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Macro Anatomical and Histological Study of Larynx Cartilage, Trachea, and Lungs in Lynx (*Lynx lynx*)

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ABSTRACT In this study the larynx, trachea, and lungs of one 1.5-year-old adult female lynx (*Lynx lynx*) were examined. The macro anatomical and histological structure of the larynx cartilages, trachea, and lungs were tried to be revealed by dissection and measurements. It was determined that the trachea had 48 cartilage rings (*cartilago trachealis*) up to the bifurcatio trachealis, the diameters of which narrow as they approach the lungs. The total tracheal length was 172.36 mm. It was seen that the cross-section of the lynx trachea resembled a circle. The hyaline cartilage structure surrounding the trachea was determined. A muscle structure called musculus transversus trachea, which closes the open ends of this cartilage and surrounds the cartilage from the outside, was detected. It was determined that the trachea consisted of tunica mucosa, submucosa, and tunica adventitia layers. The pulmo sinister in the lynx lung consisted of two main lobes, lobus cranialis, and lobus caudalis. Lobus cranialis was also divided among itself as pars cranialis and pars caudalis. Pulmo dexter was divided into four main lobes as lobus cranialis, lobus medius, lobus caudalis, and lobus accessorius. It was determined that there was connective tissue capsule surrounding the lung, respiratory bronchioles, alveoli, intraalveolar septum, and abundant blood vessels. In intraalveolar septum; capillaries, erythrocytes were determined. Type I and type II pneumocytes were seen covering the alveolar surface. In addition, it was determined that there were macrophages in the alveolar sacs.

Keywords: *Anatomy, Histology, Larynx, Lung, Lynx, Trachea.*

ÖZ

Vaşak (*Lynx lynx*)'ta Larynx Kıkırdakları, Trachea ve Akciğerler Üzerine Makroanatomik ve Histolojik Bir Çalışma

Bu çalışmada 1 adet erişkin vaşağın (*Lynx lynx*) larynx, trachea ve akciğerleri incelendi. Diseksiyon ve ölçümlerle larynx kıkırdakları, trachea ve akciğerlerin makroanatomik ve histolojik yapısı ortaya konulmaya çalışıldı. Trachea'nın bifurcatio trachealis'e kadar, çapları akciğerlere doğru yaklaştıkça daralan, 48 adet kıkırdak halkaya (cartilago trachealis) sahip olduğu tespit edildi. Toplam trachea uzunluğu 172.36 mm idi. Vaşak trachea'sının enine kesitinin daireye benzediği görüldü. Vaşak akciğerindeki pulmo sinister lobus cranialis ve lobus caudalis adında iki ana loptan oluşuyordu. Pulmo sinister'in lobus cranialis'i de kendi arasında pars cranialis ve pars caudalis olarak bölünmüştü. Pulmo dexter lobus cranialis, lobus medius, lobus accessorius ve lobus caudalis olmak üzere dört ana loptan oluşmaktaydı. Trachea'yı dışarıdan saran hiyalin kıkırdak yapısı belirlendi. Bu kıkırdağın açık olan uçlarını kapatan ve kıkırdağı dışardan saran musculus transversus trachea adlı kas yapısı tespit edildi. Trachea'nın tunika mukoza, submukoza, tunika adventisya katmanlarından oluştuğu belirlendi. Akciğeri dıştan saran bağ doku kapsülü, respiratuvar bronşiol sonları, alveoller, intraalveoler septum ve bol miktarda kan damarlarının bulunduğu tespit edildi. İntraalveolar septumlarda; kapillar damarlar, eritrositler belirlendi. Alveol yüzeyini kaplayan tip I ve tip II pnömositler görüldü. Ayrıca alveoler keselerde makrofajların olduğu belirlendi.

Anahtar Kelimeler: *Anatomi, Akciğer, Gırtlak, Histoloji, Soluk borusu, Vaşak.*

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INTRODUCTION

Lynx is the common name of medium-sized carnivorous vertebrate and wild animal species that is found in the Lynx genus from the feline (*Felidae*) family (W.W.F. 2016). The fur color is yellowish brown in summer, and gray-white in winter with distinctive black markings. They have long hind legs, and wide paws, and they are mostly active at night. The lynx is a forest cat, so it is seen in wooded and mountainous areas. They shelter in tree hollows, dens, and thick bushes. They have a 15-year lifespan. They feed on roe deer, wild goats, hares, foxes, birds, and squirrel-like rodents. They were seen in various regions in the south, especially in the Western and Eastern Black Sea Regions (Kütükçü 2016). There are four species, namely the Eurasian lynx (*Lynx lynx*), Canadian lynx (*Lynx canadensis*), Iberian lynx (*Lynx pardinus*), and lynx lynx (*Lynx rufus*). It has been reported that the Eurasian lynx subspecies *L. l. Dinniki* live in Turkey, the Caucasus mountains, northern Iran and northern Iraq (WWF 2016) and based on this information, it was thought that our research material is *L. l. Dinniki*.

