


Parasitosis caused by *Calyptospora* in *Pygocentrus nattereri* (Characiformes: Serrasalminidae) from the Sacaizal Lake in the state of Amapá, Brazil

Parasitose causada por *Calyptospora* em *Pygocentrus nattereri* do Lago Sacaizal, estado do Amapá, Brasil

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How to cite: Sena NM, Eduard J, Pereira CMB, Vilhena MPSP, Silva JFBR, Sindeaux Neto JL, Velasco, M. Parasitosis caused by *Calyptospora* in *Pygocentrus nattereri* (Characiformes: Serrasalminidae) from the Sacaizal Lake in the state of Amapá, Brazil. *Rev Bras Parasitol Vet* 2025; 34(4): e011125. <https://doi.org/10.1590/S1984-29612025070>

Abstract

Parasites of the phylum Apicomplexa are among the main etiological agents of diseases in vertebrates and invertebrates, including humans and domestic animals. In the present study, 18 specimens of *Pygocentrus nattereri* (red piranha) captured in Lagoa do Sacaizal, municipality of Pracuúba, state of Amapá, were analyzed. Of these, 6 (33%) had whitish spots on their liver lobes. Clusters of *Calyptospora* sp. oocysts measuring 18.1 ± 0.5 (17.4-18.7) μm in diameter, with four pyriform sporocysts measuring 9.0 ± 0.45 (8.3-9.8) μm in length and 4.3 ± 0.50 (3.7-5.1) μm in width, each with two sporozoites. Histopathological evaluation indicated the presence of oocysts adjacent to blood vessels and in the hepatopancreas, promoting necrosis, degeneration, and tissue fibrosis associated with inflammatory infiltrate and melanomacrophage bodies. This is the first record of coccidia parasitizing *P. nattereri*.


Keywords: Coccidiosis, fish, Brazil, histopathology, fibrosis.

Resumo

Parasitas do filo Apicomplexa estão entre os principais agentes etiológicos de doenças em vertebrados e invertebrados, incluindo humanos e animais domésticos. No presente estudo, foram analisados 18 espécimes de *Pygocentrus nattereri* (piranha vermelha) capturados na Lagoa do Sacaizal, município de Pracuúba, estado do Amapá. Destes, 6 (33%) apresentaram manchas esbranquiçadas nos lobos hepáticos. Foram observados aglomerados de oocistos de *Calyptospora* sp. medindo $18,1 \pm 0,5$ (17,4-18,7) μm de diâmetro, com quatro esporocistos piriformes medindo $9,0 \pm 0,45$ (8,3-9,8) μm de comprimento e $4,3 \pm 0,50$ (3,7-5,1) μm de largura e cada um com dois esporozoítos. A avaliação histopatológica indicou a presença dos oocistos adjacentes aos vasos sanguíneos e no hepatopâncreas, promovendo necrose, degeneração e fibrose tecidual associada a infiltrado inflamatório e corpos melanomacrófagos. Este é o primeiro registro da ocorrência de coccídio parasitando *P. nattereri*.

Palavras-chave: Coccidiose, peixes, Brasil, histopatologia, fibrose.

Received July 03, 2025. Accepted October 16, 2025.

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Assistant Editor: Maurício Laterça Martins



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Pygocentrus nattereri Kner, 1858, popularly known as “red piranha”, is a serrasalmid belonging to the most diverse group of fish, the Characiformes, with valid species of importance for regional fishing and aquaculture. The species has a wide distribution and is present in several river basins throughout South America (Nelson et al., 2016).

Organisms of the phylum Apicomplexa Levine, 1970, are among the most important etiological agents responsible for diseases in vertebrates and invertebrates, including humans and domestic animals, with significant environmental, economic, and public health implications (Silva et al., 2012). They possess an apical complex composed of specialized secretory organelles and produce resistant cysts that enable direct transmission through the food chain between predators and prey or through arthropod vectors (Votýpka et al., 2016).

