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CHARACTERISTIC DEVELOPMENTAL STAGES OF THELOHANELLUS NIKOLSKII (1979 – 1996)

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During thelohanellosis, a disease of carp fingerlings caused by Thelohanellus nikolskii, it is possible to differentiate six stages which may be described clinically and histologically using light and electron microscopy. Characteristic developmental stages were manifested during the first years of appearance of this most widely spread parasitic fish disease, while in the following years, the appearance and stages of the disease could not be resolved as regularily. This paper makes a comparison between the clinical manifestations and the results obtained using the classic histological methods (light microscopy and transparent electron microscopy). Since the mass appearance of the disease, up to date, its developmental cycle has changed considerably.

Key words: thelohanellosis, developmental stages, carp fingerlings, cysts, spores.

INTRODUCTION

In Yugoslav fishponds, thelohanellosis, caused by the myxosporidium Thelohanellus nikolskii (Ahmerov 1995), was detected for the first time in 1979 (Ćirković et al. 1983). The disease spread rapidly and reached its culmination in 1986, when it was present in almost all observed fishponds of the South Pannonian region. The intensity of the disease was similar to the description given by the Hungarian authors Jeney (1979) and Molnar (1982), and considerably greater than the case described by the Japanese authors Hosina and Hosoda (1957). The appearance of the disease was also noted in the former Yugoslav republic of Macedonia (Hristovski, unpublished). Thelohanellus was considered as a serious problem in North Korea and Argentina as well. In recent years this disease has almost disappeared in Yugoslavia (Ćirković et al., 1996), and its appearance, development and course have become atypical. Observing the disease from 1979-1996, we arrived at the conclusion that its appearance, spread and disappearance have the shape of a sinoid function. This paper describes the

typical developmental stages of the disease in the period of its most widely spread manifestation.

MATERIAL AND METHODS

Samples of carp fingerlings, used for observation of the T. nikolskii developmental cycle, were taken from fishponds in the South Pannonian region.

Sampling was performed every day from a small number of fishponds, while from others samples were taken when it was necessary to detect the presence of clinical symptoms, i. e. the disease. One hundred carp fingerlings were examined clinically, while more than twenty were taken for the laboratory examinations. The laboratory analysis of the native preparation was performed by placing the spore content between a slide and a cover slip. The slide had been previously covered with a 0.5 mm tick agar-agar layer and was stained with Lugol's solution and graphic in to achieve good contrast for photographing.

Histological examination was performed using light microscopy, as prescribed by the Institute of Parasitology, Czech Academy of Science, Ceske Budjovice.

Electron miicroscopy was carried out at the Universite des Sciences et Techniques de Languedoc, Academie de Montepelier,in the fish parasitology laboratory, in order to establish the ultrastructure of Thelohanellus nikolskii its developmental stages.

Fixation of myxosporidia for the ultrastructure study is very delicate and it can give variable results. We used several types of fixatives containing paraformaldehyde, and glutaraldehyde with or with or without osmium (OsO_4) post-fixation with the tampon of PALLADE. The best results were reached using only the osmium acid tampon according to PALLADE (pH-7,4).

Cold fixed samples were molded in araldie or in Spurr's mixture. Polymerization was performed in capsules orin silicone moulds. The spores were collected alive from the capsules, and after centrifugation the supernatan was placen in the suspension with fixative. Washing and dehydration were carried out by a series of successive centrifugations. The spore mixture appeared to be very good for moulding because, due to its low viscosity,it enabled an easy sedimentation of the spores at a small number of cycles. When a large residuum was obtained, it was transferred in small doses by a Pasteur pipette. If the elements remained scattered, we decanted the greatest part of the supernatant. After placing the residuum in the suspension, it was rinsed and put into a plastic capsule serving also as a mould.

Successive electron microscopy:

Parasites from the cysts were collected by centrifugation.

A) Fixing was performed in cold (O^0C) osmium tetraoxide (2%) by tamponing according to PALLADE at pH 7,4.

