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Species-specific features of the heart morphology of *Rana temporaria*

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During amphibian metamorphosis, the single atrium becomes divided into the right and left atria, resulting in the formation of a three-chambered heart consisting of one ventricle and two atria. The venous sinus adjoins the right atrium and receives blood returning from the body organs, whereas oxygenated arterial blood from the respiratory organs enters the left atrium. Both atria communicate with the ventricle through a common opening, causing partial mixing of blood. However, this mixing remains incomplete due to muscular trabeculae projecting from the ventricular walls, which form a system of interconnected chambers. The arterial conus with a spiral valve, represented by a longitudinal fold directing blood flow during cardiac contraction, arises from the right portion of the ventricle, where venous blood predominates. Topographically, the heart is located on the ventral side of the anterior body cavity beneath the esophagus. The lateral surfaces of the right and left atria contact the medial surfaces of the bases of the right and left lungs, while the apex of the heart is directed caudally and to the right. The macro- and histoarchitecture of the common frog heart exhibit distinctive characteristics at different levels of organization. In particular, the morphometric parameters and structure of myocardial cardiomyocytes are associated with the absence of coronary vessels, indicating that the heart lacks its own blood supply system. The heart of an adult frog is ellipsoid in shape, dorsoventrally flattened, and belongs to the elongated-expanded type, as confirmed by the development index (158.2 ± 3.9%). The absolute heart weight is 0.14 ± 0.01 g, while the relative heart weight is 0.21 ± 0.02%. The atria are completely separated by a septum and communicate with the ventricle through a common opening. The arterial conus extends from the ventricle and is positioned ventrally, whereas the venous sinus adjoins the dorsal part of the right atrium. The ventricular myocardium has a spongy structure, containing a central cavity from which additional hollow chambers extend. The muscle fibers are arranged irregularly and, together with the cavities, form a reticular structure. Contractile cardiomyocytes are mononucleated, and their longitudinal and transverse striations are less pronounced than those observed in warm-blooded animals. Cytometric analysis revealed cardiomyocytes of small (6.75 ± 0.28 μm), medium (9.34 ± 0.46 μm), and large (11.86 ± 0.52 μm) widths in the ventricular myocardium. The corresponding nuclear volumes were 32.58 ± 2.08, 48.75 ± 2.20, and 64.82 ± 2.82 μm³, respectively. Histometric analysis demonstrated that the average area occupied by muscle fibers within the ventricular myocardium was 66.8 ± 2.0%, whereas the connective tissue stroma accounted for 34.2 ± 1.9%.

Keywords: vertebrates; phylogeny; amphibians; morphometry; common frog.

Introduction

The phylogenetic development of living organisms is closely associated with environmental conditions and the evolutionary history of each species. During adaptation to specific ecological conditions, vertebrates develop adaptive mechanisms manifested at various levels of structural and functional organization, including cellular, tissue, organ, systemic, and population levels.

An essential role in these adaptive processes is played by the cardiovascular system, which ensures the transport of oxygen, nutrients, and metabolic products, thereby maintaining homeostasis. The efficient functioning of this system is a prerequisite for the survival of animals and their adaptation to changing environmental factors.

The heart is the central organ of the circulatory system in vertebrates, ensuring continuous blood circulation through the vessels and maintaining vital functions of the organism. The structural characteristics of the heart reflect the evolutionary level of animal development and are closely related to habitat conditions, lifestyle, and patterns of gas exchange (Bishopric, 2005; Bettex et al., 2014; Stephenson et al., 2017). Amphibians occupy a unique position among vertebrates because they combine features of both aquatic and terrestrial lifestyles, which has resulted in the development of specific morphological and functional characteristics of their cardiovascular system.

The common frog (*Rana temporaria* Linnaeus, 1758) is one of the most widespread representatives of the class Amphibia in the fauna of Europe (Chikhlyaev & Ruchin, 2014; Streicher et al., 2021). Owing to its wide geographical distribution, relative abundance, and important ecological role, this species is frequently used as a model organism in morphological, physiological, and ecological studies (Rodrigues et al., 2013). Nevertheless, the structural organization of the heart in the common frog remains insufficiently characterized, and its investigation is important for understanding the adaptive mechanisms of the amphibian cardiovascular system as well as the evolutionary patterns of vertebrate circulation (Navas & James, 2007).

The study of the morphological features of the common frog heart contributes to a deeper understanding of the structural and functional adaptations of the amphibian cardiovascular system and expands current knowledge of the evolutionary transformations of circulatory organs during vertebrate phylogeny.

Therefore, the aim of this study was to investigate the morphological features of the heart of the common frog (*Rana temporaria*),

determine its morphotopographical characteristics, and perform a morphometric evaluation of its principal structural components.

Materials and methods

The study of the heart morphology of the common frog as a representative of vertebrate animals was conducted in accordance with modern methodological approaches and relevant standards, including the requirements of DSTU ISO/IEC 17025:2005 (2006). Animal housing and all experimental procedures complied with the Procedure for Conducting Experiments on Animals by Scientific Institutions (Law of Ukraine No. 249, 2012) and the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. The experimental investigations were carried out at the Department of Zoology, Biological Monitoring and Nature Conservation, 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 No. 0122U002270). All studies complied with the principles of Good Laboratory Practice (GLP, 1981) and the General Ethical Principles for Animal Experiments approved by the First National Congress on Bioethics (Kyiv, Ukraine, 2001).

The object of the study was the heart of the common frog (*Rana temporaria*; n = 5), family Ranidae. Macroscopic, microscopic, morphometric, and statistical methods were employed. Clinical examination, assessment of exterior characteristics (general appearance and body weight), and interior parameters (linear measurements, absolute and relative organ weights) were performed following anatomical dissection according to established herpetological and morphological guidelines (Horalskyi et al., 2019). To minimize the influence of stress-related factors, the frogs were anesthetized before dissection using a Hypnodil solution (5–10 mL/L).

Body weight was determined using VTD-3/0.1FD electronic scales (Dneproves, Ukraine) with an accuracy of 0.1 g. The absolute heart weight and the weights of its structural components were measured using electronic laboratory balances.

The relative organ weight (RW) was calculated according to the following formula:

$$RW = AW / BW \times 100,$$

where AW is the absolute organ weight (g), and BW is the body weight of the animal (g).

Linear dimensions of the heart, including length, width, and thickness, were determined by direct measurements using a vernier caliper, while circumference was measured using a flexible measuring tape.

The heart development index (HDI) was calculated as:

$$HDI = L / W \times 100,$$

where HDI is the heart development index, L is the heart length, and W is the heart width.

For histological examination, conventional methods of tissue fixation and histological slide preparation were used (Horalskyi et al., 2019). Heart tissue samples were fixed in a 12% cooled neutral buffered formalin solution and subsequently embedded in paraffin according to standard histological procedures described by Horalskyi et al. (2019). Paraffin sections were prepared using an MC-2 sliding microtome. Section thickness ranged from 10 to 12 μm. After deparaffinization, tissue sections were stained with hematoxylin (Diapath, Italy, 2020) and eosin (Leica Biosystems, Germany, 2020) for histological evaluation. Histometric measurements of cardiac structures were performed under light microscopy using Micros microscopes (Micros, 2012) and an MBS-10 stereomicroscope (Micromed, 1998), following the recommendations of Horalskyi et al. (2019). Photomicrographs were obtained using a CAM V-200 digital camera (InterMed, China, 2017) mounted on the microscope. All images of the macroscopic heart structure were obtained by the authors following animal dissection, whereas microscopic images were acquired from permanent histological preparations stained with hematoxylin and eosin. Morphological terminology was used in accordance with the International Anatomical Nomenclature and the International Veterinary Histological Nomenclature.

Statistical analysis of the obtained data was performed using Statistica 7.0 software (StatSoft Inc., Tulsa, OK, USA). Differences between groups were evaluated using analysis of variance (ANOVA), and differences were considered statistically significant at $P < 0.05$, with Bonferroni correction applied where appropriate.

