Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Ultrastructural Characteristics of the Body Wall in Adult Helminth *Heterakis gallinarum* Schrank, 1788 (Nematoda: Heterakidae)

Fuad H. Rzayev [1,2*], Ali M. Nasirov [1], Orkhan R. Isayev [2,3], Eldar K. Gasimov [3] and Gunay F. Rzayeva [4]

[1]*Laboratory of Parasitology, Institute of Zoology, Ministry of Science and Education, Baku, Azerbaijan.*
[2]*Department of Electron Microscopy, Scientific Research Centre, Azerbaijan Medical University, Baku, Azerbaijan.*
[3]*Department of Cytology, Embryology and Histology, Azerbaijan Medical University, Baku, Azerbaijan.*
[4]*Department of Mathematical Analysis and Theory of Functions, Sumgait State University, Sumgait, Azerbaijan.*

Abstract

THE NEMATODES of the genus Heterakis are similar in appearance and a number of morphological characteristics. Therefore, determination of nematodes creates certain difficulties. *H. gallinarum* helminth is one of the widespread species of the genus. In the current study, the body wall of the parasite of the *H. gallinarum* nematode was studied at the ultrastructural level with the help of light and electron microscopic methods, and the obtained results were compared with the structures of the covering tissue of other species of the family. It was found that the bady wall of the nematode consists of 3 layers. The cuticle of the helminth is made up of 8 layers. In both individuals of the helminth, the lateral alae consist of 3 main layers. Homogeneous and fibrillar layers are many times thicker than other parts of the helminth's body. While the shape of the cuticle of both male and female *H. gallinarum* nematode is smooth throughout the body, 2 different shapes have been found around the bulb. This sign is not mentioned in other species of the family. Tubular structures were found between the basal part of the cuticle and the hypodermis of the studied helminth. The nervous system of the parasite is orthogonal, and the muscular type is polymyar. The number of muscle cells in between two ridges of the helminth varies from 19 to 28, depending on the sex of the worm and the part of the body of the helminth.

Keywords: Nematodes, *Heterakis gallinarum*, Body wall, Ultrastructure, TEM.

Introduction

The nematode *H. gallinarum*, belongs to the genus Heterakis of the family Heterakidae, is widely distributed (cosmopolitan) and is a parasite found in birds belonging to various orders (Anseriformes, Gruiformes, Strigiformes, Passeriformes) [1-7]. The ultrastructural characteristics of various organs of the helminths *H. dispar, H. spumosa* and *H. gallinarum* from the species included in the genus were studied [8-15]. *H. dispar* has been studied at the ultrastructural level in more detail than the helminths mentioned above [15-18]. There are partial ultrastructural data about *H. gallinarum* specie [8-12, 19-22]. Nevertheless, identification of the *H. gallinarum* nematode at the molecular level (through mitochondrial and ribosomal markers) and phylogenetic analysis [23-26], characteristics [27], identification through PCR [28, 29], etc. studies have been conducted. Researches have been conducted in recent years in order to determine the species diversity of domestic waterfowl helminths in the territory of Azerbaijan Republic. As a result, it became known that currently 27 species of helminths parasitize geese (*Anser anser* dom.) and ducks (*Anas platyrhynchos* dom.) in the country [30-39]. Among the nematodes, one of the species included in the Heterakidae family is the helminth *H. gallinarum*.

As a result of the analysis of the above-mentioned and literature data, it was found that there is no comprehensive research on the study of the ultrastructure of all organs of *H. gallinarum*. Taking this into account, the aim of the present

*Corresponding author: Fuad H. Rzayev, e-mail: fuad.zi@mail.ru. Tel.: +994703983296
(Received 09/03/2024, accepted 24/04/2024)
DOI: 10.21608/EJVS.2024.275741.1902
©2025 National Information and Documentation Center (NIDOC)

research was to study the ultrastructural features of the nematodes body wall using of light and electron microscopic methods.

Material and Methods

The studies conducted in the direction of studying the ultrastructure of the helminth were carried out in 2019-2023. 16 adult *H. gallinarum* nematodes (8 males and 8 females) were collected from the cecum of the host's (*Anser anser* dom.) intestines by the parasitological dissection method and were obtained from the city of Ganja (Azerbaijan) [40]. Fixed preparations of helminths were studied under a stereomicroscope MBS-9 (Russia) and a Primo Star (Carl Zeiss, Germany) light microscope. For the identification of the species, K. Ryzhikov's (1967) determinant was used [41]. In order to study the ultrastructure of the helminths, the collected adult nematodes were divided in to several parts and were immediately fixed in a solution consisting of 2.5% glutaraldehyde, 2% paraformaldehyde, 4% surcosa, 0.1% picric acid prepared in 0.1M phosphate buffer (pH=7.4). After they were postfixed in 1% osmium tetraoxide solution prepared in phosphate buffer (pH 7.4) for 1.5 hours. Araldite-Epon blocks were prepared from the material based on generally accepted protocols in electron microscopy [42, 43]. Semithin (1-2 μm) sections taken from the blocks on an EM UC7 ultramicrotome (Leica, Germany) stained with methylene blue, azure II and basic fucsin, viewed under a Primo Star (Carl Zeiss, Germany) light microscope, images from necessary sections captured with an EOS D650 (Canon, Japan) digital camera [44, 45]. Ultrathin sections of 50-70 nm thickness taken from the same blocks were first stained with 2% uranyl-acetate solution and then with 0.6% pure lead citrate prepared in 0.1N NaOH solution. Ultrathin sections were examined under a JEM-1400 (Joel, Japan) Transmission Electron Microscope (TEM) under a voltage of 80-120 kV and electrograms were recorded. The statistical analysis of the images was performed on electrograms taken in TIF format using the computer program (The TEM imaging platform) developed by the "Olympus Soft Imaging Solutions Gmbh" (Germany) [46, 47]. Data analysis was carried out with different parameters (Min, Max, mean±SD). Analyses of morphometric data were performed using the package of applied statistical programs SPSS-20, by methods of variation (averages), and univariate (one-factor) analysis of variance (One-way ANOVA). Differences were considered significant when P was $p \leq 0.05$.

Results

Helminths body wall is made up of three parts - cuticle, hypodermis and muscular layer.

Cuticle

The cuticle of *H. gallinarum* nematode, has a non-cellular structure. Cuticle is composed of 3 main layers (cortical, median or homogeneous and fibrillar or basal zones) and 8 layers in total: cortical (epicuticle, outer and inner cortical layers), homogeneous (outer and inner homogenous), basal or fibrillar (outer, inner fibrous layers and basement membrane) (Fig. 1A). The epicuticle, or outer membrane, the first layer that covers the outside of the parasite, is presented in an electron microscope at 100.000 magnification (Fig. 1B, indicated by the black arrow). A thin layer of glycocalyx (glycoprotein) on the surface of the epicuticle of the nematode *H. gallinarum* was not observed by us at higher magnifications of the TEM.

