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***Microsporidium rhabdophilum* sp. n.**
(Microsporida : Pansporoblastina),
a parasite of the nematode, *Rhabditis myriophila*
(Rhabditina : Rhabditidae)

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SUMMARY

Microsporidium rhabdophilum sp. n. (Microsporida : Pansporoblastina) is described from the microbotrophic nematode *Rhabditis myriophila* Poinar. This microsporidian normally infects the hypodermal and reproductive tissues of the nematode. The parasite possesses small uninucleate, bacilliform spores produced in pansporoblasts. The number of spores per pansporoblast is normally 8 or 16 but can range from 4 to 25. The developmental stages of the parasite are examined ultrastructurally.

RESUME

Microsporidium rhabdophilum sp. n. (Microsporida : Pansporoblastina)
parasite des nématodes *Rhabditis myriophila* (Rhabdita : Rhabditidae)

Microsporidium rhabdophilum sp. n. (Microsporida : Pansporoblastina), provenant des nématodes bacilliphages *Rhabditis myriophila* Poinar, est décrit. Cette microsporidie infecte normalement l'hypoderme et les tissus de l'appareil reproducteur du nématode. Le parasite possède de petites spores bacilliformes, uninucléées, produites dans des pansporoblastes. Le nombre de spores par pansporoblaste est normalement de 8 ou 16, mais peut varier de 4 à 25. L'ultrastructure des différents stades de développement du parasite a été étudiée.

During routine studies on the development of *Rhabditis myriophila* Poinar, 1986, individuals suffering from a microsporidian infection were discovered. A detailed examination of the parasites showed them to differ from previous microsporidians and they are described as a new species. Since the systematics of the Microsporida are in a state of flux and the present parasites of *R. myriophila* do not fall into an existing genus, placement in the collective group *Microsporidium* was considered appropriate at this time.

Materials and methods

The nematode, *Rhabditis myriophila* was originally recovered from the intestinal lumen and body cavity of the garden millipede, *Oxidis gracilis* collected in Southern California (Poinar, 1986). This protandric hermaphrodite was cultivated at 20° on nutrient agar plates seeded with bacteria associated with the millipede. Light microscope observations of infections in living and squashed nematodes were made by placing specimens in 1 % saline and examining them under

bright field and differential interference contrast microscopy.

For electron microscope studies, nematodes which had their heads and tails removed were immersed in 2.5 % phosphate-buffered (0.1 M, pH 7.2) glutaraldehyde for one hour, dehydrated and embedded in Araldite 6005. Sections were stained with saturated aqueous uranyl acetate followed by lead citrate and examined in a Philips EM 300 electron microscope.

***Microsporidium rhabdophilum* sp. n.**

(Figs 1-3)

Microspora Sprague, 1977; Microsporea Delphy, 1936;
Microsporida Balbiani, 1882, Pansporoblastina
Tuzet *et al.*, 1971.

DESCRIPTION

Spores bacilliform, tubular, straight or slightly curved; 1.28-2.56 µm long by 0.6-1.0 µm wide (Fig. 1 d, f). Polar filament uniform in diameter. Merogony not

