Z. Parasitenkd. 62, 75–84 (1980)

Zeitschrift für
Parasitenkunde
Parasitology Research

Seven New Microsporidian Parasites of Springtails (Collembola) in the Federal Republic of Germany

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Abstract. Three new species of *Nosema* (*N. lepidocyrti, N. onychiurus* and *N. petrosa*), one *Encephalitozoon* (*E. flavescens*), two species of *Thelohania* (*T. bomboschi* and *T. collembolae*) and a new genus *Auraspora* n.g. with *A. canningae* were described from Collembola in soil samples of Lower Saxony, Federal Republic of Germany.

Sieben neue Mikrosporidien-Arten der Springschwänze (Collembola) aus der Bundesrepublik Deutschland

Zusammenfassung. Obwohl die Springschwänze (Collembola) als wichtige Streuzersetzer seit langem Gegenstand bodenbiologischer Forschung sind, gab es bisher keine Hinweise auf das Vorkommen pathogener Protozoen als Parasiten dieser Insekten. Im Rahmen einer vergleichenden Bodentieruntersuchung in verschiedenen Waldstandorten Niedersachsens wurden 1320 Collembolen auf Parasitierung durch pathogene Protozoen geprüft. Dabei konnten erstmals Mikrosporidien (Microsporida, Protozoa) als Krankheitserreger von Collembolen festgestellt werden. Sie werden neu beschrieben: *Nosema lepidocyrti* sp.n. in *Lepidocyrtus lignorum* Tullberg (Tomoceridae), *Nosema onychiurus* sp.n. in *Onychiurus quadriocellatus* Gisin (Onychiuridae), *Nosema petrosa* sp.n. in *Lepidocyrtus cyaneus* Tullberg (Tomoceridae), *Thelohania bomboschi* sp.n. in *Tomocerus flavescens* Tullberg (Tomoceridae), *Thelohania collembolae* sp.n. in *L. lignorum* und *T. flavescens, Encephalitozoon flavescens* sp.n. in *T. flavescens.*

Eine Art ist keinem bekannten Genus zuzuordnen: *Auraspora* gen.n. *canningae* sp.n. in *L. lignorum.*

Der nachgewiesene Mikrosporidienbefall betrug bei *O. quadriocellatus* 3% ($n=220$), *L. lignorum* 7% ($n=130$), *T. flavescens* 8% ($n=193$) and *L. cyaneus* 6% ($n=63$).

Key words: Microsporida – *Nosema* – *Encephalitozoon* – *Thelohania* – *Auraspora* gen.n. – Collembola.

0044-3255/80/0062/0075/$02.00

Introduction

The springtails, Collembola, are a group of animals which play an important role in nature by their large numbers in soil and intense activity in transforming organic materials in the upper layers of soil. Data concerning their activity have been published by different authors during the last few years together with studies of the whole system of organisms inhabiting soils. However there are practically no data concerning their pathology and diseases. The present study contributes data on the distribution of Microsporidia (Microsporida, Protozoa) in natural populations of some Collembola in Lower Saxony, Federal Republic of Germany.

Material and Methods

During 1978 soil samples were collected from 26 different localities in mixed coniferous forests, pure beech forests, and mixed-leaf forests mainly in Lower Saxony, Federal Republic of Germany. Springtails (1,320) belonging to the four most common species were isolated from these samples in extractors in the laboratories of the Institute of Forest Zoology, University of Göttingen. Motile animals were collected and smeared in dry smears on slides, fixed with methanol, and stained with Giemsa-Romanowski. Usually the contents of the body of the springtail were used for the preparation of one slide. The infections were not visible externally. Only the more numerous infections could be fixed in situ. The animals were prepared in the usual way for embedding in Epon-Araldite and cut in ultrathin sections for study in the electron microscope. The spores were stained by the method of Weiser (1976) for differentiation of the nuclei. A small area of the stained smear was treated with a drop of 10% HCl, the drop was heated over a very small flame of a gas burner till boiling, washed with cold water, and re-stained with Giemsa for 1–2 min.

