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Ultrastructural Organization of the Muscular System Body of the Trematode Schistogonimus Rarus

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Abstract: It is known that parasitic flatworms of the class Trematoda have a unique complex life cycle, and have morphological features of adaptation, movement, and existence in the host body. Functional peculiarities of the parasitic way of life are conditioned by the developed muscular system, due to which there is movement and attachment of the snail to various organs of the host. To date, one of the main tasks of parasitology is the study of tissues and organs of parasitic worms, which can expand our ideas about the biology of trematodes and allow us to take a new look at their structure and functions. The study of the functional morphology of the trematode muscle sac allows us to elucidate the peculiarities of trematode locomotion and attachment. This article deals with the structural organization of the muscular elements of the musculocutaneous sac of the trematode Schistogonimus Rarus. On the basis of ultrastructural studies, the functional mechanisms that ensure the movement and attachment of Schistogonimus Rarus trematodes to individual organs of the host have been revealed. In addition, data were obtained on the peculiarities of the structure of individual tissues, organs, and systems. As a result of the analysis of the obtained data, it was revealed that the ultrastructural features of the muscles of the skin-muscular sack of trematodes are formed on the basis of the modification capabilities of smooth muscle cells and tissues. The results of the work can be used as a basic material for further studies on the selection of anthelmintics acting on the muscular system of trematodes, which will lead to significant effectiveness of preventive measures.

Keywords: Ultrastructure, Skin-Muscular Sac, Muscular Fibers, Elements of Muscular Cells, Tegument, Basal Lamina

Introduction

Representatives of the class Trematoda, consisting entirely of endoparasitic species, attract the attention of researchers engaged in both practical work (veterinary and medical aspects) and theoretical (fundamental) problems of parasitology. Questions of diversity of species composition, in which new species are registered annually, are of interest to science.

Researchers in biology, ecology, and systematics are interested in the study of flatworms. One class of flatworms that contains exclusively parasitic forms is the Trematoda (Li *et al*., 2022).

Parasitic flatworms in the class Trematoda have a uniquely complex life cycle with morphological features of adaptation, locomotion, and existence within the host body (Fischer *et al*., 2017; Hoai, 2020).

Schistosomes are mostly classified as avian parasites (De Santi *et al*., 2018). Their intermediate hosts can be mollusks and snails of the families Lymnaeidae, Physidae, Planorbidae, and Pleuroceridae (Brant and Loker, 2009). Schistogonimus rarus trematodes usually invade birds; mammals, including humans, may serve as accidental hosts, suffering from a cutaneous hypersensitive reaction to the parasites-cercarial dermatitis.

The use of the method of functional morphology in the study of parasites Schistogonimus Rarus allows us to identify and understand the mechanisms of their adaptation to existence in the conditions of a particular host organ. In this study, the characteristics of the body wall musculature of Schistogonimus Rarus are identified and characterized, revealing peculiarities and patterns.

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The ultrastructural peculiarity of the distribution of subcellular elements in smooth muscle cells of all layers of Schistogonimus Rarus dermal-muscular sack muscles is their restriction to peripheral parts and the entire main cell volume is occupied by fibrillar elements. Ultrastructural features of the basal plate of the tegument of trematodes are related to and depend on the restriction of helminths to the conditions of specific host organs and do not depend on their taxonomic position of helminths.

Data explaining the principles of parasitic adaptations of helminths, which allow them to resist immune, mechanical, chemical, and other types of impact on the host organism, can be obtained only by using subtle methods of studying all levels of parasitic organization.

Only this approach can provide data on the adaptation of helminth cells, tissues, and organs to endoparasitic existence. Ultrastructural and histological data under normal conditions (without exposure to anthelmintics during helminth examination) serve as a basis for the selective selection of anthelmintics (Akhmetov and Chidunchi, 2015).

The aim of the research is to study the functional characteristics of circular and longitudinal muscle fibers, as well as dorsoventral parenchyma musculature in the process of formation of a closer connection with the localization organ on the example of the trematode Schistogonimus Rarus.

Materials

As a result of incomplete helminthological dissections, the eugamic specimens of the trematode Schistogonimus rarus (Braun, 1901) in the quantity of 18 adults were collected from the bursas of Fabricius at 15 mallards (Anas platyrhynhos) (Fig. 1).

