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Migration, growth, and morphogenesis of *Dracunculus insignis* (Nematoda: Dracunculoidea)

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Larvae of *Dracunculus insignis* developed to the infective stage in experimentally infected *Cyclops vernalis* and *C. bicuspidatus thomasi* kept at 24C. The first molt occurred at 8–9 days and the second at 13–16 days. Second- and third-stage larvae are briefly described. Infective larvae were administered to raccoon (*Procyon lotor*) and mink (*Mustela vison*) and necropsies were performed at predetermined intervals for the determination of the migratory route. In raccoon, third-stage larvae were recovered from the gut wall and mesentery of the abdominal cavity on the 1st day. Larvae were found in the intercostal muscles by the 5th day and in the subcutaneous tissue of the thorax and abdomen by the 7th day. Development to fourth stage was complete by the 19th day. Sexual differences were apparent by the 34th day and worms were present in subcutaneous tissue of the thorax, abdomen, and inguinal region. Male worms were mature at 60 days and females at 65–70 days. Larvigerous females were found in the extremities as early as 120 days post infection. The prepatent period was 354 (309–410) days. Similar results were obtained from mink. Quantitative data on the distribution of worms in various locations within the final host at different times after infection are included.

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On a pu élever des larves de *Dracunculus insignis* jusqu'au stade infectieux chez des *Cyclops vernalis* et des *C. bicuspidatus thomasi* infectés artificiellement et gardés à 24 C. La première mue se produit au bout de 8–9 jours et la seconde au bout de 13–16 jours. On donne ici une brève description des larves de second et de troisème stades. Les larves infectieuses obtenues ont été injectées dans les estomacs des ratons-laveurs (*Procyon lotor*) et des visons (*Mustela vison*); des nécropsies, pratiquées à intervalles pré-determinés, ont permis de suivre les routes de migration des parasites. Chez le raton-laveur, on trouve des larves de troisième stade dans la paroi du tube digestif et le mésentère de la cavité abdominale, dès le 1er jour. Au 5ième jour, les larves atteignent les muscles intercostaux et, au 7ième jour, on les trouve dans le tissu sous-cutané du thorax et de l'abdomen. Au 19ième jour, elles ont terminé leur métamorphose en larves de quatrième stade. Les différences sexuelles se reconnaissent au jour 34; les vers se trouvent alors dans le tissu sous-cutané du thorax, de l'abdomen et de la région inguinale. Les mâles parviennent à maturité à 60 jours et les femelles à 65–70 jours. Au 120ième jour, on trouve déjà des femelles larvigères dans les extrémités. La période pré-symptomatique est de 354 (309–410) jours. On obtient des résultats similaires chez le vison. On donne ici également des données quantitatives sur la répartition des vers en divers points de l'hôte définitif, à différents moments de l'infection.
[Traduit par le journal]

Introduction

Dracunculus insignis (Leidy, 1858) Chandler, 1942 is a common parasite of raccoon (*Procyon lotor* (L.)) and mink (*Mustela vison* (Schreber)) in southern Ontario (Crichton and Beverley-Burton 1974). Larvigerous females are found most frequently in subcutaneous tissue of the limbs. Although some observations on the development of the human guinea worm, *D.*

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medinensis, in cyclopoids (Moorthy 1938) and its migration in experimentally infected mammalian hosts (Onabamiro 1956; Muller 1968*a*, 1968*b*) have been published, there has been no previous work done on any guinea worm species in its natural definitive host.

This paper describes the growth and morphogenesis of *D. insignis* within the intermediate hosts, *Cyclops vernalis* Fischer, 1853 and *C. bicuspidatus thomasi* Forbes, 1882, and during the intramammalian phase in raccoon and mink. Quantitative data on the distribution of worms in various locations within the final host at different times after infection are included.

This paper represents the third part of a study on the biology of *Dracunculus* spp. in wildlife from Ontario (Crichton 1972).

Materials and Methods

Dracunculus insignis was established in laboratory-reared mammals using infective larvae obtained from *Cyclops vernalis* and *C. bicuspidatus thomasi*. The copepods had been experimentally infected with first-stage larvae obtained from naturally infected wild raccoon and mink. Cyclopoid copepods were obtained, after dark, with a plankton net (mesh size 250 μ) from a pond at the University of Guelph.

