Egypt. J. Vet. Sci. **Vol. 55,** No.1, pp. 83-99 (2024)

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

The Effect of ZnO Nanoparticles to *Paradilepis Scolicina* Rudolphi, 1819 (Cyclophyllidea: Dilepididae) Cestode Observed First in Common Carp *Cyprinus Carpio* L., 1758) in Azerbaijan

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THE MAIN goal of this research was to determine the ultrastructural characteristics of *Paradilepis scolecina* (Rudolphi, 1819) metacestode, which was first recorded in the territory of Azerbaijan in common carp (*Cyprinus carpio* L., 1758), and the pathological changes occurring in the ultrastructure of the parasite due to the effect of ZnO nanoparticles on the helminth. For this, light, electron microscopic methods and statistical analyses were used. Nanoparticles (ZnO) at 10 mg were used *in vivo* against parasites localized in the body cavity of common carp. The helminths collected from both control and experimental groups were studied by preparing araldite-epon blocks and taking semi- and ultrathin sections. Parasite and its capsule ultrastructure of were determined and main taxonomic features compared with the data of other researchers. It was found that due to the effect of nanoparticles (ZnO), numerous destructive changes occur in the fat tissue of intestinal mesentery where the parasite is localized, in all three layers (serous, fibrillar and hyalin layers) of the capsule of helminth, and in different layers of the parasite's body wall (tegument). It was determined that at higher magnifications (>100 000) on Transmission Electron Microscope (TEM) under experimental conditions ZnO nanoparticles used are bioaccumulated in the wall of the capsule and in the body of the parasite and their size is 10-15 nm. The zinc oxide nanoparticles used have antiparasitic properties and in the future it can be used for different fish helminthiasis.

Keywords: Common carp, *Paradilepis scolecina*, ZnO nanoparticles, pathology, TEM.

Introduction

Common carp (*Cyprinus carpio* L., 1758) is a freshwater fish belonging to the order of cyprinids with a wide species composition and is easy to breed in commercial conditions. The mentioned fish is cultivated artificially in many countries of the world, including under the conditions of Azerbaijan. In addition to satisfying human demand for fish meat, the common carp perspective is an ideal model for studying animal ecology, developmental biology, and evolution [1]. Cyprinid fishes include more than 2900 species [2]. However, it is common carp that has a share of 14% among commercial fish grown in aquaculture conditions worldwide [3, 4]. Nevertheless, one of the factors directly affecting the productivity and meat quality of fish is their diseases caused by parasites.

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(Received 24/07/2023, accepted 18/08/2023)
DOI: 10.21608/EJVS.2023.224849.1547

Different fish diseases can sometimes cause mass death of fingerlings. Only in the territory of Azerbaijan, 46 species of parasites have been recorded in common carp up to the present period [5, 6]. *Paradilepis scolecina* (Rudolphi, 1819) metacestodes, which cause serious pathological changes in the host's body, are found also in common carp along with other parasites, with a wide distribution area in different countries [7-10]. The parasite *P. scolecina* metacestode life cycle occurs with two intermediate and one final hosts. First intermediate hosts are crustaceans (*Eudiaptomus graciloides* Lilljeborg, 1888), the parasite develops up to the cercoscolex stage being localized in the body cavity of copepods while the second intermediate hosts are fishes, mainly cyprinids (Cypriniformes), these helminthes pass the plerocercoid stage by parasitizing in liver, gall bladder, body cavity, and intestinal mesentery of fishes [11]. Birds, belonging to the order of pelicans (Pelicaniformes) are considered the final hosts such us *Phalacrocorax carbo* L., 1758; *Phalacrocorax pygmaeus* Pallas, 1773; *Pelecanus crispus* Bruch, 1832; *P. onocrolatus* L., 1758 of helminths. Rarely also found in *Plegadis falcinellus* L., 1766 (Ciconiiformes) and *Mareca falcata* Georgi, 1775 (Anseriformes) [12]. Larval stage of the metacestode was recorded in different species of fishes (*Alburnus chalcoides* Guld., 1772; *A. hohenackeri* Kessler, 1877; *Abramis brama orientalis* Berg, 1949; *Chondrostoma cyri* Kessler, 1877; *Neogobius melanstomus affinis* Eichwald, 1831; *Proterorhinus marmoratus* Pallas, 1814, *Benthophilus macrocephalus* Pallas, 1788; *Rutilus rutilus caspicus* Yakovlev, 1870; *Ballerus sapa bergi* Pallas, 1814) found in the Caspian Sea, Kura River, Mingachevir and Varvara reservoirs in the territory of Azerbaijan [13-16]. Adult parasitic worms were also found in the great cormorant (*P. carbo*) and the pigmy cormorant (*P. pygmaeus*) in Lankaran and Great Caucasus Natural Regions of the country [17].

Nanoparticles are another factor affecting aquatic organisms, including fish. Thus, as a result of the large-scale increase in the production and application of nanomaterials in recent years, the risk of spreading their waste into the environment has increased. The spread of nanoparticles in water bodies and their impact on the ecosystems present there is great [18]. There are some studies on the effects of different types of nanoparticles on the common carp body [18-27]. It is clear from the data that the accumulation of nanoparticles in various organs (intestine, liver, blood-vascular system), their toxicity and some pathomorphological changes caused by them have been studied. It should also be noted that, along with the changes occurring in the host's body, the introduction of nanoparticles to the endoparasites parasitizing the internal organs of fish, their bioaccumulation, and the pathological changes that the nanoparticles can cause in the structural elements are great interest. However, the research conducted in this direction is a minority [28-31]. Currently, in our country carrying out large-scale research in the direction of synthesis, practical application of nanoparticles free and with other compounds, bioaccumulation nanoparticles in various components of ecosystem and study of natural nanoparticles (ferritin) in vertebrates [31-39].

Zinc oxide is one of the widely used nanoparticles of metal oxides. In recent years, studies have been conducted to study antibacterial, antiviral, antiprotozoan, and antihelmintic properties of ZnO nanoparticles [40-44]. It was determined that destructive changes in the parasite organism, mainly in the body wall, were caused by nanoparticles use against the helminths *Toxocara vitulorum* Goeze, 1782 (Nematoda: Toxocaridae) and *Gigantocotyle explanatum* Creplin, 1847 (Trematoda: Paramphistomidae) [43, 44]. The US Food and Drug Administration considers ZnO to be generally accepted and safe [45]. In addition to the above, the toxicity of ZnO nanoparticles has been studied in fish and bacteria as separate studies [19, 40, 46-50].

The main goal of the current research is to study the effect of ZnO nanoparticles on the metacestode *P. scolecina* localized in the body cavity of common carp (*C. carpio*) using light and electron microscopic methods and to study the pathomorphological changes occurring in the body of the helminth.

