Arch. Protistenk. **126** (1982): 383–392

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Ligth and Electron Microscpic Studies on Three Microsporidians (Microsporida, Microspora) Parasitizing Springtails (Collembola, Apterygota)

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With 47 Figures

Summary

The ultrastructure of some vegetative stages and spore of the microsporidian *Nosema lepidocyrti* (WEISER and PURRINI, 1980) is discussed. Two new microsporidians *Nosema dungeri* n.sp. infecting the gut wall of the springtail *Isotoma violacea*, and *Nosema apterygotae* n.sp. invading hypodermal cells of the springtail *Orchesella flavescens* are described. The developmental cycle of new microsporidians were studied at the level of light and electron microscopy. Some data on hostparasite relationship and prevalence of infection are also given.

Introduction

Various groups of soil animals in agrobiocenoses and forests, such as springtails (Collembola, Apterygota) have recently received increasing attention of soil ecologists and biologists, since their activity largely determines soil fertility. Special attention has been given to studies of the role of industry, use of insecticides and herbicides, SO_2 immissions, and some other human activities exerting a marked negative influence on population dynamics of soil fauna, leading to the death of some animal communities, or to decrease in their activity. But, while the attention of soil ecologists and biologists has been centred on the human activities, the role of diseases caused by protozoan parasites in the dynamics of soil animal communities has been neglected. Our investigations of the pathology of forest soil fauna made during 1976—1981 show that different microorganisms belonging to Protozoa, Fungi, and Viruses are associated with soil animal communities (PURRINI 1980). The springtail-parasite associations were as common as other arthopod-parasite associations. Recent investigations of microsporidian parasites of springtails, presented in this paper, enabled me to re-examine the microsporidian *Nosema lepidocyrti* (WEISER and PURRINI 1980), at the electron microscope level. Two microsporidians, *Nosema dungeri* n.sp. and *Nosema apterygotea* n.sp. examined with light and electron microscopes are newly described.

Material and Methods

The springtails have been collected by TULLGREN funnels of litter and soil in mixed deciduous, beech, and mixed coniferous forests in Lower Saxony, Hesse and Westfalia (F.R.G.). Samples

were taken from 30 localities during the investigated period, separately from litter and soil. One sample of litter consisted of 10 l collected from 10×10 m squares, and one sample of soil consisted of 1 l of topmost 5 to 10 cm of soil, taken with a steel frame with sharpened lower edges. Specimens of springtails extracted from litter and soil were examined for microsporidian parasites by light and electron microscopy. For light microscopy, air dried smears of fresh material were prepared; fixed with methanol and stained with Giemsa stain, or semi-fine sections (1 μm thick) from blocks embedded in SPURRS medium and stained with toluidine blue. For electron microscopy, the infected specimens were fixed in glutaraldehyde (2% glutaraldehyde in 0.1 M phosphate buffer, *p*H 7.4). After washing in two changes of 0.1 M cacodylate buffer, the material was postfixed in 2% osmium tetroxide in 0.1 cacodylate buffer for 2 h, then dehydrated through 30 to 100% acetone series (each dehydration lasted 15 min); into 70% acetone containing 1% uranyl acetate, overnight. Finally, the material was embedded in SPURRS lower viscosity medium. Ultrathin sections after contrasting with uranyl acetate and lead citrate, were examined using a Philips EM 301 electron microscope.

Results

1. Description of the Parasites

a) *Nosema lepidocyrti* (WEISER and PURRINI 1980) Figs. 1—5.

The spores appeared long-oval in toluidine stained preparations, measuring 6×3.5 μm (Fig. 5).

The earliest stages seen with the light microscope were some vegetative stages of different sizes and shapes showing hardly vacuolated cytoplasm. Dividing of daughter cells occurred usually by budding (Figs. 1—3). The ultrastructure of the spore shows few internal details. There are 10 coils of the polar filament (F), two nuclei appearing to be in diplokaryon arrangement, exsporue (Ex), endospore (En), and a prominent round body (apparently belonging to the vacuole) which, occupy the posterior end of the spore (v).

