

# **Octosporea collembolae n. sp. (Microsporida, Microspora): A New Microsporidian Parasite of Springtail *Onychiurus quadriocellatus* (Onychiuridae:Collembolae)**

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The life cycle of a new microsporidium, *Octosporea collembolae*, from the fat body of naturally infected springtails, *Onychiurus quadriocellatus*, is described at light and electron microscope levels. The prevalence of infection and host-parasite relationships are discussed.

**KEY WORDS:** *Octosporea collembolae*; microsporidian; *Onychiurus quadriocellatus*; springtail; natural infection.

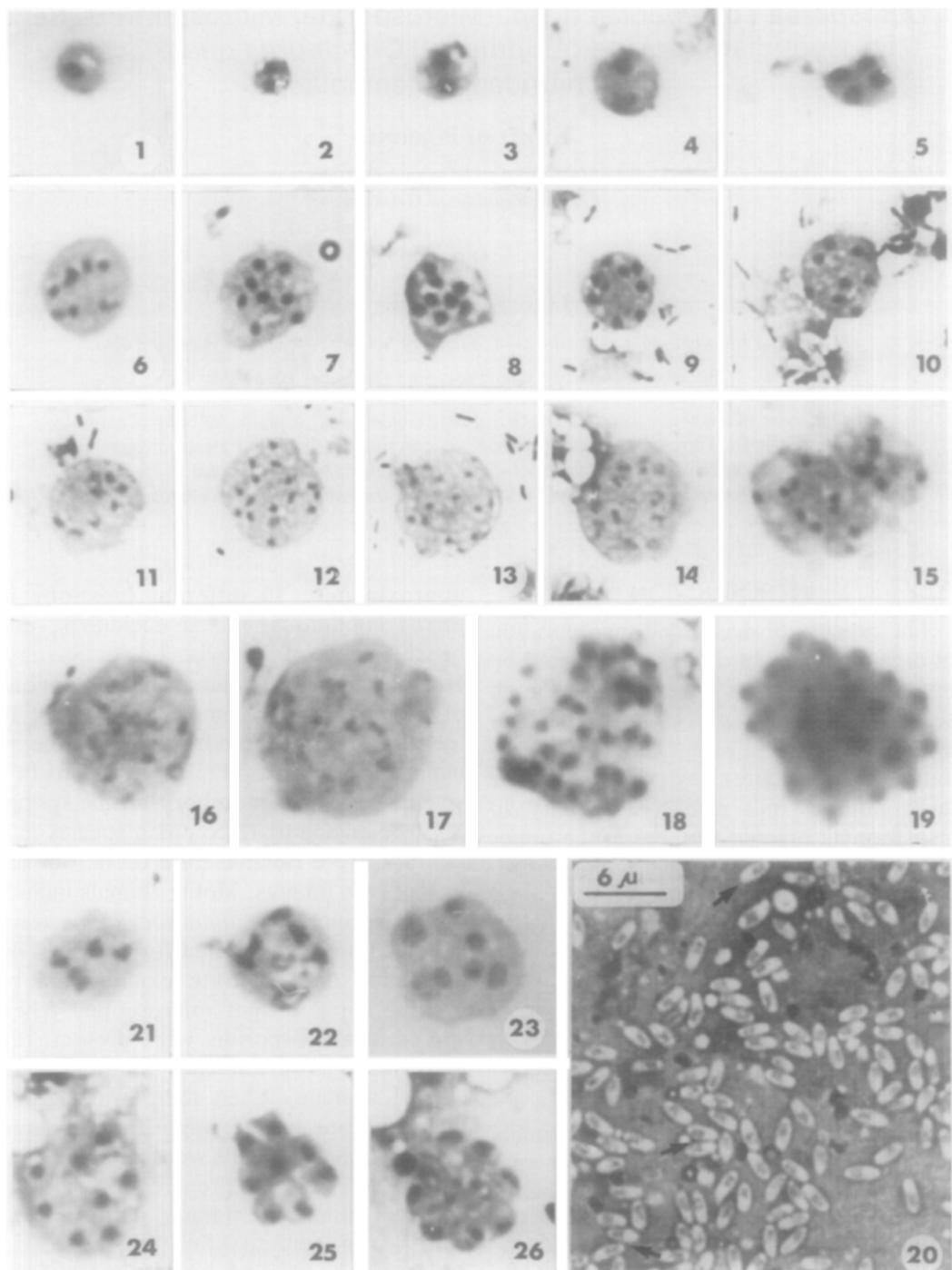
## **INTRODUCTION**

The process of decay and incorporation of organic matter into the soil, initiated by invertebrates, occurs to some degree in all soil systems. These processes are particularly rapid in forest soils. Springtails (Collembola:Apterygota), by virtue of their large number and intensive activity in transforming organic material in the upper layer of soil, are among the important invertebrates that are collectively named "decomposer organisms." However, there are few data concerning their diseases and resulting pathology. The occurrence of microsporidian parasites in springtails was first reported by Weiser and Purrini (1980) and Purrini (1980, 1982a, b). Nine species belonging to different genera have been described. A recent survey of the springtails has revealed the presence of another species in *Onychiurus quadriocellatus*. The results of the survey and description of the parasite are given.

## **MATERIAL AND METHODS**

During the period 1976-1981, samples of soil and mulch (litter), were collected from

