



Features of the microscopic structure of the lungs of *Clarias gariepinus* of the catfish family

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The vital activity of living organisms is essential for providing cells and tissues with oxygen and nutrients and the excretion of metabolic products. The body's response to environmental influences, uniting all organs and systems into a single whole, requires the regular morphofunctional activity of all body systems, including the respiratory system, which served as the purpose of the research. Using macro- and microscopic, morphometric, and statistical research methods, the results are presented on the microscopic structure of the lungs of the catfish species *Clarias gariepinus* (Burchell, 1822). Thus, in the gas exchange system of birefringent fish, represented by the *Clarias* catfish, changes occur in the process of their phylogenetic development (two circuits of blood circulation are formed), which are characteristic of amphibians, in which, in addition to gills, paired lungs are also formed, thanks to which animals can breathe atmospheric air. The lungs of the *Clarias* catfish are formed by branched anatomical structures located on the second and fourth-lung arches. These are paired pale pink morphological structures with cellular walls connected to the esophagus. The medial and lateral lobes form the right and left lungs: starting from the gill cavities, the main trunk of the medial lobe of the lungs branches out like a tree into four large branches, which are divided into medium and small, giving rise to thick-walled alveoli. The lateral lobe of the lungs begins with a separate trunk that is not connected to the medial lobe of the lungs. The lungs of *Clarias* catfish are covered with a connective tissue membrane (pleura), which is microscopically formed by three layers: outer, middle, and inner. The microscopic structure of the wall of the bronchi of the lungs is built of three membranes – outer, middle (muscular), and inner: the surface of the wall of the outer membrane is formed by epithelial cells that form elongated villi, the lamina propria of the membrane is formed by loose connective tissue, in which a network of blood vessels is located; the muscular membrane of the wall is formed by transversely striated muscle tissue, the fibers of which have a longitudinal direction; the inner membrane is thin and formed by elongated epithelial cells. The cavity of the bronchi, throughout the entire interval of the bronchial tree, ending in the pulmonary alveoli, is filled with reticular tissue. According to morphometric studies, the most prominent morphometric parameters (wall thickness, thickness of the bronchial cavity) are characteristic of the large, then the medium, followed by the small bronchi. Pulmonary alveoli have a rounded shape; microscopically, a significant network of vessels (capillaries) of the microcirculatory bed is detected in the wall of the connective tissue membrane.

Keywords: vertebrates; morphology; histological structure; morphometry.

Introduction

An essential source of food products for the world's population from livestock farming is the hydrosphere's biological resources, including the fishing industry. In recent years, the volume of production of aquatic bioresources, particularly fish products, in the world and in Ukraine has been provided by fishing and the intensive development of aquaculture.

Aquaculture is the fastest-growing sector of livestock farming and, perhaps, one of the most critical sectors of the global food system. The importance of providing humanity with food is constantly growing. Today, the development of freshwater aquaculture is one of the priority areas of the food industry. It is based on the theoretical foundations of using valuable aquatic organisms in aquaculture, considering their cultivation in marine ecosystems, to increase their productivity and the quality of food products. This is possible only with the development and introduction of new technologies in livestock farming, including in the field of fisheries.

At the same time, the cultivation of the catfish *Clarias gariepinus* is of great importance due to its biological characteristics of development and growth (Juin et al., 2017; Lawal et al., 2017; Strauch et al., 2018), namely unpretentiousness to the conditions of detention in the aquatic environment; planting density; omnivorousness; resistance to diseases; rapid growth rates, which are of crucial economic importance to the cost of production of fish products (Baßmann et al., 2017;

Ukagwu et al., 2017; Zadorozhnyi & Bekh, 2024). The African catfish (*Clarias gariepinus* (Burchell, 1822)) is found in nature throughout Africa, living in freshwater lakes, swamps, river mouths, in the rivers themselves, irrigation canals, ponds, floodplains, even if these periodically dry up (Hecht et al., 1996; Hildebrand et al., 2023). It is a bimodally breathing, omnivorous, freshwater, and thermophilic animal that can also breathe atmospheric air and breathe with its gills (Chervinski, 1984; Britz & Hecht, 1987; Tsaryk et al., 2018). For the cultivation of catfish, there must be appropriate conditions for its cultivation and maintenance: appropriate water temperature (20–30 °C), chemical composition, oxygen level, salinity, etc. (Clay, 1977; Hogenhorn & Vismans, 1980; Huisman & Richter, 1987).

There is no doubt that the introduction of scientific and innovative technologies and modern methods of fish farming will make it possible to increase the efficiency of fish production, ensure the sustainability and environmental safety of production technology processes, and reduce resource costs while maintaining high product quality.

At the same time, the use of the latest technologies in the production processes of the fish farming industry can adversely affect the morphofunctional state of the organs and systems of the fish organism during their cultivation; stress factors; mismatch of the temperature regime of the aquatic environment, chemical and physical composition of the water, etc. (Rudenko et al., 2019; Honcharova et al., 2021). One of the critical factors that affect the productive qualities of animals is the use of the latest technologies for feeding fish with com-

bined feeds to ensure the vital needs of animals regarding energy, nutrients, and biologically active substances. This leads to disruption of homeostasis in the fish organism and contributes to the emergence of new diseases of various genesis. In addition, under the influence of various technological, stressful, and adverse factors of the internal and external environment appear. When the latest technologies are used in the Gagauz fish farming industry, metabolic disorders occur in the fishes' body, which negatively affects their productivity, the formation of immunity, etc. (Kofonov et al., 2020; Kukhtyn et al., 2022; Horalskyi et al., 2023).

That is why, for the successful development of the fish farming industry, improving product quality, disease prevention and diagnosis, and organizational and economic measures, conducting in-depth research on the organism at the macro- and microscopic levels is advisable. At the same time, it is of urgent importance to study the features of the respiratory organs, including the lungs in age, breed, species aspect and under the influence of anthropogenic environmental factors on the organism (Horalskyi et al., 2019; Horalskyi et al., 2022).

In all terrestrial vertebrates, the respiratory organs include the airways and lungs, which perform essential vital functions in their body. In fish, respiration is conducted through the gills, but in bimodally breathing fish (*Clarias* catfish), in addition to gill respiration, due to the appearance of lungs in the process of phylogenetic development, pulmonary respiration is also characteristic, as a result of which they can breathe atmospheric air (Aleksienko, 2007; Melnyk et al., 2008; Sherman et al., 2009; Tsaryk et al., 2018).

That is why, with the appearance of lungs, individual features of the morphological structure of the respiratory organs are characteristic of the *Clarias* catfish, which served as the purpose of our research. Therefore, we first studied the histoarchitectonics of the lungs in bimodally breathing fish – *Clarias* catfish.

Such studies are not only of cognitive importance for morphologists and zoologists but are the basis for ichthyologists-fish breeders to prevent and diagnose diseases due to the impact of stressful and adverse environmental factors on the animal body when the fish are grown in aquaculture.

Materials and methods

The research was conducted following the rules of the international principles of the “European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes” (Strasbourg, 1986) and the “Rules for Conducting Work Using Experimental Animals,” approved by order of the Ministry of Health No. 281 of November 1, 2000 “On Measures for Further Improvement of Organizational Forms of Work Using Experimental Animals” and the relevant Law of Ukraine “On the Protection of Animals from Cruelty” (No. 3447-IV of February 21, 2006, Kyiv).