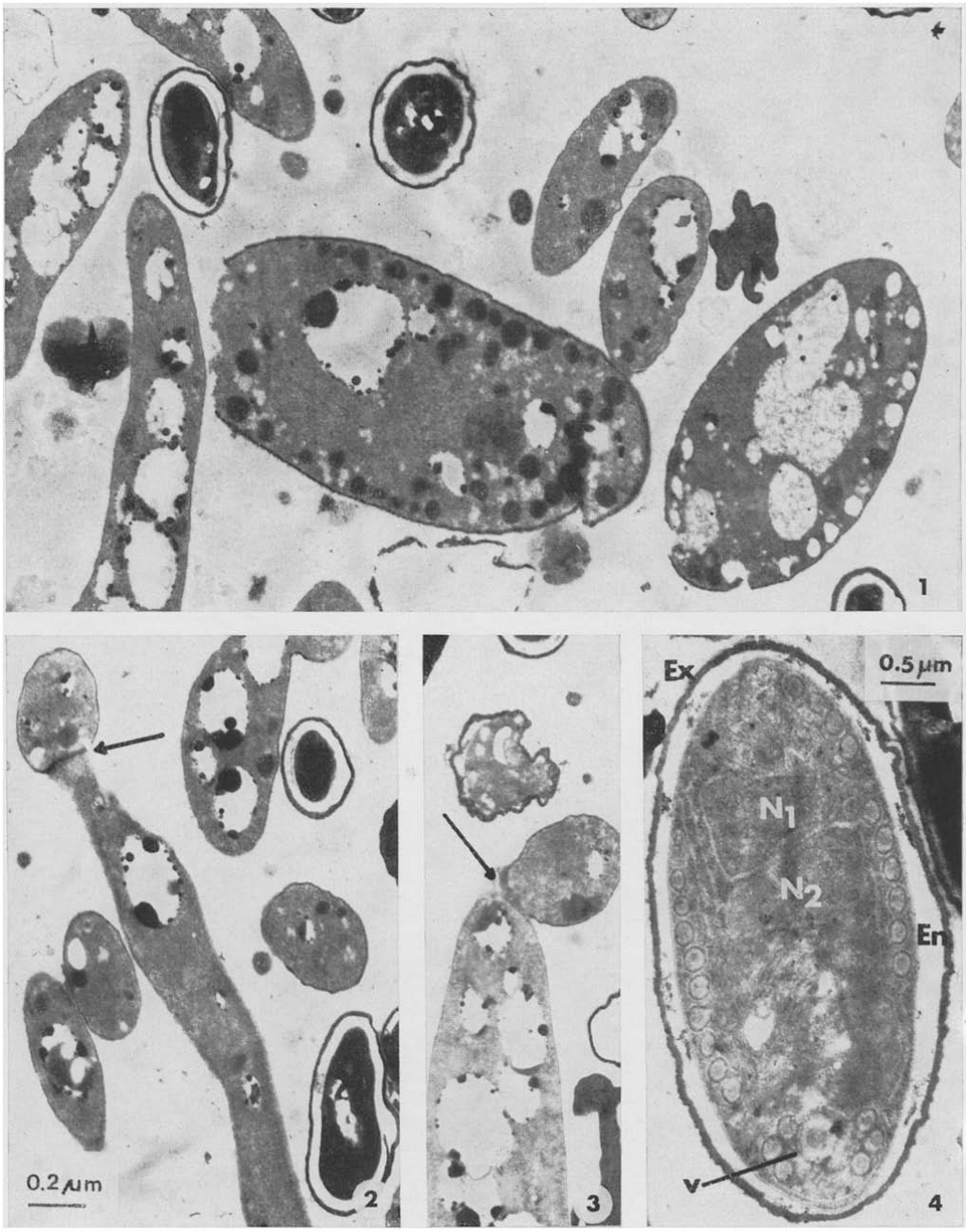
b) *Nosema dungeri* n.sp. (Figs. 6—42)

Host: *Isotoma violacea* TULLBERG 1876 (Isotomidae)

Tissue: Gut wall

Localities: Neuhaus, Melsungen, Siegerland; Mixed deciduous forests, Lower Saxony, Hesse, Westfalia (F.R.G.)

