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Research Note

Observations of *Steinernema* Nematode and Tachinid Fly Parasites in Horned Passalus Beetles, *Odontotaenius disjunctus*, from Georgia, U.S.A.

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ABSTRACT: This report describes a series of observations made on 2 parasite species infecting a collection of horned passalus beetles (*Odontotaenius disjunctus*, $n = 135$), from Georgia, U.S.A., that were collected as larvae in the wild and were reared to metamorphosis in captivity. Tachinid fly maggots emerged from 15 beetle larvae (11%) and, when they completed their development into adults, we identified them as *Zelia vertebrata*, a known but little-studied parasitoid of *O. disjunctus*. We also observed infections by *Steinernema* nematodes ($n = 11$ cases, or 8%), which killed and consumed the carcasses and which produced thousands of infective juveniles in the rearing containers. Based on morphological measurements of infective juvenile and adult worms, these nematodes were consistent with *Steinernema carpocapsae*, members of which infest the surface of soils and which infect a wide range of insects. This report is the first to describe *Steinernema* infections in *O. disjunctus*, and our observations of *Z. vertebrata* infections and prevalence will be useful for comparative purposes or future study.

KEY WORDS: horned passalus beetle, *Odontotaenius disjunctus*, *Zelia vertebrata*, *Steinernema*, captive-rearing.

The horned passalus beetle (*Odontotaenius disjunctus*) is a medium-sized, saproxylic beetle native to the eastern United States. It creates and lives in galleries in rotten hardwood logs on the forest floor and during the summer the adults raise their young in these galleries (Pearse et al., 1936). Adult horned passalus beetles are host to a wide variety of parasites including ectoparasitic mites, gut-dwelling protists, and two nematodes, one inhabiting the intestine (*Histrignathus rigidus*) and the other (*Chondronema passali*) living in the body cavity (Reinert, 1973). Parasites of young beetles are less studied, but a tachinid fly parasitoid, *Zelia vertebrata*, has been observed when wild-caught larvae are reared in captivity (Mangrum, 1942; Gray, 1946; Eoff, 1968). In this research note, we describe a series of observations on 2 parasites infecting a collection of beetle larvae originating from a site in northeast Georgia, U.S.A. These observations should prove useful for comparative purposes as well as for advancing knowledge of the parasite pressure faced by larval stages of passalus beetles.

Beetle collection and housing. On 15 July 2015, larval *O. disjunctus* (i.e., grubs) were collected from a forested site near the University of Georgia campus in Athens, Georgia, U.S.A. Larvae were extracted from rotten logs using hatchets and placed in plastic containers filled with wood pieces from the same log for transport to the laboratory. A total of 135 larvae were collected, all of which were in the 2nd or 3rd instar stage. In the laboratory each larva was placed in a 280-ml transparent plastic container to which a wood substrate was added to serve as a food source (Fig. 1). This substrate consisted of moistened hardwood from a rotten log that had been pulverized into a soft mash with a rubber mallet. Mash was added to each container to fill the bottom third to provide enough to last each larva until pupation (Davis, personal observation). Prior studies had suggested that *O. disjunctus* larvae may not be capable of feeding independently of their parents, as the adults prepare triturated wood pulp for them (Gray, 1946); however, our setup appeared to be equally effective because all larvae (except those parasitized) successfully developed into adult beetles (Figs. 2–4). All containers were inspected daily without disturbing the larvae in order to monitor survival.

Tachinid parasites. Tachinid fly maggots began emerging from beetle larvae within 1 wk of collection. The exact moment of emergence was not witnessed in any infection; rather, in each case the beetle larva was found dead with a large hole in the carcass (see Fig. 5), and when the food substrate was sifted either the fly maggot was found or, in rare cases, the already-formed puparium (Fig. 5 inset). Maggots or puparia (or both) were placed in individual (covered) Petri dishes until completion of development to adult flies. A total of 15 tachinid flies were collected, indicating that 11% of the total beetle larvae collection had been infected. Prevalence was lower than that reported from collections in Ohio (21%; Eoff, 1968) and North Carolina (33%; Gray, 1946).

