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# Differences in the anatomy of the lower respiratory tract in selected species of the order Testudines

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**Abstract:** The lower airways of turtles consist of the larynx, trachea and bronchi. Due to incomplete information about the anatomical structure of the lower respiratory tract in turtles, we decided to explore this topic more deeply. The material for this study included 95 turtle cadavers. Terrestrial tortoises were represented by 63 individuals from 8 species, aquatic and semi-aquatic turtles by 32 individuals from 11 species. The sex ratio was 54 females to 41 males. In addition to the anatomical autopsies and assessments of the macroscopic structures, numerous measurements were performed. The length, width and height of the larynx and trachea, and the length of the bronchi were measured. We found clinical significance in two parameters. The first one is the formation of the *rima glottidis*, which is significantly shorter and narrower in the aquatic turtles than in the terrestrial tortoises. It follows that for these species, we should use a smaller diameter of endotracheal cannula for intubation. The second parameter is a very short trachea in tortoises of the genus *Testudo*. The length of the trachea is only a few centimetres, which significantly increases the risk of intubation into one bronchus only.

**Keywords:** intubation of turtle and tortoise; length of trachea; *rima glottidis*; tracheal bifurcation

Turtles represent a very old group of reptiles that appeared in the Triassic period more than 220 million years ago (Li et al. 2008). All extant species of turtles belong to the Casichelydia group (Gaffney and Meylan 1988), of which there are around 360 species. The Casichelydia group is divided according to the way the head is hidden in the carapace (Rhodin et al. 2017; Lovich et al. 2018). Due to this, we distinguish between hidden-headed and hidden-throated turtles – Pleurodira and Cryptodira (Cope 1865; Gaffney et al. 1987). Turtles have recently become common patients of veterinarians and their typical problems include respiratory diseases (Schumacher 1997; Origgi and Jacobson 2000). However, information about the anatomy of the airways is poor and often contradictory. The lower airways of the turtle start right after the nasal cavity and the pharynx and end at the lungs. They consist of the larynx, trachea and bronchi (Saxena and Saxena 2008; Pyszko et al. 2013). The larynx is a simple tubular organ formed by a cricoid cartilage (*cartilago cricoidea*) and two arytenoid cartilages (*cartilago arytenoidea*). These cartilages are connected by ligaments. The

