

## THE THIRD-STAGE LARVA OF *MOLINEMA ARBUTA* (HIGHBY, 1943) (NEMATODA) AND DEVELOPMENT OF THE PARASITE IN THE PORCUPINE (*ERETHIZON DORSATUM*)

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**SUMMARY.** Development of *Molinema arbuta* was studied experimentally. Third-stage larvae from mosquitoes (*Aedes canadensis* (Theobald), *A. euedes* Howard, Dyar, and Knab, and *A. stimulans/fitchii*) and third- and fourth-stage larvae from subcutaneous tissues of the trunk of porcupines (*Erethizon dorsatum* (L.)) are described. The third moult occurred about 20 days postinfection. The fourth moult was not observed but must occur later than 28 days postinfection. The fifth stage likely migrates to the peritoneal cavity.

### ***Molinema arbuta* (Highby, 1943) (Nematoda) : description du troisième stade larvaire et développement du parasite chez *Erethizon dorsatum*.**

**RÉSUMÉ.** Le développement de *Molinema arbuta* est étudié expérimentement. Les troisièmes stades larvaires récoltés chez les moustiques (*Aedes canadensis* (Theobald), *A. euedes* Howard, Dyar, et Knab, et *A. stimulans/fitchii*) et les troisièmes et quatrièmes stades larvaires récoltés dans le tissu sous-cutané du porc-épic (*Erethizon dorsatum*) sont décrits. La troisième mue à lieu environ 20 jours après l'infestation ; la quatrième mue n'a pas été observée mais s'effectue après 28 jours. C'est probablement le cinquième stade qui fait la migration vers la cavité péritonéale.

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## Introduction

*Molinema arbuta* (Highby, 1943) Bain, Baker and Chabaud, 1982 is a parasite of the pleural, peritoneal and pericardial cavities of the porcupine (*Erethizon dorsatum* (L.)). The parasite is known to develop to the infective stage in mosquitoes (Highby 1943b) but the infective larva has not been described in detail and development in the definite host has not been investigated. The present study redescribes the infective larva and examines the development of the parasite in experimentally infected porcupines.

## Material and Methods

An infected porcupine trapped near Perth Road, Ontario was exposed to mosquitoes collected in woodlots near Guelph, Ontario and mosquitoes were maintained

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and identified as described by Bartlett (1984a). Fourteen to 18 days after feeding mosquitoes were immobilized by cold and decapitated. Heads were placed in 0.75 % physiologic saline. Third-stage larvae of *M. arbuta* which emerged were drawn into a 1 mL syringe equipped with a 22 gauge needle and inoculated subcutaneously into the tail of two 2 month old porcupines raised in captivity. Porcupine A received 120 larvae and was killed 10 days later by an overdose of sodium pentobarbital. Porcupine B received 22 larvae and 8 days later an additional 100 larvae ; this porcupine was killed 28 days after the first inoculation.

Immediately after porcupines A and B were killed, 40-60 mL of blood were collected by cardiac puncture. Erythrocytes were lysed in water and sediments were poured into Petri dishes and examined for nematodes. Carcasses were dissected and the thoracic and abdominal cavities washed with saline and the wash examined for nematodes. Skin, subcutaneous tissues, muscles, and internal organs were then examined (Bartlett 1984b).

Nematodes recovered were fixed in hot 70 % glycerin-alcohol and studied in glycerin. Specimens were deposited in the Helminth Collection of the United States National Museum (USNM) in Beltsville, Maryland 20705, U.S.A. (USNM Nos. 78564-78568).

## Results

### THIRD-STAGE LARVA FROM MOSQUITOES (*fig. 1-5*)

Larvae developed in the abdominal fat body. Third-stage larvae found in the head and labium of *Aedes canadensis* (Theobald), *A. euedes* Howard, Dyar, and Knab, and *A. stimulans*/*fitchii* 14-18 days after mosquitoes had fed on the porcupine are described below.

*General* : Cuticle with delicate transverse striations. Anterior end slightly tapered and bluntly rounded. Cephalic extremity with round oral opening, four pairs papillae, and amphids. Excretory pore inconspicuous, slightly posterior to nerve ring. Oesophagus clearly divided. One obscure, non-salient, lateral post-deirid present at about four-fifths body length. Caudal end tapered toward extremity ; extremity pointed with two sublateral, subterminal projections. Phasmids subterminal.

