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METAPARASITYLENCHUS HYPOTHENEMI N. SP. (NEMATODA: ALLANTONEMATIDAE), A PARASITE OF THE COFFEE BERRY BORER, *HYPOTHENEMUS HAMPEI* (CURCULIONIDAE: SCOLYTINAE)

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ABSTRACT: *Metaparasitylenchus hypothenemi* n. sp. (Nematoda: Allantonematidae) is described from the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Curculionidae: Scolytinae), in Chiapas, Mexico. This species differs from other members of the genus by its small size, annulated cuticle, lateral fields with 3 ridges, free-living stages with an excretory pore located between the pharyngeal gland orifices, a distinct stylet with basal swellings in free-living females, a postvulval uterine extension, a thin stylet lacking basal swellings in males, 2 separate spicules, a gubernaculum, and a peloderan bursa. Parasitic females are white, with a straight or slightly curved body and are ovoviviparous. Third-stage juveniles emerge from parasitized beetles and molt twice before reaching the adult stage. Because the coffee berry borer is the most important pest of coffee throughout the world and this parasite partially or completely sterilizes female beetles, it is worthy of further investigation as a potential biological control agent.

The coffee berry borer (*Hypothenemus hampei* (Ferrari) (Curculionidae: Scolytinae)) is the most important pest of coffee in the world (Le Pelley, 1968). Because most of the life history of the beetle occurs within the protected confines of the coffee berry, standard chemical control methods have, for the most part, been unsuccessful (Bustillo et al., 1998), with resistance arising against the most commonly used insecticide, endosulfan (Brun et al., 1989). As a result, alternative biological control methods are being investigated, including the use of pathogens and parasitoids to be included in pest management strategies against the coffee berry borer.

During studies on the biology of the coffee berry borer in field plantations in the municipality of Cacahoatán, Chiapas, parasitic nematodes were discovered in the body cavity of adult beetles (Fig. 9) and tentatively assigned to *Sphaerulariopsis* (Castillo et al., 2002). The presence of the excretory pore anterior to the nerve ring, males with well-developed stylets, cephalated spicules, a gubernaculum, and a large peloderan bursa, together with elongate-tuboid parasitic females, places the present species in *Metaparasitylenchus* Wachek 1955 (Siddiqi, 2000). Because the specimens differ from all previously described species in the genus (Wachek, 1955; Siddiqi, 2000), they are described below as a new species.

MATERIALS AND METHODS

Although over 200 infected hosts were examined during this investigation, the description was based on nematodes obtained from 10 larvae, 7 pupae, and 20 adult beetles. Nematodes (third-stage juveniles) that emerged from the body cavity of adult beetles were placed in hanging-drop slides (H_2O) and observed daily during a 2-wk period. The hanging-drop slides were placed in separate petri dishes containing wet filter paper to retard drying and held at 20 C. During this time, the nematodes matured and molted to the adult stage. Observations and photographs were made with living as well as fixed nematodes using a Nikon Optiphot Microscope. Measurements were made on nematodes that had been killed in hot (60 C) water, fixed in TAF, and processed to glycerin by the evaporation method. All measurements, including the average value and those of the range (in parentheses), are in micrometers unless otherwise specified.

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DESCRIPTION

Metaparasitylenchus hypothenemi n. sp. (Tylenchida Thorne, 1949: Sphaerularioidea Lubbock, 1861: Allantonematidae Pereira, 1931)

(Figs. 1–14)

Third-stage juvenile (Figs. 1–4, 9–11) (n = 12)*:* Total body length, 335 (302–359); greatest body width, 14 (13–16); stylet length, 11 (10–12); head to dorsal gland outlet, 20 (18–21); head to ventral gland outlet, 33 (31–35); head to excretory pore, 31 (28–33); head to nerve ring, 64 (61–67); tail length, 8 (6–9). Body small, wide, filled with storage granules; cuticle with fine transverse striae 1.0–1.2 apart; lateral fields 1.8–2.0 wide, each composed of 3 raised, thick ridges (Fig. 1, insert) (the 2 edges of each ridge appear as lines under the optical microscope, thus revealing 6 lateral lines), lateral fields extend from excretory pore to tail tip; tail tip with pair of small ventral protuberances; stylet thin, lacking basal swelling, similar in both sexes; gland orifices similar to those in free-living female; anal, cloacal, and vulval openings faint; dorsal and subventral gland nuclei present; 4-lobed cephalic region with 2 subdorsal and 2 subventral (submedian) lobes, each submedian lobe with at least 1 submedian cephalic sensilla; basal mouth plate small, 4 sided; cephalic framework quadriradiate with 2 subdorsal and 2 subventral arms; 2 circular, raised amphids in place of lateral arms.

