VETERINARSKI ARHIV 85 (2), 211-226, 2015

New aspects on the laryngeal anatomy of the bottlenose dolphin (*Tursiops truncatus*)

Hrvoje Brzica[1]*, Katarina Špiranec[1], Iva Zečević[2], Hrvoje Lucić[1], Tomislav Gomerčić[3], and Martina Đuras[1]

[1]*Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[2]*Student at Faculty of Veterinary Medicine,University of Zagreb, Zagreb, Croatia*

[3]*Department of Biology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

BRZICA, H., K. ŠPIRANEC, I. ZEČEVIĆ, H. LUCIĆ, T. GOMERČIĆ, M. ĐURAS: New aspects on the laryngeal anatomy of the bottlenose dolphin (*Tursiops truncatus*). Vet. arhiv 85, 211-226, 2015.

ABSTRACT

The mammalian larynx is an organ which serves as a barrier to prevent food from entering the respiratory system, and in most mammals it is used to produce sound. Although the general morphology of the cetacean larynx has been known for a long time, its detailed anatomy has been poorly studied, which has resulted in many inconsistencies in the available literature, particularly regarding the nature of the dorsal paired cartilages of the arytenoepiglottic tube, and the mucosal folds found in laryngeal cavity. The aim of this paper is to give a comprehensive description of the structures associated with the larynx of the bottlenose dolphin (*Tursiops truncatus*, Montagu, 1821), using the well-known laryngeal morphology of humans and various domestic mammals as guidelines. The structure of the larynx was studied in 7 bottlenose dolphins of both sexes, using the classical dissection method on fresh specimens and some conserved with 4 % water diluted formaldehyde. Although careful dissection showed distinct morphological differences between the laryngeal cartilages of the bottlenose dolphin and terrestrial mammals, most of the muscles attached to them were consistent with that of terrestrial mammals. We present structures characteristic to the larynx of the bottlenose dolphin, such as two ligaments which are not present in domestic mammals and humans. Furthermore, based on the comparison with human and animal laryngeal anatomy, we conclude that the dorsal paired cartilages of the arytenoepiglottic tube in the bottlenose dolphin most likely correspond to the cuneiform process of terrestrial mammals, and that the median and lateral folds found in the laryngeal cavity should not be identified as vocal and ventricular folds.

Key words: bottlenose dolphin, *Tursiops truncatus*, larynx, vocal folds, laryngeal ligaments, laryngeal cavity

*Corresponding author:

Hrvoje Brzica DVM., Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia, Phone: + 385 1 2390 244; E-mail: hbrzica@vef.hr

ISSN 0372-5480
Printed in Croatia

Introduction

The larynx is an organ of the respiratory system articulated to the hyoid bone, directly responsible for sound production, localized ventrally and aborally to the oral cavity and orally to the trachea (EVANS, 1993; DYCE et al., 1996). It is framed by a cartilaginous skeleton, whose individual parts are held together by joints, muscles, ligaments and membranes. Although subtle differences in the structure of various laryngeal parts do exist, the basic anatomical shape remains the same between domestic species (NICKEL et al., 1979; DYCE et al., 1996). The larynx serves as barrier which protects the lower respiratory system from foreign body invasion. In this context, the larynx of whales (Cetacea) is adapted for feeding and breathing in an aquatic environment, but despite these adaptations there are documented cases of laryngeal obstruction by fish or seaweed (BIRKUN, 1996). In addition to these obstructions, dolphins are vulnerable to gill-net strangulation due to the shape and position of the larynx. Gill-net strangulation has been reported as cause of death in 10 % of bottlenose dolphins stranded in the Croatian part of the Adriatic Sea (ĐURAS GOMERČIĆ et al., 2009). As the larynx also serves as an organ for vocalization in terrestrial mammals, during sound production the vocal, vestibular, cricoarytenoid and transversal arytenoid muscles, among others, contract and relax, regulating the width of the glottic cleft. Air, while transversing the glottic cleft, switches from linear to turbulent flow, resulting in vibrations of the vocal folds which manifests as sounds (DYCE et al., 1996).

The larynx of the bottlenose dolphin (*Tursiops truncatus*) has the same basic anatomical characteristics as the larynx of domestic mammals. However, orally and dorsally it forms a specific arytenoepiglottic tube, whose tip lies ventral to the choanae. The laryngeal tip by itself serves as a closing point, around which the palatopharyngeal muscle constricts and closes off the nasal passages (REIDENBERG and LAITMAN, 1987; HOUSER et al., 2004). In odontocetes, paired cartilages dorsally, and the epiglottic cartilage ventrally makes the framework of the arytenoepiglottic tube, whose oral rim represents the oral opening to the laryngeal cavity (GREEN, 1972; BLEVINS and PARKINS, 1973). However, in recent papers the paired dorsal cartilage of the arytenoepiglottic tube has been described as a corniculate cartilage (not listed in NAV 2012) (REIDENBERG and LAITMAN, 1987; HUGGENBERGER et al., 2008), while LISTE et al. (2006) mention only a cuneiform process. In addition to corniculate cartilages, REIDENBERG and LAITMAN (1987) describe paired cuneiform cartilages (not listed in NAV 2012) located caudal to the epiglottis and ventral to the articulations of corniculate and arytenoid cartilages.

In spite of the enormous leap in technology available for study of anatomical structures in the body (CT, SPECT/PET, MRI scans, bronchoscopy, etc.) the detailed anatomy of the odontocete larynx and its surrounding structures is still unknown, or at best ambiguous, due to many literature inconsistencies. This is clearly evident in the case of the dorsal cartilages of the arytenoepiglottic tube, or in the nature of the “vocal folds” or “laryngeal furrows”. The aim of this paper is to provide, a previously nonexistent, detailed anatomical description of the bottlenose dolphin’s laryngeal structures, obtained by classical dissection methods.

