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Use of Nucleotide Sequence Data to Identify a Microsporidian Pathogen of *Pieris rapae* (Lepidoptera, Pieridae)

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Nucleotide sequence was determined for a portion of genomic DNA which spans the V4 variable region of the small subunit ribosomal RNA gene of an unidentified microsporidium from the cabbage white butterfly, *Pieris rapae* (174 base pairs). Comparison with equivalent sequence data obtained here for two other microsporidian species, *Nosema bombycis* (240 base pairs) and *Nosema bombi* (200 base pairs), and from the GenBank database for 11 other microsporidian species suggests that the unidentified species from *P. rapae* is most closely related to some *Vairimorpha* species. Light and electron microscopic observations of the developmental stages of this parasite were in accord with this. Infection experiments conducted at 20 and 26°C demonstrated temperature-dependent dimorphism, with the production of both binucleate free spores (mean dimensions: 3.8 × 1.8 μm; 10–13 polar filament coils) and membrane-bound uninucleate octospores (mean dimensions of 3.1 × 1.9 μm). Macrospores (mean dimensions 8.0 × 2.1 μm) were also observed. Sites of infection were the gut epithelium, the Malpighian tubules, the salivary glands, and the fat body. Infections were found in all insect life stages, including the egg. This microsporidium was found to be indistinguishable from both *Nosema mesnili* (Paillot) and *Microsporidium* (*Thelohania*) mesnili (Paillot) and we propose that these species be combined and transferred to the genus *Vairimorpha* Pilley.

KEY WORDS: **microspora; *Nosema bombycis; Nosema bombi; Vairimorpha mesnili; Nosema mesnili; Microsporidium (Thelohania) mesnili; Bombyx mori,* silkworm, *Bombus terrestris,* bumble bee; *Pieris rapae,* cabbage white butterfly; ribosomal RNA; phylogeny; life cycle; dimorphism.**

INTRODUCTION

Since the first report of the extraction of DNA from microsporidian (Phylum Microspora) spores (Undeen and Cockburn, 1989), there has been an increasing number of molecular studies of this group of protoctist parasites. A majority of these have focused on determining the nucleotide sequences of ribosomal RNA (rRNA) genes and their intergenic spacer regions (e.g., Vossbrinck *et al.*, 1987, 1993; Zhu *et al.*, 1993a, b; Hartskeerl *et al.*, 1993; Malone *et al.*, 1994; Baker *et al.*, 1994). These sequences are being used to investigate theories of early eukaryote evolution (Vossbrinck *et al.*, 1987; Cavalier-Smith, 1987, 1991; Sogin *et al.*, 1989; Liepe *et al.*, 1993) and, on a more practical level, to assist in species identifications (Vossbrinck *et al.*, 1993) and to understand relationships between species (Baker *et al.*, 1994). Similarly, pulsed-field gel electrophoresis is being used to investigate molecular karyotypes (Munderloh *et al.*, 1990; Malone and McIvor, 1993; Biderre *et al.*, 1994; Kawakami *et al.*, 1994). While molecular data have yet to be incorporated as new taxonomic characters into a revised classification of the Phylum Microspora, such information can be useful for resolving particular problems with microsporidian species identifications.

In this paper, we report on the use of a partial nucleotide sequence of a rRNA gene, along with conventional microscopy and insect infection experiments, to identify a microsporidian species from the cabbage white butterfly, *Pieris rapae* (L.), which is a widespread pest of brassicas. Our earlier (unpublished) observations of this pathogen indicated that it has a dimorphic life cycle, producing both free spores and membrane-bound octospores. Conventional and molecular methods used in the present study indicate that this microsporidium is indistinguishable from both *Nosema mesnili* (Paillot) and *Microsporidium (Thelohania) mesnili* (Paillot) and we propose that these species be combined and transferred to the genus *Vairimorpha* Pilley.

MATERIALS AND METHODS

Spores of an unidentified dimorphic microsporidium (referred to as *Vairimorpha* sp.) were obtained from *P. rapae* larvae and adults collected from suburban gardens in Auckland, New Zealand. For rRNA gene se-

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quence comparisons, spores of two other species, *Nosema bombycis* and *Nosema bombi*, were also obtained. *N. bombycis* spores were sent to us by Dr. R. Ishihara, of Japan, in August 1984, and then maintained by repeatedly infecting laboratory-reared greenheaded leafrollers, *Planotortrix octo*. Spores of *N. bombi* were isolated from infected adult worker bumble bees (*Bombus terrestris*) obtained from a commercial supplier in Hastings, New Zealand.