Results

In the common frog (*Rana temporaria*), the heart is located on the ventral side of the cranial region of the body cavity beneath the esophagus, near the pharynx, and is enclosed within the pericardial cavity. Its surface is completely covered by the outer connective tissue membrane, the pericardium, which forms a thin-walled sac surrounding the heart. The organ is morphologically composed of three chambers: one ventricle and two atria, namely the right and left atria (Fig. 1a, 1b). Topographically, the lateral surfaces of the right and left atria are in contact with the medial surfaces of the bases of the right and left lungs, respectively. The apex of the heart is directed slightly to the right and caudally (Fig. 1). The right atrium is positioned caudally to the cranial surface of the ventricular base and occupies approximately the right half of its surface. Correspondingly, the left atrium occupies the left half of the cranial surface of the ventricle (Fig. 1a, 2b). The heart of the common frog is ellipsoid in shape and dorsoventrally flattened. Its cranial surface exhibits an irregular configuration (Fig. 2a, b).

The atria are completely separated by an interatrial septum; however, both atria communicate with the ventricle through a common atrioventricular opening. A sac-like conus arteriosus arises from the ventral aspect of the ventricle, whereas the sinus venosus is attached to the dorsal part of the right atrium. The right border of the ventricle gradually transitions, within its upper two-thirds, into a cardiac notch characterized by a slightly concave appearance. On both the cranial and caudal surfaces of the heart, the interventricular grooves are poorly developed and only weakly distinguishable. In the frog heart, venous blood is contained within the right atrium, whereas the left atrium contains only oxygenated arterial blood. Blood mixing occurs within the ventricle. Oxygen-rich arterial blood is preferentially directed to the brain, while the remainder of the body receives mixed blood.

According to the morphometric analysis, the absolute heart weight of the common frog was 0.14 ± 0.005 g, whereas the relative heart weight was 0.21 ± 0.02%. The absolute and relative weights of its structural components varied depending on the functional workload imposed on each chamber during the cardiac cycle. The ventricle exhibited the greatest absolute weight, reaching 0.11 ± 0.01 g (79.03 ± 4.55% of total heart weight). The absolute weight of the right atrium was significantly lower ($P < 0.01$), being 7.3-fold less than that of the ventricle and amounting to 0.015 ± 0.002 g (10.86 ± 0.30%). The lowest value was recorded for the left atrium ($P < 0.001$), with an absolute weight of 0.014 ± 0.001 g (10.11 ± 0.27%) (Table 1).

Under these morphometric conditions, the ratio of ventricular weight to total heart weight was 1:0.78, whereas the corresponding ratios for the right and left atria were 1:0.11 and 1:0.10, respectively (Table 1). These findings are associated with the greater functional demand placed on ventricular cardiomyocytes during systole. Ventricular contraction generates the pressure required to propel blood throughout the body, whereas the atria function primarily to transfer blood into the ventricle, a process requiring substantially lower mechanical workload.

According to the morphometric assessment of the heart, its length measured 9.62 ± 0.67 mm, width 6.08 ± 0.32 mm, thickness 4.30 ± 0.20 mm, and circumference 16.04 ± 1.20 mm. The thickness of the walls of the cardiac chambers differed considerably. The ventricular wall was the thickest, measuring 2.24 ± 0.38 mm. In contrast, the wall thicknesses of the right atrium (0.28 ± 0.03 mm) and left atrium (0.26 ± 0.02 mm) were similar and were almost eight times lower ($P \leq 0.001$) than that of the ventricle (Table 2).

Based on these morphometric characteristics, the heart development index of the common frog was 158.2 ± 3.9%. Therefore, the heart can be classified as ellipsoid in shape and belonging to the elongated-expanded type (Table 2).

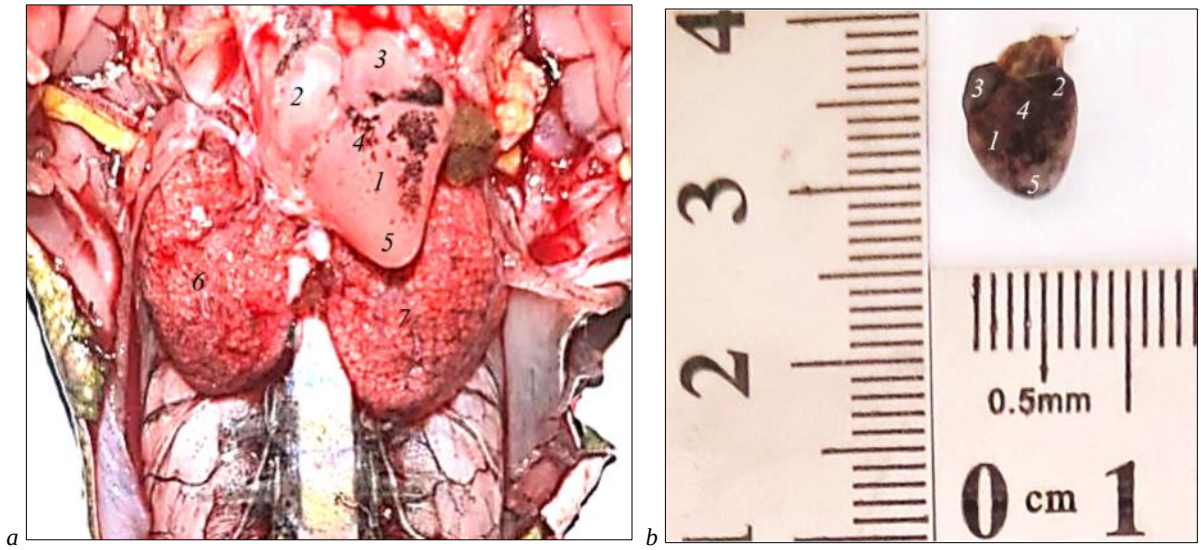

Fig. 1. Morphology of the heart of the common frog (*Rana temporaria*): *a* – cranial region of the abdominal cavity after dissection; *b* – heart after fixation in formalin; *1* – ventricle; *2* – right atrium; *3* – left atrium; *4* – base of the ventricle; *5* – apex of the ventricle; *6* – right lung; *7* – left lung; gross specimen

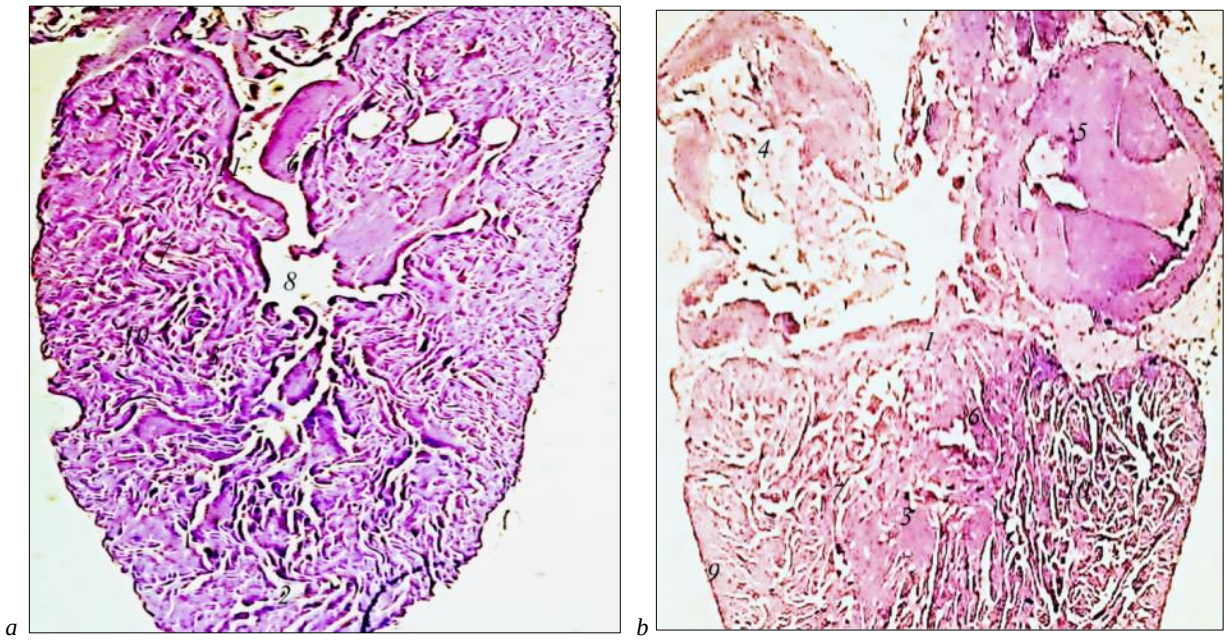

Fig. 2. Histological structure of the heart of the common frog (*Rana temporaria*) (total section): *a* – longitudinal section of the ventricle; *b* – longitudinal section of the heart; *1* – cranial surface of the heart; *2* – cardiac apex; *3* – ventricle; *4* – right atrium; *5* – left atrium; *6* – ventricular base; *7* – ventricular wall; *8* – central ventricular cavity; *9* – epicardium; *10* – myocardium; *11* – endocardium; hematoxylin and eosin stain

