Kneallhazia (=Thelohania) Solenopsae Infection Rate of Pseudacteon curvatus Flies Determined by Multiplex PCR

Authors: Valles, Steven M., Oi, David H., and Porter, Sanford D.

Source: Florida Entomologist, 92(2) : 344-349

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.092.0220

KNEALLHAZIA (=THELOHANIA) SOLENOPSAE INFECTION RATE OF *PSEUDACTEON CURVATUS* FLIES DETERMINED BY MULTIPLEX PCR

STEVEN M. VALLES, DAVID H. OI AND SANFORD D. PORTER
Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, 1600 SW 23rd Drive, Gainesville, Florida, 32608, USA

ABSTRACT

A multiplex PCR method was developed and used to determine the infection rate of *Kneallhazia solenopsae* in individual *Pseudacteon curvatus* flies in north-central Florida. Among *P. curvatus* flies infected with *K. solenopsae*, 2 amplicons were produced, one of 800 nucleotides from the *P. curvatus* 18S rRNA gene, and one of 318 nucleotides from the *K. solenopsae* 16S rRNA gene. Multiplex PCR of DNA extracted from *P. curvatus* flies was capable of detecting 117.5 ± 82.7 *K. solenopsae* spore equivalents. The mean *K. solenopsae* infection rate of *P. curvatus* from 4 sites in Gainesville and Williston, Florida, was 12.3 ± 5.0%. The *K. solenopsae* infection rate for *P. curvatus* was independent of the *K. solenopsae* infection rate observed among *S. invicta* nests from where the fly collections took place. Not all *P. curvatus* flies that developed in *K. solenopsae*-infected fire ants were positive for *K. solenopsae* upon eclosion. Among 50 *P. curvatus* flies known to develop in *K. solenopsae*-infected *S. invicta* workers, 12 (24%) were positive for *K. solenopsae* at eclosion.

Key Words: *Solenopsis invicta*, red imported fire ant, phorid fly, biological control, parasite, entomopathogen, microsporidia

RESUMEN

Se desarrollo y utilizo un método de "RCP múltiplex" para determinar la tasa de infección de *Kneallhazia solenopsae* en individuos de la mosca *Pseudacteon curvatus* en el norte-central de la Florida. Entre las moscas *P. curvatus* infectadas con *K. solenopsae*, se producieron 2 amplicaciones, uno de 800 nucleótidos del gene 18S rARN de *P. curvatus* y uno de nucleótidos del gene 16S rARN de *K. solenopsae*. El RCP múltiplex del ADN extraído de las moscas *P. curvatus* fue capaz de detectar 17.5 ± 82.7 de los equivalentes de las esporas de *K. solenopsae*. El promedio de la tasa de infección de *K. solenopsae* en las *P. curvatus* de 4 sitios en Gainesville y Williston, Florida, fue 12.3 ± 5.0%. La tasa de infeccion de *K. solenopsae* para *P. curvatus* fue independiente de la tasa de infeccion de *K. solenopsae* observado entre los nidos de *Solenopsis invicta* donde se hicieron las recolecciones de la mosca. No todas las moscas *P. curvatus* que se desarrollaron con las hormigas de fuego infectadas con *K. solenopsae* resultaron positivos para *K. solenopsae* al eclosionar. Entre las 50 moscas de *P. curvatus* que se desarrollaron con trabajadores de *S. invicta* infectadas con *K. solenopsae*, 12 (24%) fueron positivos para *K. solenopsae* al eclosionar.

Four species of *Pseudacteon* endoparasitiod flies (*P. tricuspis* Borgmeier, *P. curvatus* Borgmeier, *P. litoralis* Borgmeier, and *P. obtusus* Borgmeier) have been released and established in the USA for biological control of *Solenopsis invicta* and *S. richteri* (Graham et al. 2003; Williams et al. 2003; Porter et al. 2004; Vazquez et al. 2006; Gilbert et al. 2008). The parasitoid oviposits an egg into an adult fire ant worker and the maggot that hatches migrates to the head of the ant where it develops, pupates, and ultimately kills the host (Porter 1998).

