

Occurrence of *Dipetalonema gracile* in a wild population of woolly monkey *Lagothrix poeppigii* in the northeastern Peruvian Amazon

Ocorrência de *Dipetalonema gracile* em uma população selvagem de macaco barrigudo *Lagothrix poeppigii* no nordeste da Amazônia peruana

David Fernandez Conga^{1*}; Pedro Mayor²; Adriano Penha Furtado¹; Elane Guerreiro Giese^{1,3}; Jeannie Nascimento dos Santos¹

¹ Laboratório de Biologia Celular e Helminologia “Profa. Dra. Reinalda Marisa Lanfredi”, Instituto de Ciências Biológicas, Universidade Federal do Pará – UFPA, Belém, PA, Brasil

² Departament de Sanitat i d'Anatomia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona – UAB, Barcelona, CT, Espanha

³ Laboratório de Histologia e Embriologia Animal, Instituto de Saúde e Produção Animal na Amazônia, Universidade Federal Rural da Amazônia – UFRA, Belém, PA, Brasil

Received October 18, 2017

Accepted February 5, 2018

Abstract

Dipetalonema gracile (Rudolphi, 1809) (Filarioidea: Onchocercidae) is one of six species of filarial parasites of Neotropical non-human primates. The present study recorded the occurrence of *D. gracile*, provides morphological and morphometric data and extends the geographical distribution. Adult filariae were obtained from the thoracic and abdominal cavities of 38 specimens of woolly monkey, which were used for local human consumption, in the northeastern Peruvian Amazon. Male and female filarids were processed and analysed using light and scanning electron microscopy. Details of the cephalic papillae, post-cloacal bands and papillae, vulva, phasmid position and lateral appendages are showed by scanning electron microscopy and is recorded the occurrence of *Lagothrix poeppigii* monkey as a new host of this filaria in the Yavari-Mirin river basin, Peruvian Amazon.

Keywords: *Dipetalonema gracile*, *Lagothrix poeppigii*, woolly monkey, Peruvian Amazon.

Resumo

Dipetalonema gracile (Rudolphi, 1809) (Filarioidea: Onchocercidae), é uma das seis espécies de filárias parasitas de primatas não humanos neotropicais. O presente trabalho registra a ocorrência de *D. gracile*, proporciona dados morfológicos e morfométricos e amplia sua distribuição geográfica. Filárias adultas foram obtidas da cavidade torácica e abdominal de 38 espécimes de macaco barrigudo, que foram utilizados para consumo humano local. Essa espécie de primatas não humanos neotropicais tem distribuição ao nordeste da Amazônia peruana. Filarídeos machos e fêmeas foram processados e analisados pela microscopia de luz e microscopia eletrônica de varredura. Detalhes das papilas cefálicas, bandas e papilas pós-cloacais, vulva, posição dos fasmídeos e apêndices laterais são mostradas pela microscopia eletrônica de varredura e registra-se a ocorrência do macaco *Lagothrix poeppigii* como novo hospedeiro desta filaria na bacia do rio Yavari-Mirin na Amazônia peruana.

Palavras-chave: *Dipetalonema gracile*, *Lagothrix poeppigii*, macaco barrigudo, Amazônia peruana.

*Corresponding author: David Fernandez Conga. Laboratório de Biologia Celular e Helminologia “Profa. Dra. Reinalda Marisa Lanfredi”, Instituto de Ciências Biológicas, Universidade Federal do Pará – UFPA, Rua Augusto Correa, 1, CEP 66075-110, Guamá, Belém, PA, Brasil. e-mail: daket17@gmail.com



Introduction

The genus *Dipetalonema* (DIESING, 1861) belongs to the family Onchocercidae, and it has had other names including *Filaria* (RUDOLPHI, 1809; MOLIN, 1858) and *Acanthocheilonema* (BOULENGER, 1920). Rodents, pinnipeds and non-human primates have previously been considered to be hosts (ELEY, 1981; NOTARNICOLA et al., 2007), but phylogenetic studies conducted by Lefoulon et al. (2015) restricted *Dipetalonema* to non-human primate hosts in the Neotropics.