The respiratory system provides breathing in mammalians. The main organ of this system is the lungs and the auxiliary organs are the larynx, trachea, nasopharynx, nose, and nasal cavity (Demiraslan and Dayan 2021). Larynx is a muscular and cartilaginous organ located at the beginning of the trachea. It is responsible for the formation of sound and the transmission of inspiratory air (Evans and de Lahunta 2013). Larynx was closer to the spatium mandible in non-carnivorous animals, while it shifted towards the neck in carnivores. It connects the pars nasalis pharyngis part of the pharynx with the trachea. It is responsible for the formation of sound as well as transmitting the breathing air. The larynx skeleton is composed of cartilages called cartilagines laryngis (Bahadır and Yıldız 2014). Cartilago thyroidea, the largest larynx cartilage, is formed by the union of two lamina thyroidea. The dorsal edge of the lamina thyroidea extends anteriorly to join the cornu rostrale and posteriorly to the cornu caudale. Cornu caudale articulates with cartilago cricoidea and the fissura thyroidea is between the cornu rostrale and the lamina. The nerve called nervus laryngeus cranialis comes out of this fissura thyroidea. There is incisura thyroidea caudalis, which is shallower in carnivores is caudal of the region where the laminae join ventrally. Nervus laryngeus caudalis is extend in this region. Cartilago cricoidea is ring-shaped. The wide flat part in the dorsal is called lamina cartilaginis cricoidea, and the arch part is called arcus cartilaginis cricoidea. The dorsal projection where the laminae meet is called the crista. Cartilago arytenoidea is comb-shaped and the protruding part on its outer surface is called the processus muscularis. The downward extending part is the processus vocalis. Its protrusion, which emerges from the craniodorsal and leads caudal like a horn, is the cartilago corniculata which is not found in felis. In canis, there is a second projection, called the cartilago cuneiformis, just in front of the cartilago corniculata (Evans and de Lahunta 2013). The epiglottis is the leaf-shaped cartilage of the larynx, located at the most cranial. Its upper end, called apex, is located at cranial in carnivores. There is a projection called the petiolus epiglottidis at the base called the basis (König and Liebich 2015). The trachea extends from the cartilago cricoidea of the larynx to the bifurcatio trachea. The number of tracheal rings is 42-46 in dogs and 38-43 in cats. Cartilago trachealis join with ligamentum annulare. At the same time, the dorsal space of the cartilages is closed with the musculus trachealis (König and Liebich 2015). The lungs are divided into pulmo dexter and pulmo sinister at the level of the bifurcatio trachea. The lungs have four faces: facies costalis, facies mediastinalis, facies diaphragmatica, and facies interlobaris. It has two edges, called margo ventralis (acutus) and margo dorsalis (obtusus) (Dursun 2008). There is a notch called incisura cardiaca at the heart level of margo ventralis (König and Liebich 2015). The part of the lungs opposite the aperture thoracis cranialis is called the apex pulmonis, the part of opposite the diaphragm is called the basis pulmonis (Demiraslan and Dayan 2021). The lung parenchyma has structures that exchange CO_2 and O_2 in the respiratory air. The pulmonary airways begin with the bronchus principalis dexter and sinister at the level of the bifurcatio trachea. Each bronchus principalis is divided into bronchus lobaris, which goes to different lobes of the lungs and is named after the same lobe to name which it goes (König and Liebich 2015). Lung loping in carnivores is as follows; pulmo sinister is divided into two main lobes as lobus cranialis and lobus caudalis. Lobus cranialis is also divided into pars cranialis and pars caudalis. Pulmo dexter is divided into four main lobes as lobus cranialis, lobus medius, lobus caudalis, and lobus accessorius (Bahadır and Yıldız 2014).

In the literature review, it was seen that there were studies on the respiratory system in different animal species (Düzler et al. 2005; Perez et al. 2006; Cano and Perez 2009; Gezer İnce and Pazvant 2010; Wysocki et al. 2010; Onuk et al. 2013; Özkadif et al. 2016; Fonseca et al. 2017; Gündemir et al. 2017; Abbasabadi et al. 2021; Halıgür and Özkadif 2021). However, no anatomical and histological studies were found on the larynx, trachea, and lungs of the lynx. This study was designed in line with this perceived shortcoming. We believe that this presented study will support scientific studies on similar subjects, tracheal collapse, corneal operation, and respiratory system diseases.

MATERIAL AND METHODS

Conditional permission was obtained from the Animal Experiments Local Ethics Committee of Kafkas University (KAÜ-HADYEK/2021-175) to conduct this study.

