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Massive, disseminated and lethal cysticercosis caused by *Taenia crassiceps* (Cestoda) in captive western grey bamboo lemur (*Hapalemur occidentalis* Rumpler) in Zoo in the Czech Republic

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Abstract: *Taenia crassiceps* (Zeder, 1800), a zoonotic cestode with a wide geographical distribution, utilises canids as definitive hosts and small rodents as intermediate hosts. However, accidental infections in non-human primates, particularly in captive lemurs, have been increasingly documented. In this case report, we describe the first documented case of cysticercosis caused by the larval stage of *T. crassiceps* (metacestode; also known as *Cysticercus longicollis* Rudolphi, 1819) in a captive western grey bamboo lemur (*Hapalemur occidentalis* Rumpler). The affected female lemur showed progressive abdominal distension over a period of two months, which initially indicated pregnancy. At necropsy, multiple thin-walled metacestodes were discovered throughout the abdominal and thoracic cavities, including a large polycystic mass involving the liver and peritoneum. Histopathological analysis revealed larval cestodes with armed (hook-bearing) scoleces embedded in vascularised stromal tissue, accompanied by marked eosinophilic and macrophage infiltration and focal necrosis, particularly in the lungs. Morphological and molecular identification based on COI gene sequencing confirmed the presence of *T. crassiceps*. Despite extensive parasitological investigations, no definitive host excreting *T. crassiceps* eggs was found in the zoological facility where the lemur was kept. These results emphasise the possibility of indirect environmental transmission and highlight the susceptibility of lemurs as atypical intermediate hosts. This case confirms previous reports of lethal cysticercosis in lemurs, which is often characterised by rapid systemic spread of metacestodes. Our findings also emphasise the need for increased awareness and preventive measures to reduce the risk of parasite infections in vulnerable captive primates.

Keywords: *Cysticercus longicollis*, metacestode, PCR, genotyping, dissection, microscopy, zoo, lemur

Taenia crassiceps (Zeder, 1800) is a zoonotic tapeworm of the genus *Taenia* Linnaeus, 1758, which is widely distributed in Europe, North America and Asia (Freeman 1962). This tapeworm has an indirect life cycle with a definitive host and an intermediate host. The adult worm lives in the small intestine of domestic and wild carnivores with red fox (*Vulpes vulpes* (Linnaeus)) being the most common definitive host (Loos-Frank and Zeyhle 1982, Gori et al. 2015, Konjević et al. 2016, Schneider et al. 2025).

In addition to these typical definitive hosts, natural infections have been reported in other wild carnivores such as wolf (*Canis lupus* Linnaeus), the Arctic fox (*Vulpes lagopus* [Linnaeus]), golden jackal (*Canis aureus* Linnaeus), raccoon (*Procyon lotor* [Linnaeus]), raccoon dog (*Nyctereutes procyonoides* [Gray]), wild cat (*Felis silvestris* von Schreber), stone marten (*Martes foina* [von Schreber]) and genet cat (*Genetta genetta* [Linnaeus]) (Loos-Frank and Zeyhle 1982, Wünschmann et al. 2003, Subbotin 2009, Luzón et al. 2010, Bruzinskaite-Schmidhalter et al. 2012, Takács et al. 2014, Bouchard et al. 2021). Sporadic cases of infection have also been reported in domestic dogs (*Canis lupus familiaris* Linnaeus) and cats (*Felis catus* Linnaeus) (Wünschmann et al. 2003, Ballweber 2009).

Intermediate hosts become infected by ingesting eggs of *T. crassiceps*, which then develop into larval stages called *Cysticercus longicollis* Rudolphi, 1819 (metacestodes), which are localised in various body tissues such as muscles, subcutaneous tissue, and organs of the abdominal and thoracic cavity (Luzón et al. 2010, Alić et al. 2017, Bleyer et al. 2018, Cuccato et al. 2023).

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Table 1. List of intermediate hosts of captive non-human primates infected with metacestodes of *Taenia crassiceps* (Zeder, 1800).

