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Ultrastructure characteristic of a *Nosema* sp. (Microsporidia) from a Bulgarian population of *Euproctis chrysorrhoea* L. (Lepidoptera)

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Abstract

Ultrastructure and development of a microsporidium isolated from a natural *Euproctis chrysorrhoea* larval population in Western Bulgaria were studied. Mature environmentally resistant spores of this microsporidium possess typical diplokarya, 9–10 polar filament coils, smooth polaroplasts and wavy exospores; we consider it to be a *Nosema* species. *Nosema* sp. occurred in the fat body and silk glands of *E. chrysorrhoea* and caused hypertrophy of the silk gland cells. This species is not infective to closely related *Lymantria dispar*. This *Nosema* isolate does not appear to be one of the species previously described from *E. chrysorrhoea*.

Key words

Nosema* sp., microsporidia, ultrastructure, development, *Euproctis chrysorrhoea

Introduction

Euproctis chrysorrhoea L. is currently the most serious pest of broadleaf forests and fruit trees in Bulgaria. In the year 2000, it defoliated hundreds of hectares of forests in Southern and Central Bulgaria (National Forest Management 2000) and it is also a major pest of orchards. It has been suggested that microsporidia are among most important of the natural enemies that regulate *E. chrysorrhoea* populations (Sterling and Speight 1989).

Zwölfer (1927) was the first to describe a microsporidium from *E. chrysorrhoea*. This species, *Pleistophora* (= *Endoreticulatus*) *schubergi*, is a pathogen of the midgut tissues of *E. chrysorrhoea* and of the gypsy moth, *Lymantria dispar*. In 1957, Weiser reported that *Nosema lymantriae*, which he described from *L. dispar*, also infected the fat body and silk glands of *E. chrysorrhoea*. Lipa (1964) reported the occurrence of a *Nosema* sp. in *E. chrysorrhoea* populations from Poland. Purrini and Weiser (1975) described *Nosema kovacevici* from natural populations of *E. chrysorrhoea* from Kosovo, and in the same year Sidor *et al.* (1975) recorded microsporidian infections caused by a *Nosema* sp. in Macedonia. There are two other records of microsporidian infections in *E. chrysorrhoea* populations from Bulgaria, the *Endoreticulatus* species and the *Nosema* species (Pilarska *et al.* 2000, Solter *et al.* 2000). The above authors reported the spore size and shape for each isolate and identified the type of tissues infected; none of these microsporidia has been examined ultrastructurally and there is no photographic documentation of the infected host tissues. The aim of this paper, is to present ultrastructural data and describe the developmental stages of the *Nosema* sp. isolated from a natural *E. chrysorrhoea* larval population in Western Bulgaria.

Materials and methods

Euproctis chrysorrhoea larvae were collected from Dolni Lozen, 10 km south of Sofia, Bulgaria in 1998, 1999 and 2000, and were transported to the laboratory where they were immediately dissected and examined for the presence of microsporidian infections. Tissues of the infected larvae containing microsporidia were smeared on slides, fixed in methanol, and stained with Giemsa or contrasted with Burri ink (Vávra and Maddox 1976). Spores were immobilized by the agar method of Undeen and Vávra (1997). Spore size and shape were recorded using a LEICA Quantimet system.

For transmission electron microscopy (TEM), infected tissues were fixed in 2.5% glutaraldehyde in a 0.1 M cacodylate buffer (pH 7.2) and postfixed in 2% OsO_4. Fixed tissues were

Table I. Occurrence of *Nosema* sp. and *Endoreticulatus* sp. in the *Euproctis chrysorrhoea* population from Dolni Lozen, Bulgaria, 1998–2000

Collection date	No. collected	No. infected with *Endoreticulatus* sp.	No. infected with *Nosema* sp. in mixed infection
18.06.1998	25	23	7
15.05.1999	21	18	9
14.02.2000	59	13	5
10.05.2000	12	10	6

dehydrated in ethanol and acetone series and embedded in Epon-Araldite (Undeen and Vávra 1997). Sections were cut on an LKB ultramicrotome, stained with uranyl acetate and lead citrate, and then examined and photographed using a TESLA BS 500 electron microscope.

