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Ultrastructural Morphology of the Muscle System Organization in the Trematode *Typhlocoelium cucumerinum*

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Abstract: Trematodes, also known as flatworms, are a diverse group of parasitic organisms that live in a variety of ecosystems, from freshwater to marine habitats. Their complex life cycle often involves intermediate and definitive hosts and multiple developmental stages. Trematodes have unique morphological features that allow them to adapt, move and parasitize within host tissues and organs. The parasitic lifestyle of trematodes is facilitated by their well-developed dermal muscle sac, which allows them to move and attach to various organs of the host. The study of the biology, histology and morphology of trematodes is the main task of modern parasitology. To date, there are studies investigating the locomotor system of trematodes, but there is no clear data on the structure and functional characteristics of the structure of the trematode dermal muscle sac. This study can improve our understanding of trematode morphology and physiology, including the structure and function of the muscle system of *Typhlocoelium cucumerinum*. Ultrastructural studies revealed novel features that allow fixation and locomotion of the trematode *T. cucumerinum*. The study provided information on the unique characteristics of individual tissues, organs and systems, with emphasis on the functional features of the tegument. The studies carried out allowed the principles of structural and functional interactions between the supporting and contractile elements of its locomotor organs to be revealed. It is suggested that the basal lamina of the tegument acts as a shock absorber under compression and pressure, resulting in tight attachment to the walls of tissues and organs of wild birds. The basal lamina is a complex structure consisting of several layers. The morphology and development of the basal lamina can vary between different groups of organisms, depending on the specific localization of the organ in the host organism. Collagen filaments play a crucial role in providing structural support to the basal lamina. The results of this study may provide a basis for future research into the development of anthelmintics that target the muscle system of trematodes. Such research could greatly improve the effectiveness of preventive measures against these parasites.

Keywords: Ultrastructure, Musculocutaneous Sac, *Typhlocoelium Cucumerinum*, Muscle Cell Elements, Basal Plate

Introduction

Representatives of parasitic classes of Plathelmithes type are one of the numerous taxa of animal organisms.

As presented in the modern literature, representatives of the class trematoda mostly consist of endoparasitic species and have a uniquely complex life cycle associated with the alternation of generations and the change of animal hosts (both invertebrate and vertebrate) (Zhavoronkova and Novak, 2015; Akhmetov and Chidunchi and Akhmetov, 2015; Aleuy and Kutz, 2020). They parasitize in different organs and can cause different types of trematodes.

The muscle system of flatworms has long been considered one of the simplest among multicellular animals. The concept of a "dermal muscle sac" implies that there is no significant differentiation of muscular elements in the structure of trematodes. However, it has long been shown that the diversity of muscular elements of the dermal-muscular sac is far from being exhausted by layers of annular, longitudinal and diagonal muscle fibers (Krupenko, 2014; Fischer *et al*., 2017; Hoai, 2020; Chidunchi, 2024).

Special attention in parasitology is paid to studies related to the physiology, biochemistry, immunology, histology of organs and tissues and functional morphology of suckers (Li *et al*., 2022; Sreenayana *et al*., 2022).

Typhlocoelium cucumerinum belongs to the class trematoda and the family *Cryptogonimidae*. This parasitic worm inhabits the intestines of mammals and also parasitizes the organs of wild ducks (Marilyn *et al*., 1979). The life cycle of *T. cucumerinum* includes several developmental stages. It begins with oviposition in the intestine of the definitive host, from which the myocarditis emerges, a stage that seeks an intermediate host, various mollusks, including some species of snails. Further development occurs in the mollusks, producing metacercariae that are ready to infect the definitive host. The metacercariae, having entered the definitive host by feeding on tissues infected by intermediate hosts, then develop into mature parasites causing various pathologies.

The use of methods of functional morphology in parasitology and in particular in trematodology allows us to reveal and understand the mechanisms of adaptation of trematodes to existence in the conditions of specific organs of the host. Only this approach can provide information about the adaptation of cells, tissues and organs of helminths to endoparasitic existence. The aim of this study is to investigate the structural and morphological features of the muscular system of trematodes involved in the formation of locomotor and appendicular organs using *Typhlocoelium cucumerinum* as an example.

Materials and Methods

The metacercariae that were detected have been identified as *Typhlocoelium cucumerinum* (Rudolphi, 1808). As a result of incomplete helminthologic autopsies, sexually mature specimens of the trematode *Typhlocoelium cucumerinum* were collected from the oral cavity of 8 broad-billed ducks (Anas clypeata) in a total of 19 maritic specimens (De Santi *et al*., 2018).

