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1 **Anatomy of the upper respiratory tract in domestic birds, with**
2 **emphasis on vocalization**

3

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16

17 With 16 figures

18

19 Short title: Avian vocalization

20

21 **Abstract**

22 This work reviews the anatomy of the upper respiratory tract in domestic birds including
23 the chicken and pigeon. Non-exhaustive additional information on other bird species,
24 illustrating the extraordinary diversity in the biological class *Aves*, can be found in
25 several footnotes. The described anatomical structures are functionally considered in
26 view of avian sound production. In particular the *Syrinx* is invaluable. Its most important
27 structures are the *Labia* and the lateral and medial tympaniform membranes in non-
28 songbirds and songbirds, respectively. These structures produce sound by vibrating
29 during expiration and eventually inspiration.

30

31 **Key words:** Bird; Anatomy; Upper respiratory tract; Vocalization

32

33 ANATOMY OF THE UPPER RESPIRATORY TRACT

34 **Nasal cavity**

35 The nasal cavity (*Cavitas nasalis*) of the chicken (*Gallus gallus domesticus*) and pigeon
36 (*Columba livia domestica*) is dorsally bordered by the frontal process (*Processus*
37 *frontalis*) of the incisive bone (*Os incisivum syn. Os premaxillare*), the intermaxillary
38 process (*Processus intermaxillaris*) of the nasal bone (*Os nasale*), and the lacrimal bone
39 (*Os lacrimale*). The lateral border is delineated by the maxillary process (*Processus*
40 *maxillaris*) of the nasal bone. The floor of the nasal cavity is formed rostrally by the
41 maxillary process of the incisive bone and caudally by the palatine bone (*Os palatinum*).
42 The large orbits limit the nasal cavity caudally (Fig. 1).

43 In most avian species, the nasal openings (*Nares*) are located dorsolaterally at the
44 basis of the upper beak. In the chicken, they are enclosed by a dorsal and ventral
45 cartilaginous plate. Both plates join at their caudal borders where a shallow internal niche
46 is formed (Fig. 2). The dorsal cartilaginous plate is covered by keratinized epithelium
47 denoted as the *Operculum* (McLelland, 1990). Furthermore, it may be raised by the cere
48 (*Cera*) in some species, such as the domestic pigeon and the budgerigar (*Melopsittacus*
49 *undulatus*) (Fig. 3). In the latter species, the colour of the cere is gender-dependent.

50 The nasal openings are connected to the nasal cavity where the inhaled air is
51 filtered from the larger particles, warmed and moisturized (Fedde, 1998). In fowl, the
52 nasal cavity can be divided into the rostral *Vestibulum nasi* and the caudal *Cavum nasi*
53 *proprium*. The former structure is lined by a squamous epithelium (Nickel et al., 1977)
54 and is encircled by a curved lamella that projects mediorostrally from the caudal part of
55 the dorsal cartilaginous plate, which partly covers the nasal opening, towards the nasal

56 septum (Fig. 2). This lamella should not be mistaken for a rostral nasal concha, which is
57 absent in the chicken and pigeon (Fitzgerald, 1969). In these two bird species, as well as
58 in many other bird species, the nasal cavity is completely partitioned by the cartilaginous
59 nasal septum (*Septum nasi*) into a left and right part, which is denoted by the term *Nares*
60 *imperviae* (Fig. 4).¹

61 The inhaled air flows medial to the lamella of the upper cartilaginous plate and
62 lateral to the nasal septum to reach the nasal cavity. In most avian species, including the
63 chicken and pigeon, the nasal cavity contains a ventral and dorsal nasal concha (Fig. 5).
64 The ventral nasal concha (*Concha nasalis ventralis*) is larger, more rostrally situated and
65 consists of a thin cartilaginous lamella lined by a mucociliary respiratory epithelium (Fig.
66 6). This concha arises from the dorsolateral wall of the nasal cavity and extends in
67 caudoventral direction towards the choanae (Fig. 5). Ventral to the ventral nasal concha,
68 the nasolacrimal duct (*Ductus nasolacrimalis*) discharges into the nasal cavity by a slit-
69 like opening (Nickel et al., 1977). The smaller dorsal concha (*Concha nasalis dorsalis*)
70 originates on the ethmoid bone and projects into the nasal cavity (Fig. 5). Its rostral part
71 is covered by a mucociliary epithelium, whereas the caudal part is lined by an olfactory
72 epithelium involved in odour perception (Fitzgerald, 1969). This concha is absent in the
73 pigeon (Nickel et al., 1977).

74 The pyramidal infraorbital sinus (*Sinus infraorbitalis*) is lined by a very thin
75 mucosa and lies lateral to the nasal cavity, rostroventral to the eye and caudal to the
76 maxillary process of the nasal bone. The infraorbital sinus is connected to the cavity
77 enclosed within the dorsal nasal concha by a mediodorsal fissure, which is broad in the

¹ In aquatic birds, such as the duck (*Anas platyrhynchos*), the nasal septum presents a large aperture (*Nares perviae*) (King and McLelland, 1975).

78 duck and goose (*Anser anser*) but narrow in fowl. Communication with the nasal cavity
79 takes place through a more ventral cleft in the medial mucosal wall of the infraorbital
80 sinus (Nickel et al., 1977) (Figs. 4 and 5). The rostral compartment of the sinus is covered
81 by a squamous epithelium, whereas a ciliated cuboidal epithelium that comprises some
82 goblet cells lines the more spacious caudal part of the sinus (McLelland, 1990).

83 The nasal cavity is connected with the oral cavity through the palatine or choanal
84 cleft (*Choanae*) that presents as a slender fissure in the palate. Above this fissure lies the
85 antorbital fossa (*Fossa antorbitalis*) that contains the infraorbital sinus (Fig. 6). The
86 infundibular cleft (*Rima infundibuli* syn. *Tuba auditiva communis*) is located caudal to
87 the choanal cleft (Fig. 3). Here, both auditory tubes (*Tubae auditivae*) originate by a
88 common cavity (*Infundibulum tubarum*). The soft tissues surrounding the choanae and
89 infundibular cleft contain lymphoid tissue that corresponds with the pharyngeal tonsil
90 (Fig. 7) (Salomon and Krautwald-Junghanns, 2008; Casteleyn et al., 2010).