We fed spores of the *Nosema* sp. isolate recovered from *E. chrysorrhoea* to larvae of *Lymantria dispar* in order to determine if they were infective to this closely related species. As *L. dispar* can be reared easily in the laboratory on artificial diet, this would facilitate the study of the life cycle necessary for a more complete description of this isolate. A suspension of spores was placed on the surface of small cubes of an artificial gypsy moth diet (Bell *et al.* 1981) and fed to third instar larvae. The larvae, which were starved for 24 h prior to treatment, readily consumed the diet cubes within 24 h. Treated larvae were placed on artificial diet in 100-ml cups, 10 larvae/cup, and were reared at 25°C, 16/8 h light/dark cycle at a relative humidity of 70%.

Results

The *Nosema* species was only found in mixed infections with *Endoreticulatus* sp. in the *E. chrysorrhoea* population from Dolni Lozen. The *Endoreticulatus* also occurred in single species infections and its prevalence was therefore higher that of *Nosema* sp. (Table I).

Because the field collected larvae were at an advanced stage of the infection, the developmental stages observed in the silk glands were late merogonial stages, sporonts, sporoblasts and mature environmentally resistant spores. Compact nuclei in a diplokaryotic arrangement and large amounts of endoplasmic reticulum in the cytoplasm were present in all stages (Fig. 1).

The beginning of the sporulation cycle is characterised by a thickening of the plasmalemma and the formation of a more regular endoplasmic reticulum (Fig. 2). All sporogonial stages contained two or four nuclei (Figs. 3 and 4). In some ultrathin sections we found sporonts with more than one diplokaryon enclosed by developing coils of the polar filament (Fig. 4). Two sporoblasts developed from each sporont.

The spores of *Nosema* sp. are oval in shape (Fig. 5). Fresh spores measured 4.19 (5.45–3.38) (± 0.47) × 1.92 (2.28–1.48) (± 0.18) µm.

Ultrastructurally, the spores were diplokaryotic with 9–10 polar filament coils (Fig. 6). In some spores, the coils were arranged in 2 parallel rows (Fig. 7). The polaroplast was of a type characteristic for a *Nosema*-like microsporidium, with packed membrane lamellae. At the anterior pole of the spores there is a mushroom-shaped anchoring disc and manubrium (Fig. 8). The exospore was very often wavy (Figs. 6 and 7) and the endospore layer was relatively thick.

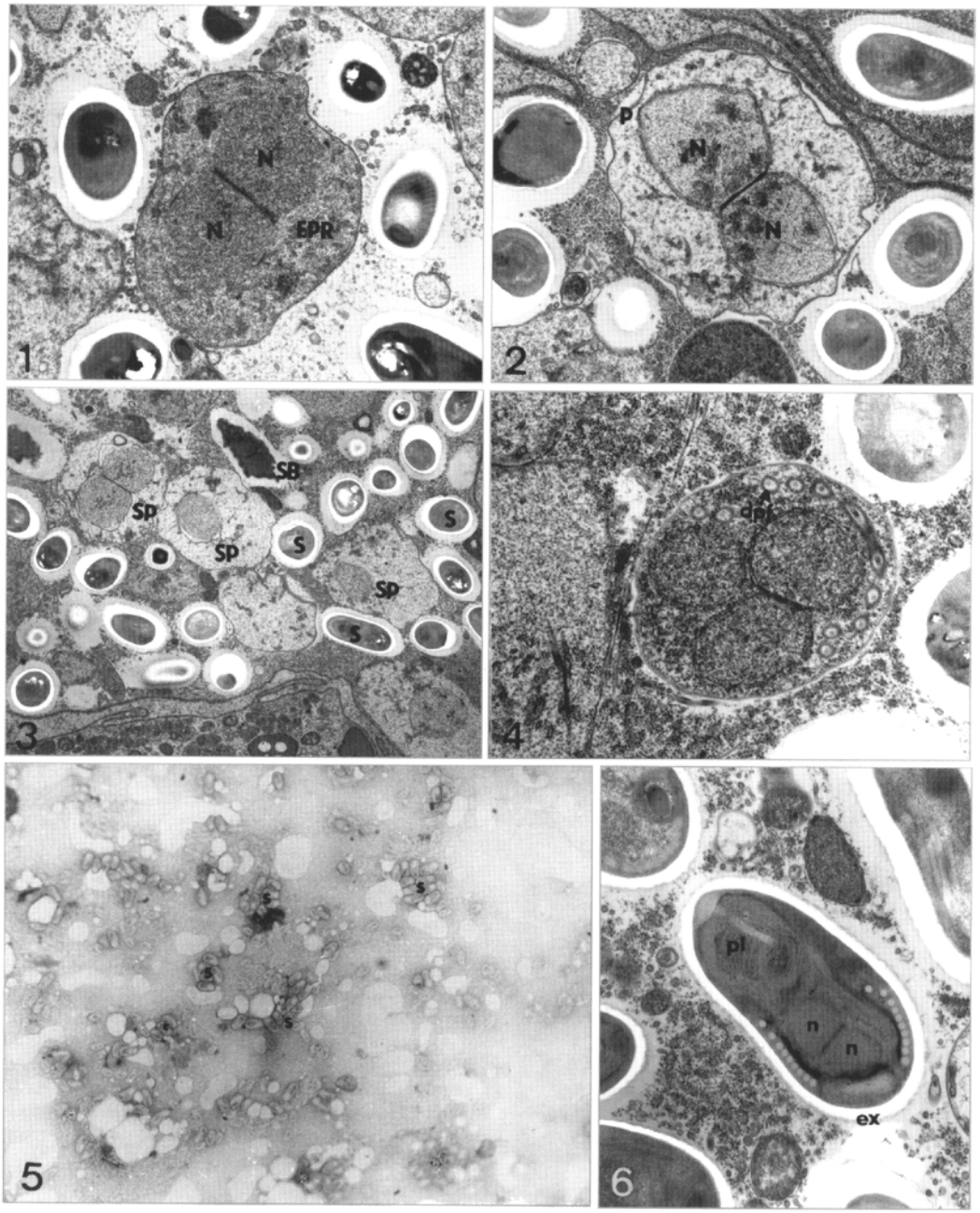
Although *Nosema* sp. is primarily a silk gland parasite, it also occurs in the fat body of its host. The infected silk glands appear swollen and white when examined macroscopically. The silk gland cells are typically hypertrophied and the nuclei are often destroyed (Fig. 9). Fat body tissues are less infected but may become heavily infected during late infection process, as described previously in *Nosema portugal* isolated from *L. dispar* (Maddox *et al.* 1999)