Purified spore suspensions were prepared for DNA studies by crushing diseased insects in sterile distilled water, filtering the homogenate through nylon mesh cloth (10-μm pore size), centrifuging at 400*g* for 15 min, and washing the resultant pellet of spores by repeated centrifugation and resuspension in water. The final suspension was purified on a discontinuous Percoll gradient at 10,000*g* for 20 min, followed by two washes in sterile distilled water. Spores were induced to germinate and DNA preparations made from the sporoplasms as described for other microsporidia (Undeen and Cockburn, 1989; Malone and McIvor, 1995).

Partial nucleotide sequences, spanning the V4 region of the small subunit ribosomal RNA (ssrRNA) gene, were obtained from the three microsporidian isolates. The V4 region, comprising two conserved regions flanking a variable region, was chosen because comparisons of microsporidian ssrRNA gene sequences have shown marked differences among species in this part of the gene (Malone *et al.*, 1994). The V4 region was defined as that lying between an internal *Sac*II site, beginning at base 392, and a conserved region of 23 bases ending at base 645 (*Vairimorpha necatrix* numbering as recorded in the GenBank database (the NIH genetic sequence database, part of the International Nucleotide Sequence Database Collaboration) Accession No. M24612). A portion of each ssrRNA gene, about 1000 base pairs long, was amplified by PCR using primers homologous to predicted conserved regions at the beginning and about ¾ of the way through the gene based on the published sequence of *V. necatrix* (Vossbrinck *et al.*, 1987). These primers had the following sequences: 5′-GTTGATTCTGCCTGACGTA-3′ (bases 7 to 25, *V. necatrix* numbering) and 5′-ACCCGACGTGCGCGTTATGT-3′ (bases 988 to 1007). The amplified DNA fragments were cloned in pBluescript (SK^+) (Stratagene) and the V4 region sequenced from the double-stranded templates with Sequenase Version 2.0 (United States Biochemical Corp.) using M13 sequencing primers and two internal primers: 5′-GTGCCAGCAGCCGCGGTAAT-3′ (bases 392 to 411) and 5′-CAAGGTCGTCATTTGATACGGC-3′ (bases 622 to 643).

Sequences of the following microsporidian species were used in our analysis: *Ameson michaelis*, L15741 (GenBank database accession number); *Encephalitozoon cuniculi*, Z19563; *Encephalitozoon hellem*, L19070; *Enterocytozoon bieneusi*, L07123; *Ichthyosporidium giganteum*, L13293; *Nosema apis*, X73894; *N. bombi*, U26158; *N. bombycis*, U26157; *Nosema trichoplusiae*, U09282; *Nosema* sp. from *Vespula* sp., L31842; *Vairimorpha* sp. from *P. rapae*, U26159; *Vairimorpha lymantriae*, L13294; *Vai. necatrix*, M24612; and *Vavraia oncoperae*, X74112. GCG DNA analysis program Version 8 (Devereux *et al.*, 1984) was used for sequence comparisons and alignments. Distance data were derived from sequence data using the DISCALC program (Weiller and Gibbs, 1993; obtained from the Australian National University) and the following pairwise distances were calculated: percentage nucleotide differences, percentage transitions, percentage transversions, transversions/length (excluding gaps), differences (excluding gaps)/length (excluding gaps), differences (including gaps)/length (including gaps), and mutations per site (with 0-, 1-, 2-, 3-, 4-, or 6-parameter corrections). Phylogenetic trees were constructed from all these data using NJTREE (Saitou and Nei, 1987; obtained from the Australian National University). Observed and patristic distances were compared for each transformation method by regression analysis using DIPLOMO (Weiller and Gibbs, 1993; obtained from the Australian National University). The "best" tree was chosen as that derived from the distance data calculation which altered the data least, i.e., that for which DIPLOMO calculated the highest correlation coefficient (R). Two protoctistans unrelated to the Phylum Microspora, *Paramecium tetraurelia*, X03772, and *Trypanosoma brucei*, M12676, were included in the sequence comparisons as taxonomic outgroups for phylogenetic tree construction.

In order to identify the microsporidium from *P. rapae* by conventional means, infected insects were examined microscopically and two consecutive infection experiments, designed to demonstrate temperature-dependent dimorphism, were carried out.

