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Ultrastructural Characteristics of the Reproductive Organs in Adult Female Helminth *Heterakis Dispar* Schrank, 1790 (Nematoda: Heterakidae)

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MORPHOLOGICAL characteristics of most of the species included in the Heterakidae family are similar. The study of the ultrastructure of helminths makes it possible to determine additional taxonomic features in the identification of species. In the present study, the ultrastructural characteristics of the organs belonging to the reproductive system of adult female nematode *Heterakis dispar* (Schrank, 1790), belonging to the Heterakidae family, with using of light and electron microscopic methods were studied. It was found that the wall of ovary, oviduct and uterus consists of two (basal lamina and epithelial), vagina has three (basal lamina, muscular and epithelial), and the wall of the ovijector consists of four layers (basal lamina, muscular and epithelial, thick cuticle in the lumen). The ultrastructural characteristics of helminth germ cells and their development (oogenesis) in different stages (oogonia, oocytes) were studied in detail. The layers of the formed eggshell of *H. dispar* nematode (uterine, vitelline, chitinous and lipid layers) were determined. In the study, a comparative analysis of the ultrastructure of reproductive organs of female *H. dispar* with nematodes *H. gallinarum* and *H. spumosa*, which belong to the Heterakidae family was given. It is appropriate to use the obtained results in specifying the systematic positions of nematodes. In the future, ultrastructural data can be used as a norm during the development of methods of combating nematodosis.

Keywords: *Heterakis dispar*, Female reproductive organs, Oogenesis, Ultrastructure, TEM.

Introduction

Parasitic nematodes are one of the main components of the biocenosis, and they have the ability to regulate the number of hosts by consuming a large amount of energy in the ecosystem [1-3]. Heterakis nematodes that parasitize domestic and wild birds. As a result of helminthological research conducted by us in the territory of the Republic of Azerbaijan, 27 species of helminths were recorded in domestic waterfowl (*Anser anser* dom. and *Anas platyrhynchos* dom.) [4-7]. Thirteen species of them (*A. acutum, A. anseris, T. tenuis, H. altaicus, H. dispar, H. gallinarum, A. galli, P. crassum, T. fissispina, H. tricolor, C. anatis E. contortus , B. obsignata*) is included in the class of nematode [8]. Three of the mentioned species (*H. dispar, H. gallinarum, H. altaicus*) belong to the Heterakis genus of the Heterakidae family. Among those species, *H. dispar* was observed with high intensity and extensiveness in domestic waterfowl in all areas of the country, causing more serious damage to their hosts [9]. Determining the taxonomic position of the species included in the Heterakidae family on the basis of the signs given in the identifiers causes certain difficulties. This situation is related to the morphological similarity of the species included in the family. The study of the ultrastructure of nematodes makes it possible to determine additional taxonomic characteristics for the identification of species. As a result of the analysis of literature data, it was found that there

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are studies on the ultrastructure of only a few species of parasitic nematodes (*H. gallinarum, H. spumosa*) belonging to the Heterakidae family [10-19]. In addition, these studies provide information on the ultrastructure of some nematode organs, rather than a complete picture. We have not found sources on the ultrastructure of *H. dispar* nematode, which is a specific parasite of domestic waterfowl and is widespread in the country. In recent years, as a result of large-scale scientific research conducted by us in the direction of studying the ultrastructure of parasites, along with other helminths [20-22], also ultrastructure of the body wall, digestive and reproductive organs of male *H. dispar* nematode have been studied [23-25]. Taking into account the above, the aim of the current research work is to study the ultrastructural characteristics of the reproductive organs of an adult female nematode *H. dispar* used light and electron microscopic methods and to compare it with the structure of other species of the same family.

Material and Methods

The studies conducted in the direction of studying the ultrastructure of the helminth were carried out in 2019-2023, and 10 individuals year-old domestic geese (*Anser anser* dom.) were obtained from the city of Shabran (41°12'18.3»N, 48°59'44.8»E) of the Republic of Azerbaijan and adult female *H. dispar* nematodes (12 individuals) were collected from the cecum of the host's intestines by the parasitological dissection method [26]. Fixed preparations of helminths were studied under a stereomicroscope MBS-9 (Russia) and a Primo Star (Carl Zeiss, Germany) microscope. For the identification of the species, K.M. Ryzhikov's (1967) determinant was used [27]. In order to study the ultrastructure of the helmintes, the collected adult females nematodes were divided in to several parts and immediately were 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 keeping the samples in that fixer for one day, they were postfixed in 1% osmium tetraoxide solution prepared in phosphate buffer (pH 7.4) for two hours. Araldite-Epon blocks were prepared from the material based on generally accepted protocols in electron microscopy [28, 29]. Semi-thin (1-2 μm) sections taken from the blocks on a EM UC7 ultramicrotome (Leica, Germany) stained with methylene blue, azure II and base fixin or toluoid blue, viewed under a Primo Star (Carl Zeiss, Germany) microscope, images from necessary sections captured with a EOS D650 (Canon, Japan) digital camera [30, 31]. 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 under a voltage of 80-120 kV and electrograms were recorded. The statistical analysis of the image was performed on electrograms taken in TIF format using the computer program (The TEM imaging platform) developed by the German company "Olympus Soft Imaging Solutions Gmbh" [32]. Data analysis was carried out with different parameters (Min, Max, mean±SD).

