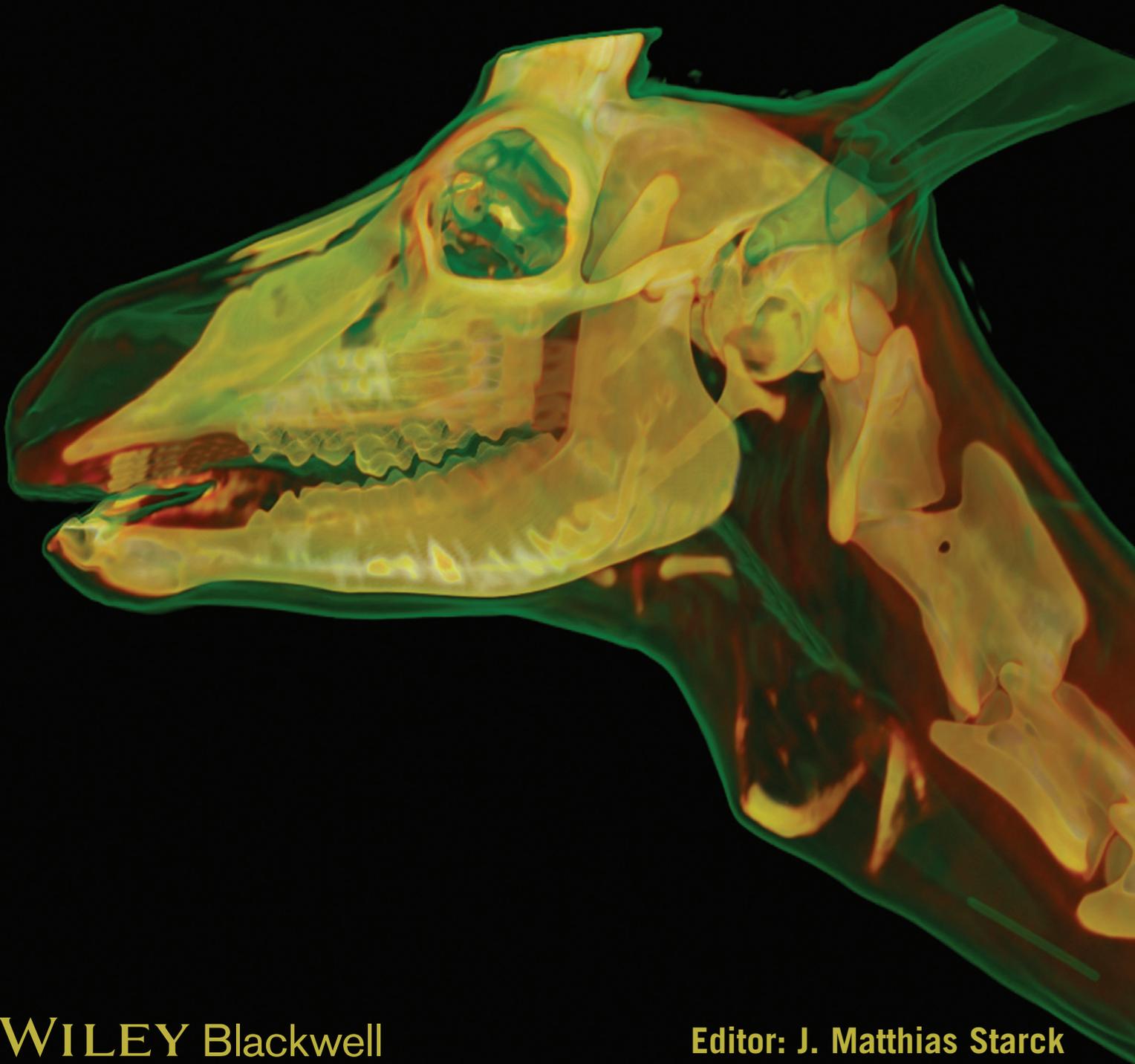


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# The Postnatal Ontogeny of the Sexually Dimorphic Vocal Apparatus in Goitred Gazelles (*Gazella subgutturosa*)

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**ABSTRACT** This study quantitatively documents the progressive development of sexual dimorphism of the vocal organs along the ontogeny of the goitred gazelle (*Gazella subgutturosa*). The major, male-specific secondary sexual features, of vocal anatomy in goitred gazelle are an enlarged larynx and a marked laryngeal descent. These features appear to have evolved by sexual selection and may serve as a model for similar events in male humans. Sexual dimorphism of larynx size and larynx position in adult goitred gazelles is more pronounced than in humans, whereas the vocal anatomy of neonate goitred gazelles does not differ between sexes. This study examines the vocal anatomy of 19 (11 male, 8 female) goitred gazelle specimens across three age-classes, that is, neonates, subadults and mature adults. The postnatal ontogenetic development of the vocal organs up to their respective end states takes considerably longer in males than in females. Both sexes share the same features of vocal morphology but differences emerge in the course of ontogeny, ultimately resulting in the pronounced sexual dimorphism of the vocal apparatus in adults. The main differences comprise larynx size, vocal fold length, vocal tract length, and mobility of the larynx. The resilience of the thyrohyoid ligament and the pharynx, including the soft palate, and the length changes during contraction and relaxation of the extrinsic laryngeal muscles play a decisive role in the mobility of the larynx in both sexes but to substantially different degrees in adult females and males. Goitred gazelles are born with an undescended larynx and, therefore, larynx descent has to develop in the course of ontogeny. This might result from a trade-off between natural selection and sexual selection requiring a temporal separation of different laryngeal functions at birth and shortly after from those later in life. *J. Morphol.* 277:826–844, 2016. © 2016 Wiley Periodicals, Inc.

**KEY WORDS:** postnatal development; vocal anatomy; sexual dimorphism; sexual selection; ruminants; Bovidae

## INTRODUCTION

The sex-specific ontogenies of the vocal organs in goitred gazelles represent a unique nonhuman

model for highlighting the evolution of an enlarged and pronouncedly descended larynx in males versus a more typically sized and only moderately descended larynx in females. The descent of the larynx from the ancestral high position close to the root of the tongue down to a slightly lower position in the upper neck region occurs in both sexes and probably evolved by natural selection whereas a more pronounced larynx descent later in ontogeny as a consequence of sexual selection is restricted to males. Therefore, investigating the vocal ontogeny of goitred gazelle may contribute to a better understanding of the evolutionary design of the vocal apparatus and related vocal production in mammals in general.

Under the influence of sexual selection, male humans and the males of several species of polygynous ruminants evolved a descended and sometimes enlarged larynx. Formerly, the overall phylogenetic and ontogenetic descent of the larynx in both sexes of humans (Negus, 1949; Lieberman, 1984; Fitch and Giedd, 1999; Fitch, 2000) had been considered as a naturally selected prerequisite for speaking ability (Lieberman, 1973, 1984; Fitch and Giedd, 1999; Fitch, 2000; Davidson,

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2003). However, there are two laryngeal descents in humans, the initial one, early in ontogeny, is common to both sexes whereas the second one, at puberty, is confined to males (Fitch and Giedd, 1999). The additional descent of the larynx in male humans during puberty is not related to speaking ability and might result from sexual selection for imposing resonant male voices generated by lowered and more closely spaced vocal tract (vt) resonance frequencies (humans: Fitch and Giedd, 1999; Collins, 2000; Feinberg et al., 2005, 2006; Puts, 2005; Rendall et al., 2005; Puts et al., 2006, 2007; ruminants: Fitch and Reby, 2001; Reby et al., 2005; Charlton et al., 2007a,b, 2008; Briefer et al., 2010; Frey et al., 2011; koala (*Phascolarctos cinereus*): Sonntag, 1921; Charlton et al., 2013a). Low resonance frequencies (formants) arise from vt elongation as a consequence of larynx descent and concomitant elongation of the pharynx, as vt resonances are inversely related to vocal tract length (vtl) (Titze, 1994; Fitch and Reby, 2001; Fitch and Hauser, 2002).

An alternative way of lowering call formants, aside from descending the larynx, is shifting from oral to nasal call emission, as nasal vts are longer than oral vts, not only in trunk-nosed mammals (saiga antelopes (*Saiga tatarica*): Frey et al., 2007; African elephants (*Loxodonta africana*): Stoeger et al., 2012) but also in species with typical nose sizes (goitred gazelles: Efremova et al., 2011a; Volodin et al., 2011; mongoose lemurs (*Eulemur mongoz*): Nadhurou et al., 2015).

Male voices with lower formants are more effective in the competition for females (humans: Collins, 2000; ruminants: Reby and McComb, 2003; Reby et al., 2005; Charlton et al., 2007a,b, 2008; Briefer et al., 2010; koala: Charlton et al., 2013b). Therefore, any enhancement of larynx descent in males that simultaneously effects a stronger lowering of vt resonance frequencies will be evolutionarily “rewarded” in terms of increased male reproductive success (Fitch, 2010).

In contrast, the initial laryngeal descent in both sexes of humans, entailing the morphological uniqueness of the human vocal tract (cf. Fitch and Giedd, 1999), most probably arose by natural selection and its evolutionary roots might reach back to nonhuman primates. A slight descent of the larynx in nonhuman primates during early ontogeny does not entail different resting positions of the larynx in adult male and female Japanese macaques (*Macaca fuscata*) (Nishimura et al., 2008) and chimpanzees (*Pan troglodytes*) (Nishimura et al., 2006). The selection pressure for laryngeal descent in both sexes might result from space reduction between lower jaw and vertebral column owing to rostral shifting of the foramen magnum.

The sexual dimorphism of larynx size and larynx position in adult goitred gazelles (*Gazella subgutturosa*) is impressive and more pronounced than in humans, whereas the vocal anatomy of neonate goitred gazelles does not differ between sexes

(humans: Negus, 1949; Lieberman, 1973, 1984; Fitch and Giedd, 1999; Fitch, 2000; Davidson, 2003; goitred gazelles: Efremova et al., 2011a,b; Frey et al., 2011; Fig. 1). The resting position of the larynx is pronouncedly lowered in adult male goitred gazelles but only to a moderate extent in adult females (Blank, 1985; Kingswood and Blank, 1996; Frey et al., 2011). The selective advantage of the moderate descent in both sexes is not yet clear but we hypothesize that it is related to the length changes of the pharynx and a concomitant higher mobility of the larynx assisting in food transport during regurgitation and swallowing of the cud (Supporting Information, Video 1). In contrast to humans, in which the position of the larynx is more or less static, the larynx in goitred gazelles is mobile in both sexes at any age (Efremova et al., 2011a,b; Volodin et al., 2011; Supporting information, Video 2), but particularly so in adult males during production of their rutting calls (Blank, 1985; Frey et al., 2011; Blank et al., 2014).

There is not only a sexual dimorphism of the vocal organs in adult goitred gazelles and other polygynous ruminants, but also a dimorphism of sexual behavior. In nature, young male goitred gazelles become interested in females at the age of about 6 months and they display mating postures at about one-year of age. However, they cannot get access to a territory of their own and start breeding before having reached 3 years of age. According to our data, this corresponds to the age at which the differences between the vocal apparatus of males and females become maximally pronounced. By contrast, more than one fourth of the females participate in the rut in their first year of life and at the age of 19–20 months, all females become involved in breeding (Blank, 1998).

Comparative studies on the vocal ontogeny in those mammalian species exhibiting a sexually dimorphic and descended larynx in adult individuals, as in goitred gazelle, have the potential for discriminating between the roles of natural and sexual selection in the evolution of this remarkable feature.

Previous works on age-related differences in nasal and oral vtls, neck circumferences and on the anatomy of the larynx and vocal folds were confined to the analysis of a few juvenile and adolescent goitred gazelles (Efremova et al., 2011a; Frey et al., 2011; Volodin et al., 2011) but included a preliminary histological investigation of the large connective-tissue vocal pads attached to the vocal folds (Efremova et al., 2011a). The present study investigates the postnatal ontogeny of the vocal apparatus in both sexes and three age classes of goitred gazelles.

## MATERIAL AND METHODS

### Specimens and Sites

Whole-body or head-and-neck specimens of 19 (11 male, 8 female) goitred gazelles, *Gazella subgutturosa*, (Güldenstaedt,

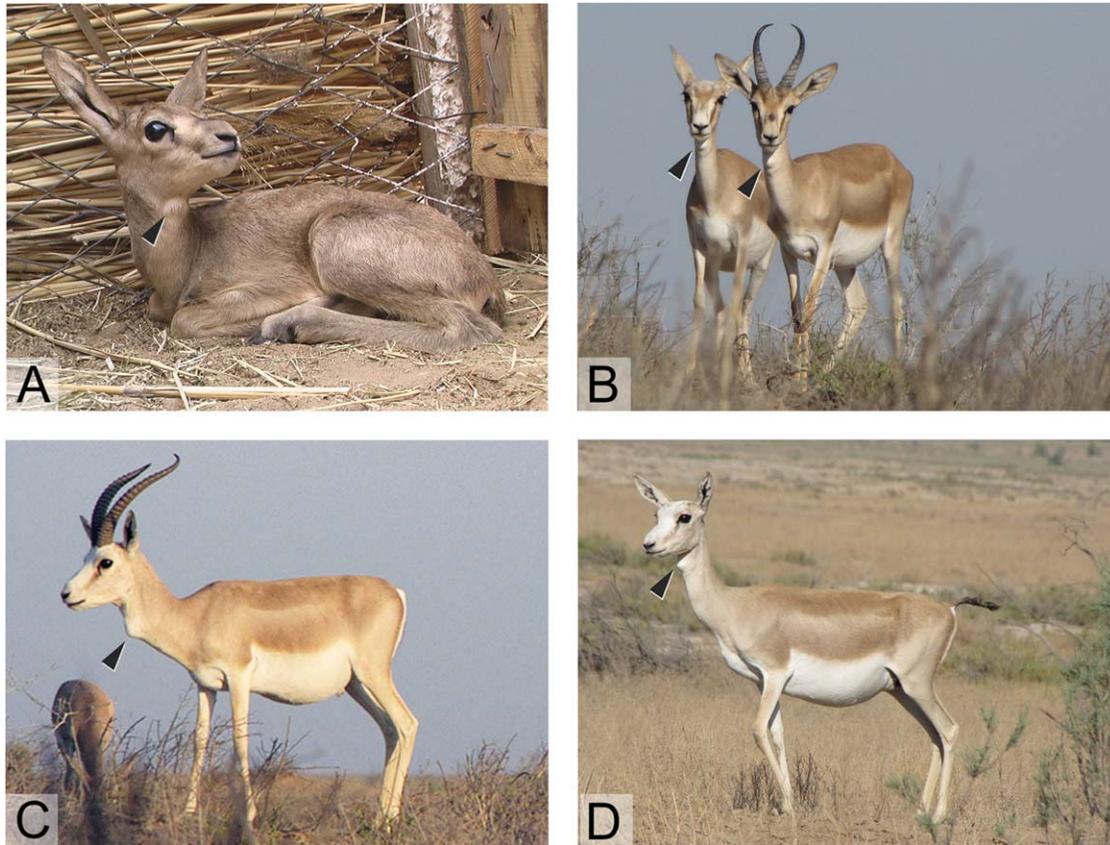


Fig. 1. *Gazella subgutturosa*. Postnatal ontogenetic life stages of goitred gazelle and respective larynx positions; black arrowhead points to the position of the larynx. Few-days-old young male (A); female and male adolescents (B); adult male (C); adult female (D). Larynx size and position are strongly sexually dimorphic in adults.

1780) were assigned to three age classes: 1–7-day neonates (2 males, 2 females), 6–9-months adolescents (3 males, 3 females) and  $\geq 24$ -months adults (6 males, 3 females). All these specimens died by natural reasons and were collected in 2008–2012,

either in a fenced 5000 hectare semidesert area or in enclosures at the Ecocenter “Djeiran” (Uzbekistan, Bukhara region, Kagan district, 39°41’N, 64°35’E; Efremova et al., 2011a; Frey et al., 2011). The specimens were frozen at  $-20^{\circ}\text{C}$  as soon as possible

TABLE 1. Mean values (mm) of the basal length of the skull, the overall ventral length of the larynx, the maximal height of the larynx, the laryngeal cartilages and the vocal folds.

