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Otterly diverse - A high diversity of *Dracunculus* species (Spirurida: Dracunculoidea) in North American river otters (*Lontra canadensis*)

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ABSTRACT

The genus *Dracunculus* contains numerous species of subcutaneous parasites of mammals and reptiles. In North America, there are at least three mammal-infecting species of *Dracunculus*. Reports of *Dracunculus* infections have been reported from river otters (*Lontra canadensis*) since the early 1900s; however, little is known about the species infecting otters or their ecology. Most reports of *Dracunculus* do not have a definitive species identified because females, the most common sex found due to their larger size and location in the extremities of the host, lack distinguishing morphological characteristics, and few studies have used molecular methods to confirm identifications. Thus, outside of Ontario, Canada, where both *D. insignis* and *D. lutrae* have been confirmed in otters, the species of *Dracunculus* in river otters is unknown. In the current study, molecular characterization of nematodes from river otters revealed a high diversity of *Dracunculus* species. In addition to confirming *D. insignis* infections, two new clades were detected. One clade was a novel species in any host and the other was a clade previously detected in Virginia opossums (*Didelphis virginiana*) from the USA and a domestic dog from Spain. No infections with *D. lutrae* were detected and neither new lineage was genetically similar to *D. jaguape*, which was recently described from a neotropical otter (*Lontra longicaudis*) from Argentina. These data also indicate that *Dracunculus* spp. infections in otters are widespread throughout Eastern North America. Currently the life cycles for most of the *Dracunculus* spp. infecting otters are unknown. Studies on the diversity, life cycle, and natural history of Dracunculidae parasites in wildlife are important because the related parasite, *D. medinensis* (human Guinea worm) is the subject of an international eradication campaign and there are increasing reports of these parasites in new geographic locations and new hosts, including new species in humans and domestic dogs.

1. Introduction

Dracunculus is a genus of subcutaneous parasites of mammals and reptiles that occur throughout much of the world. This genus includes one species (*D. medinensis*) that is of significant medical importance for humans in sub-Saharan Africa and is the subject of an international eradication campaign (Hopkins et al., 2023). Historically, most species have been reported from reptiles, but in recent years there have been increasing reports of novel species in mammals and novel mammalian hosts (e.g., *D. jaguape* in a neotropical otter (*Lontra longicaudis*), novel

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species in a Virginia opossum (*Didelphis virginiana*) from the United States and a domestic dog (*Canis familiaris*) from Spain, a novel *Dracunculus* sp. from a domestic dogs from Brazil, and a novel *Dracunculus* sp. from a jaguar (*Panthera onca*) from Brazil) (Cleveland et al., 2020; Diekmann et al., 2020; Paiva et al., 2021; Fagundes-Moreira et al., 2023; Natalini et al., 2023). There is increased interest in diseases of otters as North American river otter (*Lontra canadensis*) populations in the last 50 years have increased dramatically, largely due to river otter reintroduction campaigns that began in the 1970's in numerous US states and Canadian provinces (Raesly, 2001). River otters have been successfully restored in much of their historic range and are now widespread in eastern North America and northern Canada.

In North America, there are at least three mammal-infecting species of *Dracunculus*. One species, *D. insignis*, is a host generalist that has been reported from several wildlife hosts, including North American river otters and domestic dogs and cats (Elasser et al., 2009; Cleveland et al., 2018; Williams et al., 2018). River otters with confirmed *D. insignis* infections have been reported in Ontario, Canada and Arkansas, USA (Elasser et al., 2009; Tumlison and Surf 2018). Another species, *D. lutrae*, appears to be a host-specialist of river otters and has only been confirmed in otters in Ontario, Canada (Crichton and Beverly-Burton, 1973; Elsasser et al., 2009). The third species is an undescribed species initially detected in a Virginia opossum from Georgia, USA and subsequently from a domestic dog in Spain (Cleveland et al., 2020; Diekmann et al., 2020). There are also many reports of unidentified *Dracunculus* in river otters (Cheatum and Cook, 1948; Barding and Lacki, 2015; Tumlison and Surf, 2018).

Most reports of *Dracunculus* do not have a definitive species identified because females, the most common sex found due to their larger size and location in the extremities of the host, lack distinguishing characteristics, and few studies have used molecular methods to confirm identifications. Thus, outside of Ontario, Canada, the species of *Dracunculus* in river otters is unknown. Regardless, these reports indicate that river otters are infected with *Dracunculus* across a wide range of eastern North America, yet the species diversity and geographic distribution of *Dracunculus* spp. are poorly understood. Therefore, the goal of the current study was to genetically characterize *Dracunculus* spp. detected in river otters from the eastern United States to determine the diversity of *Dracunculus* in this broadly distributed host.

2. Materials and methods

2.1. Samples

From 2013 to 2023, large, subcutaneous nematodes were acquired from North American river otters through various sources, including necropsy of trapper-harvested animals, submissions of clinical cases to the Southeastern Cooperative Wildlife Disease Study (Athens, GA, USA), surveillance studies (e.g. Swanepoel et al., 2018; Tumlison and Surf, 2018), or from captive otters that had worms emerge. Female worms were preliminarily identified as *Dracunculus* spp. using morphology of adults and/or larvae (Cleveland et al., 2018). Origins of samples are shown in Table 1.

2.2. Molecular and phylogenetic analyses

Genomic DNA was extracted from the collected nematodes using a QIAGEN DNeasy Blood and Tissue Extraction Kit (Germantown, MD, USA) following manufacturer's directions. Portions of the 18 S rRNA (950bp) and cytochrome *c* oxidase I (*COI*) (650bp) genes were amplified using primers 18S39F and 18S977R (Olson et al., 2017) and a cocktail of three primer sets, respectively (Prosser et al., 2013). The PCR reactions contained of 5 µl of DNA added to 20 µl of a master mix containing 5 µL of 5*X* Green GoTaq® Flexi Buffer (1*X*) (Promega, Madison, Wisconsin), 2.5 µL $MgCl_2$ solution (1.5 mM), 0.5 µL dNTPs (10 µM), 0.5 µL of each primer (50 µM), 0.25 µL GoTaq® Flexi DNA Polymerase (Promega) and 10.75 µL of molecular grade water. Thermocycling conditions for the PCR amplification of both targets were 94°C for 1 min, five cycles at 94°C for 40 s, 45°Cfor 40 s, 72°C for 1 min, followed by 35 cycles at 94°Cfor 40 s, 51°C for 40 s, 72 °C for 1 min and a final extension at 72°C for 5 min.

Amplicons were detected in a 0.8% agarose gel stained with GelRed

Table 1
Source of *Dracunculus* samples from North American river otters (*Lontra canadensis*) from the United States and Canada and the molecular identification results. Genbank accession numbers are provided for unique sequences.

