

Morphology and histopathology of *Calyptospora* sp. (Apicomplexa: Calyptosporidae) in speckled peacock bass, *Cichla temensis* Humboldt, 1821 (Perciformes: Cichlidae), from the Marajó-Açu River, Marajó Island, Brazil

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Abstract Several species of coccidia are protozoan parasites that cause infection in a wide variety of animal groups. *Calyptospora* is an important genus of protozoan, which infests both freshwater and marine fish. The hepatopancreases of 150

speckled peacock bass captured on Marajó Island, Brazil were studied macro- and microscopically. Oocysts were found in 84 (56%) of the specimens in both the examination of the fresh material by compression and the analysis of histological sections stained with hematoxylin–eosin. Small, circular, homogeneous forms in negative contrast had a mean diameter of 21.2 μm , frequently with pyriform sporocysts, with a mean length of 9.2 μm and width of 3.1 μm , and a thin-walled capsule, were observed in both the hepatic and the pancreatic parenchyma, but were completely devoid of any inflammatory reaction. *Calyptospora* infections are documented for the first time in the Marajó-Açu River.

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Marajó Island is the world’s largest fluvial island, with an area of approximately 40,100 km², located in the mouth of the Amazon River in the Brazilian state of Pará. The Apicomplexa occur in a wide range of animals—from helminths to mammals—and represent a prominent potential source of economic loss, principally in birds and animals. Coccidia are common in both marine and freshwater fish (Davies and Ball 1993; Molnar 2006). Species of the genera *Eimeria* Schneider, 1875; *Epieimeria* Dykova and Lom, 1981; *Goussia* Labbe 1896; and *Calyptospora* Overstreet, Hawkins and Fournié, 1984 have been found parasitizing freshwater fish (Azevedo et al. 1993; Bekesi and Molnar 1991).

Of the five species attributed to the genus *Calyptospora* sp., only *Calyptospora serrasalmi* Cheung et al. 1986, *Calyptospora tucunarensis* Bekesi and Molnar 1991, and *Calyptospora spinosa* Azevedo et al. 1993 have been recorded infecting Brazilian fish, primarily the hepatocytes

(Casal et al. 2007). The life cycles of the coccidia which infect fish can be divided into two principal types—monoxenic and heteroxenic (involving both intermediate and paratenic hosts). The genus *Calyptospora* was initially confused with *Eimeria*, from which it is distinguished by heteroxenic life cycle and typical oocyst morphology (Davies and Ball 1993; Molnar 2006).

In the present study, the protozoan parasites of the island's speckled peacock bass, *Cichla temensis* Humboldt, 1821 (Perciformes, Cichlidae) were investigated. This territorial, ichthyophagous fish is often used to stock lakes and reservoirs, given its excellent meat and popularity among sports fishermen (Nascimento 2004). The species is consumed throughout the Amazon basin, and is commercially important.

This study is the first report of the presence of an apicomplexan species in the hepatopancreas of *C. temensis*. The morphological and histopathological aspects of the parasitism are also described. This report also represents an extension of the geographic distribution of the *Calyptospora* sp. to the Marajó-Açu River in the Brazilian state of Pará.

In 2010, a sample of 150 *C. temensis* Humboldt, 1821 (77 males and 73 females) was captured in the Marajó-Açu River (01°23'25" S 48°52'16" W) using gillnets. The specimens were dissected and, following initial microscopic confirmation, the infected hepatopancreases, containing oocysts, were collected. The histological analyses were made of the fresh specimens of the hepatopancreas, and then fixed in Davidson solution (pure formaldehyde, 95% ethanol, acetic acid, and distilled water), set in paraffin, microtomed to a thickness of 4 µm, stained with hematoxylin and eosin, and examined under a Zeiss Primo Star optical microscope. The specimens were then photographed with a Canon A610/A620 52-mm camera. The procedures for necropsy of the hosts and collection, fixation, conservation, and mounting of the parasites for identification were according to Eiras et al. (2000).

Oocysts of *Calyptospora* sp. were found in the hepatopancreases of 84 (56%) of the *C. temensis* specimens collected during the study. No significant relationship was found between infection rates and the sex of the specimens, with 51.2% of males (43 of 84 specimens) being infected, and 48.8% of the females (41 of 84). The intensity of the infection nevertheless increased during the rainy season (December to May) when much larger numbers of cysts were observed in the infected specimens. The fresh oocysts had a mean diameter of 21.2 µm, containing four pyriform sporocysts with a mean length of 9.2 µm and width of 3.1 µm, and apical sporopodia. Each sporocyst contained two sporozoites (Fig. 1).