Host (Latin name)	**Country**	**Site of infection**	**Reference/GenBank No.**
Ring-tailed lemur (*Lemur catta* Linnaeus)	Italy	liver, lungs, intestines, urinary bladder	Cuccato et al. (2023)
	Spain	subcutaneous tissue, body cavities	Luzón et al. (2010)
	Bosnia and Herzegovina	lungs, thoracic cavity	Alić et al. (2017)
	Poland	subcutaneous tissue in area of right thigh	Samorek-Pierog et al. (2022)
	Serbia	subcutaneously in knee region	Simin et al. (2023)
	Croatia	subcutaneous tissue on chest	Grbavac et al. (2024)
	China	no data	Unpublished/OP829304
Black lemur (*Eulemur macaco* [Linnaeus])	France	no data	Greigert et al. (2019)
	USA	lungs, peritoneal, pleural cavities	Dyer and Greve (1998)
Red ruffed lemur (*Varecia variegata rubra* [Saint-Hilaire])	USA	cervical subcutaneous tissue	Young et al. (2000)
Nilgiri lagur (*Semnopithecus johnii* Fischer)	India	muscles, lungs, myocardium	Bleyer et al. (2018)
Hamadryas baboon (*Papio hamadryas* [Linnaeus])	Switzerland	subcutaneously, smooth muscles, retroperitoneal cavity, spinal cord	Baer and Scheidegger (1946)
Senegal bushbaby (*Galago senegalensis* Saint-Hilaire)	Czech Republic	muscles of hind leg, connective tissue	Hofmannová et al. (2018)

Typical intermediate hosts of this tapeworm are small vertebrates that serve as prey for the definitive hosts, in particular voles (*Arvicola* Lacépède, *Microtus* Schrank, *Clethrionomys* Tilesius), wood mice (*Apodemus* Kaup), the muskrat (*Ondatra zibethicus* [Linnaeus]), the eastern chipmunk (*Tamias striatus* [Linnaeus]), the deer mouse (*Peromyscus maniculatus* Wagner), the woodchuck (*Marmota monax* [Linnaeus]) or lemmings (*Dicrostonyx* Gloger, *Lemmus* Link) (Freeman 1962, Fankhauser and Hörning 1967, Albert et al. 1972, Anderson et al. 1990, Deplazes et al. 2019, Chou et al. 2022).

Other mammals, including humans, can also serve as intermediate hosts, although the number of documented cases is relatively low (Basso et al. 2014, Delling et al. 2019, Deplazes et al. 2019, Floss et al. 2023). Among the described cases of infection in atypical hosts, there are several notable cases in non-human primates, particularly lemurs kept in captivity in zoological gardens (Table 1).

These findings raise important questions about host susceptibility and transmission dynamics, particularly in managed or captive animal populations that may come into contact with a contaminated environment. In this report, we present the first documented case of cysticercosis caused by *T. crassiceps* in a captive western grey bamboo lemur (*Hapalemur occidentalis* Rumpler), including molecular characterisation of the parasite.

MATERIALS AND METHODS

A necropsy was performed on a deceased female bamboo lemur. The morphological identification of the metacestodes was carried out under the light microscope by counting and measuring the rostellar hooks according to the criteria previously reported by Loos-Frank (2000). The specimens for histology were fixed in 4% buffered formalin and processed by the usual paraffin method. Histological sections (5 µm) were stained with hematoxylin and eosin. The collected metacestodes were washed with phosphate buffered saline (PBS) to remove dirt and tissue debris and then stored in 70% ethanol at laboratory temperature until further molecular analysis.

Total genomic DNA (gDNA) was extracted from the metacestodes in three independent replicates using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany). The extracted gDNA was stored at -20 °C. PCR protocols targeting the cytochrome c oxidase subunit I (COI) gene of *Echinococcus* spp. or other Taeniidae were performed using previously published primers (Zhang et al. 2023).

Each PCR reaction (30 µl total volume) contained 2 µl template DNA, 2.5 U Taq DNA polymerase (DreamTaq Green DNA Polymerase, Thermo Fisher Scientific, Waltham, Massachusetts, USA), 1× PCR buffer, 3 mM $MgCl_2$, 200 µM of each deoxynucleoside triphosphate (dNTP), 100 nM of each primer (Forward COIF: TTGAATTTRCCRCGTTTGAATGC and reverse COIR: RAACCYAACGACATAACATAATGA), and 2 µl of non-acetylated bovine serum albumin (BSA; 10 mg/ml; New England Biolabs, Beverly, Massachusetts, USA). DNA from *Echinococcus granulosus* (Batsch, 1786) and de-ionised water were used as positive and negative controls, respectively.

