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HISTOPATHOLOGICAL CHANGES CAUSED BY THE METACESTODES OF *NEOGRYPORHYNCHUS CHEILANCRISTROTUS* (WEDL, 1855) IN THE GUT OF THE GIBEL CARP, *CARASSIUS GIBELIO*

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Metacestodes of *Neogryporhynchus cheilancristrotus* (Wedl, 1855) were found in the gut of some gibel carp (*Carassius gibelio*) specimens from a Hungarian water reservoir. Location of metacestodes in the freshly opened gut was marked with disseminated, red-coloured, pinhead-sized nodules in the anterior part of the intestine. In histological sections, metacestodes were found in a hole inside the propria layer of the intestinal folds. The worms were in direct contact with the host tissue without being encapsulated as a result of host reaction. In some specimens with extruded rostellum the rostellar hooks were bored into the host tissue and suckers grabbed pieces of the surrounding connective tissue. Around the worms, congested capillaries and formation of macrophages were seen in the lysed connective tissue.

Key words: Gryporhynchidae, *Neogryporhynchus*, metacestodes, fish, gibel carp, histology, pathology

Metacestodes of tapeworms of the cyclophyllidean family Gryporhynchidae occur frequently in the gut, gallbladder or the abdominal cavity of fish. These cestode stages, frequently referred to as 'cysticercus', may be more common in host fishes than indicated by records in the literature. In Europe the occurrence of four species, *Paradilepis scolecina* (Rudolphi, 1819), *Neogryporhynchus cheilancristrotus* (Wedl, 1855), *Gryporhynchus pusillus* Nordman, 1832 and *Valipora campylancristrota* (Wedl, 1855) is known (Dubinina, 1987). Bona (1975), however, considered larvae identified as *G. pusillus* and *N. cheilancristrotus* to be conspecific. The morphometrics and seasonal occurrence of *N. cheilancristrotus* was studied in detail by Pietrock and Scholz (2000) in the Oder River. These authors found this parasite to be very common in blue bream. Scholz et al. (2004), who reviewed the species composition and distribution of gryporhynchid metacestodes, supposed that the number of these larval cestodes

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in other parts of the world could be much higher, as from Mexico alone the occurrence of as many as 13 species had been recorded. In Hungary the infection of fishes with gryporhynchid cestodes was first reported by Jaczó (1949), who recorded the metacestode larvae of *V. campylancristrota* (Wedl, 1855) in the gallbladder of the pike and sheatfish and described it as *Dilepis unilateralis* (Rudolphi). Molnár (1970) found another metacestode infecting the intestine of several cyprinid fishes and the weather loach, and designated it as Cysticercus *Gryporhynchus* sp. By the description and the attached photo this parasite can be identified with *N. cheilancristrotus*. Metacestodes of another gryporhynchid parasite, *P. scolecina* (Rudolphi, 1819) was found by Murai et al. (1996–1997) in the common bream *(Abramis brama)* but these stages were located in the abdominal cavity attached to the gallbladder and the intestinal serosa.

Little is known about the pathogenic importance of these cestodes. Their pathogenic effects were neglected over a long period of time, and only few papers have dealt with their veterinary importance.

The present paper describes the pathogenic effect of *N. cheilancristrotus*.

Materials and methods

Six specimens of three-year-old gibel carp (*Carassius gibelio*) 22 to 25 cm in length were received for veterinary inspection from the Tiszató water reservoir of the Tisza River on 25 November 2003. A general parasitological survey was performed. Fish killed by cutting off the head were dissected under a preparation microscope. Scrapings from the skin, gills and gut were examined under coverslip at 20- to 40-fold magnification with a Zeiss compound Jenaval microscope. Pieces of organs were squashed and examined in a similar way as the scrapings. Some of the metacestodes found in the samples were studied under coverslip alive, while some others were fixed in 10% buffered formalin or placed immediately in lactophenol to obtain a permanent preparation. The anterior part of the opened intestine was cut into some pieces, fixed between two slides with Bouin's solution for some minutes in order to make it rigid in horizontal position and fixed for further 4 h in an excess volume of Bouin's solution. Fixed samples were washed several times in 80% ethanol, embedded in paraffin wax, cut into 4 μm sections, and stained with haematoxylin and eosin. The rostellum of unfixed cestode specimens and the sections were photographed with a digital camera mounted on an Olympus BH2 microscope.

