



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

Christopher A. Cleveland^{a,b,*}, Kayla B. Garrett^{a,b}, Rebecca A. Cozad^b, Brianna M. Williams^{a,b}, Maureen H. Murray^{b,1}, Michael J. Yabsley^{a,b}

^a Southeastern Cooperative Wildlife Disease Study, Department of Population Health, College of Veterinary Medicine, Wildlife Health Building, 589 D.W. Brooks Dr, Athens, 30602, GA, USA

^b Warnell School of Forestry and Natural Resources, University of Georgia, Athens, 30602, GA, USA

ARTICLE INFO

Keywords:

Dracunculus spp
Wildlife
Definitive host
Paratenic host
Intermediate host

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 fill with large numbers of first-stage larvae that are released when females come into contact with water. The Family Dracunculidae contains two genera: *Dracunculus*, parasites of mammals and reptiles, and *Avioserpens*, 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 exists 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 in several remaining endemic countries in Africa 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.

* Corresponding author. Southeastern Cooperative Wildlife Disease Study, University of Georgia, Wildlife Health building 1082, Athens, 30602, GA, USA.
E-mail address: cleve@uga.edu (C.A. Cleveland).

¹ Current address: Davee Center for Epidemiology and Endocrinology, Urban Wildlife Institute, Lincoln Park Zoo, Chicago, IL 60614.

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. braziliensis*), 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 hosts (Crichton and Beverley-Burton, 1977; Brackett, 1938). When the host places the affected area in water the blister will erupt and the female release nearly 500, 000 first stage larvae (L1) into the water column (Muller, 1971). The release of larvae can occur multiple times during different events.

Cyclopoid copepods are a required intermediate host in the life cycle of *Dracunculus* species, although few naturally or experimentally competent species have been identified (Table 1). The intermediate host range appears broad as Old World copepod species can become infected with *D. insignis* and New World copepod species can become infected with *D. medinensis* (Sullivan et al., 1991). However, there was differential mortality among different copepods species following infection with *D. medinensis* suggesting some copepod species are better intermediate hosts than others (Bimi et al., 2005). Once L1s are consumed by copepods they develop to the infective third stage larvae (L3) (Fedchenko, 1871; Crichton and Beverley-Burton, 1975). *Dracunculus medinensis* and *D. insignis* L1 molt to L3 within 21–25 days at 24 °C (Fedchenko, 1871; Crichton and Beverley-Burton, 1975).

Definitive hosts can become infected by ingesting L3s within copepods when drinking water or, for some species, consumption of frog paratenic hosts or fish transport hosts (Fig. 1) (Brackett, 1938; Crichton

and Beverley-Burton, 1977; Eberhard and Brandt, 1995; Anderson, 2000; Eberhard et al., 2016a,b; Cleveland et al., 2017). Once ingested, L3s migrate to subcutaneous and intramuscular connective tissues of the thoracic and abdominal musculature where they undergo an additional 2 molts. Male and female nematodes fully mature to adults after 60–70 days, with fertilization occurring after maturation (Anderson, 2000). Males and unfertilized females remain in subcutaneous musculature of the abdomen and thorax, while fertilized females begin to migrate through the subcutaneous tissues, primarily to the distal extremities. Once fully gravid and larvigerous, females create swelling and an ulcerative-type lesion. This lesion occurs when the female releases uterine fluid (which may contain a few larvae), initiating an immune response from the definitive host and leading to a “thinning” of epidermis through which she can emerge (Muller, 1976; Cairncross et al., 2002).

When these lesions are exposed to water, larvae are expelled from the female into the environment and consumed by the intermediate cyclopoid 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 Eberhard, 1990).

1.4. Diagnostic features

Dracunculus spp. adults are large filiform pale yellow-white nematodes that have marked sexual dimorphism with females being considerably larger than males (Table 1). Both females and males have an atrophied intestine and females have atrophied vulva and vagina. Gravid female worms are almost completely occupied by a uterus distended with L1. The anterior end is rounded, the buccal cavity is reduced, the mouth opening is round and surrounded by variable numbers of papillae, and a thick and clearly defined peribuccal ring is present. Anterior constriction just anterior to the nerve ring may be present. The esophagus is very long with a short muscular portion and a long glandular portion. Tails are generally conical with a sharply pointed tip. Males have either equal or subequal spicules and most species have a gubernaculum (Brackett, 1938; Chandler, 1942a; Crichton and Beverley-Burton, 1975; Cairncross et al., 2002; Moravec 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., 0.3–0.9 mm), but there is considerable overlap in ranges for many species (Fig. 2). When available, length of L1s for valid *Dracunculus* spp. is provided in Table 1. When larvae develop to the infectious L3 stage in copepods, they lose the long tapering tails, become shorter and broader (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. houdemeri*, *D. doi*, *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 1
Overview of valid *Dracunculus* species from wild animals, including *D. medinensis* for comparison.^a