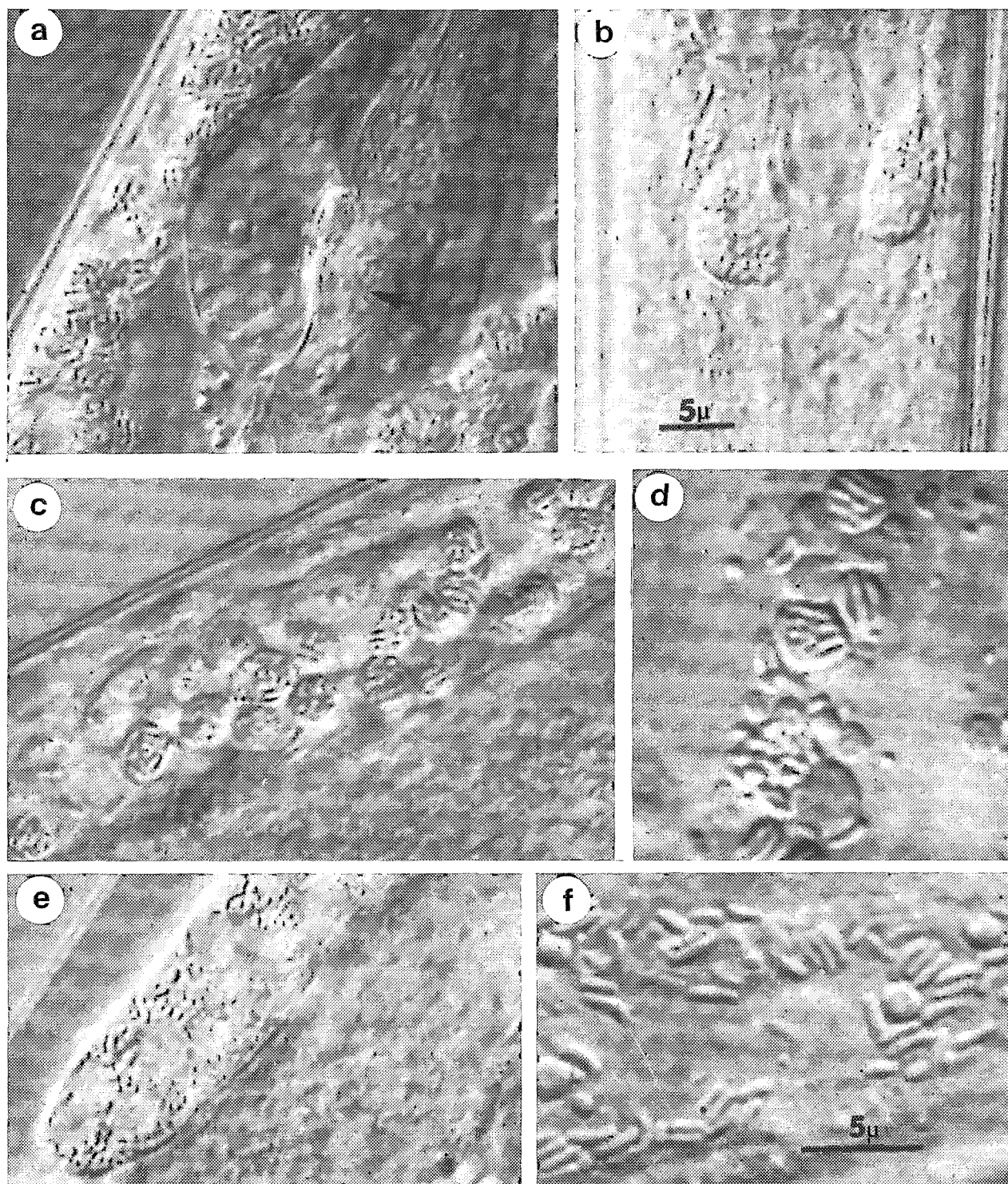


Fig. 1. Spores of the microsporidian, *Microsporidium rhabdophilum* n. sp. from the nematode, *Rhabditis myriophila*. a : Region of the basal pharyngeal bulb showing pansporoblasts with mature spores in the hypodermis and spores in the pharyngeal gland cell (arrow) (Mag. same as 1 b); b : Dauer juvenile with spores in paired sacs adjacent to the basal bulb; c : Spores in pansporoblasts maturing in the wall of the uterus and oviduct (Mag. same as 1 b); d : Pansporoblasts and spores removed from the hypodermal tissue (Mag. same as 1 f); e : Tip of ovotestis infected with spores (Mag. same as 1 b); f : Isolated spores showing typical bacilliform shape.