The African catfish were grown and kept for research in pools, using artificially created ecosystems with favorable conditions resembling their natural habitat, observing the appropriate water temperature regime and appropriate indicators of its salinity and acidity. Tap water, the source of water supply was previously settled and heated to the pools' temperature and, daily 10% of the total capacity was added to the pool. The rooms where the pools were located were darkened.

Pools with a volume of up to 500 liters, in which the catfish were grown, were equipped with technical means and appropriate devices of mechanical, biological, and bacteriological filtration systems – Eheim Professional 3 1200XL and ultraviolet sterilizers of the external type Resun UV – 08 24 W. (China). Water saturation with oxygen was measured using a radiator-type compressor Resun ASO – 001 (China).

The required water temperature (25–28 °C) for growing *Clarias* catfish was maintained by an external heater JBL ProTemp e500 (Germany) with a power of 500 W.

The density of fish stocking and the quality of the water in which the *Clarias* catfish were grown met the regulatory recommendations (Hecht et al., 1996). Hydrochemical water analysis was carried out once a day, where the following were monitored: temperature and pH level, NH₃, NH₄, NO₂, and NO₃ levels.

Compound feed was used to feed the fish, and was given twice daily – in the morning and evening.

Clinical assessment of the fish was carried out by visual daily inspection of the fish, considering their activity, mobility, appetite, etc. Experimental studies were conducted at the Department of Zoology, Biological Monitoring and Nature Conservation of Ivan Franko Zhytomyr State University, following the research topic of the department: “Animals of artificial and natural ecosystems of Ukraine” (registration number – 0122U002270).

The object of the study was the lungs of *Clarias gariepinus*, African catfish (n = 5), for which anatomical, histological, morphometric, and statistical research methods were used.

Clinical examination of freshly caught fish: assessment of exterior (appearance, body weight) and interior (linear parameters, absolute and relative organ mass) parameters, after anatomical dissection, was carried out following the recommendations of ichthyological and morphological (Horalskyi et al., 2019) manuals. To prevent the negative impact of stress factors, the fish were anesthetized with a hypnodyl solution (5–10 mL/L) before dissection.

For histological studies, generally accepted methods of fixing the material and making histological sections were used (Horalskyi et al., 2019). For this purpose, the selected lung pieces were fixed for one day or more in a 12% cooled neutral formalin solution, followed by embedding the material in paraffin according to the schemes proposed in the manual (Horalskyi et al., 2019).

A sledge microtome MS-2 was used to prepare paraffin sections. The thickness of the histological sections did not exceed 10–12 μm.

After their deparaffinization, the histological sections were stained with hematoxylin (Diapath, Italy, 2020) and eosin (LeicaGeosystems, Germany, 2020) to study the microscopic structure of the cells and tissues. The prepared histopreparations were used to determine the characteristics of the histo- and cytoarchitectonics of organs and to conduct morphometric studies.

Histometry (thickness, diameter) of structural elements was performed by light microscopy using “Micros” microscopes (Micros, Austria, 2012), according to the recommendations proposed in the manual (Horalskyi et al., 2019). The microscopic structure of the lungs was photographed with a CAM V–200 video camera (InterMed, China, 2017) mounted in a microscope.

Morphological terminology of the structural parts of the lungs is given following the International Veterinary Anatomical Nomenclature and the International Veterinary Histological Nomenclature.

Digital processing of the research results was performed statistically using the Statistica 7.0 software package (StatSoft, Tulsa, USA). Differences between values were determined using ANOVA, where differences were considered significant at $P < 0.05$ (considering the Bonferroni correction).

Results

In the African catfish, which is a representative of bimodally-breathing fish, in addition to gill breathing, there is also pulmonary breathing. That is why, in this species of fish, in addition to the gills, in their phylogenetic development, an additional respiratory organ is formed – the lungs. And so, for the African catfish, two circuits of blood circulation are characteristic: a large (somatic) and a small (pulmonary) circle of blood circulation.

According to the results of our research, the movement of blood in the large circle of blood circulation begins with the ventricle of the heart, from which blood enters the arterial cone, from there through the aorta, through the outlet gill arteries, it moves to the gills, where it is saturated with oxygen, and, already oxygenated blood through the gill arterial vessels enters the organs and tissues, where gas exchange occurs and already deoxygenated blood through the gill veins enters the right part of the atrium.

The movement of blood in the pulmonary circulation begins in the ventricle of the heart, from where it enters the arterial cone. Through the abdominal aorta and the pulmonary arteries, it enters the lungs. Gas exchange occurs in the lungs and, already saturated with oxygen, the blood enters the left half of the atrium through the pul-

monary veins. Then, from the atrium, the blood again enters the ventricle, from where the already partially mixed blood enters the arterial cone, from which it again enters the gills and lungs through the pulmonary and gill arteries, where gas exchange occurs.

The lungs of *Clarias gariepinus* are pale pink. This is a paired organ, which is built from the right and left lungs, which originate from the gill cavities, attaching to the posterior part of the gill arches (Fig. 1). Topographically, the lungs of the *Clarias* catfish are located in the right and left halves of the cranial part of the body, respectively on the sides of the mediastinum and are located in the correspondingly formed right and left paragill cavities. Both lungs are located ventrally at the base of the pharynx on both sides of the esophagus, connecting with it by the corresponding airways, thus forming an organ of air respiration (Fig. 1).

According to the morphological structure, these are cellular paired anatomical structures intertwined with a dense network of blood vessels, papillae, in which gas exchange occurs, the capillary network connected to the last pair of gill arteries. The right and left lungs consist of medial (large) and lateral (small) lobes (Fig. 1). The medial lobes of the right and left lungs are topographically located in the cranial part of the body, respectively, to the right and left of the esophagus. Their dorsal surfaces are adjacent to the lateral lobes, which laterally adjoin the branchial (fourth) arches while contacting the gill covers. The lateral lobe of the lungs borders cranially on the branchial arches (Fig. 1).

The lungs are divided into dorsal (facing the dorsal part of the body), ventral (facing the ventral part of the body), and mediastinal (facing the mediastinum) surfaces. Macroscopically, the lungs are divided into cranial, caudal, and lateral edges (Fig. 1).

Morphologically, each lung in the studied catfish, starting from the gill arches, in the form of a trunk, branches out in a tree-like (bush-like) manner, forming structures similar to the bronchial tree, as in mammals: the main branch (trunk) of the medial lobe of the lung branches into four (Fig. 2a) large branches, large ones into medium ones, which branch out into small branches, respectively, forming a tree-like structure. The lateral lobe of the lung begins with a separate trunk, which is not connected to the medial lobe of the lung (Fig. 2b).

Then the tiny branches of the bronchial tree of each lung branch into separate branches, which give rise to the formed thick-walled alveoli (Fig. 3).

Each lung in the *Clarias* catfish is covered with a connective tissue membrane (pleura), the average thickness of which is $552.6 \pm 7.9 \mu\text{m}$. According to the microscopic structure, the connective tissue membrane consists of three layers: outer, middle, and inner (Fig. 4). The outer layer of the membrane is formed by the epithelium, which, being pressed into the depth of the membrane, forms elongated glandular structures in the form of crypts, which are similar in structure to glandular structures (Fig. 4). On a longitudinal section, such glandular structures have a rounded-oval shape. They are formed by prismatic cells that form first-order leaflets; the latter, in turn, are divided into second-order leaflets. Between the epithelial cells, blood cells are found – erythrocytes and leukocytes (Fig. 5). In the lumen of the glands and around them, there is a network of tiny vessels – blood capillaries, in which blood cells are found.