Host group	Parasite	Geographic region	Known DH	Known IH	Known PH	Adult length (♂; ♀)	Left and right spicule length	Gubernaculum length	Caudal alae	L1 length
Mammals	<i>D. medinensis</i>	Historically Africa and Asia	Humans, domestic dogs, domestic ferret (E) ^b	Numerous copepod species	Various amphibians (N,E)	17–22; 200–280 (♂) and 14.2–30.1; 192–275 (Crichton and Beverley-Burton, 1973)	0.42 (0.4–0.52), 0.44 (0.41–0.52)	0.12	Absent	0.581–0.643
	<i>D. insignis</i>	United States, Canada	Raccoons (<i>Procyon lotor</i>), mink (<i>Mustela vison</i>), fisher, (<i>Martes pennanti</i>), Virginia opossum (<i>Didelphis virginiana</i>), North American river otter (<i>Lontra canadensis</i>), domestic dog, domestic cat, domestic ferret (E)	<i>Acanthocyclops vernalis</i> (E) and <i>C. bicuspidatus</i> (E)	Various amphibians (N,E)		0.46–0.495 each	0.114–0.13	NK ^c	0.664 (0.596–0.857)
	<i>D. lutrae</i>	United States, Canada	North American river otter (<i>Lontra canadensis</i>)	NK	NK	36 (32.2–40); 247 (200–290)	0.61 (0.51–0.68), 0.64 (0.59–0.72)	0.17 (0.16–0.18)	NK	0.665 (0.608–0.722)
Reptiles	<i>D. fuelleborni</i>	Brazil	Big-eared opossum (<i>Didelphis aurita</i>)	NK	NK	27–29; 465–490	0.38–0.42 each	0.088–0.1	NK	0.3–0.429
	<i>D. ophidensis</i>	United States	Common garter snake (<i>Thamnophis sirtalis</i>)	<i>C. viridis</i> (E)	Tadpoles (species not given)	Up to 16; up to 250	0.523; 0.554	0.09	NK	0.43–0.45
	<i>D. brasiliensis</i>	Brazil	Green Anaconda (<i>Eunectes murinus</i>), Brown banded water snake (<i>Helicopsis angulatus</i>)	NK	NK	NK; 130–220 mm	NK	NK	NK	0.396–0.429
	<i>D. colubereensis</i> ^{d,e}	India	Trinket snake (<i>Coelognathus</i> (= <i>Coluber helena</i>))	NK	NK	197.5; NK	0.08; 0.07	“small” one present	Absent	NK
	<i>D. alii</i> ^f	India	Checkered keelback snakes (<i>Xenochrophis</i> (= <i>Natrix piscator</i>))	NK	NK	13.09–24.4; NK	0.22–0.29; 0.23–0.3	0.05–0.07	Absent	NK
	<i>D. houdemeri</i>	Vietnam	Checkered keelback snakes	NK	NK	213	NK	NK	NK	0.33–0.363
	<i>D. doi</i>	Madagascar	Madagascar boa (<i>Acrantophis madagascariensis</i>)	NK	NK	28 mm; NK	Subequal, ~0.46	0.130	Present	0.37
	<i>D. dahomensis</i> ^{d,e}	Benin	African rock python (<i>Python sebae</i>)	NK	NK	48; NK	0.425; 0.4	NK	Absent	0.40–0.42
	<i>D. oesophagus</i>	Italy	colubrid snakes (<i>Natrix viperina</i> , <i>Natrix natrix persa</i>)	<i>C. fuscus</i> (E)	NK	17 (11.7–20); 360	0.297; 0.282	0.065	Absent	0.475–0.570
	<i>D. mulibus</i>	Australia and Papua New Guinea	Water python (<i>Liasis fuscus</i>), Papuan olive python (<i>Apodora papuana</i>)	NK	NK	23 (17–33); 240 (180–360)	0.4–0.48, 0.4–0.48	0.08–0.11	Present	0.34–0.40
Turtles	<i>D. globocapitatus</i>	United States, possibly Costa Rica	snapping turtle (<i>Chelydra serpentina</i>)	<i>C. bicuspidatus</i> (E)		16–21.7; 90–136	0.963–1.062; 0.186–0.213	Absent	NK	0.666–0.721

^a Measurements in mm unless otherwise specified.

^b N = naturally infected, E = experimentally infected.

^c NK = not known.

^d Measurement represents examination of single specimen.

^e Only based on morphology of males.

Table 2The geographic, anatomic location and prevalence of confirmed species of *Dracunculus insignis*, *D. lutrae*, and non-specified *Dracunculus* in North America.