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epiglottis is not developed in turtles and the function of this missing structure is replaced by the tongue (Fraher et al. 2010). The position and stability of the larynx is provided by the apparatus of the hyoid bone which is placed ventrally. The apparatus of the hyoid bone replaced the function of the missing thyroid cartilage, which is the largest cartilage in the mammalian larynx (Sacchi et al. 2004; Kardong 2012). The glottis is located at the base of the tongue and represents the entrance to the larynx. Unlike most snakes and lizards, the entrance to the larynx is located more caudally and, therefore, it is more difficult to access it (Schumacher 2003). The mucosa of the larynx is not uniform throughout its length. The surface of the glottis is lined with a stratified glandular epithelium. It subsequently transforms into a pseudostratified columnar epithelium with cilia in the lumen of the larynx (Sacchi et al. 2004). The trachea of turtles is a tube between the larynx and the bronchi, formed by closed cartilaginous rings. Its lining is formed by ciliated epithelium, which continues in the bronchi and lungs (Murphy et al. 2012; Davenport et al. 2014). As it passes through the neck, the trachea accompanies the oesophagus on its ventral side (Knotek et al. 1999; Pyszko et al. 2013). In *Emys orbicularis*, the trachea moves at the level of the third cervical vertebra to the left side of the neck, where the tracheal bifurcation is also located (Zehtabvar et al. 2014). The position of the trachea on the left side of the neck is also described by a study focused on the structure of the lower respiratory tract in *Chelonoidis carbonarius* (Polanco et al. 2020). Opinions on the tracheal length vary widely in the literature. Some publications say that turtles from the suborder Cryptodira have a short trachea and its bifurcation is located around the middle of the neck. In contrast to the trachea of the representatives of the suborder Pleurodira, in which the trachea is longer and the bifurcation is more caudally located (O'Malley 2005; Boyer and Innis 2019). Other studies attribute a short trachea to most terrestrial tortoises, while it is longer and the bifurcation is at the heart level in aquatic turtles (Knotek et al. 1999). Another source mentions that the trachea is very short in all species except for hidden-headed turtles and some sea turtles (Chitty 2004). At the place of the bifurcation, the trachea divides into two bronchi, which do not branch in turtles (Wyneken et al. 2008; Polanco et al. 2020). The length of the right and left bronchi is not always the same. In the European pond turtle (*Emys orbicularis*), the *bronchus dexter* is slightly longer, as it first turns ventrally from the left side of the neck from the bifurcation and only then heads towards to the right lung (Zehtabvar et al. 2014). We can also find a longer right bronchus in the species *Platysternon megacephalum*, *Chelydra serpentina*, *Macrochelys temminckii* and *Trachemys scripta*. The bronchi of these turtles are always shorter than the trachea itself (Bennett 2011; Kardong 2012). In contrast, in Hermann's tortoises (*Testudo hermanni*), the left and right bronchus are almost identical in length and significantly exceed the length of the trachea (Lambertz et al. 2010). The proximal localisation of the bifurcation increases the risk of monolateral intubation. If the endotracheal cannula is inserted too deep, it opens into one bronchus and only one lung is supplemented with oxygen or anaesthetic (Knotek et al. 1999; Boyer and Innis 2019). Due to the above-described conflicting information about the anatomical structures of some lower respiratory tract organs in turtles, we decided to examine this topic closely and compare the findings between many species.

## MATERIAL AND METHODS

### Animals

The material for this study included 95 cadavers, originating from 19 different species of the order Testudines (Table 1). The terrestrial tortoises were represented by 63 individuals from eight species, the aquatic and semi-aquatic turtles were represented by 32 individuals from eleven species. All the specimens were representatives of the suborder Cryptodira except two species from the suborder Pleurodira (*Chelodina rugosa* and *Chelus fimbriatus*). The sex ratio was almost equal with a slight predominance of females over males. There were 41 males (M) and 54 females (F).

All the turtles were divided into three groups defined by size and age. The youngest individuals (size 0–33% of the total carapace length in adulthood) were included in the group of juvenile turtles (J). The second group (S) consisted of subadult turtles (size 34–66% of the total carapace length in adulthood) and the adult individuals (67–100% of the total carapace length in adulthood) belonged to the last group (A).

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Table 1. Characteristics of the studied animals

| Turtle species | ToE | NoI | Sex | | Age category | | |
|---|---|---|---|---|---|---|---|
| | | | M | F | J | S | A |
| *Centrochelys sulcata* | Te | 6 | 4 | 2 | 4 | 2 | 0 |
| *Chelonoidis carbonarius* | Te | 3 | 1 | 2 | 2 | 1 | 0 |
| *Stigmochelys pardalis* | Te | 7 | 3 | 4 | 6 | 0 | 1 |
| *Testudo graeca* | Te | 9 | 4 | 5 | 4 | 4 | 1 |
| *Testudo hermanni* | Te | 17 | 8 | 9 | 4 | 10 | 3 |
| *Testudo horsfieldii* | Te | 10 | 4 | 6 | 2 | 6 | 2 |
| *Testudo kleinmanni* | Te | 2 | 2 | 0 | 0 | 0 | 2 |
| *Testudo marginata* | Te | 9 | 4 | 5 | 5 | 2 | 2 |
| *Cuora galbinifrons* | Aq | 1 | 0 | 1 | 0 | 1 | 0 |
| *Cuora mouhotii* | Aq | 1 | 0 | 1 | 0 | 0 | 1 |
| *Emys orbicularis* | Aq | 2 | 0 | 2 | 0 | 2 | 0 |
| *Geoemyda spengleri* | Aq | 1 | 0 | 1 | 0 | 0 | 1 |
| *Chelodina rugosa* | Aq | 3 | 2 | 1 | 0 | 3 | 0 |
| *Chelus fimbriatus* | Aq | 2 | 0 | 2 | 2 | 0 | 0 |
| *Lissemys scutata* | Aq | 3 | 2 | 1 | 0 | 3 | 0 |
| *Malaclemys terrapin* | Aq | 4 | 1 | 3 | 0 | 0 | 4 |
| *Mauremys caspica* | Aq | 1 | 0 | 1 | 1 | 0 | 0 |
| *Platysternon megacephalum* | Aq | 5 | 2 | 3 | 0 | 5 | 0 |
| *Trachemys scripta elegans* | Aq | 9 | 4 | 5 | 2 | 2 | 5 |

