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***Dracunculus* sp. PantanalBr Infection in Florida Panthers and Bobcat, Florida, USA**

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We used morphologic and genetic methods to analyze subcutaneous worms removed from endangered Florida panthers and a bobcat in Florida, USA, identifying *Dracunculus* sp. PantanalBr and several *Dirofilaria* spp. worms. *Dracunculus* sp. PantanalBr had been previously reported in a domestic dog and a jaguar in Brazil.

D*racunculus* (Spirurida:Dracunculoidea) are large subcutaneous nematodes that can be found in mammals and reptiles (1). The life cycle of the parasites involves ingestion of infected cyclopoid copepods via drinking water, although consumption of paratenic or transport hosts (amphibians, fish) may also be involved (1,2). Female *Dracunculus* nematodes

are morphologically indistinguishable by species and more commonly detected than the much smaller male nematodes, so sequence analysis is necessary for species identification (1).

Six of 15 *Dracunculus* species infect mammals, and most studies focus on the human Guinea worm, *Dracunculus medinensis*, in Africa (1). In North America, studies have reported 4 mammalian *Dracunculus* spp. nematodes: *D. insignis* (in various wild carnivores, dogs, cats), *D. lutrae* and an undescribed species (in river otters [*Lontra canadensis*]), and another undescribed species (in a Virginia opossum [*Didephis virginianus*], a river otter [USA], and a dog [Spain]) (1,3–5). Researchers have reported 3 *Dracunculus* nematode species in South America: *D. jaguape* (in a neotropical otter [*Lontra longicaudis*]), *D. fuelleborni* (in a big-eared opossum [*Didelphis aurita*]), and an undescribed species (*Dracunculus* sp. PantanalBr) (in dogs and a jaguar [*Panthera onca*] in Brazil) (6,7).

The Florida panther (*Puma concolor coryi*) is an endangered North American puma (*P. c. cougar*) subspecies restricted to South Florida. As part of mortality investigations, Florida Fish and Wildlife Conservation Commission veterinarians perform necropsies on panthers and bobcats (*Lynx rufus*). This study reports the findings related to worms collected from panthers and a bobcat, including detection of *Dracunculus* sp. PantanalBr.

We removed subcutaneous or internal parasites from 12 Florida panthers and 1 bobcat collected in Florida during 2002–2025, preserving the samples in formalin or 70% ethanol (Figure 1, panel A; Appendix 1 Table, <https://wwwnccdc.gov/EID/article/32/7/26-0514-App1.pdf>). We morphologically identified and genetically characterized all worms collected (Appendix 1).

Most worms were fragments, so we based identification on a combination of sequence analysis and morphology (characteristic first-stage larvae) (Figure 1, panel B). We noted 5 panthers and the bobcat to be infected with *Dracunculus* sp. nematodes, subsequently identifying parasites from 2 of those panthers and the bobcat as *Dracunculus* sp. PantanalBR. We identified *Dirofilaria* spp. nematodes in 7 panthers (Appendix 1).

We obtained partial *Dracunculus* cytochrome oxidase subunit I (COI) and 18S rRNA sequences from 2 Florida panthers and the bobcat. The two 657-bp COI sequences from Florida panthers were identical and were 99.7% similar to *Dracunculus* sp. PantanalBR identified in a jaguar and 98.8% similar to *Dracunculus* sp. PantanalBR detected in a dog (Appendix 2 Table 1, <https://wwwnccdc.gov/EID/article/32/7/26-0514-App2.xlsx>). The bobcat worm sequence was 99.5%

