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Host Occurrence and Geographical Distribution of *Dipetalonema* spp. (Nematoda: Onchocercidae) in Neotropical Monkeys and the First Record of *Dipetalonema gracile* in Ecuador

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ABSTRACT: Here we summarize the geographical distribution and host occurrence of *Dipetalonema* spp. in Central and South American primates, and report the first record of *Dipetalonema gracile* in Ecuador from a squirrel monkey *Saimiri sciureus*. We also provide additional data on the morphology of *D. gracile* including scanning electron microscope (SEM) photographs. The 6 species of *Dipetalonema* are known from 19 species, representing 9 genera of Platyrrhini monkeys. *Dipetalonema* spp. are widespread both geographically and in time, starting in 1809 and ranging from southern Mexico to central Bolivia and subtropical Brazil. *Dipetalonema freitasi*, *Dipetalonema yatesi*, *Dipetalonema robini*, and *Dipetalonema graciliformis* are known from only a few records from 1 or 2 localities, whereas *Dipetalonema caudispina* and *D. gracile* are known from multiple localities. *Dipetalonema caudispina* seems to be an Amazonian species, in contrast to *D. gracile*, which is widespread from 20°N (México) to 24°S (Paraguay and Brazil). Specimens of *D. gracile* in this report are similar to those presented by other authors from different host species and countries. SEM revealed a smooth cuticle on the head, and complex striations on the tail. Morphological comparisons with the other 5 species of *Dipetalonema* are also presented.

KEY WORDS: Filarioids, Onchocercidae, *Dipetalonema*, scanning electron microscopy, monkeys, geographical distribution.

Species of the genus *Dipetalonema* Diesing, 1861 sensu stricto are filarial parasites of Neotropical monkeys (Bain et al., 1982). Adult filarioids are usually located in the intraperitoneal cavity of the hosts and microfilariae invade the blood stream. It has been experimentally demonstrated that the vectors of *Dipetalonema* spp. are biting midges in the genus *Culicoides* spp. (Diptera: Ceratopogonidae) (Eberhard et al., 1979; Travi et al. 1985). As currently recognized, the genus *Dipetalonema* comprises 6 species, namely, *Dipetalonema gracile* (Rudolphi, 1809); *Dipetalonema caudispina* (Molin, 1858); *Dipetalonema graciliformis* Freitas, 1964; *Dipetalonema robini* Petit, Bain, and Roussilhon, 1985; *Dipetalonema freitasi* Bain, Diagne, and Muller, 1987; and the recently described *Dipetalonema yatesi* Notarnicola, Jimenez, and Gardner, 2007. The differential morphology of the vagina vera, the spicules, the area rugosa, the disposition of the musculature in males, plus the microfilariae, clearly separate these 6 species (Petit et al., 1985; Bain et al. 1987; Notarnicola et al. 2007). However, only *D. yatesi* has been examined with a scanning electron microscope (SEM) (Notarnicola et al., 2007).

Records of *Dipetalonema* spp. in Neotropical monkeys are widespread both geographically and in time, starting in 1809 (Rudolphi, 1809) and ranging from southern Mexico (Caballero, 1948) to central Bolivia (Karesh et al., 1998) and subtropical Brazil (Molin, 1858; Freitas, 1964). *Dipetalonema* have been reported from at least 20 species of the tribe Platyrrhini. Here we summarize the geographical distribution and host occurrence of *Dipetalonema* spp., and report the first record of *D. gracile* from Ecuador. We also provide additional data on the morphology of *D. gracile*, including scanning electron microscope photographs.