A 1.5-year-old female Eurasian lynx (*Lynx lynx*), which was brought to the Kafkas University Wildlife Rescue and Rehabilitation Center injured but could not be saved despite all the interventions, constituted our study material. The dissection process started with the larynx located at the beginning of the neck of the lynx cadaver, which was brought to the anatomy laboratory for anatomical examination. Larynx cartilages and trachea were exposed. The total trachea length, the width of the trachea at the beginning, middle and end, and the length and width of the lung lobes were measured with the help of a digital caliper (stainless steel 1- to 150-mm). For the nomenclature of anatomical terms, N.A.V. (2017) used. After the macro anatomical findings were obtained, tissue samples were taken from the head of the trachea and the tip of the lung for histological examinations and fixed in 10% formaldehyde solution. Afterwards, the routine tissue procedure was applied and it was blocked in paraffin. 5µm thick sections were taken from paraffin blocks and Crosman's triple staining and Periodic acid–Schiff (PAS) staining were performed to examine the general structure of trachea and lung tissue. The prepared sections were evaluated under the light microscope and photographed (Olympus BX43, JAPAN).

RESULTS

Macro Anatomical Results

The macro anatomical view of the larynx, trachea, and lungs of the lynx is shown in Figure 1. It was observed that the cartilages of the larynx were composed of double cartilago arythenoidea and single cartilago epiglottis, cartilago thyroidea, cartilago cricoidea. The largest cartilage in size was the thyroidea. The cornu rostrale and cornu caudale of the cartilago thyroidea were clearly identified. Incisura thyroidea caudalis, where the nervus laryngeus caudalis passes, was determined in the caudal of the region where the laminae of the cartilago thyroidea converge in the ventral. The processus muscularis of cartilago arytenoidea extending dorsally was detected. Processus vocalis was seen extending towards the cavum laryngis ventral of the same cartilage. Cartilago corniculata was not seen clearly. It was observed that the apex of the epiglottis was located. Trachea was consisted of 48 cartilago trachealis and the cross-section of the cartilago trachealis was circular in shape. Total tracheal length was 172.36 mm. Tracheal ring width was measured 12.40 mm at the beginning, 9.98 mm in the middle, and 9.23 mm at the end. The pulmo sinister in the lynx lung was divided into two main lobes as lobus cranialis and lobus caudalis. The lobus cranialis was also divided among itself as pars cranialis and pars caudalis. The pulmo dexter was divided into four main lobes as lobus cranialis, lobus medius, lobus caudalis, and lobus accessorius (Figure 1). The pulmo sinister in the lynx lung consisted of two main lobes, lobus cranialis and lobus caudalis. The lobus cranialis of the pulmo sinister was also divided among itself as pars cranialis and pars caudalis. Pars cranialis length was measured 65.75 mm, width 45.42 mm; pars caudalis as 72.61 mm in length and 54.12 mm in width. The lobus caudalis length of the pulmo sinister was 40.28 mm and the width was 34.95 mm. The pulmo dexter consisted of four main lobes: lobus cranialis, lobus medius, lobus accessorius, and lobus caudalis. Pulmo dexter's lobus cranialis was 84.04 mm long, 58.70 mm wide, lobus medius length 59.95 mm wide 30.99 mm, lobus accessorius length 36.41 mm wide 41.51 mm lobus caudalis 47.35 mm wide 38.44 mm.

Histological Results

Histological Structure of Trachea

It was determined that the C-shaped hyaline cartilage structure surrounding the trachea. It was observed that the hyaline cartilage was surrounded by the perichondrium and there were chondroblast cells just below the perichondrium. There were chondrocytes in the lacunae as we went towards the inner part of the cartilage. In addition, it was determined that the musculus transversus trachea, which consists of smooth muscle cells that close the open ends of the cartilage, surrounds the cartilage from the outside (Figure 2). It was determined that the trachea was composed of tunica mucosa, submucosa, and tunica adventitia. Lamina epithelialis was consisted of ciliated pseudostratified columnar epithelial cells and goblet cells. Lamina propria and submucosa were composed of connective tissue and in the submucosa; serous, mucous, and seromucous tracheal glands were determined. Tunica adventitia surrounded the trachea externally and had a loose connective tissue structure (Figure 3).

The Lung

The connective tissue capsule was surrounding the lung, respiratory bronchioles, alveoli, and abundant blood vessels. The connective tissue was penetrated into the lungs and the respiratory bronchioles were surrounded by the smooth muscle layer. Intraalveolar septa were separating adjacent alveoli from each other. In intraalveolar septum; capillaries, erythrocytes, type I pneumocytes which are squamous epithelial cells covering the alveolar surface, and type II pneumocytes which are less numerous and larger than type I pneumocytes, were observed. In addition, it was determined that there were macrophages (dust cells) in the alveolar sacs (Figure 4-6).