Eggs and larvae of *P. rapae* were collected from gardens and maintained in the laboratory on potted, unsprayed cabbage plants. Some of these insects were examined microscopically to check for infection. Whole eggs were squashed by pressing between two microscope slides and larvae were dissected and a sample of body tissues (fat, gut, epidermis, etc.) was taken from each and dabbed across a glass slide. These smears were air-dried, fixed in Carnoy's fluid, and stained in 10% Gurr's Improved R66 Giemsa stain in 0.02 M phosphate buffer at pH 6.9. The rest of the collected insects were maintained until pupation, adult emergence, or death and then dissected and examined (as described above) for microsporidian spores and stages. Fresh spores were measured using an image analyzer and video camera attached to a light microscope.

Both free spores and octospores (at a ratio of about 1 octospore to 50 free spores) were observed in smears made from these insects and a mixed spore suspension

was prepared from them by the method described at the beginning of this section (omitting the final Percoll gradient centrifugation step). This was used to dose second- and third-instar *P. rapae* larvae from a healthy laboratory colony. A cabbage leaf was dipped in a cooled solution of 1% gelatin and allowed to dry, and 1 ml of spore suspension was spread over each surface (top and bottom) of the leaf (approx. 2 million spores/cm^2 of leaf area). The leaf stem was placed in a flask of water, 10 larvae were placed on the leaf, and all were put into a sealed incubator at 26°C. Ten control larvae given distilled water in the place of spore suspension were also set up. All the dosed larvae from this experiment were dead after 7 days and Giemsa-stained smears and a new spore suspension were prepared from them. Octospores were not observed in smears made from these larvae.

This suspension was used to dose two further batches of 10 fourth- or fifth-instar larvae at a rate of approximately 8 million spores/cm^2 of leaf area (a higher dose was considered necessary because of the larger sizes of the larvae in this experiment). Two corresponding control batches of 10 larvae each were also set up as described above. One dosed and one control batch was maintained at 20°C and the other two at 26°C. Two larvae were removed from each batch at 4, 7, 11, and 13 days after dosing and Giemsa-stained smears made of the midgut, fat body, salivary glands, and Malpighian tubules. Heavily infected portions of gut tissue were taken from the remaining two larvae in each batch and examined via electron microscopy. For this, tissue was fixed under vacuum in 2.5% paraformaldehyde and 2% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.2 (1 hr), washed in buffer, postfixed 1% osmium tetroxide (1 hr), washed in water, dehydrated in ethanol, and embedded in L R White resin (London Resin Co. Ltd.). After polymerization, gold-colored sections were cut and stained with uranyl acetate and lead citrate.

RESULTS

Partial nucleotide sequences for the V4 regions of ssrRNA genes of the *Vairimorpha* sp. from *P. rapae*, *N. bombi*, and *N. bombycis* and were lodged in the GenBank database under Accession Nos. U26159, U26158, and U26157, respectively. Comparisons of these sequences with those from corresponding rRNA gene regions in other microsporidia are shown in Table 1 as distance data expressed as percentage nucleotide differences and as mutations per site (0-parameter correction). A phylogenetic tree based on sequence distance data transformed by a mutations per site (0-parameter correction) method is shown in Fig. 1. Of the transformation methods used, this gave the closest correlation between observed and patristic distances as determined by regression analysis ($R = 0.9862$) (DIPLOMO). This tree places the two unrelated protoctists, *Pa tetraurelia* and *T. brucei*, on a separate branch from the microsporidia and has a similar topology to the best tree (not shown) ($R = 0.9984$) derived from full-length ssrRNA sequences for 10 of the 16 species named here. (Full sequence data are not available for *I. giganteum*, *N. bombi*, *N. bombycis*, *Nosema* sp. from *Vespula* sp., *Vairimorpha* sp. from *P. rapae*, or *Vai. lymantriae*.)

TABLE 1[a]

Comparison of Partial ssrRNA Gene Sequences: Percentage Nucleotide Differences (Top Diagonal) and Mutations per Site (Bottom Diagonal)

