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Heterorhabditis megidis sp. n. (Heterorhabditidae: Rhabditida), Parasitic in the Japanese Beetle, *Popillia japonica* (Scarabaeidae: Coleoptera), in Ohio

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ABSTRACT: *Heterorhabditis megidis* sp. n. (Heterorhabditidae: Rhabditida) is described from parasitized third-stage Japanese beetle larvae, *Popillia japonica* (Scarabaeidae: Coleoptera) collected in Ohio. Distinguishing morphological characters are the large infective-stage juveniles and the presence of a pseudopeloderan bursa. Infective-stage juveniles carry and release cells of the luminescent bacterium *Xenorhabdus luminescens* in the hemocoel of host insects. A high incidence of infection was noted in certain areas of the field.

KEY WORDS: nematode taxonomy, morphology, life cycle, *Xenorhabdus luminescens*, luminescent bacterium.

While digging for diseased Japanese beetle larvae in Jeromesville, Ohio, the latter two authors came across a field containing a number of dying, reddish-colored grubs. All of the third-stage larvae in a 1-m^2 clump of green grass had been killed. Upon closer examination, the grubs were discovered to have been attacked by a nematode belonging to the genus *Heterorhabditis*. The infective stages of this species recovered from field-collected hosts were used to reinfect Japanese beetle grubs and larvae of *Galleria mellonella* in the laboratory. Material sent to the senior author was determined to be a new species of *Heterorhabditis* and a description follows.

Materials and Methods

The description presented here is based on specimens removed from *Galleria mellonella* larvae. Infected insects were maintained at 22°C and dissected on days 4 and 5 to recover the first-generation hermaphroditic females, and on days 7 and 8 to recover the males and the second-generation amphimictic females. Infective-stage juveniles were examined after they emerged from the host cadavers, approximately 14 days after initial exposure.

All nematodes were killed in hot (55°C) Ringer's, fixed in TAF, and processed to glycerin for measurements. Photographs were taken with a Nikon Optiphot microscope fitted for differential interference contrast.

The new species, together with *H. bacteriophora* and the American (NC) strain of *H. heliothidis*, were sent to John Curran for selection and comparison of restriction fragment length differences of genomic DNA. Digestion of genomic DNA from ground whole nematodes with restriction endonucleases generates a unique set of different-sized DNA restriction fragments dependent upon the base sequence of the genome. The size distribution of these restriction fragments is unique to the genotype and can be analyzed by agarose gel electrophoresis (Curran et al., 1985).

