Opis przypadku *Case report*

Atractolytocestus huronensis (Cestoda: Caryophyllidea): a new parasite of common carp in Poland and its co-occurrence with bacteria. Case report

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Summary

This study describes the first documented case of the tapeworm *Atractolytocestus huronensis* in common carp (*Cyprinus carpio*) from a small water reservoir in the Lublin region, Poland. A total of 28 tapeworms were found in the gastrointestinal tract of the examined fish. Molecular analysis of the *COX1* and *ITS2* gene regions showed a high similarity of sequences between the isolated tapeworm and previously identified *A. huronensis* strains from Slovakia, the USA, South Africa, and the UK, with similarities ranging from 97.87% to 99.25%. The *COX1* sequence has been deposited in the GenBank database under accession number PV275034, and the *ITS2* sequence is available under number PV265466. The observed clinical symptoms in the infected fish included skin redness and protrusion and redness of the anus. The gills of the fish were pale. Examination of the digestive tract revealed that its walls were edematous, and the mucous membrane showed small hemorrhages and congestion at the sites of tapeworm infestation. No visible changes were observed in the internal organs such as the liver, kidneys, and spleen. Bacterial infections were also identified, including *Aeromonas veronii* and *Shewanella putrefaciens* isolated from skin swabs, and *Aeromonas sobria*, *Pantoea agglomerans*, and *Pseudomonas anguilliseptica* identified in internal organs (kidneys and liver). This discovery represents the first report of *A. huronensis* in Poland and highlights the potential impact of parasitic and bacterial infections on the health of carp in confined water systems.

Keywords: *Atractolytocestus huronensis*, carp parasites, tapeworms

Atractolytocestus huronensis Anthony, 1958 is a monozoic (non-segmented) tapeworm belonging to the order Caryophyllidea Van Beneden (in Carus, 1863), family Lytocestidae Wardle et McLeod, 1952, and the genus *Atractolytocestus* Anthony, 1958. Currently, three species are recognized within the genus *Atractolytocestus*: *A. sagittatus* (syn. *Markevitschia sagittata* Kulakovskaya et Akhmerov, 1965), *A. tenuicollis* (syn. *Khawia tenuicollis* Li, 1964), and *A. huronensis* (type species) (38).

The geographic range of *A. sagittatus* is restricted to the Russian Far East, the Caspian Sea basin, and the Japanese lakes Suwa, Takkobu, and Biwa. *A. tenuicollis* is found exclusively in China. *A. huronensis* was first described in carp from the Huron River in Michigan, USA, by Anthony (3). Literature reports indicate that *A. huronensis* is widely distributed in Asia, Africa, America, China, Australia, and Europe (2, 12, 33, 34). In Europe, its presence has been recorded in carp in the United Kingdom, Czech Republic, Germany, Romania, Slovakia, Croatia, and Hungary (4, 5, 7, 13, 16, 17, 23, 24, 27, 29). Partial sequences of the mitochondrial cytochrome c oxidase subunit I (*COX1*) gene suggest that the introduction of the tapeworm into Europe was the result of independent introductions into Great Britain and Continental Europe (5).

A. huronensis parasitizes the intestines of freshwater fish, especially in carp farms (*Cyprinus carpio*). This species has also been detected in *Abramis brama* and *Rutilus rutilus* in Europe (14, 26). Furthermore, Dos Santos and Avenant-Oldewage (11) described the presence of juvenile forms of *A. huronensis* in *Labeobarbus aeneus* in South Africa. The development of *A. huronensis* involves two hosts. The intermediate hosts are oligochaetes, such as *Tubifex* and *Limnodrilus*, where the larval stages of the parasite develop. The presence of these organisms as transmission vectors has been confirmed both in natural environments and under experimental conditions. After ingestion of infected oligochaetes by fish, which serve as definitive hosts, the larvae settle in the fish's intestines, where they reach maturity and continue their life cycle (10, 21, 22). Tapeworms (5-12 × 0.5-1.2 mm) in the fish's digestive tract are deeply attached to the epithelium and cause local tissue necrosis (28). Mass infestations of this parasite can affect fish health and cause significant economic losses.