However, the geographical distribution of *Dipetalonema* throughout the Neotropics is imprecise because most records are from captive primates or only one host, such as *D. freitasi* in a necropsy of *Cebus capucinus* at the London Zoo (BAIN et al., 1987), *D. robini* in *Saimiri sciureus* in Guyana (PETIT et al., 1985), *D. graciliformis* in *Saguinus midas* in French Guiana (BAIN et al., 1986), and *D. yatesi* in *Ateles chamek* in the Beni region of Bolivia (NOTARNICOLA et al., 2007). In the case of *D. caudispina* and *D. gracile*, most records in the last century have been concentrated in Brazil and Guiana (FREITAS, 1943; NOTARNICOLA et al., 2008) and cover a larger number of non-human primate hosts (WEBBER, 1955; NOTARNICOLA et al., 2008).

According to Dunn & Lambrecht (1963), *D. gracile* has been reported in the Neotropics in Ecuador, Peru and Brazil as a parasite of *S. sciureus*, *Ateles paniscus* and *Saguinus nigricollis*, but like the other species of the genus, most reports are not geographically explicit. Furthermore, only the *D. gracile* specimens recorded in *S. sciureus* have complete descriptions of their morphometric data made by Bain et al. (1986) for specimens from French Guiana (49 ED, Collection of the National Museum of Natural History) and by Notarnicola et al. (2008) for specimens from the Ecuadorian Amazon (CHMLP Nro5556 and Nro5557, Collection of the Museo de La Plata).

The present study describes, for the first time, parasitism by *D. gracile* in the silvery woolly monkey, *Lagothrix poeppigii* (Schinz, 1844), which is one of the largest non-human primates found in the western Amazon and is commonly hunted for food by human subsistence hunters. In addition to extending the list of host species, this study performed an extensive morphological analysis of *D. gracile* with use of scanning electron microscopy.

Materials and Methods

Between 2009 and 2013, 38 silvery woolly monkeys (18 males and 20 females) were killed for subsistence hunting in the Yavari river basin, which is between the Yavari and Yavari-Mirin rivers (04°19'53" S, 71°57'33" W; UT-5:00) in the northeastern Peruvian Amazon in the Loreto region. According to Bodmer et al. (2003), hunting is a common activity in the area, so local hunters and residents have been incorporated into a natural resource management programme and taught to remove and identify the abdominal and thoracic organs of primate prey, preserve them in a 10% formaldehyde fixative solution and deliver them to researchers.

The protocol authorizing this study and the sample collection was approved by the Peruvian Forest and Wildlife Service (*Servicio Forestal y de Fauna Silvestre*; Committee for Ethical

Wildlife Research, protocol no. 0127-2010, 0229-2011 and 0350-2012-DGFFS-DGEFFS). The helminths were retrieved from the external surfaces of the thoracic and abdominal organs of the silvery woolly monkeys, washed in saline solution and preserved in 70% ethanol. The samples were then shipped to the Laboratory of Cell Biology and Helminthology of the Institute of Biological Sciences at the Federal University of Pará, Brazil, under import permit no. 02309-MINAGRI-SERFOR.

Following Gardner's (1996) protocol, the helminths were dehydrated in ethanol and cleared in 50% Amann's lactophenol to study their morphological and morphometric characteristics, and cross-sections of the nematodes were placed on slides with coverslips and analysed. Some of the samples were analysed using an Olympus BX41 microscope and an Olympus SZX12 stereomicroscope (Melville, New York, USA), both of which were equipped with a light chamber.

Samples were prepared for scanning electron microscopy following the protocol of Furtado et al. (2010). Male and female helminths were sectioned at 2 cm from their anterior and posterior ends, and the parts were analysed using a TESCAN Vega 3 LMU scanning electron microscope (Kohoutovice - Czech Republic). The morphometric analysis was based on 20 adult male and 20 adult female specimens. Measurements of the total length of the nematodes and the caudal area rugosa of males were taken in millimetres, and measurements of the other anatomical structures were taken in micrometres. The obtained values are presented below in the following format: mean and standard deviation followed by the range between the lowest and highest values in parentheses.

