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Biology and Histopathology of *Thelohanellus nikolskii* Achmerov, 1955 (Myxosporea, Myxozoa), a Protozoan Parasite of the Common Carp (*Cyprinus carpio*)

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Abstract. *Thelohanellus nikolskii,* a myxosporean originating from the Far East, arrived in Hungary through natural waters from neighbouring countries which it had originally reached because of the introduction of the Amur wild carp. *T. nikolskii* is a specific parasite of carp which produces large cysts on the fins of fry. The cysts usually appear on the fish at the beginning of July, and in August and September cysts containing the mature spores burst. The cysts are easily seen even by the naked eye and are surrounded by a thick capsule composed of connective tissue strengthened by cartilaginous elements. Due to the breaking down of the fins the parasite has pathological significance in fry.

Introduction

Thelohanellus nikolskii was described by Achmerov (1955) from common carp living in the river Amur. During his investigations Achmerov (1955, 1960) found four other *Thelohanellus* spp. that differed morphologically and in their location inside the host. Among the species described by Achmerov, Shulman (1962, 1966) regarded only *T. dogieli* Achmerov, 1955 as a valid species and thought the others to be synonyms of *T. dogieli* and *T. fuhrmanni* (Auerbach 1909). The occurrence of a *Thelohanellus* species of carp in Hungary and accordingly in Europe was reported for the first time by Jeney (1979), who identified the parasites developing in cysts on the fins of common carp (*Cyprinus carpio*) as *T. dogieli* and was of the opinion that this parasite had been introduced from the Far East.

Molnar and Kovacs-Gayer (1982) found a *Thelohanellus* species in the swimbladder serosa of common carp which differed from the one living on the fins, and they concluded that all the species described by Achmerov (1955, 1960) were valid, organospecific parasites, among which *T. nikolskii,* parasitic in the fins, and *T. hovorkai* Achmerov, 1960, parasitic in the serous membranes of the inner organs, occurred in Hungary.

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Little is known about the development of the *Thelohanellus* spp. Detailed information can be found only in the study by Debaissieux (1925) of a *Thelohanellus* sp. found on the roach (*Leuciscus (Rutilus) rutilus*). Debaissieux referred to this species as *Myxobolus notatus* Mavor but its identity with *Thelohanellus notatus* described in North America is rather doubtful. On the other hand, that it is a species of *Thelohanellus* can be stated without doubt.

Histological examinations on *Thelohanellus* cysts were made by Homma and Tamura (1976) who studied the lesions in the thymus of fork-tongued goby (*Ctenogobius urotaenia*).

The data on the pathology of the different *Thelohanellus* spp. are insufficient. Bauer (1948) and Petrushewski and Bauer (1948) described losses caused by *T. pyriformis* (Thelohan 1892) living in the muscles of coregonids and carps.

The aim of the present investigation was to obtain data on the prevalence, development, pathogenesis and pathological significance of carp fin thelohanellosis.

Materials and Methods

Thelohanellus infection of carp fry was demonstrated in 1979 in fish farms situated along the river Körös at the eastern border of the country. By 1981 the infection was widespread in Eastern Hungary and also appeared in some Transdanubian fish farms. By this time some *Thelohanellus* cysts had been found even on the fins of two- and three-summer-old common carp.

The examinations were carried out from 1979 to 1981 continuously on several thousand common carp raised in Hungarian fish farms. *Thelohanellus* infections of fry were surveyed and material for histopathology was fixed from young fish. The fins and internal organs of infected fish were examined in a dissecting microscope. External examinations were also made on other species of carps including *Ctenopharyngodon idella, Hypophthalmichthys molitrix, Hypophthalmichthys nobilis* and *Tinca tinca* raised in a polyculture with the common carp.

After excystation, spores were embedded in gelatine and preserved as long-term slide preparations. For histological study, infected and uninfected fins were fixed in 10% formalin or aqueous Bouin's solution, embedded in paraffin wax, cut into 4–8 μm sections and stained with haematoxylin-eosin, PAS or by Farkas-Mallory's method.

