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Ultrastructural Features of the Digestive System of the Helminth *Heterakis Dispar* Schrank, 1790 (Nematoda: Heterakidae)

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THE ultrastructural features of the digestive organs (stomodeum, intestine, and proctodeum) of the nematode *Heterakis dispar* (Schrank, 1790), beloning to the Heterakidae, which is a widespread parasite of domestic waterfowl in Azerbaijan, were studied using light and electron microscopic methods and compared with the structure of other species of the same family. The wall of the buccal cavity and pharynx are covered by cuticle, which developed from epithelial cells. Esophagus consists of three regions: procorpus, metacorpus and basal part. The wall of the lumen consists of a basement membrane, muscle cells, and a cuticle. The same cuticle is divided into cortical, homogeneous and basal layers. Between the cuticle and muscle cells at the peaks of the lumen of the esophagus, additional structures wich has not been found so far could be observed in this nematodes. The wall of the terminal bulb consists of muscle cells, glandular cells, processes of nerve cells, and the lumen is covered with a cuticle. The intestine is formed from a thick basement membrane, a single-layered epithelium with microvilli. Five zones are found in epithelial cells: basal, reticular, plasma, fibrillar and microvilli. Microvillas are located in the apical part of the epithelial cell. Unlike other nematodes family, in addition to desmosomes that connect epithelial cells, membranes also contain smooth septate junctions. The posterior section of the intestine of helminth ends in females with an anus, and in males with a cloaca.

Keywords: *Heterakis dispar*, Digestive organs, Ultrastructure, Transmission electron microscope.

Introduction

The histological and electron microscopic features of the structure of the digestive system organs of parasitic and free-living worms belonging to different families of nematodes are different, and these features, in turn, can play an important role in determining the systematic position of helminths. Species belonging to the Heterakidae family are very similar in appearance. Only *H. gallinarum* and *H. spumosa*, belonging to this family, have several literature sources for the ultrastructure of the digestive organs [1-5]. The data presented by the authors do not cover the ultrastructure of all digestive organs of helminths. Only one source has been found related to the morphology of the nematode *H. dispar* [6]. This literature source presents results obtained only from studies conducted using a light microscope. We did not find any information about the ultrastructure of the digestive organs of the parasite *H. dispar*.

As a result of helminthological studies, we registered 27 species of helminths in domestic waterfowl on the territory of the Republic of Azerbaijan (2005-2022 years) [7-11]. In all territories of study in domestic waterfowl, the nematode *H. dispar*, causing significant damage to the host organism, was superior both in intensity and extensiveness of invasion [12, 13].

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Considering the above, the goal was to study the ultrastructural features of the digestive organs of the nematode *H. dispar*, which is a helminthes of the Heterakidae family, parasitizing domestic waterfowl birds and widely distributed throughout the country, using histological and electron microscopic methods.

Material and Methods

Researches based on the studing process of the helminths ultrastructure were carried out in 2019-2022 years, and one-year-old domestic geese (*Anser anser* dom.) were obtained from the city of Shabran (41°12'18.3»N, 48°59'44.8»E), Republic of Azerbaijan. The parasitological nematodes *H. dispar* were collected from the caecum by the method of complete helminthological dissection [14]. From the collected material, permanent preparations enclosed in canadian balsam were prepared. Parasites were examined under a stereomicroscope MBS-9 (Russia) and light microscope Promo Star (Zeiss, Germany). Identification of parasite species was carried out according to the key guide [15]. To study the ultrastructure of the helminth, the collected parasites (adult males and females) were divided into several parts during the autopsy of birds and immediately were fixated in solution containing 2% paraformaldehyde, 2% glutaraldehyde and 0.1% picric acid prepared in phosphate buffer (pH 7.4). After keeping the samples in this fixative for at least a day, they were additionally fixed in a 1% solution of osmium-tetroxide prepared in phosphate buffer (pH 7.4) for 1-2 hours. Araldite-epon blocks were prepared from the material based on generally accepted protocols for electron microscopy [16]. Semithin sections (1–2 µm) were obtained with a Leica EM UC7 ultramicrotome, stained with methylene blue, azure II, toluidine blue and fuchsin [17], for further investigation by light microscope Primo Star (Zeiss, Germany) and photographed with a digital camera EOS D650 (Canon, Japan). Double-stained with uranyl acetate and lead citrate, ultrathin sections (50–60 nm) were examined under the Transmission Electron Microscope JEM-1400 (JEOL, Japan) at 80–120 kV. The morphometric analysis of the electronograms (n=60) was carried out in TIF format via a computer program (TEM Imaging Platform) developed by Olympus Soft Imaging Solutions GmbH (Germany). Data analysis (Microsoft Exel) was carried out with different parameters (Min, Max, mean±SD). Data were considered statistically significant at $p<0.05$.

Results

An ultrastructural studies of the nematode *H. dispar*, a parasite of the Heterakidae family, revealed that all internal organs, including the digestive organs, are located in a saclike pseudocoelomic cavity that surrounds the entire body and contains a fluid that is rich in organic and inorganic substances. It was found that its wall thickness is 0.066–0.084 µm (0.076±0.002 µm) (Fig. 1). The wall of the pseudocoelomic cavity of the nematode *H. dispar*, was observed using both light and electron microscopy (Figs. 1A, 1B).