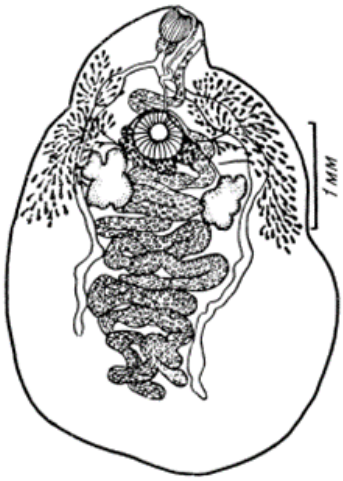

Fig. 1: Schistogonimus rarus (Braun, 1901)

General View

The body length is 2.44-4.52 mm and the width in the testis area is 1.3-2.7 mm. The cuticle is densely covered with spikes. Suction cups: Oral 0.29-0.31×0.37-0.4 mm, abdominal 0.36-0.43×0.5-0.52 mm. Pharynx 0.10-0.14×0.14-0.16 mm in length. Intestinal trunks do not reach the posterior end by 0.50-0.86 mm.

Methods

The ultrastructure of parasitic worm tissues has been studied by electron microscopy (Karupu, 1984).

To determine the characteristics of the trematode Schistogonimus Rarus muscle system, we took trematode muscle tissue fixed in 2% glutaraldehyde solution and 0.1 m cacodylate-buffered solution for 2 h at 4°C. The tissue was then washed repeatedly in cacodylate-buffered solution for 12 min each and fixed in 1% osmium tetroxide solution for 2 h. Dehydration of the studied tissues was carried out in ethyl alcohol solutions of increasing concentration: In 50% ethanol for 18 min, then in 70% ethanol for 12 h and also in 80, 90 and 96% ethanol solutions for 18 min each. At the end of the step of preparation and fixation of the examined tissues, absolute alcohol and acetone were applied and the examined tissues were immersed in each solution for 18 min (Weekly, 1975).

Dehydrated specimens were placed in a mixture of Epon Araldite resins prepared according to the specifications (Reynolds, 1963):

- Epon 812-4 g
- Araldite 502-2 g
- Epon DDSA-9 g
- Catalyst DMP-30-8 drops

The impregnation process of the tested samples was carried out according to the following scheme:

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

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

After all stages of preparation of the tissues under study, ultrathin slices of 60-100 nanometers thickness were made on Ultrotome III (LKB, Sweden). The sections were mounted on formvar substrate grids and contrasted with a 2% solution of uranyl acetate in 50% ethanol (for 10-20 min at 37°C) and plumbed 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 kilovolts.

Results

The trematode was collected from the cavity of the bursa of the Fabricius juvenile organ of young mallards. The sacciform organ does not have any physiological ducts and is a part of the immune system of young birds (Feizullaev, 1980). In adult birds, this organ vanishes. In literature, data is contained that the Schistogonimus rarus can be found in the uterus of adult birds (Ryzhikov, 1967). As is well known, the uterus in birds is represented by a tubular organ, where an egg and all its components, including the solid shell, are formed. Being parasitized by the helminth under examination, the shell gland of the host bird is affected, since the parasite disturbs the activity of the organ, feeding on the gland blood and tissues.

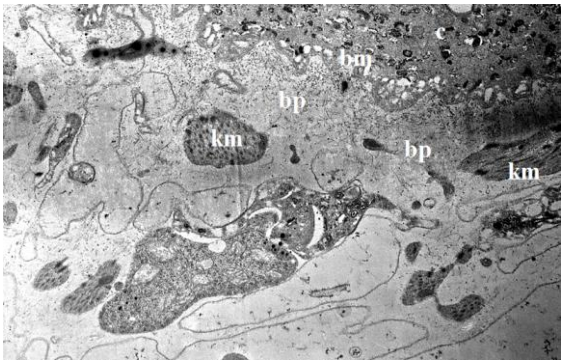

Fig. 2: Electron-diffraction pattern of the integument of the trematode Schistogonimus rarus (×9000): C cytoplasmic layer syncytium; bp-basal lamina of tegument; km-circular musculature; bm-basal membrane of tegument

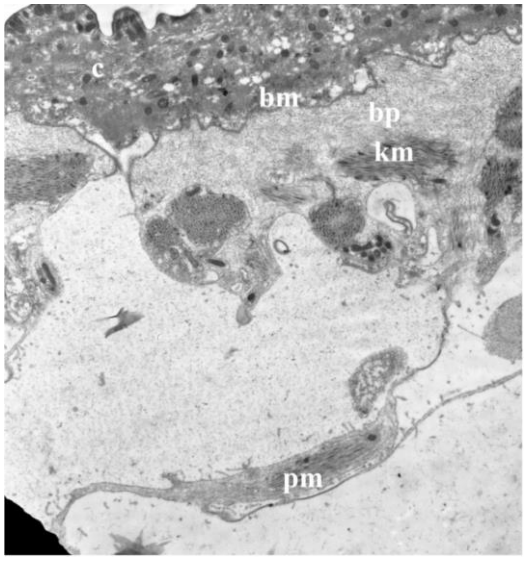

Fig. 3: Electron-diffraction pattern of the integument of the trematode Schistogonimus rarus (×12000): Bp-basal lamina of tegument; bm-basal membrane of tegument; km-circular musculature; pm-longitudinal musculature