State	County	Date	No otters positive/No. sampled (%)	Species (No.)	18 S rRNA gene	*COI* gene (Genbank accession No.)
Arkansas	various	Dec–Jan 2013 and 2014	29/184 (15.8)	*D. insignis* (14)[a]	Yes (12 worms)	Yes(PP384420–PP384424)
Florida[b]	Broward	April 2018	1/1 (100)	*Dracunculus* sp. Clade 1 (1)	Yes	Yes(PP384411)
Florida	Lee	November 2015	1/1 (100)	*Dracunculus* sp. Clade 2 (1)[c]	Yes	Yes (PP384416)
Georgia	Clarke	May 2018	1/3 (33.3)	*Dracunculus* sp. Clade 1 (1) and *D. insignis* (2)	Yes	Yes(PP384425)
Iowa	Unknown	March 2023	1/1 (100)	*D. insignis* (1)	Yes	Yes
Missouri	Unknown	February 2023	4 of unknown	*D. insignis* (4)	Yes	Yes
North Carolina	Various. Positives from Granville (1) and Pitt (1)	February–May 2017	4/38 (10.5)	*D. insignis* (7 females) and *Dracunculus* sp. Clade 1[d] (1) from Granville. *Dracunculus* sp. Clade 1 (n = 2) and *D. insignis* (n = 4) from Pitt	Yes	Yes(PP384412 (male), PP384414 PP384415)
South Carolina	Unknown	March 2017	3/3 (100)	*Dracunculus* sp. Clade 1	Yes	Yes(PP384417 PP384418 PP384419)
Pennsylvania	Bradford	February 2018	1/1 (100)	*D. insignis* (1)	No	Yes(PP384413)
Ontario, Canada[b]	Toronto	January 2017	1/1 (100)	*D. insignis* (1)	Yes	Yes

[a] All were confirmed to be *D. insignis*, but only nine were used in the phylogenetic analysis because some sequences were too short.
[b] Captive otters.
[c] Two additional worms removed but were not available for analysis.
[d] The single male worm was from Granville County, North Carolina.

(Biotium, Fremont, CA, USA). Amplicons were extracted from the gel using a QIAGEN gel extraction kit per manufacturer's directions. Bi-directional Sanger sequencing was conducted by Genewiz (South Plainfield, NJ, USA) and the sequences were edited and assembled using Geneious 10.2.6 (Biomatters Limited, Auckland, New Zealand). Related sequences were obtained from GenBank and a phylogenetic tree was constructed for the *COI* sequences in Geneious using an approximately maximum-likelihood method with FastTree v2.1 with a generalized time-reversible (GTR) model. Unique sequences were submitted to Genbank (Table 1).

3. Results

3.1. Morphology

Two species of subcutaneous nematodes were detected in river otters: *Dirofilaria lutrae* (results published in Swanepoel et al., 2018) and *Dracunculus* spp. (Fig. 1). All but one *Dracunculus* were identified as females, which were commonly found subcutaneously (SC) on the extremities and less commonly SC over the ribs and groin. The single male *Dracunculus* was from the SC area over the ribs of an adult, male river otter from Granville County, North Carolina. The male worm was missing the anterior end so the only measurements we could acquire were related to the posterior end (Fig. 2). The gubernaculum was shorter than reported for *D. lutrae* and longer than both *D. insignis* and *D. jaguape* (Table 2). The right spicule length was within the range for *D. lutrae* but longer than both *D. insignis* and *D. jaguape*. The left spicule length was within the range for all three species. The posterior tail had a round process that was similar in shape, but larger, than what is present on males of *D. lutrae* but absent from *D. insignis, D. jaguape, D. medinensis, D. fuelleborni,* and several reptile-infecting species (*D. globocephalus, D. dahomensis, D. mulbus, D. ophidensis)* (Travassos, 1934; Moorthy, 1937; Brackett, 1938; Jones and Mulder, 2007).

Most worms were acquired from otters that had been skinned by trappers or were collected by collaborators, so lesions (e.g., loss of hair, ulcers) were not apparent or noted for most individuals. However, one otter from Florida that was infected with the Clade 2 *Dracunculus* sp. was admitted for rehabilitation due to a car-strike. Twenty days after admission, a firm 2 × 2 cm firm mass was palpated in the right axillary region. The mass was aspirated and contained a heavy white blood cell count, many gram-positive cocci and nematode larvae. The next day a large nematode presumed to be an adult heartworm (*Dirofilaria immitis)* was removed from the abscess and five days later, another subcutaneous worm was removed from a small, firm abscess along the dorsum (but neither were saved). Three weeks later, another subcutaneous worm was removed from the same dorsum mass and this worm was preserved in