91

92 **Larynx**

93 The inhaled air that leaves the nasal cavity flows through the choanal cleft towards the
94 larynx (*Larynx cranialis*), which hampers the entering of larger particles into the trachea.
95 It consists of a short cylindrical structure that is composed of osseous, cartilaginous and
96 musculomembranous components (Fig. 8). Maceration of the soft tissues reveals the
97 presence of laryngeal osseous plates (Fig. 9). In addition, only the cricoid cartilage
98 (*Cartilago cricoidea*) and paired arytenoid cartilages (*Cartilago arytenoidea*) are present
99 in birds. No epiglottis nor a thyroid cartilage (*Cartilago thyroidea*) can be found (King
100 and McLelland, 1975). The cricoid is composed of a semi-circular ventral cricoid plate

101 and dorsolateral wings that are connected to the plate by means of fibrous tissue. As such,
102 it forms a ring of fibrocartilaginous/-osseous tissue. The arytenoid cartilages articulate
103 dorsally with the cricoid. They are composed of a caudal, ossified rod-like part and a thin,
104 cranial cartilaginous part that protrudes in cranial direction. The glottis (*aditus laryngis*)
105 that forms the entrance to the larynx is located in between both arytenoids on the *mons*
106 *laryngis*, which deviates the passage of fluids laterally, thus avoiding choking (Fig. 9).
107 The dimensions of the larynx can be adjusted by laryngeal muscles (Nickel et al., 1977).

108

109 **Trachea**

110 The trachea originates at the larynx (*Larynx cranialis*) and is caudally connected to the
111 *Syrinx* or *Larynx caudalis*. Along its entire length, the trachea is located in the median
112 plane and is lined by a pseudostratified ciliary epithelium that contains some goblet cells.
113 It crosses the crop dorsally and enters the thoracic cavity between the rami of the *Furcula*
114 (Fitzgerald, 1969; Nickel et al., 1977). The oesophagus initially lies dorsal to the trachea,
115 but deviates to the right towards the cranial thoracic aperture (Fig. 10).

116 The length of the trachea varies between different bird species. The trachea of the
117 chicken consists of more than 100 closed tracheal rings or *Anuli tracheales*. Since these
118 tend towards early ossification, the term *Cartilagines tracheales* seems less appropriate.
119 The consecutive tracheal rings are connected to each other by elastic membranes
120 (*Ligamenta anularia*). Moreover, the rings overlap well since they comprise a narrow and
121 a wide part that are alternately located at the dorsal and ventral side (Fitzgerald, 1969).

122 Furthermore, the tracheal rings are interconnected by several muscles. The thin,
123 striated *Musculus (M.) tracheolateralis* runs bilaterally along the entire length of the

124 trachea, thus from the *Larynx cranialis* to the *Larynx caudalis* (McLelland, 1990). In the
125 chicken and pigeon, the left and right *M. sternotrachealis* originate on the craniolateral
126 aspect of the sternum and insert ventrally on the trachea in the median plane, just cranial
127 to the syrinx (McLelland, 1990) (Fig. 11).²

128

129 **Syrinx**

130 *Definition*

131 The *Syrinx*, also called the *Larynx caudalis*, is in most bird species invaluable in sound
132 production. It is located at the bifurcation of the trachea into the primary bronchi. Its
133 anatomic position is intrathoracic at the basis of the heart, which is at the level of the first
134 and second thoracic vertebrae (Suthers et al., 1999, Larsen and Goller, 2002, Çevik-
135 Demirkan et al., 2007) (Fig. 11). Furthermore, it is surrounded by the clavicular air sac
136 (Vollmerhaus and Sinowatz, 1992).

137 According to its structural components, three syringeal types can be identified,
138 viz. a tracheal, tracheobronchial and bronchial syrinx (King, 1979). The majority of bird
139 species, including the chicken, the turkey (*Meleagris gallopavo*), the pigeon and mallard
140 possess a tracheobronchial syrinx that is characterized by modifications of both the
141 trachea and the primary bronchi (Yildiz et al., 2003; Suthers and Sollinger, 2004).³

142

143 *Osseous and cartilaginous structures*

² In several bird species belonging to the *Anserinae* (e.g. geese (genus *Anser* and *Branta*) and swans (genus *Cygnus*)), *Gruidae* (crane birds), and *Paradisaeidae* (birds-of-paradise), the trachea is elongated, allowing for the formation of curves, curls and coils (Fig. 12). This conformation enables vocalizations that contain louder, lower frequency sounds (Clench, 1978; Frith, 1994; Daley and Goller, 2004).

³ The tracheal syrinx in the order *Passeriformes* (perching birds to which the suborder of the *Oscines*, i.e. the true songbirds, belong) or the bronchial syrinx that is present in the common cuckoo (*Cuculus canorus*) shows adaptations of only the trachea or bronchi, respectively (King, 1979).

144 The syrinx is supported by a number of cartilaginous rings (*Cartilagineae syringeales*) that
145 succeed the proper tracheal rings and are consequently termed tracheal syringeal rings
146 (*Cartilagineae tracheales syringis*) (Figs. 11, 13 and 14) (King and McLelland, 1975).
147 These are closely assembled and as such form a firm cylindrical structure known as the
148 *Tympanum* (King, 1979). Furthermore, they are closed annular structures, just like the
149 proper tracheal rings. Their number varies considerably according to the species (eight in
150 the chicken, up to 78 in some members of the *Ciconiidae*) (King, 1979). Ossification is
151 possible in a few species such as the chicken, silver lark (*Larus argentatus*) and songbirds
152 (Vollmerhaus and Sinowatz, 1992).

153 The more caudal syringeal cartilaginous rings are called bronchial syringeal rings
154 (*Cartilagineae bronchiales syringis*) and arise from the tympanum and form the basis of
155 the partitioned tracheobronchial. As a consequence, they are paired (King and
156 McLelland, 1975). These cartilaginous rings are not entirely closed, but have a C-like
157 shaped profile (King, 1979, Çevik-Demirkan et al., 2007). The free margins face the
158 median plane and support the *Membrana tympaniformis medialis* or *interna* (King, 1979).
159 Where both medial tympaniform membranes join cranially, the *Pessulus*, i.e. a pyramidal
160 wedge-shaped cartilage, can be observed in many bird species including the chicken and
161 pigeon. This structure is, however, absent in the oscine songbirds (King and McLelland,
162 1975; King, 1979; Çevik-Demirkan et al., 2007) (Fig. 14).⁴

163

⁴ The *Bulla syringealis* or *Bulla tympanica* is a confined expansion of the syrinx that is present in some species (Fig. 15). The archetypal tympanic bulla of the drake is a bell-shaped extension at the left side of the syrinx that can present partial or complete ossification. It allows for the sound to resonate and as such amplifies the volume of the produced sound (King and McLelland, 1975; Vollmerhaus and Sinowatz, 1992).

