

A Light and Electron Microscopic Study of *Larssoniella resinellae* n. gen., n. sp. (Microspora, Unikaryonidae), a Parasite of *Petrova resinella* (Lepidoptera, Tortricidae) in Central Europe

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Summary: *Larssoniella* n. gen. (Microspora, Unikaryonidae) is characterized by uninucleate stages with a tuft of tubules arising in the cuticle of sporoblasts and in the posterior pole of the young spore in its exospore. *L. resinellae* n. sp., the type species, has sporoblasts and spores with distinct tubular tufts. Mature spores are smooth, tubular (4.5–5×1.7–2 µm), uninucleate, with polar filament in 10/11 coils. It infects the silk glands, Malpighian tubules, the fat body and gonads of larvae and adults of the Pine resin gall moth, *Petrova resinella*.

Key Words: Microspora; *Larssoniella* n. gen.; *Petrova*; Tortricidae.

Introduction

The Pine resin gall moth, *Petrova resinella*, is a common pest of ornamental pine in urban areas. Resin galls are formed in shoots of this year and the adults appear at the end of May till end of June. Infected shoots are in the lower parts of trees. Each gall is inhabited by a single caterpillar. A microsporidian infecting the caterpillars is described in this paper.

Material and Methods

Galls with caterpillars were collected in May and caterpillars were dissected. Individuals with the microsporidian were inspected in watermounts for localization of the infection in tissues. Dry smears were fixed with methyl alcohol. Parts of tissues were fixed in Bouin's fixative for preparation of paraffin blocks. Other fresh infected organs were fixed in 3% glutaraldehyde in cacodylate buffer (pH = 7.2) for 12 h at 4 °C. Material was washed and stored in buffer at 4 °C till refixation in 2% osmic acid in the same buffer and processed in Vestopal W. Ultrathin sections were stained in uranyl acetate and lead citrate. Semithin sections were used for histology after staining in toluidine blue. Paraffin blocks were

cut in sections 4–6 µm thick and stained with Giemsa stain. Dry smears were stained with Giemsa, parts of smears were hydrolyzed with 10% HCl and restained with Giemsa in a procedure proposed by WEISER (1976).

Results

10% of caterpillars were infected. The mortality of infected animals was low, and the infection remained in pupae and in adults. 40% of the microsporidia-positive larvae hosted larvae of hymenopteran parasites. The parasitoids were not infected.

The organs with primary development of the microsporidian were the silk glands, followed by Malpighian tubules. Later the infection enters the fat body and isolated centres appear in the connective tissue. The midgut epithelial cells and goblet cells are not infected although the infection entering per os with food had to cross the gut wall. In late pupae and adults the microsporidian spreads in the connective tissue ensheathing the gonads.

