Parasitology

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Dipetalonema graciliformis (Freitas, 1964) from the red-handed tamarins (*Saguinus midas*, Linnaeus, 1758) in French Guiana

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Research Article

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Abstract

Six *Dipetalonema* species have been reported from Neotropical monkeys, *Dipetalonema gracile*, *Dipetalonema graciliformis* and *Dipetalonema caudispina* being the dominant species found in French Guiana primates. Adult filarioids isolated from the abdominal cavity of tamarins (*Saguinus midas*) in French Guiana were morphologically and molecularly identified as *D. graciliformis*. Phylogenetic analysis based on DNA and amino acid sequences of the *cox*1 gene as well as the concatenated sequences of the *cox*1 and the 18S genes indicated that *D. graciliformis* belongs to the clade 4 (ONC4) of Onchocercidae. Blast analysis of the 18S rDNA revealed that *D. graciliformis* in the studied tamarins is conspecific with the filarioid circulating in howler monkeys (*Alouatta macconnelli*) in French Guiana, previously referred to as unidentified Onchocercidae species.

Introduction

Filarioids (Filarioidea) are heteroxenous parasitic nematodes of tissues and body cavities of many vertebrate hosts, except fish. Members of the family Onchocercidae produce blood- or skin-inhabiting microfilariae that are ingested by blood-feeding arthropods and undergo a two-stage development until the third stage (L3) is transmitted to a new receptive host. More than 30 species of filarioids have been found in wild primates and humans in the Neotropical forests (Bain *et al.*, 1982). Of these, 13 species belong to the genus *Mansonella* (*Tetrapetalonema*) (Faust, 1935) and six to the genus *Dipetalonema* (Diesing, 1861), which are restricted to platyrrhine (Neotropical) primates (Bain *et al.*, 2015; Laidoudi *et al.*, 2020). Biting midges (Diptera: Ceratopogonidae) are competent vectors of *Dipetalonema* spp. as demonstrated for *Culicoides hollens* for *Dipetalonema caudispina* (Molin, 1858) and *Dipetalonema gracile* (Rudolphi, 1809) (Eberhard *et al.*, 1979).

Overall, the genus *Dipetalonema* includes: *D. caudispina* (Molin, 1858), *Dipetalonema freitasi* Bain, Diagne and Muller, 1987, *D. gracile* (Rudolphi, 1809), *Dipetalonema graciliformis* Freitas, 1964, *Dipetalonema yatesi* Notarnicola, Jimenez and Gardner, 2007 and *Dipetalonema robini* Petit, Bain and Roussilhon, 1985 (Table S1). Adhering to the concept of morphological species, the six species above are mainly distinguished by the complex structures of the vagina vera, caudal lappets in females as well as the spicules, the area rugosa of the tail and arrangement of the musculature in males and the shape of microfilariae (Petit *et al.*, 1985; Bain *et al.*, 1987).

Dipetalonema spp. have been reported from at least 20 species of Neotropical primates, from southern Mexico to central Bolivia and subtropical Brazil and Argentina (see Table S1). The holotype of *D. graciliformis* from *Saguinus midas* in Brazil (Pará State) was deposited by Freitas and described morphologically by Bain *et al.* 1986, along with specimens collected in French Guiana. Similarly, two species of *Dipetalonema* (i.e. *D. gracile* and *D. robini*) were described in *Saimiri sciureus* from Guyana (Petit *et al.*, 1985) indicating the sympatric distribution of *Dipetalonema* spp. in this geographic region. Later on, *D. graciliformis* was found in the abdominal cavity of *Saguinus mystax* (Spix, 1823) from Amazonas State, Brazil and of *Saguinus labiatus* in Peru whereas *D. freitasi* from *Cebus capucinus* in captivity and *D. yatesi* in *Ateles chamek* from north-central Bolivia.

Here, we performed integrative studies combining morphological and molecular data on filarioid nematodes isolated from the peritoneal cavity of red-handed tamarins (*S. midas*, Linnaeus 1758).