First-stage larvae of *D. insignis* taken from female worms were concentrated in tap water in settling flasks. Copepods were infected by placing batches of about 500 in Petri dishes (diam 50 mm) containing pond water and two to three drops of the concentrated larvae taken from the bottom of the settling flasks. Ingestion of larvae by copepods was frequently observed. After 8 h copepods were transferred to, and maintained in, stacking dishes (diam 180 mm) containing fresh, well-aerated pond water to which three drops of Fosterfry (Sternco Industries, Inc., Harrison, N.J.) had been added. Dishes were covered with glass and placed in a refrigerated incubator kept at various temperatures (8–28C). Infected copepods were killed at various intervals after exposure to larvae, and the development of larvae was studied.

Copepods containing infective larvae were collected in a sieve (mesh size 149 μ). By tilting the sieve, the copepods could be washed to one side and transferred to a Petri dish. A pepsin solution (166 ml distilled H_2O, 1.33 ml HCl, 1.0 g powdered pepsin) was added and the container held at 37C for 30 min. This killed the copepods and activated the third-stage larvae, which were then counted and removed.

Locally trapped raccoons were allowed to breed in captivity, providing a source of uninfected animals. Uninfected ranch mink were obtained from colonies kept at the Ontario Veterinary College. Raccoons (two per pen) were kept in metal-framed pens (4 × 3 × 3 m) enclosed in No. 12 gauge wire each with an attached wooden nest box. Mink were kept in standard ranch-mink pens. Raccoons and mink were fed commercial mink food.

Third-stage larvae were introduced by a No. 9 stomach tube and Pasteur pipette into the stomach of animals anaesthetized with ether. Except in the case of nursing young, food was withheld from experimental animals for 12 h before infection until 12 h after infection. Animals were killed by cardiac injection (1–2 ml) of sodium pentobarbital (Nembutal, Abbott Laboratories).

The intramammalian migration was studied by examining tissues of raccoons and mink killed at various predetermined times after infection. At necropsy, up to 6 days after infection, as much blood as possible was withdrawn from the heart with a syringe. Blood was mixed with 1% sodium citrate and lysed with distilled H_2O. The mixture was placed in settling flasks and the sediment examined for worms after 2 h. In addition, the following locations were examined for larvae: visceral organs, cavities, and mesentery; skeletal muscles; subcutaneous fat and fascia; and the inner surface of the skin. Visceral organs and mesentery were soaked in physiological saline for 4–5 h, whereas muscles and fat were cut into small pieces and soaked for 10–48 h from those animals killed up to 60 days after infection. Tissues were placed in fresh saline every 2 h. 'Old' saline was placed in settling flasks for 2–3 h before the sediment was examined for larvae. Finally, tissues were digested in pepsin (see above) in 500- or 1000-ml beakers at 37C for 8–10 h. Some 66% of the pepsin digest solution was then discarded and the rest washed through a sieve (mesh size 500 μ) to remove undigested solids. Formalin (10%) and cold water were added to arrest digestion and fix any worms. To reduce the formation of foam, two drops of Octanol-1 (Fisher Scientific Co.) were added to the solution, which was then poured slowly into 500-ml cylinders tilted at 45° and allowed to settle for 3 h. Fifty percent of the solution was then decanted and the remaining contents washed once before being left to settle for 12–16 h. The sediment was then examined for worms.

About 60 days after infection, worms were large enough to be seen with the naked eye under good illumination. If worms were not recovered by visual examination the skin, fat, and quartered carcass were soaked in physiological saline for 2–3 h. The saline was then placed in settling flasks and the sediment examined for worms 2 h later.

Animals kept for 240 days or more after being given larvae had their legs shaved and were examined weekly for lesions. When open lesions were observed, saline mounts of the lesion contents were made each day to determine when larvae were first released and when larval production ceased.

The method for preserving, cleaning, and measuring worms has been described (Crichton and Beverley-Burton 1973).

Results

Development within the Intermediate Host

After ingestion by *Cyclops vernalis* and *C. bicuspidatus thomasi*, first-stage larvae penetrated in 1–2 h into the haemocoel, where development to the infective stage took place.