The binucleate cells (schizonts), measuring up to 3.5 μm in diameter, were the earliest stages in the life cycle of microsporidian (Figs. 6—8). The multiplying of nuclei in binucleate cells occurs by binary fision giving rise to tetranucleate forms (Figs. 9—17). Measurements of several tetranucleate schizonts gave the value of 3.5—5 μm in diameter. Stages with more than four nuclei (multinucleate schizonts) were not observed. Cytoplasmic division of tetranucleate stages occurred by plasmatomy, namely division of these stages into binucleate cells (Figs. 18—20). The stages represented in Figs. 6—20 are considered to belong to one closed generation of schizogony in the life cycle of microsporidian, having compact nuclei and more stained cytoplasm. Another generation was characterized by the binucleate (Figs. 22 and 23) and quadrinucleate forms (Figs. 24—28) having ring-like nuclei and less stained cytoplasm. Division of



Figs. 1–4. *Nosema lepidocyrti*. 1, 2, and 3. Ultrastructure of some vegetative cells (stages) showing hardly vacuolated cytoplasm. In Fig. 2 and 3, the typical mode of division of vegetative cells, budding, clearly visible (arrows), 4. Ultrastructure of mature spore showing exospore (Ex), endospore (En), two nuclei (N_1, N_2) in diplokaryon-like arrangement, 9 coils of the polar filament (F), and a prominent round body of the vacuole occupying the posterior pole (v). Three coils of the polar filament in both sides of the body visible.