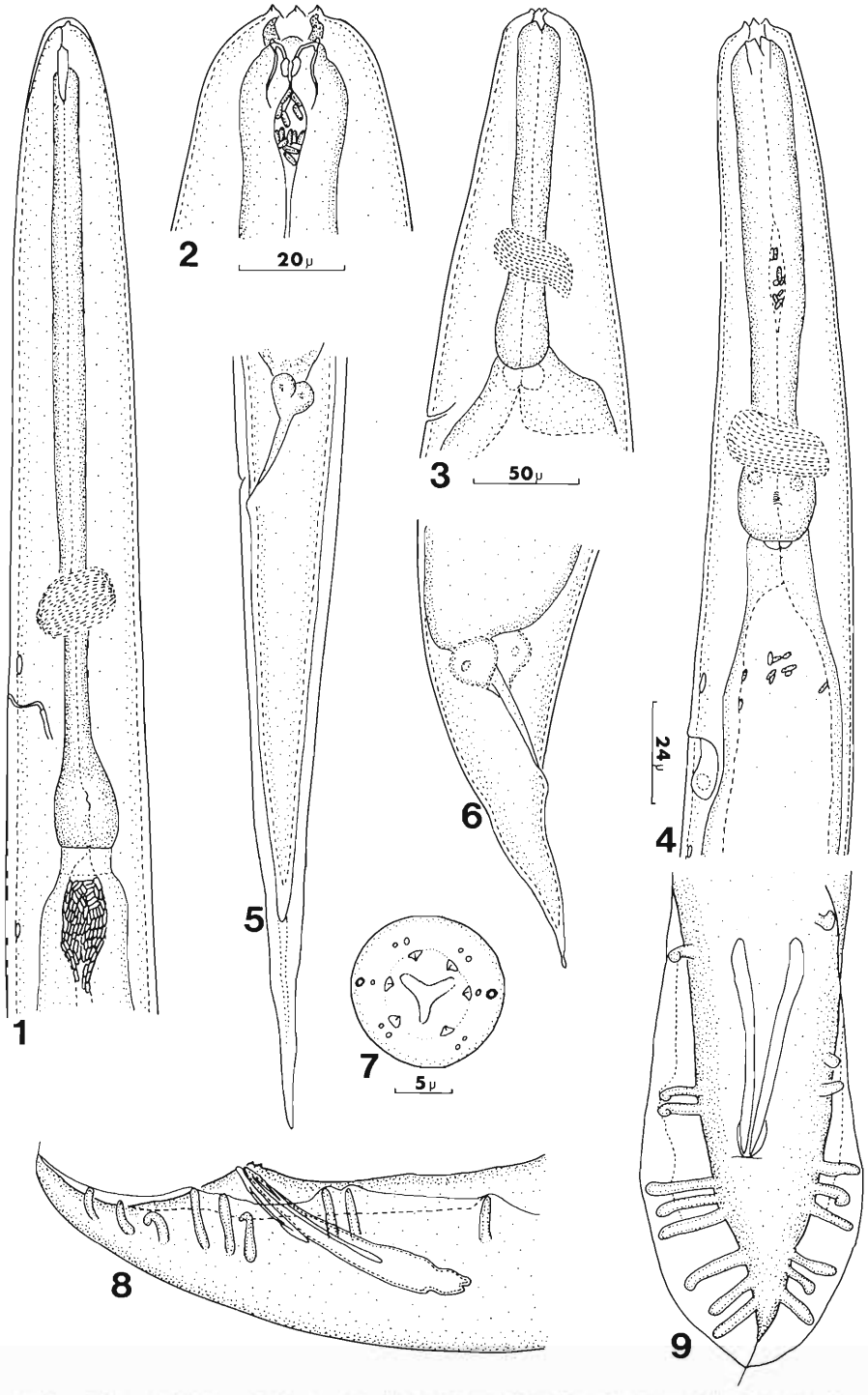
Results

In the quantitative portion of the description, all figures are given in micrometers unless otherwise specified. The first number following the character is the average value and the numbers in parentheses indicate the range.

Heterorhabditis megidis sp. n.

Heterorhabditidae Poinar, 1975; Rhabditoidea (Oerly).

DESCRIPTION: *Adults* (Figs. 2, 3, 4, 6–14): Head truncate or slightly rounded; 6 distinct protruding lips surrounding the mouth opening (in fixed mature hermaphroditic females, the lips are often withdrawn into the mouth opening); each lip bears a single labial papilla emerging at the tip; at the base of each submedial lip are 2 cephalic papillae. The lateral lips contain a single cephalic papilla and a circular amphidial opening. Cheilorhabdions represented as a refractile ring just below the lips and anterior to the pharynx. The remainder of the stoma is modified and could be interpreted as being telescoped on itself. The metarhabdions, each section bearing 1 or more fine teeth, are adjacent to the reduced pro- and mesorhabdions; telorhabdions are represented by fine elongate segments leading directly into the pharyngeal lumen. The anterior portion of pharynx encompasses all of the stoma except the cheilorhabdions. The pharynx lacks a metacorpus but contains an isthmus and pronounced basal bulb bearing some fine striations in the valve area, but not a distinct valve. Nerve ring distinct, located near the middle of the isthmus in the female, but usually on the anterior portion



