Europ. J. Protistol. 32, 116–122 (1996)
February 23, 1996

European Journal of
PROTISTOLOGY

Light and Electron Microscope Study on *Gurleya daphniae* sp. nov. (Microspora, Gurleyidae), a Parasite of *Daphnia pulex* (Crustacea, Phyllopoda)

Christoph Friedrich[1], Otmar Winder[1], Karin Schaffler[2], and Franz F. Reinthaler[2]

[1]*Institute of Zoology, Karl-Franzens-Universität Graz, Austria*
[2]*Institute of Hygiene, Karl-Franzens-Universität Graz, Austria*

SUMMARY

The life cycle of a new microsporidian species parasitizing in the epidermis of the cladoceran *Daphnia pulex* from the southeast region of Austria is described using light and electron microscopical methods. Merogonial stages have one or two single nuclei. Sporonts produce the sporophorous vesicle and normally develop into lobed sporongial plasmodia with four single nuclei. These plasmodia divide into four monokaryotic sporoblasts. The envelope of the sporophorous vesicle is of about the same thickness as the plasma membrane. Spores are pyriform, monokaryotic and measure between 3.8 × 2.3 and 4.6 × 2.6 µm. The polaroplast is composed of two parts. The anterior part is lamellar, the posterior part consists of sac-like structures. The polar filament is anisofilar and consists of 3–4 thick and 2–5 narrow coils. The spore wall is up to 415 nm thick and composed of three layers. The episporontal space contains tubules and fibres. Abnormal spores with regard to size, shape and number within the envelope frequently can be observed. Because of the differences to microsporidian species previously reported from the epidermis of cladocerans the parasite of *Daphnia pulex* is described as a new species and named *Gurleya daphniae*.

Introduction

In 1898 Doflein published a short description of *Gurleya tetraspora*, a microsporidium parasitizing the hypodermal tissue of *Daphnia maxima* [2]. About four decades later Jirovec also found a microsporidium producing four species within a sporophorous vesicle in the hypodermis of *Moina rectirostris* [4]. He supposed a misidentification of the host species by Doflein and regarded the parasites to be identical. Since this time only one *Gurleya* sp. of a cladoceran host was briefly described [3].

In 1989 during the period without ice on water two populations of the cladoceran *Daphnia pulex* were found to be infected by several species of microsporidia. One of these species was located in the epidermis of the host and showed sporophorous vesicles normally containing four spores. Life cycle stages and ultrastructure are described and the new species is compared with the previously found *Gurleya tetraspora*.

Material and Methods

The microsporidium was present in *Daphnia pulex* (de Geer), collected from March to December 1989 in two little ponds of a former brickyard and in July 1993 in a flooded meadow in the east of Graz in the southeast region of Austria. For experiments of infection cladocerans were reared in glass containers and fed on a suspension of yeast.

0932-4739-96-0032-0116$3.50-0

Impermanent squash preparations of infected tissue were stained with orcein-acetic acid-fastgreen FCF according to Kurnick and Ris [1] and revealed presporal developmental stages in peculiar quality. Entire specimens were smeared on microscope slides, airdried, fixed in methanol for half an hour and stained in Giemsa solution. To obtain sizes of fixed spores mean measurements of 30 spores of some hosts were made. Other specimens were fixed in Heidenhain's Susa (trichloracetic acid, mercury II chlorid, formaldehyde) for one day, dehydrated and embedded in paraplast. Sections were stained in Mayer's hemalum solution and eosin.

For electron microscopy infected tissue was fixed in 4% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for some hours. After washing in phosphate buffer and postfixation (overnight) in a 1:1 mixture of Heidenhain's Susa and Bouin's fixative (2.4.6-trinitrophenole, formaldehyde, acetic acid) which was diluted with distilled water the tissue was dehydrated in increasing concentrations of ethanol and embedded in LR White with accelerator. Ultrathin sections were stained with uranyl acetate and lead citrate.