B) Moulding was done in SPURR's milieu, inplastic scales, after dehydration in a series of increasing concentrations of alcohol (from 30% -100%). Repassing to SPURR's solution through alcohol was carried out progressively: 1 part of alcohol 100% + 1 part of SPURR's; then 1 part of alcohol + 2 parts of SPURR's, and finally SPURR's only.

C) After being made on the pyramidotome, few hundred angströms thick sections were sliced on an ultramicrotome (Porter Blum).They were stained at 80^0C using toluidin blue (5%) mixed with borax (0,5%), and observed through a light microscope to determine whether the desired sample zone was reached. Fine sections were placed on a copper reticule, dipped in uranic acetate and in lead citrate (Reynolds,1963).

D) The sections were observed under electron microscopes "Zeiss EM 9A" and JEM 200 CX.

RESULTS AND DISCUSSION

The first clinical symptoms in fingerlings were detected as diffuse dark discolorations that later appeared in the shape of dark transverse lines on the fins of 14 - 21 day old fingerlings. That stage is referred to as phase 1 (Figure 1).

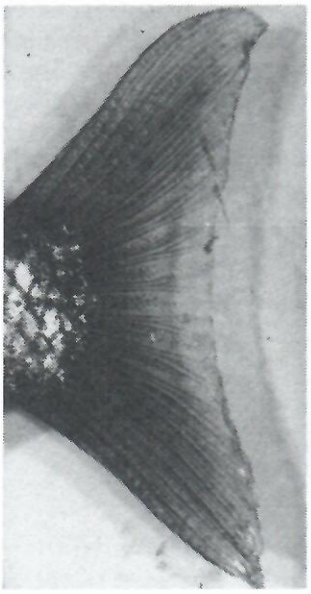

Figure 1. Phase I

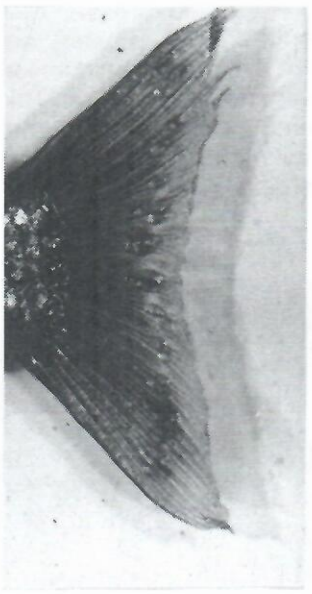

Figure 2. Phase II

In fingerlings 21 - 30 days old we noted cysts in the shape of small palpable bosses of 20-30 μm in size. That stage is referred to as phase 2 (Figure 2).

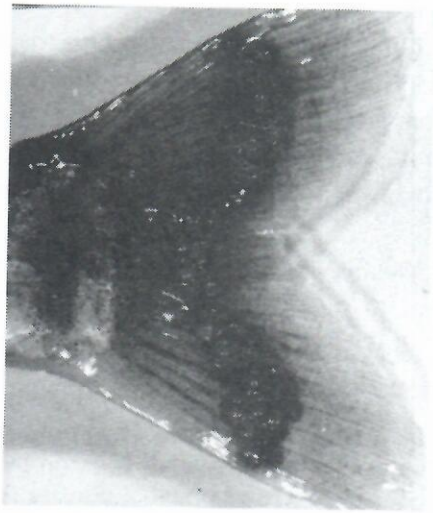

Figure 3. Phase III

Figure 4. Phase IV

Between the 30th - 37th day, we noticed the appearance of spores in the cysts, although they were dominated by developing sporogonic shapes. The size of the cysts varied from 40-70 μm. This is referred to as phase 3 (Figure 3).

Between the 37th - 45th day, the cysts were about 1 mm in size and were dominated by mature spores, although there were also some developing sporogonic shapes. This stage is referred to as phase 4 (Figure 4).

