

Pathology of *Atractolytocestus huronensis* Anthony, 1958 (Cestoda, Caryophyllaeidae) in Hungarian pond-farmed common carp

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Abstract

The caryophyllidean tapeworm *Atractolytocestus huronensis*, which was first detected in Hungary in the intestine of pond-farmed common carp (*Cyprinus carpio*) in 2001, has rapidly spread throughout the country and is now present in the common carp stock of several fish farms. This parasite has also been detected in market-size common carp imported from the Czech Republic. The cestode can infect young fry of a few weeks of age and older age groups alike. *A. huronensis* specimens measuring 0.7–1.5 cm characteristically colonise the proximal segment of the common carp intestine containing also intestinal crypts, where they are permanently attached to the intestinal wall by forcing their muscular, spear-shaped head into the gut mucosa and then changing it into a widening cone shape. The scolex, which presumably makes its way into the mucosa through an intestinal crypt, causes atrophy and disruption of the intestinal epithelium; as a result, it will be separated from the lamina propria of the mucous membrane by the basement membrane only. The basement membrane surrounds the scolex, and only a few islets of degenerated epithelium can be seen between the worm and the membrane. The gut epithelium coming into contact with the strobila of the tapeworm is flattened and the cytoplasm of epithelial cells is degenerated. In the affected areas, large numbers of cell nuclei not surrounded by cytoplasm can be seen. Some of these nuclei exhibit karyorrhexis but inflammatory changes cannot be detected. Tapeworms in the gut lumen are surrounded by numerous cellular elements including tissue cells with damaged cytoplasm, red blood cells, lymphocytes and macrophages; however, eosinophilia usually seen in cestode infections cannot be demonstrated. Up to this time no losses due to *Atractolytocestus* infection were recorded.

Key words

Cestoda, Caryophyllaeidae, *Atractolytocestus*, pathology, histology, common carp

Introduction

The detection of a caryophyllidean tapeworm hitherto unknown in Central Europe in the gut of pond-farmed common carp (*Cyprinus carpio*) in several fish farms of Hungary has been reported in another paper (Majoros *et al.* 2003). The authors have identified this parasite as the species *Atractolytocestus huronensis* Anthony, 1958, recorded mostly in North America. The European occurrence of this parasite has briefly been mentioned by Chubb *et al.* (1996), and Kirk *et al.* (2003) in Symposium abstracts, and by Hoole *et al.* (2001) who in their book on the diseases of cyprinid fishes reported, that the worm was first detected in Britain in 1993. Of the cestodes described from the gut of the common carp in Europe

only *Khawia sinensis* and *Bothriocephalus acheilognathi* have economical importance in fish farms. These latter parasites were brought into Europe during the introduction of the Amur wild carp (*Cyprinus carpio haematopterus*) and the grasscarp (*Ctenopharyngodon idellus*) (Malevitzkaya 1958, Musselius 1967). Before the introduction of the above two parasites, *Caryophyllaeus laticeps* (Pallas) parasitising the intestine of the European common carp subspecies (*Cyprinus carpio carpio*) in adult form was regarded as the commonest tapeworm species (Schäperclaus 1954). Subsequently, Russian authors (Bauer *et al.* 1969) suggested that the common carp was parasitised by *Caryophyllaeus fimbriceps* Annenkova-Chlopina, a species well distinguishable from *C. laticeps*.