The class Coccidia Leuckart, 1879 comprises a diverse group of protozoa with a parasitic lifestyle, affecting natural stocks of several fish species, with important characteristics regarding parasitic invasion and transmission ecology. *Calyptospora* Overstreet, Hawkins & Fournie, 1984, preferentially infects the hepatic region of fish. Its morphological characteristics include oocysts with four sporocysts, each containing two sporozoites (Fournie et al., 1985). The sporocysts can vary from round to ellipsoid in morphology.

Currently, eight species of *Calyptospora* have been described as infecting fish. Of these species, three occur in marine fish: *C. funduli* (Duszynski, Solangi & Overstreet, 1979) in fishes of the family Fundulidae such as *Fundulus grandis* Baird & Girard, 1853, *F. heteroclitus* (Linnaeus, 1766), *F. jenkinsi* (Evermann, 1892), *F. pulvereus* (Evermann, 1892), *F. similis* (Baird & Girard, 1853) and *Menidia beryllina* (Cope, 1867) (Atherinidae) from the coastal area of Mississippi (Fournie & Overstreet, 1993); *C. matosi* Neto, Székely, Eduard et al., 2025, identified in *Bagre bagre* (Linnaeus, 1766) (Ariidae) in Bragança (Ajuruteua beach) and Curuçá (São João do Abade district) in Pará state, Brazil (Araujo Neto et al., 2025); and *C. virescentis* Eduard, Azevedo, Pereira et al., 2025 in *Cynoscion virescens* (Cuvier, 1830) (Sciaenidae), a marine and estuarine fish from Curuçá, northeastern Pará, Brazilian coast, Brazil (Eduard et al., 2025). The greatest diversity of *Calyptospora* is found in freshwater fish, mainly in the Brazilian Amazon, according to Table 1.

This study aimed to describe the morphological and histopathological changes caused by *Calyptospora* sp. in the liver of *P. nattereri* captured from Lake Sacaizal (1°42'8.79"N 50°43'17.56"W) in the municipality of Pracuúba, Amapá State, Brazil.

Eighteen specimens of *P. nattereri* (SISBIO/ICMBio License n° 88196-1) were acquired from artisanal fishermen, placed in isothermal boxes with ice, and transported to the Morpho-molecular Integration and Technologies Laboratory at the Federal Rural University of Amazônia in Belém, Pará, for necropsy (CEUA no. 7218270723 /ID000609). Subsequently, the entire body surface, tissues, and organs were analyzed using a stereoscopic microscope and a light microscope to identify for parasites and perform morphological analyses.

The methodology of Bush et al. (1997), was used to calculate prevalence of fish examined. In the morphometric analysis, the oocysts (n = 20) and their internal structures were measured in micrometers (µm) using ImageJ version 1.46r software, and mean and standard deviation values were subsequently calculated. The Parasitized liver tissue fragments were fixed in Davidson's solution for 24 h. Subsequently, the sections were dehydrated in an increasing series of ethanol, diaphanized in xylol, and embedded in paraffin. Sections of 5 µm thickness were obtained using a microtome and stained with hematoxylin & eosin and Gomori special trichrome stain.

For scanning electron microscopy, the samples were prepared according to Azevedo et al. (1995). Visualizations and photomicrographs were obtained using a Tescan Mira3 scanning electron microscope at the Scanning Electron Microscopy Laboratory of the Research Campus of the Museu Paraense Emílio Goeldi-MPEG.

Among the 18 necropsied *P. nattereri* specimens, six presented whitish regions in the liver (Figure 1a), with the appearance of clusters distributed throughout the liver parenchyma. Optical microscopy confirmed that the clusters were coccidial oocysts, present in large numbers, forming a thin wall of connective tissue around them and distributed throughout the liver parenchyma (Figure 1b); Isolated mature spherical oocysts were also observed and measured 18.1 ± 0.5 (17.4-18.7) µm in diameter, with four pyriform sporocysts measuring 9.0 ± 0.45 (8.3-9.8) µm in length and 4.3 ± 0.50 (3.7-5.1) µm in width, and it was possible to observe the membranous veil surrounding the sporozoites (Figure 1c). SEM revealed numerous sporopods concentrated on the surface of the narrow posterior end of the sporocyst (Figure 1d).