Results

The female reproductive organs of adult nematode *H. dispar*, which belongs to the Heterakidae family, by both light and electron microscopic methods were studied. The female reproductive organs of the parasite are tube-shaped and consist of a pair of ovaries, a pair of oviducts, a pair of uteruses, a single vagina, and a single ovijector. The latter ends with a vulva of cuticular origin that opens to the external environment. In the *H. dispar* parasite, different numbers of cuticular ridges (3-7) are observed around the vulva (front and back). The vulva and ridges around it serve to spread the fully formed eggs from the body of the female individual to the environment without damage. The ovary, which is the first reproductive organ, is divided into a germinal and growth zones. Although the structure of the zones does not differ in terms of the structure of the wall, the germ cells there are at different stages of development. Figure 1A shows an image of the ovary (Ov, total size 67.41-78.87 μm (74.19±1.29 μm)) in semi-thin sections of a female individual of the nematode *H. dispar*. In the preparation viewed through a light microscope (Fig. 1A), it is clearly observed that the ovarian wall is covered from the outside with the basal lamina (Bm) and epithelial cells (Ep). Inside, the germ cells in different stages of development are observed. It is important to study the ultrastructure of the reproductive cells at all stages of the development of the reproductive organs of the female individual in order to follow the oogenesis of the studied parasitic worm *H. dispar*. Near the front and back parts of the nematode's body is the germinal zone of the ovary. Germ cells here divide intensively and are called oogonia (Oq, measuring 4.46-7.93 μm (5.42±0.397 μm)) (Fig. 1B). In the

mentioned section, the wall of the ovary is covered with basal lamina (Bm, thickness 0.090-0.112 µm (0.098±0.002 µm)) and relatively thin epithelial cells (Ep, thickness 1.16-2.93 µm (2.07±0.198 µm)).

Oogonia are relatively close to each other, and in their cytoplasm, Golgi complex, poorly developed granular endoplasmic reticulum, mitochondria (without cristae), ribosome, glycogen, fat droplets, nucleus (heterochromatin evenly distributed) are observed. The oogonia arranged around a central cytoplasmic rachis (Rx) (Fig. 1B). In the growth zone of the ovary, germ cells increase in size and become oocytes (Oo, measuring 14.77-29.17 µm (23.69±1.732 µm)). An overview of the named zone is presented in Figure 1C. Here, too, the wall of the tube consists of a basal lamina (Bm, thickness 0.22-0.27 µm (0.25±0.006 µm)) and a thin epithelial layer (Ep, thickness 3.44-6.90 µm (4.79±0.11 µm)). At the lumen of the tube, the large pyramid-shaped oocytes arranged around at the rachis (marked with a black snowflake) (Fig. 1C). Therefore, in both the germinal and growth zones of the ovary of the *H. dispar* nematode, the germ cells (oogonia and oocytes) are arranged around in the rachis (Rx). In contrast to oogonia, oocytes are pyramid-shaped and contain ribosomes, mitochondria, lipid droplets (L, size 0.98-3.67 µm (1.82±0.34 µm)) as well as I type granules (1g, diameter 1.97-3,68 µm (2.67±0.195 µm)) and II type granules (2g, sizes 1.25-2.26 µm (1.92±0.995 µm)) in their cytoplasm, are observed. I type granules are large in size, surrounded by a membrane with a large number of small granules inside. A large nucleus (4.78-7.79 µm (6.74±0.325 µm) in diameter) is located in the center of the oocyte cytoplasm (Fig.1D). II type granules are smaller than the others and consist of one granule. The epithelial layer of the growth zone of the ovary has a large nucleus (N, size 2.48-2.99 µm (2.80±0.055 µm)) in the center, and numerous mitochondria, glycogen and other organelles are observed in its cytoplasm (Fig. 1E).

After the oocytes pass through various developmental stages they enters the oviduct. A general view of the oviduct (Oi, measurements 85.46-94.21 µm (88.30±1.06 µm)) and the large oocytes within it in semithin section images are shown in Figure 2A. Figure 2B shows an electronogram of the oviduct of the parasite, where its wall, as in the ovary, consists of a basal lamina (Bm, measuring 0.29-0.57 µm (0.45±0.035 µm)), a thick epithelial layer (Ep, measuring 4.70-8.79 µm (6.38±0.41 µm)) and contains a large number of oocytes of different developmental stages (Oo, sizes 17.33-18.93 µm (18.18±0.18 µm)). The epithelial layer thicker in the oviduct than ovary. A large number of mitochondria, glycogen, ribosomes and other organelles are observed in the cytoplasm of the epithelial cell (Fig. 2B). In the oviduct, oocytes develop to a certain extent and undergo changes. A large nucleus located in the center of their cytoplasm looks like a star and is 2.71-3.51 µm (2.98±0.25 µm) in diameter (Fig. 2C). In the cytoplasm of oocytes, numerous I type (diameter 1.42-2.29 µm (1.93±0.105 µm)) and II type (diameter, 0.32-0.72 µm (0.47±0.046 µm)) granules, glycogen (diameter 0.038-0.048 µm (0.043±0.001 µm)), mitochondria (sizes 0.15-0.28 µm (0.19±0.014 µm)), lipid droplets (diameter 0.35-0.54 µm (0.44±0.021 µm)) etc. organelles are found. Ribosomes and Golgi complex are a minority in the cell cytoplasm. I type granules (1g) are mostly observed around the nucleus, while II type granules (2g) are mainly observed near the cell membrane (Fig. 2C). The oocytes in the lumen of the oviduct of the parasite communicate with each other due to membrane processes where the cells are present. Oocytes having such indented membrane processes (indicated by black arrows) are not observed in the ovary and other reproductive organs (Fig. 2D). Oocytes complete their full development in the oviduct and become a ready for fertilization. In the oviduct, the reproductive cells of the female are fertilized by the spermatozoa placed there by the male individual, and the zygote is formed first. That zygote develops into an egg.

Figure 3A shows the uterus (Ut, size 86.46-109.27 µm (95.72±2.52 µm)) of a parasitic female nematode and the fertilized oocytes therein (size 19.09-34.92 µm (29.4±1.88 µm)) is demonstrated. The uterus wall of the nematode is externally composed of a basal lamina (Bm, thickness 0.077-0.29 µm (0.22±0.013 µm)) and an epithelial layer (Ep, size 1.77-2.21 µm (2.01±0.044 µm)) are covered. In the cytoplasm of the epithelial cell, mitochondria (M, size 0.17-0.42 µm (0.25±0.032 µm)), glycogen (Ql, size 0.04-0.062 µm (0.056±0.002 µm)), granular endoplasmic reticulum, Golji complex and other organelles are observed. Fertilized oocytes (Moo) in the lumen of uterus are found (Fig.3B).