Figures 1–9. ***Heterorhabditis megidis*** **sp. n. 1. Lateral view of infective-stage juvenile (magnification same as Fig. 2). 2. Lateral view of mouth region of amphimictic female. 3. Lateral view of pharyngeal region of amphimictic female. 4. Lateral view of pharyngeal region of male. 5. Lateral view of tail of infective juvenile (mag. same as Fig. 2). 6. Lateral view of tail of amphimictic female (mag. same as Fig. 2). 7. En face view of male. 8. Lateral view of male tail (mag. same as Fig. 2). 9. Ventral view of male tail (mag. same as Fig. 2).**

of the basal bulb in the male. A double-celled valve separates the pharynx from the single-cell-thick intestine. Four coelomocytes are especially noticeable in the males, where 1 pair occurs near the tip of the testis and another pair near the reflexed portion.

Females with paired, amphidelphic ovaries, the reflexed portion of which often extends past the vulvar opening. Hermaphroditic females with sperm occurring in the proximal portion of the ovotestis; amphimictic females with sperm collected in the proximal portion of the oviduct. Vulva of the hermaphroditic female with slightly protruding lips (Fig. 10), never with a copulation plug as occurs on all mated amphimictic females (Fig. 11) that possess a reduced vulva without protruding lips. Tail pointed, normally wavy in outline, usually with a postanal swelling; rectum distinct, usually expanded and filled with bacteria when living, 3 rectal glands surround the junction of intestine and rectum (Fig. 6).

Males with a single, reflexed testis; spicules paired and separate (Figs. 8, 9, 12), slightly curved, capitulum variable, but usually well set off from the shaft and longer than broad; shaft lacking a rostrum but with a single rib that usually divides at the distal portion; spicule tips pointed; gubernaculum flat, shorter than half the spicule length (Figs. 8, 12), with both the distal and proximal portions folded dorsally. Bursa open, usually pseudopeloderan with a fine tip extending beyond the bursal membrane; a semibursa that extends only partially up the bursal papillae is also present; bursa with 9 pairs of papillae, 3 pairs anterior to the cloacal opening and 6 pairs posterior. Numbering from anterior to posterior, 1 is isolated from 2 and 3 (occasionally 2 and 3 are fused); 4, 5, and 6 normally form a group, as do 7, 8, and 9. The fifth and eighth pair are usually bent outward (laterally), whereas the remainder are straight or bent inward (ventrally).

Hermaphroditic females (Fig. 10) (N = 15): Length, 3.6 (2.4–4.9) mm; greatest width, 209 (120–333); length of stoma, 7 (5–10); width of stoma, 9 (8–11); distance from head to nerve ring, 162 (139–178); distance from head to excretory pore, 209 (193–270); length of pharynx, 229 (206–269); length of tail, 105 (95–124); body width at anus, 63 (38–86); percentage vulva, 48 (45–50); length of eggs in body, 60 (53–70); width of eggs in body, 40 (31–48); a = 17 (14–24); b = 15 (12–21); c = 34 (23–49).

Amphimictic female (Figs. 2, 3, 6, 11, 18) (N = 15): Length, 2.1 (1.5–2.5) mm; greatest width, 123 (95–140); length of stoma, 5 (4–6); width of stoma, 7 (5–8); distance from head to nerve ring, 111 (105–120); distance from head to excretory pore, 178 (158–206); length of pharynx, 160 (155–168); length of tail, 86 (70–101); body width at anus, 31 (25–38); percentage vulva, 49 (47–51); length of eggs in body, 59 (48–70); width of eggs in body, 35 (25–41); a = 17 (15–19); b = 13 (10–16); c = 24 (18–32).