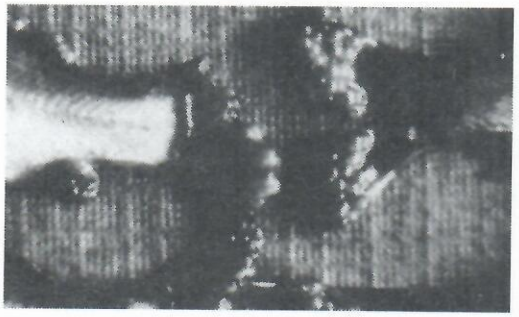

Figure 5. Phase V

Between the 45th - 60th day, the cysts assumed their maximum size of 1 - 2 mm. Most often they contained about 20.000 spores (counted in Spencer's chamber). The developing sporogonic forms were not present. This stage is referred to as phase 5 (Figure 5).

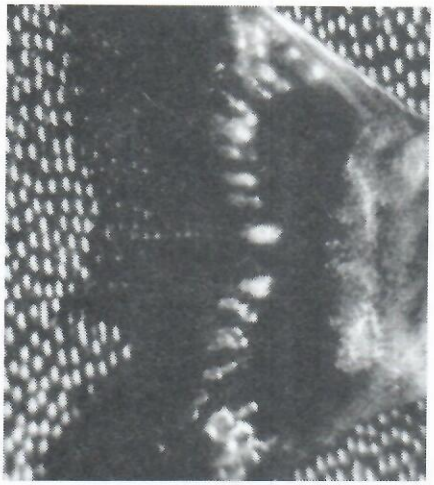

Figure 6. Phase VI

Between the 60th - 70th day, the cysts almost simultaneously dropped off. Small bosses were detectable on the fins as the result of epithelial hyperplasia. This stage is referred to as phase 6 (Figure 6).

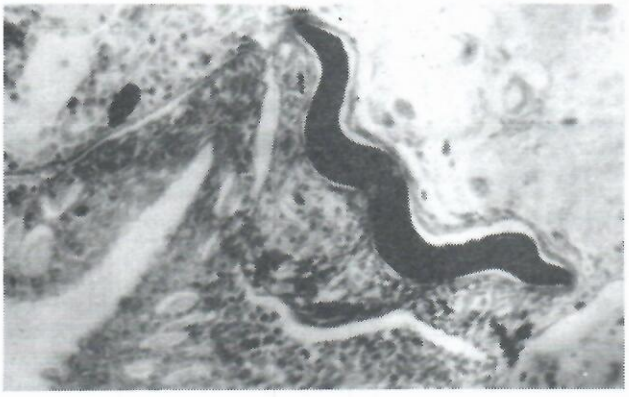

Figure 7. First histological changes detectable in phase II.

An exception to the previously described developing cycle could be found in those pounds where the disease appeared for the first time. In that case there

were no mass infections and the first clinical symptoms could be detected in fingerlings 60-90 days old. Since 1989 we have detected cysts on 10% of the examined fingerlings during the autumn capture, and, in several ponds even during the spring capture (in the next vegetative period). Twice we have detected the presence of cysts on two-year-old carp fish (1994). Cysts containing myxosporidium spores have not been found in three-year-old carp fish and other fishes reared with them in poly-culture.

The disease infected more than 90% of the fingerlings. The magnitude of losses caused by the disease was directly dependent on the size of the fingerlings at the moment the disease appeared.

The first histological analysis was done during the second phase of the disease, when the cysts were about 40 μm in size (Figure 7). Cysts containing developing sporogenic forms were detected on the outer surface of the fin ray. The cells of the host and those of the parasites were differently stained with hematoxylin-eosin.

In the third phase, when the cysts were 40 - 70 μm in diameter, their endoplasm contained a small number of mature spores besides the dominating sporogenic forms. As early as this phase we detected discernible deformations of the fin ray tissue whose cells were pressed by the cysts in the developing stage (Figure 8).

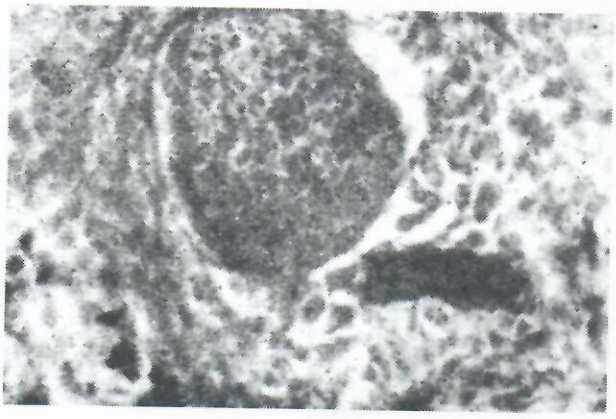

Figure 8. Commencement of sporogenesis in phase III.

