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Light and Electron Microscopic Studies on the Microsporidian  
*Pleistophora schubergi neustriæ* n. subsp.  
(Microsporida: Phylum Microspora)  
Parasitizing the Larvae of *Malacosoma neustriæ* L.  
(Lymantriidae, Lepidoptera)

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With 4 Plates

**Key words:** Microsporida: *Pleistophora schubergi neustriæ*, Lepidoptera: *Malacosoma neustriæ*, butterfly: natural infection.

### Summary

The life cycle: The schizogony (merogony) and sporogony of *Pleistophora schubergi neustriæ* n. subsp. from larvae of *Malacosoma neustriæ* was investigated. The midgut epithelium is the usual site of infection of the parasite. The ultrastructure of pansporoblasts and spores is also discussed.

### Introduction

ZWÖLFER (1927) first reported the presence of a microsporidian, resembling that causing “pebrine” in silkworms, *Euproctis chrysorrhœa* L. (Lymantriidae, Lepidoptera). He named the parasite *Pleistophora schubergi*. Later, the microsporidian has been reported as infections to various other Lepidoptera, by VEBER (1956, 1957, and 1963); GÜNTHER (1956); WEISER (1957, and 1961); ISSI and NILOVA (1967); VEREMTCHUK and ISSI (1968); ISSI (1968, 1969, and 1971); ISSI and CHERVANSKAYA (1969); LIPA (1963, and 1971); NORDIN and MADDOX (1972, and 1974); KAYA (1973); WILSON (1978). The descriptions by the above mentioned authors contained very limited data on the life cycle of the parasite by light microscopy. The parasite was not studied by electron microscope.

While conducting studies on pathogenic agents of some insect pests at the Institute of Forest Zoology in München and Göttingen (Federal Republic of Germany), I also found *P. schubergi* infecting natural populations of five lepidopteran species: *Euproctis chrysorrhœa* (PURRINI 1979); *Erranis defoliaria* (PURRINI, SKATULLA 1979), and recently in *Stilpnobia salicis*, *Aporia crataegi*, and *Malacosoma neustriæ*. In June and July of 1980 I had an opportunity to collect hundreds of specimens of two populations of *M. neustriæ* (infesting fruit trees of *Prunus* sp. in two outbreaks of the pest in Lower Saxony, Federal Republic of Germany) in which infections by *P. schubergi*

and polyhedrosis-viruses were observed. Some young infections found in twenty-two specimens of the examined hosts enabled me to study the life cycle of *P. schubergi* in detail.

The present paper concerns the light and electron microscopic studies of the life cycle of the parasite, undertaken in order to validate its taxonomic status and to establish the characteristics which differentiate it from other sub-species of *P. schubergi* described from other lepidopterans.

### Material and Methods

In 1980, hundreds of larvae of *M. neustria*, infesting fruit trees of *Prunus* sp. in Lower Saxony (Federal Republic of Germany) together with other lepidopteran hosts, were collected and examined for pathogens. For light microscopy the gut, Malpighian tubules, and fat body were dissected from the insects. The distribution of pathogens in the organs was recorded. The gut, the principal site of infection by the parasite *P. schubergi* found in *M. neustria*, was fixed in BOUIN's solution for histological sections, 4  $\mu\text{m}$  thick, which were stained with HEIDENHAIN's Iron Haematoxylin. Dry smears, fixed with methanol and stained with Giemsa stain, were also prepared. For electron microscopy, the gut was dissected and fixed at temperature 6 °C overnight in phosphate buffered glutaraldehyde (2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4). The gut (cut into small pieces) was transferred into distilled water (5 minutes), followed by two washes (10 minutes each) in 0.1 cacodylate buffer contained 5% sucrose. The material was postfixed in 2% osmium tetroxide in veronal acetate buffer, dehydrated through a 30–90% acetone series, and three changes of absolute acetone (each steps lasting 15 minutes) and embedded in SPURR's medium. Sections were stained with uranyl acetate and lead citrate and examined with PHILIPS 301 electron microscope.

### Results

#### Descriptions of the life cycle of *Pleistophora schubergi neustriæ* n. subsp.

1. Light Microscopy. Figures 1–38 represent various stages of schizogony and sporogony in the life cycle of *P. schubergi neustriæ* in epithelial cells of the gut of *M. neustria*, observed in Giemsa and Heidenhain's iron hematoxylin stained preparations.