FIG. 1-5. — Larvae from experimentally infected mosquitoes.

FIG. 6-15. — Larvae from experimentally infected porcupines.

*Fig. 1.* — Anterior end, third-stage female, lateral view. *Fig. 2.* — Œsophageal-intestinal junction, third-stage female. *Fig. 3.* — Posterior end, third-stage female, lateral view. *Fig. 4.* — Posterior extremity, third-stage male, ventral view. *Fig. 5.* — Posterior end, third-stage male, lateral view. *Fig. 6.* — Posterior end, third-stage male, 10 days, lateral view. *Fig. 7.* — Anterior extremity, third-stage female, 10 days, lateral view. *Fig. 8.* — Genital primordium, third-stage female, 10 days, lateral view. *Fig. 9.* — Vulvar and vaginal primordia, moulting third-stage female, 20 days, lateral view. *Fig. 10.* — Posterior extremity, fourth-stage male, 28 days, ventral view. *Fig. 11.* — Posterior end, fourth-stage female, 28 days, lateral view. *Fig. 12.* — Anterior end, fourth-stage female, 28 days, lateral view. *Fig. 13.* — *En face*, fourth-stage female, 28 days. *Fig. 14.* — Partial cross section at level of glandular Œsophagus of female, 28 days. *Fig. 15.* — Posterior end, fourth-stage male, 28 days, lateral view.

