Morphological and molecular characterization of a new microsporidian species from the predatory mite *Metaseiulus occidentalis* (Nesbitt) (Acari, Phytoseiidae)

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Abstract

A new microsporidian species is described from the predatory mite *Metaseiulus* (formerly *Typhlodromus* or *Galendromus*) *occidentalis* (Nesbitt) (Acari, Phytoseiidae). The ultrastructure of this new species is presented together with the first molecular characterization for a microsporidium of mites. All stages of this new microsporidium are haplokaryotic and develop in direct contact with the host-cell cytoplasm. Sporogony is disporoblastic and spores are formed in eggs, immature stages, and adults of *M. occidentalis*. There are two morphological classes of spores, one with a short polar filament (3–5 coils) that measured $2.53 \times 1.68\,\mu m$ and one with a longer polar filament (8–9 coils) that measured $3.14 \times 1.77\,\mu m$. Horizontal transmission of this new species occurs by cannibalism of eggs and other stages and perhaps involves the spores with the long polar filament. Spores with the short polar filament may play a role in autoinfection and vertical (transovarial) transmission that is highly efficient in transferring the microsporidium from adults to progeny. Analysis of the small subunit ribosomal DNA indicated that this species from *M. occidentalis* is most closely related to the *Nosema/Vairimorpha* clade of microsporidia. A conflict between the morphological and molecular data is discussed. The species is compared to previously described microsporidia of arachnids resulting in creation of *Oligosporidium occidentalis* n. sp. in the family Unikaryonidae. © 2002 Elsevier Science (USA). All rights reserved.

Keywords: Acari; *Metaseiulus occidentalis*; Microsporidia; Unikaryonidae; *Oligosporidium*

1. Introduction

Diseases of pest mites are often lauded as promising biological control agents but diseases of predatory mites used as biological control agents are undesirable because of economic losses caused during culture and poor performance in the field (Van der Geest et al., 2000). Microsporidia are insidious pathogens that produce chronic infections characterized by a general debilitation of the host and are efficiently transmitted both horizontally and vertically (Becnel and Andreadis, 1999). Because of these attributes, microsporidia can rapidly establish and spread within colonies relatively unnoticed until virtually all individuals are infected and productivity and performance are reduced. Mites in the family Phytoseiidae are important predators of spider mites in various agroecosystems (Huffaker et al., 1970) and microsporidia have been reported from several species including *Phytoseiulus persimilis* (Bjørnson and Keddie, 2000), *Neoseiulus cucumeris*, and *Amblyseius barkeri* (Beerling and van der Geest, 1991). Recently, a species of microsporidia has been isolated from the phytoseiid *Metaseiulus* (formerly *Typhlodromus* or *Galendromus*) *occidentalis* (Nesbitt) that has a negative impact on the overall fitness of this predator (Olsen and Hoy, 2002). Here we present an ultrastructural and molecular description of this new microsporidian species and discuss its taxonomic classification and placement.

2. Materials and methods

The new microsporidium was described using standard techniques (Undeen and Vávra, 1997). Air-dried

smears from infected mites were fixed in methanol and stained for 10 min with 10% Giemsa stain buffered at pH 7.4. Fresh spores from *M. occidentalis* were measured using a Vickers A.E.I. Image Splitting Micrometer. Fresh squashes of *M. occidentalis* and the prey mites *Tetranychus urticae* (Koch) were examined for the presence of spores.

Processing mites for ultrastructural studies was difficult due to their small size and thick cuticles. Nymphs and adults of *M. occidentalis* were restrained by placing them onto double-sided sticky tape with a brush followed by the addition of a drop of 2.5% glutaraldehyde at room temperature (RT, approximately 25 °C) onto each specimen. Finely pulled capillary tubes were used to pierce the cuticle of the submerged individuals to allow for better exchange of fixatives and plastics. These individuals were immediately transferred to 2.5% glutaraldehyde plus 1% acrolein at 60 °C and then placed onto an orbital shaker at RT for 2 h. Specimens were post-fixed in 1% osmium tetroxide at RT, dehydrated through an ascending ethanol and acetone series, and embedded in Epon–Araldite. Thin sections were post-stained with 50% aquatic uranyl acetate at 60 °C followed by lead citrate, examined, and photographed with a Hitachi H-600 electron microscope at 75 kV. We were not able to process infected mite eggs for ultrastructural examination and therefore all information for stages in eggs was obtained from fresh squashes and Giemsa-stained preparations.

Genomic DNA was extracted from 20 infected females of the COS-Chico strain of *M. occidentalis* (Hoy, 1984) using PUREGENE reagents following manufacturer's suggested protocol (Gentra Systems, Minneapolis, MN) and the pellet was resuspended in 20 µl water. Small subunit ribosomal RNA gene sequence from *Vairimorpha necatrix*, available in GenBank (Accession No. Y00266) (Vossbrinck et al., 1987), was used to design primers (30-mers, Mic-F, 5′-CACCAGGTT GATTCTGCCTGACGTAGACGC-3′ and Mic-R, 5′-GATCCTGCTAATGGTTCTCCAACAGCAACC-3′). Long PCR was performed in a 50 µl volume containing 1 µl mite DNA preparation, 50 mM Tris (pH 9.2), 16 mM ammonium sulfate, 1.75 mM MgCl$_2$, 350 µM dATP, dGTP, dCTP, dTTP, 800 pmol of primers (Mic-F and Mic-R), 1 U of *Pwo*, and 5 U of *Taq* DNA polymerases (Barnes, 1994). The Long PCR was carried out using three linked profiles:

(i) 1 cycle consisting of denaturation at 94 °C for 2 min.

