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Co-infection with filarial nematodes in *Sapajus macrocephalus* and *Cebus albifrons* (Primates: Cebidae) from the Peruvian Amazon

D.F. Conga[1], P. Mayor[2], A.P. Furtado[1], E.G. Giese[1,3] and J.N. Santos[1]

[1]Laboratório de Biologia Celular e Helmintologia 'Profa. Dra. Reinalda Marisa Lanfredi', Universidade Federal do Pará (UFPA), Instituto de Ciências Biológicas, Rua Augusto Correa 01, Guamá, Belém, Pará 66075-110, Brazil; [2]Departament de Sanitat i d'Anatomia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, Edifici V, E-08193 Bellaterra, Barcelona, Spain and [3]Laboratório de Histologia e Embriologia Animal, Instituto de Saúde e Produção Animal na Amazônia, Universidade Federal Rural da Amazônia (UFRA), Av. Perimetral, 2501, Montese, Pará 66077-901, Brazil

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Author for correspondence:
D.F. Conga, E-mail: daket17@gmail.com

Abstract

Dipetalonema caudispina (Molin, 1858) and *D. gracile* (Rudolphi, 1809) (Filarioidea: Onchocercidae) are two of six known species of filarial nematodes that parasitize Neotropical non-human primates. Adult filariae were collected from the thoracic and abdominal cavities of 38 of 44 specimens of *Sapajus macrocephalus* (Spix, 1823) and nine of ten specimens of *Cebus albifrons* (Humboldt, 1812) (Primates: Cebidae), distributed in the Yavarí-Mirín river basin and used locally for human consumption. Co-occurrence of *D. caudispina* and *D. gracile* is reported for the first time, with a prevalence of 18.5% (10 of 54 hosts examined). Our finding of *D. caudispina* and *D. gracile* in cebids from the Peruvian Amazon constitutes a new geographical record for both filariae, two new host records for *D. caudispina,* and the first report of *D. gracile* in *S. macrocephalus*. In addition, we provide morphometric data for *D. caudispina*, complementing the original description, as well as scanning electron microscopy details on the structure of the area rugosa and number of caudal papillae in males.

Introduction

Dipetalonema caudispina was described by Molin (1858), who initially named the species *Filaria caudispina* and subsequently revised the taxonomy and considered it to be *Filaria gracilis* Rudolphi, 1809 (Boulenger, 1920); it was validated as *D. caudispina* by Freitas (1943). The occurrence of *D. caudispina* in Brazil was reported by Molin (1858) from the following species: *Ateles paniscus* (L.) (syn. *Cebus paniscus* Fischer) (Atelidae); *Sapajus apella* (L.) (syn. *Cebus apella* Linnaeus) (Cebidae); *Brachyteles arachnoides* (G.) (syn. *Cebus arachnoides* Geoffroy) (Atelidae); *Saimiri sciureus* (L.) (syn. *Callithrix sciureus* Geoffroy) (Cebidae); *Lagothrix lagotricha* (H.) (syn. *Cebus Lagothrix* E. Geoffroy) (Atelidae); *Alouatta seniculus* (L.) (syn. *Cebus ursinus* Linnaeus) (Atelidae); *Leontopithecus rosalia* (L.) (syn. *Jacchus rosalia* Fischer) (Callitrichidae). It was also reported in *Ateles paniscus* from French Guiana by Bain *et al*. (1986). Molin reported *D. caudispina* in non-human primates from Brazil but did not provide quantitative or morphometric data on the helminths found, nor the condition of the hosts and accurate geographical records. Moreover, some observations by Freitas (1943) suggest mistaken records of parasitism by filariae in some hosts mentioned by Molin (1858).

Dipetalonema gracile (Rudolphi, 1809), on the other hand, has been reported in Bolivia, Ecuador, Mexico, Colombia, Venezuela, Panama, Paraguay, Peru and Brazil as a parasite of several non-human primates (Notarnicola *et al.*, 2008). However, complete morphometric data were provided only for specimens of *D. gracile* diagnosed from *S. sciureus* by Bain *et al.* (1986) in specimens from French Guiana, and for specimens from the Ecuadorian Amazon by Notarnicola *et al.* (2008). Until now, the presence of filariae of the same genus in the same host has not been reported. This work records for the first time the co-infection with *D. caudispina* and *D. gracile* in two species of cebid primates, *Sapajus macrocephalus* (Spix, 1823) and *Cebus albifrons* (Humboldt, 1812), in free-living conditions in the Peruvian Amazon, where these primates are used for human consumption and harvested through subsistence hunting. As well as expanding the host range for *D. caudispina*, and the geographical range for both *D. gracile* and *D. caudispina*, we provide additional morphological data for the latter species based on scanning electron microscopy (SEM).