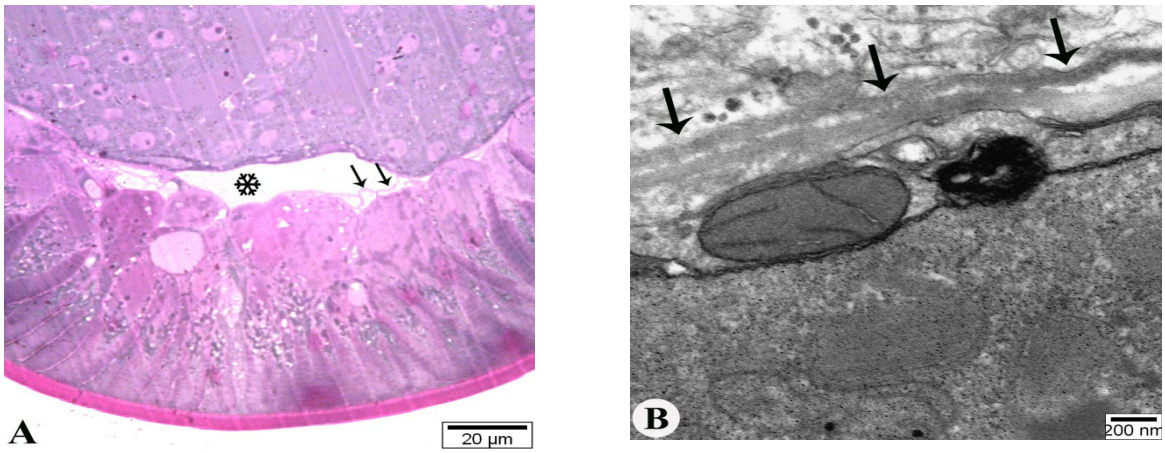

Fig. 1. Light (A) and electron microscopic (B) images of the wall of the pseudocoelomic cavity of the nematode H. dispar. A: light microscopic photograph of a semi-thin section (1 µm), two-layer staining by D'Amico method; B: Electron microscopic photograph of an ultra-thin section (50–70 nm) stained by uranyl acetate and lead citrate. Designations: black snowflake - cavity fluid, double black arrows - general view of the wall of the pseudocoelom, black arrow - basement membrane of the pseudocoelomic cavity.

The digestive system of the nematode *H. dispar* is divided into three main sections: 1. stomodeum - buccal cavity (stoma), pharynx, esophagus; 2. intestine; 3. proctodeum - posterior part of intestine. The structures of these organs are composed of various types of differentiated cells. The first part of the digestive organs of the helminth, located in the anterior part of the body and surrounded the buccal cavity from the outside, has 3 equal-sized lips of cuticular origin. The lumen of the buccal cavity and pharynx is lined with a cuticle, which originates from epithelial cells. The esophagus of a parasitic worm has a relatively complex structure and it is divided into three sections: anterior - the procorpus, which has a cylindrical structure; metacorpus - which has finger-like processes of the cuticle at the lumen; and basal section - bulb. Light microscopic image of the metacorpus section of the esophagus (diameter 70.05-76.29 µm (73.80±0.56 µm)) is shown in Fig. 2A. It was revealed that the lumen of the esophagus has a triangular shape (diameter - 19.67-28.06 µm (23.62±1.17 µm)) and, in addition, three peaks can be traced (diameter - 5.05-8.79 µm (6.37±0.51 µm)). On the electronogram shown in Fig. 2B, from the outside to the inside of the esophagus, the basement membrane is first traced (thickness - 0.283-0.340 µm (0.307±0.007 µm)), then specialized lateral muscle cells are localized at the peaks of the lumen (length - 25.44-26.28 µm (25.88±0.09 µm)), which are structurally differ from somatic cells in the muscle layer of the parasite. Along the edges of the lumen peaks, specialized radial muscle cells (length 26.65-27.62 µm (26.92±0.098 µm)) which are clearly visible. And the lumen of the esophagus extends through the cuticle. According to the statistical calculations the difference between the lengths of specialized radial and lateral muscle cells is very small (1.04±0.008 µm). In addition to what has mentioned in the esophagus, there are glandular cells which are involved in the formation of its wall (their drains open into the lumen), processes of nerve cells and esophagal valves with a cellular structure that prevent the return of food. The thick basement membrane that surrounds the wall of the esophagus from the outside is connected to the fibrils inside the muscle cells by hemidesmosomes (indicated by black arrows) (diameter 0.0014-0.0018 µm (0.0017±0.0005 µm)) (Fig. 2C). As a result of an ultrastructural study of muscle cells, a large number of active mitochondria, glycogen, fibrils, as well as ribosomes, the endoplasmic reticulum, and the holgi complex were observed in their cytoplasm (Fig. 2D). The lumen of the esophagus is covered with a cuticle layer, the total thickness of which is 0.085–0.138 µm (0.108±0.006 µm) (Fig. 2E). It, in turn, consists of a dense cortical layer (thickness - 0.018-0.022 µm (0.020±0.0004 µm)), a relatively thick and nondense middle (homogeneous) layer (thickness - 0.040-0.101 µm (0.059±0.007 µm)) and consists of a basal layer (thickness - 0.018-0.024 µm (0.021±0.0008 µm)). At all three peaks of the lumen between the cuticle and muscle cells, additional structures with a cellular structure (thickness - 0.52-0.65 µm (0.58±0.016 µm)) were also observed on electronograms obtained in the direction of studying the ultrastructure of the esophagus of the nematode *H. dispar* (Fig. 2E). On the border with that part, dense bodies are also noted, which are involved in the formation of muscle fibers (filaments) in muscle cells. A large number of filaments are also observed around them (Fig. 2E).