(ii) 10 cycles each consisting of denaturation at 94 °C for 10 s, annealing at 65 °C for 30 s, and extension at 68 °C for 1 min.

(iii) 25 cycles each consisting of denaturation at 94 °C for 10 s, annealing at 65 °C for 30 s, and extension at 68 °C for 1 min, plus an additional 20 s added for every consecutive cycle.

Recombinant DNA techniques, including restriction digests, ligation, bacterial transformation, and plasmid DNA preparation, were performed by standard methods (Sambrook et al., 1989). Long PCR products were purified using QIAquick PCR Purification Columns (QIAGEN, Valencia, CA) and cloned into the plasmid pCR2.1-TOPO using the procedure suggested by the manufacturer (Invitrogen, Carlsbad, CA). DNA sequencing was performed at the University of Florida Interdisciplinary Core Facility for Biotechnology Research using a Perkin–Elmer Applied Biosystems ABI PRISM Automated DNA Sequencer. DNA sequences were aligned by using CLUSTAL W, transported into MacClade and PAUP 4.0b8 to generate phylogenetic trees.

3. Results

Infected mites did not exhibit any external or gross signs of infection. Development of this microsporidium occurred in eggs, larvae, nymphs, and adults of *M. occidentalis*. Immature stages and mature spores were found in the cytoplasm of cecal cells (Fig. 1) (Figs. 1–6), lyrate organ cells, ganglia, epithelial cells, muscle, within the ovary, and in developing and mature eggs. There was no interfacial envelope as presporulation and sporulation stages were all in direct contact with the host-cell cytoplasm. No sporophorous vesicle was present at any point in the developmental cycle.

All life cycle stages had isolated nuclei. The earliest stages observed were spherical or ovoid uninucleate schizonts limited by a simple plasmalemma (Fig. 2). Schizogony was a series of binary divisions producing numerous schizonts. The nuclei and cytoplasm of schizonts were fairly electron dense with typical microsporidian cytoplasmic organelles (ribosomes, endoplasmic reticulum, Golgi bodies, spindle plaques, and associated polar bodies). The final schizonts transformed directly into spherical uninucleate sporonts (Fig. 3). Early sporonts were characterized by a thickening of the plasmalemma and a less electron dense cytoplasm. Sporogony was by binary fission with each sporont dividing once to produce two uninucleate sporoblasts (Fig. 4). Sporogenesis began with the concurrent formation of the exospore and polar filament followed by cell elongation (Fig. 5). Development in the eggs followed the same pattern as that observed in nymphs and adults with all stages being uninucleate and division by simple binary fission.