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Materials and methods

From 2009 to 2013, 44 large-headed capuchins *S. macrocephalus* (30 males, 14 females) and ten white-fronted capuchins *C. albifrons* (four males, six females) were harvested by subsistence hunters in the north-eastern Peruvian Amazon at the Yavarí-Mirín river basin (04°19′53″S, 71°57′33″W) in the region of Loreto, Peru. As subsistence hunting is a common activity in the region, hunters and local residents were included in a natural resource management programme and advised on the removal and identification of abdominal and thoracic organs and their preservation in 10% formaldehyde fixative solution.

Helminths were collected, preserved in 70% ethanol and sent to the Laboratory of Cell Biology and Helminthology of the Biological Sciences Institute of the Federal University of Pará (UFPA) under an import license to Brazil (No. 02309-MINAGRI-SERFOR). Helminths were cleared in 50% Aman lactophenol (Gardner, 1996) and examined under an Olympus BX41 microscope and an Olympus SZX12 stereo microscope (Olympus Corporation, Tokyo, Japan). SEM samples were prepared following the protocol of Furtado *et al.* (2010) and analysed under a VEGA3 LMU microscope (TESCAN, Brno, Czech Republic) at the Laboratory of the Federal Rural University of the Amazon (UFRA). Specimens of *D. caudispina* (one male, MPEG 0115, and one female, MPEG 0116) and *D. gracile* (two males, MPEG 0117–0118 and two females, MPEG 0119–0120), were deposited in the Invertebrate Collection of Museu Paraense Emílio Goeldi (MPEG, Belém, Pará state, Brazil).

Table 1. Morphometric comparison of the descriptions of male and female *Dipetalonema caudispina* found in *Sapajus macrocephalus* and *Cebus albifrons* (present study) and in *Ateles paniscus* by Freitas (1943) and Bain *et al.* (1986). All measurements are in μm except body length and area rugosa length (in mm).

Host	*Sapajus macrocephalus* (n = 7) and *Cebus albifrons* (n = 3)		*Ateles paniscus* (n = 1)		*Ateles paniscus* (n = 1)	
Locality	Yavarí-Loreto, Peru		Cuminá-Pará, Brazil		Troits Sauts, French Guiana	
Source	Present study		Freitas (1943)[a]		Bain *et al.* (1986)	
	Male (n = 7)	Female (n = 9)	Male (n = 3)	Female (n = 3)	Male (n = 1)	Female (n = 1)
Body length (mm)	75.2 (59–92)	143.5 (116–165)	113–127	310–320	123	295
Body width	160 (133–203)	259 (190–326)	360–370	620–680	290	250
Buccal capsule length	10 (7–12)	11 (9–13)			11	12
Buccal capsule width	14 (13–16)	16 (13–18)				
Nerve ring from apex	152 (131–165)	159 (139–190)	220	160–230	180	200
Excretory pore from apex	218 (205–235)	211 (181–268)				
Oesophagus, total length	1766 (1347–2373)	2276 (1360–3333)	2240–2820	2560–3530	3000	2720
Muscular portion, length	424 (397–453)	368 (219–553)	270	390–510	500	800
Glandular portion, length	1342 (949–1920)	2644 (1579–3875)				
Vulva from apex		441 (267–763)		380–530		420
Vagina length		280 (249–361)		430		460
Vagina width		53 (46–61)				130
Ovijector length		3220 (2400–3867)		4520		9000
Left spicule length	657 (603–736)		950–1050		1030	
Handle length	291 (245–349)		460		450	
Lamina length	366 (333–432)					
Lamina–handle ratio	1–1.3 (1.1–1.8)		1.06–1–1.28		270	
Right spicule length	194 (160–237)		220–280			
Spicular ratio	1 : 3.4 (2.9–3.9)		1 : 4			
Gubernaculum length	23 (20–30)		31–37		42	
Area rugosa length (mm)	6.3 (4.7–7.9)				13,9	
Tail length	323 (262–373)	536,6 (427–683)	430–480	460–500	420	800
Caudal lappets, length	7 (7–8)	12 (9–17)			5	10
Caudal lappets from tip of tail	18 (16–25)	19 (10–27)			33	28
Microfilaria, body length		173 (161–179)				180–190
Microfilaria, body width		5 (4–7)				3.5–4.5

[a]Measurements of *D. caudispina* provided by Freitas (1943) converted from micrometers to millimeters.

