

**An anatomopathological study of hepatic coccidiosis (*Calyptospora* sp.) in the
Acará-pixuna, *Aequidens plagiozonatus* Kullander, 1984
from the Brazilian state of Pará**

[*Estudo anatomopatológico de coccidiose hepática (Calyptospora sp.) em Acará-pixuna, Aequidens plagiozonatus Kullander, 1984, no Brasil, estado do Pará*]

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ABSTRACT

The present study focuses on the anatomopathological alterations provoked by parasitism by *Calyptospora* sp. in 40 specimens of *Aequidens plagiozonatus* collected in Pará, Brazil. Examinations of the fresh material by compression of the hepatopancreas and histological sections showed immature forms and oocysts characteristic of the genus *Calyptospora*, in addition to a large quantity of melanomacrophagic centers spread throughout the organ. No significant inflammation of the hepatic tissue was observed. The melanomacrophagic centers and the compression of the hepatocytes are part of the response of the host to the parasite. This study represents the first record of parasitism by *Calyptospora* in *A. plagiozonatus*.

Keywords: fish, acará-pixuna, parasite, coccidio, *Calyptospora*, pathology

RESUMO

Descreveram-se as alterações anatomopatológicas provocadas pelo parasitismo por Calyptospora sp. em 40 espécimes de Aequidens plagiozonatus, provenientes do município de Peixe-boi, Pará, Brasil. Foram encontradas formas imaturas e oocistos característicos do gênero Calyptospora, nos exames frescos por compressão e cortes histológicos, além de um grande número de centros melanomacrofágicos dispersos por todo o órgão. Digna de nota foi a ausência de inflamação significativa no tecido hepático. Centros melanomacrofágicos e compressão dos hepatócitos estão envolvidos na resposta do hospedeiro ao parasito. Este é o primeiro registro de ocorrência de parasitismo por Calyptospora sp. na espécie estudada.

Palavras-chave: peixe, acará-pixuna, parasita, coccidio, Calyptospora, patologia

INTRODUCTION

The acará-pixuna (*Aequidens plagiozonatus* Kullander, 1984) was first described from the River Plate basin in Paraguay. This fish feeds on small crustaceans and smaller fish, and, like other cichlids, is relatively aggressive. Its main characteristics are its distinct parental care

behavior, attractive colors, and small size, all of which contribute to its increasing popularity as an ornamental fish (Kullander, 2006).

The phylum Apicomplexa Levine, 1970, is a group of micro-organisms which includes pathogenic and parasitic forms that occur in a wide variety of organisms, including bivalve mollusks and fish (Matos *et al.*, 2004). The

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parasites of this phylum found in Amazonian fish include *Calyptospora spinosa* (Calyptosporidae), found in the liver of the Jacundá, *Crenicichla lepidota* (Azevedo et al., 1993/1995), *Calyptospora tucunarensis* in *Cichla ocellaris*, the speckled peacock bass (Bekesi and Molnar, 1991), and *Calyptospora* sp. in the Pirarucu, *Arapaima gigas* (Bonar, 2006).

Spores of the coccidian *Calyptospora* sp. can be observed under light microscopy, infesting the liver, forming oocysts with four sporocysts, each containing two sporozoites. Cells in different stages of the life cycle are commonly observed, in some cases forming parasitophorous vacuoles, with rounded to elliptical sporozoites visible under transmission electron microscopy (Azevedo et al., 1993).

Histopathological studies may provide useful insights into the principal and secondary causes of diseases in fish populations, with the aim of evaluating the impacts on the tissue, biochemistry, physiology, behavior or metabolism of these aquatic organisms resulting from their exposure to external agents (Teixeira, 2008). The present study describes the anatomopathological effects provoked by the parasitism of *A. plagiozonatus* by *Calyptospora*.

MATERIAL AND METHODS

A total of 40 specimens of *A. plagiozonatus* were collected in the municipality of Peixe-Boi (01°07'17.65"S, 47°18'48.35"W) in the Brazilian state of Pará. The specimens had a mean total length of 13.4±1.5cm, and mean weight of 66.0±19.2g. They were captured using cast nets and gillnets. Following the collection of biometric data, the animals were anesthetized with MS222 (Sigma), and then necropsied for the detailed examination of their internal organs.