Larvae in copepods kept at 8 and 15C showed no development 60 days after infection. At 17C a few fully developed second-stage larvae were recovered in 35 days, while at 20C third-stage

TABLE 1

Dimensions (μ) of second- and third-stage larvae of *Dracunculus insignis* taken from experimentally infected copepods

	Second-stage larvae	Third-stage larvae	
	Raccoon‡	Raccoon‡	Mink‡
No. specimens	6	20	20
Length	598 (558–629)	633 (595–703)	646 (601–682)
Width (maximum)	19 (18–20)	14.5 (13.0–16.0)	14.0 (13.0–16.0)
Nerve ring*	74 (67–86)	90 (75–104)	90 (74–108)
%†	12.4 (11.1–13.7)	14.2 (11.9–17.2)	13.9 (12.2–15.9)
Excretory pore*	88 (75–100)	106 (86–115)	107 (89–121)
%	14.7 (12.5–15.9)	16.8 (13.7–18.8)	16.5 (14.6–17.8)
Oesophagus	186 (154–210)	311 (255–366)	336 (246–380)
%	31.2 (24.5–37.6)	49.0 (40.1–54.6)	52.1 (40.5–59.2)
Position of g.p.*	357 (329–424)	399 (329–483)	425 (316–406)
%	60.9 (55.8–70.4)	62.9 (51.7–74.5)	65.9 (48.8–78.5)
Length of g.p.	17 (15–18)	20 (13–27)	18 (13–27)
Anus*	491 (466–518)	566 (518–632)	578 (543–608)
%	82 (79.3–84.4)	89.3 (86.6–91.1)	89.5 (87.7–92.0)
Tail	107 (92–130)	67 (53–80)	68 (55–84)
%	17 (15.6–20.7)	10.7 (8.9–13.4)	10.6 (9.2–12.3)

NOTE: Average values followed by the range.
*Distance from anterior extremity; g.p. = genital primordium.
†% = The position of the relevant structure from the anterior extremity expressed as a percentage of the total body length.
‡Origin of first-stage larvae.

larvae were first found in 29 days. At 24C the first molt occurred at 8–9 days and the second at 13–16 days.

First-stage larvae have been described previously (Crichton and Beverley-Burton 1973). Second- and third-stage larvae (Table 1) of *D. insignis* have transverse cuticular striations that are more closely spaced than those of first-stage larvae. The tail of the third-stage larva was short, blunt, and trifid. The oesophagus was divided into a short anterior muscular portion and a longer glandular posterior portion. The nerve ring was immediately behind the point of demarcation between the two portions. The lumen of the oesophagus was narrow and the oesophageal–intestinal valve was well developed. The intestinal wall had numerous brown irregularly arranged granules and the rectum was long and tubular. The genital primordium contained eight cells and was sausage-shaped.

Transmission to Raccoon

Guinea worms were recovered from 33 (86.8%) of 38 raccoons given infective larvae. Of some 9320 infective larvae given to 30 raccoons, 404 (4.3%) developing larvae and adult worms were recovered. The average number of worms recovered from the 33 experimentally infected raccoons was 12.3 per animal.

Migration and Morphogenesis

The time after infection of necropsy, the recovery sites, and numbers of worms recovered from 21 raccoons are given in Tables 2 and 3. Worms recovered from the thorax, abdomen, and inguinal region were found in the subcutaneous tissue, while those recovered from the lower parts of the limbs were often found in the intramuscular connective tissue. The various developmental stages recovered at necropsy are recorded below.

Third-stage Larvae (7 h – 6 Days)

Seven raccoons were examined during this time period and all the larvae recovered were still at third stage, with a pointed head and with no visible signs of the molt to fourth stage.

The Molt to Fourth Stage (7–14 Days)

At 7 days the head of the larva was dome-shaped compared with the characteristically pointed head of the third stage, and the double cuticle was visible at the anterior and posterior extremities. At 8 days the head was distinctly dome-shaped and the double cuticle was more obvious. By 14 days, although the molt was not complete, the third-stage cuticle was detached along its entire length and the larvae measured 620–1017 μ long.

TABLE 2

Early migration of *Dracunculus insignis* in experimentally infected raccoon

	Time of necropsy								
	7h	19h	41h	4d 12h	4d 17h	5d 17h	5d 19h	6d 17h	7d 21h
No. larvae administered	300	250	150	215	260	600	250	715	430
No. worms recovered and location									
Duodenum	6	1							
Stomach	1								
Abdominal cavity		5	1		1				
Diaphragm						1			
Intercostal muscles						3			
Subcutaneous tissue of thorax and abdomen								23	17
% recovered	2.3	2.4	0.7	0.0	0.4	0.7	0.0	3.2	4.0

NOTE: d = days; h = hours.

The Molt to Adult (19–60 Days)

A single larva (1220 μ long) was recovered at 19 days. It was fourth stage, with the trifid tail (characteristic of third-stage larvae) replaced by a blunt tail with several conical projections.

By 34 days sexual differences were apparent. The females (4.5–10.3 mm long) had an obviously pointed tail within the fourth-stage cuticle and a colorless intestine. In the males (7.6–8.4 mm long) the testis, spicules, and gubernaculum were visible.

