American Journal of Primatology

WILEY

AMERICAN JOURNAL OF PRIMATOLOGY

RESEARCH ARTICLE OPEN ACCESS

Detection of Filariid Infections in Mexican Primate Populations Through qPCR

Brenda Solórzano-García[1] | Norberto Colín García[1] | Filippo Aureli[2,3] | Gerardo Pérez-Ponce de León[1]

[1]Escuela Nacional de Estudios Superiores unidad Mérida (ENES-Mérida, UNAM), Mérida, Yucatán, Mexico | [2]Instituto de Neuroetología, Universidad Veracruzana, Xalapa, Veracruz, Mexico | [3]Research Centre in Evolutionary Anthropology and Palaeoecology, Liverpool John Moores University, Liverpool, UK

Correspondence: Brenda Solórzano-García (brenda_solorzano@yahoo.com.mx)

Received: 10 April 2024 | **Revised:** 2 September 2024 | **Accepted:** 19 September 2024

Funding: This study was funded by the grant PAPIIT-UNAM No. IA202623.

Keywords: Filariae | howler monkey | molecular diagnostics | spider monkey | vector

ABSTRACT

Filariae are parasitic nematodes of high veterinary and medical importance, responsible for some acute tropical diseases. They are transmitted through the bite of hematophagous vectors such as biting midges and blackflies. Filariae are among the most prevalent vector-borne parasitoses in Neotropical primates in which severe infections can cause inflammatory reactions and tissue damage. Given the location inside the host (peritoneal cavity, bloodstream, and lymphatics), the detection of filariid nematodes is challenging and is mostly postmortem; hence the scarcity of studies on the prevalence of filariae in wild primate populations. Here, we report the prevalence of filariid infections in free-ranging populations of Geoffroy's spider (*Ateles geoffroyi*) and black howler (*Alouatta pigra*) monkeys across southern Mexico, using a combination of noninvasive sampling and molecular diagnostic techniques. Fecal samples were screened for filariid DNA by qPCR protocols. A total of 88 samples were examined with an overall prevalence of 26%. Filariae were slightly more common in spider monkeys compared to howler monkeys. This study constitutes the first report of the prevalence of infection of filariid nematodes in populations of wild spider monkey across southern Mexico, and the first reporting of filariae in black howler monkeys, as part of a new era of primate parasitology and the diagnostics of parasite infections in light of the everyday more affordable molecular tools.

1 | Introduction

Filariae are thread-like parasitic nematodes of the superfamily Filarioidea that inhabit tissues, peritoneal cavity, bloodstream, and lymphatic nodes of their hosts (Anderson 2000; Morales-Hojas 2009). They exhibit viviparity with indirect life cycles involving hematophagous arthropods as intermediate hosts and vertebrates as final hosts (Lefoulon et al. 2015). Female worms release microfilariae (L1) that invade the host bloodstream; microfilariae are then ingested by the arthropod during blood meals, reaching its gut where they undergo two maturation stages (L3); the infective larvae are then passed to the vertebrate host during arthropod feeding bites, where they mature into adult worms (Lima et al. 2016). This group of nematodes holds high medical and veterinary importance, with several filariases considered as tropical neglected diseases in humans (Lima et al. 2016; Mediannikov and Ranque 2018).

In the American continent, filariid infections have been reported from southern Mexico to Argentina in a diverse group of terrestrial vertebrates including humans and nonhuman primates (Lefoulon et al. 2015; Notarnicola, Pinto, and Navone 2008). Neotropical

Abbreviations: bp, base pairs; DNA, deoxyribonucleic acid; L1, larvae stage 1; L3, larvae stage 3; NP, neotropical primates; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction.