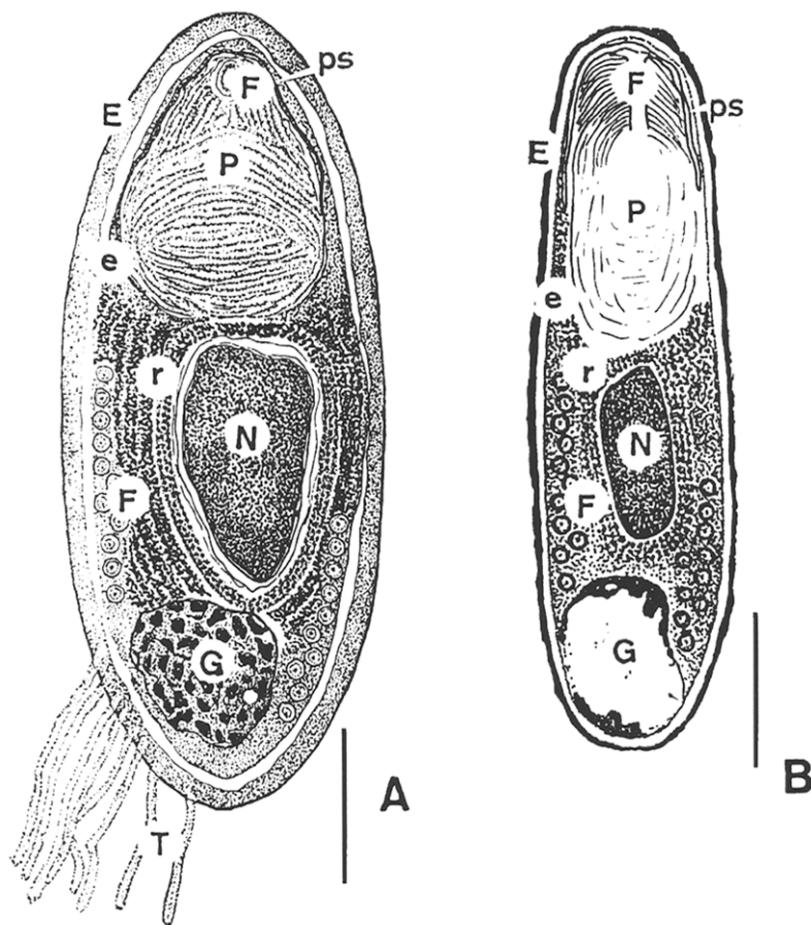


Fig. 1. Spores of *Larssoniella resinellae*. A young spore; B mature spore. E – exospore, e – endospore, F – polar filament, G – Golgi, N – nucleus, P – polaroplast, ps – polar sac, r – ribosome basket, T – tubules tuft. Bar = 1 μm . Drawing after details of ultrathin sections.

In detail, the silk gland is infected in flat infiltrations of the central parts of the cells (Figs. 2 and 3), without hypertrophy of the infected cells and without direct contact of the pathogen with the internal channel of the gland. Vegetative stages in some cases adhere to the nuclei of glandular cells. Vegetative stages and spores are disposed in a system of confluent cavities (Fig. 3, V). The silk in the gland does not contain any spore material.

The Malpighian tubules are exposed to a heavy infection and mature spores fill the whole cytoplasm of the epithelial cells. The infection in its early stage is visible as opaque white spots. In the final stage a mass of spores fills the space between the outer and interior membrane of the tubules (Fig. 6). Some tubules in infected caterpillars are completely filled with spores, other tubules are free of infection.

The development in the fat body is much reduced, minute centres of development are spread over the tissue, but they do not fill up the infected cells at the end of larval stage. The fat globules in infected cells remain unchanged.

Hemocytes (plasmacytocytes, granulocytes) of infected larvae seen in smears and in sections do not harbour

phagocytized stages of the microsporidian. In cases where parasitoids were present, some spores were in teratocytes accompanying the development of the parasite. There was no evidence of immunological interactions (nodule formation, deposits of melanin) in these cases, and no nodules were formed in the fat body and the body cavity. Neither segmental muscles nor circular muscles of the midgut were infected.

In pupae and adult moths the microsporidian invades connective tissues, especially the sheets of the gonads and spores were present in egg follicles. Maturing eggs were not destroyed. Spores were present also among the bundles of sperms in the spermatophores.

As evident from sections (Figs. 2 and 3) the developmental stages of the microsporidian are in irregular vacuoles. Schizonts are uninucleate oval stages, 2.5–3 μm in diameter, binucleate stages are only dividing schizonts. Some schizonts adhere to cell nuclei (Fig. 3, N) and eventually enter the nuclei. Contacted nuclei are not hypertrophic. Uninucleate sporoblasts are elongated, 2–3×4–5 μm . Spores are single, elongated to tubular, 1.7–2×4.5–5 μm . Dried on smears the spores are broader than fresh in watermounts, 2–2.5 μm . In HCl-hydrolysed material a single elongated nucleus is

