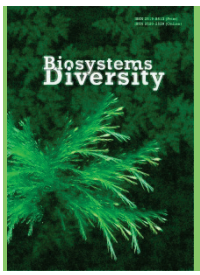

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Ultrastructural characteristics of the reproductive organs of adult male helminth *Heterakis dispar* (Nematoda: Heterakidae)

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Nematodes belonging to the Heterakidae family are similar in appearance and their identification causes certain difficulties. For this reason, there is a need to define new taxonomic characteristics. On the other hand, despite the fact that the nematode *Heterakis dispar* Schrank, 1790, included in the same family, has a wide distribution area, there is little information about the ultrastructure of the parasite. Research on the ultrastructure of the reproductive system of this helminth hase not been conducted. Parasitic worms collected from the caeca of birds were identified, following which, based on the accepted methods of electron microscopy, blocks were prepared, cut, stained, pictures and electrograms were prepared and described. In the present study, the ultrastructural characteristics of the reproductive organs (testis, seminal vesicle, vas deferens and ejaculatory duct) of the adult nematode *H. dispar*, a specific parasite of domestic waterfowl, were studied with the help of light and electron microscopic methods. The obtained results were compared with the structure of other studied species (*H. gallinarum* Gmelin, 1790 and *H. spumosa* Schneider, 1866) of the Heterakidae family. Firstly, it was revealed that the reproductive organs of the male nematode *H. dispar* are located in the pseudocoelomic cavity, which is considered a taxonomic sign of this family. As in other species of the family, the testis, seminal vesicle, and wall of the vas deferens of the male nematode *H. dispar* consist of a basement membrane and an epithelial layer. In addition to the basement membrane, muscular and epithelial layers, the wall of the ejaculatory duct is also composed of glandular cells in the front part of the duct. It was revealed that, in the lumen of the tubular reproductive organs of the adult male nematode *H. dispar*, germ-cells turn into spermatogonia in the germinal zone and spermatocytes in the growth zone of the testis, spermatids in the seminal vesicle, incomplete formed spermatozoa in the vas deferens, and fully formed spermatozoa in the ejaculatory duct of the parasite. The spicules of the parasite nematode, which are the secondary sexual organs consist of a thick cuticle on the outside, and hypodermal origin cells and processes of nerve cells (dendrites and axons) on the inside. Two processes are found on the spicules, which is not observed in other nematodes of the same family.

Keywords: *Heterakis dispar*; adult male nematode; reproductive organs; ultrastructure; transmission electron microscope.

Introduction

The reproductive potential of tapeworms (nematodes) depends on the speed of their development and the number of eggs they lay. Nematodes include free-living and parasitic species. Tapeworms that are parasitic in animals have the ability to lay more eggs. Most of the currently known species of nematodes are heterosexual (males and females) and differ from each other in appearance, size and structure of the reproductive organs. The reproductive organs of both male and female individuals are tubular and have a unique ultrastructure. Those features, in turn, play an important role in determining the systematic positions of helminths. In order to study the ultrastructure of the reproductive organs of adult male individuals of nematodes belonging to the Heterakidae family with a wide species composition, there are only literatures on the helminths *H. gallinarum* and *H. spumosa* (Baker, 1973; Bogoyavlensky et al., 1978; Bird & Bird, 1991; Mehlhorn & Harder, 1997). The nematode *H. dispar*, another representative species of the genus, is selected from the 27 species of parasites recorded by us in domestic waterfowl in the territory of our country due to its high extensity and intensity of infection (Rzayev, 2021, 2023a; Rzayev et al., 2021). In recent years, the same result has been recorded in domestic waterfowl in other countries (Zhang et al., 2012; Borah et al., 2018; Rukambile et al., 2020; Yevstafieva et al., 2020, 2022; Elshahawy et al., 2021). Although there are studies on the ultrastructure of the body wall and digestive organs of that parasitic worm (Rzayev, 2023b), data on the ultrastructure of the reproductive organs of adult males of the nematode *H. dispar* have not been found. There is only one source on the histological study of the structure of the reproductive system of adult female *H. dispar* (Nasirov et al., 2008). Nevertheless, molecular identification of genes and phylogenetic analysis of the *H. dispar* nematode were performed (Bobrek et al., 2019; Geo et al., 2019; Elshahawy et al., 2021).

Taking into account the above, the aim of the present study, with the help of histological and electron microscopic methods, was to study the ultrastructural characteristics of the reproductive organs of adult male nematode s *H. dispar*, a specific parasite of domestic waterfowl and belonging to the Heterakidae family.

Matherial and methods

All the experimental part of this work on domestic waterfowl was carried out in accordance with the requirements of the international principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986). Experiments of this research work were performed after the approval from the Ministry of Health of Azerbaijan Republic, Ethics Committee of Azerbaijan Medical University, Azerbaijan (No: EP 0039).