The microscopic examination of the hepatopancreas revealed the presence of innumerable colorless, homogeneous, circular oocysts with thin walls, centralized by slightly acidophilic sporocysts, which are frequently pyriform (Figs. 2 and 3), with characteristics of the coccidian *Calyptospora*. Infection

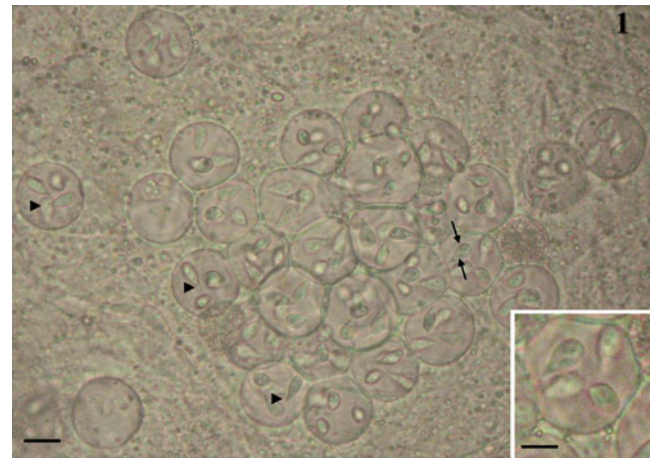


Fig. 1 *C. temensis*. Hepatopancreas. Oocysts of *Calyptospora* sp. containing four sporocysts (insert. scale bar: 10 µm) which present apical sporopodia (arrowhead). Each sporocyst contains two sporozoites (arrow). Fresh material. Scale bar: 10 µm

was limited to the hepatic and pancreatic tissue of all the specimens analyzed. No inflammatory reaction or tissue lysis was observed.

Parasitic diseases are increasingly being considered an economic problem in aquaculture (Paperna 1991). Fish coccidia have received special attention in the last decade, mainly due to the pathogenic concern of some species (Jendrysek et al. 1994).

Desser and Lianxiang (1984) reported the presence of *Eimeria laureleus* in *Perca flavescens* naturally infected from Lake Sasajeum, Ontario. Two species of coccidia, *Eimeria sparis* and *Goussia sparis*, were found in the gut of *Sparus aurata* in Spain (Sitjà-Bobadilla et al. 1996). El-Mansy (2008) reported the coccidian parasite *Goussia cichlidarum* on the swim bladder of tilapias in Egypt.

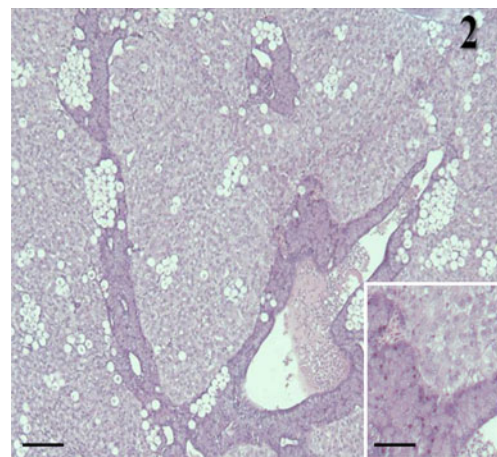


Fig. 2 *C. temensis*. Hepatopancreas. *Calyptospora* sp. foci with sporocysts that are often pyriform. Note the absence of any inflammatory reaction (insert: higher magnification. Scale bar: 40 µm). HE. Scale bar: 100 µm

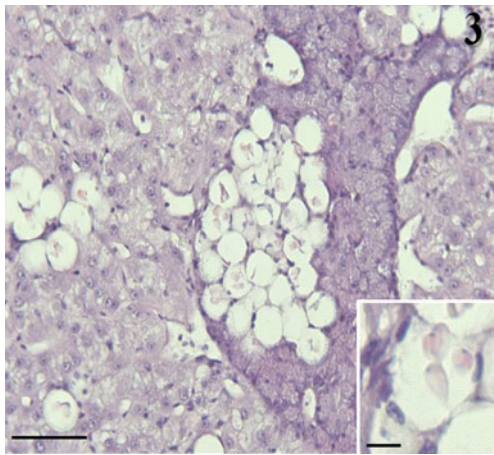


Fig. 3 *C. temensis*. Detail of the oocysts of *Calyptospora* sp. shown in Fig. 2. HE. Scale bar: 50 μ m (insert: higher magnification. Scale bar: 10 μ m)

Calyptospora has already been reported from a number of different geographic areas. Parasite originally described as *Eimeria* may also belong in the genus *Calyptospora*; the species *Eimeria harpodoni* was described from *Harpodon nehereus* in India by Setna and Bana (1935). The species *Calyptospora funduli* was described in *Fundulus grandis* in Mississippi (USA) by Duszynski et al. (1979). Fournie et al. (1985) described *Calyptospora empristica* in *Fundulus notti*, also from Mississippi.

