The wild world of Guinea Worms: A review of the genus Dracunculus in wildlife

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ABSTRACT

Nematodes are an extremely diverse and speciose group of parasites. Adult dracunculoid nematodes (Superfamily Dracunculoidea) occur in the tissues and serous cavities of mammals, fish, reptiles, amphibians and birds. Of the dracunculid group, perhaps best known is Dracunculus medinensis, the human Guinea Worm. Considerable work has been done on D. medinensis; however recent infections in peri-domestic dogs and the finding of naturally-infected paratenic hosts (previously unreported for D. medinensis) indicate we still have much to learn about these parasites. Furthermore, among eight species in the Old World and six species in the New World there is a lack of general life history knowledge as well as questions on species occurrence, host diversity, and transmission dynamics. Herein, we provide a comprehensive review of the genus Dracunculus, in order of a theoretical evolutionary progression from reptilian to mammalian hosts. Species descriptions, where available, are provided but also show where gaps occur in our knowledge of various species. Additionally, many first reports of Dracunculus spp. were done prior to the development and use of molecular tools. This is especially important for this group of parasites as speciation based on morphology is only applicable to males of the genus, and males, given their size, are notoriously difficult to recover from definitive hosts. Therefore, we also discuss current molecular tools used in the investigation of this group of parasites. Given recent host-switching events, the dracunculids are of increasing importance and require further work to expand our understanding of this genus.

1. Introduction

Adult dracunculoid nematodes (Superfamily Dracunculoidea) occur in the tissues and serous cavities of mammals, fish, reptiles, amphibians, and birds (Chabaud, 1960; Petter and Planelles, 1986). Many of the species in this group are similar in that females come into contact with water. The Family Dracunculidae contains two genera: Dracunculus, parasites of mammals and reptiles, and Avioserpen, parasites of birds. There are 14 valid species of Dracunculus but most knowledge about these parasites stems from research on the medically important D. medinensis, also known as the African Guinea worm. Considerable literature and reviews exist for this species (Muller, 1971, 1976; Cairncross et al., 2002; Ruiz-Tiben and Hopkins, 2006; Eberhard et al., 2014). The life cycle of D. medinensis has been well studied and documented, due in large part to the long history of human infections and an eradication campaign initiated by the World Health Assembly in 1981 and spearheaded in 1986 by The Carter Center (Ruiz-Tiben and Hopkins, 2006). However, despite a long history of epidemiologic and public health research, recent infections in peri-domestic dogs and the finding of naturally infected paratenic hosts (previously unreported for D. medinensis) indicate we still have much to learn about these parasites (Eberhard et al., 2014, 2016a,b; Cleveland unpublished data).

This review focuses on Dracunculus species other than D. medinensis, with an emphasis on D. insignis, which is being used as a model parasite for studies to assist the Guinea Worm Eradication Program.
1.1. Genus Dracunculus

Nematodes in the genus *Dracunculus* are large subcutaneous parasites of mammals and reptiles (snakes and turtles) with most described species being from snakes. The females of *Dracunculus* spp. are some of the longest nematodes with recorded lengths up to 100 cm (Cairncross et al., 2002). Morphologically, female *Dracunculus* spp. are very similar and molecular characterization is needed for definitive identification. Males are considerably smaller (16–40 mm), but they have several morphological features that can be used to distinguish the different parasite species (Crichton and Beverley-Burton, 1973; Cairncross et al., 2002). Unfortunately, males are rarely detected and have never been described for some species. This is particularly problematic for hosts that may be infected with more than one dracunculid (e.g., river otters (*Lontra canadensis*)) or with parasites detected in novel hosts.

1.2. Species of Dracunculus

The highest diversity of described *Dracunculus* species occurs in the Old World. To date, eight species have been described with seven occurring in snakes endemic to Europe, Africa, Asia, and Australia (Table 1). Although most *Dracunculus* species described are from snakes, the most widely known and studied species is *D. medinensis*, the human Guinea worm, which also happens to be the only Old World mammalian species (Eberhard et al., 2014). Although *D. medinensis* was historically widespread in Africa and South Asia, through considerable management and eradication efforts, the total number of countries with endemic transmission in either humans or dogs has been reduced from 21 to 3 resulting in a decrease of human cases from 3.5 million in 1986 to 30 in 2017 (Molyneux and Sankara, 2017; https://www.cartercenter.org/health/guinea_worm/case-totals.html). Numerous studies and reviews on recent developments related to this parasite have been published (Eberhard et al., 2014; Eberhard et al., 2016a; b; Cleveland et al., 2017).

In the New World, a lower diversity of *Dracunculus* species has been reported, but more species have been described from mammals (Tables 1 and 2). There are at least 2 species of *Dracunculus* that infect snakes (*D. ophidensis* and *D. brazilianus*), 1 from a snapping turtle (*D. globocephalus*), and 3 from mammals (*D. insignis*, *D. lutrae*, and *D. fuelleborni*).

1.3. General life cycle

Adult female *Dracunculus* mature in the subcutaneous tissues of the definitive vertebrate host where they will form blisters primarily on the distal extremities; however, reports of these lesions are rare in reptile species being from snakes. The females of *Dracunculus* spp. are some of the longest nematodes with recorded lengths up to 100 cm (Cairncross et al., 2002). Morphologically, female *Dracunculus* spp. are very similar and molecular characterization is needed for definitive identification. Males are considerably smaller (16–40 mm), but they have several morphological features that can be used to distinguish the different parasite species (Crichton and Beverley-Burton, 1973; Cairncross et al., 2002). Unfortunately, males are rarely detected and have never been described for some species. This is particularly problematic for hosts that may be infected with more than one dracunculid (e.g., river otters (*Lontra canadensis*)) or with parasites detected in novel hosts.

When these lesions are exposed to water, larvae are expelled from the female into the environment and consumed by the intermediate cyclopid copepod host (Fig. 1). Female nematodes will then senesce and may be pulled out of tissue by the affected animal or will retreat subcutaneously and calcify. Male and unfertilized female nematodes can survive for 330 days; however, it is proposed that this estimate is conservative (Crichton and Beverley-Burton, 1973, 1974; 1977; Brandt and Little, 2004).

First stage larvae released from female worms have extremely long, tapered tails and their overall length can vary between species (i.e., 0.24–0.608 mm, although lengths of most species are unknown), and develop a trilobed tail (Fig. 2) (Anderson, 2000; Muller, 1971).

2. *Dracunculus* species of squamates

Currently there are nine formally described *Dracunculus* species in snakes. The highest diversity has been reported in Eurasia and Africa where *D. coluberensis, D. alii, D. houedemert, D. dai, D. dahomensis,* and *D. oesophageus* occur. A single species, *D. mulbus*, has been reported from Oceania. In the Americas, two species (*D. ophidensis* and *D. brasiliensis*) have been described. Also, worldwide, several uncharacterized species have been reported from numerous snake species.