A = adult; Aq = aquatic; F = female; J = juvenile; M = male; NoI = number of individuals; S = subadult; Te = terrestrial; ToE = type of environment

## Receipt of the cadavers

No wild turtle carcasses were used for this work. Most cadavers came from private breeders and also from the dissecting rooms of the Department of Anatomy, Histology and Embryology from the University of Veterinary Sciences Brno. Another important source was the Avian and Exotic Animal Clinic (also from the University of Veterinary Sciences Brno) and private veterinary clinics from Brno and Prague. Moreover, some turtles were also obtained from a few zoos in the Czech Republic. None of the animals used for our study died or were euthanised because of respiratory disease. No turtle was killed for this study.

## Animal dissection

In order to identify each cadaver, the subjects were carefully photographed, measured, and weighed before the necropsy. The measured parameters included the length and width of the carapace, the height of the carapace and the length of the plastron (Figure 1).

For the autopsy itself, the turtles were fixated in the dorsal position. The first step was the separation of the plastron from the carapace using an oscillating saw at the junction on both sides. The plastron was dissected including a small portion of the surrounding skin using a scalpel and scissors. In the next step, the body cavity was opened by removing the abdominal wall and serosa together with the muscle of the pectoral and pelvic girdle. This was followed by removal of the heart and gastrointestinal tract for easier orientation in the body cavity. Then the trachea with bronchi was completely uncovered by careful preparation. According to the need to maintain the position of the larynx at the caudal end of the oral cavity, the lower jaw was separated at the joint with the rest of the head and the surrounding skin was cut with scissors. In the next step, the lower airways were removed along with the lower jaw and lungs.

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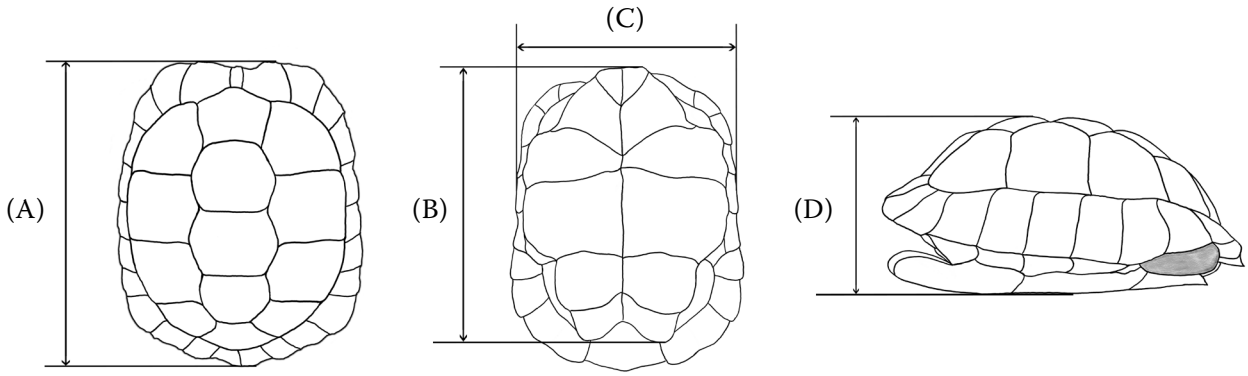