Results

Pathology

The microsporidium developed in the epidermis (Fig. 4) of *Daphnia pulex* and caused a white colouration of the host organism visible to the naked eye. Early stages of infection were recognized from small white points especially in the posterior part of the body. From these foci the parasites spread out, the infected areas became greater and fused together. Completely white *Daphnia* specimens were rare because most of the infected animals died when about one half to two thirds of their shell surface was white. Apart from exceptional cases only *Daphnia* specimens distinctly longer than 1 mm originating from the ponds were parasitized. In infection experiments the microsporidium could be transmitted to other *Daphnia pulex* specimens. For this, parasitized cladocerans were crushed and slowly dispersed in water containing *Daphnia pulex* from a microsporidian-free culture.

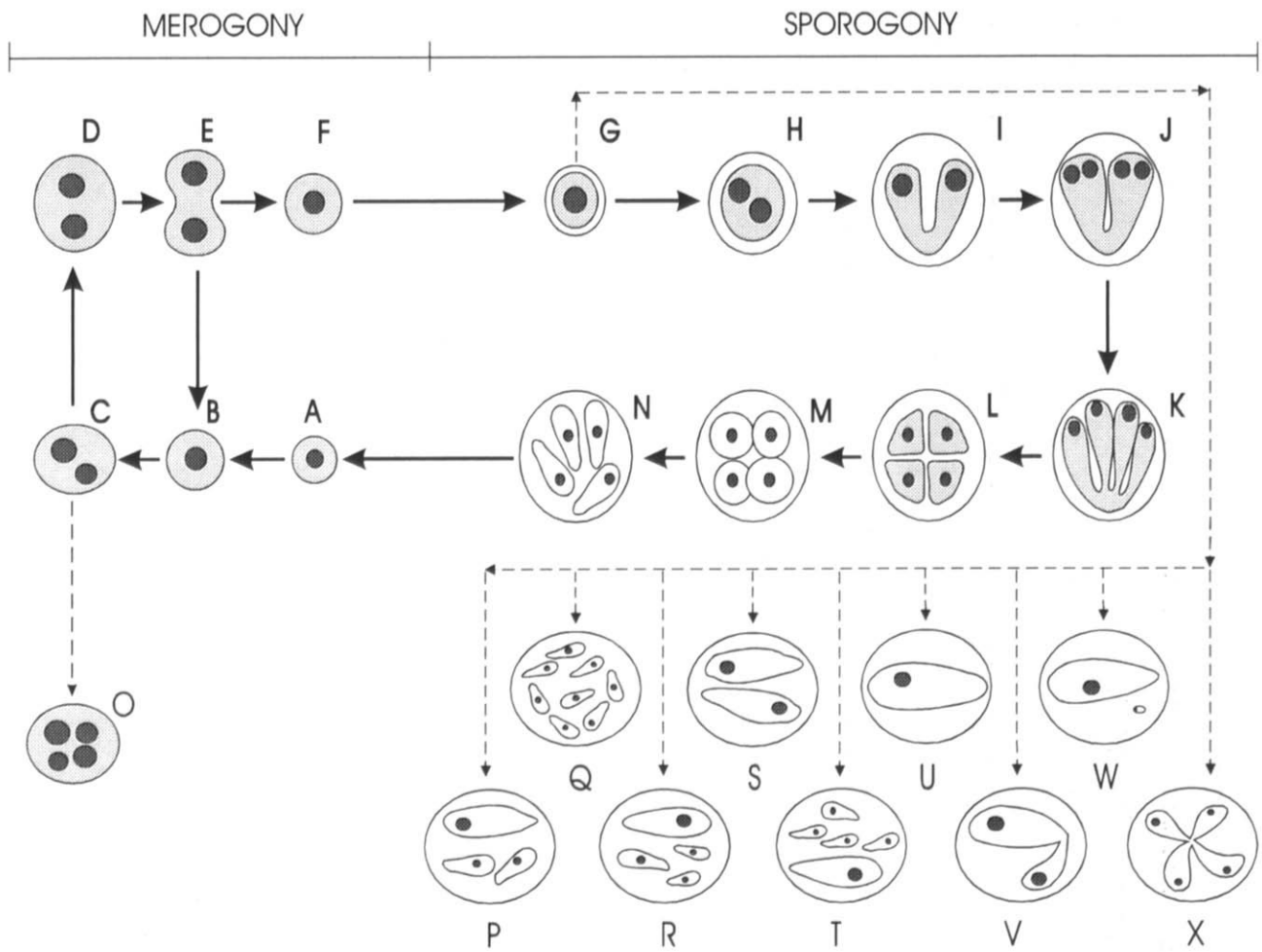

Fig. 1. Sketch of the life cycle of *Gurleya daphniae* sp. nov. A–F. Merogonial stages. G–N. Sporogonial stages. O–X. Final stages of anomalous development.

