

Light and Electron Microscope Study of *Thelohania solenopsae* n. sp. (Microsporida: Protozoa) in the Red Imported Fire Ant, *Solenopsis invicta*^{1,2,3}

J. D. KNELL, G. E. ALLEN

Department of Entomology and Nematology, University of Florida, Gainesville, Florida 32611

AND

E. I. HAZARD

Insects Affecting Man Laboratory, Agricultural Research Service, U. S. Department of Agriculture,
Gainesville, Florida 32611

Received May 13, 1976

A new species of Microsporidia, *Thelohania solenopsae*, is described from the red imported fire ant, *Solenopsis invicta*. The *Thelohania* infections are localized in the fat body of workers. Meronts causing infections of progeny are found in the ovaries of queens. Spores occur only in adult ants and only vegetative stages are present in larvae and pupae. Both uninucleate octospores (eight spores within a pansporoblast membrane) and binucleate free spores (spores developing in isolation) are formed.

INTRODUCTION

Since its introduction some 30-35 years ago, the red imported fire ant, *Solenopsis invicta*, has spread dramatically throughout the southeastern United States and now occupies nine states from the Carolinas to Texas (Lofgren, et al., 1975). Although estimates of economic impact vary, the ants' aggressive nature and a painful sting make the ants a nuisance of some consequence. To date the only effective chemical control has been Mirex bait. Due to the appearance of Mirex residues in nontarget organisms, the use of this chemical has been severely restricted in estuarine and prime wildlife habitats (Ruckelshaus, 1972). These restrictions and the absence of a suitable

alternative chemical control have triggered the search for potential biological control agents.

Allen and Buren (1974) were the first to report a microsporidian infection in ants and identified it as a species of *Thelohania*. The microsporidium was discovered in collections of *S. invicta* taken in and around the city of Cuiabá, Mato Grosso, Brazil, during trips made in 1971, 1973, and 1975. Similar organisms have been isolated from seven other species in the *S. saevissima* complex in Argentina, Brazil, Paraguay, and Uruguay. This paper is a description of the microsporidium found in *S. invicta* from Cuiabá.

MATERIALS AND METHODS

Light microscopy. Meronts and sporonts were detected by smearing the abdomens of *Solenopsis invicta* workers and brood on glass slides and staining with Giemsa's stain. Air-dried smears were fixed in 95% methanol for 5 min, stained with 10%

¹ Florida Agricultural Experiment Station Journal Series No. 6101.

² Support for this research was provided in part by U. S. Department of Agriculture Cooperative Agreement No. 12-14-7001-160.

³ Mention of a pesticide or a proprietary product does not constitute recommendation or endorsement by the University of Florida.

Giems'a solution, made with phosphate buffer, pH 7.41, for 11 min, and washed with tap water.

Wet smears of adult abdomens on cover-slips were fixed in aqueous Bouin's solution for 6 hr, washed in 70% ethanol overnight, mordanted in iron alum for 4 hr, stained in aqueous Heidenhain's hematoxylin overnight, destained in iron alum to the desired intensity, and mounted on glass slides.

Fresh spores were measured with an A.E.I. Cook image-splitting micrometer at 1000 \times . Giems'a-stained stages were measured with an A.O. ocular micrometer at 1000 \times .

Electron microscopy. Abdomens of infected *S. invicta* workers were cut into 1-mm³ pieces and fixed in 4% gluteraldehyde in 0.1 M sodium phosphate buffer, pH 7.2, at 8°C for 24 hr. They were rinsed in phosphate buffer, pH 7.2, postfixed in 1% OsO₄, dehydrated in graded ethanol solutions, infiltrated using an extended schedule (Endo, pers. comm.), and embedded in Spurr's (1969) low viscosity medium. Thin sections, 60–90 nm, were cut on a Sorvall MT-2 ultramicrotome with a diamond knife and stained with uranyl acetate and lead citrate (Venable and Coggeshall, 1965). Sections were examined and photographed with a Hitachi 125-E electron microscope at an accelerated voltage of 50 kV.