Species	Host	Geographic location	Anatomic location	Prevalence (%)	Species confirmation method	Reference	
<i>D. insignis</i>	River Otter (<i>Lontra canadensis</i>)	Arkansas, USA	Subcutaneous and intermuscular fascia of carpal and tarsal areas	12/184 (6.5)	molecular	Tumilson and Surf, 2018	
	Raccoon (<i>Procyon lotor</i>)	Ontario, Canada	NA	NA	NA	molecular	Elsasser et al., 2009
		Ontario, Canada	Subcutaneous tissues in the inguinal area, thorax, abdomen and fascial layers of the lower legs	253/553 (45.75)	morphology via male specimens	Crichton and Beverly-Burton, 1974	
		Ontario, Canada	Subcutaneous tissues in the inguinal area, left and right axillary areas	1/1 (100)	morphology via male specimens	Gibson and McKiel, 1972	
		Maryland, USA	Subcutaneous fascia of legs	1/1 (100)	morphology via male specimens	Chitwood, 1950	
<i>D. lutrae</i>	River Otter	Ontario, Canada	NA	NA	molecular	Elsasser et al., 2009	
		Ontario, Canada	Subcutaneous tissues of thoracic, abdominal, inguinal areas and intermuscular fascia of legs	178/203 (87.7)	morphology via male specimens	Crichton and Beverly-Burton, 1974	
		Ontario, Canada	Connective tissue beneath latissimus dorsi, subcutaneous tissues of thoracic, abdominal, inguinal areas and intermuscular fascia of legs	NA	morphology via male specimens	Crichton and Beverly-Burton, 1973	
<i>Dracunculus</i> sp. ^a	Badger (<i>Taxidea taxus</i>)	Iowa, USA	Subcutaneous and intermuscular fascia of carpal and tarsal areas	2/24 (8.3)	NA	Wittrock and Ulmer, 1974	
	Beaver (<i>Castor canadensis</i>)	Kansas, USA	Connective tissue beneath latissimus dorsi	2/63 (3)	NA	McKown et al., 1995	
	Fischer (<i>Martes pennanti</i>)	New Hampshire, USA	Subcutaneous and intermuscular fascia of carpal and metatarsal areas	37/748 (4.9)	NA	Carlson and Vito, 1984	
	Marten (<i>Martes americana</i>)	Ontario, Canada	NA	1/405 (0.2)	NA	Seville and Addison, 1995	
	Mink (<i>Neovison vison</i>)	Ontario, Canada	Connective tissue beneath latissimus dorsi, subcutaneous tissues of thoracic, abdominal, inguinal areas and intermuscular fascia of legs	14/42 (33)	NA	Schulte-Hostedde and Elsasser, 2011	
			Arkansas, USA	Subcutaneous and intermuscular fascia of carpal and metatarsal areas	35/507 (6.9)	NA	Tumilson et al., 1984
			Ohio, USA	Subcutaneous and intermuscular fascia of carpal and metatarsal areas, tail musculature	3/3 (100)	NA	Crites, 1963
			Minnesota, USA	Subcutaneous and intermuscular fascia of carpal and metatarsal areas	3/3 (100)	NA	Huggins, 1958
			New York, USA	Intermuscular fascial layers of lower leg and ankles; subcutaneous position over pectoralis major	NA	NA	Cheatum and Cook, 1948
			Minnesota, USA	hind leg	2/72 (2.7)	NA	Erickson, 1946
			Iowa, USA	NA	NA	NA	Benbrook, 1940
			Wisconsin, USA	NA	NA	NA	Chaddock, 1940
			Nebraska, USA	NA	NA	NA	Chitwood, 1933
			Muskrat (<i>Ondatra zibethicus</i>)	Ontario, Canada	Right inguinal region, left and right axillary regions	1/1 (100)	NA
	NA	NA			NA	Fyvie, 1969	
	Minnesota, USA	Subcutaneous and intermuscular fascia of carpal and metatarsal areas			1/1 (100)	NA	Huggins, 1958
	Maryland, North Dakota, USA	NA			NA	NA	Dikmans, 1948
	Maryland, USA	Subcutaneous fascia			3/64 (4.7)	NA	Alexander et al., 1972
	Opossum (<i>Didelphis virginiana</i>)						Cheatum and Cook, 1948
	Otter	Raccoon	Florida, USA	Subcutaneous and intermuscular fascia	9/54 (16.7)	NA	Keeling et al., 1993
			Arkansas	fascia of carpal and metatarsal areas	4/30 (13)	NA	Richardson et al., 1992
			Kentucky, USA	Intermuscular fascial layers of lower leg and ankles	10/70 (14.3)	NA	Cole and Shoop, 1987
			Tennessee, Kentucky, USA	NA	20/145 (13.8)	NA	Smith et al., 1985
Illinois, USA			Subcutaneous and intermuscular fascia of carpal and metatarsal areas	2/245 (0.8)	NA	Snyder and Fitzgerald, 1985	
Arkansas			Subcutaneous and intermuscular fascia of carpal and metatarsal areas	35/507 (6.9)	NA	Tumilson et al., 1984	
Georgia, USA			NA	1/148 (0.7)	NA	Price and Harman, 1983	
Connecticut, USA			NA	NA	NA	Diters and Ryan, 1980	
Nebraska, Kansas, Missouri, USA			Subcutaneous and intermuscular fascia of carpal and metatarsal areas, facial areas	4/4 (100)	NA	Ewing and Hibbs, 1966	
North Carolina, South Carolina, Florida, USA			Subcutaneous tissue and muscle fascia	14/209 (6.7), 8/34 (23.5), 6/19 (31.6)	NA	Harkema and Miller, 1964	

(continued on next page)

Table 2 (continued)

Species	Host	Geographic location	Anatomic location	Prevalence (%)	Species confirmation method	Reference
		Ohio, USA	Subcutaneous and intermuscular fascia of carpal and metatarsal areas, tail musculature	3/3 (100)	NA	Crites, 1963
		Florida, USA	fascia of carpal and metatarsal areas	2/2 (100)	NA	Layne et al., 1960
		South Dakota, USA	Subcutaneous and intermuscular fascia of carpal and metatarsal areas	1/1 (100)	NA	Huggins, 1958
		Michigan, USA	Subcutaneous fascia of thigh	NA	NA	Wilson, 1958
		New York, USA	Intermuscular fascial layers of lower leg and ankles; subcutaneous position over pectoralis major	NA	NA	Cheatum and Cook, 1948
		New Hampshire, USA	Intermuscular fascial layers of lower leg and ankles	2/4 (50)	NA	Miller et al., 1946
		Texas, USA	Subcutaneous and intermuscular fascia of carpal and metatarsal areas	6/15 (40)	NA	Chandler, 1942
	Short-tailed Weasel (<i>Mustela erminea</i>)	Minnesota, USA	Subcutaneous and intermuscular fascia of tibia on hind legs	1/1 (100)	NA	Goble, 1942
	Silver Fox (<i>Vulpes fulva</i>)	Iowa, USA	Subcutaneous and intermuscular fascia of carpal and tarsal areas	NA	NA	Benbrook, 1932
	Striped skunk (<i>Mephitis mephitis</i>)	Ontario, Canada	Subcutaneous and intermuscular fascia of carpal and metatarsal areas	1/125 (0.8)	NA	Webster and Casey, 1970
		Grand Island, Nebraska, USA	Rear leg	1/1 (100)	NA	Ewing and Hibbs, 1966
		New York, USA	Intermuscular fascial layers of lower leg and ankles; subcutaneous position over pectoralis major	NA	NA	Cheatum and Cook, 1948
		Minnesota, USA	skin	1/15 (6.7)	NA	Erickson, 1946

^a 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*.

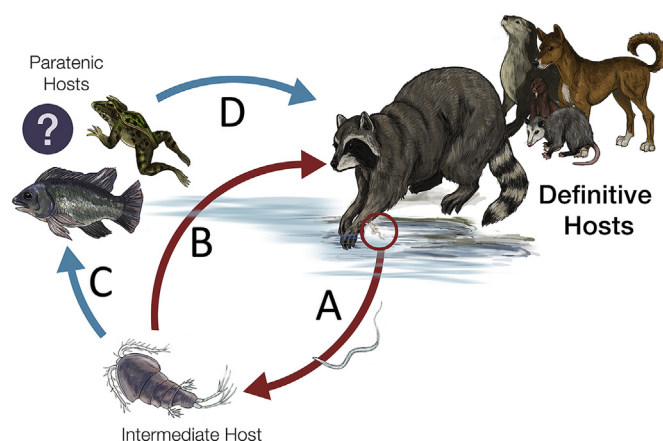


Fig. 1. Life-cycle of *Dracunculus insignis* in wildlife and domestic dogs. Arrows in red represent transmission from (A) definitive hosts to intermediate hosts (Cyclopid copepods) and (B) transmission to definitive hosts via consumption of intermediate hosts. Arrows in blue represent (C) transmission 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.)