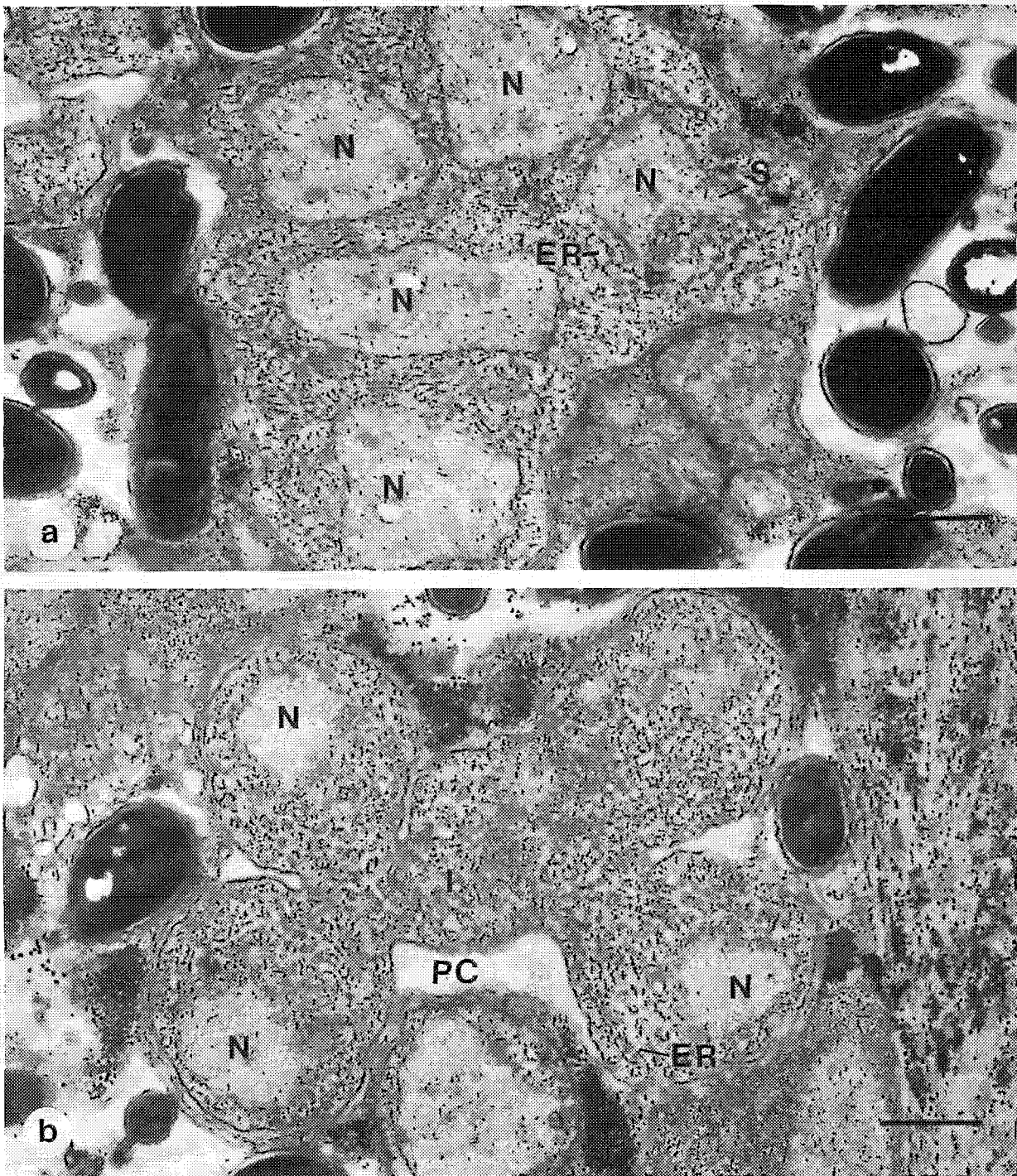


Fig. 2. The sporogonial plasmodia of *M. rhabdophilum* n. sp. Both magnification lines represent 0.5 µm. *a* : The plasmodium consists of a multinucleate cell in which portions of the spindle apparatus (S) are still present within the nuclei. The sporogonial plasmodium is surrounded by multiple layers of membranes, but no pansporoblast cavity is present. The cytoplasm is fairly undifferentiated containing free ribosomes and sparse rough endoplasmic reticulum (ER); *b* : This plasmodium has divided into four distinct cells still interconnected by a central isthmus (I). The cytoplasm contains a prominent rough endoplasmic reticulum (ER), some golgi and is surrounded by two membranes. The inner membrane is thickened. The outer membrane is faint and can best be seen in the indentations between cells. The pansporoblast cavity is evident (PC). (N = nuclei).

observed. Sporoblasts uninucleate, formed within a semi-persistent to persistent pansporoblastic membrane. Spore number per pansporoblast variable, ranging from 4 to 25, but most frequently 8 or 16. Sporogonial plasmodia (the earliest stages observed) undergo multiple fission, during which a rosette stage occurs (Fig. 1 *a, b*).