Research carried out in the direction of studying the ultrastructure of the helminth was carried out in 2019–2022 on the territory of the Republic of Azerbaijan. Ten one year old domestic geese (*Anser anser* dom.) previously identified as infected with helminths *H. dispar* were obtained from

the city of Shabran (41°12'18" N, 48°59'45" E) and brought to the Laboratory of Parasitology of the Institute of Zoology and examined by method full parasitological dissection (Dubinina, 1971). *H. dispar* nematodes were collected from the cecum of the birds. Fixed preparations of helminths were studied under a stereomicroscope MBS-9 and a light microscope Primo Star (Carl Zeiss, Germany). For the identification of the species, Ryzhikov's (1967) identification guide was used. In order to study the ultrastructure of the helminths, the collected adult male nematodes were divided into several parts and immediately were fixed in a solution consisting of 2.5% glutaraldehyde, 2% paraformaldehyde, 4% surcosa, 0.1% picric acid prepared in 0.1 M 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 using general methods adopted in electron microscopy (Kuo, 2014; Yushin et al., 2021). Semi-thin (1–2 μm) sections taken from the blocks on a Leica EM UC7 (Leica, USA) ultramicrotome, stained with methylene blue, azur II and basic fucsin or toluoid blue (D'Amico, 2005), were viewed under a Primo Star (Zeiss, Germany) microscope. Images of the necessary sections were taken with a digital camera EOS D650 (Canon, Japan). Ultrathin sections (50–70 nm) of the blocks, double-stained with uranyl acetate and lead citrate, were examined under the Transmission Electron Microscope JEM-1400 (JEOL, Japan) at a voltage of 80–120 kV. The morphometric analysis of the electronograms was carried out in TIF format via a computer program (TEM Imaging Platform) developed by Olympus Soft Imaging Solutions GmbH (Germany) (Agayeva et al., 2020). Data analysis was carried out with different parameters (Min, Max, mean ± SD).

Results

During the study of the ultrastructural structure of the reproductive organs of the nematode *H. dispar*, which belongs to the Heterakidae family, it was found that the reproductive organs of adult male helminth are tubular and consist of four parts, namely, the testis, the seminal vesicle, the vas deferens and the ejaculatory duct. In addition, the parasite also has secondary sexual organs, which include two spicules of the same length, bursa, sensilla, copulatory muscles, and supplements. Each organ has its own ultrastructure and functions. The first stage of spermatogenesis occurs in the testis. The wall of the testis (Te) of the nematode *H. dispar* ultrastructurally consists of a basement membrane (Bm) on the outside and a thin single layer of epithelial cells (Ep) on the inside (Fig. 1A). The total size of the testis was 59.37–66.20 (63.04 ± 0.59) μm.

Germ-cells go through several stages of development in different parts of the reproductive organs of an adult male individual until they become fully formed spermatozoa. The testis is divided into two regions. The germinal zone is located in the anterior part, and the growth zone is located in the posterior part. Germ-cells develop into spermatagonia in the germinal zone and in the growth zone become spermatocytes. They differ from each other according to their ultrastructural characteristics. Thus, in the cytoplasm of the spermatogonia cell, membrane-less nucleus, nucleolus, granular endoplasmic reticulum, ribosome, mitochondria are noted, while in spermatocytes, in addition to the listed structures, fibrillar bodies and membrane organelles are also observed.

On the electrogram obtained by us from ultrathin sections, muscle cells (Mc) and processes of nerve cells (Nc) are observed near the wall of the pseudocoelomic cavity (Ps) (Fig. 1B). As a result of statistical calculations, it was revealed that the thickness of the basement membrane (Bm) was 0.087–0.116 (0.102 ± 0.003) μm, and the diameter of the thin epithelial layer (Ep) was 0.357–1.146 (0.722 ± 0.086) μm that form the wall of the testis. In addition, a large number of spermatocytes (Sq) (length – 8.733–9.336 (9.187 ± 0.062) μm) were traced in the lumen of the testis (Fig. 1B, C, D). It was determined that the thickness of the basal membrane (Psm) of the wall of the pseudocoelomic cavity was 0.083–0.112 (0.099 ± 0.003) μm. It, in turn, is located alongside the processes of the muscle cells (MCc) that innervate the nerves in the dorsal and ventral cords (Fig. 1C). It was observed that there were a lot of mitochondria, glycogen, ribosome in the cytoplasm of the epithelial cells that make up the wall of the testis. A spermatocyte (Sq) was located in the growth zone of the testis and it consisted of a large and round nucleus (N) with a diameter of 4.928–5.490 (5.296 ± 0.072) μm and a nucleolus (Nu) of 1.137–1.294 (1.217 ± 0.016) μm inside (Fig. 1D). In addition to the above, in the cytoplasm of spermatocytes, mitochondria (M) (0.246–0.401 (0.340 ± 0.018) μm), granular endoplasmic reticulum (Er) (0.026–0.070 (0.048 ± 0.005) μm), Golgi complex (Hk) (0.190–0.341 (0.239 ± 0.016) μm), formed nuclear membrane (indicated by black arrow), nucleus (N), free fibrillar bodies (Fb) (0.318–0.448 (0.369 ± 0.013) μm), ribosomes (0.016–0.022 (0.019 ± 0.001) μm), etc. and organelles were observed (Fig. 1E). Apart from fibrillar bodies, membrane organelles (Mo) (0.187–0.251 (0.229 ± 0.007) μm) were also found in the cytoplasm of the spermatocyte. They were found in the form of a complex compound (Fig. 1F).