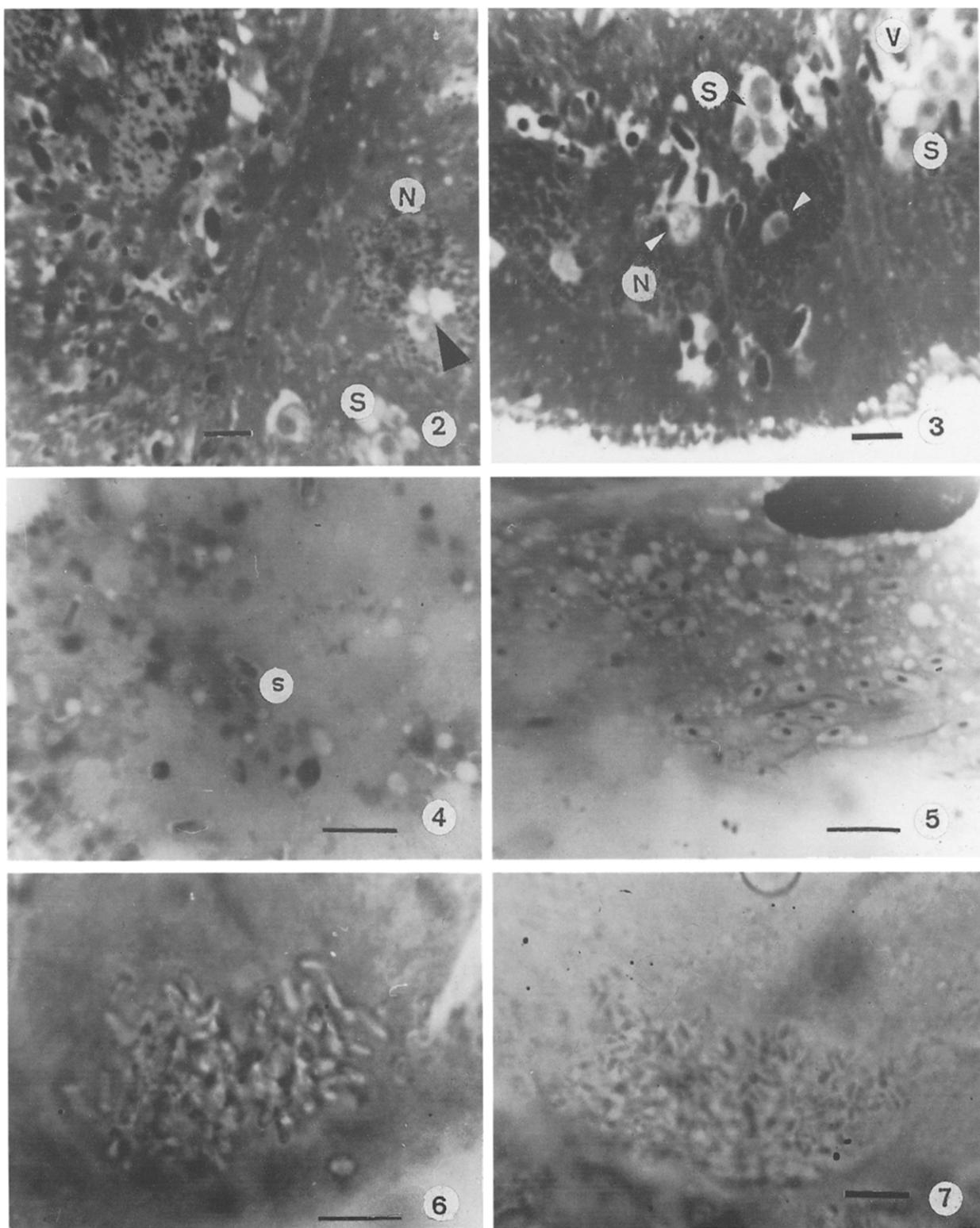


Fig. 2. Schizonts (S) and spores of *Larsoniella* in the infected silk gland. In one nucleus (N) of the host cells developing schizonts (arrowhead). Bar = 5 µm.