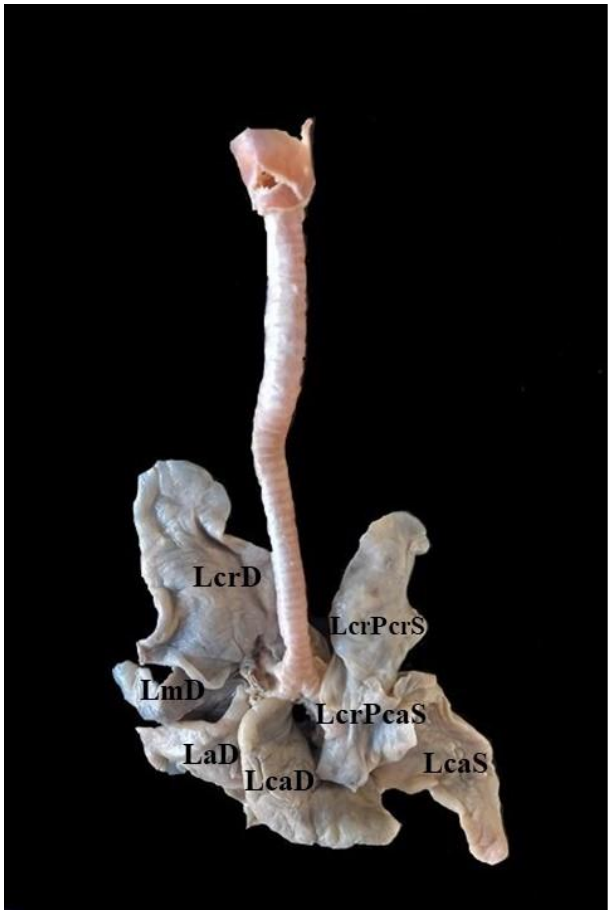

Figure 1: Larynx, trachea and lung in lynx (LcrD: Lobus cranialis of pulmo dexter, LmD: Lobus medius of pulmo dexter, LcaD: Lobus caudalis of pulmo dexter, LaD: Lobus accessorius of pulmo dexter, LcrPcS: Pars cranialis of lobus cranialis of pulmo sinister, LcrPcaS: Pars caudalis of lobus cranialis of pulmo sinister, LcaS: Lobus caudalis of pulmo sinister.

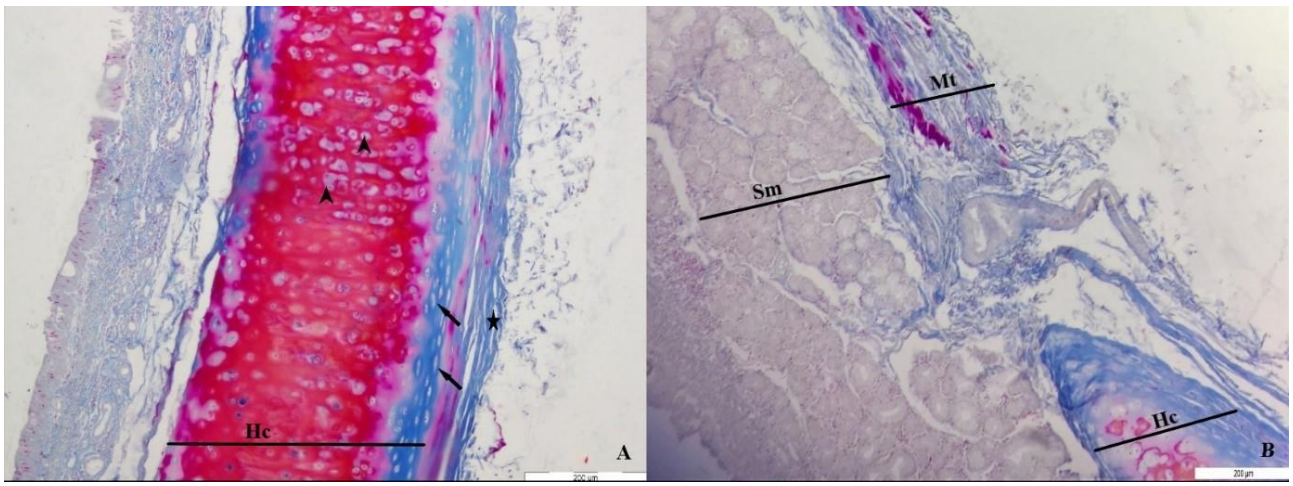

Figure 2: Lynx trachea (A, B). Hc: hyaline cartilage, Sm: Submucosa, Mt: musculus transversus, Chondroblasts (arrows), chondrocytes (arrowheads), and perichondrium (stars). Triple staining.