Light Microscopic Appearance of the Life Cycle

After application of mechanical pressure spores of fresh smears extruded their polar filaments. In some cases a little swelling which might have been caused by the sporoplasm was recognized near the end of the extruded polar tube.

The most immature developmental stages observed in squash preparations on *Daphnia pulex* were rounded monokaryotic cells with diameters between 2.6 and 4 µm (Figs. 1A and 1B). Before division into two monokaryotic cells (Fig. 1F) they changed from a rounded to an oval and consecutively dumb-bell-like shape (Figs 1C, 1D, 1E). Sometimes voluminous rounded plasmodia with four nuclei were found (Fig. 10. No signs of fragmentation of these plasmodia could be observed.

At the beginning of the sporongy an envelope, the sporophorous vesicle, was formed around the monokaryotic rounded sporont (Fig. 1G). These sporophorous vesicles measured 5–9.3 µm in diameter. A nuclear division (Fig. 1H) was followed by cytoplasmic fission and resulted in a two-lobed plasmodium with two isolated nuclei (Fig. 1I). Sporophorous vesicles of these plasmodia were slightly oval or rounded. Further nuclear divisions took place at the ends of the two-lobed plasmodia (Fig. 1J) and were also followed by cytoplasmic fissions resulting in four-lobed plasmodia, each lobe containing a single nucleus (Fig. 1K). These sporogonial plasmodia divided into four monokaryotic sporoblasts of irregular and later rounded shape (Figs. 1L and 1M). They developed into four monokaryotic pyriform spores (Figs. 1N, 2 and 3) with a smooth surface looking somewhat angular at the anterior end. Mature sporophorous vesicles were round with up to 10.5 µm in diameter, or oval, measuring 10.2–11.2 × 8.6–9.3 µm. Measurements taken from smears revealed a variation in the sporesizes from parasitized *Daphnia pulex* of the same pond depending on water temperature. The mean spore-size at a water temperature of 18 °C (July 1989) was 4.6 × 2.6 µm, at a water temperature of 4 °C (November 1989) it was 3.8 × 2.3 µm.

Sporophorous vesicles from hosts caught at the same time were sometimes found to contain only one great spore (up to 13.9 × 5.3 µm, Fig. 1U), two spores of the same size (7.3 × 3.4 µm, Fig. 1S) or eight small spores of the same size (3.3 × 1.6 µm, Fig. 1Q) instead of four equivalent spores (4.6 × 2.6 µm) which was most frequently seen. Spores of different shape and irregular size as well as corresponding presporal stages were not unusual. There existed sporophorous vesicles containing one very large and one small spore (Fig. 1W), one large and two small spores (Fig. 1P), one large and four small spores (Fig. 1T), or one large, one medium-sized and two small spores (Fig. 1R). Sometimes spores connected two by two or four by four were observed (Figs. 1V and 1X). Abnormal spores with regard to shape and number of appearance seemed to be more numerous in cladocerans kept in containers at high abundance and at room temperature (about 20 °C) than *Daphnia* specimens obtained from the ponds.

Ultrastructural Investigation

Preparation of parasitized *Daphnia pulex* for electron microscopy encountered difficulties. Usual fixation in glutaraldehyde and embedding in Epon or Spurr did not lead to acceptable results. Therefore some experiments were made applying LR White which was just at disposal and resulted in the method mentioned above. Ultrathin sections were not of best quality and revealed destructions because of shrinkage, but observations made with the light microscope (merogonial and sporogonial stages, no diplokarya) were confirmed.

