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MICROSCOPIC ARCHITECTURE OF THE RESPIRATORY AND CONDUCTING SYSTEM OF THE LUNG OF THE NILE MONITOR (*VARANUS NILOTICUS*)

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ABSTRACT:

Using semi-thin sections, the present investigation examined the microscopic characteristics of the lungs of the Nile monitor (*Varanus niloticus*). The lungs were composed of Intrapulmonary conducting airways and respiratory faveoli. Intrapulmonary airways originate from the terminal portion of the bronchus, which extends into the lung to create the bronchial tree. The bronchus was lined with pseudostratified ciliated epithelium composed of both ciliated and non-ciliated cells, and it was supported by plates of hyaline cartilage. The central lumen is surrounded by contractile fibers that contain smooth muscle cell bundles and are covered by ciliated and non-ciliated cells. The central lumen communicates with the faveoli. Separating adjacent faveoli are pulmonary trabeculae covered with various cell types, including type I pneumocytes, type II pneumocytes, and pulmonary macrophages. Some substantial pulmonary bronchi were also supported by small cartilage plate granules and lined with ciliated epithelium. Type I pneumocytes were flat cells, whereas type II pneumocytes had cuboidal cells with vacuolated cytoplasm. Surface irregularity and vacuolated cytoplasm were features of pulmonary macrophages. In addition, the connective tissue of the pulmonary septa contained immune cells, such as Mast and Eosinophils. In conclusion, the microstructure of the lung of the Nile monitor closely resembles that of other reptile species. However, the distinction between intrapulmonary cartilage palates and pulmonary septa raises the concept of species differentiation. In addition, the discovery of various types of pulmonary immune cells enhances the Nile monitor's ability to persist in a variety of environments by enhancing its pulmonary immunity.

KEYWORDS: Nile monitor, *Varanus niloticus*, Histochemical investigations, Pulmonary trabeculae

1. INTRODUCTION

The Nile monitor, *Varanus niloticus*, is a member of the monitor family (Varanidae) and can be found throughout sub-Saharan Africa. They can be found in countries such as Egypt, Sudan, Kenya, Tanzania, and South Africa. They have also been introduced to parts of Florida in the United States (South African National Biodiversity Institute, n.d.). *Varanus niloticus* are inhibiting aquatic environments and closely associated with water bodies such as rivers, swamps, lakes, and wetlands. They are strong swimmers and are often observed in or near water (Smithsonian National Zoo, n.d.). While Nile monitors have a strong affinity for water, they also inhabit adjacent terrestrial habitats. These include savannas, grasslands, and forests, often near water sources (IUCN Monitor Lizard Specialist Group, n.d.). Despite being semi-aquatic lizards, Nile monitors are skilled swimmers who establish paths, burrow, and reproduce in terrestrial habitats. The semi-aquatic species have adapted their respiratory system to live underwater. They dig their burrows for safety and reproduction, but they will often enter and improve the burrows of other species (Pernetta, 2009).

The lungs of the lizard are simple hollow sacs. They receive bronchial branches, which arise from the trachea. The bronchi open into the lungs without bronchioles. The lungs contain internal folds lined with faveoli (small sacs) for an increased surface area. A few sizable septae further divide the lungs into interconnected chambers in more highly developed lizards. There are species differences in the structure of the lung among lizards. Monitor lungs are multichambered with bronchioles that

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extend to form a faveolus terminal. While the lungs of chameleons are hollow, the edges of their lungs have finger-like extensions that can swell their bodies when responding to predators. Certain species of chameleons have an accessory lung lobe that extends from the anterior trachea cranial to the forelimbs (Divers & Mader, 2005).

The Nile monitor, or Varanus niloticus, belongs to the family Varanidae (Dowell et al., 2016; Nussbaum, 2002). The Nile monitor is considered a semi-aquatic lizard. The semi-aquatic species has the respiratory adaptation to live underwater (Quaglietta, 2018). The current study aims to investigate the microscopic features of the lung of the Nile monitor (*Varanus niloticus*) using a semi-thin technique. The obtained results are important as they provide basic knowledge that can contribute to our better understanding of the lung structure and habits of that species.

2. MATERIALS AND METHODS

Ethical approval

IAACUC-KSU-2022-27 is the approval number from Kafr Elsheikh University's Institutional Aquatic Animal Care and Use Committee, which oversaw the procedures of this study.