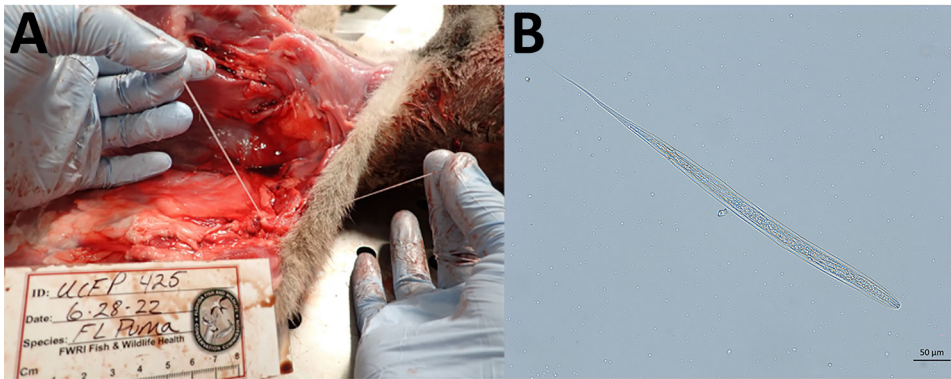


Figure 1. *Dracunculus* sp. PantanalBR nematode samples collected in investigation of *Dracunculus* sp. PantanalBR infection in Florida panthers and a bobcat, Florida, USA. A) Female *Dracunculus* sp. PantanalBR nematode was detected in subcutaneous tissues of a Florida panther (*Puma concolor coryi*). B) First-stage larvae of female *Dracunculus* sp. PantanalBR from a Florida panther. Original magnification $\times 200$.