At 60 days the fourth-stage cuticle was present in the females, although, in some, it was obviously detached along its entire length. The tail carried 10 small conical projections and the intestine was dark brown. The males had completed the final molt. The seminal vesicle contained sperm and the spicules and gubernaculum were amber-colored and fully developed. The intestine and rectum were not confluent.

Adults (77–270 Days)

At 77 days the females had completed the final molt and measured 87–125 mm long. Ova and a vaginal plug (indicating that fertilization had occurred) were present and the intestine was atrophied. The males were mature with typical adult morphology. The lumen of the intestine was visible anteriorly but posteriorly the intestine was atrophied.

At 90 days the fertilized females still contained ova but by 120 days larvae were present within the uterus. In the males the intestine appeared to be completely atrophied. Subsequently all fertilized females were larvigerous and showed an increase in size to 246 (200–310) mm at about 270 days.

Lesion Formation (300–365 Days)

Most females were found, with the head closely applied to the tissue, moving toward the external surface of the leg. The uterus, packed with active larvae, was either pressed against the cephalic extremity or, as the lesion formed, was prolapsed through a rupture in the body wall in the head region thus releasing the larvae. The females died after larval expulsion and were then usually resorbed by the host. In one experimental raccoon two or three female worms had become calcified. Males recovered maintained a typical adult morphology.

At 480 days a single female (95 mm long) was recovered from subcutaneous tissue of the trunk. The worm contained ova but there was no vaginal plug and the intestine was atrophied.

The combined observations of migration and morphogenesis can be summarized as follows. Female worms matured about 65–70 days after infection and males at about 60 days. Fertilization occurs while worms are in connective tissue of the trunk region, where the males, which measure 24.3 (19.4–30.1) mm long, remain. After fertilization the females migrate to the lower extremities and increase in size to 246 (200–310) mm. Unfertilized females do not complete their migration and remain in the trunk region, measuring only about 100 mm in length.

Transmission to Mink

Guinea worms were recovered from 18 (58.1%) of 31 mink given infective larvae. Of some 4895 infective larvae given to 31 mink, 53 (1.1%) were recovered. The average number of worms recovered from the 18 experimentally infected mink was 2.9 per animal.

TABLE 3

Location of *Dracunculus insignis* in experimentally infected raccoons (14–270 days)

	Time of necropsy, days											
	14	19	34	60	77	90	120	150	180	210	240	270
No. larvae administered	270	110	300	210	130	210	250	250	250	210	210	300
No. worms recovered and location												
Thorax	7		2 ♂♂ 5 ♀♀	1 ♂ 1 ♀	7 ♀♀	2 ♂♂ 2 ♀♀	3 ♂♂	1 ♀	1 ♂	8 ♂♂ 1 ♀	3 ♂	
Abdomen	6	1	1 ♂ 5 ♀♀	5 ♂♂ 4 ♀♀	3 ♂♂ 8 ♀♀	5 ♂♂ 5 ♀♀	3 ♂♂	4 ♂♂ 1 ♀	2 ♂♂	2 ♂♂ 1 ♀		1 ♂
Inguinal region				4 ♂♂ 1 ♀	4 ♂♂	1 ♀	1♂			3 ♂♂ 1 ♀		1 ♂
Legs					13 ♀♀	2 ♀♀	7 ♀♀	9 ♀♀	16 ♀♀	12 ♀♀		6 ♀♀
% recovered	4.8	0.9	4.3	7.6	26.9	8.1	5.6	6.0	7.6	13.3	1.4	2.7

TABLE 4

Location of *Dracunculus insignis* in experimentally infected mink (13–270 days)

	Time of necropsy											
	5d 16h	13d	30d	64d	90d	120d	120d	150d	180d	210d	240d	270d
No. larvae administered	210	205	210	210	210	210	210	210	210	210	400	50
No. worms recovered and location												
Thorax		3		1 ♀	1 ♀	1 ♀		1 ♂				
Abdomen		2		1 ♂ 4 ♀♀	1 ♀	1 ♂ 3 ♀♀				1 ♂	1 ♂	1 ♂
Inguinal region				1 ♀	1 ♀	1 ♀			1 ♂ 1 ♀	1 ♀	2 ♀♀ 3 ♀♀	1 ♀
Legs							? ♀♀*					
% recovered	0.0	2.4	0.0	3.3	1.4	2.9	?	1.0†	1.0	1.0	1.5	4.0

NOTE: d = days; h = hours.
*Animal examined by palpation only.
†In addition, one ♀ was found at 150 days in the abdominal cavity.