Liver fragments were set in a fresh preparation between slide and coverslip for examination. When parasitism was confirmed, the liver was fixed in Davidson solution (95% ethanol, formaldehyde and acetic acid, distilled water), and processed using the standard techniques for setting in paraffin, staining with hematoxylin and eosin and Gutierrez's trichrome for examination. The specimens were photographed under a Zeiss Primo Star microscope coupled to a Canon A610/A620 52mm camera.

RESULTS AND DISCUSSION

The prevalence of infection by *Calyptospora* in *A. plagiozonatus* was 35.2%. Under optical microscopy, *Calyptospora* oocysts (with four sporocysts) were observed forming parasitic vacuoles (Figure 1A), in addition to immature forms (Figure 1B). These vacuoles were found throughout the livers of the infected specimens, and in the intra-hepatic pancreatic tissue, or exocrine pancreas, in the complex known as the hepatopancreas (Figure 1C).

Macroscopically, the coloration of the livers varied from pale whitish to darker superficial areas. The lighter coloration was observed in the livers in which *Calyptospora* oocysts were identified microscopically. These mature oocysts were relatively rare, and were found in local agglomerations, within a well-defined area delimited by an acidophilic wall. These morphological features were observed in 35% of the infected specimens.

These areas of darker coloration in the livers were rich in immature coccidians. The histopathological analysis revealed the presence of a large number of melanomacrophagous centers widely distributed throughout the organ. These centers are very dark in color, varying from chestnut-yellow to brown (Figure 1C), and were common in 65% of the infected specimens analyzed.

Cheung et al. (1986) observed that infections with *Calyptospora* in redeye piranha, *Serrasalmus niger*, caused a variety of lesions, including grayish blemishes on the hepatic lobes, visible macroscopically, which were consistent with the features observed in the present study. It is interesting to note that, in the present study, melanomacrophagic centers were also observed throughout the hepatopancreas of a specimen of *A. plagiozonatus* that was not infected with coccidians. Flores-Lopes and Malabarba (2007) recorded these centers in a variety of sizes and concentrations, coated externally with a thin layer of conjunctive tissue and melanin pigments, as observed in the present study (Figure 1D).