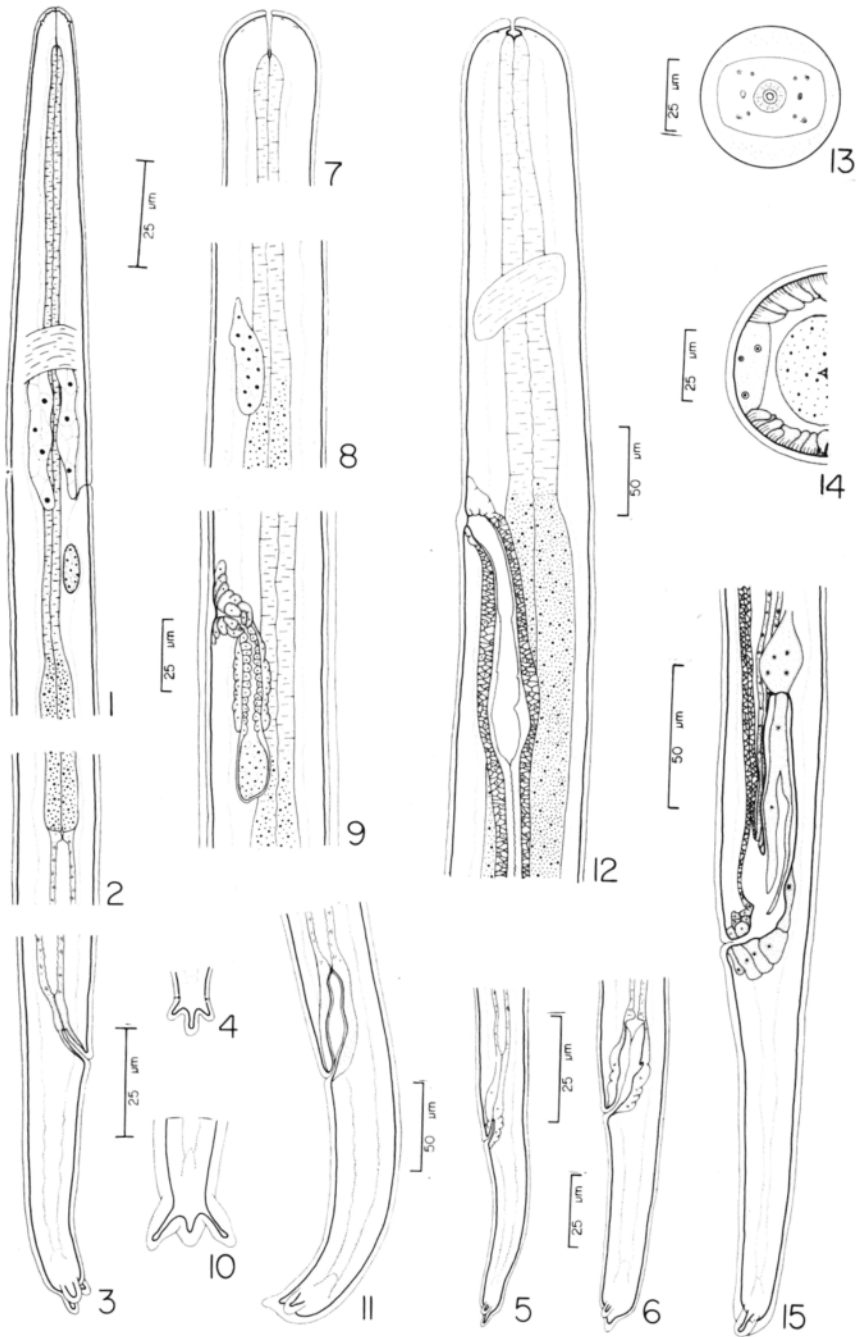


FIG. 1-15. — *Molinema arbuta* (Highby, 1943).

*Male* (N = 10, mean followed by range in parentheses) : Length 1.1 (1.0-1.2) mm. Maximum width 21 (18-22)  $\mu\text{m}$ . Nerve ring 84 (70-100)  $\mu\text{m}$  from anterior extremity. Muscular oesophagus 180 (135-220)  $\mu\text{m}$  long, glandular oesophagus 360 (290-440)  $\mu\text{m}$  long. Genital primordium oval, 600 (500-675)  $\mu\text{m}$  from anterior extremity. Anus 59 (53-65)  $\mu\text{m}$  from posterior extremity.

*Female* (N = 10, mean followed by range in parentheses) : Length 1.1 (1.0-1.2) mm. Maximum width 21 (20-24)  $\mu\text{m}$ . Nerve ring 84 (70-90)  $\mu\text{m}$  from anterior extremity. Muscular oesophagus 185 (170-220)  $\mu\text{m}$  long, glandular oesophagus 375 (340-400)  $\mu\text{m}$  long. Genital primordium oval, 130 (110-150)  $\mu\text{m}$  from anterior extremity. Anus 58 (50-52)  $\mu\text{m}$  from posterior extremity.

#### DEVELOPMENT IN THE PORCUPINE

Nematodes were recovered from subcutaneous tissues of the trunk. Seven third-stage larvae were recovered from porcupine A. Nine larvae were recovered from porcupine B; two were moulting third-stage females and four were young fourth-stage larvae (2 ♂, 2 ♀), all presumably from the 20 day infection; three were older fourth-stage larvae (1 ♂, 2 ♀) and presumably from the 28 day infection.

##### *Third-stage larva (fig. 6-8)*

*General* : Similar to third-stage larva from mosquito except anterior end of body slightly bulbous.

*Male* (N = 3, measurements of each given) : Length 1.5, 1.7, 1.8 mm. Maximum width 28, 33, 30  $\mu\text{m}$ . Genital primordium slightly elongate.

*Female* (N = 4, measurements of each given) : Length 1.5, 1.5, 1.6, 1.7 mm. Maximum width 29, 35, 35, 38  $\mu\text{m}$ . Genital primordium slightly elongate with anterior end attached to ventral hypodermis.

##### *Moulting third-stage larva (fig. 9)*

*General* : Cuticle loosened at extremities and along entire length of body.

*Female* (N = 2, measurements of each given) : Length 2.6, 2.1 mm. Maximum width 50, 50  $\mu\text{m}$ . Genital primordium differentiated into vulvar and vaginal portions.

##### *Fourth-stage larva (fig. 10-15)*

*General* : Similar to third-stage larva from mosquito and porcupine except anterior end of body of uniform width with bluntly rounded extremity. Two deirids visible posterior to nerve ring. Preoesophageal ring present.