In Poland, two genera of tapeworms from the family Lytocestidae are present: *Caryophyllaeides* Nybelin, 1922 and *Khawia* Hsü, 1931 (31). To the best of the authors' knowledge, the occurrence of tapeworms from the genus *Atractolytocestus* has not been previously reported in Poland. This paper aims to fill this knowledge gap by presenting the first report of *A. huronensis* in Poland and analyzing the potential implications of this discovery for local aquatic ecosystems and the fishing industry.

Material and methods

In May 2023, one diseased common carp (*C. carpio*) weighing 1.2 kg was brought for examination to the Department of Biology and Fish Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Poland. Diseased fish were euthanized in accordance with American Veterinary Medical Association (AVMA, 2020) (1). After euthanasia, a necropsy was performed, during which the gastrointestinal tract was removed, and the intestines were examined for the presence of intestinal parasites. The isolated tapeworms were preserved in 10% neutral buffered formalin for morphological analysis and in 96% ethanol for further molecular studies. Microphotographs of the parasites were taken using an Evolution 100 microscope (Delta Optical, Poland), operated with DLT-Cam Pro software (Delta Optical, Poland). Preliminary identification was based on the taxonomic keys of Pojmańska (31), and further identification followed those of Oros et al. (27), Králová-Hromadová et al. (19), Bazsalovicsová et al. (5). All research procedures were conducted in accordance with institutional guidelines and the regulations of the Ethics Committee, ensuring compliance with the current standards regarding animal welfare.

Genomic DNA was extracted from isolated tapeworm using the Genomic Mini Kit for genomic DNA purification from various sources (A&A Biotechnology, Poland), according to the manufacturer's instructions and described by Pastuszka et al. (29). For genetic identification, two gene loci were amplified by PCR: the internal transcribed spacer 2 (*ITS2*) gene and the cytochrome c oxidase subunit 1 (*COX1*) gene, using the primer pairs described previously (5, 19).

For the amplification of the partial *COX1* gene, the forward primer CFCYT2 (5'-ACTAAGTGTTTTCAAAA-3'), which binds to the tRNA-Trp (Tryptophane), and the reverse primer CRCYT2 (5'-CCAAAAAACCAAAACAT-3') were applied as described Bazsalovicsová et al. (5). For amplification of the complete ribosomal *ITS2* spacer, the 5.8S-2 (5'-GTCGATGAAGAGCGCAGC-3') (18) and *ITS2* (5'-AGGAGGCGAATCACTAT-3') (9) primers were applied as described Bazsalovicsová et al. (5). The PCR conditions for amplifying both gene clusters were as follows: an initial denaturation at 94°C for 5 minutes, followed by 40 cycles consisting of 1 minute of denaturation at 94°C, 1 minute of anneling at 50°C and 2 minutes of extension at 72°C, with a final extension at 72°C for 7 minutes. The PCR products were visualized on a 1.5 agarose gel stained with Simply Safe (EURx, Poland), and the amplicons were purified using the Gel-Out Kit (A&A Biotechnology, Poland), according to the manufacturer's protocol. Sanger sequencing was conducted by Genomed (Warsaw, Poland). The resulting sequences were assembled using FinchTV 1.4.1 and BioEdit software, followed by identification through comparison using the BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences were also submitted to the GenBank database through NCBI (https://www.ncbi.nlm.nih.gov).

For phylogenetic analysis, sequence alignment was performed using ClustalW, and cluster analysis was carried out with MEGA11. Phylogenetic trees were constructed using the neighbour-joining method with 500 bootstrap iterations, incorporating both the obtained sequences and their closest matches. Evolutionary distances were calculated using the Maximum Composite Likelihood method.

