Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Features of lung morphology in the grass frog (*Rana temporaria*)

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Article info

Received 01.03.2026
Received in revised form 30.03.2026
Accepted 02.05.2026

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Horalskyi, L. P., Tsanko, I. V., Romaniuk, R. K., Gutyj, B. V., Tsekhmistrenko, S. I., & Dmytruk, V. S. (2026). Features of lung morphology in the grass frog (Rana temporaria). Regulatory Mechanisms in Biosystems, 17(3), e26061. doi:10.15421/0226061

One of the essential functions of the organism is to provide cells and tissues with oxygen and nutrients and to remove metabolic products, which is ensured by the respiratory organs. The present study reports the results concerning the morphotopography and morphological structure of the lungs of the grass frog (*Rana temporaria familiaris* Linnaeus, 1758). The lungs of the frog are located in the cranial part of the body cavity, medially adjacent to the esophagus and ventrally bordering the right and left atria of the heart. Laterally, the right lung is adjacent to the right lobe of the liver, whereas the left lung borders the cardiac part of the stomach. The lungs are paired organs of dark pink color formed by the right and left lungs, representing elongated, oval-shaped sac-like structures. Their absolute mass is 0.12 g, and the relative mass is 0.18%. According to the lung development index, which averages 42% (right lung – 43.1%, left lung – 41.8%), the lungs of the grass frog belong to the expanded-shortened type. The lung walls are formed by three layers: the outer layer, consisting of a thin layer of simple squamous epithelium and a layer of loose connective tissue; the middle layer, formed by dense regular connective tissue containing reticular and collagen fibers; the inner layer, lined with simple single-layered epithelium under which there is a layer of loose connective tissue. The walls of the right and left lungs form folds and villous projections that branch into the depth of the organ cavity, thereby increasing the respiratory surface of the lungs. The volume of the right lung is 216 mm³, while the volume of its cavity is 157 mm³; the ratio of the cavity volume to the total lung volume is 0.73%. In the left lung, the morphometric parameters are similar: the lung volume is 199 mm³, the cavity volume is 147 mm³, and the ratio of the cavity volume to the total lung volume is 0.41%, which indicates the absence of pronounced asymmetry between the left and right lungs.

Keywords: vertebrates; phylogeny; amphibians; respiratory organs; morphometry.

Introduction

In biology, the broadest concept encompassing the fauna and animal population of a particular territory (or aquatic area) is the animal world, which represents one of the most important components of the natural environment and an important object of research in modern zoology. During the historical development (phylogeny) of the animal world, including the evolutionary pathways of individual species and taxonomic groups of organisms that reflect their common origin and evolutionary relationships, living organisms undergo evolutionary transformations. This process has led to the emergence of the modern diversity of animals, ranging from the simplest to the most complex forms of life.

Evolutionary changes in animals during their historical development are directly correlated with the environmental conditions in which they live, as well as with the structural features of the organism itself, determined by its previous phylogenetic history.

An important direction of zoogeographical research aimed at characterizing the taxonomic composition of fauna within a specific region is faunistics. Therefore, the study of environmental factors affecting fauna and their changes over time is of considerable scientific importance (Liang et al., 2019; Itsukushima & Kano, 2024). Adapting to particular environmental conditions, representatives of vertebrate animals, including the grass frog, undergo adaptive changes that enable them to respond to environmental influences. These changes directly affect the morphofunctional state of the organism and involve all levels of structural and functional organization, including subcellular, cellular, tissue, organ, and population levels (Channing & Willems, 2018; Southwell et al., 2018).

Adapting to environmental conditions in terrestrial or aquatic habitats, living organisms require coordinated and efficient functioning of all body systems, including the respiratory organs. In morphofunctional terms, these organs are closely interconnected and ensure vital physiological processes. The most important of these processes is gas exchange, which involves the exchange of gases between the organism and the environment through inhalation of atmospheric oxygen and the release of carbon dioxide produced during metabolic processes (Davies & Moores, 2010; Maina, 2014; Horalskyi et al., 2025). Therefore, an important task in modern biology is the study of the structural and functional characteristics of the respiratory organs from comparative phylogenetic and species-specific perspectives, as well as under the influence of anthropogenic environmental factors. This served as the basis for the aim of the present study.

Accordingly, we investigated the morphology of the lungs and performed histological and cytomorphometric evaluation of their structural components in the grass frog as one of the representatives of vertebrate animals.

Materials and methods

Experimental studies were conducted at the Department of Zoology, Biological Monitoring and Nature Protection, Faculty of Natural Sciences, Zhytomyr Ivan Franko State University, within the framework of the research project "Animals of Artificial and Natural Ecosystems of Ukraine" (state registration number 0122U002270). The conducted studies complied with the requirements of the Good Laboratory Practice (GLP, 1981) guidelines and the provisions of the General Ethical Principles of Animal Experiments, approved by the First National Congress on Bioethics (Kyiv, 2001).

The research object was the lungs of the grass frog (n = 5) belonging to: Class Amphibia, Family Ranidae, Species *Rana temporaria familiaris* Linnaeus, 1758. The grass frog is an amphibious animal and one of the representatives of the genus brown frogs. It is a medium-sized species with morphology, lifestyle, and behavior typical of frogs. Macroscopic, microscopic, morphometric, and statistical methods were used in the study.

Clinical examination of animals, evaluation of exterior parameters (external appearance and body weight) and interior parameters (linear measurements, absolute and relative organ weight) after anatomical dissection were performed according to the recommendations of batrachological (herpetological) and morphological manuals (Horalskyi et al., 2019). To prevent the negative effects of stress factors, frogs were anesthetized prior to dissection using a Hypnodil solution (5–10 ml/L).

Body weight was determined using VTD–3/0.1FD "Dneproves" scales with an accuracy of 0.1 g.

The absolute mass of the lungs (and their structural elements) was determined using electronic scales. The relative organ weight (RW) was calculated using the following formula: $RW = AM/BM \times 100\%$, where AM – absolute organ mass, BM – body mass of the animal.

Linear parameters of the organ (length, width, and thickness) were measured directly using a vernier caliper.

