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NOTE

The Black Imported Fire Ant, *Solenopsis richteri,* Infected with *Thelohania solenopsae*: Intracolonial Prevalence of Infection and Evidence for Transovarial Transmission

The microsporidium *Thelohania solenopsae* (Microsporida: Thelohaniidae) was described from the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), in Brazil (Knell *et al.,* 1977). A *Thelohania* sp., probably *T. solenopsae*, was also found infecting colonies of the black imported fire ant, *Solenopsis richteri,* in Argentina. Previous studies showed that this microsporidium might be a growth limiting factor of *S. richteri* colonies in Argentina and a good candidate for the biological control of both species of imported fire ants in the United States (Briano, 1993; Briano *et al.,* 1995a,b). The objective of this work was to quantify the intracolonial prevalence of *T. solenopsae* in the different castes and stages within diseased colonies of *S. richteri* in Buenos Aires Province, Argentina.

Seventy-six fire ant colonies were excavated in 1992 in pastures near Saladillo (180 km southwest of Buenos Aires), Argentina. In the laboratory the ants were separated from the soil by flotation (Banks *et al.,* 1981). To detect the infected colonies, a sample of approximately 1000 workers was ground in a glass tissue grinder with 2 ml of water; one drop of the aqueous extract was examined by phase-contrast microscopy (400×) for the presence of spores. From the 76 colonies excavated, 41 (54%) were infected. The infected colonies were maintained individually in plastic trays (40 × 30 × 15 cm) at 5°C until examination.

Varying numbers of individuals of the different castes and stages were randomly sampled from each of the infected colonies (Table 1). Each individual was examined separately for the presence of vegetative stages and/or spores (meiospores and free spores) of *T. solenopsae.* The following myrmecophiles found within the colonies were also examined: queens of the parasitic ant *Solenopsis daguerrei* (Hymenoptera: Formicidae), nymphs and adults of *Blissus parasigaster* (Hemiptera: Lygaeidae), adults of *Myrmecaphodius* sp. (Coleoptera: Scarabaeidae), adults of *Myrmecosaurus* sp. (Coleoptera: Staphylinidae), and many unidentified mites.

The vegetative stages of the microsporidium were detected by microscopic examination (400 and 1000×) of Giemsa-stained smears of whole individuals. Meiospores and free spores were quantified in major and

TABLE 1
Number (%) of Individuals of *Solenopsis richteri* and Myrmecophiles Infected with *Thelohania solenopsae*

	Number of individuals examined	Number of (%) individuals infected with		
		Vegetative stages	Meiospores	Free spores
Castes or stages				
Eggs	372	74 (20)	0	0
Larvae I–II–III	102	20 (20)	0	0
Larvae IV	113	50 (44)	0	0
Pupae	128	58 (45)	1 (1)	0
Minor workers	140	0	114 (81)	1 (0.7)
Major workers	140	0	133 (95)	9 (6.4)
Winged females	73	0	55 (75)	0
Males	15	0	12 (80)	0
Wingless females	40	0	18 (45)	0
Queens	85	1 (1)	29 (34)	0
Myrmecophiles				
S. daguerrei	8	0	1 (14)	0
B. parasigaster	100	0	1 (1)	0
Myrmecaphodius	18	0	0	0
Myrmecosaurus	10	0	0	0
Mites	23	0	0	0

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minor workers ($n = 42$). Spores from workers were extracted individually in water with a glass tissue grinder and counted with a haemocytometer (phase-contrast microscope, 400×).

Vegetative stages of *T. solenopsae* were found in eggs, larvae, pupae, and queens in 1 to 45% of the individuals examined (Table 1). The presence of vegetative stages in eggs confirmed, for the first time, transovarial (vertical) transmission of this microsporidium in *S. richteri*.

Meiospores were found in all mature stages of *S. richteri,* from 34 to 95% (Table 1). The mean number of meiospores was 6.7×10^6 per major worker (range: 1×10^4–2.9×10^7) and 9×10^5 per minor worker (range: 1×10^4–5.4×10^6) (two-sample t test; $t = -6.82$; $df = 40$; $P < 0.0001$). Similar meiospores were also found in one queen of *S. daguerrei* and in one adult bug of *B. parasigaster* (Table 1). The extremely low number of individuals infected suggests that they may not be hosts of the pathogen but have merely ingested spores.

Free spores were extremely rare; they were found in nine major workers (Table 1). The mean number of free spores was 8×10^4 per major worker (range: 3×10^4–1.4×10^5). They were also found in only one minor worker (1×10^4 spores).

The intracolonial prevalence of the microsporidium reported here for *S. richteri* agrees with the findings by Knell *et al.* (1977) of *T. solenopsae* in *S. invicta*. However, ultrastructural examination and molecular sequencing of this microsporidium from *S. richteri* are in progress to determine whether it is identical to *T. solenopsae* as described by Knell *et al.* (1977) from *S. invicta* in Brazil. The host-specificity of *T. solenopsae* must be investigated but attempts to transmit these pathogens have been unsuccessful (Jouvenaz, 1983; Briano *et al.,* unpublished data). Since the microsporidium apparently occurs in congeneric hosts (*S. invicta* and *S. richteri*) over a wide geographic range, the possibility exists that this pathogen, though morphologically appearing to be a single species, is actually a complex of two or more sibling species.

This report has documented a very high intracolonial prevalence of *T. solenopsae* within diseased colonies of *S. richteri* in Argentina. A detrimental effect of this pathogen on colonies of the black imported fire ant in Argentina was reported by Briano *et al.* (1995a,b). Before considering the introduction of *T. solenopsae* into the United States, however, its taxonomic status must be clarified. In addition, even though vertical transmission may be a highly efficient means of transmission, it is unlikely to be the sole mechanism for transmission. The mechanisms for the horizontal transmission of *T. solenopsae* within populations of fire ants must be determined. This is currently under investigation.

KEY WORDS: *Solenopsis richteri; Thelohania solenopsae;* fire ants; microsporidia; biological control.

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