Due to the spherical shape of the oocysts containing four pyriform sporocysts, each with two sporozoites, these were identified as oocysts of an unidentified species in the genus *Calyptospora*, with a prevalence of 33.3%.

Histopathological findings showed grouped oocysts compressing the hepatocytes and cells of the intrahepatic pancreas, along with melanomacrophage bodies containing pigment granules with a yellowish-brown color and

Table 1. Freshwater hosts of *Calyptospora*. Measurements in micrometers (µm).

Order of Fish	Host	Location	Parasite species	Site of infection	Oocyst diameter	Sporocysts		Reference	
						Shape	Length		Width
Characiformes	<i>Pygocentrus nattereri</i> Kner, 1858 - Serrasalminidae	Lake Sacaizal, Pracuúba, Amapá, Brazil	<i>Calyptospora</i> sp.	Liver	18.1	Pyriiform	9.0	4.3	Present study
	<i>Serrasalmus striolatus</i> (Steindachner, 1908) -Serrasalminidae, S. <i>rhombeus</i> (Linnaeus, 1766) - Serrasalminidae	Amazon River, Belém and lagoon region of the "Dois Irmãos" Zoo, Recife, Brazil	<i>Calyptospora</i> <i>serrasalmi</i>	Liver	25.5	Pyriiform	11.8	6.0	Casal et al. (2007)
	<i>Triportheus guentheri</i> (Garman, 1890) - Triporthetidae	Three Marias Reservoir, São Francisco River, Brazil	<i>Calyptospora</i> sp.	Liver	24.5	Elipsoidal	11.5	4.5	de Albuquerque & de Carvalho Brasil-Sato (2010)
	<i>Tetragonopterus chalceus</i> Spix & Agassiz, 1829 - Characidae			Intestine					
	<i>Tetragonopterus chalceus</i> Spix & Agassiz, 1829 - Characidae	Three Marias Reservoir upper São Francisco, Minas Gerais, Brazil.	<i>Calyptospora</i> sp.	Liver	-	-	-	-	de Albuquerque et al. (2016)
	<i>Triportheus guentheri</i> (Garman, 1890) - Characidae								
	<i>Hoplias malabaricus</i> (Bloch, 1794) - Erythrinidae	Curiaú river, Macapá, Amapá, Brazil	<i>Calyptospora</i> <i>serrasalmi</i>	Liver	23.4	Pyriiform	8.2	4.0	Silva Negrão et al. (2019)
	<i>Triportheus Angulatus</i> (Spix & Agassiz, 1829) -Triporthetidae	Tocantins River, Maranhão, Brazil	<i>Calyptospora</i> <i>gonzaguensis</i>	Liver, Gallbladder, adipose tissue	19.6	Elipsoidal	9.2	3.9	Silva et al. (2020)
	<i>Serrasalmus rhombeus</i> (Linnaeus, 1766) - Serrasalminidae	Grass River, Ipixuna of Pará, Pará, Brazil	<i>Calyptospora</i> sp.	Liver	17.4	Pyriiform	8.3	4.3	Oliveira et al. (2021)
	<i>Hoplias malabaricus</i> (Bloch, 1794) - Erythrinidae	Três Marias reservoár, São Francisco river, Minas Gerais, Brazil	<i>Calyptospora</i> sp.	Liver	-	-	-	-	Duarte et al. (2023)

Table 1. Continued...