As a result of the study of the layer between the cuticle and muscle cells, it was found that various cellular elements are concentrated in its cytoplasm, and is divided into 2 different parts: nondense and dark-colored (marked with a black asterisk, thickness - 0.51-0.58 µm (0.53±0.007 µm)) in the cytoplasm with mitochondria, and light but dense with myofibrils (marked with a snowflake, thickness - 0.49-0.54 µm (0.52±0.005 µm)) (Fig. 2E). These components resemble the structure of a muscle cell and are probably a modified form of the muscle cell. If we take into account that muscle cells are involved in the formation of the cuticle in this part, then it can be considered appropriate to locate this layer there. The fingerlike processes of the cuticle (marked with black arrows) are observed from the peaks of the triradiate lumen of the esophagus towards the center (Fig. 2F). The last and enlarged part of the esophagus, located in the posterior region, is the bulb, where the muscle cells are large and numerous. Many fibers are traced in these cells. Glandular cells (one dorsal and 2 subventral - located between the lateral muscle cells) and processes of nerve cells are also observed in anterior and posterior part of the bulb. Between the esophagus and intestines there is a esophageal-intestinal valve, consisting of several cells of epithelial origin, which prevents food from moving backward. The lumen of the bulb is also covered with a cuticle.

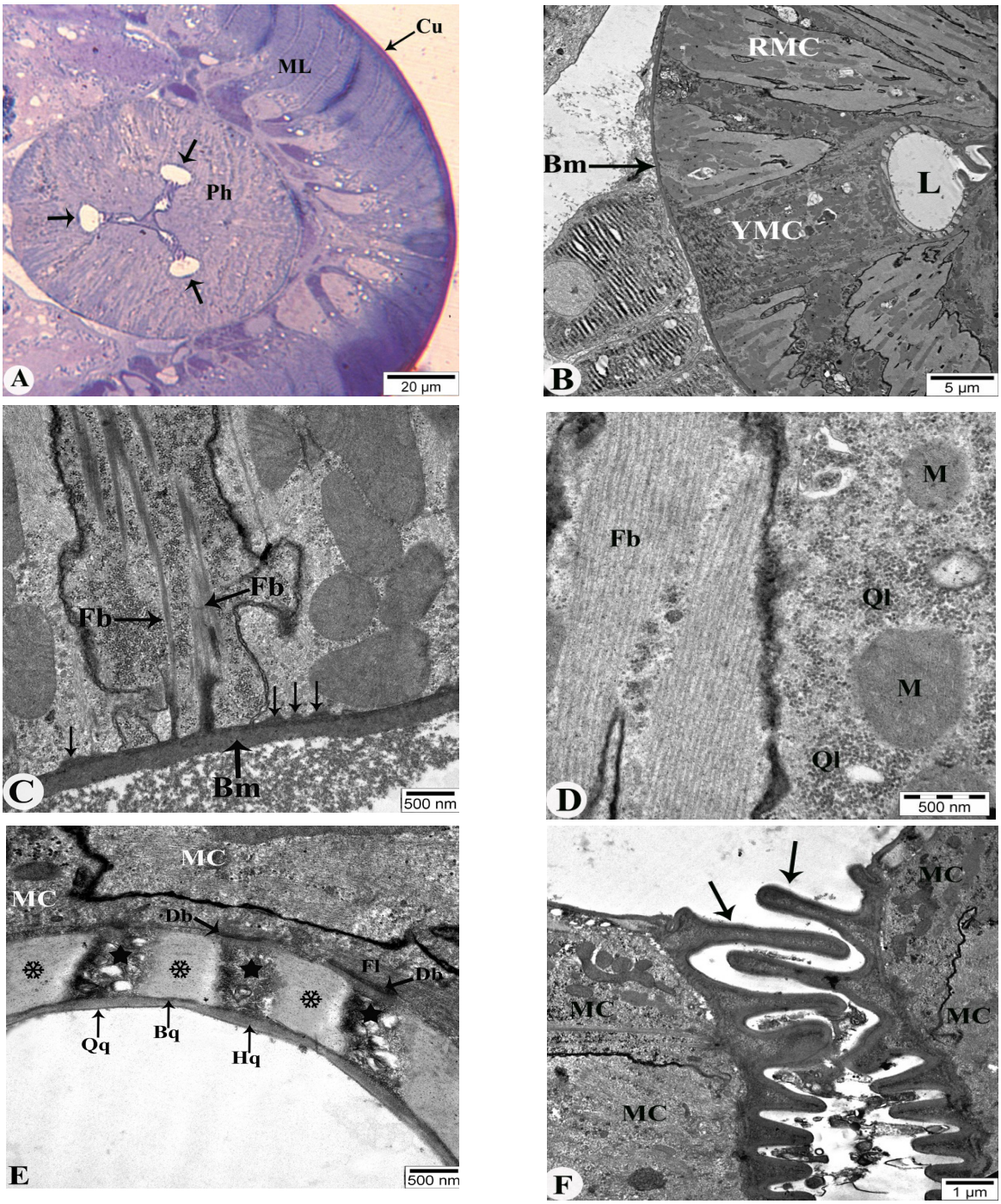

Fig. 2. Light (A) and electron microscopic (B-F) images of the metacorpus part of the esophagus of the parasitic nematode *H. dispar*. A: light microscopic photograph of a semi-thin section (1 µm), two-layer staining by D'Amico method; B, C, D, E, F: Electron microscopic photograph of an ultra-thin section (50–70 nm) stained by uranyl acetate and lead citrate. A - general image under a light microscope, B - ultrastructural features of the esophagus, C - ultrastructure of the basement membrane, D - structural elements of the muscle cell, E - ultrastructural features of the cuticle, F - finger-like processes of the cuticle - indicated by a black arrow; Designations: Cu - cuticle, ML - muscle layer, Ph - esophagus, Bm - basement membrane, L - lumen, RMC - radial muscle cells, YMC - lateral muscle cells, Fb - fibrils, M - mitochondria, Ql - glycogen, MC - muscle cell , Db - dense bodies, Fl - filament, Qq - cortical layer, Bq - basal layer, Hq - middle or homogeneous layer.

