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Ultrastructure and Pathology of *Microsporidium phytoseiuli* n. sp. Infecting the Predatory Mite, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae)

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Ultrastructure and pathology of *Microsporidium phytoseiuli* n. sp. infecting the predatory mite *Phytoseiulus persimilis* Athias-Henriot is described using light and transmission electron microscopy. Infected mites showed no gross, external symptoms. All observed stages of the parasite had unpaired nuclei. Schizonts were commonly observed within nuclei of digestive cells of the ventriculus and within the cytoplasm of cells lining the cecal wall and in muscle tissue underlying it. Sporoblasts and spores occurred in the nuclei and cytoplasm of digestive cells within the ventriculus, in cortical regions of the sub- and supraesophageal ganglia, within the cecal wall and muscle tissue, and in parenchyma cells underlying the cuticle. Mature spores were also observed in developing eggs within gravid females. These were broad- to elongate-ovoid, measured 4.33 ± 0.35 × 1.27 ± 0.15 μm (electron micrographs), 5.37 ± 0.46 × 2.22 ± 0.17 μm (fixed and stained), and 5.88 ± 0.34 × 2.22 ± 0.19 μm (fresh) and had an isolfilar polar filament coiled 12 to 15 times within the posterior two-thirds. Within cells, individual spores appeared to be in direct contact with host cytoplasm, while groups of spores were infrequently observed within interfacial envelopes. Groups of 4, 8, to more than 16 spores were observed by light microscopy, while 8 was the maximum observed by electron microscopy. No spores were observed in *Tetranychus urticae*, a mite used as food during this study.

KEY WORDS: **Acari; *Phytoseiulus persimilis;* biological control; microsporidia; pathology; ultrastructure.**

INTRODUCTION

The predatory mite *Phytoseiulus persimilis* Athias-Henriot is mass-reared by commercial insectaries and shipped to growers worldwide. Growers use this mite for biological control of the two-spotted spider mite, *Tetranychus urticae* Koch, in commercial greenhouses and on field crops. *P. persimilis* is an effective predator and its ability to control pest mite populations in greenhouses is well documented (Scopes, 1985). However, recent grower complaints regarding poor performance of this predator in Alberta greenhouses led to the discovery of several potential pathogens associated with it, including microsporidia (Steiner, 1993a).

Microsporidia have been reported from both aquatic and terrestrial mites. Other phytoseiid hosts include *Neoseiulus cucumeris* (formerly *Amblyseius*) (Oudemans) and *Amblyseius barkeri* (Hughes), predators used for biological control of western flower thrips, *Frankliniella occidentalis* Pergande, and onion thrips, *Thrips tabaci* Lindeman, in commercial greenhouses (Beerling *et al.*, 1993; Steiner, 1993a). Microsporidia reduced productivity of mass-rearings of these mites (Beerling and van der Geest, 1991) and were implicated in reducing fecundity and longevity in *P. persimilis* (see Steiner, 1993b).

Based on spore morphology alone, we have observed three distinct microsporidia in *P. persimilis*, obtained from three different, commercial insectaries. The ultrastructure and pathology of one of these microsporidia are described from light and transmission electron microscopy data.

MATERIALS AND METHODS

Mite Rearing

P. persimilis for this study were obtained from a supplier of biological control agents in Europe in December 1993 and reared at the Alberta Environmental Centre (Vegreville, AB). Colonies of mites were maintained in cages on bean plants (*Phaseolus vulgaris* L.) infested with two-spotted spider mites, *T. urticae*. Cages were placed within greenhouses under controlled environmental conditions (18L:6D; 25°C:20°C).

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Light Microscopy

Fresh smears of whole *P. persimilis* and *T. urticae* were prepared in salt solution (150 mM NaCl; 2 mM $CaCl_2$; 3 mM KCl) and measurements of fresh spores were obtained by the agar cushion method of Hostounský and Žižka (1979). Permanent smears of whole mites were fixed in methanol for 10 min, stained in 15% Giemsa buffer (pH 6.9) for 2 hr, dehydrated in an ethanol series, and mounted in Permount.

Transmission Electron Microscopy

Adult female *P. persimilis* were placed in fixative consisting of 1% paraformaldehyde and 1.5% glutaraldehyde in cacodylate buffer (pH 7.4) for 24 to 48 hr. Fixative was replaced with 0.12 M cacodylate buffer (pH 7.2) with three changes in 60 min. Tissue was postfixed for 2 hr in 1% osmium tetroxide in 0.12 M cacodylate buffer.