MATERIAL AND METHODS

In the Helminthological Collection from the Museo de Zoología de la Universidad Católica del Ecuador (QCAZ), we found a vial with several unidentified filarioids from a squirrel monkey *Saimiri sciureus* (Linnaeus) (Primates: Cebidae) collected in the Ecuadorian Amazon by F. Campos and D. Tirira. Parasites were preserved in 70% ethanol. For light microscopy worms were cleared in lactophenol. The anterior end of 1 of the worms was cross-sectioned to get an apical view. Microfilariae were obtained from the uteri of mature females. For scanning electron microscopy (SEM), specimens were dehydrated in ethanol series, dried with the critical point technique, coated with gold, and examined with a JEOL JSM 6360 LV electron microscope. Measurements are presented in micrometers, unless otherwise stated, as the mean ± the standard deviation followed by range values in parentheses.

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Table 1. Hosts and localities of the 6 species of *Dipetalonema*. Type host and type locality in bold.

Filarioids	Host species	Locality—Country	Reference
D. caudispina	***Ateles paniscus***	**Brasilia; Volta do Campo dos Veados; Fazenda do Padre Battista—Brazil**	Molin (1858) in Freitas (1943)
	Cebus apella	Matto dentro; Matogrosso; Marabitanas; Villa Maria; Registo do Rio Araguay; Serra Arimani—Brazil	Molin (1858) in Freitas (1943)
	Brachyteles arachnoides	Ipanema—Brazil	Molin (1858) in Freitas (1943)
	Saimiri sciureus	Marabitanas; Barra do Rio Negro; Salto Theotonio—Brazil	Molin (1858) in Freitas (1943)
	Lagothrix lagotricha	Rio Xie Içanna; Rio Vaupe; Salto Theotonio—Brazil	Molin (1858) in Freitas (1943)
	Alouatta seniculus	Rio Mamoré; Ipanema—Brazil	Molin (1858) in Freitas (1943)
	Leontopithecus rosalia	Pará—Brazil	Molin (1858) in Freitas (1943)
	Ateles paniscus	Pará—Brazil	Freitas (1943)
	Ateles paniscus	Pará; Mato Grosso—Brazil	Freitas (1964)
	*Saimiri sciureus**	?	Eberhard et al. (1979)
	Ateles paniscus	Trois Sauts—French Guyana	Bain et al. (1986)
D. freitasi	***Cebus capucinus***	**Necropsy at the Society Garden of London**	Bain et al. (1987)
D. yatesi	***Ateles chamek***	**45 km North Yacuma (14°42.5′S; 67°04′W), Beni—Bolivia**	Notarnicola et al. (2007)
D. robini	***Saimiri sciureus***	**Guyana**	Petit et al. (1985)
	Saimiri sciureus,† *Saimiri boliviensis*‡	Lowland forest of Loreto—Peru	Dunn and Lambrecht (1963) after Bain et al. (1986)
	Cebus sp.§	Surinam	van Thiel (1926) after Bain et al. (1986)
D. gracile	***Cebus capucinus***	?	Rudolphi (1809)
	Saimiri sciureus	Necropsy at the Society Garden of London	Boulenger (1920)
	Cebus apella, *C. capucinus*, *Lagothrix lagotricha*, *Ateles paniscus*	Necropsy at the New York Zoological Park—U.S.A.	McClure (1932)
	Cebus capucinus	Darien; Chagres Valley; Chiriqui Province—Panama	MacCoy (1936)
	Ateles geoffroyi	Chagres Valley; Chiriqui Province—Panama	MacCoy (1936)
	Ateles fusciceps	Darien- Panama	MacCoy (1936)
	Saimiri sciureus	Florencia (Caqueta)—Colombia	Caballero (1947)
	Aotus lemurinus, *Saimiri oerstedii*, *Ateles fusciceps*, *A. geoffroyi*, *Cebus capucinus*	Panama	Caballero (1947)
	Ateles paniscus, *Lagothrix lagotricha*	Amazonas—Brazil	Freitas (1964)
	Saimiri sciureus	Pará—Brazil	Freitas (1964)
	Cebus apella	Mato Grosso—Brazil; Paraguay	Freitas (1964)
	Cebus sp.	Sao Pablo—Brazil; Bolivia	Freitas (1964)
	Undetermined monkey	Goiás—Brazil	Freitas (1964)
	Saimiri sciureus	Guyana	Bain et al. (1986)
	*Saguinus mystax**	?	Eberhard et al. (1979)
	Cebus apella, *Cebus albifrons*, *Saimiri sciureus*	Forest of the northern part of Colombia	Dunn and Lambrecht (1963)
	Ateles paniscus, *Saguinus nigricollis*, *Lagothrix lagothricha*, *Saimiri sciureus*	Lowland forest of Loreto—Peru	Dunn and Lambrecht (1963)
	Ateles geoffroyi	Paval; Mapastepec (Chiapas); Campeche—Mexico	Lamothe-Argumedo et al. (1997)