The intestine of the *H. dispar* nematode, like that of other nematodes, it is of endodermal origin, is generally divided into three regions: anterior or ventricular part, the midintestinal part, and the posterior or prerectal part. They merge into each other without any perceptible boundaries. The total intestinal diameter is 111.54-116.86 μm (115.45±0.54 μm). Its outer wall consists of a thick basement membrane (thickness - 0.44-0.46 μm (0.450.003± μm)). Then, single-layer epithelial cells are located inside (length - 27.97-33.26 μm (31.870.56± μm)). In the apical part of these cells, microvillas are located (length - 3.854.27- μm (4.060.4± μm); width - 0.120.20- μm (0.140.011± μm)) (Fig. 3A, 3B, 3C). It is noted that the intestine of the nematode *H. dispar* has a different shape in different parts of the body. Thus, Fig. 3A shows that the intestinal lumen is triangular, while Fig. 3B shows that it is hexagonal. Both light and electron microscopic images clearly show that the intestine is in a pseudocoelomic cavity along with other internal organs (Fig. 3A, 3B, 3C; indicated by a snowflakes). Epithelial cell nuclei is also observed in semithin and ultrathin sections (Fig. 3A, 3B, 3C). As a result of electron microscopic studies, it was found that the ultrastructure of intestinal epithelial cells of the nematode *H. dispar* are divided into 5 regions: 1 - basement membrane, 2 - reticular zone, 3 - main part (plasmatic), 4 - terminal zone (fibrillar), 5 - microvillas. Each region of the intestinal epithelial cell has unique ultrastructural features that differ from others. Information about each of them is given separately. Fig. 3D shows the basement membrane and reticular zone of the intestinal cell. The basement membrane of a cell is thicker than the membranes of other organs. In the reticular region, there are structures with a triple membrane which are not found in other parts of the cell and entering the basement membrane (shown by black arrows). They increase the area of the plasmolemma, forming a unique network, and, most likely, are involved in the metabolism and transport of substances into the body cavity of the nematode (Fig. 3D). The reticular layer is observed in areas of epithelial cells close to the basement membrane in all three sections of the intestine. The next third and main part of the intestine, which is considered the largest in area, contains the nucleus, nucleolus, granular endoplasmic reticulum, non-granular endoplasmic reticulum, Golgi complex, glycogen, ribosomes, various lysosomal bodies, electron dense granules, lipids, vacuoles, mitochondria, elements of the cytoskeleton, basal bodies, membrane-like vesicles, and other organelles (Fig. 3E). The nucleus, located in the center of the cytoplasm of the intestinal cell, has a predominantly round, sometimes oval shape (diameter - 6.58-7.37 μm (7.080.079± μm)). The cytoplasm contains more mitochondria, glycogen, granular endoplasmic reticulum and ribosomes. On Fig. 3F shows the last two layers of the epithelial cell, the area containing the microvilli and the terminal region which connects them to the epithelial cytoplasm. Microvillas are located throughout the intestine in that part of the epithelium which leads to the lumen. Microvillas are attached to fibrils in the terminal part of the intestinal cell. Outside, they are covered with a transparent mesh substance called the glycocalyx. Its main function is to protect microvillas from physical and toxic influences, including playing the role of a filter. The terminal part (marked with a white asterisk, thickness 1.58–1.71 μm (1.660.016± μm)) consists of small granules and fibrils, no other organelles are observed here (Fig. 3F). Various intercellular connections (junction) are observed between intestinal epithelial cells of the nematode *H dispar*. Our electron microscopic studies revealed dense contacts of intestinal cells with desmosomes in the terminal region of the cell or in the apical part of microvilli (Fig. 3F). In addition, the smooth membranes of epithelial cells create a barrier junction with each other (smooth septate junction, marked with a white snowflake), and this situation leads to the formation of stronger bonds between the two cells (Fig. 3F). The posterior part of the intestine of the nematode *H. dispar* ends with sphincter (muscle) cells and opens into the rectum in females and into the cloaca in males. The cells that form the wall of the cloaca are also involved in the formation of copulatory organs that are part of the male reproductive system. Unlike the intestines, the rectum in females and the cloaca in males are covered not with microvilli, but with cuticle. In females, in the organization of the wall of the rectum, covered with a cuticle, along with muscle cells, three glandular cells take part. The rectum ends with the ventral anus. The cloacal wall of the male *H. dispar* nematode consists of a basement membrane, a single-layer epithelial cell, and a cuticle from the lumen (Fig. 4). On fig. 4A shows a light microscope image of the male cloacal lumen and a pair of equally sized spicules around it. An ultrastructural study of the cloacal wall revealed that its wall is innervated by processes of

nerve cells (Fig. 4B). The thickness of the cuticle of the cloaca is 1.00-1.35 µm (1.205±0.035 µm) and consists of 3 layers: 1-cortical, thickness 0.018-0.023 µm (0.020±0.0006 µm), 2 - middle or homogeneous layer, thickness 1.061.17- µm (1.130.012± µm), 3 - basal layer, thickness 0.011-0.014 µm (0.012±0.0003 µm) (Fig. 4C). It has been established that the cuticle covering the wall of the cloaca from the side of the lumen is thicker in many times (~10) than that observed in the esophagus. Figure 4B shows that the processes (length - 0.47-0.78 µm (0.57±0.032 µm)) penetrate from the basal part of the cuticle to the cytoplasm of the epithelial cell. These processes are observed in the hypodermal ridges that make up the body wall of the nematode *H. dispar*, as well as in the area around the male bursa between the hypodermis and the cuticle. This fact once again proves that both cuticles are identical in origin and that the cuticle on the wall of the cloaca is connected with the cuticle covering the outside of the body of the parasite. Hence, their ultrastructure are similar. Mitochondria, glycogen, ribosomes, smooth and granular endoplasmic reticulum, vacuoles and microtubules are observed in the cytoplasm of an epithelial cell (length - 16.18-19.26 µm (18.230.32± µm)), which forms the wall of the cloaca (Fig. 4C). The epithelial cell is externally lined with a basement membrane (thickness 0.034–0.053 µm (0.04±0.002 µm)) (Fig. 4D). Compared to the basement membrane of the intestinal wall, the basement membrane of the cloacal wall is thinner in many times. Male nematodes have 6 rectal glandular cells that open into the lumen of the cloaca. In addition to those mentioned, muscle fibers are traced around the cloaca and sphincter. Some of them are attached to the muscle layer of the parasite, and the other part is attached to the outer cuticle of the body. It was also found that the processes of nerve cells (dendrites and axons) are innervated outside the epithelial layer of the cloaca bordering the basement membrane (Fig. 4D).