Minnesota (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).

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 subgravid female and part of a gravid female recovered from subcutaneous tissue and mesentery of one anaconda (*Eunectes murinus*) from Brazil were used to describe *D. brasiliensis* (Moravec and Santos, 2009). Although most female *Dracunculus* spp. are morphologically similar, the shape of the female caudal end of *D. brasiliensis* differs from other *Dracunculus* spp. in that it is broad with a widely-rounded tip and the excretory pore located just posterior to the nerve ring. Additionally, the L1 of *D. brasiliensis* are considerably larger than most snake-infecting *Dracunculus* spp. (Moravec and Santos, 2009). Recently, *D. brasiliensis* was reported in a Brown-banded water snake (*Helicops angulatus*) from Matto Grosso state, Brazil (Quirino et al., 2018). Description of a male of this species is still needed.

Uncharacterized *Dracunculus* spp. have been reported from a number of free-ranging Central American, South American, and Caribbean snake species including free-ranging anaconda from Brazil and Venezuela, Pearl Island boa (*Boa constrictor sabogae*) from Panama, and boas (*B. constrictor* and reported as *Corallus enydris*) from Trinidad (Muller, 1971; Calle et al., 1994; Moravec and Santos, 2009; USNM 1345047 and 1345052). In addition, unidentified *Dracunculus*

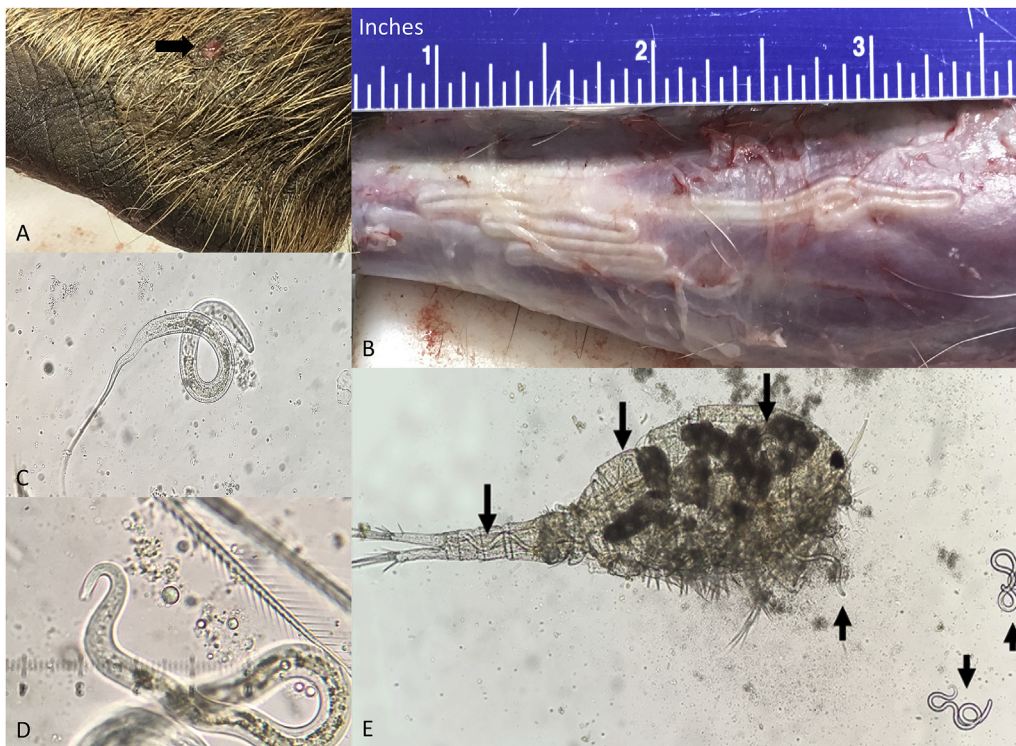


Fig. 2. *Dracunculus insignis* infection in definitive host raccoon (*Procyon lotor*) and intermediate host (Cyclopoid copepod): (A) lesion from emergence of gravid female *D. insignis* (marked by arrow); (B) in situ photograph of patent *D. insignis* infection in forelimb of raccoon; (C) first-stage larvae (L1) of *D. insignis* (0.596–0.857 mm); (D) third-stage larvae (L3) of *D. insignis* (0.434–0.605 mm) with trilobed tail, a distinguishing morphologic characteristic; (E) a copepod infected with *D. insignis* (L3s marked with arrow).

nematodes have been recovered from several captive snakes that had been imported from South America including boas (*B. constrictor* and *B. mexicana*), an anaconda, and a bushmaster (*Lachesis muta*) (Kotlan and Raitaits, 1923; Moravec, 1966; USNM 1338673 and 1335517).

2.1.3. *Dracunculus* spp. from Eurasia

The highest diversity of squamate *Dracunculus* spp. have been reported from Eurasia, including *D. oesophageus* (Desportes, 1938), from Europe and *D. coluberensis*, *D. alii* (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. alii* 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. viperina*), 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 inglisii* 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* (Wijová 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 (Turkhud, 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*, *Hastospiculum*) 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).

2.1.4. *Dracunculus* spp. from oceania

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).

2.1.5. *Dracunculus* spp. from Africa

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 (*Acrantophis madagascariensis*) (Chabaud, 1960). Females of this parasite were subsequently detected in a captive Madagascar tree boa (*Sanzinia madagascariensis*) in a Paris Zoo (Vaucher and Bain, 1973). Larvae from the tree boa were

infective to *Cyclops strenuus* and developed to L3 in 13 days at 22–25 °C. These infective larvae were given to a ball python (*Python regius*), which developed an infection with two male nematodes (Vaucher and Bain, 1973; Moravec, 2006).

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. sipidon*) 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 (Burse 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 linjanticus* and *Mesocyclops aequatorialis similis* from Cameroon, Africa were experimentally susceptible (Sullivan et al., 1991). Susceptibility can vary as *Mesocyclops 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* (Cheatum 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) (Crichton and Beverley-Burton, 1977; Andersen, 2000).