Results

The Hosts

Of the four species involved, *Onychiurus quadriocellatus* Gisin was the most common in samples from all localities, occurring mainly in mixed-leaf forests. The infection rate of 220 inspected animals was only 3%. In *Tomocerus flavescens* Tullberg, occurring in pure beech forests, mixed-leaf forests, and mixed coniferous forests, 8% of 193 inspected specimens were infected by Microsporidia. Investigations of 130 specimens of *Lepidocyrtus lignorum* Fabr. and 63 specimens of *Lepidocyrtus cyaneus* Tullberg revealed infection rates of 7% and 4% respectively. Both species were mainly found in mixed-leaf forests.

Distribution of microsporidian infections was limited to 5 out of the 26 localities investigated. There was no relationship to soil type or environmental peculiarities. Since the extraction technique for soil arthropods reveals only live, motile specimens the frequency and distribution of microsporidia in springtails may be less restricted, however. As to the parasite spectrum of the different hosts, *Tomocerus* was infected by 3 species; *L. lignorum* by two other species. *L. cyaneus* however, appeared to be the host of only one and *Onychiurus* that of one other parasite species.

Microsporidian Infections

Seven different microsporidians were found in the material investigated. Most of them were infections in cells of the fat body, some were localized in muscles and others in male gonads. Two of the species collected belonged to the genus *Thelohania,* three were members of the genus *Nosema,* one was close to the genus *Encephalitozoon* (or *Pérezia* of the recent revision of French authors), and one belonged to a genus which is not yet included in the system of Microsporidia. All species found are new. Due to the minuteness of the host bodies and conditions of collecting the description could not be based on a very large quantity of material and mainly stages of sporogony were documented in our study. Further investigations of the ultrastructures underlying the structures described using the optical microscope need to be performed.

1. *Nosema lepidocyrti* sp.n. (Plate I, 1–11, Plate III, 1). Host: *Lepidocyrtus lignorum* Fabricius 1781 (Fam. Tomoceridae, Entomobryomorpha, Collembola). Locality: Mixed-leaf forests, Bergen (Lower Saxony, Federal Republic of Germany), 1978.

Oval, elongated spores were 6 µm long and 3.5–3.7 µm wide, thin-walled, with very small deformities which occurred during desiccation on slides. The germ inside the spore stained well as a dark cone, with the rare appearance of a posterosome (Golgi granules) only in early sporoblasts. In some young spores two nuclei were visible. In HCl-treated spores the nuclei were stained as two round fine corpuscles arranged longitudinally on the side of the spore. On other occasions the nuclei were in the posterior pole beside each other. This was also the position of the nuclei in the sparse sporoblasts which were among the spores spread irregularly in the smears of tissues. The spores were not grouped together inside membranes. The host cells ruptured easily releasing the spores.

2. *Nosema onychiurus* sp.n. (Plate I, 16–20, Plate III, 2). Host: *Onychiurus quadriocellatus* Gisin 1947 (Fam. Onychiuridae, Poduromorpha, Collembola). Locality: Mixed-leaf forests, Bergen and Rosengarten (Lower Saxony, Federal Republic of Germany), 1978.

Thin-walled spores are long and oval in fresh preparations and oval in stained preparations, 2.5–3.5 µm long and 1.2–2.0 µm wide, with a deep anterior vacuole (polaroplast), and an intensively stained minor spherical posterosome. Differentiation of the nuclei is rather difficult, as they are very small and are easily dissolved when the HCl is too hot. The nuclei are close together at the posterior end, usually beside each other. Spores were seen only in the fat body in the material collected.

3. *Nosema petrosa* sp.n. (Plate I, 32–38, Plate III, 3). Host: *Lepidocyrtus cyaneus* Tullberg 1871 (Fam. Tomoceridae, Entomobryomorpha, Collembola). Locality: Mixed-leaf forests, Bergen (Lower Saxony, Federal Republic of Germany), 1978.