All layers of the cuticle of the nematode *H. gallinarum* were statistically counted. The main parameters are presented here: epicuticle 14.35-20.88 nm (16.69±0.63 nm), outer cortex 132.41-202.29 nm (155.96±4.9 nm), inner cortex 167.02-238.24 nm (204.61±5.45 nm), outer homogeneous 306.28-418.99 nm (358.26±8.61 nm), inner homogeneous 654.30-765.50 nm (714.06±7.56 nm), outer fibrous 712.59-849.27 nm (773.43±10.49 nm), inner fibrous 292.47-369.42 nm (325.97±6.09 nm), basement membrane 18.74-25.33 nm (22.65±0.51 nm). The total thickness of the cuticle is 2575.02-2761.54 nm (2649.68±13.26 nm), the thickness of the cortical layer is 341.63-431.53 nm (377.92±6.60 nm), the homogeneous (middle layer) layer its thickness was 1043.87-1176.09 nm (1083.88±9.90 nm), and the thickness of the fibrillar or basal layer was 1065.31-1190.52 nm (1134.02±8.37 nm).

During the ultrastructural study of the cuticle of *H. gallinarum*, it was revealed that its structure is different in male and female individuals of the helminth, as well as in different of the body parts of adult individuals. Thus, in both light and electron microscopic studies by TEM, it was determined that there are a pair of lateral alae (La) on the sides of the nematode's body from the front to the back of the parasite body, which serve the direction of movement of the nematodes (Fig. 1C). As a result of the electron microscopic study of the lateral alae, it was found that they are slightly different from the structure of the cuticle of the helminth. In the electrogram presented (Fig. 1D), the lateral alae is revealed to be composed of 3 main layers – cortical (C), homogeneous (H) and fibrillar (F) in contrast to the cuticle (not 8 layers) in other parts of parasite body. Those layers were measured and the results are as follows: the cortical layer was 527.10-715.91 nm (609.32±11.30 nm), the homogeneous layer was 1473.29-2810.58 nm (2234.22±107.5 nm), and the fibrillar layer It was 1098.81-4217.97 nm (2555.66±272.55 nm). The total height of the lateral alae was determined to be 14600-16700 nm

(15900±290 nm). The homogeneous and fibrillar layer is thicker in the lateral alae. It should also be noted that the homogeneous layers here consist of two shares (Fig. 1D). After the mentioned layers, as a continuation of the cuticle, there are two thick fibrillar layers (the total thickness of both of them is 3414.50-4134.14 nm (3786.57±56.33 nm)) and it ends with a basement membrane. It was observed that the two fibrillar layers under the lateral alae are thicker than those under the cuticle. Those fibrillar layers provide additional strength to the lateral alae.

The lateral alae become wider around the bursa in the posterior part of male individuals of the nematode *H. gallinarum*. As a result of the electron microscopic study of the alae here, it is revealed that the homogeneous layer is several times thicker.

The shape of the cuticle of the helminth was not the same throughout the body during the electron microscopic examination of the body wall of the nematode. Thus, while the shape of the cuticle is smooth throughout the body of both male and female parasite individuals, two different forms that differ from each other were found in the front part of (at the level of the bulb and areas close to it) the helminth. The height of the cuticle found here was 2-3 times thicker than other parts of the body (4800-8500 nm) (Fig. 1E and 1F). As for the shapes of the cuticle, in the electrogram presented in Figure 1E, depressions (height 3100-3200 nm) located at a distance of 7600-7800 nm from each other and directed towards the inner cortical layer of the cuticle are clearly visible. In addition, 5-6 small depressions (height 400-700 nm) are also observed in the raised area between those deep depressions. The noted small depressions extend to the end of the outer cortical layer (Fig. 1E). Another form of cuticle found around the bulb of the *H. gallinarum* nematode is presented in Figure 1F. Here, cuticular bumps are noted at a distance of 4800-5900 nm from each other (Fig. 1F).

Hypodermis

The second layer of the body wall of the nematode *H. gallinarum* is the hypodermis (also called epidermis and sub-cuticle), which is closely involved in cuticle formation, helminth growth and development, and has a syncytial structure. We studied the hypodermis and its structural elements at the ultrastructural level with the help of light and electron microscopic methods. The hypodermis, located between the muscular layer and the cuticle, is of connective tissue origin, rich in various organelles, and forms thickenings (hypodermal ridges) in the lateral alae, dorsal, and ventral parts of the helminth. In total, they consisted of four: 2 lateral, 1 ventral and 1 dorsal hypodermal ridge. Light microscope images of the hypodermis (Hy), lateral (Ln), dorsal (Dn), and ventral (Vn) ridges in semithin (1 μm) sections are presented in Fig. 2A and 2B. In semi-thin sections, the length of the lateral ridges is 61370-77510 nm (69650±1240 nm), the length of the dorsal ridges is 37860-41290 nm (39620±320 nm), the length of the ventral ridges is and its length was 43770-57110 nm (50240±1720 nm). It can be seen from figures 2A and 2B that the hypodermal ridges divide the muscular layer of the helminth into 4 equal parts and touch the wall of the pseudocoelomic cavity (pseudocoelom), where the internal organs are located. The helminth hypodermis was also examined by electron microscopy and images are presented in Fig. 2C and 2D.

During the ultrastructural examination of the hypodermis, various cytoplasmic structures, including mitochondria (M), glycogen (G), granular endoplasmic reticulum, vacuoles, ribosomes, fibrils (F) were observed here (Fig. 2C and 2D). Mitochondria and glycogen predominated in the hypodermis. Filaments in the cytoplasm provide strength to the skin-muscle by forming a connection between the cuticle and the muscular layers. The total thickness of the hypodermis was determined to be 3406-5154 nm (4226±124.5 nm).

Between the basal part of the cuticle of *H. gallinarum* and the hypodermis layer, tubular structures were found that extended throughout the body and were brightly stained in the obtained electrograms (Fig. 2C, indicated by white arrows). The thickness of those structures were 133.01-191.02 nm (159.14±4.88 nm). At higher magnifications of the transmission electron microscope, dense fibrils and microtubules were observed inside the tubular structures. These structures are also likely to provide additional strength to the skin-muscle sac. In addition to the above, tubular structures are bordered by hemidesmosomes (Hd) in the walls of the hypodermis (Fig. 2D). Nuclei were not found in the hypodermic layer of the helminth (except for the area with ridges) in both male and female individuals of the helminth. On the border with the muscular layer (ML) in the lateral ridges (Ln), oval-shaped nuclei (N) and a nucleus inside them were observed (Fig. 2E). The size of the nuclei was 2800-3300 nm (3100±60 μm). In addition to the mentioned processes (Mc) of the basal membranes of the cuticle towards the hypodermal cytoplasm were also observed at the border of the lateral canals of the *H. gallinarum* nematode with the covering tissue. Their length is 600–800 nm (700±20 nm) (Fig. 2F). Ultrastructural examination of the hypodermis of the nematode *H. gallinarum* revealed the presence of an excretory canal (Ik) (two in each parasite) in the center of the lateral ridges (Fig. 3A). Having a cellular structure, the cytoplasm is relatively dark in color and rich in various types of

organelles, including ribosomes, mitochondria, granular endoplasmic reticulum, vacuoles, and numerous canals leading to the lumen. The cytoplasm is surrounded by a membrane. The diameter of the tubular excretory canal is 6930-11660 nm (8930±430 nm), and the diameter of the lumen is 570-2410 nm (1270±180 nm). Harmful or unnecessary substances collected in the body of the helminth are located in the vacuole in the cytoplasm of the lateral ridges. They approach the membrane of the secretory canals and enter the cytoplasm of the cell by endocytosis. They, in turn, pass through the canals in the cytoplasm of the excretory canal to its exit, and from there they are removed from the body through the duct located in the front part of the parasite's body. The excretory canal is innervated by processes of nerve cells along with muscle cells in the muscular layer.