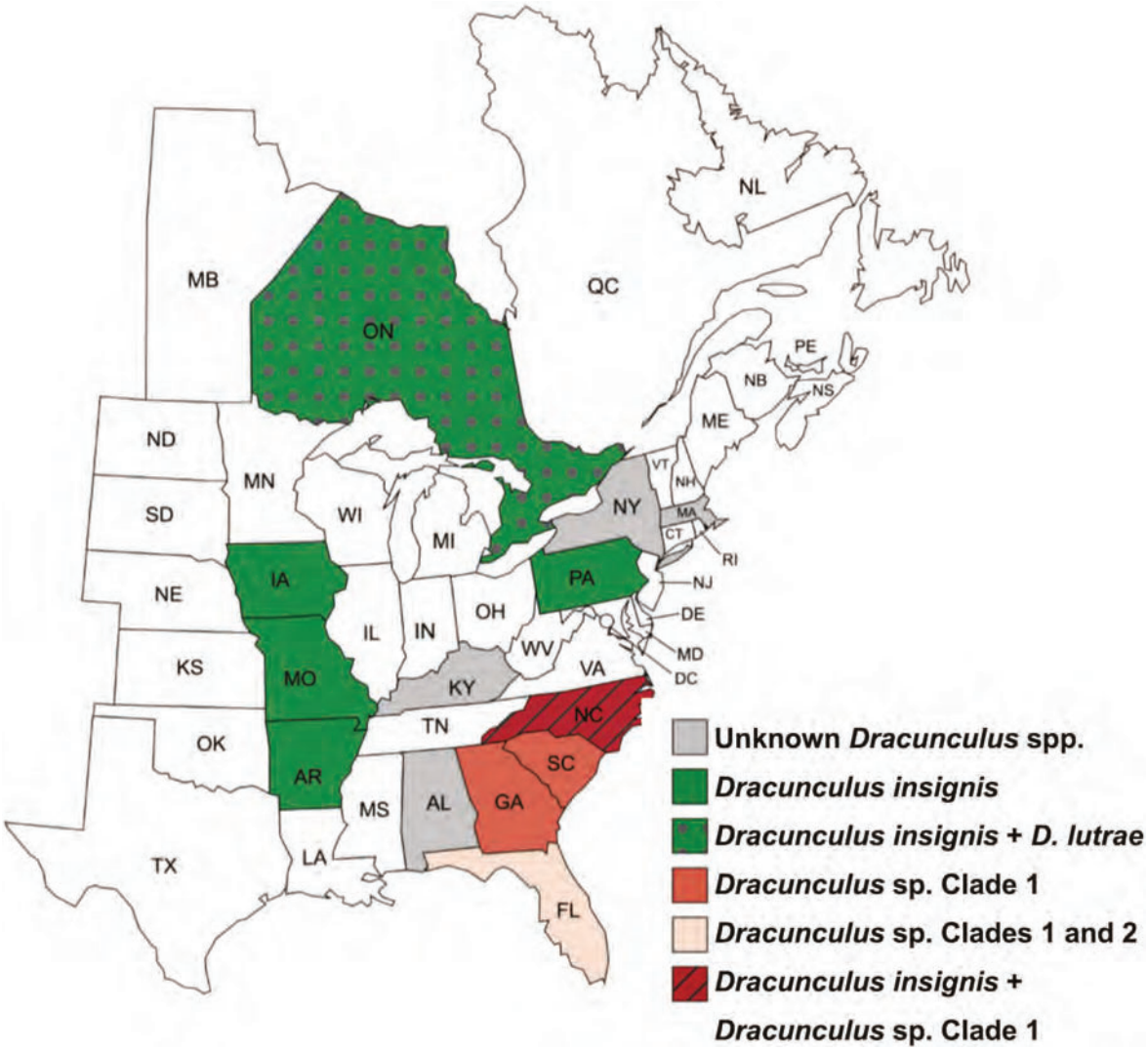

Fig. 1. Distribution of *Dracunculus* spp. in North American river otters (*Lontra canadensis*) in North America. Species identifications are based on molecular identification or male morphology.

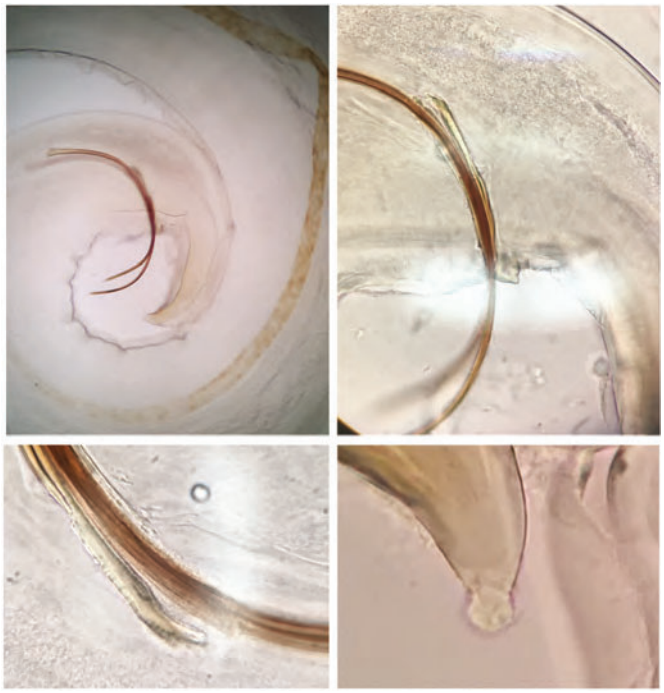

Fig. 2. Posterior end of a male worm of a Clade 1 *Dracunculus* sp. (NC-otter8C) from a North American river otter (*Lontra canadensis*) from North Carolina showing the paired spicules (A), gubernaculum (B, C), and the bulbous posterior end of the tail (D).

ethanol for identification in this study (Fig. 3). Additionally, swellings and cysts containing large number of worms that often were deep in the joints were noted for several *D. insignis*-infected otters from Arkansas (reported in Tumlison and Surf, 2018) and in otter paws acquired from Missouri (Fig. 4).

Measurements were acquired for 15 first-stage larvae (L1) from three female worms (2 from Clade 1 and 1 from Clade 2) from three different otter hosts. The length of L1 from FL15-33,934 (Clade 2) was 640.2 μm (range 603.9–653.4 μm) and width was 19.2 μm (range 17.5–20 μm). The lengths of L1 from FL-Terry and GA-OTT1 (Clade 1) were 634.6 μm (range 603.9–643.5 μm) and 644.5 μm (594–693 μm), respectively, and widths were 20 μm (range 17.5–21.25 μm) and 20.7 μm (18.75–22.5 μm), respectively.

3.2. Molecular characterization

A portion of the 18 S rRNA gene was obtained from all but two worms from Arkansas and one worm from Pennsylvania (Table 1). Sequences were 100% identical to many sequences from mammal-infecting *Dracunculus* spp. such as *D. lutrae* from Canada (JF934737), a *Dracunculus* sp. V3104 from a river otter from Iowa (DQ503457), *D. insignis* from a Northern raccoon (*Procyon lotor*) from Georgia (AY947719), *Dracunculus* sp. Toledo from a Spanish dog (MT311138), and a *Dracunculus* sp. from a human in Vietnam (MW685454). The sequences were 96.6% similar to *D. oesophageus* (AY852269) from a snake host (*Natrix natrix*) from Slovakia (Wijová et al., 2006).

The *COI* sequences were more variable and were acquired from all worms included in the study. Thirty-two worms from Arkansas, Georgia, Iowa, Missouri, and Pennsylvania and Ontario, Canada (Table 1) were identified as *D. insignis* (97.8–100% similarity to numerous *D. insignis* sequences in Genbank) (Table 1, Fig. 1). Phylogenetically, these worms grouped with other *D. insignis* sequences with no apparent host or geographic clustering (Fig. 5). The remaining nine worms were

Table 2
Morphology of gubernaculum and spicules from a male *Dracunculus* sp. Clade 1 from a North American river otter (*Lontra canadensis*) from North Carolina, USA compared with other *Dracunculus* spp. from otters. Measurements given in μm.