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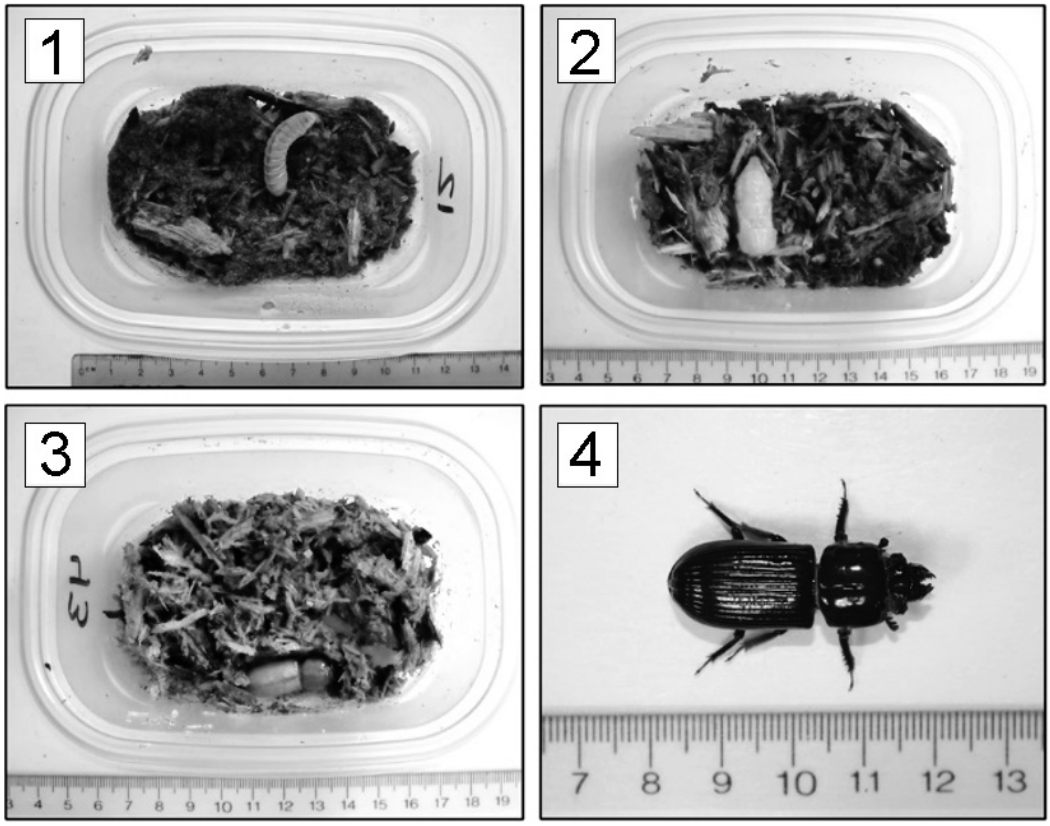
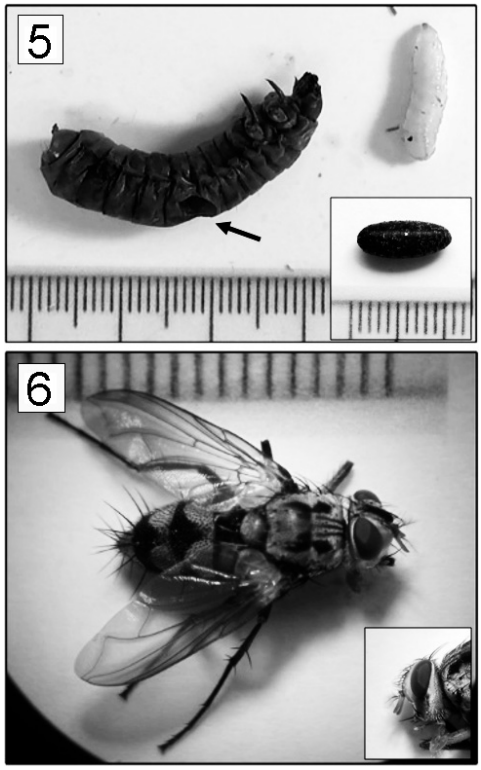


Figures 1–4. Rearing conditions and stages of *Odontotaenius disjunctus*. The food substrate consisted of pulverized, moistened wood from a rotten log. Four developmental stages are depicted including: **1.** larval form; **2.** pupa, recently formed; **3.** soft-shelled adult; and **4.** fully formed adult beetle.