	Napi	Vnec	Nves	Vsp	Vlym	Nbb	Nbom	Ntri	Ecun	Ehel	Ebie	Vonc	Amic	Igig	Ptet	Tbru
Napi	—	1.6	9.4	40.6	44.3	29.5	37.6	11.0	33.9	35.3	38.7	45.8	46.9	69.3	63.6	61.7
Vnec	0.016	—	7.9	39.4	43.2	28.7	37.6	11.0	33.9	35.0	38.3	45.8	46.6	69.5	64.3	62.0
Nves	0.050	0.033	—	37.0	41.6	26.0	42.4	18.0	38.5	39.6	41.5	49.4	51.8	69.3	63.1	65.1
Vsp	0.132	0.115	0.088	—	63.1	38.9	55.9	47.1	61.2	62.0	64.8	71.5	72.5	83.6	81.1	78.6
Vlym	0.058	0.040	0.045	0.099	—	51.7	62.2	50.3	60.7	60.8	62.7	67.8	69.0	61.7	73.0	74.6
Nbb	0.105	0.095	0.105	0.118	0.138	—	36.6	27.8	53.6	54.8	58.9	62.5	61.8	73.5	68.9	70.9
Nbom	0.148	0.148	0.170	0.264	0.171	0.080	—	30.5	56.6	56.5	61.4	63.7	62.9	78.0	76.4	67.1
Ntri	0.106	0.106	0.136	0.224	0.157	0.080	0.048	—	37.8	37.6	43.2	47.1	47.2	69.5	66.9	62.9
Ecun	0.209	0.209	0.227	0.322	0.247	0.295	0.313	0.259	—	10.5	35.2	38.1	40.5	64.7	62.7	56.0
Ehel	0.219	0.215	0.234	0.328	0.252	0.305	0.304	0.250	0.093	—	36.0	39.7	40.8	64.9	61.7	57.4
Ebie	0.302	0.298	0.300	0.413	0.308	0.404	0.412	0.356	0.309	0.312	—	42.9	48.5	69.7	64.3	64.6
Vonc	0.335	0.335	0.347	0.488	0.372	0.415	0.413	0.353	0.365	0.373	0.375	—	36.5	59.7	58.0	59.5
Amic	0.347	0.343	0.377	0.503	0.391	0.401	0.396	0.353	0.389	0.384	0.436	0.353	—	64.4	59.9	61.7
Igig	0.394	0.398	0.412	0.530	0.537	0.446	0.447	0.401	0.432	0.427	0.476	0.367	0.432	—	68.3	76.4
Ptet	0.439	0.450	0.454	0.556	0.446	0.500	0.506	0.492	0.521	0.500	0.524	0.474	0.496	0.484	—	59.4
Tbru	0.472	0.476	0.496	0.569	0.462	0.490	0.528	0.490	0.493	0.503	0.565	0.543	0.562	0.597	0.430	—

[a] Abbreviations are as follows: Napi (*Nosema apis*), Vnec (*Vairimorpha necatrix*), Nves (*Nosema* sp. from *Vespula* sp.), Vsp (*Vairimorpha* sp. from *Pieris*), Vlym (*Vairimorpha lymantriae*), Nbb (*Nosema bombi*), Nbom (*Nosema bombycis*), Ntri (*Nosema trichoplusiae*), Ecun (*Encephalitozoon cuniculi*), Ehel (*Encephalitozoon hellem*), Ebie (*Enterocytozoon bieneusi*), Vonc (*Vavraia oncoperae*), Amic (*Ameson michaelis*), Igig (*Ichthyosporidium giganteum*), Ptet (*Paramecium tetraurelia*), Tbru (*Trypanosoma brucei*).