Free-living female (Fig. 7) (n = 10)*:* Total body length, 403 (389–421); greatest body width, 15 (13–17); stylet length, 11 (10–12); head to dorsal gland outlet, 21 (19–23); head to ventral gland outlet, 36 (34–37); head to excretory pore, 26 (23–30); head to nerve ring, 66 (65–67); tail length, 19 (17–21); tail tip to vulva, 48 (43–53); % vulva, 88 (82–93); length postuterine sac, 7 (6–8).

Body small, slender, with transverse annulations and lateral fields similar to those in third-stage juvenile; stylet distinct, the distal third often bent slightly dorsal (seen best in living specimens); stylet composed of 3 longitudinal columns (1 dorsal and 2 subventral), which bend slightly outward at base, giving the base a swollen appearance; stylet guiding ring present, located in the upper third of stylet (conus region); dorsal pharyngeal gland opening about 1 stylet length behind stylet base; subventral gland opening located approximately 3 stylet lengths behind head; pharyngeal glands extending posteriorly, normally

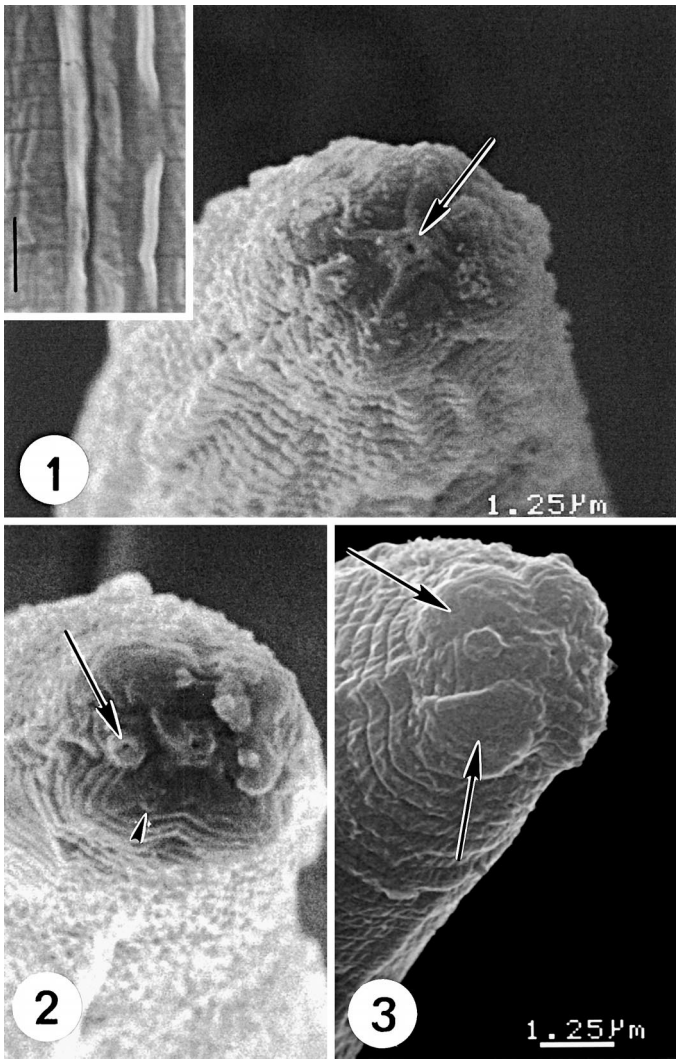

FIGURE 1–3. Scanning electron micrographs of the cephalic region of third-stage juveniles of *Metaparasitylenchus hypothenemi* n. sp. **1.** Enface view showing square basal mouth plate (arrow) and quadriradiate cephalic framework. Note annulated cuticle. Insert shows section of the lateral field with 3 raised ridges. Bar for insert = 1.4 µm. **2.** Enface view showing circular amphid (arrow) and cephalic sensilla on submedian lobe of labial region (arrowhead). Magnification same as in Figure 1. **3.** Lateral-apical view showing 2 submedial lobes (arrows) of the labial region with an amphid between them.