164 *Ligaments*

165 A connective tissue strand links the medial aspects of the rostral parts of the left and right
166 primary bronchi. This *Ligamentum interbronchiale* is called *Bronchidesmus* by King and
167 McLelland (1975). The cavity enclosed by the tracheal bifurcation cranial to this ligament
168 is the *Foramen interbronchiale* (King, 1979) (Fig. 14).

169

170 *Membranes*

171 The *Membrana tympaniformis lateralis* or *externa* is located bilaterally in the lateral
172 walls of the syrinx at the level of the tracheobronchial junction (Fitzgerald, 1969). It can
173 either extend from the *Tympanum* to the first bronchial rings, as in the chicken and the
174 pigeon (Vollmerhaus and Sinowatz, 1992; Yildiz et al., 2003; Gaban-Lima and Höfling,
175 2006; Pierko, 2007) (Fig. 14), or can be composed of multiple short membranes that are
176 located in between the successive bronchial syringeal rings (King, 1979). It lacks in the
177 duck while its presence is controversial in perching birds (King, 1979).⁵

178 The *Membrana tympaniformis medialis* bilaterally bridges the *Pessulus* and the
179 medial side of the primary bronchi. It dorsoventrally connects the medial free margins of
180 the bronchial syringeal rings (Yildiz et al., 2003; Kabak et al., 2007). Both membranes
181 form the lateral walls of the *Foramen interbronchiale* (Fig. 14).

182 The *Labia* are paired elastic strings located within the lateral and medial
183 tympaniform membranes (King, 1979; Suthers and Zollinger, 2004; Çevik-Demirkan et

⁵ In the tracheal syrinx type, the membrane is modified and accordingly termed *Membrana trachealis* (King, 1979).

184 al., 2007) (Fig. 14). These strings are in many cases the major source of sound (Frank et
185 al., 2006).^{6, 7}

186

187 *Muscles*

188 The description below makes use of the nomenclature presented in the Nomina
189 Anatomica Avium (King, 1979). An alternative terminology that complicates the
190 comprehension of the anatomy of the muscles involved in sound production can,
191 however, be found in several literature reports. Nevertheless, two important clusters of
192 muscles can be distinguished, i.e. the *Mm. tracheales* and the *Mm. syringeales*. The latter
193 consist of intrinsic syringeal muscles, which have their origin and insertion on the syrinx.
194 Extrinsic syringeal muscles have an attachment beyond the syrinx (King, 1979) and
195 include some of the tracheal muscles.

196 The *Mm. tracheales* comprise the *M. tracheolateralis*, *M. sternotrachealis*, *M.*
197 *cleidotrachealis* and *M. sternohyoideus*. The presence of this entire repertoire in a
198 particular species is exceptional. In most non-songbirds, which possess a
199 tracheobronchial syrinx type, only the *M. tracheolateralis* is described. Moreover,

⁶ Labia lack in some bird species, such as the silver lark and the long-legged buzzard (*Buteo rufinus*) (King, 1979; Kabak et al., 2007).

⁷ In addition to the here-described syringeal components, specific structures may be present in other avian species. In the common quail (*Coturnix coturnix*), the mucosal membrane that covers the *Pessulus* protrudes into the tracheal lumen. As a result, a *Membrana semilunaris* is formed (Fitzgerald, 1969; Frank et al., 2006). Since this structure cannot vibrate, it may play no role in sound production, in contrast to what was previously accepted (Frank et al., 2006). The *Membrana tracheosyringealis* is a paired membrane that is especially present in *Columbiformes* and *Psittaciformes* (King, 1979). This membrane covers the inner wall of the trachea caudally and attaches to the *Tympanum* and the first bronchial rings (Gaban-Lima and Höfling, 2006). The *Valvula syringealis* that is present in diving ducks (*Aythiinae*) and mallards (*Anatinae*) is a caudally oriented valve that originates on the inside of the lateral wall of the right primary bronchus. During diving, the contraction of the sternotracheal muscle allows for this valve and the medial tympaniform membranes to protrude into the bronchial lumina and almost entirely block the airflow through both primary bronchi (Frank et al., 2006).

200 Ratites, *Ciconiidae*, *Cathartidae* and some *Galliformes* lack tracheal muscles (King,
201 1979; Brackenbury, 1980; Gaunt, 1983; Yildiz et al., 2003; Frank et al., 2006; Çevik-
202 Demirkan et al., 2007). The chicken and the pigeon possess both the *M. sternotrachealis*
203 and *M. tracheolateralis* that both are extrinsic syringeal muscles (King, 1979;
204 Brackenbury, 1980; Vollmerhaus and Sinowatz, 1992) (Fig. 16). Most domestic bird
205 species such as the chicken and pigeon lack intrinsic syringeal muscles. However, some
206 smooth muscle cells are present in the inter-bronchial area in the duck (Frank et al.,
207 2006).⁸

208

209 VOCALIZATION

210 **Introduction**

211 Mammals most commonly produce sound by means of their vocal cords that are
212 bilaterally present in the larynx. Although the latter structure is also found in birds, vocal
213 cords are lacking in these animals. Most bird species rely on their syrinx to vocalise.⁹

214 According to the species, the vocal repertoire of birds varies from simple sounds
215 to more complex songs (Marler and Hamilton, 1966). Several functions can be attributed
216 to the birdsong, such as territory marking and socialisation of the offspring in female

⁸ *Passeriformes*, *Psittaciformes* and the oilbird (*Steatornis caripensis*) possess well-developed *Mm. syringeales* in addition to the tracheal muscles. Syringeal muscles are short and poorly delineated. They include the *Mm. tracheobronchialis dorsalis*, *ventralis et brevis* and the *Mm. syringealis dorsalis et ventralis* (King and McLelland, 1975; King, 1979; Sherwood et al., 2004; Gaban-Lima and Höfling, 2006).