In the fourth phase the cysts were about 1 mm in size. The mature spores dominated them, although the developing sporogenic forms were still present. The structure of the host tissue in places where the cysts developed was completely deformed (Figure 9) and, of the original structure of the fin ray, there were only amorphous shapes left. In the fifth phase the cysts assumed their maximum size of 1 - 2 mm, and they contained only the mature spores without the developing sporogenic forms. The interior of a cyst was separated from the spore wall by a thin membrane (Figure 10). In this phase the cysts began to burst and were replaced by connective tissue. During the next ten days most of the cysts dropped off.

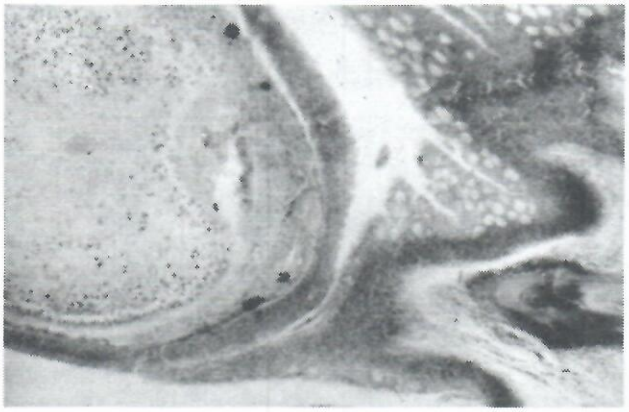

Figure 9. Late Sporogenesis in phase IV.

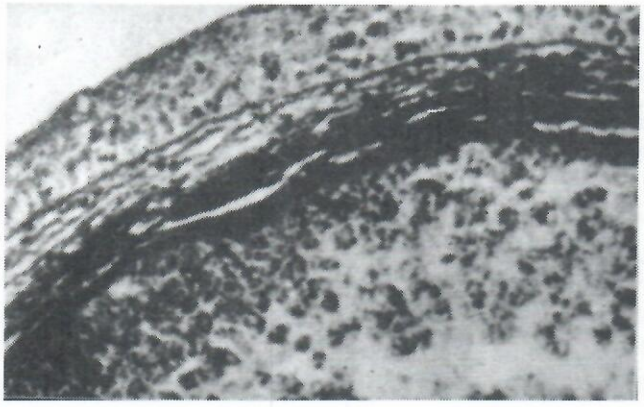

Figure 10. Mature spores in phase V.

In the sixth phase there were no more cysts. However there were discernible fin ray deformations, followed by thickening of their epithelium. Full restitution was completed by the end of the vegetative period of the same year, with the exception of those fingerlings that suffered greater fin damage due to their poor condition (Figure 11).

During examination of the ultrastructure (using electron microscopy) in the first phase of the disease, which was characterized by dark discolorations of the tail fins, we detected chromotaphore cells with thick melanin pigment granulations (Figure 12).

In the second phase, small bosses were observable under the light microscope, which were the young plasmodium and connective tissue capsule in formation. This could be better resolved in the electron micrograph (Figure 13).

Figure 11. Restitution of the affected host tissue in phase VI.

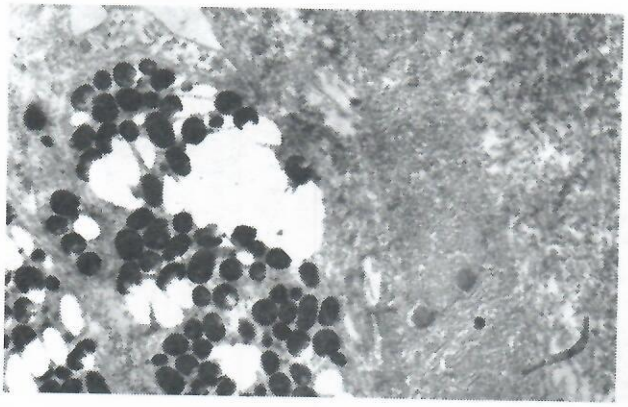

Figure 12. The wall of the cyst with connective tissue fibers.