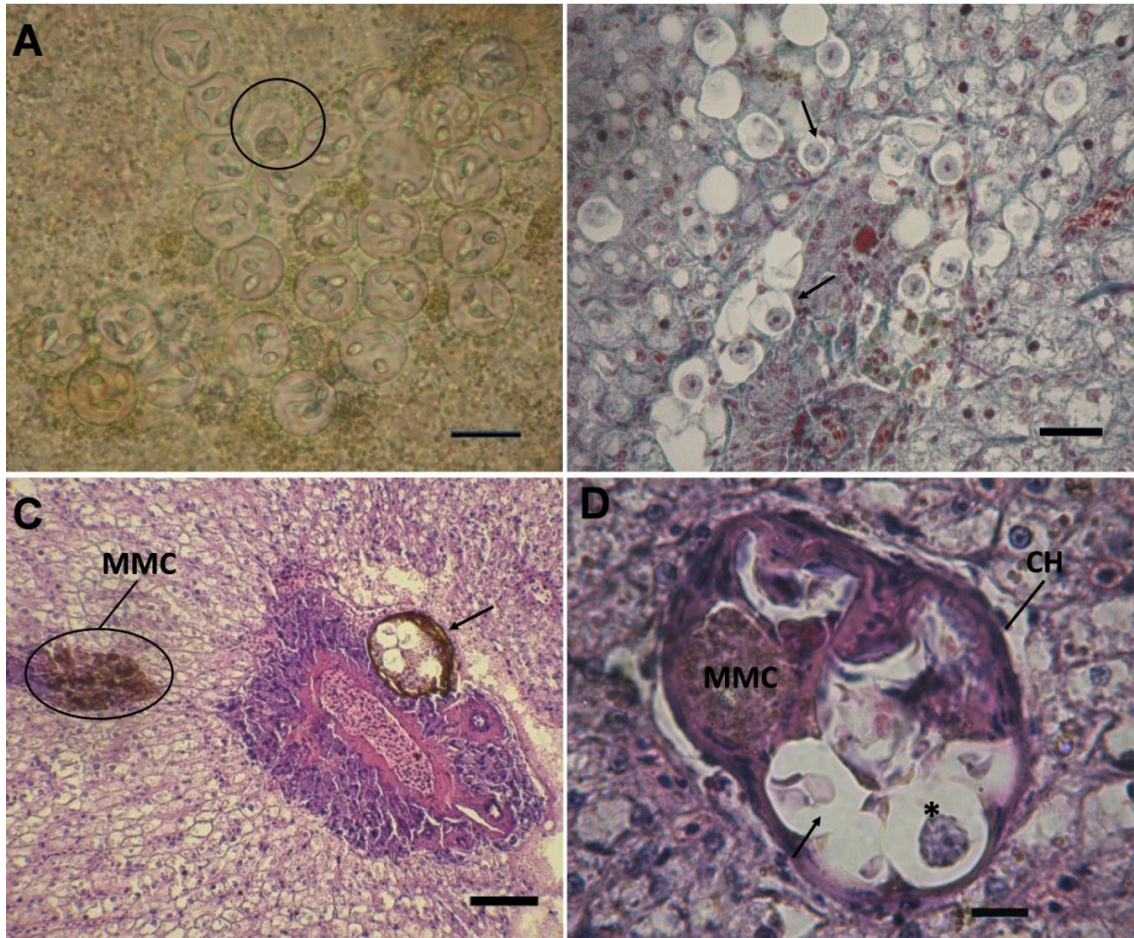


Figure 1. *Aequidens plagiozonatus*. Hepatopancreas. *Calyptospora* sp. Oocysts **A**- Predominance of mature sporocysts (arrow) observed in a fresh preparation under optical microscopy. Note the presence of an immature sporocyst (circled). **B**- Immature sporocysts located among the hepatocytes (arrow) (Gutierrez trichromatic stain). **C**- Oocyst (arrow) located in the hepatopancreas. Note the presence of the melanomacrophagic centers (MMC). (HE stain). **D**- Oocyst containing mature (arrow) and immature (*) sporocysts, a hyaline capsule (HC) and melanomacrophagic centers (MMC). Scale: 20µm.

In fish, these melanomacrophagic centers are normally found in the hepatic tissue (Manera *et al.*, 2000), and are made up of cells full of dark chestnut pigmentary granules, as observed under routine (HE) staining. An excess of these bodies may indicate an inflammatory reaction of the cells, the processing of antigens and/or exposure to an external agent. Some authors have also proposed that these bodies may be useful as bio-indicators of environmental pollution or degradation (Couillard and Hodson, 1996).

Agius and Roberts (2003) interpreted the melanomacrophagic centers as depositories for resistant pathogens and parasitic spores. Some

studies have also shown that these centers are associated with elevated resistance to mycobacteria and parasites of the genus *Myxobolus* (Roberts, 2001), and this was confirmed in the present study, although in this case, the features enveloped the *Calyptospora* oocysts (Fig. 1D). In some cases, in addition to the mature sporocysts, unsporulated oocysts were observed dispersed among the hepatocytes, or even grouped among the hepatic cells.

Azevedo *et al.* (1993) described alterations caused by *Calyptospora* in fish, observed using transmission electron microscopy, including nuclear hypertrophy and the destruction of the

cytoplasm of the host's cells. Békesi and Molnar (1991) also observed degradation of the liver in histological sections. However, the agglomerations of parasites observed in the present study rarely provoked a marked response in the host, but rather a slight compression of the hepatocytes adjacent to the location of the parasite. This is consistent with the findings of Bonar *et al.* (2006), who noted an absence of any significant inflammation of the hepatic tissue infected by *Calyptospora* in *Arapaima gigas*.