⁹ Since the syrinx is absent in some members of the *Ratites* it is not surprising that they are deficient in voice production. However, some species generate sound using the beak. Several species of woodpeckers (*Picidae*) drum on tree-trunks to mark their territory. Storks (*Ciconiidae*), on the other hand, clatter by rapidly opening and closing the beak. Besides the beak, feathers can generate sound as well. This is the case in a.o. the white-bearded manakin (*Manacus manacus*), the common snipe (*Gallinago gallinago*) and hummingbirds (*Trochilidae*). The sound can either originate from wing snapping or from air turbulence around the feathers (Vuilleumier, 2002, Clark et al., 2011).

217 birds, and the luring of a mating partner in male birds. Additionally, it plays a role in the
218 reproductive cycle and the recognition of family members (Marler and Hamilton, 1966;
219 Gorissen and Eens, 2005). The text below focuses on sound production in the syrinx.

220

221 **The mechanism of sound generation**

222 In non-songbirds, such as the chicken and the pigeon, the lateral tympaniform membranes
223 are responsible for the produced sound (Elemans et al., 2008). The classical model states
224 that these membranes are initially relaxed, but when the air flows through the syrinx
225 during expiration, the membranes present a cyclic vibration that is the result of the
226 Bernouilli forces created by the airflow and the elastic restoring forces of the membrane
227 itself (Gaunt, 1983; Ballintijn and Ten Cate, 1998). In this model, the pitch of the
228 generated sound depends on the tension of the membranes, whereas the volume is related
229 to the amplitude of the vibration (Gaunt, 1983). The structures that can influence these
230 parameters are described below.¹⁰

¹⁰ Extrapolating the classical model to songbirds holds that the medial tympaniform membranes are the sound generating structures since songbirds do not possess lateral tympaniform membranes. However, the fact that songbirds are still able to produce sound after their medial tympaniform membranes have been cut surgically could militate against the validity of the classical model in these bird species (Goller and Larsen, 1997b). After surgery, the sound is of lower intensity but the individual sounds are still mutable (Goller and Larsen, 1997b; 2002). Because of this observation, endoscopic research has promoted the labial hypothesis or the pulse-tone model that is most probably valid for a large number of songbird species (Goller and Larsen, 1997a; Suthers and Zollinger, 2004). Prior to the production of the sound, the syrinx is displaced in cranial direction and stretched. Consequently, the bronchi and the medial tympaniform membranes are stretched (Goller and Larsen, 2002). The Bernouilli forces that arise during expiration result in the subsequent displacement of the lateral and medial labia towards the lumina of the bronchi (Goller and Larsen, 1997a; 2002; Larsen and Goller, 2002). Due to their elasticity, a self-oscillating system, referred to as a pneumatic valve, is set in place (Goller and Larsen, 2002).

An alternative hypothesis is the whistle hypothesis that is based on aerodynamic principles. This model aims to clarify the production of tonal sounds in the collared dove (*Streptopelia decaocto*) (Ballintijn and Ten Cate, 1998; Goller and Larsen, 2002). When air travels at high velocity through a narrow passage, i.c. the syringeal opening (Fig. 14), turbulences arise in the airflow that, as in a whistle, act as sound generators (Gaunt, 1983; Ballintijn and Ten Cate, 1998). However, recent studies have rejected this model because the fundamental frequency of the produced tones failed to shift upwards when birds inhaled a lighter gas. Moreover, it was observed in the ring dove (*Streptopelia risoria*) that the tracheal and syringeal muscles have a major impact on the sound production (Elemans et al., 2004; 2006).

231

232 **Influence of the musculature**

233 Non-songbirds usually have no or only minor intrinsic syringeal muscles (King, 1979;
234 Frank et al., 2006). The sternotracheal and tracheolateral muscles, both extrinsic syringeal
235 muscles, have antagonistic mechanical effects. The tracheolateral muscle indirectly
236 abducts or tenses the lateral tympaniform membrane by retracting the syrinx in cranial
237 direction. The sternotracheal muscle, on the other hand, directly adducts or relaxes the
238 membrane by drawing the trachea caudally (Gaunt and Gaunt, 1977; Suthers and
239 Zollinger, 2004; Elemans et al., 2006). During the trill of the ring dove, the lateral
240 tympaniform membranes are adducted and abducted in and out the syringeal lumen at a
241 frequency of 24 Hz (Elemans et al., 2004; 2006).¹¹

242

243 **Influence of the respiration**

244 In the majority of avian vocalizations, the sound is produced during expiration (Suthers et
245 al., 1999; Suthers and Zollinger, 2004). This can especially be appreciated when
246 observing the crowing of the rooster, which is limited in time. The ring dove and the
247 zebra finch, however, can also generate inspirational sounds (Gaunt et al., 1982; Goller

¹¹ In songbirds, the *M. tracheolateralis*, which ends in front of the syrinx (McLelland, 1990), stretches the syrinx and fits it in the appropriate position for sound production (Goller and Larsen, 1997a; Larsen and Goller, 2002). The *M. syringealis dorsalis* and the *M. tracheobronchialis dorsalis* adduct the medial and lateral labia, which results in the narrowing of the syringeal aperture (Elemans et al., 2006). This is important for the timing of the vocalisation within the appropriate phase of the respiratory cycle (Larsen and Goller, 2002; Suthers and Zollinger, 2004). The *M. tracheobronchialis ventralis* has the opposite function (Larsen and Goller, 2002). The lateral part of the *M. syringealis ventralis* mainly influences the tension on the labia. As a result, this muscle has impact on the pitch of the produced sound (Suthers and Zollinger, 2004). Finally, the *M. sternotrachealis* holds the syrinx in a stable position during the strong contractions of the intrinsic syringeal muscles that are necessary for the generation of high tones (Goller and Suthers, 1996).