The helminth has a phylloid strongly applanate body; well-developed oral and abdominal suckers are present. The tegument has chitinous hooks on the anterior third part of the body (Sonin, 1985; Shigin, 1993).

As long as the helminth under study was collected by us from the bursa of Fabricius at birds, it would be logical to suppose that the absence of any appreciable physical influence on the trematode promoted a lack of development of the muscular layers of the body.

In our opinion, the transition to parasitizing exactly in this organ, possibly, began evolutionary recently. As is well known, natural physical movements occur there, such as the wall peristalsis and the movement of the formed egg, which can be a kind of "banishing" factor, which the helminth should resist and thereby have well-developed muscular layers of the body. In the case of the helminths found in the bursa of Fabricius, such "banishing" factors are absent; in this connection, the helminth has no such adaptive capabilities.

The upper border of the basal lamina body of the trematode Schistogonimus Rarus of the tegument is limited by the three-layer basal membrane. The presence of three layers of the basal membrane of the syncytial layer of the tegument is caused by the classical organization described for the fixed living membranes. In the composition of this membrane, the upper and lower layers are defined, which can be described as a couple of electron-dense layers having an electron-brighter layer between (Fig. 2).

The basal lamina of the tegument is expressed very weakly; this is typical, especially for the lower border of this layer of the skin-muscular sac. On the electron-diffraction patterns, the lower border of layers is occasionally absent. Very little of the fibrous material is differentiated in the structure of the basal lamina and the collagenous fibers composing the body of the basal lamina are the evidence of the aforesaid statement. The impression is given that this is a supporting structure; particularly, the basal lamina is absent or is very tender and does not provide any important supporting function for the muscles of the helminth's body.

Circular Muscular Fibers

At the trematode under examination, the circular muscular fibers are located sufficiently far from each other and this is not accidental; they are virtually not differentiated on the histological preparations (Fig. 1).

In the composition of the circular muscular complex, there are mostly one or two muscular fibers. The plasmic membrane of the circular fibers is very thin and is barely differentiated. At the periphery of the fiber, under the plasmic membrane, the nuclei of muscular cells were discovered. Evidently, the nucleus

has a curved form (Fig. 3); there are very few mitochondria on the electron-diffraction patterns of the circular muscles. The glycogen grains were discovered in the undermembrane area, in the perimembrane layer.

The thick protofibrils on the electron-diffraction patterns of the transverse sections point to the absence of the special order in their arrangement (Fig. 4). The ratio between the protofibrils and the myosin fibrils is 1×4-5.

Longitudinal Muscular Fibers

The longitudinal muscular fibers on the electron-diffraction patterns look more developed than the circular fibers. According to the morphological characteristics, the plasmic membrane of the muscular fiber is the same as that of the circular muscles and looks like a single structure. On the electron-diffraction patterns of the transverse sections, it is obvious that the plasmic membrane of the muscular fiber has more fibrillar structures than the circular fibers (Fig. 5).

The nuclei of muscular cells are localized at the periphery under the plasmic membrane (Chidunchi and Akhmetov, 2015). The glycogen grains are present in the perinuclear area. At the periphery of the muscular fibers, the mitochondria were discovered.

There are few thick protofibrils on the transverse sections as well as in the circular fibers and there are considerably more myosin protofibrils than in the circular fibers.

Dorsoventral Parenchymal Musculature

The developed back-front (dorsoventral) muscles discovered on the electron-diffraction patterns became an organizational peculiarity of the muscular system of the described trematode (Fig. 6). Apparently, this group of muscles is the most developed in the composition of the muscular system of the helminth. In our opinion, the dorsoventral muscular group of the helminth promotes more intimate contact with the surface of the organ of localization; possibly, this is connected with the tegument nutrition. Detailed examination of a number of electrograms reveals areas resembling Z-zones of transverse striated muscles. This is probably the only muscle group in the body of the described trematode that shows a similar morphological feature of transverse striated muscles. The described situation may indicate the exceptional importance of this muscle group for the motor actions of the described helminth.