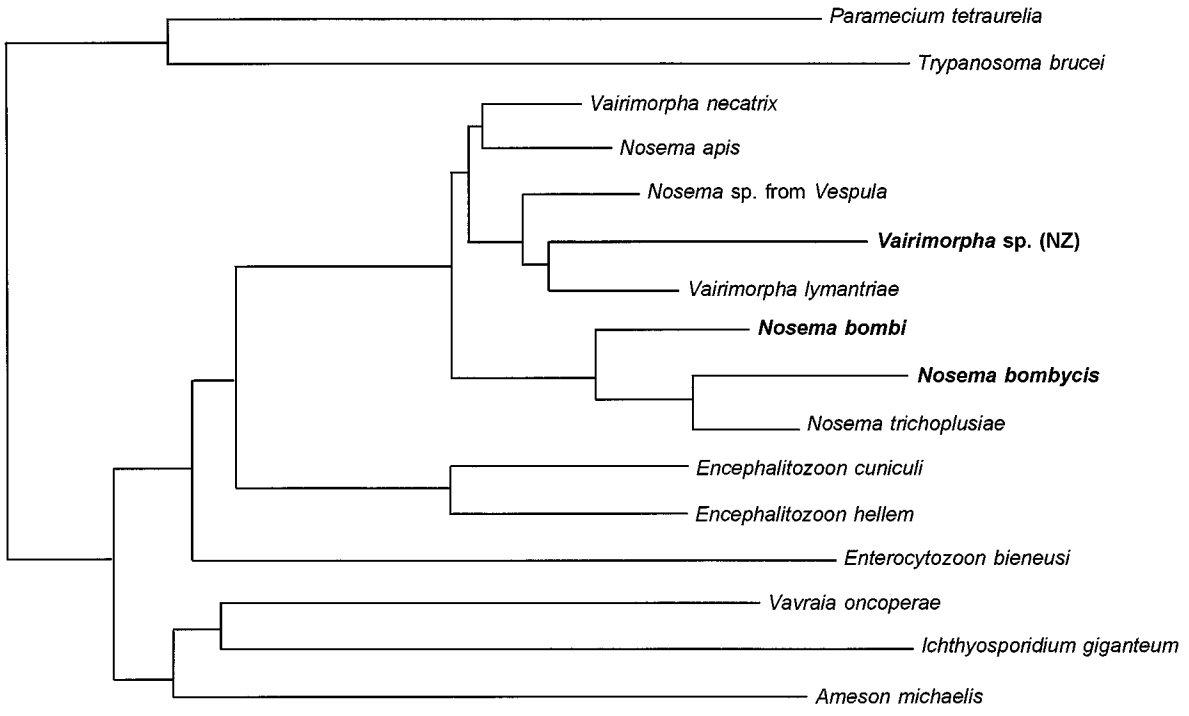


FIG. 1. Unrooted phylogenetic tree inferred from partial nucleotide sequences of ssrRNA genes of 14 microsporidian species and 2 other protoctistans using the neighbor-joining method.

Although percentage nucleotide differences are commonly used for tree construction, and this transformation method gave the "best" tree with full ssrRNA microsporidian data, regression analysis and visual comparison with the full sequence tree showed that this was not an appropriate method to use with V4 region sequence data ($R = 0.9500$).

The tree in Fig. 1 places the *Vairimorpha* sp. from *P. rapae* closest to *Vai. lymantriae,* a dimorphic microsporidian pathogen from the lepidopteran, *Lymantria dispar.* These microsporidia are both grouped with a parasite of wasps (*Vespula* sp.) which also infects lepidopteran larvae in the laboratory and which is tentatively identified as a *Nosema* sp. (D. L. Anderson and J. A. Ninham, personal communication). *N. apis* and *Vai. necatrix* appear on another nearby branch to these microsporidia. *N. bombi* is placed in a somewhat separate group, along with the lepidopteran-infecting *Nosema* species, *N. bombycis* and *N. trichoplusiae, Pa. tetraurelia* and *T. brucei* form an expected outgroup, and *Vau. oncoperae, I. gignateum,* and *A. michaelis* appear to be quite separate from the other microsporidia. The two *Encephalitozoon* species are closely linked and in the same major grouping as the *Nosema* and *Vairimorpha* species, with *Ent. bieneusi* linked but on a separate branch from all of these species.

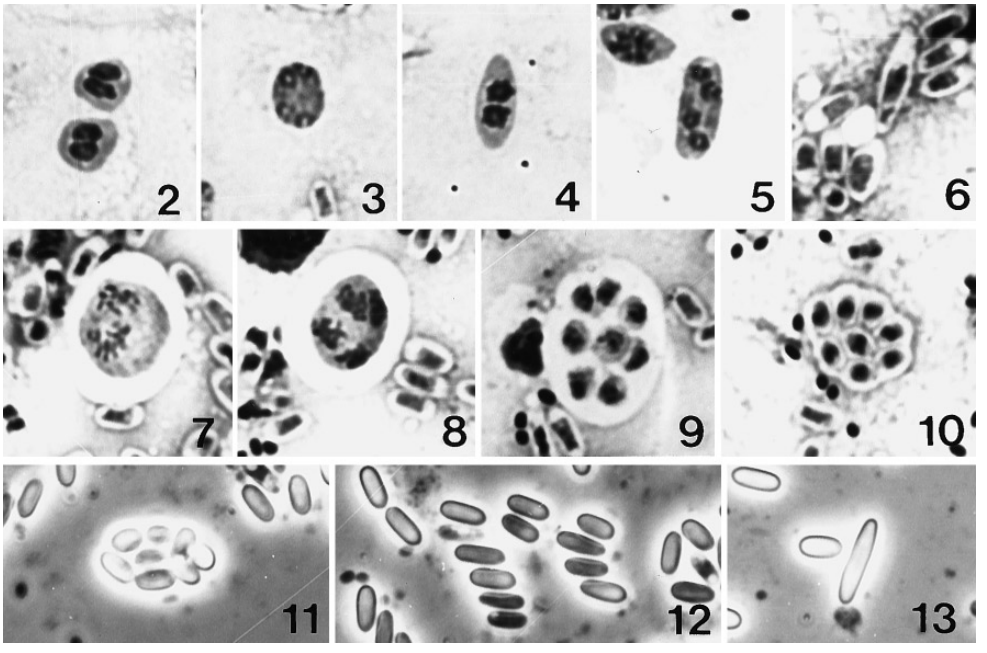
Microsporidian spores were observed in fresh preparations or Giemsa-stained smears made from 3/6 eggs, 3/9 larvae, 3/17 pupae, and 6/10 adults of *P. rapae* collected from the field. Fresh spore dimensions are given in Table 2. Elongate, somewhat fusiform macrospores occurred at a ratio of about 1 macrospore to every 130 free spores and membrane-bound octospores at a ratio of about 1 octospore to every 50 free spores in preparations made from field-collected material.