Material and Methods

Selection of research objects and development of parameters

The research was carried out during 2021-2023 at the Parasitology laboratory of the Institute of Zoology of the Ministry of Science and Education of the Republic of Azerbaijan and at the Mingachevir Scientific Experiment Base of the Institute. Fish length (L), mass (P), fullness factor (F), spawning productivity were determined according to the rules adopted in ichthyology [51, 52]. In order to investigate the possible bioaccumulation of ZnO nanoparticles

in the body of the cestode, which is a part of the ecosystem, at the ultrastructural level, common carp fingerlings (24 fishes one-year-old - 0+) were used as biomodels.

As a parasitological object, *P. scolecina* metacestode, which spreads widely in common carp grown in pools in the research area and causes serious damage to the host's body, was chosen. The larval stage of this tapeworm (cestode) is found in fishes.

Information about nanoparticles

ZnO (99,8%, <30 nm, Product #: 8417DX) nanoparticles purchased from Skyspring Nanomaterials Inc., Houston TX, USA, were used in the experiments.

Describing the experiment

In Mingachevir Scientific Experiment Base of the Institute of Zoology, the length of the fingerlings were measured, divided into 2 groups of 12 each, and placed in 2 circular pools (control and experimental) with the same volume (50 liters). The average length (L) of fingerlings placed in the pools was 6.8 cm, and the average weight (P) was 4.8 g. The volume of water (50 liters), hydrochemical indicators of water (t^0 – 22-24^{0}C , O_2 – 8,3-8,5 mg/l and pH=7.4-7.5) and the amount of daily food ration of fishes (10 g) of the swimming pools were checked during the experiment. Fishes belonging to the first group (control) were fed only with artificial feed, while in the feeding of fishes belonging to the second group (experiment), respectively, 10 mg of ZnO nanoparticles were added to the daily feed ration. The exposure of experiments lasted for 3 days [27]. Experiments were repeated 3 times under *in vivo* conditions.

Complete parasitological dissection of fish

It should be noted that before the experiments were carried out, the common carp in the pools in the study area were previously examined and it was determined that they were highly infected with the metacestode *P. scolecina*. After the end of the experiments, both control and experimental fish were examined by the method of complete parasitological dissection [53-55]. A part of the helminth larvae (inside the capsule) found in the intestinal mesentery of the dissected fishes was collected, fixed and stained. During the working process, the parasites were observed under a Primo Star (Zeiss, Germany) light microscope, and pictures of the necessary parts of helminthes were taken with an EOS D650 (Canon, Japan) digital camera. The parasite was identified with the help of "Key to parasites" [7, 56]. The developmental stage of the cestode found in the fish was also determined [57].

Light and electron microscopic studies

Helminths collected from fish in the control and experimental groups were included in the fixator to study their ultrastructure. More specifically, the samples were fixed in 0.1M phosphate buffer (pH=7.4) from 2.5% glutaraldehyde, 2% paraformaldehyde, 4% surcosa, and 0.1% picric acid. The fixed materials were submitted to the Electron Microscopy Department of the Scientific Research Center of Azerbaijan Medical University for studying by electron microscopic methods. 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 [58]. 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 fuchsin or toluoid blue, viewed under a Primo Star (Zeiss, Germany) microscope [59]. Images of necessary sections were taken with a digital camera EOS D650 (Canon, Japan). The 50-70 nm ultra-thin sections taken from the same blocks and stained with lead citrate, uranyl-acetate were studied under a JEM-1400 (JEOL, Japan) transmission electron microscope under a voltage of 80-120 kV and electrograms were taken.

Determination of nanoparticles.

In the histograms obtained during the study of electrograms taken from ultrathin sections prepared from unstained blocks using the "Intensity profile" computer program, the length of the structures drawn on the horizontal line (nm) and the gray value are given in the vertical direction. It should be noted that, as in other black-and-white photographs, the intensity of the image in electrograms depends on the degree of shade of gray color, and the weakest intensity fluctuates between black and the highest intensity. The digital camera (Veletta) used has the ability to carry 14 bits of information per pixel in the side camera, and since the intensity range of the recorded electrograms is 2^{14} = 16384, it has the ability to accurately distinguish the specified number of shades of gray from each other [37, 60]. These indicators make it possible to

accurately determine the location of the used nanoparticles in living organisms.

Statistical analysis

The statistical analysis of the image was performed using the computer program (The TEM imaging platform) developed by the German company "Olympus Soft Imaging Solutions Gmbh" on images taken in TIF and JPEG format. In this study, during the determination of the *P. scolecina*, the total length, the diameter of the suckers, the hooks, the scolex, as well as the measurements of the capsule and parenchyma (tegument) of the helminth during TEM studies were carried out, and parameters such as Min, Max, X and Mean±SD were calculated.

Ethical rules

All the experiments were performed after the approval from the Ministry of Health of Azerbaijan Republic, Ethics Committee of Azerbaijan Medical University, Azerbaijan (No: EP 0040).

Results

During the complete parasitological analysis of common carp caught from the pools of the Zoological Institute's Experimental Base, the plerocercoid stage of the metacestode *P. scolecina* was found in the body cavity (intestinal mesenteries) of fish. Intensity of infection of helminth larvae with fish in the study area was 1-24 individuals, and total infection rate was 42%. This parasite was recorded for the first time in common carp grown in aquaculture conditions in the territory of Azerbaijan (Fig. 1: A-F). The length of the oval-shaped capsule of the parasite was 0.808-1.086 mm (0.919±0.017 mm), the width was 0.536-0.716 mm (0.635±0.012 mm) (Fig.1: A-C). Individuals of the plerocercoid stage of the parasite were located inside the capsule, their length was 0.560-0.868 mm (0.645±0.017 mm), and their width was up to 0.384-0.459 mm (0.414±0.008 mm) (Fig. 1: B-C). In some preparations the location of the larva attached to the wall of the capsule has also been noted. In some cases, parasites are able to leave the capsule (Fig. 1: D). The pear-shaped scolex of the metacestode was located in the front part of the body and was determined to be 0.344-0.360 mm (0.352±0.002 mm) in size. Invaginate the scolex into the helminth is observed. The scolex is equipped with 4 oval-shaped suckers (0.091-0.116 mm (0.106±0.002 mm)) (Fig. 1: B-C). There are 20 hooks (10 large and 10 small) at the tip of the rostellum. The length of large hooks is 0.112-0.120 mm (0.116±0.001 mm) (blade - 0.058-0.060 mm (0.059±0.0003 mm), handle - 0.050-0.055 mm (0.053±0.0005 mm)), and the length of small hooks is 0.077-0.081 mm (0.079±0.0003 mm) (blade - 0.038-0.041 mm (0.039±0.0003 mm), handle - 0.036-0.039 mm (0.037±0.0003 mm)) (Fig. 1: E-F).