RESULTS

Light Microscopy

Stages of the microsporidium infect the fat body of workers, males, and queens of the red imported fire ant. Meronts contain one, two, or four unpaired nuclei (Figs. 1–5) or one or two diplokarya (Figs. 6–8). Mononucleate meronts are round cells measuring 4.9 μm in diameter with irregular or round nuclei measuring 3.0 μm in diameter. Bi- and tetranucleate meronts are round with oval or reniform nuclei and are 5.8 μm in diameter, with a 2.0- μm nucleus. Binucleate meronts are often seen dividing

(Fig. 4). The nuclei of multinucleate meronts may partially split (Fig. 4) or remain in close proximity after division (Figs. 3, 5) thus superficially resembling diplokarya. Meronts with one diplokaryon are round and measure 5.3 μm in diameter with the nuclei measuring 3.4 μm . Cells bearing two diplokarya are oval in outline (Fig. 7) and are occasionally seen dividing (Fig. 8). Meront cytoplasm stains dark blue in Giems'a-stained smears with nuclei staining dark red. These stages occur only in larvae and pupae; only sporonts may be found in adult workers and males. Mononucleate meronts may also be detected in smears of infected queens (Fig. 1) in which sporonts and octospores are also seen.

Sporonts contained one, two, four, or eight unpaired nuclei (Figs. 10–14) or one diplokaryon (Fig. 9). They are round or oval cells 8 μm in diameter and do not stain as intensely with Giems'a solution as do meronts. The nuclei of mononucleate cells are round and measure 3.6 μm in diameter. Those of bi-, tetra-, and octonucleate cells are more irregular in shape and smaller in size, but the cell increases in diameter. Stages of the sporulation sequence are found only in late-instar larvae, pupae, and young adults.

The fat body of infected workers contains both free spores and octospores, i.e., eight spores in a pansporoblast membrane. Heavily infected fat body cells undergo hypertrophy, forming cysts which emerge from severed gasters (Fig. 26). Fresh free spores (Fig. 17, arrow) are elongate oval in shape and measure $1.85 \pm 0.16 \times 4.93 \pm 0.58 \mu\text{m}$ with a range of $1.70\text{--}2.23 \mu\text{m}$ wide $\times 6.47\text{--}4.20 \mu\text{m}$ long. Octospores (Figs. 16, 17) are pyriform in shape, measure $1.95 \pm 0.20 \times 3.32 \pm 0.48 \mu\text{m}$ and range from 1.42 to 2.38 μm wide $\times 2.85$ to 3.68 μm long. The two spore types occur in a ratio of 57.2 octospores/1.0 free spore. The polar filament extrudes under mechanical pressure while a few spores extrude the polar filament in distilled water, pH 5.8 (Fig. 19). Occasionally large octospores