Two classes of uninucleate spores were produced differing primarily in the length of the polar filaments and the presence in one of a large posterior vacuole (Fig. 6). One class of spores was most common in nymphs and young adults and was characterized at the light level by the presence of a prominent posterior vacuole (Fig. 6). It
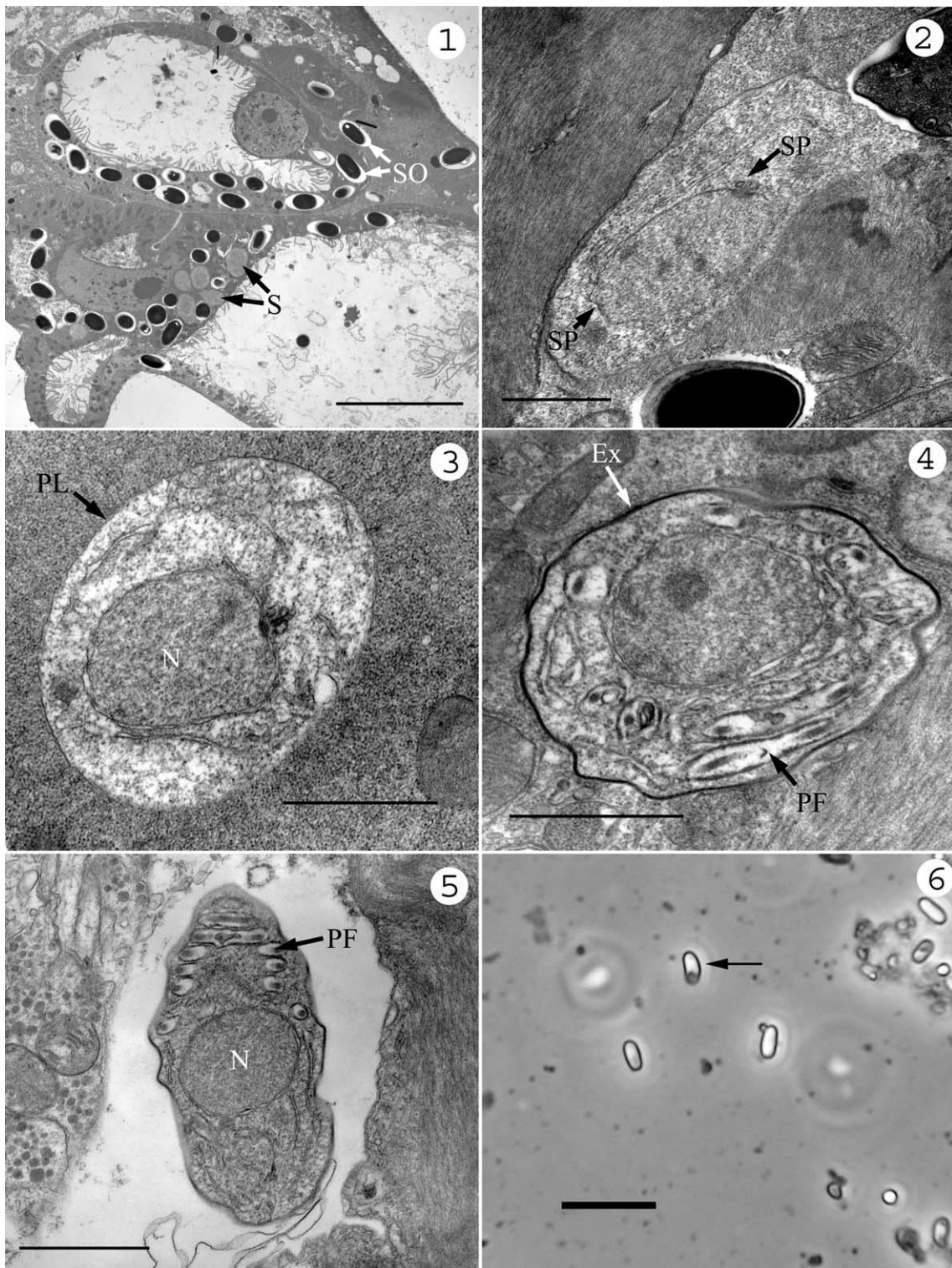
Figs. 1–6. Developmental stages of *O. occidentalis* n. sp. in adult *M. occidentalis*. (1) Sporonts (S) and spores (SO) in caecal wall cells. (2) Schizont. Note the spindle plaques (SP) on the nuclear envelope in preparation for division. (3) Sporont characterized by a thickened plasmalemma (PL) and less dense cytoplasm. N, nucleus. (4) Early sporoblast as indicated by formation of the exospore (EX) and primordium of the polar filament (PF). (5) Elongate sporoblast with a single centrally located nucleus (N). PF, polar filament. (6) Fresh spores, one with a large posterior vacuole (arrow).