In our experiment, ZnO nanoparticles were applied *in vivo* against *P. scolecina* metacestode, which is localized in the capsule form in the intestinal mesentery in the body cavity of common carp. In addition to the above, tapeworm larvae collected from fish in the control group were studied by light and electron microscopic methods, and the ultrastructural features of the capsule and the parasite inside it were described. Figures 2A and 2B show semi-thin sections (1 µm) of *P. scolecina* metacestodes. Here, the capsule (C) surrounded by fat tissue (Ft) and the parasite (P) are clearly visible. Figure 2A shows a general view of the capsule (C), and Figure 2B shows all three layers (marked with numbers 1, 2, 3 in Fig. 2:B). From Figures 2A and 2B, it can be seen that tegument of the parasite located inside the capsule are very close, sometimes confluent with capsule wall, while other parts are quite far apart. It was found that fluid (marked with snowflakes) accumulates in large areas between the parasite and the last inner layer of the capsule (Fig. 2: A-B).

In order to determine the number of layers of the parasite's capsule and its structural elements, the electron microscope method was also used. Figure 2C presents a general view of the capsule of the metacestode of *P. scolecina*. It is clear from this that the capsule is composed of 3 different layers with different structures and the total thickness is 17.87-24.67 µm (21.92±0.46 µm). The first layer surrounding the capsule (marked 1 in Fig. 2: C) is called the serous layer of host origin and its thickness is 3.16-7.34 µm (4.97±0.32 µm). The second one is of parasitic origin and is called the fibrillar layer (marked with 2 in Fig. 2: C) and its total thickness was 11.93-19.58 µm (14.99±0.52 µm). The inner third layer is called the hyaline layer (marked with 3 in Fig. 2: C) and its thick is 0.98-1.79 µm (1.32±0.06 µm). Among all three layers, the hyaline is the thinnest in thickness. In addition to the above, Figure 2C also shows fluid (marked with a snowflake) in the space between the hyaline layer and the parasite's tegument (T). Figures 2D and 2E show

the ultrastructural features of the serous layer. Thus, it was known that the serous is composed of 5-6 layers of cells. They are connected to each other by desmosomes (marked as D in Fig. 2: E). In the center of the cells, there is a large elongated nucleus (marked with N in Fig. 2: D), mitochondria in the cytoplasm (marked with M in Fig. 2: E), dimensions - length 0.2-0.67 µm (0.41±0.03 µm), width 0.14-0.31 µm (0.23±0.01 µm) and abundant vesicles (marked V in Fig. 2: E), in diameter 0.05–0.10 µm (0.07±0.003 µm) are observed. The fibrillar layer, in turn, is composed of numerous small fibrils and other connective tissue elements (labeled 2 in Fig. 2: D). The hyaline layer is structureless (labeled 3 in Fig. 2: D). The hyaline layer of the parasite's covering tissue is dense on the sides and distant on the back, where fluid is observed (marked with a snowflake in Fig. 2: F).

Along with the capsule of the parasite, the ultrastructure of the larval stage of the helminth was also studied by electron microscopic method (Fig. 3). Figure 3A shows a general view of the parasite (P) inside the capsule. Here the covering tissue of the metacestod is clearly visible. Figure 3B shows a general view of the tegument (T). At higher magnifications, the tegument is revealed to be covered externally with tusk-shaped microtriches (Tm) (Fig. 3: C). Below the microtrichia is a layer of distal cytoplasm (Dc). A large number of disc-shaped bodies (Db) of various sizes are observed inside the distal cytoplasm (Fig. 3: D). The distal cytoplasm is terminated by the basal lamina (Bl) and is 0.1–0.37 µm (0.20±0.02 µm) thick (Figures 3:C and 3:E). Electrongrams obtained at higher magnifications (80 000) of the electron microscope clearly show that the basal lamina (Bl) is composed of two layers, consisting of dark (in the part near the distal cytoplasm) and light (in the part near the muscle cells) parts (Fig. 3: E). Several layers of muscle cells (Mc) are located below the basal lamina (Fig. 3: B-C, and 3: E). Muscle cells are rich in fibrils and active mitochondria (M) are observed in their cytoplasm (Fig. 3: E). Muscles close to the basal layer are circular, and longitudinal muscle cells are located below it. Under the muscular layer, tegumental cyton cells (Tc) are found (Fig. 3: F). Thus, it was determined by the electron microscopic method that the tegument of the *P. scolecina* metacestode located inside the capsule was composed of microtrichia, distal cytoplasm, basal lamina, muscular layers and tegumental cyton cells from the outside to the inside. After ZnO nanoparticles were fed to fish for 3 consecutive days, the fish were dissected and the larvae of *P. scolecina* metacestodes parasitized in the intestine mesentery were collected and examined by both light and electron microscopic methods (Fig. 4). From the semi-thin (1µm) section images in Figures 4A and 4B, it is clear that ZnO nanoparticles induced significant pathomorphological changes in both the capsule and the parasite (P). Thus, the fat tissue where the parasite is localized has been destroyed and dispersed. In all three layers of the capsule (serous, fibrillar, hyaline), the formation of edema fluid and the formation of spaces between the layers (marked with an asterisk in Fig. 4: A) were observed. The parasite is noted to compressed within the capsule and move towards the center (Fig. 4: A). The body wall (tegument) of the metacestode itself is observed to be thickened and disintegrated in many places (Fig. 4: B). Pathological changes in the fat tissue, the capsule, and the body of the parasite were detected by the electron microscopic method (Fig. 4: C-F). Figure 4C shows the adipose tissue around the parasite capsule. Here, the wall of the fat cells is damaged and the organelles in the cytoplasm are scattered outside. The structure of almost all cytoplasmic elements is destroyed. The membrane of the nuclei is damaged, the chromatin is unevenly distributed (Fig. 4: C). In addition to the above, damage to the capsule wall was also investigated by electron microscopy (Fig. 4: D-E). Figure 4D shows the ultrastructural features of the serous layer. While in the control group, the serosa consisted of 5-6 layers of cells, most of them were destroyed due to the effect of nanoparticles, some of them were thickened due to the effect of edema fluid, and others were sharply thinned. In the serous layer, the nuclei of the cells and the organelles in the cytoplasm were damaged (Fig. 4: D). In addition, it is observed that the walls of the membrane structures in the cytoplasm of the cells are destroyed and the structural elements inside are absent. The distance between the bundles of fibrils in the capsule's fibrillar layer (marked with 2) has increased and the layer itself has thickened. Changes are also observed in the structure of the fibrils themselves (Fig. 4: E). Less pathological changes were recorded in the hyaline layer with acellular structure (marked with 3 in Fig. 4: E) compared to others. After the application of ZnO nanoparticles *in vivo*

to fish, serious pathomorphological changes were observed in the body wall (tegument) and internal organism of the parasites. Vacuolation in the cytoplasm of cells in different layers of the tegument, damage to membrane structures and turning them into myelin-like bodies, formation of edema fluid between cells, etc. changes were noted (Fig. 4: F).