Materials and methods

Due to the sporadic nature of obtaining specimens, the study was performed over the course of several years, ranging from 2001 to 2012. Bottlenose dolphins used for this research were obtained and processed as reported previously (LUCIĆ et al., 2010; VUKOVIĆ et al., 2011). Five larynxes were studied in situ, after which they were incised with the surrounding tissue and either fixed with 4 % formaldehyde or frozen to be studied later in greater detail. All incisions and following preparations were made using standard anatomical tweezers and a No 15 surgical blade. Scissors were used to open up windows through the trachea and laryngeal cartilages.

In order to obtain laryngeal cartilages free of surrounding tissue, the larynxes removed from two dolphins (one adult, and one juvenile) were left in water for approximately one month to macerate. The water was changed daily to prevent the development of an unpleasant odor.

Photographs were taken using a Canon EOS40D digital camera, and were edited using Adobe Photoshop CS4 for optimal presentation of various structures. Exceptions are Figs 1 and 4, which were taken with an analog camera, and digitalized using Adobe Photoshop CS4.

This study was approved by annual permits of the Croatian Ministry of Environmental and Nature Protection.

When possible, we followed the nomenclature found in Nomina Anatomica Veterinaria (ANON., 2012).

Results

Laryngeal region. The laryngeal region and larynx of the bottlenose dolphin are situated more orally when compared to terrestrial mammals. The laryngeal tip/spout is held in place by the palatopharyngeal muscle, just below the choanae, while its caudal parts are located ventrally to the skull base. After removing skin and blubber and revealing the surrounding musculature, by carefully palpating the caudodorsal apex of the thyrohyoid we determined that it is not articulated with the oral part of the plate (lamina) of the thyroid cartilage, and it is free in the surrounding tissue, covered by sternohyoid muscle.

Laryngeal cartilages. The arytenoid cartilage (Fig. 1A, b) is a paired cartilage, laying ventrally to the lamina of the cricoid cartilage, to which it is articulated through the cricoarytenoid joint. The articulation facet (arrow) is found on the medial face of the arytenoid cartilage on its dorsal margin. The oral and ventral margins are fused with the cuneiform cartilages (a) with fibrous tissue. The wider part of the cuneiform cartilages is positioned orally to the arytenoid cartilage and dorsally to the epiglottic cartilage, making up the cartilaginous basis for the dorsal part of the arytenoepiglottic tube. Its aboral, narrower part lies laterally and ventrally to the arytenoid cartilage.

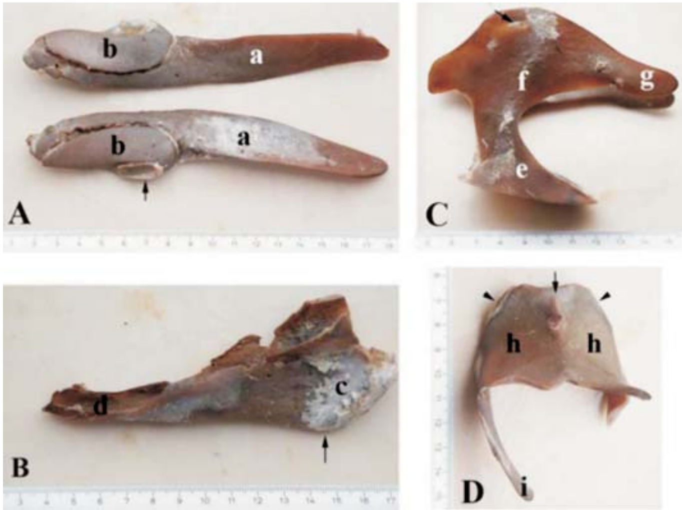

Fig. 1. Laryngeal cartilages of the bottlenose dolphin A. Medial face of paired cuneiform cartilages of the arytenoepiglottic tube (a) with arytenoid cartilages (b) fused together with fibrous connection. Arrow - articulation facet for the cricoarytenoid articulation. B. Left side of the epiglottic cartilage. Strong base of the epiglottic cartilage (c) bears an oral ridge (arrow) facing the hyoid bone. Tip (d) of the epiglottic cartilage represents ventral part of the arytenoepiglottic tube. C. Thyroid cartilage. Ventral body (e) of the thyroid cartilage bears the thin base of the stronger plates (f). Aborally the plate bears the facet for the fibrous connection with the crycoid cartilage (g). The foramen on the dorsal margin of the plate of the thyroid cartilage (arrow). D. Cricoid cartilage. The single plate of the cricoid cartilage (h) bears the strong median ridge (arrow) and articulation facets for the cricoarytenoid articulation (arrowheads). Ventrally from both sides of the plate, arches extend (right arch is broken) (i).

The epiglottic cartilage (Fig. 1B) is triangular in shape, laterally flattened and resembling a keel, with an aboral base (c) which lies orally to the thyroid cartilage. The oral part of the base represents the most ventral part of the lingual surface of the whole cartilage shaped as a small ridge (arrow). The laryngeal surface of the epiglottic cartilage is concave throughout its length, with thinly extended lateral borders, reaching its widest point at its lateral margins (arrowhead) on its aboral part. The epiglottic cartilage forms the cartilaginous skeleton for the ventral part of the arytenoepiglottic tube.