Merogonial life cycle stages showed one or two isolated nuclei (Fig. 5). Sporonts (Fig. 6) also were

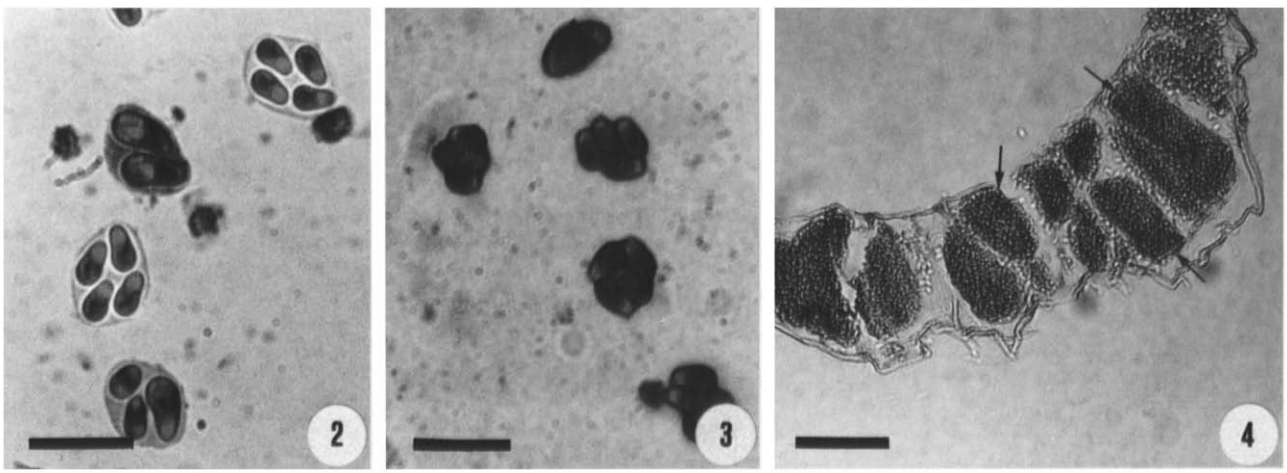

Fig. 2.–4. Light microscopic aspect of spores and parasitized tissue. – Fig. 2. Sporophorous vesicles containing two, three or four spores (squash preparation). – Fig. 3. Sporophorous vesicles containing one or four spores (Giemsa-stained smear). – Fig. 4. Section of host epidermis packed with masses of parasitic stages (arrows). Scale bars: Figs. 2–3 = 10 µm; Fig. 4 = 50 µm.