approximately 30 different locations in mixed coniferous, mixed deciduous, and beech forests of Bavaria, Lower Saxony, Hessa, and Westphalia (Federal Republic of Germany). Each sample consisted of 10 liter of mulch collected from a 10-m<sup>2</sup> site by hand and 1 liter of soil taken by means of a spade. Beside other soil organisms, springtails, belonging to 12 common species in 7 families, were isolated from these samples by Tullgren funnels. Motile animals including the springtail, *O. quadriocellatus*, were collected and investigated for parasites by dissection and separate examination of organs, using a light microscope. When spores of microsporidia were present, the material was dried and stained with Giemsa's stain. When there was a high infection rate in the population, a large number of host animals were fixed in aqueous Bouin's fluid and prepared for histological sectioning and staining with Heidenhain's iron hematoxylin. The nuclei in spores were stained by the method of Weiser (1976). A small area of the stained smear was covered with a drop of 10% HCl, heated over a low gas flame, washed with



cold water, and restained with Giemsa's for 1–2 min.

For electron microscopy, usually the whole body of an infected specimen was fixed in phosphate-buffered glutaraldehyde (2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.5) at room temperature (2 hr), washed in buffer, postfixed in 1% osmium tetroxyde (1 hr), dehydrated in an acetone series of 30–100%, washed in propylene oxide (5 min), and embedded in Spurr's low-viscosity resin. Sections were cut with glass knives on a Reichert OmU 2 ultramicrotome, stained with uranyl acetate and lead citrate, and observed in a Philips 301 electron microscope.

## RESULTS

### *Octosporea collembolae* n. sp. from *Onychiurus quadriocellatus*

*Light microscopy* (Figs. 1–28). Earliest stages noted were small rounded uni- or binucleate meronts (Figs. 1–4), which measured 1.5–4.5  $\mu\text{m}$  in diameter. Larger stages with four to eight and more nuclei (Figs. 6–10, 15, 21–23) measuring 5–8  $\mu\text{m}$  were also probably meronts. Division stages were not positively identified. Figure 15 depicts either two adjacent meronts or the final phase of plasmotomy of a large meront with about 14 nuclei.