Following fixation, mites were placed in distilled water for 10 min and dehydrated in the following ethanol series: 50% (30 min), 70% (30 min), 90% (30 min), 100% (60 min), followed by propylene oxide: absolute ethanol (1:1) (30 min), propylene oxide (60 min), propylene oxide:low-viscosity Spurr resin (16 hr), and low-viscosity Spurr resin (24 hr). Each solution was changed three times. An individual mite was placed in Spurr resin within a flat mold and cured for a minimum of 16 hr in a 60°C oven.

Ultrathin gold sections, approximately 100 nm thick, were cut with a diamond knife using an LKB Nova Ultramicrotome. Sections were placed on Formvar-coated grids and stained with prefiltered, 4% uranyl acetate for 20 min in a 60°C oven and then with lead citrate for 6 min at room temperature. Sections were examined with an Hitatchi H-600 transmission electron microscope at 75 kV.

RESULTS

Presporal Stages

Distinct developmental stages of the microsporidium were not observed in light microscopic examination of stained preparations. All stages observed with the electron microscope had unpaired nuclei, with only one nucleus observed in any individual. The earliest stage observed was the schizont. Rounded-to-ovoid schizonts measured 1.9–2.9 µm (Fig. 1). The nucleus occupied a large portion of schizonts and cisternae of rough endoplasmic reticulum and Golgi apparatus were commonly observed within them. Cytoplasm contained densely packed ribosomes. Several schizonts were commonly observed within a single host cell (Fig. 2). Those developing within host cell nuclei were typically in chromatin-rich regions adjacent to the nuclear membrane.

Individual cells suspected of being sporonts contained rough endoplasmic reticulum, a single Golgi apparatus, and abundant, loosely packed, ribosomes. These cells, although presumed to be sporonts, could not be confidently distinguished from schizonts. Plasmodia were not observed, suggesting that both schizogony and sporogony may be the process of binary division.

Sporoblasts were more elongate than earlier stages and measured up to 5.6 µm long, making them comparable in size to mature spores. Each was surrounded by a double-layered, electron-dense plasma membrane. Sporoblasts were often observed with a caudal prolongation (Fig. 3). These appeared to lack a double-layered membrane, likely an artifact resulting from incomplete fixation.

The Mature Spore

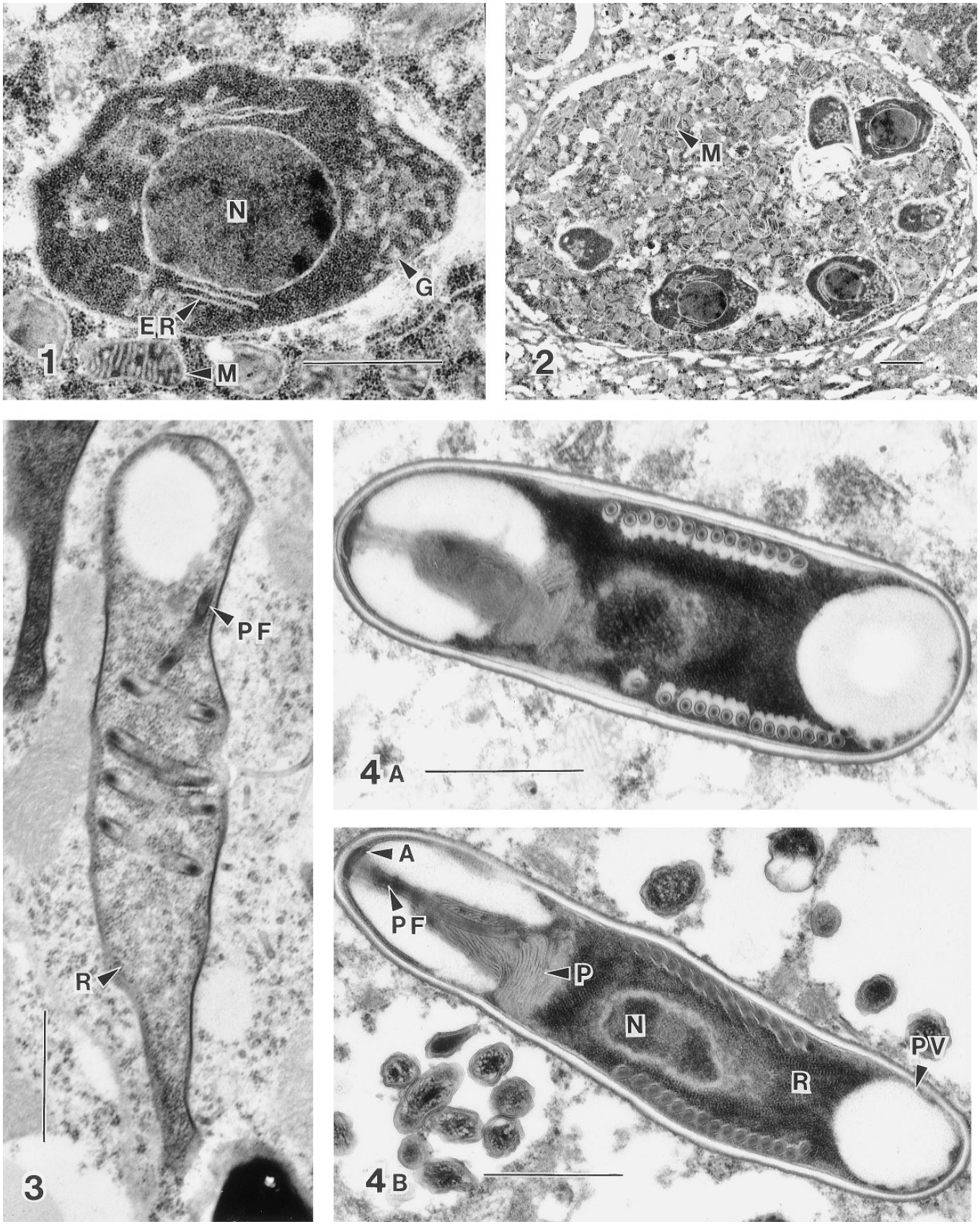
Mature spores were broad- to elongate-ovoid (Figs. 4A and 4B) and measured $4.33 \pm 0.35 \times 1.27 \pm 0.15$ µm in electron micrographs ($n = 13$). Fresh spores measured $5.88 \pm 0.34 \times 2.22 \pm 0.19$ µm ($n = 30$), while those fixed in methanol and Giemsa stained measured $5.37 \pm 0.46 \times 2.22 \pm 0.17$ µm ($n = 30$).