Results

In the first part of the opened gut pinhead-sized small red nodules bulging over the level of epithelium were seen with the unaided eye. In the scrapings a single metacestode of *N. cheilancristrotus* was found in each nodule. Two out of the six gibel carp specimens examined were heavily infected with metacestodes and harboured 41 and 44 nodules, respectively; the other four fish showed a relatively low-intensity infection with 7 to 11 parasites.

The 400 to 720 μm long and 220 to 380 μm wide metacestodes had a central constriction, which divided the body into an anterior and a posterior half (Fig. 1). In the anterior half there were four large suckers and an extruded or invaginated rostellum with two rows of rostellar hooks composed of 10 larger and 10 smaller hooks (Fig. 2). The length of the larger hooks varied between 55 to 62 μm and that of the smaller hooks between 38 to 40 μm.

Histological findings

Metacestodes were located in the lamina propria of the intestinal folds of the gut (Fig. 3), forming a hole inside the damaged host tissue. In some cases the worms had invaginated rostellum (Fig. 4), while in other cases the rostellum was extruded (Figs 3, 5 and 6). In extruded position it was seen that the suckers and the rostellar hooks fixed the worm to the host tissues, breaking off pieces of the latter (Figs 6 and 7). The parasite was surrounded by fractured and lysed connective tissue cells showing epitheloid character (Figs 5 and 6). Cells lining the hole around the worm became degenerated, and macrophages and erythrocytes were commonly found among them. Inside the suckers grabbed fragments of host tissues were seen (Fig. 5) and in this part the original structure of the lysed cells could no longer be recognised. The rostellar hooks that attached to structure-less cell debris condensed the nuclei of the host tissue into a mass (Fig. 6). Inside the proliferated tissue of the attacked intestinal folds dilated capillaries filled by red blood cells and sera were seen (Fig. 7). The cytoplasm of degenerated histiocytes had undergone lysis, and among the histiocytes the remnants of capillaries and less frequently foreign body-type giant cells were found (Fig. 8).

Discussion

The taxonomic position of cyclophyllidean metacestodes in fish is poorly studied. The importance of a more thorough study of the question was recognised only a short time ago, and data on the distribution of the known species have been reported only recently by Scholz et al. (in press). In Europe, where the majority of related works have been published, it is still not known whether the occurrence of three or four species is probable. Authors addressing this question