The wide oval spores measuring 3.5–4.0 by 2.5–2.8 µm have a very thick wall, which appeared to be 0.3 µm thick in Giemsa stained smears. The spores were single in the remains of the fat body. Only a few details of the morphology of the interior were visible. After HCl-treatment two well staining nuclei were

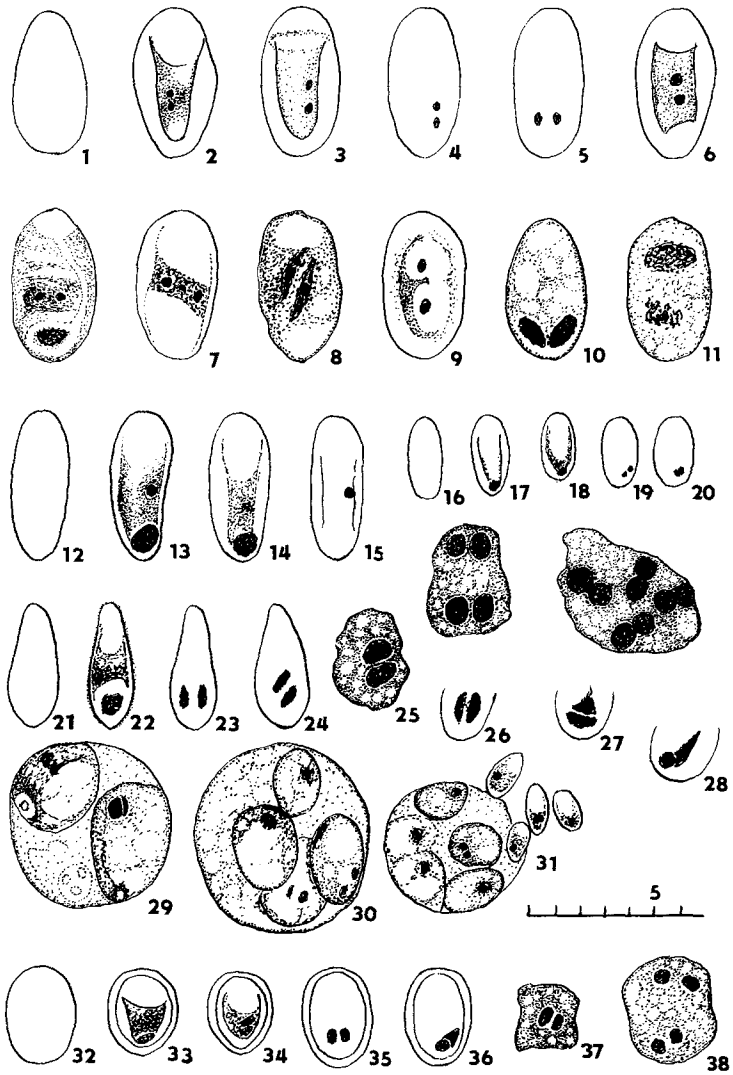

Plate I, Figs. 1–38. Figs. 1–11. *Nosema lepidocyrti:* **1** fresh spore, **2, 3** Giemsa stain, **4, 5** nuclei in spores, **7–11** different stages in spore maturation. **Figs. 12–15.** *Encephalitozoon flavescens:* **12** fresh spore, **12, 14** Giemsa stain, **15** nucleus in a spore after HCl-treatment. **Figs. 16–20.** *Nosema onychiurus:* **16** fresh spore, **17, 18** Giemsa stain, **19, 20** nuclear staining. **Figs. 21–31.** *Auraspora canningae:* **21** fresh spore, **22** Giemsa stained spore, **23, 24, 26, 27, 28** different types of nuclei in spores, **29, 30, 31** anomalous sporogony. **Figs. 32–38.** *Nosema petrosa:* **32** fresh spores, **33, 34** Giemsa stained spores, **35, 36** nuclei inside the spores, **37, 38** sporonts

visible (Plate I, 32–38). In Giemsa stained preparations of spores not hydrolysed with HCl the nuclei were hidden behind a more distinct posterosome. The nuclei were usually of different size and shape, one was more rounded and the other rod-shaped or spheroidal. Only binucleate sporonts or late schizonts and quadrinucleate early schizonts could be identified on smears.