contained a short isofilar polar filament composed of 3–5 coils which were arranged such that the first turn began near the midpoint of the spore and then angled to the posterior part of the spore (Figs. 7 and 11) (Figs. 7–13). The polar sac-anchoring disk complex was subterminal with a bipartite lamellar polaroplast (Fig. 8). Fresh
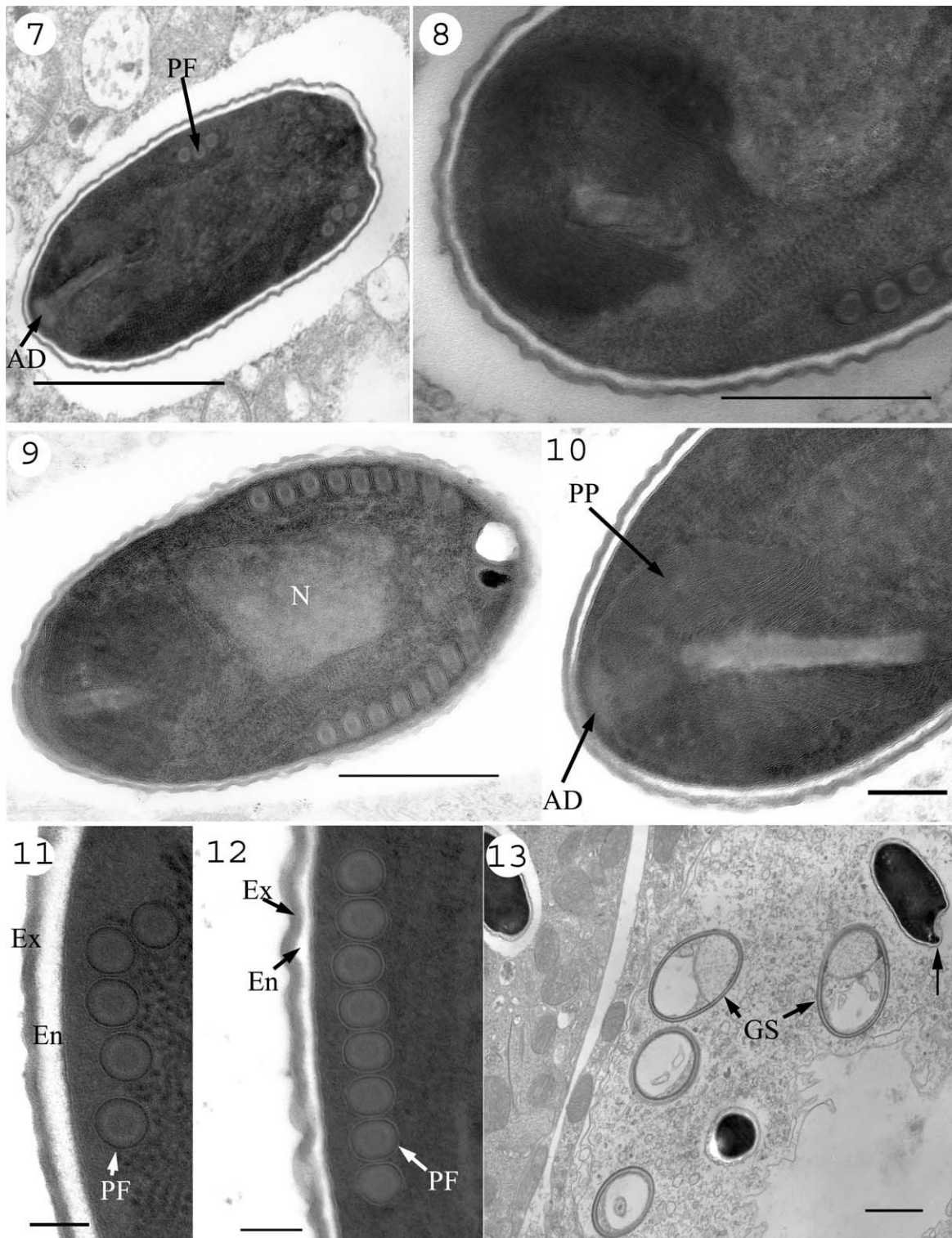
Figs. 7–13. Spores of *O. occidentalis* n. sp. in adult *M. occidentalis*. (7) Uninucleate spore with a polar filament (PF) making 3–4 turns. AD, anchoring disk. (8) Bipartite lamellar polaroplast of a spore with a short polar filament. (9) Uninucleate spore with a polar filament making 8–9 turns. N, nucleus. (10) Subterminally positioned anchoring disk (AD) and the bipartite lamellar polaroplast (PP) of a spore with a long polar filament. (11–12) Details of the spore wall (EX, exospore; EN, endospore) and polar filament coils (PF) of a spore with a short polar filament (11) and a longer polar filament (12). (13) Intracellular germinated spores (GS). Note that the mature spore (arrow) is of the short polar filament class with a collapsed posterior vacuole.

spores with the large posterior vacuole (Fig. 6) measured $2.53 \pm 0.06\,\mu m \times 1.68 \pm 0.06\,\mu m$ (mean ± standard error, $n = 8$). Spores with short polar filaments were quite transitory, apparently germinating intracellularly to spread the infection to other tissues (Fig. 13). In older adult mites, another class of spore was observed which contained an isofilar polar filament arranged in 8–9 coils around the nucleus in the posterior half of the spore (Figs. 9 and 12). The polar sac-anchoring disk complex was also subterminal with a bipartite lamellar polaroplast (Fig. 10) and a small posterior vacuole. Fresh spores measured $3.14 \pm 0.07\,\mu m \times 1.77 \pm 0.03\,\mu m$ ($n = 24$). Both spore types were oblong-ovoid with a rugose spore wall composed of three layers; an external unlayered electron dense exospore, a thicker median lucent endospore layer, and an internal plasmalemma (Figs. 11 and 12).

Fresh spores from eggs measured $3.06 \pm 0.07\,\mu m \times 1.70 \pm 0.03\,\mu m$ ($n = 32$) did not have a large posterior vacuole and were not significantly different from the size of the larger spore found in older adult mites.

A 1.2 kb DNA band was readily amplified from genomic DNA preparations of infected *M. occidentalis* by Long PCR using the microsporidian ssrRNA-primers (Mic-F and Mic-R) and cloned into pCR2.1-TOPO plasmid. Identical 1240 bp DNA sequences were obtained from two independent clones (pAJ110 and pAJ111) (GenBank Accession No. AF495379). A BLAST search of the GenBank database with the sequence obtained from infected *M. occidentalis* has detected close matches to other microsporidia sequences: *Nosema vespula* (Accession No. U11047), *N. portugal* (AF033316), *N. oulemae* (U27359), *N. ceranae* (U26533), *N. apis* (U26534), *N. bombycis* (L39111), *N. trichoplusiae* (U09282), *N. furnacalis* (U26532), *N. granulosis* (AJ011833), *Vairimorpha lymantriae* (AF033315), *V. necatrix* (Y00266), *Encephalotozoon cuniculi* (Z19563), *E. hellem* (AF118142), *E. intestinalis* (L19567), *Nucleospora*