The organ development index was determined as the ratio of the total length to the width using the following formula: $ODI = L/W \times 100$, where L – organ length, W – organ width.

Histological studies were performed using generally accepted methods of fixation and preparation of histological sections (Horalskyi et al., 2019). Selected lung tissue samples were fixed in 12% cooled neutral formalin followed by paraffin embedding according to the protocols described by Horalskyi et al. (2019).

Paraffin sections were prepared using a sliding microtome MS-2. The thickness of histological sections did not exceed 10–12 μm.

For histological examination of cells and tissues, the prepared sections were deparaffinized and stained with hematoxylin (Diapath, Italy, 2020) and eosin (Leica Geosystems, Germany, 2020).

Histometric measurements of lung structural elements were performed using light microscopy with Micros microscopes (Micros, Austria, 2012) according to the recommendations described by Horalskyi et al. (2019).

The volume of the lungs and their cavities was calculated using the following formula: $V = \pi/6 \times A \times B2$, where V – lung (or cavity) volume, π – 3.14, A – length, B – width.

The ratio of the cavity volume to the total lung volume was determined using the formula: $VcVl \times 100$, where Vc – cavity volume of the lungs, Vl – total lung volume.

Photographs of the microscopic structure of the lungs were taken using a CAM V-200 digital camera (InterMed, China, 2017) mounted on a microscope. All macroscopic images of the lungs were obtained by the authors during anatomical dissection, whereas microscopic images were obtained from prepared permanent histological slides after hematoxylin and eosin staining.

Morphological terminology of lung structural components was used in accordance with the International Anatomical and International Veterinary Histological Nomenclature.

Statistical analysis of numerical data was performed using Statistica 7.0 software (StatSoft, Tulsa, USA). Differences between values were determined using ANOVA, and differences were considered statistically significant at $P < 0.05$, taking into account the Bonferroni correction.

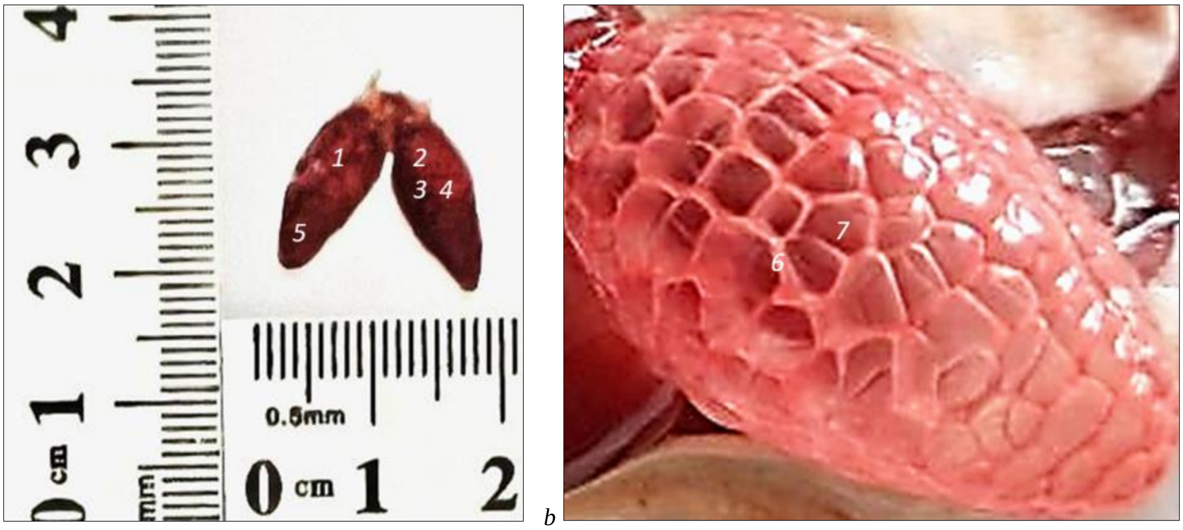
Results

The lungs of the frog are paired respiratory organs of dark pink color that ensure gas exchange. Topographically, the lungs are located in the cranial part of the body cavity (Fig. 1). Medially, the lungs are adjacent to the esophagus, while ventrally they contact and border the right and left atria of the heart.

Laterally, the right lung is adjacent to the right lobe of the liver, whereas the left lung borders the cardiac part of the stomach (Fig. 1).

The right and left lungs represent paired thin-walled, elongated, alveolar sac-like structures with a large internal volume (Fig. 1). In shape, they are somewhat narrowed at their base. In the middle part, each lung expands, forming cavity-like structures (reservoirs) with a large internal lumen. Caudally, each lung gradually narrows and ends with a pointed apex directed dorsally toward the body (Fig. 2).

Fig. 1. Dissection of a female grass frog: *1* – heart; *2* – right lung; *3* – left lung; *4* – lung in the state of inspiration; *5* – lung in the state of expiration; *6* – left lobe of the liver; *7* – right lobe of the liver; *8* – gallbladder in the central lobe of the liver; *9* – stomach; gross specimen



a *b*

Fig. 2. Macroscopic structure (*a*) and alveolar structure (*b*) of the lungs of a female grass frog (*Rana temporaria*): *1* – right lung; *2* – left lung; *3* – lung base; *4* – middle part of the lungs; *5* – lung apex; *6* – lung in the form of a thin-walled air-filled sac; *7* – alveolar cells; gross specimen

When filled with air, the lungs increase in size, and their surface acquires an alveolar (honeycomb-like) appearance (Fig. 2), resembling the swim bladder of bony fishes, which performs hydrostatic functions.

According to the results of organometric studies, the absolute mass of the lungs of the grass frog is 0.12 ± 0.02 g, while the relative mass is 0.18 ± 0.04%. The absolute mass of the right lung is 0.061 ± 0.004 g (53.81 ± 8.68%), whereas the left lung weighs 0.059 ± 0.018 g (46.19 ± 8.5%). Thus, the absolute mass of the right lung is 1.03 times greater than that of the left lung (Table 1).