Kneallhazia (=Thelohania) solenopsae is an intracellular microsporidian parasite that infects *S. invicta* and *S. richteri* (Sokolova & Fuxa 2008). *Kneallhazia solenopsae* is widespread among *S. invicta* in the USA and this infection causes decreased colony vigor and ultimately colony elimination (Oi & Williams 2003). The life cycle and natural transmission mechanisms of *K. solenopsae* are largely unknown (Sokolova & Fuxa 2008). *Kneallhazia solenopsae* appears limited to mostly polygyne *S. invicta* colonies in the USA, but is found in monogyne and polygyne colonies equally in South America where it is native (Oi et al. 2004; Valles & Briano 2004; Milks et al. 2008). Lack of an intermediate host has been proposed to explain the social form limitation (greater *K. solenopsae* infection in polygyne colonies) of *K. solenopsae* infections in North America (Oi et al. 2008). Recently Oi et al. (2008) reported the presence of *K. solenopsae* in several *Pseudacteon* decapitating fly species (*obtusus*, *cultellatus* and *curvatus*) suggesting that these endoparasitic flies may play a role in the life cycle or transmission of the microsporidian to fire ants.

The objectives of this study were to develop a multiplex PCR method capable of detecting *K. so-*

lenopsae in *Pseudacteon* flies and use the method to determine the infection rate of *K. solenopsae* among field-collected *Pseudacteon curvatus*. These experiments provide new descriptive data on the relationships between *K. solenopsae* and *Pseudacteon* flies as well as the *Pseudacteon* flies and their host, *S. invicta*.

Materials and Methods

DNA was extracted from individual *Pseudacteon* flies by the method of Valles et al. (2002). Individual flies were homogenized in 150 µL of digestion buffer (50 mM Tris-HCl, pH 8, 4% sodium dodecyl sulfate, and 5% 2-mercaptoethanol) in a 1.5-mL microcentrifuge tube with a disposable plastic pestle for 15 s and the mixture was incubated at 100°C for 15 min. The mixture was allowed to cool on ice for 1 min, followed by the addition of 200 µL of phenol:chloroform:isoamyl alcohol (Tris-HCl-saturated, pH 8). The tube was inverted several times to mix the contents and then centrifuged at room temperature for 5 min at 16,000*g*. The supernatant was removed and nucleic acids precipitated with iced-cold isopropanol (750 µL) and the pellets were washed twice with 500 µL of 70% ethanol. Pellets were dried for 5 min at 37°C, and suspended in 30 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8).

Multiplex PCR was conducted with DNA preparations from *Pseudacteon* flies to detect the presence of a portion of the *K. solenopsae* 16S rRNA gene (Valles et al. 2002) and a portion of the *Pseudacteon* 18S rRNA gene to verify satisfactory DNA extraction and amplification (i.e., an internal positive control). Amplifying the 18S rRNA gene of *Pseudacteon* serves as a method to eliminate false negatives from failed PCR reactions.

Oligonucleotide primers for the *Pseudacteon* flies were designed to conserved regions of the *Pseudacteon* 18S rRNA gene. DNA was purified from flies of a known species (n = 3) which was used as template to amplify a 1,288 nucleotide region of the 18S rRNA gene. Amplification was accomplished with an oligonucleotide primer set designed to conserved regions from an alignment of Diptera sequences for this gene in GenBank (primer 51, 5'GACTCATCCGAGGCCCCGTAATC and primer 54, 5'CGGGCGGTGTGTGCAAAGG). Amplicons from each of 4 fly species (*P. obtusus*, *P. cultellatus*, *P. curvatus*, *P. litoralis*) were agarose gel-purified, ligated into the pCR4-TOPO vector, transformed into TOP10 competent cells (Invitrogen, Carlsbad, CA), and sequenced by the Interdisciplinary Center for Biotechnology Research (University of Florida). The sequences were then aligned with the Vector NTI software suite (Invitrogen) and oligonucleotide primers designed to conserved regions to permit amplification from any of the fly species (but not the ant host).