The thyroid cartilage (Fig. 1C) is elegant and slim, and it represents the cartilaginous base for the lateral and ventral part of the laryngeal wall. The ventral part of the cartilage is extended orally and aborally (e). Dorsally, a lateral plate arises on both sides (f). The plates are narrow and slim at their bases, while their dorsal, wafer-like parts are extended orally and aborally into short and long horns, respectively. The aboral horn bears the articular facet for the cricoid cartilage (g). The dorsal margin bears a small foramen at its center (arrow).

The cricoid cartilage (Fig. 1D) represents the aboral, lateral and dorsal basis for the laryngeal wall. The main body of the cartilage forms a dorsal plate (h) which has a median notch on its oral and aboral margins. Laterally to the oral and aboral notch plate extends in two wide and short processes which carry articulation facets for the cricoarytenoid and cricothyroid joint respectively (arrowheads). A small median ridge is present on the oral part of the plate (arrow). Lateral arches extend ventrally from the plate, but they do not fuse ventrally, thus failing to close a full ring (i).

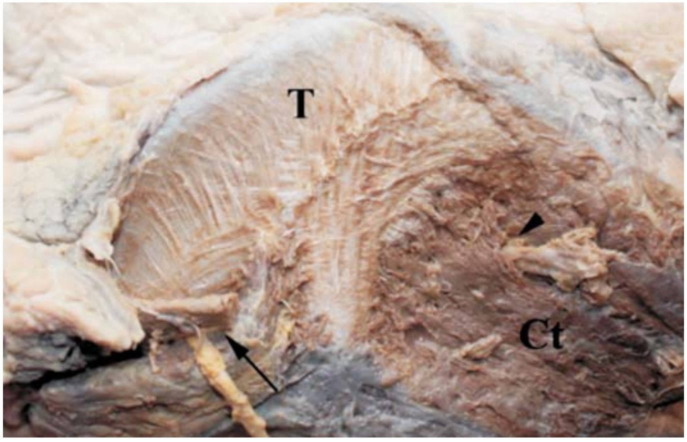

Fig. 2. A close up of the left lateral laryngeal wall. On the thyroid cartilage (T) tendinous enhancements are present as strong fibers running dorsoventrally. Cranial (arrow) and caudal (arrowhead) laryngeal nerves enter the laryngeal wall beneath the cranial parts of the thyroid cartilage and going through the cricothyroid muscle, respectfully.

Laryngeal membranes and ligaments. As the laryngeal skeleton is formed by relatively thin and sensitive cartilages (excluding the epiglottic and arytenoid), strong bundles of tendinous fibers support the whole laryngeal structure (Fig. 2). One bundle starts orally and laterally on the plate of the cricoid cartilage and widens towards the aboral margin of the plate of the thyroid cartilage. The second bundle is located only on the aboral part of the thyroid cartilage, between the body and the plate of the respective side. The third bundle is located on the oral margins of the cricoid cartilage (not shown).

A strong ligament connects the ventral margin of the arytenoids and cuneiform cartilages with the lateral margins of the base of the epiglottic cartilage (Fig. 3, arrows).

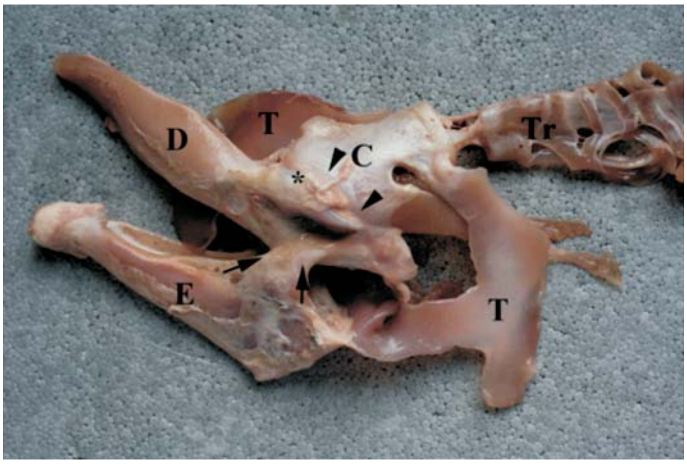

Fig. 3. Laryngeal cartilages and ligaments, left lateral aspect. The left plate of the thyroid cartilage has been folded caudally. An exceptionally strong ligament connects the lateral margins of the epiglottic cartilage (E) and caudoventral parts of the arytenoid (A) and dorsal (D) cartilages (arrows). Cricoarytenoid ligament (arrowheads), cricoarytenoid joint (asterisk), thyroid cartilage (T), cricoid cartilage (Cr), trachea (Ta).

The cricoarytenoid ligament (Fig. 3, arrowheads) connects the oral margins of the cricoid cartilage to the arytenoids cartilages, aborally to the cricoarytenoid joint.

There is a small, but strong ligament connecting the medial ridge of the cricoid cartilage plate and the dorsal aboral margin of the cuneiform cartilages (Fig. 5, arrowheads)

The cricothyroid ligament is exposed after removal of the cricothyroid muscle and it fully closes the space between cricoid and thyroid cartilage, making contact with the cricotracheal ligament through the spaces between the arches of the cricoid cartilage (not shown).

Muscles of the larynx and the laryngeal region. The sternothyroid muscle begins on the craniodorsal parts of the sternum and it laterally covers the cricothyroid muscle (Fig. 4, St). Muscle fibers run cranially and dorsally, ending on the thick part of the plate of the thyroid cartilage, ventrally to the cricopharyngeal and thyropharyngeal muscles. To some extent the fibers of the sternothyroid muscle are interwoven with the thyropharyngeal muscle.

