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Pleistophora carabidorum n. sp. (Microsporida, Microspora) —
a New Microsporidian Parasitizing Reproductive Organ
of Beetle *Amara aenea* DEG. (Carabidae, Coleoptera)

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With 35 Figures

Key words: Microsporidia; *Pleistophora carabidorum*; Coleoptera; *Amara aenea*; Natural infection

Summary

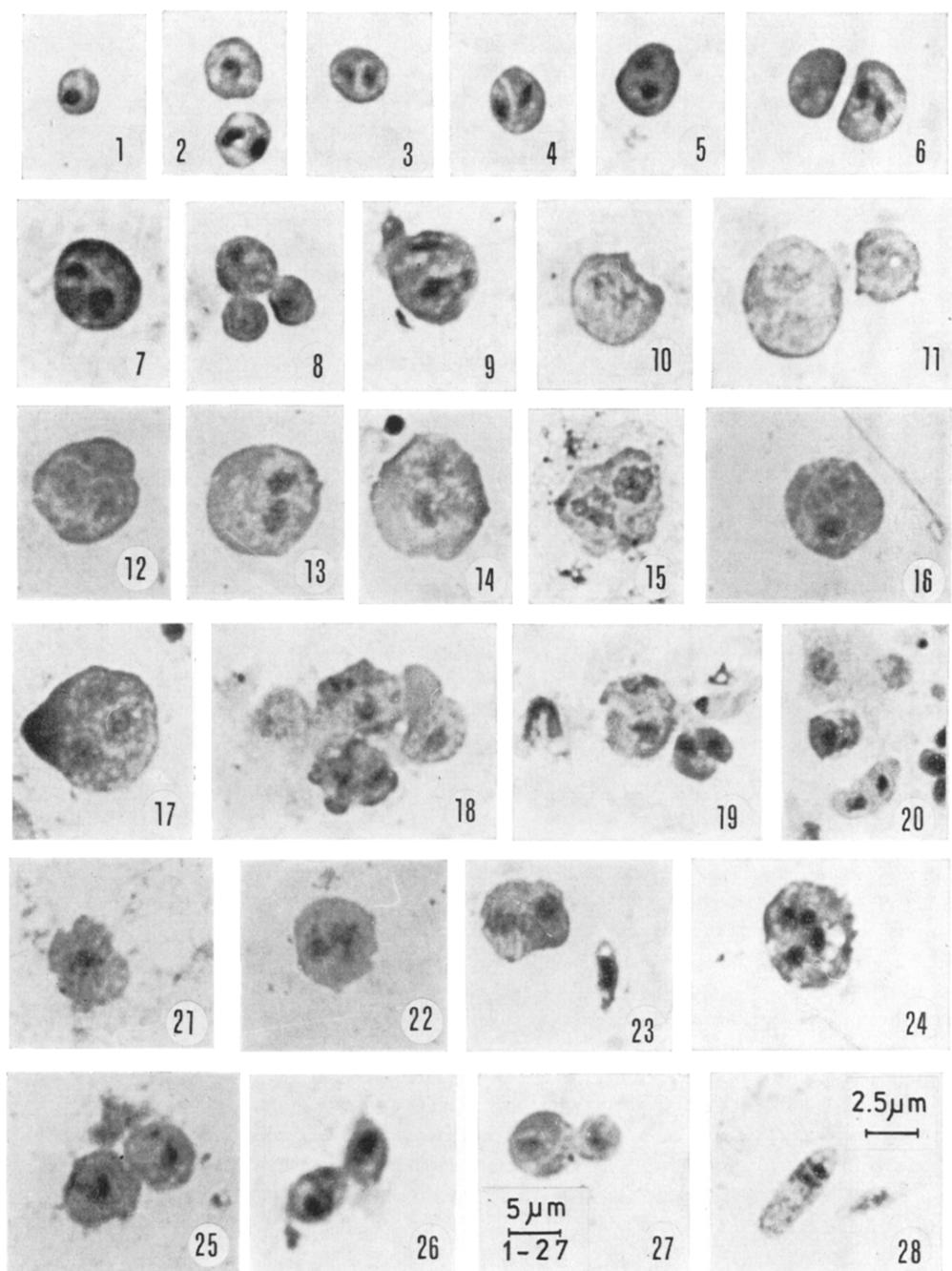
A new microsporidian species *Pleistophora carabidorum* is described from adults of the beetle *Amara aenea* collected in spruce stands in Lower Saxony (Federal Republic of Germany). The infection was localized in gonads of male animals. The parasite's life cycle incorporating some early stages (schizonts) and sporogony is described by means of light and electron microscopy. Some data on the host-parasite relationship are also discussed.