Table 1. Continued.

Filarioids	Host species	Locality—Country	Reference
	Cebus olivaceus, *Cebus* sp.	Rio Orinoco—Venezuela	Lamothe-Argumedo et al. (1997)
	Cebus sp.	Aguara—Venezuela	Lamothe-Argumedo et al. (1997)
	Ateles chamek	Parque Nacional Noel Kempff Mercado—Bolivia	Karesh et. al. (1998)
	Cebus apella	Reserva de Mbaracayú, Departamento Canindeyú—Paraguay	Present study
	Saimiri sciureus	Rio Indillana (00°26′S; 78°40′W), Parque Nacional Yasuni, Orellana—Ecuador	Present study
D. graciliformis	***Saguinus midas***	**Abaelé, Para—Brazil**	Freitas (1964)
	Saguinus midas	French Guyana	Bain et al. (1986)

* Eberhard et al. (1979) described the microfilaria and the larval stages from a natural infected monkey.
† Originally described as *D. gracile*; after Bain et al. (1986) revision this host showed mixed infection with *D. gracile* and *D. robini*.
‡ Microfilariae originally described as *D. gracile*, and adults and microfilariae as *D. caudispina*; after Bain et al. (1986) revision it is *D. robini*.
§ Originally mentioned as *D. gracile*.

RESULTS

Six species of *Dipetalonema* have been reported from 19 species, representing 9 genera of platyrrhine monkeys (Table 1). Host names follow Wilson and Reeder (2005). In case of synonymy we also cross-checked the locality where the host was collected and the known geographical distribution of the host species in question. Figure 1 presents locality records from those references that provided enough data to place the collection locality on a map. Those names without any reference of the state and which correspond with 2 or more distant places were omitted. Table 1 summarizes the host occurrence of the genus *Dipetalonema*. Despite the fact that several collection localities were rather vague, the picture of the geographical and host distribution of *Dipetalonema* (Fig. 1) clearly shows that this genus is Neotropical.