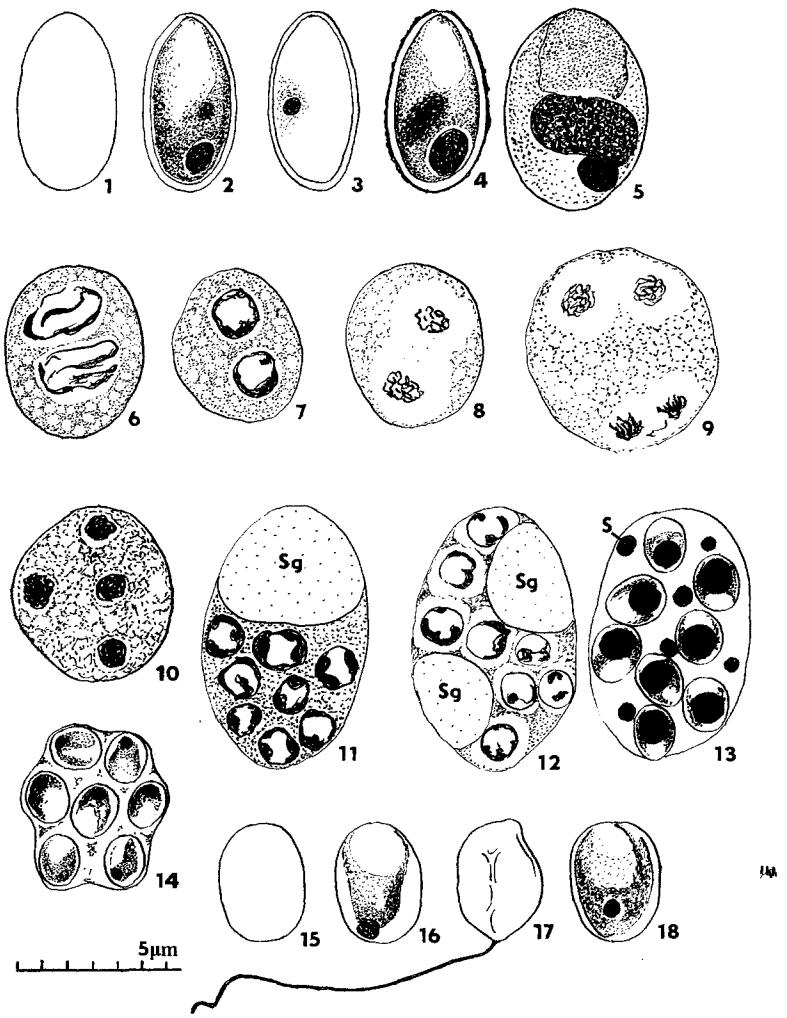

Plate II, Figs. 1–18. Figs. 1–14. *Thelohania collembolae:* **1** fresh spore, **2, 4** Giemsa stained spores, **3** nuclear staining, **5** sporoblast with visible nucleus and dark posterosome, **6–10** early sporogonial stages, **11–12** plasmodia with secretion granules (*Sg*), nuclei of future sporoblasts concentrated in the other stages; **13** pansporoblast with young spores. Secretory granules changed into spherical siderophilic granules, **14** mature octosporous pansporoblast. **Figs. 15–18.** *Thelohania bomboschi:* **15** fresh spore, **16** Giemsa stained spore, **17** spore with extruded polar filament, **18** staining of the nucleus inside the spore

4. *Encephalitozoon flavescens* sp.n. (Plate I, 12–15, Plate III, 4). Host: *Tomocerus flavescens* Tullberg 1871 (Fam. Tomoceridae, Entomobryomorpha, Collembola), muscles. Locality: Pure beech and mixed-leaf forests. Wünneberg and Bergsdorf (Westphalia, Lower Saxony, Federal Republic of Germany), 1978.