Thick collagen fibers dominated the structure of the connective tissue of the cyst wall. In the cytoplasm of the young plasmodium there were numerous mitochondria, Golgi, smooth endoplasmic reticulum and lipid droplets. Plasmodium was separated from the wall by a thin membrane of homogeneous structure containing a number of pinocytotic canals.

At the end of the second and the beginning of the third phase, generative cells in pairs (the first stage of sporogenesis) were observable. Their chromatin material appeared in the shape of two big bladder-like nuclei with corresponding nucleolus. Thick homogeneous cytoplasm contained numerous ribosomes, Golgi, short segments of smooth endoplasmic reticulum and mitochondria with developed cristae. A thick structure forming microtubules represented a bridge between the two generative cells (Figure 14).

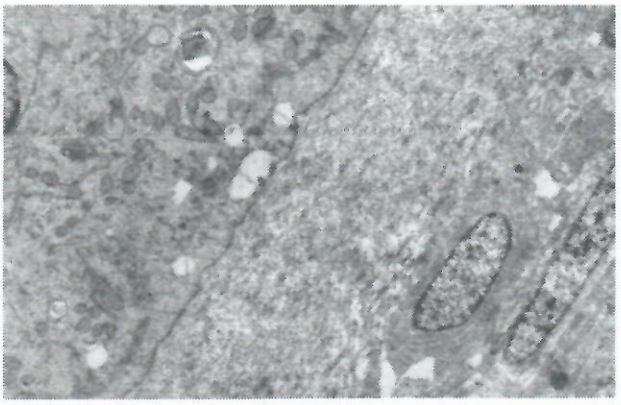

Figure 13. The wall of the cyst with the homogenous structure of connective tissue fibers enclosing a young plasmodium.

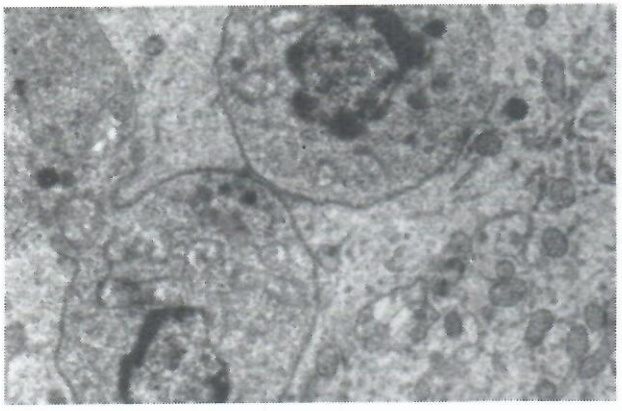

Figure 14. Generative cells in pairs, connected by an intracellular bridge.

In the next phase of the sporogenesis (in the third clinical phase there were also a small number of mature spores), one generative cell is enclosed by another cell - pericyte, as can be seen in Figure 15. The cytoplasmic properties of the enclosed cell and of the pericyte, resemble the previous phase of sporogenesis, except for the "central dense body" in the nucleus, with a few similar satellite bodies around it, i. e. on the periphery of the nucleus.

The enclosed generative cell then divided into 4 cells. One of them degenerates, the second gives the sporoplasm and the two remaining generate a polar capsule and valves, which can be seen in Figure 16. In Figure 17 we can see the moment of cell division, with four, or five observable cells. The young forming spore is shown in Figure 18, where there are four cells, two of which belong to the spore and the other two are valvular cells. In the cytoplasm of the