The vagina of a female individual of the nematode *H. dispar* was also studied and its wall was found to consist of three layers (basal lamina, muscular and epithelial layers) rather than two layers like the wall of the previous reproductive organs. Figure 4 shows the wall of the vagina (Va, diameter 62.69-73.10 µm (68.47±1.18 µm)) and the developing eggs (sizes, 19.68-35.40 µm (26.45±2.18 µm)) image obtained

from semi-thin preparations is presented. Here basal lamina (Bm), muscular layer (Mc, thickness 3.79-9.98 µm (7.33±0.70 µm)), epithelial layer (Ep, thickness 5.26-7.97 µm (6,52±0.34 µm)) is clearly distinguished. The developing eggs move through the vagina.

In the female reproductive system of the *H. dispar* nematode, the vagina in turn opens into the ovijector. A general light microscope image of the named organ is shown in Figure 5A. At the same time, the ultrastructure of the ovijector is presented in Figure 5B. In both images, the ovijector consist (Yc, dimensions 63.64-80.32 µm (73.32±2.09 µm)) with a basal lamina (Bm), muscular (Mc, dimensions 5.17-21.51 µm (11.90±1.60 µm)) and surrounded by epithelial layers (Ep, size 2.64-3.51 µm (3.11±0.107 µm)). However, unlike the another reproductive organs of the female *H. dispar* nematode, there is also a layer of cuticle (Cu, size, 0.30-0.69 µm (0.56±0.042 µm)) over the epithelial layer (Fig, 5A and 5B). That cuticular layer extends to the vulva of the parasite and merges with the cuticle that covers the body from the outside. Muscle cells are rich in glycogen, mitochondria and fibers. A large number of cuticular processes are observed at the border of the epithelial cells with cuticle in the lumen of the ovijector. Glycogen, mitochondria, ribosomes, granular endoplasmic reticulum and other organelles are noted in the cytoplasm of the epithelial cell.

Thus, the ultrastructural study of the reproductive organs of the adult female nematode H. dispar showed that the wall of the tube-shaped ovary, oviduct and uterus is composed of two (basal lamina and epithelial layer), the vagina is composed of three (basal lamina, muscular and epithelial layers), the ovijector consists of 4 layers (basal lamina, muscular and epithelial layers, thick cuticle in the lumen).

In most species of nematodes, mostly representatives of separate sexes, females continue their generation by laying eggs. After fertilization, they go through various stages of development to become a fully formed egg. In addition to studying the ultrastructure of the nematode H. dispar, which belongs to the Heterakidae family, through electron and light microscopes, we also studied the structural features of the egg of the parasitic worm.

After oocytes are fertilized, changes in their structure occur. After successive divisions in the zygote, the shell of the egg begins to form. In Figure 6A, semi-thin sections of eggs forming in the uterus of a female nematode *H. dispar* are presented. Here, the egg's thin shell and membrane-enclosed I type granules and their granular cytoplasm are clearly visible. The above-mentioned I type granules were not in the center of the cytoplasm, but in areas close to the eggshell (Fig. 6A, 6B, 6C). The reason for this is the involvement of I and II type granules in the cell cytoplasm in the formation of the egg shell. It was determined that the fully formed egg of the *H. dispar* nematode studied by us consists of 4 layers (the total thickness of the egg wall is 2.85-3.05 µm (2.96±0.021 µm)) (Fig. 6D). The egg is covered from the outside by the uterine layer (marked with 1 in Fig. 6D) synthesized by the epithelial cells that make up the uterine wall, and its thickness is 0.32-0.38 µm (0.36±0.003 µm). The cytoplasm of the egg does not participate in the synthesis of this layer. The second layer of the egg from the outside to the inside is called the vitelline layer and its thickness is 0.23-0.28 µm (0.25±0.006 µm). It takes its development from the oolemma of the egg (marked 2 in Fig. 6D). The third or middle layer is the thickest layer and is called chitinous layer, and its thickness is 1.94-2.14 µm (2.01±0.021 µm). Its main function is to provide rigidity to the eggshell (marked with 3 in Fig. 6D). The last, fourth layer is called the lipid layer (0.20-0.30 µm thick (0.23±0.013 µm)) and ensures the impermeability of the eggshell (marked with 4 in Fig. 6D). The lipid layer is bounded by the thin membrane of the egg cell. If we look at the development dynamics of the layers of the egg, the vitelline layer develops first, then the chitinous layer, the third lipid layer, and finally the uterine layer, which surrounds the egg from the outside, develops. The electrogram presented in Figure 6C shows that the immature egg has only three layers (vitelline, chitin, and lipid). Thus, as a result of the study of the ultrastructure of the nematode *H. dispar* by electron microscopic methods, it was found that the shell of the fully formed egg of the parasite consists of 4 layers (uterine, vitelline, chitinous and lipid layers).

Discussion

The study of the ultrastructural features of the nematodes belonging to the Heterakidae family, which are similar in appearance, leads to the discovery of additional taxonomic features in the designation of species. The ultrastructural characteristics of the reproductive organs of an adult female worm parasite *H. dispar* were studied