Sample Collection

Nile monitors (*Varanus niloticus)* obtained from the animal market in Cairo. Three adult male Nile monitors were kept in controlled conditions for two days in the animal facilities of Egypt's South Valley University's (SVU) Histology Department laboratory. On the third day, a trained veterinary professional 60–120 mg/kg of sodium pentobarbital intraperitoneally (Shaker & Ibrahium, 2021; Abd-Elhafeez, *et al.*, 2023).

All the animals appeared healthy based on how healthy they looked. The snout-to-vent length was about 38 cm, and the weight was about 10±2 kg. Three Nile monitors were used to obtain samples, which were then processed for histological examination using semi-thin sections (Moustafa *et al*., 2013). samples from different regions of the lung were extracted and washed using saline (Sodium chloride; NaCl) and then fixed at 4°C overnight. Representative samples (2.0–3.0 mm length) will be fixed in Worbel- Moustafa fixative for 24 h (Abd-Elhafeez *et al.*, 2017a; Abd-Elhafeez *et al*., 2017b).

The procedures for the semi-thin section, according to Soliman *et al.* (2022), were achieved as follows: The samples were washed four times for 15 minutes with a 0.1M sodium phosphate buffer (pH 7.2) before being fixed for 2 hours at 4°C with 1% osmic acid in a 0.1M sodium phosphate buffer. The samples were washed three times for 20 minutes with 0.1M phosphate buffer (pH 7.2) before being dried with increasing concentrations of ethanol: 50% for 30 minutes, 70% overnight, 90% for 30 minutes, 100% I for 30 minutes, and 100% II for 60 minutes. The dried samples were set in resin (Epon–Araldite) in the following way: propylene oxide (Merck, Darmstadt, Germany) for 30 minutes, Epon–propylene oxide (ratio 1:1) for 30 minutes, and Epon for 3 hours. In an incubator-shaker set to 60°C, 5 mL of Epon812 (Polysciences, 3Eppelheim, Germany) was completely mixed with 5 mL of Araldite and 12 mL of dodecenylsuccinic anhydride (DSAA). This mixture formed the Epon resin. 50% of the samples were polymerized with the help of the mixture and an activator called 2,4,6-Tris (dimethylaminomethyl) phenol, 1.5% (DMP30). The blocks were kept in an incubator for 3 days at 60°C on day 1, 70°C on day 2, and 75°C on day 3. Toluidine blue was used to stain semithin (1 µm) slices cut with an Ultracut E ultramicrotome (Reichert, Leica, Germany). Toluidine blue was prepared as follows: 1g sodium tetraborate (borax), 1g Toluidine blue, and 100 ml DW.

Additional specimens embedded in resin were utilized for histochemical investigations. Resin was dissolved by treating resin sections for 15 minutes with a saturated alcoholic solution of sodium hydroxide (Abd-Elhafeez & Soliman, 2017b; Lloyd, 2001).

The semithin sections were stained with Hematoxylin-Eosin - (H&E) for general histological examinations. In addition, the PAS stain was used goblet cells and the basement membrane. The staining procedures were used based on Bancroft et al., (Suvarna et al., 2018) the Lietz Dialux 20 Microscope. Using a Canon digital camera (Candison Powershot A95), photographs were captured.

3. RESULTS

The general architecture of the lung of the Nile monitor

The intrapulmonary airways originate from the terminal portion of the bronchus, which extends into the lung to form the bronchial tree within the lung parenchyma (Figures 1A-C). In the bronchial epithelium, three cell categories were identified: ciliated cells, non-ciliated cells, and cartilage platelets help support the bronchus. The cartilage matrix was metachromatic with toluidine blue (Figure. 2A) and positively stained with PAS (Figure. 2 B). Some large pulmonary bronchi were additionally supported by cartilage platelets and lined with ciliated epithelium. Contractile trabeculae containing bundles of smooth muscle cells and a pseudostratified bronchus-type epithelium surround the central lumen. This type of epithelium is composed of ciliated and non-ciliated cells. The central lumen provides access to several generations of bronchi and bronchioles. Separating the adjacent faveoli is a pulmonary septum or trabeculae. The serous membrane of the pleura covers thelungs(Figure1B,C).