A study of the ultrastructure was conducted by the method of transmission electron microscopy (Karupu, 1984). The ultrathin sections were prepared on the method of Weekly (1975). Transmission electron microscopy was used to study the ultrastructure of organs and cells (Salnikova *et al*., 2016).

To characterize the muscle system of trematodes *Typhlocoelium cucumerinum*, the material was fixed in 2% glutaraldehyde solution and 0.1 m cacodylate buffer solution for 2 h at 4°C. Then it was fixed in 1% osmium tetroxide solution for 2 h. Dehydration of the studied tissues was carried out in ethyl alcohol solutions of increasing concentration: 50% ethanol for 18 min, then in 70% ethanol for 12 h and then in 96% ethanol solution for 60 min. Upon completion of the preparation and fixation step, absolute alcohol and acetone were applied and the examined tissues were immersed in the solution for 18 min (Weekly, 1975).

The dehydrated samples were placed in a mixture of Epon 812 and Araldite 502 resins prepared according to specifications (Reynolds, 1963).

The impregnation process of the investigated specimens was carried out according to the scheme:

- 4 h in a mixture of resins: Absolute acetone 1:3
- 4 h in resin mixture: Absolute acetone 1:1
- 4 h in resin mixture: Absolute acetone 3:1

The samples were then placed in a fresh resin mixture for polymerization, which was carried out at 60°C for two days.

Then, ultrathin slices with a thickness of 60-100 nm were fabricated. Sections were mounted on formvar substrates and contrasted with 2% uranyl acetate solution in 50% ethanol (for 10-20 min at 37°C) and plum citrate (at room temperature for 3-10 min) according to the instructions of Reynolds (1963). The images obtained were examined on a JEM-100 CXII electron microscope (JEOL, Japan) with an aperture of 25-30 μm at an accelerating voltage of 80 km.

Results

The trematode *Typhlocoelium cucumerinum* parasitizes in different parts of respiratory organs and sometimes can be met in the oral cavity of water birds (Ryzhikov, 1967; Sonin, 1985). In our case, the helminthes were collected from the oral cavity of ducks. Localizing in the oral cavity, the trematode fixes on the surface of the palatal part. In the latter case, the level of fixation is very high and therefore, during collecting helminths at the freshly bagged birds it is necessary to make certain efforts to unstick them from the place of localization. The marks in the shape of the mosaically located foveolas are left on the place of fixation and are mostly expressed in the median area of the place of attachment of the trematode (Chidunchi and Akhmetov, 2015; Shigin, 1993; Correa *et al*., 2010).

Under the detailed examination of the ultrastructure of the body musculature of the trematode under study, it was determined that the basal membrane of the tegument is developed. This appears in its electron density prominent in comparison with the electron density of the basal layers of the cytoplasmic layer of the tegument and the subjacent layers of the basal lamina of the tegument. A study of the electron-diffraction patterns indicates that the basal membrane is closely coupled with the basal lamina and its collagenic fibers contact tightly with the basal lamina, as

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if "ingrowing" into it. In any case, the borders of the basal lamina are sharply discriminated; it has a three times higher electron density than the layer of the basal lamina (Fig. 1).

Practically on all sides, at the periphery, the circular muscular fibers are surrounded by the layer of the basal lamina. On the electron diffraction patterns, the plasmalemma covering the circular fibers looks like a very thin sustained formation; the collagenic fibers of the basal lamina are closely adjacent to it (Fig. 2). The described above situation can be explained by us only with the fact that the circular muscular fibers can contract and relax i.e., be in motion irrespective of other muscular fibers, particularly, of the fibers of the longitudinal musculature. The collation of the sizes of the circular and longitudinal muscles points to the fact that the section of the circular muscles yields violently to the section of the longitudinal muscles in size.

Immediately under the plasmalemma covering the circular muscular fibers an area with electron-bright characteristics was discovered. In the layer with the electron bright properties the electron-dense granular material, which is differentiated by us as glycogen, is localized. The glycogenic grains can be single or can form linear agglomerations, although we assume that in view of the fact that the electron diffraction pattern is a section such linear agglomerations can be formed up spatially in very different directions. We note that the glycogenic grains are not marked in the layer of the basal lamina. This can be evidence of the fact that glycogen does not arrive at the muscles from the external layers of the tegument, particularly, from the syncytial layer. Therefore, it is logical to suppose that the tegument nutrition is not typical for the described helminth; the presence of such nutrition is mentioned by many authors, e.g., at the intestinal trematodes Fasciola hepatica (Lepitzki *et al*., 1994; Marilyn *et al*., 1979, Krupenko and Dobrovolskij, 2015).