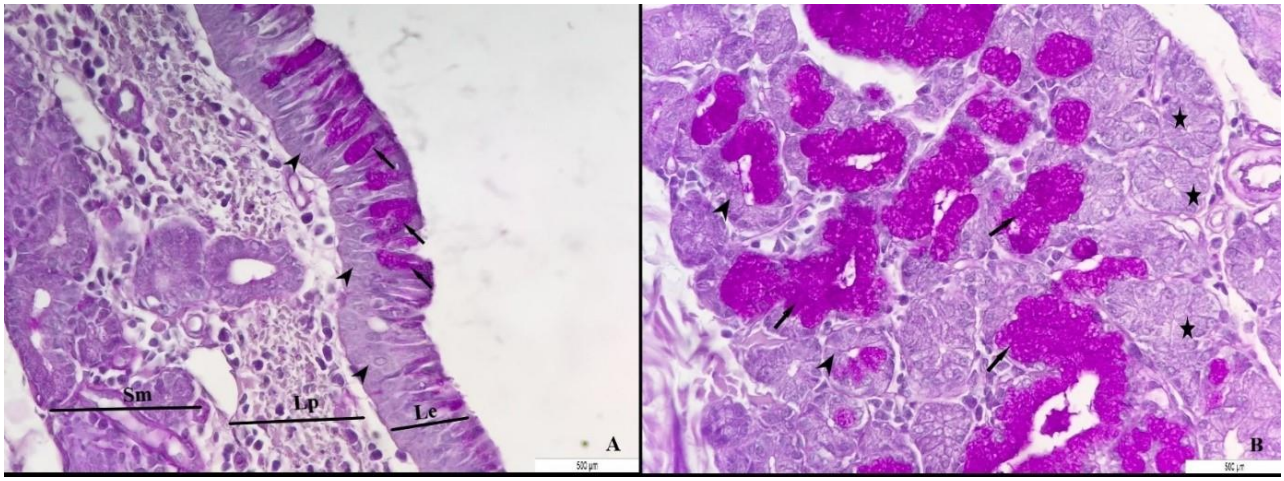

Figure 3: Lynx trachea (A, B). Le: Lamina epithelialis, Lp: Lamina propria, Sm: Submucosa. A: Epithelial cells (arrowheads) and goblet cells (arrows). B: Serous glands (stars), mucous glands (arrows), and seromucous (arrowheads) glands in the submucosa. PAS staining.

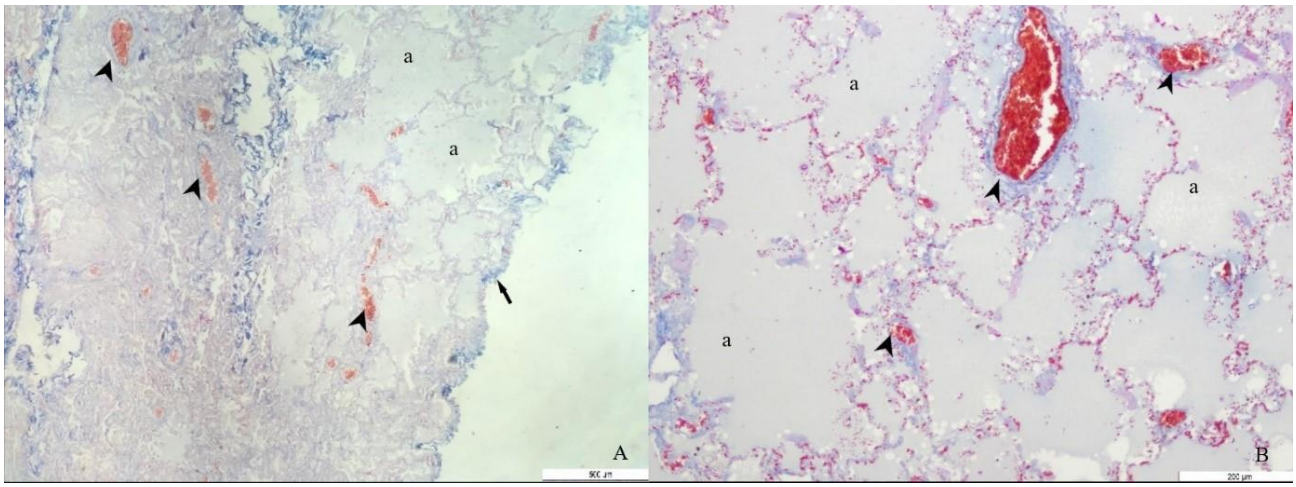

Figure 4: Lynx lung (A, B). Capsule (arrow), blood vessel (arrowhead), alveoli (a). Triple staining.