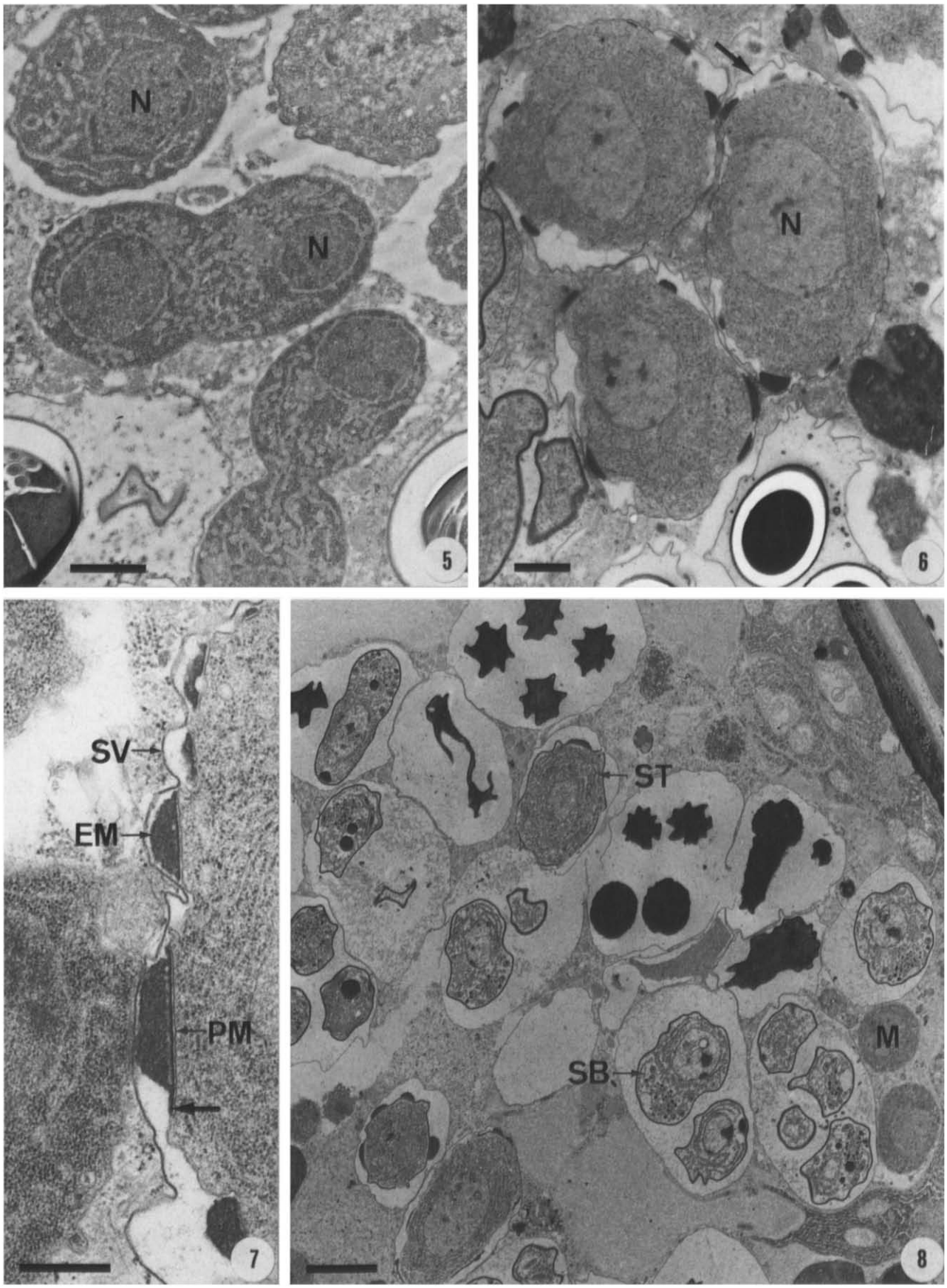

Fig. 5.–8. Ultrastructure of some developmental stages. – Fig. 5. Merogonial stages with isolated nuclei. – Fig. 6. Monokaryotic sporonts surrounded by the envelope of the sporophorous vesicle (arrow). – Fig. 7. Secretions of granulated electron-dense material on the surface of a sporont and initiation of the exospore (arrow). – Fig. 8. Different developmental stages of *Gurleya daphniae* sp. nov. within the host epidermis. EM = electron-dense material, M = merogonial stage, N = nucleus, PM = plasma membrane, SB = sporoblast, ST = sporont, SV = envelope of the sporophorous vesicle. Scale bars: Figs. 5–6 = 1 μm; Fig. 7 = 0.5 μm; Fig. 8 = 3 μm.