Male (Figs. 4, 7–9, 12–14) (N = 15): Length 1.0 (0.8–1.1) mm; greatest width, 47 (44–50); length of stoma, 3 (2–4); width of stoma, 4 (3–6); distance from head to nerve ring, 104 (96–112); distance from head to excretory pore, 156 (139–176); length of pharynx, 128 (122–134); reflection of testis, 138 (117–230); length of tail, 39 (35–43); width at cloaca, 26 (22–31); length of spicules, 49 (46–54); width of spicules, 6 (5–8); length of gubernaculum, 21 (17–24); width of gubernaculum, 1.1 (0.3–1.6); a = 19 (18–22); b = 8 (7–9); c = 26 (23–31).

Infective juveniles (Figs. 1, 5, 15–19) (N = 15):

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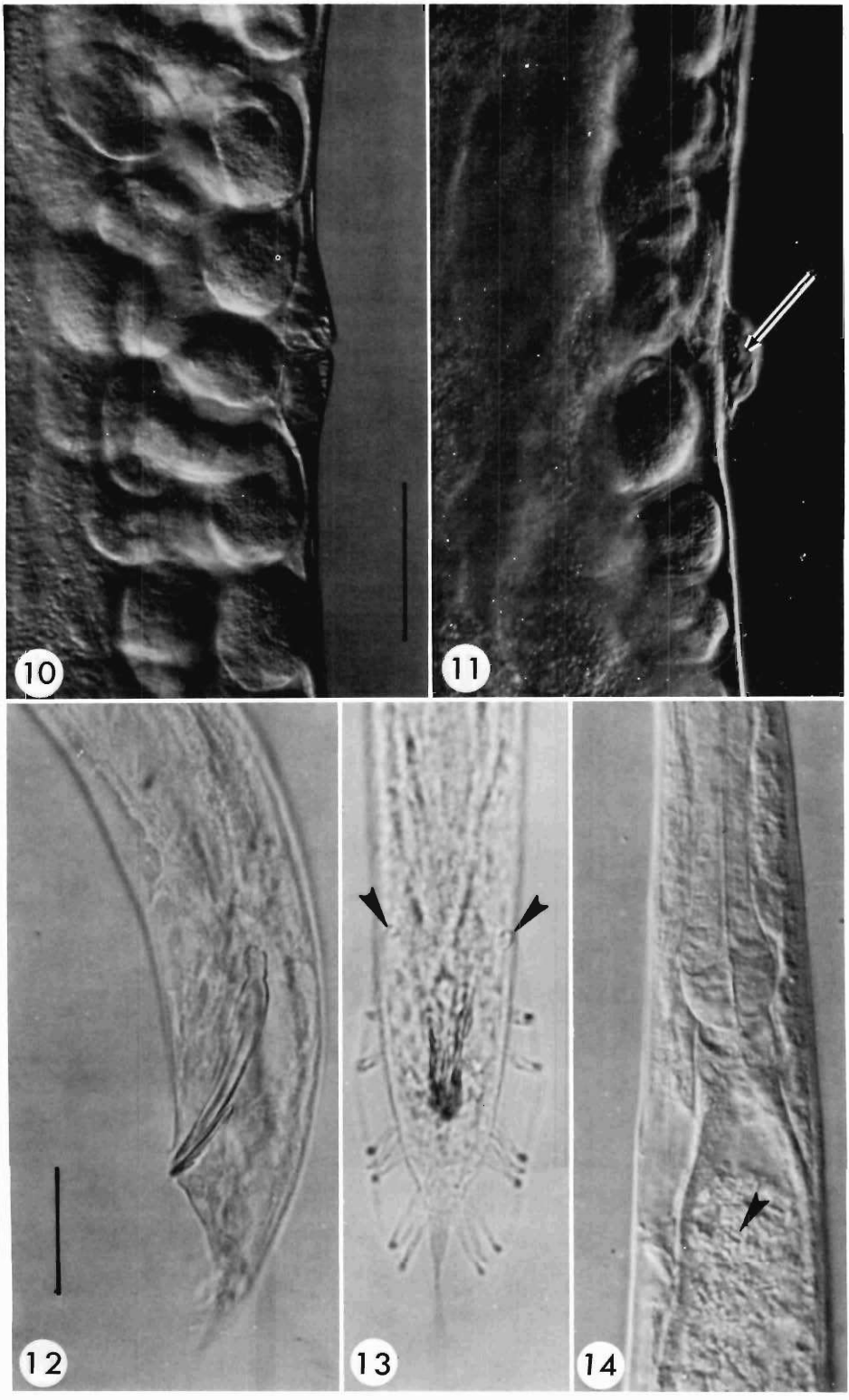