In smears of springtails infected with *Thelohania collembolae* sp.n. (see below) there were scattered muscle strands with spores distributed singly or in groups of irregular number. Spores did not produce digestive vacuoles around them; they were all oriented more or less longitudinally; and only maturing and

mature spores were present. Spores are elongated and tubular, 5.5–6.0 μm long and 2.5 μm wide. In Giemsa stained smears a clearly visible posterosome, stained vividly red, spherical, and 0.5–0.8 μm in diameter was located at the furthest point of the posterior end of the sporoplasm. The anterior polaroplast vacuole was clearly visible. After HCl-hydrolysis only one spherical, very small (0.4 μm) nucleus was stained in the centre of the spore, slightly laterally. It is impossible at the moment to place this microsporidian correctly into a genus and our localisation of it in the genus *Encephalitozoon* is provisional due to the uninucleate spore which appeared to be single without any visible covering membrane.

5. *Thelohania bomboschi* sp.n.[1] (Plate II, 15–18, Plate III, 5, 6, 11). Host: *Tomocerus flavescens* Tullberg 1871 (Fam. Tomoceridae, Entomobryomorpha, Collembola), fat body. Locality: Mixed-leaf forests, Bergsdorf, Rosengarten, and Wildpark (Lower Saxony, Federal Republic of Germany), 1978.

This microsporidian fills the lobes of the fat body. Single octosporous pansporoblasts were usually 16 μm in diameter with the spores in an irregular cluster, stuck togehter with the remains of the cytoplasm of the plasmodium. Young pansporoblasts or younger sporonts were not observed. Prosporoblasts when separated singly from the cluster, showed a well-formed posterosome, a large nucleus, and a faintly stained polaroblast filling the anterior third of this stage.

The fine structure of young stages (sporoblasts) is presented in Plate III, Fig. 11. Ultrathin sections of mature sporoblast indicate that the thickness of the surface membrane (*m*) increases with maturity. Fine granular sporoplasm appears to be evenly distributed inside the cells, including the irregularly shaped nuclei (*n*), which are covered by a double membrane. A portion of some granular, more electron-dense bodies in the centre of the nucleus is also evident. Formation of a polar filament (*f*) occurs in the posterior part of the sporoblasts, while vacuoles are irregularly scattered in the sporoplasm.

Light microscopy shows that late maturating spores have an oval, well staining red posterosome situated slightly laterally in the posterior end. The spore wall is thick, refringent, and 0.3 μm in cross-section. On its surface are plasmatic coagula, staining red with Giemsa. Nuclear staining with HCl demonstrates one round nucleus 0.5 μm in diameter, slightly to one side of the centre of the spore. Dimensions of the spores are 6–7 by 4.0–4.5 μm.

6. *Thelohania collembolae* sp.n. (Plate II, 1–14, Plate III, 7, 8, 9). Hosts: *Tomocerus flavescens* Tullberg 1871, and *Lepidocyrtus lignorum* Fabricius (Fam. Tomoceridae, Entomobryomorpha, Collembola), fat body. Locality: Mixed-leaf and coniferous forests, Wünneberg, Rosengarten (Westphalia, Lower Saxony, Federal Republic of Germany), 1978.

The stages in sporogony of this microsporidian are very common in all infected animals. The youngest stages were binucleate sporonts with large nuclei and chromosomal figures. The nuclei divide and produce quadrinucleate and octonucleate stages. The early octonucleate plasmodia are oval and during the subsequent formation of future sporoblasts around the nuclei a large secretory granule is formed in the cytoplasm. This occurs inside the persisting pansporoblastic membrane. Later during this process two to three large granules are

[1] The *Thelohania bomboschi* sp.n. is dedicated to Prof. Dr. Siegfried Bombosch, Head of the Institute of Forest Zoology, University of Göttingen

