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Note

First field report of a nematode (Tylenchida: Sphaerularioidea) attacking the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) in the Americas

The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae), is the most important pest of coffee throughout the world and is present in most coffee producing countries (Le Pelley, 1968). The insect is very difficult to control due to its cryptic life cycle which involves egg laying inside the coffee berry, followed by the emergence of adult females that are already inseminated by their siblings. Once a female emerges from the berry, it promptly locates another berry and starts boring a hole, thus, limiting the time in which control techniques can be implemented. Among these, chemical control is limited due to human and environmental concerns, the lack of financial incentives to use this type of control as a result of the prevailing low coffee prices (Cárdenas, 2001), and reports of resistance to endosulfan, one of the most commonly used insecticides against the coffee berry borer (Brun et al., 1989). Several biological control agents, including parasitoids and fungal entomopathogens, have been reported in the field as natural enemies of the coffee berry borer (Murphy and Moore, 1990; Vega et al., 1999), but reports on nematodes are much more limited. The only field report of coffee berry borer infection by a nematode comes from India, where *Panagrolaimus* sp. (Rhabditida: Panagrolamidae) was found attacking the coffee berry borer (Varaprasad et al., 1994). In the laboratory, *Heterorhabditis* sp. and *Steinernema* sp. have been shown to cause high coffee berry borer mortality (Allard and Moore, 1989; Castillo and Marbán-Mendoza, 1996), but these nematodes have never been recovered from field-collected insects. Here we report on a different nematode species attacking the coffee berry borer in a Mexican coffee plantation.

While studying the biology of the coffee berry borer in field plantations in the municipality of Cacahoatan, Chiapas, it was noticed that some adult insects collected at "Rancho El Paraíso" (N 15°00′27.6″; W 92°09′51.2″; 564 m above sea level; Fig. 1) were infected with nematodes. This led to additional sampling with the objective of identifying the nematode, determining parasitization rates during the coffee cycle, and determining its effect on the insect. Samples consisted of 100 coffee berries exhibiting the characteristic entry hole bored by the insect; these were randomly collected in the plantation where the nematode was observed for the first time. Sampling was done on a monthly basis (1997) and twice in June; however, during April and May, when berries are not present on the plant, samples were taken from the soil (only 50 berries were collected due to their scarcity). Berries were dissected in the laboratory to determine the number of coffee berry borers within the berry. To determine survival and fecundity of parasitized and unparasitized insects, these were individually disinfected in 2% formol to avoid fungal contamination and placed individually in glass vials (7×1.5 cm) containing coffee berry borer artificial diet (Villacorta and Barrera, 1993).

Parasitized coffee berry borers were dissected under the stereoscope to obtain the nematodes. Male nematodes were never isolated, suggesting that females are the only ones that parasitize the insect and that males are free living, as has been reported for members of the Sphaerularioidea (Siddiqi, 2000). The lack of males has been a limiting factor in the identification of the specimens as these are necessary for taxonomic identification. Nevertheless, based on the morphological characteristics observed in the females, the nematode was placed in the genus *Sphaerulariopsis* sp. nov. An interesting aspect of this nematode's biology is the production of an uterium, i.e., an everted uterus containing the eggs (Fig. 2A). Once the uterus is everted, the female shrivels and dies (Siddiqi, 2000). This phenomenon has also been reported for other Sphaerulariids, e.g., *Sphaerularia bombi* infesting bumblebees and bark beetles (Khan, 1957) and *Tripius sciarae* (Bovien) infesting sciarid flies (Poinar, 1965).

Out of 1000 berries collected on 11 dates, 640 were insect free. A total of 543 insects was obtained from 360 berries containing coffee berry borers, with 59 of these parasitized with nematodes (10.9% infection rate), and 9 of these dead at sampling time (15.3% mortality; Table 1). The coffee berry borer exhibits a skewed sex ratio (10:1) favoring females; all insects parasitized with nematodes were females. Nematodes were found as adults and juveniles in a range of 6 (April)–163 (July)

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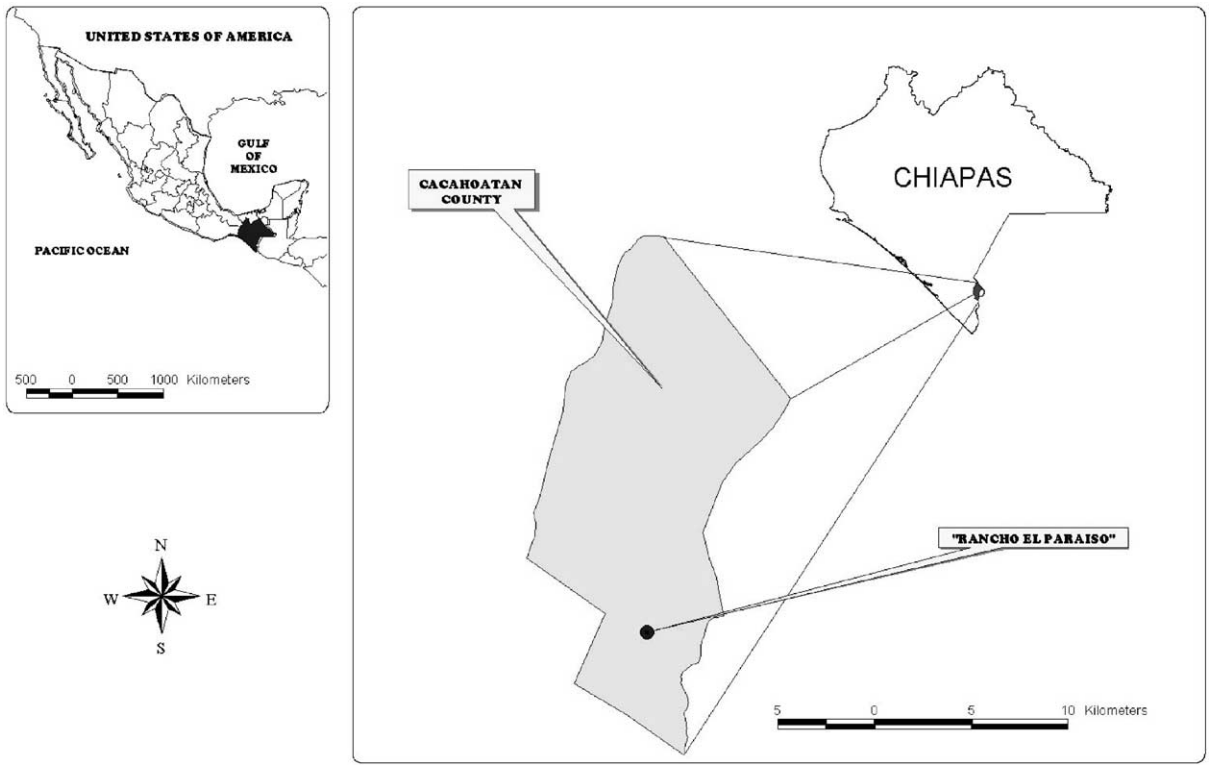