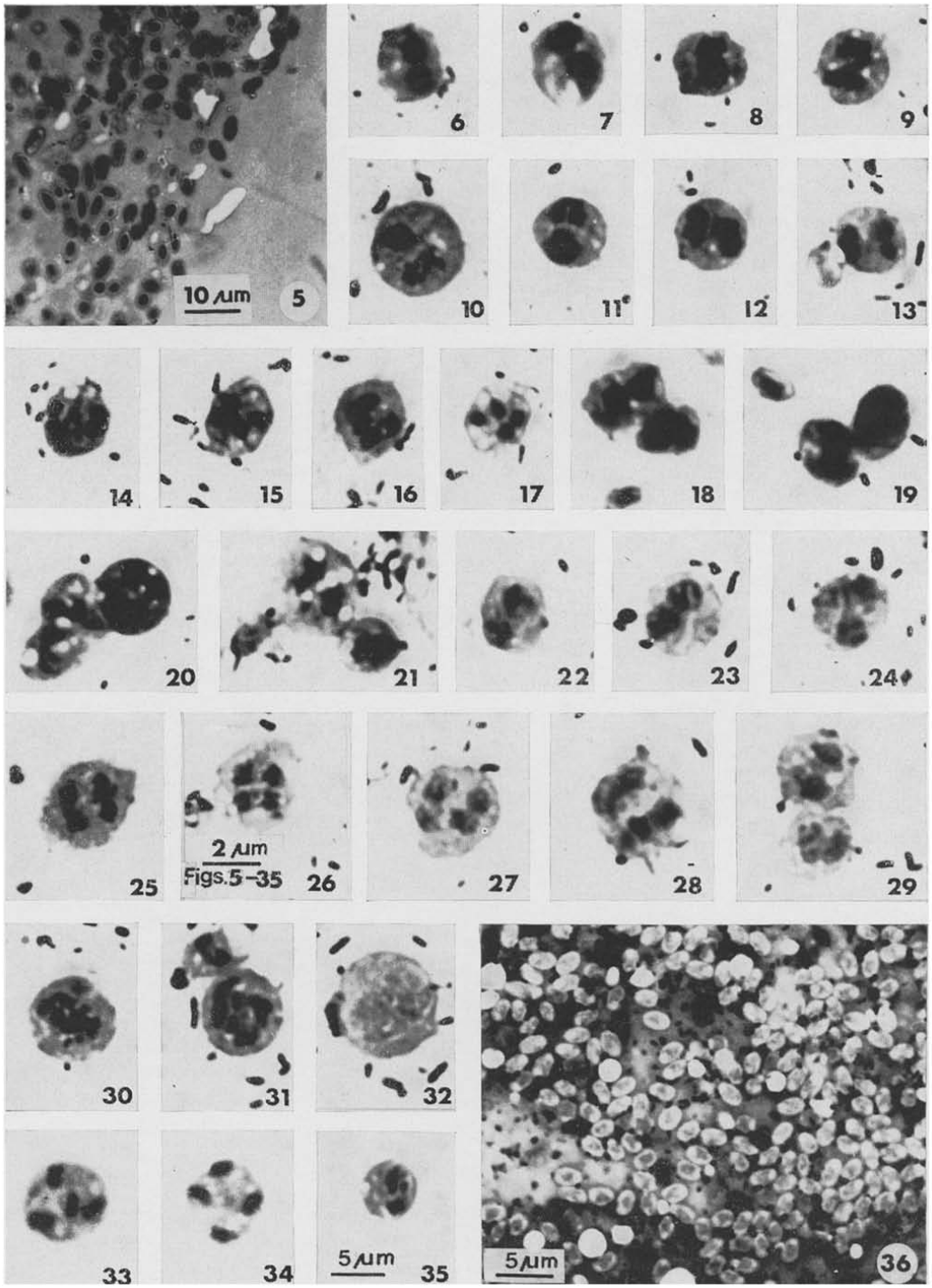


Fig. 5. *Nosema lepidocyrti*, mature spore in preparation stained with toluidine blue (semi-fine section 1 μm thick).

the quadrinucleate cells gave rise to binucleate stages (Fig. 29). The stages presented in Figs. 30—32, where chromosomal differentiation is discernible have been interpreted as stages intermediate to sporogony. The early quadrinucleate sporonts were round bodies with rod-shaped nuclei, measuring 4 μm in diameter. Two binucleate sporoblasts result from division of the sporont. The young sporoblast was ovoid, measuring 4—5 $\times$ 3.4 μm. Mature spores in Giemsa stained preparations were ovoid, measuring 2—2.5 $\times$ 1.3—1.8 μm. Some of them measured 3.0—3.5 $\times$ 2.0 μm.

The ultrastructure of the sporoblasts and spore is represented in Figs. 37—42. The sporoblasts were considerably larger than the mature spore and always ovoid. The irregularly shaped sporoblasts in Fig. 37 probably are a shrinking artifact. The surrounding membrane, unilayered at the initial stages (Figs. 37 and 38) became double layered (Figs. 39 and 40) with sporoblast maturation. An agregation of some "cisternae-like" dark stained bodies (probably belonging to Golgi apparatus) represents a primordium of the future polaroplast. It is always situated in aerea containing the basal portion of the polar filament. Two vacuoles were also seen in the inner structure of sporoblast as shown in Fig. 40 (V, v). The ultrastructure of the spore shows on outer envelope consisted of exospore (Ex), thick endospore (En), 18 coils of the polar filament (F), and two centraly situated nuclei (N_1, N_2) (Fig. 41). The polar filament in cross sections was almost circular with an outer electron lucent layer enclosing more than 6 other layers of contrasting density (Fig. 42).

c. *Nosema apterygotae* n.sp. (Figs. 43—47)

Host: *Orchesella flavescens* BOURLET, 1839 (Entomobryidae)

Tissue: Hypodermal cells

Locality: Rosengarten; Beech Forests, Lower Saxony (F.R.G.), 1981

The spores in Giemsa stained preparations were long-oval, sometimes appearing pear-like form, with an intensively stained posterosome. They measured 4.5—5.0 $\times$ 2.5 to 3.0 μm in stained preparations (Fig. 43).