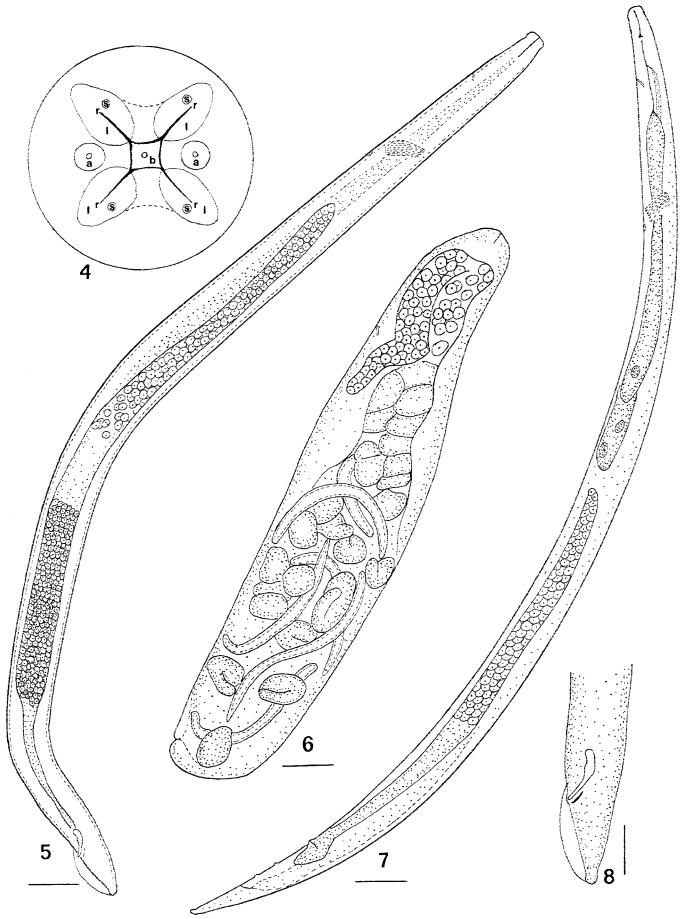

FIGURE 4–8. *Metaparasitylenchus hypothenemi* n. sp. **4.** Schematic drawing of cephalic region of third-stage juvenile (based on scanning electron micrographs). a = amphid, b = basal mouth plate, l = lobes of labial region, r = rays of cephalic framework, s = sensilla. **5.** Lateral view of free-living male. Bar = 17 µm. **6.** Lateral view of parasitic female. Bar = 40 µm. **7.** Lateral view of free-living female. Bar = 17 µm. **8.** Detail of male tail. Bar = 11 µm.

reaching and sometimes overlapping anterior portion of ovary; excretory pore opening between dorsal and ventral gland orifices; hemizonid posterior to excretory pore; vulval and anal openings distinct; vulval lips present; uterus with postuterine extension, which is less than body width at vulva; ovary outstretched; tail short, rounded, tip often slightly offset; 2 ventrally located small papillae arranged in tandem between anus and vulva; molts twice (single or double) after leaving host to become infective stage.

Free-living male (Figs. 5, 8, 12, 14) (n = 10)*:* Total body length, 293 (271–353); greatest body width, 16 (14–17); stylet length (measured along the axis of the curvature), 10 (11–12); head to excretory pore, 24 (23–26); head to nerve ring, 60 (56–64); tail length, 18 (16–19); spicule length, 10 (9–11); gubernaculum length, 3 (2.5–3.3); bursa length, 23 (21–24). Body shorter but wider than female; body with transverse striations and lateral fields similar to those of third-stage juvenile (but lateral ridges less prominent); stylet similar in length to that of female but base not swollen; pharyngeal glands not visible; testis outstretched or occasionally reflexed slightly at tip, seminal vesicle filled with developing sperm; spicules separate, slightly curved in lateral view, cephalated, with a slight ventral arch; gubernaculum short, straight; bursa peloderan, wide, reaching tail tip, cloacal opening circular; tail tip slightly offset; final 2 molts shed simultaneously.

Mature parasitic female (Figs. 6, 13) (n = 10)*:* Total body length, 510 (488–748); head to excretory pore, 8 (6–11); stylet length, 11 (10–12); greatest body width, 71 (63–79); length of eggs (n = 10), 125 (115–144); width of eggs (n = 10), 76 (64–88). Body white, straight or slightly curved (with dorsal side out); head cone absent, stylet present, normally not withdrawn into body; excretory pore near head end; vulva subterminal; anus not visible; ovoviviparous.