Measure	Neonates		Adolescents		Adults	
	Male	Female	Male	Female	Male	Female
Skull, basal length	96.0 $\pm$ 8.5 (n = 2)	98.8 $\pm$ 8.8 (n = 2)	153.7 $\pm$ 5.8 (n = 3)	145.0 $\pm$ 9.9 (n = 2)	191.9 $\pm$ 7.6 (n = 5)	179.3 $\pm$ 1.5 (n = 3)
Larynx, overall ventral length	36.0 $\pm$ 1.4 (n = 2)	40.2 $\pm$ 4.2 (n = 2)	81.1 $\pm$ 4.0 (n = 3)	68.3 $\pm$ 4.6 (n = 2)	133.6 $\pm$ 18.6 (n = 4)	100.0 $\pm$ 4.8 (n = 3)
Larynx, max. height	20.0 $\pm$ 1.4 (n = 2)	20.7 $\pm$ 0.7 (n = 2)	33.1 $\pm$ 4.1 (n = 3)	31.1 $\pm$ 0.2 (n = 3)	54.0 $\pm$ 6.1 (n = 4)	43.3 $\pm$ 2.9 (n = 3)
Cart. thyr., ventral length	24.0 $\pm$ 5.7 (n = 2)	27.4 $\pm$ 2.5 (n = 2)	44.1 $\pm$ 3.7 (n = 3)	39.7 $\pm$ 3.6 (n = 3)	71.3 $\pm$ 10.0 (n = 3)	50.1 $\pm$ 0.5 (n = 3)
Cart. cric., ventral length incl. spine	13.5 $\pm$ 0.7 (n = 2)	17.0 $\pm$ 2.8 (n = 2)	32.5 $\pm$ 0.7 (n = 2)	27.5 $\pm$ 6.4 (n = 2)	42.7 $\pm$ 9.1 (n = 3)	39.0 $\pm$ 6.6 (n = 3)
Cart. cric., spine length	9.5 $\pm$ 0.7 (n = 2)	13.5 $\pm$ 3.5 (n = 2)	27.0 $\pm$ 2.0 (n = 3)	24.7 $\pm$ 6.8 (n = 3)	34.0 $\pm$ 8.7 (n = 3)	32.0 $\pm$ 6.2 (n = 3)
Plic. voc., dorsoventral length	7.0 $\pm$ 1.4 (n = 2)	7.7 $\pm$ 1.8 (n = 2)	14.1 $\pm$ 2.6 (n = 3)	12.1 $\pm$ 0.1 (n = 3)	28.2 $\pm$ 4.6 (n = 6)	16.6 $\pm$ 2.2 (n = 3)
Plic. voc., rostrocaudal length	5.0 $\pm$ 0 (n = 2)	5.5 $\pm$ 0.7 (n = 2)	11.8 $\pm$ 3.9 (n = 2)	9.4 $\pm$ 0.9 (n = 2)	17.0 $\pm$ 3.4 (n = 4)	10.2 $\pm$ 2.1 (n = 3)

Neonates 1–7 days; Adolescents 6–9 months; Adults  $\geq 24$  months

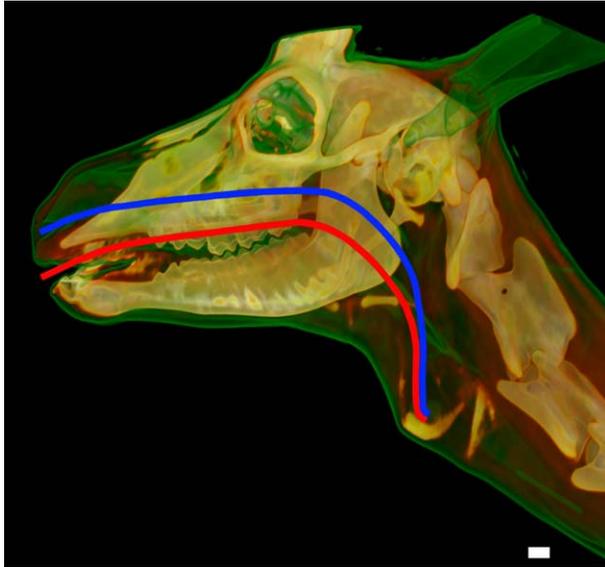


Fig. 2. *Gazella subgutturosa*. Measurements of the oral vocal tract length (red line) and the nasal vocal tract length (blue line) in goitred gazelles. CT-based 3D reconstruction of an adult male head and neck. Left lateral view. Scale bar 10 mm.

after death in a position resembling the resting head-to-neck position in live animals. In adult male specimens, the horns were cut off close to their bases to facilitate retention of a close-to-natural resting position of head and neck during the dissections. Four specimens (1 male and 1 female adolescents and 2 male adults) were used in previous publications (Efremova et al., 2011a; Frey et al., 2011). These specimens were dissected in the Ecocentre “Djeiran.” Skeletal parts of the adolescents and one adult male were later transferred to the Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, whereas the dissection of the second adult male was only documented photographically. The transferred skeletal parts are stored in the IZW morphological collection and can be accessed by sending a short request to the second author (RF).

The age was known for 10 of the 11 male and for 6 of the 8 female specimens; the remaining three adolescent specimens were aged using the basal lengths of their skulls (Table 1) and by their dentition status. As reference values we used the skull basal lengths of 61 goitred gazelles of known age (Mambetjumaev, 1970), linear body dimensions (Kingswood and Blank, 1996) and the tables of dentition changes along the ontogeny of domestic goats (*Capra aegagrus* f. *hircus*) (Nickel et al., 1987).

### Computer Tomographic and Anatomical Investigation

For obtaining non-invasive anatomical information prior to our invasive macroscopic dissections, five male and four female deep frozen specimens (four neonates, three adolescents, two adults) were scanned in a 64-slice spiral Computer Tomograph (CT) Aquilion CX (Toshiba Medical Systems Corp., Shimoishigami, Japan) at the IZW. The postmortem in situ positions of the vocal organs were registered by means of virtual serial sections (Multiplanar reconstructions obtained with the settings: 120.0 kV, 120.0 mA, slice thickness 0.6 mm) and in 3D-reconstructions established by Vitrea 2 software (Toshiba Medical Systems Corp., Shimoishigami, Japan) and by Osirix v. 3.9.4 64-bit (Pixmeo Sàrl, Bernex, Switzerland).

For anatomical dissections, executed partly in the Ecocentre “Djeiran” and partly in the Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, the specimens were thawed at +5°C for the duration of 24–48 hours. We began dissections on

the left side, removing the anatomical structures layer by layer, and then the right side was dissected in an identical manner. The larynges, head-and-neck glands, most muscles, hyoid apparatus, soft palate, tongue, ears and the trachea of all specimens were fixed in 4% formalin and stored in the IZW morphological collection. The horns and the skeletal parts were manually cleaned and also stored in the IZW morphological collection. Photos of consecutive dissection steps were taken with a Nikon D70S digital camera (Nikon Corp., Tokyo, Japan) on a compact flash (CF) card, fed to a PC and processed with Adobe Photoshop 5.5 or CS4 (Adobe Systems Inc., San Jose, CA). A comparison of photo, video and CT-images, taken from live and dead animals of the same age and sex in lateral profiles suggested that the post-mortem position of the larynx approximately corresponded to its resting position in live animals.

The larynx measurements (Supporting Information S-Figs 1,2) were taken on the excised larynges. The maximum height of the larynx (the distance between the most ventral point of the thyroid cartilage and the most dorsal point of the larynx) and the height of the thyroid cartilage (the distance between the most ventral and the most dorsal points of the thyroid cartilage), were measured using electronic calipers (Aerospace, Brüder Mannesmann Werkzeuge GmbH, Remscheid, Germany) with 0.5 mm precision (Supporting Information S-Fig. 1A,B). The ventral length of the cricoid cartilage (from the rostral edge of the cricoid arch to the caudal edge of the cricoid arch) and the length of the spine of the cricoid cartilage (from the caudal edge of the cricoid arch to the caudal tip of the spine) were measured with a ruler with 1 mm precision (Supporting Information S-Fig. 1A). The ventral length of the larynx (from the rostral notch of the thyroid cartilage along the ventral midline of the larynx up to the caudal tip of the spine of the cricoid cartilage) and the ventral length of the thyroid cartilage (from its rostral notch along the ventral midline to its caudal edge) were measured with a string with  $\pm 1$  mm precision (Supporting Information S-Fig. 1B). After cutting the entire larynx mediosagittally in two halves, the maximal dorsoventral length and the maximal rostrocaudal length of the right and left vocal folds were measured with calipers. The dorsoventral length was measured along the medial surface of the vocal folds facing the glottis, from their ventral attachment to the thyroid cartilage dorsally up to their attachment to the vocal process of the arytenoid cartilage. The maximal rostrocaudal length was measured at the maximal rostrocaudal diameter in the dorsal third of the vocal fold, from its rostral edge to its caudal edge (Supporting Information S-Fig. 2). For most specimens, the vt and vocal fold measurements were done by two researchers (KE and RF), one after the other.

For measurements of the hyoid apparatus, the lengths of the tympanohyoid, ceratohyoid, and basihyoid were taken along their lateral midlines with a ruler. The lengths of the stylohyoid, epihyoid and thyrohyoid were taken with a string (Supporting Information S-Fig. 3).

After exposure of the left half of the mandible, that is, after removal of the temporalis, masseter and buccal muscles, the oral and nasal vtls were taken with a string measure ( $\pm 1$  mm precision). The oral vt was measured from the approximate position of the vocal folds along the pharyngeal and oral cavities up to the edges of the lips (Fig. 2, red line), and the nasal vt from the approximate position of the vocal folds along the pharyngeal and nasal cavities up to the nostrils (Fig. 2, blue line). We selected this stage of the dissection for measuring vt because the main muscles and other structures, contributing to the suspension of the larynx (e.g., the hyoid apparatus), were still undamaged and, thus, the larynx retained a position close to its natural resting position in the live animal. At the same time, the larynx and the mandible were well visible and not covered by overlaying musculature. This allowed the most accurate determination of the course and length of both oral and nasal vt.

The length of the trachea, from the caudal edge of the cricoid cartilage to the first rib, was measured using a string. We also

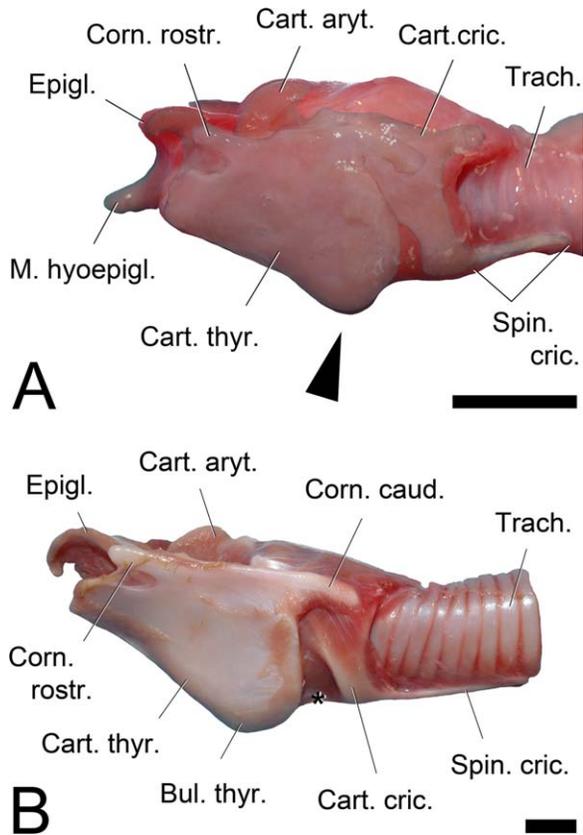


Fig. 3. *Gazella subgutturosa*. Larynx, cricoid spine and rostral part of the trachea of a one-day-old neonate male goitred gazelle (A) and of an adult female (B); the arrowhead in (A) points to the prominence of the thyroid cartilage; the asterisk in (B) marks the position of the cricothyroid ligament. (A) is an overlay of the larynx and the laryngeal cartilages. Left lateral view. Scale bars in (A) and (B) 10 mm, respectively. Bul. thy., thyroid bulla; Cart. ary., arytenoid cartilage; Cart. cric., cricoid cartilage; Cart. thy., thyroid cartilage; Corn. caud., caudal horn of thyroid cartilage; Corn. rostr., rostral horn of thyroid cartilage; Epigl., epiglottis; M. hyoepigl., hyoepiglottic muscle; Spin. cric., cricoid spine; Trach., trachea.

counted the number of tracheal cartilages along the same distance. The external dorsoventral and transverse diameters of the tracheal cartilages at the rostral end of the trachea (where it connected to the larynx) and at the level of the caudal end of the cricoid spine were measured with a ruler.

For few specimens, some measurements could not be taken because structures were partly damaged. Measurements were conducted three times for each structure, recorded in Excel (Microsoft Corp., Redmond, WA) and then the mean  $\pm$  SD values were calculated. Anatomical terms are in accordance with *Nomina Anatomica Veterinaria* (NAV, 2005, 2012).

## RESULTS

As the structures of the vocal apparatus of goitred gazelle have already been published elsewhere, the subsequent description has been reduced to the necessary minimum to avoid repetition of published information. For further data see Efremova et al. (2011a) and Frey et al. (2011).

## Laryngeal Cartilages

The overall ventral lengths of the larynx of neonates, adolescents and adults are given in Table 1. Dorsally, the thyroid cartilage has two rostral horns and two caudal horns (Fig. 3). The rostral horns connect the larynx to the thyroid cartilage of the hyoid apparatus and the caudal horns connect it to the cricoid cartilage via the paired cricothyroid articulation.

The shape and size of the larynx are primarily determined by the enlarged thyroid cartilage with its ventral prominence. This thyroid bulla prominence is already well noticeable in the youngest individuals (Fig. 3A). The overall ventral length of the thyroid cartilage (Table 1) gradually increases with age. However, its length relative to the overall length of the larynx, including the spine of the cricoid cartilage (Fig. 3; Supporting Information S-Fig. 4), slightly decreases from neonates to adults, comprising 66.7% in neonate males, 68.2% in neonate females, 54.4% in adolescent males, 58.1% in adolescent females, 53.4% in adult males and 50.1% in adult females. The maximum height of the larynx also decreases with age relative to the overall ventral length of the larynx, comprising 55.6% in neonate males, 51.5% in neonate females, 40.8% in adolescent males, 45.5% in adolescent females, 40.4% in adult males and 43.3% in adult females. The shapes of the cartilages remain more or less the same from neonates to adults (Fig. 4).

The most peculiar trait of the larynx of any sex and age is the long cartilaginous cricoid spine (Fig. 3; Supporting Information S-Fig. 4). The spine extends along the ventral midline of the trachea and is attached to it by resilient connective tissue. At its caudal end, the spine has a small semicircular extension, which, particularly in adult males, contacts an oblique slide face on the trachea (Supporting Information S-Fig. 4). From neonates to adults, the spine length increases threefold (Table 1), covering the first five to seven tracheal cartilages in neonates and the first six to eight cartilages in other ages.

The epiglottis is attached to the thyroid cartilage *via* a thyroepiglottic ligament. This thyroepiglottic ligament and the surrounding tissues, including the fibroelastic membrane of the larynx and the paired, dorsally located aryepiglottic folds, are highly elastic in adult males. As a consequence, strong manual extension of the laryngeal vestibule increases the distance from the rostral edge of the epiglottis to the rostral edge of the thyroid cartilage more than twice (Fig. 5). In contrast, the extensibility of the laryngeal vestibule in neonates is much restricted.