Table 1
Morphometric parameters of the absolute heart weight and the weights of its structural components in the common frog (*Rana temporaria*) (x ± SD, n = 5)

Parameter	Absolute mass, g	Relative mass, %
Heart	0.14 ± 0.01	0.21 ± 0.02
Ventricle (relative to total heart mass)	0.11 ± 0.01	79.03 ± 4.55
Right atrium (relative to total heart mass)	0.015 ± 0.002	10.86 ± 0.30
Left atrium (relative to total heart mass)	0.014 ± 0.001	10.11 ± 0.27
The ratio of the mass of the ventricle to the absolute mass of the heart	1:0.78	
Ratio of right atrial mass to absolute heart mass	1:0.11	
The ratio of the mass of the left atrium to the absolute mass of the heart	1:0.10	

The ventricle is the most developed morphological component of the heart in the common frog. It is a hollow muscular chamber whose cranial portion is markedly expanded, forming the base of the heart. The caudal portion of the ventricle is rounded in shape and forms a prominent cardiac apex (Fig. 3a).

Table 2
Linear parameters of the heart of the common frog (*Rana temporaria*) (x ± SD, n = 5)

Parameter	Value
Absolute heart mass, g	0.140 ± 0.005
Relative heart mass, %	0.21 ± 0.02
Heart length, mm	9.62 ± 0.67
Heart width, mm	6.08 ± 0.32
Heart thickness, mm	4.30 ± 0.20
Heart circumference, mm	16.0 ± 1.2
Heart development index, %	158.2 ± 3.9
Ventricular wall thickness, mm	2.24 ± 0.38
Right atrial wall thickness, mm	0.28 ± 0.03***
Left atrial wall thickness, mm	0.26 ± 0.02***

Fig. 3. Gross structure of the heart of the common frog (*Rana temporaria*) after formalin fixation: *a* – longitudinal section; *b* – transverse section; *1* – ventricle; *2* – right atrium; *3* – left atrium; *4* – ventricular base; *5* – ventricular apex; *6* – ventricular wall; *7* – ventricular cavity; *8* – epicardium; *9* – myocardium; *10* – endocardium; gross specimen

The ventricular wall consists of three layers: the inner layer (endocardium), the middle muscular layer (myocardium), and the outer layer (epicardium) (Fig. 3a, 3b).

The middle layer of the ventricular wall, the myocardium, exhibits a characteristic spongy structure. Histological examination of longitudinal heart sections stained with hematoxylin and eosin revealed a central ventricular cavity from which numerous additional hollow chambers extend into the myocardial wall. These chambers are bounded by numerous muscular trabeculae, resulting in the formation of intertrabecular spaces (Fig. 4a, 4b).

The myocardial trabeculae of the ventricular wall display an irregular and disorganized arrangement in hematoxylin- and eosin-stained sections (Fig. 4a, 4b). Some intertrabecular spaces are slit-like in shape and, together with the larger hollow chambers, form a reticular network within the myocardium (Fig. 4a, 4b).

Numerous trabeculae extend from the ventricular apex toward the ventricular base and are arranged parallel to one another. The slit-like spaces formed between them communicate directly with the central ventricular cavity (Fig. 4a, 4b). In addition, some of the larger trabeculae are interconnected through anastomosing branches, creating an intricate three-dimensional network. This trabecular architecture, together with the elongated cavities and chambers, modifies the internal relief of the ventricle and increases its effective volume.

Within the intertrabecular spaces, additional longitudinally oriented muscular trabeculae are present. These structures originate from the ventricular wall and attach to the leaflets of the atrioventricular valve. They are flattened in shape and considerably larger than the surrounding trabeculae.

Thus, the ventricular myocardium of the common frog possesses a distinctly spongy organization. The ventricular lumen is divided into a central cavity and numerous accessory chambers bounded by muscular trabeculae. These trabeculae perform a functional role analogous to that of papillary muscles, which are characteristic of mammals but have not yet evolved as distinct anatomical structures in the heart of the common frog. Muscle fibers within the ventricular myocardium are frequently arranged parallel to one another, forming bundles of varying thickness that can be classified as small, medium, and large (Fig. 5a). The width of these bundles ranges from 18.73 ± 1.32 μm to 48.64 ± 1.73 μm, whereas bundles of medium caliber measure 34.12 ± 1.44 μm in width (Fig. 5a; Table 3). In transverse sections, the bundles of myocardial fibers exhibit irregular profiles of various shapes, including triangular, oval, elongated, and V-shaped configurations (Fig. 5b, 5c). These histological structures are externally surrounded by connective tissue layers, which are also clearly visible between individual myocardial fibers. The intermuscular connective tissue septa vary considerably in both width and shape (Fig. 5c, 5d).

Histometric analysis demonstrated that muscle fibers occupied, on average, 66.8 ± 2.03% of the ventricular myocardium, whereas connective tissue accounted for 34.2 ± 1.9% of the myocardial area (Table 3).

Table 3
Histometric characteristics of the ventricular myocardium in the common frog (*Rana temporaria*) (x ± SD, n = 5)

Parameter	Value
Small-caliber myocardial bundles, μm	18.73 ± 1.32
Medium-caliber myocardial bundles, μm	34.12 ± 1.44
Large-caliber myocardial bundles, μm	48.64 ± 1.73
Area occupied by muscle fibers, %	66.80 ± 2.03
Area occupied by connective tissue stroma, %	34.20 ± 1.93

The principal structural components of the myocardial layer are contractile myocytes, namely cardiomyocytes, which are interconnected to form myocardial fibers of varying thickness. Based on their width, cardiomyocytes were classified into small (6.75 ± 0.28 μm), medium (9.34 ± 0.46 μm), and large (11.86 ± 0.52 μm) categories. The corresponding nuclear volumes were 32.58 ± 2.08 μm³, 48.75 ± 2.20 μm³, and 64.82 ± 2.82 μm³, respectively (Table 4).

Within the sarcoplasm of cardiomyocytes, both transverse and longitudinal striations can be distinguished. The transverse striation is associated with the orderly arrangement of actin and myosin filaments, whereas the longitudinal striation reflects the organization of myofibrils. The surface of each cardiomyocyte is clearly delineated by the plasmalemma.

The nuclei of the contractile cardiomyocytes are centrally located and exhibit oval, elongated, or rod-shaped profiles. A distinct nuclear envelope (karyolemma) surrounds the nucleus. Within the nucleoplasm, small nucleoli and heterochromatin aggregates are clearly visible.

The myocardial layer is lined internally by a simple squamous endothelium, which forms the endocardium, a thin inner connective tissue layer of the cardiac wall (Fig. 5a). Externally, the myocardium is covered by the outer connective tissue membrane of the heart, the pericardium (Fig. 5b).