An isofilar polar filament coiled 12 to 15 times in the posterior two-thirds of the spore (Figs. 4A and 4B). The base of its anchoring disc measured 150 nm, with long, slender arms each measuring approximately 250 nm in length (Fig. 5). A lamellar polaroplast occupied approximately one-third of the anterior region of the mature spore. Lamellae in the posterior portion of the polaroplast were more loosely packed than those in the anterior part. Large cavities in the polaroplast are artifacts, probably resulting from incomplete fixation. All spores had a relatively large posterior vacuole occupying about one-fourth of the spore.

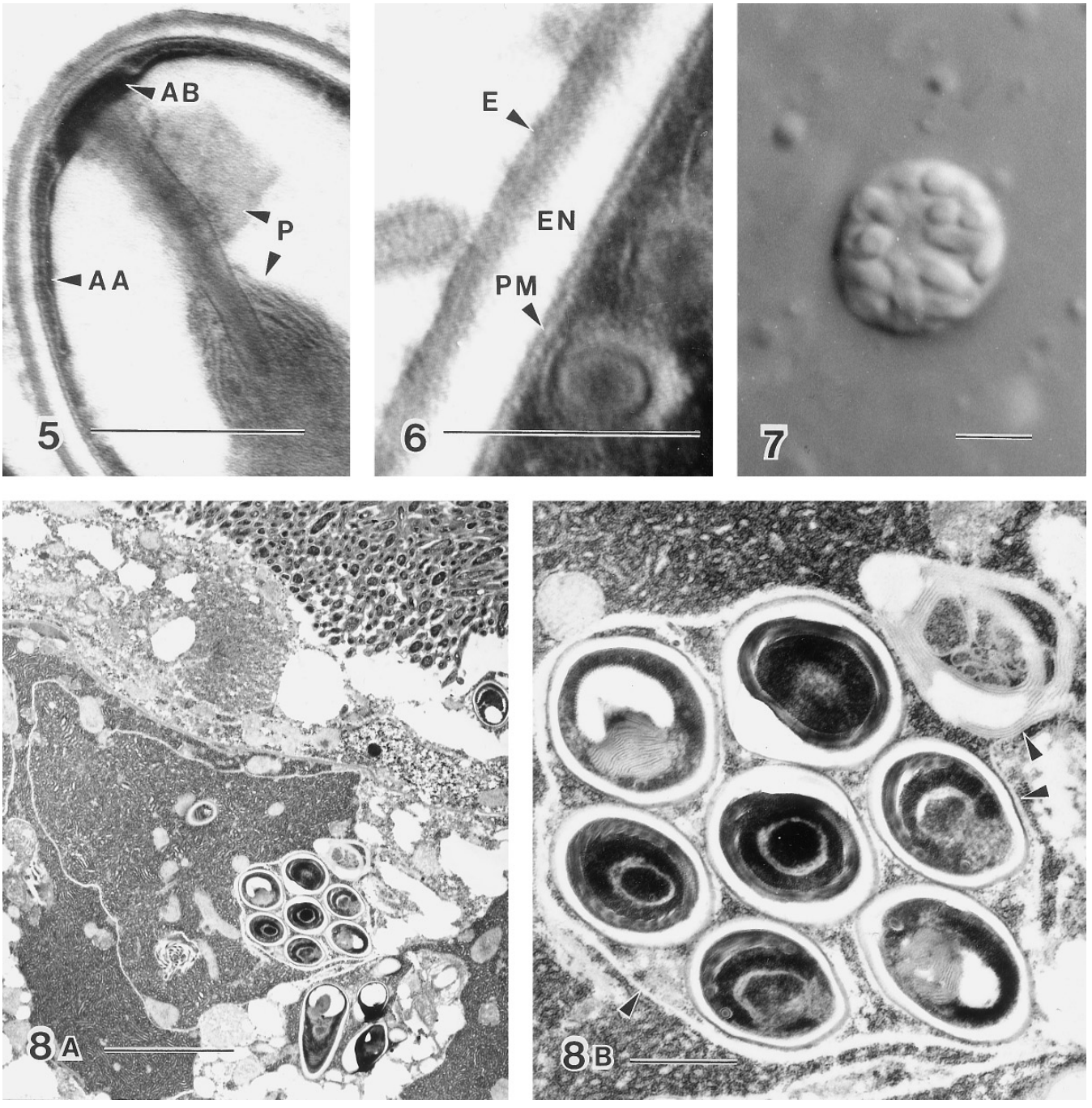
Spore walls were relatively uniform in thickness but thinner at the anterior end. Measurements from micrographs showed them to be 89–101 nm thick and to consist of three layers: an innermost plasma membrane, a translucent endospore layer measuring 54–65 nm, and an electron-dense exospore measuring 30–36 nm (Fig. 6).