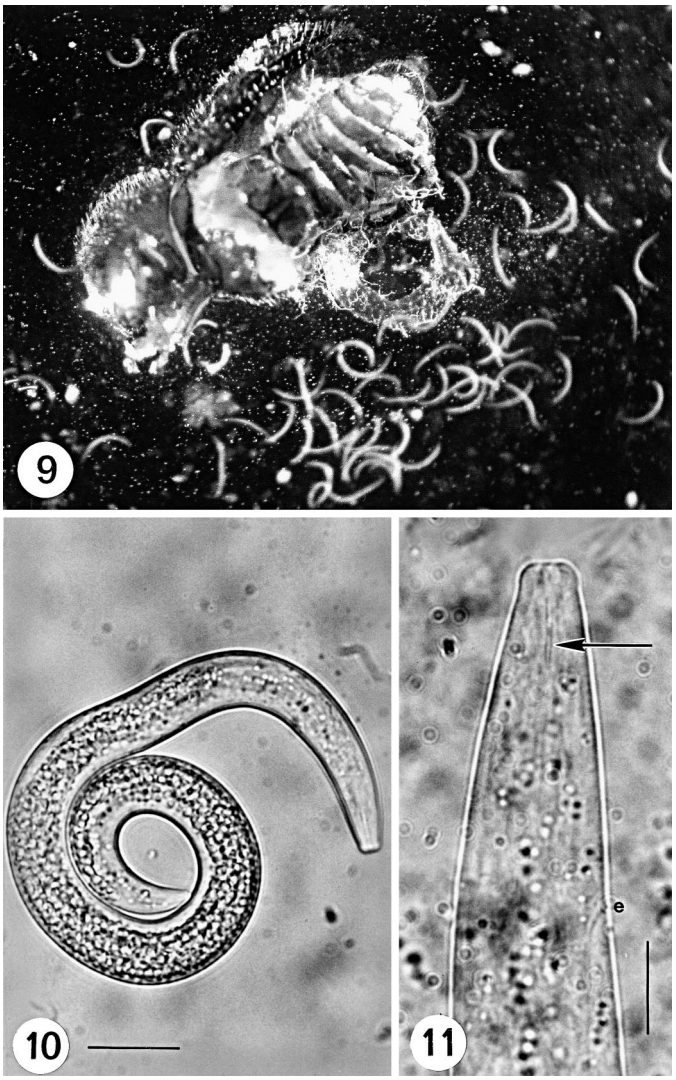

FIGURE 9–11. *Metaparasitylenchus hypothenemi* n. sp. **9.** Third-stage juveniles released from the body cavity of an adult coffee berry borer. Length of beetle = 1.7 mm. **10.** A third-stage juvenile soon after emerging from the host. Note storage granules filling gut cells. Bar = 20 μm. **11.** Anterior end of third-stage juvenile showing stylet (arrow) lacking basal swellings. Note excretory pore (e). Bar = 10 μm.