	Dracunculus sp. Clade 1 (NC otter 8)	*D. lutrae*	*D. insignis*	*D. jaguape*
Gubernaculum	155	170 (160–180)	120 (120–130)	133
Right spicule	620	640 (590–720)	480 (430–520)	549
Left spicule	520	610 (510–680)	480 (420–510)	526
Reference	Present study	Crichton and Beverley-Burton (1973)	Crichton and Beverley-Burton (1973); Cleveland et al. (2018)	Natalini et al. (2023)

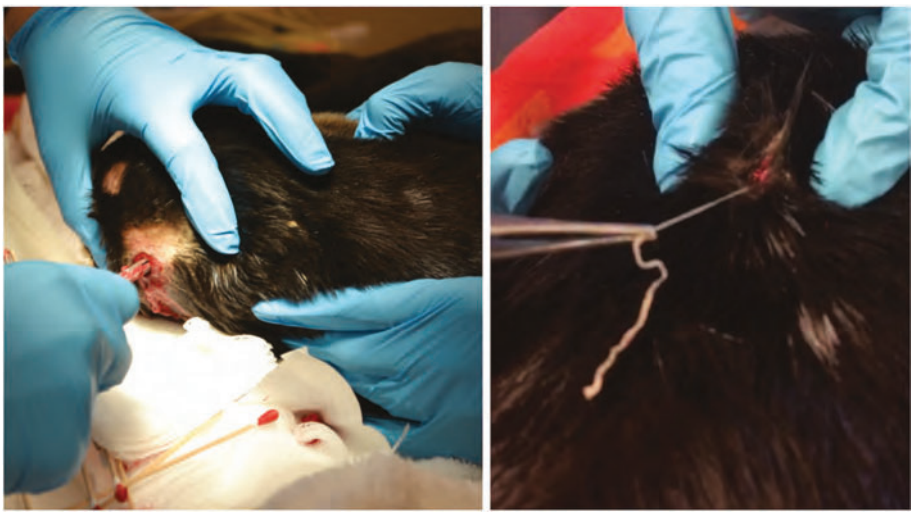

Fig. 3. Surgical removal of a Clade 2 *Dracunculus* sp. (FL15-33934) from a North American river otter (*Lontra canadensis*) from Florida, USA.

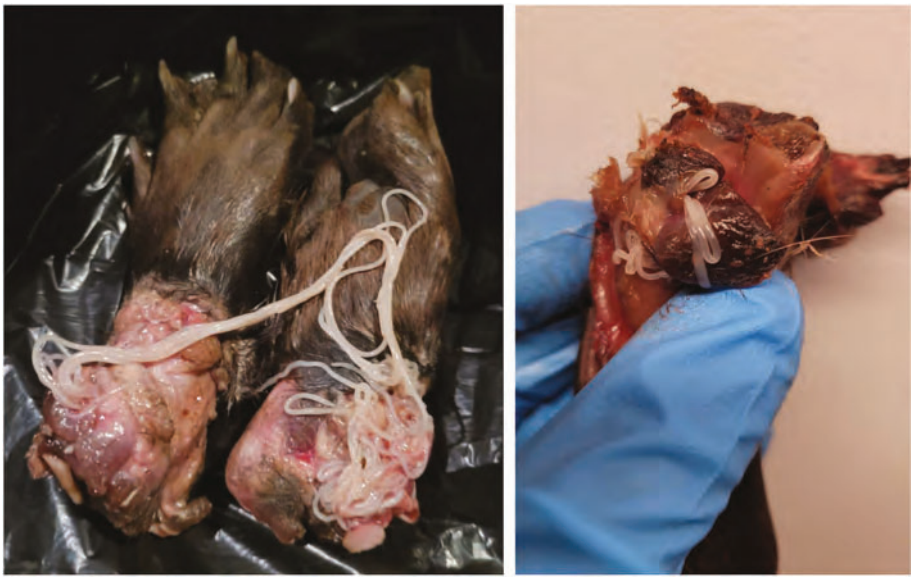

Fig. 4. Large clusters of *Dracunculus insignis* in paws (A) and joint (B) of North American river otter (*Lontra canadensis*) from Missouri, USA.