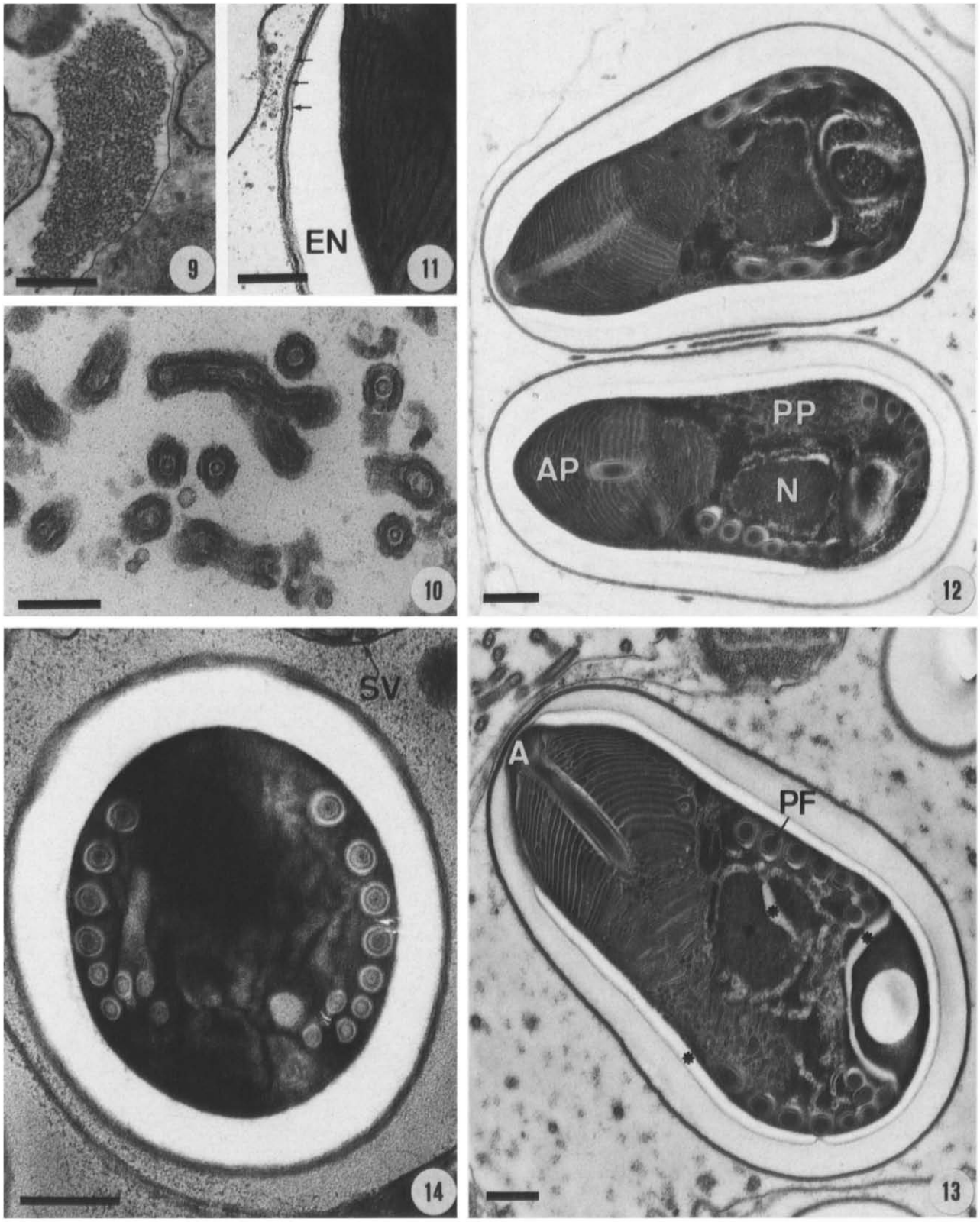

Fig. 9.–14. Spores and structures of the episporontal space. – Fig. 9. Fibrous material within the episporontal space. – Fig. 10. Tubules between sporoblasts or spores and the envelope of the sporophorous vesicle. – Fig. 11. Wall of a mature spore. The exospore consists of three layers marked by arrows. – Figs. 12.–13. Longitudinally sectioned spores. Destructions (asterisks) are effects of shrinkage. – Fig. 14. Spore section revealing the anisofilar polar filament consisting of at least eight layers. A = anchoring disc, AP = anterior polaroplast, EN = endospore, N = nucleus, PF = polar filament, PP = posterior polaroplast, SV = envelope of the sporophorous vesicle. Scale bars: Fig. 9 = 1 µm; Fig. 10 = 200 nm; Fig. 11 = 250 nm; Figs. 12–14 = 0.5 µm.

monokaryotic and produced the envelope of the sporophorous vesicle. During development of the sporont accumulations of electron-dense granulated material (Fig. 7) were deposited on the surface of the plasmamembrane and the secretion of the exospore began at specific points. Sometimes the electron-dense material was also observed in sporogonial plasmodia. Nuclear divisions led to formation of plasmodia which divided into monokaryotic sporoblasts (Fig. 8). Fibrous material (Fig. 9) and tubules (Fig. 10) were observed between the envelope of the sporophorous vesicle and sporoblasts or spores.