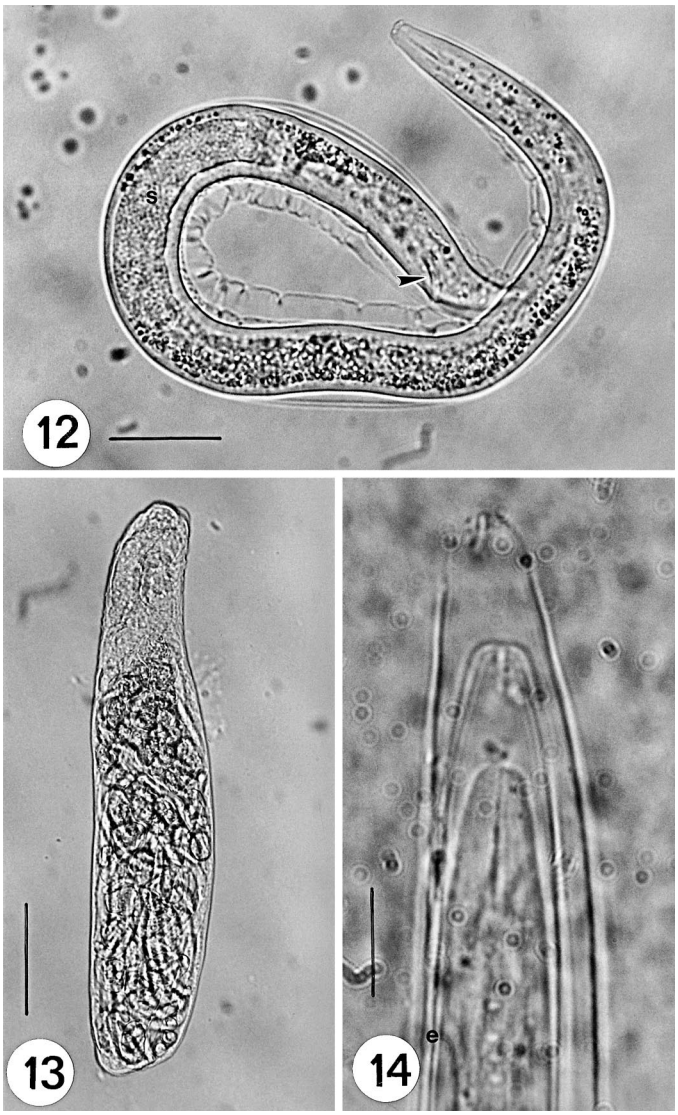

FIGURE 12–14. *Metaparasitylenchus hypothenemi* n. sp. **12.** Male in process of shedding its third- and fourth-stage cuticles simultaneously. Note cuticles surrounding the nematode, spicule (arrowhead) and sperm (s) in the seminal vesicle. Bar = 20 μm. **13.** Parasitic, ovoviviparous female showing straight body and eggs and juveniles in her uterus. Bar = 102 μm. **14.** Head of male undergoing double molt. Note that only the conus regions of the stylet remain on the shed cuticles, the absence of basal swellings on the stylet and the excretory pore (e). Bar = 10 μm.

Taxonomic summary

Type host: Hypothenemus hampei (Ferrari) (Curculionidae: Scolytinae). The parasites occur in the body cavity of the larva, pupa, and adult stages. The most noticeable effect of the parasite on the scolytid is partial or complete sterilization. Further details on infection levels and pathological effects are given by Castillo et al. (2002).

Type locality: Municipality of Cacahoatán, Chiapas, Mexico.

Holotype: Free-living female (USDANL #T-575t) and allotype (free-living male USDANL #T-576t) deposited in the USDA Nematode Laboratory, Beltsville, Maryland. Paratypes (free-living females and juveniles) deposited in El Colegio de la Frontera Sur, Carretera Antiguo Aeropuerto, km. 2.5, Tapachula 30700, Chiapas, Mexico.

Etymology: ''Hypothenemi'' is taken from the genus of the host insect, *Hypothenemus*.

Remarks

In his diagnosis of *Metaparasitylenchus*, Siddiqi (2000) stated ''no postvulval uterine sac.'' However, several of the species listed by Siddiqi as belonging to *Metaparasitylenchus*, including the type species, *Metaparasitylenchus telmatophili* (Wachek), possess a postvulval uterine sac, as does the species described in this study. Therefore, an amended diagnosis of *Metaparasitylenchus* follows: ''Members of the family Allantonematidae possessing males; free-living stages with the

excretory pore at the level of or anterior to the nerve ring; free-living females with a distinct stylet, usually knobbed or swollen at the base; free-living females may or may not possess a post-vulval uterine sac; free-living males possess a stylet (may be as long as in female but usually thinner and lacking basal knobs or swellings), 2 separate spicules, a gubernaculum, and a peloderan bursa; parasitic females with a cylindroid, straight, arcuate, or coiled body; parasitic females white (occasionally with yellow-brown deposits); stylet of parasitic female similar in structure and size to that of free-living female; third-stage juveniles normally emerge from hosts and molt twice before reaching the adult stage.''