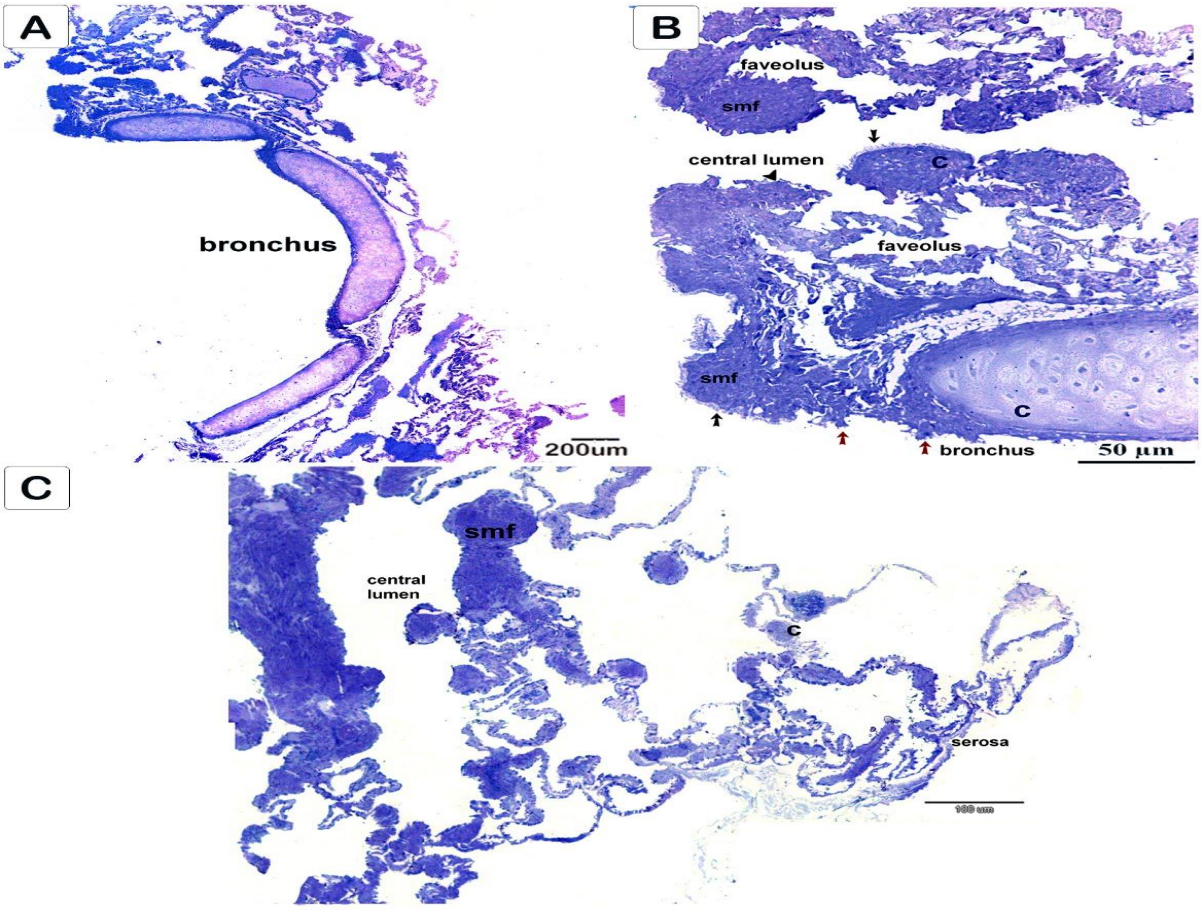

Figure 1: General Architecture of the Lung of the Nile Monitor. Semithin sections stained with toluidine blue. The intrapulmonary airways consist of a central lumen that was continuous with the bronchus. The bronchial epithelium had cytoplasmic projections (A). The bronchus is supported by cartilage platelets (C). Some large pulmonary trabeculae were also supported by small cartilage platelets (C) and are covered by ciliated epithelium. The central lumen is bounded by contractile trabeculae, which contain bundles of smooth muscle fibers (smf) and are covered by a pseudostratified bronchus-type epithelium. This type of epithelium is composed of ciliated and non-ciliated cells. The central lumen opens into the foveoli. Adjacent faveoli are separated by a pulmonary septum, or trabeculae. The lung is covered by serosa.