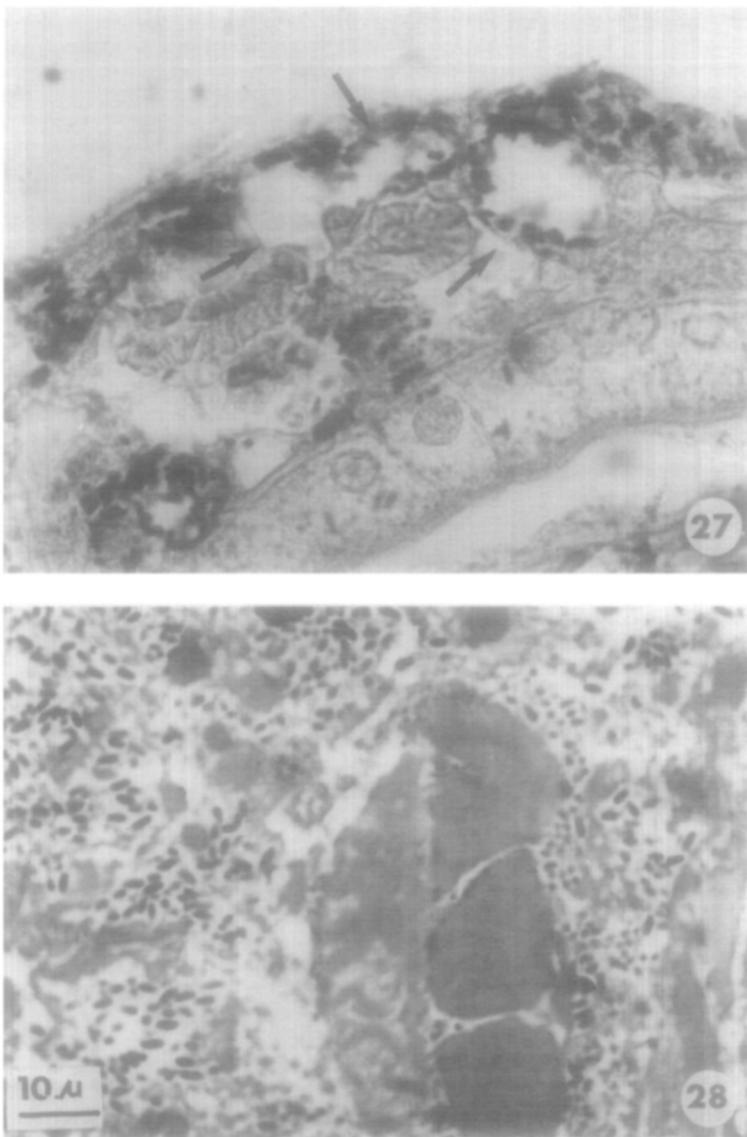
Other stages, the nuclei of which are smaller and often shows signs of division, are attributed to the sporogonic cycle (Figs. 11–19). There are sporogonial plasmodia, measuring 8–16  $\mu\text{m}$ , with often vacuolated cytoplasm (Figs. 11–14) and stained less deeply than that of the meronts. The nuclei

were about half the size of the meront nuclei, some being V shaped or double. The number of nuclei ranged from 8 up to probably 32 in the largest plasmodia (Figs. 16, 17). At the time of division of the plasmodia into sporoblasts (Figs. 18, 19, 24) the nuclei are again rounded and are as large as those of meronts. Two octonucleate pansporoblasts with morula-like protrusions are presented in Figures 25 and 26. The individual sporoblasts with a single (Fig. 25) or two nuclei (Fig. 26) were formed in each protrusion. In preparations stained with Giemsa's, the spores were elongate ovoid and measured 2.5–3.2  $\times$  1.0–1.5  $\mu\text{m}$ . Weiser's method of restaining with Giemsa's (after hydrolysis with 10% HCl) was not efficient in demonstration of nuclei in spores.

*Electron microscopy* (Figs. 29–36). All stages seen by electron microscopy were surrounded by a thick surface coat and were separated from the host cell cytoplasm by a fine membrane, the pansporoblast membrane, which hold the stages together in groups (Figs. 29–32). Even the earliest stages, represented by two adjacent individuals, were apparently bound by a pansporoblast membrane (Fig. 29, arrows). These individuals contained abundant ribosomes patchily distributed. The endomembrane system was not well preserved and, although membranes could not be seen round them, the areas lacking in ribosomes may correspond to the vacuolated cytoplasm seen in light micrographs and a slightly denser area may represent the nucleus (Fig. 29).

Division within the pansporoblast membrane gives rise to sporoblasts with vacuo-

Figs. 1–26. Life cycle of the microsporidian *Octospora collembolae* n. sp. parasitizing the springtail *Onychiurus quadriocellatus*. (1) Uninucleate meront. (2)–(4) Binucleate meronts. (5) Tetra-nucleate meront. (6)–(10) Octonucleate meronts. (15) Possible division of phase of large meront. (11)–(14) Multinucleate sporonts (sporogonial plasmodia); (16)–(19). Larger sporonts with nuclei which ranged in number from 8 probably up to 32; in the largest plasmodia (Figs. 18 and 19) the nuclei were again rounded and as large as those of the meronts; (21)–(23) Tetranucleate (Fig. 21) and octonucleate stages (Figs. 22 and 23) are probably meronts; the division of nuclei in the stages is visible. (24) Octonucleate sporont with rounded nuclei and as large as those of the meronts; (25) and (26) Octonucleate morula-like pansporoblasts; the individual sporoblasts with single (Fig. 25) or double nuclei (Fig. 26) are visible in each protrusion. (20) Mature spores; in some spores double nuclei visible (arrows). Figs. 1–26. Giemsa's stain.