However, in all other species studied in this study, such Z-shaped areas of contractile structures are formed in smooth muscle cells. We can explain this only by the conditions in the host organ and accordingly, by the function of the body muscles.

The plasmic membrane covering the back-front fibers is very thin and is barely differentiated on the electron-diffraction patterns. The nuclei and mitochondria are situated in the under-membrane area and are immediately adjacent to it, as well as in other muscular groups. On the preparations, there are less large protofibrils and more myosin protofibrils. The situation described above can be evidence of the exclusive significance of this muscular group for performing emotional acts of the helminth.

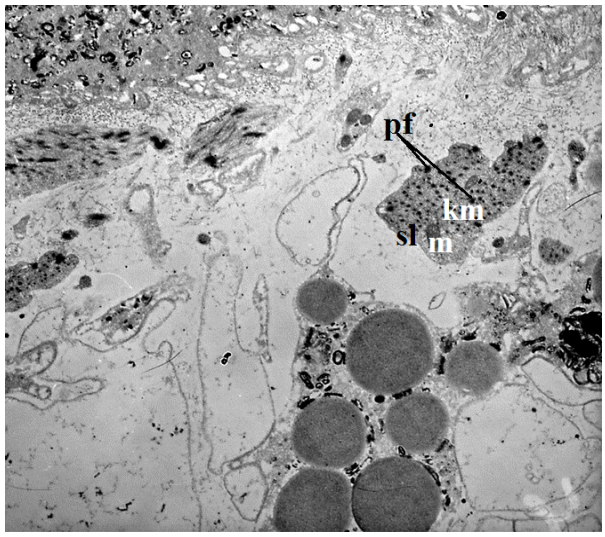

Fig. 4: Electron-diffraction pattern of the integument of the trematode Schistogonimus rarus (×9000): Km-circular musculature; pf-protofibrils; m-mitochondria; sl-plasmic membrane

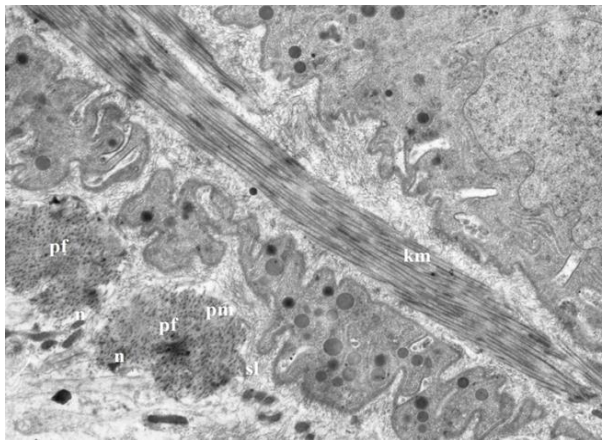

Fig. 5: Electron-diffraction pattern of the longitudinal tissue of the trematode Schistogonimus rarus (×10000): Km-circular musculature; pf-protofibrils; m-mitochondria; sl-plasmic membrane; n-nucleus; c-cytoplasmic layer syncytium; pm-longitudinal musculature

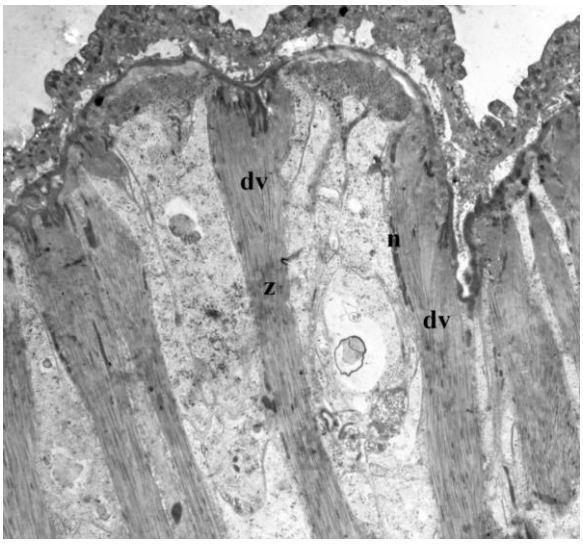

Fig. 6: Electron-diffraction pattern of the integument of the trematode Schistogonimus rarus (×13000): Dv-dorsoventral muscles; z-z-shaped muscular zone; n-nucleus

Discussion

Zoonotic trematodes are small parasites that live mainly in the intestines of birds and mammals (Correa *et al*., 2010; Anh *et al*., 2010). However, depending on the organ of localization, they can also be found in other organs, such as the fabrycium pouch, oral cavity, and circulatory system organs (Chidunchi and Akhmetov, 2015). Currently, due to the high diversity of species and indistinguishable morphology, many species have not been accurately classified and studied. Initially, (Jousson *et al*., 1999) proposed the use of genetic markers or restriction fragment length polymorphism for molecular identification (Jousson *et al*., 1999; Li *et al*., 2022).