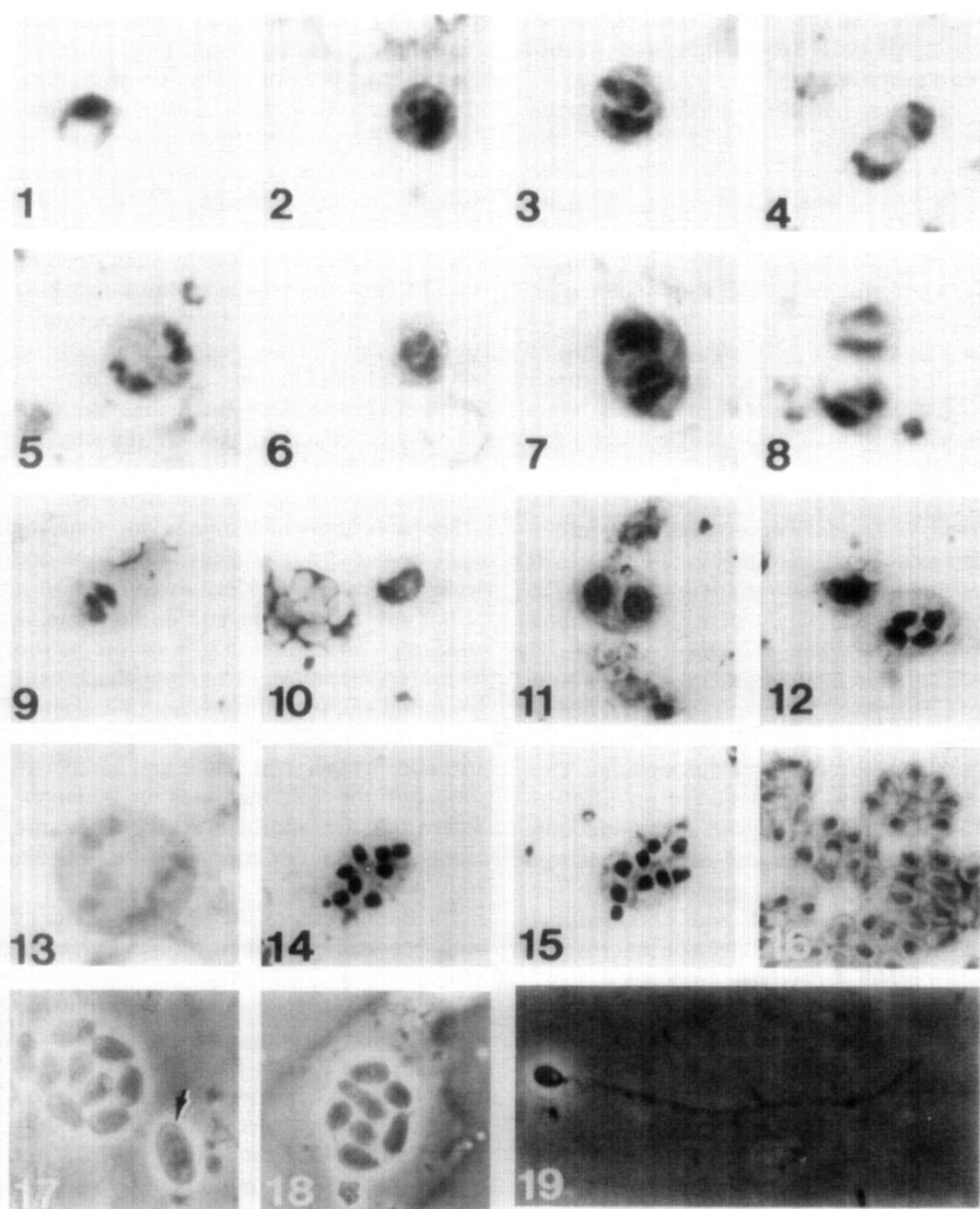


FIG. 1. Meront with one nucleus from infected queen. Giemsa's stain, 900 \times .

FIG. 2. Meront with one nucleus from larval worker. Giemsa's stain, 900 \times .

FIG. 3. Binucleate meront from larval worker. Giemsa's stain, 900 \times .

FIG. 4. Dividing binucleate meront from larval worker. Giemsa's stain, 900 \times .

FIG. 5. Tetranucleate meront from larval worker. Giemsa's stain, 900 \times .

FIG. 6. Meront with one diplokaryon from larval worker. Giemsa's stain, 900 \times .

FIG. 7. Meront with two diplokarya from larval worker. Giemsa's stain, 900 \times .

FIG. 8. Meront with two diplokarya dividing from larval worker. Giemsa's stain, 900 \times .

FIG. 9. Diplokaryon-bearing sporont from adult worker. Giemsa's stain, 900 \times .

FIG. 10. Sporont with one nucleus from adult worker. Giemsa's stain, 900 \times .

(macrospores) are formed when one nucleus fails to make a division (Fig. 18).

Electron Microscopy

Sequence forming octospores. Due to the rapidity of sporulation in adults, the earliest stages seen under the electron microscope in this study were multinucleate sporonts enclosed by a pansporoblastic membrane (Fig. 20). At this stage the only recognizable organelles are the nuclei (Fig. 20, N) and sparse endoplasmic reticulum (Fig. 20, Er). Traces of granular metabolic products are seen at the periphery of the pansporoblast membrane (Fig. 20, mp). This material is more abundant in earlier stages but traces are seen in the early sporoblast stage (Fig. 21). Early sporoblasts show a reticulate-appearing polar filament primordium, usually identified as part of the Golgi complex (Fig. 21, PFP) and a few coils of the polar filament (Fig. 21, PF). At this stage only the electron-dense outer spore coat layer (exospore) is present (Fig. 21, OL). The mature spore is distinguished by the presence of a thick electron-transparent inner spore coat (endospore) (Fig. 22, IL). The polar filament of mature octospores has nine to eleven coils around the nucleus (Fig. 22, PF).

The polar filament consists of an electron-dense outer membrane and an inner core separated by an electron-transparent zone (Fig. 22, PF). Octospores have a single nucleus bordered by one to four rows of polyribosomes (Fig. 22, PS). The posterior end of the spore contains an amorphous body corresponding to the vacuole (Fig. 22, V). This structure is usually separated from

the nucleus by an irregular clear area which is undoubtedly an artifact (Fig. 22). Anteriorly the spore contains a lamellate polaroplast (Fig. 22, P).