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Table 1. Comparative measurement (in μm unless specified) of adult females of *Dipetalonema graciliformis* (Freitas, 1964) from our study with the six *Dipetalonema* species

Species	*Dipetalonema graciliformis* (Freitas, 1964) from the current study		*D. graciliformis* (Freitas, 1964)	*Dipetalonema yatesi* (Notarnicola, Jimenez and Gardner, 2007)	*Dipetalonema robini* (Petit Bain, and Roussilhon, 1985)	*Dipetalonema freitasi* (Bain Diagne and Muller, 1987)	*Dipetalonema caudispina* (Molin, 1858)	*Dipetalonema gracile* (Rudolphi, 1819)
Type host	*Saguinus midas*		*S. midas*	*Ateles chamek*	*Saimiri Sciureus*	*Cebus capucinus*	*Atels panicus*	*S. sciureus*
Geographical origin	French Guiana		French Guiana	North central Bolivia	Guyana	Captive monkeys (London zoological garden)	French Guiana and Brazil	Guyana
	Female/4							
Specimens	Mean (S.D.)	[Range]	Female	Female	Female	Female	Female	Female
Body length (mm)	227 (93.5)	[152–339]	[255–330]	[239.6–254.2]	[130–152]	[210–230]	[295]	[167–210]
Body width	366.6 (55.8)	[250.5–486.6]	[340–350]	[339–575]	[250–430]	[450–460]	[220–250]	[400–450]
Buccal capsule length	8.1 (0.6)	[6.9–8.8]	[12]	[5–9]	–	[10–11]	[12]	[10]
Buccal capsule width	15.8 (0.9)	[14.5–17.6]	–	[17–21]	–	–	–	–
Nerve-ring from anterior extremity	178.4 (25.3)	[145.8–230.6]	[225]	[226–300]	[170–225]	[230–230]	[200]	[155]
Excretory pore from anterior extremity	262.4	[262.4]	–	–	–	–	–	–
Oesophagus length	2900	[2900]	[3360–3700]	[3175–4787]	[3920–4700]	[2000–2040]	[2720]	[3950–5500]
Muscular portion length	392 (24.6)	[322.6–432.7]	[430–550]	[350–750]	[700–1050]	[425–440]	[800]	[720]
Glandular portion length	2500	[2500]	[2930–3150]	–	–	[1575–1600]	[1980]	–
Tail length	278.4 (40)	[241.3–321.2]	[250–300]	[635–810]	[660]	[510–590]	[800]	[640–850]
Caudal lappets length	14 (0.6)	[13.4–15.3]	[12]	[10]	[8–11.]	[9–10]	[10–12]	[7–12]
Lateral lappets from tip of tail	13.1 (0.4)	[11.6–14.1]	[8–14]	–	–	[6–6]	[8–10]	[5]
Vulva from anterior extremity	754.9 (93.1)	[667.6–853.7]	[760–850]	[588–1636]	[830–980]	[650–670]	[400–420]	[1150–1600]
Vagina vera length	209.5 (7.1)	[216.7–202.5]	[280]	[269]	[200]	[380–390]	[350–460]	[300]
Vagina vera width	71 (7.5)	[63.9–78.8]	[110–120]	[92]	–	[80–100]	[100–130]	[110–150]
Microfilaria body length	116.6 (1.9)	[113–119]	[93–115]	[158–172]	[150–186]	[100–107]	[180–190]	[125–145]
Microfilaria body width	5 (0.2)	[4.7–5.4]	[3.5–4]	[4.8–6]	[4–4.8]	[3.5–4]	[3.5–4]	[2.5–3.3]

Table 2. Comparative measurement (in μm unless specified) of adult males of *D. graciliformis* (Freitas, 1964) from our study with the six *Dipetalonema* species