Spores were occasionally observed within envelopes of unknown origin. These are referred to as interfacial envelopes after the definition of Sprague *et al.* (1992). Fresh smear preparations examined by light microscopy revealed few interfacial envelopes, some of which persisted as sporophorous vesicles. These often contained 4, 8, to more than 16 spores (Fig. 7).

Both individual spores and spores within interfacial envelopes were observed by transmission electron microscopy. Individual spores, apparently in direct contact with host cytoplasm, were observed throughout all infected mite tissues, including supra- and subesophageal ganglia, muscle tissue, paranchyma, digestive cells of the ventriculus, and cells lining the cecal lumen.



FIGS. 1–4. Developmental stages of the microsporidium in *P. persimilis*. **FIG. 1.** Schizont. **FIG. 2.** Schizont in an unidentified host cell, showing numerous host mitochondria. **FIG. 3.** Sporoblast, showing early development of the polar filament. **FIG. 4.** (A and B). Mature spores, showing variability in shape and size. Scale bars, 1 μm. Abbreviations for all figures are: A, anchoring disc; AA, arms of anchoring disc; AB, base of anchoring disc; E, exospore; EN, endospore; ER, rough endoplasmic reticulum; ES, evacuated spore; G, Golgi apparatus; HN, host cell nucleus; M, mitochondrion; MF, muscle fiber; N, monokaryon; P, polaroplast; PF, polar filament; PM, plasma membrane; PV, posterior vacuole; R, ribosomes.



FIGS. 5–8. Characteristics of mature spores. **FIG. 5.** Polar filament/anchoring disc detail. **FIG. 6.** Spore wall detail. **FIG. 7.** Sporophorous vesicle containing many spores (fresh smear preparation, light microscopy). **FIG. 8.** Spores within an interfacial envelope within a parenchyma cell, underlying host cuticle (A). Interfacial envelope (arrowheads) enveloping eight spores (B). Scale bars: Fig. 5, 0.5 µm; Fig. 6, 0.25 µm; Figs. 7 and 8A, 5 µm; Fig. 8B, 1 µm.

Interfacial envelopes, observed only in parenchyma cells underlying the cuticle (Figs. 8A and 8B), contained up to eight spores.

Pathology

Microsporidian spores were not observed in prey mites (n = 500) taken directly from rearing colonies nor in partially consumed mites (n = 200) exposed to infected *P. persimilis*. Microsporidian-infected *P. persimilis* showed no gross external signs or symptoms, although some gravid females were unable to oviposit. Infected mites often appeared lethargic, and, occasionally, moribund mites rapidly retracted their legs and twitched when disturbed.

Schizonts occurred in digestive cells, in the cytoplasm of cells lining the cecal wall or in its underlying muscle tissue. Schizonts were commonly observed within nuclei of digestive cells of the ventriculus in the chromatin-rich region of a nucleus adjacent to the nuclear membrane (Fig. 9), but occasionally outside nuclei of these cells. Sporont-like cells and sporoblasts occurred within nuclei of digestive cells, and mature spores in both cytoplasm and nuclei. Evacuated spores, remnants of spores after sporoplasm discharge, were occasionally observed in digestive cell nuclei. Sporont-like cells, sporoblasts, and mature spores were not restricted to digestive cells as these were commonly observed in cells lining the ceca. Both mature spores and sporoblasts were observed simultaneously within individual cells.