Introduction

An extensive survey of diseases of forest pests, particularly weevils such as *Brachyderes incanus* L. and *Hylobius abietis* L. (Curculionidae, Coleoptera) has recently been undertaken in Institute of Forest Zoology, University of Goettingen and Institute of Applied Zoology, Ludwig-Maximilians-University, Munich (PURRINI 1980, 1981). Some coleopterans of the families Chrysomelidae and Carabidae, among them 6 specimens of *Amara aenea* DEG. (Carabidae), were also collected during the investigation period of 1980—1982. The male gonads of two specimens of *A. aenea* were found to be infected by the microsporidian parasite but not destroyed. According to the literature, beetles of the family Carabidae are mostly predators, playing an important role in nature. Some of them, approximately 25 species, such as *A. aenea*, are phytophags. Our knowledge of some predators and gregarines as factors limiting the population density of Carabidae is based on Schwenke's Textbooks (SCHWENKE 1974). Only two species of parasitoid diptera and one of hymenoptera attacking carabids, and some species of gregarines living in the gut lumen of their hosts are known so far. This is the first report on natural infection caused by microsporidian parasites of Carabidae.

Material and Methods

A large number of adult weevils *B. incanus* (450) and *H. abietis* (806) together with 6 specimens of the beetles *Amara aenea* were collected in spruce stands and brought to the laboratories. All specimens collected were examined for parasites. The specimens of *A. aenea* were either dead or dying. The male gonads of 2 of the collected beetles were infected by a microsporidian. The material was not good for histology but dry smears were prepared for identification of the pathogen, stained with Giemsa's stain. The spores in smears were hydrolysed for the staining of nuclei after WEISER (1976). A minor fragment of the gonads was used for electron microscopy. This material



was fixed in 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.5 (2 h), and postfixed with osmium tetroxide, dehydrated in acetone series, and embedded in Spurr's low viscosity resin. Ultrathin sections were cut with a REICHERT OmU 2 ultramicrotome, stained with uranyl acetate/lead citrate, and examined with a Philips 301 electron microscope.

Results

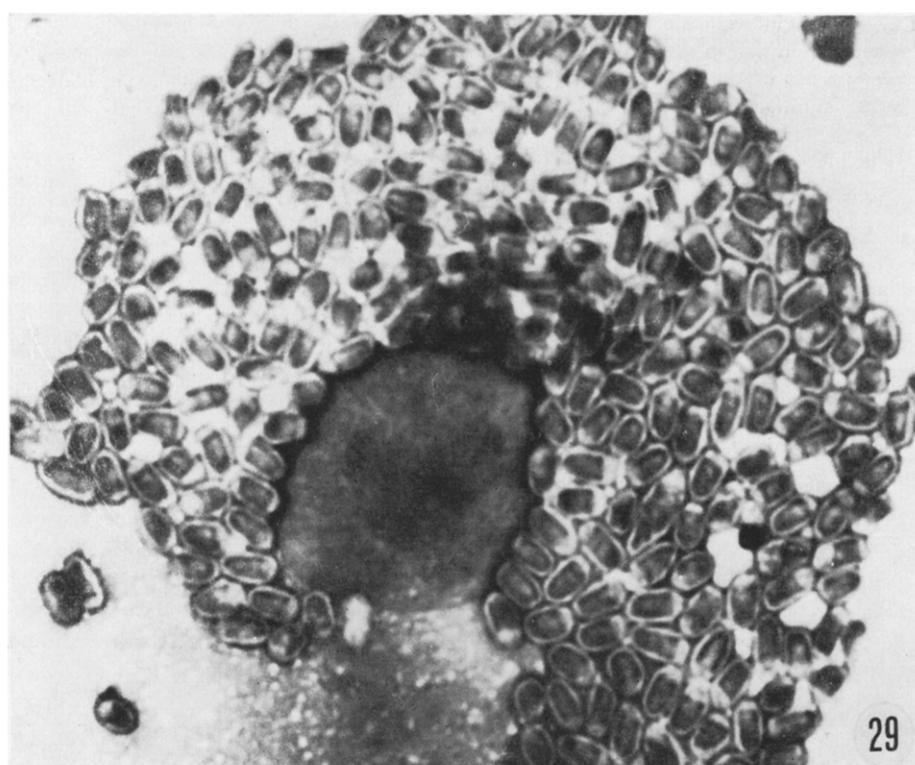
a) Light microscopy (Figs. 1—30)