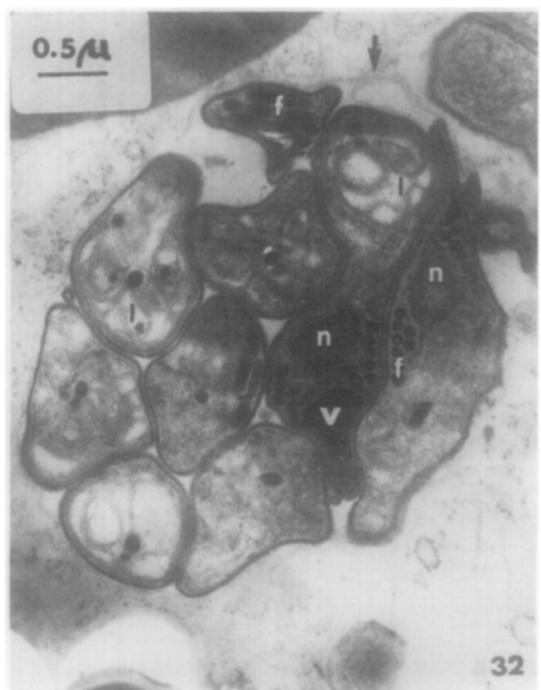
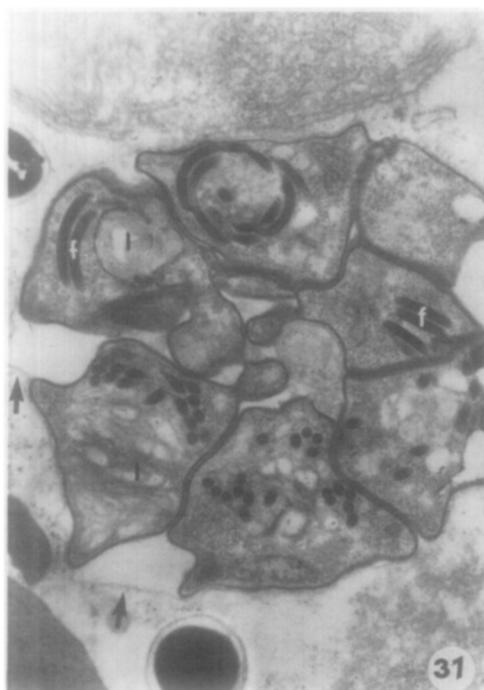
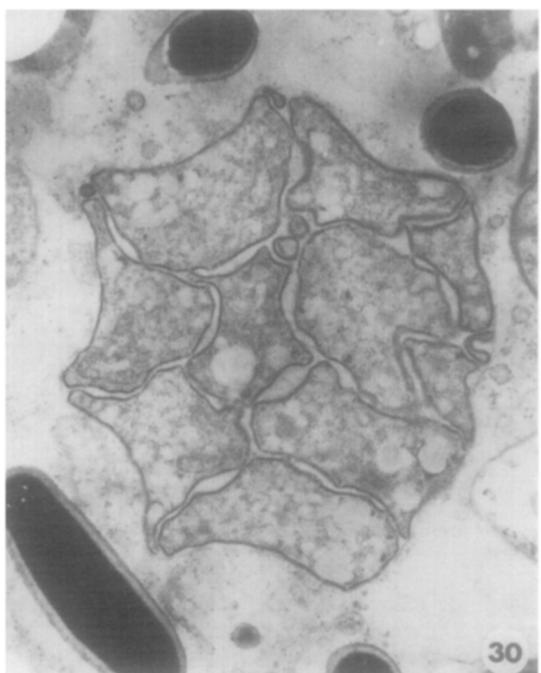
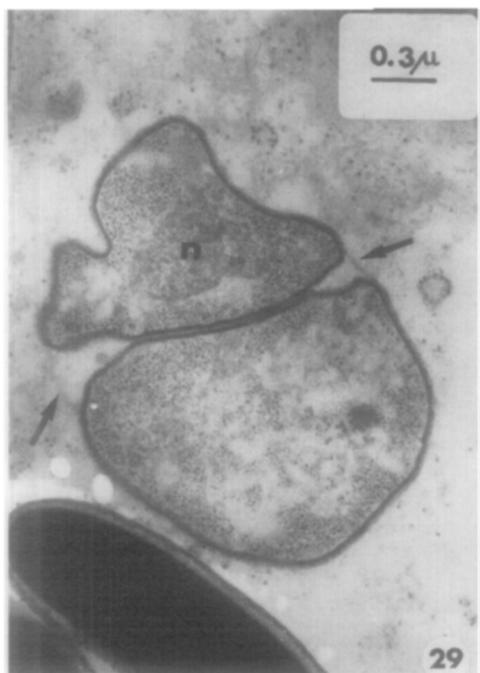


FIGS. 27 and 28. Tissue of *Onychiurus quadriocellatus* showing pathological effect of *Octospora collembolae*. (27) Hypertrophied and vacuolated isolated cells (arrows) of the fat body invaded by parasites. (28) Fat body destroyed by massive invasion of parasites; Figs. 27 and 28 are of sections 4- $\mu$ m thick stained with Heidenhain's iron hematoxylin.

