

1 **The remarkable vocal anatomy of the koala (*Phascolarctos***  
2 ***cinereus*): insights into low-frequency sound production in**  
3 **a marsupial species**

4 Roland Frey<sup>1</sup>, David Reby<sup>2</sup>, Guido Fritsch<sup>1</sup>, Benjamin D. Charlton<sup>3</sup>,

5 <sup>1</sup> Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany;

6 <sup>2</sup> University of Sussex, School of Psychology, Brighton, UK;

7 <sup>3</sup> Animal and Environment Research Group, Department of Biology, Anglia Ruskin  
8 University, Cambridge, U.K.;

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10 **Key words:** marsupials, mating calls, bellow vocalisation, sexual selection, descended  
11 larynx, intra-thoracic sternohyoid and sternothyroid muscles, extra-laryngeal velar  
12 vocal folds, laryngeal vocal folds

13

14 **Abstract**

15 Koalas are characterised by a highly unusual vocal anatomy, with a descended larynx  
16 and velar vocal folds, allowing them to produce calls with disproportionately low  
17 frequencies. Here we use advanced imaging techniques, histological data, classical  
18 macroscopic dissection and behavioural observations to provide the first detailed  
19 description and interpretation of male and female koala vocal anatomy. We show that  
20 both males and females have an elongated pharynx and soft palate, resulting in a  
21 permanently descended larynx. In addition, the hyoid apparatus has a human-like  
22 configuration in which paired dorsal, resilient ligaments suspend the hyoid apparatus  
23 from the skull, while the ventral parts tightly connect to the descended larynx. We also  
24 show that koalas can retract the larynx down into the thoracic inlet, facilitated by a  
25 dramatic evolutionary transformation of the ventral neck muscles. First, the usual  
26 retractors of the larynx and the hyoid have their origins deep in the thorax. Second,  
27 three hyoid muscles have lost their connection to the hyoid skeleton. Third, the  
28 genioglossus and geniohyoid muscles have greatly increased in length. Finally, the  
29 digastric, omohyoid and sternohyoid muscles, connected by a common tendinous  
30 intersection, form a guiding channel for the dynamic down-and-up movements of the  
31 ventral hyoid parts and the larynx. We suggest that these features evolved to  
32 accommodate the low resting position of the larynx and assist in its retraction during  
33 call production. We also confirm that the edges of the intra-pharyngeal ostium have  
34 specialised to form the novel, extra-laryngeal velar vocal folds, which are much larger  
35 than the true, intra-laryngeal vocal folds in both sexes, but more developed and  
36 specialised for low frequency sound production in males than in females. Our findings  
37 illustrate that strong selection pressures on acoustic signalling not only lead to the

38 specialisation of existing vocal organs, but can also result in the evolution of novel  
39 vocal structures in both sexes.

## 40 **Introduction**

41 Examining vocal anatomy is an important step towards understanding the  
42 proximate factors that shape the vocal signals of a given species. The link between  
43 vocal anatomy and vocal output has been thoroughly investigated in humans (e.g.  
44 speech production and operatic singing; Némai & Kelemen, 1933; Doscher, 1994;  
45 Titze, 1994; Lieberman et al, 2001; Dayme, 2009; Fitch, 2010; Koda et al, 2012).  
46 However, over the last 25 years the generalisation of the source-filter theory has also  
47 allowed researchers to explicitly link vocal anatomy and acoustic output in non-human  
48 mammals. For example, studies combining behavioural, morphological and acoustic  
49 data have revealed that red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) have  
50 a descended and retractable larynx that lowers vocal tract resonances in their  
51 respective sexual calls (Fitch & Reby, 2001, McElligott et al 2006), that male saigas  
52 (*Saiga tatarica*) and southern elephant seals (*Mirounga leonina*) extend the nasal tract  
53 to lower resonance frequencies in their rutting calls (Frey et al, 2007, Sanvito et al  
54 2007) and have also provided key insights into sound production in Savanna elephants  
55 (*Loxodonta africana* - Herbst et al 2012, 2013), North American wapitis (*Cervus*  
56 *canadensis* - Reby et al, 2016), and koalas (*Phascolarctos cinereus* - Charlton et al  
57 2011, 2013).

58 According to the source-filter theory, mammals produce vocal signals via a two-  
59 stage process (Fant, 1960): firstly, a source signal is generated as air expelled from  
60 the lungs passes through the larynx, causing the vocal folds to open and close, and  
61 breaking up the airstream to produce a sound wave. The rate that the vocal folds open  
62 and close determines the fundamental frequency (F0), responsible for the perceived

63 pitch of the vocalisation. The supra-laryngeal vocal tract then acts as a resonator,  
64 selectively diminishing certain frequencies of the source signal and enhancing other  
65 frequencies termed formants before the filtered signal radiates out through the mouth  
66 or nostrils. Because source and filter characteristics of mammal vocal signals are  
67 produced by the larynx and vocal tract, respectively; variation in these features can be  
68 directly predicted from the laryngeal and vocal tract anatomy of the caller (for a review  
69 see: Taylor et al 2016).

70       The koala (*Phascolarctos cinereus*) is a specialized arboreal folivore that inhabits  
71 the open forests and woodlands of eastern and southeastern Australia dominated by  
72 trees of the genus *Eucalyptus* (Lee and Carrick, 1989). Koalas are the only mammals  
73 that consume eucalypt foliage as a dominant component of their diet (Tyndale-Biscoe,  
74 2005). This relatively low-energy diet means that koalas spend a large proportion of  
75 their time resting, only coming to the ground for brief periods in order to move between  
76 trees (Martin and Handasyde, 1999). During the annual breeding season, however,  
77 both sexes roam over much larger areas in order to locate a suitable mating partner  
78 and vocal activity increases (Ellis et al, 2009). In particular, males produce a high rate  
79 of extremely low-pitched bellow vocalizations when most copulations are predicted to  
80 occur (Ellis et al, 2011), indicating that these calls are important in reproductive  
81 contexts. Bellows are produced in a characteristic two-stage calling posture, gradually  
82 changing from pronounced extension to marked flexion of the head and neck region  
83 (Charlton et al, 2011; Fig. 1).

84       Recent studies examining the information content and function of male koala  
85 bellows have shown that formants are important cues to male identity and size that are  
86 used in both inter- and intra-sexual contexts (Charlton et al, 2011a; Charlton et al,  
87 2011b; Charlton et al, 2012c; Charlton et al, 2012a; Charlton et al, 2012b; Charlton et

88 al, 2013). Taken together, these findings indicate that identity- and size-related formant  
89 information is functionally relevant to male koalas in their natural environment, and that  
90 sexual selection favours males able to produce lower formants in their bellows.  
91 Interestingly, the formant pattern of male bellow exhalation phases and this species'  
92 well-developed sternothyroid muscle indicate that koalas extend the vocal tract just  
93 prior to call production by lowering the larynx (Charlton et al, 2011b). In addition,  
94 anatomical studies have revealed that male koalas have an additional set of vocal folds  
95 outside of the larynx, the 'velar vocal folds' (VVF), which are used instead of the intra-  
96 laryngeal vocal folds to produce the extremely low F0 of bellow vocalisations (Charlton  
97 et al, 2013). As the VVFs are much larger than the laryngeal vocal folds they can  
98 oscillate periodically at much lower frequencies (Charlton et al, 2013).

99 Female koalas produce bellow vocalizations when they are in oestrous (Smith,  
100 1980) and it has been shown that they also possess adaptations of the soft palate  
101 (velum) similar to those found in males (Curry et al 2016). Female koala bellows have  
102 a mean F0 of 31.3 Hz (Charlton, 2015) which suggests that female koalas, like males,  
103 use VVFs to produce disproportionately low-pitched calls, although not as low as those  
104 of males. The formant spacing of the inhalation sections of female bellows is 423.5 Hz  
105 (Charlton, 2015). Applying the following equation  $eVTL = c/2\Delta F$ , in which eVTL is the  
106 estimated vocal tract length of the caller,  $c$  = the speed of sound in warm air (350 m/s)  
107 and  $\Delta F$  is the formant spacing, yields an estimated vocal tract length of 41.3 cm. This  
108 is clearly a much longer vocal tract than expected for an animal the size of a koala,  
109 and indicates that female koalas, like males, possess adaptations that allow them to  
110 elongate their vocal tract. Female koalas also produce individually distinctive squawks,  
111 screams, squeaks and wails (collectively termed "rejection calls") when they rebuff  
112 male copulation attempts (Charlton, 2015). The mean F0 of these calls (792.1 Hz) is

113 substantially higher than the mean F0 of the inhalation sections of female bellows (31,3  
114 Hz – Charlton 2015), indicating that the rejection calls are more likely to be produced  
115 by the laryngeal vocal folds.

116 In this study, we combined histological investigations with measurement data derived  
117 from dissections and computed tomography (CT) imaging to comprehensively  
118 document male and female koala vocal anatomy. Our specific aims were to: 1) provide  
119 a detailed description of the koala's vocal anatomy; 2) identify key differences in  
120 laryngeal and vocal tract anatomy between the sexes; 3) examine the tissue  
121 composition of the koala's remarkable velar vocal folds; and 4) determine *in situ*  
122 whether male koalas can retract their larynx into the thoracic inlet. Our findings will  
123 provide additional insights into the production mechanisms of male and female koala  
124 vocal signals.

125

## 126 **Material and Methods**

### 127 ***Specimens***

128 Whole-body specimens: One adult female and one adult male koala (weighing  
129 5.9 and 7.6 kg, and having overall body lengths of 680 and 750 mm, respectively) were  
130 dissected. The animals were euthanized as part of normal hospital practice at Moggill  
131 Koala Hospital, Bellbowrie, Queensland, Australia. A second adult male was used for  
132 demonstrating the 'retracted' larynx in a virtual 3D reconstruction based on a CT scan  
133 (S-Tab. 1). The specimens were stored deep-frozen (-20° C) before shipment to the  
134 Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany, for anatomical  
135 investigations. No animals were killed expressly for the purpose of this study.

136        Excised larynges: A total of 18 excised larynges (9 male, 9 female), including the  
137 part of the soft palate with the intra-pharyngeal ostium, were collected from dead and  
138 euthanized individuals at Moggill Koala Hospital during the 2011 breeding season  
139 (October-December). The specimens were stored deep-frozen (-20° C) before  
140 shipment to the Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany,  
141 for anatomical investigations. In addition, the larynges of the two dissected whole body  
142 specimens (1 male, 1 female) were included in this series. For measurements, the  
143 larynges were individually defrosted and mediosagittally cut into two halves. Larynx  
144 and vocal fold dimensions were measured in the half-larynx specimens whereas velar  
145 vocal fold dimensions were measured in the undivided soft palate parts. Overall  
146 dimensions of the larynx and velar vocal folds were documented photographically.

147

#### 148        ***Computer tomographic (CT) and anatomical investigations***

149        To obtain as much information as possible about skeletal and soft tissue prior to  
150 our macroscopic dissections, the male and female deep-frozen specimens were  
151 scanned using a 64-slice spiral Computer Tomograph Aquilion CX (Toshiba Medical  
152 Systems Corp., 1385 Shimoishigami, Otawara-shi, Tochigi 324-8550, Japan) at the  
153 IZW. The post-mortem *in situ* positioning of the vocal anatomy was documented using  
154 virtual serial sections (MPRs obtained with the settings: 120.0 kV, 120.0 mA, slice  
155 thickness 0.6mm) and 3D-reconstructions established by Vitrea 2 software. A second  
156 male was slowly defrosted in a refrigerator at +5° C over a period of 48 hours. Once  
157 the specimen had fully thawed, its larynx was pushed back into the thoracic inlet by  
158 external manipulation and fixed using a plastic strap. It was then refrozen and the  
159 plastic strap was removed in order to CT scan with the larynx in a fully retracted state  
160 (Fig. 2).

161 For all anatomical dissections conducted at the IZW the specimens were thawed  
162 at +5°C for a period of 24-48 hours and then submersed in water to facilitate  
163 macroscopic dissection and photographic documentation. This technique improves the  
164 diagnosis of structural details whilst also allowing for the dissection of non-preserved  
165 fresh specimens over a comparably long time period (cf. Frey et al 2007, 2008, 2011).  
166 The water in the dissection tank was continuously replaced by cool water (about 16°C)  
167 between consecutive dissection steps. Photographs were taken whilst the specimen  
168 was placed in a larger tank (using a Nikon D70s digital camera, Nikon Corp., Tokyo,  
169 Japan) and transferred to a computer for image processing (Adobe Photoshop 5.5 and  
170 CS4). Specimens were kept overnight in water at temperatures ranging between +2  
171 and +4°C (refrigerator: Philipp Kirsch GmbH, Okenstrasse 103, 77652 Offenburg,  
172 Germany).

