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# Light- and Electron Microscopic Studies on a New Microsporidian, *Pleistophora tanzaniae* n. sp. (Microsporida: Microspora) Parasitizing the *Oryctes monoceros* Oliv. (Scarabaeidae, Coleoptera)

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With 17 Figures

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## Summary

The life cycle of a new microsporidian, *Pleistophora tanzaniae* n. sp. parasitizing natural population of rhinoceros beetle, *Oryctes monoceros* Oliv. in Tanzania is described by means of light- and electron microscopy. The midgut epithelium was the usual site of infection of the parasite. Some data on the ultrastructure of spore are given. The membraneous structure of the polaroplast in ultrathin sections is discussed.

## Introduction

The rhinoceros beetle, *Oryctes monoceros* Oliv. is one of the major pests of coconut palms in Tanzania. So far, except for eugregarine, *Stictospora curdistana* (Théodoridès 1961; Tuzet et al. 1967; Huger 1968) no protozoan parasite of *Oryctes*-fauna have been described. Our investigations on disease agents in a natural population of *O. monoceros* in Tanzania revealed the occurrence of 6 new protozoan species: one neogregarine of the genus *Ophryocystis*, and 5 microsporidians of the genera, *Pleistophora*, *Nosema*, *Toxoglugea*, and *Oryctospora* gen. n. This paper reports on results of the light- and electron microscopic examinations of *Pleistophora*. The results of examinations on neogregarine and other microsporidians will be published elsewhere (Purrini 1986; Purrini and Kohring 1986; Purrini et al. 1986).

Some data on host-parasite relationship are also given.

## Material and Methods

During the period from November 1983 to July 1984 thousands of samples (7,000 third instar larvae, and 2,500 of adults) of rhinoceros beetle, *O. monoceros* infesting coconut- and wild palms in Tanzania were collected and brought to the laboratories of the National Coconut Development Programme (NCDP) in Mlingano (Tanga, Tanzania). The weak and dead specimens were immediately examined for parasites by dissection of all organs using light microscopy. Distribution of disease-agents in the organs was recorded. For light microscopy air dried smears were fixed with methanol and stained with Giemsa's stain. For electron microscopy the pieces of infected gut were fixed in glutaraldehyde (2 % glutaraldehyde in 0.1 M phosphate buffer, *p*H 7.5), postfixed in 2 % osmium tetroxide in the same buffer for 2 h, then dehydrated through 30—100 % acetone

series; into 70 % containing 1 % uranyl acetate, overnight. Finally, the material was embedded in Spurr's low viscosity medium (resin).

Ultrathin sections, after staining with uranyl acetate/lead citrate, were examined using a Philips EM 301 electron microscope at the Institute of Microbiology, University of Göttingen.

## Results

*Pleistophora tanzaniae* n. sp. from *Oryctes monoceros*

### Light Microscopy

Early stages noted were octonucleate meronts divided into two round tetranucleate cells (Fig. 1). Larger stages with nuclei which showed signs of division were also probably meronts (Figs. 2 and 3). Some stages with ring nuclei represented in Figs. 4 and 5a, were attributed to the sporogonic cycle (sporonts, sporogonial plasmodia).

Division of the sporogonial plasmodia gave rise to pansporoblasts with 8, 12, 16, 32, 48 and 64 sporoblasts which mature into spores (Figs. 6—13). The sizes of sporogonial plasmodia and pansporoblasts ranged between 10 to 30 $\mu$m in diameter. In Giemsa stained preparations the spores were oval and measured 3.5—4.5 $\times$ 2.8—3.2 $\mu$m; maximum 5.0 $\times$ 3.5 $\mu$m.

### Electron Microscopy

At the electron microscope level only the mature spores could be observed (Figs. 14—17). They were stained with little contrast. The ultrastructure of the spore in Fig. 14 showed a single nucleus (N), a tube of the polar filament in longitudinal section (F) and a sporoplasm containing electron-dense material. Other ultrastructural features were not observed.