Discussion

Parasitic nematodes, including the studied parasitic worms belonging to the Heterakidae family, have a pseudocoelomic cavity covering the internal organs (digestive and reproductive organs) in the form of a sack throughout the body on the border with the integumentary tissue. It differs from the body cavity in its origin (not fully developed from the mesodermal layer) [18]. As a result of an electron microscopic analisys of the wall of the pseudocoelomic cavity of the free-living nematode *Caenorhabditis elegans*, belonging to the Rhabditidae family, it was found that it cannot be attributed to cells of mesodermal, endodermal, or ectodermal origin [4]. And from the parasitic nematodes, information on the structure of the pseudocoelomic cavity is available in the nematode *Ascaris lumbricoides* [19]. It should also be noted that although the ultrastructure of representatives of some families of parasitic worms (Capillariidae, Trichostrongylidae) have been studied, there is no information about their pseudocoelomic cavity [20-22]. Some authors note that the digestive and reproductive organs of the nematode *H. spumosa* belonging to the family Heterakidae are located in the pseudocoelomic cavity filled with fluid [5]. A basement membrane was identified, which constitutes the wall of the pseudocoelom, covering the internal organs of the nematode *H. dispar* studied by us (Fig. 1). This pattern is also reflected in the studies of other scientists [4].

Although the digestive system of nematodes mainly consists of three parts, the morphological features are variable and different in species belonging to different families [4]. For example, one of the main features of species belonging to the family Heterakidae is that the posterior part of the esophagus is enlarged and consists of a well-developed bulb. The available literature contains partial information on the ultrastructure of the digestive organs of only 2 species of the family Heterakidae (*H. gallinarum* and *H. spumosa*) [3, 5]. Regarding the nematode *H. dispar*, only one source was found, where the helminth was studied only by the histological method [6]. Information about the ultrastructure of the digestive organs of this parasite was not found. All these authors emphasize that the digestive system of the studied nematodes consists of the anterior - stomodeum, middle - intestine, and posterior - proctodeum parts [3-6]. It has been established that the digestive organs of above mentioned nematode *H. dispar*, whose ultrastructure has been studied by us, also consist of the 3 parts. It has been noted that the esophagus of another nematode, *H. gallinarum*, belonging to the Heterakidae family, consists of two main parts - procorpus and metacorpus [23]. It can be seen that the anterior part of the metacorpus is thin and long, while the posterior part expands and turns into a bulb. It has been established that the nematode *H. dispar* studied by us, belonging to the same family, the esophagus consists of the same parts - procorpus,

