

First report of *Dipetalonema gracile* in a captive Marcgrave's capuchin monkey (*Sapajus flavius*) in northeastern Brazil: Scientific communication

Primeira descrição de *Dipetalonema gracile* em macaco-prego-galego (*Sapajus flavius*) do nordeste do Brasil: Comunicação científica

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Highlights

Dipetalonema gracile in *Sapajus flavius*.

Microfilariae cause a inflammatory reaction in lymph nodes and spleen.

Filarial nematode *D. gracile* may be found in the abdominal cavity of *S. flavius*.

Abstract

Parasitic infections are important concern to the Wildlife Conservation Biology, particularly in endangered species. Herein, we report a parasitism by *Dipetalonema gracile* Rudolphi, 1809 (Spirurida, Filarioidea, Onchocercidae), in the peritoneal cavity of a captive Marcgrave's capuchin monkey (*Sapajus flavius*) that died at the Wild Animal Screening Center (CETAS) of the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA) in the municipality of Cabedelo, state of Paraíba, northeastern Brazil. The necropsy revealed two filarial worms *D. gracile* in the abdominal cavity. Exudates, thin fibrin

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layers and fibrous adhesions were also present in the mesentery and spleen capsule. The mesenteric, mandibular, and tracheobronchial lymph nodes were enlarged. Multiple small nodules were seen in the spleen parenchyma. Microscopic examination of the lymph nodes and spleen revealed markedly and diffuse inflammatory reaction, with edema, plasma cells, eosinophils, histiocytes, lymphocytes and rare multinuclear giant cells, with obliteration of the normal histological architecture of the organ. This is the first report of *D. gracile* parasitism in Marcgrave's capuchin monkeys, a critically endangered species. Studies of this nature significantly contribute to the knowledge of the parasitic fauna of endangered species, in addition to helping to formulate conservation strategies (*in situ* and *ex situ*) and records of new hosts and new areas of occurrence of parasites.

Key words: Conservation. Endoparasites. Filarial worms. Neotropical primates.

Resumo

Infeções parasitárias são uma questão importante para a Medicina e Biologia da Conservação. É descrito o parasitismo por *Dipetalonema gracile* Rudolphi, 1809 (Spirurida, Filarioidea, Onchocercidae) na cavidade peritoneal de um macaco-prego-galego (*Sapajus flavius*) que morreu no Centro de Triagem de Animais Silvestres (CETAS) do Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA), município de Cabedelo, Paraíba, Brasil. A necropsia revelou dois espécimes de parasitas na cavidade abdominal. Exsudatos, finas camadas de fibrina e aderências fibrosas também estavam presentes no mesentério e na cápsula do baço. Os linfonodos mesentéricos, mandibulares e traqueobrônquicos estavam aumentados. Vários pequenos nódulos foram vistos no parênquima do baço. O exame microscópico dos linfonodos e baço revelou reação inflamatória grave e difusa, com edema, células plasmáticas, eosinófilos, histiócitos, linfócitos e raras células gigantes multinucleares, com obliteração da arquitetura histológica normal do órgão. Este é o primeiro relato de parasitismo por esse nematódeo em macaco-prego-galego, uma espécie criticamente ameaçada da Mata Atlântica do Rio Grande do Norte, Paraíba e Pernambuco. Estudos dessa natureza contribuem significativamente para o conhecimento sobre a fauna parasitária de espécies ameaçadas de extinção, além de auxiliar na formulação de estratégias de conservação (*in situ* e *ex situ*) e registros de novos hospedeiros e novas áreas de ocorrência de parasitas.

Palavras-chave: Conservação. Endoparasitos. Nematódeo filarial. Primatas neotropicais.

Sapajus flavius (Marcgrave's capuchin monkey, blond capuchin monkey) occurs in fragments of Atlantic Forest in the states of Rio Grande do Norte, Paraíba, Pernambuco and Alagoas, northeastern Brazil (Valença-Montenegro, Bezerra, Martins, & Fialho, 2015). It is critically endangered and requires *in situ* and *ex situ* conservation actions (International Union for Conservation of Nature and Natural Resources [IUCN], 2011).

Wild animals are hosts to a wide range of parasites, which can act as primary or opportunistic agents for disease. In both cases, parasites represent an obstacle to management programs and to recovery of populations of endangered species. Wild animals living in captivity are frequently affected by parasitic diseases, with morbidity and mortality depending on parasite species, parasite load and the host's nutritional

status, immune response, and physiological condition (Godoy & Cubas, 2011). Therefore, reintroduction protocols require correct identification of the parasitic fauna (IUCN, 2011).

Various species of filarial nematodes of the genera *Dipetalonema* and *Tetrapetalonema* have been described in New World primates, including in endangered species (Muniz-Pereira, Vieira, & Luque, 2009; Vanderhoeven, Notarnicola, & Agostini, 2017; Milstein et al., 2020). To date, six species of *Dipetalonema* have been described in Brazil: *D. gracile*, *D. caudispina*, *D. graciliformis*, *D. robini*, *D. freitasi* and *D. yatesi* (Muniz-Pereira et al., 2009). Microfilariae of *D. gracile* develop to the infectious stage (L3) in arthropods of the genus *Culicoides*, thus acting as vectors in enzootic areas. The adult parasites live in the peritoneal cavity of primates. Accordingly, this study aimed to describe the lesions caused by *Dipetalonema* sp. and the morphologic features of the parasite found in a Marcgrave's capuchin monkey.