The spore-wall showed in a high magnification (Fig. 15) consisted of a more or less thick electron-dense exospore (Ex), thick electron-transparent endospore (En), and thin cytoplasmic membrane of spore content (Cm).

The sections of mature spores demonstrated that the polar filament was formed in a double row of coils with 18 to 19 turns. In cross sections (Fig. 16, high magnification) the polar filament showed more than 8 concentric layers, proportion of which varied somewhat along the longitudinal axis similar to all microsporidia (VAVRA 1977). The polaroplast, which in our ultrathin sections was well preserved, is composed of two parts of membranous systems: The anterior portion (A) with tightly parallel-formed membranes and the posterior portion (B) with widely parallelspaced out membranes (Fig. 17). Some, more or less large, vesicles scattered throughout the polaroplast could also be observed (Vs).

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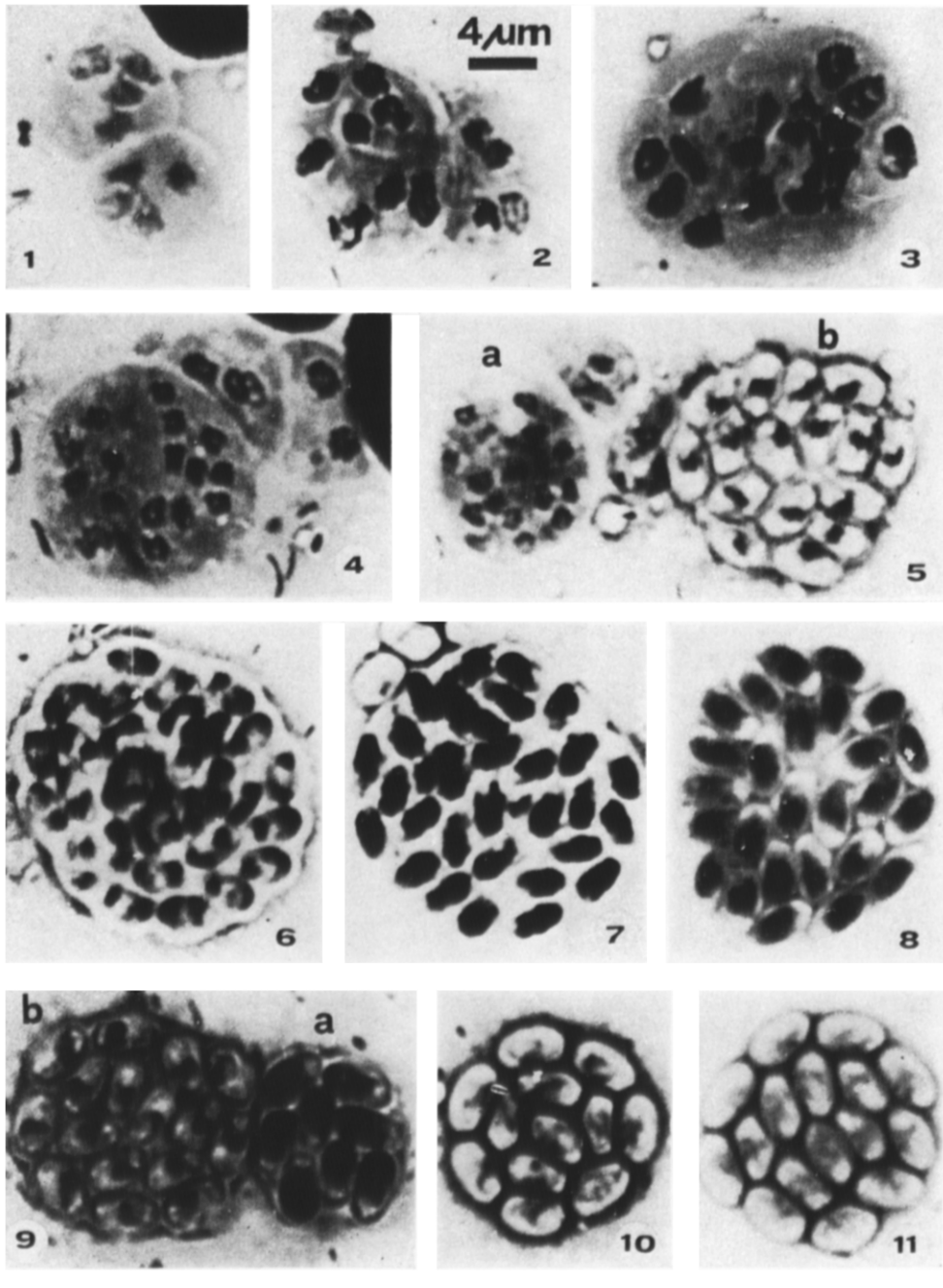
Figs. 1—13. *Pleistophora tanzaniae* n. sp. from *Oryctes monoceros* OLIV. Light Microscopy, Giemsa staining.