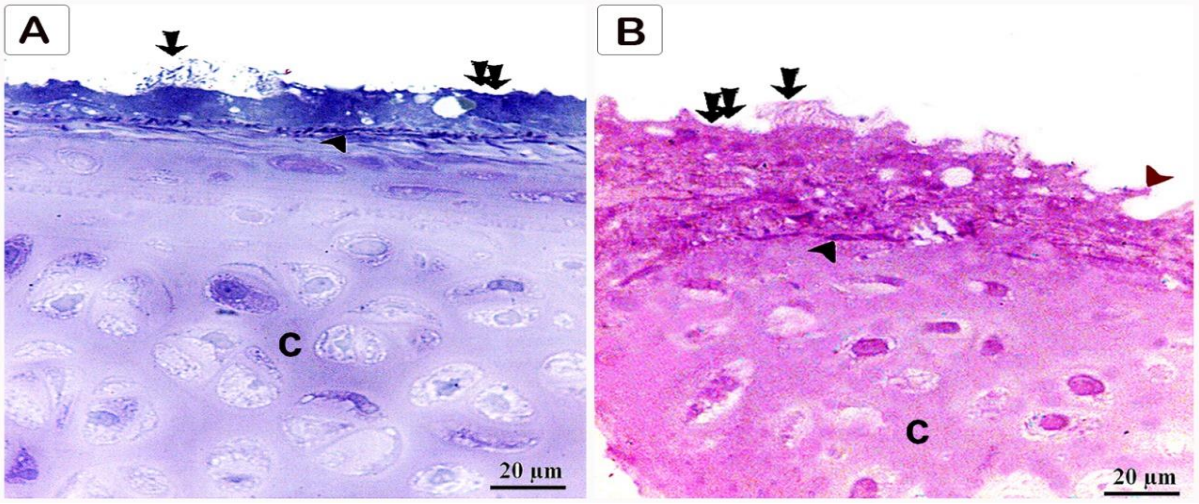

Figure 2: Microscopic Features of the Bronchial Wall, Semithin sections stained by methylene blue (A) and PAS (B) The bronchus is supported by cartilage platelets (C). The cartilage matrix displays metachromatic characteristics when stained with methylene blue and by PAS. The bronchial epithelium consisted of ciliated cells (arrows), and non-ciliated cells (double arrows) had cytoplasmic projections (red arrowhead). Note that the basal lamina contained collagen fibers (black arrowheads).

The Microscopic Features of the Contractile and Pulmonary Trabeculae

Using semithin sections stained with toluidine blue, the microscopic characteristics of contractile and pulmonary trabeculae were examined. The contractile trabeculae consisted of an inner core of smooth muscle bundles covered by an epithelium of pseudostratified bronchus type. This variety of epithelium is made up of both ciliated and non-ciliated cells (Figure 3A, B). A pulmonary septum, or trabeculae, separates adjacent faveoli. The pulmonary trabeculae are covered by various types of cells, including type I pneumocytes, type II pneumocytes, and vacuolated pulmonary macrophages located within the favolar space. There are blood capillaries adjacent to the pulmonary epithelium (Fig. 3C, Fig. 4A, B). Mast cells with metachromatic granules were identified in the stroma of the pulmonary septa using semithin sections stained with methylene blue. Serous pleura covered the outer surface of the lung (Fig. 4C). Several types of immune cells were detected in the pulmonary septa and pulmonary spaces such as the macrophage which has granular cytoplasm that contained phagocytic inclusions(Fig. 5).