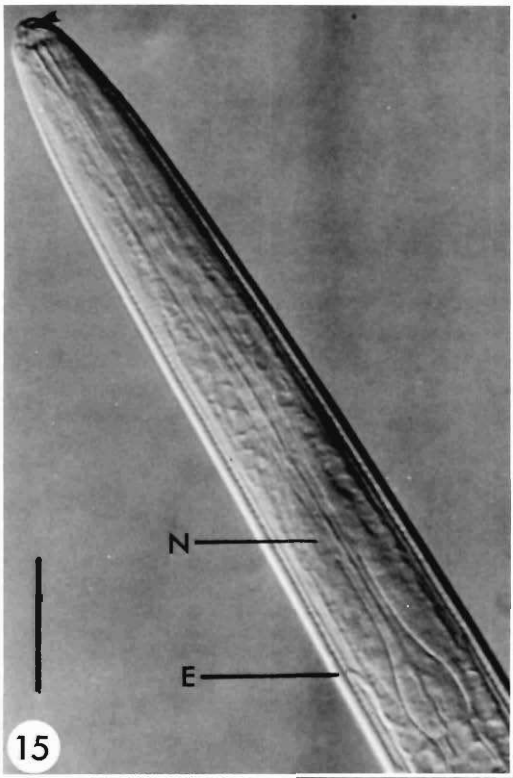
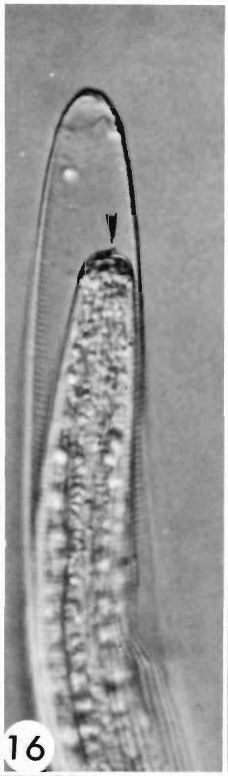
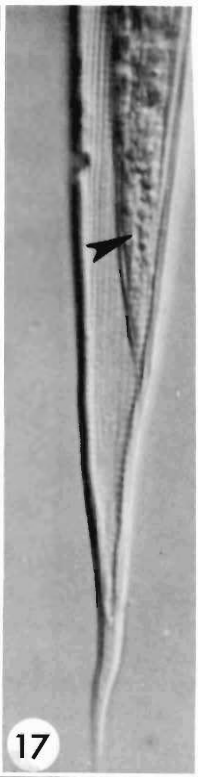
Figures 10–14. ***Heterorhabditis megidis*** **sp. n. 10. Vulva of hermaphroditic female. Note slightly protruding lips. Bar represents 60 μm. 11. Vulva of amphimictic female. Note attached copulation plug (arrow) and absence of vulva lips (magnification same as Fig. 10). 12. Lateral view of male tail. Bar represents 24 μm. 13. Ventral view of male tail, showing nine pairs of papillae and pseudopeloderan bursa. Arrows show first pair of papillae (mag. same as Fig. 12). 14. Portion of pharynx and intestine of male. Note ingested bacteria (arrow) (mag. same as Fig. 12).**