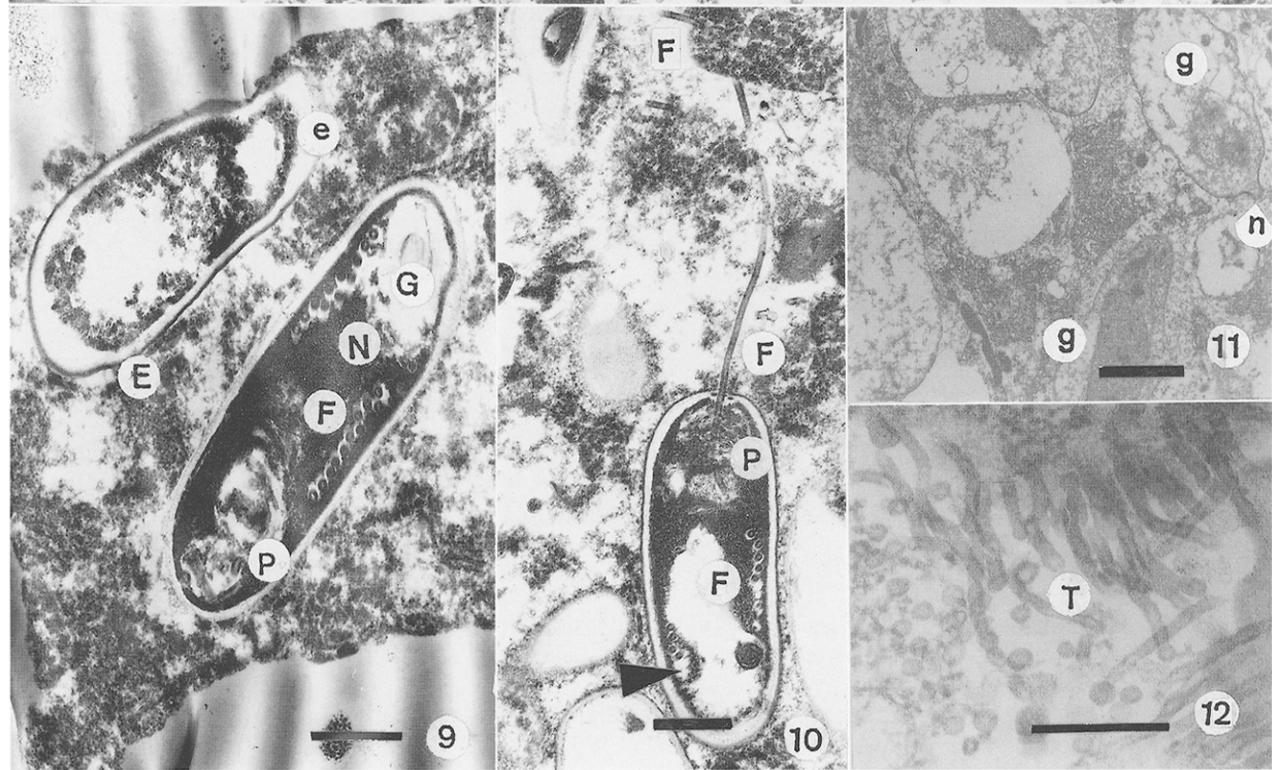
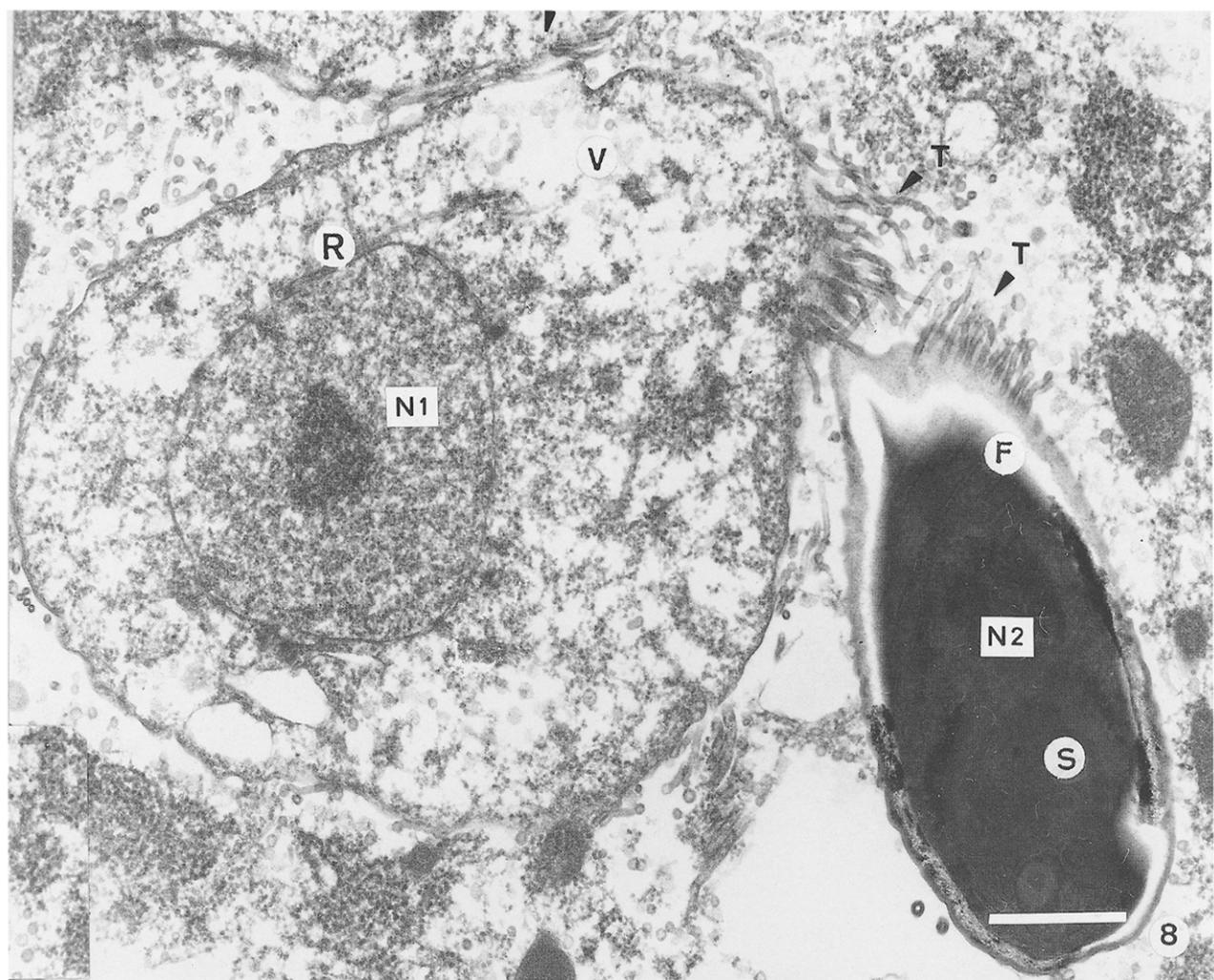
Fig. 3. Schizonts of *Larsoniella* (S) in lysed vacuoles (V) close and inside of nuclei of the silk gland cells (N). Bar = 5 µm.