2.1. Squamate Dracunculus species by region

2.1.1. *Dracunculus ophidensis* (Brackett, 1938) and other species from North America

*Dracunculus ophidensis* was first described from garter snakes (*Thamnophis sirtalis*) from southern Michigan and subsequently found in...
<table>
<thead>
<tr>
<th>Host group</th>
<th>Parasite</th>
<th>Geographic region</th>
<th>Known DH</th>
<th>Known IH</th>
<th>Adult length (♂; ♀)</th>
<th>Left and right spicule length</th>
<th>Gubernaculum length</th>
<th>Caudal alae</th>
<th>L1 length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammals</td>
<td>D. medinensis</td>
<td>Historically Africa and Asia</td>
<td>Humans, domestic dogs, domestic ferret (E)†</td>
<td>Numerous copepod species</td>
<td>0.42 (0.40–0.52)</td>
<td>0.44 (0.41–0.52)</td>
<td>0.12</td>
<td>Absent</td>
<td>0.581–0.643</td>
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<td></td>
<td>Various amphipods</td>
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<td></td>
<td></td>
<td>(N,E)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>D. insignis</td>
<td>United States, Canada</td>
<td>Raccoons (Procyon lotor), mink (Mustela vison), Virginia opossum (Didelphis virginiana), North American river otter (Lontra canadensis), domestic dog, domestic cat, domestic ferret (E)</td>
<td>Acanthocyclops renfieldi (E) and C. bicuspidatus (E)</td>
<td>0.46–0.495 each</td>
<td></td>
<td>0.114–0.13</td>
<td>NK†</td>
<td>0.664</td>
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<td></td>
<td>Various amphipods</td>
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<td>(N,E)</td>
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<tr>
<td></td>
<td>D. lutrae</td>
<td>United States, Canada</td>
<td>North American river otter (Lontra canadensis)</td>
<td>NK</td>
<td>36 (32.2–40); 247</td>
<td>(200–290)</td>
<td>0.61 (0.51–0.68),</td>
<td>0.17 (0.16–0.18)</td>
<td>0.665</td>
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<td></td>
<td></td>
<td>0.64 (0.59–0.72)</td>
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<tr>
<td>Reptiles</td>
<td>D. fuelleborni</td>
<td>Brazil</td>
<td>Big-eared opossum (Didelphis aurina)</td>
<td>NK</td>
<td>27–29; 465–490</td>
<td></td>
<td>0.38–0.42 each</td>
<td>0.088–01</td>
<td>0.3–0.429</td>
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<td></td>
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<td></td>
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<td>C. viridis (E)</td>
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<tr>
<td></td>
<td>D. brasiliensis</td>
<td>Brazil</td>
<td>Green Anaconda (Eunectes murinus), Brown banded water snake (Helicops angulus)</td>
<td>NK</td>
<td>130–220 mm</td>
<td></td>
<td>NK</td>
<td>NK</td>
<td>0.396–0.429</td>
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<tr>
<td></td>
<td>D. coluberensis</td>
<td>India</td>
<td>Trinket snake (Cophyphatus (= Coluber helena))</td>
<td>NK</td>
<td>197.5; NK</td>
<td>0.08; 0.07</td>
<td>&quot;small&quot; one present</td>
<td>Absent</td>
<td>NK</td>
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<tr>
<td></td>
<td>D. alii</td>
<td>India</td>
<td>Checkered keelback snakes (Xenochrophis (= Natrix piscator))</td>
<td>NK</td>
<td>13.09–24.4; NK</td>
<td>0.22–0.29; 0.23–0.3</td>
<td></td>
<td>Absent</td>
<td>NK</td>
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<tr>
<td></td>
<td>D. houdemeri</td>
<td>Vietnam</td>
<td>Checkered keelback snakes</td>
<td>NK</td>
<td>21.3</td>
<td></td>
<td>NK</td>
<td>Subequal, −0.46</td>
<td>0.33–0.363</td>
</tr>
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<td></td>
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<td></td>
<td>Madagascan boa (Acranthurus madagascarensis)</td>
<td>28 mm; NK</td>
<td></td>
<td></td>
<td>NK</td>
<td>Present</td>
<td>0.37</td>
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<tr>
<td></td>
<td>D. doi</td>
<td>Madagascar</td>
<td></td>
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<tr>
<td></td>
<td>D. dahomensis</td>
<td>Benin</td>
<td>African rock python (Python sebae)</td>
<td>NK</td>
<td>48; NK</td>
<td>0.425; 0.4; 0.297; 0.282</td>
<td>0.065</td>
<td>Absent</td>
<td>0.40–0.42</td>
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<tr>
<td></td>
<td>D. oesophagus</td>
<td>Italy</td>
<td>colubrid snakes (Natrix viripina, Natrix matrix persa)</td>
<td>NK</td>
<td>17 (11.7–20); 360</td>
<td>0.4–0.48; 0.4–0.48</td>
<td>0.08–0.11</td>
<td>Present</td>
<td>0.34–0.40</td>
</tr>
<tr>
<td></td>
<td>(= D. ricci)</td>
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<td></td>
<td>D. malbas</td>
<td>Australia and Papua New Guinea</td>
<td>Water python (Liiasis fuscus), Papuan olive python (Apodora papuana)</td>
<td>NK</td>
<td>23 (17–33); 240</td>
<td>(180–360)</td>
<td>0.4–0.48</td>
<td>Absent</td>
<td>NK</td>
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<tr>
<td>Turtles</td>
<td>D. globocephalus</td>
<td>United States, possibly Costa Rica</td>
<td>snapping turtle (Chelydra serpentina)</td>
<td>C. bicuspidatus (E)</td>
<td>16–21.7; 90–136</td>
<td>0.963–1.962; 0.186–0.213</td>
<td></td>
<td>Absent</td>
<td>0.666–0.721</td>
</tr>
</tbody>
</table>
Table 2
The geographic, anatomic location and prevalence of confirmed species of *Dracunculus insignis*, *D. lutrae*, and non-speciated *Dracunculus* in North America.