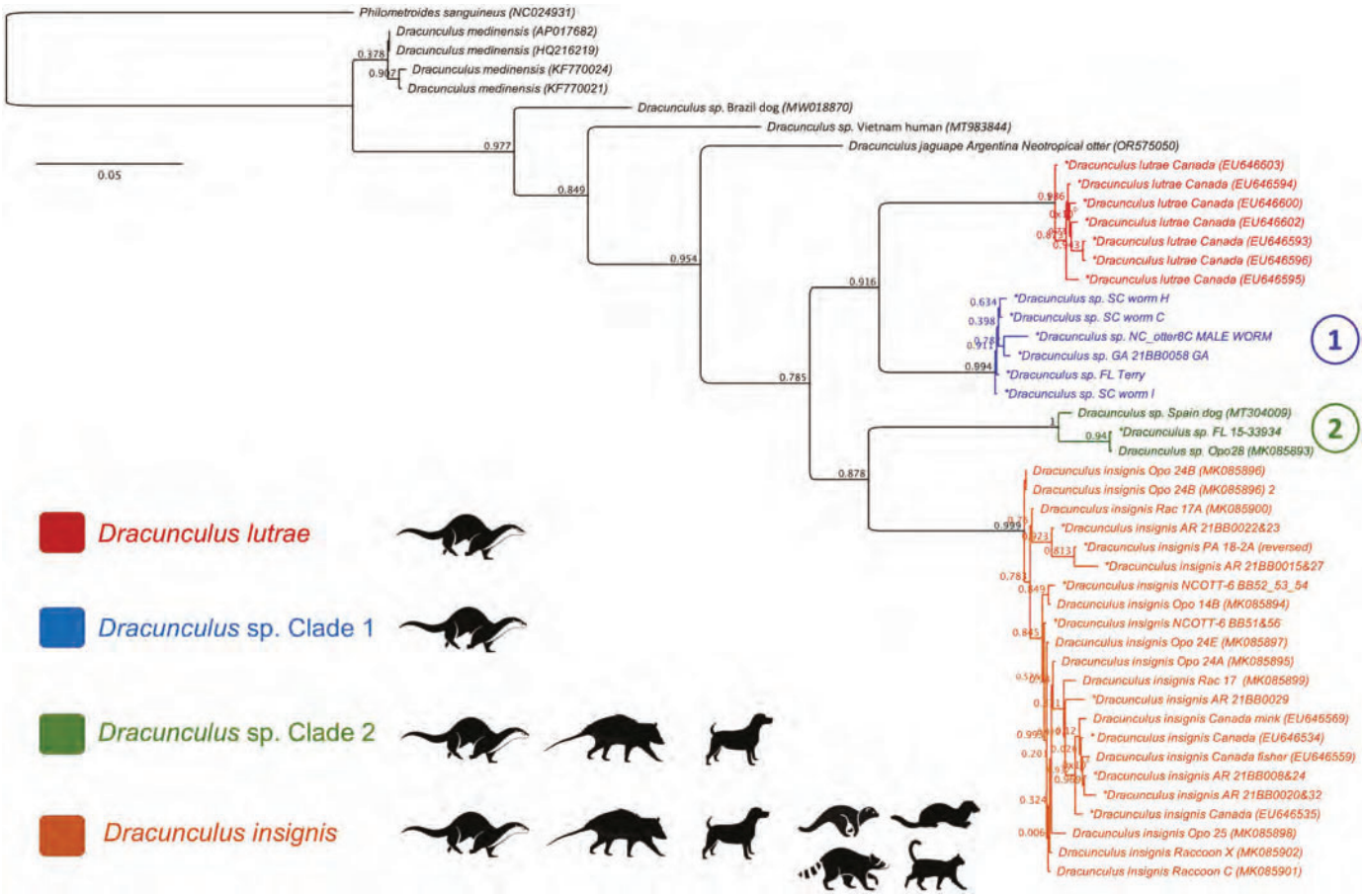

Fig. 5. Genetic relationships of *Dracunculus* spp. from North American river otters (*Lontra canadensis*) compared with other *Dracunculus* spp. based on partial cytochrome *c* oxidase subunit 1 gene sequences. The text in bold in the figure represents specimens analyzed in this study. Sequences with an asterisk (*) were derived from river otters (current and previous studies).

confirmed to be *Dracunculus,* but phylogenetically were separated into two unique clades (Clades 1 and 2 on Fig. 5). Clade 1 included sequences from eight worms collected from otters from Florida, Georgia, South Carolina, and North Carolina. Clade 2 included sequences from a single worm collected from an otter from Florida and previously reported sequences from a Virginia opossum from Georgia and a domestic dog from Spain (Cleveland et al., 2020; Diekmann et al., 2020). Clade 1 was genetically most similar to *D. lutrae* (92.4–93% similarity) and

phylogenetically it grouped as a sister clade to *D. lutrae* (Table 3, Fig. 5). Clade 2 was most similar to *D. insignis* (90.3–92.8% similarity) and also was a sister group to *D. insignis* (Table 3, Fig. 5). Clade 1 and Clade 2 were only 89.9–90.8% similar. Percent similarities among all individual samples included in the study and select mammal-infecting *Dracunculus* spp. are shown in Supplementary File 1. Two otters were co-infected with *D. insignis* and *D.* sp. Clade 1.

4. Discussion

Dracunculus infections have been reported from river otters since the early 1900s; however, little is known about the species infecting otters or their ecology (Cheatum and Cook 1948). Historically, parasites detected in otters were called *D. insignis* or were just called *Dracunculus* sp. because species identification was assumed or males were not available for species confirmation (Cheatum and Cook 1948; Toll, 1961; Lauhachinda 1978; Tumlison et al., 1984; Barding and Lacki, 2015). In the current study, molecular characterization of nematodes from river otters showed that there is a high diversity of *Dracunculus* species infecting this host. *Dracunculus insignis* was previously reported in river otters, but two new clades were detected in the current study, with Clade 1 being a novel species in any host. In addition, none of the lineages we detected were genetically similar to *D. jaguape*, which was recently described from a neotropical otter from Argentina (Natalini et al., 2023). Most *Dracunculus* species have been reported from only a single host species, but river otters and domestic dogs currently have the highest known diversity of *Dracunculus* species using a single definitive host species (three for each). The Virginia opossum is the only other host known to be infected with more than one *Dracunculus* spp. (Cleveland et al., 2018).

None of the nematodes in river otters from the eastern United States were confirmed to be *D. lutrae.* This species was initially described during a study of Dracunculidae in wild mammals in Ontario, Canada (Crichton and Beverley-Burton (1973). Males of *D. lutrae* were distinct from other mammal-infecting species based on overall length and length of the gubernaculum and spicules. Subsequent studies, based on morphology of males, found that *D. lutrae* was common in otters in southern Ontario, but was absent from otters from northern Ontario (Crichton and Beverley-Burton, 1974). A molecular-based project in Ontario confirmed that *D. lutrae* was distinct from *D. insignis* and common in otters (Elsasser et al., 2009). The lack of detection of *D. lutrae* in the eastern United States suggests that this parasite has a limited distribution in otters (Fig. 1). However, more surveillance of parasites in otters from northeastern and upper midwestern states is needed to better understand prevalence and distribution.

The host generalist parasite, *D. insignis*, was detected in a high percentage (29/38, 76%) of *Dracunculus* individuals we tested from otters. The natural host range is large and includes many species of mustelids (river otters, American mink (*Neogale vison)*, and fishers (*Pekania pennanti*)), procyonids (Northern raccoons), marsupials (Virginia opossums), canids (domestic dogs), and felids (domestic cats) (Crichton and Beverley-Burton, 1974; Elsasser et al., 2009; Cleveland et al., 2018, 2020; Williams et al., 2018). The host range is likely larger than has been described, but few studies find males or conduct molecular confirmation of species. For example, unidentified worms presumed to be *D. insignis* have been reported from a marten (*Martes americana*), an ermine *(Mustela erminea)*, four striped skunks (*Mephitis mephitis*), a captive silver fox (*Vulpes vulpes),* two badgers (*Taxidea taxus*), four muskrats (*Ondatra zibethicus*), and two beavers (*Castor canadensis*) (Cleveland et al., 2018). The geographic distribution of *D. insignis* is also large and includes many states in the eastern US and Ontario, Canada. Despite the large distribution (Fig. 1), little intraspecific genetic variation has been noted in the *COI* gene target by our study and Elsasser et al. (2009).

Eight worms from three states in the southeastern US were identified as a novel clade of *Dracunculus* related to *D. lutrae* (Clade 1), although given the high divergence of the *COI* gene target, it likely represents a new species. The one male worm that we found was from this clade. The morphology of the male (i.e., gubernaculum and spicules) also suggests that this clade represents a novel species. The larvae from two worms from this clade were generally shorter than both *D. insignis* and *D. lutrae,* but were within the range of both (596–857 μm and 608–772 μm, respectively). However, the L1 were considerably longer than those from *D. fuelleborni* (300–429 μm), *D.* sp. Pantanal (488–614 μm), and *D.* sp. Jaguar (507–583 μm), but considerably shorter than *D. jaguape* (668–771 μm) from the South American otter. Similar to *D. lutrae*, this clade has only been reported from river otters. Other hosts are unknown, but relatively few specimens from wildlife and domestic dogs have been genetically characterized in the southeastern United States, so additional work is needed to better understand this species (Williams et al., 2018; Cleveland et al., 2020).