separately. It has been known that the reproductive system of a female individual consists of an ovary, an oviduct, an uterus, a vagina, and an ovijector. The morphology of the reproductive organs of some free-living nematodes (Tylenchida, Araeolaimida) was studied by histological and electron microscopic methods, and several taxonomic characters were also determined [33, 34]. On the other hand, there are also data on the ultrastructure of the reproductive organs of parasitic nematodes belonging to other families [18, 35-37]. There are some literature sources on the ultrastructure of reproductive organs of *H. gallinarum* and *H. spumosa* from other parasitic nematodes belonging to the Heterakidae family [16, 19]. The literature data on the morphological structure of the female reproductive organs of *H. dispar* nematode was found in only one source [38]. Brief information about the structure of reproductive organs of the parasite was given in that research work by only histological method. Thus, as a result of the analysis of literature data, it was found that the female reproductive system of the other two species belonging to the Heterakidae family and whose ultrastructure was studied (*H. gallinarum* and *H. spumosa*) consists of the above-mentioned organs, as in the case of the nematode *H. dispar* [16]. The ovary of the *H. dispar* nematode studied by us was divided into germinal and growth zones, and it was found that both are composed of a basal lamina and an epithelial layer. However, it was determined that the germinal zone of the basal lamina was 2.55 times thinner, and the epithelial layer was 2.3 times thinner than the growth zone. In other species included in the family (*H. gallinarum* and *H. spumosa*), the wall of the ovary is also composed of a basal lamina and an epithelial layer, and the muscular layer is not noted in any species. In the nematode *H. gallinarum*, the ovary is divided into the germinal and growth zones. The basal lamina forming the wall of the helminth ovary is thicker (0.4 μm), and the epithelial layer (1.7-2.5 μm) is almost the same as in the nematode *H. dispar* [13, 16, 18].

In nematodes (*H. gallinarum, H. spumosa* and *H. dispar*) it is observed that germ cells are arranged around a central cytoplasmic rachis in both germinal and growth zones. The wall of the oviduct of the nematode *H. dispar* consists of a basal lamina and an epithelial layer, as in the ovary. In the other two species (*H. gallinarum* and *H. spumosa*) in which the ultrastructure of the female genital organs was studied and included in the same family, the wall of the oviduct was composed of a basal lamina and an epithelial layer [13, 16]. The basal lamina of the oviduct wall of H. dispar nematode is 2 times thinner than *H. gallinarum* parasite. The epithelial layer is also thick (7.5-10 μm) in the nematode *H. gallinarum*. *H. dispar, H. gallinarum* and *H. spumosa* nematodes belonging to the Heterakidae family all have a basal lamina and an epithelial layer in uterus wall. The basal lamina of the uterus wall of the nematode *H. dispar*, is 4 times thinner than that of the helminth *H. gallinarum*, and the epithelial layer is 2.5 times thinner. The ultrastructural characteristics of the vagina of the H. dispar nematode, unlike the ovary, oviduct and uterus, consist of 3 layers (basal lamina, muscular and epithelial layers) instead of two. As a result of the analysis of the literature data, it was found that the vagina wall of the *H. gallinarum* and *H. spumosa* nematodes, whose structure was previously studied, also consists of the mentioned 3 layers [16]. When comparing the dimensions of the layers of the vagina, it was determined that the epithelial layer is thicker (7.5-10.5 μm) in the nematode *H. gallinarum* than in the helminth *H. dispar*. Among the reproductive organs of an adult female, the ovijector has a more complex structure, which consists of 4 layers (basal lamina, muscular, epithelial and cuticle at the lumen). The structure of these layers is also noted in the previously studied nematodes *H. gallinarum* and *H. spumosa*. The difference is that in the nematode *H. gallinarum*, the layers of the ovijector are thicker in size [16]. In general, as a result of the obtained data and the analysis of literature, it was found that the wall (including layers) of the reproductive organs of the adult female of the nematode *H. gallinarum* is thicker than that of the helminth *H. dispar*. In the research carried out by us, all reproductive organs (five organs) that make up the female reproductive system of the *H. dispar* nematode have a basal lamina and an epithelial layer. Epithelial layer in the oviduct and vagina was thicker than in other reproductive organs.

The cuticle in the lumen of the ovijector connects with the cuticle that covers the body wall of the helminth from the outside. Around the vulva, this layer thickens and forms cuticular ridges. In our previous studies, it was noted that different number of cuticular ridges (3-7) were observed around the vulva (front and back) in *H. dispar* parasite [39]. In addition, a large number of cuticular processes are observed at the border of the cuticle in the orifice of the ovijector. This kind of morphological feature is also repeated at the border of the hypodermal cells or lateral hypodermal ridges

with the cuticle that forms the body wall of the H. dispar nematode and covers the body from the outside [23]. These processes were not found among the helminths belonging to the Heterakidae family and whose ultrastructure was studied.

During the study of the ultrastructure of the reproductive system of an adult individual of the nematode *H. dispar*, various stages of the development of the reproductive cells in the organs (oogenesis) were also monitored. Thus, it became known that the oogonia develop into pyramid-shaped oocytes in the ovary. Apart from the organelles found in oogonia, I and II type granules are also found in their cytoplasm. Oocytes are 5 times larger than oogonia. Those oocytes undergo certain development in the oviduct and pass into the uterus. In the uterus, oocytes complete their development and become fertilized and become a zygote. The egg and its shell are formed in vagina. It develops into a fully formed egg in the ovijector. Among the other species included in the Heterakidae family, the nematodes *H. gallinarum* and *H. spumosa* whose oogenesis has been studied at the ultrastructural level are. As a result of the analysis of literature data, it was found that oogenesis in all three species of helminths (*H. gallinarum, H. spumosa* and *H. dispar* - studied by us) follows a similar course. Oogonia and oocyte stages of germ cells are detected in all three nematodes. Although those cells are similar in terms of structure, both oogonia and oocytes are larger in *H. gallinarum* and *H. spumosa* nematodes [13, 16].

The process of eggshell formation takes place in the fertilized germ cells of the female nematode *H. dispar*. It was found that the wall of the fully developed egg is composed of 4 layers. The chitinous layer is many times thicker than the others (Fig. 6D). In general, these layers differ from each other according to their structure, development, composition. The formation of the egg shell occurs as it interacts with the reproductive organs (uterus, vagina, ovijector). In addition to the mentioned, the cytoplasmic elements inside the egg (I and II type granules) are directly involved in the formation of the shell. This information is also reflected in the results obtained in the studies of other scientists [10, 13, 35, 36]. Thus, the characteristics of the eggshell formation of the parasitic worm H. gallinarum belonging to the Heterakidae family are also mentioned sources [10]. In the literature on the study of the ultrastructure of the nematode *H. spumosa*, there is information not about how many layers the egg shell consists of, but only about the shell being composed of 3 membranes [16]. Although the egg shell of *H. gallinarum* helminth is shown to be composed of 3 layers, it is also noted that there is a lipid assembly between the cytoplasm and the egg shell [13, 16]. So, even if the authors observed the lipids, they did not show it as a separate layer forming the egg wall in that parasite (*H. gallinarum*). In *H. dispar* nematode, whose egg wall was studied at the ultrastructural level by us, the lipid layer was separately identified as the 4th layer (Fig. 6D). In general, in many nematodes in which the egg wall is studied by electron microscopic methods, there is a lot of information about the egg shell being composed of 4 layers [18, 35, 36].