Order of Fish	Host	Location	Parasite species	Site of infection	Oocyst diameter	Sporocysts			Reference										
						Shape	Length	Width											
Cicliformes	<p><i>Cichla ocellaris</i> Bloch & Schneider, 1801 - Cichlidae</p> <p><i>Crenicichla lepidota</i> Heckel, 1840 - Cichlidae</p> <p><i>Cichla temensis</i> Humboldt, 1821 - Cichlidae</p> <p><i>Aequidens plagiazonatus</i> Kullander, 1984 - Cichlidae</p> <p><i>Cichla piquiti</i> Kullander & Ferreira, 2006 - Cichlidae</p> <p><i>Geophagus proximus</i> (Castelnau, 1855)</p> <p><i>Cichla monoculus</i> Agassiz, 1831 - Cichlidae</p> <p><i>Mayaheros urophthalmus</i> (Günther, 1862) - Cichlidae and <i>Parachromis friedrichsthalii</i> (Heckel, 1840) - Cichlidae</p>	<p>Reservoirs: Curfi and Jaguaribe River Basins - State of Ceará, Serido River Basin - State of Rio Grande do Norte, and Experimental Ichthyological Research Center Fish Farming, Pentecoste, Brazil</p> <p>Amazon River, Belém, Brazil</p> <p>Marajó-Açu River, Marajó Island, Pará, Brazil</p> <p>Municipality of Peixe-Boi, Pará, Brazil</p> <p>Estreito hydroelectric reservoir - Tocantins River, Maranhão, Tocantins, Brazil</p> <p>Curiaú river, Macapá, Amapá, Brazil</p> <p>Lake Sacaizal, Pracuúba, Amapá, Brazil</p> <p>Three water sources (Baldiosera, Muuch and San Crisanto), Yucatán, México</p>	<p><i>Calyptospora tucunarensis</i></p> <p><i>Calyptospora spinosa</i></p> <p><i>Calyptospora</i> sp.</p> <p><i>Calyptospora</i> sp.</p> <p><i>Calyptospora paranaidji</i></p> <p><i>Calyptospora serrasalmi</i></p> <p><i>Calyptospora</i> sp.</p> <p><i>Calyptospora mexicanus</i></p> <p><i>Calyptospora</i> sp.</p>	<p>Liver</p> <p>Liver, testes and ovaries</p> <p>Liver</p> <p>Hepatopancreas</p> <p>Liver</p> <p>Liver, gallbladder and heart</p> <p>Liver</p> <p>Liver</p> <p>Liver</p> <p>Liver and gallbladder</p> <p>Hepatocytes and pancreatic acinar cells</p> <p>Liver</p>	<p>24.3</p> <p>22.3</p> <p>21.2</p> <p>-</p> <p>22.1</p> <p>22.5</p> <p>21.0</p> <p>25.3</p> <p>21.0</p> <p>25.3</p>	<p>Ellipsoidal</p> <p>Ellipsoidal</p> <p>Pyriform</p> <p>-</p> <p>Ellipsoidal</p> <p>Pyriform</p> <p>Pyriform</p> <p>Pyriform</p> <p>Pyriform</p> <p>Pyriform</p>	<p>8.3</p> <p>9.3</p> <p>9.2</p> <p>-</p> <p>9.7</p> <p>9.0</p> <p>8.7</p> <p>13.7</p> <p>9.2</p> <p>13.7</p>	<p>3.7</p> <p>3.8</p> <p>3.1</p> <p>-</p> <p>4.6</p> <p>4.0</p> <p>4.9</p> <p>5.4</p> <p>4.5</p> <p>5.4</p>	<p>Békési & Molnár (1991)</p> <p>Azevedo et al. (1993)</p> <p>Santiago et al. (2012)</p> <p>Videira et al. (2013)</p> <p>Silva et al. (2019)</p> <p>Silva Negrão et al. (2019)</p> <p>Amoras et al. (2024)</p> <p>Colunga- Ramírez et al. (2025)</p> <p>Silva et al. (2012)</p> <p>Colunga- Ramírez et al. (2025)</p> <p>Fournie et al. (1985)</p> <p>Bonar et al. (2006)</p>										
										Siluriformes	<p><i>Brachyplatystoma vaillantii</i> (Valenciennes, 1840) - Pimelodidae</p> <p><i>Rhamdia guatemalensis</i> (Günther, 1864) - Heptapteridae</p>	<p>Paracauari River, in Salvaterra, Marajó Island, Pará, Brazil</p> <p>San Crisanto water spring, Yucatán, México</p>	<p><i>Calyptospora</i> sp.</p> <p><i>Calyptospora mexicanus</i></p>	<p>Liver</p> <p>Liver and gallbladder</p>	<p>21.0</p> <p>25.3</p>	<p>Pyriform</p> <p>Pyriform</p>	<p>9.2</p> <p>13.7</p>	<p>4.5</p> <p>5.4</p>	<p>Silva et al. (2012)</p> <p>Colunga- Ramírez et al. (2025)</p>
										Osteoglossiformes	<p><i>Arapaima gigas</i> (Schinz, 1822) - Arapaimidae</p>	<p>Manaus, Brazil</p>	<p><i>Calyptospora</i> sp.</p>	<p>Liver</p>	<p>19.0</p>	<p>Pyriform</p>	<p>9.0</p>	<p>4.0</p>	<p>Bonar et al. (2006)</p>