The *Endoreticulatus* sp. isolated from *E. chrysorrhoea* produced heavy infections in the midgut cells of *L. dispar,* but the *Nosema* sp. did not infect tissues of *L. dispar*. As *E. chrysorrhoea* is difficult to rear in laboratory colonies, no larvae were available to conduct the infection experiments with the natural host. For this reason, it was impossible to obtain any ultrastructural data or other information on the early developmental stages of the infection with *Nosema* sp.

Discussion

Diplokarya were present in all merogonial and sporogonial stages and in the spores and thus we consider the new microsporidian isolate from the Bulgarian population of *E. chrysorrhoea* to be a *Nosema* species. It does not form uninucle-

Fig. 1. Late merogonial stage of *Nosema* sp. with nuclei (N) in a diplokaryotic arrangement and endoplasmic reticulum (EPR) (× 5,500). **Fig. 2.** Early sporogonial stage of *Nosema* sp. with a diplokaryon. A thickening of the plasmalemma (P) is observed (× 4,500). **Fig. 3.** Sporonts (SP), sporoblasts (SB) and spores of *Nosema* sp. (× 2,300). **Fig. 4.** Sporogonial stage with presumably two diplokaryons and developing coils of polar filament (dpf) in a silk gland host cell (× 6,000). **Fig. 5.** Giemsa stained spores (s) (× 550). **Fig. 6.** Mature enviromental spore of *Nosema* sp. Diplokaryon (n), polaroplast (pl) and a wavy exospore (ex) are visible (× 9,900)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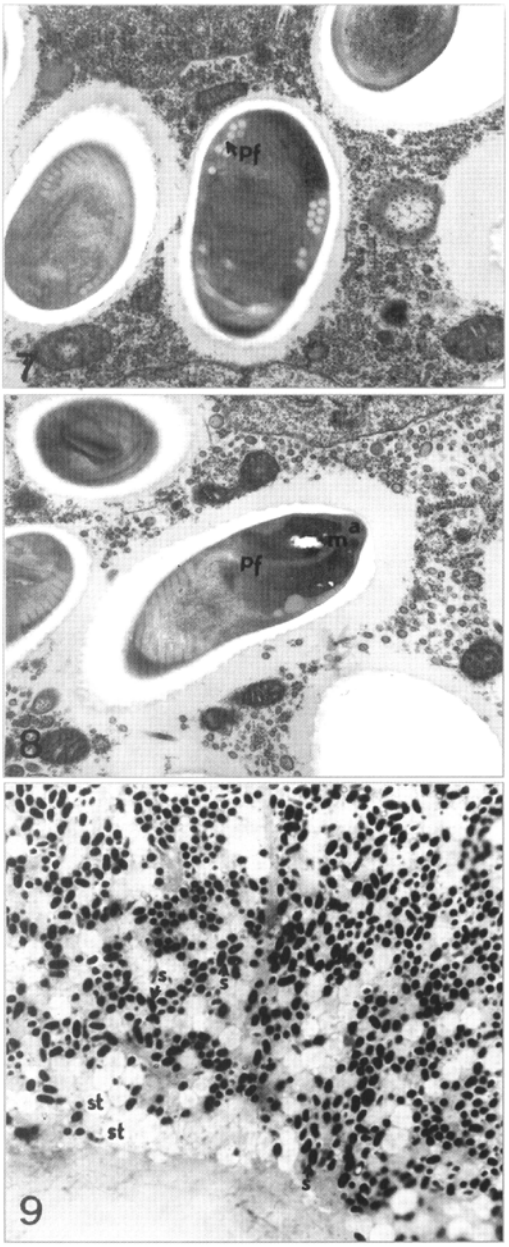