Results

Onchocercidae (CHABAUD & ANDERSON, 1959)

Dipetalonema gracile (RUDOLPHI, 1809)

Description

(Figures 1-4, Table 1)

Long pale body with fine transverse striations and thickening on the sides along the length of the body beginning at the nerve ring and ending near the tip of the tail in both sexes (Figure 3A and E). The ratio of the size of females to males is 1:1.7. The cephalic structure has a smooth quadrangular shape with four cephalic papillae and four labial papillae located around a simple, small mouth composed of four dorsal and four ventral papillae; the amphids are arranged between the cephalic papillae (Figures 1B, 2B, 3A and 4A). The oesophagus is divided into the muscular region (anterior and short) and the glandular region (posterior and long), and the nerve ring is located in the muscular region of the oesophagus. Posteriorly to the nerve ring there is a small, discrete excretory pore (Figures 1A and 2A). In both males and females, the posterior extremity ends in a conic tail accompanied by caudal appendages on either side with the phasmid at the base (Figure 3F).

Male (description based on 20 adult specimens). Total body 91 (62-105), small buccal capsule 5 (3-7) × 13 (9-13) (Figures 1B and 3A); nerve ring 196 (179-232), excretory pore 284 (211-347) (Figure 1A), total length of oesophagus 2568 (2227-3120), muscular oesophagus 415 (347-579), width

of body at the intestine 270 (190-337) (Figure 1D), testicles beginning at the level of the oesophagus/intestine transition (Figures 1C and D). Posterior extremity of male with 6-8 spiral twists and small longitudinal striations in the form of transverse bands with microstriations on the ventral caudal surface forming the area rugosa 12 (7-16) (Figure 3B). Post-cloacal bands arranged in two rows, the right shorter than the left (Figure 3D). Left spicule measured 951 (813-1064) (Figure 1F) and subdivided into the proximal handle 251 (219-301), membranous alae 351 (283-560) and flagellum 349 (173-493); the latter two regions forming the lamina 700 (595-803). Right spicule, simple 192 (160-221) and gubernaculum small 18 (16-19). Handle/lamina ratio = 2.8, and right spicule/left spicule ratio = 5; tail 298 (267-320). Seventeen sessile caudal papillae (Figures 1E and 3C, F) arranged in three pairs plus a single central pre-cloacal papilla, a pair of ad-cloacal papillae and two pairs of post-cloacal papillae. Four papillae at the end of the tail in an irregular formation and the caudal

appendages measuring 3 (3-5) with the distance from the tip of the tail to the phasmids at the base of the lateral caudal appendages measuring 22 (21-27); in addition, fine irregular striations are observed around the caudal papillae and appendages (Figure 3F).

Female (description based on 20 adult specimens). Body length 160 (115-191), width at the level of the oesophagus/intestine 349 (274-416) (Figure 2C), small buccal capsule 6 (4-8) × 13 (10-13) (Figure 2B); nerve ring 199 (147-237), excretory pore 300 (253-384), length of the oesophagus 3069 (2693-3507), muscular oesophagus 467 (369-721) (Figure 2A). Vulva posterior to the muscular oesophagus 776 (547-1021) (Figure 4C). Sinuous vagina vera measuring 242 (199-305) × 63 (52-82) (Figure 2E). Ovíjector 4388 (3733-5760) directed towards the posterior extremity. Uterine microfilariae 109 (104-126) × 4 (3-7) (Figures 2D and 4B). Tail measuring 419 (338-494) (Figure 4B), and posterior extremity of the tail with caudal appendages 7 (7-11) with the distance between the tip of the tail and the phasmids at the base of the caudal appendages measuring 17 (11-20) and ending in a digitiform projection (Figures 2F and 4D).