The PCR cycling conditions were as follows: initial denaturation at 95 °C for 5 minutes; 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 45 seconds and extension at 72 °C for 60 seconds; followed by a final extension at 72 °C for 7 minutes in a PCR thermocycler. PCR amplicons were visualised by electrophoresis on a 2% agarose gel stained with ethidium bromide. Positive PCR products were then sequenced in both directions by SeqMe s.r.o. (Czech Republic).

Raw nucleotide sequences were manually edited using ChromasPro 2.1.4 (Technelysium Pty Ltd., South Brisbane, Australia), aligned to each other and to reference sequences from GenBank (https://www.ncbi.nlm.nih.gov) using the online server MAFFT version 7 (http://mafft.cbrc.jp/alignment/software/) and trimmed using BioEdit v7.0.5 (https://bioedit.software.informer.com/7.0/). Phylogenetic analyses were performed using the neighbour-joining (NJ) method implemented in the Molecular Evolutionary Genetics Analysis software MEGA X (https://www.megasoftware.net/) after determining the most appropriate substitution model and calculating relevant parameters. The sequence obtained in this study was deposited in GenBank (PV789700).

This case report presents a female western bamboo lemur (*H. occidentalis*) born on 30 April 2018 at the Parc zoologique de Paris and transferred to Jihlava Zoo on 5 December 2021. No significant health problems were observed throughout her captivity. In the second half of 2024, a pregnancy was suspected as the abdomen was visibly distended and swaying. The female was housed together with a male western bamboo lemur in an enclosure outside the exhibition. After one day of inappetence and apathy, an ultrasound examination of the abdomen was carried out.