TYPE HOST

Rhabditis myriophila Poinar, 1986 (Rhabditida : Rhabditidae).

TYPE LOCALITY

Infected nematodes were recovered from garden millipedes, *Oxidis gracilis* collected from Azusa, California.

LIGHT MICROSCOPE OBSERVATIONS

Infected hermaphrodites of *R. myriophila* were recovered from 12 day old nutrient cultures at a ratio of one diseased to twenty apparently healthy individuals. The most obvious sign of microsporidan infection was pansporoblasts containing mature spores in various nematode tissues. In hermaphrodites, developing spores were observed in the pharyngeal glands and hypodermis, especially the tissue surrounding the basal bulb (Fig. 1 *a*), the wall of the uterus, oviduct (Fig. 1 *c*) and ovotestis (Fig. 1 *e*). The hypodermis and ovotestis were the tissues most commonly infected. Occasionally, spores could be found in the intestinal cells.

Dauer juveniles found on exhausted nutrient agar plates sometimes contained spores in a pair of pouches on either side of the collapsed basal pharyngeal bulb (Fig. 1 *b*). Whether these pouches represent nervous tissue or expanded pharyngeal gland cells that have hypertrophied as a result of the infection is not known.

Heavy infections involving the entire reproductive system would result in sterility. In such individuals, eggs were lacking. Mortality of *R. rhabdophila* occurred when the hypodermis of hermaphrodites and third and fourth stage juveniles was infected throughout.

ELECTRON MICROSCOPE OBSERVATIONS

Meronts with a single limiting unit membrane were not observed. Uninucleate sporonts surrounded by two membranes were observed in the cytoplasm of infected cells. The cytoplasm was largely undifferentiated and only free ribosomes and some rough endoplasmic reticulum were observed. The host cell membranes were closely appressed to the surface of the sporonts. Plasmodia were observed with two to five nuclei in the plane of sectioning (Fig. 2 *a*). The nuclei frequently had portions of the spindle apparatus connected to the outer nuclear membrane. Microtubules were present in the

nucleoplasm as well as dense patches of nuclear material considered to be chromosomal. The cytoplasm contained rough endoplasmic reticulum as well as some membrane-bound packets of cytoplasm. Small vacuoles were occasionally observed. At this stage the host cell was still closely appressed to the outer surface but additional membranes were present between the host and the plasmodium. In later stages of division, the inner membrane of the sporogonial plasmodium had increased in electron density and rosettes with individual cells were visible within a cavity (Fig. 2 *b*) bounded by the pansporoblast membrane. Division of the plasmodium seemed to be equal from a central isthmus and a maximum of four connected cells were noted in any one plane of sectioning. However, many pansporoblasts appeared to contain additional cells either previously separated or in some cases still connected to another cell in the process of dividing. Fully separated sporoblasts were observed within a well-defined pansporoblastic cavity. The maximum number of sporoblasts found in the plane of sectioning was eight. These sporoblasts were in various stages of morphogenesis (Fig. 3 *a, b*). Newly derived sporoblasts were ovoid and contained more dilated rough endoplasmic reticulum. Nuclei were lobulate. Patches of Golgi vesicles were present as well as vacuoles. The inner surface membrane was thickened, uniformly dense and in some instances the presence of two membranes was suggested. An outer fine membrane was present. In contrast to the complete separation of sporoblasts just described, in some sporoblasts maturation appeared to progress while the individual cells were still interconnected. In these cases it was difficult to accurately count the number of sporoblasts, but more than eight portions of sporoblasts were frequently present within a pansporoblast cavity. The maturing cells, both those that finished division and those still interconnected contained profiles of developing polar filaments, polar caps, polaroplasts and posterior vacuoles. The polar filament was observed in clear cytoplasmic areas around the nucleus. Three to four cross-section profiles could be observed on either side of the nucleus (Fig. 3 *b*). All cells were separated before elongation occurred. Elongation of the sporoblast into a bacilliform shape appeared to be associated with the positioning of the polar cap (anchoring disc and polar aperture) and polaroplast at the anterior end of the cell (Fig. 3 *a, b*). The posterior vacuole was retained in the opposite end of the cell and the nucleus maintained a central position.