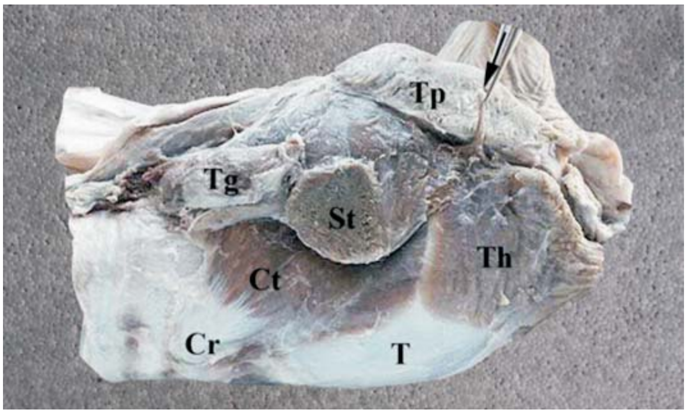

Fig. 4. Lateral view of the right laryngeal wall: thyroid gland (Tg), cricothyroid muscle (Ct), laryngeal insertion of the sternothyroid muscle (St), thyrohyoid muscle (Th), thyropharyngeal muscle (Tp), a ventral part of the arch of the cricoid cartilage (Cr) and body of the thyroid cartilage (T). The arrow is pointing to the cranial laryngeal nerve.

The thyrohyoid muscle begins orally to the cricothyroid muscle on the cranial margin of the ventral part of the plate and body of the thyroid cartilage (Fig. 4, Th). Its fibers run orally towards the thyrohyoid of the hyoid bone, where they separate into two distinct ends. The dorsal muscle head ends on the free apex of the thyrohyoid, while the ventral muscle head ends on the ventral part of the thyrohyoid.

The cricopharyngeal muscle begins at the aboral corner of the cricoid cartilage on the angle between the plate and the arch, with a substantial amount of interweaving muscle fibers with the thyrohyoid muscle. It runs between the cricoid cartilage and the lateral pharyngeal wall.

The thyropharyngeal muscle is located orally to the cricopharyngeal muscle (Fig. 4, Tp). It starts along the dorsal margin of the thyroid cartilage, continuing all the way to the oral end of the plate. There is a particularly strong head, which starts ventrally on the arches of the cricoid cartilage next to the cricothyroid muscle. The fibers of the cricopharyngeal and thyropharyngeal muscles interweave just a few millimeters dorsally to their insertion points on the laryngeal cartilages. Dorsal to the plates of the thyroid cartilage, the muscle fibers which originated on the medial sides of the plates merge with the main body of the thyropharyngeal muscle, with fibers originating aborally on the cricothyroid ligament, and orally as far as the lateral corners of the base of the epiglottic cartilage. It is partially merged with the medial fibers of the cricothyroid muscle. The fibers continue orally and dorsally from these insertion points and with the cricopharyngeal muscle run towards and participate in forming the pharyngeal wall.

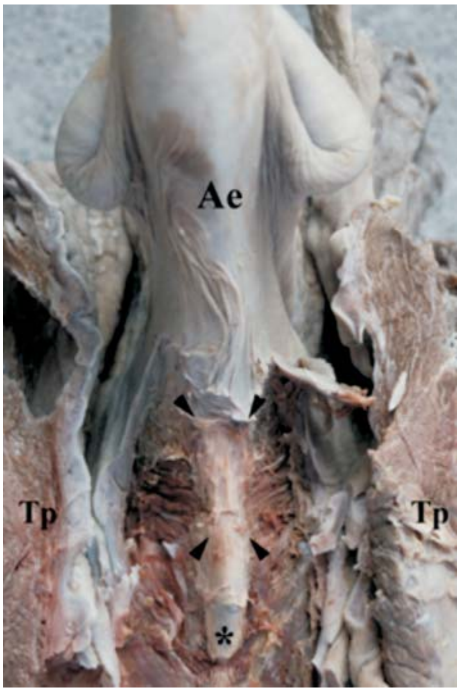

Fig. 5. Dorsal laryngeal wall, after the removal of the pharyngeal mucosa. The ligament connecting median ridge of the cricoid cartilage (asterisk) to the cuneiform cartilages is marked by arrowheads. Note the transversal arytenoid muscle to the left and right of the ligament. Arytenoepiglottic tube (Ae), thyropharyngeal muscle (Tp).

The cricothyroid muscle begins on the arches of the cricoid cartilage, continues dorsally and orally, terminating at the aboral margin of the plates of the thyroid cartilage. Some fibers continue medially from the main muscle mass and terminate on the medial surface of the plate of the thyroid cartilage (Fig. 4, Ct). Numerous blood vessels continue their way through this muscle, while passing through the cricothyroid ligament as well, extending further into the larynx.

The dorsal cricoarytenoid muscle has its insertion point on the aboral border and the medial line of the cricoid cartilage plate, merging with the same muscle on the other side. The fibers continue orally and laterally, terminating on the lateral side of the arytenoid cartilage above the cricoarytenoid articulation and ventral to the transversal arytenoid muscle.

The lateral cricoarytenoid muscle begins on the dorsal aboral border of the arches of the cricoid cartilage. Its fibers run orally towards the lateral sides of the arytenoid cartilages, terminating orally to the cricoarytenoid articulation.

The transversal arytenoid muscle (Fig. 5) is located orally to the cricoid cartilage, and its fibers run transversally on the dorsal margin of the arytenoid cartilages, and to some extent, on the cuneiform cartilages. Some fibers attach to the ligament found dorsally to the muscle (vide supra). It covers the space closed by the arytenoid cartilages when moved caudally towards the cricoarytenoid articulation.

The hyoepiglottic muscle (Fig. 6, He) begins orally in the middle of the ridge on the lingual surface of the epiglottic cartilage. Its fibers run orally and ventrally to the hyoid bone.