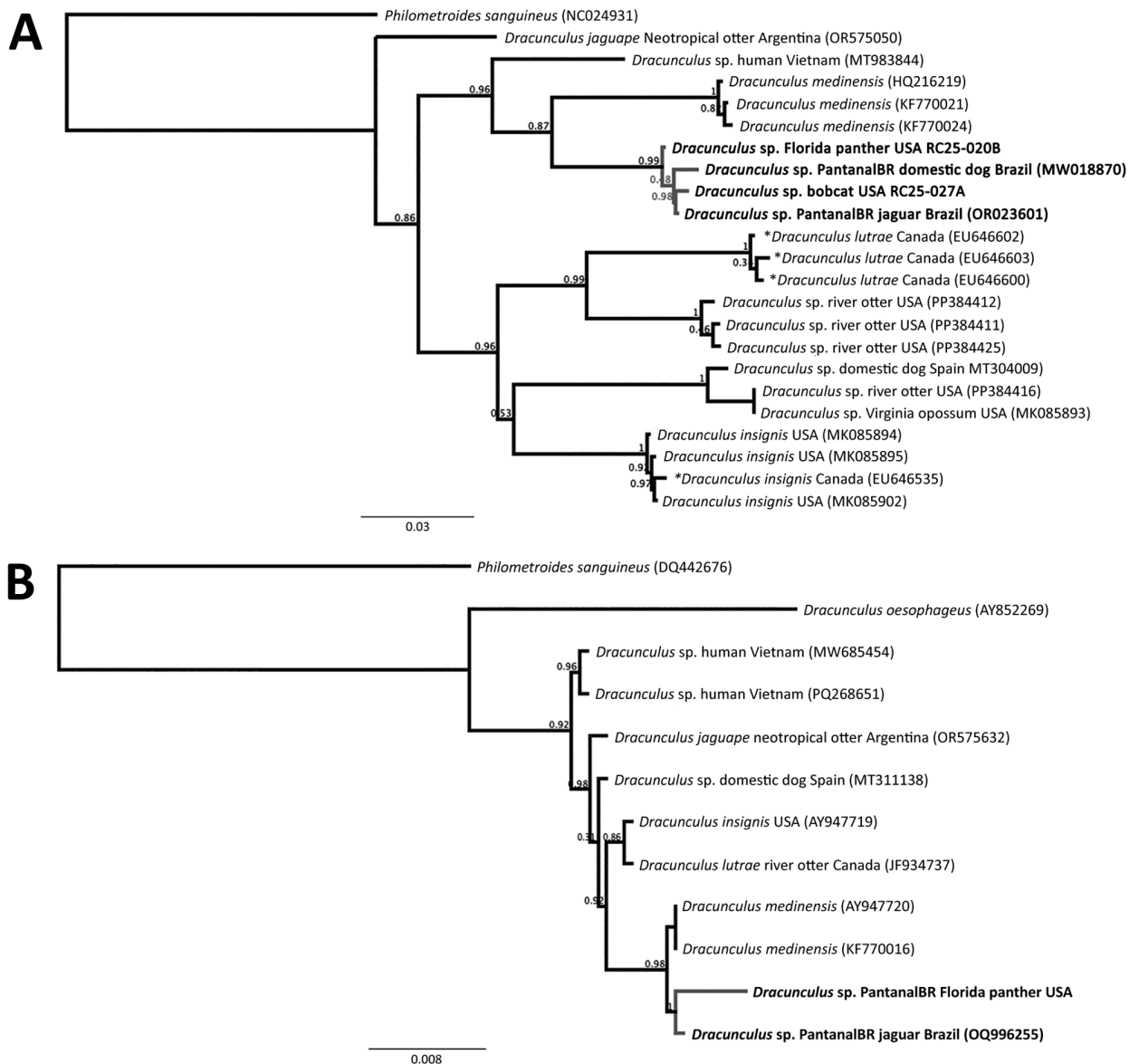


Figure 2. Phylogenetic tree of *Dracunculus* sp. PantanalBR nematodes collected from Florida panthers and a bobcat, Florida, USA. A) Genetic relationships of *Dracunculus* sp. PantanalBR from a Florida panther (*Puma concolor coryi*) and a bobcat (*Lynx rufus*) compared with other *Dracunculus* spp. based on partial cytochrome c oxidase subunit 1 gene sequences. B) Genetic relationships of *Dracunculus* sp. PantanalBR from Florida panther compared with other *Dracunculus* spp. based on partial 18S rRNA gene sequences. Boldface text represents specimens analyzed in this study. Scale bars indicate substitutions per site.

(654/657 bp) similar to the Florida panther sequences. Phylogenetically, the Florida panther and bobcat worm sequences grouped with the 2 *Dracunculus* sp. PantanalBR sequences (Figure 2, panel A). The 18S rRNA sequences (956 base pair) from the 2 Florida panthers and bobcat were identical and 99.9% similar to *Dracunculus* sp. PantanalBR (806/807 bp) (Appendix 2 Table 2). Phylogenetic analysis produced a similar tree to the COI gene (Figure 2, panel B). Larvae from *Dracunculus* sp. PantanalBR from 1 panther measured 601.67 µm long and 25.56 µm wide.

Our data confirm *Dracunculus* sp. PantanalBR nematodes in North America. A prior report of female *Dracunculus* nematodes in Florida panthers was reported as *D. insignis* in 2 Florida panthers from Monroe County in 1989–1990; however, that investigation included no genetic analysis to confirm species (8). Thus, it is unknown if Florida panthers are hosts for *Dracunculus* sp. PantanalBR and *D. insignis* nematodes, although *D. insignis* nematode infects domestic cats (5). Before our report, researchers reported 3 *Dracunculus* nematode species in Florida, including 2 undescribed *Dracunculus* clades in 2 river otters and unspiciated female *Dracunculus* nematodes in a domestic dog and raccoons (1,3,4). However, because *Dracunculus* sp. PantanalBR infects dogs and *D. insignis* nematode is only presumed to occur in Florida, worms from dogs and cats should be genetically characterized to determine species.

The *Dracunculus* sp. PantanalBR life cycle is unknown, but *Dracunculus* nematode species use copepods as intermediate hosts, and some species may use aquatic paratenic hosts (1,2). Further studies are needed to determine if this parasitic species is transmitted through ingestion of copepods or through a paratenic host. We discovered the subcutaneous parasites in the animals we studied during routine necropsy, and no lesions were noted, but researchers have observed ulceration and edema in other *Dracunculus*-infected hosts (1,3–7). *Dracunculus* infections can cause lameness in some hosts, but observation of clinical signs in free-ranging wildlife might be difficult.