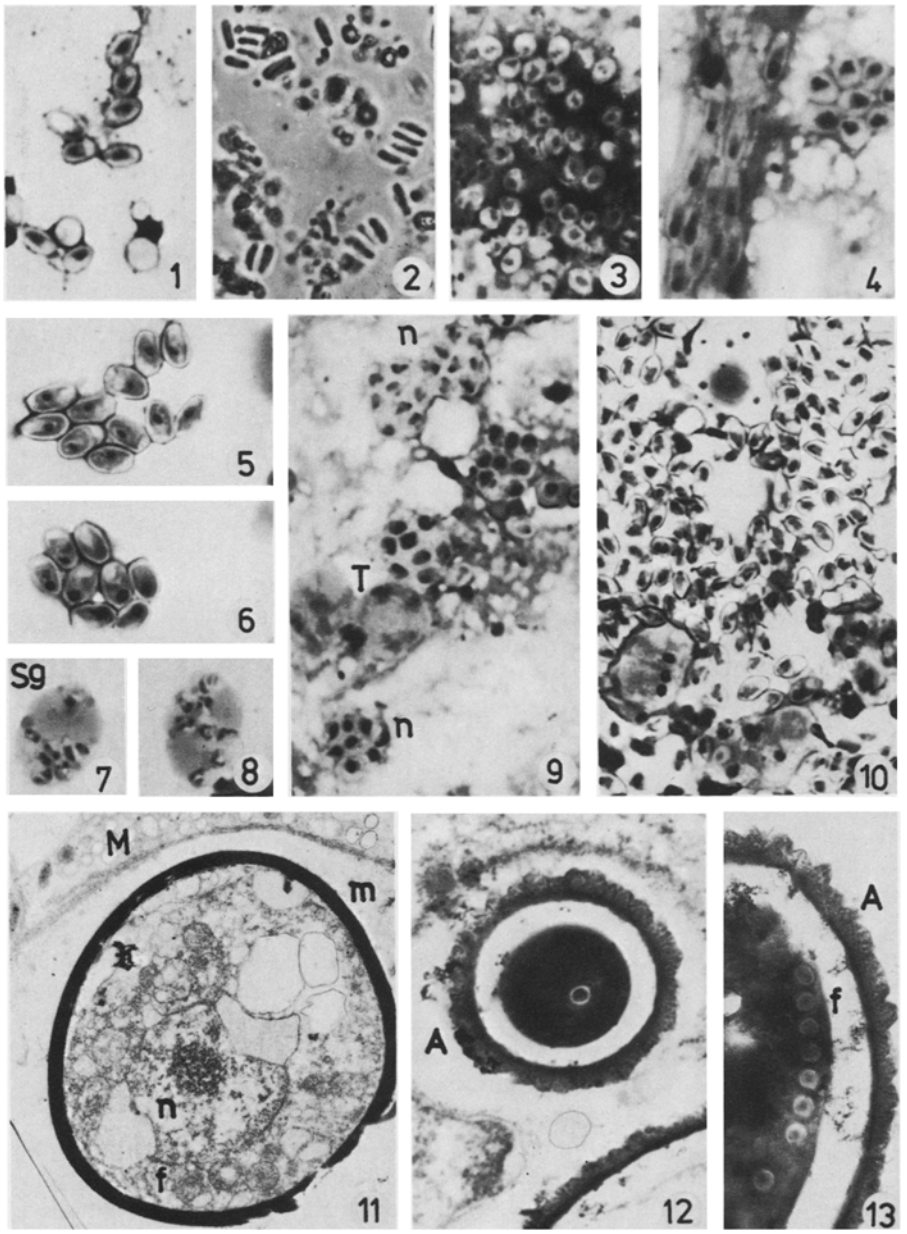

Plate III, Figs. 1–13. Figs. 1–10. 1, 3–10. Giemsa stain, **2** fresh, ca. ×4,000. **Figs. 11–13.** Electron microscopy, **11** ×14,200, **12** ×30,000, **13** ×60,000. **Fig. 1.** *Nosema lepidocyrti:* mature spores. **Fig. 2.** *Nosema petrosa:* mature spores. **Fig. 3.** *Nosema onychiurus:* mature spores. **Fig. 4.** *Encephalitozoon flavescens:* mature spores, and *T. collembolae:* pansporoblast. Mixed infection. **Figs. 5, 6.** *Thelohania bomboschi:* mature spores. **Figs. 7–9.** *Thelohania collembolae:* **7, 8** plasmodia with secretory granules (*Sg*), **9** tetranucleate plasmodia (*T*) and pansporoblasts. In pansporoblasts after HCl-hydrolysis the rod-shaped nuclei (*n*) and siderophilic granules are evident. **Fig. 10.** *Auraspora canningae:* plasmodia and mature spores. **Fig. 11.** *Thelohania bomboschi:* sporoblast, showing the membrane of pansporoblasts (*M*), membrane of sporoblasts (*m*), the nucleus (*n*), and formation of polar filaments (*f*). **Figs. 12, 13.** *A. canningae:* ultrathin sections of the spore showing the episporal aura (*A*), the thick endosporium, and cross-section of the polar filament (*f*)

formed, with staining characteristics analogous to those of *Thelohania fibrata* in blackflies (Maurand and Bouix, 1969), where granules appear in the same period of development. Chromatin clumps in nuclei are concentrated on nuclear walls. Later the secreted masses disappear during spore maturation. At the stage when prominent posterosomes are present in pansporoblasts, there are spherical siderophilic granules among the spores; usually less than eight in each pansporoblast. Finally when the spores are mature, the pansporoblast wall closes an empty space and shrinks around the spores which are held together irregularly. The oval early pansporoblasts (plasmodia) are 16–18 µm long and 10–16 µm wide, their secretory granules take up about half of the oval body. The final pansporoblast with maturing spores is almost spherical, 12 µm in diameter. As a rule there are not free spores outside the pansporoblasts except after intensive smearing of the body content. Spores are wide, oval, thin-walled, and are sometimes deformed after drying to the slide. The cone of the germ inside the spore has a broad anterior vacuole and a spherical posterosome at the posterior end. After