## Vocal Folds and Vocal Pads

The laryngeal cavity contains massive vocal folds, which extend between the vocal process of the

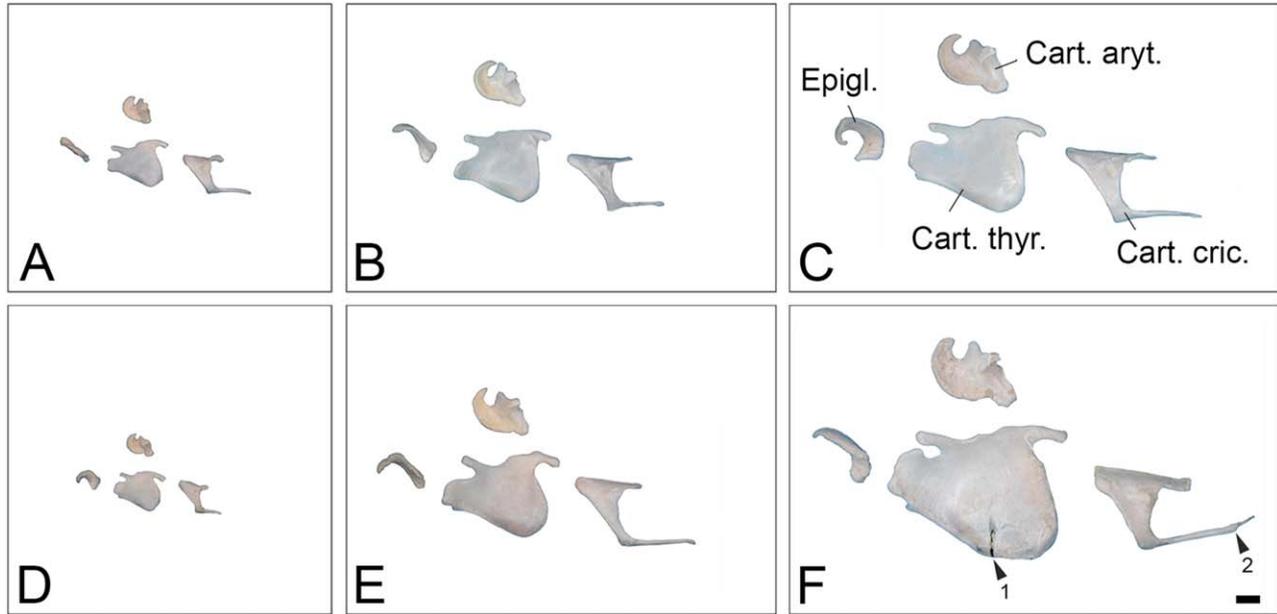


Fig. 4. *Gazella subgutturosa*. The ontogeny of the laryngeal cartilages. Females (ABC); Males (DEF). Neonates (AD); Adolescents (BE), Adults (CF). The overall shape of the cartilages is more or less retained throughout postnatal ontogeny. 1 = damage of thyroid cartilage, 2 = healed fracture of cricoid spine. Left lateral view, rostral is to the left. Scale bar 10 mm. Cart. aryt., arytenoid cartilage; Cart. cric., cricoid cartilage; Cart. thy., thyroid cartilage; Epigl., epiglottis.

arytenoid cartilage and the internal surface of the laryngeal prominence (Supporting Information S-Figs 5, 6). Dorsoventral and rostrocaudal lengths of the vocal folds are almost identical between sexes in neonates and not pronouncedly different in adolescents. However, in adult males these measures exceed those in adult females 1.7-fold (Table 1).

Large ellipsoid vocal pads are attached to the laterocaudal surface of the vocal folds (Supporting Information S-Fig. 6). The connective-tissue nature of the vocal pads has been confirmed histologically elsewhere (Efremova et al., 2011a).

**Hyoid Apparatus**

The tympanohyoids, stylohyoids, and epihyoids suspend the hyoid apparatus from the skull base. The ceratohyoids connect the suspending elements to the basihyoid and the thyrohyoids establish the connection of the hyoid apparatus to the larynx. In adults three paired elements, stylohyoids, epihyoids, and ceratohyoids, are fully ossified. The thyrohyoids are only partly ossified, their caudal ends remain cartilaginous. Completely nonossifying elements of the hyoid apparatus are the tympanohyoids; they remain fully cartilaginous and retain their flexibility even in adults. The tympanohyoid contacts the tympanic bulla laterally and its dorsal end attaches to the styloid process of the temporal bone.

With increasing age, the styloid process and the tympanohyoid become more and more embedded in the tympanic bulla and the relative length of the tympanohyoid shortens relative to the overall

size of the hyoid apparatus. In neonates, the tympanohyoid is not imbedded into the tympanic bulla and its length relative to the total length of all elements of the hyoid apparatus comprises 13% in males and 14% in females. In adolescents, the proportion of the non-imbedded part of the tympanohyoid comprises 7% in males and 6% in females, whereas in adults this proportion decreases to 4% in males and 5% in females (Table 2; Fig. 6).

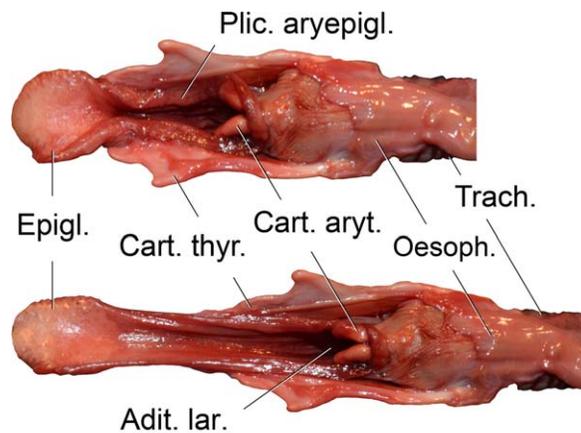


Fig. 5. *Gazella subgutturosa*. The highly extensible laryngeal vestibule of an adult male goitred gazelle. The epiglottis in the resting state (top) and the epiglottis-thyroid cartilage connection plus the aryepiglottic folds manually extended by fixing the epiglottis and pulling the trachea and oesophagus in the opposite direction (bottom). Dorsal view, rostral is to the left. Scale bar 10 mm. Adit. lar., laryngeal entrance; Cart. aryt., arytenoid cartilage; Cart. thy., thyroid cartilage; Epigl., epiglottis; Oesoph., oesophagus; Plic. aryepigl., aryepiglottic fold; Trach., trachea.

TABLE 2. Mean lengths (mm) of the individual elements of the hyoid apparatus.

Measure	Neonates		Adolescents		Adults	
	Male (n = 2)	Female (n = 2)	Male (n = 2)	Female (n = 3)	Male (n = 4)	Female (n = 2)
Tympoh.	8.0 ± 0	10.0 ± 1.4	7.7 ± 2.5	5.3 ± 0.6	4.8 ± 0.5	6.0 ± 1.4
Styloh.	31.0 ± 1.4	34.5 ± 2.1	50.3 ± 4.2	44.7 ± 1.5	57.8 ± 1.0	52.5 ± 0.7
Epih.	3.5 ± 2.1	5.5 ± 0.7	9.7 ± 1.5	10.3 ± 3.1	11.5 ± 1.9	10.5 ± 0.7
Ceratoh.	5.5 ± 2.1	6.0 ± 2.8	12.0 ± 2.6	9.7 ± 2.3	16.5 ± 1.3	13.5 ± 0.7
Basih.	4.5 ± 0.7	5.5 ± 0.7	9.0 ± 0	7.3 ± 1.2	9.0 ± 0.8	8.0 ± 0
Thyroh.	10.5 ± 2.1	10.0 ± 1.4	21.0 ± 1.7	19.7 ± 0.6	37.5 ± 5.0	27.5 ± 2.1
Total	63.0 ± 8.5	71.5 ± 3.5	109.7 ± 5.7	97.0 ± 5.3	137.0 ± 6.7	118.0 ± 1.4
Tympoh./Total	12.7	14.0	7.0	5.5	3.5	5.1
Thyroh./Total	16.7	14.0	19.1	20.3	27.4	23.3

Total = total length of hyoid apparatus; Tympoh./Total = the ratio of the length of the tympanohyoid to the total length of the hyoid apparatus (%), Thyroh./Total = the ratio of the length of the thyrohyoid to the total length of the hyoid apparatus (%). Neonates 1–7 days; Adolescents 6–9-months; Adults ≥ 24 months.

Unlike the tympanohyoid, the relative total length of the thyrohyoid (ossified plus cartilaginous part) increases with age. In male and female neonates, the length of the thyrohyoid comprises 17% and 14% of the total length of all elements of the hyoid apparatus, respectively (Table 2; Fig. 6; Supporting Information S-Fig. 3). In male and female adolescents, the corresponding length of the thyrohyoid comprises about 19–20% whereas in adult males, the thyrohyoid comprises 27% and in adult females 23% of the total length of all elements of the hyoid apparatus (Table 2; Fig. 6). A sexual dimorphism of the hyoid apparatus occurs

only in adults: the total length of all elements of the hyoid apparatus is 16% higher in males (137 mm) than in females (118 mm) as a consequence of the different lengths of the thyrohyoids, which are 37% longer in males (37.5 mm) than in females (27.5 mm) (Table 2; Fig. 6).

The hyoid apparatus and the larynx are connected by paired resilient thyrohyoid ligaments between the cartilaginous ends of the thyrohyoids and the rostral horns of the thyroid cartilage (Supporting Information S-Fig. 7). In neonates, the rostral horns of the thyroid cartilage either overlap the caudal end of the thyrohyoid or are located at

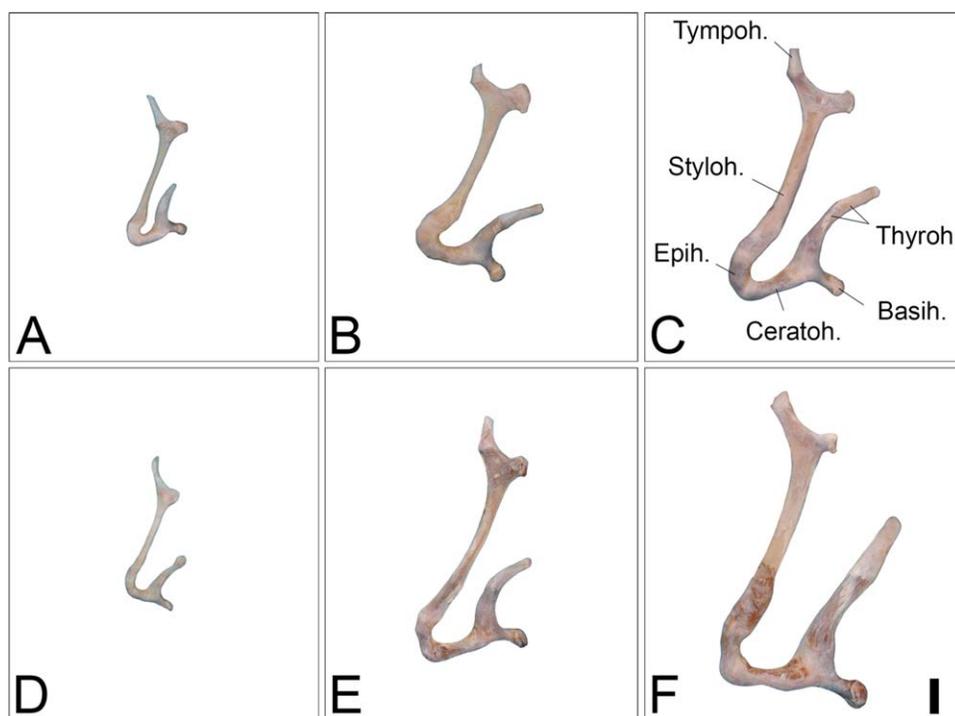


Fig. 6. *Gazella subgutturosa*. The ontogeny of the hyoid apparatus. Females (ABC); Males (DEF). Neonates (AD); Adolescents (BE), Adults (CF). Left lateral view, rostral is to the left. Scale bar 10 mm. Basih., basihyoid; Ceratoh., ceratohyoid; Epih., epihyoid; Styloh., stylohyoid; Thyroh., thyrohyoid; Tympoh., tympanohyoid.

TABLE 3. Mean values (mm) of the lengths of the oral and nasal vocal tract, soft palate, thyrohyoid ligament and hyoepiglottic muscle.

Measure	Neonates		Adolescents		Adults	
	Male	Female	Male	Female	Male	Female
Oral vt length	110.5 ± 13.4 (n = 2)	123.5 ± 2.1 (n = 2)	197.0 ± 9.8 (n = 3)	178.7 ± 22.6 (n = 3)	292.7 ± 30.9 (n = 6)	240.7 ± 3.1 (n = 3)
Nasal vt length	126.5 ± 13.4 (n = 2)	140.5 ± 4.9 (n = 2)	214.0 ± 14.0 (n = 3)	199.0 ± 19.3 (n = 3)	315.3 ± 30.0 (n = 6)	258.0 ± 5.3 (n = 3)
Palat. mol., resting length	25.5 ± 2.1 (n = 2)	28.1 ± 0.6 (n = 2)	45.9 ± 5.3 (n = 2)	43.6 ± 0.8 (n = 2)	75.4 ± 8.0 (n = 3)	67.3 ± 4.6 (n = 3)
Lig. thyroh., resting length	3.0 ± 0 (n = 2)	2.5 ± 0.7 (n = 2)	no data	no data	32.2 ± 20.0 (n = 2)	14.0 ± 2.8 (n = 2)
Lig. thyroh., max. extended length	10.5 ± 0.7 (n = 2)	10.0 ± 0 (n = 2)	no data	no data	73.3 ± 10.4 (n = 3)	28.5 ± 2.1 (n = 2)
M. hyoepigl., resting length	7.3 ± 1.1 (n = 2)	11.0 ± 1.4 (n = 2)	18.5 ± 0.7 (n = 2)	18.5 ± 6.4 (n = 2)	42.5 ± 10.6 (n = 2)	22.0 ± 4.4 (n = 3)
M. hyoepigl., max. extended length	15.5 ± 0.7 (n = 2)	16.5 ± 0.7 (n = 2)	28.5 ± 4.9 (n = 2)	31.5 ± 2.1 (n = 2)	60.0 ± 7.1 (n = 2)	34.0 ± 6.9 (n = 3)

Neonates 1–7 days; Adolescents 6–9-months; Adults ≥ 24 months.

the same level and the thyrohyoid ligament is short (Supporting Information S-Fig. 8A). In adolescents, the rostral horns of the thyroid cartilage and the caudal ends of the thyrohyoids do not overlap; the distance between these structures is longer, and the length of the connecting thyrohyoid ligament is increased accordingly. Thyrohyoid ligament length further increases in adults but much less in females than in males (Supporting Information S-Fig. 8B).

In neonates, the resting length of the thyrohyoid ligament of 2.5–3.0 mm can be manually extended up to 10–10.5 mm (Table 3). Regrettably, reliable data on the lengths of the thyrohyoid ligament are not available for adolescents. In adult males, the resting length of the thyrohyoid ligament varies from 18 to 55 mm, and maximal manual extensions range from 65 to 85 mm. In adult females, the resting length of the thyrohyoid ligament is less than half of that in adult males and length at maximal extension is 2.5-fold less than in adult males (Table 3). Length increase by maximal manual extension is similar in adult males and females (56% vs. 51%).

### Oral and Nasal Vocal Tracts

The oral vocal tract (vt) of goitred gazelles extends from the vocal folds along the vestibule of the laryngeal cavity, the oropharynx the fauces, the oral cavity and the oral vestibule and ends at the lips surrounding the oral opening. The nasal vt extends from the vocal folds along the nasopharynx, through the choanae, along the nasal cavity and the nasal vestibule and ends at the nostrils (Fig. 2). The nasal vocal tract length (vtl) constantly exceeds the oral vtl in all age and sex classes (Table 3). The position of the larynx shifts caudally with increasing age and the pharynx including the soft palate elongates concomitantly

(Table 3). This entails a gradual caudally directed shift of the intrapharyngeal ostium from neonates to adults.

Inside the pharynx, the oral and nasal vts are separated by the soft palate, which divides the pharyngeal cavity into a dorsally located nasal and a ventrally located oral compartment (Supporting Information S-Fig. 9). The soft palate is perforated by the intrapharyngeal ostium, that is, the only, oval-shaped connection between the nasal and the oral part of the pharynx (Supporting Information S-Fig. 10). The soft palate, including the intrapharyngeal ostium, is highly elastic and extensible as demonstrated by manual retraction of the larynx. Even after taking into account skull size differences between males and females (Table 1), a slight sexual dimorphism of the resting length of the soft palate is noticeable in adults but not in neonates and adolescents (Table 3).

### Muscles Retracting the Larynx and Hyoid Apparatus

The sternothyroid and sternohyoid muscles (Table 4) have a common origin from the manubrium of the sternum and are fused along their caudal third (Supporting Information S-Fig. 11). The sternothyroid muscle, representing an immediate retractor of the larynx, terminates on the lateroventral surface of the thyroid cartilage. The sternohyoid muscle terminates on the ventrocaudal area of the basihyoid. It retracts the basihyoid and the thyrohyoids and, by this action, the sternohyoid muscle supports caudal movements of the larynx. The hyoepiglottic, thyropharyngeal, crico-pharyngeal, caudal stylopharyngeal, and thyrohyoid muscles are considered to be protractors of the larynx (Tables 3 and 4). Both the retractor and protractor muscles also contribute to suspending

TABLE 4. Origin, termination and functions of the major muscles effecting the movements of the larynx and hyoid apparatus of the goitred gazelle.