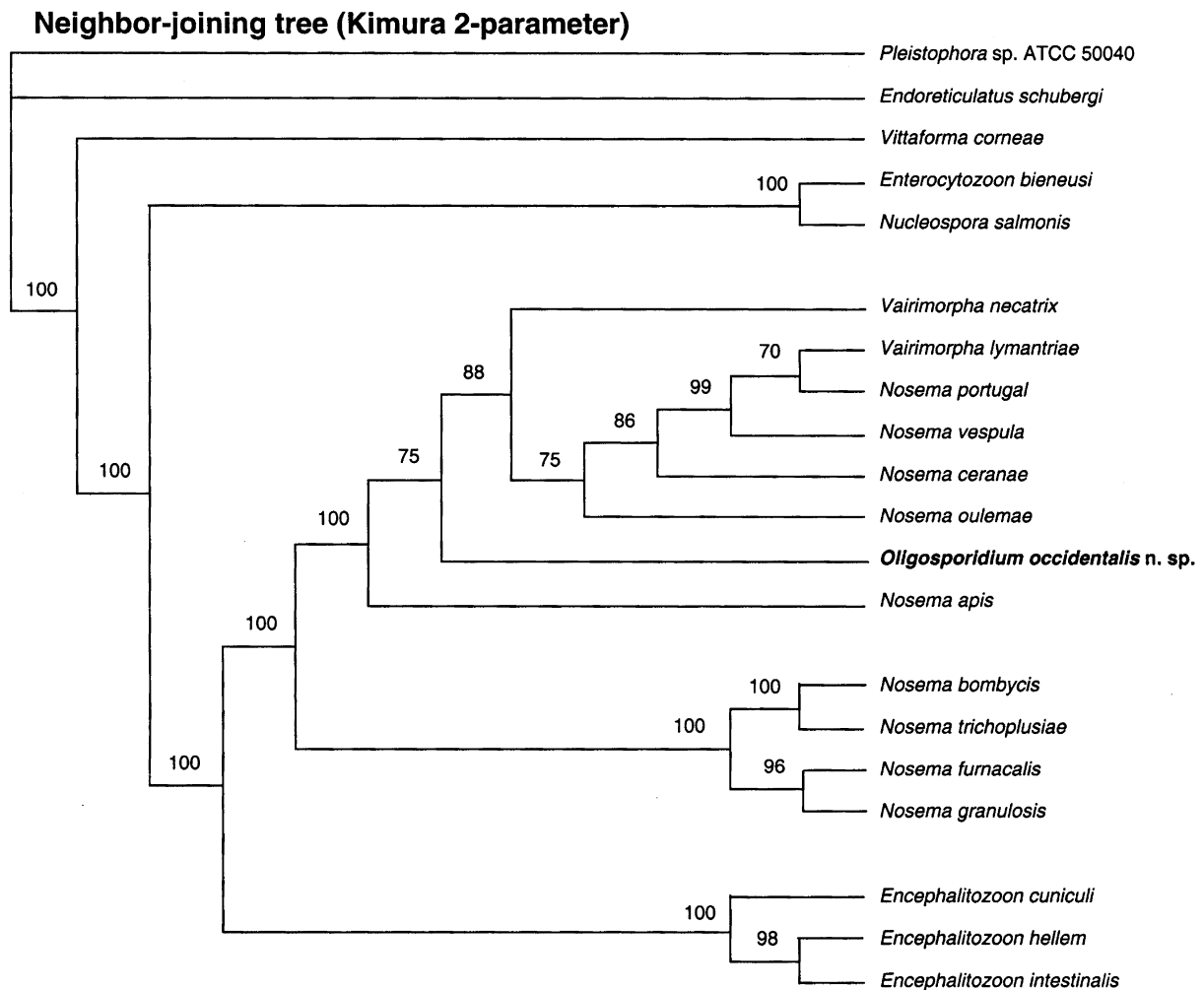Fig. 14. A Neighbor-joining tree generated for microsporidia using a CLUSTAL W alignment of ssrRNA gene sequences with the optimality criteria set for distance and Kimura 2-parameter in PAUP 4.0b8. Bootstrap values detected for 100 replicates are shown before the nodes. *E. schubergi* and *Pleistophora* are used as outgroup. The microsporidium *O. occidentalis* n. sp., from *M. occidentalis* is shown in bold.

Table 1
Pairwise distance between microsporidian species for ssrRNA sequences was obtained using PAUP 4.0b8 with optimality criteria set for distance and Kimura 2-parameter algorithm