Table 4
Histometric characteristics of ventricular myocardial cardiomyocytes in the common frog (*Rana temporaria*) (x ± SD, n = 5)

Cardiomyocyte category	Cardiomyocyte width, μm	Nuclear volume, μm³
Small-sized	6.75 ± 0.28	32.58 ± 2.08
Medium-sized	9.34 ± 0.46	48.75 ± 2.20
Large-sized	11.86 ± 0.52	64.82 ± 2.82

Thus, the cardiovascular system of the common frog (*Rana temporaria*) represents a more advanced stage of phylogenetic development than that of fishes, owing to the evolution of pulmonary respiration, the establishment of the pulmonary (minor) circulation, and the formation of a three-chambered heart. The morphoarchitecture of this organ exhibits distinct features at the organ, tissue, and cellular levels. During metamorphosis, the single atrium of amphibians becomes divided into right and left atria, resulting in the formation of a three-chambered heart composed of one ventricle and two atria. The sinus venosus adjoins the right atrium and receives venous blood returning from the body, whereas oxygenated arterial blood from the respiratory organs enters the left atrium. Both atria communicate with the ventricle through a common atrioventricular opening, which results in partial mixing of blood. However, this mixing is incomplete owing to the presence of muscular trabeculae projecting from the ventricular walls, which subdivide the ventricular lumen into interconnected chambers.

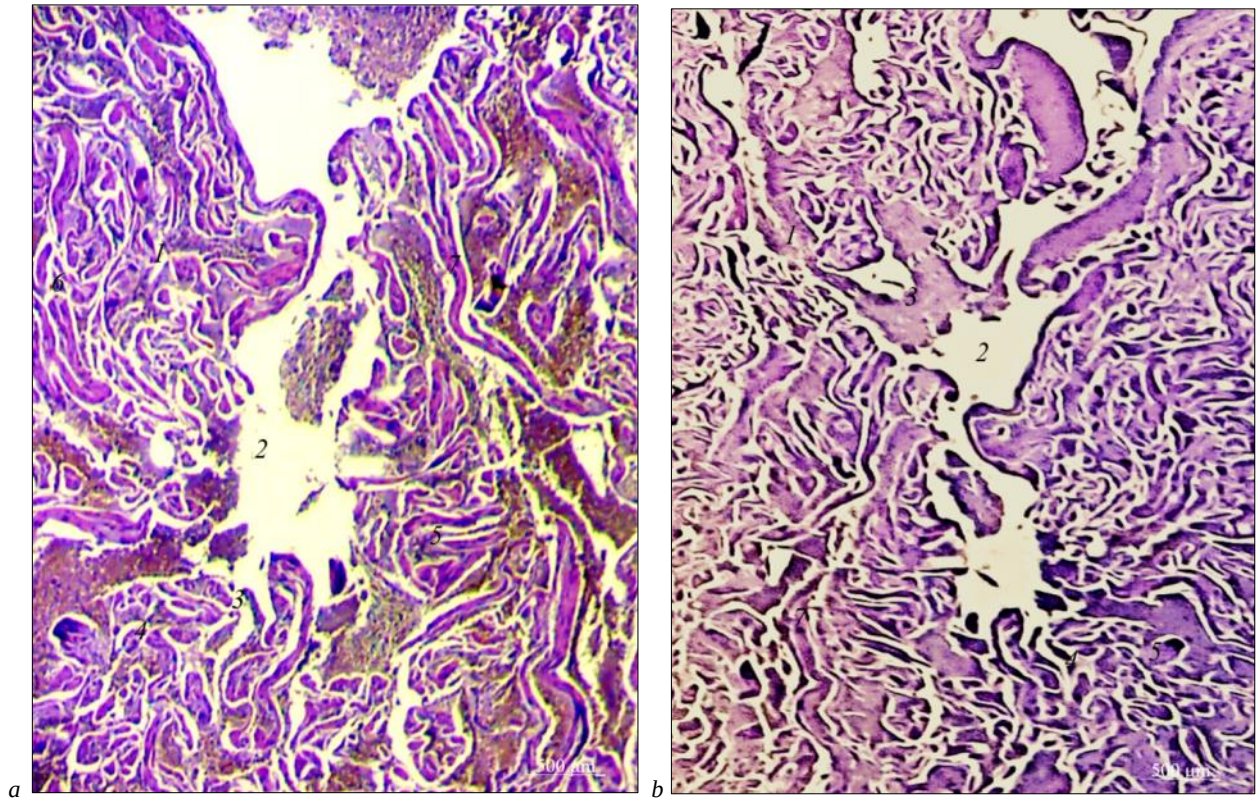

Fig. 4. Histological structure of the ventricular myocardium in the common frog (*Rana temporaria*): *a, b* – representative sections of the ventricular wall; *1* – ventricular wall; *2* – central ventricular cavity; *3* – accessory cavities; *4* – slit-like spaces; *5* – transverse trabeculae; *6* – parallel arrangement of trabeculae; *7* – longitudinal trabeculae; hematoxylin and eosin stain

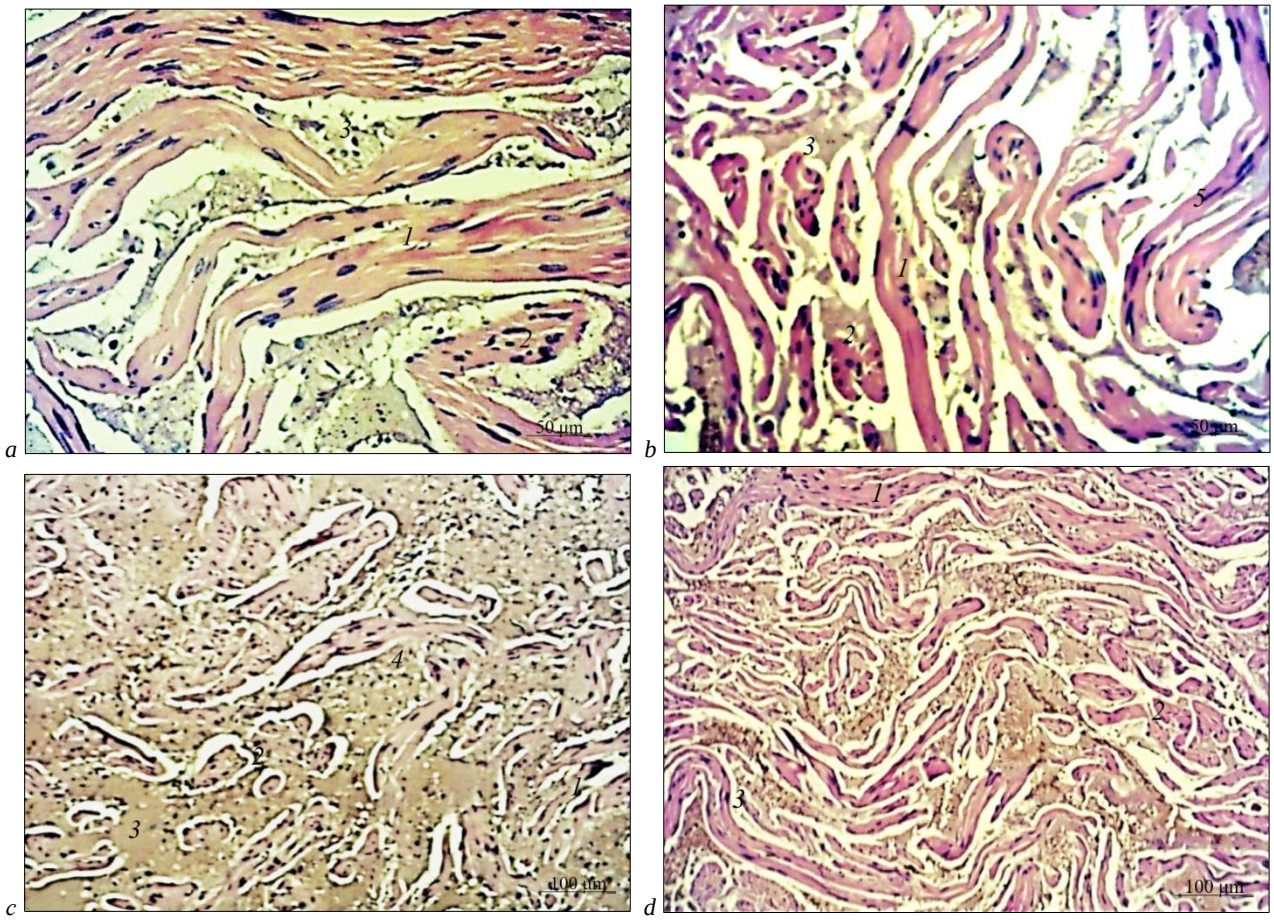

Fig. 5. Histological features of the ventricular myocardium in the common frog (*Rana temporaria*): *a–d* – representative sections of the ventricular wall; *1* – longitudinally sectioned myocardial fibers; *2* – transversely sectioned myocardial fibers; *3* – intermuscular connective tissue; *4* – V-shaped bundle of myocardial tissue; *5* – elongated bundle of myocardial tissue; hematoxylin and eosin stain