<table>
<thead>
<tr>
<th>Species</th>
<th>Host</th>
<th>Geographic location</th>
<th>Anatomic location</th>
<th>Prevalence (%)</th>
<th>Species confirmation method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. insignis</em></td>
<td>River Otter (<em>Lontra canadensis</em>)</td>
<td>Arkansas, USA</td>
<td>Subcutaneous and intermuscular fascia of carpal and tarsal areas</td>
<td>12/184 (6.5)</td>
<td>molecular</td>
<td>Tumlison and Surf, 2018</td>
</tr>
<tr>
<td></td>
<td>Racoon (<em>Procyon lotor</em>)</td>
<td>Ontario, Canada</td>
<td>Subcutaneous tissues in the inguinal area, thorax, abdomen and fascial layers of the lower legs</td>
<td>NA</td>
<td>molecular morphology via male specimens</td>
<td>Elsasser et al., 2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ontario, Canada</td>
<td>Subcutaneous tissues in the inguinal area, left and right axillary areas</td>
<td>1/1 (100)</td>
<td>morphology via male specimens</td>
<td>Gibson and McKiel, 1972</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maryland, USA</td>
<td>Subcutaneous fascia of legs</td>
<td>1/1 (100)</td>
<td>morphology via male specimens</td>
<td>Chitwood, 1950</td>
</tr>
<tr>
<td><em>D. lutrae</em></td>
<td>River Otter</td>
<td>Ontario, Canada</td>
<td>Subcutaneous tissues of thoracic, abdominal, inguinal areas and intermuscular fascia of legs</td>
<td>NA</td>
<td>molecular</td>
<td>Elsasser et al., 2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ontario, Canada</td>
<td>Connective tissue beneath latissimus dorsi, subcutaneous tissues of thoracic, abdominal, inguinal areas and intermuscular fascia of legs</td>
<td>NA</td>
<td>molecular morphology via male specimens</td>
<td>Crichton and Beverly-Burton, 1974</td>
</tr>
<tr>
<td></td>
<td>Badger (<em>Taxidea taxus</em>)</td>
<td>Iowa, USA</td>
<td>Subcutaneous and intermuscular fascia of carpal and tarsal areas</td>
<td>2/24 (8.3)</td>
<td>NA</td>
<td>Wittrock and Ulmer, 1974</td>
</tr>
<tr>
<td></td>
<td>Beaver (<em>Castor canadensis</em>)</td>
<td>Kansas, USA</td>
<td>Connective tissue beneath latissimus dorsi</td>
<td>2/63 (3)</td>
<td>NA</td>
<td>McKown et al., 1995</td>
</tr>
<tr>
<td></td>
<td>Fischer (<em>Martes pennanti</em>)</td>
<td>New Hampshire, USA</td>
<td>Subcutaneous and intermuscular fascia of carpal and metatarsal areas</td>
<td>37/748 (4.9)</td>
<td>NA</td>
<td>Carlson and Vito, 1984</td>
</tr>
<tr>
<td></td>
<td>Marten (<em>Martes americana</em>)</td>
<td>Ontario, Canada</td>
<td>Connective tissue beneath latissimus dorsi, subcutaneous tissues of thoracic, abdominal, inguinal areas and intermuscular fascia of legs</td>
<td>1/405 (0.2)</td>
<td>NA</td>
<td>Seville and Addison, 1995</td>
</tr>
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<td>Mink (<em>Neovison vison</em>)</td>
<td>Ontario, Canada</td>
<td>Subcutaneous and intermuscular fascia of carpal and tarsal areas</td>
<td>14/42 (33)</td>
<td>NA</td>
<td>Schulte-Hostedde and Elsasser, 2011</td>
</tr>
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<td></td>
<td>Arkansas, USA</td>
<td>Subcutaneous and intermuscular fascia of carpal and metatarsal areas</td>
<td>35/507 (6.9)</td>
<td>NA</td>
<td>Tumlison et al., 1984</td>
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<td></td>
<td>Ohio, USA</td>
<td>Subcutaneous and intermuscular fascia of carpal and metatarsal areas, tail musculature</td>
<td>3/3 (100)</td>
<td>NA</td>
<td>Crites, 1963</td>
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<td></td>
<td>Minnesota, USA</td>
<td>Subcutaneous and intermuscular fascia of carpal and metatarsal areas</td>
<td>3/3 (100)</td>
<td>NA</td>
<td>Huggins, 1958</td>
<td></td>
</tr>
<tr>
<td></td>
<td>New York, USA</td>
<td>Intermuscular fascial layers of lower leg and ankles; subcutaneous position over pectoralis major</td>
<td>NA</td>
<td>NA</td>
<td>Cheatum and Cook, 1948</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minnesota, USA</td>
<td>Hind leg</td>
<td>2/72 (2.7)</td>
<td>NA</td>
<td>Erickson, 1946</td>
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<tr>
<td></td>
<td>Iowa, USA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Benbrook, 1940</td>
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<tr>
<td></td>
<td>Wisconsin, USA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Chaddock, 1940</td>
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<td></td>
<td>Nebraska, USA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Chitwood, 1933</td>
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<td></td>
<td>Ontario, Canada</td>
<td>Right inguinal region, left and right axillary regions</td>
<td>1/1 (100)</td>
<td>NA</td>
<td>Gibson and McKiel, 1972</td>
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<td></td>
<td>Minnesota, USA</td>
<td>Subcutaneous and intermuscular fascia of carpal and metatarsal areas</td>
<td>1/1 (100)</td>
<td>NA</td>
<td>Fyvie, 1969</td>
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<td></td>
<td>New York, USA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Huggins, 1958</td>
<td></td>
</tr>
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<td>Muskrat (<em>Ondatra zibethicus</em>)</td>
<td>New York, USA</td>
<td>Intermuscular fascial layers of lower leg and ankles; subcutaneous position over pectoralis major</td>
<td>3/64 (4.7)</td>
<td>NA</td>
<td>Dikmans, 1948</td>
<td></td>
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<td>Opossum (<em>Didelphis virginiana</em>)</td>
<td>New York, USA</td>
<td>Intermuscular fascial layers of lower leg and ankles; subcutaneous position over pectoralis major</td>
<td>3/64 (4.7)</td>
<td>NA</td>
<td>Alexander et al., 1972</td>
<td></td>
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<td></td>
<td>Cheatum and Cook, 1948</td>
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<tr>
<td>Raccoon</td>
<td>Florida, USA</td>
<td>Subcutaneous and intermuscular fascia of carpal and metatarsal areas</td>
<td>9/54 (16.7)</td>
<td>NA</td>
<td>Keeling et al., 1993</td>
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<td></td>
<td>Arkansas</td>
<td>4/30 (13)</td>
<td>NA</td>
<td>Richardson et al., 1992</td>
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<td>Kentucky, USA</td>
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<td>Connecticut, USA</td>
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<td>4/4 (100)</td>
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<td>North Carolina, South Carolina, Florida, USA</td>
<td>Subcutaneous tissue and muscle fascia</td>
<td>14/209 (6.7), 8/34 (23.5), 6/19 (31.6)</td>
<td>NA</td>
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(continued on next page)
Table 2 (continued)

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<tr>
<th>Species</th>
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<th>Geographic location</th>
<th>Anatomic location</th>
<th>Prevalence (%)</th>
<th>Species confirmation method</th>
<th>Reference</th>
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<td>Ohio, USA</td>
<td>Subcutaneous and intermuscular fascia of carpal and metatarsal areas, tail musculature fascia of carpal and metatarsal areas</td>
<td>3/3 (100)</td>
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<td>Layne et al., 1960</td>
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<td>Huggins, 1958</td>
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<td>NA</td>
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<td>Cheatum and Cook, 1948</td>
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<td>Ewing and Hibbs, 1966</td>
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<td>New York, USA</td>
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<td>NA</td>
<td>Cheatum and Cook, 1948</td>
<td></td>
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<tr>
<td>Minnesota, USA</td>
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<td>1/15 (6.7)</td>
<td>NA</td>
<td>Erickson, 1946</td>
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</table>

\* Given the hosts and range, most of these reports are likely *D. insignis*; however, only female worms were collected and thus definitive identification could not be made despite many of the studies publishing them as *D. insignis*.