In the adult male reproductive system of the nematode *H. dispar* and also in most species, in the process of spermatogenesis, after the spermatocyte stage, the germ cells undergo a certain developmental stage and turn into spermatids. This stage occurs in the seminal vesicle, which is another reproductive organ in the nematode *H. dispar*. We have studied the ultrastructural features of the seminal vesicle and the spermatids located in its lumen. In the images obtained from the semi-thin sections of the seminal vesicle (SV), it was found that its wall measured 72.0–76.43 (74.62 ± 0.49) μm and that it consisted of epithelial cells (Ep), which are thicker than the epithelial layer of the testis (Fig 2A). Figure 2B shows a general view of the seminal vesicle by transmission electron microscopy, and it is also shown to be surrounded by the pseudocoelomic cavity fluid. The thickness of the basal membrane (Bm), which forms the wall of the seminal vesicle (SV), was 0.143–0.195 (0.165 ± 0.006) μm. The diameter of the thick single-layered epithelial layer (Ep) was 5.316–8.031 (6.796 ± 0.378) μm and the size of the spermatids (St) located in its lumen was 5.257–8.693 (7.083 ± 0.452) μm. In the cytoplasm of the epithelial cell, numerous mitochondria (M) (0.377–0.628 (0.486 ± 0.032) μm), glycogen (Ql) (0.035–0.063 (0.049 ± 0.003) μm), one large nucleus (N) in the center of the cell (diameter 3.472–3.744 (3.586 ± 0.031) μm) and within it a nucleolus (diameter 0.463–0.644 (0.555 ± 0.020) μm) were registered (Fig. 2C). A general view of the spermatid located in the lumen of the nematode seminal vesicle is shown in Figure 2D. Here, the cell's cytoplasm, nucleus (N) (diameter 3.858–4.165 (4.106 ± 0.032) μm) and its inner nucleolus (Nu) (0.617–0.761 (0.679 ± 0.018) μm) are shown. As a result of the ultrastructural examination of the spermatid cytoplasm, it was found that there are a large number of mitochondria (M) (0.313–0.569 (0.366 ± 0.028) μm), while Golgi complex (Hk) (0.285–0.329 (0.301 ± 0.005) μm), fibrillar bodies (Fb) (0.303–0.422 (0.393 ± 0.013) μm), membranous organelles (Mo) (0.312–0.413 (0.365 ± 0.013) μm), granular endoplasmatic reticulum, ribosomes and other organelles are also observed (Fig. 2E, F). Fibrillar bodies and membranous organelles form numerous complexes (Fig. 2F).

One of the features distinguishing the spermatids observed in the seminal vesicle of the nematode *H. dispar* from the spermatocytes in the testis is the presence numerous fibrillar bodies, and membranous organelles form numerous complexes. Neither cilia nor axoneme are found in the structure of the nematode *H. dispar* during the spermatogenesis of germ cells, starting from the spermatagony stage until the stage when they form spermatocytes, spermatids, unformed spermatozoa and fully formed spermatozoa.

The vas deferens of the studied nematode *H. dispar* has a tubular structure, it is finished with basement membrane from the outside, and cells of epithelial origin from the inside. Unformed spermatozoa in the lumen of vas deferens are observed.

The ultrastructural study of the ejaculatory duct of the adult male nematode *H. dispar* revealed that it is morphologically different from other reproductive organs. In addition to basement membrane and epithelial cells, the duct was found to consist of glandular cells in the anterior part and muscle cells in the posterior part. The ejaculatory duct, in turn opens ventrally into the cloaca. Already fully formed spermatozoa are observed here.

The spicules, are of the same length, which are considered to be one of the secondary sexual organs of the adult nematode *H. dispar* and they are located dorsally in the upper part of the cloaca. They participate in the fertilization of female nematodes. As a result of the research conducted by us, it was found that around the spicules there are muscle cells whose