The earliest stage of microsporidian observed with electron microscope was the diplokaryon stage presumed to be precursor of the zygote or sporont (Fig. 44). Its large nuclei were similar to the pairs shown by VAVRA (1977) for *Pleistophora debaisieuxi*, having the typical nuclear membrane and being flattened in the aerea of mutual contact. Except for ribosomes which were very common, no other structure

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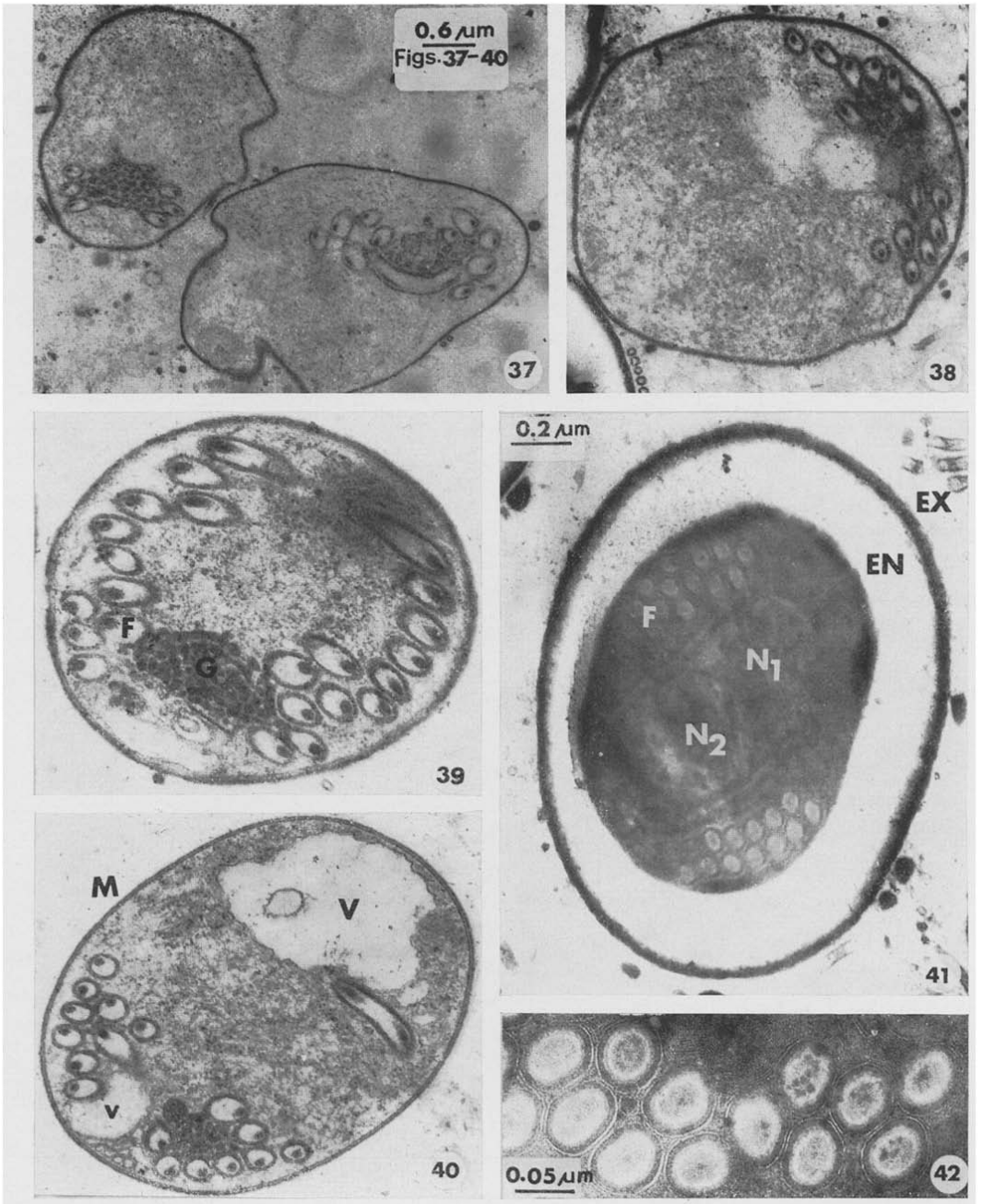
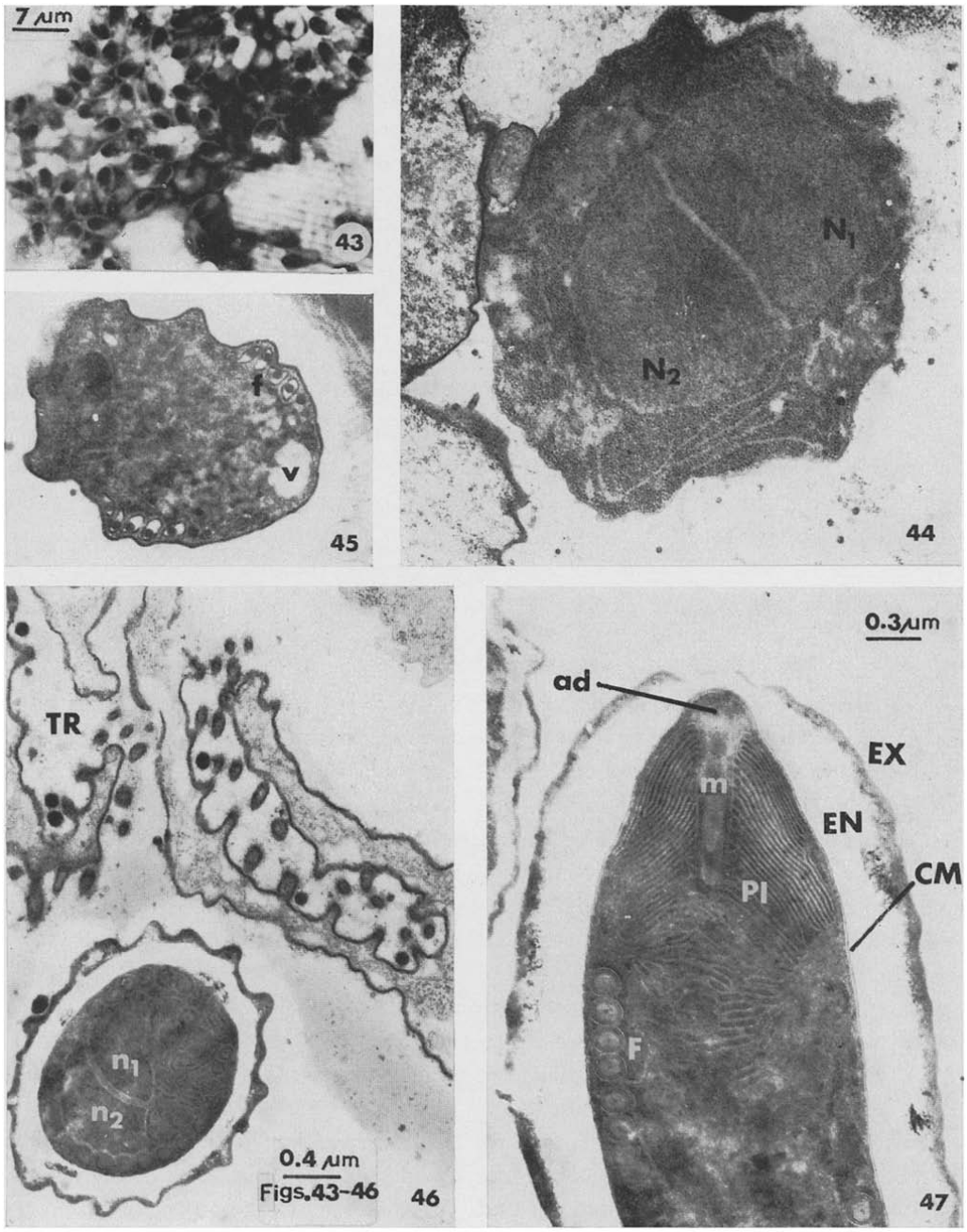
Figs. 6—36. *Nosema dungeri* n.sp. (light microscopy), 6—20. Schizonts with compact nuclei and more stained cytoplasm, 6—8. Binucleate schizonts, 9—13. Nuclear division in binucleate schizonts giving rise to formation of tetranucleate stages, 14—17. Tetranucleate schizonts, 18—21. Dividing teranucleate schizonts, 22—28. Schizonts with ring nuclei and less stained cytoplasm, 22 and 23. Binucleate schizonts, 24—28. Tetranucleate schizonts, 29. Dividing teranucleate schizont, 30—32. Three vegetative cells with nuclear region, in which "chromosome-like" bodies are present. They are considered to belong to the presporogonic stages in the life cycle of the microsporidian, 33 and 34. Two tetranucleate sporonts, 35. Binucleate sporoblast, 36. Mature spores; Figs. 6—36: Giemsa stain.