HCl-treatment one spherical nucleus, 0.5–0.8 µm in diameter is stained in the posterior third of the spore. The polar filament which was sometimes extruded during preparation of the smears is short, 10 to 15 µm long. The secretory bodies are dissolved during the HCl-hydrolysis for nuclear staining and instead of one body several round siderophilic spherules are formed, similar to these appearing in late sporogony. Mature spores when free from pansporoblasts are broad, oval, thin-walled, 4.5–4.8 µm long, and 3.5–3.8 µm wide.

7. *Auraspora canningae* gen. et sp.n.[2] (Plate I, 21–31, Plate III, 10, 12, 13). Host: *Lepidocyrtus lignorum* Fabricius 1781. Gonads of males. Locality: Mixed-leaf forests, Bergsdorf and Wildpark (Lower Saxony, Federal Republic of Germany) 1978.

This microsporidian has two sequences of developmental stages which differ so much that it is difficult to connect them together in any logical way. Both sequences appear in all cases collected. The sporogonial sequence corresponds to the morphology of a *Nosema:* scarce oval sporonts or sporoblasts, ending in piriform spores with both ends slightly constricted. The spores are thin-walled and during drying on smears they undergo different deformations. They are 4–5 µm long and 2–2.5 µm wide with a visible round posterosome, 0.5 µm in diameter. In some cases a faint granule stained red with Giemsa appears at the fixing point of the polar filament in the anterior end. The anterior (polaroblast) vacuole is not very deep, taking up less than one third of the spore length. When treated with HCl and stained for nuclei, all mature spores show two well stained nuclei. These nuclei are elongated, 0.5 µm long and 0.2–0.3 µm wide, and are situated in the posterior third of the spore but in different positions. In many cases one nucleus is hemispherical and the other nucleus, which is cone-shaped, sits on its flat side. Of the stages which precede the mature spore, binucleate sporoblasts, quadrinucleate and octonucleate plasmodia can be observed, but the number and frequency does not correspond to the number

[2] The *Auraspora canningae* gen.n. sp.n. is dedicated to Dr. Elizabeth Canning, Imperial College Field Station, Ascot (London), Berks., England

of maturing spores and the infection must have an early stage where the classical line of development changes to the anomalous line.