Conclusion

The reproductive system of an adult female nematode *H. dispar*, belonging to the Heterakidae family, was studied by light and electron microscopic methods. It was determined that the tube-shaped ovary, oviduct, and uterus wall have two (basal lamina and epithelial layer), the vagina has three (basal lamina, muscular and epithelial layers), and the ovijector has 4 layers (basal lamina, muscular, epithelial and cuticle layers). Germ cells and their development (oogenesis) in different stages (oogonia, oocytes) of the adult female *H. dispar* nematode and egg formation are described. The layers (uterine, vitelline, chitinous and lipid layers) that make up the fully formed egg wall have been determined. The obtained results were compared with the characteristics of other species included in the Heterakidae family and whose ultrastructure was studied.

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Funds statement

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Ethical consideration

This sudy were performed after the approval from the Ethics Committee of Azerbaijan Medical University (Ministry of Health of Azerbaijan Republic), Baku, Azerbaijan.

Conflict of interest

The author claims that there are no competing interests.

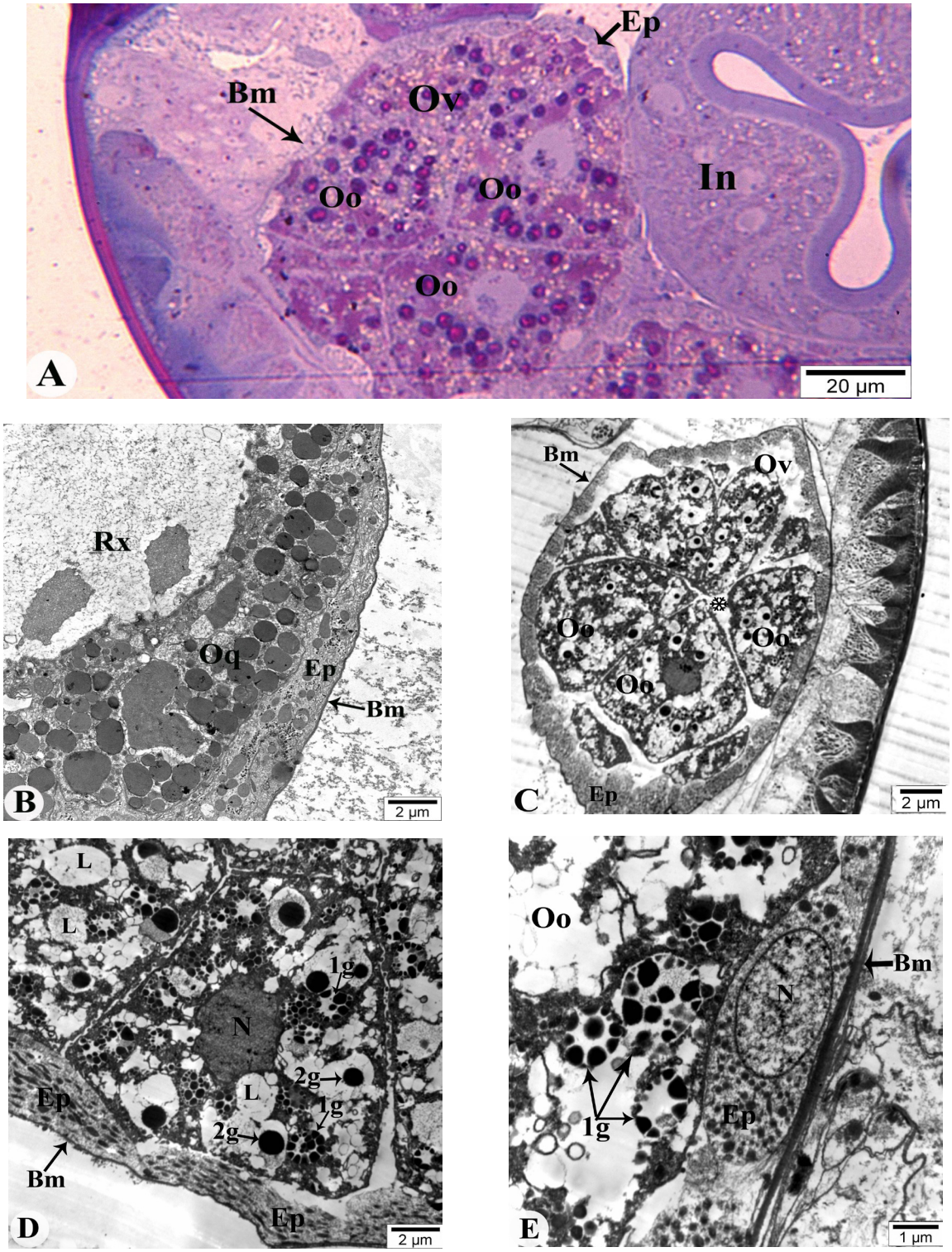

Fig. 1. Structural characteristics of the ovary of adult female nematode *H. dispar*. A - general view of the ovary under a light microscope, semi-thin section (1 µm), two-layer staining by D'Amico method, B - ultrastructural features of the ovarian germinal zone, C - ultrastructural features of the ovarian growth zone, D - ultrastructural features of the cytoplasm of the oocyte, E - ultrastructural characteristics of the ovarian wall (explanation is given in the text). Ultrathin sections (50-70 nm), stained by uranyl acetate and lead citrate. Designations: Bm – basal lamina, Ep – epithelium, In – intestine, N – nucleus, Oo – oocyte, Ov – ovary, Oq – oogonia, L – lipid granules, Rx – rachis, 1g – I type granules, 2g – II type granules.