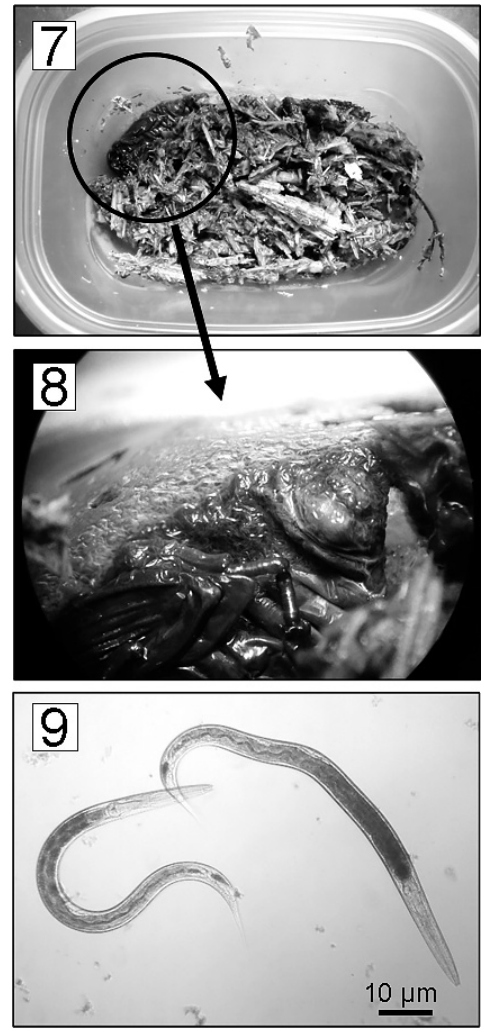
The tachinid maggots were white, rugous in appearance, and approximately 12–15 mm long (Fig. 5). The puparia were dark brown, approximately 10–12 mm long and 3–4 mm wide. Adult flies generally emerged from the puparia within 2 wk (Fig. 6). Based on the morphological characteristics of the maggots, puparia, and the overall appearance of the adults, including the wing venation pattern, these were identified as *Zelia vertebrata*, a known parasitoid of *O. disjunctus* (Mangrum, 1942; Eoff, 1968).

Steinernema *sp.* Within 2 wk of collection, additional mortality not due to Tachinid flies (i.e., no holes in the carcass) was observed in the beetle larva, pupae, or both. Inspection of the carcasses using a dissecting microscope revealed large numbers of nematodes (Figs. 7–8). Given that the nematodes were apparently feeding off of the beetle carcass, it was assumed they were a type of *Steinernema*. The affected containers were segregated for monitoring and to determine the species (see below). In all cases, the nematodes consumed the carcass within 1–2 wk, leaving only parts of the exoskeleton. Moreover, thousands of infective juveniles, which displayed the waving, nictitating behavior characteristic of members of the *Steinernema* (Lewis et al., 2006), were observed throughout the containers. A total of 11 beetles (8%) were infected.

Identification of species of *Steinernema* requires measurement of both infective juveniles and adults from an active infection (Nguyen and Smart, 1996). Ten randomly selected infective juveniles were separated from 1 container with the aid of a dissecting microscope, using fine forceps, and mounted on glass microscope slides. Images of the worms for measurement were obtained under × 100 (Fig. 9) using image analysis software (ImageJ, https://imagej.nih.gov/ij/index.html). The average length was 543 µm (range = 425–635). Ten adult nematodes were collected from 1 beetle larvae during the midst of an active infection and these were mounted on glass slides for



Figures 5, 6. **5.** Carcass of beetle larva from which a Tachinid maggot emerged. Note the large hole in the carcass (arrow). Inset shows puparium of the Tachinid. **6.** Adult Tachinid fly, *Zelia vertebrata*. Inset shows fly mouthparts.



Figures 7–9. Infection of beetle pupa with *Steinernema* nematodes. **7.** Infections killed the host which, on closer inspection, could be seen covered with thousands of live nematodes that consumed the carcass. **8.** Surrounding substrate in the container was infiltrated with infective juveniles after carcass was devoured. **9.** Infective juveniles which displayed waving, nictitating behavior.

measurement of their spicules. The average spicule length was 65 µm (range = 55–74). Further, male nematodes had a prominent posterior mucron and females did not have a double-flapped epiptygma. Based on a taxonomic key to the genus (Nguyen and Smart, 1996), these measurements and characteristics are consistent with *Steinernema carpocapsae*. *Steinernema carpocapsae* is well-studied because of its applications for biocontrol of insect pests (e.g., Journey and Ostlie, 2000) and is a known parasite of soil- and surface-dwelling beetles (Forschler and Gardner, 1991).

It should be noted that the *Steinernema* infections observed by us likely originated either as the result of the presence of infective juveniles in the mash–food substrate used to rear the grubs or from their presence in the original wood used during transport to the laboratory. While these juveniles are usually associated with soils, it is possible the nematodes had crawled from the soil to the log before we used it. In fact, we often find passalus beetles at the bottom of logs (where the log meets the soil surface), likely because passalus beetles prefer moist conditions (Davis, personal observation), thus providing opportunity for contact with soil-dwelling nematodes. In any case, this report is the first observation of *Steinernema* nematodes infecting *O. disjunctus*.

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