More than 100,000 magnifications of the transmission electron microscope were used to obtain visual images of the presence of ZnO nanoparticles as the cause of the pathological changes occurring in the body of *P. scolecina* metacestode (Fig. 5 and 6). As a result, nanoparticles were identified inside the fat tissue around the capsule of the parasite (Fig. 5: A), in the serosa covering the capsule from the outside (Fig. 5: C), in the completely damaged fibrillar layer (Fig. 5: E) and in the last hyaline layer (Fig. 5: G). Along with the images of the nanoparticles, their histograms for each are also given (Fig. 5: B, D, F, H). Here, it was determined that the size of ZnO nanoparticles in all layers is 10-15 nm, with the degree of gray value varying between 4900-5300. In the same direction, studies were conducted on the parasite located inside the capsule. Thus, images of nanoparticles were obtained in the microtriches surrounding the tegument from the outside (Fig. 6: A) and inside the parasite (Fig. 6: C). Their histograms were also produced separately (Fig. 6: B, D). It was determined that the size of the ZnO nanoparticles in the parasite's tegument and internal body is 10-12 nm, varying between the degree of gray value 5300-5400. Thus, the size of the ZnO nanoparticles observed in *P. scolecina* metacestodes was 10-15 nm, and the degree of gray value varied from 4900-5400.

Discussion

The tapeworm *P. scolecina* is a widespread metacestode and spends one stage of its development (larval stage - plerocercoid) in fish, mainly in cyprinid. As a result of the analysis of literature data, it was found that *P. scolecina* parasite is similar to other two species (*Valipora campylancristrota* Wedl, 1855 and *Neogryporhynchus cheilancristrotus* Wedl, 1855) of tapeworms in a number of characteristics [61]. Later, as a result of a more detailed study of the species, the localization of the parasites in hosts, the formation of the capsule, the size of the hooks, etc. it was determined that *P. scolecina* metacestod differs according to taxonomic characteristics [62, 63, 64, 65, 66]. The taxonomic parameters that will define the species were statistically calculated as the mentioned parasite was recorded for the first time in the common carp in the territory of Azerbaijan water bodies. Those results are compared with the data of other researchers who describe in detail the larval stage of the metacestode *P. scolecina* and are placed in Table 1.

As can be seen from Table 1, a number of taxonomic characters of *P. scolecina* metacestode (on 10 characters) and the indicators of their statistical results were generally similar in different researchers. Only in Chukalova's (2008) studies, the size of the *P. scolecina* metacestode was smaller than others for one characteristic (capsule width and length) [67]. All other measurements (other parameters – measurments of the larva, diameter of suckers, thickness and length of rostellum, different sizes of large and small hooks) are similar. Comparing the literature data and the results obtained from our own research, as our measurements are basically similar to the statistical figures shown by previous researchers, it gave a strong reason to say that the parasite noted in common carp grown in aquaculture conditions in Azerbaijan is a metacestode of *P. scolecina*. One of the main taxonomic features in the determination of species is the number of hooks and their sizes, which were completely similar in all researchers and in our results.

Data on the normal histological structure of the larval form (plerocercoid) of the metacestode *P. scolecina*, which has a wide distribution area (Europe, Asia, Africa and America), parasitizes fish were scarce [63, 66, 68]. In those sources, the larvae of the helminth *P. scolecina*, which are parasitic in the internal organs of fish, including the liver, are described inside the capsule. As a result of the histological examination of the capsule, the authors found that it consists of 3 layers [63]. As a result of the study of the *P. scolecina* metacestode localized in the intestinal mesentery in the body cavity, as a result of the light and electron microscopic method, the capsule consists of the same number of layers and their structural elements are described in detail (Fig. 2). We have not found any literature on the previous study of *P. scolecina* metacestode, including its capsule, at the ultrastructural level. But the capsule of another species of same order Cyclophyllidea (*Mesocestoides* sp.) was studied by electron microscopic method and it was shown that it consists of those layers

(except for some morphological features) [69]. Therefore, the structural characteristics of the capsule of the *P. scolecina* parasite are identical to the morphological characteristics of other species belonging to the order Cyclophyllidea, to which the species belongs. The ultrastructure of *P. scolecina* metacestod was studied for the first time. Therefore, the ultrastructural features of the parasite's tegument were compared with the structures of other species included in the order (Cyclophyllidea). It should also be noted that ultrastructure of some tapeworms of other species (*Taenia crassiceps* Zeder, 1800, *Microsomacanthus microskrjabini* Spassky et Jurpalova, 1964, *Hymenolepis stylosa* Rudolphi, 1809, *Branchiopodataenia pacifica* Spassky & Jurpalova, 1968) included in the order were studied [70-73]. Summarizing these data, it is noted that the tegument of the studied tapeworms consists of microtriches of different sizes and shapes, distal cytoplasm, basal lamina and muscular layers from the outside to the inside. During the electron microscopic study of *P. scolecina* metocestodes, the above-mentioned structures were identified and described in the tegument (Fig. 3). The structural elements in the layers of tegument differed from other studied species according to their size and some morphological characteristics. Currently, nanoparticles are widely used in various fields of science, including biology and medicine, and positive results are obtained [74]. Many properties of nanoparticles allow them to be used as a method of combating some pathogens. The analysis of literature data has shown that nanoparticles of metal oxides show antiparasitic properties and destroy parasites by creating pathomorphological changes in their body. Thus, different metal nanoparticles were used against tapeworm larvae and it was found that the tegument and scolex of the parasites were completely damaged [75-78]. On the other hand, nanoparticles applied against monogenetic worms damaged their body wall and caused the death of the parasite in a short time [29]. As a result of the effect of nanoparticles used against the *P. scolecina* metacestod, the pathological changes in the fat tissue where the parasite is localized, the layers of the capsule and the body of the parasite itself were observed and explained in detail (Fig. 4). So, it is proven once again that various metal nanoparticles have anthelmintic properties.