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; Diters 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. insignis* 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. insignis* 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 filarid worms (e.g., *Filaria taxidae* 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. insignis*

Several species, including raccoons, rhesus macaques (*Macaca mulatta*), mink, and domestic ferrets (*Mustela putorius furo*), have been experimentally-infected with *D. insignis* (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 develop into adult worms. Exposure of two domestic dogs (*Canis lupus familiaris*) and a single marten (*Martes americana*) to *D. insignis*-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. insignis*, 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 Beverley-Burton, 1977).

In one study, captive bred raccoons were inoculated with L3 recovered from digested copepods (Crichton and Beverley-Burton, 1975). Raccoons were euthanized at various time points to examine parasite migration. Overall, 30 of 33 (87%) 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 μ m 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. insignis* recovered from experimentally infected paratenic hosts (*Lithobates pi-piens* and *L. clamitans*) were fed to a captive-born raccoon. Upon necropsy, the infected raccoon had larvigerous female *D. insignis* 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. insignis*, 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. insignis* but fewer ingested larvae develop to larvigerous females.

4.2.3. Rhesus macaques

A Rhesus macaque was experimentally exposed to 400 L3 of *D. insignis* 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. insignis* 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; Broderon et al., 1991). Collectively, these studies show that domestic ferrets are appropriate definitive hosts for both *D. insignis* and *D. medinensis* (Eberhard et al., 1988, 2016; Brandt and Eberhard, 1990).

Initially, ferrets were exposed to *D. insignis*-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 intraperitoneal (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. insignis* 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 chemoprophylactic 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 amocarazine (CGP 6140)) 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. ophidensis*—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*), stonecatfish (*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 niloticus*). 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. pipiens* 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. pipiens* and *L. clamitans* were negative (Crichton and Beverley-Burton, 1977). However, a natural infection was recently detected in one of eight southern leopard frogs (*L. sphenoccephalus*) 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* (Elsasser et al., 2009). Recently, 184 river otters (*Lontra canadensis*) from Arkansas were examined for the presence of *Dracunculus* species (Tumilson 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

D. lutrae have been reported in several locations in North America including Kentucky, Alabama, New York, and Ontario, Canada (Table 2) (Cheatum and Cook, 1948; Crichton and Beverly-Burton, 1974; Fleming et al., 1977; Kimber and Kollias, 2000; Elsasser et al., 2009; Barding and Lacki, 2015). To date, no studies have been conducted on possible paratenic hosts, but based off research conducted on *D. insignis*, it is assumed that fish and/or frogs could be involved in transmission.

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 *Dracunculus* spp. 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.

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 superfamily 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 Dracunculoidea 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) who included the 18S rRNA sequences from *D. medinensis*, *D. oesophageus* (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 obturans*, *Philometra ovata*, *Philonema oncorhynchi*, *Skrjabillanus scardinii*, and *Molnaria intestinalis* from fish, and *Micropleura 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 confirmed to be different species based on analysis of the cytochrome c oxidase I (COI) gene (Elsasser et al., 2009; Laetsch et al., 2012; Cleveland 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 educational and personal losses and has recently been the subject of a highly successful eradication campaign that has reduced the number of endemic 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 epidemiology' 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 records 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 because of lack of available samples preserved appropriately for molecular 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 suggests that they may represent the ancestral host of the genus. The breadth of wildlife definitive hosts parasitized by the numerous dracunculids 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 nematodes. Considering these intriguing dynamics of transmission and host-parasite relationships, large gaps in basic parasitological knowledge still exist, and further research is needed.

Acknowledgements

We would like to thank S.G. Sapp (Creepy Collie Creative) for creating the graphical abstract and life-cycle image for this review. The Carter Center funded this research; a full listing of supporters is available at <http://www.cartercenter.org/donate/corporate-government-foundation-partners/index.html>. Additional support was provided by the wildlife management agencies of the Southeastern Cooperative Wildlife Disease Study member states through the Federal Aid to Wildlife Restoration Act (50 Stat. 917) and by a US Department of the

Interior Cooperative Agreement. Additional support for C.A.C. was provided by the ARCS Foundation (Atlanta Chapter).