The nervous system of the nematode *H. gallinarum* studied by histological and electron microscopic methods is orthogonal and consists of a nodular pharyngeal ring and columns separated from it and directed towards both the anterior and posterior parts of the parasite. Those columns are located in the dorsal and ventral ridges of the hypodermis. Nerve columns are connected to each other through commissures. Nerve cells also innervate the digestive and reproductive organs. Figures 3B and 3C present neural columns (SS) located in the ventral (Vn) and dorsal ridges (Dn) and processes (Sc) of nerve cells innervating other organs and tissues. Figure 3D shows the dendrite (Dt) and axons (Ax) structures of the nerve column at high magnification of the electron microscope. It should be noted that only microtubules and neurofilaments are found in the cytoplasm of axons, and mitochondria and glycogen are found in the cytoplasm of dendrites in addition to the above mentioned.

Muscular layer

The last, third - muscular layer of the body wall of nematodes worms is located between the hypoderm and the wall of the pseudocoelom. While there are several types of muscle cells, the muscle layer of the nematode *H. gallinarum* has been found to contain somatic muscle cells. The muscular layer of the helminth is divided into 4 equal parts with the help of lateral, ventral and dorsal ridges. It was found that the *H. gallinarum* nematode studied by us consists of 3 parts in the images obtained from both light and electron microscopes of the cells in the muscular layer: sarcomere (Sa) composed of bundles of muscle fibers; glycogen, mitochondria, nucleus (including nucleolus), ribosome, granular endoplasmic reticulum, vacuole, etc. organelle-rich plasmatic part or sarcoplasm (Sp); muscle cell processes (Ec) that enter into synaptic contact with neurons in the dorsal or ventral ridges. Glycogen is observed in the cytoplasm of the processes of muscle cells (Fig. 4A, 4B, and 4C). In general, the thickness of the muscular layer was 34190-54830 nm (47970±1400 nm). The number of muscle cells located between the ridges was different depending on the genus of the helminth and the part of the body. Thus, it was found that the number of these muscle cells in *H. gallinarum* nematode varies from 19 to 28 in total. According to the number of muscle cells located between the ridges, the muscle layer of tapeworms is divided into several types. In the *H. gallinarum* nematode whose ultrastructure was studied, the muscle cells of polymyar type. The length of the sarcomere (Sa) of the muscle cell of the parasite, i.e. the unfolding part, was 26790-50810 nm (42310±1760 nm). According to the structure of the sarcomere itself, there are several types. The helminth sarcomere examined by electron microscopy surrounds the muscle cell in a U-shaped form rising from the sides to the sarcoplasm and is of coelomyar type (Fig. 4A and 4B). The height of sarcoplasm (Sp) was determined to be 11940-22420 nm (17280±880 nm).

During the ultrastructural study of the sarcomere of the muscle cell of the nematode *H. gallinarum*, it was found that it consists of two (right and left) fibrillar columns (Fig. 4B). Columns, in turn, are composed of numerous fibrillar bundles (Fd) (Fig. 4D). They are separated from each other by dense bodies (Db) (Fig. 4E). The thickness of dense bodies was 86.13-308.43 nm (211.41±13.77 nm). They participate in the synthesis of fibrils. Fibrillar bundles located in the sarcomere are in turn thick (marked with 1, sizes – 14.12-19.14 nm (16.45±0.38 nm)) and thin (marked with 2, sizes – 5.14-9.98 nm (7.11±0.37 nm)) separated into filaments (Fig. 4E). Comparison of the obtained morphometric measurements shows that thick filaments are 2.3 times bigger than others (thin filaments). The muscular layer is demarcated from the hypodermis and neighboring muscle cells by a basement membrane (Bm) (size 37.39-64.59 nm (49.02±2.70 nm)) (Fig. 4D). Apart from the structural elements mentioned, mitochondria are also found between the filaments in the sarcomere (Fig. 4D and 4E). The sarcoplasmic part of the muscle cell of the helminth is directed towards the pseudocoelom cavity of the worm and ends with a basal lamina . The body wall of *H. gallinarum* nematode was studied with the help of light and electron microscopic methods, and it was determined that muscle cells have complex ultrastructural elements.

Discussion

The analysis of literature data on the ultrastructural structure of the body wall of nematodes shows that the covering tissue of

nematodes is composed of cuticle, hypodermis and muscular layer. Cuticle, in turn, consists of 3 main layers: cortical, homogeneous and fibrillar. The cuticle of nematodes completely covers the parasite from the outside (some internal organs, for example, the digestive system, the reproductive of male and female parasites) and develops from hypodermal cells. It was found that the nematodes body wall consists mostly of three main layers [15, 48, 49, 50, 51], as well as in *H. gallinarum* helminth, whose ultrastructure was studied by us. Therefore, the covering tissue of the nematode *H. gallinarum* is similar to the generally accepted structure of nematodes.

Many species are included in the Heterakidae family, only *H. dispar, H. spumosa* and *H. gallinarum* nematodes have literature data on the ultrastructure of the body wall [8, 11, 14, 15]. It was found that the cuticle of all three mentioned parasites, as well as the cuticle of the *H. gallinarum* nematode studied by us, consists of 8 layers. As a result of the analysis of the received statistical data, it was found that the cortical layer of the *H. gallinarum* nematode is 14.4% of the total cuticle, the homogeneous layer is 41.0%, and the fibrillar layer is 44.6%. The epicuticle of some free-living nematodes (*Caenorhabditis elegans*), including *H. dispar*, has been shown to be covered externally by a thin glycocalyx or glycoprotein layer [15, 52, 53]. This layer was not observed even in high magnifications of the obtained electronograms related to the cuticle of *H. gallinarum* nematode. It should also be noted that during the ultrastructural study of the helminth cuticle, the homogeneous layer around the bursa of male individuals of *H. gallinarum* nematode is thicker than other parts of the parasite's body, as in adult male prasite of *H. dispar* nematode [15]. In addition, as a result of the ultrastructural study of the lateral alae extending along the body of the helminth, it consists of 3 main layers (cortical, homogeneous and fibrillar), instead of 8 layers as in the cuticle (Fig. 1D). The mentioned homogeneous and fibrillar layers are many times thicker than the layers of the same name located in the cuticle. Those signs were also identified in *H. dispar*, another species of the Heterakidae family [15]. However, as a result of the comparison of both light and electron microscopic images, it is known that *H. gallinarum* nematode differs from *H. dispar* nematode due to the shape of lateral alae. It was determined that the shape of the cuticle in different parts of the bodies of helminths is variable in the species whose ultrastructure was studied. Thus, it was found that the cuticle around the bulb of both adult male and female *H. gallinarum* nematode is not smooth, but has two different forms (Fig. 1E and 1F). In adult *H. dispar* nematodes males, in the anterior part of the body, the shape of the cuticle is smooth. In the area where the seminal vesicle is located, the cross sections of the helminth show depressions located at the same distance (4 μm) from each other. These depressions extend to the homogeneous cuticle layer. In the posterior part of the body, in the area where the spicules are located, the cuticle has a wave shape [15]. There is information in the literature that the tubular structures noted in the basal part of the cuticle of *H. gallinarum* nematode (Fig. 2C) are also found in nematodes belonging to other families [54, 55]. Those tubular structures were also noted in the nematode *H. dispar*, another representative of the family of the same name [15]. In the literature, the ultrastructure of *H. spumosa*, another parasite belonging to the same family, was clearly visible in the electrograms shown during the study of the changes in their structures under normal conditions and after the effect of drugs. However, about these structures were not reported in the text of the article [14]. Thus, in the researches conducted by us and as a result of the analysis of literature data, tubular structures are found in the basal part of the cuticle in all 3 species (*H. gallinarum, H. dispar* and *H. spumosa*) whose ultrastructure was studied and are included in the Heterakidae family. Taking into account the mentioned, these structures can be shown as a characteristic feature of the species belonging to the *Heterakis* genus.