173 The specimens were dissected starting from the left side, and the anatomical  
174 structures were removed layer by layer. The right side was dissected in an identical  
175 manner. The larynges, head-and-neck glands, most muscles, hyoid apparatus, soft  
176 palate, tongue, ears and the trachea of the specimens were all fixed in 4% formalin  
177 and stored in the IZW morphological collection. The skeletal parts were cleaned and  
178 also stored in the IZW morphological collection. Photos of consecutive dissection steps  
179 were taken with a Nikon D70S digital camera (Nikon Corp., Tokyo, Japan) on a CF  
180 card, fed to a PC and processed with Adobe Photoshop 5.5 and CS4 (Adobe Systems  
181 Inc., San Jose, CA, USA).

182

### 183 ***Micro-CT investigation of excised larynges***

184 In order to obtain *in-situ* information on laryngeal cartilages and soft tissues, one  
185 male and one female excised larynx (both earlier cut into two halves for



186 measurements) were subjected to Micro-CT investigation prior to invasive  
187 macroscopic dissection. The deep-frozen larynges were slowly defrosted at about 5°  
188 C for approximately 24h, and put into 4% formalin for 3 days to achieve soft fixation  
189 and avoid autolysis during the long scanning procedure. Subsequent storage in  
190 Phosphate Buffered Saline (PBS) for 5 days served to wash out the formalin.  
191 Immediately before scanning, a larynx was transferred to a 50 ml falcon tube filled with  
192 FomblinY®Perfluoropolyether lubricant (Solvay GmbH, Hannover, Germany. This oil  
193 is MR-inert, thus giving contrast only to the tissue, and avoids dehydration of the  
194 specimen during scanning. The scanning was conducted using a 7T PharmaScan  
195 70/16 US (Bruker BioSpin, Ettlingen, Germany) with Bruker software Paravision 5.1  
196 and a linear 38 mm volume resonator (Bruker BioSpin). Scanning was performed with  
197 the following settings: T2\_TurboRARE\_3D with TR/TE = 1500/32.5ms, rare factor 6,  
198 averages 6; FOV (Field of View) = 46 x 30 x 25.8mm; Matrix size = 306 x 200 x 172;  
199 image resolution = 150 µm x 150 µm x 150 µm; Scan time for the male and female  
200 larynx: 14h11m24s, respectively.

201

## 202 ***Histology***

203 Following *in-situ* dissection in water, the VVFs of the whole body male specimen  
204 were excised and preserved in 10% neutral buffered formalin. Longitudinal and cross  
205 sections (5 µm in thickness) of the VVFs were then stained with haematoxylin-eosin  
206 for a general overview, Masson/Goldner Trichrome as a collagen fibre stain, and  
207 Elastica-Van Gieson as an elastic fibre stain. Photographs of representative  
208 histological slides were taken with a Keyence VHX 1000 Digital Microscope (Keyence  
209 Deutschland, Neu-Isenburg, Germany) using a VH-Z00R, RZ ∞ - 50, Zoom 5-50x lens

210 and a VH-Z20R, RZ x20-x200 lens. Photoshop version CS4 was used to process the  
211 images.

212

### 213 ***Laryngeal retraction***

214 At key stages of the dissection, the larynx was manually retracted to document  
215 laryngeal mobility and extension of the pharynx, including the soft palate and the velar  
216 vocal folds. Retraction was achieved by fastening a string to the caudal end of the  
217 trachea, manually pulling the string through an opening in the abdominal wall, and  
218 attaching the string to the wall of the dissection tub using a clamp. Simultaneously, the  
219 head and neck were extended and pulled forward by a string fastened to an incisor  
220 tooth and attached to the opposite wall of the dissection tub by using another clamp.

221 Once the dissections were complete, the specimens were subjected to bacterial  
222 skeletonization. For this purpose, the skeletal parts were first put into appropriately  
223 sized tubs filled with cold water for several days to wash the blood out. The specimens  
224 were then transferred to a heated tank filled with water and kept at 38°C until the soft  
225 parts had been fully decomposed by bacteria. The bacterial skeletonization process  
226 took approximately one month. After this, the remaining bones were kept in the same  
227 tank at the same temperature in a concentrated solution of washing powder for an  
228 additional month before the skeletal parts were cleaned, dried, and used for graphic  
229 2D-reconstructions.

230 Anatomical terms are in accordance with Nomina Anatomica Veterinaria  
231 (Schaller 2012; NAV, 2005, 2012).

232

233            **Abbreviations**

234	Adit. lar.	=	laryngeal entrance
235	App. hyo.	=	hyoid apparatus
236	Arc. pal. phar.	=	palatopharyngeal arc
237	Arc. zyg.	=	zygomatic arc
238	Basih.	=	basihyoid
239	Bifurc.	=	tracheal bifurcation
240	Bul. tymp.	=	tympanic bulla
241	Cart. aryt.	=	arytenoid cartilage
242	Cart. cost. IV	=	4 <sup>th</sup> costal cartilage
243	Cart. cric.	=	cricoid cartilage
244	Cart. postaryt.	=	postarytenoid cartilage
245	Cart. proaryt.	=	proarytenoid cartilage
246	Cart. thyр.	=	thyroid cartilage
247	Cart. trach. I, IV	=	1 <sup>st</sup> , 4 <sup>th</sup> tracheal cartilage
248	Cav. nasi	=	nasal cavity
249	Cav. or.	=	oral cavity
250	Ceratoh.	=	ceratohyoid
251	Choan.	=	choanae (internal nares)
252	Clav.	=	clavicle
253	Cont. Mand.	=	contour of lower jaw
254	Cor	=	heart
255	Corn. caud.	=	caudal horn of thyroid cartilage
256	Corn. rostr.	=	rostral horn of thyroid cartilage
257	Cost. I , III, IV	=	1 <sup>st</sup> , 3 <sup>rd</sup> , 4 <sup>th</sup> rib
258	Crist. med.	=	dorsomedian crest of soft palate
259	C1, C7	=	1 <sup>st</sup> , 7 <sup>th</sup> cervical vertebra
260	Diaphr.	=	diaphragm
261	Epigl.	=	epiglottis
262	Intsect. tend.	=	tendinous intersection

263	IPO	=	intra-pharyngeal ostium
264	Lar.	=	larynx
265	Lig. hyo:	=	hyoid ligament
266	Ling.	=	tongue
267	LVBs	=	laryngeal vocal folds
268	Man. sterni	=	sternal manubrium
269	Meat. ac. ext.	=	external acoustic meatus
270	M. ceratoh.	=	ceratohyoid muscle
271	M.cricphar.	=	cricopharyngeus muscle
272	M. digastr.	=	digastric muscle
273	M. digastr. vent. caud.	=	caudal belly of digastric muscle
274	M. digastr. vent. rostr.	=	rostral belly of digastric muscle
275	M. genioh.	=	geniohyoid muscle
276	M.geniogllar.	=	genioglossolaryngeal muscle
277	M. hyoceph.	=	hyocephalic muscle (united M. styloh.,
278			M. stylphar.caud., M.hyophar.)
279	M. hyogl. (sin.)	=	(left) hyoglossus muscle
280	M. hyophar.	=	hyopharyngeus muscle
281	M. omoh.	=	omohyoid muscle
282	M. parotaur.	=	parotidoauricularis muscle
283	M. sternceph.	=	sternocephalic muscle
284	M. sternoh. (sin., dex.)	=	(left, right) sternohyoid muscle
285	M. sternthyr. (sin., dex.)	=	(left, right) sternothyroid muscle
286	M. stylogl.	=	styloglossus muscle
287	M. styloh.	=	stylohyoid muscle
288	M. stylphar. caud.	=	caudal stylopharyngeal muscle
289	M. thyroh.	=	thyrohyoid muscle
290	M. thyrophar	=	thyropharyngeus muscle
291	M <sup>4</sup>	=	4 <sup>th</sup> upper molar
292	Nar. (sin.)	=	(left) nostril
293	Nasal vt.	=	nasal vocal tract

294	Nasophar.	=	nasopharynx
295	Oesoph.	=	oesophagus
296	Oral vt.	=	oral vocal tract
297	Orophar.	=	oropharynx
298	Os pteryg.	=	pterygoid bone
299	Os rostr.	=	rostral bone
300	Palat. mol.	=	soft palate
301	Phar.	=	pharynx
302	Plic. voc.	=	vocal fold
303	Proc. parac.	=	paracondylar process
304	Proc. voc.	=	vocal process of arytenoid cartilage
305	Proc. xiph.	=	xiphoid process
306	Pulm.	=	lungs
307	Scap.	=	scapula
308	Stern.	=	sternum
309	Sulc. med.	=	dorsomedian trench of soft palate
310	Thyroh.	=	thyrohyoid
311	Trach.	=	trachea
312	T1, T4	=	1 <sup>st</sup> , 4 <sup>th</sup> thoracic vertebra
313	Vest. lar.	=	laryngeal vestibule
314	Vest. nasi	=	nasal vestibule
315	VTL	=	vocal tract length
316	VVF(s) (dex.)	=	(right) velar vocal fold(s)

317

## 318 **Results**

### 319 Nostrils, nasal vestibulum and nasal cavity

320 The nostrils of both sexes are obliquely oriented, from laterodorsal to ventromedial in  
321 frontal view. In lateral view, starting from the nares, the narrow, tube-like nasal

322 vestibulum first courses 80° dorsally towards the nasal dorsum before it bends sharply  
323 in a caudal direction to connect to the larger volume of the nasal cavity proper.  
324 Caudally, the upward portion of the nasal vestibulum is supported by a small rostral  
325 bone (*Os rostrale*) at the rostral end of the nasal septum, resting mediosagittally on the  
326 incisive bone (Fig. 3, 8). From the most dorsal point of the nasal vestibulum, at eye  
327 level, the air passage runs slightly oblique caudoventrally (10°-15°) and almost straight,  
328 along the ventral nasal meatus and the nasopharyngeal meatus up to the choanae.

### 329 Choanae

330 The osseous choanae for both sexes are located comparatively far rostrally, at a level  
331 just caudal to the last molars (M<sup>4</sup>/M<sub>4</sub>). In lateral view, the lateral osseous laminae of  
332 the palatine and pterygoid bones have a very oblique ventral edge (caudally inclined  
333 by about 45°) as a foundation for anchoring the thick soft palate. As a consequence of  
334 the great dorsoventral height of the soft palate at this level, the nasopharyngeal meatus  
335 is narrow dorsoventrally and transversely and courses horizontally along the skull  
336 base, passing by the long pterygoids and the auditory bulla, before sharply bending  
337 ventrally towards the nasopharynx at a level between the auditory bulla and the  
338 paracondylar processes.

### 339 Cheek pouches

340 Cheek pouches are present in both the male and the female (S-Fig. 1). They are  
341 positioned immediately caudal to the upper lip and the mouth angle, between the nostril  
342 and the eye. Their opening is obliquely oriented, from the small upper canine  
343 rostr dorsally, to the first premolars P<sup>3</sup>/P<sub>3</sub> caudoventrally, following the contour of the  
344 upper lip. The dimensions of the cheek pouches for the female and male were:  
345 rostrocaudal length ~28/~35, dorsoventral height ~20/~25 and transverse width

346 ~15/~20 mm, respectively (S-Table 2). The caudoventral half of the cheek pouches  
347 contains a layer of buccal salivary glands on its lateral surface and is covered by the  
348 buccinator muscle.

### 349 Pharynx

350 The upper pharynx is very spacious and extensible (S-Fig. 2; S-Video 1). It connects  
351 the caudal ends of the nasal and oral cavities to the larynx and oesophagus. The  
352 resting position of the head is approximately at a right angle to the longitudinal axis of  
353 the body. This results in a pronounced bending of the upper pharynx, the oropharynx  
354 in particular, while the head is kept in its typical resting position. In contrast, neck  
355 extension, as observed in the initial phase of call production, will straighten the  
356 pharynx. Due to the short snout and the short hard palate, the choanae are positioned  
357 rather far rostrally, between the upper last molars ( $M^4$ ), and about half way along the  
358 length of the head from a lateral view. The rostroventral, lateral and caudoventral parts  
359 of the pharyngeal wall receive terminating fibres from the genioglossolaryngeal muscle  
360 (see below and S-Tab. 7). The mucosa of the nasopharynx resembles the respiratory  
361 mucosa in the respiratory region of the nasal cavity. The nasopharyngeal mucosa is  
362 finely plicated, pliable and highly elastic. The mucosa of the oropharynx resembles the  
363 cutaneous mucosa of the oral cavity. The oropharyngeal mucosa is corrugated and  
364 also pliable and highly elastic. Internally, the oropharynx is completely separated from  
365 the nasopharynx, except for one connection just rostral to the laryngeal entrance, the  
366 intra-pharyngeal ostium (IPO). The soft palate is large, thick, and of triangular shape  
367 when viewed from a mediosagittal perspective. Thickness is maximal close to the  
368 choanae and tapers caudally towards the IPO. Inside the triangular space, between  
369 the roof of the oropharynx and the floor of the nasopharynx, the palatopharyngeal

370 muscle surrounds the IPO on both sides (S-Tab. 7). When the pharynx is manually  
371 extended (Fig. 4, 5) the IPO remains close to the epiglottis and the laryngeal entrance.