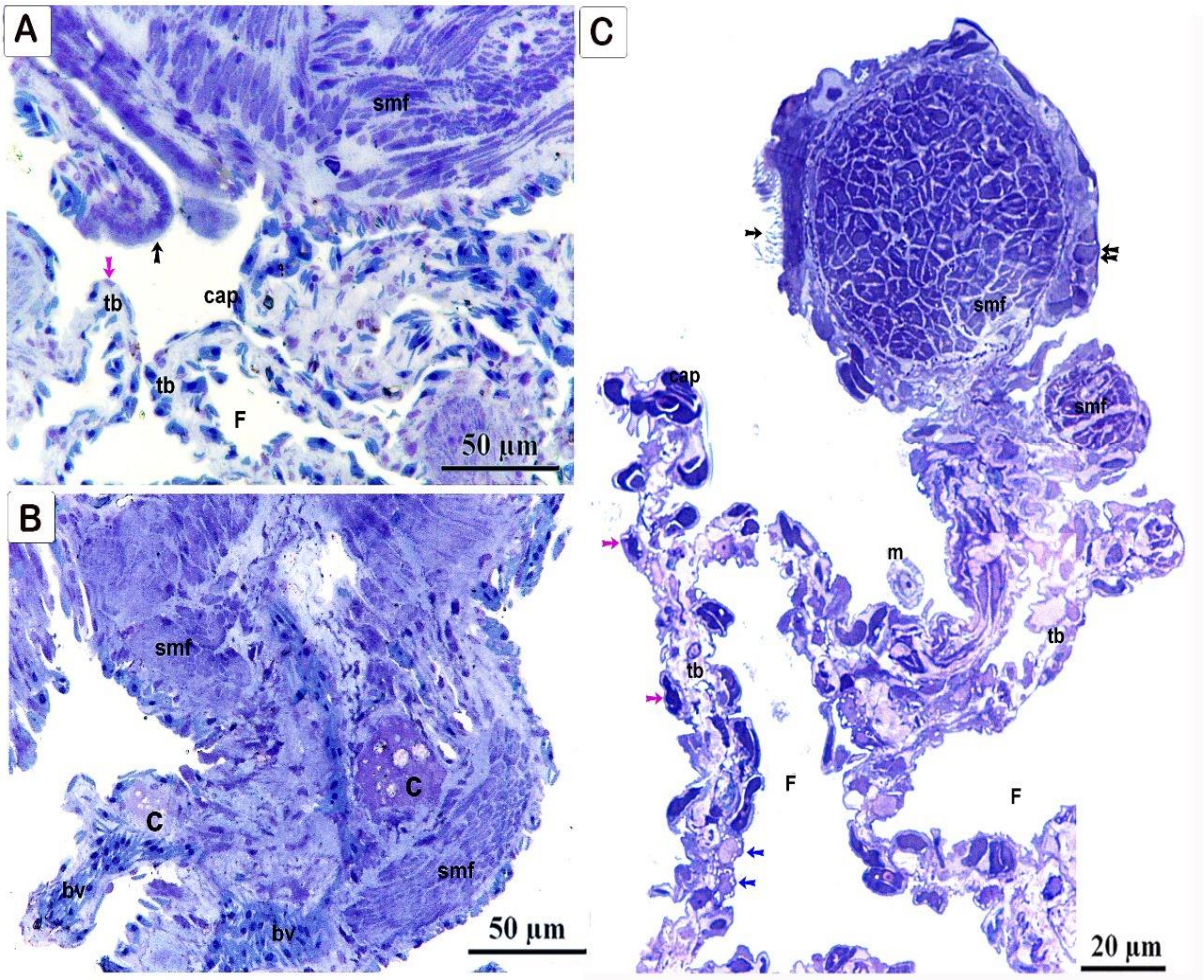

Figure 3: Microscopic Features of the Contractile and Pulmonary Trabeculae, Using toluidine blue Semithin sections were stained with toluidine blue. A pseudostratified bronchus-type epithelium covered the contractile trabeculae, which contains bundles of smooth muscle cells. This type of epithelium contains ciliated (black arrows) and non-ciliated cells (double arrows). Small cartilage platelets (C) also support some sizable contractile trabeculae. A pulmonary septum, or trabeculae (tb), separates the adjacent faveoli (F). Different cell types, such as type I pneumocytes (pink arrows), type II pneumocytes (blue arrows), and pulmonary macrophages (m) that are present in the foveolar space and have vacuolated cytoplasm, cover the pulmonary trabeculae. Note Capillaries (cap).