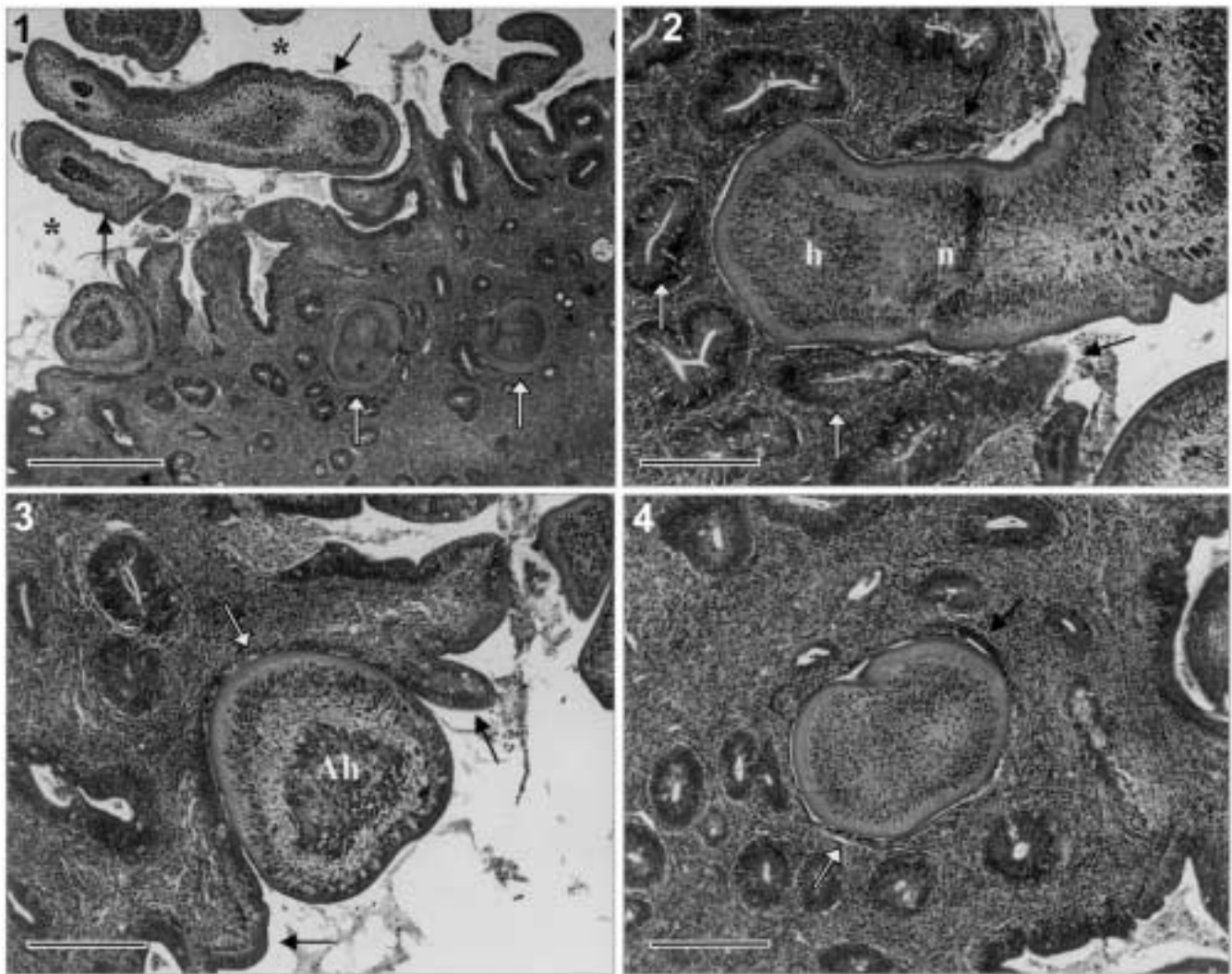
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This paper describes the pathological changes induced by the scolex of *A. huronensis* piercing into, and by its body adhering closely to, the intestinal wall of common carp.

Materials and methods

The test material comprised common carp regularly submitted to the Central Veterinary Institute for examination in the

framework of health surveys, as well as common carp (*Cyprinus carpio* L.) specimens taken from some infected farms selected for sampling. Fish were sent to the laboratory alive and killed just before examination. During the parasitological examination the intestines were cut open and examined under stereomicroscope. The tapeworms were collected, placed into 0.65% isotonic saline solution, freed from the adhering mucus by gentle shaking, and their measurements were taken. After taking photographs with an Olympus C4040