The remaining worm was present in a unique group (Clade 2) that was first recognized by Cleveland et al. (2020) when a single sample from a Virginia opossum from Georgia grouped outside the *D. lutrae* and *D. insignis* clusters. Subsequently, Diekmann et al. (2020) detected a nearly identical sequence (98.3% similar) for a worm from a domestic dog from Spain. The hypothesis was that it could have been introduced to Spain by the establishment of invasive North American raccoons or paratenic hosts; however, no *Dracunculus* was detected from raccoons in the region where the infected dog lived (Sanjuán et al., 2022). In the current study, only one otter from southern Florida was infected with a worm from this clade and it was a female so there is currently no data on the morphology of male worms from this clade. Measurements of L1 from a female from an otter from this study were similar to larvae from two worms from clade 1 and overlapped with both *D. insignis* and *D. lutrae*. There are many unknowns related to this clade, including the natural host (only three reports, each in a different host), geographic range, and natural history. The finding of another worm from this lineage in North America further supports the suggestion this parasite was introduced to Spain, although the mechanism is unknown. Because this clade was found in a domestic dog in Spain, worms from dogs in the United States should be genetically characterized to determine if this parasite also infects dogs in the United States; however, a recent study of worms from dogs and cats only detected *D. insignis* (Williams et al., 2018).

In general, morphology is useful for the identification of helminth parasites, especially for male worms. For *Dracunculus* spp., females have few, if any, distinguishing morphological features but are by far the most common parasite sex detected because of their size and location. Therefore, molecular characterization is needed to definitively identify parasites to species. As has been noted in previous studies, we found the

Table 3
Interspecific variation among the four groups of *Dracunculus* spp. from otters and *Dracunculus medinensis*. The percent similarity is shown in white at the top of the chart and percent differences are shown in grey on the bottom of chart.

	D. lutrae	*Dracunculus* sp. Clade 1	*Dracunculus* sp. Clade 2	*D. insignis*	*D. jaguape*	*D. medinensis*
D. lutrae		92.4–93	90.1–90.7	89.8–91.4	90.4–91.1	89–90
Clade 1	7–7.6		89.9–90.8	90.3–92.8	91.4–91.9	87.9–89.9
Clade 2	9.3–9.9	9.2–10.1		90.0–92.4	89.7–90.1	88.3–89.7
D. insignis	8.6–10.2	7.2–9.7	7.6–10		90.1–91.1	89.1–91.4
D. jaguape	8.9–9.6	8.1–8.6	9.9–10.3	8.9–9.9		90.7–91.5
D. medinensis	10–11	10.1–12.1	10.3–11.7	8.6–10.9	8.5–9.3	

18 S rRNA gene of limited use in distinguishing species as sequences of both new clades were identical to several *Dracunculus* spp., including *D. lutrae* and *D. insignis*, while distinct from others such as *D. jaguape* and *D. medinensis* (Bimi et al., 2005; Thach et al., 2021; Natalini et al., 2023). The *COI* gene target had more utility, with 7–12% sequence differences between several mammal-infecting *Dracunculus* species. Both novel clades were >7–10% different than either *D. lutrae* or *D. insignis*, which was similar to differences between several established species and supports that the novel clades represent unique species. The length of L1 can be used to distinguish some *Dracunculus* spp.; however, few of the mammal-infecting parasite species vary in L1 length and often females do not contain any larvae (e.g., Spain specimen, Diekmann et al., 2020), which limits the use of this feature to distinguish species. In addition, fixation can alter the length of larvae and the lifecycle point at which adult females are removed from hosts can cause variation in maturity of larvae (e.g., in this current study, worms were removed from captive otters or trapped animals, so worms were not emerging). Finally, otters can be infected with other subcutaneous parasites (e.g., *Dirofilaria lutrae* and *Spirometra*), so careful examination is needed to ensure accurate identification (Swanepoel et al., 2018; Yabsley, unpublished).

Some *Dracunculus* spp. are known to use copepod intermediate hosts, but the lifecycle of most *Dracunculus* spp. is unknown. Both *D. medinensis* and *D. insignis* use multiple species of copepods as intermediate hosts but, to date, no intermediate host has been identified for *D. lutrae* or either of these two novel clades (Cleveland et al., 2018). Recently, fish and amphibians have been reported to serve as transport or paratenic hosts for the related parasites *D. medinensis* and *D. insignis* (Eberhard et al., 2016a; Cleveland et al., 2019). The importance of these paratenic or transport hosts in the maintenance of these parasites in nature is unknown, but some epidemiologic data and modeling suggests they may facilitate transmission (Eberhard et al., 2014; Richards et al., 2020; Gonzalez Engelhard et al., 2021; Vinson et al., 2021). Fish can serve as transport hosts because they consume a large number of copepods and, if the fish are eaten by a definitive host when infected copepods are passing through their gastrointestinal tract, transmission can occur to the definitive host (Eberhard et al., 2016b; Cleveland et al., 2017; Box et al., 2021a, 2021b). Tadpoles consume fewer copepods compared to fish, but larvae remain infectious in their tissues for a long period of time, which can facilitate transmission during and after the peak transmission season (Eberhard et al., 2016b; Cleveland et al., 2017; Box et al., 2021a; Box et al., 2021b). Because the diet of otters includes primarily fish as well as amphibians, it is possible that these hosts may be important for transmission of *D. lutrae, D. jaguape*, and other otter infecting *Dracunculus* spp. (Tumlison and Karnes, 1987; Reid et al., 1994). The aquatic lifestyle of otters may also expose them to more infected copepods.

5. Conclusions

In summary, we detected three species of *Dracunculus* from North American river otters in the eastern United States, bringing the total diversity of *Dracunculus* spp. in river otters to four (Fig. 1). It is unknown why river otters host such a high diversity of *Dracunculus* spp. and serve as definitive host for all four mammal-infecting species in North America. We did not detect *D. lutrae,* so this parasite may be restricted to northern parts of North America. We also detected two novel species of *Dracunculus* in otters, one of which is now known to infect three host species in the United States and Spain. The effects of historic translocations of otters on parasite prevalence and diversity is unknown. None of the parasites we detected were closely related to *D. jaguape*. The pathogenicity of these parasites in river otters is poorly studied. These parasites do not appear to cause significant disease or mortality, but cysts may be large, associated with joints, and there is evidence that otters experience irritation during cyst formation and worm emergence (Tumlison and Surf, 2018; Tumlison et al., 2018). These data hopefully will stimulate interest in the life cycle of Dracunculidae parasites because there are increasing reports of these parasites in new geographic locations and new hosts, including new species in humans (Cleveland et al., 2018; Thach et al., 2021). In addition, the medically important species, *D. medinensis*, is the subject of an international eradication campaign (Hopkins et al., 2023). Life cycle and natural history data from these other species can potentially inform eradication efforts and further our understanding of this poorly studied group of parasites.