cytoplasm is rich in fibrils. They ensure the movement of the spicules (back and forth). The both spicules of the nematode H. dispar have small processes along the surface. The structural features of the nematode spicules (S) were studied by both light and electron microscopic methods (Fig. 3A). The presented electrogram shows the spicules (S) of the nematode *H. dispar*, the specialized muscle cells (Mc) surrounding them, the cloaca (Kl) and its lumen (L). It should be noted that spicules are located in spicular sacs (Sk), which are filled with fluid (Fig. 3A, B). The diameter of the spicular sac surrounded by muscle cells is 15.846–17.354 (16.850 ± 0.182) μm, and the diameter of the spicule itself is 14.318–16.624 (15.924 ± 0.238) μm. The spicule sac (snowflake) is lined with a cuticle (Cu1) and its thickness is 0.452–0.888 (0.659 ± 0.048) μm (Fig. 3C). The spicule also consists of a cuticle (Cu2) 1.934–3.315 (2.619 ± 0.171) μm thick and two lateral processes (Sx) (1.409–4.353 (2.900 ± 0.124) μm (Fig. 3C). In addition to the outer covering of nematode spicules with cuticle, cellular structures inside were found. Here, nerve cell processes (Fig. 3D) and cells of hypodermal origin are encountered (Fig. 3E). In the cytoplasm of dendrites (Dt) mitochondria (M) (0.209–0.340 (0.266 ± 0.012) μm), granular endoplasmic reticulum (Er) (0.032–0.055 (0.046 ± 0.003) μm), Golgi complexes (Hk) (0.174–0.242 (0.216 ± 0.009) μm), ribosomes and numerous microtubules are found (Fig. 3D). In the cytoplasm of hypodermal cells (Hc), cytoplasmic bodies (Sc) with sizes of 0.283–0.347 (0.319 ± 0.006) μm were detected (Fig. 3E). In addition, the cytoplasm of the hypodermal cell (Hc) also contains mitochondria (M) (0.125–0.223 (0.180 ± 0.010) μm), granular endoplasmic reticulum (Er) (0.030–0.044 (0.037 ± 0.002) μm), while ribosomes and other organelles were also identified (Fig. 3E). The location of nerve cells inside the spicules also indicates that they are sensory organs. During the study of the male reproductive organs of the nematode *H. dispar*, muscle cells and nerve cell processes around the spicules sacs also were found (Fig. 3F). Figure 3F shows the spicular sac (marked with a snowflake) and nerve cell processes (Dt-dendrites and Ax-axons) and muscle cells (Mc). The cytoplasm of muscle cells is rich in glycogen and mitochondria (Fig. 3F). The function of the processes of the nerve cells located here is to innervate the muscle cells that ensure the up and down movement of the spicules, which are considered secondary sexual organs. In addition to the spicules, the secondary sexual organs of the adult male *H. dispar* nematode includes the copulatory bursa and associated lateral, dorsal, ventral rays, and cuticular ridges. They develop from the hypodermal layer and consist mainly of cuticle.

Discussion

There is literature on the study of the structure of adult male reproductive organs of only two nematodes (*H. gallinarum*, *H. spumosa*) belonging to the Heterakidae family and four species (*Caphalobellus papilliger*, *Aspiculuris tetraptera*, *Enterobius vermicularis*, *Oxyuris curvula*) belonging to the Oxyurata suborder (Hulinska, 1968; Baker, 1973; Bogoyavlensky et al., 1978; Bird & Bird, 1991; Mehlhorn & Harder, 1997). From the analysis of the results of the authors' researches, it is known that in all studied species (except *C. papilliger* nematode), belonging to the same suborder, the reproductive organs of the adult male consist of the testis, the seminal vesicle, the vas deferens and the ejaculatory duct. The reproductive organs of the adult male nematode *H. dispar*, which belongs to the same family, whose ultrastructure was studied by us, have a tubular structure and also consist of four parts.

Except for the body wall of the helminth, the digestive organs, and also the reproductive organs, are located inside the pseudocoelomic cavity. No information about this was found in the literature on the structure studied nematodes of the Heterakidae family. However, it is known that the pseudocoelomic cavity was discovered for the first time in the species belonging to this family and it is considered a taxonomic sign. The structure, composition and functions of the pseudocoelomic cavity of nematode *Caenorhabditis elegans* were studied in more detail (Bird & Bird, 1991).

The ultrastructural features of the reproductive organs were studied in the testis of the nematode *H. dispar*, which are divided into germinal part and growth parts, as in the case of the helminths *H. gallinarum* and *H. spumosa*, which belong to the same family. The wall of the testis is covered with a basement membrane and a thin epithelial layer from the outside in both parts (germinal and growth). The difference is that in the *H. dispar* nematodes studied by us, the thickness of the basement membrane is 0.10 ± 0.003 μm and the height of the epithelial cell is 0.70 ± 0.086 μm. In *H. gallinarum* and *H. spumosa* helminths, respectively, the basement membrane is 0.4 and 0.6 μm, and the height of the epithelial layer is 1.3–1.6 and 1.2–1.8 μm (Baker, 1973; Bogoyavlensky et al., 1978; Bogoyavlensky et al., 1982). As a result of the comparison of the obtained statistics with the literature data, it is shown that the testis wall of the nematode *H. dispar* is thinner than the other two studied nematode species same family.