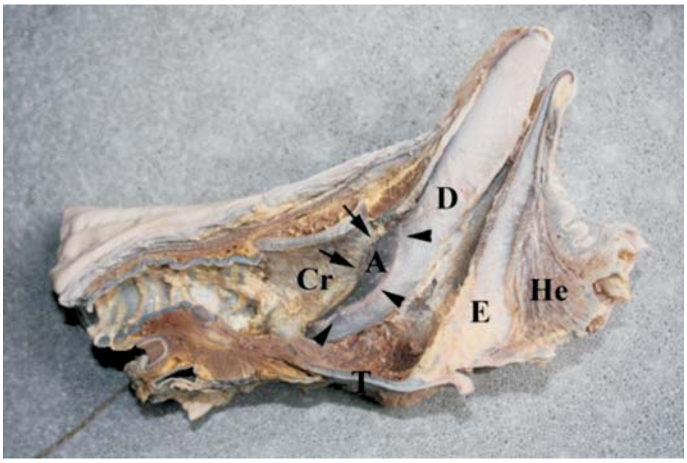

Fig. 6. Median view of the larynx, after the removal of the mucosa. The cricoid (Cr) cartilage is evident as a lateral ring on the caudal part of the whole structure. It is articulated with the dorsal surface of the arytenoid cartilage (A) with a synovial joint (T) and epiglottic cartilage (E) which can be seen respectively ventrally and rostrally on the larynx. Note the singular muscle mass of the thyroarytenoid muscle between the thyroid and dorsal/arytenoids cartilages. Hyoepiglottic muscle (He).

The thyroarytenoid muscle (Fig. 6) is located on the lateral side of the laryngeal cavity, and medial to the thyroid cartilage plate, as a single, wide muscular mass. The muscle fibers run dorsoventrally, connecting the whole length of the thyroid cartilage body to the lateral and ventral parts of the arytenoid cartilages. Medially, ventrally and aborally to this muscle, a venous plexus can be found.

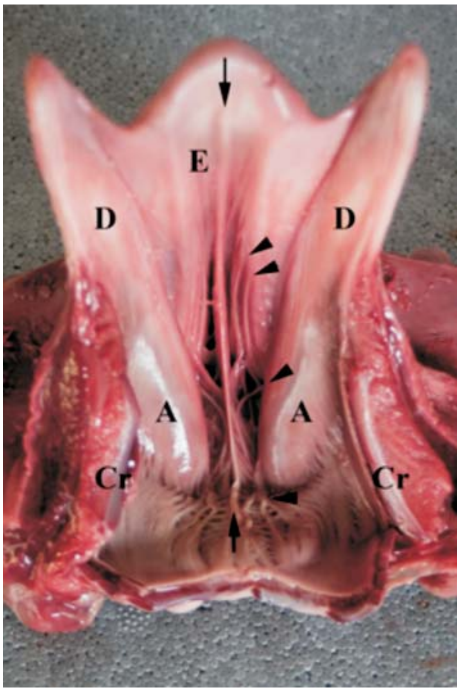

Fig. 7. Exposed laryngeal cavity, using a dorsomedian incision. A single median (arrows) and numerous lateral (arrowheads) folds are visible on the floor of the bottlenose dolphin's laryngeal cavity. The median fold spans from the apex of the epiglottic cartilage almost to the tracheal rings. By means of numerous lateral folds it is indirectly attached to the lateral margins of the epiglottic cartilage, and the paired dorsal (cuneiform) cartilage. Note the relatively smaller folds aborally to the arytenoid cartilages and glottic cleft. Epiglottic cartilage (E), cuneiform cartilage (D), arytenoid cartilage (A), cricoid cartilage (Cr).

Laryngeal cavity. Aborally to the oral entrance of the laryngeal cavity (Fig. 6 and 7), the relatively long but extremely tight space of the arytenoepiglottic tube represents the laryngeal vestibule. The space of the vestibule tightens even more in the region of the arytenoid cartilage (Fig. 6 and 7, A) where the laryngeal glottic cleft is formed. Mucosa of the laryngeal floor in the region of the glottic cleft forms longitudinal folds. A single median fold (arrow) is clearly identifiable with several lateral ones of varying height and length (arrowheads). Between these folds, approximately 130 - 160 small sacs or cavities are present (Fig. 7). These small cavities are most numerous orally to the cleft, giving the mucosa on that side a particularly 'spongy' look. All these cavities actually represent a multitude of small diverticules of the laryngeal cavity.

Aborally to the laryngeal cleft, the space of the laryngeal cavity abruptly opens up and reaches the diameter of the trachea. Ventral laryngeal folds fan out, giving the impression as if the arytenoid cartilages have pinched the mucosa, thus creating the folds.

Discussion

As was previously reported (REIDENBERG and LAITMAN, 1987) the larynx of the bottlenose dolphin is located more rostrally and dorsally than in domestic mammals, with the laryngeal tip and aditus laryngis lying just ventrally to the choanae, surrounded by the palatopharyngeal muscle. The bottlenose dolphin's larynx is held in its proper place partly by the extrinsic laryngeal muscles (strenothyroid, thyrohyoid, cricopharyngeal and thyropharyngeal), which (taking into account the specific shape and size of the cartilages) correspond to those found in domestic mammals (NICKEL et al., 1979; HERMANSON and EVANS, 1993) and humans (JOHNSTON and WHILLIS, 1947). The only exception can be found in the thyropharyngeal muscle: while in domestic mammals it originates only on the lateral side (NICKEL et al., 1979), in the bottlenose dolphin it has insertions on the lateral and medial sides of the thyroid lamina. Two additional origination points of this muscle - on the epiglottic cartilage and on the cricothyroid ligament, add to the differences between dolphins and domestic mammals.