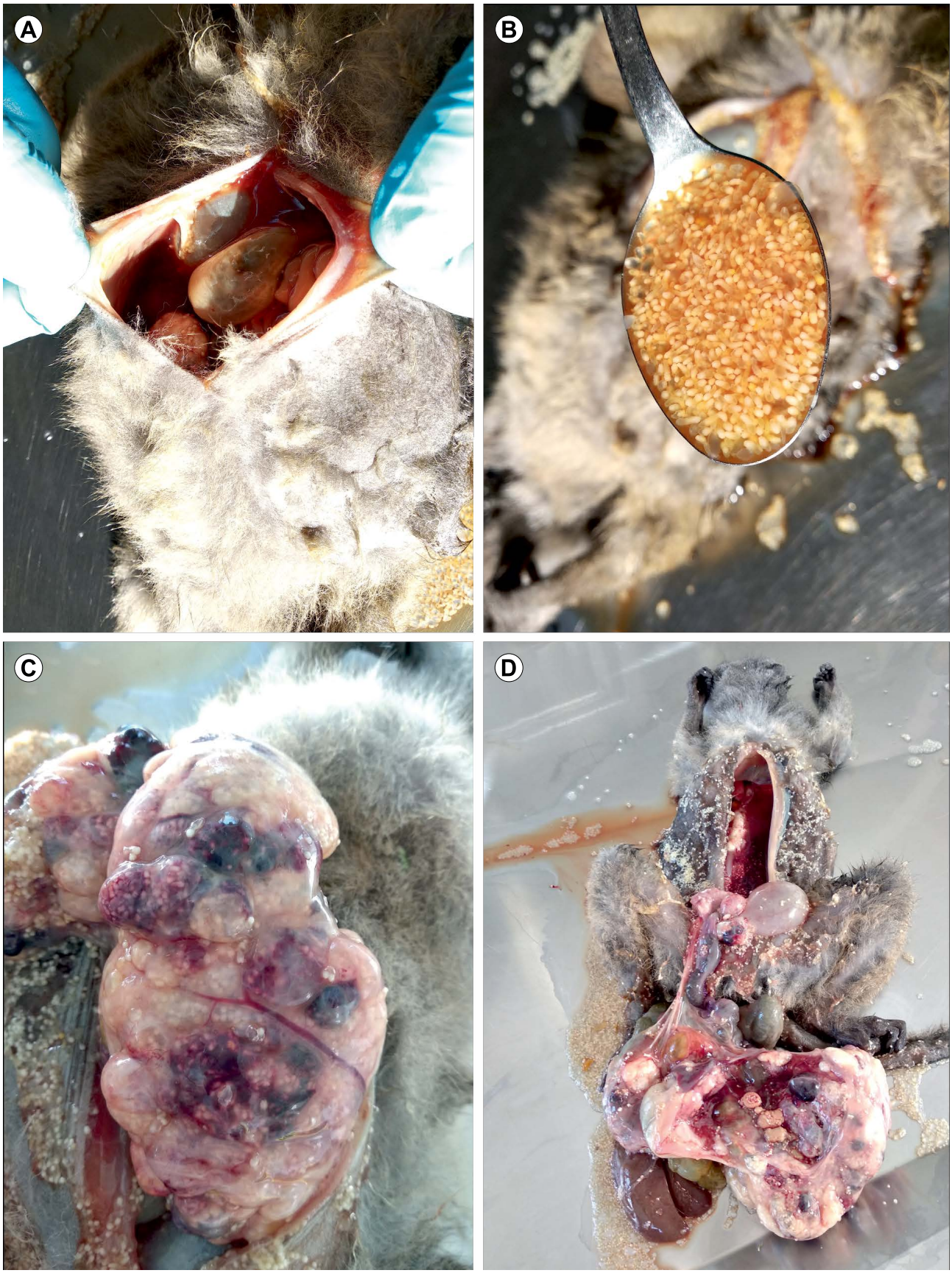

Fig. 1. Metacestodes of *Taenia crassiceps* (Zeder, 1800) in a captive western grey bamboo lemur (*Hapalemur occidentalis* Rumpler). **A** – view into the thoracic cavity of a bamboo lemur during necropsy; smooth, thin-walled cystic structures containing clear fluid are visible within the cavity, freely situated among the organs and surrounded by hemorrhagic exudate; **B** – numerous free small vesicles corresponding to *T. crassiceps* metacestode collected from abdominal and thoracic cavityl; **C**, **D** – multilobulated cystic mass in the abdominal cavity of a bamboo lemur; the lesion contains numerous translucent vesicles and hemorrhagic areas, with visible *T. crassiceps* metacestodes scattered across the serosa.