The seminal vesicle wall of the *H. dispar* nematode consists of two layers (basal membrane and thick epithelium) as in the previously studied helminths *H. gallinarum* and *H. spumosa*. The sizes of those layers differ by species. Thus, the basal membrane of the nematode *H. gallinarum* is 0.4 μm, the height of the epithelial layer is 1.6–2.5 μm, and the diameter of the nucleus of the epithelial cell is 4.1 μm. Respectively the sizes of the parasitic worm *H. spumosa* were shown to be 0.6–0.8, 2.4–3.0 and 1.0 μm, respectively (Bogoyavlensky et al., 1982). Although the thickness of the basement membrane of the seminal vesicle wall of the nematode *H. dispar* studied by us is 2–3 times thinner than that of other studied nematodes, the epithelial layer is several times thicker. The size of the epithelial cell nuclei was found to be almost the same as *H. gallinarum* and more than three times larger than that of the nematode *H. spumosa*.

The wall of the helminth's vas deferens consists of a basement membrane and an epithelial layer, as in other species of the Heterakidae family. But in the nematode *H. gallinarum*, there are cytoplasmic processings in the apical part of the epithelial cell, which are directed towards the lumen (Bogoyavlensky et al., 1982). The above-mentioned signs were not observed in the epithelial cells of the wall of the vas deferens of the nematode *H. dispar* studied by us. It should also be noted that these structures were not detected in the helminth *H. spumosa* (Bogoyavlensky et al., 1982).

As a result of the ultrastructural study of the nematode *H. dispar*, the ejaculatory duct wall consists of a basement membrane, glandular cells in the front part, and a muscular layer in the back part and an epithelium in the lumen, while in the nematodes *H. gallinarum* and *H. spumosa* it is composed of a basement membrane, muscular and epithelial layers.

Thus, as a result of the ultrastructural study of the reproductive organs of the adult male nematode *H. dispar*, it was found that the wall of the testis, seminal vesicle, and the wall of the vas deferens consists of a basal membrane and an epithelial layer, as in other species of the family. In addition to basement membrane, muscular and epithelial layers, the wall of the ejaculatory duct is also composed of glandular cells in the front part of the duct. Ultrastructural characteristics of germ-cells of *H. dispar* nematodes at different stages of development (spermatogenesis) were also studied in the research carried out by us.

Thus, in the adult male helminth testis, germ cells were spermatogonia in the germinal zone, spermatocytes in the growth zone, spermatids in the seminal vesicle, incomplete formed spermatozoa in the vas deferens, and fully formed spermatozoa in the ejaculatory duct. The general course of spermatogenesis and the organelles in the cytoplasm of the germ-cells of the male nematodes are similar to those of other free-living and parasitic nematodes studied. The difference is in the sizes and morphological structures of the germ-cells in different stages (Afanasiev-Grigoriev & Yushin, 2009; Yushin & Ryss, 2011; Yushin & Malakhov, 2014; Yushin et al., 2014; Slos et al., 2020). In nematodes, spicules – secondary reproductive organs of males are different in all species, being considered one of the main taxonomic characters in the determination of species (Rammah & Hirschmann,1987; Baran, 2011; Basyoni & Rizk, 2016). The structure of the spicules of the nematode *H. dispar*, whose ultrastructure was studied by us, was also studied and described. There are literature data on the ultrastructure of the spicules of the male nematode *H. gallinarum* and other helminth species from parasitic nematodes belonging to the Heterakidae family (Lee, 1973; Croll & Wright, 1976; Wright, 1978; Bird & Bird, 1991). As a result of the analysis of literature data, it was found that cells of hypodermal origin and processes of nerve cells (dendrites and axons) are observed inside the spicules of various types of nematodes studied until now.