Summary

- Filariid DNA in primate host feces was detected via qPCR.
- Filariid infections were frequent in wild Mexican primate populations.
- Prevalence of filariae was slightly higher in spider monkeys than in howler monkeys.

primates (NP) are frequently parasitized by members of the family Onchocercidae belonging to two genera, *Dipetalonema* and *Mansonella* (Solórzano-García and Pérez-Ponce de León 2018), although the presence of *Brugia* sp. has been reported in red howler monkeys (*Alouatta macconnelli*) from French Guiana (Laidoudi et al. 2020). Biting midges of the genus *Culicoides* (Ceratopogonidae) and blackflies (Simuliidae) act as intermediary hosts of these filariid species (Lefoulon et al. 2015). Filariae are among the most prevalent parasitic infections in NP (Conga et al. 2022; Shaffer et al. 2022; Travi, Eberhard, and Lowrie 1985), with symptoms of disease being mild or even absent (Travi, Eberhard, and Lowrie 1985).

A significant amount of the reports on filariae in NP come from opportunistic necropsies of carcasses derived from subsistence hunting, through integrative approaches of local communities and researchers collaborating in conservation and wildlife management programs (Conga et al. 2018; Conga, Mayor, Furtado, et al. 2019; Conga, Mayor, Giese, et al. 2019; Shaffer et al. 2022; Zárate-Rendón et al. 2022). These studies have been extremely relevant in determining the diversity of filariae species that can infect NP, their evolutionary history, their ecological and epidemiological dynamics, as well as potential zoonotic risk factors. Unfortunately, the feasibility of such studies is restricted to certain geographic locations, hence certain NP species and populations.

The advance of molecular techniques has facilitated the study of parasitic diseases in a wide range of wildlife species. Specifically, PCR and qPCR have proven effective in detecting filariid DNA from different sources of host samples such as blood, skin, and feces (Gaillard et al. 2020; Laidoudi et al. 2020; Sandri et al. 2021). A study on parasitic diversity using molecular diagnostics documented the presence of various protozoa and several species of nematodes, including filariae, in feces of African humans and nonhuman primates (Medkour et al. 2020). Likewise, molecular screening of blood samples revealed the presence of three species of filariae infecting Guyanan red howler monkeys, *A. macconnelli* (Laidoudi et al. 2020).

Geoffroy's spider monkeys (*Ateles geoffroyi*) and black howler (*Alouatta pigra*) inhabit tropical forest of southern Mexico; both primate species are considered endangered by national and international authorities (IUCN 2022; SEMARNAT 2010). While there is substantial information regarding the parasitic fauna of Mexican primates, particularly, for gastrointestinal parasites, data on other types of endoparasites such as filariae are very scarce, limited to a few sporadic reports (see Solórzano-García and Pérez-Ponce de León 2018). In this study, we assessed the presence of filariid infections in wild populations of these two primate species at 11 localities in southern Mexico, employing a combination of noninvasive sampling and qPCR techniques for parasite diagnostics. This constitutes the first survey of filariid nematodes in wild Mexican primates, and the first effort to assess how frequent these vector-borne infections are occurring in the two species, contributing to the understanding of the parasitic fauna of these NP species and their ecological interactions.

2 | Methods

This study was carried out entirely by noninvasive sampling techniques without sedation, capture, or manipulation of any primate individual, in accordance with Mexican law (SEMARNAT collection permit: FAUT-0168), and the Principles for the Ethical Treatment of Nonhuman Primates of the American Society of Primatologists, as well as the ASP.
Fecal samples were collected from several wild howler and spider monkey individuals belonging to different groups across southeastern Mexico (Figure 1). Fecal material was collected right after deposition, preserved in RNAlater buffer and kept at ambient temperature in the field. Once in the laboratory, samples were kept at −20°C until processing.

Genomic DNA extraction was performed using the Quick-DNA Fecal/Soil Microbe MiniPrep Kit (Zymo Research), according to manufacturer's instructions. To test extraction quality, a standard PCR was carried out in all samples for the amplification of a fragment of the mitochondrial cox1 gene, employing the universal primers (Folmer et al. 1994). Only cox1 positive samples were used in further qPCR procedures.