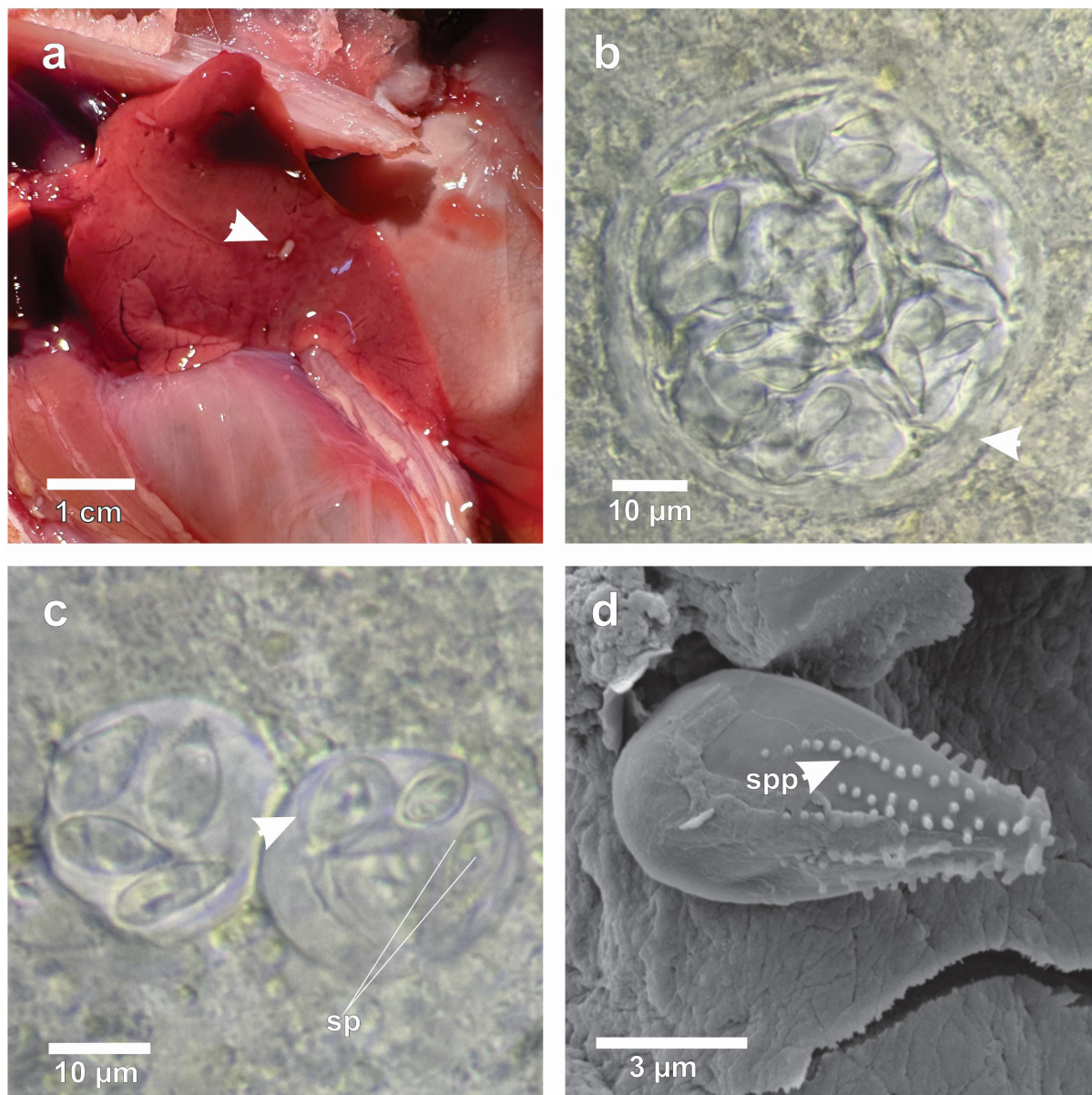


Figure 1. Macro and microphotographs of *Calyptospora* sp. infection in *Pygocentrus nattereri*. (A) Liver showing a whitish region with a cystic appearance. (B) Clusters of oocysts surrounded by a thick wall with a fibrous appearance observed under light microscopy. (C) Oocysts containing 4 sporocysts, sporozoites (sp) inside and the membranous veil (arrowhead). (D) Scanning electron microscopy micrographs of a sporocyst of *Calyptospora* sp. with emphasis on sporopods (spp).

inflammatory infiltrate (Figure 2a). The oocyst clusters were surrounded by a connective tissue wall with fibroblasts that clearly defined a single cystic layer (Figure 2b). We also observed tissue degeneration and compressive necrosis (Figure 2c and 2d), with liver cells exhibiting pyknotic and irregular nuclei and a lacy cytoplasm, particularly notable were emphasizing oocysts in the vicinity of blood vessels (Figure 2e).

Records exist of *Calyptospora* species parasitizing teleost fish from the families Pimelodidae, Cichlidae, Arapaimidae, Triportheidae, and Serrasalminidae, infecting the livers of these hosts and causing histopathological changes in the tissues (Silva et al., 2020; Oliveira et al., 2021). In the present study, *Calyptospora* sp. infection was recorded in the liver of *P. nattereri* with a prevalence of infection similar to that recorded in *Serrasalmus rhombeus* Linnaeus, 1766 (33.0%). However, *Serrasalmus striolatus* (Steindachner, 1908) higher prevalence values of *Calyptospora* at 53.3%, followed by *Cichla temensis* Humboldt, 1821, at 56%, *Brachyplatystoma vaillantii* (Valenciennes, 1840) at 60%, *Crenicichla lepidota* Heckel, 1840, at 63% and *S. rhombeus*, at 80%.