Sequence forming free spores. Sporonts are distinguished in electron micrographs by the presence of diplokarya (Fig. 23, N). No other organelles are seen at this time (Fig. 23). Sporoblasts show developing spore organelles such as the polar filament (Fig. 24, PF) and polar cap (Fig. 24, PC) and a diplokaryon (Fig. 24, N). As in the octospores, only the exospore has developed at this time (Fig. 24, OL). Mature free spores are similar in ultrastructure to octospores except for the presence of the paired nuclei (Fig. 25, N). The spore coat is composed of an outer exospore (Fig. 25, OL) and an inner endospore (Fig. 25, IL). The polar filament (Fig. 25, PF) is coiled nine to eleven times around the nuclei. The nuclei are bordered by one to five layers of polyribosomes anteriorly and one layer posteriorly (Fig. 25, PS). Free spores (Figs. 24, 25) tend to be more ovoid in outline than octospores which are distinctly pyriform.

DISCUSSION

Hazard and Oldacre (1975) have created the Family Thelohaniidae to include microsporidia having eight uninucleate spores enclosed by a pansporoblastic membrane (octospores). During sporoblast formation the cytoplasm of the sporont constricts around each nucleus giving it a lobed appearance (endogenous budding), and granular metabolic products are secreted which are retained by the pansporoblast membrane. There are eleven genera in this

FIG. 11. Binucleate sporont from adult worker. Giemsa's stain, 900 \times .

FIG. 12. Tetranucleate sporont from adult worker. Giemsa's stain, 900 \times .

FIG. 13. Tetranucleate sporont dividing into octonucleate form from adult worker. Giemsa's stain, 900 \times .

FIG. 14. Octonucleate sporont from adult worker. Giemsa's stain, 900 \times .

FIG. 15. Developing sporoblasts within pansporoblastic membrane from adult worker. Giemsa's stain, 900 \times .

FIG. 16. Octospores in pansporoblast membrane from adult worker. Giemsa's stain, 900 \times .

FIG. 17. Octospores and free spore (arrow) from adult worker. Fresh mount, 900 \times .

FIG. 18. Pansporoblast with six normal and one aberrant octospores from adult workers. Fresh mount, 900 \times .

FIG. 19. Spore with extruded polar filament. Fresh mount, 900 \times .