References

- Alexander, A.D., Flyger, V., Herman, Y.F., McConnell, S.J., Rothstein, N., Yager, R.H., 1972. Survey of wild mammals in a Chesapeake Bay area for selected zoonoses. *J. Wildl. Dis.* 8 (2), 119–126.
- Anderson, R.C., 2000. *Nematode Parasites of Vertebrates: Their Development and Transmission*, second ed. CABI Publishing, CAB International, Wallingford, Oxon, U.K., pp. 650.
- Barding, E.E., Lacki, M.J., 2015. Occurrence of nematodes (*Dracunculus* spp.) in re-introduced river otters in Kentucky. *J. Ky. Acad. Sci.* 75, 94–96.
- Benbrook, E.A., 1940. The occurrence of the Guinea-worm, *Dracunculus medinensis*, in a dog and in a mink, with a review of this parasitism. *J. Am. Vet. Med. Assoc.* 96 (755), 260–262.
- Beverly-Burton, M., Crichton, V.F.J., 1976. Attempted experimental cross infections with mammalian Guinea worms, *Dracunculus* spp. (Nematoda: Dracunculoidea). *Am. J. Trop. Med. Hyg.* 25, 704–708.
- Bimi, L., Freeman, A.R., Eberhard, M.L., Ruiz-Tiben, E., Pieniazek, N.J., 2005. Differentiating *Dracunculus medinensis* from *Dracunculus insignis*, by the sequence analysis of the 18S rRNA gene. *Ann. Trop. Parasitol.* 99, 511–517.
- Bolette, D.P., 1998. *Hastospiculum spiralis* n. sp. (Nematoda: Diplotriaenoidea: Dicheilonematinae), from an Indonesian mangrove monitor, *Varanus indicus* (Reptilia: Sauria: Varanidae). *J. Parasitol.* 84, 366–369.
- 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.
- Brandt, F.H., Eberhard, M.L., 1990. Distribution, behavior, and course of patency of *Dracunculus insignis* in experimentally infected ferrets. *J. Parasitol.* 76, 515–518.
- Brandt, F.H., Eberhard, M.L., 1991. Inoculation of ferrets with ten third-stage larvae of *Dracunculus insignis*. *J. Parasitol.* 77 (5), 786–787.
- Burse, C.R., Brooks, D.R., 2011. Nematode parasites of five species of turtles from the Area de Conservacion Guanacaste, Costa Rica, with description of a new species of *Falcausta*. *Comp. Parasitol.* 78, 107–119.
- Cairncross, S., Muller, R., Zagaaria, N., 2002. Dracunculiasis (Guinea worm disease) and the eradication initiative. *Clin. Microbiol. Rev.* 15 (2), 223–246.
- Calle, P.P., Rivas, J., Munoz, M., Thorbjarnarson, J., Dierenfeld, E.S., Holstrom, W., Braserton, W.E., Karesh, W.B., 1994. Health assessment of free-ranging anacondas (*Eunectes murinus*) in Venezuela. *J. Zoo Wildl. Med.* 25, 53–62.
- Carlson, B.L., Vito, G.S., 1984. *Dracunculus insignis* in Fishers in New Hampshire. *J. Am. Vet. Med. Assoc.* 185 (11), 1372.
- Chabaud, A.G., 1960. Deux nematodes parasites de serpents malgaches. *Mém. Inst. Sci. Madagascar* 14, 95–103.
- Chaddock, T.T., 1940. Diseases of mink. *Am. Fur Breeder* 12 (10), 6–8.
- Chandler, A.C., 1942a. The helminths of raccoons in east Texas. *J. Parasitol.* 28, 255–268.
- Chandler, A.C., 1942b. The Guinea worm, *Dracunculus insignis* (Leidy 1858), a common parasite of raccoons in east Texas. *Am. J. Trop. Med.* 22, 153–157.
- 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.
- Chitwood, B.G., 1933. Does the Guinea-worm occur in north America? *J. Am. Med. Assoc.* 100, 802–804.
- Chitwood, B.G., 1950. The male of *Dracunculus insignis* (Leidy, 1858) Chandler, 1942. *Proc. Helm. Soc. Wash.* 17 (1), 14–15.
- 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 (9), 1590–1592.
- Cole, R.A., Shoop, W.L., 1987. Helminths of the raccoon (*Procyon lotor*) in western Kentucky. *J. Parasitol.* 73, 762–768.
- Crichton, V.F.J., Beverly-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.
- Crichton, V.F.J., Beverley-Burton, M., 1975. Migration, growth, and morphogenesis of *Dracunculus insignis* (Nematoda: Dracunculoidea). *Can. J. Zool.* 63 (2), 105–113.
- Crichton, V.F.J., Beverley-Burton, M., 1977. Observations on the seasonal prevalence, pathology, and transmission of *Dracunculus insignis* in the raccoon in Ontario. *J. Wildl. Dis.* 13, 273–280.
- Crites, J.L., 1963. Dracontiasis in Ohio carnivores and reptiles with a discussion of the Dracunculid taxonomic problem (Nematoda: Dracunculidae). *Ohio J. Sci.* 63, 1–6.
- Deshmukh, P.G., 1969. Two new species of the genus *Dracunculus* Reichard, 1759 from snakes. *Folia Parasitol.* 16, 105–110.
- Deshmukh, P.L., 1970. *Dracunculus ricci* sp. n. from a snake *Natrix natrix*. *Riv. Parasitol.* 31, 143–146.
- Desportes, C., 1938. *Filaria oesophagea*, Polonio 1859, parasite of the grass-snake is a *Dracunculus*, closely related to the guineaworm. *Ann. Parasitol. Hum. Comp.* 16, 205–326.
- Dikmans, G., 1948. Another case of Guinea worm, *Dracunculus* sp., infestation in a dog in the United States. *Proc. Helm. Soc. Wash.* 15, 39–40.
- Diters, R.W., Ryan, M.J., 1980. Vegetative endocarditis with generalized bacterial embolism in association with phlegmon induced by *Dracunculus insignis* in a raccoon. *Cornell Vet.* 70, 213–217.
- Eberhard, M.L., Ruiz-Tiben, E., Wallace, S.V., 1988. *Dracunculus insignis*: experimental infection in the ferret, *Mustela putorius furo*. *J. Helminthol.* 62 (3), 265–270.
- Eberhard, M.L., Brandt, F.H., Ruiz-Tiben, E., Hightower, A., 1990. Chemoprophylactic drug trials for treatment of dracunculiasis using the *Dracunculus insignis*-ferret model. *J. Helminthol.* 64 (2), 79–86.
- Eberhard, M.L., Brandt, F.H., 1995. The role of tadpoles and frogs as paratenic hosts in the life cycle of *Dracunculus insignis* (Nematoda: Dracunculoidea). *J. Parasitol.* 81 (5), 792–793.
- Eberhard, M.L., Ruiz-Tiben, E., Hopkins, D.R., Farrell, C., Toe, F., Weiss, A., Withers Jr., 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 (1), 61–70.