It should also be noted that during the use of ZnO nanoparticles against various species of parasites, we did not find any information on the bioaccumulation of nanoparticles in their body. In the current study, the bioaccumulation of ZnO nanoparticles in all three layers of the parasite's capsule and the helminth's tegument was determined with the help of electron microscopic methods and "intensity profile" software (Fig. 5 and 6). As a result of the analysis of literature data, it was found that metal oxide nanoparticles each have their own degree of gray value during bioaccumulation in living organisms. It is known that SiO - 5500-5600, Fe/Ni biometal - 5000, AlNPs - 5000-6400, Fe3O4 nanoparticles show a gray value between 5200-5600. In addition to those mentioned, the diameter of the nanoparticles collected in the living organism was up to 20 nm [35, 38, 39]. The size of ZnO nanoparticles in the destroyed capsule and tegument of the *P. scolecina* metacestode parasitizing fishes did not exceed 10-15 nm. The degree of gray value of nanoparticles was determined to be between 4900-5400. The obtained data show that ZnO nanoparticles in such small sizes can cause pathological changes in the parasite's body by bioaccumulating it.

Conclusion

The parasite *P. scolecina* cestode larva (plerocercoid) was identified for the first time in common carp grown under aquaculture conditions in Azerbaijan. Therefore, the taxonomic characteristics of that species were described and compared with the data of other authors. This helminth causes serious damage to the host organism, affecting its fish production and flesh quality, which in turn affects commercial fish farms. Nanoparticles caused serious pathomorphological changes in the capsule and in the body of the parasite. It was determined that nanoparticles with a size of 10-15 nm are bioaccumulated in all three layers of the capsule and in the tegument of the parasite. The ZnO nanoparticles used have antiparasitic properties and in the future it can be used (freely or in combination with other anthelmintic compounds) for fish helminthiasis.

Funds statement:

This work was self-financial supported by the author.

Conflict of interest:

The author claims that there are no competing interests.

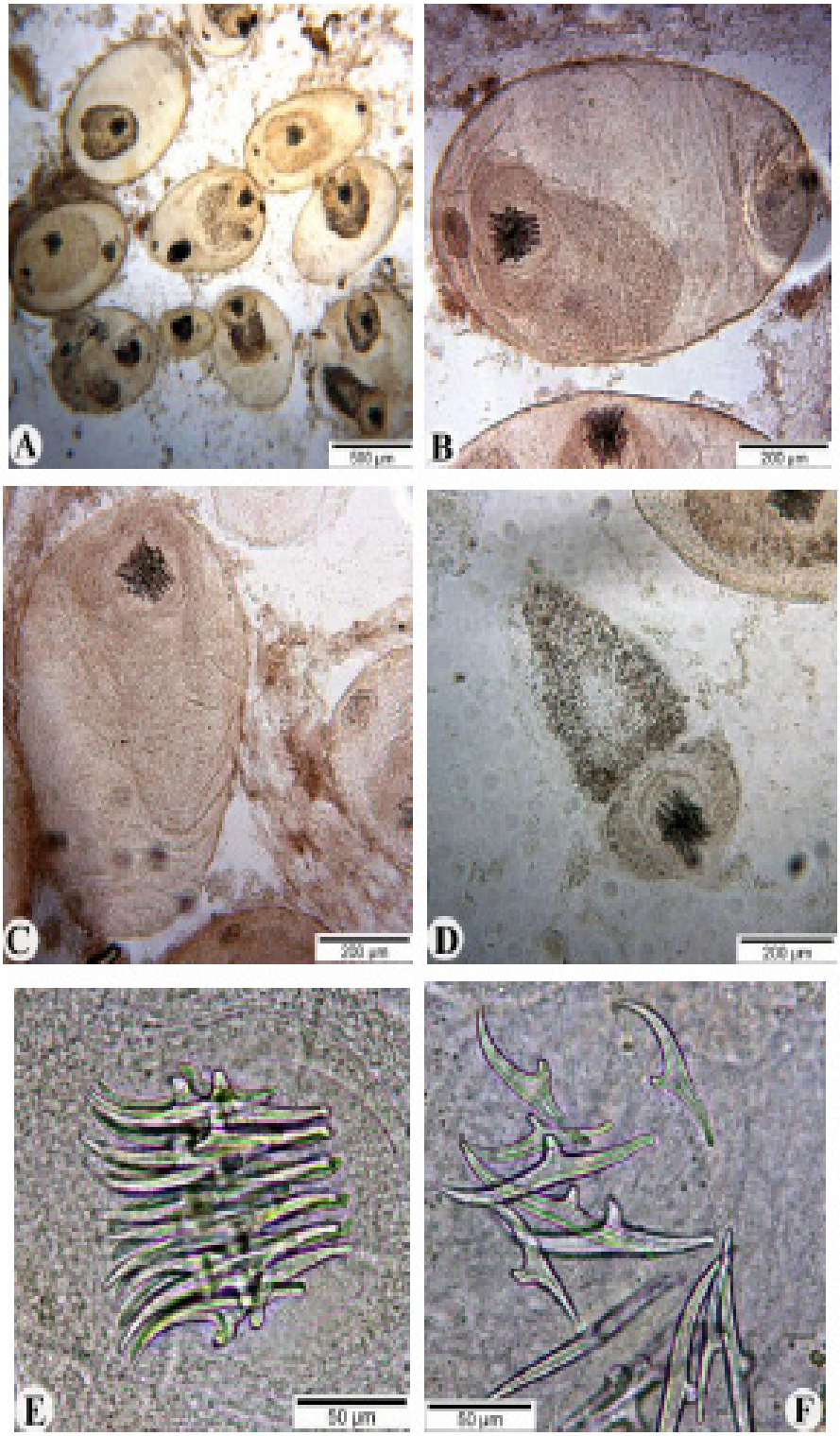

Fig. 1. Larval stage of the metacestode P. scolecina found in the intestinal mesentery of common carp. A- General view of the parasite, B, C – individuals inside the capsule, D – free individual leaving the capsule, E – all hooks at the tip of the scolex (20 pieces), F – large and small hooks (separately).