There are currently 12 species in *Metaparasitylenchus* (Siddiqi, 2000). The majority of these were described by Wachek (1955), who provided a key for their separation. The present species differs from *M. telmatophili* (Wachek), *Metaparasitylenchus strangaliae* (Wachek), *Metaparasitylenchus rhizophagi* (Wachek), *Metaparasitylenchus helmidis* (Wachek), and *Metaparasitylenchus cossini* (Wulker), in which the excretory pore in the free-living females is found behind the opening of the subventral pharyngeal glands. The new species differs from *Metaparasitylenchus cryptophagi* (Wachek), in not having the anterior end of the mature parasitic female normally sunk into the body. The free-living stages of *Metaparasitylenchus mycetophagi* (Wachek) have a smooth cuticle (annulated in *Metaparasitylenchus hypothenemi* n. sp.). Also, the vulva of *M. mycetophagi* lacks lips, and the male has a weakly knobbed stylet and a long slender, bent gubernaculum, all of which differ from the condition in *M. hypothenemi* n. sp. The new species differs from *Metaparasitylenchus guadeloupensis* Laumond & Mauléon, 1982 in having parasitic females under 1 mm in length (over 1.5 mm in *M. guadeloupensis*). Also, *M. guadeloupenis* has no postuterine sac, and the distance from the tail to the anus in the free-living female is more than half the distance from the tail terminus to the vulva (it is less than half the distance in *M. hypothenemi* n. sp.). *Metaparasitylenchus boopini* (Wachek) has a larger parasitic female (over 1 mm in length), a larger male (from 580 to 644 μm in length), and a larger free-living female (over 500 μm in length) than *M. hypothenemi* n. sp. Also, the male of *M. boopini* has a knobbed stylet, and the free-living stages have a smooth cuticle. *Metaparasitylenchus hypothenemi* n. sp. differs from *Metaparasitylenchus leperisini* (Massey, 1974) in having a much longer tail in the free-living stages, with the distance from the tail terminus to the anus more than half the distance from the tail terminus to the vulva. The male of *M. leperisini* also has prominent stylet knobs. *Metaparasitylenchus oschei* (Rühm) (Rühm, 1956), which is known only from parasitic females, has larger parasitic females (over 2 mm in length) than *M. hypothenemi* n. sp. and parasitizes members of Rhizophagidae (Coleoptera).

DISCUSSION

The life cycle of *M. hypothenemi* n. sp. appears to have a typical allantonematid development, with infection initiated by the fertilized free-living females. Fertilization probably occurs in the coffee berry. The ratio of free-living males to females was approximately 1:2. Entry into the host larvae is probably by direct penetration of the cuticle. After reaching the hemocoel, the females began to enlarge and the eggs initiate development. The parasitic females are carried into the pupal and adult stages of the beetle. The eggs hatch inside the parasitic female, and the first molt is initiated within the egg with the cast cuticle remaining in the eggshell or being carried out of the egg and deposited in the uterus of the parasitic female. A second molt occurs inside the host beetle after the juveniles leave the parasitic female. Exit from the host by third-stage juveniles occurs through the alimentary and genital tracts of adult beetles. The nematodes molt 2 more times before attaining sexual maturity. The females shed the third and fourth cuticles either separately or simultaneously, whereas the males always shed them simultaneously (Figs. 12, 14). All juvenile stages, including the first, are equipped with a stylet.

Metaparasitylenchus species parasitize only Coleoptera, especially wood-boring beetles. It is interesting that *M. hypothenemi* n. sp. has many characters of the closely related *Formicitylenchus* Poinar (2003), including an excretory pore anterior to the nerve ring, an ovoviviparous, parasitic female, a free-living female with basal stylet swellings, and a male lacking basal stylet swellings. However, the parasitic females of *Formicitylenchus* are yellow and not white, and the male has a short, crenulate leptoderan bursa. Although the insect hosts of *Formicitylenchus* are carpenter ants, it is possible that this latter clade evolved from a *Metaparasitylenchus* line as a result of environmental host selection (Poinar, 2003).

This is the first description of a tylenchid parasite of the coffee berry borer. In India, some populations of this beetle are parasitized by an undescribed species of *Panagrolaimus* (Varaprasad et al., 1994). A species of this genus has also been found in association with *H. hampei* in Chiapas and is currently under study. It would be interesting if the Indian and Mexican species were conspecific, which brings up the question of the origin of *M. hypothenemi* n. sp. Because the coffee berry borer is endemic to Central Africa (Le Pelley, 1968), did it bring the nematode from the Old World or was the infection acquired in the New World?

Metaparasitylenchus hypothenemi n. sp. appears to be widely distributed in coffee plantations in Chiapas, with infected beetles recovered from 11 of 24 sampled sites. The nematode has also been recovered from parasitized coffee borer beetles in Honduras (A. Castillo, pers. obs.). The effects of the nematode on the coffee berry borer were discussed previously (Castillo et al., 2002) and include partial or complete sterilization, depending on the number of parasitic females present. The sterility effect has been used to detect parasitized beetles in the field. When infested coffee berries are found with female beetles lacking progeny, the insects are usually found to be parasitized by *M. hypothenemi* n. sp. Because the coffee berry borer is such an important pest of coffee and *M. hypothenemi* n. sp. partially or completely sterilizes the female beetles, it is worthy of further investigation as a potential biological control agent.

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