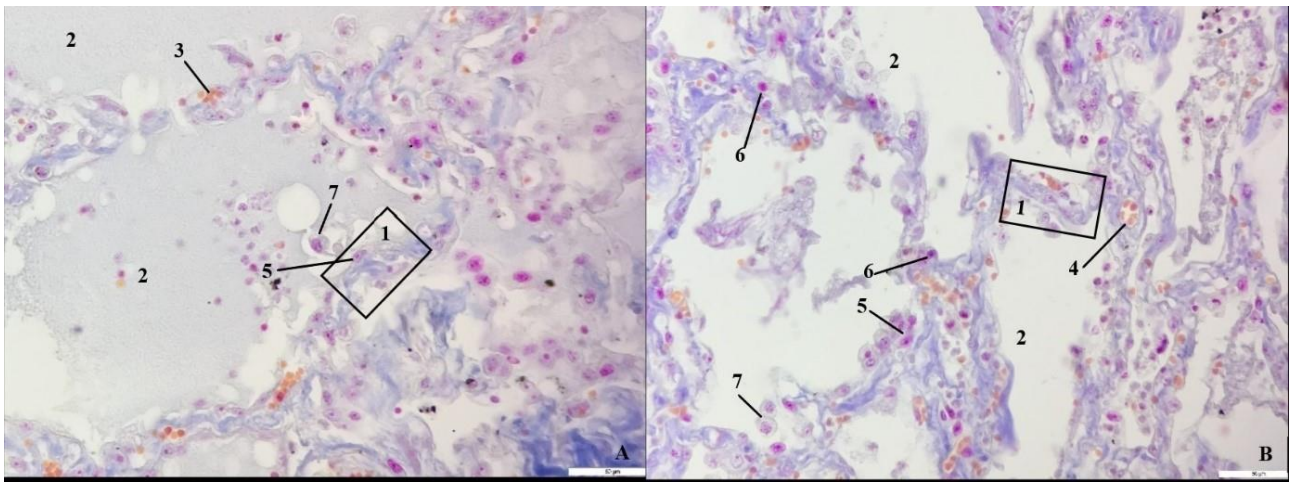

Figure 5: Lynx lung (A, B). Alveolar septum (1), alveoli (2), erythrocytes (3), capillary (4), Type 1 pneumocyte (5), Type 2 pneumocyte (6), macrophage (7). Triple staining.

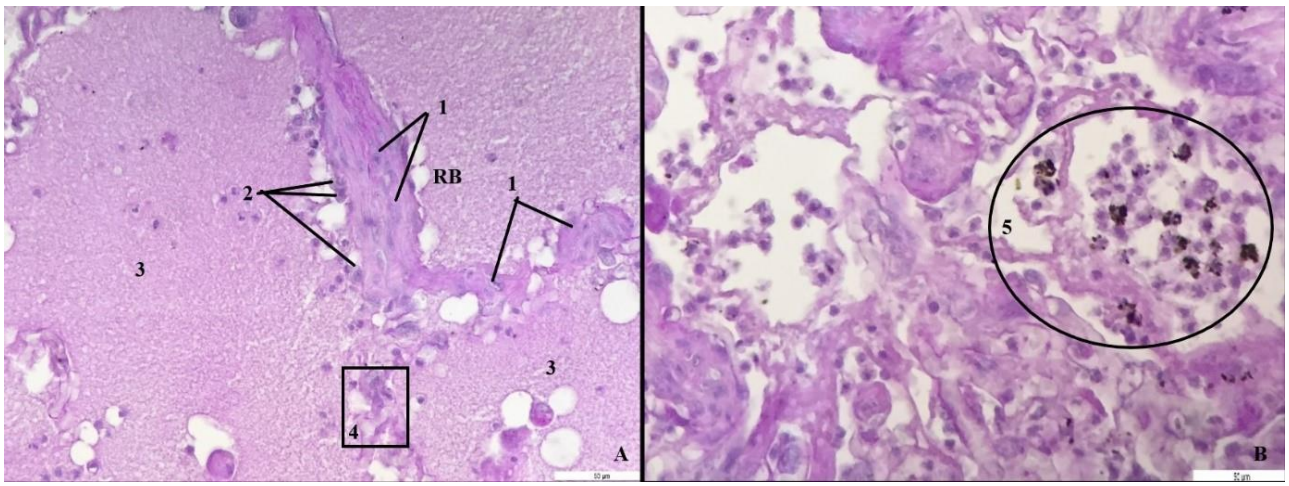

Figure 6: Lynx lung (A, B). Respiratory bronchiole ends (RB), smooth muscle layer (1), clara cells (2), alveoli (3), alveolar septum (4), dust cells (5). PAS staining.