Swabs from the skin, liver, and trunk kidney were collected aseptically for bacterial isolation and characterization. The samples were streaked onto tryptic soy agar (TSA) (Sigma-Aldrich, USA) and incubated at 28°C. The resulting colonies were identified using MALDI-TOF MS (matrix-assisted laser desorption/ionization – time-of-flight mass spectrometry) (Bruker Daltonik GmbH, Bremen, Germany), as described by Pastuszka et al. (29). Mass spectra of the bacteria were analyzed using the MALDI Biotyper 3.1. The interpretative score criteria were applied as recommended by the manufacturer: a score ≥ 2.300 indicates highly probable species-level identification; a score from 2.000 to 2.299 indicates probable species and secure genus identification; a score between 1.700 and 1.999 indicates probable genus identification; and a score < 1.700 indicates unreliable identification.

Results and discussion

This study describes the first case of *Atractolytocestus huronensis* tapeworms found in carp (*C. carpio*) kept in a small water reservoir in the Lublin region during the summer season. The investigation revealed the presence of 28 *A. huronensis* tapeworms in the gastrointestinal tract of the examined fish (Fig. 1). This is the first documented record of this tapeworm species in Poland. The external examination of the diseased fish revealed emaciation, congestion on the body surface, at the base of the pectoral and pelvic fins, and around the anus, which was swollen (Fig. 1). The gills of the fish were pale. Examination of the digestive tract revealed that its walls were edematous, and the mucous membrane showed small hemorrhages and congestion at the sites of tapeworm infestation (Fig. 1). No visible changes were observed in the internal organs such as the liver, kidneys, and spleen.

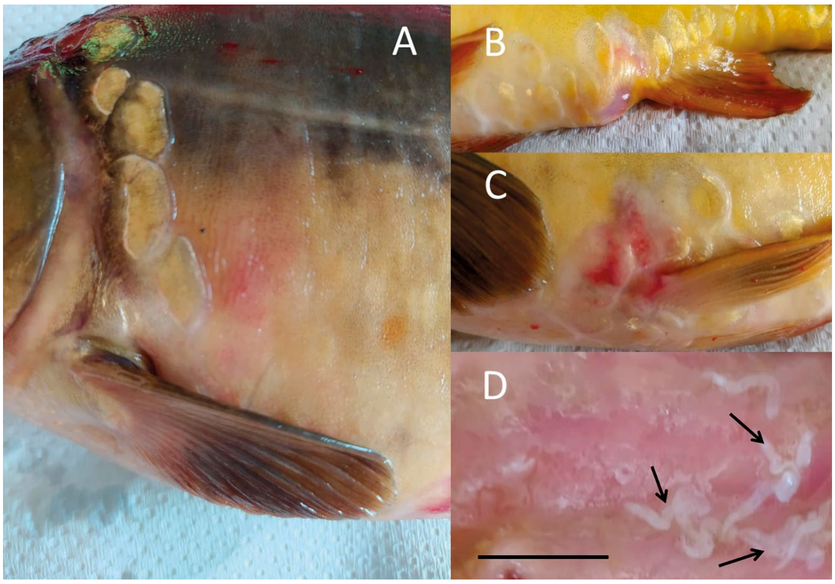

Fig. 1. Changes on the body surface and tapeworms in the digestive tract of affected fish. Skin redness (A). Anus swelling (B). Redness at the base of the pelvic fin (C). Tapeworms in the intestine (black arrows) (D). Scale bar = 10 mm

The *COX1* gene and the *ITS2* region were amplified from isolated tapeworm. BLAST analysis revealed that the *COX1* sequence is highly similar to that of *A. huronensis* (HM480475 strain isolated from *C. carpio* in Slovakia, KP635010 from *C. carpio* in South Africa, and HM480479 from *C. carpio* in United Kingdom), with the highest identities (99.25%, 99.10%, and 98.79%, respectively) (Tab. 1, Fig. 2). This sequence was deposited in the GenBank nucleotide sequence database under accession number PV275034. The *ITS2* sequence is also highly similar to that of *A. huronensis* (FJ475103 strain isolated from *C. carpio* in Slovakia, FJ475098 from *C. carpio* in Slovakia, HM480461 from *C. carpio* in USA, with the highest identities (98.03, 97.87, and 97.87, respectively)) (Tab. 2, Fig. 3). The sequence was deposited in the GenBank nucleotide sequence database under accession number PV265466.