The middle layer of the connective tissue membrane (pleura) is formed by loose connective tissue, where the vessels of the hemomicrocirculatory bed are located – arterioles, venules, and capillaries. The inner layer of the membrane is the thinnest, formed by polygonal, elongated cells (Fig. 4).

Histologically, the structure of the bronchial tree of the lungs in the *Clarias* catfish has certain microscopic features in common with those of mammals. Thus, the microscopic structure of the wall of the bronchi (large, medium, and small) of the lungs of the *Clarias* catfish consists of three membranes: outer, middle, and inner (Fig. 6a, 6b).

The surface of the outer membrane of the bronchial wall is formed by prismatic epithelial cells, which, being pressed into its depth, form vertically elongated villi. After the epithelial layer, there is a lamina propria, formed by loose connective tissue, in which a network of vessels of the microcirculatory bed is found: arterioles, venules, capillaries, and large vessels (arteries, veins), the branching of which occurs in the longitudinal direction of the bronchial tree, along which arterial and venous blood flows in the small (pulmonary) circle of blood circulation (Fig. 6).

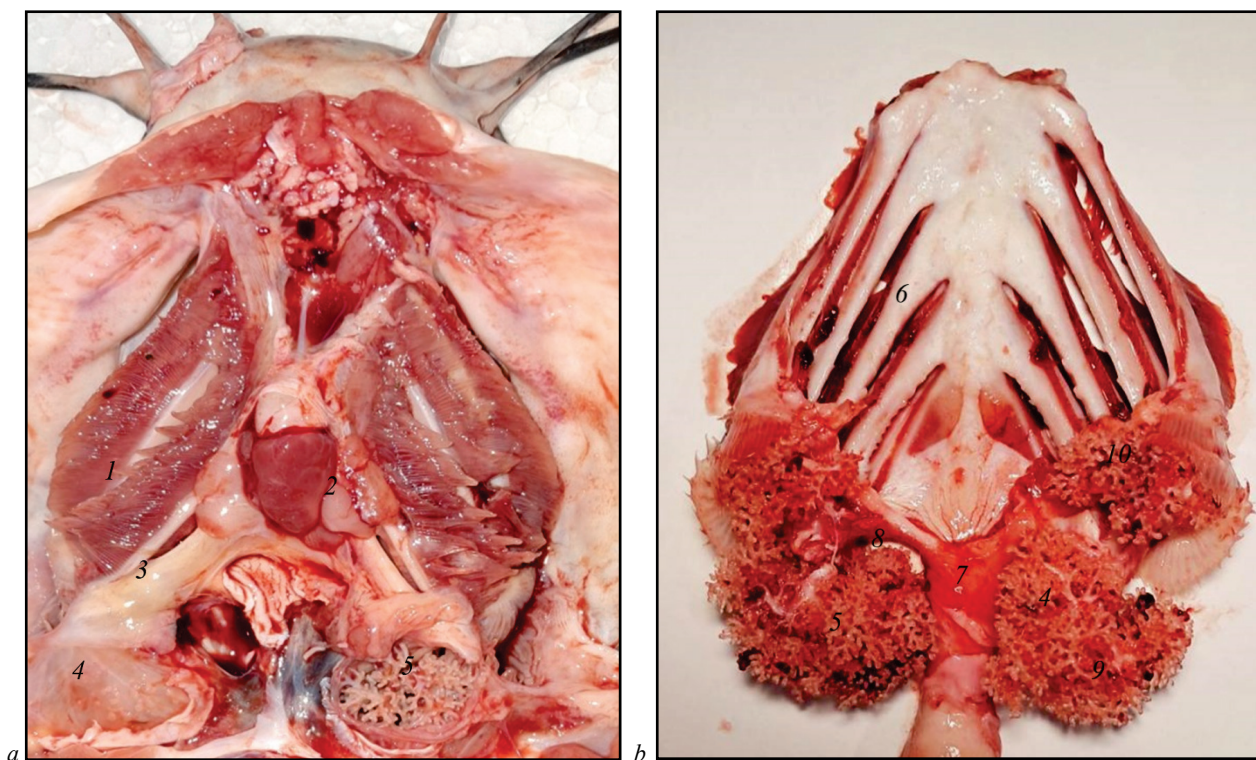


Fig. 1. Topography of the lungs of *Clarias* catfish: *a* – morphotopography of the lungs (cranial part of the body); *b* – morphology of the lungs of the *Clarias* catfish: 1 – gills; 2 – heart; 3 – humeral bones; 4 – left lung (covered with pleura); 5 – right lung; 6 – gill arches; 7 – esophagus; 8 – connection of the lungs with the esophagus; 9 – medial lobe of the lung; 10 – lateral lobe of the lung; macroscopic preparation

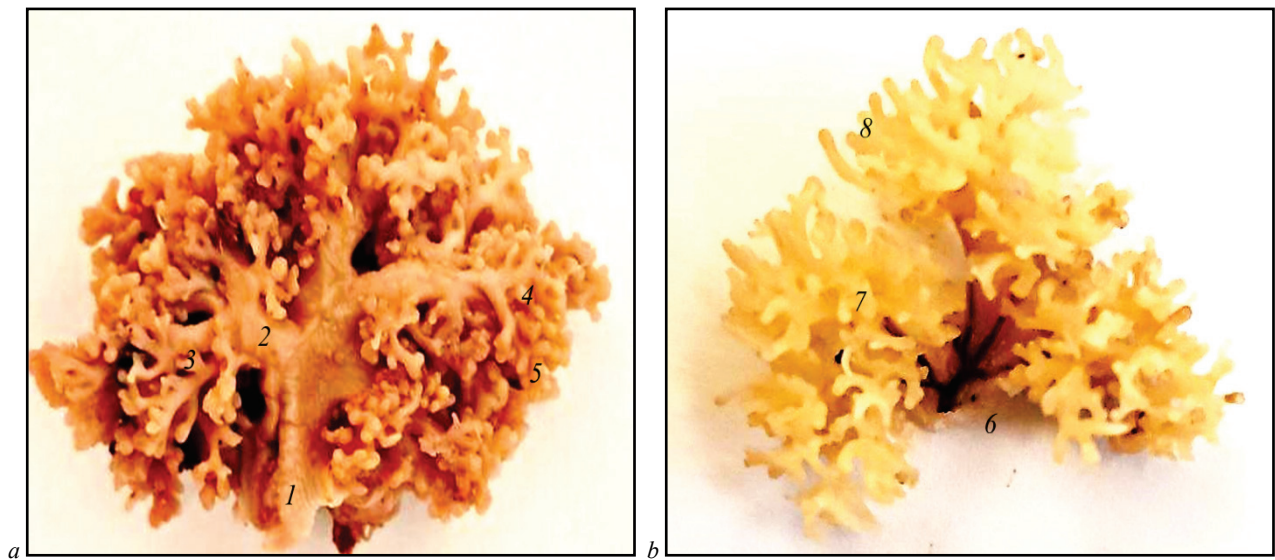


Fig. 2. Medial (a) and lateral (b) lobe of the lung: a – main trunk; 1 – main branch; 2 – large branch; 3 – middle branch; 4 – small branch; 5 – alveoli; 6 – branching of blood vessels of the bronchial tree; 7 – lung parenchyma; 8 – alveoli; macroscopic specimen