Oligonucleotide primers specific to the 16S rRNA gene (Moser et al. 1998; Moser et al. 2000; Valles et al. 2002; accession number: AF031538) of *K. solenopsae* (P1: 5'CGAAGCATGAAAGCGGAGC and P2: 5'CAGCATGTATATGCACTACTGGAGC) and the 18S rRNA gene of *Pseudacteon* flies as described above (P800: 5'GTAGTACACCTATACATTGGGTTCGTACATTACTCTA and P801: 5'ATAAGTTTCAACGCTATAATCCTGAAAGCATC) were included in the same reaction. Multiplex PCR was conducted by the hot start method in a PTC 100 thermal cycler (MJ Research, Waltham, MA) under the following optimized temperature regime: 1 cycle at 94°C for 2 min, then 35 cycles at 94°C for 15 s, 55°C for 15 s, and 68°C for 40 s, followed by a final elongation step of 5 min at 68°C. The reaction was conducted in a 25 µL volume containing 2 mM $MgCl_2$, 200 µM dNTP mix, 0.5 units of Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, CA), 0.4 µM of primers P1 and P2, 0.05 µM of primers P800 and P801, and 50 to 200 ng of the genomic DNA preparation (depending on the experiment). PCR products were separated on a 1% agarose gel and visualized by ethidium bromide staining. For all experiments, positive (DNA mixture purified from *P. curvatus* and *K. solenopsae*) and negative (non-template) controls were run alongside treatments.

Limits of detection of *K. solenopsae* were determined by combining DNA preparations of *K. solenopsae*-negative *Pseudacteon* DNA and DNA prepared from a known quantity of purified *K. solenopsae* spores (Valles et al. 2002). The fly DNA was held constant at 200 ng (generally 0.5 fly equivalents) and *K. solenopsae* DNA was diluted geometrically from 8 ng (15,060 spore equivalents) to 0.0078 ng (14 spore equivalents). Altogether, 10 dilutions of the stock solution (15,060 spore equivalents) were completed. Multiplex PCR was conducted as described above.

To determine the *K. solenopsae* infection rate of *P. curvatus*, field collections of flies were conducted by disturbing fire ant mounds and collecting flies by aspiration that were attracted to the ants (Porter et al. 2004). A hand-held mechanical aspirator (Clarke Mosquito Control, Roselle, Illinois) was used by adding a fine mesh screen (52 × 52 mesh) to contain the flies. Flies were returned to the laboratory where they were segregated by species and sex. DNA was extracted from flies individually. Collections were made from 4 sites in Alachua County, Florida, with varying *K. solenopsae* infection rates among the *S. invicta* community: Site 1, Whitehurst pasture (southeast gate), Williston, Florida, *K. solenopsae* infection rate of *S. invicta*: 0/20 nests = 0%, flies collected 7 and 12 Nov 2008; Site 2, University of Florida, near the microbiology building, *K. solenopsae* infection rate of *S. invicta*: 5/17 nests = 29%, flies collected 2 Oct 2008; Site 3, field by former University of

Florida poultry unit, *K. solenopsae* infection rate of *S. invicta*: 18/28 = 64%, flies collected 25 Sep 2008 and 16 Oct 2008; Site 4, Hilton Hotel, NW 34th Avenue, Gainesville, FL, *K. solenopsae* infection rate of *S. invicta*: 12/19 nests = 63%, flies collected 6 Nov 2008.