Species	*D. graciliformis* (Freitas, 1964) from the current study		*D. graciliformis* (Freitas, 1964)	*D. yatesi* (Notarnicola, Jimenez and Gardner, 2007)	*D. robini* (Petit Bain and Roussilhon, 1985)	*D. freitasi* (Bain Diagne and Muller, 1987)	*D. caudispina* (Molin, 1858)	*D. gracile* (Rudolphi, 1819)
Type host	*S. midas*		*S. midas*	*A. chamek*	*S. sciureus*	*C. capucinus*	*Ateles panicus*	*S. sciureus*
Geographical origin	French Guiana		French Guiana	North central Bolivia	Guyana	Captive monkeys (London zoological garden)	French Guiana and Brazil	Guyana
	Male							
Specimens	Mean	[Range]	Male	Male	Male	Male	Male	Male
Body length (mm)	107.9 (4)	[104–112]	[132.0]	[81.9–111.9]	[69.5–77]	[78–100]	[123]	[76–104]
Body width	311.3 (5.6)	[305.8–317]	[350]	[272–333]	[280–300]	[255–260]	[290]	[280–350]
Buccal capsule length	9.2	[9.2]	[10]	[6–10]	–	[10–13]	[11]	[12]
Buccal capsule width	17.7	[17.7]	–	[20–22]	–	–	–	–
Nerve-ring from anterior extremity	207.4	[207.4]	[200]	[150–250]	[240]	[215–260]	[180]	[225]
Oesophagus length	3100	[3100]	[3.000]	[2242–2999]	[3700–4400]	[1700–2100]	[3000]	[3200–5700]
Muscular portion length	407.3	[407.3]	[450]	[388–550]	[565]	[455–455]	[500]	[1000–1070]
Glandular portion length	2.7	[2.693]	[2550]	–	–	[1245–1645]	–	[3720]
Tail length	249.8 (34.3)	[217.8–286.5]	[580]	[340–440]	[305–380]	[325]	[420]	[320–400]
Left spicule length	1.2 (0)	[1212–1234]	[1130]	[1020–1270]	[1000–1100]	[985–1040]	[1030]	[900–1220]
Handle length	276.1 (11)	[265,3–287,3]	[250]	[270–340]	[290]	–	[450]	[300]
Lamina length	946.9 (22.1)	[925.1–969.2]	[880]	–	–	–	–	–
Membranous alae length	336.7 (13)	[324–349.9]	[330]	[400–500]	–	[400–430]	–	–
Flagella length	610.4 (9.5)	[601–620]	[550]	[370–480]	–	–	–	–
Right spicule length	244.9 (11.1)	[234.1–256.2]	[260]	[180–260]	[240–245]	[240–250]	[270]	[210–290]
Lamina to handle ratio	2.9 (0.7)	[2.3–3.6]	[3.5]	–	–	–	–	–
Spicule length ratio	4.9 (0.3)	[4.7–5.2]	[4.3]	[4.2–5.7]	–	–	–	–
Gubernaculum length	22.9 (0.3)	[22.7–23.2]	[30]	[20–30]	–	[35]	[42]	[23]
Area rugosa length	1200	[1203]	[1200]	[900–1602]	[9000–9600]	[800]	[13 900]	[9500]