### 372 Soft palate

373 The overall dimensions of the soft palate in the female and male were: resting length  
374 – 50/70, extended length – 75/100 and dorsoventral thickness at the choanae 15/20  
375 mm, respectively (S-Tab. 3). The entire soft palate is remarkable: its roof (= the mucosa  
376 of the dorsal surface of the soft palate) has a deep (~ 2 mm) median trench along its  
377 entire length in both sexes, although it is less prominent in the female. In the male, its  
378 transverse width in the resting position is only 1-2 mm, i.e. it is almost closed, whereas  
379 in the bilaterally extended state it is 6-8 mm wide, and becomes progressively wider  
380 towards the IPO. There is a median, serrated crest along the trench that terminates  
381 about 10 mm rostral to the IPO (Fig. 6A). The rostrocaudal length of the crest is ~ 20  
382 mm and tapers rostrally. Maximum dorsoventral height of the crest is ~ 3 mm. Lateral  
383 to the median trench, the mucosa is flat and smooth and the two halves of these  
384 elevated surfaces of the floor of the nasopharynx are both ~ 5 mm wide (transversely).  
385 The surface of the median trench is covered with small knobs or caruncles. The lateral  
386 flat surfaces are a light whitish/greenish colour, whereas the mucosa of the median  
387 trench is dark reddish/brownish. The mucosa of the median trench is supplied with  
388 many tiny blood vessels.

389 In addition, the male and female IPO is not a simple opening between the dorsal and  
390 ventral compartments of the pharynx, as it is in other mammals (S-Fig. 3). Instead, its  
391 medial edges form two large, longitudinally oriented, voluminous folds, which protrude  
392 ventrally into the caudal part of the oropharynx (laryngopharynx) (Fig. 6B). These folds  
393 are termed 'velar vocal folds' (VVF's - Charlton et al 2013).



394 The mucosa of the ventral surface of the soft palate possesses a shallow median  
395 depression, about 9 mm wide rostrally, that narrows to 3 mm halfway along the  
396 rostrocaudal length. Along its caudal third, the depression widens to form a triangle  
397 that merges with a broad, caudally open horseshoe-shaped fold, which encompasses  
398 the velar vocal folds. Its longitudinal limbs, 6 mm wide rostrally, taper in caudal direction  
399 and fuse with the ventrocaudal parts of the velar vocal folds (VVF) (Fig. 6B). The  
400 palatopharyngeal arc consists of a narrow fold surrounding the IPO, the VVFs, and the  
401 horseshoe-shaped fold, before fusing with the dorsocaudal wall of the pharynx caudal  
402 to the VVFs. On each side there is a rostrally closed blind space of about 15 mm in  
403 rostrocaudal length (destroyed on the left side when cutting into the pharynx), between  
404 the horseshoe-shaped fold medially and the palatopharyngeal arc laterally. The entire  
405 mucosa of the ventral surface of the soft palate is finely plicated transversely (Fig. 6B).

#### 406 Velar vocal folds

407 VVFs occur in the male and the female. Rostral to the IPO, the lateral flat ventral  
408 surfaces of the soft palate rise up to form two longitudinally oriented, voluminous folds  
409 medially along the edges of the IPO (Fig. 6B). These velar vocal folds are clearly  
410 distinct from the surrounding soft palate. They narrow the IPO considerably, restricting  
411 it to a slit-like opening that is slightly wider rostrally than caudally. Laterally, the velar  
412 vocal folds are encompassed by a horseshoe-shaped fold and, further laterally, by the  
413 palatopharyngeal arc. Caudally, each VVF fuses with the respective limb of the  
414 horseshoe-shaped fold. The velar vocal folds protrude ventrally into the  
415 laryngopharynx (Fig. 3). The dimensions of the VVFs for female and male (n = 10,  
416 respectively) are: resting length -  $26.2 \pm 2.59$  versus  $32.5 \pm 2.8$ , resting depth –  $11.7 \pm 1$   
417 versus  $14.9 \pm 2.13$ , resting width –  $7.6 \pm 0.74$  versus  $10.7 \pm 1.32$  and maximally extended  
418 length –  $43.6 \pm 5.1$  versus  $51.4 \pm 6.29$  mm. (S-Tab. 4).

419 Histological examination of the VVFs reveals that they are differentiated from the  
420 surrounding soft palate. Longitudinal and cross sections (5 µm thickness) show a three  
421 layered structure. The superficial thin layer consists of a cornifying stratified squamous  
422 epithelium. The intermediate layer comprises two sublayers: a subepithelial thin layer  
423 of collagenous and elastic fibres running mostly parallel to the surface of the VVFs;  
424 and a thicker layer of very loosely organised short and mostly single collagenous fibres,  
425 intermingled with few, mostly single elastic fibres. This sublayer contains numerous  
426 small, thin-walled vessels, most likely venules or lymphatic vessels and nerves. The  
427 deepest layer, constituting the base of the VVFs, is composed of a scaffolding of larger  
428 bundles of collagenous and elastic fibres, and striated muscle fibre bundles  
429 interspersed with groups of mucinous glands, larger blood vessels and nerves. The  
430 collagen and elastin fibres within this deep layer are mostly longitudinal in  
431 arrangement. The basal deep layer makes the transition to the larger muscle bundles,  
432 the larger, mostly transversely arranged, scaffolding of collagenous and elastic fibres,  
433 larger aggregations of mucinous glands, and larger blood vessels and nerves of the  
434 soft palate proper (Fig. 7).

435 Although the structure of the VVFs is broadly similar in males and females, there are  
436 a few key differences. The VVFs of the dissected male specimen are larger than those  
437 of the female (S-Tab. 4), and the second (b) sublayer of the intermediate layer appears  
438 to contain a higher overall density of collagenous fibres, which are organised in thicker  
439 bundles in the male than in the female. In the male there is also a large, longitudinally  
440 oriented bundle of elastic fibres, running parallel to the free edge of the VVFs in the  
441 intermediate layer. This bundle appears to be lacking in the female VVFs. The deep  
442 layer of the VVFs also appears to be more muscular in the male than in the female.

443 Hyoid apparatus

444 The koala's hyoid apparatus is suspended from the skull base by a pair of highly  
445 resilient hyoid ligaments. Each of these hyoid ligaments consists of several sub-  
446 ligaments that run parallel to one another. The dorsal attachment to the skull base is  
447 located between the auditory bulla and the paracondylar process. This attachment is  
448 supported by a small occipitohyoid muscle. Ventrally, the hyoid ligaments connect to  
449 the lateral ends of the ceratohyoids. Other parts of the suspension apparatus, for  
450 example, the tympanohyoids, stylohyoids, and epihyoids, are lacking. Interestingly, the  
451 hyoid ligaments of the male appear to be more elastic than those of the female: resting  
452 length versus maximally extended length in the female 30/60 mm, (100% extension)  
453 and 40/100 mm in the male, (150% extension) (S-Tab. 5). The ceratohyoids are short,  
454 cartilaginous, and fused rostrally to the transversely oriented osseous basihyoid.  
455 Caudolaterally, the basihyoid connects to two thyrohyoids that are directed  
456 caudodorsally (S-Fig. 4). The larger rostral part of the thyrohyoids is ossified whereas  
457 the caudal ends are cartilaginous. The cartilaginous part of the thyrohyoids connects  
458 to the rostral horn of the thyroid cartilage (Fig. 8).

#### 459 Thyrohyoid membrane

460 The thyrohyoid membrane extends between the thyroid cartilage and the thyrohyoid  
461 laterally, and the thyroid cartilage and the basihyoid ventrally. It is short and rather  
462 tough, with a ventral length that exceeds its lateral length. Rostrocaudal resting length  
463 for the female and the male are 2-3/3-4 mm laterally and 6-7/10-11 mm ventrally;  
464 maximally extended lengths are ~4/~5 mm laterally and 8-9/12-13 mm ventrally (S-  
465 Tab. 6). Due to the stiffness of the thyrohyoid membrane, when the larynx is manually  
466 retracted the ventral parts of the hyoid apparatus (ceratohyoids, basihyoid,  
467 thyrohyoids) stay close to the larynx as it descends down the neck towards the thorax.  
468 The highly elastic hyoid ligament permits the larynx to be fully retracted into the thoracic

469 inlet. The laryngeal entrance and the ventral parts of the hyoid apparatus are then  
470 located at the level of the manubrium.

#### 471 Musculature

472 The results of the ventral neck musculature dissection, including presumed muscle  
473 functions, are summarized in S-Table 7. Instances where muscles deviate greatly from  
474 the typical mammalian pattern are shortly listed below:

- 475 1) Three muscles, the digastric, the omohyoid and the sternohyoid, share a  
476 common tendinous intersection. In the koala the omohyoid and sternohyoid  
477 muscles have entirely lost their connection to the hyoid apparatus (Fig. 9).
- 478 2) The caudal fibres of the genioglossus muscle are of considerable length and  
479 do not terminate on the basihyoid. Instead they terminate on the lateral wall  
480 of the oropharynx and on the thyrohyoid membrane close to the rostral edge  
481 of the thyroid cartilage. As a consequence of this, it is henceforth called a  
482 genioglossolaryngeal muscle (Fig. 5).
- 483 3) The geniohyoid muscle is greatly extended in length and bunches up to form  
484 undulating reserve bends during the resting state of the larynx. It passes  
485 through a guiding arch provided by the hyoglossus muscle (S-Fig. 5).
- 486 4) The sternohyoid and sternothyroid muscles are deeply anchored in the thorax.  
487 The truly intrathoracic origins of both muscles arise from the dorsal surface of  
488 the sternum and the first few costal cartilages (Fig. 10).
- 489 5) The mylohyoideus and hyoglossus muscles have completely lost their  
490 attachment to the hyoid apparatus. In the koala the hyoglossus muscle forms  
491 a type of guiding arch for the long geniohyoid muscle (S-Fig. 5).
- 492 6) The stylohyoid, caudal stylopharyngeus, and hyopharyngeus muscles are  
493 difficult to compare with the corresponding muscles of placental mammals

494 because an osseous suspension of the hyoid apparatus to the skull is lacking  
495 in the koala. MacAlister (1872) and Young (1882) realised this and combined  
496 them as the styloid group. We found that the main portion of this muscle sheet  
497 originates from the thyrohyoid, and terminates on the base of the skull,  
498 caudally adjacent to the attachment of the hyoid ligament. We therefore  
499 suggest that these three muscles are grouped together under a new name: the  
500 hyocephalic muscle (Fig. 11).

## 501 Larynx

### 502 Larynx dimensions

503 Overall measurements of the larynx for female (n = 9) and male (n = 10) koalas are:  
504 maximal rostrocaudal length  $25.2 \pm 1.66$  versus  $28.2 \pm 2.68$  mm, maximal transverse  
505 width  $22.0 \pm 2.13$  versus  $25.2 \pm 2.68$  mm and maximal dorsoventral height  $16.7 \pm 0.78$   
506 versus  $19.5 \pm 1.62$  mm (S-Table 8).

### 507 Laryngeal cartilages

508 In both sexes, the cartilages of the larynx comprise the epiglottis, the thyroid cartilage,  
509 the cricoid cartilage, the two arytenoid cartilages, the proarytenoid cartilage, resting on  
510 the rostral commissure of the medial processes of the arytenoid cartilages, and a small  
511 postarytenoid cartilage located caudal to the commissure of the medial processes of  
512 the arytenoid cartilages (Fig. 12). The thyroid and cricoid cartilages are fused  
513 ventromedially (as previously noted, e.g., by Symington 1898; Schneider 1964 - S-Fig.  
514 6). The vocal process of the arytenoid cartilage is crescent-shaped, rostrally directed,  
515 and protrudes considerably in a ventral direction towards the laryngeal lumen. The  
516 vocal process extends far rostrally, up to the level of the proarytenoid cartilage and  
517 approaching the laryngeal entrance (Fig. 12). On either side of the cricoid, the paired

518 dorsal cricothyroid connection is established by a synchondrosis, which is located  
519 dorsal to the caudal laryngeal nerve. Laterally, the proarytenoid, the arytenoids, the  
520 postarytenoid, and the cricoid cartilage(s) are covered by the thyroid lamina and the  
521 broad rostral and long caudal horns of the thyroid cartilage.