The Florida panther is restricted to southern Florida, and the source of *Dracunculus* nematodes in the population is unknown. Genetic testing of panthers in Florida identified a unique lineage in the Everglades National Park that appeared to be of South American origin (9,10). Researchers presumed the origin of this unique genotype was the introduction of 7 captive pumas in the 1950–1960s to the park, all of which were brought from Central America (9,10). Although that history suggests a possible introduction route for *Dracunculus* sp. PantanalBR nematodes, additional surveillance of canids

and felids in the Americas is needed to further clarify distribution and risk for infection.

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***Ancylostoma ceylanicum* Hookworm, Rural Papua New Guinea, 2020**

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We conducted a cross-sectional study of zoonotic hookworm *Ancylostoma ceylanicum* in humans in Western Province, Papua New Guinea, confirmed by internal transcribed spacer sequencing. Overall hookworm prevalence was 54.9%; *A. ceylanicum* hookworms were present in 3.3% of specimens. One Health approaches are needed for hookworm control in Papua New Guinea.

Hookworm infections pose a major public health challenge in Papua New Guinea (PNG). Historic national estimates suggest that up to three quarters of the population may be infected (1); a 2025 study reported prevalence $\geq 80\%$ for the anthropophilic hookworm species *Necator americanus* in Madang Province (2). In 2018, the zoonotic hookworm *Ancylostoma ceylanicum* was confirmed in a

migrant worker returning from Manus Island by using molecular methods (3). However, since that initial report, no data have been published on the prevalence of *A. ceylanicum* hookworm among local populations in PNG. We report molecular evidence of locally acquired *A. ceylanicum* infections in a rural community in PNG, alongside a high overall prevalence of hookworm infection.

We conducted a cross-sectional study in collaboration with the Balimo District Hospital (Balimo, PNG) during January 2020. We recruited community members >16 years of age through convenience sampling from Balimo, which is situated in the Delta Fly District of the Western Province of PNG (Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/32/7/25-1657-App1.pdf>). We preserved fecal specimens in sodium-acetate 5% formalin (SAF) and separately in guanidinium thiocyanate within 8 hours of submission. We examined SAF-preserved specimens for hookworm ova by microscopy, using direct unconcentrated and ethyl acetate-concentrated fecal smears.

We extracted genomic DNA from guanidinium thiocyanate-preserved fecal specimens using the Zymo Quick-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research Corporation, <https://www.zymoresearch.com>). We detected 3 hookworm species, *N. americanus*, *A. ceylanicum*, and *A. duodenale*, using TaqMan quantitative PCR (qPCR) (Integrated DNA Technologies, <https://sg.idtdna.com>) (Appendix Table). We Sanger sequenced all *Ancylostoma* qPCR-positive samples targeting the internal transcribed spacer region, using custom primers (forward 5'-GAATGCCGCCTTACTGCTTG-3' and reverse 5'-CGATTCAGCAGCAACAACGAG-3') (Appendix).

Among the 122 participants who submitted a fecal specimen, microscopy detected hookworm ova in 28 (22.9%), whereas qPCR identified 64 (52.5%) as positive. Combining both methods yielded an overall hookworm prevalence of 54.9% (67/122) (Table). Three (4.5%) samples that tested positive by microscopy were negative by qPCR. *N. americanus* was the predominant hookworm species detected by qPCR, identified in 64 (52.5%) participants. We detected *Ancylostoma* spp. hookworm in 4 (3.3%) samples; all *Ancylostoma*-positive participants were also infected with *N. americanus*. All sequences identified in this study (GenBank accession nos. PV530493–6) formed a distinct cluster with *A. ceylanicum* sequences, including the positive template control isolate (accession no. PV530497) and the PNG isolate previously identified from the migrant worker (accession no. LC036567), confirming all 4 cases as *A. ceylanicum* hookworm