The earliest stages were uni- and binucleate schizonts with a dense cytoplasm, measuring 2.5—9 μm in diameter (Figs. 1—8). Tetranucleate stages with irregular, band-shaped nuclei, measuring 9 μm in diameter were also present (Fig. 9). Schizonts appeared to divide by plasmotomy (Fig. 6) and budding (Fig. 8). The schizonts changed to broad stages with a large nucleus and chromatin organized in chromosomes or in granulated groups (Figs. 10—17). Sporonts with one (Fig. 21), 2 (Figs. 22 and 23), 4 (Fig. 24) and more nuclei were formed during sporogony. During the next divisions of nuclei the irregular fragments and spherical buds on their surface produced spherical sporoblasts with a single spherical nucleus in the centre of the stage, measuring 4—5 \times 3 μm (Figs. 25—27). The sporoblasts did not form isolated groups in the mass of sporogonal stages. This indicates that they separate and mature individually, not as a whole pansporoblast. There was no sign of a pansporoblastic membrane or of groups of sporoblasts and spores sticking together. Groups of spores in irregular numbers filled the infected cells and these formed pseudocysts with masses of spores (Fig. 29). The nuclei of the plasmodium and the sporoblasts were spherical, rather compact and relatively minute, usually less than 1 μm in diameter.

The spores were oval, with equally rounded ends. Their size varied; a minor type of 3.0 \times 2.0 μm and a major 1 of 5.0 \times 2.0 μm (Figs. 29 and 30). There was no difference in the microscopical structure of both types and they were mixed together in the smears and in infected cells. A thick spore wall closed the spore, being of equal thickness over the whole body. Inside of Giemsa stained spores, an icecream cone-like sporoplasm with a very distinct apical polaroplast vacuole was remarkable. The border of the sporoplasm was distinct, ending with a straight transversal line. A single nucleus was visible in the central part of some spores, 1 μm in diameter. There was no metachromatic granule in the area of the posterosome (Golgi), neither in sporoblasts nor in spores. There was no posterior vacuole in the spore. The polar filament was extruded only in a few instances and measured 50 μm , but this might not be its entire length.

Figs. 1—30. *Pleistophora carabidorum* n. sp. infecting male gonads of the beetle *Amara aenea* (Light microscopy, Giemsa's stained preparations).

1—9. Schizogonial stages with dense stained cytoplasm; 1. Uninucleate schizont; 2. Uni- and bi-nucleate schizonts; 3—5, and 7. Binucleate schizonts; 6. Tetranucleate schizont during division by plasmotomy; 8. Tetranucleate schizont during division by budding; 9. Tetranucleate schizont with band-shaped nuclei; 10—17. Cells with large nuclei and chromatin organized in chromosome-like structures; considered to be intermediate stages giving rise to sporogonial plasmodia; 18—20. Sporogonial plasmodia producing spherical and oval uninucleate sporoblasts; 21. Uninucleate sporont; 22 and 23. Binucleate sporonts; 24. Tetranucleate sporont; 25—27. Dividing sporonts; 28. Young spore; 30. Testes invaded by microsporidian spores; 29. The trophic cell of testes changed into a pseudocyst filled with masses of microsporidian spores.



b) Electron microscopy (Figs. 31—35)

On ultrathin sections the oval spores present an irregular, corrugated surface (Fig. 32). The exospore is very thin and equal in all parts of the spore. The endospore is rather massive, 0.2 μm thick, also on the anterior end (Figs. 32 and 33). A thin cytoplasmic membrane closes the spore content (Fig. 35). The polar filament which is visible in all our figures forms 10—15 coils. The diameter of the polar filament in cross sections gradually diminishes, but there is no sign of thicker and thinner regions as it is the case in some other genera. The posterosome disappeared after the formation of the polar filament and there are no remains of this structure or its product. In a section of an aberrant spore where the nucleus is situated in the posterior part, a structure is present which may be a Golgi apparatus (Fig. 31). It is spherical, with circular tubules and lacunae, 0.5 μm in diameter. The single nucleus is spherical, dense, without a specific arrangement of chromatin (Figs. 31 and 33). The spore has a thick lining of lamellae of the endoplasmic reticulum which is parallel to the length of the spore on one side but coiled in minute curls on the other. They are populated with rows of ribosomes arranged in tight spirals (Fig. 35). The centre of the spore is filled with free ribosomes (Figs. 34, 35).