In order to determine the rate of *K. solenopsae*-infection in *P. curvatus* that developed in *K. solenopsae*-infected *S. invicta* workers, we exposed *S. invicta* workers from 2 *K. solenopsae*-infected *S. invicta* colonies to *P. curvatus* in the field. Each colony contained approximately 10,000 adult worker caste ants, and the *K. solenopsae* infection rate was 90 and 60%, which was determined by examining wet mounts of 10 individual ants per colony for the presence of spores under phase-contrast microscopy (400X). Colonies were held in separate trays (56 cm L × 44 cm W × 13 cm D) and exposed to *Pseudacteon* flies at the poultry unit site. To attract flies, trays were placed near *S. invicta* nests that were disturbed by opening them and crushing some of the ants that streamed out of the nests. In addition, a harborage consisting of a dental plaster (Castone, Dentsply, York, PA) disk (14.5 cm diameter) moistened with water was moved at roughly 10 min intervals to different locations in each tray to keep ants exposed to flies as they moved between harborage locations. About 20-30 flies per tray were observed attacking ants or hovering in trays during arbitrary observations over the exposure period. Colonies were exposed 2.5 h (12:30-3:00 pm EST) on a sunny day with air temperature of about 26°C and an occasional slight breeze.

After 5 d, adult ants were separated from brood and held in rearing containers. Ten d after exposure to the flies, dead ants were removed from the containers twice daily and placed on moistened dental plaster to await decapitation. Separating dead ants prevented live ants from moving decapitated heads. From 10-16 d after fly exposure, ant heads that could be matched definitively with bodies (head partially attached to body or adjacent to body) were labeled on a separate dental plaster block. Individual headless bodies were frozen and subsequently examined for *K. solenopsae* spores by microscopy. Heads that came from *K. solenopsae*-infected bodies were grouped together and held for emergence. Adult flies that emerged were aspirated, frozen, and immediately preserved in 95% ethanol. Since the majority of the eclosed flies were *P. curvatus*, only this species was segregated by sex and tested for the presence of *K. solenopsae* by multiplex PCR.

Results and Discussion

Amplification with oligonucleotide primers P51 and P54 yielded a significant portion of the 18S rRNA gene from the 4 *Pseudacteon* species. In each case, a 1,288-nucleotide amplicon was produced. These sequences have been deposited in the GenBank database under the following accession numbers: *P. curvatus* (EU851871), *P. litoralis* (EU851872), *P. obtusus* (EU851869), and *P. cultellatus* (EU851870). The *Pseudacteon* sequences exhibited significant identity (99.5%) with each other. An alignment of these sequences with the 18S rRNA sequence for *S. invicta* (AY334566) was used to develop oligonucleotide primers that would amplify the 18S gene of any of the 4 *Pseudacteon* fly species equally while not recognizing or amplifying the ant 18S rRNA gene. Thus, oligonucleotide primers P800 and P801 exclusively recognized and amplified DNA (small subunit 18S rRNA gene) from *Pseudacteon* flies. DNA prepared from *S. invicta* and *K. solenopsae* (3 independent preparations) failed to yield an amplicon with these primers (data not shown). However, the DNA from these samples did yield an amplicon with other (species-specific) oligonucleotide primers indicating that failure was not the result of insufficiencies in the PCR conditions or the DNA preparation (i.e., that the oligonucleotide primers were specific for the *Pseudacteon* 18S rRNA gene). An 800-nucleotide amplicon was consistently produced from DNA prepared from *Pseudacteon* flies or from *S. invicta* workers parasitized by *Pseudacteon* larvae.

Among *Pseudacteon* flies infected with *K. solenopsae*, 2 amplicons were produced, 1 of 800 nucleotides (Fig. 1, lane 1) from the *Pseudacteon* 18S rRNA gene, and 1 of 318 nucleotides (Fig. 1, lane 2) from the *K. solenopsae* 16S rRNA gene. The multiplexing (2 simultaneous reactions within the same tube) provides an internal positive control that eliminates false negatives as a result of insufficiencies in the PCR reaction or DNA preparation. Regardless of the *K. solenopsae* infection status, an amplicon should always be generated from the *Pseudacteon* fly 18S rRNA gene, which provides an internal control to verify that the PCR reaction worked. However, a false negative would still be possible if oligonucleotide primers specific for the *K. solenopsae* 16S rRNA gene were omitted from the reaction mixture, but the *Pseudacteon* oligonucleotide primers were not.