In 2013, a male Marcgrave's capuchin monkey that was being kept at the Wild Animal Screening Center (CETAS) of the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA) in the municipality of Cabedelo, state of Paraíba, Brazil, died of an unknown cause and was subjected to necropsy. and was subjected to necropsy. The procedures were approved and performed under the regulations of the Brazilian Institute for the Environment and the Renewable Resources (Sisbio/IBAMA, protocol number 35565-1) and the Ethics Committees for Animal Experimentation at the Universidade Federal da Paraíba, Brazil (protocol number 0710/12).

A complete forensic necropsy was performed. The veterinary pathologist systematically examined the carcass. Samples of all organs and tissues were collected and placed in 10% neutral buffered formalin until histopathologic investigation. The samples were processed routinely for histopathology: samples were embedded in paraffin, sectioned at a thickness of 3 μ m and stained with hematoxylin and eosin (H&E) (Fischer, Jacobson, Rose, & Zeller, 2008). The histopathological lesions were described, including identification and description of the parasite structures associated with the lesions.

Specimens of nematodes were found at necropsy. These were fixed in AFA (ethanol 70 °GL, 37% formalin and glacial acetic acid) and were further processed and identified as previously described (Amato, Böeger, & Amato, 1991; Anderson, Chabaud, & Willmott, 2009). The specimens were deposited in the Parasite Collection of the Parasitology Laboratory (LAPAR) of the Universidade Federal Rural de Pernambuco (UFRPE).

The monkey carcass was underweight. The necropsy revealed two specimens of parasites in the abdominal cavity. Exudates, thin fibrin layers and fibrous adhesions were also present in the mesentery and spleen capsule (Figure 1.C, D).

The mesenteric, mandibular, and tracheobronchial lymph nodes were enlarged. Multiple small nodules were seen in the spleen parenchyma. Microscopic examination of the lymph nodes and spleen revealed markedly and diffuse inflammatory reaction, with edema, plasma cells, eosinophils, histiocytes, lymphocytes and rare multinuclear giant cells,

with obliteration of the normal histological architecture of the organ (Figure 1.A). Microfilariae of approximate length 125 µm were associated with this lesion (Figure 1.B, C, D). In the other evaluated organs, no lesions were observed (Figure 1.C, D).

The adult parasites were identified as filarial nematode *D. gracile*. The specimens found were large and filiform, with a long and tapered tail. The male (M) had four pairs of preanal papillae and markedly unequal thin spicules; the female (F) had a vulva located near to the glandular portion of the esophagus. Were measured: body length (M- 81 mm and F- 195 mm), esophagus length (M- 3,25 mm and F- 3,65 mm), distance of the vulva to the anterior end (F- 0,85 mm), length of the big spicule (M- 1,01 mm) and length of the less spicule (M- 0,23 mm).

A diagnosis of lymphadenitis associated with parasitism by *D. gracile* was established. It was determined from the characteristic macro and microscopic lesions, presence of two specimens of nematodes in the abdominal cavity and characterization of the parasite based on morphological aspects. In Brazil, parasitism by *D. gracile* has been reported in *C. capucinus*, *Lagothrix lagotricha*, *Callithrix jacchus*, *Ateles paniscus*, *Brachyteles arachnoides*, *Leontopithecus chrysopygus*, *Leontopithecus rosalia*, *Saguinus bicolor* (Muniz-Pereira et al., 2009), *Cebus cay* and *Saimiri sciureus* (Correa, Bueno, Soares, Fabiano, & Muniz-Pereira, 2016).

Dipetalonema gracile is pathogenic for squirrel monkeys and may be found free in the peritoneal cavity or bound up in the

mesentery (Zhang et al., 2020), as observed in the present case. However, to the best of our knowledge, this is the first report of *D. gracile* parasitism in *S. flavius*. Although there are no reports of mortality among primates affected by *D. gracile*, our findings reinforce the importance of periodic parasitological surveillance for monitoring animal health status, particularly in wildlife species.

Under experimental conditions, infective larvae of *D. gracile*, which had developed in *Culicoides hollensis*, were inoculated into four laboratory-born squirrel monkeys (*Saimiri sciureus*). Slight capsular fibrosis was observed on the spleen of two of the animals, but fibrous adhesions were absent. The authors concluded that level of microfilaremia did not correlate directly to the number of adult parasites recovered (Travi, Eberhard, & Lowrie, 1985), like observed in the blond capuchin monkey. However, the spleen lesions were more severe in the present case.

Additionally, ulcerative enteritis and peritonitis associated with *Molineus torulosus* parasitism in *S. flavius* has been described (Bacalhao et al., 2016). However, we did not observe granulomas or ulcers in the intestine. The absences of lesions in the other evaluated organs, confirmed the involvement *D. gracile*.