Fig. 1. Dividing octonucleate meront; Fig. 2 and 3. Multinucleate stages showing signs of nuclear division are probably meronts; Figs. 4 and 5a. Multinucleate stages with ring nuclei are probably sporonts; Figs. 5b, 9b and 11. Pansporoblasts, each of them containing 16 mature spores; Figs. 6 and 7. Pansporoblasts, each of them containing 32 maturating spores; Fig. 8. Pansporoblast containing 24 mature spores; Fig. 9a. Pansporoblast containing 8 mature spores; Fig. 10. Pansporoblast containing 12 mature spores; Fig. 12. Pansporoblast containing 64 mature spores; Fig. 13. Pansporoblast containing 48 mature spores.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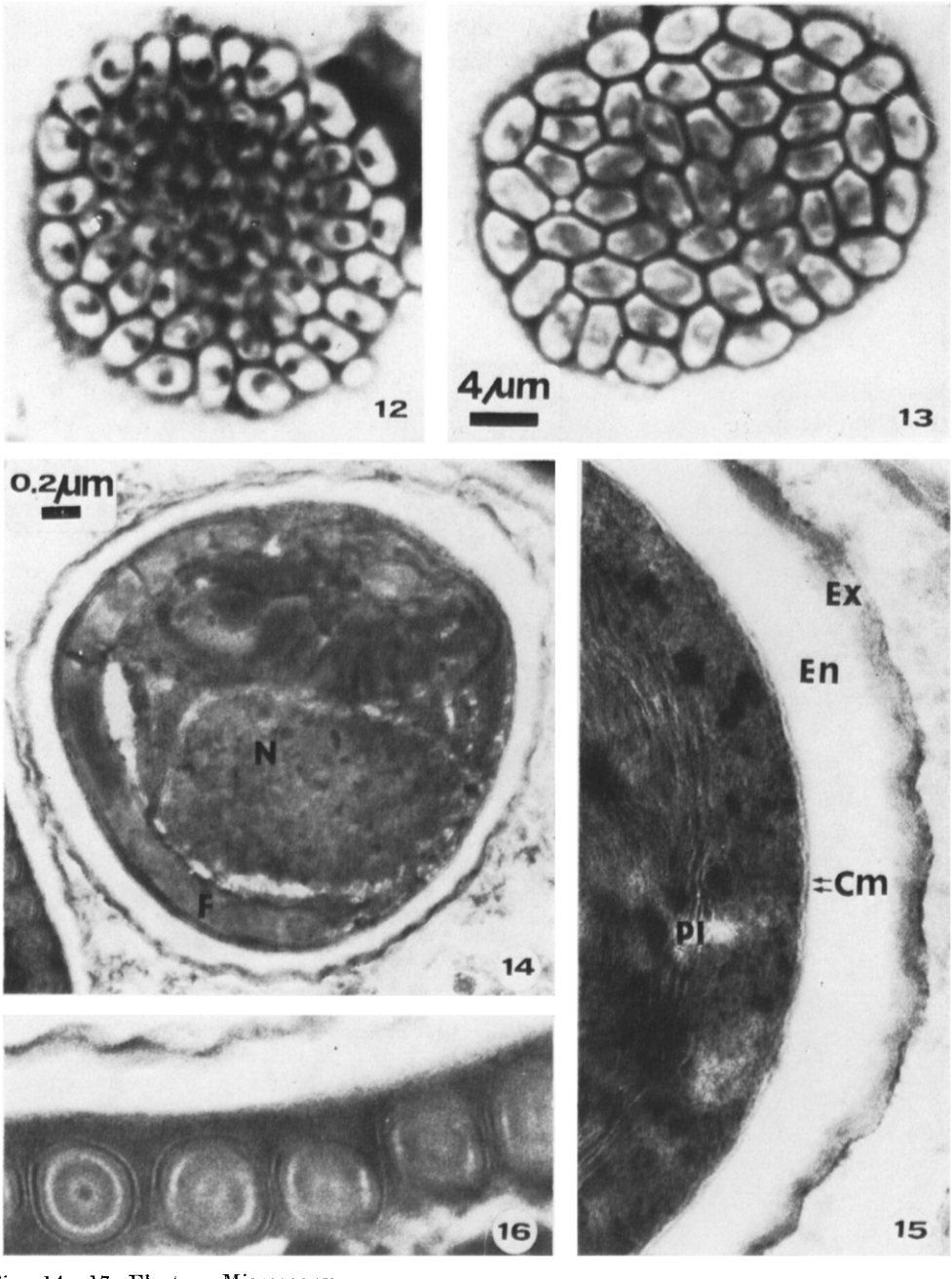


