thyrohyoids in Table 3) are relatively longer in both sexes of red and fallow deer than in the sika deer male and female. Probably, this difference in proportion reflects an increased potential for unfolding and extending the hyoid apparatus ventrocaudally associated with a mobile larynx in both sexes of red and fallow deer, thereby facilitating call-synchronous caudal movements of the larynx. Possibly, the slightly higher normalized values of total RC thyrohyoid length in males compared to females (males, females: 0.22, 0.21 in red deer and 0.22, 0.19 in fallow deer, Table 3) faintly reflect the strong sexual dimorphism in terms of laryngeal mobility and vocal tract extension between the sexes. In contrast, vocalizing with a permanently high-positioned, mostly immobile, larynx allows a smaller size of the thyrohyoids (male, female: 0.16, 0.16, Table 3) in sika deer.

As the hyoepiglottic muscle connects the hyoid apparatus to the epiglottis, it might also be considered as an indicator of larynx descent. However, the relative length of this muscle is much greater in male red deer than in male fallow deer, although their laryngeal descent is similar. This might reflect a difference in the position of the epiglottis, the size of the laryngeal vestibulum, and the resilience of the epiglottis/thyroid cartilage connection in both species. The laryngeal vestibulum in male fallow deer is large (Figures 4 and 10) and there is a considerable distance between the base of the epiglottis and the rostral edge of the thyroid cartilage. In addition, this region is particularly flexible in fallow males. Together, this might allow a relatively high position of the epiglottis despite a pronounced descent of the remaining parts of the larynx (Figure 4). As a consequence, this would allow the relative length of the hyoepiglottic muscle in the male fallow deer to stay shorter than in the male red deer with a small vestibulum and a short and less resilient epiglottis/thyroid cartilage connection. In red deer and fallow deer, females have shorter relative hyoepiglottic muscle lengths than their male counterparts, with the largest sexual dimorphism present in red deer. In the male and female sika deer, the relative length of the hyoepiglottic muscle is particularly short and not sexually dimorphic because a descent of the larynx is lacking in both sexes.

It does not seem possible to predict larynx position and vocal tract extensibility from the structure of the hyoid apparatus in polygynous deer. Apparently, the typical hyoid apparatus of ruminants that is firmly connected to the skull base and, thus, stays close to the skull during down movements of the larynx, accommodates even extreme extensions of the pharynx without the need for pronounced anatomical transformations of the hyoid apparatus itself. However, this mode of vocal tract extension, apart from an extensible pharynx, requires a highly resilient connection between the hyoid apparatus and the larynx: the thyrohyoid ligament.

A different mode of extension occurs in those species, in which ventral parts of the hyoid apparatus (mostly basihyoid and thyrohyoids) do not stay close to the skull base but move down together with the larynx, such as in the roaring cats (Pantherinae; Weissengruber et al., 2002) and the koala (Frey et al., 2018). In these species, the hyoid apparatus underwent strong transformations by evolving a highly resilient ligament (an epihyoid ligament and a hyoid ligament, respectively) between the skull base and the basihyoid, that is, within the suspension part of the hyoid apparatus. In addition, this extension mode also requires an extensible pharynx (Frey et al., 2018; Weissengruber et al., 2002).

4.2 | Source-related anatomy: Larynx dimensions, larynx shape, vocal folds

At the level of the source, we observed strong differences in the over-all dimensions of the underlined larynx, as well as in the macrostructure, micro-structure, and dimensions of the vocal folds. As expected, the male fallow deer had a larger larynx size than the other specimens relative to body size, most evident by the amazing dorsoventral height of the thyroid cartilage, as well as long, thin rostral horns and a large epiglottis and cricoid cartilage (Table 5; Figures 10 and 11). Correspondingly, the male fallow deer also displayed relatively larger-sized intrinsic laryngeal muscles (dorsal cricoarytenoid muscle, thyroarytenoid muscle) and fat pads compared to the other specimens (Table 5; Figure 12; Figure S2). In contrast, sika deer of both sexes displayed relatively small larynges with short, thick rostral horns, and smaller laryngeal cartilages and intrinsic laryngeal muscles and fat pad, relative to body size. In line with our expectations, the relative size and shape of most source-related anatomical features in male and female red deer and female fallow deer was intermediate to that of the male fallow deer and sika deer of both sexes. As expected, there is little sexual dimorphism in laryngeal size and shape between the sexes except for fallow deer where the male was larger on a relative scale than the female in nearly all levels of source-related anatomy (Table 5).