Mature tetraspores (Figs. 12, 13 and 14) contained an anisofilar polar filament with 6–9 coils which were mostly arranged in a single layer in the posterior half of the spore. In the posterior region of the spore the sections sometimes could form a second layer. At least eight concentrically arranged subunits could be distinguished in transverse sections of polar filament coils. In some spore-sections the filament seemed to widen successively from the posterior to the anterior coils increasing with a third to a half in width (Fig. 13). Posterior coils measured 95–140 nm, anterior coils 190–215 nm in diameter. Other sections revealed a constriction of the polar filament dividing this organelle into 3–4 thick and 2–5 narrow coils (Fig. 14). The polaroplast (Figs. 12 and 13), composed of two parts, proceeded far in posterior direction and ended about one fifth from the posterior pole. The anterior part consisted of lamellae which seemed to be anastomosing at some points. The posterior part was vesicular. The nucleus was not exactly located in the centre of the spore. The posterosome region as well as the nucleus, the zone around the polar filament, and the spore wall were seriously damaged by shrinkage (Figs. 12 and 13). The smooth spore wall exhibited three layers (Fig. 11): the internal approximately 10 nm thick plasma membrane, the electron-translucent endospore with a thickness of up to 350 nm in the posterior region of the spore but very thin at the anterior pole and the exospore consisting of three different zones totally measuring 45–55 nm. The internal layer of the exospore was moderately electron-dense and seemed to be limited against the endospore by a thin more electron-dense zone. The median clearly separated double-layer was covered with a less electron-dense surface layer.

Discussion

The microsporidian described in this paper belongs to the genus *Gurleya*. Criteria are the mode of division during sporogony, the shape of the spores, the number of spores within sporophorous vesicles, the number of nuclei in different developmental stages, and the absence of diplokaryotic life cycle stages [5]. Only the anisofilar polar filament and the construction of the polaroplast do not correspond to Larsson's postulation in his identification key [5].

Divergences concerning spore sizes, spore shapes and the number of spores within sporophorous vesicles were already reported for a *Gurleya* sp. parasitizing *Macrocyclops albidus* [8].

Spore sizes can differ within one host species as mentioned by Larsson who found different spore sizes of *Thelohania muelleri,* a parasite of *Rivulogammarus pulex,* collected from different localities [6]. According to Maddox and Luckmann microsporidian spore sizes may depend on environmental temperature, a fact confirmed by our investigation [7].

In 1974 Green [3] briefly described *Gurleya vavrai* form *Daphnia longispina* without mentioning tissue specificity of this microsporidium.

Microsporidian species named *Gurleya tetraspora* were described Doflein [2] and Jirovec [4]. Doflein found a microsporidian species with sporophorous vesicles containing four oval spores in the hypodermal tissue (epidermis) of *Daphnia maxima* in Bavaria. Infected host specimens showed brownish patches. The spore surface was covered with longitudinal grooves which could be interpreted as artifacts of preparation [9]. Jirovec discovered a microsporidian species with tetrasporous sporophorous vesicles in hypodermal cells of *Moina rectirostris* in Bohemia. Spores were rounded at one end and slightly tapering towards the other end. They measured 2.8–3.4 × 1.4–1.6 µm. Infected hosts were milky white. Jirovec did not mention the grooves observed by Doflein. The present species was found in the epidermis of *Daphnia pulex*. Pyriform spores are of different size and do not have ornamented surfaces. Infected hosts also are white.

The species of this investigation, as well as the species described by Doflein and by Jirovec infect the same tissue but different host species [2, 4]. There exists a clear difference between the spores found in *Daphnia maxima* and the spores out of *Moina rectirostris* and *Daphnia pulex* supposing that the longitudinal grooves of the spore surfaces of *Gurleya tetraspora* described by Doflein were no artifacts. *Daphnia maxima* infected by *Gurleya tetraspora* showed brownish patches whereas both *Gurleya*-infected species, *Moina rectirostris* and *Daphnia pulex*, were white. Spore shapes described by Doflein and Jirovec are slightly different from *Gurleya* sp. found in *Daphnia pulex*.