Figure 1. The length of the carapace (A) and plastron (B) were measured at the longest site
Measurement of the carapace width (C) was made at the border between the pectoral and abdominal keratin plastron plates. The last measurement determined the height of the carapace (D) at the largest arch of the carapace

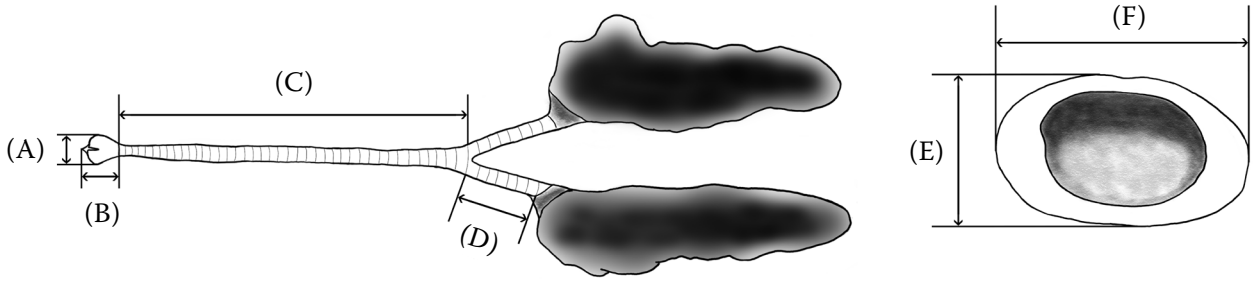


Figure 2. Measurement of the width (A) and length (B) of the larynx, length (C), height (E) and width (F) of the trachea and length (D) of the bronchi

### Measurement of the lower respiratory tract

The measurement of the individual respiratory tract followed the careful removal of the whole tract. A digital calliper (model Extol Premium 8825227, Extol; Madal Bal a.s., Zlín, Czech Republic) with a measurement accuracy to a hundredth of a millimetre was used for the measurement. The first measured parameter was the distance from the outer edge of the rostral end of the mandible to the entrance to the larynx. Subsequently, the length, width and height of the larynx and trachea and the length of the bronchi were measured (Figure 2). The last parameter was the number and completeness of the rings forming the trachea and bronchi.

## RESULTS AND DISCUSSION

The macroscopic structures of the lower respiratory tract varied slightly and in their proportions only. The larynx of all the examples was composed of three cartilages – two arytenoid cartilages (*cartilago arytenoidea sinistra et dextra*) and one cricoid cartilage (*cartilago cricoidea*). The entrance to the larynx (glottis) was localised at the base of the tongue and the *rima glottidis* had the appearance of a narrow slot which was orientated rostrocaudally. In the aquatic and semi-aquatic turtles, this slit was approximately two times shorter and narrower than in the terrestrial tortoises (Figure 3). We believe that a shorter and narrower entrance into the larynx of the aquatic turtles represents their protective adaptation mechanism against the entry of water into the lower respiratory tract. The trachea as well as the bronchi were formed by closed cartilaginous rings, which were connected to each other by a connective tissue. In almost all the individuals, we found several rings, whose construction was incomplete. Some rings were split and formed Y-shaped structures, others were approximately horseshoe-shaped and ended freely in the wall of the trachea or bronchi (Figure 4). At the site of the bifurcation, the trachea branched into two bronchi and continued toward the lungs. The location of the tracheal bifurcation was not the same in all the species (Figure 5), also, the length of the bronchi was variable. In the turtles with a lon-