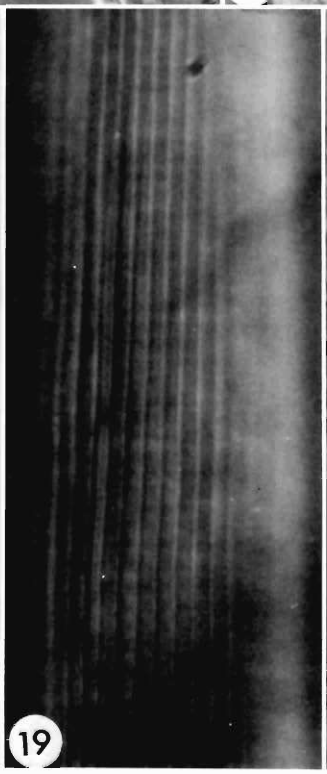
Figures 15–20. ***Heterorhabditis megidis*** **sp. n. 15. Third-stage infective juvenile inside second-stage cuticle. Note dorsal hook on head of third-stage juvenile (arrow). E = excretory pore, N = nerve ring. Bar represents 24 μm. 16. Infective-stage juvenile pulled away from enclosing second-stage cuticle. Note dorsal head hook (arrow) (magnification same as Fig. 15). 17. Tail of third-stage infective juvenile (arrow) inside second-stage cuticle (mag. same as Fig. 15). 18. Infective-stage juveniles inside amphimictic female. Bar represents 120 μm. 19. Striations on second-stage cuticle enclosing third-stage infective juvenile. 20. Cells of** ***Xenorhabdus luminescens*** **released by infective juveniles in the hemolymph of a wax moth larva. Bar represents 12 μm.**











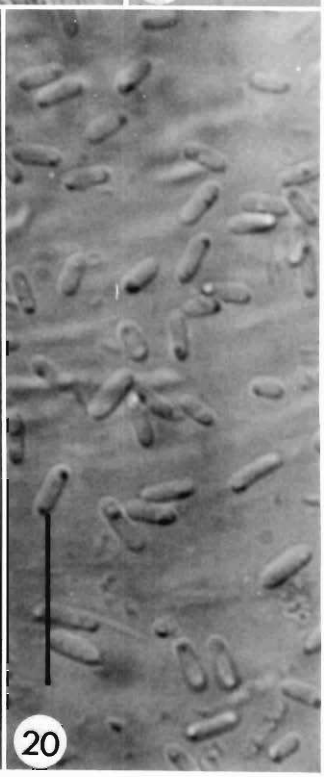


Table 1. Comparison of the infective stages of *H. megidis* with other species and strains of *Heterorhabditis*.

Species/ strain	Body length	Distance from head to excretory pore	Length of pharynx	Reference
H. megidis	768 (736–800)	131 (123–142)	155 (147–160)	Present study
H. bacteriophora	570 (520–600)	104 (94–109)	125 (119–130)	Poinar (1975)
H. heliothidis				
American (NC)	644 (618–671)		133 (130–139)	Khan et al. (1976)
New Zealand	685 (570–740)	112 (94–123)	140 (135–147)	Wouts (1979)
Cuban	615 (540–700)		128 (109–152)	Hernandez and Mráček (1984)

Length, 768 (736–800); greatest width, 29 (27–32); distance from head to nerve ring, 109 (104–115); distance from head to excretory pore, 131 (123–142); length of pharynx, 155 (147–160); length of tail, 119 (112–128); body width at anus, 19 (17–21); a = 26 (23–28); b = 5.0 (4.6–5.0); c = 6.5 (6.1–6.9). The infective stage is a third-stage juvenile inside the second-stage cuticle. The second-stage cuticle is strongly ribbed longitudinally, but also shows weak cross striations (Fig. 19) and is closely appressed to the third-stage juvenile. If the infectives that have just emerged from an insect are placed directly in 70% alcohol, the third-stage juvenile contracts and pulls away from the second-stage cuticle (Figs. 16, 17). The head of the third-stage juvenile bears a minute dorsal tooth (Figs. 1, 15, 16) that is probably used to rasp through tissue and aid entry into a host. In second-generation amphimictic females, the eggs hatch inside the females and develop to infective stages within the female body (Fig. 18).