Multiplex PCR of *K. solenopsae* was capable of detecting 117.5 ± 82.7 spore equivalents in DNA extracted from *Pseudacteon* flies (Fig. 1, lane 10). Although *K. solenopsae* meiospores are monokaryotic, the 16S rRNA gene is typically present in multiple copies within the genomes of microsporidia, either as tandem repeats (Kawakami et al. 1994; Belkorchia et al. 2008) or found discontinuously on multiple chromosomes (Biderre et al. 1997). Indeed, the small subunit rRNA element was found on every chromosome of *Nosema bombycis* (Liu et al. 2008) and is thought to be present in multiple copies in all microsporidia (O'Mahony et al. 2007). Thus, the true limit

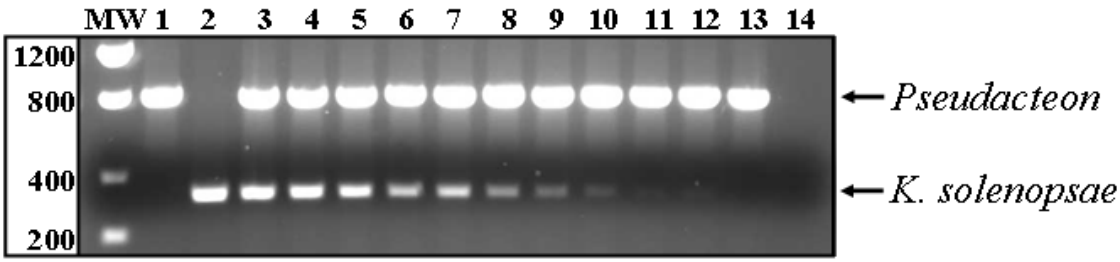

Fig. 1. Agarose gel electrophoresis of amplicons generated by multiplex PCR to determine the detection limits for *K. solenopsae* in *Pseudacteon* flies. Preparations of *K. solenopsae*-negative *Pseudacteon* DNA and DNA prepared from a known quantity of purified *K. solenopsae* spores were mixed and used as template for the PCR. The fly DNA was held constant at 200 ng (generally 0.5 fly equivalents) and *K. solenopsae* DNA was diluted geometrically from 8 ng (15,060 spore equivalents, lane 3) to 7.8 pg (14 spore equivalents, lane 13). MW = molecular weight standards (sizes indicated on the left). Lane positions are as follows: lane 1 = *Pseudacteon* DNA only, lane 2 = *K. solenopsae* DNA only, lane 3 = 15,060 spore equivalents, lane 4 = 7,530 spore equivalents, lane 5 = 3,765 spore equivalents, lane 6 = 1,883 spore equivalents, lane 7 = 941 spore equivalents, lane 8 = 471 spore equivalents, lane 9 = 235 spore equivalents, lane 10 = 117 spore equivalents, lane 11 = 59 spore equivalents, lane 12 = 29 spore equivalents, lane 13 = 14 spore equivalents, lane 14 = non-template control.

of detection of the assay is unknown because the number of copies of the 16S rRNA gene in *K. solenopsae* is not known currently. However, based on spore equivalents, this level of detection is commensurate with similar studies whose objective was to determine infection levels of microsporidia in their hosts (Leiro et al. 2002; Valles et al. 2002).