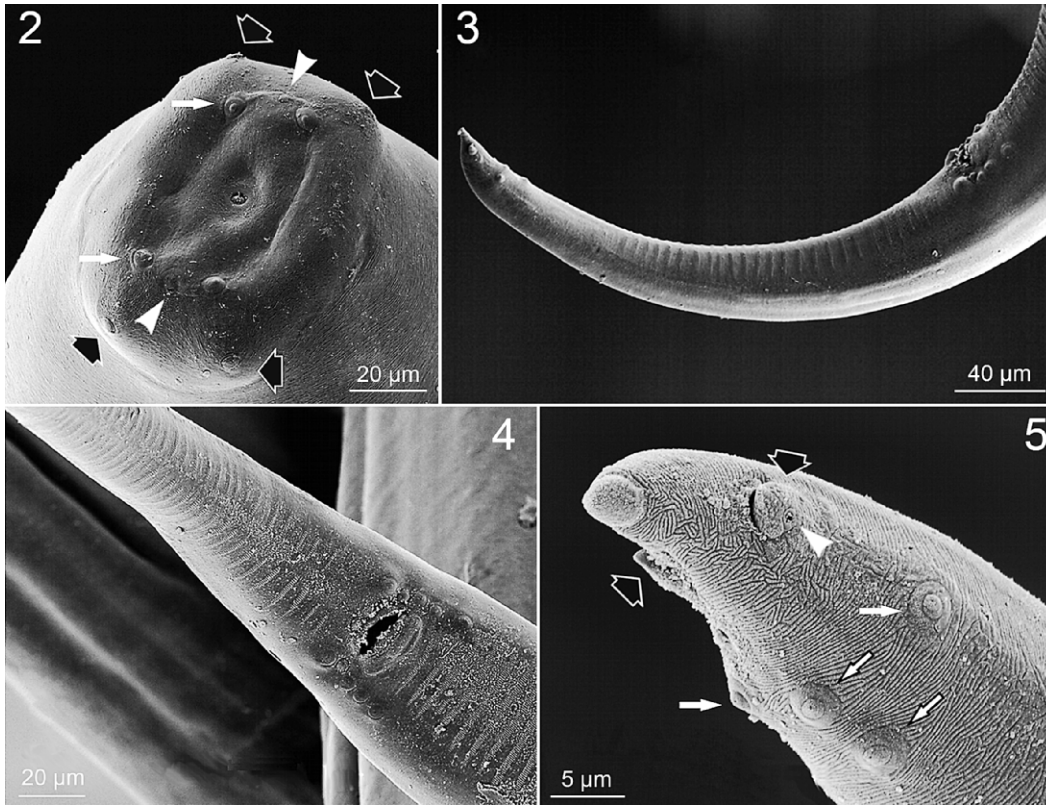
Filarioids found in the QCAZ correspond to *D. gracile* (Rudolphi, 1809). Here we present a brief description.

Dipetalonema gracile (Rudolphi, 1809) (Figs. 2–5)

General: Head cuticle smooth. Head papillae located in a cephalic plate forming a rectangle, flattened dorsoventrally; with 4 inner labial papillae and 4 outer cephalic papillae (Fig. 2). Esophagus divided into muscular and glandular portions. Vulva at the level of the esophagus. Caudal extremity with lappets (Fig. 5). Caudal papillae distributed as follows: 5 pairs of adcloacal papillae arranged in 2 rows on both sides of the cloaca, 1 precloacal, 1 submedian postcloacal pair, and 2 submedian and 1 pair of subventral papillae at the caudal extremity (Figs. 4, 5). Gubernaculum present. *Right spicule:* Lamina with cuticular axes displaying a subterminal elbow, distal extremity bifid with pleat membranous alae. *Left spicule:* Handle shorter than lamina; lamina consisting of a membranous alae and a terminal filament or flagella. Lengths of membranous alae and flagella similar. *Area rugosa:* Extending through the coiled region including the tail, consisting of rows of small longitudinal crest (Figs. 4, 5). Precloacal area rugosa consisting of 1 ventral band, postcloacal area with 2 lateroventral bands. Left band extends more posterior than the right. *Vagina vera:* a sinuous tube with 2 median valves.

Male: $n = 7$. Body length 88.7 ± 5.1 (78.6–95) mm; width 268 ± 15 (243–288). Labial papillae in a rectangle of 70 by 25 and cephalic papillae in a rectangle of 95 by 39; other male 55 by 28 and 82 by 52, respectively; buccal capsule length 11 ± 1.7 (11–12), width 20.3 ± 0.6 (20–21); nerve ring 229 ± 6 (225–234) from apex; esophagus 3.6 ± 446 (3–4.1) mm long, muscular portion ($n = 2$) 699 long; excretory pore ($n = 1$) 300 from the anterior extremity; tail 307 ± 37 (247–349) long; left spicule 900.5 ± 104 (741–1,000) long, with handle 272 ± 24 (240–300) long; membranous alae of 1 specimen 290 long and flagella 400 long; right spicule 230.5 ± 38 (167–282) long; spicular ratio 4 ± 0.89 (3.2–5.9); gubernaculum 35 ± 10 (25–51) long. Area rugosa 8.7 ± 1.5 (6.6–10) mm long. Lateral lappets 48, 45,