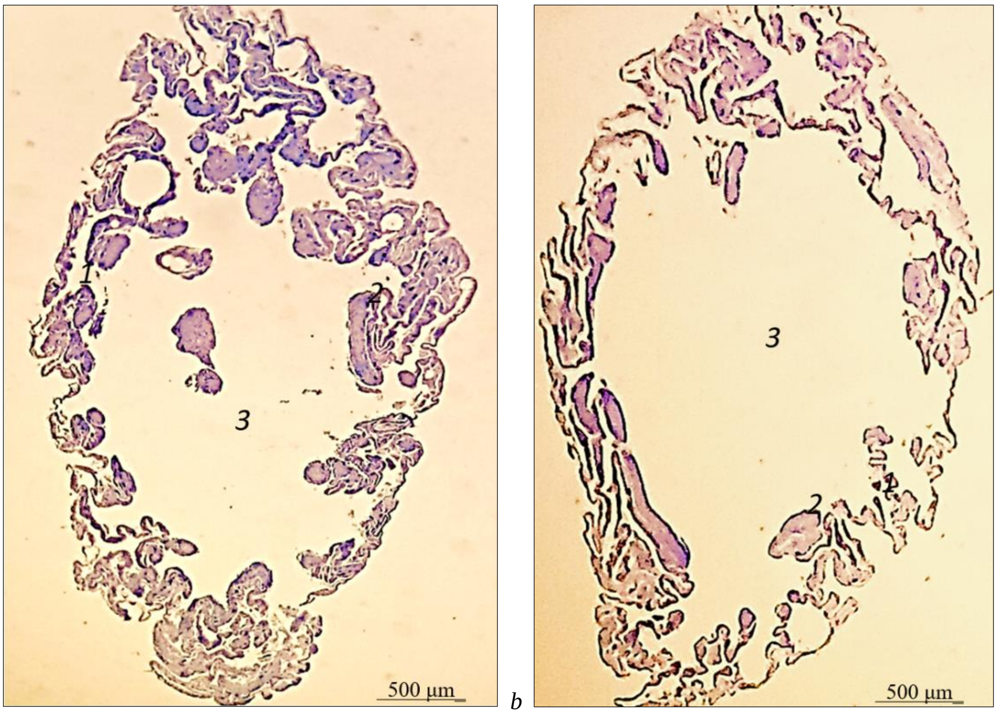
The microscopic structure of the lungs of the grass frog in longitudinal section has an oval-elongated shape, with a lung cavity of a corresponding shape located inside the organ (Fig. 3). According to the results of histological studies, the lungs of the studied animals have a relatively small gas-exchange surface. However, due to the folded structure of the lung wall, the respiratory surface area available for gas exchange increases.

Microscopically, the lung wall of the grass frog consists of three layers: the inner (mucosal) layer, the middle (fibrous connective tissue) layer, and the outer (serous, pleural) layer (Fig. 4).

Table 1
Morphometry of structural components of the lungs of the grass frog (x ± SD, n = 5)

Morphometric parameters	Indicators	
	absolute mass, g	relative mass, %
Right and left lungs	0.12 ± 0.02	0.18 ± 0.04
Right lung	0.061 ± 0.004	53.81 ± 8.68
Left lung	0.059 ± 0.018	46.19 ± 8.50

The mucosal layer is formed by an epithelial plate composed of simple squamous epithelium. The apical surface of the epithelial cells is in direct contact with the external environment, namely with the lung cavity (Fig. 4). Beneath the epithelium lies the lamina propria, which consists of a delicate layer of loose connective tissue containing vessels of the hemomicrocirculatory bed.



a *b*

Fig. 3. Longitudinal section of the microscopic structure of the lungs of the grass frog (*Rana temporaria*): *a* – left lung; *b* – right lung; *1* – lung wall; *2* – folds of the lung wall; *3* – lung cavity; hematoxylin and eosin staining