Fig. 1. Map showing location in Mexico where parasitized insects were collected.

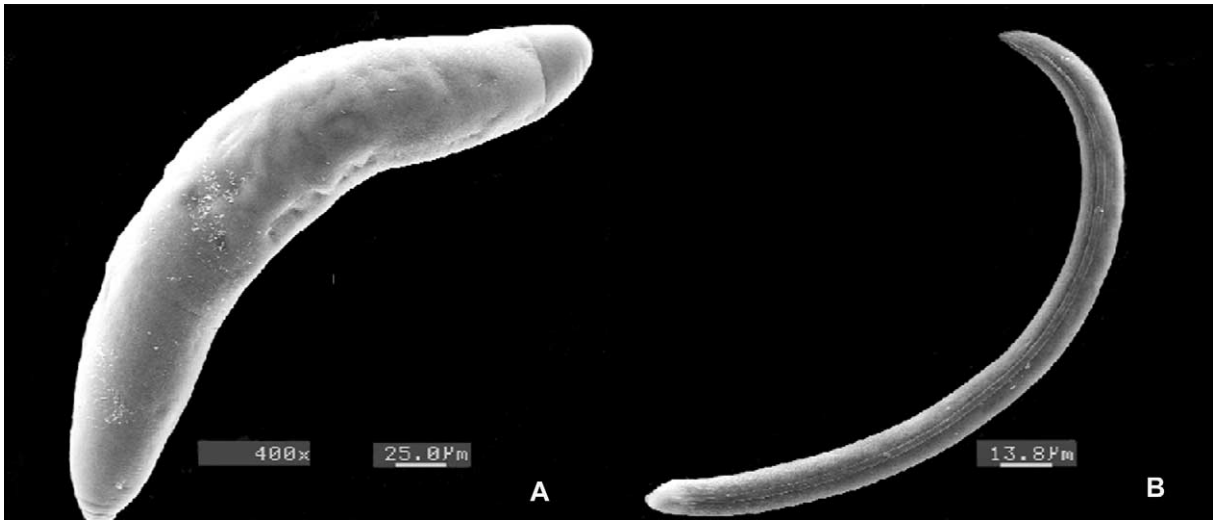

Fig. 2. Microphotographs of the nematode found parasitizing the coffee berry borer: (A) uterium; (B) infective juvenile.

per parasitized insect and an overall average of 55 nematodes per insect. Fecundity of coffee berry borers was significantly reduced in individuals infected with nematodes ($\chi^2 = 30.4$, $df = 1$, $p < 0.001$). The average number of eggs laid by coffee berry borers was lower when parasitized with nematodes (mean = 1.7 eggs) than in non-parasitized insects (mean = 10.7 eggs) which leads us to believe that nematodes progressively affect reproductive organs and reduce reproductive capacity. A reduction in fecundity has also been reported for other insects infected by Sphaerulariid nematodes (Ashraf and Berryman, 1970a,b; Kaya, 1984; Thong and Webster, 1975). We lack data to determine whether nematodes have an effect on longevity, although the low

Table 1
Survival and fecundity of coffee berry borer infected with nematodes

Sampling date (1997)	Parasitized insects		Parasitized insects		Mean # eggs laid per	
	Alive	Dead	With progeny	w/o progeny	Parasitized insect	Unparasitized insect
March 17	0	0	0	0	0	23
April 4	0	0	0	0	0	4
146	6	0	1	5	0.8	10.3
June 5	7	0	3	4	6.5	33.3
June 25	11	2	2	9	1.3	11.8
July 11	14	5	7	7	4.3	6.5
August 1	4	2	0	4	0	4.5
September 5	5	0	3	2	6	9.3
October 24	2	0	0	2	0	7.1
November 21	1	0	0	1	0	8.2
December 5	0	0	0	0	0	0

level of mortality in parasitized insects (15.3%) suggests that this nematode does not cause high mortality. No nematodes were detected in insects originating from berries collected from the leaf litter, although we believe this is where infection occurs. Assuming this is correct, and since all parasitized insects were females, we hypothesize that females are responsible for carrying the nematode up to the berry once they leave coffee berries within the leaf litter, since males do not fly and never leave the berry (Le Pelley, 1968).

Even though we still do not know the potential for nematodes to act as significant biological control agents against the coffee berry borer, this report expands on the number of natural enemies attacking this insect. This discovery constitutes the first record of a nematode attacking the coffee berry borer under natural conditions in the Americas and the second on a worldwide basis.

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