Another peculiarity of the circular fibers is the fact that the fibers are situated at a certain distance from each other. This can be seen in the electron diffraction patterns made on the sections of the front and central parts of the body of the trematode.

The mitochondria, as well as the nuclei, are situated at the periphery of the muscular fibers; frequently they have elongated along the fiber's structure; cases when the mitochondria have the rounded or close to the rounded shape of the section, apparently, can be explained with the fact that the mitochondria are actually filiform and coiled in very different planes and during receiving the ultrathin sections for electron microscopic research can be cut athwart the long axis of the mitochondrion (Fig. 3).

In the muscular fibers of the trematode areas reminding of the z-shaped areas of the cross-striated musculature of other animals, are discovered (Fig. 4).

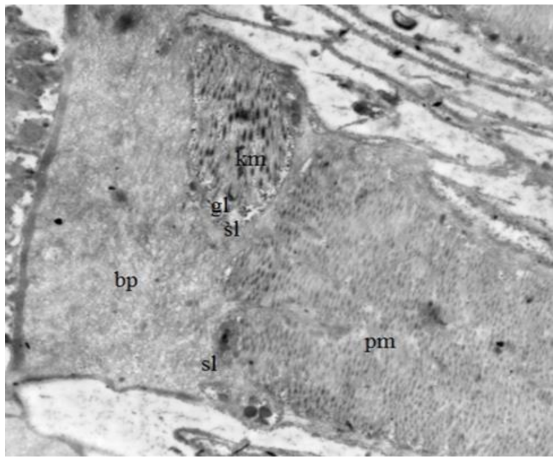

Fig. 1: Electron-diffraction pattern of muscles of trematode *Typhlocoelium cucumerinum* (×10000): Bp-Basal lamina of tegument; km-circular musculature; pm-longitudinal musculature; gl-glycogen; sl-sarcolemma

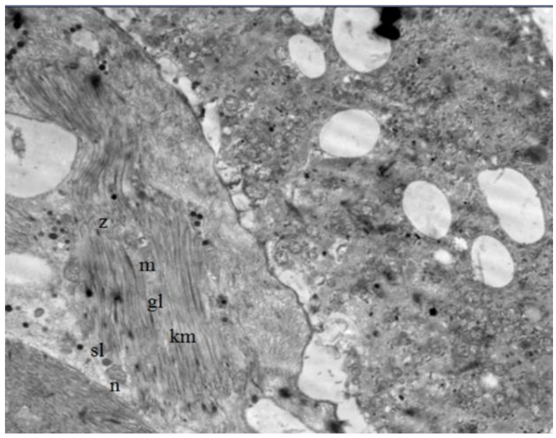

Fig. 2: Electron-diffraction pattern of the integument of the trematode *Typhlocoelium cucumerinum* (×12000): Bp-Basal lamina of tegument; bm-basal membrane of tegument; km-circular musculature; pm-longitudinal musculature

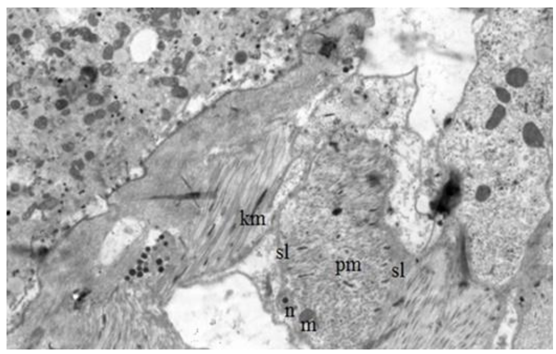

Fig. 3: Electron-diffraction pattern of circular fiber of *Typhlocoelium cucumerinum* (×12000): N-nucleus; km - circular musculature; pm-parenchymal musculature; sl - sarcolemma; z-z-shaped muscular zone