DISCUSSION AND CONCLUSION

The respiratory system is tasked with exchanging oxygen and carbon dioxide and delivering oxygen to all tissues of the body. This system consists of two parts. These are the part where the air is transmitted and respiration, and the part where gas exchange, takes place (Gartner and Hiatt 2014). The trachea, the part through which air is conducted, is a tubular organ that extends from the larynx to the primary extrapulmonary bronchi (Banks 1993). Gartner and Hiatt (2014) reported that 15-20 horseshoe-shaped (C-ring) rings formed the trachea, Halıgür and Özkadif (2021) found that trachea rings were 42-48 in foxes, Dabanoğlu et al. (2001) found as 36-45 in dog. In the present study, it was determined that the trachea consisted of 48 rings. When we compare the number of cartilage rings detected with the literature, it is seen that it is closer to the fox. They (Halıgür and Özkadif 2021) reported the trachea length as 154.16± 0.60 mm in male foxes and 137.28± 0.62 mm in female foxes. The total tracheal length was 172.36 mm in our study so; we can say that the lynx trachea is longer than the fox trachea.

Haligür and Özkadif (2021) was measured the diameter of the cartilago trachealis as 13.37±0.38 mm in male foxes and 12.28±0.23 mm in female foxes. In the present study, the tracheal ring width in the female lynx was measured as 12.40 mm at the beginning, 9.98 mm in the middle, and 9.23 mm in the last part. Initial values of tracheal ring width were close to Haligür and Özkadif (2021). The open ends of the C-rings are connected posteriorly by smooth muscle (*musculus trachealis*). With the contraction of this muscle, the lumen of the trachea narrows, thereby increasing the speed of airflow (Gartner and Hiatt 2014). Musculus trachealis was located inside the cavity of the tracheal cartilages in horses, pigs, and ruminants but in cats and dogs outside the cavity (Bacha and Bacha 2012). Tracheal lumen is lined by pseudostratified ciliary columnar epithelium known as respiratory epithelium. This epithelium also contains DNES cells, about 30% of goblet cells. Goblet cells are single-celled glands that produce mucinogen, a mucus substance that is released onto the surface of the wet epithelium (Gartner and Hiatt 2014). The lamina propria and submucosa which are not clearly separated from each other, are located under the epithelium (Bacha and Bacha 2012). The lamina propria and lamina muscularis consist of prominent elastic fibers believed to replace the mucosa (Banks 1993). In the submucosa, there are tubuloacinar seromucous glands that open into the lumen via channels lined with ciliary cells, mucus-secreting cells, and various intermediate cells. The tubular parts of the tracheal glands are lined with mucus-secreting cells, while the acinar parts are lined with serous secretory cells. These glands are abundant in the proximal

parts of the trachea of almost all domestic mammal species (Eurell and Frappier 2006). It was observed that the C-shaped non-continuous cartilage rings provided the opening of the lumen, the cartilage was thicker in the anterior than in the posterior, and was continuous with the perichondrium in the monkey trachea. The lamina propria is thin, the submucosa is thick, and there are mucous and seromucous glands. The perichondrium of the cartilage fuses with the connective tissue in the submucosa and there are plenty of blood vessels (Gartner and Hiatt 2014). In the pig trachea, the ends of the cartilage rings are not joined and are interconnected by connective tissue. The musculus trachealis is inside the cartilage. The epithelial layer of the mucosa is typically composed of ciliated epithelium, including goblet cells (Banks 1993). Carnivorous trachea is lined by goblet cell, ciliated, and pseudostratified columnar epithelium. The lamina propria is in the form of a longitudinal band of elastic fibers. The musculus trachealis (with a smooth structure) is located outside the C-shaped cartilaginous cavity (Bacha and Bacha 2012). C-shaped hyaline cartilage surrounding the trachea externally, musculus transversus trachea, outside the cartilage rings; lamina epithelialis, ciliated pseudostratified columnar epithelial cells, and goblet cells, loose connective tissue in the lamina propria. Serous, mucous, and seromucous glands in the submucosa and loose connective tissue adventitia surrounding the trachea were determined in lynx.