Muscle	Origin	Insertion	Function
M. occipitohyoideus	Laterally from Paracondylar process	Angle of Stylohyoid	Draws stylohyoid angle Dorsocaudally
M. geniohyoideus	Rostromedially from body of mandibula	Basihyoid	Draws basihyoid rostrally
M. stylohyoideus	Lateroventral surface of stylohyoid	Basihyoid	Draws basihyoid dorsally
M. stylopharyngeus caudalis	Medially from dorsal half of stylohyoid	Dorsal rim of thyroid cartilage	Protracts the larynx
M. hyoepiglotticus	Basihyoid, ceratohyoid	Ventral surface of epiglottis	Changes shape of epiglottis and draws it ventrally
M. omohyoideus	Deep neck fascia, transverse processes of neck vertebrae I-V	Basihyoid	Fixes basihyoid and draws it dorsocaudally
M. thyropharyngeus	Lateral surface of thyroid cartilage	Pharyngeal raphe	Protracts the larynx
M. cricopharyngeus	Lateral surface of cricoid cartilage	Pharyngeal raphe	Protracts the larynx
M. thyrohyoideus	Lateroventral surface of thyroid cartilage	Thyrohyoid	Retracts the basihyoid, protracts the larynx
M. sternothyroideus	Sternal manubrium	Thyroid cartilage	Retracts the larynx
M. sternohyoideus	Sternal manubrium	Basihyoid	Retracts the basihyoid

Functions were taken from Nickel et al. (1984, 1987) and Schaller (1992).

the larynx in its low resting position, probably by small-amplitude contractions and relaxations.

The highly elastic, V-shaped hyoepiglottic muscle, connecting the hyoid apparatus to the epiglottis, can be manually extended to maximally 1.5–2 times its resting length (Table 3). In neonates and adolescents, a sexual dimorphism is lacking, whereas in adults, both resting length and maximally extended length of the hyoepiglottic muscle in males considerably exceed those in females (Table 3). The length of the thyrohyoid muscle, which connects the thyroid cartilage to the hyoid apparatus (Supporting Information S-Fig. 11), does not differ between neonate and adolescent males and females but a pronounced sexual dimorphism occurs in adults. In adult females the length of this muscle is almost 1.5 times less than in adult males (Table 3).

The digastric muscle takes its origin from the paracondylar process of the occipital bone and terminates on the ventral edge of the molar part of the lower jaw. The stylohyoid muscle originates from the angle of the stylohyoid and terminates on the basihyoid. Commonly, the stylohyoid muscle courses lateral to the digastric muscle. However, in some specimens, the stylohyoid muscle is subdivided into two portions close to the stylohyoid angle, thereby forming a slit-like opening through which the caudal part of the digastric muscle passes. Origin and termination of the stylohyoid muscle remain undivided though (Supporting Information S-Fig. 12).

### Pharyngeal Nerves

The superficial laryngeal and pharyngeal nerves, representing branches of the vagus nerve (X), are commonly well distinguishable: the cra-

nial laryngeal nerve splits into an external branch to the cricothyroid muscle and an internal branch to the laryngeal mucosa, which enters the larynx at the rostral end of the thyroid cartilage; the laryngopharyngeal branches of the laryngopharyngeal nerve from the cranial cervical ganglion supplies the caudal constrictors of the pharynx. In the resting state, these nerves follow a slightly sinuous course alongside the short pharynx in neonates of either sex. In adult males, however, these nerves are packed in densely meandering loops

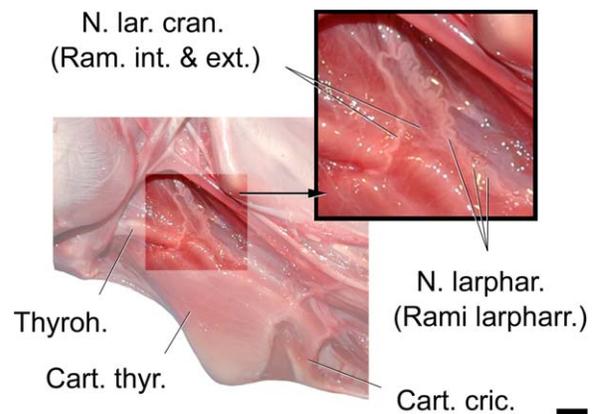


Fig. 7. *Gazella subgutturosa*. The cranial laryngeal nerve and the branches of the laryngopharyngeal nerve, supplying the larynx and the caudal pharyngeal constrictors, in an adult male goitred gazelle. In the resting state, these nerves are packed in densely meandering loops. Overall view (left) and detail (right). Left lateral view, rostral is to the left. Scale bar 10 mm. Cart. cric., cricoid cartilage; Cart. thy., thyroid cartilage; N. lar. cran. (Ram. int. & ext.), cranial laryngeal nerve (internal and external branch); N. larphar. (Rami larpharr.), laryngopharyngeal nerve (laryngopharyngeal branches), Thyroh., thyrohyoid.

TABLE 5. Mean dimensions of the trachea (mm).

Measure	Neonates		Adolescents		Adults	
	Male (n = 2)	Female (n = 2)	Male (n = 2)	Female (n = 2)	Male (n = 3)	Female (n = 3)
Length of trachea	107.0 ± 17.0	125.5 ± 2.6	194.8 ± 6.8	217.7 ± 3.8	231.3 ± 23.0	256.7 ± 7.6
Number of tracheal cartilages	49.0 ± 0	45.5 ± 3.5	47.0 ± 5.7	45.0 ± 0	47.0 ± 2.6	50.8 ± 7.6
Dorsoventral external Ø at rostral end	8.8 ± 0	9.0 ± 0	12.5 ± 3.5	13.5 ± 0.7	15.7 ± 3.1	16.0 ± 1.0
Transverse external Ø at rostral end	7.0 ± 2.8	9.0 ± 1.4	17.0 ± 0	13.5 ± 2.1	21.0 ± 3.6	21.0 ± 1.0
Dorsoventral external Ø caudal to cricoid spine	9.5 ± 3.5	10.0 ± 0	17.0 ± 4.2	17.0 ± 1.4	21.0 ± 1.0	20.3 ± 1.2
Transverse external Ø caudal to cricoid spine	8.0 ± 0	8.5 ± 0.7	21.0 ± 1.4	20.5 ± 0.7	25.7 ± 0.6	25.7 ± 2.1

Ø = diameter. Neonates 1–7 days; Adolescents 6–9 months; Adults ≥ 24 months.

while the larynx is in its resting position (Fig. 7). In adolescents and adult females, corresponding with their intermediate length of the pharynx, higher resting position of the larynx and, compared to adult males, much less pronounced capability of momentary larynx retraction, the course of those nerves is intermediate between that of neonates and adult males.

### Trachea

The trachea consists of longitudinally connected tracheal cartilages in form of incomplete, dorsally open rings connected to each other by circular annular ligaments and by the membranous wall covering the resulting longitudinal dorsal gap along the total length of the trachea. A sexual dimorphism of the length of the trachea or the number of tracheal cartilages cannot be substantiated at any age (Table 5). In neonates, the diameter of the tracheal cartilages at the rostral end of the trachea does not differ from the diameter of the remaining part of the trachea and comprises approximately 8–9 mm throughout (Table 5). In adults, the dorsoventral diameter of the first four to five tracheal cartilages is reduced by 20–30% compared to the remaining part of the trachea and they are tilted caudally at an angle of 20 degrees, approximately. This causes a slight dorsal bending of the trachea relative to the larynx. Dorsally, the first tracheal cartilages are covered by the thin and flexible, caudal portion of the cricoid plate (Fig. 3B).

### DISCUSSION

This is the first study of a nonhuman mammal with a descended larynx that quantitatively documents the progressive development of sexual dimorphism of the vocal organs along ontogeny. To our knowledge, the ontogenetic development of the vocal organs in mammals has only been investigated in humans (e.g., Fitch and Giedd, 1999; Lieberman et al., 2001). The vocal organs are sexually dimorphic in adult goitred gazelles yet isomorphic in male and female neonates. Sexually dimorphic features in adults comprise: larynx size,

permanent larynx position, momentary larynx retraction, vocal fold size, thyrohyoid length, thyrohyoid ligament length, pharynx length, soft palate length, vocal tract length, hyoepiglottic muscle length, width of sternothyroid muscle, meandering of laryngeal and pharyngeal nerves. Divergence of the ontogenetic vocal development began in six to nine months-old adolescents and then developed progressively towards full expression in adults. The pronounced sexual dimorphism of the vocal organs in adults appears to be mainly a consequence of a high selection pressure on males for the production of effective rutting calls (Frey et al., 2011). Therefore, larynx and vocal fold enlargement and additional larynx descent in male goitred gazelles may serve as a model for the corresponding events in male humans (cf. Fitch and Giedd, 1999). These male-specific secondary sexual features in goitred gazelle appear to have evolved by sexual selection for the acoustic attraction of females and the acoustic deterrence of rivals. These acoustic functions are effected by a low fundamental frequency and by low, closely spaced formant frequencies. Corresponding features in male humans may serve similar functions and, thus, are not related to speech ability as already suggested by Fitch and Giedd (1999). Apparently, sexual selective pressures towards imposing “deep” male voices are similar in male goitred gazelles and male humans.

By contrast, natural selective pressures, responsible for the moderate laryngeal descent in both sexes may differ between goitred gazelle and humans. In humans, natural selection may have led to the evolution of the unique “two-tube” vt morphology that is favorable for speech production (Lieberman et al., 1969; Lieberman, 1975, 1984; Fitch and Giedd, 1999). In goitred gazelle, the evolutionary driving force for the moderate larynx descent in both sexes has not yet been identified. It might be related to supportive length changes of the pharynx, involving higher laryngeal mobility, in the context of regurgitation and swallowing of the cud (Supporting Information, Video 1). But further research is needed for clarifying this issue.

Apparently, larynx descent in female goitred gazelles reaches a moderate end state at the end of adolescence whereas larynx descent in males continues well into adulthood until their pronounced end state is reached. As a consequence, the postnatal ontogenetic development of the vocal organs takes considerably longer in males than in females.

In addition, the sexual dimorphism of the vocal organs in goitred gazelle might relate to the different ages at which females and males reach “social sexual maturity” and to differing male and female reproductive strategies in polygynous species (Clutton-Brock and Harvey, 1978; Blank 1985, 1998).

### Larynx

**Larynx size.** In a comparative perspective and consistent with previous findings, the larynx of goitred gazelles is prominently enlarged relative to body size, at any age and in either sex (Güldenstaedt, 1780; Efremova et al., 2011a,b; Frey et al., 2011; Volodin et al., 2011). A sexual dimorphism of larynx size was lacking in neonates, but developed in the course of adolescence and was most evident in adults. In adolescents both overall larynx size and the sizes of thyroid and cricoid cartilages were larger by 10–15% in males than in females. In addition, the cricoid spine length was longer by 8.5% in males than in females. In adults, a pronounced sexual dimorphism had established even when taking into account a 5% difference of linear body dimensions between sexes (Kingswood and Blank, 1996): males exceeded females by 25% in overall larynx size, by 30% in thyroid cartilage size and by 12% in larynx height. A sexual dimorphism of the cricoid spine length had disappeared in the adults.

**Vocal folds.** A sexual dimorphism of the vocal folds was not yet detectable in neonates but developed in adolescents. Males exceeded females by about 14% in dorsoventral and 20% in rostrorocaudal vocal fold length. Sexual dimorphism in adults was still more pronounced as males exceeded females by about 59% in dorsoventral and 40% in rostrorocaudal vocal fold length. The actual sexual dimorphism of the vocal folds in adolescents and adults may be slightly less than the above figures as a sexual dimorphism of linear body dimensions of about 5% in adults and less than 5% in adolescents are reported (Kingswood and Blank, 1996). However, a sexual dimorphism of skull size in adult goitred gazelles of known age could not be substantiated (Mambetjumaev, 1970).

### Fundamental Frequency

Male goitred gazelles produce a fundamental frequency as low as 22 Hz during emission of their rutting calls (Frey et al., 2011). In other rumi-

nants, in which males possess vocal fold lengths comparable to that of male goitred gazelles (saiga antelopes: 25 mm, Frey et al., 2007; Rocky Mountain elk *Cervus elaphus nelsoni*: 30 mm, Riede and Titze, 2008; Frey and Riede, 2013; and Iberian red deer *C. e. hispanicus*: 30 mm, Frey et al., 2012) the fundamental frequency of male rutting calls, despite mostly larger body size, is higher than in the rutting calls of goitred gazelles (saiga antelopes: 45 Hz, Frey et al., 2007; Rocky Mountain elk: above 1000 Hz, Riede and Titze, 2008; Frey and Riede, 2013; Iberian red deer: 186 Hz, Frey et al., 2012). Although these male rutting calls are produced in a particular, comparable behavioral context, such a wide range of f<sub>0</sub> frequencies may result from different phonation mechanisms (cf. Frey and Riede, 2013), which evolved under different selective pressures in different species or subspecies.

Sexual dimorphism of larynx size and larynx position in goitred gazelles probably arose as a consequence of sexual selection for effective low-frequency rutting calls in males (Frey et al., 2011; Blank et al., 2014). Male rutting calls assist in attracting female mating partners and deterring rival males acoustically. Female goitred gazelles do not produce rutting calls and rarely vocalize during the rut. Their larynges are distinctly smaller than those of the males. An even greater sexual dimorphism of the larynx than in goitred gazelle has been reported for Mongolian gazelle (*Procapra gutturosa*) (Frey and Riede, 2003; Frey et al., 2008b). As in goitred gazelle, only the males exhibit acoustic display during the rut (Frey et al., 2008a). The larynx of female Mongolian gazelles is only about half the size of the male larynx and, as female goitred gazelles, they are mostly silent during the rut.

In contrast, sexual dimorphism of the larynx in polygynous red deer and North American elk, both species with very prominent male acoustic rutting display, is poorly expressed (Frey et al., 2012; Frey and Riede, 2013). Male and female vocalizations are similar in fundamental frequency (Volodin et al., 2015) and hinds are capable of producing call patterns that strongly resemble stag rutting calls (Feighny et al., 2006; Volodin et al., 2016). Taken together, the above quoted results demonstrate that sexual selection for male acoustic display during the rut does not obligatorily lead to the evolution of sexually dimorphic vocal organs.

Despite their smaller larynx, adult female and young goitred gazelles also produce very low-frequency contact calls, for example, in spring during the postnatal nursing period. The vocal fold length of 12–17 mm in adolescent females and of 15–19 mm in adult female goitred gazelles (this study) is comparable to those of adult female (12.5–21.5 mm) and male (17–28.9 mm) humans (Kahane, 1982; Titze, 1994; Roers et al., 2009).

However, the fundamental frequencies of goitred gazelle contact calls are lower than those of human voices even in only 2-weeks-old goitred gazelles (goitred gazelles: 93 Hz and 113 Hz for males and females, respectively, Efremova et al., 2011a; humans: 107–129 Hz and 189–210 Hz for males and females, respectively, Monsen and Engebretson, 1977; Lass and Brown, 1978; Rendall et al., 2005; Apicella et al., 2007; Evans et al., 2008). A potential explanation for this surprising result may come from the peculiar large vocal pads attached to the vocal folds of goitred gazelles.

**Vocal pads.** The large vocal folds of goitred gazelles gain additional size and mass by large connective tissue vocal pads attached to them. Apparently, this assists the vocal folds of goitred gazelles in producing fundamental frequencies as low as 17–28 Hz in male rutting roars (Frey et al., 2011; for amphibians cf. Gridi-Papp et al., 2006; Ryan and Guerra, 2014). Such a low fundamental frequency matches the 13–26 Hz fundamental frequency of the rumbles of African elephants (McComb et al., 2003) despite an enormous disparity of body size. Body mass of African elephants is 4,000–6,300 kg in adult males and 2,200–3,500 kg in adult females (Laurson and Bekoff, 1978; Herbst et al., 2013) whereas body mass of adult male goitred gazelles is  $28.1 \pm 1.7$  kg,  $n = 3$  (Supporting Information S-Table 1). Larynx size and vocal fold length also differ substantially. Larynx height in an adult female African elephant was 112 mm (Herbst et al., 2013) but 54 mm in an adult male goitred gazelle (this study). Correspondingly, the length of the vocal folds in an adult female African elephant was 104 mm (Herbst et al., 2013) but 28–32 mm in adult male goitred gazelles (Frey et al., 2011; this study).