Figs. 37–42. *Nosema dungeri* n.sp. (electron microscopy) 37–40. Ultrastructure of the sporoblasts showing outer envelope (being uni-layered at initial stages, Figs. 37 and 38, it became double-layered with sporoblast maturation, Figs. 39 and 40), some dark vesicles, presumably belonging to the Golgi apparatus (G), various number of coils of the polar filament (F), a large vacuole (V), and a small one (v), 41. Ultrastructure of the spore showing more or less thick exospore (Ex), thick endospore (En), two nuclei (N_1, N_2), and 16 coils of the polar filament, 42. The polar filament in a high magnifications showing more than 6 layers in cross sections.



Figs. 43—47. *Nosema apterygotae* n.sp. 43. Mature spores in Giemsa stained preparations. A dark stained posterosome inside the spore remarkable (light microscopy). Figs. 44—47: Electron microscopy; 44. Ultrastructure of the diplokaryon stage with nuclear component represented by two adjacent nuclei in close relationship. Its cytoplasm filled with ribosomes visible, 45. Ultrastructure of the sporoblast showing coils of the polar filament (f), and a small vacuole (v), 46. Ultrastructure of mature spore in cross section; the main features to note are rugose structure of outer spore-envelope and two nuclei (n_1, n_2) in diplokaryon arrangement, TR. Tracheoles, 47. Ultrastructure of the spore in longitudinal section showing exospore (EX), thick endospore (EN), cytoplasmic membrane of spore content (CM), anchoring disc of the polar filament (ad), manubroid part of the polar filament (m), lamellar structure of polaroplast (Pl) and 8 coils of the polar filament (F).

could be observed in diplokaryons cytoplasm. The sporoblast show the presence of 5 coils of the polar filament (f) and a vacuole (v) (Fig. 45). The ultrastructure of mature spore in cross section shows the exposure with rugose surface, the endospore, two nuclei (n_1, n_2) in diplokaryon arrangement, and 8 coils of the polar filament (Fig. 46). In longitudinal section (Fig. 46) the spore wall consists of a relatively thin exospore (Ex), thick endospore (En), and a membranous layer of spore content (CM). There are 8 coils of the polar filament. The anterior part of the spore is occupied by extrusion apparatus consisting of the anchoring disc (ad), manubroid part (m) of the polar filament (F), and lamellar polaroplast (Pl).

2. Host-Parasite Relationship and Prevalence of Infection

The infection caused by microsporidian parasites did not induce any apparent external symptoms in their hosts. Only after dissection the hypertrophied guts and hypodermal cells filled with masses of the spores could be observed with light microscope. The infection rate caused by *N. dungeri* in 150 inspected specimens of springtail *Isotoma violacea* was 10% and of *N. apterygotae* in 100 specimens of springtail *Orchesella flavescens* was 5%.

Discussion

New species of microsporidian parasites and new host records have regularly been added until present time, when more than seven hundred species are known, almost all of invertebrate groups as common hosts. There were none recorded from soil arthropods, and until 1980 there was no confirmation that any occurred in springtails. The first report on microsporidians infecting springtails was given by WEISER and PURRINI (1980) describing seven species by means of light microscopy. Two new species described here show that springtail-microsporidian associations are more common than expected. New informations about the ultrastructure of these species are now presented.

The fine structure of the spore containing two nuclei in *Nosema lepidocyrti*, as typical feature of microsporidians close to the genus *Nosema* NAEGELI 1857 (VAVRA 1977), confirms our earlier investigations of named species made with the light microscope.

Two newly found microsporidians in springtails: *I. violacea* and *O. flavescens* conform to the genus *Nosema*, which is characterized by disporoblastic sporogony giving rise to isolated spores. *Nosema dungeri* and *Nosema apterygotae* possess these characteristics and therefore, belong to the genus *Nosema*. Two schizogonial generations appeared in *N. dungeri*. In *N. apterygotae*, although schizogony was terminated, there is an evidence of occurrence of the diplokaryon stage. The diplokaryotic organisation is typical of the genus *Nosema*.