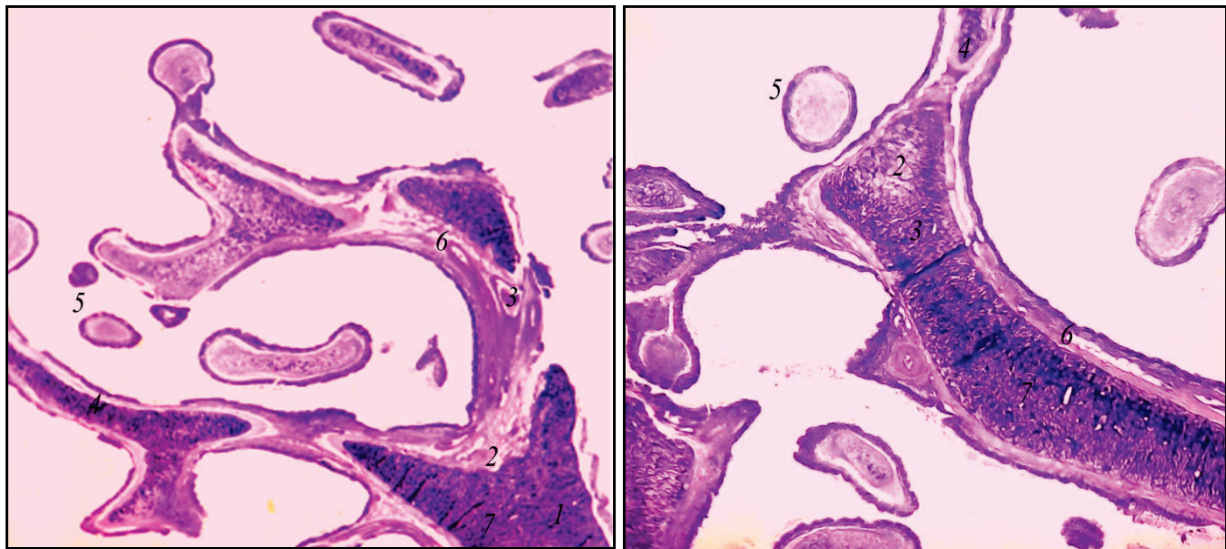


Fig. 3. Branching of the bronchi of the lungs of *Clarias catfish*: 1 – trunk (bronchus) of the large bronchus; 2 – bifurcation; 3 – trunk of the middle bronchus; 4 – trunk of the small bronchus; 5 – alveoli; 6 – wall of the bronchus; 7 – reticular tissue of the bronchial cavity; hematoxylin and eosin (total section)

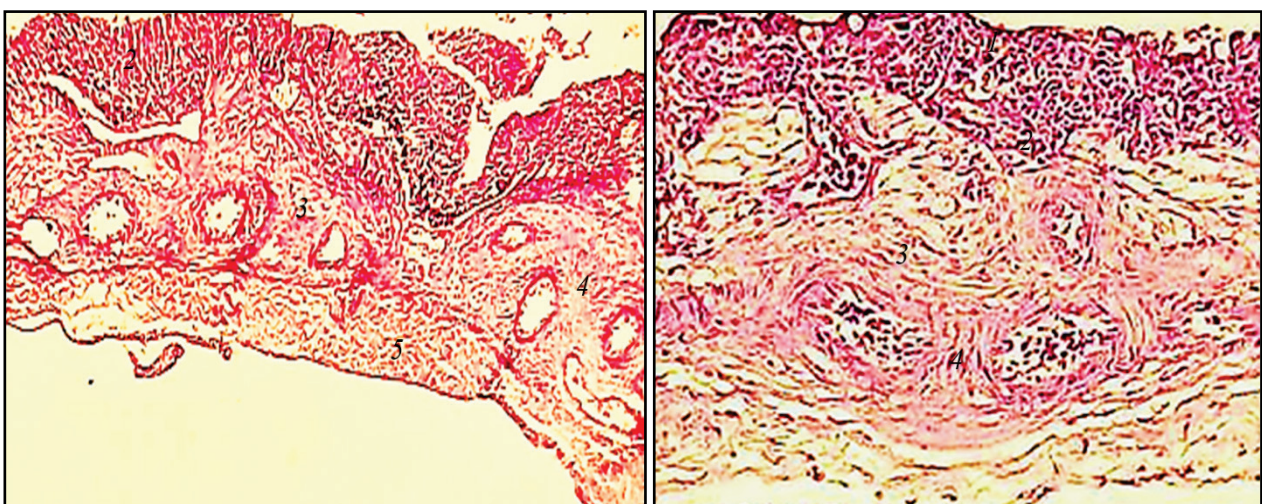


Fig. 4. Microscopic structure of the pleura of the lungs: 1 – epithelial layer; 2 – glandular structures; 3 – middle layer; 4 – microcirculatory vessels; 5 – inner layer; hematoxylin and eosin

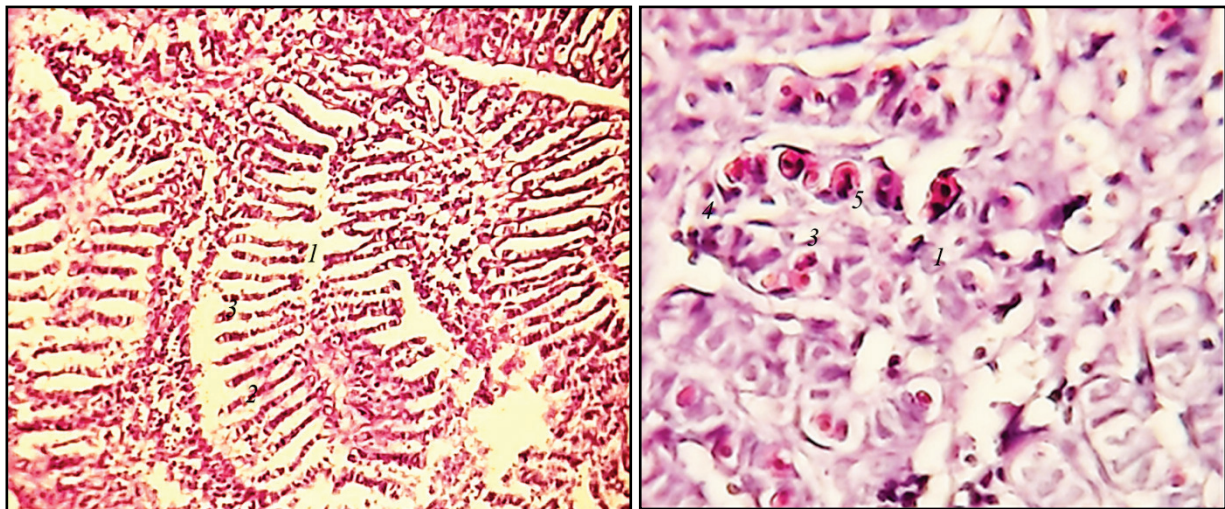


Fig. 5. Fragment of the microscopic structure of the connective tissue lining of the bronchial wall: 1 – glandular structures; 2 – first-order leaflets; 3 – second-order leaflets; 4 – epithelial cells; 5 – erythrocytes; hematoxylin and eosin