Compliance with ethical standards

The examination of animals for parasites was included in a protocol reviewed and approved by the University of Georgia, Institutional Animal Care and Use Committee (UGA IACUC) (A2013 07–003, A2014 10–018, A2018 02–010, A2020 11–010).

Authors' contributions

M.Y. and C.C. wrote the main manuscript text, M.Y., C.C., H.B., R.S, A.G., J.B., R.T., A.S., C.D., C.O., and J.B. carried out field work and submitted parasites, K.G., A.T., L.S., M.G., E. Box. and S.C. performed the molecular analysis. All authors reviewed and contributed to the final manuscript

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Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property. We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from myabsley@uga.edu.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2024.100922.

References

Barding, E.E., Lacki, M.J., 2015. Occurrence of nematodes (*Dracunculus* spp.) in reintroduced river otters in Kentucky. J. Ky. Acad. Sci. 75, 94–96.

Bimi, L., Freeman, A.R., Eberhard, M.L., Ruiz-Tiben, E., Pieniazek, N.J., 2005. Differentiating *Dracunculus medinensis* from *D. insignis*, by the sequence analysis of the 18S rRNA gene. Ann. Trop. Med. Parasitol. 99, 511–517.

Box, E.K., Cleveland, C.A., Garrett, K.B., Grunert, R.K., Hutchins, K., Majewska, A.A., Thompson, A.T., Wyckoff, S.T., Ehlers, C., Yabsley, M.J., 2021a. Copepod consumption by amphibians and fish with implications for transmission of *Dracunculus* species. Int. J. Parasitol. Parasites Wildl. 15, 231–237.

Box, E.K., Yabsley, M.J., Garrett, K.B., Thompson, A.T., Wyckoff, S.T., Cleveland, C.A., 2021b. Susceptibility of anurans, lizards, and fish to infection with *Dracunculus* species larvae and implications for their roles as paratenic hosts. Sci. Rep. 11, 11802.

Brackett, S., 1938. Description and life history of the nematode *Dracunculus ophidensis* n. sp., with a redescription of the genus. J. Parasitol. 24, 353–361.

Cheatum, E.L., Cook, A.H., 1948. On the occurrence of the North American Guinea worm in mink, otter, raccoon, and skunk in New York State. Cornell Vet. 38, 421–423.

Cleveland, C.A., Eberhard, M.L., Thompson, A.T., Smith, S.J., Zirimwabagabo, H., Bringolf, R., Yabsley, M.J., 2017. Possible role of fish as transport hosts for *Dracunculus* spp. larvae. Emerg. Infect. Dis. 23, 1590–1592.

Cleveland, C.A., Garrett, K.B., Cozad, R.A., Williams, B.M., Murray, M.H., Yabsley, M.J., 2018. The wild world of Guinea Worms: a review of the genus *Dracunculus* in wildlife. Int. J. Parasitol. Parasites Wildl. 7, 289–300.

Cleveland, C.A., Eberhard, M.L., Thompson, A.T., Garrett, K.B., Swanepoel, L., Zirimwabagabo, H., Moundai, T., Ouakou, P.T., Ruiz-Tiben, E., Yabsley, M.J., 2019. A search for tiny dragons (*Dracunculus medinensis* third-stage larvae) in aquatic animals in Chad, Africa. Sci. Rep. 9, 375.

Cleveland, C.A., Eberhard, M.L., Garrett, K.B., Thompson, A.T., Swanepoel, L., Miller, E. A., Stephens, O.L., Yabsley, M.J., 2020. *Dracunculus* species in meso-mammals from Georgia, United States, and implications for the Guinea worm eradication Program in Chad, Africa. J. Parasitol. 106, 616–622.

Crichton, V.F., Beverley-Burton, M., 1973. *Dracunculus lutrae* n. sp. (Nematoda: Dracunculoidea) from the otter, *Lutra canadensis*, in Ontario, Canada. Can. J. Zool. 51, 521–529.

Crichton, V.F.J., Beverley-Burton, M., 1974. Distribution and prevalence of *Dracunculus* spp. (Nematoda: Dracunculoidea) in mammals in Ontario. Can. J. Zool. 52, 163–167.

Diekmann, I., Alnassan, A.A., Globokar, M., Pantchev, N., Kurzrock, L., Hernandez, L., Lopez, J., Ruano, R., Herrero, S., von Samson-Himmelstjerna, G., Krücken, J., 2020. Canine *Dracunculus* nematode infection, toledo, Spain. Emerg. Infect. Dis. 26, 1860–1863.

Eberhard, M.L., Ruiz-Tiben, E., Hopkins, D.R., Farrell, C., Toe, F., Weiss, A., Withers, P. C., Jenks, M.H., Thiele, E.A., Cotton, J.A., Hance, Z., Holroyd, N., Cama, V.A., Tahir, M.A., Mounda, T., 2014. The peculiar epidemiology of dracunculiasis in Chad. Am. J. Trop. Med. Hyg. 90, 61–70.

Eberhard, M.L., Cleveland, C.A., Zirimwabagabo, H., Yabsley, M.J., Ouakou, P.T., Ruiz-Tiben, E., 2016a. Guinea Worm (*Dracunculus medinensis*) infection in a wild-caught frog. Chad. Emerg. Infect. Dis. 22, 1961–1962.

Eberhard, M.L., Yabsley, M.J., Zirimwabagabo, H., Bishop, H., Cleveland, C.A., Maerz, J. C., Bringolf, R., Ruiz-Tiben, E., 2016b. Possible role of fish and frogs as paratenic hosts of *Dracunculus medinensis*. Chad. Emerg. Infect. Dis. 22, 1428–1430.

Elsasser, S.C., Floyd, R., Hebert, P.D., Schulte-Hostedde, A.I., 2009. Species identification of NorthNorth American Guinea worms (nematoda: Dracunculus) with DNA barcoding. Mol. Ecol. Resour. 9, 707–712.

Fagundes-Moreira, R., Bezerra-Santos, M.A., May-Junior, J.A., Baggio-Souza, V., Rampim, L.E., Sartorello, L.R., Lia, R.P., Soares, J.F., Otranto, D., 2023. The jaguar (*Panthera onca*) as a potential new host of *Dracunculus* sp. Parasitol. Res. 122, 2951–2956.

Gonzalez Engelhard, C.A., Hodgkins, A.P., Pearl, E.E., Spears, P.K., Rychtář, J., Taylor, D., 2021. A mathematical model of Guinea worm disease in Chad with fish as intermediate transport hosts. J. Theor. Biol. 521, 110683.