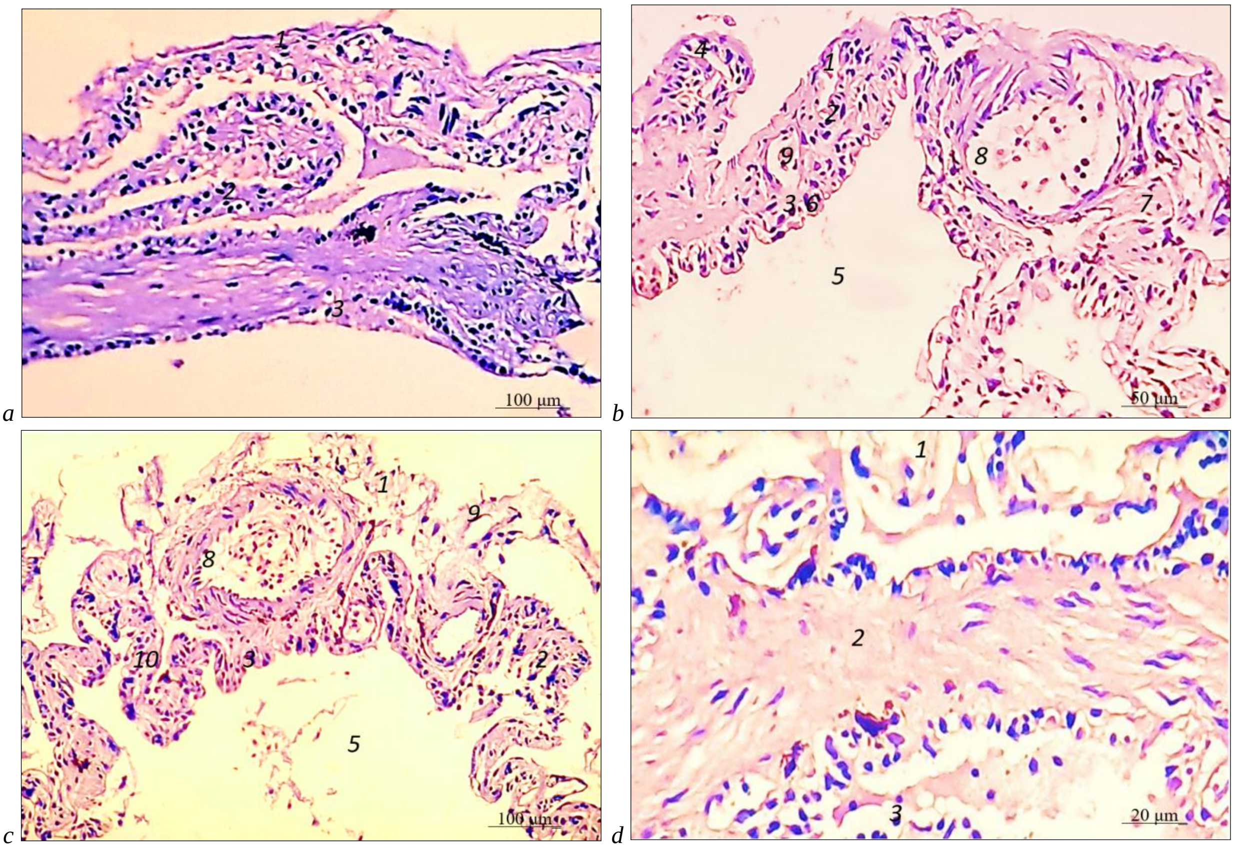


Fig. 4. Fragment of the microscopic structure of the frog lung wall (*a*, *b*, *c*, *d*): *1* – outer (serous) layer of the lung wall; *2* – middle (fibrous connective tissue) layer; *3* – inner (mucosal) layer; *4* – epithelial layer of the outer membrane; *5* – lung cavity; *6* – epithelial layer of the inner membrane; *7* – connective tissue; *8* – artery; *9* – capillary; *10* – fold of the lung wall; hematoxylin and eosin staining

Adjacent to the outer layer is the middle connective tissue layer. The latter is formed by dense regular connective tissue, which consists of collagen and reticular fibers closely arranged to each other. A small number of fibrocytes are observed between the fibers, whose nuclei have an elongated rod-like shape (Fig. 5). Due to this structural organization, the lung wall acquires strength and elasticity, which are necessary to ensure effective gas exchange during respiration.

The outer (serous) layer is formed by a thin layer of loose connective tissue covered with simple squamous epithelium, the cells of which lie on the basement membrane and have an elongated uniform shape, while their nuclei are located at approximately the same level (Fig. 5). Within the layer of loose connective tissue that contacts the fibrous connective tissue layer, a network of microcirculatory vessels is observed, including arterioles, venules, and capillaries (Fig. 5), as well as arteries and veins whose branches extend longitudinally and conduct arterial and venous blood within the pulmonary circulation.

According to histometric studies, the length of the right lung in the grass frog is 13.08 ± 0.51 mm, the width is 5.64 ± 0.32 mm, and the thickness is 3.40 ± 0.48 mm. With these linear parameters, the volume of the right lung is 216.34 ± 5.17 mm³, and the volume of its cavity is 157.16 ± 6.39 mm³. The ratio of the cavity volume to the total lung volume is 0.730 ± 0.0021% (Table 2).

The morphometric parameters of the left lung are similar to those of the right lung, although there is a slight tendency toward smaller values. Thus, the length of the left lung is 12.82 ± 0.99 mm, the width is 5.36 ± 0.37 mm, and the thickness is 3.23 ± 0.42 mm. Accordingly, the volume of the left lung is 198.53 ± 7.75 mm³, the volume of its cavity is 147.28 ± 5.38 mm³, and the ratio of the cavity volume to the total lung volume is 0.740 ± 0.036%, indicating the absence of asymmetry in the structure of the lungs of the grass frog (Table 2).

Based on the linear parameters, namely the ratio of lung length to width, the lung development index in the grass frog averages 42%, specifically 43.10 ± 3.06% for the right lung and 41.82 ± 3.37% for the left lung. Therefore, the lungs belong to the expanded – shortened type.

Table 2
Morphometry of structural components of the lungs of the grass frog (x ± SD, n = 5)

Morphometric parameters	Indicators	
	right lung	left lung
Lung length, mm	13.08 ± 0.51[b]	12.82 ± 0.99[a]
Lung width, mm	5.64 ± 0.32[b]	5.36 ± 0.37[a]
Lung thickness, mm	3.40 ± 0.48[b]	3.23 ± 0.42[a]
Lung development index, %	43.10 ± 3.06[b]	41.82 ± 3.37[a]
Lung volume, mm³	216.34 ± 5.17[b]	198.53 ± 7.75[a]
Lung cavity volume, mm³	157.16 ± 6.39[b]	147.28 ± 5.38[a]
Ratio of cavity volume to total lung volume, %	0.730 ± 0.0021[a]	0.740 ± 0.036[a]

The lung wall in the grass frog forms folds (Fig. 5) and villi (Fig. 5) that extend deep into the lung cavity. Due to this histoarchitectural organization, the lung wall has a folded structure that significantly increases the respiratory surface area available for gas exchange. All layers of the lung wall participate in the formation of folds, whereas the middle and inner layers participate in the formation of villi. The lung villi have an elongated finger-like shape, clearly distinguished by a basal region (base) connected to the outer lung layer, an elongated body, and an apical surface of narrowed form that contacts the lung cavity.

Externally, the villi are covered by the mucosal layer, which contributes to their formation through its protrusions. The outer covering of the villi consists of simple epithelium, beneath which lies a delicate layer of loose connective tissue (Fig. 6).

Within the connective tissue layer of the villi, a large number of blood vessels are observed, forming an extensive capillary network where gas exchange occurs, including the diffusion of oxygen from air into the blood and carbon dioxide from the blood into the air. As a result, gas exchange between the animal organism and the external environment is ensured.