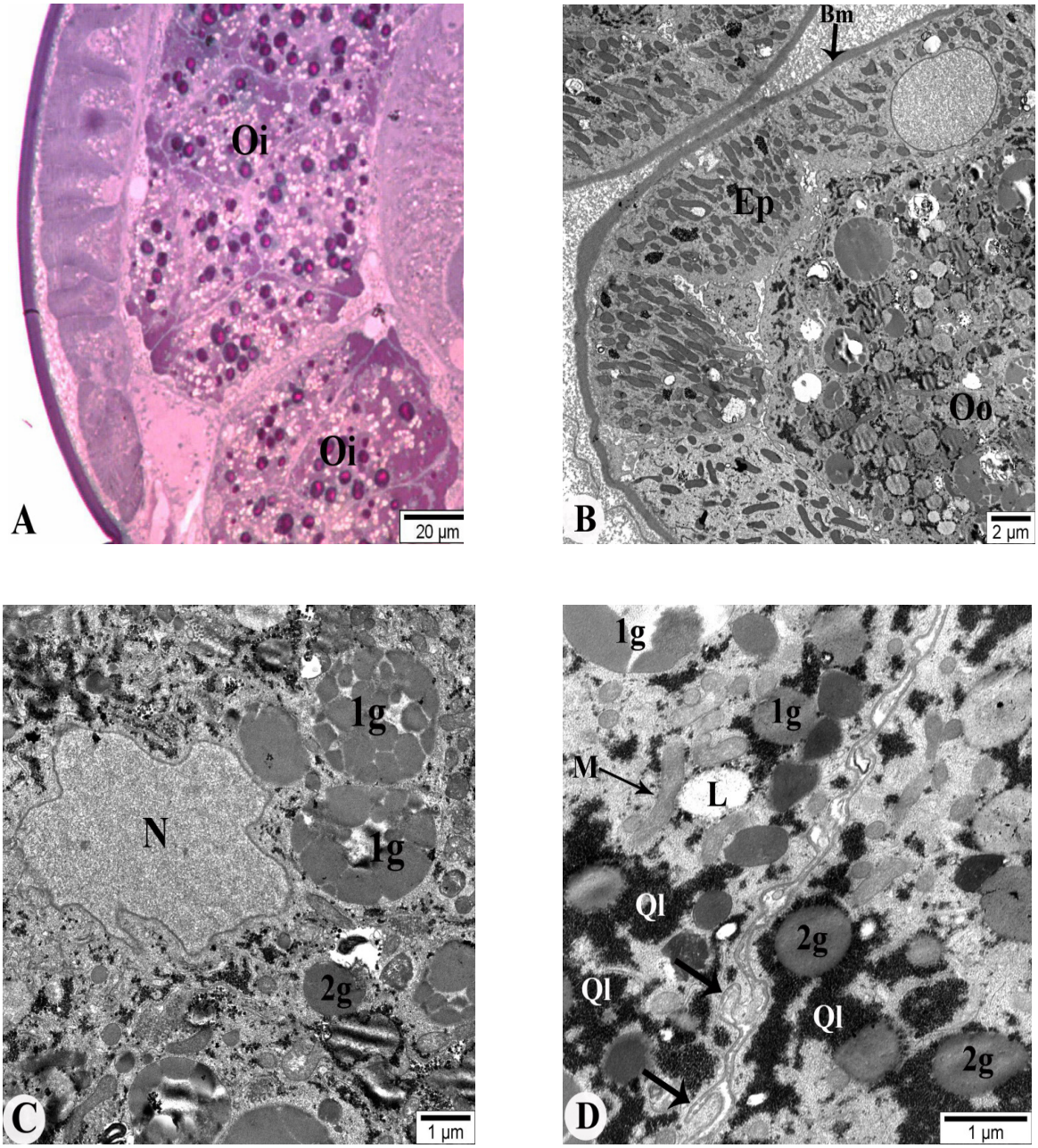

Fig. 2. Structural characteristics of the oviduct of adult female nematode *H. dispar*. A - general view of the oviduct under a light microscope, semi-thin section (1 µm), two-layer staining by D'Amico method, B- electron microscopic view of the wall of the oviduct, C and D - ultrastructural features of the cytoplasm of the oocyte in the oviduct (explanation is given in the text).Ultrathin sections (50-70 nm), stained by uranyl acetate and lead citrate. Designations: Oi – oviduct, Bm – basal lamina, Ep – epithelium, N – nucleus, Ql – glycogen, M – mitochondria, Oo – oocyte, 1g – I type granules, 2g – II type granules.