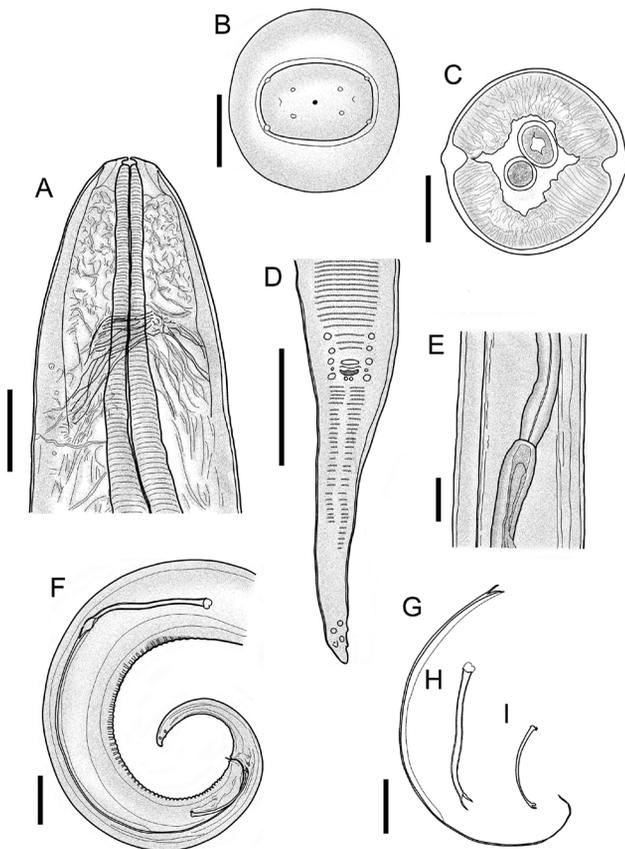


Figure 1. Morphology of *D. gracile* Male: (A) Cephalic end, lateral view with details of the nerve ring, excretory pore and muscular oesophagus, Bar = 50 µm; (B) Cephalic end, apical view. Bar = 50 µm; (C) Cross section of the body at the level of the intestine and testis. Bar = 100 µm; (D) Ventral view of posterior end with details of the caudal papillae of the cloacal band. Bar = 100 µm; (E) Lateral view with detail of the testes and oesophagus-intestine. Bar = 100 µm; (F) Posterior end, lateral view with details of the spicules and cloaca. Bar = 10 µm; (G) Membranous alae. Bar = 10 µm; (H) Flagella. Bar = 10 µm; (I) Right spicule. Bar = 10 µm.

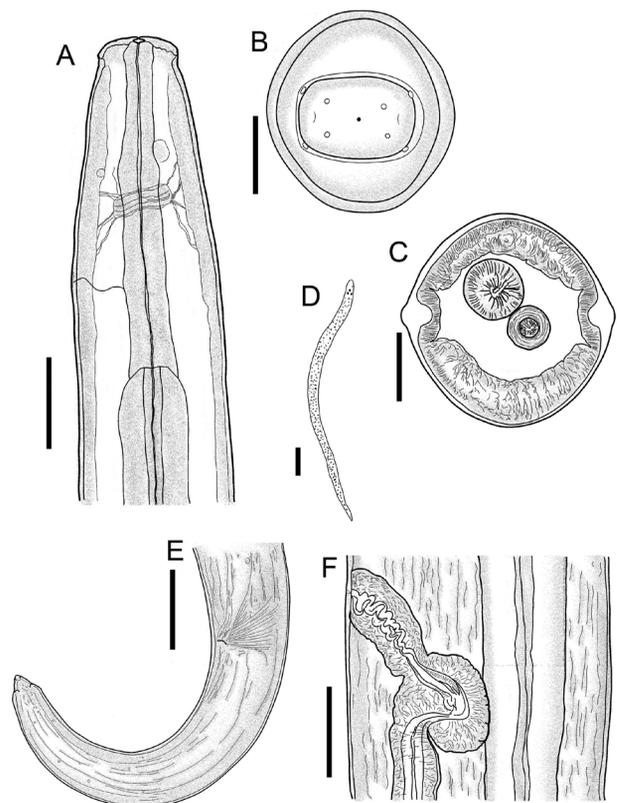


Figure 2. Morphology of *D. gracile* Female: (A) Cephalic end, lateral view with details of the nerve ring, excretory pore, glandular and muscular oesophagus. Bar = 100 µm; (B) Cephalic end, apical view. Bar = 50 µm; (C) Cross section of the body at the level of the intestine and ovijector. Bar = 100 µm; (D) Uterine microfilaria. Bar = 10 µm; (E) Posterior end with details of the lappets and anal aperture. Bar = 100 µm; (F) Lateral view with details of the vulvar aperture and vagina. Bar = 100 µm.