In infection experiments, dimorphic development, in which both free spores (Figs. 6 and 12) and membrane-bound octospores (Figs. 10 and 11) were produced, was observed at 20 but not at 26°C. Macrospores (Figs. 6 and 13) were produced at both temperatures. At 26°C, spherical binucleate and tetranucleate meronts (Figs. 2 and 3), binucleate and tetranucleate sporonts (Figs. 4

TABLE 2

Vairimorpha sp. Spore Dimensions (μm, Fresh Preparations)

	Mean ± standard error of mean	Range	*n*
Free spores	3.84 ± 0.03 × 1.82 ± 0.01	2.82 to 5.39 × 1.47 to 2.30	151
Octospores	3.14 ± 0.06 × 1.86 ± 0.03	2.42 to 4.48 × 1.33 to 2.35	59
Macrospores	7.95 ± 0.12 × 2.14 ± 0.03	6.20 to 10.45 × 1.50 to 2.60	51



FIGS. 2–13. Photomicrographs of life cycle stages of *Vairimorpha* sp. from *P. rapae* (Figs. 2–10, Giemsa-stained smears; Figs. 11–13, fresh preparations, phase-contrast; Bar, 5 μm). (2) Binucleate meronts. (3) Tetranucleate meront. (4) Binucleate sporont. (5) Tetranucleate sporont. (6) Mature free spores and elongate macrospore. (7) Membrane-bound binucleate meront. (8) Membrane-bound tetranucleate meront. (9) Eight uninucleate sporoblasts within a membrane. (10) Eight octospores within a membrane. (11) Membrane-bound mature octospores. (12) Mature free spores. (13) Macrospore and free spores.

and 5), and binucleate sporoblasts and free spores (Fig. 6) were observed in Giemsa-stained smears made from gut epithelial tissue of *P. rapae* 4 days after dosing with *Vairimorpha* sp. Nuclei in these stages were always arranged in pairs. Some had the hemispherical shape and close apposition typical of a diplokaryon, others were not so closely associated and had a more spherical shape, and some had a ring-like configuration (e.g., Figs. 3 and 5), perhaps reflecting the various stages of nuclear division. Distinctly uninucleate stages were not observed. After 7 days, numerous meronts, sporonts, and free spores were also observed in the Malpighian tubules and the salivary glands. By 11 days, the fat body had become infected in a similar manner. At 20°C, parasite development was slower, with a few meronts, sporonts, and free spores first appearing in the gut epithelium 4 days after dosing, but only in significant numbers in this tissue after 7 days. Free spores, meronts, and sporonts were first observed in the Malpighian tubules and the fat body 7 days after dosing and in the salivary glands after 11 days. At 11 days, vegetative stages with 2, 4, or 8 isolated nuclei and bounded by an outer membrane (Figs. 7–9) were observed in all tissues examined along with mature octospores (Fig. 10).

Electron microscopy did not reveal any unusual features in the fine structure of the microsporidium from *P. rapae*. Meronts were bounded by a simple plasmalemma and contained scattered rough endoplasmic reticulum and diplokaryotic nuclei. Sporonts had a similar ultrastructure, but the plasmalemma was generally thickened by the external, electron-dense coating typical of microsporidian sporonts. Spindle plaques were observed in some nuclei. We did not obtain ultrathin sections of any of the membrane-bound stages preceding octosporous development. Free spores were thick-walled and binucleate, with between 10 and 13 coils of the polar filament (median = 12 coils, $n = 38$). There was insufficient longitudinally sectioned material for us to make observations on the numbers of polar filament coils in macrospores or octospores. Ultrathin sections of macrospores revealed more than two diplokaryotic nuclei, suggesting that such spores were teratogonic and probably the result of a failure of the cytoplasm to cleave after the final nuclear division before spore formation.