Figure 1. Geographic distribution of *Dipetalonema caudispina*, *Dipetalonema yatesi*, *Dipetalonema robini*, *Dipetalonema gracile*, and *Dipetalonema graciliformis*. (Collection localities: **1.** Mato Grosso, Brazil (BR). **2.** Registo do Rio Araguay, Maranhao and Pará, BR. **3.** Marabitanas; Barra do Rio Negro; Rio Xie; Rio Içanna; Rio Uapé, Amazonas, BR. **4.** Rio Mamoré, Rondonia, BR. **5.** Ipanema, Minas Gerais, BR. **6.** Pará, BR. **7.** French Guiana. **8.** Beni, Bolivia (BOL). **9.** Loreto, Perú (PE). **10.** Surinam. **11.** Darien, Panamá (PA). **12.** Chiriqui, PA. **13.** Florencia, Caquetá, Colombia (CO). **14.** Amazonas, BR. **15.** Paraguay. **16.** Sao Pablo, BR. **17.** BOL. **18.** Goias, BR. **19.** North CO. **20.** Paval; Mapastepec, Chiapas, México (ME). **21.** Campeche, ME. **22.** Rio Orinoco, Venezuela (VE). **23.** Aragua, VE. **24.** Parque Nacional Noel Kempff Mercado, Santa Cruz, BOL. **25.** Reserva de Mbaracayú, Canindeyú, PA. **26.** Rio Indillana, Orellana, Ecuador. **27.** Abaelé, Pará, BR.).



Figures 2–5. Scanning electron photography of *Dipetalonema gracile*. **2.** Female apical view showing the inner labial papillae (thin white arrows), the outer cephalic papillae (black arrows) and the lateral amphids (triangular white arrows). **3.** Male tail showing the area rugosa and papillae. **4.** Posterior extremity of male tail showing the cloacal papillae and the postcloacal area rugosa consisting of 2 subventral bands separated in the median ventral line. **5.** Posterior extremity of a male tail showing the striations of the cuticle, the pair of median papillae (thin white and black arrows), the lateral pair of papillae (thin white arrows), the caudal lappets (width black arrows) and the phasmid opening (triangular white arrow).

36, 30 from the tip of tail; phasmids located at 21, 31, 17, 18 of the distal extremity.

Female: $n = 7$. Body length 149.29 ± 26 (128.9–192.9) mm; width 347 ± 32 (303–390). Labial papillae in a rectangle of 55 by 40; cephalic papillae in a rectangle of 120 by 80; other female 82 by 40 and 125 by 62; another female 78 by 30 and 130 by 50, respectively; buccal capsule length 12.5 ± 4.3 (10–19); width 24.7 ± 2.6 (21–27); nerve ring 263 ± 73 (190–364) from apex; esophagus 3.5 ± 579 (2.7–4.1) mm long, muscular portion 2.7 mm (1 specimen); tail 771 ± 219 (609–1,210) long. Vulva 1.4 ± 298 (0.9–1.9) mm from anterior end; vagina vera with a long sinuous tube 250 long and 150 wide; ovijector 2.6 mm long (1 specimen). Caudal lappets 9 long; located at 27 from tip tail; phasmids at the base of the lappet.

Microfilariae: From uteri. Body fusiform, sheathed. Anterior extremity tapered, tail conic. Length ($n = 4$): 109, 83, 105, 120; width 4.

Taxonomic summary

Host: Squirrel monkey *Saimiri sciureus* Linnaeus (Primates: Cebidae) from the abdominal cavity.

Locality: Orellana Province, Río Indillana, Parque Nacional Yasuni (north limit), Ecuador (00°26′S; 78°40′W) (200 m). Nematodes collected in June 1993 by Diego Tirira and Felipe Campos.