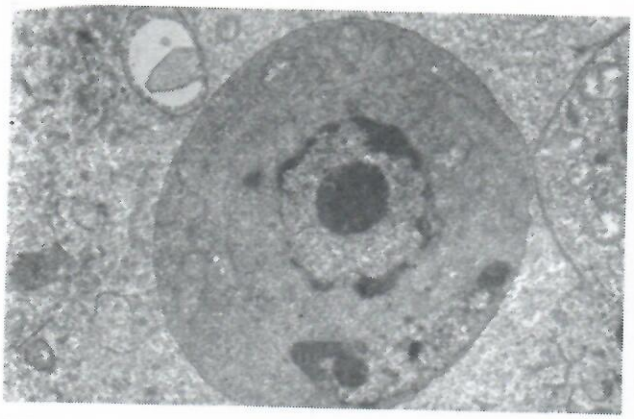

Figure 15. Initial stadium of sporogenesis with pericyte and generative cell.

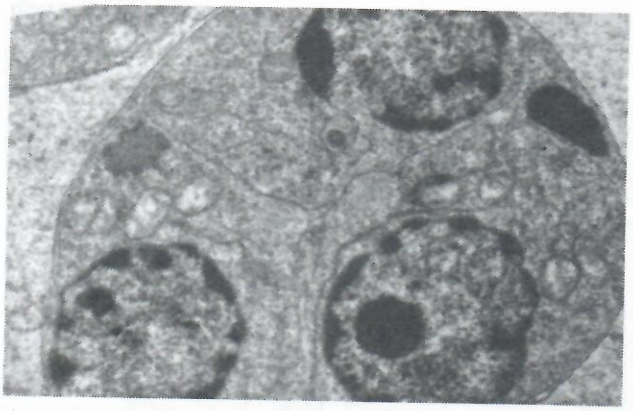

Figure 16. Tetracellular sporoblast.

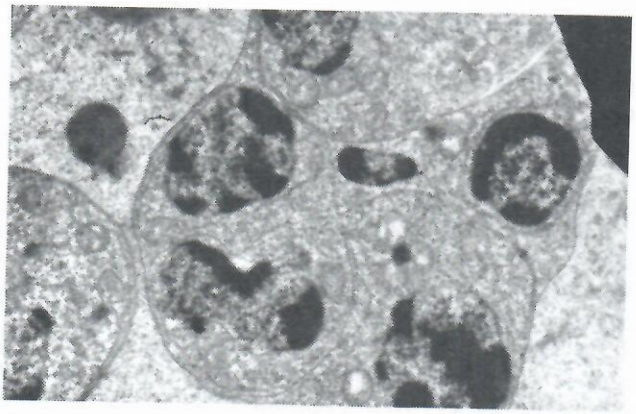

Figure 17. Sporoblast with two capsulogenic cells connected with dense body.

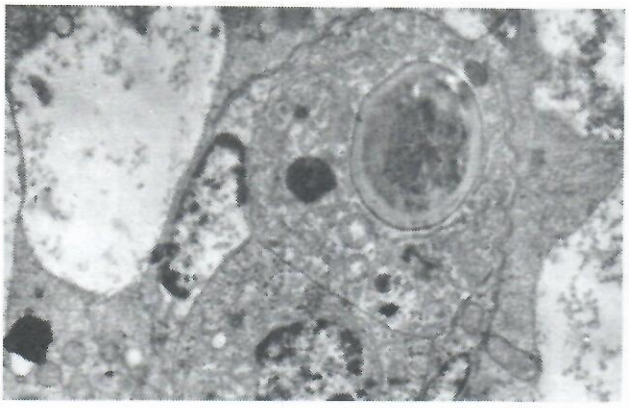

Figure 18. Young spore in formation.

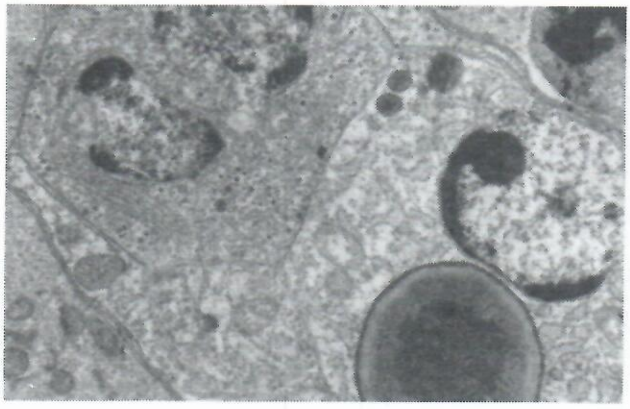

Figure 19. Formation of the polar capsule.