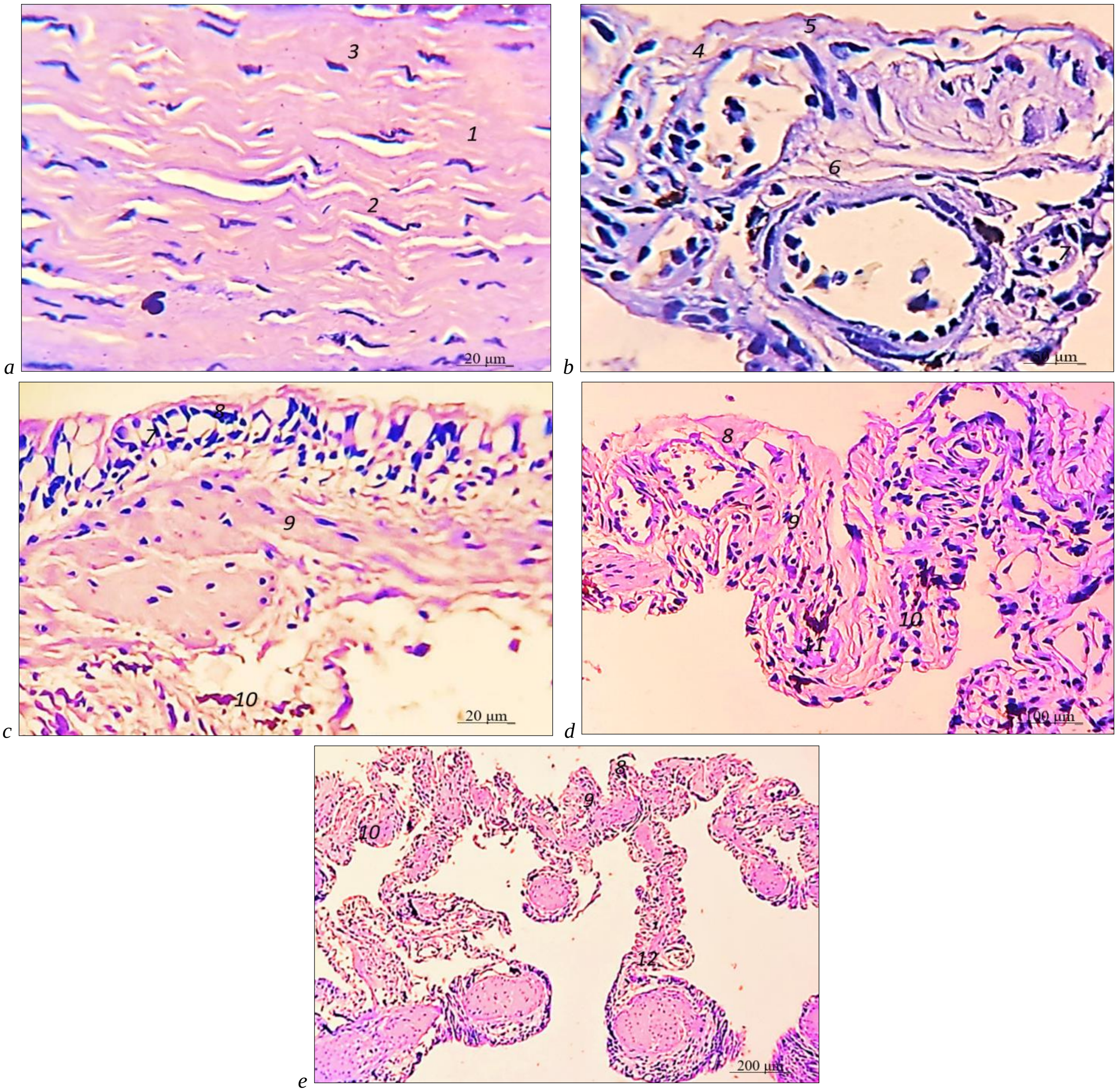


Fig. 5. Microscopic structure of the frog lung wall: *a* – fragment of the microscopic structure of the middle layer; *b*, *c* – fragments of the microscopic structure of the lung wall; *d*, *e* – fragments of the microscopic structure of the frog lung wall: *1* – dense regular connective tissue; *2* – bundles of collagen fibers; *3* – fibrocyte nuclei; *4* – surface of the lung wall; *5* – epithelial layer; *6* – connective tissue; *7* – capillaries; *8* – serous layer of the lung wall; *9* – fibrous connective tissue layer; *10* – mucosal layer; *11* – fold of the lung wall; *12* – villi of the lung wall; hematoxylin and eosin staining

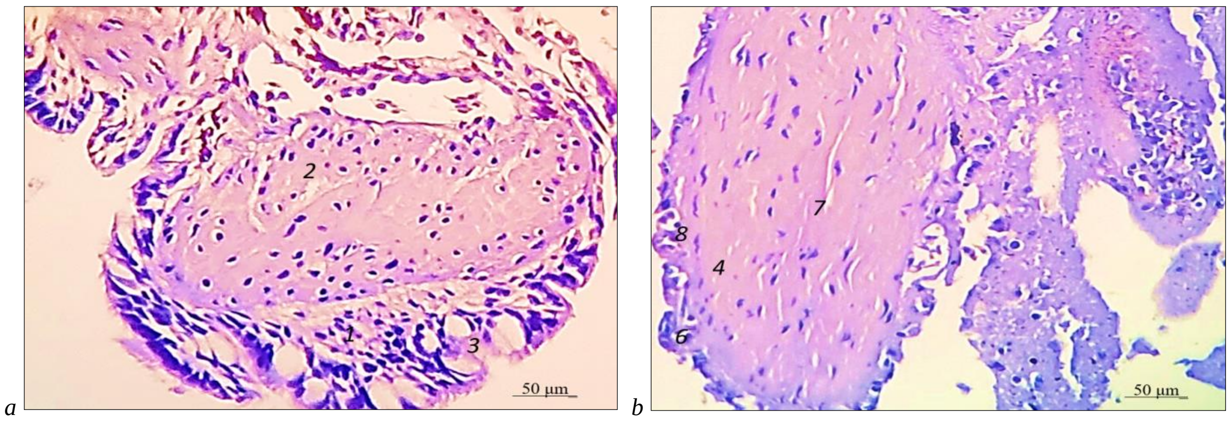


Fig. 6. Fragment of the microscopic structure: *a* – apical surface of a villus of the grass frog; *b* – lung villi of the grass frog: *1* – mucosal layer; *2* – fibrous connective tissue layer; *3* – capillary network; *4* – villus; *5* – villus wall; *6* – epithelial plate of the villus; *7* – dense connective tissue of the villus cavity; *8* – capillaries; hematoxylin and eosin staining

Microscopically, the cavity (body) of the villi, similarly to the middle layer of the lung wall, is composed of dense regular connective tissue, which represents a protrusion of the middle layer of the lung wall and, together with the inner wall layer, participates in the formation of the villi (Fig. 6).