Fig. 7. Environmental spore of *Nosema* sp. with coils of polar filament (pf) arranged in two rows (× 9,900). **Fig. 8.** Spore of *Nosema* sp. with mushroom-shaped anchoring disc (a) and manubrium (m) attached to the polar filament (pf) (× 9,900). **Fig. 9.** Semithin sections of silk glands heavily infected with *Nosema* sp. spores (s) and sporogonial stages (st) after staining with toluidine blue (× 550)

ate spores in an octosporous sporulation cycle, which would be characteristic for placement in the genus *Vairimorpha* (Pilley 1976).

Microsporidia in the genus *Nosema* have two sporulation sequences, a primary and a secondary cycle (Iwano and Ishihara 1991; Iwano and Kurti 1995; Solter and Maddox 1998a, b). Primary spores are distinguished by a thin exospore and fewer coils of the polar filament and are considered to be important for the spread of the infection within the host tissues. They occur for a brief period following oral infection. We did not detect the presence of primary spores because only later developmental stages were observed. We were not successful in our attempts to produce infections in an alternative host (*L. dispar*). This isolate, therefore, is not an *L. dispar* pathogen, despite its similarity to other isolates in the *L. dispar* from the *Nosema* group (Maddox *et al.* 1999, Solter *et al.* 2000).

The examined spores possess typical features of environmental spores of the lymantriid *Nosema* described by Maddox *et al.* (1999). They have a similar polaroplast and 9–10 isofilar polar filament coils. We can not explain the presence of two parallel rows of polar filament coils in several mature spores that we observed. It is possible, that these spores may represent another species of microsporidia that had also infected these field-collected hosts.

Lipa (1964) found that the size of the *Nosema* sp. from Poland varied from 4.5 to 8.2 µm in length and 2.0 to 3.5 µm in width. He also presented data about the infection of the midgut, fat body, Malpighian tubules, silk glands, hemocytes and hypodermis. According to Purrini and Weiser (1975), *Nosema kovacevici* also showed great variability in spore length, from 2.5 to 6.2 µm. Sidor *et al.* (1975), however, recorded 4.2 × 2.6 µm spore size for the *Nosema* sp. from Macedonia and showed that it infected the fat body, hypodermis, hemocytes and midgut of *E. chrysorrhoea.* In our material the maximum length of *Nosema* sp. spores was 5.45 µm.

It was difficult to compare our results concerning the site of infection with those described by Lipa (1964), Purrini and Weiser (1975), and Sidor *et al.* (1975). At the time these papers were published, the existence of two spore types, primary and environmental spores, was not yet recognised. It is not clear, therefore, whether the hypodermis, hemocytes and midgut tissues reported as infected were populated by the primary or the environmental spores.

Our results on the morphology and developmental life cycle of the Bulgarian *E. chrysorrhoea* microsporidium suggest that this *Nosema* isolate has not been described. Further investigations of the general life cycle as well as molecular phylogenetic data are needed in order to provide a formal description of this isolate as a new species.

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