Filariid DNA was screened by qPCR on 1:5 DNA dilutions, using general primers for filariae (Pan-Fil-28S) targeting a fragment of ~151 bp of the large ribosomal subunit (Laidoudi et al. 2020): qFil-28SF 5′-TGTTTGAGATTGCAGCCCA-3′; qFil-28SR 5′-GTTTCCATCTCAGCGGTTTC-3′; and the probe FAM-5′-CAAGTACCGTGAGGGAAAGT-3-TAMRA. The qPCR screening was performed in a Step-One RT-PCR system (Applied Biosystems). Reactions were performed in 12.5 μL final volume, containing 1 μL of diluted DNA template, 5.5 μL of PATH-ID Master Mix (Applied Biosystems), 0.3 μL of each primer (10 μM), and 0.3 μL of the probe (10 μM) per reaction.

The TaqMan cycling conditions included two hold steps at 50°C for 2 min, followed by 95°C for 10 min, and 40 cycles of two steps each (95°C for 15 s and 60°C for 1 min). Negative and positive controls (from a previously extracted DNA from a filariid nematode) were used in each qPCR screening cycle.

To further corroborate that the amplified products were indeed filaria and not just unspecific amplification, qPCR positive samples were subjected to a standard PCR for the amplification of ~500 and ~470 bp of the cox1 and 12S genes, respectively, using the filariid specific primers and conditions (Laidoudi et al. 2020). PCR products were treated with Exo-SAP-IT (Thermo Scientific), according to the

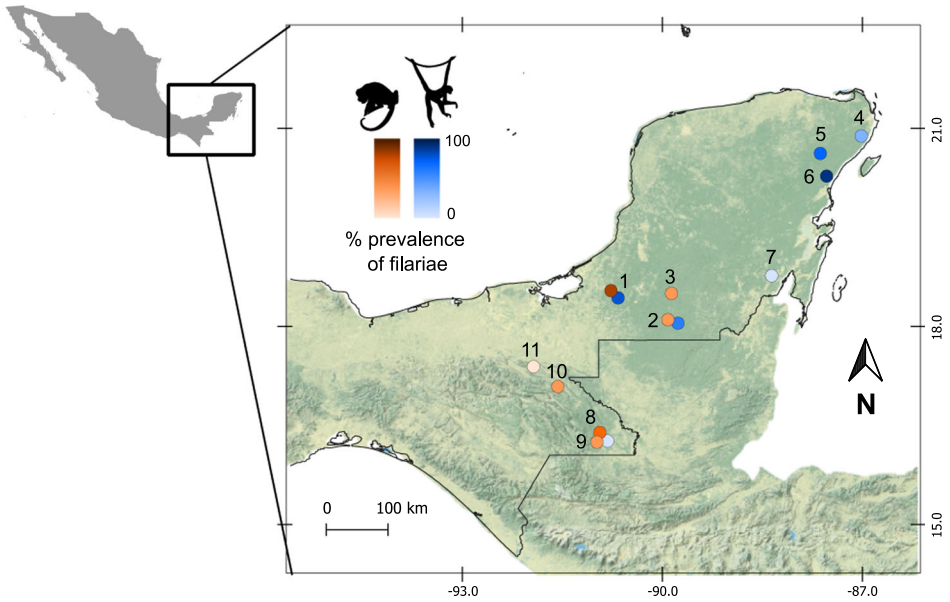

FIGURE 1 | Sampling sites and presence of filariae in Mexican primate populations. Ramp color indicate percentage of samples positive to filariid DNA. Orange = howler monkeys (*Alouatta pigra*); blue = spider monkey (*Ateles geoffroyi*). Numbers correspond to localities in Table 1: (1) Escárcega, (2) Calakmul, (3) Conhuas, (4) Puerto Morelos, (5) Punta Laguna, (6) Tulum, (7) Bacalar, (8 and 9) Reforma Agraria, (10) Metzabok, and (11) Palenque.

manufacturer's instructions, and sequenced at the sequencing facility of the Instituto de Biología, UNAM.