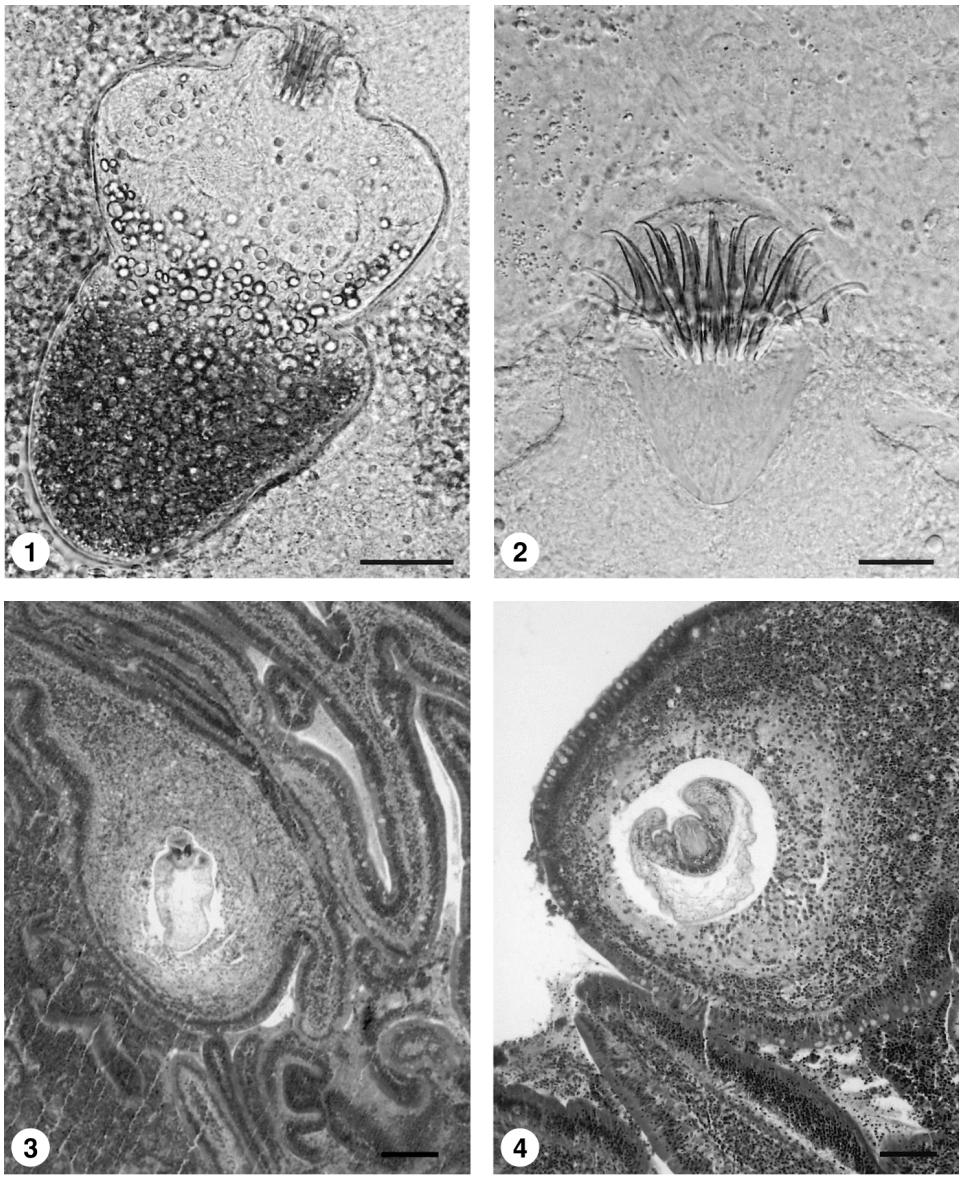

Fig. 1. Unfixed specimen of *Neogryporhynchus cheilancristrotus* metacestode with extruded rostellum. Scale bar: 40 µm; *Fig. 2.* Rostellar hooks of *N. cheilancristrotus*. Scale bar: 60 µm; *Figs 3–8*. Histological section of the gut of a gibel carp stained with haematoxylin and eosin (H.&E.); *Fig. 3. N. cheilancristrotus* metacestode bored into the propria layer of an intestinal fold. Scale bar: 200 µm; *Fig. 4. N. cheilancristrotus* metacestode with intruded rostellum located in a hole inside the propria layer of an intestinal fold. Scale bar: 100 µm