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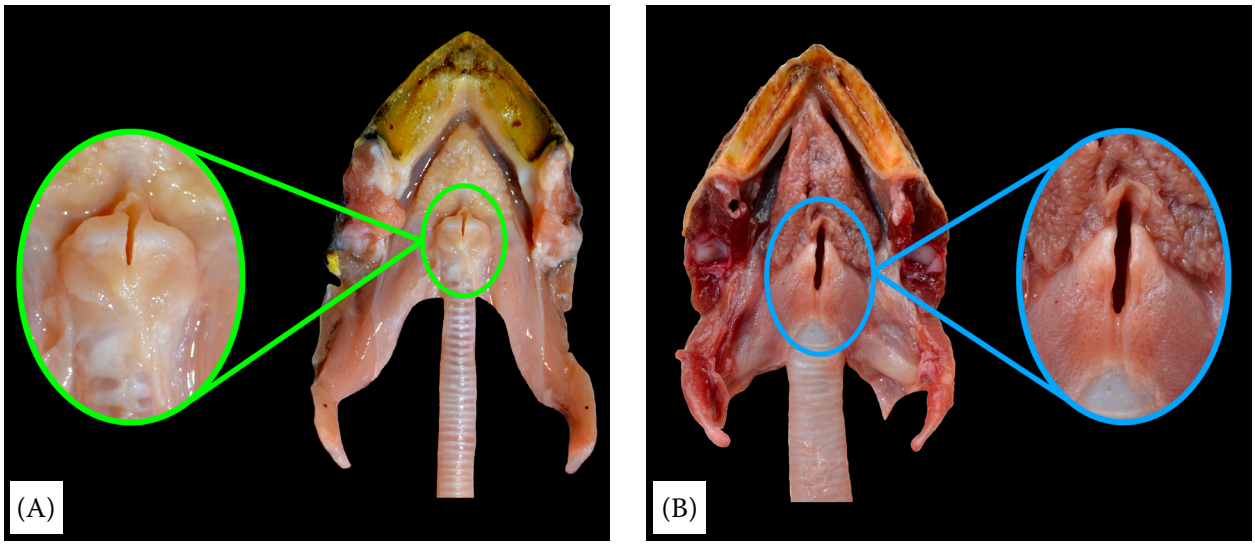


Figure 3. Appearance of the *rima glottidis* in the aquatic and terrestrial species
(A) Lower jaw with tongue, larynx and part of trachea – Red-eared slider (*Trachemys scripta elegans*), dorsal view. (B) Lower jaw with tongue, larynx and part of trachea – African spurred tortoise (*Centrochelys sulcata*), dorsal view