The folds of the lung wall, together with the villi, combine into an integrated structure while maintaining their functional properties and often form hollow conglomerates of round or oval shape (Fig. 7). These structures are separated from one another by septa attached to the outer wall of the lungs. The connective tissue septa located between these morphological structures consist of connective tissue containing collagen and elastic fibers, which provide mechanical strength to the lung walls.

The surface of the walls of such hollow conglomerates borders the lung cavity. Similar to the surface of the inner lung layer, it is formed by a single layer of squamous epithelial cells. These cells are outlined by a clearly defined cell membrane which, after staining with hematoxylin and eosin, forms a thin wavy orange-colored border on the apical surface of the epithelial cells. This border contacts the lung cavity, while basally the epithelial cells are connected with a delicate layer of loose connective tissue.

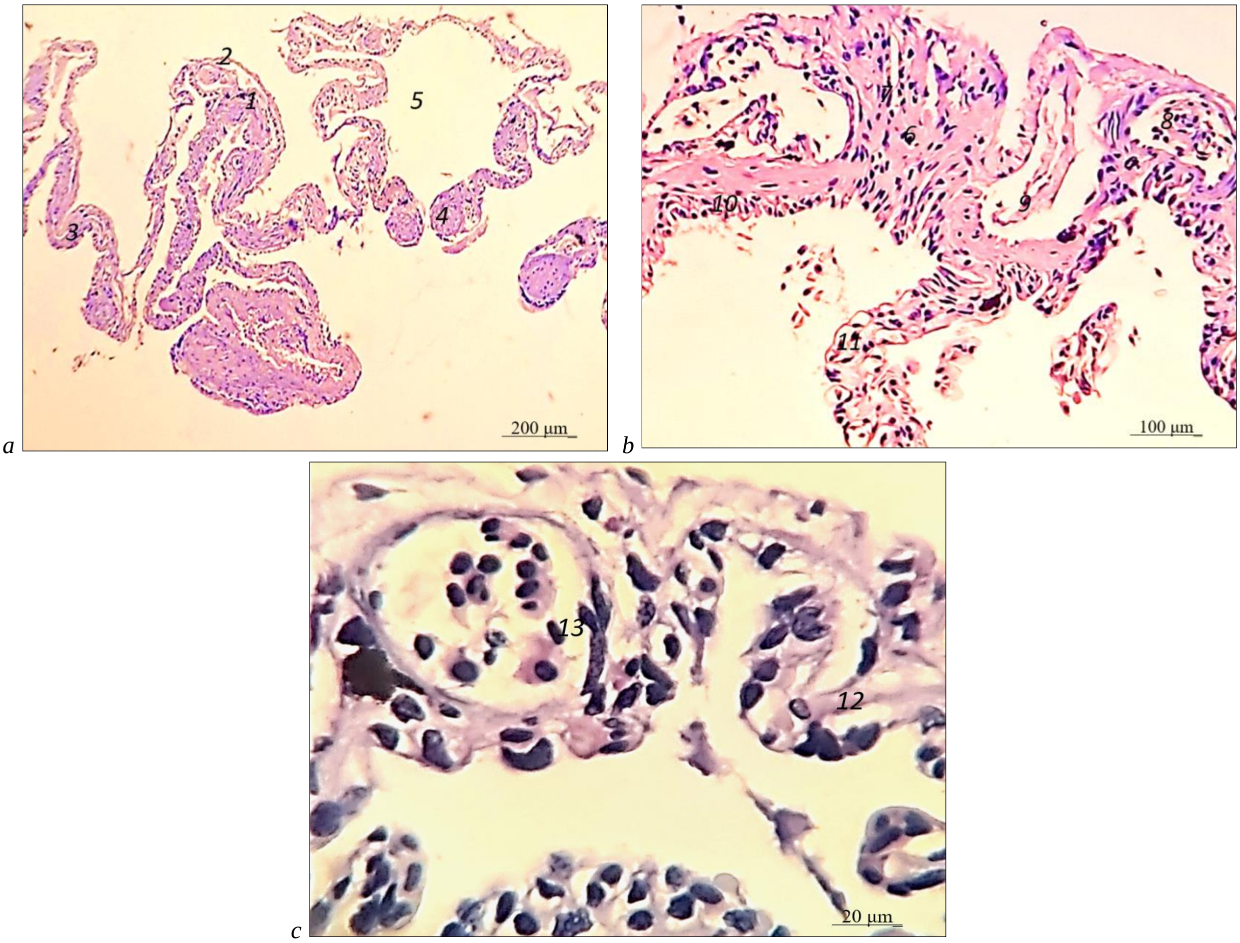


Fig. 7. Fragment of the microscopic structure of the frog lung wall (*a*, *b*, *c*): *1* – outer layer of the lungs; *2* – epithelial layer; *3* – folds of the lung wall; *4* – villus; *5* – hollow conglomerate; *6* – wall of the hollow conglomerates; *7* – connective tissue; *8* – artery; *9* – vein; *10* – epithelial layer of pulmonary structures; *11* – border (apical margin); *12* – surface of the wall of the hollow lung conglomerate; *13* – capillary; hematoxylin and eosin staining

Within the connective tissue, a dense network of blood vessels and capillaries arranged as a branched vascular mesh is observed, which performs the gas exchange function (Fig. 7). Such a structure of the lung wall, formed by a thin layer of loose connective tissue covered by squamous epithelial cells, ensures maximum proximity between inhaled air and the blood vessels. At the same time, the dense capillary network located extremely close to the surface of the hollow structures promotes rapid and efficient gas exchange.

According to the results of our histometric studies, the thickness of the right lung wall in the grass frog is 216.6 ± 4.66 µm. The thickness of the inner (mucosal) layer is 61.2 ± 1.27 µm, the middle (fibrous connective tissue) layer is 106.1 ± 2.91 µm, and the outer (serous) layer is 49.3 ± 1.52 µm (Table 3).