Because *K. solenopsae* was detected in several *Pseudacteon* species present in the USA (Oi et al. 2008), our original intention was to examine the infection rate of *K. solenopsae* in all *Pseudacteon* species collected from the field in north-central Florida. Unfortunately, the combination of a low infection rate of *K. solenopsae* and the displacement of other *Pseudacteon* species previously present in this area of Florida by *P. curvatus* (SDP, unpublished) forced us to change our objective to determine the *K. solenopsae* infection rate in *P. curvatus* only. The mean *K. solenopsae* infection rate (Table 1) of *P. curvatus* from the 4 sites in Gainesville and Williston, Florida, was 12.3 ± 5.0%, a value close to the infection rate we estimated previously (9 to 13%) with pooled groups of flies reared in the laboratory (Oi et al. 2008). The infection rate for *P. curvatus* was independent of the infection rate among *S. invicta* colonies in the immediate area of the fly collections. For example, the *K. solenopsae* infection rate among field-collected flies was 15.6% in areas where *K. solenopsae* was not detected among *S. invicta* colonies and 14.5% in areas with high *K. solenopsae* infections among *S. invicta* mounds (>60%). It is possible that the *K. solenopsae* infection rate was higher, but could not be detected because of limitations with the assay. Also, the *K. solenopsae* infection rate in *P. curvatus* would be expected to be lower because not all ants serving as host for flies would be infected with *K. solenopsae*. In other words, the *K. solenopsae* intra- or inter-colony infection rate is not 100%. Indeed, *K. solenopsae* infection rates of adult *S. invicta* workers among field colonies can vary widely, from less than 1 to 88% (Briano et al. 1996; Cook 2002; Oi et al. *in press*). Furthermore, as shown in Table 2, not all *P. curvatus* flies that develop in *K. solenopsae*-infected fire ants are themselves positive for *K. solenopsae* upon eclosion. However, the *K. solenopsae* infection rate was higher among flies completing development within *K. solenopsae*-infected fire ant workers (24% males + females) compared with those flies sampled directly from the field (12.3% females only). An additional consideration is the possibility that many flies reared in infected ants do not develop successfully into adults; however, this does not appear to be a primary explanation because fly production rates

TABLE 1. *KNEALLHAZIA SOLENOPSAE* INFECTION AMONG INDIVIDUAL FIELD-COLLECTED *PSEUDACTEON CURVATUS FLIES* IN NORTH-CENTRAL FLORIDA.

Collection Date	Sex	*n*	Number of flies infected	Infection rate (%)	Site
7/12 Nov 2008	♀	32	5	15.6	Whitehurst
25 Sep/16 Oct 2008	♀	49	7	14.3	Poultry
2 Oct 2008	♀	63	3	4.8	Microbiology
6 Nov 2008	♀	48	7	14.6	Hilton Hotel

TABLE 2. *KNEALLHAZIA SOLENOPSAE* INFECTION AMONG INDIVIDUAL, *PSEUDACTEON CURVATUS* FLIES THAT COMPLETED DEVELOPMENT IN *K. SOLENOPSAE*-INFECTED *S. INVICTA* WORKER ANTS.

Sex	*n*	Number of flies infected	Infection rate (%)
♀	25	2	8.0
♂	25	10	40.0

from groups of infected workers is about the same as those from uninfected colonies (Oi et al. *in press*). In this study, 79% ($n = 63$) and 72% ($n = 66$) of the ant heads from *K. solenopsae*-infected bodies from each colony had evidence of adult eclosion, while uninfected parasitized ants had an eclosion rate of 77% ($n = 22$; sum of both colonies).

Parasatoid infection by host microsporidia has been reported and in several instances these infections reduced the vigor and effectiveness of the parasitoid (Brooks et al. 1993; Hoch et al. 2000). Whether *K. solenopsae* is actually infecting cells of *Pseudacteon* flies and, if so, its possible effect on the parasitoid remain unanswered, although as mentioned above (Oi et al. *in press*) flies from infected ant colonies appear healthy and abundant. There is no doubt, however, that *Pseudacteon* flies possess *K. solenopsae* in some form. The more relevant question from our perspective is whether *Pseudacteon* flies are part of the normal life cycle of *K. solenopsae* and if the flies somehow vector the parasite to *S. invicta*. We have studies currently under way to address these questions.

