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# NOTE

# Discovery of *Thelohania solenopsae* from the Red Imported Fire Ant, *Solenopsis invicta*, in the United States

The red imported fire ant *Solenopsis invicta* (Hymenoptera: Formicidae) was introduced into the United States from South America in the 1930s. It infests over 112 million hectares in the United States and is a serious medical and agricultural pest (Lofgren, 1986). During the last several decades numerous surveys have been made in South America and throughout the southern United States for potential biological control organisms of fire ants (Jouvenaz *et al.*, 1977, 1981). A limited number of organisms have been identified mainly in South America. Porter *et al.* (1992) indicated that fire ants are much more abundant in the United States than in South America, which is consistent with the hypothesis that *S. invicta* was introduced without any major natural enemies (Jouvenaz *et al.*, 1977). This suggests that North American fire ant populations may be amendable to classical biological control techniques.

The microsporidium *Thelohania solenopsae* (Microsporida: Thelohaniidae) was described from *S. invicta* in Brazil (Knell *et al.*, 1977). It was also found infecting colonies of the black imported fire ant *Solenopsis richteri* in Argentina. Based on a comparative morphological and molecular study, Moser (1995) concluded that the microsporidia from the red and black imported fire ants were conspecific. This obligate intracellular pathogen was discovered by Allen and Buren (1974) in alcohol-preserved specimens of *S. invicta* collected in Mato Grosso, Brazil, in 1973. Jouvenaz *et al.* (1981) found it to be one of the most common pathogens in fire ants in Brazil and Argentina. Field studies on populations of *S. richteri* in Argentina indicated that reduced densities of this ant were associated with the presence of this pathogen (Briano *et al.*, 1995a,b).

During a survey of 10 polygyne field-collected colonies, we discovered a microsporidium in workers of *S. invicta* found along a roadside (US 441) by Payne's Prairie 8 km south of Gainesville, Florida, USA. Individuals of different castes and stages were randomly sampled from each of the infected colonies. Individual worker ants and queens were examined for the presence of microsporidian spores by smearing the contents of each gaster on a microscope slide, adding a drop of water, placing a coverslip over the mixture and examining under the microscope. Vegetative stages of the microsporidium were examined with Giemsa-stained smears of 4th instar larvae and workers of imported fire ants by microscopic examination (400 and 1000×).

The microsporidian species isolated from *S. invicta* in Florida was determined to be dimorphic with features similar to those described for *T. solenopsae* (Knell *et al.*, 1977). The predominant sporulation sequence in adult workers was octosporoblastic and resulted in the formation of eight spores (meiospores) within a sporophorous vesicle (Fig. 1). Meiospores ($n = 25$) had a length and width (mean ± SE) of 3.72 ± 0.05 and 2.01 ± 0.02 $\mu$m, respectively. Moser (1995) reported meiospore measurements for *T. solenopsae* of 4.1 ± 0.31 × 2.32 ± 0.14 from *S. richteri* workers from Argentina. An unexpected number of abnormal (irregular shaped) meiospores were also observed (Fig. 1). The second sporulation sequence involved the formation of binucleate (free) spores as previously described for *T. solenopsae* (Knell *et al.*, 1977). Free spores were rare (<1%) and were not measured. Meiospores and free spores were not present in larvae or pupae with only vegetative development observed.

In addition to the morphological data, we determined the sequence of the 16s rRNA gene of the Florida microsporidium using the protocols of Moser (1995). Analysis of the 16s rRNA genes of microsporidia has proven to be useful in taxonomic studies of the microsporidia (Baker *et al.*, 1995). Sequence comparisons of the 16s rRNA genes of *T. solenopsae* (Moser, 1995) and the microsporidium found in *S. invicta* populations in Florida were almost identical (unpublished observations). Therefore, as indicated by the species diagnostics described above, we believe we have the first evidence of *T. solenopsae* infection in *S. invicta* in the U.S.