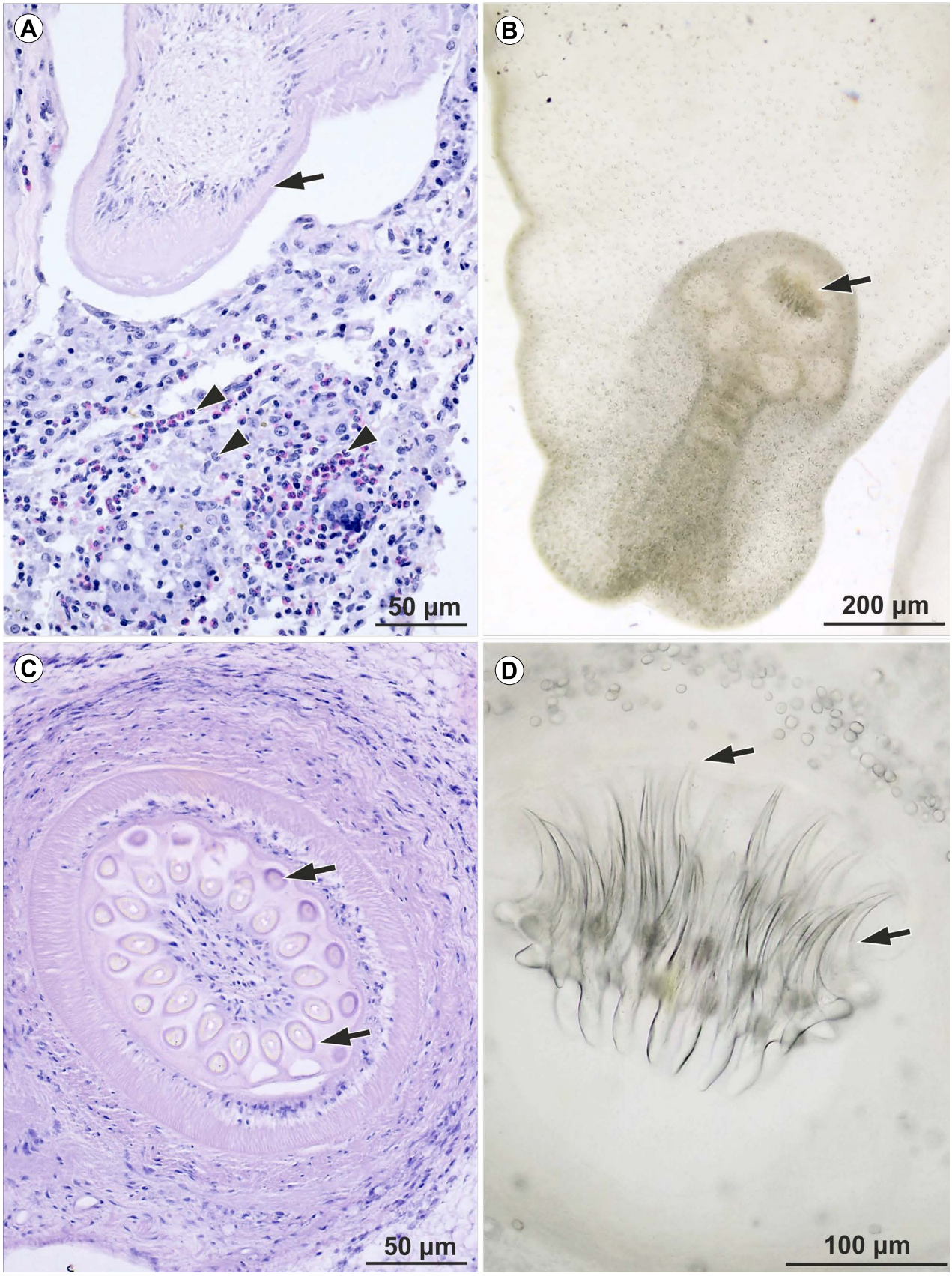

Fig. 2. Representative images illustrating the pathological, morphological, and molecular features of infection with *Taenia crassiceps* (Zeder, 1800) in a western grey bamboo lemur (*Hapalemur occidentalis* Rumpler). **A** – histological section of lung tissue showing the wall of a metacestode (arrow) and marked infiltration of the pulmonary parenchyma by eosinophilic granulocytes and macrophages (arrowhead); **B** – whole mount of metacestode observed under a light microscope, displaying an invaginated scolex with visible hooks (arrow); **C** – histological section of the scolex stained with hematoxylin and eosin; rostellar hooks are visible in the central part of the section (arrow); **D** – overlying hooks of the rostellum (arrow).