There are literature data on the study of the ultrastructure of the hypodermis of *H. gallinarum, H. dispar* and *H. spumosa*, species of the Heterakidae family [8, 14, 15, 51]. Information about the location of hemidesmosomes between the basal part of the cuticle and the hypodermis is given in the literature [13, 56]. The same hemidesmosomes were found in the nematode *H. dispar* [15], as well as in the nematodes *H. gallinarum* (Fig. 2D). In the nematode *H. gallinarum*, the sizes of the ridges that form thickenings in the hypodermis differ from each other. Thus, the lateral ridges are larger in size than the ventral and dorsal ridges In addition, the hypodermal ridges become thinner when they reach the level of the muscular layer, and widen again in the direction towards the pseudocoelomic cavity (Fig. 2E). The mentioned was also found in *H. dispar* nematode [8, 15]. There are various data on the number, shape and location of nuclei in the hypodermis of nematodes [57]. Thus, it is known that *H. dispar* parasite has 2 large nuclei in each of the lateral ridges [15]. Another source shows the presence of 1-2 large or several small nuclei in the hypodermal ridges of the *H. gallinarum* nematode [8]. During the ultrastructural study of the hypodermis of the nematodes *H. gallinarum*, two nuclei were observed in the ridges. The presence of cytoplasmic bodies in the hypodermis of the nematode *H. dispar* is shown [15]. They are protein assemblies of different sizes formed as a result of

expression of recombinant proteins. Cytoplasmic bodies are also found in other nematodes [55]. They were not observed in the hypodermis of *H. gallinarum* nematode. In addition to the above, at the border between the ridges of the nematode and the covering tissue, processes of the basal membranes of the cuticle directed towards the hypoderma are observed (Fig. 2F). Those protrusions were also found in the nematode *Wuchereria bancrofti*, which is a parasite in humans, and in the nematode *H. dispar*, a species of the Heterakidae family [15, 58]. The orientation of the basement membrane towards the hypodermis suggests that it provides strength to both by multiplying the contact area of the cuticle with the hypodermis. The ultrastructure of the excretory channal passing through each of the alae located on the lateral ridges of nematodes and having a cellular structure has been studied in the species *Cystidicoloides ephemeridarum, Trichostrongylus colubriformis, Haemonchus placei, Nematodirus battus* [59-61], and in the species of *H. spumosa* and *H. dispar* from the genus Heterakis [14, 15]. Based on the results obtained from literature sources and personal research, it was found that the structural elements of the excretory channel of the nematode *H. gallinarum* are the same as in other species included in the genus (Heterakis). There are some literature data on the ultrastructural structure of nerve columns located in the dorsal and ventral hypodermal ridges of nematodes. The ultrastructural characteristics of the neural columns of the nematodes *Cystidicoloides ephemeridarum* belonging to the family Cystidicolidae, *Procamallanus halitrophus* belonging to the family Camallanidae, *Trichostrongylus colubriformis* belonging to the family Trichostrongylidae and *Haemonchus placei* are given [55, 59, 61]. About the ultrastructure of the species belonging to the genus Heterakis, there are literature data on the study of *H. spumosa* and *H. dispar* species [14]. The ultrastructural features of the nerve columns of the nematode *H. gallinarum* studied by us corresponded with the structure of the species included in the genus.

As a result of the analysis of literature data, it was found that there are several types of muscle cells depending on their location in the body of nematodes: somatic and specialized muscle cells [13]. Specialized muscle cells are mainly found around the digestive (mouth, pharynx, esophagus, bulb, etc.) and reproductive organs (in both male and female individuals). The conducted studies showed that the muscular system of *H. gallinarum* nematode is composed of somatic cells. According to the data of some researchers, there is a basal lamina between the hypodermis and the muscular layer [55]. Other authors report that there is a basement membrane at the boundary between the hypodermis layer and muscle cells [13, 62]. It is noted that the nematode *H. dispar*, which belongs to the Heterakidae family, has a basement membrane [15]. It was found that the nematode *H. gallinarum* studied by us is bounded by the hypodermis layer and other muscle cells through the basement membrane (Fig. 4D). Usually, there is information that the muscle cells of most species of nematodes consist of three parts (sarcomere, sarcoplasm, muscle processes). But the lack of muscle cell processes of some species of nematodes is also noted [8, 48, 49]. Parasitic helminths belonging to the Heterakidae family, including *H. dispar*, *H. spumosa* and *H. gallinarum* species, also have processes of muscle cells [8, 14, 15]. During the ultrastructural examination of the body wall of the nematode *H. gallinarum*, we observed the presence of cytoplasmic processes of muscle cells (Fig. 4C). Based on the literature data [8, 14, 15] and the results obtained from our own research, it was found that the muscle layer of all species belonging to the Heterakidae family and whose ultrastructure was studied is polymyar type (Fig. 4A). Among the species whose ultrastructure is studied, the number of muscle cells in nematodes is different in parasites, which are divided into 4 equal parts due to ridges. In the *H. dispar* nematode, their number is 19-28, and in the *H. gallinarum* species, up to 120 in total (in the cross-section of the entire parasite) [8, 15]. In the *H. gallinarum* nematode studied by us, it was found that there are 17-26 muscle cells between each ridge.

There are several types of the sarcomere part of the muscle cell of tapeworms. There are known, platymyar, coelomyar and circomyari types of sarcomeres [13]. There is information that the sarcomere of *H. dispar* and *H. spumosa* species, which belong to the Heterakidae family and whose ultrastructure of muscle cells is studied, is of the coelomyar type [14, 15]. The same type of sarcomere was also noted in the nematode *H gallinarum*. It is known that mitochondria are found in the sarcoplasm of muscle cells. In addition to the sarcoplasm of the studied nematode *H. gallinarum*, a small number of mitochondria are found among the fibrils in the sarcomere (Fig. 4D and 4E). The same process was observed during the ultrastructural study of the nematode *H. dispar* [15]. Based on the obtained results, it was once again confirmed that muscle cells perform the function of both support and movement of helminths. Thus, the body wall of the *H. gallinarum* nematode and its constituent layers were studied at the ultrastructural level using light and electron microscopic methods.