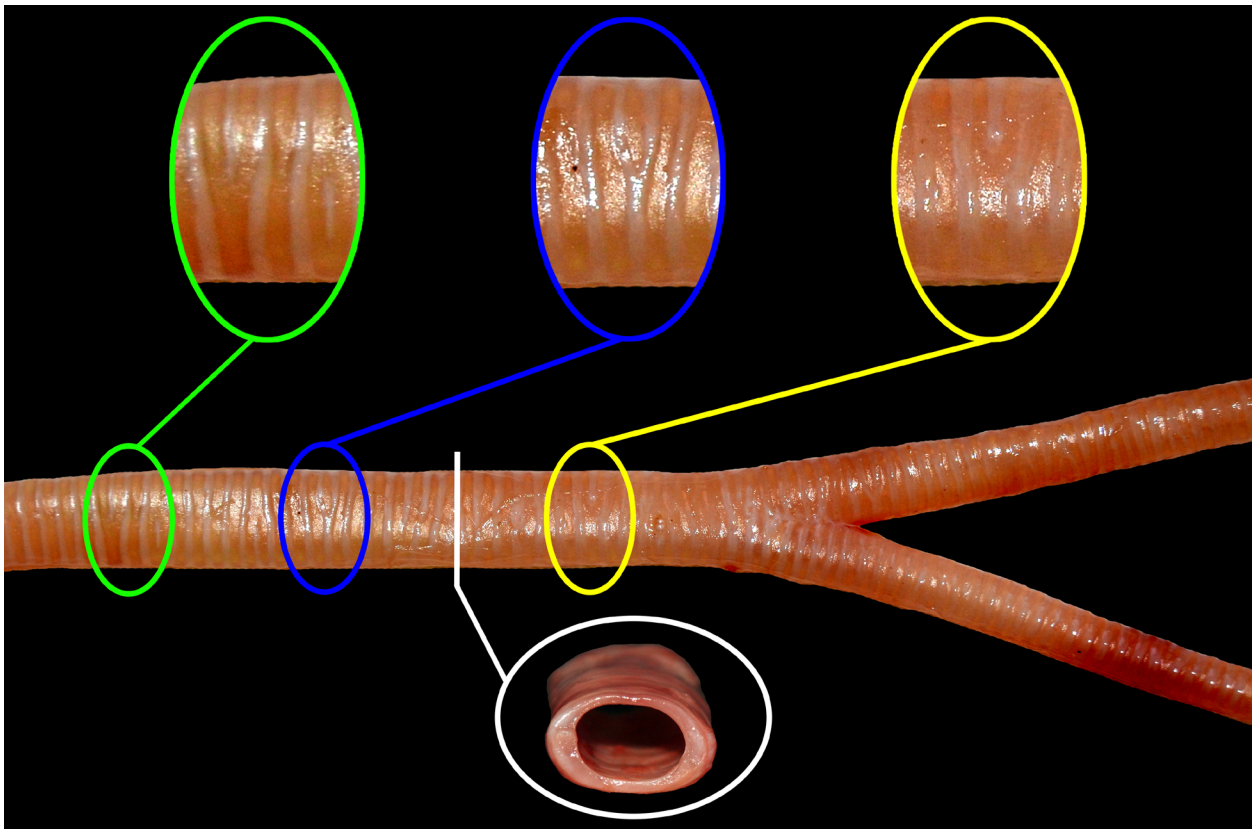

Figure 4. Incomplete cartilaginous tracheal rings (green, blue and yellow circles) of the red-eared slider (*Trachemys scripta elegans*), dorsal view
White circle – a picture of the flattening trachea

ger trachea, it is usually located in the left side of the neck (including the bifurcation), as reported by Zehtabvar et al. (2014) and Polanco et al. (2020). The measurement of the trachea included the determination of its length, width and height. In the species under study, we confirmed information