3 | Results

A total of 88 samples, 40 from howler monkeys and 48 from spider monkeys, were screened through qPCR, only two samples from spider monkeys did not yield any cox1 product and were not subjected to qPCR. Filariid infections were detected in 26% of the samples; a slightly higher percentage of infection was observed in spider monkeys than howler monkeys (Table 1). Sample size in each spider monkey locality ranged from 4 to 10 screened samples, with positive cases observed in 20%–80% of the samples; no positive samples were detected in individuals from Chiapas neither south Quintana Roo (Table 1, Figure 1). Sample size in each howler monkey locality ranged from 5 to 10 screened samples; filariid DNA positive cases were detected in 20% to 60% of the samples; no positive samples were detected in individuals from northern Chiapas (Table 1, Figure 1).

Standard PCR yielded positive amplification for both markers, corroborating the filariid detection with an identity of 86%–90% for 12S and 89%–96% for cox1 with *Brugia* spp., *Mansonella* spp., and *Dipetalonema* spp. when compared to records in the GenBank database. The obtained chromatograms showed several overlapping peaks suggesting coinfection with two or more species of filariae in most of the sequenced samples.

4 | Discussion

Filariid infections were frequent in both primate species with an overall 26% of positive samples. Positive cases of filariae were slightly more common among spider monkeys compared to howler monkeys. This tendency has been reported in studies on other species living in South America (Conga et al. 2022; Notarnicola, Pinto, and Navone 2008; Shaffer et al. 2022), suggesting that spider monkeys are more prone to suffer filariid infections than howler monkeys.

High prevalence of infection was observed in almost every sampled locality (up to 80%) with a couple of exceptions where no positive cases were detected. This coincides with values reported in other studies applying molecular detection of filariid DNA in host feces (Gaillard et al. 2020; Medkour et al. 2020). This detected prevalence of infection was nonetheless lower than previous reports of 100% positive cases in spider monkeys (*Ateles paniscus*, *Ateles chamek*, and *Ateles belzebuth*) from Guyana and Amazonia (Conga et al. 2022; Shaffer et al. 2022) via direct observation through necropsies, and 89% via qPCR from howler monkeys blood samples (*A. macconnelli*) from French Guiana (Laidoudi et al. 2020). Thus, it is possible that the infection parameters presented here are underestimated due to the sensitivity of the methods regarding the nature of the samples giving the non-gastrointestinal habitat of the parasites within the host (Gaillard et al. 2020; Medkour et al. 2020).

Several species of filariid nematodes have been reported to infect NP (Conga et al. 2018; Conga, Mayor, Furtado, et al. 2019; Conga, Mayor, Giese, et al. 2019; Notarnicola, Pinto, and Navone 2008; Vanderhoeven, Notarnicola, and Agostini 2017; Zárate-Rendón et al. 2022). In Mexico, only two reports exist of the onchocercid nematode *Dipetalonema gracile* in spider monkeys from Campeche and Chiapas (Caballero 1948; Lamothe-Argumedo et al. 1997); hence, some of the infections detected here probably correspond to this parasite species. For *A. pigra*, to our knowledge, there are no records of filariid nematodes; therefore, this study constitutes the first report of filariae in this species. Additionally, the observed overlapping peaks in both cox1 and 12S amplicons indicate the presence of more than one species of filariae in both host species. Mixed infections of different species of filariae occupying the same individual host have been reported frequently in spider and howler

TABLE 1 | Prevalence of filariid infection in howler and spider monkeys at 11 localities across southeastern Mexico, detected via qPCR screening.