As spore maturation continued, the cytoplasm of the cell became dense. Ribosomes increased in numbers and became arranged in tight spirals around the outer portion of the cell in the area below the polaroplast (Fig. 3 *c*). The polar cap was located beneath the multiple layers of surface membranes and consisted of a dense outer layer, the anchoring disc surrounding a cleared inner area and the polar aperture. The

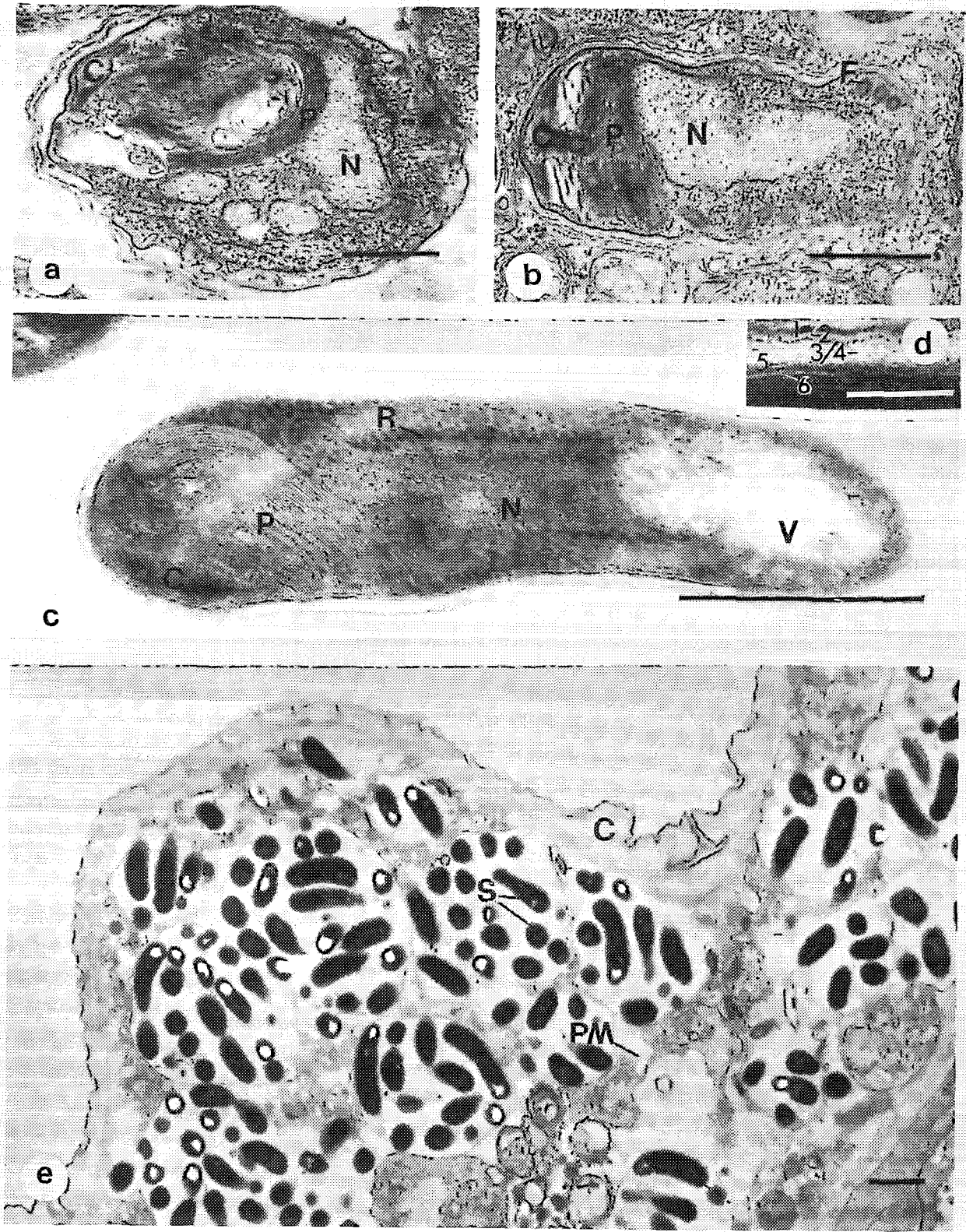


Fig. 3. *a* : An ovoid-shaped sporoblast. The polar cap (C) is positioned beneath the plasma membrane. The membranous stacks of the polaroplast (P) are associated with the nucleus (N); *b* : This sporoblast has attained the bacilliform shape. The polar cap (C) is in position with polaroplast membranes (P) between it and the nucleus (N). In the posterior portion of the cell the polar filament (F) is seen in cross section; *c* : A mature spore is shown with a posterior vacuole (V), nucleus (N), polaroplast (P), polar cap (C), and ribosomes (R). (Picture is underdeveloped to show cytoplasmic details); *d* : A high magnification picture of the spore coat shows the layers of the exospore (1, 2, 3) endospore (4) and surface membranes (5, 6) (6 is difficult to see due to electron dense cytoplasm immediately below); *e* : Pansporoblast showing the pansporoblast membranes (PM) and variable numbers of spores (S). C, cuticle of *Rhabditis myriophila*. (Magnification lines represent 0.5 μ m in *a-c*, 100 nm in *d* and 1 μ m in *e*.)

manubroid portion of the polar filament, similar in diameter to the rest of the polar filament, terminated in the polar aperture. From the anterior portion of the cell the wide polar filament extended posteriorly, displaced toward the periphery of the cell. The polaroplast, consisting of stacked membranous lamellae, formed around the manubroid portion of the polar