Contrary to theoretical exclusion of vocal fold mass as a determinant of fundamental frequency (Titze, 2011; Riede and Brown, 2013), we suggest re-evaluation of an increase of vocal fold mass, for example, by the addition of a large vocal pad to each of the vocal folds as in goitred gazelle, as a feature that assists in generating lower frequencies. As the vocal pads are already present at birth and increase in size ontogenetically (Efremova et al., 2011a; Frey et al., 2011; this study), this would provide a plausible explanation for the production of lower than expected fundamental frequencies on the basis of vocal fold length in the course of ontogeny. In túngara frogs (*Physalaemus pustulosus*), fibrous masses that are attached to the vocal folds lowered the fundamental frequency and contributed to the complexity of male advertising calls (Gridi-Papp et al., 2006; Ryan and Guerra, 2014).

Histological investigation of the vocal pads in juvenile goitred gazelles showed that the vocal pads mainly consist of collagen and elastic fibers, embedded in an intercellular matrix with few

interspersed fat cells and small blood vessels. The outermost layer of the vocal pads is made up by stratified squamous epithelium (Efremova et al., 2011a). Similar vocal pads, mainly composed of collagen and elastic fibers, were described for the large vocal folds of takins (*Budorcas taxicolor*, Frey and Hofmann, 2000) and Mongolian gazelles (Frey and Gebler, 2003; Frey et al., 2008a,b). Vocal pads with a higher amount of fat were described for saigas (Frey et al., 2007) and for some large felid species of the genus *Panthera*, the so-called “roaring cats” (Peters and Hast, 1994; Klemuk et al., 2011). The gain in size and mass of the vocal folds may not only decrease producible fundamental frequencies but also results in a decrease of the minimal subglottal air pressure from the lungs necessary for the onset of sustained vibrations of the vocal folds, thus decreasing the costs of phonation. For tigers, the minimal subglottal pressure level was as low as 0.2–0.3 kPa, that is, several times lower than in other species (Titze et al., 2010; Klemuk et al., 2011).

Another potential acoustic function of the vocal pads of goitred gazelles might be a desynchronization of vocal fold vibrations, resulting in noisy, hoarse-sounding, atonal calls as a result of the chaotic mode of vocal fold oscillation (cf. Berry et al., 1994, 2006; Eysholdt et al., 2003). Confirmation comes from the pathophysiology of the vocal folds. Inflammatory processes including Reinke’s edema and allergies, often accompanied by a swelling of the vocal folds, that is, by an increase in volume and mass, cause desynchronized asymmetric vibration modes and irregular configurations of the glottis, which, among other effects, lower the fundamental frequency (Ng et al., 1997; Jackson-Menaldi et al., 1999; Colton et al., 2006; Zhang and Jiang, 2004, 2008; Mirza et al., 2010).

**Spine of the cricoid cartilage.** A unique morphological trait of all sex and age classes in goitred gazelle is the spine of the cricoid cartilage, previously interpreted as a structure preventing excessive dorsoventral bending of the larynx-trachea junction at retraction of the larynx during male rutting calls (Frey et al., 2011). Although much less pronounced than in adult males, retraction of the larynx occurs in all other sex and age classes during the production of contact calls (Supporting Information Video 2). Therefore, the spine of the cricoid cartilage cannot be considered as a completely sexually selected trait. Although the spine was longer in adolescent males than in adolescent females (this study), this sexual dimorphism of cricoid spine length had disappeared in adults.

Most bending of the larynx-trachea junction is to be expected at maximal retraction of the larynx almost down to the sternum in adult males while less bending should occur at less retraction. So, perhaps the above functional explanation for adult

males also applies to adult females and younger individuals of either sex. Restricted retraction of the larynx would produce less dorsoventral stress at the larynx–trachea junction and, therefore, would require accordingly shorter cricoid spines. Nevertheless no other species with a retractable larynx possesses a cricoid spine. Hence, we have to assume specific strains at the larynx–trachea junction in goitred gazelle that do not occur in other species with a retractable larynx. A potential source of such specific strains may result from the pronounced caudal inflation of the thyroid cartilage into a prominent thyroid bulla. The considerable difference between the rostral and the caudal height of the larynx may impart a stronger angular momentum to the larynx, which is dampened by the cricoid spine and its sliding along the obliquely oriented slide face situated medioventrally on the trachea. Alternatively, the spine may have a different function.

### Larynx Mobility and Vocal Tract Elongation

Sex differences in vt morphology were practically lacking in neonate goitred gazelles and were only weakly expressed in adolescents, whereas in adults the lengths of the oral and nasal vts and of the thyrohyoid ligament and the hyoepiglottic muscle were all considerably shorter in females than in males (Table 3). Correspondingly, laryngeal descent is least expressed in neonates, increases along ontogeny in both sexes, reaches a moderate end state in adult females and is maximal in adult males. In live goitred gazelles, the larynx descends during vocalization and yawning and elevates during swallowing and regurgitation (Efremova et al., 2011a,b; Frey et al., 2011).

When taking resting length and maximally extended length of the thyrohyoid ligament as a proxy for the capability of larynx retraction, it becomes clear from Table 3 and from observation of live individuals that already neonates of goitred gazelles can retract the larynx to a certain extent (Volodin et al., 2011). At this age there is no difference in larynx retraction between the sexes. In adults, however, a pronounced sexual dimorphism is evident. Maximal retraction of the larynx in adult males exceeds that in adult females about 2.5-fold (Table 3). Although data for adolescents are lacking, we may reasonably assume that their capability of larynx retraction is intermediate between neonates and adults.

In all terrestrial mammals protraction of the larynx for swallowing pushes the laryngeal entrance into the intrapharyngeal ostium, allowing the food to pass around the laryngeal entrance into the oesophagus without the danger of choking (Pommerenke, 1928; Bosma, 1957; Storey, 1968; Donner et al., 1985; Jean, 2001; Miller, 1982, 2002, 2008; Nishino, 2013; Shaker et al., 2013). The cranial

laryngeal nerve is involved in the afferent input related to swallowing (Doty, 1951; Doty and Bosma, 1956; Roman and Car, 1970; Miller, 1972; Jean and Car, 1979; Jean, 2001; Steele and Miller, 2010), thus indicating a close integration of nutritional, respiratory and vocalizing functions of the larynx. As the intra-pharyngeal ostium descends together with the larynx by elongation of the pharynx including the soft palate, the intrapharyngeal ostium and the laryngeal entrance retain their close spatial proximity. Thus, the degree of larynx protraction for swallowing does not differ markedly between species with non-descended and descended larynx but the site of protraction has shifted caudally in the latter.

### Hyoid Apparatus

The hyoid apparatus of goitred gazelles showed an overall size increase in both sexes from neonates to adults as the result of body growth. Disproportional length changes occurred in the thyrohyoid.

In both sexes the relative size of the thyrohyoid increases towards adulthood. In addition to this general tendency, there is a sexual dimorphism of thyrohyoid proportion as the relative elongation of the thyrohyoid is more pronounced in adult males than in adult females (Table 2; Fig. 6). The relative elongation of the thyrohyoid in males might reflect higher demands on the suspension apparatus of the larynx both by increasing permanent descent to a lower resting position and by increasing capability of further momentary descent. Furthermore, relative larynx size and mass differs between sexes and the larger larynx in adult males may require a suspension apparatus more resistant to loading by weight or by pull than that of adult females or of younger individuals of either sex with smaller larynges. The elongated thyrohyoid of males provides a longer lever arm that can resist increased demands with reduced muscle force.

A sexual dimorphism of the osseous hyoid parts has also been demonstrated in humans (Lekšan et al., 2005; Mukhopadhyay, 2010; Kothapalli et al., 2015). The larger dimensions in male humans probably correlate with their larger larynx size. An additional sexual dimorphism of the age up to which ontogenetic increases in hyoid dimensions occur has been reported: in females till the age of 17 but in males till the age of 35 (Mukhopadhyay, 2010).

Compared to domestic ruminants (Nickel et al., 1984; Saber and Hofmann, 1985), in both sexes of goitred gazelles the thyrohyoid was elongated and its caudal part remained cartilaginous even in adults (Fig. 6; Supporting Information S-Fig. 7). Across ages, the connection between this flexible and resilient part of the thyrohyoid and the rostral

horns of the thyroid cartilage was not established by a synovial thyrohyoid articulation, as in many other ruminants, but by an extensible, resilient thyrohyoid ligament.

The tympanohyoid that connects the hyoid apparatus to the skull is flexible and completely visible in neonates but its dorsal part becomes gradually embedded into the lateral wall of the tympanic bulla with increasing age, particularly so in adult males. Thus, the visible part of the tympanohyoid is relatively longer in neonates than in adolescents and adults and in adult females it is less embedded than in adult males. In adult males, the relative shortening of the tympanohyoid by its dorsal inclusion in the wall of the tympanic bulla may avoid excessive caudal rotation of the hyoid apparatus and decrease the tension in the hyoid-skull connection during production of the rutting calls. Mechanical demands on the hyoid-skull connection in adult females can be expected to be less as a result of only moderate larynx retraction.

### Thyrohyoid Ligament

Evaluation of the ontogenetic development of the thyrohyoid ligament is impaired by the lack of data for adolescents (Table 3). In neonates, the thyrohyoid ligament is short and of almost equal length in males and females. It can be manually extended 3.5–4 times its resting length. Resting length increase towards adulthood differs markedly between the sexes: more than 10-fold in males but only sixfold in females (Table 3). This coincides with the externally visible lower resting position of the larynx in males and the higher position in females. The extensibility of the thyrohyoid ligament decreases from about 3.5-fold in neonates to about twofold in adults. As a consequence of the much higher resting length in males, the length at maximal extension is also clearly (about 2.5-fold) higher in males than in females. This coincides well with call-synchronous pronounced momentary larynx retraction in males and only moderate larynx retraction in females during vocalization.

An elongated and resilient thyrohyoid connection also occurs in male red and fallow deer, which also retract their permanently descended larynx further down during production of their rutting calls (Reby and McComb, 2003; Frey et al., 2008a, 2011, 2012; Frey and Riede, 2013). Adult saiga antelopes, which are assumed to lack pronounced retraction of their undescended larynx, also possess a thyrohyoid ligament, though very short, in both sexes (Frey et al., 2007). In large felids with descended and retractable larynx, for example, lions (*Panthera leo*), tigers (*Panthera tigris*), and jaguars (*Panthera onca*), it is the epihyoid, intercalated between the stylohyoid and the ceratohyoid, that has been evolutionarily transformed into a ligament (Weissengruber et al., 2002). As a conse-

quence, the cerato-, basi- and thyrohyoids are retracted together with the larynx during call production. In contrast, cerato-, basi- and thyrohyoids are not retracted in those species possessing a thyrohyoid ligament, as, for example, goitred gazelles. A similar ‘intra-hyoideal ligament’ has also been documented for the koala (Charlton et al., 2013a).

### Connections between Epiglottis and Thyroid Cartilage and between Epiglottis and Hyoid Apparatus

The thyroepiglottic ligament, connecting the epiglottis with the thyroid cartilage medioventrally, and the surrounding lateral and dorsal tissues, including the thyrohyoid membrane and the aryepiglottic folds, are extraordinarily elastic in goitred gazelles but particularly so in adult males. In anatomical specimens of adult males this soft tissue connection was extensible more than two times the resting length (Fig. 5). Similarly, the hyoepiglottic muscle, connecting the base of the epiglottis to the hyoid apparatus, is highly extensible.

The connections between the epiglottis and the thyroid cartilage and between the epiglottis and the hyoid apparatus did not gain resilience with age in both sexes. For the hyoepiglottic muscle, manual extensions demonstrated that the maximum extension remains at 1.5–2 times the resting length from neonate to adulthood (Table 3). Therefore it appears that the increasing extensibility of the connection between the epiglottis and the hyoid apparatus requires corresponding longitudinal growth of the hyoepiglottic muscle. This is substantiated by our data (Table 3).

The resting length of the strongly extensible hyoepiglottic muscle, connecting the base of the epiglottis to the hyoid apparatus, increases with increasing age in all age classes but did not differ between the sexes from neonates via adolescents until the onset of adulthood. In neonates, the resting length of this muscle was even larger in females than in males but this may result from our small sample size per age class. In adults, however, both the resting length and the maximally extended length of males were almost double of those in females (Table 3). Apparently, this sexual dimorphism develops between adolescence and adulthood and it corresponds with sexually dimorphic larynx positions and different capabilities of momentary larynx retraction in adult males and females.

The high extensibility of both the hyoepiglottic muscle and the thyroepiglottic connection might play a role in facilitating momentary retraction of the larynx in all ages of goitred gazelle. High extensibility may reduce resistance to momentary larynx descent and, thus, contribute to the efficiency of the sternothyroid muscle in terms of vt elongation during call production. In addition,

these elastic connections might facilitate maintaining the respiratory position of the laryngeal entrance inside the intra-pharyngeal ostium during activities other than the production of oral calls, e.g. (head down/head up) feeding or locomotion. Furthermore, the contracting hyoepiglottic muscle might act as a synergist of the thyrohyoid muscle in protracting the larynx up to the resting position subsequent to retraction and call production. And it might aid in stabilizing the resting position of the larynx during diverse activities by isotonic contractions.

In male Mongolian gazelles, the hyoepiglottic muscle appears to be extended even in-between calls, because of the low mid-neck resting position and the weight of the large larynx (Frey et al., 2008a). This may also apply to adult male goitred gazelles and, to a lesser extent, to adult females, adolescents and neonates.

### **Thyrohyoid, Sternohyoid, and Sternothyroid Muscles**

The permanent low resting position of the larynx in adult male goitred gazelles is associated with the evolutionary elongation of the pharynx, including the soft palate. Further momentary descent of the larynx during call production is effected by muscle action. The main muscles responsible for the retraction of the larynx are the sternothyroid and sternohyoid muscles while the thyrohyoid muscle, the caudal constrictors of the pharynx (thyropharyngeal and cricopharyngeal muscles), and the hyoepiglottic muscle are involved in returning the larynx up again to its resting position.

Considering the different resting positions of the larynx in adult males and females, one could expect corresponding length differences of those muscles suspending the larynx in the throat or ventral neck region and maneuvering the larynx down and up the ventral neck contour. Muscles rostral to the larynx (e.g., thyrohyoid and hyoepiglottic muscles) should be shorter in females than in males and the main retractor of the larynx, connecting it to the sternal manubrium (the sternothyroid muscle — Table 4), should be longer in females than in males. Unfortunately, the lengths of the sternothyroid muscle could not be measured in all individuals as in several specimens the sternal origin of the sternothyroid muscle was not preserved. However, the lengths of the hyoepiglottic and thyrohyoid muscles were measured and provided evidence for the appearance of a marked sexual dimorphism during postnatal ontogeny. Sex differences were lacking in neonates but later on the lengths of these muscles increased much more in males than in females (see Table 3 and above). Therefore, we may reasonably assume that the length of the sternothyroid muscle is also sexually

dimorphic, although the length of this muscle should decrease in males as its position is caudal to the larynx.

Similar to Mongolian gazelles (Frey et al., 2008a,b), the sternothyroid and sternohyoid muscles were noticeably wider dorsoventrally in adult male goitred gazelles than in adult females (observed during the dissections by KE and RF but not measured). This sexual dimorphism of the main retractors of the larynx in adults corresponds with an increasing size difference of the larynx, with an increasing difference of larynx resting position and with an increasing difference in the degree of momentary larynx retraction towards adult male and female goitred gazelles.

The origin of the initially fused sternothyroid and sternohyoid muscles of goitred gazelles, as that of Mongolian gazelles (Frey et al., 2008a,b) and red deer (Frey et al., 2012; Frey and Riede, 2013), does not extend into the thorax but is solely from the sternal manubrium. By contrast, in the 'roaring felids' (lions, tigers etc.) the sternothyroid muscle evolutionarily extended its origin caudally into the thorax and onto the dorsal surface of the sternum (Weissengruber et al., 2007).