248 and Daley, 2001). During expirational sound production, the expiratory muscles generate
249 a specific airflow and pressure that are bilaterally present in the syrinx, even though the
250 sound might be produced unilaterally, a phenomenon known as lateralisation of the sound
251 production (Suthers and Zollinger, 2004).¹²

252

253 **Influence of the suprasyringeal structures**

254 The sound waves that are generated in the syrinx are modified through their way
255 out by the trachea, the oropharyngeal-oesophageal cavity, the tongue and the beak
256 (Suthers et al., 1999; Beckers et al., 2004; Riede et al., 2006). The trachea mutes or
257 amplifies some frequencies. As mentioned above, the tracheas of geese and swans, crane
258 birds and birds-of-paradise present curves, loops or windings (Fig. 12) that allow for
259 generating louder sound of lower frequency (Clench, 1978; Frith, 1994; Daley and
260 Goller, 2004). The larynx and the hyoid bone can modify the volume of the initial
261 segment of the oesophagus (Riede et al., 2006). When low frequency sounds are
262 produced, the oesophagus and the oropharynx present a single cavity, i.e. the
263 oropharyngeal-oesophageal cavity. Its volume decreases when higher frequency sounds

¹² Songbirds possess two independent sound sources because the musculature of each syringeal side is innervated by the ipsilateral *Nucleus n. hypoglossi* (Suthers et al., 1999; Wild et al., 2000). Both sources can function simultaneously or independently (Suthers et al., 1999). Some bird species switch between both sides or use both sides simultaneously (Suthers, 2001). Since the tones that are produced in the right side of the syrinx, which has a smaller diameter bronchus (Luine et al., 1980), have a higher frequency, the bird can cover a wider range of frequencies (Suthers, 2001). This two-voice system is also present in non-songbirds such as the king penguin (*Aptenodytes patagonicus*) and the emperor penguin (*Aptenodytes forsteri*) that are able to produce specific calls that can be recognized individually (Aubin et al., 2000).

Other species have a preference for one side of the syrinx (Wild et al., 2000; Suthers, 2001; Wade et al., 2002). If, for example, the left side of the syrinx produces sound during expiration, the right side is closed, but it opens after each syllable for a short inspiration. The longer the syllable, the longer the duration of the inspiration is (Suthers and Zollinger, 2004). When a high number of sound elements has to be generated, which is typical in the canary (*Serenus*), a pulsating respiration is performed. Sound is produced by repeatedly opening and closing one side of the syrinx, allowing the passage of air pulses that are each associated with one syllable (Suthers et al., 1999). The advantage of the mini-respiration lies in the fact that the bird can continue singing for longer periods.

264 are generated (Suthers, 2006). The degree of opening of the beak during vocalization is
265 positively correlated with the frequency (Suthers et al., 1999). The tongue might seem of
266 minor importance since it is a rather rigid structure that is not suitable for articulation, as
267 in humans (Wild, 2004). However, it was recently demonstrated in northern cardinals
268 (*Cardinalis cardinalis*) that tongue elevation lowers the resonance frequency below 2
269 kHz by reducing the area of the passage from the oral cavity into the beak (Suthers et al.,
270 2016). The tongue of *Psittaciformes* is exceptional as it can change the amplitude and
271 frequency of the sound. This partly explains their capacity of mimicking the human
272 speech (Beckers et al., 2004).

273

274 CONCLUSIONS

275 Vocalization in birds is the result of the flawless synchronisation of the respiration and
276 the muscle activation in the upper respiratory tract. In most species, the sound is created
277 during expiration by the vibrations of the membranes and *Labia* that are present in the
278 syrinx. Although the main principles of sound production in birds are well known, it
279 should be noticed that variations exist in the fine-tuning of the singing between species.
280 Moreover, the anatomy of the sound producing organs is variable as well.

281

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285

286 **Conflict of interest statement**

287 None declared.

288

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408 **Figures**

409 **Fig. 1.** Left lateral view of the skull of a chicken showing the bony limitations of the
410 nasal cavity. 1 = incisive syn. premaxillary bone, 1' = frontal process, 1'' = maxillary
411 process, 2 = maxillary bone, 3 = nasal bone, 3' = intermaxillary process, 3'' = maxillary
412 process, 4 = lacrimal bone, 5 = palatine bone

413

414 **Fig. 2.** Left ventrolateral views of the left nasal opening of a chicken. A: The dorsal (1)
415 and ventral (2) cartilaginous plate surround the nasal opening. Where they join caudally,
416 a niche (asterisk) is formed. A lamella (3) branches off from the dorsal cartilaginous
417 plate, which is also called the operculum. B: After detaching the operculum rostrally, the
418 lamella becomes obvious.

419

420 **Fig. 3.** Rostral view of the head of a pigeon showing the cere (1) at the basis of the upper
421 beak which borders the nasal openings caudally (2). The widely-opened beak allows for
422 inspection of the choanal cleft (3) and the infundibular cleft (4). Adapted from Casteleyn
423 et al. (2011b)

424

425 **Fig. 4.** Left lateral view of the head of a chicken. The cartilages surrounding the nasal
426 opening have been removed showing the cartilaginous nasal septum (1). The infraorbital
427 sinus (2) is visible in between the maxillary process of the nasal bone and the eye. The
428 probe shows the cleft through which the infraorbital sinus communicates with the nasal
429 cavity.

430

431 **Fig. 5.** Left lateral view of the head of a chicken visualising the nasal cavity. A: The
432 cartilages of the nasal opening and the maxillary process of the nasal bone have been
433 removed, showing the nasal septum (1) and the antorbital fossa (2) that lies dorsal to the
434 palatine cleft. The infraorbital sinus (3) has been opened. B: After removal of the nasal
435 septum, the medial aspect of the right nasal cavity becomes visible. The lamella of the
436 right operculum (4), the large ventral nasal concha (5) and the small dorsal nasal concha
437 (6) can be observed. The level at which the histological cross-section of the nasal cavity
438 shown in Fig. 6 is made is indicated by the double arrow.

439

440 **Fig. 6.** Histological cross section of the nasal cavity of the chicken (decalcified specimen;
441 original size: 125 mm x 90 mm) at the level of the corners of the beak. 1 = nasal septum,
442 2 = antorbital fossa, 3 = dorsal nasal meatus, 4 = cavity of the dorsal nasal concha, 5 =
443 infraorbital sinus, 6 = nasolacrimal duct, 7 = frontal bone, 8 = palatine bone, 9 = jugal
444 bone

445

446 **Fig. 7.** Longitudinal histological section (haematoxylin-eosin staining) of the palate of a
447 chicken showing the presence of lymphoid tissue in the nasal mucosa bordering the
448 choanal and infundibular clefts. Some lymphoid follicles are indicated by the arrows.
449 Adapted from Casteleyn et al. (2010)

450

451 **Fig. 8.** Dorsal view of the larynx (1) and cranial segment of the trachea (2) of the
452 chicken. The slit-like laryngeal entrance that is flanked by the protruding arytenoid
453 cartilages (asterisks) is indicated by the arrow.