Additionally, skin reddening and anus swelling were observed on the surface of the affected fish (Fig. 1). Bacterial isolates from the skin swabs of the diseased fish included *Aeromonas veronii* and *Shewanella putrefaciens*, while *Aeromonas sobria*, *Pantoea agglomerans*, and *Pseudomonas anguilliseptica* were found in the internal organs (kidney and liver) (Tab. 3).

The co-occurrence of pathogenic bacteria and parasites exacerbates the pathogenic effect, leading to chronic infections that can be difficult to control without appropriate combination therapies involving antiparasitic drugs and antibiotics. In the absence of

Tab. 1. Sequences deposited in GenBank to which the cytochrome c oxidase subunit I (*COX1*) gene, partial coding sequence (cds) obtained from *A. huronensis* isolate AH 01 showed the highest degree of identity

Accession number of nucleotide sequence	Description	Geographical location	Host	Percent identity (%)
HM480475	*Atractolytocestus huronensis* mitochondrial *COX1* gene, partial cds	Slovakia	C. carpio	99.25
KP635010	*Atractolytocestus huronensis* mitochondrial *COX1* gene, partial cds	South Africa	C. carpio	99.10
HM480479	*Atractolytocestus huronensis* mitochondrial *COX1* gene, partial cds	United Kingdom	C. carpio	98.79

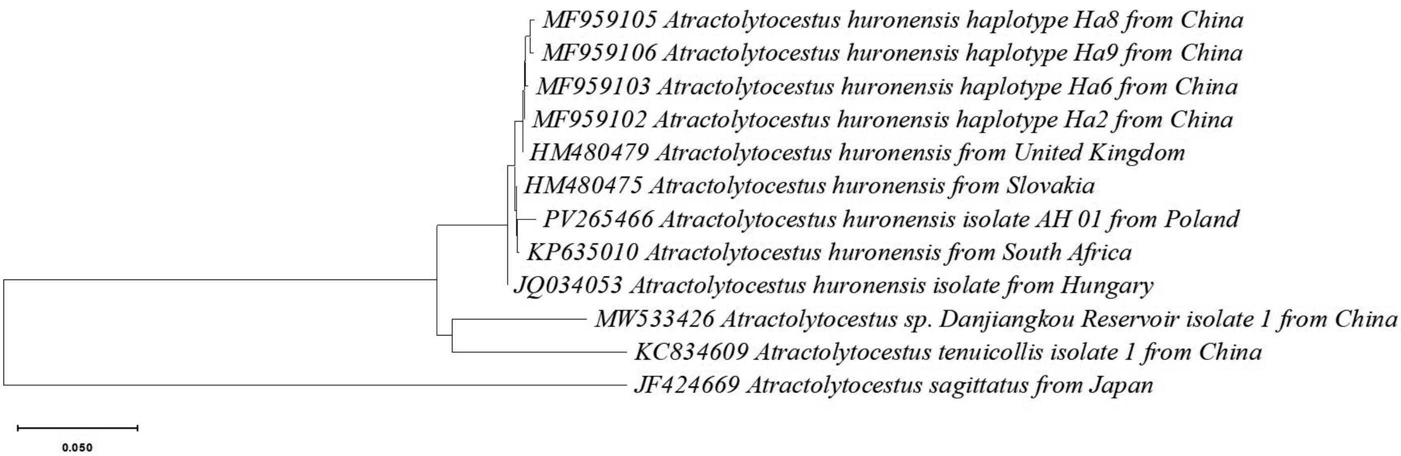

Fig. 2. Phylogenetic tree based on the partial coding sequence of cytochrome c oxidase subunit 1 (*COX1*) gene of *A. huronensis* AH 01 isolated from the isolated from common carp (*C. carpio*) intestines and the partial coding sequence of cytochrome c oxidase subunit I (*COX1*) gene of 11 other, closely related *Atractolytocestus* representatives deposited in the GenBank database

Tab. 2. Sequences deposited in GenBank to which the internal transcribed spacer 2 (*ITS2*), partial sequence obtained from *Atractolytocestus huronensis* isolate AH 01 showed the highest degree of identity