*Male* (N = 3, measurements of each given, the first two measurements represent specimens from the 20 day infection and the measurement in parentheses the specimen from the 28 day infection) : Length 4.5, 5.0 (8.6) mm. Maximum width 56, 60 (70)  $\mu\text{m}$ . Nerve ring 140, 150 (160)  $\mu\text{m}$  from anterior extremity. Muscular oesophagus 270,

295 (270)  $\mu\text{m}$  long, glandular oesophagus 1 000, 1 140 (1 400)  $\mu\text{m}$  long. Posterior reproductive tract opening into ventral side of rectum. Spicular pouches thinwalled, opening into dorsal side of rectum. Spicules in early stage of development. Spicular retractor muscle present at anterior terminus of each spicular pouch. Anus 146, 144 (170)  $\mu\text{m}$  from posterior extremity.

*Female* ( $N = 4$ , measurements of each given, the first two measurements represent specimens from the 20 day infection and the two measurements in parentheses specimens from the 28 day infection) : Length 3.5, 4.5 (10.6, 11.1) mm. Maximum width 54, 58 (84, 88)  $\mu\text{m}$ . Nerve ring 140, 140 (160, 160)  $\mu\text{m}$  from anterior extremity. Muscular oesophagus 265, 260 (276, 300)  $\mu\text{m}$  long, glandular oesophagus 950, 1 040 (1 380, 1 300)  $\mu\text{m}$  long. Vulva 255, 270 (286, 310)  $\mu\text{m}$  from anterior extremity, not patent. Anterior vaginal lumen large. Posteriorly, vaginal walls and lumen narrowing and reproductive tract bifurcating. Anus 124, 130 (164, 150)  $\mu\text{m}$  from posterior extremity.

#### *Adult nematodes*

Adult *M. arbuta* were found in the peritoneal cavity of the porcupine on which mosquitoes had engorged. Illustrations of the anterior extremity of the body (*fig. 16*, *17*), the vulva (*fig. 18*), and a cross section of the body (*fig. 19*) of the female are included herein to supplement the descriptions by Highby (1943a) and Anderson (1953).

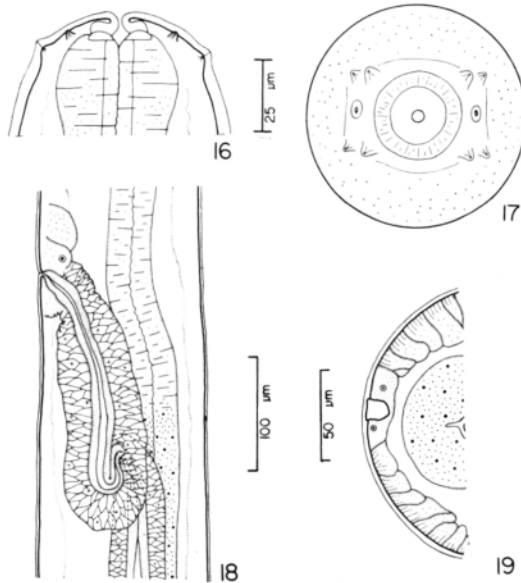


FIG. 16-19. — *Molinema arbuta* (Highby, 1943) from wild-caught porcupines.

*Fig. 16.* — Anterior extremity, female, lateral view. *Fig. 17.* — *En face*, female. *Fig. 18.* — Vulva and anterior vagina, lateral view. *Fig. 19.* — Partial cross section at level of glandular oesophagus of female.

## Discussion

*Molinema arbuta* probably develops to the fifth stage in subcutaneous tissues of the trunk of the porcupine, as third- and fourth-stage larvae were recovered in this site. The fifth stage probably migrates to the definitive site. Development in subcutaneous tissues, followed by migration to the final site, characterizes *Dirofilaria immitis* (Leidy, 1856) and *Dirofilaria scapiceps* (Leidy, 1886); moreover, migration by the fifth stage is common within the Filarioidea (Bartlett 1984b).

Third-stage larvae apparently moulted to the fourth stage at about 20 days, a later time than other filarioids in which development has been studied (Bartlett 1984b). It is not known when *M. arbuta* moults from the fourth to the fifth stage, although it must be after 28 days. There is little uniformity among filarioid species in the time of the fourth moult although some are known to moult as early as 12 days (Bartlett 1984b) and others as late as 50-70 days (Orihel 1961; Eberhard and Orihel 1981).

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