filament in the anterior third of the cell (Fig. 3 c). Their appearance was uniformly lamellar. The nucleus was located in the mid-portion of the cell with the posterior vacuole adjacent (Fig. 3 c). The wall of the mature spore was approximately 50 nm thick. The exospore (Fig. 3 d) was composed of a dense outer layer, a transparent middle layer and an inner dense layer. The inner and outer layers sometimes structurally resembled unit membranes. The exospore surface was slightly wrinkled. In some areas small vesicles and larger tubular profiles were observed budding at the exospore. In cross section, the tubular profiles were observed to consist of an outer layer (a unit membrane?) followed by a clear zone, then an inner layer (membrane?) surrounding a dense innermost matrix. Total diameter of the tubules and vesicles varied due to the thickness of the inner matrix. The endospore (Fig. 3 d) was approximately 25 nm thick and contained fine flocculent material. There were apparently two membranes surrounding the spore cytoplasm. The inner membrane was difficult to resolve due to the dense underlying cytoplasm.

Sections of 468 pansporoblasts revealed a variable number of included spores (Fig. 4). The number of observed spores would depend on where in the pansporoblast the section passed. Although spores were frequently aligned parallel to each other; many pansporoblasts, especially those with large numbers of spores, contained randomly arranged spores. Since most spores were slightly curved, it is possible that they could be counted twice. In cross sectioned material, eight spores were frequently observed. The maximum number of spores counted in a single pansporoblast was 25.

Discussion

This is the first report of a definite microsporidan infection in a microbotrophic nematode and the first EM study of a microsporidan infection of a nematode.