Hopkins, D.R., Weiss, A.J., Yerian, S., Sapp, S.G.H., Cama, V.A., 2023. Progress toward eradication of dracunculiasis - worldwide, January 2022-June 2023. MMWR Morb. Mortal. Wkly. Rep. 72, 1230–1236.

Jones, H.I., Mulder, E., 2007. *Dracunculus mulbus* n. sp. (nematoda: spirurida) from the water python *Liasis fuscus* (serpentes: boidae) in northern Australia. Syst. Parasitol. 66, 195–205.

Lauhachinda, V., 1978. Life History of the River Otter in Alabama with Emphasis on Food Habits. Ph.D. Dissertation. Auburn University, Auburn, Alabama, p. 169.

Moorthy, V.N., 1937. A redescription of *Dracunculus medinensis*. J. Parasitol. 23, 220–224.

Natalini, M.B., Notarnicola, J., Monje, L.D., Vallejos, A.B., Gavier, F.S., Kowalewski, M. M., 2023. A new *Dracunculus* species (Nematoda: dracunculoidea) in neotropical otters (*Lontra longicaudis*) from Argentina: morphological and molecular characterization. Int. J. Parasitol. Parasites Wildl. 22, 234–242.

Olson, M., Harris, T., Higgins, R., Mullin, P., Powers, K., Olson, S., Powers, T.O., 2017. Species delimitation and description of *Mesocriconema nebraskense* n. sp. (Nematoda: criconematidae), a morphologically cryptic, parthenogenetic species from North American grasslands. J. Nematol. 49, 42–66.

Paiva, F., Piazzalunga, P.S., Pereira, F.B., Borghesan, T.C., Soares, P., Tavares, L.E.R., 2021. Dracunculiasis in a domestic dog in Brazil. Parasitol. Res. 120, 1271–1377.

Prosser, S.W.J., Velarde-Aguilar, M.G., León-Règagnon, V.A., Hebert, P.D.N., 2013. Advancing nematode barcoding: a primer cocktail for the cytochrome c oxidase subunit I gene from vertebrate parasitic nematodes. Mol. Ecol. Resour. 13, 1108–1115.

Raesly, E.J., 2001. Progress and status of river otter reintroduction projects in the United States. Wildl. Soc. Bull. 29, 856–862.

Reid, D.G., Code, T.E., Reid, A.C.H., Herrero, S.M., 1994. Food habits of the river otter in a boreal ecosystem. Can. J. Zool. 72, 1306–1313.

Richards, R.L., Cleveland, C.A., Hall, R.J., Tchindebet Ouakou, P., Park, A.W., Ruiz-Tiben, E., Weiss, A., Yabsley, M.J., Ezenwa, V.O., 2020. Identifying correlates of Guinea worm (*Dracunculus medinensis*) infection in domestic dog populations. PLoS Neglected Trop. Dis. 14, e0008620.

Sanjuán, C.G., Aguirre, J.I., Villaverde, S., Banda, E., Hernandez, S.M., Yabsley, M.J., 2022. Surveillance for gastrointestinal, subcutaneous, and ectoparasites of invasive North American raccoons (*Procyon lotor*) in central Spain. Vet. Parasitol. Reg. Stud. Reports. 36, 100793.

Swanepoel, L., Cleveland, C.A., Olfenbuttel, C., Dukes, C.G., Brown, D., Brown, J.D., Surf, A., Tumlison, R., Yabsley, M.J., 2018. Prevalence and genetic characterization of *Dirofilaria lutrae* Orihle, 1965 in North American river otters (*Lontra canadensis*). Vet. Parasitol. Reg. Stud. Reports. 14, 187–190.

Thach, P.N., van Doorn, H.R., Bishop, H.S., Fox, M.S., Sapp, S.G.H., Cama, V.A., Duyet, L. V., 2021. Human infection with an unknown species of *Dracunculus* in Vietnam. Int. J. Infect. Dis. 105, 739–742.

Toll, W.G., 1961. The Ecology of the River Otter (*Lutra canadensis*) in the Quabbin Reservoir of Central Massachusetts. M.S. Thesis. University of Massachusetts, Amherst, Massachusetts, p. 45.

Travassos, L., 1934. *Dracunculus fuelleborni* n. sp., parasito de *Didelphis aurita* Wied. Mem. Inst. Oswaldo Cruz 28, 235–237.

Tumlison, R., Karnes, M., 1987. Seasonal changes in food habits of river otters in southwestern Arkansas beaver swamps. Mammalia 51, 225–231.

Tumlison, R., Surf, A., 2018. Prevalence, structure, and distribution of novel parasite cysts containing *Dracunculus* species in river otters (*Lontra canadensis*) from Arkansas. J. Parasitol. 104, 319–321.

Tumlison, R., Smith, R., Hudspeth, J., Polechla, P.J., King, A.W., 1984. Dracunculiasis in some Arkansas carnivores. J. Parasitol. 70, 440.

Tumlison, R., Surf, A., May, H., 2018. Distribution of *Dracunculus* sp. infection in river otters (*Lontra canadensis*) in Arkansas. J. Ark. Acad. Sci. 72. Article 32.

Vinson, J.E., Park, A.W., Cleveland, C.A., Yabsley, M.J., Ezenwa, V.O., Hall, R.J., 2021. Alternative transmission pathways for Guinea worm in dogs: implications for outbreak risk and control. Int. J. Parasitol. 51, 1027–1034.

Wijová, M., Moravec, F., Horák, A., Lukes, J., 2006. Evolutionary relationships of Spirurina (Nematoda: chromadorea: Rhabditida) with special emphasis on dracunculoid nematodes inferred from SSU rRNA gene sequences. Int. J. Parasitol. 36, 1067–1075.

Williams, B.M., Cleveland, C.A., Verocai, G.G., Swanepoel, L., Niedringhaus, K.D., Paras, K.L., Nagamori, Y., Little, S.E., Varela-Stokes, A., Nemeth, N., Wyrosdick, H., Tucker, A., Deal, L., Gauthier, D., Prouty, S., DeAngelo, C., Marsh, A., Piepgras, D., Cook, L.H., Milliren, K.B., Becker, J.S., Lyons, C., Clark, J., Stumph, J., Borst, M.M., Tucker, K.L., Ward, A., Baird, E.M., Burke, K.A., Camp, J.W., Davis, C.A., Yabsley, M. J., 2018. *Dracunculus* infections in domestic dogs and cats in North America; an under-recognized parasite? Vet. Parasitol.: Reg. Stud. Reports. 13, 148–155.