Microsporidia	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. *Pleistophora*	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2. *E. schubergi*	0.007	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3. *V. comeae*	0.132	0.128	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–ddd	–	–
4. *E. bineusi*	0.339	0.341	0.334	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5. *N. salmonis*	0.332	0.331	0.318	0.179	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6. *V. necatrix*	0.452	0.456	0.435	0.461	0.469	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7. *V. lymantriae*	0.447	0.451	0.432	0.461	0.466	0.041	0	–	–	–	–	–	–	–	–	–	–	–	–	–
8. *N. portugal*	0.447	0.451	0.431	0.460	0.465	0.039	0.003	0	–	–	–	–	–	–	–	–	–	–	–	–
9. *N. vespula*	0.449	0.454	0.430	0.467	0.469	0.038	0.003	0.003	0	–	–	–	–	–	–	–	–	–	–	–
10. *N. ceranae*	0.456	0.460	0.440	0.476	0.479	0.047	0.022	0.025	0.022	0	–	–	–	–	–	–	–	–	–	–
11. *N. oulemae*	0.453	0.457	0.436	0.464	0.461	0.034	0.022	0.021	0.019	0.035	0	–	–	–	–	–	–	–	–	–
12. *O. occidentalis*	0.450	0.455	0.434	0.461	0.463	0.056	0.046	0.044	0.044	0.051	0.047	0	–	–	–	–	–	–	–	–
13. *N. apis*	0.452	0.456	0.444	0.461	0.457	0.065	0.060	0.056	0.057	0.064	0.055	0.058	0	–	–	–	–	–	–	–
14. *N. bombycis*	0.495	0.500	0.474	0.485	0.481	0.230	0.224	0.227	0.228	0.229	0.229	0.233	0.233	0	–	–	–	–	–	–
15. *N. trichoplusiae*	0.486	0.491	0.466	0.474	0.472	0.217	0.212	0.215	0.216	0.217	0.217	0.213	0.222	0.010	0	–	–	–	–	–
16. *N. furnacalis*	0.492	0.497	0.458	0.478	0.468	0.219	0.213	0.214	0.215	0.216	0.216	0.210	0.220	0.034	0.025	0	–	–	–	–
17. *N. granulosis*	0.494	0.496	0.460	0.473	0.472	0.218	0.216	0.218	0.218	0.221	0.216	0.209	0.221	0.035	0.025	0.010	0	–	–	–
18. *E. cuniculi*	0.485	0.486	0.473	0.448	0.475	0.486	0.481	0.481	0.483	0.486	0.476	0.477	0.489	0.529	0.511	0.504	0.508	0	–	–
19. *E. hellem*	0.423	0.426	0.428	0.418	0.436	0.406	0.408	0.406	0.407	0.409	0.405	0.402	0.405	0.446	0.430	0.436	0.444	0.237	0	–
20. *E. intestinalis*	0.417	0.424	0.427	0.408	0.418	0.401	0.415	0.411	0.412	0.417	0.403	0.390	0.397	0.444	0.432	0.435	0.443	0.225	0.096	0

Distance measured using a scale of 0–1.

salmonis (U78176), *Enterocytozoon bieneusi* (AF024657), and *Vittaforma corneae* (U11046). Sequences from *Endoreticulatus schubergi* (L39109) and *Pleistophora* sp. ATCC 50040 (U10342) were identified as outgroups and included for the analysis.

Phylogenetic analyses were conducted using a total of 1323 aligned characters (alignment deposited into EMBL database, Accession No. ALIGN-000348). The sequence divergences detected between closely related microsporidia species varied from as low as 0.003% between *N. vespula* and *N. portugal* to as high as 52.9% between *E. cuniculi* and *N. bombycis* (Table 1). The sequence divergence detected between the microsporidium of *M. occidentalis* and the closely related *N. apis* from the honey bee *Apis mellifera* was 5.8%. A Neighbor-joining tree was generated using PAUP 4.0b8 with optimality criteria set for the distance and Kimura 2-parameter (Fig. 14). Bootstrap values for the Neighbor-joining tree were above 70% for all the branches and therefore strong support exists for this tree. The sequence obtained from infected *M. occidentalis* clustered within the *Nosema/Vairimorpha* clade of microsporidia. The ssrRNA gene sequence obtained from infected *M. occidentalis* was unique and the phylogenetic analysis supports the conclusion drawn from the morphological data that this microsporidium is a new species (Fig. 14).

4. Discussion

Microsporidia from several different genera produce spores of two classes; the first or early spore has a short polar filament responsible for autoinfection and the other with a longer polar filament is responsible for infection between hosts. Examples of microsporidia with two classes of spores include *N. bombycis* (Iwano and Ishihara, 1991), *N. apis* (deGraaf et al., 1994), *N. muscidifuracis* (Becnel and Geden, 1994), *V. necatrix* (Solter and Maddox, 1998), and *Edhazardia aedis* (Johnson et al., 1997). In all of these cases, the two classes of spores have been diplokaryotic. This new species in *M. occidentalis* seems to be the first species for which these two classes of spores are uninucleate, although they are otherwise similar in morphology and function to the previously described spores. It has been suggested that the differences between these two classes of spores can be explained as different degrees of maturation and that the production of early spores may be a normal phase of the microsporidian life cycle (Larsson, 1999). We agree with the latter conclusion that production of an early spore may be a part of the developmental cycles of many microsporidia but the suggestion that the early spores are immature and mature variants of the same spore type requires additional study.