Four laryngeal cartilages of cetaceans (epiglottic, thyroid, cricoid, and arytenoids) have been previously described (REIDENBERG and LAITMAN, 1987; REIDENBERG and LAITMAN, 1988; SMITH et al., 1999; HUGGENBERGER et al., 2008). These four cartilages show modifications when compared to domestic mammals: the ventral space between the arches of the cricoid cartilage, and fibrous enhancements of the thyroid cartilage, (Fig. 1C, Fig. 2), the latero-lateral flattening of the epiglottic cartilage (Fig. 1B) (JOHNSTON and WHILLIS, 1947; NICKEL et al., 1979; REIDENBERG and LAITMAN, 1987). More dramatic changes are visible in the morphology of the arytenoids cartilages. Our research has shown that the arytenoid cartilages in bottlenose dolphin lack muscular and vocal processes (Fig. 1A) which serve as attachment points for the ventricular and vocal muscles and vestibular and vocal ligaments, respectively, in dogs and horses (NICKEL et al., 1979), for vestibular and vocal ligaments in pigs (NICKEL et al., 1979), and for fibers that correspond to the vestibular ligament and vocal ligament in oxen (NICKEL et al., 1979), which are all integral parts of the vocal folds.

The homology of the paired cartilages orally to the arytenoids is still unclear. GREEN (1972) and BLEVINS and PARKINS (1973), and GREEN et al. (1980) described them as cuneiform because of their relative position to the arytenoid and epiglottic cartilages which corresponds to the position of the cuneiform process in the dog (EVANS, 1993) and horse (NICKEL et al., 1979). More recent papers concerning the cetacean larynx report that these cartilages articulate with the arytenoids on the superior-rostral surface, characterizing them as corniculates (SCHNEIDER, 1964; REIDENBERG and LAITMAN, 1987; HUGGENBERGER et al., 2008). Here, we can show that these cartilages articulate with the inferior surface of the arytenoids, leaving the dorsal surface free for attachment to the cricoid cartilage (Fig. 6, arrows). Furthermore the corniculate process provides a foundation for the arytenoepiglottic fold (connecting arytenoid with epiglottis) in most domestic mammals (NICKEL et al., 1979), while in humans (JOHNSTON and WHILLIS, 1947) and dogs (EVANS, 1993), the cuneiform process is also an integral part of the arytenoepiglottic fold and is closer to the epiglottic cartilage than the corniculate process. In addition, BLEVINS and PARKINS (1973) describe a small corniculate cartilage positioned aborally, while REIDENBERG and LAITMAN (1987), describe small paired cuneiform cartilages aborally to the epiglottic and inferior to the arytenoid cartilages. We were not able to find these cartilages in our specimens. Considering all this, there is more supporting evidence to define the dubious, dorsal cartilages as cuneiforms, or at the very least support them as cuneiform processes, as was suggested by LISTE et al. (2006). In order to precisely determine the nature of the cartilage, research should be made on much younger or even neonate animals.

Reflecting the peculiarities of the cartilage form, the laryngeal ligaments show some differences as well. The ligament that connects the ventral margin of the arytenoid and cuneiform cartilages with the lateral margins of the base of the epiglottic cartilage is not found in domestic mammals. Furthermore, the ligament connecting the cricoid and cuneiform cartilages of the arytenoepiglottic tube cannot be found in humans or domestic mammals (JOHNSTON and WHILLIS, 1947; NICKEL et al., 1979)

Some discrepancies between the available literature data and our specimens regarding the intrinsic laryngeal muscles were found. The cricohyoid muscle of the dolphin was described by GREEN (1972), as beginning on the thyroid cartilage and ending on the lateral side of the arches of the cricoid cartilage. This muscle has never been reported either in humans (JOHNSTON and WHILLIS, 1947) or in domestic mammals (NICKEL et al., 1979), and we believe it was most likely confused with the corresponding cricothyroid muscle. A previous report (BLEVINS and PARKINS, 1973) describes a single cricoarytenoid muscle, while we found two distinct muscles:; the dorsal and lateral cricoarytenoid muscles, which correspond to the morphology of domestic mammals (NICKEL et al., 1979). While the thyreoarytenoid muscle in dogs and horses has two distinct parts - vocal and ventricular muscles, each enclosed in separate membranaceous folds, other species of domestic mammals have a single thyreoarytenoid muscle (NICKEL et al, 1979) while, in humans only a vocal muscle is present as an integral part of the thyreoarytenoid muscle (JOHNSTON and WHILLIS, 1947). Our results indicate that in bottlenose dolphins a single thyreoarytenoid muscle is present, without its distinct parts, thus corresponding to animals such as swine and cows. Although REIDENBERG and LAITMAN, (1988), report on collagen and elastic fibers which may be homologous to vocal and vestibular ligaments, we did not find the corresponding vocal and vestibular ligament, enclosed within the respective muscles in the vocal and vestibular fold. In humans (JOHNSTON and WHILLIS, 1947) and all domestic mammals, even small ones such as dogs (NICKEL et al., 1979), these ligaments are relatively large and unmistakable to the naked eye, which is not the case in odontocetes.