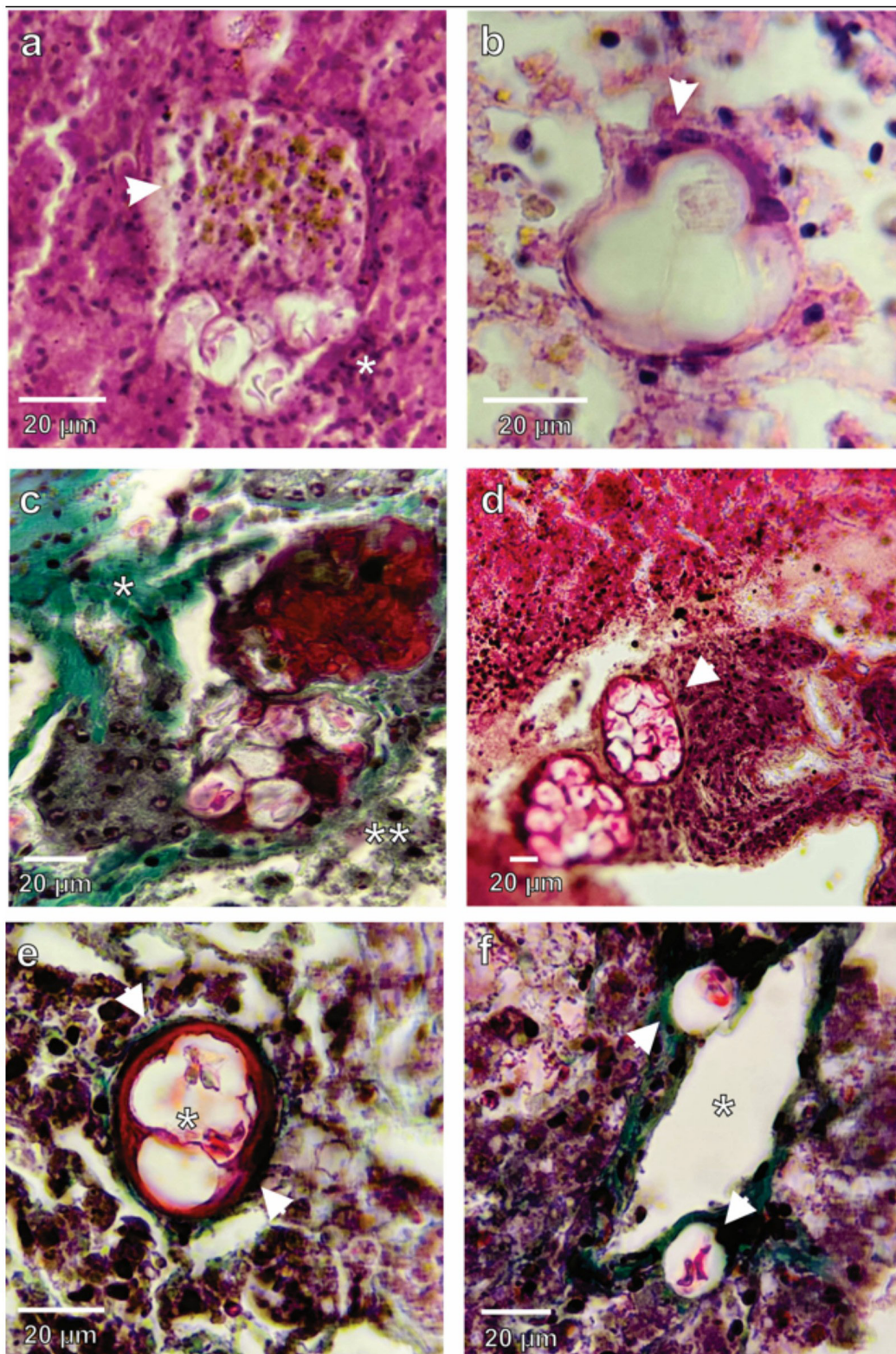


Figure 2. Microphotographs of histological sections of liver tissue from *Pygocentrus nattereri* infected by *Calyptospora* sp. A and B – Histological liver sections stained with HE. (A) Grouped oocysts compressing the hepatocytes and the presence of melanomacrophagic cells (arrowhead) full of yellowish pigment granules and inflammatory infiltrate (*). (B) Clusters of oocysts surrounded by a wall of fibroblasts (arrowhead). C, D, E, and F – Gomori trichrome staining: (C) Parasites of the genus *Calyptospora* causing fibrosis. Green coloring indicates the presence of collagen fibers (*) and degeneration (**) in the liver parenchyma and intrahepatic pancreas. (D) Oocysts clustered in the hepatopancreas region. (E) Cluster of oocysts in the intrahepatic pancreas region surrounded by a thick, fibrous wall (arrowhead). (F) Oocysts close to the blood vessel of the liver parenchyma (*).