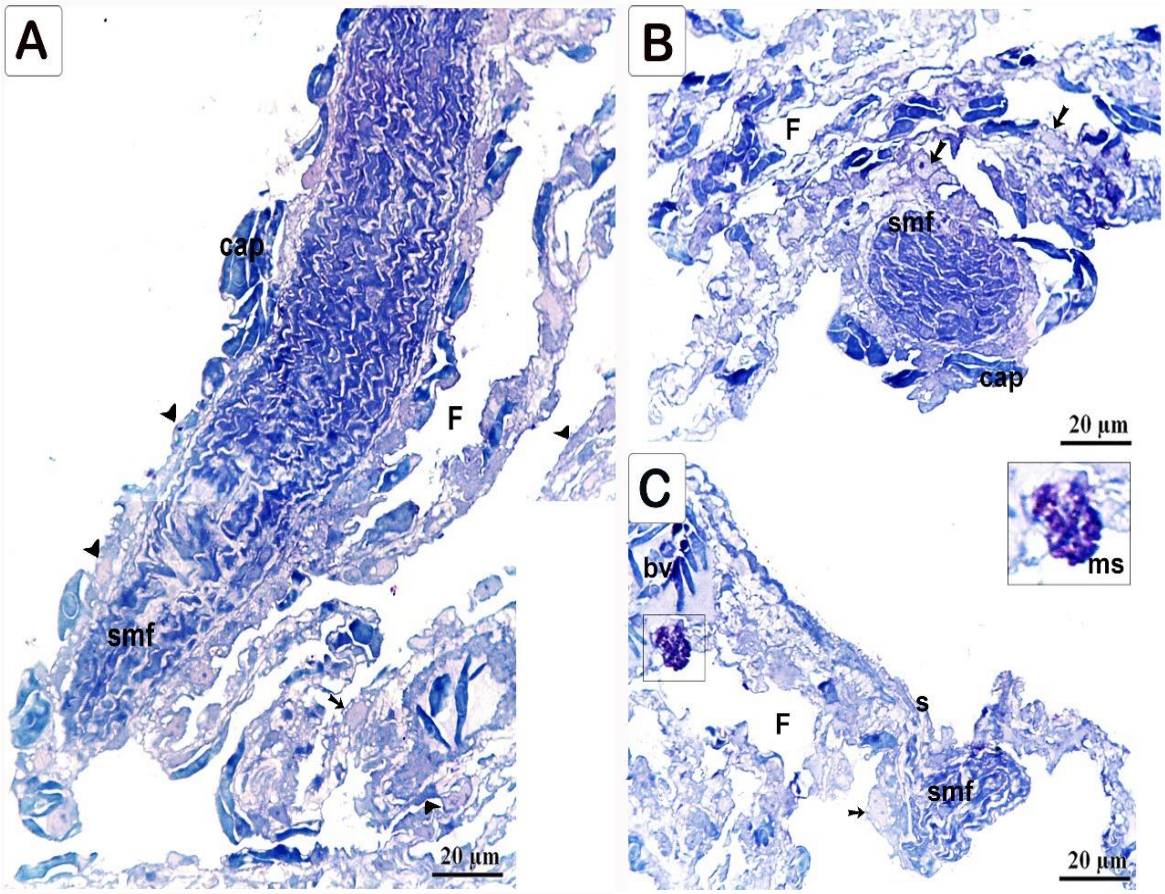

Figure 4: Microscopic Features of the Contractile and Pulmonary Trabeculae Using methylene blue. The contractile trabeculae contain bundles of smooth muscle cells (smf) and are covered by type I pneumocytes (arrowheads) and capillaries (cap). Note faveoli, type II pneumocytes (arrows). The Serosa (s) type is present. The pulmonary septa contained *mast cells (ms)*, which has metachromatic granules.

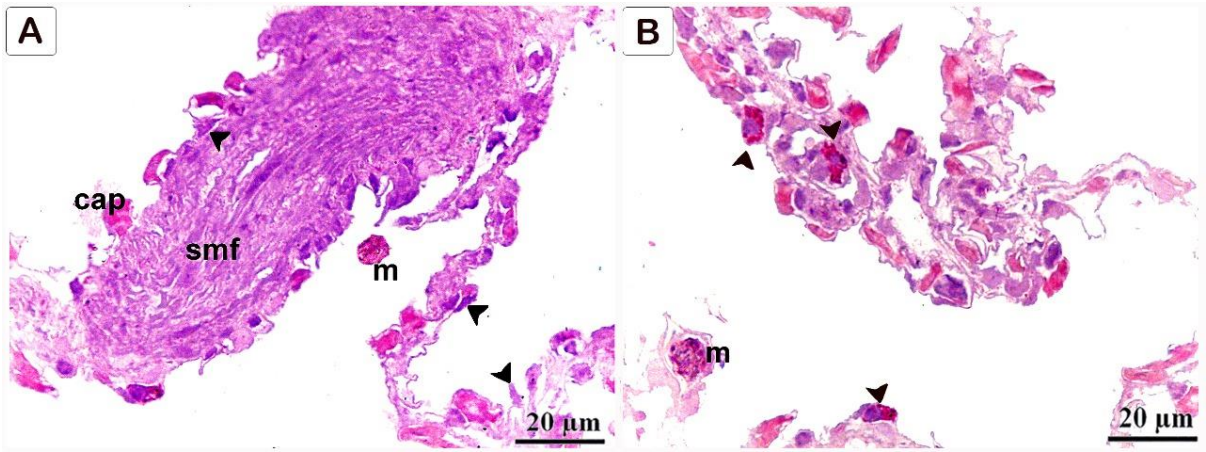

Figure 5: Microscopic Features of the Contractile and Pulmonary Trabeculae
Using H&E, Semithin sections were stained by H&E. The contractile trabeculae which contain bundles of smooth muscle cells (smf) and are covered by type I pneumocytes (black arrowheads) and capillaries (cap). Several types of immune cells were detected in the pulmonary septa and pulmonary spaces such as the macrophage (m) which has granular cytoplasm that contained phagocytic inclusions.