Similar histometric parameters were characteristic of the left lung: wall thickness – 221.2 ± 3.97 µm, inner layer thickness – 63.8 ± 1.36 µm, middle layer – 110.9 ± 3.42 µm, and outer layer – 46.5 ± 2.01 µm. The absence of significant differences in the histometric parameters between the left and right lungs confirms the absence of asymmetry in the lung structure of the grass frog.

With such histometric dimensions of the lung wall, its structural elements (folds and villi, which give the wall an uneven relief) have different sizes. The folds of the right lung have a leaf-like shape, with a width of 468.9 ± 9.95 µm and a length of 703.1 ± 20.86 µm. The villi, in turn, have a greater length (1339.4 ± 21.12 µm) and a smaller width (342.7 ± 10.2 µm) compared with the folds. Similar values for the linear parameters of folds and villi were observed in the left lung (Table 3). Thus, gas exchange in the grass frog occurs between air and blood within the hollow lung structures and on the surface of the lung walls where the vascular network is located. The efficiency of gas exchange depends on the total surface area of the lungs.

Due to the unique structural organization of the lung wall, formed during phylogenetic development (including a well-developed arterial and venous vascular system and a complex network of hemomicrocirculatory vessels and capillaries) oxygen diffuses from air into the blood, while carbon dioxide diffuses from the blood into the air due to concentration gradients, ensuring efficient gas exchange in the organism.

Oxygen from the air contained within the lung cavity diffuses through the thin walls of the hollow structures (villi and folds) and capillaries into the blood, whereas carbon dioxide from the blood diffuses into these cavities and is subsequently expelled from the body.

Table 3
Morphometry of structural components of the lungs of the grass frog (x ± SD, n = 5)

Morphometric parameters	Indicators	
	right lung	left lung
Thickness of lung wall, μm	216.6 ± 4.7[a]	221.2 ± 3.9[b]
Thickness of mucosal layer, μm	61.2 ± 1.3[a]	63.8 ± 1.4[b]
Thickness of fibrous connective tissue layer, μm	106.1 ± 2.9[a]	110.9 ± 3.4[b]
Thickness of serous layer, μm	49.3 ± 1.5[b]	46.5 ± 2.0[a]
Thickness of folds, μm	468.9 ± 9.9[b]	454.6 ± 9.3[a]
Length of folds, μm	703.1 ± 20.9[b]	687.5 ± 16.0[a]
Thickness of villi, μm	342.7 ± 10.2[a]	361.8 ± 11.0[b]
Length of villi, μm	1339.4 ± 21.1[b]	1287.9 ± 25.4[a]

Discussion

The phylogeny of the respiratory organs in amphibians represents an evolutionary transition from aquatic respiration (gills) to terrestrial respiration (lungs and skin), corresponding to their dual aquatic-terrestrial lifestyle (Tsaryk et al., 2018).

Respiration in representatives of the class Amphibia is cutaneous-pulmonary, while the trachea and bronchi are absent. The main respiratory organs in amphibians are the lungs, which are hollow sac-like structures with a relatively small respiratory surface area. Amphibian lungs are surrounded by a dense network of blood vessels and capillaries, through which gas exchange occurs (Maina, 1989; Tattersall et al., 2013; Guangming et al., 2020).

At the early stages of amphibian phylogenetic development, the primary type of respiration was branchial respiration, which was provided by specialized structures with a dense network of blood vessels – the gills. These ensured respiration in the aquatic ancestors of amphibians and in their larvae by absorbing oxygen dissolved in water. This represents a rudimentary feature of the evolutionary transition from an aquatic lifestyle (Burggren & West, 1982; Jørgensen, 2000).

An additional respiratory organ enabling gas exchange on land is the skin, which is rich in blood vessels. The mucus secreted by skin glands keeps the skin moist and facilitates respiration. Later, during the phylogenetic development of amphibians and their transition from aquatic to semi-terrestrial habitats, lungs appear during metamorphosis as specialized organs of aerial respiration (Santin & Hartzler, 2016; McWhinnie et al., 2021; Horalskyi et al., 2025).

The lungs are paired sac-like structures that absorb oxygen from atmospheric air. Their development as organs begins with the appearance of paired outgrowths of the intestine in their fish-like ancestors. The ability for cutaneous respiration develops due to the presence of thin, highly vascularized skin, which also secretes mucus that facilitates gas exchange and protects against desiccation (Jørgensen, 2000; Perry et al., 2001; Maina, 2002).

In adult amphibians, the exchange of oxygen and carbon dioxide between the blood and atmospheric air occurs in three different body regions: the skin (cutaneous respiration), the lungs (pulmonary respiration), and the mucous membranes of the oral cavity and pharynx (buccopharyngeal respiration). In these regions, blood circulates in capillaries that are separated from the external environment only by a thin aqueous membrane, which is necessary for blood aeration (Burggren & West, 1982; Tattersall et al., 2013).

The significance of these three types of respiration varies among amphibian species. In some species (e.g., certain salamanders), pulmonary respiration is absent and gas exchange occurs entirely through cutaneous and buccopharyngeal respiration. Other amphibian species possess both lungs and external gills. In the grass frog, the respiratory organs include the lungs, skin, and mucous surface of the oropharyngeal cavity (Bodegas et al., 1995).

Considering the complex stages of phylogenetic development of respiratory organs in amphibians, we investigated the morphofunctional state of the lungs as the primary respiratory organ of the grass frog. According to our results, the lungs of the grass frog are located in the cranial part of the body cavity. Medially, the lungs contact the esophagus, while ventrally they border the right and left atria. Laterally, the right lung contacts the right lobe of the liver, whereas the left lung is adjacent to the cardiac part of the stomach.

Based on macroscopic and microscopic observations, the lungs of the grass frog exhibit certain morphological features compared with those of lungfish, animals considered transitional forms in the evolutionary shift from aquatic to terrestrial life.

In *Clarias* catfish species, each lung is covered by a connective tissue membrane (pleura) consisting of three layers: outer, middle, and inner. Macroscopically, the right and left lungs consist of a large medial lobe and a smaller lateral lobe. Cranial, caudal, and lateral margins can be distinguished in each lung, as well as dorsal, ventral, and mediastinal surfaces (Horalskyi et al., 2025).