In adult male goitred gazelles, the sternothyroid muscle is capable of very strong contraction for momentary retracting the larynx down towards the thoracic inlet during production of the rutting calls. At the same time, the pharyngeal muscles and the hyoepiglottic muscle are strongly extended. The shortening of the sternothyroid muscle during rutting calls was estimated as high as 2.5 times the resting length, approximately (Frey et al., 2011). These data suggest that the strongly contracting muscles of goitred gazelles, involved in the process of larynx retraction, might have a peculiar structure of the sarcomeres to allow their extraordinary alternating shortening and extension. Possibly, their structure resembles that of supercontracting muscles in invertebrates (Hoyle et al., 1965; Leyton and Ullrick, 1970) or in the hyoglossal muscles of certain chamaeleons (Rice, 1973; Herrel et al., 2001, 2002).

### **Pharynx and Nerves**

In those species with a permanently descended larynx in the adults, including goitred gazelle, the elongation of the pharynx and the soft palate, entailing an elongated vt, emerge as a consequence of the evolution of a low resting position of the larynx (Frey et al., 2008a, 2011, 2012). The larynx gradually descends in the course of the ontogenetic development. For demonstrating this descent, the length of the soft palate can be taken as a proxy to pharynx length (Table 3). Pharynx length did not yet differ between the sexes in neonates and adolescents. Neonates, representing a model for the ancestral high position of the larynx,

have a short pharynx, adult females, representing a model for a slightly descended larynx, have a moderately elongated pharynx while adult males, representing a model for a pronouncedly descended larynx, have a strongly elongated pharynx. Larynx position and pharynx length in female and male adolescents are intermediate in comparison with neonates and adults (Table 3). Pharynx length in all age classes may be larger in live animals, but particularly in adult males with their large larynx, as the pull of the intrathoracic parts of the oesophagus (digestive tract), trachea (lungs) and large blood vessels (aorta, cranial caval vein, heart) plus the pull of the larynx induced by gravity are lacking in the anatomical specimens.

Apart from the sexually dimorphic permanent elongation of the pharynx resulting from the evolutionary descent of the larynx, momentary retraction of the larynx during vocalizations causes an additional short-term extension of the pharynx and its accompanying blood vessels and nerves (Frey et al., 2011, 2012). Generally, the capability of larynx retraction increases with age in both sexes, though to different degrees. In contrast to connective tissue, muscular tissue and blood vessels, nerves cannot be extended. Therefore, the evolution of momentary larynx retraction requires the elongation of the respective nerves and the formation of reserve loops that are straightened in the moment of retraction and reformed when the larynx returns to its resting position. Loops and pronounced bending of the nerves in the pharyngeal region, that is, between the skull base and the larynx, of adult male goitred gazelle have been demonstrated in this (Fig. 7) and a preceding study (Frey et al., 2011) and were also described for red deer (Frey et al., 2012).

As expected, the meandering course of the involved laryngeal and pharyngeal nerves did not yet occur in male and female neonates. This is in agreement with their high larynx position, that is, the short distance between the skull base, from which these nerves emerge, and the limited ability of larynx retraction in neonates. Nerve bending increases with age in both sexes of goitred gazelle but does not yet differ markedly between sexes in adolescents. In adults however, the formation of loops is much more pronounced in males than in females in accordance with the sexual dimorphism of momentary larynx retraction.

### Trachea

In view of the sexually dimorphic resting positions of the larynx in adult males and females, one could expect a longer trachea in females than in males. Although the trachea was shorter in males across all ages, even in neonates, the differences were non-significant (Table 5). Probably, the severing of the trachea from its thoracic attachments

and the incompleteness of the trachea in several of the anatomical specimens might have obscured existing differences in tracheal length between sexes. Further studies of more individuals by considering the entire length of the trachea, from the larynx to the bifurcation, might reveal a sexual dimorphism of this trait in goitred gazelles.

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## Supplementary online material

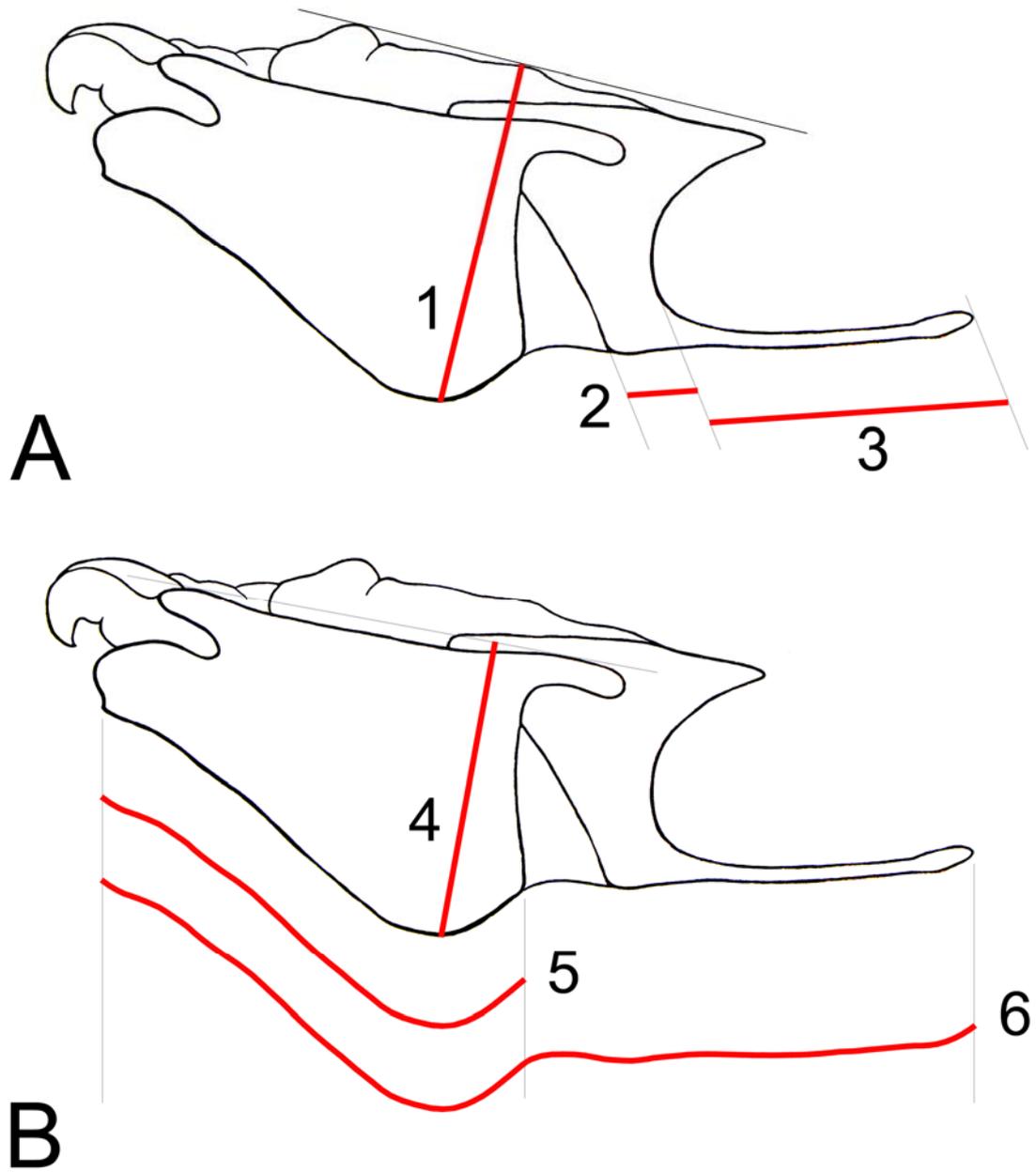
### The postnatal ontogeny of the sexually dimorphic vocal apparatus in goitred gazelles (*Gazella subgutturosa*)

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Guido Fritsch, Natalia V. Soldatova, Elena V. Volodina

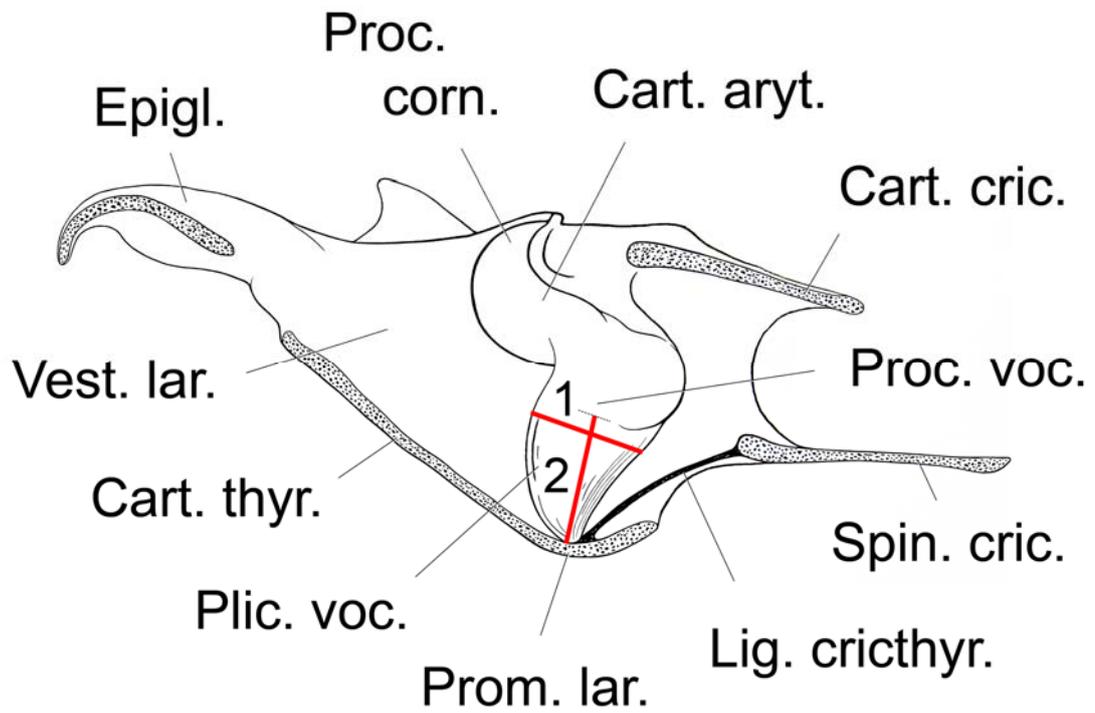
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**Supplemental S-Table 1.** Body mass of adult male goitred gazelles, taken from live animals.

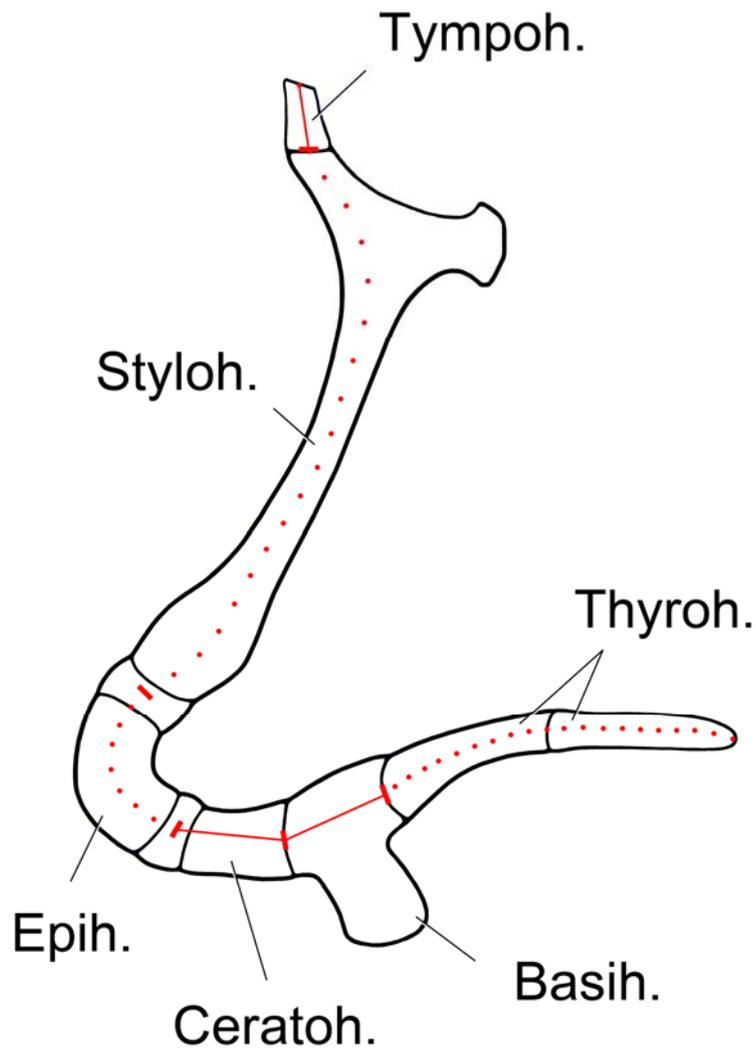
Date	Individual	Age	Body mass (kg)
04.05.2008	Galamus	4 years	29.0
10.05.2009	Bembi	3 years	26.1
03.06.2010	Bogdan	3 years	29.2



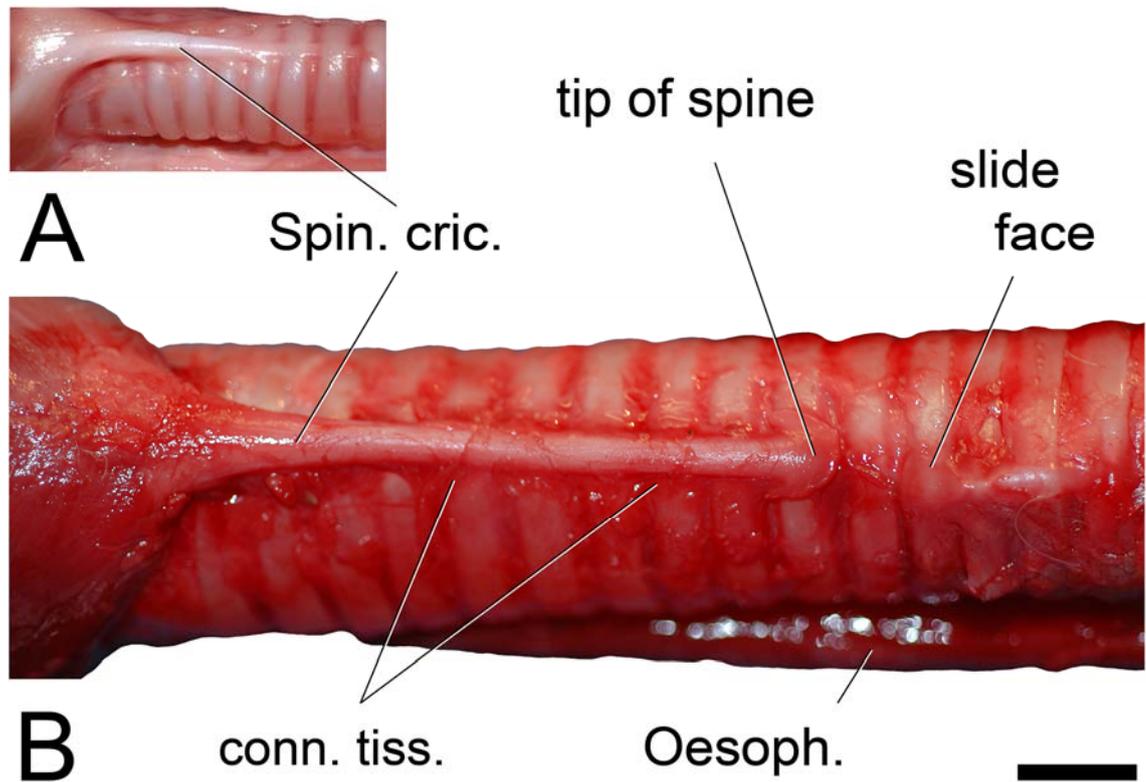
**S-Fig. 1.** *Gazella subgutturosa*. Measurements done on the intact excised larynx of goitred gazelles. Left lateral view). A: maximum height of the larynx (1); ventral length of cricoid cartilage (2); length of the spine of the cricoid cartilage (3); B: height of the thyroid cartilage (4); ventral length of the thyroid cartilage (5); ventral length of the entire larynx (6).