Conclusion

The *H. gallinarum* nematode, which has a wide distribution area, can cause serious pathologies in

the body of its hosts by being encountered with high intensity in poultry farms. Parasites included in the genus Heterakis are similar in appearance, so their determination creates certain difficulties. Therefore, the body wall of the *H. gallinarum* nematode was studied by light and electron microscopic methods, and comparative characteristics with the structures of the cover tissue of other species of these family were given. The cuticle that covers the helminth from the outside consists of 8 layers. In both individuals (male and female) of the parasite, the lateral alae are composed of 3 main layers. Here, homogeneous and fibrillar layers are many times thicker than other parts of the helminth's body. Unlike other species of the family whose ultrastructure was studied, while the shape of the cuticle of both male and female individuals of the nematode *H. gallinarum* was smooth throughout the body, 2 different shapes were found around the bulb. Tubular structures were noted between the basal part of the cuticle and the hypodermis. In the hypodermis of the helminth, dorsal, ventral and 2 lateral ridges are found, the latter of which are 2 times larger than the others. In addition, the ultrastructural features of the excretory canal and nerve elements located in the hypodermal ridges are also described. The nervous system of the helminth is orthogonal. The muscular system is of polymyar type and the number of muscle cells varies from 19 to 28 depending on the sex and body part of the helminth.

Funding statement

This work was self-financial supported by the authors.

Ethical consideration

This study was performed after the approval from the Ethics Committee of Azerbaijan Medical University (Ministry of Health of Azerbaijan Republic), Baku, Azerbaijan

Conflict of interest

The authors claims that there are no competing interests

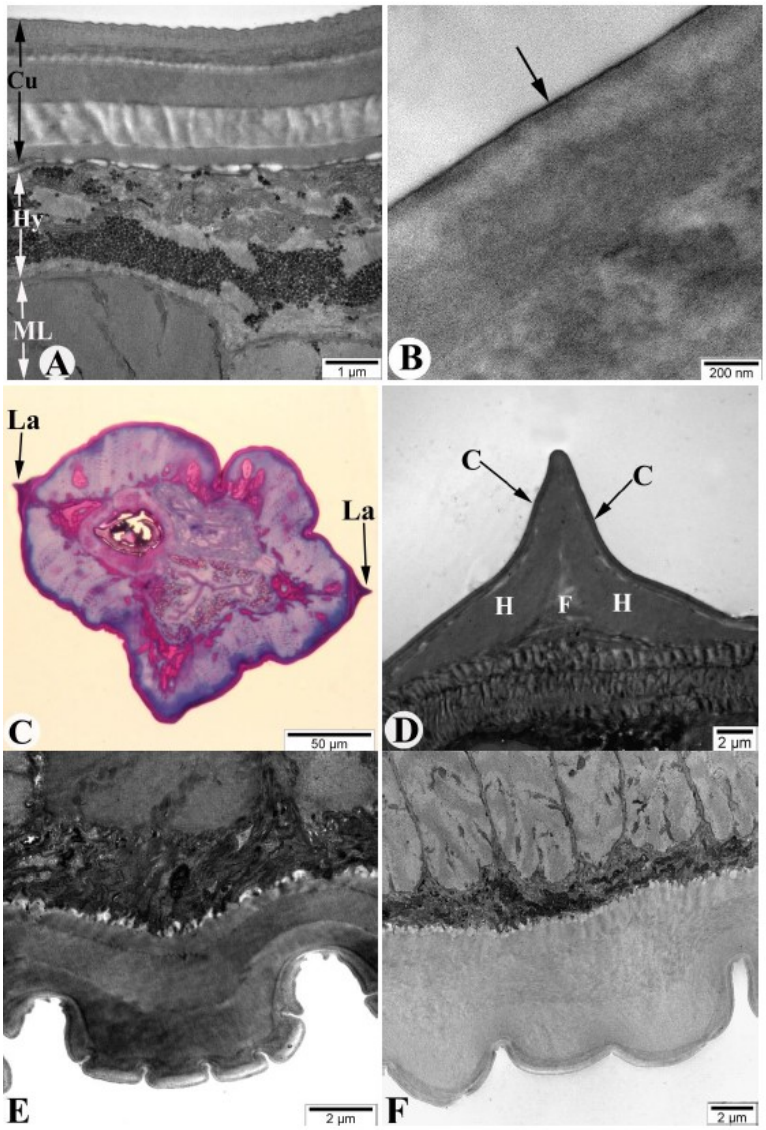

Fig. 1. Light and electron microscopic images of the cuticle and lateral alae of *H. gallinarum*. A- layers of the cuticle, B - epicuticle indicated by a black arrow, C - double lateral alae of the cuticle, D - ultrastructure of the lateral alae, E, F - different forms of the cuticle around the worm bulb. Designations: Cu-cuticle, Hy-hypodermis, ML-muscular layer, La- lateral alae, C – cortical layer, H – homogeneous layer, F – fibrillar layer. (C) - Semithin section (1 µm), Morikawa et al. (2018) one-step staining, (A, B, D) - ultrathin sections (50-70 nm), stain: uranyl acetate and Pb citrate.