Infected digestive cells sloughed into the cecal lumen contained developmental stages and mature spores, in addition to food particles and bacteria (Fig. 10). Bacteria were not observed in all specimens examined and are therefore considered secondary pathogens.

Numerous mature spores were observed within cells of the cecal wall. Once infected with microsporidia, these cells hypertrophy and break away from the ceca, damaging the integrity of the cecal wall (Fig. 11). Severe infection caused extensive damage to the cecal wall, including loss of all microvilli and occasional secondary infection by opportunistic bacteria (Fig. 12). Spores were also observed within muscle fibers (Fig. 13), within the cortex of the sub- and supraoesophageal ganglia (Fig. 14), and within developing eggs of gravid females in direct contact with yolk.

DISCUSSION

The microsporidium in *P. persimilis* had unpaired nuclei for all stages of development observed. Spores were broad- to elongate-ovoid, with single ones apparently in direct contact with host cytoplasm. Interfacial envelopes contained 4, 8, to more than 16 spores. Variability in spore shape, infrequent occurrence of interfacial envelopes (which may be an artifact due to incomplete fixation), and the presence of schizonts in both nuclei and cytoplasm of host cells raise the possibility that more than one species of microsporidia was present.

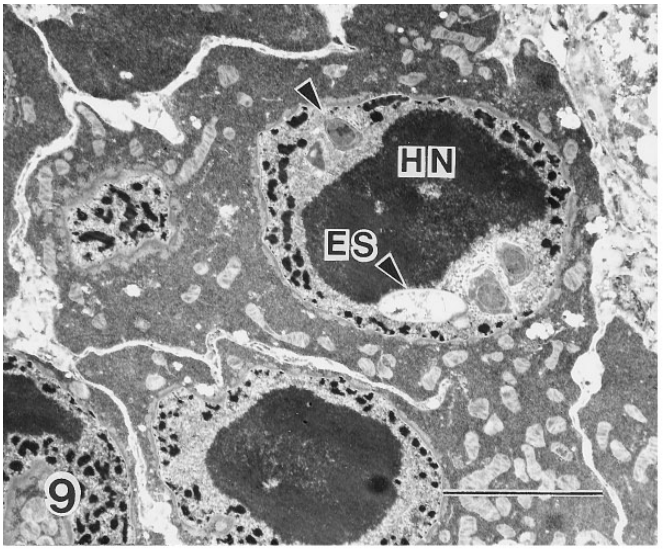
Host cell nuclei are rarely invaded by microsporidia (Canning, 1990), although several exceptions have been observed. *Nosema apis* Zander (in the honeybee, *Apis mellifera* L.), *Nosema fumiferanae* (Thom.) (in spruce budworm, *Choristoneura fumiferana* (Clemens)), and *Nosema bombycis* Naegeli (in the silkworm, *Bombyx mori* (L.)) have been observed within cell nuclei of their respective hosts (Tanada and Kaya, 1993). *Nosema* sp. were detected in nuclei and cytoplasm of midgut muscle cells in the parasitic wasp *Pediobius foveolatus* (Crawford) (Chapman and Hooker, 1992), and *Steinhausia mytilovum* Field, a microsporidium infecting the nurse cells of ovaries and oenocytes of the mussel, *Mytilus edulis* L., occasionally infects host nuclei (Sprague *et al.*, 1992). *Enterocytozoon salmonis* Chilmonczyk, Cox, and Hedrick and *Microsporidium rhabdophilia* Modin develop exclusively within cell nuclei of salmonid fish (Chilmonczyk *et al.*, 1991).

Although infected nuclei often hypertrophy, protein synthesis necessary for cells to grow and accommodate maturing parasites continues (Canning, 1990). In *P. persimilis*, infection of digestive cell nuclei was clearly observed but their premature death may be insignificant, as undifferentiated cells in the ventriculus are continuously maturing and replacing them. In starved, uninfected mites, few mature digestive cells are present in the gut (Chant, 1985). Shortly after ingestion of food, undifferentiated cells mature and become more vacuolated. These absorb food particles and are eventually sloughed into the cecal atrium. Degeneration of mature cells occurs rapidly and sloughed ones are replaced continually during digestion (Akimov and Starovir, 1974).

Evacuated spores observed in the nuclei of digestive cells are evidence of autoinfection. Sloughing of infected digestive cells into the cecal lumen may disseminate microsporidia throughout the digestive system. The presence of schizonts in the cecal wall and in its underlying muscle tissue supports the assumption that autoinfection of the ceca does occur. Extensive damage and numerous mature spores within the cecal wall further indicate autoinfection. Secondary infection by opportunistic bacteria is occasionally observed when wall damage is extensive.

