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FIRST REPORT OF A MERMITHID NEMATODE INFECTING *PIEZODORUS GUILDINII* AND *ACROSTERNUM HILARE* (HEMIPTERA: PENTATOMIDAE) IN THE UNITED STATES

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Stink bugs (Hemiptera: Pentatomidae) are important economic pests of many agricultural crops, and have become one of the most difficult pest complexes to control in field crops, vegetables and fruit trees (McPherson & McPherson 2000). Stink bugs usually attack developing fruiting structures, and direct damage to the fruit is caused by insertion of their stylets to feed on plant fluids. In soybeans (*Glycine max* (L.) Merr.), stink bug feeding can cause delayed maturity and reduce seed quality, yield and germination (Underhill 1934; Chyen et al. 1992).

The adoption of an early soybean production system has resulted in an increase in stink bug populations (Baur et al. 2000). The stink bug complex in the south contains the native species *Euschistus servus* (Say) (brown stink bug), *Nezara viridula* (L.) (southern green stink bug) and *Acrosternum hilare* (Say) (green stink bug). (Taxonomists have proposed changing the name *A. hilare* to *Chinavia hilaris* (Say), but the Entomological Society of America has not approved this yet [David Rider, personal communication].) Historically, the southern green stink bug has had the greatest economic impact on southern USA soybean producers (Panizzi & Slansky 1985). Prior to 2000, *Piezodorus guildinii* (Westwood) (redbanded stink bug) had never been an economic threat to soybean production in the U.S., although it has long been a serious pest in South America (Panizzi 1985). The redbanded stink bug has currently spread throughout Louisiana and is the dominant stink bug species, comprising 59 to 72% of the total stink bugs found in soybean throughout the growing season (Temple et al. 2011a).

Stink bug management can require multiple applications of broad-spectrum organophosphate or pyrethroid insecticides. The redbanded stink bug is very tolerant of many products available for stink bug control on soybean and, consequently, insecticide applications have significantly increased (Temple et al. 2011b). Further, these insecticides applications negatively impact natural enemies, and may lead to population resurgences of other soybean pests (Panizzi & Slansky 1985).

As a result, we are always watchful for possible biological control organisms that may reduce stink bug populations.

During the summer of 2009, approximately 100 stink bugs were collected for bioassays from Louisiana soybean fields using 38-cm-diam sweep nets and returned to the laboratory. After about 24 h, mermithid nematodes were found to have emerged from field-collected adult, *A. hilare* and *P. guildinii*. The nematode from *P. guildinii* emerged on 6 Oct 2009, and had been collected from Ben Hur Research Station in Baton Rouge (30°22'14.3538"N -91°9'52.236"W) (Figs. 1A and 1B). The nematode collected from *A. hilare* emerged on 30 Sep 2009 (Fig. 1C). It was collected at Macon Ridge Research Station in Winnsboro (32°8'1.6542" N -91°41'57.5484" W). An additional specimen was discovered in a 1992 insect collection emerging from a 4th instar nymph of *A. hilare* (Fig. 1D) that had been preserved in 90% alcohol. This sample was collected 23 Sep 1992 from the Rice Research Station in Crowley (30°4'22.0416"N -92°20'54.2112"W). The rainfall for the 4 wk before emergence for all nematode infections was approximately 4.5-cm above average. The increased precipitation may have made the environment more conducive to the nematode's survival.

Pictures of the nematode infecting the *A. hilare* adult were taken using a differential interference contrast (DIC) optics microscope (Olympus BX51, Olympus Corp. USA) (Fig. 2A, 2B) before being identified to genus via DNA sequence analysis. The nematode DNA was extracted following methods described in Dorris et al. (2002). Species identification was conducted by amplifying 18S rDNA following Vandergast & Riderick (2003) using the primers 18S-5F (5'GCGAAAGCATTTC-CCCAAGAA) and 18S-9R (5'GATCCTTCCG-CAGGTTACCT). PCR products were treated with EXOSAP-it (GE Healthcare, USA) prior to sequencing. Sequencing was conducted by the University of Arizona Genomic Analysis and Technology Core Facility, and the sequence will be submitted to the GenBank. The analysis and the caudal appendage on the posterior end of the