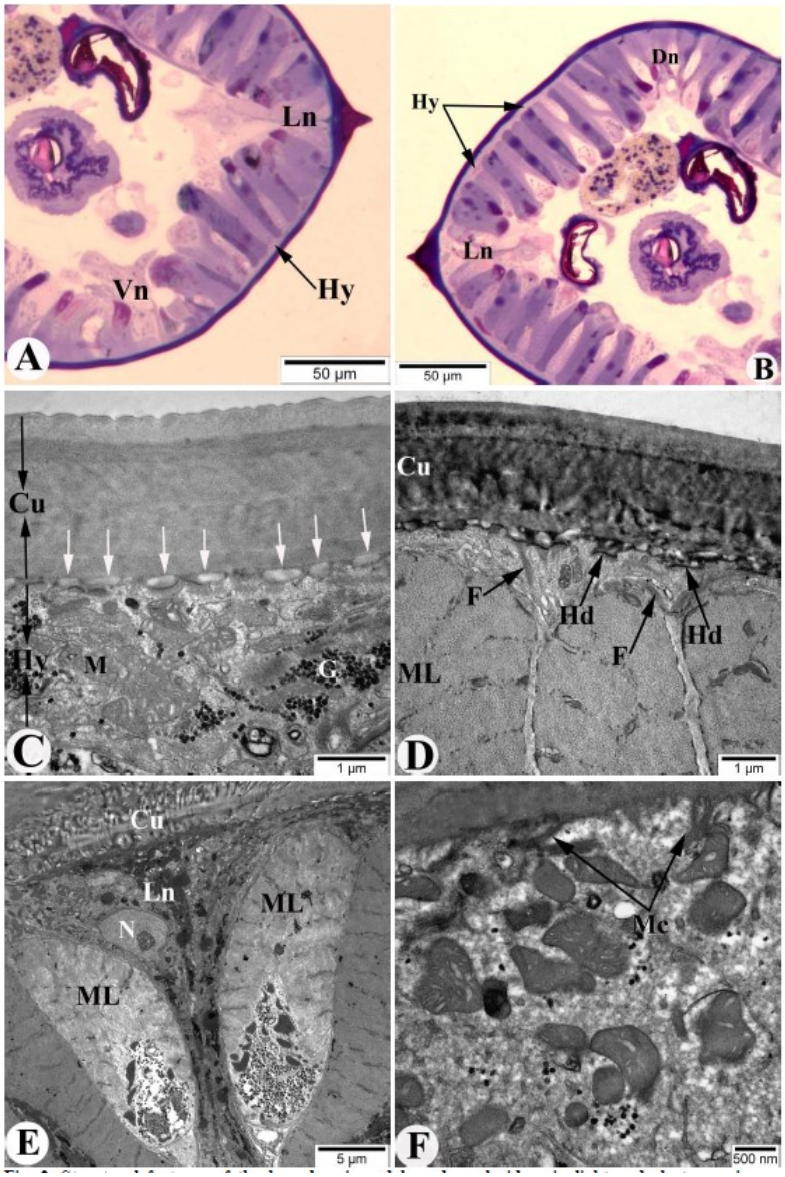

Fig. 2. Structural features of the hypodermis and hypodermal ridges in light and electron microscopes of nematode *H. gallinarum*. A, B - hypodermis and all ridges, C, D - hypodermis and tubular structures and hemidesmosomes, E - nucleus in the lateral ridge, F - processes of basement membrane. Designations: Dn - dorsal ridge, Vn – ventral ridge, Ln – lateral ridge, Hy – hypodermis, M – mitochondria, G – glycogen, Cu – cuticle, N – nucleus, ML – muscular layer, Mc – processes of basement membrane. (A, B) - semi-thin section (1 µm), Morikawa et al. (2018) one-step staining, (C, D, E, F) - ultrathin sections (50-70 nm), stain: uranyl acetate and Pb citrate.

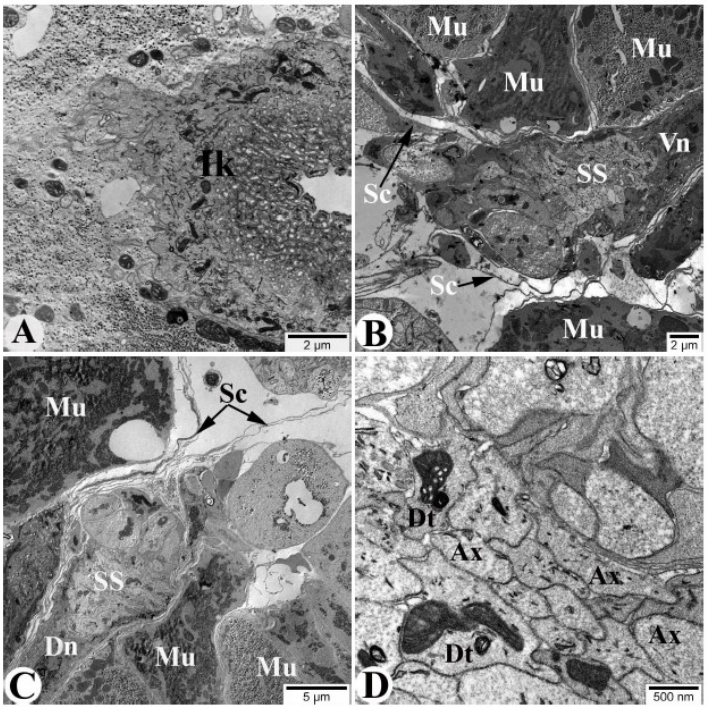

Fig. 3. Ultrastructural features of excretory canal and neural columns of nematode *H. gallinarum*. A – general view of the excretory canal, B – nerve column located in the ventral ridge, C – nerve column located in the dorsal ridge, D – nerve cells, Designations: Dn - dorsal ridge, Vn - ventral ridge, Ik - excretory canal, Mu - muscle cell, SS – nerve column, Sc – nerve processes, Dt – dendrite, Ax – axon, ultrathin sections (50-70 nm), stain: uranyl acetate and Pb citrate.

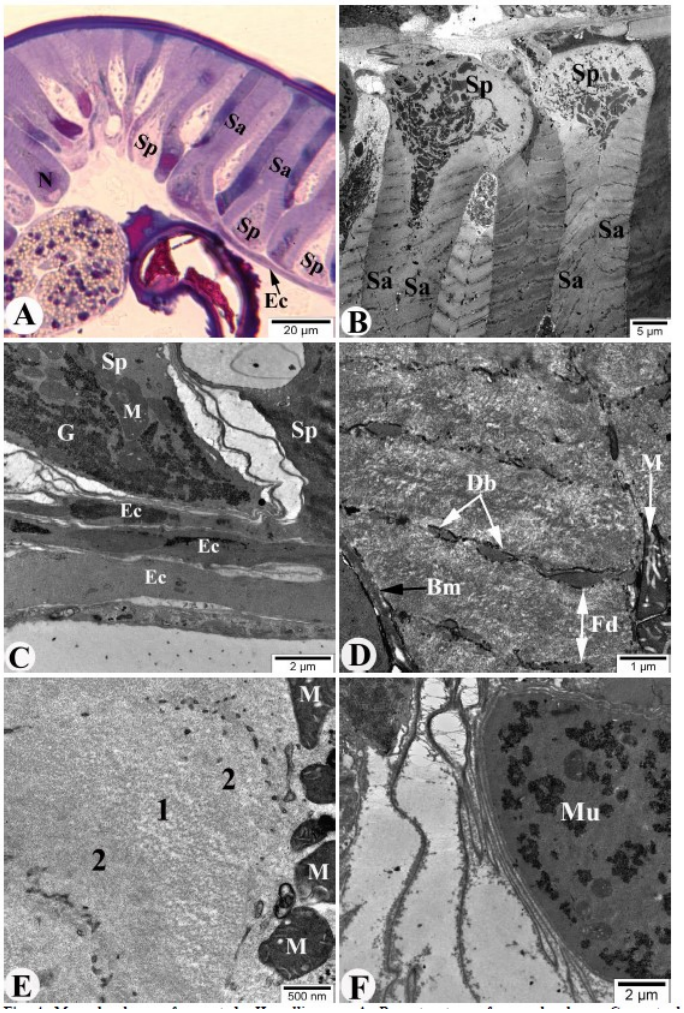

Fig. 4. Muscular layer of nematode *H. gallinarum*. A, B – structure of muscular layer, C - cytoplasmic processes, D, E – structure of sarcomere, F - the basal lamina of the sarcoplasm. Designations: Sa - sarcomere, Sp - sarcoplasm, Ec - cytoplasmic processes, N - nucleus, G - glycogen, M - mitochondria, Bm - basement membrane, Db - dense bodies, Fd - fibrillar bundles, Mu - muscle cell, 1 – thick filaments, 2 – thin filaments. (A) - semithin section (1 µm), Morikawa et al. (2018) one-step staining, (B-F) - ultrathin sections (50-70 nm), stain: uranyl acetate and Pb citrate.