ACKNOWLEDGMENTS

The assistance of C. Strong and E. Mena is appreciated. We thank J. Pridgeon and M.Y. Choi (USDA, Gainesville, FL) for reviewing an earlier version of the manuscript.

REFERENCES CITED

BELKORCHIA, A., BIDERRE, C., MILITON, C., POLONAIS, V., WINCKER, P., JUBIN, C., DELBAC, F., PEYRETAILLADE, E., AND PEYRRET, P. 2008. *In vitro* propagation of the microsporidian pathogen *Brachiola algerae* and studies of its chromosome and ribosomal DNA organization in the context of the complete genome sequencing project. Parasitol. Internat. 57: 62-71.

BIDERRE, C., PEYRETAILLADE, E., DUFFIEUX, F., PEYRET, P., METENIER, G., AND VIVARES, C. 1997. The rDNA unit of *Encephalitozoon cuniculi* (Microsporidia): complete 23S sequence and copy number. J. Eukaryot. Microbiol. 44: 76S.

BRIANO, J. A., PATTERSON, R. S., BECNEL, J. J., AND CORDO, H. A. 1996. The black imported fire ant, *Solenopsis richteri*, infected with *Thelohania solenopsae*: Intracolonial prevalence of infection and evidence for transovarial transmission. J. Invertebr. Pathol. 67: 178-179.

BROOKS, W. M. 1993. Host-parasitoid-pathogen interactions, pp. 231-272 *In* N. E. Beckage, S. N. Thompson, and B. A. Federici [eds.], Parasites and Pathogens of Insects Vol. 2: Pathogens. Academic Press, San Diego, CA.

COOK, T. J. 2002. Studies of naturally occurring *Thelohania solenopsae* (Microsporida: Thelohaniidae) infection in red imported fire ants, *Solenopsis invicta* (Hymenoptera: Formicidae). Environ. Entomol. 31: 1091-1096.

GILBERT, L. E., BARR, C. D., CALIXTO, A. A., COOK, J. L., DREES, B. M., LEBRUN, E. G., PATROCK, R. J. W., PLOWES, R. M., PORTER, S. D., AND PUCKETT, R. T. 2008. Introducing phorid fly parasitoids of red imported fire ant workers from South America to Texas: Outcomes vary by region and by *Pseudacteon* species released. SouthWest Entomol. 33: 15-29.

GRAHAM, L. C., PORTER, S. D., PEREIRA, R. M., DOROUGH, H. D., AND KELLEY, A. T. 2003. Field releases of the decapitating fly *Pseudacteon curvatus* (Diptera: Phoridae) for control of imported fire ants (Hymenoptera: Formicidae) in Alabama, Florida, and Tennessee. Florida Entomol. 86: 334-339.

HOCH, G., SCHOPF, A., AND MADDOX, J. V. 2000. Interactions between an entomopathogenic microsporidium and the endoparasitoid *Glyptapanteles liparidis*. within their host, the gypsy moth larva. J. Invertebr. Pathol. 75: 59-68.

KAWAKAMI, Y., INOUE, T., ITO, K., KITAMIZU, K., HANAWA, C., ANDO, T., IWANO, H., AND ISHIHARA, R. 1994. Identification of a chromosome harboring the small subunit ribosomal RNA gene of *Nosema bombycis*. J. Invertebr. Pathol. 64: 147-148.

LEIRO, J., IGLESIAS, R., PARAMA, A., ARAGORT, W., AND SANMARTIN, M. L. 2002. PCR detection of *Tetramicra brevifilum* (Microspora) infection in turbot (*Scophthalmus maximus* L.) Musculature. Parasitol. 124: 145-151.

LIU, H., PAN, G., SONG, S., XU, J., LI, T., DENG, Y., AND ZHOU, Z. 2008. Multiple rDNA units distributed on all chromosomes of *Nosema bombycis*. J. Invertebr. Pathol. 99: 235-238.