Migration and Morphogenesis

The time after infection of necropsy, the recovery sites, and numbers of worms recovered from 12 mink are given in Table 4. Worms recovered from the thorax, abdomen, and inguinal region were found in the subcutaneous tissue, while those recovered from the lower parts of the limbs were often found in the intramuscular connective tissue. The various developmental stages recovered at necropsy are recorded below.

The Molt to Fourth Stage (13 Days)

At 13 days, larvae (840–884 μ long) had molted to the fourth stage, and had blunt tails bearing conical projections. Two other larvae (611 and 794 μ long) carried the third-stage cuticle, which was still intact.

The Molt to Adult (64–365 Days)

At 64 days sexual differences were apparent, although the molt was incomplete in females. The males had molted and the spicules and gubernaculum were amber-colored. All males found at subsequent necropsies were fully mature.

By 90 days the females were mature and contained ova but vaginal plugs were not observed. Males were not recovered from this particular host. The lumen of the intestine was visible anteriorly. At 120 days the females were less advanced than those recovered at 90 days, as the fourth-stage cuticle was still present covering the pointed tail of the preadult. Palpation of the legs of another mink 120 days after infection indicated that female worms, which were presumably fertilized and larvigerous, had migrated to the tarsal region. At 150 days a single female (47 mm long) was recovered in the abdominal cavity. Ova were present but there was no vaginal plug. At 180 days a single female (93 mm long) was found and a vaginal plug was present, and although the worm was not yet larvigerous, it had migrated to the dorsal surface of the hind foot. Similarly at 210 days a fertilized female (53 mm long) had migrated to the hind leg but was without larvae. At 240 days, four females (95–140 mm long), of which three had migrated to the hind limbs, contained ova and had vaginal plugs, while another female (22 mm long) was still at the fourth stage. At 270 days the single female had migrated to the hind leg and was larvigerous.

As late as 365 days a single unfertilized female (48 mm long) was recovered from one mink. The worm contained ova. A single male was recovered from another mink at 365 days. Sperm were visible in the seminal vesicle, and the intestine was atrophied.

These observations are summarized. Female worms probably mature at about the same time as in raccoon (65–75 days). However, some worms showed delayed development. In one case females were detected, by palpation, in the hind limbs only 120 days after infection. Females recovered from the extremities were usually larvigerous and measured 220 (192–275) mm long. Males developed to maturity at about 64 days after infection, when they measured 18.0 (14.2–19.7) mm long.

Prepatent Period

The prepatent period is defined as the time interval from administration of infective larvae to the definitive host until first-stage larvae are detected in a cutaneous lesion. In raccoon, the average prepatent period was 354 (309–410) days. Females release larvae for 5 to 7 days after formation of the open lesion. All female worms, within an individual animal, did not produce lesions at the same time; for example, one raccoon had different female worms passing larvae into lesions at 324, 360, 380, and 410 days post infection, and when killed 410 days after infection, five additional larvigerous females were recovered.

Discussion

In common with other dracunculoids, larvae of *D. insignis* develop from first to third stage in cyclopoid copepods. This development is temperature dependent. Muller (1971) stated that *D. medinensis* failed to develop in copepods kept at temperatures below 19C. In contrast, present studies show that *D. insignis* developed to the second stage in 35 days in copepods kept at 17C. The rate of development of *D. insignis* and *D. medinensis* from first to third stage in copepods kept at 24C was similar.

Most larvae of *D. insignis* completed their development at about the same time. However, in some copepods containing two or more larvae, a few did not complete their development. Others showed no obvious development beyond the first stage and some were found dead in the haemocoel. This failure to develop and consequent mortality may have resulted from damage inflicted by the copepod during ingestion or by

overcrowding. Moorthy (1938) observed a similar delayed development of *D. medinensis* larvae in copepods in which more than five larvae were present.

First- and third-stage larvae of *D. insignis* are similar to those of *D. medinensis* as figured by Moorthy (1938). The caudal extremity of *D. insignis* third-stage larvae is trifid and the genital primordium always consisted of eight conspicuous cells. Moorthy (1938) found that the genital primordium in third-stage larvae of *D. medinensis* consisted of six to eight cells.