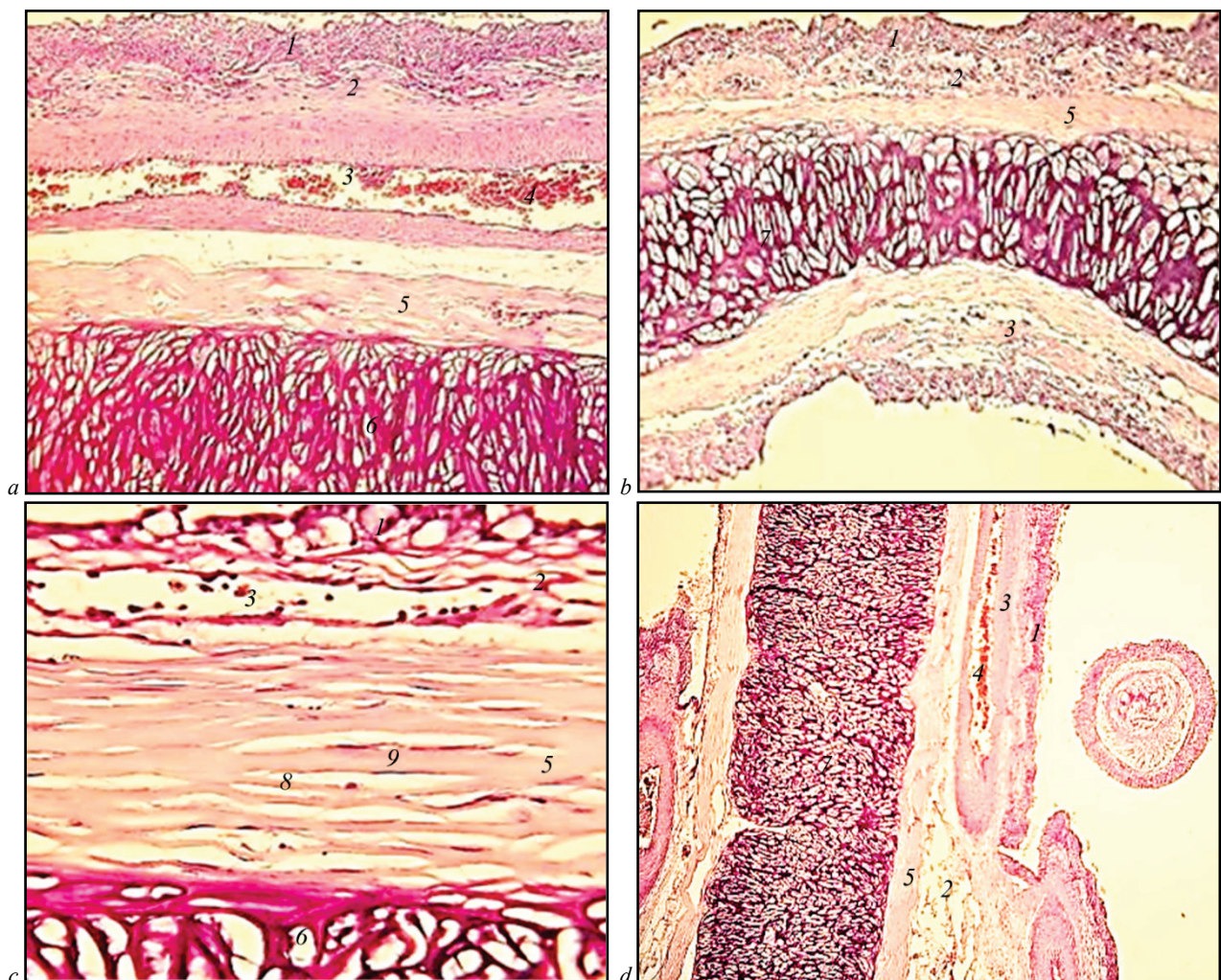


Fig. 6. The microscopic structure of the bronchi of the lungs of *Clarias catfish*: a – fragment of the microscopic structure of the large bronchus of the lungs; b – the microscopic structure of the middle bronchus of the lungs; c – a fragment of the microscopic structure of the wall of the middle bronchus of the lungs; d – the microscopic structure of the middle bronchus of the lungs: 1 – the outer shell of the wall; 2 – loose connective tissue; 3 – blood vessel; 4 – lumen of the vessel; 5 – muscular membrane of the wall; 6 – reticular tissue; 7 – lumen of the bronchus filled with reticular tissue; 8 – muscle fibers; 9 – nuclei of muscle fibers. Hematoxylin and eosin

The middle (muscular) membrane of the bronchial wall is highly developed; it is formed from transversely striated muscle tissue, the fibers of which have a longitudinal direction (Fig. 6). Their muscle fibers have different thicknesses: large, medium, and thin. Between the fibers is intermuscular connective tissue. The nuclei of the muscle fibers are located on the periphery of the fibers themselves (Fig. 6c).

The inner membrane of the bronchial wall is formed by an extremely thin, inconspicuous layer formed by elongated epithelial cells, which are in internal contact with the reticular tissue that fills the bronchial cavity.

The pulmonary alveoli, on a cross-section, have a rounded shape. Their wall is built according to the same principle as that of the bron-

chi (Fig. 7). However, in their wall of the connective tissue membrane, a significant network of vessels (capillaries) of the microcirculatory bed is found; perhaps it is there that gas exchange occurs, where oxygen, by diffusion, through the walls of the alveoli and blood capillaries from the air passes into the blood, and carbon dioxide from the blood enters the vessels.

In the *Clarias* catfish, the lumen of the bronchi, throughout the entire length of the bronchial tree, ending in the pulmonary alveoli, is filled with reticular tissue. Its reticular cells, together with the reticular fibers that are intertwined with each other, form a mesh, supporting framework (base) of the cavity of the bronchial tree, starting from the

large bronchi and ending in the pulmonary alveoli, occupying their cavity (Fig. 8). Due to this unique structure of the bronchial tree, the bronchi in the *Clarias* catfish do not collapse.

According to the results of our histometric studies of the bronchial tree of the lungs of the *Clarias* catfish, the thickness of the large bronchi is $1701 \pm 31 \mu\text{m}$. The thickness of their wall is $402 \pm 15 \mu\text{m}$: the thickness of the outer shell is $221 \pm 9 \mu\text{m}$, the middle shell is $169 \pm 6 \mu\text{m}$, the inner shell is $8.8 \pm 0.3 \mu\text{m}$. At the same time, the thickness of the bronchial cavity, filled with reticular tissue, is $903 \pm 17 \mu\text{m}$ (Table 1).