MILKS, M. L., FUXA, J. R., AND RICHTER, A. R. 2008. Prevalence and impact of the microsporidium *Thelohania solenopsae* (Microsporidia) on wild populations of red imported fire ants, *Solenopsis invicta,* in Louisiana. J. Invertebr. Pathol. 97: 91-102.

MOSER, B. A., BECNEL, J. J., MARUNIAK, J., AND PATTERSON, R. S. 1998. Analysis of the ribosomal DNA sequences of the microsporida *Thelohania* and *Vairimorpha* of fire ants. J. Invertebr. Pathol. 72: 154-159.

MOSER, B. A., BECNEL, J. J., AND WILLIAMS, D. F. 2000. Morphological and molecular characterization of the *Thelohania solenopsae* complex (Microsporidia: Thelohaniidae). J. Invertebr. Pathol. 75**:** 174-177.

OI, D. H., AND WILLIAMS, D. F. 2003. *Thelohania solenopsae* (Microsporidia: Thelohaniidae) infection in reproductives of red imported fire ants (Hymenoptera: Formicidae) and its implication for intercolony transmission. Environ. Entomol. 32: 1171-1176.

OI, D. H., VALLES, S. M., AND PEREIRA, R. M. 2004. Prevalence of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) infection in monogyne and polygyne red imported fire ants (Hymenoptera: Formicidae). Environ. Entomol. 33: 340-345.

OI, D. H., PORTER, S. D., VALLES, S. M., BRIANO, J. A., AND CALCATERRA, L. A. 2008. *Pseudacteon* decapi-

tating flies (Diptera: Phoridae): potential vectors of the fire ant pathogens *Kneallhazia* (=*Thelohania*) *solenopsae* (Microsporidia: Thelohaniidae) and *Vairimorpha invictae* (Microsporidia: Burenellidae)? Biol. Control *in press*.

O'MAHONY, E., TAY, W. T., AND PAXTON, R. J. 2007. Multiple rRNA variants in a single spore of the microsporidian *Nosema bombi*. J. Eukaryot. Microbiol. 54: 103-109.

PORTER, S. D. 1998. Biology and behavior of *Pseudacteon* decapitating flies (Diptera: Phoridae) that parasitize *Solenopsis* fire ants (Hymenoptera: Formicidae). Florida Entomol. 81: 292-309.

PORTER, S. D., NOGUEIRA DE SÁ, L. A., AND MORRISON, L. W. 2004**.** Establishment and dispersal of the fire ant decapitating fly *Pseudacteon tricuspis* in North Florida. Biol. Control 29: 179-188.

SOKOLOVA, Y. Y., AND FUXA, J. R. 2008. Biology and lifecycle of the microsporidium *Kneallhazia solenopsae* Knell Allan Hazard 1977 **gen. n., comb. n**., from the fire ant *Solenopsis invicta*. Parasitol. 135: 903-929.

VALLES, S. M., OI, D. H., PERERA, O. P., AND WILLIAMS, D. F. 2002. Detection of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) in *Solenopsis invicta* (Hymenoptera: Formicidae) by multiplex PCR. J. Invertebr. Pathol. 81: 196-201.

VALLES, S. M., AND BRIANO, J. A. 2004. Presence of *Thelohania solenopsae* and *Vairimorpha invctae* in South American populations of *Solenopsis invicta*. Florida Entomol. 87: 625-627.

VAZQUEZ, R. J., PORTER, S. D., AND BRIANO, J. A. 2006. Field release and establishment of the decapitating fly *Pseudacteon curvatus* on red imported fire ants in Florida. BioControl 51: 207-216.

WILLIAMS, D. F., OI, D. H., PORTER, S. D., REREIRA, R. M., AND BRIANO, J. A. 2003. Biological control of imported fire ants (Hymenoptera: Formicidae). American Entomol. 49**:** 150-163.