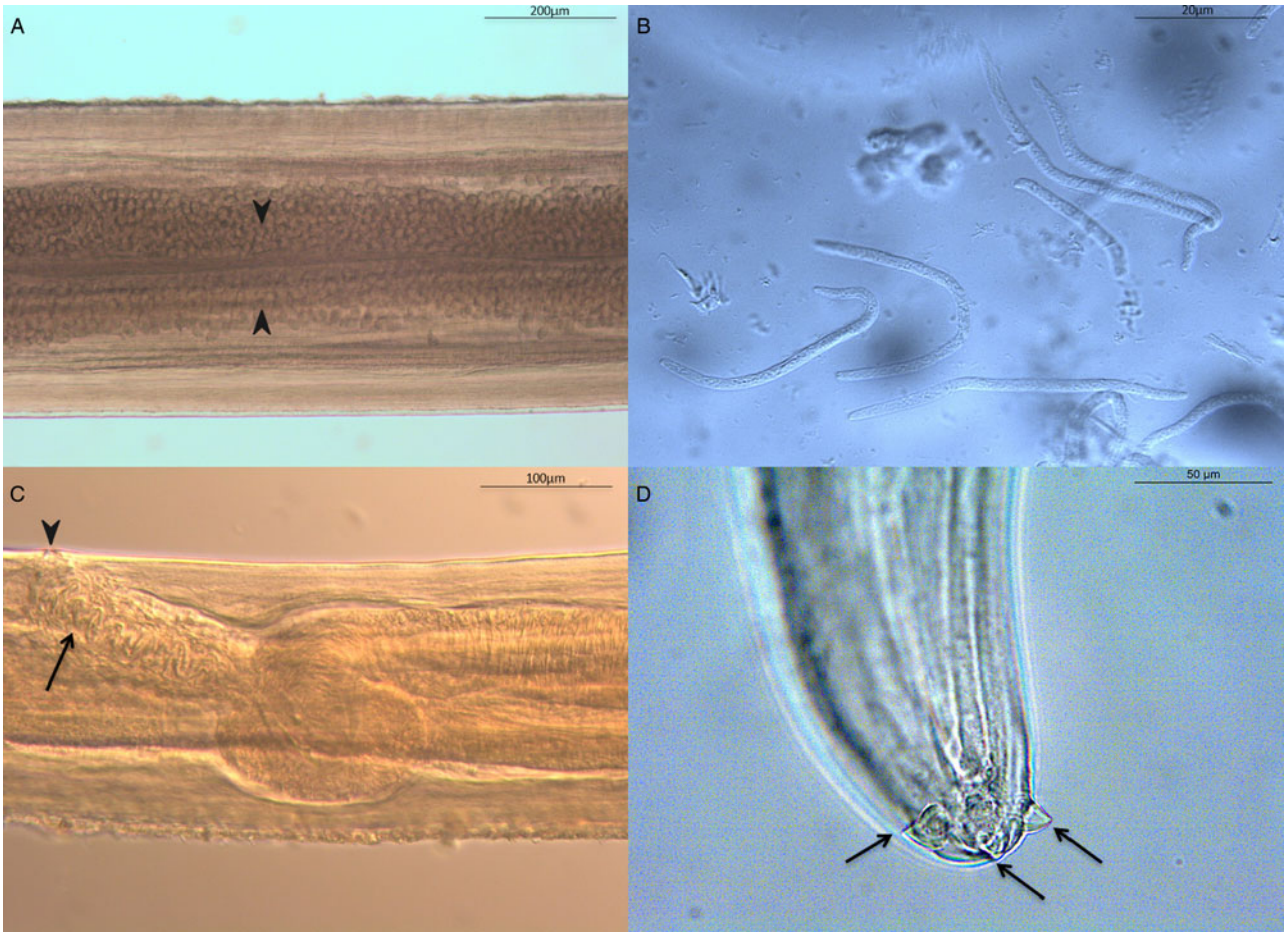

Fig. 1. Morphology of *Dipetalonema graciliformis* female. (A) Anterior part of female body showing ovary structure to the mid-body (arrows). (B) Uterine microfilaria. (C) Lateral view of the anterior region showing: genital opening (arrowhead), vagina vera (anterior end) with sinuosis duct (arrow). (D) Posterior end (ventral view) with details of the lappets.

Materials and methods

Adult filarioid worms (nine females and five males) were retrieved from the peritoneal cavities (Fig. S1) of two of the four red-handed tamarins found road killed on the edge of the national road 1, near the coast of northern French Guiana. Collected worms were identified at the species level based on morphological criteria. In addition, three molecular loci (i.e. *cox*1, 18S and 28S genes) were amplified, sequenced and deposited in the GenBank database under the accession numbers: MW357380-357394, MW355926-355940 and MW355911-355925 for the *cox*1, 18S and 28S genes, respectively (see Supplementary File S1 for details).

Results

Species identification and general morphological description

All filarioids were identified as *D. graciliformis*, according to Petit *et al.* (1985) and Bain *et al.* (1986). The comparative measurements of adult females and males from the current study and the other *Dipetalonema* species are detailed in Tables 1 and 2. Body whitish with fine transverse cuticular striation (Fig. S2A). Anterior end rounded (Fig. S2B). Buccal capsule small with head cuticle smooth cephalic region with smooth quadrangular shape (Fig. S2B). Oral opening small with four labial papillae arranged on a laterally elongated rectangle and four cephalic papillae located around oral opening. The oesophagus is divided in an anterior muscular part (short) (Fig. S2C) and posterior glandular portion (long) (Fig. S2C). The posterior end tapering to a point (Fig. S2D). Nerve ring located at half-length of muscular oesophagus, the excretory pore is visible behind the nerve ring. Vulva at the level of the oesophagus. Ovaries are located at the mid-body (Fig. 1A). Microfilaria (Fig. 1B) were obtained from the uteri (Fig. 1C) of a mature female. The female presented with extremity with three well developed petaloid appendage triangular shape with side base (Fig. 1D).

Caudal extremity in male spirally coiled accompanied by caudal papillae (Fig. S3A). The male presents single testis at the level of the oesophago-intestinal junction. The paired spicules are unequal in size being the left longer than the right one (Fig. S3A); the left spicule subdivided into the proximal handle (Fig. S3B) and lamina (Fig. S3C), being the latter formed by a membranous alae and a flagellum (Fig. S3D). Laterally (Fig. S4A), it is possible to observe: three pair of the pre-cloacal papillae, a single central pre-cloacal papilla, a pair of ad-cloacal papillae and one pair of post-cloacal papillae (the second pair was not visible) plus four papillae at the end of the tail near to the caudal appendages shaped like a nipple (Fig. S4B). A very small gubernaculum (Fig. S4C) is of navicular shape with post cloacal bands present. Posterior extremity with two spiral twist with small longitudinal striae as transverse band on ventral surface representing the area rugosa (Fig. S4D) formed by rows of short longitudinal crest. Body size ratio of female to male 1:1.8.