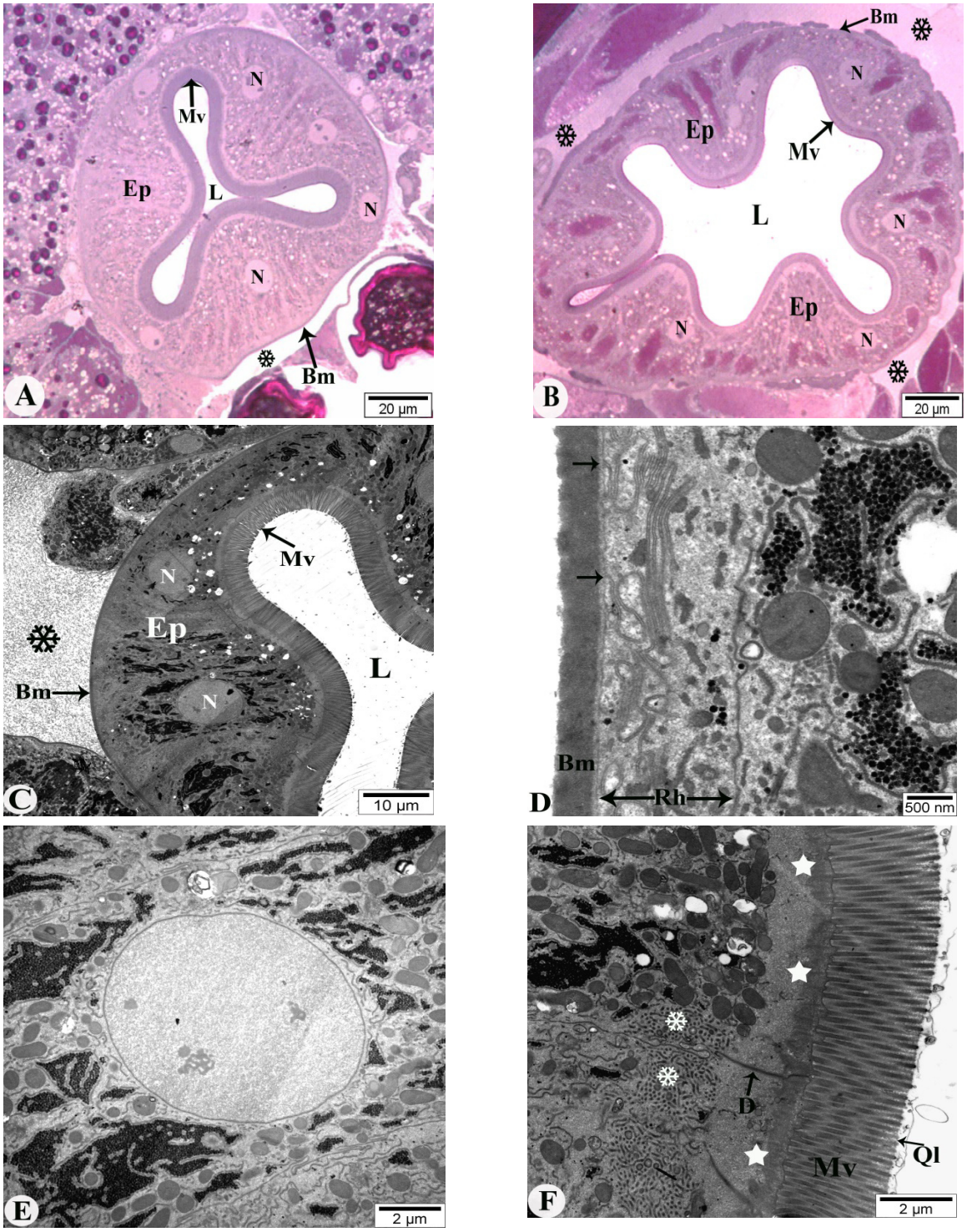

Fig. 3. Structural features of the intestine of the nematode *H. dispar*. A, B: light microscopic photograph of a semi-thin section (1 µm), two-layer staining by D'Amico method; C, D, E, F: electron microscopic photograph of an ultra-thin section (50–70 nm) stained by uranyl acetate and lead citrate. A and B – general view of the intestine in a light microscope, C - general view of the intestine in an electron microscope, D - features of the ultrastructure of the basement membrane of the intestinal wall and the reticular layer of the epithelium, E - features of the ultrastructure of the cytoplasm of the intestinal epithelium, F - ultrastructural features of the intestinal microvillas and terminal region. Designations: Ep - epithelium, Mv - microvilli, L - lumen, N - nucleus, Bm - basement membrane, Rh - reticular region, Ql - glycocalyx, D - desmosome.