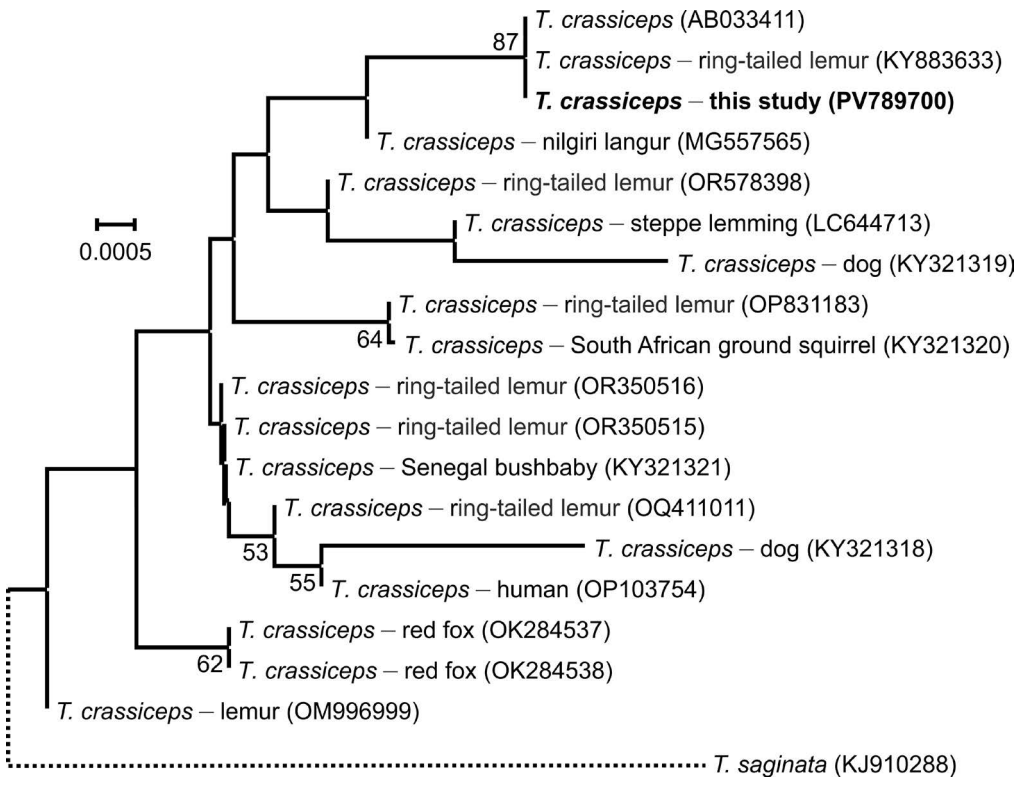

Fig. 3. Phylogenetic relationships of metacestodes of *Taenia crassiceps* (Zeder, 1800) from captive western grey bamboo lemur (*Hapalemur occidentalis* Rumpler) (bolded) inferred by the Neighbor-joining analysis of the partial sequence of cytochrome c oxidase subunit 1 (COI). Bootstrap supports and posterior probabilities higher than 50% are shown.

Radiographs of the thorax and abdomen were also taken, and free peritoneal fluid was collected by fine needle aspiration.

Blood samples were taken for routine biochemical and haematological tests. The aspirated fluid was sent to the State Veterinary Institute in Jihlava for cytological examination. An exploratory laparotomy was scheduled for the next day, but the female died before the procedure could be performed. The animal's nutritional status was classified as average, the skeletal system was assessed as firm and free of deformities, and the joints of the limbs showed no signs of inflammatory changes.

RESULTS

On 1 November 2024, at the age of 6 years and 6 months, the female died suddenly. The initial assumption that it was a pregnancy-related death was not confirmed. At necropsy, a large accumulation of serous, yellow-grey fluid was found in the abdominal cavity, together with numerous vesicle-like structures 1–3 mm in diameter and yellow in colour (Fig. 1A,B). Some of the larger metacestodes had thin transparent walls. In addition, irregular white to yellowish spongy metacestodes were observed. From the visceral peritoneum caudal to the stomach and from the visceral surface of the liver, a large oval polycystic structure approximately 10 × 14 cm in size emerged, consisting of cystic cavities filled with serous fluid, with similar cystic structures freely distributed in the abdominal cavity (Fig. 1C,D).

Smaller metacestodes of the same character were also detected in the thoracic and pelvic cavities, particularly in the cranial thoracic opening, along the ventral border of the thoracic cavity and bilaterally near the rectum. Tiny cystic foci were also observed in the parenchyma of the cranial lobe of the left lung. The lungs were aerated and focally hyperaemic; hydropericardium and dilatation of the right ventricle were present.

The liver was fragile, light brown in colour, with signs of passive congestion in the spleen and kidneys. The stomach was empty and the small intestine was partially adherent in a compact fold. The lumen was locally compressed and the mucous membrane was covered with mucus. The colon contained a small amount of formed brown faeces.

Histopathological examination of the structures from the abdominal cavity and lungs revealed multiple metacestodes with thin walls consisting of compact membranes with loosely arranged, often vascularised stroma (Fig. 2A). Numerous cestode larvae with armed scoleces were found in the metacestodes. The periphery of the metacetodes, particularly in the lung parenchyma, was infiltrated with eosinophils and macrophages (Fig. 2A) and focal necrosis was observed. The spongy structures consisted of necrotic material with fragments of metacestodes.

Under light microscopy, vesicles with a central white spot and a developing hook-bearing scolex inside were observed (Fig. 2B), confirming their identity as metacestodes. A total of 32–34 hooks were observed on the scoleces (Fig. 2C), which had a curved, arched claw (blade) of the hook that was longer than the base (handle, Fig. 2D). Large hooks ranged from 177 to 189 µm and small hooks ranged from 130 to 151 µm in length. The budding observed in some metacestodes and the number and size of hooks on the scoleces indicated the presence of the larval stage of the tapeworm *Taenia crassiceps*.