Host species	State	Locality map ID	% of positive samples
Ateles geoffroyi	Campeche	1	50—(2/4)
		2	22—(2/9)
	Quintana Roo	4	20—(2/10)
		5	43—(3/7)
		6	80—(4/5)
		7	0—(0/4)
	Chiapas	9	0—(0/9)
		Subtotal	27—(13/48)
Alouatta pigra	Campeche	1	60—(3/5)
		2	20—(1/5)
		3	20—(1/5)
	Chiapas	8	20—(2/10)
		9	40—(2/5)
		10	20—(1/5)
		11	0—(0/5)
		Subtotal	25—(10/40)
Total			26—(23/88)

monkeys (Bain et al. 2015; Conga et al. 2018, 2022; Laidoudi et al. 2020); thus, the occurrence of co-infections in the examined samples it is not surprising. Further molecular analyses, including species-specific markers and a combination of techniques, are needed to identity the filariid nematodes species that infect these two primate species in Mexico.

Since filariases are vector-borne parasitoses, the frequency of these infections could be related to vector richness and abundance. Twenty-seven species of biting midges are reported to inhabit the regions where this study was carried out, four of which have been associated with filariid nematodes (Mendez-Andrade and Ibáñez-Bernal 2023). Three of these four vector-biting midge species are present in Campeche and Quintana Roo (*Culicoides furens*, *Culicoides phlebotomus*, *Culicoides barbosai*), while only one species has been reported in Chiapas (*Culicoides diabolicus*) (Mendez-Andrade and Ibáñez-Bernal 2023). The higher diversity of filariae associated *Culicoides* could explain the high prevalence of filariid nematodes observed in monkey populations from Campeche and the low prevalence in Chiapas.

According to previous reports of filariids in neotropical nonhuman primates, it seems that the infection does not have a detrimental effect on host performance; however, intense infections could indeed induce host inflammatory responses and tissue damage (Travi, Eberhard, and Lowrie 1985). Alternative physiological, endocrine, and genetic markers of host health remain needed to assess the actual effect of filariae in these primate species. The advances in molecular technologies allow for the detection of different pathological and parasitological agents affecting wildlife populations following noninvasive sampling methods, such as the one we presented here. Through molecular diagnostics, we were able to detect the presence of an understudied vector-borne parasitosis in wild Mexican primates, which otherwise would require host sacrifice. A continuous and robust survey of wildlife parasites and pathogens will contribute to our better understanding of the disease-health ecological cycles, and to identify risk factors associated with transmission among primates or even zoonotic events. This is extremely relevant for health monitoring of endangered species in which capturing, manipulation, and even sacrifice is not feasible or desired.

Author Contributions

Brenda Solórzano-García: conceptualization (lead); formal analysis (lead); funding acquisition (lead); investigation (lead); writing–original draft (lead). **Norberto Colín García:** investigation (equal); resources (equal); writing–review and editing (equal). **Filippo Aureli:** investigation (equal); writing–review and editing (equal). **Gerardo Pérez-Ponce León:** resources (equal); writing–review and editing (equal).

Acknowledgments

We thank local people for their support during field work, and Emanuele Cissello, Amor Aline Saldaña and Ruben Mateo for their invaluable cooperation in sample collection. We also thank directors and staff of the Jardín Botánico Dr. Alfredo Barrera Marín in Puerto Morelos, the Centro de Investigación y Transferencia de Tecnología Forestal "El Tormento." Campeche, La Puerta de Calakmul Hotel, the Otoch Ma'ax Yetel Kool protected area, and Los Árboles Tulum residents for facilitating field work. We are grateful to Laura Márquez and Nelly López who performed sequencing services at the LANABIO—IB facilities. This project was funded by the grant PAPIIT-UNAM No. IA202623 to BSG.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Anderson, R. C. 2000. *Nematode Parasites of Vertebrates. Their Development and Transmission*, 2nd ed. CABI Publishing.