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Results

A detailed analysis of the reproductive structures and other taxonomic characteristics of 345 filarial specimens from *S. macrocephalus* and 260 filarial specimens from *C. albifrons* allowed the identification of two species co-parasitizing these two hosts in the Amazon basin of the Yavarí-Mirín River in the Peruvian Amazon: *D. caudispina* and *D. gracile*. Both male and female specimens of *D. caudispina* examined during this study were smaller in body size compared to descriptions by Freitas (1943) and Bain *et al.* (1986) (table 1). According to studies of experimental infections on the development of the nematode under the influence of the host, these body size variations may be influenced by the acquired immunity and the age of the host (Chylinski *et al.*, 2009). These effects probably occur with more dynamism in free-living animals.

The presence of a discrete excretory pore in both sexes had been reported by Notarnicola *et al.* (2008) for *D. gracile* but not for *D. caudispina*. For this reason it was decided to examine specimens of *D. caudispina* from the helminthological collection of Instituto Oswaldo Cruz (CHIOC, Rio de Janeiro, Brazil); specimens deposited under number CHIOC 9027 had been studied by Freitas (1943) and Bain *et al.* (1986). The excretory pore of *D. caudispina* collected from *S. macrocephalus* and *C. albifrons* is immediately posterior to the nerve ring, at a mean distance of 218 µm from the anterior end in males and 211 µm in females; this is similar to the measurements obtained from specimens from *A. paniscus* deposited under CHIOC 9027, in which the excretory pore is immediately posterior to the nerve ring and situated at a distance of 251 µm from the anterior end in a single male and at a mean distance of 229 µm (195–259 µm) in six females.

Male specimens of *D. caudispina* from *S. macrocephalus* and *C. albifrons* differed in the number and arrangement of caudal papillae (fig. 1B) compared to those described by Freitas (1943) and Bain *et al.* (1986). Both earlier studies described 12 circular papillae differing in size and a median pre-cloacal papilla differing in shape, in addition to four papillae near the end of the tail. The present SEM study revealed one additional pair of papillae in the caudal region (fig. 1C, F) of three specimens studied, situated between the two pairs of caudal papillae directly following the cloaca and the posteriormost group of four caudal papillae (fig. 1A, E); these additional papillae could be an intraspecific variation observed in these three. Another important characteristic of male *D. caudispina* from *S. macrocephalus* and *C. albifrons* are the postcloacal bands, located on the left side only and which stop at a level that is approximately as far removed from the posteriormost group of caudal papillae as that group is removed from the tail tip, similar to the specimens of *D. caudispina* examined by Bain *et al.* (1986). SEM micrographs of this structure are provided for the first time (fig. 1D, E). Specimens of *D. gracile* examined in the present study (supplementary table S1) match descriptions of *D. gracile* provided by Bain *et al.* (1986) and Notarnicola *et al.* (2008) in having a sinuous vagina, a left spicule that is divided into three parts and a left postcloacal band that extends further posteriorly than the right one.

Twenty filariae of *D. caudispina* were recovered from *S. macrocephalus* and five from *C. albifrons*, with a mean intensity of infection of 2.9 (1–6) in *S. macrocephalus* and 1.7 (1–2) in *C. albifrons*. The overall prevalence of *D. caudispina* was 18.5% (15.9% in *S. macrocephalus*, 7/44 hosts examined, four males and three females, and 30.0% in *C. albifrons*, 3/10 hosts examined, only in males). Of *D. gracile*, 325 filariae were recovered from *S. macrocephalus* and 255 from *C. albifrons*, with a mean intensity of infection of 10.5 (1–61) in *S. macrocephalus* and 28.3 (1–114) in *C. albifrons*. The overall prevalence of *D. gracile* was 74.1% (70.5% in *S. macrocephalus*, 31/44 hosts examined, 21 males and ten females, and 90.0% in *C. albifrons*, 9/10 hosts examined, four males and five females). In general, the combined prevalence of *D. caudispina* and *D. gracile* was 87% (47/54 hosts examined), with a prevalence of mixed infection of 18.5% (7/44 hosts examined, four males and three females, from *S. macrocephalus*; 3/10 hosts examined, males only, from *C. albifrons*).