Destruction and sloughing of cells of the cecal wall may lead to reinfection of the same host and infection of new ones. Once in the cecal atrium, spores may be excreted with metabolic waste onto foliage and provide inoculum necessary for horizontal transmission. Mature spores have also been observed in developing eggs within gravid females and these may provide a means for vertical transmission.

Microsporidia previously reported to infect aquatic and terrestrial mites are listed in Table 1. The first reported occurrence of microsporidiosis in mites was by Weiser (1956) of *Nosema steinhausi* Weiser in the phytophagous mite *Tyrophagus noxius* Zakhvatkin. A second species, *N. sperchoni* Lipa, was identified from a single specimen of the water mite, *Sperchon* sp. (see Lipa, 1962). A highly pathogenic microsporidium, *Gurleya sokolovi* Issi and Lipa was found in hemocytes, nerve cells, and adipose tissue of the water mite, *Limnochares aquatica* L. (Issi and Lipa, 1968), and a second microsporidium, *Napamichum aequifilum* Larsson, was reported to infect the same mite (Larsson, 1990). Hazard and Oldacre (1976) described *Cryptospo-*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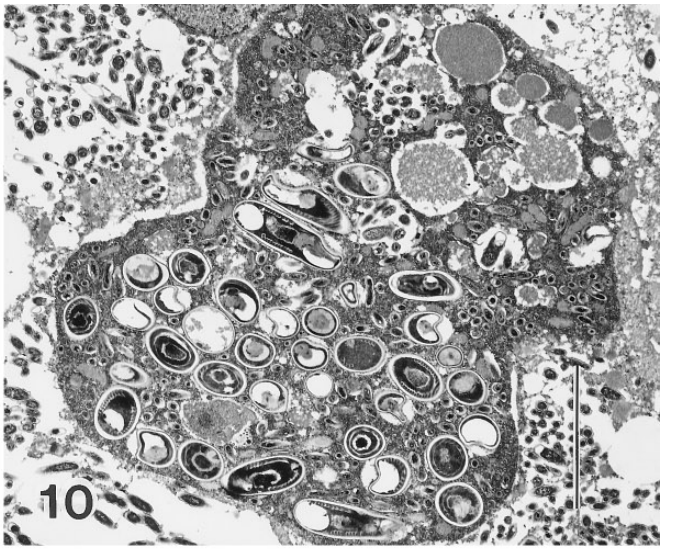
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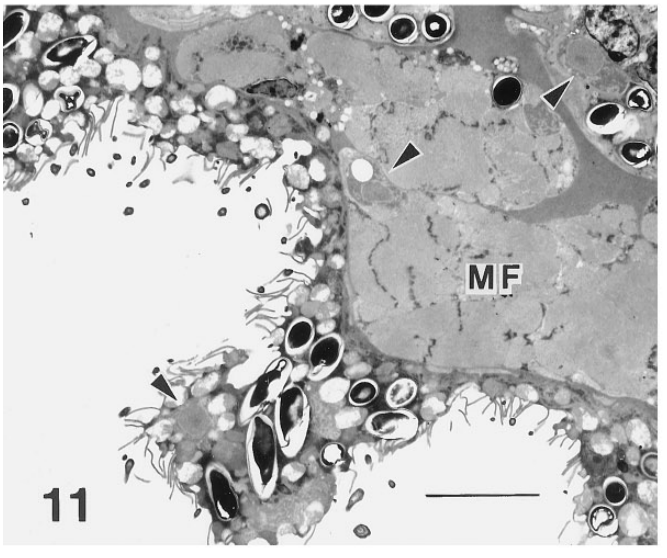
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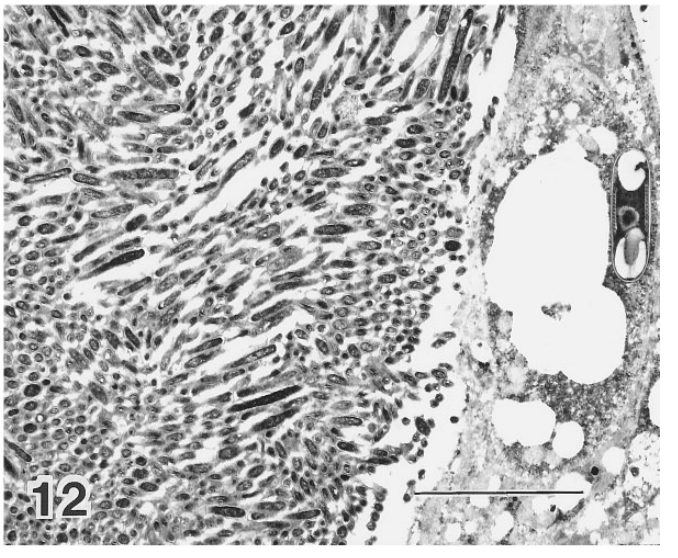
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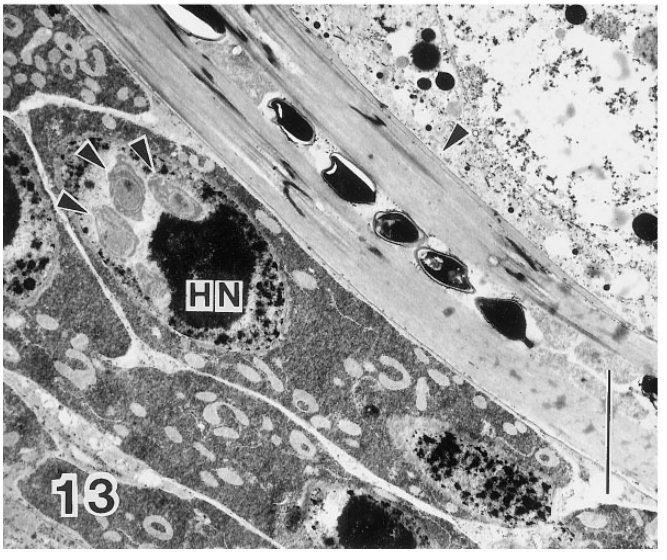
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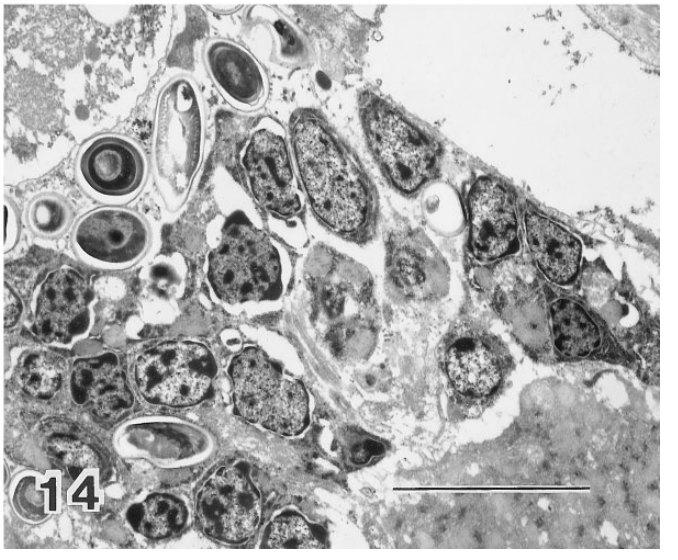
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TABLE 1
Microsporidia Reported in Aquatic and Terrestrial Mites