Results

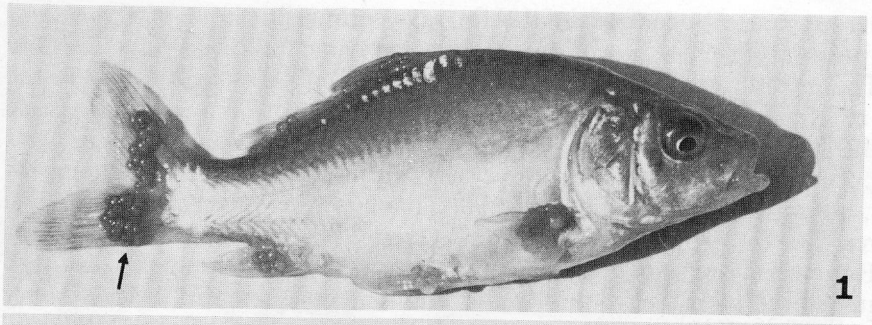
T. nikolskii was found exclusively in common carp in which it was restricted to the fins. Thick-walled cysts, 1–2 mm in diameter (Fig. 1) and containing

Fig. 1. Common carp fry infected with *Thelohanellus nikolskii* cysts on the fins. Actual size

Fig. 2. Common carp fry infected with young stage of *T. nikolskii*. Upper fish: characteristic black transversal line on the caudal fin. Lower fish: the tips of the caudal fin have broken down at the site of the developing cysts. ×2

Fig. 3. Section of the fin. Young cysts surrounded by perichondral cells. *a* cartilaginous fin rays *b* perichondral cells *c* cysts *d* connective tissue *e* epithelium. HE ×100

Fig. 4. Part of a developing *T. nikolskii* cyst. *a, b* endoplasma of the cyst with mono- *a* and polynuclear *b* developmental cell stages *c* ectoplasma *d* extracellular surface coat *e* perichondral cells *f* connective tissue *g* cartilaginous islets in the cyst wall *h* epithelium *i* pigmented area. HE ×700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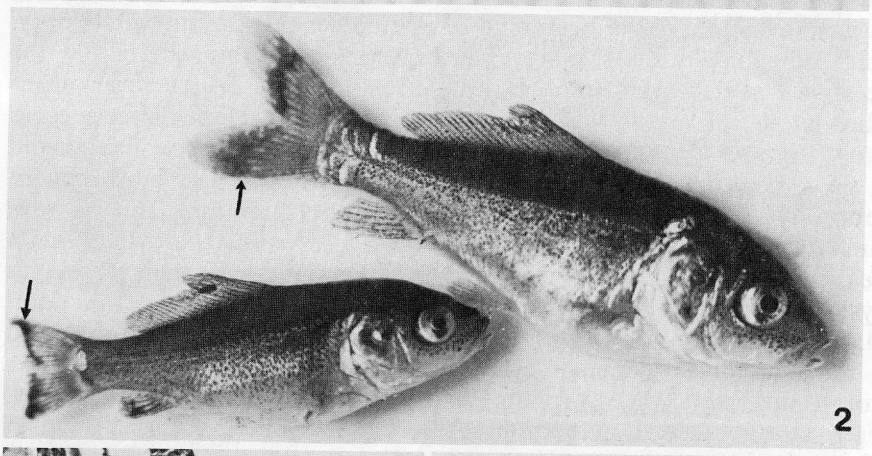
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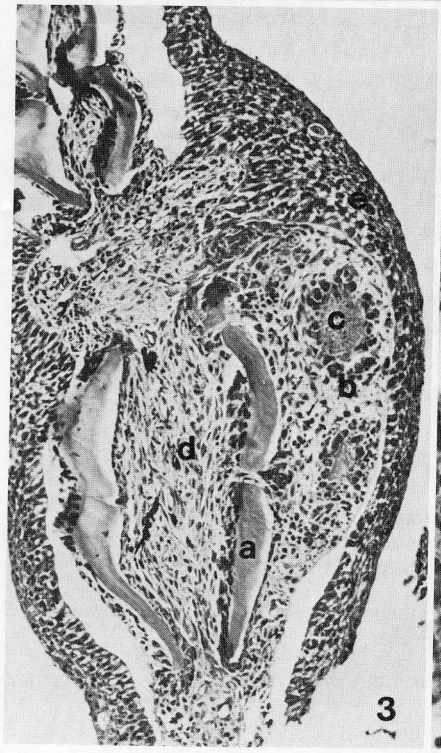
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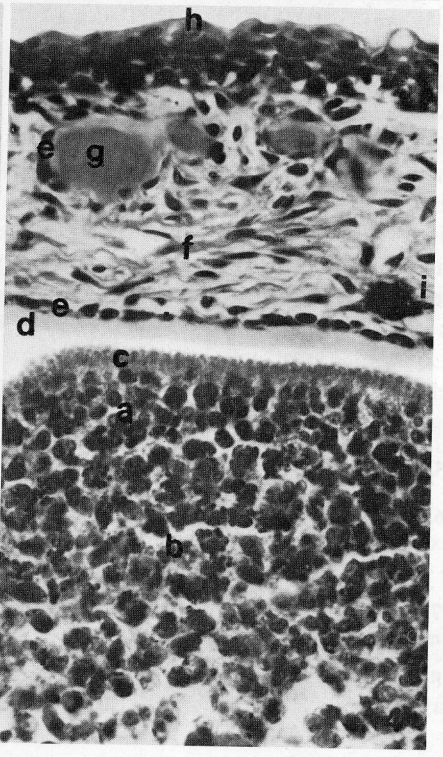
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numerous spores, occurred in the fin rays, primarily those of the cauda and pectoral fins. The fins of heavily infected fish contained up to 50 cysts