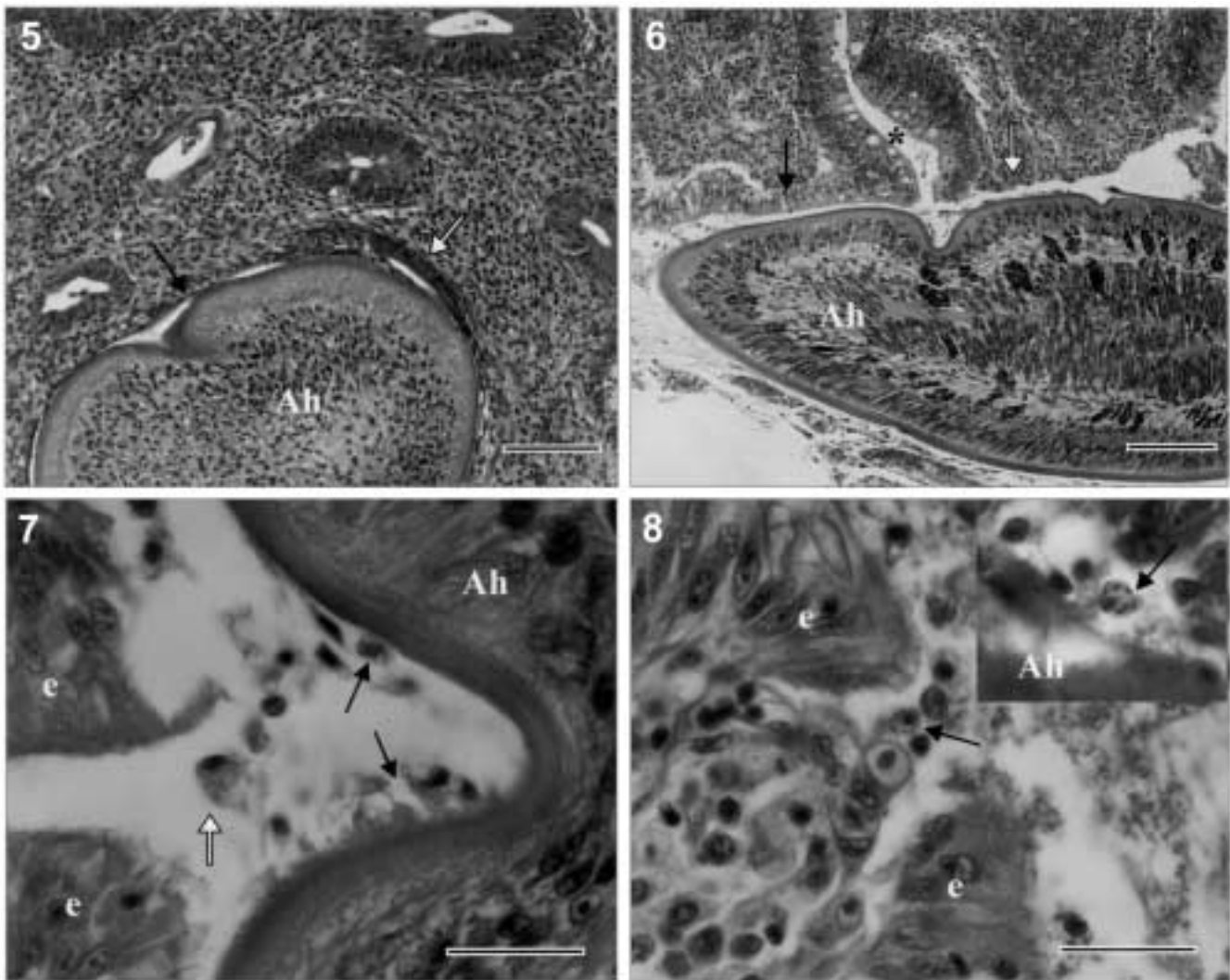
Figs 1–4. Histological sections made from the foregut of common carp infected with *Atractolytocestus huronensis*. Haematoxylin and eosin staining: **1.** In the gut lumen (*) transsected parts of the non-segmented strobilae of *A. huronensis* specimens (arrows), while in the crypt-containing mucosa layer of the gut wall cross-sectioned parts of the worm scolex (white arrows) can be seen. Scale bar = 500 μ m. **2.** Longitudinal section of the head (h) and neck (n) of *A. huronensis* having penetrated into the deep layers of the mucosa containing crypts (white arrows). At the site of penetration the injured epithelium (arrows) is still discernible, but the scolex is separated from the lamina propria of the mucosa by the basement membrane only. Scale bar = 200 μ m. **3.** Transverse section of the body of *A. huronensis* (Ah) in the area of penetration into the mucosa. Close to the surface the intestinal folds are still covered by relatively intact epithelium (arrow), at the bottom of the fold the epithelium becomes thinner then disappears (white arrow), and the worm gets close to the propria. Scale bar = 200 μ m. **4.** Cross-section of the head of an *A. huronensis* tapeworm having penetrated into the intestinal mucosa. The worm is surrounded by a thin layer of host origin, formed from the basement membrane. Within the membrana basalis formed zone, here and there shreds of the epithelium of the disrupted crypt (arrow) or its remnants (white arrow) can be seen. Scale bar = 200 μ m