Molecular phylogenetic analyses

All polymerase chain reaction amplifications yielded the DNA amplicon of expected size from all the 14 specimens examined. Sequence alignment showed the identity of all DNA amplicons

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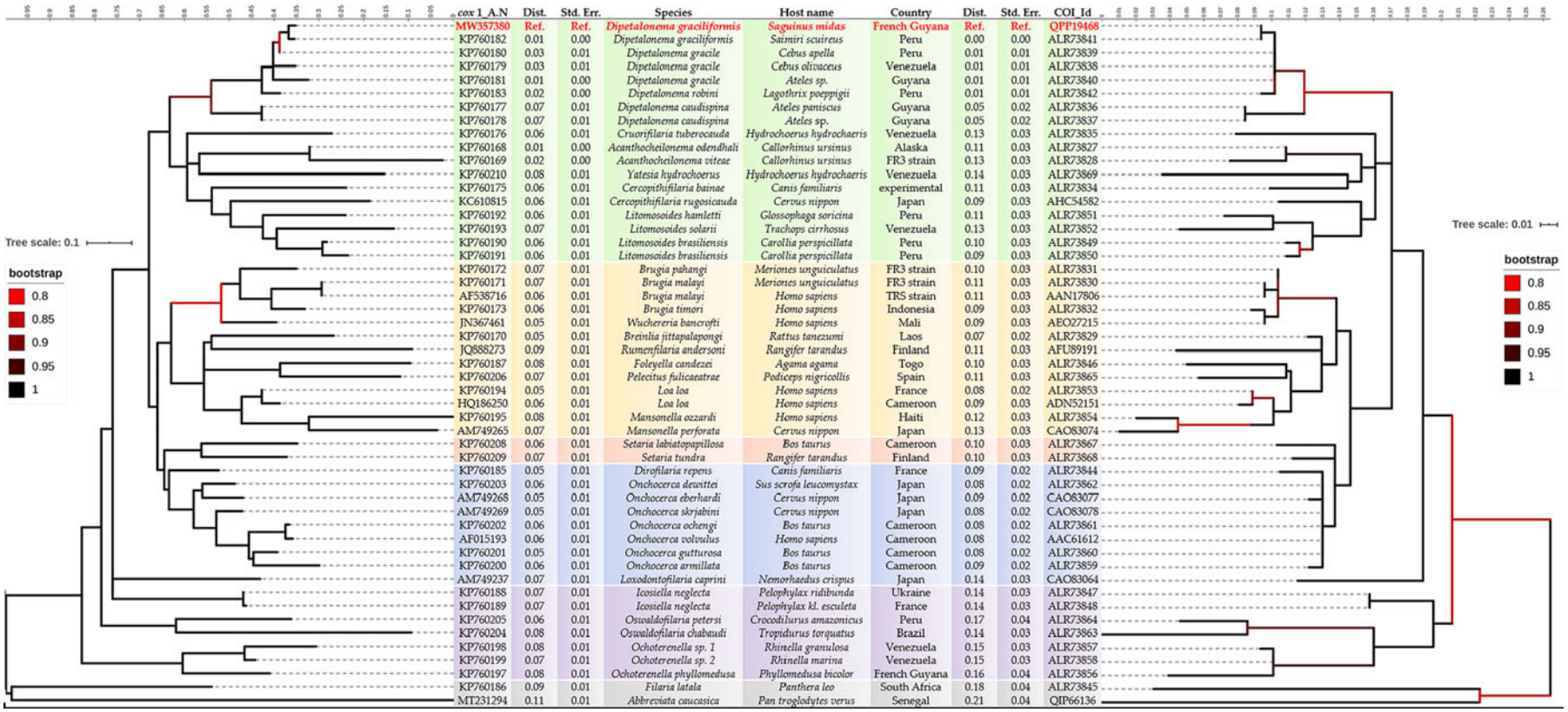