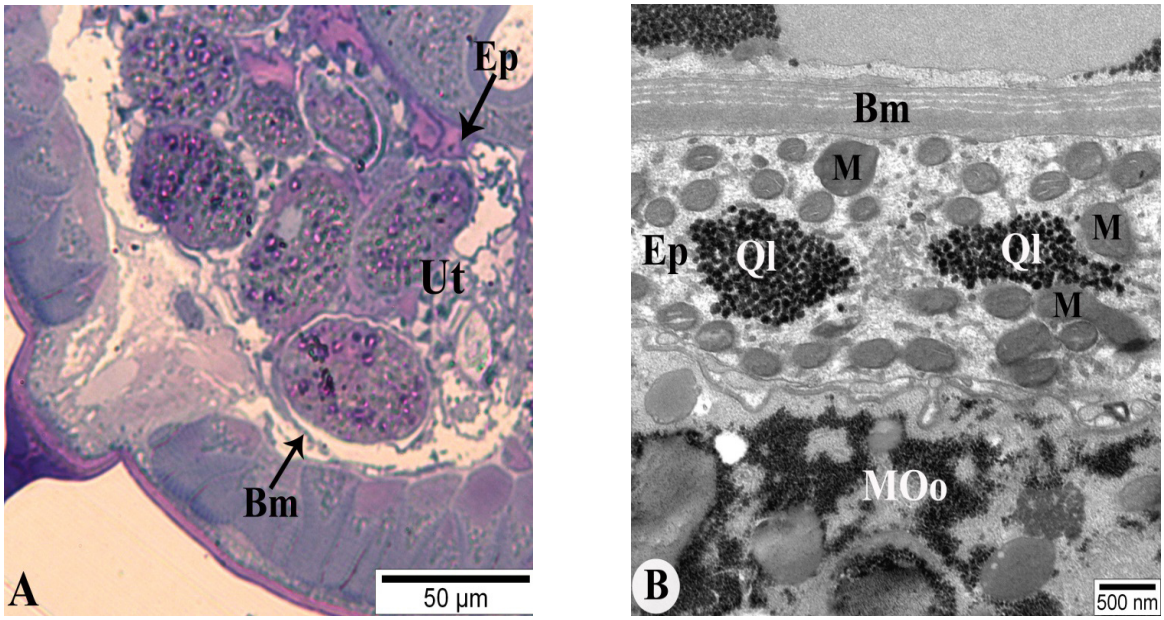

Fig. 3. Structural characteristics of uterus of adult female nematode *H. dispar*. A - general view of uterus under light microscope, semi-thin section (1 µm), two-layer staining by D'Amico method, B- electron microscopic features of uterus wall (explanation is given in the text). Ultrathin sections (50-70 nm), stain: uranyl acetate and Pb citrate. Designations: Ut - uterus, Bm - basal lamina, Ep - epithelium, Ql - glycogen, M - mitochondria, MOo - fertilized oocyte.

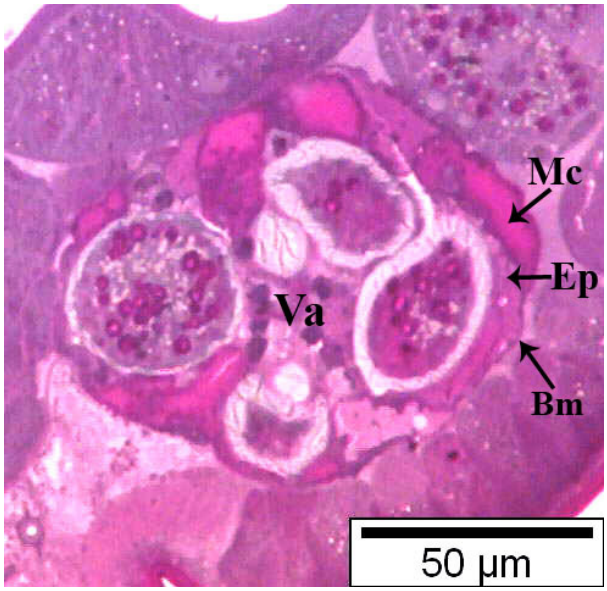

Fig. 4. Structural features of the vagina of adult female nematode *H. dispar* (explained in the text), semi-thin section (1 µm), two-layer staining by D'Amico method. Designations: Va – vagina, Mc – muscular layer, Bm – basal lamina, Ep – epithelium

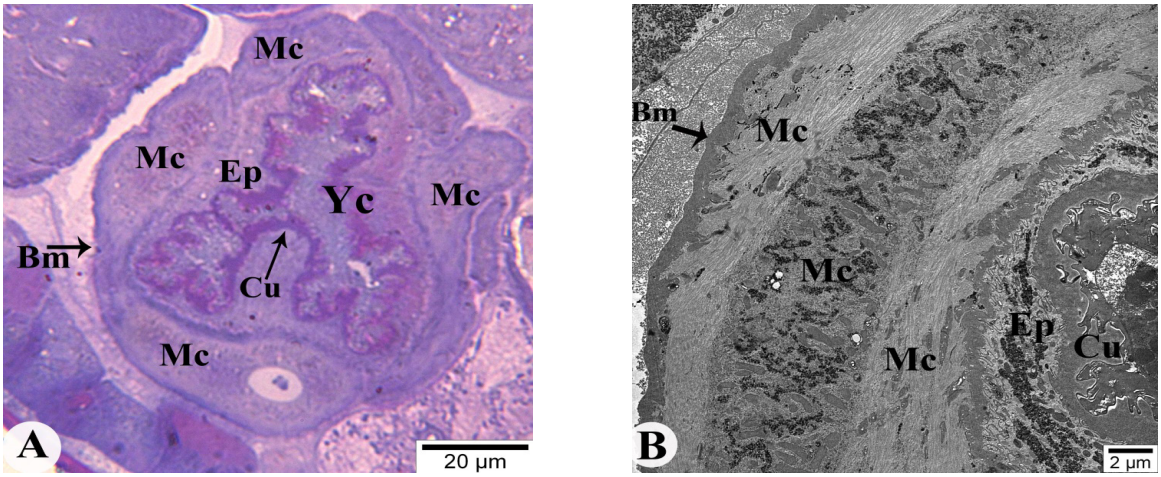

Fig. 5. Structural characteristics of the ovijector of adult female nematode *H. dispar*. A - general view of ovijector under light microscope, semi-thin section (1 µm), two-layer staining by D'Amico method, B- electron microscopic image of ovijector (explanation is given in the text). Ultrathin sections (50-70 nm), stain: uranyl acetate and Pb citrate. Designations: Yc - ovijector, Cu - cuticle, Bm - basal lamina, Ep - epithelium, Mc - muscular layer.