The anomalous line appears with different stages scattered among the spores. It is represented by spherical stages in a common membrane of uniform, or more often of different size. There are giant sporonts with very small (in relation to their size) nuclei. The round pansporoblasts of this series were 15–20 µm in diameter, the oval stages in pansporoblasts with two stages were 10×5–7 µm long, those with four oval stages were 6–7×4–5 µm, and in cases with more than four "sporoblasts", the oval bodies were about 5 µm long. We never found a clear final series where sporoblasts and spores appeared in any regular number in the pansporoblasts.

It is possible that the anomalous series is a teratogenic byproduct of the development of the microsporidian. However it is strange that the teratogenic sequence appears in every animal infected with the "normal" series. The ultrathin sections of the spores of this microsporidian show thick-walled spores with a thin exosporium and thick endosporium. On the surface of the exosporium there is a foamy layer of an episporal secretion (aura in Latin, the original root of the proposed generic name). The polar filament with 8–9 coils is of equal thickness throughout its length; the polaroplast is of the normal lamellate structure.

The definition of the proposed new genus *Auraspora* is as follows: Sporogony of the normal series with thick-walled, piriform spores, with a surface covering of foamy episporal secretion, binucleate, with prevailing dimorphy of the nuclei, spores mature singly. The second sequence of sporogony with teratological uninucleate sporoblasts is in the pansporoblast.

Discussion

There are no data in the literature concerning microsporidia attacking Collembola and this series of observations fills this gap and describes seven more common species. In general, most species are morphologically identical with similar genera in other arthropods. The material was collected only as dry smears and due to the rare occurrence of the infected specimens and their very small size, it was not possible to prepare ultrathin sections and follow the development of the stages in the electron microscope. Therefore all possible morphological diagnostic markers, especially in spore structures, were used to support the descriptions.

In three instances the picture differed: In the case of *"Encephalitozoon"* only the organisation of the spore with one nucleus characterizes the affiliation of this species with the genus and there is no information concerning the nuclear conditions in schizogony and sporogony before sporulation. Spores are single when mature and this is also typical for the genus. *Thelohania collembolae,* a second aberrant form, belongs, due to its plasmatic secretions during sporogony, to a group of microsporidia characterized in the same way as *Thelohania fibrata, T. bracteata,* or *T. varians* in blackflies. The mucopolysaccharides which are formed there, are a product of differentiation of plasmodia into prosporo-

blasts and at the end of that period they disappear or are reduced and reabsorbed in the maturing sporoblasts. The plasmatic secretions increase the pansporoblastic membrane during this development and so the membrane is visualized during that stage, but the membrane must also be present during pansporoblast formation by this system.

The situation with *Auraspora* is rather complicated. The binucleate spore with nuclei of different shapes resembles the arrangement of Culicosporidae, and in this family two types of spores are formed after two types of sporogonial development (Weiser, 1977). Against the affiliation with this family is the formation of a minor, lamellate polaroplast, formation of an isofilar polar filament, and the absence of mature spores of different organisation in the second morphological sequence. Compared with *Variiomorpha,* another bi-morphic genus, there are binucleate spores but the nuclei are different and there are no uninucleate spores in any octosporous pansporoblast.

Further investigation of the diseases of Collembola should concentrate on ultrastructural features of the microsporidia.

Acknowledgements. The authors wish to thank Dr. Dr. habil. W. Dunger, State Museum of Natural History, Görlitz, German Democratic Republic for the determination of Collembolan materials, Prof. Dr. F. Mayer, Institute of Microbiology, Göttingen, and U. Hofacker, Institute of Forest Zoology, Göttingen, for their help during the electron microscope studies.

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Received May 8, 1979