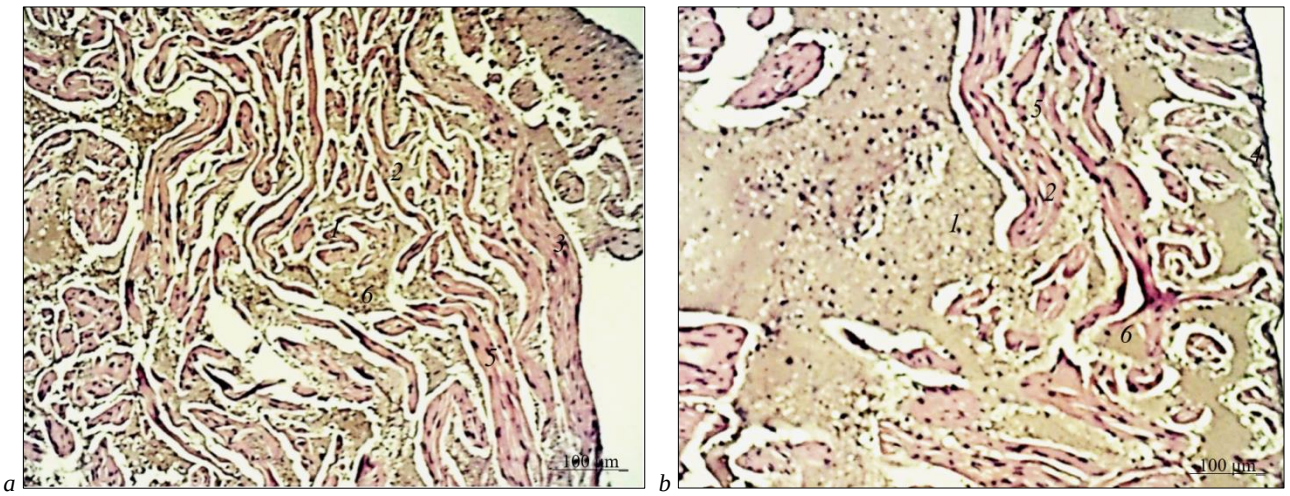

Fig. 6. Histological structure of the ventricular wall in the common frog (*Rana temporaria*): *a*, *b* – representative sections of the ventricular wall; *1* – ventricular wall; *2* – myocardium; *3* – endocardium; *4* – pericardium; *5* – myocardial fibers; *6* – intermuscular connective tissue; hematoxylin and eosin stain

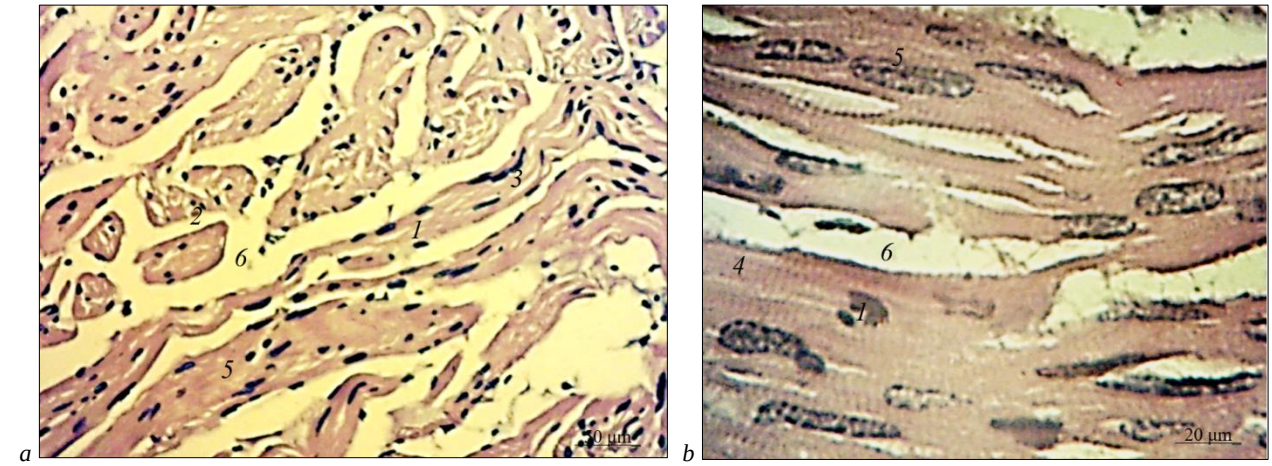

Fig. 7. Histological structure of ventricular myocardial fibers in the common frog (*Rana temporaria*): *a*, *b* – representative histological sections; *1* – longitudinally sectioned myocardial fibers; *2* – transversely sectioned myocardial fibers; *3* – longitudinal striation; *4* – transverse striation; *5* – nuclei of myocardial fibers (cardiomyocytes); *6* – intermuscular connective tissue; hematoxylin and eosin stain

The conus arteriosus arises from the right side of the ventricle, where venous blood predominates, and contains a spiral valve represented by a longitudinal fold that directs and distributes blood flow during cardiac contraction. Therefore, both structurally and functionally, the cardiovascular system of the common frog and its central organ, the heart, occupy an intermediate evolutionary position between those of fishes and fully terrestrial vertebrates.

Discussion

The cardiovascular system plays a pivotal role in regulating the functions of organs and organ systems and is actively involved in maintaining trophic, respiratory, and excretory processes (Zhurenko et al., 2018; Goralskyi et al., 2021; Horalskyi et al., 2023). Through the systemic and pulmonary circulatory pathways, oxygen, nutrients, hormones, and other biologically active substances are delivered to tissues, while metabolic by-products are removed from the organism (Svendsen et al., 2019; Horalskyi et al., 2023; Rahulia et al., 2023).

Consequently, the investigation of the cardiovascular system, particularly the heart, remains one of the major areas of modern morphological research. Comparative studies of cardiac structure in vertebrates provide valuable insights into the evolutionary transformations that have occurred during phylogenesis and contribute to a better understanding of structural and functional adaptations in normal and experimental conditions (Horalskyi et al., 2022; Horalskyi et al., 2025).

Recent studies have significantly expanded current knowledge regarding the morphoarchitecture of the heart and its components at the organ, tissue, cellular, and subcellular levels in birds and mammals (Hnatiuk et al., 2016; Hnatiuk & Slabyi, 2016; Hnatiuk et al., 2017). These investigations have established quantitative relationships among morphometric and histological parameters of the heart and identified significant correlations that contribute to a deeper understanding of cardiac anatomy and development. Experimental studies of the cardiovascular system in vertebrates, particularly domestic animals, have revealed previously unknown structural and functional features, emphasizing the need for further comparative investigations involving different species, breeds, age groups, and physiological states (Horalskyi et al., 2022, 2024; Rahulia et al., 2023).

In contrast, considerably fewer studies have focused on the morphology of the heart in fishes, amphibians, and reptiles. As a result, information regarding the structural organization of the cardiovascular system in these vertebrate groups remains relatively limited. This situation highlights the importance of further investigations aimed at clarifying the evolutionary pathways and adaptive mechanisms underlying cardiovascular development.

The common frog (*Rana temporaria* Linnaeus, 1758) is a semi-aquatic amphibian belonging to the brown frog group (Ranidae) and represents a suitable model for studying evolutionary adaptations of the cardiovascular system. Owing to its ecological characteristics and intermediate phylogenetic position between aquatic and terrestrial vertebrates, this species provides valuable information concerning the structural modifications associated with the transition from aquatic to terrestrial life. The present study demonstrated that the heart of the common frog is located on the ventral side of the cranial body cavity

beneath the esophagus. The lateral surfaces of the right and left atria are in contact with the medial surfaces of the bases of the right and left lungs, respectively, whereas the cardiac apex is directed caudally and slightly to the right. The right atrium adjoins the cranial surface of the ventricular base and occupies approximately the right half of this region, while the left atrium occupies the corresponding left half.