Similar patterns were observed in the vocal folds. As predicted from mammalian vocal production principles (Elemans et al., 2015; Taylor & Reby, 2010), Figure 15 illustrates an inverse relationship between measured dorsoventral vocal fold length in the specimens and minimum f_o in the mating or contact calls produced by these species (Table 1). As expected from the vocalizations, the male fallow deer had the longest dorsoventral length, over 1.5 times longer than that of the male red deer and over 3.3 times longer than that of the male sika deer, as well as the longest rostrocaudal length (Table 5). In comparison, the male and female sika deer and the female fallow deer had the shortest vocal fold lengths and the male and female red deer had intermediate vocal fold lengths. Interspecific and intersexual patterns of dorsoventral vocal fold length were similar in absolute and relative measures across all specimens. Additionally, the vocal fold of the male fallow deer was slack and fleshy in comparison to the more rigid and structured vocal folds that extended in a tensed fashion from the thyroid prominence to the vocal process in all of the other specimens (Figure 12). Again, no strong sexual dimorphism was present in the vocal fold dimensions or macrostructure except in the fallow deer.

The histological analysis of male vocal fold microstructure confirmed the substantial difference between the fallow deer and the red and sika deer: Fallow deer had considerably longer dorsoventral vocal fold lengths relative to other vocal fold dimensions (vocal ligament diameter and rostrocaudal length, thyroarytenoid muscle diameter), in comparison to red and sika deer (Table 6; Figure 13). The vocal ligament of the red and sika deer shared a sharp-edged triangular appearance in contrast to the long and less defined vocal ligament of the fallow deer. Additionally, our analysis of elastic and collagen fibre content revealed that male red deer and male sika deer had similar,
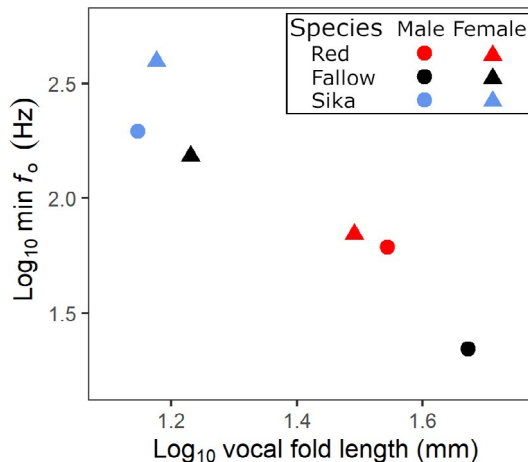

FIGURE 15 Average minimum fundamental frequency (minf_o) against vocal fold size (mm) in red deer, fallow deer and sika deer. This log-log graph depicts the measured $\log_{10}$ dorsoventral vocal fold length of each specimen (Table 5) versus the $\log_{10}$ minf_o of vocalizations produced by males and females of each species based on published literature (Table 1)

high concentrations of elastic fibres compared to collagen (E:C ratios: ≈3.3:1 in red deer and ≈3.4:1 in sika deer), while male fallow deer showed higher concentrations of collagen (E:C ratio: ≈0.8:1; Table 7).

Taken together, the macro- and micro-anatomy of the male fallow deer larynx are likely to reflect adaptations for producing a very low f_o. The elongated larynx with its long vocal folds and intrinsic muscles allows for the production of lower frequencies (cf. Herbst et al., 2012; Titze, 2000). Furthermore, the flaccid nature of the vocal folds and vocal ligament, larger fat pad, less mineralized cartilages, and the narrow angle of the laminae of the thyroid cartilage indicate that male fallow deer have limited potential to apply high tension to their vocal folds during phonation.

In contrast, male and female red deer and sika deer and female fallow deer share anatomical characteristics that have a higher potential for achieving greater call-synchronous vocal fold tension and production of higher frequencies (cf. Riede & Brown, 2013; Titze, 2000; Titze et al., 2016), such as shorter and stiffer vocal folds, a sharper vocal ligament (males), more mineralized cartilages, and a wider thyroid cartilage angle. In comparison to the male fallow deer, these characteristics likely allow for higher vocal fold tension through increased stability and more favourable leverage (cf. Lesch et al., 2021). Furthermore, the higher ratio of elastic:collagen fibre concentration in male red deer and male sika deer compared to the male fallow deer could allow for increased stretching and recoil functionality and higher control of the vocal folds during phonation (Moore & Thibeault, 2012). Many of these anatomical features are more pronounced in both sexes of sika deer compared to the male and female red deer and female fallow deer (e.g., relatively smaller, more compact larynx, smaller vocal folds, higher ossification, wider thyroid lamina angle, etc.), allowing for an even higher potential for increased vocal fold tension and

high frequency vocalizations. As expected, there was little sexual dimorphism in red deer and sika deer. As a result of the strong sexual differences of larynx size and structure in fallow deer, the corresponding features in female fallow deer appeared closer to red and sika deer than to male fallow deer.