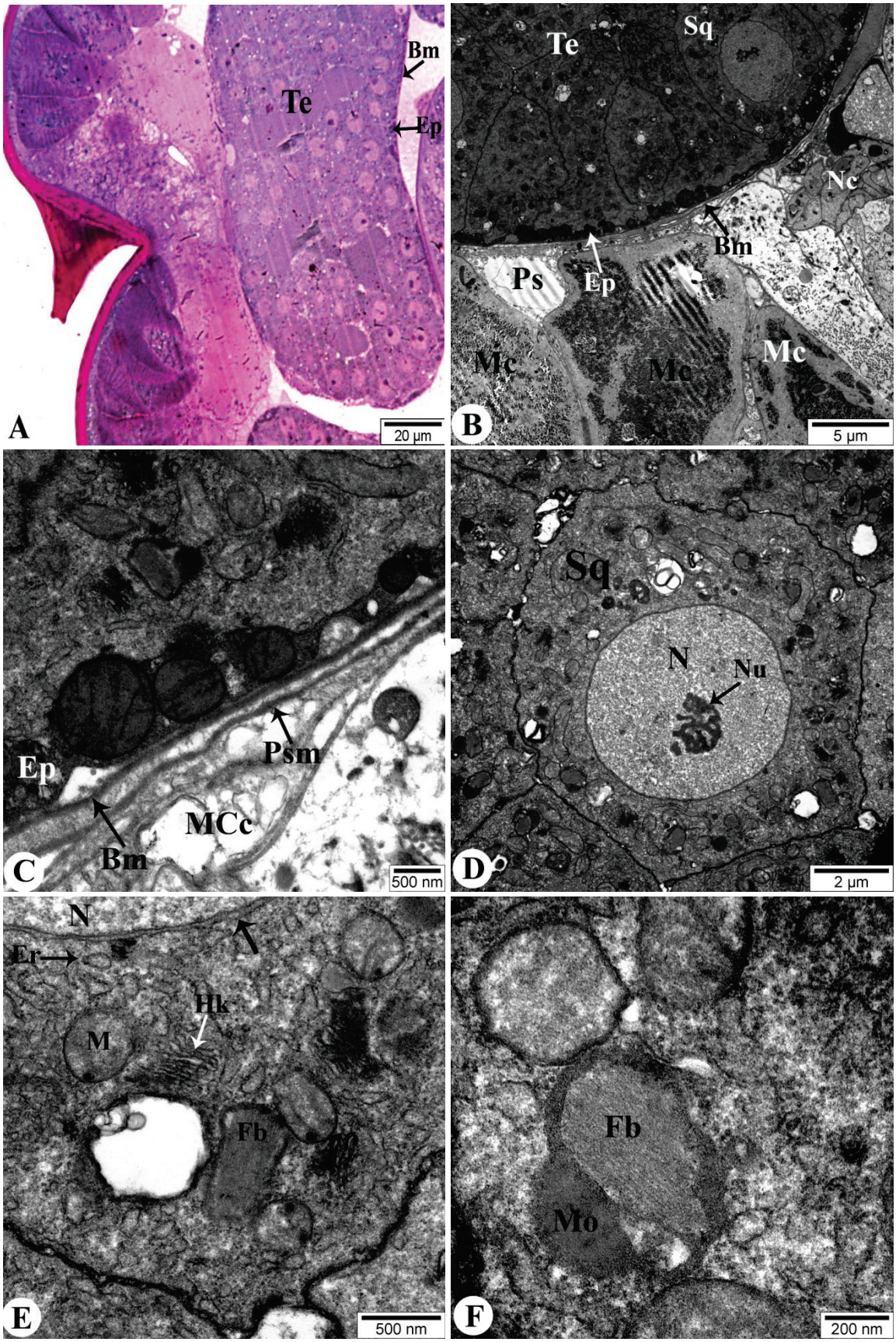

Fig. 1. Structural characteristics of testis of the adult male nematode *H. dispar*: *a* – general appearance of testis under light microscope, semi-thin section (1 µm), D'Amico double staining, *b* – electron microscopic image of testis, *c* – ultrastructural features of testis wall, *d* – general view of spermatocyte, *e* and *f* – ultrastructural characteristics of cytoplasmic structures of spermatocyte; ultrathin sections (50–70 nm), staining: uranyl acetate and Pb citrate; Te – testis, Ep – epithelial cells, Bm – basement membrane, Nc – processes of nerve cells, Sq – spermatocyte, Mc – muscle cells, Ps – pseudocoelomic cavity, MCc – processes of the muscle cells, Psm – pseudocoelomic cavity membrane, N – nucleus, Nu – nucleolus, Er – granular endoplasmic reticulum, M – mitochondria, Hk – Golgi complex, Fb – fibrillar bodies, Mo – membranous organelles