This is the first report of *D. gracile* parasitism in *S. flavius*. Studies of this nature contribute significantly towards the body of knowledge about the parasitic fauna of endangered species and help underpin formulation of conservation strategies (*in situ* and *ex situ*) and records of new hosts and new areas of occurrence of parasites.

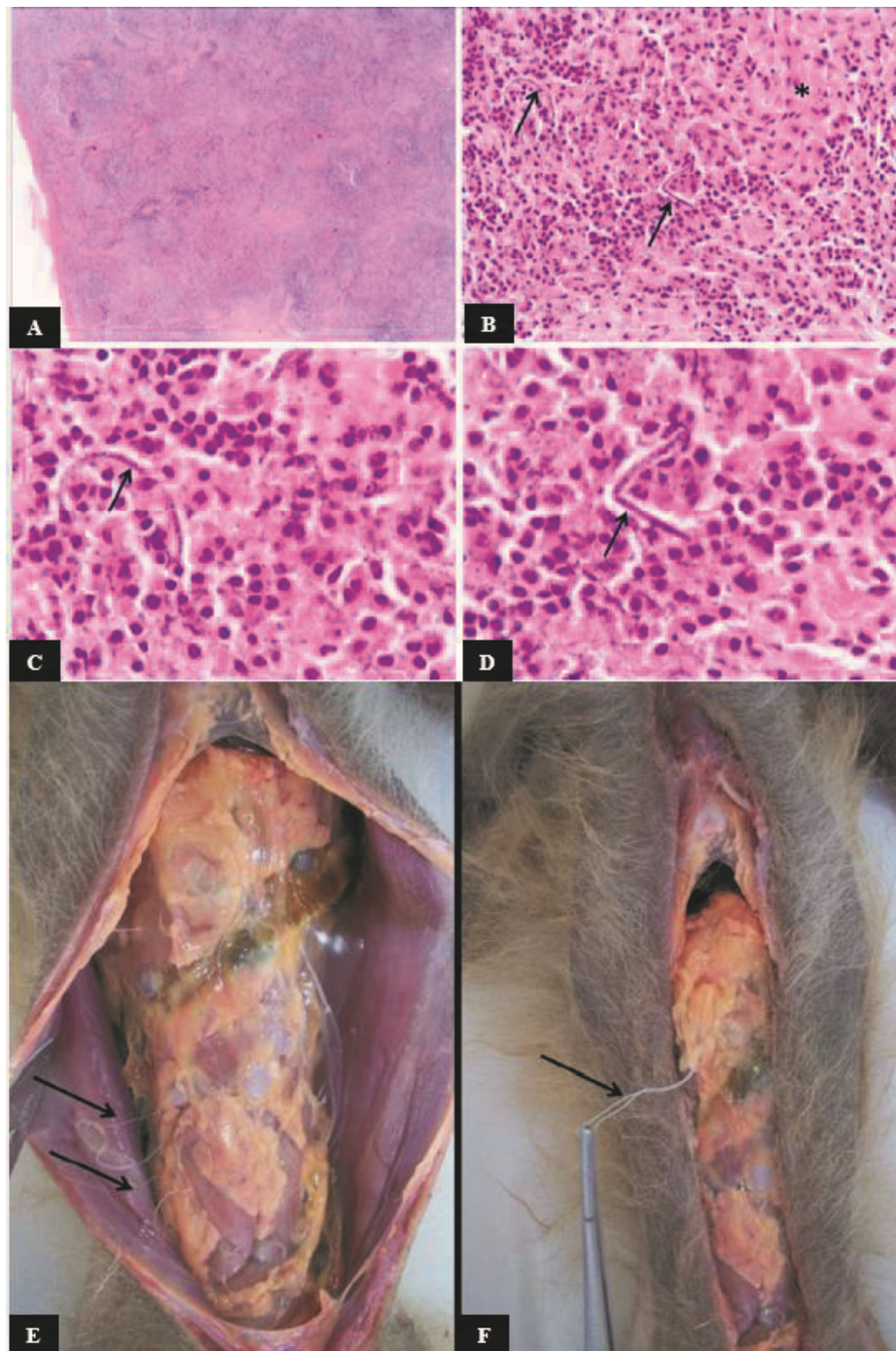


Figure 1. Splenitis caused by parasitism by *Dipetalonema gracile* in a captive Marcgrave's capuchin monkey (*Sapajus flavius*).

(A) The normal structure of the spleen was completely altered due to diffuse inflammation and fibrosis of the parenchyma. Hematoxilín and eosin, obj. 4x. (B) Spleen parenchyma presents fibrosis (asterisk) and many plasma cells, histiocytes and lymphocytes surround extracellular microfilariae (arrows). (C, D) Microfilariae in a maximum optical magnification (arrows). Hematoxilín and eosin, obj. 100x. (E) Nematodes free in the peritoneal cavity of monkey. (F) Macroscopic detail of the worm.

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