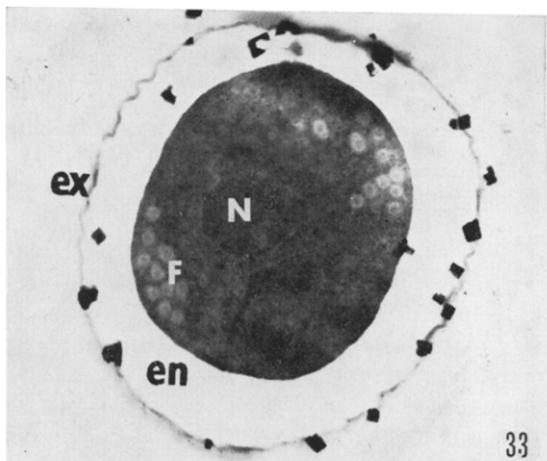
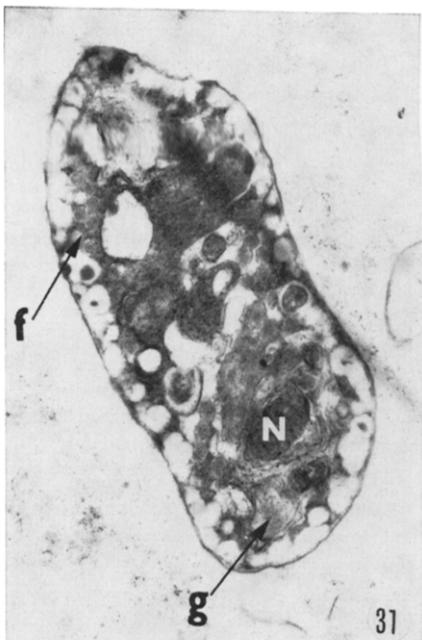
c) Host-Parasite Relationship

The infected tissue were male gonads. They were white, containing developmental stages and masses of spores of the microsporidian. The infected cells were primarily identified as trophic cells of the testes which were invaded and hypertrophied. During further development the parasite destroyed the whole structure of the testes; they were breakable (Fig. 30). Individual cells were changed into pseudocysts and filled with masses of spores (Fig. 29).

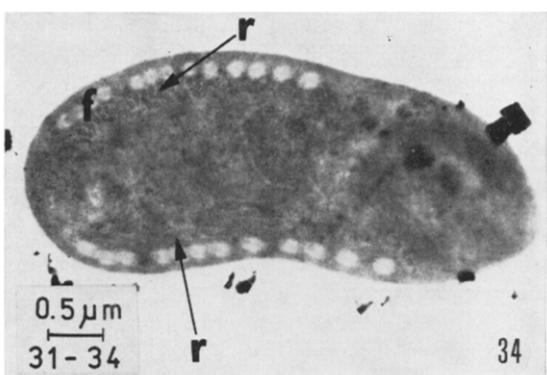
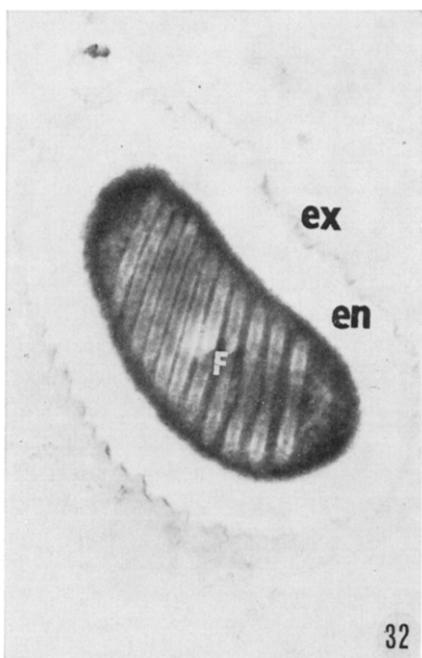
Discussion