N. dungeri and *N. apterygotae* should be compared with microsporidians listed in Table 1. *Nosema dungeri* n.sp. is proposed because of striking differences from *N. lepidocyrti* and *N. onychiurus* in its development, size of spores, and host-parasite relationships; from *N. onychiurus* also in its site of infection. *Nosema apterygotae* n.sp. is

Table 1. Comparison of known Microsporidian parasites of Springtails (Collembola, Apterygota)

Parasite	Host	Site of	Infection Spore (in μm)	Author
Nosema lepidocyrti	*Lepidocyrtus lignorum*	gut wall	6×3.5—3.7	Weiser and Purrini (1980)
Nosema onychiurus	*Onychiurus quadriocellatus*	fat body	2.5—3.5×1.2—2.0	Weiser and Purrini (1980)
Nosema petrosa	*Lepidocyrtus cyaneus*	fat body	3.5—4.0×2.5—2.8	Weiser and Purrini (1980)
Encephalitozoon flavescens	*Tomocerus flavescens*	muscles	5.5—6.0×2.5	Weiser and Purrini (1980)
Thelohania bomboschi	*Tomocerus flavescens*	fat body	6.0—7.0×4.0—4.5	Weiser and Purrini (1980)
Thelohania collembolae	*Tomocerus flavescens*	fat body	4.5—4.8×3.5—3.8	Weiser and Purrini (1980)
Auraspora canningae	*Lepidocyrtus lignorum*	male gonads	4.0—5.0×2.0—2.5	Weiser and Purrini (1980)
Nosema dungeri n.sp.	*Isotoma violacea*	gut wall	2.0—2.5×1.3—1.8	this paper
Nosema apterygotae n.sp.	*Orchesella flavescens*	hypodermal cells	4.5—5.0×2.5—3.0	this paper

proposed for differences from *N. lepidocyrti*, *N. onychiurus*, and *N. dungeri* in size of the spore, site of infection and host-parasite relationships.

The present investigations of ultrastructure of the spore of microsporidians newly recorded did not show any exceptional features compared with microsporidians known from other Arthropods. However, it could be noted that vegetative cells of *N. lepidocyrti* seen with the light microscope show hardly vacuolated cytoplasm. It is suggested that vacuolation might signify degeneration of these cells, due to food deficiency in the host-cell which is important for normal development of intracellular parasites. The host springtail *L. lignorum* is a small animal (0.5—0.7 cm), and there is a limited food reserve for pathogenic microorganisms of great reproductive capacity, such as microsporidians.

Zusammenfassung

Die Ultrastructur einiger vegetativer Stadien und Sporen der Mikrosporidie *Nosema lepidocyrti* Weiser and Purrini (1980) wurde untersucht. Zwei Mikrosporidien-Arten, *Nosema dungeri* n.sp. aus dem Springschwanz *Isotoma violacea* und *Nosema apterygotae* n.sp. aus dem Springschwanz *Orchesella flavescens* werden neu beschrieben. Sie befallen das Darmepithel *(N. dungeri)* und Hypoderm *(N. apterygotae)* der Wirte. Der Lebenszyklus der neuen Mikrosporidien-Arten wird auf Grund licht- und elektronenmikroskopischer Untersuchungen beschrieben. Über die Infektionsrate der Mikrosporidien wird auch berichtet.

Acknowledgements

I wish to thank Prof. Dr. FRANK MAYER, Institute of Microbiology, University of Göttingen for his help with EM examination, to Mrs. BIRGIT SCHREIBER, and Mrs. URSULA HOFACKER, Institute for Forest Zoology, University of Göttingen, for their help in sectioning and staining the objects.

Remarks

Nosema dungeri n.sp. (Microsporida: Phylum Microspora) of springtail *Isotoma violacea* is deidcated to Prof. Dr. WOLFRAM DUNGER Staatliches Museum für Naturkunde, Görlitz-Forschungsstelle, DDR - 8900 Görlitz, Am Museum 1.

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