Figs. 14—17. Electron Microscopy.

Fig. 14. Transversal section of mature spore showing a single nucleus (N) and a tube of the polar filament in longitudinal section (F); Fig. 15. Transversal section of mature spore (high magnification) showing more or less thick electron-dense exospore (Ex), thick electron-transparent endospore (En), thin cytoplasmic membrane of spore content (Cm), and membraneous polaroplast (Pl); Fig. 16. Cross section of the polar filament (high magnification) consisting of several concentric layers;

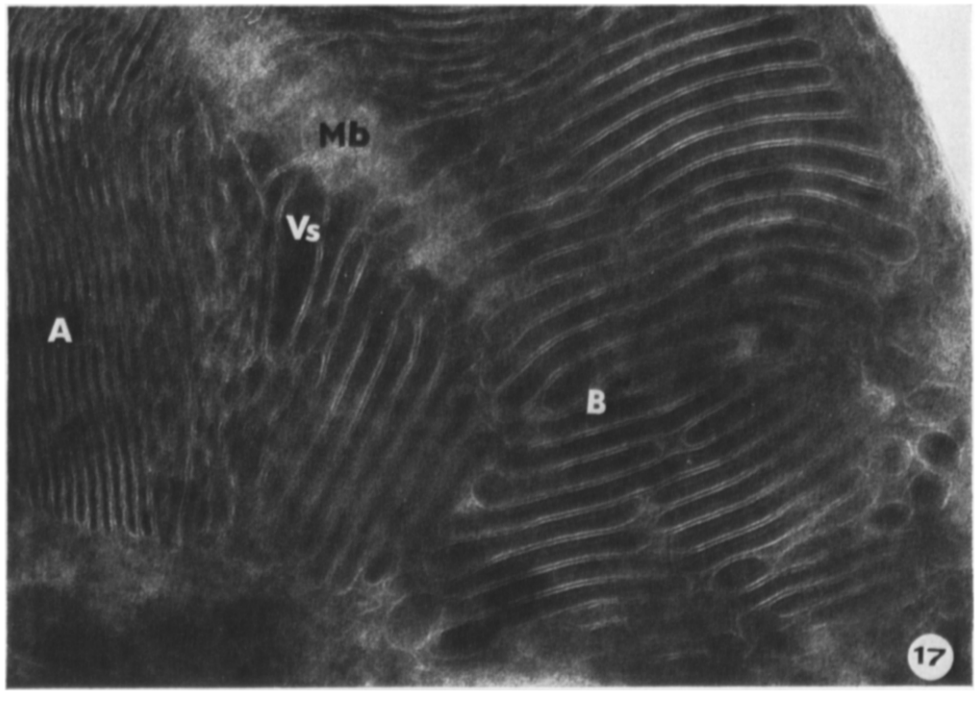


Fig. 17. Longitudinal section of mature spore showing membraneous structure of the polaroplast composed of a portion with tightly parallel-formed membranes (A) and a portion with widely parallel-spaced out membranes (B). Note the presence of some, more or less larger vesicles (Vs) in portion B of the polaroplast. Mb, Manubrium part of the polar filament.

### Host-parasite relationships and prevalence of infection

Except for collected specimens of the rhinoceros beetle, *O. monoceros* from coconut palms, some specimens (50 third instar larvae and 20 adults) were also collected from wild palms at the localities Mwarongo (Tanga Region) and Kifaru (Kilimanjaro Region). Among them three (3) adult beetles were found to be infected by microsporidian, *P. tanzaniae*. The infected tissue was the midgut-epithelium. After dissection, the gut appeared to be white coloured and hypertrophied. Individual cells changed into pseudocysts filled with mature spores could be observed under light microscope. Laboratory infection surveys with the microsporidian, *P. tanzaniae*, using as hosts the reared adults of *O. monoceros* from coconut palms, were not successful.

## Discussion

Microsporidia in which sporogonial plasmodia give rise to a variable and usually large number of spores within pansporoblast membranes have recently been separated into three genera from the original single genus *Pleistophora*.