Parasites identified as *D. ophidensis* have been reported from the mesentery of the Blackbelly garter snake (*Thamnophis melanogaster*) from several states in Mexico, indicating that this parasite may be widespread in garter snakes (Pérez-Ponce de León et al., 2001; Jiménez-Ruiz et al., 2002; Moravec, 2006). *Dracunculus ophidensis* also has been reported from northern water snakes (*Nerodia sipedon*) from Maryland, Minnesota, and Pennsylvania, and a plain-bellied water snake (*Nerodia erythrogaster*) from Michigan. However, confirmation in these other species requires molecular characterization or detection of male nematodes (Mirza and Roberts, 1957; USNPC 1362086; Mirza, 1957; Moravec, 2006).

Unidentified *Dracunculus* spp. have been detected in a captive gopher snake (*Pituophis catenifer*) and a captive Florida kingsnake (*Lampropeltis getula floridana*) from The National Zoological Park (Washington D.C.) (Mirza and Roberts, 1957; USNPC 1342730).

### 2.1.2. *Dracunculus brasiliensis* (Moravec and Santos, 2009) and other species from central and South America

A single sub gravid female and part of a gravid female recovered from subcutaneous tissue, serous membranes, and body cavity of infected snakes, and during summer months, gravid females were present in visible subcutaneous swellings (Brackett, 1938; Moravec, 2006). Infections were only detected during the summer months, with signs of infection disappearing by fall or early winter (Brackett, 1938). The arrangement of genital papillae and length of spicules in males are the most reliable characteristics used to differentiate *D. ophidensis* from other *Dracunculus* species. Spatial variation has been noted in *D. ophidensis* prevalence with a higher prevalence found in Minnesota compared with Michigan (Brackett, 1938).

**Fig. 1.** Life-cycle of *Dracunculus insignis* in wildlife and domestic dogs. Arrows in red represent transmission from (A) definitive hosts to intermediate hosts (Cyclopoid copepods) and (B) transmission to definitive hosts via consumption of intermediate hosts. Arrows in blue represent (C) transmission from intermediate hosts to paratenic and transport hosts (amphibians and fish) via consumption of infected copepods and (D) transmission to definitive hosts via consumption of paratenic/transport hosts. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Minneso (Brackett, 1938). Nematodes were found in the subcutaneous tissue, serous membranes, and body cavity of infected snakes, and during summer months, gravid females were present in visible subcutaneous swellings (Brackett, 1938; Moravec, 2006). Infections were only detected during the summer months, with signs of infection disappearing by fall or early winter (Brackett, 1938). The arrangement of genital papillae and length of spicules in males are the most reliable characteristics used to differentiate *D. ophidensis* from other *Dracunculus* species. Spatial variation has been noted in *D. ophidensis* prevalence with a higher prevalence found in Minnesota compared with Michigan (Brackett, 1938).
The highest diversity of squamate Dracunculus spp. have been reported from Eurasia, including D. oesophageus (Desportes, 1938), from Europe and D. coluberensis, D. ali (Deshmukh, 1969), and D. houdemeri (Hsü, 1933) from Asia (Table 1). Many of these remain poorly described and studied, with the latter three having only been described based on individuals of one sex. Furthermore, from the checkered keelback snake (Xenochrophis (=Natrix) piscator), D. ali was described from only male nematodes in India and D. houdemeri was described from only subcutaneous female nematodes in Vietnam (Hsü, 1933; Deshmukh, 1969). No other life history traits are known, and additional morphological and molecular work is needed to better define the validity of these two species from keelback snakes.

The best studied of this group is D. oesophageus which was described from colubrid snakes (Moravec, 2006). A male specimen of D. oesophageus originally described as Filaria oesophagea, was detected in the esophagus of a viperine snake Natrix maura (=N. vipersin), thus the specific epithet. Based on morphologic analysis of parasites detected in the grass snake (Natrix natrix persa) the parasite was transferred to the genus Dracunculus and renamed D. oesophageus (Desportes, 1938; Deshmukh, 1970). Parasites from N. natrix described as Pesteria inglesi by Tadros (1966) are presumed to be D. oesophageus. The copepod C. fuscus is a confirmed experimental intermediate host (Desportes, 1938). The tails of the L3 have the typical tricuspid tail similar to D. medinensis. This species is also the only squamate Dracunculus spp. for which there is any genetic data available; phylogenetic analysis of ~1,600bp of the 18S rRNA gene from two specimens from the mesenteries of N. natrix from Slovakia indicated that this parasite was related to D. medinensis and the fish parasite Philometra obturans which is in the same order as Dracunculus (Wojová et al., 2005).

An unidentified female Dracunculus species was reported emerging from the head of a cobra (Naja tripudians) that was held in captivity. Recovered larvae were used to infect copepods and underwent growth (from 300 µm to 600 µm). Subsequent inoculation of snakes with the unidentified Dracunculus species failed, possibly due to the use of copepods that had only been infected for six days and larvae that had not yet molted to the infectious L3 stage (Turkud, 1920).

The only report of Dracunculus in a lizard species was by Mirza and Basir (1937), but this report is suspect. In that study, 60% of monitor lizards (Varanus sp.) in India were infected in the body cavity and in subcutaneous tissues with up to 15 worms. However, identification was based only on one damaged female worm measuring 68 cm long. Although originally identified as D. medinensis, no additional morphologic or molecular work was conducted so it is possible that this parasite is one of the commonly reported filarial worms (e.g., Oswaldofilaria, Hастospiculun) in Varanus spp. (Bolette, 1998; Rataj et al., 2011). For example, in 2017 several large subcutaneous nematodes were collected from V. niloticus in Chad, Africa and were identified as filarial worms (Onchocercidae) based on molecular analysis of the cytochrome c oxidase I (COI) (Cleveland and Yabsley, unpublished data).

The only species from Oceania is D. mulbus, which was originally described from nematodes collected from the body cavity of the water python (Liasis fuscus) in northern Australia (Table 1) (Jones and Mulder, 2007). This parasite was found in 22% of pythons examined and a maximum of 14 individual nematodes were detected; males were collected from around the heart, lungs, and liver and females from the mesenteries. This parasite has also been reported from the Papuan olive python (Apodora papuana) from Papua New Guinea (Moravec and Gibson, 2007).

There are two described Dracunculus species from Africa (Table 1). Dracunculus doi (Chabaud, 1960) was originally described using a male specimen from the Madagascar ground boa (Acroantophis madagascariensis) (Chabaud, 1960). Females of this parasite were subsequently detected in a captive Madagascar tree boa (Sanastria madagascariensis) in a Paris Zoo (Vaucher and Bain, 1973). Larvae from the tree boa were...
These infective larvae were given to a ball python (Python regius), which developed an infection with two male nematodes (Vaucher and Bain, C.A. Cleveland et al. IJP: Parasites and Wildlife 7 (2018) 289–300).

Dracunculus dahomensis (Neumann, 1895) Moorthy, 1937 was originally described from the lymphoid tissues of a captive African rock python (Python sebae) from Benin (then Dahomey) (Neumann, 1895). Males of this species were redescribed by Moorthy (1937). No other life history traits are known for this parasite (Moravec, 2006).