Specimens deposited: Four males, 1 posterior male extremity, 9 females and 1 anterior extremity at the Helminthological Collection of the Museo de Zoología de la Universidad Católica del Ecuador (QCAZ); and 2 males and 2 females at the Museo

Table 2. Morphology of males of 6 species of *Dipetalonema*. Measurements correspond to 1 specimen, to 2 if separated with semicolon, or to more than 3 if separated by a dash.

	*D. caudispina**	*D. freitasi*†	*D. yatesi*‡	*D. robini*§	*D. gracile**	*D. graciliformis**
Body length	123	78; 100	81.9–111.1	77; 69.5	76–104	132
Esophagus	3,000	1,700; 2,100	2,242–2,999	3,700; 4,000	4,400–5,700	3,000
Tail	420	325; 360	340–440	305; 380	320–400	580
Left spicule	1,030	985; 1,040	1,020–1,260	1,000; 1,100	900–1,220	1,130
Handle¶	450	430; 400	270–340	290; 290	300	250
Lamina–handle ratio¶	1.2	1.29; 1.6	2.7	2.7; 2.4	2.8	3.5
Flagella	Absent	Absent	Length similar to the membranous alae	Length similar to the membranous alae	Longer than the membranous alae	Much longer than the membranous alae
Right spicule	270	250; 240	210–260	240; 245	210–290	260
Postcloacal area rugosa	One ventral band on the left side	Absent	Two ventral bands, right band shorter than the left	One ventral band on the median line	Two ventral bands, right band shorter than the left	Two ventral bands, right band shorter than the left

* Measurements taken from Bain et al. (1986).
† Measurements taken from Bain et al. (1987).
‡ Measurements taken from Notarnicola et al. (2007).
§ Measurements taken from Petit et al. (1985).
¶ Measure obtained from Bain et al. (1986) from the specimen 49ED.

de La Plata, Argentina CHMLP Nro5556 and SEM stub CHMLP Nro5557.

DISCUSSION

The morphology of the specimens we studied agrees with *D. gracile* (Freitas, 1943; Caballero, 1947; Bain et al., 1986). SEM revealed that the cuticle is smooth on the head, but complexly striated on the tail (Figs. 2, 5). Phasmids open at the base of the lappets, as in *D. freitasi*, *D. graciliformis*, and *D. yatesi* (Bain et al., 1986, 1987; Notarnicola et al., 2007). The lappets are nipple-shaped. Tables 2 and 3 summarize the measurements and some diagnostic character of the 6 known species of *Dipetalonema*. These filarioids can be clearly identified by their morphology and measurements. One of the characters, the length of the left spicule, is similar among the 6 species; however, there are differences between the lengths of the different portions. The length of the handle differs in size among the different species, which is clearly denoted by the handle–lamina ratio (Table 2). The lamina consists of a membranous alae in *D. caudispina* and *D. freitasi*; and by a membranous alae and a terminal flagella in the remaining 4 species. Although the membranous alae and the flagella are of similar lengths in *D. robini* and *D. yatesi*, in *D. gracile* and *D. graciliformis* the flagella are longer, and the membranous alae are shorter. The postcloacal area rugosa is absent in *D. freitasi*, which is similar to other filarioid species (e.g., *Litomosoides*). Although the postcloacal area rugosa occupies the ventral side in *D. robini*, in *D. caudispina* it is displaced to the left side. In *D. yatesi*, *D. gracile*, and *D. graciliformis* 2 subventral bands interrupted in the median ventral line are present, with the right band shorter than the left one (Table 2). Another important characteristic in females is the position and shape of the vulva. The opening is near the anterior end in *D. caudispina* and *D. freitasi*, but is more posterior in the remaining species (Table 3). As Bain et al. (1987) stated, the shape of the vagina vera is a simple tube in these 2 species, becoming a sinuous tube in the remaining species.