Morphologically, the lungs of the grass frog are elongated paired alveolar sac-like structures. They are slightly narrowed at their base, expand in the middle part, and narrow again caudally, ending in a pointed apex directed dorsally, forming reservoirs with a large internal lumen. The lung wall of the grass frog consists of three layers: outer, middle, and inner. The outer layer is formed by a thin layer of simple squamous epithelium and a layer of loose connective tissue containing arteries, veins, and microcirculatory vessels. The middle layer consists of dense regular connective tissue. The inner layer, appearing as a marginal lining, is covered by simple epithelium and a delicate layer of loose connective tissue containing vessels of the microcirculatory bed involved in gas exchange, where oxygen diffuses from air into the blood and carbon dioxide diffuses from blood into the air through the capillary walls (Abalaka et al., 2021; Ogunwole et al., 2021; Hassan et al., 2023).

Morphometric studies are of great importance in morphological research, as they provide quantitative characteristics of organ structure and functional state (Horalskyi et al., 2020). According to our morphometric results, the absolute mass of the lungs in the grass frog is 0.12 ± 0.02 g, while the relative mass is 0.18 ± 0.04%.

Based on the linear parameters of the lungs (right lung length – 13.08 ± 0.51 mm, width – 5.64 ± 0.32 mm; left lung length – 12.82 ± 0.99 mm, width – 5.36 ± 0.37 mm), and considering the ratio of length to width, the lung development index averages 42% (right lung – 43.10 ± 3.06%, left lung – 41.82 ± 3.37%). Therefore, the lungs belong to the expanded–shortened type.

Histometric analysis showed that the volume of the right lung is 216.34 ± 5.17 mm³, while the volume of its cavity is 157.16 ± 6.39 mm³, with the cavity volume representing 0.73 ± 0.0021% of the total lung volume. Similar morphometric parameters were found in the left lung (lung volume – 198.53 ± 7.75 mm³, cavity volume – 147.28 ± 5.38 mm³, ratio – 0.74 ± 0.036%), indicating no significant asymmetry between the lungs.

The lung wall forms folds and villi that extend into the organ cavity. All layers of the lung wall participate in the formation of folds, whereas only the middle and inner layers participate in the formation of villi. The folds also form hollow rounded or oval structures separated by connective tissue septa.

Histometric analysis demonstrated that the fibrous connective tissue layer is the thickest component of the lung wall, followed by the mucosal layer and the serous layer. The predominance of the fibrous connective tissue layer provides the lung wall with mechanical strength and elasticity, which is necessary for effective respiration. Thus, morphological and morphometric analyses show that the lungs of the grass frog have a relatively small respiratory surface area, which is compensated by the folded structure of the internal lung wall, increasing the effective surface for gas exchange. This structural organization reflects the evolutionary adaptations of amphibians.

Due to the relatively thin lung wall and limited supporting structures compared with mammalian lungs, amphibian lungs remain highly elastic and capable of significant expansion. As a result, the lung volume of the grass frog can increase almost twofold or more, depending on functional load, and morphologically resembles the thin-walled swim bladder of many bony fishes.

During diving, the lungs may fill with air and increase in volume as the folds expand, forming a thin-walled sac that may perform a hydrostatic function, similar to that observed in fish. During this time, pulmonary respiration may be partially replaced by cutaneous respiration, which is characteristic of amphibians.

The skin of frogs plays an important role in gas exchange, providing up to half of the required oxygen, and in some species living in cold environments or underwater, cutaneous respiration may become the primary respiratory mechanism. This is possible because frog skin is thin, moist, and richly supplied with capillaries, allowing oxygen absorption both from air and water.

Therefore, in amphibians such as the grass frog, evolutionary changes in the respiratory system reflect adaptations associated with the transition from aquatic to terrestrial environments. Compared with fish, which represent a lower stage of phylogenetic development, amphibians possess a more complex respiratory system in which paired lungs appear in addition to gills, enabling animals to breathe atmospheric air.

Conclusions

During the phylogenetic development of the grass frog (*Rana temporaria*), a representative of amphibians, certain structural changes occur in the respiratory organs associated with the improvement of the respiratory system due to the appearance of lungs. The lungs of the grass frog are paired dark-pink organs consisting of the right and left lungs and representing elongated alveolar sac-like structures.

Topographically, the lungs are located in the cranial part of the body cavity: medially they are adjacent to the esophagus, while ventrally they border the right and left atria of the heart. Laterally, the right lung contacts the right lobe of the liver, whereas the left lung is adjacent to the cardiac part of the stomach. According to morphometric analysis, the absolute lung mass is 0.12 ± 0.02 g, and the relative mass is $0.18 \pm 0.04\%$. Considering the ratio of lung length to width, the lungs belong to the expanded–shortened type, as indicated by the lung development index, which averages 42% (right lung – $43.10 \pm 3.06\%$, left lung – $41.82 \pm 3.37\%$).

The histoarchitectonics of the lung wall consists of three layers: outer, middle, and inner. The outer layer is formed by a thin layer of simple squamous epithelium and a layer of loose connective tissue containing arteries, veins, and vessels of the microcirculatory bed. The middle layer is composed of dense regular connective tissue consisting of reticular and collagen fibers, between which fibrocytes with elongated nuclei are located. The inner layer is lined with simple epithelium, beneath which there is a delicate layer of loose connective tissue containing vessels of the hemomicrocirculatory bed.

The lung wall forms folds and villi that extend into the cavity of the organ. All layers of the lung wall participate in the formation of folds, whereas the middle and inner layers are involved in the formation of villi. The folds of the lung wall form rounded or oval hollow structures separated by connective tissue septa.

According to histometric analysis, the volume of the right lung is 216.34 ± 5.17 mm³, while the volume of its cavity is 157.16 ± 6.39 mm³, and the ratio of cavity volume to total lung volume is $0.73 \pm 0.0021\%$. The morphometric parameters of the left lung are similar to those of the right lung: the volume is 198.53 ± 7.75 mm³, the cavity volume is 147.28 ± 5.38 mm³, and the ratio of cavity volume to total lung volume is $0.74 \pm 0.036\%$, which indicates the absence of asymmetry between the right and left lungs.

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