- Eberhard, M.L., Yabsley, M.J., Zirimwabagabo, H., Bishop, H., Cleveland, C.A., Maerz, J.C., Bringolf, R., Ruiz-Tiben, E., 2016a. Possible role of fish and frogs as paratenic hosts of *Dracunculus medinensis*. *Chad. Emerg. Infect. Dis.* 22 (8), 1428–1430.
- Eberhard, M.L., Cleveland, C.A., Zirimwabagabo, H., Yabsley, M.J., Ouakou, P., Ruiz-Tiben, E., 2016b. Guinea worm (*Dracunculus medinensis*) infection in a wild-caught frog. *Chad. Emerg. Infect. Dis.* 22 (11), 1961–1962.
- Elsasser, S.C., Floyd, R., Hebert, P.D.N., Schulte-Hostedde, A.I., 2009. Species identification of North American Guinea worms (Nematoda: *Dracunculus*) with DNA barcoding. *Mol. Ecol. Resour.* 9, 707–712.
- Erickson, A.B., 1946. Incidence of worm parasites in Minnesota Mustelidae and host lists and keys to North American species. *Am. Midl. Nat.* 36 (2), 494–509.
- Ewing, S.A., Hibbs, C.M., 1966. *Dracunculus insignis* (Leidy, 1858) in dogs and wild carnivores in the Great Plains. *Am. Midl. Nat.* 76, 515–519.
- Fedchenko, A.P., 1871. Concerning the structure and reproduction of the Guinea worm (*Filaria medinensis* L.). *Am. J. Trop. Med. Hyg.* 20 (4), 511–523.
- Fleming, W.J., Dixon, C.F., Lovett, J.W., 1977. Helminth parasites of river otters (*Lutra canadensis*) from southeastern Alabama. *Proc. Helm. Soc. Wash.* 44, 131–135.
- Fyvie, A., 1969. Manual of Common Parasites, Diseases and Anomalies of Wildlife in Ontario. Ontario Ministry of Natural Resources, pp. 84.
- Gardner, S.L., 2001. Worms, Nematoda. Faculty Publications from the Harold W. Manter Laboratory of Parasitology. pp. 78.
- Gatschet, T.P., Schmidt, G.D., 1974. A redescription of *Dracunculus globocephalus* Mackin, 1927 (Nematoda: Dracunculidae), a parasite of turtles. *Helminthologia* 15, 835–838.
- Gibson, G.G., McKiel, D.A., 1972. *Dracunculus insignis* (Leidy, 1858) and larval *Eustrongylides* sp. in a muskrat from Ontario. *Canada. Can. J. Zool* 50 (6), 897–901.
- Goble, F.C., 1942. The Guinea-worm in a Bonaparte weasel. *J. Mammal.* 23 (2), 221.
- Harkema, R., Miller, G.C., 1964. Helminth parasites of the raccoon, *Procyon lotor* in the southeastern United States. *J. Parasitol.* 50 (1), 60–66.
- Hsi, H.F., 1933. On *Dracunculus houdemeri* n. sp., *Dracunculus globocephalus*, and *Dracunculus medinensis*. *Z. Parasitenkd.* 6 (1), 101–118.
- Huggins, E.J., 1958. Guinea worms in a raccoon from South Dakota and in Mink from Minnesota. *J. Parasitol.* 44, 41–42.
- Jiménez-Ruiz, F.A., García-Prieto, L., Pérez-Ponce de León, G., 2002. Helminth infra-community structure of the sympatric river snakes *Thamnophis eques* and *Thamnophis melanogaster* from the Mesa Central of Mexico. *J. Parasitol.* 88, 454–460.
- 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 (3), 195–205.
- Keeling, N.G., Roelke, M.E., Forrester, D.J., 1993. Subcutaneous helminths of the raccoon (*Procyon lotor*) in Southern Florida. *J. Helm. Soc. Wash.* 60, 115–117.
- Kimber, K.R., Kollias, G.V., 2000. Infectious and parasitic diseases and contaminant-related problems of North American river otters (*Lontra canadensis*): a review. *J. Zoo Wildl. Med.* 31 (4), 452–472.
- Kotlán, S., Raitsits, E., 1923. Rare nematode-caused disease (dracontiasis) in giant snake (*Boa constrictor*). *The Nature [A természeti]* 19, 121–129.
- Laetsch, D.R., Heitinger, E.G., Taraschewski, H., Nadler, S.A., Blaxter, M.L., 2012. The phylogenetics of Anguillicolidae (Nematoda: Anguillicolioidea), swimbladder parasites of eels. *BMC Evol. Biol.* 12 (1), 60.
- Layne, J.N., Birkenholz, D.E., Griffo, J.V., 1960. Records of *Dracunculus insignis* (Leidy 1858) from raccoons in Florida. *J. Parasitol.* 46, 685.
- Long, J.L., 2003. *Introduced Mammals of the World*. CABI Publishing, Wallingford, UK.
- Macklin, J.G., 1927. *Dracunculus globocephalus* n. sp., from *Chelydra serpentina*. *J. Parasitol.* 14, 91–94.
- McKown, R.D., Veatch, J.K., Robel, R.J., Upton, S.J., 1995. Endoparasites of beaver (*Castor canadensis*) from Kansas. *J. Helminthol. Soc. Wash.* 62 (1), 89–93.
- Miller, G.S.J.R., Kellogg, R., Charters, E.M., 1946. Guinea worm infestation of raccoons in New Hampshire. *J. Mammal.* 27, 179–187.
- Mirza, M.B., Basir, M.A., 1937. A report on the Guinea-worm found in *Varanus* sp., with a short note on *Dracunculus medinensis*. *Proc. Indiana Acad. Sci.* 5, 26–32.
- Mirza, M.B., Roberts, L.S., 1957. A redescription of *Dracunculus* from a snake, *Natrix si-pedon* Linn. *Z. Parasitenkd.* 18, 40–43.
- Mirza, M.B., 1957. On *Dracunculus reichard*, 1759, and its species. *Z. Parasitenkd.* 18, 44–47.
- Molyneux, D., Sankara, D.P., 2017. Guinea worm eradication: progress and challenges—should we beware of the dog? *PLoS Neglected Trop. Dis.* 11 (4), e0005495.
- Moorthy, V.N., 1937. A redescription of *Dracunculus medinensis*. *J. Parasitol.* 23 (2), 220–224.
- Moravec, F., 1966. The First record of dracunculosis in the anaconda *Eunectes murinus* L. *Folia Parasitol.* 13, 281–283.
- Moravec, F., Little, M.D., 2004. Redescription of *Dracunculus globocephalus* Mackin, 1927 (Nematoda: Dracunculidae), a parasite of the snapping turtle *Chelydra serpentina*. *Folia Parasitol.* 51, 339–345.
- Moravec, F., 2006. Dracunculoid and Anguillicoloid Nematodes Parasitic in Vertebrates. Academia, Prague, pp. 634 ISBN 80-200-1431-4.
- Moravec, F., Gibson, D.I., 2007. The first record of *Dracunculus mulbus* (Nematoda: Dracunculidae) in the papuan olive python *Apodora papuana* (Ophidia: Boidae).