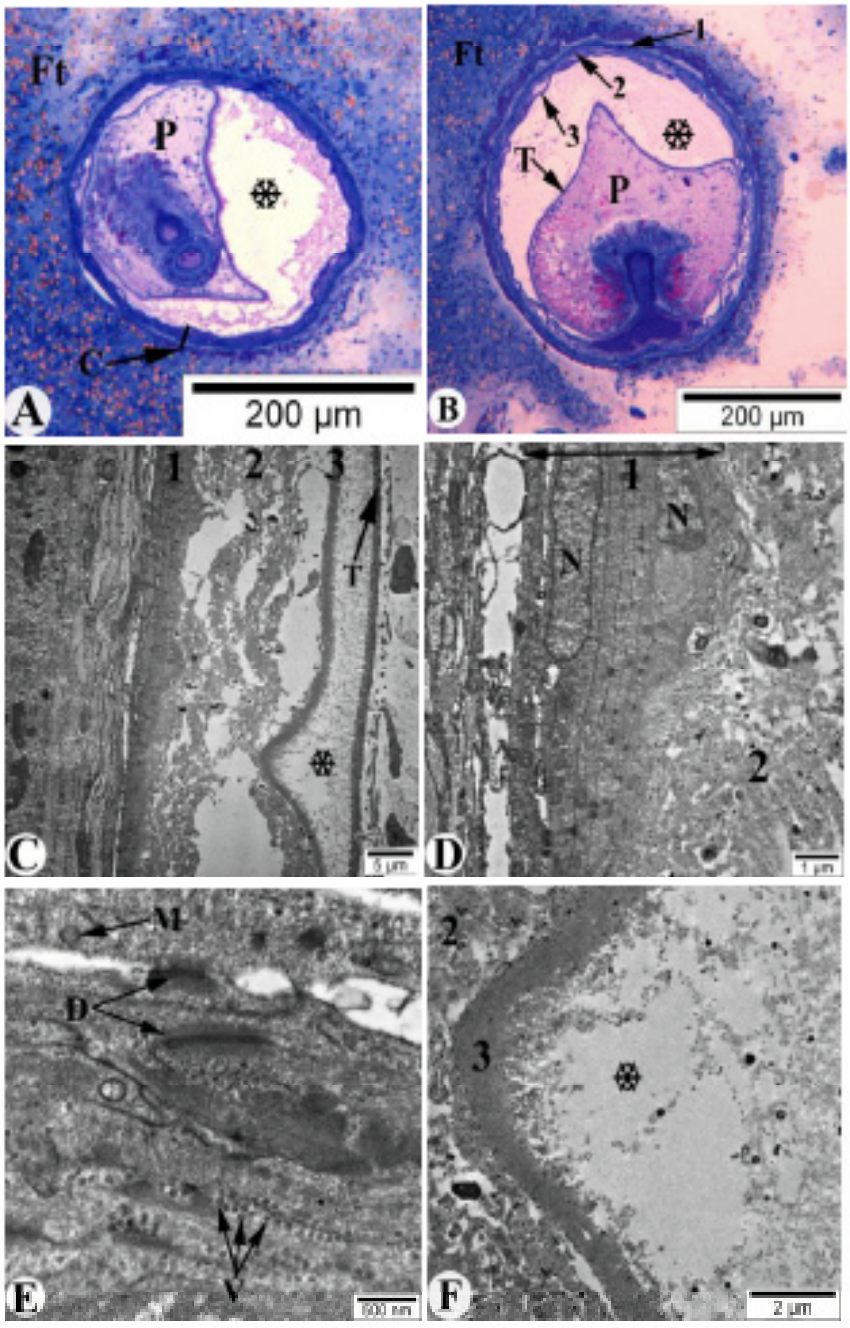

Fig. 2. Light and electron microscopic structural features of the capsule of metacestode *P. scolecina* in norm. A-B – light microscopic photograph of a semi-thin cross section (1 µm), two-layer staining by D'Amico method; C–F – electron microscopic photograph of an ultra-thin section (50–70 nm) stained by uranyl acetate and lead citrate. Designations: C – capsule, P – parasite, N – nucleus, Ft – fat droplets, T – tegument, M – mitochondria, D – desmosome,V – vesicles, 1 – serous layer, 2 – fibrillar layer, 3 – hyalin layer, Snowflake - fluid (the explanation is given in the text).

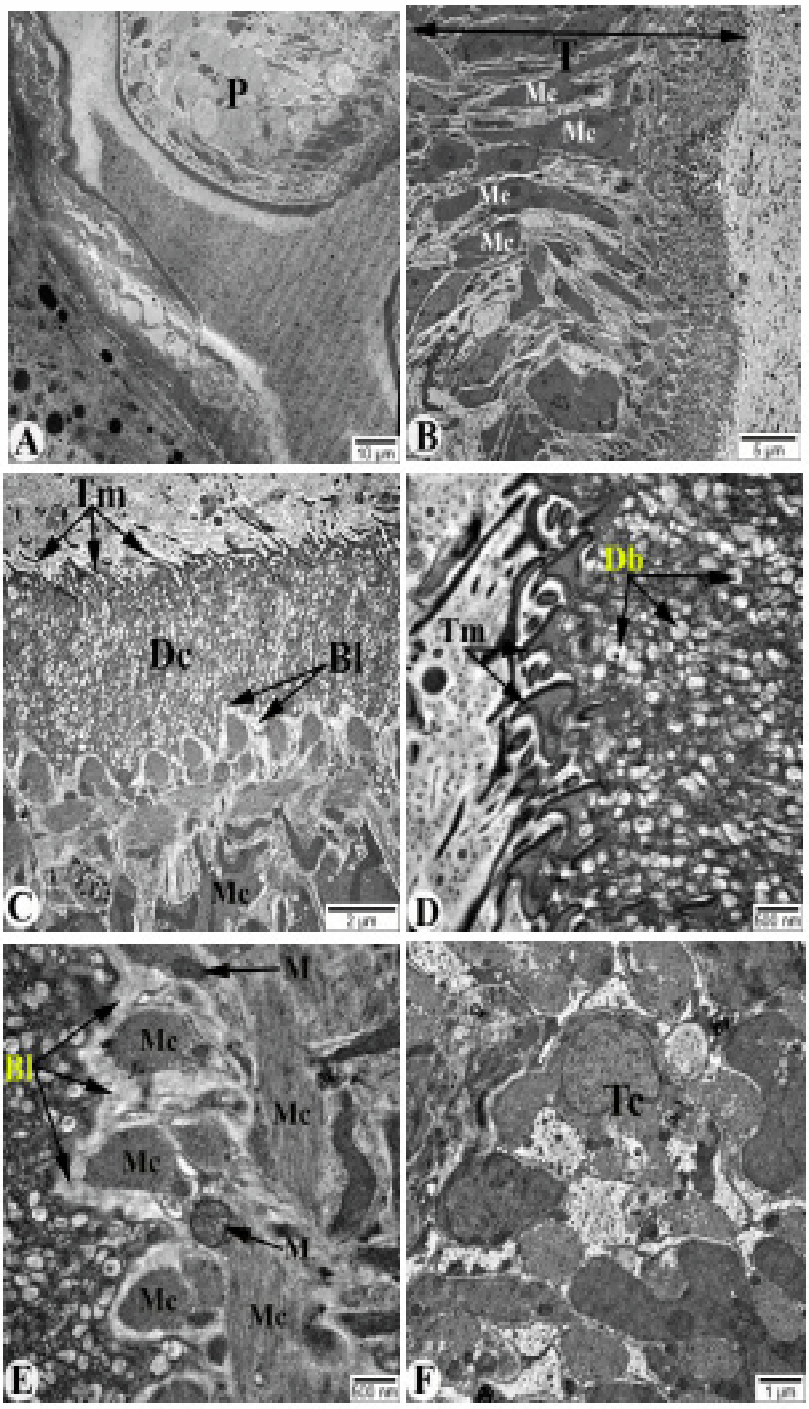

Fig. 3. Light and electron microscopic structural features of the larvae of metacestode *P. scolecina* in norm. A–F – electron microscopic photograph of an ultra-thin section (50–70 nm) stained by uranyl acetate and lead citrate. Designations: P – parasite, T – tegument, Mc - muscle cell, Tm – tusk-shaped microtriches, Dc – distal cytoplasm, Bl – basal lamina, Db – disc-shaped bodies, M – mitochondria, Tc – tegumental cytons (the explanation is given in the text).