4. DISCUSSION

This study examined the microscopic characteristics of the lungs of the Nile monitor (*Varanus niloticus*). Intrapulmonary conducting airways and respiratory faveoli make up the lungs. Intrapulmonary airways originate from the terminal portion of the bronchus and extend into the lung to form the central lumen. The bronchus was lined with pseudostratified ciliated epithelium composed of ciliated and non-ciliated cells and is supported by platelets of hyaline cartilage. Contractile trabeculae containing bundles of smooth muscle cells and ciliated and non-ciliated cells surround the central lumen. The central lumen provides access to the foveoli. Separating adjacent faveoli is a pulmonary septum or

trabeculae lined with various cell types, including type I pneumocytes, type II pneumocytes, and pulmonary macrophages. Some large pulmonary trabeculae were additionally supported by cartilage platelets and were covered by ciliated epithelium. Pneumocytes of type I were flat cells, whereas type II pneumocytes were cuboidal and had vacuolated cytoplasm. The surface of pulmonary macrophages was irregular, and their cytoplasm was vacuolated. In addition, immune cells, such as mast cells and eosinophils, were present in the connective tissue of the pulmonary septa.

The pulmonary epithelium of all Iguana consists of ciliated cells, type I and type II pneumocytes. Type I pneumocytes have a squamous morphology and are involved in gas exchange, whereas type II pneumocytes are cuboidal cells with lamellar bodies that secrete pulmonary surfactant. The air-blood barrier is made up of type I pneumocytes, a basement membrane, and vascular endothelial cells (Peixoto *et al.*, 2018).

In *Rhacodactylus leachianus* (Reptilia: Gekkonidae), type I pneumocytes are the respiratory capillaries that form the air–blood barrier, whereas type II lack lamellar bodies (Perry *et al.*, 1989).

In contrast to the cellular structure of Rainbow water snake (*Enhydris enhydris*) lungs, alveolar ducts, alveolar sacs, and alveoli constitute the respiratory airways. Type 1 alveoli epithelial cells, type 2 alveoli cells, muscle bundles, connective tissue, capillary vessels, and macrophage cells line the latter. (Zainuddin *et al.*, 2020).

Thus, the microstructure of the lung of the Rainbow water snake is more similar to that of mammals than that of reptiles. The respiratory epithelium of the lung of the toad, Melanophryniscus stelzneri, comprises a type of pneumocyte that exhibits the characteristics of both type I and type II alveolar cells in mammals. Pneumocytes have an irregular shape and cytoplasmic processes. The cytoplasm includes lamellar bodies and their precursors, dense bodies, and multivesicular bodies. In addition, neuroepithelial structures are present over the septa (Hermida *et al.*, 2003)

The respiratory structure of the Nile monitor resembles that of nonavian sauropsid amniotes (reptiles). The sauropsid lung contains a central pulmonary lumen and a series of subunits called faveoli (ediculae). Separating the faveoli are pulmonary septae (Ruben *et al.*, 1997).

Nile monitors have well-developed lungs that allow them to breathe air. They are obligate air breathers, meaning they must come to the water's surface to take in oxygen. When submerged, they hold their breath, and a build-up of carbon dioxide triggers their need to surface and breathe

It is important to note that while Nile monitors possess certain adaptations for their semi-aquatic lifestyle, they still primarily rely on air-breathing and must regularly return to the water's surface to breathe. Their time spent underwater is limited compared to fully aquatic species.

CONCLUSION

In conclusion, the microstructure of the lung of the Nile monitor is closely related to that of other reptilian species. The distinction of intrapulmonary cartilage palates within the pulmonary septa, however, raises the concept of species differentiation. In addition, the discovery of diverse forms of pulmonary immune cells enhances the Nile monitor's ability to adapt to various environments.

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