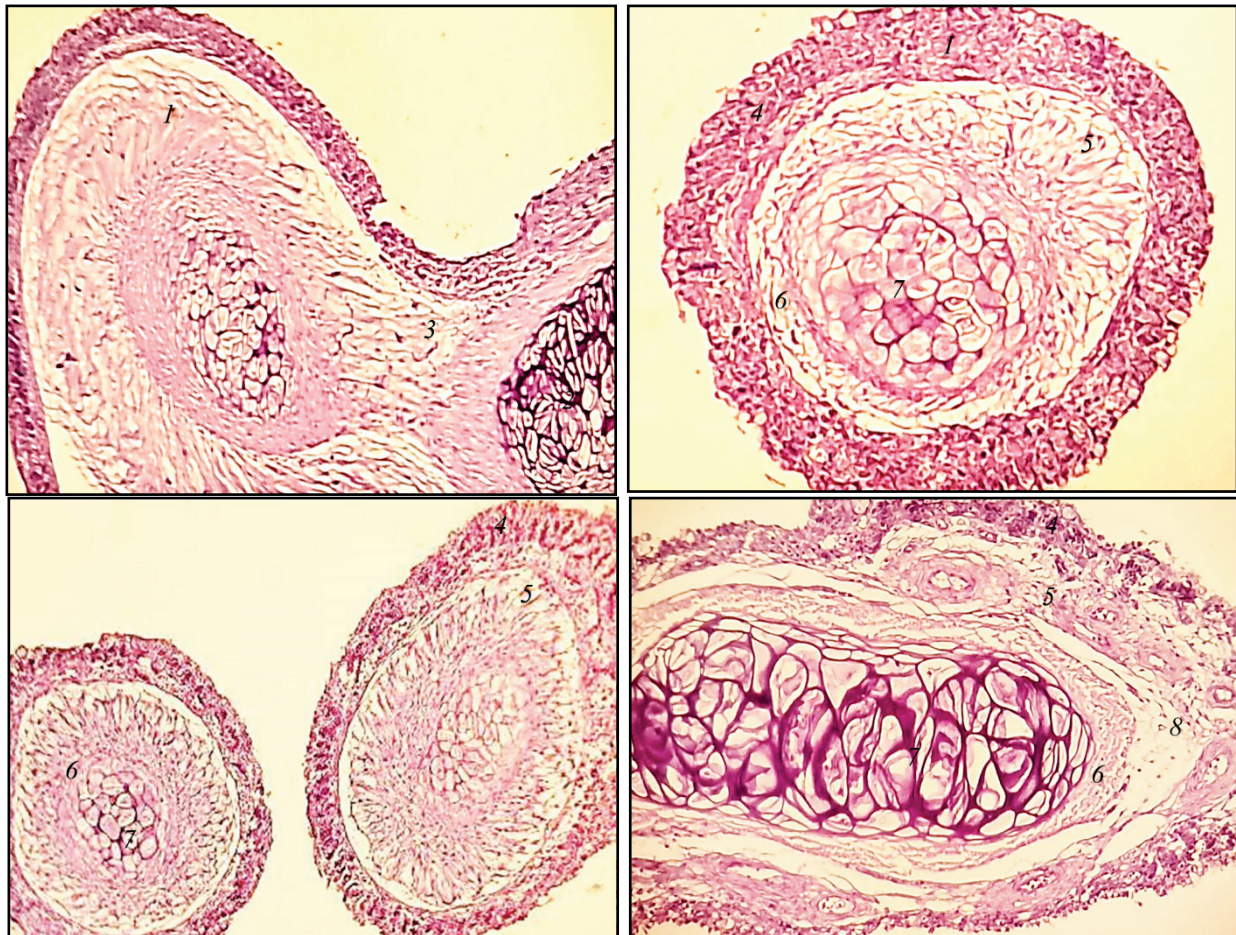


Fig. 7. Microscopic structure of the pulmonary alveolus of *Clarias* catfish: 1 – alveolus; 2 – fragment of a small bronchus; 3 – the place of branching of a small bronchus into the alveolus; 4 – the outer membrane of the wall; 5 – loose connective tissue; 6 – the muscular membrane of the wall; 7 – the lumen of the alveolus filled with reticular tissue; 8 – capillary network; hematoxylin and eosin

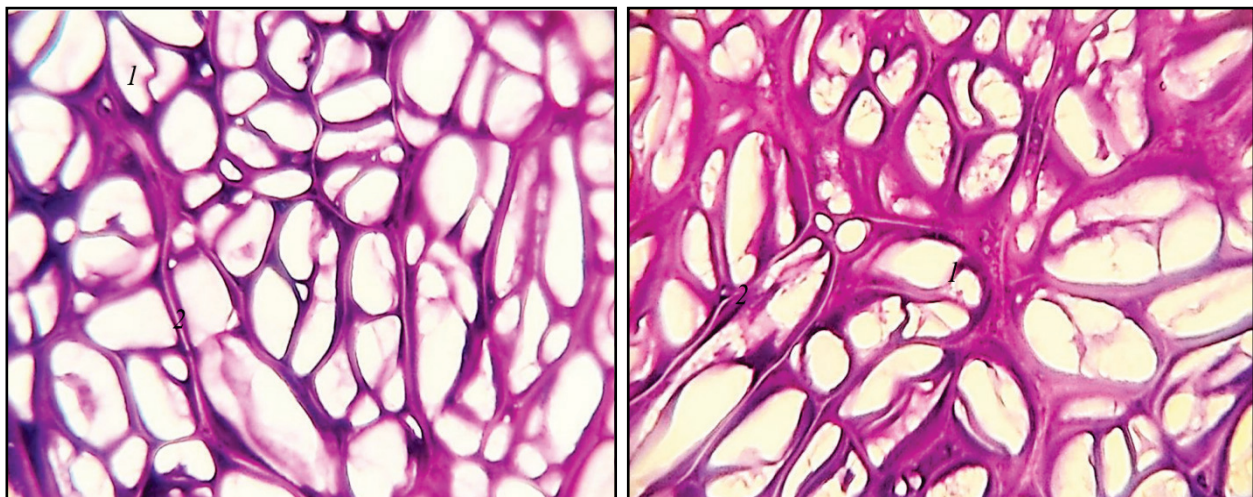


Fig. 8. Reticular tissue in the bronchial cavity of the lungs of *Clarias* catfish: 1 – reticular cell; 2 – reticular fibers; hematoxylin and eosin

The indicators of the structural components of the middle bronchi are significantly ($P < 0.05$) lower compared to such indicators in the large bronchi (Table 1). Thus, the thickness of the average bronchi of the lungs is $1299 \pm 17 \mu\text{m}$, and the thickness of their wall is $313 \pm 11 \mu\text{m}$: the outer shell is $174 \pm 6 \mu\text{m}$, the middle shell is 132 ± 5 , the inner shell is 6.8 ± 0.3 . The thickness of the cavity in such bronchi is $678 \pm 12 \mu\text{m}$. Similar morphometric changes, towards a significant decrease, were also found for the morphometric parameters of the small bronchi, compared with such indicators in the medium ($P < 0.05$) bronchi. At the same time, the thickness of the small bronchi of the lungs is $600.2 \pm 9.97 \mu\text{m}$, respectively, and the wall thickness is $163 \pm 5 \mu\text{m}$. With such morphometric parameters, the thickness of the outer shell of the wall of the small bronchi is $105 \pm 3 \mu\text{m}$, the thick-

ness of the middle shell is $52 \pm 1 \mu\text{m}$, and the thickness of the inner shell is $4.0 \pm 0.2 \mu\text{m}$. At the same time, the thickness of the bronchial cavity filled with reticular tissue is $313 \pm 11 \mu\text{m}$ (Table 1).