Because of the differences to the microsporidian species described by Doflein and Jirovec the parasite found in *Daphnia pulex* in the ponds at Graz is considered to be a new species and named *Gurleya daphniae* sp. nov..

Description: Gurleya daphniae sp. nov.

Presporal stages. Merogonial stages with one or two single nuclei; sporonts produce sporophorous vesicle and normally develop into lobed sporogonial plasmodia with four single nuclei; sporogonial plasmodia divide into four monokaryotic sporoblasts.

Spores. Monokaryotic, pyriform and looking somewhat angular at the anterior end; dimensions (fixed and

Giemsa-stained) from 3.8 × 2.3 to 4.6 × 2.6 µm (depending on water temperature); spore wall up to 415 nm in diameter, triple-layered exospore; polar filament anisofilar with 3–4 thick and 2–5 narrow coils; anterior region of the polaroplast lamellar, posterior part with sac-like structures; nucleus approximately in the centre of the spore.

Sporophorous vesicle. Normally four pyriform spores, often anomalous concerning size, shape and number of spores within the envelope; episporontal space with tubules and fibrous material between the microsporidian cells.

Host. Daphnia pulex (de Geer) (Phyllopoda, Daphniidae).

Affected host tissue. Epidermis.

Locality. Ponds at Graz, Styria, Austria.

Deposition of slides.

Two slides (inventory numbers 3424, 3425) were deposited at the "Naturhistorisches Museum Wien" (Austria).

Deviation of name. Daphniae referring to the genus of the host organism.

Acknowledgements

We thank Prof. O. Kepka, Institute of Zoology, Prof. E. Marth and Prof. W. Sixl from Institute of Hygiene, K.-F.-University at Graz, for kind support of our investigations. Advice was given by Dr. J. I. R. Larsson and Prof. J. Vávra. The investigations were partially financed by research grants from the „Jubiläumsfonds der Österreichischen Nationalbank" (project number 4847).

References

1 Adam H. und Czihak G. (1964): Arbeitsmethoden der makroskopischen und mikroskopischen Anatomie. Gustav Fischer Verlag, Stuttgart.
2 Doflein F. (1898): Studien zur Naturgeschichte der Protozoen. II. Ueber Myxosporidien. Zoologische Jahrbücher, Abteilung für Anatomie und Ontogenie der Thiere, *11,* 281–350.
3 Green J. (1974): Parasites and epibionts of Cladocera. Transactions of the Zoological Society, London, *32,* 417–515.
4 Jirovec O. (1942): Zur Kenntnis einiger Cladoceren-Parasiten II. Zool. Anz. *140,* 281–350.
5 Larsson J. I. R. (1988): Identification of microsporidian genera (Protozoa, Microspora) – a guide with comments on the taxonomy. Arch. Protistenk. *136,* 1–37.
6 Larsson R. (1983): On two microsporidia of the amphipod *Rivulogammarus pulex* – light microscopical and ultrastructural observations on *Thelohania muelleri* (Pfeiffer, 1895) and *Nosema rivulogammari* n. sp. (Microspora, Thelohaniidae and Nosematidae). Zool. Anz., *211,* 299–323.
7 Maddox J. V. and Luckmann W. H. (1966): A microsporidian disease of the Alfalfa Weevil, *Hypera postica,* J. Invertebr. Pathol. *8,* 543–544.
8 Maurand J., Fize A., Michel R. et Fenwick B. (1972): Quelques données sur les Microsporidies parasites de Copépodes Cyclopoides des eaux continentales de la région de Montpellier. Bull. Soc. Zool. France, *97,* 707–717.
9 Weiser J. (1961): Die Mikrosporidien als Parasiten der Insekten. Monogr. angew. Entomol. *17,* 1–149.

Key words: Gurleya daphniae – Microsporidia – Development – Parasitism – *Daphnia pulex*

Address for Correspondence: Karin Schaffler, Institute of Hygiene, Universitätsplatz 4, A-8010 Graz, Austria