The earliest stages recognized were round, oval or irregularly-shaped, bi- and tri-nucleate schizonts, measuring 2–3  $\mu\text{m}$  in diameter (Figs. 1–4). Uninucleate forms were not observed. Nuclear multiplication produced stages: all numbers of nuclei, odd and even, up to 32, were produced (Figs. 1–11, and 23–25). Nuclear divisions were not synchronous and nuclei were isolated; they were not in diplokaryon arrangement. Cytoplasmic division was by plasmotomy, namely division of a multinucleate stages into smaller multinucleate parts (Figs. 12–22). Measurements of several multinucleate schizonts gave values of 4–5.5  $\mu\text{m}$  in diameter for tetranucleate schizonts; 5–7  $\mu\text{m}$  for schizonts with six nuclei; 2–5  $\mu\text{m}$  with five; 6.5–7  $\mu\text{m}$  for schizonts with eight nuclei; 7  $\mu\text{m}$  with ten; 7–10  $\mu\text{m}$  with twelve; 10  $\mu\text{m}$  with twenty; 10–12 with twenty four and 15–18  $\mu\text{m}$  in diameter for schizonts with 32 nuclei. Schizonts with more than 32 nuclei were not observed. We consider the stages represented in Figures 1–25a to belong to one closed generation of the schizogony in the life cycle of the parasite. They all had a compact nuclei that stained deeply with Giemsa stain.

The forms represented in Figures 25b—35 are considered to be developmental stages of other generation of the schizogony (merogony) in the life cycle of the parasite. Large multinucleate stages of this generation (Figs. 26—31, 32a, and 34a) were possibly undergoing a kind of plasmotomy division producing cells (meronts) with a "ribon-like" form. They were of different sizes, measuring sometimes up to 250  $\mu\text{m}$  long (Figs. 32b, 33, 34b, and 35). Some of these cells represented in Figures 27—30, may probably be (after CANNING, personal communications) the host-cells. Because the intermediate stages could not be clearly observed by light microscope due to the minuteness of these cells, no attempt has yet been made to place the schizogonial generations in sequence.

Large multinucleate, maturing sporonts, called plasmodia were occasionally seen, apparently dividing (Figs. 36 and 37). When the sporont is mature, cytoplasmic cleavage take place and results in a pansporoblast containing different numbers of sporoblasts, or spores. The largest one, similar to that illustrated in Figure 38 contained 128 spores. The most common spores were ovoid or round-oval in shape, measuring 2.5  $\times$  1.5  $\mu\text{m}$ . Some of them were larger, 3  $\times$  1.8  $\mu\text{m}$ .

2. Electron Microscopy. The pansporoblasts containing sporoblasts were the youngest stages observed with the electron microscope. They were externally limited by a very thin unit type membrane (Figs. 39 and 40).

The ultrastructure of the spore of *P. schubergi neustriæ* found in gut epithelial cells of *M. neustria* is represented in Figures 41—46. In longitudinal sections of the spore, with the spore wall consisting of a more or less thick exospore, 15—25 nm (Ex), a thick endospore, 160—20 nm (En) and cytoplasmic membrane of the spore content (Cm), the spore has a surface where the exospore were more or less rugose. The polar filament (F) is seen as a single row of coils, or with final two to six coils. More than six layers of coil are clearly visible in Fig. 46 (high magnification). The anterior half of the spore was occupied by extrusion apparatus consisting of the anchoring disc of the polar tube (ad), manubroid part of the filament (mf) and lamellar polaroplast (Pl). Other ultrastructural features of the spore were sporoplasm containing electron dense material and ribosomes. In intrasporal sporoplasm lay only one (single) nucleus covered with a double-layered membrane. The nucleus was spherical or oval shaped, measuring 600—800 nm in diameter (Figs. 41 and 42). The posterior vacuole (Pv) in Figure 44 shows as an ovoid body in the posterior end of the spore, filled with some clear and dark bodies of uncertain nature. The presence of a membranous layer covering the vacuole can only be supposed.