digital camera mounted on a stereomicroscope, within a short time 1 to 3 cm long pieces of the proximal intestinal segments containing the tapeworms were fixed for 4 hrs in Bouin's solution partly in their entirety, partly in unopened condition and partly after having been cut open longitudinally and coiled up. After several cycles of washing with 80% ethanol and embedding in paraplast the pieces of gut were sectioned in different planes. The 5 µm thick sections were stained with haematoxylin and eosin solutions. Photomicrographs were taken

with an Olympus DH-10 digital camera mounted on an Olympus BH2 microscope.

Results

After their first detection in July 2001, specimens of the cestode *A. huronensis* were found on several occasions during the dissection of common carp submitted for routine diagnostic



Figs 5–8. Histological sections made from the foregut of common carp infected with *Atractolytocestus huronensis*. Haematoxylin and eosin staining: **5.** The scolex (Ah) is separated from the connective tissue cells of the lamina propria by the basement membrane of eosinophilic staining (arrow), which can be found in its original function, i.e. at the base of the epithelial cells (white arrow), only in the area where epithelial remnants of disrupted crypts occur. Scale bar = 30 µm. **6.** Cross-section of a body part of *A. huronensis* (Ah) located in the gut lumen, adhering to the intestinal wall. At some sites of attachment only flattening of the epithelium (arrow) while in other areas complete absence of the epithelium (white arrow) can be observed. In the intestinal crypt (*) the epithelium is relatively intact. Scale bar = 100 µm. **7.** Note cells with degenerated cytoplasm (arrows) that got into the gut lumen through the injured epithelial surface (e) and adheres to the tegument of the worm (Ah). Among them a monocyte (white arrow) can also be seen. Scale bar = 20 µm. **8.** Cells of lysed cytoplasm have got into the gut lumen through the parasite-induced injury of the intestinal epithelium (e). The nucleus of one of the cells clearly shows signs of necrosis by karyorrhexis (arrow). Scale bar = 20 µm. Inset: Necrosis by karyorrhexis in a host cell located close to the worm tegument (Ah). Scale bar = 20 µm

examination from fish farms located in different areas of Hungary. These worms were equally found in common carp fry having already changed over to feeding on benthos and in several years old common carp. During the survey conducted in 2002, fish from 14 ponds of 9 fish farms proved to be infected. In some ponds the infection had low prevalence and intensity while in others the prevalence of infection came close to 100% and the intensity varied between 1 and 32 worms per fish (average 14). The parasite was detected on several occasions also in market-size common carp imported from the Czech Republic and not placed out into ponds, in addition to samples submitted from Hungarian pond farms.

The tapeworms removed from the gut wall and placed into physiological saline were 7–15 mm in length and 1–1.5 mm in width in live stage. With the exception of their cephalic end, their body was dorsoventrally flattened. The scolex was cylindrical in cross-section and tapered off in apical direction like a cone. It was separated from the strobila by a tapering muscular neck. The surface of the scolex was smooth, and no suckers, bothridia or carnation-like widened structures could be seen on it. The scolex of moving worms was sometimes narrowed and assumed a spear shape, while at other times it had shrunk into a mushroom-shaped structure.