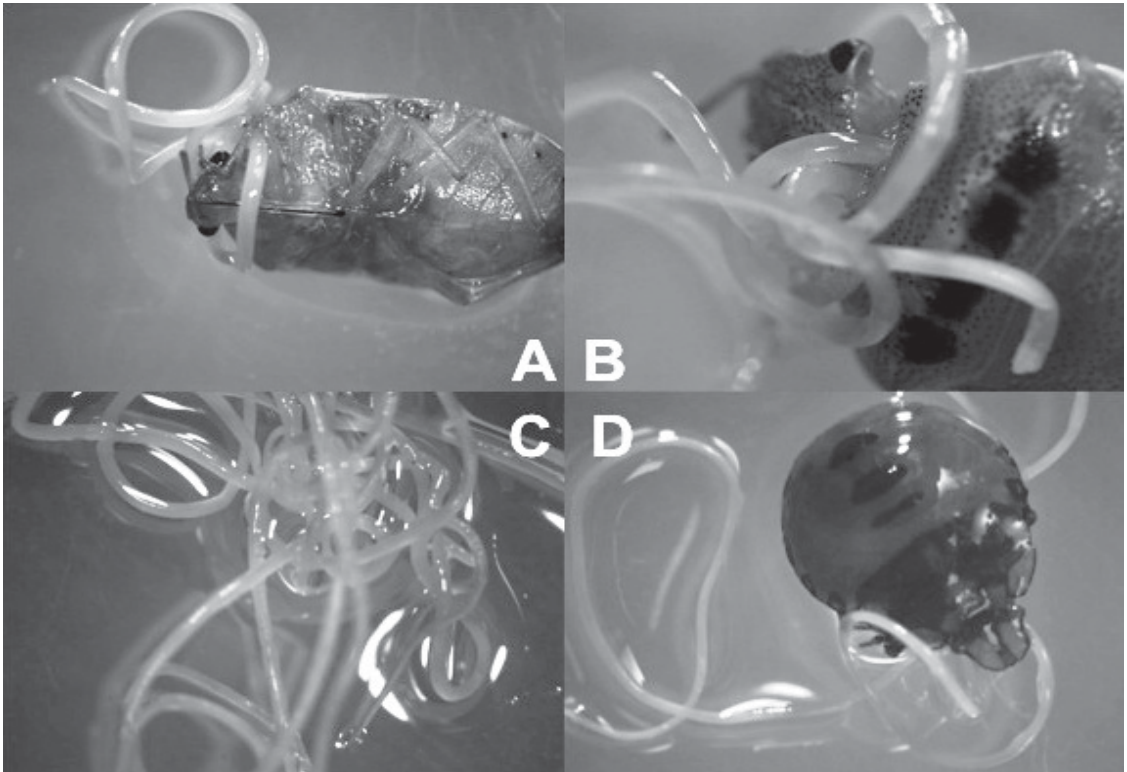


Fig. 1. Nematodes infesting Louisiana stink bugs. Figs. 1A, 1B. The mermithid nematode that emerged from *Piezodorus guildinii*. Fig. 1C. The mermithid nematode that emerged from an *Acrosternum hilare* adult. Fig. 1D. The *A. hilare* nymph from a 1992 insect collection with a mermithid nematode.

nematode (Fig. 2B) indicate that the nematode belongs in the genus *Hexameris*. A Basic Local Alignment Search Tool (BLAST) search through GenBank denoted that the sequence (696 bp) has the closest match to *Hexameris agrotis* Wang et al. (RefSeq DQ_530350.1) 18S ribosomal RNA gene. However, further studies, both at the molecular and morphological levels, are needed to complete the identification of this mermithid species.

Since the 1930s, nematodes have been utilized as insect biological control agents (Smart 1995). Nematodes have been used to control pests in multiple environments including aquatic habitats, plant canopies, and soil surfaces. Location, humidity, and temperature are the most significant factors for nematode survivorship (Arthurs et al. 2004). Nematodes require high humidity and cool temperatures to survive, therefore, they are generally applied to control aquatic and soil inhabiting insects. However, some nematodes have been found in foliar habitats. This environment is more stressful and can result in mortality because high temperatures and sunlight can lead to desiccation. Nonetheless, studies have reported successful attempts in the use of ento-

mopathogenic nematodes to reduce populations of pests belonging to the Lepidoptera, Diptera and Coleoptera (Popiel 1992; Arthurs et al. 2004).

Interest in improving the management of pests of many fruiting crops with biological control agents has resulted in research examining the susceptibility of stink bugs to nematodes. The southern green stink bug was reported as being very susceptible to Steinernematidae under laboratory conditions (Wassink & Poinar 1984). Also, mermithids were discovered infesting *N. viridula* (*Pentatomimermis* sp.) in Russia (Rubtsov 1977) and India (Bhatnagar et al. 1985), and *P. guildinii* (*Hexameris* or *Mermis* sp.) in Uruguay (Riberiro & Castiglioni 2008). These documented nematode populations were small and did not reduce field populations of stink bugs. A survey in Louisiana of parasitoids infesting *N. viridula* by Fuxa et al. (2000) indicated that only about 2% were infected with a mermithid nematode.

The genus *Hexameris* has been found infesting other insects, such as the brown planthopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) (Satpathi 2009), brown-tail moth, *Euproctis chrysorrhoea* (L.) (Lepidoptera: Lymantriidae) (Nikdel et al. 2008), *Leptinotarsa decemlineata*



Fig. 2. Nematode infesting *Acrosternum hilare* adult as seen with a differential interference contrast optics microscope. 2A. Anterior end of the mermithid nematode. 2B. Posterior end of the mermithid nematode showing the caudal appendage that is common in the genus *Hexamermis*

(Coleoptera: Chrysomelidae) (Bozhkov & Kaitazov 1976), and the horned beetle, *Diloboderus abderus* Stur (Coleoptera: Scarabaeidae) (Achinelly & Camino 2008). Other stink bugs have also been infected with *Hexamermis* sp. These include *Rhaphigaster nebulosa* Poda (Manachini & Landi 2003) and the bark bug, *Halys dentatus* Fabricius (Dhiman & Yadav 2004). *Halys dentatus* was discovered to have about 9% parasitism by nematodes during a parasitoid survey in India (Dhiman & Yadav 2004). Parasitism levels are typically low for these insects, but may be a consistent contributor to natural mortality. Future research needs to focus on incidence and life cycles in relation to stink bug parasitism.

SUMMARY

A mermithid nematode emerged from stink bugs, *Acrosternum hilare* and *Piezodorus guildinii*, collected from Louisiana soybean fields. Photographs of this parasite are presented. An analysis conducted by amplifying 18S rDNA revealed that the nematode belongs to the genus *Hexamer-*

mis. A BLAST search indicated that the sequence (696 bp) has the closest match to *Hexamermis agrotis* Wang et al. 18S ribosomal RNA gene. This is the first report of a *Hexamermis* sp. infecting *A. hilare* worldwide and a mermithid infecting *P. guildinii* in the United States.

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