## Discussion

Subspecies differentiation of the microsporidian found in larvae of *M. neustria* has been based upon the host range, site of infection and morphology of developmental stages and spores in relationships to other subspecies listed in Table 1. Up to now, five subspecies of the microsporidian *Pleistophora schubergi* ZWÖLFER (1927) parasitizing different species of Lepidoptera have been described. The list given in the Table 1, is an attempt to bring together informations on the named subspecies. They are as

Table 1. Comparison of described subspecies of the *Pleistophora schubergi* ZWÖLFER 1927

Parasites	Authors	Hosts	Site of infection	Vegetative stages ( $\mu\text{m}$ )	Sporulation stages ( $\mu\text{m}$ )	Spores ( $\mu\text{m}$ )	Localities
<i>Pleistophora schubergi schubergi</i>	ZWÖLFER, 1927	<i>Euprotis chrysorrhoea</i>	midgut epithelium	uninucleate cells, plasmodia: $20 \times 3 - 5$	pansporoblast: 10	$2.5 \times 1.5$	Germany
<i>P. schubergi aporidae</i>	WEBER, 1956	<i>Aporia crataegi</i>	midgut epithelium	schizonts: 2 – 3; plasmodia: 4 – 6	pansporoblast: no data of the size	$2 \times 1.5$	ČSSR, UdSSR
<i>P. schubergi pandemis</i>	WEBER, 1957	<i>Pandemis corylana</i> , and <i>E. c.</i>	midgut epithelium	schizonts: 2 – 3; plasmodia: 5 – 10	pansporoblast: 6 – 7.5	$4 - 5 \times 2.5$	Poland
<i>P. schubergi balbiani</i>	WEBER, 1963	<i>Anthera pernyi</i> , and <i>E. c.</i>	midgut epithelium	no data	no data	$2.5 \times 1.5$	ČSSR
<i>P. schubergi lymantriae</i>	WEISER, 1957	<i>Hypenantria cunea</i>	midgut epithelium	no data	no data	$2.5 \times 1.5$	ČSSR
<i>P. schubergi noctuidae</i>	VEREMTCHUK; ISSI (1968)	<i>Agrotis segetum</i> , and <i>E. c.</i>	midgut epithelium	no data	no data	$2.5 \times 1.5$	UdSSR
<i>P. schubergi neustriæ n. sp.</i>	this paper	<i>Malacosoma neustria</i>	midgut epithelium	two generations of the schizogony: diff. sizes	pansporoblasts, sporonoids, sporoblasts: diff. sizes	$2.5 \times 1.5$ ; some spores: $3 \times 1.8$	Federal Republic of Germany

reviewed by the authors identical in the site of infections and spore sizes, measuring  $2.5 \times 1.5 \mu\text{m}$ . However, the measurements given in original publications were larger  $3 \times 2 \mu\text{m}$ , for the spores of *P. schubergi pandemis* (VEBER 1957) and smaller  $2 \times 1.5 \mu\text{m}$  for the spores of *P. schubergi aporiae* (VEBER 1956). Some macrospores, measuring  $4-5 \times 2.5 \mu\text{m}$ , observed by VEBER (1957) has also been reported. In their original descriptions the authors gave some data on developmental stages of listed subspecies. Of the schizogony ZWÖLFER (1927) reported on uninucleate cells, which later produced plasmodia, and VEBER (1956, 1957) on schizonts, being  $2-3 \mu\text{m}$  in diameter, which later produced plasmodia, measuring  $4-6 \mu\text{m}$ , or  $5-10 \mu\text{m}$  in diameter. The measurements of the pansporoblasts given in their publications were  $10 \mu\text{m}$  in diameter (ZWÖLFER 1927) and  $6-7.5 \mu\text{m}$  (VEBER 1956, 1957). Further informations on the life cycle of listed subspecies are lacking (Table 1).

The parasite described here conforms in its characteristics features to the genus *Pleistophora* GURLEY (1893), as re-defined and summarized by CANNING and NICHOLAS (1980). Two developmental phases in its life cycle: The schizogony (merogony) and sporogony, were established. In schizogonial phase studied at light microscope level it could be determined existence of two structurally different generations. One generation is characterized by formation of the schizonts (initially round cells), which later growth producing multinucleate plasmodia, up to 32 nuclei. All forms and sizes (up to  $18 \mu\text{m}$  in diameter) of schizonts in this generation were observed. Division was by plasmotomy. Other generation is characterized by formation of round or irregularly shaped and deeply stained cells. A final division of these cells give rise to the large "ribon-like" stages with vesicular nuclei, measuring up to  $250 \mu\text{m}$  long.