The infection first became apparent in 5- to 6-week-old carp fry at th end of June or in early July, when a black transverse line became visibl on the fins of heavily infected fish indicating the location of young cyst (Fig. 2). The first spores in these cysts were detected at the beginning o July, but at this time the cysts contained mainly developmental stages whil spores were only found in the centre.

By the beginning of August most cysts contained only spores. In mid August some of the cysts had burst and only the remains of the empt cysts were evident. By the beginning of September most cysts were empty Within individual fish stocks the infection was synchronized so that th same stages of development were seen in all the fish. In fry population hatched late in the season the symptoms of invasion developed later accord ing to their age, and among these fry and in two- or three-summer-ol fish, spore-bearing cysts occurred occasionally even at the beginning o October. The infected fins showed significant deformation. The fin ray became distorted and in some small fish the fins even broke down (Fig. 2) In heavy infections the fins of small fish broke down during the early stage of cyst development. In such cases primarily the caudal fins became dam aged. In 7- to 8-week-old fry fin damage was also observed but it occurre in a more advanced stage of cyst development and mainly affected th pectoral and pelvic fins. Carp that had lost their fins swam abnormally After the cysts burst, regeneration of the fins depended on the growth o the fish and the temperature of the water. In fast-growing carp population where the developmental cycle was completed by the middle of August the only evidence of infection in surviving fish was distortion and loca thickening of the cartilageous fin rays. By this time the epithelial damag of the fins was repaired and the fins had grown significantly. Howeve in cases where the infection continued until the end of September, the en of the feeding-growing period of the fish, evidence of the emptied cyst persisted until the next spring. The level of infection was much lower i older fish than in the fry. The cysts in the older fish were larger tha those of the fry, but the measurements of the spores were the same i both age groups.