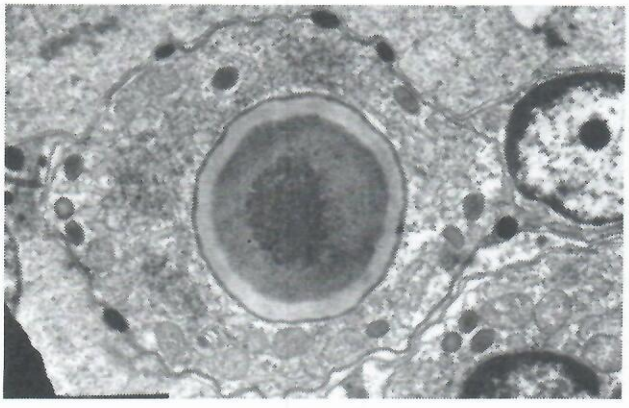

Figure 20. Details at the "seam" level showing a desmosome.

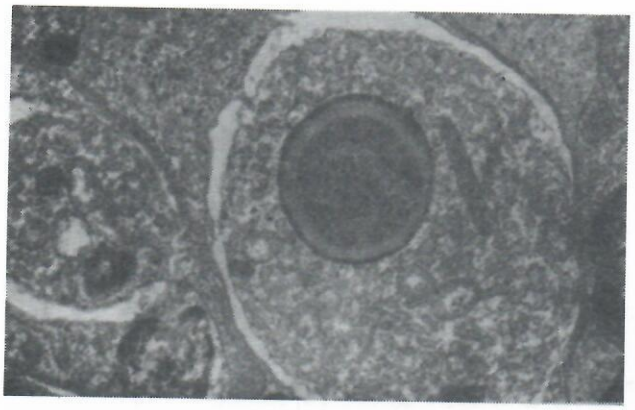

Figure 21. Commencement of polar fiber generation beside the encapsulated primordium.

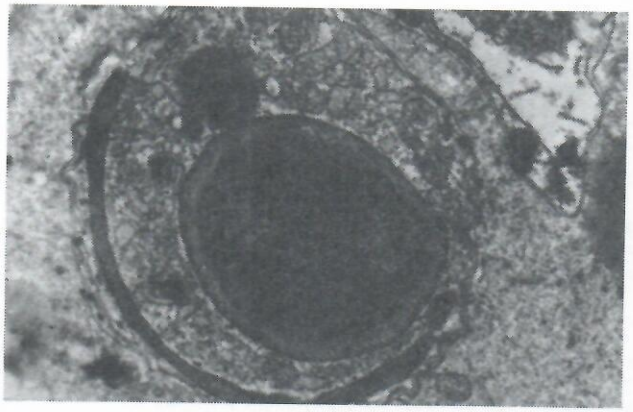

Figure 22. Well-expressed polar fiber.

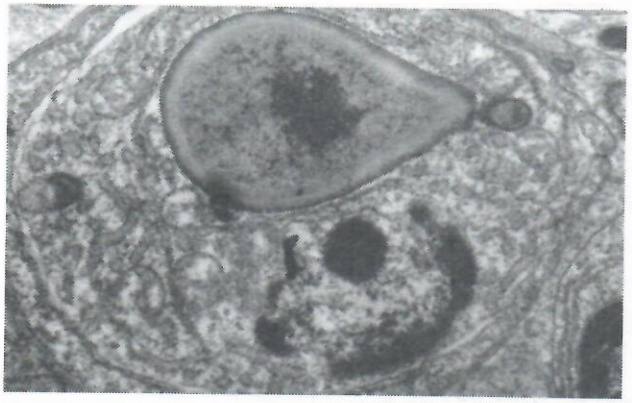

Figure 23. Elongation of the primordium with degenerating polar capsule.