Fig. 4. Spores and vegetative stages of *Larsoniella* in dry smear stained with Giemsa. In spores (s) terminal vacuoles. Bar = 10 µm.

Fig. 5. Spores of *Larsoniella* with nuclei stained after HCl hydrolysis. Bar = 10 µm.

Fig. 6. Mature spores in Malpighian tubules. Bar = 10 µm.

Fig. 7. Spores on the gonads of adult *P. resinella*. Bar = 10 µm.



stained in the central part of the spore (Fig. 5). The variability in size of the spores is evident in Figs. 4 and 5. The stages of the microsporidian are usually stained with difficulties.

In electron micrographs the youngest stage is the early schizont (Fig. 11) with an evident nipple, an elongated stage with thin covering membrane and single nucleus close to the nipple. Next to this stage is the elongated schizont in the same frame. There is not difference in the structure of the plasmatic membrane between schizonts and early sporonts except that on one pole of the broad oval uninucleate sporont a zone of long microtubules is formed. The tubules are 50 nm in diameter and up to 1 µm long in groups (bands) of 200 and more, fixed in distances of 100–200 nm in the membrane of the sporont (Fig. 8, T). The tufts of tubules are not closed by any membrane and they are formed only on the pole which in further development forms the posterior pole of the spore. The tubules are in the young spore arising in the plastic layer of the exospore (Fig. 8), during the maturation of the spore they disappear. Mature spores have a rigid endospore, 100–150 nm thick, and the exospore forms an electron-dense granulated layer, 50 nm thick. The interior of mature spores is dense, the structures are difficult to differentiate. The polar filament is anchored in a flat disc (Figs. 1 and 10), protected by a thin polar sac (ps in Fig. 1). Its posterior end shows 10/11 coils. The single oval nucleus is situated in the central part, enclosed in a basket of polyribosomes. The Golgi system is in the posterior end of the young spore, in the mature spore it is usually empty, and the spore is depressed in this area (Figs. 1 and 9, G). The polaroplast is rather long, filling the first part of the spore (Figs. 1 and 9, P). It is composed of parallel lamellae in both parts of the complex. In sections a certain number of spores is empty (Fig. 9), in some cases the polar filament is extruded (as result of fixation?) (Fig. 10).

Discussion

The solitary life of the caterpillars of *Petrova resinella* does not support the transmission with fecal materials where spores are present from infected Malpighian

tubules. Transmission via the egg provides localized infections in limited populations. Parasitoids do not participate in the transmission, and there is no direct coincidence between infections and parasitism.

There is also no evidence of any larval mortality connected with the microsporidian, and the old galls contain just remains of larvae and pupae infected with parasitoids.

In the invaded silk glands empty zones are formed around developing stages of the microsporidian (Fig. 3, V) and in electron micrographs is no evidence of any parasitophorous vacuole with any limiting membrane. In other infections of silk glands by microsporidia, such as *Vairimorpha ephestiae* in *Galleria mellonella* (see WEISER & PURRINI 1985), the infected cells are hypertrophic, the spores are released from bursting cells into the channels of the glands. There is no vacuolization of the cells and the nuclei remain unchanged. In an analogous infection of salivary glands of midges with *Helmichia glandulicola* (see WÜLKER & WEISER 1991) the infected cells are hypertrophic, all filled with spores and with some hypertrophy of their nuclei with polythene chromosomes. In this second case there is no vacuolization around the developing microsporidian. In all three cases the contact relations between the microsporidian and the host cells are different.

In the development of the microsporidian in *Petrova resinella* early stages, analogous to the gametes observed by HAZARD et al. (1985) in *Culicospora magna* and in *Microsporidium* sp. in *Aedes aegypti*, have a nipple-like structure (Fig. 11, n). Another stage presented in the same figure (Fig. 11, g) is morphologically very close to the gamonts of *Hazardia milleri* reported in the same publication. The gamont-like stages, as first steps in development of the microsporidian after release of the planont, were recognized earlier only in the Amblyosporidae, but are evidently common also in other groups of microsporidia. Further development is a schizogony, nuclei are all single, diplokarya are not present and the typical stages for this microsporidian are late schizonts, eventually sporonts, with the tuft of tubules. The ultrastructures of these tubules are close to tubules appearing in *Lanatospora tubulifera* presented by BRONNVALL & LARSSON (1995) for *Janačekia debaisieuxi* (LARSSON 1983). In both

Fig. 8. Sporont and young spore of *L. resinellae*. N1 – nucleus with nucleolus, T – tuft of cuticular tubules. R – endoplasmic reticulum, V – vacuolized area close to the tubules. S – young spore with tuft of tubules (T) arising in the posterior pole of the spore. F – coiled polar filament, N2 – nucleus of the spore. Bar = 1 µm.