Currently, there are no records of *Calyptospora* infection in *P. nattereri*. However, such infections have been reported in other species of the Serrasalmidae family, including *Calyptospora serrasalmi* parasitizing *S. striolatus* and *S. rhombeus* (Casal et al., 2007). The morphology of the oocysts and the arrangement of the sporopods in the sporocysts of *Calyptospora* sp. were similar to those of *C. serrasalmi* described by Whipps et al. (2012).

Studies on parasitism by *Calyptospora* have frequently reported the presence of whitish regions in the livers of host fish (Azevedo et al., 1993; Velasco et al., 2012; Videira et al., 2013; Oliveira et al., 2021). Furthermore, *Calyptospora* can damage the liver of fish at the cellular level, causing nuclear hypertrophy, destruction of the cytoplasm, and even severe degeneration of the organ (Békési & Molnár, 1991; Azevedo et al., 1993), which can lead to host death due to liver failure (Bonar et al., 2006). In the present study of *P. nattereri*, the presence of oocysts in the vicinity of blood vessels, fibrosis around clusters of oocysts in the hepatopancreas, tissue degeneration, and compressive necrosis were recorded, as reported in *S. rhombeus* by Oliveira et al. (2021).

The presence of inflammatory infiltrates and melanomacrophagic cells near the clusters of oocysts found in the liver of *P. nattereri* demonstrates the establishment of an infection process and the host's immunological response to parasitism (Bonar et al., 2006). These changes caused by *Calyptospora* have been recorded in the livers of *Cichla ocellaris* Bloch & Schneider, 1801 (Békési & Molnár, 1991), *C. temensis* (Velasco et al., 2012), *Aequidens plagiozonatus* Kullander, 1984 (Videira et al., 2013), *Cichla piquiti* Kullander & Ferreira, 2006 (Silva et al., 2019) and *S. rhombeus* (Oliveira et al., 2021). However, there are cases in which, despite the occurrence of histopathological changes caused by *Calyptospora* in the hepatopancreas of the host no serious inflammatory processes have been observed in the tissue, such as those described in *Arapaima gigas* Cuvier, 1829 (Bonar et al., 2006) and *C. temensis* (Santiago et al., 2012).

This is the first documented case of coccidiosis caused by *Calyptospora* sp. in *P. nattereri* from Pracuúba municipality, Amapá state. These infections result in significant liver disease. Oocysts form fibrous cysts, that trigger recruitment of inflammatory cells and melanomacrophage bodies to the site of infection. Consequently, the liver parenchyma undergoes degeneration. These findings demonstrate that infection with *Calyptospora* parasites can cause liver damage and potentially affect the overall physiology of the host.

Financial support

No financial support was received for this research.

Data availability

Data supporting the results of this study are available upon request to the corresponding author.

Ethics declaration

The analysis of the specimens presented was authorized by the UFRA Ethics Committee for the Use of Animals in Research (CEUA 7218270723) and by a license from the Brazilian Institute for the Environment and Renewable Natural Resources, IBAMA (SISBIO/ICMBiolicense number 88196-1)

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Nayana Moraes de Sena: Writing - original draft, conceptualization, data curation, research, methodology. Jhonata Eduard: supervision, writing - review, and editing. Camila Maria Barbosa Pereira: formal analysis, research, methodology. Maria do Perpetuo Socorro Progene Vilhena: review and methodology. José Francisco Berrêdo Reis da Silva: methodology. José Ledamir Sindeaux-Neto: formal analysis, research, methodology, project administration,

writing - review, and editing. Michele Velasco: research, methodology, project administration, supervision, validation, visualization, writing - review, and editing.

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