Several attempts were made to amplify ssrRNA gene sequence from microsporidia-infected *M. occidentalis*

females. The primers (19-mer, 5′-GTTGATTCTGCC TGACGTA-3′ and 20-mer, 5′-TGTATTGCGCGTGC AGCCCA-3′) and a Standard PCR procedure (Saiki, 1989) suggested by Malone and McIvor (1996) were used initially to amplify microsporidian DNA from infected *M. occidentalis*, but no PCR products could be obtained. This finding prompted us to introduce two modifications to the assay:

(i) the primer length was increased; 30-mers (Mic-F and Mic-R) were designed from the *V. necatrix* ssrRNA gene and

(ii) the Long PCR procedure was used (Barnes, 1994). These modifications allowed amplification of the ssrRNA sequence from infected *M. occidentalis* and eliminated the need for concentrating the spores by centrifugation (Adler et al., 2000). The Long PCR procedure has been used to amplify *Wolbachia* bacterial DNA from a wide variety of arthropods and the greening bacterium *Liberobacter asiaticum* DNA from infected citrus leaves more efficiently than Standard PCR (Hoy et al., 1999; Hoy et al., 2001; Jeyaprakash and Hoy, 2000). Long PCR was 5–6 orders of magnitude more efficient than Standard PCR in amplifying these microbial DNAs when they were present mixed in with genomic DNA of eukaryotes (Hoy et al., 2001; Jeyaprakash and Hoy, 2000). Therefore, Long PCR may be useful for amplification of microsporidial DNA from other infected arthropods.

Microsporidia from mites have been assigned to a number of different genera, with most species assigned to *Nosema*, *Pleistophora* or the unclassified genus *Microsporidium*. Single microsporidian species found in mites are also known from *Cryptosporina*, *Gurleya*, *Napamichum*, *Thelohania*, and the new genus *Intexta* a species with atypical cytology, classified in the family Chytridiopsidae (Bjørnson et al., 1996; Larsson et al., 1997). There is a paucity of information on the biological and morphological features of the previously described species with typical cytology and to date none of these have been characterized at the molecular level. The rather comprehensive information on the life cycle, cytology, and molecular characterization assembled for this new species presents a dilemma in generic placement because the morphological and molecular data conflict.

The new species described here exhibits developmental and morphological features typical for the family Unikaryonidae that includes the genera *Oligosporidium*, *Orthosomella*, *Unikaryon*, *Canningia*, and *Larssoniella*. These genera have unpaired nuclei in all stages (no diplokarya), develop in direct contact with the cytoplasm (no interfacial envelope), and sporogony is disporoblastic for *Unikaryon*, *Oligosporidium*, *Canningia*, and *Larssoniella* and polysporoblastic in *Orthosomella* (Sprague et al., 1992). Only one class of spore has been reported for species in these genera. Canning (1990) considered *Oligosporidium* as a junior

subjective synonym of *Unikaryon* but Sprague et al. (1992) chose to retain the genus. The type species *Unikaryon piriformis* Canning, Lai & Lie, 1974 was described from a trematode and Weiser et al. (1995) suggested that species from trematodes be assigned to *Unikaryon* (and provisionally species from chrysomelid beetles and Crustacea) and created *Canningia* to contain species from bark beetles (Weiser et al., 1995). *Larssoniella resinellae* was described from *Petrova resinella* (Lepidoptera, Tortricidae) and is distinguished from other members of this group by the presence of tufts of tubules on immature spores (Weiser and David, 1997). *Oligosporidium arachnicolum* was described from the ovaries of a spider (Arachnida, Araneae) (Codreanu-Bălcescu et al., 1981). Considering strictly the developmental and morphological features of the new species described here, placement into either *Unikaryon* or *Oligosporidium* could be easily justified, requiring only minor modifications of the genus description for the occurrence of two classes of spores. However, the molecular data for this new species indicate a close phylogenetic relationship with the *Vairimorpha/Nosema* group. All members of this group possess diplokaryotic stages and spores. The *Vairimorpha* spp. also have a meiotic sequence producing uninucleate meiospores, but the role of these spores in the life cycle of *Vairimorpha* spp. and developmental sequences with unpaired nuclei are unknown.

There are several recent examples where morphological and molecular data are in conflict. *Vittaforma corneae*, isolated from a human, is diplokaryotic throughout development with polysporous sporogony but molecular data show that it is most closely related to *Endoreticulatus schubergi*, an entomophilic species that has unpaired nuclei throughout development and polysporous sporogony (Nilsen et al., 1998). Two entomophilic species, *Nosema locustae* (diplokaryotic throughout development with disporous sporogony) and *Cystosporogenes operophterae* (unpaired nuclei throughout development and polysporous sporogony) cluster together but with poor bootstrap support (Cheney et al., 2001). A recent molecular phylogeny based on rDNA concluded, "the diplokaryon is not a suitable synapomorphy to separate microsporidians into different classes" (Nilsen et al., 1998; Sprague et al., 1992). Since there are no molecular data available for any members of the family Unikaryonidae for comparison with our data, any conclusions about what the molecular data mean relative to the taxonomic value of classical characters such as the number of nuclei are premature, particularly at the family and genus levels. Additional molecular data on microsporidia from other mites and arachnids, as well as members of the Unikaryonidae, may help establish better relationships as has recently occurred for microsporidia from fish (Nilsen et al., 1998).