Fig. 9. Mature spores of *L. resinellae* with thin exospore (E) without tubules and rigid endospore (e). Polaroplast (P) with polar filament (F) activated, with 11 coils. Nucleus (N) not distinct, Golgi in empty vacuole. Bar = 1 µm.

Fig. 10. Mature spore with polar filament extruded during fixation procedure. Polaroplast (P) is activated, and filament (F) is extruded with its apical end. Uncoiling starts with last coils (arrowhead). Bar = 1 µm.

Fig. 11. Early schizonts (gamonts) (g) of *L. resinellae*, one with nipple (n). Bar = 2 µm.

Fig. 12. Structure of tubules on sporoblasts of *L. resinellae* (T). Bar = 1 µm.

microsporidia tubular structures are arising from the exospore, and they persist for some periods as a surface cover of spores. In the cited cases the tubular structures are closed under a membranous cover. In our microsporidian the tubules are not closed in any membranous cover neither in the sporont nor in the young spore. In contrary, deposits of secretions in pansporoblasts of octospores of *Vairimorpha ephestiae* are temporary products secreted from sporonts during divisions of the plasmodium in octospore formation, and their direct origin on the surface of developing sporonts is not visible (WEISER & PURRINI 1985).

With uninucleate spores, a regular cover of a thin exospore and endospore, an isofilar polar filament in 11–12 coils adhering to the spore wall, and with the tuft of tubules arising on the sporont and early spores, the microsporidian in *Petrova resinella* does not fit in any described genus of microsporidia. In *Janačekia* and *Lanatospora* the tubules are distributed over the whole surface and closed in a covering membrane. Spores of *Hirsutosporos* (BATSON, 1983), with four tufts of lateral filaments used for floating, and *Ringueletium* (GARCIA, 1990), with appendages distributed over the whole spore surface, have diplokarya. We propose therefore to present the microsporidian in *Petrova resinella* as a type species for a new genus. The definition is as follows:

Genus *Larsoniella* gen. n.

Schizogony uninucleate, schizonts simple, in cavities in the cytoplasm of host cells. Sporonts with tufts of tubules arising in the membrane of the stages and maintained on the exospore of young spores on their posterior end. Mature spores long oval, endospore thin, exospore thin, regular, without remains of tubules. Polar filament isofilar, coiled in 10–12 coils. Anchoring disc flat, polar sac thin, adhering to spore wall. Polaroplast lamellar in both parts, anterior and posterior. Nucleus single, oval in central part of the spore. Posterior end with Golgi, later with vacuole. Infecting silk glands, Malpighian tubules and gonads of Lepidoptera. The genus belongs to the family Unikaryonidae SPRAGUE.

The generic name is proposed in honour of Dr. RONNY LARSSON, the specialist in microsporidia taxonomy at the Lund University, Sweden.

Type species: *Larsoniella resinellae* sp. n.

Larsoniella resinellae sp. n.

Type host and site of location: *Petrova resinella* (L.) (Lepidoptera, Tortricidae), silk glands, Malpighian tubules, fat body, gonads. In larvae, pupae, adults and eggs.

Locality: Ornamental pine, urban areas Prague, Třeboň, Czech Republic.

Transmission: Peroral with infected feces; infected eggs.

Interface: Cytoplasm of host cells, adhering to nuclei, irregular empty spaces with vegetative stages and spores, without membrane.

Development: Round uninucleate schizonts 3–4 µm in diameter. Stages corresponding with gamets, with nippule present. Sporonts without apparent thickening of outer membrane. Sporoblasts and young spores with posterior tuft of tubules 50 nm in diameter, up to 1 µm long. Up to 200 tubules in one tuft. Tubules disappear during spore maturation. Mature spores long oval to tubular 4.5–5×1.7–2 µm, with posterior vacuole visible in fresh spores. After HCl hydrolysis an elongated single nucleus is distinctly visible. Polar filament is isofilar, coiled posteriorly in 10/11 coils, with inapparent flat anchoring disc and thin polar sac. Polaroplast in both parts composed of flat lamellae.

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