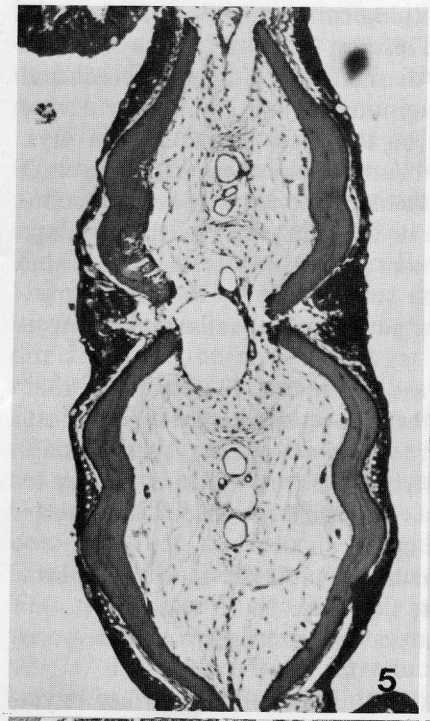
Losses were often observed in infected carp populations, but they coul never be attributed exclusively to *Thelohanellus* infection.

Fig. 5. Normal structure of the fin. Transversal section. HE ×80

Fig. 6. Semimatured cysts in the fin. *a* cyst of parasite origin shrunken and detached fro the extracellular surface coat *b* extracellular surface coat *c* connective tissue with cartilagino islets *d* epithelium *e* broken pieces of cartilaginous fin rays. HE ×80

Fig. 7. Regeneration of cysts after bursting. *a* Epithelium grows into the lumen. *b* Hyali elements are still present. HE ×80

Fig. 8. Signs of a survived infestation with *T. nikolskii*. See outgrowths ↗ on the cartilagino fin rays. ×20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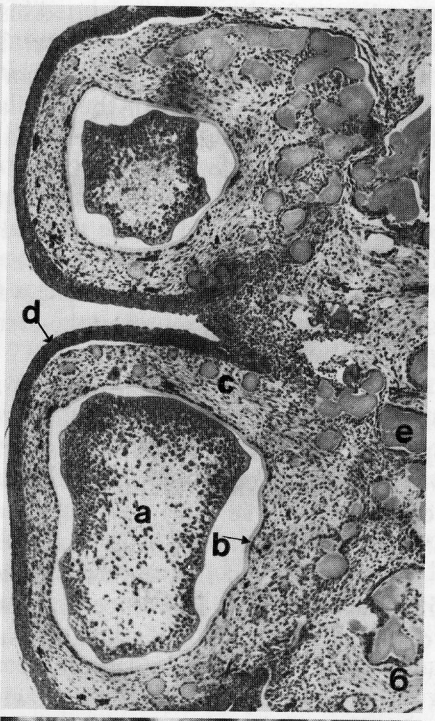
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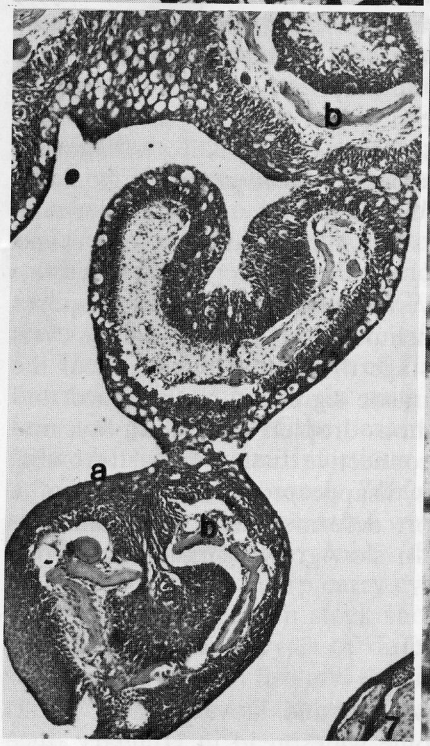
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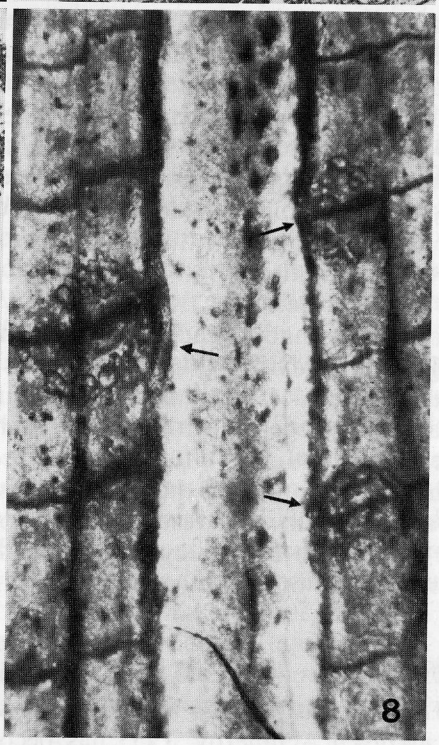
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The earliest infection was diagnosed histologically in the fins of a 5-week-old carp fry at the beginning of July. This fry exhibited the symptoms shown in Fig. 2. *Thelohanellus* cysts with a diameter of 25–35 μm were found attached to the outer surface of the cartilaginous fin rays. With rare exceptions developing cysts were also detected on the inner surface of fin rays. Inside the cysts the endoplasm contained round, uninuclear developmental stages. Between the host cells and the ectoplasm of the parasite an extracellular surface layer developed which stained in the same way as the cartilage of the fin rays, red with haematoxylin-eosin and Giemsa's stain and blue with Farkas-Mallory's stain. The cyst was surrounded by the same large-nucleated, intensely staining, monolayered perichondral cells which constituted also the perichondrium (Fig. 3).