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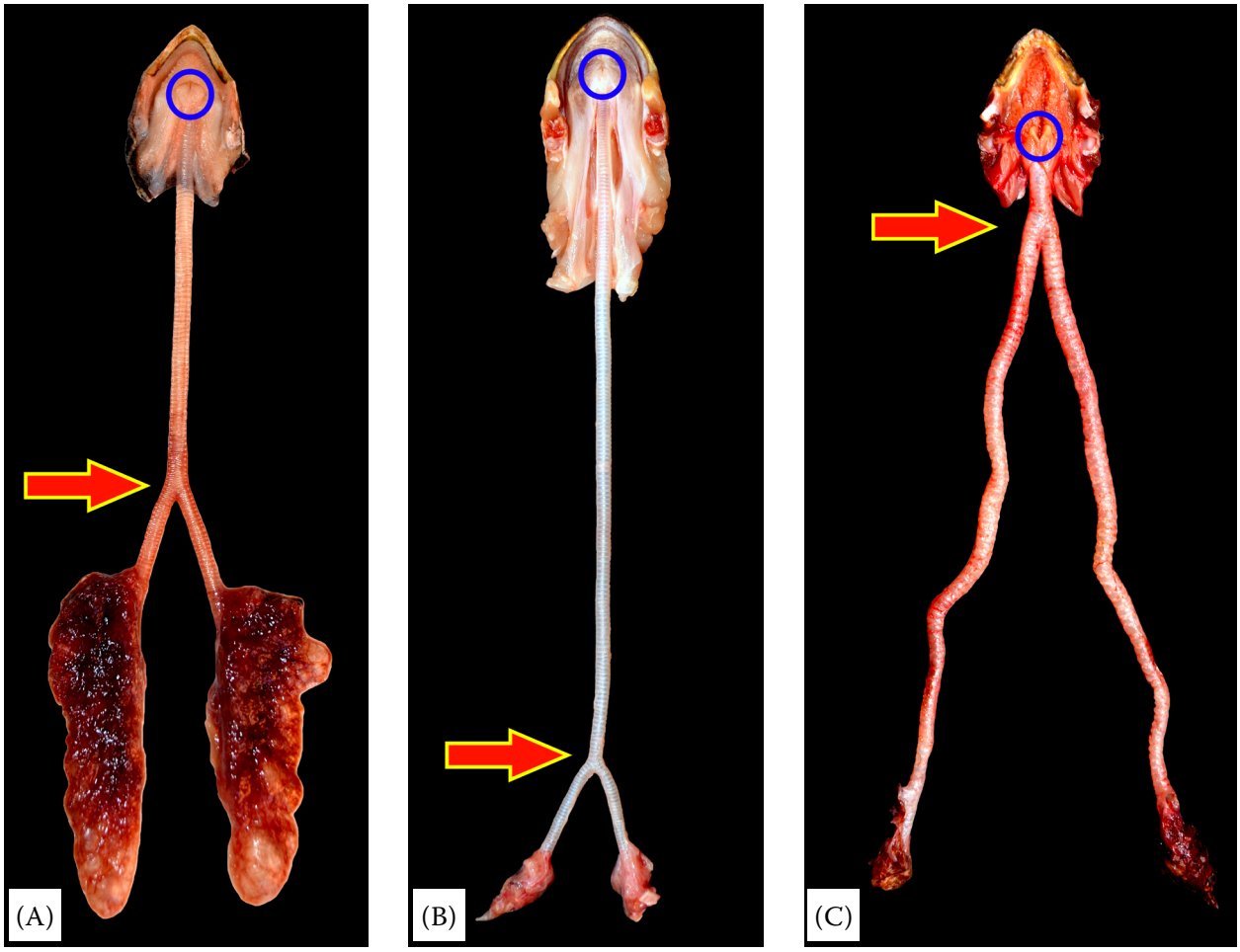


Figure 5. Comparison of the tracheal length in three species of turtles

(A) Lower respiratory tract – Caspian turtle (*Mauremys caspica*), dorsal view. (B) Lower respiratory tract – Northern snake-necked turtle (*Chelodina rugosa*), dorsal view. (C) Lower respiratory tract – Marginated tortoise (*Testudo marginata*), dorsal view. Blue circle – *glottis*, arrow – bifurcation of the trachea

from the literature that the tracheas of Pleurodira turtles are very long (Chitty 2004; O'Malley 2005; Boyer and Innis 2019). In both species from this suborder, the tracheal length reached more than 85% of the carapace length. The individuals of the species *Chelodina rugosa* even had a longer trachea than the carapace itself. In the case of turtles from the suborder Cryptodira, the literature reports a relatively short trachea with a bifurcation localised in the middle of the neck (Lambertz et al. 2010; Zehtabvar et al. 2014). However, we can disprove this information in many species. The tracheal length of the species we studied from this suborder was highly variable. We found a very short trachea in all the individuals of the genus *Testudo*. The trachea of some turtles from this genus did not even reach 5% of the length of the carapace (the average was 8% for the whole genus) and its bifurcation was located just behind the head. The other studied species, also belonging to Cryptodira, had a long trachea with a bifurcation located in the heart area (in some animals, it reached up to 58% of the length of the carapace). Rather, we can partially agree with the sources that attribute a short trachea to most terrestrial tortoises (Knotek et al. 1999; Boyer and Innis 2019). We explain their claim by the fact that the majority of the most commonly bred species of turtles in Central and Eastern Europe belong to the genus *Testudo* (Figure 5). In these turtles, caution during intubation is necessary because with careless insertion, an endotracheal cannula can easily be inserted into one bronchus only. In turtles of the genera *Centrochelys*, *Chelonoidis* and *Stigmochelys* as well as in the aquatic and semi-aquatic turtles of the suborder Cryptodira, we discovered a long trachea. In almost all the studied