4.2.1 | Evolutionary context

While our observations confirmed clear correspondence between the anatomy of the vocal organs and the acoustic signals, they also inform our **understanding of the possible evolutionary pathways for a low, extendable, relatively large larynx in red and fallow deer versus a high, immobile, small larynx in sika deer.** Two explanations for these broad interspecific differences are possible:

Option A

The ancestor of the Cervinae had a small, high, immobile larynx similar to sika deer. In this scenario, selection for low f_o and formant utilization in male red deer and male fallow deer drove convergent evolution for increased larynx size and lower, more mobile larynges in these species.

Option B

The ancestor of the Cervinae had a large, low and mobile larynx. Here, selection for high f_o in male sika deer or a recent relative would have driven adaptations of a high positioned yet small larynx.

The evidence presented here suggests that Option B is the more parsimonious explanation. This is supported by phylogenetic reconstructions inferred from male vocal behaviour in the Cervidae family (Cap et al., 2008). Indeed, while examination of acoustic data indicates that male sika deer are unlikely to exhibit pronounced larynx retraction, the rostral portions of their sternohyoid muscles are reduced to a similar extent as in red deer and terminate on the thyroid cartilage instead of on the basihyoid (Figure 8). This anatomical feature, in combination with the fact that males of all other documented Cervinae have a descended and mobile larynx (Fitch & Reby, 2001; McElligott et al., 2006; Reby & McComb, 2003b; Reby et al., 2016; Frey & Riede, 2013), suggests that sika may derive from an ancestor with a descended larynx. Otherwise the conforming strap muscle morphology in sika deer and larynx-retracting species of the Cervinae would be hard to explain. This indicates a potential evolutionary reversal of larynx position in this species (an ascent of the larynx concomitant with a decrease in larynx size) as a secondary specialization for high frequency moaning. Apparently, an evolutionary reversal of strap muscle morphology, that is, a rostrally directed growth of the sternohyoid muscle and re-connection to the hyoid apparatus, did not occur. Indeed, a short VT may favour source/filter tuning in a call with a very high f_o and thus increase the signal's intensity. Alternatively, selection pressures for the exaggeration of apparent body size may have been relaxed as f_o is too high to achieve the spectral density required for the expression of vocal tract resonances in these calls. Further comparative investigations including a

wider range of polygynous and monogamous cervids are needed to explore these possibilities.

Interestingly, wapiti (*Cervus canadensis*), a *Cervus* species that is more closely related to sika deer than western red deer (Heckeberg, 2020; Ludt et al., 2004), emit bugle vocalizations during the rut that contain both a whistle component with a very high fundamental frequency (g_0) as well as a low fundamental frequency (f_0) produced by their proportionately large, low-positioned and mobile larynx (Frey & Riede, 2013; Reby et al., 2016). The presence of the high g_0 component may indicate that the closely related sika deer and wapiti (or previous ancestors to both) experienced selection for calls with a high fundamental frequency but achieved it through different means: a small larynx and, consequently, short vocal folds producing a high f_o in sika deer versus rapid air flow through a supraglottic constriction producing a high g_o whistle in wapiti (Reby et al., 2016).

Across all three studied species, females show similar interspecific anatomical patterns to the males, except that there is less difference in relative measures between the female fallow deer and female red deer than observed between their male counterparts. This stems partially from the low sexual dimorphism in red deer and the high sexual dimorphism in fallow deer: female fallow deer do not possess the highly exaggerated larynx size, shape and mobility, nor the large, flaccid vocal folds and narrow thyroid lamina angle observed in male fallow deer. Compared to the female red deer and female fallow deer, female sika deer have a relatively high-positioned, small larynx with a wide thyroid lamina angle and short vocal folds. Yet, the sternohyoid/sternothyroid structure of female sika deer is very similar to male sika deer (rostral portion of sternohyoid muscle completely reduced), suggesting an ancestral lower larynx position for the female sika deer, too.