DISCUSSION

Percentage nucleotide differences among the sequences of V4 regions of the ssrRNA genes of the microsporidian species shown in Table 1 vary more widely (1.6 to 83.6%) than those calculated for full-length microsporidian ssrRNA sequences (13.0 to 52.7%, not shown). This is a region of the gene which varies considerably in both sequence and overall length from species to species (Malone *et al.*, 1994), particularly when microsporidia from very different hosts (e.g., fish

and bees) are considered. Microsporidian ssrRNA sequences also vary among species more than those of some other groups of organisms, such as the eubacteria, where species may differ by only one or two nucleotides (Dams *et al.*, 1988; Barry *et al.*, 1990). This wide diversity among the microsporidia may be the result of their long evolution as obligate parasites of a wide range of host animals (Vossbrinck *et al.*, 1987).

Several microsporidia have been found to cause natural infections in the cabbage white butterfly species, *Pieris brassicae, P. rapae,* and *Pieris canidia* (see Sprague, 1977 and Table 3). Of these, the New Zealand microsporidium from *P. rapae* most closely resembles both *N. mesnili* (Paillot, 1918a) and *M.(T.) mesnili* (Paillot, 1924a). It does not produce the large pansporoblasts containing 20 to 30 spores described by Veber (1956) for *Pleistophora schubergi aporiae.* Lipa (1963) gives insufficient details for comparison with an unidentified *Nosema* sp. from *P. rapae,* but its spores appear to be somewhat broader than those of our isolate. Likewise, the spores of the unidentified *Thelohania* sp. described from this insect by Laigo and Paschke (1966) are larger than the octospores of the New Zealand parasite. These authors depict both octospores and free spores in their Fig. 1, but do not comment on this and they do not discount the possibility that the species they describe is a synonym of *T. mesnili.*

N. mesnili was first described as *Perezia mesnili* by Paillot (1981a), who noted that it appeared to be "une Microsporidie intermédiaire entre les *Nosema* et les *Perezia*." Based on synonymies proposed by Weiser (1961) and Hostounský (1970), Sprague (1977) treated *N. mesnili, Nosema polyvora* Blunck, 1952, *Perezia pieris* Paillot, 1924b, and *Perezia legeri* Paillot, 1918b as identical species. The spores and vegetative stages of *N. mesnili* described and illustrated by Paillot (1918a) are very similar in size and form to those of the disporous pathway in the New Zealand microsporidium. However, we did not observe Paillot's "chaînettes" of meronts. The free spore ultrastructure of our species is also similar to that described by Sokolova *et al.* (1988) and Cheung and Wang (1995) for *N. mesnili.* Although *N. mesnili* has largely been reported from *P. brassicae* (e.g., Khansen *et al.*, 1982), infections have also been noted in *P. rapae* (Voronin, 1982; Haji-Mamat and Tamashiro, 1988).

The octosporous developmental stages noted in the New Zealand microsporidium at 20°C closely resemble those of *M. (T.) mesnili,* as first described by Paillot (1924a) and later by Blunck (1954) and Lipa (1963). Sprague (1977) placed this species into the collective group, *Microsporidium,* as there was no pansporoblast membrane mentioned in its first description, even though later authors did note the presence of this characteristic. Dual infections of *P. brassicae* with both *N. mesnili* and *M. (T.) mesnili* have been recorded (Voronin, 1982).

In our infection experiments, both octospores and free spores were produced in larvae fed only free spores at 20°C, and only free spores were produced in larvae fed both types of spores at 26°C. This suggests that the New Zealand microsporidium is a single species displaying the temperature-dependent dimorphism first described by Pilley (1976) as a distinguishing characteristic of the genus *Vairimorpha.* Our nucleotide sequence data also suggest a closer affinity to *Vairimorpha* species than to the lepidopteran-infecting *Nosema* species.

We therefore conclude that the microsporidium infecting *P. rapae* in New Zealand is indistinguishable from both *N. mesnili* and *M. (T.) mesnili* and propose that these species be combined and placed in the genus *Vairimorpha.* Pilley (1976) and Sprague *et al.* (1992) list the characters typical of this genus.

Vairimorpha mesnili (Paillot, 1918a) comb. nov.

Perezia mesnili Paillot, 1918, *C. R. Soc. Biol.* **81,** 66.