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individuals, the trachea was dorsoventrally flattened and its height was noticeably smaller than the width (Figure 4), this corresponds to the information from the literature (Saxena and Saxena 2008; Murphy et al. 2012). The width of the trachea in terrestrial tortoises averaged 3.18% of the length of the carapace, in the case of aquatic and semi-aquatic species it was 2.75% of the length of the carapace. Due to this, for endotracheal intubation, it is important to choose the diameter of the cannula matching the height of the trachea and not to its width, in order to avoid any wall damage. Significant interspecies differences were also found in the bronchial length (Figure 6). In the individuals of the genus *Testudo*, the bronchi were significantly longer than the trachea. In some individuals, the ratio of both lengths was 1 : 10 in favour of the bronchi. In the other terrestrial species, the length of the bronchi and trachea was almost the same. The semi-aquatic and aquatic turtles, on the other hand, proved to have significantly longer trachea than bronchi. Also, the mutual length of both bronchi was diverse in the different species. We discovered a significantly longer right bronchus in the turtles *Cuora galbinifrons*, *Cuora mouhotii* and *Centrochelys sulcata*. On the contrary, there was a significantly longer left bronchus in *Lissemys scutata* and *Chelonoidis carbonarius*. In the other studied species, there were no major differences between the length of the right and left bronchus. The numbers of cartilaginous rings strengthening the tracheal wall and bronchi corresponded to the lengths of the structures. We counted the lowest number of cartilaginous rings in the genus *Testudo* (on average 15) and the most in the representatives of the suborder Pleurodira (the number reached


Figure 6. Comparison of the length of the right and left bronchi between different species of turtles
(A) Significantly longer right bronchus in an Indochinese box turtle (*Cuora galbinifrons*). (B) Approximately the same length of both bronchi in a Caspian turtle (*Mauremys caspica*). (C) Significantly longer left bronchus in a Burmese flapshell turtle *(Lissemys scutata*)

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167 rings). In the studied turtles, only the proportion of incomplete rings differed, but it was not directly proportional to the length of the organs (Figure 4). In our samples, we found, on average, 15.6% incomplete rings on the trachea for terrestrial species, and 11.53% for aquatic and semi-aquatic turtles. For bronchi, the number of incomplete rings was much higher, which confirmed information from the literature (Bennett 2011; Kardong 2012). We recorded the highest number in the aquatic and semi-aquatic turtle species, where the incomplete rings formed up the half of the rings. Among the terrestrial species, the incomplete rings accounted for less than a quarter of the total.

We found differences in the arrangement of the lower respiratory tract between terrestrial and aquatic turtles in several parameters. These differences were mainly in the size of the entrance to the larynx, the length of the trachea and the variable ratio between the length of the trachea and the bronchi. A very short trachea was found in all the studied individuals of the genus *Testudo*, in the other terrestrial turtles, the length of the trachea and bronchi was approximately the same. In contrast, in all the aquatic species, the trachea was longer than the bronchi. We mainly found a clinical significance in two parameters. The first was the entrance to the glottis, which is shorter and narrower in the aquatic turtles than in the terrestrial species (Figure 3). In these species, we should select a smaller endotracheal cannula diameter for intubation. The second parameter was the very short trachea in tortoises of the genus *Testudo* (Figure 5). The length of the trachea is only a few centimetres, which increases the risk of intubation into one bronchus only.

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## Conflict of interest

The authors declare no conflict of interest.

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