522 The epiglottis is spatula-shaped in dorsal view. Medioventrally, its base  
523 connects to the rostral edge of the thyroid cartilage. Its laryngeal surface faces the  
524 laryngeal vestibule whereas the ventral third of its lingual surface serves as the origin  
525 of the hyoepiglottic muscle. The dimensions of the epiglottis for female (n = 8) and  
526 male (n = 9) koalas were: rostrocaudal length  $10.1 \pm 0.92$  versus  $11.0 \pm 0.77$  mm and  
527 transverse width  $12.2 \pm 1.08$  mm versus  $14.8 \pm 1.85$  mm (S-Table 9). The colour of the  
528 epiglottic cartilage is yellow, suggesting a composition of elastic cartilage (Fig. 12A).  
529 Cuneiform processes are lacking in both sexes.

530 The thyroid cartilage has a short, ventrally-curved rostral horn that establishes  
531 the cartilaginous connection to the thyrohyoid of the hyoid apparatus. In contrast, the  
532 caudal horn is long and straight, and protrudes caudally in parallel to the longitudinal  
533 axis of the larynx. The laminae of the thyroid cartilage are of trapezoid shape in lateral  
534 view, their ventral rostrocaudal length exceeding the dorsal by about one third. A large  
535 fat pad is sandwiched between the thyroid lamina laterally and the thyroarytenoid and  
536 ceratocricothyroid muscles medially. A thyroid bulla is not observed in the koala.

537 The cricoid cartilage has almost the same rostrocaudal dimensions dorsally and  
538 ventrally, i.e. the dorsal cricoid lamina is very short. In the dorsal third, the left and right  
539 rostral edges of the cricoid cartilage bulge out rostrally and form the articular surfaces  
540 for connection with the arytenoid cartilages. Together with the ventrally and dorsally  
541 fixed connections to the thyroid cartilage, the bilateral bulging creates a boomerang-  
542 shaped opening between the rigidly coupled thyroid and cricoid cartilages, its convex

543 shape being directed rostrally. In lateral view, the caudal edge of the cricoid cartilage,  
544 to which the first tracheal cartilage connects, is of concave shape.

545 The arytenoid cartilages are comparatively large and articulate with the  
546 dorsorostral bulges of the cricoid cartilage. Rostrally, the arytenoid cartilages extend  
547 upwards to form the caudal boundary of the laryngeal entrance. Each arytenoid  
548 cartilage possesses three well-developed processes: a lateral muscular process, a  
549 (dorso)medial process, and a ventrally directed vocal process. Corniculate processes  
550 of the arytenoid cartilages are lacking, and left and right arytenoid cartilage are linked  
551 to each other via their medial processes by connective tissue. The dorsoventral extent  
552 of the arytenoid cartilage is considerable. The vocal process protrudes widely in a  
553 ventral direction, and causes a slight prominence of the laryngeal mucosa at its ventral  
554 tip, to which the dorsal end of the vocal fold attaches.

555 The proarytenoid cartilage, which is also termed procricoid cartilage in the older  
556 literature, is a homologue of the interarytenoid cartilage in placental mammals  
557 (Symington 1898; Schneider 1964, p. 42). It is located rostral to where the medial  
558 processes of the arytenoid cartilages connect and its caudal surface is intimately linked  
559 to this transverse junction by connective tissue.

#### 560 Intrinsic laryngeal muscles

561 The cricothyroid muscle is lacking, possibly as a result of the ventral fusion of the  
562 thyroid and cricoid cartilages, and the concomitant restriction in mobility between these  
563 two cartilages. The thyroarytenoid muscle is a homologue of the corresponding muscle  
564 of placental mammals (Schneider 1964, p. 34). It originates medioventrally from the  
565 thyroid lamina and its fibres course laterodorsally, before terminating on the muscular  
566 process of the arytenoid cartilage ventrally. The thyroarytenoid muscle does not  
567 connect to the vocal process of the arytenoid cartilage.

568 The lateral cricoarytenoid muscle is also a homologue of the corresponding muscle of  
569 placental mammals (Schneider 1964, p. 34). It originates from the laterodorsal surface  
570 of the cricoid cartilage, where it is partly covered by the ceratocricicoarytenoid muscle.  
571 Its fibres course obliquely rostr dorsally and terminate lateroventrally on the muscular  
572 process of the arytenoid cartilage.

573 The aryprocricoid muscle in the koala is a homologue of the transverse arytenoid  
574 muscle of placental mammals (Schneider 1964, p. 35). It originates from the arcuate  
575 crest of the arytenoid cartilage and most of its fibres terminate on the proarytenoid  
576 cartilage, whereas the most rostral fibres fuse with those of the contralateral muscle.

577 The cricoprocricoarytenoid muscle originates from the dorsal edge of the cricoid  
578 cartilage and terminates laterocaudally on the procricoid cartilage.

579 The koala's ceratocricicoarytenoid muscle is a homologue of the dorsal cricoarytenoid  
580 muscle of placental mammals (Schneider 1964, p. 44). It originates from the medial  
581 surface of the caudal horn of the thyroid cartilage, the lateral surface of the cricoid  
582 cartilage, and the laterodorsal parts of the cricoid lamina. The muscle terminates  
583 dorsally on the muscular process and the arcuate crest of the arytenoid cartilage. We  
584 assume that the function of the ceratocricicoarytenoid muscle is to dilate the glottis.

#### 585 Laryngeal entrance and cavity

586 The laryngeal entrance is surrounded by the epiglottis rostrally, the aryepiglottic folds  
587 laterally, and the arytenoid cartilages caudally. A large pad of small glands is laterally  
588 attached to each of the aryepiglottic folds, and mostly covered by the thyroid laminae.  
589 The laryngeal cavity comprises a vestibule extending from the entrance of the larynx  
590 to the vocal folds, the glottis between the vocal folds, and an infraglottic space,  
591 extending from the vocal folds to the exit of the larynx at its junction with the trachea.



592 As a consequence of the large vocal process of the arytenoid cartilages and the  
593 relatively short vocal folds, the ratio of the intercartilaginous to intermembraneous  
594 portions of the glottis is approximately 50:50.

#### 595 Laryngeal vocal folds

596 The laryngeal vocal folds (LVFs) are rostrally inclined against the longitudinal axis of  
597 the larynx by about 30°, from craniodorsally to caudoventrally. Ventrally, the LVFs  
598 attach to the dorsal surface of the fused thyroid and cricoid cartilages, close to the  
599 cranial edge of the cricoid arch in a paramedian plane. Dorsally, the LVFs attach to the  
600 tip of the vocal process of the arytenoid cartilage. The dimensions of the LVFs for  
601 female (n = 9) and male koalas (n = 10) respectively are: dorsoventral resting length  
602  $8.0 \pm 0.57$  versus  $9.8 \pm 0.88$  mm, rostrocaudal resting length  $0.8 \pm 0.1$  versus  $1.0 \pm 0.13$   
603 mm, transverse resting width  $0.5 \pm 0.12$  versus  $0.8 \pm 0.16$  mm and maximally extended  
604 dorsoventral length  $9.8 \pm 0.39$  versus  $11.8 \pm 0.94$  mm (S-Tab. 10).

#### 605 Laryngeal mobility

606 The resting position of the larynx is approximately at the level of cervical vertebrae 4-  
607 6 in an adult male koala (Fig. 8). The larynx can be manually retracted down into the  
608 thoracic inlet in both sexes. At maximal retraction, the ceratohyoids and the basihyoid  
609 are close to the level of the first rib, and the maximally extended hyoid ligament runs  
610 more or less parallel to the ventral edge of the hypaxial neck musculature. Larynx  
611 movements down and up the ventral neck region are facilitated by a highly flexible  
612 double gliding layer of connective tissue that dorsally separates the pharynx, larynx  
613 and oesophagus from the hypaxial neck musculature (longus capitis, longus colli  
614 muscles) (S-Fig. 7). The medial retropharyngeal lymph nodes are located on the dorsal  
615 wall of the pharynx at the level of the first tracheal rings, contacting the dorsal gliding

616 layer. The lateral retropharyngeal lymph nodes are located a bit more cranially, at the  
617 level of the osseous hyoid apparatus in its resting position, and in the groove between  
618 the nasopharynx and the neck musculature. Ventrally, the muscular channel or  
619 guidance for any potential craniocaudal movements of the pharynx, larynx and  
620 oesophagus, provided by the inter-linked digastric, omohyoid and sternohyoid  
621 muscles, is lined with a connective tissue layer. In addition, there is a fat cushion  
622 between the styloglossus muscle laterally and the genioglossolaryngeal muscle  
623 medially, and a short connective tissue canal at the thoracic inlet, through which the  
624 larynx can move. This canal is attached to the clavicle, the carotid artery, the neck  
625 fascia and the sternal manubrium. This gliding space continues inside the thorax,  
626 dorsal to the heart and the large blood vessels at the base of the heart, which are both  
627 covered by the pericardial pleura. Movement of the larynx, oesophagus and trachea  
628 inside the thorax is further guided by the thoracic portions of the hypaxial neck  
629 musculature (longus colli muscle) dorsally and the sternohyoid and sternothyroid  
630 muscles ventrally. A gliding space for minor cranio-caudal movements of the  
631 intrathoracic portions of the sternohyoid and sternothyroid muscles along the  
632 craniodorsal surface of the sternum is provided by the common connective tissue  
633 sheath interlinking these muscles, ventrally, and by the dorsally adjacent pericardial  
634 pleura.

635

### 636 Vocal tract length

637 Oral vocal tract length (hereafter VTL) was taken as the distance from the lips to the  
638 LVFs, and the nasal VTL was measured from the tip of the nostrils to the LVFs. The  
639 dimensions of the koala's nasal and oral vocal tract lengths during different stages of  
640 the dissection of one female and one male koala are: resting nasal vtl with angled head

641 160-165 versus 185-190 mm, maximally extended nasal vtl with angled 185-190  
642 versus 230-240, maximally extended nasal vtl with head and neck extended (male only  
643 255-260 mm; resting oral vtl with angled head 140-145 versus 160-170 mm, maximally  
644 extended oral vtl with head and neck extended (male only) 210-220 mm (S-Tab. 11).  
645 The laryngeal vocal folds are not involved in the production of male koala bellow  
646 vocalizations, and it is presumed that the glottis is open during call production (Charlton  
647 et al 2013). Accordingly, we also provide additional measurements of vtl from the  
648 nostrils and lips to the VVFs (a) and from the nostrils and lips down to the tracheal  
649 bifurcation (b) at maximal extension in the male specimen: nasal vtl ~220 mm (a) and  
650 ~ 295 mm (b), oral vtl ~190 mm (a) and ~270 mm (b) (S-Tab. 11).

651

## 652 Nerves

653 The nerve supply to the pharynx, larynx, and associated muscles did not appear to  
654 differ between the male and female adult koala specimens. The hypoglossal nerve  
655 emerges from the skull base ventral to the external acoustic meatus, between the  
656 auditory bulla rostrally and the paracondylar process caudally. This nerve curves  
657 laterally around the hyoid ligament obliquely in a rostral direction, and along the ventral  
658 edge of the styloglossus muscle. It then enters the hyoglossus muscle laterally before  
659 subdividing into 4 branches: three branches curve dorsally along the rostral edge of  
660 this muscle to innervate the tongue and supply the intrinsic tongue muscles. The most  
661 ventral of these branches supplies the rostral part of the genioglossolaryngeal muscle.  
662 This branch is undulating, while the dorsal two are not. The most ventral branch of the  
663 hypoglossal nerve is also an undulating nerve that supplies the geniohyoid muscle.

664 Motor nerve supply to the omohyoid, sternohyoid and sternothyroid muscles is  
665 provided by the ventral ramus of the first cervical nerve (N. cervicalis I). As a

666 consequence of the long sternohyoid and sternothyroid muscles, the associated nerve  
667 fibres are of corresponding length. The lengths of the more or less straight branches  
668 to the caudal, intrathoracic parts of the sternohyoid and sternothyroid muscles are  
669 around 150 mm in the female and around 200 mm in the male. In contrast, branches  
670 to the cranial parts of the sternohyoid and sternothyroid muscles form caudally convex  
671 reserve loops that are slightly undulating.

672         The glossopharyngeal nerve emerges from the skull base close to the  
673 paracondylar process. It traverses the caudal stylopharyngeal muscle in a rostral  
674 direction and provides a small branch to this muscle. The pharyngeal branches of the  
675 glossopharyngeal nerve to the dorsal wall of the pharynx were not dissected. The  
676 larger lingual branch of the glossopharyngeal nerve runs ventrally along the rostral  
677 border of the caudal stylopharyngeal muscle and medial to the hyoid ligament before  
678 sharply bending rostrally towards the lateral wall of the oropharynx and the tongue. On  
679 the way it subdivides into a dorsal branch leading to the soft palate and a ventral branch  
680 leading to the root of the tongue. All of these branches of the glossopharyngeal nerve  
681 are strongly undulating nerves.