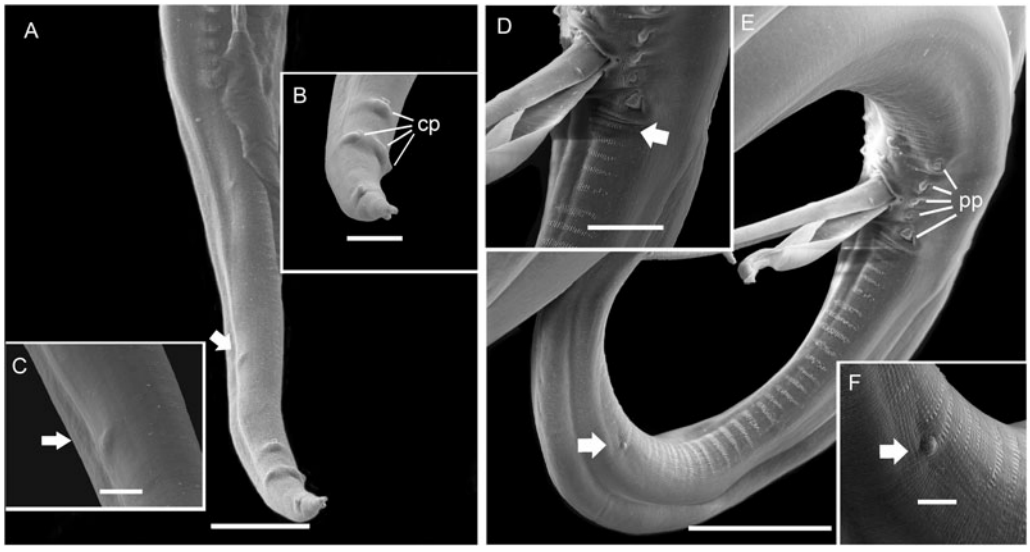

Fig. 1. Scanning electron micrographs of male *Dipetalonema caudispina*. (A) Posterior end, with detail of lateral caudal papilla (arrow); scale bar: 5 µm. (B) Posterior end, with details of group of four caudal papillae (cp); scale bar: 20 µm. (C) Posterior end, with detail of lateral caudal papilla (arrow); scale bar: 5 µm. (D) Posterior end, ventral view with detail of left postcloacal band of area rugosa (arrow); scale bar: 20 µm. (E) Posterior end, ventral view with detail of left postcloacal band, cloacal papillae (pp) and lateral caudal papilla (arrow); the posterior end of the right spicule is broken; scale bar: 50 µm. (F) Posterior region, lateral caudal papilla; scale bar: 5 µm.

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Discussion

It is noteworthy that *D. caudispina* was not observed as an independent parasite in any host but was always associated with *D. gracile*. This is the first record of co-infection with these two filariae. Occurrences of co-infections of helminths are common in wild hosts because they are exposed to these parasites in nature, but the difficulty in collecting specimens makes it difficult to record them. These co-infections could have effects not only on the host but also on the parasites themselves, their number and pathogenicity. The distribution of *Dipetalonema* spp. and other filarial species is important, as it complements data on the diversity of primate hosts in the Amazon region and provides epidemiological evidence of natural infections caused by this parasite in wildlife populations.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X18000287

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Conflict of interest. None.

Ethical standards. The research and sample collection authorization protocol used in this study was approved by *Servicio Forestal y de Fauna Silvestre* of Peru (Ethics Committee for research on wild animals; No. 0229-2011-DGFFS-DGEFFS).

References

Bain O, Petit G and Rosales L (1986) Filaires de Singes sud-américains. *Bulletin du Museum National de Histoire Naturelle, Section A* **8**, 513–542.

Boulenger CL (1920) Filariid worms from mammals and birds in the Society's Garden, 1911–1915. *Proceedings of the Zoological Society of London* **4**, 491–505.

Chylinski C, Boag B, Stear MJ and Cattadori IM (2009) Effects of host characteristics and parasite intensity on growth and fecundity of *Trichostrongylus retortaeformis* infections in rabbits. *Parasitology* **136**, 117–123.

Freitas JFT (1943) Estudos sobre nematodeos filarideos *Dipetalonema caudispina* (Molin, 1858). *Memorias do Instituto Oswaldo Cruz* **38**, 361–372.

Furtado AP, Melo FTV, Giese EG and Santos JN (2010) Morphological redescription of *Dirofilaria immitis*. *The Journal of Parasitology* **96**, 499–504.

Gardner SL (1996) Essential techniques for collection of parasites during surveys of mammals. pp. 291–298 *in* Wilson D, Cole R, Nichols JD, Rudran P and Foster M (*Eds*) *Measuring and monitoring biological diversity: standard methods for mammals*. Washington, DC, Smithsonian Institution Press.

Molin R (1858) Versuch einer Monographie der Filarien. *Sitzungsb. Denkschriften der Kaiserlichen der Wissenschaften, Wien. Mathematisch-Naturwissenschaftliche* **28**, 365–461.

Notarnicola J, Pinto CM and Navone GT (2008) Host occurrence and geographical distribution of *Dipetalonema* spp. (Nematoda: Onchocercidae) in Neotropical monkeys and the first record of *Dipetalonema gracile* in Ecuador. *Comparative Parasitology* **75**, 61–68.

Rudolphi CA (1809) *Entozoorum sive vermium intestinalium historia naturalis*. Vol. 2.