- Helminthologia 44, 118.
- Moravec, F., Santos, C.P., 2009. *Dracunculus brasiliensis* sp. n. (Nematoda:Dracunculidae) from the anaconda, *Eunectes murinus* (Ophidia: Boidae). Parasitol. Res. 104, 589–592.
- Muller, R., 1971. *Dracunculus* and dracunculiasis. Adv. Parasitol. 9, 73–151.
- Muller, R., 1976. The Pathology of Experimental *Dracunculus* Infection and its Relevance to Chemotherapy. Pathophysiology of Parasitic Infections. Academic Press, New York, New York USA, pp. 133–147.
- Neumann, G., 1895. Sur une Filaire (*Filaria dahomensis*, n. sp.) du Python de Natal, voisine de la Filaire de Médine. Bull. Soc. Zool. France. 20. pp. 123–127.
- Petter, A.J., Planelles, G., 1986. Un nouveau genre de Dracunculidae (Nematoda) parasite d'Amphibien. Bull. Mus. Natl. Hist. Nat., Paris, 4e sér 8, 123–132.
- Pérez-Ponce de León, G., Jiménez-Ruiz, A., Mendoza-Garfías, B., García-Prieto, L., 2001. Helminth parasites of garter snakes and mud turtles from several localities of the Mesa Central of Mexico. Comp. Parasitology 68, 9–20.
- Price, R.L., Harman, D.M., 1983. Helminths from the raccoon, *Procyon lotor* litoreus nelson and goldman, 1930, on St. Catherines Island, Georgia. Proc. Helminthol. Soc. Wash 50, 343–344.
- Quirino, T.F., Ferreira, A.J.M.G., Silva, M.C., Silva, R.J., Morais, D.H., Ávila, R.W., 2018. New records of helminths in reptiles from five states of Brazil. Braz. J. Biol. 78 (4).
- Rataj, A.V., Lindtner-Knific, R., Vlahović, K., Mavri, U., Dovč, A., 2011. Parasites in pet reptiles. Acta Vet. Scand. 53, 33.
- Richardson, D.J., Owen, W.B., Snyder, D.E., 1992. Helminth parasites of the raccoon (*Procyon lotor*) from North-central Arkansas. J. Parasitol. 78, 163–166.
- Ruiz-Tiben, E., Hopkins, D.R., 2006. Dracunculiasis (Guinea worm disease) eradication. Adv. Parasitol. 61, 275–309.
- Schulte-Hostedde, A.I., Elsasser, S.C., 2011. Spleen mass, body condition, and parasite load in male American mink (*Neovison vison*). J. Mammal. 92 (1), 221–226.
- Seville, R.S., Addison, E.M., 1995. Nongastrointestinal helminths in marten (*Martes americana*) from Ontario, Canada. J. Wildl. Dis. 31 (4), 529–533.
- Siegler, H.R., 1946. Guinea-worm infestation of raccoons in New Hampshire. J. Mammal. 27, 179.
- Smith, R.A., Kennedy, M.L., Wilhelm, W.E., 1985. Helminth parasites of the raccoon (*Procyon lotor*) from Tennessee and Kentucky. Am. Soc. Parasitol. 71, 599–603.
- Snyder, D.E., Fitzgerald, P.R., 1985. Helminth parasites from Illinois raccoons (*Procyon lotor*). J. Parasitol. 71, 274–278.
- Sullivan, J.J., Bishop, H.S., Hightower, A.W., 1991. Susceptibility of four species of copepods, from areas of endemic *Dracunculus medinensis*, to the North American *D. insignis*. Ann. Trop. Med. Parasitol. 6, 637–643.
- Tadros, G., 1966. On *Pesteria inglisi* gen. nov., sp. nov., from the English grass snake (*Tropidonotus natrix*). J. Vet. Sci. United Arab Rep 3, 55–64.
- Travassos, L., 1934. *Dracunculus fueleborni* n. sp., parasito de *Didelphis aurita* Wied. Mem. Inst. Oswaldo Cruz 28, 235–237.
- Tumilson, R., Smith, R., Hudspeth, J., Polechla, P.J., King, A.W., 1984. Dracunculiasis in some Arkansas carnivores. J. Parasitol. 70, 440.
- Tumilson, R., Surf, A., 2018. Prevalence, structure, and distribution of novel parasite cysts containing *Dracunculus* species in River Otters (*Lontra canadensis*) from Arkansas. J. Parasitol. 4 (103), 319–321.
- Turkhuđ, D.A., 1920. Dracontiasis in animals, with notes on a case of Guinea-Worm in a cobra. Indian J. Med. Res. 7 (4).
- Vaucher, C., Bain, O., 1973. Développement larvaire de *Dracunculus doi* (Nematoda), parasite d'un Serpent malgache, et description de la femelle. Ann. Parasitol. 48, 91–104.
- Webster, W.A., Casey, G.A., 1970. The occurrence of *Dracunculus* (Leidy, 1858) chandler, 1942 in a skunk from Ontario, Canada. J. Wildl. Dis. 6 (1) 71–71.
- Wijová, M., Moravec, F., Horák, A., Modrý, D., Lukes, J., 2005. Phylogenetic position of *Dracunculus medinensis* and some related nematodes inferred from 18S rRNA. Parasitol. Res. 96, 133–135.
- Wijová, M., Moravec, F., Horák, A., Lukeš, 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 (9), 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. (Ahead of Print).
- Williams, R.W., 1953. Helminths of the snapping turtle, *Chelydra serpentina*, from Oklahoma, including the first report and description of the male of *Capillaria serpentina* Harwood, 1932. Trans. Am. Microsc. Soc. 72 (2), 175–178.
- Wilson, W.D., 1958. The Guinea Worm, *Dracunculus insignis* (Leidy 1858) Chandler 1942, in a raccoon, *Procyon lotor*, from Michigan, a new location. Am. Midl. Nat. 59, 256.
- Wittrock, D.D., Ulmer, M.J., 1974. Helminths of badgers, *Taxidea taxus* (Schreber), in northwest Iowa. Iowa State J. Res. 48, 319–327.