454

455 **Fig. 9.** Craniolateral view of a macerated larynx of a chicken showing the laryngeal
456 cartilages that are highly ossified in this specimen. The entrance to the larynx is indicated
457 by the arrow. The cricoid is composed of the ventral cricoid plate (1) and the dorsolateral
458 wings (2). The fibrous tissue that connects both parts has been macerated. Its localisation
459 is indicated by the asterisk. The paired arytenoid cartilage consists of a rod-like caudal
460 part (3) and a thin cartilaginous cranial part (4).

461

462 **Fig. 10.** Ventral view of the neck of a chicken showing the localisation of the trachea (1),
463 oesophagus (2) and crop (3).

464

465 **Fig. 11.** Ventral views of the thoracic cavity of a pigeon after removal of the sternum. A:
466 The syrinx presents an intrathoracic localisation at the level of the first two thoracic
467 vertebrae. It is covered ventrally by the basis of the heart. B: After caudal retraction of
468 the heart, the syrinx (boxed) that is located at the tracheal bifurcation becomes visible. (1
469 = trachea, 2 = heart, 3 = lungs, 4 = liver, asterisks = sternotracheal muscles, arrows =
470 tracheolateral muscles) Adapted from Casteleyn et al. (2011a)

471

472 **Fig. 12.** Left craniolateral view of the cranial thoracic aperture of a goose demonstrating
473 the tracheal elongation that is visible as a ventral loop. Adapted from Casteleyn et al.
474 (2011a)

475

476 **Fig. 13.** Ventral view of the chicken syrinx (boxed) that is located in between the trachea
477 (1) and the left and right primary bronchi (2 and 2', respectively). The sternotracheal
478 muscles are indicated by the asterisks. Adapted from Casteleyn et al. (2011a)

479

480 **Fig. 14.** A: Longitudinal histologic section of the syrinx of a chicken (after Casteleyn et
481 al., 2011a). B: Schematic representation of the syrinx of the blackbird (*Turdus merula*)
482 adapted from King (1979) and Vollmerhaus and Sinowatz (1992). (1 = trachea, 2 =
483 *Tympanum*, 3 = *Pessulus*, 4 = medial *Labia*, 5 = lateral tympaniform membranes, 6 =
484 lateral *Labia*, 7 = medial tympaniform membranes, 8 = interbronchial ligament, 9 =
485 primary bronchi, asterisks = sternotracheal muscles)

486

487 **Fig. 15.** Ventral view of the syrinx of a drake located in between the trachea (1) and the
488 left and right primary bronchi (2 and 2', respectively). The sternotracheal muscles are
489 indicated by the asterisks. The syringeal bulla (3) is clearly visible as a voluminous
490 extension at the left side of the syrinx. Adapted from Casteleyn et al. (2011a)

491

492 **Fig. 16.** Ventral view of the trachea of a pigeon showing the left and right sternotracheal
493 muscle (1 and 1', respectively) and the left and right tracheolateral muscles (2 and 2',
494 respectively). The syrinx (boxed) is located at the tracheal bifurcation. Adapted from
495 Casteleyn et al. (2011b)