The worms were located in the foregut, often in the most proximal 3 to 5 cm segment thereof, piercing their scolex into the deep layers of the mucous membrane. That part of the intestine is characterised by the presence of numerous intestinal crypts located close to one another, in addition to intestinal folds generally typical of the gut of cyprinids. Presumably through the intestinal crypts, the worms forced their way deep into the lamina propria of the intestinal mucosa with their scolex, and occasionally they reached even the submucosa. There they became permanently attached and could be removed only with a forceps or by scraping off the mucosa.

In histological sections made from the foregut the non-segmented strobilae of the worms sectioned in more or less longitudinal direction could be seen in the gut lumen and the cross-sectioned pieces of the cephalic part and neck of the worms in the intestinal wall (Fig. 1). The cephalic part and thin neck of the worms, abounding in muscle cells and distinguishable from the body of the worm by its eosinophilic staining, was always found in the deeper layers of the intestinal mucosa (Figs 2 and 4). In such cases the disrupted columnar epithelium was still discernible at the site of worm penetration which presumably took place through an intestinal crypt. However, in the deeper layers the scolex was surrounded only by the basement membrane, which separated it from the lamina propria of the intestinal mucosa (Fig. 2). At the site of worm penetration into the mucous membrane (Fig. 3) the damage of the epithelium was well visible. In areas more distant from the point of entry the gut wall was still covered by an intact epithelium which gradually became narrower, and at the site of penetration only epithelial debris, extravasated red blood cells and damaged connective tissue cells could be found. The scolex having penetrated into the mucosa was surrounded by small islets constituted by shreds of epithelium or

tissue debris of epithelial origin, which replaced the previously intact epithelium of the intestinal crypts. In places where the basement membrane was damaged, the scolex came into direct contact with the damaged connective tissue cells and blood vessels of the mucosa (Fig. 4). By higher magnification it was clearly seen that the scolex of the parasite and the propria cells of the mucosa were separated only by the basement membrane showing eosinophilic staining. The integrity of this layer was interrupted only by remnants of the crypt epithelium, where the basement membrane occurred in its original location, at the base of the epithelial cells (Fig. 5). In places more distant from the attachment points of scolices, where the less eosinophilic segments of the strobila containing vitelline glands and sexual products were located, the epithelium appeared mostly intact. However, in some places coming into direct contact with the body of the worm thinning, balloon-like transformation and degeneration of the epithelium were observed. Moreover, in one case the complete absence of epithelium was noticed in a segment where the worm tegument was in direct contact with the connective tissue cells and damaged capillaries of the lamina propria (Fig. 6). In intestinal segments infected with the tapeworm, especially in areas surrounding epithelial injuries, large numbers of cells with degenerated cytoplasm and exposed nuclei occurred freely in the gut lumen. These had probably originated from the damaged epithelium, connective tissue and red blood cells (Figs 7 and 8). A few lymphocytes and monocytes (Fig. 7) could only occasionally be recognised among these structures. Here and there, these cells released into the gut lumen through the damaged epithelium were adhered directly to the tegument of the worm; however, their type could not be determined (Fig. 7). Despite the lytic disruption of the cytoplasm, in some degenerated cells disruption of the nucleus by karyorrhexis could also be observed (Fig. 8 and inset).

Discussion

Two parasitic species of the genus *Atractolytocestus*, *A. huronensis* Anthony, 1958 reported from Canada and the United States and *A. sagittatus* (Kulakovskaya and Akhmerov, 1965) described from the Far East are known at present. The authors first describing them (Anthony 1958, Kulakovskaya and Akhmerov 1965) originally detected both species in common carp (*Cyprinus carpio*). Of the two species, *A. huronensis* has been recorded in Hungary and in common carp originating from the Czech Republic (Majoros *et al.* 2003). As a result of fish movements and poorly controlled fish transportations that are being carried out all over the world today, the transcontinental translocation of parasites to a new biotope has become a common event (Bauer and Hoffmann 1976, Molnár 1987). Introduction of a given parasite into a new continent is especially likely in the case of fish species bred as ornamental fish. The European common carp (*Cyprinus carpio carpio*) is particularly at risk in this respect, as it has an ornamental variation known as 'koi', which has been bred from the Asian sub-