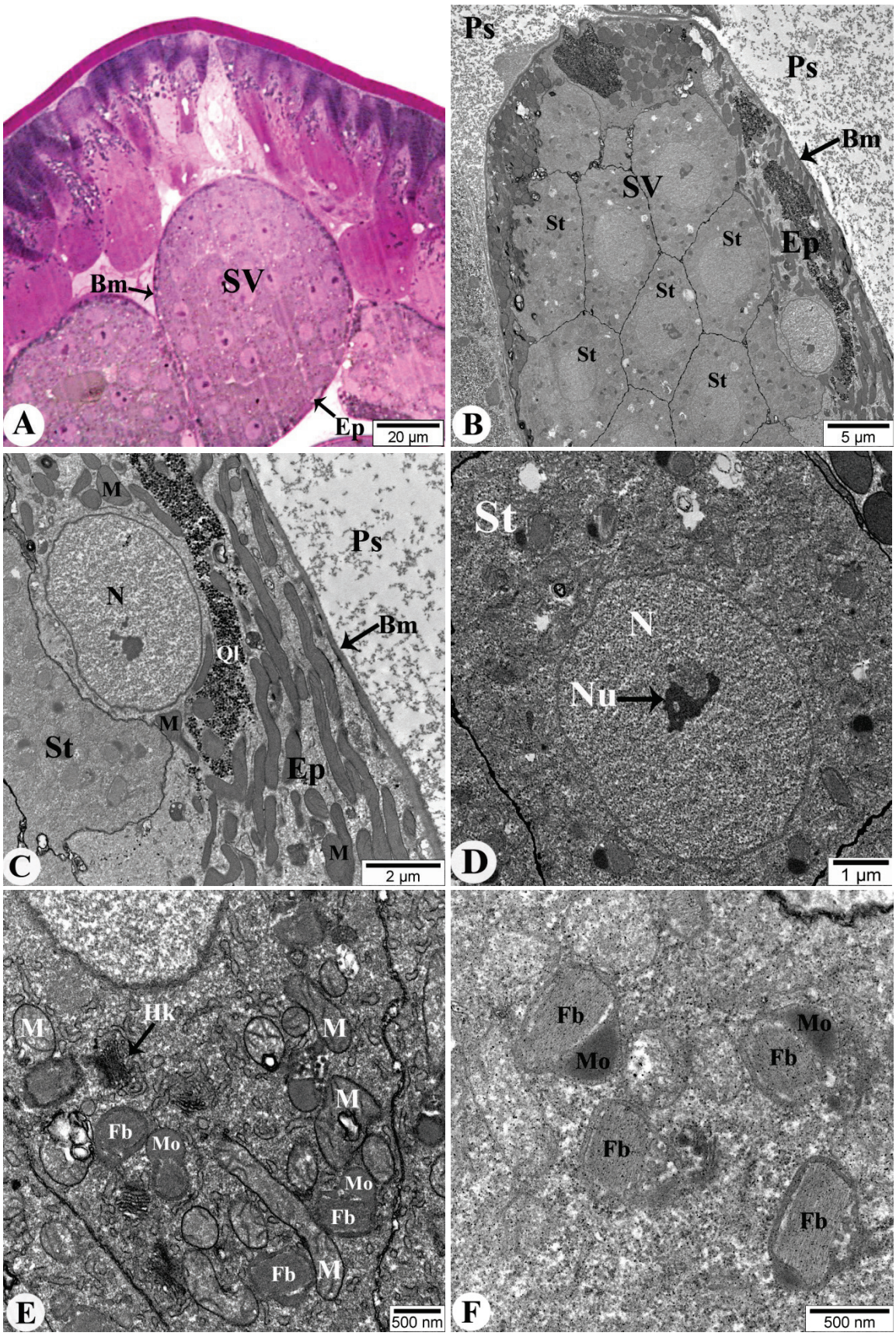

Fig. 2. Structural characteristics of the seminal vesicle of adult male nematode *H. dispar*: *a* – general view of the seminal vesicle under a light microscope, semi-thin section (1 µm), D'Amico double staining, *b* – electron microscopic image of the seminal vesicle, *c* – ultrastructural features of the seminal vesicle wall, *d* – general view of the spermatid, *e* and *f* – ultrastructural features of the cytoplasmic structures of the spermatid; ultrathin sections (50–70 nm), staining: uranyl acetate and Pb citrate; SV – seminal vesicle, Ep – epithelial cells, Bm – basement membrane, St – spermatid, Ps – pseudocoelomic cavity, N – nucleus, Nu – nucleolus, M – mitochondria, Hk – Golgi complex, Fb – fibrillar bodies, Mo – membranous organelles, Ql – glycogen