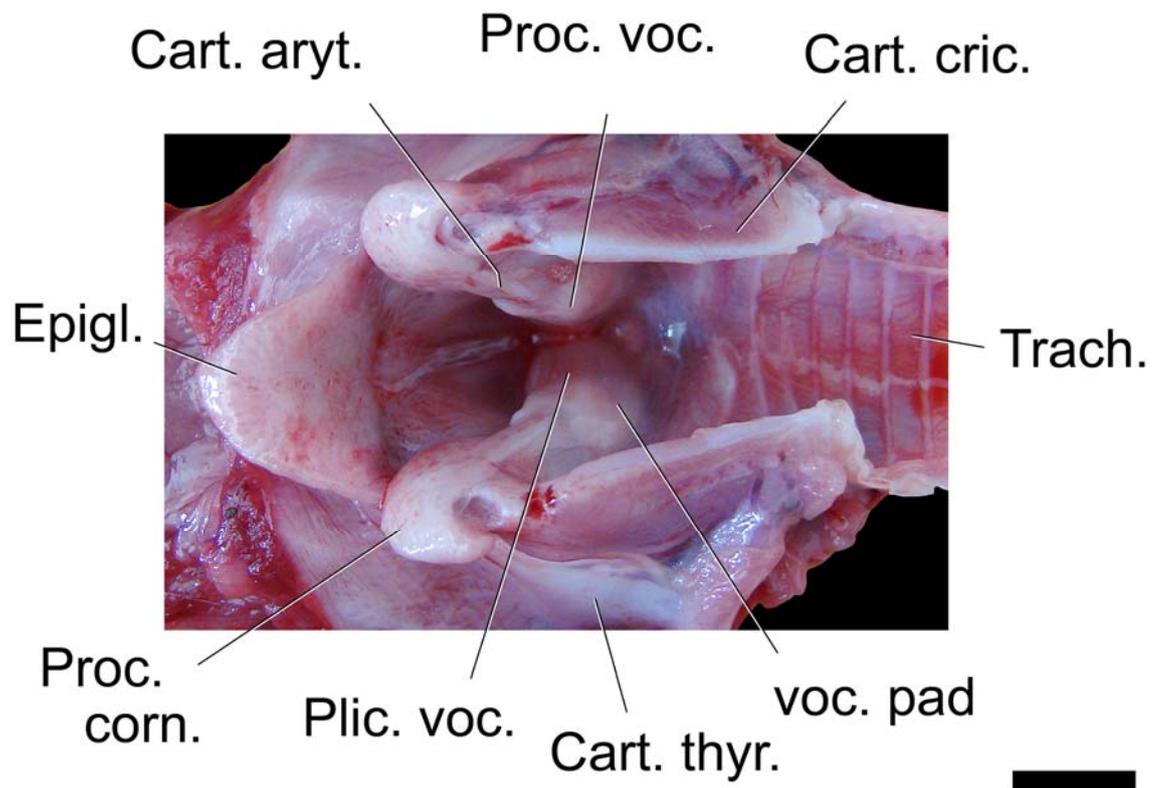
**S-Fig. 2.** *Gazella subgutturosa*. Measurements done on the vocal folds of goitred gazelles. Mediosagittal section of the larynx, right half, medial view. The rostro-caudal (1) and dorso-ventral (2) lengths of the vocal fold are indicated in red. Cart. aryt., arytenoid cartilage; Cart. cric., cricoid cartilage; Cart. thy., thyroid cartilage; Epigl., epiglottis; Lig. cricthy., cricothyroid ligament; Plic. voc., vocal fold; Proc. corn., corniculate process of arytenoid cartilage; Proc. voc., vocal process of arytenoid cartilage; Prom. lar., laryngeal prominence; Spin. cric., cricoid spine; Vest. lar., laryngeal vestibulum.



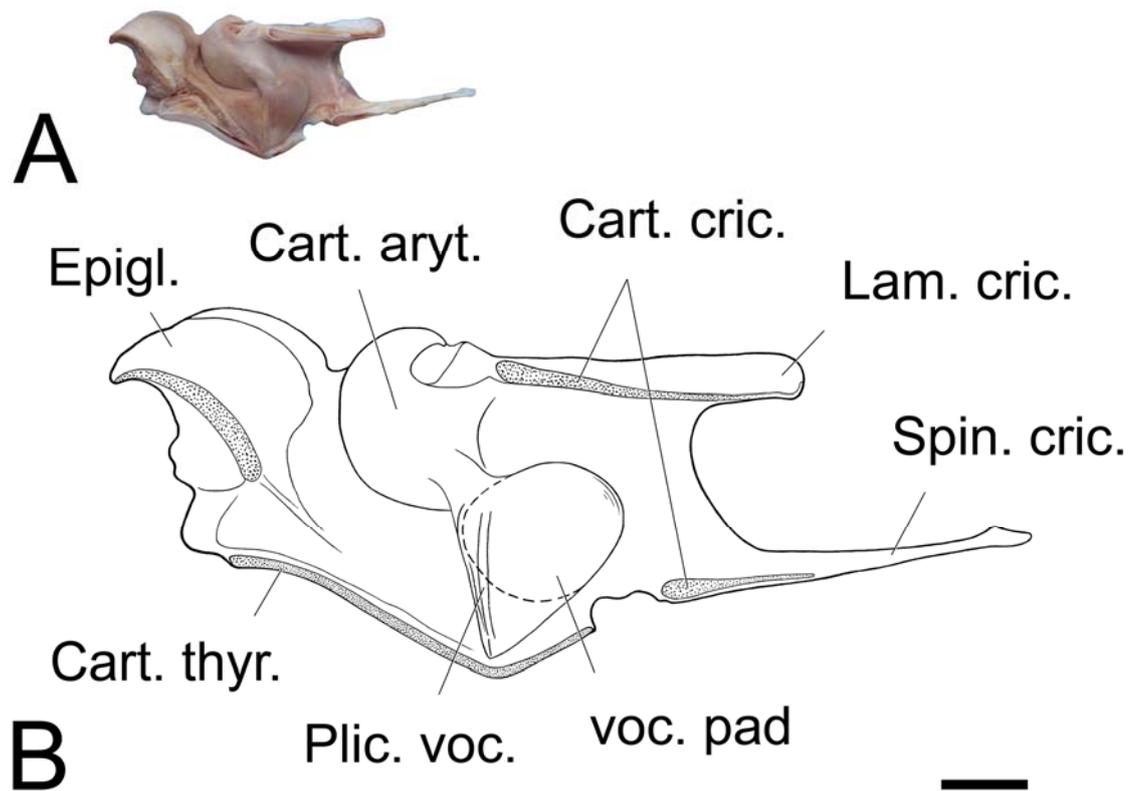
**S-Fig. 3.** *Gazella subgutturosa*. Measurements of the hyoid apparatus of an adult female goitred gazelle. Left lateral view. Ruler measurements are indicated by straight solid red lines; string measurements are indicated by curved dotted red lines. Basih., basihyoid; Ceratoh., ceratohyoid; Epih., epihyoid; Styloh., stylohyoid; Thyroh., thyrohyoid; Tympoh., tympanohyoid.



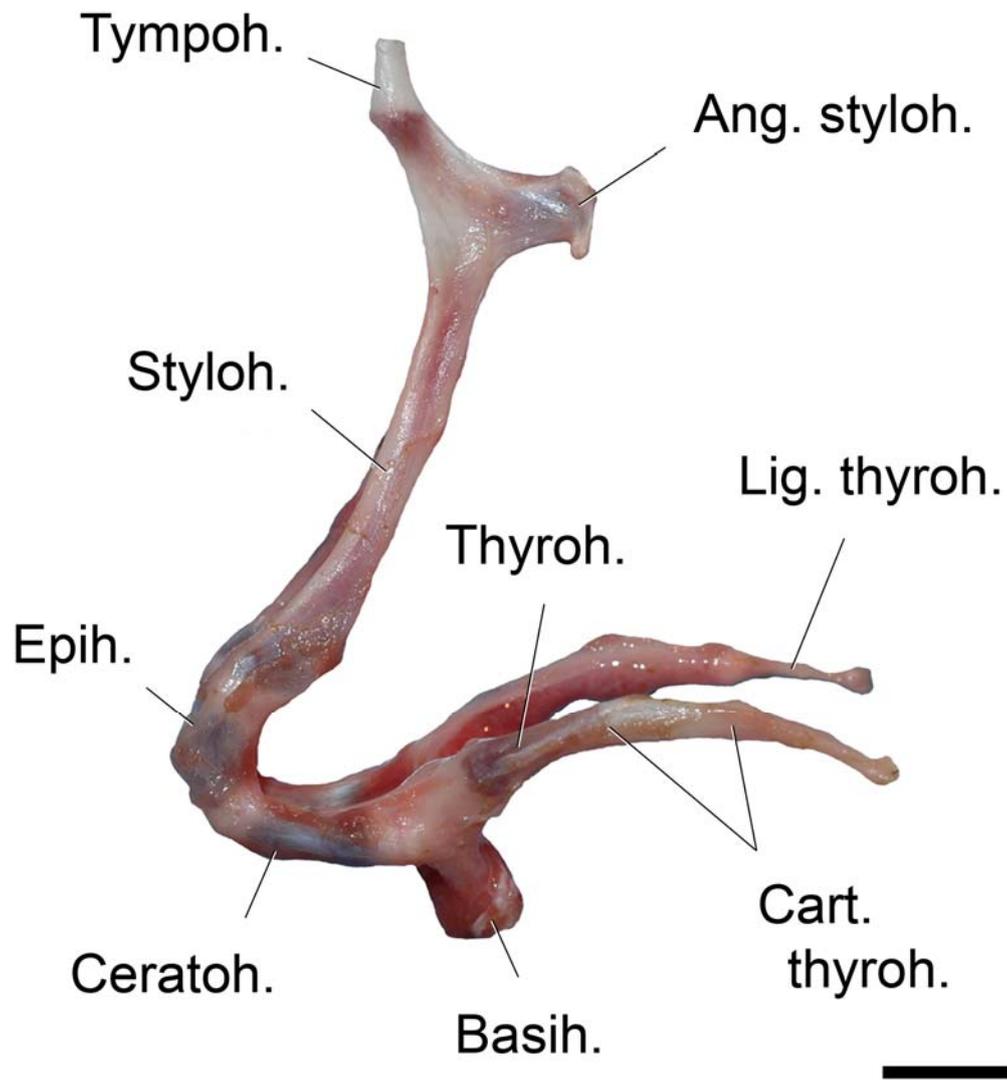
**S-Fig. 4.** *Gazella subgutturosa*. The spine of the cricoid cartilage of a neonate male (A) and an adult male (B) goitred gazelle. Ventral view of the caudal end of the larynx and the cranial end of the trachea, cranial is to the left. Scale bar 10 mm. Oesoph., oesophagus; Spin. cric., cricoid spine.



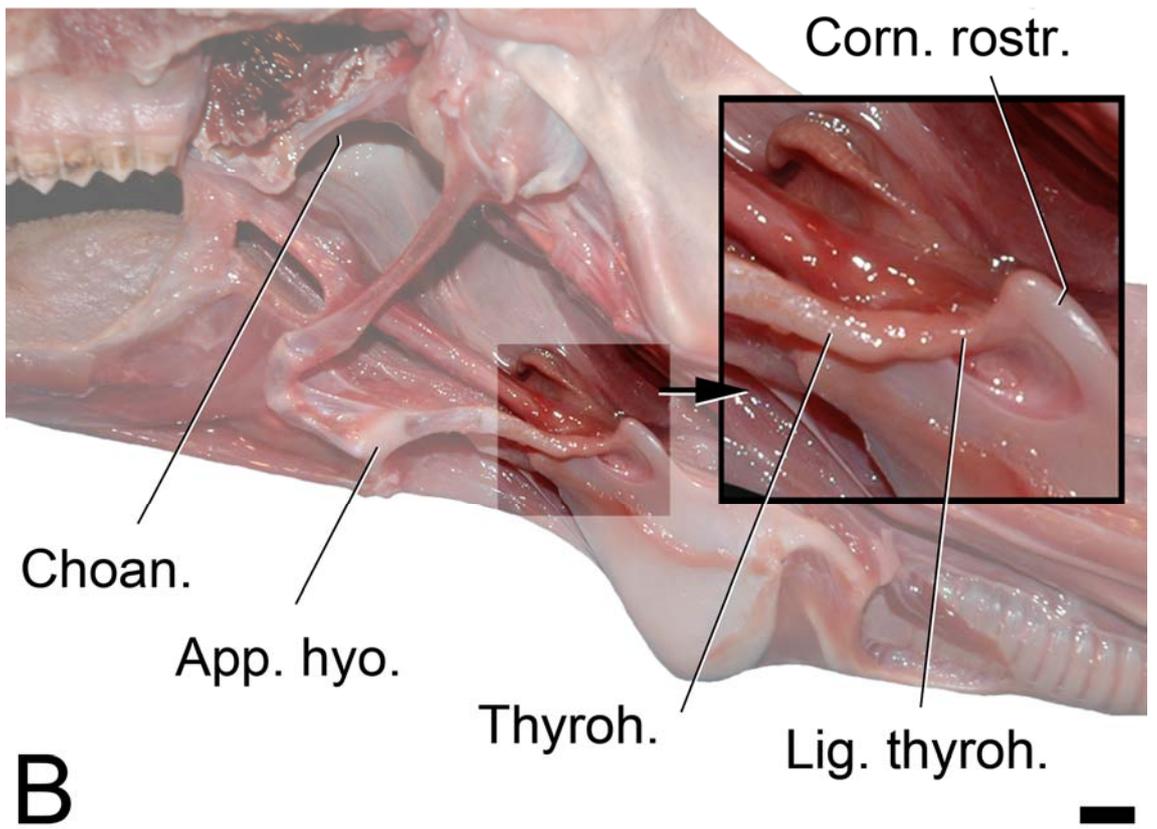
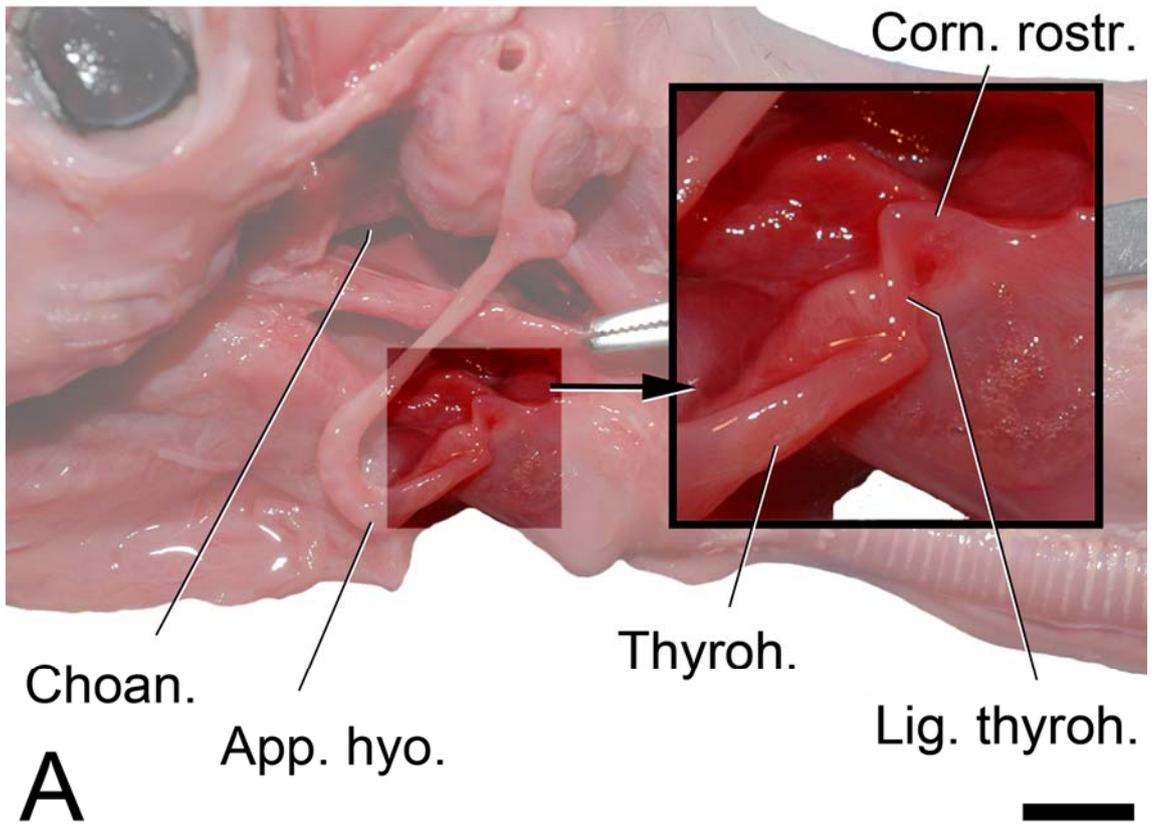
**S-Fig. 5.** *Gazella subgutturosa*. The larynx and vocal pads of a neonate male goitred gazelle. The larynx has been dorsally split between the arytenoid cartilages and medio-sagittally along the cricoid plate and the thyroid cartilage set under bilateral pull to provide insight into the laryngeal cavity (vocal pads). The epiglottis and the ventral parts of the larynx are intact. Dorsal view, rostral is to the left. Scale bar 10 mm. Cart. aryt., arytenoid cartilage; Cart. cric., cricoid cartilage; Cart. thyr., thyroid cartilage; Epigl., epiglottis; Plic. voc., vocal fold; Proc. corn., corniculate process of arytenoid cartilage; Proc. voc., vocal process of arytenoid cartilage; Trach., trachea; voc. pad, vocal pad.