species (*Cyprinus carpio haematopterus*). Therefore, the introduction of this parasite was not unexpected, though that of the species *A. sagittatus*, which had already become naturalised in the eastern part of Europe, seemed much more likely. The species *A. sagittatus* was described by Kulakovskaya and Akhmerov (1965) by the name of *Markevitschia sagittata* from the river Amur. Subsequently its occurrence was reported by Demshin and Dvoryadkin (1981), who detected *M. sagittata* in Amur wild carp introduced to the Astrakhan territory of the Soviet Union. This species differs from *A. huronensis* by the large number of its testes. Namely, the latter species possesses only 8–14 testes and, according to Jones and Mackiewicz (1969), is unique in its triploidy and parthenogenesis. The appearance of the species in Britain and Central Europe is difficult to explain, as common carp and cyprinids are only rarely imported from America, while the importation of Far Eastern cyprinids, primarily coloured carp (koi), is an everyday activity.

Data on the pathological mechanism of caryophyllid cestodes were presented by Mackiewicz *et al.* (1972), who made a comparative pathology study on the mode of attachment and scolex morphology of 15 caryophyllid species among them *A. huronensis*. These authors found that caryophyllid species without attachment organs as *A. huronensis* and *Hunterella nodulosa* could cause considerable pathology at the attachment point. They reported about some mechanical displacement and epithelial loss adjacent to the scolex proper and a narrow eosinophilic interface layer at the neck region which we identify with lamina propria.

Apart from the above study, the role played by this parasite in pond farms and the pathological changes caused by it in common carp can mostly be revealed by studies on the similarly caryophyllidean species *Caryophyllaeus* and *Khawia* occurring in similar location and on *Bothriocephalus acheilognathi*, the commonest tapeworm of carp.

Vikhman and Kapustina (1975) reported that in *Khawia sinensis* infection the location of the strobila along the intestinal wall resulted in mucoid degeneration of the intestinal epithelium, increase of mucus secretion, and infiltration by leukocytes. According to Karanis and Taraschewski (1993), in *Caryophyllaeus laticeps* infection of cyprinids the scolices of the worms caused local compression of the host's gut epithelium at their site of attachment, where vacuolation of the epithelial cells and rupture of the brush border could be observed. The appearance of large masses of eosinophilic granulocytes could also be seen in the affected segment of the gut. The above authors also observed granuloma formation in chronic cases. Morley and Hoole (1995) saw similar changes in common carp intestine colonised by *K. sinensis*. Around the attachment site of the worm only minimal changes, characterised by flattening of the epithelium, were seen. Electron microscopic examination revealed disappearance of the microvilli of epithelial cells and compression of the mucosa. In more chronic cases vacuolation and detachment of the epithelial cells also occurred. As a sign of cellular host response, lymphocytes, macrophages and eosinophilic granulocytes

entered the gut lumen and accumulated on the tegument of the worm and in the space between the gut and the worm. Hoole and Nisan (1994) observed similar pathological and cellular changes limited to areas around the scolex attachment site in *B. acheilognathi* infection of the common carp. In the present case, massive macrophage and lymphocyte infiltration of the gut lumen could not be demonstrated by the histological techniques applied, and these examinations did not confirm the appearance of eosinophilic granulocytes either. It appears that the scolex of *Khawia* and *Caryophyllaeus* species having a broad interface with the host and that of *Bothriocephalus* species carrying bothridia can ensure worm attachment without causing substantial damage to the intestinal epithelium. At the same time, in the case of the species *Anantrum histocephalum* having a cap-like scolex and a thin neck, Jensen and Heckmann (1977) observed that the cephalic end of the worm was embedded in the gut wall and a fibrous capsule was formed. Similar locations involving the deep layers of the gut wall have been reported by Heckmann (2000) also for *B. acheilognathi*. In that case, penetration of the scolices into the hepatic parenchyma was observed; however, this latter observation should be accepted with some reserve, as other authors have not reported similar cases, and worm cross-sections detected in deep layers of the gut wall and in the liver in histological sections were more likely attributable to a simultaneously existing proteocephalid infection. In the present case, the attachment of *A. huronensis* in the intestine resembles the mechanism of attachment of *A. histocephalum*. Namely, as reported by Kulakovskaya and Akhmerov (1965), Demshin and Dvoryadkin (1981) as well as Scholz *et al.* (2001), the morphology of the cephalic end of *A. sagittatus* varies from a narrowing spear shape to a mushroom-like widened structure. In our opinion, the former shape enables penetration into crypts of the intestinal mucosa while the latter facilitates attachment in the deep layers of the intestinal wall. Our results indicate that, despite its smaller size, species *A. huronensis* may have a more important pathological role than *K. sinensis*, and it can cause much more severe histopathological changes than the *Caryophyllaeus*, *Khawia* or *Bothriocephalus* species reported from the European common carp earlier. Namely, due to its form of attachment, *A. huronensis* gives rise to local tissue destruction in addition to the epithelial lesions caused by the species listed above. By widening into a mushroom-like shape its scolex lodged deep in the intestinal crypts, *A. huronensis* rather securely attaches itself to the intestinal epithelium and can be removed from there only with difficulty. The scolices, which are much wider than the crypts, mechanically disrupt the crypt epithelium and the capillaries running beneath them, and thereby cause much more severe histopathological changes at the site of penetration than *Caryophyllaeus* and *Khawia* species adhering to the epithelium more superficially and loosely, with a broad interface, and than *Bothriocephalus* species that attach themselves to the epithelium only by the suction effect of the bothridia. It appears that, despite the obvious epithelial injuries, the *Atractolytococestus* scolex having penetrated the epithelium does not