In 6-week-old fish the cysts reached a diameter of 40–60 μm. In their endoplasm, in addition to the uninuclear cells, polynuclear pansporoblasts also appeared, and sometimes spores were detected (Fig. 4). At this stage of development the cysts were separated from the cartilaginous fin ray by loose connective tissue that grew between the perichondral cell layers surrounding both the perichondrium and the cysts.

In 7- to 8-week-old fish the cysts measured 1 mm and their endoplasm was filled with spores. In the ectoplasm, however, there was still a thin layer of uninuclear cells and a slightly larger one consisting of pansporoblasts.

Externally the cyst was covered with an intercellular surface layer, 8–12 μm thick, surrounded by perichondral cells. Over this layer loose connective tissue layer, 80–150 μm thick, was seen, which was covered with multilayered epithelium. In the vicinity of developing cysts the cartilaginous structure of the fin rays degenerated; the rays lost their original structure (Fig. 5) and disintegrated into amorphous fragments. Small cartilaginous islets covered with perichondral cells developed even in areas far from the fin rays and became embedded in loose connective tissue of the cyst wall (Fig. 6). In addition to cartilaginous islets small pigmented areas were found in the connective tissue.

In 8- to 10-week-old fish the cysts were completely filled with spores and some cysts had already burst. Epithelium grew into the empty cysts and filled their cavities (Fig. 7). At this stage the hyaline substance of the intercellular surface layer around the parasite significantly thickened and gradually grew together with the cartilaginous droplets around fin rays and with cartilageous islets situated in the connective tissue. In fast-growing fish the discontinuity of the skin of fins disappeared and the signs of a survived infection were restricted to short deformed sections of fin rays at the site of the former cysts (Fig. 8). In slow-growing populations this stage was found only in overwintered fish.

Discussion

The *Thelohanellus* species identified by Molnar and Kovacs-Gayer (1982) as *T. nikolskii* Achmerov, 1955 has become widespread in Hungary since

it was first detected by Jeney (1979) and has become a common parasite of common carp fry. The present investigations have proved the hypothesis of Achmerov (1955, 1960) that this parasite is a host- and organospecific species.