The lungs, located in the rib cage, serve as the main organ of the respiratory system. The visceral leaf of the capsule-shaped pleura surrounds the lungs externally. This connective tissue capsule enters the organ and divides the lungs into lobes and lobules (Girgin et al. 2010). The pulmo sinister of carnivores is divided into two main lobes, lobus cranialis and lobus caudalis. Lobus cranialis is also divided among as pars cranialis and pars caudalis. Pulmo dexter is divided into four main lobes as lobus cranialis, lobus medius, lobus caudalis, and lobus accessorius (Hazıroğlu and Çakır 2018; Demiraslan and Dayan 2021). In a study of lung typing (Voyevoda et al. 1992), the right lung lobe mostly (70%) consists of four lobes in arctic foxes: cranial, medial, caudal, and infracardiac. In our study, the right lung consisted of four lobes. Only the lobus accessorius of pulmo dexter was named lobus infracardiaca in the other study. Voyevoda et al. (1992) stated that the left lung lobe in dogs mostly consists of three lobes as cranial, medial, and caudal. In the lynx, the left lung lobe consisted of three lobes in total. But the nomenclature was different. In our study, while the lobus cranialis of the left lung lobe was divided into pars cranialis and pars caudalis, Voyevoda et al. (1992) named the left lung lobe in the dog as cranial, caudal, and medial. We think that this difference is due to the different branching of the bronchi.

The structure of the lungs is generally examined in two parts as air-conducting pipes and respiratory tissue. The airways are the bronchi and bronchioles. It begins where the trachea splits in the thoracic cavity and continues to the respiratory tissue (Girgin et al. 2010). Bronchioles are air-conducting ducts of 1 mm or less in diameter. Larger bronchioles represent branches of segmental bronchi. These ducts branch repeatedly to form terminal bronchioles. Terminal bronchioles also branch to form respiratory bronchioles. Respiratory bronchioles form a transition zone in the respiratory system and are involved in air conduction and gas exchange. They have a narrow diameter. The first sections contain ciliated cubic epithelium and clara cells. Clara cells predominate in its distal. Rarely brush cells and dense nucleated granule cells are also present along the length of the respiratory bronchiole (Ross and Pawlina 2016). Respiratory bronchioles are well developed in cats and dogs (Bacha and Bacha 2012). The alveoli are surrounded by capillary networks. Capillaries are attenuated, non-perforated, continuous endothelial cells, very close to type I pneumocytes. The alveoli are separated from each other by walls of varying thickness known as interalveolar septa. Macrophages, known as dust cells, are usually seen in the interalveolar septa. Dust cells differentiate from monocytes and enter the lungs through the bloodstream (Gartner and Hiatt 2014). Each alveolar duct divides into three or more alveolar sacs. There are no smooth muscles in the sacs. Thin squamous epithelial cells (type I alveolar cells) are abundant in the alveoli, while surfactant-producing type II alveolar cells are rare (Bacha and Bacha 2012). Type I pneumocytes are not capable of dividing. They are fairly thin squamous cells and occupy most (95%) of the alveolar surface. Cells, also called type II pneumocytes or septal cells, are cubic in shape. It is found interspersed between type I pneumocytes and tends to collect at septal junctions. Type II pneumocytes occupy only 5% of the alveolar air surface (Ross and Pawlina 2016). In the monkey, the two alveoli are separated by an interalveolar septum. There are capillaries containing abundant erythrocytes in the septum. The entire alveolar surface consists of an abundance of type I pneumocytes and a smaller number of type II pneumocytes. The interalveolar septa are thick, containing blood vessels and connective tissue elements, including macrophages known as dust cells. At the entrance to the alveoli are smooth muscle cells that look like knobs in the dog, the alveoli are composed of highly attenuated endothelial cells, type I pneumocytes, and an intervening basal lamina. Cytoplasm is scarce in both cell types. The air space of the alveoli is empty, while the capillary lumen contains red blood cells (Gartner and Hiatt 2014). In the lynx lung; it was determined that the connective tissue capsule surrounding the lung from the outside entered the inside of the organ and divided the organ into lobes. It was observed that the respiratory bronchiole endings were surrounded by the smooth muscle layer. In addition, intraalveolar septums separating adjacent alveoli from each other were detected. In intraalveolar septum; capillary vessel nuclei, erythrocytes, type I pneumocytes with squamous epithelial cells and covering the entire alveolar surface were seen interspersed with type II pneumocytes. Also, it was determined that there were macrophages (dust cells) interspersed in the alveolar sacs and lung tissue.

In conclusion, Lynxes are endangered wild animals. Therefore, very few studies have been found on lynxes (Arı et al. 2018; Arı and Uslu 2021). The larynx, trachea and lungs of the wild animal lynx were analyzed anatomically and histologically. We believe that this presented study will support scientific studies on similar subjects (Akgün et al. 2018; Osorio-Echeverri et al. 2019) cornage operation with tracheal collapse, and approach to respiratory system diseases (Masseaua and Reinero 2019).

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest for this study.

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AUTHOR CONTRIBUTIONS

Idea / Concept: GKD
Supervision / Consultancy: EKS
Data Collection and / or Processing: ŞYA, GKD
Analysis and / or Interpretation: ŞYA, GKD
Writing the Article: ŞYA, GKD, EKS
Critical Review: ŞYA, GKD, EKS

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