2.2. Intermediate and paratenic hosts of squamate Dracunculus spp.

As in experimental infection trials with other Dracunculus species, copepods have been used as experimental intermediate hosts (Brackett, 1938; Muller, 1971) (Table 1). Emergence of adult female nematodes from snake hosts has rarely been reported; however, adult females are present in subcutaneous masses and Brackett reported that gravid D. ophidensis responded to water in the same manner as D. medinensis. Therefore, it is likely that transmission to copepods is similar to the mammalian Dracunculus spp. (Brackett, 1938). Experimentally, an undescribed Dracunculus sp. detected in boas from Trinidad developed in C. vernalis to the infective third stage larvae in 12–14 days at 25 °C and D. doi developed to L3 in Cyclops strenuus in 13 days at 22–25 °C, thus development appears to be similar to that of mammalian Dracunculus spp. (Muller, 1971; Vaucher and Bain, 1973).

Experimental studies show that paratenic amphibian hosts may be involved in the life cycle of some squamate Dracunculus species. Copepods infected with D. ophidensis were readily ingested by tadpoles of an unreported frog species and larvae were noted in the tadpole body cavity (Brackett, 1938). Feeding of these tadpoles to two garter snakes and a water snake (N. sipedon) resulted in infection of one snake of each species (Brackett, 1938). However, there have been no reports of natural infections in aquatic paratenic hosts.

3. Dracunculus species in chelonians

To date, D. globocephalus is the only species reported from chelonians. The only two known hosts are the common snapping turtle (Chelydra serpentina) in the United States and the South American snapping turtle (Chelydra acutirostris) in Costa Rica. However, the report from Costa Rica was based on morphology of a single worm of unknown sex (Bursey and Brooks, 2011).

Dracunculus globocephalus was first described by Macklin (1927) from the mesenteries and body cavity of common snapping turtles from Oklahoma and Illinois, USA. Since its first description, D. globocephalus has been re-described by two groups and detected in numerous states (Table 1) (Williams, 1953; Gatschet and Schmidt, 1974; Moravec and Little, 2004). Morphologic features that can be used to distinguish D. globocephalus from other Dracunculus spp. are the presence of markedly uneven spicules (0.8 mm and 0.2 mm in length), the absence of a gubernaculum, and the placement of male caudal papillae (Moravec and Little, 2004).

4. Dracunculus species in mammals

4.1. Dracunculus insignis

Dracunculus insignis was initially described as Filaria insignis by Leidy in 1858 from a female nematode in the foot of a raccoon (Procyon lotor) from Pennsylvania, USA (Chandler, 1942a). Morphologically similar parasites detected in raccoons from Texas were noted to be similar to Dracunculus fuelleborni from a big-eared opossum (Didelphis aurita) in South America, based on the size and cephalic structures of the female nematodes. At that time, the morphologic characteristics of males, which are needed for definitive identification, were not available (Travassos, 1934; Chandler, 1942b). A male specimen of D. insignis was first detected in a raccoon in Dorchester County, Maryland (Chitwood, 1950) and was noted to be morphologically like D. medinensis but was differentiated based on the number and arrangement of genital papillae and length of gubernaculum (Chandler, 1942a; Chitwood, 1950). Although this review focuses on wildlife hosts, D. insignis also infects domestic dogs and domestic cats in the United States and Canada. A recent review was published (Williams et al., 2018).

4.1.1. Intermediate hosts for Dracunculus insignis

To date, there have been no reports of wild D. insignis-infected copepods; however, the intermediate host range appears to be large as both Acanthocyclops vernalis and Cyclops bicuspidatus from North America as well as Cryptocyclops littiniectus and Mesoecyclops equatorium similis from Cameroon, Africa were experimentally susceptible (Sullivan et al., 1991). Susceptibility can vary as Mesoecyclops leuckarti leuckarti from Pakistan and Thermocyclops emini from Cameroon were partially refractory to infection (Sullivan et al., 1991). Dracunculus insignis larvae develop to L3 in 21–25 days at 24 °C (Crichton and Beverley-Burton, 1975). As noted earlier, development is regulated by water temperature and infected copepods kept at 8 °C and 15 °C showed no development 60 days post infection (DPI) (Crichton and Beverley-Burton, 1975).

4.1.2. Natural infections of D. insignis in wildlife

4.1.2.1. Infections in wild raccoons. Raccoons are the most common host for D. insignis (Cheatam and Cook, 1948; Long, 2003). Although the classic transmission route for D. medinensis is the ingestion of copepods infected with L3 in drinking water, it has been suggested that this is unlikely to be the primary route for D. insignis among raccoons (Muller, 1971; Crichton and Beverley-Burton, 1977). Instead, transmission via consumption of an amphibian paratenic host or fish transport host containing D. insignis L3 seems more likely, although this has not been evaluated beyond showing the capability of transmission (Fig. 1).

Most reports of D. insignis in raccoons have been based on detection of adult females which cannot be identified to species (Fig. 2); thus, these infections cannot be definitively said to have been with D. insignis. To date, however, no other Dracunculus sp. has been detected in raccoons (either by identification of males or genetic characterization) (Chitwood, 1950; Crichton and Beverley-Burton, 1975; Elsasser et al., 2009). Infections have been noted in raccoons in numerous states in the United States (primarily East of Texas/South Dakota line) and in Ontario province, Canada (Table 2). The prevalence of infections in raccoons from Ontario, Canada, where a good proportion of surveillance work has been conducted, was 69% (154/223) (Crichton and Beverley-Burton, 1974). In the southeastern United States at a site with endemic D. insignis transmission in raccoons, a prevalence of 36% (35/98) has been detected (Cleveland and Yabsley, unpublished data).

Several studies have noted a marked seasonality in the prevalence of infection and stage of development of D. insignis. The highest prevalence has been reported in the spring (April–June) at multiple locations throughout the United States (Texas, New Hampshire, Tennessee, and Kentucky) and Canada (Ontario). However, the lower prevalence noted in other seasons may be related to the lack of detection of males and immature females, as female worms in the fall are mostly in the subcutaneous tissues of the abdomen or thorax and are small and immature (Chandler, 1942a; Siegler, 1946; Crichton and Beverley-Burton, 1974; Crichton and Beverley-Burton, 1977; Ditters and Ryan, 1980; Smith et al., 1985). A study of D. insignis in raccoons at a site in the southeastern United States with endemic transmission found that naturally-infected individuals sampled in February–March had gravid females subcutaneously or had post emergence scarification, indicating a late winter to early spring emergence (Cleveland and Yabsley, unpublished data).
4.1.2.2. Infections in other wild species. In addition to raccoons, infections with parasites presumed to be *D. insigne* have been reported in multiple wild carnivore species (e.g., skunks (*Mephitis* spp.), coyotes (*Canis latrans*), foxes (*Vulpes* spp.), Virginia opossums (*Didelphis virginiana*), and rarely rodents (i.e., muskrat (*Ondatra zibethicus*) and North American beaver (*Castor canadensis*)) (Table 2). Except for a study in Ontario Canada, none of the worms detected in these other wild species have been confirmed to species by examination of males or through molecular confirmation. In Ontario, fisher (*Martes pennanti*), mink (*Neovison vison*), and North American river otter (*Lontra canadensis*) have been confirmed as hosts for *D. insigne* through molecular characterization (Elsasser et al., 2009). This lack of species confirmation may be important for species that are known to harbor more than one *Dracunculus* sp. (e.g., river otters), are hosts for other subcutaneous filarial worms (e.g., *Filaria taxidæ* in mustelids and raccoons), or unusual hosts (e.g., rodents, none of which were infected with mature larvigerous females so may be dead-end hosts) (Gibson and McKiel, 1972; McKown et al., 1995).