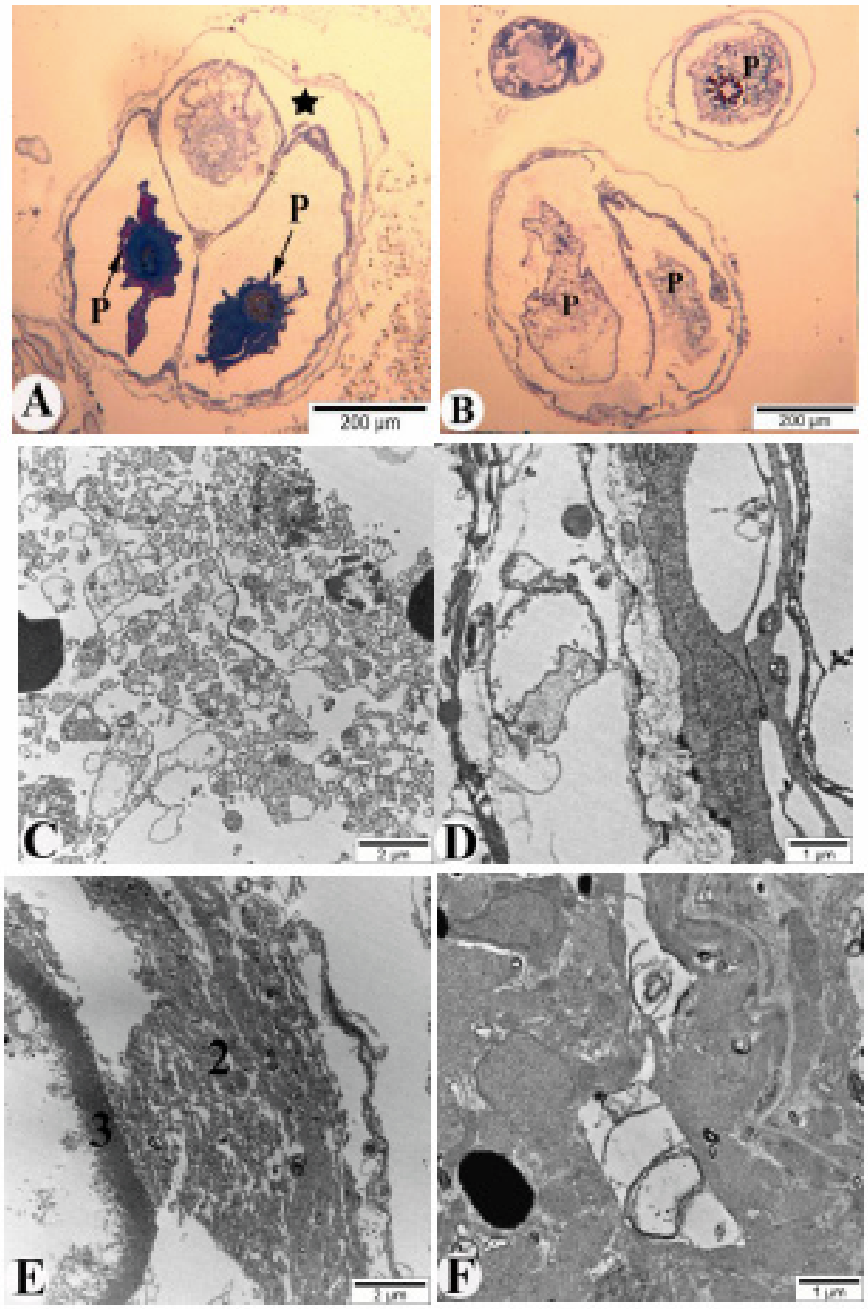

Fig. 4. Pathomorphological changes of *P. scolecina* larvae and its capsule wall due to the effect of ZnO nanoparticles. A-B – light microscopic photograph of a semi-thin cross section (1 µm), two-layer staining by D'Amico method; C–F – electron microscopic photograph of an unstained ultra-thin cross section (50–70 nm). Designations: P – parasite, 2 – fibrillar layer, 3 – hyalin layer, star – edema (the explanation is given in the text).