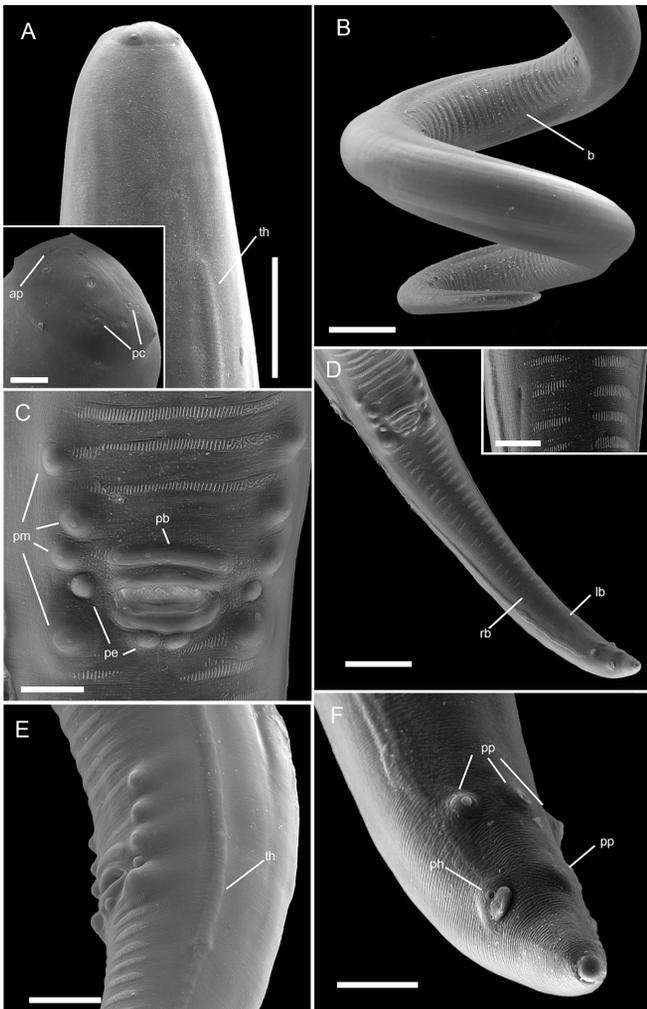


Figure 3. Ultrastructure of *D. gracile* Male: (A) Cephalic end, lateral and apical view. Bar = 100 μ m. Details of the papillae (pc), amphids (ap) and lateral thickening (th). Bar = 20 μ m; (B) Posterior end, ventral view with details of the bands (b) in the area rugosa. Bar = 100 μ m; (C) Posterior end, ventral view with details of the cloacal papillae (pm, pe and pb). Bar = 10 μ m; (D) Posterior end, ventral view. Bar = 50 μ m. Details of the post-cloacal bands (rb and lb). Bar = 10 μ m; (E) Posterior end, lateral view with details of the lateral thickening (th). Bar = 10 μ m; (F) Posterior end, ventral view with details of the lappets (ph) and caudal papillae (pp). Bar = 10 μ m.

Taxonomic summary

Host: *Lagothrix poeppigii* Schinz (1844) (Primates: Atelidae).

Site of infection: filariae observed among the organs of the abdominal and thoracic cavities.

Locality: Yavari-Mirin and Yavari river basin, Peruvian Amazon (S0 04°27.5'; W0 71°45.9').

Prevalence: 100% (38 examined hosts, 18 males and 20 females).

Mean infection intensity: 30.6 (3-108).