References

1. McDonald, M.E. Catalogue of helminths of waterfowl (Anatidae). Washington, D.C.: U.S. Dept. of the Interior, Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife (1969).
2. Smogorzhevskaya, L.A. Helminths of waterfowl and wading birds of the fauna of Ukraine. Kiev, Nauka Dumka, (1976).
3. Gicik, Y., and Arslan, M.O. The prevalence of helminths in the alimentary tract of geese (*Anser anser domesticus*) in Kars District, Turkey. *Veterinary Research Communications*, **27**, 391–395 (2003).
4. Zhang, Sh., Bu, Y., Huang, G., Wen, Q. and Zhang, L. A checklist of parasitic nematodes (Nematoda) from birds (Aves) in China. *Zootaxa*, **3446**, 1–31 (2012).
5. Begum, A., Mukutmoni, M. and Akter, F. Parasite diversity in domestic duck on *Anas platyrhynchos domesticus* from Munshiganj, Dhaka. *Bangladesh Journal of Zoology*, **47**(1), 121–128 (2019).
6. Al-Lahaibi, B.Y., Hasan, M.H. and Al-Taee A.F. Incidence of internal parasites of the slaughtered local breeds of ducks and geese. *Iraqi Journal of Veterinary Sciences*, **35**(1), 39–44 (2021).
7. Parlak, Y. and Gürler, A.T. Gastro-intestinal and Respiratoric System Helminths of Domestic Geese in Samsun and Districts. *Turkiye Parazitoloji Dergisi*, **47**(1), 49–52 (2023).
8. Bogoyavlensky, Yu.K. Structure and functions of integumentary tissues of parasitic nematodes. Moscow, Nauka, (1973).
9. Bogoyavlensky, Yu.K., Bogolepova, I.I., and Onushko, N.V. Microstructure of tissues of parasitic nematodes. Moscow, Nauka, (1983).

10. Lee, D.L. Structure and function of the intestinal-cloacal junction of the nematode Heterakis gallinarum. *Parasitology*, **70**, 389–396 (1975).

11. Wright, K.A. and Hui, N. Post-labial sensory structures on the cecal worm, *Heterakis gallinarum*. *The Journal of Parasitology*, **62**(4), 579–584 (1976).

12. Zmoray, I. and Guttekova, A. Ultrastructure of intestinal cells of *Heterakis gallinarum*. *Angewandte Parasitologie*, **19**(2), 106–111 (1987).

13. Bird, F.A. and Bird, J. The structure of Nematodes.2 nd edition. Academic Press, San Diego, (1991).

14. Mehlhorn, H. and Harder, A. Effects of the synergistic action of febantel and pyrantel on the nematode *Heterakis spumosa*: a light and transmission electron microscopy study. *Parasitology Research*, **83**, 419–434 (1997).

15. Rzayev, F.H. Ultrastructural features of the body wall of the helminth Heterakis dispar (Schrank, 1790) (Nematoda: Heterakidae). *Parasitologia*, **57**(1), 20–37 (2023).

16. Rzayev, F.H. Ultrastructural features of the digestive system of the helminth *Heterakis dispar* Schrank, 1790 (Nematoda: Heterakidae). *Egyptian Journal of Veterinary Sciences*, **54**(3), 503–514 (2023).

17. Rzayev, F.H. Ultrastructural characteristics of the reproductive organs of an adult female helminth *Heterakis dispar* Schrank, 1790 (Nematoda: Heterakidae). *Egyptian Journal of Veterinary Sciences*, **55**(1), 135–146 (2024).

18. Rzayev, F.H., Gasimov, E.K., Nasirov, A.M. and Seyidbeyli, M.I. Ultrastructural characteristics of the reproductive organs of adult male helminth *Heterakis dispar* (Nematoda: Heterakidae). *Biosystems Diversity*, **31**(2), 202–208 (2023).

19. Lee, D.L. The structure and development of the spermatozoon of *Heterakis gallinarum* (Nematoda). *Journal of Zoology*, **164**, 181–188 (1971).

20. Lee, D.L. Evidence for a sensory function for the copulatory spicules of nematodes. *Journal of Zoology*, **169**, 281–285 (1973).

21. Lee, D.L. and Lestan, P. Oogenesis and egg shell formation in *Heterakis gallinarum* (Nematoda). *Journal of Zoology*, London, **164**, 189–196 (1971).

22. Wright, K.A. Labial sense organs of the nematode, *Heterakis gallinarum*. *The Journal of Parasitology*, **63**(3), 528–539 (1977).

23. Bazh, E.K. Molecular identification and phylogenetic analysis of *Heterakis gallinae* from native chickens in Egypt. *Parasitology Research*, **112**, 3557–3560 (2013).

24. Wang, B.J., Gu, X.B., Yang, G.Y., Wang, T., Lai, W.M., Zhong, Z.J. and Liu, G.H. Mitochondrial genomes of *Heterakis gallinae* and *Heterakis beramporia* support that they belong to the infraorder Ascaridomorpha. *Infection, Genetics and Evolution*, **40**, 228–235 (2016).

25. Mlondo, S., Tembe, D., Malatji, M.P., Khumalo, Z.T.H. and Mukaratirwa S. Molecular identification of helminth parasites of the Heterakidae and Ascarididae families of free-ranging chickens from selected rural communities of KwaZulu-Natal province of South Africa. *Poultry Science*, **101**, 1–10 (2022).

26. Abdel-Gaber, R., Kamel, R., Maher S. and Fergani Y.A. Morphological and molecular studies of the nematode parasite *Heterakis gallinarum* (Heterakidae) infecting the cattle egret *Bubulcus ibis* (Ardeidae). *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, **75**(6), 1096–1106 (2023).

27. Nabil, A., Sarra, F., Osama, B.M., Abdulaziz, A. and Lilia, B. Molecular characterization of the nematode *Heterakis gallinarum* (Ascaridida: Heterakidae) infecting domestic chickens (*Gallus gallus domesticus*) in Tunisia. *Turkish Journal of Veterinary and Animal Sciences*, **42**, 388–394 (2018).

28. Cupo, K.L. and Beckstead, R.B. PCR detection of *Heterakis gallinarum* in environmental samples. *Veterinary Parasitology*, **271**, 1–6 (2019).

29. Biswas, P.G., Ohari Y., Mohanta, U.K. and Itagaki T. Development of a multiplex PCR method for discriminating between *Heterakis gallinarum, H. beramporia*, and *H. indica* parasites of poultry. *Veterinary Parasitology*, **295**, 1–5 (2021).

30. Rzayev, F.H. The comparative analysis of mixed invasions of the domestic waterfowl in different ecological areas of Azerbaijan. *Proceedings of the Institute of Zoology*, **31**(2), 136–144 (2013).

31. Rzayev, F.H., Mehraliyeva, G.A. and Ibrahimova, N.E. Comparative characteristics of the helminth fauna of domestic waterfowl in the south-eastern part of Azerbaijan. *Ecological Bulletin,* **2**(32), 101–106 (2015).

32. Seyidbeyli, M.I. and Rzayev, F.H. Systematical review of helminth fauna of waterfowl poultry in the territory of Babek region of Nakhchivan AR. *Journal of Entomology and Zoology Studies*, **6**(1), 1668–1671 (2018).

33. Seyidbeyli, M.I., Maharramov, S.H. and Rzayev, F.H. Causes of similarity of helminth fauna of domestic waterfowls and wild birds in Nakhchivan AR, parasites specificity. *The Journal of Agrarian Science*, **1**(1), 58–63 (2019).