682         The vagus nerve emerges from the skull base together with the  
683 glossopharyngeal nerve. The oesophageal branch of the vagus nerve is a strongly  
684 undulating nerve and courses caudally along the nasopharynx towards the caudal  
685 constrictor muscles of the pharynx and the oesophagus. At the level of the thyroid  
686 cartilage it subdivides into three branches supplying the thyro- and cricopharyngeal  
687 muscles and the vestibulum of the oesophagus (Fig. 13). A further branch of the vagus  
688 nerve, the cranial laryngeal nerve, is also strongly undulating. It courses caudally along  
689 the caudal edge of the hyocephalic muscle and pierces the thyropharyngeal muscle  
690 before entering the larynx with its ventral branch. The strongly undulating lingual

691 branch of the glossopharyngeal nerve, the cranial laryngeal nerve, and the  
692 oesophageal branch of the vagus nerve appear to be enclosed in hose-like connective  
693 tissue sheaths (Fig. 13).

#### 694 Trachea

695 In both sexes all the tracheal cartilages between the cricoid cartilage and the bronchial  
696 cartilages are dorsally separated by a ~ 5 mm wide gap that is bridged by elastic  
697 connective tissue i.e. they do not touch dorsally. This membranous wall continues  
698 dorsally onto the main bronchi. The number of tracheal cartilages (from cricoid to  
699 bifurcation) was 17 in the female and 18 in the male. Both the resting and the maximally  
700 extended lengths of the trachea did not differ appreciably between male and female: ~  
701 40 mm and ~ 70 mm, respectively.

#### 702 Sexual dimorphism of vocal anatomy and acoustic values

703 In order to evaluate the measured differences of vocal features and acoustic values  
704 between males and females, intersexual body size differences have to be considered  
705 (Tab. 1)

706

#### 707 **Discussion**

708 This study provides the first comprehensive description of the koala's vocal anatomy.  
709 Although anatomical peculiarities of the vocal organs and the associated musculature  
710 in koalas were noted by the classical morphologists (MacAlister 1872; Young 1882;  
711 Symington 1898; Sonntag 1921), they were mostly considered in a taxonomic context.  
712 By combining detailed anatomical data obtained using modern imaging and  
713 histological techniques, systematic dissections, behavioural observations, and

714 published acoustic data, we are now able to provide a comprehensive and functional  
715 account of the koala's unusual vocal anatomy.

#### 716 A simple, but descended larynx and a specialised hyoid apparatus

717 Although most features of the koala larynx correspond to those of other marsupials, a  
718 rostral thyroid bulla, generally present in marsupials, is lacking in the koala (Schneider  
719 1964, p. 9). One of the key differences between the marsupial larynx and the larynx  
720 of placental mammals is the characteristic ventral fusion of the thyroid and cricoid  
721 cartilages observed in the former. This longitudinal cartilaginous connection is derived  
722 and arises postnatally (Symington 1898; Schneider 1964, p. 8, 14). The greatly  
723 restricted mobility between both cartilages is likely to explain why the marsupial larynx  
724 lacks a cricothyroid muscle (Schneider 1964, p. 26). In placental mammals, the mutual  
725 mobility of thyroid and cricoid cartilages and coordinated activities of the thyroarytenoid  
726 and cricothyroid muscles represent a major means of tensioning the laryngeal vocal  
727 folds (LVFs), thereby increasing the range of producible fundamental frequencies (F0)  
728 (Titze 1993; Titze et al 2016). As a consequence of the stiffened thyroid/cricoid  
729 connection and the lacking cricothyroid muscle in the marsupial larynx, the potential  
730 F0 range and control over F0 is either reduced or differently achieved in marsupials.

731         The 50:50 ratio between the intercartilaginous and intermembraneous portions  
732 of the koala glottis indicates a large vocal process of the arytenoid cartilage, which  
733 widely protrudes rostroventrally along the inner laryngeal wall (Schneider 1964, p. 17).  
734 In our specimens we found that male LVFs were ~ 10 mm and female LVFs were ~ 8  
735 mm long. Considering the LVFs and their predicted F0 frequency of ~ 400 Hz (Charlton  
736 et al 2013), the LVFs are probably only involved in the production of the higher F0  
737 vocalizations produced by this species. In addition, both male and female koalas the  
738 LVFs are rostrally inclined, and dorsally attach to the rostrally directed vocal process.

739 A comparable rostral inclination occurs in wild boar (*Sus scrofa*); however, in this  
740 placental species the vocal process is caudally directed (Schneider 1964, p. 64f, fig.  
741 66). The koala larynx does not possess lateral laryngeal ventricles and, as a result,  
742 vestibular folds are also lacking (Fig. 12). The lack of a thyroid bulla and lateral  
743 laryngeal ventricles means that there are virtually no intra-laryngeal resonance spaces.

744 Interestingly, in the koala, all the dorsal suspensory elements of the hyoid  
745 apparatus (except the ceratohyoids) have been evolutionarily transformed into a highly  
746 resilient hyoid ligament. Due to the short cartilaginous connection between the  
747 thyrohyoid and the larynx and the short and tough thyrohyoid membrane, it is likely that  
748 the ventral cartilaginous and osseous parts of the hyoid apparatus, i.e. the  
749 ceratohyoids, the basihyoid and the thyrohyoids, are retracted together with the larynx  
750 towards the thoracic inlet during call production (see 'Laryngeal mobility, calling  
751 posture and the VVFs'). Because the sternohyoid muscles have lost their attachment  
752 to the hyoid apparatus, they cannot support the sternothyroid muscles in retracting the  
753 ventral parts of the hyoid apparatus and larynx towards the sternum (see 'Muscles and  
754 nerves of the vocal tract').

#### 755 A novel organ for low frequency production

756 The most spectacular evolutionary transformation of the koala's vocal organs has  
757 occurred in the region of the intra-pharyngeal ostium (IPO). In both sexes, the lateral  
758 edges of the IPO have been transformed into two large, longitudinally oriented,  
759 voluminous folds that protrude ventrally towards the laryngeal entrance and medially  
760 towards the midline (cf. McCurry et al 2016 Fig. 6; S-Fig. 3). These extra laryngeal  
761 folds have previously been termed 'velar vocal folds' or VVFs because they look  
762 remarkably similar to large laryngeal vocal folds (Charlton et al 2013). This previous  
763 study also confirmed that VVFs function as extra-laryngeal acoustic sources in male

764 koalas (Charlton et al 2013), allowing them to produce the extremely low F<sub>0</sub> of bellow  
765 vocalisations (circa 27 Hz). While we confirm here that female koalas also possess  
766 VVFs, in our specimens the male has larger VVFs (Tab. 5), which protrude further into  
767 the laryngopharynx than in the female, indicating that these structures are significantly  
768 more developed in males than in females.

769 Owing to their position and orientation, the VVFs can only function as a sound  
770 source during nasal inhalation phases of vocal production. This function requires  
771 tensioning and closure of the IPO, which can be described as a ‘velar glottis’. When  
772 we extended the pharynx in our specimens the IPO remained close to the laryngeal  
773 entrance, confirming that the VVFs are ideally located for sound production during  
774 nasal inhalations. The VVFs are also heavily wrinkled in their resting (relaxed) state,  
775 which might provide the reserve tissue necessary for allowing the dramatic length  
776 changes accompanying larynx retraction and tensioning of the soft palate, including  
777 the VVFs and the closure of the IPO prior to vocal production. Functionally, this would  
778 correspond to the tensioning and bringing together of the LVFs by muscle-induced  
779 movements of the arytenoid cartilages inside the larynx.

780 It is also worth noting that narrowing of the IPO by the VVFs is expected to  
781 impede nasal respiration. Therefore, except prior to vocal production, the VVFs should  
782 narrow the IPO as little as possible. As the VVFs cannot be pivoted laterally by  
783 cartilage-muscle-induced pulling, the change between respiratory and phonatory  
784 position must be achieved by a different mechanism. Shrinking of the VVFs after vocal  
785 production might be facilitated by their distinct wrinkles in the resting position.  
786 Additionally, expansion of the dorsal trench and the longitudinal plication of the soft  
787 palate, and relaxation of the palatopharyngeal muscle (the constrictor of the IPO) might  
788 be involved in keeping the IPO open. These features may also play a role in bellow-



789 synchronous pharynx expansion (S-Fig. 2; S-Video 1). Opening and closing of the IPO  
790 might also be regulated by coordinated contractions of the strap muscles (inducing  
791 length changes) and palatopharyngeal muscle (inducing width changes).

792 Our histological examination reveals that the VVFs are organised into three  
793 discrete layers, approximating the structure of placental mammal LVFs (Hirano et al  
794 1981; Kurita et al 1983; Hirano & Kakita 1985), although on a larger scale. The  
795 structure of the VVFs can be interpreted as a body-cover complex, in which the third,  
796 deep layer (muscle bundles and connective tissue scaffolding) represents the 'body'  
797 and the upper two layers (epithelium and sub-epithelial loosely organised connective  
798 tissue) the cover. Hence, the VVFs consist of a robust basal region near their transition  
799 to the soft palate and a soft and deformable apical region close to their free edge  
800 flanking the IPO. Accordingly, the VVFs, like the LVFs, can be described as a layer-  
801 structured oscillator (Hirano & Kakita 1985).

802 In addition, we found that male VVFs contain more collagen fibres than female  
803 VVFs, and have a large bundle of elastin fibres in the lower portion of the second layer  
804 of the VVFs. In humans, significantly higher levels of collagen are found in the male  
805 vocal fold (Chan et al 2007) and testosterone is known to increase the collagen/elastin  
806 ratio of tissue (Fischer & Swain 1980). If we consider that the levels of collagen and  
807 elastin could contribute differentially to VVF elasticity, testosterone exposure during  
808 development, or the dramatic increase in testosterone that is known to occur in male  
809 koalas just prior to the breeding season (Mitchell 1990; Allen 2010), could facilitate low  
810 frequency, periodic oscillation of the VVFs during call production by increasing the  
811 stiffness of these structures, allowing males to produce their extremely low F0 bellow  
812 vocalisations.

813 Muscles and nerves of the vocal tract

814 Several muscles of the koala's throat and ventral neck region deviate considerably  
815 from the typical mammalian pattern (cf. Nickel et al 1979). Although this has been  
816 noted previously, to our knowledge, no functional explanation has been proposed so  
817 far for this unique arrangement in the koala (MacAlister 1872; Young 1882; Sonntag  
818 1921, 1922). Recent findings (Charlton et al 2013), along with observations of vocal  
819 behaviour, now allow us to propose a functional explanation. We suggest that the  
820 unusual, muscular organisation of the koala's throat and ventral neck region facilitates  
821 the dynamic movement of the larynx-hyoid complex during production of bellow  
822 vocalisations. The main retractor of the larynx and the ventral hyoid parts, the strap-  
823 like sternothyroid muscle, has elongated and extended its origin deep into the thorax.  
824 Pronounced contractions of this muscle would produce a strong pull on the larynx and  
825 ventral hyoid parts, resulting in the caudal movement of these structures down into the  
826 thorax. In the koala, unlike other mammals, the caudal movement of the larynx-hyoid  
827 complex is not restricted by connections to the tongue and lower jaw because the  
828 mylohyoid and hyoglossus muscles are not attached to the hyoid apparatus, and  
829 because the genioglossolaryngeus and geniohyoid muscles have been greatly  
830 elongated (Fig. 14).

831 We suggest that the return of the larynx and ventral hyoid parts to their resting  
832 position is achieved by the resilience of the hyoid ligament and of the pharynx, which  
833 become heavily extended during larynx and ventral hyoid retraction, and the constrictor  
834 muscles of the pharynx, the hyocephalic muscle, and strongly elongated extrinsic  
835 tongue muscles. Concomitant shortening of the pharyngeal walls would be supported  
836 by contractions of the middle and caudal pharyngeal constrictors and the hyocephalic  
837 muscle, all of which are elongated during the extension phase. The  
838 genioglossolaryngeus muscle, which extends from the tongue down to the thyrohyoid

839 membrane and the rostral edge of the thyroid cartilage, would then be able to contract  
840 and return the larynx to its resting position (around the 4<sup>th</sup> cervical vertebra). The  
841 prominent, and greatly extended strap-like geniohyoid muscle, would additionally  
842 support the mobility of the larynx and ventral hyoid parts down and up the ventral neck  
843 region. For example, during the retraction phase, i.e. during contraction of the  
844 sternohyoid muscle, the undulations of the geniohyoid muscle will be stretched and  
845 contraction of the geniohyoid muscle during the protraction phase will restore the  
846 undulations. In addition, because the fibres of the hyoglossus muscle diverge and  
847 connect to the tendinous intersection between omohyoid, sternohyoid and digastric  
848 muscle, it forms an arch around the long geniohyoid muscle. This arrangement  
849 ensures, analogous to the retinacula of certain tendons of limb muscles (cf. Clavero et  
850 al 2005; Numkarunarunrote et al 2007; Robertson et al 2007), that the geniohyoid  
851 muscle is kept in the correct position as the larynx is maximally retracted and then  
852 returned to its resting position. Protraction of the larynx and ventral hyoid parts will  
853 further be assisted by contraction of the hyocephalic muscle, which suspends these  
854 elements from the skull base (Fig. 14).