cox 1_A.N	Dist.	Std. Err.	Species	Host name	Country	Dist.	Std. Err.	COI_Id
MW357380	Ref.	Ref.	*Dipetalonema graciliformis*	*Saguinus midas*	French Guyana	Ref.	Ref.	QPP19468
KP760182	0.01	0.00	*Dipetalonema graciliformis*	*Saimiri scuireus*	Peru	0.00	0.00	ALR73841
KP760180	0.03	0.01	*Dipetalonema gracile*	*Cebus apella*	Peru	0.01	0.01	ALR73839
KP760179	0.03	0.01	*Dipetalonema gracile*	*Cebus olivaceus*	Venezuela	0.01	0.01	ALR73838
KP760181	0.01	0.00	*Dipetalonema gracile*	*Ateles sp.*	Guyana	0.01	0.01	ALR73840
KP760183	0.02	0.00	*Dipetalonema robini*	*Lagothrix poeppigii*	Peru	0.01	0.01	ALR73842
KP760177	0.07	0.01	*Dipetalonema caudispina*	*Ateles paniscus*	Guyana	0.05	0.02	ALR73836
KP760178	0.07	0.01	*Dipetalonema caudispina*	*Ateles sp.*	Guyana	0.05	0.02	ALR73837
KP760176	0.06	0.01	*Cruorifilaria tuberocauda*	*Hydrochoerus hydrochaeris*	Venezuela	0.13	0.03	ALR73835
KP760168	0.01	0.00	*Acanthocheilonema odendhali*	*Callorhinus ursinus*	Alaska	0.11	0.03	ALR73827
KP760169	0.02	0.00	*Acanthocheilonema viteae*	*Callorhinus ursinus*	FR3 strain	0.13	0.03	ALR73828
KP760210	0.08	0.01	*Yatesia hydrochoerus*	*Hydrochoerus hydrochaeris*	Venezuela	0.14	0.03	ALR73869
KP760175	0.06	0.01	*Cercopithifilaria bainae*	*Canis familiaris*	experimental	0.11	0.03	ALR73834
KC610815	0.06	0.01	*Cercopithifilaria rugosicauda*	*Cervus nippon*	Japan	0.09	0.03	AHC54582
KP760192	0.06	0.01	*Litomosoides hamletti*	*Glossophaga soricina*	Peru	0.11	0.03	ALR73851
KP760193	0.07	0.01	*Litomosoides solarii*	*Trachops cirrhosus*	Venezuela	0.13	0.03	ALR73852
KP760190	0.06	0.01	*Litomosoides brasiliensis*	*Carollia perspicillata*	Peru	0.10	0.03	ALR73849
KP760191	0.06	0.01	*Litomosoides brasiliensis*	*Carollia perspicillata*	Peru	0.09	0.03	ALR73850
KP760172	0.07	0.01	*Brugia pahangi*	*Meriones unguiculatus*	FR3 strain	0.10	0.03	ALR73831
KP760171	0.07	0.01	*Brugia malayi*	*Meriones unguiculatus*	FR3 strain	0.11	0.03	ALR73830
AF538716	0.06	0.01	*Brugia malayi*	*Homo sapiens*	TRS strain	0.11	0.03	AAN17806
KP760173	0.06	0.01	*Brugia timori*	*Homo sapiens*	Indonesia	0.09	0.03	ALR73832
JN367461	0.05	0.01	*Wuchereria bancrofti*	*Homo sapiens*	Mali	0.09	0.03	AEO27215
KP760170	0.05	0.01	*Breinlia jittapalapongi*	*Rattus tanezumi*	Laos	0.07	0.02	ALR73829
JQ888273	0.09	0.01	*Rumenfilaria andersoni*	*Rangifer tarandus*	Finland	0.11	0.03	AFU89191
KP760187	0.08	0.01	*Foleyella candezei*	*Agama agama*	Togo	0.10	0.03	ALR73846
KP760206	0.07	0.01	*Pelecitus fulicaeatrae*	*Podiceps nigricollis*	Spain	0.11	0.03	ALR73865
KP760194	0.05	0.01	*Loa loa*	*Homo sapiens*	France	0.08	0.02	ALR73853
HQ186250	0.06	0.01	*Loa loa*	*Homo sapiens*	Cameroon	0.09	0.03	ADN52151
KP760195	0.08	0.01	*Mansonella ozzardi*	*Homo sapiens*	Haiti	0.12	0.03	ALR73854
AM749265	0.07	0.01	*Mansonella perforata*	*Cervus nippon*	Japan	0.13	0.03	CAO83074
KP760208	0.06	0.01	*Setaria labiatopapillosa*	*Bos taurus*	Cameroon	0.10	0.03	ALR73867
KP760209	0.07	0.01	*Setaria tundra*	*Rangifer tarandus*	Finland	0.10	0.03	ALR73868
KP760185	0.05	0.01	*Dirofilaria repens*	*Canis familiaris*	France	0.09	0.02	ALR73844
KP760203	0.06	0.01	*Onchocerca dewittei*	*Sus scrofa leucomystax*	Japan	0.08	0.02	ALR73862
AM749268	0.05	0.01	*Onchocerca eberhardi*	*Cervus nippon*	Japan	0.09	0.02	CAO83077
AM749269	0.05	0.01	*Onchocerca skrjabini*	*Cervus nippon*	Japan	0.08	0.02	CAO83078
KP760202	0.06	0.01	*Onchocerca ochengi*	*Bos taurus*	Cameroon	0.08	0.02	ALR73861
AF015193	0.06	0.01	*Onchocerca volvulus*	*Homo sapiens*	Cameroon	0.08	0.02	AAC61612
KP760201	0.05	0.01	*Onchocerca gutturosa*	*Bos taurus*	Cameroon	0.08	0.02	ALR73860
KP760200	0.06	0.01	*Onchocerca armillata*	*Bos taurus*	Cameroon	0.09	0.02	ALR73859
AM749237	0.07	0.01	*Loxodontofilaria caprini*	*Nemorhaedus crispus*	Japan	0.14	0.03	CAO83064
KP760188	0.07	0.01	*Icosiella neglecta*	*Pelophylax ridibunda*	Ukraine	0.14	0.03	ALR73847
KP760189	0.07	0.01	*Icosiella neglecta*	*Pelophylax kl. esculeta*	France	0.14	0.03	ALR73848
KP760205	0.06	0.01	*Oswaldofilaria petersi*	*Crocodilurus amazonicus*	Peru	0.17	0.04	ALR73864
KP760204	0.08	0.01	*Oswaldofilaria chabaudi*	*Tropidurus torquatus*	Brazil	0.14	0.03	ALR73863
KP760198	0.08	0.01	*Ochoterenella sp. 1*	*Rhinella granulosa*	Venezuela	0.15	0.03	ALR73857
KP760199	0.07	0.01	*Ochoterenella sp. 2*	*Rhinella marina*	Venezuela	0.15	0.03	ALR73858
KP760197	0.08	0.01	*Ochoterenella phyllomedusa*	*Phyllomedusa bicolor*	French Guyana	0.16	0.04	ALR73856
KP760186	0.09	0.01	*Filaria latala*	*Panthera leo*	South Africa	0.18	0.04	ALR73845
MT231294	0.11	0.01	*Abbreviata caucasica*	*Pan troglodytes verus*	Senegal	0.21	0.04	QIP66136