Specimens deposited: three males (No. MPEG 071-073) and three females (No. MPEG 074-076). Voucher specimens deposited in the Invertebrate Collection of the *Museu Paraense Emilio Goeldi* (MPEG, Belém, Pará, Brazil).

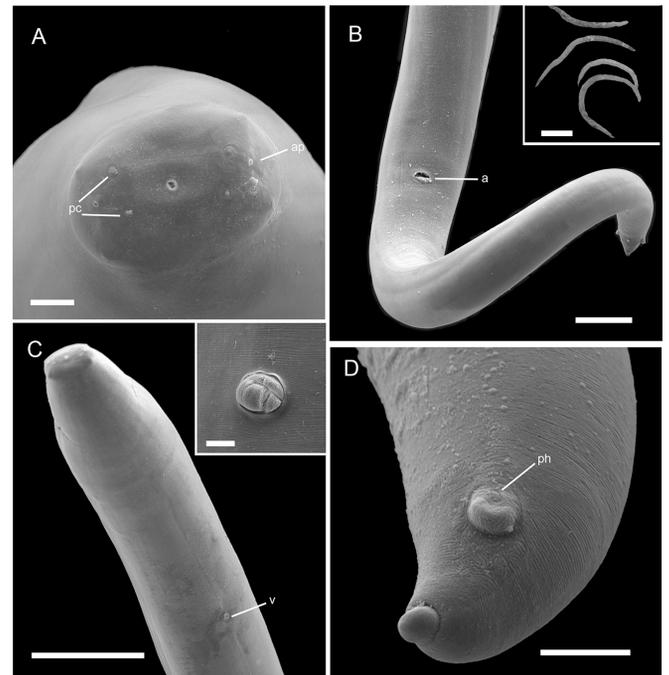


Figure 4. Ultrastructure of *D. gracile* Female: (A) Cephalic end, apical view with details of the papillae (pc) and amphids (ap). Bar = 20 μ m; (B) Posterior end, ventral view with details of the anal aperture (a). Bar = 50 μ m. Uterine microfilaria. Bar = 20 μ m; (C) Anterior end, lateral view. Bar = 200 μ m. Details of the vulvar aperture (v). Bar = 10 μ m; (D) Posterior end with detail of the lappets and phasmids (ph). Bar = 10 μ m.

Discussion

Detailed analysis of the structures of male and female specimens found in 38 *L. poeppigii* hosts revealed their compatibility with *D. gracile*, initially denominated *Filaria gracilis* by Rudolphi (1809). Characteristics such as the difference in the size of the spicules and the divided oesophagus placed the specimens within the genus *Dipetalonema*, which was originated described by Diesing (1861). There are currently six filariae species of the genus *Dipetalonema* that are exclusive to the peritoneal and thoracic cavities of Neotropical primates: *D. caudispina*, *D. graciliformis*, *D. yatesi*, *D. gracile*, *D. freitasi* and *D. robini*. Specific denomination is possible due to differences in the morphology of the reproductive organs of these six species.

Among the female specimens of the six *Dipetalonema* species, the shape of the vagina is distinctive; *D. caudispina*, *D. yatesi* and *D. freitasi* have a simple, straight vaginal canal that is structurally less complex than those found in the other three species, *D. robini*, *D. graciliformis* and *D. gracile*; the latter two have vaginas with similar sinuous shapes. The vulvar opening of the *D. gracile* from *L. poeppigii* has well-developed labias as well as caudal appendages, of which those at the end of the tail have phasmids at the base. These characteristics are shown in detail for the first time by scanning electron microscopy, which also shows the lateral thickening along the body in both sexes of the nematode. The uterine microfilariae

Table 1. Morphometric comparison of the descriptions of male and female *Dipetalonema gracile* specimens found in *Lagothrix poeppigii* (present study) and in *Saimiri sciureus* by Bain et al. (1986) and Notarnicola et al. (2007).