855         The three muscles that are connected to each other by a tendinous intersection  
856 (digastric, omohyoid and sternohyoid muscles), together with their contralateral  
857 counterparts, appear to function as a longitudinal muscular guiding channel, along  
858 which the pharynx, the ventral hyoid parts, and the larynx and trachea are able to glide  
859 in a rostrocaudal direction during their dynamic down and up movements. As the  
860 koala's omohyoid and sternohyoid muscles have lost their connection to the hyoid  
861 apparatus, they would not be involved in moving the ventral hyoid parts. In contrast,  
862 the sternohyoid muscle is likely to be very effective as a lateral guide bar because it  
863 extends dorsally from the sternum and costal cartilages up to the rostral portion of the

864 digastric muscle. Strong extension of the head and neck, as observed during bellow  
865 production, probably facilitates this guiding by stabilising, tensioning, and extending  
866 the channel along a straight line from the angle of the lower jaw down into the thorax.  
867 Rostrocaudal gliding movements of the ventral hyoid parts and the larynx are further  
868 facilitated by the double connective tissue layer intercalated between the hypaxial neck  
869 musculature and the cervical vertebrae dorsally and the pharynx, larynx and  
870 oesophagus ventrally (S-Fig. 7). In addition, because the digastric muscles terminate  
871 on the lower jaw, they are likely to act as an anchor for the guiding channel. However,  
872 in contrast to laryngeal movements in ruminants with a descended larynx (Frey et al  
873 2008; 2011; 2012), the short and weak thyrohyoid muscle of koalas cannot assist in  
874 returning the larynx to its resting position because the ventral parts of the hyoid  
875 apparatus, including the thyrohyoid, descend together with the larynx during the  
876 retraction phase. Hence, the most parsimonious explanation for the profound  
877 evolutionary transformation of the koala's throat and ventral neck musculature is that  
878 it facilitates the retraction and return of the larynx and ventral parts of the hyoid  
879 apparatus during the production of this species' bellow vocalisations. The phylogenetic  
880 distance between koalas and ruminants (marsupial vs. placental species) suggests  
881 independent evolution of a descended and mobile larynx and the respective muscular  
882 organisation of these species reinforce this notion.

883         As the muscles involved in extensive rostrocaudal excursions of the larynx  
884 elongated and underwent considerable length changes during protraction and  
885 retraction of the larynx over evolutionary time, the nerves supplying these muscles had  
886 to elongate correspondingly. In order to accommodate the length changes of their  
887 target muscles these nerves evolved two different modes of adjusting to shortly and  
888 profoundly changing muscle lengths. First, the nerves to the rostral portions of the

889 ribbon-like strap muscles (*M. sternohyoideus*, *M. sternothyroideus*) evolved large,  
890 caudally sagging loops, which can be stretched during muscle extension (e.g. by head  
891 and neck extension, or by strong muscle contraction during larynx retraction). Second,  
892 the nerves to the muscles in the region of the pharynx evolved strongly undulating  
893 branches, which can also be stretched during extensions of the pharynx. Additionally,  
894 the strongly undulating nerve branches evolved hose-like connective tissue sheaths to  
895 facilitate stretching and rewinding of the nerves (Fig. 13). Similar undulating nerves  
896 have been documented in ruminants with a descended and mobile larynx, e.g. Iberian  
897 red deer (*Cervus elaphus hispanicus*) and goitred gazelle (*Gazella subgutturosa*) (Frey  
898 et al 2011, 2012).

#### 899 Laryngeal mobility, calling posture and the VVFs

900 In ruminant species with a descended and mobile larynx (e.g. red deer, fallow deer,  
901 Mongolian gazelle, goitred gazelle) the larynx is retracted, thereby elongating the  
902 pharynx (the acoustic filter), and the LVFs (the acoustic source) produce the  
903 fundamental frequency at exhalation. In koalas the low fundamental frequency is  
904 produced by the pharyngeal VVFs, and hence, laryngeal retraction must have a  
905 different function.

906 In species that produce low fundamental frequencies using the LVFs, e.g.  
907 elephants (Herbst et al 2012) sound production requires adduction of the arytenoid  
908 cartilages, i.e. closure of the glottis, and tensioning of the LVFs by means of intrinsic  
909 laryngeal muscle contractions. Then, and only then, can sustained periodic oscillations  
910 of the LVFs be achieved by an air stream (typically produced by exhalation from the  
911 lungs). While the structure of the VVFs clearly differs from that of the LVFs, the glottis-  
912 like cleft in between these novel structures also has to be closed, and tension applied  
913 to the VVFs before periodic oscillations can be induced (by an inhalatory airstream in

914 koala bellows (Charlton et al 2011b, 2013). Since the VVFs are not directly connected  
915 to any skeletal parts, closure of the 'velar glottis' and tensioning of the VVFs must be  
916 achieved in a quite different way from that of the LVFs.

917         The koala's VVFs are part of the pharynx. Therefore, to produce a bellow  
918 vocalisation both vocal tract elongation and tension on the VVFs have to be achieved  
919 simultaneously by appropriate adjustment of the pharynx. The lowering of the formants  
920 is produced by larynx retraction (as in ruminants with a descended and mobile larynx,  
921 for example: Frey et al 2008, 2011, 2012). In contrast to these ruminants, however  
922 (see above), the tension on the VVFs that is required to produce a source signal is  
923 effected by the same action. Strong extension of the pharynx will tend to bring the  
924 median edges of the VVFs together, thereby closing the 'velar glottis'. Simultaneously,  
925 strong extension of the pharynx, including the soft palate, will set the VVFs under  
926 tension. As such, laryngeal retraction leading to the extension of the pharynx seems to  
927 be the most likely mechanism for producing low F<sub>0</sub> and formants in the koala (Charlton  
928 et al 2013). As a consequence of this coupled mode of sound production and formant  
929 lowering, and in contrast to ruminants with a descended mobile larynx and an intra-  
930 laryngeal sound source, production of the lowest frequencies in koalas requires  
931 maximal vocal tract extension, i.e. maximal extension of the acoustic filter. At low  
932 pharynx tension, the 'velar glottis' would not close and the VVFs would not be  
933 sufficiently tensed for producing the very low extra-laryngeal source frequencies.

934         As in male ruminants with a descended larynx, the extension of the pharynx in  
935 koalas is supported by a characteristic calling posture involving pronounced head and  
936 neck extension (Fig. 1 – for ruminants cf. Frey et al 2008, 2011, 2012). However, in  
937 contrast to ruminants, male koalas produce their mating calls in a characteristic two-  
938 stage posture, gradually changing from pronounced extension (position 1) to distinctive

939 flexion (position 2) of the head and neck region (Charlton et al, 2011). In the first  
940 position, the neck is extended and the head turned upward so that the longitudinal axis  
941 of the head is held in parallel to the longitudinal axis of the body (Fig. 1A). In the course  
942 of the call, along with numerous vocal in- and exhalations, the head is successively  
943 lowered until, as the very low F0 and formants are produced at the end of the call, it  
944 reaches the second position (Fig. 1B). Here, the longitudinal axis of the head is either  
945 held at an obtuse angle relative to the longitudinal axis of the body, or the chin is pulled  
946 a bit more ventrally towards the sternum (Charlton 2011b). Presumably, position 1  
947 involves pronounced larynx retraction down to the thoracic inlet, thereby effecting  
948 longitudinal extension of the pharynx. In position 2, the angular position of the head  
949 will raise the base of the skull, to which the rostral portion of the pharynx is attached,  
950 thereby achieving the utmost of pharynx extension. During previous experiments on  
951 koala cadavers we found that position 2 was best for setting the VVFs under  
952 appropriate tension to produce this species' characteristically low F0 (Charlton et al  
953 2013).

#### 954 Sexual dimorphism of vocal anatomy versus acoustics

955 Using head length as a proxy for overall body size, male Queensland koalas are  
956 around 9% larger than females (Ellis & Bercovitch 2011). In the current study we found  
957 that male head length (measured from the occipital ridge to the tip of the nasal bone:  
958 Charlton et al 2011) was around 10% greater than female head length (Tab. 1), which  
959 accords well with the findings of Ellis and Bercovitch on 35 male and female koalas  
960 (2011), and suggests that our relatively small sample of six males and females was  
961 representative of the Queensland koala. Male body length was around 4% greater  
962 than female body length in our sample (Tab. 1). Accordingly, if we assume that male

963 koalas are between 4-10% larger than females, then the size dimorphism in vocal  
964 anatomy is greater than expected (Tab. 1).

965         When we consider how size dimorphism in vocal anatomy relates to acoustic  
966 differences between male and female koalas, we find that the 15-16% longer male  
967 vocal tract corresponds extremely well to the documented 16% lower  $\Delta F$  of male  
968 koalas when compared to females (Tab. 1) (Charlton et al 2011, Charlton 2015). Since  
969 differences in male and female vocal tract length closely track differences in male and  
970 female formant spacing, these findings lend further support to the notion that the  
971 broadband frequency components that have been identified in previous studies  
972 (Charlton et al 2011, Charlton 2015) are indeed formants. The production of the  
973 extremely low formant frequencies in male and female bellows remain unexplained.  
974 We suggest that koalas use the VVFs to simultaneously excite resonances in the oral  
975 and nasal vocal tract, including “sub-laryngeal” spaces like the trachea and the main  
976 bronchi. Future studies that use precise geometrical data to predict the centre  
977 frequencies of the oral and nasal vocal tracts (including the sub-laryngeal air spaces)  
978 are now required to confirm how koalas produce such low formant frequencies for their  
979 size.

980         Because koalas produce the exceptionally low F0 of bellow vocalisations using  
981 their VVFs (Charlton et al 2013), we would also expect bellow F0 dimorphism to track  
982 VVF dimorphism. We found that male VVFs were 24% longer than female VVFs, yet  
983 male bellow mean F0 (of 28.7 Hz) is only 8% lower than the female bellow mean F0  
984 (of 31.3 Hz) (Tab. 1). However, much closer correspondence between VVF sexual  
985 size dimorphism and differences in male and female minimum bellow F0 was found  
986 (Tab. 1), illustrating that the minimum producible F0 is ultimately constrained by the  
987 length of the oscillating structure generating the sound (in this case the VVFs).



988 Interestingly, male LVFs are 22% longer than females, which is far above the ~4-10%  
989 size difference expected from overall body size differences between the sexes. Data  
990 on male and female F0 from the same call type that is likely to be produced using the  
991 larynx are now required to determine how this large difference between male and  
992 female LVF length corresponds to acoustic differences.

993

994

995 **Acknowledgements**

996 We cordially thank Dipl. Ing. Susanne Mueller, Labmanager at the Charité Core Facility  
997 7T Experimental MRIs, Centrum für Schlaganfallforschung Berlin, Charité University  
998 Medicine Berlin, Berlin, Germany, for performing the Micro-CT scanning of a male and  
999 a female koala larynx. We further thank Doris Krumnow, Department of Wildlife  
1000 Diseases at the IZW, who prepared the histology slides and Gudrun Wibbelt, same  
1001 Department, one of the pathologists of the IZW, Berlin, for crucial support in the  
1002 interpretation of those slides. We also want to thank all of the staff at Moggill Koala  
1003 Hospital, especially Allan McKinnon and Peter Theilemann for helping us to obtain the  
1004 specimens. A Scientific Purposes Permit (WISP12601313) approved the study.  
1005 Benjamin D. Charlton was financially supported by a Leverhulme Trust Early Career  
1006 Fellowship.

1007 **References**

- 1008 Allen CD, de Villiers DL, Manning BD, Dique DS, Burridge M, Chafer ML et al (2010)  
1009 Seasonal reproduction in wild and captive male koala (*Phascolarctos cinereus*)  
1010 populations in south-east Queensland. *Reprod Fertil* 22, 695-709.
- 1011 Andersson M (1994) *Sexual selection*. Princeton, Princeton University press, 599 pp.
- 1012 AZA Koala SSP Veterinarian Manual (2009), 28 pp.
- 1013 Berillis P (2013) The role of collagen in the aorta's structure. *The Open Circulation*  
1014 *and Vascular Journal (TOCVJ)* 6, 1-8.
- 1015 Briscoe NJ, Krockenberger A, Handasyde KA, Kearney MR (2015) Bergmann meets  
1016 Scholander: geographical variation in body size and insulation in the koala is related  
1017 to climate. *J Biogeogr* 42, 791-802.
- 1018 Cembrano J, Lillo M, Val J, Mardones J (1960) Influence of sex difference and  
1019 hormones on elastin and collagen in the aorta of chicken. *Circ Res* 8, 527-529.
- 1020 Chan RW, Fu M, Young L, Tirunagari N (2007) Relative contributions of collagen and  
1021 elastin to elasticity of the vocal fold under tension. *Ann Biomed Eng* 35, 1471-1483.
- 1022 Charlton B (2015) The acoustic structure and information content of female koala  
1023 vocal signals. *PLoS One* 10, e0138670.
- 1024 Charlton BD, Ellis WAH, Brumm J, Nilsson K, Fitch WT (2012c) Female koalas prefer  
1025 bellows in which lower formants indicate larger males. *Anim Behav* 84, 1565-1571.
- 1026 Charlton BD, Ellis WAH, Larkin R, Fitch WT (2012a) Perception of size-related  
1027 formant information in male koalas (*Phascolarctos cinereus*). *Anim Cogn* 15, 999-  
1028 1006.