Fig. 2. Comparative phylograms showing the position of *D. graciliformis* (indicated in red) among Onchocercidae Clades. ML inferences were generated from (A) the partial (431-bps) nucleotide sequences of the cox1 gene and (B) 159-aa from their translated (COX) protein sequences. The axis shows the global distance observed throughout the trees. Branches are colour-coded according to the bootstrap's values. The identity of each taxa is colour-coded according to the Onchocercidae Clades. Outgroup taxons are showing in grey. GenBank accession numbers, protein Id, species name and hosts are indicated at each node. The number of base substitutions per site between *D. graciliformis* isolated in the current study and the other Onchocercidae members are shown for both cox1 and COX1 sequences. Log-likelihood were −6216.4 and −1793.4 for the cox1 and COX1 sequences, respectively.

for each gene. No gaps were found in the alignment of the *cox*1 gene against the reference mitogenome of *Acanthocheilonema viteae* (HQ186249), and when translated, there were no stop codons in the amino acid sequences, suggesting the absence of co-amplified numts. BLASTn analysis revealed that the nucleotide and aminoacid sequences were 98.65 and 100% homologous with those from *D. graciliformis* (GenBank accession number: KP760182, Protein Id. ALR73841, voucher 220YU ind1 MNHN) suggesting that nucleotide mutations are silent. In addition, the BLASTn analysis of the 18S rDNA sequence revealed 100% of identity with the GenBank sequence (GenBank accession number: MT336175) of a genotype isolated from howler monkey in the same area, previously referred to as an unidentified Onchocercidae species (Laidoudi *et al.*, 2020).