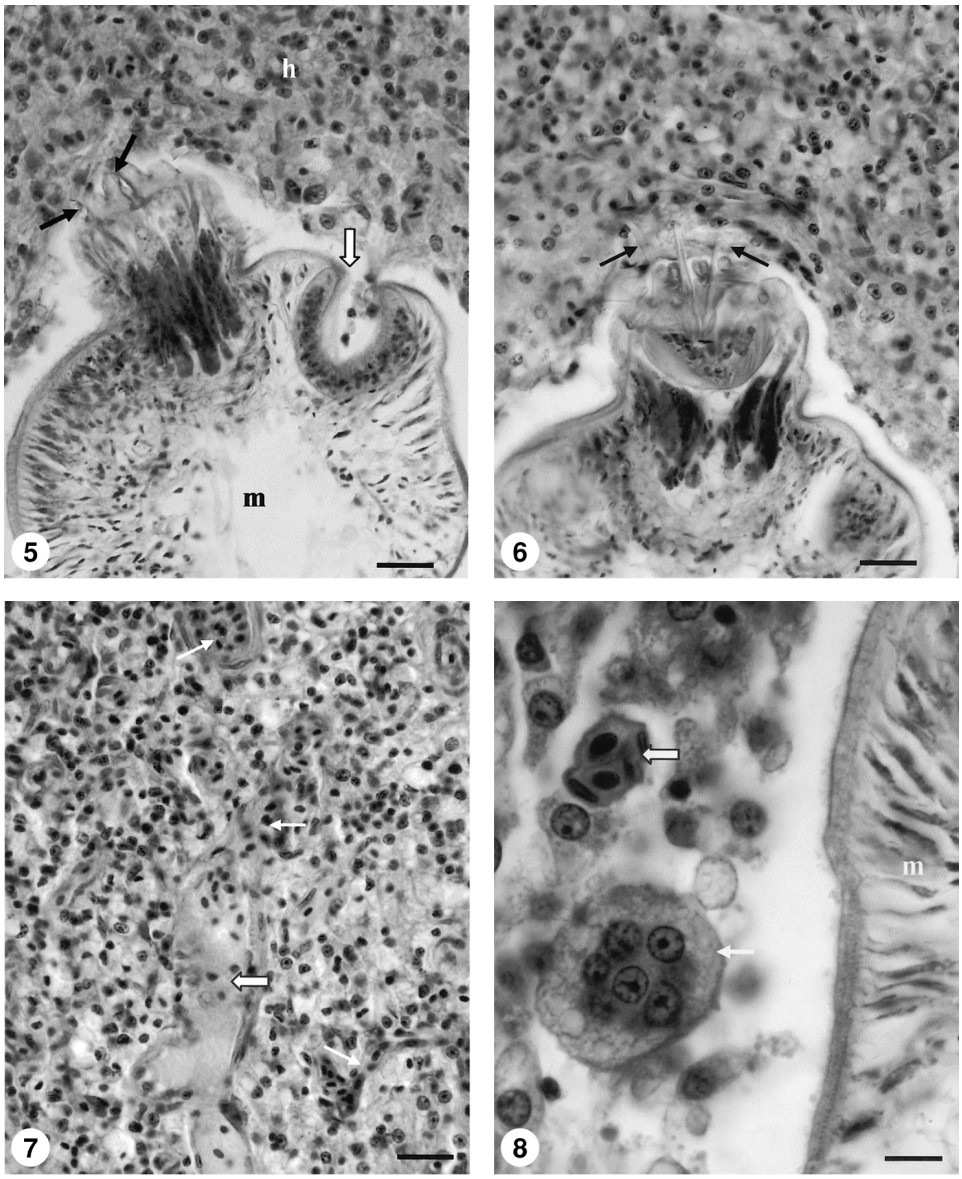

Fig. 5. Anterior end of a *N. cheilancristrotus* metacestode. Rostellar hooks bored into the propria damage connective tissue cells (arrow). One of the suckers (arrowhead) is tearing off a piece of tissue. m = metacestode, h = host tissue. Scale bar: 30 µm; *Fig. 6.* Anterior end of a *N. cheilancristrotus* metacestode. Rostellar hooks (arrows) are bored deep into the propria. Scale bar: 33 µm; *Fig. 7.* Histological changes in the attacked propria layer around the metacestode. Congested capillaries filled with accumulated erythrocytes (arrows) and sera (arrowhead). Scale bar: 33 µm; *Fig. 8.* Changes in the host tissue. A foreign-body-type giant cell (arrow) and cross-section of a dilated capillary (arrowhead) in the disintegrated propria around the metacestode (m). Scale bar: 10 µm

(Kozicka, 1971; Dubinina, 1987; Scholz et al., 2004) agree that the shape and measurements of rostellar hooks are the most appropriate tools for identification of gryporhynchid metacestodes. The shape and measurements of hooks of specimens found in this study and also of those found by Molnár (1970) correspond to the data given by Dubinina (1987) for *N. cheilancristrotus* but seem to be somewhat larger than those given by Kozicka (1971) and Scholz et al. (2004). Although there are uncertainties in the exact identification, the species found has been tentatively designated as belonging to *N. cheilancristrotus*. As the main objective of this paper is to examine the pathological effect of the metacestodes, no detailed taxonomic studies have been performed. The author of this paper is of the opinion that, owing to the use of molecular biological methods instead of the lengthy morphological examinations and the difficult experimental infections, substantial progress can be made in clarifying the taxonomy of gryporhynchid metacestodes in the near future.