4.2. Experimental infections of hosts with *D. insigne*

Several species, including raccoons, rhesus macaques (*Macaca mulatta*), mink, and domestic ferrets (*Mustela putorius furo*), have been experimentally-infected with *D. insigne* (Beverly-Burton and Crichton, 1976). Regardless of the inoculation route or dose, not all individuals of host species developed patent infections in these studies. Furthermore, a relatively low percentage of L3 used to expose definitive hosts developed into adult worms. Exposure of two domestic dogs (*Canis lupus familiaris*) and a single marten (*Martes americana*) to *D. insigne*-infected *Cyclops vernalis* (n = 250, 210, and 220 respectively) did not result in infection; however, natural infections have been reported in both hosts (Beverly-Burton and Crichton, 1976).

4.2.1. Raccoons

Because raccoons are considered to be the primary wildlife host for *D. insigne*, experimental trials have been conducted to document the transmission, pathologic lesions associated with infection, and possible changes in behavior of infected raccoons (Miller et al., 1946; Cheatum and Cook, 1948; Wilson, 1958; Crichton and Beverly-Burton, 1977).

In one study, captive bred raccoons were inoculated with L3 recovered from digested copepods (Crichton and Beverly-Burton, 1975). Raccoons were euthanized at various time points to examine parasite migration. Overall, 30 of 33 (91%) raccoons became infected and only 4.3% of 9,320 larvae administered to the raccoons were recovered with the average number recovered being 12.3 worms/raccoon. At 7 h post inoculation, 2.3% of inoculated larvae were detected within the duodenum and stomach (a similar percent recovery was noted at 19 h) but of the 16 larvae recovered, 1 was from the duodenum whereas the other 5 worms were located in the abdominal cavity. At 4 days post inoculation, all worms were recovered from the abdominal cavity while some worms were detected in diaphragm and intercostal muscles on day 5. By days 6 and 7, worms were detected in the subcutaneous tissues of the thorax and abdomen. At 19 days the single larvae detected was a L4 and was 1,220 mm long with a blunt tail. By 34 DPI, females were 4.5–10.3 mm long and males were 7.6–8.4 mm long and had developed spicules and a gubernaculum.

By 60 days, the fourth stage cuticle was nearly shed, tails had 10 small conical projections, and the intestine had become dark brown. Males had already shed their L4 cuticle and the seminal vesicle contained sperm. By 77 days, females were 87–125 mm long and mated (vaginal plug present). The intestine in males had begun to atrophy and appeared completely atrophied by day 120. By 90 days females contained ova which had developed to L1 by 120 days. By 270 days, females were 200–310 mm in length. From 300 to 365 days females began to create lesions into which the uterus would sometimes rupture releasing larvae into the lesion (Fig. 2). Females died after larvae were released and some were resorbed by the host, while others became calcified. Males were also found, indicating that males could likely persist between transmission seasons. At 480 days, a small (95 mm) immature female was found in the subcutaneous tissue of the trunk providing evidence that not all female worms mature and become gravid and these unfertilized females likely do not migrate to the extremities.

Experimental studies have also been conducted to examine the ability of frogs to transmit infection to raccoons. Larvae of *D. insigne* recovered from experimentally infected paratenic hosts (*Lithobates pipiens* and *L. clamitans*) were fed to a captive-born raccoon. Upon necropsy, the infected raccoon had larvigerous female *D. insigne* present in all legs and lesions from female worms preparing for larval release (Crichton and Beverley-Burton, 1977). Localized edema, inflammation, and thickened cells typically surrounded ulcer formation in the host. Upon death of worms subcutaneously, the affected extremities bore small superficial scars (Crichton and Beverley-Burton, 1977). Bouts of inactivity (30–60 min) and distress, difficulty moving, and favoring a leg were all observed in the infected individual.

4.2.2. Mink

In the same study in which raccoons were inoculated with *D. insigne*, Crichton and Beverley-Burton (1975) similarly exposed captive-bred mink. Compared with the raccoons, a lower percentage of exposed mink became infected (18/31, 58%), the number of larvae recovered was lower (53/4895 larvae, 1.1%) and the worm burdens were lower (2.9 worms/infected mink). In addition, development of most worms was slower and fewer worms matured and became larvigerous. A male was detected in a mink euthanized at day 365. Collectively, these data show that mink can be definitive hosts for *D. insigne* but fewer ingested larvae develop to larvigerous females.

4.2.3. Rhesus macaques

A Rhesus macaque was experimentally exposed to 400 L3 of *D. insigne* via a stomach tube (Beverly-Burton and Crichton, 1976). The primate was necropsied at 180 DPI and nine gravid female nematodes from the subcutaneous tissues and a single male nematode from the connective tissue were recovered (Beverly-Burton and Crichton, 1976). This finding in a non-human primate raises the possibility that *D. insigne* has the potential to be zoonotic; however, no infections in humans have ever been reported.

4.2.4. Domestic ferret

Because raccoons, mink, and rhesus macaques are not amenable to laboratory studies on *Dracunculus* due to husbandry costs and handling difficulty, domestic ferrets were evaluated as a suitable animal model (Eberhard et al., 1988; Brandt and Eberhard, 1990, 1991; Broderson et al., 1991). Collectively, these studies show that domestic ferrets are appropriate definitive hosts for both *D. insigne* and *D. medinensis* (Eberhard et al., 1988, 2016; Brandt and Eberhard, 1990).

Initially, ferrets were exposed to *D. insigne*-infected copepods to evaluate susceptibility (Eberhard et al., 1988). Ten of the 18 (56%) ferrets developed infections with 44 worms (Eberhard et al., 1988). Gravid females were recovered as early as 128 DPI and by 190 DPI, 93% (14/15) of female worms contained larvae. Most (87%, 13/15) worms were recovered from the legs (Eberhard et al., 1988). Examination of various inoculation routes showed that intraepithelial (IP) inoculation resulted in the highest recovery rate of adult nematodes compared to gavage and subcutaneous inoculation routes (Brandt and Eberhard, 1990). Therefore, another study was conducted to examine the IP route of transmission using a low number (n = 10) of *D. insigne* L3 (Brandt and Eberhard, 1991). Of 10 ferrets exposed, one died of unrelated causes early in the study, and 67% (6/9) became infected. Recovery rate increased from 6.5% to 21% compared to the Eberhard et al. (1988) study. Although the IP route of exposure does not mimic natural infections, this work further establishes that a small number of
D. insignis larvae are sufficient to establish infection in definitive hosts.