34. Seyidbeyli, M.I., Maharramov, S.H. and Rzayev, F.H. Comparative analysis of mixed invasions in domestic waterfowls in Nakhchivan AR. *Scientific publications of Nakhchivan State University, Series of Natural and Medical Sciences*, **3**(100), 209–212 (2019).

35. Rzayev, F.H., Nasirov, A.M. and Gasimov, E.K. A systematic review of the parasites (Plathelminthes: Cestoda) of the domestic goose (*Anser anser* dom.). *The Journal of V. N. Karazin Kharkiv National University, Series "Biology"*, **36**, 58–73 (2021).

36. Rzayev, F.H. Cestodes (Plathelminthes: Cestoda) of domestic waterfowl. *Advances in Biology & Earth Sciences*, **6**(2), 133–141 (2021).

37. Rzayev, F.H. A systematic review of flukes (Plathelminthes: Trematoda) of domestic goose (Anser anser dom). *Biosystems Diversity*, **29**(3), 294–302 (2021).

38. Rzayev, F.H., Nasirov, A.M. and Gasimov, E.K. A systematic review of tapeworms (Plathelminthes, Cestoda) of domestic ducks (*Anas platyrhynchos* dom). *Regulatory Mechanisms in Biosystems,* **12**(2), 353–361 (2021).

39. Rzayev, F.H. Helminths of domestic waterfowl of Azerbaijan and influence on them of plants with anthelmintic properties. Baku, Zangazurda, (2023).

40. Dubinina, M.N. Parasitological study of birds of the Academy of Sciences. Methods of Parasitological Research. Leningrad, Nauka, (1971).

41. Ryzhikov, K.M. Key to helminths of domestic waterfowl. Moscow, Nauka, (1967).

42. Kuo, J. Electron microscopy: methods and protocols. Totowa, Humana Press, (2014).

43. Yushin, V.V., Claeys, M., Leunissen, J.L.M. and Zograf, J.K. Electron Microscopy Techniques. Techniques for work with plant and soil nematodes. Boston, CABI, (2021).

44. D'Amico, F. A polychromatic staining method for epoxy embedded tissue: a new combination of methylene blue and basic fuchsine for light microscopy. *Biotechnic Histochemitry*, **80**(5–6), 207–210 (2005).

45. Morikawa, Sh., Sato, A. and Ezaki, T. A simple, one-step polychromatic staining method for epoxy-embedded semithin tissue sections. *Microscopy*, **67**(6), 331–344 (2018).

46. Agayeva, N.J., Rzayev, F.H., Gasimov, E.K., Mamedov, Ch.A., Ahmadov, I.S., Sadigova, N.A, Khusro, A., Al-Dhabi, N.A. and Arasu, M.V. Exposure of rainbow trout (*Oncorhynchus mykiss*) to magnetite (Fe_3O_4) nanoparticles in simplified food chain: Study on ultra-structural characterization. *Saudi Journal of Biological Sciences*, **27**(2), 3258–3266 (2020).

47. Rzayev, F.H., Gasimov, E.K., Agayeva, N.J., Manafov, A.A., Mamedov, C.A., Ahmadov, I.S., Khusro, A., Valan Arasu, M., Sahibzada, M.U.K., Al-Dhabi, N.A., and Choi, K.C. Microscopic characterization of bioaccumulated aluminium nanoparticles in simplified food chain of aquatic ecosystem. *Journal of King Saud University – Science*, **34**(1), 1–8(2022).

48. Seyidbeyli, M.I., Rzayev, F.H. and Gasimov, E.K. Ultrastructural features of the body wall of the helminth *Trichostrongylus tenuis* (Mehlis, 1846) (Nematoda: Trichostrongylidae). *Parazitologiya*, **54**(5), 402–412 (2020).

49. Rzayev, F.H., Seyidbeyli, M.I., Maharramov, S.H. and Gasimov, E.K. Forms and ultrastructural features of the lateral alae of the helminth *Trichostrongylus tenuis* Mehlis, 1846 (Nematoda: Trichostrongylidae). *The Journal of V. N. Karazin Kharkiv National University*, **34**, 112–119 (2020).

50. Rzayev, F.H. To studying of morphology of nematode Ganguleterakis dispar (Schrank, 1790). *News of Azerbaijan National Academy of Science*, **65**(2), 141–146 (2010).

51. Nasirov, A.M., Bunyatova, K.S., Kaziyeva, N.Sh. and Rzayev, F.H. Micromorphology of tissues of the nematode *Ganguleterakis dispar* (Schrank, 1790). *Proceedings of the IV Congress of the Russian Society of Parasitologists*, *Saint-Petersburg, Lemma*, **2**, 208–210 (2008).

52. Cox, G.N., Kusch, M. and Edgar, R.S. Cuticle of *Caenorhabditis elegans*: its isolation and partial characterisation. *Journal of Cell Biology*, **90**, 7–17 (1981).

53. Page, A.P. and Johnstone, I.L. The cuticle, WormBook, ed. The *C. elegans* Research Community, WormBook, (2007).

54. Peixoto, C.A., Kramer, J.M. and De Souza, W. *Caenorhabditis elegans* cuticle: a description of new elements of the fibrous layer. *Journal of Parasitology*, **83**, 368–372 (1997).

55. Cardenas, M.Q., Souza, D.W. and Lanfredi, R.M. Ultrastructure of *Procamallanus (Spirocamallanus) halitrophus* (Nematoda: Camallanidae) parasite of flounder. *Parasitology Research*, **97**, 478–485 (2005).

56. Neuhaus, B., Brescianit, J., Christensen, Ch.M., and Frandsen, F. Ultrastructure and Development of the Body Cuticle of *Oesophagostomum dentatum* (Strongylida, Nematoda). *The Journal of Parasitology*, **82**(5), 820–828 (1996).

57. Frantova, D., Brunanska, M., Fagerholm, H. and Kihlström, M. Ultrastructure of the body wall of female *Philometra obturans* (Nematoda: Dracunculoidea). *Parasitology Research*, **95**, 327–332 (2005).

58. Oliveira-Menezes, A., Noroes, J., Dreyer, G. and Lanfredi, R. Ultrastructural analysis of *Wuchereria bancrofti* (Nematoda: Filarioidea) body wall. *Micron*, **41**, 526–531 (2010).

59. Smith, K. and Harness, E. The ultrastructure of the adult stage of *Trichostrongylus colubriformis* and *Haemonchus placei*. *Parasitology*, **64**, 173–179 (1972).

60. Martin, J. and Lee, D.L. *Nematodirus battus*: structure of the body wall of the adult. *Parasitology*, **86**, 481–488 (1983).

61. Frantova, D. and Moravec, F. Ultrastructure of the body wall of *Cystidicoloides ephemeridarum* (Nematoda, Cystidicolidae) in relation to the histopathology of this nematode in salmonids. *Parasitology Research*, **91**, 100–108 (2003).

62. Lecroisey, C., Segalat, L., and Gieseler, K. The *C. elegans* dense body: anchoring and signaling structure of the muscle. *Journal of Muscle Research and Cell Motility*, **28**, 79–87 (2007).