The parasite described here conforms in its characteristic features to the genus *Pleistophora* GURLEY 1893 (SPRAGUE and VAVRA 1977). Two developmental phases, schizogony and sporogony, were observed. Only a few schizonts could be observed; they were examined with a light microscope. They divided by plasmotomy and budding. The sporogonic phase was examined at light and electron microscope levels. The sporogonial plasmodia divide by stepwise division through multinucleate segments into uninucleate sporoblasts which give rise to uninucleate spores. The microsporidian from the beetle *Amara aenea* belongs to a species which does not form pansporoblasts, having always single sporoblasts and spores during their maturation.

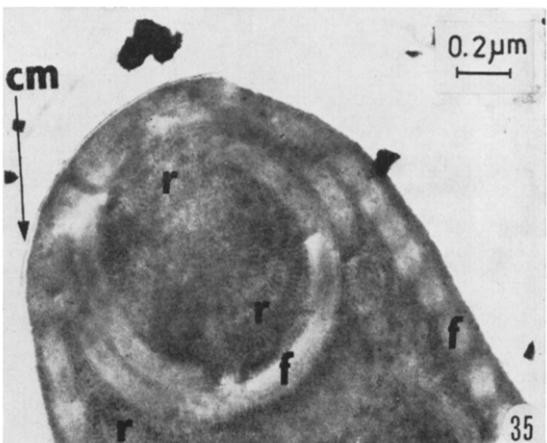
The spores seen with the electron microscope consisted of a coat composed of two outer layers, 8—16 coils of the polar filament, a single nucleus, and cytoplasm with abundant ribosomes. It is interesting that despite of this mass of ribosomes as mentioned in *Nosemaoides vivieri*, *Octosporea musca-domestica* (VAVRA 1976), and *Nosema hylobii* (PURRINI 1981), there was no apparent nucleolus in the nucleus of the microsporidian spore during sporogenesis. Other structural features were not apparent in the mature spore. The polar filament was cut in more than 37 cross sections in different regions of the degenerated spore. A layer of vacuoles lines the whole surface of the spore membrane. They are of equal size and there is no distinct polarisation or irregularity in their distribution. They indicate that all over the spore membrane there is communication with the exterior, which brings about interaction with the interior of the spore. The anterior pole with the ending of the polar filament does not represent any specific communication with the exterior.



33



34



35

Figs. 31—35. *Pleistophora carabidorum* n. sp. (Electron microscopy).

31. Degenerate early spore showing more than 37 cross sections of the polar filament (f), nucleus (n), and a structure which seems to be Golgi apparatus (g); 32. Mature spore showing exospore (ex) with rugose surface, thick endospore (en) and circular arrangement of coils of the polar filament (f); 33. Transversal section of mature spore showing thin exospore (ex), thick endospore (en), single nucleus (n) and coils of the polar filament (f); 34. Longitudinal section of mature spore showing coils of the polar filament. Cytoplasm contains electron dense material and polyribosomes (r). Other structural features were not apparent. 35. A part of mature spore in a high magnification, showing the polar filament (f) crossing the anterior vacuole, cytoplasmic membrane of spore content (cm), and cytoplasm containing abundant polyribosomes (r).

(Dark-stained bodies in Figs. 33—35 are staining artifacts.)

The parasite infecting male gonads of the beetle *Amara aenea* differs in the size and shape of spores, site of infection and host-parasite relationships from other microsporidian species described from Coleoptera. We proposed for it the name *Pleistophora carabidorum* n. sp.

Zusammenfassung

Es wurde eine neue Mikrosporidie, *Pleistophora carabidorum* n. sp., im Laufkäfer *Amara aenea* DEG. aus Nadelmischwäldern Niedersachsens gefunden. Der Parasit befallt die männlichen Gonaden des Wirtes. Der Entwicklungszyklus (Schizogonie und Sporogonie) der Mikrosporidie wurde licht- und elektronenmikroskopisch untersucht. Über die Parasit-Wirt-Beziehungen wird auch berichtet. Dies ist die erste Beobachtung einer Mikrosporidie als Parasit bei Laufkäfern (Carabidae).

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