Accession number of nucleotide sequence	Description	Geographical location	Host	Percent identity (%)
EJ475103	*Atractolytocestus huronensis* isolate 3 clone 5 *ITS2*, complete sequence	Slovakia	*C. carpio*	98.03
FJ475098	*Atractolytocestus huronensis* isolate 3 clone 5 *ITS2*, complete sequence	Slovakia	*C. carpio*	97.87
HM480461	*Atractolytocestus huronensis* isolate 3 clone 5 *ITS2*, complete sequence	USA	*C. carpio*	97.87

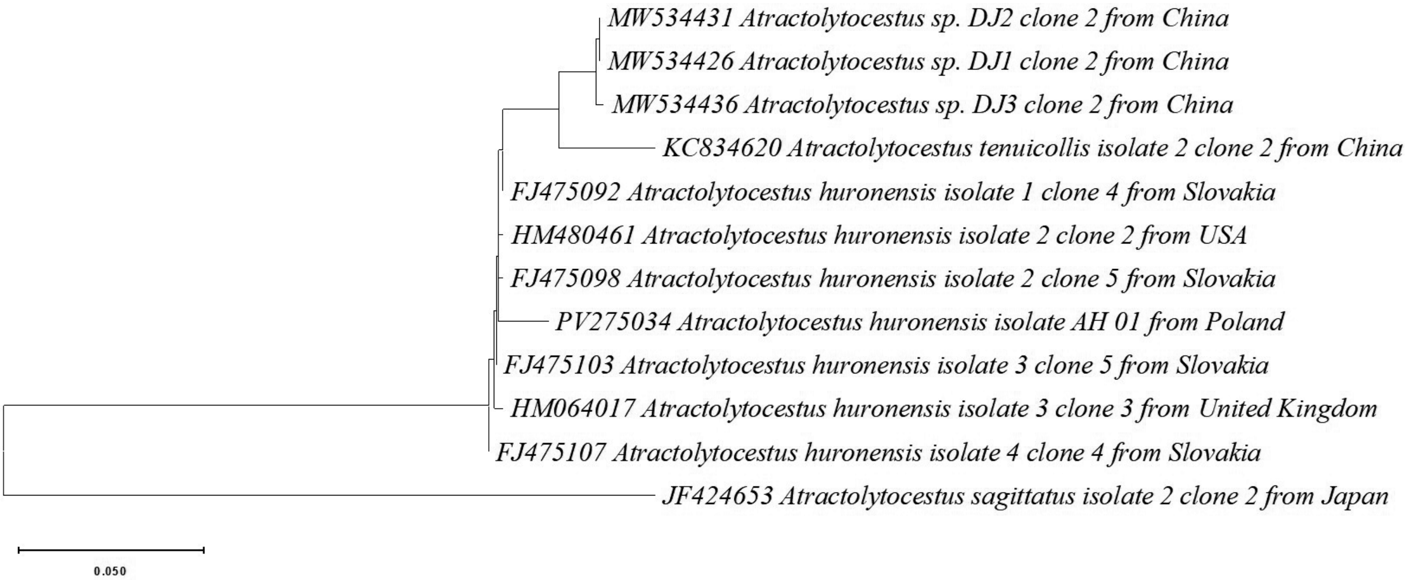

Fig. 3. Phylogenetic tree based on the partial coding sequence internal transcribed spacer 2 gene of *A. huronensis* AH 01 isolated from the isolated from common carp (*C. carpio*) intestines and the partial sequence internal transcribed spacer 2 gene of 11 other, closely related *Atractolytocestus* representatives deposited in the GenBank database

such treatment, the fish may experience prolonged stress, which affects their growth, health condition, and can lead to mass losses in aquaculture.

The presence of bacteria in the internal organs of the infected carp may be related to their translocation from the gastrointestinal tract. In fish infected with tapeworms, bacterial translocation could occur through: 1) mechanical damage to the mucosal lining, facilitating the entry of bacteria into the bloodstream, 2) changes in the gut microbiota composition favoring the development of opportunistic bacteria capable of translocation, 3) modulation of the immune system (immunosuppression or shifting the response toward Th2), 4) secretion of metabolites or enzymes weakening tight junctions between enterocyte cells, thereby facilitating bacterial translocation (35, 37). The bacteria isolated from the internal organs in this study are commonly found in environmental waters and can cause infections in both fish and humans, particularly under stressful conditions.