Bain, O., Y. Mutafchiev, K. Junker, et al. 2015. "Review of the Genus *Mansonella* Faust, 1929 *Sensu Lato* (Nematoda: Onchocercidae), With Descriptions of a New Subgenus and a New Subspecies." *Zootaxa* 3918: 151–193. https://doi.org/10.11646/zootaxa.3918.2.1.

Caballero, C. E. 1948. "Estudios Helmintológicos De La Región Oncocercosa De México Y De La República De Guatemala, 4ta Parte: Filarioidea III." *Anales Del Instituto de Biología, Universidad Nacional Autónoma de México* 19: 137–151.

Conga, D. F., H. R. El Bizri, C. González Crespo, et al. 2022. "Environmental Predictors of Filarial Infection in Amazonian Primates." *Acta Tropica* 235: 106670. https://doi.org/10.1016/j.actatropica.2022.106670.

Conga, D. F., P. Mayor, A. P. Furtado, E. G. Giese, and J. N. Santos. 2018. "Occurrence of *Dipetalonema gracile* in a Wild Population of Woolly Monkey *Lagothrix poeppiigii* in the Northeastern Peruvian

Amazon." *Revista Brasileira de Parasitologia Veterinária* 27: 154–160. https://doi.org/10.1590/S1984-296120180014.

Conga, D. F., P. Mayor, A. P. Furtado, E. G. Giese, and J. N. Santos. 2019. "Coinfection With Filarial Nematodes in *Sapajus macrocephalus* and *Cebus albifrons* (Primates: Cebidae) From the Peruvian Amazon." *Journal of Helminthology* 93: 375–378. https://doi.org/10.1017/S0022149X18000287.

Conga, D. F., P. Mayor, E. G. Giese, and J. N. dos Santos. 2019. "First Report of Filarial Nematodes in Free-Living Pitheciid Primates." *Systematic Parasitology* 96: 257–264. https://doi.org/10.1007/s11230-019-09838-y.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. "DNA Primers for Amplification of Mitochondrial Cytochrome C Oxidase Subunit I From Diverse Metazoan Invertebrates." *Molecular Marine Biology and Biotechnology* 3: 294–299.

Gaillard, C. M., S. D. Pion, H. Hamou, et al. 2020. "Detection of DNA of Filariae Closely Related to *Mansonella perstans* in Faecal Samples From Wild Non-Human Primates From Cameroon and Gabon." *Parasites & Vectors* 13: 313. https://doi.org/10.1186/s13071-020-04184-1.

Laidoudi, Y., J. L. Marié, D. Tahir, S. Watier-Grillot, O. Mediannikov, and B. Davoust. 2020. "Detection of Canine Vector-Borne Filariasis and Their *Wolbachia* Endosymbionts in French Guiana." *Microorganisms* 8: 770. https://doi.org/10.3390/microorganisms8050770.

Laidoudi, Y., H. Medkour, A. Levasseur, B. Davoust, and O. Mediannikov. 2020. "New Molecular Data on Filaria and Its Wolbachia From Red Howler Monkeys (*Alouatta macconnelli*) in French Guiana—A Preliminary Study." *Pathogens* 9: 626. https://doi.org/10.3390/pathogens9080626.

Lamothe-Argumedo, R., L. García-Prieto, D. Osorio- Sarabia, and G. Pérez-Ponce de León. 1997. *Catálogo de la Colección Nacional Helmintos*. Mexico city, Mexico: Instituto de Biología, UNAM.

Lefoulon, E., O. Bain, J. Bourret, et al. 2015. "Shaking the Tree: Multi-Locus Sequence Typing Usurps Current Onchocercid (Filarial Nematode) Phylogeny." *PLoS Neglected Tropical Diseases* 9: e0004233. https://doi.org/10.1371/journal.pntd.0004233.