Microsporidia from phytoseiid mites appear to have normal microsporidian cytology and all stages are reported to be uninucleate throughout development. The unnamed forms from *Neoseiulus cucumeris* and *Amblyseius barkeri* (Beerling and van der Geest, 1991) were assigned to the family Pleistophoridae but little information other than spore size is available. One named (*Microsporidium phytoseiuli*) and two unnamed species (designated species A and B) have been described from *Phytoseiulus persimilis* (Bjørnson and Keddie, 2000; Bjørnson et al., 1996). *M. phytoseiuli* has a number of biological and morphological similarities to the microsporidium from *M. occidentalis*. Both microsporidia cause systemic infections in the host and are horizontally and vertically transmitted (Bjørnson and Keddie, 2001). The uninucleate spores of *M. phytoseiuli* measured $5.88 \times 2.22\,\mu m$ with a polar filament with 12–15 coils. This difference in size and length of the polar filament distinguishes *M. phytoseiuli* from the spores with a long polar filament described for the present species but may represent a similar type of spore. Poor fixation of *M. phytoseiuli* spores prevents a more detailed comparison and while spores with a short polar filament were not reported, "evacuated spores" were observed suggesting the presence of a spore type responsible for autoinfection (Bjørnson et al., 1996). Occasional groups of spores within "envelopes of unknown origin" were also reported but the significance of these is unknown.

Species A and B from *P. persimilis* (Bjørnson and Keddie, 2000) were obtained from colonies with separate origins (North America and Israel, respectively). The spore sizes of these two isolates were similar but ultrastructurally species A had a polar filament arranged in 7–10 coils while species B had 2–4 coils. The spore size and two classes of spores of both species A and B are very similar to the microsporidium from *M. occidentalis* but the limited information and the poor fixation of species A and B spores make it impossible to determine the relationship among these microsporidia.

Considering the available diagnostic information and in particular the recent changes to the family Unikaryonidae made by Weiser and David (1997), the most conservative approach is to assign the new speices described here to the genus *Oligosporidium*. As mentioned above and elsewhere (Larsson, 1999), species in several genera appear to be restricted to a particular group of hosts and we suggest that species of microsporidia from arachnids that satisfy the diagnostic characteristics for the family Unikaryonidae be assigned to *Oligosporidium*. We assign the new species described here the specific name *Oligosporidium occidentalis* n. sp. Furthermore, we suggest that *Microsporidium phytoseiuli* may also be a member of this genus based on the current information for this species.

5. *Oligosporidium occidentalis* n. sp. (Figs. 1–14)

Type host: Metaseiulus occidentalis (Nesbitt) (Acari, Phytoseiidae)

Transmission: Spreads within the host by autoinfection. Horizontal transmission occurs by cannibalism (Olsen and Hoy, 2002) of eggs and larvae (laboratory) that contain spores. Vertical (transovarial) transmission is highly efficient in transferring the microsporidium from adults to progeny (Olsen and Hoy, 2002).

Site of infection: Infection was systemic in eggs, larvae, nymphs, and adults. Immature stages and mature spores were found in the cytoplasm of cecal cells, lyrate organ cells, ganglia, epithelial cells, muscle, within the ovary, and in developing and mature eggs.

Interface: Presporulation and sporulation stages were in direct contact with the host-cell cytoplasm. No sporophorous vesicle was present at any point in the developmental cycle.

Other parasite host-cell relations: Hypertrophy of infected caecal cells.

Development: All life cycle stages had isolated nuclei. Schizogony was a series of binary divisions producing numerous schizonts. Sporogony was by binary fission with each sporont dividing once to produce two uninucleate sporoblasts.

Spores: Two classes of uninucleate spores were produced. The first, found primarily in nymphs and young adults, had a large posterior vacuole and measured $2.53 \pm 0.06\,\mu m \times 1.68 \pm 0.06\,\mu m$ (fresh). The polar filament was isofilar and arranged in 3–5 coils. The polar sac-anchoring disk complex was subterminal with a bipartite lamellar polaroplast. The second class of spores, found primarily in older adult mites, had a small posterior vacuole and measured $3.14 \pm 0.07\,\mu m \times 1.77 \pm 0.03\,\mu m$ (fresh). The polar filament was isofilar and arranged in 8–9 coils. The polar sac-anchoring disk complex was subterminal with a bipartite lamellar polaroplast. Both spore types were oblong-ovoid with a rugose spore wall composed of three layers; an external unlayered electron dense exospore, a thicker median lucent endospore layer, and an internal plasmalemma.

Type locality: Colony originally isolated from multiple locations of California, Oregon, and Washington states and crossed to generate a COS-Chico strain, as described by Hoy (1984).

Etymology: Named after the host, *M. occidentalis.*

Molecular characterization: The ssrRNA gene sequence obtained from infected *M. occidentalis* was unique and the phylogenetic analysis indicated that *O. occidentalis* is most closely related to the *Vairimorpha/Nosema* clade of microsporidia.

Deposition of type specimens: Syntypes have been deposited in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, DC (USNM No. 1004212). Additional slides and specimens embedded in plastic resin are held at the Center for Medical, Agricultural, and Veterinary Entomology, USDA, Gainesville, FL.

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