The histological findings obtained in this study show that *N. cheilancristrotus* is a truly pathogenic species causing degeneration and inflammation in the intestinal wall. While the majority of cestodes inhabiting the gut of fish live in the lumen of the gut and attach more or less firmly to the gut epithelium with their scolices, *N. cheilancristrotus* intrudes into deeper layers of the gut wall and, breaking through the epithelium, is located in the lamina propria of the mucosa layer. Karanis and Taraschewski (1993) described that, of the large-sized cestodes of cyprinids, the scolex of *Caryophyllaeus laticeps* caused only local compression of the host's gut epithelium and at the site of attachment of these cestodes vacuolation of the epithelial cells and rupture of the brush border could be observed. In a similar way only minor changes were found in common carp infected by *Khawia sinensis*. Morley and Hoole (1995) saw only minimal changes around the attachment site of the worm, characterised by flattening of the epithelium. In more chronic cases vacuolation and detachment of the epithelial cells also occurred. Studying the pathological and cellular changes occurring around the site of scolex attachment in *Bothriocephalus acheilognathi* infection of the common carp, Hoole and Nisan (1994) found no substantial damage to the intestinal epithelium. However, Mackiewicz et al. (1972), who made a comparative pathological study on the mode of attachment and scolex morphology of 15 caryophyllid species, found that some cestodes, among them *Hunterella nodulosa* and *Monobothrium ulmeri*, could break through the intestinal epithelium and, as a result, the scolices of these worms resided in deeper layers of the mucosa. A similar way of host–parasite interface has been recently registered by Molnár et al. (2003), who found that the scolices of *Atractolytocestus huronensis*, a parasite newly introduced to Hungary, intruded deeply under the epithelial layer. There are, however, great differences between the location of *N. cheilancristrotus* metacestodes and that of *A. huronensis*. While the scolices of *A. huronensis* pushed the epithelium at the interface into the deeper layers, caused

mechanical damage to the epithelial cells only, and were always demarcated from the host's propria by the remnants of the basement membrane, the metacestodes of *N. cheilancristrotus* bore themselves deep into the propria and came into direct contact with connective tissue cells and capillaries. The pathological effect of metacestodes was characterised by mechanical damage of the cells surrounding the metacestode, by lysis of these cells and by the formation of giant cells. Cestodes do not have an intestinal tract; therefore, the damage caused in the tissues cannot serve the purpose of enabling direct feeding of the cestode; however, it cannot be ruled out that the mechanical and probably enzymatic rupture of the host cells might provoke a release of nutrients from the host cells, which nutrients are necessary for the metacestode to survive. Cases when host tissue was found in the suckers or hooks were bored in the tissue might represent the process of introduction, while cases when larvae were found in the parasitic hole with invaginated rostellum might represent a resting situation. Although minor extravasation of red blood cells was frequently seen, no haemorrhage was found around the worms. Macroscopic changes of the gut result probably from the dilatation of capillaries and cell proliferation inside the propria layer of the intestinal folds. Up to this time, the pathogenic effect of gryporhynchid metacestodes in the intestine has been studied thoroughly by Körting (1984), who found metacestodes of a closely not identified dilepidid (gryporhynchid) species in a similar location, deep in the subepithelial layer of the gut of common carp and tench. The above author described haemorrhage, necrosis and cellular host reaction, and attributed this infection to enteritis observed in the target fishes. Infection with gryporhynchid cestodes is rather common in the gut, abdominal cavity and gallbladder of fish living in the natural waters of Hungary. Since the first paper (Molnár, 1970) was published, these larval stages have continuously been detected in unpublished faunistic studies and veterinary surveys. Only a single case was thoroughly examined, when intensive infection and encapsulation of *Paradilepis scolecina* (Rudolphi) was observed in the abdominal cavity of the common bream. The host reaction against the two related species is rather different. While *N. cheilancristrotus* infection was characterised by degenerative processes without an observable host response, *P. scolecina* always evoked a host reaction which resulted in the formation of a connective tissue capsule around metacestodes. The absence of a connective tissue capsule around metacestodes in the gut wall is rather unique as other parasites such as the migrating larvae of the nematodes *Anguillicola crassus* or *Raphidascaris acus* are always intensively demarcated from the surrounding tissues by granuloma formation and fibrotic capsules (Molnár, 1994; Valtonen et al., 1994). Although in the observed case no general symptoms of disease were recorded, histological evidences show that gryporhynchid metacestodes should be regarded as pathogenic parasites of fishes, and their systematics and host–parasite relationship deserve closer attention.

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