Several authors have commonly observed mixed infection of these filarioids (i.e., *D. gracile* and *D. robini* in *S. sciureus* in Dunn and Lambrecht [1963], ascribed by Bain et al. [1987]), and also between *Dipetalonema* and *Tetrapetalonema* species (Petit et al., 1985). Because adults of *Tetrapetalonema* species are parasites of tissues, they are clearly separated from *Dipetalomena* spp. as well as by morphological characters, but microfilariae could be confused. Microfilariae of *Dipetalomena* are shorter and wider than those of *Tetrapetalonema* (length of 100 to 245 μm; width of 3.5 to 5.5 μm in *Dipetalonema* spp. versus a length of 395–435 μm; width of 1.8–3 μm in *Tetrapetalonema* spp.).

Dipetalonema gracile has been reported from many hosts and localities. Bain et al. (1986) redescribed the species, based on specimens from Rudolphi (1809) and new specimens collected from *Saimiri*

Table 3. Morphology of females and microfilaria (Mf) of the 6 species of *Dipetalonema*.

	*D. caudispina**	*D. freitasi*†	*D. yatesi*‡	*D. robini*§	*D. gracile**	*D. graciliformis**
Body length	295	230; 210	237.3–254.2	130; 152	167–210	255; 330
Esophagus	2,720	2,000; 2,040	3,569–4,787	3,920; 4,700	3,950–5,500	3,360; 3,700
Tail	800	510; 590	710–810	660	640–850	250; 300
Vulva from anterior end	400; 420	670; 650	750–1,636	830; 980	1,150–1,600	760; 850
Shape of the vagina vera	A simple tube	A simple tube with an elbow	A simple tube with thin walls	A tube with 2 valves	A sinuous tube	A sinuous tube
Phasmid position	On the side of the lappet	At the base of the lappet	At the base of the lappet	On a small tubercle	At the base of the lappet	At the base of the lappet
Mf body length	183–188	100–107	158–172	150–186	125–145	93–115
Mf body width	3.5–4.5	3.5–4	4.8–7.1	4–4.8	2.5–3.3	3.4–4
Mf tail	Attenuated, tip tail anucleated	Attenuated, with nuclei	Attenuated, tip tail anucleated	Attenuated, tip tail anucleated	Rounded, with nuclei	Rounded, with nuclei

* Measurements taken from Bain et al. (1986).
† Measurements taken from Bain et al. (1987).
‡ Measurements taken from Notarnicola et al. (2007).
§ Measurements taken from Petit et al. (1985).

sciureus (Linnaeus) from Guyana, and clarified its synonymies. The type host is *Cebus capucinus* (Linnaeus) (specimens from Rudolphi, 1809), but the type locality remains unknown. Later, MacCoy (1936), Caballero (1947), Freitas (1964), and Dunn and Lambrecht (1963) extended the host and the locality records (Table 1). In Paraguay, Freitas (1964) did not report a locality, however the finding of 2 females and 1 male of *D. gracile* from *Cebus apella* in the Reserva de Mbaracayú, Departamento Canindeyú—in the east of Paraguay—confirms its presence (material collected by Joe Cook, deposited in the parasite collection of the Harold W. Manter Laboratory (HWML48479). This is the first record of *D. gracile* from Ecuador.

As presently known, the geographic distributions of *D. freitasi*, *D. yatesi*, *D. robini*, and *D. graciliformis* are restricted to 1 or 2 localities (Table 1), whereas the remaining species (*D. caudispina* and *D. gracile*) are more broadly distributed. It seems that *D. caudispina* is an Amazonian species, in contrast to *D. gracile* which is distributed from 20°N (México) to 24°S (Paraguay and Brazil), mainly along the forested highlands bordering the east of the Andes (Fig. 1).

Species of *Dipetalonema* in Platyrrhini monkeys apparently have low host specificity, in contrast to the findings in pinworms by Hugot (1998), where the oxyurid parasites coevolved with the monkeys.

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