The diameter of the alveoli of the lungs of the *Clarias* catfish is $625 \pm 11 \mu\text{m}$. The diameter of the alveoli cavity filled with reticular tissue is $393 \pm 8 \mu\text{m}$. The thickness of the wall of the alveoli is $186 \pm 7 \mu\text{m}$: the thickness of the outer shell is $118 \pm 3 \mu\text{m}$, the thickness of the middle shell is $59.5 \pm 1.4 \mu\text{m}$, the thickness of the inner shell is $4.17 \pm 0.18 \mu\text{m}$ (Table 1). The average volume of the alveoli is 94770 ± 909 thousand μm^3 , the average volume of the cavity is 27862 ± 413 thousand μm^3 . The ratio of the volume of the alveoli cavity to the volume of its wall is 0.4164 (Table 2).

Table 1
Morphometry of structural components of the bronchial tree ($x \pm \text{SD}$)

Morphometric indicators	Structural components of the bronchial tree		
	large bronchi	middle bronchi	small bronchi
Bronchus thickness, μm	1701 ± 31^c	1299 ± 17^b	600 ± 10^a
Thickness of the bronchial cavity, μm	903 ± 17^c	678 ± 12^b	313 ± 11^a
Bronchial wall thickness, μm	402 ± 15^c	313 ± 9^b	163 ± 5^a
Thickness of the outer membrane of the bronchial wall, μm	221 ± 9^c	174 ± 6^b	105 ± 3^a
Thickness of the middle layer of the bronchial wall, μm	169 ± 6^c	132 ± 5^b	52 ± 1^a
Thickness of the inner lining of the bronchial wall, μm	8.8 ± 0.3^c	6.8 ± 0.3^b	4.0 ± 0.2^a

Note: letters indicate significant differences between the subgroups within one line ($P < 0.05$) according to the Tukey's test.

Table 2
Morphometry of structural components of the pulmonary alveoli of the *Clarias* catfish ($x \pm \text{SD}$)

Morphometric indicators	Digital values
Thickness of the wall of the connective tissue membrane (pleura) of the lungs, μm	553 ± 8
Diameter of the pulmonary alveolus, μm	625 ± 11
Diameter of the cavity filled with reticular tissue, μm	393 ± 8
Vesicle wall thickness, μm	186 ± 7
Thickness of the outer wall shell, μm	118 ± 3
Thickness of the middle wall shell, μm	59.5 ± 1.4
Thickness of the inner wall shell, μm	4.17 ± 0.18
Average volume of the pulmonary alveolus, thousand μm^3	94770 ± 909
The average volume of the vesicle cavity filled with reticular tissue, thousand μm^3	27862 ± 413
The ratio of the volume of the vesicle cavity to the volume of its wall	0.4164

Thus, in the process of phylogenetic development of the *Clarias* catfish, which is representative of the air-breathing fish, in the structure of the respiratory organs, specific changes occur in the respiratory system, which provides gas exchange between the animal's body and the environment. This is manifested by the improvement of the structure of the respiratory apparatus, where, in addition to gills, paired lungs are also formed, thanks to which animals can breathe atmospheric air.

According to our morphological studies, the lungs of the *Clarias* catfish differ in macro- and microscopic structure from those of vertebrates, amphibians, reptiles, birds, and mammals.

Discussion

The respiratory organs of bony fish are gills. In addition, up to 10% of gas exchange in fish occurs through the skin. In addition, certain parts of the intestine and the swim bladder can participate in gas exchange (DeLaney et al., 1983; Melnyk et al., 2008; Huang et al., 2008). In air-breathing fish, represented by the *Clarias* catfish, pulmonary breathing is also characteristic in addition to gill breathing. With a cellular structure, the swim bladder is rebuilt into two lungs, which, if necessary, can breathe atmospheric air (Melchenkov et al., 2011; da Silva et al., 2017; Tsaryk et al., 2018). According to studies (Melchenkov et al., 2011), the lungs are more important for the vital activity of the *Clarias* catfish than the gills. In addition, pulmonary respiration in the *Clarias* catfish, which, in addition to gills, have paired lungs formed during phylogenetic development, allows these animal species to survive without water for up to two days, or much longer in turbid water with low oxygen content, as well as to move

along the surface of the terrestrial environment. At the same time, in scientific and literary sources (Melchenkov et al., 2011), manuals, textbooks on fish anatomy, vertebrate zoology, etc. (Aleksiienko, 2007; Melnyk et al., 2008; Sherman et al., 2009), the features of the macro- and microscopic structure of the lungs in air-breathing fish are almost not described, or their morphological structure is presented only in the form of schematic images.

Therefore, we conducted a study of the morphology of the lungs in air-breathing fish, the representative of which is the African catfish. In bony fish, which have a two-chambered heart and a single circulatory system, blood enters the venous sinus via the gill veins, then moves to the atrium, ventricle, and arterial cone, from where it flows through the abdominal aorta to the gills, where gas exchange occurs (Fishman et al., 1985; Farmer, 1999; Amelio & Garofalo, 2023). From the gills, arterial blood flows through vessels to organs and tissues, where it gives off oxygen and is saturated with carbon dioxide, transforming into venous blood, from where deoxygenated blood returns to the heart via veins (Florindo et al., 2004; Hillman et al., 2013).

According to our research, in addition to gill breathing, the *Clarias* catfish also has pulmonary breathing, which contributed to the emergence of a minor (pulmonary) circulatory system in this species, in addition to the extensive (somatic) circulatory system: the movement of blood in the sizeable circulatory system begins with the ventricle of the heart and ends in the right half of the atrium; the movement of blood in the large circulatory system begins with the ventricle of the heart and ends in the left half of the atrium.

The lungs in mammals are located in the thoracic cavity and appear like a cone. Such a morphotopography of the lungs and their shape, which is similar to a truncated cone and somewhat compressed from the sides, is associated with their placement in the thoracic cavity, which gives the right and left lungs such a shape as a whole (Amin-Naves et al., 2004; Barreto & Volpato, 2004; Cieri, 2019; Gam et al., 2020). This is because the lungs, in their natural state, together with the heart and other organs of the thoracic cavity (aorta, esophagus, thymus, etc.), generally reproduce the shape of the thoracic cavity (Smits et al., 1994; Katz, 1996; Vanderelst et al., 2012).

According to our studies, the lungs of the *Clarias* catfish are paired cellular structures, pale pink in color, which are located in the formed right and left paragill cavities, attaching to the posterior part of the gill arches.

In mammals, the left and right lungs are divided into cranial, middle, and caudal lobes by deep incisions from the ventral edge: the right lung also has an additional lobe (Maina & van Gils, 2001; Roux, 2002; Horalskyi et al., 2022). In the *Clarias* catfish, according to research, each lung is covered with a connective tissue membrane (pleu-

ra), which is formed by three layers: external, middle, and internal. Macroscopically, the right and left lungs are built from large (medial) and small (lateral) lobes. Each lung has a cranial, caudal, and lateral margin and a dorsal (facing the dorsal part of the body), ventral (facing the ventral part of the body), and mediastinal (facing the mediastinum) surface.

Compared to other vertebrate classes, mammals' lungs are more morphofunctionally perfect. The main features of their structure in mammals are the differentiation of lung tissue into the airways – the bronchial tree (main, large, medium, small bronchi) and the respiratory department – the alveolar tree (alveolar ducts, alveolar sacs, alveoli), where gas exchange occurs (Torday & Rehan, 2004).

In the branching of the bronchial tree in mammals, almost all of its structural components, up to the lobular bronchi, conform to the primary type, only the lobular bronchi branch according to the dichotomous type. In this case, the trachea at the bifurcation point is divided into two primary bronchi, which then branch into large, medium bronchi and terminal bronchioles (Horsfield, 1976; Scrivani & Percival, 2023).