1029 Charlton BD, Ellis WAH, McKinnon AJ, Brumm J, Nilsson K, Fitch WT (2011a)  
1030 Perception of male caller identity in koalas (*Phascolarctos cinereus*): acoustic  
1031 analysis and playback experiments. PLoS ONE 6.

1032 Charlton BD, Ellis WAH, McKinnon AJ, Cowin GJ, Brumm J, Nilsson K, Fitch WT  
1033 (2011b) Cues to body size in the formant spacing of male koala (*Phascolarctos*  
1034 *cinereus*) bellows: honesty in an exaggerated trait. J Exp Biol 214, 3414-3422.

1035 Charlton B, Frey R, McKinnon A, Fritsch G, Fitch W, Reby D (2013). Koalas use a  
1036 novel vocal organ to produce unusually low-pitched mating calls. Curr Biol 23,  
1037 R1035-R1036.

1038 Charlton BD, Reby D (2016) The evolution of acoustic size exaggeration in terrestrial  
1039 mammals. Nat Commun 7, 12739.

1040 Charlton BD, Reby D, Ellis WAH, Brumm J., Fitch WT (2012b) Estimating the active  
1041 space of male koala bellows: propagation of cues to size and identity in a Eucalyptus  
1042 forest. PLoS ONE 7.

1043 Charlton BD, Whisson DA, Reby D (2013) Free-ranging male koalas use size-related  
1044 variation in formant frequencies to assess rival males. PLoS ONE 8.

1045 Charlton BD (2015) The acoustic structure and information content of female koala  
1046 vocal signals. PLoS ONE 10(10): e0138670. doi:10.1371/journal.pone.0138670, 19  
1047 pp.

1048 Clavero JA, Golanó P, Fariñas O, Alomar X, Monill JM, Esplugas M (2003) Extensor  
1049 mechanism of the fingers: MR imaging - anatomic correlation. Radiographics 23,  
1050 593-611.

1051 Dayme MB (2009) Dynamics of the singing voice. 5<sup>th</sup> edition. Wien, New York,  
1052 Springer, 252pp. ISBN 978-3-211-88728-8

1053 Doscher B (1994) The functional unity of the singing voice. 2<sup>nd</sup> edition. Lanham &  
1054 London, Scarecrow Press, 352 pp. ISBN 0-8018-2708-5

1055 Ellis WAH, Bercovitch FB (2011) Body size and sexual selection in the koala. Behav  
1056 Ecol Sociobiol 65, 1229-1235.

1057 Ellis WAH, Melzer A, Bercovitch FB (2009) Spatiotemporal dynamics of habitat use  
1058 by koalas: the checkerboard model. Behav Ecol Sociobiol 63, 1181-1188.

1059 Ellis WAH, Bercovitch FB, FitzGibbon S, Roe P, Wimmer J, Melzer A, Wilso R (2011)  
1060 Koala bellows and their association with the spatial dynamics of free-ranging koalas.  
1061 Behav Ecol 22, 372-377.

1062 Fant G. (1960). Acoustic Theory of Speech Production. The Hague: Mouton.

1063 Fischer GM, Swain ML (1980) Influence of contraceptive and other sex steroids on  
1064 aortic collagen and elastin. Exp Mol Pathol 33, 15-24.

1065 Fitch WT (2010) The evolution of language. Cambridge, Cambridge University Press,  
1066 624 pp.

1067 Fitch WT, Reby D (2001) The descended larynx is not uniquely human. Proc R Soc  
1068 London, Series B: Biological Sciences 268, 1669-1675.

1069 Flower WH (1885) An introduction to the osteology of the mammalia. Marsupial  
1070 hyoids, 238-241 including 3 figures. 3<sup>rd</sup> edition. London, MacMillan & Co, 383 pp.

1071 Frey R, Volodin I, Volodina E (2007) A nose that roars: anatomical specializations  
1072 and behavioural features of rutting male saiga. J Anat 211, 717-736.

1073 Frey R, Gebler A, Olson KA, Odonkhuu, Fritsch G, Batsaikhan N, Stuermer IW  
1074 (2008) Mobile larynx in Mongolian gazelle: Retraction of the larynx during rutting  
1075 barks in male Mongolian gazelle (*Procapra gutturosa* PALLAS, 1777). *J Morphol* 269,  
1076 1223-1237.

1077 Frey R, Volodin I, Volodina E, Soldatova NV, Juldachev ET (2011) Descended and  
1078 mobile larynx, vocal tract elongation and rutting roars in male goitred gazelles  
1079 (*Gazella subgutturosa* Gldenstaedt, 1780). *J Anat* 218, 566-585.

1080 Frey R, Volodin I, Volodina E, Carranza J, Torres-Porras J (2012) Vocal anatomy,  
1081 tongue protrusion behaviour and the acoustics of rutting roars in free-ranging Iberian  
1082 red deer stags (*Cervus elaphus hispanicus*). *J Anat* 220, 271-292.

1083 Herbst CT, Stoeger AS, Frey R, Lohscheller J, Titze IR, Gumpenberger M, Fitch WT  
1084 (2012) How low can you go? Physical production mechanism of elephant infrasonic  
1085 vocalizations. *Science* 337, 595-599.

1086 Herbst CT, Švec JG, Lohscheller, Frey R, Gumpenberger M, Stoeger AS, Fitch WT  
1087 (2013) Complex vibratory patterns in an elephant larynx. *J Exp Biol* 216, 4054-4064.

1088 Hirano M, Kurita S, Nakashima T (1981) The structure of the vocal folds. In: Vocal  
1089 fold physiology (Stevens KN, Hirano M, eds), 33-43. Tokyo: University of Tokyo  
1090 Press.

1091 Hirano M, Kakita Y (1985) Cover-body theory of vocal fold vibration, 1-46. In: Speech  
1092 science (Daniloff RG, ed). London, Taylor & Francis.

1093 Jackson S (2003) Australian mammals: biology and captive management. CSIRO  
1094 Publishing, 147-151.

- 1095 Koda H, Nishimura T, Tokuda IT, Oyakawa C, Nihonmatsu T, Masataka N (2012)  
1096 Soprano singing in gibbons. *Am J Phys Anthropol* 149, 347-355.
- 1097 Kratzing JE (1984) The anatomy and histology of the nasal cavity of the koala  
1098 (*Phascolarctos cinereus*). *J Anat* 138, 55-65.
- 1099 Kurita S, Nagata K, Hirano M (1983) A comparative study of the layer structure of the  
1100 vocal fold. In: *Vocal fold physiology: contemporary research and clinical issues* (Bless  
1101 DM, Abbs JH, eds), 3-21. San Diego: College Hill Press.
- 1102 Lee AK, Carrick FN (1989) Phascolarctidae. In *Fauna of Australia*, vol. 1B Mammalia  
1103 (ed. Dyne GR, Walton DW), 31 pp.; pp. 740-754. Canberra: Australian Government  
1104 Publishing Service.
- 1105 Lieberman DE, McCarthy RC, Hiiemae KM, Palmer JB (2001) Ontogeny of postnatal  
1106 hyoid and larynx descent in humans. *Arch Oral Biol* 46, 117-128.
- 1107 MacAlister A (1872) The muscular anatomy of the koala. *Ann Mag Nat Hist* 10, 127-  
1108 134.
- 1109 Martin R, Handasyde KA (1999) *The koala: natural history, conservation and*  
1110 *management*. Sydney (Australia): UNSW press.
- 1111 Martin R, Handasyde K (1999) *The koala – natural history, conservation and*  
1112 *management*. Malabar, Florida, Krieger Publishing, 132 pp.
- 1113 Martin R, Handasyde K, Krockenberger A (2008) Koala, *Phascolarctos cinereus*. *The*  
1114 *mammals of Australia*, 3<sup>rd</sup> edition (Van Dyck S, Strahan R eds), 198-201. Chatswood,  
1115 NSW. New Holland Publishers.

1116 McCurry MR, Quayle MR, Cally J, Adams JW (2016) Velar vocal folds are present in  
1117 female and immature male koalas (*Phascolarctos cinereus*). Aust Mammal 38, 232-  
1118 233.

1119 McElligott AG, Birrer M, Vannoni E. (2006) Retraction of the mobile descended larynx  
1120 during groaning enables fallow bucks (*Dama dama*) to lower their formant  
1121 frequencies. J Zool 270, 340-345.

1122 Mitchell P (1990) Social behaviour and communication of koalas. In: Biology of the  
1123 koala (Lee AK, Handasyde KA, Sanson GD, eds), 151-170. Chipping Norton, NSW:  
1124 Surrey Beatty and Sons.

1125 NAV - Nomina anatomica veterinaria, 5th edition (2005) prepared by ICVGAN,  
1126 authorized by WAVA 2003. Hannover, Ghent, Columbia MO and Sapporo, XVII &  
1127 166 pp.

1128 NAV - Nomina anatomica veterinaria, 5th edition, revised version (2012) prepared by  
1129 ICVGAN, authorized by WAVA 2003. Hannover, Ghent, Columbia MO and Sapporo,  
1130 XVII & 160 pp.

1131 Némai J, Kelemen G (1933) Beiträge zur Kenntnis des Gibbonkehlkopfes. Z Anat  
1132 Entw Gesch 100, 512-520.

1133 Nickel R, Schummer A, Seiferle E (1979) The Viscera of the Domestic Mammals. 2<sup>nd</sup>  
1134 revised edition. Berlin, Hamburg. Paul Parey

1135 Numkarunarunrote N, Malik A, Aguiar RO, Trudell DJ, Resnick D (2007) Retinacula  
1136 of the foot and ankle: MRI with anatomic correlation in cadavers. AJR (Am J  
1137 Roentgenol) 188, W348-W354.



- 1138 Peters W (1961) Methoden zur Herstellung von Aufhellungspräparaten. Zool Anz  
1139 167, 233-240.
- 1140 Piechocki R (1967) Makroskopische Präparationstechnik. Teil I Wirbeltiere. 2.  
1141 überarbeitete und ergänzte Auflage. X. Herstellung von Aufhellungspräparaten, 305-  
1142 346. Leipzig. Akademische Verlagsgesellschaft Geest & Portig.
- 1143 Reby D, Wyman MT, Frey R, Passilongo D, Gilbert J, Locatelli Y, Charlton BD (2016)  
1144 Evidence of biphonation and source-filter interactions in the bugles of male North  
1145 American wapiti (*Cervus canadensis*). J Exp Biol 219, 1224-1236.
- 1146 Robertson BL, Jamadar DA, Jacobson JA, Kalume-Brigido M, Caoili EM, Margaliot Z,  
1147 De Maeseneer MO (2007) Extensor retinaculum of the wrist: sonographic  
1148 characterization and pseudotenosynovitis appearance. AJR (Am J Roentgenol) 188,  
1149 198-202).
- 1150 Schaller O (ed) (2012) Illustrated Veterinary Anatomical Nomenclature. 3<sup>rd</sup> revised  
1151 edition. Stuttgart: Enke in MVS Medizinverlage, 620 pp. ISBN 978-3-8304-1086-7
- 1152 Schneider R (1964) Der Larynx der Säugetiere. 1-128. Helmcke J-G, Lengerken Hv,  
1153 Starck D, Wermuth H (eds) Handbuch der Zoologie – eine Naturgeschichte der  
1154 Stämme des Tierreiches. Bd. 8, 35. Lieferung. Berlin. Walter de Gruyter.
- 1155 Smith M (1980a). Behaviour of the koala, *Phascolarctos cinereus* (Goldfuss), in  
1156 captivity. III. Vocalizations. Australian Wildlife Research 7, 13-34.
- 1157 Smith M (1980b). Behaviour of the koala, *Phascolarctos cinereus* (Goldfuss), in  
1158 captivity. IV. Scent-marking. Australian Wildlife Research 7, 35-40.
- 1159 Sonntag CF (1921) The comparative anatomy of the koala (*Phascolarctos cinereus*)  
1160 and vulpine phalanger (*Trichosurus vulpecula*). Proc Zool Soc London 39, 547-577.