The intramammalian migration route of *D. insignis* has not been studied previously. Larvae migrated from the duodenum, across the abdominal cavity, and into the thoracic and abdominal musculature. About 7 days after larvae were administered to raccoons, they were found in the subcutaneous tissue and had begun to molt. The cuticle was shed in the interval 14–19 days post infection. In raccoons the final molt had started by 34 days and was completed in male worms at 60 days and in female worms at 65–70 days post infection. In mink, male worms had shed the fourth-stage cuticle at 64 days and females at 90 days post infection. However, the presence of females in the legs of mink 120 days after infection suggests that the development may be similar to that in raccoon. The presence of fertilized females in the extremities of raccoons 77 days post infection indicates that they reached maturity and became fertilized at 65–70 days. Results indicate that the fourth stage takes the longest to complete and extends over a longer interval in females than in males. A fourth-stage larva was found in mink 270 days post infection.

Unfertilized and larvigerous females differed in size. An unfertilized female worm recovered from a raccoon 480 days post infection measured 95 mm long. This was in contrast to larvigerous females recovered 120 days post infection, which were about 200 mm in length.

Neither Onabamiro (1956) nor Muller (1968*a*) found *D. medinensis* molting to the fourth stage in the definitive host, but Muller suggested it occurs 15–22 days after infection. Onabamiro (1956) observed the molt from fourth stage to adult 43 days after infection.

Moorthy (1938) and Onabamiro (1956) suggested that the vaginal plug in females is composed of mucus and is an indication that the worms are fertilized. The presence or absence of this plug was our basis for determining whether small females of *D. insignis* were fertilized.

Muller (1968*b*) concluded that females of *D. medinensis* move to the extremities of monkeys 240–300 days post infection; they are filled with eggs at 240 days and larvae at 300 days. This development and migration is slower than that of *D. medinensis* in dogs (Moorthy and Sweet 1936, 1938) and *D. insignis* in raccoon and mink.

The prepatent period of *D. insignis* in raccoon (309–410 days) is similar to that of *D. medinensis* (321–360 days). The extended prepatent period of *D. insignis* (>410 days) in some animals probably resulted from different times of fertilization of the females. An unfertilized female worm was recovered from an experimentally infected raccoon 480 days after infection. If fertilization is still possible at 480 days or later, the prepatent period might exceed 800 days. This may explain the case cited by Chitwood (1933) of a man from the northern United States who developed a guinea worm lesion 6 years after leaving an endemic country. Results from the present study indicate that males can live for at least 365 days and probably longer. Thus, some males recovered from natural infection might be 2 or 3 years old. The longevity of males and females is interesting in view of the fact that the digestive tract is atrophied. The estimation of the prepatent period in man varies from 300 to 420 days and is based chiefly on accounts of travellers who have made a short visit to an endemic region (Muller 1971).

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CHITWOOD, B. G. 1933. Does the guinea-worm occur in North America? J. Am. Med. Assoc. **100**: 802–804.

CRICHTON, V. F. J. 1972. The biology of *Dracunculus* spp. (Dracunculoidea: Dracunculidae) in wildlife from Ontario. Ph.D. Thesis, University of Guelph, Guelph, Ontario.

CRICHTON, V. F. J., and M. BEVERLEY-BURTON. 1973. *Dracunculus lutrae* n. sp. (Nematoda; Dracunculoidea)

from the otter, *Lutra canadensis*, in Ontario, Canada. Can. J. Zool. **51**: 521–529.

——— 1974. Distribution and prevalence of *Dracunculus* spp. (Nematoda: Dracunculoidea) in mammals in Ontario. Can. J. Zool. **52**: 163–167.

MOORTHY, V. N. 1938. Observations on the development of *Dracunculus medinensis* larvae in cyclops. Am. J. Hyg. **27**: 437–460.

MOORTHY, V. N., and W. C. SWEET. 1936. A note on the experimental infection of dogs with dracontiasis. Indian Med. Gaz. **71**: 437.

——— 1938. Further notes on the experimental infection of dogs with dracontiasis. Am. J. Hyg. **27**: 301–310.

MULLER, R. 1968*a*. Studies on *Dracunculus medinensis* (Linnaeus). 1. The early migration route in experimentally infected dogs. J. Helminthol. **42**: 331–338.

——— 1968*b*. Experimental dracontiasis in animals. Parasitology, **58**: 7–8.

——— 1971. *Dracunculus* and dracunculiasis. Adv. Parasitol. **9**, 73–151.

ONABAMIRO, S. D. 1956. The early stage of the development of *Dracunculus medinensis* (Linnaeus) in the mammalian host. Ann. Trop. Med. Parasitol. **50**: 157–166.