The obtained findings support the concept that evolutionary transformations of vertebrate organs and organ systems are closely associated with environmental conditions and the phylogenetic history of the organism. During the course of vertebrate evolution, progressive structural modifications have occurred within the cardiovascular system, enabling more efficient circulation and improved oxygen transport. These adaptations are particularly evident in amphibians, whose cardiovascular system exhibits features characteristic of both aquatic and terrestrial modes of life. The structural organization of the common frog heart observed in the present study reflects these evolutionary adaptations and confirms its intermediate position between the simpler circulatory system of fishes and the more advanced cardiovascular systems of fully terrestrial vertebrates.

The evolution of the cardiovascular system has been characterized by several major trends, including the emergence of the heart and an increase in the number of its chambers, the differentiation of blood vessels arising from the heart, and improvements in blood oxygenation efficiency. In primitive organisms, the transport of nutrients and respiratory gases occurs primarily through diffusion across cells directly exposed to the external environment. During vertebrate evolution, fishes developed a two-chambered heart consisting of one atrium and one ventricle. Alternating contractions and relaxations of the atrial and ventricular myocardium ensure blood circulation throughout the vascular system (Spaink et al., 2014; Martins et al., 2021; Chan et al., 2022).

In fishes, venous blood passes through the heart and is transported via the ventral aorta to the gills, where gas exchange occurs and the blood becomes oxygenated. In air-breathing fishes, such as the African sharptooth catfish (*Clarias gariepinus*), additional morphological adaptations of the cardiovascular system have evolved in association with the development of aerial respiration alongside branchial respiration (Horalskyi et al., 2025).

Most reptiles possess a three-chambered heart composed of two atria and one ventricle, whereas birds and mammals have a four-chambered heart that completely separates oxygenated and deoxygenated blood, thereby preventing their mixing within the circulatory system. In amphibians, including the common frog (*R. temporaria*), the evolution of pulmonary respiration resulted in the appearance of the pulmonary (minor) circulation. During metamorphosis, the originally single atrium becomes divided into right and left chambers, leading to the formation of a three-chambered heart. The sinus venosus adjoins the right atrium and receives venous blood from the systemic circulation, whereas oxygenated blood returning from the respiratory organs enters the left atrium. Both atria communicate with a common ventricle through a shared atrioventricular opening, resulting in partial blood mixing. However, complete mixing is prevented by the complex arrangement of muscular trabeculae within the ventricular wall, which subdivide the ventricular lumen into interconnected chambers. Furthermore, the conus arteriosus originates from the ventricle and contains a spiral valve that directs blood flow during cardiac contraction, thereby contributing to the functional separation of blood streams.

Morphometry represents one of the principal approaches in modern morphology for evaluating structural development at the cellular, tissue, and organ levels. It provides an objective quantitative assessment of biological structures and enables a more comprehensive understanding of organogenesis, morphogenesis, and evolutionary transformations of organs and tissues (Dzau et al., 2006; Hnatiuk & Slabyi, 2016; Hnatiuk et al., 2017). Quantitative morphometric methods facilitate the interpretation of structural adaptations and allow meaningful comparisons among species, developmental stages, and environmental conditions (Stakhurska & Pryshliak, 2014; Dunaievska et al., 2023; Horalskyi et al., 2024).

Particularly informative are measurements of absolute and relative organ mass, which reflect not only the degree of organ development and morphofunctional maturity (Mits et al., 2016), but also provide valuable comparative indicators for assessing organ growth patterns and developmental indices (Horalskyi et al., 2019; Horalskyi et al., 2024). In the present study, these parameters contributed to a comprehensive evaluation of the structural organization and morphofunctional characteristics of the heart in the common frog.

The absolute and relative masses of the cardiac chambers are closely associated with the functional workload imposed on each anatomical structure during the cardiac cycle. In the present study, the ventricle exhibited the greatest absolute mass, reaching 0.11 ± 0.01 g ($79.03 \pm 4.55\%$ of total heart mass). In contrast, the absolute mass of the right atrium was significantly lower ($P < 0.01$), being 7.3-fold less than that of the ventricle and amounting to 0.015 ± 0.002 g ($10.86 \pm 0.30\%$). The lowest value was recorded for the left atrium ($P < 0.001$), whose absolute mass was 0.014 ± 0.001 g ($10.11 \pm 0.27\%$).

These findings can be explained by the greater mechanical workload performed by ventricular cardiomyocytes during systole. Ventricular contraction generates the pressure required to propel blood throughout the circulatory system and ensure tissue perfusion. Consequently, the ventricle possesses the highest mass ratio relative to the total heart mass (1:0.78). In contrast, the right and left atria function primarily as receiving chambers that transfer blood into the ventricle, requiring substantially less contractile force. Accordingly, the atrium-to-heart mass ratios were markedly lower, amounting to 1:0.11 for the right atrium and 1:0.10 for the left atrium.

Linear dimensions, including length, width, thickness, and circumference, are among the principal morphological criteria used to assess organ growth and development. These parameters allow the determination of organ shape, developmental indices, and other morphometric characteristics (Mits et al., 2016; Dukhnytskyi et al., 2024).

Heart size, shape, and mass are species-specific characteristics influenced by taxonomic affiliation, age, sex, body conformation, and physiological status. Moreover, the ratio between the greatest heart length and its basal width is commonly used as an indicator of cardiac shape in vertebrates (Shevchenko, 2018).

According to the morphological studies of Nazarova and Kramar (2021), the heart of most frog species exhibits an ellipsoid shape (94%), whereas a spherical form occurs in approximately 4% of individuals. A conical heart shape has been reported only rarely (2%) (Nazarova & Kramar, 2021).

The present study demonstrated that the heart of the common frog measured 9.62 ± 0.67 mm in length, 6.08 ± 0.32 mm in width, and 4.30 ± 0.20 mm in thickness, with a circumference of 16.04 ± 1.20 mm. Based on these measurements, the heart development index was calculated as $158.22 \pm 3.94\%$, indicating that the heart of the common frog is ellipsoid in shape and belongs to the elongated-expanded type.

An important indicator of cardiac functional status is the thickness of the ventricular and atrial walls, which collectively determine the structural organization of the heart (Vlasenko & Kuzmenko, 2010). In the present study, the ventricular wall was substantially thicker (2.24 ± 0.38 mm) than the walls of either atrium. The right and left atrial walls measured 0.28 ± 0.03 mm and 0.26 ± 0.02 mm, respectively, and did not differ markedly from one another.

The fundamental structural and functional units of the myocardium are cardiomyocytes. Through their coordinated contraction, these cells generate blood flow within the closed circulatory system, thereby ensuring the transport of oxygen and nutrients to tissues and the removal of metabolic waste products (Ben-Shachar et al., 1985; Goralskyi et al., 2021; Horalskyi et al., 2022). Together with the connective tissue stroma, cardiomyocytes form the myocardial layer of the heart. Histometric analysis revealed that myocardial fibers occupied $66.8 \pm 2.0\%$ of the ventricular myocardium, whereas connective tissue accounted for $34.2 \pm 1.9\%$ of the myocardial area. This predominance of contractile tissue reflects the functional specialization of the ventricle as the principal pumping chamber of the amphibian heart.

In the common frog, the ventricular myocardium exhibits a characteristic spongy organization. A central ventricular cavity occupies the interior of the ventricle, from which numerous accessory chambers extend into the myocardial wall. These chambers are bounded by muscular trabeculae that form a complex system of intertrabecular spaces. The myocardial trabeculae are arranged irregularly, and together with the accessory chambers create a reticular three-dimensional architecture of the ventricular myocardium.

Myocardial fibers are frequently arranged in parallel bundles of different sizes (small, medium, and large). In transverse sections, these bundles display irregular profiles that are commonly triangular, oval, elongated, or V-shaped. Such structural organization contributes to the formation of a highly specialized myocardial architecture adapted to the functional requirements of the amphibian heart.