Using the ferret model system, Eberhard et al. (1990) investigated possible chemophylactic treatment on D. insignis infections with the goal of informing the eradication efforts of D. medinensis in Africa. Five compounds (diethylcarbamazine (DEC), albendazole (ALBZ), ivermectin (IVER), metrifonate (INN), and amocarzine (CGP 61400)) were evaluated on ferrets exposed to 100 infected copepods. Ferrets were treated at 60 and 90 DPI. Necropsies were performed between 7 and 11 months post-infection and no significant difference of worm recovery rates was found between treatment and control groups. To date, there has been no effective anthelmintic preventative or treatment identified for use on Dracunculus species.

4.3. Paratenic hosts of D. insignis

Until recently, no natural infections of a paratenic host for D. insignis had been identified despite data from several experimental studies suggesting that amphibians, and possibly fish, may serve as paratenic hosts (Fig. 1) (Eberhard et al., 2016a,b; Cleveland et al., 2017). Interestingly, the first evidence suggesting that amphibians can serve as paratenic hosts came from an experimental study with D. ophidiensis—one of the more poorly studied Dracunculus species (Brackett, 1938). Several potential paratenic hosts have been experimentally evaluated for D. insignis including several species of crayfish, fish, and amphibians (Crichton and Beverley-Burton, 1977; Eberhard and Brandt, 1995; Eberhard et al., 2016a,b; Cleveland et al., 2017).

4.3.1. Fish

Exposure trials with various species of fish suggest they are generally refractory to infection, but some species may act as paratenic or transport hosts (Cleveland et al., 2017). In one study, of the seven species of fish (white suckers (Catostomus commersonii) rainbow trout (Oncorhynchus mykiss) common shiner (Luxilus cornutus) brown bullhead (Ameiurus nebulosus) bluntnose minnow (Pimephales notatus), stonecat catfish (Noturus flavus) and brindled madtom catfish (N. miurus)) exposed to 10–200 D. insignis L3s, 40% (2/5) white suckers and 33% (1/3) rainbow trout had 1–2 larvae recovered 6–11 DPI; however, sample sizes were generally low (Crichton and Beverley-Burton, 1977). Similarly, the single study that exposed eight Northern Clearwater crayfish (Orconectes propinquus) with 10–20 D. insignis L3s failed to establish any infection (Crichton and Beverley-Burton, 1977). These data contrast sharply with adult amphibians (Lithobates spp. and African clawed frogs (Xenopus spp.)) which generally have high infection rates and 45–90% recovery rate of D. insignis larvae. Regardless, these data suggest a need for further investigation into the role of certain fish species to serve as paratenic hosts.

Recently, work has been conducted to investigate the possibility that fish may serve as a short-term transport host in the transmission of Dracunculus spp. (Cleveland et al., 2017). Three species of fish were allowed to feed on D. insignis- and D. medinensis-infected copepods; Mosquitofish (Gambusia affinis) Fathead minnows (Pimephales promelas) and Nile tilapia (Tilapia nilotica). The fish were euthanized and fed to domestic ferrets within 3 hours of ingesting copepods. Three ferrets were given D. insignis-fed fish and one ferret was given D. medinensis-fed fish. Two of the D. insignis ferrets became infected, as did the single D. medinensis-exposed ferret. The results of this study were important to the three remaining endemic countries where D. medinensis is still transmitted as The Carter Center Guinea Worm Eradication Program has suggested fish entrails be buried or burned to prevent their ingestion by dogs (Eberhard et al., 2014). These results indicate yet another potential route of transmission for Dracunculus species.

4.3.2. Amphibians

Several studies have shown that tadpoles and adult frogs of numerous species are experimentally susceptible to D. insignis, including initial work that was conducted via oral inoculation on Northern leopard frog (Lithobates pipiens) and Green frog (Lithobates clamitans) tadpoles and adults (Crichton and Beverley-Burton, 1977). The low recovery rate of D. insignis larvae from tadpoles (0–7%) was attributed to difficulty in orally inoculating small tadpoles. A subsequent study which allowed African clawed frogs (X. laevis) and American bullfrogs (L. catesbeianus) tadpoles to ingest D. insignis-infected copepods from small dishes of water, a more natural route of infection, obtained similar results (Eberhard and Brandt, 1995).

To investigate the ability of larvae to survive and persist through metamorphosis, Xenopus spp. tadpoles were infected with D. insignis and then held for 2–4 weeks at which time larvae were recovered from tissues of adults (Eberhard and Brandt, 1995). Similarly, examination of experimentally-infected adult X. laevis at regular time periods indicated live D. insignis could be recovered up to eight months post infection (Cleveland and Yabsley, unpublished). In contrast to tadpoles, oral inoculation of adult frogs resulted in a larval recovery rate of 45–90%, further supporting the role of amphibians as potential hosts of D. insignis (Eberhard and Brandt, 1995). One study also noted that L3s recovered from L. ppiens were longer (570–698 μm, mean 629 μm) than L3s recovered from copepods (434–605 μm, mean 554 μm); however, this finding has not been further investigated (Crichton and Beverley-Burton, 1977).

Larvae recovered from tadpoles were capable of developing in two species of vertebrate definitive hosts; raccoons and ferrets. Larvae (n = 250 L3s) from orally-inoculated Lithobates spp. were given to a captive-reared raccoon, which harbored infection with 13 male and 27 female mature D. insignis 167 DPI (Crichton and Beverley-Burton, 1977). Similarly, one of two ferrets fed tadpoles exposed to D. insignis-infected copepods became infected with one male worm after seven months (Eberhard and Brandt, 1995).

Collectively, these data indicate amphibians can act as experimental hosts for D. insignis and that infection can be maintained through metamorphosis and for at least eight months post infection. However, critical data on the potential role of amphibians in the natural cycle of Dracunculus are generally lacking. Despite the experimental findings to support their role, the occurrence of natural infections is likely rare and thus difficult to detect. However, surveillance has been limited and further study is needed to identify sylvatic transmission of Dracunculus. In one study in Ontario, Canada, 45 wild-caught L. ppiens and L. climitans were negative (Crichton and Beverley-Burton, 1977). However, a natural infection was recently detected in one of eight southern leopard frogs (L. sphenoecephalus) from central Georgia/upper coastal plains Georgia where the prevalence of D. insignis in raccoons 36% (Cleveland and Yabsley, unpublished data). Additionally, two amphibians of 240 surveyed from Chad, Africa were detected harboring D. medinensis L3s (Eberhard et al., 2016a,b; Cleveland and Yabsley, unpublished data). These findings support the role of amphibians as paratenic hosts for Dracunculus and will hopefully stimulate further research.