TABLE 3

Microsporidia Described from *Pieris* spp.

Microsporidian species	Spore sizes	Site of infection	Host insects	References
Nosema (Perezia) mesnili	3–4 × 1.5–2 μm	Malpighian tubules, silk glands, fat body, hemocytes	*P. brassicae* *P. rapae* *P. canidia*	Paillot (1918a) Tanada (1953) Cheung and Wang (1995)
Microsporidium (Thelohania) mesnili	2.5–3.5 × 1.5–2 μm (from illustration)	Fat body	*P. brassicae*	Paillot (1924a)
Pleistophora schubergi aporiae	2 × 1.5 μm	Gut	*P. brassicae* *P. rapae*	Veber (1956) Issi (1969)
Nosema sp.	4.5–6 × 2.5 μm	Not given	*P. rapae*	Lipa (1963)
Thelohania sp.	5.6–6.8 × 3.1–3.8 μm	All major tissues	*P. rapae*	Laigo and Paschke (1966)
New Zealand isolate	2.8–5.4 × 1.5–2.3 μm (free spores) 2.4–4.5 × 1.3–2.4 μm (octospores)	Gut, Malpighian tubules, salivary glands, fat body	*P. rapae*	Present study

Perezia legeri Paillot, 1918, *C. R. Soc. Biol.* **81,** 187.

Perezia pieris Paillot, 1924, *C. R. Soc. Biol.* **90,** 1255.

Thelohania mesnili Paillot, 1924, *C. R. Soc. Biol.* **90,** 501.

Nosema mesnili Blunck, 1952, *Trans. 9th Int. Congr. Entomol. Amsterdam 1952,* 432; Weiser, 1961, *Monogr. Angew. Entomol.* **17,** 1; Hostounský, 1970, *Acta Entomol. Bohemoslav.* **67,** 1.

Nosema polyvora Blunck, 1952, *Trans. 9th Int. Congr. Entomol. Amsterdam 1952,* 432.

Microsporidium mesnili Sprague, 1977, *Comp. Pathobiol.* **2,** 309.

Hosts. Lepidoptera: *Pieris rapae, Pieris brassicae, Pieris canidia.* Hymenoptera: *Apanteles glomeratus, Apanteles rubecula, Hyposoter ebenius, Pimpla instigator.* Nematoda: *Neoaplectana* sp., *Neoaplectana agriotis.*

Transmission. Per os and via the egg.

Site of infection. Gut epithelium, Malpighian tubules, salivary glands, and fat body.

Interface. Meronts and disporous sporonts in direct contact with host cytoplasm. Octosporous sporulation within interfacial envelope.

Other parasite–host cell relations. Infected cells become packed with parasite. Xenoma not observed.

Haplophase. Uninucleate cells not observed.

Merogony. Binary division of diplokaryotic cells to produce binucleate and tetranucleate cells precedes disporous development. Octosporous development preceded by multiple fission of binucleate stage with isolated nuclei, within an envelope, to become plasmodium with eight isolated nuclei.

Transition to sporogony. For disporous development, binucleate diplokaryotic meront undergoes a shape change and its cell membrane gains an outer coating of electron dense material to become a fusiform/ovoid sporont. For octosporous development, plasmodium with eight isolated nuclei undergoes cytoplasmic cleavage to form eight uninucleate sporonts simultaneously, still contained within an envelope.

Sporogony. For disporous development, the fusiform/ovoid diplokaryotic sporont undergoes nuclear division followed by cytoplasmic cleavage to produce two ovoid binucleate sporoblasts. For octosporous development, each of eight uninucleate sporonts within an envelope becomes an ovoid uninucleate sporoblast.

Spores. Binucleate free spores oblong/ovoid, mean dimensions: 3.8 × 1.8 µm, $n = 151$ (range 2.8–5.4 × 1.5–2.3 µm), 10–13 coils of polar filament. Uninucleate octospores ovoid, mean dimensions: 3.1 × 1.9 µm, $n = 59$ (range 2.4–4.5 × 1.3–2.4 µm). Teratogonic macrospores elongate fusiform, mean dimensions: 8.0 × 2.1 µm, $n = 51$ (range 6.2–10.4 × 1.5–2.6 µm).

Locality. Specimens described here collected from Auckland, New Zealand.

Deposition of specimens. Two slides of a New Zealand isolate of this parasite are deposited in the Museum of National History at the Smithsonian Institution, Washington, DC, with the Catalog Nos. USNM 47814 and USNM 47815.

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