These are *Pleistophora* Gurley, 1893; *Vavraia* Weiser, 1977; and *Polydispyrenia* Canning and Hazard, 1982. The first two are characterized by nuclei isolated throughout development and thick pansporoblast membranes (sporophorous vesicle walls) derived from secreted amorphous material already present on the merogonic stages. They differ in the mode of division of the sporogonial plasmodium directly

into uninucleate sporoblasts by multiple fission in *Vavraia* and by stepwise division via multinucleate segments in *Pleistophora*. The genus *Polydispirenia* exhibits nuclei in diplokaryon arrangement in merogony, which separate and undergo meiosis to give the isolated haploid nuclei of the sporoblasts. The pansporoblast wall arises external to the sporogonial plasmodium at the time of meiosis as a fine, membraneous layer thickened externally by electron dense secretions.

The polysporous parasite of rhinoceros beetle, *O. monoceros* belongs to the genus *Pleistophora* in having isolated nuclei throughout development and stepwise division of the sporogonial plasmodia via multinucleate segments. The name *Pleistophora tanzaniae* n. sp. is proposed for it.

The spore seen with electron microscope consisted of spore wall (exospore, endospore, and cytoplasmic membrane of spore content), single nucleus, polar filament and polaroplast. The polaroplast in our ultrathin sections showed a system of membranes (previously reported as lamellar-system) which in the anterior part (portion) were tightly and in the posterior part widely parallel-formed membranes. In both parts, some vesicles (more or less large sites) were scattered throughout the polaroplast.

## Zusammenfassung

Der Lebenszyklus einer neuen Mikrosporidie, *Pleistophora tanzaniae* n. sp., wurde an Material aus natürlichen Populationen von Nashornkäfern, *Oryctes monoceros*, auf Grund von licht- und elektronenmikroskopischen Beobachtungen beschrieben. Der Parasit befällt das Mitteldarm-Epithel des Wirtes. Die Ultrastruktur der Spore wurde auch elektronenmikroskopisch untersucht.

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