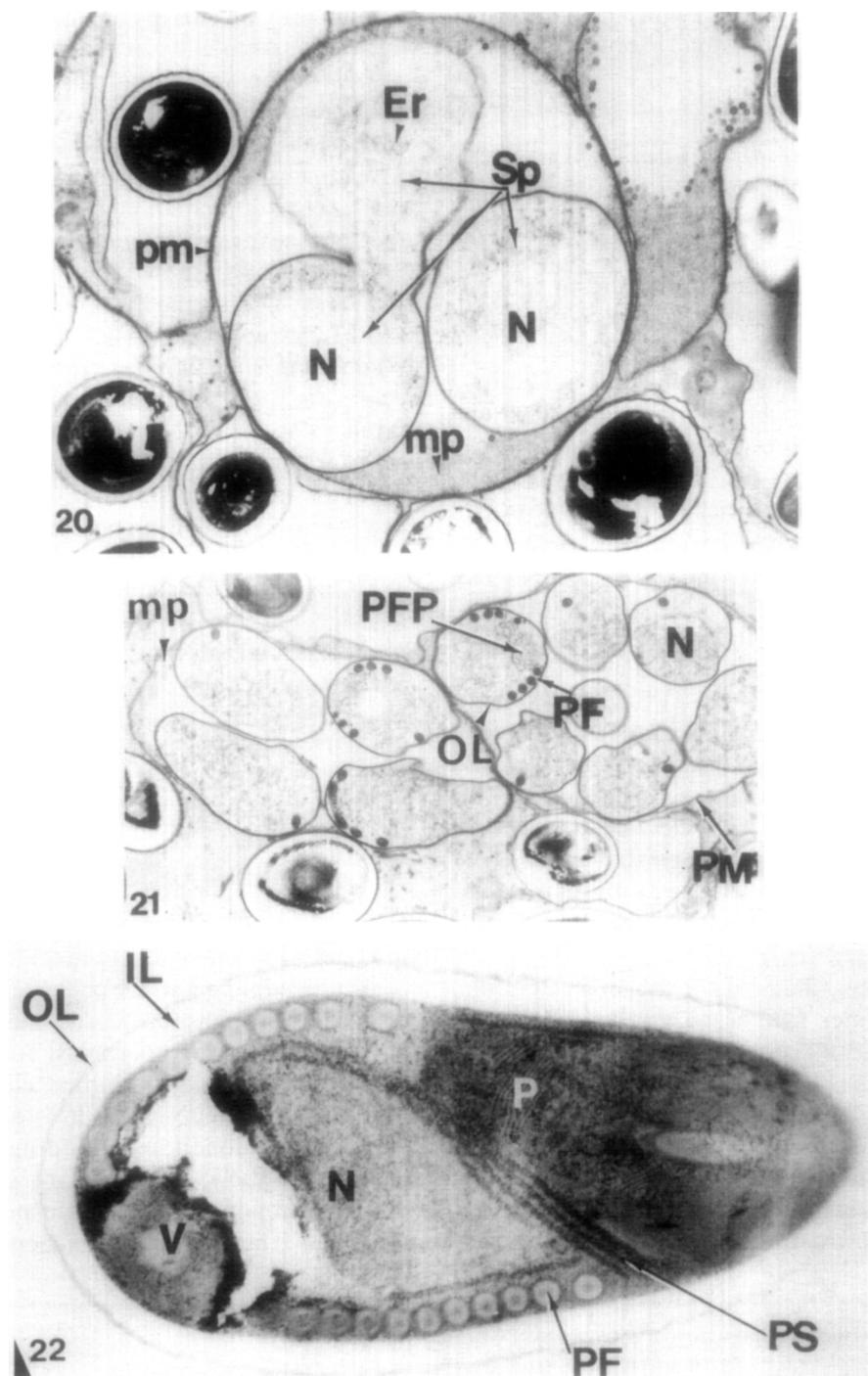


FIG. 20. Sporont in process of endogenous budding. Er, endoplasmic reticulum; N, nucleus; pm, pansporoblast membrane; sp, sporoblasts; mp, metabolic products. 11,000 \times .

FIG. 21. Developing sporoblasts in pansproblast membrane. N, nucleus; PF, polar filament; PFP, polar filament primordium; PM, pansporoblast membrane; OL, exospore; mp, metabolic products. 5100 \times .

FIG. 22. Mature octospore. IL, endospore; OL, exospore; N, nucleus; P, polaroplast; PF, polar filament; PS, polyribosomes, V, vacuole. 38,000 \times .