Symbiotic bacteria: The infective-stage juveniles of *H. megidis* normally carry bacterial cells of *Xenorhabdus luminescens* in their intestines (Fig. 1). These cells are very characteristic in shape and can be found in the hemolymph of insects attacked by the nematodes (Fig. 20). They produce a red pigment and impart a red color to the infected host. They are also luminescent and freshly killed insects or agar plates with 1–2-day-old cultures are luminous in the dark.

TYPE LOCALITY: Mohican Hills Golf Course, Jeromesville, Ohio.

TYPE HOST: The Japanese beetle, *Popillia japonica* Newm. (Scarabaeidae: Coleoptera). Infection occurred in third-instar larvae on 17 and 18 October 1985.

TYPE SPECIMENS: Holotype (male) and allotype (hermaphroditic female) deposited in the Nematology Collection at the University of California, Davis, California.

DIAGNOSIS: There are only two adequately described species in the genus *Heterorhabditis*. One of these is the type species, *H. bacteriophora* Poinar, 1975, and the other is *H. heliothidis* (Khan, Brooks, and Hirschmann, 1976). The latter species is composed of American or NC (Khan et al., 1976), New Zealand (Wouts, 1979), and Cuban (Hernandez & Mráček, 1984) strains. *Heterorhabditis hoptha* (Turco, 1970) was described from specimens collected from the Japanese beetle in 1938 in Moorestown, New Jersey. However, because of the inadequate description, this and the other recorded species of *Heterorhabditis* listed by Poinar (1979) in his synopsis of the genus must remain nomina dubia.

Differences between the infective stages of *H. megidis, H. bacteriophora,* and the various strains of *H. heliothidis* are listed in Table 1. The average length of *H. megidis* infectives, as well as the average distance from the head to excretory pore and average length of the pharynx, separates this species from previously described species or strains of *Heterorhabditis*.

In the males, the ratio of the length of the gubernaculum to the length of the spicule distinguishes *H. megidis* (less than 0.5) from *H. bacteriophora* and the American strain of *H. heliothidis* (0.5 or greater). Also, the pseudopeloderan bursa, with the tail tip slightly protruding beyond the bursal rim, separates *H. megidis* from the other two species and strains of *Heterorhabditis*. The semibursa described here in *H. megidis* was not reported in the other two species, but could have been overlooked because of its delicate structure.

DNA ANALYSIS: Characterization by Dr. Curran of genomic DNA fragments of *H. heliothidis, H. bacteriophora,* and *N. megidis* resulted in bands of restriction fragments that showed a clear distinctness between *H. megidis* and the other two species. Because these bands represent multiple copies of respective DNA sequences and the restrictive fragment length dif-

ferences between such bands can be used as diagnostic characters (Curran et al., 1985), these results support the conclusion that *H. megidis* is a distinct species.

Discussion

The life history of *H. megidis* is similar to that reported for other species of the genus. Infective-stage juveniles enter the host and develop into hermaphroditic females, which then produce male and female progeny. These forms mate and the amphimictic females produce young, which normally develop into infectives inside their bodies. Contrary to Wouts's (1979) conclusion that the infectives of the New Zealand strain of *H. heliothidis* are second-stage juveniles, the infectives of *H. megidis* are clearly third-stage juveniles enclosed in a tightly fitting second-stage cuticle. This second-stage cuticle is closely appressed to the third-stage juvenile, thus making it appear that the infective is a second-stage juvenile. Wouts (1979) also assumed that the males of the New Zealand strain of *H. heliothidis* do not feed. However, the males of *H. megidis* ingest bacteria and have a normal alimentary tract (Figs. 4, 14).

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