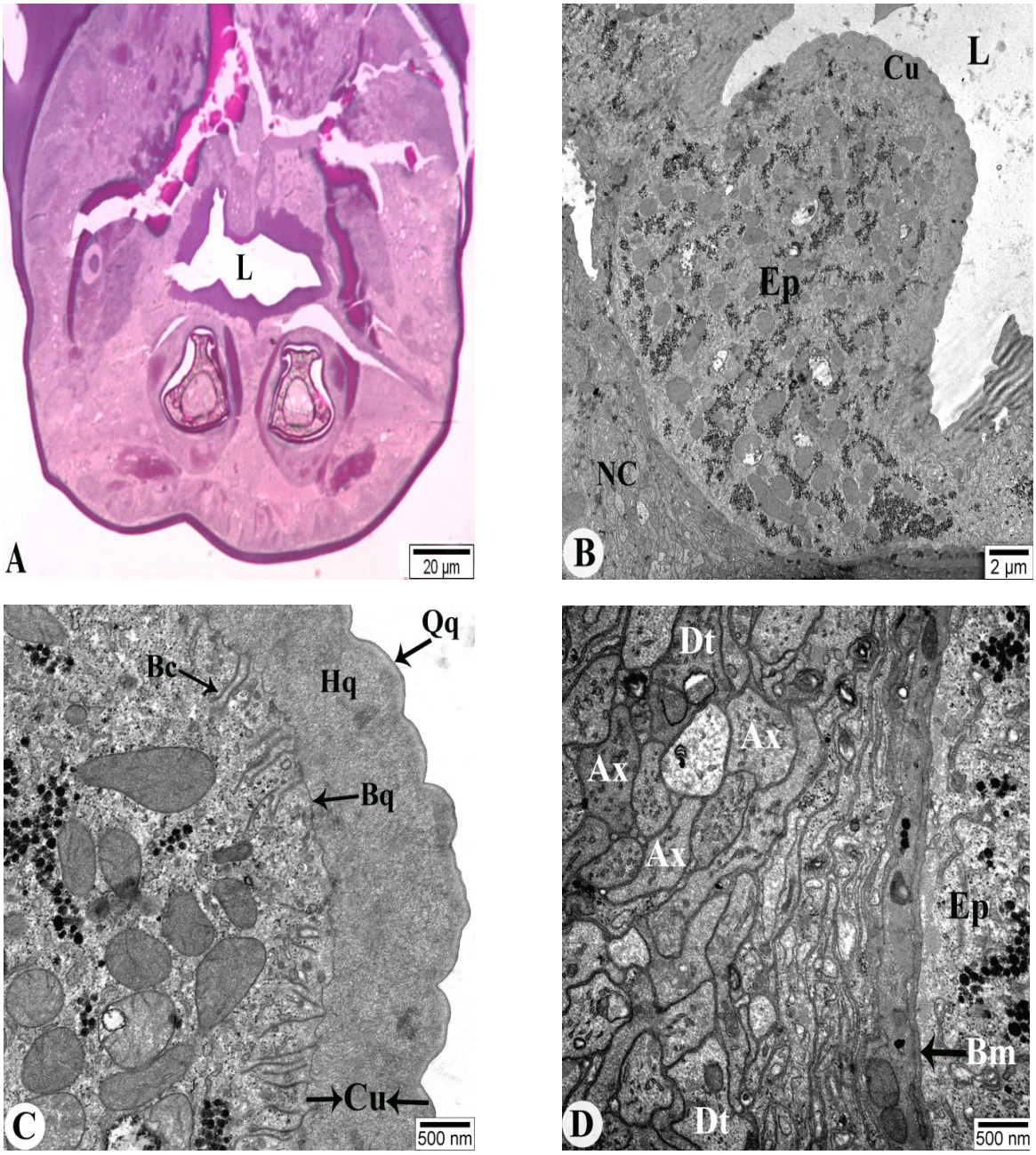

Fig. 4. Features of the structure of the cloaca of the male nematode *H. dispar*. A: light microscopic photograph of a semi-thin section (1 μm), two-layer staining by D'Amico method; B, C, D: electron microscopic photograph of an ultra-thin section (50–70 nm) stained by uranyl acetate and lead citrate. A – general view of the cloaca in a light microscope, B – general view of the cloacal wall in an electron microscope, C – ultrastructure of the cuticle in the lumen of the cloacal wall, D – basement membrane of an epithelial cell and the processes of nerve cells innervating it. Designations: Ep - epithelium, L - lumen, Bm - basement membrane, Cu - cuticle, NC - processes of nerve cells, Qq - cortical layer, Hq - middle or homogeneous layer, Bq - basal layer, Bc - basal processes of cuticle, Dt - dendrite, Ax - axon.

metacorpus and basal part (bulb). The researcher showed that the cells that make up the wall of the procorpus of the esophagus of the nematode *H. gallinarum* do not have nuclei, while they do exist in the metacorpus and bulb [23]. Nuclei were not found in any of the three parts of the esophagus of the studied nematode *H. dispar* (Fig. 2). There is literature evidence that the lumen of the esophagus of all studied helminths, including the nematode *H. dispar*, is covered with a cuticle [3, 6, 23]. Unlike previous studies, for the first time among the species included in the family, the ultrastructural features of the cuticle were studied and it was determined that it consists of 3 layers (Fig. 2E), and statistical calculations were also carried out. The fact that the cuticle located in the lumen of the esophagus has such a structure was also observed in the nematodes *Leptonemella juliae* (Nematoda, Adenophorea) belonging to other families [24]. In nematodes, the hypodermis is involved in the secretion of the cuticle that covers the body of the parasite from the outside. But the cuticle, which covers the internal organs of the worm (buccal cavity, pharynx, reproductive organs, etc.), develops mainly, unlike others, at the expense of epithelial cells. The cuticle in the lumen of the esophagus develops from epithelial cells, as well as from muscle cells [25]. Studies by some scientists have shown that the thickness of the cuticle at the peaks located in the lumen of the esophagus of nematodes of other families is the cause of its keratinization or sclerotization [25]. On the electronograms associated with the cuticle in the lumen of the esophagus of the nematode *H. dispar*, the ultrastructure of which was studied by us, none of the above mentioned morphological features was observed by other authors. On the contrary, the thickening of the cuticle at the peaks of the lumen is associated with the presence of an additional layer located between the cuticle and the muscle layer, which has mitochondria and dense fibrils (Fig. 2E). On the other hand, fingerlike processes of the cuticle were also found in the lumen of the esophagus (Fig. 2F). Information about this was not found in any of the species included in the family. In general, it is noted that these fingerlike processes of the cuticle perform the function of retaining bacteria penetrating the worm's body [25]. In the course of our studies, in addition to those mentioned, we have reason to say that these finger-like processes are involved in grinding food in the esophagus. In nematode *H. gallinarum* belonging to the family Heterakidae, in the bulb, the basal part of the esophagus, relatively large muscle cells and the presence of several glandular cells can be traced [3, 23]. In *H. dispar* nematodes, it was also found that the bulb wall consists of large muscle cells and 3 glandular cells. There are literature data on the ultrastructural structure of the intestine, which is the second part of the digestive system, in nematodes of the Ascaridiida and other families, as well as in *Ascaris suum, A. lumbricoides, Parascaris equorum, Oxyuris equii, Strongylus equinus, Ancylostoma caninum, C. elegans, Bursaphelenchus xylophilus* etc. [3, 26, 27]. There is information on the intestinal ultrastructure of the nematode species *H. gallinarum* and *H. spumosa* belonging to the family Heterakidae, and only on the morphological structure of the parasite *H. dispar* [1, 3, 5, 6, 23, 28]. As a result of the analysis of literature data, it was found that the intestine of parasitic nematodes has a tubular shape and the wall consists of a basement membrane, a single-layer epithelial cell, which ends in microvilli in the apical part. The number of epithelial cells varies depending on the studied species [3]. Only one source mentions that the nematode *H. spumosa* belonging to the Heterakidae family has a thin layer of muscles in the intestinal wall [5]. In our and other studies, no muscle layer was found in the intestinal wall of nematodes. In species belonging to the same family, the shape of the intestinal lumen may be different. In the nematode *H. gallinarum*, the intestinal lumen has a three-, four-, and sometimes pentagonal shape [3, 23], the nematode *H. dispar,* whose ultrastructure was studied by us, has triangular and hexagonal shapes. None of the species belonging to the Heterakidae family has data on the division of intestinal epithelial cells into 5 different regions. Such information can be traced only in some species of the family Ascaridiidae [3]. The intestinal epithelial cells are connected to each other by desmosomes (Fig. 3F). In addition, in the nematode *H. dispar*, smooth septate junction were also found at the border between the cells of the intestinal epithelium (marked by a white snowflake), which was not observed in other species of the family (Fig. 3F). These structures were observed only in the free-living nematode species *C. elegans* belonging to the Rhabditidae family [29].

The last - the third part of the digestive organs of the nematode *H. dispar* refers to the posterior intestine (proctodeum). Features of the ultrastructure of this part are different depending on the sex of the parasite (male and female).