The contractile myocytes (cardiomyocytes) forming the myocardial fibers vary in width from 6.75 ± 0.28 to 11.86 ± 0.52 μm. These dimensions are considerably smaller than those reported for mammals (Horalskyi et al., 2024), which may be associated with species-specific physiological characteristics and the unique mechanism of myocardial oxygen supply in amphibians.

The morphological organization of the common frog heart, including its morphometric characteristics and myocardial histoarchitecture, appears to be closely related to the absence of coronary vessels. Unlike mammals and birds, the amphibian heart lacks a well-developed coronary circulation. Consequently, the myocardium retains a spongy (trabecular) organization that facilitates oxygen diffusion directly from the blood contained within the ventricular lumen to the cardiomyocytes.

Under these conditions, oxygen delivery occurs primarily through diffusion rather than through a dedicated coronary vascular network. In addition, amphibian cardiomyocytes differ from those of mammals in possessing a single centrally located nucleus and a lower proportion of cytoplasmic volume occupied by myofibrils. Furthermore, the transverse striation of cardiomyocytes is less pronounced than that observed in endothermic vertebrates, which is consistent with the histological findings obtained in the present study.

Overall, the heart of the common frog conforms to the general structural plan characteristic of amphibians and resembles that described for the marsh frog (*Pelophylax ridbundus*) and other anuran species. Both species possess a three-chambered heart composed of two atria and a single ventricle. The right atrium receives venous blood from the systemic circulation, whereas the left atrium receives oxygenated blood from the lungs and skin. Although blood is partially mixed within the ventricle, the trabecular organization of the myocardium and the presence of the spiral valve contribute to a relative separation of blood streams.

Nevertheless, certain species-specific differences are evident. The marsh frog generally possesses a larger and more elongated heart, which may reflect its more aquatic lifestyle and increased oxygen demands during prolonged periods in water. In contrast, the common frog has a relatively smaller and more compact heart. The comparatively greater ventricular wall thickness relative to overall heart size may represent an adaptation that enhances circulatory efficiency under conditions of variable locomotor activity and terrestrial habitat use. Therefore, both structurally and functionally, the cardiovascular system of the common frog occupies an intermediate evolutionary position between the simpler circulatory system of fishes and the fully separated cardiovascular systems of terrestrial vertebrates. These findings further support the concept that the amphibian heart represents an important transitional stage in the evolution of vertebrate circulation.

Conclusions

The circulatory system of amphibians occupies an intermediate evolutionary position between that of fishes and terrestrial vertebrates. Compared with fishes, which represent the most numerous group of vertebrates, the common frog (*Rana temporaria* Linnaeus, 1758) exhibits a more advanced level of phylogenetic development due to the emergence of a second (pulmonary) circulation and a three-chambered heart. During metamorphosis, the originally single atrium becomes divided into right and left atria, resulting in the formation of a three-chambered heart composed of one ventricle and two atria.

The morphotopography, macrostructure, and histoarchitecture of the common frog heart exhibit distinct features at the organ, tissue, and cellular levels. The morphometric characteristics and myocardial histoarchitecture are associated with the absence of coronary vessels, indicating that the heart lacks an independent coronary blood supply system.

Topographically, the heart is located on the ventral side of the cranial body cavity beneath the esophagus. The lateral surfaces of the right and left atria are in contact with the medial surfaces of the bases of the corresponding lungs, while the cardiac apex is directed caudally and slightly to the right.

In adult frogs, the heart is ellipsoid in shape, dorsoventrally flattened, and belongs to the elongated-expanded type, as indicated by its heart development index (158.2 ± 3.9%). The absolute heart mass is 0.14 ± 0.005 g, whereas the relative heart mass is 0.21 ± 0.02%. The atria are completely separated by an interatrial septum and communicate with the ventricle through a common atrioventricular opening. The conus arteriosus originates from the ventral aspect of the ventricle, whereas the sinus venosus adjoins the dorsal part of the right atrium.

The ventricular myocardium exhibits a characteristic spongy architecture. A central ventricular cavity occupies the interior of the ventricle and gives rise to numerous accessory chambers. Myocardial fibers are arranged irregularly and, together with these chambers, form a reticular structural network. Contractile cardiomyocytes contain a single centrally located nucleus, and their longitudinal and transverse striations are less pronounced than those observed in endothermic vertebrates.

Cytometric analysis revealed three populations of ventricular cardiomyocytes based on cell width: small (6.75 ± 0.28 μm), medium (9.34 ± 0.46 μm), and large (11.86 ± 0.52 μm). The corresponding nuclear volumes were 32.58 ± 2.36, 48.75 ± 3.17, and 64.82 ± 4.02 μm³, respectively. Histometric analysis demonstrated that myocardial fibers occupied 66.8 ± 2.0% of the ventricular myocardium, whereas connective tissue stroma accounted for 34.2 ± 1.9% of the myocardial area.

References

Ben-Shachar, G., Arcilla, R. A., Lucas, R. V., & Manasek, F. J. (1985). Ventricular trabeculations in the chick embryo heart and their contribution to ventricular and muscular septal development. Circulation Research, 57(5), 759–766.

Bettex, D. A., Prêtre, R., & Chassot, P. G. (2014). Is our heart a well-designed pump? The heart along animal evolution. European Heart Journal, 35(34), 2322–2332.

Bishopric, N. H. (2005). Evolution of the heart from bacteria to man. Annals of the New York Academy of Sciences, 1047, 13–29.

Chan, J. T. H., Kadri, S., Köllner, B., Rebl, A., & Korytář, T. (2022). RNA-Seq of single fish cells – seeking out the leukocytes mediating immunity in teleost fishes. Frontiers in Immunology, 13, 798712.

Chikhlyaev, I., & Ruchin, A. (2014). The helminth fauna study of European common brown frog (*Rana temporaria* Linnaeus, 1758) in the Volga Basin. Acta Parasitologica, 59(3), 459–471.

Dukhnytskyi, V. B., Horalskyi, L. P., Sokolyuk, V. M., Gutyj, B. V., Ishchenko, V. D., Ligomina, I. P., Kolesnik, N. L., & Dzhmil, V. I. (2024). Morphofunctional changes in the internal organs of laying hens affected by chronic thiamethoxam intoxication. Regulatory Mechanisms in Biosystems, 15(3), 578–586.

Dunaievska, O. F., Horalskyi, L. P., Sokulskiy, I. M., Radzikhovskyi, M. L., & Gutyj, B. V. (2023). Influence of protein-vitamin mineral supplements on the splenic morphometric parameters of quails. Regulatory Mechanisms in Biosystems, 14(2), 242–247.

Dzau, V. J., Antman, E. M., Black, H. R., Hayes, D. L., Manson, J. E., Plutzky, J., Popma, J. J., & Stevenson, W. (2006). The cardiovascular disease continuum validated: Clinical evidence of improved patient outcomes: Part I: Pathophysiology and clinical trial evidence (risk factors through stable coronary artery disease). Circulation, 114(25), 2850–2870.

Goralskyi, L., Ragulya, M., Sokulskyi, I., Kolesnik, N., & Goralska, I. (2021). Morphological and morphometrical characteristics of cattle heart structure.

Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies, Series: Veterinary Sciences, 23(103), 145–151.

Hnatiuk, M. S., & Slabyi, O. B. (2016). Morfometrychna otsinka osoblyvostei remodeliuvannia kamer lehenevoho sertsia z riznymy typamy krovopostachannia [Morphometric assessment of the remodeling features of the pulmonary heart chambers with different types of blood supply]. Zdobutky Klinichnoi i Eksperymentalnoi Medytsyny, 1, 17–20 (in Ukrainian).

Hnatiuk, M. S., Slabyi, O. B., & Tatarchuk, L. V. (2016). Yaderno-tsytoplazmatychni vidnoshennia u kardiomiotsytakh ta endoteliotsytakh shlunochkiv lehenevoho sertsia [Nuclear-cytoplasmic relationships in cardiomyocytes and endothelial cells of the ventricles in cor pulmonale]. Klinichna Anatomiia ta Operatyvna Khirurhiia, 15(55), 67–70 (in Ukrainian).