4.4. Dracunculus lutrae

Dracunculus lutrae was first described from specimens collected from otters from Ontario, Canada (Table 2) (Crichton and Beverley-Burton, 1973). A molecular study conducted on Dracunculus from various wildlife species in Ontario, Canada revealed that 18 otters were infected with D. lutrae but two other otters were infected with D. insignis (Elssasser et al., 2009). Recently, 184 river otters (Lontra canadensis) from Arkansas were examined for the presence of Dracunculus species (Timlison and Surf, 2018). Twelve otters were found to have cysts on the distal extremities consistent with Dracunculus infections. An individual nematode from each otter was genetically characterized and all were D. insignis. These data indicate that the distribution of D. lutrae and D. insignis in otters may be constrained by a latitudinal gradient, with D. lutrae occurring above 45° N and D. insignis occurring below 45° N. Few studies have assessed the prevalence and distribution of D. lutrae in otters, but unidentified Dracunculus spp. or molecularly unconfirmed
Being extracted from de characteristics on female worms and the rare occurrence of male specimens (Moravec, 2006; Wijová et al., 2005). This is problematic for the Dracunculus superfamily Dracunculoidea has at least 166 recognized species, with including the Dracunculoidea, that are underrepresented (Gardner, 2001; Bimi et al., 2005; Wijová et al., 2005; Elsasser et al., 2009). The there have been no con assertion as opossums from Georgia (USA) were infected with D. fuelleborni has yet to be documented. Despite erroneous older reports that assumed worms detected in opossums were D. fuelleborni, it is not known to occur in North America. Our recent genetic data supports this assertion as opossums from Georgia (USA) were infected with D. insignis (Alexander et al., 1972; Cleveland unpublished data). Thus, to date, there have been no confirmed reports of D. fuelleborni since its initial description.

4.5. Dracunculus fuelleborni

Dracunculus fuelleborni was first reported from the subcutaneous connective tissue of a big-eared opossum from Rio, Brazil (Travassos, 1934). Both male and female nematodes were obtained and, morphologically, the female resembled D. insignis, yet was nearly twice the length of recorded D. insignis specimens (Table 1). Morphometrics of spicules and gubernaculum of males confirmed D. fuelleborni was a distinct species. However, this parasite has not been genetically characterized so the relationship between D. fuelleborni and other Dra- cunculus spp. has yet to be documented. Despite erroneous older reports which had 0.33% (n = 23 worms).

5. Molecular epidemiology of Dracunculus spp.

Molecular characterization has allowed for the investigation of the phylogenetic relationships of many parasites, including nematodes. However, among the parasitic representatives there are some groups, including the Dracunculoidea, that are underrepresented (Gardner, 2001; Bimi et al., 2005; Wijová et al., 2005; Elsasser et al., 2009). The superfamilly Dracunculoidea has at least 166 recognized species, with most species having only morphologic descriptions (i.e., no gene sequence data) and many only having been reported once (see section 3) (Moravec, 2006; Wijová et al., 2005). This is problematic for the Dra- cunculoidea because of the limited distinguishing morphologic characteristics on female worms and the rare occurrence of male specimens being extracted from definitive hosts.

The first study to investigate the phylogenetic relationships of Dracunculus spp. was by Wijová et al. (2005) which included the 18S rRNA sequences from D. medinensis, D. oesophagus (from Natrix natrix) and Philometra obturans, a related dracunculid parasite from fish. These three parasites formed a clade within the Spirurida. A subsequent study, also using 18S rRNA gene sequences, included D. insignis as well as many other members of the Dracunculoidea including Philometra ob- turans, Philometra ovata, Philomena oncorhynchi, Skrjabillamus scardinii, and Molnaria intestinalis from fish, and Micropleurania australiensis from the Australian freshwater crocodile (Crocodylus johnsoni) (Wijová et al., 2006). The three Dracunculus spp. formed a clade with Philometra spp. as a sister clade. The 18S rRNA gene sequence has also been obtained for a specimen of D. lutrae from an otter from Ontario, Canada and it was identical to D. insignis. Additional 18S rRNA sequences of D. lutrae from the southeastern US are also identical to D. insignis sequences even though these two species are morphologically distinct and were con- ferred to be different species based on analysis of the cytochrome c oxidase I (COI) gene (Elsasser et al., 2009; Laetsch et al., 2012; Cle- veland and Yabsley, unpublished).

These initial studies focused on the 18S rRNA gene which, although useful in examining relationships among large groups of nematodes, could not distinguish some species of Dracunculus. Thus, this gene target is not useful for investigating intraspecific variation. An increasingly common target for ‘barcoding’ is the COI gene, and Elsasser et al. (2009) used this target to study D. insignis and D. lutrae samples from several hosts (fishers, mink, raccoon and otter) from Ontario, Canada. Sequences were obtained for 82 of the 92 worms investigated. The two species were easily distinguished and it was shown that D. insignis was a host-generalist and was detected in all samples species, including otters while D. lutrae was specific to otters (Elsasser et al., 2009). In addition, D. insignis had minimal intraspecific variability (0.02% (n = 59 worms), especially compared to D. lutrae which had 0.33% (n = 23 worms).

6. Conclusions

The human Guinea worm, D. medinensis, has afflicted people throughout Africa and parts of Asia for centuries, causing significant morbidity among those infected. Infection results in significant educa- tional and personal losses and has recently been the subject of a highly successful eradication campaign that has reduced the number of en- demic countries and the number of annual cases (Molyneux and Sankara, 2017). Yet, despite vast amounts of history and knowledge, this parasite has now been described as having a ‘peculiar epide- miology’ with infections in domestic dogs becoming more prevalent than at any time in the past. This suggests that there has been a change in transmission mode (Eberhard et al., 2014) and has led to several field and laboratory-based studies that have confirmed the ability of this parasite to utilize amphibian paratenic hosts and the recognition that fish may serve as either paratenic or transport hosts (Eberhard et al., 2016a; b; Cleveland et al., 2017). As noted in this review, several members of the genus were known to infect amphibians, so this new finding for D. medinensis is not surprising and many questions remain regarding the importance of amphibians in natural transmission or maintenance of the parasite in the environment (Fig. 1). Although parasites should be studied for their intrinsic value, the emerging concern surrounding D. medinensis combined with the discovery of a possible ‘novel’ transmission pathway (that was highly suggested from past studies with D. insignis and other species) should highlight how research on parasites of non-medical importance can be informative to related parasites and should be encouraged.

Because this group of parasites is often overlooked due to the limited pathological importance to their definitive hosts, there are many basic evolutionary and ecological questions outstanding. This lack of knowledge is especially highlighted by the fact that the historical re- cords of many Dracunculus species are still based on morphological identification, typically of females, which is not accurate to distinguish most species. Molecular phylogenetics may hold promise in addressing questions on species diversity and host specificity, but is limited be- cause of lack of available samples preserved appropriately for mole- cular work and the need for extensive field studies to collect new parasite specimens. The application of molecular analyses is especially important among the reptilian species of Dracunculus which, given their diversity compared to mammalian-infecting Dracunculus species sug- gests that they may represent the ancestral host of the genus. The breadth of wildlife definitive hosts parasitized by the numerous dra- cunculids discussed herein, along with the potential role of paratenic hosts in transmission of these nematodes, exemplify unique complex life-cycles highlighting both host-specialist and host generalist nema- todes. Considering these intriguing dynamics of transmission and host-parasite relationships, large gaps in basic parasitological knowledge still exist, and further research is needed.

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