Author	Host	Microsporidium	Spore dimensions (μm)
Weiser (1956)	*Tyrophagus noxius*	*Nosema steinhausi*	2.2–3 × 1–1.5
Davies (1960)	*Sperchon* nr. *jasperensis*	*Nosema* sp.	(?)
Lipa (1962)	*Sperchon* sp.	*Nosema sperchoni*	4.5–5.5 × 3.0–3.5
Issi and Lipa (1968)	*Limnochares aquatica*	*Gurleya sokolovi*	6.9–7.5 × 3.5–4.4 (fresh) 4.5–6.6 × 1.9 (fixed/stained)
Hazard and Oldacre (1976)	*Piona* sp.	*Cryptosporina brachyfila*	1.80–1.91 × 0.53–0.80
Purrini and Bäumler (1976)	*Rhysotritia ardua*	*Nosema ptyctimae*	4.4–4.8 × 1.6–1.8
Purrini and Weiser (1981)	Oribatid mites	*Pleistophora oribatei*	2.0–2.5 × 1.0–1.5
		Pleistophora cephei	3.0–3.5 × 2.0–2.5
		Pleistophora platynothri	4.5–5.0 × 2.5
		Pleistophora dindali	2.0–2.5 × 1.8–2.0
		Thelohania microtritiae	1.8–2.0 × 1.5
		Nosema acari	4.0–5.5 × 2.2–2.5
		Nosema steganacari	3.5–4.0 × 2.0–2.5
		Nosema führeri	2.5–3.0 × 2.0
Lipa (1982)	*Euzetes seminulum*	*Nosema euzeti*	3.1–4.3 × 1.9–2.5 (fixed/stained) 3.2–4.7 × 1.9–2.6 (fresh)
Larsson (1990)	*Limnochares aquatica*	*Napamichum aequifilum*	5.4–5.8 × 3.0 (unfixed) 4.0–5.0 × 2.8–3.0 (fixed/stained)
Beerling *et al.* (1993)	*Amblyseius cucumeris* *Amblyseius barkeri* *Acarus siro* *Tyrophagus putrescentiae*	Fam: Pleistophoridae	1.8 × 0.9

rina brachyfila Hazard and Oldacre in adipose tissue of *Piona* sp., a water mite.