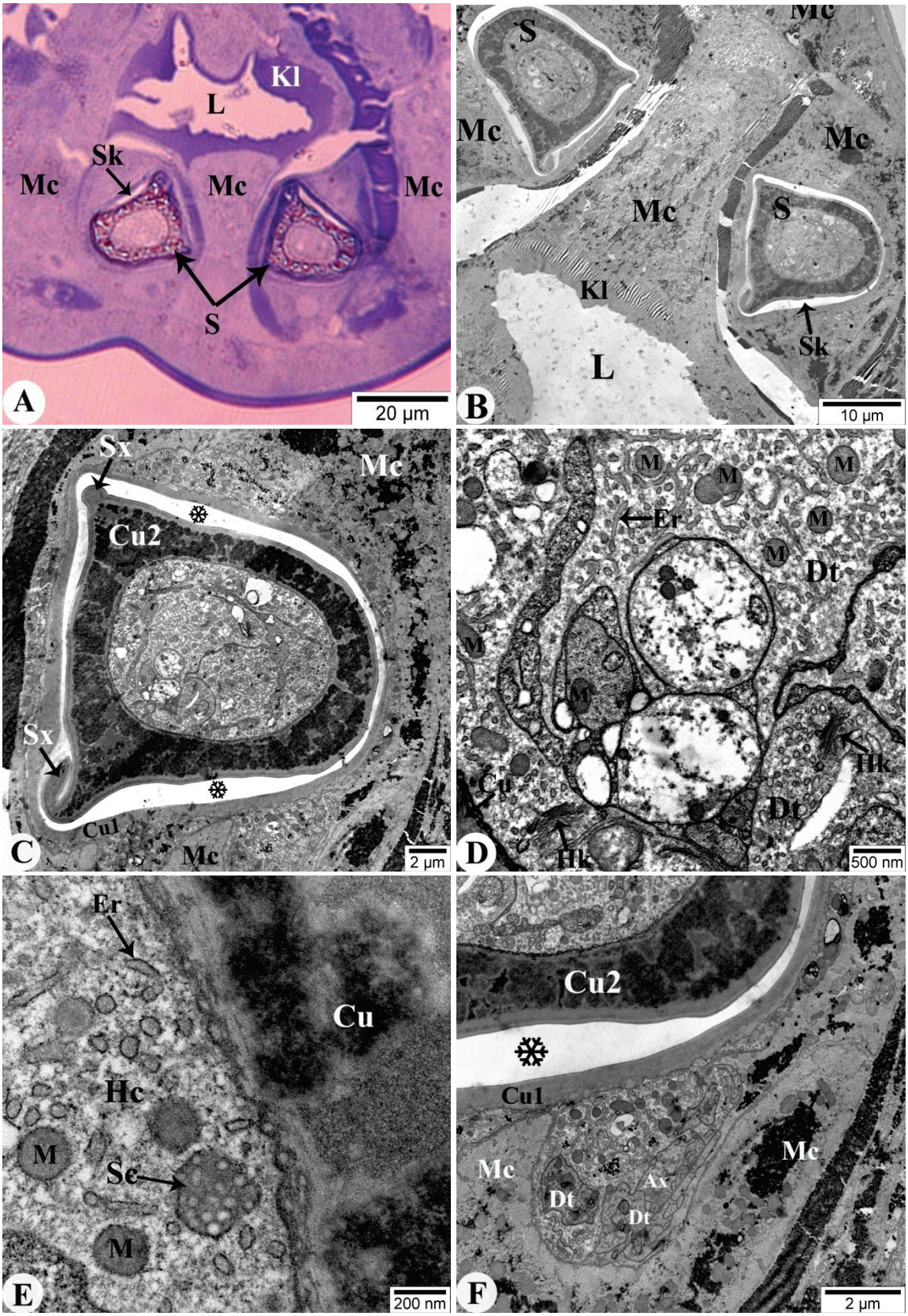

Fig. 3. Structural characteristics of spicules of nematode *H. dispar*: *a* – general appearance of spicules under a light microscope, semi-thin section (1 μm), D'Amico double staining, *b* and *c* – electron microscopic image of spicules, *d* – ultrastructural features of nerve cells inside spicules, *e* – ultrastructural characteristics of hypodermal cells inside spicules, *f* – ultrastructural characteristics of nerve cells around the spicular sac; ultrathin sections (50–70 nm), staining: uranyl acetate and Pb citrate; Kl – cloaca, L – lumen of the cloaca, Mc – muscle cells, Sk – spicular sac, S – spicula, Sx – processes of the spicula, Cu2 – cuticle of the spicula, Cu1 – cuticle of the spicule sac, M – mitochondria, Hk – Golgi complexes, Er – granular endoplasmic reticulum, Sc – cytoplasmic bodies, Dt – dendrites, Ax – axon, Hc – hypodermal cells

The above-mentioned researchers came to the conclusion that those spicules are sensory organs. The ultrastructure of the spicules of the nematode *H. dispar* showed that the structure of the spicules of other studied species is basically the same. The presence of two processes of the spicule

of the parasite on the sides was noted by us for the first time in histological studies (Rzayev, 2010). In the present study, the ultrastructural characteristics of those processes are described in detail. Neither *H. gallinarum* nor *H. spumosa* helminths have been reported to have these processes on the spicules. In addition to the above, during the study of the ultrastructure of the hypodermal layer that forms the skin-muscle sac of the *H. dispar* nematode and is located under the cuticle, cytoplasmic bodies found there (in the hypodermis and in the cytoplasm of the lateral ridges) were also identified in the cytoplasm of the hypodermal cells inside the spicule. Although both structures are different in location, they are the same in origin (hypodermis), so it is not surprising that the structural elements (organelles) in their cytoplasm are similar.

Conclusion

Thus, during the ultrastructural study adult male nematodes *H. dispar*, it was found that the tubular reproductive organs located in the pseudocoelomic cavity, consist of four parts. The walls of the testis, the seminal vesicle and the vas deferens consist of a basement membrane and an epithelial layer. The wall of the ejaculatory duct is composed of basal membrane from the outside, glandular in the front part, muscular layers in the back part, and epithelial cells in the lumen. The spicules, which are the secondary sexual organs and of the same length, consist of a thick cuticle on the outside, and hypodermal cells and processes of nerve cells on the inside. The ultrastructural characteristics of the germ-cells of male helminths at different stages of development (spermatogenesis) were studied. It was determined that, in the adult male nematode *H. dispar*, germ-cells turn into spermatogonia in the germinal zone and spermatocytes in the growth zone of the testis, spermatids in the seminal vesicle, incompletely formed spermatozoa in the vas deferens, and fully formed spermatozoa in the ejaculatory duct.

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