In Brazil, a number of *Calyptospora* species have been described from Amazonian fish species—*C. serrasalmi* in *Serrasalmus niger* by Cheung et al. (1986), *C. tucunarensis* in *Cichla ocellaris* by Bekesi and Molnar (1991), *C. spinosa* in *Crenicichla lepidota* by Azevedo et al. (1993), and *Calyptospora* sp. in *Arapaima gigas* by Bonar et al. (2006). *Calyptospora* was also recorded in *Triporthus guentheri* and *Tetragonopterus chalcus* in Minas Gerais, Brazil by Albuquerque and Brasil-Sato (2010).

More than half of the specimens analyzed in this study were infected with *Calyptospora*. Studies, such as those of Azevedo et al. (1993) and Bonar et al. (2006), reported infection rates of 63% and 85.71%, respectively for *Calyptospora*, whereas Albuquerque and Brasil-Sato (2010) reported a much lower rate of less than 10%. In the present study, the infection rate increased during the rainy season (December to May). Fournie and Solangi (1980) observed that infection rates were associated with the abundance of intermediate hosts within the study area. Given this, the continuous monitoring of fish species throughout the course of the year would be necessary for a better understanding of infestation patterns in *C. temensis*.

The high rates of extraintestinal infection by coccidia in fish are the principal difference in comparison with homeothermic

organisms (Davies and Ball 1993). *Calyptospora* infects primarily the hepatic tissue of fish (Azevedo et al. 1993; Bekesi and Molnar 1991; Casal et al. 2007; Cheung et al. 1986; Fournie et al. 1985). In the present study, infections were recorded exclusively in the hepatic and pancreatic tissue of the specimens analyzed.

The innumerable circular oocysts observed in the hepatopancreases of the specimens were characteristic of the coccidian *Calyptospora*, i.e., homogeneous, colorless, homogeneous, thin-walled, centralized by slightly acidophilic sporocysts, and often pyriform, as described by Albuquerque and Brasil-Sato (2010), Azevedo et al. (1993); Bekesi and Molnar (1991); Casal et al. (2007); Cheung et al. (1986) and Fournie et al. (1985). Based on a comparison with the ecological and morphometric data presented by Bekesi and Molnar (1991), the parasite observed in the present study appears to be *C. tucunarensis*, although this can only be confirmed reliably using transmission and scanning electron microscopy and molecular analyses.

As in the present study, Bonar et al. (2006) confirmed the absence of significant inflammation of the hepatic tissue of *A. gigas* infected with *Calyptospora*. However, Cheung et al. (1986) found that infections by *Calyptospora* caused a variety of lesions in *S. niger*, including grayish patches observed macroscopically in the hepatic lobes and severe degeneration of the liver found in the histological analysis (Bekesi and Molnar 1991). Azevedo et al. (1993) report that hepatic infection by coccidia in fish is characterized by marked alterations of the host cells, such as nuclear hypertrophy and the destruction of the cytoplasm, visible in optical microscopy. Albuquerque and Brasil-Sato (2010) found that in fish, as in other vertebrates, when the infection by coccidia occurs under natural conditions, the effects on the equilibrium of the parasite–host relationship are greatly reduced.

Few studies are available on the life cycle of *Calyptospora*. In one of these studies, Azevedo et al. (1995) provide the first description of the life cycle of *C. spinosa* in the Amazonian vertebrate host *C. lepidota*. Freshwater prawns—*Macrobrachium* spp.—are probably the intermediate host of this protozoan (Albuquerque and Brasil-Sato 2010), and the transfer occurs when the primary host ingests infected prey. *Macrobrachium* prawns are found in the Marajó-Açu, so it is possible that these crustaceans play a role in the biological cycle of the *Calyptospora* parasite observed during the present study.

This is the first report of infection by *Calyptospora* with no histopathological alterations in *C. temensis* from the Marajó-Açu River on Marajó Island in Brazil. This is also the first report of coccidiosis in both the tissues of the hepatopancreas simultaneously. Infection rates increased during the rainy season, between December and May. This record from the Marajó-Açu River also represents an extension of the geographic distribution of the genus *Calyptospora*.

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