capsulogenic cell, the rounded "capsular plasmodium" appears. Valvular cells are extremely flattened. Sporogenesis advances further and in Figure 19. we can detect the nucleus degeneration and development of the "capsulogenic primordium", the result of which is formation of only one polar capsule by which the genus Thelohanellus is characterized. The cytoplasm is dominated by granulated endoplasmic reticulum, Golgi, mitochondria and cisternae formed by smooth endoplasmic reticulum. In Figure 20 we can see details of the rounded capsulogenic primordium with three clearly visible zones. Since the section was made at the "seam" level, we can see the desmosome made up of a large number of microtubules. The commencement of the polar fiber development can be seen in Figures 21. and 22., while in Figure 23 we can see the elongation of the "capsulogenic primordium" and the further degeneration of the polar capsule. The stage before mature spore formation can be seen in Figure 24. In the basis

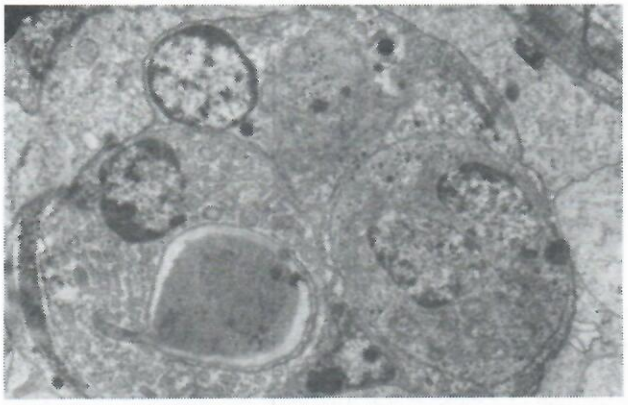

Figure 24. Extenal tubules forming from the base of the primordium.

Figure 25. Mature polar capsule.

of the capsulogenic cell the other tubule is formed which is not visible in the next stage (Figure 25). The polar fiber is considerably enlarged, and the dense core of "capsulogenic primordium", characteristic for the previous stages, is not visible. On the aperture of the polar capsule we can see the protein plug reminiscent of a cork.

In the 4thand 5th clinical phase, the mature spores are dominant. In the 6th stage the cysts have already dropped off, and we can see only the abundant connective tissue as the result of the host's defense reaction to the presence of parasites.

Hoshina and Hosoda (1957) describe the same disease caused by T. cyprini. In our case, the time needed for detecting the first symptoms was approximately the same as was described in their research, while the duration cycle was much shorter. Molnar (1982) gives a detailed study of the biology and histopathology of the disease caused by Thelohanellus nikolskii and,in co-operation with other authors (Deser et al. 1983), he also describes the ultrastructure of the developing cycle of T. nikolskii. In their research there is no comparison of the clinical stage with the histological research, which is the aim of this paper. Cycle changing, appearance and duration of the disease in our ponds depended on the implementation of preventive disinfection, the use of a furan preparation (Ćirković 1996) and, presumably, on the immunological mchanisms of the fish, which have not yet been thoroughly examined.

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KARAKTERISTIČNE FAZE RAZVOJA THELOHANELLUS NIKOLSKII (1979-1996)

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SADRŽAJ

U toku obolenja mladunaca šarana, telohaneloze, izazvane sa Thelohanellus nikolskii, razlikujemo šest faza opisanih klinički i histološki (svetlosna i elektronska mikroskopija). Karakteristične faze razvoja ispoljavale su se u prvim godinama pojavljivanja ove najmasovnije parazitske bolesti riba, dok u kasnijim godinama pojava bolesti i njen tok nisu se više mogli prema simptomima podeliti u faze koje bi se ispoljile kao zakonitost. U radu su pravljena poređenja kliničkog ispoljavanja sa nalazima do kojih smo dolazili služeći se klasičnim histološkim metodama odnosno svetlosnom mikroskopijom i transparentnom elektronskom mikroskopijom. Od perioda masovnog pojavljivanja bolesti do danas razvojni ciklus oboljenja se značajno izmenio.