Following the discovery of the microsporidium, we used a survey procedure to collect samples of workers which involved placing glass scintillation vials (30 ml) coated with Fluon (to prevent ants from escaping) into fire ant mounds for 20 min (Banks *et al.*, 1981). After the discovery of infected workers, 30 colonies were excavated from the Payne's Prairie area and taken to the laboratory where ants were separated from the soil by flotation (Banks *et al.*, 1981). To detect the percentage of colonies infected, a sample containing 50–100 workers was ground in a glass tissue grinder with ca. 1



0022-2011/98

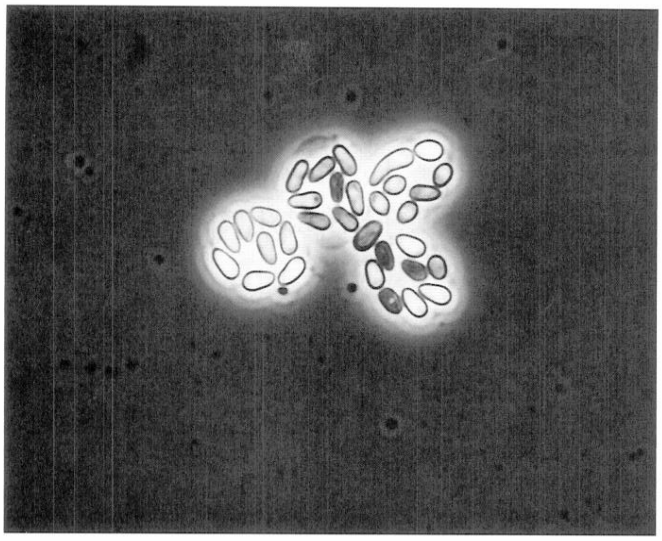

**FIG. 1.** Photomicrograph of meiospores of the microsporidium found in *Solenopsis invicta* workers (fresh, magn. 500×).

ml of water; one drop of the aqueous extract was examined by phase-contrast microscopy (400×) for the presence of spores.

A total of 379 colonies was excavated in north central Florida and 86 (23%) of these were infected. This is not unlike the infection rate in Argentina which over a 4-year period, ranged from 22.4 to 35.7% (Briano *et al.*, 1995b). Within infected colonies, workers (43 of 60) had the highest infection rate (72%) followed by larvae (54% of 78) and queens (31% of 16). We also examined polygynous colonies from Hurley, MS, the site from which polygynous colonies were first reported in the U.S. (Glancey *et al.*, 1973) (3 of 8 colonies were infected), Gulfport, MS (1 of 8 colonies was infected), and Thorndale, TX (5 of 7 colonies were infected). In addition, other ant species were also examined: *S. geminata* (15 colonies), *Dorymyrmex bureni* (9), *Pheidole metallescens* (1), *Pheidole moerens* (1), *Camponotus floridanus* (1), *Trachymyrmex septentrionalis* (1), and *Brachymyrmex depilis* (1); all were negative for the microsporidium.

At the present time, only polygynous *S. invicta* colonies have been found to be infected with *T. solenopsae*. One explanation may be that this microsporidium is horizontally transmissible and because polygynous colonies, unlike monogyne colonies, are not aggressive toward each other, workers and brood move frequently between colonies. Thus, infected colonies could easily infect healthy colonies in a polygynous fire ant population when infected workers and brood are moved to a healthy colony. This would not occur in monogyne populations. We have no direct evidence for transovarial (vertical) transmission of this microsporidium in *S. invicta* in the U.S. However, it could occur similar to the transovarial transmission of *T. solenopsae* in *S. richteri* in Argentina which is from infected queens to progeny leading to infected workers (Briano *et al.*, 1996).

This report documents for the first time a microbial pathogen in *S. invicta* in the U.S. The detrimental effects on *S. invicta* field colonies are not known at present, but of the original 30 field-collected colonies that were infected and returned to the laboratory, all are completely without brood and have only a few workers and queens remaining after 6 months. This is in contrast to healthy field-collected colonies that have not only survived in our laboratory for several years without loss of brood but have increased in size.

The origin of this microsporidium in the U.S. is unknown. Additional morphological and molecular studies are underway as well as transmission testing to determine the mechanisms involved in the spread of this pathogen within fire ant populations in North America.

Key Words: *Solenopsis invicta; Thelohania solenopsae;* fire ants; microsporidia; biological control.

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