Hnatiuk, M. S., Slabyi, O. B., & Tatarchuk, L. V. (2017). Prostorova kharakterystyka kamer sertsia doslidnykh tvaryn z riznymy typamy vehetatyvnoi rehuliatsii [Spatial characteristics of heart chambers of experimental animals with different types of autonomic regulation]. Biomedical and Biosocial Anthropology, 28, 35–39 (in Ukrainian).

Hnatiuk, M. S., Slabyi, O. B., Tatarchuk, L. V. (2017). Kilkisnyi morfolohichnyi analiz deiakykh ultrastruktur skorotlyvykh kardiomiotsytiv pravoho shlunochka lehenevoho sertsia [Quantitative morphological analysis of some ultrastructures of contractile cardiomyocytes of the right ventricle in cor pulmonale]. Visnyk Naukovykh Doslidzhen, 3(88), 119–123 (in Ukrainian).

Horalskyi, L. P., Demus, N. V., Sokulskyi, I. M., Gutyj, B. V., Kolesnik, N. L., Pavliuchenko, O. V., & Horalska, I. Y. (2023). Species specifics of morphology of the liver of the fishes of the Cyprinidae family. Regulatory Mechanisms in Biosystems, 14(2), 234–241.

Horalskyi, L. P., Khomych, V. T. & Kononskyi, O. I. (2019). Osnovy histolohichnoyi tekhniky i morfofunktsional'ni metody doslidzhennia u normi ta pry patolohiyi [Basics of histological technique and morphofunctional methods of research in the norm and in pathology]. Polissia, Zhytomyr (in Ukrainian).

Horalskyi, L. P., Ovdiiuk, O. V., Gutyj, B. V., & Brygadyrenko, V. V. (2025). The features of the morphology of the heart of *Clarias gariepinus* (Siluriformes, Clariidae). Regulatory Mechanisms in Biosystems, 16(1), e25019.

Horalskyi, L. P., Ragulya, M. R., Kolesnik, N. L., Sokulskyi, I. M., & Gutyj, B. V. (2024). Peculiarities of macro- and cytometric assessment of morphological structures of the domestic pig heart. Regulatory Mechanisms in Biosystems, 15(1), 55–61.

Horalskyi, L. P., Ragulya, M. R., Glukhova, N. M., Sokulskiy, I. M., Kolesnik, N. L., Dunaievska, O. F., Gutyj, B. V., & Goralska, I. Y. (2022). Morphology and specifics of morphometry of lungs and myocardium of heart ventricles of cattle, sheep and horses. Regulatory Mechanisms in Biosystems, 13(1), 53–59.

Horalskyi, L. P., Rahulia, M. R., Kostiuk, V. K., & Sokulskyi, I. M. (2024). Vyznachennia obiemu kardiomiotsytiv ta yikh yaderno-tsytoplazmatychnoho vidnoshennia [Determination of cardiomyocyte volume and their nuclear-cytoplasmic ratio]. Naukovo-Metodychnyi Tsentr Vyshchoyi ta Fakhovoyi Peredvyshchoyi Osvity, Kyiv (in Ukrainian).

Horalskyi, L. P., Sokulskiy, I. M., Kolesnik, N. L., Gutyj, B. V., Romaniuk, R. K., Pavliuchenko, O. V., Shevchuk, S. Y., & Maksymenko, Y. V. (2024). Morphology and morphometric features of the cerebellum of poultry. Regulatory Mechanisms in Biosystems, 15(4), 679–687.

Horalskyi, L., Ragulya, M., Kolesnik, N., & Sokulskyi, I. (2023). Peculiarities of organometry and morphoarchitectonics of the heart of the Domestic ram (*Ovis aries* L., 1758). Ukrainian Journal of Veterinary Sciences, 14(4), 40–56.

Horalskyi, L., Sokulskyi, I., Ragulya, M., Kolesnik, N., & Ordin, Y. (2023). Morphology, organo- and histometric features of the heart and lungs of a sexually mature domestic dog (*Canis lupus familiaris* L., 1758). Scientific Horizons, 26(12), 9–21.

Martins, B. O., Franco-Belussi, L., Siqueira, M. S., Fernandes, C. E., & Provete, D. B. (2021). The evolution of red blood cell shape in fishes. Journal of Evolutionary Biology, 34(3), 537–548.

Mits, I. R., Denefil, O. V., & Andriishyn, O. P. (2016). Morfolohichni zminy vnutrishnikh orhaniv u tvaryn riznoyi stati, yaki zaznaly khronichnoho stresu [Morphological changes of internal organs in animals of different sexes with chronic stress]. Visnyk Naukovykh Doslidzhen, 3, 107–110 (in Ukrainian).

Navas, C. A., & James, R. S. (2007). Sexual dimorphism of *extensor carpi radialis* muscle size, isometric force, relaxation rate and stamina during the breeding season of the frog *Rana temporaria* Linnaeus 1758. The Journal of Experimental Biology, 210(4), 715–721.

Nazarova, D. I., & Kramar, S. B. (2021). Morfolohichna budova sertsia zhaby u postnatal'nomu ontohenezi [Morphological structure of the frog heart in postnatal ontogenesis]. Norwegian Journal of Development of the International Science, 56(2), 68–71 (in Ukrainian).

Rahulia, M., Horalskyi, L., Sokulskyi, I., & Kolesnik, N. (2023). Osoblyvosti morfoarkhitektoniky ta morfometriyi sertsia krolia (*Oryctolagus cuniculus* L. 1758) [Features of morphoarchitectonics and morphometry of the rabbit heart (*Oryctolagus cuniculus* L. 1758)]. Ahrarnyi Visnyk Prychornomoria, 108, 51–62 (in Ukrainian).

Rodrigues, N., Betto-Colliard, C., Jourdan-Pineau, H., & Perrin, N. (2013). Within-population polymorphism of sex-determination systems in the common frog (*Rana temporaria*). Journal of Evolutionary Biology, 26(7), 1569–1577.

Shevchenko, I. V. (2018). Morfolohichni osnovy morfohenezu sertsia u rannomu postnatal'nomu rozvytku v normi [Morphological basis of cardiac morphogenesis in early postnatal development in normal condition]. Visnyk Problem Biolohiyi i Medytsyny, 145, 340–344 (in Ukrainian).

Spaink, H. P., Jansen, H. J., & Dirks, R. P. (2014). Advances in genomics of bony fish. Briefings in Functional Genomics, 13(2), 144–156.

Stakhurska, I. O., & Pryshliak, A. M. (2014). Morfometrychna kharakterystyka kamer sertsia tvaryn riznoyi stati [Morphometric characteristics of heart chambers of animals of different sexes]. Visnyk Problem Biolohiyi i Medytsyny, 106, 269–272 (in Ukrainian).

Stephenson, A., Adams, J. W., & Vaccarezza, M. (2017). The vertebrate heart: An evolutionary perspective. Journal of Anatomy, 231(6), 787–797.

Streicher, J. W., Wellcome Sanger Institute Tree of Life Programme, Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective, Tree of Life Core Informatics collective, & Darwin Tree of Life Consortium (2021). The genome sequence of the common frog, *Rana temporaria* Linnaeus 1758. Wellcome Open Research, 6, 286.

Svendsen, M. B. S., Johansen, J. L., Bushnell, P. G., Skov, P. V., Norin, T., Domenici, P., Steffensen, J. F., & Abe, A. (2019). Are all bony fishes oxygen regulators? Evidence for oxygen regulation in a putative oxygen conformer, the swamp eel *Synbranchus marmoratus*. Journal of Fish Biology, 94(1), 178–182.

Vlasenko, R. P., & Kuzmenko, L. P. (2010). Zoolohiia khrebetnykh [Vertebrate zoology]. Vydavnytstvo Zhytomyrskoho Derzhavnoho Universytetu imeni Ivana Franka, Zhytomyr (in Ukrainian).

Zhurenko, O., Karpovskiy, V., Danchuk, O., & Kravchenko-Dovga, Y. (2018). The content of calcium and phosphorus in the blood of cows with a different tonus of the autonomic nervous system. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences, 20(92), 8–12.