Due to the specific morphology of the larynx in the bottlenose dolphin, the laryngeal cavity represents a direct link between the choanae and the trachea. The laryngeal cavity with its vestibular and vocal folds is the key of sound production in terrestrial mammals (DYCE et al., 1996), while in odotontocetes a larger part of sound production takes place in the nose, i.e. as phonic lips and melon (FRANKEL, 2002). The available literature is still not unanimous regarding the existence of vestibular and vocal folds in cetaceans in general. While some deny their existence (REIDENBERG and LAITMAN, 1987; FRANKEL, 2002), REIDENBERG and LAITMAN (1988), and GUREVICH (2006) describe the medial fold on the laryngeal floor as a vocal fold, and the numerous lateral folds as vestibular folds. In addition, they also describe numerous sacs found between the laryngeal folds as a lateral laryngeal sac. We have clearly shown that the midline fold starts near the tip of the epiglottic cartilage and continues aborally to the region of the thyroid cartilage or even further, so it cannot be described as a vocal fold which spans between the arytenoids and thyroid cartilage. Furthermore, in domestic mammals and humans the vocal folds are paired (JOHNSTON and WHILLIS, 1947; NICKEL et al., 1979), while in dolphin the fold in question is single. The situation is similar regarding the lateral folds. Although they pass by, and extend over the arytenoid cartilages, they begin in the epiglottic and thyreoid region (Fig. 7, arrowheads). Regarding differences in morphology, we can also conclude that the position of the folds in the bottlenose dolphin makes them unable to regulate the span of the glottis cleft, as is the case with the vestibular and vocal folds in terrestrial mammals. As was shown by TSANG et al. (2002) using a bronchoscopy technique, the bronchoscope could only be passed through the arytenoepiglottic tube during respiration, while for the rest of the time it was firmly shut. In addition, our own studies revealed a glottis cleft framed by arytenoids and cartilages that we believe are cuneiforms. Furthermore, ligaments that provide the tension of the folds are absent in bottlenose dolphin, and possible turbulent air flow cannot cause the vibration of the cartilagenious tissue, tightly covered with mucosa. Taking all this into account, it is our opinion that ventral folds found in the larynx of the bottlenose dolphin cannot be identified with the vocal and vestibular folds present in domestic mammals, and that a more appropriate name could be laryngeal furrows, as proposed by HUGGENBERGER et al. (2008).

In summary, we conclude that: a) bottlenose dolphin lacks m. vocalis and m. ventricularis, as distinct parts of the m. thyroarytenoideus, b) organized ligaments that can be associated with lig. vocalis and lig. ventricularis do not exist in the bottlenose dolphin, c) the ventral median and lateral folds of the laryngeal mucosa should not be identified as vocal or vestibular folds, d) the glottic cleft is framed with cartilages, rather than with vocal and vestibular folds as in terrestrial mammals, e) the paired dorsal cartilages of the arytenoepiglottic tube most likely correspond to the cuneiform process of terrestrial mammals, and f) there are two distinct ligaments not present in humans and domestic mammals: one connecting the median ridge of the cricoid cartilage to the dorsal (cuneiform) cartilages, and the other connecting the lateral margins of the epiglottic cartilage and arytenoid/cuneiform cartilages.

Acknowledgements

This study was funded by the Ministry of Science, Education and Sport of the Republic of Croatia (Project No. 053-0533406-3640) and Gesellschaft zur Rettung der Delphine, Munich, Germany.

References

ANONYMOUS (2012): Nomina Anatomica Veterinaria. 6th ed., The World Association of Veterinary Anatomists, Zürich, Ithaca.

BIRKUN, A. (1996): Complexities of by-catch diagnosis in Black Sea cetaceans. In: Diagnosis of by-catch cetaceans: Proceedings of the second European Cetacean Society workshop on cetacean pathology (Kuiken, T., Ed.). European Cetacean Society Newsletter no. 26, pp. 12-15.

BLEVINS, C. E., B. J. PARKINS (1973): Functional anatomy of the porpoise larynx. Am. J. Anat. 138, 151-164.

DYCE, K. M., W. O. SACK, C. J. G. WENSING (1996): Veterinary Anatomy. W.B. Saunders Company. Philadelphia. 856 p.

ĐURAS - GOMERČIĆ, M., A. GALOV, T. GOMERČIĆ, D. ŠKRTIĆ, S. ĆURKOVIĆ, H. LUCIĆ, S. VUKOVIĆ, H. ARBANASIĆ, H. GOMERČIĆ (2009): Bottlenose dolphin (*Tursiops truncatus*) depredation resulting in larynx strangulation with gill-net parts. Mar. Mammal. Sci. 25, 392-401.

EVANS, H. E. (1993): The respiratory system. In: Miller's Anatomy of the Dog. (Evans, H. E., Ed.), W. B. Saunders Company, Philadelphia. pp. 472-479.

FRANKEL, A. S. (2002): Sound production. In: Perrin WF, Würsig B, Thewisen JGM, editors. Encyclopedia of Marine Mammals. (Perrin, W. F., B. Würsig, J. G. M. Thewisen, Eds.), London: Academic Press. Pp. 1126-1138.

GREEN, R. F. (1972): Observations on the anatomy of some cetaceans and pinnipeds. In: Mammals of the Sea. Biology and medicine. (Ridgway, S. H., Ed.), C.C. Thomas Publisher, Springfield, USA, pp. 247-297.

GREEN, R. F., S. H. RIDGEWAY, W. E. EVANS (1980): Functional and descriptive anatomy of the bottlenose dolphin nasolaryngeal system with special reference to the musculature associated with sound production. In: Animal Sonar Systems (Busnel, R. G., J. F. Fish, Eds.), Plenum Publishing Corporation, pp. 199-238.

GUREVICH, M. D. (2006): Computational acoustic modeling of cetacean vocalizations PhD Thesis, Stanford (CA), Stanford University. Available from: Proquest, document ID: 1232405461, ISBN: 9780542894275.

HERMANSON, T. F., H. E. EVANS (1993): The muscular system. In: Miller's Anatomy of the Dog. (Evans, H. E., Ed.), W. B. Saunders Company, Philadelphia. pp. 259-384.