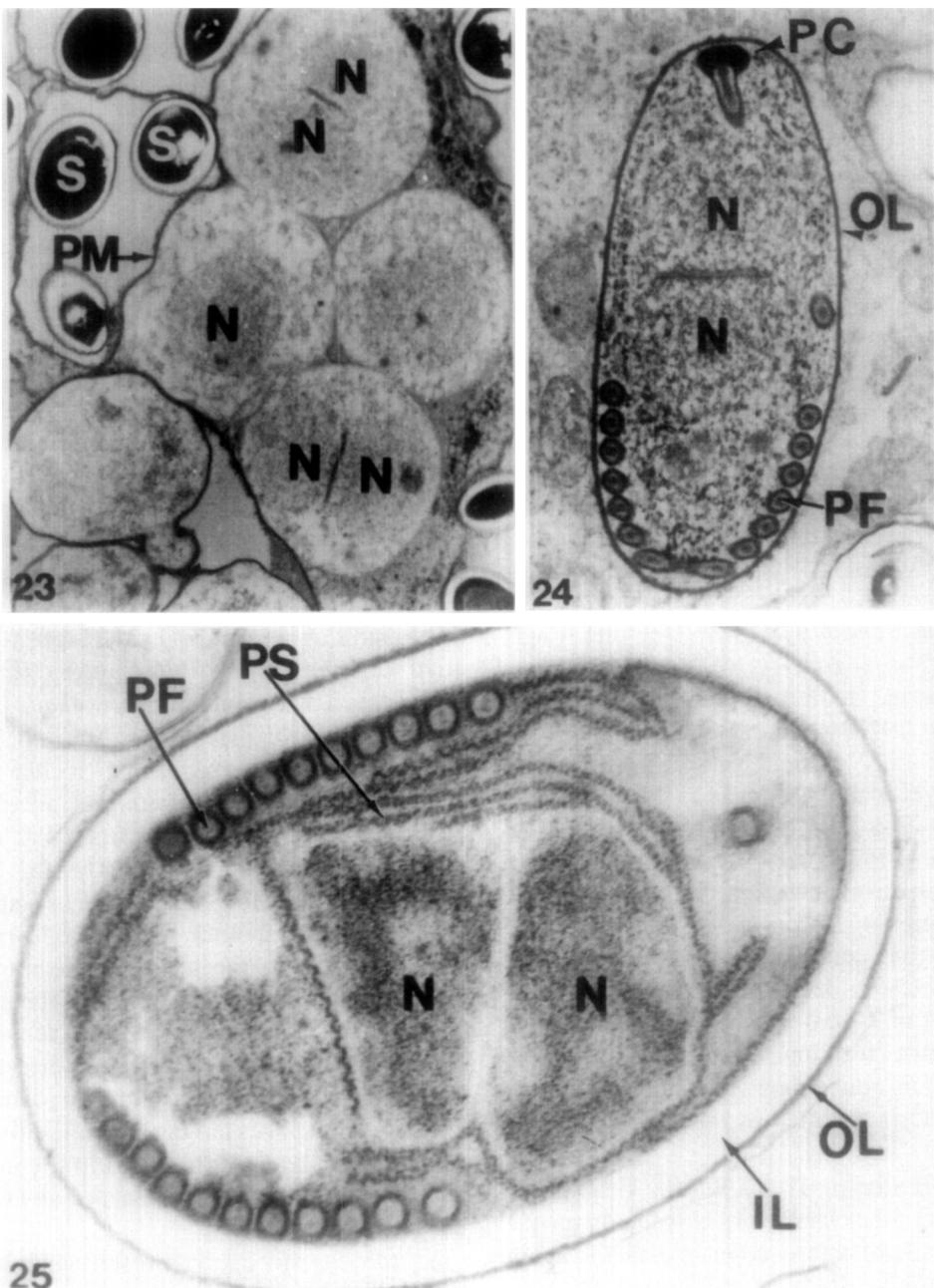


FIG. 23. Diplokarya-bearing sporonts. N, nucleus; PM, pansporoblast membrane; S, octospores. 6900 \times .

FIG. 24. Developing sporoblast with paired nuclei. N, nucleus; OL, exospore; PC, polar cap; PF, polar filament. 15,500 \times .

FIG. 25. Mature free spore. N, nucleus; IL, endospore; OL, exospore; PF, polar filament; PS, polyribosomes. 38,000 \times .

family, eight of which are new. Genus separation is based on polar filament structure, pansporoblast shape and persistence, spore shape, and sporont morphology. The

microsporidium under consideration here meets all of the family criteria. It produces octospores (Fig. 17) and sporoblasts by endogenous budding (Figs. 20, 21), and it

secretes metabolic products retained by the pansporoblast membrane (Figs. 20, 21, mp). It is included in the genus *Thelohania* because the polar filament is of nearly uniform diameter throughout (Figs. 22, 25) and the octospores are oval to pyriform in shape when fixed with Bouin's solution and stained with Heidenhain's hematoxylin and because the spores lack appendages.

Hazard and Oldacre (1975) list six species in the genus *Thelohania*, all from decapod crustaceans. Two of them, *T. duorara* and a microsporidium identified by Maurand and Vey (1973) as *T. contejeani*, have been studied with the electron microscope and may be differentiated from *T. solenopsae* on the basis of ultrastructure. The mature pansporoblasts of *T. contejeani* contain microtubules having a moniliform core (Maurand and Vey, 1973; Vey and Vago, 1973). Pansporoblasts of *T. duorara* contain large quantities of thin filaments (Kelley, 1975) while those of *T. solenopsae* contain only granular metabolic products which gradually disappear as the octospores mature (Figs. 20, 21, mp). Neither Maurand and Vey (1973) nor Vey and Vago (1973) show electron micrographs of the mature octospores of *T. contejeani*, therefore they cannot be compared to *T. solenopsae*. Kelley (1975) has shown that the polar filament of *T. duorara* has thirteen to fourteen coils forming three rows, whereas that of *T. solenopsae* has nine to eleven coils and is oriented in one row (Figs. 22, 25, PF).