Host Location Author	<i>L. poeppigii</i> (n = 38)		<i>S. sciureus</i> (n = 1)		<i>S. sciureus</i> (n = 1)	
	Peru (Loreto) Present study		French Guiana Bain et al. (1986)		Ecuador Notarnicola et al. (2008)	
	Male (n = 20)	Female (n = 20)	Male (n = 6)	Female (n = 4)	Male (n = 7)	Female (n = 7)
Parameters/Filarids						
Body length (mm)	91 ± 10 (62-105)	160 ± 20 (115-191)	76-104	167-210	88.7 ± 5.1 (78.6-95)	149.29 ± 26 (128.9-192.9)
Body width	270 ± 33 (190-337)	349 ± 37 (274-416)	280-350	400-450	268 ± 15 (243-288)	347 ± 32 (303-390)
Buccal capsule length	5 ± 1 (3-7)	6 ± 1 (4-8)	12	10	11 ± 1.7 (11-12)	12.5 ± 4.3 (10-19)
Buccal capsule width	13 ± 1 (9-13)	13 ± 1 (10-13)	-	-	20.3 ± 0.6 (20-21)	24.7 ± 2.6 (21-27)
Nerve ring	196 ± 14 (179-232)	199 ± 24 (147-237)	225	155	229 ± 6 (225-234)	263 ± 73 (190-364)
Excretory pore	284.3 ± 31.2 (210.5-347.4)	300 ± 35 (253-384)	-	-	300	-
Oesophagus	2568 ± 234 (2227-3120)	3069 ± 237 (2693-3507)	4400-5700	3950-5500	3600 ± 446 (3000-4100)	3500 ± 579 (2700-4100)
Muscular oesophagus	415 ± 53 (347-579)	467 ± 80 (368-721)	--	720	699	2700
Vulva (from anterior end)	-	776 ± 125 (547-1021)	-	1150-1600	-	1400 ± 298 (900-1900)
Vagina length	-	242 ± 31 (199-305)	-	300	-	250
Vagina width	-	63 ± 7 (52-89)	-	150	-	150
Ovjector	-	4388 ± 553 (3733-5760)	-	10000	-	2600
Left spicule	951 ± 61 (813-1064)	-	900-1220	-	900.5 ± 104 (741-1000)	-
Handle	251 ± 19 (219-301)	-	300	--	272 ± 24 (240-300)	-
Lamina	700 ± 46 (595-803)	-	-	-	690	-
Membranous alae	351 ± 63 (283-560)	-	-	-	290	-
Flagella	349 ± 78 (173-493)	-	300	-	400	-
Right spicule	192 ± 16 (160-221)	-	210-290	-	230.5 ± 38 (167-282)	-
Lamina-handle ratio	2.8 ± 0.1 (2.4-3.1)	-	-	-	-	-
Spicular length ratio	5 ± 0.5 (4.1-6.1)	-	-	-	4 ± 0.89 (3.2-5.9)	-
Gubernaculum length	18 ± 1 (16-19)	-	23	-	35 ± 10 (25-51)	-
Area rugosa length (mm)	12 ± 2 (7-16)	-	9.5	-	8.7 ± 1.5 (6.6-10)	-
Tail length	298 ± 14 (267-320)	419 ± 45 (338-494)	320-400	640-850	307 ± 37 (247-349)	771 ± 219 (609-1210)
Caudal lappets length	3 ± 1 (3-5)	7 ± 1 (7-11)	-	7-12	-	9
Lateral lappets from tip of tail	22 ± 2 (21-27)	17 ± 4 (11-20)	18-30	20-50	36-48	27
Microfilaria body length	-	109 ± 3 (104-126)	-	125-145	-	83-109
Microfilaria body width	-	4 ± 1 (3-7)	-	2.5-3.3	-	4

of *D. gracile* from *L. poeppigii* have morphometric values similar to those found by Notarnicola et al. (2008) and Bain et al. (1986).

The structure of the left spicule of *D. gracile* described from *L. poeppigii* is similar to that described by Bain et al. (1986), with the division into three structures: the handle, membranous alae and flagellum; the latter two comprise what is termed the lamina, which has a ratio of 2.8 relative to the handle. Additionally, the membranous alae is larger, on average, than that in the only existing data reported by Bain et al. (1986). The right spicule is a simple structure with a mean length ratio of five relative to the left spicule, which was similar to the findings of Yeh (1956) and Notarnicola et al. (2008) and should be considered an important taxonomic trait in the species description.