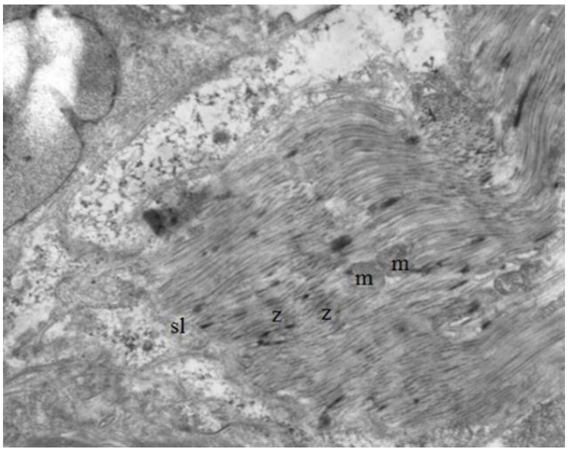

Fig. 4: Electron-diffraction pattern of circular fiber of *Typhlocoelium cucumerinum* (× 12000): N-nucleus; km-circular musculature; pm-parenchymal musculature; sl-sarcolemma; z-z-shaped muscular zone

Fig. 5: Electron-diffraction pattern of trematode *Typhlocoelium cucumerinum* (× 10000): T1-longitudinal musculature of the first type, which consists of two fibers; T2-longitudinal musculature of the second type, which consists of more than two fibers; m-mitochondrion; n-nucleus; km-circular musculature; pm-longitudinal musculature; sl-sarcolemma

Longitudinal Muscular Fibers

As well as the circular fibers, the longitudinal muscular fibers of the skin-muscular sac of the helminth have close connections with the basal lamina of the tegument. The fibers composing the body of the basal lamina are tightly connected with the plasmalemma covering the longitudinal musculature. Close connections of the muscular fibers with the basal lamina, apparently, are evidence of sufficiently active work of this muscular layer.

The electron-diffraction pattern of the longitudinal muscular fibers indicates that the sectional area of the fibers of this layer exceeds the sectional area of the circular fibers by several times. At the same time, the longitudinal fibers are elongated in the median direction in the section, i.e., the sizes of measurement in one direction exceed the measurement of the section of the fiber in the other direction.

The plasmalemma covering the longitudinal muscular fibers is thin. In the undermembrane area such subcellular elements as: The nuclei, the mitochondria and the granular material, which we differentiate as glycogene, are localized (Fig. 5).

Discussion

The functional morphology of smooth muscle elements in trematodes varies depending on their location in the musculature of the helminth body. Our study showed that these features are developed to varying degrees depending on the layer to which they belong (Scott *et al*., 1982a-b).

The basal lamina is a widely distributed feature in epithelial tissues, smooth muscle cells and some organs of higher organisms. The development of the basal lamina is most prominent on the basal side of the epithelium. Electron microscopy has revealed that the basal lamina may consist of both a true basal membrane and adjacent fibrillar connective tissue components. In helminth species, the basal lamina can reach an average thickness of 80-300 nm and can be morphologically divided into layers such as the basal membrane and the fibrillar layer (Mansour *et al*., 2023).

The basal lamina of the dermal muscular sac of trematodes is situated beneath the basal membrane of the syncytial layer of the tegument and extends to the musculature layers. Collagen filaments are a crucial component of the basal lamina of all multicellular organisms, including trematodes. They provide structural support for the basal lamina and their density and orientation determine its stability. The basal lamina is commonly referred to as the 'shiny layer' or 'fibrous layer' at the light microscopy level, leading to the interpretation that it is homogeneous. However, it is important to note that this may not accurately reflect basal lamina development in different trematode species. The development of the basal lamina seems to depend more on the localization of a particular organ in the host than on the taxonomic position of the trematode species. For instance, trematodes that inhabit the host's gastrointestinal tract typically have a more developed basal plate than those inhabiting other organs (Mordvinov *et al*., 2021; Avgustinovich *et al*., 2021, Ponomarev *et al*., 2024).

The ultrastructure of muscle fibers in the locomotor sac of Plathelminthes is not well understood. Previous studies have mainly focused on the fine morphology of cestode musculature. However, studies on the ultrastructure of the musculature of the dermal muscle sac and muscle elements in trematodes are limited. Existing studies only report the presence of muscle fibers in various layers of the helminth body. Further research has been conducted on the ultrastructure of the body and sucker muscles in tapeworms. The ultrastructure of muscle fibers in the locomotor sac of Plathelminthes has not been extensively studied. Previous research has primarily focused on the fine morphology of the musculature in cestodes. However, the ultrastructure of the musculature of the dermal-muscle sac and muscle elements in trematodes has been studied to a limited extent. The available studies only describe the presence of muscle fibers in different layers of the helminth body. Additional studies were conducted on the ultrastructure of the body and sucker muscles in tapeworms.