Differentiating *T. solenopsae* octospores from the other species of this genus on the basis of light microscopy is difficult as all have similar spore shapes (oval to pyriform). Two species, however, have considerably smaller spores than *T. solenopsae*. *Thelohania contejeani* has octospores measuring $3.5-4.0 \times 2.0 \mu\text{m}$ (Maurand and Vey, 1973), and *T. petroliithis*, $3 \times 2 \mu\text{m}$ (Sprague, 1970). Spore dimensions of the other four species are similar to the dimensions of *T. solenopsae*.

Until ultrastructural studies are made on

T. giardi, *T. maenadis*, *T. paguri*, and *T. petroliithis*, these species may be separated from *T. solenopsae* on the basis of host specificity. The six *Thelohania* species listed by Hazard and Oldacre (1975) are all parasites of decapod crustaceans. Generally, the primary infection site is the musculature, although other tissues including heart, brain, and connective tissue may be involved. The only exception is *T. paguri* which occurs in the abdominal cavity of the hermit crab, *Eupagurus bernhardis*. The spores of *T. solenopsae* occur only in the abdominal fat body of the red imported fire ant, *S. invicta*.

A characteristic feature of *T. solenopsae* is the presence of octospores and free spores developing simultaneously in the same tissues of the host. The authors have noted this condition in approximately 50 *S. invicta* colonies, thus the changes that it represents a mixed infection are slight. Both the genera *Amblyospora* Hazard and Oldacre, 1975 [type *A. californica* (Kellen and Lipa, 1960)] and *Parathelohania* Codreanu, 1966 [type *P. legeri* (Hesse, 1904)] have two developmental cycles (Hazard and Oldacre, 1975). However, in these genera the octospore sequence occurs exclusively in and is fatal to the larvae while the sequence producing free spores remains dormant in the oenocytes of larval females. The free spores then develop in the adult female and are ultrastructurally distinct from the octospores having a comparatively thin spore wall and differences in the number of polar filament coils (Hazard and Oldacre, 1975).

T. solenopsae is at present the only dimorphic member of the genus *Thelohania*. The type species, *T. giardi* Henne-guy, 1892, is poorly known, and no ultrastructural work has been done on it. Ultrastructural studies of *T. contejeani* and *T. duorara* have not revealed the presence of free spores; however, the free spores of *T. solenopsae* are extremely difficult to locate in thin sections. In addition, the work of Kelley (1975) shows stages with diplo-

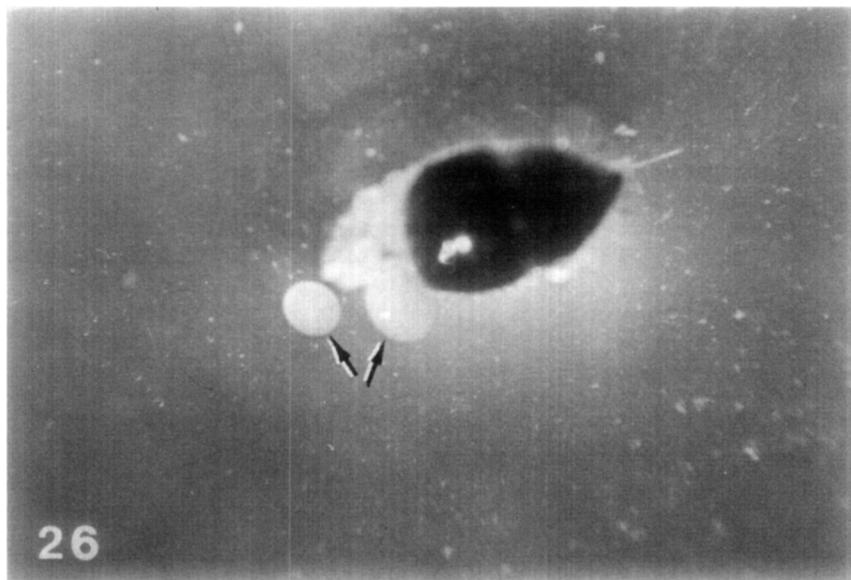


FIG. 26. Severed fire ant gaster showing characteristic cysts (arrows) composed of masses of *Thelohania* spores. 30 \times .

karya side by side with developing octospores similar to Figure 23, and Maurand and Vey (1973) mention vegetative stages in Giemsa-stained smears of *T. contejeani*. Thus, it is possible that *T. giardi* may be dimorphic, and the microsporidium from fire ants has been placed in the genus *Thelohania* pending a careful ultrastructural study of the type species.