The results of the present study indicate that the melanomacrophagic centers are involved in the response of the host to the parasite. This is the first record of parasitism by *Calyptospora* in *A. plagiozonatus*.

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REFERENCES

- AGIUS, C.; ROBERTS, R.J. Melanomacrophage centres and their role in fish pathology. *J. Fish Dis.*, v.26, p.499-509, 2003.
- AZEVEDO, C.; MATOS, P.; MATOS, E. Morphological Data of *Calyptospora spinosa* n. sp. (Apicomplexa, Calyptosporidae) Parasite of *Crenicichla lepidota* HEÜKEL, 1840 (Teleostei) from Amazon River. *Eur. J. Protistol.*, v.29, p.171-175, 1993.
- AZEVEDO, C.; MATOS, P.; MATOS, E. Ultrastructural data on sporogony of the coccidian parasite *Calyptospora spinosa* from the liver of the Amazonian fish, *Crenicichla lepidota* Heckel. *J. Fish Dis.*, v.18, p.475-79, 1995.
- BÉKÉSI, L.; MOLNAR, M. *Calyptospora tucunarensis* n. sp. (Apicomplexa: Sporozoea) from the liver of Tucunaré *Cichla ocellaris* in Brazil. *Syst. Parasitol.*, v.18, p.127-132, 1991.
- BONAR, C.J.; POYNTON, S.L.; SCHULMAN, F.Y. *et al.* Hepatic *Calyptospora* sp. (Apicomplexa) infection in a wild-born, aquarium-held clutch of juvenile arapaima *Arapaima gigas* (Osteoglossidae). *Dis. Aquat. Org.*, v.70, p.81-92, 2006.
- CHEUNG, P.J.; NIGRELLI, R.F.; RUGGIERI, G.D. *Calyptospora serrasalmi* sp.nov. (Coccidia: Calyptosporidae) from liver of the Black piranha, *Serrasalmus niger* Schomburgk. *J. Aquat. Sci.*, v.4, p.54-57, 1986.
- COUILLARD, C.M.; HODSON, P.V. Pigmented macrophage aggregates: a toxic response in fish exposed to bleached-kraft mill effluent? *Environ. Toxicol. Chem.*, v.15, p.1844-1854, 1996.
- FLORES-LOPES, F.; MALABARBA, L.R. Alterações histopatológicas observadas no fígado do lambarí *Astyanax jacuhiensis* (cope, 1894) (Teleostei, Characidae) sob influência de efluentes petroquímicos. *Biociências*, v.15, p.166-172, 2007.
- KULLANDER, S.O. Cichlid Fishes from the La Plata Basin. Part V. Description of *Aequidens plagiozonatus* sp. n. (Teleostei, Cichlidae) from the Paraguay River. *Syst. Zool. Scr.*, v.13, p.155-159, 1984.
- KULLANDER, S.O. Ichthyological Exploration of Freshwaters: An international journal for field-orientated. *Ichthyology.*, v.17, p.289-398, 2006.
- MANERA, M.; SERA, R.; ISANI, G.; CARPENÉA, E. Macrophage aggregates in gilthead sea bream fed copper, iron and zinc enriched diets. *J. Fish Biol.*, v.57, p.457-465, 2000.
- MATOS, E.; CASAL, G.; MATOS, P. *et al.* Microrganismos Parasitos de Animais Aquáticos da Amazônia. In: RANZANI-PAIVA, M.J.T.; TAKEMOTO, R.M.; LIZAMA, M.A.P. *Sanidade de organismos aquáticos*. São Paulo: Editora Varela, p.158-178, 2004.
- ROBERTS, R.J. *Fish Pathology*, 3rd ed. Philadelphia: W.B. Saunders, PA. p.96, 2001.
- TEIXEIRA, P.A.N. *Avaliação da histopatologia hepática em boga (Chondrostoma polylepis) e barbo (Barbus bocagei) capturados no Rio Vizela*. 2008. 54f. Dissertação (Mestrado em Tecnologia Ambiental) Universidade de Trás-dos-Montes e Alto Douro.