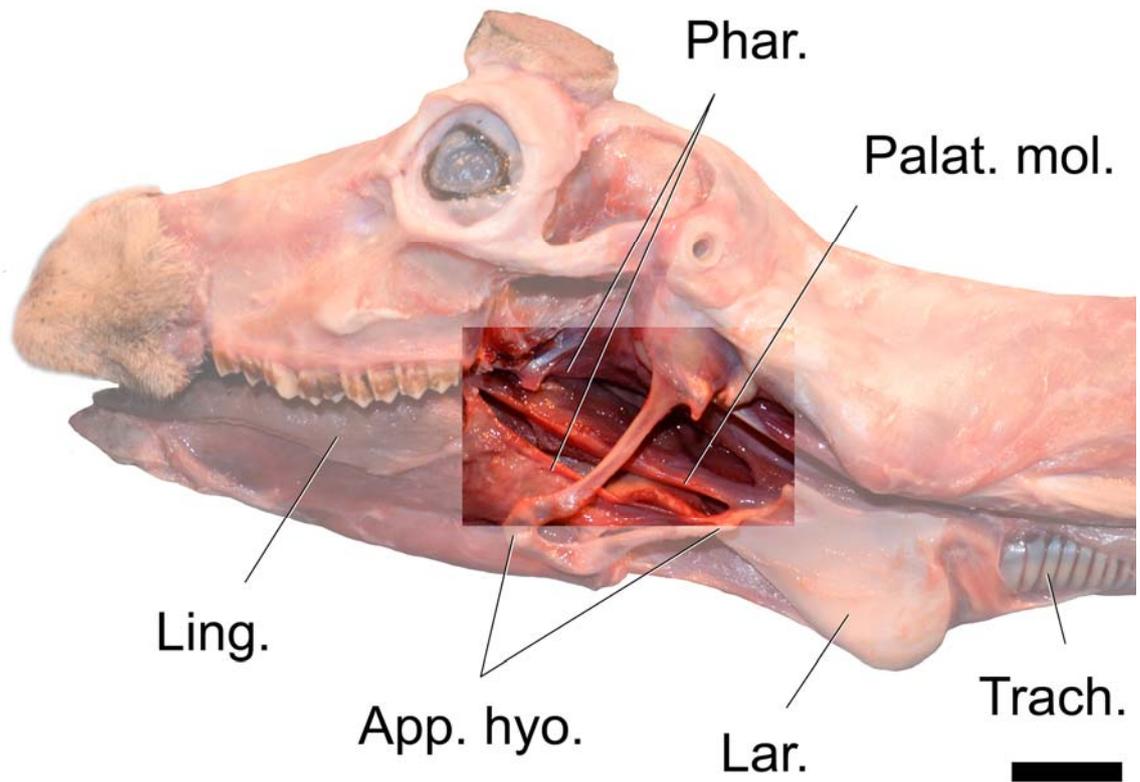
**S-Fig. 6.** *Gazella subgutturosa*. Laryngeal cavity and vocal fold of an adult female goitred gazelle. A: Dissection photo; B: Drawing of relevant features. Mediosagittal section. Right half of larynx, medial view. Scale bar 10 mm. Cart. aryt., arytenoid cartilage; Cart. cric., cricoid cartilage; Cart. thy., thyroid cartilage; Epigl., epiglottis; Plic. voc., vocal fold; Lam. Cric., cricoid lamina; Spin. cric., cricoid spine; voc. pad, vocal pad.



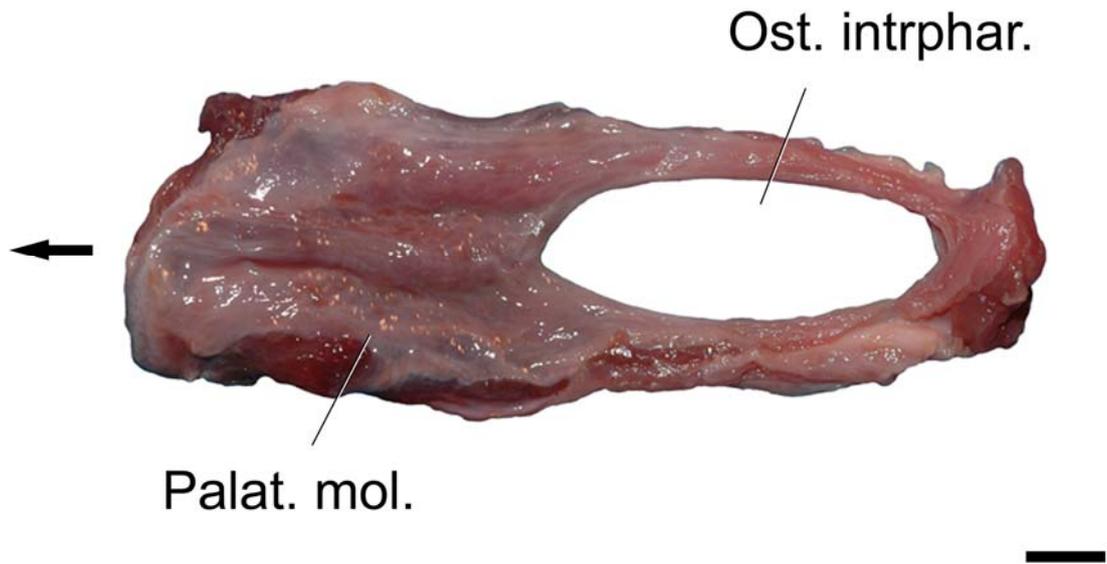
**S-Fig. 7.** *Gazella subgutturosa*. The hyoid apparatus and the thyrohyoid ligaments of an adult female. The thyrohyoid ligaments are still connected to the cartilaginous parts of the thyrohyoids. Left lateral view. Scale bar 10 mm. Ang. styloh., angle of stylohyoid; Basih., basihyoid; Cart. thyroh., thyrohyoid cartilage; Ceratoh., ceratohyoid; Epih., epihyoid; Lig. thyroh., thyrohyoid ligament; Styloh., stylohyoid; Thyroh., thyrohyoid; Tymphoh., tympanohyoid.



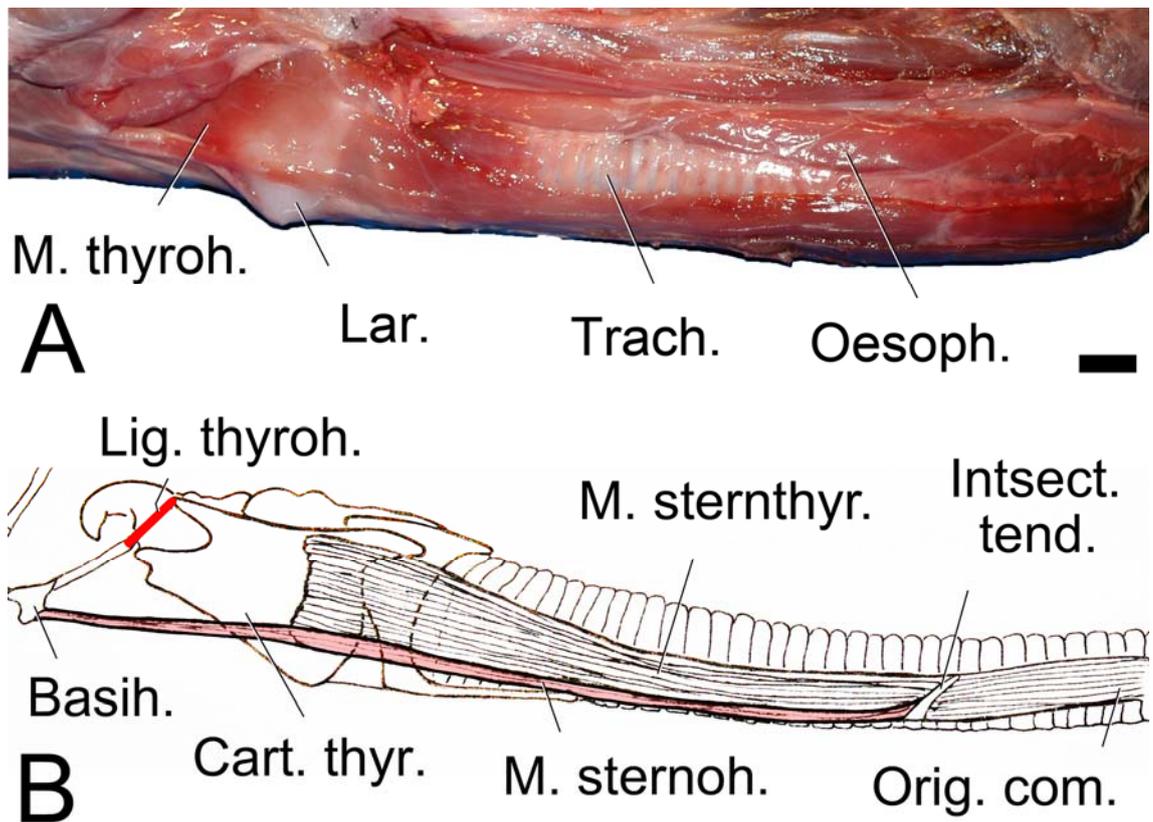
**S-Fig. 8.** *Gazella subgutturosa*. Thyrohyoid connection in a neonate male (A) and in an adult male (B). In the neonate, the thyrohyoid and the rostral horn overlap and the thyrohyoid ligament is very short. Left lateral view. Scale bar 10 mm. App. hyo., hyoid apparatus; Choan., choanae (internal nares); Corn. rostr., rostral horn of thyroid cartilage; Lig. thyroh., thyrohyoid ligament; Thyroh., thyrohyoid.



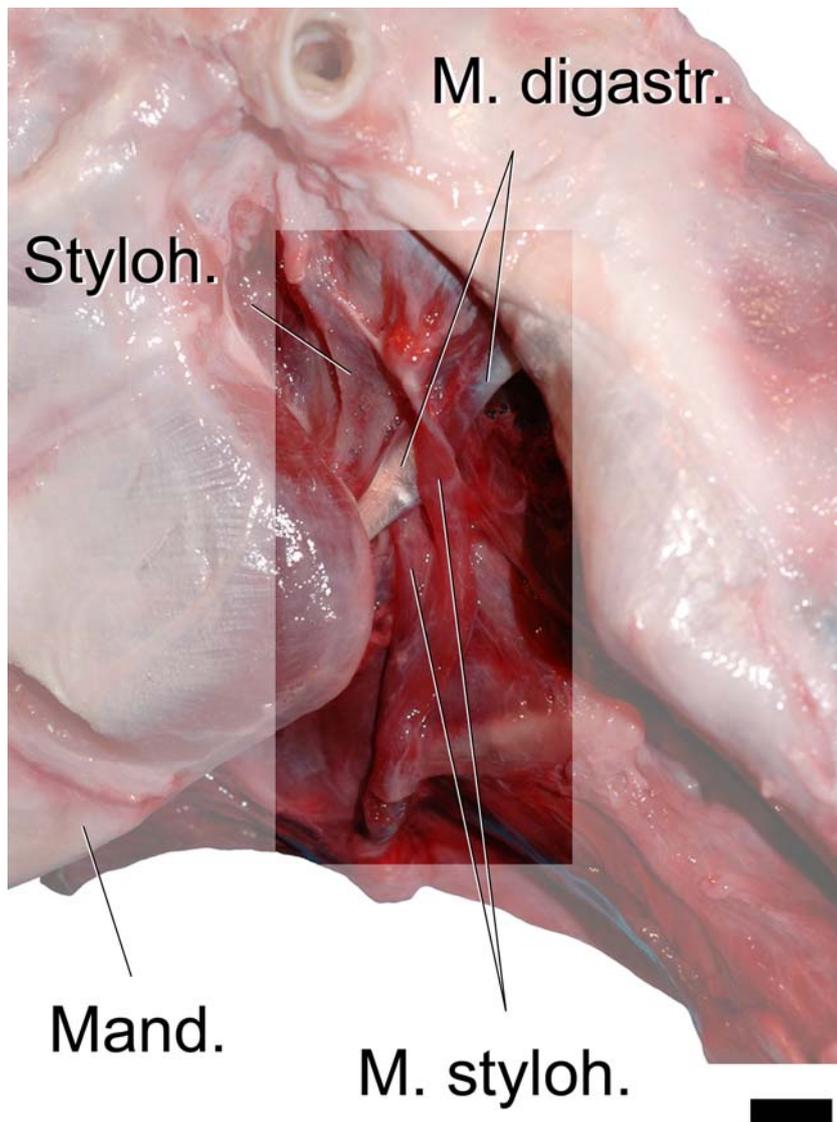
**S-Fig. 9.** *Gazella subgutturosa*. The dorsal and ventral compartments of the pharynx in an adult male goitred gazelle. The pharynx has been opened laterally, exposing the long soft palate. Left lateral view. Scale bar 30 mm. App. hyo., hyoid apparatus; Lar., Larynx; Ling., tongue; Palat. mol., soft palate; Phar., Pharynx; Trach., trachea.



**S-Fig. 10.** *Gazella subgutturosa*. The soft palate of an adult male goitred gazelle and the intra-pharyngeal ostium. This opening is the sole connection between naso- and oropharynx. Dorsal view, rostral is to the left (arrow). Scale bar 10 mm. Ost. intrphar., intrapharyngeal ostium; Palat. mol., soft palate.



**S-Fig. 11.** *Gazella subgutturosa*. Sternothyroid and sternohyoid muscles of an adult male. Both muscles have a common origin from the sternal manubrium (not shown) and are fused along their initial portion. The site where they divide into two separate muscles is cranial to the tendinous intersection. Dissection photo (top); drawing of crucial features (bottom). Left lateral view. Scale bar 10 mm. Basih., basihyoid; Cart. thyr., thyroid cartilage; Intsect. tend., tendinous intersection (M. sternthyr./M. sternoh.); Lar., Larynx; Lig. thyroh., thyrohyoid ligament; M. sternoh., sternohyoid muscle; M. sternthyr., sternothyroid muscle; M. thyroh., thyrohyoid muscle; Oesoph., oesophagus; Orig. com., common origin (of M. sternthyr. and M. sternoh.); Trach., trachea.



**S-Fig. 12.** *Gazella subgutturosa*. Stylohyoid muscle and caudal portion of the digastric muscle of an adult male. In some specimens, the stylohyoid muscle was not uniform but subdivided into two bundles, thus forming a passage for the caudal portion of the digastric muscle. Origin and termination of the stylohyoid muscle remained single, respectively. Left lateral view, rostral is to the left. M. digastr., digastric muscle; M. styloh., stylohyoid muscle; Styloh., stylohyoid.

**Supplemental Video 1.** Larynx movements in an adult female goitred gazelle during rumination, swallowing, regurgitation and immediate swallowing of liquid after regurgitation. Swallowing: rapid ascent and descent of larynx. Regurgitation: slow ascent and incomplete descent of the larynx followed by immediate swallowing (rapid ascent and descent of the larynx).

**Supplemental Video 2.** Juvenile male goitred gazelle retracts its larynx during vocalization.