The maximum liklihood trees inferred from *cox*1 (Fig. 2A), COX1 (Fig. 2B) and the concatenated sequences (*cox*1 and 18S) (Fig. S5) produced a similar topology and very similar posterior bootstrap values. The trees provide evidence that investigated filarioids are an integral part of the Onchocercidae clade 4 (ONC4) (Fig. 2A and 2B and Fig. S5). This organism clustered with *D. graciliformis* (GenBank accession number: *cox*1: KP760182, COX1: Id. ALR73841, 18S: KP760131, voucher 220YU ind1 MNHN) in all phylograms. Similarly, the lowest interspecific nucleotide pairwise distance was observed with *D. graciliformis* (Fig. S5). BLAST search revealed that Onchocercidae members, in particular those of clade 4 (ONC4) lacked the representative 28S in both GenBank and Worm–Parasite databases. The 28S ML tree allowed the comparison of *D. graciliformis* with members representing three Onchocercidae (ONC2, ONC3 and ONC5) clades. The phylogram supported the divergence of *D. graciliformis* from these clades (Fig. S6).

Discussion

Neotropical primates are commonly parasitized by filarioid nematodes of the genera *Dipetalonema* and *Mansonella* (Bain *et al.*, 2015; Laidoudi *et al.*, 2020). Many of these primates are threatened due to anthropic pressures such as the illegal wildlife trade (Bezerra-Santos *et al.*, 2021). These parasites are living in various tissues and cavities outside the gastrointestinal tract (Strait *et al.*, 2012), and they may induce pathologies that involve pleuritis, fibrinopurulent peritonitis and fibrinous adhesion, resulting in the entrapment of worms (Baker, 2018). Thus, understanding the impact of parasitic helminths on their primate hosts is an aspect that should not be overlooked as a vital part of primate conservation. Studies on parasitic nematodes of primates are scant in the Neotropics as it is difficult to obtain parasites from these highly mobile, arboreal and protected animals. The filarioids reported herein were recovered from the abdominal cavity of tamarins found road-killed, which documents the high value of such a material for studies in pathogens of wildlife. The current study emphasizes the usefulness of the combined morphological and molecular-based approaches in identifying filarioid parasites. Morphological and molecular data prove the conspecificity of the retrieved material with *D. graciliformis*, confirming the presence of this species in free-ranging red-handed tamarins (Bain *et al.*, 1986) and howler monkeys (Laidoudi *et al.*, 2020).

The genus *Dipetalonema* is restricted to Neotropical primates (Lefoulon *et al.*, 2015) with a high species diversity observed in a wide range of host species (Table S1). Morphologically, *D. graciliformis* is close to *D. gracile*, though females of *D. graciliformis* are longer, present shorter oesophagus and tail and slightly shorter microfilariae with two obtuse extremities (Bain *et al.*, 1986). The male of *D. graciliformis* can be distinguished from *D. gracile* by its caudal region which is coiled on three turns of spires and a longer flagellus in the longer spiculum (Bain *et al.*, 1986).

The *cox*1 analysis allowed us to discriminate *D. graciliformis*. We used three different methods for the *cox*1 analysis, which confirmed that the filarioids studied clustered with *D. graciliformis* isolated from *Saimiri sciureus* in Peru (GenBank accession number: KP760182). The *cox*1 gene is an efficient DNA barcode for filarioid species which is due to its low nucleotide distances (from 0 to 0.02) within filarioid species (Ferri *et al.*, 2009) and a larger variation between congeneric species (i.e. 0.098–0.2) (Casiraghi *et al.*, 2001; Ferri *et al.*, 2009). In addition, the *cox*1 has been successfully used to monitor the distribution of emerging nematodes, such as the case of *Thelazia callipaeda* eyeworm (Otranto *et al.*, 2021). On the other hand, the 18S rRNA gene has been proven a proper target to reconstruct the phylogenetic history of nematode clades (Blaxter *et al.*, 1998). Despite the absence of representative 18S rDNA data with high query cover, phylogenetic analysis from the concatenated partial 18S and *cox*1 sequences clustered the filarioids from the current study with *D. graciliformis* (GenBank accession number: KP760182 and KP760131 for the *cox*1 and the 18S, respectively). In contrast, the analysis of the 28S was not sufficiently conclusive, which is due to the lack of representative data from GenBank and Worm databases.

In the current study, we provide detailed morphological data on *D. graciliformis* isolated from red-handed tamarins from French Guiana, as well as analyses of nuclear and mitochondrial molecular markers. The analysis of 18S sequences from examined nematodes proven their identity with previously reported undescribed onchocercid filarioids from howler monkeys from the same area (Laidoudi *et al.*, 2020). Further studies are needed to clarify the epidemiology, circulation and host diversity of these parasites as well as their possible impact on the fitness of infected individuals of Neotropical primates.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0031182021000901

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