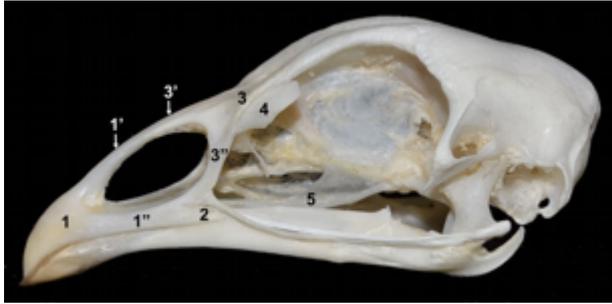


Fig. 1



Fig. 2

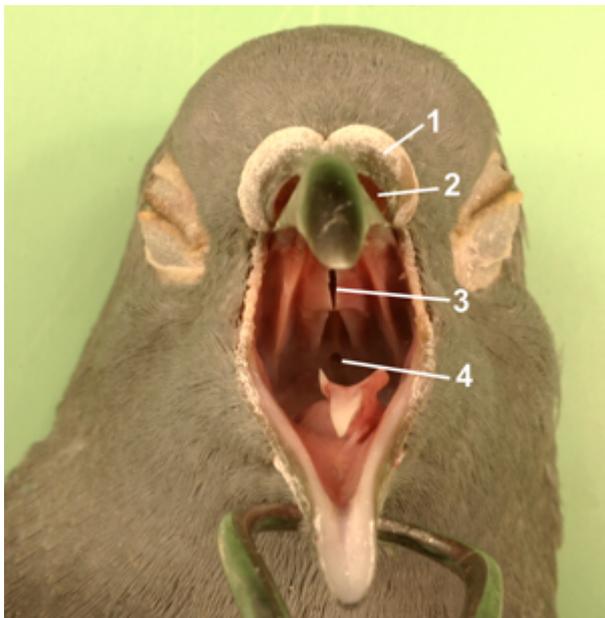


Fig. 3



Fig. 4

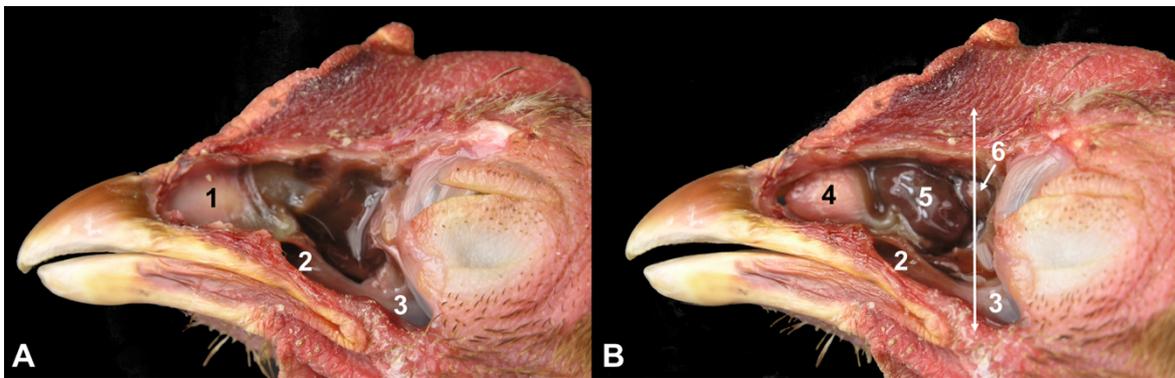


Fig. 5



Fig. 6

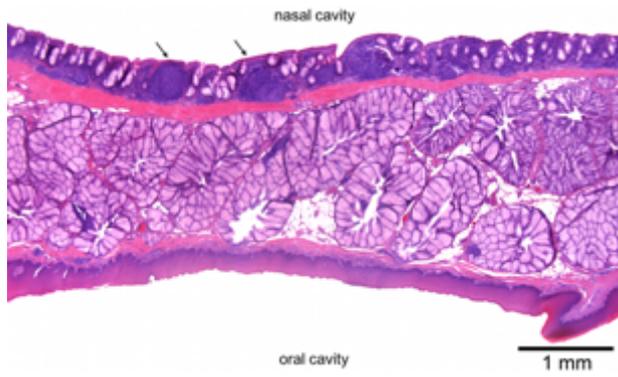


Fig. 7

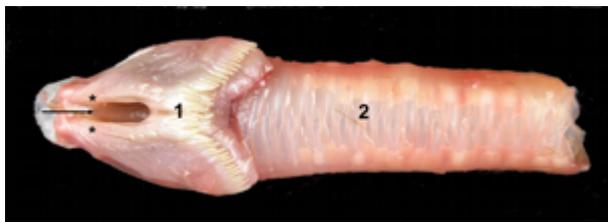


Fig. 8

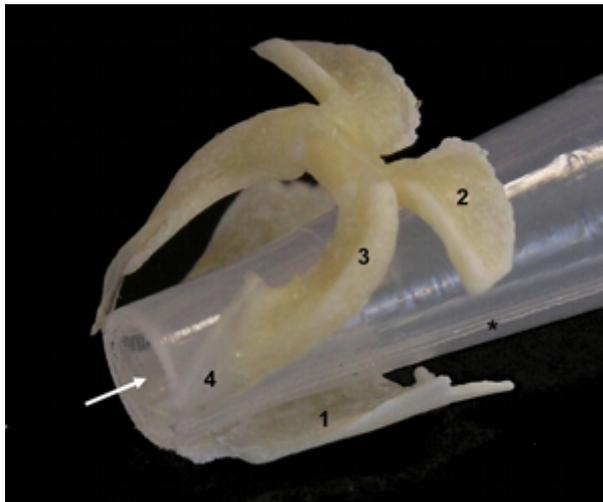