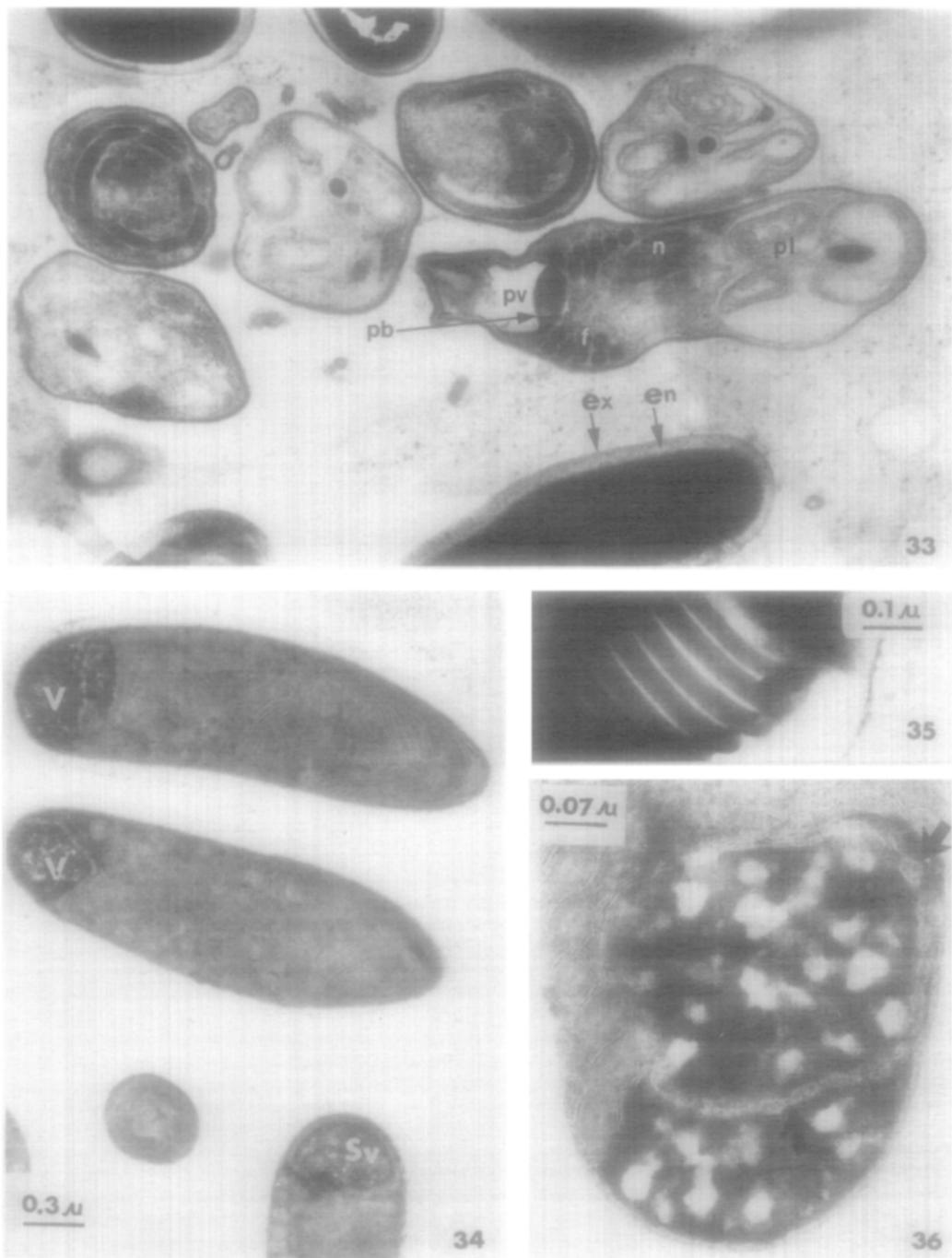
lated cytoplasm (Fig. 30). Internal structures of sporoblasts, as shown in Figure 30, are not very distinct and there is no sign of any attachment of cells to each other or to the pansporoblastic membrane. The same membrane closes another pansporoblast with eight maturing sporoblasts, depicted in Figures 31 and 32. The sporoblasts are characterized by the primordia of the polar

filament, polaroplast, vacuole, and nucleus (Figs. 31, 32).