This microsporidium was originally discovered by Dr. W. F. Buren who noticed large cysts in the partially cleared gasters of alcohol-preserved workers of *S. invicta* (Fig. 26). Such cysts cannot be seen in noncleared ants. Thus, infected individuals may be detected only through microscopical examination. Infected *Solenopsis* spp. are readily determined under the dissecting microscope by severing the gaster just behind the petiole releasing the characteristic cysts (Fig. 26). Cyst numbers average four to six per gaster. However, as many as 22 variable-sized bodies have been observed in a single specimen. The exact effect of *T. solenopsae* on its host is not known at this time. Infected colonies may be as large as healthy ones in the field but cannot be maintained in the

lab as long as can noninfected colonies. Thus, the effect of this microsporidium appears to be one of debilitation caused by destruction of the adult fat body.

Thelohania solenopsae n. sp.

Host. The red imported fire ant *Solenopsis invicta*.

Type locality. University campus, Cuiabá, Mato Grosso, Brazil.

Site of infection. Fat body of workers, males and queens and ovaries of queens.

Derivation of name. Named after its host.

Vegetative stages. Meronts occur in larvae and pupae and in the ovaries of queens. They have one, two, or four unpaired nuclei or one or two diplokarya.

Sporulation stages. Sporonts are found in late pupae and young adult workers, males, and queens. The cells contain one, two, four, or eight nuclei or a single diplokaryon. Sporonts with eight nuclei produce eight sporoblasts within a pansporoblast membrane by endogenous budding.

Spores. Two types of spores are produced, pyriform uninucleate octospores

and oval binucleate free spores. Typically eight octospores are contained within a pansporoblast membrane. Free spores develop in isolation.

Type material. Holotype slides have been sent to the United States National Museum. Paratype material has been sent to Brazil for proper disposition.

ACKNOWLEDGMENTS

The authors wish to acknowledge the contributions of Ms. Suzanne Hickman in the preparation of the manuscript and Mrs. Susan Avery, Mrs. Janet Colbert, and Mr. Darrell Anthony for their assistance in electron microscopy.

REFERENCES

- ALLEN, G. E., AND BUREN, W. F. 1974. Microsporidian and fungal diseases of *Solenopsis invicta* Buren in Brazil. *J. N. Y. Entomol. Soc.*, **32**, 125-130.
- CODREANU, R. 1966. On the occurrence of spore or sporont appendages in the Microsporidia and their taxonomic significance. In "Proceedings 1st International Congress on Parasitology, 1964," pp. 602-603. Pergamon Press, New York.
- HAZARD, E. I., AND OLDACRE, S. W. 1975. Revision of microsporidia (Protozoa) close to *Thelohania*, with descriptions of one new family, eight new genera, and thirteen new species. *U. S. Dept. Agr. Tech. Bull.*, 1530, 104 pp.
- KELLEY, J. 1975. "A Description of the Histological Structure of Normal and Microsporidian-Infected Pink Shrimp, *Penaeus duorarum* Burkenroad." Ph.D. Dissertation, University of Miami, Miami, Fla.
- LOFGREN, C. S., BANKS, W. A., AND GLANCEY, B. M. 1975. Biology and control of imported fire ants. *Annu. Rev. Entomol.*, **20**, 1-30.
- MAURAND, J., AND VEY, A. 1973. Etudes histopathologique et ultrastructurale de *Thelohania contejeani* (Microsporida, Nosematidae) parasite de l'ecrevisse *Austropotamobius pallipes* Lereboullet. *Ann. Parasitol. (Paris)*, **48**, 411-421.
- RUCKELSHAUS, W. D. 1972. Products containing Mirex; determination and order. *Fed. Reg.* **37** (130), 299-300.
- SPRAGUE, V. 1970. Some protozoan parasites and hyperparasites in marine decapod Crustacea. In "A Symposium on Diseases of Fishes and Shellfishes" (S. F. Snieszko, ed.). *Amer. Fish. Soc. Spec. Publ.* **5**, 416-430.
- SPURR, A. R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.*, **26**, 31-43.
- VENABLE, J. H., AND COGGESHALL, R. 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.*, **25**, 407-408.
- VEY, A., AND VAGO, C. 1973. Protozoan and fungal diseases of *Austropotamobius pallipes* Lereboullet in Frances. *Ann. Hydrobiol. I.N.R.A.*, **3**, 59-64.