According to the results of the macro- and microscopic structure of the lungs, the bronchial tree in the *Clarias* catfish has certain features comparable to those of mammals. In *Clarias* catfish (starting from the gill arches), each lung branches in a tree-like manner, forming structures similar to those of the bronchial tree, similar to those of mammals. At the same time, the main trunk of the medial lobe of the lung is divided into four large branches, which branch into medium, the last into small branches, forming a tree-like structure. The small branches of the bronchial tree of each lung branch into separate branches, giving rise to thick-walled alveoli. The lateral lobe of the lungs is formed by an individual trunk, which is not connected to the medial lobe of the lung and has a corresponding branching of the bronchial tree (Tongo & Erhunmwunse, 2022).

The bronchi of mammals, depending on their caliber, compared with those of locomotor fish, have different morphological features, they are built of mucous, fibrocartilaginous membranes and adventitia. In mammals, the structure of the bronchial wall contains a fibrocartilaginous membrane: in the wall of the main bronchi, in the form of continuous cartilage rings; in the wall of the large bronchi, in the form of separate, different in size and shape, cartilage plates – cartilage islands; in the wall of the medium bronchi, in the form of separate islands formed by hyaline cartilage (in places elastic cartilage). This feature of the structure of the bronchial wall of mammals creates the conditions under which the bronchi do not collapse (McFawn & Mitchell, 1997).

In the *Clarias* catfish, according to the results of our studies, the microscopic structure of the bronchial wall is formed by three membranes – outer, middle, and inner. The surface of the outer membrane of the bronchial wall is formed by epithelial cells. The latter, pressing into the depth of the membrane, form elongated villi. Then, after the epithelial plate, there is the plate itself, formed by loose connective tissue, where a network of vessels is located – arterioles, venules, capillaries, and large vessels, through which blood flows in the pulmonary circulation. The bronchial wall's muscular (middle) membrane is highly developed and formed by transversely striated muscle tissue, the fibers of which have a longitudinal direction. The inner membrane of the bronchial wall is relatively thin and formed by elongated epithelial cells. A similar microscopic structure is characteristic of the medium and small bronchi, which end in bronchial alveoli with a rounded shape. Their wall is formed similarly to that of the bronchi. In the connective tissue membrane of the alveoli wall, there are many vessels of the hemomicrocirculatory bed, which participate in gas exchange, whereby diffusion through the walls of the alveoli and blood capillaries takes place, oxygen from the air enters the blood, and carbon dioxide from the blood enters the vessels (Abalaka et al., 2021; Ogunwole et al., 2021; Hassan et al., 2023).

Thus, according to the results of the literature, in animals of the mammalian class, specific features of the structure of the bronchial wall (the presence of a fibrocartilaginous membrane) create conditions under which the bronchi do not collapse. In the *Clarias* catfish, according to the results of our studies, the lumen of the bronchi,

throughout the entire length of the bronchial tree, ending in the pulmonary alveoli, is filled with reticular tissue. Reticular tissue is a mesh supporting framework for soft organs, such as lymphatic tissue, spleen, lymph nodes, bone marrow, liver, etc. It is built of reticular cells (reticulocytes) and reticular fibers. Reticulocytes have a stellate or elongated shape with many long processes and a light nucleus. The processes of the cells, tightly connecting, form a mesh-like mass, which, together with the network of reticular fibers, constitutes the skeleton (stroma) of the hematopoietic organs etc. (Lütge et al., 2021).

Therefore, due to such a unique morphological structure of the bronchi and pulmonary alveoli in the *Clarias* catfish, the lumen of which is filled with reticular tissue, their walls do not collapse, which creates appropriate conditions for constant air movement in the cavity of the bronchial tree.

Based on histometric studies and mathematical analysis, which are often used in morphology, various histometric parameters of the bronchial tree have been established, depending on the size of the bronchi, their functional load during the movement of air through the respiratory tract of the lungs towards the pulmonary alveoli, to ensure the supply of oxygen to the body and its use in oxidative reactions. At the same time, according to the results of morphometry, the most considerable morphometric parameters (bronchial thickness, bronchial wall thickness, bronchial cavity thickness) are characteristic of large bronchi, then for medium-caliber bronchi, and the smallest indicators are characteristic of small bronchi.

Of great importance in morphology is the study of morphometric criteria (area, volume, the ratio of specific indicators, etc.), the characteristics of which are becoming increasingly relevant in morphological research in normal and pathological conditions and are highly visual indicators by which one can judge the structure and functional state of organs and tissues.

Histometric studies, which are often used in histology, allow us to clearly and reliably analyze not only quantitative changes at the tissue level but also provide an opportunity to conduct a detailed, in-depth analysis of the histoarchitectonics of the organism, namely the respiratory organs, including the lungs in the process of individual development and phylogenesis. This is not by chance since the mathematical analysis of the structures of morphological objects has received recognition as a method distinguished by objectivity and reliability.

That is why the quantitative histometric studies of lungs conducted from the standpoint of assessing their plasticity in animals of different levels of organization, namely representatives of the order Siluriformes, catfish, with various ecological and functional organizations, which are characterized by a moderate degree of activity and their living in an aquatic (semi-aquatic) environment, allowed us to identify specific patterns, trends and criteria regarding the structural organization of the lungs, their histoarchitectonics at the tissue level of organization.

Conclusion

The lungs of the *Clarias* catfish are paired, with pale pink anatomical structures with cellular walls that connect to the esophagus, providing atmospheric respiration. The medial and lateral lobes form the right and left lungs: the medial lobes are located in the cranial part of the body, respectively to the right and left of the esophagus; the lateral lobes adjoin the dorsal surfaces of the medial lobes, cranially bordering the branchial arches. The lungs of the *Clarias* catfish are covered with a connective tissue membrane (pleura), $552.6 \pm 7.9 \mu\text{m}$ thick, which consists of three layers: outer, middle, and inner.

Starting from the gill cavities, the main trunk of the medial lobe of the lungs branches tree-like into four large branches, large ones into medium ones, and the last ones into small ones, which are divided into separate branches, giving rise to thick-walled vesicles. The lateral lobe of the lung begins with an individual trunk, which is not connected to the medial lobe of the lung. Morphologically, the lungs have dorsal, ventral, mediastinal surfaces and cranial, caudal, and lateral edges.

The microscopic structure of the bronchial wall of the lungs of the *Clarias* catfish consists of three membranes – outer, middle (mus-

cular), and inner. The surface of the wall of the outer membrane is formed by epithelial cells that form elongated villi. The lamina propria of the outer membrane is formed by loose connective tissue in which there is a network of blood vessels. The muscular membrane of the bronchial wall is formed by transversely striated muscle tissue, the fibers of which have a longitudinal direction. The inner membrane of the bronchial wall is thin and formed by elongated epithelial cells.

The cavity of the bronchi, throughout the entire span of the bronchial tree, ending in the pulmonary alveoli, is filled with reticular tissue, the reticular cells of which, together with the reticular fibers (intertwining with each other), form a supporting mesh frame (base) of the cavity of the bronchial tree, due to which the bronchi do not collapse. The pulmonary alveoli, on a cross-section, have a rounded shape. In the wall of the connective tissue membrane, a significant network of vessels (capillaries) of the microcirculatory bed is found.

Authors declare no conflict of interests.

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