The first description of microsporidiosis in oribatid mites was recorded by Purrini and Bäumler (1976) with *Nosema ptyctimae* Purrini and Bäumler in the fat body and nephrocytes of *Rhysotritia ardua* C. L. Koch and *Nosema euzeti* Lipa was reported to infect both larvae and adults of *Euzetes seminulum* (O. F. Müller) (Lipa, 1982).

In 1981, Purrini and Weiser found 12 species of moss mites to be infected with eight distinct microsporidia in three genera. Four belonged to the genus *Pleistophora* Gurley, 3 to *Nosema* Naegeli and a single species to *Thelohania* Henneguy. Microsporidia in the Family Pleistophoridae Doflein have been reported to infect *N. cucumeris* (formerly *Amblyseius*) and *A. barkeri*, two phytoseiid mites used for biological control of western flower thrips, *F. occidentalis*, and onion thrips, *Thrips tabaci*, in commercial greenhouses. In this instance, the phytophagous mites *Acarus siro* L. and *Tyrophagus putrescentiae* (Schrank), which serve as food for *A. cucumeris* and *A. barkeri* in mass rearing, were infected with the same microsporidian species (Beerling and van der Geest, 1991; Beerling *et al.*, 1993). Microsporidia were not detected in prey mites used in our study.

Only *Pleistophora platynothri* Purrini and Weiser, in the oribatid mite *Platynothrus peltifer* C. L. Koch (Purrini and Weiser, 1981), has uninucleate spores with measurements similar to those found in *P. persimilis* (Table 1). The microsporidium in *P. persimilis*, therefore, represents a previously undescribed species. Incomplete information on the life cycle and development of this microsporidium makes it difficult to ascribe it to an existing genus. For this reason we have assigned this microsporidium to the collective group *Microsporidium* and given it the specific name *phytoseiuli*.

Taxonomic Summary

Type host. *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae).

Transmission. Unknown. Evidence of autoinfection in digestive cell nuclei and ceca. Mature spores observed in developing eggs within gravid females may provide a means for vertical transmission.

FIGS. 9–14. Pathology caused by the microsporidium in *P. persimilis*. **FIG. 9.** Development of schizonts (arrowheads) in nucleus of digestive cell in ventriculus. **FIG. 10.** Digestive cell containing microsporidian spores and bacteria, sloughed off into cecal atrium. **FIG. 11.** Microsporidian spores within cells of cecal wall, causing cell hypertrophy and sloughing of cells into cecal atrium. Schizonts (arrowheads) observed in cells of cecal wall and muscle fibers underlying the cecal wall. **FIG. 12.** Secondary infection of bacteria in ceca and destruction of cecal wall. **FIG. 13.** Mature spores within muscle fiber. Schizonts in digestive cell nucleus (arrowheads). **FIG. 14.** Microsporidian spores in cortex of subesophageal ganglion. Scale bars, 5 μm.

Site of infection. Nuclei of digestive cells (ventriculus), cecal wall, muscle tissue, cortical regions of sub- and supraesophageal ganglia, parenchyma cells underlying the cuticle, and within developing eggs.

Interface. Schizonts observed almost exclusively in digestive cell nuclei: sporont-like cells, sporoblasts, and spores in direct contact with host cytoplasm. Groups of 4, 8, to more than 16 spores occasionally observed within envelopes of unknown origin.

Other parasite–host cell relations. Parasites occur in both host cell nucleus and cytoplasm. Hypertrophy of infected cecal wall cells.

Development. All stages observed had unpaired nuclei. Schizogony and sporogony not recognized.

Spore. Broad- to elongate-ovoid. Fresh spores are $5.88 \pm 0.34 \times 2.22 \pm 0.19$ µm, Giemsa-stained spores are $5.37 \pm 0.46 \times 2.22 \pm 0.17$ µm, and spores from transmission electron micrographs are $4.33 \pm 0.35 \times 1.27 \pm 0.15$ µm. Spores are uninucleate. An isofilar polar filament coils 12 to 15 times. Polaroplast lamellar. Spore walls relatively uniform in thickness (89–101 nm) but thinner at the anterior end. Exospore moderately developed (30–36 nm) and endospore relatively thick (54–65 nm). Spores occasionally observed within envelopes of unknown origin. Groups of 4, 8, to more than 16 spores.

Type locality. Biological control agents obtained from a commercial insectary in Europe.

Deposition of type specimens. Retained by the author (S.B.).

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