come into direct contact with the lamina propria of the mucous membrane. The flexible collagenic elements of the basement membrane surround the scolex also in areas devoid of epithelium, and accompany it to the deeper layers of the mucosa. Obviously, smaller or larger injuries may occur also on the basement membrane, as suggested by the presence of cell debris in the gut lumen. The effect exerted by the body of the worm on the host's epithelium cannot be fully explained. In the majority of cases the epithelium surrounding the worm showed no changes and its brush border was also normally developed. The epithelium was flattened in all cases when the body of the worm was pressed directly to it. Direct contact between the worm surface and an area devoid of epithelium was observed in a single case only; therefore, this loss of epithelium cannot be attributed to the worm with certainty, and a possible histological technical defect cannot be ruled out. At the same time, it is known that tapeworms ingest nutrients by digesting the intestinal content and partially by damaging the intestinal wall with the help of their proteolytic enzymes while they protect themselves from the effect of host-produced proteolytic enzymes with their protease inhibitors (Matskási 1978, 1984). Mackiewicz *et al.* (1972) supposed that proteolytic enzymes or other lytic secretion played a role in pronounced tissue reaction. On the basis of the degeneration of the epithelial layer and plasmolysis of cells of the lamina propria we cannot exclude that the proteolytic enzymes of *A. huronensis* cause more substantial tissue damage than that reported by Matskási (1984) for *B. acheilognathi*. Therefore, in addition to deprivation of the host of nutrients and the mechanical destructive effect of the scolex, sometimes local tissue damage caused by the digestive enzymes released by the worm should also be reckoned with. In an intensive infection, the epithelial changes and local tissue injuries caused by *A. huronensis* are important pathological factors already in themselves; however, the possibility of complications due to facultative pathogenic bacteria should also be taken into account.

According to the results of our studies, besides Hungary the parasite is widespread also in the Czech Republic, as demonstrated by its frequent detection during the routine examination of market-size common carp imported from that country. With the appearance of *A. huronensis* in Hungary a new parasite has been added to the parasite fauna of cultured common carp, and in the short time that has elapsed since its detection the rapid spread of this tapeworm in the Hungarian fish farms could be demonstrated. The type of reproduction and site of colonisation of the parasite are identical with those of the already naturalised *Khawia sinensis*. Only time can tell whether an antagonism like that observed between *K. sinensis* and *Caryophyllaeus fimbriceps* will develop between the two parasites, or rather a combined damage-causing effect of the two cestodes will have to be reckoned with. Namely, according to our unpublished observations, since the appearance of *K. sinensis* in Hungary it has almost completely displaced the previously common species *C. fimbriceps*, which can now be detected only in fish stocks living in natural waters.

More data are necessary to evaluate economical importance of *A. huronensis* and possible losses in pond farms.

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