Phylogenetic analysis of a partial sequence of the cytochrome c oxidase subunit I (COI) gene, obtained from three independent DNA isolations and amplifications, confirmed the presence of *T. crassiceps* (Fig. 3). All sequences obtained from the different isolations in this study were identical to each other and matched a COI sequence previously reported from a ring-tailed lemur from a zoo in Bosnia and Herzegovina (Fig. 3).

DISCUSSION

In this study, a combination of molecular and morphological data allowed a convincing identification of *Taenia crassiceps* in a captive female western grey bamboo lemur (Loos-Frank 2000). This tapeworm and its larval stage, *Cysticercus longicollis*, usually circulate between definitive hosts, especially foxes, and their prey, mainly small rodents. However, a number of mammals can inadvertently become intermediate hosts after ingesting the parasite's eggs. Captive non-human primates, especially lemurs, seem to be particularly susceptible.

The case presented in this report is a further addition to the more than ten documented cases of *T. crassiceps*-induced cysticercosis in lemurs kept in zoological facilities (Table 1). In intermediate hosts, metacestodes usually form in the subcutaneous tissue and body cavities after ingestion of oncospheres (Freeman 1962). The parasite reproduces asexually by both exogenous and endogenous budding, which enables it to spread rapidly throughout the host organism within a few weeks (Willms and Zurabian 2010).

Experimental studies have shown that the metacestode burden in mice infected with *T. crassiceps* increases significantly between the fourth and eighth week after infection (Pereira et al. 2016, Jiménez et al. 2023). The female lemur described in this study developed a pronounced abdominal enlargement over the course of two months, simulating pregnancy. The gestation period in this species is around 137–140 days (Eppley et al. 2020). Due to the rapid progression of cysticercosis, we estimate that the infection occurred about 2–3 months before death.

Although the source of infection remains unclear, the lemur must have somehow come into contact with food, water or materials contaminated with the faeces of a definitive host. However, parasitological examination of all carnivores housed in the zoological garden did not reveal the presence of taeniid eggs or proglottids in the faeces of any of the animals examined (data not shown).

Fatal cysticercosis caused by *T. crassiceps* in lemurs is not uncommon. Similar cases have been reported in several captive lemurs showing a consistent clinical picture, including a sudden deterioration followed by the discovery of disseminated metacestodes in the lungs, thoracic and abdominal cavities, with extensive visceral organ involvement and metacestodes free-floating in the peritoneal fluid (Dyer and Greve 1998, Alić et al. 2017, Deplazes et al. 2019, Samorek-Pierog et al. 2022). A similar course of infection has also been described in other non-human primates infected with larval stages of *Taenia martis* (Zeder, 1803), *Versteria* spp. or *Echinococcus* spp. (Federer et al. 2016, Deplazes et al. 2019).

The histopathological examination in our case showed pronounced eosinophilic and macrophage inflammatory infiltrates with focal necrosis, especially in the lung tissue displaced by the larval structures. The metacestodes were thin-walled and embedded in an abundantly vascularised stroma, suggesting active proliferation and progression of the infection. These findings are consistent with previously published cases in which eosinophilic infiltration and fibrotic encapsulation were also noted (Samorek-Pierog et al. 2022, Grbavac et al. 2024). The observed systemic infection with a strong tissue immune response may reflect the immunological status of the host. It has been shown that hosts with suppressed Th1 immune responses are more susceptible to rapid and lethal dissemination of the parasite (Hofmannová et al. 2018, Díaz-Zaragoza et al. 2020, Jiménez et al. 2023).

The results of this and other studies suggest that lemurs and other non-human primates are highly susceptible to infection with *T. crassiceps* and cannot effectively control the parasite's multiplication. Although the source of infection has not yet been conclusively determined, this problem deserves increased attention when keeping endangered primate species. Contamination of the environment with parasite eggs remains a plausible risk, even if no definitive hosts have been identified. Eggs of various taeniid species, including *T. crassiceps*, have been found on vegetables and fruit intended for human consumption but used as food for primates in zoos (Federer et al. 2016). Therefore, protection protocols designed to protect both animals and keepers should include staff training and the implementation of strict hygiene measures when handling animals, feed and faeces.

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