T. nikolskii was described by Achmerov (1955) from Amur wild carp (*Cyprinus carpio haematopterus*), and *T. cyprini* Hoshina and Hosoda 1957, a synonym of the aforementioned species, was also found on the same carp subspecies raised in Japanese carp farms. It is unquestionable that *T. nikolskii* was introduced to Europe by this carp subspecies. Ivasik and Karpenko (1967) reported that the Amur wild carp had been introduced to the western part of the Ukraine first from Bielorussia in 1955, then directly from the river Amur in 1963. Also Ivasik et al. (1970) reported that the Amur wild carp and its hybrids were cultured together with the European mirror carp in the pond farms of the Transcarpathian region of the Ukraine belonging to the water system of the river Tisza. Among the parasites of the Amur wild carp four species, *Dactylogyrus achmerowi* Gussev 1955, *Khawia sinensis* Hsü 1935, *T. nikolskii* Achmerov 1960, have been identified in Hungary though the fish itself has not been introduced (Molnar 1976; Molnar and Buza 1975; Molnar and Kovacs-Gayer 1982). The appearance of *T. nikolskii* was associated with the water system of the river Körös whose source is in Rumania. At the beginning of the 1960s several fish species were introduced into Rumania from Chinese natural waters but I have no exact data on the possible introduction of the Amur wild carp. In my opinion *T. nikolskii* was introduced into Hungary by free-living common carp inhabiting the river Körös, and its rapid spread through the country was undoubtedly connected with the transport of carp fry.

T. nikolskii seems to be a specific parasite of common carp. Until now it has not been recorded in grass carp, bighead, silver carp or tench although these carp are often examined in diagnostic laboratories. *T. nikolskii* occurs exclusively on the fins of both young and mature fish. In addition to the spore morphology this fact also indicates the necessity to accept – in contrast to the opinion of Shulman (1962, 1966) – the original data of Achmerov (1955, 1960) which regarded *T. nikolskii* as a valid species.

The developmental cycle of *T. nikolskii* is completed within a relatively short time, 8–10 weeks. In spite of this, according to our observations, only one generation of the parasite develops annually. The spores excreted from the fish become infectious after a comparatively long period spent outside the host. Obviously *Thelohanellus* spores must undergo "maturing and hibernation" in the same way as hypothesized by Hoffman and Putz (1971) for *Myxosoma cerebralis*. The synchrony of infection is a characteristic feature. Affected carp carry cysts of the same developmental stage, and all the carp in a certain stock show the same stage of infection at a given time. The fact that cysts of different developmental stages do not occur simultaneously on a fish and that in fry hatched later in the season infection evolves later, raises the question as to whether protective immunity is developed.

Present knowledge about the pathological significance of the parasite is not satisfactory. In the case of fry, the ability of movement is undoubtedly impaired by the deformation and detachment of the fins. In pond farms, however, where the acquisition of natural food is of minor significance, infected fish may survive. The fact that fins are organs of lesser vital importance is underlined by the findings that there are no significant differences in the growth rate of the affected and healthy fry. Fin damage seems to be of greater significance if the fins of small fish break down in the period of early cyst development (Fig. 2), because at this time bacterial and fungal infections may aggravate the process. In two- or three-summer-old fish, *Thelohanellus* infection is of no significance.

The histological findings connected with *Thelohanellus* infection are highly characteristic. Cyst development begins on the surface of the cell layer covering the cartilaginous fin rays. Growing cysts are situated in loose connective tissue which forms a thick, host-derived wall around the cyst. The appearance of cartilaginous islets in the connective tissue forming the cyst wall is also characteristic. The cyst distorts the cartilaginous fin rays though it is not directly connected with them. The structure of the fin rays situated adjacent to the cysts undergoes alteration. Affected fin rays become fragmented presumably because the statical balance of the fin is lost due to the pressure caused by the large cysts. The cartilaginous islets located in the fibrous wall of the cyst presumably cannot be connected with the disintegration of fin rays seen at the base of the cyst. These islets probably strengthen the cyst wall and are the products of perichondral cells carried away by connective tissue. The formation of an intercellular surface coat consisting of a comparatively thick hyaline material around the ectoplasm of the cyst is particularly surprising. This is produced by the same cells that surrounded the cyst at the place of its formation. This hyaline capsule provides considerable stability for the large cysts and – together with the fibrous capsule – prevents their premature rupture. The mode by which host-parasite relationship is established across this comparatively thick intercellular material, requires closer investigation.

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