HOUSER, D. S., J. FINNERAN, J. CARDER, W. VAN BONN, C. SMITH, C. HOH, R. MATTREY, S. RIDGEWAY (2004): Structural and functional imagining of bottlenose dolphin (*Tursiops truncatus*) cranial anatomy. J. Exp. Biol. 207, 3657-3665.

HUGGENBERGER, S., M. A. RAUSCHMANN, H. H. A. OELSCHLÄGER (2008): Functional morphology of the hyolaryngeal complex of the Harbor Porpoise (*Phocoena phocoena*): implications for its role in sound production and respiration. Anat. Rec. 291, 1262-1270.

JOHNSTON, T. B., J. WHILLIS (1947): Gray's Anatomy: descriptive and applied. Longmans, Green and Co. London, New York, Toronto. 1597 p.

LISTE, F., J. PALACIO, V. RIBES, A. ÁLVAREZ-CLAU, L. FERNÁNDEZ DOMÍNGEZ, J. M. CORPA (2006): Anatomic and computed tomographic atlas of the head of the newborn bottlenose dolphin (*Tursiops truncatus*). Vet. Radiol. Ultrasoun. 47, 453-460.

LUCIĆ, H., S. VUKOVIĆ, V. POSAVAC, M. ĐURAS GOMERČIĆ, A. GALOV, D. ŠKRTIĆ, S. ĆURKOVIĆ, H. GOMERČIĆ (2010): Application of dual energy X-ray absorptiometry method for small aniimals in measuring bone mineral density of the humerus of bottlenose dolphins (*Tursiops truncatus*) from the Adriatic Sea. Vet. arhiv 80, 299-310.

NICKEL, R., A. SCHUMMER, E. SEIFERLE (1979): The Viscera of the Domesic Mammals, Berlin, Hamburg: Paul Parey. p. 401.

SMITH, T. L., B. S. TURNBULL, D. F. COWAN (1999): Morphology of the complex laryngeal gland in the Atlantic bottlenose dolphin, *Tursiops truncatus*. Anat. Rec. 254, 98-106.

REIDENBERG, J. S., J. T. LAITMAN (1987): Position of the larynx in odontoceti (toothed whales). Anat. Rec. 218, 98-106.

REIDENBERG, J. S., J. T. LAITMAN (1988) Existence of vocal folds in the larynx of odontoceti (toothed whales). Anat. Rec. 221, 884-891.

SCHNEIDER, R. (1964): Der Larynx der Säugetiere. In: Handbuch der Zoologie, 8 Band, 35 Lieferung (Helmcke, J. G., H. V. Lengerken, D. Starck, H. Wermuth, Eds.), de Gruyter, Berlin pp. 1-128.

TSANG, K. W., R. KINOSHITA, N. ROUKE, Q. YUEN, W. HU, W. K. LAM (2002): Bronchoscopy of cetaceans. J. Wildl. Dis. 38, 224-227.

VUKOVIĆ, S., H. LUCIĆ, M. ĐURAS GOMERČIĆ, A. GALOV, T. GOMERČIĆ, S. ĆURKOVIĆ, D. ČKRTIĆ, G. DOMITRAN, H. GOMERČIĆ (2011): Anatomical and histological characteristics of the pituitary gland in the bottlenose dolphin (*Tursiops truncatus*) from the Adriatic Sea. Vet. arhiv 81, 143-151.

Received: 30 January 2014
Accepted: 25 November 2014

BRZICA, H., K. ŠPIRANEC, I. ZEČEVIĆ, H. LUCIĆ, T. GOMERČIĆ, M. ĐURAS: Novi pogledi na anatomiju grkljana dobrog dupina (*Tursiops truncatus*). Vet. arhiv 85, 211-226, 2015.

SAŽETAK

Grkljan sisavaca je organ koji predstavlja zaštitu od ulaska hrane u dišni sustav, a većini sisavaca služi i za proizvodnju zvuka. Iako je općenita anatomija grkljana kitova odavno poznata, detalji su slabo proučavani što je za posljedicu imalo razne nedosljednosti u dostupnoj literaturi, pogotovo na temu prirode dorzalnih parnih hrskavica aritenoepiglotične cijevi i nabora sluznice u grkljanskoj šupljini. Cilj ovog rada je iznijeti potanki opis struktura grkljana dobrog dupina (*Tursiops truncatus*, Montagu, 1821), pri čemu se kao smjernice rabe dobro upoznata anatomija grkljana domaćih sisavaca i čovjeka. Građa grkljana se proučavala na 7 dobrih dupina oba spola služeći se klasičnim metodama sekcije na svježim uzorcima i arhivskim uzorcima uščuvanim u 4 % -tnom formaldehidu razrijeđenim u vodi. Iako je pažljiva sekcija pokazala očigledne morfološke razlike u hrskavicama između dobrog dupina i kopnenih sisavaca, većina mišića koji se prihvaćaju za njih su odgovarajući kopnenim sisavcima. Iznosimo strukture koje su karakteristične za grkljan dobrog dupina kao što su: dva ligamenta koji nisu prisutni u čovjeka i domaćih sisavaca. Također, na temelju usporedbe s ljudskim i životinjskim grkljanima, zaključujemo da dorzalne, parne hrskavice aritenoepiglotične cijevi najvjerojatnije odgovaraju kuneiformnom izdanku domaćih sisavaca, a da se medijani i lateralni nabori sluznice grkljanske šupljine ne bi trebali poistovjećivati s glasnicama.

Ključne riječi: dobri dupin, *Tursiops truncatus*, pas, grkljan, vokalni nabor, grkljanski ligamenti, grkljanska šupljina