Thus, in most nematodes, in both individuals, common and similar structures, sphincter muscles or intestinal rectal valve are observed between the intestine and proctodeum [1, 4]. In the cytoplasm of the above-mentioned cells there are fibrils, with the help of which these muscles have the ability to contract. It has been noted that the posterior part of the intestine is covered with a cuticle in both males and females of all species in which the proctodeum (*H. gallinarum* and *H. dispar*), belonging to the Heterakidae family, has been studied [3, 6, 30]. According to the researchers, the same cuticle found in the nematode *H. gallinarum* is considered to be a continuation of the cuticle covering the body wall of the parasitic worm. In addition, it was shown that the cuticle in the lumen of the female nematode consists of only one homogeneous layer [30]. As a result of an ultrastructural study of the cuticle of the cloaca of the nematode *H. dispar* studied by us, it was found that it consists of 3 layers - cortical, homogeneous and basal. In addition, cuticle processes that penetrate into the cytoplasm of the epithelial cell of the nematode *H. dispar* were found, but were not found in other members of the family, the ultrastructure of which was studied. However, in the nematode *H. dispar*, these processes were also observed between the cuticle and hypodermis in thebody wall, which has the same origin. These basal processes have also been found in the nematode *Wuchereria bancrofti*, which belongs to the Onchocercidae family and causes serious human disease [18].

Conclusion

For the first time, the ultrastructural features of the digestive organs of the nematode *H. dispar* was studied and it was found that the organs are located in the pseudocoelomic cavity of the body and they are divided into 3 parts - stomodeum, intestine and proctodeum. The esophagus consists of the procorpus, metacorpus and basal part. Its lumen has a triangular shape and is divided into three peaks. Its wall consists of a basement membrane, lateral and radial muscle cells, and a cuticle. Muscle cells are connected to the basement membrane through hemidesmosomes. The cuticle, in turn, consists of cortical, homogeneous and basal layers. Between the cuticle and muscle cells in all three peaks of the lumen of the esophagus, additional structures with a cellular structure were observed. In the bulb, the muscle cells are large in size and number. Also, glandular cells and processes of nerve cells are observed on the anterior and posterior parts of bulb. The lumen of the bulb is also covered with a cuticle. The intestine originates from the endoderm and is divided into the ventricular, middle, and prerectal regions. The intestinal wall consists of a thick basement membrane, a single-layer epithelium with microvilli in the apical part of the cell. The epithelial cell is divided into 5 parts, differing in structure. In addition to desmosomes, membranes of epithelial cells also contain smooth septate junctions between themselves. The intestine ends with sphincter cells and opens into the rectum in females and into the cloaca in males. The lumen of both is covered with a cuticle. The wall of the cloaca consists of a basement membrane, an epithelial layer, and a cuticle. The cuticle, in turn, consists of 3 layers. From the basal part of the cuticle towards the cytoplasm of the epithelial cell, processes with a 3-layer membrane are introduced.

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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.

References

1. Lee, D.L. Structure and function of the intestinal-cloacal junction of the nematode *Heterakis gallinarum*. *Parasitology,* **70**, 389-396 (1975).
2. 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).
3. Bogoyavlenskiy, Yu. K., Bogolepova, I.I. and Onushko, N.V. Microstructure of tissues of parasitic nematodes. Moscow, Nauka, (1982).

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

5. 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. *Parasitol Res*., **83**(5), 419–434 (1997).

6. 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).

7. 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).

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

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

10. 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).

11. 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*, *Biology*, **36**, 58–73 (2021).

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

13. Eyvazov, A. (Ed.). *The taxonomic spectrum of the fauna of Azerbaijan (Protozoa and Helminthes)*. Baku, Elm ve Tahsil, (2022).

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

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

16. 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, 152-176 (2021).

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

18. Basyoni, M.M.A. and Rizk, E.M.A. Nematodes ultrastructure: complex systems and processes. *Journal of Parasitic Diseases*, **40**(4), 1130–1140 (2016).

19. Harris, J.E. and Crofton, H.D. Structure and function in the nematodes: Internal pressure and cuticular structure in *Ascaris*. *Journal of Experimental Biology*, **34**(1), 116–130 (1957).

20. Nasirov, A.M. Microstructure of Capillariid tissues (Nematoda: Capillariidae). Baku, Sabah (1996).

21. 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).

22. 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, Series Biology,* **34**, 112–119 (2020).

23. Bogolepova, I.I. Morphofunctional features of the intestines of parasitic nematodes. Proceedings of the meeting on parasites and diseases of fish. Moscow, 14-15 (1979).

24. Hoschitz, M., Bright, M. and Ott, J.A. Ultrastructure and reconstruction of the pharynx of *Leptonemella juliae* (Nematoda, Adenophorea). *Zoomorphology,* **121**, 95–107(2001).

25. Altun, Z.F. and Hall, D.H. Alimentary System, Pharynx. In *Worm Atlas*. (2009).

26. McGhee, J.D. *The C. elegans intestine, WormBook,* ed. The *C. elegans* Research Community, WormBook, (2007).

27. Ekino, T., Kirino, H., Kanzaki, N. and Shinya, R. Ultrastructural plasticity in the plant-parasitic nematode, *Bursaphelenchus xylophilus*. *Scientific Reports*, **10**(1), 11576 (2020).

28. Sharipova, A.A. Morphological study of the digestive system of the nematode *Ganguleterakis spumosa* (Schneider, 1866). *Actual Questions of Modern Parasitology*, **84**, 120-124 (1975).

29. Herndon, L.A., Wolkow, C.A. and Hall, D.H. *The aging intestine*. In *Worm Atlas*. (2018).

30. Bogolepova, I.I. and Chernoozerskaya, N.G. Micromorphological structure of the posterior intestine of *Heterakis gallinarum*. Measures to combat helminthiases and other parasitic diseases of agricultural animals. *Moscow*, **37**, 11-16 (1974).