1161 Sonntag CF (1922) On the myology and classification of the wombat , koala, and  
1162 phalangers. Proc Zool Soc London 1922, 863-896.

1163 Spalteholz W (1914) Über das Durchsichtigmachen von menschlichen und tierischen  
1164 Präparaten. 2. Auflage. Leipzig. S. Hirzel, 93pp.

1165 Symington J (1898) The marsupial larynx. J Anat Physiol 33, 31-49.

1166 Taylor AM, Charlton BD, Reby D (2016) Vocal Production by Terrestrial Mammals:  
1167 Source, Filter and Function. In Vertebrate Sound Production and Acoustic  
1168 Communication. New York, Springer. 229-259.

1169 Titze IR (1994) Principles of Voice Production. Englewood Cliffs, Prentice Hall, 354  
1170 pp.

1171 Tyndale-Biscoe H (2005) Life of marsupials. Collingwood: Csiro Publishing.

1172 Wells KD (2001) The energetic cost of calling in tree frogs. In: Ryan MJ (ed) Anuran  
1173 communication. Washington, DC. Smithsonian Institution Press, 45-60.

1174 Wells KD, Schwartz JJ (2006) The behavioural ecology of anuran communication. In:  
1175 Narins PM, Feng AS, Fay RR, Popper AK (eds) Hearing and sound communication in  
1176 amphibians. New York. Springer, 44-86.

1177

1178 Young AH (1881) Anatomy of the koala (*Phascolarctos cinereus*). J Anat Physiol 15,  
1179 466-474.

1180 Young AH (1882) The muscular anatomy of the koala (*Phascolarctos cinereus*), with  
1181 additional notes. J Anat Physiol 16, 217-242.

1182

1183 **Tables**

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<b>Table 1. Sexual dimorphism of body size, vocal anatomy and acoustic variables</b>						
LVFs = laryngeal vocal folds, VVFs = velar vocal folds, VTL = vocal tract length						
oral and nasal VTL measured from the lips and nostrils to the glottis, respectively, with the larynx in its resting state, head angled and pharynx opened. ^ denotes values taken as the midpoint between two measures. Acoustic values from Charlton et al (2011) and Charlton (2015). In all cases sexual dimorphism is calculated by dividing male over female measures.						
	Male (n)	Female (n)	Male	Female	Dimorphism	
<b>Anatomy</b>						
Body length (mm)	6	6	685	657	1.04	
Head length (mm)	6	6	139	127	1.10	
LVFs (mm)	10	9	9.8	8	1.22	
VVFs (mm)	10	10	32.5	26.2	1.24	
oral VTL	1	1	165	142	1.16	
nasal VTL	1	1	187	162	1.15	
<b>Acoustics</b>						
Bellow mean F0	20	23	28.7	31.3	0.92	
Bellow minimum F0	20	23	9.9	12.3	0.80	
Bellow ΔF	20	23	354	423	0.84	

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1188 **Figure legends**

1189 **Fig. 1:** Two-stage calling posture of an adult male koala while producing a bellow  
 1190 vocalisation. Initial stretched posture, head, neck, and thorax extended (A); flexed  
 1191 posture towards the end of the call, head angled, neck and thorax less extended (B).  
 1192 Landmarks used for the red longitudinal head line are the mouth opening and the  
 1193 visible ear base, which is close to the atlantooccipital articulation. Landmarks used for  
 1194 the red longitudinal body line are the visible ear base and (less reliable) the assumed  
 1195 position of the cranial edge of the pelvis. In A the head line is in straight continuation  
 1196 of the body line (modified from Charlton et al, 2011)

1197 **Fig. 2:** Initial calling posture and full larynx retraction demonstrated by a CT-based 3D  
1198 reconstruction of the skeleton in an adult male specimen. Prior to CT scanning, the  
1199 larynx had been externally fixed in a maximally retracted position. Left lateral view.

1200 **Fig. 3:** The nasal cavity of an adult male koala. 3D reconstruction of the skeleton of  
1201 the upper body; left foreleg, clavicle and shoulder removed; virtual sagittal section of  
1202 the skull and lower jaw; background: a photo of the same specimen. Tongue, soft  
1203 palate and hyoid ligament manually reconstructed for full larynx retraction. Left lateral  
1204 view. Note the presence of a small rostral bone riding on the incisive bone and lending  
1205 support to the tip of the nose (similar to European wild boar, *Sus scrofa*).

1206 **Fig. 4:** Fully extended pharynx in an adult male koala. Multi Planar Reconstruction  
1207 (MPR) based on a CT scan of an adult male, in which the larynx had been externally  
1208 pushed back and fixed to simulate maximal larynx retraction. The ventral parts of the  
1209 hyoid apparatus and the larynx are located at the thoracic inlet. Note the capacious  
1210 naso- and oropharynx and the long soft palate. The IPO (indicated by the red ellipse)  
1211 is also situated far caudally, opposite the laryngeal entrance.

1212 **Fig. 5:** Dissection stage at which full retraction of the ventral hyoid parts and the larynx  
1213 down into the thoracic inlet had been simulated. Maximal extension of the pharynx; the  
1214 hyoid ligament, and the genioglossolaryngeal muscle are exposed. Scale bar 10 mm

1215 **Fig. 6:** The excised soft palate of an adult male koala: dorsal view with part of right  
1216 lateral wall of nasopharynx (A), and ventral view (B). A prominent feature of the dorsal  
1217 surface is the narrow median trench passing caudally into the IPO. A prominent feature  
1218 of the ventral surface is the IPO with the large VVFs protruding into the laryngopharynx.  
1219 The asterisk marks a steel pin inserted into the right blind space lateral to the VVFs.  
1220 Scale bar 10 mm

1221 **Fig. 7: Upper panel:** Histological sections of the male VVFs. The cornifying stratified  
1222 squamous epithelium and the underlying, parallel network of elastic fibres (black) and  
1223 collagenous fibres (pink) of the upper intermediate layer (A); high density of thicker  
1224 bundles of collagenous fibres in the lower intermediate layer (B); longitudinally oriented  
1225 large bundle of elastic fibres, parallel to the free edge of the VVFs in the lower  
1226 intermediate layer (C); longitudinal muscle fibre bundles of the deep layer near the  
1227 laterodorsal base of the VVFs (D).

1228 A, B and D: transverse sections, C: sagittal section. A, C and D: Elastica van Gieson  
1229 staining, D: Masson/Goldner Trichrome staining. In each subfigure the arrow points  
1230 towards the highlighted feature.

1231 **Fig. 7: Lower panel:** Histological sections of the female VVFs. The cornifying stratified  
1232 squamous epithelium and the upper intermediate layer of collagenous and elastic  
1233 fibres (A); the mostly longitudinally arranged muscle fibre bundles, collagenous and  
1234 elastic fibres inside the VVF stand out against the mostly transversely oriented thick  
1235 muscular bundles of the soft palate in the region of the VVFs (B); Detail of the deepest  
1236 layer of the VVF (C), illustrating the scaffolding of collagenous and elastic fibres  
1237 interspersed with muscle fibre bundles and mucinous glands; soft palate immediately  
1238 caudal to the VVFs containing transversely arranged larger bundles of elastic fibres  
1239 (D).

1240 A, D: transverse sections; B, C: sagittal sections. A, B, C, D: Elastica van Gieson  
1241 staining. In each subfigure the arrow points towards the highlighted feature.

1242 **Fig. 8:** CT-based 3D reconstruction of the hyoid apparatus in an adult male koala. The  
1243 position of the left hyoid ligament is indicated by the yellow bar. The left clavicle, ribs  
1244 and shoulder girdle have been removed virtually to expose the *in situ* position of the

1245 hyoid apparatus and larynx. The white asterisk marks the cartilaginous connection  
1246 between the hyoid apparatus and the larynx. The black asterisk marks the cartilaginous  
1247 longitudinal ventral fusion of thyroid and cricoid cartilages that is typical for marsupials.  
1248 The resting position of the larynx is approximately at the level of the cervical vertebrae  
1249 4-6.

1250 **Fig. 9:** Superficial muscular triangle covering the ventrolateral neck region, consisting  
1251 of the two bellies of the digastric muscle rostrally, the sternohyoid muscle ventrally,  
1252 and the V-shaped omohyoid muscle laterally. A tendinous intersection links these three  
1253 muscles. Position of lower jaw indicated by white contour. In the koala the omohyoid  
1254 and sternohyoid muscles have completely lost their connection to the hyoid apparatus.  
1255 The three muscles are laterally flanking the pharynx, the ventral hyoid parts, the larynx,  
1256 trachea and oesophagus. Scale bar 10 mm

1257 **Fig. 10:** Intra-thoracic origin of the sternohyoid and sternothyroid (strap) muscles in a  
1258 female (A) and a male (B) adult koala. (A) shows the resting position of the larynx  
1259 whereas in (B) the neck has been extended and the larynx pulled caudally (red arrows)  
1260 to simulate its retraction down into the thoracic entrance. The origins of the two  
1261 muscles extend down to the level of the 3<sup>rd</sup> and 4<sup>th</sup> costal cartilage. The sternohyoid  
1262 muscle courses ventrally, contacting the inner thoracic wall, and is covered dorsally by  
1263 the sternothyroid muscle. *In situ* the intrathoracic portions of both muscles are covered  
1264 by a sheath of connective tissue. The sternothyroid muscle is the main retractor of the  
1265 larynx while the sternohyoid muscle, due to its tendinous connection to the digastric  
1266 and omohyoid muscles, can guide the extensive up-and-down movements of the  
1267 larynx, the ventral hyoid parts and the pharynx during bellow production. Scale bar 10  
1268 mm, respectively

1269 **Fig. 11:** The hypocephalic muscle in an adult female koala. Left lateral view. The  
1270 hypocephalic muscle (probably emerging from the fusion of the stylohyoid muscle  
1271 rostrally, the caudal stylopharyngeal muscle (middle), and the hyopharyngeal muscle  
1272 caudally) originates from the cerato-, basi- and thyrohyoid and rostradorsally from the  
1273 thyroid cartilage, and mainly terminates on the base of the skull, caudally adjacent to  
1274 the attachment of the hyoid ligament. As a result of this topographical relationships the  
1275 hypocephalic muscle can assist in protraction of the larynx subsequent to momentary  
1276 retraction during bellow production. Scale bar 10 mm

1277 **Fig. 12:** Right half of the larynx of an adult male koala (A) and an adult female koala  
1278 (B). Medial view. Dissection photo, showing the mucous membrane relief of the  
1279 laryngeal cavity, including the laryngeal vocal fold (LVF), and the relative positions of  
1280 the laryngeal cartilages. The thyroid and cricoid cartilages are ventrally fused by a  
1281 longitudinal cartilaginous keel (A); translucent specimen (B) prepared by using the  
1282 Spalteholz technique (Spalteholz 1914; Peters 1961; Piechocki 1967). + = rostral horn,  
1283 o = caudal horn of thyroid cartilage. Scale bars 10 mm, respectively

1284 **Fig. 13:** Undulating nerves supplying the musculature of the pharynx, which is  
1285 subjected to considerable length changes during extensive rostrocaudal excursions of  
1286 the ventral hyoid parts and the larynx. The undulating nerve branches are ensheathed  
1287 by flexible hose-like connective tissue tubes (three red arrows), which facilitate  
1288 stretching and rewinding. Asterisks mark two steel pins used for separating the nerves.  
1289 Left lateral view. Scale bar 10 mm

1290 **Fig. 14:** Reconstruction of the vocal anatomy of an adult male koala in six layers while  
1291 producing the very low frequencies during the nasal inhalatory sections of the final  
1292 stage of bellow vocalisations. The larynx is maximally retracted down into the thoracic  
1293 inlet; the hyoid ligament is maximally extended and the sternothyroid muscle maximally

1294 contracted. Left lateral view. Parotidoauricularis muscle terminates on sternal  
1295 manubrium (A); Y-shaped tendinous intersection connects digastric, omohyoid and  
1296 sternohyoid muscle, the latter with intrathoracic origin (B); the genioglossolaryngeal  
1297 muscle terminates on pharynx wall, thyrohyoid and thyrohyoid membrane (C); the  
1298 geniohyoid muscle is markedly elongated and kept in place by passing through an arch  
1299 formed by the hyoglossus muscle and the rostral branch of the Y-shaped tendinous  
1300 intersection; the stylohyoid muscle connects the thyrohyoid to the skull base; the  
1301 sternothyroid muscle has an intrathoracic origin (D); the hyopharyngeus muscle and  
1302 the caudal constrictors of the pharynx connect the thyrohyoid and the larynx to the  
1303 pharynx; first rib removed (E); nasal and oral vocal tract, naso- and oropharynx and  
1304 soft palate maximally extended; VVFs opposite to the laryngeal entrance; complete  
1305 (longitudinally compressed) trachea from the larynx to the bifurcation (F).

1306