In red deer and fallow deer the relative thyrohyoid muscle length is larger in the males than in the females, whereas the relative lengths of the sternohyoid and sternothyroid muscles are smaller in the males than in the females as a consequence of the lower larynx position in the males (Table 3). In sika deer the situation is reversed: the relative thyrohyoid muscle length is larger in the female than in the male, whereas the relative lengths of the sternohyoid and sternothyroid muscles are smaller in the female (Table 3). Although the intersexual differences are small, this reversal might indicate a selective pressure in the males to ascend their larynx as high as possible (for acoustic reasons), potentially even higher than the resting position in the female.

4.3 | Speculation on mating systems as evolutionary drivers for interspecific and intersexual differences in polygynous deer

In these polygynous species, mating systems are characterized by high reproductive skew amongst males. Reproductive strategies vary by species, with red deer males employing harem-defense (Clutton-Brock et al., 1982) while sika deer males exhibit territory-defense

(Minami et al., 2009; Miura, 1984). However, fallow deer populations exhibit a wide variety of mating systems, from location-based single territories (Apollonio et al., 1992) and established leks (Apollonio et al., 1992; Clutton-Brock et al., 1988) to non-lekking populations where males defend harems of females rather than territories (Moore et al., 1995). Fallow deer males invest heavily in vocal signalling, with very high rates of vocalizations, up to over 3000 groans per hour during the rut (McElligott & Hayden, 1999 cf. Payne & Pagel, 1997). The male fallow deer groan affects the behaviour of rival males (McElligott & Hayden, 1999; Pitcher et al., 2015) and potentially females. Pitcher et al. (2015) showed that male fallow deer attend to f_o and formants in male groans, with lower f_o and formants being more threatening. The efficient communication of body size at short distance is facilitated by a low f_o increasing the size-related resolution in formant frequencies. Accordingly, the evolution of **a large, low-resting and mobile larynx with a flaccid vocal fold structure in male fallow deer, concomitant with a strong sexual dimorphism in vocal anatomy size, shape and mobility,** is likely to reflect adaptation to produce a disproportionately low f_o in this relatively small species. The enlargement of the larynx and the corresponding elongation of the vocal fold in fallow deer males, combined with a specialization of the vocal fold histology, supports the production of low-f_o, "pulsed" groans.

In red deer, successful males defend harems (groups of females) from rival males (Clutton-Brock et al., 1982). Clues to selection pressures on vocalizations, and consequently vocal anatomy, can be derived from experimental studies on male competition and mate choice contexts. Male red deer roars with lower formants are more intimidating to rival males (Reby et al., 2005) and more attractive to oestrous females (Charlton et al., 2007). In contrast, roars with higher f_o are more attractive to oestrous females (Reby et al., 2010) but provoke no change in agonistic responses of rival males (Garcia et al., 2013). Together, these observations suggest that selection for the production of disproportionately low formants has led to the evolution of low-resting, mobile larynges. Selection for the production of a higher f_o (associated with a higher reproductive success and female preferences in this species (Reby et al., 2010; Reby & McComb, 2003b)), potentially leads to the evolution of a disproportionately small larynx. However, we found that relative larynx and vocal fold size are comparable between the two sexes. This suggests constraints on f_o (which must remain relatively low in order to highlight vocal tract resonances, preserving the saliency of size-related variation in formant frequencies) may counteract selection pressure for a high f_o in this species. Red deer are consequently characterized by a stronger **sexual dimorphism in filter anatomy rather than in source anatomy**. Interestingly, Corsican red deer, the smallest subspecies of red deer, do have higher formants than Western European red deer (as expected from body size) but produce roars with a disproportionately lower f_o, as the f_o of male calls is twice as low as that of female calls (Kidjo et al., 2008). This large vocal sexual dimorphism indicates selection for lower frequencies through an enlarged larynx, potentially similar to male fallow deer, and may reflect relaxation of selection pressures for long-distance signalling.

In sika deer, males typically defend established territories from rival males and attempting to prevent passing females from leaving their area (Minami et al., 2009; Miura, 1984). Although male sika deer produce a variety of long- and short-range call types during the rut, the moan, given primarily by dominant males in the presence of both rivals and females, is the most prevalent. This loud long-range call is thought to potentially function as an advertisement of fighting ability to both rivals and passing females (Minami, 1997; Minami & Kawamichi, 1992) and as a territory advertisement. 'Howls' are the second type of loud calls emitted by dominant males during the rut: these less common calls have moderately higher f_o (min-max f_o: 183–2164 Hz), shorter durations (mean ± SE: 2.27 ± 0.27 s) and louder amplitudes, and are thought to mainly function as longer-distance territorial advertisements (Minami & Kawamichi, 1992; cf. Yen et al., 2013).