The studies revealed that in certain flatworm taxa, there is differentiation of unspecialized cambial structures for the formation and renewal of specialized cells, including muscle cells. The research suggests the existence of a single multipotent cambial element capable of transforming its morphology and function according to specific cell system vectors. The statement implies that the flatworm type is in the initial phase of tissue organization, which is regarded as an ancient trait.

In trematodes, the distribution of contractile elements and nuclei in muscle cells differs from that observed in cestodes. The central part of muscle cells contains contractile elements, while the nuclei are located in the periphery. This difference in localization may be due to the fact that previous studies on trematodes have focused mainly on the body musculature rather than the attachment apparatus.

Subcellular elements such as mitochondria and ribosomes are present at the periphery of muscle cells in all layers of helminth musculature, indicating the existence of a specific functional unit within the cells. The structural and functional characteristics of smooth muscle in the studied trematodes vary and may not depend on a particular muscle layer. These characteristics are influenced by the conditions in the localization organ.

In conclusion, the basal lamina is a complex structure composed of different layers. Its development and morphology may vary among different groups of organisms and depend on the specific localization of the organ in the host. Collagen filaments are crucial in providing structural support to the basal lamina. Additional studies using electron microscopy are necessary to gain a better understanding of the morphological and functional characteristics of the basal lamina in various species and tissue types.

Research on cestodes and trematodes offers valuable insights into the detailed morphology and distribution of muscle cells and subcellular components. However, further studies are necessary to fully comprehend the structural and functional characteristics of these muscle fibers and their significance in the overall biology of flatworms.

Conclusion

Trematodes are exclusively endoparasitic species that attract the attention of researchers both for practical (veterinary and medical aspects) and theoretical (fundamental) problems of parasitology. Questions of species diversity are of interest to science and new species are being recorded every year. The biology and ecology of trematodes are also important areas of research. The study of the adaptive mechanisms of trematodes is important to understand their adaptation to specific host species and the development of their organs under specific environmental conditions. It is very important to determine the cellular, organ and organismal adaptations of trematodes to their parasitic existence, as this has both theoretical and practical implications for science.

T. cucumerinum has been found in the oral cavity, where active processes of respiration and feeding take place (Li *et al*., 2020; Assis *et al*., 2021).

Ultrastructural methods of research fully confirmed the initial assumptions about the well-developed musculature of the trematode body, thanks to which a dense and firm attachment in the host organism occurs. In addition, this method allowed us to reveal the principles of structural and functional interactions between the supporting and contractile elements of their locomotor organs.

The study of the muscular system consisting of smooth muscle cells allowed us to determine its basic morphological organization. Using the method of ultra-thin electron microscopy, we determined the mechanisms that allow smooth muscle cells to perform effective motor actions in the skin-muscle sack of trematodes. The appearance of Z-shaped zones in smooth muscle cells was found to be an intracellular mechanism.

The principles of interaction of supporting and contractile elements of locomotive organs in different organisms were revealed during the research. It was revealed that the basal lamina of the tegument performs the function of a shock absorber under compression and pressure, which leads to strong attachment to tissues and organs of wild birds. At the same time, the basal lamina is a complex structure consisting of several layers, the morphology and development of which depend on the specific location of the organ in the host organism. Collagen filaments play an important role in the structural support of the basal lamina.

The organization of the locomotor apparatus in trematodes is both morphologically and functionally flexible and is influenced by factors related to the conditions of the localizing organ. This flexibility is independent of the taxonomic position of the helminths or their hosts.

The results obtained on the ultrastructure and morphology of the musculature of *T. cucumerinum* are a good addition to the already available knowledge on the peculiarities of the locomotor system and the morphology of the musculoskeletal sac of trematodes. The generalized results can be used in the selection of effective anthelmintics for the treatment and prevention of diseases caused by *T. cucumerinum*.

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Author's Contributions

Irina Yurievna Chidunchi: Supervised the study, conducted the literature review and drafted the manuscript.

Ainagul Balgauovna Kaliyeva and Natalya Petrovna Korogod: Conducted fieldwork and also contributed to manuscript written.

Shynar Zhanybekovna Arynova: Performed comparative analysis of the data obtained.

Ethics

This article contains original, unpublished material. The corresponding author acknowledges that other authors have reviewed and approved this manuscript; no ethical issues are involved.

Conflicts of Interest

The authors declare no conflict of interest.

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