S. putrefaciens is a Gram-negative, facultatively anaerobic, rod-shaped bacterium that can cause infections in fish, particularly when skin damage or wounds are present, leading to putrefactive processes in tissues. In humans, it can cause skin infections, soft tissue infections, and, in rare cases, bloodstream infections, especially in immunocompromised individuals or as a result of contact with contaminated water (25).

A. veronii is a Gram-negative opportunistic bacterium that can cause diseases in fish, such as enteritis, ulcers, and skin infections. In humans, it can cause wound infections, peritonitis, and, less frequently, bacteremia, particularly in individuals with a weakened immune system (30).

A. sobria is a Gram-negative opportunistic bacterium that can cause infections in fish, especially when their immunity is weakened, leading to diseases such as skin ulcers and wound infections. In humans, it can cause gastrointestinal infections, wound infections, and, in rare cases, bacteremia (30).

Tab. 3. Identification results using MALDI-TOF-MS for isolated bacteria from diseased fish (*C. carpio*)

Isolate	MALDI-TOF MS identification		Site of isolation
	Score values	Species ID (based on MALDI Biotyper 3.1 software)	
Cc1/23	2.214	*Aeromonas sobria* CECT 4245T DSM	Liver
Cc2/23	2.087	*Aeromonas veronii* CECT 4199T DSM	Skin
Cc3/23	2.222	*Pantoea agglomerans* DSM 3493T DSM	Trunk kidney
Cc4/23	1.841	*Pseudomonas anguilliseptica* DSM 12111T HAM	Liver
Cc5/23	2.102	*Shewanella putrefaciens* CCM 2803 CCM	Skin

P. agglomerans (formerly known as *Erwinia herbicola* or *Enterobacter agglomerans*) is a Gram-negative bacterium found in both plant and aquatic environments, which can cause soft tissue infections following trauma induced by vegetation. Loch and Faisal (20) reported the isolation of *P. agglomerans* from the kidney of an infected feral brown trout (*Salmo trutta*) caught in Gilchrist Creek, Lake Huron watershed, Michigan, USA. Similarly, Saticioglu et al. (32) isolated *P. agglomerans* from diseased rainbow trout as a pathogenic agent. In humans, this bacterium can cause skin infections, osteoarticular infections, osteomyelitis, and, more rarely, bacteremia, especially in cases involving contaminated infusion fluids (8).

P. anguilliseptica is a Gram-negative bacterium that is the etiological agent of red skin disease in many fish species, such as *Anguilla japonica*, *A. anguilla*, *Acanthopagrus schlegeli*, *Plecoglossus altivelis*, *Clupea harengus*, *Sparus aurata*, *Dicentrarchus labrax*, *Scophthalmus maximus*, and salmonid fish. It causes skin diseases and internal organ damage (6).

A. huronensis is a unique parthenogenetic tapeworm widely distributed in Europe, reported in countries such as Hungary, Slovakia, Czech Republic, Germany, Croatia, and Romania (4, 5, 13, 15, 16, 36). Its presence in Poland may be the result of infected carp being transported across borders for trade purposes, suggesting a potential threat to local fish populations. The spread of this species is facilitated by the frequent transportation of carp, which are popular in aquaculture and consumption.

The findings of this study emphasize the need for monitoring the health of carp populations in Poland, with particular attention to potential threats posed by the introduction of foreign parasite species. The introduction of *A. huronensis* into Poland could have negative consequences for local aquatic ecosystems and the fishing industry. Further research is necessary to better understand the dynamics of this tapeworm's spread and to develop effective preventive and intervention strategies.

This study provides new data on the occurrence of *A. huronensis* in Poland. It also highlights the need for monitoring and protecting the health of carp populations in the context of the global spread of foreign parasite species.

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