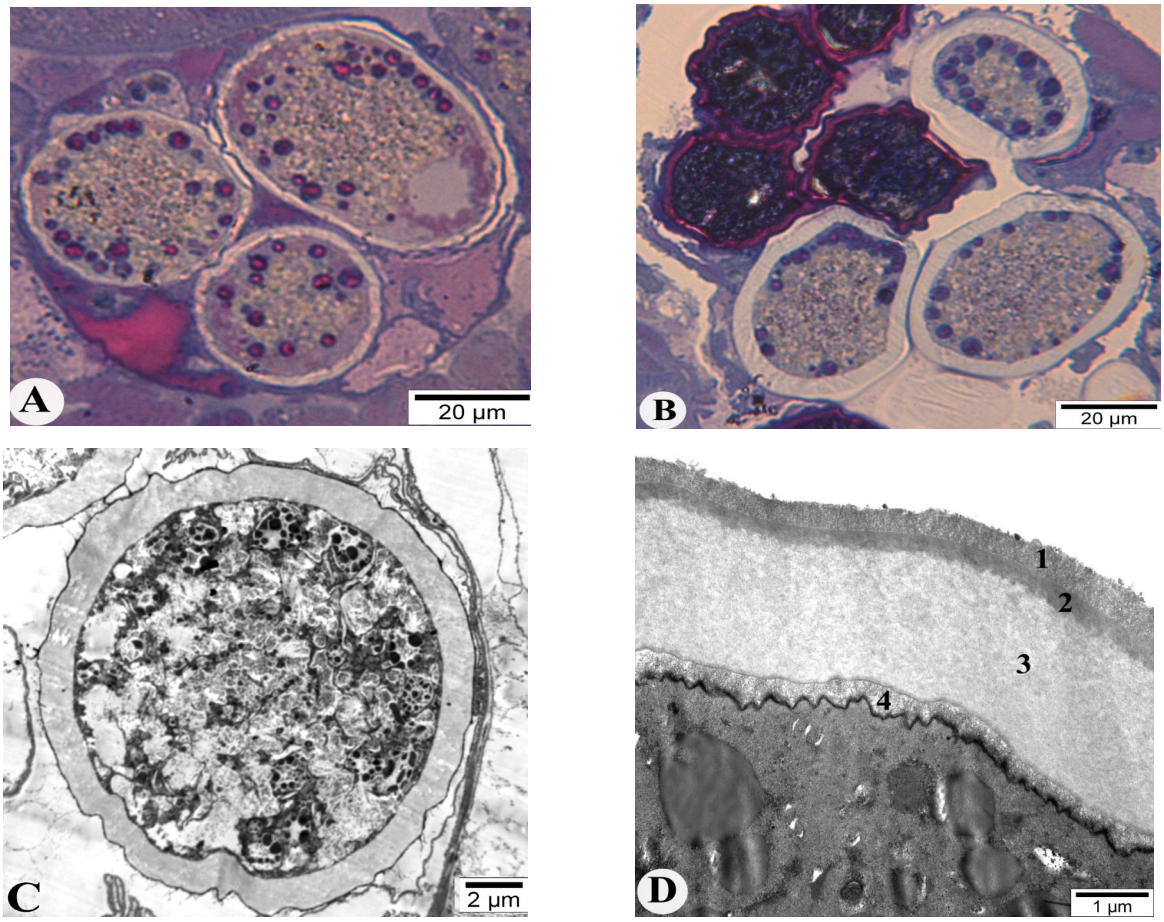

Fig. 6. Structural characteristics of the egg of adult female nematode H. dispar. A and B - a general view of different developmental stages of the egg under a light microscope, semi-thin section (1 µm), two-layer staining by D'Amico method, C - electron microscopic image of the egg, D - electron microscopic image of the layers of the egg shell (explanation is given in the text). Ultrathin sections (50-70 nm), stain: uranyl acetate and Pb citrate. Designations: 1 – uterine layer, 2 – vitelline layer, 3 – chitinous layer, 4 – lipid layer.

References

1. Bongers, T., and Ferris, H. Nematode community structure as a bioindicator in environmental monitoring. *Trends in Ecology and Evolution*, **14**(6), 224–228 (1999).

2. Lok, J.B. Signaling in parasitic nematodes: Physicochemical communication between host and parasite and endogenous molecular transduction pathways governing worm development and survival. *Current Clinical Microbiology*, **3**(4), 186–197 (2016).

3. Viney, M. How can we understand the genomic basis of nematode parasitism? *Trends in Parasitology*, **33**(6), 444–452 (2017).

4. 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).

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

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

7. 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).

8. Eyvazov, A. (ed.) Taxonomic spectrum of the Azerbaijan fauna. Protozoa and helminths. Baku, Institute of Zoology, (2022).

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

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

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

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

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

14. Bogoyavlensky, Yu.K. Structure and functions of integumentary tissues of parasitic nematodes. Moscow, Nauka, (1973).

15. 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).

16. Bogoyavlensky, Yu.K., Bogolepova, I.I., and Onushko, N.V. Microstructure of tissues of parasitic nematodes. Moscow, Nauka, (1983).

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

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

19. 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).

20. 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).

21. 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* (2022).

22. 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).

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

24. 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 (2023c).

25. 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).

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

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

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

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

30. 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).

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

32. 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).

33. Geraert, E. The female reproductive system in nematode systematics. *Annales de la Societe royale Zoologique de Belgique*, **110**(2), 73–86 (1980).

34. Yushin, V.V., and Malakhov, V.V. Ultrastructure of the female reproductive system of the free-living marine nematode *Enoplus demani* (Nematoda: Enoplida). *Fundamenthal applied Nematology*, **20**(2), 115–125 (1997).

35. Foor, W.E. Ultrastructural Aspects of Oocyte Development and Shell Formation in *Ascaris lumbricoides*. *The Journal of Parasitology*, **53**(6), 1245–1261 (1967).

36. Adamson, M.L. Ultrastructural observations on oogenesis and shell formation in *Gyrinicola batrachiensis* (Walton, 1929) (Nematoda: Oxyurida). *Parasitology,* **86**, 489-499 (1983).

37. MacKinnon, B.M. An ultrastructural and histochemical study of oogenesis in the trichostrongylid nematode *Heligmosomoides polygyrus*. *The Journal of Parasitology*, **73**(2), 390–399 (1987).

38. 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).

39. 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).