In the male specimen, the cloacal papillae present marked differences, being 12 of circular format with difference in size and a papilla in format of central bar. These structures are more noticeable in scanning electron microscopy and were not highlighted in the studies of Bain et al. (1986) and Notarnicola et al. (2008). Another important male characteristic are the spiral twists in the area rugosa, which were found to be six to eight in number; these structures were larger in the present study than those found by Bain et al. (1986) and Notarnicola et al. (2008). This trait may vary given the difficulty of measuring that region of the body due to the twists in the tail.

A discrete excretory pore was observed in both sexes of the *D. gracile* parasite of *L. poeppigii* in this study, which was not previously reported. However, the location of the excretory pore relative to the anterior extremity coincides with the value described by Notarnicola et al. (2008) in the female. Compared to the data presented by Bain et al. (1986) and Notarnicola et al. (2008), additional morphometric differences were also observed in the present study, including the larger size of the ovijector and smaller sizes of the buccal capsule, oesophagus, gubernaculum, caudal appendages, distance of the vulva and nerve ring from the anterior extremity, and a significantly smaller tail in the female. However, there was no significant difference in the total body size found by those authors and in this study. These variations can be attributed to the inclusion of a much larger number of specimens in this study, which resulted in more complete morphometric data.

Dunn & Lambrecht (1963) reported finding specimens of *D. gracile* in some primate hosts in the Peruvian Amazon without providing the correct taxonomic identification. These authors reported *A. paniscus*, *T. nigricollis* (*S. nigricollis*), *S. sciureus* and *S. boliviensis* (DUNN & LAMBRECHT, 1963) as hosts without specifying exact geographical locations. However, it's already known that the distribution of *D. gracile* or other species of filariae may be much wider, given that there are more than 40 species of primates in Peru and large geographical areas that have not yet been studied. Other initial reports for *D. gracile* or synonyms in the Americas (BOULENGER, 1920; WEBBER, 1955) are based on zoo animals or individual cases for which the precise geographic origin of the host, and hence the parasite, is unknown, hampering efforts to assemble an ideal and accurate record. The 38 infected hosts in our study represent a wide geographic distribution of filariasis in non-degraded natural areas and are not limited to representing hosts in captivity.

Due the great diversity of hosts and the difficulty of reaching large geographic areas for research, gaps in our knowledge of natural infections of filariae in Neotropical primates remain. Therefore, *in situ* studies are needed to gain an accurate view of the distribution of filariae in natural areas. We have presented the occurrence of *D. gracile* as a parasite in the thoracic and peritoneal cavities of *L. poeppigii* under natural infection conditions with additional details about its morphometry and morphology as well as images of its ultrastructure. This is the first record of its presence in this host and in the Amazonian region of northeastern Peru.

Acknowledgements

The authors thank the people of Nueva Esperanza, all of whom actively participated in the data collection, demonstrating that community participation is an important step in the development of wildlife management strategies. Institutional support was provided by the *Servicio Forestal y de Fauna Silvestre (SERFOR)* - *Ministerio de Agricultura* of Peru. This work was made possible by the institutional support of the *Fundació Autònoma Solidària* of the *Universitat Autònoma de Barcelona*. We thank to the Laboratory of Animal Histology and Embryology (LHEA/*Universidade Federal Rural da Amazônia*) for providing access to the scanning electron microscope (Vega 3/TESCAN). This study was funded by the CAPES Foundation of the Ministry of Education of Brazil (grant CAPES - Basic Parasitology/2010), grant PAPQ 2016-PROPEP/UFPA and the National Council for Scientific and Technological Development (CNPq) (CNPq Productivity Research Grant to Santos, JN). This work is part the doctoral dissertation of Conga, D.F. of the Post Graduate Program in Biology of Infectious and Parasitic Agents, ICB-UFPA

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