Fig. 9

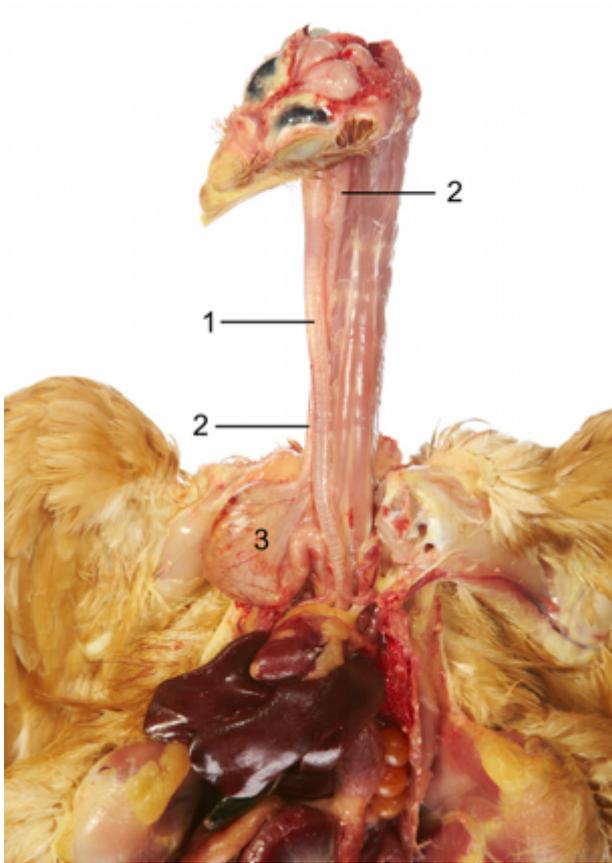


Fig. 10

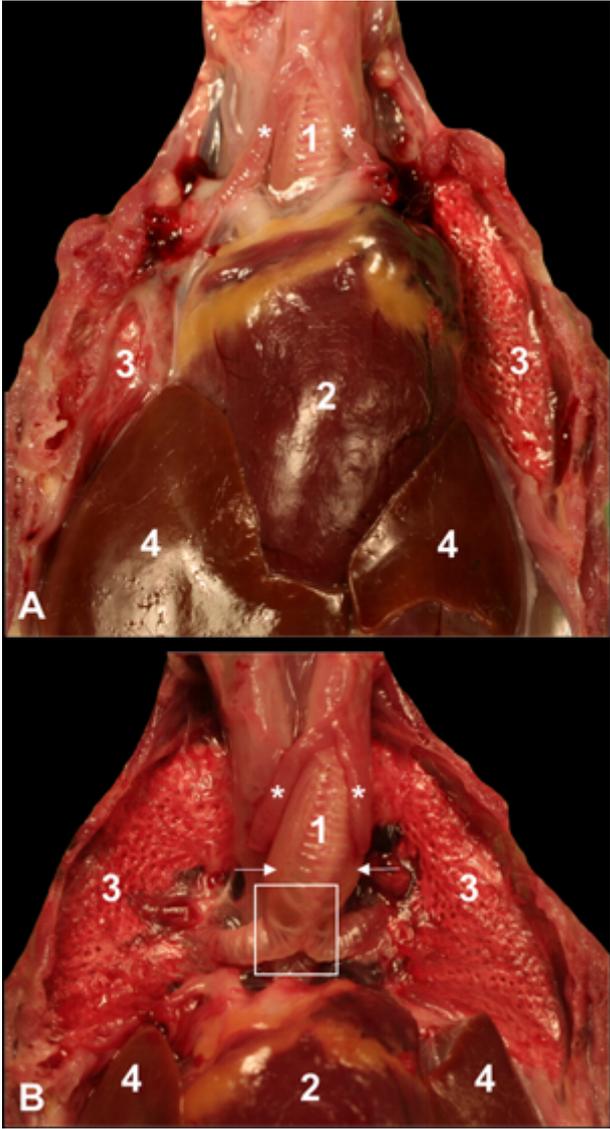


Fig. 11

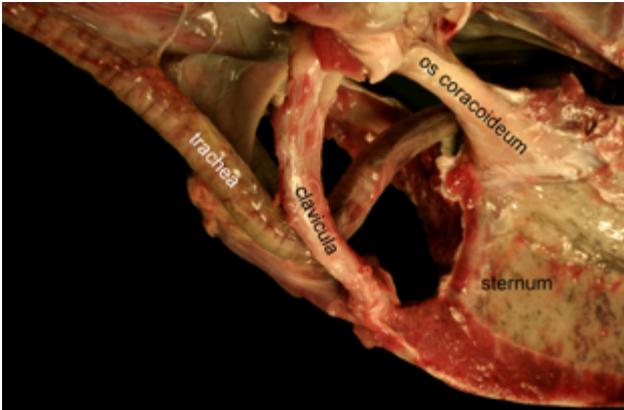


Fig. 12

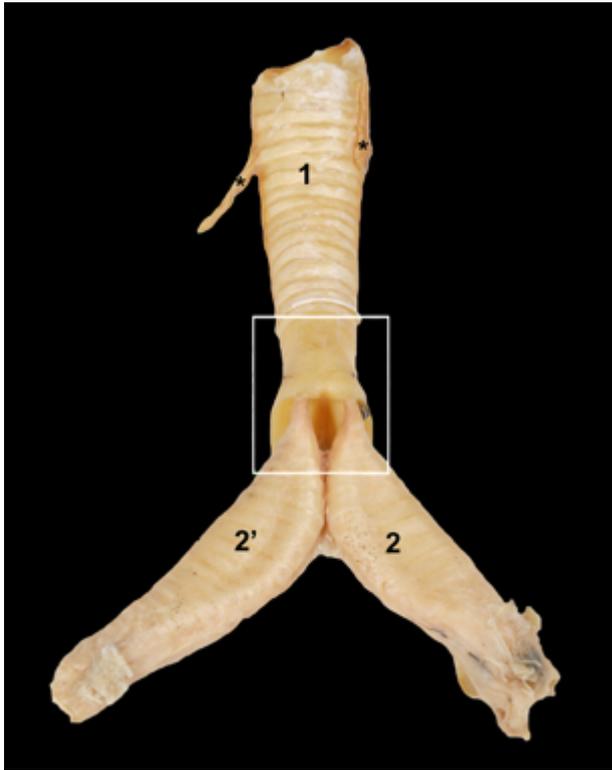


Fig. 13

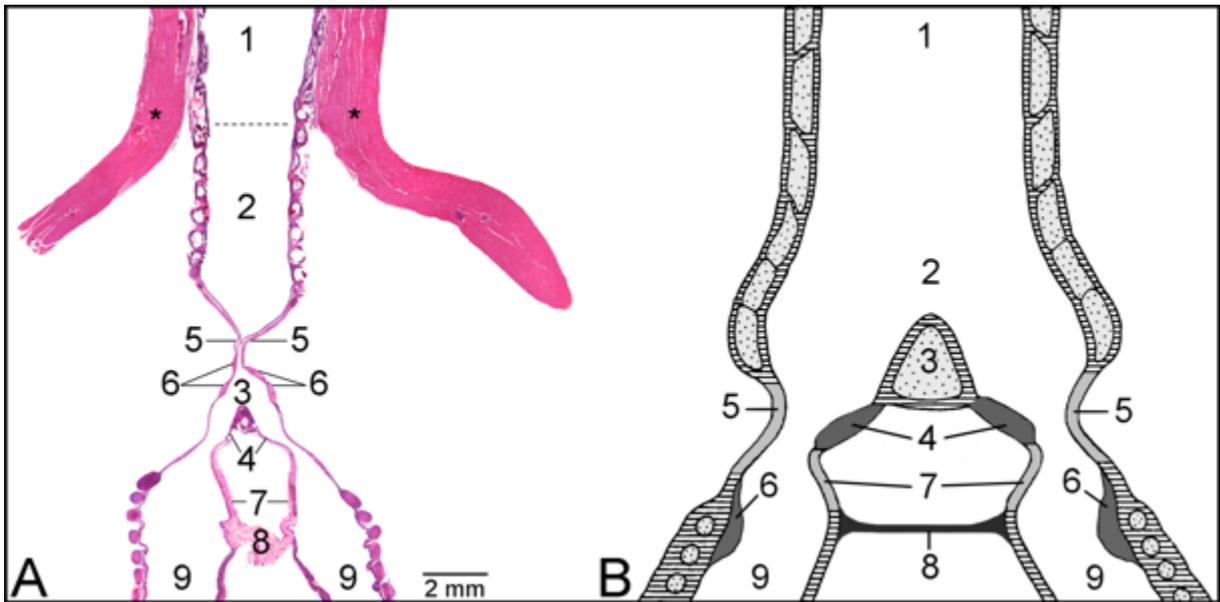


Fig. 14



Fig. 15

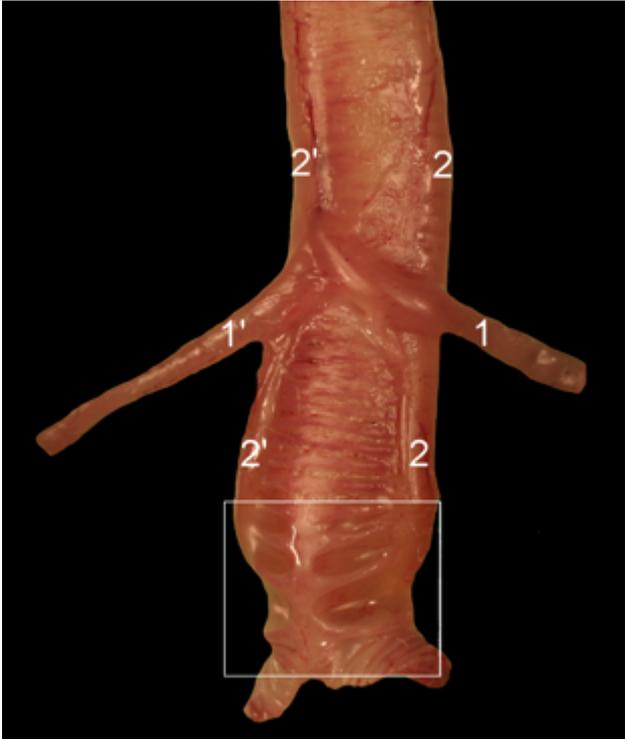


Fig. 16