Lima, N. F., C. A. Veggiani Aybar, M. J. Dantur Juri, and M. U. Ferreira. 2016. "*Mansonella ozzardi*: A Neglected New World Filarial Nematode." *Pathogens and Global Health* 110: 97–107. https://doi.org/10.1080/20477724.2016.1190544.

Mediannikov, O., and S. Ranque. 2018. "Mansonellosis, the Most Neglected Human Filariasis." *New Microbes and New Infections* 26: S19–S22. https://doi.org/10.1016/j.nmni.2018.08.016.

Medkour, H., I. Amona, Y. Laidoudi, et al. 2020. "Parasitic Infections in African Humans and Non-Human Primates." *Pathogens* 9: 561. https://doi.org/10.3390/pathogens9070561.

Mendez-Andrade, A., and S. Ibáñez-Bernal. 2023. "An Updated Catalogue of Biting Midges of the Genus Culicoides Latreille, 1809 (Diptera, Ceratopogonidae) of Mexico and Their Known Distribution By State." *ZooKeys* 1167: 1–47. https://doi.org/10.3897/zookeys.1167.102858.

Morales-Hojas, R. 2009. "Molecular Systematics of Filarial Parasites, With an Emphasis on Groups of Medical and Veterinary Importance, and Its Relevance for Epidemiology." *Infection, Genetics and Evolution* 9: 748–759. https://doi.org/10.1016/j.meegid.2009.06.007.

Notarnicola, J., C. M. Pinto, and G. T. Navone. 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. https://doi.org/10.1654/4284.1.

Sandri, T. L., A. Kreidenweiss, S. Cavallo, et al. 2021. "Molecular Epidemiology of *Mansonella* Species in Gabon." *Journal of Infectious Diseases* 223: 287–296. https://doi.org/10.1093/infdis/jiaa670.

SEMARNAT. 2010. *Norma Oficial Mexicana NOM-059-ECOL-2010, Protección Ambiental-Especies Nativas de México De Flora y Fauna Silvestres-Categorías De Riesgo y Especificaciones Para su Inclusión, Exclusión o Cambio-Lista de Especies en Riesgo*. México: Diario Oficial de la Federación.

Shaffer, C. A., M. S. Milstein, L. L. Lindsey, et al. 2022. "Spider Monkey Cotton": Bridging Waiwai and Scientific Ontologies to Characterize Spider Monkey (*Ateles paniscus*) Filariasis in the Konashen Community Owned Conservation Area, Guyana." *International Journal of Primatology* 43: 253–272. https://doi.org/10.1007/s10764-021-00272-w.

Solórzano-García, B., and G. Pérez-Ponce de León. 2018. "Parasites of Neotropical Primates: A Review." *International Journal of Primatology* 39: 155–182. https://doi.org/10.1007/s10764-018-0031-0.

"The IUCN Red List of Threatened Species. Version 2022-2." IUCN, 2022. https://www.iucnredlist.org.

Travi, B. L., M. L. Eberhard, and R. C. Lowrie. 1985. "Development of *Dipetalonema gracile* in the Squirrel Monkey (*Saimiri sciureus*), With Notes on Its Biology." *Journal of Parasitology* 71: 17–19. https://about.jstor.org/terms.

Vanderhoeven, E., J. Notarnicola, and I. Agostini. 2017. "First Record of *Dipetalonema robini* Petit, Bain & Roussilhon 1985 (Nematoda: Onchocercidae) Parasitazing *Sapajus nigritus* in Northeastern Argentina." *Mastozoología Neotropical* 24: 483–488.

Zárate-Rendón, D. A., M. N. Salazar-Espinoza, S. Catalano, et al. 2022. "Molecular Characterization of *Dipetalonema yatesi* From the Black-Faced Spider Monkey (*Ateles chamek*) With Phylogenetic Inference of Relationships Among Dipetalonema of Neotropical Primates." *International Journal for Parasitology. Parasites and Wildlife* 17: 152–157. https://doi.org/10.1016/j.ijppaw.2022.01.005.