Stages in the maturation of the spores are represented in Figures 33-36. The number of coils of the polar filament is about eight (Figs. 33, 35). In one young spore (Fig. 33), a single nucleus is located in the region posterior to the polaroplast and just within the anterior turns of the polar filament.



Figs. 29-32. Electron micrographs of *Octospora collembolae*. (29) Dividing stage, probably a sporont, with a thick surface coat and surrounded by a thin pansporoblast membrane (arrows); ribosomes and nucleus (n) are visible. (30) Octosporous pansporoblast. Internal structure of sporoblasts not distinct. (31) and (32) Octospororous pansporoblasts closed in a thin plasmatic membrane (arrows). The sporoblasts are characterized by the primordium of the polar filament (f), polaroplast (l), nucleus (n), and vacuole (v).



Figs. 33-36. Electron micrographs of *Octospora collembolae*. (33) Sporoblasts separated from sporogonial plasmodium within fine pansporoblast membrane. A mature spore with well-developed exospore (ex) and endospore (en) is also present. The sporoblasts show the membranes of the future polaroplast (pl), nuclear material (n), eight coils of the polar filament (f), posterior vacuole (pv), and posterior body of the vacuole (pb). (34) Mature spores showing well-differentiated single vacuole (sv) and bipartite vacuole (V). (35) Spiral arrangement of the polar filament. (36) Posterior vacuole traversed by cytoplasm (arrows) giving bipartite appearance.

There is a conspicuous posterior vacuole occupying one-fifth to one-quarter of the spore length. Mature spores are stained with little contrast; they are elongate, three times longer than broad (Fig. 34). The exospore is thin, but is differentiated late in spore maturation. The part which in most spores is not stained, the endospore, is stained in young (nearly mature) spores (Figs. 29, 30, 33). The vacuole in the spores as shown in Figures 34 and 35 is darker than the rest of sporoplasm. It contains electron-dense material with lucent patches and is often split into two by a layer of cytoplasm.

#### *Prevalence of Infection and Host-Parasite Relationships*

Of the twelve springtail species involved, *Onychiurus quadriocellatus* was the most common in samples from all localities. The infection rate in approximately 1300 specimens was 30%. However, the extraction method for soil animals reveals only motile specimens. If heavily infected specimens are not as active as healthy ones, they would not have been able to pass through the funnels and move from the litter to the sampling glasses with the flow of water. Thus, heavily infected specimens may have remained in the litter, escaping detection and the true prevalence may have been higher.

As known from the literature, usually the diseased tissues of arthropods appear milky white. However, in our case the healthy springtails are milky white, and under light microscope it was observed that the diseased ones were transparent (crystal glass-like), with small dispersed white areas filled with spores of the parasite. The microsporidian invading springtails, *O. quadriocellatus* is localized in the cells of the fat body (Figs. 27, 28). In case of heavy infections, the invaded fat body, containing masses of spores, was totally destroyed (Fig. 28).

#### DISCUSSION

The assignment of this microsporidian to the genus *Octosporea* was especially complicated. It was supported by the light mi-

croscope observation of double nuclei in spores stained with Giemsa's. However, this finding could not be confirmed after hydrolysis of spores with HCl followed by Giemsa restaining, nor by ultrathin sectioning. The most important feature found both in light and electron microscope preparations was the occurrence of characteristic octonucleated stages and of octosporous pansporoblasts. In addition to these stages, groups of spores which might have included 16 or 32 spores in one mass, were observed. Our species resembles more the majority of known *Octosporea* spp. listed by Sprague (1977). These usually include two nuclei, elongate oval or tubular spores with a sporogonial cycle including stages usually containing 8, 16, or 32 spores. In contrast to some pansporoblastic microsporidia of the genera *Octosporea* (see Ormières et al., 1976) and *Pleistophora* (see Canning and Nicholas, 1980; Canning and Hazard, 1982; Purrini 1982a, b), the membrane surrounding the pansporoblast was especially thin and not visibly differentiated in our samples. We propose the name *Octosporea collembolae* n. sp. for our newly detected species.

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