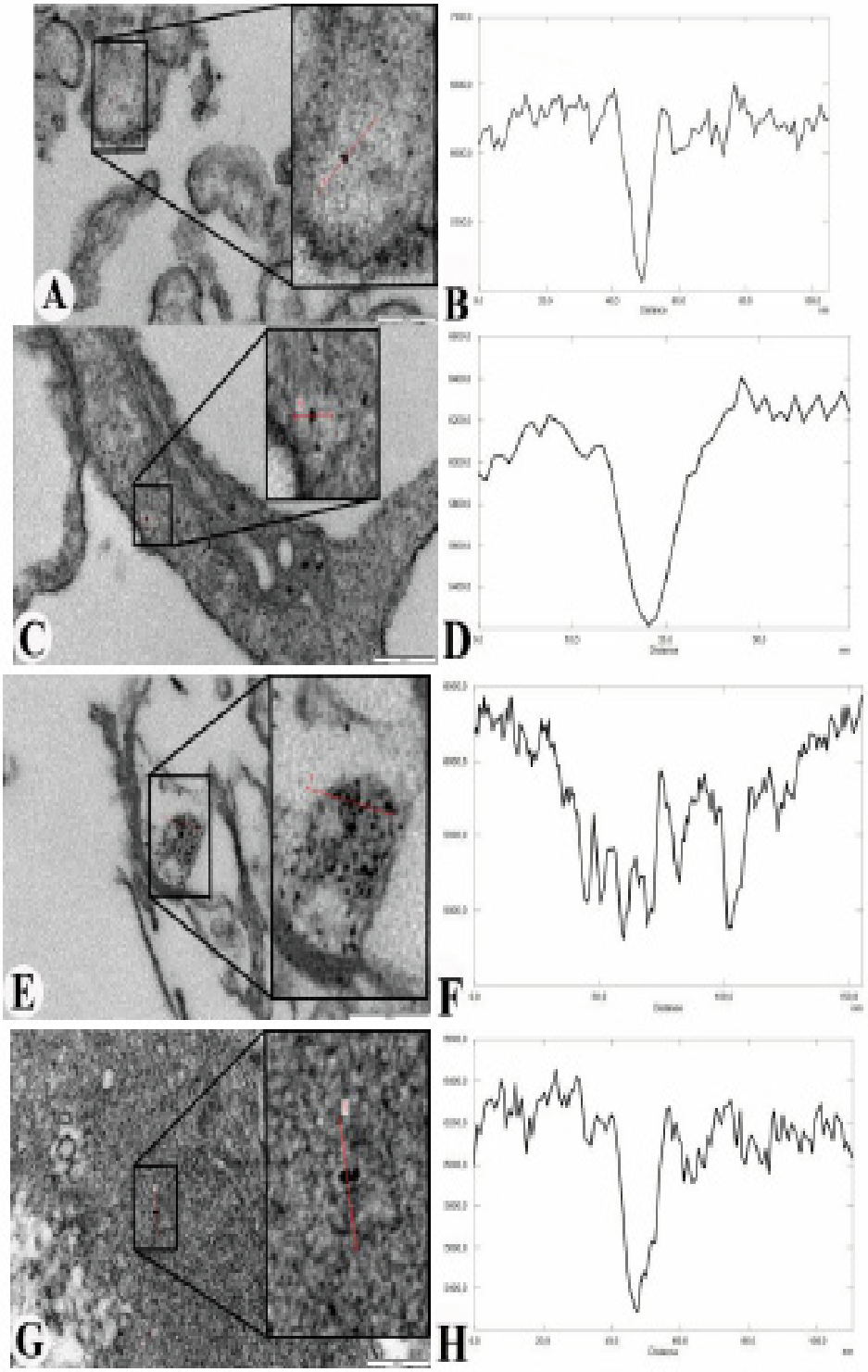

Fig. 5. Bioaccumulation of ZnO nanoparticles in parasite capsule (A, C, E, G) and corresponding histograms (B, D, F, H). A, C, E, G – electron microscopic photograph of an unstained ultra-thin cross section (50–70 nm) (the explanation is given in the text).

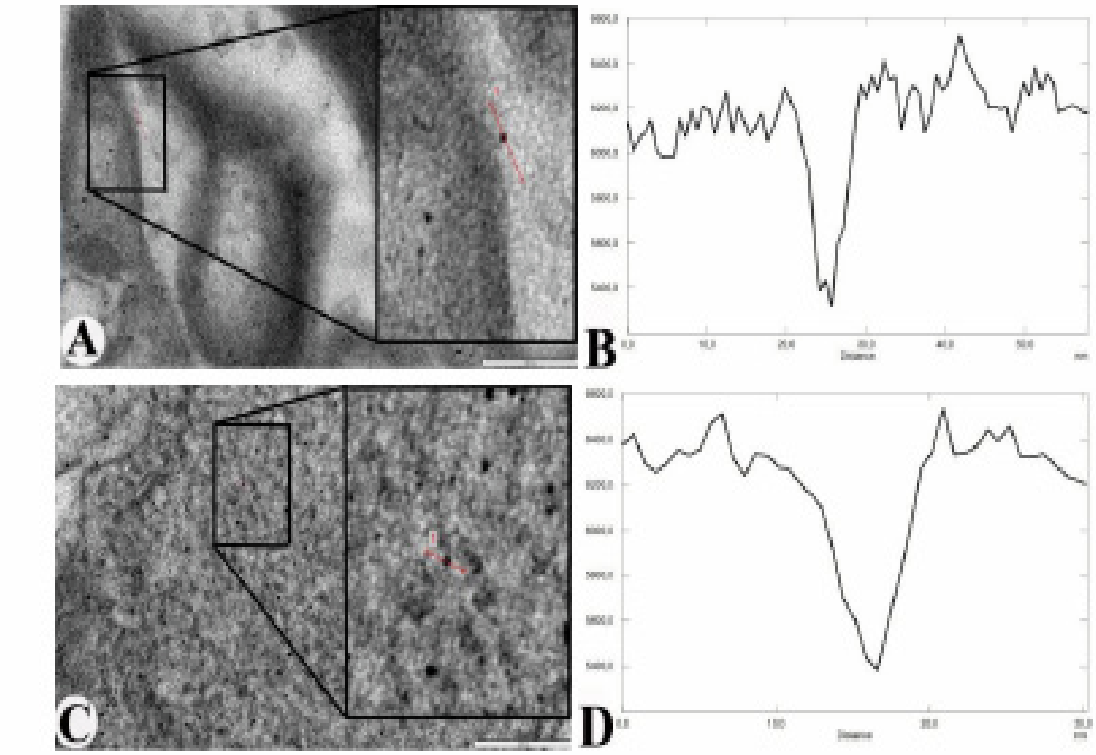

Fig. 6. Bioaccumulation of ZnO nanoparticles in parasite (A, C) and corresponding histograms (B, D). A, C – electron microscopic photograph of an unstained ultra-thin cross section (50–70 nm) (the explanation is given in the text).

TABLE 1. Taxonomic characters and its measurements (mm) of *P. scolecina* metacestodes recorded by various researchers (including our own data)

Taxonomic characters and measurements		Bauer, 1987	Scholz, 1989	Chukalova, 2008	Williams et al., 2012	Scholz et al., 2018	Own data
Capsula		0.56-1.31 0.30-0.76	0.9-1.21 0.63-0.72	0.425-0.510 0.340-0.442	-	-	0.808-1.086 0.536-0.716
Parasite (larvae)		0.30-0.75 0.17-0.56	0.60-0.86 0.39-0.62	0.374-0.748 0.17-0.442	-	0.63-0.83 0.36-0.48	0.56-0.868 0.384-0.459
Suckers		0.08-0.11	0.11-0.124 0.092-0.10	0.08-0.09	-	0.80-0.108 0.64-0.94	0.091-0.116
Rostellum		-	0.18-0.21 0.19-0.23	-	-	0.142-0.17 0.13-0.158	-
Large hooks	Lenght	0.09-0.12	0.106-0.115	0.102-0.125	0.103-0.107	0.106-0.115	0.112-0.120
	Blade	-	0.055-0.060	-	0.056-0.060	0.056-0.064	0.058-0.060
	Handle	-	0.050-0.055	-	0.048-0.052	0.049-0.055	0.050-0.055
Small hooks	Lenght	0.06-0.08	0.075-0.080	0.059-0.082	0.072-0.077	0.074-0.080	0.077-0.081
	Blade	-	0.038-0.041	-	0.039-0.042	0.039-0.045	0.038-0.041
	Handle	-	0.036-0.039	-	0.034-0.037	0.036-0.040	0.036-0.039

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