Observational studies indicate that male sika deer attend to loud calls from other males, often emitting loud calls of their own, but females do not appear to directly respond (Minami, 1997). However, in sika deer it remains to be investigated how rival males or oestrous females respond to changes in conspecific loud call frequencies. Interestingly, playbacks have demonstrated that oestrous females show no discrimination between conspecific male moans, male red deer roars, or male red × sika hybrid sexual calls that are acoustically intermediate to the parent species (Long et al., 1998; Wyman et al., 2014, 2016). While these results suggest that mate choice is unlikely to exert a strong selective pressure on the f_o of moans, specific experiments are clearly needed to further investigate this possibility. Alternatively, the territorial nature of the sika deer mating system may favour increased glottal efficiency and propagation, rendering higher f_o tonal calls more adaptive than calls with lower f_o and salient, lowered formants. Our observations that sika deer have a non-descended, immobile and small larynx capable of producing a high f_o with **very low sexual dimorphism in relative vocal anatomy size support this contention**. Interestingly, there is some evidence for a **potential reversal of typical sexual dimorphism** in some features: the female has slightly longer absolute and relative lengths of the thyrohyoid muscle and shorter sternohyoid/sternothyroid muscles than the male, suggesting a marginally lower larynx position, and a longer absolute and relative dorsoventral larynx height, suggesting a marginally longer glottal space for the vocal folds in the female.

As noted above, sika deer males display anatomical adaptations indicating that the larynx of their ancestors may have been descended and/or mobile, a trait that is no longer expressed in the extant species. We suggest that at some stage during the species' evolution, adaptive pressures favouring the production of high-frequency moaning may have "switched-off" the impact of androgens on the differential growth and specializations of the male vocal anatomy. While the sika male body is clearly affected by testosterone when approaching physical sexual maturity (as expressed by their larger body size, increased antler growth, etc.), this would explain why the vocal anatomy remains similar to female sika in both size and morphology. Interestingly, the fact that in the high-f_o

moaning male sika deer, the larynx is not descended (despite being very closely related to red deer), whereas both roaring male red deer and low-f_o groaning male fallow deer have a descended larynx further emphasizes that, at least in deer, the male larynx position is clearly related to vocalizing (and not feeding, breathing or other functions).

5 | CONCLUSION

To conclude, our investigations of the vocal anatomy of three polygynous deer species provides convincing evidence for a strong correspondence between the anatomical features of a species' vocal system and the acoustics of its vocal behaviour. These findings support existing studies that highlight such correspondences across other mammalian species (e.g., Borgard et al., 2020; Charlton & Reby, 2016; Garcia, Herbst, et al., 2017; Lesch et al., 2021). In a context of sexual selection, in which specific selection pressures immediately affect reproductive success, the males of polygynous deer species may be particularly susceptible to dramatic evolutionary transformations and even partial reversals regarding their vocal system (for similar mechanisms in the evolution of songbirds see Friis et al., 2021). For instance, in male sika deer, our findings suggest that sexual selection may have led to secondary evolutionary transformations that complicate the correspondence between vocal anatomy and acoustic output. Here, we found indications of the retention of anatomical features from the preceding primary adaptation (e.g., strap muscle morphology that formerly served vocal tract extension despite lacking vocal tract extension on the new evolutionary pathway) and indications of the evolution of novel features during adaptation to the new evolutionary pathway (e.g., an ascent and a decrease of larynx size when selection switches from low f_o to high f_o). For these and other reasons, for example, the flexibility and dynamics of the vocal system (cf. Boë et al., 2019; Fitch, 2010), it is not possible to reliably or exactly reconstruct the acoustic output from the anatomy and vice versa. Therefore, when aiming at a comprehensive explanation of a species' vocal system, the vocal anatomy and the vocalizations plus the evolutionary history and relationships of the respective species need to be investigated.

AUTHOR CONTRIBUTIONS

RF and MW share joint first authorship on the manuscript. The concept and design of the study was developed by DR, MW and RF. Data acquisition was conducted by RF, MW, DR, YL and MS. Data analysis and interpretation was conducted by RF, MW, DR and MJ. Manuscript development was performed by RF, MW and DR with contributions from MS, MJ and YL.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.