Previous authenticated reports of microsporidan infections in nematodes are limited to two. The first was made by Kudo and Hetherington (1922) who described *Thelohania reniformis* from the gut epithelial cells of *Protospirura muris*, a parasite of the house mouse. Interestingly, these authors also could not document a schizogonic (merogonial) cycle. The second report is by Veremitchuk and Issi (1970) regarding an infection in populations of the insect parasitic nematode, *Neoaplectana carpocapsae* (Agriotes strain). Nematodes

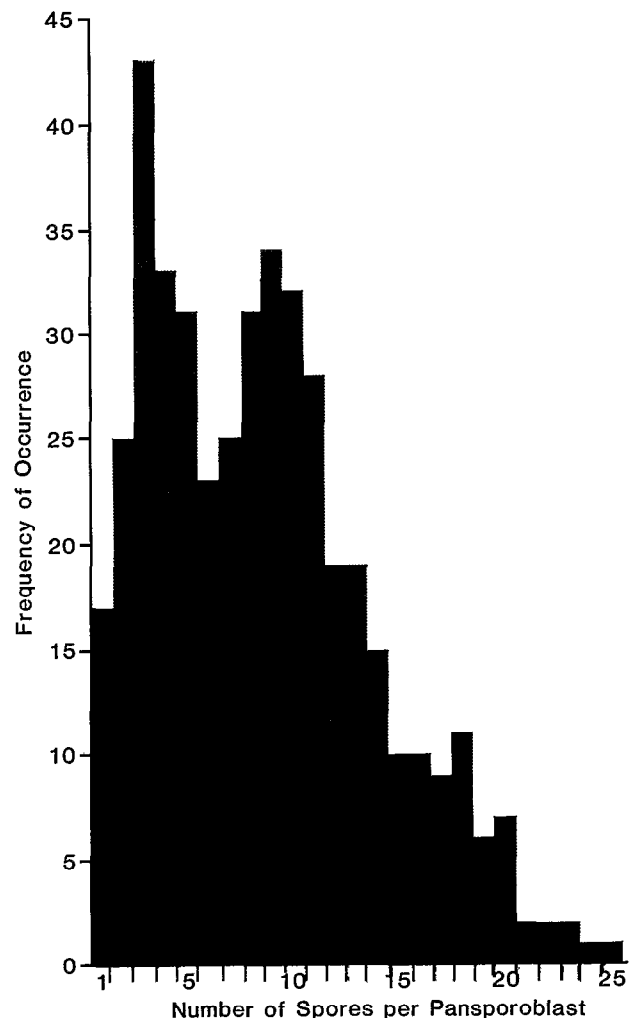


Fig. 4. Frequency of occurrence of spores of *Microsporidium rhabdophilum* n. sp. per pansporoblast in electron micrograph sections of 468 pansporoblasts in the nematode, *Rhabditis myriophila*.

were allowed to enter caterpillars of *Pieris brassicae* infected with the microsporidan *Nosema mesnili* and *Agrotis segetum* infected with the microsporidan, *Plistophora schubergi*. After the nematodes had developed for a period in the infected caterpillars, they were examined and microsporidan spores were observed in the nematode's body cavities. All other reports describing microsporidan infections in nematodes are incomplete since there is no electron microscopic evidence that the parasite was a microsporidium (Hopper, Meyers & Cefalu, 1970) or even a protozoan in some cases (Sprague, 1977).

The report by Veremitchuk and Issi (1970) brings up an interesting point. In nature, *R. myriophila* is associated with the millipede *Oxidis gracilis* (Poinar, 1986) and we considered the possibility that *R. myriophila* had acquired the infection by feeding on dead millipedes. We examined tissues of living and dead specimens of *O. gracilis* but could find no evidence of a microsporidian infection. Thus for the present, *M. rhabdophilum* can be considered as a primary parasite of *R. myriophila*.

The presently described microsporidium possesses several unique features. First, the size of the spores makes it one of the smallest described microsporidians from an invertebrate host. Second, the apparent range in spore number per pansporoblast is unusual in comparison with other members of the phylum. Although we describe only a sporogonial cycle, it is possible that merogony does occur but was missed because by the time we could determine if a nematode was infected, spores were already forming and the merogonial cycle could have been terminated.

The significance of the spore filled sacs in the dauer stage of *M. rhabdophilum* is that it offers a method of transport and distribution for the microsporidian. In nature, dauer juveniles would leave the millipede cadaver, and enter the alimentary tract of another living host. After the host died and the nematodes initiated development on the cadaver, the spores would be

released into the environment or even infect the dauer carrier. It is interesting that no other sign of infection was noted in dauer juveniles that carried the microsporidian spores.

ACKNOWLEDGMENTS

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