The sporogonic phase has been examined at light- and electron-microscope levels. As far, as we could determine, the sporogony of the parasite of *M. neustria* was poly-sporous. The sporogonial plasmodia (sporont) divided by stepwise division through multinucleate segments into uninucleate sporoblasts, which give rise to uninucleate spores. This is one of the important feature of the microsporidians belonging to the genus *Pleistophora*, as reported by CANNING and NICHOLAS (1980). In this way variable numbers of sporoblasts and of spores were formed from each sporont. The pansporoblasts containing sporoblasts and spores were oval or spherical in shape, measuring up to  $45 \mu\text{m}$  in diameter.

The common spores of the microsporidian from *M. neustria* were similar in shape and sizes to that of subspecies listed in Table 1. The spore seen with the electron microscope, consisted (as mentioned before) of a coat composed of three outer layers, filament, lamellar polaroplast, posterior vacuole, nucleus and cytoplasm containing abundant ribosomes. Our electron microscopic studies revealed with certainty the evidence of a single nucleus in mature spore. This represent other important feature of the microsporidians closed to the genus *Pleistophora*.

On the bases of the presented characteristics in comparison to other subspecies shown in Table 1, we consider the parasite of *M. neustria* as a new subspecies, for which the name *Pleistophora schubergi neustriæ* n. subsp. is proposed.

## Zusammenfassung

Der Lebenszyklus: Schizogonie (Merogonie) und Sporogenie der Mikrosporidie, *Pleistophora schubergi neustriæ* n. subsp. wurde an Material aus den Larven von *Malacosoma neustria* L. untersucht. Der Parasit befallt das Mitteldarm-Epithel des Wirtes. Zwei Stadien der Sporogenie der Mikrosporidie: die Pansporoblasten und die Sporen wurden auch elektronenmikroskopisch untersucht.

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### Explanation of Plates

Plates 1–4. Life cycle of *Pleistophora schubergi neustriæ* n. subsp. parasitizing the midgut epithelium of *Malacosoma neustria*.

#### 1. Light microscopy (Figures 1–38)

Figs. 1–25, and 36–38: Giemsa stain; Figs. 26–35: Heidenhain's iron haematoxylin; magnifications: 1–37: ca. 3,200 $\times$ ; 38: ca. 1,600 $\times$ .

Figures 1–25a: Schizonts (meronts) closed to one generation with deeply stained compact or ring nuclei.

1–3. Schizonts with 3 nuclei; 4. One binucleate schizont and one schizont with 5 nuclei; 5. Schizont with 8 nuclei; 6. Schizont with 10 nuclei; 7. Schizont with 8 nuclei; 8 and 9. Schizonts with 12 nuclei; 10 and 11. Schizonts with 10 nuclei; 12–22. Schizonts with various number of nuclei during division. Division by plasmotomy is clearly visible; 22. Multinuclear plasmodia with 32 nuclei; 23. Schizont with 10 ring nuclei; 24. Schizont with 24 ring nuclei; 25a. Schizont with 12 nuclei; 25b. Sporogonial plasmodia (sporont).

Figures 25b–35: Schizonts closed to other generation of the schizogony (merogony) with not clearly stained nuclei.

31, 32a, and 34a. Schizogonial stages before division into "ribon-like" forms; 32b, 33, 34b, and 35. Schizogonial stages "ribon-like" form with ring nuclei, up to 250  $\mu\text{m}$  long; 36 and 37. Sporogonial plasmodia (sporonts) apparently dividing; 38. Pansporoblast containing 128 mature spores.

#### 2. Electron microscopy (Figs. 39–46)

39 and 40. Two pansporoblast containing sporoblasts. Thin unit type membrane of both pansporoblasts remarkable; 13,600 $\times$ ; 41–44. Ultrastructure of the spore, exospore (Ex), endospore (En), cytoplasmic membrane of the spore content (Cm), nuclei (N). Single nucleus in mature spore covered by double layered membrane visible in Figures 41 and 42; filament (F); lamellar polaroplasts (Pl), anchoring disc of polar filament (ad), manubroid part of the polar filament (mf), posterior vacuole (Pv); 45. Longitudinal section of polar filament; 46. Transversal section of polar filament in high magnification, magnifications: 41: 41,600 $\times$ , 42: 39,200 $\times$ , 43: 39,200 $\times$ , 44: 30,400 $\times$ , 45: 30,400 $\times$ , 46: 84,000 $\times$ .