Therefore, the present study on the ultrastructural morphology of Schistogonimus rarus sheds light on the physiology, morphology, and ecology of trematodes of this species.

Discussing the data of the electron microscopic research of the muscular fibers of different layers of the skin-muscular sac of the trematode, we arrive at a conclusion that the contractile elements of muscular cells and the nuclei can be situated in the same area, but at the same time, the nuclei localize at the periphery of muscular cells. The contractile elements of muscular cells fill the bulk of the central part. In comparison with the data obtained by Kolářová *et al*. (1999), who was studying the ultrastructure of single muscular groups at three species of cestodes and discovered the nucleusiferous and contractile parts in their muscular cells, we did not determine a similar situation in our observations. Possibly, such morphology is connected with the fact that we were examining the musculature of the body, not of the fixing apparatus.

By results of our works, we reach a conclusion that the bulk of muscular cells is occupied with the contractile elements; the quantity of the larger central fibrils and the protofibrils situated around them is different subject to their belonging to a definite muscular layer (Chidunchi and Akhmetov, 2015). Discussing the electron microscopic peculiarities of distribution of other subcellular elements, the mitochondria, the ribosomes, and their complexes, in particular, it is necessary to mark their localization at the periphery in muscular cells of all layers of the helminth's musculature. Such localization of the cellular elements is noticed by many authors in different groups of the Plathelminthes (Bisserova and Kuperman, 1998). The presence and the structural affiliation with a definite area of muscular cells of the subcellular structures mentioned can suggest an idea of the presence of a specific functional (Matthews, 1973; Chapman, 1973; Krupenko, 2014; Krupenko and Dobrovolskij, 2015; Pearson, 1959).

Discussing the ultrastructural peculiarities of muscular cells of the trematode, it is possible to say that it corresponds to the description of the slow nonstriated musculature. As is well-known, the energy extraction in such fibers is connected with the process of anaerobic glycolysis; earlier this was noticed in research of Soprunov (1987) Possibly, this is connected with a sufficiently low-activity and fixed existence of helminths. In our opinion, there are common generalities specific to muscular cells of some classes within the phylum Plathelminthes, the classes Trematoda and Cestoda, in particular. The peculiarities described above were discovered by Lumsden and Specian (1980), Specian at the tapeworm helminths.

Conclusion

According to the results of our work, we conclude that the main volume of muscle cells is occupied by contractile elements, the number of larger central fibrils and protofibrils located around them varies depending on the affiliation to a certain muscle layer. Discussing electron-microscopic features of the distribution of other subcellular elements, in particular, mitochondria, ribosomes, and their complexes, it is necessary to note their localization on the periphery in muscle cells of all layers of helminth muscle. The presence and structural restriction of the mentioned subcellular structures to a specific region of muscle cells may indicate the presence of a specific functional block.

Structurally functional features of smooth muscle in the trematodes studied by us are developed to different degrees and depend on belonging to a particular layer of muscle of the helminth body. Structural changes in smooth muscle, from which the body muscles of

trematodes are formed, are manifested under the influence of specific factors and conditions in the organ of localization. For example, in the trematode Schisthogonimus rarus from the sac-like organ of the avian Fabrycium pouch, muscle cells do not form features of transverse striated muscles, such as Z-shaped areas of contractile elements. The appearance of Z-shaped structures in smooth muscle cells is an apomorphic trait that, in the further evolution of multicellular tissues, may have been the starting point for the appearance of transverse striated muscles in more highly organized animal taxa.

Based on the ultrastructural studies of the trematode Schisthogonimus rarus and the analysis of the localization peculiarities of adult helminths in the organs of the host, we could make the following assumptions: Since the studied helminth was collected from the bursa of Fabricius in birds, it is logical to assume that the absence of any perceptible physical influence on the trematode promoted the lack of development of the muscular layers of the helminth body. Possibly, the transition to parasitism in this organ began evolutionary recently. The trematode Schisthogonimus rarus has developed abdominal and oral suckers and chitinous spines on the surface of the tegument, at the expense of which the attachment occurs.

Acknowledgment

I thank the publisher for giving me the opportunity to publish my research.

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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.

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